SEXUAL AND ASEXUAL PROPAGATION TECHNIQUES FOR DOMESTICATION OF *ALLANBLACKIA STUHLMANNII* (ENGL.) ENGL. IN NORTH-EASTERN TANZANIA

MOSES RUKWARO MUNJUGA

MASTER OF SCIENCE

(Landscape Planning and Conservation)

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Sexual and Asexual Propagation Techniques for Domestication of *Allanblackia stuhlmannii* (Engl.) Engl. in North-Eastern Tanzania

Moses Rukwaro Munjuga

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DECLARATION

This thesis is my original work and has not been presented for a degree award in any other University.

Signature…… Date……

Moses Rukwaro Munjuga

This research thesis has been submitted for examination with our approval as Supervisors.

Signature……..…… Date.……

Prof. Wariara Kariuki

Department of Horticulture, JKUAT

Signature…….**.....** Date…….

Prof. J. B. Njoroge Mukundi

Department of Horticulture, JKUAT

Signature…… Date……

Dr. Ramni Jamnadass

World Agroforestry Centre (ICRAF)

DEDICATION

To my wife, Mary and my children, James, Samuel and Anthony. You're the source of my inspiration.

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
ANR	Amani Nature Reserve
a.s.l.	Above sea level
EFSA	European Food safety Authority
EU	European Union
FAO	Food and Agriculture Organization
GA_3 or **GA**	Gibberellic acid
Ha	hectares
H_2SO_4	Sulphuric acid
ICRAF	World Agroforestry Centre (formally International Centre for Research in Agro-Forestry)
IUCN	International Union for the Conservation of Nature (The World Conservation Union)
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KNO_3	Potassium nitrate
KOH	Potassium hydroxide
NDTL	Novel Development Tanzania Limited
PGR	Plant Growth Regulators
RBD	Randomised Block Design

ABSTRACT

Allanblackia stuhlmannii (Engl.) Engl. is a fruit-bearing tree species in the family *Clusiaceae* growing in eastern Arc Mountains, Tanzania. The seed contains edible, high quality oil used for production of food and cosmetics and is desired by oil-manufacturing companies. Exploitation of this species in the wild for oil production with little or no attention to its conservation and with current lack of propagation knowledge, will threaten it and its habitat. To satisfy the nut demand for industrial use, domestication which is currently constrained by propagation techniques due to poor and long germination and rooting periods, poor scion-rootstock healing, dioecism and slow growth of the species, is necessary. There is therefore need to develop sexual and asexual propagation methods that would allow the multiplication and selection of superior genotypes. The objective of the present study was to investigate the requirements for seeds germination and success rate of grafting in *Allanblackia stuhlmannii* under two nursery conditions, that is, highland and lowland environments. This research investigated the factors affecting seeds germination and success rate of grafting under nursery conditions in *Allanblackia stuhlmannii*. The first experiment evaluated methods for seed germination including: (i) sowing methods, (ii) application of germination enhancers, and (iii) physical treatments of seeds. The second experiment assessed three grafting techniques and two rootstock types under nursery conditions using scions from five mother tree sources. The fruits were collected from the ground and the seeds extracted after storing fruits for 6 weeks. For the seed germination study, a randomized complete block design with a factorial split plot arrangement with 25 seeds per treatment in each of 16 plots per block or site was adopted. The treatments were seeds sowing methods (sand in a room, sand in nursery and polybags in a room), seeds source trees (L19, L26, L28 and L62), scarification (intact or removal of seed coat) and chemical treatments (GA_3, Accelegrow^{TM}, fungicide and water). For the grafting study, five mother trees were used as scions sources (L19, L26, L28, L62 and L64), three grafting methods (top cleft, T-budding and side veneer), and two rootstocks types (rootstocks with/without leaves) were tested and monitored under nursery conditions. Two nurseries; Longuza (lowland at 180m a.s.l.) and Kwamkoro (highland at 995m a.s.l.)

located at Amani Nature Reserve block in the East Usambara Mountains, Tanzania were used as study sites. Longuza nursery recorded the highest seed germination with 77.8%. The mean germination rate was 56.5% with scarified seeds recording 62.8% and un-scarified seeds at 50.2%. The type of sowing method influenced the rate and capacity of seeds germination but seeds scarification and seeds sources (mother trees) had no significant effect on the germination. There was a significant difference in germination between nurseries regardless of environmental (light or dark) conditions that seeds were sowed in. Grafted seedlings achieved survival of top cleft (43%), side veneer (14%) and T-budding (0%) methods, 8 weeks after grafting. There was no significant difference on survival of grafts between rootstocks with or without leaves. However, there were significant differences in survival of scions between grafting methods and scions source (mother trees). From the results, seeds with their coats removed tended to be higher germination by 22.5% compared to seeds with their coats was left intact especially when incubated in polythene bags. Hence, scarification is necessary for rapid and uniform seed germination. The Longuza nursery had higher germination capacity by 42.6% compared to Kwamkoro nursery, hence providing ideal conditions for seeds propagation in this species. In the grafting experiments, top-cleft tended to have a higher healing by 29% compared side-cleft, hence a superior graft method in this species. This research provides input into development of propagation protocols towards domestication, conservation and management for this species.

Key words: grafting, seed, germination, dormancy, scion, rootstock, seedling, oil, East Usambara

CHAPTER ONE

INTRODUCTION

Background information

The total area of land covered by forests and woodlands in Tanzania is estimated to occupy 33.5 million ha which consist of high closed forests, closed and open Miombo woodlands and coastal mangrove. Out of the 33.5 million ha, 16 million ha are set aside and gazetted as production and protection forest and woodland reserves, 2 million ha as national parks and 16 million ha are unreserved forest lands (Vatn, Vedeld, Pétursson, & Stenslie, 2009). This unreserved forest is under heavy pressure from conversion to other land-use systems such as agriculture, wildlife protection, grazing, land settlement, recreation and industrial activities. Due to poor management of forest on general lands, deforestation in the country has escalated to 130,000-500,000 ha/year (FAO, 2006). It is estimated that between 1990 and 2005, Tanzania lost 14.9% of its forest cover equivalent to 6,184,000 ha (Mwakaje, King'ori, Temu, Lokina & Chalu, 2010). The main drivers of deforestation are clearing for agriculture and overgrazing, which account for more than 80% of forest cover loss; others includes wildfires, mining and wood extraction for charcoal, timber, poles and fire wood (Vatn *et al*., 2009).

Nationally, six land uses can be identified including, small scale farming (4.1%), large scale agriculture (0.6%), grazing land (46.9%), forest and woodland (40.4%), urban development land (1.7%) and inland waters (6.3%). It is estimated that of all deforestation factors, shifting cultivation accounts for more than 50% of the deforestation in the dense forest areas in Tanzania (Mwampamba & Schwarts, 2011). Clearing forests for shifting cultivation can contribute to climate change, biodiversity loss, species extinction, reduced wood and non-wood forest product supply, flooding, siltation, soil degradation and change of forest vegetation from primary to secondary and eventually to grassland (Holden, 2001, Mertz *et al*., 2009). Due to increased population pressure, high demand of cereals and growth of urban markets for forest products shifting cultivation has been intensified with fallow period reduced from 25

years to less than 3 years (Mwampamba & Schwarts, 2011). This has also fragmented forests especially those with high biological importance that supports hundreds of unique and endemic species like *Cephalosphaera usambarensis* (Warb.) Warb. and *Allanblackia stuhlmannii* (Engl.) Engl. (Brooks *et al*., 2002). They are also used by local people to support their livelihoods (Burgess & Clarke, 2000). Thus these forests are not only biotic carbon stocks, but also provide important biological and economical co-benefits. They also have a valuable role in conserving biodiversity and providing multiple benefits from the natural ecosystems (Burgess, Balmford, & Cordeiro, 2007).

Forests have been described as the most essential *biomes* of the planet earth because they play an important role in the earth's biophysical system, and support human well-being (Sunderlin *et al*., 2005). Across the globe, forests have been recognized for meeting the needs of the people for timber, food, medicines and fibre (Sunderlin *et al*., 2005; Akinnifesi *et al*., 2008). Traditionally, the forests sustained the livelihoods of people, first through hunting and gathering and then through shifting cultivation. However, forests are often cleared extensively to satisfy the needs and aspirations of the rapidly expanding population and their increasing materialism. Forests are also the domain of thousands of plants and animals species, which live in complex and dynamic ecosystems, bound intricately together through food chains and life cycles. The loss of forests in developing countries is mainly due to expansion of subsistence agriculture, although commercial timber extractions, creation of new settlements and infrastructure developments, as well as bush fires have all contributed to this effect (Sunderlin *et al*., 2005; Vatn *et al*., 2009). Traditional food crop farming systems in tropics of Africa often exclude tree crops in the landscape. The forests are increasingly under pressure to feed and sustain their families on a small area of land and pay for health care and the education of their children (Sunderlin *et al*., 2005). The removal of original vegetation from forests has immensely contributed to deforestation and biodiversity loss. This has led to landscapes dominated by exotic species, mainly commercial monocultures such as: oil palm plantations for example in Ivory Coast, coconut plantations in coastal East Africa and plantation forestry of eucalyptus in farm forestry of East Africa.

However, stabilization of the fragile small-holder food crop farming systems through integration of high value indigenous fruit trees could lead to sustained productivity (Akinnifesi *et al*., 2008).

Global inventories of food crops (Shackleton *et al*., 2002; Cunningham & Shackleton, 2004) list astonishingly high numbers of edible plants, typically exceeding several thousand species that are either cultivated or collected from the wild. A compilation of useful plants in East Usambara (Reyes, Luukkanen, & Quiroz, 2006) recognizes many edible species for this mountain block alone. Each edible plant species itself may be the source of a multitude of foods, depending on intra-specific or varietal diversity, the use versatility of the species' edible parts, cultural preferences and post-harvesting procedures (drying, curing, fermentation, extraction of particular constituents, etc.), that typically have co-evolved with particular plant varieties suited for such procedures (Herman, 2009). Many of these indigenous trees species, which have little importance as timber trees, are becoming increasingly recognized as valuable sources of raw materials for various food and industrial uses. These uses include extraction of vegetable oils from the seeds for various purposes, alkaloids from several parts of the plants for medicinal purposes and fibre for pulp. In sub-Saharan Africa, a number of native forest tree species of economic importance are dioecious and this poses particular challenges for management, which requires investigation of propagation strategies and regeneration potential (Asfaw & Tadesse, 2001; Shackleton *et al*., 2002). Agroforestry has been part of traditional polycultural systems practiced by the people in which the trees are protected or planted for domestic requirements (Akinnifesi *et al*., 2008). Indigenous fruit and nut trees are important components in this system. Knowledge about plant propagation for many African trees of high economic potential such as *Allanblackia* species is scant (Akinnifesi *et al*., 2008) and often absent.

The *Allanbackia stuhlmannii* (Engl.) Engl. plant is one of the nine species of the family *Clusiaceae*. All the nine species are restricted to the African rainforest; with *A. stuhlmannii* limited to the Eastern-Arc Mountains in Tanzania (Bamps, Robson & Verdcourt, 1978; Mpanda, Munjuga, Ndangalasi & Cordeiro, 2009; Neondo *et al*., 2011). It is an evergreen dioecious fruit tree which is a potentially important source

of alternate income to farmers in its range and could be rapidly domesticated for continuous seeds supply. The seed of *A. stuhlmannii* yields oil used for centuries by local inhabitants for food and soap production and that was adopted during World War I as an alternative edible fat to butter by German soldiers in the region (Monela, Kajembe, Kaoneka & Kowero, 2001; Neondo *et al*., 2011). The species can also be used as shade tree and for timber (Schulman, Junikka, Mndolwa & Rajabu, 1998; Mwaura & Munjuga, 2007; Mpanda *et al*., 2009). The bark concoction or tea has been used traditionally for many years as herbal medicine to treat different ailments such as coughs, colds and infected throat (Schulman *et al*., 1998; Mwaura & Munjuga, 2007; Jamnadass *et al*., 2010). The oil is used as cooking oil and also as liniment for joint pains and coughs (Schulman *et al*., 1998; Jamnadass *et al*., 2010). Other possible uses include the treatment of HIV/AIDS as guttiferone F, an HIV-inhibitor, has been reported from bark extracts of *A. stuhlmannii* (Fuller, Blunt, Boswell, Cardellina & Boyd, 2003). Recently, there has been increased international interest by Unilever and other commercial enterprises in turning *Allanblackia* into a significant commercial species for edible oil production that can be used in healthy food products in global food markets (Amanor, Ghansah, Hawthorne & Smith, 2008). The most important benefit derived from the species is the seed oil with great potential for incorporation in manufacture of margarine and cosmetics. This edible oil has significant potential in the global food market as a raw material for the production of healthy spreads that are low in trans-fats (Ochieng, 2007; Asaah, Tchoundjeu & van Damme, 2012). The oil from the nuts has a unique composition containing 52-58% stearic acid and 39-45% oleic acid (FAO, 1983; Axtell *et al*., 1992; Atangana, Tchoundjeu, Asaah, Simons & Khasa, 2006; Jamnadass *et al*., 2010) is of high commercial importance, with considerable tree-to-tree variation in fatty acid profiles (Atangana *et al*., 2011). Its composition and resulting high melting point (34°C) makes the fat a valuable raw material that can be used without transformation to improve the consistency of margarines, cocoa butter substitutes and similar products (Amanor *et al*., 2008; Atangana *et al*., 2011). The fatty acid profile of the oil has been reported to lower plasma cholesterol levels (Sartika, 2011), thereby reducing the risk of heart attacks.

Unilever estimates that the potential market for *Allanblackia* oil is more than 100,000 tons annually (Jamnadass *et al*., 2010), provided the right quality standards are met. So far, all *Allanblackia* production is derived from wild harvesting, i.e. seeds collected from naturally occurring trees in forests and on farms (Amanor *et al.*, 2008; Akinnifesi *et al*., 2008). Over the past ten years, supply chains based on wild harvesting have been established in Ghana, Tanzania and Nigeria with fair returns to farmers, collectors, and local processors. Presently, about 10,000 smallholder farmers from the three countries earn approximately US$ 250,000 in total per nut collection season (Egyir, 2008). For example, 54 collection centres have been established and are actively involved in the collection and quality control of seeds in Tanzania with about 6500 farmers collecting the nuts from the wild since 2004 (Table 1.1). On average, oil yield is about one-third of seed dry weight (Attipoe, van Andel & Nyame, 2006; Wilfred, Adubofuor, & Oldham, 2010). From this, a total direct income from *Allanblackia* nut sales have increased from US$ 4,000 in 2004 to US$110,000 in 2008 with 120 tonnes of oil shipped to Netherlands (Pye-Smith, 2009). The market price of *Allanblackia* is comparable with palm oil, being sold for about USD 650/t for refined oil in Europe (Pye-Smith, 2009). Wild collection alone cannot meet the minimum requirement to sustain the *Allanblackia* oil business as demand exceeds the supply. These markets provide an opportunity for income generation in ways that are understood and adopted by local people. Consequently, the current increasing interest of the produce for market value and the need for its sustainability can be addressed through domestication strategy by integrating these species into their current farming systems.

Since *Allanblackia* trees did not have an economic value in the past, farmers have cut most of the trees and thus decimated their original tree populations (Amanor *et al.*, 2008). Towards this goal, plant breeding and tree improvements are essential components of the production supply chain and integration into agroforestry systems. Unfortunately, these indigenous tree species that can be domesticated are little known outside their natural range and have attracted little scientific interest internationally in the past. Concerted efforts are needed to develop better policy and methods for sustainable land use practices to meet and addresses the needs of farmers

to preserve the environment and develop species that are of economic value. Enhancing current agroforestry practices of farmers is one way of taking a step forward to alleviate the growing socio-economic and mitigating environmental problems. Integration of this multipurpose wild fruit tree species in farmlands or managing existing trees in their natural habitat is important to address the deficit of *Allanblackia* oil supply. This will however demand development of reliable propagation methods and protocols for mass production of desirable genotypes.

Year	**Allanblackia seeds traded (kg)**	**Smallholder farmers involved**		
		Female	**Male**	**Total**
2004	42,790	295	274	569
2005	152,400	1753	1318	3071
2006	357,562	2958	2657	5615
2007	86,022	867	987	1854
2008	447,264	3250	2950	6200
2009	107,028	1096	884	1980
2010	533,635	3310	2534	5844
2011	269,360	1314	1179	1878
2012	208,068	1878	1509	3387

Table 1.1: Quantities of Allanblackia seeds produced and farmers involved in collection and on-farm tree planting in Tanzania (*Source: NDTL records, 2013*)

Agroforestry practices come in many forms and seek to address many of the problems associated with land use degradation, declining livelihoods, poor nutrition and health. Over the last 10 years, one agroforestry initiative that has become significant is the move to domesticate indigenous food and medicinal plants – especially the trees that used to be important in traditional land use systems and culture. The domestication of indigenous trees, and in particular, fruit and nut species is seen as an incentive for farmers to adopt agroforestry (Akinnifesi *et al*., 2008).

