Beyond vegetative propagation of indigenous fruit trees: case of *Dacryodes edulis* (G. Don) H. J. Lam & *Allanblackia floribunda* Oliv.

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Beyond vegetative propagation of indigenous fruit trees:
case of *Dacryodes edulis* (G. Don) H. J. Lam
and *Allanblackia floribunda* Oliv.

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Front cover photographs by Ebenezar Asaah

Front cover photos: (top), old fruiting *Dacryodes edulis* tree and a young, 4 years old fruiting stem cutting intercropped with maize, and (below) an old fruiting *Allanblackia floribunda* tree and a young graft of *A. floribunda* carrying its first fruit after 4 years

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DEDICATION

To the glory of God,

To my parents (Pa Peter Fonyam & Ma Esther Angwi Asaah),

who never lived to see the fruits of their labour, and

To all tree crop domesticators and farmers from whom I have always drawn my inspiration.

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LIST OF ABBREVIATIONS

AFTP:	Agroforestry Tree Products
AGB:	Above Ground Biomass
AIDS:	Acquired Immune Deficiency Syndrome
ANODE:	Analysis of deviance
ANOVA:	Analysis of variance
BGB:	Below Ground Biomass
C:	Carbon
CARPE:	Central Africa Environment Programme
CFA:	Financial Cooperation in Central Africa (*Coopération financière en Afrique centrale*)
CO_2:	Carbon dioxide
DBH:	Diameter at Breast Height
DRC:	Democratic Republic of Congo
EU:	European Union
FAO:	Food and Agricultural Organization
GHG:	Green House Gases
GLM:	General Linear Model
HIV:	Human Immunodeficiency Virus
IAA:	Indole Acetic Acid
IAASTD:	Assessment of Agricultural Knowledge, Science and Technology for Development
IBA:	Indole-3-Butyric Acid

ICRAF:	World Agroforestry Centre
IPCC:	Intergovernmental Panel on Climate Change
IUCN:	International Union for the Conservation of Nature
LULUCF:	Land Use, Land-Use Change and Forestry
MDG:	Millennium Development Goal
PPP:	Private Public Partnership
PROTA:	Plant Resources of Tropical Africa
RD:	Root Density
RH:	Relative Humidity
RLD:	Root Length Density
RS:	Root to Shoot ratio
RWD:	Root Weight Density
Sed:	Standard error of difference of means
SNV:	The Netherlands Development Organization
ST:	Side tongue
ST-Al:	Side tongue + aluminium foil
ST-NPP:	Side tongue + non-perforated plastic
ST-PP:	Side tongue + perforated plastic
TREE:	Tropical forest cover monitoring in the humid tropics – TREES project
UNDP:	United Nations Development Programme
VPD:	Vapour Pressure Deficit
WWF:	World Wide Fund for Nature

ABSTRACT – RESUME – SAMENVATTING

Abstract

The importance of domesticating and integrating high value fruit trees in agricultural landscapes by farmers is increasingly being recognized in the humid tropics. However in an agroforestry context, the growth attributes of intercropped trees above and belowground determine whether the association will be complementary or competitive. The focus of the present research was on two African indigenous fruit tree species (*Dacryodes edulis* G. Don) H. J. Lam (Burseraceae) and *Allanblackia floribunda* Oliv. (Clusiaceae), which are currently under domestication, and have high food, income and environmental security values. On *D. edulis,* the thrust was to assess and compare the structural and fine rooting systems together with the aboveground growth attributes of fruiting trees propagated sexually and vegetatively, while on *A. floribunda,* the focus was on how to reduce the long juvenile phase of about 10-12 years before first fruiting to less than 5 years through grafting techniques.

The results of the investigations on *D. edulis* indicate that trees of seed origin were characterized by a tap root, reaching depths of about 1.20 m, whereas, trees of cutting origin showed three strong vertical roots ($d > 5$ mm) with the longest reaching depths of 1.31 m. Similarly, trees of marcot origin were observed to have thick, relatively short prominent vertical roots, reaching depths of 1.15 m. In addition, trees of seed origin had greater fine root density (RD) ($P \leq 0.001$) than trees of vegetative origin (cuttings and marcots) in the upper soil stratum (0-30 cm). Trees of seed origin were also shown to have an exponential distribution pattern for fine root density and root length within depth to 80 cm. In contrast, the distribution pattern of fine roots of trees of vegetative origin (cuttings and marcots) were quadratic for the same variables which increased in the 20-30 cm soil depth stratum before declining steadily to a depth of 80 cm. Furthermore, shoot density, defined as, number of shoots per tree, and height differed significantly ($p=0.004$ and $p=0.005$, respectively) amongst tree origins. Trees of seed and cutting origins had single-stem shoots whereas marcots had 6 shoots per tree on average. Trees of cutting origin grew tallest, with mean height of 8.4 ± 2.2 m compared to 6.7 ± 0.9 m and 7.6 ± 1.9 m for trees of marcot or seed origins respectively. Mean carbon (C) sequestration estimated using allometric models differed significantly ($p= 0.014$) between trees of vegetative origin and those of seed origin, with 10 years old *D. edulis* trees of cutting and marcot origins sequestrating averagely 26.8 ± 19.1 Mg C ha^{-1} and 21.74 ± 12.8 Mg C ha^{-1} respectively over 10 years, compared to 13.10 ± 9.4

Mg C ha^{-1} for trees of seed origin. In summary, the results from the studies on *D. edulis* suggest that vegetative propagation on the species, besides reducing the long juvenile phase to fruiting and maintaining trueness in the transfer of desirable traits over generations, also results in trees that are apparently less competitive for belowground resources. In addition, vegetative propagated trees of *D. edulis* were shown to have apparently stable root system and the trees bigger in stature and higher in carbon sinks than trees of seed origin.

In parallel, *A. floribunda* scions were taken from female trees, and grafted onto rootstocks using side tongue, top cleft, side veneer, and whip-and-tongue methods under nursery conditions. In a separate experiment, side tongue and inverted 'T' budding methods were also tested *in situ* on young *A. floribunda* wildings growing under semi-deciduous and evergreen tree covers. In addition, the effects of protecting side tongue grafts with non-perforated, translucent plastic, perforated translucent plastic and aluminium foil were assessed. *A. floribunda* was shown to be amenable to grafting both under nursery and field (*in situ*) conditions. Under nursery conditions, side tongue grafts were significantly more successful (80.0 ± 6.3 %), than grafts of side veneer (52.5 ± 7.9 %), top cleft (55.0 ± 7.9 %) and whip–and-tongue (50.0 ± 7.9 %). The success of side tongue grafts was further increased (86.7 ± 6.2 %) under evergreen shade when grafts were protected by non-perforated translucent plastic. A grafted *A. floribunda* tree transplanted in the field in 2007 flowered and carried a single fruit to maturity after 4 years, thereby reducing the long juvenile period to first fruit production of about 10-12 years reported in literature to less than 5 years.

In conclusion, the effect of propagation methods on the belowground and aboveground growth attributes and their C storage potential studied in the present research on *D. edulis*, have not yet been studied and published anywhere according to the authors knowledge. Similarly, the reduced long juvenile period to first fruit production to less than 5 years in *A. floribunda* grafts reported in this research is the first published prove of concept on this species according to the author's knowledge. Therefore, the research results presented in this thesis should be considered as pilot case studies aimed at obtaining insights into the effect of vegetative propagation methods on the above and belowground growth and development of improved planting materials of these two African indigenous fruit trees (*Dacryodes edulis* and *Allanblackia floribunda*) under domestication.

Key words: *Allanblackia floribunda*, carbon sequestration, cuttings, *Dacryodes edulis,* diversification, domestication, fine roots, grafting, growth attributes, macrots, structural roots, tree of seed origin, vegetative propagation.

Résumé

L'importance de la domestication et de l'intégration des arbres fruitiers dans les systèmes agroforestiers par les producteurs est très reconnue dans la zone des tropiques humides. Cependant dans un contexte agroforestier, les attributs de croissance souterraine et aérienne des arbres associés déterminent si l'association sera complémentaire ou compétitive. La présente étude s'est focalisée sur deux espèces fruitières africaines indigènes de grande valeur nutritive, alimentaire et environnementale qui sont *Dacryodes edulis* (G. Don) H. J. Lam (Burseracée) et *Allanblackia floribunda* Oliv. (Clusiacée) et dont le processus de domestication est en cours. Sur *D. edulis*, le travail à consisté à étudier et à comparer d'une part les systèmes de distribution des grosses (d ≥ 2 mm) et fines (d < 2 mm) racines et d'autre part les attributs de croissance des parties aériennes des arbres produits par multiplication générative et végétative. Sur *A. floribunda*, l'étude s'est focalisée sur la façon de réduire la longue période juvénile (avant la première fructification) qui varie de 10 à 12 ans à moins de 5 ans en passant par la technique de greffage.

Les résultats des investigations sur *D. edulis* indiquent que les arbres issus des semis ont été caractérisés par des racines pivotantes qui vont jusqu'à 1,20 m de profondeur, tandis que les racines des plants issus des boutures montrent 3 racines verticales (d > 5 mm) solides qui peuvent s'allonger jusqu'à 1,31 m. Similairement, les arbres issus des marcottes portent des racines épaisses relativement courtes et verticales pouvant atteindre 1,15 m de profondeur. En plus, les arbres issus du semis ont une densité de fines racines significativement plus grande (RD) (p ≤ 0,001) que ceux des arbres issus de la multiplication végétative (boutures et marcottes) dans des strates supérieures du sol (0 – 30 cm). Les arbres issus de semis ont aussi montré une distribution exponentielle de la densité des racines fines et de la longueur des racines dans les 80 premiers centimètres du sol. Par contre, la distribution des racines fines des arbres issus de la multiplication végétative (boutures et marcottes) a été quadratique pour les mêmes variables qui augmentent dans les 20 à 30 premiers centimètres du sol avant de diminuer de façon régulière jusqu'à la profondeur 80 cm. En outre, la densité de rejets, entendu par nombre de rejets par arbres et la hauteur diffèrent significativement (p = 0,004 et p = 0,005 respectivement) entre les origines des arbres. Les arbres issus des semis et des boutures ont une seule tige (rejet) tandis que les marcottes ont 6 rejets en moyenne par arbre. Les arbres issus des boutures vont plus haut, avec une moyenne de 8,4 ± 2,2 m comparé à 6,7 ± 0,9 m et 7,6 ± 1,9 m pour les arbres issus du marcottage et du semis respectivement. Les

quantités moyennes de carbone (C) séquestrées est estimées par les modèles allométriques diffèrent significativement (p = 0.014) entre les arbres issus de la multiplication végétative et ceux issus du semis. Les arbres de 10 ans des boutures et des marcottes séquestrent respectivement en moyenne 26,8 ± 19,1 Mg C ha-1 et 21,74 ± 12,8 Mg C ha-1, comparé à 13,10 ± 9,4 Mg C ha-1 pour les arbres issus des semis. En résumé, les résultats de ces études sur *D. edulis* évoquent que la multiplication végétative en plus du fait qu'elle réduit la phase juvénile de fructification ,permet le maintien du patrimoine héréditaire d'une génération à l'autre, elle donne aussi des arbres apparemment moins compétitifs pour les éléments nutritifs du sol et a un système racinaire plus stable, une stature plus grande et une quantité de carbone séquestrée plus importante que celle des semis.

En parallèle, les greffons d'*A. floribunda* ont été récoltés sur les arbres femelles et greffés sur les porte-greffes en utilisant les techniques de fente de côté, de couronne, de l'anglaise simple et de l'anglaise compliquée. Dans un autre essai, les techniques de fente de côté et de « T » inversé ont été testées *in situ* sur les jeunes *A. floribunda* qui poussent en milieu réel sous un couvert d'arbres semis décidus. En plus, l'effet de la protection du greffage en fente de côté à l'aide d'un plastique transparent non perforé, d'un plastique transparent perforé et de l'aluminium a été déterminé. Il a été montré qu'*A. floribunda* est favorable au greffage aussi bien en pépinière qu'en champ *(in situ)*. En pépinière, le greffage en fente de côté réussit significativement (80,0 ± 6.3 %) plus que le greffage à l'anglaise simple (52,5± 7,9 %), le greffage en couronne (55,0 ± 7,9 %) et le greffage à l'anglaise compliqué (50,0 ±7,9%). Le taux de réussite du greffage en fente de côté a été plus élevé (86,7 ± 6.2 %) sous couvert forestier sempervirent sur les greffes couverts par les plastiques transparents non perforés. Un plant issu du greffage d'*A. floribunda* transplanté au champ en 2007 a déjà fleuri et a donné un fruit qui est arrivé à maturité. Ce qui justifie la réduction de la longue période juvénile de 10-12 ans (telle que citée dans la littérature) à moins de 5 ans.

En conclusion, l'effet des méthodes de propagation sur les attributs de la croissance souterraine et aérienne et de leur potentiel de stockage du carbone étudié ici sur *D. edulis* n'a pas encore fait l'objet d'une étude ou d'une publication quelconque selon la littérature disponible. Similairement, la réduction de la période juvénile d'*A. floribunda* à moins de 5 ans par le greffage rapporté dans cette étude est la première publication qui prouve ce constat sur cette espèce. A cet effet, les résultats présentés dans cette thèse pourraient être considérés comme un cas pilote visant à obtenir un aperçu de l'effet des méthodes de

multiplication végétative sur la croissance souterraine et aérienne du matériel amélioré de ces deux espèces fruitières indigènes d'Afrique (*Dacryodes edulis* et *Allanblackia floribunda*).

Mots clés : *Allanblackia floribunda*, arbre issu de semis, attribut de croissance, boutures, *Dacryodes edulis*, domestication, fine racine, greffage, marcotte, multiplication végétative, séquestration du carbone, structure racinaire.

Samenvatting

Het belang van de domesticatie en de integratie door landbouwers van hoogwaardige fruitbomen in landbouwgebieden wordt in toenemende mate erkend in de vochtige tropen. De associatie in een agroforestry context kan echter complementair of competitief zijn afhankelijk van zowel de boven- als ondergrondse groeikenmerken van de tussengeteelde bomen. Voorliggend onderzoek focust op twee inheems Afrikaanse fruitboomsoorten (*Dacryodes edulis* G. Don) H. J. Lam (Burseraceae) en *Allanblackia floribunda* Oliv. (Clusiaceae), die momenteel worden gedomesticeerd en een grote nutritionele, economische en ecologische waarde hebben. Bij *D. edulis* werd gepoogd om de structurele haarwortels evenals de bovengrondse groeikenmerken bij generatief en vegetatief vermeerderde bomen te beschrijven en te vergelijken. Bij *A. floribunda* werd getracht om aan de hand van enten de lange juveniele fase van ongeveer 10 tot 12 jaar voor de eerste vruchtzetting, te reduceren tot minder dan 5 jaar.

De onderzoeksresultaten op *D. edulis* tonen aan dat bomen die uit zaad werden vermeerderd, gekenmerkt worden door een penwortel met een diepte tot 1,20 m, terwijl bomen die door stekken werden vermeerderd drie sterke vertikale wortels vertoonden (d > 5 mm), waarbij de langste tot 1,31 m diep reikte. Ook de gemarcoteerde bomen hadden dikke, relatief korte en prominente, vertikale wortels tot een diepte van 1,15 m. Daarenboven hadden bomen die uit zaad werden vermeerderd een hogere dichtheid aan haarwortels in het bovenste stratum (0 – 30 cm) ($P \leq 0{,}001$) dan de vegetatief vermeerderde bomen (stekken en marcoteringen). Het haarwortelstelsel van de generatief vermeerderde bomen vertoonden een exponentieel verdelingspatroon en een wortellengte tot een diepte van 80 cm. Het verdelingspatroon van de haarwortels van de vegetatief vermeerderde bomen echter, was kwadratisch waarbij de dichtheid en de lengte toenam in het stratum van 20 – 30 cm diepte, maar dan geleidelijk aan daalde tot een diepte van 80 cm. Bovendien verschilden de scheutdichtheid, gedefinieerd als het aantal scheuten per boom, en de hoogte significant ($p = 0{,}004$ en $p = 0{,}005$, respectievelijk) tussen de vermeerderingsoorsprongen van de bomen. De bomen die door middel van stekken werden vermeerderd werden het hoogst; gemiddeld 8,4 ± 2,2m in vergelijking met 6,7 ± 0,9 m en 7,6 ± 1,9 m voor gemarcoteerde bomen en generatief vermeerderde bomen respectievelijk. De gemiddelde koolstof (C) sequestratie, geschat aan de hand van allometrische modellen, verschilde significant ($p = 0{,}014$) tussen vegetatief en generatief vermeerderde bomen, waarbij

tienjarige, gestekte en gemarcoteerde *D. edulis* bomen respectievelijk gemiddeld 26,8 ± 19,1 Mg C ha-1 en 21,74 ± 12,8 Mg C ha-1 sequestreerden. De resultaten van het onderzoek op *D. edulis* suggereren dat vegetatieve vermeerdering van de soort resulteert in een kortere juveniele fase, behoud van gewenste kenmerken in de overgang tussen verschillende generaties; in grotere bomen die minder competitief zijn voor ondergrondse grondstoffen, met een stabieler wortelstelsel en die een grotere carbon sink vormen dan bomen die door middel van zaad werden vermeerderd.

Terzelfdertijd werden enten van *A. floribunda* genomen van vrouwelijke bomen en geënt op onderstammen in kwekerijen door middel van zijdelings enten, spleetenten, zijdelingse plakent en de verbeterde engelse griffel. In een ander experiment werd zijdelings enten en omgekeerde 'T'-oculatie in situ toegepast op jonge, wilde A. floribunda bomen die groeiden onder semi-bladverliezend en immergroen bladerdek. Bovendien werd het effect nagegaan van de bescherming van de zijdelingse enten met ongeperforeerd, doorzichtig plastiek, met geperforeerd, doorzichtig plastiek en met aluminiumfolie. Het enten van *A. floribunda* bleek succesvol zowel in de kwekerij als in het volleveld (in situ). In de kwekerij had zijdelings enten significant meer succes (80,0 ± 6,3 %) dan zijdelings plakenten (52,5 ±7,9 %), spleetenten (55,0 ± 7,9 %) en verbeterde engelse griffel (50,0 ± 6,3 %). Het welslagen van het zijdelings enten verhoogde bovendien (tot 86,7 ± 6,2 %) onder immergroen bladerdek wanneer de enten werden beschermd door ongeperforeerd, doorzichtig plastiek. Geënte *A. floribunda* bomen die in 2007 in het veld werden overgeplant, bloeiden en vertoonden vruchtzetting en rijpe vruchten na 4 jaar, wat de lange juveniele fase van 10 tot 12 jaar, zoals vermeld in de literatuur, aanzienlijk reduceerde.

Het effect van de vermeerderingsmethodes op de boven- en ondergrondse groeikenmerken en het opslagpotentieel voor C van *D. edulis*, zoals beschreven in deze studie, werd voor zover gekend niet eerder bestudeerd noch gepubliceerd. Dit geldt ook voor de studie naar de reductie van de lange juveniele fase tot de eerste vruchtzetting tot minder dan 5 jaar bij het enten van *A. floribunda*. De hier gepresenteerde onderzoeksresultaten moeten bijgevolg worden beschouwd als piloot- en gevalstudies die tot doel hadden om inzichten te verwerven in het effect van de vegetatieve vermeerdering op de onder- en bovengrondse groei en de ontwikkeling van verbeterd plantmateriaal bij deze twee inheemse, Afrikaanse fruitsoorten (*Dacryodes edulis* en *Allanblackia floribunda*).

Key words: *Allanblackia floribunda*, koolstofsequestratie, stekken, *Dacroydes edulis*, diversificatie, domesticatie, haarwortels, enten, groeikenmerken, marcoteren, structurele wortels, generatieve vermeerdering, vegetatieve vemeerdering.

