DOMESTICATION OF THE SHEA (*Vitellaria paradoxa* C.F. GAERTN) TREE: DEVELOPING IMPROVED PROPAGATION TECHNIQUES FOR ACCELERATED PLANT GROWTH.

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DECLARATION

I declare that the work in this thesis was carried out of me, and that, it has never been submitted either in part or whole for any other degree elsewhere. Besides the references made, which l have duly acknowledged, this thesis is the result of my own investigation.

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DEDICATION

I dedicate this thesis to my wife, Priscilla Yeboah and three beautiful daughters, Abigail Owusua Yeboah, Eunice Dedo Yeboah and Esther Tiaa Yeboah as a motivation

for them to reach higher heights in their endeavours

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ABSTRACT

The shea (*Vitellaria paradoxa* Gaertn) tree which grows wild is a multi – purpose species highly valued for oil obtained from its seed which is similar to that of cocoa butter. The wild nature of the shea tree, slow growth and long gestation period does not make its development attractive thus hampering its domestication. However, worldwide, vegetative propagation methods have been used as a means for domesticating endangered and wild species which will give a promising future to the shea industry. A series of field and laboratory experiments were carried out at the Cocoa Research Institute Sub-station, Bole and KNUST, Kumasi from 2012 to 2014. The objectives of the study were to (i) assess the rooting performance of air-layered stems under dry and wet conditions (ii) determine the effect of propagating structures and seedling types on the weaning and field survival of rooted propagules (iii) assess the role of some endogenous growth promoting substances and application of some exogenous growth regulators on rooting of air-layered stems (iv) investigate the physiological and environmental effects of scions and rootstocks on grafting success (v) study the biochemical constituents and anatomical features of shoots and rootstocks used for grafting, layering and rooted cuttings . A series of factorial experiments in randomized complete block design were used for the field studies. The data were analyzed using Analysis of Variance (ANOVA), regression and correlation analysis. Rooting of the air-layered shoots was better in both number and length of the roots with the application of 10,000 ppm indolebutyric acid and using *Sphagmum* moss as rooting medium than the other treatments. Ambient low temperatures (22-24^{o}C) had significant positive effect on rooting of air-layered shoots. Weaning of propagules showed high survival for the rooted cuttings in the mist propagator (93.3 %) comparable to that of the seedlings (100 %). Plant height and stem girth of the rooted cuttings were however similar for all the propagating structures during the weaning period of three months. In comparison with the normal (seedling) plantlet, the rooted cuttings

in the mist propagator recorded better growth in terms of leaf production, plant height and stem girth. There was a significant relationship between the canopy architecture of the selected tree and the root production of the layered shoots expressed as $Y_{(rooting)} = 113.87 - 23.697 X_{(canopy\ spread)}$; $R^2 = 0.89$; $P < 0.002$; $n = 9$. There was also a significant relationship between field survival of propagules and the month of establishment expressed as Y (percent survival) = -2844 + 0.070 X (month); $p < 0.001$; $R^2 = 0.68$; $n = 90$. For field establishment, planting at a soil depth of 52 cm was suitable for transplanting weaned propagules as it produced the highest survival, biggest girth and highest number of leaves. Biochemical studies on stem portions showed the presence of nitrogen, protein, simple sugars, total free phenols and auxins which played very significant role in vegetative propagation (air-layering, cuttings and grafting) in producing high rooting and graft success. Anatomical studies revealed that some growth hormones and medium promoted cell differentiation (vascular tissues, xylem, cambium and phloem) in the shoots to enhance rooting. The best wounding method on scions to yield significant graft success and enhanced growth was pre-curing whilst rejuvenated shoot was preferred to young plants as root stocks. Grafting using the top cleft technique with partially sprouted scions significantly gave the best graft success. Coppicing trees in May/June produced the highest number of shoots within sixty days after coppicing as well as the highest graft success. Stages of wound healing and graft union formation were also revealed through the anatomy (differentiation of the xylem phloem and cambial tissues) of the grafts which were indicative of compatibility or incompatibility. Conclusions of the study indicate that the top cleft grafting technique results in the highest graft success.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABBREVIATIONS

ACC	-	1-aminocyclopropane-1-carboxylic acid
ABA	-	Abscisic acid
ANOVA	-	Analysis of Variance
BA	-	Benzyl adenine
BAP	-	Benzylaminopurine
CBE	-	Cocoa butter equivalent
CDP	-	Cashew Development Project
C/N	-	Carbohydrate-to-nitrogen ratio
CPPU	-	N-(2-chloro-4-pyridyl) N'-phenylurea
CRIG	-	Cocoa Research Institute of Ghana
DFSC	-	Danida Forest Seed Centre
DNA	-	Deoxyribonucleic
2, 4-D	-	2, 4-dinitrophenoxyacetic acid
FAO	-	Food and Agricultural Organization
GMT	-	Greenwich mean time
HNO_3	-	Nitric Acid
H_2SO_4	-	Sulphuric Acid
IAA	-	Indoleacetic acid
IBA	-	Indolebutyric acid
TFP	-	Total Free Phenol
ICRAF	-	International Centre for Research in Agro Forestry
2iP	-	2-isopentyadenine
L	-	laevo-rotatory
LSD	-	Least Significant Different
masl	-	metres above sea level

NAA		Naphtheleneacetic acid
Na_2CO_3	-	Sodium Carbonate
pH	-	Acid Strength
V	-	*Vitellaria*
RNA	-	Ribonucleic acid
w/v	-	Weight to volume
OC	-	Organic carbon
Ca	-	Calcium
N	-	Nitrogen
Mo	-	Molybdenum
As	-	Arsenic
Mn	-	Manganese
Zn	-	Zinc
B	-	Boron
K	-	Potassium
P	-	Phosphorus
Fe	-	Iron
Cu	-	Copper
Cd	-	Cadmium
Mg	-	Magnesium
Hg	-	Mercury
Pb	-	Lead
TDZ	-	Thiodizuron

CHAPTER ONE

1.0 INTRODUCTION

The shea (*Vitellaria paradoxa* C. F. Gaertn) tree is an important tree species which plays a significant socioeconomic role in Sub-Saharan Africa. It is a major component of woody flora of the Sudan and Guinea savanna vegetation zones. The species forms an almost unbroken belt in the semi-arid regions of seventeen countries and stretches from Senegal to Kenya (Bonkoungou, 1987). The fruits from the tree are an important source of income for women in many populations of the Semi-arid region of West Africa. The pulp contains large quantities of proteins and mineral and the kernel is rich in fatty acids (oleic, stearic, linoleic and palmitic) (Wiesman and Maranz, 2001). The wood is used for firewood, charcoal, construction, furniture and as pounding mortars (Abbiw, 1990 ; Dalziel, 1937). In Ghana, the bark is used in various mixtures for the treatment of stomach ache (Bennett- Lartey and Asare, 2000). In 2003, shea butter became one of the six vegetable fats allowed by the EU to serve as a Cocoa Butter Equivalent (CBE). In Ghana, in 2011, exports of crude shea butter and raw sheanut yielded 27,611,980 USD and 25,086,810 USD, respectively (Ghana Export Promotion Council, 2012). With the potential output of over 100,000 metric tons of sheanut per annum, Ghana could earn a significant amount of revenue by improving the production and the quality of the produce.

Despite its economic and environmental benefits, the shea tree has been neglected by mainstream domestication (Leakey and Newton, 1994). However, not long had there been renewed interest in the shea tree (Boffa *et al.*, 1996) such that the International Centre for Research in Agro Forestry (ICRAF) has prioritized it as a major tree species for the improvement of livelihoods in the Sahel zone (Bonkoungou, 1997). The tree species has also gained recognition on the list of tree species constituting the African forest genetic resource

priorities (FAO, 1977). Existing populations still remain essentially unmanaged and are regarded as wild trees. However, over the last few decades, there has been renewed interest in the shea tree. The main constraints to domestication of the shea tree are its slow growth, long juvenile phase and large yield-variability (Adu-Ampomah *et al*., 1995). In addition, there is a high degree of variation in fruit and nut production, nut and fruit size, pulp sweetness, oil content and butter quality (Maranz *et al*., 2004 ; Hall *et al*., 1997). The sheanut tree are its slow growth, long juvenile phase and large yield-variability. Efforts at domestication requires that germplasm is conserved whiles enhancing the fruit production of the species. Presently an acceptable approach used for domesticating endangered tree species is by some vegetative propagation methods which include rooting, air-layering and grafting. These methods allows for the production of individuals of the same genetic constitution and also facilitates the multiplication of desirable genotypes in the reproductive phase (Hartmann *et al*., 2002; Grolleau, 1989). This is achieved by taking shoots from ontogenetically mature crowns of large trees with a resultant effect of a significant shortening of the juvenile phase (Hartmann *et al*., 2002 ; Hackett, 1985). Vegetative propagation methods can therefore be used to enhance domestication of the shea tree. Consequently, the objectives of this study were to;

1. Assess the rooting performance of air-layered stems under dry and wet conditions.
2. Assess the role of some endogenous growth promoting substances and application of some growth regulators on rooting of air-layered stems
3. Study the biochemical constituents and anatomical features of shoots and rootstocks used for grafting, layering and rooting.
4. Determine the effect of propagating structures and plantlet types on the weaning and field survival of rooted propagules.

5. Investigate the physiological and environmental effects of scions and rootstocks on grafting success.

CHAPTER TWO

2.0 LITERARURE REVIEW

2.1 Distribution and Species Description.

2.1.1 Distribution of the Shea tree.

Shea trees grow in a large part of Sub-Saharan Africa, occurring naturally in a 5,000 km long stretch from Senegal to Kenya with a width of 500 km. Its distribution spans over seventeen countries, namely, Senegal, Guinea, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Central African Republic, Congo, Mali, Burkina Faso, Niger, Chad, Uganda, Kenya and Sudan (Fig 2.1). In Ghana, the shea tree is predominantly found in the three Northern regions (Upper East, Upper West, and Northern) where the climate is mostly dry. Sparse population of the tree may be found in northern Brong Ahafo and northern Volta regions. Typically the shea belt lies in the rainfall zone of 600 and 1400 mm per annum (DFSC, 2000).

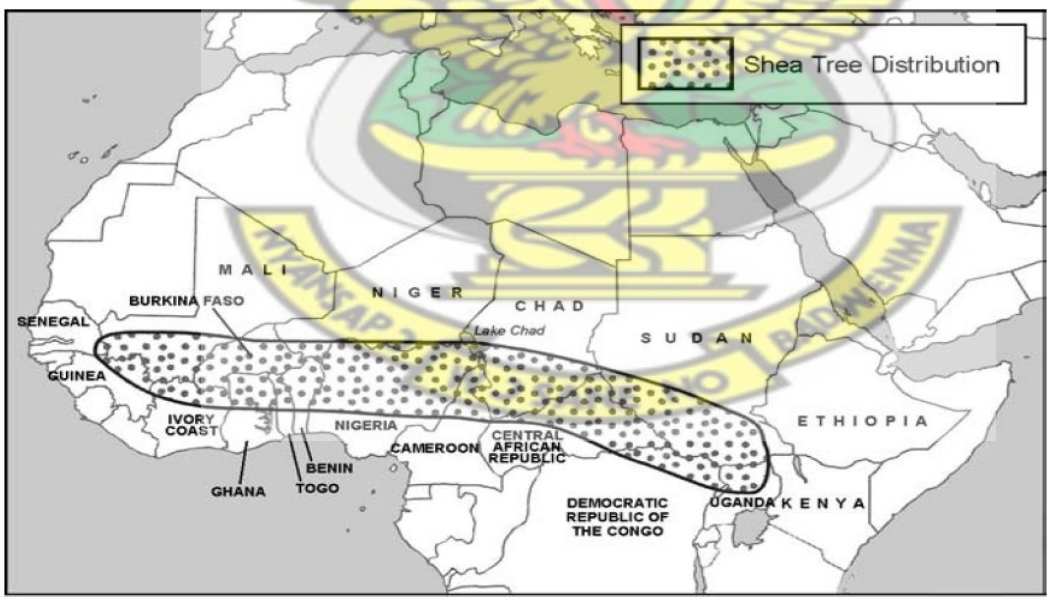

Figure 2. 1 Distribution of the shea tree in Africa. (Elias and Carney, 2007)

2.1.2 Morphology

Shea is normally a small to medium-sized tree, perennial and deciduous with its size apparently controlled by external conditions. In savanna areas subject to regular fires, its height rarely exceeds 10-15 m. Tree heights of 15-20 m are more common in annually cultivated fields. In protected areas, individual trees may be as tall as 25 m, while, in the most severe environments, mature trees do not exceed 7 m in height (Amin, 1990). In mature trees of subsp. *paradoxa*, the trunk is short, usually 3 - 4 m and can reach 8 m (Vivien, 1990) with a diameter ranging from 0.3 m - 1.0 m (Chevalier, 1943). Shoots are initially pubescent and reddish, but soon become glabrous (Chevalier, 1948). Flower-bearing shoots are characteristically stout and blunt (1.0 - 1.5 cm diameter), bearing many leaf scars (Chevalier, 1943). Desmarest (1958) observed that the amount of shade cast by shea trees ranged from very light to very dense and was dependent on tree shape. Heckel (1897) described mature sheanut trees as having round, spindle-umbrella or broom-like crowns. It has also been suggested that the tree size or form is associated with certain agronomic characteristics (Hall *et al*., 1996 ; Desmarest, 1958; Ruyssen, 1957). Ruyssen (1957) also observed the following for the subsp. *paradoxa*; crown radius ranged between 5.5 m for spherical crowns and 9.4 m for umbrella-shaped crowns. Franklin and Hiernaux (1991) recorded crown radii or crown diameter of 4.15 m for tree of 38 cm mean diameter at breast height (dbh).

The architecture of subsp, *paradoxa* is reported by Hallé *et al.* (1978) to conform to the dominant model of the Sapotaceae (Pennington, 1991). Both the main stem and the branches show rhythmic growth, the branches being modular and plagiotropic. The trunk is short and stout, and the main branches large, gnarled and wide-spreading. Boffa (1995) found crown and stem diameters of trees of subsp. *paradoxa* in Burkina Faso to be directly and significantly related. This was confirmed by Schreckenberg's (1996) allometric relationship determined from 60 trees in Benin. For subsp. *paradoxa*, Delolme (1947) distinguished three

stem and branch growth patterns. The first pattern, rapid apical growth, is typical of very young trees. The second, starting at 5-6 years of age is represented by the development of axillary branches extending 15-20 cm yr^{-1}. Such growth occurs only once a year, except in young and particularly vigorous plants, where it may occur two or three times. The third type of growth occurs in old trees or under difficult growing conditions and is very slow, approximately 2-3 cm yr^{-1}; apical buds elongate, forming characteristics barrel-shaped internodes per year.

2.1.3 Foliage

Phyllotaxy is spiral. The majority of leaves are borne at the ends of the branches, 20-30 leaves being crowded into the terminal 5 cm of the shoot (Lely, 1925), but some also develop along branches, and are widely spaced (Chevalier, 1943). Similar descriptions are given for both subsp. *paradoxa* (Aubre'ville, 1964) and subsp. *nilotica* (Eggeling and Dale, 1951). The leaf margins are usually undulate with variations in leaf length (1-30 cm, usually 10-25 cm) and leaf width (2-19, cm usually 4-12 cm) (Amin, 1990 ; Ruyssen, 1957). Petioles are 3-10 cm, exceptionally 17.5 cm, long. The leaves of subsp. *nilotica* tend to be wider than those of subsp. *paradoxa*. Hemsley (1968) describes the leaves of subsp. *nilotica* as being oblong to ovate-oblong in shape, 10-25 cm in length and 4.5-14 cm wide.

2.1.4 Bark and Roots

There is variation concerning the colour of the bark. Colours reported range from almost white (Lely, 1925), through ash grey (Chevalier, 1943), dark grey to almost black (Eggeling, and Dale, 1951 ; Lely, 1925), dark brown (Amin, 1990). Little information is known about the effect of age and environment on the colour of the bark. The bark is rough and deeply

fissured longitudinally and horizontally into thick, more or less square, rectangular or diamond- shaped scales, even on small branches (Kennedy, 1936 ; Lely, 1925).

The square scales of subspecies *nilotica* have a width of 4-6 cm (Eggeling and Dale, 1951). The more rectangular scales of subsp. *paradoxa* are 1.5-2.5 cm in width (minimum 0.5 and maximum 4.5 cm), and 2 – 4 cm in length (minimum 0.5 and maximum 10.5 cm).

The subsp. *paradoxa* has a taproot which usually penetrates to a depth of 0.7-1.0 m (Bonkoungou, 1987 ; Ruyssen, 1957), but depths of up to 2 m been reported (Breman and Kessler, 1995). Ruyssen (1957) measured seedling taproot lengths of 20 cm, two months after germination, and estimated that full length (60-70 cm) was reached within the first few years, after which development occurred solely in lateral roots. Bonkoungou (1987) reported tap roots 20 cm long in 6 month-old seedlings and 65 cm long in 18 month-old seedlings. Lateral roots are shallow. Delolme (1947) found lateral roots of 20 cm from the stem in trees of 48 cm diameter breast height.

2.1.5 Inflorescence and flowers

The flowers are hermaphrodite and develop in fascicles during the dry season, in the axils of scale leaves, at the extremities of dormant twigs, from buds formed two years previously (Chevalier, 1948). Flowering occurs when the tree is leafless (Andrews, 1952 ; Chevalier, 1943). The inflorescence is a dense fascicle, 5.0-7.5 cm, in diameter (Lely, 1925). Together, the fascicles at the end of a flowering twig usually contain 30-40 flowers, though 80-100 have been recorded (Ruyssen, 1957; Eggeling and Dale, 1951; Chevalier, 1948). Individual flowers are white or creamy-white in colour, about 1.5 cm in diameter and subtended by scarious, brown, ovate or lanceolate bracteoles, which are abscised before flower opening (Chevalier, 1943 ; Greenwood, 1929).

There are no consistent differences between subspecies in the numbers of floral parts, but subsp. *paradoxa* flowers tend to be smaller than those of subsp. *nilotica*, notably with respect to the sepals, filaments and styles. However, there is a wide variation in the size of floral parts within each subspecies (Hall *et al.*, 1996). Some trees consistently produced high proportions of flowers (up to 80 %) with unequal numbers of sepals and petals. The four outer sepals are 9-14 mm long x 3.5-6.0 mm wide, and bear indumentums similar to that of the pedicel. The inner parts are slightly smaller and more finely pubescent (Hemsley, 1968), and greenish in colour, often with a pink tinge (Amin, 1990 ; Ruyssen, 1957). The corolla is as long as the calyx, with eight oblong-lanceolate lobes, which are glabrous to pilose. It consists of filaments which are filiform. The dark red anthers are dorsally attached, oblong, mucronate and double cavity of the ovary. They are erect during aestivation, becoming reflexed after anthesis. The anthers and filaments in subsp. *paradoxa* are 3.2-5.0 mm and 7-9 mm long respectively. In subsp. *nilotica*, their respective lengths are 3.5- 4.5 mm and 10-12 mm (Hemsley, 1961).

The flower contains a single ovoid, pubescent superior ovary with 5-10 locules (usually 6-8), each containing a single, anatropous ovule (Aubréville, 1964 ; Chevalier, 1943). The style is slender, variable in length (8-12 mm in subsp. *paradoxa*, and 12-15 mm in subsp. *nilotica*) and persistent (Hemsley, 1961). In some flowers, the style extrudes from the unopened bud, but not in others. This has been interpreted as dimorphic heterostyly (Chevalier, 1948) but work suggests continuous variation in style length (Osei-Amaning, 1996).

