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Genetic diversity of baobab (*Adansonia digitata* L.) along an elevation transect in Kenya

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DECLARATION

I, Anna Chládová, hereby declare that this thesis, submitted in partial fulfilment of requirements for the master degree in Faculty of Tropical AgriSciences of the Czech University of Life Sciences Prague, is wholly my own work written exclusively with the use of the quoted sources.

In Prague 2016

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Anna Chládová

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ABSTRACT

Adansonia digitata L. (Malvaceae) is a huge multipurpose tree of the savannahs of sub-Saharan Africa, with high economic potential for local communities. The edible fruits and leaves are known for their high nutritional values and can be used fresh or processed. However, a high intra-specific variability regarding morphology, genetics and nutritional content of baobab and its products is documented for several African regions, while data for Kenya is largely lacking. This study aimed at documenting the genetic and morphological variability of baobab accessions in Kenya and at checking the presence of the newly described diploid baobab species *Adansonia kilima*. Samples were collected from 204 baobab trees from seven populations defined by geographical distance in South-eastern and Coastal Kenya at altitudes of 6-1,058 m asl. Leaf or bark samples for genetic diversity assessment were collected from all 204 trees, while leaves only from 65 and fruits from 76 trees (all in inland locations) for morphological analyses based on the publication 'Descriptors for Baobab'. Nine microsatellite loci were used to assess genetic variation and results analysed with specific software because of the tetraploid nature of baobab.

Overall genetic diversity was high and all loci were polymorphic. The mean gene diversity was 0.803 and observed heterozygosity was 0.907. Analysis of molecular variance (AMOVA) revealed low variation among populations (12.4%) and high variation within populations (87.6%). Bayesian clustering and Principal Coordinate Analysis divided the accessions into two clusters, one with only inland and one with coastal accessions. Although the presence of *Adansonia kilima* was previously postulated for Kenya, flow cytometry did not detect any among the analysed samples as only tetraploids were observed. Regarding morphological characteristics, no differences among the fruit accession from inland populations were found (no fruits were collected in coastal areas). Leaf morphological data showed significant differences between inland and coastal populations with longest leaflets and leaf petioles in accession from the Coast, thus confirming the results obtained for genetic analysis.

This study contributes to the overall knowledge of the genetic diversity of baobab in Kenya and can contribute to the development of germplasm conservation strategies and domestication programs for baobab.

Keywords: baobab, *Adansonia digitata*, genetic diversity, microsatellites (SSR), morphology

ABSTRAKT

Adansonia digitata L. (Malvaceae) je mohutný víceúčelový strom ze savan subsaharské Afriky, s vysokým ekonomickým potenciálem pro místní obyvatele. Jeho jedlé plody a listy jsou známé pro svou vysokou nutriční hodnotu a mohou být využity čerstvé nebo zpracované. Studie zabývající se morfologií, genetikou a nutričním složením baobabů jsou převážně zaměřeny na západoafrické regiony, zatímco údaje pro Keňu do značné míry chybí. Cílem této studie bylo zdokumentování genetické a morfologické variability baobabu v Keni a ověření přítomnosti nově objeveného druhu *Adansonia kilima*. Vzorky byly sebrány z 204 stromů ze sedmi populací v nadmořské výšce 6-1058 m n. m. Všech těchto 204 vzorků (kůry nebo listů) bylo podrobeno analýze pro zjištění genetické diverzity. Pro morfologickou analýzu byly použity listy z 65 stromů a plody ze 76 stromů (tedy pouze vzorky sebrané ve vnitrozemí) a byly vyhodnoceny na základě publikace 'Descriptors for Baobab'. Devět mikrosatelitních lokusů bylo použito k posouzení genetické variability a výsledky byly vyhodnoceny pomocí speciálních softwarů uzpůsobených tetraploidní povaze baobabu.

Byla zjištěna vysoká genetická diverzita a všechny lokusy byly vysoce polymorfní. Průměrná genetická diverzita (He) byla 0,803 a očekávaná heterozygotnost (Ho) byla 0,907. Analýza molekulární variance (AMOVA) ukázala nízkou variabilitu mezi populacemi (12,4%), ale vysokou v rámci populace (87,6%). Bayesiánské shlukování a analýza hlavních koordinát rozdělily všechny populace do dvou klastrů: jeden složený pouze z vnitrozemských populací a druhý obsahující pouze pobřežní populace. Ačkoli přítomnost nového druhu *Adansonia kilima* byla v Keni potvrzena, průtoková cytometrie nezjistila přítomnost *A. kilima* mezi analyzovanými vzorky. Co se týče morfologických charakteristik, nebyly zjištěny žádné rozdíly mezi plody v rámci vnitrozemských populací (v pobřežních oblastech nebyly sebrány žádné plody). Morfologie listů ukázala významné rozdíly mezi vnitrozemskými a pobřežními populacemi. V pobřežních oblastech mají stromy delší listy a listové řapíky než u vnitrozemských populací. Morfologické údaje korelují s genetickými výsledky. Tato studie přispívá k celkovým znalostem genetické diverzity baobabu pro východní Afriku, a může přispět ke konzervačním účelům a k domestikaci tohoto druhu v Keni.

Klíčová slova: baobab, *Adansonia digitata*, genetická diverzita, mikrosatelity (SSR), morfologie

TABLE OF CONTENTS

1 INTRODUCTION 1
2 LITERATURE REVIEW 3
2.1 Population genetics of tropical trees 3
2.1.1 Maintenance of genetic diversity 3
2.1.2 Genetic diversity of *Adansonia digitata* L. across Africa 4
2.1.3 Tree domestication 5
2.1.4 Assessing genetic diversity by molecular markers 7
2.2 Baobab (*Adansonia digitata* L.) 9
2.2.1 Taxonomy 9
2.2.2 Ploidy of Adansonia digitata 11
2.2.3 Origin and distribution 12
2.2.4 Ecology 14
2.2.5 Morphology 14
2.2.6 Phenology 16
2.2.7 The use - Baobab and society 17
2.2.8 Chemical composition 19
2.2.9 Cultivation 20
2.2.10 Pests and diseases 22
2.2.11 Marketing 23
2.3 Study area 24
2.3.1 Climate of Kenya 24
3 OBJECTIVES 26
4 MATERIALS AND METHODS 27
4.1 Sampling locations 27

4.2 Tree sampling and data collections 30
4.3 Genetic analyses 32
4.3.1 DNA extraction 33
4.3.2 SSR analyses 34
4.4 Determination of ploidy - Flow cytometry 37
4.5 Data analyses 38
5 RESULTS 39
5.1 Morphological evaluation 39
5.1.1 Tree morphological evaluation 39
5.1.2 Fruit morphological evaluation 42
5.1.3 Leaf morphological evaluation 46
5.2 Genetic analysis 48
5.3 Ploidy 55
6 DISCUSSION 57
6.1 Morphological comparison 57
6.2 Discussion to genetic analyses 58
6.3 Ploidy and species identification 61
7 CONCLUSION 62
8 REFERENCES 63

LIST OF TABLES

Table 1. Taxonomy of *Adansonia digitata* L. 10
Table 2. List of *Adansonia* species 11
Table 3. Chemical composition of baobab fruit pulp 20
Table 4. Agro-ecological zones of selected study sites 29
Table 5. Number of sampled trees, fruits and leaves 30
Table 6. SSR primers used for analysis 35
Table 7. PCR composition 36
Table 8. PCR profile 36
Table 9. Tree height, crown diameter and DBH 41
Table 10. Fruit qualitative data 43
Table 11. Fruit quantitative traits 45
Table 12. Leaf qualitative data 46
Table 13. Leaf quantitative data 47
Table 14. Gene diversity and allele frequencies per loci 48
Table 15. Gene diversity and allele frequencies per populations 49

LIST OF FIGURES

Figure 1. Gaps in the knowledge related to baobab domestication 6
Figure 2. Distribution of Adansonia digitata in Africa. 13
Figure 3. Baobab tree by Louise Jasper 15
Figure 4. Climate diagrams of four regions in Kenya 25
Figure 5. Map of Kenya 28
Figure 6. Tree shape, tree growth habit, and trunk shape 40
Figure 7. DBH size-class distribution of 195 baobabs 41
Figure 8. Fruit shape 42
Figure 9. Output from the program Structure 51
Figure 10. Evaluation of the similarity coefficient 51
Figure 11. Principal Coordinate Analysis 52
Figure 12. Spatial autocorrelation using Moran's I 54
Figure 13. Representative example of flow cytometer histogram 55
Figure 14. Photos of young baobab seedlings 56

1 INTRODUCTION

A multipurpose tree *Adansonia digitata* L. is, one of the most important indigenous fruit trees of sub-Saharan Africa, with great social and economic importance (Sidibé and Williams, 2002; Wiehle et al., 2014). Published studies focusing on the species *A. digitata* are mainly concentrated to Western Africa (Assogbadjo et al., 2006; Assogbadjo et al., 2009; Kyndt et al., 2009). Baobab research in Eastern Africa is lagging behind, however is slowly progressing. Up to now, investigation of genetic diversity of baobab in East Africa has been done in Sudan and Malawi (Munthali et al., 2013; Wiehle et al., 2014). Therefore, this study was focused to examine genetic diversity of baobabs in Kenya to increase our knowledge in this region. In general, the investigation of plant genetic diversity is crucial for conservation of germplasm and to set strategies for domestication. The stored genetic resources should be used for crop improvement and help solve food and nutritional security. The genetic diversity is considered the key pillar of biodiversity and diversity within species, between species, and of ecosystems (Govindaraj et al., 2015).

The African baobab is supposed to be domesticated in the Kenya. Domestication is a process undertaken by humans when plants are selected, bred and adapted for increasing of production and quality during cultivation. The domestication of annual crops began over 10,000 years ago, however most of the tree species are still found in the wild stage or early stage of domestication. Tree domestication may help with diversifying of farming systems, bring additional income to farmers and enhance nutrition intake of local communities (Jamnadass et al., 2012). The importance of molecular marker characterization in the context of agroforestry tree domestication is notable. Using molecular markers, it is possible to assess genetic variation among geographically distributed trees and consequently selection of superior trees for domestication may be easier, since the level of genetic diversity is known. In case of low genetic variation in already known populations, molecular markers can be used to discover highly diverse natural population suitable for tree improvement (Dawson et al., 2012).

There are known eight species of genus *Adansonia*, however only *Adansonia digitata* occurs in mainland Africa, the other species are native to Madagascar and one to Australia. Pettigrew et al. (2012) described a new species *Adansonia kilima* sp. nov. of mainland Africa. Considering the genetic aspect, *Adansonia digitata* is tetraploid in contrast to eight other *Adansonia* species all of which are diploid, even the newly described species *A. kilima*.

The main objective of my thesis was to assess morphological and genetic diversity of baobab along an elevation transect in South-East Kenya with the aim of germplasm conservation and baobab domestication in this region. Additionally, the presence or absence of new *Adansonia* species was tested.

2 LITERATURE REVIEW

2.1 Population genetics of tropical trees

2.1.1 Maintenance of genetic diversity

The morphological differences among individuals are usually assigned to site environment. Nevertheless, differences were recognized within same-age trees at same location, which led to the conclusion that the variation is based on genetic origin (Bosch et al., 2004). The importance of genetic diversity is notable; when a population of a species contains a large gene pool then it has a greater probability of surviving and thriving than a population with limited genetic variability (NGA, 1999). Investigation of plant genetic diversity is crucial to preserve genetic material for long period. Wider plant genetic resources may be maintained *in situ* (e.g. grown by farmers themselves) or *ex situ* in gene bank, DNA library etc. for further improvements of food and nutritional security. These stored genetic resources should serve for crop improvement and help solve food and nutritional security. In general, genetic diversity is considered the key pillar of biodiversity and diversity within species, between species, and of ecosystems (Govindaraj et al., 2015).

Billions of people (more than 25% of the world's population) rely on forest resources; especially in developing countries trees may provide food, fuel, shelter, medicine, income, improve soil, reduce erosion, etc. Due to overexploitation, inappropriate agricultural practices (as slash and burn), overgrazing by livestock, etc. the loss of forest biodiversity is notable (FAO, 2014). High level of genetic diversity of tropical tree species is important for avoiding an inbreeding depression and allowing trees to adjust to new environments (Dawson et al., 2009). The knowledge about the genetics of tropical trees is still limited, many tree species are subjected to poor germplasm collection practice. Support of seed and seedling exchange network among farmers should be enhanced to preserve proper genetic variation (Dawson et al., 2009).

2.1.2 Genetic diversity of *Adansonia digitata* L. across Africa

Research concerning baobab in Africa was mainly focused on West African countries, the knowledge about East Africa is rather limited. Wiehle et al. (2014) were the first to publish a study from East Africa where genetic and morphological variability of *A. digitata* was assessed. It was found out that genetic diversity is balanced, and even higher variability was detected in homesteads (positive effect of human intervention) in case of Sudan. On the contrary in Malawi, southeast Africa, low mean genetic diversity and moderate genetic differentiation among five populations was observed (Munthali et al., 2013). The SSR markers developed by Larsen et al. (2009) were used in the Sudan and Malawi research. More research was performed in West Africa. Samples from Benin, Ghana, Burkina Faso, and Senegal were divided into eleven populations and genetic diversity was assessed by AFLP method. It revealed relatively high levels of genetic structuring. The levels of genetic diversity were comparable among all populations (Kyndt et al., 2009). In Benin, six baobab populations distributed in three climatic zones showed substantial degree of genetic structuring that was revealed by AFLP markers (Assogbadjo et al., 2006). Samples from three West African countries, Benin, Ghana and Senegal were subjected to AFLP analyses to reveal if genetic fingerprinting correlate with the traditional morphological identification of *A. digitata*. This theory was not confirmed therefore they concluded that morphotype-based approach in the collection of genetic variation for conservation or domestication programs is not recommendable (Assogbadjo et al., 2009). Chloroplast DNA fragments obtained from individuals covering the entire extant distribution range of the species were analysed to reconstruct the phylogeographical history of the species and detect the centre of origin. Phylogeographical pattern showed three main baobab groups; two are assigned to West Africa and one group to southern and eastern Africa (Pock Tsy et al., 2009).

2.1.3 Tree domestication

Tree domestication is a process of selection, management and propagation of trees by humans i.e. scientists, authorities, farmers, companies etc. Not much attention is being paid to tree species present in agricultural landscape (agroforestry), in comparison to research concerning natural forest management, commercial tree improvement and forest plantation. Tree domestication in agroforestry should improve tree products such as timber, fruits, or medicine (Simons and Leakey, 2004). Additionally, it should improve local environment by reducing erosion, improving soil fertility, or increasing species biodiversity.

There are several steps to be performed during tree domestication. After choosing the right tree for our purpose, the next step is to determine species variation. It includes field trials (studying germination process in laboratories or establishing vegetative propagation protocols, and field experiments), provenance and progeny trials, studying the relationship between phenotype, genotype and environment and molecular marker characterisation of genetic diversity. Afterwards, it should be possible to obtain quality germplasm (seed, seedlings and clones) and deliver it to farmers (Dawson et al., 2012).

One of the priority tress for domestication from Sahel region of Sub-Sahara Africa is the baobab whose domestication process has already begun in West Africa. In Mali, fruit traits were examined (as fruit morphological traits, content of vitamin C, calcium, iron etc.) to establish selection criteria for domestication. It was found out that fruit and pulp weights and pulp fraction were influenced mainly by environmental factors (Simbo et al., 2013). There is a research group in Burkina Faso, Mali and Niger, where undergoing baobab domestication by research actions in plant physiology, population genetics, tree breeding, food science, and socioeconomics is done. Jensen et al. (2011) proposed knowledge gaps related to baobab that is necessary to overcome to properly domesticate this species (Figure 1). Comparative study of West and Southeast African countries (Mali and Malawi) was performed in order to help select superior materials for cultivation. Significant differences between countries in fruit characteristics were observed. It was also described that in hotter environment the pulp percentage is lower, fruits are more spherical with

smaller seeds than in wetter conditions, where higher pulp percentage is present (Cuni Sanchez et al., 2011). Domestication process and breeding of many plants usually decreased their genetic pool, thus lowering their resilience to environmental stresses. The knowledge of genetic diversity of any species could help us to maintain its genetic resources and set up strategies for its domestication. The crucial step for domestication of *A. digitata* is to determine genetic diversity that would allow scientists to choose representative individuals for breeding purposes.