One of the main challenges in agroforestry is to address the lack of genetically superior seed sources for tree propagation (Akinnifesi *et al*., 2008). Most studies on the domestication of fruit trees to date have focused on the selection of desirable tree characteristics and their capture by vegetative propagation practices (Leakey, Newton & Dick, 1994; Tchoundjeu *et al*., 2006), or by their selection and subsequent breeding of superior individuals (Tchoundjeu *et al*., 2006). In this respect any domestication effort of a species has to involve making a decision as to whether to use sexual or vegetative propagation to achieve tree seedling production and improvement of tree, fruit and nut characteristics. In forestry research, it is typical to find foresters adopting seed-based breeding approaches, while in horticulture it is common to find horticulturists adopting clonal propagation and the development of cultivars (Akinnifesi *et al*., 2008). Mckey *et al*., (2010) emphasised that perennial plants under domestication are often propagated clonally, which in addition to reducing their long juvenile phase to fruiting, further decreases the number of sexual cycles separating domesticated individuals from their wild progenitors. Hence tree domestication processes involve a gradual modification of production systems and/or conditions.

Through agroforestry, risk aversion can also be achieved by diversification of the agro-ecosystems through the introduction of other species and food crops in ways that would provide food, income and other agro-ecological system functions (nutrient recycling, erosion control, habitat for flora and fauna, carbon sequestration, etc.) (Akinnifesi *et al*., 2008). However, the cultivation of *Allanblackia* species on farm is constrained by propagation success (both sexual and vegetative propagation techniques) (Jamnadass *et al*., 2010). According to Bhojwani & Razdan (1996) and Silvertown (2008), a shift from sexual to clonal reproduction allowed for the reproduction of individuals with superior features (traits) by eliminating uncertainty in the transmission of favoured traits over reproductive cycles associated with sexual reproduction. Diversification of clonal production population is crucial in the domestication process for pest/disease risk aversion and/or to avoid poor performance of individual trees as a result of inbreeding depression. Vegetative propagation (grafting, budding and marcotting) has also been used to achieve early

fruiting and tree dwarfing (Tchoundjeu *et al*., 2006; Akinnifesi *et al*., 2008). For example, grafted *Uapaca kirkiana* began to produce fruits after only 2-3 years, while those derived from seedlings took 12-15 years before fruiting (Akinnifesi *et al*., 2008). Similarly, in other *Allanblackia* species, rooting of leafy stem cuttings, grafting and air layering were used (Anegbeh, Iruka & Nkirika, 2006; Atangana *et al*., 2006; Ofori *et al*., 2008), resulting in first flowering on one to two years old grafts (Ofori *et al.*, 2008, Asaah *et al*., 2011). This can also be developed in *A. stuhlmannii* with an aim of producing trees with superior fruit traits and to shorten juvenile phase from seedling to flowering and fruiting of a tree. The overall goal of this study was therefore to develop seed germination and grafting protocols for *Allanblackia stuhlmannii* so as to improve seedling production for its wider cultivation in Tanzania.

Statement of Research Problem

Allanblackia stuhlmannii fruit production faces a number of challenges in relation to growth and a long juvenile phase, fruit production variability and lack of knowledge on the propagation and silviculture of the species (Akinnifesi *et al*., 2008). According to Jamnadass *et al*., (2010) *Allanblakia* species are characterized by a predominance of remnant old trees and a lack of regeneration in the Eastern Arc Mountain forest. Female trees are often retained when clearing land for agriculture, but planting is still rare. The juvenile phase of the naturally regenerated trees varies between 10 and 15 years. Whilst fruit production may commence at 12 to 15 years, full production is only reached after 20 years (Mwaura & Munjuga, 2007). Fruit production varies from one year to another, from place to place and between individual trees and this could be under the influence of environmental factors and genetic variability as has been reported by Akinnifesi *et al*., (2008). This variation in the production could be a disincentive for farmers to plant *A. stuhlmannii* in the future unless more productive planting stocks are developed. To date, efforts aimed at domesticating the species have not progressed significantly due to lack of suitable propagation techniques and cultivation knowledge (Jamnadass *et al*., 2010). Wild harvesting has been the main source of seeds for oil production (Egyir, 2008; Amanor *et al*., 2008). Due to the continued expansion of *Allanblackia* market from increased demand for oils, its

natural regeneration may be disrupted, leading to a threat to its conservation. With the provision of a guaranteed market, there is potential for farmers to cultivate the species and reduce the pressures on forests. Domestication is therefore required urgently to enhance fruit production, reduce pressure on the wild trees and conserve the genetic resources of this species.

The use of seeds and seedlings in *Allanblackia* domestication is limited by its recalcitrant nature, seed dormancy and limitations in seed storage technology. It has been observed that the development or degree of dormancy changes during the lifetime of the seed, usually as a response to external conditions (Schmidt, 2000; Finch-Savage & Leubner-Metzger, 2006). Furthermore, where *Allanblackia* seedlings may be required for planting, there is a problem in determining sex of the seedlings raised or planted as this species is dioecious meaning that it has separate male and female plants. Seed germination is slow and may take place over a lengthy period of time, therefore, there is need to develop methods that could enhance seed germination. To address the slow and erratic seed germination and the dioecy phenomenon in the reproductive biology of the species, several reports indicated that vegetative propagation may be used to overcome this problem (Leakey *et al*., 1994; Akinnifesi *et al*., 2008; Ofori *et al*., 2008). When seeds germinate, their growth is usually slow to attain sizable seedlings required as rootstocks for grafting and can take over three years (Ofori *et al*., 2008). Furthermore, desirable genotypes can also be conserved through vegetative propagation (Ofori, Cobbinah, & Appiah-Kwarteng, 2001; Akinnifesi *et al*., 2008) such as grafting and marcotting. However, successful grafting depends on factors such as the environmental conditions, the origin of the scions, compatibility of scions with rootstocks and the physiological state of the mother trees (Ofori *et al*., 2008).

Protection and planting of *Allanblackia* trees will not only contribute to income generation but will also be an incentive for improved ecosystem management and biodiversity conservation (Jamnadass *et al*., 2010). A large scale cultivation of *Allanblackia* spp. aims to promote it as a commercial crop (Egyir, 2008; Jamnadass *et al*., 2010) and turn it into a significant income earner for tropical rainforest Africa. Domestication of *Allanblackia* has the potential to provide on-farm source of

cultivated fruit for commercial and domestic use thereby contributing to poverty reduction of rural communities (Egyir, 2008; Jamnadass *et al*., 2010) while reducing the pressure on primary forests where its exploitation is currently taking place. When carefully selected, the species can improve food availability in rural areas, as well as provide much needed cash and other valuable products (Egyir, 2008). Breeding and selection of superior clones with high and regular fruit production over years could lead to rapid and substantial genetic improvement in yield and oil production. However, farmers tend to look for short term benefits and will appreciate the use of grafting due to its ability to shorten the gestation period (Ofori *et al.*, 2008; Asaah *et al*., 2011).

Considering the commercial importance of this unexploited cash crop and the potential of *Allanblackia* tree cultivation, there is great need for improvement of this species and to develop varieties suitable for cultivation under varied agro-climatic conditions. The use of grafting in *A. stuhlmannii* can therefore be an essential propagation method to capture and multiply the desirable characteristics of selected trees. Such techniques can be used to graft wildings in the natural environment and on-farm. Once the best scions have been obtained, suitable environmental conditions should be provided to the scions and rootstocks during and after the suitable grafting technique is performed (Ofori *et al*., 2008).

It is expected that this work will provide an opportunity towards conservation of this species and enhance the income generation for farmers by developing suitable seed and vegetative propagation techniques to represent the first step in the process of domestication. Thus the work will contribute towards developing appropriate protocols for propagation (seed germination and grafting) for sustainable supply of *Allanblackia* nuts, thereby helping to reduce food insecurity, poverty as well as enhancing ecosystem services.

Objectives of the study

The general objective of the study was to investigate the requirements for seed germination and grafting methods for successful propagation of *Allanblackia stuhlmannii* under two nursery conditions.

The specific objectives:

(i) To assess the effect of seed source and seed pre-treatment techniques on germination of *Allanblackia stuhlmannii* under varied nursery environmental conditions.

(ii) To determine the effect of scion source and grafting technique on healing and survival of grafted seedlings of *Allanblackia stuhlmannii* under nursery conditions.

Hypotheses

The hypotheses tested were;

- Seeds with their testa removed and treated with GA_3 and Accelegrow™ will germinate faster than untreated seeds.
- Success rate of top cleft grafting is higher than side veneer and T-budding methods.
- Rootstocks with leaves have higher survival rate than those without the leaves.

CHAPTER TWO

LITERATURE REVIEW

Botanical Description of *Allanblackia stuhlmannii*

Allanblackia stuhlmannii (Engl.) Engl. is an evergreen, dioecious forest tree that grows in size from medium size to a fairly large tree of about 35 to 45 m high with a straight cylindrical bole and drooping branches and belongs to the family *Clusiaceae*. *Allanblackia* trees (Plate 1.1a) are especially abundant in African wet forests, which are important biodiversity hotspots threatened by deforestation and land conversion. The leaves are simple, opposite and deep green 5-19.5 cm long by 1.2-7 cm wide. Female flowers are large, unisexual and succulent with ovoid ovaries growing singly in the axils up to 5cm across when expanded and have 6-8 cm long pedicels with five pinkish (Plate 1.1b) to creamish (Figure 1.1c) petals. The male flowers (Plate 2.1a) are in terminal racemes, crowded towards the apex of the drooping branches with club-like, waxy and flattened stamen-bundles. The trees have the largest fruits of all rainforest plants, and one tree can produce up to 300 fruits per season. In Tanzania, fruits take more than 1 year to develop and mature in the period between December and March. The fruit is brown or red-brown 10–35 cm long and 10-20cm wide with tough flesh (Plate 2.1b), producing yellow latex hanging on long pedicels and have many berry-like seeds (40–100 seeds per fruit). The pods can only be collected when they are ripe and have fallen to the ground, so harvesters have to compete with rodents and other animals that feed on the seeds. The seeds (Plate 2.1c) are brittle-shelled, four-angled, about 4 cm long and 3 cm wide, embedded in a gelatinous pulp (Mugasha, 1980; Schulman *et al*., 1998; Mwaura & Munjuga, 2007). The seeds have a high content of edible fat and are traditionally harvested on a subsistence basis for domestic use as cooking oil and for soap-making.

Allanblackia stuhlmannii is listed in the IUCN Red List as vulnerable because of their small and severely fragmented areas of distribution and declining habitat. The species is found on seaward slopes and valley bottoms of evergreen sub-montane and montane forest, restricted to the Eastern Arc Mountains, Tanzania between 500-1800m above sea level (Mugasha, 1980; Schulman *et al*., 1998; Mwaura & Munjuga,

2007). Average annual rainfall in its habitat is 1100–2400 mm with more than 180 rainy days. The species is found on mostly acidic clay soils derived from granite, gneiss or siliceous rock. Under natural conditions, trees first flower when about 12 years old (Jamnadass *et al*., 2010). Flowering is during the short rainy season in November–February. Pollination is done by short-tongued insects, birds and bats. Rodents and monkeys feed on the fruits and may disperse the seeds (Mpanda *et al*., 2009; Jamnadass *et al*., 2010). The tree is associated with tree species like *Cephalosphaera usambarensis, Parinaria excelsa, Albizia gummifera, Beilschmiedia kweo, Diospyros abyssinica, Englerodendron usambarense* and *Drypetes gerrardii* (Mugasha, 1980; Mwaura and Munjuga, 2007; Mpanda *et al*., 2009).

a **b** **c**

Plate 1. 1: (a) Heavily fruited Allanblackia trees next to a tea estate, (b) female flower of red morph, (c) female flower of cream morph

a **b** **c**

Plate 1. 2: (a) Male flower buds and open flowers showing yellow pollen, (b) fruit on a tree and (c) extracted clean seeds

Allanblackia stuhlmannii can be propagated by seed, but the seeds are recalcitrant. There are about 120 dry seeds per kg. Well-matured fruits are kept for about 2 weeks

to allow the pulp to become soft and to make extraction of the seed easy. Fruits may be kept for up to 3 months if covered with banana leaves in a cool place. After sowing seeds take about 3 months to start germination, but may take more than 7 months to start (Ofori *et al*., 2011). In natural forest, its regeneration is poor due to seed eaters such as the rodents, excessive seed collection for oil extraction and sporadic, prolonged germination exposing the seeds to detrimental environmental elements (Mwaura & Munjuga, 2007).

Due to the characteristics of its seeds, *Allanblackia* is of high potential interest to the development of rural communities in tropical Africa, where the trees occur naturally in the wild (wet forests, often protected areas) as well as scattered on smallholder farms and commonly-owned lands of rural communities. A mature tree may yield up to 150 fruits or up to 50 kg fat per year, and approximately 3 kg of seeds are needed to produce 1 kg of *Allanblackia* oil (i.e., ~15 kg of oil/tree). Seeds are extracted from the fruits by crushing them between the hands and rubbing them clean. The seeds are then dried to avoid the development of moulds before being transported to the buying centres, where seeds are graded (Amanor *et al*., 2008). Fat is extracted locally by traditional methods, or seeds are dried and sold to extraction plants. Traditionally, the seeds are dried and crushed; the resulting mass is mixed with water and boiled until the fat separates and floats to the surface from where it is scooped off (Jamnadass *et al*., 2010; Mwaura & Munjuga, 2007).

In addition to seed oil, local communities have long valued this plant as a shade tree, for fuelwood, timber and as herbal medicine (Schulman *et al*., 1998; Mugasha, 1980). The pressed cake is bitter and contains tannins, but is sometimes used as animal feed. Due to its commercial value for oil production, the genus *Allanblackia* was recognized by FAO as a crop of high potential interest for the development of rural communities (FAO, 1983; Jamnadass *et al*., 2010). *Allanblackia* seed oil has been approved by European Food Safety Authority for use in yellow fat and cream based spreads as it meets the quality criteria of edible vegetable oils (Table 2.1) and the limits for potential contaminants set in the EU (Jamnadass *et al*., 2010). Fatty acid compositions from *A. floribunda*, *A. parviflora* and *A. stuhlmannii* showed no

significant differences between the two species (Atangana *et al*., 2011). Refined seed oil is semi-solid at room temperature.

Lauric acid (C12:0)	< 1 %
Myristic acid (C14:0)	< 1 %
Palmitic acid (C16:0)	< 2 %
Palmitoleic acid (C16:1)	< 1 %
Stearic acid (C18:0)	45 – 58 %
Oleic acid (C18:1)	40 – 51 %
Linoleic acid (C18:2)	< 1 %
γ-Linolenic acid (C18:3)	< 1 %
Arachidic acid (C20:0)	< 1 %
Free fatty acids	max. 0.1 %
Trans fatty acids	max. 0.5 %
Peroxide value	max. 0.8 meq/kg
Iodine value	< 46 g / 100 g
Unsaponifiable matter	max. 0.1 %
Saponification value	185 – 198 mg KOH/g

Table 2.1: Composition of refined *Allanblackia* seed oil (Source: *EFSA, 2007)*

Allanblackia species have a history of use in the treatment of certain medical conditions. Extracts of the leaves, the stem bark and the root bark are reported to be used alone or in combination with other plants to treat ailments such as upper respiratory tract infections, dysentery, diarrhoea and toothache. In phytochemical studies of the heartwood of *A. floribunda* and the root bark of *A. stuhlmannii* compounds belonging to the benzophenone (e.g. hydrocortin, guttiferones), the xanthone (e.g. 1,3,5-trihydroxyxanthone, 4,5-dihydro-1,6,7-trihydroxy-4´,4´,5´-trimethoxy-furano (2,3:3,4)-xanthone) and the biflavonoid (e.g. morelloflavone, volkensiflavone) families have been identified (Locksley and Murray, 1971; Fuller *et al*., 2003; Nkengfack, Azebaze, Vardamides, Fomum & Van Heerden, 2002). These

compounds are reported to exhibit a range of activities including cytotoxic, anti-inflammatory, anti-microbial properties (Nkengfack *et al*., 2002; Neondo *et al.*, 2011).