2.1.6 Fruits, Nuts and Kernels

The shape of the fruit is oblong, ellipsoid berry (Pennington, 1991), borne on the pedicle 1.5-3.0 cm long (Aubréville, 1964) thickened at the distal end, where the reflexed calyx persist as

the fruit develops. Greenwood (1929), in Ghana and Ruyssen (1957), in Mali, distinguished two different fruits shapes, these being round (round to ovoid) and long (oval to elliptical). The fruit length is most commonly 4-5 cm (occasionally up to 8) and the diameter slightly less (2.5-5.0). In subsp. *paradoxa*, the diameter of the fruit is never less than 50% of the length (Vivien, 1990). Weight varies from 10-57 g but is usually 20-30 g (Adu-Ampomah *et al*., 1995 ; Ruyssen, 1957). The pericarp is 4-8 mm (Aubréville, 1950). Fruit size appears to be similar in subsp. *nilotica*; 5.0-6.4 cm in length and 3.8-4.5 cm in width (Eggeling and Dale, 1951). The number of seeds within the fruit will determine the fruit size and shape (Hemsley, 1961). The inner structure of the sheanut fruit is as described; the berry contains a seed which consist of a testa enclosing an embryo. In literature, the berry is described as the fruit. Work done by Nyarko *et al.* (2012) showed some morphological traits of leaves and fruits between and within the shea populations which may show some variations in the species. The seed is commonly referred to as the nut (in French, *noix*) but also fruit (Greenwood, 1929). The testa is described as the shell (Kershaw and Hardwick, 1986 ; Ruyssen, 1957 ; Delolme, 1947 ; Greenwood, 1929).

The seed with the testa is termed as nut and assumes the shape of the fruit (oblong, ellipsoid) (Pennington, 1991). Ruyssen (1957) describes the nut of subsp. *paradoxa* as spherical, ovoid or fusiform whilst Eggeling and Dale, (1951) describes that of sheanut (*nilotica*) as oval. Individual nuts are 2.8-5.0 cm long and 2.2-3.5 cm in diameter, in both subspecies (Amin, 1990 ; Vivien, 1990 ; Hemsley, 1968 ; Vuillet, 1911). The testa is lignified and sclerotic, dark or pale brown in colour with a broad, pale adaxial hilum almost covering one side (Corner, 1976 ; Vuillet, 1911).

The average fresh weight of the nut is 8-10 g, although extreme values of 4.7 g and 16.0 g have been recorded, which make shea nuts among the heaviest oilseeds available (Kershaw and Hardwick, 1986 ; Delolme, 1947). The fresh nut contains contains 43-68 % moisture by

weight (Greenwood, 1929 ; Vuillet, 1911). According to Vuillet (1911), 100 kg of fresh nuts when dried and decorticated will yield 29-47 kg of dry kernels. Dried kernels form 60-70 % of the nut by weight.

The kernels consist of two thick and fleshy, closely adpressed cotyledons which are buff, orange salmon pink, or black in colour (Kershaw and Hardwick, 1981 ; Vaughan, 1970). The colour varies from tree to tree, and is also dependent on the degree of ripeness at harvest, moisture content and storage conditions (Kershaw and Hardwick, 1981 ; Vuillet, 1911). The embryo is well developed and aligned with the long axis of the nut. The nut has no endosperm (Pennington, 1991). The oil-rich kernels are the most valuable part of the shea tree, and when fresh, represent 15-20 % of the whole fruit weight. In Mali, kernel weight of subsp. *paradoxa* at 7 % moisture content was most commonly 4.4-5.7 g, though extreme values of 2.6 g and 6.6 g were recorded (Ruyssen, 1957). In Benin, Schrekenberg (1996) found that weights of dried kernels ranged 3.9-6.2 g.

Kershaw and Hardwick (1981) give a range of 0.43-7.90 g for parcels of dried kernels from various parts of West Africa. The fat content varies widely among individual trees, and within and among populations and years. The quality of fat produced also varies; some kernels yielding solid fat and other oil (Masters, 1992 ; Kershaw and Hardwick, 1981).

2.2 Ecology

2.2.1 Environmental factors in distribution

2.2.1.1 Elevation

The major occurrence of shea spp. is between 100 m – 600 m above sea level whilst few can be found in the range of 100 m-1300 m above sea level. For the subsp. *nilotica*, higher altitudes of 650 m-1600 m is prevalent. This shows that the shea (*nilotica*) is mostly found in the mountainous areas. The 600 m elevation for shea (*paradoxa)* is located around the Fouta

Djalon Mountain, Guinea, the Jos Plateau, Nigeria and the Adamoua Plateau, Cameroun (Osei-Amaning, 1996).

2.2.1.2 Rainfall, potential evaporation and water deficit

The subspecies lie within fairly narrow climatic limits. Subsp. *paradoxa* occurs where the mean annual rainfall falls between 600 mm and 1400 mm: occurrences outside this range are few and under rainfall only slightly more or less than these values (Osei-Amaning, 1996). Mean annual potential evapo-transpiration estimates for meteorological stations within the range are from about 1400 mm to about 2300 mm. Typically, 5-7 months can be regarded as dry (<50 mm mean rain fall). Assuming a soil storage capacity of 100 mm of available water, a soil moisture deficit prevails for 7-8 months in most of the range- sometimes longer (9-10 months) at the northern limit or more briefly (5-6 months) towards the southern limit.

Subsp. *nilotica* is associated with more equable conditions: mean annual rainfall of 900 mm-1400 mm; estimated mean annual potential evapotranspiration of 1500 mm-2000 mm; 3-5 dry months; 4-7 months with a soil moisture deficit (Osei-Amaning, 1996).

2.2.1.3 Temperature

Shea *(paradoxa)* occurs in high temperature zones because of its low altitude (< 600 metres above sea level) with a mean annual temperature of 24-30^{o}C. The mean annual temperatures where the trees occur are lower (21-22^{o}C). This can be located around the Guinea highlands and Jos Plateau in Nigeria. In Ghana the location of the trees has a mean annual temperature of 24-34^{o}C. In the range of subsp. *nilotica* all of which is >400 elevation, the highest mean annual temperature does not exceed 28^{o}C. Despite elevations comparable with those where subsp. *paradoxa* occurs on the West African highlands, meteorological data indicates mean annual temperature not lower than 22^{0}C (Osei-Amaning, 1996) .

2.3 Biology

2.3.1 Phenology

From various research (Lovett and Haq, 2000) a wide variation exists in both within and between the shea populations. Leaf fall, flushing, flowering and fruiting are noted principally as dry season events in the literature. Leaf fall occurs mostly at the beginning of the dry season (Irvine, 1961) and appears to be strongly affected by the timing of the last rains (Delolme, 1947). A wide variation therefore exist within and between shea populations. Schreckenberg (1996) observed in Benin that, some were completely leafless for short period (two weeks), but this is not a common phenomenon. Some trees are never completely defoliated, having an indistinguishable transition from old to new foliage (Ruyssen, 1957 ; Coull, 1928).

Populations of subsp. *paradoxa* may flower twice within a year. In Central Nigeria, the occurrence at a second flowering in certain trees has been reported in July, normally the period at fruit maturation (Anon, 1975). Picasso (1984) reports that certain trees at Koupela, Burkina Faso, also flower twice which permits a second harvest in October to November. Once flowering is over, there is a flush of leaves as the flowers senesce, one or two new shoots developing from vegetative axillary buds just below the inflorescence (Lely, 1925). In Yendi, Ghana, it was noted that if new leaves were produced before the onset of flowering, fewer flowers were produced (Coull, 1928). Flowering can last for 30 -75 days (Chevalier, 1948).

Ruyssen (1957) reported that fire has a ‘quickening effect’ on both flowering and leaf flush in the Savanna trees. Shea trees which lose their flowers prematurely through burning, can commence flowering again within 2-4 weeks, although only those parts affected demonstrate this early flowering (Chachu, 1982). Burning of the bush during flowering is reported to have a negative effect on yield (Abbiw, 1990 ; Ruyssen, 1957 ; Aubréville, 1950 ; Chevalier,

1948), and harmattan winds have been known to cause flower abscission (Leplaideur, 1987 ; Ruyssen, 1957). Fruit development is a 4-6 month process (Ladipo, and Kio, 1987 ; Ruyssen, 1957; Coull, 1928). Delolme (1947) stated that production occurs in a 3-year cycle, whereas Depommier and Fernandes (1985) observed a 3-4 year cycle in Central African Republic.

2.4 Vegetative Propagation

Vegetative propagation is a fundamental occupation of humankind to produce offspring that will be exactly alike to the original plant. Naturally, a successful propagation method is the one that will help in the transmission of all characteristics of the original mother plant to its offspring (Hartmann *et al*., 2002).

The major activity that occurs in vegetative propagation is cell division by mitosis which involves division of tissues used for growth. There are various methods of vegetative propagation which can be used to produce offspring that are true-to-type to the parent plant. Some of the types of vegetative propagation which can be used in the shea to facilitate domestication are, rooted cuttings, air-layering, grafting and budding.

2.4.1 Cutting and layering propagation

These two types of vegetative propagation are almost similar i.e. production of adventitious roots on cuttings.

Propagation by rooted cuttings is the most important means for clonal regeneration of many horticultural crops: ornamentals, fruits, nuts, and vegetables. Adventitious root formation is a prerequisite to successful cutting propagation. Vegetative propagation of forest planting stock through adventitious rooting is one of the most exciting emerging technologies in forestry (Hartmann *et al*., 2002). Scientists have done a lot of research on the rooting of sheanut stem cuttings which has yielded rooting successes (between 40-80 % rooting success) (Yeboah *et al*., 2010 ; Yeboah *et al*., 2009a ; Yeboah *et al*., 2009b ; Opoku-Ameyaw *et al*., 2000).

Layering is a form of rooting cuttings in which adventitious roots are initiated on a stem still attached to the plant. The rooted stem (layer) is then detached, transplanted and becomes a separate plant on its own roots. Most of the techniques (application of hormones, basal wounding, sucrose application, anatomical consideration etc.) used for rooting sheanut stem cuttings are the same as those for rooting layers (Opoku-Ameyaw, *pers comm*. 2010).

2.4.2 Grafting and budding propagation

Grafting is the joining of two pieces of living plant tissues together in such a manner that they will unite and grow as a composite plant. Budding is a form of grafting. However, the scion is reduced in size and usually contains only one bud.

Fruit trees are generally grafted because of the difficulty in propagating by cuttings and the superiority and high value of the grafted crop. Grafting is among the most expensive propagation techniques even surpassing micro propagation, however, yields good results. The horticulture and forestry industries have sought to develop clonal propagation systems that avoid labour-intensive graftage. Yet, traditional and highly efficient grafting and budding systems are essential for the propagation of many woody plant species. New markets continue to require grafted and budded plants for improved plant quality, fruit yield, and superior forms and better adaptation to greater ecological ranges (Hartmann *et al*., 2002).

2.4.2.1 Genetic limits of grafting

Since one of the requirements for a successful graft union is the close matching of the callus-producing tissues near the cambium layers, grafting is generally confined to the dicotyledons in the angiosperms and to gymnosperms. Both have vascular cambium layer existing as a continuous tissues between the xylem and the phloem.

Monocotyledons (monocots) are difficult to graft with high success because the vascular tissues are scattered throughout the stem rather than the continuous vascular cambium of the

dicots. However, there are cases of successful graft unions between monocots (Hartmann *et al*., 2002) where the meristematic conditions of the intercalary tissues are made use of (Muzik, 1958). It is of importance to identify plants that are capable of combining easily. There is no definite rule that can predict this statement, however, if the plant materials are similar botanically, the chances of graft success is high (Jayawickrama *et al*., 1991).

A graft success can be achieved if a scion is grafted onto a plant within the same clone. In fruit tree and nut crops, different clones within a species can always be grafted together without difficulty. There are some exceptions in some conifers which are difficult to graft with success, for example, red maple (*Acer rubra*), and red oak (*Quercus rubra*) (Copes, 1969).

For plants in different species but in the same genus, grafting is successful in some cases but unsuccessful in others. Grafting between genera within a family and between families is mostly unsuccessful.

2.4.2.2 Graft incompatibility in plants

Graft incompatibility occurs when there is a mechanical/anatomical mismatch (between scion and rootstock), poor craftsmanship, adverse environmental conditions and diseases (Hartmann *et al*., 2002). Sometimes, mutual physiological influences between tissues of the scion and stock can also result in graft failure (incompatibility).

There are different types of incompatibility which mostly occur through anatomical flaws. These are non-translocatable incompatibility, translocatable incompatibility and modification of cells and tissue. Non-translocatable incompatibility includes graft combinations in which a mutually compatible interstock (bridge) overcomes the incompatibility of the scion and rootstock. The interstock prevents physical contact of the rootstock and scion and affects the physiology of the normally incompatible scion and rootstock. Moore, (1982 ; 1984a)

demonstrated that membrane filters placed between the graft partners demonstrated that physical contact is not necessary to develop compatible grafts. An example of nontranslocatable incompatibility is 'Barlett' ('Williams') pear grafted directly onto dwarfing quince rootstock. When mutually compatible 'Old Home' or ('Buerre Hardy') is used as an interstock, the three graft combination is completely compatible and satisfactory tree growth takes place (Roberts and Blaney, 1967 ; Mosse, 1958).

Translocatable incompatibility includes certain graft/rootstock combination in which the insertion of a mutually compatible interstock does not overcome incompatibility. Apparently, some biochemical influence moves across the interstock and causes phloem degeneration. This can be recognized by the development of a brown line or necrotic area in the bark at the rootstock interface. Consequently, carbohydrate movement from the scion to the rootstock is restricted at the graft union (Hartmann *et al*., 2002).

The lignifications processes of cell wall are important in the formation of strong unions in pear–quince graft. Inhibition of lignin formation and the establishment of a mutual middle lamella results in weak graft unions. In compatible pear-quince graft combinations, the lignin in cell walls at the graft union is comparable to adjacent cells outside (Errea, 1998). Conversely, adjoining cell walls in the graft union of incompatible combinations contain no lignin and are interlocked only by cellulose fibres.

2.4.2.3 Rootstock effects on scion growth and vigour

Rootstocks have a profound influence on the growth of scions. Dwarfing rootstocks reduce the amount of scion dry weight. This effect is achieved by a reduction in both the rate at which vegetative shoots grow (extend) and the time period over which they grow. Compared to an invigorating rootstock, a dwarfing rootstock directs a greater proportion of dry weight into fruit. Scions on dwarfed rootstocks might be expected to have less leaf area than those on

vigorous rootstock. The effects of a reduction in leaf area means that scion shoots on dwarfing rootstocks would produce less assimilates relative to scions on vigorous rootstock (Tubbs, 1973a: 1973b).

Differences in the way leaves are oriented on the tree may also influence their ability to intercept solar radiation for photosynthesis and fruit production. These factors may contribute to the reduction in tree size associated with dwarfing rootstocks (Atkinson and Else, 2001).

2.4.2.4 Rootstock effects on nutrient uptake and translocation

Plants store minerals and other nutrients in different organs such as roots, stems, leaves and/or fruits. The organs have a considerable influence on the uptake and translocation of mineral nutrients in plants and plays essential role in physiological processes such as growth, transduction and development (Flowers and Colmer, 2008 ; Wang *et al*., 2006).

The influence of the rootstock on the mineral content in the aerial plant parts was attributed to the physical characteristics of the root system, such as lateral and vertical development, which resulted in enhanced uptake of water and minerals (Jang, 1992 ; Heo, 1991), this being one of the main motives of the widespread use of grafted rootstocks (Lee, 1994). However, in grafted fruit trees, no effect of the rootstock on leaf mineral content was found (Chaplin and Westwood, 1980) and in these cases, more influence of the scion on leaf mineral content was observed. Tagliavani *et al*. (1993) suggested that the vigour of both the scion and the root system had an important role in the uptake and translocation of nutrients in grafted fruit trees, while in pistachio (*Pistachia vera* L.), grafted plant changes of rootstock had an influence on the leaf content of certain essential minerals (Brown *et al*., 1994).

The contents of macro and micronutrients are therefore affected by the rootstock and scion characteristics. However depending on the element and environmental conditions, the effect of the rootstock and/or scion may change.

2.4.2.5 Rootstock-scion interaction as a barrier to water flow

Water and nutrient uptake could be increased in grafted plants as a result of the enhancement of vigour by the rootstock system and its effects on plant yield (Ruiz *et al*., 1997).

Thus, water relations in the rootstock-scion system, as well as the influence of the graft union on water transport to the aerial parts, have been studied with special emphasis on the search for plants' ability to withstand environment changes. Plants form callus at the interface, which enables water to flow from the rootstock to the scion when the callus develops vascular bundles (Moore, 1984a, and b). Insufficient connection of vascular bundles between the scion and the rootstock decreases the water flow (Torii *et al*., 1992).

When water absorption by roots is suppressed at the graft interface, stomatal conductance and scion growth decreases (Oda *et al*., 2005 ; Atkinson and Else, 2001).

Thus, hydraulic architecture becomes of fundamental importance, since the sustained flow of water controls many plant processes such as growth, mineral nutrition, photosynthesis and transpiration. Graft incompatibility can induce undergrowth or overgrowth of the scion, which can lead to decreased water and nutrient flow through the graft union and cause wilting of the plant (Davis *et al*., 2008). Grafting incompatibility usually occurs at the early stages, when vascular connections are forming, but it can appear as late as the fruiting stage, when the plant has a high demand for water and nutrients.

It has been reported that the supply of warm water to the graft union of grafted tomato and egg plants improved the storage quality, reducing water stress at the beginning of the storage when vascular tissues of the rootstock and scion are not connected yet (Shibuya, *et al*., 2007 ; Tokuda *et al*., 2006). This reduction of water stress by a high graft union temperature is due to improved water absorption by the scion (Shibuya, *et al*., 2007).

Thus, to avoid physiological wilt, the promotion of root development and improved management of watering are important in grafted plants.

2.4.2.6 Rootstock effects on flowering

One of the great advantages of using rootstocks is that they induce precocious flowering. Not only are the flowers produced earlier in the trees life cycle, but also large numbers of flowers are produced per unit tree size. This effect is particularly evident with rootstocks that dwarf scion (Atkinson and Else, 2001).

Rootstocks may influence the number of flowers on a tree through changes in scion architecture, particularly with respect to branch angle (orientation) and shoot development. The production of new shoots may be modified such that flower bud induction is favoured rather than vegetative shoots. Developmental changes of this type can be seen on 1- year old wood as an increased production of short shoots and flower buds. Rootstocks may also induce increases in the number and size of flowering spurs on older wood.

2.4.3 Factors affecting vegetative propagation

2.4.3.1 Environmental conditions affecting vegetative propagation

Environmental factors seriously affect vegetative propagation on the responsiveness of the plant through absorption, translocation, plant developmental stage, nutritive conditions and many others. The most striking aside what is mentioned is that of temperature. It is well known that maximal auxin effectiveness is obtained at high temperatures. Rice (1948) observed a greater of auxin in his trial on beans. Temperature and light conditions bear on the nutritional status of the plant.

These two factors strongly influence the carbohydrate level of the plant, and accordingly they do not only affect absorption and translocation of the auxin but they also simultaneously alter the sensitivity of the plant to the auxin after absorption and translocation have been effected (Leopold, 1960). For rooting of cuttings and graft union healing, moderate to low

temperatures (24-27^{o}C) can enhance cell processes whilst temperatures above 28^{o}C can retard development.

Temperature also has a pronounced effect on the production of callus tissues. In apple grafts, little, if any, callus is formed below 0^{o}C or above 40^{o}C and higher, callus production is retarded and cell injury increases with higher temperatures. Cell death occurs around 40^{o}C. Too high a temperature to induce rapid callus development of bench-grafted plants can deplete needed carbohydrate reserves, which limit field survival (Davies *et al*., 1980).

Following bench grafting of grapes, a temperature of 24 to 27^{o}C is about optimal; 29^{o}C or higher results in profuse formation of a soft type of callus tissue that is easily injured during transplanting.

In some instances, it has been found that relative humidity (moisture and water relations) influences auxin effectiveness (Hitchcock and Zimmerman, 1935). One can deduce that humidity may have an effect on auxin activity in the unusual situation where translocation of the auxin is taking place in the xylem, as when the auxin has been introduced into the vascular system. In addition, relative humidity may alter the rate of drying of droplets of auxin solution on the leaves. In this way, low humidities may somewhat impair absorption of auxin. The cambium of graft partners and parenchyma cells comprising the important callus tissue, are thinned walled and tender, with no provision for resisting desiccation. If exposed to drying air, they will be killed. This was found to be the case in studies of the effect of humidity on the healing of the apple grafts. Air moisture levels below the saturation point inhibited callus formation. Desiccation of cell increased as the humidity dropped (Hartmann *et al.,* 2002). High humidity in structures used for vegetative propagation controls evapotranspiration in grafts and cuttings (Yeboah *et al*., 2010a).