PHD THESIS OUTLINE

The research work reported in this thesis focused on two indigenous fruit tree species in Africa (*D. edulis* and *A. floribunda*) and is presented in two parts divided into eight chapters. The first part of the thesis is dedicated to work on *D. edulis* and the second part centres on *A. floribunda*.

The first chapter introduces the study and covers the background of the study in terms of current trends in natural resource degradation and the need to adapt current agricultural practices to produce more crops from less land as the single most significant way of jointly achieving mitigation and adaptation to projected climatic changes. The need for agroforestry and the domestication of indigenous fruit trees to promote food, income and employment, and environmental services is also discussed. The concepts of tree domestication and vegetative propagation techniques as used in the improvement of indigenous fruit trees are also reviewed. The chapter ends with the thesis problem statement, and the main objectives of the research conducted within the framework of this thesis.

The first part of the thesis begins from chapter two, which is a monograph on *D. edulis.* It reviews information on the socio-economic values, botany, ecology, distribution, propagation, cultivation, domestication status, and research needs of *D. edulis*. The chapter ends with research questions on below and aboveground growth and development of *D. edulis* trees of seed and vegetative origins. The experimental work on below and aboveground growth attributes that constitutes the core of the research conducted on *D. edulis,* is presented in chapters 3, 4, and 5. The effect of propagation methods on structural root development of *D. edulis* trees of seed and vegetative (cutting and marcot) origins, and their implication on relative stability of the different tree types are presented in the third chapter. Fine root distribution as affected by propagation methods of *D. edulis* trees of seed and vegetative origins and their relative potential complementary/competitive nature for belowground resources within an agroforestry context is developed in the fourth chapter. The fifth chapter presents the aboveground growth attributes of *D. edulis* trees of seed and vegetative origins as affected by propagation methods, and quantifies through allometric regression models, carbon stored as live biomass in both above and belowground parts of the trees over 10 years.

The second part of the thesis begins with chapter 6, with a review on the socio-economic values, botany, ecology, distribution, propagation, domestication status and research needs of *A. floribunda.* The research focus on this species was to develop a suitable vegetative propagation method that could reduce the long juvenile period to fruiting in *A. floribunda*. The amenability of *A. floribunda* to grafting technique is developed in the seventh chapter. The results obtained from the grafting experiments are discussed and their implications for the domestication of the species are presented in the same chapter. The eighth and final chapter of the thesis starts with a summary of the findings with general discussions on *D. edulis* rooting characteristics and aboveground growth attributes as affected by propagation methods. Similarly, a summary of the findings and comments on the amenability of *A. floribunda* to grafting are presented. The chapter ends with a general conclusion, recommendations and suggestions for further research work on both *D. edulis* and *A. floribunda*.

CHAPTER 1

1 GENERAL INTRODUCTION

1.1 Background

Despite a considerable increase in global food production over the last few decades, the world's efforts to meet the Millennium Development Goal (MDG) of reducing hunger by half by the year 2015 appears to be beyond reach. According to UN estimates (FAO, 2009), the number of people suffering from chronic hunger has increased from under 800 million in 1996 to over a billion in 2009 with most of the world's hungry people residing in South Asia and Sub-Sahara Africa. These regions have large rural populations, widespread poverty and extensive areas of low agricultural productivity due to steadily degrading natural resource bases, weak markets and high climatic risks. Most of these rural poor people are also dependent on forests, remnant woodlands, homestead trees, and on mixed agricultural and forestry production systems for their subsistence, fuel wood, food and fodder needs (FAO, 2009). Global gross forest cover loss was estimated to be 1,011,000 km^2 between the years 2000 and 2005, representing a 3.1% loss per year (Hansen et al., 2010). At continental level, Africa suffered the second largest net loss in forests with 4.0 million hectares cleared annually, largely due to subsistence activities with Nigeria and Sudan being the two largest losers of natural forest during the 2000-2005 period (Butler, 2005).

According to a recent study of deforestation in Central Africa, Duveiller et al. (2008), estimated that between 1990 and 2000, net annual deforestation rate in Cameroon equalled 0.14 %, net deforestation being the difference between average yearly gross deforestation (0.20 %) and gross reforestation (0.06 %). Although this rate of deforestation puts Cameroon in second place after Democratic Republic of Congo (DRC) in the Congo Basin, it generally remains low, as does the average annual deforestation rate in the Congo Basin (0.16 %). These figures, however, differ from FAO estimates for Cameroon, which assign an annual rate of deforestation of 1% (FAO, 2007). This difference apparently stems from the fact that Duveiller et al. (2008), only considered deforestation data from the dense forest areas of Cameroon; mainly in the south and east regions. These two regions are sparsely populated with 7 to 13 inhabitants per km^2 (Neba, 1999). Meanwhile, FAO's figures were based on deforestation data of all woody vegetation types (savanna, suburban forest and forest-savanna mosaics) in Cameroon. The mountainous forest-savanna transition zones of Cameroon (Figure 1.1) have high population densities exceeding 150 inhabitants per km^2 with a very active farming sector and a corresponding higher rate of deforestation than what is obtained in dense forest areas (Neba, 1999). The main driver to deforestation in these areas is agriculture, which accounts for more than 80 % of forest cover loss in Cameroon (CARPE, 2005). This includes not only slash-and-burn shifting cultivation, long recognized as a key driver of deforestation in the dense forest areas (Ndoye and Kaimowitz, 2000), but also cash cropping with most notably cocoa (*Theobroma cacao)* (Sunderlin et al., 2000). Cocoa has been a major cash crop promoted by the government of Cameroon since independence up to the 1980s with considerable impact on forest integrity in Cameroon (World Bank, 2008 cited by Eba'a Atyi et al., 2009). According to the same report, cocoa cultivation was less profitable in the 1990s as a result of less attractive global market prices. However, with the current trend, Cameroon revitalized cocoa planting since 2005 at the expense of forests.

Land degradation is one of the most serious problems facing global agriculture as it affects two billion hectares (38% of the world's cropland). Consequently, many smallholder farmers in the tropics are trapped in poverty and hunger, together with malnutrition (Leakey and Asaah, In press). With little, if any, land remaining for the expansion of farming, the only option is to use the available land more efficiently.

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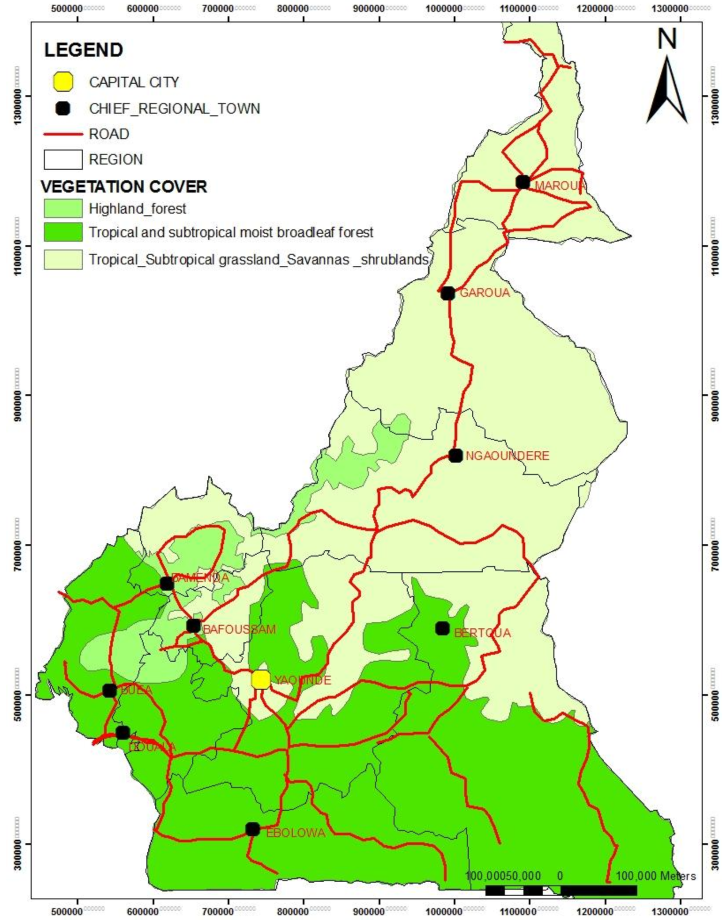

Figure 1.1 Vegetation map of Cameroon

Source: Redrawn from WWF Biomes of Cameroon, 2006 (by Precilia Ngaunkam)

For example, Africa is the only continent where cereal production per capita has steadily decreased since the early 1960s (St. Clair and Lynch, 2010).

It is against this background that agricultural products and agro-ecosystem services (these are the collection of physical, environmental, economic and social factors that affect a cropping enterprise) will continue to be threatened as human demands on these functions continue to mount. Four sources of threats to agro-ecosystem functions can be distinguished. The first threat originates from competition for other land uses – urbanization and especially expanding croplands. Second are the threats related to growing global demand for wood. Third are threats exogenous to forests such as climate variability and loss of biodiversity; and lastly the abandonment of degraded land. Faced with the fierce competition between needs for forest products and increases in agricultural land, it is essential to produce more food through intensification and diversification, rather than expansion of currently available arable land area. This postulation was one of the conclusions of the International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD) in a recent review on the state of global agriculture *vis-à-vis* sustainable rural development (McIntyre et al., 2009). According to this report, economic, social and environmental sustainability could be achieved if agriculture were redirected towards multifunctionality (Kiers et al., 2008) in recognition of the 'inescapable interconnectedness of agriculture's different roles and functions'. IAASTD recognized the functions of multifunctional agriculture to be: the production of food and non-food commodities; delivery of environmental services; the improvement of rural livelihoods, and the upholding of traditional crops and local culture. Thus, if multifunctional agriculture were to be adopted on a large scale, future agriculture will be as much about enhancing livelihoods, health and nutrition of rural households and restoring natural capital, as about increasing food security and economic growth.

Agroforestry is a delivery mechanism of multifunctional agriculture (Leakey, 2010) as the latter allows to and actually does better address the issues of: declining soil fertility, the rehabilitation of degraded land; restoration of above and belowground biodiversity; sequestration of carbon; and protection of soils and watersheds. Additionally, agroforestry provides unique outputs not often provided by other systems which include but is not limited to: (i) provision of indigenous products from underutilized species for food and markets to generate income and enhance nutritional/health status; (ii) restoration of integrated agro-ecosystems of diverse plant species with the potential of developing into natural woodlands and forests; and (iii) maintain linkages with human cultures through the availability of traditional foods and other products important to local people (Leakey, 2010; Termote et al., 2011).

In agroforestry, the domestication of underutilized and indigenous trees was initiated in the mid 1990s by the World Agroforestry Centre (ICRAF) and its partners. This programme aimed at improving the quality and yield of products from traditionally important species that used to be gathered from forests and woodlands. In addition to meeting the everyday needs of local people, these products are widely traded in local and regional markets. Underutilized crops therefore have the potential to become new cash crops for income generation and to counter malnutrition and disease by diversifying staple food/food energy sources and dietary uptake of micro-nutrients that boost the immune system among others. These indigenous tree species also play an important role in enhancing agro-ecological functions and, through carbon sequestration, can help to counter climate change.

1.2 Plant species domestication

Domestication is a complex process akin to evolution in which human use of plant and animal species leads to morphological and physiological changes that create a distinction between domesticated taxa from their wild ancestors (Purugganan and Fuller, 2009). Domestication can also be viewed as the selection process conducted by humans to adapt plants and animals to the needs of humans, whether as farmers or consumers (Gepts, 2002). Interestingly, the process of domestication has been conducted for more than 13,000 years (i.e., since the last ice age), and occurred independently in several regions (Gepts, 2002). Purugganan and Fuller (2009) recognise at least six regions of domestication, including Mesoamercia, the southern Andes (including the eastern piedmonts), the Near East, Africa (probably the Sahel and the Ethiopian highlands), Southeast Asia and China.

In spite of the geographic and climatic diversity in domestication centres, there is a remarkable similarity in the set of traits which were used to select completely unrelated types of crops (Table 1.1). According to Gepts (2004), many seed-propagated crops under domestication have been selected mainly for their traits in seed dispersal and dormancy. The same author maintains that domesticated varieties are characterized by a lack of seed dispersal at maturity. For example, in cereal crops, selection is focus on individual plants with a tough rachis that prevent the disarticulation of the inflorescence and the release of seeds. Whereas, in the wild relatives, an abscission layer is formed between each successive seed insertion site such that at maturity, this layer causes the rachis to break and subsequently the dispersal of seeds. Furthermore, seeds of varieties under domestication exhibit little of no dormancy compared to the wild relatives. While dormancy in the seeds from wild relatives prevent premature germination during adverse growth conditions like drought, the lack of seed dormancy in varieties under domestication will on the contrary promote uniform germination and population, hence uniform harvest.

In perennial crops, the selection of individuals under domestication is focused towards individuals with a more compact growth habit, having fewer and shorter branches as opposed to tall single stem wild relatives. All of these changes in plant growth habit have been report to have a positive impact on the harvest index (ratio of harvested part to overall aboveground biomass) and size of the fruit or grain (Donald, (1968) cited by Gepts (2004)). Another feature that has widely been reported (Elias and Mckey, 2000; Gepts, 2004) to have change in crop evolution is the reproductive system. The latter authors emphasize that the reproductive system of most species under domestication has changed towards increase selfing (as in tomatoes and peppers) or a replacement of sexual reproduction by vegetative propagation in order to maintain trueness in type of cultivated individuals faced with possibilities of outcrossing with wild relatives. The fruits of such individuals could be more tasty as the case in *Chysophyllum ciaimito* in Panama where fruit from trees under domestication were found to have much pulp, less acid, larger in size and higher in sugar content as compared to those obtain with wild trees (Parker et al., 2010). Waruhiu et al. (2004) also reported that *D. edulis* fruits from trees on farms in Cameroon and Nigeria were 66% larger than those obtain from trees in the wild.

The repeated independent selection of similar 'desirable traits' in different crops under domestication that have render them unable to grow and reproduce successfully for more than a few seasons in natural environments, away from the care of humans who provide adequate management to reduce competition from pest and diseases, these shared traits (seed dispersal, seed dormancy, gigantism in harvest parts, determinate and synchronized growth, increased harvest index and change in flavour) have been recognized and described as 'domestication syndrome' (Darwin, 1859; Gepts, 2004; Hammer, 1984 and Harlan, 1992). According to Harlan (1992), the origins of these desirable traits were probably spontaneous mutations occurring in wild plant populations. These traits selected by man at various growth stages of wild plants, as well as after harvest could have been deleterious in the wild (Gepts, 2002). Hence, fully domesticated crops can have difficulties surviving in the wild without human intervention. Partially domesticated crops (i.e. those that exhibit some but not all of the above-mentioned domestication syndrome traits) show an exception to this. Among these partially domesticated species are fruit trees like apples (*Malus domestica*) in which it is sometimes difficult to distinguish between wild and natural populations (Ladizinsky, 1999).

A tree is normally considered 'wild' when it grows spontaneously in self-maintaining populations in a natural or semi-natural ecosystem and when it can exist independently of direct human action (FAO, 1999). In contrast it is considered to be domesticated when it has been selected purposefully for specific genetic characteristics, and when it is propagated and cultivated in managed agro-ecosystems (Leakey and Newton, 1994). Crop domestication has been limited to less than 0.05% of all plant species and about 0.5% of edible species (Leakey and Tomich, 1999). According to FAOStat (2010), out of a total of 400,000 flowering plants species, less than 200 have been domesticated as food and feed plants, and just 12 species provide 75% of the food eaten. The domestication of oranges and apples for instance, goes back about 3,000 years in China and central Asia, respectively (Simmonds, 1976). Domestication gave rise to food surpluses, and led to craft specialisation, and the development of arts, social hierarchies, civilizations and the origin of states (Purugganan and Fuller, 2009).

Domestication could therefore be considered as a multidimensional process in which a progressively closer interaction between people and plant resources takes place (Table 1.2). Most studies on the domestication of fruit trees till date have focused on the selection of desirable tree characteristics and their capture by vegetative propagation practices (Leakey and Newton, 1994; Leakey et al., 1996), or by their selection and subsequent breeding of superior individuals (Roshetko and Evans, 1999). Other studies however view domestication as essentially involving a process of change in exploitation systems (Michon and De Foresta, 1996; Wiersum, 1996; Michon, 2005). This line of thought considers the domestication process of perennial species as co-evolutionary between species and production systems. While the domestication of agricultural crops required 'open field' environmental conditions, a tree domestication process does not necessarily require similar conditions. This is due to the ecological nature of tree species which are basically forest species rather than 'open field' species. In addition, trees have a long juvenile phase, are obligate outcrossers, experience high rate of intra and inter specific gene flow and frequently reproduces both sexually and asexually (Vallejo-Marim et al., 2010). Thus, the route to tree domestication involves a gradual modification of production systems and/or conditions (from forest to cultivated fields) (Michon and De Foresta, 1997; Wiersum, 1997).

Table 1.1 Domestication syndrome traits

	Trait selected		
Selection stage	**General**	**Specific trait**	**Crop examples**
Seedling	Increase in seedling vigour	Loss of seed dormancy	Many crops Tomatoe Cassava Maize Legumes
Reproductive system	Increase selfing (multiplication) rate	Loss of seed dispersal	Fruit trees
	Amenability to vegetative propagation	More compact growth habit	Wheat, barley, maize
		Increase in number or size of inflorescences	Maize, amaranth
		Increase in number of seeds per inflorescence, pulp volume, oil content/quality, active ingredients	Legumes, rice, tree crops
		Change in photoperiod sensitivity	Many crops
		Colour, size, taste, texture	
Harvest or post-harvest	Increase in yield and shelf life Consumer appeal	Reduction in toxic substances	Cassava, lima bean etc..

Source: Adapted from Harlan (1992) and Gepts (2002)

As a consequence, the domestication process of fruit and nut trees should not only include changes in tree characteristics but also changes in forest ecosystems and human-plant interactions.

Table 1.2. Crop domestication in the sense of a process of increasing people-plant interaction

1. Modification of a plant's biological characteristics
   - human-induced change in a plant's morphological characteristics and genetic make-up
2. Modification of the biophysical environment in which a plant is growing in order to stimulate its production through:
   - homogenization of a species population composition by selective removal of undesirable species and stimulation of desirable species;
   - control of pest and diseases;
   - homogenization and enhancement of the physical growth conditions.
3. Acculturation of a crop to a social management environment through:
   - increase adaptation of crop species to human needs;
   - incorporation of a species in a human-controlled production environment through the formulation of access rules for crop utilization and management.

Source: Wiersum, (2007)

One of the main challenges in agroforestry is to address the lack of genetically superior seed sources for tree propagation (Simons, 1996). In this respect any domestication effort of a species has to involve making a decision as to whether to use sexual or vegetative propagation to achieve tree seedling production and improvement of tree, fruit and nut characteristics. In forestry research, it is typical to find foresters adopting seed-based breeding approaches, while in horticulture, it is common to find horticulturists adopting clonal propagation and the development of cultivars (Bhojwani and Razdan, 1996; Leakey and Akinnifesi, 2008). Mckey et al. (2010), emphasised that perennial plants under domestication are often propagated clonally, which in addition to reducing their long juvenile phase to fruiting, further decreases the number of sexual cycles separating domesticated individuals from their wild progenitors.

When Simons and Leakey (2004) advanced a definition of domestication used for agroforestry trees, they proposed that it should encompass both the socio-economic and biophysical processes involved in the identification and characterisation of germplasm resources; the capture, selection and management of genetic resources; and the regeneration and sustainable cultivation of the species in managed ecosystems.

Domestication is reportedly stimulated, when demand for certain species products exceeds supply (Leakey and Asaah, In press). Sometimes, modern agriculture that clears land for monocultures can exacerbate the process. This probably explains the recent interest in domesticating tree crops from wild forest species in the tropics, as deforestation has increased in proportion to population growth. In Cameroon for example, forest is being destroyed at an annual rate of 1% (FAO, 2007) as a consequence of unsustainable agricultural practices (slash-and-burn shifting cultivation). This accounts for more than 80% of forest cover loss (CARPE, 2005). This has made indigenous and culturally important species a scarce resource, even though they remain much in demand.

