PHENOTYPIC CHARACTERIZATION, REPRODUCTIVE BIOLOGY AND VEGETATIVE PROPAGATION OF *ADANSONIA DIGITATA* L. WILD POPULATIONS IN MALAWI

MSc (FORESTRY AND ENVIRONMENTAL MANAGEMENT) THESIS

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DECLARATION

I the undersigned hereby declare that this thesis is my own original work which has not been submitted to any other institution for similar purposes. Where other people's work has been used acknowledgements have been made.

Objective two has been published as:

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Herbert Jenya

Signature: 

Date: 28th May, 2018

CERTIFICATE OF APPROVAL

We the undersigned certify that this thesis represents the student's own work and effort and has been submitted with our approval.

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DEDICATION

This work is dedicated to my wife Elizabeth, my son Isaac and to all my family members.

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ABSTRACT

Baobab *(Adansonia digitata* L.) is a multipurpose indigenous fruit tree with high nutritional and economic value and contributes significantly to livelihoods of local people, particularly in areas where it occurs. For this study, phenotypic characterization of five selected wild baobab populations in Malawi was compared using fruit and seed traits among fruit shapes and tree characteristics. Furthermore, age and existence of fruits in baobab trees over years were determined. Amenability of the baobab tree to vegetative propagation was investigated using grafting, cuttings and air layering. Lastly, the study identified mating systems in *A. digitata*. Results revealed the presence in Malawi of 13 unique fruit shapes of which only three (ellipsoid, oblong, ovate), could be found in all the studied populations. There were significant differences ($P \leq 0.001$) for fruit and seed traits among the fruit shapes. Seed coat colours found were; light brown, brown spotted, dark brown and grayish brown while seed shapes were; very-reniform, reniform and oblong. Tree characteristics varied from one to another. Age estimates ranged from 48 years to 180 years with a diameter at breast height (dbh) of 104.09 cm and 422 cm respectively. Fruit shapes were distributed across all ages with ellipsoid shape found across all ages. Significant differences were observed for grafting success between grafting methods ($P \leq 0.003$) and ortets ($P \leq 0.001$) in October and November. No callusing and rooting were observed for treatments propagated using cuttings and air layering. Mating system results showed bud becoming fully opened at around 19:21 pm. Buds in all the treatments flowered (100 %). Fruit abortion was high in cotton cloth (95.7%), followed by mosquito net (87.5%) and chicken wire (78.3%). Fruit production was highest in control treatment (33%). Vigorous fruit growth was only attained in the control treatment. High tree diversity revealed in this study can be used in

selection and cultivation of the species by promoting grafting of trees possessing desirable attributes for farmers to benefit. It is envisaged that the information generated could help formulate conservation, management, improvement and domestication strategies, which are important for projects promoting the sustainable use and domestication of the African baobab in the agroforestry systems.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANODE	Analysis of deviance
ANOVA	Analysis of variance
CRD	Completely randomized design
DBH	Diameter at breast height
GLM	Generalized linear model
LSD	Least significant difference
m.a.s.l	Meters above sea level
MAR	Mean annual rainfall
US$	United States Dollar
CV	Coefficient of Variation

DEFINITION OF TERMS

Characterization

Grouping of individuals with distinctive features capable of being described or measured; e.g. colour, size, performance. A character of a given individual will have a certain phenotype as determined by the individual's genotype and environment.

Domestication

The process whereby a population of living organisms is changed at the genetic level, through generations of selective breeding, to accentuate traits that ultimately benefit the interests of humans.

Grafting

Grafting is a special type of plant propagation, in which a part of a plant (scion) is joined to another plant (rootstock) for the two parts to grow together and form a new plant (Chimera).

Morphology

The branch of biology dealing with the study of the form and structure of organisms and their specific structural features.

Ortet

The original plant from which a clone is started through rooted cuttings, grafting, or tissue culture, or other means of vegetative propagation.

Rootstock

The below-ground or lower part of a tree, sometimes including part of the stem and some branches, which will form the root system of the new plant after grafting.

Ramet

A physiologically distinct organism that is part of a group of genetically identical individuals derived from one progenitor, as a tree in a group of trees that have all sprouted from a single parent plant.

Scion

The aerial part of a tree that will form the crown of the new plant after grafting.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

1.1.1 Taxonomy and distribution of African Baobab

Adansonia digitata L. belongs to the subfamily Bombacoideae in the Malvaceae family, which has eight species (Wickens and Lowe, 2008). The African baobab tree is deciduous and characterised by its massive size (reaching a height of 18-25 m), has huge branches and swollen trunk that can grow up to more than 10 m in diameter (Wickens, 1982; Baum, 1995; Sidibé and Williams, 2002; Bosch et al., 2004). As reported by Kehlenbeck et al. (2015) the members of this family are popularly known as baobab tree, bottle tree, or monkey bread tree in English. According to Pătrut et al. (2007) the tree has a life span of more than 1, 275 years.

The African baobab is native to semi-arid sub-Saharan Africa (Wickens, 1982; Yazzie et al., 1994; Sidibé and Williams, 2002), due to its high levels of drought tolerance at both the seedling and adult stages (De Smedt et al., 2012) and became iconic for Sudano-Sahelian savannahs (Sidibé and Williams, 2002; Diop et al., 2006) and Sahelian tropical grasslands (Diop et al., 2006). It is distributed in a large area and the species can be found in most of sub-Sahara Africa's semi-arid and sub-humid regions as well as in western Madagascar (Diop et al., 2006). It extends from northern Transvaal and Namibia to Ethiopia, Sudan and fringes of the Sahara (Gebauer et al., 2002; Sidibé and Williams, 2002).

1.1.2 Social and Economic Importance of African Baobab

The baobab tree is one of the important and under-utilized indigenous fruit trees owing to its various end uses (Cuni-Sanchez et al., 2011). The fruit pulp is presently exported to European and US markets as a food additive (De Smedt et al., 2011) and the seeds are used to produce oil for numerous cosmetic applications (Gruenwald and Galiza, 2005; Venter and Witkowski, 2013). At the national level, both formal and informal trade are growing (Munthali, 2012). Numerous commercial products found locally include: organic baobab pulp, sweets, ice lollies, juice, jam, coffee substitute, soap, and lip balm. Products found on the international market include: soft drinks, milk drinks, ice drinks, and natural fruit smoothies (Gruenwald, 2009), breakfast cereals, cereal bars, granola, crunches, probiotic yogurts, and smoothies (Gruenwald and Galizia, 2005). Edible parts of the African baobab supply vitamins, minerals, proteins, and energy that are not commonly obtained from the cereal-dominated diets of drylands of Africa (Muthai et al., 2017). The plant parts such as roots, bark, leaves, and seeds are used to treat various ailments, such as constipation, diarrhoea, dysentery, fever, intestinal inflammations, low iron content in the blood, smallpox, diseased teeth and gum, wounds, coughs, asthma and respiratory problems, eye complaints, lower blood pressure, malaria, and tumours (Arbonnier, 2004; Assogbadjo, 2006; Wickens and Lowe, 2008; Buchmann et al., 2010). Manufactured cosmetic products include shower and non-shower products (soaps, gels, face lift creams, body moisturizers, shampoos and hair conditioners (Munthali, 2012).

The commercialization of Baobab products is contributing to the improvement of livelihood in many communities where the tree is abundant (Kamatou et al., 2011; Matambo, 2015). For example, in Zimbabwe, Luckert at al. (2014) reported that households generate US $350 to US $1500 per year from direct or indirect participation in the baobab trade. Unfortunately, trade is reliant on dwindling natural populations due to rapid deforestation, population growth, and increases in commodity consumption. Consequently, this is affecting the sustainability of the baobab trade and the equity of supply chains (Carney and Rosomoff, 2010). Trade can be sustained only through the improved management of existing wild populations and domestication.

1.1.3 Phenotypic Variations studies on African Baobab

Knowledge in species variation in domestication is an essential step for selecting plant material with desired traits (Santos et al., 2012). Studies of phenotypic variations of baobab have been performed on fruits and seeds in Malawi and West Africa (Sidibe and Williams, 2002; Assogbadjo et al., 2005; Assogbadjo et al., 2006; Cun-Sanchez et al., 2011; Munthali et al., 2012). There is still paucity of information on the variations of fruit and seed traits among different fruit shapes and on the variations in baobab tree characteristics (bark colour, bark texture, tree shape, growth habit, trunk shape) in wild populations. Also there is dearth of information on the existence of fruit shapes in baobab trees over time (in years). Fruit shapes affect productivity and may influence the choice of consumers (Rashidi and Seyif, 2007). Additionally, fruit shape is a valuable economical parameter that determines price and productivity and its diversity gives chance to develop ideotypes during domestication (Vihotogbé et al., 2013). Use of vegetative propagation is envisaged to be the only avenue to maintain the true breed or variety of a specific fruit.

1.1.4 Propagation of the African Baobab

Previously artificial planting was a challenge due to poor seed viability. High germination rate have recently been achieved following pretreatment (Esenowo, 1991; Diop et al., 2005; Falemara et al., 2014; Niang et al., 2015). At present, people are discouraged to plant the species at large scale due to long precocity ranging from 8 to 23 years (Sidibe and Williams, 2002). Recent information has reported a possibility of reducing juvenile phase through vegetative propagation from 23 years to about 3 to 5 years (ICUC, 2002; Sidibe and Williams, 2002). Furthermore, the species has shown to have trees within the population which do not produce fruits despite having normal flowering (Venter and Witkowski, 2011). Therefore, propagation by seed cannot help to control the presence of non-fruit bearing trees.

Vegetative propagation of baobab trees has shown that top and side grafting methods can achieve high success rates of about 85 % (Kalinganire et al., 2007). Propagation through cuttings has also been reported (Assogbadjo, 2008; Kalinganire et al., 2007). Many questions on the vegetative propagation of baobab still linger on how to achieve high success rates which include: (1) When is the right time of the year for grafting? (2) What is the right size for rootstock and scion? (3) What is the right environment for grafting? (4) Whether air layering is possible using different substrates? (6) Whether individual tree variation is a factor in grafting? and (7) At what age of a tree can propagation by cuttings be successful?

1.1.5 Mating Systems in African Baobab

Generally, mating system influences reproduction in plants such as fruit productivity (quantity and quality) (Nangolo, 2016). However, contradicting information is reported on the mating system of baobab with some authors allege that it is effectively pollinated by bats (Baum, 1995) while others have reported insects (Munthali, 2012) and wind or a combination of several pollination agents (Venter, 2012). Mating system should be investigated to generate information that will assist in the management of wild and artificially planted tree populations. *A. digitata* is a hermaphrodite (Baum, 1995). Several hermaphroditic plants generally produce abundant flowers but have poor fruit set (Stephenson, 1981). Low fruit set is attributed to sparse distribution in the population (Årgren, 1996) and pollen limitation during fertilization (Burd, 1994; Burd et al., 2009).

1.2 Research problem

Both formal and informal trade in baobab products are currently taking place in southern Africa, contributing to the economic improvement of rural communities. Baobab products are also traded in Malawi (Munthali, 2012) and there is potential for commercialization. Unfortunately, current supply cannot be sustained because of a dwindling natural resource base which has resulted in low supply of baobab products on the growing market. To ensure sustainable supply, there is thus a need to domesticate baobab. This requires a good understanding of the reproductive biology and vegetative propagation techniques of a species, which for the baobab is currently scanty in Malawi (Munthali, 2012).

However, the initial step would be to understand the phenotypic variations of the fruits, seeds, and tree descriptors in order to select superior ideotypes for domestication. Despite studies on the phenotypic variation of the baobab fruits in Malawi by Munthali et al. (2012) and Cun-Sanchez et al. (2011) there is still a dearth of information on how different fruit traits are related to different fruit shapes in the wild. There is also, scarcity of information on the phenotypic variation of the tree descriptors in the wild. Even though there have been reports of successfully propagating baobab using vegetative propagation techniques in other countries, such as grafting and cuttings (Assogbadjo, 2008; Kalinganire et al., 2008; Anjarwalla et al., 2016), the information is scanty in Malawi. Vegetative propagation could help enhance precocity and maintain selected genotypes as outlined in Figure 1.1. Studies on aspects of the reproductive biology of baobab are scarce with limited observations of bat pollination in West and East Africa (Baum, 1995). To date, breeding system studies in Malawi have not given conclusive results (Munthali, 2012). However, mating system studies could help to understand how the population structures of baobab are built, consequently leading to improvement in the management of these populations.

Information on phenotypic characterization, reproductive biology, and vegetative propagation is vital for optimizing the use of baobab trees for the improvement of livelihoods for many communities, which is one of the cornerstones of the Malawi Forest Policy (Government of Malawi, 2016). Therefore, it is essential that the current resource base is managed sustainably to ensure its long-term productivity (Venter and Witkowski, 2013). Further, the generated information can spur domestication, improvement, and management in order to sustain tree productivity in agroforestry systems.

1.3 Objectives

1.3.1 General Objective

The overall aim of the study was to investigate phenotypic variation, vegetative propagation, and reproductive biology of *A. digitata* in Malawi.

1.3.2 Specific Objectives

- To characterize baobab based on fruit shape, seed morphology and tree descriptors in different wild populations.
- To investigate amenability of *A. digitata* to vegetative propagation.
- To determine the mating systems in *A. digitata* in Karonga population.

1.3.3 Hypotheses

- There is no rich diversity in baobab in Malawi for fruit shapes, seed morphology and tree descriptors and also that baobab fruit morphology does not influences fruit productivity.

- Success in baobab vegetative propagation is not a factor of timing, types of cuttings, and types of grafting.
- There is no diversity in mating systems in *A. digitata*.

1.4 Justification of the Study

Several reports indicate that baobab has poor recruitment in most areas where it exists and this is evidenced by populations with positively skewed stem diameters (Dhillion and Gustad, 2004; Assogbadjo et al., 2005; Chirwa et al., 2006; Edkins et al., 2008; Venter and Witkowski, 2010). There is need to artificially balance the population structure of the baobab trees. Farmers can be encouraged to plant the baobab trees only when the fruiting precocity is shortened, unlike the case where seedlings are used for propagation. It is estimated that it takes between 8 and 23 years before the baobab reach maturity (Sidibé et al., 1996; Sidibé and Williams, 2002). However, Swanepoel (1993) explained that baobab trees in their natural semi-arid environment may only start flowering at 125-189 years of age. Thus, vegetative propagation techniques using mature trees are one way of assuring speedy and sustainable supply of baobab resource for a variety of current and potential end-uses at domestic, national, and international spheres. For instance, grafting can reduce the time taken by the baobab tree to start producing fruits to 3-5 years (Sidibe and Williams, 2002). Furthermore, an adequate understanding of the reproductive biology of this plant will help to understand the causes of sterility in some trees within the same population. Therefore, information gathered will help to understand the baobab flowering phenology and pollination process, which are lacking at the moment. A summary of key issues that the study investigated in phenotypic variation, reproductive biology and vegetative propagation of *A. digitata* is shown in Figure 1.1.