Sexual propagation

A seed is a ripened ovule, which consists of an embryo, stored food and a seed coat (Schmidt, 2000; Hartmann, Kester, Davies & Geneve, 2002). Propagation by seed is a means of continuation of life and assuring of plant species survival. A seed is said to have germinated when the radicle emerges through the seed coat under favourable conditions (Hartmann *et al*., 2002; Bentsink & Koornneef, 2008). The classical work of Toole *et al*., (1956), Crocker *et al*., (1957) and other pioneers contributed to the knowledge regarding several aspects of seed research. In many cases, even when all conditions for germination are optimal, seeds still fail to germinate. Seeds that behave in this way are termed as being dormant. This dormancy has to be broken before seeds can germinate. In the last few decades, sophisticated aspects of seed research have thrown more light on the dormancy and viability factors in seed germination (Hartmann *et al*., 2002; Bentsink, & Koornneef, 2008). Germination of seeds depends on the origin and genotypes. Nonetheless both internal (seed moisture content, viability of the embryo, and food reserves) and external factors (nature of seed coat, water availability, temperature and light intensity) need to be at optimum levels (Sacande, Hoekstra, van Aelst, & De Vos, 2000; Hartmann *et al*., 2002). Although scientists have long realized that dormancy mechanisms exist (Geneve, 2003; Bentsink & Koornneef, 2008), reviews of the literature have shown that dormancy is one of the least understood processes in seed biology (Hilhorst, 1995; Alouani & Bani-Aameur, 2004; Bentsink & Koornneef, 2008). In the last few years, there has been a significant increase in the number of publications on seed dormancy, thus enhancing understanding of these processes (Finch-Savage & Leubner-Metzger, 2006; Bentsink & Koornneef, 2008).

Seed Dormancy

Seed dormancy has been defined as the failure of an intact, viable seed to complete germination under favourable conditions (Bewley, 1997; Baskin & Baskin, 2004). It is an ecologically adaptive mechanism that helps plants to survive the period

between seed maturation and the establishment of the next generation as a seedling (Geneve, 2003; Bentsink & Koornneef, 2008), thus equipping the seed to sustain extended periods of unfavourable conditions. Numerous studies have been performed to better understand how germination is controlled by various environmental factors. However, very little is known about the process by which the embryo emerges from the seed to complete germination and how embryo emergence is blocked in dormant seeds (Bewley, 1997; Baskin, Zackrisson, & Baskin, 2002; Baskin & Baskin, 2004; Bentsink & Koornneef, 2008). Several schemes for classifying seed dormancy have been presented. Baskin & Baskin (2004) divided seed dormancy into 5 types as explained below:

Physiological Dormancy

Physiological dormancy of seeds is due to decreased activity of the embryo, which, together with the restriction to gas exchange imposed by the seed covers, produces a double mechanism of inhibition (Nikolaeva, 1977; Hartmann *et al.* 2002; Baskin & Baskin, 1998; Baskin & Baskin, 2004; Bentsink & Koornneef, 2008). Among physiological types of dormancy one can also distinguish the type of dormancy which can be broken by the action of low temperatures together with those cases of delayed germination, which can be overcome by dry storage and which is related to the phenomenon of light sensitivity. Physiological dormancy is divided into three types: non-deep, intermediate and deep physiological dormancy.

Non-deep dormancy is typical of freshly collected seeds in the majority of species from temperate zones. With removal or even disturbance of seed covers, non-deep dormant seeds would germinate and give rise to normal seedlings. Nonetheless, the fact that embryos are unable to overcome the retarding action of the covers testifies to their reduced activity. Non-deep dormancy, as a rule, gradually disappears in the course of after-ripening during dry storage of seeds, by short periods of moist pre-chilling, or by applications of various growth stimulators (Geneve, 2003; Rodríguez, Toorop, & Benech-arnold, 2011).

Intermediate type of physiological dormancy is observed in seeds that are rather diverse in terms of their biological properties. Seeds with intermediate dormancy

require a sufficiently prolonged stratification, whereas treatment with gibberellic acid (GA_3) may, under defined conditions, stimulate germination (Geneve, 2003; Baskin & Baskin, 2004; Bentsink & Koornneef, 2008).

Deep physiological dormancy is typical for embryos, which, although beginning to germinate, show a retarded and abnormal growth, whereas intact seeds acquire the ability to germinate only when exposed to a sufficiently prolonged cold stratification at 1 to 7°C (Hartmann *et al.* 2002; Baskin & Baskin, 1998, and 2004; Bentsink & Koornneef, 2008).

Morphological Dormancy

Morphological dormancy is where embryo is under development and undifferentiated. Thus, the embryo simply need time to grow to full size and then germinate (radicle protrusion) (Baskin & Baskin, 2004; Copete, Herranz, Ferrandis, Baskin & Baskin, 2011). This process takes place in the seeds following their separation from the parent plant, usually under conditions of warm stratification and takes from several days to 4 months (Copete *et al*., 2011).

Morpho-physiological Dormancy

In the majority of cases, the under development of the embryo is associated with the presence of the physiological inhibition mechanism, this giving rise to morpho-physiological dormancy type (Hidayati *et al*., 2012). Thus, in order for the seeds to germinate, embryo growth and radicle emergence require a considerably longer period of time than in seeds with morphological dormancy (Baskin & Baskin, 2004; Hidayati *et al*., 2012).

Physical Dormancy

Physical dormancy (hard seed coat) is caused by one or more water-impermeable layers of palisade cells in the seed or fruit coat. Impermeability to water is conceivably related to changes occurring in the fine structure of the hilum (Cook *et al*., 2008). Percentage of hard seed shows considerable variability depending on the species, degree of maturity, ripening conditions and storage time (Nikolaeva, 1977; Alouani and Bani-Aameur, 2004). Seed dormancy breaking under both natural and artificial (except mechanical scarification) conditions has been assumed to involve

the formation of an opening in a specialized anatomical structure on the seed coat, through which water moves to the embryo (Baskin *et al.*, 2002; Cook *et al*., 2008).

To obtain a rapid and uniform imbibition, seeds are subjected to various chemical and physical treatments which includes temperature treatment such as heating, chilling, drastic temperature shifts, or brief immersion of seeds in boiling water (Hartmann *et al*., 2002; Baskin and Baskin, 2004; Ribeiro, Pedrosa, & Borghetti, 2013). Frequently, treatment of seeds with concentrated sulphuric acid, grinding with abrasives or sand (scarification), or shaking (impaction) are used. Duration of the treatment is chosen experimentally, and immersion in sulphuric acid may last from 15 minutes to 24 hours (Nikolaeva, 1977; Baskin *et al.*, 1998; Baskin and Baskin, 2004).

Combinational Dormancy

In seeds with combinational dormancy, the seed coat is physically water impermeable and the embryo is physiologically dormant. Embryos of freshly matured seeds have some conditional dormancy which is overcome by stratification before seed sowing (Baskin *et al.*, 1998; Baskin & Baskin, 2004; Kimura & Islam, 2012).

Breaking seed dormancy

Successful seed germination depends on numerous internal and external factors. These include cold and warm stratification, scarification, growth regulator application, etc. (Bewley, Black, & Black, 1994; Baskin & Baskin, 2004; Kimura & Islam, 2012).

Scarification

Scarification is any process of breaking, scratching, or mechanically altering the seed coat or endocarp to make it permeable to water and gases. In the wild, scarification can be by cold and soil particles, by damage to the seed coat by rolling along the soil surface, by passing through the digestive system of animals and washing by water (Baskin and Baskin, 2004; Hartmann *et al*., 2002). During floods after a heavy rain, seeds are washed with sand and gravel and their coats are scarified accordingly (Gutterman, 1993). Mechanical scarification such as chipping hard seed coats or

endocarp by rubbing with sandpaper, cutting with a file, or cracking with a hammer to expose the inner parts of the seed are simple methods (Baskin & Baskin, 2004; Hartmann *et al*., 2002; Kimura & Islam, 2012).

Several mechanical and chemical methods such as freezing, mechanical scarification, using boiling water, sulphuric acid, citric acid, and hydrogen peroxide have been used in attempts to crack, remove, or soften the endocarp (Bewley *et al.*, 1994; Baskin & Baskin, 1998; Can *et al*., 2009; Hartmann *et al.*, 2002; Kimura & Islam, 2012). In acid scarification, seeds are scarified with concentrated acid such as suphuric or nitric acid in order to dissolve the exocarp, mesocarp and endocarp (Ali, Tanveer, Nadeem, & Asghar, 2011) but duration of soaking, depends on the kind of seed (Nasir, Summrah, Allah-Bakhsh & Mohammad-Nawaz, 2001; Hartmann *et al.*, 2002; Pipinis, Milios, Smiris, & Gioumousidis, 2011; Kimura & Islam, 2012).

Chemical Agents

Growth regulators, such as auxins, gibberellins, cytokinins (usually kinetin or benzyladenin), abscisic acid (ABA) and ethylene have the potential to remove seed dormancy and regulate seed growth, development and eventual germination (Hartmann *et al.*, 2002; Ren & Guan, 2008; Schwechheimer, 2008; Zoghi, Azadfar, & Kooch, 2011). Seeds deficient in ABA during development, lack primary dormancy once dispersed. Conversely, an over expression of ABA synthesis genes during seed development enhanced dormancy and hindered germination (Finch-Savage & Leubner-Metzger, 2006). Gibberellic acid (GA) promotes germination, reduces many of the environmental requirements for germination, and has a role in counteracting the inhibitory effects of ABA, hence a shift to low ABA:GA ratios (Kucerna *et al*., 2005; Ren & Guan, 2008; Zoghi *et al*., 2011) is required for fast seed germination.

Environmental Factors

Environmental signals, including temperature and light have immense effect on dormancy and seed germination ability (Hilhorst, Bentsink & Koonneef, 2006; Kucerna *et al*., 2005; Zoghi *et al*., 2011). Heat increases the catalytic activity of enzymes. Light is perceived to enhance seed germination through increase of bioactive GA concentration (Hilhorst *et al*., 2006; Zoghi *et al*., 2011). Though not

really a form of dormancy, undesirable temperatures used for germination can be partially or completely inhibitory. The range of temperatures required by some seeds, can be very narrow and specific. Under natural conditions necessary changes take place gradually under varying combinations of aeration, moisture, temperature, and light. By duplicating key conditions of the natural environment in the laboratory or nursery, dormant seeds can be induced to germinate with a reasonable length of time (Krugman, Stein & Schmitt, 1974; Penfield, Josse & Halliday, 2010).

Seed germination

Allanblackia species is currently a highly desired tree by farmers, but an extended germination period causes production to be expensive, and the processes of seed dormancy and germination are poorly understood. *Allanblackia* seed dormancy may be influenced by morphological, chemical and physiological factors. This morpho-physiochemical combinational dormancy may be complex and may result from interplay of a rudimentary embryo, chemical inhibitors, mechanical or physical barriers (Msanga, 1987; Mwaura & Munjuga, 2007; Ofori *et al*., 2011). It also appears that the seed embryo is normally not fully developed and needs time after fruit ripening to develop. Breaking dormancy in this species requires development of seed germination techniques that target morpho-physiochemical factors controlling seed dormancy.

Vegetative propagation

Vegetative propagation techniques offer the opportunity to produce reliable and adequate supply of planting stocks. Many tropical tree species have been successfully propagated vegetatively by stem cuttings (Akinnifesi *et al.*, 2008) and through grafting (Ofori & Foli, 1997; Mng'omba *et al*., 2008; Ofori *et al*., 2008; Asaah *et al*., 2011). Problems faced by *Allanblackia* rooting cuttings include plagiotropism, the slow rooting rate of cuttings and the poor quality of the roots resulting in low number of roots per cutting. Besides difficulties mentioned above, most of the big fruited trees such as jackfruit when propagated by the cuttings produce seedlings with surface adventitious weak roots, which is not suited to the nature of such trees (Jamaludheen, Kumar, Wahidand & Kamalam, 1997). Abd El-Zaher (2008) also reported that most of the physiologically active roots of the

jackfruit tree were concentrated within a 30-75 cm depth, hence easy to be uprooted by wind. Grafting methods is a suitable, easy and cheap procedure for propagating fruit trees where produced plants maintain good traits of the parents, produce a disease free plants and speed fruiting. For the production of high quality oil and faster tree growth, it is essential to start by selecting superior clones/trees from which the scions are to be taken. Vegetative propagation through grafting in many tree species is markedly affected by several factors such as propagation environment, post-severance treatments (leaf area), stock plant factors (scion origin and environment), pre-severance stockplant environment, stockplant management, phase change (ontogenetic and physiological ageing), and genetic variation (Hartmann *et al*., 2002; Mng'omba *et al*., 2008). Further to the above factors, successful propagation by grafting depends on factors such as the state of rootstocks and skills of the grafter (Mng'omba *et al*., 2008). Several studies have also demonstrated different levels of incompatibility between scions and rootstocks (Mng'omba *et al*., 2008) due to natural genetic variation. Positions from which scions are taken also influence the overall quality of graft and subsequent growth habit (Hartmann *et al*., 2002).

Grafting Techniques

Numerous grafting techniques have been described in literature, but all existing grafting methods fall into two categories: approach grafting and detached scion grafting (Garner, 1993; Hartmann *et al*., 2002). Approach grafting is where the scion and the rootstock are not totally severed from the parent plant until a union is formed, such as inarching and bridge grafting. Detached scion grafting involves complete severance of the scions before the union is formed, such as cleft grafting, whip-and-tongue grafting, and bud grafting also known as budding. The success of grafting methods depends on season, age of both rootstock and scion and cultivar or seedling type (Jiang *et al*., 2010). The simple, highly efficient and most widely used grafting techniques in trees are cleft grafting, whip-and-tongue grafting; T-budding, and chip budding (Wertheim and Webster, 2003). It has been reported that cleft grafting is easier to use (Kulwal & Tayde, 1989; Sanou *et al*., 2004) and more

successful than other methods of grafting (Ram, 1997; Sanou *et al.*, 2004; Abd El-Zaher, 2008).

Grafting of scions from reproductively matured trees was found to considerably shorten the juvenile phase (Sherman & Lyrene, 1983; Abd El-Zaher, 2008). In *A. floribunda* and apple, flowering occurred on 4 years (Asaah *et al*., 2011) and 2-4 years (Hackett,1985) old grafts, respectively. However, in *A. stuhlmannii* production of grafted seedlings, it was reported that grafting was a difficult process due to the low rate of callus formation and survival (Mugasha, 1980). In other species, according to Gandev (2007), this was caused by the presence of high concentration of phenolic compounds in plant tissues and the compounds oxidation from wounding species, which was also reported as a major obstacle to micro-propagation of *A. stuhlmannii* tree (Neondo *et al*., 2011). For this reason, different methods of propagation have been investigated in *Allanblackia* species, because propagation with cuttings and grafting was found to be difficult due to plagiotropism and/or poor rooting (Atangana & Khasa, 2008). Hence, germinating seeds is the most popular technique in the production of seedlings in this species. Success in grafting may however vary depending upon physiological condition of the rootstock, scion, and season, as reported by Alam, Mortuza, Uddin, Sarker, & Barman (2006). *A. floribunda* trees planted in 2007 in Cameroon have started producing fruits after 4 years and farmers have started grafting using their own scions (Asaah *et al*., 2012). This model from Cameroon can be applied to *A. stuhlmannii* in Tanzania (Asaah *et al*., 2011).

CHAPTER THREE

EFFECT OF SOWING METHOD AND GERMINATION PROMOTERS ON THE *ALLANBLACKIA STUHLMANNII* SEED GERMINATION UNDER LIGHT AND DARK ENVIRONMENT

Introduction

Seed germination is an important stage in the life cycle of any plant, affecting seedling development, survival, and population dynamics. Germination begins with seed water uptake and terminates with the elongation of the embryonic axis from the seed coat (Bewley *et al*., 1994; Bewley, 1997; van Klinken, Lukitsch, & Cook, 2008). Germination events and subsequent establishment are controlled by nuclear and maternal genetics, and current and maternal environments (Baskin & Baskin, 2004; Bentsink & Koornneef, 2008). Genotypic inheritance can increase plant fitness to local habitats by adaptation, which enables seeds to germinate at the right time and the right place. Phenotypic variation, on the other hand, may increase the diversity of seed germination in time (Baskin & Baskin, 2004; Van Klinken *et al*., 2008), maintaining a soil seed bank. Environment regulates seed dormancy release, seed germination rate and capacity, and seed deterioration and mortality. The microclimate that directly surrounds seeds in a seedbed determines the seed germination process; therefore, seedbed conditions affect seed germination timing and its landscape pattern, seedling establishment, and eventually population dynamics (Baskin & Baskin, 2004; Bentsink & Koornneef, 2008).

