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UNIVERSITY OF ABOMEY-CALAVI
FACULTY OF AGRONOMIC SCIENCES



SCHOOL OF ENVIRONMENT MANAGEMENT

4th Batch Licence-Master-Doctorate



Developing best agro-ecological practices for African
baobab tree *Adansonia digitata* L. leaves production
in smallholders farming systems in Benin

DISSERTATION

To obtain Master Degree of Agronomics Sciences
OPTION: Forestry and Natural Resources Management

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REPUBLIQUE DU BENIN
UNIVERSITE D'ABOMEY-CALAVI
FACULTE DES SCIENCES AGRONOMIQUES



ECOLE D'AMENAGEMENT ET GESTION DE L'ENVIRONNEMENT

4^{ème} Promotion Licence-Master-Doctorat



Développement de pratiques agro-écologiques pour la
production de feuilles du baobab africain *Adansonia digitata*
L. dans les petits systèmes d'exploitation agricole au Bénin

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Certification

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Dedication

*To the Almighty God for granting me his limitless care, love and blessings
all along the way, may this work serve to the glory of your holy name.*

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List of abbreviations

%	: Percentage
°C	: Degree Celcius
ADB	: African Development Bank
AGRN	: Natural Resources Management
AIC	: Akaike Information Criterion
ANOVA	: Analysis of Variances
ATS	: Agroforestry tree species
Cm	: Centimeter
Cv	: Coefficient of variation
FAO	: Food and Agriculture Organization of the United Nations
FSA	: Faculty of Agronomics Sciences
G	: Gramme
GRG	: Graduate Research Grant
Ha	: Hectare
HDI	: Human Development Index
ICRAF	: World Agroforestry Centre
IER	: Institut d'Economie Rurale
IFAD	: International Fund for Agricultural Development
INSAE	: Institut National de la Statistique et de l'Analyse Economique
Kg	: Kilogramme
kJ	: Kilojoule
km ²	: Square Kilometer
LABEF	: Laboratory of Biomathematics and Forestry Estimations
m	: Meter
max	: Maximum
min	: Minimum
MRECD	: Ministère des Relations Extérieures de la Coopération et du Développement
mm	: Millimeter
NGOs	: Non-Governmental Organizations
RUFORUM	: Regional Universities Forum for Capacity Building in Agriculture
SDGs	: Sustainable Development Goals
SCUC	: Southampton Centre for Underutilised Crops
T	: Ton
UAC	: University of Abomey-Calavi
UN	: United Nations
UNDP	: United Nations Development Programme
WFP	: World Food Programme

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Abstract

African baobab (*Adansonia digitata* L.) is an agroforestry species with high potential in the fight against food insecurity and malnutrition in Africa. Pulp and leaves of baobab are the two key harvested products which highly contribute to farmers, especially women income and households food security in sub-Saharan Africa. Overexploitation of these products, combined with the low natural regeneration of the species, threatens the survival of the remaining populations of the African baobab. Among sustainable alternatives for conservation is the domestication/cultivation of the species for its leaves and fruits.

This study focused on the cultivation of baobab seedlings for their leaves. It : (a) assessed baobab seedlings [a1] growth response, [a2] leaves biomass response, and [a3] roots' growth and biomass responses to variation of dose of compost - poultry dropping - cow dung, sowing density, and leaves harvesting frequency.

Agricultural experimentations were used for assessing baobab seedlings growth, leaves biomass and root growth and biomass in two biogeographical zones of Benin (Guineo-Congolese and Sudano-Guinean zones). Data of the experimentation (number of germinated seeds, of leaves, branches and rootlets, total height and basal diameter of the seedlings, fresh and dry weights of harvested leaves, of tap root and rootlets, Index of leaf area and total length and basal diameter of tap root and rootlets) were analyzed using linear and generalized linear mixed effects models. From our field experimentation producing *A. digitata* seedlings with 300 kg/100 m² of poultry dropping or 900 kg/100 m² of cow dung with 20 × 20 cm sowing density and monthly harvests yielded the optimum growth of seedlings, roots and its biomass and 15 × 15 cm for leaves biomass production. The average leaves biomass production was 41.62 ± 1.16 kg of dry matter per 100 m² (equivalent to 157.29 ± 4.38 kg of fresh leaves per 100 m²).

We suggested that further studies (i) assess profitability of baobab fresh leaves production, (ii) identify the potentials pests and the effective pest control approaches, (iii) evaluate on the anti-nutritional properties of harvested leaves as the plant respond to stress due to frequent harvesting, (iv) assess on the protection methods of baobab seedlings against the adventitious plants, and (v) assess chemicals components of baobab seedlings roots for its probable use in food and medicine.

Key words: *Adansonia digitata*, seeds, seedling, leaves, root biomass

Résumé

Baobab africain (*Adansonia digitata* L.) est une espèce agroforestière de haute potentialité dans le combat contre l'insécurité alimentaire et la malnutrition en Afrique. La pulpe et les feuilles du baobab sont les deux produits clés moissonnés qui contribuent fortement aux revenus des agriculteurs particulièrement des femmes et à la sécurité alimentaire des ménages en Afrique subsaharienne. La surexploitation de ses organes ajoutée à la faible régénération naturelle de l'espèce menacent les populations restantes. Une des solutions alternatives durables pour la conservation est la domestication/cultivation de l'espèce pour ses feuilles et fruits.

Cette étude est concentrée sur la culture des plantules de baobab pour leurs feuilles. Elle a : (a) évalué la dynamique de croissance des plantules de baobab [a1], la biomasse foliaire produite [a2], et [a3] le développement et la biomasse des racines suivant différentes doses de compost - fiente de volaille - bouse de vache, densités de semis, fréquences de récolte des feuilles.

L'expérimentation agricole a été utilisée pour évaluer la dynamique de croissance et la biomasse foliaire et racinaire des plantules de baobab dans deux zones bio-géographiques du Bénin (zones Guinéo-Congolaise et Sudano-Guinéenne). Des données de l'expérimentation (nombre de graines germées, de feuilles, branches et racines secondaires, hauteur totale et diamètre au collet des plantules, poids frais et sec des feuilles récoltées, de racines pivotantes et secondaires, indice de surface foliaire et longueur totale et diamètre au collet des racines pivotantes et secondaires) ont été analysées en utilisant les modèles linéaires et les modèles linéaires généralisés à effets mixtes.

De l'expérimentation pour la culture des plantules de *A. digitata*, l'application de 300 kg/100 m² de fiente de volaille ou 900 kg/100 m² de bouse de vache avec 20 × 20 cm densité de semis et les récoltes mensuelles ont rapporté la croissance optimale des plantules, des racines et ses biomasses et 15 × 15 cm pour la production optimale de biomasse foliaire. La production moyenne de biomasse foliaire est 41,62 ± 1,16 kg de matière sèche par 100 m² (équivalent à 157,29 ± 4,38 kg de feuilles fraîches par 100 m²).

Nous suggérons que de futures études (i) évaluent la rentabilité de la production des feuilles fraîches de baobab, (ii) identifient les potentiels parasites des plantules et les techniques de lutte efficace, et (iii) clarifient les propriétés anti-nutritionnelles des feuilles récoltées en réponse au stress lié à la fréquence des récoltes, iv) évaluent les méthodes de protection des plantules de baobab contre les adventices, et (v) évaluent les composantes chimiques de racines des plantules de baobab pour son usage probable dans l'alimentation et la médecine.

Mots clés: *Adansonia digitata*, graines, plantules, feuilles, biomasse racinaire

1.

General introduction

1. General introduction

1.1. Background

Food and nutritional insecurity is one of the major challenge worldwide. Approximately 12 percent (842 million) suffer from chronic hunger in the world (FAO, FIDA and WFP, 2013). Africa's population is projected to rise from 1 billion to 1.6 billion by 2030, and by 2050 it would have doubled (ADB, 2011). According to UNDP (2015), Sub-sahara Africa is the region in the world where HDI is the lowest (0.502), showing the precarious situation of this region. In Benin, region of Atakora and Donga in the North and Couffo and Mono are the most affected by Food insecurity. It has also been showed that in these regions, the most vulnerable households are those which have agriculture as single source of income. The challenge of feeding all those undernourished households is further challenged by a mixture of contemporary issues including: Land degradation, Deforestation, Loss of biodiversity and Increased risks of climate change (Hughes and Haq, 2003).

Because it is a global concern, a number of priorities have been set and defined as Sustainable Development Goals (SDGs) among which: End poverty for all, Freedom from hunger, Sustainable consumption and production, Sustainable ecosystems, Action on climate change. Agroforestry tree species (ATS) have been identified as key to help reaching this goal. Indeed, Agroforestry is centred on species-rich, low-input agricultural techniques including a diverse array of new indigenous tree crops, rather than on high-input monocultures with only a small set of staple food crops (Leakey, 2001a, 2001b). ATS have substantial potential to reach SDGs. Indeed, these trees bear fruits that are not highly researched and which are generally ignored by the commercial sector (Hughes and Haq, 2003). They are used traditionally for their food, fibre, fodder, oil or medicinal properties, but have yet to be adopted by large-scale agriculturalists for agri-business. They have the potential to contribute to food security, nutrition, health, income generation (Jamnadass *et al.*, 2009) and environmental services. They also help diversify farming systems (Dawson *et al.*, 2009). The rising human population has increased pressure on some of them, so that the wildy available resources are no longer capable of supporting the rising demand. It therefore becomes a must to Shift to cultivation/plantation instead of only saving Agroforestry tree species (ATS) in croplands/traditional agroforestry systems. Poor value chain promotion, limited market development, inadequate policies and lack of knowledge on cultivation, breeding, etc. are the probems which limited the optimal use of ATS includes. In front of the multiple degradations which the environment undergoes, it is significant to set up actions going in the direction of its protection. Wild edible plants have traditionally been

used by rural communities in Sub-Saharan Africa for subsistence and trade (Sinha and Brault, 2005). However, the wildy available resources from some tree species seem not to be sufficient to sustain the growing demand, since large numbers of these people are facing chronic food insecurity even in growing season. This suggests that people should not rely on only collection of wild resources, instead they should shift to their domestication. As such, it becomes essential to develop and experiment sustainable production methods using ecologically sound agricultural practices and beneficial to farmers.

One of the most important trees in the region is the multipurpose Baobab (*A. digitata* L.), an agroforestry tree species very common in Africa (Kyndt *et al.*, 2009). Regional consultations organised by the International Centre for Underutilised Crops (ICUC) have accorded high priority to enhanced research and development of baobab (Sidibé and Williams, 2002). Bioversity International classified baobab tree among the 10 top agroforestry tree species to be conserved and domesticated in West Africa (Eyog Matig *et al.*, 2002) largely because of its value in food preservation. Also in Benin, Assogbadjo *et al.* (2017) classified baobab tree among the 10 top priority Non timber Forest Products (NTFP) species to be valued due to its economic potential and the regional importance. National research efforts, especially in Benin, Nigeria, Burkina-Faso, Mali and Senegal have provided data on food values, agronomy, ethnobotany knowledge, ecology and genetic diversity of baobab. In many African countries, especially in West Africa, rural people rely on the variety of nutritional and medicinal products provided by the tree (Buchmann *et al.*, 2010; De Caluwé *et al.*, 2009). In Benin, baobab is the second common wild tree providing leafy vegetables after *Vitex doniana* and is followed by *Moringa Oleifera* as the third important tree in this category (Dansi *et al.*, 2008). It is ranked as the first most important traditional leafy vegetables in the arid zone of Benin (Dansi *et al.*, 2008). The species has great economic importance; a three months market investigation showed that commercialisation of baobab pulp generates up to US\$ 30,000 per year for 130 households of Malanville (north of Benin) involved in that business (Codjia *et al.*, 2003). Every part of the baobab is used: roots, bark, wood, leaves, flowers, capsules, gum, seeds, and fruits (Wickens and Lowe, 2008; Buchmann *et al.*, 2010). Among the 300 uses that have been identified, the tree provides nutritious food, livestock fodder, fibre, medicine, and income to local people (Wickens, 1982; Sidibé and Williams, 2002; De Caluwé *et al.*, 2010; Buchmann *et al.*, 2010). The baobab tree also provides important ecosystem services: it adds organic matter and nutrients through leaf-fall, it reduces soil temperature and water loss (Amundson *et al.*, 1995) and it attracts birds and large mammals that add nutrients to the soil with their droppings

(Wickens and Lowe, 2008). Moreover, chemical analysis of parts contents showed the presence of proteins, amino acids, iron, vitamins C, A, E and F (abundant compared to daily needs) within its organs, especially leaves (Sidibé and Williams, 2002; De Caluwé *et al.*, 2010). Pruning in baobab is so high that only a few fruits are produced per tree as a result of reduced photosynthesis. The leaves are generally consumed fresh in rainy season but in dried form (common practices in the arid zone) in dry season (Dansi *et al.*, 2008). As such, fresh leaves are collected, dried, pounded and sieved into powder in the rainy season when they are abundant for use during the dry season when fresh leaves are scarce (Dansi *et al.*, 2008). However, it is preferred fresh (Dansi *et al.*, 2008), but non availability in dry season constrains local people to dry the leaves for use in dry season. This practice leads to forced harvest of all the leaves that may affect fruit and seed production of the species as found for some tropical species. In the north and central Benin, baobab is said, when regularly consumed, to give stoutness and facilitate good growth, dentition and bones' solidification in the children. According to certain communities, baobab leaves have curative, regulative and stimulative properties besides food qualities and are sometimes used as nutraceutical (Dansi *et al.*, 2008).

According to the World Agroforestry Centre and Bioversity International, the multipurpose baobab tree is expected to play a major role in future crop diversification programs and in the development of West-African agroforestry systems. This was also expressed by the rural people in semi-arid West Africa, who selected baobab among top species that should deserve more attention in future domestication programs (Eyog Matig *et al.*, 2002; FAO, 2014). The domestication and cultivation of the baobab is necessary to protect natural stands and to provide a sustainable source of food, medicine and income to local population (Sanchez, 2010a). Domestication seeks to capture and multiply trees with desirable characteristics, thus taking advantage of the variations found in the wild (Pye-Smith, 2010). While preserving the characteristics that are important to local or international customers, domestication also reduces the time of first fructification (Chikamai and Tchatat, 2009). Domestication and cultivation can not only increase the harvested volume, but also they can ensure reliability and quality of supply, aspects identified to be key factors determining the long-term viability of a given product in international trade (Chikamai and Tchatat, 2009). The big challenge the species is facing is a high risk of extinction in the wild where it is threatened by bush fire (seedlings), grazing and lack of natural regeneration (Assogbadjo *et al.*, 2010). Conservation and domestication strategies of baobab genetic resources consist in the proper management option to obtain sustainable benefits for present and future generations. Consequently, finding a

pathway for propagating baobab in rural areas, especially in smallholder farming systems to satisfy the daily demand of baobab leaves will not only allow protecting the species but also help in fighting food insecurity and poverty. Previous research evidenced genetic differentiation between populations of the species in the three climatic zones of Benin (Assogbadjo *et al.*, 2009). Plant spacing for vegetable cultivation is an important criterion for attaining maximum vegetative growth and an important aspect of crop production for maximizing the yield. Optimum plant spacing ensures judicious use of natural resources and makes the intercultural operations easier. It helps to increase the number of leaves, branches and healthy foliage. Densely planted crop obstruct the proper growth and development. On the other hand, wider spacing ensures the basic nutritional requirements but decrease the total number of plants as well as total yield. Yield may be increased for any crop up to 25% by using optimum spacing in leafy vegetable (Bansal *et al.*, 1995). Investigations of the effect of cutting frequency on biomass production have been undertaken and differences in production due to various cutting regimes have been found with many species such as *Leucaena leucocephala* and *Gliricidia sepium* (Guevarra *et al.*, 1978; Latt *et al.*, 2000). Baobab tree can withstand drought, it has no important pest or diseases and it does not require extra watering (SCUC, 2006; Sidibé and Williams, 2002). Declining soil fertility remains the fundamental reason for declining smallholder food production in sub-Saharan Africa (Gruhn *et al.*, 2000; Smaling *et al.*, 1997). Fertilizer application in appropriate time, appropriate dose and proper method is the prerequisite of crop cultivation (Islam, 2003). Organic manures from domestic breeding or compost from domestic organic waste are hypothesized to provide sustainable manure for sustainable production in smallholder farming systems (Assogba-Komlan, 2001). Also, organic manures from domestic breeding or compost from domestic organic waste are hypothesized to provide sustainable manure for sustainable production in smallholder farming systems (Assogba-Komlan, 2001). However, animal rearing in Benin is cow-based in both semi-arid and arid zones whereas it is poultry-based in the humid zone. This would thus influence the type of dung or droppings available for the propagating tests but also adoption of the options that will be further developed.

1.2. Research objectives and hypotheses

1.2.1. Objectives

This study aims at developing agro-ecological practices to improve the production of the daily used baobab leaves by building capacity of local farmers and NGOs on how to propagate and grow *A. digitata* seedlings in small garden plots using environmentally friendly practices.

Specifically, this study focuses on:

- (i) Assessing the combine effect of organic manure (compost versus animal excrements), dose of organic manure, density of sowing and frequency of leaves harvesting on *A. digitata* seedlings growth;
- (ii) Assessing the combine effect of organic manure (compost versus animal excrements), dose of organic manure, density of sowing and frequency of leaves harvesting on *A. digitata* leaves production;
- (iii) Assessing the combine effect of organic manure (compost versus animal excrements), dose of organic manure, density of sowing and frequency of leaves harvesting on the growth and biomass of roots of *A. digitata* seedlings.

1.2.2. Hypotheses

H (i): Optimum combination of organic manure (compost versus animal excrements), dose of organic manure, sowing density and frequency of leaves harvesting on *A. digitata* seedlings growth is identified;

H (ii): Optimum combination of organic manure (compost versus animal excrements), dose of organic manure, sowing density and frequency of leaves harvesting on *A. digitata* leaves production is identified;

H (iii): Optimum combination of organic manure (compost versus animal excrements), dose of organic manure, sowing density and frequency of leaves harvesting on the growth and biomass of roots of *A. digitata* seedlings is identified.

1.2.3. Research questions

Q (i): What is the combination of the various factors (dose of organic manure, sowing density and leaves harvesting frequency) which gives the optimal germination rate, number of ramifications, height and basal diameter of baobab seedlings?

Q (ii): What is the combination of the various factors (dose of organic manure, sowing density and leaves harvesting frequency) which gives the optimal number of leaves, morphological traits of the leaf and dry weight of yield of baobab seedlings?

Q (iii): What is the combination of the various factors (dose of organic manure, sowing density and leaves harvesting frequency) which gives the optimal growth and morphological traits of tap roots and rootlets and the dry weight of roots biomass of baobab seedlings?

1.3. Outline of the dissertation

This dissertation is composed of six (08) parts. The first part (General introduction) presents the background and objectives of the study. The second part describes the study area in particular the physical characteristics and the socio-economic data and brief description of *A. digitata*. The third part presents a review of the technical route on baobab leaves production at early stage. The fourth part assesses the morphological variation of baobab fruits and seeds traits across three biogeographical zones in Benin. The fifth part assesses the growth responses of baobab seedlings to variation of dose of organic manure, sowing density and leaves harvesting frequency. The sixth part assesses the leaves biomass responses of baobab seedlings to variation of dose of organic manure, sowing density and leaves harvesting frequency. The seventh part assesses the growth and biomass responses of roots of baobab seedlings to variation of dose of organic manure, sowing density and leaves harvesting frequency. The conclusion and perspectives are presented at the eighth part.

The parts 3, 4, 5, 6 and 7 are written in the format of scientific papers. Then, a short introduction, methods and principal results are presented. Each one of these parts was discussed and concluded.

2.

Description of *A. digitata* and study area

2. Description of *A. digitata* and study area

2.1. Description of *A. digitata*

2.1.1. Taxonomy description

Baobab tree (Figure 2.1a), Kingdom: Plantae; Phylum: Tracheophyta; Class: Magnoliopsida; Order: Malvales; Family: Malvaceae; Genus: *Adansonia*; Species: *digitata*; Botanical name: *A. digitata*; English name: African baobab (Bosch *et al.*, 2004).

Synonyms: *Adansonia baobab* Gaertn., *Adansonia digitata* var. *congolensis* A. Chev., *Adansonia integrifolia* Rafin., *Adansonia scutula* Steud., *Adansonia Situla* Spreng., *Adansonia somalensis* Chiov., *Adansonia sphaerocarpa* A. Chev., *Adansonia sulcata* A. Chev., *Baobabus digitata* Kuntze., *Ophelus sitularius* Lour. (Assogbadjo and Loo, 2011).

The tribe, which is pantropical, includes *Bombax* and *Ceiba* producing fruit fibres used as kapok. The family includes about 30 genera, 6 tribes and about 250 species (Baum, 1995). A number of these species are used locally for leaves, wood, fruits, seeds or gum (Rahul *et al.*, 2015).

A. digitata is a massive deciduous tree, up to 20-30 m tall with a diameter up to 2-10 m at adult age (Rahul *et al.*, 2015). African baobab is a very long-living tree. It normally lives for about 500 years, but it is believed that some trees are up to 5000 years old (Namratha and Sahithi, 2015). The trunk is often of vast girth. The bark is smooth, reddish brown to grey, soft and possesses longitudinal fibers. *A. digitata* is highly branched. *A. digitata* produces an extensive lateral root system until 50 m from the trunk. The roots tips are often in the form of tubers. But the main roots of old trees are relatively shallow and rarely extend beyond 2 m depth. Therefore they are very sensitive to strong winds and can be uprooted by storm (Sidibé and Williams, 2002). Leaves of young trees are usually simple. Adult trees begin each season by producing simple leaves followed by 2-3-leaflets leaves; mature leaves (20 cm diameter with about 5 to 9 leaflets) appear later. The inflorescence of baobabs consists of a single flower, situated in the axils of leaves near the tips of reproductive branches. The flowers are white, large, pendulous, solitary or paired in the leaf axils, showy (Wickens, 1982). Flowering begins about the end of dry season or just before the first rains; often when the first leaves appear. The flowers open in late afternoon and fall the next day at dawn. The flowers emit a sulfur fragrance that attracts particularly the bats which play the role of pollinators (Wickens and Lowe, 2008).

The history of known references to African baobab is well documented (Baum, 1995). The binomial name of *A. digitata* was given by Linnaeus, the generic name honouring Michel

Adanson who had been to Senegal in the eighteenth century to describe baobab (Adanson, 1771).

The African baobab is named a very large number of local names. A selection of important ones is shown in Table 2.1.

Table 2.1 Common names for African baobab

Language	Common name
English	African baobab, baobab, monkey bread tree, Ethiopian sour gourd, cream of tartar tree, senegal calabash (fruit), upside-down tree
French	Baobab, pain de singe (fruit), arbre aux calebasses, arbre de mille ans, calebassier du Sénégal
Fon/ goun	Kpassa
Yorouba/ nagot	Osché
Dendi	Kôô
Bariba	Sônbu
Ditammari	Moutomu

Other local names can be found in Assogbadjo and Loo (2011); Rahul *et al.* (2015).

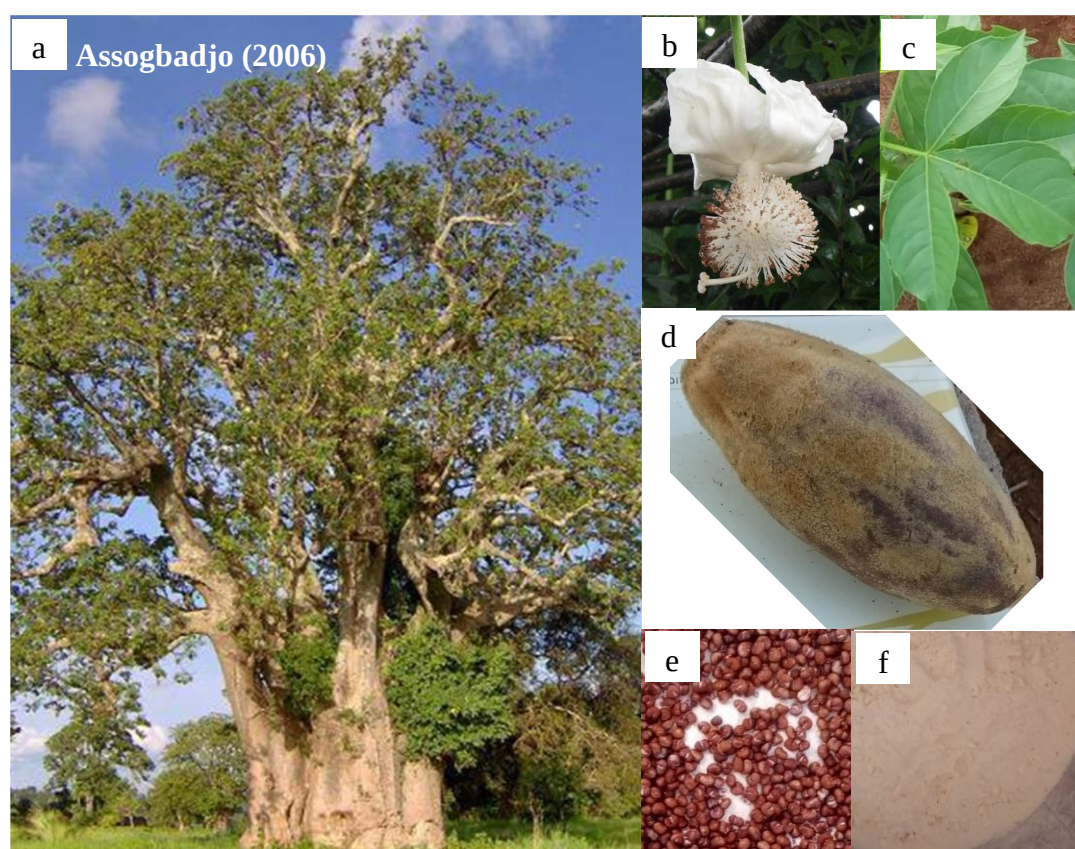


Figure 2.1 Baobab tree and its various organs
Baobab tree (a) (Assogbadjo, 2006), Flower (b), Leaves (c), Fruit (d), Seeds (d), Pulp (f)

2.1.2. Geographic distribution and Ecology

The African baobab (*A. digitata*) occurs naturally in most countries of Sahara as a scattered tree in the savannah, and is also present in human habitation. *A. digitata* is distributed in the dry climatic zone (above 150–250 mm precipitation) in the North Soudanian zone and is an important species in the dry tropical zone south of the Sahara (Wickens, 1982; Diop *et al.*, 2005) (Figure 2.2). The species is also found in more humid sites (1,400–1,600 mm) as for example Benin (Assogbadjo, 2006), Togo, Angola, etc. The tree has been introduced in many countries used as an ornamental plant. It is also known as the dead-rat tree (from the appearance of the fruits), monkey-bread tree (the dry fruit as food for monkeys), upside-down tree (the bare branches looked like roots) and cream of tartar tree (the acidic taste of the fruits) (Sidibé and Williams, 2002). The distribution of *A. Digitata* is not continuous, but rather scattered as revealed by Jensen *et al.* (2011) in Burkina Faso, Mali and Niger, examples from Sudan as reported by Wickens and Lowe (2008) and in southern Africa (Anthony Cunningham, personal communication; www.phytotrade.com).

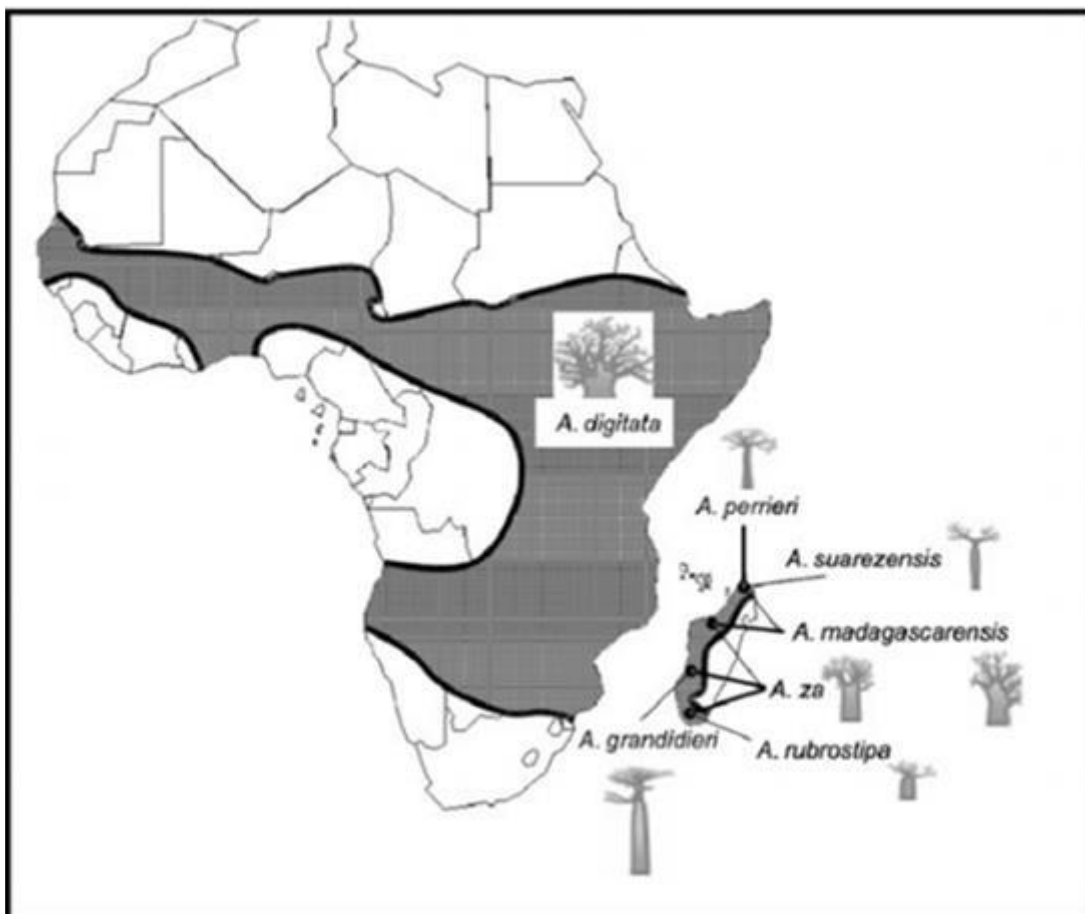


Figure 2.2 Distribution of *Adansonia spp* in Africa and Madagascar (Diop *et al.*, 2005)

2.1.3. Socioeconomic importance

Traditional information

The various parts of the plant (leaves, bark and seeds) are used as a panacea, that is, to treat almost any disease and specific documented uses include the treatment of malaria, tuberculosis, fever, microbial infections, diarrhoea, anaemia, dysentery, toothache, etc. (Table 2.2) (Adanson, 1771; Watt and Breyer-Brandwijk, 1962; Nguta *et al.*, 2010). The leaves and fruit pulp are used as febrifuge as well as an immune stimulant (De Caluwé *et al.*, 2010; El-Rawy *et al.*, 1997).

In India, it is reported that baobab pulp is used externally with buttermilk for the relief of diarrhea and dysentery, while the young leaves are crushed and used to treat painful swellings (Sidibé and Williams, 2002).

In some countries in West Africa, the leaves, fruit pulp and seeds are the main ingredients in sauces, porridges and beverages (De Caluwé *et al.*, 2010; Chadare *et al.*, 2009; Yusha'u *et al.*, 2010). Recently, baobab has been referred to as a “super fruit” based on its nutritional profile (e.g. vitamin, fatty acid, mineral) (Gruenwald, 2009). The nutritional value of baobab is only briefly discussed since a comprehensive report on the nutritive aspects is already available (De Caluwé *et al.*, 2010; Chadare *et al.*, 2009). The major interest in baobab products is as a result of its ascorbic acid and dietary fiber content. The level of vitamin C contained in fruit pulp is high and can range from 2.8 to 3 g/kg (Vertuani *et al.*, 2002). It was noted that baobab fruit pulp has very high vitamin C content (280–300 mg/100 g), which is seven to ten times more than oranges (51 mg/100 g) (Manfredini *et al.*, 2002; Täufel *et al.*, 1993). One study demonstrated that the consumption of 40 g of baobab pulp provided 100% of the recommended daily intake of vitamin C in pregnant women (19–30 years) (Chadare *et al.*, 2009). The ascorbic acid content was evaluated in the fruit of *A. digitata* (Diop *et al.*, 1988); and it was found to contain 337 mg/100 g of ascorbic acid (Eromosele *et al.*, 1991; Gebauer *et al.*, 2002). Sidibé and Williams (2002) recommended that baobab leaves should be stored as whole leaves rather than ground leaf powder in order to preserve the high vitamin content.