Interestingly, some innovative farmers have reacted to deforestation and rarity in the supply of traditional tree products by starting to select and manage useful trees or growing them within their farms. This approach to farmer-driven domestication in which species are brought into a managed environment through planting or retention, is indicative of the convinced that it is worth investing in indigenous fruit species. For example, *Bactris gasipaes* (peach palm) is an underutilized food crop, cultivated by small-scale farmers in the Amazon forest. Some farmers prefer starchy fruits for flour, while others prefer oily fruits for cooking. Over the years, these farmers have been able to develop visual descriptors to select palms for oil (red waxy coated fruits) and palms for flour (red or yellow not waxy fruits) (Weber et al., 2004). Likewise, anthropic selection of *Vitellaria paradoxa* (sheanut tree) by local farmers in Ghana eliminated unwanted tree species on-farm, leaving only sheanut trees that were selected for tree growth characteristics (tree size, growth, health status, age to fruiting) and yield parameters (nut size, oil content etc.) (Lovett and Haq, 2000). Similarly, Asaah et al. (2003), and Leakey et al. (2004a) reported that farmers in Cameroon and Nigeria where selecting and multiplying *Irvingia wombolu* and *I. gabonensis* (bush mango) trees respectively, that have 44% large kernels over similar other trees of the same species (particularly in south-eastern Nigeria). Farmers in southern Cameroon have also been reported to select particular trees for their large fruit size as well as other characteristics such as taste and yield (Schreckenberg et al., 2006). Such selective planting by farmers in Cameroon and Nigeria, has been reported by Waruhiu et al. (2004), to result in *D. edulis* fruits from trees on farms being 66% larger than those obtain from trees in the wild.

These are strategies developed by farmers in order to be self-sufficient for food, micro-nutrients, medicines and all their other day-to-day needs (Tchoundjeu et al., 2008). These actions by farmers to retain natural seedlings on farms and in homegardens, and to eliminate trees with products (fruits/nuts) with less desirable attributes as they open up land to cultivate other crops, and the parallel sowing and/or dispersal of seeds of the more delicious fruits they eat, close to the homestead has been suggested to be a form of 'commensal' domestication (Leakey and Asaah, In press). This commensal approach to domestication constitutes one of the building blocks of the pathway to participatory tree domestication. Participatory tree domestication combines agricultural science and technology with traditional knowledge as an integral package (Tchoundjeu et al., 2006). The domestication of agroforestry trees could therefore be considered as a necessary step to promote sustainable agriculture through diversification with species which generate income in local and distant markets, improve diets and health, meet domestic needs, and restore functional agro-ecosystems, as well as empowering local communities (Leakey, In press).

1.3 Domestication strategies for agroforestry trees

Agroforestry practices are widespread in the tropics and used by more than 1.2 billion people (FAO, 2005). These systems generate products that are important for the livelihoods of millions of people in developing countries. The area under agroforestry worldwide has not been exactly determined, but is estimated that over one billion hectares (46%) of farmland have more than 10% tree cover, thus concerning about 30% of all rural people worldwide (Zomer et al., 2009).

In agroforestry systems, different species fulfil diverse functions as providing food, medicine, fodder, timber and income generation from the sales of surplus food stuffs, cash crop products and extracted AFTPs such as fruits, nuts, leaves, bark, etc. to billions of farmers. Even though most of such land use systems are "rich" in species diversity, they are poor in high value economic species, and therefore, hardly contribute to the well-being of the farmers beyond the subsistence level. In Cameroon for example, about 17% of the population still live on less than $1 per day in rural areas, 50 % on less than $2 per day in peri-urban areas, and 40% are under the national poverty line in urban areas (UNDP, 2008).

Much evidence suggests that poverty drives deforestation (Zwane, 2007) and the loss of biodiversity (including primates) (Naughton-Treves et al., 2011). A rise in poverty occurred at the end of the 1980s in most sub-Sahara African countries following recurrent fluctuations in prices of cash crops (cocoa, coffee and rubber) on the world market with disproportionate effects on the more vulnerable rural poor of which women, youths and elderly were particularly hard hit. The World Bank's structural adjustment programme coupled with price liberalization and currency devaluation presented both challenges and opportunities for the rural poor, as these were accompanied, respectively, by price rises for basic commodities and cheapening of potential export products (cash crops). Despite increased export opportunities, the combination of a weak technological environment, and weak price control and regulatory supply mechanisms generally made export crop farmers increasingly uncompetitive and vulnerable. As a result, relatively developed economies in West and Central Africa, like Cameroon, collapsed.

Against this background, there is an urgent need to diversify farmers' livelihood options through the development of sustainable poverty reduction and tree crop management strategies, such as tree domestication. Fruit tree domestication can be considered as a linear process from collection of fruits and seeds in natural forests to cultivation of improved trees species in specialised tree production systems such as monocrop plantations (Wiersum, 2008). This specialisation makes it possible to minimize competition by other crops and optimize commercial production, and thus make the most efficient use of improved tree species. Verheiji (1991) argued that in mixed production systems such as homegardens, its strength lies in its stability rather than peak performance. However, according to Leakey et al., (2005), this perception is changing with increased recognition that the process of tree domestication does not only concern the adjustment (evolution) of tree species characteristics to improved quality and yield to the expectation of users, but also to social and environmental concerns. Thus, while monoculture tree plantations could result in optimal yields when intensively and professionally managed, under other conditions they have several limitations such as increased susceptibility to pests and diseases and/or, limited biodiversity among others. Specialized forms of monocrop cultivation do not fit into existing smaller-scale farming systems in tropics. Small-scale farming is focused on multifunctional production for subsistence and commercial products. Such multi-species arrangement according to Schroth et al., (2004), would provide synergistic ecological processes, such as:

- circulation of nutrients as a result of the presence of a root filter against nutrient loss;
- plant protection as a result of the presence of buffers against damaging agents such as pests and diseases; and
- protection by vegetative barriers against potential degrading forces such as rain, run-off or strong winds.

Two principal pathways are generally distinguished in the domestication strategy for most agroforestry species in small-holder farming systems in the tropics (Figure 1.2). These include domestication implemented on farm by farmers (Phase 1), who bring trees into cultivation themselves (Leakey et al., 2004a) or through genetic improvement programmes on research stations (Leakey and Simons, 1998). Increasingly, scientific approaches are also being introduced into on-farm domestication through the application of participatory approaches to tree improvement (Phase 2) whereby researchers mentor and advise farmers, and sometimes implement and monitor joint on-farm research. Among the advantages of the use of participatory approaches are the benefits of building on tradition and culture while promoting rapid adoption by growers to enhance livelihood and environmental benefits (Tchoundjeu et al., 2006, 2008). The bottom-line is that both domestication pathways should strive to meet traditional as well as emerging market opportunities for the species.

For the domestication strategy to be of benefit to the target farm population, it should be based on participatory approaches in decision making on both choices of species for domestication and the domestication approach to use (Leakey et al., 2003; Tchoundjeu et al., 1998). The use of participatory approaches has been observed to be an incentive to farmers adopting tree domestication as farmers see this as an opportunity to raise themselves out of poverty, malnutrition and hunger through enhanced livelihoods, food and nutritional security (Asaah et al., 2011b). This sets a solid foundation for participatory processes and ensures that domestication is a *farmer-driven* process with a local market focus in order to also ensure that farmers will be able to sell their products (Leakey and Simons, 1998; Scherr, 2004; Simons and Leakey, 2004; Tchoundjeu et al., 1998, 2006, 2008; Termote et al., 2011).

The first participatory step involves an exercise in priority setting, in which farmers list their preferred species for domestication (Franzel et al., 1996; 2008). This is to ensure that the outputs of the domestication process are relevant to farmers' needs and by so doing stimulates their active interest and involvement. Fascinatingly, almost everywhere in the world where this priority setting has been done, farmers have selected familiar and locally-marketed indigenous fruits and nuts as their top priority (Leakey and Asaah In press). This is probably because most traditionally important products are no longer readily available in the wild and are important domestically to rural people because of their cultural and nutritional value.

The second step in a participatory approach to domestication is aimed at empowering local communities, promoting food self-sufficiency, generating income and employment, and enhancing nutritional benefits. By providing knowledge and training, farmers are assisted to develop the right skills to set up tree nurseries and apply simple researched, adapted and adoptable approaches to nursery management, mother tree selection and horticultural techniques of vegetative propagation (rooting of stem cuttings, grafting/budding, marcotting). Together, these two steps to participatory domestication probably also explain the rapid adoption by rural communities (Tchoundjeu et al., 2006; 2010). For example, after about 12 years, the number of communities that engaged in this approach under ICRAF's guidance in Cameroon has grown from 2 pilot villages to 485 villages centred on five Rural Resource Centres and involving about 7100 farmers (Asaah et al., 2011b).

Another important element of the village-level participatory domestication strategy is its recognition of the rights of local people to their indigenous knowledge and traditional use of native plant species in conformity with the Convention on Biological Diversity (Simons and Leakey, 2004). Protection of farmers' intellectual property rights is needed to ensure that participatory domestication by local farmers can be recognized as a valuable model of biodiscovery (recognizing and patenting farmers' indigenous best practices) rather than biopiracy by expatriate or local entrepreneurs. According to Lombard and Leakey (2010) until global negotiations create an effective means of protecting the intellectual property rights of farmers they remain at risk of being exploited.

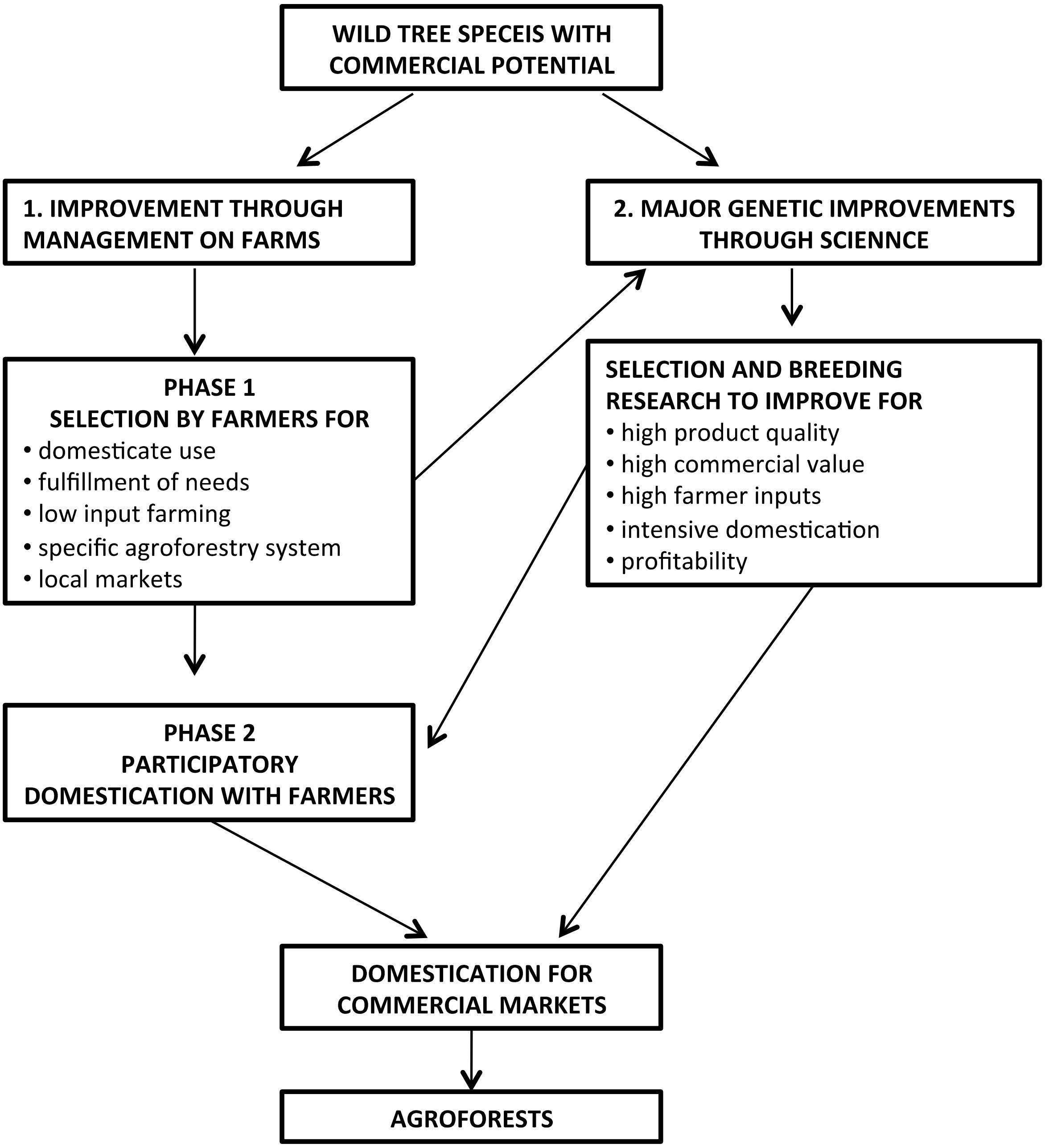

Figure 1.2. Two pathways for the domestication of agroforestry tree products

Source: Leakey and Akinnifesi (2008)

1.4 Vegetative propagation

Vegetative propagation is defined as the regeneration of new individuals from vegetative organs such as stems, roots, leaves, buds and even single cells (Jaenicke, 1996). Vegetative propagation can also be considered as a phenomenon of regeneration of differentiated, somatic cells of plants to produce a new organ and restore body parts that have been lost by injury or autotomy (selfamputation of body parts) (De Klerk, 2000). Plants have a remarkable capability to replace lost parts and may even grow a complete, fully new organism from a single somatic cell cultured *in vitro*. De Klerk (2000), distinguishes three types of regeneration in plants: (1) caulogenesis (adventitious shoot formation); (2) rhizogenesis (adventitious root formation); and (3) somatic embryogenesis (adventitious embryo formation).

For the purpose of producing plants that are true-to-type, the domestication strategy adopted in agroforestry tree domestication is the clonal propagation approach based on well-known horticultural techniques of vegetative propagation (Leakey, 2004b; Tchoundjeu et al., 1998; 2000) applied in a simple, robust and low-tech manner (Leakey et al., 1990), so as to be appropriate for implementation in remote areas of tropical countries which lack reliable supplies of running water or electricity but also other basic resources.

Vegetative propagation is a powerful means of capturing existing genetic traits and fixing them over generations so that they can be used as the basis for a genetic 'variety' or 'cultivar' development process (Leakey, In press). The advantage of using clonal propagules outweighs those of seedlings especially when the products are of high nutritional or income value, or when the tree has a long juvenile phase before first fruiting, and when seeds are scarce, difficult to germinate or difficult to store (Leakey and Akinnifesi, 2008; Tchoundjeu et al., 2006; 2008). The resultant uniformity in the eventual crop is advantageous in terms of maximizing quality, matching market specifications and increasing (if this has been a selection criteria) productivity, but it also increases the risks of pest and disease problems. Therefore, risk aversion through diversification of initial clonal production population is a crucial component in the domestication strategy adopted. However, through agroforestry, risk aversion can also be achieved by diversification of the agro-ecosystem by introducing other species and food crops in order to improve the overall agro-ecological system functions (Leakey, 2010).

1.4.1 Propagation by stem cuttings

Cuttings are portions of stems, roots or leaves that are detached from plants and used to clonally multiply new plants (Hartmann et al., 2002). Two major groups of cuttings can be distinguished: i.e. soft and hardwood cuttings. As a result of morphological differences among these two types of cuttings, the factors responsible for their rooting differ. For example, leafy stem cuttings of softwood species depend on photosynthates produced during propagation conditions, whereas leafless hardwood cuttings rely on hydrolysis of carbohydrates stored within the stem (Leakey, 2004b).

In a review of the physiology of vegetative propagation, Leakey (2004b) identified propagation environment, post severance treatment, stockplant factor and management as key determinants for the rooting ability of cuttings under propagation.

Propagation environment

These are essentially the enabling conditions within a propagation system that encourage physiological activities (photosynthesis and transpiration) in leaves by minimizing stress of leaf tissues from transpiration and respiration, while also ensuring meristematic activities (mitosis and cell differentiation) prevail in the stem.

Meristematic differentiation is a prerequisite to adventitious root formation and to successful rooting of cutting under propagation. Commercial propagators successfully regulate environmental conditions to maximize rooting (i.e. intermittent mist and/or fog systems, temperature and light manipulation) (Hartmann et al., 2002). A low tech propagation system, 'non-mist propagation, developed in the 80s is currently widely used in rural communities in West and Central Africa for successful cutting multiplication. Non-mist propagators are constructed following a design based on that of Howland (1975), further modified as described by Leakey and Longman (1988) and Leakey et al. (1990). The basic principle underlying all these propagation systems is need to make water available at both the base and leaf of the cutting in a shady cool environment with low vapour pressure deficit (VPD) as this will minimize stress from water (Leakey, 2004b). While all propagation systems are reportedly effective, they however vary in their cost and technical sophistication. The non-mist propagation system can be distinguished from the others as a low-cost and low tech system, adaptable to rural settings in the tropics where pipe-borne water and energy are in short supply.

A non-mist propagator consists of a wooden frame enclosed in clear polythene sheet so that the base of the propagator is watertight (3 x 1 x 1 m^3) (Figure 1.3). The base of a non-mist propagator is covered by a thin layer of fine river sand to prevent the polythene from being punctured by stones, then successive layers of small stones and gravel that trap air and create a convectional pressure overlaid by the rooting medium (to a depth of 10 cm on top of gravel). According to Leakey (2004) and Mesen et al. (1997), anoxia at the cutting base encourages rotting and poor rooting, hence the need to create optimal air-filled pore space (air:water ratio) of the rooting medium through various particle sizes. In this way, the rooting medium does not only hold the cutting firm in the propagation unit, but also provides moisture to the cutting base and allows respiration of the tissues.

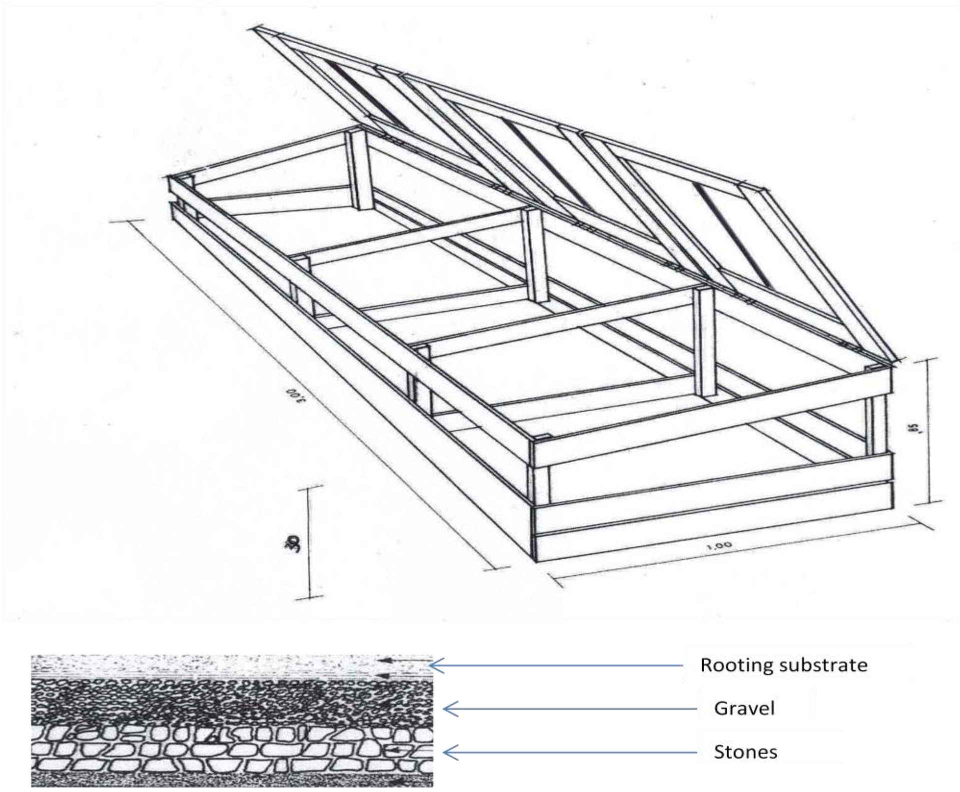

Figure 1.3. Non-mist propagator for rooting of stem cuttings (Redrawn and modified from Leakey et al., 1990 by EK Asaah and Z Tchoundjeu)

In a non-mist propagation system, air humidity is maintained by the provision of a water table beneath the rooting medium, resulting in a permanently humid environment (RH = 80-

90 %) and moist substrate throughout the propagation period. Thus, cutting bases have a constant source of water while the leaves are in a cool, environment with a low vapour pressure deficit. Relative humidity and temperature of both ambient air and propagation medium provide stress-free growing conditions approximately similar to field conditions in humid lowlands of central Africa. Non-mist propagators are sited under a shade house roofed with corrugated iron sheets and translucent plastic sheets to provide the proper low irradiance environment for photosynthesis and root development in cuttings.