Allanblackia stuhlmannii naturally reproduces by seed but the major difficulty in its propagation is related to seed germination. Seeds of *Allanblackia* species can take several months to germinate (Ofori *et al*., 2011; Mwaura & Munjuga, 2007). In *Allanblackia* species, seed coat is usually thick and hard, offering a barrier to water imbibition to embryo. Thus, seed dormancy is suspected to be caused by multiple factors such as embryo and physical dormancy (Ofori *et al*., 2011). In many species, embryo dormancy is released by hormonal treatments using cytokinins, gibberellins or auxins (Kucera, Cohn & Leubner-Metzger, 2005; Ren & Guan, 2008; Zoghi *et al*.,

2011). The phenomenon of seed dormancy is an innate seed property that defines the environmental conditions in which the seed is able to germinate (Finch-Savage & Leubner-Metzger, 2006; Van Klinken *et al*., 2008). An understanding of dormancy mechanisms is of ecological and economic importance.Various environmental components, such as temperature, light, pH and soil moisture, have been known to influence seed germination (Chachalis & Reddy 2000; Koger *et al.*, 2004 Gupta *et al*., 2011). Seed dormancy and germination are regulated by a complex interaction of environmental, edaphic, physiological, and genetic factors (Radosevich, Holt, & Ghersa, 1996; Van Klinken *et al*., 2008). There are several methods used to free seeds from dormancy and initiate early germination depending on the type of seed dormancy. For instance, exogenous application of growth regulators such as auxin, gibberellins, cytokinins and chemicals such as Accelegrow^{TM}, potassium nitrate or thiourea have been found to enhance seed germination of some fruit trees and crops (Hartmann *et al*., 2002; Ren & Guan, 2008; Zoghi *et al*., 2011). Due to their hard exocarps, *Allanblackia* seeds are often thought to have physical dormancy as it offers a barrier to imbibition's (Mugasha, 1980; Msanga, 1987; Ofori *et al*., 2011). Considering the importance of the species and the problem posed by the delayed seed germination, our study aimed at identifying a simple and effective method for improving seeds germination in *A. stuhlmannii*.

Materials and Methods

Study site

The study was conducted at Amani Nature Reserve (ANR) (Figure 3.1), located in North Eastern Tanzania, Tanga region (5°5'S and 5°14'S latitude and 38°40'E and 38°32'E longitude). ANR is renowned as the single most important biodiversity site of the Eastern Arc Mountains due to its high biodiversity per unit area (Newmark, 2002).

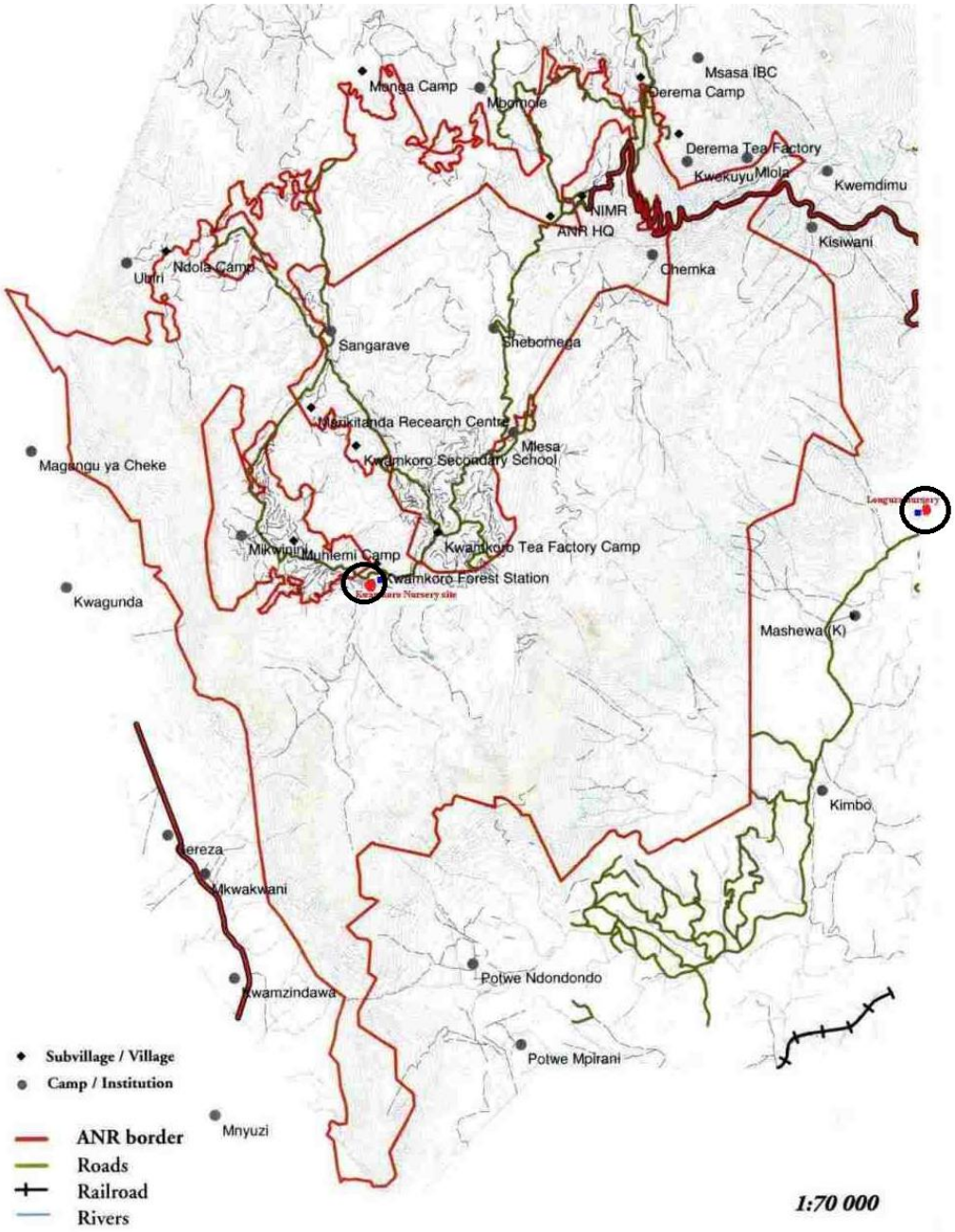


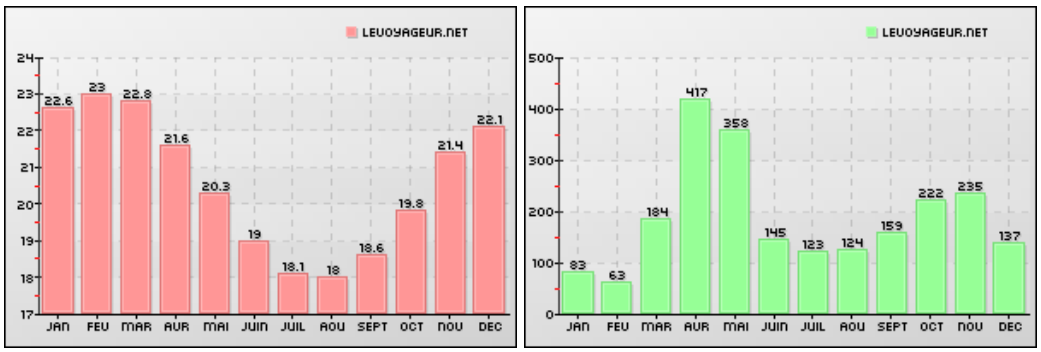
Figure 3. 1: Map of Amani Nature Reserve Range showing nursery location (circled red dots)

Site One: Kwamkoro nursery

The vegetation at Kwamkoro (1050 m a.s.l.) is mainly composed of sub-montane forest. Two main forest types characterize Amani range: secondary forest which predominate the Amani community area while primary forest is to be found in much of Kwamkoro and Monga area at 1050m a.s.l. (Newmark, 2002). The mean

maximum temperature varies between 16–26°C while minimum temperatures range from 10–18°C (Reyes *et al*., 2006). ANR has bi-modal rainfall with a mean annual rainfall of up to 2,262 mm at Kwamkoro station (995m a.s.l.) (Figure 3.2). The rainfall is sustained throughout the year by constant flow of moist currents from the nearby Indian Ocean. Humidity is also high registering up to 87% in the morning and 77% at midday during wet months which provides warm conditions that further facilitate the fast growth of plants. The dry periods (January-February and June-September) register low rainfall of about 100mm (Hamilton & Bensted-Smith, 1989).

The geology of Amani range comprises ancient crystalline rocks, which belong to the Precambrian Usagara system. These are dominated by gneiss, with lesser amounts of granulites and amphibolites (Hamilton & Bensted-Smith, 1989). Generally Amani range soils are acidic clay to clay-loams of varying depth (over 150 cm), good nutrients and well drained. The colour varies from dark reddish brown in the topsoil to yellowish red and red brown in the subsoil, often having a clay layer in the subsoil (Newmark, 2002. Local altitude seems to greatly influence soil character such that, at high altitude (e.g., 1050m a.s.l.), the soils are rather acidic ferrasols (pH 4) but the pH tends to be neutral (pH 7) as we move to lower altitude (300m a.s.l.) (Hamilton and Bensted-Smith, 1989; Reyes *et al*., 2006).



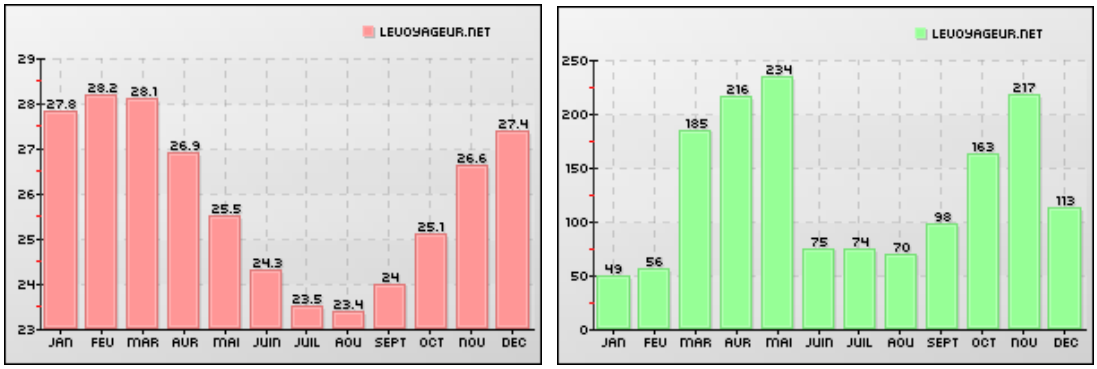
a **b**

Figure 3.2: (a) Average temperature (°C) and (b) rainfall (mm) distribution at Kwamkoro nursery (*Source*: http://www.levoyageur.net/weather.php)

Site Two: Longuza nursery

Longuza which is a lowland forest is predominantly secondary forest composing of teak plantation forest. It is located at the foothills of the Amani range between 4°55' S and 38°40' E at 180-300 metres above sea level. The mean maximum temperature varies between 26–32°C while minimum temperatures range from 15–20°C (Pedersen, Hansen, Mtika & Msangi, 2007). The mean annual rainfall is 1548 mm with a dry spell between June and September (Figure 3.3). The natural vegetation in the dominant canopy is moist-deciduous to evergreen lowland forest characterised by tree species such as *Cephalosphaera usambarensis*, *Beilschmedia kweo*, *Newtonia buchananii*, *Milicia excelsa*, *Antiaris usambarensis* and *Khaya anthotheca* (Pedersen *et al*., 2007).

The Amani Nature Reserve is surrounded by 18 villages, out of which 11 villages are in lowland and the remaining 7 villages are in highland. The estimated average population density in Amani area is 132 people per km^2 but some villages have over 300 people per km^2. Farming (both food and cash crops) is the main economic activity with average farm size of 2.7 hectares. The main food crops cultivated are maize, cassava, bananas and beans, while the main cash crops are sugarcane, cardamom, cinnamon, cloves and black pepper. Tea estates (privately owned) are major employers in the upper plateau of Amani (Reyes *et al*., 2006).



a **b**

Figure 3.3: (a) Average temperature (°C) and (b) rainfall (mm) distribution at Longuza nursery (*Source*: http://www.levoyageur.net/weather.php)

Study materials

Five trees were used to be source of the experimental materials in this study. All the five trees were located on farm as remnant trees left after removing other vegetation to pave way for agriculture. The five mother trees used in this study were coded L26, L28, L62, L19 and L64 according to a previous unpublished study on their molecular genetics conducted by ICRAF in 2005. The letter 'L' was coined from the person (Lars Schimdt) who marked the trees and conducted study them. L26 and L62 were trees previously cut and coppiced to grow to mature trees again whereas L19, L28 and L64 were trees which had never been cut. Another distinction was that L62 and L19 were cream flowered tree type located on-farm whereas L26, L28 and L64 were pink flowered tree type located on the forest edge. The assumption of selecting these tree was that materials from the two flowered tree types may behave differently during seed germination and grafting trials.

Experimental design

In the Amani Nature Reserve, *Allanblackia stuhlmannii* trees simultaneously flower and fruit from December to March each year. A study with 4 mother trees seed sources, 2 physical seed coat treatment, 4 chemical treatments and 3 sowing methods in a factorial split plot arrangement was laid out at Kwamkoro and Longuza nurseries at the fruiting season of December 2009 to March 2010. The experiment was an incomplete randomized block design (RBD) with incubation or sowing methods as 'main plot' factor with one replicate at each nursery or blocks (Plate 3.1 and 3.2). The 'sub-plot' factors per nursery were 4 mother trees (L26, L28, L62 and L19) as seed sources, 2 seed scarification methods (with and without seed coat) and 4 chemical pre-treatments (control, GA_3, Accelegrow™ and fungicide). These sub-plot factors form 32 combinations (4 mother trees seed sources by 2 seed scarification treatments by 4 chemical treatments). However, due to practical constraints at the time of experimentation and to ensure homogeneity within each sowing method only 16 of these combinations could be evaluated at each location per sowing method. The design ensured partially balanced and orthogonal comparisons by ensuring 4 replications of each genotype, 8 replications of each scarification treatment and 4 replications of each chemical treatment in each location by sowing method (main plots). This enabled comparison of all main effects, two factor and three factor

interactions. Four factor interactions between the three sub-plot factors and the main plot sowing method were not possible due to partial confounding and insufficient remaining residual degrees of freedom. This was considered less important than ensuring lower level interactions could be evaluated.

During the fruiting season, four individual trees were sampled and selected to be the seed sources for this study and the fresh fruits were collected from the ground. From each of four mother trees, 30-50 mature fruits were collected from the ground, selected to be free of diseases, and kept separately for each tree. Fruits were stored in the nursery for four weeks to soften their pulp for ease of extraction of seeds by hand and for post-maturation.

1200 seeds were sampled from each of the four trees. Half of the extracted seeds were pre-treated or scarified by removing the whole seed coat with a knife in such a way that the embryo and cotyledons were not injured while the other half had their seed coats left intact. Hence the physical or scarification treatments were categorised as seeds with seed coat or seeds without seed coat. After scarification or physical treatments, seed lots was divided into four portions and each portion assigned to each of the treatment sub-plots and treated with prepared chemicals by soaking until they were covered and left standing. This was done as follows: treatment with GA_3 at 2000 ppm for 30 minutes, treatment with Accelegrow^{TM} at 30 ml per litre of water for 10 minutes, treatment with Ivory M72 (fungicide) at 30 grams per litre of water for 10 minutes, and soaking in water for 15 minutes (control). The concentrations of GA_3 were based on a previous study in the laboratory (Ofori *et al*., 2011) whereas Accelegrow™ was based on recommendations from the manufacturer, Biosciences international, USA. The reason for using the fungicide as treatment was to test whether fungal contamination was affecting the germination in this species, despite the seeds being treated with germination promoters. Each class of the treated seed lot was divided into three sowing methods and sown as follows: 25 seeds were sown on black bags in a room (dark environment); 25 seeds sown on sand in a room (dark environment), and 25 seeds sown on sand in a nursery bed (light environment). Hence, a ‘plot’ was formed of 25 seeds from a specific seed sources, scarification method and chemical treatment applied.

The effects of pre-sowing treatments were assessed periodically by counting germinated seeds. Data for cumulative germination was recorded weekly until experiment termination. The scoring of each sowing method (block), seed scarification (whole plots), and 4 seed treatment levels (sub-plots) was used as the variables of analysis. The numbers of seeds germinated were scored when the radicle was equal to or more than 1 cm. At the termination of experiment (after 120 days), all un-germinated seeds were inspected for viability by cutting test method (where seeds were cut into two to verify whether they are dead or alive).

Means of seed germination, cumulative germination, and un-germinated seed percentage was calculated for each fortnight to explore possible 'treatment' variation. Prior to statistical analysis, the percent was arc-sine transformed to normalize the data (Webster and Oliver, 1990). Analysis of variance (ANOVA) was performed on different parameters using GenStat (GenStat, 2009). Bonferroni Test was applied for means comparison.