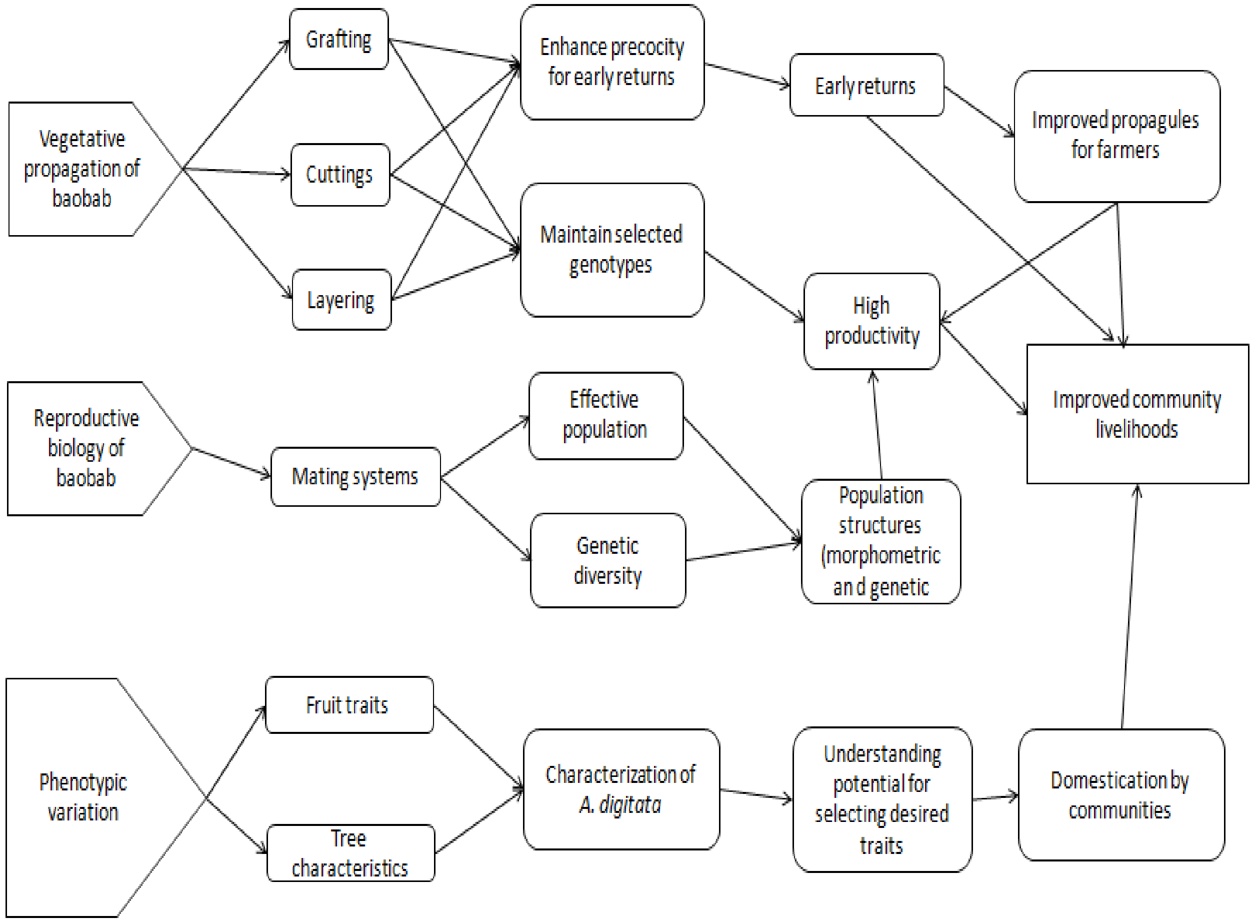


Figure 1.1: Conceptual model of studies on characterization, vegetative propagation and reproductive biology of baobab

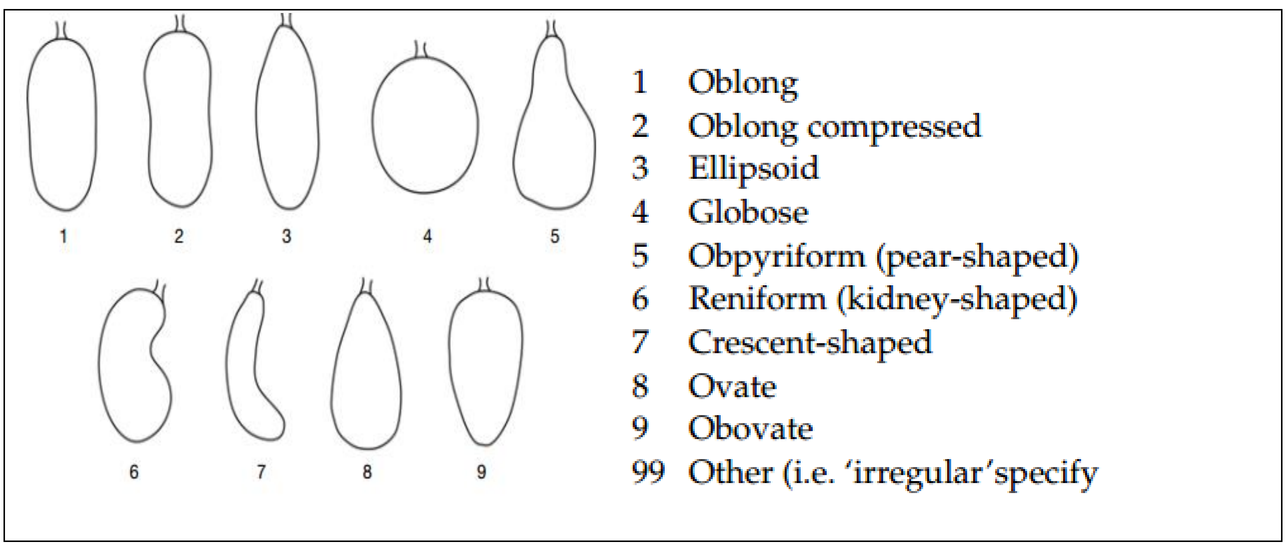
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Phenotypic Variations Observed on African Baobab (*Adansonia digitata*)

Fruit traits such as diameter, shell thickness and colour, fruit weight, and shape give a good indication of phenotypic variability (Martínez De Laraet al., 2004). Evidence exists of a number of local types of baobab trees differing in habit, vigor, size, quality of fruits, and vitamin content of the leaves (Gebauer et al., 2002). Oral knowledge in Mali frequently classify baobab in several types by taking into account bark color, pulp and leaf taste, or height and girth of the tree (Sidibé and Williams, 2002). Codjia et al. (2001) have reported that in Benin, trees are mainly distinguished due to their fruits' shape while in Sudan it is known that baobabs from different areas have different fruits in terms of size, shape, and sweetness (Gebauer et al., 2002).

Phenotypic variations of baobab fruit shapes have been described in descriptors of baobab (Kehlenbeck et al., 2015) and also in Sudan (Gurashi and Kordafani, 2014), (Figures 2.3 and 2.4 respectively).



(Source: Kehlenbeck et al., 2015)

Figure 2.1: Nine baobab fruit shapes form the descriptors of baobab

Gurashi and Kordafani, (2014), describes the presence of 12 fruit shapes in Sudan (Figure 2.4) as opposed to 9 fruit shapes described in the descriptors on baobab by Kehlenbeck et al. (2015) (Figure 2.3).

(Source: Gurashi and Kordafani, 2014)

Figure 2.2: Twelve baobab fruit shapes identified in Sudan

Baobab fruit sizes have shown to differ between localities as presented in literature by different authors as follows: in Senegal, two different ranges of fruit length have been reported; 16.8 to 26 cm (Soloviev et al., 2004) and 20 cm to 30 cm (Gebauer et al., 2002). Assogbadjo et al. (2005) reported a variation in fruit length of 16.8 cm to 20.7 cm in Benin. To the contrary, baobab fruits in Malawi have shown to be small in terms of fruit length compared to those from West Africa with a variation ranging from 11.9 cm to 16.5 cm (Munthali et al., 2012). Variations have also been observed on fruit weight of baobab where reports indicate that they range between 167 g to 348 g in Senegal (Soloviev et al., 2004), 203g to 275g in Benin (Assogbadjo et al., 2005) and 125.8g to 162.9g in Malawi (Munthali et al., 2012). Phenotypic variations of other baobab fruit variables are outlined in Table 2.1.

Table 2.1: Phenotypic variations of some baobab fruit variables observed in some countries

Country	fruit width (cm)	Number of seed	Seed weight (g)	Pulp weight (g)	Single seed weight (g)	Authors
Malawi	6.8 to 7.6	86 to 111	49.5 to 66.0	28.7 to 41.5	0.37 to 0.58	Munthali et al., 2012
Malawi	7.7 to 10.0	42 to 249	21 to 124.5	-	0.5 to 0.6	Cun-Sanchezi et al., 2011
Mali	7.5 to 10.0	121 to 241	48.4 to 96.4	-	0.4 to 0.5	Cun-Sanchezi et al., 2011
Mali	-	-	52.0 to 87.0	32.0 to 77.0		De Smedt et al., 2011

There seem to be a relationship between different variables in a baobab fruit. Cuni-Sunchez et al. (2011) have reported correlations between pulp and fruit weight in Mali and Malawi. Using Spearman’s rank correlation coefficients, they found that, in Mali, fruit weight was correlated with pulp weight (R = 0.93, P = 0.01) and the same in Malawi was (R = 0.86, P = 0.01). The authors concluded that the heavier the fruit of baobab, the higher the pulp weight. The authors have suggested that when selecting heavy fruits, more attention should be given to fruit diameter than to fruit length. They further suggested that the higher pulp percentage of elongated fruits found in Malawi suggests that fruit shape could be a verifiable tool in selecting trees with high pulp weight under field conditions, since it is an easily measurable trait.

2.2 Vegetative Propagation in Fruit Trees

Vegetative propagation may act as a quick method where farmers can benefit from a selection of superior genotypes unlike the use of classical breeding (Akinnifesi et al., 2008). Propagation by vegetative means may be useful in the following ways: propagation of the parent plant at an early stage of growth, preservation of genotypes by clone banks, transfer of additive and non-additive characters, evaluation of genotypes and their interaction with the environment through clonal testing, and the potential to get greater uniformity of the tree crops (Thirunavoukkarasu and Gurumurti, 1998). Propagation by vegetative methods can be in the form of budding, grafting, cutting, suckering or layering (Gubbuk et al., 2011).

2.2.1 Propagation through the use of Grafting

Grafting is the method of purposively combining the buds or branches of one plant with the roots and base of another plant. The upper component is the ‘scion’, and the lower is the ‘rootstock’ or ‘stock’. ‘Autografting’ is grafting between the same plants of the same species; ‘homografting’ is grafting between different plants of same species; ‘heterografting’ is grafting between plants of different species (YouQun, 2011). The grafting combinations used in agricultural production are commonly compatible, as both scion and rootstock survive after grafting and grow normally until flowering (YouQun, 2011). Most hetero-grafting combinations are incompatible as either scion or rootstock, and both may die after grafting. Grafting is commonly performed at plant stems using flat, cleft, and approach methods, among which cleft grafting is the most commonly used. In cleft grafting, the scion base is cut into a wedge shape. The rootstock is first sliced horizontally and then cut into a cleft. The scion is inserted into the cleft in the rootstock. Flat grafting is commonly used in studies of graft union development. In flat grafting, both the scion and the rootstock are cut into flat surfaces, and are grafted together. In approach grafting, the cambium of the scion and rootstock are exposed by cutting the epidermis and cortex. The surfaces are then combined (YouQun, 2011). To successfully perform grafting, the precise docking of anatomical structures between scion and rootstock is very important. Dislocation of vascular system and cambium often leads to graft failure or delayed development (Kollmann et al., 1985).

During formation of the graft union, many researchers have observed callus proliferation (from both the rootstock and the scion), callus bridge formation, differentiation of new vascular tissue from callus cells and the production of secondary xylem and phloem (Moore, 1983; Hartmann et al., 2002). A low or incorrect callus formation between the rootstock and scion could lead to defoliation, reduction of scion growth and low survival of grafted plants (Oda et al., 2005; Johkan et al., 2009). Thus, the vascular connection in the rootstock–scion interface may determine water and nutrient translocation, affecting other physiological traits. In addition, the influence of the rootstock on nutrient and water uptake was attributed principally to physical characteristics, such as lateral and vertical development of roots, or greater uptake ability (Ballesta et al., 2010). Insufficient connection of vascular bundles between the scion and the rootstock decreases the water flow (Torii et al., 1992). When water absorption by roots was suppressed at the graft interface, stomatal conductance and scion growth decreased (Atkinson and Else, 2001; Oda et al., 2005). Thus, hydraulic architecture becomes of fundamental importance, since the sustained flow of water controls many plant processes such as growth, mineral nutrition, photosynthesis and transpiration (Ballesta et al., 2010).

2.2.2 Propagation through the use of Cuttings

Various factors, both endogenous and exogenous, may affect the rooting capacity of different species and may include the following: type of substrate, growth regulators, environmental factors, plant age, physiological condition of the mother plant, type of cutting, time of cutting, and the action of oxidizing agents specific to each plant (Trevisan et al., (2008).

Both endogenous and exogenous factors can interact with each other to influence propagation success. For instance, *Dovyalis hebecarpa* cuttings propagated in autumn from lower positions had a higher rooting percentage than those from the upper portions (14.7 and 3.1 %, respectively), while the spring cuttings from upper portions gave better results than lower positions (71.2 and 53.1 %, respectively) (Almeida et al., 2008). Each factor can influence propagation success as an independent factor as has been the case in Annonaceae family, where summer cuttings performed better than autumn and winter cuttings (40.4 and 18.0% respectively) (Scaloppi Junior and Martins, 2003).

Callus rooting species are relatively more difficult to root than species that root from bark (OuYang et al., 2015). It has been noted that the physiological conditions of the stock plants (Mitchell et al. 2004), time and place of cutting collection (Jurásek and Martincová, 2004), and environmental conditions (Ragonezi et al., 2010) significantly affect cutting propagation (OuYang et al., 2015) in most plants. Initial cutting size is a very important factor involved in rooting ability and growth performance of a plant (Vigl and Rewald 2014). Cuttings with a larger diameter and longer length result in better survival and growth under normal conditions (Vigl and Rewald 2014). But Leakey (2004) has reported that cutting size is not a simple variable as it is also affected by cutting origin and age. An interaction between cutting length and cutting diameter may be critical factors that affect rooting (Tchoundjeu and Leakey,, 1996). Adventitious rooting is a complex developmental process that can be affected by internal and external factors (Leakey, 2004). Auxins play a critical role in the formation of adventitious root by increasing initiation of the root primordium and growth via cell division (Fogaça and Fett-

Neto, 2005). Auxins promote starch hydrolysis and mobilize sugars and nutrients to the cutting base (Das et al., 1997).

2.2.3 Propagation through the use of Air Layers

Air layering is a technique that is frequently used to obtain plants of a relatively large size from plants that are difficult to root (Ramírez-Malagón et al., 2014). Different percentages of rooting have been reported for air layering systems in other woody species. Nunez-Elisea et al. (2000) have reported a 73.3% from *Annona glabra*, 67.3% was observed for *Litchi chinensis* (Rahman et al., 2002), and 79.3% was documented for *Anacardium occidentale* (Lopes et al., 2005). It is well known that rooting depends on the intrinsic characteristics of the plant material (genotype, physiological conditions, phenological stage, etc.) and extrinsic factors (environmental conditions, growth regulators, fertilization, etc.) (Felker et al., 2005; Schwambach et al., 2005; Felker, 2008). It has also been noted that juvenile tissues respond better to auxins because they have higher sensitivity than adult tissues and this sensitivity leads to a higher cell division activity and root formation (Vidal et al., 2003).

2.3 Mating System

Flower buds of *A. digitata* appear with or shortly after leaf buds (Wickens and Lowe, 2008). According to Breitenbach and Breitenbach (1974), bud opening starts by swelling in the late afternoon, opens mainly between 6.30 and 7.30 pm, and finish opening by 8.30–9.00 pm. Each flower takes some 20 minutes to unfold, and a further 30 minutes of jerky movements to smooth out any remaining wrinkles. By early morning the staminal bundle is fully exposed and thereafter the petals gradually closed. By late afternoon the flower is completely wilted. Therefore, flowers have a 24 hour life and a functional pollination life of 16–20 hours.

Adansonia digitata flowers are hermaphroditic (Baum, 1995). Hermaphroditic flowers extend from being full self-compatibility to full self-incompatibility. Breeding and pollination systems both determine the reproductive success of plants (Kearns et al., 1998) and fruit production depends on the effectiveness of pollination (Nangolo, 2016). Breeding system has been observed to affect the genetic composition of the progeny produced (Lowe et al., 2005) and influences the effective population size (Eckert et al., 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Phenotypic Characterization of Fruit Shape, Seed Morphology and Tree Descriptors

3.1.1 Study Site

Fruit samples were collected from five baobab populations of Chikwawa, Karonga, Mangochi, Neno, and Salima. The study populations were selected based on the distribution of the species in different silvicultural zones (A, BA, L and J) (Hardcastle, 1978) (Table 3.1; Figure 3.1). Furthermore, the populations were selected on the abundance of trees and prevailing trade in Baobab products. According to Hardcastle (1978), silviculture zone L has a mean annual rainfall (MAR) of 1,600 mm with predominantly weathered ferralitic soils. Silviculture zone BA has an MAR ranging between 710 and 850 mm and is characterized by calcimorphic soils overlaying vertisols. Silviculture zone J has an MAR ranging between 1,200 and 1,600 mm and is characterized by ferralitic soils, while silviculture zone A has an MAR ranging between 710 and 840 mm with predominantly vertisols.