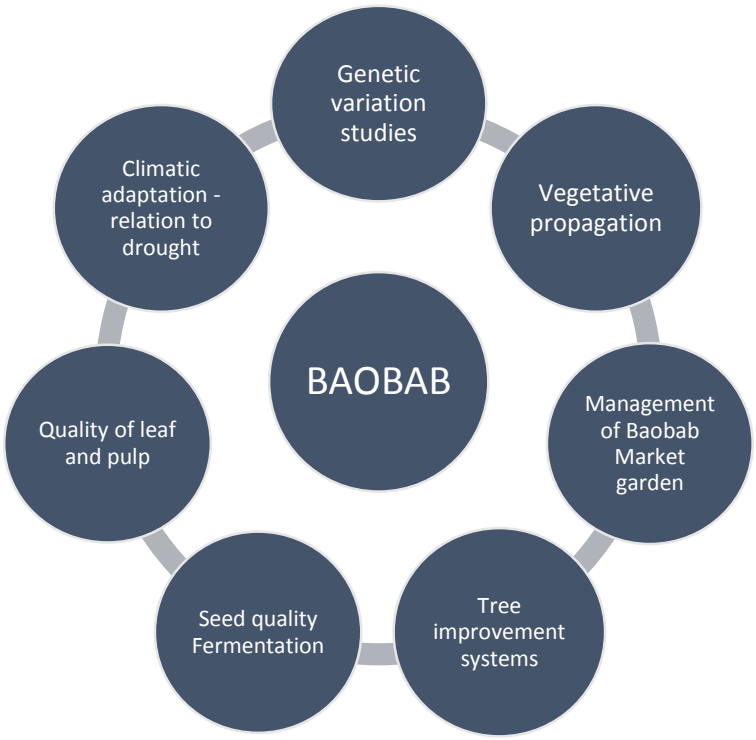


Figure 1. Gaps in the knowledge related to baobab domestication (Jensen et al., 2011)

2.1.4 Assessing genetic diversity by molecular markers

The assessment of genetic diversity may be performed by following techniques: morphological markers, biochemical markers, molecular markers. The most widely used, are molecular markers. Definition of molecular marker is following: "*A molecular marker can be defined as a genomic locus, detected through probe or specific starter (primer) which, in virtue of its presence, distinguishes unequivocally the chromosomic trait which it represents as well as the flanking regions at the 3´ and 5´ extremity*." (Govindaraj et al., 2015). There are two types of molecular markers: dominant and codominant. Codominant means that it is possible to recognize homozygotes and heterozygotes, dominant markers do not allow this and therefore are not as informative (Govindaraj et al., 2015). Codominant markers are more appropriate for molecular ecological studies (Larsen et al., 2009).

Microsatellites or simple sequence repeats (SSR) are molecular markers based on PCR (Polymerase Chain Reaction) with attributes such as hypervariability, multiallelic nature, codominant inheritance, reproducibility, relative abundance, extensive genome coverage, chromosome specific location and much more (Parida et al., 2009). SSR are tandem repeated motifs of base pairs (bp) which frequently occur in genomes of prokaryotic and eukaryotic organisms, present in both coding and noncoding regions distributed throughout the nuclear genome (Zane et al., 2002; Kalia et al., 2011). In plants there are mainly present AT bp repeats, whereas in animals AC repeats are more common. This fact is probably the main genome characteristic that differentiates between plants and animals (Powell et al., 1996). The evolution of SSR is in fact any change in SSR by increasing or decreasing repeat number (Kalia et al., 2011). This increasing or decreasing of bp number is caused by single-stranded DNA slippage, double stranded DNA recombination, mismatch/double strand break repair, and retrotransposition (Wang et al., 2009). These variations in lengths of the microsatellites show polymorphism (Kalia et al., 2011). The application of SSR in plant sciences is huge: gender identification, hybridization and breeding, transgenics, population genetics, taxonomic and phylogenetic studies, functional genomics, genome mapping,

diversity and cultivar analysis etc. (Kalia et al., 2011). Such a large range of applications makes microsatellites the most widely used molecular markers nowadays.

In this study, SSR-primers (Microsatellites) are used to evaluate genetic diversity. Also the impact of anthropogenic influence and climatic fluctuations may be detected by differentiations between populations (Larsen et al., 2009).

2.2 Baobab (*Adansonia digitata* L.)

"Baobab trees are living monuments, the oldest natural things in Africa, outlasting every plant and animal around them. These trees have evolved formidable resilience in order to survive in some of the driest, rockiest areas of old continent." (Watson, 2007). In Namibia, investigation by radiocarbon dating of baobab called Grootboom was done. Results indicated an age of about 1,275 years. However, Grootboom collapsed unexpectedly in late 2004 (Patrut et al., 2007). Patrut et al. (2013) found out by radiocarbon investigation that there is even an older specimen, it is Glencoe baobab in South Africa with age 1835 ± 40 years which makes it as the oldest tree among angiosperms. *A. digitata* L. is a multipurpose tree, one of the most important indigenous fruit trees of sub-Saharan Africa, with great social and economic importance (Sidibé and Williams, 2002; Wiehle et al., 2014). Large number of vernacular names are known according to wide geographical distribution of this species; in English language it is known as Baobab, monkey-bread tree, dead-rat tree or upside down tree. In Swahili language, which is second official language in Kenya after English, are used names mbuyu and majoni (Kehlenbeck et al., 2015).

2.2.1 Taxonomy

The name baobab is probably coming from the 16th century when the Venetian herbalist and physician Alpino described fruits with medicinal properties. He used name Bahobab, probably derived from the Arabic "bu hibab," meaning "many-seeded fruit". Linnaeus used the name Adansonia, in honour of Michel Adanson, botanist who firstly described *A. digitata* in detail, in the mid-18th century. The species name digitata (hand-like) is connected to the shape of the leaves (Baum, 1995; Kamatou et al., 2011).

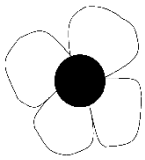
Nowadays *Adansonia digitata* L. belongs to family *Malvaceae* based on APG taxonomy classification (Table 1). However, in some literature sources can still be found family *Bombacaceae* which follows the Cronquist taxonomic classification system (Bosch et al., 2004).

Table 1. Taxonomy of *Adansonia digitata* L. (Missouri Botanical Garden, 2015)

Class	*Equisetopsida* C. Agardh
Subclass	*Magnoliidae* Novák ex Takht.
Superorder	*Rosanae* Takht.
Order	*Malvales* Juss.
Family	*Malvaceae* Juss.
Genus	*Adansonia* L.
Species	*Adansonia digitata* L.

There are known eight species of genus *Adansonia* (Table 2). Native species in mainland Africa is ***A. digitata*** L. from where it has been distributed throughout the tropics by humans. ***A. gregorii*** F. Muell., is the only Australian species endemic to the Kimberley region. Six species are endemic to Madagascar: ***A. grandidieri*** Baill., ***A. suarezensis*** H. Perrier***, A. rubrostipa*** Jum. and H. Perrier, ***A. za*** Baill., ***A. madagascariensis*** Baill., ***A. perrieri*** Capuron. Such a species distribution makes Madagascar the centre of diversity (Baum, 1995; Pettigrew et al., 2012). In addition, Pettigrew et al. (2012) described a new species ***Adansonia kilima* sp. nov.** of mainland Africa. To distinguish *Adansonia digitata* from other species of the genus is not a difficult task owing to clear diagnostic characters as the pendulous flower, the globose buds and broad petals. Moreover, the rounded crown and irregularly distributed branching are also useful diagnostic characters (Sidibe and Williams, 2002).

Table 2. List of *Adansonia* species with chromosome number, basic flower characteristics and pollination (n= the gametic or haploid number), modified from (Baum and Oginuma, 1994; Pettigrew et al., 2012)

Species	2n	Size and colour of flower	Pollination
Adansonia perrieri	88		Hawk moth pollinated
A. madagascariensis	88		
A. za	88		
A. rubrostipa	88		
A. suarezensis	88		Mammal pollinated
A. grandidieri	88		
A. gregorii	88		No information
A. kilima	88		Mammal pollinated
A. digitata	160		

2.2.2 Ploidy of Adansonia digitata

Adansonia digitata is presumed to be autotetraploid unlike other *Adansonia* species which are diploids (Pettigrew et al., 2012). Autotetraploid organisms have four sets of chromosomes, which are produced by the duplication of the chromosomes of a single diploid species. It is known that polyploidization has an effect on plant morphology; number of chromosomes is higher than in diploid individuals, which influences the size of the nucleus as well as the size of the whole cell. Therefore, increases in size of plant organs, as

flowers, stomata, pollen etc., are notable for polyploid species. It was found out that *Adansonia kilima* (diploid) differs from *A. digitata* (tetraploid) by having flowers half the size, smaller and denser stomata, smaller and more densely spinose pollen, fewer free staminal filaments (Pettigrew et al., 2012). Besides distinguishing two African baobab species by morphological characteristics, revealing ploidy can be done by flow cytometry (FC), which is a method of measuring nuclear DNA content in plants. Accuracy, speed and reliability makes FC the prevailing method for ploidy level analyses (Dolezel et al., 2007). Looking for the diploid species *Adansonia kilima* in this study was performed by FC. Hypothetically, it should be possible to distinguish *A. digitata* and *A. kilima* from resulting histograms of fluorescence intensities (Dolezel et al., 2007).

2.2.3 Origin and distribution

Considering the genetic aspect, *Adansonia digitata* is tetraploid in contrast to other species all of which are diploid (Table 2), even a newly described species *A. kilima*. There are two hypotheses trying to describe the origin of this genus (Pettigrew et al., 2012). The first one says that the original species of *A. digitata* were diploid with the centre of origin in Africa and tetraploidy was evolved after the divergence of the Australian and Malagasy lineages. The second hypothesis describes that *A. digitata* ancestors are not from Africa and tetraploidy was evolving during or after dispersal to Africa. Latest research has discovered a new diploid species *A. kilima* in Africa, which may be diploid progenitor line thus the first hypothesis could be empirically supported (Pettigrew et al., 2012). Pock Tsy et al., (2009) studied chloroplast DNA of 344 individuals across Africa to reconstruct the phylogeographical history of *A. digitata* and suggested that centre of origin is in West Africa.

Baobab is associated with the savannah, especially the drier parts. It occurs naturally in most countries south of the Sahara (Figure 2), however it is absent in Liberia, Uganda, Djibouti and Burundi. Nevertheless, there are some extensions of the distribution into forest areas, e.g. Gabon and Democratic Republic of the Congo, probably associated with human habitation. In other countries, baobab is grown mainly as an ornamental tree like in

Mauritius, Java, New Caledonia, Hawaii, the Philippines, Cuba, the USA (Florida), etc. (Wickens, 1982).

Baobab in Kenya is distributed below the 1,000 m a.s.l. elevation (Wickens, 1982). Mainly in *Acacia-Commiphora* bushland and scrub and the semi-desert grasslands of northern Kenya. It is also reported from the Kenyan coastal zone on breccia in association with *Ehretia petiolaris, Fagara chalybea, Grewia plagrophylla* and *Heeria mucronata* (Wickens, 1982). Sidibe and Williams (2002) mentioned that populations are coastal as well as scattered in lowland bush and scrub.

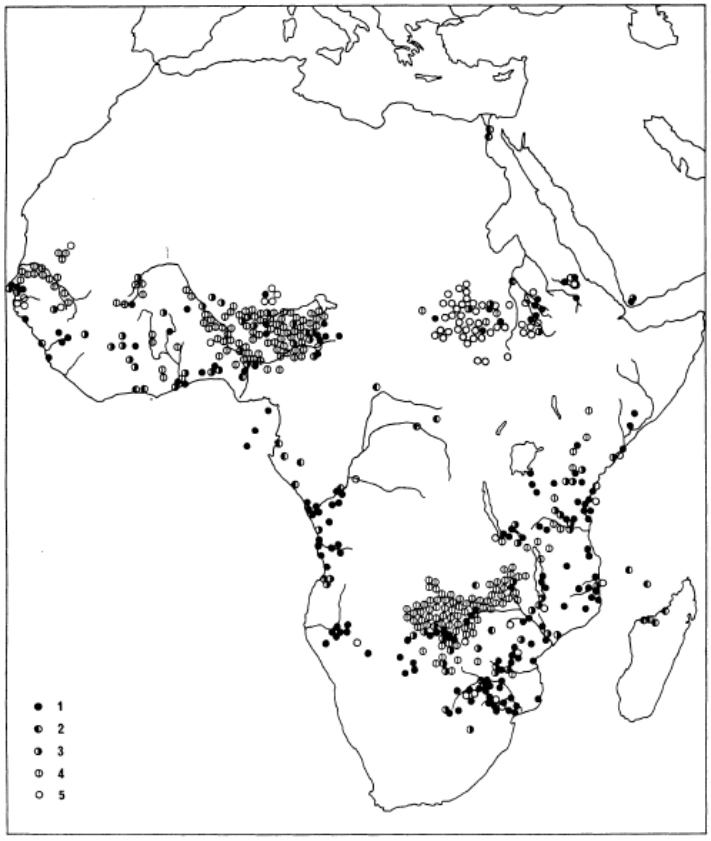


Figure 2. Distribution of Adansonia digitata in Africa and neighboring areas.

1 Distribution based on Herbarium and flora records; 2 Specimens known to be cultivated or introduced; 3 Distribution based on published and unpublished photographs; 4 Distribution base on the Kew 'Baobab Survey' information 5; Records obtained from travel literature, maps. (Wickens, 1982)

2.2.4 Ecology

The baobab is widespread throughout the hot, drier regions of tropical Africa. It survives well in dry climates and is resistant to fire, however seedlings and small trees are susceptible to fire (Gebauer et al., 2002). It occurs on a wide range of vegetation types; including scrub, wooded savannah, dry areas, and semiarid to sub-humid tropics south of the Sahara. Evidence from the coastal areas is also reported e.g. in Tanzania (FAO, 1993). It grows best at altitudes of 450–600 m.a.s.l. with an annual rainfall of 300–500 mm. However, it is also common in areas with an annual rainfall of 200–800 mm. In the southern part of its area of distribution it is found mostly on north facing slopes that protect trees against cold southern winds because even mature trees can be killed by severe frost (Bosch et al., 2004). Water economy of baobab is considered very effective; in literature they are often cited as water storing trees. The wood can contain about 64% water by volume. The daily loss of water is only about 2% of their total water content (Fenner, 1980). Due to their efficient water economy, baobabs are also highly adapted to drought. The trunk of the tree contracts when the environment becomes dry and it expands in the wet season; there is marked increase in the circumference of a baobab after heavy rainfall. It was reported that the thick bark is fire resistant and has regenerative powers (Sidibe and Williams, 2002).

Sandy top soils overlaying loamy substrates are preferred, but baobab is absent on deep sands for reason of incapacity to get enough moisture, even though it can tolerate poorly drained heavily-textured soils. Baobab withstands both acid and calcareous soils. It is also found on rocky hillsides, on sites receiving run-off, or where water accumulates (FAO, 1993; Gebauer et al., 2002).

2.2.5 Morphology

The baobab is a massive deciduous tree with typically swollen and stout trunk reaching to height of 18-25 m and producing a rounded crown with a stiff branching habit (Figure 3). The trunk diameter is up to 10 m, some old individuals reach girth up to 28 m. Trunk shape is usually tapering and abruptly bottle-shaped. Young branches are tomentose (having hairs), rarely glaborous (having no trichomes). The bark is smooth, reddish brown or greyish

and it can be described as wrinkled like an elephant's skin. Below the outer layer of the bark lies a green layer which has probably photosynthetic activity when the tree has shed its leaves (Wickens, 1982). Hollow centres in trunks of old and large baobabs are often occurring due to natural causes or as a result of human intervention. The root system is extensive with high water holding capacity. It is relatively shallow (<1.8 m) but well spread which is the best adaptation system for the climate where baobab grows; exploiting the low annual rainfall, most of which falls in the form of infrequent heavy showers (Gebauer et al., 2002).