Studies on the effect of light environment during propagation of leafy stem cutting according to Mesen et al. (1997), confirm that photosynthesis occurs on cuttings grown under propagation systems. The same study further relates rooting ability to lower irradiance depending on the leaf area of the cutting.

Post-severance treatment

There is a large body of contradictory literature on effects of post-severance treatment on the rooting ability of cuttings. According to Leakey (2004b), the absence of clear principles defining the rooting process makes it difficult for researchers to understand the contradictory literature. However, the following post-severance factors have been advanced by the same author to be critical for the rooting of cuttings i.e. auxin application, cutting leaf area and cutting volume (length and node diameter).

Auxins are root growth promoting and regulatory substances and most commonly utilized to enhance rooting in stem cuttings (Hartmann et al., 2002). The effects of auxins include promotion of starch hydrolysis, and attraction of sugars and nutrients towards the cutting base. Stem cuttings treated to auxin root more rapidly thus producing more roots and sometimes higher rooting percentages. For example, Tchoundjeu et al. (2002), reported that *Prunus africana* stem cuttings treated with Indole-3-Butyric Acid (IBA) had a higher rooting percentage (85%) compared to (40%) for untreated cuttings at the end of the experiment (10 weeks). Similarly, the mean number of roots was also influenced by auxin concentration and leaf area. Interestingly, some species seem to be difficult-to-root and are not very responsive to auxin application. Rooting percentage, was neither enhanced nor the number of roots per cutting increased. Examples of such species include: *A. floribunda* (Atangana et al., 2006), *Biallonella toxisperma* (Ngo Mpeck and Atangana, 2007) and *Annickia chlorantha* (Ngo Mpeck et al., 2009). However, this situation may be due to other factors being a greater constraint. The principle here is that auxins enhance rooting, but only in cuttings which have been preconditioned to root by their stockplant environment/management and are in an appropriate rooting environment.

Leaf area and the physiological processes within the leaf are major factors determining the rooting of softwood stem cuttings (Hartmann et al., 2002; Leakey, 2004b). Leafless cuttings often die. Leafy softwood cuttings have to be capable of undergoing photosynthesis to produce the necessary sugars to promote root initiation and elongation. This is achieved by obtaining the right balance between photosynthesis and water loss through transpiration. If leaf area is too small, rooting will be constrained by available photosynthate, while if too large water stress will occur causing leaf abscission or stomatae closure. Both outcomes prevent on-going photosynthesis.

Leaf area is also an interactive variable with the level of irradiance, cutting length, and applied auxins all which impact on the overall rooting ability of cuttings. A negative relation was reported by Leakey and Tchoundjeu (1999), on rooting of cuttings with reduced stem length, a confirmation that cutting length is important for storage of new assimilates.

Cutting volume (length and cutting diameter) have been reported to be strongly linked to rooting ability as both cutting length and diameter provide storage for new assimilates until the roots become active and form an active sink for carbohydrates (Leakey, 2004b).

Stockplant factors

This refers to the cutting origin and the stockplant environment. Two major sources of variation can be distinguished within-shoot and between shoot factors. Furthermore, these sources of variation can be further influenced by recurrent vegetative growth phases (Tchoundjeu and Leakey, 2000). Within-shoot factor accordingly to Leakey (2004b), results from numerous gradients of variation from top to bottom of a shoot in terms of leaf area, leaf carbon balance, age of nodes, internode length, diameter, lignification, nutrient content etc. Whereas between shoot factors, refers to the relative position and number of lateral branches, plant height, and the pruning regimes of the stockplant (Leakey, 2004b). These within and between shoot variables can be manipulated by stockplant management and are also sensitive to the stockplant environment, especially with regard to light (irradiance and quality) and nutrients.

Stockplant management. Management is essential for the maintenance of sustainable rooting and is achieved by pruning, fertilization and regulation of the light environment (level of irradiance (quantity) and wavelength (quality – especially the red:far-red ratio). These components and light environment have been reported to promote appropriate morphological and physiological conditions of shoot growth and to pre-condition the subsequent rooting ability of shoots (Leakey 2004b). Cutting failure to root for example, can also be attributed to the use of inappropriate plant tissues (old woody tissues which are starch filled) or too young and sensitive to water stress, or incapable of photosynthesising (below compensation point, water stress) or are infected by pests and diseases or under sub optimal propagation environment.

An additional aspect of stockplant management is associated with the transition between the juvenile (vegetative) and mature (reproductive) phases of growth in trees. Leakey (2004b), maintains that a major challenge is how to resolve the threshold of phase transition between when new shoots in the stockplant have capacity to develop flowers and fruits and under which the shoots are still juvenile. In general, juvenile material from seedlings or coppice shoots from stumps or stockplants when managed optimally are considered easy–to-root as stem cuttings due to their young ontogenetic, physiological and chronological age (Hartmann et al., 2002). In contrast, ontogenetically mature shoots are typically difficult to root because of their physiological age (lack of vigour). Hartmann et al. (2002), maintain that cuttings of *Ficus pumila* (easy-to-root) undergo cell divisions and root primordia formation more quickly than matured cuttings (difficult-to-root) under optimal auxin treatments. Once root primordia are formed, it takes 7-9 days to reach maximum rooting in both easy-to-root and difficult-to-root plants of this species. Likewise, in *Agathis australis* **(**Kauri) cuttings root primordia formation was variable in cuttings from different-age stockplants, but once root primordia were formed, roots emerged consistently within the third and fourth weeks (Davies et al., 1982).

There is great interest now in propagating many agroforestry fruit and nut species selected from natural populations or obtained from breeding programmes using stem cuttings in non-mist propagators probably due to the low cost and technology requirements of this system. For example, *A. floribunda* (Atangana et al., 2006; 2007); *D. edulis* (Mialoundama et al., 2002); *Irvingia gabonensis* (Shiembo et al., 1996), *Prunus africana* and *Pausinystalia johimbe* (Tchoundjeu et al., 2002, 2004) have been successfully multiplied through stem cuttings. Mialoundama et al. (2002), reported sawdust as a suitable rooting medium giving the best result (95% rooting of stem cuttings after 12 weeks) for *D. edulis* in a low technology non-mist polythene propagator developed by Leakey et al. (1990). Similarly, recent studies on *A.*

floribunda stem cuttings recorded 68.7% rooting at 25 weeks in the non-mist propagator after important factors (rooting substrate – river sand; clone and cutting leaf area – 25 cm^2) had been optimised. These stems cuttings were obtained from coppiced juvenile shoots and this rooting percentage did not change till the end of the experiment at 38 weeks (Atangana et al., 2006).

Propagation by stem cuttings is a relatively easy process and can provide in a short time a large number of clones (or cultivars), each of which retains the genetic traits of the original trees from which the cutting was obtained. Rooting of stem cuttings is thus a technique for clonal propagation that is adaptable to trees where large numbers of planting stocks are needed.

1.4.2 Propagation by marcotting (air layering)

Marcotting (or air layering), is a technique in which an aerial stem is girdled and enclosed in a rooting medium to produce roots on the upper part of branch while still on the tree (Hartmann et al., 2002). This technique has been developed and used successfully for the propagation of *D. edulis* (Mampouya et al., 1994; Kengue and Tchio, 1994). Mialoundama et al. (2002), recommend that *D. edulis* marcots should be set on horizontal branches with diameter above 4 cm. Mbondo (2002), cited by Kengue (2002), reported on the contrary that irregular rooting was observed to characterise marcotted plants derived from horizontal and sub-horizontal branches of *D. edulis*.

In a recent study on the effect of branch orientation (orthotropic, oblique or plagiotropic branches) on root distribution around the base of rooted marcots of *D. edulis*, it was discovered that plagiotropic and oblique branches ($<45^0$ from the orthotropic branch) have a greater tendency to develop irregularly distributed roots (ICRAF, unpublished data).

Although there have been few studies to critically evaluate the factors important in marcotting, this technique produces clonal propagules possessing the same characteristics as those of parent plants and are early fruiting (Kengue et al., 1990; Kengue, 2002). Even though this technique presently gives the best results for *D. edulis* (Kengue, 2002), it cannot be use for mass production since it is done on large branches of *D. edulis* (with diameters of up to 4 to 5 cm) leading to high pruning of trees, and a very low rate of multiplication. However, it should be used to capture and replicate the phenotype of a superior individual mother tree of *D. edulis* with desirable fruit characteristics. The resultant marcot can then be planted and managed to resprout copiously providing vegetative growth (shoots) which can then be multiplied by stem cuttings (Mialoundama et al., 2002). Eighty percent success on air layering has been reported for *D. edulis* (Kengue, 1990; Kengue and Tchio, 1994).

Marcotting techniques are still being tested on *Allanblackia.* It is hoped that the necessary conditions for successful rooting and management of *Allanblackia* marcots could be established, using similar approaches described above for *D. edulis.*

1.4.3. Propagation by grafting

Grafting is the union of a root system (rootstock) with a shoot system (scion) in such a manner that they will unite and subsequently grow and develop as a composite (compound) plant (Hartmann et al., 2002). The rootstock may be a seedling, cutting, or micro-propagated plant. The scion is composed of a short piece of detached shoot from a selected tree containing several dormant buds which, when united with the rootstock, comprises the upper portion of the graft and from which will grow the new main stem and its branches (Hartmann et al., 2002). Scions are usually collected from the mature part of the crown of mother trees, so that the cultivar has the capacity to flower and fruit immediately. This is important to ensure early productivity as a result of a phase change in the scion which though juvenile has the capacity to flower and fruit. It is the genetic potential of the scion which determines the characteristics of the cultivar. Any shoots forming on the unselected rootstock should be regularly removed because it competes with the selected scion for nutrients and if allow to grow into maturity could produced fruit/nuts of undesired quality.

Grafting *D. edulis* trees with scions from adult trees has not produced encouraging results. Some variants of grafting (approach grafting) have produced some success (12%-50%) but need greater skill just like in rooting of cuttings and marcots (Kengue, 2002).

Meanwhile and contrarily to *D. edulis,* grafting has proven to be very successful in *Allanblackia* species. For example, Ofori et al. (2008) reported grafting success of 80% using cleft grafting as opposed to 50% for side veneer grafting on *A. parviflora*. However, the orientation of the branch from where a scion is obtained is also crucial. Successful *Allanblackkia* species grafts with scions obtained from orthotropic and oblique branches grow up right whereas, successful grafts with scions obtain from plagiotropic branches grow laterally (personal observation).

1.4.4 *In vitro* in propagation

In vitro propagation is the culture of plant cells (callus, cells, protoplast) or organs (stems, roots, embryos) in aseptic culture vessels (like test tubes) under controlled environment and in sterile nutritive growth medium (Hartmann et al., 2002). This is a capital intensive and high technology approach to vegetative propagation which is not compatible with the participatory domestication strategy being developed for agroforestry trees. Through *in vitro* propagation, calli and adventitious roots have been formed from shoot tips and auxiliary buds of young seedlings of *D. edulis* (Youmbi and Benbadis, 2001). This has not gone beyond laboratory research for *D. edulis* whereas similar research on *Allanblackia* is still to be reported.

1.5 Constraints to tree domestication

A key constraint to the domestication of agroforestry trees is that they are predominantly out-breeders therefore a constant mating of related/unrelated parents trees of the same species with diverse attributes. This means that gains resulting from crosses of parent trees in any given trait of interest for example size, colour, pulp, etc. are on average small because of the wide range of intra-specific variation in the progeny arising from uncontrolled cross-pollinations. Additionally, the long juvenile phase to fruiting of many tree species (10-20 years), means that breeders have to wait multiple years (sometimes decades) before fruits can be evaluated, selected and cultivated. However, these problems can be circumvented through clonal multiplication (rooting of stem cuttings, marcotting, grafting etc.) of individual trees of a given species with superior characteristics.

Until the last decade, trees in the past have been reported to be very difficult to propagate by stem cuttings (Leakey and Asaah, In press). This perception was probably as a result of limited knowledge of the underlying principles determining success in rooting of stem cuttings, grafting or macrotting indigenous and culturally important species. According to Leakey (2004b), this was mainly the result of poor experimental reporting leading to a confused and controversial research literature on vegetative propagation techniques (rooting of cuttings, grafting, budding and marcotting). These techniques provide an alternative means of capturing phenotypic variation, but they too have some technical problems (*e.g.* graft incompatibility reported in *Uapaca kirkiana* (Mng'omba et al., 2007), rootstock dominance, etc.), as well as requiring special skills to properly apply them (Hartmann et al., 2002). Though there are no correct figures, the limited number of people in developing countries with appropriate skills in clonal propagation may be a constraint to scaling-up and a wider adoption of participatory tree domestication in the future (Asaah et al., 2011b; Simons and Leakey, 2004). According to Leakey and Asaah (In press), the overriding factor that has probably delayed the domestication of indigenous fruit and nut trees has been the limited interest of colonialists and development agencies in indigenous tree products that did not appeal to 'western' tastes. Consequently, they were neither promoted by early European settlers, nor were they a target of donors.

1.5.1 Managing genetic variability and cultivar development in tree domestication

Typically and/or ideally, only the best 'individuals' are brought into domestication programmes, so domestication is generally considered to reduce the genetic diversity of the species being domesticated, creating the so-called 'domestication bottleneck' and/or inbreeding depression (Cornelius et al., 2006; Miller and Gross, 2011). This is probably true in situations where the domesticated plant replaces or starts dominating the wild origin (domestication syndrome), (Harlan, 1992). However, this is probably not the case at the current level of domestication of agroforestry trees (Leakey and Asaah, In press). Evidence from phenotypic characterisation studies on *Irvingia gabonensis* (Atangana et al., 2002) and *D. edulis* (Waruhiu et al., 2004) in Cameroon and Nigeria, reveal a high frequency of intraspecific variation in measured fruit traits (fruit length, width, pulp thickness) in wild and planted village populations (about 80%). Interestingly, this variation is greatest at within village level, while variation between villages is only modest; suggesting that genetic diversity at the species level can be maintained by village-level domestication. In the same way, cultivar development at village level will also minimize loss of genetic diversity as selected cultivars developed in different villages will all have for example large fruits (for *D. edulis*) or large oily nuts (*Allanblackia*), whereas at the same time they will be genetically diverse in all unselected traits, such as pest and disease resistance, etc., especially when wild populations remain present nearby. This could therefore be another advantage of implementing a participatory domestication strategy independently in different villages.

Additionally, modern molecular techniques are useful in the development of a more informed strategy for the maintenance of genetic diversity. Within the geographic range of a particular species they can be used to identify 'hotspots' of intraspecific diversity (e.g.

Assogbadjo et al. (2006), suggested *in* or *ex situ* conservation options for *Adansonia digitata*, and Lowe et al. (2000), for *Irvingia* species. Pauku et al. (2010), reported in *Barringtonia procera* in Solomon Island that genetic diversity within 5 sampled populations was 87% whereas between populations was 13%. This suggests that trees selected for their large kernels are most likely not to be unrelated, so providing opportunities to develop superior cultivars without severely narrowing intraspecific genetic diversity. With a good understanding of intraspecific variation in all traits of importance for selection and improvement to meet different market opportunities, clonal approaches can then be used for improvements in yield and quality traits (Leakey et al., 2005ac; Miller and Gross, 2011). Furthermore, hotspots which should, whenever possible, be protected for *in situ* genetic conservation, or be the source of germplasm collections if *ex situ* conservation is required can be identified

1.5.2 Advantages and concerns of vegetative propagation

Leakey and Akinnifesi (2008), Leakey and Newton (1994) and Tchoundjeu et al. *(*2006), advanced advantages of vegetative propagation of which eight apply to *D. edulis* and *A. floribunda.* Concomitantly, there are certain concerns and/or limitations associated with vegetative propagation which should be considered seriously in the formulation of a species domestication strategy (Table 1.3).

Table 1.3. Advantages and concerns associated with vegetative propagation based on reviews of Leakey, 1991; Leakey and Akinnifesi, 2008 and Tchoundjeu et al., 2006

Advantages	**Concerns and/or limitations**
Cloning of individual trees from a wild population with a rare combination of attributes (or traits) such as large fruit/nut size, oiliness, precocity, early fruiting, delayed or extended fruiting season.	The risk of reducing the genetic diversity of the species through the widespread proliferation of only a few cultivars, which consequently, increase its susceptibility to pest and disease outbreaks amongst others. However, adequate genetic diversity can be maintained through conservation of wild sources, regular introduction of new clones and the generation of new genotypes through hybridization (Leakey 1991).

Cloning will result in plants of high uniformity in their yields and quality of products to meet market demands.	Possible root structure defects through initiation of insufficient numbers of roots during propagation. Such defects can be mitigated by proper stockplant management and the implementation of well-researched and robust propagation methods that ensure that new plants have a radially-distributed plate of roots to provide stability (Tchoundjeu and Leakey, 2000).
The superiority of clonally-produced cultivars often justifies the extra cost and care required to ensure quality and productivity, especially when the risks of market saturation are minimal.	The cost of producing plants vegetatively can be several times as high as that of producing seedlings.
Plant multiplication will continue in the event there are problems of poor and/or erratic seed supply by shy seeder. For example, off-season *D. edulis* trees are sometimes seedless or seeds are not physiologically matured, while *Allanbackia* trees had bi-annual productivity.	The propagation environment is somewhat sophisticated in terms of structures and equipment especially for *in vitro* or micro-propagation.
Through cloning, production of elite trees within genetically diverse wild populations.	All vegetative propagation techniques are more labour-intensive than generative propagation methods often exceeding 80% of total production costs (Davies, 1994) compared to seedling production.
The genetic gains through cloning are higher per generation compared to a seed orchard/seedling-based tree improvement programme.	
Through cloning, tree species with seeds of short viability, low germination rate etc., can still be multiplied.	
Clonal multiplication 'fixes' genetic gains (such as rare characteristics combined in hybrid mother trees produced through breeding elite trees) over generations.	

1.6 Problem statement

The belowground and aboveground growth attributes of vegetative/clonal propagules seem to be different from those of plants grown from seeds and are not well-studied or understood. Belowground tree growth attributes (rooting pattern and distribution) are very important for water and nutrient uptake. Knowledge of rooting/root distribution and the development of root systems is essential to understand the ecological niche of tree species in order to optimize their productivity in various silvicultural and agroforestry systems (Huxley, 1983; Von Maydell, 1987; Toky and Khosla, 1989a). Akinnifesi et al. (2004) and Vogt and Persson (1991), classified tree root systems into 2 main components based on the root diameter which are: (i) the main structural roots component (diameter > 2 mm) for support and anchorage of the plant; and (ii) the fine roots component (diameter ≤ 2 mm), which consist of long exploratory branching roots with root hairs for water and nutrient uptake.