Although *A. digitata* is mostly regarded as a fruit-bearing forest tree, it is a multipurpose and widely-used species with medicinal properties, numerous food uses of various plant parts, bark fibers and root that are used for a variety of purposes. Centuries ago the products were traded: it was well known in Cairo markets in the sixteenth century (Sidibé and Williams, 2002).

Table 2.2 Traditional medicinal uses of *A. digitata* in different types of aliments (Kamatou *et al.*, 2011)

Aliments	Part(s) used	Preparation	References
Diarrhoea, fever, inflammation, kidney and bladder diseases, blood clearing, asthma	Leaves	Decoction, infusion	Brendler <i>et al.</i> (2003)
Malaria, fever	Leaves	Mixed with water	Watt and Breyer-Brandwijk (1962)
Toothache, gingivitis	Leaves	Decoction	Tapsoba and Deschamps (2006)
Diaphoretic, fever remedy	Leaves	Decoction	Abbiw (1990)
Diaphoretic, kidney and bladder diseases, asthma, insect bites	Leaves	Decoction	Wickens (1982)
Fever, diarrhoea	Seeds	Mixed with water	Watt and Breyer-Brandwijk (1962)
Microbial diseases	Fruits	Decoction	Jumelle (1909)
Anaemia	Bark	Aqueous extract	Adesanya <i>et al.</i> (1988)
Coughs	Powdered seeds	Decoction	Watt and Breyer-Brandwijk (1962)
Wound healing	Stem bark	Decoction	Baillon (1876)

Leaves of *A. digitata*

Leaves are 2-3-foliate at the start of the season and they are early deciduous, of which more mature ones are 5-7(-9) foliate. Leaves are alternate at the ends of branches or occur on short spurs on the trunk. Leaves of young trees are often simple. Leaflets are sessile to shortly petiolulate with great variation in size. Overall mature leaf size may reach a diameter of 20 cm and the medial leaflet can be 5-15 × 2-7 cm, leaflet elliptic to ovate-elliptic with acuminate apex and decurrent base. Margins are entire and leaves are stellate-pubescent beneath when young becoming glabrescent or glabrous. Stipules are early caducous, subulate or narrowly triangular, 2-5mm long, glabrous except for ciliate margins (Figure 2.1c) (Sidibé and Williams, 2002).

The leaves contain 13-15% protein, 60-70% carbohydrate, 4-10% fat and around 11% fibre and 16% ash (De Caluwé *et al.*, 2010). Energy value varies from 1180-1900kJ/100g of which 80% is metabolized energy. The leaves are rich in pro-vitamins A and C (Zahra'u *et al.*, 2014).

The highest level of pro-vitamin A was detected in young leaves especially when used as dried material (Sidibé *et al.*, 1996), expressed in retinol equivalent it was between 9 and 27 mg/kg. Nordeide *et al.* (1996) recorded that the level of pro-vitamin A was about one-third the content

in *Amaranthus* dried leaves. Becker (1983) noted the absence of vitamin C but a significant content of vitamin B2.

In terms of mineral content, baobab leaf is said to be an excellent source of calcium, iron, potassium, magnesium, manganese, molybdenum, phosphorus, and zinc (Yazzie *et al.*, 1994). The leaves of baobab tree are a staple for many populations in Africa, especially the central region of the continent (Yazzie *et al.*, 1994; Gebauer *et al.*, 2002). During the rainy season when the baobab leaves are tender, people harvest a fresh batch of leaves. During the last month of the rainy season, leaves are harvested in great abundance and are dried for domestic use and for marketing during the dry season. The leaves are typically sun-dried and either stored as whole leaved or pounded and sieved into a fine powder (Gebauer *et al.*, 2002). Young leaves are widely used, cooked as spinach, and frequently dried, often powdered and used for sauces over porridges, thick gruels of grains, or boiled rice (Sidibé and Williams, 2002).

2.1. Study area

2.2.1. Geographic location

Benin is a West African country located between latitude 6°10'N-12°25'N and longitude 0°45'E-3°55'E (Figure 2.3, Table 2.3). It covers a land area of 114,763 km².

Three climate zones can be distinguished in Benin (Adjanohoun *et al.*, 1989): (i) the humid zone from the coast up to 7°N with two rainy seasons alternating with a long dry season (December-February). The short dry season (July-August) rarely exceeds two months. (ii) The sub-humid zone: between the latitudes 7° and 9°N, with a tendency to one rainy season and one dry season; (iii) the semiarid zone between 9°N-12°25'N, characterized by unimodal rainfall regime. The rainy season lasts seven months (on average) and covers the April-October period with a maximum around August or September.

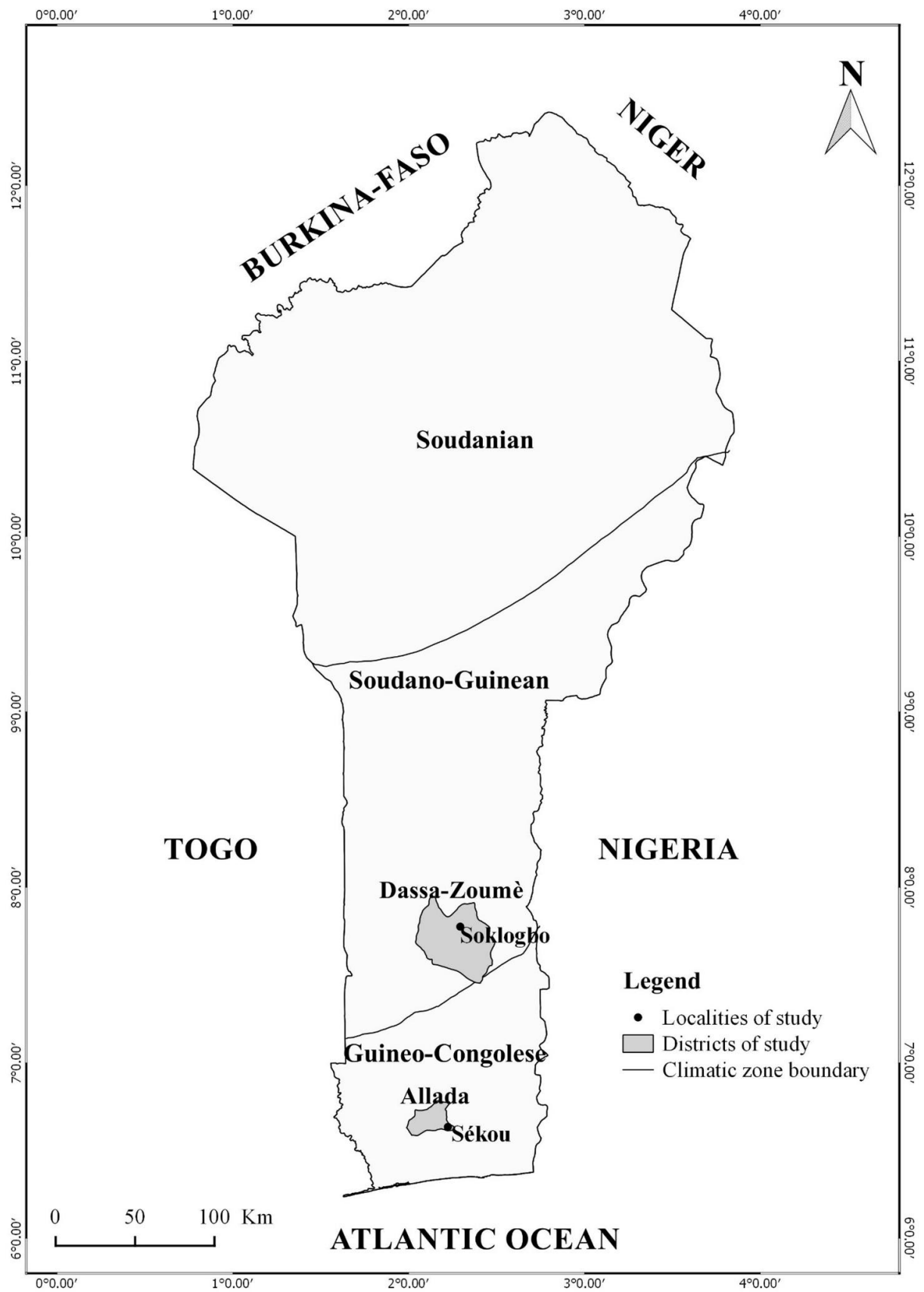


Figure 2.3 Map showing the sites of study

2.2.2. Climate and hydrography

Benin has tropical weather conditions all year round. Mean annual rainfall varies from 785 to 1485 mm. Its lowest values are recorded in the south-western section and in the north (787-950 mm). The highest precipitations (1200-1500 mm) are recorded from southeast Benin as well as the Bassila-Djougou corridor. Mean annual temperatures range from 26°-28°C and may exceptionally reach 35° to 40°C in northern localities such as Kandi and Malanville. Annual temperature amplitude is low in the south (5°-10°C) while it is higher (11°-13°C) in the northern part (from 8°N northwards) (Adomou *et al.*, 2006; Kamagate *et al.*, 2007). In the southern part of the country minimum temperatures are 20-25 degrees Celsius. In the northern part of the country it may be a few degrees colder from November to February. However, temperatures lower than 12 degrees Celsius are uncommon.

Benin has several inland water among which the most important are Pendjari in the North-West (380 km), Couffo in South-East (170 km), Ouémé in the Southern and Central part (450 km) and Mono in Western part (100 km). Some of these rivers have significant tributaries. Those are: Mékrou (410 km), Alibori (338) and Sota (250 km) for Niger River, and with Zou (150 km) for Ouémé river.

2.2.3. Soils and vegetation

Four major soil types with characteristic vegetation are distinguished in the territory of Benin (Willaine and Volkoff, 1967): (1) ferralitic soils covered by semi-deciduous forest, (2) ferruginous soils covered by dry forest, woodland, and savanna, (3) vertisol in the depression of Lama covered by a dry semi-deciduous forest, and (4) hydromorphic soils covered by swamp and riparian forests.

2.2.4. Population and economy

The Republic of Benin has an estimated population of 10 880 000 inhabitants in 2015 (UN, 2015) and slightly female-biased (50.12%). The development index of UNDP was 0.485 placing it at 167th position from 188 countries in 2015 (UNDP, 2016). The gross product per habitant was 745.47 Dollars US in 2013 (INSAE, 2015). With the support of its partners, Benin has been able to make important economic and structural reforms and sustain its economic growth rates over the last decade (World Bank, 2015). Growth has been primarily driven by trade and agriculture. The country economy is overwhelmingly agricultural, and most workers (> 70%) engaged in extensive agriculture.

Poverty remains widespread in Benin, with national poverty rates of 37.5% in 2006, 35.2% in 2009 and 36.2% in 2011 (World Bank, 2015). Female-headed households experience lower

levels of poverty (28%, compared to 38% for male-headed households), though women remain more vulnerable and continue to suffer from a lack of economic opportunities.

Table 2.3 Characteristics of the three biogeographic zones within which sites were selected for data collection

Parameters	Guineo-Congolean zone	Sudano-Guinean zone	Sudanian zone
Location	6°25' -7°30' N	7°30' -9°45' N	9°45' -12°25' N
Rainfall regime	Bimodal	Unimodal	Unimodal
Rainfall (mm)	1200	900-1110	<1000
Temperature (°C)	25-29	25-29	24-31
Relative humidity (%)	69-97	31-98	18-99
Climate type	Humid tropical	Humid tropical	Dry tropical
Vegetation	savannas and gallery forests	Mosaics of woodlands	Fallows and small forest patches
Density of population (Inhabitant.km ⁻²)	>70	31-70	7-30

Source : White (1983)

3.

Knowledge on *A. digitata* leaves production: a review

(In preparation for submission to *FRUITS*)

3. Knowledge on *A. digitata* leaves production

Abstract

Baobab (*Adansonia digitata* L.) is an agroforestry species with high potential in fighting against food insecurity and malnutrition in Africa. Pulp and leaves of the species are the two key harvested products. Overexploitation of those organs added to low natural regeneration of the species put at risk the remaining population of the species in the wild. The current review is an attempt to summarize and critical examine the technical routes for the species leaves production, from germination to leaves harvesting. Results showed diverse seed pre-treatments (physical, chemical and thermic) but seed scarification with a razor blade was often considered safe and yielded good germination rates (60 -100 %). There was also a large variation of sowing density (10 cm × 20 cm to 250 cm × 250 cm). The substrates used are often enriched with compost or manure (44.44 - 6250 kg per 100 m²) and even chemical manure but we found a critical lack of precision on doses used. Frequency of leaves harvesting varied from every two weeks to every eight weeks. Harvesting techniques consist in collecting all leaves but sparing the terminal buds. Leaves biomass lacks consistent data, making hard to derive a consistent trend. However, harvested leaves biomass for a sowing density of 15 cm x 15 cm yielded 400 kg / 100 m² / month for the first two months after germination and 200 kg / 100 m² / month from the 3rd month. We conclude that further experimentations are still needed to optimize the baobab leaves production. Suggested foci include (i) providing consistent data on leaves biomass in relationships to technic routes, (ii) elucidating profitability of the activity, (iii) identifying the potentials devastating of the seedlings and the effective fight techniques, and (iv) clarifying on the anti-nutritional properties of harvested leaves as the plant responses to stress due to frequent harvesting.

Key words: Baobab leaves, seed pre-treatment, density of sowing, fertilization, harvesting frequency

3.1. Introduction

Baobab (*Adansonia digitata* L.) is an African wild edible tree species which belongs to the family Malvaceae. *Adansonia digitata* is a multi-purpose tree of which the products have numerous food uses and medicinal properties. The pulp, the seeds, and the leaves are all utilized and are essentially wild-gathered foods, daily consumed and commercialized by rural populations in Africa. Various plant parts (*e.g.* leaves, bark, fruit, pulp), have traditionally been used for immuno-stimulant, anti-inflammatory, analgesic, insect repellent and pesticidal properties, in the treatment of diarrhoea and dysentery in many African countries, and have been evaluated as a substitute for imported western drugs (Ramadan *et al.*, 1994; El-Rawy *et al.*, 1997). Baobab products (*e.g.* seed oil, fruit pulp) are increasingly being commercialised and exported around the world leading to increased pressure on its natural populations (Sidibé and Williams, 2002). The tuberous taproot of seedlings and young saplings are also eaten, especially in times of famine. Recently, the European Commission authorised baobab fruit pulp as a novel food (Buchmann *et al.*, 2010) while it was approved in 2009 by the Food and Drug Administration as a food ingredient in the United States of America (Addy, 2009). Due to the high demand for commercial baobab products in EU and United States, this tree with its edible fruits needs to be conserved and treasured (Sanchez *et al.*, 2010a). Leaves of young trees are often simple, they are glabrous to tomentose, and highly appreciated for food (Sidibé and Williams, 2002), because their leaves are particularly rich in calcium, and they are known to contain good quality proteins (Chadare *et al.*, 2009). For example, the average daily consumption of baobab leaves is estimated to 11 - 50 g per person (Sidibé *et al.*, 1998; Dhillon and Gustad, 2004). In 1988–1989 in the region of Ségou (Mali) alone, 3,600 tonnes of dried leaves were consumed, corresponding to 2.49 kg per person per year (Savard *et al.*, 2006). All the leaves are harvested from wild populations and the intensity and traditional methods for collection of leaves have negative feedback on the species reproduction, hence persistence (Assogbadjo, 2006; Korbo *et al.*, 2013). The increasing awareness of these drawbacks added to the increasing demand of baobab leaves have led to trials on the production of baobab leaves as vegetables at early stage (Eyog Matig *et al.*, 2002; Assogbadjo, 2006; Pye-Smith, 2010; Korbo *et al.*, 2013). Literature reviews on baobab by FAO (1982), Sidibé and Williams (2002), ICUC (2004), Diop *et al.* (2005), Gruenwald and Galizia (2005), Assogbadjo (2006), Kalinganire *et al.* (2007), Teklehaimanot (2008), Chadare *et al.* (2009), De Caluwé *et al.* (2010), Kaboré *et al.* (2011), Kamatou *et al.* (2011), Jamnadass *et al.* (2011), Stadlmayr *et al.* (2013), Zahra'u *et al.* (2014), Namratha and Sahithi (2015) and Gebauer *et al.* (2016) provide substantial information on the species taxonomy, distribution, properties, utilization, agronomy,

agroecology, nutritional value of their food products and commercial importance. However, these reviews did not provide any knowledge on the state of arts of the various technics used for baobab leaves production.

This study aimed to present a synthesis review of the various technics reported on baobab leaves production and suggest future research prospects to advance the field. In this study the following key question was posed: What are the technical routes for baobab leaves production at early stage?

3.2. Methods

Numerous literature searches were conducted, primarily using Google™ and Google Scholar™. A variety of terms and combinations of terms were used including ‘baobab’, ‘*Adansonia digitata*’, ‘baobab production’, ‘baobab domestication’, ‘baobab seedlings’, ‘baobab leaves production’, and ‘baobab seeds germination’. For each baobab leaves cultivation parameter (seed pre-treatment, irrigation, harvesting technics, density of sowing, fertilization, harvesting frequency), various technics were noted according to each authors. Yields variation were also extracted to compare different technics identified by authors in synoptic tables (see tables 3.1 & 2). We also included articles that focused on germination ability of baobab but not necessary aiming at production of its leaves. This was done since those articles provide basic information on germination which is a key step for the species cultivation.

3.3. Results

3.3.1. Brief description of the scope of examined articles

From the raw articles initially collected, thirty five (35) articles were considered and examined in this review. Several studies focused on the development of suitable technics for baobab leaves production as vegetable to ensure the availability of fresh leaves of the species throughout the year. Most of these studies were conducted in Mali, Nigeria, Burkina Faso, Senegal, Benin, India, Niger, Madagascar, Denmark, Italy, Malawi and Southern Africa (Figure 3.1). These studies focused on examining effects of seed pre-treatment, fertilization, density of sowing, irrigation, harvesting frequency and harvesting technics on the variation in the yield of baobab leaves (Figure 3.2).

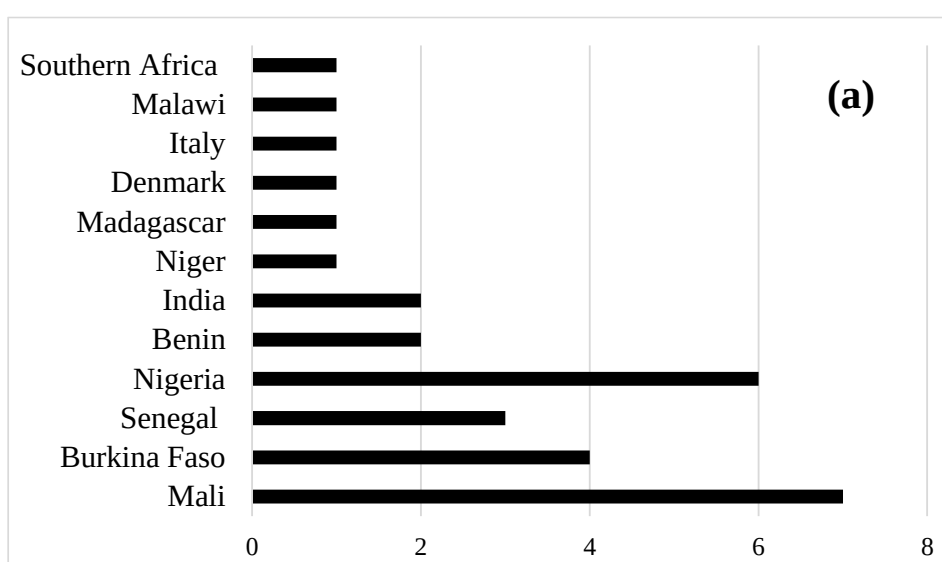


Figure 3.1 Study trend on baobab leaves production per region

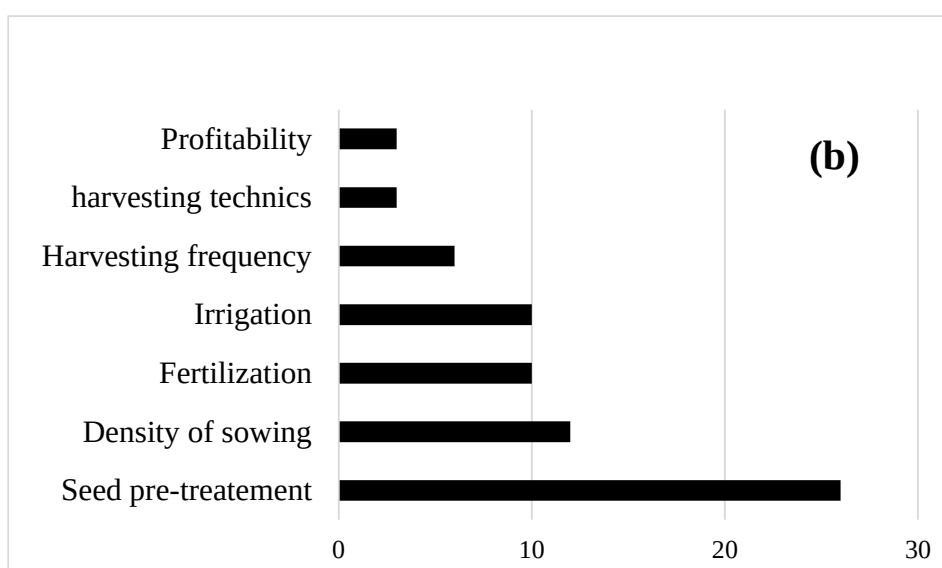


Figure 3.2 Study trend focused on the technics of baobab leaves production

3.3.2. Seed pre-treatment

In natural conditions, low seed germination is often mentioned for the African baobab (Esenowo, 1991; Sidibé and Williams, 2002), thus leading to test of several pre-treatments methods. As a consequence, several technics have been tested. Maghembe *et al.* (1994), IER/ICRAF (2001), Sidibé and Williams (2002), Savard (2003), Assogbadjo (2006), Savard *et al.* (2006), Olivier *et al.* (2006), Razanameharizaka *et al.* (2006), SCUC (2006), Kalinganire *et al.* (2007), Munthali *et al.* (2008), Assogbadjo *et al.* (2010) reported that seed scarification with a razor blade can lead to high germination rate (almost 100 %). Lower germination percentage (85 – 92 %) was reported for cutting the seed coat and then soaking in cold water for 72 h (Maghembe *et al.*, 1994; SCUC, 2006), putting the seeds in a container of boiling water for 48 h (58 – 96 %) (Maghembe *et al.*, 1994; Vogt, 1996; IER/ICRAF, 2001; Savard, 2003; Olivier *et al.*, 2006; Savard *et al.*, 2006; Kalinganire *et al.*, 2007), or boiling the seeds in a container for 5 to 7 min (> 90 %) (Razanameharizaka *et al.*, 2006).

Other seed treatments such as with sulphuric acid for 6 to 12 h (Esenowo, 1991; Danthu *et al.*, 1995; IER/ICRAF, 2001; Sidibé and Williams, 2002; Savard, 2003; Savard *et al.*, 2006; Olivier *et al.*, 2006; Kalinganire *et al.*, 2007; Gahane and Ogje, (2013); Van den Bilcke *et al.* 2013; Niang *et al.*, 2015), or putting the seeds in concentrated in 98% sulphuric acid for 1 h (Falemara *et al.*, 2013; Falemara *et al.*, 2014; Usman and Asan, 2017), in 95% sulfuric acid for 45 min (Bationo, 2003; Bonneville *et al.*, 2007; Bationo *et al.*, 2009), or in nitric acid for 15 min (IER/ICRAF, 2001; Sidibé and Williams, 2002; Savard, 2003; Savard *et al.*, 2006; Olivier *et al.*, 2006; Razanameharizaka *et al.*, 2006; Kalinganire *et al.*, 2007) were reported in the literature with up to 98% germination. However, these treatments have the drawbacks of being dangerous to be handled by non-specialists with some environments issues if not well managed.

In vitro propagation was reported by Singh *et al.* (2010), Fasola and Okerenkporo (2012), N'Doye *et al.* (2012) and Rolli *et al.* (2014) (Table 3.1).

Table 3.1 Reported technics for baobab seed pre-treatment

Technics	Germination	Authors
– Scarification/cutting the seed coat	60 - 100 %	– Maghembe <i>et al.</i> (1994); IER/ICRAF (2001); Sidibé and Williams (2002); Savard (2003); Assogbadjo (2006); Savard <i>et al.</i> (2006); Olivier <i>et al.</i> (2006); Razanameharizaka <i>et al.</i> (2006); SCUC (2006); Kalinganire <i>et al.</i> (2007) ; Munthali <i>et al.</i> (2008); Assogbadjo <i>et al.</i> (2010)
– Cutting the seed coat and then soaking in cold water for 72 h	85 – 92 %	– Maghembe <i>et al.</i> (1994); SCUC (2006)
– Putting the seeds in a container of boiling water for 48 h	58 – 96 %	– Maghembe <i>et al.</i> (1994); Vogt (1996); IER/ICRAF (2001); Savard (2003); Savard <i>et al.</i> (2006); Olivier <i>et al.</i> (2006); Kalinganire <i>et al.</i> (2007)
– Boiling the seeds in water for 5 to 7 min	> 90 %	– Razanameharizaka <i>et al.</i> (2006);
– Putting the seeds in concentrated sulphuric acid for 6 to 12 h	68 - 98 %	– Esenowo (1991); Danthu <i>et al.</i> (1995); IER/ICRAF (2001); Sidibé and Williams (2002); Savard (2003); Savard <i>et al.</i> (2006); Olivier <i>et al.</i> (2006); Kalinganire <i>et al.</i> (2007); Gahane and Ogie (2013); Van den Bilcke <i>et al.</i> 2013; Niang <i>et al.</i> (2015)
– Putting the seeds in nitric acid for 15 min	86 - 90 %	– IER/ICRAF (2001); Sidibé and Williams (2002); Savard (2003); Savard <i>et al.</i> (2006); Olivier <i>et al.</i> (2006); Razanameharizaka <i>et al.</i> (2006); Kalinganire <i>et al.</i> (2007)
– Putting the seeds in concentrated 98% sulphuric acid for 1 h	62 - 85 %	– Falemara <i>et al.</i> (2013); Falemara <i>et al.</i> (2014); Usman and Asan (2017)
– Putting the seeds in concentrated 95% sulfuric acid for 45 min	80 %	– Bationo (2003); Bonneville <i>et al.</i> (2007); Bationo <i>et al.</i> (2009)
– In vitro propagation	-	– Singh <i>et al.</i> (2010) ; Fasola and Okerenkporo (2012) ; N'Doye <i>et al.</i> (2012) ; Rolli <i>et al.</i> (2014)

3.3.3. Fertilization

In Burkina Faso, the substrates are enriched with compost or manure 44.44 kg per 100 m² per month (Bationo, 2003; Bonneville *et al.*, 2007; Bationo *et al.*, 2009). In Mali, the substrates are enriched with 6250 kg manure per 100 m² (SCUC, 2006) or 100 kg of compost or manure per 100 m² (Kalinganire *et al.*, 2007). IER/ICRAF (2001), Sidibé and Williams (2002), Savard (2003), Savard *et al.* (2006), Olivier *et al.* (2006) reported without, dose precision, the use of chemical fertilizers, bi- or tricalcium phosphate, potassium chloride and urea with 46 % N ; (Table 3.2).

3.3.4. Density of sowing

Two propagation techniques were identified: transplantation and direct sowing. Transplantation consists in passing the seeds in nursery followed by transplantation on prepared plots. Kalinganire *et al.* (2007) proposed the use of 10 × 25 cm in nursery and transplanted with 250 × 250 cm (Sidibé and Williams, 2002). In direct sowing, sowing is carried out on prepared plots. Numerous sowing densities were used: 15 x 15 cm (Bationo, 2003; Bonneville *et al.*, 2007; Bationo *et al.*, 2009); 10 × 20 cm (IER/ICRAF, 2001; Savard, 2003; Savard *et al.*, 2006; Olivier *et al.*, 2006); 50 x 20 cm (SCUC, 2006; Assogbadjo *et al.*, 2010; (Korbo *et al.*, 2013) ; (Table 3.2).

3.3.5. Irrigation

Growth of seedlings require watering morning and evening (IER/ICRAF, 2001; Savard, 2003, Savard *et al.*, 2006; Olivier *et al.*, 2006; SCUC, 2006; Kalinganire *et al.*, 2007). Some 444.44 litres of water are sprayed on plots of 100 m² per day (Bationo, 2003; Bonneville *et al.*, 2007; Bationo *et al.*, 2009). Assogbadjo *et al.* (2010) reported watering once a day, preferably in the evening from sowing/transplanting day ; (Table 3.2).

3.3.6. Harvesting frequency

Seedlings raised in nursery are transplanted when they are aged 3 to 4 months. Then, the first harvest began 45 days after transplantation, and every 2 to 3 weeks (Kalinganire *et al.*, 2007). After direct sowing of seeds on prepared plots, Bationo (2003), Bonneville *et al.* (2007) and Bationo *et al.* (2009) reported 40th days, for the first harvest followed monthly harvests. IER/ICRAF (2001) reported first harvest 6 weeks after sowing, then between 3 and 8 weeks. Also, the first harvest began 45th days after sowing, and every 2 to 3 weeks (Kalinganire *et al.*, 2007), or every month (Korbo *et al.*, 2013). Overall, the first harvest took place between the

40th and the 45th day after sowing/transplanting. The following harvests take place between every 3 to 8 weeks but the most common is every month; (Table 3.2).

3.3.7. Harvesting technics

Two harvesting technics of baobab seedlings leaves were identified. The first technic which intensifies the production of fresh leaves and produces vigorous plant stock for agroforestry schemes, consists in harvesting all leaves but sparing the terminal buds (Bonneville *et al.*, 2007; Bationo *et al.*, 2009). The second technic consists in cutting the plants 15 cm above ground (Kalinganire *et al.*, 2007); (Table 3.2).

3.3.8. Yields variation

Very large variation of the biomass production was found, presumably due to differences in technics and local environment conditions but also the genetic material. Leaf production is estimated to be 400 kg / 100 m² / month for the first two months after germination; and in average of 200 kg / 100m² / month from the 3rd month (Bonneville *et al.*, 2007; Bationo *et al.*, 2009). Korbo *et al.*, 2013 reported that the average leaf biomass is of 30 kg / 100m² / month after germination; (Table 3.2).

Table 3.2 Reported technics for baobab leaves production

Density of sowing	Harvesting frequency	Fertilization	Variation in yields	Region	Reference
15 x 15 cm	40 days after establishment and every month	44.44 kg of compost or manure per 100 m ² per month	-	Burkina Faso	Bationo (2003)
10 x 20 cm	Six weeks after planting, then between 3 and 8 weeks	Manure or bi- or tricalcium phosphate , potassium chloride and urea with 46 % N (<i>Dose not precise</i>)	-	Mali	IER/ICRAF (2001); Savard (2003), Savard <i>et al.</i> (2006); Olivier <i>et al.</i> (2006)
20 x 50 cm	Within the first year of planting	6250 kg manure per 100 m ²	-	Mali	SCUC (2006)
10 × 25 cm / 250 × 250 cm	45 days after establishment; The plants are cut to 15 cm from the ground and it every 2 to 3 weeks	100 kg of compost or manure per 100 m ²	-	Mali	Kalinganire <i>et al.</i> (2007)
15 x 15 cm	40 days after establishment and Every months; Harvesting sparing the terminal buds	44.44 kg of compost or manure per 100 m ² per month	400 kg / 100 m ² / month for the first two months after germination; 200 kg / 100 m ² / from the 3 rd month	Burkina Faso	Bonneville <i>et al.</i> (2007); Bationo <i>et al.</i> (2009)
20 x 50 cm	45 days after establishment and Every months	-	300 kg / 100 m ² / month	Mali, Niger and Burkina Faso	Korbo <i>et al.</i> (2013)

3.4. Discussion

Baobab seeds' pre-treatment

This review shows that the reported numerous technics fall within chemical and mechanical seed pre-treatments. Esenowo (1991) and Danthu *et al.* (1995) revealed that seeds' pre-treatment consisting of scarification or with concentrated sulphuric acid or nitric acid led to germination of more than 90% in less than 24 hours and emergence is 4-6 days after sowing, and all those that will germinate will have emerged by 18 days (Sidibé and Williams, 2002). However usage of such dangerous products even if possible in laboratory, are not advisable for farmers. Studies by Assogbadjo *et al.* (2010) in Benin showed contrasting results in that scarification had negative effect in breaking seed dormancy. Thus, baobab seeds from Benin do not seem to have a strict physical dormancy compared to the Malagasy ones (Razanameharizaka *et al.*, 2006) and the observed differences may be due to a physiological response lead by genetic and/or environmental factors. The scarification (cutting the seed coat) with a razor blade (Razanameharizaka *et al.*, 2006; Assogbadjo, 2006) is not adapted to a production of great scale (hundreds of seed to be sown). It would be thus preferable to employ the mechanical methods of seeds pre-treatment in bulk: cutting the seed coat and then soaking in cold water for 72 hours (SCUC, 2006), or putting the seeds in a container of boiling water for 48 hours (IER/ICRAF, 2001), for several minutes (Esenowo, 1991), or boiling the seeds in water for 5 to 7 minutes (SCUC, 2006). Alternatively, one may initiate the manufacturing of the instrument adapted to the manual scarification of seeds.