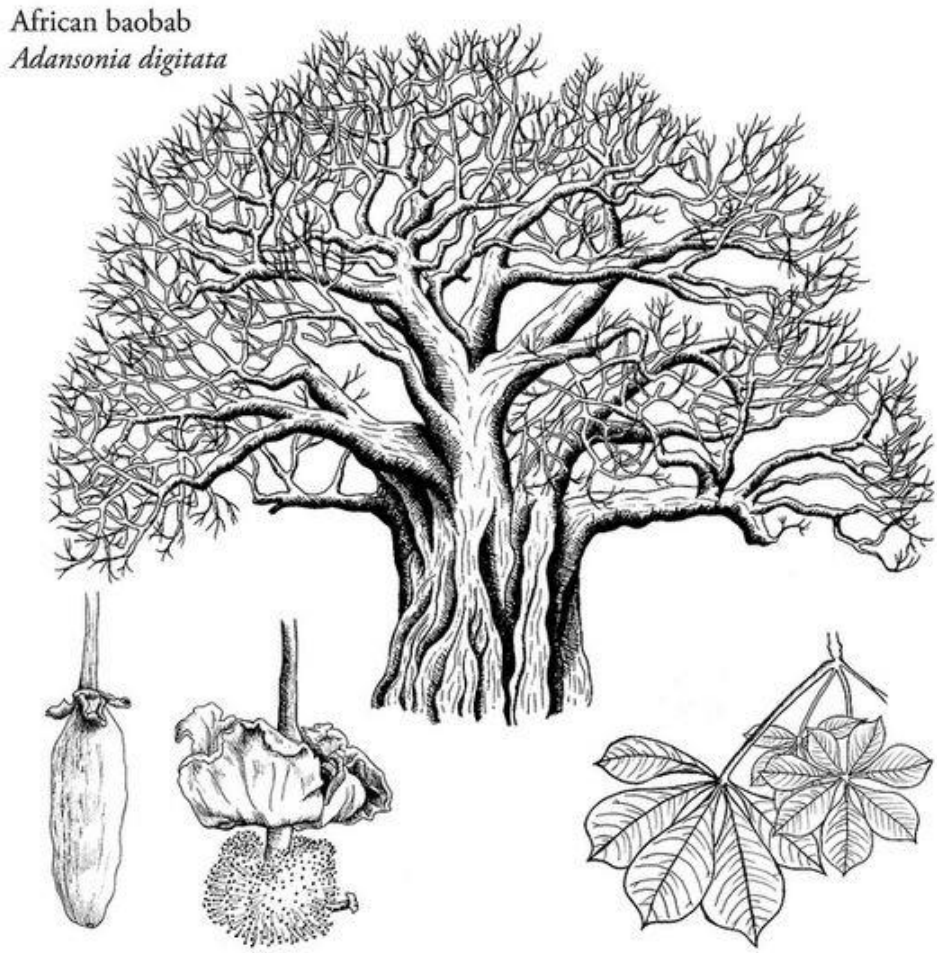


Figure 3. Baobab tree by Louise Jasper

Digitate (hand-shaped) **leaves** are 2-3-foliate in young stage, more mature ones are 5-7(-9)-foliate; the size of medial leaflet is 5-15 x 2-7 cm. Leaflet shape is elliptic to obovate-elliptic, apex is acuminate and base is decurrent, margins are usually entire. Leaflet is sessile or shortly petiolate. Leaf hairiness, observed on the upper side of the leaf, can occur. Simple leaves are occurring on young plants. Leaves alternate on branches; leaf petiole length is up to 16 cm long (Sidibe and Williams, 2002; Kehlenbeck et al., 2015).

Very showy bisexual **flowers** are solitary or paired in leaf axils. Pedicels are usually very long; 15-90 cm. Flower bud is globose with conical to apiculate apex. Calyx consist of 5 cup-shaped hairy sepals. White corolla has 5 partite, petals overlapping and are hairy inside. The androecium is made up of 720-1,600 stamens forming a tube. The ovary is 5-10 locular, conical to globose. The style exceeds (about 15 mm) the anthers. The stigma is white with 5-10 lobes which are irregular (Sidibe and Williams, 2002).

In general, the **fruits** are very variable in size and shape; usually globose to ovoid but sometimes oblong cylindrical indehiscent capsules, which hang on a long stalk. The range of size is 7.5-54 cm long x 7.5-20 cm wide. Woody pericarp (shell) is 8-10 mm thick, outer layer is covered by velvety greenish-brown hairs. Inside, the capsule contains a dry mealy pulp. In the pulp the reniform seeds are embedded. One fruit contains about 100 brownish seeds (Gebauer et al., 2002; Sidibe and Williams, 2002).

2.2.6 Phenology

Four principal growth phases are recognized in the development of *A. digitata*: sapling phase (up to 10–15 years), cone phase (up to 60–70 years), bottle phase (up to 200–300 years) and an old age phase (up to 500–800 years). The growth is fast at the initial phase, especially in the cone phase, but older trees grow very slowly (Von Breitenbach, 1985). The trunk thickens rapidly, attaining 4-5 m in diameter after 100 years. Swollen trunk and digitate leaves do not characterize seedlings and saplings of baobabs, as in the case of adult trees. Sapling phase has typically simple leaves with combination 2-foliolate and 3-foliolate leaves. Adult trees have characteristic digitate leaves, however observation in Sudan by Wickens (1982) describe that first leaves in a season are simple, then shed and replaced by

typically digitate ones. In Kenya, the leafy period appears to be confined to the rainy season (Wickens, 1982). The baobab spends only four months of the year in leaf. Therefore, the photosynthesis takes place also in the green layer just below outer surface of the bark (Gebauer et al., 2002). However, a study carried out in Benin observed that the baobab retains its leaves almost throughout the year (Assogbadjo et al., 2005).

First flowering reported in South Africa was at 16-17 years, in Zimbabwe at 22-23 years (Wickens, 1982). Different ages of first flowering may be a reflection of differences in climatic regimes. Flowers can occur during both wet and dry seasons. Flowering time is not defined accurately, except for the height of the dry season, flowers can be found anytime throughout the year whether leaves are present or not. The buds begin to open from the late afternoon, fully open flowers are found the following morning; the calyx and corolla lobes curl back to expose the stamens. The lifespan of the flowers is not more than 24 hours with pollination period of 16-20 hours. The pendulous flowers of baobabs are adapted for pollination by bats, which are attracted by strong carrion smell, however pollination by bluebottle, nocturnal moths or wind pollination may also be possible. Fruits are ripe five to six months after flowering (Wickens, 1982).

2.2.7 The use - Baobab and society

African people have been using baobab for centuries. It is strongly connected to their lives, traditions and knowledge. Many stories relating to the baobab have grown up. A lot of them explain the bizarre appearance of the tree; one Arab legend about upside-down tree recounts the story that the devil pulled out the tree and thrust the branches into the soil and left the roots in the air. Another story from the Kilimanjaro tells of vain, fat and wrinkled baobab beside a pool. It was jealous of the more colourful neighbouring trees reflected in the water and continuously complained to God, who got angry and seized the baobab and planted it upside down (Wickens, 2008).

Baobab is a multi-purpose tree providing mainly food, medicine, and fibre. Almost all tree parts (fruit pulp, seeds, leaves, flowers, roots, bark) are edible (Rahul et al., 2015). The following text summarizes the main utilization of the tree:

The pulp is eaten fresh; it is added to gruels or ground pulp serves to make refreshing drinks. For retaining the high vitamin C content, it is important not to boil the pulp. Fruit pulp is usually sundried and can be stored for relatively long periods in appropriate containers. Storage is improved by the use of sodium metabisulphite. Fresh **leaves** are consumed directly as vegetable or are used for cooking. They can be also dried, powdered and in this form added to several meals. The leaves should be dried in shade (not full sun) and preservation of whole leaves than powdered ones is preferable to conserve high levels of pro-vitamin A (Sidibe and Williams, 2002). The survey, done in Mali, confirmed that leaves are consumed preferably in dry season than rainy season. The results also indicated that leaves are more consumed in rural areas (Nordeide et al., 1996). Young shoots, roots, raw flowers are eaten as well (Bosch et al., 2004). Thickening agent in soups is the main utilization of the **seeds**. Fermented seeds are used as flavouring agent. Roasted seeds are eaten as a snack or may be used as substitute for coffee. Seeds are also a source of cooking oil. Oil is nowadays used also in cosmetics (Sidibe and Williams, 2002). The research published by Modiba et al. (2014) reported the production of biodiesel from baobab seed kernel oil, its fuel properties and evaluation of its potential.

There is a notable amount of non-food products from baobab. Bark provides strong **fibre** which is widely used for making rope, basket nets, snares, fishing lines etc. Fibres from disintegrated wood or root bark may be also extracted. In East Africa, red or green **dyes** (from roots and bark) are extracted for decorations. From woody **fruit shells** pots for food and drink or decorative purposes are made. In Tanzania, fruit shells are used as fuel instead of baobab wood, which is not suitable as source of fuel. Baobab leaves can be fed to livestock as well (Sidibe and Williams, 2002).

Medicinal purposes; traditional healing practices are connected to baobab in Sub-Saharan Africa. It has been used to treat ailments such as diarrhoea, malaria, and microbial infections. Antimicrobial, antiviral, anti-oxidant and anti-inflammatory activities are also known for baobab (Kamatou, et al., 2011). Due to its excellent antioxidant activity mediated by high vitamin C content in fruit pulp (which is seven to ten times higher than the vitamin

C content of oranges), it may help to prevent a variety of serious diseases (Kamatou, et al., 2011). The most widely used plant part is traditionally bark; its decoction can serve as quinine substitute, antipyretics, prophylactics, etc. Powdered leaves can be used to treat dysentery, fatigue, insect bites, guinea worm, and internal pains. Seeds and seed oil may be also used (Sidibe and Williams, 2002).

2.2.8 Chemical composition

Baobab fruit pulp has low water content which brings benefits - there is no need for processing. It is also generally low in fat. It is well known for high vitamin C content. Nevertheless, values among individuals vary considerably which may be due to different genetic and environmental factors as well as different ripeness stages of the fruit samples. Baobab pulp has very high energy, available carbohydrate, fibre, ash, vitamin C, calcium, magnesium and potassium contents (Table 3) (Stadlmayr et al., 2013). There is a high antioxidant activity in fruit pulp and leaves but more significant in the pulp than in the leaves. The leaves contain good quality proteins and are considered rich in calcium. The seeds (whole seeds and the kernels) have a relatively high lipid content (Chadare et al., 2009).

The research done by Assogbadjo et al. (2012) in Benin, investigated the biochemical composition of baobab (pulp, leaf and seed) in relation to soil types and tree provenances. It was demonstrated that physiochemical characteristics of the soil had significant influence on the nutritive value of baobab parts. Highly basic soils, which are rich in carbon, clay, fine silt and organic matter have probably positive effect on concentration of iron, potassium, vitamin C, carbohydrates, zinc, proteins and lipids. Nevertheless, the same soils influence negatively the concentration of magnesium, calcium, vitamin A and fibre in baobab parts. Reverse effect was found for gross silt and sandy soils.

Table 3. Chemical composition of baobab fruit pulp (Gebauer et al., 2002)

Constituents (dry weight basis)	
Total soluble solids (%)	79.3
Alcohol insoluble solids (%)	57.3
Total sugars (%)	23.2
Reducing sugars (%)	19.9
Total pectin (% galacturonic acid)	56.2
Protein (% N)	2.6
Fat (%)	0.2
Crude fibre (%)	5.7
Ash (%)	5.3
Ascorbic acid (mg/100 g)	300.0
Iron (mg/100 g)	8.6
Calcium (mg/100 g)	655.0
Phosphorus (mg/100 g)	50.8
Moisture	6.7
pH	3.3

2.2.9 Cultivation

Baobabs are still not widely planted despite their economic value, because of the long maturation period before trees start to produce the fruits and due to difficulties in germination of untreated seeds (Wickens, 2008). Naturally, the seeds, after falling of the fruit to the ground and freeing via termites, are dispersed by animals e.g. monkeys, squirrels and rats. Humans, birds, large animals or water systems take part in seed dispersal as well. Transplanting natural seedlings has been a traditional method of propagation. However, there is not a high occurrence of seedlings in the wild due to intensive browsing by livestock or environmental conditions such as severe drought. Therefore, seed collection is required.

It can be done by collecting fruit from the ground, climbing the trees or using poles and sticks (Sidibe and Williams, 2002).

Because of a hard seed coat, seeds require pre-treatments before sowing. An effective technique is to soak the seeds in concentrated sulphuric acid for 6-12 hours to scarify the seed coat. After this treatment the germination rate is more than 90% (Danthu et al., 1995). Esenowo (1991) used scarification with HNO_3 or H_2SO_4 and reached 86% and 98% germination rate, respectively. Trials done by the Forest Research Institute in Mali gave germination rates of 92% and more by using sulphuric acid for 90 minutes followed by water rinsing for 24 hours (Sidibe and Williams, 2002). Alternatively, manual scarification by cutting, or cutting followed by soaking seeds in cold water, or boiling the seeds for a few minutes in water is possible and is reported to enhance germination, however there is a risk of damage (SCUC, 2006). Subsequently, seeds are sown in beds, pots or polybags. Emergence is usually 4-6 days after sowing. When young plants reach age of 3-4 months (at height 40-50 cm) they are ready for transplanting. Planting should be managed at the beginning of the rainy season. Recommended spacing is 10x10 m, hole size is 60x60x60 cm. Protection against animal grazing and fire is necessary (Sidibe and Williams, 2002). Methods of vegetative propagation were also reported. Promising results were accomplished by the use of stem cuttings and grafting (Sidibe and Williams, 2002); grafted trees have lower height and shorter time of first flowering.

There is little requirement for management. Organic and mineral fertilizers can be applied; however, mineral fertilizers are costly and not always available in rural areas. However, application of organic fertilizers as manure, compost, green legume manure is recommended, especially for young trees. Mature trees demand very little pruning. If trees are mainly planted for leaf production, more attention has to be paid to shortening branches at the end of rainy season. Irrigation of trees is not necessary, nevertheless if present, better growth and shorter dormant period were observed (SCUC, 2006). First harvest of fruits depends on the planting technique; when trees are propagated via seed, first bearing of fruit may take 10-23 years. A tree propagated by grafting will start to bear

some fruits as early as 3-4 years (SCUC, 2006). Harvesting of leaves is done by climbing to the tree using tools sickle, dolé or hand. Fruits are harvested using dolé, or sticks or picking fruit by hand (Sidibe and Williams, 2002).

There are scarce data for baobab yield. According to Arum (1989) baobab produces as much as 200 kg of fruit per season, however great variation among trees occurs. This variation is connected to environmental conditions, genotypes but also age of the trees. Adult trees produce 8 times more fruit than sub-adult trees (Venter and Witkowski, 2011). Different land-use systems were compared to baobab production. It was discovered that there is no significant difference among systems, but if there are baboons, fruit loss can reach up to 85%. Baobab present in villages and fields produce slightly more fruits than in plains, rocky outcrops and natural reserves (Venter and Witkowski, 2011).

Some agroforestry practices are used during baobab cultivation. Intercropping with pearl millet, or other cereals or vegetable can be employed. Advantages of this method are weed control, aeration of soil and diversifying of production, main disadvantage is competition between species. Other possible agroforestry practice may be using baobabs as windbreaks (SCUC, 2006).

2.2.10 Pests and diseases

Serious pests or diseases are not recorded to affect baobabs. Nevertheless, insect attacks and some fungal and viral diseases have been noticed. In West Africa, many baobabs close to cotton or cocoa plantations have been eliminated because baobabs are hosting pests that affect these plantations. Elephants and livestock can seriously damage trees by browsing, affecting more young plants (Wickens, 1982; Sidibe and Williams, 2002). Some of the pests are following: the cotton bollworms (*Heliothis armigera*, *Diparopsis castanea* and *Earias biplaga*), cotton-stainer bugs (*Dysdercus* spp., *Odontopus* spp.), flea beetles (*Padagrica* spp.). Also mealybugs may be present (Wickens, 1982).