There is some evidence to suggest that the photoperiod under which the stock plants are handled exerts an influence in growth (rooting, graft union formation. etc.) (Moe and

Anderson, 1988 **;** Grange, 1985 ; Christiansen *et al*., 1980 ; Johnson and Roberts, 1971). This could be a photosynthetic or morphogenic effect. If light alteration influences photosynthesis, it may be related to carbohydrate accumulation, with best response (rooting/grafting) obtained under that condition promoting increased carbohydrates.

High carbohydrate level sustains a plant material during rooting and graft union formation as observed by Hartmann *et al*. (2002).

2.4.3.2 Physiological factors influencing vegetative propagation

Several physiological factors within the stem cutting are very important in the rooting of cuttings and successful graft formation. Some of them are polarity, girdling, leaves and buds. Auxins and other plant substances move from the apex to the base where they influence their effect. This movement of the substances from the apex to the base results in rooting and graft union which indicates that the stem is fixed in the rootstock or placed in the medium with the apex pointing upwards.

Girdling or otherwise constricting the stem, blocks the downward translocation of carbohydrates, hormones and other substances responsible for rooting or graft formation. The base where the constriction has been done accumulates high levels of these mentioned substances thereby promoting rooting and graft formation (Hare, 1977).

The presence of leaves and buds as well as age of material being used have a strong influence on rooting of cuttings and layers and graft union formation. High levels of auxins are produced in leaves and buds during rooting and promote wound healing (Hartmann *et al.,* 2002). Old (hardwood stem cuttings) materials may not be suitable because the cells are not meristematic hence resulting in low callus formation, poor grafting success and rooting. Noggle and Fritz (1983) were able to identify the level of auxins in the vegetative parts of a plant (Table 2.1). Leaves play a very important role in vegetative propagation.

They provide certain nutritive materials beneficial to graft formation and rooting. The dependence of the plant on the carbohydrate supply has been amply demonstrated (Pearse, 1943), and other materials such as vitamins and nitrogenous materials are supplied by the leaves as well. The beneficial effect of leaves may be proved by spraying sugar solution on the foliage (Yeboah *et al*., 2009a ; Langston, 1954). The presence of buds on stem for rooting and grafting has a strongly promotive effect. lek Van der (1934) found that rooting/graft success were almost absent if all the buds were removed from woody stem cuttings/scions. Buds produce high levels of auxins to enhance graft/rooting success.

Table 2. 1 Level of auxins in a typical plant – In relative units

Part	Level
Apical bud	10
Apical leaf	8
Normal leaf	6
Axillary shoot	9
Axillary bud	2
Stem	2
Main root	2
Root tip	4
Main tap root	3

Info: Noggle and Fritz (1983)

2.4.3.3 Hormonal influence affecting vegetative propagation

All classes of growth regulators- auxins, cyctokinins, gibberellins, ethylene polyamines and phenolics affect rooting / grafting either directly or indirectly (Davies and Moser, 1980). However, auxins have the greatest effect on graft success and root formation. It is also widely used in a lot of experiments for growth and development.

Previous studies over the years on the physiology of auxin action showed that auxin was involved in such varied plant activities as cell division leading to stem growth, adventitious root formation (Haissig and Davis, 1994; Thimann and Koepfli, 1935; Thimann and Went, 1934; Went, 1934) lateral bud inhibition by apical dominance, formation of abscission layer on leaves and fruit, activation of cambial growth and shoot formation and wound healing in graft formation.

Indoleacetic acid (IAA) is naturally occurring and have considerable auxin activity (Haissig and Davis, 1994). It is synthesized from the amino acid L- tryptophan in leaf primordia, young leaves and developing seeds. IAA moves from cell to cell in a polar gradient (i. e., from tip to base). Apart from the IAA, two other synthetic auxins, indolebutyric acid (IBA) and α-naphthaleneacetic acid (NAA) were developed which were more effective for rooting than the naturally occurring/synthetic IAA. These are being widely used for rooting stem cuttings and for rooting tissue-culture produced micro cuttings.

It has been confirmed in propagation that rooting/graft formation is fully dependent upon either applied or endogenous auxin (Gasper and Hofinger, 1989 ; Stromquist and Hansen, 1980 ; Haissig, 1972; Maini, 1968;). IBA, although less abundant than IAA, is also naturally occurring substance in plants (Ludwig-Muller and Epstein, 1994). In *Arabidopsis*, endogenously formed IAA is more readily transported than endogenously formed IBA. IAA also conjugates via amide bonds, while IBA conjugates form ester bonds. In apple (*Malus*),

when IBA is applied to stem cuttings or micro cuttings to stimulate rooting, it is in part, converted to IAA (Vander Krieken *et a*l., 1992). IBA may also enhance rooting via increased internal-free IBA, or synergistically modify the action of IAA or endogenous synthesis of IAA; IBA can enhance tissue sensitivity for IAA and increase rooting (Vander Krieken *et al*., 1993).

Cytokinins (plant growth regulator) play very important roles in cell division especially bud development (Davies and Moser, 1980). Coconut milk, which is liquid endosperm, as well as yeast extract were found to promote cell division in callus tissues when applied in an agar medium under sterile conditions.

Adenine, a component of DNA and RNA, produced the same response. An extract from autoclaved fish sperm DNA yielded a compound that stimulated cell division in the presence of auxins. Kinetin is representative of a group of hormones that have been named cytokinins (Landis, 1993). Other natural cytokinins include zeatin, isopentenyadenine (2IP), and synthetic substances such as benzyladenine (BA or BAP). Other substances with cytokinin activity include thiourea, diphenylurea, thidizuron (TDZ) and N-(2-chloro-4-pyridyl) n'-phenylurea (CPPU).

The interaction of auxin and cytokinin is one of the primary relationships in plant propagation. A high auxin/cytokinin ratio favours rooting, a high cytokinin/auxin ratio favours shoot formation and a high level of both favours callus development (Hartmann *et al*., 2002).

Gibberellins were discovered from rice infected with a fungus, *Gibberella fujikouri*. An ingredient was extracted from the fungus and its chemical structure was determined. The most important product from the forms of gibberelllins is Gibberellic acid. They occur in high concentration in developing seeds, stem apices, particularly in the leaf primordial, roots, fruits and tubers and function in the germination and control of dormancy. They are

transported within the plant in the xylem and phloem. In the plant they promote shoots elongation through the increase of both cell division and elongation (Hartmann *et al*., 2002).

Abscisic acid (growth inhibitor), among the other natural occurring materials, play an important role in plant activities (George, 1993). Abscisic acid (ABA) is synthesized from mevalonic acid either directly or from the breakdown of carotenoid pigments. Biosynthesis occurs in chloroplast and in all organs of higher plants and in xylem sap, but its function apparently depends on its concentration. ABA has a major function in controlling water relations through the stomates, as well as playing a role in food storage reserves.

Horticulturally, ABA is involved in the dormancy of buds and seeds, abscission and plant response to stress, particularly moisture. ABA controls stomatal closure, controls water and ion uptake by roots and affects leaf senescence and abscission (Hartmann *et al*., 2002).

Ethylene with a very simple structure can have a profound effects on plant grow at high concentration, senescence and abscission in leaves and fruits, flowering, lateral bud stimulation, latex production and flower induction. In propagation, ethylene can induce adventitious roots, stimulate germination and overcome dormancy.

Auxin sometimes stimulates ethylene production, but the effect is limited to vegetative organs. Wounding and stress, however, consistently result in an increase in ethylene. Naturally occurring ethylene is involved in the maturity of fruits and is widely used to induce ripening in commercial storage. A liquid chemical (2-chloroethylphosphoric acid called ETHEPHON) is absorbed by plant tissue where it breaks down to ethylene. Ethylene is synthesized from amino acid methionine via a pathway that includes 1-aminocyclopropane-1-carboxylic acid (ACC). Exogenous carbohydrate, light, cytokinins, auxins and carbon dioxide enhance the process (Hartmann *et al*., 2002).

Certain other naturally occurring substances are considered by some to show hormonal action. Polyamines, such as putrescine, spermidine and spermine, synthesized form the amino

acids arginine and ornithine, are widespread in plants. Others classed as plant hormones by some include jasmonate, brassionsteroids, phenolics and silicylate. Myo-inositol is usually classed as a vitamin and used in tissue culture (Hartmann *et al*., 2002).

2.4.3.4 Nutritional factors influence on vegetative propagation

Carbohydrate and minerals are the two main nutritional factors. The factors include carbohydrate levels as well as mineralization that influence vegetative propagation by rooted cuttings and grafting.

Kraus and Kraybill (1918) hypothesized the importance of the carbohydrate-to-nitrogen (C/N) ratio in plant growth and development.

Carbohydrate performs a lot of functions to enhance rooting in cuttings. The carbohydrate pools of sugars (soluble carbohydrates) and storage carbohydrates (starches or insoluble carbohydrates) are important to rooting as building blocks of complex macromolecules, structural elements, and energy sources (Greenwood and Berlyn, 1973; Haissig, 1986; Struve, 1981). Although stock plant carbohydrate content and rooting may sometimes be positively correlated (; Henry *et al*., 1992 ; Hambrick *et al*., 1991), carbohydrates do not have a regulatory role in rooting. A positive correlation between carbohydrate content and rooting may reveal that the supply of current photosynthate is insufficient for optimal rooting (Veierskov, 1988). High C/N ratios in tissues of cuttings promote rooting but do not accurately predict the degree of rooting response (Struve, 1981). Cuttings use stored carbohydrates in root regeneration, but only in small amounts. Differences in C/N ratios are mainly due to nitrogen rather than carbohydrate content. Nitrogen has been negatively correlated to rooting (Hambrick *et al*., 1991), which suggests that the correlation between high C/N ratios and rooting may be due to low nitrogen levels.

In grafting to a dwarfing rootstock, a lot partitioning to a greater portion of carbon to reproductive areas (spurs, spur leaves, fruit) and less to the tree branch and frame dry weight, compared with non-dwarfing rootstocks (Strong and Miller-Azarenko, 1991).

The greater water and nutrient uptake of the vigorous rootstock contributes to production of new vegetative growth, which is a competing sink with reproductive growth. The rootstock affects the partitioning of dry matter between above- and below- ground tree components.

Vigorous rootstocks accumulate more dry matter in the shoot and root system than dwarfing rootstock (Atkinson *et al*., 1999 ; Stutte *et al*., 1994).

Generally, carbohydrates enhances the translocation of auxins to the zone where rooting/graft union is taking place.

Minerals in plants play vital roles in promoting growth and development. Plants store minerals and other nutrients in different organs such as roots stems, leaves and / or fruits. These organs have a considerable influence on the uptake and translocation of mineral nutrients in plants and thus play an essential role in physiological processes such as growth, signal transduction and development (Flowers and Colmer, 2008 ; Wang *et al*., 2006).

A lot of mineral nutrients are found in stem cuttings of most plants. In a study of poinsettia where mineral element concentration was analyzed during the developmental stages of rooting, iron (Fe), molybdenum (Mo) and copper (Cu) increased in the basal portion of stem cuttings during early root initiation while phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) decreased (Svenson *et al.*, 1995). During root primordial elongation and root emergence, iron (Fe), copper (Cu), molybdenum (Mo), magnessium (Mg), manganese (Mn), boron (B) and zinc (Zn) concentration continued to increase at the bases, but P and K concentrations remained low compared to when cuttings were initially inserted into the propagation medium. The importance of nitrogen (N) in root initiation is supported by

nutritional studies on rooting of cuttings and importance of N in nucleic acid and protein synthesis (Blazich, 1988).

The importance of N on root initiation and development also relates to such factors as carbohydrate availability C/N ratio and hormonal interaction.

Zn can promote the formation of the auxin precursor, tryptophan and the formation of auxin (IAA) from tryptophan. Conversely, Mn acts as an activator of the IAA-oxidase enzyme system and B may enhance IAA-oxidase activity, thus regulating endogenous auxin levels (Jarvis, 1986; Weiser and Blaney, 1967). Higher endogenous auxin levels are required for early rooting initiation than for later root development. If root initiation is related to the relative activity of IAA and IAA-oxidase, then rooting may be correlated with changes in relative Zn, Mn and B concentration at the site of root initiation during the developmental stages of *de novo* rooting.

Almost all the mineral nutrients mentioned play similar roles in grafting to enhance wound healing for graft success.

The type of rootstock will however, determine the effectiveness of a mineral in inducing union formation in grafting. Apple rootstocks affect Ca, Mg, Mn and B uptake, but there is no apparent direct relationship of mineral status with rootstock vigour, productivity or spur characteristics (Hirst and Ferree, 1995). The influence of the rootstock on the mineral content in aerial plant parts was attributed to the physical characteristics of the root system, such as lateral and vertical development, which resulted in enhanced uptake of water and minerals (Jang, 1992 ; Heo, 1991).This being one of the main motives for the widespread use of grafted rootstocks in propagating fruit trees (Lee, 1994). However, in grafted fruit trees, no effect of the rootstock on leaf mineral content was found (Chaplin and Westwood, 1980) and in these cases, more influence of the scion on leaf nutrient content was observed. Tagliavani *et al*. (1993) suggested that the vigour of both scion and root system had an important role in

the uptake and translocation of nutrients in grafted fruit trees. Christensen (1968) also observed that the rootstock play a very important role in nutrient translocation.

He found new and small differences in scion nutrition caused by rootstock. The scion has also been shown to affect the stock mineral concentrations of bark (Haas and Halma, 1929) and roots (Wallace *et al.,* 1952 ; Colby, 1935).

Excessive levels of heavy metals in agricultural land constitute an increasing serious threat not only for intact plant growth and yield, but also for environment and human health (Hong Bo *et al*., 2010 ; Clemens, 2006 ; Gratao *et al*., 2005 ; An *et al*., 2004 ; Raskin *et al*., 1997).

Some heavy metals are toxic to plants at very low concentrations, while others may accumulate in the plant tissues up to a certain level without visible symptoms or yield reduction (Vekleij *et al*., 2009 ; Clemens, 2001 ; Moustakas *et al*., 2001). Non-nutrient heavy metals such as cadmium (Cd), arsenic (As), lead (Pb) and mercury (Hg), which are harmful to both plants and humans are introduced to agricultural ecosystem from various sources, including industry, reclaimed wastewater and soil amendment originating from various sources (Diacono and Montemurro, 2010; Gupta *et al*., 2010). Although the problem of heavy metal contamination in fruit vegetables is currently not widespread, some recent reports are worrying. In a survey conducted in Japan (Arao *et al*., 2008), approximately 7% of eggplant (*Solanum melongina* L) fruit contain Cd at concentrations exceeding the internationally fruity vegetable plant processes, including water transport, nitrogen metabolism, oxidative phosphorylation in mitochondria, photosynthesis and chlorophyll content (Freng *et al*., 2010 ; Lopez-Millan *et al*., 2009 ; Djebali *et al*., 2005 ; Burzynski and Klobus, 2004). The impact of Cd on plant growth and development depends not only on its concentration in the external medium, but also on the plant genotype, plant part and duration of exposure (DalCorso *et al.,* 2008 ; Zhang and Shu, 2006 ; Clemens, 2001 ; Moustakas *et al*., 2001).

Recent research has indicated that some rootstocks of fruit vegetable may restrict the uptake of heavy metals (Mori *et al.*, 2009 ; Arao *et a*l., 2008; Rouphael *et al*., 2008a). Hence, grafting fruit vegetable onto appropriate rootstocks, may limit the heavy metal accumulation in the aerial parts, thereby mitigating their adverse effects on crop performance and human health.

2.5 Biochemical factors

2.5.1 Biochemical basis for adventitious root formation

The basis for root formation implies that there are root-promoting and root-inhibiting substances produced in plants and their interaction is thought to be involved in rooting. This indicates that the difficult-to-root cuttings lack the appropriate root-promotion substances or have high levels of root-inhibiting substances. Scientists in the early 1950s reported that endogenous chemical inhibitors retarded rooting in selected plant species. This was observed for grapes cultivars. Difficult-to-root hardwood cuttings of wax flower (*Chamaellaucium uncinatum*) have a cinnamic acid derivative that inhibits rooting, while no detectable levels of this phenolic compound were found in easy-to-root softwood cuttings (Cuir *et al.*, 1993). Cuttings of difficult- to-root mature eucalyptus (Crow *et al.*, 1971; Paton *et al.*, 1970), chestnut (Vieitez *et al.*, 1987) and Dahlia cultivars (Biran, 1973 ; Biran and Halevy, 1973) also had higher rooting inhibitors than easy-to-root forms.

Hess (1968 ; 1962) observed that cultivars of chrysanthemum and *Hibiscus rosa-sinensis* contained greater nonauxin rooting stimuli than their difficult-to-root forms. He termed these nonauxin rooting stimuli rooting co-factors, which was a modification of the rhizocaline that biochemical factors, other than just auxin, were controlling rooting. These rooting co-factors were naturally occurring substances that appeared to act synergistically with IAA in promoting rooting. Rooting co-factors have been found in some cultivars through isolation

(Kling *et al*., 1988). Fadl and Hartmann (1967) and Fadl (1967) isolated an endogenous root-promoting factor from basal sections of hardwood cuttings of an easy rooted pear cultivar ('Old Home').

Extracts from basal segments of similar cuttings of a difficult-to-root cultivar ('Bartlett') treated with IBA, did not show this root-promoting factor. The action of these phenolic compounds in root promotion was theorized to be in protecting the root-inducing, naturally occurring auxin-IAA-from destruction by the enzyme, IAA-oxidase (Hackett, 1970). Jarvis (1986) attempted integrating the biochemical and developmental anatomy of adventitious root formation by examining the four developmental stages of rooting. His premise was that (a) the initial high concentrations of auxin needed in early rooting events are later inhibitory to organization of the primordium and its subsequent growth - hence the importance of regulating endogenous auxin concentration with the IAA oxidase/peroxidase enzyme complex playing a central role (i.e., IAA oxidase metabolizes or breaks down auxin); and (b) IAA oxidase activity is controlled by phenolics (o-diphenols are inhibitory to IAA oxidase), while borate complexes with o-diphenols result in greater activity- and hence a reduction of IAA to levels that are optimal for the later organization stages of rooting. Work done on the biochemical composition of the nut (Quainoo *et al*., 2012): fat and moisture content, may have relationship on vegetative growth of the plant and need to be investigated.

2.5.2 Biochemical mechanism of grafting incompatibility

One proposed biochemical mechanism concerns incompatibility combinations of certain pear cultivars on quince stock (Gur *et al*., 1968).

The incompatibility is caused by a cyanogenic glucoside, prunasin, normally found in quince, but not in pear tissues. Prunasin is translocated from the quince into the phloem of the pear.

The tissues break down the prunasin in the region of the graft union, with the hydrocyanic acid as one of the decomposition products.

The presence of the hydrocyanic acid leads to a lack of cambial activity at the graft union, with pronounced anatomical disturbances in the phloem and xylem at the resulting union. The phloem tissues are gradually destroyed at and above the graft union. Conduction of water and materials is seriously reduced in both xylem and phloem. The presence of cyanogenic glucosides is restricted to a relatively few genera. This reaction is not general for all species.

2.5.3 Lignification of cell and tissues in grafting partners

Phenolic compounds are also involved in graft incompatibility (Errea, 1998) and are widespread in plants and present in the biochemical responses of tissues to wounding. They play a role in lignification (Buchloh, 1960) which occurs in graft formation.

2.5.4 Cell recognition of the grafting partners

The graft union of plant parts requires some sort of cellular recognition or cellular communication. Evidence available suggests that in the graftage of *Cucumis* and *Cucurbita,* changes in protein banding may be due to polypeptides migrating symplastically across the graft union via the connecting phloem (Tiedemann and Carsens-Behrens, 1994). Translocation of signaling molecules, such as polypeptides in the phloem could be significant in cell recognition and compatibility between the graft partners (in graft incompatibility phloem degeneration frequently takes place at the graft union). Pectin fragments formed during the adhesion process of grafting may act as signaling molecules- and influence cell recognition. In Sikta spruce, the bead-like projections from callus formed during graftage are in part composed of pectins, proteins, carbohydrates and fatty acids. These bead-like

projections besides binding or cementing cells, may serve a more active role in cell recognition and the successful merging of tissues of the grafting partners (Miller and Barnett, 1993).