3.1.2 Fruit Sampling and Data collection

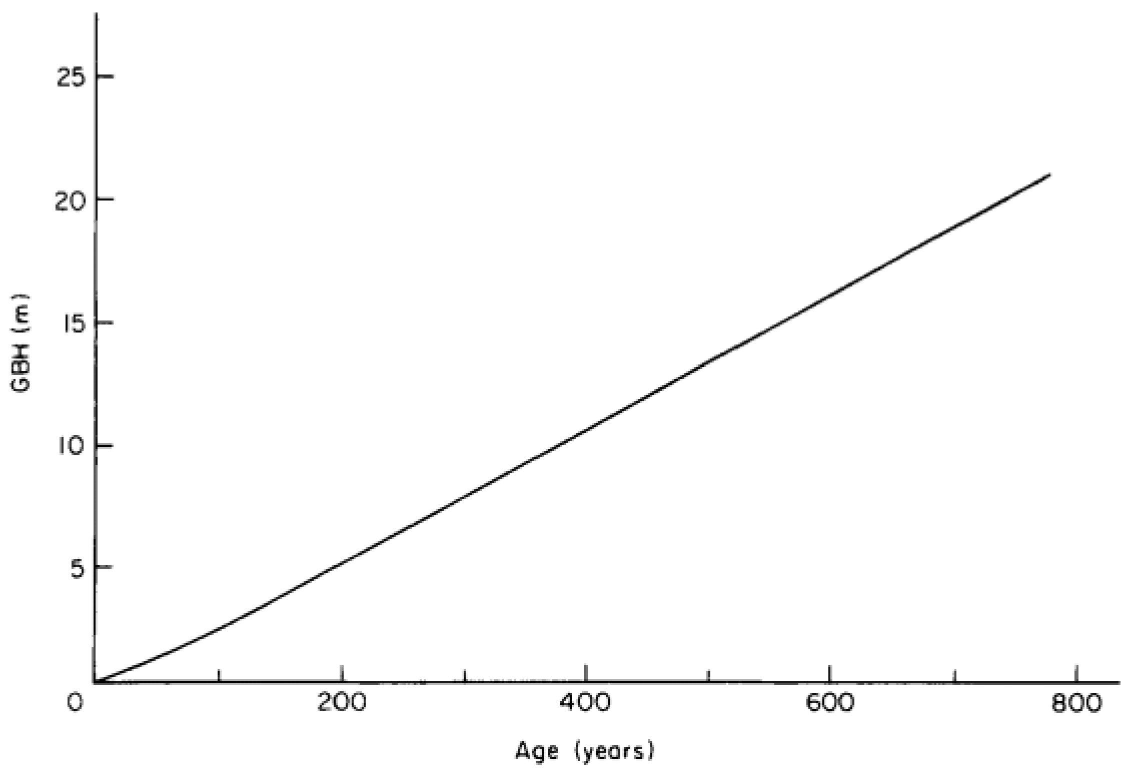
Fruits were collected at the peak of fruit maturity, between March and May of 2016. Dry fruits (10 per tree) were randomly collected from trees spaced at a minimum distance of 100 m (Munthali et al., 2012). A total of 135 trees were characterized from the five populations, comprising 21 trees from Chikwawa, 24 from Karonga, and 30 each from Mangochi, Neno and Salima (Table 3.1).

Table 3.1: Location, physical description of populations and number of trees sampled

Population	Coordinates	Silviculture zone*	Altitude (m)	Mean annual rainfall (mm)	Mean annual temperature (°C)	Soil type
Chikwawa (21)**	16°02'S, 34°50'E	A	<200	710-850	>25	Vertisols
Karonga (24)**	09°56΄S 33°56΄E	L	475-1000	>1600	23-25	Ferrisols dominant; regosols
Mangochi (30)**	14°25'S, 35°16'E	BA	200-1200	710-850	20-25	Alluvial calcimorphic soils above the vertisols
Neno (30)**	15°25'S, 34°40'E	J	900-1500	1200-1600	19-21	Sandy ferrallitic
Salima (30)**	13°47'S, 34°28'E	BA	200-1200	710-850	20-25	Alluvial calcimorphic soils above the vertisols

*Adopted from Hardcastle (1978), ** number of trees sampled

The fruit shape descriptions followed descriptions by Gurashi and Kordofani (2014) and Kehlenbeck et al. (2015). From each fruit shape, subsamples of five fruits were randomly selected for the assessment of fruit weight, seed weight, and pulp weight. Fruits were weighed to the nearest 0.1 g with an electronic scale (TREE LW Measurements HRB 10001-High Resolution Balance 10000 g x 0.1 g). Fruit shells were cracked and the fruit content (pulp + seeds) was removed and weighed. Pulp was separated from seeds by washing with tap water, and seeds were then air dried and weighed again. Pulp content was calculated by subtracting the seed weight from the fruit content (pulp + seeds). Seeds were counted to determine the seed number. Single seed weight was calculated by dividing the combined seed weight by the number of seeds in the fruit (Cuni-Sanchez et al., 2011). A subsample of ten seeds was randomly selected for the measurement of seed length, width and thickness using a micro-calliper (Appendix 13) to the nearest mm. Seed shape and colour were visually assessed in the laboratory using a guide by Kehlenbeck et al. (2015) and colour chart respectively. Tree descriptors were assessed visually in the field following a guide by Kehlenbeck et al. (2015). The relationship between girth at breast height (GBH) and estimated age was calculated following a method devised by Barnes (1980), (Figure 3.1), by correlating the GBH reading to that of age using a ruler.



(Source: Barnes, 1980)

Figure 3.1: Relationship between GBH and estimated age of baobab

Fruit diversity was determined using the Rėnyi diversity profile in the Biodiversity R package (Kindt and Coe, 2005). The fruit diversity was determined in terms of fruit shape. Fruit and seed trait data were also subjected to analysis of variance.

3.1.3 Statistical analysis

Data obtained were tested for normality and homogeneity. After the two criteria were met, all the data were subjected to analysis of variance (ANOVA) using MINITAB for Windows Release 16.1 and means were separated with Fischer's least significant difference (LSD) at the 0.05 level. The following two-way nested model was used:

$$Y_{ijk} = \mu + P_i + F_{i(j)} + e_{ijk}$$

Where:

Y_{ijk} is the observed value for the i^{th} provenance and j^{th} fruit shape;

μ is the overall mean;

P_i are fixed effects of i^{th} provenance

F_j are fixed effects of j^{th} fruit shape within i^{th} provenance

e_{ijk} is the random residual effects, $e_{ijk} \sim N(0, \sigma e^2)$.

Qualitative data on tree characteristics (bark colour, bark texture, tree shape, growth habit and trunk shape) and seeds (shape and colour) were analysed using descriptive statistics. Scatter plots were used to show the age estimate using diameter at breast height and the existence of fruit shapes over time (age in years).

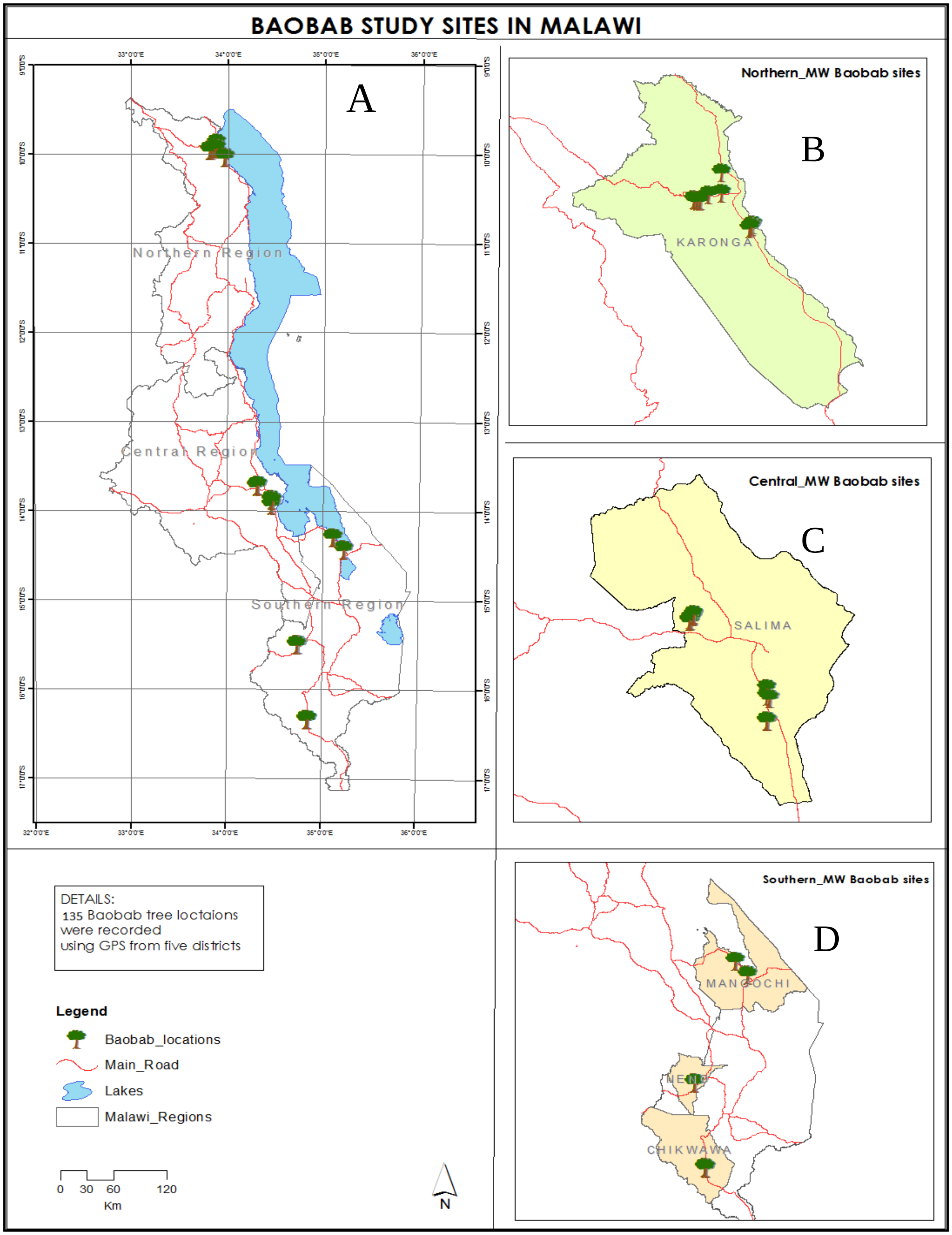


Figure 3.2: A= Map of Malawi showing baobab fruits collection sites, B=baobab collections sites in Karonga, C=baobab collection sites in Salima, D=baobab collection sites in Southern Region

3.2 Amenability of *A. digitata* L. to Vegetative Propagation

3.2.1 Study Sites

Scions and cuttings were collected from Karonga at two time intervals (October 5, 2016 and November 26, 2016). Grafting and rooting of cutting trials were conducted in the greenhouse and shed net at Forestry Department, Mzuzu University, between October 6, 2016 and November 27, 2016. Mzuzu University is located at a latitude of 11° 28’ S and longitude of 34° 01’ E, with an altitude of 1270 m above sea level and average annual temperatures ranging from 13.5°C to 24°C. Air layering trials were established in the field on October 5, 2016 and November 26, 2016 in Karonga District.

3.2.2 Sampling and Data Collection

3.2.2.1 Grafting

Scions of 50 mm to 120 mm diameter were harvested from three ortets (Tree 1, Tree 2, and Tree 3) from Karonga district. Scions were collected in the morning and kept wet in hessian sacs, ready for transportation to Mzuzu University. Upon arrival (approximately after 4 hours driving) in Mzuzu, the samples were stored in the shade net with cool temperature (10- 18 ^{o}C) at night. Grafting was done the following day from 7:30 am to 5:00 pm. Only one person performed the grafting. Ten grafts were top cleft and ten grafts were side veneer for each ortet (Figure 3.2). The mean diameter for scions was 85 mm and 30 cm long. Grafting was done on one year old rootstocks growing in polythene tubes of size (40 cm long by 20 cm wide) filled with fertile black Miombo soil mixed with sand (2:1, w/w). The rootstocks had been raised at Forestry Department nursery, Mzuzu University in 2015. Seeds used to raise the rootstocks were collected in Karonga, Likoma, Salima, Mangochi and Neno and then bulked before being sawn in the nursery.

Figure 3.2: Grafting methods: Top cleft (A) and side veneer (B)

The study was arranged in a three-factorial design (factor 1 = grafting time; factor 2 = grafting method; factor 3 = mother tree) with two replicates of 5 ramets per treatment for October and November with each of the two grafting methods and three mother trees. A sum of 60 grafted baobab plants were obtained per grafting time and a total of 120 grafted baobab plants were obtained for the whole experiment. The grafted baobab plants were watered when need be to maintain field capacity. Grafting success (Figure 3.3 and Appendix 4) was monitored continuously and final assessment was done at six months after establishment. The data collected included grafting success, length of new shoot, and number of leaves. Shoot length (mm) was measured using a linear tape to the nearest mm.

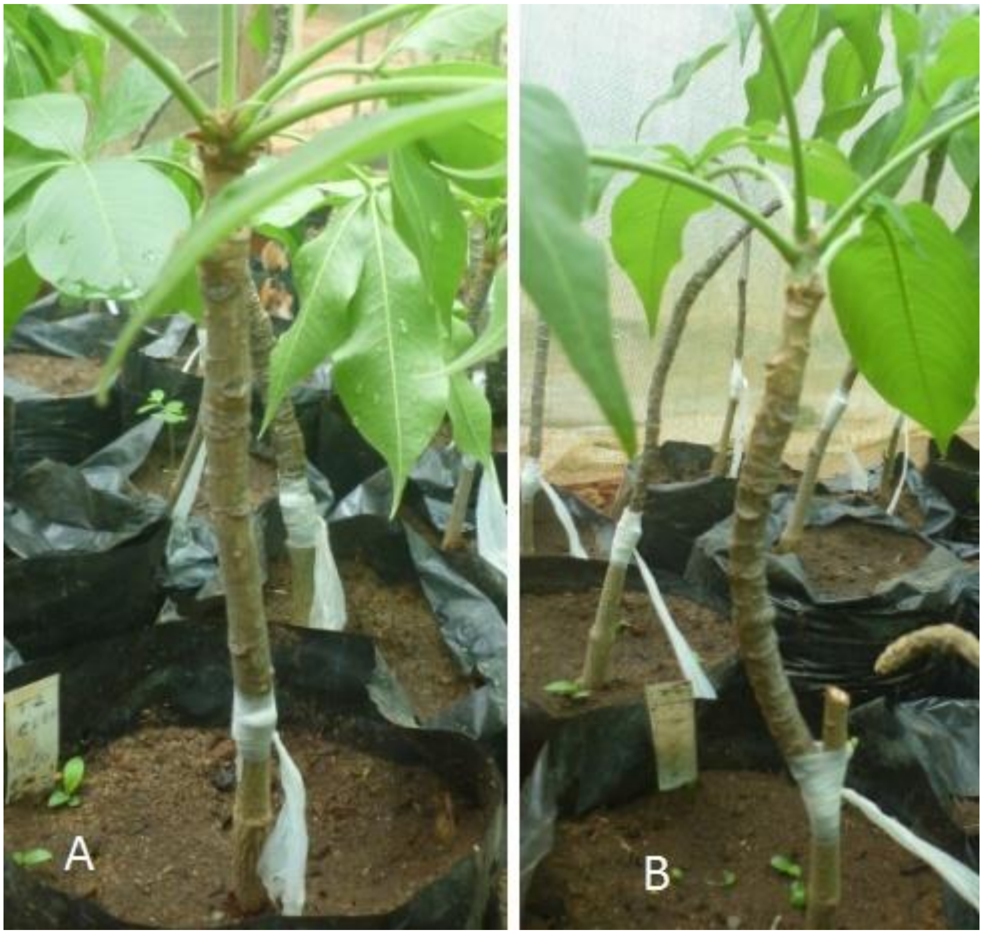


Figure 3.3: Baobab plants showing successful top-cleft grafts (A) and side veneer grafts (B).

3.2.2.2 Rooting of cuttings

Cuttings in polythene tubes

Rooting ability of baobab cuttings from Karonga was tested in October and November, 2016. A total of 405 cuttings were used in the experiments consisting of three diameter categories (small = 3cm, medium = 4cm and large = 5cm) and three length categories (10, 20, 30 cm). Three ortets were used (T1, T2 and T3). Cuttings were kept moist in wet hessian sacs and planted in the shade net a day after collection. Cuttings were planted in black polythene tubes (40cm long and 20cm wide) (Appendix 3), filled with natural Miombo soil mixed with sand (2:1, w/w). Cuttings were planted at a depth of 7 cm. Watering was done only to field capacity. The average shade net temperature during the experiment was 18 $^{\circ}$C with a 50:50 percentage of shade and light.

The experiment was laid in a Completely Randomized Design (CRD) with 27 treatments combinations, 5 cuttings per treatment, and replicated 3 times (Table 3.2). The effects of stem length, stem diameter, cutting source (ortet), and propagation time on rooting and shooting were recorded in terms of mean number of roots produced per cutting and number of shoots produced per cutting. Shoot counts were taken once every week for the whole study period from the day the first buds sprouted.

Cuttings in non-mist propagator

Tender (soft) cuttings of baobab from Karonga were collected from tips of branches in the month of October and November, 2016. A total of 270 cuttings were used in the experiment. Three lengths (10, 20, 30cm) and a basal diameter of 50–120mm from three mother trees (T1, T2 and T2) were planted in non-mist polythene propagators (Appendix 5) in a CRD (Table 3.3). The experiment was replicated two times for October and November with ten cuttings per treatment. The propagators were constructed following a design based on that of Howland (1975) modified by Leakey et al. (1990). The data collected were on number of shoots, length of taproot, mean number of roots, average root length, percentages of rooting, and survival of cuttings.