2.2.11 Marketing

Considering marketing, fresh leaves, fruits, craft products and fibres (from bark) are sold in local markets in many African countries. The price vary considerably due to seasonality, here are provided some examples from Sahel region (SCUC, 2006):

- Fresh leaves 0.06-0.18 USD per kg, sold during rainy season
- Dried leaves 0.09-0.18 USD per kg sold in local market
- Whole fruits 0.18-0.46 USD per kg in local markets, 6.4 USD per kg for export
- Pulp powder 0.73-0.91 USD per kg

Baobab has a high potential for international market and may bring benefits to both sides; for local people increasing considerably their income and for consumers representing new source of highly nutritive products. Extracted oil from seeds serves as a substance for cosmetic products. The fruit pulp is usually sold powdered and can be used for different culinary purposes (SCUC, 2006). In Eastern Africa, baobab is considered as underutilized (North et al., 2014). In Kenya, large quantities of fruit are harvested and sold in coastal areas, semi-arid areas of east part of the country. Coloured pulp is sold as snack in big towns like Nairobi, Mombasa, Malindi, etc. (Mbora et al., 2008). However, further research steps are still required to fully recognize the contribution to local food security and livelihoods in East Africa. These steps are already happening; the project BAOFRUIT, funded by the German Federal Ministry of Education and Research, had a following objective: “Exploration of sustainable use and commercialization of products based on baobab to improve food and nutrition security and to combat rural poverty in East Africa” (North et al., 2014). Dried baobab fruit pulp has been accepted, in 2008, as a novel food ingredient in the European Union and for the US market. This allowed growing market of baobab products is in EU and the USA (North et al., 2014)

2.3 Study area

The study was done in the coastal and inland regions of the Republic of Kenya that is a country located in East Africa with the total area of 580,370 km^2. It is bordered by Uganda to the west, Tanzania to the south, Somalia and Indian Ocean to the east, Ethiopia to the north and South Sudan to the northwest. The population is about 45 million people. It is divided into 8 provinces and 70 districts. The altitude varies from sea level to Mt. Kenya with 5,199 meters above sea level. Arid and semiarid areas cover approximately 80% of the country, while 17% are estimated as land with high agriculture potential and only 3% is covered by forest. Main sector of Kenya's economy is agriculture, which employs 74% of the economically active (FAO, 2015).

2.3.1 Climate of Kenya

Kenya is located in East Africa. Therefore, the climate is tropical, however due to the higher altitudes, south-west part of the country is more moderate. The coast meets with the Indian Ocean and does not reach high altitudes, the climate there is humid and hot. In central highland regions occur considerably lower temperatures than in the coast (15° C for the highest point of central highland region and 29 °C for the coast region) (McSweeney et al., 2010). The average annual rainfall is 630 mm, however with high variations; in the dry north areas it is around 200 mm and on the slopes of Mt. Kenya annual rainfalls can reach 1,800 mm (FAO, 2015). The migration of Inter-Tropical Convergence Zone (ITCZ) is responsible for seasonal rainfall. ITCZ is a narrow belt of very low pressure, which is created by mixing of trade wind from north and south hemisphere, and results in heavy precipitations. The ITCZ changes its position throughout the year, in Kenya it migrates southwards in October to December (occurrence of short rains) and returning northwards from March to May (occurrence of long rains). The precipitation during this season is from 50-200 mm per month, however, it fluctuates every year due to Indian Ocean sea surface temperatures and fluctuations of precipitations are also influenced by El Niño (brings higher rainfall) and La Niña (brings drier periods) (McSweeney et al., 2010). To support the statements mentioned above, climate diagrams of four regions are provided (Figure 4).

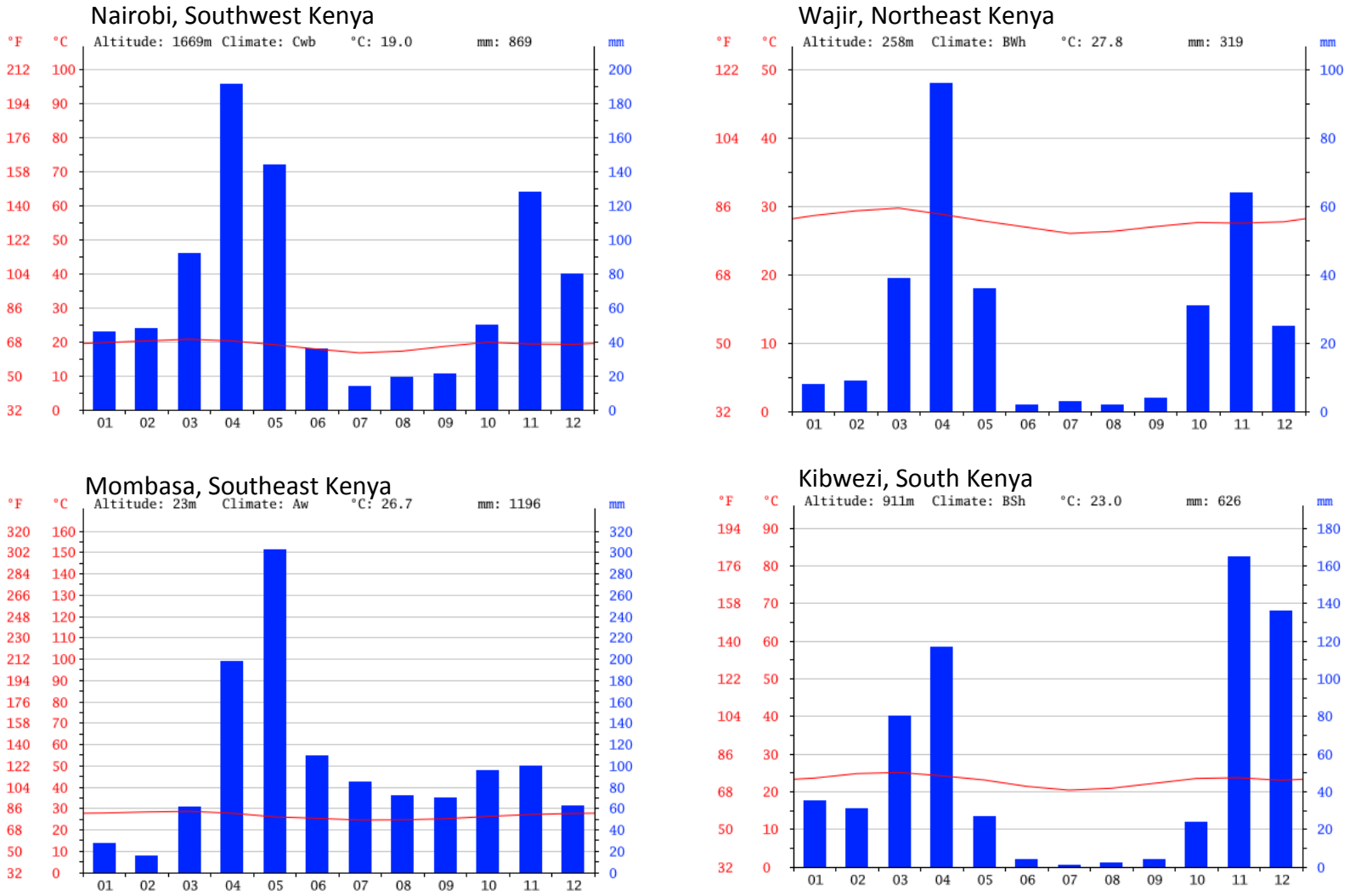


Figure 4. Climate diagrams of four regions in Kenya: Nairobi, Mombasa, Wajir and Kibwezi. Source: http://en.climate-data.org/

3 OBJECTIVES

The main objective of the thesis was to determine the level of genetic diversity of baobab trees in two different ecological zones of South-Eastern Kenya.

The specific objectives were:

1. To evaluate the genetic and morphological diversity among and within population from different geographical regions.

2. To examine the relationship of trees inside population and to assess relationship of trees growing close together.

3. To assess morphological characteristics and correlate them with genetic diversity.

4. To verify the presence or absence of new species - *Adansonia kilima*.

We set following research questions:

- How is the genetic diversity within and across populations?
- What is the relationship among trees growing close together? How much are trees related to each other?
- How is the correlation between morphological tree characteristics and genetic diversity?
- If an occurrence of *A. kilima* in Kenya is confirmed, what is its distribution?

4 MATERIALS AND METHODS

4.1 Sampling locations

The study was carried out in two geographically and ecologically different regions: the Eastern inland province - Makueni County and in coastal province - Taita Taveta Country, Kwale County and Kilifi County (Figure 5). Sampling was done on 64 selected farms by Kehlenbeck and Waruhiu (2014) and in Chyulu Hills National Park with the guidance of government stakeholders as Kenya Forestry Research Institute (KEFRI), Kenya Forest Service (KFS) and Ministry of Agriculture (MoA), The Kenya Wildlife Service (KWS), locals and key informant. In Makueny Country, sampling was done around Kibwezi and Mtito Andei towns and in Chyulu Hills National Park; in Taita Taveta Country around Voi; in Kwale Country around Diani Beach; in Kilifi Country around Kilifi and Malindi towns. Therefore, we have established seven populations: Kibwezi, Mtito Andei, Chyulu Hills National Park and Voi that were identified as inland populations and Diani Beach, Kilifi and Malindi identified as coastal populations. Table 4 provides the agro-ecological zones to each collecting site (besides Chyulu Hills, because it is a national park) to describe climate and most common crops to regions.

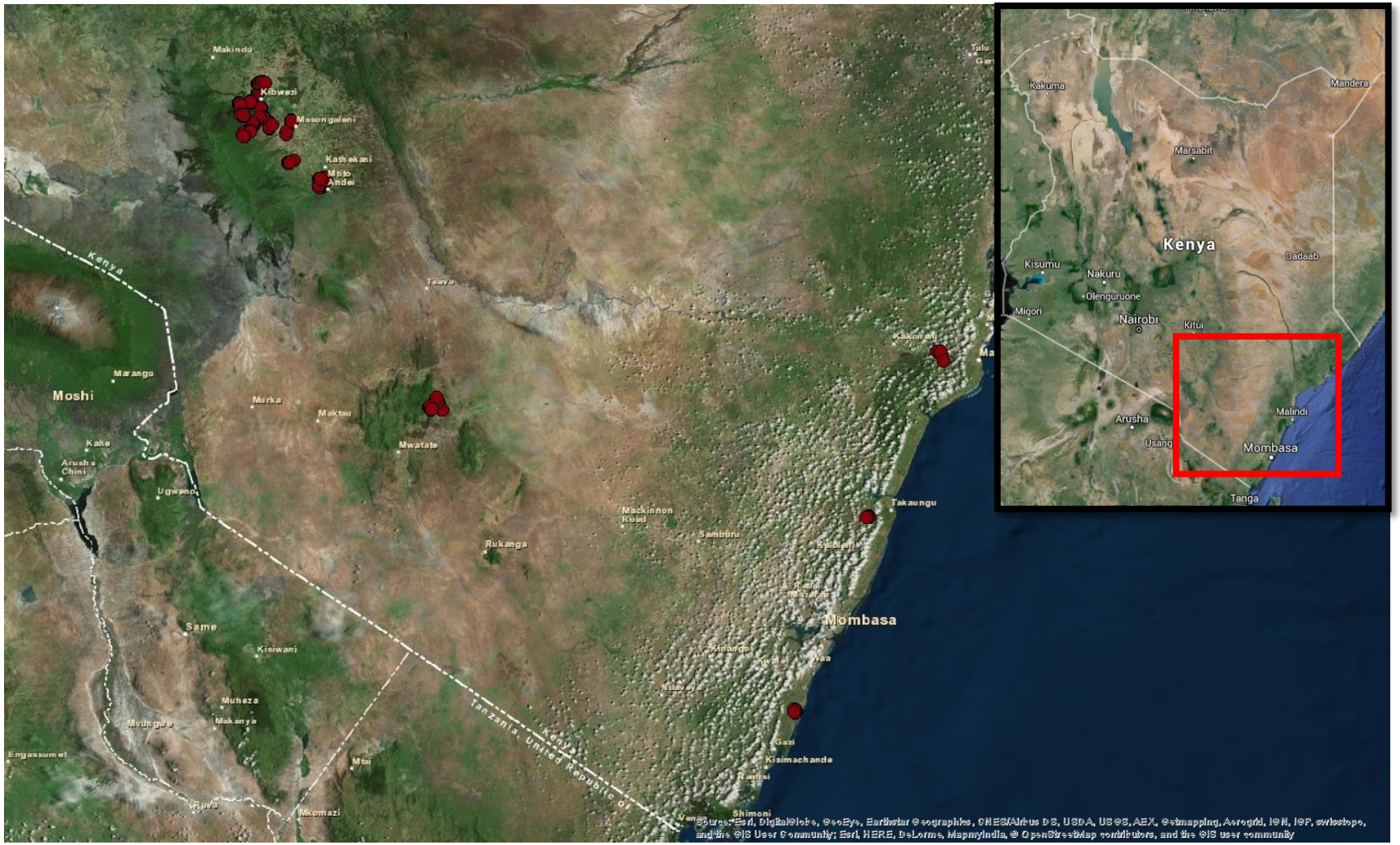


Figure 5. Map of Kenya (upper- right corner) with the study site marked in red and map of sampled trees (red circles)

Table 4. Agro-ecological zones of selected study sites

Location	Climate	Altitude (m.a.s.l)	Agro-ecological zone*	Soil	Average annual rainfall (mm)	Mean annual Temperature (°C)
Kibwezi$_{in}$	Hot semi-arid	911	Livestock-Millet Zone	Luvisols, Ferralsols	< 700	23
Mtito Andei$_{in}$	Hot semi-arid	731	Livestock-Millet Zone	Luvisols, Ferralsols	< 700	23
Voi$_{in}$	Hot semi-arid	550	Inner Lowland Livestock-Millet Zone	Luvisols	500-600	25.1
Diani$_{co}$	Tropical savanna	31	Coconut-Cassava Zone	Luvisols to Acrisols, Ferralsols	> 1100	26.5
Kilifi$_{co}$	Tropical savanna	8	Cashewnut-Cassava Zone	Luvisols	1000-1100	26
Malindi$_{co}$	Tropical savanna	12	Cashewnut-Cassava Zone	Arenosols, Ferralsols, Luvisols, Planosols, Vertisols	1000-1100	26.3

*Agro-ecological zones of sampling locations (Jaetzold et al, 2012a,b), $_{in}$= inland population, $_{co}$= coastal population

Livestock-Millet Zone – with weak and a very short to short cropping season; yield potential for 1st rainy season is for proso millet, hog millet, green gram; for 2nd rainy season is for maize, millets, sorghum, cowpeas, chickpeas, dolichos beans, groundnuts, pumpkins; for whole year: castor, sisal, cassava, yeheb nuts
Inner Lowland Livestock-Millet Zone - with a (weak) very uncertain and a (weak) very short to short cropping season; yield potential for 1st rainy season is for foxtail, proso and hog millet, cowpeas, green grams, bambara groundnuts; for 2nd rainy season is for pearl millet, sorghum, proso millet, foxtail millet, black and green grams, moth beans, cowpeas, chick peas, rai (oilseed), mung beans, French beans, bambara groundnuts; for whole year: sisal, castor, yeheb nuts, opuntia, cassava, neem trees
Coconut-Cassava Zone - with a medium to long cropping season and a very uncertain one; yield potential for 1st rainy season is for maize, white sorghum, sweet potatoes, cowpeas, dolichos beans, winged beans, roselle; nearly all vegetables, esp. chillies, brinjals, tomatoes, onions, kales, cabbages; for whole year: coconuts, cassava, bixa, mangoes, bananas, pawpaws, avocadoes, sisal, pineapples, guavas, senna, castor, citrus
Cashewnut-Cassava Zone - with a medium cropping season, followed by intermediate rains, and towards inland with a (weak) very uncertain 2nd rainy season; yield potential for 1st rainy season is for maize, sorghum, sweet potatoes, kenaf, sunflower, soya beans, dolichos beans, kales, onions, okra, aubergines, sweet pepper, egg plants, chillies, Chinese cabbage, water and sweet melons, cucumbers, pumpkins, zucchini, mchicha; for whole year: cashew nuts, cassava, sisal, mangoes, castor

4.2 Tree sampling and data collections

In total, we have collected data of 204 individual trees that were divided into seven populations (Table 5), from July to August 2015. There were two expeditions; the first expedition was done to 64 preselected farms along Mombasa road and Coast area and the second expedition was done in Chyulu Hill National Park. The preselection of 64 farms was done by Kehlenbeck and Waruhiu (2014); there was already known GPS location of one baobab on each farm and additionally, tissue samples from two other trees closest to the known tree was collected. The total number of sampled trees from the first expedition was 150 individuals. During the second expedition to Chyulu Hills National Park, another 54 trees were sampled.