2.6 Anatomical factors

2.6.1 Graft union development - Isolation layer formation and initial adhesion between scion and stock.

Wounding during the grafting process can lead to the formation of isolation layer, also known as a necrotic layer. Observation under an electron microscope has indicated that the isolation layer is composed of the remaining cell walls of cutting-induced dead cells surrounding substances with high electron density (Tiedemann, 1989; Moore and Walker, 1981). The initial adhesion between scion and rootstock has been observed to occur one day after grafting, and is related to a pronounced dictyosome activity in cells near the graft interface (Moore and Walker, 1981).

During the early stages of grafting, the isolation layer is distributed over the whole graft interface. With the formation of the callus, the isolation layer is disrupted in the vascular area, and is then gradually extended. Thus, scions and rootstock are able to directly connect. The isolation layer disappears mainly because of growth pressures caused by the callus dividing at both sides of the graft union and by callus absorption (Wang, 2011).

2.6.2 Formation of the wound callus

Once the two components of the graft (stock and scion) are in intimate contact, the cambial region is capable of meristematic activity to produce parenchymatic cells which soon intermingle and interlock, producing the callus tissue that fills the space between the two

components connecting the scion and rootstock (Hartmann *et al.*, 2002; Wang and Kollmann, 1996 ; Errea *et al.*, 1994).

Two to three days after grafting, the wound callus is formed by division of the parenchyma cells from cambium, phloem, xylem and pith near the wounded surface. In herbaceous plants, callus cells are first formed at the vascular bundles and cortex with only few in the pith (Tiedemann, 1989 ; McCully, 1983; Stoddard and McCully, 1983). Mixing of the wound cells of both graft partners can lead to further connection between scion and rootstock.

Yeoman was the first to document structural events correlated with changes in wall cells occurring during graft formation in Solanaceae describing a cell recognition mechanism in which opposing cells of graft partners touch (Yeoman, 1984 ; Yeoman *et al.*, 1978).

The basis of this recognition system is that protein molecules released from the plasmalemmas combine to form a complex with catalytic activity that subsequently initiates a developmental sequence resulting in the formation of a successful graft. Further works studying the formation of callus tissue implied some of these compounds in the mechanism of adhesion of the graft partners. Wart-like projections on the cell wall surface have been reported in callus cells at the union (; Barnett and Weatherhead, 1988 ; Jefree and Yeoman, 1983). Adhesion between cells of the scion and rootstock is aided by a "cement" or binding material, which projects beadlike projections from the callus that consist of homogenous matrix made up of a mixture of pectin, carbohydrate, protein and fatty acids and a fibril/vesicular component comprised mainly of carbohydrate and pectins (Miller and Barnett, 1993).

2.6.3 Differentiation into new cambial cells

From the newly formed callus, new cambial cells are differentiated forming a continuous cambial connection between rootstock and scion (Hartmann *et al.*, 2002; Barnett and

Weatherhead, 1988). During this step, the first signs of incompatibility could appear due to the failure of either procambial differentiation of the callus cells or a precise spatial orientation of the procambium, which could imply a form of cellular communication in the establishment of an appropriate grafting union (Moore and Walker, 1981; Moore, 1984).

In incompatible combinations, the abnormal function of the newly formed cambium results in an involution at the cambial area of incompatibility **(**Mosse, 1962 ; Herrero, 1951).

Diverse phenolic compounds have been implicated in these processes of division, development and differentiation into new tissues (Mossella and Macheix, 1979).

In callus cultures *Prunus annum*, it has been shown that levels of pruning limits the proliferation and differentiation of the cells (Feucht *et al*., 1988). Similar results were also observed also in *P. annum* with other compounds like chlorogenic acid that might inhibit the development of the callus and the stimulation of cell division (Jordan *et al*., 1980). In *Prunus armeniaca* an accumulation of some phenolic compounds related to problems in differentiation of the callus have been detected in early steps of graft establishment (Errea *et al*., 1994). Again in *P. annum*, experiments involving callus growth from root segments show other compounds, such as flavonols, catequins and proanthocyanidins that affect cell division intensity (Feucht and Nachit, 1977).

Similarly it has been observed that, the accumulation of the flavonoid isovitexin affects the normal process of cell differentiation producing growth abnormalities (van Brederode and Steyns, 1985).

An observed accumulation of phenols in the cambium tissues of some graft combinations can induce disorganization at the sub cellular level and may be implicated in cellular damage and alter the complete phloem cambium system around the graft union, as has been observed in apricot grafts (Errea *et al*., 1994). The detection of these accumulations in the early stages of cell division and differentiation into new cambial cells are of particular interest since they

could prevent the formation of a continuous cambial connection between stock and scion at this stage of graft establishment.

2.6.4 Differentiation into new xylem and phloem

In the final stage of the graft establishment process, new vascular tissue is produced from the new cambium. Most authors consider a graft union to be successful and complete when several functional phloem and xylem connections cross the graft interface (Schöning and Kollmann, 1997; Wang and Kollmann, 1996; Gebhardt and Goldbach, 1988; Moore, 1984; Yeoman, 1984). However, it has been observed that incompatible grafts can grow for several years without any external indications of incompatibility, denoting the presence of functional vascular connections in incompatible grafts (Hartmann *et al*., 2002 ; Mosse, 1962). In these cases, the differences between compatible and incompatible grafts lays in the presence of a portion of the tissue in incompatible grafts that cannot differentiate into cambium and vascular tissue. This results in the existence of wide areas at the union similar to undifferentiated callus cells (Errea *et al*., 1994a). The lack of cambial activity in some areas of the graft union could affect the activity on the new xylem and phloem formed, causing discontinuities in the cambium and the formation of a parenchymatous line interrupting the vascular connection (Hartmann *et al*., 2002).

Phenolic compounds are involved in these steps due to their important role as cross linking agents between polysaccharides (Fry, 1988 ; Markwalder and Neukon, 1976) and their implications in the lignin synthesis pathway (Tomaszewski, 1962 ; Buchloh, 1960). The lignifications of the cell wall could be the main event leading to the formation of a solid union and resulting in a compatible graft (Feucht and Schmid, 1979 ; Buchloh, 1962).

In compatible grafts, a network wound vessel members develop between vascular bundles of stock and scion, and lignifications of the wound regenerative tissue is produced until a complete xylem bridge is established. In incompatible grafts, lignifications occurs within isolated areas of the pith, but no vascular connection is observed (Gebhardt and Goldbach, 1988).

An important substance implicated in the development of compatible unions is auxin, which is released from vascular strands of the stock and scion and induces the differentiation of vascular tissues (Aloni, 1987 ; Moore, 1984; Quessada and Macheix, 1984). Its translocation from the root system has been studied in apples and has been related to graft incompatibility, since a supra and basipetal movement of auxin can organize the morphogenic pattern of the entire plant body (Zajaczowski *et al*., 1983) even accelerating the formation of a successful graft (Shimomura and Fugihara, 1977). Polyphenols with an orthohydroxy group can act as auxin protectors (Feucht and Schmid, 1979) and can modulate the IAA- oxidase level enzyme that degrades auxin and regulated by phenols (Feucht and Kahn, 1973).

On the other hand, Gebhardt and Feucht (1982) reported a lack of compatibility with a pronounced accumulation of polyphenols above the graft union, which are known to affect auxin transport (Stenlid, 1976). Low auxin content in incompatible combinations may then affect the differentiation of xylem and phloem and also lignifications (Macheix, *et al*., 1986 ; Roberts, 1976). The destruction of auxin might explain the failure of vascular restitution in incompatible systems (Wang and Kollmann, 1996). Asante *et al*. (2002) postulated the stepwise graft union formation in cashew which similarly followed a basic pattern described in other species.

2.6.5 Adventitious root formation in cuttings and air-layers

There are two types of adventitious roots formation. These are preformed roots and wound-induced roots.

2.6.5.1 Preformed roots

Preformed root initials and primordial develop naturally on stems while they are still attached to the parent plant and roots may or may not emerge prior to severing the stem piece. The roots generally lie dormant until the stem are made into cuttings and placed under environmental conditions favourable for further development and emergence of the primordial as adventitious roots (Smith and Wareing, 1972).

2.6.5.2 Wound- induced roots

These type of roots develop only after the cutting is made, in response to wounding in preparing the cutting. In effect, they are considered to be formed *de novo* (Davies, 1985). Any time living cells at the cut surface are injured and exposed, a response to wounding begins (Cline and Neely, 1983). The outer injured cells die, a necrotic plate forms, the wound is sealed with a corky material (suberin), and the xylem may plug with gum. The plate protects the cut surfaces from desiccation and pathogens.

Living cells behind this plate begin to divide after a few days and a layer of parenchyma cells form callus which develops into a wound periderm. Certain cells in the vicinity of the vascular cambium and phloem begin to divide and initiate *de novo* adventitious roots.

Adventitious roots in stem cuttings of woody perennial plants usually originate from living parenchyma cells, in the young, secondary phloem, but sometimes in vascular rays, cambium, phloem, callus, or lenticels (Harbage *et al.*, 1994; Lovell and White, 1986 ; Ginzburg, 1967). Two patterns of adventitious root formation emerge: direct root formation of cells in close

proximity to the vascular system (i.e., generally more easy-to-root species); and indirect root formation, where non- directed cell divisions, including callus formation, occur for an interim period before cells divide in an organized pattern to initiate adventitious primordial (i.e., generally more difficult-to-root species) (Geneve, 1991; Lovell and White, 1986).

2.7 Tree husbandry and management

2.7.1 Propagation of shea tree

2.7.1.1 Propagating of shea tree by seed

Shea *(paradoxa)* is readily propagated by seed (Delolme, 1947 ; Holland, 1922). The seed size varies considerably, being 150-300 seeds/kg (Maydell, 1986). The seeds are available for planting only if picking is done early in the morning to avoid collection by women for butter processing. Like many seeds with high oil content, shea nuts have a short period of viability. With fresh seed, the germination rate can be over 90 % (Booth and Wickens, 1988), though considerable variation has been observed; some seed sources have been found to give only approximately 40 % success (Ruyssen, 1957). As germination potential falls very rapidly, seed should be sown as soon as possible after harvesting, preferably within the first week, but at most within a month (Ruyssen, 1957). Short viability means that seed cannot be stored for any length of time, which is a problem when it comes to the collection and dissemination of 'improved' seeds. Osei-Amaning (1996) found that seed germination in shea (*paradoxa*) was facilitated by removal of the pericarp prior to sowing, confirming the findings of Ruyssen (1957), who obtained very poor germination levels where intact fruit were sown on the soil surface.

Raising of seedlings by direct sowing can be done in the field. This avoids the difficulties associated with transplanting the seedlings with their large taproots, and reduces seedling

production costs (Irvine, 1961; Bonkoungou, 1987). However, the seed is very vulnerable to rodent attack and large losses may be incurred (Maydell, 1986 ; Delolme, 1947).

Germination of shea (*paradoxa*) seeds sown directly in the field tends be low, sometimes only 40-50 % (Bonkoungou, 1987).

Unlike sowing seeds for forestry species which last 3 - 6 months at the nursery, shea takes a longer period of care. Seed should be sown into beds at 20 cm x 15 cm spacing, or into large polythene pots, in soil, at a depth of 1.5 cm (; Maydell, 1986 ; Ruyssen, 1957). The seed should be sown with the scar facing downwards (Vivien, 1990). In the subsp. *paradoxa*, there may be a long delay of between seven weeks to five months before shoots emerge from the soil. (Delolme, 1947). Mostly, shoots appear within two months of sowing (Delolme, 1947). Frimpong *et al*. (1987) tested the influence of different media (top soil, sand and a 1:1 sand: soil mixture) and soil temperature (32^{o}C and 36^{o}C) on germination. Differences were, not significant, although the highest germination rate occurred in the top soil at the higher temperature.

Seedling establishment using bare roots gave poor results (Delolme, 1947) but has been recommended for sandy soils by Picasso (1984).

In this case, transplanting should be rapidly done during the rainy season with the roots being protected from desiccation during transit, with pruned stem and all leaves removed. Delolme (1947), in Cote D'Ivoire obtained poor survival rates with balled planting stock of subsp. *paradoxa* when seedlings were planted out during the rainy season. The establishment was very successful (86-96%) when dormant plants were transplanted during the dry season and given sufficient water to allow for root development before leaf expansion. Pits, 25cm in diameter and 50 cm deep were dug and filled with 15 litres of water; the top soil was then added and mixed in with the water to form a thick paste. Plants with a root ball 35-40 cm deep and 15 cm in diameter, were then placed in each pit and the root ball covered with 5-10

cm fine soil in order to reduce evaporation. This approach does not need watering again. Mulching seedlings reduces soil moisture loss and also moderates soil temperature, thus minimizing stress after transplanting (Osei-Amaning, 1996). For the establishment of plantations, Vuillet (1911) recommended that wild seedlings, 0.5-0.75 cm in height, could be transplanted with a large root ball, in order to minimize damage to the tap root and hence stress to the plants which would have lowered survival rates. However, Delolme (1947) successfully transplanted wild seedlings of sheanut (*paradoxa*) 0.9-1.5 cm in height, by using the same method as outlined above for transplanting nursery-grown seedlings.

2.7.1.2 Propagating of shea tree by cuttings

The shea tree can be propagated by cuttings to produce adventitious roots (Yeboah *et al*., 2009). The following factors can positively enhance the rooting performance of the cuttings; (a) the suitable woodtype for setting is the semi-hardwood (b) cuttings should be dipped in indolebutyric acid hormone at a concentration of 10,000 ppm to enhance good rooting (c) the propagating bin/pit can be used for cuttings to achieve high rooting (d) irrigating cuttings in a structure is done once during the heavy rains and twice when the rainfall reduces (e) fungal infection on the cuttings should be controlled during the rainy period by using Dithane M45 fungicide at 3,000 ppm and spraying once every two weeks (f) the number of leaves to be retained on the apex of the cuttings to enhance rooting is six (g) rice husk medium is suitable for setting cuttings to achieve high success. After setting the cuttings under the necessary micro climatic conditions, signs of rooting is observed after 29 days. Rooting also depends on the mother plant (genotype) where they were harvested from. Selection of cuttings therefore should be done from desirable shea trees for good rooting. The rooted cuttings should be allowed on the rooting bed seven days before potting them in polythene bags (26 cm x 40 cm) for hardening. Unlike bareroot seedlings (Hall *et al*., 1997), exposure of adventitious

roots of a rooted cuttings beyond one minute can cause injury and death to the roots (Amissah *et al.,* 2013; Akakpo *et al*., 2013 ; Yeboah *et al*., 2010 a & b ; Yeboah *et al*., 2009 a & b ; Opoku-Ameyaw *et al.*, 2000).

Biochemical studies have shown the importance of some plant substances to the rooting success eg soluble sugars and total free phenols which provide energy for the cuttings in the rooting bed and protect the endogenous hormone from being oxidized /destroyed. Nutrients like zinc, manganese, calcium and magnesium positively enhance rooting by regulating the levels of the enzyme IAA-oxidase and prevent them from destroying the auxin, IAA (Amissah *et al.,* 2013; Akakpo *et al*., 2013 ; Yeboah *et al*., 2009b)

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Anatomically, the presence of suberized discontinuous schlerenchma cells and root primordial is an indication of promising rooting according to Yeboah *et al*. (2009b).

Low temperature range of 24 – 29^{o}C can promote rooting by enhancing the translocation of auxins and soluble plant substances to the rooting zone. This occurs in the rainy season when humidity is high.

Transplanting of the rooted cuttings can be done (in the rainy season) by potting them in polythene bags (26 cm x 40cm) and hardening them in the mist or pit propagator at the nursery for nine months (during the dry season). The hole depth for transplanting is similar that of cashew seedlings (52 cm x 52 cm x 52 cm) (Cashew Develpoment Project, 2008).

2.7.1.3 Propagating of shea tree by grafting

Shea tree can be propagated on wild young shoots using five grafting methods, side cleft, whip and tongue, top cleft, chip budding anf side veneer (Haby *et al*., 2004) with the greatest in the study being side cleft grafting (success – 86.1 %). Under Savanna conditions, the

suitable period for grafting to achieve high success is in May which is the end of the dry season/onset of the rainy season. The success of seedling grafting ranges between 50-70 % (Cocoa Research Institute *pers com*, 2010). For high grafting success, scions should be harvested from recently flushed, green shoots from the crown of mature large plus- shea trees. This should be done during the overcast days or early in the morning to minimize physiological stress. In this way, the juvenile phase of the trees is greatly shortened (Hackett, 1985). Shoot length should be between 5-10 cm long and should be free from diseases and unlignified and the young wild plant which acts as a rootstock should have a girth of 1.5 – 3.0 cm and height of 50 – 150 cm high (Haby *et al.*, 2004)

2.7.2 Sheanut as a multi-purpose tree species

The shea tree is important for the livelihoods of the rural population as it has been for over centuries (Lovett & Haq, 2000). Almost every part of the tree has its use, for example: the fruit is eaten by humans and other domestic animals e.g. sheep, cattle and pigs when ripe (Fleury, 1981) and the leaves are used as fodder and serve as an ingredient for making alkaline and paint (Lovett & Haq 2000). The wood is durable, and can be used for household items, such as furniture, tool handle, pestles and mortars or as building poles and fuelwood eg charcoal (Abbiw, 1990 ; Dalziel, 1937). The wood has been described as dull red to deep rich red with purple tinge, very hard and heavy, resistant to termites, difficult to work but taking a good polish, (720 to 1280 kg per m^3 air dry) (Booth and Wickens, 1988 ; Bolaji, 1980; Eggeling, 1940). Millogo- Rasolodimby (1989) observed the importance of the nectar from the flowers for honey by bees. Despite a significant reduction in crop yields below the canopies of the tree, the long term existence of sheanut on agricultural land has also been shown to be beneficial to land management by reducing wind speeds, preventing soil erosion,

providing shade, increasing soil fertility and improving the microclimate near trees (Jonsson *et al.,* 1999 ; Boffa, 1995; Kater *et al.,* 1992; Kessler, 1992).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The field experiment were conducted at the Cocoa Research Institute of Ghana (CRIG) Substation, Bole (9^o 03'N, 2^o 23'N, 600 m.a.s.l.) in the Northern region of Ghana which falls within the Guinea Savanna belt of West Africa. The area has a uni-modal rainfall pattern with one maximum rainfall (600-1400) mm which occurs from May to September (Hall *et al.,* 1996) and mean annual temperature ranging between 24 to 29 ^oC and mean relative humidity between 88 to 20 %.The months of January through to March record very high day temperatures with low humidity particularly, in January. The area experiences single rainfall maxima with the highest occurring in September followed by June. The months of December and January do not normally record rains (Table 3.1).

Table 3. 1 Climatic data from October, 2012 to September, 2013.

Month	Mean Temperature °C		Mean Humidity %		Total
	Max	Min	9.00am	3.00pm	Rainfall
October 2012	32.0	22.2	82.2	59.2	78.7
November	33.8	21.6	73.4	50.7	15.9
December	34.7	19.2	44.8	27.2	0.0
January 2013	35.1	20.1	30.4	20.1	0.0
February	36.6	20.5	50.1	31.0	31.8
March	35.3	23.3	73.1	45.1	111.0
April	34.3	22.9	76.1	59.0	105.9
May	33.3	22.8	76.5	60.6	117.3
June	29.8	23.6	88.0	78.0	157.2
July	29.8	23.2	78.0	71.0	121.7
August	28.6	22.1	87.5	73.4	113.6
September	30.3	24.5	85.6	68.0	215.4
September	30.3	24.5	85.6	68.0	215.4

3.2 STUDY EXPERIMENTS

Six field experiments and two laboratory experiments were conducted during the study.

3.2.1 FIELD EXPERIMENTS

A series of factorial experiments were carried out from January to December, 2013 in a randomized complete block design to investigate rooting success, weaning and establishment, graft union formation, biochemical and anatomical studies.

3.2.1.1 Experiment One

To determine the rooting response of air-layered shea (V paradoxa) trees to media and hormonal application in two contrasting environments.