Table 3.2: Treatment combinations for cutting experiment planted in polythene tubes

Treatment	Description	Ortets/Trees
V1	Large diameter (5cm), 30cm long	T1
V2	Large diameter (5cm), 30cm long	T2
V3	Large diameter (5cm), 30cm long	T3
V4	Large diameter (5cm), 20cm long	T1
V5	Large diameter (5cm), 20cm long	T2
V6	Large diameter (5cm), 20cm long,	T3
V7	Large diameter (5cm), 10cm long	T1
V8	Large diameter (5cm), 10cm long	T2
V9	Large diameter (5cm), 10cm long,	T3
V10	Medium diameter (4cm), 30cm long	T1
V11	Medium diameter (4cm), 30cm long	T2
V12	Medium diameter (4cm), 30cm long	T3
V13	Medium diameter (4cm), 20cm long	T1
V14	Medium diameter (4cm), 20cm long	T2
V15	Medium diameter (4cm), 20cm long	T3
V16	Medium diameter (4cm), 10cm long	T1
V17	Medium diameter (4cm), 10cm long	T2
V18	Medium diameter (4cm), 10cm long	T3
V19	Small diameter (3cm), 30cm long	T1
V20	Small diameter (3cm), 30cm long	T2
V21	Small diameter (3cm), 30cm long	T3
V22	Small diameter (3cm), 20cm long	T1
V23	Small diameter (3cm), 20cm long	T2
V24	Small diameter (3cm), 20cm long	T3
V25	Small diameter (3cm), 10cm long	T1
V26	Small diameter (3cm), 10cm long	T2
V27	Small diameter (3cm), 10cm long	T3

T1 = Tree 1, T2 = Tree 2, T3 = Tree 3

Table 3.3: Treatment combination for cutting trial established in non-mist propagator

Treatment	Description	Ortets/Trees
V1	Long (30cm)	T1
V2	Long (30cm)	T2
V3	Long (30cm)	T3
V4	Medium (20cm)	T1
V5	Medium (20cm)	T2
V6	Medium (20cm)	T3
V7	Short (10cm)	T1
V8	Short (10cm)	T2
V9	Short (10cm)	T3

T1 = Tree 1, T2 = Tree 2, T3 = Tree 3

3.2.2.3 Air layering

Air layering was tested on five ortets in the month of October and November 2016 in the field in Karonga. Two rooting mediums (top soil and moss (*Leucobryum madagassum*)) (Appendix 2) were investigated. Each treatment was replicated three times in each of the five ortets. Moisture was checked and water added every two weeks wherever necessary. The trial was closed at six months. Data collected was on percentage rooting of the air layers.

3.2.3 Data Analysis

Grafting success data (in the binary form: '1' for grafting success and '0' for not successfully grafted) was analyzed using descriptive statistics for percentages of per tree, per grafting method, and then per grafting month success. Secondly, due to the binomial distribution of the data, an analysis of deviance (ANODE) using Generalized Liner Model (GLM) procedures of Genstat 4th Edition with the logit function as the link function was carried out to assess the effect of grafting date and grafting methods on graft success probability. Below is the equation of the fitted model.

$$\text{Logit}(p_i) = \log\left(p_i / (1 - p_i)\right)$$

= constant, grafting time, mother tree and grafting method effect,

where p_i, the probability of success of a seedling grafted with method *i*, is computed as the ratio of successful grafts over the total number of grafts per date, mother and method (n = 60, 20 and 30 respectively).

Shoot growth (length) and number of leaves data was analyzed using paired t-test in MINITAB 16.1 in order to test whether there were significant differences between means during propagation period (October and November) and between grafting methods. Before analysis data for the number of leaves were normalized using arcsine transformation (Fowler et al., 2008), percentage callusing and rooting of the cuttings and air layers were analyzed in MINITAB 16.1 using descriptive statistics.

3.1 Mating Systems of *A. digitata* L. in Karonga District

3.1.1 Study Site

The study was conducted in Karonga District (Table 3.1).

3.1.2 Data Collection

The mating systems study was done following the methodology by Ngulube et al. (1998) and Munthali, (2012). The studies were conducted from November 2016 to March 2017. Four treatments were investigated as follows:(i) Selfing and apomixes, where six green buds per tree irrespective of size were covered with cotton cloth to deter any pollination agents (insects, bats and wind); (ii) Wind pollination, where six green buds were covered with green mosquito net (Appendix 7) to inhibit access by insects, bats and other animals; (iii) Insect pollination, where six green buds were covered with a chicken wire mesh to deter bats and other mammals; and

(iv) Natural pollination, where labels were tied to six buds per tree to act as a control. Each treatment was replicated six times in each of five trees.

Flowering process and fruit development were observed and recorded initially every month up to fruiting stage using a scale of 0 to 8 as described by Ngulube et al. (1998) and Munthali (2012). In this scale: (0) represented small buds, inflorescence sepals closed (bud stage closed); (1) Large buds, inflorescence sepals cracked (opening); (2) Sepals and petals fully open; (3) Inflorescence petals, anthers, stigma, turned brown; (4) Inflorescence petals, anthers, stigma wilting; (5) Inflorescence petals, anthers, stigma fallen off; (6) Bud fallen before sepals open; (7) Fruit forming; and (8) Fruit aborted. Floral anthesis was recorded using a Bushnell wildlife camera with night vision (Appendix 6) on a flower bud which was about to start split open (reach anthesis) from 25th November, (15:35:01) to 26th November, 2016 (07:52:15).

3.1.3 Data Analysis

Data on number of fruits aborted and number of fruits developed were subjected to descriptive analysis using MINITAB 16.1 statistical package in order to calculate percentages.

CHAPTER FOUR

4.0 RESULTS

4.1 Phenotypic Characterization of Fruit Traits and Tree Descriptors

4.1.1 Diversity and Distribution of Fruit Shapes

4.1.1.1 Diversity of fruit shapes

Thirteen fruit shapes were found in this study (Figure 4.1). The fruit shapes found were; ellipsoid, oblong, ovate, obovate, fusiform, high spheroid, globose, ellipsoid pointed, oblong compressed, oblong pointed, rhomboid, spheroid emarginate, and clavate.

KEY: 1=Ellipsoid 2=Oblong 3=Ovate 4=Obovate 5=Fusiform 6=High spheroid 7=Globose 8=Ellipsoid pointed 9=Oblong compressed 10=Oblong pointed 11=Rhomboid 12=Spheroid emarginate 13=Clavate

Figure 4.1: Diversity of fruit shapes found in five populations of Karonga, Salima, Neno, Mangochi and Chikwawa Districts in Malawi.

Fruit shape richness was classified into three groups. The highest diversity was found in Neno and Chikwawa ($H\alpha$=2.30) followed by Karonga ($H\alpha$=2.08). The least diversity was found in Mangochi and Salima ($H\alpha$=1.96) (Figure 4.2).

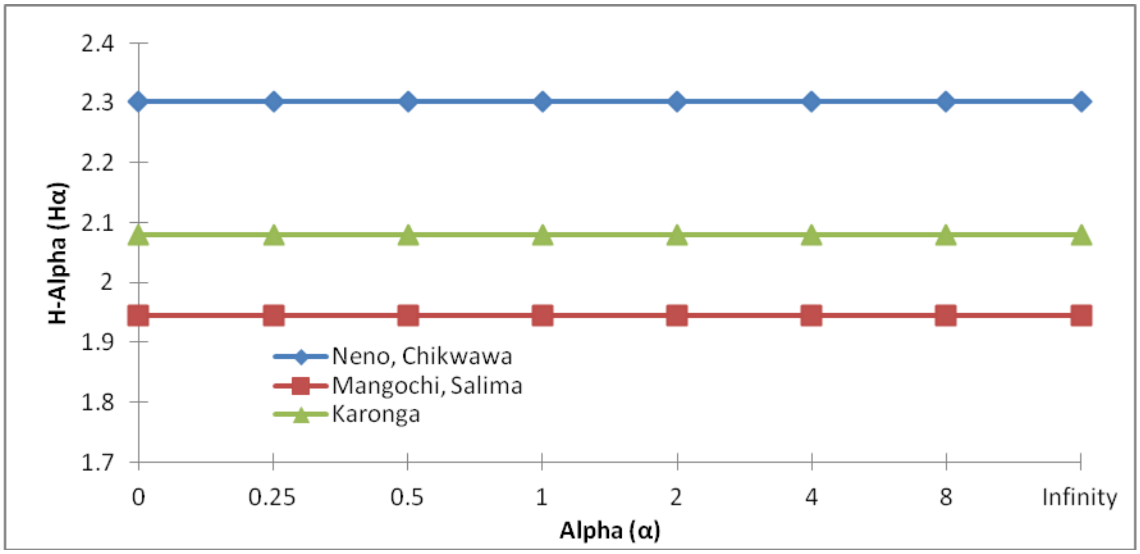


Figure 4.2: Rẻnyi diversity profiles for fruit shape in the wild populations

4.1.1.2 Distribution of fruit shapes across populations

The number of fruit shapes varied between populations (Table 4.1). Three fruit shapes (ellipsoid, oblong, ovate) were found in all the populations. The frequency of ellipsoid fruits ranged from 16 to 43%, oblong from 6 to 24%, and ovate from 7 to 24%. Ellipsoid pointed fruits were found in four populations (Karonga, Mangochi, Neno, and Salima) with a frequency ranging from 3 to 7%. Obvate fruits occurred in four populations (Chikwawa, Karonga, Mangochi, and Neno) with a frequency varying from 3 to 14%. Fusiform fruits were found in three populations (Chikwawa, Mangochi, and Neno) with a frequency ranging from 10 to 15%. Spheroid fruits were found in three populations (Chikwawa, Karonga, and Salima) with a frequency ranging from 3 to 12%. Oblong pointed fruits were found in two populations (Chikwawa and Neno) with a frequency ranging from 5 to 6%. Rhomboid fruits were found in two populations (Chikwawa and Karonga) with a frequency ranging from 5 to 12%. Clavate fruits were found in two populations

(Chikwawa and Neno) with a frequency ranging 6 to 10%. Spheroid emarginate fruits were only found in Mangochi with a frequency of 3%.

Table 4.1: Fruit shape occurrence, non-occurrence (-) and percentages in the sampling sites

	Percentage frequency of fruit shapes in the five populations				
Fruit shape	***Chikwawa***	**Karonga**	**Mangochi**	**Neno**	**Salima**
Clavate	*10*	—	—	6	—
Ellipsoid	*25*	16	38	30	43
Ellipsoid pointed	—	3	7	3	3
Fusiform	*15*	—	10	13	—
High spheroid	—	16	—	6	10
Oblong	*15*	12	21	6	24
Oblong compressed	—	—	—	6	7
Oblong pointed	*5*	—	—	6	—
Obovate	*10*	10	14	3	—
Ovate	*10*	19	7	24	10
Rhomboid	*5*	12	—	—	—
Spheroid	*5*	12	—	—	3
Spheroid emarginate	—	—	3	—	—

4.1.2 Variation of Fruit Traits between Fruit Shapes

4.1.2.1 Fruit weight

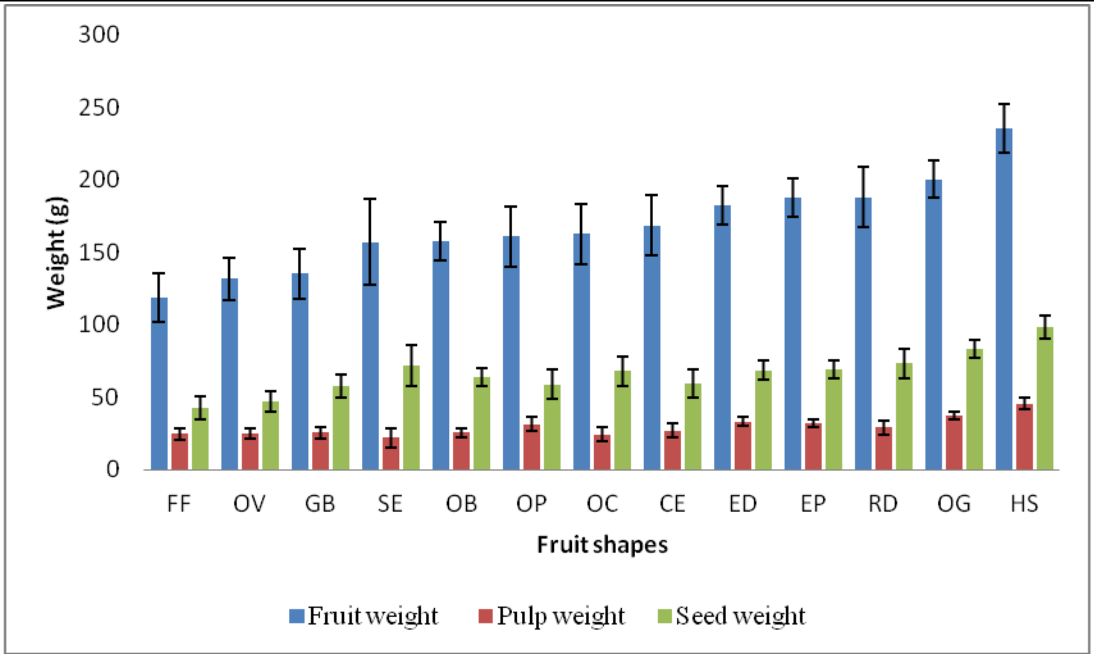
Results of variation in fruit weight among the thirteen fruit shapes are presented in Figure 4.3. There was a significant difference ($P \leq 0.001$) in fruit weight among the 13 fruit shapes. The heaviest fruit were found in high spheroid fruits (235.5±17.1 g), oblong fruits (200.5±13.2 g) and rhomboid fruits (188.0±20.9 g). Intermediate-weight fruits were found in ellipsoid pointed fruits (187.4±13.2 g) and ellipsoid fruits (182.4±13.2 g). While lightest fruits were found to be fusiform fruits (118.7±17.1 g) and ovate fruits (131.5±14.8 g). The range of fruit weight was 22 g to 407 g with a coefficient of variation (*CV*) of 38.9%.

4.1.2.2 Pulp weight

Pulp production varied significantly ($P \leq 0.001$) among fruit shapes (Figure 4.3). Shapes that were best pulp producers were high spheroid (45.3±3.9 g) and oblong (37.2±3.0 g). Shapes that were intermediate pulp producers were ellipsoid (33.0±3.0 g), ellipsoid pointed (32.0±3.0 g), oblong pointed (31.4±4.8 g), rhomboid (29.0±4.8 g) and clavate (27.0±4.8 g). Shapes that were lowest pulp producers were spheroid emarginate (22.0±6.7 g), oblong compressed (24.1±4.8 g), ovate (24.8±3.4 g) and obovate (25.4±3.0 g). The mean pulp production was 30.23 g per fruit shape. The range of pulp production was 3.0 g to 130.0 g. The *CV* was 49.9%.

4.1.2.3 Seed weight

Seed weight differed significantly ($P \leq 0.001$) among the fruit shapes (Figure 4.3), with high spheroid (98.1±8.1 g) and oblong (83.4±6.3 g) fruits having the heaviest seed weight. The rest of the shapes were not different. The seed weight ranged from 4 g to 163 g with a *CV* of 47.2%.



Key: CE=Clavate, ED=Ellipsoid, EP=Ellipsoid pointed, FF=Fusiform, OG=Oblong, OP=Oblong pointed, OB=Obovate, OV=Ovate, RD=Rhomboid, GB=Globose, HS=High spheroid, SE=Spheroid emarginate, OC= Oblong compressed.

Figure 4.3: Variation of fruit traits among the fruit shape of *Adansonia digitata*

4.1.3 Variation of Seed Traits among the Fruit Shapes

4.1.3.1 Single-seed weight

A summary of the results of variations in seed weight among the thirteen fruit shapes is presented in Table 4.2. The single-seed weight was significantly different ($P \leq 0.001$) among the fruit shapes. The heaviest seed weight was found in the following fruit shapes: clavate (0.59±0.03 g), spheroid emarginate (0.58±0.04 g), rhomboid (0.55±0.03 g), ellipsoid pointed (0.54±0.02 g), obovate (0.53±0.02 g), oblong (0.52±0.02 g) and oblong compressed (0.51±0.03 g). The lightest single-seed weight was recorded in the ovate (0.43±0.02 g) and spheroid (0.45±0.03 g) fruit shapes. The mean seed weight was 0.50 g with the heaviest seed weighing 0.57 g, and the lightest seed weighing 0.35 g, and the coefficient of variation (*CV*) was 20.7%.

4.1.3.2 Seed length

Results of variations in seed length are presented in Table 4.2. Seed length differed significantly ($P \leq 0.001$) among the fruit shapes, with clavate (12.4±0.2 mm), rhomboid (12.4±0.2 mm) and ellipsoid pointed (12.1±0.1 mm) having the longest seeds, while the shortest seeds were found in ovate (11.1±0.2 mm) and spheroid (11.1±0.2 mm). The average seed length was 11.69 mm, the range was 9.8 mm to 14.2 mm and the *CV* was 6.2%.

4.1.2.3 Seed width

The seed width varied significantly ($P \leq 0.001$) between the fruit shapes (Table 4.2). Seeds in clavate (9.6±0.2 mm, spheroid emarginate (9.4±0.3 mm) and oblong compressed (9.3±0.2 mm) fruit shapes had the biggest width as opposed to seeds in ovate (8.2±0.1 mm) and spheroid (8.2±0.2 mm) fruits, which had the smallest width. The mean seed width was 8.83 mm, the range was 7.1 mm to 11.2 mm and the *CV* was 6.9%.