The spatial and temporal distribution of roots in multispecies agro-ecosystems is known to vary with species, tree husbandry and edaphic site factors (Akinnifesi et al., 1995; Schroth 1995; 1998; van Noordwijk and Purnomosidhi, 1995). According to van Noordwijk and Purnomosidhi (1995), desirable root architecture requirement differs for sequential and simultaneous agroforestry systems.

Fine root length, for example, is a relevant estimator of the rooting system for water and nutrient uptake (Akinnifesi et al., 2004; Anderson and Ingram, 1993). According to Schroth (1995) and van Noordwijk and Purnomosidhi (1995), data on root abundance as a function of soil depth are needed to get an idea about the ability to compete for belowground water and nutrients. Decrease in fine root volume of karité (*Vitellaria paradoxa*) and néré (*Parkia biglobosa*) trees in the 0-20 cm soil stratum following root pruning during the cropping season has been reported to reduce tree x crop competition for belowground resources resulting in yield increase of companion crops (Bayala et al., 2004). Several authors have examined the distribution of structural primary roots (Akinnifesi et al., 1999a; Asaah et al., 2010a; Coutts 1983; Toky and Khosla, 1989). Other works have described and compared the fine root distribution on: 8 tree species in India (Chaturvedi and Das, 2003); 13 multipurpose trees in Nigeria (Akinnifesi et al., 1999a); *Grevillea robusta* and *Gliricidia sepium* in Kenya (Odhiambo et al., 2002); and *Senna siamea* in three regions of Togo (Vanlauwe et al., 2002). Till date, little or no research work has been done on indigenous trees under domestication in Africa to understand the rooting system of transplanted trees of respectively vegetative and seed origins.

Interestingly, the author is not aware of any published studies that have rigorously quantified the effect of propagation methods on the aboveground growth attributes of any indigenous fruit tree under domestication in Africa, beyond their fruiting characteristics. Meanwhile, the aboveground parts of these trees are made of carbon (C) stored in the stem(s) and branches. Most tree products (fruits, nuts, vegetables, spices, bark, oils, resin, etc.) for which the trees are grown for, can be harvested with negligible impact on overall C stock of that system.

Trees in agricultural landscapes constitute major potential C sinks capable of absorbing and storing large quantities of atmospheric C in live biomass if trees are integrated into farming systems and judiciously managed together with crops and/or animals (Albrecht and Kandji, 2003; Nair et al., 2009; Soto-Pinto et al., 2010; Takimoto et al., 2008a). It is in this vein that agroforestry was recently recognized as a greenhouse gas mitigation strategy under the Kyoto Protocol for biological C sequestration (Nair et al., 2009). Watson et al. (2000) reported that C sequestration rates ranging from 1.5 to 3.5 Mg C ha^{-1} y^{-1} and a tripling of C stocks in a 20-year period, to 70 Mg C ha^{-1} was achievable in mature agroforestry systems. Albrecht and Kandji (2003) maintain that the C sequestration potential in agroforestry systems could range between 29 and 53 Mg ha^{-1} in the humid tropics of Africa.

That notwithstanding, agroforestry providing a dual function of strengthening food security and C sequestration to fight climate change is still little understood. Consequently, it was necessary to initiate this study aimed at getting insights on the effect of propagation methods often used in clonal propagation of fruits trees, on their aboveground growth attributes. Knowledge on individual tree's aboveground growth attributes is vital in designing and managing agroforestry systems and their inherent agro-ecosystems services. In addition, fruit trees displaying high carbon sinks, would be an incentive to promote tree crop cultivation, as farmers would not only gain from the tree products, but could also gain extra revenue from the C stock in their trees offered by various environmental service reward mechanisms.

In the first part of this thesis, the species *D. edulis* is presented (chapter 2) and the effect of propagation methods on structural and fine roots system and/or distribution of fruiting *D. edulis* trees of seed and vegetative origins (cutting and marcot) examined to assess their relative stability and competitive/complementary nature towards belowground resources (chapters 3 and 4, respectively). The effect of propagation methods on aboveground growth attributes and their relative potential to sequestrate atmospheric C into live biomass are examined and presented in chapter 5.

Diversification of clonal production population is crucial in the domestication process for pest/disease risk aversion and/or to avoid poor performance of individual trees as a result of inbreeding depression. Through agroforestry, risk aversion can also be achieved by diversification of the agro-ecosystems through the introduction of other species and food crops in ways that would provide food, income and other agro-ecological system functions (nutrient recycling, erosion control, habitat for flora and fauna, carbon sequestration, etc.) (Leakey, 1999; 2010). In the second part of this thesis (Chapters 6 and 7), the focus is on *Allanblackia floribunda* Oliv. (Clusiaceae), a species with potential for diversification of farming systems in an agroforestry context.

Within the last decade *Allanblackia* has become a subject of international interest to Unilever and other commercial enterprises as the seeds contain edible oil that can be used in healthy food products. Their particular interest lies in the oil obtainable from its seed which has significant potential in the global food market as a 'raw material' for the production of healthy spreads that are low in trans-fats (Ochieng, 2007). Unilever estimates that the potential market for *Allanblackia* oil is more than 100,000 tons annually, provided the right quality standards are met.

A. floribunda has a long juvenile phase to fruiting of at least 12 years in addition to a very slow germination rate of less than 5% (Vivien and Faure, 1996). Recently, however, new techniques developed in Ghana and Tanzania on *A. parviflora* and *A. stuhlmannii* have greatly improved nursery germination success (Ofori et al., 2011; Munjuga unpublished).

Allanblackia is an allogamous species; thus, vegetative propagation techniques are needed to capture certain desirable fruit or tree traits so as to produce planting materials having the same genetic characteristics as the mother trees. Recent studies by Atangana et al. (2006) on *A. floribunda* leafy stem cuttings recorded 68.7% rooting success at 25 weeks after setting up the experiment with cuttings obtained from juvenile shoots of a coppiced *A. floribunda* tree. These figures did not change till the end of the experiment at 38 weeks. Though successful, this approach to vegetative propagation is slow, and only a few roots are produced per cutting.

The cultivation of *Allanblackia* species on farm is therefore constrained by propagation success (both sexual and vegetative propagation techniques) (Munjuga et al., 2008). According to Bhojwani and Razdan (1996), a shift from sexual to clonal reproduction allowed for the faithful reproduction of individuals with superior features (traits) by eliminating uncertainty in the transmission of favoured traits over reproductive cycles associated with sexual reproduction. Vegetative propagation (grafting, budding and marcotting) has also been used to achieve early fruiting and tree dwarfing (Akinnifesi et al., 2008; 2009). For example, grafted *Uapaca kirkiana* began to produce fruits after only 2-3 years, while those derived from seedlings took 12-15 years before fruiting (Akinnifesi et al., 2008; 2009).

A study was therefore designed to assess the amenability of grafting scions from ontogenetically mature crowns of *A. floribunda* trees on seedlings and wildings under nursery and *in situ* conditions respectively with an aim of producing trees with superior fruit traits and of shorten juvenile phase to flowering and fruiting.

1.7 Objective

The main objective of this research was to assess: (i) belowground and aboveground growth attributes of fruiting *D. edulis* trees of seed and vegetative origins (cuttings and marcots), and (ii) the amenability of vegetative propagation techniques (grafting) in the reduction of the long juvenile phase to first fruiting in *A. floribunda*. These two African indigenous fruit/nut tree species produce valuable food products which are marketable and which also have agro-ecological importance.

PART ONE

CHAPTER 2

2. MONOGRAPH OF *DACRYODES EDULIS* (G. DON) H. J. LAM

Abstract

Dacryodes edulis (G. Don) H.J. Lam, also called African plum or safou is an evergreen tree indigenous to the central Africa and Gulf of Guinea regions. The tree has a relatively short trunk and a dense crown. The bark is pale gray and rough with droplets of resin exudates. *D. edulis'* preferred habitat is a shady humid tropical forest environment. However, it adapts well to variations in soil type, humidity, temperature, and day length. Its natural range extends from Angola in the south, Nigeria in the north, Sierra Leone in the west and Uganda in the east. It is also cultivated in Malaysia. *D. edulis* is cultivated mainly for its fruit which varies in shape (oblong, ellipsoidal, oval or conical) and sometimes reaches lengths of 18 cm. The fruit is rich in lipids, essential oils, vitamins and minerals. It can be eaten raw, cooked in hot water and/or roasted in hot ash or grilled in an oven. *D. edulis* fruits are highly commercialized with transactions known to occur both at national and international levels. The fruits of *D. edulis* therefore constitute an important source of nutritious food, income and employment, and enhance livelihood to farmers, transporters and traders in regions where it grows.

Key words: *Dacryodes edulis*, domestication, genetic variation, rooting systems, vegetative propagation

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Asaah Ek, Tchoundjeu, Foundjem-Tita D, Tsobeng A, Van Damme P (Submitted). *Dacryodes edulis* (G. Don) H. J. Lam. In Atlas on African Agrobiodiversity: Indigenous Fruits & Vegetables, by PROTA (Plant Resource of Tropical Africa). An invited book chapter.

2.1 Introduction

Dacryodes edulis belongs to the Burseraceae family. It is an evergreen tree indigenous to the central Africa and Gulf of Guinea regions. The genus name is derived from the Greek word '*Dakruon*' (a tear) in reference to the resin droplets that appears on the bark surface of its species. The species specific name '*edulis*' means edible (Anonymous, 2011a). The genus *Dacryodes* comprises about 40 species, occurring in the American, Asian and African tropics. In Africa, about 20 species have been described (Anonymous, 2011b). The species has been described under different names, now considered synonyms. These include*:*

Pachylobus edulis G. Don (1832),

Canarium edulis Hook (1849),

Canarium mubafo Ficalho (1881),

Canarium saphu Engl. (1893),

Pachylobus saphu Engl (Engl) (1896),

Pachylobus edulis G. Don var *preussi* Engl. (1898),

Canarium manfeldianum Engl.(1910),

Pachylobus edulis G. Don var. *glabra* A. Chev (1916),

Pachylobus edulis G. Don var *sylvestris* A. Chev (1916), and

Dacryodes edulis (G. Don) H. J. Lam (1932).

2.2 Economic importance of *D. edulis*

The fruit of *D. edulis* is the economic part that gives its true economic value to the species and explains why the tree is cultivated and/or under domestication (Tchoundjeu and Leakey, 2002; Kengue, 2002; Waruhiru et al., 2004; Anegbeh et al., 2005; Schreckenberg et al., 2006). The fruit is rich in lipids, proteins, minerals and vitamins making it an excellent snack to consumers.

The economic importance of *D. edulis* fruits is quite pronounced in Central Africa in general, and Cameroon, in particular. *D. edulis* is the most-collected agroforestry tree product (AFTP) (quantity-wise) and the most commercialized AFTP in southern Cameroon (Ndoye et al., 1998; Tabuna, 1999). In 1999, 2,324 t of the fruit were sold for a total amount of about US$ 1.5 million in 9 big markets in Cameroon (Awono et al., 2002). This quantity represented only about 14-23% of total production in the national territory as Isseri and Temple (2000) estimated the national production at 10,000 to 16,000 t. In Nigeria, 70% of fruits are home-consumed whereas the average market price per ton of fruits ranges from US$ 300-700 (Ajibesin, 2011).

D. edulis fruits have entered the international market. Tabuna (1999), reported that in 1998 Europe (France and Belgium), imported 105 tons of *D. edulis* fruits of which 100 tons came from Cameroon, 3 tons from Democratic Republic of Congo (DRC) (Congo Kinshasa) and 2 tons from the Republic of Congo (Congo Brazzaville) with volumes increasing yearly. More in general, the principal importing countries of *D. edulis* are Belgium, France and United Kingdom from countries such as Cameroon, Nigeria, Republic of Cameroon, Democratic Republic of Congo, and Central Africa Republic. The estimated potential market in these importing countries was about 120,000 people (Awono et al., 2002). *D. edulis* fruits therefore constitute an important source of food, income and employment, and enhance livelihood to farmers, transporters and traders (Schreckenberg et al., 2006).

2.3 Nutritional importance of *D. edulis*

The contribution of fruits to a healthy and nutritious diet, the world over is a well-established fact. *D. edulis* is a tree cultivated widely for its edible and nutritious fruits (Table 2.1). Generally, the fruit may be cooked in hot water, or roasted/baked in an oven at about 50ºC. The cooked fruit can be eaten with maize, plantain, cassava, cocoyam, bread, etc. Unlike other oily fruits, both *D. edulis* fruit pulp and seed oil contain the same fatty acids and physicochemical properties (Tables 2.2 and 2.3) (Silou, 1999; Ajayi and Adesanwo, 2009) with the seeds containing as much as 18-70% oil made of polyunsaturated fatty acid (Gunstone and Norris, 1982).

Table 2.1. Proximate composition of *D. edulis*

	Mean %	
	Pulp	Seed
Moisture	18.6	20.6
Ash	3	2.6
Protein	1.93	1.4
Fat	11.94	10.44
Fibre	47.5	48.5
Carbohydrate	17.03	16.46

Source: Ajayi and Oderinde, 2002

The fruit pulp of *D. edulis* is rich in lipids, with oil content determined on dry basis reported to range from 30-60% (Silou and Kama Niamayoua, 1999). Kinkela et al. (2006), maintains that oil content in *D. edulis* pulp could be as high as 70%. However, Ikhuoria and Maliki (2007) reported lower oil content from *D. edulis* fruit pulp in Nigeria with values as low as 23.2%. According to Omogbai and Ojeaburu (2010), *D. eduis* pulp oil content is considerably higher when compared to other fruits such as apple (*Malus domestica*) with 0.4%, guava (*Psidium guajava*) 0.4%, banana (*Musa paradisiaca*) 0.39% and pawpaw (*Carica papaya*) with traces of oil.

The lipids yields numerous fatty acids (Table 2.2) such as palmitic acid (30-62%), oleic acid (18-60%), linoleic acid (15-24%) and stearic acid (1.3-5.5%) (Omoti and Okiy, 1987; Obasi and Okoli, 1993; Silou and Kama Niamayoua, 1999; Mbofung et al., 2002; Kinkela et al., 2006; Ikhuoria and Maliki, 2007). Arachidonic acid was recently identified in *D. edulis* fruit pulp and seed and reported as an important fatty acid (Ajayi and Adesanwo, 2009).

Table 2.2 Fatty acid profile of *D. eduis'* pulp and seed

Fatty acid	Fatty acid name	Mean %	
		Pulp	Seed
$C_{16:0}$	Palmitic acid	17.18	43.16
$C_{18:0}$	Stearic acid	14.84	4.59
$C_{18:1}$	Oleic acid	40.45	21.97
$C_{18:2}$	Linoleic acid	23.17	12.63
$C_{20:0}$	Arachidic acid	2.1	11.56
$C_{20:1}$	Gadoleic acid	0.91	0.21
$C_{20:2}$	Eicosadienoic acid	0.84	3.21
$C_{20:3}$	Eicosatrienoic acid	0.51	1.72
$C_{22:0}$	Behenic acid	-	0.32
$C_{22:1}$	Erucic acid	-	0.27
$C_{22:2}$	Brassic acid	-	0.12
$C_{24:0}$	Lignoceric acid	-	0.06
$C_{26:0}$	Cerotic acid	-	0.18
Saturated		34.12	59.87
Unsaturated		65.88	40.13

Source: Ajayi and Oderinde, 2002

Mbofung et al. (2002) reported a 49-58% variation in the fatty acid content of *D. edulis* fruits from Cameroon, Republic of Congo, Democratic Republic of Congo, and Gabon with some consistency observed in samples from Equatorial Guinea. The latter fatty acid values reported for countries within the Congo basin forest in Central Africa are higher than those reported for Nigeria (30.35 - 35.60%) in West Africa (Omogbai and Ojeaburu, 2010).

Table 2.3 Physicochemical properties of *D. eduis'* pulp and seed

Physicochemical properties	Mean %	
	Pulp	Seed
Acid value	5.61	5.61
Free fatty acid (as oleic acid)	2.82	2.81
Saponification value	179.52	179.52
Ester value	173.91	173.91
Iodine number	19.87	31.5
Peroxide value	30	20

Source: Ajayi and Adesanwo, 2009

D. edulis fruit pulp and seed oil contain important polyunsaturated fatty acid such as linoleic acid, which are relevant to human food in the prevention of cardiovascular disorders. The rich content of oleic acid in the oil gives it oxidative stability which is important for its use as frying oil (Ikhuoria and Maliki, 2007). Thus oil from *D. edulis* fruit pulp and seed can be exploited commercially as vegetable oil for home use as well as other industrial applications.

The protein content of *D. edulis* fruit pulp is reported to vary between 2.89-4.16% (Omogbai and Ojeaburu, 2010). Though this figure is generally low, the latter pulp could still make significant contribution in starchy diet thereby ameliorating protein malnutrition. For example Mbofung et al. (2002), reported a 39% increase in protein contents of biscuits when ordinary margarine in the biscuit recipe was substituted by *D. edulis* pulp oil.

The fruit pulp of *D. edulis,* following a chemical analysis of 100 g samples from five states in Nigeria, is a rich source of mineral elements such as sodium, potassium, calcium, phosphorus, magnesium, iron, copper, and zinc (Table 2.4) (Omogbai and Ojeaburu, 2010). Thus, *D. edulis* pulp is highly nutritive if consumed. For instance, elements in the pulp like sodium are required for the regulation of acid-base equilibrium, maintenance of osmotic balance, whereas it protects against dehydration in the body. Potassium is the chief cation of the intracellular fluid and is also involved in protein synthesis. Other elements like iron and calcium also present in *D. edulis* fruits are vital for blood formation as well as providing hardness and/or strength to bones and teeth (Omogbai and Ojeaburu, 2010).

Table 2.4 Mineral composition of *D. edulis* (ppm)

	Mean %	
	Pulp[&]	Seed[$]
Mg	280.15 - 287.65	4.85
Fe	3.43 - 358	6.2
Zn	3.65 - 3.81	0.55
Cu	0.38 - 0.45	4.85
Ca	347.50 - 354.62	0.74
Na	692.55 - 698.40	1.36
K	540.81 - 549.23	85.15
P	692.55 - 698.40	-

Source: [&]Omogbai and Ojeaburu 2010; [$]Ajayi and Adesanwo, 2009

2.4 Health benefits of *D. edulis*

Decreasing the rate of malnutrition and prevalence of underweight children are two important MDGs. Thus, raising awareness of the nutritional value of indigenous fruit could help to attain these goals. *D. edulis* fruit, for example, contains 66% more fat than peanuts (recommended as a high fat food by FAO) (Barany et al., 2004).

The entire plant of *D. edulis* has pharmaceutical properties that are variously exploited by many African communities (Kengue, 2002). The bark of the plant has long been reported in Gabon to treat wounds (Walker and Silas, 1961). In RDC, a concoction of the bark is taken as oral treatment against leprosy and it is also gargled as mouth-wash for the treatment of tonsillitis (Bouquet, 1969). In the western parts of Cameroon, the bark is crushed and used in concoctions against dysenteries while in central Cameroon the bark is used to treat toothache (Mapogmetsem, 1994). The leaves are boiled in combination with *Lantana camara*, *Cymbopogon citratus* and *Persea americana* yielding a steam bath taken to treat fever/headaches (Bouet, 1980) and malaria in Republic of Congo (Diafouka, 1997). The leaves made into a plaster have been recently reported to treat snake bites in South West Cameroon (Jiofack et al., 2010). The leaves are also crushed and the resultant juice used to treat skin diseases such as scabies, ring worm, rashes, while twigs from branches are sometimes used as chewing sticks (Igoli et al., 2005; Ajibesin et al., 2008; Okwu and Nnamdi, 2008). The leaves and seed are used in Nigeria for animal feed (Adewusi, 2004).