The objective was to develop an air-layering technique using soilless medium in combination with IBA hormone for rooting of shea tree seedlings. The experiment was set out as a 2 x 3 factorial arrangement in a randomized complete block design with three replications. The first factor was media type at two levels; *sphagnum* moss (3g) and palm fibre (3g). The second factor was indole-butyric acid (IBA) at three concentration levels; 0 ppm (water as control), 5,000 ppm and 10,000 ppm. There were a total of six treatment combinations, replicated three times. Shea trees in fruition (ageing between 35 to 50 years) were selected for the air-layering experiment. The bark of the semi-hardwood shoots were removed in a girdle width of 2 - 3 cm and at a distance of 15 – 18 cm from the growing tip (Hartmann *et al.*, 2002). Twenty stems were girdled per treatment. Each girdled portion was sprayed with the required IBA concentration, covered with the media type and tightly secured with a wrapping of white polythene sheet to ensure that the set up was air-tight. Occasionally, each set up was injected with 3 ml of water using a syringe and needle to ensure the availability of moisture during the rooting process (Hartmann *et al.*, 2002). The experiment was carried out in both wet and dry seasons. Samples of the treated layered shoots were harvested for biochemical analysis to determine simple sugars and total free phenols.

Data collected included (i) climatic parameters (ii) number of rooted layered shoots (iii) number and length of developed roots (iv) moisture content of media using moisture meter and (v) mineral (zinc, calcium, nitrogen, potassium and phosphorus) content of the media and (vi) fungal infection on roots.

An analysis of variance (ANOVA) was performed on the data using Gentstat version 9. Least significant differences (LSD) were calculated and the probability of treatment means being significantly different was set at P = 0.05.

3.2.1.2 Experiment two

The effect of propagating structures and propagules on the weaning survival responses of plantlets.

The objective of the experiment was to determine the effect of propagating structures and plantlet types on the weaning of rooted propagules. The design was 3 x 2 factorial arrangements in a randomized complete block design with three replications. The first factor was propagating structure at three levels viz; mist propagator (6.0 m x 7.0 m x 5.0 m), propagating pit (3.0 m x 0.9 m x 0.2 m) and lath house. The second factor was propagules at two levels viz; normal and rooted plantlets. The mist propagator was a structure of wood covered with transparent polythene sheet. Spraying nozzles were fixed on top of the structure to spray water in the form of mist. The mist propagator was kept under a shade net to provide 50 % shade. The propagating pit was a rectangular pit with dimensions 3.0 m x 0.9 m x 0.2 m. A flexible metal dome made of a flexible wire was placed on top of the rectangular pit before covering with a transparent polythene sheet. The structure was also kept under a shade net to provide 50 % shade.

The lath house was used as the control structure in the experiment. It was made of a metal shed covered with shade net to provide 50 % shade.

Normal plantlets – seeds harvested from selected trees were nursed in polythene bags 30 cm long x 17 cm wide filled with top soil. At the same time, cuttings of pencil size thickness and 13 – 15 cm long were harvested from the same trees and set in pit propagators using indolebutyric acid (IBA) as root inducing hormone and rice husk as rooting medium.

Rooted plantlets were harvested potted into polythene bags (with same dimension) filled with top soil 60 days after setting. Both potted plantlet types were kept in the mentioned structures from July to last week of September for the weaning studies.

The propagules were arranged in the structures described for the weaning. The lath house was covered with a shade net while, the mist and pit propagators were fully covered with transparent polythene sheet under a bigger shed covered with shade net.

Watering was done on the plantlets each structure at 06.00 hrs and 14.00 hrs GMT. For the mist propagator, water was sprayed in the form of mist whereas for the two other structures (propagating pit and lath house) a watering can was used to provide water for the plantlets.

Data were collected on (i) ambient weather parameters – The environmental (ambient) weather data was collected for 7 months (May to December) whilst weather data in the structures with the propagules were collected for 3 months (July to September). Both data were collected using data loggers which were kept in a small wooden frame and placed in the structures with the propagules.

(i) percentage of survived propagules (ii) leaf production of propagules (iii) stem girth of propagules. (iv) plant height of propagules – All these parameters (i, ii, iii and iv) were collected at the end of the weaning studies in September.

An analysis of variance (ANOVA) was performed on the data using Gentstat version 9. Least significant differences (LSD) were calculated and the probability of treatment means being significantly different was set at P = 0.05.

3.2.1.3 Experiment three

Field establishment of weaned propagules as affected by transplanting hole depth and plantlet types.

The objective of the experiment was to determine the effect of transplanting hole depth and plantlet types on the field establishment of weaned propagules. The design was 3 x 2 factorial arrangement in a randomized complete block design with three replications. The length and width of each transplanting hole was maintained at 52 cm x 52 cm. The first factor, was transplanting hole depth at three levels viz; 26cm depth; 39 cm depth; 52cm depth (based on work done on cashew by the Cashew Development Project, 2008). The second factor was plantlet types at two levels viz; normal plantlets; rooted cuttings. The plantlets types (rooted cuttings and normal seedlings) were prepared as described in Experiment two.

After weaning, the plantlets were hardened for five weeks. The hardening was done by bringing them out of the propagators and keeping them under an open shed covered with net for two weeks. they were then moved from the shed and placed under a shea tree without net for another two weeks and finally placed in the open for one week before sending them to the field for transplanting in October the same year.

Data were collected on (i) the weather (ii) number of survived plants (iii) plant height and (iv) stem girth six months after field establishment (v) soil moisture taken using Moisture Meter; an instrument made of a hollow box (to contain the soil sample) with an attached electronic scale to record moisture levels (for 4 weeks), (vi) soil temperature taken after transplanting

using Earth Thermometers which were buried into the ground at different depths to record temperatures in the mornings and afternoons(for 4 weeks).

Statistical analysis

Data were analyzed by analysis of variance (ANOVA), using Gentstat version 9. Least significant differences (LSD) were calculated and the probability of treatment means being significantly different was set at $P = 0.05$.

3.2.1.4 Experiment four

The effect of wounding of (girdling and pre-curing) scions and rootstock type on grafting success.

The objective was to determine the effect of different wounding treatments to scions and type of rootstock on grafting success.

The experiment was laid out as a 2 × 2 factorial arranged in a randomized complete block design with three replications. The first factor was rootstock type at two levels; i.e. (a) young plant and (b) rejuvenated shoots. The young plants were naturally growing wild seedlings of 2 cm – 5 cm girth and 50 cm -150 cm height (Plate 3.1) (Haby *et al*., 2004). The rejuvenated shoots comprised shoots developed from coppiced mature trees of 16 cm – 25 cm girth and 50 cm height (Plate 3.2) (Haby *et al*., 2004). The second factor was wounding treatment at three levels; i.e. (a) control (no wounding), (b) pre-curing and (c) girdling. The pre-curing was done by cutting the leaves at the apical meristem and leaving the petioles only (Plate 3.3). Girdling involved the removal of the bark of width, 2 - 3 cm and 13 – 15 cm long (the growing tip) of the semi-hardwood shoots (Plate 3.4) (Hartmann *et al*., 2002). Both the girdled and pre-cured shoots were retained on the tree for 4 days (to accumulate enough food) before harvesting for grafting.

Type of rootstocks and wounded scions

Plate 3.1: Rootstock (Young plant)

Plate 3.2: Rootstock (Rejuvenated)

Plate 3.3: Pre-cured scion on intact

Plate 3.4: Girdled scion on intact

There were 20 girdled and pre-cured stems per treatment. There were a total of six treatment combinations, replicated three times. The top cleft grafting method as described by Hartmann *et al*. (2002) was applied. By this grafting technique, the rootstocks (slitting vertically down

into two equal halves to about 3 – 5 cm long) were first prepared before the preparation of the scions (cutting the lower end of the shoot to form a sharp wedge of length, 3 - 5 cm long). The scions and rootstocks were matched in size so that the cambial tissues were juxta-posed; after fixing the scion. The scion and rootstock were tied together with a grafting polythene tape, 25 cm in length and 1 cm in width. The union was covered with a plastic polythene cap, of length between 20–22 cm to prevent desiccation. The union after grafting were monitored once every three days to ensure that shoots growing below the union were removed to enhance fast wound healing and apical bud sprout.

Wounded scions were harvested (from the tagged trees scions were harvested for grafting) for biochemical analysis to determine the levels of simple sugars, IAA, protein and total free phenols. Additional data collected included

(i) climatic parameters (ii) percent graft success (iii) percent infection rate (iv) vegetative growth measurement (girth, height and leaf development).

An analysis of variance (ANOVA) was performed on the data using Gentstat version 9. Least significant differences (LSD) were calculated and the probability of treatment means being significantly different was set at P = 0.05.

3.2.1.5 Experiment five

To determine the grafting success as affected by rootstock types, bud development stage of scions and grafting methods.

The objective of the experiment was to determine the combined effect of rootstock type, bud development stage of scions and grafting method on the success of grafting. The experiment was carried out out as 2 x 2 x 3 factorial arrangement in a randomized complete block design with three replications. The first factor was rootstock type at two levels; young plant and

rejuvenated shoots. The second factor was grafting methods at two levels, side grafting and top cleft grafting and the third factor was scions with apical bud at different stages of bud development; dormant apical bud (Plate 3.5), partially sprouted apical bud (Plate 3.6) and fully sprouted apical bud (Plate 3.7). There were 12 treatment combinations replicated three times. The scions were harvested the same day and grafted on the rootstocks using the different grafting methods as described in experiment four by Hartmann *et al*. (2002).

The side grafting was slightly different from the top cleft in the rootstock preparation. Chiping was made at the side of the rootstock a little into the xylem cells. The scion preparation for the side grafting was the same as the top cleft method. The tying and covering of the side grafting were the same as the top cleft grafting. After grafting, the grafts were monitored once every three days to ensure that shoots growing below the union were removed to enhance fast wound healing.

Data collected were (i) climatic parameters (ii) graft success (ii) determination of levels of endogenous plant substances (simple sugars, nitrogen, IAA, protein and total free phenols).

An analysis of variance (ANOVA) was performed on the data using Gentstat version 9. Counts data were square root transformed. Least significant differences (LSD) were calculated and the probability of treatment means being significantly different was set at $P = 0.05$.

Bud development stage of scions

Plate 3.5: Scion with dormant apical bud

Plate 3.6: Scion with partially sprouted apical bud

Plate 3.7: Scion with fully sprouted apical bud

3.2.1.6 Experiment six

Determination of the best period for coppicing shea tree to produce shoots for high grafting success.

The objective of this experiment was to determine the most suitable period for coppicing to produce shoots to achieve high graft success. Top cleft grafting technique (Hartmann *et al*., 2002) was used in this experiment at two-monthly intervals.

The one- treatment experiment was arranged in a randomized complete block design with ten plants per treatment and replicated three times. The single treatment (period) was done once every two months in the year with coppicing of the selected trees prior to grafting as stated:

Treatment Period	**Month of coppicing**	**Month of grafting**
January – February	1st week in January	Last week in February
March – April	1st week in March	Last week in April
May – June	1st week in May	Last week in June
July – August	1st week in July	Last week in August
September – October	1st week in September	Last week in October
November – December	1st week in November	Last week in December

The rejuvenated shoots developed on the stumps (for grafting) 45 - 60 days after coppicing. The top cleft grafting method and the rejuvenated shoots recommended in experiment five were used for experiment six.

Data collected after 3 months of grafting were; (i) number of shoots developed (ready for grafting) after 2 months (ii) number of successful grafts after 3 months (iii) concentration of endogenous plant substance (IAA, protein, nitrogen, phenols and sugars) in scions and rootstocks before grafting and leaves of successful graft after six months of grafting.

An analysis of variance (ANOVA) was performed on the data using Gentstat version 9. Least significant differences (LSD) were calculated and the probability of treatment means being significantly different was set at P = 0.05.

3.2.2 LABORATORY STUDIES

The laboratory analysis were conducted at the Physiology and Soil Science Laboratory, CRIG, Tafo and Soil Science Laboratory (Department of Crop Science) KNUST, Kumasi to determine the concentration of the various plant substances (proteins, soluble sugars, nitrogen, IAA and total free phenols) minerals (zinc, manganese, magnesium and calcium) that are related to graft union formation and and rooting of air-layers. A total of four biochemical analysis were carried out.

3.2.2.1 Extraction and estimation of the IAA content.

The auxin content was measured according to the method of Donate-Correa *et al.* (2004). One gram from the stem cuttings/scions was weighed in duplicate. Sample A was extracted with 5.0 ml 35 % perchloric acid (CAS 7601-90-3) and sample B with 5.0 ml of modified Salkowski Reagent and kept in the dark for 1 hour as described (Donate-Correa *et al.*, 2004). The solutions were centrifuged at 10,000 rpm for 15 mins. The supernatant was then collected and optical density of the solution was read at 530 nm. The relative auxin content was determined by subtracting the extinction of adjusted A from extinction of adjusted B, using the IAA standard curve and is expressed as $\mu g g^{-1}$ fresh wt. Each value presented is the mean for replicates.

3.2.2.2 Extraction and analysis of soluble sugars and soluble proteins.

Cutting/scion sample were lyophilized, ground in a mill (0.42 mm screen) and analyzed for reserved compounds. The samples (100 mg) were weighed, homogenized and extracted 3 times with 80 % (v/v) ethanol at 80^{o}C for 5 mins. After centrifugation for 5 mins at 1300 x g, the ethanolic supernatants (soluble carbohydrate fraction) were combined and the volume was measured (Dubois *et al*., 1956). From the residue, soluble proteins were extracted three times with phosphate buffer (0.2M, pH 4.9), centrifuged as stated above, and the supernatants were pooled and measured three times with Coomasie Blue reagent by a spectrophotometer at 595 nm. Bovine albumin was used as the standard (Bradford, 1976). 5 ml cold water and 6.5 ml perchloric acid 52 % (v/v) were added to the residue material used for sugar analysis and mixed for 15 mins. Following the method given elsewhere (McCready *et al*., 1950) absorption of the sample was recorded at 630 nm. Starch concentrations of the samples were calculated from the calibration graph drawn from glucose standard solutions by multiplying 0.92 (McCready *et al*., 1950).

3.2.2.3 Bioassay for total free phenolics.

Total phenolics constituents in fresh stem cuttings/scions were determined using the Folin-Ciocalteu reagent and gallic acid as standard (Slinkard and Singleton, 1977). Briefly, 50 mg of fresh stems of sheanut was homogenized in 2.5 ml ethanol and flask was kept in a water bath at 25^{o}C for 24 h with continuous shaking. After filtration, 1 ml of supernatant solution was taken in volumetric flask, 1 ml ethanol and 5 ml. distilled water and 1 ml. Folin-Ciocalteu reagent was added and flask was shaken thoroughly. After 3 min, 3 ml of solution 2 % (w/v) $NaCO_3$ was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The procedure was repeated to all standard gallic acid solutions (0-100 µg/0.1 ml) and standard curve was obtained. Total phenolic

compounds of the sample were calculated using the calibration curve by using gallic acid standard. The assay was carried out in triplicate.

3.2.2.4 Estimation of mineral in cuttings, scions and leaves from grafts.

One (1.0) g of the plant parts (finely ground and oven dried) was weighed into a digestion tube. To that quantity, 15 ml of Nitric acid (HNO_3) was added to digest it. The digestion was done for another hour at 200 ^oC. The digested sample in the tube was allowed to cool under fume chamber until no fumes evolved, after which it was then filtered through Whatman No. 42 filter paper into 250 ml volumetric flask. The solution (filtrate) was topped up with distilled water to the 250 ml mark and the volumetric flask was shaken by hand to ensure a thorough mix of the contents. The concentrations of the elements were determined on an Atomic Absorption Spectrophotometer (AAS) – Spectra AA 220 FS model; Varian brand. The content of the element in the plant material was calculated using the formula below:

$$\text{Element (mg/kg)} = \frac{\text{Concentration read on AAS x Final Volume after digestion}}{\text{Weight of sample}}$$

For Nitrogen in the plant materials the Kjeldhal Method as quoted by Warner and Jones (1967) in Axford (1974) project was used. One (1.0) g of oven-dried plant material (finely grinded) was weighed into a smaller digestion tube, after which about 0.5 g of catalyst was added to the sample in the tube. 12.0 ml of concentrated sulphuric acid ($H_2SO_{4)}$ of nitrogen (N) free was added to the sample under fume chamber. The tube was then put in a digester under the fume chamber and digested for two (2) hours at 350 ^0C. The digested sample in the tube was allowed to cool under fume chamber until no fumes evolved. The smaller tube containing the digest was washed and rinsed about three times with distilled water into bigger

tube for distillation. The distilled sample which contained the ammonia compounds was then collected in a receiver flask and titrated with 0.02 N H_2SO_4 which had previously been standardized with borax (salt of boric acid or sodium borate), till just a colour was observed (from green to blue).

The percentage Nitrogen in the sample was then calculated using the relation below;

$$\% \text{ Nitrogen} = \frac{\text{Titre value of sample (ml)} \times \text{Normality of acid (0.02)} \times 1.401}{\text{Weight of sample (g)}}$$

Data collected included:

1. Concentration of soluble sugars and proteins in cuttings and scions
2. Concentration of total free phenols in cuttings and scions
3. Concentration of auxins, and proteins in cuttings and scions
4. Concentration of nitrogen, calcium, magnesium, potassium and phosphorus in cuttings/scions

Statistical analysis: An analysis of variance (ANOVA) was performed on the data using Gentstat version 9. Least significant differences (LSD) were calculated and the probability of treatment means being significantly different was set at P = 0.05.

3.3 ANATOMICAL STUDIES

Anatomical features of the shoots were determined by the method of Ruzin (1999) at Forestry Research Institute of Ghana, Fumesua - Kumasi. Shoot samples were cut into sections with the following chemicals for the mounting process; ethanol, safranin O dye, Canada balsam and Clove oil. The following cells were examined under the electron microscope; presence of xylem, phloem and cambium vessels (vascular tissues) in graft union formation and in rooting of layers. The base of sample cuttings about 3 cm were cut and placed in a beaker of water and boiled for 5-6 hours to soften the tissues. The sliding microtome (Reichert-Jung, Heidelberg, Germany) was adjusted to cut sections to a thickness of 15 microns. The cut sections were placed in discs containing different concentrations of ethanol (45 %, 50 %, 70 % , 80 % and 95 %) to absorb water from the section simultaneously without shrinking. The dried sections were then placed in a crucible containing Safranin O Red (dye) solution to colour the cells for easy identification. The Canada balsam and Clove oil were used to harden and mount the sections on slides and finally placed in an oven for the mounted sections to dry. The slides with the mounted sections were examined on a National compound microscope with attached digital camera to observe the various cells.

CHAPTER FOUR

4.0 RESULTS

The results presented in this Chapter follow the order of experiments stated in Chapter three of the thesis.

4.1 Determining the rooting response of air-layered shea trees to media and hormonal application in two contrasting environments.

4.1.1 *Effect of media type and IBA concentration on rooting of air-layered shoots in wet and dry seasons.*

There was significant ($P < 0.05$) interactive effect of IBA concentration and media type on percent rooting of layered shoots in the wet season. Layered shoots sprayed with 10,000 ppm IBA and covered with *Sphagnum* moss produced 73.3 % of rooted shoots, significantly greater than the other treatment combinations. The least percentage of rooted shoots was produced by either palm fibre (3.3 %) or *Sphagnum* (6.7 %) media sprayed with water as control (Table 4.1). No data on the air-layers were in the dry season. The experiment ended abruptly due to the death of all the air-layers.

Table 4.1: Effect of media and IBA concentration on percentage rooting of air- layered shoots in the wet season.

	Percent rooted shoots (%)		
	Type of Media		
IBA (ppm)	Palm fibre	*Sphagnum* moss	Mean
Water (Control)	3.3	6.7	5.0
5000	30.0	33.0	31.7
10,000	46.7	73.3	60.0
Mean	26.7	37.8	

LSD (5%): Media= 9.7; IBA concentration= 11.9; Media x IBA concentration= 16.8

4.1.2 *Effect of type of medium and IBA concentration on mean root length in the wet season.*

There was also significant ($P < 0.05$) IBA concentration level x media type interaction on root length of the layered shoots in the wet season. Layered shoots sprayed with 10,000 ppm and covered with both *Sphagnum* moss and palm fibre were similar. However, *Sphagmum* moss produced 2.5 times longer roots than the mean of the shortest roots produced by either media sprayed with water as control (Table 4.2). No rooting occurred for the air-layers in the dry season.