Fertilization

Chemical fertilizer (IER/ICRAF, 2001; Savard, 2003, Savard *et al.*, 2006; Olivier *et al.*, 2006) are not always hands-on; moreover, they contribute to soil and land degradation if not properly used (Rehm *et al.*, 1995). An alternative would be organic matter from either domestic dust composting or animals' dungs.

Organic matter (Bationo, 2003; SCUC, 2006; Bonneville *et al.*, 2007; Kalinganire *et al.*, 2007; Bationo *et al.*, 2009) needs to be added during planting (Sidibé and Williams, 2002) and it is advised that planting is done at the beginning of the rainy season. Supplying organic matter (Compost or organic manure) promoted further seedlings growth after 11 days of germination (Assogbadjo *et al.*, 2010). Organic manures from domestic breeding or compost from domestic organic waste are hypothesized to provide sustainable manure for sustainable production in smallholder farming systems (Assogba-Komlan, 2001). In this context, biological agriculture offers new prospects. Indeed, a husbandry which implies the consequent contribution of organic fertilizers such as the crop waste products or different standard from composts, could improve

soil fertility (Weber *et al.*, 2007). The use of composts produced by organic waste improves their properties, makes available nutrients and reduces the risks of pollution (Douglas *et al.*, 2003), improves soil structure, increases the water holding capacity and in nutrients, stimulate the microbial activity and increase the outputs (Kowaljow and Mazzarino, 2007). Doses of organic manure in horticulture often varies between 10 t/ha to 40 t/ha (Olaniyi, 2008; Akparobi, 2009). This often leads to an excellent yield and rapid growth of cultivated vegetables.

Density of sowing

There was a large variation of sowing density (10 cm × 20 cm to 250 cm × 250 cm). Fang *et al.* (1999), Pinkard and Neilsen (2003) reported that, when spacing between trees is reduced, biomass that is allocated to branches and foliage usually decreases to the benefit of the stem. Root growth influences the provisioning of nutritive elements of a plant, and consequently, its growth and yield. It follows that the growth of the roots is correlated positively with the yield (Blomme *et al.*, 2003). Plant spacing for vegetable cultivation is an important criterion for attaining maximum vegetative growth and an important aspect of crop production for maximizing the yield. Optimum plant spacing ensures judicious use of natural resources and makes the intercultural operations easier. It helps to increase the number of leaves, branches and healthy foliage. Densely planted crop obstruct the proper growth and development. On the other hand, wider spacing ensures the basic nutritional requirements but decrease the total number of plants as well as total yield. Yield may be increased for any crop up to 25% by using optimum spacing in leafy vegetable (Bansal *et al.*, 1995). For example, Bationo (2003), Bonneville *et al.* (2007), Bationo *et al.* (2009) reported that 15 x 15 cm sowing density yielded 400 kg per 100 m² per month for the first two months after germination compared to 50 x 20 cm (SCUC, 2006; Assogbadjo *et al.*, 2010; Korbo *et al.*, 2013) which provided a yield relatively higher of 300 kg per 100 m² per month. These results join the idea according to which the variation of the density of sowing induces the variation of the yield. For this purpose, it is important to define optimum density of sowing to guarantee the profitability of the sector.

Harvesting frequency

Frequency of leaves harvesting varied from every two weeks to every eight weeks. Harvesting techniques consist in collecting all leaves but sparing the terminal buds. Investigations of the effect of cutting frequency on biomass production have been undertaken and differences in production due to various cutting regimes have been found with many species such as *Leucaena leucocephala* and *Gliricidia sepium* (Guevarra *et al.*, 1978; Latt *et al.*, 2000). Leaves biomass lacks consistent data, making hard to derive a consistent trend.

Yields variation

As highlighted in this review, only a few studies have been carried out on technical route of baobab leaves production based on plots (Table 3.2). Several studies (IER/ICRAF, 2001; Bationo, 2003; Savard, 2003, Savard *et al.*, 2006; Olivier *et al.*, 2006; SCUC, 2006; Bonneville *et al.*, 2007 ; Bationo *et al.*, 2009; Assogbadjo *et al.*, 2010; Korbo *et al.*, 2013) mentioned direct sowing of seeds on prepared plots except Sidibé and Williams (2002) and Kalinganire *et al.* (2007) who suggested that seedlings should have reached a height of 40 - 50 cm in nursery before transplanting with 250 × 250 cm.

The variation of the yield observed would be related to the different technics used. Assogbadjo *et al.* (2010) reported that next to environmental factors, genetic factors can also have an influence on seed germination traits. The variation in germination rate could be due to seed size and provenances (Assogbadjo, 2006; Assogbadjo *et al.*, 2010). Thus, part of this variation, due to genetic differences between provenances has been demonstrated Sanchez *et al.* (2010b) who showed that provenances from Benin differed in their leaf morphology, provenances from areas with low precipitation showing more xerophytic characters than provenances from more humid areas.

3.5. Conclusion

This paper has reviewed the literature on baobab leaves production whose primary purpose is production of food and sometimes herbs for home consumption. We found that reported data show considerable variation in the technics applied for baobab leaves production. Further research is however needed to optimize the baobab leaves production. In particular future research should (i) provide consistent data on leaves biomass in response to technical routes used, clearly showing how yield of leaves is related to sowing density, harvesting frequency, type of fertilizers (chemical and organic) and its doses, etc., (ii) elucidate profitability of the activity, (iii) identify the potentials devastating of the seedlings and the effective fight techniques, and (iv) clarify the anti-nutritional properties of harvested leaves as the plant responses to stress due to frequent harvesting.

4.

Morphological variation of baobab fruits and seeds traits across three biogeographical zones in Benin

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*“Morphological variation of baobab fruits and seeds traits in smallholders farming systems
in Benin: a preliminary study for baobab leaves production”*

4. Morphological variation of baobab fruits and seeds traits across three biogeographical zones in Benin

Abstract

Baobab (*Adansonia digitata* L.) leaves are an important non-timber forest products (NTFP) in rural areas and increasingly in urban areas in Africa. However, the overharvesting of the leaves constitutes a serious threat for natural stands of baobab. This paper presents the results found on baobab capsules and seeds collected from three biogeographical zones (Sudanian, Sudano-Guinean and Guineo-Congolese zones) in Benin. Thirty to forty fruits were collected from thirty trees in each of the three bio-geographical zones. Seeds were extracted and conserved for one month before sowing. Observations on fruits and seeds characteristics showed that fruits from the Sudanian zone were bigger in size (median diameter - 89.9 cm and length - 19.9 cm) and heavier with the heaviest seeds (weighing 46.60 ± 6.98 g) than seeds from the others zones. The Sudano-Guinean zone had intermediate values of the morphological traits except for the seeds weight which intermediate value was observed in the Guineo-Congolese zone (44.89 ± 6.38 g) per 100 seeds. These variations could have effect on seedlings growth and worth to account for when assessing effects of abovementioned factors for baobab leaves production.

Key words: *Adansonia digitata*, morphological variation, baobab fruits, baobab seeds, biogeographical zones

4.1. Introduction

Baobab tree (*Adansonia digitata* L., family Malvaceae) is a prime candidate for domestication in the semi-arid regions of Africa (Matig *et al.*, 2006). Ecologically, the baobab tree can withstand drought more easily than many annual crops. It has no significant pests or diseases and it does not require water or fertiliser (Sidibé and Williams, 2002). The importance of baobab to human livelihood is reviewed by several authors such as Assogbadjo (2006), Teklehaimanot (2008), Kalinganire *et al.* (2007), Chadare *et al.* (2009), De Caluwé *et al.* (2010), Kamatou *et al.* (2011), Jamnadass *et al.* (2011), Zahra'u *et al.* (2014), Namratha and Sahithi (2015) and Gebauer *et al.* (2016). In addition, baobab is currently a crop of high economic value internationally (Gruenwald and Galiza, 2005; De Smedt *et al.*, 2011). Baobab oil, extracted from the seed, is used in the cosmetics industry and is also sold internationally (Gruenwald and Galiza, 2005). Baobab fruit pulp has been approved for sale in the EU (2008/575/EC) and USA (GRAS Notice No. GRN 000273) (De Smedt *et al.*, 2011). With a wide range of geographical distribution, certain plant characteristics represent morphotypes (Sidibé and Williams, 2002). Across geographical range of baobab, the major phenotypic variation reported include leaf taste, size, form and colour of fruits, seed colour, pulp taste and colour, bark colour, height and width of trees (Sidibé and Williams, 2002; Assogbadjo, 2006) and fruit traits (Smedt *et al.*, 2011).

The domestication and cultivation of the baobab tree is necessary to protect the natural stands of this species and to provide a sustainable source of income to local populations (Sanchez, 2011). Katsvanga *et al.* (2007) have also reported high variability in fruit traits among sites largely attributed to climatic, edaphic, genetic, and cultural factors. The extent to which individuals develop within the species is also a function of the mechanism of seed dispersal (Chung *et al.*, 2002). According to Dawson *et al.* (2009), unless a wide genetic base within the species is maintained, trees are vulnerable to inbreeding depression ultimately reducing individual fitness and raises the chances of population and or species extinction.

Knowledge of variation in any species is prerequisite in agroforestry (Dawson *et al.*, 2009) and any tree improvement programme (Zobel and Talbert, 1984). Successful domestication requires identification and use of good genetic germplasm. Earlier studies on baobab reported important phenotypic variations within populations for fruit traits in Benin (Assogbadjo *et al.*, 2005; Chadare *et al.*, 2009; De Caluwé *et al.*, 2009) and in West and East Africa (Sanchez *et al.*, 2011). In Mali, De Smedt *et al.* (2011) reported considerable phenotypic variability in fruit traits of baobab. Parkouda *et al.* (2012) found large geographical variation in vitamin C and sugar content in fruit pulp, in leaf production (Korbo *et al.*, 2013). Studies on the genetic

diversity of natural baobab populations in Benin revealed genetic differentiations between populations originating from different climatic zones (Assogbadjo, 2006; Kyndt *et al.*, 2009). The results of previous studies pointed to a certain level of physical isolation between baobab populations from the three climatic zones of Benin, presuming a genetic structuring between them (Assogbadjo, 2006; Kyndt *et al.*, 2009).

The current study was undertaken to assess morphological variation of baobab fruits and seeds traits across three biogeographical zones in Benin. In this study the following key question was posed: What is the morphological variability of baobab fruits and seeds traits across three biogeographical zones in Benin?

4.2. Material and methods

4.2.1. Study areas

The study was conducted in three bio-geographical zones of Benin (Guineo-Congolese or humid zone in the south, Sudano-Guinean or semi-arid zone in the center and and Sudanian zone or semi-arid in north) (Dansi *et al.*, 2008). Benin is a West-African country of 114 763 km² located between 6°25'N-12°30' and 0°45'E - 4°E. The resident population is about 10 880 000 inhabitants in 2015 (UN, 2015) and slightly female-biased (50.12%). The development index of UNDP was 0.485 placing it at 167th position from 188 countries in 2015 (UNDP, 2016). The gross product per habitant was 745.47 Dollars US in 2013 (INSAE, 2015).

3.2.2. Sampling and data collection

The baobab capsules were collected from 30 trees randomly chosen in each zone. Thirty to forty capsules were collected from each tree. These capsules were measured for their morphological traits (total length, median diameter and weight). They were then broken and seeds were extracted. For each baobab tree, five random samples of 100 seeds were selected and measured for their weight.

3.2.3. Data analysis

Descriptive statistics (minimum-min, maximum-max, and coefficient of variation-cv) of each morphological trait of capsules and seeds were calculated. Bio-geographical zones were compared using analysis of variance followed by a Student-Newman and Keuls tests. Finally, a correlation analysis was performed to examine relationship between seeds weight and capsules morphological traits. All analyses were implemented in the R software version 3.4.0 (R Core Team 2017).

4.3. Results

4.3.1. Morphological variation of fruits traits across biogeographical zones

Table 4.1 summarizes the range of each morphological parameter and its variability (assessed by the coefficient of variation, cv). The lowest values of weight, length and median diameter of the fruits were observed in the Guineo-Congolese zone while the highest values of these parameters were observed in the Sudanian zone. Capsules from the Sudano-Guinean zone had the intermediate values. Globally, the variation was quite higher for the weight of the fruits (cv=40.39%) than their length (cv=18.35%) and median diameter (cv=15.41%). The highest variation for the weight, length and median diameter of the fruits were observed in the Sudano-Guinean zone, followed by the Guineo-Congolese zone. Capsules from the Sudano-Guinean are highly diverse regarding these 3 parameters. For the weight of 100 seeds, the lowest value was observed in the Sudano-Guinean zone and the highest value in the Sudanian zone. The variation in general was not high suggesting that the seeds variability is almost the same regarding their weight in the 3 zones. However, the Sudano-Guinean has the highest value (cv=15.49%), followed by the Sudanian (cv=14.99%), and then the Guinean (cv=14.22%, Table 4.1).

Table 4.1 Minimum (min), maximum (max) and coefficient of variation (cv) of the weight, length, median diameter of the capsules of baobab and of the weight of 100 seeds of baobab for the study zones

Zones	Weight of capsules (g)			Length (cm)			Median diameter (cm)			Weight of 100 seeds (g)		
	Min	Max	cv	Min	Max	cv	Min	max	cv	min	max	Cv
Guineo-Congolese	9.80	709.00	75.029	6.20	41.00	30.931	9.06	126.00	20.398	30.63	57.58	14.22
Sudano-guinean	10.00	802.00	83.08	6.40	30.50	31.23	25.98	130.06	27.513	28.87	58.20	15.50
Sudanian	56.00	784.00	40.39	8.90	35.00	18.35	56.20	261.00	15.41	31.62	62.36	14.99
Whole sample	9.80	802.00	69.19	6.20	35.00	30.25	9.06	261.00	24.13	28.87	62.36	15.45

There was a significant variation of the median diameter (df=2, F=475, p-value<0.001), total length (df=2, F=500.5, p-value<0.001) and weight (df=2, F=409.8, p-value<0.001) among the biogeographical zones. The mean value of the median diameter was higher in the Sudanian zone (mean=89.29, sd=13.76) but lower in the Sudano-Guinean zone (mean=67.82, sd=18.66). Capsules from the Sudano-Guinean zone were in average of 75.77 cm (sd=15.46). For the length of capsules, the highest value was observed in the Sudanian zone (mean=19.94, sd=3.66) while the lowest was observed in the Guineo-Congolese zone (mean=14.55, sd=4.50). Intermediate value was observed in the Sudano-Guinean zone (mean=14.84, sd=4.64) (Figure 4.1). Similar trend was observed for the weight of capsules (Figure 4.2).

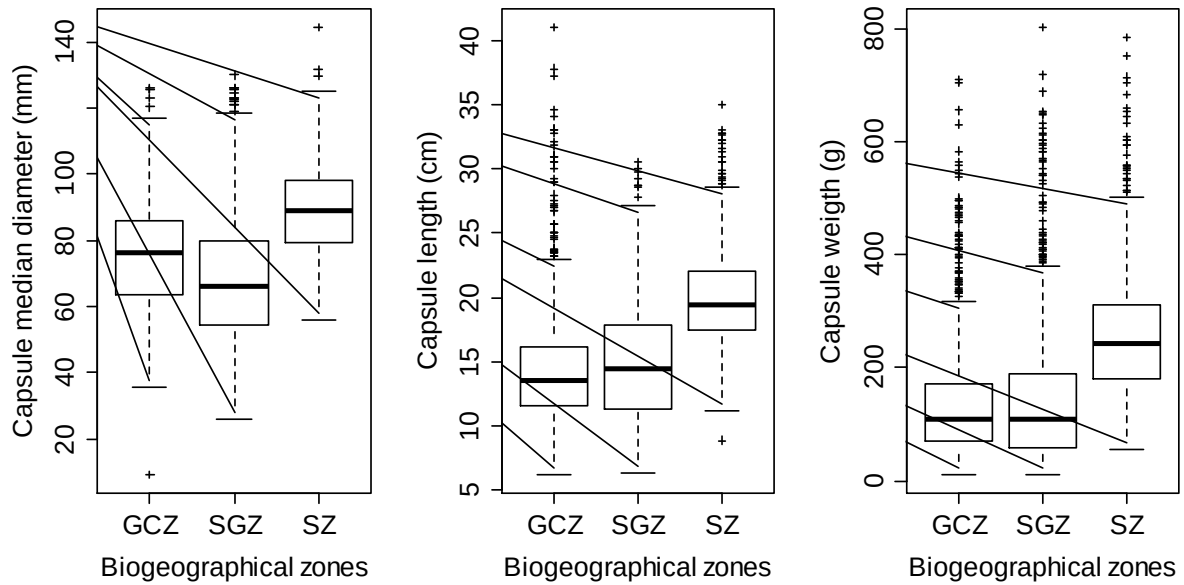


Figure 4.1 Variation of the capsules traits across the biogeographical zones

4.3.2. Variation of seed weight across biogeographical zones

The weight of 100 seeds was significantly different ($F=17.36$, $df=2$, $p\text{-value}<0.001$) among the zones. Heaviest seeds were observed in the Sudanian zone (46.60 ± 6.98 g) while the lightest seeds were observed in the Sudano-Guinean zone (42.08 ± 6.52 g) (Figure 4.2). Seeds from the Guineo-Congolese zone weighted in average 44.89 ± 6.38 g / 100 seeds.

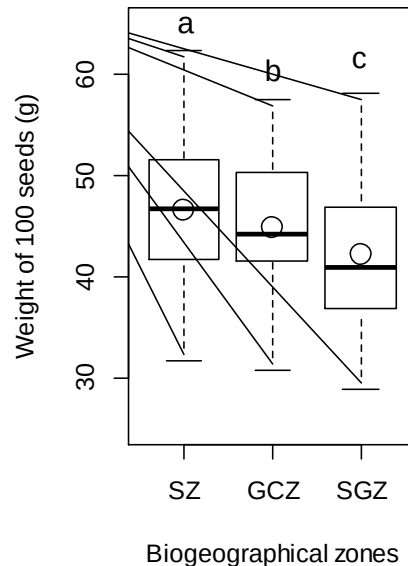


Figure 4.2 Variation of 100 seeds weight across the three biogeographical zones

SZ=Sudanian zone, GCZ=Guineo-Congolese zone and SGZ=Sudano-Guinean zone. Boxplot with different letter indicates significant differences of means. Dot circle of each boxplot indicates mean values.

4.3.3. Correlation between capsules traits and seeds weight

Figure 4.3 shows the correlation plot between capsules' morphological traits and seeds weight. Only significant (p -value <0.05) correlations were displayed. They were strong correlations between capsules weight and capsule diameter (0.81), capsules weight and capsule length (0.74) than between capsule length and capsule diameter (0.45) (Figure 4.3). Although significant, the correlations between seeds weight and capsules traits (diameter and weight) were weak. The correlation (0.26) between seeds weight and capsules median diameter (Figure 4.3) was higher than the correlation between seeds weight and capsules weight (0.21). These correlations suggest that the higher the median diameter of capsules or the heavier the capsules, the heavier were the seeds.

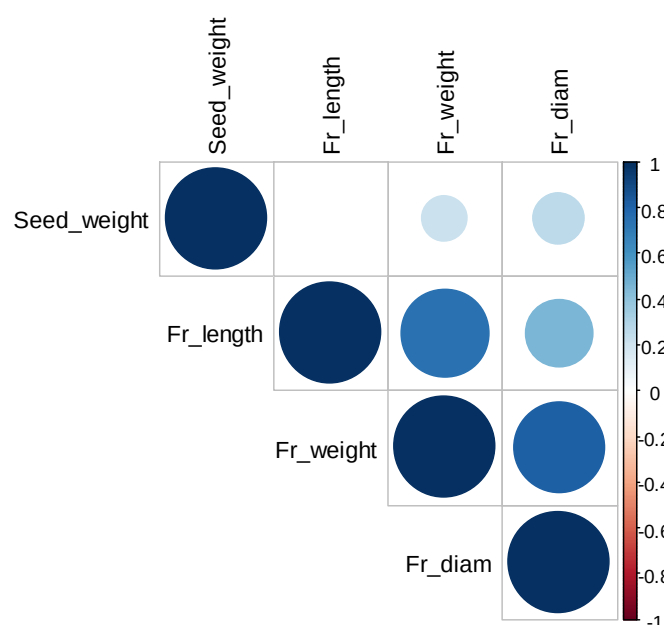


Figure 4.3 Correlation plot between morphological traits of capsules and seeds weight *only significant (p -value < 0.05) correlations are displayed*

4.4. Discussion

Current findings, with regards to morphological traits of capsules, suggest that larger and heavier capsules are found in the drier zone while smaller and lighter capsules are found in the wetter zone. These findings were previously reported by Assogbadjo *et al.* (2010) arguing that the observed variation in baobab fruits is probably influenced by both genetic and environmental factors (temperature and rainfall).

Our study also revealed that the seeds were heavier in the Sudanian zone than in the Guineo-Congolese zone which in turn has seeds that were in average heavier than the ones from the Sudan-guinean zone. Several scholars found a close relationship between environment (particularly rainfall) and seed weight. Some studies revealed that plants growing in areas with lower rainfall tend to develop both lower fruit and seed weights (Raddad, 2007; De Smedt *et al.*, 2011). Our data suggests that this does not apply to baobab since the heaviest seeds were observed in the driest area. This difference may be due to the fact that the heaviest seeds were observed in the heaviest and largest capsules that were mostly observed in the Sudanian and Guineo-Congolese zones (Figures 4.1 & 2). Assogbadjo *et al.* (2005) found that weight of baobab kernels is inversely related to drought: the drier the provenances, the lower the kernels weight. Therefore, the observed trend in the weight of seeds could be due to the differences in the remaining part of the seeds (overall seed weight – kernel weight). The latter was estimated to 2/3 of the total seed (Assogbadjo A.E., field observation).

In addition, the variation in baobab provenances with respect to their seed morphometric traits could be due to the fact that these species grow over a wide range of climatic conditions (Niang *et al.*, 2015). Taking into account the potential adaptation of baobab to the latitudinal gradients in Benin (Assogbadjo *et al.*, 2010), the use of local seed provenances is often recommended in restoration and conservation strategies because they are thought to be better adapted to local habitat conditions. It has to be noted that next to environmental factors, also genetic factors can have an influence on seed germination traits.

4.5. Conclusion

This paper has assessed morphological variation of baobab fruits and seeds traits across three biogeographical zones in Benin. From this, it can be concluded that fruits from the Sudanian zone were bigger in size and heavier with the heaviest seeds than seeds from the others zones. The Sudano-Guinean zone had intermediate values of the morphological traits except for the seeds weight which intermediate value was observed in the Guineo-Congolese zone.

5.

Growth responses of baobab seedlings to variation of
dose of organic manure, sowing density and leaves
harvesting frequency

(In preparation for submission to *Agroforestry Systems*)

5. Growth responses of baobab seedlings to variation of dose of organic manure, sowing density and leaves harvesting frequency

Abstract

This study was conducted to assess the dose of organic manure (0, 10, 20 and 30 tons/ha for compost and poultry dropping in Guineo-Congolese zone, and 0, 10, 20 and 30 tons/ha for compost and 0, 30, 60 and 90 tons/ha for cow dung in Sudano-Guinean zone), sowing density (15 × 15 cm, 20 × 20 cm, and 30 × 30 cm) and leaves harvesting frequency (15, 22 and 30 days) on the germination rate, the number of ramifications, height and basal diameter growth of *A. digitata* seedlings. The experiment was conducted on-station in the Guineo-Congolese and Sudano-Guinean zones of Benin using a split-split plot design with three replicates. Data were analysed using linear, linear mixed effects for longitudinal data with a normal distribution for errors and generalized linear mixed effects models for longitudinal data with a Poisson error distribution models. The results showed that higher germination rate, greater number of ramifications and higher height and basal diameter growth were obtained when *A. digitata* seedlings were produced applying 30 t/ha of poultry dropping or 90 ton/ha of cow dung with 20 × 20 cm sowing density and 30 days as leaves harvesting frequency. This combination provided the optimum growth of *A. digitata* seedlings. Further research is necessary to investigate effect of different doses of two organic manures (compost of organic waste and poultry dropping), sowing densities and leaves harvesting frequencies on the number of leaves, morphological traits of the leaves and dry weight of yield of *A. digitata* seedlings.

Key words: Baobab seedling, growth, density of sowing, organic matter, harvesting frequency

5.1. Introduction

The multipurpose tree, *Adansonia digitata* L. is an agroforestry tree species very common in Africa (Kyndt *et al.*, 2009). Regional consultations organised by the International Centre for Underutilised Crops (ICUC) have accorded high priority to enhanced research and development of baobab (Sidibé and Williams, 2002). Bioversity International classified baobab tree among the 10 top agroforestry tree species to be conserved and domesticated in West Africa (Eyog Matig *et al.*, 2002) largely because of its value in food preservation. National research efforts, especially in Benin, Nigeria, Burkina-Faso, Mali and Senegal have provided data on food values, agronomy, ethnobotany knowledge, ecology and genetic diversity of baobab (Codjia *et al.*, 2003; Diop *et al.*, 2005; Assogbadjo, 2006; Kyndt *et al.*, 2009; Assogbadjo *et al.*, 2010). In many African countries, especially in West Africa, rural people rely on the variety of nutritional and medicinal products provided by the tree (De Caluwé *et al.*, 2009; Buchmann *et al.*, 2010). In Benin, baobab is the second common wild tree providing leafy vegetables after *Vitex doniana* and is followed by *Moringa Oleifera* as the third important tree in this category (Dansi *et al.*, 2008). It is ranked as the first most important traditional leafy vegetables in the arid zone of Benin (Dansi *et al.*, 2008). The specie has great economic importance. A three months market investigation showed that commercialisation of baobab pulp generates up to US\$ 30,000 per year for 130 households of in Malanville district (north of Benin) involved in that business (Codjia *et al.*, 2003). Every part (roots, bark, wood, leaves, flowers, capsules, gum, seeds, and fruits) of the baobab is used: (Wickens and Lowe, 2008; Buchmann *et al.*, 2010). Among the 300 uses that have been identified. Baobab provides nutritious food, livestock fodder, fibre, medicine, and income to local people (Wickens, 1982; Sidibé and Williams, 2002; De Caluwé *et al.*, 2009; Buchmann *et al.*, 2010). The baobab tree plays also important role in the ecosystem balance: it adds organic matter and nutrients through leaf-fall, it reduces soil temperature and water loss (Amundson *et al.*, 1995) and attracts birds and large mammals that add nutrients to the soil with their droppings (Wickens and Lowe, 2008). Moreover, chemical analysis of parts contents showed the presence of proteins, amino acids, iron, vitamins C, A, E and F (abundant compared to daily needs) within its organs, especially leaves (Sidibé and Williams, 2002; De Caluwé *et al.*, 2010). Pruning in baobab is so high that only a few fruits are produced per tree due to the reducing of the photosynthesis activity. The leaves are generally consumed fresh in rainy season but in dried form (common practices in the arid zone) in dry season (Dansi *et al.*, 2008). As such, fresh leaves are collected, dried, pounded and sieved into powder in the rainy season when they are abundant (Dansi *et al.*, 2008). However, fresh leaves are preferred (Dansi *et al.*, 2008), but its non-availability in dry season constrains local people to dry them for their

use in dry season. This practice leads to forced harvest of all of the leaves that affect fruit and seed production of the species as found for some tropical species. In the north and centre of Benin, it is said that, when baobab regularly consumed, it gives stoutness and facilitate good growth, dentition and bones' solidification for children. According to local people, baobab leaves have curative, regulative and stimulative properties besides food qualities and are sometimes used as neutraceutical (Dansi *et al.*, 2008).

According to the World Agroforestry Centre and Bioversity International, the multipurpose baobab tree is expected to play a major role in future crop diversification programs and in the development of West-African agroforestry systems. This was also expressed by the rural people in semi-arid West Africa, who selected baobab among top species that should deserve more attention in future domestication programs (Eyog Matig *et al.*, 2002; FAO, 2014). The domestication and cultivation of the baobab is necessary to protect natural stands and to provide a sustainable source of food, medicine and income to local population (Sanchez *et al.*, 2010a). Domestication seeks to capture and multiply trees with desirable characteristics, thus taking advantage of the variations found in the wild (Pye-Smith, 2010). While preserving the characteristics that are important for local or international customers, domestication also reduces the time of first fructification (Chikamai and Tchatat, 2009). Domestication and cultivation can not only increase the harvested volume, but also the reliability and quality of supply, aspects identified to be key factors determining the long-term viability of a given product in international trade (Chikamai and Tchatat, 2009).

The species is facing a high risk of extinction in the wild where it is threatened by bush fire (seedlings), grazing and lack of natural regeneration (Assogbadjo *et al.*, 2010). Thus leading to test of several pre-treatments methods (Assogbadjo, 2006; Olivier *et al.*, 2006; Savard *et al.*, 2006; SCUC, 2006; Kalinganire *et al.*, 2007; Assogbadjo *et al.*, 2010; Falemara *et al.*, 2014; Niang *et al.*, 2015). Conservation and domestication strategies of baobab consist in the proper management option to obtain sustainable benefits for present and future generations. Consequently, finding a pathway for propagating baobab in rural areas, especially in smallholder farming systems to satisfy the daily demand of baobab leaves will not only allow protecting the species but also help in fighting food insecurity and poverty. Plant spacing for vegetable cultivation is an important criterion for achieve maximum vegetative growth. Optimum plant spacing ensures judicious use of natural resources and makes the intercultural operations easier. It helps to increase the number of branches. Densely planted crop obstruct the proper growth and development. On the other hand, wider spacing ensures the basic nutritional requirements but decrease the total number of plants as well as total yield (Bansal *et*

al., 1995). Investigations of the effect of cutting frequency on biomass production have been undertaken and differences in production due to various cutting frequencies have been found with many species such as *Leucaena leucocephala* and *Gliricidia sepium* (Guevarra *et al.*, 1978; Latt *et al.*, 2000). Baobab tree can withstand drought, it has no important pest or diseases and it does not require extra watering (Sidibé and Williams, 2002; SCUC, 2006). Declining soil fertility remains the fundamental reason for declining smallholder food production in sub-Saharan Africa (Smaling *et al.*, 1997; Gruhn *et al.*, 2000). Fertilizer application in appropriate time, appropriate dose and proper method is the prerequisite of crop cultivation (Islam, 2003). Also, organic manures from domestic breeding or compost from domestic organic waste are hypothesized to provide sustainable manure for sustainable production in smallholder farming systems (Assogba-Komlan, 2001). However, animal rearing in Benin is cow-based in semi-arid zone and poultry-based in the humid zone. This would thus influence the type of dung or droppings available for the propagating tests but also adoption of the options that will be further developed. The overall objective of this research is to develop best agro-ecological practices to improve the growth of baobab seedlings. Specifically, the study aims at assessing: i) the germination rate and number of ramifications, and ii) the height and basal diameter of baobab seedlings in relationships to variation of dose of organic manure, sowing density and leaves harvesting frequency in Guineo-Congolese and Soudano-Guinean zones in Benin. In this study the following key question was posed: What is the combination of the various factors (dose of organic manure, sowing density and leaves harvesting frequency) which gives the optimal germination rate, number of ramifications, height and basal diameter of baobab seedlings?