The resin from the bark has long been reported to treat parasitic skin diseases and jiggers in Nigeria (Hutchinson, 1963) whereas when applied in lotions and body creams it smoothens the skin (Ekpa, 1993). The resin is also used in some communities as incense and is believed to send off evil spirits in Nigeria (Sofowora, 2008).

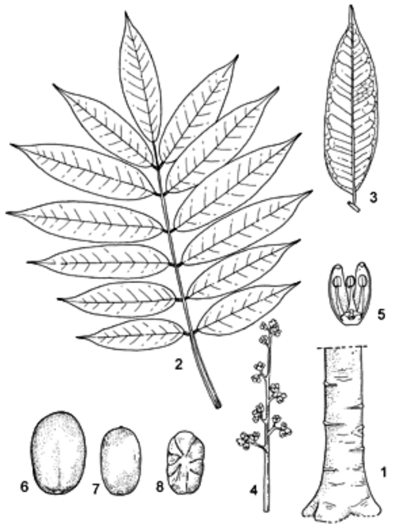
Essential oils have been extracted from various parts of *D. edulis*, fruit and leaves (Onocha et al., 1999) and the bark (Okwu and Ighodaro, 2009). According to Obame et al. (2008), these essential oils have shown potent antibacterial effects against *Staphylococcus aureus*, *Bacillus cereus, Escherichia coli*, *Salmonella enteric* and *Proteus mirabilis* and to have antifungal effects against *Candida albicans*. This probably explains the various uses of *D. edulis* in traditional medicine.

The high content in fatty acids (50%) and amino acids (10%) makes *D. edulis* an important source of vegetable oils used in pharmaceutical products (Mbofung et al., 2002).

2.5 Botany, ecology, geographical distribution of *D. edulis*

2.5.1 Botanical description of *D. edulis*

D. edulis is a gynodioecious (i.e. the species has male, female and hermaphrodite individual trees), small to medium-sized tree that grows up to 20–25 m tall with a bole up to 70–90 cm in diameter (Kengue, 2002). Leaves are alternate and imparipinnate, whereas stipules are absent. The petiole is up to 7.5 cm long with 11–19 leaflets. The inflorescence is an axiliary panicle up to 40 cm long, with cymose ramifications, ferruginous-tomentose, with flowers often in clusters of 3 surrounded by bracts. Often, entire shoot tips become floriferous with reduced leaves, giving the impression of a terminal inflorescence (Figure 2.1).



1. base of bole
2. leaf
3. leaflet
4. inflorescence
5. male flower in longitudinal section
6. fruit
7. endocarp
8 seed

3-18 cm

Redrawn and adapted by W. Wessel-Brand

Figure 2.1. Botanical features of *Dacryodes edulis*

Source: Anonymous 2011b

Three types of flowers occur on two tree types. One tree type is the female tree which bears predominantly female flowers and sometimes hermaphrodite flowers at varying proportions. The second tree type is a male tree which bears male flowers.

The fruit is a monospermous drupe characterized by an extreme diversity in form, dimensions, colour and taste. Major fruit forms are oblong, ellipsoidal, sub-globular, oval or conical. Diameter generally varies from 3-18 cm (Kengue, 2002) (Figure 2.2). Some fruits have longitudinal ridges on their surfaces. Epicarp colour is pink in young fruits, and gradually turns to dark-blue, whitish green at maturity. The fruit has an oily, fleshy mesocarp which also is the edible part. Mesocarp (pulp) colour can be pink, whitish, greenish, yellowish or other similar colours. Within the fruit, one can find one or two seeds (kernels) enclosed in a smooth membranous tegument.

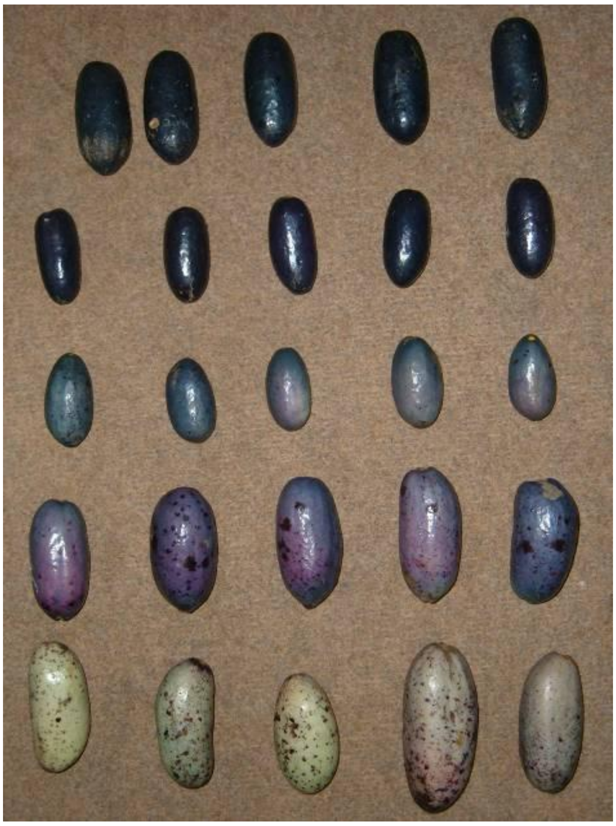


Figure 2.2. Diversity in fruits of *D. edulis* (Photo by EK Asaah)

2.5.2 Ecology, geographical distribution of *D. edulis*

D. edulis is an oliferous fruit tree found in equatorial and humid tropical climates. It originated from Central Africa and more precisely from the Gulf of Guinea (Cameroon and Nigeria) (Silou, 1994; Auberville, 1962). Older literature limited its geographical occurrence to the forest regions of Central Africa (Auberville, 1962). The current distribution as a result of human activities extends beyond its zone of origin to: Central African Republic (CAR), Gabon, Republic of Congo and Democratic Republic of Congo, then as far as Uganda in East Africa and Angola and northern Zimbabwe in the south of Africa (Bourdeault, 1971) (Figure 2.3). Humans have extended its current distribution not only within Africa but also to tropical Asia (Kengue, 2002). In Central Africa, it is a dominant fruit tree species in homegardens, cocoa and coffee agroforests, fallow land, and crop fields especially in Cameroon, Gabon, Congo, Democratic Republic of Congo and southern Nigeria (Figure 2.4). The species is also sparsely distributed in secondary forests. In the humid tropics of West and Central Africa, *D. edulis* manifests high climatic plasticity.

D. edulis can develop under light shade (hemi-sciaphile) but prefers open areas (heliophile) (Kengue, 2002). According to the latter author, temperature and rainfall are the two major climatic factors that influence growth and development of the tree. The tree develops well under an average temperature range of 23 - 25 °C, and with an annual rainfall range of 1,400 - 4,000 mm. Very high rainfall encourages vegetative development to the detriment of fruit production. Average altitude for best performance is 1,000 m.

D. edulis grows on various soil types. Nonetheless it prefers slightly acidic, deep ferallitic and evolved volcanic soils with exploitable thick and humic horizons (Kengue, 2002). Where there is a well-marked dry season, it is found only in gallery forests and on wet soils.

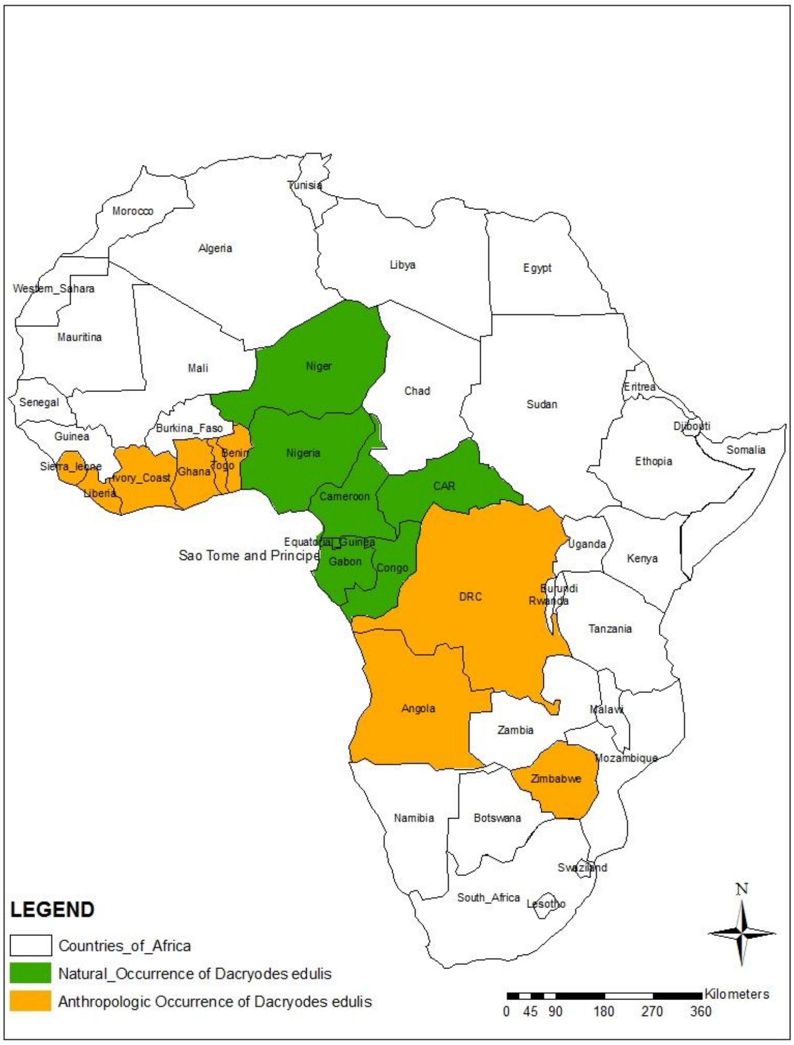


Figure 2.3. *Dacryodes edulis* distribution: natural and planted populations in Africa. Redrawn by Precilia Ngaunkam from Kengue, 2002 and PROTA database, accessed 02/09/2011.

Figure 2.4. Typical *D. edulis* tree found within a homestead in Cameroon, (Photo by EK Asaah)

2.6. Cultivation/domestication/management practices

2.6.1 Cultivation of *D. edulis*

In spite of the numerous values of *D. edulis*, monocultures or plantations of the species are rarely found. It is common to find *D. edulis* trees randomly planted in agroforestry systems, homegardens, food crop fields, coffee and cocoa plantations as shade crop for cocoa and coffee. Most of the planting materials are wildings or seedlings and to a lesser extent selected material produced vegetatively (cutting and marcots). The recommended planting density is 100 trees per hectare (i.e. is 10x10 m^2). Management of trees is minimal, often limited to slashing the weeds around each tree. When the tree is integrated in food crops fields, it benefits from the crop weed management (Figure 2.5).

Figure 2.5. Fruiting marcot of *D. edulis* tree in a maize field in Cameroon, (Photo by EK Asaah)

2.6.2 Genetic variation in *D. edulis*

Phenotypic characterization studies on *D. edulis* fruit traits (fruit length, width and pulp thickness) (Waruhiu et al., 2004) in wild and planted village populations in Cameroon and Nigeria, observed a frequent occurrence (about 80%) of intraspecific variation in the above-measured fruit traits. The observed tree-to-tree variation in fruit size was 3-to-10-fold (Waruhui et al., 2004; Anegbeh et al., 2005). Contrary to Okafor (1983), who claims the existence of two varieties, var *edulis* with large fruits and var. *parvicarpa* with small fruits, there is continuous variation in the fruits and thus there is no indication of any distinct varieties. Interestingly, this variation is greatest at within village level, while variation between villages is modest, suggesting that genetic diversity at species level can be maintained by village level domestication (Waruhui et al., 2004; Anegbeh et al., 2005).

Presently, most of the trees on farms are old and very tall and do not produce adequate fruits. This therefore means that *D. edulis* fruit production has to be increased. The options are either to rehabilitate old D. edulis trees to increase yields on farm or to replace the old trees with improve planting materials with shorter juvenile phase to first fruit production, and that will yield quality fruits of desired traits. All these, require that appropriate technologies and policies are developed that could complement current domestication efforts on *D edulis* and encourage the use of improved planting materials (derived from vegetative propagation methods) for any new plantings of the species.

2.6.3 Domestication of *D. edulis*

Domestication studies have been in progress for *D. edulis* since the 1950s (Philippe, 1957) using vegetative propagation to create cultivars. As an agroforestry species, the desire has been to rapidly acquire higher yields, early fruiting and better quality fruit/nut products. Through cloning, cultivars can be developed that could offer more uniform products that would better meet market demands. Several vegetative propagation techniques can be utilized to achieve this, including the use of stem cuttings, marcots and, to a limited extent, grafting and *in vitro* techniques (Table 2.5).

The decision to pursue vegetative propagation options in the domestication of *D. edulis* warrants the implementation of an appropriate strategy, such as that described by Leakey and Akinnifesi (2008) with regard to: (i) the methods of propagation to adopt; (ii) the type of technology that is appropriate to achieve genetic improvement; and (iii) whether to use juvenile or mature tissues, or both.

2.6.3.1 Methods of propagation and the cloning process in *D. edulis*

Propagation of *D. edulis* is fundamentally by seed. However, research has also focused on the development of vegetative propagation techniques for the species.

Table 2.5. Summary of propagation methods applicable to *D. edulis*

Propagation techniques	**Success rate**	**Reference**
Seed germination	>90%	Kengue, 1990
Rooting of stem cuttings	< 90%	Mialoundama et al., 2002
Marcotting (or air layering)	< 80%	Kengue and Tchio, 1994
Grafting	>10%	Damesse et al. (2001) cited by Kengue, 2002
		Youmbi and Benbadis, 2001
In vitro	N/A	

Legend: N/A = not available

2.6.3.1.1 Sexual propagation

Propagation using freshly extracted seed from mature *D. edulis* fruit results in a germination rate in the order of 95% to 100% (Kengue and Nya Ngatchou (1990) cited by Kengue, 2002). The major inconvenience associated with seed propagation is that seedlings are genetically heterogeneous, each seed having inherited different parts of the genetic codes of its parent trees, with segregation of genes among the progeny. This is particularly true for species that are outbreeders (allogamy) with mating of unrelated parents in contrast to species that could undergo self-fertilization (autogamy). In addition, seed-propagated trees of *D. edulis* have a long juvenile phase of at least 7 years before they produce their first fruits (Kengue, 2002).

2.6.3.1.2 Vegetative propagation

Early research on *D. edulis* indicated that it was difficult to propagate by vegetative methods (Phillippe, 1957). However, vegetative propagation methods have been developed, improved and used successfully in the multiplication of *D. edulis* by air layering (Kengue and Tchio, 1994), rooting of stem cuttings (Mialoundama et al., 2002) and to a limited extent, grafting (Damesse et al. (2001), cited by Kengue, 2002). At an experimental level, micropropagation (*in vitro* culture; Youmbi and Benbadis, 2001) can also be used with success. Rooting of stem cuttings and marcotting are the recommended vegetative propagation techniques that are promoted for the multiplication of superior individual mother trees for *D. edulis* (Asaah et al., 2007).

2.6.3.1.2.1 Propagation by stem cuttings

Mialoundama et al. (2002), in a study on the rooting of stem cuttings of *D. edulis*, reported sawdust is a suitable rooting medium giving the best result (95% rooting of stem cuttings after 12 weeks) in a low technology, non-mist polythene propagator developed by Leakey et al. (1990). In a separate study reported by the same authors, auxin application (IBA, NAA and a mixture of both) did not impact on the rooting ability of either treated or untreated cuttings of *D. edulis.*

2.6.3.1.2.2 Propagation by marcotting (air layering)

This technique has been developed and used successfully for the propagation of *D. edulis* (Kengue and Tchio, 1994; Mampouya et al., 1994; Mialoundama et al., 2002). The marcotting technique produces clonal propagules possessing the same characteristics as those of parent plants and is early fruiting (Kengue et al., 1990; Kengue, 2002). The technique could be used to replicate a superior individual mother tree of *D. edulis* with desirable mother tree/fruit characteristics. Eighty percent success on air layering has been reported for *D. edulis* (Kengue, 1990; Kengue and Tchio, 1994). The resultant marcot can then be planted and managed to resprout copiously providing vegetative growth (shoots) which can be multiplied by stem cuttings (Mialoundama et al., 2002).

2.6.3.1.2.2 Propagation by grafting

Grafting young *D. edulis* trees with scions from adult trees has not produced encouraging results. Only a variant of grafting (approach grafting) has produced some success (12%-50%) (Kengue, 2002).

2.6.3.1.2.3 *In vitro* propagation

Through *in vitro* propagation, calli and adventitious roots have been formed from shoot tips and auxiliary buds of young seedlings of *D. edulis* (Youmbi and Benbadis, 2001). This has not gone beyond laboratory research, however.

2.7 Conclusion

Tree crops are attracting increasing attention as important components of sustainable integrated agriculture. Species like *D. edulis* under domestication, and in response to purposeful man-led selection, now have reduced juvenile phase to first fruit production, larger fruit size, extended fruiting season, and less competitive rooting system for belowground resources if intercropped in an agroforestry context with companion crops, all brought about by vegetative propagation methods. Thus, establishing long-lived perennials like *D. edulis* of vegetative origin will provide food, and generate income through sales of fruits and could enhance the provision of ecological services (erosion control, habitat for flora and fauna, nutrient recycling, carbon sequestration and help to counter climate change). Furthermore, filling the niches under the trees through intercropping with useful and marketable annuals – herbs, cereals and grain legumes would be important for the further improvement and diversification of the livelihoods options of rural populations, and overall profitability and value of the system. This could lead to higher yields, increase rural incomes, resulting in greater food security and avoided emissions through the integration and retention of useful trees supplying diverse products in agricultural landscapes in the humid tropics of Africa where the species grow naturally.

2.8 Research focus on *D. edulis*

2.8.1 Effect of propagation methods on root system and tree stature in *D. edulis*

In spite of the interest in the domestication of agroforestry trees through vegetative propagation, there is a scarcity of information about the effect of vegetative propagation methods on root architecture and root distribution of trees of vegetative origin (cuttings, marcots) and on the aboveground growth attributes of the trees. Meanwhile, the lack of a clear understanding of the changes in tree stature, root distribution and potential interaction that could occur as a result of propagation methods could be a major obstacle to better design and management of agro-ecosystems and therefore farmers' livelihoods.

According to Akinnifesi et al. (2004), the distribution of plant roots in agroforestry system soils depends on plant genotype, soil properties, nutrient status, and plant vigour among others factors. For example, Vanlauwe et al. (2002) reported that *Senna siamea* exhibited different root distribution patterns in the north, central and south savannah regions of Togo. The effect of genotype on lateral and tap root development was reported by Akinnifesi et al.

(1999b) among 13 trees species grown in Ibadan, Nigeria with a 10-fold difference in lateral root volume per m^3 between *Dialium guineense* and *Nauclea latifolia*. In parallel, changes in aboveground tree growth attributes have been reported to have a positive impact on the harvest index (the ratio of the harvested part to overall aboveground biomass) and size of the fruit or grain (Donald, (1968) cited by Gepts, 2004; Akinnifesi et al., 2009).

We are not aware of any published studies that have rigorously quantified the effect of propagation methods on both the below and aboveground growth attributes of trees of seed and vegetative origins of any indigenous fruit tree in Africa under domestication. Consequently, a study was conducted to examine the rooting system of five-year old fruiting *D. edulis* trees of seed and vegetative origins (cutting and marcot) on the one hand, and to identify distinctive growth attributes in tree morphology and architecture in 10 years old *D. edulis* trees of seed and vegetative origins. A secondary objective was to quantify the mean amount of carbon stocks (C stocks) and potential CO_2 sequestration ability of *D. edulis* trees of seed and vegetative origins (cutting and marcot) in Cameroon.