Results

Seed germination behaviour

Germination of *A. stuhlmannii* seeds started with secondary (seed) root emerging from the distal end of the seed. The shoot was observed to emerge later at the proximal end of the seed followed by the emergence of a primary (main) root from the base of that shoot (Plate 3.3). Subsequently, the seed root degenerated and eventually the primary root developed adventitious roots that took over as the main root system of the young seedling.

a **b**

Plate 3.1: (a) Seed beds in the nursery and (b) seed beds in a room

a b

Plate 3.2: (a) Seeds sown in hanged polybags in a room and (b) germinated seeds on black polybags inserted in blue polybags

a b

Plate 3.3: (a) Germinated seeds with long seed (secondary) roots and (b) germinated seeds showing seed roots on distal side and radicle (primary root) and plumule on proximal sides

Effect of nursery location on seed germination

The average seed germination for both nursery sites was 56.5% at 4 months after sowing. The result of the experiment revealed that Longuza nursery had cumulative mean percentage germination of 77.8% whereas Kwamkoro had 35.2% (Figure 3.4). There was a significant difference in the seed germination between the two nurseries ($p<0.001$). The seed germination in light and dark environment was 57.6% and 49.1% respectively and the difference was not significant ($P > 0.05$). Fungal infections were observed on seeds but this was more on seeds sowed on polythene

bags (75%) in dark environment at highland (Kwamkoro) nursery during the wet season.

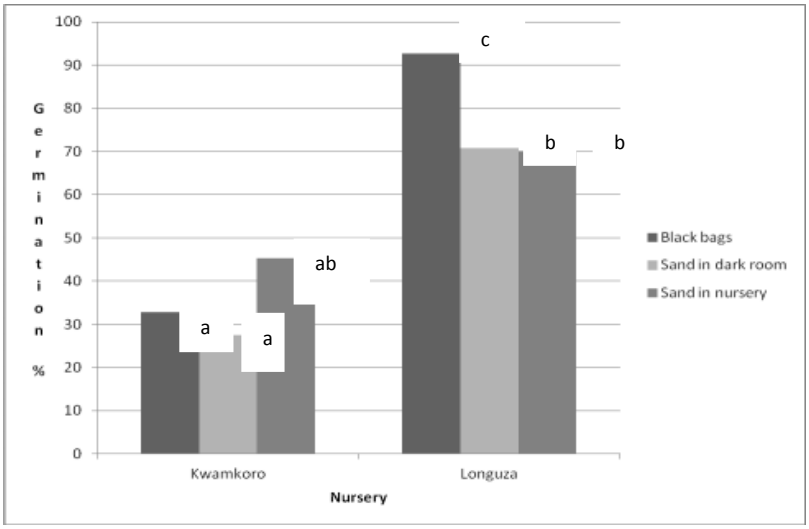


Figure 3.4: Effect of nursery location and sowing methods on seed germination (bars marked with the same letter are not significantly different, p>0.05).

Effects of seed scarification on seed germination

At Longuza there was highest germination percentage with non-scarified seeds at 78.7% and scarified seeds at 77% compared to scarified and non-scarified seeds at Kwamkoro with 48.7% and 21.7% respectively. There was significant differences on seed germination between scarified and un-scarified seeds ($p<0.001$) (Figure 3.5). However, at Longuza nursery the difference in the total germination between the scarified and un-scarified was minimal. At Kwamkoro (highland) nursery, there was a clear difference in seeds germination between scarified and un-scarified seeds treatments. There were no significant differences between the interactions of seed scarification and seed sources ($p=0.591$), GA_3 and Accelegrow^{TM} treatment ($p=0.752$) or sowing methods ($p=0.620$). The results showed that mother seed sources, seed treatment with chemicals or sowing methods used did not affect the germination capacity on the scarified or non-scarified seeds.

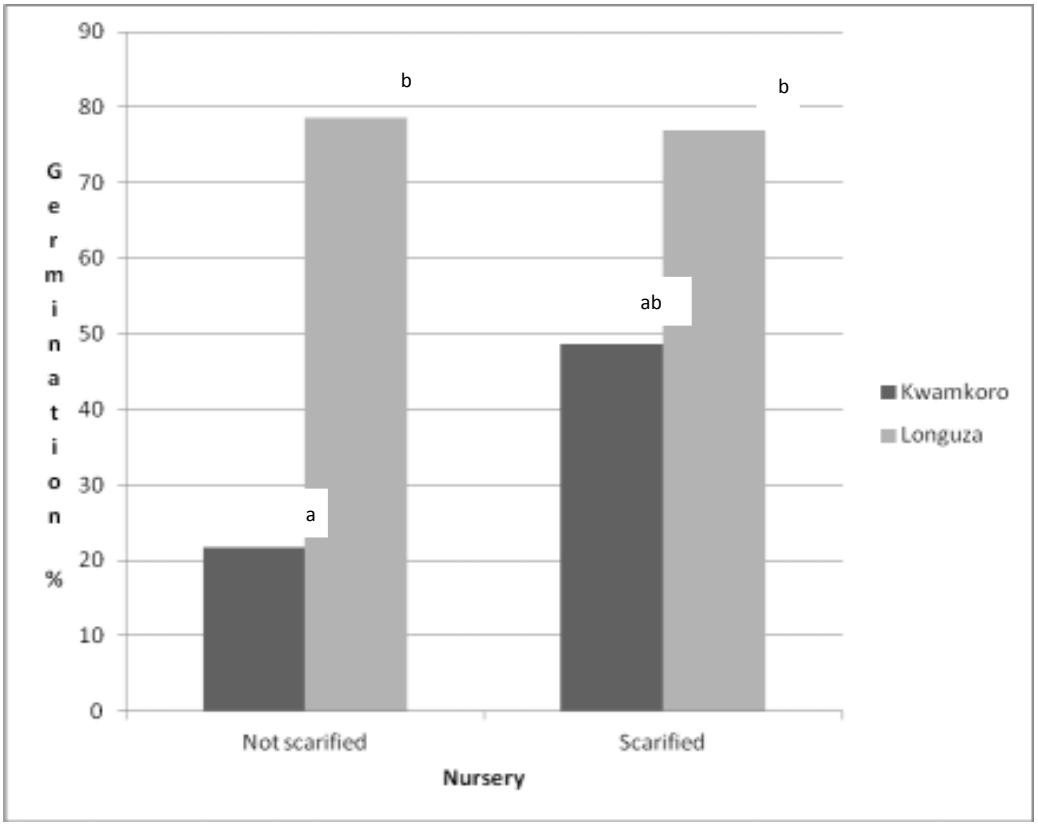


Figure 3.5: Effect of pre-germination treatments of seed coat removal on seed germination at each nursery location (bars marked with the same letter are not significantly different, p>0.05).

Effect of sowing methods on seed germination

On average, seeds sown in black bags had 62.8% germination, followed by sand in nursery with 57.6% and finally seeds sown in sand under dark room environment (49.1%) for both nurseries (Figure 3.6). The sowing method influenced seed germination percentage differently with seeds in Longuza nursery attaining the highest germination of all the three sowing methods used with black bags 92.8%. Germination for the seeds sown on sand, under dark room environment and nursery condition at Longuza nursery were 70.75% and 70% respectively and were not significantly different (p= 0.618). However, germination for seeds sown on sand, in dark room environment and nursery condition between nurseries were significant differences (P<0.001). The highest germination at Kwamkoro was recorded on sand under nursery conditions with 45.3%, followed by black bags with 32.8 % and finally on sand under dark room environment (27.5%). Overall, interactions between

sowing methods and scarification methods (P=0.62), chemical treatments (P=0.813), mother seed sources (P=0.114) were not significant.

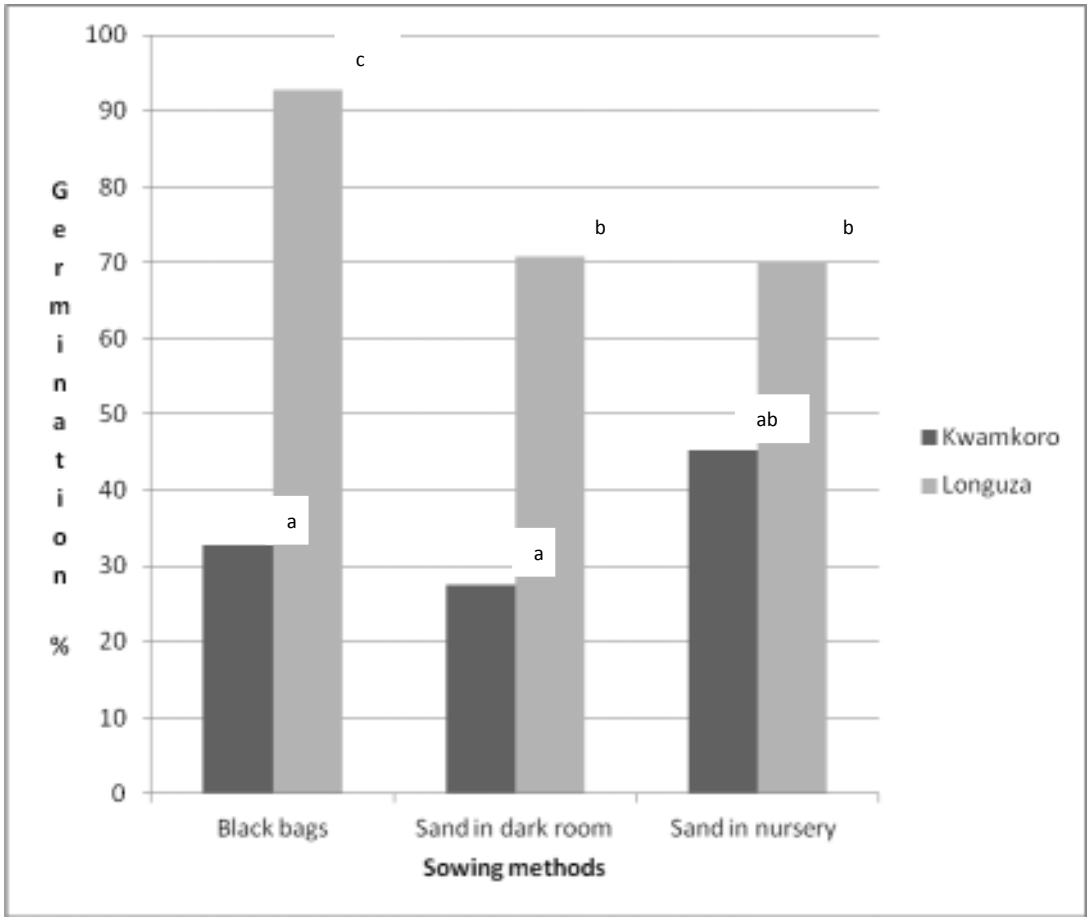


Figure 3.6: Effect of sowing methods on seed germination at each nursery (bars marked with the same letter are not significantly different, p>0.05).

Effect of chemical treatments on germination

The germination percentage did not vary significantly among the chemical treatments; GA_3 and Accelegrow^{TM} versus control (seeds soaked in water) towards germination promotion in the same nursery. Nor were there significant differences in germination between Accelegrow with fungicide treatment and control (seeds soaked in water) against fungal infection in the same nursery. Germination of seeds pretreated with Accelegrow had a slightly higher germination (57.8%) compared to others followed by control (56.2%), fungicides (56.3%) and GA_3 (55.7%) for both nurseries (Figure 3.7). Pre-soaking treatment of seeds with Accelegrow^{TM} or GA_3 did not improve germination. Treatment of seeds with Accelegrow^{TM} or fungicide did

not have an effect on fungal growth. The use of germination promoters or fungicide in this study to improve germination did not result in significantly better germination than the control (P=0.930).

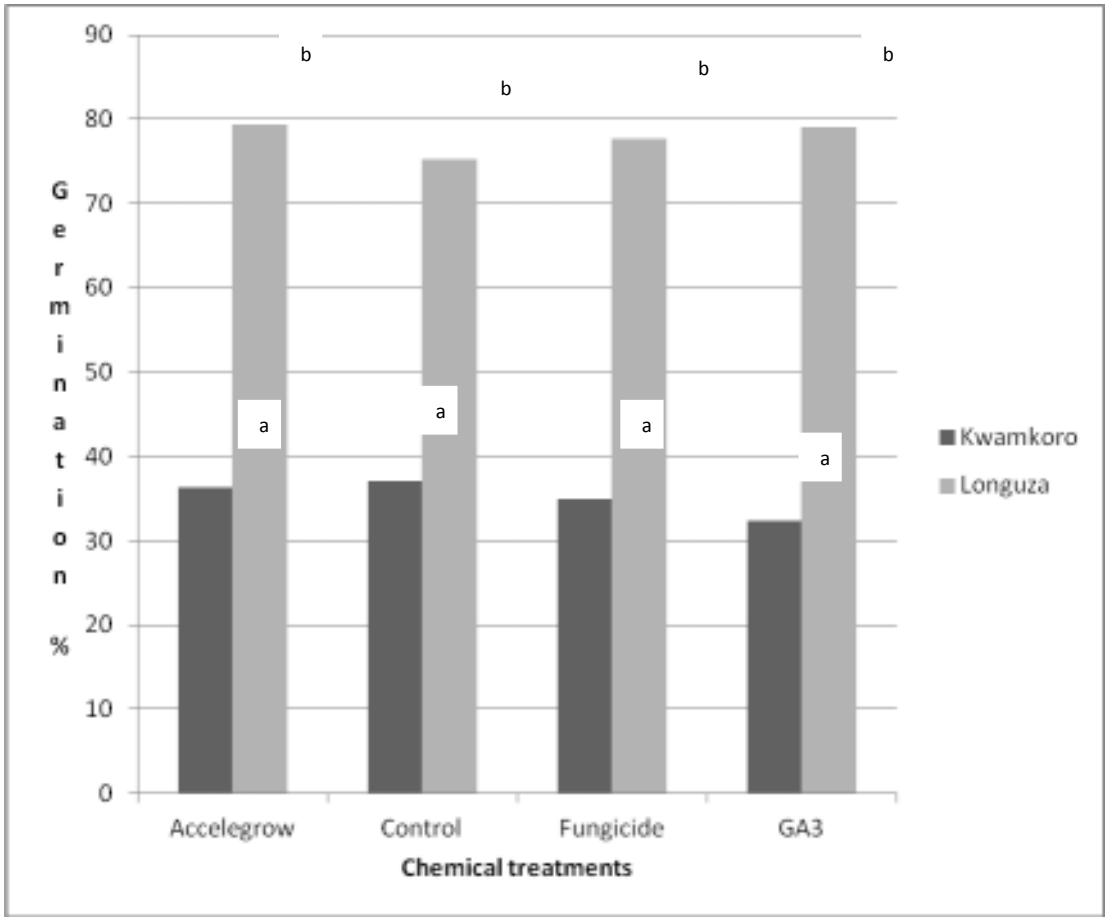


Figure 3.7: Effect of chemical treatment on seed germination (bars marked with the same letter are not significantly different, p>0.05).

Mother tree as seed source effect on seed germination

Seeds from coppiced trees tended to have higher germination percent (L62-58% and L26-58.7%) than the un-coppiced sources (L28 – 55.3% and L19 – 54%) at each nursery location (Figure 3.8). Mother tree effect as seed source was not significantly different for seed germination (P = 0.361) but its interaction on seed sources and chemical treatments on seeds was significant (P=0.005). Seed germination of various mother tree sources was not significantly different irrespective of dormancy-breaking method.