5.2. Material and methods

5.2.1. Study areas

The study was conducted in two bio-geographical zones of Benin (Sekou in the Guineo-Congolese or humid zone in the south and Soclogbo in the Sudano-Guinean or semi-arid zone in the center). Indeed, Benin is a West-African country of 114 763 km² located between 6°25'N-12°30' and 0°45'E - 4°E. The resident population is about 10 880 000 inhabitants in 2015 (UN, 2015) and slightly female-biased (50.12%). The development index of UNDP was 0.485 placing it at 167th position from 188 countries in 2015 (UNDP, 2016). The gross product per habitant was 745.47 Dollars US in 2013 (INSAE, 2015).

5.2.2. Sampling and data collection

Data were collected on the seedlings of *A. digitata* (Figure 5.1) obtained with the seeds collected in each bio-geographical zones (Guineo-Congolese and Sudano-Guinean zones). The experiment was conducted in a factorial trials using a split-split plot design with three replications. The sites area were first divided into two main plots depending on the type of organic manure (one for compost and one for poultry dropping in Guineo-Congolese zone or cow dung in Sudano-Guinean zone). Each main plot was then divided into 4 subplots corresponding to the four doses of each type of organic manure (0, 10, 20 and 30 tons/ha for compost and poultry dropping in Guineo-Congolese zone and 0, 10, 20 and 30 tons/ha for compost and 0, 30, 60 and 90 tons/ha for cow dung in Sudano-Guinean zone). The 9 (3 × 3) combinations of densities (15 × 15 cm, 20 × 20 cm and 30 × 30 cm) and harvest frequencies (every 15, 22 and 30 days; starting 30 days after planting) were randomized per dose. In order to increase the germination rate, seeds were pre-treated using a container of boiling water for 48 h (IER/ICRAF, 2001; Savard, 2003; Olivier *et al.*, 2006; Savard *et al.*, 2006; Kalinganire *et al.*, 2007). Four seeds were sown to approximately 2 cm of depth per seed hole with 7 plants per row and 4 rows of planting per experimental unit (1.1 × 2 m of size). Next to sprouting, the seedlings were thinned out and only the most vigorous seedling was kept per seed hole. Watering frequency was 10 litres early in the morning per plot per day and weeding was done if need be. Factors tested were summarized in Table 5.1.

After establishment, number of germinated seeds were counted every three days for only the first 30 days; plant total height and basal diameter were recorded for the entire experiment (every three days for the first 30 days and every week for the following 180 days); number of branches was counted every week and frequencies of total leaves harvest were followed (every 15th, 22nd and 30th days according to the treatments) for six months (180 days) next to the

general harvest at 30th day after establishment on the 10 middle plants of the plots except the border plants.

Germination rate of the seeds on an unspecified date i was calculated by using equation 1.

$$R_i = \frac{n_i}{N} \times 100 \quad (\text{Eq. 1})$$

R_i is the germination rate at date i , n_i the number of seed hole with germinated seed and N the total number of seed holes.

Table 5.1 Factors tested in the frame of the experiment and their modalities

Factors	Types	Nature	Modalities
Type of organic matter	Fixed	Categorical	Compost of organic waste <i>versus</i> organic manure from animals: poultry for the Guineo-Congolese zone and cow for the Sudano-Guinean zone
Doses of organic matter	Fixed	Quantitative	- 0 (control), 10, 20 and 30 tons/ha for the compost and the poultry dropping - 0 (control), 30, 60 and 90 tons/ha for the cow dung
Density of sowing	Fixed	Quantitative	15 × 15 cm, 20 × 20 cm and 30 × 30 cm
Frequency of leaves harvest	Fixed	Quantitative	Every 15, 22 and 30 days; starting 45 days after planting
Time (days)	Random	Quantitative	
Block	Random	Quantitative	

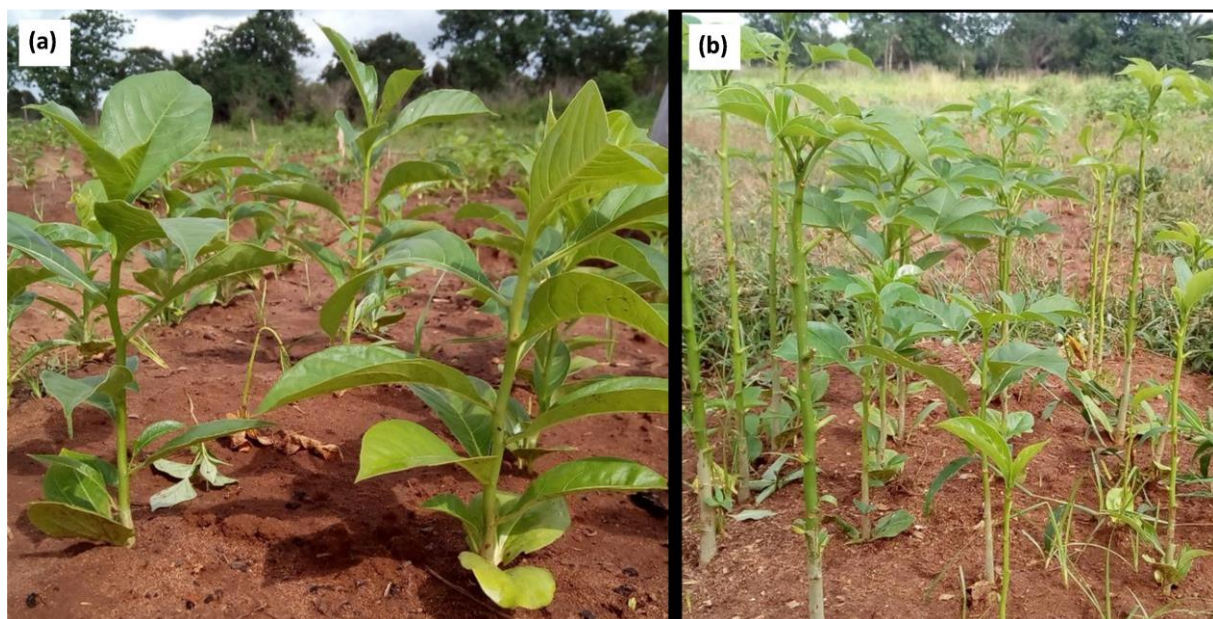


Figure 5.1 Baobab seedlings, before harvest (a) and after harvest (b)

5.2.3. Data analysis

Two biogeographical zones were considered for the study. The type of organic manure was different for all these sites. Hence, data analysis was applied separately to the sites (Guineo-Congolese and Sudano-Guinean zones).

To assess the influence of dose of organic manure, sowing density and leaves harvesting frequency on the germination rate, growth dynamic in height and basal diameter linear mixed effects models for longitudinal data with a normal distribution for errors were used. The models were run with the packages “nlme” (Pinheiro *et al.*, 2017). Linear models were performed on specific leaf area, leaf shape and specific dry weight. And generalized linear mixed effects models for longitudinal data with a Poisson error distribution Poisson errors were used to assess the influence of dose of organic manure, sowing density and leaves harvesting frequency on the number of ramifications using the package “MASS” (Venables and Ripley, 2002). Anova was used to test overall significance of factors. Model selection was based on AIC (Akaike Information Criterion ; the low values are preferred) using the package “bbmle” (Bolker and R Core Team, 2017). Least square means (lsmeans) of each variable were extracted and multiple comparisons among them were estimated using the package “lsmeans” (Lenth, 2016). The lsmeans were used to draw figures using the package “ggplot2” (Wickham, 2009) for showing the evolution trend of each of them according to the factors considered. All statistical analyses were carried out in R 3.4.0 (R Core Team, 2017). All factors (organic matter, doses of organic matter, sowing density and frequency of leaves harvest) were considered fixed. Only the significant ($p < 0.05$) factors and interactions were carried along this study next to model simplification.

5.3. Results

5.3.1. Germination rate of baobab seeds in relationships to variation of dose of organic manure, sowing density and leaves harvesting frequency in Guineo-Congolese and Soudano-Guinean zones

In Guineo-Congolese zone, the effect on germination rate depend on time, type of organic matter, their dose and sowing density. Moreover, some of the interactions between these factors were also significant (Time $\times$ Matter, Matter $\times$ Dose, Time $\times$ Density, Matter $\times$ Density, Dose $\times$ Density and Time $\times$ Matter $\times$ Dose), showing that differences among times were not similar across matter, doses and densities and vice-versa ($P < 0.05$; Table 5.2). In general, seed germination started the 6th day and reached its maximum after 24 days. Figure 5.2a shows that seeds sown on 30 ton/ha of compost of organic waste plots gave the highest germination rate ($94 \pm 0.07\%$). Seeds sown on unenriched plots (0 ton/ha) gave the lowest germination rate ($36 \pm 0.06\%$). In addition, 20×20 cm and 15×15 cm densities of sowing showed the highest germination rate ($88 \pm 0.07\%$) and the lowest germination rate ($38 \pm 0.05\%$) with 30×30 cm density of sowing (Figure 5.2b). Seeds sown on poultry dropping plots also had low germination rate. High dose of compost of organic waste (30 ton/ha) and 20×20 cm and 15×15 cm densities of sowing presented the highest germination rate.

In Soudano-Guinean zone, it wasn't significant effect of the time on germination rate of baobab seeds. It was affected by only two mains (type of organic matter and density of sowing; $P < 0.05$; Table 5.2). Seed germination started the 24th day and increased over 30th day. Figure 2a shows that seeds sown by 20×20 cm density of sowing gave the highest germination rate ($21.34 \pm 0.07\%$) and the lowest germination rate ($14.12 \pm 0.07\%$) with 30×30 cm density of sowing (Figure 5.3a). In addition, on cow dung plots gave the highest germination rate ($24.41 \pm 0.07\%$) and the germination rate ($10.70 \pm 0.07\%$) on compost of organic waste plots (Figure 5.3b). Seeds sown on cow dung plots and 20×20 cm density of sowing presented the highest germination rate. The lowest germination rate on compost of organic waste plots and 30×30 cm density of sowing.

Table 5.2 Effect of significant factors and interactions on the germination rate of baobab seeds in Guineo-Congolese and Soudano-Guinean zones

Source	df	Germination rate			
		Guineo-Congolese zone		Soudano-Guinean zone	
		F-value	P-value	F-value	P-value
Time	4	19.20	<0.001	-	-
Matter	1	136.91	<0.001	166.82	<0.001
Dose	3	14.38	<0.001	-	-
Density	2	35.82	<0.001	15.54	<0.001
Time: Matter	4	2.65	0.031	-	-
Matter: Dose	3	9.76	<0.001	-	-
Time: Density	8	2.26	0.021	-	-
Matter: Density	2	14.29	<0.001	-	-
Dose: Density	6	7.48	<0.001	-	-
Time: Matter: Dose	12	1.92	0.027	-	-

df=degree of freedom, F-value=Fisher statistic, P-value Probability of significance. Only significant effects are presented in the table.

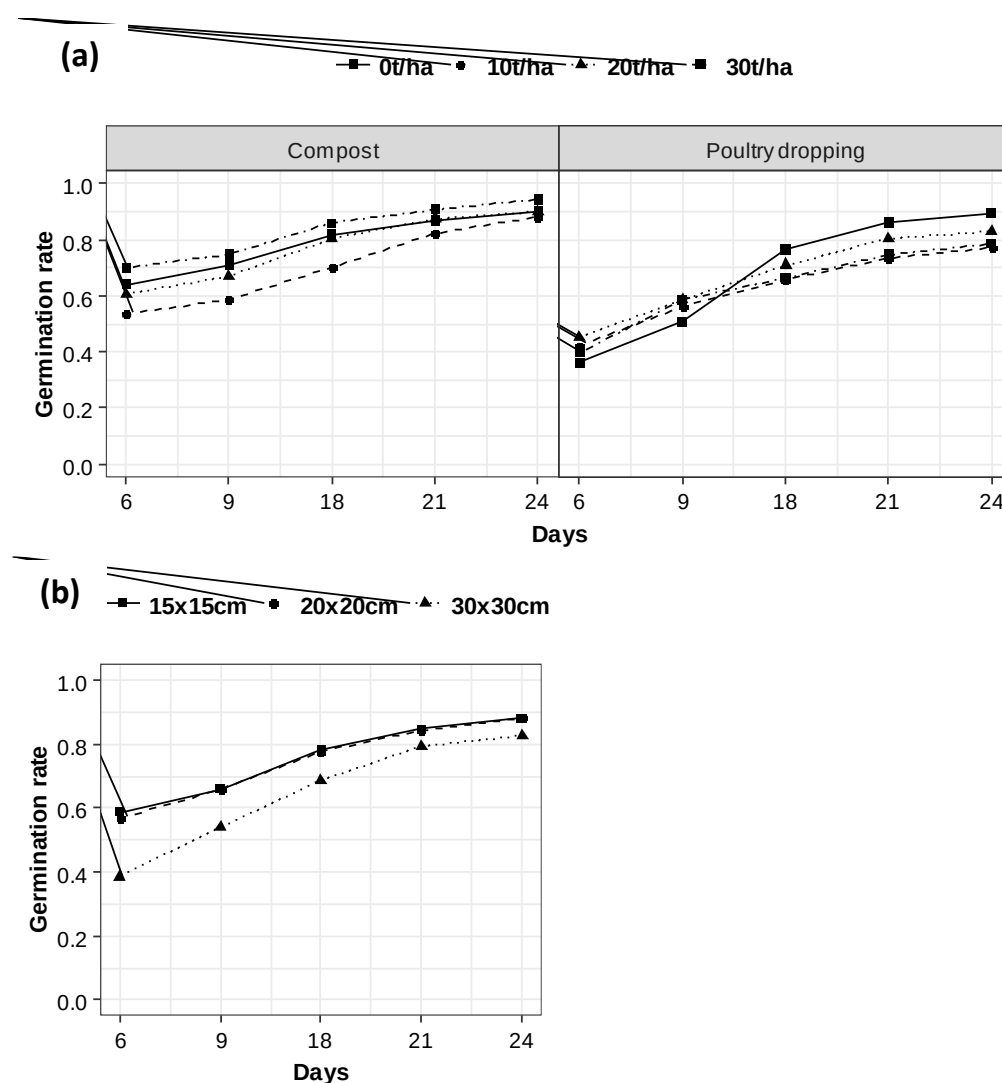


Figure 5.2 Evolution trend of germination rate of baobab seeds in Guineo-Congolese zone according to the time, the organic matter type and their dose (a), and the time and sowing density (b)

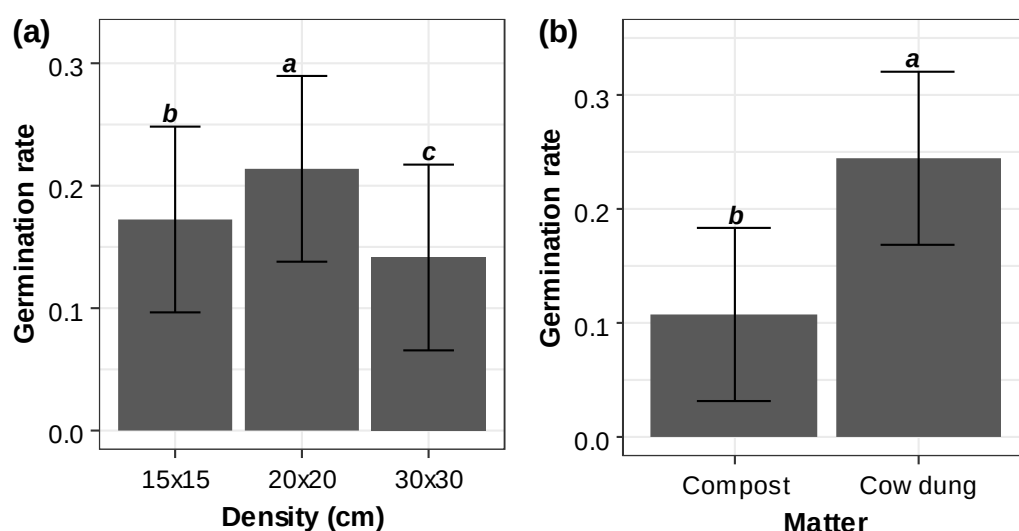


Figure 5.3 Evolution trend of germination rate of baobab seeds in Soudano-Guinean zone according to the sowing density (a), and the organic matter type (b)

5.3.2. Growth dynamic in height and in basal diameter of baobab seedlings in relationships to variation of dose of organic manure, sowing density and leaves harvesting frequency in Guineo-Congolese and Soudano-Guinean zones for the first thirty days after sowing

In Guineo-Congolese zone, a significant effect of the time, dose of organic matter and sowing density of on the height growth of baobab seedlings for the first thirty days after sowing was noted. In addition, the interactions between Time $\times$ Matter, Time $\times$ Dose, Matter $\times$ Dose, Time $\times$ Density, Matter $\times$ Density, Dose $\times$ Density, and Time $\times$ Matter $\times$ Dose were significant, showing that differences among times were not similar across matter, doses and densities and vice-versa ($P < 0.05$; Table 5.3). Accordingly, 30 ton/ha of poultry dropping and of compost of organic waste showed the highest height (13.12 ± 0.43 and 12.21 ± 0.43 cm) and the lowest with unenriched plots (0 ton/ha) (9.83 ± 0.43 cm; Figure 5.4a). In addition, 15×15 cm and 20×20 cm sowing densities showed the highest height (12.15 ± 0.41 and 11.85 ± 0.41 cm) and the lowest for 30×30 cm sowing density (9.91 ± 0.41 cm; Figure 5.4b). This indicates that, in Guineo-Congolese zone a higher dose of poultry dropping (30 ton/ha), with 15×15 cm and 20×20 cm sowing densities presented the highest height, while, the lowest height with unenriched plots (0 ton/ha) and 30×30 cm sowing density.

It wasn't significant effect of the time on the growth of basal diameter for the first thirty days after sowing. A significant effect on the basal diameter growth of baobab seedlings for the first thirty days after sowing was noted for the organic matter type, their dose and sowing density. The interactions between Matter $\times$ Density and Dose $\times$ Density were significant, showing that differences among matter were not similar across doses and densities and vice-versa (Table 5.3). In particular, application of compost of organic waste showed the highest basal diameter

for 15×15 cm (3.87 ± 0.02 mm) and 20×20 cm (3.83 ± 0.02 mm) sowing density and the lowest for 30×30 cm sowing densities (3.54 ± 0.02 mm). Intermediate growth value of basal diameter was showed by using poultry dropping (Figure 5.5a). 30 ton/ha of organic matter and 20×20 cm sowing density showed the highest basal diameter (4.02 ± 0.03 mm) and 10 ton/ha of organic matter and 30×30 cm sowing density the lowest (3.55 ± 0.04 mm; Figure 5.5b). This indicates that, in Guineo-Congolese zone a higher dose of organic matter (30 ton/ha), with 20×20 cm sowing density presented the highest basal diameter while, the lowest basal diameter with low enriched plots (10 ton/ha) and 30×30 cm sowing density.

In Soudano-Guinean zone, a significant effect of the time, dose of organic matter, sowing density and interaction between Dose $\times$ Density on the height growth of baobab seedlings for the first thirty days after sowing was noted ($P < 0.05$; Table 5.4). Accordingly, after seeds germination, seedlings height grown faster (from to cm in six days; Figure 5.6a). 90 ton/ha cow dung showed the highest height (11.85 ± 0.41 cm) and the lowest on 10 ton/ha of compost of organic waste plots (9.91 ± 0.41 cm) for 30×30 cm sowing density (Figure 5.6b). This indicates that, Soudano-Guinean zone average higher dose of cow dung (90 ton/ha), with 30×30 cm sowing density presented the highest height, while, the lowest height with 10 ton/ha of compost of organic and 30×30 cm sowing density.

A significant effect on the basal diameter growth of baobab seedlings for the first thirty days after sowing was noted for the time and dose of organic matter ($P < 0.05$; Table 5.4). In particular, after seeds germination, seedlings basal diameter grown faster (from to cm in six days; Figure 5.6c). Application of high dose compost of organic waste plots (30 ton/ha) and of cow dung plots (90 ton/ha) showed the highest basal diameter (3.87 ± 0.02 and 3.87 ± 0.02 mm) and the lowest on 10 ton/ha of compost of organic waste plots (9.91 ± 0.41 cm; Figure 5.6d). This indicates that, Soudano-Guinean zone a higher dose of organic matter (30/90 ton/ha) presented the highest basal diameter, while, the lowest basal diameter with low enriched plots (10 ton/ha).

Table 5.3 Effect of significant factors and interactions on the height and basal diameter of baobab seedlings for the first thirty days after sowing in Guineo-Congolese zone

Source	df	First 30 days			
		Height		Basal diameter	
		F-value	P-value	F-value	P-value
Time	4	291.46	<0.001	-	-
Matter	1	-	-	10.02	0.001
Dose	3	72.19	<0.001	20.22	<0.001
Density	2	173.35	<0.001	40.10	<0.001
Time: Matter	4	10.94	<0.001	-	-
Time: Dose	12	6.30	<0.001	-	-
Matter: Dose	3	21.18	<0.001	-	-
Time: Density	8	11.51	<0.001	-	-
Matter: Density	2	10.59	<0.001	9.25	<0.001
Dose: Density	6	5.30	<0.001	4.23	<0.001
Time: Matter: Dose	12	3.86	<0.001	-	-

df=degree of freedom, F-value=Fisher statistic, P-value=Probability of significance, First 30 days=First thirty days after sowing. Only significant effects are presented in the table.

Table 5.4 Effect of significant factors and interactions on the height and basal diameter of baobab seedlings for the first thirty days after sowing in Soudano-Guinean zone

Source	df	First 30 days			
		Height		Basal diameter	
		F-value	P-value	F-value	P-value
Time	2	188.82	<0.001	36.68	<0.001
Dose	7	13.61	<0.001	10.886	<0.001
Density	2	8.88	<0.001	-	-
Dose: Density	14	4.76	<0.001	-	-

df=degree of freedom, F-value=Fisher statistic, P-value=Probability of significance, First 30 days=First thirty days after sowing. Only significant effects are presented in the table.

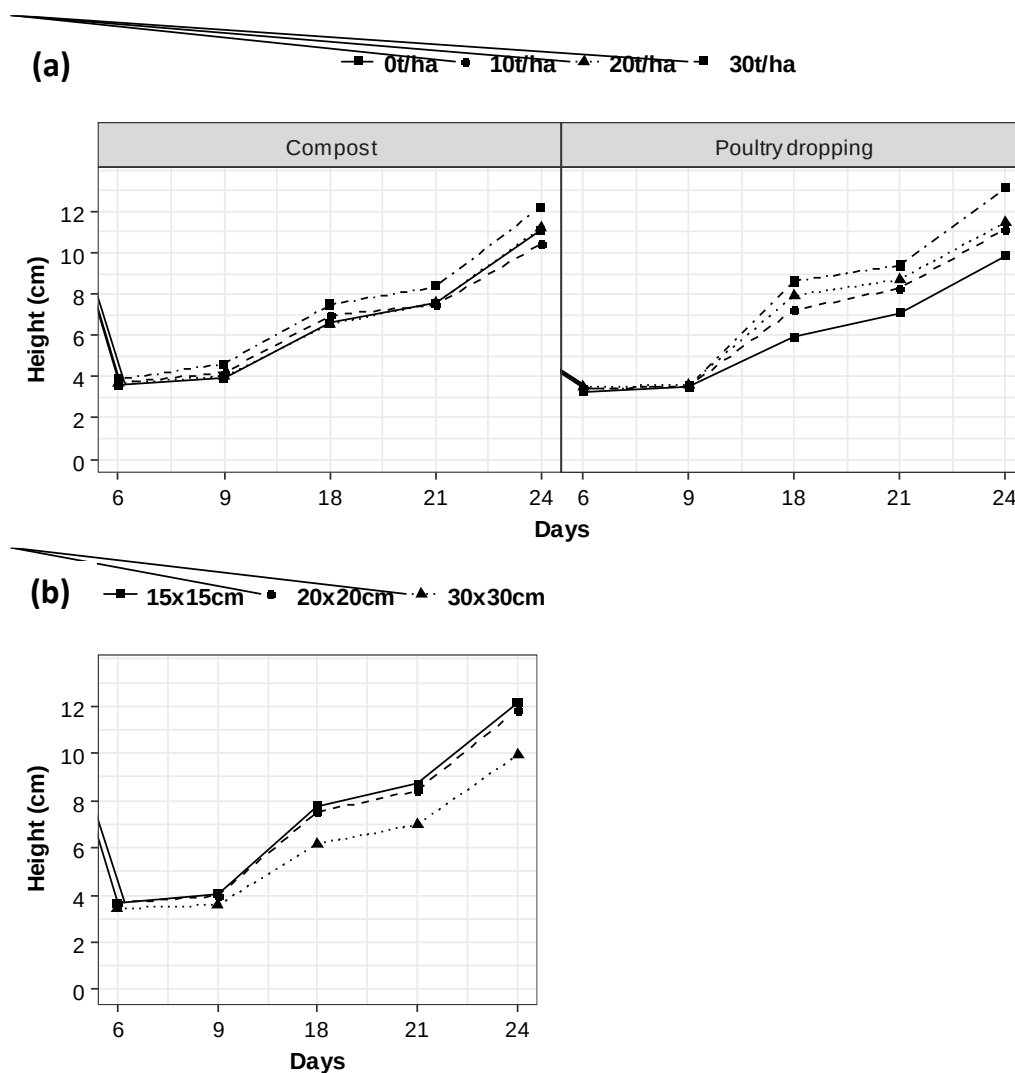


Figure 5.4 Evolution trend of the height for the first 30 days after sowing in Guineo-Congolese zone according to the time, organic matter type and their dose (a) and the time and sowing density (b)

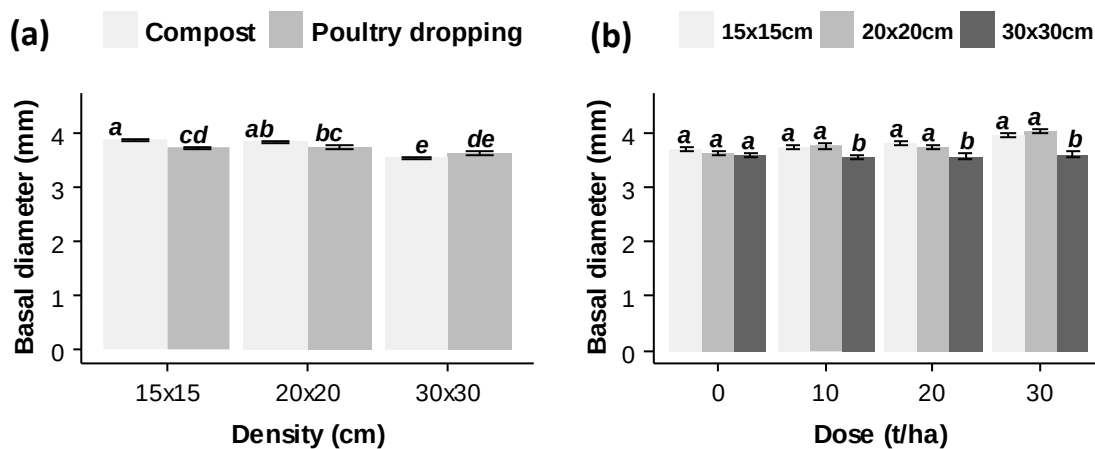


Figure 5.4 Evolution trend of the basal diameter for the first 30 days after sowing in Guineo-Congolese zone according to the organic matter type and sowing density (a) and the dose and sowing density (b)

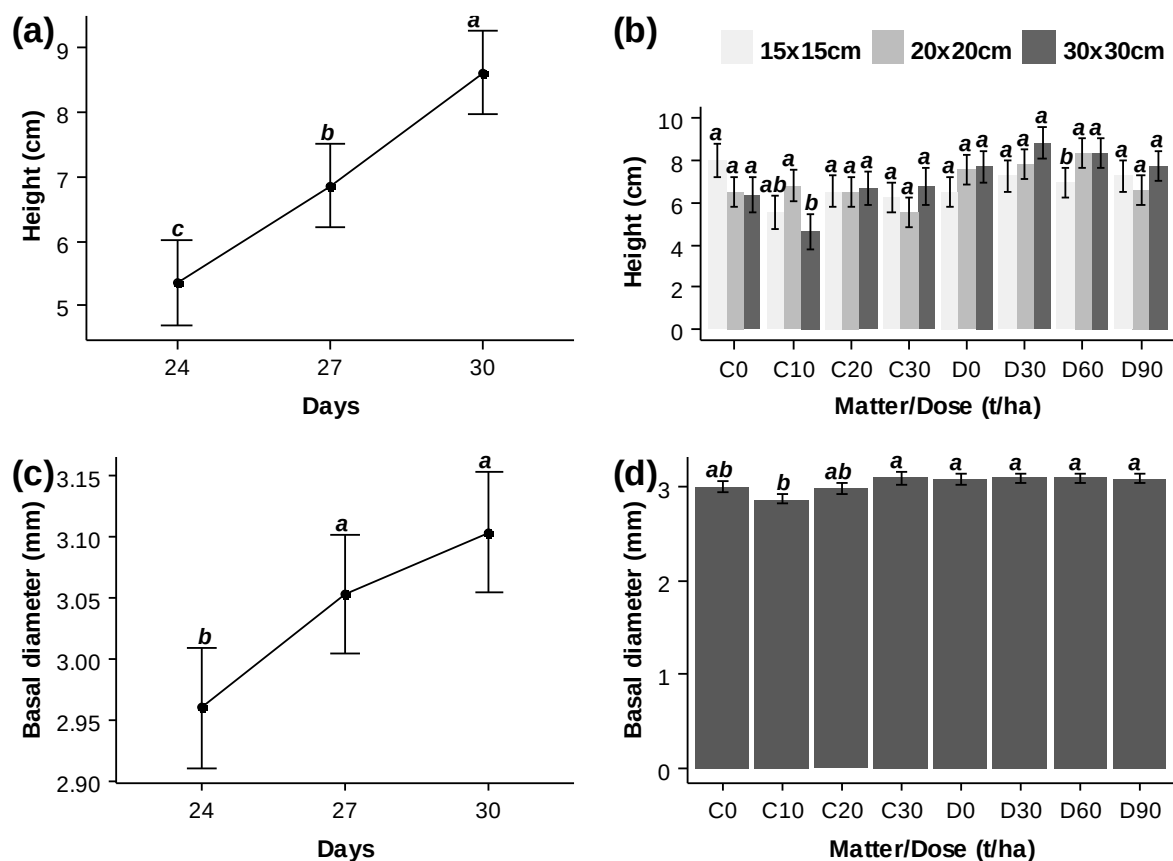


Figure 5.5 Evolution trend of the height and the basal diameter for the first 30 days after sowing in Soudano-Guinean zone according to the time respectively (a) and (c), the dose of organic matter type and sowing density (b) for height, and dose of organic matter type (d). C=Compost, D=Cow dung

5.3.3. Growth dynamic in height and in basal diameter of baobab seedlings in relationships to variation of dose of organic manure, sowing density and leaves harvesting frequency in Guineo-Congolese and Soudano-Guinean zones next to thirtieth days after sowing

In Guineo-Congolese zone, a significant effect of type of organic matter, dose of organic matter, sowing density and harvesting frequency on the height growth of baobab seedlings next to thirtieth days after sowing was noted. In addition, the interactions between Matter $\times$ Dose, Matter $\times$ Frequency, Dose $\times$ Density, Dose $\times$ Frequency, and Density $\times$ Frequency were significant, showing that differences among doses were not similar across matter, densities and harvesting frequencies and vice-versa. However, effect of time and sowing density was not significant ($P < 0.05$; Table 5.5). Accordingly, 30 ton/ha of poultry dropping showed the highest height (33.41 ± 0.22 cm) and the lowest with low enriched plots (10 ton/ha) and unenriched plots (0 ton/ha) (22.89 ± 0.21 and 23.24 ± 0.20 cm; Figure 5.7a). Poultry dropping with 30 days frequency of leaves harvest presented the highest height (31.54 ± 0.18 cm) whereas the lowest was obtained for compost of organic waste and 15 days frequency of leaves harvest ($23.13 \pm$

0.18 cm; Figure 5.7b). 30 ton/ha of organic matter and 20 × 20 cm sowing density showed the highest height (33.67 ± 0.25 cm) and low enriched plots (10 ton/ha) and unenriched plots (0 ton/ha) and 30 × 30 cm sowing density the lowest (21.38 ± 0.27 and 21.63 ± 0.26 cm; Figure 5.7c). In addition, 30 ton/ha of organic matter and 30 days frequency of leaves harvest presented the highest height (34.27 ± 0.25 cm) whereas the lowest was obtained unenriched plots (0 ton/ha) and 22 and 15 days frequencies of leaves harvest (21.54 ± 0.26 and 21.73 ± 0.25 cm; Figure 5.7d). 20 × 20 cm sowing density and 30 days frequency of leaves harvest presented the highest height (30.90 ± 0.22 cm) and 30 × 30 cm sowing density and 15 days frequency of leaves harvest the lowest (20.49 ± 0.24 cm; Figure 5.7e). This indicates that, in Guineo-Congolese zone a higher dose of poultry dropping (30 ton/ha), with 20 × 20 cm sowing density and 30 days frequency of leaves harvest presented the highest height next to thirtieth days after sowing. While, the lowest height presented with unenriched plots (0 ton/ha), 30 × 30 cm sowing density and 15 days frequency of leaves harvest.