2.8.2 Research questions

- How do propagation methods affect the structural root architecture of *D. edulis* trees of seed and vegetative (cutting and marcot) origins?
- What effect would propagation methods have on the fine root distribution of *D. edulis* trees of seed and vegetative (cutting and marcot) origins?
- Do propagation methods affect aboveground growth attributes, C stock and CO_2 sequestration potential of *D. edulis* trees of seed and vegetative (cutting and marcot) origins?

2.8.3 Objectives

- Determine the effect of propagation methods on structural root architecture of fruiting *D. edulis* trees of seed and vegetative origins (cuttings and marcots),
- Determine the effect of propagation methods on fine root distribution of mature *D. edulis* trees of seed and vegetative (cutting and marcot) origins,
- Identify the variability in tree growth attributes, biomass yield, C stock and CO_2 sequestrated in 10 year old *D. edulis* trees of seed and vegetative (cutting and marcot) origins.

2.8.4 Hypotheses

- *D. edulis* trees of seed and vegetative origins (cutting and marcot) differ in structural root architecture.
- *D. edulis* trees of seed and vegetative origins (cutting and marcot) have similar fine root distribution patterns.
- *D. edulis* trees of seed and vegetative origins differ in aboveground tree growth attributes, C stock and CO_2 sequestration potential.

CHAPTER 3

3. EFFECT OF PROPAGATION METHODS ON THE STRUCTURAL ROOT ARCHITECTURE OF AFRICAN PLUM (*DACRYODES EDULIS* (G. DON) H. J. LAM.) OF SEED AND VEGETATIVE (CUTTING AND MARCOT) ORIGINS

Abstract

Root morphology of 5-year-old trees of *Dacryodes edulis* (G. Don) H. J. Lam. (Burseraceae) of seed and two vegetative (cutting and marcot) origins were assessed in Cameroon. Root system of *D. edulis* trees of seed and vegetative origins were totally excavated and root morphology described and quantified. Trees of seed origin were characterized by a tap root, reaching depths of about 1.20 m. Opposing to this, trees of cutting origin showed three strong vertical roots (d > 5 mm) with the longest reaching depths of 1.31 m. Trees of marcot origin were observed to have thick, relatively short prominent vertical roots, reaching depths of 1.15 m. At fixed lateral distances of 50 cm from tree bases, trees of seed and marcot origins recorded high root densities at soil depths of 0–20 cm; both differed significantly (p = 0.03) from those of trees of cutting origin. At a soil depth of 60–100 cm, trees of cutting origin recorded the highest root density which, however, differed significantly (p = 0.02) from those of trees of both seed and marcot origins. *D. edulis* trees of seed origin had a tap root system, whereas trees of vegetative origin (cuttings and marcots) had developed prominent adventitious roots growing vertically (sinker roots) into greater depths and a wider/deeper lateral root spread than seedlings. This could be a vital adaptation to the absence of a tap root in trees of vegetative origins so as not to compromise the acquisition of soil-based resources (water and dissolved ions) and anchorage.

Keywords: cuttings, domestication marcots, propagation root morphology, vegetative propagation

This chapter is drawn from the following publication: Asaah E.K., Tchoundjeu Z., Wanduku T.N., Van Damme P. (2010). Understanding structural roots system of 5-year-old African plum tree of seed and vegetative origins (*D. edulis* (G. Don) H. J. Lam). *Trees Structure and Function* 24:789-796.

3.1 Introduction

Dacryodes edulis (G. Don) H. J. Lam (Burseraceae), an evergreen humid tropical forest species from Africa, now has been introduced in numerous African farm settings where it is particularly planted in association with cash crops like cocoa and coffee, in home-gardens and other tree crop fields (Ayuk et al., 1999). It is highly valued for its fruits (Mialoundama et al., 2002) in the humid zones of West and Central Africa. Vegetative methods of propagation through leafy stem cuttings and marcotting have been developed and used successfully (Mampouya et al., 1994; Kengue and Tchio, 1994; Mialoundama et al., 2002). Vegetative propagules are increasingly becoming more attractive to users due to the added advantage of capturing desirable traits, quicker fruit yields and their potential to resolve problems of seasonality in *D. edulis* fruit production (Mng'omba et al., 2008).

Some knowledge of the structural development of root systems is essential to understand the ecological niche of tree species in order to optimize their productivity in various silvicultural and agroforestry systems (Huxley, 1983; Von Maydell, 1987; Toky and Khosla, 1989a). Studies on root systems should concentrate more on root architecture and distribution than on individual root numbers. Root system architecture influences anchorage and is an important factor in guaranteeing tree stability (Coutts, 1983a). Studies on root architecture do not include root hairs but are concerned with the entire root system or a large subset of the root system of an individual plant (Lynch and Nielsen, 1996). Parameterization and validation of any species' root architecture model requires the excavation of complete root systems, or at least large representative parts of them, but on a smaller scale than in purely descriptive methods (Salas et al., 2004). Bohm (1979) and Vogt and Persson (1991) are of the opinion that tree root systems consist of a main structural root component with diameters > 2 mm, which form the base for support and anchorage of the plant, complemented by a fine root component with root diameters ≤ 2 mm which consists of long exploratory branching systems, that include root hairs that play a role in nutrient and water uptake of the plants.

D. edulis trees of seed origin have been described to have a tap root system, whereby the central root develops from the root primordium, while *D. edulis* trees of vegetative origin (marcots) from nurseries have been reported to have an adventitious root system (Kengue, 2002) with roots developing from preferentially the basal end of the cut surface (Davies et al., 1982). As a result, marcots, cuttings and vegetatively propagated plants in general are alleged to suffer from lodging due to the absence of a tap root (Kengue, 2002).

In the absence of adequate information on the rooting system of tropical trees that form an important part of tropical agroforestry systems, there exist information gaps in the quest to improve the value of existing and/or newly to-be-designed agroforestry systems. The present study describes and compares root morphology and architectural characteristics of *D. edulis* trees of both seed and vegetative origin (cuttings and marcots) in view of assessing their relative suitability for introduction in agroforestry systems.

Specifically, the study tested the following key hypotheses:

- *D. edulis* trees of seed and vegetative origins (cutting and marcot) differ in structural root architecture.
- *D. edulis* trees of seed and vegetative origins differ in root morphology.

3.2 Material and methods

3.2.1 Study site

The study was implemented in a *Dacryodes edulis* (G. Don) H. J. Lam. (Burseraceae) orchard established in 1999 and 2001 respectively by the World Agroforestry Center (ICRAF) in the equatorial humid forest zone of Cameroon. This orchard is situated near Mbalmayo (3^{o} 40' N, 11^{o} 00'E) at 650 m above sea level, with 1,500 hours/annum of insolation, and a bimodal distributed total annual rainfall of 1,200 - 2,500 mm (Figure 3.1).

The orchard was originally set up as a comparative growth trial between *D. edulis* trees of seed and vegetative (juvenile cuttings and marcots) origin from the same mother plant of at most three months difference in age. Trees of both seed and vegetative origins were raised in black-perforated polybags carrying about 3 kg rich top soil from a fallow mixed with river sand at a ratio of 2:1 and put on a raised platform to facilitate air pruning of roots at ICRAF's nursery for 6 months before transplanting into the field in 2001. The trial was established as a randomized complete block design of 10 replicates each, laid out as single tree plots. Trees were transplanted in holes of 40x40x40 cm^3, filled back to the depth of 30 cm with a mixture of compost and top soil at a spacing of 10x10 m^2. Tree density per hectare is thus 100. The soils of Mbalmayo fall under the ferrasol major soil grouping with soil unit defined as a xanthic ferrasol (FAO, 1991). Weeds were manually controlled in the plots using cutlasses. In order to minimize injury on the trees, weeds adjacent to trees were hand-weeded.

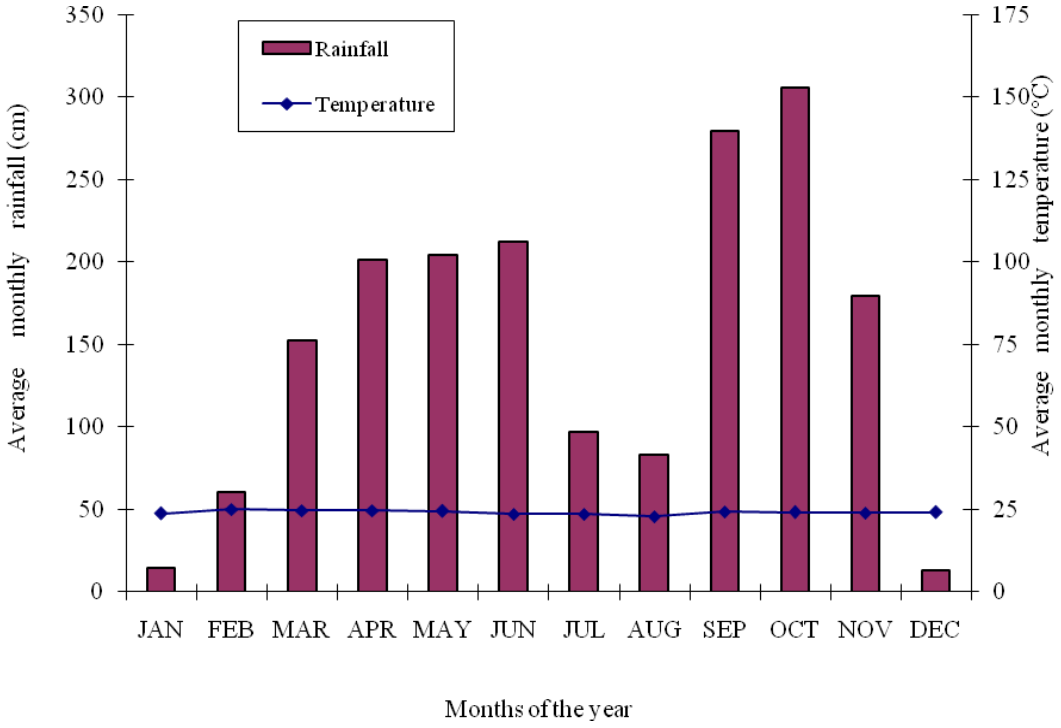


Figure 3.1. Average monthly rainfall and temperature in Mbalmayo, Cameroon 1999-2007

Source: Institute of Agricultural Research for Development (IRAD), Cameroon

Ring weeding was done around each tree every other month during the rainy season and the entire plot slashed quarterly. During the dry season, trees were mulched with weeds cleared from adjacent plots. Neither fertilizers nor herbicides were used for either soil fertility or weed management, respectively.

3.2.2 Tree selection

From a plot of 45 *D. edulis* trees distributed into 15 trees each of seed, cutting and marcot origins planted in 1999, the root system of three randomly selected trees of seed, cutting and marcot origins, respectively were excavated in order to assess their rooting systems. Each tree constituted a replicate. In a similar study in which both fine and structural root systems of 13 multiple wood species commonly found in agroforestry system in Nigeria were evaluated, Akinnifesi et al. (1999a) used a destructive sampling method and excavated two trees from each of the 13 trees species in two of the three replicates of the study. In a separate study to map root systems of urban trees *in situ*, Stokes et al. (2002) adopted a non-destructive method using Ground Penetrating Radar (GPR). Three trees were also randomly sampled to generate raw data used in modelling 3 dimensional rooting systems of the sampled trees.

3.2.3 Structural root assessment

Root densities were evaluated on profile walls using a metal frame of 1 x 1 m^2 demarcated into grids of 10 x 10 cm^2 with a rubber band and hung on the wall (Figure 3.2). Only roots with diameter of > 2 mm were considered, and separated from finer roots using a calliper measured to the nearest millimetre. This was followed by root morphology description by carefully exposing and following the course of all major roots (vertical and horizontal) for each tree thus excavated within a radius of 2 m around the tree trunk (Figures 3.3 **A** and **B**).

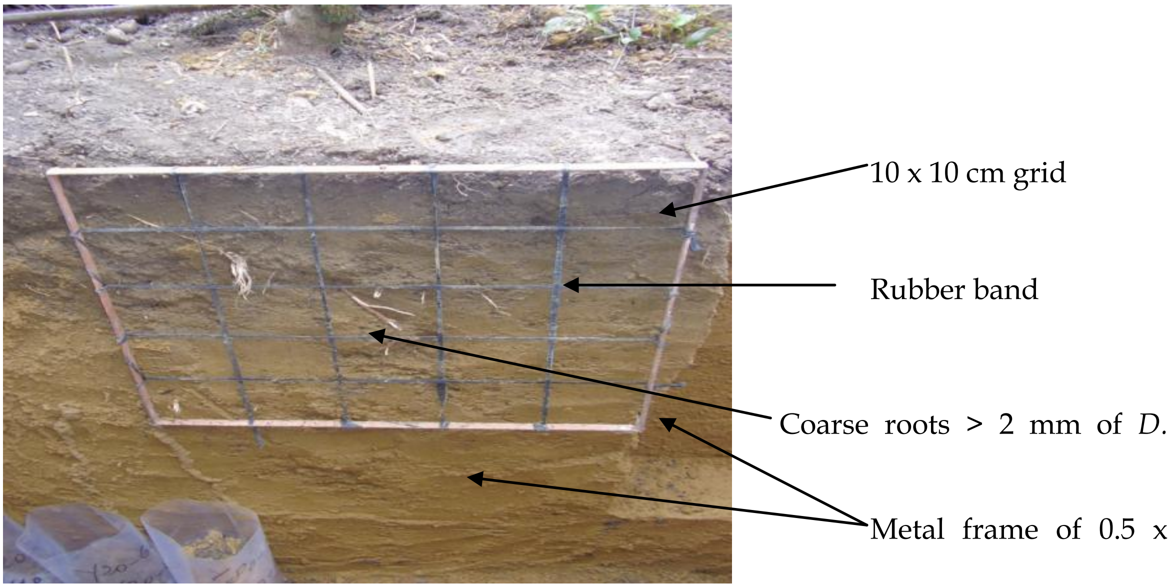


Figure 3.2. Typical frame on profile wall from where structural roots (> 2 mm) of *D. edulis* trees were assessed (Photo by EK Asaah)

Roots having angles with a horizontal plane ≤ 45^0 were considered as horizontal roots while those with angles ≥ 45^0 were classified as vertical. A longitudinal representation of the root system of each tree type was drawn on a graph paper of size 297 x 210 mm using the following scale: depth of 1:10 cm and lateral distance of 1.5: 25 cm. Lateral roots were monitored by exposing all structural roots at 0 to 50 cm top soil depth at all sides of the tree surveyed, and their length followed to a diameter of 5 mm from the tree trunk. This description was followed by total excavation of the entire root system and subsequently re-arranging it as far as possible into its original position in the laboratory.

Figure 3.3. Vertical (A) and horizontal (B) distribution of a typical *D. edulis* tree root system of vegetative and seed origins respectively (Photos by EK Asaah)

Roots originating from the main root, irrespective of their sizes, were designated as primary roots, secondary and tertiary roots, respectively. In trees of vegetative origin (cuttings and marcots), there was no distinctive main root so the most prominent and central roots were considered as the main roots. Main roots for each propagule type were stretched and their lengths and diameters at 10 cm intervals measured.

Crown spread (diameter), for each selected tree was measured using a measuring tape (± 1cm). A root spread to crown-spread ratio was calculated as a proxy for lateral root spread from tree base. Data of coarse root number per depth (0-20, 20-60 and 60-100 cm), and lateral distance from tree base was analyzed using GenStat 9.1 and significantly different means were separated by the standard errors of the differences of means. The reconstituted rooting systems were drawn on graph paper.

3.3 Results

3.3.1. Structural root density of *D. edulis* trees of seed and vegetative origin

At fixed lateral distances from the tree bases to 50 cm along the tree row, trees of seed origin recorded the highest root density at a soil depth of 0 to 20 cm, which was similar to root density values of trees of marcot origin but differed significantly ($p = 0.032$) from trees of cutting origin. However, the situation was opposite at a soil depth of 20 to 60 cm where trees of marcot origin were observed to have the highest root density that differed very significantly ($p < 0.01$) from that of trees of seed and cutting origins. Furthermore, at a soil depth of 60 to 100 cm, trees of cutting origin recorded the highest root density values, which differed significantly ($p = 0.02$) from those of trees of seed and marcots origins (Table 3.1).

Table 3.1.Mean root density at various soil depths, measured at lateral distances from tree bases to 50 cm along rows of *D. edulis* trees of seed and vegetative origins

	Depth (cm)		
Tree origin	0-20	20-60	60-100
	no. roots/m^2		
Seed	23.3_a	4.2_c	5.0_b
Cutting	6.7_b	15.0_b	18.3_a
Marcot	25.0_a	17.5_a	4.2_b
P-Value	0.03	<0.01	0.02
S.e.d	4.71	0.68	3

Means with the same letter in a column are not significantly different according to the standard error of the differences of mean (s.e.d).

It was also noticed that at lateral distances of 50 - 100 cm along the tree row, trees of seed origin recorded the highest root density, which differed significantly ($p = 0.02$) from those of trees of cuttings and marcots origins. However, at a soil depth of 20 to 60 cm, fewer roots were observed; density values for trees of seed origin and marcots were similar and both differed significantly ($p = 0.05$) from those of cuttings (Table 3.2).

Table 3.2. Mean root density at various soil depths measured at lateral distances of 50 cm to 100 cm along rows of *D. edulis* trees of seed and vegetative origins.

Tree origin	Depth (cm) 0-20	Depth (cm) 20-60
	No. roots/m²	
Seed	25.0_a	4.0_a
Cutting	10.0_b	2.5_b
Marcot	11.7_b	5.0_a
P-value	0.02	0.05
S.e.d	3.19	0.68

Means with the same letter in a column are not significantly different according to the standard error of the differences of mean (s.e.d)

3.3.2 Structural root morphology and lateral root spread of *D. edulis* trees of seed and vegetative origins

Trees of seed origin were characterized by a central tap root, reaching depths of about 1.2 m. Lateral roots were mainly concentrated in the upper 20 cm of the soil and their diameters were observed to decrease progressively with length (Figure 3.7). Several secondary roots branched vertically downwards, sometimes reaching depths of 90 cm. The longest lateral roots reached a length of 5.11 m (Table 3.3) with a crown spread of 5.82 m.

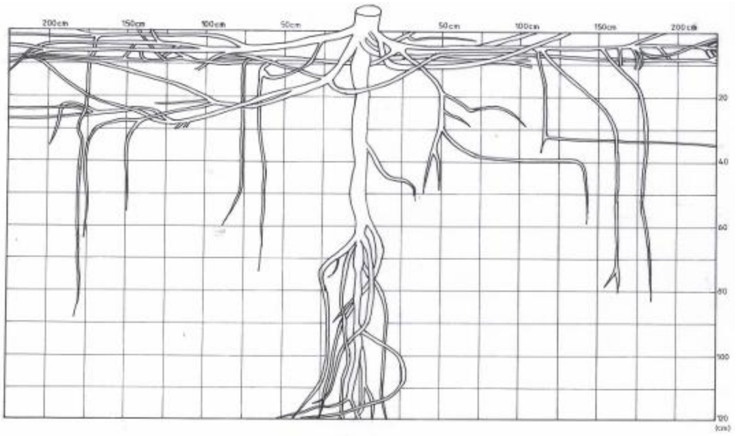


Figure 3.4. Root morphology and distribution pattern of a typical five years old *D. edulis* tree of seed origin.

Trees of cutting origin showed three strong vertical roots (sinker roots) (d > 5 mm) which rapidly dissipated into secondary and tertiary roots of lesser diameter with the longest reaching depths of 1.31 m. Lateral roots branched within the upper 50 cm of the soil and gradually curved towards the soil surface with increasing distance from the trunk (Figure 3.5). Maximum lateral root spread was 5.73 m, with a root spread to crown spread ratio of 0.94. Few secondary roots branched vertically downwards, reaching depths of 50 cm (Table 3. 2 and 3. 3).