Table 4.2: Effect of media and IBA concentration on mean root length on air-layered shoots in the wet season.

Mean root length on shoots (cm)			
	Type of Medium		
IBA (ppm)	Palm fibre	*Sphagnum* moss	Mean
Water (Control)	2.8	4.5	3.7
5000	3.7	4.8	4.4
10,000	7.9	9.0	8.2
Mean	4.6	6.1	

LSD (5%): Media= 2.3; IBA concentration= 2.8; Media x IBA concentration= 4.0

4.1.3 *Effect of media type and IBA concentration on number of developed roots on air-layered shoots in the wet season.*

A similar interaction trend was also observed for the number of developed roots such that layered shoots sprayed with 10,000 ppm IBA and either covered with *Sphagnum* moss or palm fibre was not significantly ($P < 0.05$) different from each other. But the *Sphagmum* moss had greater number of roots (three times more) than the mean of the least number of roots produced by either media sprayed with water or 5,000 ppm IBA (Table 4.3).

Table 4.3: Effect of media and IBA concentration on number of developed roots on air-layered shoots in the wet season.

	Number of developed roots on shoots		
	Type of Media		
IBA (ppm)	Palm fibre	*Sphagnum* moss	Mean
Water (Control)	2.0	2.5	2.3
5000	5.0	3.5	4.3
10,000	6.0	7.5	6.8
Mean	4.3	4.5	

LSD (5%) Media= 1.7 (ns); IBA concentration= 2.0; Media x IBA concentration= 2.2

4.1.4 *Relationship between tree canopy architecture of air-layered shoots.*

There was a significant relationship between the canopy of the selected tree and the root production of the layered shoots expressed as $Y_{(rooting)} = 113.87 - 23.697\ X_{(canopy\ spread)}$; $R^2 = 0.89$; $P < 0.002$; $n = 9$. The spread of the tree canopy was negatively related to the roots developed on the layered shoots and explained 89 % of the variation in the rooting of the air-layered shoots. This implies that the more closed the canopy, the better the rooting of the air-layered shoots.

4.1.5 *Effect of different media on fungal infection of roots in air-layered shoots.*

Fungal infection of roots on layered shoots was significantly ($P < 0.05$) lower under *Sphagnum* moss media than under palm fibre media. Root fungal infection was 5.5 times greater under palm fibre media than under *Sphagnum* moss media (Table 4.4)

Table 4. 4: Percent fungal infection on roots of air- layered shoots under different media

Media	Infection (%)
Palm fibre	36.7
Sphagnum moss	6.7
LSD (5%)	23.9

4.1.6 Relationships between IBA, simple sugars and total free phenols.

There were highly significant and positive correlations between IBA and simple sugars as well as between IBA and total free phenols (Table 4.5).

Table 4. 5: Correlation between IBA and simple sugars and total free phenols.

	Correlation coefficient (r)	
	Simple sugars	Total free phenols
IBA	0.99	0.98
	$P < 0.0001$;n = 9	$P < 0.0002$; n = 9

4.1.7 *Effect of climatic parameters (temperature) on rooting of air-layered trees.*

Atmospheric temperature, both day maximum and day minimum, significantly affected rooting of the air-layered trees. Relatively low temperature (22-24^{o}C) levels affected rooting to a greater extent than high temperatures (27 – 29^{o}C) such that 82 % (Eqn 2) of the variation

in the rooting of the layered trees was affected by low temperatures as compared to 65 % effect by high temperatures (Eqn 1).

Rooting = 5250 – 175.0 Temp (high) R^2 = 64.5%, P < 0.009, ……. Eqn 1

Rooting = 836 – 34.2 Temp (low) R^2 = 82%, P < 0.001, ……. Eqn 2

Rooting of layered shoots was however not affected by the environmental relative humidity (high or low).

4.1.8 Nutrient composition and moisture content of media for air-layering.

Sphagnum moss contained significantly higher levels of nitrogen (N), organic carbon (OC), phosphorus (P) and zinc (Zn) than the palm fibre (Table 4.6). The moss contained 13 times more nitrogen, 2 times more organic carbon, and 1.3 times more zinc than the palm fibre. Conversely, phosphorus content was 1.3 times greater in the palm fibre than in the moss. There were no significant differences in the calcium and potassium contents in the media. Both media had similar alkaline reactions. In terms of moisture, *Sphagnum* moss contained 3.2 times more moisture than the palm fibre, the difference being significant (Table 4.6).

Table 4. 6: Nutrient and moisture contents of the media for the air-layering.

Media	Nutrient level (%)						pH	Moisture content (%)
	Ca	K	N	OC	P	Zn		
Sphagmum moss	0.5	1.0	1.3	38.9	4.2	4.9	8.5	9.4
Palm fibre	0.5	0.9	0.1	18.3	5.6	3.0	8.4	2.9
LSD (5%)	0.2 (ns)	0.1 (ns)	0.01	0.8	0.8	0.1	0.7(ns)	1.1

4.1.9 *Hormonal effects on cell differentiation in air- layered shoots.*

There was no pronounced differentiation in the shoot which was neither sprayed with IBA hormone nor covered with any media. Only the cambium and phloem cells showed some expansion leaving the rest of the organs (pith, xylem, pericycle, cortex and epidermis) intact (Plate 4.1). Covering with media alone resulted in a little differentiation in the cambium, phloemnd the pericycle cells in the shoot (Plate 4.2). Further, only spraying the shoot with 5,000 IBA without a media covering resulted in differentiation of cells from the xylem to the cortex (Plate 4.3). For shoots sprayed with 5,000 ppm IBA and covered with media, differentiation occurred in the xylem, cambium, phloem and pericycle with a slight differentiation in the cortex and epidermis (Plate 4.4). For shoots sprayed with 10,000 ppm IBA without media covering, there was comparatively more differentiation in the xylem, cambium, phloem and pericycle as compared to the differentiation in the cortex and epidermis (Plate 4.5). Finally, for shoots sprayed with 10,000 ppm IBA and covered with

medium, all the cells (xylem, cambium, phloem, pericycle, cortex and epidermis) were fully differentiated except the pith cells (Plate 4.6).

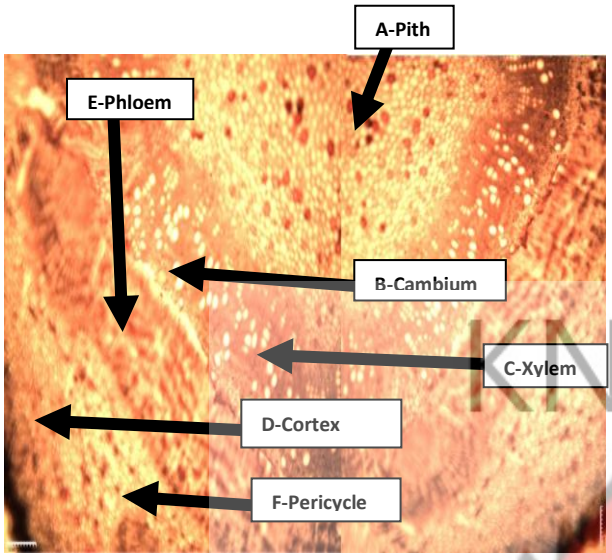


Plate 4.1: Cells in air- layered shoot neither sprayed with IBA nor covered with medium (Mag – 4)

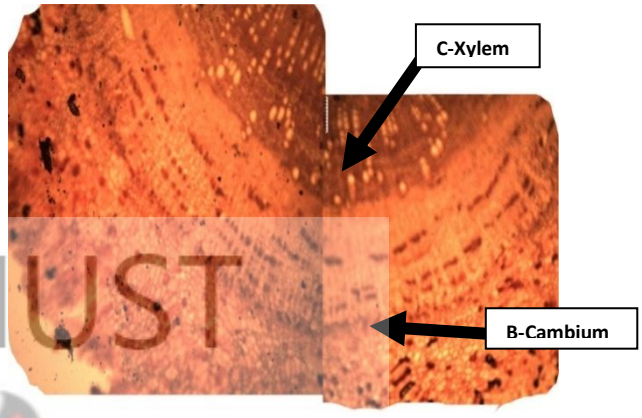


Plate 4.2: Cells in air-layered shoot not sprayed with IBA but covered with medium (Mag – 4)

A- Pith cell B – Cambium cell C – Xylem cell D – Cortex

E – Phloem cell F – Pericycle cell **MEDIUM – *Sphagmum* moss**

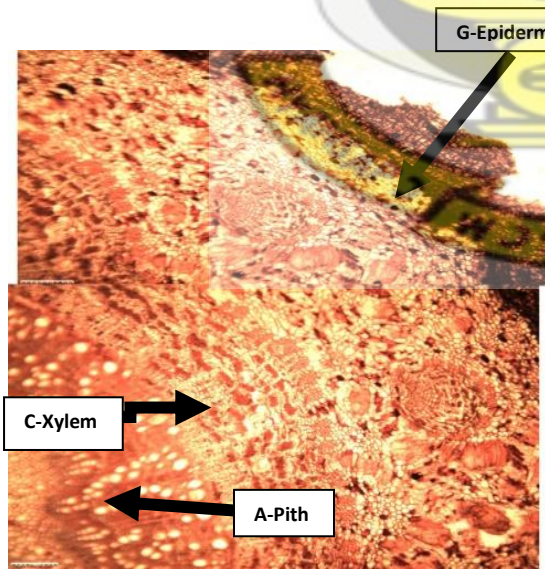


Plate 4.3: Cells in air-layered shoots sprayed with 5,000 IBA without medium (Mag – 4)

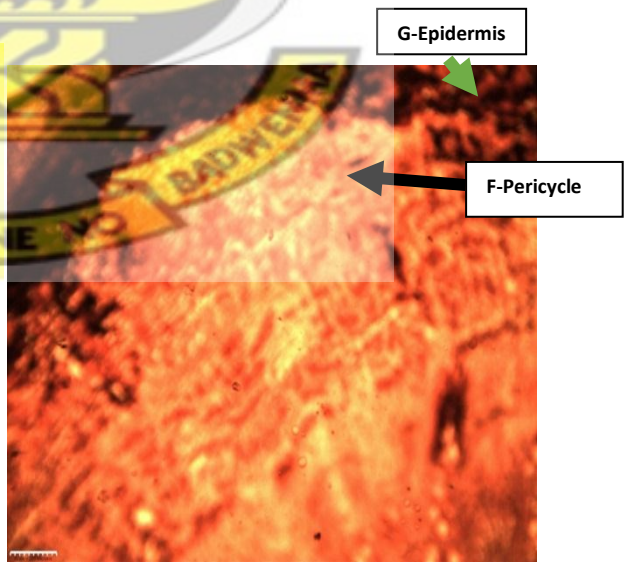


Plate 4.4: Cells in air-layered shoots sprayed with 5,000 ppm IBA with medium (Mag – 4)

A- Pith cell C – Xylem cell F – Pericycle cell G – Epidermis

MEDIUM – *Sphagmum* moss

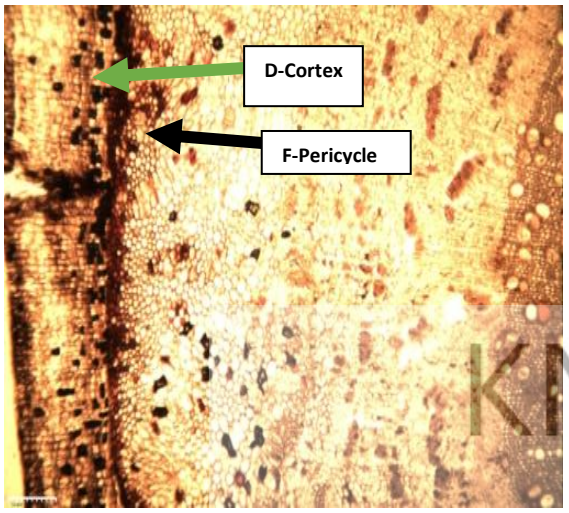


Plate 4.5: Cells in air- layered shoots sprayed with 10,000 ppm IBA without medium (Mag – 4)

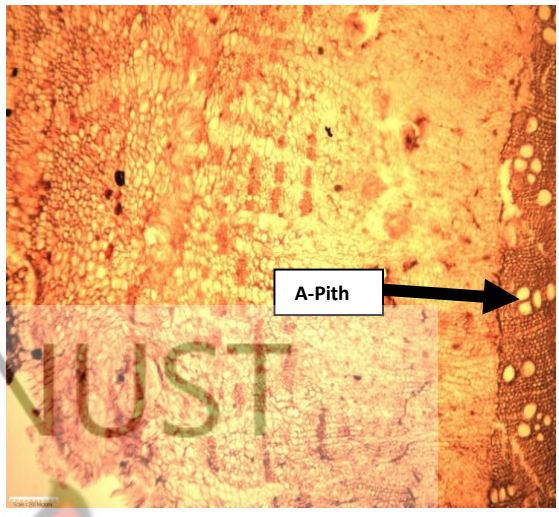


Plate 4.6: Cells in air-layered shoots sprayed with 10,000 ppm IBA with medium (Mag – 4)

A- Pith cell D– Cortex F – Pericycle cell **MEDIUM - *Sphagmum* moss**

4.2 The effect of propagating structures and propagules on the weaning and field survival of plantlets.

4.2.1 *Mean morning and afternoon humidity in different structures.*

Humidity in the mist propagator and propagating pit in the mornings were similar and significantly higher than in the lath house. However, in the afternoon, humidity in the propagating pit was significantly higher than that of the mist propagator and the lath house. In both morning and afternoon, the lath house had the lowest humidity (Table 4.7).

Table 4.7: Mean morning and afternoon humidity in three different propagating structures.

Propagating Structures	Mean humidity at 9.00 am (%)	Mean humidity at 3.00 pm (%)
Mist Propagator	87.5	60.2
Propagating Pit	82.6	69.2
Lath House	61.1	42.4
LSD (5%)	5.42	4.96

4.2.2 Weaning response of propagules.

4.2.2.1 *Effect of propagating structures and propagules on the percent survival of plantlets during weaning.*

There was a significant ($P < 0.05$) effect of propagating structure and plantlet type interaction on the survival rate of propagules during the weaning period of three months (Table 4.8). Normal plantlet as propagules, produced the highest survival rate of 100 % which was significantly higher than the survival rates of the cuttings in both the lath house and propagating pit. The rooted cuttings in the mist propagator was not significantly different from the survived plantlets in any of the structures. Generally, the mist propagator resulted in a significantly higher rate of survival of propagules than the lath house, though similar to the survival rate of the propagating pit (Table 4.8).

Table 4.8: The effect of propagating structures and propagules on the percent survival of plantlets at the end of three- month period of weaning.

Structure	Percent survival % Plantlet type		
	Rooted cuttings	Normal Plantlet	Mean
Lath House	50.0	100.0	75.0
Propagating Pit	76.7	100.0	88.4
Mist Propagator	93.3	100.0	96.7
Mean	73.3	100.0	
LSD (5%): Structure =19.7; Plantlet types = 19.2; Structure x Plantlet types= 21.3			

4.2.2.2 *Effect of propagating structures and propagules on leaf production of plantlets during weaning.*

There was a significant ($P < 0.05$) effect due to propagating structure x plantlet type interaction on the production of leaves of the propagules during weaning (Table 4.9). Rooted cuttings in the mist propagator produced the highest number of leaves (which was about eleven times greater than the leaves produced by normal plantlets in the lath House) though it did not significantly differ from the propagating pit.

Comparing the plantlet types, the rooted cuttings produced significantly greater number of leaves, 4.8 times than the normal plantlets. In addition, both mist propagator and propagating pit resulted in the production of more leaves than the lath house (Table 4.9).

Table 4.9: The effect of propagating structures and propagules on leaf production of plantlets at the end of three- month period of weaning.

Propagating Structure	Number of leaves		
	Plantlet t type		
	Rooted cuttings	Normal Plantlet	Mean
Mist Propagator	122.3	24.7	73.5
Propagating Pit	103.0	22.3	62.7
Lath House	49.7	10.7	30.2
Mean	91.7	19.2	

LSD (5%) Structure= 16.3; Plantlet types= 13.3; Structure x plantlet types= 21.1

4.2.2.3 *Effect of propagating structures and propagules on stem girth of plantlets during weaning.*

There was also a significant ($P < 0.05$) interaction between structure and plantlet type on stem girth of the propagules. Girth of rooted cuttings in the three structures were similar but higher than the plantlets in the lath house (Table 4.10). The rooted cuttings in the mist propagator produced 4.4 times bigger stem girths than the normal plantlets in the lath house which had the smallest stem girt4.10: The effects of propagating structures and propagules on stem girth of plantlets at the end of a 3- month weaning period.

Table 4.10: The effect of propagating structures and propagules on stem girth of plantlets at the end of three- month period of weaning.

Propagating Structure	Stem Girth (mm)		Mean
	Plantlet type		
	Rooted cuttings	Normal Plantlets	
Mist Propagator	2.6	1.2	1.9
Propagating pit	2.2	1.2	1.7
Lath house	1.7	0.6	1.2
Mean	2.2	1.0	

LSD (5%): Structure = 1.0; Plantlet type = 1.1; Structure x Plantlet type = 1.3

4.2.2.4 *Effect of propagating structures and propagules on plant height of plantlets during weaning.*

Plant height of propagules was significantly ($P < 0.05$) affected by structures x plantlet type interaction (Table 4.11). The height of the rooted cuttings in the mist propagator, propagating pit and lath house were similar but significantly higher than plantlet height that in the lath house. The rooted cuttings in the mist propagator produced the tallest plants, 1.4 times and 1.9 times taller than the normal plantlets in the propagating pit and lath house. Plantlets generally weaned in the lath house were the shortest.

Table 4.11: The effect of propagating structures and propagules on plant height of plantlets at the end of three- month period of weaning.

Propagating Structure	Plant Height (cm)		Mean
	Plantlet type		
	Rooted cuttings	Normal Plantlets	
Mist Propagator	5.5	5.0	5.3
Propagating pit	4.9	4.0	4.5
Lath house	4.3	2.9	3.6
Mean	4.9	4.0	

LSD (5%): Propagating Structure = 1.2; Plantlet type = 1.3; Propagating Structure x plantlet type= 1.5

4.3 Field establishment of weaned propagules.

4.3.1 *Effect of hole depth on soil temperature and moisture in the dry season*.

There were significant differences between the hole depths on soil temperature and moisture. Soil temperature was lowest at 52 cm depth, significantly lower than those at 39 cm and 26 cm depths (Table 4.12). Conversely, soil moisture was highest at 52 cm depth, significantly greater than at 26 cm depth yet similar to moisture at 39 cm depth.

Table 4.12: Effect of hole depth on soil temperature and soil moisture averaged over six months dry season.

Hole depth	Mean Soil Temperature ^{o}C	Mean soil Moisture %
26 cm depth	29.1	43.0
39 cm depth	27.4	45.6
52 cm depth	24.2	47.0
LSD (5%)	2.49	1.62

4.3.1.1 *Effect of hole depth and propagules on survival of transplanted weaned plantlets in the dry season.*

There was a significant hole depth x seedling type interaction on the percent survival of transplanted propagules in the dry season (Table 4.13). Normal plantlets planted at a depth of 52 cm produced the highest percentage of survived transplants significantly different from the rooted cuttings planted at depths of 39 cm and 26 cm respectively but did not differ from the other treatments. Generally, at each depth, the normal plantlets produced significantly greater percentage of survived transplants than the corresponding rooted cuttings (Table 4.13).

Table 4.13: Effect of hole depth on the survival of transplanted plantlet in the dry season.