A significant effect of the time, type of organic matter, dose of organic matter, sowing density and harvesting frequency on the basal diameter growth of baobab seedlings next to thirtieth days after sowing was noted. In addition, the interactions between Time × Matter, Matter × Dose, Time × Density, Time × Frequency, Matter × Density, Dose × Density, Dose × Frequency, and Density × Frequency were significant, showing that differences among times were not similar across matter, doses, densities and frequencies and vice-versa ($P < 0.05$; Table 5.5). Accordingly, 30 days frequency of leaves harvest presented the highest growth of basal diameter whereas the lowest was obtained for 15 days frequency of leaves harvest (Figure 5.8a). 20 × 20 cm sowing density showed the highest growth of basal diameter and the lowest for 30 × 30 cm sowing density (Figure 5.8b). 30 ton/ha of organic matter showed the highest growth of basal diameter and the lowest with low enriched plots (0-20 ton/ha; Figure 5.8c). In addition, 30 ton/ha of poultry dropping showed the highest growth of basal diameter (9.94 ± 0.13 mm) and the lowest with low enriched plots (10 ton/ha) and unenriched plots (0 ton/ha) (7.35 ± 0.13 and 7.63 ± 0.12 mm; Figure 5.8d). This indicates that, in Guineo-Congolese zone a higher dose of poultry dropping (30 ton/ha), with 20 × 20 cm sowing density and 30 days frequency of leaves harvest presented the highest growth of basal diameter next to thirtieth days after sowing. While, the lowest basal diameter presented with unenriched plots (0 ton/ha), 30 × 30 cm sowing density and 15 days frequency of leaves harvest.

In Soudano-Guinean zone, a significant effect of dose of organic matter, sowing density and harvesting frequency on the height growth of baobab seedlings next to thirtieth days after sowing was noted. In addition, the interactions between Dose × Density, Dose × Frequency,

and Density $\times$ Frequency were significant, showing that differences among doses were not similar across densities and harvesting frequencies and vice-versa. However, effect of time and sowing density were not significant ($P < 0.05$; Table 5.6). Accordingly, high dose of cow dung (60-90 ton/ha) and 20×20 cm sowing density showed the highest height (28.85 ± 0.35 cm) and the lowest with unenriched plots (0 ton/ha) and 15×15 cm sowing density (14.78 ± 0.35 cm; Figure 5.9a). High dose of cow dung (60-90 ton/ha) with 30 days frequency of leaves harvest presented the highest height (30.10 ± 0.35 cm) whereas the lowest was obtained with unenriched plots (0 ton/ha) and 15 days frequency of leaves harvest (15.12 ± 0.33 cm; Figure 5.9b). 20×20 sowing density and 22 days frequency of leaves harvest and 30×30 cm sowing density and 30 days frequency of leaves harvest presented the highest height respectively 21.96 ± 0.22 and 21.57 ± 0.30 cm, and the lowest for 15×15 cm sowing density and 15 days frequency of leaves harvest (17.28 ± 0.25 cm; Figure 5.9c). This indicates that, in Soudano-Guinean zone a higher dose of cow dung (60-90 ton/ha), with 20×20 cm sowing density and 30 days frequency of leaves harvest presented the highest height next to thirtieth days after sowing. While, the lowest height presented with unenriched plots (0 ton/ha), 15×15 cm sowing density and 15 days frequency of leaves harvest.

A significant effect of the time, dose of organic matter, sowing density and harvesting frequency on the basal diameter growth of baobab seedlings next to thirtieth days after sowing was noted. In addition, the interactions between Time $\times$ Dose, Dose $\times$ Density, Dose $\times$ Frequency, and Density $\times$ Frequency were significant, showing that differences among times were not similar across doses, densities and frequencies and vice-versa ($P < 0.05$; Table 5.6). Accordingly, high dose of cow dung (60-90 ton/ha) showed the highest growth of basal diameter and the lowest with unenriched plots (0 ton/ha; Figure 5.10a). 20×20 cm sowing density and 22 and 30 days frequency of leaves harvest presented the highest height respectively 6.64 ± 0.23 and 6.50 ± 0.23 mm, and 15×15 cm sowing density and 15 days frequency of leaves harvest the lowest (5.41 ± 0.23 mm; Figure 5.10b). This indicates that, in Soudano-Guinean zone a higher dose of cow dung (60-90 ton/ha), with 20×20 cm sowing density and 30 days frequency of leaves harvest presented the highest growth of basal diameter next to thirtieth days after sowing. While, the lowest basal diameter presented with unenriched plots (0 ton/ha), 15×15 cm sowing density and 15 days frequency of leaves harvest.

Table 5.5 Effect of significant factors and interactions on the height and basal diameter of baobab seedlings next to thirtieth days after sowing in Guineo-Congolese zone

Source	df	After 30 days			
		Height		Basal diameter	
		F-value	P-value	F-value	P-value
Time	13	-	-	131.85	<0.001
Matter	1	243.27	<0.001	562.60	<0.001
Dose	3	456.59	<0.001	405.66	<0.001
Density	2	366.49	<0.001	241.91	<0.001
Frequency	2	459.45	<0.001	540.00	<0.001
Time: Dose	39	-	-	1.71	0.003
Matter: Dose	3	77.24	<0.001	55.66	<0.001
Time: Density	26	-	-	2.96	<0.001
Time: Frequency	26	-	-	10.60	<0.001
Matter: Density	2	47.02	<0.001	7.25	<0.001
Dose: Density	6	18.18	<0.001	24.19	<0.001
Dose: Frequency	6	10.19	<0.001	5.25	<0.001
Density: Frequency	4	12.14	<0.001	4.24	0.002

df=degree of freedom, F-value=Fisher statistic, P-value=Probability of significance, After 30 days=next to thirtieth days after sowing. Only significant effects are presented in the table.

Table 5.6 Effect of significant factors and interactions on the height and basal diameter of baobab seedlings next to thirtieth days after sowing in Soudano-Guinean zone

Source	df	After 30 days			
		Height		Basal diameter	
		F-value	P-value	F-value	P-value
Time	25	-	-	277.65	<0.001
Dose	7	318.66	<0.001	500.67	<0.001
Density	2	119.59	<0.001	199.44	<0.001
Frequency	2	44.60	<0.001	81.76	<0.001
Time: Dose	175	-	-	2.34	<0.001
Dose: Density	14	21.43	<0.001	28.59	<0.001
Dose: Frequency	14	23.69	<0.001	26.15	<0.001
Density: Frequency	4	6.83	<0.001	3.74	0.004

df=degree of freedom, F-value=Fisher statistic, P-value=Probability of significance, After 30 days=next to thirtieth days after sowing. Only significant effects are presented in the table.

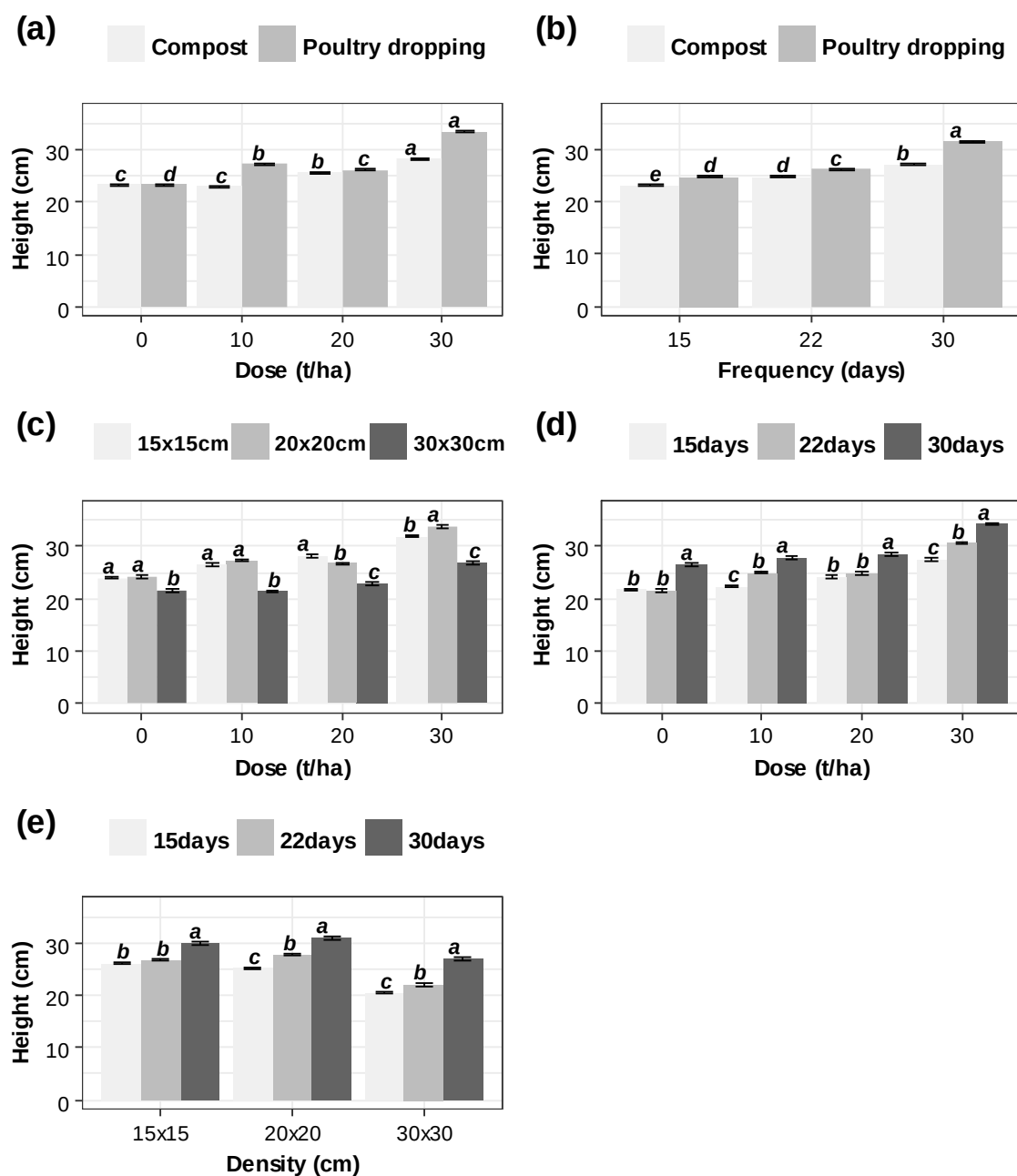


Figure 5.6 Evolution trend of the height after 30 days next to the sowing in Guineo-Congolese zone according to the organic matter type and their dose (a), the organic matter type and frequency of leaves harvest (b), the dose and sowing density (c), the dose and frequency of leaves harvest (d), and the sowing density and frequency of leaves harvest (e)

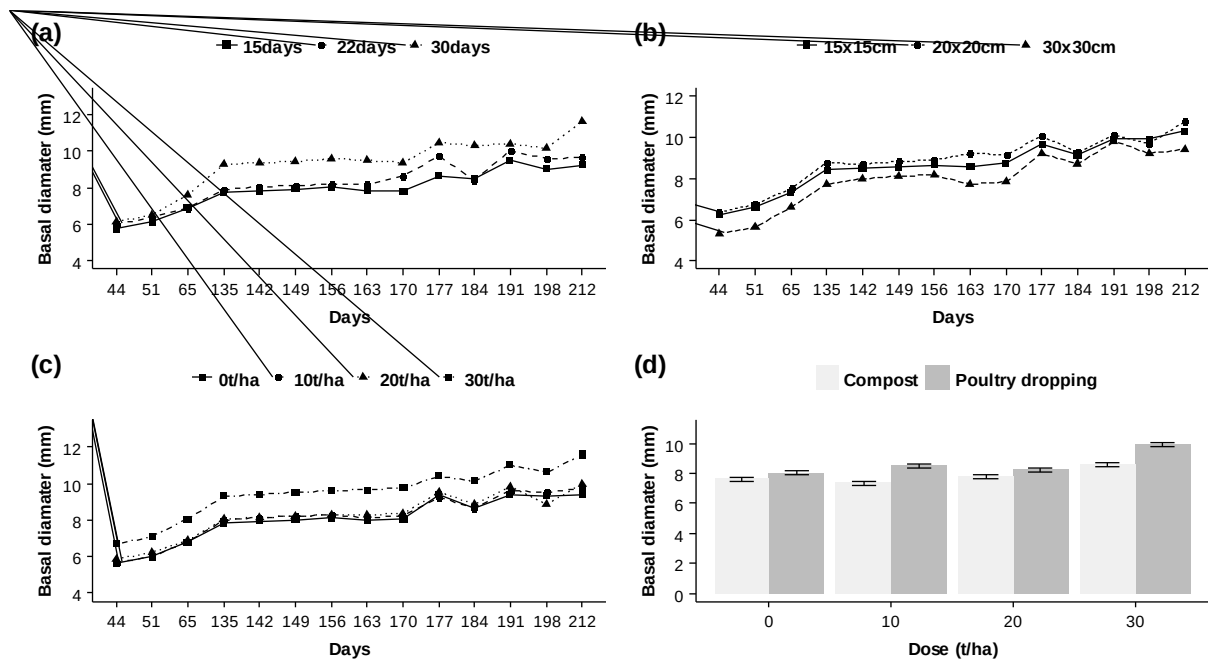


Figure 5.7 Evolution trend of the basal diameter after 30 days next to the sowing in Guineo-Congolese zone according to the time and frequency of leaves harvest (a), the time and sowing density (b), the time and dose of organic matter type (c), and the organic matter type and their dose (d)

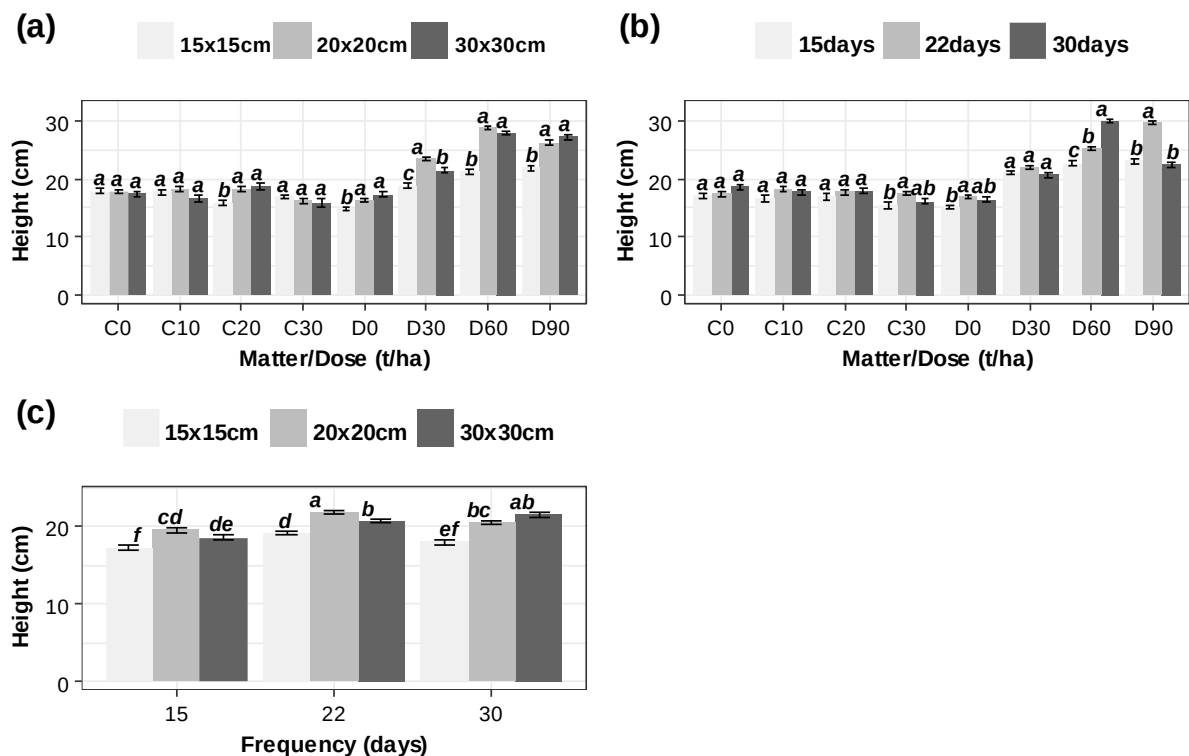


Figure 5.8 Evolution trend of the height after 30 days next to the sowing in Soudano-Guinean zone according to the dose of organic matter type and sowing density (a), the dose of organic matter type and frequency of leaves harvest (b), and the sowing density and frequency of leaves harvest (c). C=Compost, D=Cow dung

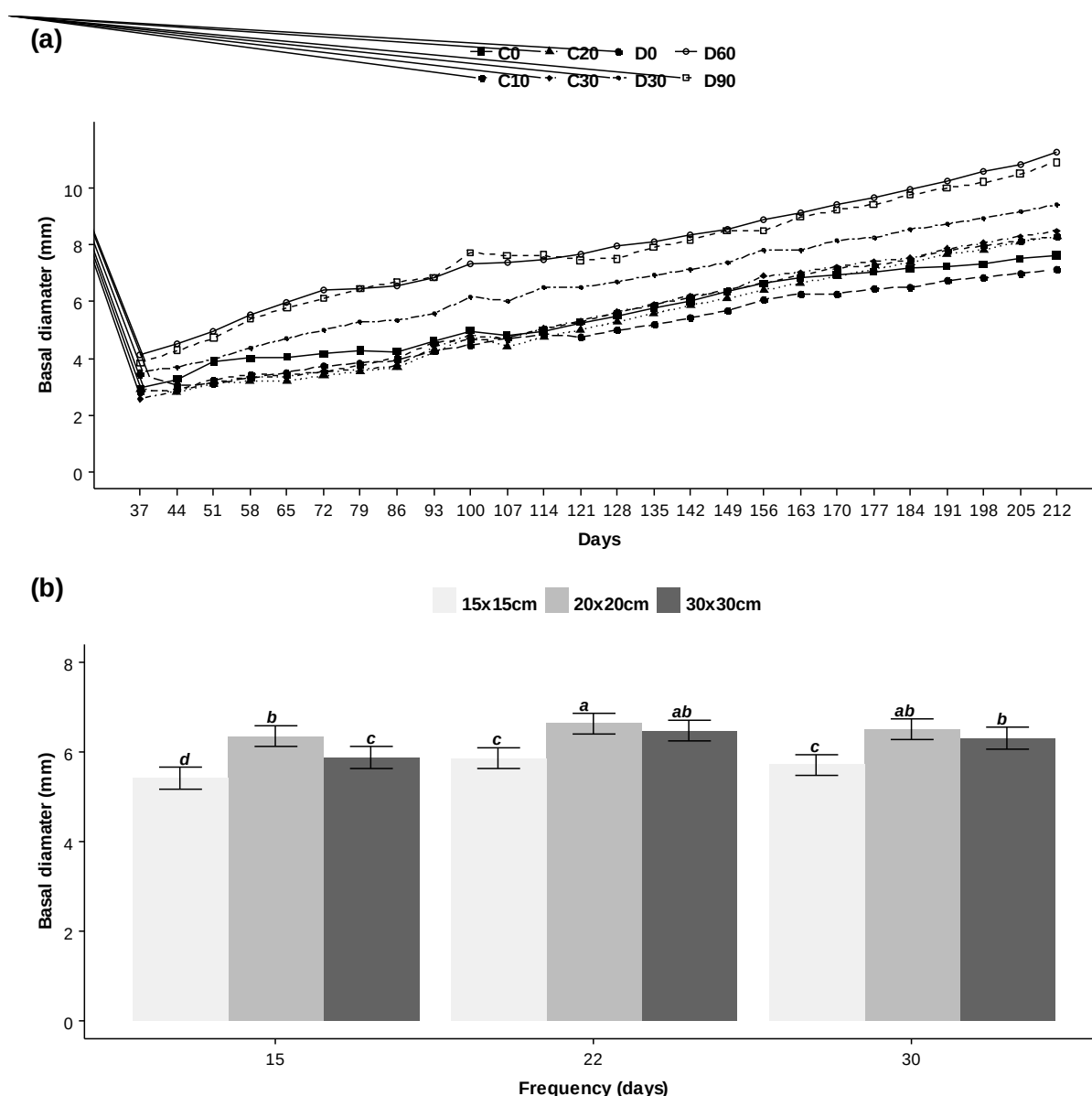


Figure 5.9 Evolution trend of the basal diameter after 30 days next to the sowing in Soudano-Guinean zone according to the time and dose of organic matter type (a), and the sowing density and frequency of leaves harvest (b). C=Compost, D=Cow dung

5.3.4. Evolutionary dynamics of number of ramifications of baobab seedlings in relationships to variation of dose of organic manure, sowing density and leaves harvesting frequency in Guineo-Congolese and Soudano-Guinean zones

In Soudano-Guinean zone, a significant effect of the dose of organic matter, sowing density and harvesting frequency on the number of ramifications of baobab seedlings was noted. In addition, the interactions between Dose $\times$ Density, Dose $\times$ Frequency and Density $\times$ Frequency were significant, showing that differences among doses were not similar across densities and harvesting frequencies and vice-versa ($P < 0.05$; Table 5.7). In particular, 90 ton/ha of cow dung and 20×20 cm sowing density showed the greatest number of ramifications (0.99 ± 0.03 per

plant) and zero ramification with unenriched plots (0 ton/ha) and 30 × 30 cm sowing density (Figure 5.10a). In addition, 90 ton/ha of cow dung and 30 days frequency of leaves harvest presented the greatest number of ramifications (0.73 ± 0.03 per plant) and zero ramification with unenriched plots (0 ton/ha), compost of organic waste and 15 days frequency of leaves harvest (Figure 5.10b). 20 × 20 cm sowing density showed the greatest number of ramifications (0.34 ± 0.02 per plant) and the least for 15 × 15 cm sowing density (0.05 ± 0.02 per plant) for 15 days frequency of leaves harvest (Figure 5.10c). This indicates that, in Soudano-Guinean zone a higher dose of cow dung (90 ton/ha), with 20 × 20 cm sowing density and higher frequency of harvesting (30 days) presented the greatest number of ramifications. While, the least number of ramifications presented with unenriched plots (0 ton/ha), compost of organic waste, 15 × 15 cm sowing density and 15 days frequency of leaves harvest.

In Guineo-Congolese zone, a significant effect of organic matter type, dose, sowing density and harvesting frequency on the number of ramifications of baobab seedlings was noted. In addition, the interactions between Matter × Dose, Matter × Density, Matter × Frequency, Dose × Density, and Dose × Frequency were significant. These showing that differences among matter were not similar across doses, densities and harvesting frequencies and vice-versa ($P < 0.05$; Table 5.7). Accordingly, 30 ton/ha of poultry dropping showed the greatest number of ramifications (2.27 ± 0.04 per plant) and the least with unenriched plots (0 ton/ha) (0.83 ± 0.04 per plant; Figure 5.11a). In addition, poultry dropping and 30 days frequency of leaves harvest presented the greatest number of ramifications (1.75 ± 0.03 per plant) and the least for compost of organic waste and 15 days frequency of leaves harvest (1.00 ± 0.03 per plant; Figure 5.11b). 15 × 15 cm and 20 × 20 cm sowing densities with poultry dropping showed the greatest number of ramifications (1.69 ± 0.03 and 1.59 ± 0.03 per plant) and the least for 15 × 15 cm and 30 × 30 cm sowing densities with compost of organic waste (1.03 ± 0.03 and 1.09 ± 0.03 per plant; Figure 5.11c). Figure 5.11d showed the greatest number of ramifications (2.11 ± 0.05 per plant) for 30 ton/ha of organic matter and 20 × 20 cm sowing density, and the least (0.97 ± 0.05 and 0.98 ± 0.05 per plant) for 10 ton/ha of organic matter and unenriched plots (0 ton/ha) and 30 × 30 cm sowing density. Likewise, 30 ton/ha of organic matter and 22 and 30 days frequencies of leaves harvest presented the greatest number of ramifications (2.03 ± 0.05 and 2.01 ± 0.05 per plant) and the least for unenriched plots (0 ton/ha) with 22 and 15 days frequencies of leaves harvest (0.91 ± 0.05 and 0.96 ± 0.05 per plant; Figure 5.11e). This indicates that, in Guineo-Congolese zone a higher dose of poultry dropping (30 ton/ha), with 20 × 20 cm sowing density and higher frequency of harvesting (30 days) presented the greatest number of ramifications. While, the least number of ramifications presented with unenriched plots (0 ton/ha), 10 ton/ha

of organic matter, compost of organic waste, 30 × 30 cm sowing density and 15 days frequency of leaves harvest.

Table 5.7 Effect of significant factors and interactions on the number of ramifications of baobab seedlings in Guineo-Congolese and Soudano-Guinean zones

Source	Number of ramifications					
	Guineo-Congolese Zone			Soudano-Guinean Zone		
	df	Chisq	P-value	df	Chisq	P-value
Matter	1	204.69	<0.001	-	-	-
Dose	3	162.79	<0.001	7	161.60	<0.001
Density	2	6.98	<0.001	2	41.34	<0.001
Frequency	2	24.82	<0.001	2	3.49	0.030
Matter: Dose	3	12.03	<0.001	-	-	-
Matter: Density	2	11.58	<0.001	-	-	-
Matter: Frequency	2	3.02	0.048	-	-	-
Dose: Density	6	7.91	<0.001	14	29.35	<0.001
Dose: Frequency	6	5.87	<0.001	14	4.24	<0.001
Density: Frequency	-	-	-	4	13.45	<0.001

df=degree of freedom, F-value=Fisher statistic, P-value Probability of significance, GCZ= Guineo-Congolese Zone, SGZ= Soudano-Guinean Zone. Only significant effects are presented in the table.

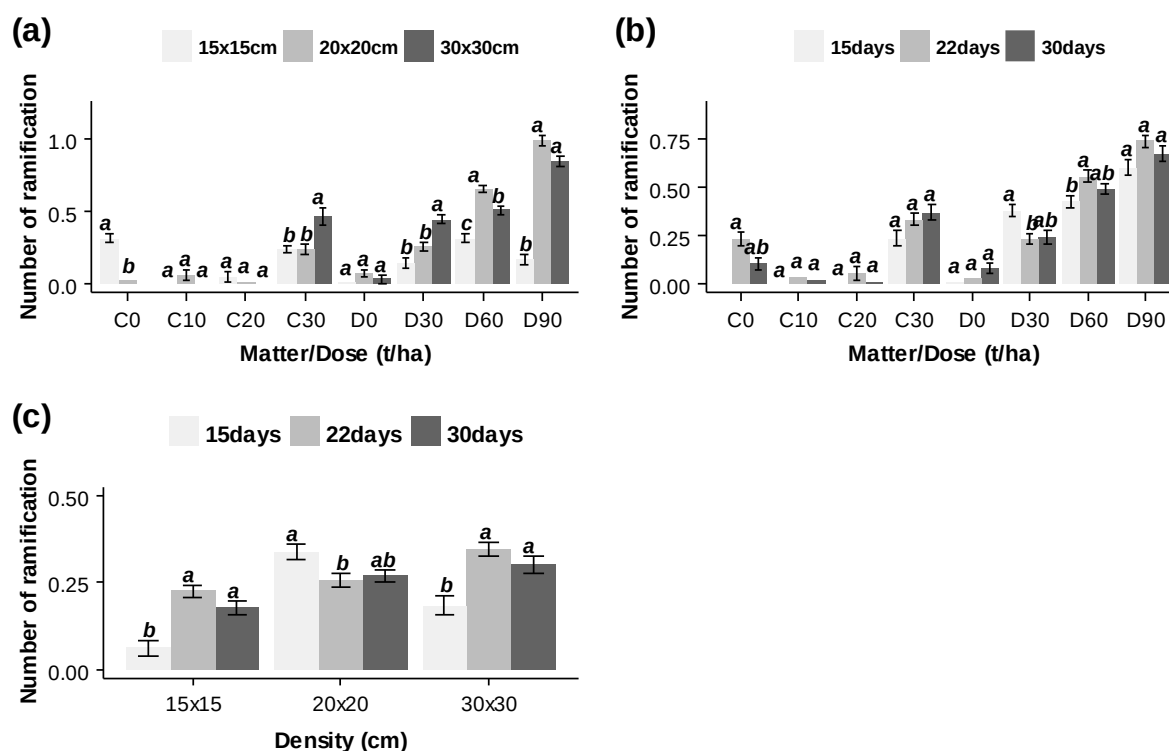


Figure 5.10 Evolution trend of the number of ramifications in Soudano-Guinean zone according to the dose and sowing density (a), the dose and frequency of leaves harvest (b), and the sowing density and frequency of leaves harvest (c). C=Compost, D=Cow dung

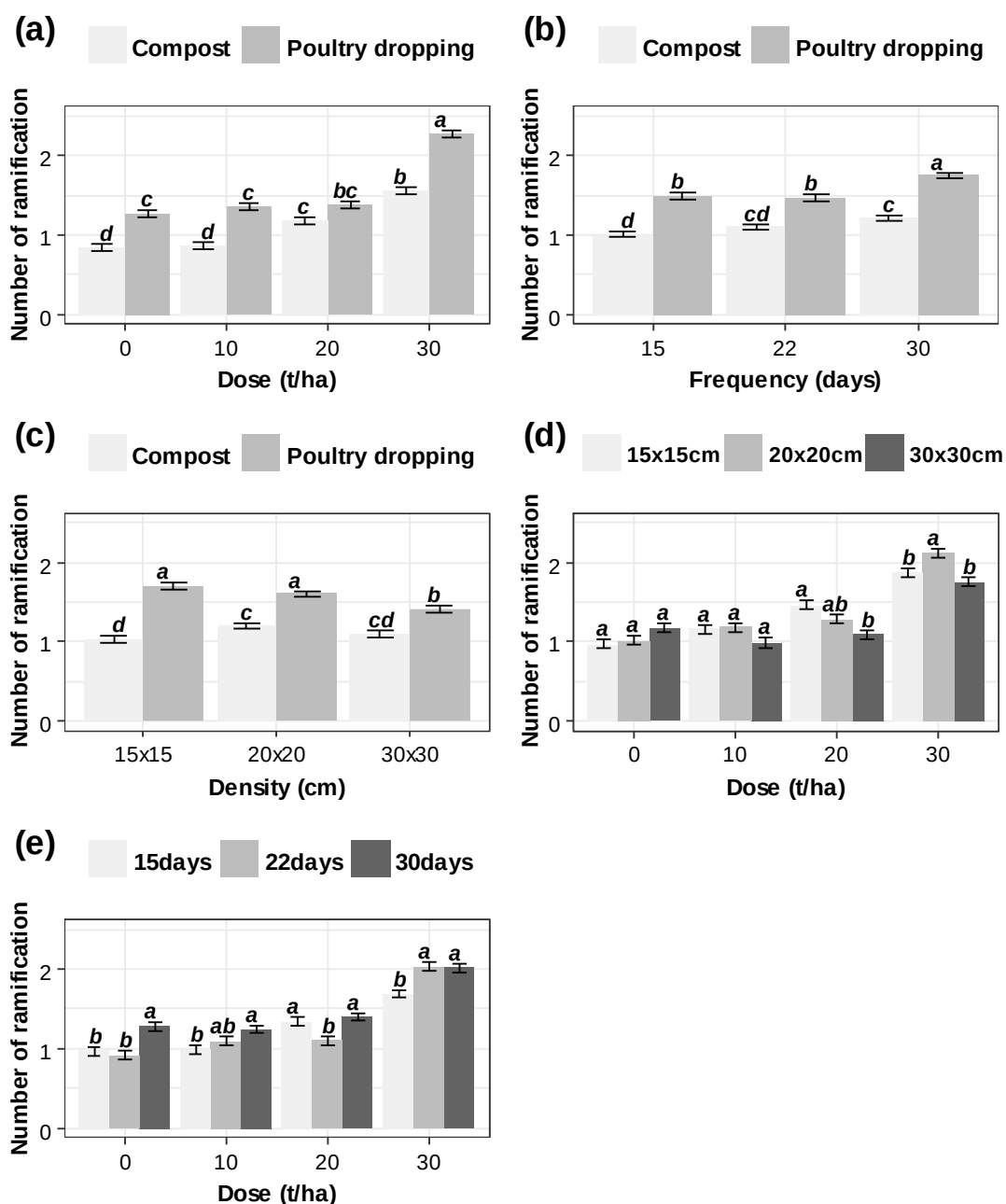


Figure 5.11 Evolution trend of the number of ramifications in Guineo-Congolese zone according to the organic matter type and their dose (a), organic matter type and frequency of leaves harvest (b), the sowing density and organic matter type (c), the dose and sowing density (d), and the dose and frequency of leaves harvest (e). No Ramification= Number of ramifications

5.4. Discussion

This study investigated effect of different doses of two sources of organic manures, density of sowing and leaves harvesting frequencies on the germination rate, growth of the number of ramifications, height and basal diameter of *A. digitata* seedlings in Guineo-Congolese and Soudano-Guinean zones of Benin.