4.1.2.4 Seed thickness

There were significant variations ($P \leq 0.001$) in seed thickness among the fruit shapes (Table 4.2). Clavate (7.36±0.13 mm), rhomboid (7.32±0.13 mm) and ellipsoid pointed (7.14±0.08 mm) fruits produced the thickest seeds. Fusiform (6.71±0.11 mm) and oblong pointed (6.67±0.13 mm) fruits produced seeds that were less thick. The range of seed thickness was from 5.9 mm to 8.4 mm, and the *CV* was 5.9%.

Table 4.2: Variation of seed traits among the fruit shapes of *Adansonia digitata L*

Fruit shape	Single seed weight (g)	Seed length (mm)	Seed width (mm)	Seed thickness (mm)
CE	0.59±0.03a	12.4±0.2a	9.6±0.2a	7.36±0.13a
ED	0.50±0.02bcd	11.8±0.1bcd	8.8±0.1cd	6.97±0.08bcd
EP	0.54±0.02abc	12.1±0.1ab	9.0±0.1bcd	7.14±0.08abc
FF	0.47±0.03cd	11.7±0.2bcd	8.8±0.2cd	6.71±0.11e
OG	0.52±0.02abc	11.9±0.1bc	9.1±0.1bc	6.98±0.08bcd
OP	0.48±0.03bcd	11.3±0.1de	8.6±0.2de	6.67±0.13e
OB	0.53±0.02abc	11.6±0.2cd	8.8±0.1cd	7.13±0.08abcd
OV	0.43±0.02d	11.1±0.2e	8.2±0.1e	6.90±0.09cde
RD	0.55±0.03abc	12.4±0.2a	8.8±0.2cd	7.23±0.13ab
SD	0.45±0.03d	11.1±0.2e	8.2±0.2e	6.75±0.11de
HS	0.47±0.03cd	11.4±0.2de	8.7±0.2d	6.95±0.11bcde
SE	0.58±0.04ab	11.8±0.3bcd	9.4±0.3ab	7.00±0.18abcde
OC	0.51±0.03abcd	11.5±0.2cde	9.3±0.2abc	6.86±0.13cde
Grand mean	0.50	11.69	8.83	6.98
Minimum	0.35	9.8	7.1	5.9
Maximum	0.57	14.2	11.2	8.4
P-value	<0.001	<0.001	<0.001	<0.001
L.S.D.	0.08	0.55	0.46	0.31
CV (%)	20.7	6.2	6.9	5.9

Note: Mean values are followed by standard error of the mean; Mean followed by different letters within a column significantly differ ($P<0.05$); CE=Clavate, ED=Ellipsoid, EP=Ellipsoid pointed, FF=Fusiform, OG=Oblong, OP=Oblong pointed, OB=Obovate, OV=Ovate, RD=Rhomboid, SD=Spheroid, HS=High spheroid, SE=Spheroid emarginate, OC= Oblong compressed.

4.1.4 Variation in Seed Colour and Seed Shape

4.1.4.1 Seed colour

High seed diversity was found existing in the five populations. Four seed colours (Figure 4.3 and Figure 4.4) were found among the fruit shapes.

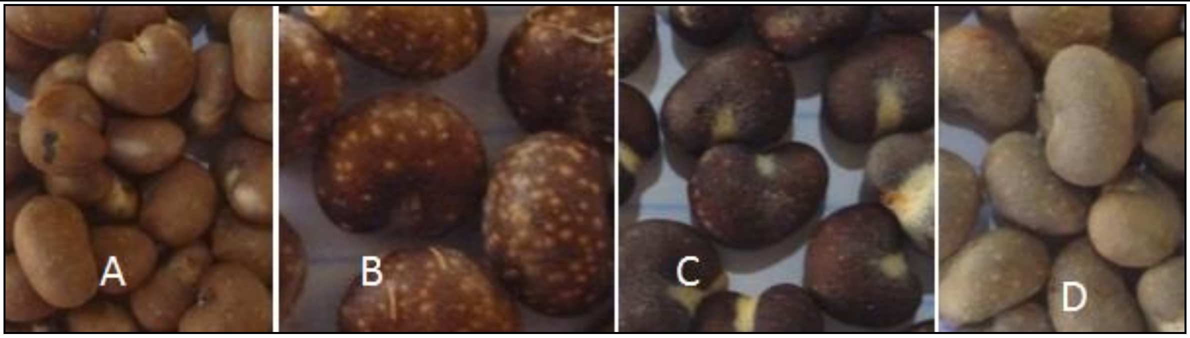


Figure 4.4: Seed colours, A=Light brown, B=Brown, C=Dark brown and D=Greyish brown

The seed colours included the following; brown (46%), dark brown (35%), light brown (16%), and greyish brown (3%).

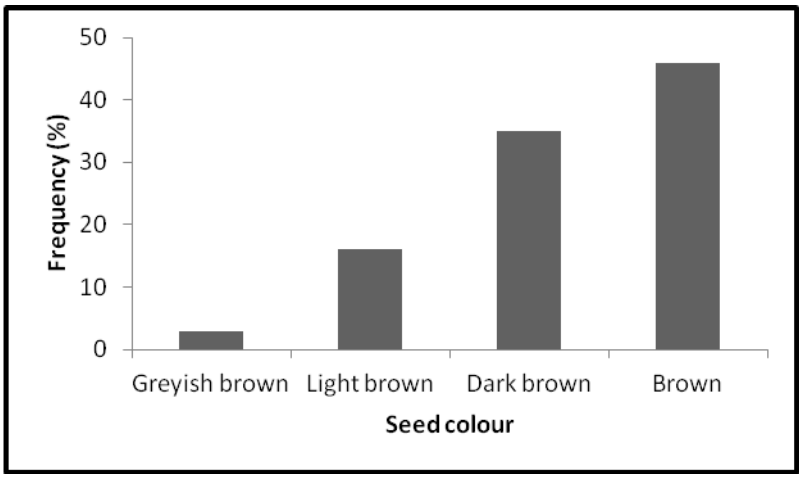


Figure 4.5: Frequency of seed colours among the fruit shapes

4.1.4.2 Seed shape

Three seed shapes were found (Figure 4.5 and Figure 4.6). The predominant shape was reniform (55%) followed by very reniform (42%).

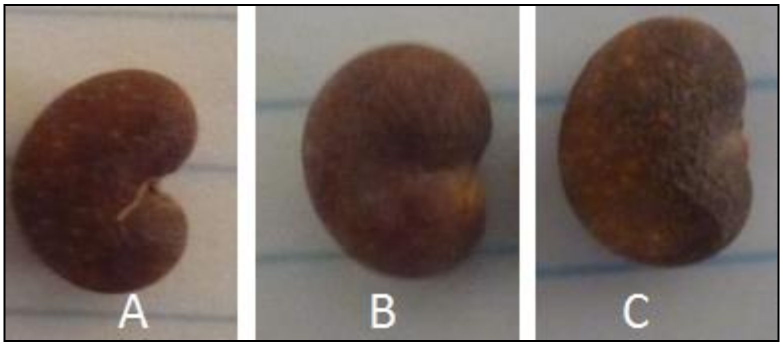


Figure 4.6: Seed shape, A=Very-reniform, B=Reniform and C= Oblong

The least shape was oblong seed (3%). All the seeds had spots in varying density on their seed coats. Light brown seed (A), dark brown seed (C) and greyish brown seed (D) were light spotted while brown seed (C) were densely spotted.

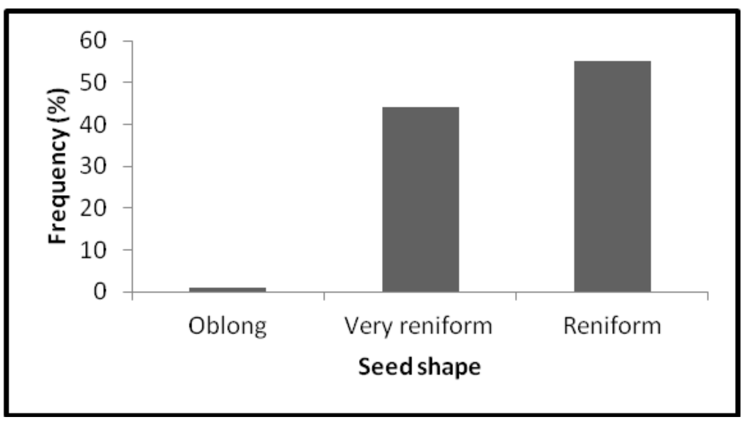


Figure 4.7: Frequency (%) of seed shapes among the fruit shapes

4.1.5 Variation in Baobab Bark, Growth Habit and Trunk

Two bark colours were found. The silver-grey bark trees were preponderant (94%). Reddish-brown bark trees were less common (6%). Two bark textures were found. Smooth bark texture was dominant (92%) whilst rough bark texture constituted 8 % of the trees. Three tree shapes were found. Most of the trees were roundish (56%), followed by semi-circular trees (33%).

Ellipsoid trees were the minority (11%). Three growth habits were found. Spreading growth habit (Appendix 15) was prevalent (65%), followed by dropping growth habit (20%). Erect growth habit (Appendix 14) was the least frequent (15%). Four trunk shapes were found of which three are shown in Figure 4.7. Conical shaped trees were predominant (56%), followed by cylindrical shaped trees (21%) and concave shape trees (17%). Bell-shaped trees were less common (7%).

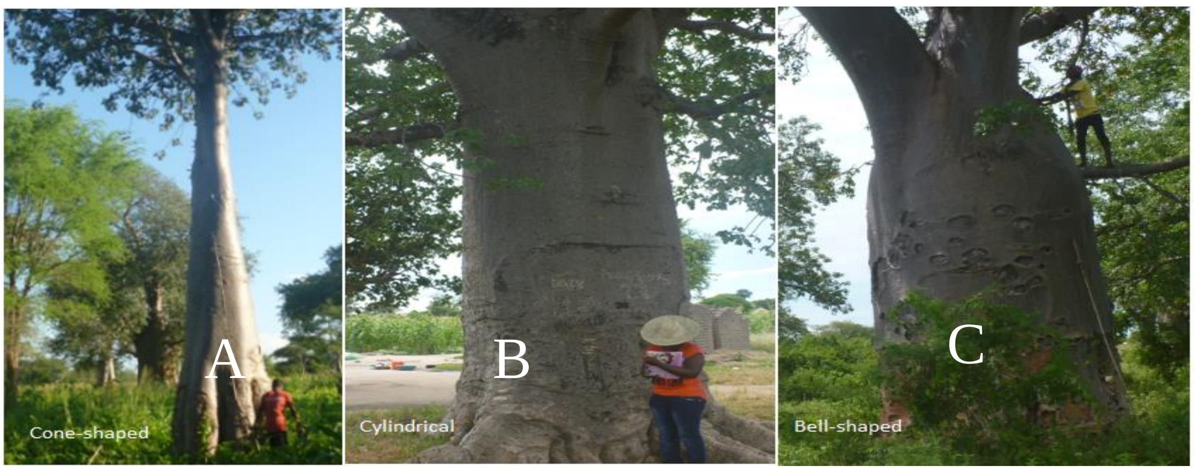


Figure 4.8: Baobab trunks; A= Cone-shaped, B= Cylindrical, C= Bell-shaped

4.1.6 Diameter at Breast Height Growth and Age of baobab Trees

The youngest baobab tree was estimated to have an age of 48 years with a diameter at breast height (dbh) of 104.09 cm, and the oldest baobab tree was estimated to have an age of 180 years with a dbh of 422 cm. Fruiting baobab trees with a dbh of less than 100 cm or more than 450 cm were not found in this study (Figure 4.8) and all the trees were observed to be producing fruits from 48 years of age to 180 years.

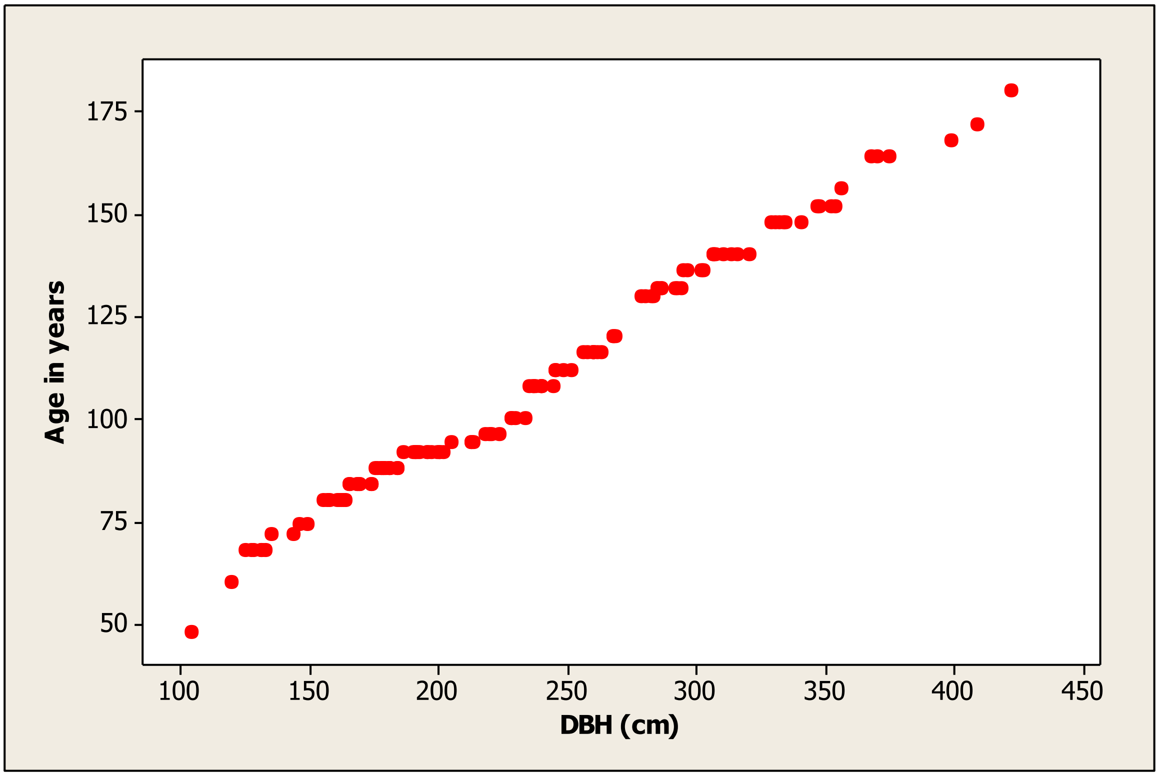
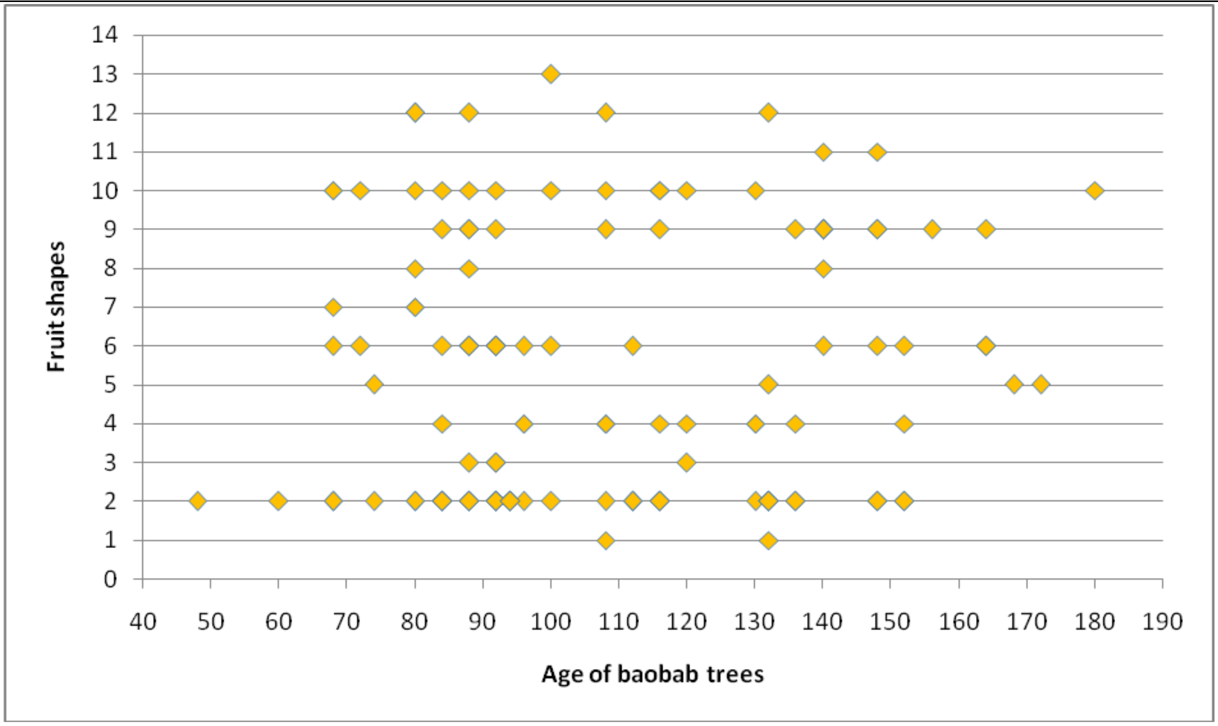


Figure 4.9: Scatter plot of age of sampled trees based on diameter breast height

4.1.7 Existence of Fruit Shapes over Time (Age in Years)

Fruit shapes were found to be distributed across all ages in this study (Figure 4.9). Only oblong compressed shapes were found in trees which were less than 100 years of age while rhomboid shapes were found in trees which were above 130 years of age. Ellipsoid shapes were found in all age categories compared to all other shapes.