Table 5. Number of sampled trees, fruits and leaves for genetic and morphological evaluation. $_{in}$= inland population, $_{co}$= coastal population

Populations	Code	Tree morphological data and tissue samples for DNA analyses	Fruits samples for morphologycal analyses	Leaf voucher specimen for morphologycal analyses
Kibwezi	KIB_{in}	80	50	20
Mtito Andei	MTI_{in}	20	10	10
Voi	VOI_{in}	24	16	3
Chyulu	CHY_{in}	54	0	6
Diani	DIA_{co}	9	2	9
Kilifi	KIL_{co}	11	0	11
Malindi	MAL_{co}	6	0	6
Total		204	78	65

Firstly, we have collected morphological data using prepared baobab descriptor (Kehlenbeck et al., 2015) (Appendix I). The descriptor contains information about tree location i.e. elevation, coordinates, distance from human settlement, habitat, topography, and about the tree itself i.e. tree condition, tree height, crown diameter, trunk

circumference, bark colour, bark texture, tree shape, tree growth habit, trunk shape (Kehlenbeck et al., 2015). Additionally, a photo of each tree was taken (Appendix II).

In total, fruits from 78 trees (38%) were sampled and characterized, most of them from inland populations. During the collection, the average appearance of ten randomly selected fruits on each tree was considered, including average shape, size, and health aspects of the fruits, one to two fruits were collected and evaluated in the Seed laboratory of World Agroforestry Centre (ICRAF), Nairobi in Kenya, according to Kehlenbeck et al. (2015). Firstly, external morphological traits were assessed and the whole fruit was weighed. Afterwards, the fruit was cracked and internal traits were assessed, in this stage all fruits parts i.e. shell, fibre, pulp and seeds, were weighed separately. Fruit pulp and seeds were separated using mortar and pestle followed by seed washing in hot water and drying. To determine more precise pulp weight, following calculations were required: sum of fibre, pulp, seed and shell weight was calculated and obtained value was subtracted from fruit weight, the resulting difference was considered to be fruit pulp remaining on the seed, fibre, and shell which was not possible to remove due to strong adherence, so final pulp weight was calculated as sum of resulting difference and pulp weight separated by mortar and pestle. Quantitative data was assessed i.e. fruit length, fruit diameter (measured at the widest point of the fruit then turned 90° and measured again, it means two values were obtained and then mean was calculated), fruit shape ratio (length divided by diameter), fruit pedicel length, fruit weight, fibre weight, pulp weight, seed weight, shell weight, pulp percentage (pulp weight divided by fruit weight and multiplied by 100), fruit shell thickness, number of seeds per fruit. Qualitative data that were further assessed are fruit shape, fruit apex shape, fruit pedicel insertion, fruit neck prominence, fruit beak type, fruit shell hairiness, colour of hairs on the fruit skin, fruit ground colour, fruit cross section outline, fruit shell surface texture, fruit shell hardness to crack, fibre colour, adherence of fibre to fruit shell, texture of fibres in fruit, adherence of pulpy seed to fibre, pulp colour of fresh fruit, adherence of fruit pulp to seed, pulp texture of ripe fruit, pulp sweetness, pulp sourness, pulp bitterness, pulp aroma/scent. For colour determination, colour codes from the Royal Horticultural Society were used

(Kehlenbeck et al., 2015). Additionally, a photo of each fruit was captured to preserve the fruit's appearance and to compare them between each other (Appendix III).

Samples of leaves for voucher specimen were collected from 65 trees (32%) among all 204 sampled individuals. The low number was caused due to the fact that sampling was done off leafy period. Leaves from 65 trees were originated 40% (n=26) from coastal area and 60% (n=39) from inland area. Fresh leaves were cut off, placed between newspapers with proper labelling and press under moderate pressure using wooden frame with straps. Newspapers were exchanged 2 to 3 times to eliminate leaves decay. Already dry leaves were kept in newspapers and transported to CULS in Prague, where they were transformed to proper herbarium specimens. Qualitative and quantitative data were also observed and measured; i.e. number of leaflets, leaflet blade shape, leaflet apex shape, leaflet base shape, leaflet margin, mature leaf hairiness, leaf petiole length, leaflet petiole length, leaflet length, leaflet width (Kehlenbeck et al., 2015).

Last, we have collected tissue samples from each individual tree (n=204) for further genetic analysis. Preferably, healthy leaves were collected from trees however the presence of leafy period vary from tree to tree, therefore in some cases it was necessary to get bark samples as an alternative source of DNA. Samples were inserted to permeable paper bags with proper labelling, stored in sealed plastic boxes with silica gel and transported for analysis at CULS Prague, Czech Republic.

4.3 Genetic analyses

The DNA extraction and PCR (polymerase chain reaction) were done in Laboratory of Molecular Biology at Faculty of Tropical AgriSciences, Czech University of Life Sciences, Prague. Fragment analysis by capillary electrophoresis of the PCR products was performed in Laboratory of Molecular Genetics at Faculty of Environmental Science, Czech University of Life Sciences, Prague.

4.3.1 DNA extraction

For DNA extraction, modified CTAB method was chosen (Doyle and Doyle 1987; Saghai-Maroof et al, 1984) as the method performed better concentration and purity of DNA compared to extraction by DNA extraction Kit (Invisorb Spin Plant Mini Kit). Modified CTAB protocol for *A. digitata* was recommended by Dr. Kathleen Prinz from University of Jena, Germany. The baobab tissue was put into microtubes, with 0.5 g of dried tissue, and ground to a fine powder using mechanical grinder (Silamat S6) and purified sand. To the ground plant material, 500 µl of CTAB buffer and 10 µl of β-mercaptoethanol were added and mixed on vortex. Then the samples were incubated for 30 minutes at 65°C, during incubations they were mixed three times by inverting tubes. After incubation, 600 µl of chloroform-isoamylalcohol (24:1) was added and put into vortex for 15 minutes to mix. Samples were centrifuged for 15 minutes at 9,000 rpm. After centrifugation, two phases were created, an upper aqueous phase – supernatant, containing DNA, and a lower chloroform phase containing proteins, lipids and secondary metabolites. Between the upper and the lower phase, there was formed an interface from cell debris, degraded proteins, etc. (Doyle and Doyle 1987). The upper supernatant was transferred by broad tips into new microtubes and cold isopropanol was added. Solution was mixed gently and incubated for one hour in freezer at -19°C to precipitate DNA. Then the samples were again centrifuged for 15minutes at 14,000 rpm. The supernatant was discarded, 500 µl of 70% ethanol was added and centrifuged for 15minutes at 14,000 rpm. The supernatant was discarded carefully again and created pellets, attached to microtubes walls, were let to dry at room temperature. After the pellets were completely dry, 100 µl of TE buffer and 5 µl of RNAse were added. Samples were incubated overnight at room temperature and afterwards, DNA concentration and purity was measured on NanoDrop™ Spectrophotometer. As extracted DNA was in high concentrations, dilution was necessary, final concentrations for further PCR analyses range from 8.3 to 38.1 ng/µl. The ratio of absorbance at 260 nm and 280 nm (260/280), which determines purity of DNA, ranged from 0.68 to 2.00 with mean value of 1.50.

4.3.2 SSR analyses

Eighteen microsatellite primer pairs were developed for *A. digitata* and other baobab species by Larsen et al. (2009) and ten of them were chosen for genetic fingerprinting of collected samples (Table 6). All primers were synthesized by Generi Biotech. To successfully run the PCR, it was necessary to determine the optimal concentration of $MgCl_2$ and the annealing temperature of the primer pairs. Six different concentrations of $MgCl_2$ (1 mM, 1.25 mM, 1.5 mM, 2 mM, 2.5 mM, 3 mM) and eight annealing temperatures in a gradient from 49 to 59°C were tested for each primer pair. The PCR products were visualized by electrophoresis on 1% agarose gel stained by EtBr (ethidium bromide), and the most visible bands were chosen. The best results were obtained with $MgCl_2$ concentration of 2.5 mM and annealing temperature of 58°C for all primer pairs.

Then the primer pairs were further tested with sixteen samples to determine if extracted DNA is suitable for analysis. Gel electrophoresis results showed satisfactory amplifications of loci. After this testing, all ten primers were chosen for further analysis based on their size range and ability to amplify under our laboratory conditions (Table 6). Those ten primers (only forwards) were synthesized again with fluorescent labelling, four colours were used: 6-FAM (Generi Biotech), PET, NED, VIC (Thermo Fisher Scientific). Two primer mixes (PM) were established, each primer was diluted to concentration of 10 mM. Primer mix one (PM1) consisted of six primers; Ad05, Ad08, Ad09, Ad13, Ad17, Ad18, from each primer (forward and reverse) was taken 0.2 µl, which means 2.4 µl of primers and refilled with 2 µl PCR H_2O to get final volume of 4.4 µl of PM1. PM2 contained four primers; Ad01, Ad06, Ad11, Ad15, from each primer (forward and reverse) was taken 0.2 µl, i.e. 1.6 µl of primers and refilled with 2.8 µl PCR H_2O. PCR reaction mixture had a total volume of 12 µl per well. It consisted of 1 µl of DNA, 4.4 µl of primer mix, 0.9 µl of TaqBuffer (Fermentas), 0.9 µl of 2 mM dNTP Mix (Thermo Scientific), 0.9 µl of 2.5 mM $MgCl_2$ (Fermentas), 0,7 µl (0.5 u/µl) of Taq DNA Polymerase and remaining volume was refilled with PCR H_2O (Table 7). Profile of PCR reaction is described in Table 8. PCR was run with all 204 samples and it had to be done separately for PM1 and PM2.

Table 6. SSR primers used for analysis (Larsen et al., 2009)

Locus name	Primer sequence 5´- 3´		Motif	Size range (bp)	Dye
	F	R			
Ad01	CATTGCCAGGAATGCTTTTGC	GGATTGCCAGGTCTACTAC	$(AG)_{19}$	96–125	6-FAM
Ad05	CTCAACAAGGTTCGGATGTCGTATG	GTCTGCCGGGTGTTTTGCATG	$(CA)_{12}(CT)_{12}$	295–319	VIC
Ad06	TGCATCAGCTTTCACTCCAGAC	GCCACCCATAAAACCCAATCC	$(TC)_{19}$	129–154	PET
Ad08	TCTAAAGCCTGTAAGGAAAAATGGG	TTCTCCGTTCACTCTGTACTTCC	$(GAA)_{14}$	267–296	6-FAM
Ad09	TACCACTTCTCCAGATGCTAC	ACTGGCTAGAGATGCGTTG	$(AAG)_{11}$	190–209	NED
Ad11	ATCAGCCATTCTGCATACCTGC	TAGGCACAAAACTGAGATGCACAG	$(CA)_{13}(AT)_{6}$	118–181	VIC
Ad13	CCCCACTTCAGATCAAGTAAGTC	GCTGTATTTCTGAGCCTGAGAAG	$(AC)_{14}$	305–330	PET
Ad15	TGAAGAGACAAAGCAAGAAG	CATGACATCTCCTTGAACC	$(GAA)_{14}$	130–161	NED
Ad17	GCGCCTTAGAAAGGACTTGTTAGAG	GCCAACAGCCTTAGTAGTCCAAG	$(AC)_{14}$	174–215	6-FAM
Ad18	ACCGCTTCCGTTCTCATTCC	ACCACCACTACACCGTCATTG	$(TG)_{17}$	257–291	NED

Table 7. PCR composition

Substance	Concentration	Volume [µl]
Template DNA	8.3 to 38.1 ng/µl	1
Primer Mix (I. or II.)	10 mM each primer	4.4
dNTP	2 mM	0.9
MgCl2	2.5 mM	0.9
TaqBuffer		0.9
DNA Polymerase	0.5 u/µl	0.7
H_2O		3.2

Table 8. PCR profile

Step	Temperature [°C]	Time [min]	Cycles
Denaturation	95	2:00	
Denaturation	95	0:30	
Annealing	58	1:00	
Elongation	72	2:30	36
Elongation	72	7:00	

Afterwards, PCR products were mixed in a ratio of 1:2 to create a multiplex for final step of genetic analysis, which was fragment analysis by capillary electrophoresis. Such prepared PCR products were mixed with GeneTrace 500 LIZ size standard (Carolina Biosystems) and analysed by Gene Analyser 3500 (Applied Biosystems). Allele size was determined using GeneMarker software.

4.4 Determination of ploidy - Flow cytometry

Verification of the presence or absence of *Adansonia kilima* was performed by flow cytometry. This method requires fresh leaf material (Dolezel et al., 2007). Therefore, seeds obtained from 78 fruits were brought from Kenya to Czech Republic. Seeds were sown in Botanical Garden of the Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague. The seed scarification by sandpaper was done before sowing because the seed coat of baobab is very thick. Seedlings were grown from November 2015 until March 2016 when young plants reached approximately 20 cm of height.

Out of 78 samples, only 32 germinated and developed suitable seedlings. From each seedling, one mature leaf was taken for analysis. Samples were prepared and measured using protocol from Dolezel et al. (2007): firstly, small amount (20 mg) of fresh leaf tissue of baobab and internal standard (*Zea mays* L.) were placed in Petri dish together with 1 ml of OTTO I.[1], chopped finely and mixed. Homogenate was filtered to remove cell fragments and 1 ml of OTTO II[1] with 4´,6-diamidino-2-phenylindole (DAPI) was added. Such prepared samples were then placed into CyFlow Space flow cytometer (Partec GmbH, Műnster, Germany) and measured with relative fluorescence intensity of at least 3,000 nuclei. The data were analysed using FloMax software, 2.4d (Partec GmbH, Műnster, Germany). Resulting histograms of DNA content formed two peaks (standard and baobab) and the ratio of their positions determined the genome size of baobab samples (Dolezel et al., 2007).

[1] Otto I solution: 0.1 M citric acid; Otto II solution: 0.4 M $Na_2HPO_4 \cdot 12H_2O$.

4.5 Data analyses

Morphological data of each tree, fruit and leave measurements were inserted into excel spreadsheets and analysed statistically using STATISTICA software. Firstly basic descriptive statistics were done. ANOVA (Analysis of variance) and the Newman and Keuls test were used to describe and differentiate morphological data among populations.

To evaluate genetic data, several software were used. As baobab is a tetraploid species, specific approaches for data evaluation had to be used. Polyploid microsatellite data can be handled as binary data (used software FAMD, GenAlEx) or special software designed for polypoid species can be used as in our case Polysat and SPAGeDi.

FAMD (Fingerprinting Analysis with Missing Data, Schlüter and Harris 2006) was used to calculate AMOVA (Analysis of molecular variance). In GenAlEx (Peakall and Smouse, 2012) frequency-based population genetic analysis was calculated. In R (R Core Team, 2014) using package POLYSAT (Clark and Jasieniuk, 2011) designed to handle polyploids, PCA (Principal Coordinate Analysis) was performed and input files to software SPAGeDi and STRUCTURE were prepared. In SPAGeDi (Hardy and Vekemans, 2002) gene diversity and allele frequencies per locus and populations was computed. Also Moran's I index of spatial autocorrelation was computed using SPAGeDi. The program Structure 2.3.4 (Pritchard et al., 2000) was used to determine the spatial structure and partition within tree populations.

5 RESULTS

5.1 Morphological evaluation

5.1.1 Tree morphological evaluation

Basic morphological and geographical data were assessed for all 204 trees. 60% of the trees were collected within less than 100 m far from human settlement, mainly were found on the field associated with other agriculture crops as corn (*Zea mays)* or pigeon pea (*Cajanus cajan)* or in woodland/shrubland habitat. Topography was in 80% flat, 14% of trees were present on undulating landscape. Tree sampled at the highest altitude 1,058 m.a.s.l. was from Chyullu population, at the lowest altitude 6 m.a.s.l. in Diani population. Among trees collected from inland population the altitude range was from 657-1,058 m.a.s.l. with mean 904 m.a.s.l., for coastal population 6-54 m.a.s.l. with mean 28 m.a.s.l.

Most of the trees (184) were mature-vigorous, ten trees were young, eight trees were mature-non vigorous or dying: trees were effected by parasitic plants (e.g. *Ficus* sp.), or there was a human or natural intervention. Two trees were found dead (one cut down by farmer, second fell down naturally).

The bark of the trees was mostly grey, smooth or rough. Most frequent shape was roundish and semi-circular (Figure 6), growth habit was spreading and most frequent trunk shape was cone-shaped. Mean tree height was reported 12.2 m, crown diameter 21.9 m and DBH (Diameter at breast height) 2.3 m (Table 9). Stem diameter ranged from 0.2-4.8 m DBH. DBH size-class distribution (Figure 7) showed normal distribution among 195 trees, most of the trees belong to class in range 2-3 m. Interestingly, some of the trees had multi-stemmed nature, from all sampled trees 20 trees were indicated to have two stems, four trees have three stems, three trees four stems and two trees were reported to have six and seven stems, respectively. Baobabs have the capacity to generate new stems periodically and mainly old trees have multi-stemmed nature (Patrut et al., 2015).