Effect of fertilizer

In Guineo-Congolese zone, baobab seed germination started the 6th day and reached its maximum after 24 days. High dose of compost of organic waste (30 ton/ha) presented the highest germination rate (94 %). Seeds sown on poultry dropping plots also had low germination rate. This rate of emergence of the seedlings is lightly higher than the rate obtained by IER/ICRAF (2001); Savard (2003); Olivier *et al.* (2006); Savard *et al.* (2006); Kalinganire *et al.* (2007). Indeed, with the same technics for baobab seed pre-treatment as in this study, these authors obtained 58-90% in Mali by putting the seeds in a container of boiling water for 48h. In Soudano-Guinean zone, seeds sown on cow dung plots presented the highest germination rate (24 %). The lowest germination rate was obtained on compost of organic waste plots. While it is possible that non-germinated seeds were still viable, they were not particularly vigorous. The percentage of germination was generally higher for baobab seeds from Guineo-Congolese zone than for those from Sudano-Guinean zone. This might be due to differences in temperature and rainfall between the relatively dry and hot Sudanian zone and the colder and more humid Guinean climate. In many species from dry and hot regions, the loss of dormancy rate increases with the temperature to which the seeds are exposed (Baskin and Baskin, 2001). It is then logical that in our experiments, executed in the humid and colder Guinean zone the highest germination rate is observed for the seeds from this particular zone. Seeds collected in dryer climates are probably germinating less efficiently because the applied conditions fail to break their dormancy. This indicates a strong response of baobab germination to ecological conditions like humidity and temperature. It has to be noted that next to environmental factors, also genetic factors can have an influence on seed germination traits (Assogbadjo *et al.*, 2010). Wulff (1995) and Gutterman (2000) reported that maternal factors, such as position of the seed in the fruit/tree and the age of the mother plant influence seed germination ability. Then, the zone with high rainfall reduce the average germination time.

Next to germination, significant differences were also observed in baobab seedling growth among the different climatic zones and between the substrates on which the seeds were sown. Both in Guineo-Congolese and Soudano-Guinean zones a higher dose of organic matter (30 ton/ha of poultry dropping and 30-90 ton/ha of cow dung), presented the highest height and

basal diameter of baobab seedlings for the first thirty and next to thirtieth days after sowing and greatest number of ramifications, while, the lowest height and basal diameter and the least number of ramifications by application of low dose of organic matters (0-10 ton/ha of compost of organic waste). Emerging seedlings mainly depend on seed reserve for initial growth, which explains why seedling height and diameter increased with approximatively the same trend after seed germination. Regarding the substrate, it is not surprising to notice that the seedlings grow faster when organic matter is supplied. Rai and Tripathi (1982) and Parker *et al.* (2006) reported a positive influence of large seed size and seed reserve on the establishment and early growth of seedlings.

Effect of density of sowing

The germination rate, the highest height and basal diameter of baobab seedlings for the first thirty and next to thirtieth days after sowing and greatest number of ramifications were generally higher for 20 × 20 cm sowing density in both Guineo-Congolese and Sudano-Guinean zones. This sowing density of the seedlings growth joined the one obtained by Assogba-Komlan *et al.* (2015) for *Lactuca sativa* L and *Amaranthus* sp. Especially, in Soudano-Guinean zone 30 × 30 cm sowing density presented the highest and the lowest heights of baobab seedlings for the first thirty days after sowing. Optimum seedlings growth required optimum sowing density (Badi *et al.*, 2004; Maboko and Du Plooy, 2009; Žnidarčič *et al.*, 2011). Then, too high or too low sowing density (Maboko, 2013) can result in lower growth and biomass of leaves. It was revealed that with closer sowing density seedlings growth showed increasing trend. DeBell *et al.* (1996), Henskens *et al.* (2001) and Alcorn *et al.* (2007) reported that reduced spacing between plants reduced development of ramifications. Similar results were reported by Niederwieser (2001) on *Beta vulgaris* L. It was revealed that, at closer spacing, *Beta vulgaris* L. seedlings were forced to grow upright and faster due to the competition for sunlight. In contrast, the wider spaced plants resulted in leaves growing more horizontal with an open growing pattern (Maboko and Du Plooy, 2013). The slowest growth and weak germination rate in Sudano-Guinean zone might be due to the environmental factor of this zone. The very large variation of germination rates recorded between the two zones could justify the differences observed in growth and productivity. This would explain the reason of divergence of the results. The low rate in Soudano-Guinean zone thus does not make it possible to make comparison between the zones. It would thus be necessary to pass by the nursery to ensure 100% emergence of the seedlings. Hence, the results in Guineo-Congolese zone would be priority on the choice of the technical routes.

Effect of harvesting frequency

Both in Guineo-Congolese and Soudano-Guinean zones, a higher frequency of leaves harvest (30 days) presented the highest height and basal diameter of baobab seedlings next to thirtieth day after sowing and greatest number of ramifications. Results revealed no significant effect of frequency of leaves harvest on germination rate, height and basal diameter of baobab seedlings for the first thirty day after sowing (previous leaves harvest). As Latt *et al.* (2000) found on other plant models such as *Gliricidia sepium*, *Leucaena leucocephala*, the seedlings growth increases by raising harvesting frequency. In fact, the high harvest frequency reduces the photosynthesis activity and oblige the plant to grow faster to get more sunlight. Assogba-Komlan *et al.* (2015) reported same frequency of leaves harvest for *Lactuca sativa* L.

5.5. Conclusion

Globally, the results showed that higher germination rate, greater number of ramifications and higher height and basal diameter growth were obtained when *A. digitata* seedlings were produced applying 30 t/ha of poultry dropping or 90 ton/ha of cow dung with 20 × 20 cm sowing density and 30 days leaves harvesting frequency intervals. This combination provided the optimum growth of *A. digitata* seedlings.

Further research is necessary to investigate effect of different doses of two sources of organic manures, sowing density and leaves harvesting frequencies on growth of the number of leaves, morphological traits of the leaf and dry weight of yield of *A. digitata* seedlings.

6.

Leaves biomass responses of baobab seedlings to
variation of dose of organic manure, sowing density
and leaves harvesting frequency

(In preparation for submission to *Forests, Trees and Livelihoods*)

6. Leaves biomass responses of baobab seedlings to variation of dose of organic manure, sowing density and leaves harvesting frequency

Abstract

Demand for biological vegetables strongly increases. This production requires a reflexion increased on the farming techniques as well as regular observations of the plants and their environment. It requires very broad competences on the techniques and plannings of production. This study aimed to assess the growth of the number of leaves, morphological traits of the leaf and dry weight of yield of baobab seedlings to variation of dose (0, 10, 20, and 30 tons/ha) of organic manure (Compost of organic waste vs organic manure), sowing density (15×15 cm, 20×20 cm, and 30×30 cm) and leaves harvesting frequency (15, 22 and 30 days). The experiment was conducted on-station in the Guineo-Congolese zone of Benin using a split-split plot design with three replicates. Data were analysed using linear, linear mixed effects for longitudinal data with a normal structure for errors and generalized linear mixed effects models for longitudinal data with a Poisson structure for errors models. The results showed that more biomass and greatest number of leaves growth were obtained when *A. digitata* seedlings were produced applying 30 t/ha of poultry dropping with 15×15 cm sowing density and monthly harvests next to sowing. This combination provided the highest production of leaves biomass (41.62 ± 1.16 Kg of dry matter per 100 m^2 equivalent to 157.29 ± 4.38 Kg of fresh leaves per 100 m^2), a less tapering leaf and the lowest specific leaf area and specific dry weight of *A. digitata* seedlings. And optimum growth of the number of leaves of *A. digitata* seedlings for low dose of organic matter (10-20 ton/ha of poultry dropping). Further research is necessary to assess on the protection methods against the bio-attackers and control of adventitious plants.

Key words: Baobab leaves, density of sowing, compost, organic matter, harvesting frequency

6.1. Introduction

African baobab (*Adansonia digitata* L.), is a deciduous stem-succulent tree native to the dry regions of tropical Africa (Sidibé and Williams, 2002; Wickens and Lowe, 2008) . In total, more than 300 uses have been reported for this species, with the most important ones being related to food, medicine and income generation (Wickens and Lowe, 2008; De Caluwé *et al.*, 2009; Buchmann *et al.*, 2010). The highly nutritious fruits, seeds and leaves (Nordeide *et al.*, 1996) are frequently consumed by rural people in both western and south-eastern Africa, thereby supplementing the local diet and improving food security (Wickens and Lowe, 2008; Buchmann *et al.*, 2010).

The leaves contain 13-15% protein, 60-70% carbohydrate, 4-10% fat and around 11% fibre and 16% ash (De Caluwé *et al.*, 2010). Energy value varies from 1180-1900kJ/100g of which 80% is metabolized energy. The leaves are rich in pro-vitamins A and C (Zahra'u *et al.*, 2014). The highest level of pro-vitamin A was detected in young leaves especially when used as dried material (Sidibé *et al.*, 1996), expressed in retinol equivalent it was between 9 and 27 mg/kg. Nordeide *et al.* (1996) recorded that the level of pro-vitamin A was about one-third the content in *Amaranthus* dried leaves. Becker (1983) noted the absence of vitamin C but a significant content of vitamin B2. In terms of mineral content, baobab leaf is said to be an excellent source of calcium, iron, potassium, magnesium, manganese, molybdenum, phosphorus, and zinc (Yazzie *et al.*, 1994).

The leaves of baobab tree are a staple for many populations in Africa, especially the central region of the continent (Yazzie *et al.*, 1994; Gebauer *et al.*, 2002). During the rainy season when the baobab leaves are tender, people harvest a fresh batch of leaves. During the last month of the rainy season, leaves are harvested in great abundance and are dried for domestic use and for marketing during the dry season. The leaves are typically sun-dried and either stored as whole leaved or pounded and sieved into a fine powder (Gebauer *et al.*, 2002). Young leaves are widely used, cooked as spinach, and frequently dried, often powdered and used for sauces over porridges, thick gruels of grains, or boiled rice (Sidibé and Williams, 2002).

The species is facing a high risk of extinction in the wild where it is threatened by bush fire (seedlings), grazing and lack of natural regeneration (Assogbadjo *et al.*, 2010). Thus leading to test of several pre-treatments methods (Savard, 2003; Assogbadjo, 2006; Olivier *et al.*, 2006; Savard *et al.*, 2006; SCUC, 2006; Kalinganire *et al.*, 2007; Assogbadjo *et al.*, 2010; Falemara *et al.*, 2014; Niang *et al.*, 2015). Conservation and domestication strategies of baobab genetic resources consist in the proper management option to obtain sustainable benefits for present and future generations. Consequently, finding a pathway for propagating baobab in rural areas,

especially in smallholder farming systems to satisfy the daily demand of baobab leaves will not only allow protecting the species but also help in fighting food insecurity and poverty. Plant spacing for vegetable cultivation is an important criterion for attaining maximum vegetative growth and an important aspect of crop production for maximizing the yield. Optimum plant spacing ensures judicious use of natural resources and makes the intercultural operations easier. It helps to increase the number of leaves and healthy foliage. Densely planted crop obstruct the proper growth and development. On the other hand, wider spacing ensures the basic nutritional requirements but decrease the total number of plants as well as total yield. Yield may be increased for any crop up to 25% by using optimum spacing in leafy vegetable (Bansal *et al.*, 1995). Investigations of the effect of cutting frequency on biomass production have been undertaken and differences in production due to various cutting regimes have been found with many species such as *Leucaena leucocephala* and *Gliricidia sepium* (Guevarra *et al.*, 1978; Latt *et al.*, 2000). Baobab tree can withstand drought, it has no important pest or diseases and it does not require extra watering (Sidibé and Williams, 2002; SCUC, 2006). Declining soil fertility remains the fundamental reason for declining smallholder food production in sub-Saharan Africa (Smaling *et al.*, 1997; Gruhn *et al.*, 2000). Fertilizer application in appropriate time, Also, organic manures from domestic breeding or compost from domestic organic waste are hypothesized to provide sustainable manure for sustainable production in smallholder farming systems (Assogba-Komlan, 2001). However, animal rearing in Benin is poultry-based in the humid zone. The overall objective of this research is to develop best agro-ecological practices to improve the leaves biomass production on baobab seedlings. Specifically, the study aims at assessing: i) the number of leaves for the first 30 days after sowing, ii) the dry weight of yield, and iii) the morphological traits and dry weight of the leaf of baobab seedlings in relationships to variation of dose of organic manure, sowing density and leaves harvesting frequency in Benin. In this study the following key question was posed: What is the combination of the various factors (dose of organic manure, sowing density and leaves harvesting frequency) which gives the optimal number of leaves, morphological traits of the leaf and dry weight of yield of baobab seedlings?

6.2. Material and methods

6.2.1. Study areas

The study was conducted in the humid zone (Guineo-Congolese) in south Benin (Dansi *et al.*, 2008). Indeed, Benin is a West-African country of 114 763 km² located between 6°25'N-12°30' and 0°45'E - 4°E. The resident population is about 10 880 000 inhabitants in 2015 (UN, 2015) and slightly female-biased (50.12%). The development index of UNDP was 0.485 placing it at 167th position from 188 countries in 2015 (UNDP, 2016). The gross product per capita was 745.47 Dollars US in 2013 (INSAE, 2015).

6.2.2. Sampling and data collection

Data were collected on the seedlings of *A. digitata* (Figure 6.1) established with the seeds collected in the same bio-geographical zone (Guineo-Congolese zone). The experiment was conducted in a factorial trials using a split-split plot design with three replications under on-station conditions. The area was first divided into two main plots depending on the type of organic manure (one for compost and one for poultry dropping). Each main plot was then divided into 4 subplots corresponding to the four doses of each type of organic manure (0, 10, 20 and 30 tons/ha for compost and poultry dropping). The 9 (3 × 3) combinations of densities (15 × 15 cm, 20 × 20 cm and 30 × 30 cm) and harvest frequencies (every 15, 22 and 30 days; starting 30 days after planting) were randomized per dose. In order to increase the rate of germination, seeds were pre-treated using a container of boiling water for 48 h (IER/ICRAF, 2001; Savard, 2003; Olivier *et al.*, 2006; Savard *et al.*, 2006; Kalinganire *et al.*, 2007). Four seeds were sown to approximately 2 cm of depth per seed hole with 7 plants per row and 4 rows of planting per experimental unit (1.1 × 2 m of size). Next to sprouting, the seedlings were thinned out and only the most vigorous seedling was kept per seed hole. Watering frequency was 10 litres early in the morning per plot per day and weeding was done if need be. Factors tested were summarized in Table 5.1.

After establishment, number of leaves were counted every three days for only the first 30 days. Next to the general harvest at 45th day after establishment, leaves biomass harvested in each experimental unit were weighed fresh and after drying in an oven at 105° C during 48h until constant weight (Figure 6.1).

For the leaves morphology and the index of leaf area, next to the last leaves harvest to the 230th day, 3 leaves were picked by seedling on each of the 10 seedlings (among the 30 seedlings) randomly selected (are on the whole 720 seedlings) per each treatment. The length and the width of each leaf were measured. Leaf shape was deduced by using shape index of leaf

obtained by the ratio of leaf length over their width (Hounkpevi *et al.*, 2016). In absence of sophisticated material, specific leaf area of the leaves selected was given by using the approach of Kindomihou (2005). Indeed, the greatest full rectangular section of leaf was extracted and the width and the length of the section were measured. The dry weight of the aforementioned section was obtained after drying in oven at 105°C during 48h until constant weight. The specific leaf area was obtained by equation 2.

$$SLA_i = \frac{L_i \times W_i}{Dry\ weight_i} \times 100 \quad (Eq. 2)$$

SLA_i is the specific leaf area of leaf i ($cm^2.g^{-1}$), L_i and W_i are respectively the length and the width of the rectangular section of leaf i (cm).

The specific dry weight of the leaf was obtained by ratio total dry weight of the leaf over their total fresh weight. Data were collected on the 10 middle plants of the plots except the border plants.

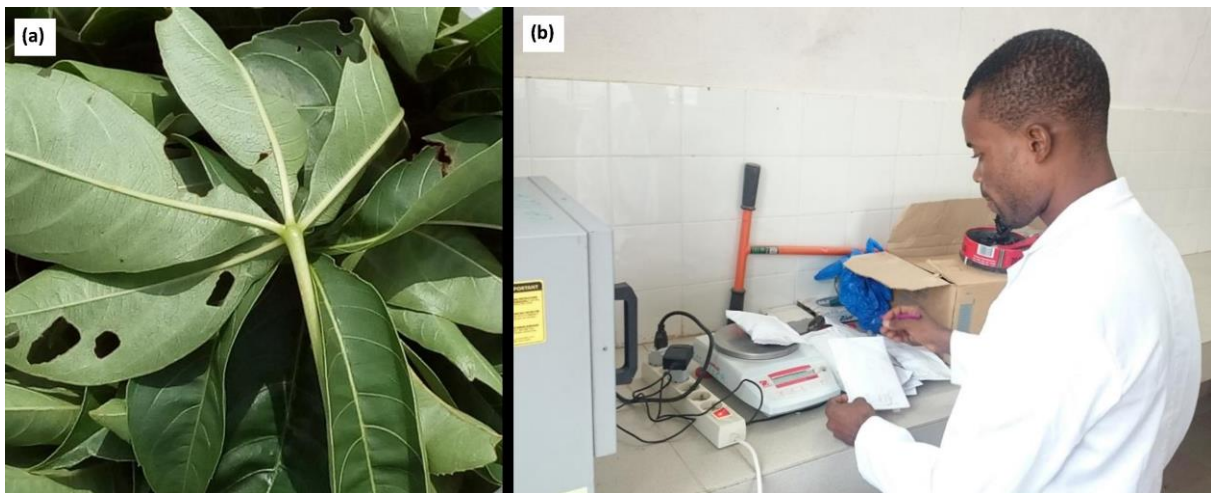


Figure 6.1 Baobab leaves biomass harvested (a) and their drying in oven (b)

6.2.3. Data analysis

To assess the influence of dose of organic manure, sowing density and leaves harvesting frequency on the yields (dry weight of harvested leaves) linear mixed effects models for longitudinal data with a normal structure for errors were used. The models were run with the packages “nlme” (Pinheiro *et al.*, 2017). Linear models were used of the variables (specific leaf area, leaf shape and specific dry weight). And generalized linear mixed effects models for longitudinal data with a Poisson structure for errors were used to assess the influence of dose of organic manure, sowing density and leaves harvesting frequency on the number of leaves using the package “MASS” (Venables and Ripley, 2002). Anova was used to test overall significance of factors. Model selection was based on AIC (the low values are preferred) using the package “bbmle” (Bolker and R Core Team, 2017). Least square means of each variable

were extracted and multiple comparisons among them were estimated using the package “lsmeans” (Lenth, 2016) and used to draw figures using the package “ggplot2” (Wickham, 2009) showing the evolution trend of each of them according to the factors considered. All statistical analyses were carried out in R 3.4.0 (R Core Team, 2017). All factors (organic matter, doses of organic matter, density of sowing and frequency of leaves harvest) were considered fixed. Only the significant ($p < 0.05$) factors and interactions were carried along this study next to model simplification.

6.3. Results

6.3.1. Evolutionary dynamics of number of leaves of baobab seedlings and leaf biomass production in relationships to variation of dose of organic manure, sowing density and leaves harvesting frequency

Number of leaves of baobab seedlings was affected by time, type of organic matter, their dose and density of sowing. Moreover, some of the interactions between these factors were also significant (Time $\times$ Matter, Matter $\times$ Dose, Matter $\times$ Density, Dose $\times$ Density and Time $\times$ Matter $\times$ Dose) were significant, showing that differences among times were not similar across matter, doses and densities and vice-versa ($P < 0.05$; Table 6.1). In general, number of leaves was roughly constant ($2.0.5 \pm 0.08$ per plant) from 6th to 9th day and from 7.77 ± 0.33 to 8.70 ± 0.36 number of leaves at 24th days per plant after sowing and grown progressively until leaves harvest. 15×15 cm and 20×20 cm densities of sowing with compost of organic waste showed the greatest number of leaves (1.69 ± 0.03 and 1.59 ± 0.03 per plant) and the least for 30×30 cm density of sowing with poultry dropping (Figure 6.2a). 10 ton/ha of poultry dropping showed the greatest number of leaves (4.72 ± 0.06 per plant) and the least with 20 ton/ha compost of organic waste and 15 days frequency of leaves harvest (3.95 ± 0.06 per plant; Figure 6.2b). This indicates that, 10 ton/ha of poultry dropping and 15×15 cm density of sowing presented the greatest number of leaves. While, the least number of leaves presented with unenriched plots (0 ton/ha), 20 ton/ha compost of organic waste and 30×30 cm density of sowing.

Significant effect of organic matter type, density of sowing and harvesting frequency on the yield dry weight was noted. In addition, the interactions between Matter $\times$ Density and Density $\times$ Frequency were significant, showing that differences among matters were not similar across densities and frequencies and vice-versa ($P < 0.05$; Table 6.1). Accordingly, poultry dropping and 30 days frequency of leaves harvest showed the highest yield dry weight (29.15 ± 0.95 Kg of dry matter per 100m^2) and compost of organic waste and 15 days frequency of leaves harvest the lowest (8.66 ± 0.69 Kg of dry matter per 100m^2 ; Figure 6.3a). Likewise, 15×15 cm density of sowing and 30 days frequency of leaves harvest presented the highest yield dry weight (41.62 ± 1.16 Kg of dry matter per 100m^2) and the lowest value (3.00 ± 0.85 Kg of dry matter per 100m^2) with 30×30 cm density of sowing and 15 days frequency of leaves harvest (Figure 6.3b). This indicates that application of poultry dropping, closed density of sowing (15×15 cm) and higher frequency of harvesting presented (monthly harvests next to sowing) presented the highest yield dry weight.

Table 6.1 Effect of significant factors and interactions on the number of leaves and yield dry weight

Source	df	Number of leaves		Yield dry weight	
		<i>Chisq</i>	<i>P-value</i>	<i>F-value</i>	<i>P-value</i>
Time	4	3606.48	<0.001	-	-
Dose	3	87.22	<0.001	-	-
Matter	1	94.12	<0.001	8.97	<0.002
Density	2	201.69	<0.001	295.63	<0.001
Frequency	2	-	-	202.63	<0.001
Time: Matter	4	22.01	<0.001	-	-
Matter: Dose	3	28.71	<0.001	-	-
Matter: Density	2	40.26	<0.001	-	-
Matter: Frequency	2	-	-	10.48	<0.001
Density: Frequency	4	-	-	22.88	<0.001
Time: Matter: Density	16	33.20	0.006	-	-

df=degree of freedom, Chisq = Chi square statistic, F-value=Fisher statistic, P-value Probability of significance. Only significant effects are presented in the table.

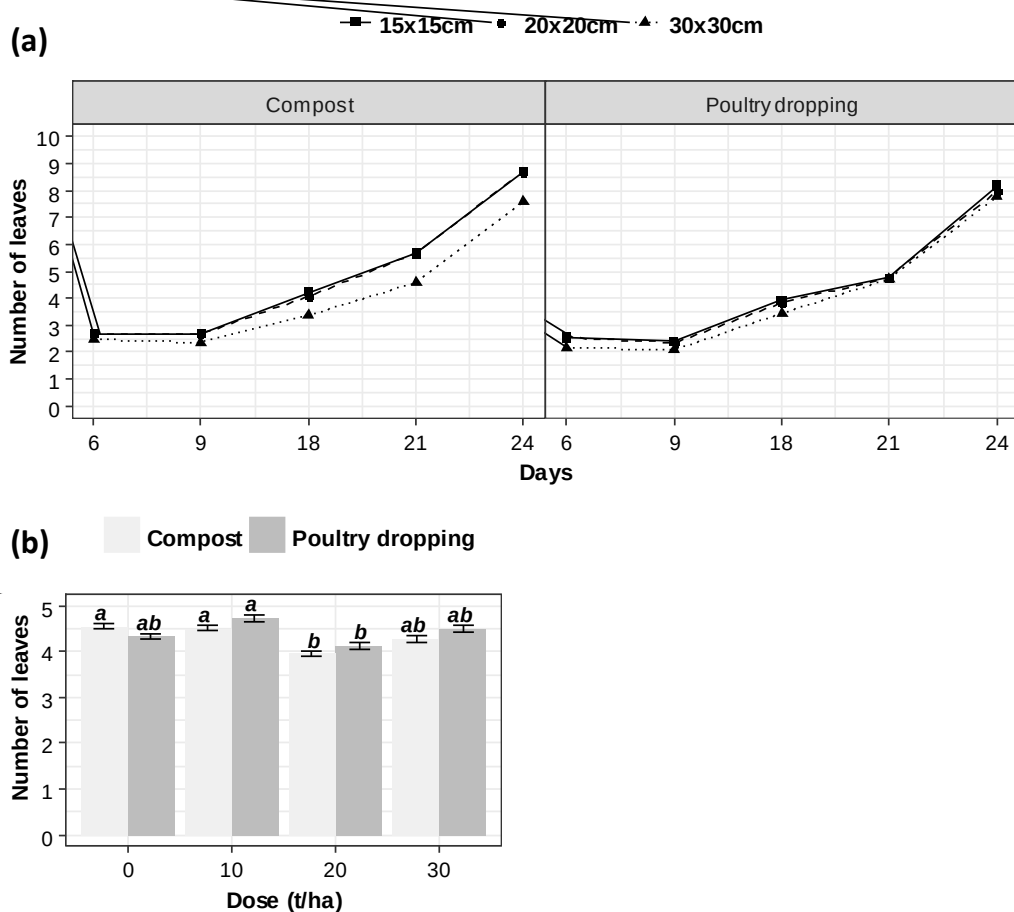


Figure 6.2 Evolution trend of number of leaves of baobab seedlings according to the time, the organic matter type and density of sowing (a), and the organic matter type and density of sowing their dose (b)

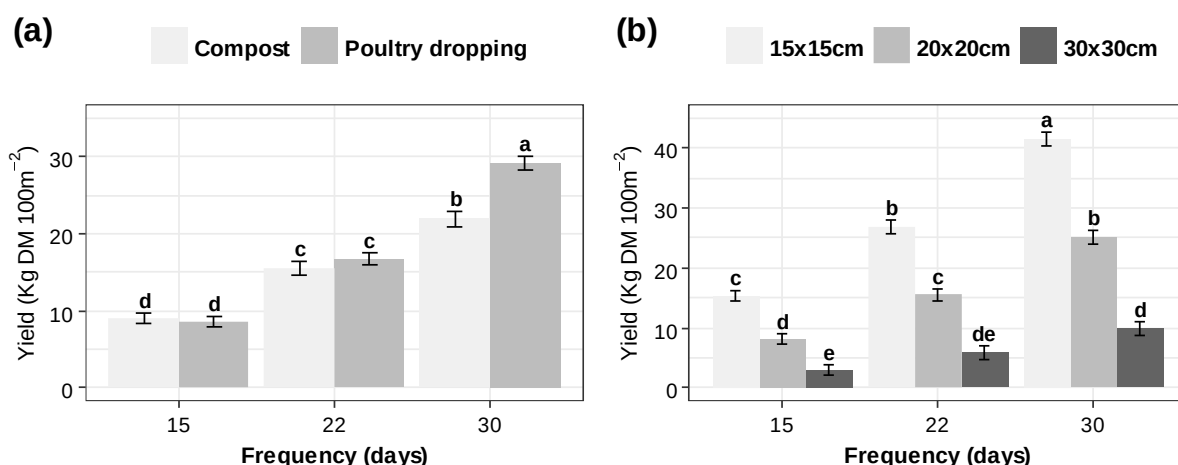


Figure 6.3 Evolution trend of leaf biomass production (Kg of dry matter per 100m²) of baobab seedlings according to the organic matter type and frequency of leaves harvest (a), and the density of sowing and frequency of leaves harvest (b)

6.3.2. Morphological traits and specific dry weight of the leaf of baobab seedlings in relationships to variation of dose of organic manure, sowing density and leaves harvesting frequency

Shape of leaf, specific leaf area and specific dry weight of leaf were affected by doses of organic matter and density of sowing. ($P < 0.05$; Table 6.2). In particular, Figure 6.4a,b,c showed the highest values of leaf shape (0.41 ± 0.05), specific leaf area ($13.22 \pm 1.85 \text{ cm}^2 \text{ g}^{-1}$), and specific dry weight (0.03 ± 0.01) for unenriched plots (0 ton/ha) and the lowest values of leaf shape (0.14 ± 0.06), specific leaf area ($3.98 \pm 1.93 \text{ cm}^2 \text{ g}^{-1}$), and specific dry weight (0.01 ± 0.01) for 20 ton/ha of organic matter. In addition, Figure 6.4d,e,f showed the highest values of leaf shape (0.48 ± 0.05), specific leaf area ($15.35 \pm 1.72 \text{ cm}^2 \text{ g}^{-1}$), and specific dry weight (0.04 ± 0.01) for $30 \times 30 \text{ cm}$ density of sowing, and the lowest values of leaf shape (0.15 ± 0.05), specific leaf area ($4.34 \pm 1.56 \text{ cm}^2 \text{ g}^{-1}$), and specific dry weight (0.01 ± 0.01) for $15 \times 15 \text{ cm}$ density of sowing. This indicates that roughly high dose of organic matter (20 ton/ha), and closed density of sowing ($15 \times 15 \text{ cm}$) presented a less tapering leaf and the lowest specific leaf area and specific dry weight. Likewise, for the same leaf area, the seedlings leaf for 20 ton/ha of organic matter and $15 \times 15 \text{ cm}$ density of sowing have a biomass more raised than the seedlings leaf for unenriched plots (0 ton/ha).

Table 6.2 Effect of significant factors and interactions on the morphological traits and dry weight of the leaf

Source	df	Leaf shape		Specific leaf area		Specific dry weight	
		F-value	P-value	F-value	P-value	F-value	P-value
Dose	3	4.18	0.006	5.35	0.001	3.57	0.013
Density	2	9.94	<0.001	11.33	<0.001	8.24	<0.001

df=degree of freedom, F-value=Fisher statistic, P-value Probability of significance. Only significant effects are presented in the table.