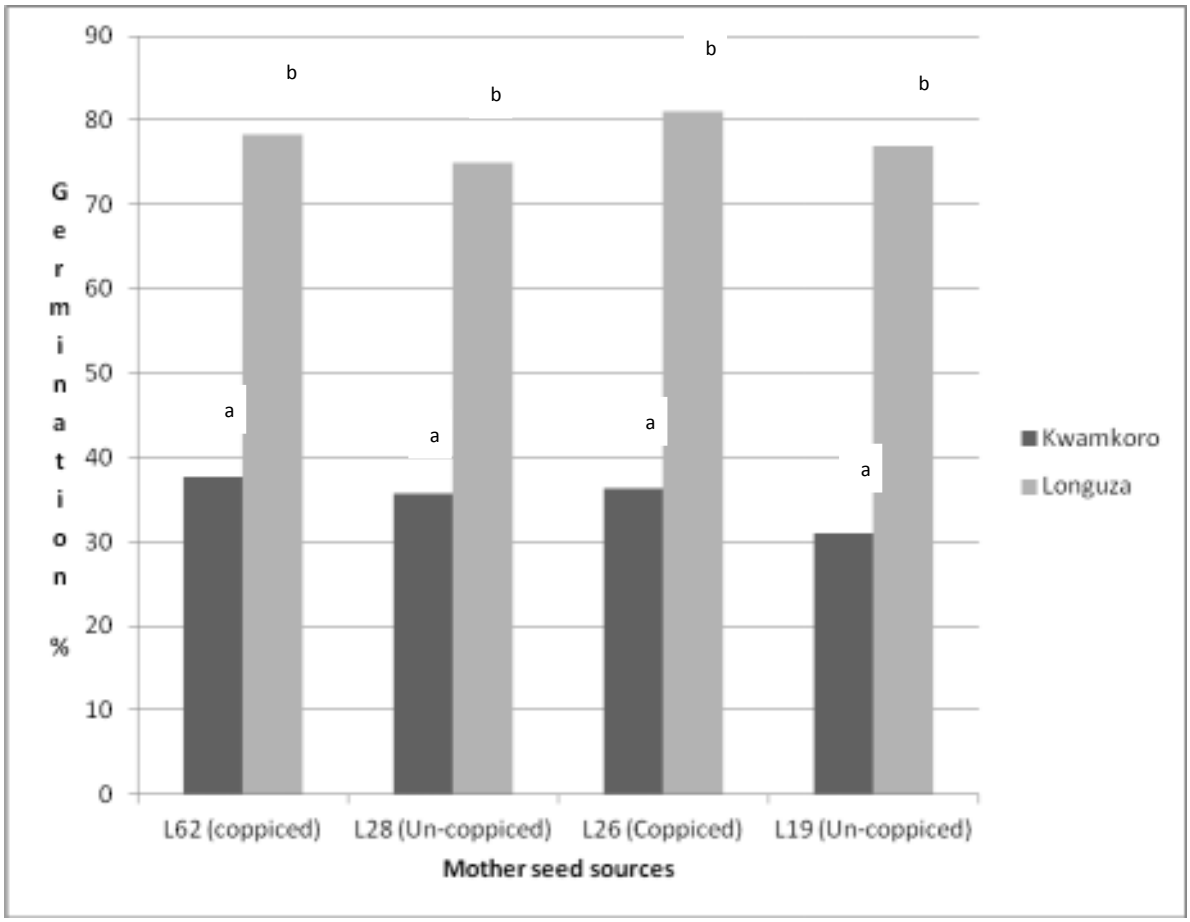


Figure 3.8: Effect of mother tree as seed source on seed germination at each nursery location (bars marked with the same letter are not significantly different, p>0.05).

Seed germination rate

Generally, seed germination was higher at Longuza than at Kwamkoro for all the treatments. At Longuza, seed germination ranged from 70% to 92.5%, with seeds incubated in black polybags being the highest while seeds sowed in sand in a dark environment and nursery conditions attaining 70% germination after 9 weeks. Similarly, seed germination at Kwamkoro ranged from 27.5% to 45%, with seeds sown on sand under nursery conditions being the highest at week 9 (Figure 3.9). In both nurseries, the seeds started to germinate after 14 days with scarified seeds showing earlier germination which was completed within 63 days. The germination rate was highest at 6th week after sowing on both nurseries in all the treatments (Figure 3.10). At the lowland nursery, germination rate was highest in seeds with

seed coats unlike the highland nursery where seeds without seed coat had the highest germination. Germination rate was significantly different between the scarification methods and nursery location (p=<0.001). However, there were no significant interactions in germination rates between sowing methods and chemical application (GA_3 and Accelegrow^{TM}) to seeds.

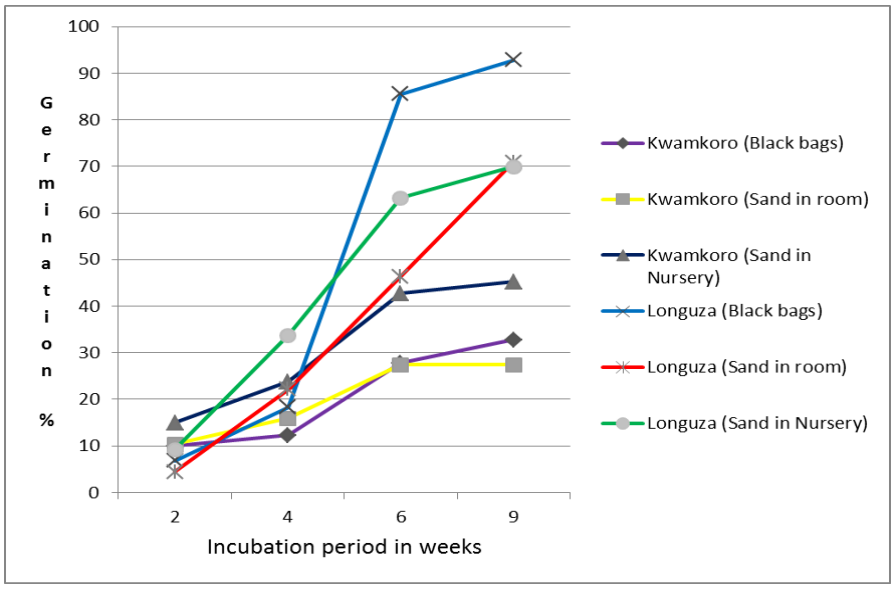


Figure 3.9: Cumulative seed germination (%) of three sowing methods under two nursery conditions up to the ninth week

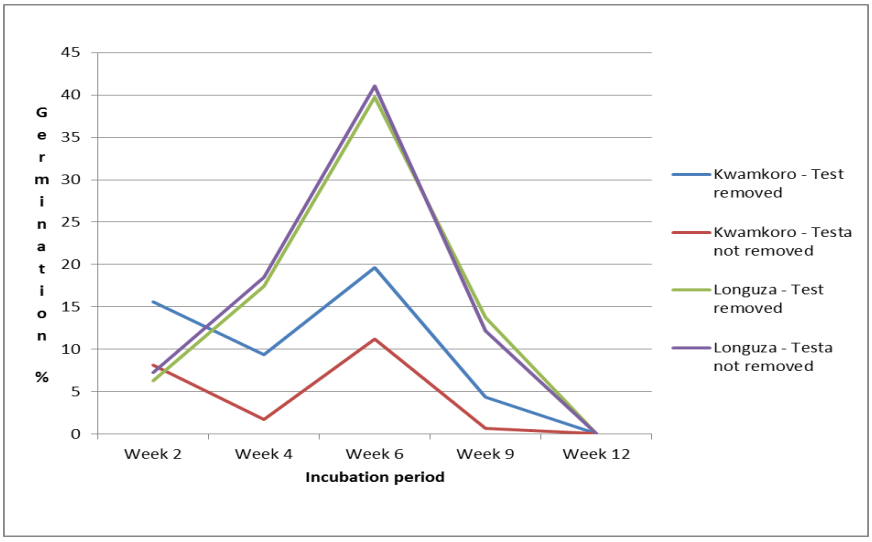


Figure 3.10: Seed germination percentage (%) of two scarification methods at each assessment time on both nurseries

Discussion

Seed germination characteristics:

The behaviour of *Allanblackia* species seeds germination was such that seeds showed the growth of two types of roots: in addition to the primary root, a secondary root (seed root) crosses the seed lengthwise. In *A. stuhlmannii*, the secondary root normally emerged first on distal end of seed; however, the emergence of this secondary root alone did not guarantee the shoot growth. When potted at the stage of radicle emergence only, seeds took a long time for shoot to emerge, root growth sometimes was impaired resulting to death of the plant. Most often, root growth preceded shoot emergence and therefore shoot could be allowed to emerge before potting. In *A. parviflora*, it was observed that when both shoot and root had emerged, the seedlings are suitable for potting as they grow fast than when radicle or shoot only had emerged (Ofori *et al*., 2011). If the seeds with shoot and secondary root only were potted it took longer for seedling to emerge from the soil surface. Similar behaviour had been reported in other species in the same family such as in *Garcinia kola* (Anegbeh *et al*., 2006; Kanmegne & Omokolo, 2008; Di Stefano, Marín & Díaz, 2006) where the seed develop seed (secondary) root first on the distal end, followed by shoot (plumule) and radicle (main root) on the base of plumule on proximal side of seed (Plate 3.3a & b). It was observed that it was necessary to pot the seedlings when both main root and shoot had emerged. Di Stefano *et al*., (2006) reported that the role of this secondary root was to assist the seeds to survive and promote the seedling growth through the use of seed food reserve. Seeds should be aligned in the right position (primary and secondary roots facing down) when potting to hasten the shoot emergency and subsequent growth contributing to healthy rootstocks or seedlings (Ofori *et al*., 2011).

Effect of nursery location on seed germination

Natural regeneration of plant species depends upon the production of viable seeds, subsequent germination and successful establishment of seedlings. Previous studies have suggested that freshly collected seeds possess very low viability both in field and nursery condition (Mugasha, 1980). It was necessary to examine if seeds pre-treatment could enhance seed germination. Hard seed coat is known to inhibit

imbibition of water resulting in poor germination. The differences in seed germination percentage in the study at the two locations may reflect different environmental conditions supplied during germination, which may affect seed physiological and metabolic characteristics. The differences in seed germination were possibly because of differences in temperature regimes between the two sites (Figure 3.2 and 3.3). But it was not possible to distinguish whether the variations in germination responses occurred due to differences in physiological or environment factors (Özer, 2006).

Effects of seeds scarification on seed germination

Several types of seed dormancy have been described including physical, chemical and physiological (Schmidt, 2000). Mechanical constraint, including prevention of water and oxygen uptake, and retention or production of chemical inhibitors are some of the possible mechanisms that cause the strong inhibitory effect of the seed coat on seed germination (Gunaga & Vasudeva, 2011). Several efforts using the methods from the literature have been made to break seed dormancy of *A. stuhlmannii* (Munjuga *et al*., 2008) with little success. Similar studies on *A. parviflora* (Peprah *et al*., 2009) suggested that seed coat scarification was an essential pretreatment to improve seed germination. Hartmann *et al.*, 2002 reported that the seed coat removal and conditions at which the seeds are sown, determine the speed of germination. Unlike the lowland nursery (Longuza) where germination was minimal differences between seeds treatment (78.7% and 77%), the results in the highland nursery confirmed that seeds exhibit dormancy due to their hard seed coat where scarification significantly enhanced seed germination. Breaking down impermeability of the seed coat by scarification resulted in a considerable increase in the germination percentage (50 to 62.8%). The effectiveness of seed coat removal treatment led to the assumption that part of seed dormancy mechanism is external or coat-imposed as reported by Ofori *et al*., (2011) in *Allanblackia parviflora*.

The variation in seed germination was minimal between the scarified and un-scarified seeds in the lowland nursery unlike the highland nursery where variation was significant different (Fig 3.5). However, these results suggest that other factors such as warmer temperature at lowland nursery, where temperature is exceeds 30°C

during the day, could have influenced seed germination beside seed coat removal. Hence, warmer temperatures may be necessary to promote seed germination by overcoming other forms of dormancy in the seeds (Ribeiro *et al*., 2013). Again, removal of pericarp (seed coat) itself is expensive and cumbersome as it requires more labor.

Effect of sowing methods and media on seed germination

In the appraisal of media effect, sand did not significantly affect the germination or the germination time. The reason is that sand is porous and water percolates faster through the medium leading to scarcity of moisture in the media for seed germination. The role of medium is to provide favourable environment especially moisture and air requirement for seed germination (Schmidt, 2000). However, seeds sown on sand, which has a low water-holding capacity, dried faster but the moisture level was not a limiting factor in this experiment as watering of beds was done daily. The differences in the germination capacity between the sowing environment and nursery sites could be explained in terms of fungal contamination through watering as reported by Peprah *et al*., (2009) and the differences in temperature regimes between the two nurseries. In the highland nursery, the seeds were severely attacked by fungal infection due to dampness which was contributed by the coolness of environment as opposed to the lowland which was warmer. The sowing methods or main effect (P=0.618) and its interaction was not significantly different for seed germination irrespective of dormancy-breaking methods. The results showed that the use of black bags in lowland nursery was the best method but sand as a sowing medium would be preferred under nursery condition due to less beds maintenance and its availability in the area. The germination values obtained from light conditions were similar at the same incubation location and sowing method. Therefore, it was supposed that presence or absence of light has no effect on both germination percentage and germination speed of *Allanblackia* seeds.

Effect of chemical treatments on germination

Exogenous GA has been applied to many species for stimulating germination and to increase embryo growth potential (Hartmann *et al*., 2002). Also other chemicals such as Thiourea and KNO_3 have been known to stimulate germination by reducing the

preventive effect of the seed coat in seeds of many species (Hartmann *et al*., 2002). Among the mechanisms, the accumulation of abscisic acid (ABA) during maturity of fruits (Hartmann *et al*., 2002), pericarp thickness and seed coat are considered to contribute to dormancy. In the present study, seed treated with germination stimulants (GA_3 and Accelegrow^{TM}) did not influence germination capacity. Both these chemicals were unable to break dormancy of seeds in this species. This could be due to embryo dormancy or its excessively hard seed coat especially after extraction of seed from the fruit (Msanga, 1987) that affected seed germination.

Effect of mother tree as seed source on seed germination

The interaction of chemical treatments and seed sources (mother trees) had positive effects on seed germination. However, seed source had a direct effect on germination probably due to differential sensitivity to fungi contamination. Seed source had an indirect effect on germination because senescent fruits collected beneath tree canopy might have had a variable proportion of physiologically immature seeds in the fruit which is also dependent on the mother tree (Alouani & Bani-Aameur, 2004). Moreover, because of differential trees precocity, ripe fruits may be more or less exposed to unfavourable conditions which would hinder seed viability and germination (Mycock *et al*., 1995; Smith and Berjak., 1995). After collection of fruits from the ground, sorting physiological mature fruits and storing them for post-harvest maturity may improve germination (Ofori *et al*., 2011). Some seeds might be highly dormant while others are nearly non-dormant influencing germination uniformity. Consequently, the observed effect of the treatments on hard seeds was likely due to the alteration of the physical properties of the seed coat rather than a direct effect on the physiological processes underlying mother tree dormancy (Hartmann *et al*., 2002).

Seed germination rate

The steepness of the cumulative seeds germination curves (Figures 3.9) showed differences in germination over time, periods of seed germinability and periods at which germination remained constant as well as when maximum germination was attained. The steepness of the curves also revealed how fast or otherwise the biologically relevant parameters namely: the final germination percentage and the

mean germination time rate of germination which informed the dynamics of the germination process attained at the incubation period. Generally, the cumulative germination over time appeared as stretched S-shaped curves resembling typical cumulative germination of a population of seeds over time (Hartmann *et al*., 2002).

The behaviour of *Allanblackia* seeds was such that the emergence of the radicle alone did not guarantee the shoot growth. When potted at the stage of radicle emergence, it took a long time for the shoot to emerge and sometimes the root growth was impaired resulting in death. Most often, root growth was observed to precede shoot emergence, therefore, more time should be given for the shoot to emerge before the seedling is potted. It was reported in other studies that when both shoot and root emerged or shoot alone emerged, it was the best time to pot seedlings rather than when only the seed root had emerged. In this study it was observed that *Allanblackia* germination usually started with the seed root emergence at the distal end of the seed. The shoot later emerged at the proximal end of the seed with a strong primary root emerging from the base of that shoot. Subsequently, the secondary (seed) root degenerated and eventually the primary root took over as the main root of the young seedling. The pattern of germination observed in this species was also reported in other genera like *Garcinia* in the family *Clusiaceae* (Di Stefano *et al*., 2006).

Conclusion

From the results, it was found out that seed coat removal improved seed germination by 27% in the highland nursery, however, in the lowland nursery seed coat removal did not have any effect on germination. Seeds with their coats removed and incubated in polythene bags tended to have higher germination by 22.5% compared to seeds with their coats intact. The time to germination was reduced by 10% by seed coat removal at the highland nursery hence increasing the germination rate of *A. stuhlmannii*. Light or dark environment results reported here did not support the argument that seeds required light or dark environment to germinate. However, the sowing environment and sowing methods may not always behave the same to seeds germination in this species even within the same nursery, during incubation period. The use of chemical treatments did not promote or speed up germination in this

species. The Longuza nursery had a higher germination compared to Kwamkoro nursery by 42.6%, hence providing ideal conditions for seeds propagation in this species. The temperature regimes at the lowland nursery, where the average daily temperature exceeded 26.6°C, did not vary much between day and night time which may have played a significant role in promoting seed germination. The results fundamentally agreed with suggestions that seed dormancy problems are indeed associated with environmental condition specifically temperature regimes (Ribeiro *et al*., 2013). Further research is required to explore and understand the *A. stuhlmannii* seed dormancy and how to break it, so as to attain uniform sized seedlings of a certain age in the nursery. These initial findings, hopefully, will help to direct further research to promote domestication and the conservation of this neglected indigenous tree species to aid its large scale cultivation on-farm.