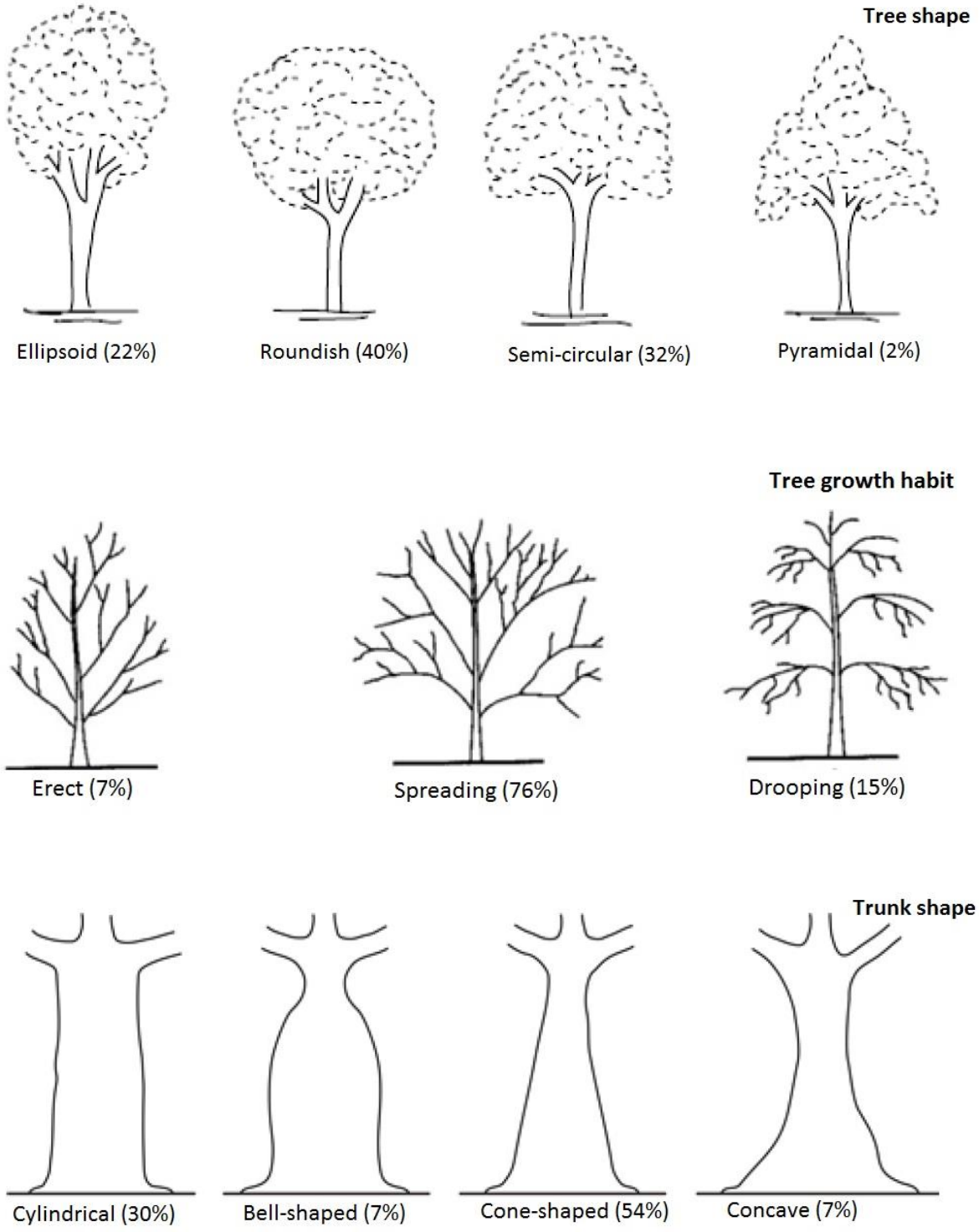


Figure 6. Tree shape, tree growth habit, and trunk shape, with relative frequencies of 204 trees observed

Table 9. Mean, maximum and minimum values for tree height, crown diameter and DBH (Diameter at breast height) (n=204)

Descriptor	Mean	Min	Max
Tree height [m]	12.2	3.5	21.0
Crown diameter [m]	21.9	2.0	36.5
DBH [m]	2.3	0.2	4.8

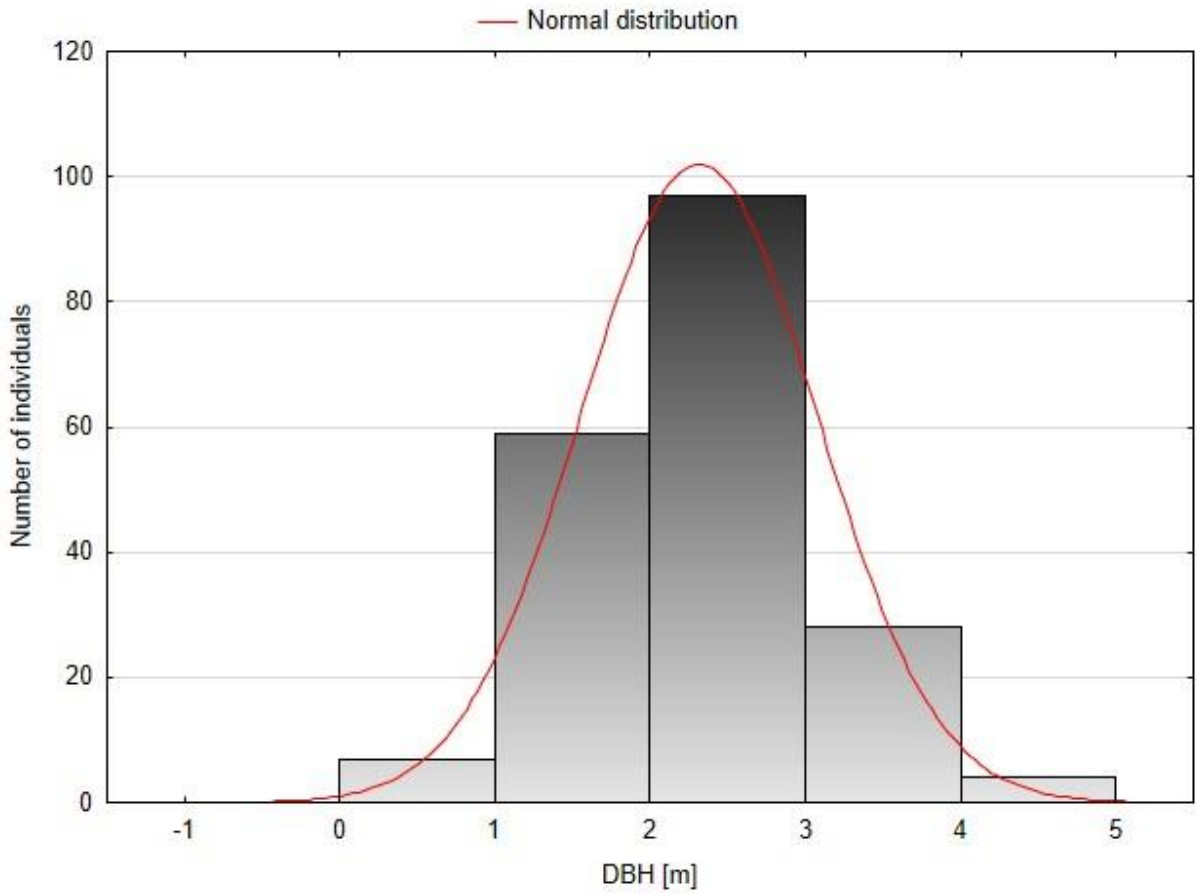


Figure 7. DBH size-class distribution of 195 baobabs (*Adansonia digitata* L.)

5.1.2 Fruit morphological evaluation

Fruit morphological data were assessed for 78 trees, predominantly from inland population, because among the coastal populations the fruits were not ripe. Also, there were no fruits from the Chyulu population because it is a National Park and all fruits were probably eaten by animals (e.g. elephants, baboons).

The most characteristic fruit shape was oblong, ellipsoid and obovate (Figure 8). Other fruit characteristics such as fruit apex shape, pedicel insertion, neck prominence, beak type, cross section outline and their relative frequencies calculated for three populations are present in Table 10. Fruit apex shape was mostly acute or obtuse with absent or perceptible beak, the pedicel insertion was vertical and fruit has mostly slight prominent fruit neck. Fruits were mostly evenly hairy, colour of hair were yellow-green (codes from the Royal Horticultural Society: 152A, 152B). Fruit ground colour was primarily brown (code: 200A).

Figure 8. Fruit shape with their relative frequencies for 78 sampled trees

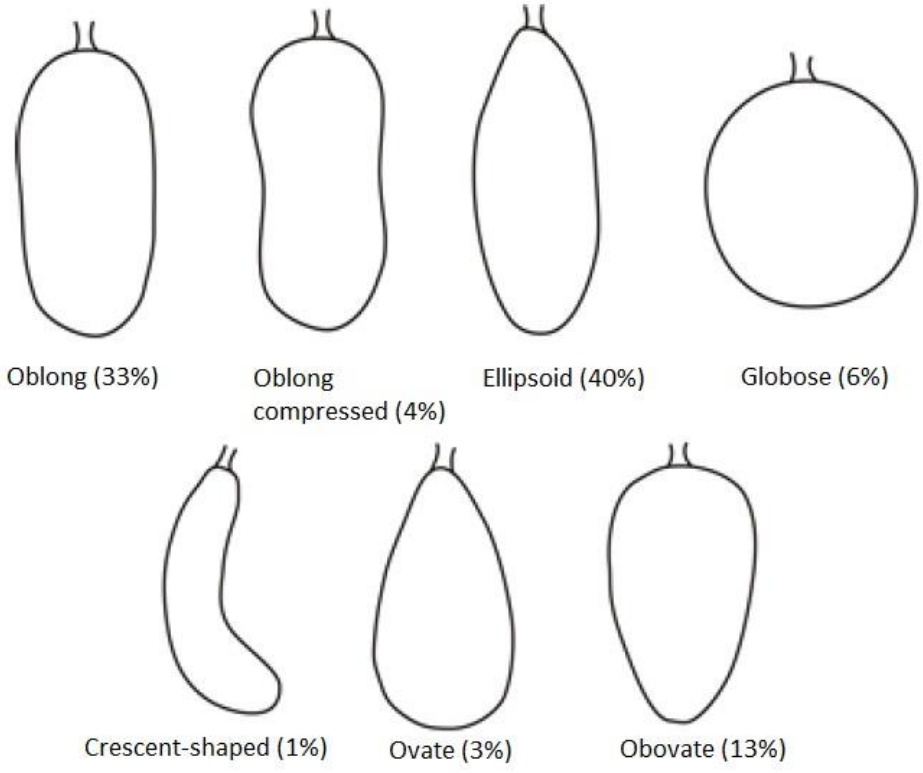


Table 10. Fruit qualitative data of inland populations expressed by their relative frequencies [%] assessed only for inland populations (Kibwezi, Mtito Andei, Voi)

Descriptor	Categories	KIB (n=50)	MTI (n=10)	VOI (n=16)
Fruit apex shape	1 Acute	40	30	50
	2 Obtuse	30	40	25
	3 Round	14	10	6
	4 Depressed	14	20	19
Fruit pedicel insertion	1 Vertical	60	70	88
	2 Slightly oblique	24	30	13
	3 Oblique	16	0	0
Fruit neck prominence	0 Absent	10	40	31
	1 Slightly prominent	50	40	38
	2 Prominent	32	20	25
	3 Very prominent	8	0	6
Fruit beak type	0 Absent	40	30	31
	1 Perceptible	36	40	31
	2 Pointed	20	30	25
	3 Prominent	4	0	13
Fruit cross section outline	1 Not contoured	38	20	38
	2 Shallowly contoured	48	60	56
	3 Deeply contoured	14	20	6
Pulp sweetness	0 Absent	18	30	0
	3 Slightly sweet	57	70	81
	5 Sweet	20	0	19
	7 Very sweet	4	0	0
Pulp sourness	0 Absent	22	20	0
	3 Slightly sour	59	40	63
	5 Sour	14	40	38
	7 Very sour	4	0	0

The internal fruit traits are following: the most common number of segments was 6, 7 or 8. Inside the fruits, yellow-white pulp is present (code: 158A or 158B) in which seeds are embedded. Adherence of fruit pulp to seed was predominantly firm/strong. The pulp is held in the fruit by greyed-orange fibre. Adherence of fibre to fruit shell was detected as intermediate to strong and adherence of pulpy seed to fibre was weak to intermediate. From organoleptic point of view most characteristic fruit pulp taste is slightly sweet, or slightly sour to sour, absent bitterness and with mild aroma. The comparison of fruits taste among three populations is described by relative frequencies in Table 10, however there were not found significant differences among populations.

Mean values of fruits quantitative data per population are present in Table 11. Data did not show significant differences among populations except Diani (DIA) population that possessed longer fruits with higher number of seeds, however, there were collected only two fruits, so there is a questions if the data are representative for whole population. Fruit shape ration ranged from 1.7 (Mtito Andei) to 2.8 (Diani). The heaviest parts of fruit are shell and seeds. Percentage of seeds from total fruit weight ranged from 32 to 38.3 %. Pulp percentage ranged from 16.1 to 18.4 %.

Table 11. Fruit quantitative traits for four sampled populations of baobab. Mean values per populations. Numbers with the same letters within a same row are not significantly different at 0.05 level, (n=number of individuals per population), $_{in}$= inland population, $_{co}$= coastal population

Descriptor	**KIB $_{in}$** (n=50)	**MTI $_{in}$** (n=10)	**VOI $_{in}$** (n=16)	**DIA $_{co}$** (n=2)
Length [cm]	13.5[a]	11.5[a]	14.0[a]	20.3[b]
Diameter [cm]	7.2[a]	7.3[a]	7.6[a]	7.3[a]
Fruit shape ratio	1.9[a]	1.7[a]	1.9[a]	2.8[b]
Pedicel length [cm]	8.8[a]	7.9[a]	7.1[a]	13.2[b]
Fruit weight [g]	117.9[a]	115.8[a]	116.9[a]	154.3[a]
Seed weight [g]	48.0[a]	42.6[a]	40.9[a]	51.9[a]
Shell weight [g]	47.3[a]	50.3[a]	52.2[a]	76.7[a]
Total pulp weight [g]	20.4[a]	21.0[a]	21.3[a]	24.8[a]
Pulp percentage [%]	17.6[a]	18.4[a]	18.3[a]	16.1[a]
Seed percentage [%]	38.3[a]	34.2[a]	32.0[a]	33.9[a]
Shell thickness [cm]	0.52[a]	0.55[a]	0.60[a]	0.55[a]
Number of seeds	81.5[a]	67.3[a]	71.8[a]	180.0[b]

5.1.3 Leaf morphological evaluation

Leaf morphological data from 65 trees were evaluated using qualitative and quantitative traits. Qualitative data showed that the most typical leaf had 5 leaflets, with elliptic blade, apiculate leaflet apex shape, acuminate leaflet base shape, entire margins and were hairless. Slight differences were found between coast and inland populations (Table 12). Inland leaves have prevailingly five leaflets and are always without hairs. In the coastal regions, individuals have more often seven leaflets and can be hairless or with hairs.

Table 12. Leaf qualitative data expressed by relative frequencies [%] for inland and coastal populations

Descriptor	**Categories**	**Inland (n=39)**	**Coastal (n=26)**
Number of leaflets	2 Three leaflets	8	0
	3 Five leaflets	78	31
	4 Seven leaflets	8	62
	5 Six leaflets	6	7
Leaflet blade shape	1 Elliptic	41	62
	2 Oblong	18	8
	4 Obovate	28	23
	6 Orbicular/Obovate	13	7
Mature leaf hairiness	0 Absent	100	54
	1 Slightly hairy	0	31
	2 Hairy	0	15
Leaflet apex shape	1 Acute	26	15
	2 Apiculate	39	58
	3 Cuspidate	5	12
	4 Obtuse/Rounded	11	0
	5 Acuminate	18	15
Leaflet base shape	2 Attenuate	31	35
	3 Acuminate	69	65

Quantitative data were assessed to see if there is a difference among seven populations and among inland and coastal populations. Results showed that inland trees have significantly smaller leaves with shorter petioles than coastal individuals (Table13).