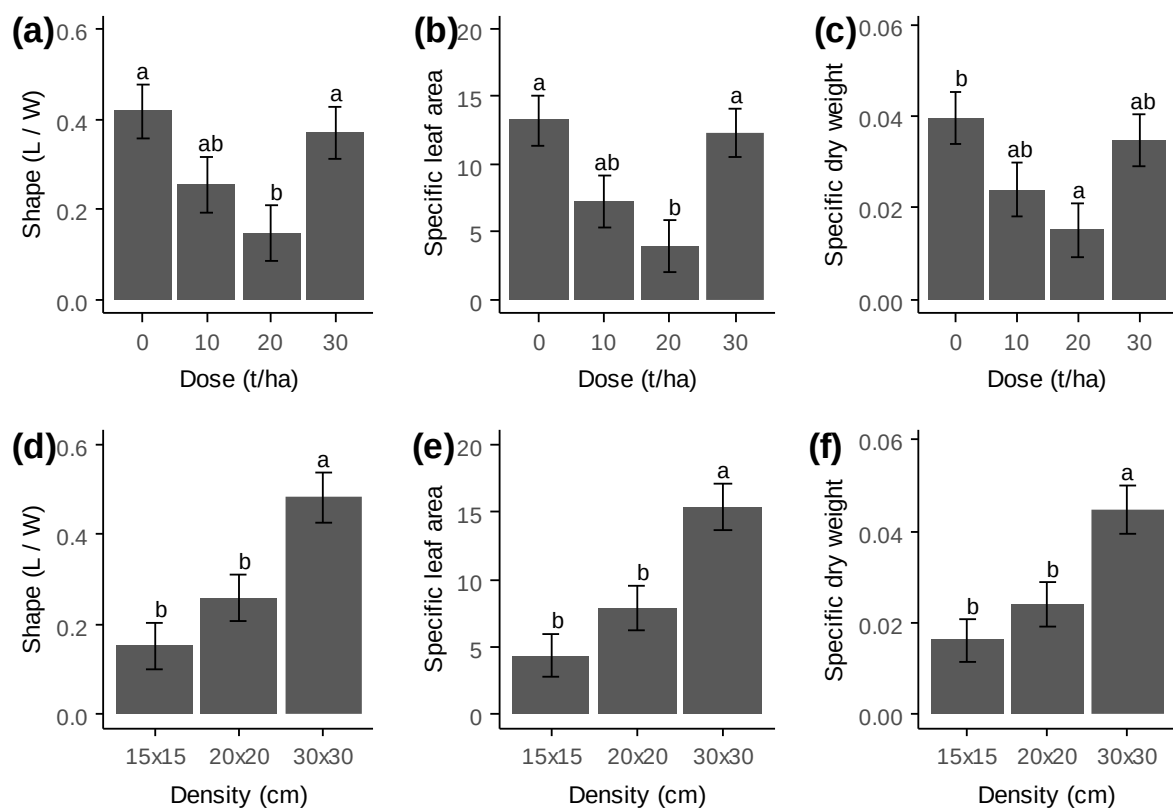


Figure 6.4 Evolution trend of the leaf shape, specific leaf area ($\text{cm}^2 \text{g}^{-1}$), and specific dry weight according to the dose of organic matter respectively (a,b,c), and density of sowing respectively (d,e,f). L=length, W=width

6.4. Discussion

This study investigated effect of different doses of two sources of organic manures, density of sowing and leaves harvesting frequencies on growth of the number of leaves, morphological traits of the leaf and dry weight of yield of *A. digitata* seedlings in Benin.

Effect of fertilizer

Results showed that, the growth of number of leaves was greatest by application of low dose of organic matter (10 ton/ha of poultry dropping) and least with unenriched plots (0 ton/ha) and compost of organic waste. High dose of organic matter (30 ton/ha of poultry dropping) presented the highest yield dry weight. And roughly high dose of organic matter (20 ton/ha of poultry dropping) presented a less tapering leaf and the lowest specific leaf area and specific dry weight. These results joined the idea according to which the organic matter, is the best basic fertilizer (Giller *et al.*, 2002). MRECD (2002) specifies that the nitrogen is an essential factor of growth of the seedlings, especially on the level of the leaves and stems. The maximum number of leaves/plant recorded for organic matters would be due to the highest level of nitrogen which ensure favorable condition for the growth of baobab seedlings. Similar findings were observed by Rincon *et al.* (1998), Tittonell *et al.* (2003) and Boroujerdnia and Ansari (2007) on *Lactuca sativa* L. It was revealed that with the increase of dose of organic matters application, dry weight/plant increase due to more availability of nutrients among the plants during vegetative growth. Similar results were also obtained by Tittonell *et al.* (2003) and Mahmoudi (2005). The lowest values provided by the smallest dose of compost of organic waste and poultry dropping and unenriched plots (0 ton/ha) on the biomass and number of leaves growth, would be related on the low nitrogen content of the compost used, which could induce the competition between the seedlings due to the availability of the nitrutifs elements. Then, under low-nitrogen conditions, biomass allocated to leaves may decrease (Poorter and Nagel, 2000; Proe *et al.*, 2002; Hegazy *et al.*, 2008). Under low-fertilizer conditions, the increase in specific leaf area (specific leaf area, the ratio of leaf area to leaf dry mass) may compensate for a decrease in biomass allocation to leaves (Maroof Tahsin, 2010). Optimum level of fertilizer Sodkowski and Rekowska (2003) ensured maximum vegetative growth. These results contradicted the ones of Larwanou *et al.* (2014), which revealed that fertilizers do not have any significant difference on the growth parameters especially for biomass at early stage of baobab development. Also, Chia *et al.* (2008) reported that soil type can affect seedling performance of baobab. The average leaves biomass production was 41.62 ± 1.16 kg of dry matter per 100 m² per month (equivalent to 157.29 ± 4.38 kg of fresh leaves per 100 m² per month). This yield is weak compared to 300 kg per 100 m² per month obtained by Korbo *et al.*

(2013) resulting by mineral fertilizers. This variation observed explains the immediate disponibility of the nutritive elements of mineral fertilizers after spreading, contrary to the slow effect of organic fertilizers which must be mineralized before relieving the nutritive elements (Aliyu, 2000; Kaya *et al.*, 2001).

Effect of density of sowing

Results revealed that, the maximum yield dry weight, the greatest number of leaves, a less tapering leaf and the lowest specific leaf area and specific dry weight were obtained with the closer spacing from 15 × 15 cm density of sowing. The least number of leaves, a more tapering leaf, the highest specific leaf area and specific dry weight and the highest yield dry weight were obtained with 30 × 30 cm density of sowing. Plant spacing plays an important role to optimise yield (Badi *et al.*, 2004; Maboko and Du Plooy, 2009; Žnidarčič *et al.*, 2011). Too high or too low density of sowing (Maboko, 2013) can result in lower growth and biomass of leaves. It was revealed that with closer density of sowing leaf growth and biomass showed increasing trend. In case of larger spacing, plant compete for light being leaf growth and biomass decrease. According to Benomar *et al.* (2011), specific leaf area was significantly and negatively associated with spacing between trees. Sodkowski and Rekowska (2003) reported largest leaf from closer spacing. It was revealed that with the increases of spacing leaf breadth showed increasing trend. In case of closer spacing plant compete for light and with the time being leaf breadth decreases. Then, enough space for vertical and horizontal expansion in the optimum spacing that leads for production of maximum number of leaves per plant than the closer spacing (Steingrobe and Schenk, 1994). That idea joined Sharma *et al.* (2001) which reported that with the optimum spacing, yield dry weight of plant showed increasing trend because of less competition for nutrients among the plants during growth stages.

Effect of harvesting frequency

Results showed that, a higher frequency of leaves harvest (every 30 days or monthly harvests next to sowing) presented the highest yield dry weight. It wasn't significant effect of frequency of leaves harvest on number of leaves, shape of leaf, specific leaf area and specific dry weight of leaf. Harvest frequencies of the leaves strongly influence the yield. Generally the greater the interval between harvest, the greater the yield of each crops is high (Rahajanirina *et al.*, 2016). This result follows on logically from numerous studies of other plant models such as *Gliricidia sepium*, *Leucaena leucocephala* (Latt *et al.*, 2000). These results joined the ones of Maboko and Du Plooy (2013) which revealed that, harvesting matured leaves of *Beta vulgaris* encourages regrowth of new leaves and increases yield. Same frequency of leaves harvest was reported by B.A. Bationo (2003), Bonneville *et al.* (2007), B. A. Bationo *et al.* (2009) and

Korbo *et al.* (2013) on *A. digitata* in Mali, Niger and Burkina, and Assogba-Komlan *et al.* (2015) on *Lactuca sativa* in Benin.

6.5. Conclusion

The results showed that more biomass of leaves growth were obtained when *A. digitata* seedlings were produced applying 30 t/ha of poultry dropping and low dose of poultry dropping (10-20 ton/ha) for greatest number of leaves growth, with 15 × 15 cm sowing density and monthly harvests next to sowing. This combination provided the optimum growth of the number of leaves and biomass of *A. digitata* seedlings. The average leaves biomass production was 41.62 ± 1.16 kg of dry matter per 100 m² (equivalent to 157.29 ± 4.38 kg of fresh leaves per 100 m²). Further research is necessary to assess on the protection methods against the bio-attackers and control of adventitious plants.

7.

Growth and biomass responses of roots of baobab seedlings to variation of dose of organic manure, sowing density and leaves harvesting frequency

(In preparation for submission to *Scientia Horticulturae*)

7. Growth and biomass responses of roots of baobab seedlings to variation of dose of organic manure, sowing density and leaves harvesting frequency

Abstract

Baobab organs contribute highly to food security especially in sub-Saharan Africa. The root of *Adansonia digitata* L. seedlings is reputed to be edible when roasted for use as a coffee substitute. As root growth influences the provisioning of nutritive elements of a plant, and consequently, its growth and yield, it is correlated positively with the yield. This study aimed to assess the growth and biomass responses of roots of baobab seedlings to variation of dose (0, 10, 20, and 30 tons/ha) of organic manure (compost vs poultry droppings), sowing density (15 × 15 cm, 20 × 20 cm, and 30 × 30 cm) and leaves harvesting frequency (15, 22 and 30 days). The experiment was conducted on-station (Sekou) in the humid region of Benin using a split-split plot design with three replicates. Seedlings were uprooted at 240 days after sowing and morphological and biomass data were collected on taproots and rootlets. Data were analysed using linear and Poisson generalized linear models. Results showed that more biomass and greatest root growth were obtained from baobab seedlings when applying 30 ton/ha of poultry dropping with 20 × 20 cm sowing density and 30 days leaves harvesting frequency intervals. This combination provided the optimum growth and biomass of roots of *A. digitata* seedlings. Further research is necessary to assess chemicals components for its probable use in food and medicine.

Key words: *Adansonia digitata*, root biomass, baobab seedling, taproot, rootlet

7.1. Introduction

In many African countries, especially in West Africa, rural people rely on the variety of nutritional and medicinal products provided by Baobab (*Adansonia digitata* L.) tree (De Caluwé *et al.*, 2009 ; Buchmann *et al.*, 2010). Almost all organs of African baobab is used: roots, bark, wood, leaves, flowers, capsules, gum, seeds, and fruits (Assogbadjo, 2006; Wickens and Lowe, 2008; Buchmann *et al.*, 2010). Among the 300 uses that have been identified, the tree provides nutritious food, livestock fodder, fibre, medicine, and income to local people (De Caluwé *et al.*, 2009; Buchmann *et al.*, 2010). Baobab plays also a key role in traditional medicine and religious beliefs, which often consider the tree as sacred (Jensen *et al.*, 2011). The sprout of seedlings can be eaten like asparagus. The root of very young trees is reputed to be edible which can be roasted for use as a coffee substitute (Bosch *et al.*, 2004).

In our days, the species is facing a high risk of extinction in the wild where it is threatened by bush fire (seedlings), grazing and lack of natural regeneration (Assogbadjo *et al.*, 2010). Thus leading to test of several pre-treatments methods (Savard, 2003; Assogbadjo, 2006; Olivier *et al.*, 2006; Savard *et al.*, 2006; SCUC, 2006; Kalinganire *et al.*, 2007; Assogbadjo *et al.*, 2010; Falemara *et al.*, 2014; Niang *et al.*, 2015). However, differences in the regeneration performance can be attributed to differences in drought tolerance as drought had been cited among factors limiting growth, development and productivity of plants (Blum, 1997).

Root growth influences the provisioning of nutritive elements of a plant, and consequently, its growth and yield (Blomme *et al.*, 2003). It follows that the growth of the roots is correlated positively with the yield (Blomme *et al.*, 2003). Crown architecture plays an important role in short-rotation forestry productivity (Ceulemans *et al.*, 1990; Wu and Stettler, 1994). For example, a strong relationship was found between crown and stem diameter for several species (Foli *et al.*, 2003; Hemery *et al.*, 2005). This relationship may offer substantial information that is required to optimize spacing between plants (Hall, 1994; Hemery *et al.*, 2005). Crown architecture determines the spatial distribution of foliage and, consequently, solar radiation interception efficiency (Mitchell *et al.*, 1992), which may explain the variation in growth responses (Cannell, 1980; Nelson *et al.*, 1981; Hall, 1994; Harper, 2008). When spacing between trees is reduced, biomass that is allocated to branches and foliage usually decreases to the benefit of the stem (Fang *et al.*, 1999; Pinkard and Neilsen, 2003). Plasticity of rooting patterns in response to competition likely plays a role in nutrient uptake when resources are limited. For example, maximum rooting depth is expected to increase under high competitive stress, to enhance access to soil nitrogen (Casper and Jackson, 1997). Investigations of the effect of cutting frequency on biomass production have been undertaken and differences in production

due to various cutting regimes have been found with many species such as *Leucaena leucocephala* and *Gliricidia sepium* (Guevarra *et al.*, 1978; Latt *et al.*, 2000). Baobab tree can withstand drought, it has no important pest or diseases and it does not require extra watering (Sidibé and Williams, 2002; SCUC, 2006). Declining soil fertility remains the fundamental reason for declining smallholder food production in sub-Saharan Africa (Smaling *et al.*, 1997; Gruhn *et al.*, 2000). Organic manures from domestic breeding or compost from domestic organic waste are hypothesized to provide sustainable manure for sustainable production in smallholder farming systems (Assogba-Komlan, 2001). However, animal rearing is poultry-based in Benin humid zone. The overall objective of this research is to assess effect of dose of organic manure (compost versus animal excrements), sowing density and frequency of leaves harvesting on the growth and biomass of roots of *A. digitata* seedlings. Specifically, the study aims at assessing: i) the growth and morphological traits of tap roots and rootlets, and ii) the dry weight of roots biomass of baobab seedlings in relationships to variation of dose of organic manure, sowing density and leaves harvesting frequency in Guineo-Congolese zone in Benin. In this study the following key question was posed: What is the combination of the various factors (dose of organic manure, sowing density and leaves harvesting frequency) which gives the optimal growth and morphological traits of tap roots and rootlets and the dry weight of roots biomass of baobab seedlings?

7.2. Material and methods

7.2.1. Study areas

This study was conducted in the humid zone (Guineo-Congolese) in south Benin. Indeed, Benin is a West-African country of 114 763 km² located between 6°25'N-12°30' and 0°45'E - 4°E. The resident population is about 10 880 000 inhabitants in 2015 (UN, 2015) and slightly female-biased (50.12%). The development index of UNDP was 0.485 placing it at 167th position from 188 countries in 2015 (UNDP, 2016). The gross product per habitant was 745.47 Dollars US in 2013 (INSAE, 2015).

7.2.2. Sampling and data collection

Data were collected on the roots of seedlings of *A. digitata* (Figure 7.1) established with the seeds collected in the same bio-geographical zone (Guineo-Congolese zone). The experiment was conducted in a factorial trial using a split-split plot design with two replications. The site was first divided into two main plots depending on the type of organic manure (one for compost and one for poultry dropping). Each main plot was then divided into 4 subplots corresponding to the four doses of each type of organic manure (0, 10, 20 and 30 tons/ha for compost and poultry dropping). The 9 (3 × 3) combinations of densities (15 × 15 cm, 20 × 20 cm and 30 × 30 cm) and harvest frequencies (every 15, 22 and 30 days; starting 30 days after planting) were randomized per dose. In order to increase the rate of germination, seeds were pre-treated using a container of boiling water for 48 h (IER/ICRAF, 2001; Savard, 2003; Olivier *et al.*, 2006; Savard *et al.*, 2006; Kalinganire *et al.*, 2007). Four seeds were sown to approximately 2 cm of depth per seed hole with 7 plants per row and 4 rows of planting per experimental unit (1.1 × 2 m of size). Next to sprouting, the seedlings were thinned out and only the most vigorous seedling was kept per seed hole. Watering frequency was 10 litres early in the morning per plot per day and weeding was done if need be. Factors tested were summarized in Table 5.1.

30 days after the last general leaves harvesting at 240th day, 10 seedlings (among the 20 seedlings) were randomly selected (for a total of 720 seedlings) per treatment. Each selected seedling was completely excavated. Cutting was done just at the beginning of the tap root, which permitted to isolate the aerial part of the root. Total length and basal diameter of the tap root, the number of rootlets and their total length and basal diameter were recorded. The shape of each tap root was obtained through calculation of the shape index which is the ratio of the total length to the basal diameter (Sanchez *et al.*, 2011). The higher the value of this index, the more the tap root is tapering. Total fresh weight of the tap and whole roots were also measured.

This was then put in envelopes for drying in oven at 105°C during 48h until constant weight to obtain their dry weight.



Figure 7.1 Root of baobab seedlings

7.2.3. Data analysis

To assess the influence of dose of organic manure, sowing density and leaves harvesting frequency on the length and basal diameter of tap roots and rootlets linear models were used. Generalized linear model was used with a Poisson structure for errors to assess the influence of dose of organic manure, sowing density and leaves harvesting frequency on the number of rootlets using the package “MASS” (Venables and Ripley, 2002). Anova was used on each model to test overall significance of factors. Model selection was based on AIC (Akaike Information Criterion ; the low values are preferred) using the package “bbmle” (Bolker and R Core Team, 2017). Least square means of each variable were extracted and multiple comparisons among them were performed using the package “lsmeans” (Lenth, 2016) and used to draw figures using the package “ggplot2” (Wickham, 2009) showing the evolution trend of each of them according to the factors considered. All statistical analyses were carried out in R 3.4.0 (R Core Team, 2017). All factors (organic matter, doses of organic matter, sowing density and frequency of leaves harvest) were considered fixed. Only the significant ($p < 0.05$) factors and interactions were carried along this study next to model simplification.

7.3. Results

7.3.1. Growth and morphological traits of tap roots of baobab seedlings in relationships to variation of dose of organic manure, sowing density and leaves harvesting frequency

There was a significant effect (main factor and their interaction) of type of organic matter and harvesting frequency on the length of tap root indicating that differences of length of tap root between matter depends on the harvesting frequency. A significant difference exists also among doses of organic matter ($P < 0.05$; Table 7.1). However, effect of sowing density was not significant. In particular, compost of organic waste with 30 days frequency of leaves harvest presented the highest length of tap root (15.19 ± 0.55 cm) whereas the lowest was obtained for poultry dropping and 15 days frequency of leaves harvest (8.39 ± 0.61 cm; Figure 7.2a). The highest dose of organic matter (30 ton/ha) showed the highest length of tap root (13.38 ± 0.44 cm) and intermediate dose of organic matter (20 ton/ha) the lowest (11.49 ± 0.47 cm; Figure 7.2c). High dose of organic matter (30 ton/ha) and higher frequency of harvesting (30 days) presented the longest length of tap root. And, small dose of organic matter (0-20 ton/ha) and 15 days frequency of leaves harvest presented the shortest length of tap root.

A significant effect of type of organic matter, doses of organic matter, sowing density and harvesting frequency on the basal diameter of tap root was noted. In addition, the interactions between Matter $\times$ Dose, Dose $\times$ Density, and Dose $\times$ Frequency were significant, showing that differences among doses were not similar across matter, densities and harvesting frequencies and vice-versa ($P < 0.05$; Table 7.1). Accordingly, 30 ton/ha of poultry dropping showed the highest basal diameter of tap root (11.22 ± 0.26 mm) and the lowest with 10 ton/ha of compost of organic waste (8.17 ± 0.27 mm; Figure 7.2e). In addition, 20×20 cm sowing density and 30 ton/ha of organic matter showed the highest basal diameter of tap root (11.62 ± 0.31 mm) and the lowest (8.40 ± 0.32 mm) with 15×15 cm sowing density and unenriched plots (0 ton/ha; Figure 7.2f). Likewise, 20 ton/ha of organic matter with 30 days frequency of leaves harvest presented the highest basal diameter of tap root (12.47 ± 0.32 mm) and the lowest (7.55 ± 0.34 mm) with unenriched plots (0 ton/ha) and 15 days frequency of leaves harvest (Figure 7.2g). This indicates that a higher dose of poultry dropping (30 ton/ha), 20×20 cm sowing density and higher frequency of harvesting (30 days) presented the largest basal diameter of tap root. And, 10 ton/ha of compost of organic waste or even unenriched plots (0 ton/ha), 15×15 cm sowing density and 15 days frequency of leaves harvest presented the shortest basal diameter of tap root.

Shape of tap root was affected by type of organic matter, doses of organic matter and the interaction of matter and harvesting frequency ($P < 0.05$; Table 7.1). Application of compost of organic waste with 15 days frequency of leaves harvest showed a more tapering tap root (1.89 ± 0.10) and the less tapering by using poultry dropping with also 15 and 30 frequency of leaves harvest respectively (1.05 ± 0.11) and (1.19 ± 0.09 ; Figure 7.2b). Tap root on unenriched plots (0 ton/ha) was more tapering (1.63 ± 0.08) than enriched plots, 20 ton/ha of organic matter showed a less tapering tap root (1.21 ± 0.08) followed by 30 ton/ha of organic matter (1.39 ± 0.08 ; Figure 7.2d). This indicates that roughly high dose of poultry dropping (20-30 ton/ha) apart from harvesting frequency presented a large shape of tap root.

Table 7.1 Effect of significant factors and interactions on the morphological traits of the tap roots

Source	df	Length of tap root		Basal diameter of tap root		Shape of tap root	
		<i>F-value</i>	<i>P-value</i>	<i>F-value</i>	<i>P-value</i>	<i>F-value</i>	<i>P-value</i>
Matter	1	15.84	<0.001	8.66	0.003	19.74	<0.001
Dose	3	2.74	0.042	24.07	<0.001	4.29	0.005
Density	2	-	-	5.58	0.003	-	-
Frequency	2	18.68	<0.001	106.66	<0.001	-	-
Matter: Dose	3	-	-	13.29	<0.001	-	-
Matter: Frequency	2	10.66	<0.001	-	-	4.30	0.001
Dose: Density	6	-	-	4.00	0.000	-	-
Dose: Frequency	6	-	-	3.95	0.000	-	-

df=degree of freedom, F-value=Fisher statistic, P-value Probability of significance. Only significant effects are presented in the table.

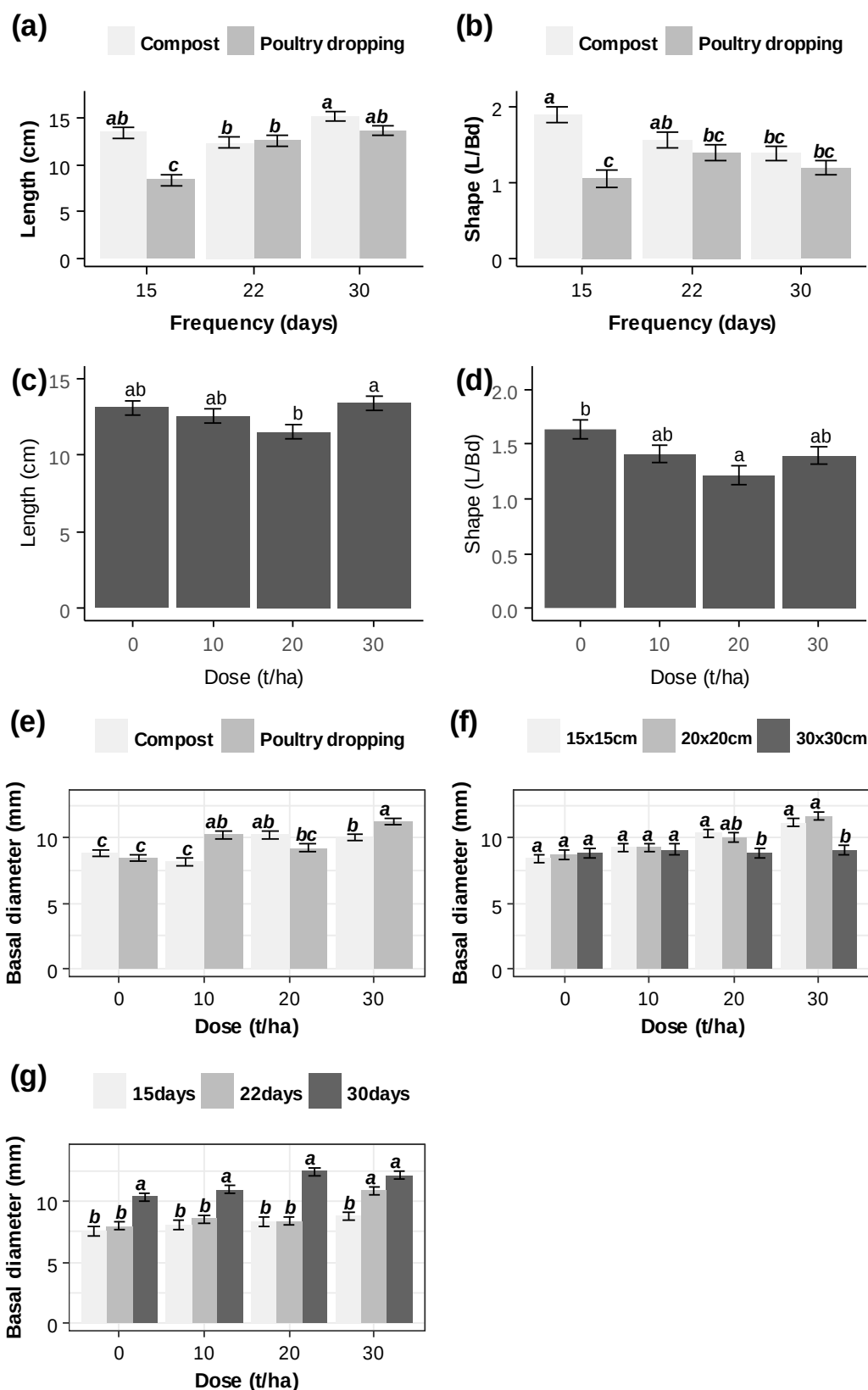


Figure 7.2 Evolution trend of the tap roots growth of the length and shape according to organic matter type and frequency of leaves harvest, and dose respectively (a,b) and (c,d) and basal diameter according to organic matter type and their dose (e), the dose and density of sowing (f) and the dose and frequency of leaves harvest (g). Bd=Basal diameter, L=Length

7.3.2. Growth and morphological traits of rootlets of baobab seedlings in relationships to variation of dose of organic manure, sowing density and leaves harvesting frequency

There was a significant effect of doses of organic matter, sowing density and harvesting frequency on the number of rootlet. In addition, the interactions between Matter $\times$ Dose, Matter $\times$ Density, Matter $\times$ Frequency, Dose $\times$ Density, and Density $\times$ Frequency were significant, showing that differences among type of organic matter were not similar across doses, densities and harvesting frequencies and vice-versa ($P < 0.05$; Table 7.2). Accordingly, 30 ton/ha of poultry dropping with 20×20 cm sowing density showed the greatest number of rootlet (6.65 ± 0.20) and the least for compost of organic waste with 30×30 cm sowing density (3.21 ± 0.21 ; Figure 7.3a). 30 days frequency of leaves harvest with 20 ton/ha of compost of organic waste presented the greatest number of rootlet (7.73 ± 0.20) and 15 days frequency of leaves harvest the least (3.09 ± 0.15) by using 10 ton/ha of compost of organic waste (Figure 7.3b). Figure 7.4a showed the greatest number of rootlet (6.08 ± 0.15) for 20 ton/ha of organic matter and unenriched plots (0 ton/ha) the least (3.84 ± 0.19) for 30×30 cm sowing density. Likewise, 20×20 cm sowing density with 30 days frequency of leaves harvest presented the greatest number of rootlet (6.47 ± 0.13) and 30×30 cm sowing density with 15 days frequency of leaves harvest presented the least (2.99 ± 0.20 ; Figure 7.4b). And, 30 ton/ha of poultry dropping showed the greatest number of rootlet (5.57 ± 0.13) and its 20 ton/ha the least (2.99 ± 0.20 ; Figure 7.4c). This indicates that a higher dose of poultry dropping (30 ton/ha), average dose of compost of organic waste (20 t/ha), with 20×20 cm sowing density and higher frequency of harvesting (30 days) presented the greatest number of rootlet while least number with unenriched plots (0 ton/ha), 10 ton/ha of compost of organic waste, 30×30 cm sowing density and 15 days frequency of leaves harvest.

A significant effect (main factor and their interaction) of type of organic matter and harvesting frequency on the length of rootlet ($P < 0.05$) indicating that differences of length of rootlet between matter depends on the harvesting frequency. In addition, the interactions between Matter $\times$ Dose, Matter $\times$ Frequency and Dose $\times$ Frequency were significant, showing that differences among type of organic matter were not similar across doses and harvesting frequencies and vice-versa ($P < 0.05$; Table 7.2). In particular, 30 ton/ha of poultry dropping showed the highest length of rootlet (4.11 ± 0.15 cm) and 10 ton/ha of poultry dropping the lowest value (3.09 ± 0.19 cm; Figure 7.5a). In addition, using a compost of organic waste with 22 days frequency of leaves harvest presented the highest length of rootlet (5.30 ± 0.11 cm) and the lowest value (2.89 ± 0.18 cm) by using a compost of organic waste with 30 days frequency of leaves harvest (Figure 7.5b). 30 days frequency of leaves harvest with 10 ton/ha

of compost of organic waste presented the highest length of rootlet (5.64 ± 0.22 cm) and 15 days frequency of leaves harvest with 10 ton/ha of poultry dropping the lowest (2.33 ± 0.33 cm; Figure 7.5c). Rootlet length provided by high dose of poultry dropping (30 ton/ha) or with 30 days frequency of leaves harvest are longer and the smaller length of rootlet by 10 ton/ha poultry dropping with 15 days frequency of leaves harvest.

Basal diameter of rootlet was affected by type of organic matter, doses of organic matter and harvesting frequency. In addition, the interactions between Matter $\times$ Dose, Dose $\times$ Density and Dose $\times$ Frequency were significant, showing that differences among type of organic matter were not similar across doses and harvesting frequencies and vice-versa ($P < 0.05$; Table 7.2). Application of 30 ton/ha of poultry dropping showed the highest basal diameter of rootlet (0.94 ± 0.03 mm) and unenriched plots (0 ton/ha) the lowest (0.55 ± 0.03 mm; Figure 7.6a). Figure 7.6b showed the highest basal diameter of rootlet (0.87 ± 0.04 mm) with 30×30 cm sowing density and 30 ton/ha of organic matter, and the lowest (0.54 ± 0.04 mm) for 15×15 cm sowing density and unenriched plots (0 ton/ha). In addition, 30 days frequency of leaves harvest with 20 and 30 ton/ha of organic matter presented the highest length of rootlet respectively (0.90 ± 0.03 and 0.86 ± 0.03 mm) and 15 days frequency of leaves harvest with 10 ton/ha of organic matter the lowest (0.50 ± 0.05 mm; Figure 7.6c). This indicates that 30 ton/ha of poultry dropping with 30×30 cm sowing density and 30 days frequency of leaves harvest presented the largest basal diameter of rootlet, while smaller with unenriched plots or lower organic matter (0 - 10 ton/ha) with 15×15 cm sowing density and also 15 days frequency of leaves harvest.

Table 7.2 Variability in the morphological traits of the rootlets

Source	df	Number of rootlet		Length of rootlet		Basal diameter of rootlet	
		F-value	P-value	F-value	P-value	F-value	P-value
Matter	1	-	-	26.22	<0.001	9.81	0.001
Dose	3	13.29	<0.001	-	-	12.47	<0.001
Density	2	22.31	<0.001	-	-	-	-
Frequency	2	141.50	<0.001	85.50	<0.001	9.34	<0.001
Matter: Dose	3	28.50	<0.001	4.98	<0.001	10.51	<0.001
Matter: Density	2	5.59	0.003	-	-	-	-
Matter: Frequency	2	56.80	<0.001	4.87	0.007	-	-
Dose: Density	6	13.58	<0.001	-	-	2.20	0.024
Dose: Frequency	6	-	-	3.24	0.003	2.99	0.006
Density: Frequency	4	6.14	<0.001	-	-	-	-

df=degree of freedom, F-value=Fisher statistic, P-value Probability of significance. Only significant effects are presented in the table.