CHAPTER FOUR

EFFECTS OF SCIONS, ROOTSTOCK TYPE AND GRAFTING METHODS ON THE GRAFTING SUCCESS UNDER NURSERY CONDITION

Introduction

Efforts to domesticate and increase the productivity of indigenous fruit trees in Africa have been hampered by poor seed germination, slow growth rate and the long-time taken to first fruiting (Akinnifesi *et al*., 2008; Kwesiga & Mwanza, 1984). *Allanblackia* could be regarded as one of those important genera, which provide economic or environmental benefits but have been neglected in mainstream domestication (Akinnifesi *et al*., 2008). Seed germination has been quite difficult. Furthermore, *Allanblackia* is dieocious and germinated seed may either be male or female (Perpah *et al*., 2011) and the sex ratio cannot be controlled. Secondly planted seedlings take more than 6 years to fruit (Ofori *et al.,* 2011). Developing genetically uniform material derived from selected desirable individuals to capture interested traits could help to address some of these problems. Grafting has been shown to reduce the period between seedlings planting and tree fruiting in *A. floribunda* (Asaah *et al*., 2011). Similarly, a two year old *A. parviflora* flowered within 2 years after grafting in Ghana, although the tree did not bear fruits (Ofori *et al.,* 2011). It appears that grafting method could be employed to shorten the period between seedling planting and tree fruiting in *A. stuhlmannii* as reported by Hartmann *et al*., (2002) in many species. The methods of grafting and environmental conditions are however important as grafting has yielded low success rate in many indigenous species tried in the past (Mhango, Akinnifesi, Mng'omba & Sileshi, 2000).

In 1980, grafting of *A. stuhlmannii* wildings in Amani nursery resulted in 10% success (Mugasha, 1980) and this poor rate of success was attributed to the rotting of rootstock-scion union due to high humidity at the time of grafting and healing, health condition of wilding at the time of grafting, and to the abundance of latex which prevented contact between cambial cells of the scion and the rootstock. Four years after establishment of field trial with the grafted seedlings resulted to stunted seedlings growth and poor survival rate. Again, no fruits were produced by the end

of the three months assessment period (Mugasha, 1980). However, in other *Allanblackia* species, grafting has proven to be very successful. For example, Ofori *et al*., (2008) reported grafting success of 80% and 50% in *A. parviflora* in Ghana using cleft and side veneer grafting respectively. The current study was therefore undertaken to evaluate success rate of various grafting methods in *A. stuhlmannii*.

Materials and methods

Study site, materials and experimental design

The study was conducted at Amani Nature Reserve (ANR) (Figure 3.1). The experiments were set up at the two localities of a highland area at Kwamkoro and lowland area at Longuza. Healthy fruiting healthy looking prolific trees (big fruit, large number of fruits, fruiting each fruiting season and large kernel) in the farms were identified in Amani Nature reserve block between November 2009 and February 2010 as sources of scions. Five trees including 2 coppiced and 3 un-coppiced trees were marked and used for this study. The five mother trees were coded L26 and L62 were previously cut and coppiced to grow to mature trees again whereas L19, L28 and L64 were trees which have never been cut. L62 and L19 were cream flowered tree type located on-farm whereas L26, L28 and L64 were pink flowered tree type located on the forest edge. The scion shoots, collected from the desired donor plants (mature trees) consisted of a short section of 10 to 20 cm long branches, containing about three to six nodes of well-developed bud eyes. These scion shoots were orthotropic in nature or growing 45^0 to the trunk of the tree and had the same diameter as rootstocks. All the leaves were removed from the collected shoots to minimise the loss of moisture, making sure the buds were not injured while removing leaves. Scions were collected from these trees either early in the morning or in light rain conditions and kept in a moist Snaplock® bag, base downwards to avoid desiccation.

a b

Plate 4.1: (a) Rootstocks or seedlings ready for grafting and (b) grafted seedlings

a b

Plate 4.2: (a) Scion source mother trees and (b) top-cleft grafted seedling sprouted

a **b**

Plate 4.3: (a) Top-cleft grafted healed seedlings and (b) a side-veneer grafting seedling

Two year old rootstocks were selected from seedlings in the nursery on the basis of growth vigour, disease free and absence of parasites. The selected six-hundred rootstocks were divided in two categories; one category of seedlings had their leaves removed and the other category had their leaves left intact. Five scion sources, three grafting methods (top cleft, budding and side veneer), and two rootstocks pre-treatment methods (rootstocks with/without leaves) were tested at Kwamkoro and Longuza nurseries as replications or blocks in a randomized complete block design. 30 plots were laid out in each nursery site, each with 10 seedlings (Plate 4.1 to 4.3). The standard procedures for grafting was used as described by Hartmann *et al*., (2002) as follows: (i) the scions, budwood and the rootstocks were cleaned with 10% sodium hypochlorite, (ii) the scion or bud and rootstock were matched in size, so that the cambial tissues are juxtaposed, (iii) after inserting the scions, the scion or bud and the root stock were tied together by wrapping with transparent plastic strips, and iv) the grafted seedlings was covered with clear polythene bag for 14 days to increase the relative humidity and avoid leaf dehydration. The grafting success

(survival) was scored as 0 = dead or 1= alive. The assessment was made for four months after grafting for graft success and survival percentage.

Prior to statistical analysis, the data was arc-sine transformed (Webster & Oliver, 1990) and analysis of variance (ANOVA) was performed on different parameters using GenStat (GenStat, 2009). The significant differences of the means of treatments were compared by Bonferroni Test.

Results

Effect of nursery site on grafting survival

The survival rate of grafts was generally poor with Kwamkoro (highland nursery) getting 20.7% overall as compared to Longuza (lowland nursery) with 17.3% (Figure 4.1). However there was no statistical significant difference on the grafting success between the two sites (p = 0.581).

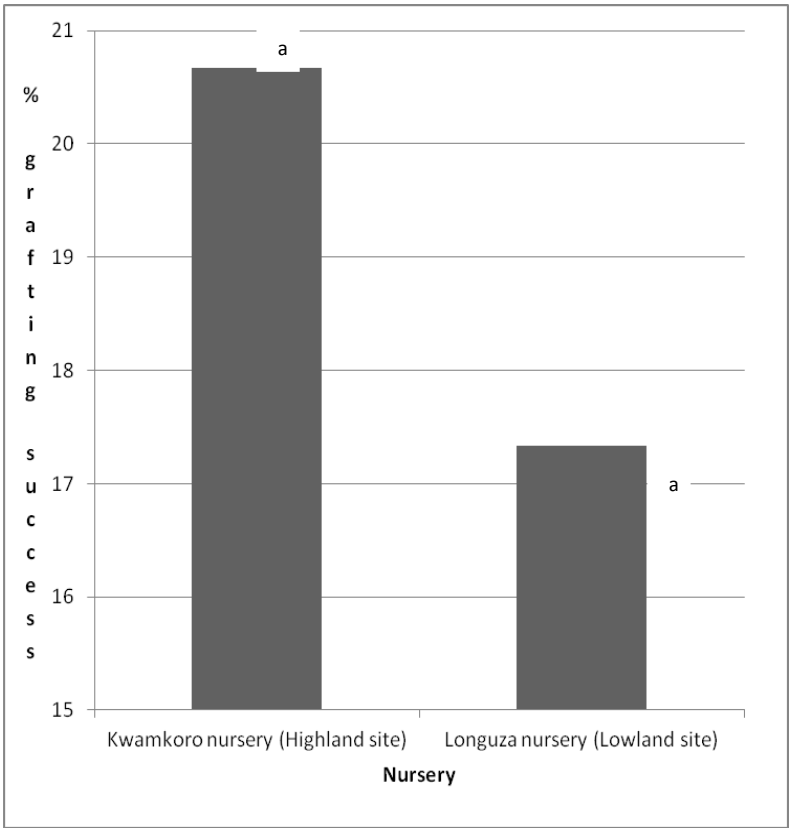


Figure 4.1: Effects of nursery location on grafting success (bars marked with the same letter are not significantly different, p>0.05).

Effect of grafting methods on grafting survival

Top cleft method resulted in higher success rate at Kwamkoro (50%) compared to Longuza (35%); whereas the side cleft was more successful at Longuza (15%) compared to Kwamkoro (11%) (Figure 4.2). Budding on the other hand was not successful in either nursery. There were significant differences between grafting methods ($p<0.001$) and the interaction between grafting methods and scion of coppiced sources ($p<0.001$) (Appendix 2).

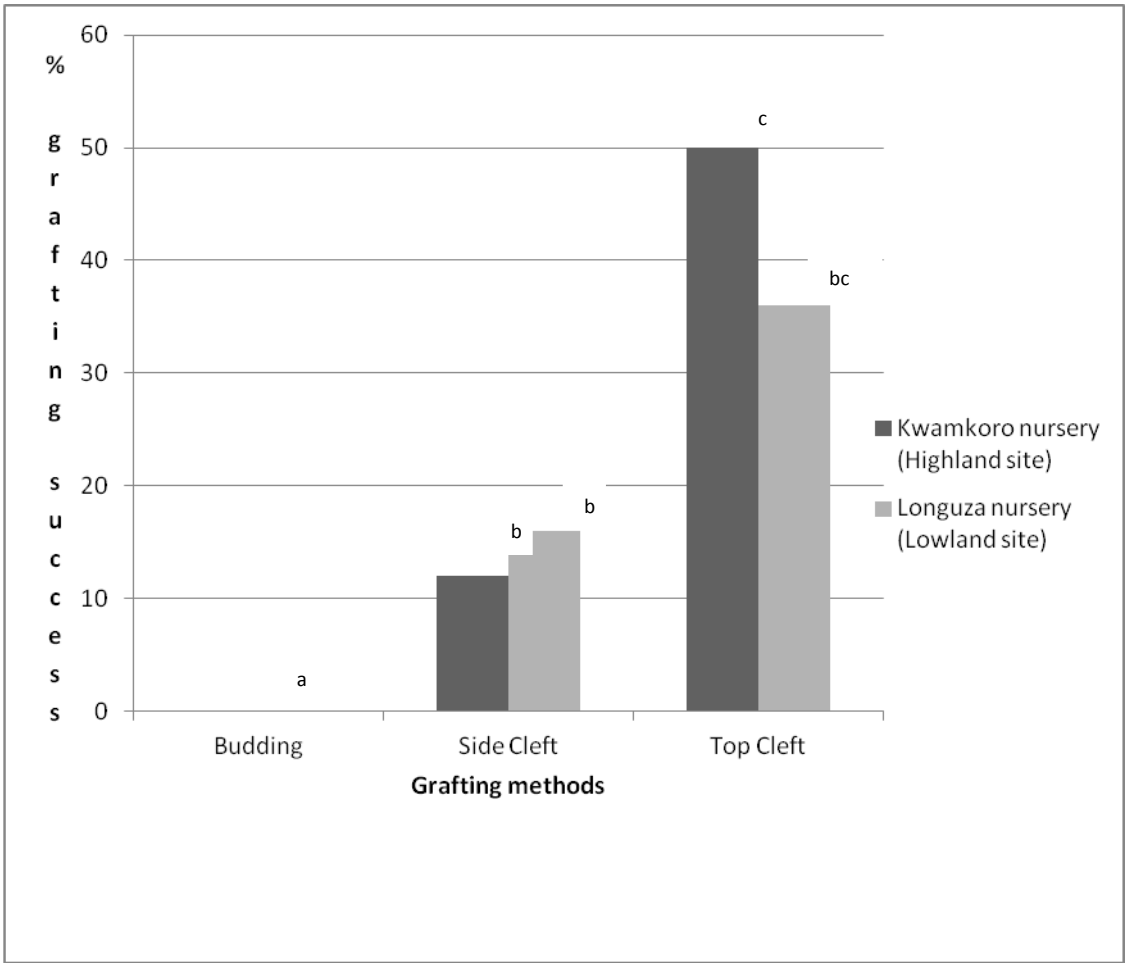


Figure 4.2: Effects of grafting methods on grafts survival as influenced by nursery site (bars marked with the same letter are not significantly different, p>0.05).

Effect of scion sources on grafting survival

Grafting success between scions from coppiced and un-coppiced trees was significantly different (P<0.001). Success of scions from coppiced trees (mean percentage of L62=40% and 30% and L26=30% and 37% at Kwamkoro and Longuza, respectively) was higher than scions from un-coppiced trees (mean percentage of L28=7% and 10%, L19=27% and 10% at Kwamkoro and Longuza, respectively and L63=0% for both nurseries). Mother tree scion source effect on grafting success was significant as shown in Figure 4.3 with coppiced source recording the higher percentage.

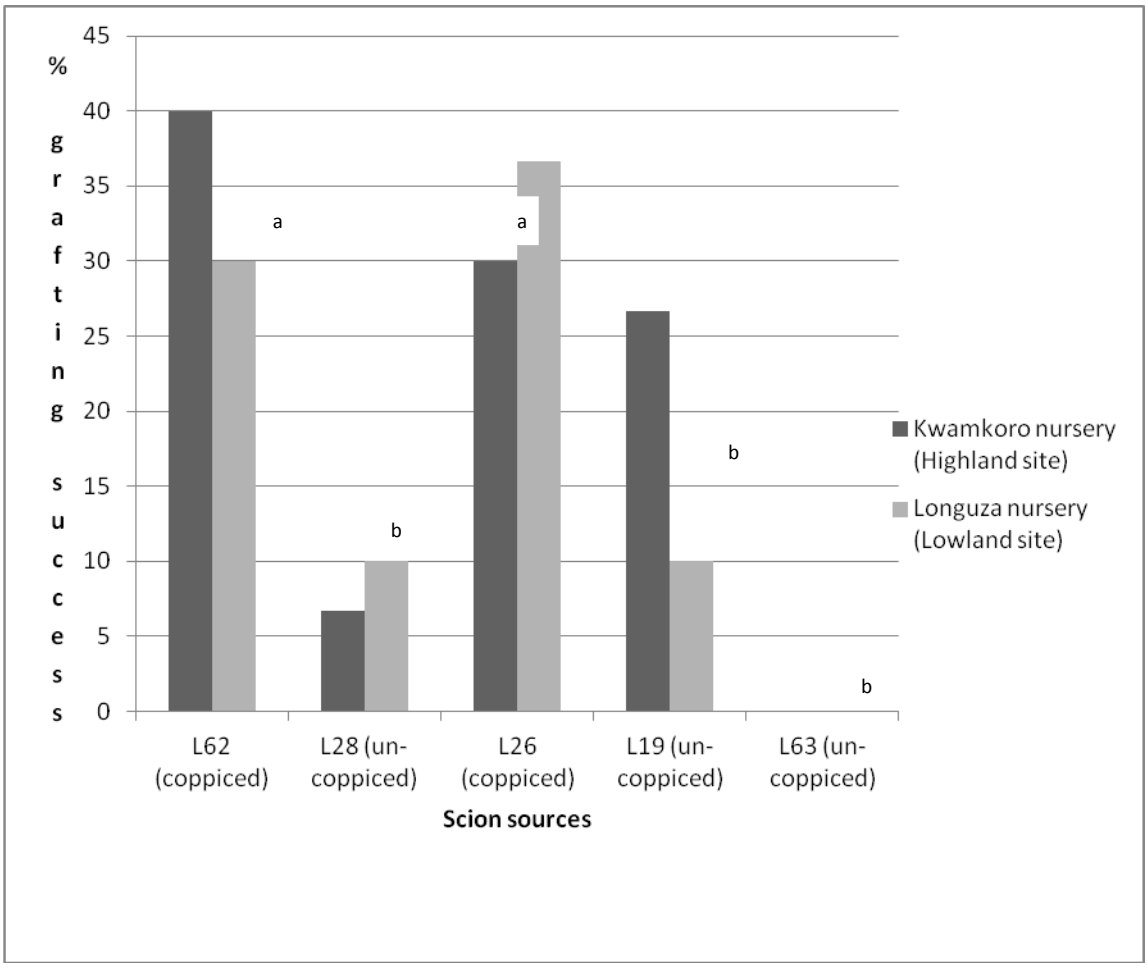


Figure 4.3: Effects of scions sources on grafts success (bars marked with the same letter are not significantly different, p>0.05).

Effect of rootstock treatment on grafting survival

Rootstocks with leaves intact achieved a slightly higher grafting success rate (22.0%) than rootstocks without leaves (16.0%). Rootstocks with leaves intact grafted at Kwamkoro attained the highest grafting success at 22% while 21% was achieved at Longuza nursery compared to rootstocks without leaves whose survival rate was 19% and 14% at Kwamkoro and Longuza nurseries, respectively (Figure 4.4). Removal of leaves on the rootstock had no influence on grafting success (P=0.076).