Hole depth (cm)	% survival of transplanted propagules		
	Plantlet type		
	Rooted cuttings	Normal Plantlets	Mean
26 cm depth	60.0	80.0	70.0
39 cm depth	65.0	85.0	75.0
52 cm depth	75.0	90.0	82.5
Mean	66.7	85.0	

LSD (5%) Hole depth = 16.1 (ns); Plantlet type = 18.2 (ns); Hole depth x Plantlet type = 19.0

4.3.1.2 *Relationship between field survival and period of field establishment of propagules.*

There was a significant relationship between field survival of propagules and the month of establishment. The month of establishment accounted for 68 % of the variation in the percent survival of the propagules. The relationship was expressed as

$Y_{(\text{percent survival})} = -2844 + 0.70\ X_{(\text{month})}$ $P < 0.001$; $R^2 = 0.68$; $n = 90$

4.3.1.3 *Effect of hole depth and propagules on stem girth of transplanted plantlets six months after field establishment.*

There was a significant ($p < 0.05$) effect of hole depth x plantlet type interaction on stem girth of propagules six months after field establishment. The rooted cuttings transplanted at a hole depth of 39 cm and 52 cm were similar in girth but bigger than plantlets transplanted in the same hole sizes (Table 4.14). Generally, rooted cuttings produced bigger stem girth of propagules as compared to normal plantlets. Additionally, propagules (rooted cuttings) transplanted at a depth of 52 cm produced bigger stem girth than those transplanted at a shallow depth of 26 cm (Table 4.14).

Table 4.14: Effect of hole depth and propagules on the stem girth of transplanted plantlets six months after field establishment.

Hole depth (cm)	Stem girth (mm)		Mean
	Plantlet type		
	Rooted cuttings	Normal Plantlets	
26 cm depth	1.3	0.6	1.0
39 cm depth	2.4	0.6	1.5
52 cm depth	3.4	0.7	2.1
Mean	2.4	0.6	

LSD (5%): Hole depth = 1.0; Plantlet type = 1.1; Hole depth x Plantlet type = 1.3

4.3.1.4 *Effect of hole depth and propagules on plant height and leaf production of transplanted plantlets six months after field establishment.*

There was no significant difference in the height of propagules six months after field establishment. However, there was significant ($P < 0.05$) effect of hole depth x plantlet type interaction on leaf production. Rooted cuttings transplanted at a hole depths of 52 cm and 39 cm were similar in number of produced leaves. Cuttings transplanted in hole depth 52 cm produced more leaves (three times) than the normal plantlet in a 26 cm hole depths. Plantlets in 26 cm and 39 cm depths were similar in girth (Table 4.15). Furthermore, the rooted cuttings in each of the hole depths produced significantly more leaves that the normal plantlet in both 26 cm and 39 cm hole depths, the exception being normal plantlets in 52 cm hole depth (Table 4.15).

Table 4.15: Effect of hole depth and propagules on the number of leaves of transplanted plantlets six months after field establishment.

Hole depth (cm)	Number of leaves produced		
	Plantlet type		
	Rooted cuttings	Normal Plantlets	Mean
26 cm depth	4.0	1.9	3.0
39 cm depth	4.5	2.4	3.5
52 cm depth	5.7	3.7	4.7
Mean	4.7	2.7	

LSD (5%): Hole depth = 1.2 (ns); Plantlet type= 1.2; Hole depth x Plantlet type = 1.5

4.4 The effect of scion wounding types (girdling and pre-curing) and different rootstock types on grafting success.

4.4.1 *Effect of rootstock type and scion wounding type on graft success.*

There was significant ($P < 0.05$) rootstock x scion wounding interaction for graft success (Table 4.16). Pre-cured scions grafted onto rejuvenated shoots recorded 76.7 % graft success which was significantly higher than the other treatments. The least graft success was observed in pre-cured scions grafted onto young plants. The rejuvenated shoots generally gave higher graft success than the young plants.

Table 4.16: Combined effect of rootstock type and scion wounding type on graft success.

	Graft success (%)		
	Type of wounding		
Rootstock type	Girdle	Pre-cure	Mean
Rejuvenated shoot	35.0	76.7	55.9
Young plant	20.0	6.7	13.4
Mean	27.5	41.7	

LSD (5%) Rootstock type= 30.6; Wounding type= 32.6 (ns);
Rootstock type x wounding type= 33.3

4.4.2 *Rate of fungal infection on wounded scions before and after grafting.*

Significant fungal infection occurred for the wounded scions before harvesting and grafting. Scions which were girdled had significantly higher ($P < 0.05$) infection rate than the pre-

cured scions. The infection rate of the girdled shoots after grafting was more than 73 % of the pre-cured shoots after grafting (Table 4.17).

Table 4.17: Rate of infection on wounded scions before and after grafting

Wounded type on Scions	Infection rate (%)		
	Before grafting	After grafting	Mean
Pre-cure	1.1	3.0	2.1
Girdle	8.7	25.9	17.3
Mean	4.9	14.5	
LSD (5%)	6.6	6.6	

4.4.3 *Effect of rootstock type and scion wounding type on the girth of scion six months after grafting.*

There was significant ($P < 0.05$) wounding x rootstock interaction on stem girth. Pre-cured scions grafted onto rejuvenated shoots produced the biggest girth of 7.4 mm, significantly greater than the girdled shoots (Table 4.18). The smallest scion girth was recorded by the shoots that were not wounded and grafted onto the young plants. Among root stock types, pre-cured wounding produced the greatest scion girth increase, significantly different from the girdle wounding and no wounding.Girdle wounding also resulted in significantly higher girth increase than no wounding as control.

Table 4.18: Girth of scions as affected by rootstock type and wounding type six months after grafting.

Wounding type	Girth (mm)		Mean
	Rootstock type		
	Young Plant	Rejuvenated shoots	
Control	2.7	3.0	2.9
Girdle	4.6	5.1	4.9
Pre-cure	5.1	7.4	6.3
Mean	6.2	7.8	

LSD (5%) Rootstock type= 0.6; Wounding type = 1.3; Rootstock type x wounding type= 1.5

4.4.4 *Effect of rootstock type and scion wounding type on the height of scion six months after grafting.*

There was significant rootstock type x wounding type interaction for height of scions, six months after grafting (Table 4.19). The pre-cured scions grafted onto rejuvenated shoots produced significantly the tallest scions, though it did not differ significantly from the girdled shoots grafted onto the same rootstock. The shoots which were not wounded and grafted onto young plants gave the least height. Generally, scions from the rejuvenated shoots were taller than those from the young plants. Wounding alone did not affect the height of the scions produced.

Table 4.19: Height of scions as affected by rootstock type and wounding type six months after grafting.

Wounding type	Height of scions (cm)		Mean
	Rootstock type		
	Young Plant	Rejuvenated shoots	
Control	19.6	32.5	26.1
Girdle	27.5	37.2	32.4
Pre-cure	23.6	45.9	34.8
Mean	23.6	36.2	

LSD (5%) Rootstock type= 8.9; Wounding type= 9.1 Rootstock type x wounding type= 12.0

4.4.5 *Effect of rootstock type and scion wounding type on the leaf production of scion six months after grafting.*

Wounding x rootstock interaction significantly ($P < 0.05$) affected leaf production over the 6-month period with pre-cured scions grafted onto rejuvenated shoots produced the greatest number of leaves, significantly ($P < 0.05$) greater than the wounded scions grafted onto young plants (Table 4.20). The least leaf production was recorded by non-wounded scions grafted onto young plants. The pre-cured scions used for grafting produced 1.4 and 2.2 more leaves than the girdle and non-wounded scions (Control), respectively. The rejuvenated shoots produced 1.8 more leaves than young plants.

Table 4.20: Leaf production of scions as affected by rootstock type and wounding type six months after grafting.

Wounding type	Leaf production		Mean
	Rootstock type		
	Young Plant	Rejuvenated shoots	
Control	12.0	25.0	18.5
Girdle	20.0	38.0	29.0
Pre-cure	28.0	55.0	41.5
Mean	20.0	39.3	

LSD (5%) Rootstock type= 1.4; Wounding type= 1.6; Rootstock type x wounding type= 1.8

4.4.6 *Levels of biochemical substances in wounded scions.*

The pre-cured scions contained significantly more nitrogen (5.8 times) and more protein (1.8 times) than the girdled scions (Table 4.21). On the contrary, IAA concentration in the girdled scion was 1.3 times higher than in the pre-cured scion. For soluble sugars and total free phenols, similar concentrations were found in both pre-cured and girdled scions (Table 4.21).

Table 4.21: Concentration of biochemical substances in wounded scions.

Wounded scions	Nitrogen (%)	Protein (%)	IAA %	Soluble sugars (mg/g)	Total free phenols (mg/g)
Girdled	0.21	1.31	0.004	5.33	7.23
Pre-cured	1.22	2.41	0.003	5.03	6.90
LSD (5%)	0.009	0.06	0.0037	0.42	1.2

4.4.7 *Levels of biochemical substances in different rootstocks.*

Rejuvenated shoots contained significantly higher levels of nitrogen (2.2 times), protein (14 times), soluble sugars (1.4 times), IAA (3 times) and total free phenols (1.3 times) than young plants (Table 4.22).

Table 4.22: Concentration of biochemical substances in different rootstocks.

Rootstock	Nitrogen (%)	Protein (%)	Soluble sugar (mg/g)	(IAA) %	Total Free Phenols (mg/g)
Rejuvenated shoots	0.76	1.14	4.8	0.003	5.5
Young plant	0.34	0.08	3.5	0.001	4.1
LSD (5%))	0.01	0.9	1.2	0.0004	1.2

4.4.8 *Wounding effect on cell development in grafting.*

Differentiation did not occur in the pre-cured scions. However, a pronounced differentiation was observed in the xylem and cambium cells with moderate cell division in the girdled scions (phloem, pericycle cortex and epidermis), (Plates 4.7 & 4.8).

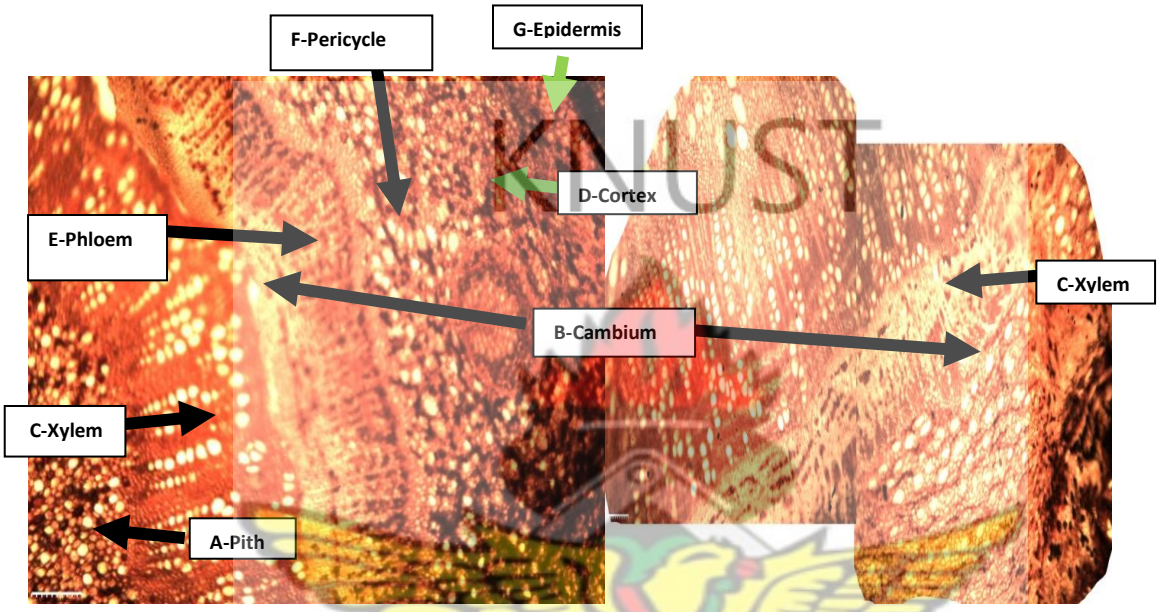


Plate 4.7: Cells in intact pre-cured scion before grafting (Mag – 4)

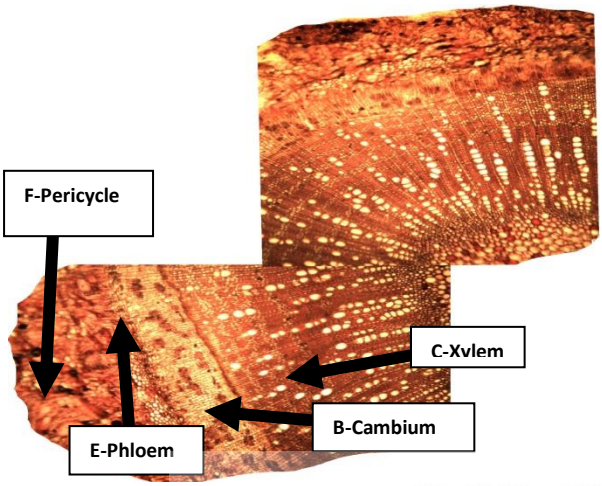
Plate 4.8: Cells in intact girdled scion before grafting (Mag – 4)

A- Pith cell B – Cambium cell C – Xylem cell D – Cortex

E – Phloem cell F – Pericycle cell G - Epidermis

4.4.9 *Cell development in rootstocks.*

The vascular cells (xylem, phloem and cambium) as well as cortex and epidermis in the young plant showed expansion without any differentiation (Plate 4.9) while for the rejuvenated shoots, differentiation occurred in the phloem, cambium, cortex and epidermis cells with expansion in the xylem and cortex cells (Plate 4.10).



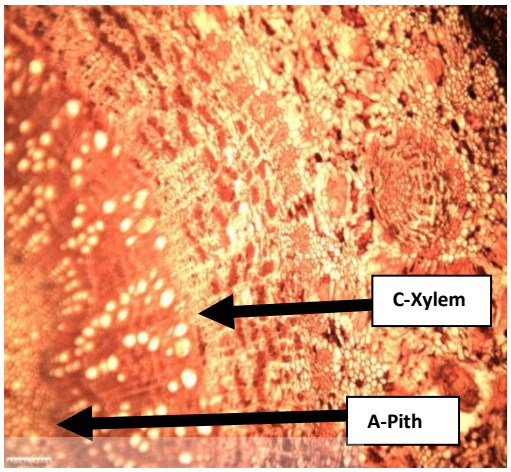


Plate 4.9: Cell in young plant (rootstock) (Mag – 4)

Plate 4.10: Cells in rejuvenated shoots (rootstock) (Mag – 4)

A- Pith cell B – Cambium cell C – Xylem cell E – Phloem cell

F– Pericycle cell

4.5 Grafting success as affected by rootstock types, bud development stage of scions and grafting methods.

4.5.1 *Effect of grafting method, rootstock types and bud development stage of scions on grafting success.*

There was no significant ($P < 0.05$) grafting method x bud development stage x rootstock type interaction on graft success. However, graft method x bud stage development interaction was significant ($P < 0.05$) for graft success. Using top cleft grafting technique with partially sprouted bud scions recorded 70.0% graft success significantly greater than the other treatment combinations. The least graft success of 28.3% which was significantly lower than the other treatments was observed in scions with fully sprouted buds and grafted using side grafting technique (Table 4.23). There was also a significant grafting method x rootstock interaction on graft success (Table 4.24).

Grafting on rejuvenated shoots as root stock using the top cleft technique significantly gave high (71.1 %) graft success with the lowest being side grafting on young plant rootstock (30.0%).

Table 4.23: Interactive effects of grafting method and bud development stage of scion on graft success.

Graft method	Percent graft success (%)			Mean
	Bud development stage			
	Dormant	Partially sprouted	Fully sprouted	
Top cleft	48.8	70.0	51.7	56.8
Side	33.3	30.0	28.3	30.6
Mean	41.1	50.0	40.0	
LSD (5%) Graft method = 7.2; Bud development stage = 8.8; Graft method x Bud development stage = 12.4				

Table 4.24: Interactive effects of grafting method and rootstock type on graft success.

Graft method	Percent graft success (%)		Mean
	Rootstock type		
	Rejuvenated shoots	Young plant	
Top cleft	71.1	42.2	56.8
Side	31.1	30.0	30.6
Mean	51.1	36.1	
LSD (5%) Graft method= 7.2; Rootstock type= 7.2; Grafting method x Rootstock type= 10.2			

4.5.2 *Levels of biochemical substances in buds at different developmental stages.*

Partially sprouted buds contained significantly higher levels of IAA (1.3 times), total free phenols (1.3 times) and protein (1.3 times) than in dormant buds (Table 4.25). The fully sprouted buds contained 2.5 times more nitrogen than the dormant buds, though similar in concentration with the partially sprouted buds. The concentration of sugars was similar for all the bud types. (Table 4.25).

Table 4.25 : Concentration of biochemical substances in buds at different stages of development.

Type of bud	IAA (%)	Total free phenol (mg/g)	Sugars (mg/g)	Protein (%)	Nitrogen (%)
Dormant	0.003	1.4	4.8	0.9	0.53
Partially sprouted	0.004	1.7	5.1	1.2	1.10
Fully sprouted	0.003	1.5	5.0	1.0	1.32
LSD (5%)	0.0005	0.26	0.56 (ns)	0.24	0.26

4.6 Determination of the best period for coppicing to produce shoots and subsequent graft success.

4.6.1 *Effect of coppicing to produce shoots and subsequent graft success.*

Sprouting of shoots on the stumps was significantly ($P < 0.05$) affected by the period of coppicing (Table 4.26). The May – June period significantly recorded the highest sprouts of of 90.3% at sixty days after grafting (DAG) as well as the highest subsequent graft success of 85.8%. The least sprout percentage coppiced plants was recorded in the July - August period while the lowest graft success was recorded in the March – April period.

Table 4.26: Effect of period of coppicing to produce shoots and subsequent graft success.

Period of coppicing	Percent of sprouts by 60 DAG (%)	Graft success (%)
January – February	80.0	70.0
March – April	60.0	33.3
May - June	90.3	85.9
July - August	46.7	36.7
September - October	53.3	36.7
November - December	66.7	51.3
LSD (5%)	15.6	25.2

4.6.2 *Relationship between shoot sprout and graft success.*

There was also a significant relationship between graft success and the percentage of shoot sprouts such that 69 % of the variation in the success of the graft was explained by the quantity of shoot sprouts (Eqn 4).

$Y\ (_{\text{graft success}}) = 1.0432\ X\ (_{\text{sprouts}}) - 13.992;\ R^2 = 0.69;\ p < 0.001$…… Eqn 4

4.6.3 *Levels of plant substances in scions for grafting at different periods*.

There were significant differences in the levels of plant substances in the scion harvested for grafting during the various periods. High levels of IAA (0.004 %) and protein (2.3%) were recorded in May/June, total free phenols (1.4 %) in Jan. / Feb., and soluble sugars (7.2 mg/g) in Sept. /Oct. The lowest levels for IAA (0.001 %) were in March-April and August-

September whereas total free phenols (0.6 mg/g), protein (0.7 %) and soluble sugar (3.0 mg/g) were lowest only in March-April (Table 4.27).

Table 4.27: Levels of plant substances in scions for grafting at different periods.

Period	IAA (%)	Total free phenol (mg/g)	Protein (%)	Soluble sugar (mg/g)
(Jan – Feb)	0.003	1.40	1.25	5.20
(Mar–April)	0.001	0.62	0.70	2.10
(May –Jun)	0.004	1.22	2.30	7.00
(Jun – Jul.)	0.002	0.90	1.30	4.90
(Aug. – Sept.)	0.001	1.15	1.00	1.0
(Nov - Dec)	0.003	1.20	1.90	6.00
LSD (5%)	0.0005	0.26	0.24	0.56

4.6.4 *Levels of nutrients in leaves of grafted and non-grafted rootstocks after six months of graft success.*

Leaves of grafted rejuvenated shoots contained significantly higher levels of Ca, Zn, Mn and N than the leaves of the other graft types, six months after graft success (Table 4.27). The least concentration of nutrients were found in the leaves of the non-grafted young plants. In general, the leaves of the grafted rootstocks contained more nutrients than those of the non-grafted (Table 4.28).

Table 4.28: Nutrient concentration of leaves of grafted and non-grafted rootstocks six months after graft success.