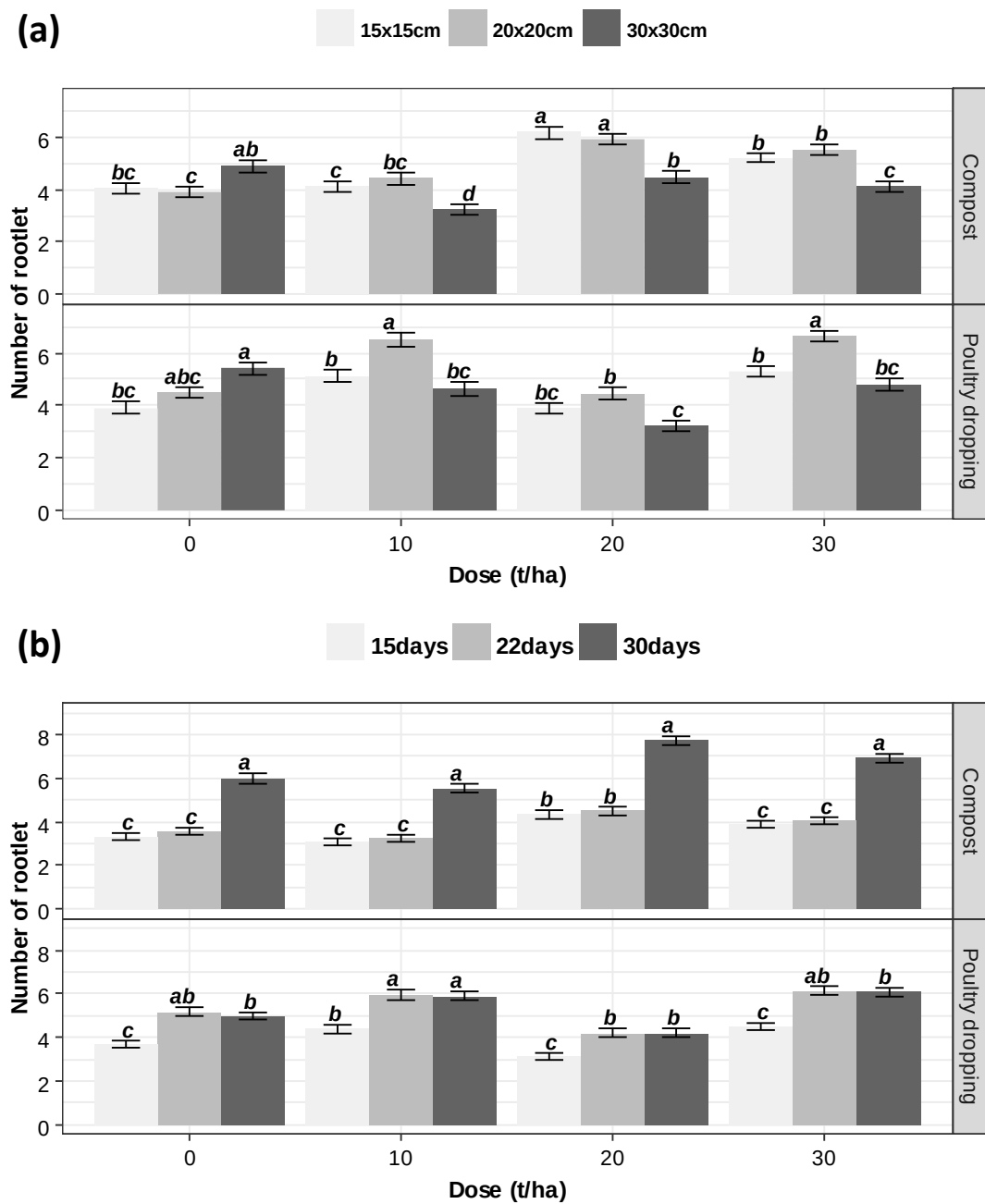


Figure 7.3 Evolution trend of rootlets number according to the organic matter, their dose and density of sowing (a) the organic matter, the dose and frequency of leaves harvest (b) with contrast within dose

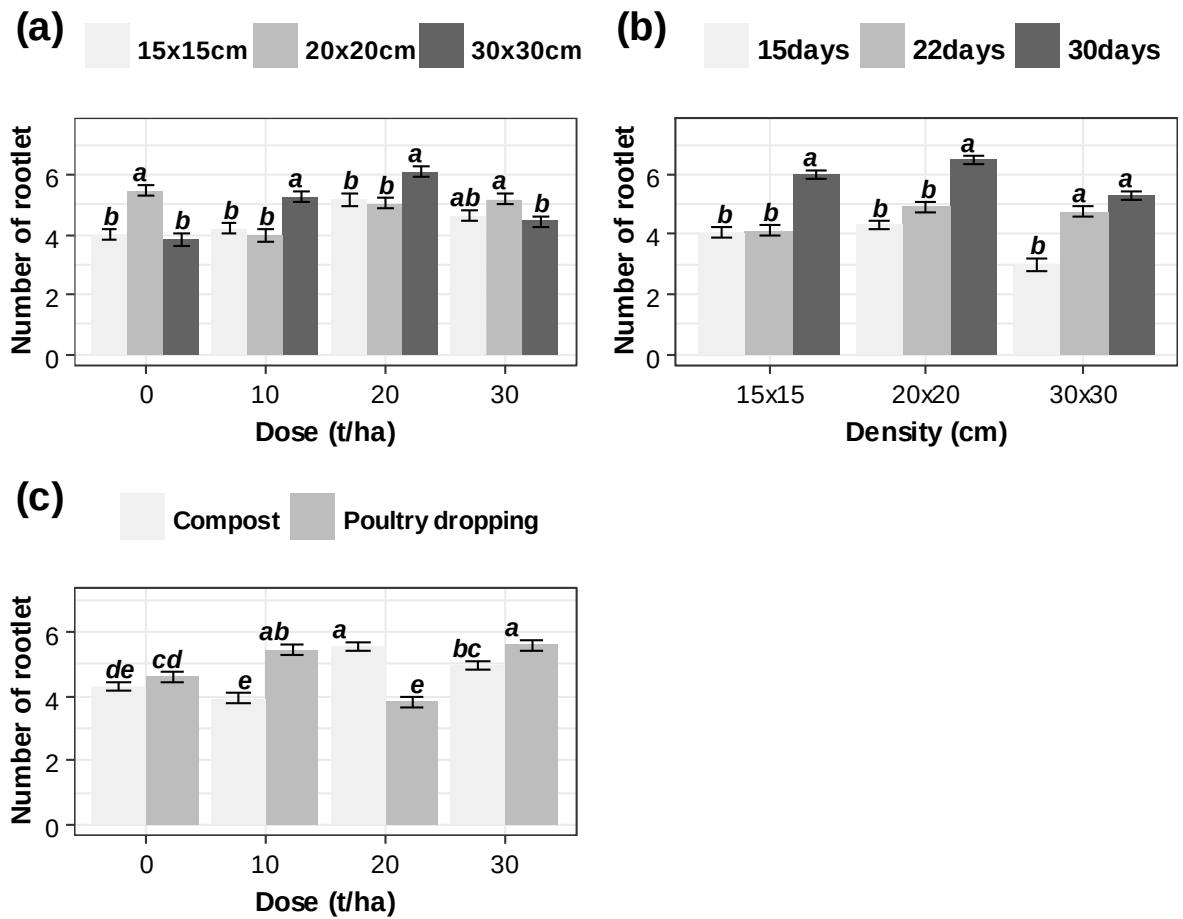


Figure 7.4 Evolution trend of rootlets number according to the dose of organic matter and density of sowing (a), the density of sowing and frequency of leaves harvest (b), and the organic matter type and their dose (c)

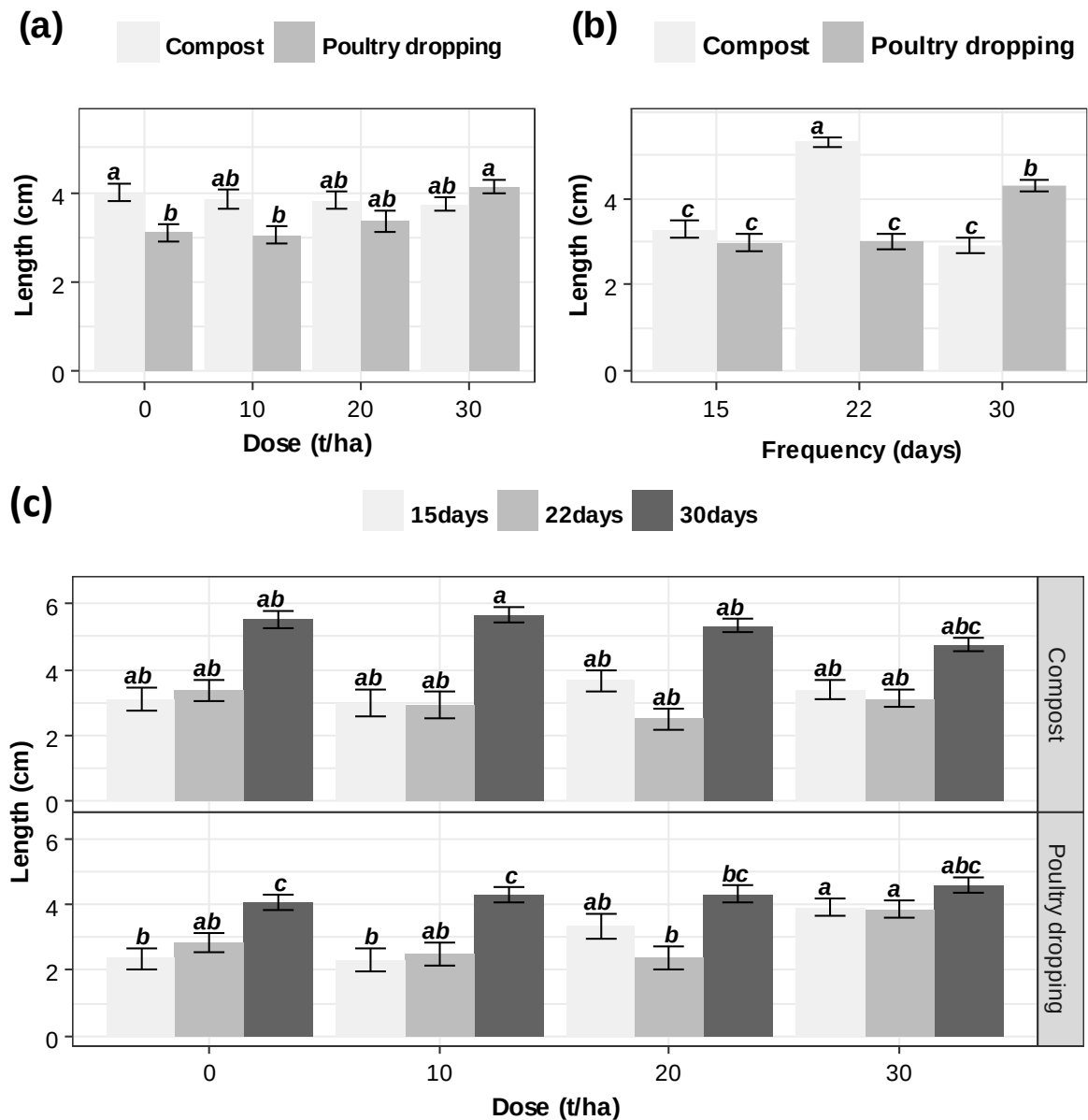


Figure 7.5 Evolution trend of the length of the rootlets according to the organic matter type and their dose (a) the organic matter type and frequency of leaves harvest (b), and the organic matter type, the dose and frequency of leaves harvest (c) with contrast within the frequency of leaves harvest

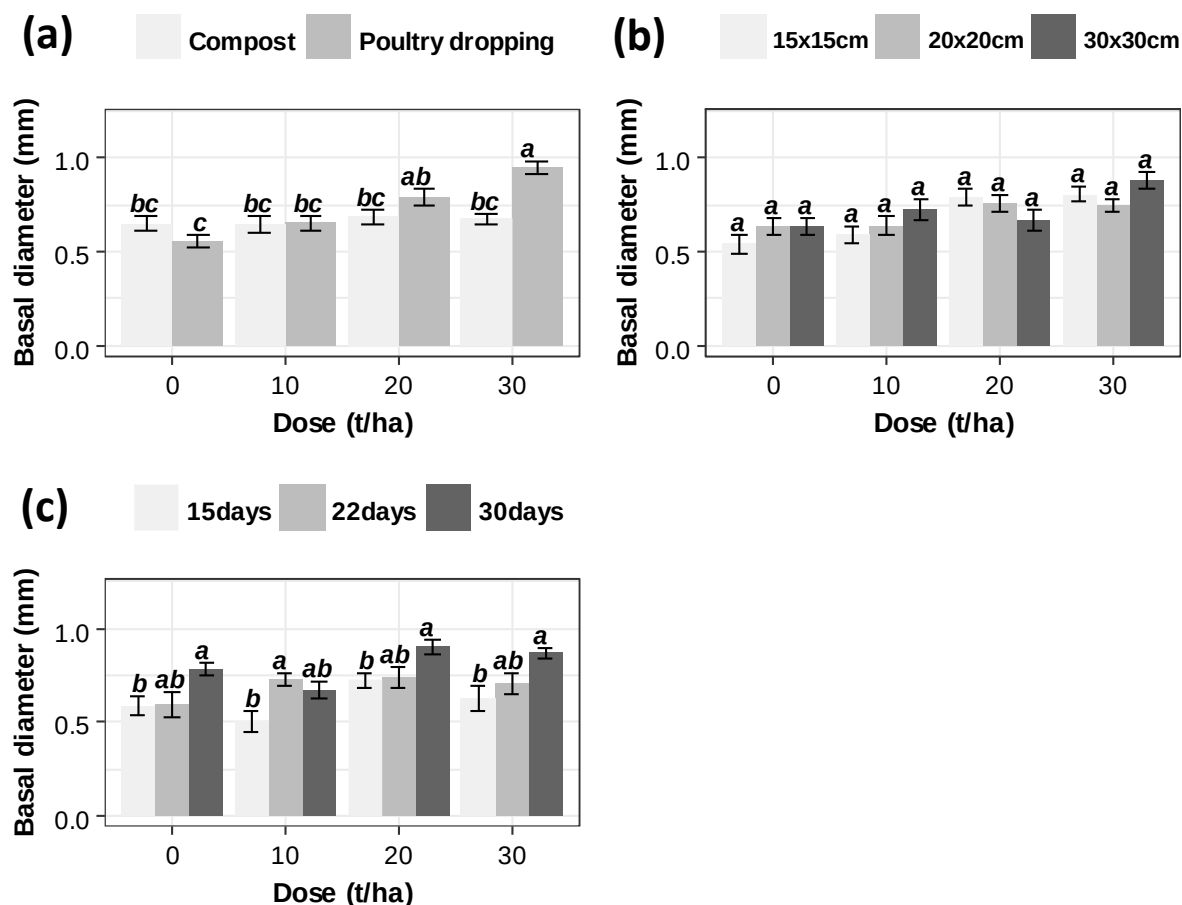


Figure 7.6 Evolution trend of the basal diameter of the rootlets according to the organic matter type and their dose (a), the dose and density of sowing (b), and the dose and frequency of leaves harvest (c)

7.3.3. Dry weight of roots biomass of baobab seedlings in relationships to variation of dose of organic manure, sowing density and leaves harvesting frequency

There was significant effect of type of organic matter, doses and harvesting frequency on the tap and whole root dry weight ($P < 0.05$). In addition, the interactions between Matter $\times$ Dose, Matter $\times$ Density, Dose $\times$ Density, and Dose $\times$ Frequency were significant, showing that differences among type of organic matter were not similar across doses, densities and harvesting frequencies and vice-versa ($P < 0.05$; Table 7.3).

In particular, 30 ton/ha of poultry dropping showed the highest dry weight (1.82 ± 0.11 and 1.86 ± 0.11 g per plant) respectively for the tap and whole root, and 10 ton/ha of compost of organic waste the lowest values respectively 0.80 ± 0.11 and 0.81 ± 0.11 g per plant (Figure 7.7; 7.8a). Figure 7.7, 7.8b showed the highest dry weight (1.37 ± 0.09 and 1.39 ± 0.09 g per plant) respectively for the tap and whole root for poultry dropping with 20×20 cm sowing density, and the lowest values respectively 0.92 ± 0.10 and 0.94 ± 0.10 g per plant for compost of organic waste with 30×30 cm sowing density. In addition, 20×20 cm sowing density and 30 ton/ha

of organic matter showed the highest dry weight (1.85 ± 0.12 and 1.88 ± 0.13 g per plant) and unenriched plots (0 ton/ha) with 15×15 cm sowing density the lowest values respectively 0.71 ± 0.13 and 0.72 ± 0.13 g per plant (Figure 7.7; 7.8c). For the tap root dry weight 30 ton/ha of organic matter and 22 and 30 days frequencies of leaves harvest presented the highest values respectively 1.99 ± 0.12 g per plant and 1.96 ± 0.12 g per plant, and the lowest value for unenriched plots (0 ton/ha) and 15 days frequency of leaves harvest (Figure 7.7d). And for the whole root dry weight 30 ton/ha of organic matter and 30 days frequency of leaves harvest presented the highest value (2.03 ± 0.13 g per plant), and the lowest value by unenriched plots (0 ton/ha) and 15 days frequency of leaves harvest (Figure 7.8d).

This indicates that 30 ton/ha of poultry dropping with 20×20 cm sowing density and three and 30 days frequency of leaves harvest presented the heaviest dry weight of tap and whole root. While lightest were observed with unenriched plots (without organic manure addition), with 15×15 cm or 30×30 cm sowing density and 15 days frequency of leaves harvest.

Table 7.3 Variability in tap and whole root dry weight

Source	Df	Dry weight of tap root		Dry weight of whole root	
		F-value	P-value	F-value	P-value
Matter	1	11.18	<0.001	10.46	0.001
Dose	3	16.00	<0.001	16.31	<0.001
Frequency	2	77.75	<0.001	80.82	<0.001
Matter: Dose	3	3.66	0.012	3.89	0.008
Matter: Density	2	3.02	0.049	3.03	0.049
Dose: Density	6	2.77	0.011	2.65	0.015
Dose: Frequency	6	5.75	<0.001	5.46	<0.001

df=degree of freedom, F-value=Fisher statistic, P-value Probability of significance. Only significant effects are presented in the table.

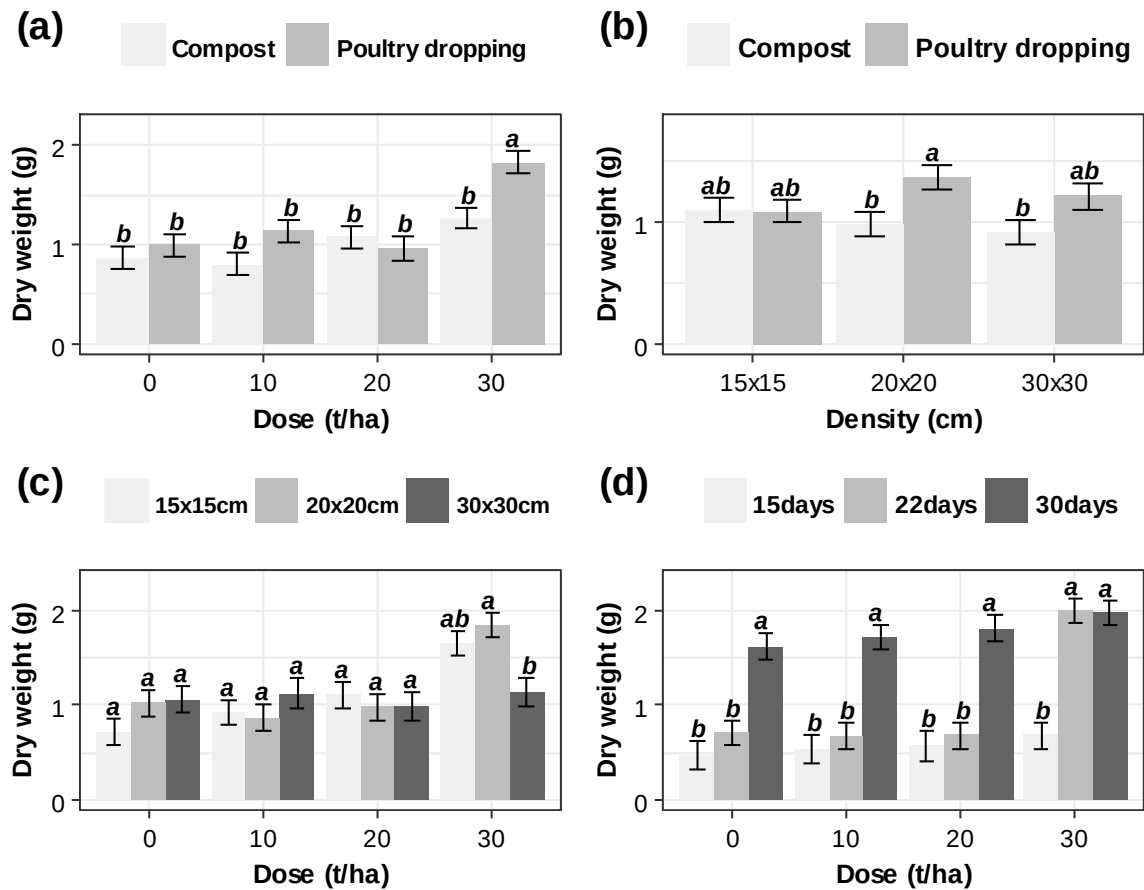


Figure 7.7 Evolution trend of the dry weight of the tap roots according to the organic matter type and their dose (a), the organic matter type and density of sowing (b), the dose and density of sowing (c) and the dose and frequency of leaves harvest (d)

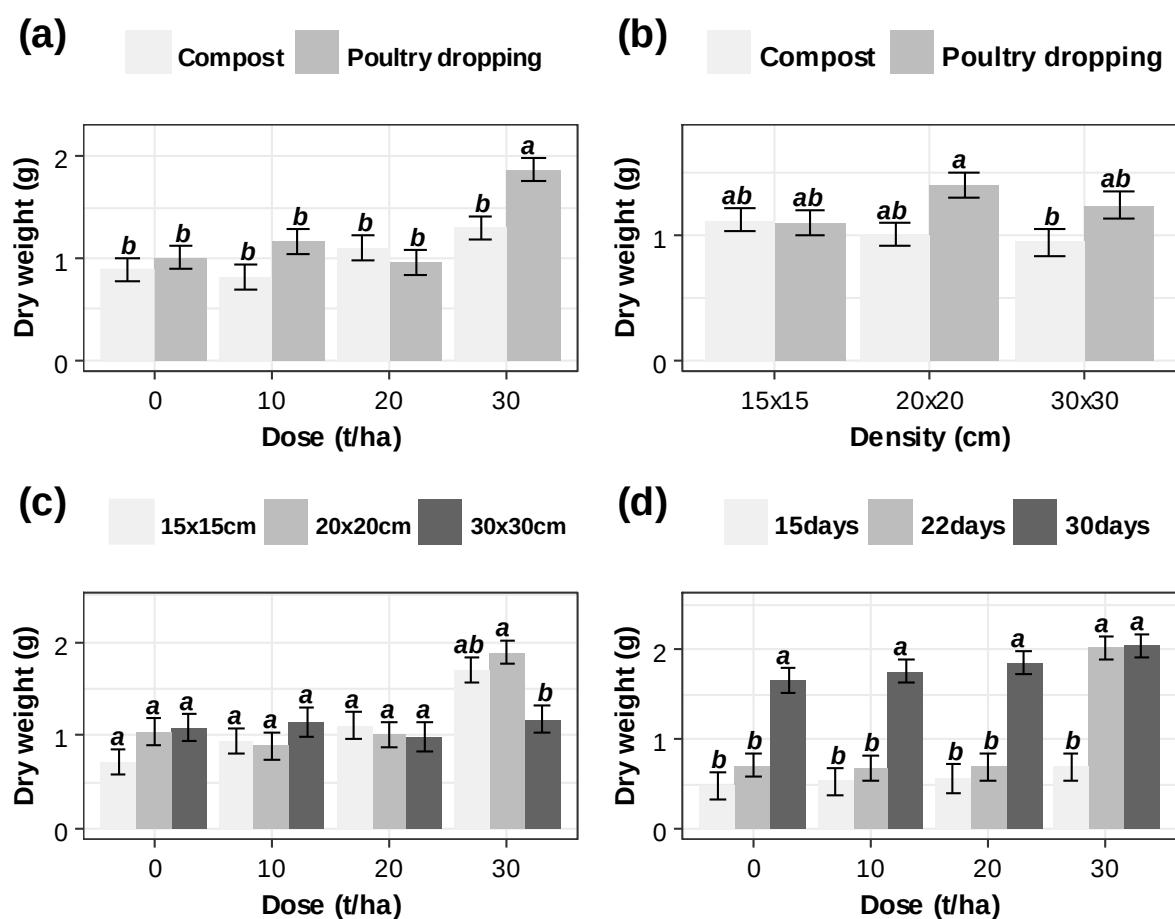


Figure 7.8 Evolution trend of the dry weight of the whole roots according to the organic matter type and their dose (a), the organic matter type and density of sowing (b), the dose and density of sowing (c) and the dose and frequency of leaves harvest (d)

7.4. Discussion

This study investigated effect of different doses of two sources of organic manures (compost of organic waste and poultry dropping), density of sowing and leaves harvesting frequencies on growth and morphological traits of tap roots and rootlets and their biomass dry weight of *A. digitata* seedlings in humid zone of Benin.

Effect of fertilizer

Results showed that a higher dose of poultry dropping (30 ton/ha) presented the longest tap root and rootlet, the largest basal diameter of tap root and rootlet, shape of tap root, the greatest number of rootlet and the heaviest dry weight of tap and whole root. While 10 ton/ha of compost of organic waste and poultry dropping or even unenriched plots (0 ton/ha) presented the smallest rootlet, the shortest basal diameter of tap root and rootlet, the least number and lightest dry weight of tap and whole root. Poultry dropping releases minerals gradually, which can ensure their availability the moment of the effective need by the plant (Le Villio *et al.*, 2001; Ouattara, 2009). Poultry dropping is a manure of quality, in particular for its strong content of nitrogen, element essential with the development of the plants (MRECD, 2002). This joined the idea according to which the organic matter, is the best basic fertilizer (Giller *et al.*, 2002). Indeed, MRECD (2002) specifies that the nitrogen is an essential factor of growth of the seedlings, especially on the level of the leaves and stems. These results about matter type and dose are in agreement with those obtained by Akparobi (2009) and Olaniyi (2008). Also, joined Biaou (2010); Tchabi *et al.* (2012), which worked on *Lactuca sativa* L and *Daucus carota*. Our results are in conformity with those of Ouattara (2009), which precise that in the systems of fertilization, the poultry dropping could be applied before sowing and into only once like fertilizer in a program aiming at the increase in the number of seeds of *Lagenaria siceraria*. It could be deducted that other the secondary minerals, in particular elements (sulphur, magnesium, calcium) and trace elements (copper, zinc, iron, etc), of which the presence in the animal manure, in particular poultry, was shown by Nys *et al.* (2003), would be essential for the good development and the production of *A. digitata* seedlings. However, these authors underline risks of phytotoxicity of these dejections beyond a certain threshold. The lowest values provided by the smallest dose of compost of organic waste and poultry dropping and unenriched plots (0 ton/ha) on the biomass and root growth, would be related on the low nitrogen content of the compost used, which could induce the competition between the seedlings due to the availability of the nitrutifs elements. These last observations would justify the slower growth which can go until the complete destruction of the root system (Villain, 2002). This strategy is undoubtedly more the result of an optimization for the transport of water

and minerals elements. The roots of structure have of course like function to carry the fine roots and to convey with the stems the nutritive elements and water (Danjon and Fourcaud, 2009).

Effect of density of sowing

Results showed that 20×20 cm sowing density presented the largest basal diameter of tap root, the greatest number of rootlet and the heaviest dry weight of tap and whole root. And, 30×30 cm sowing density presented the largest basal diameter of rootlet. While 15×15 cm sowing density presented the shortest basal diameter of tap root and rootlet and the lightest dry weight of tap and whole root. Least number of rootlet and, also the lightest dry weight of tap and whole root were provided for 30×30 cm sowing density. Plant spacing plays an important role to optimise yield (Badi *et al.*, 2004; Maboko and Du Plooy, 2009; Žnidarčič *et al.*, 2011). Blomme *et al.* (2003) reported that root growth influences the provisioning of nutritive elements of a plant, and consequently, its growth and yield. Too high or too low sowing density (Maboko, 2013) can result in lower growth and biomass of root. It's noticed that, more the seedlings are close (15×15 cm) from/to each other, less their roots developed and same result is observed when these seedlings are more distant (30×30 cm) from/to each other. This fall in growth and dry biomass is explained by the fact why the competition would be strong when they are too close and weak and even null when they are too distant from/to each other, which would be prejudicial for the seedlings development. These last observations would justify the slower growth which can go until the complete destruction of the root system (Villain, 2002). This strategy is undoubtedly more the result of an optimization for the transport of water and minerals elements. The fraction of biomass that is allocated to roots is predicted to increase under conditions of nitrogen limitation and to decrease under competition for light. Under intraspecific competition (plant density), both nitrogen and light presumably decrease. 20×20 cm sowing density presented more biomass and greatest root growth. These results explain on the other hand that, the air competition can have a strong effect on anchoring of the roots (Danjon and Fourcaud, 2009). Similar results were reported by Niederwieser (2001) on *Beta vulgaris*. However, plasticity of rooting patterns in response to competition likely plays a role in nutrient uptake when resources are limited. For example, maximum rooting depth is expected to increase under high competitive stress, to enhance access to soil nitrogen (Casper and Jackson, 1997). It should be stressed that, result showed no significant effect of sowing density on the length of tap root and rootlet and shape of tap root.

Effect of harvesting frequency

Results indicated that higher frequency of harvesting (30 days) presented the longest and the largest tap root and rootlet, the greatest number of rootlet and the heaviest dry weight of tap and

whole root. While 15 days frequency of leaves harvest presented the shortest tap root and rootlet, least number of rootlet and the lightest dry weight of tap and whole root. Harvest frequencies of the leaves strongly influence growth and biomass production of root. This, would be related on the regular pressures caused by the frequencies of harvest. Generally the greater the interval between harvest, the greater the yield of each crops is high (Rahajanirina *et al.*, 2016). This result follows on logically from numerous studies of other plant models such as *Gliricidia sepium*, *Leucaena leucocephala* (Latt *et al.*, 2000). Effect of harvesting frequency on biomass production have been undertaken and differences in production due to various cutting regimes have been found with many species such as *Leucaena leucocephala* and *Gliricidia sepium* (Guevarra *et al.*, 1978; Latt *et al.*, 2000). It should be stressed that, result showed no significant effect of harvesting frequency on the shape of tap root.

7.5. Conclusion

The results showed that more biomass and greatest root growth were obtained when *A. digitata* seedlings were produced applying 30 t/ha of poultry dropping with 20 × 20 cm sowing density and 30 days leaves harvesting frequency intervals. This combination provided the optimum growth and biomass of roots of *A. digitata* seedlings. Further research is necessary to assess chemicals components for its probable use in food and medicine.

8.

Conclusion and perspectives

8. Conclusion and perspectives

Baobab organs contribute highly to food security especially in sub-Saharan Africa. Pulp and leaves of the species are the two key harvested products. Demand for biological vegetables strongly increases. This production requires a reflexion increased on the farming techniques as well as regular observations of the plants and their environment. It requires very broad competences on the techniques and plannings of production. Globally, results showed that higher growth of seedlings, roots and its biomass was obtained when *A. digitata* seedlings were produced applying 30 t/ha of poultry dropping or 90 t/ha of cow dung with 20 × 20 cm sowing density and monthly harvests and 15 × 15 cm for leaves biomass production. These combinations provided the optimum growth of seedlings, roots and leaves biomass production of *A. digitata* seedlings.

Further research should (i) elucidate profitability of the activity, (ii) identify the potentials devastating of the seedlings and the effective fight techniques, (iii) clarify on the anti-nutritional properties of harvested leaves as the plant responses to stress due to frequent harvesting, (iv) assess on the protection methods of baobab seedlings against the adventitious plants, and (v) assess chemicals components of baobab seedlings roots for its probable use in food and medicine.

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