Key: 1=Clavate, 2=Ellipsoid, 3=Ellipsoid pointed, 4=Fusiform, 5=High spheroid, 6=Oblong, 7=Oblong compressed, 8=Oblong pointed, 9=Obovate, 10=Ovate, 11=Rhomboid, 12=Spheroid, 13=Spheroid emarginate

Figure 4.10: Existence of fruit shapes across different ages of sampled trees

4.2 Amenability of *A. digitata* to Vegetative Propagation

4.2.1 Variation in Grafting Success between Two Months for Grafting Methods

Results on grafting success of *A. digitata* using two grafting methods; top cleft and side veneer are shown in Table 4.3. There were significant differences ($P \leq 0.003$) (Appendix 9) in grafting success (%) between the two grafting methods (top cleft and side veneer) in the month of October and November. Top cleft in October attained the highest grafting success rate of 66.6 ± 3.33%, whilst side veneer attained 63.3 ± 12.0%. In November, top cleft attained grafting success of 33.3 ± 16.7% whilst side veneer achieved 30.0 ± 17.3 % in the month of November. Mean grafting success in October was 65.00 ± 7.64 % and in November was 31.7 ± 16.4 %.

Table 4.3: Grafting month and grafting method on grafting success of *A. digitata* plants

Grafting month	Grafting method	Grafting success (%)
October (06/10/16)	Top cleft	66.6 ± 3.3*
	Side veneer	63.3 ± 12.0
November (27/11/16)	Top cleft	33.3 ± 16.7
	Side veneer	30.0 ± 17.3

Note: n = 20 per grafting method, * = grafting success is followed by the standard error of the mean.

4.2.2 Variation in Grafting Success between Two Months for Three Ortets

There was significant difference ($P \leq 0.001$) (Appendix 10) in grafting success between ortets (Table 4.4). In the month of October, scions collected from tree 2 produced the best success rate (75 ± 5.00 %) while scions from tree 1 were the least performers (50 ± 10.00%) in terms of grafting success. In contrast, scions from tree 1 performed well (55 ± 5.00%) in November and scions from tree 3 were the poorest with a zero result in the month of November. On average, grafting success in October was 65.0 ± 7.64 % whilst in November was 31.7 ± 16.4 %.

Table 4.4: Variation in grafting success (%) between two months for the tree ortets

Grafting month	Ortets	Grafting success (%)
October (06/10/16)	Tree 1	50 ± 10.00*
	Tree 2	75 ± 5.00
	Tree 3	70
November (27/11/16)	Tree 1	55 ± 5.00
	Tree 2	40 ± 10.00
	Tree 3	0

Note: n = 10 per ortet, * = grafting success is followed by the standard error of the mean

4.2.3 Variations in Growth of *A. digitata* Plantlets after Grafting Success

4.2.3.1 Shoot Growth

Results of shoot growth of *A. digitata* plantlets propagated in the month of October and November are shown in Figure 4.10. There was significant difference (t = 3.62, $P \leq 0.001$) (Appendix 11) in shoot growth attained by grafts propagated in October and November. Shoots for grafts propagated in the month of October (3.0±0.497 cm) out grew shoots of grafts propagated in the month of November (1.21±0.104 cm).

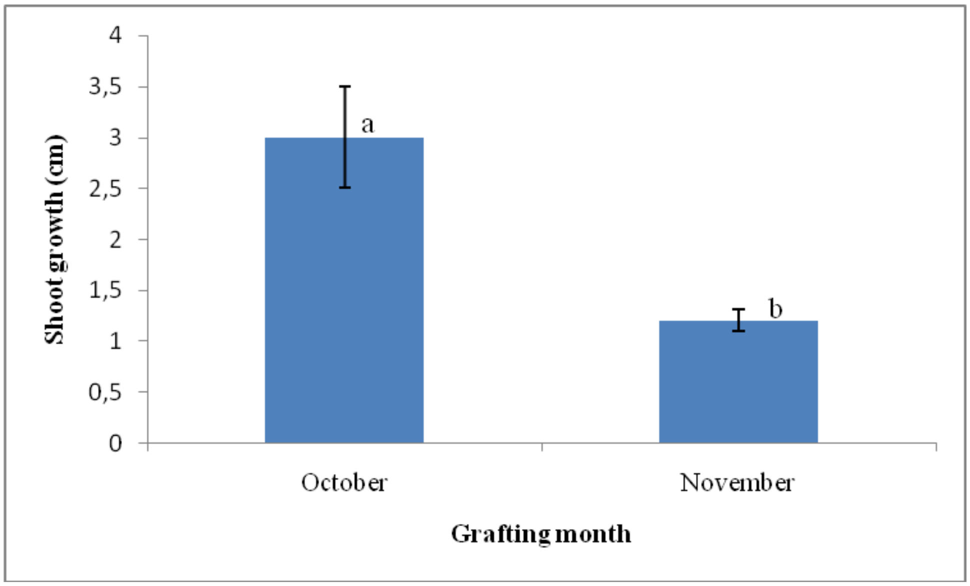


Figure 4.11: Variation of shoot growth between grafting months.

Letters show significant difference ($p < 0.05$) of shoot growth between grafting months.

Results of variations in shoot growth between side veneer and top cleft in the months of October and November are presented in Figure 4.11. No significant difference (t = -0.47, P = 0.640) (Appendix 11) was observed in shoot growth between side veneer and top cleft in the month of October. Shoot growth for side veneer was 3.30 ± 0.7 cm and shoot growth for top cleft was 2.70 ± 0.713 cm. No significant difference (t = -0.40, P=0.690) (Appendix 11) was observed in shoot growth between side veneer and top cleft in the month of November. Shoot growth for side veneer was 1.24 ± 0.166 cm and shoot growth for top cleft was 1.17 ± 0.122.

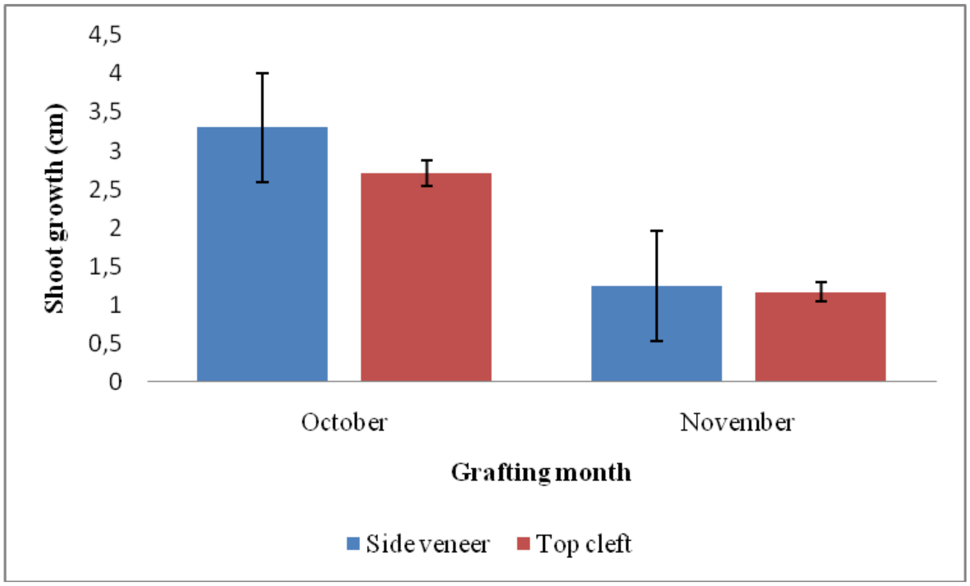


Figure 4.12: Variations in shoot growth between grafting methods

4.2.3.2 Number of leaves

Variation of number of leaves per baobab plantlet between the month of October and November are presented in Figure 4.12. There were significant differences (t = 2.66, P = 0.01) (Appendix 12) in the number of leaves for baobab plants propagated in October and plants propagated in November. Mean number of leaves for baobab plants propagated in October was 5 leaves and 3 leaves for plants propagated in November.

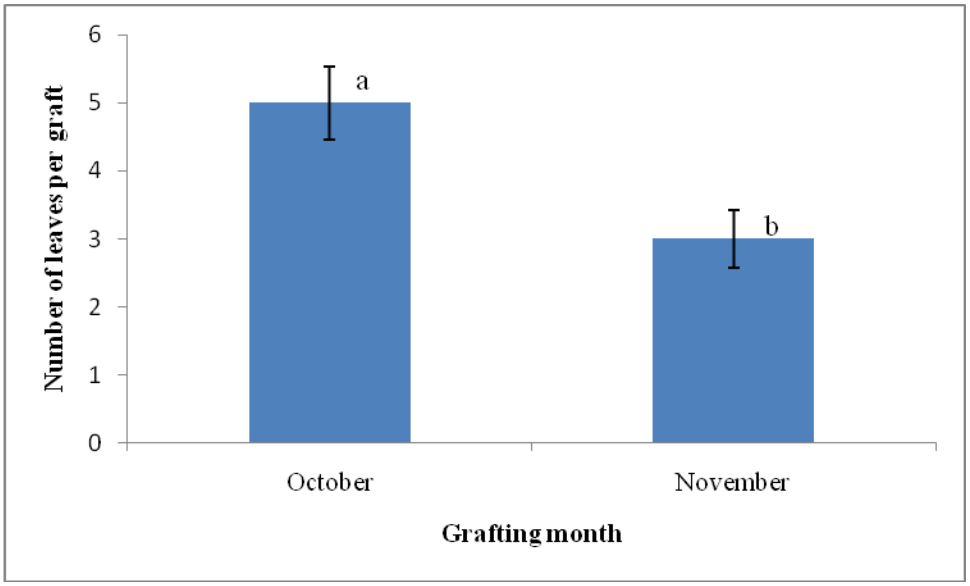


Figure 4.13: Variation of number of leaves per plantlet between grafting months. Letters show significant difference ($p < 0.05$) of number of leaves between grafting months.

Variation results of number of leaves for baobab plantlets propagated using side veneer and top cleft are presented in Figure 4.12. There was no significant difference ($t = -0.59$, $P = 0.562$) (Appendix 12) in the number of leaves for plantlets propagated using side veneer and plantlets propagated using top cleft in the month of October. Average number of leaves for plantlets propagated using side veneer was 4 leaves, and 5 leaves for plantlets propagated using top cleft. There was no significant difference ($t = 0.75$, $P = 0.462$) (Appendix 12) in the number of leaves for plantlets propagated using side veneer and top cleft in the month of November. The mean number of leaves for plantlets propagated using side veneer was 3 leaves, and 2 leaves for plantlets propagated using top cleft.

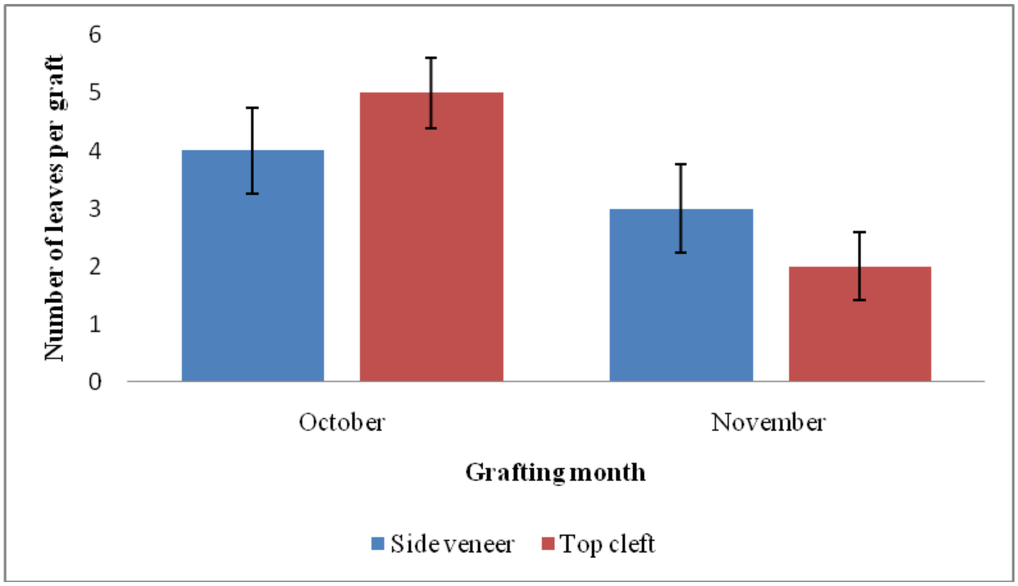


Figure 4.14: Variation of number of leaves per plantlet between grafting methods

4.2.4 Propagation of *A. digitata* by Cuttings

There was no callusing and no rooting of cuttings in all the treatments both in the polythene tubes and poly-propagators. Sprouted cuttings did not produce roots (Figure 4.13).

Figure 4.15: Baobab cutting with shoots but no roots

4.2.5 Propagation of *A. digitata* by Air Layering

Rooting of air layers failed (0%) in both treatments in two month periods of October and November in all the five ortets. There was no success in air layering in this experiment. No roots were observed after five and four months of conducting the trials (Figure 4.14).

Figure 4.16: Baobab air layer without roots five months after establishment

4.3 Mating system in *A. digitata* in Karonga District

4.3.1 Floral Activities of baobab

Events that were captured with a Bushnell wildlife camera with night vision from bud splitting to flowering are presented in Table 4.5. At 16:37 pm an insect was observed on a bud which was close to opening (splitting) (Figure 4.15). By 16:58 pm the bud had started to open (split) (Figure 4.16). The bud was fully opened at around 19:21 pm (Figure 4.17). The flowering took about 2 hours and 23 minutes to reach anthesis (fully open). In the morning (5:15 am) an insects was captured flying near the opened corolla. Further, the pollen dust caused the corolla to appear brownish at 05:15 am. The corolla, anthers, and stigma were vividly separated by 05:15 am (Figure 4.18). No bats were captured in the tree at the time of flowering.

Table 4.5: Events captured during bud splitting into flower

Time	Activity
16:37 PM	Captured insect near the tip of the about to open bud
16:58 PM	Bud starts splitting
19:21 PM	Bud fully open after 2 hours 23 minutes
5:15 AM	Capture insect flying near the opened corolla Brownish anthers may be due to dust of pollen Stigma with three lobes distended outside the anthers
7:53 AM	Closed the observation, no bats visited the anthers during the capturing period after 15 hour 16 minutes

Figure 4.17: Insect visitation of the bud

Figure 4.18: Bud starts splitting

Figure 4.19: Bud completely opened

Figure 4.20: Insect visiting the flower or Insect flying close to the corolla

4.3.2 Variation in Flowering and Fruit Set in Four Mating Systems

The results on baobab flowering and fruit set in four treatments in mating systems are shown in Table 4.6. Buds in all the treatments flowered (100 %). All flowers developed into fruits for control (24) and mosquito net treatments (24), whilst one flower each aborted for cotton cloth (23) and Chicken wire (23) treatments. The highest fruit abortion was in Cotton cloth (95.7 %), followed by mosquito net (87.5%), and chicken wire (78.3%). The lowest fruit abortion was in the control (66.7%). The highest fruit production was in Control treatment (33 %) whilst the lowest was in the cotton cloth treatment (4.3 %). Vigorous fruit growth was only attained in the control treatment (Figure 4.19 D). The other fruits in chicken wire (Figure 4.19 A), covered with cotton (Figure 4.19 B) and mosquito net (Figure 4.19 C) treatments were under-developed and unhealthy (dying) in case of Figure 4.19 B and C.