Grafted rootstock type	Nutrient level (%)			
	Calcium	Zinc	Manganese	Nitrogen
Leaves of grafted rejuvenated shoots	1.1	2.7	1.8	1.9
Leaves of non-grafted rejuvenated shoots	0.7	1.5	1.2	1.3
Leaves of grafted young plant	0.8	2.1	1.7	1.4
Leaves non-grafted young plant	0.6	1.5	1.0	1.3
LSD (5%)	0.2	0.3	0.2	0.2

CHAPTER FIVE

5.0 DISCUSSIONS

5.1 Effect of IBA concentration and media type on rooting of air-layered shoots in wet and dry seasons.

Rooting of the air-layered shoots was better in both number and length of the roots with the application of 10,000 ppm of IBA as compared to the 5,000 ppm and control. This finding was also observed in the anatomy of the shoots treated with IBA at 10,000 ppm concentration. Yeboah *et al.* (2009a; 2009b; 2010a; 2010b) recorded similar findings when higher concentrations of IBA (> 5,000 ppm) were applied to shea tree cuttings. This implies that the greater the concentration of IBA, the better the cell performance and its efficiency of action. Similar findings were also made by Henrique *et al.* (2006) using paclobutrazol (PBZ) on *Pinus caribea* cuttings. As an auxin, IBA, most probably enhanced the translocation of carbohydrate and other endogenous plants substances and nutrients to the rooting zone for cell division, root initiation and development of the air-layered shoots. Additionally, the nutrient translocation ability of IBA might have resulted in more mineral nutrients being translocated from the *Sphagmum* moss, (with higher N, Zn, and O.C content) than from the palm fibre (higher P content), for better rooting of the air-layered shoots. Each of these nutrient elements played a unique role in the improved rooting of the shoots. For instance, nitrogen being involved in protein synthesis and RNA production signaled adventitious root development through cell organization (Jain and Nanda, 1973; Cakmak, 2000). Furthermore, the synthesized protein catalyzed the division of the vascular cells (xylem, phloem and cambium) and enhanced the rooting process (Errea, 1998). Zinc also played a role in auxin synthesis (Hartmann *et al*., 2002). Phosphorus, on the other hand, aided healthy root growth by enhancing the translocation of carbohydrates whereas organic carbon served as energy and raw material sources for microbial biomass (Noggle and Fritz, 1983). Besides the nutrients,

the higher moisture availability in the *Sphagmum* moss medium as compared with the palm fibre medium reduced stress on the plant and probably enhanced biochemical and physiological functions leading to enhanced metabolic activities during the rooting process (Hutcheon *et al.*, 1973). Additionally, the high moisture content (water holding capacity) might have provided a congenial regime for efficient functioning of micro-organisms that were involved in organic matter decomposition and nutrient release (Hutcheon *et al.*, 1973). Infection on rooted layers in the *sphagmum* moss medium was very negligible yielding higher rooting compared to the palm fibre which recorded low rooting. The poor rooting with the palm fibre was a result of the interruption of the movement of some plant substances responsible for cell function by the fungi (Hartmann *et al*., 2002; Armstrong, 1992; James, 1993). As regards the other IBA + media combined treatments, some level of cell (especially in the xylem, phloem, cambium) expansion and differentiation were also observed, except the control where shoots were without both IBA and medium.

In the present study, rooting was better in the wet season than the dry season, probably due to the mean moderate temperatures experienced in the respective seasons. In the wet season, low mean ambient temperatures were experienced and this could be partly responsible for the high rooting of the air-layered shoots. Contrarily, the low rooting of the air-layered shoots in the dry season could be partly attributed to the high mean ambient temperatures experienced during this period of the year. Hartmann *et al*. (2002) indicated that high temperatures, greater than 32^{o}C, hasten evapo-transpiration rate, slow down cell processes, cause injury to cells and eventually impede rooting.

5.2 Effect of synthesized biochemical products on rooting and root protection.

In the present study, the air-layered shoots treated with 10,000 ppm IBA and covered with *Sphagmum* moss which recorded the highest rooting also synthesized the highest levels of sugar and phenols. Earlier biochemical studies on the rooting of shea tree (Akakpo *et al*., 2013; Amissah *et al.,* 2013) and other crops (Das *et al*., 1997; Gabryszewska, 2011) clearly indicate that sugars and phenols play a role in root formation thus supporting the results of this present study. For the sugars, the IBA bound sugar molecules were translocated to the base of the shoots to provide energy for the rooting process (Woodward and Bartel, 2005; Das *et al*., 1997); whiles for the phenols, IBA bound phenol molecules were translocated to the base to prevent infection of the developed shoots as well as protection against the oxidation of the IBA by IAA-oxidase (Hartmann *et al*., 2002; Pandey and Pathak, 1981). Hartmann *et al*. (2002) also indicated that the type of shoot selected (dormant or lateral) influenced the level of endogenous substances and nutrients in the shoot which were important for root initiation and development. In the present study, better rooting were observed from air-layered shoots selected from laterals in a dense canopy. Yidana (1994) observed the shoots in a dense canopy store more food than photosynthesizing resulting in their high rooting performance when air-layered or used as cuttings in a propagator.

5.3 Effect of environmental temperature on rooting of air-layered shoots.

Ambient temperature had a significant effect on rooting of layered shoots. The extent of the effect was however more pronounced by low temperatures than high temperatures during the study period. Low to moderate temperatures have been reported to decrease bud elongation in advance of root initiation as well as decrease water loss from the leaves thus increasing the moisture status in the system for the layers to gain the potential benefit to root (Hartmann *et al*., 2002). Furthermore, a temperature of 22 – 24 ^{o}C has been found to be suitable for carbohydrate metabolism, cell division and root initiation (Moore *et al.*, 1975). Sivaci and

Yalcin (2008) also indicated that temperature was one of the factors that affected the seasonal changes of some important endogenous growth regulators in apples.

5.4 Effects of propagating structures and propagules on the survival of weaned plantlets.

Survival of the rooted cuttings in the mist propagator was very high and comparable to that of the plantlets. The lath house on the other hand, recorded the least survival of the rooted cuttings. The micro humidity and temperature around the cuttings could be the reason for the observed differences. This is because in the mist propagator due to the frequent provision of water from the mist spray, the humidity around the cuttings was always very high coupled with a lowering of the temperature around the cuttings. This observation is corroborated by Hartmann *et al*. (2002) who indicated that high humidity and low ambient temperature are suitable micro climate for the survival and development of cuttings because they ensure low evapo-transpiration and prevent moisture stress in the cuttings. As regards the plantlets, the presence of their deep taproot systems in the polythene bags enabled them to withstand the harsh ambient weather conditions.

In the lath house, humidity was low, the least amongst the propagating structures and therefore probably accounted for the low survival of the rooted cuttings. During the weaning period, leaf production of the rooted cuttings in the mist propagator was about 2.5 times greater than similar rooted cuttings in the lath house and 6.5 times more than the mean number of leaves of the plantlets across the propagating structures. The very high humidity in the mist propagator has been implicated as a possible factor enhancing leaf formation and production. This is because it promotes the breaking of leaf buds through the provision of endogenous substances such as cytokinins which allow them to mature and break (Kramer and Kozlowski, 1979; Noggle and Fritz, 1983; Malik and Srivastava, 2002). Moreover,

Hartmann *et al*. (2002) indicated that humidity management was an effective tool in enhancing plant growth because it ensured the presence of moisture in the environment which enhanced rapid cell processes like cell division, vitamins production, protein synthesis, auxin synthesis and DNA production. Plant height and stem girth of the rooted cuttings were however similar for all the propagating structures during the weaning period of three months. In comparison to the seedlings, the rooted cuttings in the mist propagator recorded better growth in terms of leaf production, plant height and stem girth.

5.5 Effects of propagating structures and propagules on field establishment of weaned plantlets.

The period of field establishment coincided with good rains which facilitated the high survival rate of the transplanted propagules. It was therefore not surprising that the month of field establishment significantly accounted for 68 % of the variation in the survival of the transplanted propagules. Generally, the percent survival of the transplanted plantlets was significantly higher than the rooted cuttings due mainly to the rooting system of the propagules; plantlets with a long tap root whereas the rooted cuttings had adventitious roots. Consequently, the plantlets were better able to explore and draw on the soil resources than the rooted cuttings with the shallow adventitious roots.

Naturally, the sheanut tree has a long tap root with very limited secondary and tertiary roots. By this root systems, it draws more on soil resources from the deeper layers than the superficial layers and this in part accounts for its slow growth in its natural habitat. In the present study, once established, the transplanted rooted cuttings performed better in terms of growth than the plantlets as observed in its recording of 5.6 times bigger stem girth and 1.7 times more leaves than the plantlets across all the hole depths. This could be due to the fact that the rooted cuttings had a better root system in terms of having more secondary and

tertiary roots six months after establishment thus enhancing the ability of the rooted cuttings to explore both the superficial and deeper layers of the soil to facilitate growth. Among the rooted cuttings, transplanting into a hole of 52 cm depth produced significantly better growth in stem girth and leaf production than transplanting into a 26 cm deep hole. This could be explained by the fact that the 52 cm deep hole facilitated the development of a long tap root, a natural tendency of sheanut tree, than the more shallow 26 cm deep hole depth which impeded root development and growth. Furthermore, at the deepest depth of 52 cm, soil temperature was lowest whiles soil moisture was greatest. Moderate soil temperatures help the root system of the plants to function well in absorption of water and nutrients (Haskell *et al.*, 2010) while high soil moisture helps the roots to extract water to the shoots for growth and enhanced photosynthetic activity (Jackson, 1968; Hartmann *et al*., 2002). The high soil moisture at the 52 cm hole depth might have also provided a conducive environment for efficient functioning of soil microorganisms that were involved in soil biogeochemical processes including organic matter decomposition and synthesis of hormones and organic acids which aid nutrient release from soil for enhanced uptake by plants (Hutcheon *et al*., 1973).

5.6 Effect of rootstock type and scion wounding type on graft success.

The results obtained showed that pre-cured shoots grafted onto rejuvenated shoots gave the best graft union formation. The rejuvenated shoots according to Hartmann *et al.* (2002) were preferred to young plant in graft formation, because of the rapid development of the shoots. He further explained that the large root volume of the stumps of rejuvenated shoots hastened the translocation of plant substances and perpetuated the breaking of the apical buds. Biochemical analysis of the shoots developed on the stumps (rootstock) showed high levels of nitrogen, protein, soluble sugars, IAA and total free phenols which might have played important roles in cell functions to enhance wound healing of the grafts. Though pre-cured scions achieved high graft success than the girdled scions, its auxin (IAA) content (0.003%) was lower than the girdle (0.004%). This may be explained by the fact that the presence of leaves on the girdled scions enhanced continuous basi-petal movement of plant substances to the area of the girdle and increased the auxin level and other plant substances (Hartmann *et al*., 2002; Das *et al*., 1997) at the base as long as it was still attached to the parent plant with leaves. However the level of the substances in the upper portion of the girdled shoots would be lower than at the base (in the rootstock). On the other hand, in the pre-cured shoots where all the apical leaves were removed, translocation of the substances to the base was drastically reduced with the shoots storing a lot of these substances. This resulted in a uniform distribution of the plant substances in the pre-cured shoot, especially, in the apical meristem (bud). Graft union formation was faster in the girdled shoot (15 – 25 days after grafting) than in the pre-cured shoots (30 - 40 days after grafting), although the girdled shoot had a slow apical bud sprout compared with the pre-cured shoots which had a faster apical bud sprouts and consequently higher graft success. Anatomically, observations in the girdled shoots revealed rapid cell division in the xylem, cambium, phloem, pericycle, cortex and epidermis which probably accounted for the fast wound healing at the base of the graft union.

Contrarily, in the pre-cured shoots, there was expansion of the vascular cells which might have contributed to the faster bud development for growth. Yeoman and Brown (1976), indicated that the expansion of the cells was due to the formation of protein complexes with catalytic activity resulting in the formation of successful grafts. This expansion of the cells observed in the pre-cured scion most probably explained the enhancement of the wound healing process.

The high graft success in the pre-cured scions could also be explained by its lower observed fungal infection level as compared to that of the girdled scion. This is because fungal infection can interrupt the movement of sap and other plant nutrients (by blocking their pathway) which play a major role in graft formation (James, 1993; Armstrong, 1992).

Findings in the present study suggest that fast wound healing accompanied by high graft success could be achieved using pre-cured scions as against girdled scions. These findings corroborate the use of pre-cured scions as the most preferred choice in cashew vegetative propagation with very high rates of graft success (Cashew Development Project, 2008).

5.7 Grafting success as affected by rootstock types and bud development stage on scions and grafting methods.

Among the grafting types, the top cleft technique achieved a high success (top cleft – 71.1%; side – 31.1 %) in graft union formation. However, Sanou *et al*. (2004) reported that in a similar grafting study, the side cleft technique led to a higher (86.1 %) graft success than the top cleft technique (78.1 %). No scientific reason was assigned by Sanou *et al*. (2004) for this finding.

The probable reason for better success (85.7%) for top cleft could be due to a good vascular contact as observed by Hartmann *et al.* (2002).Furthermore, in top cleft grafting, because the scions are directly fixed into the rootstock there is a large potential cambial contact (Bashiru,

1998) which is better than the contact achieved with the side grafting. Additionally, top cleft grafting gives a firm support to the scion after take as compared to the side which can slip off with the least disturbance or when the scion is bigger than the rootstock. In recent times, top cleft grafting technique is the most commonly used technique in the development of cashew because of its high success rate (Cashew Development Project, 2008).

Among the different scion buds, the partially sprouted type gave better graft union formation than the others. This finding is explained by the biochemical analysis which showed high levels of auxin (IAA), total free phenols, proteins and nitrogen in the sprouted buds than the others. Auxin (IAA) accelerates the formation of a successful graft through cell division, growth and development (Aloni, 1987). The basipetal movement of the auxin causes conjugation of the auxin with other endogenous plant substances like carbohydrates, growth co-factors like phenols and nutrients and together move to the graft zone to enhance rapid wound healing and union formation. Yeboah *et al.* (2009a; b; 2010a; b) also made similar observations of auxins in rooting of stem cuttings. As regards the phenols and phenolic compounds, which are triggered by various stress situations (Hartmann *et al.*, 2002) such as wounding and infections, they act as auxin precursors (Feucht and Schmid, 1979) and also modulate the levels of IAA-oxidase which degrades auxin. Nitrogen on the other hand, is responsible for DNA production and synthesis of proteins which play important roles in graft union formation (Hartmann *et al.*, 2002). The synthesized protein catalyzes the division of the vascular cells (xylem, phloem and cambium) and enhances the wounding process (Errea, 1998).

In terms of the rootstocks, rejuvenated shoots performed better than the young plants in graft success. The shoots which developed from a stump with larger root volume could have received a lot of plant substances which resulted in the graft success. This finding was also reflected in the biochemical analysis of the rootstocks (rejuvenated shoots and young plants)

and leaves of the successful grafts on the rootstocks (rejuvenated and young plants). High levels of endogenous plant substances and minerals were recorded for the rejuvenated shoots. This could be attributed to the translocation of the plant substances which enhanced cell processes and consequently led to a graft union formation. Hartmann *et al.* (2002) reported that the presence of high sugar levels in rootstocks was an indication of the energy production and sustainability during the healing process. It also played a role in the basipetal translocation of other plant substances to the union zone for rapid cell division which culminates in successful graft formation. Axford (1974) compared the mineral nutrition of Cherry tree scions grafted onto different rootstocks and inferred that vigorously growing rootstocks easily permit the movement of endogenous plant substances to the grafting zone to promote graft success which is observed from the rejuvenated shoot. The finding could also be explained anatomically where the rejuvenated shoots showed complete cell differentiation, a feature not found in the young plant which showed only cell expansion. The observed cell differentiation in the rejuvenated shoots could be due to the presence of IBA that causes differentiation in the vascular tissues thereby leading to callus formation and subsequent wound healing i.e. graft success (Hartmann *et al*, 2002).

5.8 Determination of the best period for coppicing of shea to produce shoots for high grafting success.

Among the periods investigated, May/June produced the most developed shoots within sixty days after coppicing as well as the highest graft success.

These findings corroborates those of Yeboah *et al*. (2009a) who indicated that the best period for plant growth and development by vegetative propagation was May /June when rainfall was continuous and less destructive. Generally, during the May/June period the high moisture

status of the soils promote easy translocation of nutrients and other endogenous substances to the stump to enhance the development of more shoots (Luckwill and Cuttings, 1970) through nutrient dissolution for easy plant uptake (Hutcheon *et al*., 1973). Furthermore during this period, the moderate ambient temperatures (22^{o}C to 29^{o}C) prevent cell injury and death, enhance callus formation and restore carbohydrate levels for energy and further development of the cells to promote graft success (Hartmann *et al*., 2002).

Leaf analysis in the present study, over the same period, showed appreciable levels of nitrogen, zinc, phosphorus, manganese and magnesium which might have played certain roles in cell division after being absorbed by the rootstock. For example, the absorbed nitrogen could have been involved in protein synthesis and DNA production which promote graft formation (Jain and Nanda, 1972; Cakmak, 2000). Zinc could also have played a role in auxin synthesis which enhanced cell division (Hartmann *et al*., 2002). Phosphorus, on the other hand, could have aided the easy translocation of carbohydrate and other substances leading to a healthy union formation (Noggle and Fritz, 1983). Performing the same function, Manganese and Magnesium play a role in moderating the levels of IAA-oxidase which prevent the destruction of IAA (Hartmann *et al*., 2002). Recent research has indicated that some rootstocks of fruit vegetables may restrict the uptake of heavy metals (Arao *et al*., 2008; Rouphael *et al*., 2008; Mori *et al*., 2009) and therefore grafting fruit vegetable onto such rootstocks, may limit the heavy metal accumulation in the aerial parts, thereby mitigating their adverse effects on crop performance. Graft success depended on the rate at which shoots sprouted and matured on the stumps. The development of the sprouted shoots on the stumps indicated that growth was rapid and this depended on the nutrient status of the stump that developed the shoots. In the present study, the biochemical analysis of the developed shoots on the stumps showed a high concentration of nutrients and plant substances (nitrogen, proteins, simple sugars, total free phenols and IAA) which were responsible for energy

production, protection and cell division (Aloni, 1987; Yeoman and Brown, 1976). Anatomically, the shoots developed on the stumps had complete differentiated cells (xylem, cambium, phloem, pericycle, cortex and epidermis) which could have enhanced rapid wound healing. These observations could account for the positive relationship between sprouting and graft success.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS.

6.1 Conclusions

The results of the rooting experiment showed that girdled air-layered shoots with the application of 10,000 ppm IBA and covered with sphagmum moss in the wet season resulted in the highest rooting success. Selected shoots from trees with dense canopy gave better rooting than the open canopy. In the biochemical analysis, plant substances and nutrients such as sugars, total free phenols, nitrogen and phosphorus played important roles in the rooting success of air-layered shoots. To achieve good growth, propagules (rooted cuttings) should be weaned in the mist and pit propagating units in the nursery. A hole depth of 52 cm is suitable for transplanting rooted cuttings to achieve high survival as well as successful establishment and good growth.

The grafting results showed that grafting could be achieved by pre-curing (wounding) scions and grafting onto rejuvenated shoots developed from coppiced trees. This technique gave rapid with uniform branching and compact canopy formation. Grafting scions with partially sprouted buds onto rejuvenated shoots using the top cleft grafting technique gave high success and improved growth over a short period of time. Anatomically, wounded scions revealed complete rootstock/scion compatibility and advanced stages of cell differentiation that resulted in fast callus formation and rapid wound healing. The month May/June was the suitable period for coppicing tress to produce shoots and enhanced high graft success and was biochemically characterized by the production of high levels of plant substances and nutrients such as auxins, protein, sugars, phenols, nitrogen, phosphorus, zinc, magnesium and manganese which enhanced the growth and development of successful grafts.

6.2 Recommendations for future research.

1. Growth enhancement studies may be carried out aimed at reducing the period which would make the seedling ready for grafting for the production of clonal materials for plantation establishment.

2. Growth regulators at different levels and in combination with each other need to be applied under different environmental conditions to assess the possibility of early fruiting of the shea nut tree.

3. Agronomic studies such as intercropping with food crops and application of manures/fertilizers could be carried out to determine their effects in reducing the gestation period of the shea nut tree to make its cultivation attractive to farmers.

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