Table 13. Leaf quantitative data expressed by mean values among seven populations and among inland and coat populations. Numbers with the same letters within a column are not significantly different at p=0.05. in= inland population, co= coastal population

Descriptor/ population	N	Leaf petiole length [cm]	Leaflet length [cm]	Leaflet width [cm]	Leaf shape ratio
KIB_{in}	20	10.5^{a}	9.6^{ab}	3.9^{a}	2.5^{ab}
MTI_{in}	10	8.9^{a}	10.0^{ab}	4.3^{a}	2.4^{ab}
VOI_{in}	3	9.6^{a}	8.8^{a}	3.5^{a}	2.6^{ab}
DIA_{co}	9	12.7^{a}	13.3^{c}	5.6^{b}	2.4^{ab}
KIL_{co}	11	12.7^{a}	13.3^{c}	5.7^{b}	2.4^{ab}
MAL_{co}	6	10.3^{a}	12.1^{bc}	5.8^{b}	2.1^{a}
CHY_{in}	6	8.0^{a}	9.0^{a}	3.2^{a}	2.9^{b}
Inland	39	9.7^{a}	9.5^{a}	3.9^{a}	2.3^{a}
Coastal	26	12.1^{b}	13.0^{b}	5.7^{b}	2.5^{b}

5.2 Genetic analysis

For genetic analyses 189 individuals divided in seven populations were assessed. In total 204 individuals were sampled; however, 15 samples were excluded because of unsuccessful amplification during PCR reaction. Nine microsatellite loci were evaluated (Table 14). At the beginning 10 SSR-primer were used for evaluation, but one locus failed during fragment analysis by capillary electrophoresis and showed results only for 50% of samples therefore was excluded for further calculations. A total of 117 alleles were scored among all loci with multilocus average of 13 alleles. Locus Ad06 showed the highest number of alleles (21). Gene diversity ranged from 0.6707 to 0.8925 (average 0.8032). Gene diversity was the highest for locus Ad06. Observed heterozygosity ranged from 0.796 to 0.968 (average 0.907). Observed heterozygosity was higher than gene diversity, which suggest high number of heterozygotes among sampled individuals. Mean inbreeding coefficient (Fi) was -0.085.

Table 14. Gene diversity and allele frequencies per loci

Locus	NA	Ae	AR	He	Ho	Fi
Ad01	11	6.59	6.58	0.8483	0.961	-0.109
Ad05	6	3.66	4.54	0.7266	0.854	-0.152
Ad06	21	9.3	8.46	0.8925	0.951	-0.041
Ad08	15	6.39	7.07	0.8434	0.968	-0.123
Ad09	7	3.04	4.41	0.6707	0.85	-0.115
Ad11	14	4.23	5.78	0.7635	0.796	-0.023
Ad13	20	8.12	7.61	0.8768	0.933	-0.024
Ad17	13	5.03	5.6	0.8012	0.91	-0.08
Ad18	10	5.14	5.73	0.8053	0.94	-0.112
Multilocus average	13	5.72	6.2	0.8032	0.907	-0.085

NA: observed number of alleles, Ae: Effective number of alleles, AR: Allelic richness, He: gene diversity corrected for sample size, Ho: observed heterozygosity, Fi: individual inbreeding coefficient

Table 15. Gene diversity and allele frequencies per populations using two approaches (binary data and allele sizes). The minimum and maximum values are marked in bold.

	Coast/ inland	N	Polymorphic Loci	Private Bands	H	I	NA	Ae	AR	He	Ho	Fi
Kibwezi	in	78	81.10%	3	0.153	0.252	11	5.22	2.99	0.7876	0.913	-0.120
Mtito	in	19	67.72%	3	0.156	0.251	9	5.7	3.01	0.7945	0.933	-0.146
Voi	in	23	64.57%	0	0.144	0.232	9	4.73	2.9	0.7696	0.911	-0.141
Chyulu	in	43	81.89%	2	**0.163**	**0.267**	11	**6.0**	**3.08**	0.8109	**0.938**	-0.135
Diani	co	9	50.39%	0	**0.116**	**0.190**	7	**3.99**	**2.68**	0.7124	**0.788**	-0.015
Kilifi	co	11	46.46%	1	0.124	0.196	6	4.2	2.71	0.7206	0.836	-0.078
Malindi	co	6	39.37%	1	0.124	0.190	5	4.79	2.8	0.7531	0.812	**0.049**
Inland	in	163	73.82%	2	0.154	0.251	10	5.41	2.99	0.7907	0.924	-0.136
Coast	co	26	45.41%	0.7	0.121	0.192	6	4.33	2.73	0.7287	0.812	-0.015
Mean		**189**	61.64%		0.140	0.226	13	5.72	3.04	0.803	0.907	-0.085

N: number of individuals, Private Bands: number of bands unique to a single population, H: Expected Heterozygosity (Nei's genetic diversity), I: Shannon's Information Index. Those values were calculated from binary data in GenAlEx 6.5 (Peakall and Smouse, 2012). NA: observed number of alleles, Ae: Effective number of alleles, AR: Allelic richness, He: gene diversity corrected for sample size, Ho: observed heterozygosity, F: individual inbreeding coefficient. Those data are evaluated in SPAGeDi (Hardy and Vekemans, 2002).

Gene diversity and allele frequencies were also computed per populations using software GenAlEx 6.5 for binary data (Peakall and Smouse, 2012) and SPAGeDi for allele sizes (Hardy and Vekemans, 2002) (Table 15). All loci were highly polymorphic (mean value 61.64%), except for Malindi population, which can be explained by small number of individuals (6). The highest number of private bands were reported in Kibwezi and Mtito populations. Overall gene diversity was high for all populations. Two approaches, H and He, showed the same results i.e. the highest gene diversity was reported for Chyulu and the lowest in Diani. Comparison of inland and coastal populations showed slightly higher values for inland accession, however gene diversity is still high for both groups. The lower number of coastal accession can be partly explain by smaller number of sampled individuals. But for example gene diversity (He), which is corrected for sample size, still showed slightly higher values for inland group.

Analysis of molecular variance (AMOVA) calculated for seven populations revealed low variation among populations (12.37 %) and high variation within populations (87.63 %). ANOVA calculated for two groups (inland and coastal populations) showed 27.43 % variance among groups and 72.57 % within groups.

Bayesian clustering using STRUCTURE software differentiate individuals into two clear clusters (Figure 9), consisting of only inland individuals and smaller of only coastal individuals. Figure 10 is demonstrating the similarity between runs in STRUCTURE, it shows the greatest similarity coefficient for K = 2. Thus, populations were assigned into two clusters with 100% probability.

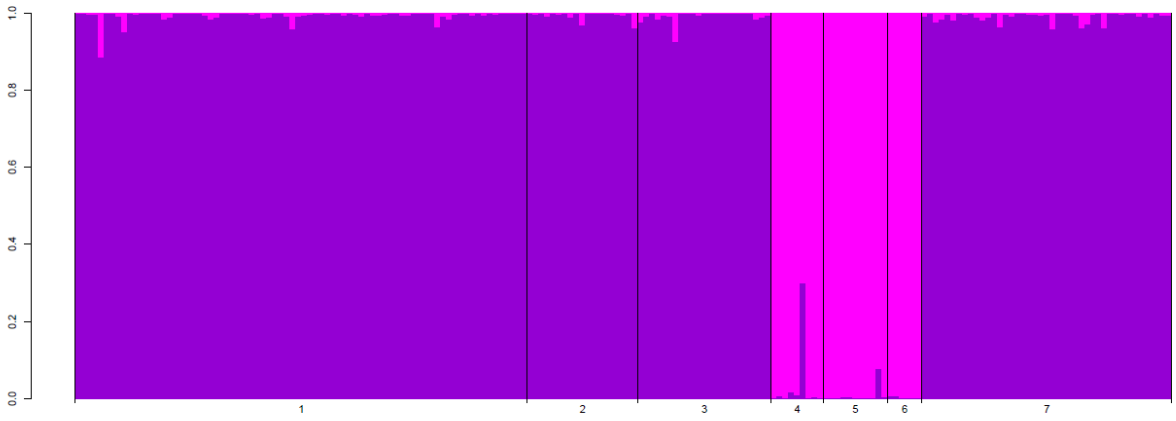


Figure 9. Output from the program Structure. Bayesian clustering revealed two main clusters: inland (violet) and coastal populations (pink). Populations: 1 Kibwezi (in), 2 Mtito Andei (in), 3 Voi (in), 4 Diani (co), 5 Kilifi (co), 6 Malindi (co), 7 Chyulu (in)

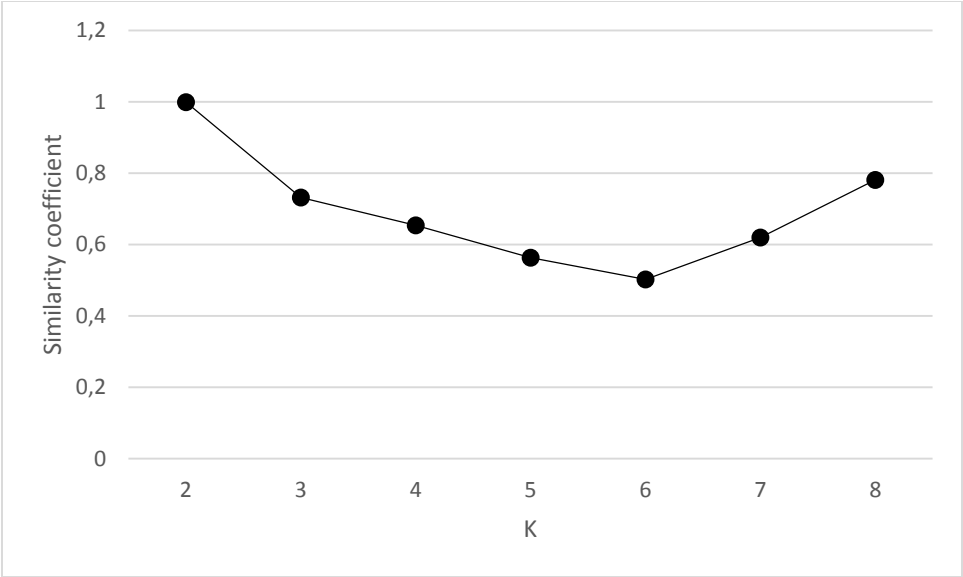


Figure 10. Evaluation of the similarity coefficient for various runs in the STRUCTURE software showed the greatest similarity to K = 2. It means that populations were assigned into two clusters with 100% probability

Similar results as Bayesian clustering showed PCA (Principal Coordinate Analysis) (Figure 11). We can clearly differentiate inland and coast groups and also conclude that inland populations do not group into distinct populations (as we grouped them based on geographical distance) but as one big population.

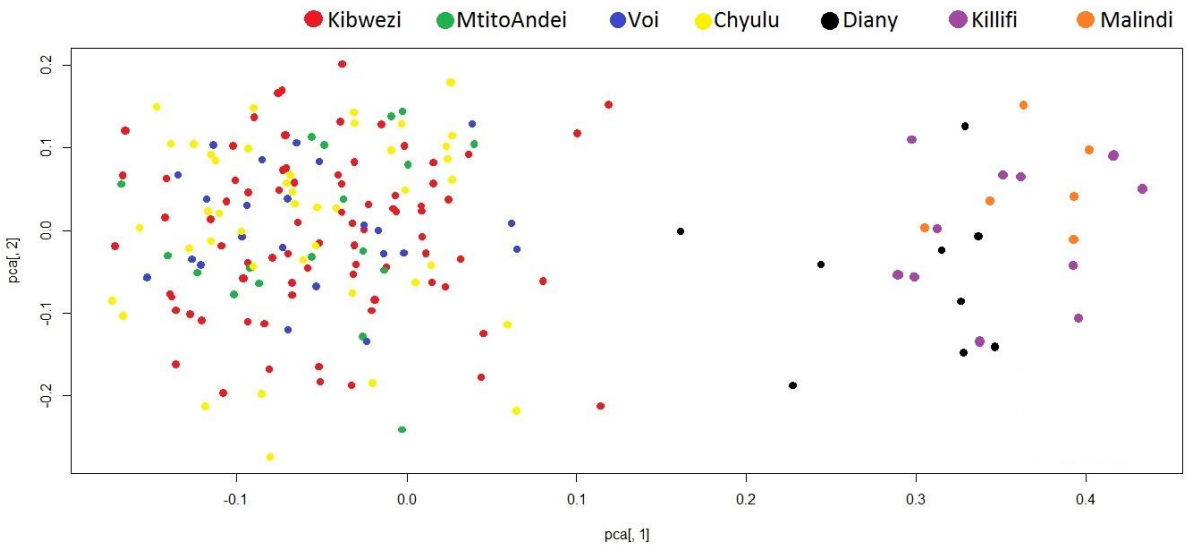


Figure 11. Principal Coordinate Analysis (PCA) with Bruvo distance. On the left side, there are highlighted by different colours inland populations of Kibwezi, Mtito Andei, Voi and Chyulu. On the right side, there is a group of coastal individuals from Diani, Kilifi and Malindi populations

Genetic differentiation G_{st} and pairwise spatial distance was computed among populations (Table 16). The smallest genetic distance was revealed between Mtito Andei and Chyulu populations and the highest between Voi and Diani. G_{st} values among inland population range from -0.005 to 0.006 and coastal populations range from 0.010 to 0.052. These results indicate low genetic differentiation within inland population, low to medium-low within coastal population.

Table 16. Pairwise spatial and genetic distances. Genetic differentiation G_{st} computed as pairwise values between locations (below diagonal) and pairwise spatial distance (in km) between populations (above diagonal). $_{in}$= inland population, $_{co}$= coastal population

	KIB$_{in}$	**MTI$_{in}$**	**VOI$_{in}$**	**DIA$_{co}$**	**KIL$_{co}$**	**MAL$_{co}$**	**CHY$_{in}$**
KIB$_{in}$		29	114	270	243	238	**8**
MTI$_{in}$	-0.003		85	241	216	215	33
VOI$_{in}$	0.004	0.006		160	150	170	116
DIA$_{co}$	0.099	0.106	**0.114**		71	132	**274**
KIL$_{co}$	0.097	0.100	0.108	0.010		61	248
MAL$_{co}$	0.098	0.102	0.112	0.052	0.019		245
CHY$_{in}$	0.000	**-0.005**	0.006	0.096	0.096	0.100	

Moran's I index of spatial autocorrelation was computed in SPAGeDi software, which was primarily designed to express the spatial genetic structure. Spatial distance intervals were set and Moran´s I relationship coefficient was computed as the correlation between individual allele frequencies (Hardy and Vekemans, 2002). Moran´s I was firstly computed for all individuals, but the results showed that individuals are the most correlated in interval 60-80 km i.e. individuals are most similar at a distance of 60-80 km, which seemed to be rare. Thus, Moran´s I was computed separately for inland and coastal individuals. Results in Figure 12 show that inland individuals are the most correlated at distance 100 m, but there are probably also individuals that are very similar but distance between may be 0.9 km, 2 km, or 4-7km. Interestingly, coastal individuals seem to be the most correlated at distance of 3 km.