Table 4.6: Number and percentage of inflorescences (in parentheses) that developed flowers, fruits and aborted of four different mating treatments

Treatment	Number of buds flowered (Score 2 to 5)	Number of fruits formed (Score 7)	Fruits aborted (score 8)	Fruits developed
Chicken wire	30	23	18 (78.3 %)	5 (21.7 %)
Cotton cloth	30	23	22 (95.7 %)	1 (4.3 %)
Mosquito net	30	24	21 (87.5 %)	3 (12.5 %)
Control	30	24	16 (66.7 %)	8 (33.3 %)

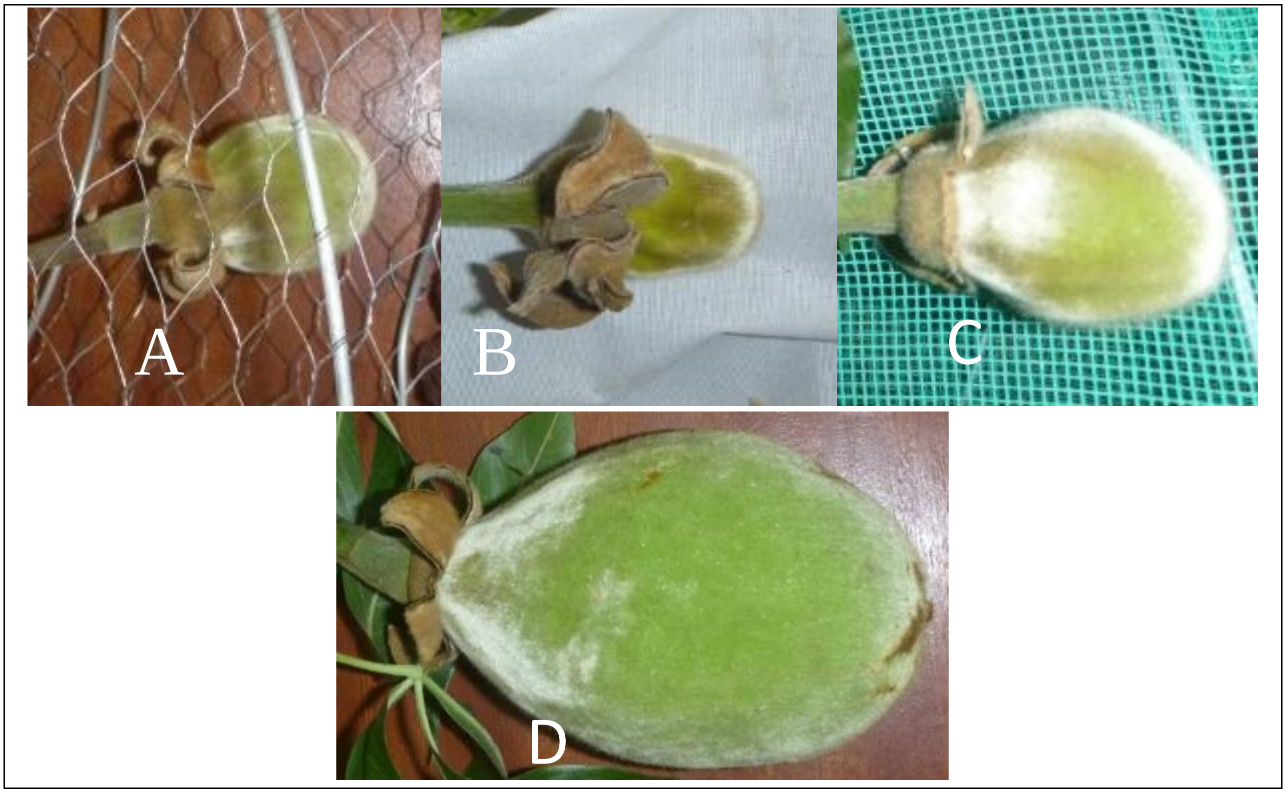


Figure 4.21: Fruit development following different treatments (A=Covered with Chicken mesh wire, B=Covered with cotton cloth, C=Covered with net D=Control (open pollination)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Phenotypic Characterization of Fruit Traits and Tree Descriptors

5.1.1 Diversity and Distribution of Fruit Shapes

The centre of diversity for African baobab is in West Africa (Tsy et al., 2009). The 13 different fruit shapes of ellipsoid, oblong, ovate, obovate, fusiform, high spheroid, globose, ellipsoid pointed, oblong compressed, oblong pointed, rhomboid, spheroid emarginate, and clavate (Figure 4.1) found implied existence of high diversity of fruit shapes for *A. digitate*. The fruit diversity found in the present study has revealed higher diversity than what Wickens (1982) reported previously. Wickens (1982) described baobab fruit in the southern Africa as being uniform (globose or ovoid) which is not the case. The present results imply the existence of high diversity of fruit shapes in Malawi although located far from the centre of diversity (in fringes of diversity). The present results are similar to those reported by Gurashi and Kordofani (2014) and Gebauer and Luedeling (2013) in Sudan. Gurashi and Kordofani (2014) reported 12 fruit shapes namely ellipsoid, high spheroid, ovate, obovate, oblong pointed, fusiform, globose, spheroid emarginate, ellipsoid pointed, clavate, and crescent shaped. Oblong compressed and rhomboid reported in Malawi have not been reported in Sudan by Gurashi and Kordofani (2014). The findings may confer that the alleles controlling fruit shape are widely distributed in the species distribution range. It could also be hypothesised that the founder effect has had very little effect on the gene frequency controlling fruit shape as the species has been spreading. In addition it could be suggested that minimal or no genetic drift has affected the population in fruit shape as the species has been spreading away from the centre of diversity (colonising new places). The occurrence of the fruit shapes in far distant areas on the globe could be a demonstration of strong genetic control of the trait (fruit shape). Currently there is need to investigate the inheritance pattern of the trait focusing on simple dominance effect, additive genetic effect and epistasis.

The present study has revealed ellipsoid fruits being the most prevalent across the five sampled populations in high frequencies (Table 4.1). Similar results have been reported by Gurashi and Kordofani (2014) in Sudan with ellipsoid fruits being the most abundant fruit in North Kordofan region. Suggesting that the alleles controlling ellipsoid shape might occurr at highest frequency across the entire species' geographical range. One fruit shape (spheroid emarginate) occurred in a specific site, thus Mangochi only. The variation in occurrence of the fruit shapes may signify the existence of rare alleles in operation in different sites. Rare alleles could have evolved due to mutations in different ecological conditions.

Fruit shape was consistent in individual trees. For instance, no two fruit types were found in a single tree, in the study period. Such results are subtracting the effect of simple dominance effect controlling the shape of the fruits. The inheritance pattern in the species appears to be complex in nature. However, it portrays high maternal effect on the fruit shape. Results could be utilised in clonal forestry where special ideotypes are envisaged in agroforestry systems. The results may also mean that through vegetative propagation, such as grafting which has been successful, the genotype could be maintained. Fruit characteristics have also been reported to be influenced by environmental factors (Zunzunegui et al., 2010). The present results suggest low or minimal environmental influence on the fruit shape. This is vital because it shows that once seed is moved from one area to another, fruit production may not significantly change. Usually in horticulture, seedling production for mass propagation is discouraged due to aberration (segregation of the seed) which causes the quality of products to vary from one generation to another. Fortunately the present results show that to be the opposite for baobab. There is still need to comprehend the inheritance pattern in fruit shape as it controls both qualitative and quantitative traits. Gene mapping could be employed to exactly identify the alleles that control the fruit shape. The other question that requires an answer is how much tree differentiation there is between trees of different fruit shapes from different localities. The present results are different from the findings in Benin where low capsule variation was found (Assogbadjo et al., 2011). Perhaps low variation in Benin is a result of founder effect or genetic drift which tends to favour certain allele combinations. The results here found Neno, Chikwawa and Karonga with highest fruit diversity than Salima and Mangochi. The variation from one locality to other could be attributed to founder effects, natural selection, anthropogenic effects (human selection) and mutation forces (Stuessy et al., 2014; Saro et al., 2015). For instance, anthropogenic intervention through selection could have existed on baobab because most trees are relics in farm land. Thus, preferred fruit types could have been retained for human consumption. Furthermore, baobab grows in cotton growing areas where it has been associated as a host for the pests of the crop. Likely, some un-preferred trees could have been felled to give way for the crop causing un-intended directional selection in fruit characteristics. Another parameter concerning fruit shape is the influence of “male” trees in the populations. Male trees usually flower massively in synchrony (same time with female trees that produce fruit abundantly) and yet they themselves do not produce fruits. Genetically, the contribution of pollen (genes) from the “male” trees to fruit shape diversity is not known. This knowledge is required to manage the wild populations and domesticated trees. Otherwise, currently, “male” trees are considered useless in the ecosystem where pressure for agricultural land is unbearable. The “male” trees do not produce fruits despite having hermaphroditic flowers. Further, they do mimic the other normal female trees in flowering behaviour.

5.1.2 Variation of Fruit Traits among the Fruit Shapes

Largest variations have been found to exist for fruit traits assessed (fruit weight, pulp weight, seed weight) among fruit shapes. Taking the best and lowest mean values of the fruit traits, the difference was of high magnitude: seed weight (4,075%), pulp weight (3,433.3%) and fruit weight (1,850%). The presence of high values of coefficient of variation in fruit weight (38.9 %), pulp weight (49.9 %) and seed weight (47.2 %), implied that there is a lot of variation in the wild population. Similar variation has been reported on baobab fruit weight among fruit shapes in Sudan (Gurashi and Kordofani, (2014). The presence of variations provides the possibility to derive improved cultivars from the wild populations for the purposes of domestication (Gouwakinnou et al., 2010). In the process of domestication, the observed variability may help to identify and select new cultivars through a selection system which may target commonly used fruit components of the species i.e. pulp weight and seed weight (Bantiono et al., 2008). Pulp was best produced by high spheroid and oblong fruits while seeds were best produced by high spheroid fruits. Therefore, appropriate elite trees bearing high spheroid and oblong fruits can be selected for mass production in order to increase production of pulp and seeds.

5.1.3 Variation of Seed Traits among Fruit Shapes

Variations have also been uncovered in seed traits (Table 4). Considering the best and the lowest mean of the four seed traits, the study observed that the greatest variations occurred in single seed weight (162.9%), followed by seed width (157.7%), and the least variation was observed in seed length (144.9%) and seed thickness (142.4%). Variations in seed length, seed width, and weight among populations have previously been reported to exist in other species such as *Cordia Africana,* (Loha et al., 2006), *Albizia lebbek* (Bhat and Chauhan, 2002), *Pongamia pinnata* (Shivanna et al., 2010) and *Dalbergia melanoxylon* (Amri, 2014). High coefficient of variation

(CV=20.7%) in single-seed weight shows there is high variation in the wild population on this trait. Hence, both genetic and environmental factors are controlling this trait. Linkies et al. (2010) reported seed weight being strongly influenced by environmental factors such as soil, temperature, light quality, water availability, and altitude. Environmental factors in turn influence the ability of the plant to provide resources to embryos (Tremayne and Richards, 2000). Low coefficients of variation for seed thickness (5.9 %), seed length (6.2 %) and seed width (6.9 %) shows there is low variation in the wild population probably due to strong genetic effects (Munthali et al., 2012).

5.1.4 Variation in Seed Colour and Seed Shape

Results from this study have revealed the presence of four seed coat colours. Seed coat colours mostly reported are dark brown and reddish black (Kehlenbeck et al., 2015; Rahul et al., 2015). In Benin, Assogbadjo, (2008) found three seed coat colours though no colour names were mentioned. More seed coat colours have been reported to be found in Sudan namely: black, violet, brown, dark brown, reddish and white brown (Gurashi et al., 2017). Therefore, Sudan and Malawi have baobab which is diverse in seed coat colours. More than four seed coat colours have been reported in other plant species such as soybean (Song et al., 2016). In soybean seed coat colour can be used to predict presence of phytochemicals. Black or brown seeds are said to accumulate flavonoids and anthocyanins within the epidermal layer of the seed coat (Dixon and Sumner, 2003). Therefore, further research in baobab should look at the possibility of using seed coat colour to predict antioxidant properties of the species. The antioxidants play a role in maintaining optimal cellular functions and thus systemic health and well-being (Kurutas, 2015). Seed coat pigmentation plays an important role in seed dormancy and germination (Ochuodho and Modi, 2008) in other species but is not yet tested in baobab. In *Vicia sativa*, seed coat colour is used to distinguish between hard-seeded and soft-seeded varieties (Büyükkartal et al., 2013). Seed coat colour in *Brassica rapa* is used to predict oil content in seed (Bagheri et al., 2013). Further research in baobab should evaluate the possibility of determining seed oil quantity and quality using seed coat colour. Descriptors of baobab by Kehlenbeck et al. (2015) describe three seed shapes of baobab seeds. Similarly our study has found the three seed shapes to exist in Malawi. This may imply that Malawian population might have a full range of alleles controlling seed colour.

5.1.5 Variation in Baobab Bark, Growth Habit and Trunk

The result showed that bark colour in Malawi is not diverse as compared to other parts of Africa. In Burkina Faso and Mali five different bark colours have been reported namely: dark grey, grey, light grey, reddish, and reddish grey (Parkouda et al., 2012). Variations were also observed on other baobab tree characteristics such as bark texture, tree shape, growth habit, and trunk shape. Variations in bark colour could be attributed to founder effects and natural selection. The variations in other tree characteristics such as bark texture, tree shape, growth habit and trunk shape apart from being described in baobab descriptors by Kehlenbeck et al. (2015) have not been studied elsewhere. Therefore, it is difficult to say how rich the diversity is for these other baobab characteristics in Malawi. However, the presence of variation within the species is of paramount importance, indicating a great potential for further improvement through the development of cultivars from elite trees using horticultural techniques (Leakey et al. 2008).

5.1.6 Diameter at breast height growth and age of baobab trees

All the sampled (assessed) trees were above 48 years of age. The oldest trees were about 180 years old. The population is considered young (Dhillion and Gustad, 2004) since baobab can live for 1,275 years (Patrut et al., 2007). Thus, the trees still have a long way to go producing fruits if nothing catastrophic occurs. In southern Africa, baobab trees should start fruiting at about 8 to 23 years (Sidibé et al., 1996; Sidibé and Williams, 2002). Present findings did not sample fruits from very young trees. This could be as a result of poor natural regeneration of the species as manifested by populations having positively skewed stem diameters (Venter & Witkowski, 2010). This is a matter of concern for sustainable production of the fruits. Large trees are dying due to wild fire or tumbling from wind pressure (Gebauer et al., 2016). Hence, there is need to artificially increase the number of trees.

5.1.7 Existence of Fruit Shapes over Time (Age in Years)

Ellipsoid fruit shape has been the common fruit shape in all age categories in this study. The dominancy of ellipsoid fruit shapes suggests that this shape might be the original fruit shape of the baobab tree. Presence of different fruit shapes (Figure 4.1) may be due to mutation, which could have changed the genes controlling the occurrence of fruit shapes in the baobab. For instance, in tomato, changes in fruit shape are contributed by mutations in four genes: *SUN* and *OVATE* regulating fruit elongation, and *LOCULE NUMBER* (*LC*) and *FASCIATED* (*FAS*) regulating locule number and flat fruit shape (Rodríguez et al., 2011). It will also be necessary in future to determine genes in *A. digitata* that could be responsible for fruit shapes and also evaluate the mutations. Movement of germplasm from its original place may have caused mutation to happen. In tomato, domestication has seen the emergence of variable fruit shapes (Paran and van der Knaap, 2007). In this study, baobab trees with the age of between 48 and 60 years have only ellipsoid fruit shape. While other fruit shapes appear after 60 years. It is not known why other fruit shapes are coming in after 60 years of age. Therefore, future studies are critical to investigate the alleles that actually control fruit shape and the inheritance pattern. The knowledge will be important in baobab breeding program.

5.2 Amenability of *A. digitata* to Vegetative Propagation

5.2.1 Variation in Grafting Success between Grafting Methods

The study found that baobab is amenable to grafting techniques. Both methods (top cleft and side veneer) were successful in the month of October. The level of success reported here for the month of October (top cleft (66.6%) and side veneer (63.3%) is in agreement with what Anjarwalla et al. (2016) reported for top cleft (71%) and side veneer (55%). In contrast Kalinganire et al. (2008) reported a greater success rate of 85% for both top and side grafting methods. Success rate of top cleft (80%) has also been reported to be greater than side veneer (50 %) for *Allanblackia parviflora* in Ghana (Ofori et al., 2008). In this study the success rate of top cleft grafting method may be attributed to the fact that it is easy to make a cambium to cambium fusion between the scion and the rootstock (Mannan et al., 2006; Kalinganire et al., 2008). In many species, top cleft has been reported to be successful than any other grafting methods (Hibbert-Frey et al., 2010). The reasons why top cleft is more successful are not clear and further research is required to explore the phenomenon. High grafting success rate has also been attributed to reduced dehydration at the grafting union (Hartmann et al., 2002).

The grafting success for October was 51 % better than that of November for both top cleft and side veneer. Akinnifesi et al. (2008) reported the best time for conducting grafting and scion collection to be from August to December for baobab in Southern Malawi. Taylor et al. (1996) found September and October as the best time for grafting *Sclerocarya birrea*. The results here have found that October is the best time for grafting baobab with scions from Karonga comparing to November. Baobab populations in the country differ in their phonological events. Successful grafting in October could be as a result of an accumulation of auxin in the trees prior to shooting. Accumulation of auxin is effective in inducing differentiation of the vascular elements in the tissues (Hartman et al., 2002). Starting of meristematic activity helps the scion rootstock union to be established quickly (Sanou et al., 2004; Yelleshkumar et al., 2010). Therefore, further research should help find appropriate time for grafting baobab in different populations.