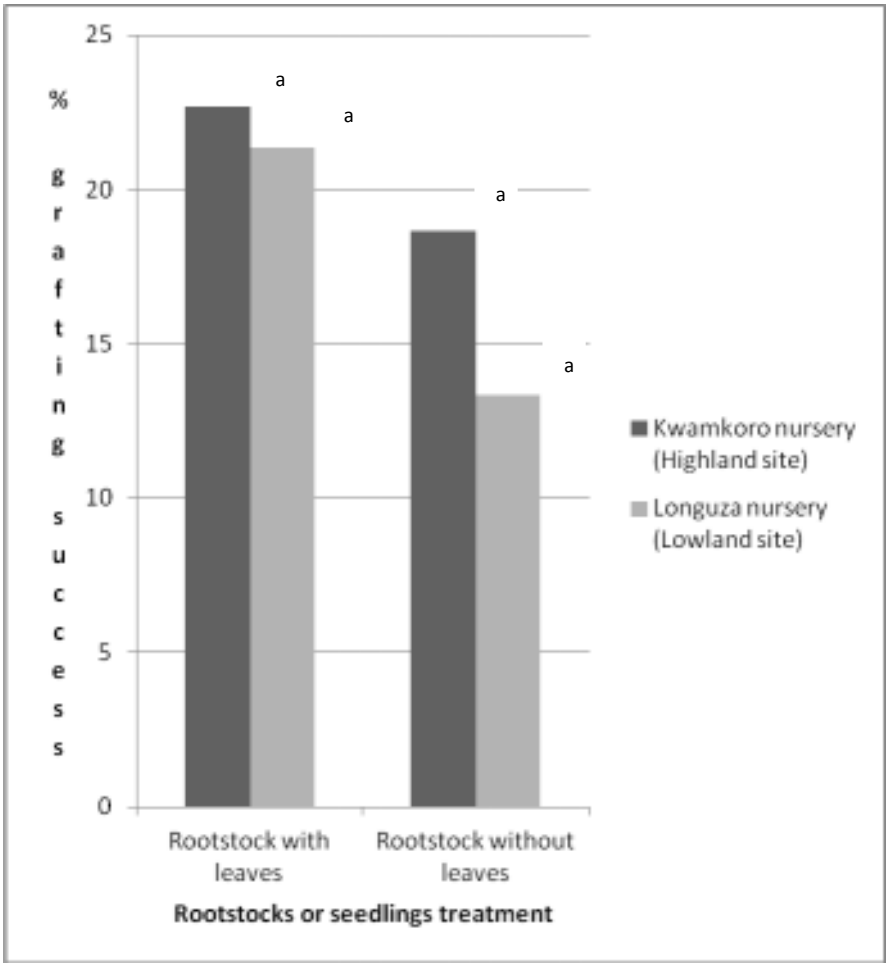


Figure 4.4: Effects of rootstock types on grafting success (bars marked with the same letter are not significantly different, p>0.05).

Discussion

Effect of nursery site on grafting success

Grafting success was rather low at both sites with the highest graft healing being 43% in top cleft graft. On average, Kwamkoro (highland) nursery which has cooler

temperatures had 20.7% graft healing compared to 17.3% at Longuza (lowland) nursery which had relatively warmer temperatures (exceeding 30°C during the day). Warmer temperature at Longuza nursery could have caused the scions to dry up considering the average daily temperature exceeded 26.6°C. Grafting success at Kwamkoro nursery could also be attributed to the wet weather condition at the time of grafting rootstocks as earlier reported by Mugasha (1980) in *A. stuhlmannii* and Ofori *et al.*, (2008) in *A. parviflora* where the scions should remain fresh and healthy. Ofori *et al.*, (2008) also stated that too much moisture may hampered grafting success through moulding on the grafts wound. Reduced grafting success in both nurseries may have been due to wet condition during the rainy season and high humidity in the month of November, which could have promoted mould growth on the graft union. However, this is contrary to other species where moderate temperature and high relative humidity are major factors related to success of grafting (Ram, 1997; Yelleshkumar, Swamy, Patil, Kanamadi, & Kumar, 2008). From the literature, disease had been reported to be one of the most common causes of grafting failure (Leakey 1985; Hartmann *et al*., 2002). It was earlier reported that in many indigenous fruit trees, the best time for grafting was usually during the transition from dry to rainy season when the meristematic activity was starting in order for the scion- rootstock union to establish quickly (Leakey 1985; Yelleshkumar *et al*., 2008). In other species like avocado seasonality effect has been discussed as a contributing factor in the success rate of grafting (Edossa, 2006). In East Usambara, good success rate in grafting can be achieved after cold season and before the short rainy season at end of July to September before the trees start flushing flower buds.

Effect of grafting methods on grafting survival

The results of this study indicated that *A. stuhlmannii* could be successfully grafted using the top cleft grafting method. This method of top cleft grafting method in *A. stuhlmannii* was previously attempted in the Amani with little success (Mugasha, 1980) and similar studies were also conducted on *A. parviflora* in Ghana (Ofori *et. al.,* 2008) with good success. In many species, it has been reported that cleft grafting is more successful than other methods of grafting (Ram, 1997; Sanou *et al.,* 2004) because it is easier to use (Kulwal & Tayde, 1989; Sanou *et al.,* 2004). The ease of

using top-cleft method might be the critical factor in determining the healing success of grafts. The reasons for this are unclear and probably relate to the ease of developing good contact between the cambium in the rootstock and scion, the prevention of desiccation and other factors not examined here. Therefore, top cleft method is a superior grafting technique for this genus as confirmed by Ofori *et al*. (2008) and this demonstrates the potential benefit if the technique can be perfected. However, more tests and practice was required to perfect the art.

Effect of scion sources on grafting survival

Significant differences observed between scion source types (coppiced or un-coppiced mother sources) may be due to the vigour of juvenile material from coppiced sources which contributed to active growth period of mother trees with higher level of nutrients in scion shoots. Better survival of grafts from coppiced mother sources could correlate to higher cell activity (Hartmann *et al*., 2002) for active growth at the on-set of flowers bud formation. Poor success rate on un-coppiced material could be attributed to old age of the mother scion source or dormant stage after they had used most of their food reserves for flowers and fruit production. This in turn may be due to decreased synthesis of endogenous auxin and mobilization of reserved food material promoting healing of the scion-rootstock union (Akinnifesi *et al*., 2008). Low success of grafting in un-coppiced material may also be attributed to the reduced rate of division of cambial cells, their differentiation and consequent development in healing of stock scion union (Hartmann *et al*., 2002). Presence of high concentration of phenolic compounds in *Allanblackia* shoot tissues and their oxidation by wounding (Neondo *et al*., 2011) during grafting might result in a low rate of callus formation hence low grafting success rate (Gandev, 2007). These compounds, therefore, might have interfered with the healing process between the scions and the rootstock.

Effect of rootstock type on grafting survival

Removal of leaves or leaving them intact on rootstocks did not improve the survival of scions. The absence of significant difference between the two methods of pre-treating rootstock suggests that the rootstock without leaves is as good as the one with leaves. Unlike *A. stuhlmannii*, Medagoda & Weerawardana (2005) reported that

Macadamia species produced higher grafts success rate when scions were grafted on rootstocks when all the leaves from the rootstocks were removed. The presence of leaves is thought to help the seedling photosynthesis increasing food supply to plant hence improving the healing of rootstock/scion union. The graft success could be improved when rootstock selection was considered and based on desirable growth attributes of rootstocks as reported in *Uapaca kirkiana* and *Strychnos cocculoides* (Simons, 1987; Akinnifesi *et al*., 2008). Furthermore, proper alignment of scions with rootstocks cambium tissues determines the graft success (Pina & Errea, 2005).

Conclusion

Based on the results of this study, it was concluded that the use of scions from coppiced mother sources for the cleft grafting technique was the best option to graft *A. stuhlmannii* since it had a higher healing by 29% compared to side-cleft grafting. Although low success rate was achieved with the best graft union healing being at 43%, grafting is a very promising technique for propagating *Allanblackia*; however, what is required is to perfect the art to improve on the graft take and success. The time of grafting in this species might be an important aspect as this might influence the scion cambial vigour and healing success. Any type of *Allanblackia* rootstocks can be used in grafting as long as they are healthy since the use of rootstocks with or without leaves did not influence the survival of grafting. The cool weather condition experienced in the highland nursery may have improved the grafting success but very wet conditions may encourage fungal growth leading to low survival rate. From the results, it is recommended that cleft grafting technique should be used for *A. stuhlmannii* propagation using scions from coppiced mother sources. However, further investigation is required to understand the scion-rootstock union and scion source cambial characteristics. This can further be applied to *in-situ* grafting especially on-farm or in a natural environment.

CHAPTER FIVE

GENERAL CONCLUSION AND RECOMMENDATIONS

Conclusion

Two different propagation techniques for *Allanblackia stuhlmannii* tree were the main focus of this study. This is because mass multiplication and conservation of any species depend on reliable propagation methods. Domesticating or managing *A. stuhlmannii* tree successfully in its natural habitats hinges on better propagation methods. Generally, many wild fruit trees are sexually propagated (i.e., from seeds), but a few have been successfully propagated by vegetative methods. Each tree species is unique in its requirements for successful propagation and these must be established. The purpose of these studies was to develop a method to increase germination percentage, shorten germination time, provide more synchronous germination, and result in more efficient seed propagation techniques for production of rootstocks for grafting. Hence, the results from this study provided a basic understanding of *A. stuhlmannii* seed and grafting requirements for which very little information was available.

Results of this study demonstrated that *A. stuhlmannii* could be successfully propagated using seeds. The results also suggested that *A. stuhlmannii* seeds exhibit dormancy. Seed treatment by seed coat removal prior to sowing increased germination rates by 26% at Kwamkoro contrary to lowland nursery where seeds with seed coat intact had more germination by 1.7% than seeds with seed coat removed. This contradicts the effectiveness of seed coat scarification as an effective method; suggesting that germination in this species primarily depends on other factors to promote seed germination. Therefore, *A. stuhlmannii* seeds can be sown with or with seed coat removed as long as other seed germination requirement (temperature, seeds health and maturity) are optimised. However, removal of seed coat without damaging endosperm or embryo was observed to promote germination by 27% in the highland nursery. In addition to seed testa and endosperm layer surrounding the embryo, the growth potential, health and maturity of the embryo are also an important factors to overcome the constraint of surrounding these structures

and thereby affecting the dormancy state of such seed. On the other hand, use of chemicals to promote germination did not have any effects in this species.

Although temperature was not a quantifiable parameter in this study, it was interesting to note that lowland nursery where the mean daily temperature was higher than the highland nursery site, all seed pre-treatments with or without seed coat removed was observed to have higher germination success by 42.6%. The differences in temperatures between nurseries sites significantly influenced seed germination. More research is required to understand the dormancy in this species and how to break it in a controlled condition and in the nursery with varying temperature regimes.

The study demonstrated that *A. stuhlmannii* tree is amenable to grafting technique. The top-cleft method was a superior grafting method as it attained a higher success (43%) compared to other techniques tested in *Allanblackia* species. However, there were still low healing and survival rates compared to most fruit tree species. This method could be used as basis for genetic improvement efforts of this species through selection of superior provenances from the wild. Effect of grafting time on survival of *A. stuhlmannii* needs further investigation to optimise the developed protocol. Furthermore, improvement in survival of grafted seedlings using various grafting methods needs to be evaluated further.

Results of grafting experiments show that rootstocks with or without leaves did not affect the rate of graft healing. However, more research is required geared towards testing grafting on rootstocks with different age classes on different seasons and with growth promoter chemicals such as auxins and gibberellins to determine the best time to graft this species. This research provides input into development of propagation protocols towards domestication, conservation and management for this species, as well as to aid in its production in farming systems.

Recommendations

The following recommendations are suggested as a means of continuing the development of *Allanblackia stuhlmannii* propagation program:

- Lack of quality seedlings for rootstock production and low seed germination and viability has been a problem. Therefore, farmers should be advised and supported to acquire seeds either from local known trees or other known sources for quality germplasm. Based on the seed germination recommendations from this study, temperature is an important factor in developing seed germination protocol for *Allanblackia* spp. in Tanzania. More research is required to understand dormancy in this species and how to break dormancy in a controlled condition with varying temperature regimes.

- Grafting is still a potential propagation method due to dwarfing effects imposed by stocks that bear fruit in shorter time after planting. However, low survival rate of grafting is a threatening problem, and hence selection of suitable vigorous scion/stock combinations is recommended for successful grafting. Shortage of scions/planting has been a limiting factor to scale out planting. The use of scions from locally available trees with the desirable traits such as short trees, more fruit production, fruiting all the years and many large seeded fruits are recommended especially from coppiced fruiting trees. Development of such propagation methods for selection and breeding programs to develop new varieties which are earlier flowering and have fruits in all years is necessary. Finally, it is recommended that the top-cleft grafting method be considered as the main propagation method in the *Allanblackia* seedlings. Further research on grafting should be carried out using different shading or covering effects, time of grafting or seasons, method of grafting, age of scion and defoliation of scion these influence the grafting success. Further studies are required to understand the physiological characteristics of *Allanblackia* trees and how they affect the grafting success. Thus future studies need to examine the time of grafting or grafting seasons, effect of temperature on grafting take and management of the grafting environment to minimise physiological stress and increase the grafting success. Finally, more research is required to explore the possibility of grafting seedlings to produce orthotropic planting materials since the materials produced from grafted

seedlings have plagiotropic behaviour which is not suitable for bearing big fruits as found in this species.

- Scientific studies on different vegetative propagation methods for *A. stuhlmannii* tree species have not yet been done. There is need to evaluate air layering, budding and micro-grafting as potential vegetative propagation methods for *A. stuhlmannii* tree species. Furthermore, field survival assessment of grafting, air layers and budded or grafted *A. stuhlmannii* trees will be required. Therefore, such studies will assist propagators in selecting a feasible and reproducible propagation method for *A. stuhlmannii* tree species.

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APPENDICES

Appendix 1: Analysis of variance for seed experiments

Variate: AcrSine

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Location stratum					
Treatment.Scarification_Method.Clone	9	18988.54	2109.84		
Residual	-8	0.00			
Location.Sowing_Method stratum					
Sowing_Method	2	1730.61	865.30	0.62	0.618
Residual	2	2794.67	1397.34	27.01	
Location.Sowing_Method.Plot_Num stratum					
Treatment	3	34.74	11.58	0.22	0.878
Scarification_Method	1	1849.71	1849.71	35.76	<.001
Clone	3	178.52	59.51	1.15	0.361
Sowing_Method.Treatment	6	149.12	24.85	0.48	0.813
Sowing_Method.Scarification_Method	2	51.06	25.53	0.49	0.620
Treatment.Scarification_Method	3	62.67	20.89	0.40	0.752
Sowing_Method.Clone	6	653.03	108.84	2.10	0.114
Treatment.Clone	9	2134.77	237.20	4.59	0.005
Scarification_Method.Clone	3	101.99	34.00	0.66	0.591
Sowing_Method.Txtment.Scarification_Method	6	303.18	50.53	0.98	0.474
Sowing_Method.Treatment.Clone	18	1323.77	73.54	1.42	0.248
Sowing_Method.Scarification_Method.Clone	6	316.93	52.82	1.02	0.449
Treatment.Scarification_Method.Clone	9	186.65	20.74	0.40	0.916
Residual	15	775.97	51.73		
Total	**95**	**31635.93**			

Appendix 2: Analysis of variance for grafting experiments

Variate: arcsine

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r**	**F pr**
Nursery stratum	1	135.0	135.0	0.78	
Nursery.*Units* stratum					
Grafting method	2	13919.9	6959.9	40.12	<.001
Scion sources	4	7947.6	1986.9	11.45	<.001
Seedling treatment	1	473.0	473.0	2.73	0.109
Grafting method.Scion sources	8	9829.0	1228.6	7.08	<.001
Grafting method.Seedling treatment	2	1464.1	732.0	4.22	0.025
Scion sources.Seedling treatment	4	1112.0	278.0	1.60	0.200
Grafting method.Scion sources.Seedling treatment	8	3663.2	457.9	2.64	0.026
Residual	29	5030.5	173.5		
Total	59	43574.2			

Grafting methods

Comparison-wise error rate = 0.0167

Grafting Method	Mean
Budding	0.00 a
Side Cleft	15.06 b
Top Cleft	37.09 c

Scion sources

Comparison-wise error rate = 0.0050

Mother scion sources	Mean
L63	0.00 a
L28	11.07 a
L19	15.96 ab
L26	28.84 b
L62	31.06 b