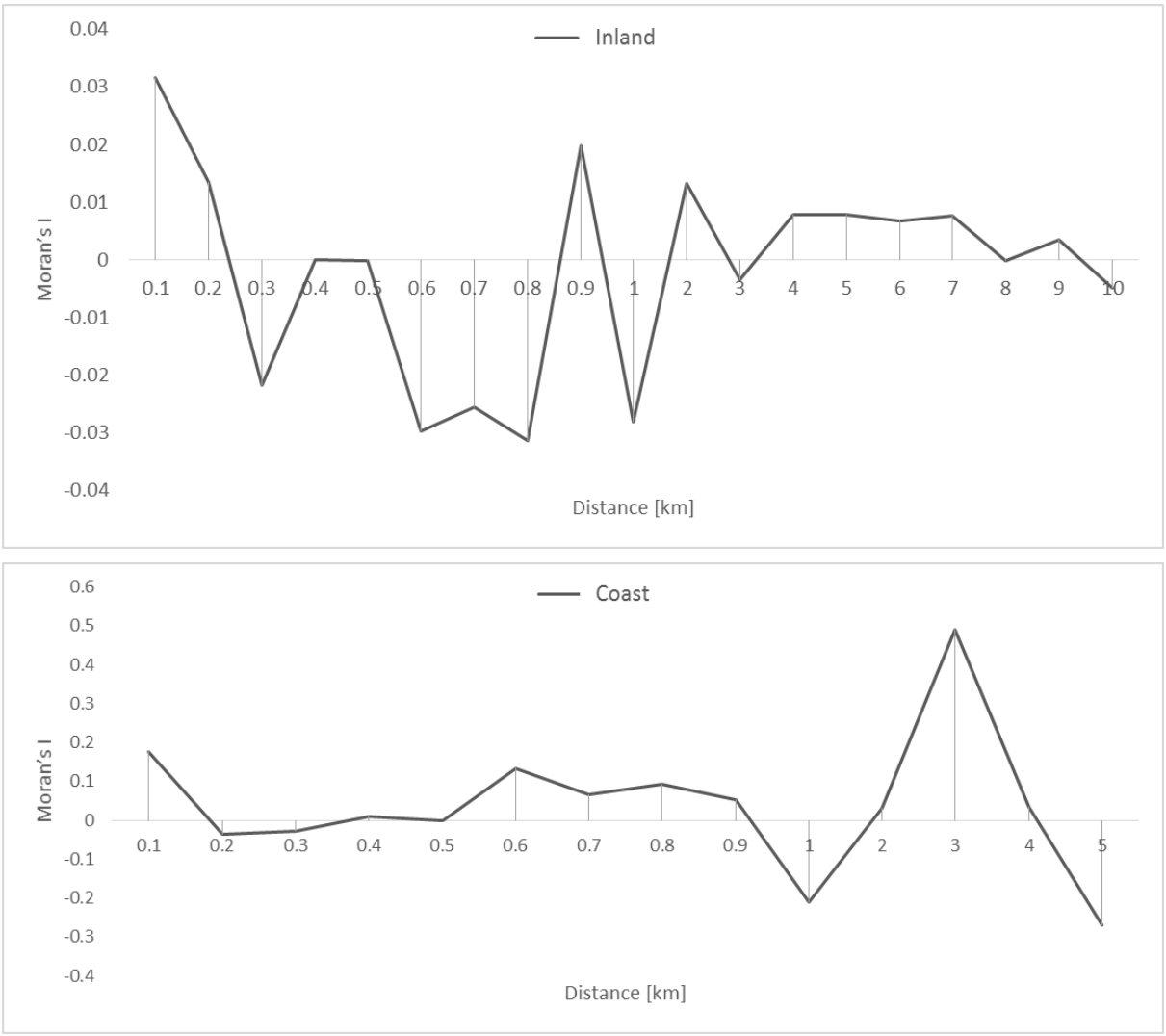


Figure 12. Spatial autocorrelation using Moran's I. Upper graph was computed for inland individuals and it describe that individuals were most correlated for 100 meters (0.1 km). Lower graph was computed for coastal individuals and demonstrate correlation for 3 km. Values were calculated over all alleles with 9999 permutations

5.3 Ploidy

Samples from four populations i.e. Kibwezi (19 samples), Mtito Andei (3), Voi (8) and Diani (2) were analysed for their ploidy using flow-cytometry measurements. In total, 32 samples were assessed for ploidy level to reveal presence or absence of *Adansonia kilima.* The ratio of peaks (with mean value 1.183) detected no significant differences among samples. Such results clearly detected only one ploidy (Figure 13). Photo of young seedling is provided in Figure 14. However, flow cytometer only calculates nuclei, so it was not known if we deal with di- or tetra- ploidy. For this reason, flow cytometry results were compared to fragment analysis results where some samples showed clearly their tetraploid nature. We could conclude that all analysed samples are tetraploids.

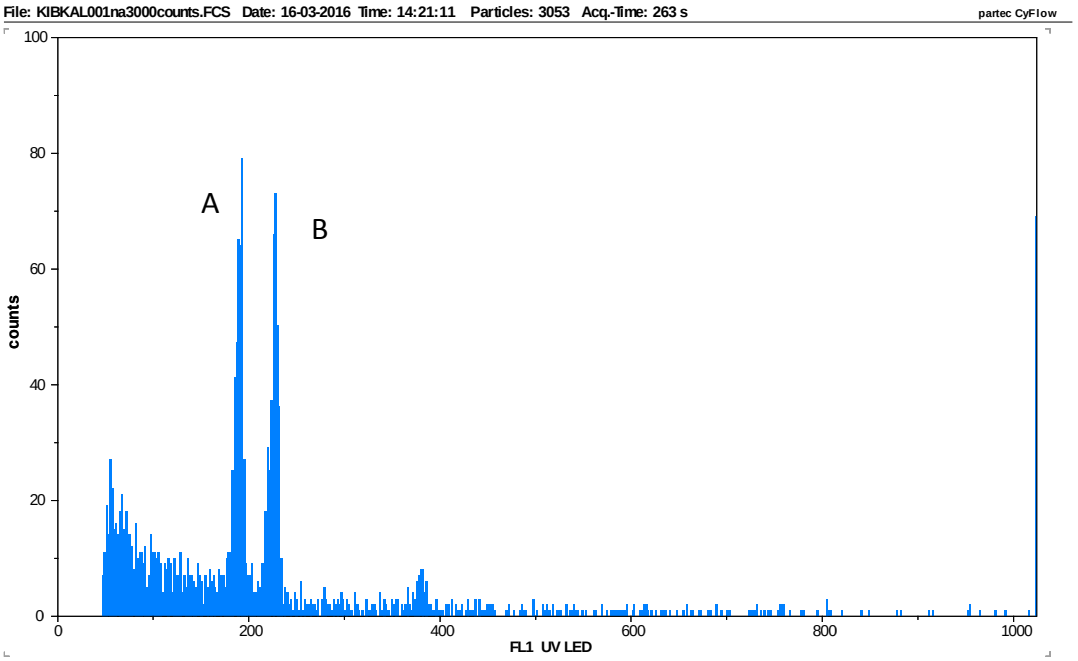


Figure 13. Representative example of flow cytometer histogram: A is standard (*Zea mays*), B is baobab

Figure 14. Photos of young baobab seedlings which were grown for flow cytometry analyses

6 DISCUSSION

6.1 Morphological comparison

Diameter of the trunk at breast height (DBH) measured among 195 trees ranged from 0.2-4.8 m. In Sudan, stem diameter among 240 trees ranged from 0.06 to 4.77 m (Gebauer and Luedeling, 2013). In Kenya, most common size class was 2-3 m, while in Sudan 1-1.25 m. Typical fruit shape reported was oblong, ellipsoid and obovate. Fruit shape ratio (1.7-1.9, assessed for inland populations only) was smaller compared to Sudanese populations (1.82-2.38) (Wiehle et al., 2014), which points out shorter fruits in inland Kenya. The mean fruit length ranged from 11.5 to 20.3 cm. If we consider only inland populations, the range is only 11.5-14 cm (from the coastal area were collected only two fruits, which were significantly longer). However, based on our own observation of trees with unripe fruits in the coastal region, we can conclude that coastal fruits are longer than inland fruits. Fruit length in Sudan was reported 13.9-16.5 cm (Wiehle et al., 2014), 15.7-22.2 cm in Mali and 12.9-17.6 cm in Malawi (Cuni Sanchez et al., 2011). Munthali et al. (2012) reported length of 11.9-16.5 cm. Very long fruits (16.59-22.71 cm) were found in Benin populations, which were located in an area with annual precipitation of approximately 900–1110 mm (Assogbadjo et al., 2006). It seems that baobab capsule length may be influenced by annual precipitation and monthly precipitation distribution. In our study, inland individuals with smaller fruits are growing in condition with four dry months and annual precipitation < 700 mm, whereas coastal environment has only two drier months and precipitation more than 1,000 mm. But to confirm this theory, more coastal fruits should be collected and compared to inland fruits.

Fruit morphological results did not show significant differences among inland populations. However, if the coastal fruits had been collected, the results would probably have distinguished coastal and inland populations (it was already mentioned that bigger fruits were observed). Leaf morphological data, collected for both coastal and inland accession, revealed significant differences between those two groups. The morphological data correlates with genetic results. Inland populations did not reveal differentiation to more populations, we could say that there is one big population with high level of gene flow. Clear

differentiation to coast and inland groups was observed for both morphological and genetic results.

6.2 Discussion to genetic analyses

High genetic variation within a one species is important to enhance its adaptability to new environments, to avoid inbreeding depression, to establish domestication, conservation and breeding programmes (Sreekumar and Renuka, 2006; Dawson et al., 2009). The genetic diversity in this study was high among and within all populations (mean He=0.8). Similar results were gained for Sudanese populations, where substantial level of genetic variation (mean He=0.65) was reported (Wiehle et al., 2014). West African studies (Assogbadjo et al., 2006; Assogbadjo et al., 2009; Kyndt et al., 2009) concerning *A. digitata* used AFLP markers, therefore not all results could be directly compared to our microsatellite data. Only Sudanese and Malawian studies were using microsatellites; Malawian data were treated as binary data only (0, 1) and for Sudanese data there were used two approaches: tetraploid and binary matrices. Nei's genetic diversity was 0.12-0.16 in case of Kenya. For Sudanese population, similar results were obtained (0.11-0.15) (Wiehle et al., 2014). In Malawi, population gene diversity was reported slighter higher (0.12-0.18) (Munthali et al., 2012).

The percentage of polymorphic loci was ranging from 39.37% to 81.89% with mean 61.64% in our study. In the studies from West Africa (Assogbadjo et al., 2006; Assogbadjo et al., 2009; Kyndt et al., 2009) higher values were obtained, i.e. 91.2-94.9%, 94.1-100%, 41.7-96.1% respectively. While Munthali et al. (2012) reported percentage of polymorphic loci lower from 39 to 59% with mean 48%. High values of the percentage of polymorphic loci indicate high genetic diversity, however this result may be influenced by number of samples per population or probably the fact of using different methods i.e AFLP and microsatellites.

The AMOVA (Analysis of molecular variance) results revealed low genetic variation among seven populations (12.37 %) and high variation within populations (87.63 %). However, in Sudan, it was 7.1% among and 92.9% within populations (Wiehle et al., 2014), thus our results show slightly higher differentiation among populations. Across eleven populations in Benin, Ghana, Burkina Faso, and Senegal, AMOVA reported 20.68% variation among and

79.32% within populations (Kyndt et al., 2009). Austerlitz et al. (2000) and Kyndt et al. (2009) assume, that high levels of pollen flow and multi-generational populations of long living trees are responsible for high levels of within-population genetic diversity and low among-populations, which is also supported by our results.

The genetic differentiation (G_{ST}) was revealed low to medium-low within inland populations and within coastal populations. However, between inland and coastal populations, substantially higher G_{ST} values were observed. This fact is probably associated with very low or no gene flow between coastal and inland populations. This seems to be improbable as the distance between Voi (inland population which is closest to coast) and Kilifi (coastal population closest to inland) populations was only 160 km, but reported G_{ST} value was high 0.11, comparing two inland population Kibwezi and Voi, where distance is 114 km and G_{ST} 0.004. In Sudan, Gst values were ranging from 0.025 to 0.048 and in Malawi 0.009 to 0.056 (Munthali et al., 2012; Wiehle et al., 2014). Our results support the hypothesis of very low gene flow between coastal and inland populations

The Bayesian clustering and principal coordinate analysis divided all individuals into two clearly separated clusters: again coastal and inland populations. This genetic structure exposing two distinct groups can be explained by different origin of inland and coastal populations or, more probably, it even suggests the existence of two different species. If we consider that one of the population could belong to one of the eight other *Adansonia* species (Baum, 1995; Pettigrew et al., 2012), it should be confirmed by its ploidy, as it is known that the other species are diploids. However, our flow cytometry and capillary electrophoresis results revealed only one ploidy – tetraploidy, both among coastal and inland trees, rejecting this theory. We could speculate that there could possibly be a new species or there is overlooked already known species of *Adansonia* in the coastal areas of Kenya. But how can be explained the origin of "*new-coastal*" species? It could have been introduced by humans to the coast thousand years ago from other region, the new species might have differentiated by influence of climatic conditions or elevation. We can also consider that the analysis revealing new species was not appropriate. In any case, further

research should be undergone. In Benin, baobabs across three different climatic zones were examined by AFLP. Their principal coordinate analysis showed similar results as in our case i.e. coastal populations (from Guinean climatic zone) were clearly divided from inland populations. Guinean zone (coastal zone) is characterized by mean annual rainfall of 1,200 mm, mean annual temperature 25–29 °C and the relative humidity 69–97 % (Assogbadjo et al., 2006). It is very similar to the Kenyan coast with annual rainfall more than 1,100 mm and annual temperatures 26°C. Assogbadjo et al. (2006) assigned coastal baobabs to the Dahomey-Gap, it means that the savannas interrupted the forest block and reach the sea coast. However, the study from Benin was the first baobab genetic research and the authors were not paying much attention to coastal populations. What if the coastal baobabs are creating new species across African coast? To confirm "*new-coastal*" species, further research is needed. One approach could be to collect coastal samples from Kenya, Benin or alternatively from other coastal areas and compare baobabs from humid, coastal regions across Africa.

Based on morphological observation, we consider that trees sampled close together (according to our methodology) may be genetically different because of different fruit shapes (Appendix III). For that reason, Moran's I index of spatial autocorrelation was assessed. Inland populations did not fully confirm our theory. Most of the trees are similar to distance of 100 m but some trees are similar while distance between them is only 0.9 km, 2 km, or 4-7km. Coastal individuals are the most similar at a distance of 3 km. It may be explained by seed dispersal via zoochory (by animals) or anthropochory (by humans). Monkeys, especially baboons, and also humans can transport the seeds even for kilometers (Wickens, 1982; Wiehle et al., 2014). It would explain the similarity of the trees, which are genetically close but the distance between them can be even several kilometers.

6.3 Ploidy and species identification

Although the presence of *Adansonia kilima* was confirmed in Kenya (Pettigrew et al., 2012), in our study it was not identified. It may be caused by small amount of samples subjected to flow cytometry analysis, which is connected with poor seed germination. Only 41% of the seed samples germinated, therefore it can be concluded, that the scarification method was not as effective as we expected. Scarification by treating seeds in concentrated sulphuric acid for 6-12 hours should be used to get germination rate of up to 90% (Danthu et al., 1995).

Flow cytometry is a fast, convenient and relatively cheap method for detecting ploidy in comparison to other methods i.e. chromosome counts, Feulgen microdensitometry, by estimating relative nuclear DNA content (Doležel et al., 2007a). Anatomy and chemistry of plant tissues are diverse among plants, therefore no single isolation buffer works well with all species (Doležel and Bartoš, 2005). In this study it was confirmed, that it is possible to use flow cytometry for *Adansonia* species and detect its ploidy by using buffers OTTO I and II as described in protocol from Doležel et al. (2007).

7 CONCLUSION

This study revealed high levels of genetic diversity of *Adansonia digitata* L. accessions collected in inland and coastal regions of South-eastern Kenya. In total, 189 individuals were genetically analysed using nine microsatellite loci, which were polymorphic. Bayesian clustering and principal coordinate analysis revealed two clearly distinct groups of individuals: coastal and inland. Our results even suggest the existence of a new coastal species, however, this statement has to be confirmed by more in-depth studies. Genetic differentiation showed sufficient gene flow within inland and within coastal populations, however, very low gene flow between those two populations was detected, which again supports our theory of two species. The relationship of trees growing close together seemed to have common values within inland population (trees were mostly correlated at the distance of 100 m) but unusual for coastal population (trees were mostly correlated at 3 km). The morphological data assessed for fruits (only for inland individuals) did not show any significant differences. For leaf morphology, the accessions from the coast had longer leaflets and petioles than that of the inland accessions. These results again correlate with the genetic evaluation. The occurrence of newly discovered species *Adansonia kilima* was not confirmed among our sampled individuals in Kenya, although its presence is confirmed in this country.

Our results could be used to determine the potential and select the best mother trees for baobab domestication and to integrate results of this research into the selection criteria of superior baobab individuals in Kenya. Attention should be also paid to conservation strategies, and viable populations should be maintained. It can be done preferably in-situ: on-farm practice for conservation of genetic diversity. We also recommend more intensive sampling and analysis in coastal areas across Africa, to confirm the possible existence of a new species of *Adansonia*.

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