5.2.2 Variation in Grafting Success between Ortets

Grafting success varied from one ortet to another. The difference could be genetic or environmental effects. Zero grafting in case of tree three in November is suspected to be due to small scions that were used. The observation showed that scions about (80 mm) have higher success of grafting than small scions (60 mm). Similarly, Anjarwalla et al. (2016) found variation in grafting success from one ortet to another in Kenya. The authors attributed variation in grafting success between ortets to better compatibility of the mother tree with the rootstock based on some genetic differences between the individual trees. The differences in grafting success among ortets could also be attributed to differences in the age of the tree, although that has not been measured in this study. Ortets with high grafting success are hypothesized to have higher cellular activities (Hartmannet al., 2002).

5.2.3 Variation in Growth on *A. digitata* Plants after Grafting Success

Significant difference in shoot growth and number of leaves has been observed only between the grafting months. Obviously, this could be due to differences in the time of grafting. October grafts attained the highest growth in shoot length and number of leaves due to early establishment as compared to November grafts and presence of nutrition in the stock (Akinnifesi et al., 2008). The absence of significant differences in shoot growth between the grafting method and scion source is in agreement with the findings of Anjarwalla et al. (2016) in Kenya. The authors have observed that this trait is highly variable by nature, due to the differences in phenological stages and conditions of rootstocks and scions at the time of grafting. Therefore, in most grafting studies shoot growth (shoot length and number of leaves) is not put into consideration.

5.2.3 Propagation of *A. digitata* by Cuttings

Rooting failed in all treatments for cutting trial. Assogbadjo (2008) and Kalinganire et al. (2008) reported that it is possible to vegetatively propagate baobab through cuttings. In the present study the finding is that propagation of baobab through cuttings is a challenge. The time for scion collection was the right one, soon before shooting. Furthermore, rooting was tested at favourable environmental conditions in the poly-propagator and polythene tubes in the nursery. The expectation was that rooting would take place as reported in literature. The current findings may imply that baobab may not root easily as portrayed in literature. More investigation is supposed to be done on quality of scion such as age of ortet and position of cutting. Rooting hormones enhance rooting in difficult to root plants (Rufai et al., 2016; Hammo et al., 2015; Hossain et al., 2013; Trevisan et al., 2008). Thus, type and concentration and mode of application of hormones should be tested in rooting cuttings in baobab. Furthermore, rooting medium also influences rooting of cuttings (Konto, 2016; Eed and Burgoyne, 2014; Amissah and Monney, 2012; Trevisan et al., 2008). Different rooting substrates should be investigated in rooting of baobab. Akinnifesi et al. (2008) have reported that most Miombo indigenous fruit tree species root easily with juvenile stem cuttings than mature cuttings. Assogbadjo (2008) also reported successful rooting from pruned branches of the 24 month old baobab seedlings. The cuttings in this study were collected from mature baobab trees. Thus, age of the ortet could be a hindrance in rooting in the present study. However, it is difficult to base easiness of vegetative propagation of baobab based on research done on seedlings. Seedlings are immature and mass propagation of baobab plants through seedlings cannot solve problems of long juvenile stage (8-23 years) which is a major factor discouraging people from artificially planting baobab through seedlings. Research should be carried out to find out the rooting of “juvenile scions” collected from pruned branches. Furthermore, since it is possible to easily propagate baobab through grafting, orchards could be established and designed for scion collection for vegetative propagation through cuttings. Breakthroughs on cutting propagation will ease propagation because grafting requires a lot of resources and grafting experts.

5.2.4 Propagation of *A. digitata* by Air Layers

Although air layering is taken as a viable technique for species that have difficulties in rooting (Entelmann et al., 2014), in this study, neither callus nor roots were formed with moss or sub-soil as substrates. It was observed that in some cases the wound healed very quickly instead of callusing or forming roots. Healing may be attributed to retention of strands of bark, during bark removal (Appendix 1), which may accelerate the re-growth of the bark (Mialoundama et al., 2002). Factors such as stage of development of the plant, location in the branch where rooting will be induced, carbon/ nitrogen ratio (C/N) and environmental factors dependent on the time of implementation, as well as the substrate used (Ligarreto-Morenoet al., 2013) have been reported to assist in air layering success. Further research should look into these factors in order to increase the chances of success in air layering. Application of auxin (rooting hormones) could also improve the success rate (Brennan and Mudge, 1998) but no auxin was used in the current study.

5.3 Mating system in *A. digitata*

5.3.1 Floral Activities of *Adansonia digitata*

The results show that anthesis takes place around 16:58 to 19:21 pm. At these hours you would be expecting visitation of bats if the species is pollinated by bats. No other animal was captured landing or visiting the anthers and stigma. This casts doubts on pollination by insects, bats, as reported by Baum (1995) and Djossa et al. (2015). Around 5:00 am, an insect was captured flying close to the corolla (Figure 4.18). It is not possible to conclude on the role of the insect. Munthali (2012), reported several types of diurnal insect species that visit the flower but not necessarily landing on the stigma. The research should continue to record the insects that visit .m of insects that hatched out of mature fruit. It was suspected that the pest laid eggs at the time of flower opening. There is need to exactly know what time the insects invade the flower. Results from the camera showed insect visitation near the tip of the about to open flower at 16:37 pm (Table 4.5). Furthermore, Munthali (2012) noted that insects dwell in an about to open flower. We presume such tiny ants could not be captured even with a sophisticated camera. Therefore, there is need to find out if the ants are the major pollination agents of the baobab flowers, their role in a mature flower and during inflorescence development when do they enter the baobab flower. The results of the camera supports the findings of Munthali (2012) and Venter (2012) that bat pollinators are not the only agents of pollination in baobab species. The present results cannot yet conclusively determine the pollinators of baobab in Karonga. Further, the results show that bracket recommendations on pollination agents of plant species growing in different localities have little or no merit. From the present results, no nocturnal flower visitors were captured in the tree and flower itself. Results could be pointing at the possibility of wind pollination as a major pollination agent in the species.

5.3.2 Fruit Set Produced by Different Mating System of *Adansonia digitata*

Fruit development was observed in all the four treatments. However the quality of fruits varied among the treatments. Optimum fruit development was obtained in control treatment (flower not obstructed) (Figure 4.19). Poor fruits developed in treatments with chicken wire (Appendix 8), mosquito net, and cotton cloth. The present results supports the results of Munthali (2012) who found normal fruit growth in the control (unobstructed) whilst in mosquito net, fruit development was suboptimal. Munthali (2012) found out that in cotton cloth covered treatments, there were no fruits (100 % fruit abortion). It is currently unclear as to why there is suboptimal fruit growth in obstructed treatments. Maybe it could be as a result of inadequate cross-pollination due to pollinator visitation patterns (Venter et al., 2017). The results cast doubts as to whether wind is the effective pollinator of the species. The only major conclusion that can be drawn is that the species seem to be self-incompatible, which sets in late (Munthali, 2012). At present, the pollination mechanism of baobab species still remains a myth in Malawi. Future studies should continue using a camera to capture nocturnal and diurnal floral visitors for many days. Secondly, it is proposed that controlled pollination should be tested to see the effect of cross pollination. Massive flower and fruit abortion seen in most trees could be attributed to self-pollination. A lot of pollen dust close to the elongated stigma was seen in a picture after anthesis (Figure 4.18). Maybe the species is able to control self-pollination by having the stigma mature at different time with the pollen grain. Wickens and Lowe (2008) found similar results in *Adansonia gregorii*, un-pollinated and self-pollinated flowers aborted within 38 days, while 75% of cross-pollinated flowers had optimal fruit growth. Pollination studies in *Tectona grandis*, which is also hermaphroditic, had similar findings where no self-pollinated flowers develop into mature fruits, but many fruits, developed to different sizes before they were aborted (Tangmitcharoen and

Owens, 1997). The understanding of mating system is fundamental for the management and improvement of the species. Further, the knowledge is vital to comprehend how baobab interacts with other organisms in the ecosystem.

It has been reported that in higher plants such as *A. digitata*, self-incompatibility is a mechanism to avoid inbreeding (del Carmen Mandujano et al., 2010). Treatments that were left for natural pollination produced optimal growth, manifesting importance of effective pollination in the species. Bud treatments covered with chicken wire, mosquito net, and cotton cloth had suboptimal growth showing the need of maintaining the ecosystem balance if the species is to continue producing fruits. However, the present study is unable to pinpoint the exact hindering factor in chicken wire, mosquito net, and cotton cloth treatment that caused stunted fruit growth. Djossa et al. (2015) attributed limited pollination success in *A. digitata* for caged flowers being due to self-pollination and cross-pollination by insects other than bats. In this species the effective pollinators are reported to be fruit bats (Baum, 1995). In our results, it is doubtful that normal fruit growth was as a result of bat pollination since the Camera did not capture any visitation. In our results high abortion rate of developed fruits within the first few months could indicate strong presence of endogamic depression (Hernández-Montero et al., 2016) especially for treatments which did not give chance to out-crossing such as obstructed buds with mosquito net and cotton cloth.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results of this study have revealed rich diversity in fruit shapes which compare well with other populations in Sudan and Kenya. The results may imply that the fruit characteristics may have not been affected much with founder effect, genetic drift, and mutation as it has been spreading from the centre of diversity in West Africa. This means that Malawi has fruit types on which baobab trade can flourish. The other advantage is that the country has enough diversity upon which a domestication process can be dependent. Furthermore, the results seem to show no need for major infusion of breeding materials once a tree breeding programme is initiated. The diversity in fruit shape was variable from one locality to another, but the difference was not significant. Most of the fruit shapes occurred in multiple localities. There was no population that contained all the fruit types. Differences in diversity at population level means that all the populations should be conserved since they harbour different alleles controlling fruiting in the species. The study has not established the cause of the fruit variation at population and individual levels. However, the difference could strongly be attributed to genetic effects because it was observed that, within the same microclimate, different fruit types were found. This eliminates the previous hypotheses that fruit characteristics were heavily dependent on climatic conditions, especially the amount of rainfall. Further studies should be done to understand the inheritance pattern of fruit shape by looking at simple dominance, over dominance, incomplete dominance, or epistasis and additive genetic effect (polygenic). It is envisaged that, through gene markers, different alleles in action could be identified in a fruit shape gene. A lot of variation in seed shape and colour has been discovered in the study. At present, the genetic role on the seed traits in population structuring, especially adaptive, reproductive behaviour and nutrition is yet to be understood. Although the age profile of the fruiting trees could be classed as young trees in the study, there is need to deliberately multiply the trees rather than continue relying on the ageing trees for the booming baobab trade at national and international levels.

The results have shown possibility of vegetatively propagating baobab through both top cleft and side veneer grafting in October soon before bud burst. The technology is not very involving such that it could be disseminated to farmers for them to mass propagate the species. Vegetative propagation will see reduction in precocity period to 3-4 years from about 8-23 years. So far, propagation through cuttings and air layers has challenges to be solved before the technologies could be deemed achievable. Results in reproductive biology have shown the importance of effective pollination to achieve optimal fruit growth. Any obstruction to pollination results in suboptimal baobab fruit growth. This sends out a serious warning against any disturbance to ecosystem services, otherwise baobab productivity will be adversely affected. Unfortunately, the present study cannot conclusively ascertain the pollination agents that attain optimal growth. Study of pollinating agents of baobab requires systematic investigation to achieve a breakthrough on reproductive biology (mating system) in Malawi. The information is needed for proper management of the wild and planted trees and tree improvement.

6.2 Recommendations

1. Rooting hormones should be tested in rooting of cuttings and air layering.
2. Pollinating agents for baobabs occurring in different localities should be identified in Malawi.

3. Genetic markers should be used to identify alleles that control fruit shapes.
4. The relationship between tree trunk characteristics and fruit characters should be investigated.
5. The contribution of "Male" trees to population structure (genetic and morphometric) should be studied.

6.3 Limitations of the study

1. No meteorological data was collected during the study period. This data could have been important in regulating the frequency of watering in air layering trials in Karonga District.
2. Studies on fruit shapes were only done in one season and only at the peak of fruit maturity. Had it been that it was done towards the end of the peak period or in two succeeding seasons, may be other fruit shapes could have emerged in a single tree.
3. Propagation by use of cuttings and air layers were done without the use of hormones. May be the use of hormones could have enhanced rooting of both cuttings and air layers.

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APPENDICES

(Photo: Jenya, 2016)

Appendix 1: Removing bark on a baobab branch for air layering trial

(Photo: Jenya, 2016)

Appendix 2: Air layers in a baobab tree using sub-soil and moss as a substrate

(Photo: Jenya, 2016)

Appendix 3: A Baobab cutting sprouting shoots in a polythene tube

(Photo: Jenya, 2016)

Appendix 4: Successful Baobab grafts

(Photo: Jenya, 2016)

Appendix 5: Baobab cuttings in a non-mist poly-propagator

(Photo: Jenya, 2016)

Appendix 6: Night vision camera used to capture floral activities of Baobab bud splitting

(Photo: Jenya, 2016)

Appendix 7: Baobab bud caged with a net to deter insects and mammal pollination

(Photo: Jenya, 2016)

Appendix 8: Baobab fruit being developing in a chicken wire cage

Regression analysis

Response variate: Compatible
Binomial totals: Total_trees
Distribution: Binomial
Link function: Logit
Fitted terms: Constant + Grafting_date + Grafting_method + Grafting_date.Grafting_method

Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	3	13.76	4.586	4.59	0.003
Residual	8	25.48	3.185		
Total	11	39.24	3.567		

Dispersion parameter is fixed at 1.00.

Appendix 9: Analysis of deviance for of grafting methods

Regression analysis

Response variate: Compatible
Binomial totals: Total_trees
Distribution: Binomial
Link function: Logit
Fitted terms: Constant + Grafting_date + Scion_source + Grafting_date.Scion_source

Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	5	37.122	7.4244	7.42	<.001
Residual	6	2.116	0.3527		
Total	11	39.238	3.5671		

Dispersion parameter is fixed at 1.00.

Appendix 10: Analysis of deviance for ortets

Paired T-Test and CI: October, November (Shoots)

```
Paired T for October - November

                 N      Mean     StDev   SE Mean
October         60     1.917     2.866     0.370
November        60     0.467     0.676     0.087
Difference      60     1.450     3.105     0.401

95% CI for mean difference: (0.648, 2.252)
T-Test of mean difference = 0 (vs not = 0): T-Value = 3.62 P-Value = 0.001
```

Paired T-Test and CI: Top cleft, Side veneer (October shoots)

```
Paired T for Top cleft - Side veneer

                 N      Mean     StDev   SE Mean
Top cleft       30     1.733     2.791     0.510
Side veneer     30     2.100     2.975     0.543
Difference      30    -0.367     4.247     0.775

95% CI for mean difference: (-1.952, 1.219)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.47 P-Value = 0.640
```

Paired T-Test and CI: Top cleft, Side veneer (November shoots)

```
Paired T for Top cleft - Side veneer

                 N      Mean     StDev   SE Mean
Top cleft       30     0.433     0.626     0.114
Side veneer     30     0.500     0.731     0.133
Difference      30    -0.067     0.907     0.166

95% CI for mean difference: (-0.405, 0.272)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.40 P-Value = 0.690
```

Appendix 11: T-test analysis for shoot growth

Paired T-Test and CI: October, November (leaves)

```
Paired T for October - November

                  N      Mean     StDev   SE Mean
October          60     4.633     4.129     0.533
November         60     2.567     3.301     0.426
Difference       60     2.067     6.014     0.776

95% CI for mean difference: (0.513, 3.620)
T-Test of mean difference = 0 (vs not = 0): T-Value = 2.66 P-Value = 0.010
```

Paired T-Test and CI: Top cleft, Side veneer (October leaves)

```
Paired T for Top cleft - Side veneer

                  N      Mean     StDev   SE Mean
Top cleft        30     4.367     4.081     0.745
Side veneer      30     4.900     4.229     0.772
Difference       30    -0.533     4.981     0.909

95% CI for mean difference: (-2.393, 1.327)
T-Test of mean difference = 0 (vs not = 0): T-Value = -0.59 P-Value = 0.562
```

Paired T-Test and CI: Top cleft, Side veneer (November leaves)

```
Paired T for Top cleft - Side veneer

                  N      Mean     StDev   SE Mean
Top cleft        30     2.867     3.371     0.615
Side veneer      30     2.267     3.258     0.595
Difference       30     0.600     4.407     0.805

95% CI for mean difference: (-1.046, 2.246)
T-Test of mean difference = 0 (vs not = 0): T-Value = 0.75 P-Value = 0.462
```

Appendix 12: T-test analysis for number of leaves

(Photo: Jenya, 2016)

Appendix 13: Measuring seed length using a micro calliper

(Photo: Jenya, 2016)

Appendix 14: Baobab tree growth habit (erect)

(Photo: Jenya, 2016)

Appendix 15: Baobab tree growth habit (spreading)