Table 3.3. Lateral root spread (cm) and aerial characteristics of five year old *D. edulis* trees of seed and vegetative origins

Tree origin	Lateral root spread (m)	Root collar diameter (cm)	Aerial crown spread (m)	Root spread/crown ratio
Seedlings	5.11_{a}	14.3_{ab}	5.82_{a}	0.88
Cuttings	5.73_{a}	21.1_{a}	6.10_{a}	0.94
Marcots	5.36_{a}	12.1_{b}	3.90_{b}	1.37
P-value	0.65	0.11	0.024	
S.e.d.	0.11	3.36	0.51	

Means with the same letter(s) in a column are not significantly different according to the standard error of the differences of means (s.e.d.).

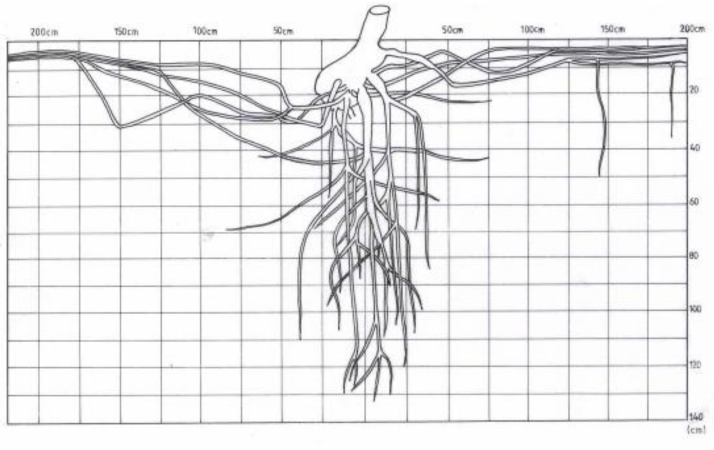


Figure 3.5. Root morphology and distribution pattern of a typical five years old *D. edulis* tree of cutting origin

Trees of marcots origin were observed to have developed thick, relatively short prominent vertical roots, reaching depths of 1.15 m. Lateral roots branched within the upper 20 cm of the top soil and extended obliquely with increase in distance from the trunk, reaching a maximum spread of 5.36 m with a crown spread of 3.9 m (Table 3.3). Also, diameters of these roots decreased sharply with length within the first 50 cm. Sinker roots were not very prominent. However, we observed a distinct central vertical root from where secondary roots radiated (Figure 3.9).

Figure 3.6. Root morphology and distribution pattern of a typical five years old *D. edulis* tree of marcot origin

3.3.3 Number of primary, secondary and tertiary roots on *D. edulis* trees of seed and vegetative origins

The number of primary, secondary and tertiary roots on *D. edulis* trees of seed and vegetative origins did not differ significantly at $p \leq 0.05$ (Table 3.4).

Table 3.4. Primary, secondary and tertiary roots of five year old *D. edulis* trees of seed and vegetative origins

Tree origin	**Primary**	**Secondary**	**Tertiary**	**Total**
Seedlings	20.7_a	76.0_a	65.3_a	162
Cuttings	19.7_a	75.0_a	51.7_a	146
Marcots	14.3_a	66.7_a	48.7_a	130
P-Value	0.62	0.8	0.59	
s.e.d.	6.59	15.09	16.29	

Means with the same letter(s) in a column are not significantly different according to the standard error of the differences of means (s.e.d.)

3.3.4 Main structural root diameter of *D. edulis* trees of seed and vegetative origins

Trees of cutting origin recorded the largest main root diameters at root lengths of 10 cm, 20 cm and 30 cm, respectively, which was followed by trees of seed origin, and lastly by trees of marcots origin. However, main root diameter of trees of cutting origin at 20 cm depth was similar to that of trees of seed origin, but differed ($p \leq 0.03$) from those of trees of marcots origin. The situation was opposite at root lengths of 40 and 50 cm where trees of seed origin were largest in main root diameters, followed by trees of cutting origin and lastly by trees of marcot origin. At root length of 60 cm and beyond, trees of cutting origin again emerge with largest root diameter which differed significantly from trees of marcots origin at 70 cm and 110 cm ($p \leq 0.05$ and $p \leq 0.01$, respectively). At all root lengths, trees of marcot origin showed the smallest main root diameter values (Figure 3.7).

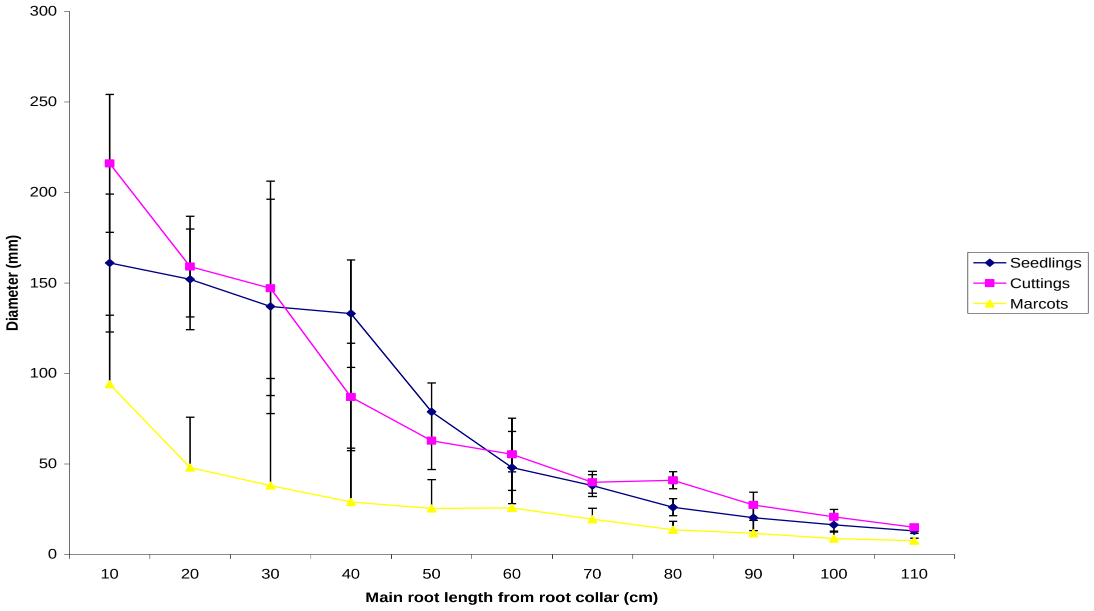


Figure 3.7. Main root diameters (mm) of five year old *D. edulis* trees of seed and vegetative origins

3.3.5 Structural root depth (length) of main root of *D. edulis* trees of seed and vegetative origin

No significant differences were recorded in main rooting depth and length for trees of seed and vegetative (cutting and marcot) origins (Figure 3.8).

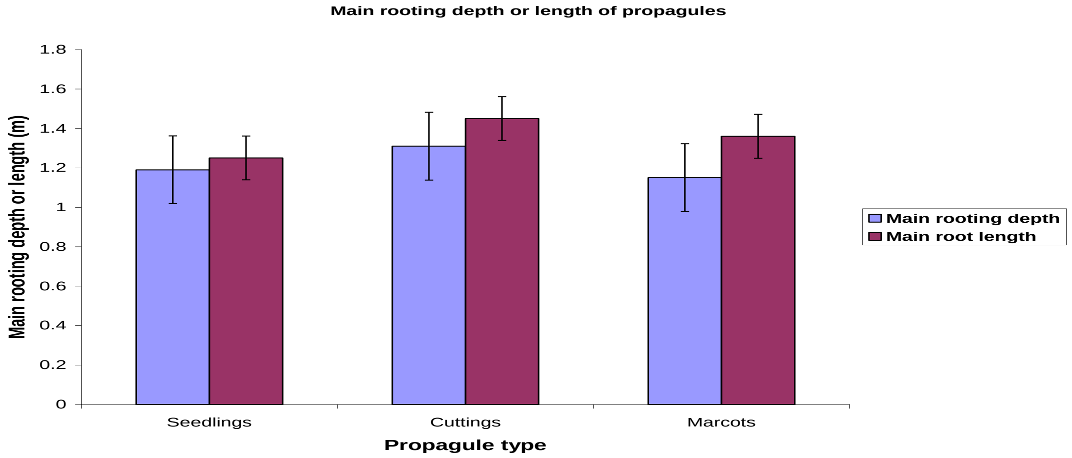


Figure 3.8. Main root depth (cm) and length (cm) of five year old *D. edulis* trees of seed and vegetative origins

3.4 Discussion

Trees of seed origin (n= 3) had a well-developed tap root characterized by a symmetrical root system, different from trees of vegetative origin (cuttings and marcots) that rather presented a system of vertically arranged, thick and deep adventitious roots, resembling tap roots. The former develop from the radical which grows as the primary root giving rise to the secondary and tertiary roots, whereas the latter adventitious roots are roots induced from stem cuttings. Adventitious roots were characterized by an asymmetrical spread with roots oriented sometimes predominantly in one direction. In this respect, it has been recognized that where main roots are poorly developed or even absent at one side, tree stability will be reduced (Coutts, 1983a; Nielsen, 1990; 1991).

The above differences in rooting systems of trees of seed and vegetative origins might have been due to the method of tree propagation as the root system of trees of seed origin develop directly from root primordia (Hartmann et al., 2002), while the root system of trees of vegetative origin develop indirectly and/or arise from buds in the parenchyma tissue not directly associated with apical meristems (Davies et al., 1982; De Klerk, 2001; Zimmermann and Brown, 1971).

This therefore suggests the rooting system of *D. edulis* trees of seed origin develops from the primary root that emerges from the seed, whereas those of trees of vegetative origin (cuttings and marcots) are derived from adventitious roots at the stem bases. The root system of *D. edulis* trees of vegetative origin could thus be liken to the rooting system of Sitka spruce (*Picea sitchensis*), that was reported to have woody roots radiating from the stem base at shallow angles (Coutts, 1983b) from which numerous vertical roots descend. In some species such as Scot pine (*Pinus sylvestris*) there often is a dominant vertical root, as large as or larger than the large radiating roots (Köstler et al., 1968). Similarly, main root length (depth) in *D. edulis* was not significantly different between trees either of seed or vegetative origins.

While root systems vary widely both within and between species (Krasilnikov, 1968; Weaver, 1958), and a wide variation in branching forms can be found within a species depending on age (Köstler et al., 1968), site conditions (Pomerleau and Lotie, 1962) and genotype (Akinnifesi et al., 2004; Zobel, 1996), the observed development of dormant vertical roots in *D. edulis* trees of vegetative origin, could be an important adaptation to the absence of a tap root so as not to compromise the acquisition of soil-based resources (principally, water and dissolved ions) and anchorage. The anchorage potential of trees with deeply-located lateral roots has been described by Coutts (1983a), to result from stiffening and reinforcing effect under wind tension.

Lateral root spread ranged from 5.11 m in trees of seed origin to 5.73 m in trees of cutting origin. Akinnifesi et al. (1999), reported lesser spread of roots of 13 multipurpose trees in Nigeria at 4 years of age ranging from less than 1 m to more than 2 m. Similarly, in a study of root development in wild jack trees (*Artocarpus hirsutus*), Jamaludheen et al. (1997), reported that lateral root spread rarely went beyond 2.3 m during the first 10 years of tree growth. In another study, at 5 years of age, lateral root spread varied between 8 m in *Pithecellobium dulce*, and 1.7 m in *Syzygium cumini* (Chaturvedi and Das, 2003). Roots of *Senna siamea* at 4.5 years of age extended laterally to about 9 m from the stem in a semi-arid region of Kenya (Rao et al., 1993).

Lateral root spread values of *D. edulis* trees of seed and cuttings origins were lower than crown spread while the reverse was true for trees of marcot origin. Similarly, lower root spread compared to crown spread was reported by Toky and Bisht (1992) for 6-year-old trees of *Acacia catechu, A. nilotica, Albizia lebbeck, Azadirachta indica, Dalbergia sissoo, Melia azedarach, Morus alba, Zizyphus mauritiana* and *Leucaena leucocephala*.

Total number of primary roots (roots emerging from the main root) ranged from 14 to 21 in trees of *D. edulis*. Similar short number observations in total number of primary roots (4 to 11 roots) were reported for many coniferous trees (*Pinus resinosa*) (Fayle, 1975ab), *Tsuga heterophylla*, *Thuja plicata* and *Pseudotsuga menziesii* with the number ranging from 3 to 8 (Eis, 1974). Higher ranges of primary root patterns from 8 to 44 roots have been observed in 12 tree species (*Acacia catechu, A. nilotica, Albizia lebbeck, Azadirachta indica, Dalbergia sissoo, Melia azedarach, Morus alba, Prosopis cineraria, Zizypus mauritiana, Populus deltoides, Eucalyptus tereticornis* and *Leucaena leucocephala*) (Toky and Bisht, 1992).

The total number of primary roots of *D. edulis* trees, irrespective of tree origin (seed, cutting and marcot), overlaps between both short and longer figures cited above, suggesting that *D. edulis* primary roots fall in a medium range of primary root patterns for tree species.

Main root depth of *D. edulis* trees irrespective of vegetative or seed origin ranged between 1.0 to 1.5 m. Similar observations have been reported for tap roots of multipurpose trees specieslike *Triplochiton scleroxylon*, *Albizia niopoides* and *Tetrapleura tetraptera* (Akinnifesi et al., 1999).

3.5 Conclusion

Methods of tree propagation can influence root system configuration of mature trees. *D. edulis* trees of seed origin were shown to consist of a tap root system, while trees of vegetative origin (cuttings and marcots) had adventitious root morphology. *D. edulis* trees of vegetative origin had a wider and deeper root spread compared to trees of seed origin.

Large-sized tap roots may have a greater capacity to penetrate compacted soils and can better resist or tolerate uprooting damage by wind and survive drought conditions.

The adventitious rooting systems of trees of vegetative origin (especially cuttings) were observed to have significantly deeper vertical (main) root compared to the tap root of trees of seed origin. *D. edulis* trees of vegetative origin therefore have developed dominant vertical roots which could be an important adaptation to the absence of a tap root so as to optimize the acquisition of soil-based resources (principally, water and dissolved ions) and anchorage.

Through this study, we have attempted to describe, quantify and establish the existence of morphological differences in the root system and distribution of *D. edulis* trees of seed and vegetative origin (cutting and marcot). Therefore, further studies are recommended so as to relate these morphological differences of the rooting system to various belowground functional attributes like water and nutrient uptake.

CHAPTER 4

4. EFFECT OF PROPAGATION METHODS ON FINE ROOT DISTRIBUTION IN *DACRYODES EDULIS* (G. DON) H. J. LAM TREES OF SEED AND VEGETATIVE (CUTTING AND MARCOT) ORIGINS

Abstract

Belowground tree growth attributes determines whether associations will be complementary or competitive in an agroforestry context. A study on fine root (d ≤ 2 mm) distribution patterns of *D. edulis* based on root density (RD), root length density (RLD) and root weight density (RWD) was conducted to evaluate the effect of propagation methods on fine root distribution. Results showed that *D. edulis* trees of seed origin had greater RD ($P \leq 0.001$) than trees of vegetative origin (cuttings and marcots) in the upper soil stratum (0-30 cm). Similarly, in the uppermost soil stratum (0-10cm), RLD and RWDs varied significantly ($p<0.01$). Trees of seed origin had an exponential distribution pattern for fine RD, RLD and RWD with depth to 80 cm. In contrast, the distribution pattern of fine roots of trees of vegetative origin (cuttings and marcots) were quadratic for the same variables which increased in the 20-30 cm soil depth stratum before declining steadily to a depth of 80 cm. The findings of this study suggest, that *D. edulis* trees of vegetative origin (cuttings and marots) are likely to be less competitive than trees of seed origin when intercropped with shallow rooted annual plants in an agroforestry system for belowground resources.

Key words: agroforestry, fine roots, indigenous fruit tree, propagation methods

This chapter is drawn from the following publication: Asaah E.K., Wanduku T.N., Tchoundjeu Z., Kouodiekong L., Van Damme P. (In press). Do propagation methods affect the fine root architecture of African Plum *(Dacryodes edulis* (G. Don H. J. Lam)? *Trees Structure and Function* 00:00-00

4.1 Introduction

Dacryodes edulis (G. Don) H. J. Lam. (Burseraceae) is a priority agroforestry species in the humid zone of West and Central Africa (Franzel et al., 1996) where it is indigenous to countries of the Gulf of Guinea (Kengue, 2002). The fruits are easy to prepare and highly nutritious. They are an important source of food and edible oil (Silou, 1994; Umoti and Okyi, 1987). They are widely marketed both locally and regionally (Awono et al., 2002) and even, to a small extent, internationally (Tabuna, 1999). Consequently, these fruits are an important source of income for vulnerable and marginalized people (women and the poor). This value is further enhanced by the overlap of the fruit maturing season with the start of the school year (Schreckenberg et al., 2002; 2006) so providing parents the most-required financial resources from fruit sells to prepare school-going children. Agroforestry tree products such as these are also beneficial because of their easy access and limited need for capital investment (Ayuk et al., 1999). Conventionally, *D. edulis* is grown from seed (Kengue, 1997) as shade trees in cocoa and coffee farms as well as a fruit crop in home gardens and food crop fields (Ayuk et al., 1999). In this way, farmers have initiated the domestication of this tree, especially in Cameroon (Leakey et al., 2004a).

Trees on-farm are also a source of livelihood diversification strategy for farmers through the provision of tree products and ecosystem services such as fruit, food, fodder, firewood, and timber (Albertin and Nair, 2004; Rice, 2008). Thus, the establishment of productive and useful perennials like *D. edulis* trees would help to restore agro-ecological functions and enhance the provision of environmental services (carbon sequestration, help to counter climate change) while also generating income. Meanwhile, filling the niches under the trees through intercropping with useful and marketable annuals – herbs and other species would be important for further improvement of livelihoods options of rural communities, and the overall profitability and value of their farming systems. According to Leakey (2010), all these creates a more sustainable and multifunctional approach to land use.

In 1995, *D. edulis* was chosen by farmers in West and Central Africa, as a priority species for more intensive domestication (Tchoundjeu et al., 2002) through a programme of genetic selection (Leakey et al., 2002; 2005) and vegetative propagation using stem cuttings and marcotting (Kengue and Tchio, 1994; Mampouya et al., 1994; Mialoundama et al., 2002) in order to preserve parent material characteristics. However, it is commonly thought that trees of vegetative origin have an undesirable root system and are prone to wind-blow due to the absence of a tap root. However, Asaah et al. (2010) reported that mature *D. edulis* trees of vegetative origin have well-developed primary lateral and deep sinker roots which confer stability. The present study extends this assessment of rooting system structure and distribution with a focus on the fine roots.

Knowledge of rooting/root distribution as influenced by management and site conditions is necessary for the optimization of agroforestry systems (Ong and Leakey, 1999; Schroth, 1995). The spatial and temporal distribution of roots in multispecies agro-ecosystems is known to vary with species, tree husbandry and edaphic site factors (Akinnifesi et al., 1995; Schroth, 1995; 1998; van Noordwijk and Purnomosidhi, 1995). According to van Noordwijk and Purnomosidhi (1995), desirable root architecture requirement differs for sequential and simultaneous agroforestry systems.

Akinnifesi et al. (2004); Vogt and Persson (1991), have classified tree root systems into 2 main components based on the root diameter which are: (i) the main structural roots component (diameter > 2 mm) for the support and anchorage of the plant, and (ii) the fine roots component (diameter ≤ 2 mm), which consist of long exploratory branching roots with root hairs for water and nutrient uptake. It's these fine roots which allow trees to compete with crops in agroforestry systems (Smucker et al., 1995). Information on root distribution of different species in agroforestry systems is important to understand the belowground root interactions between trees and crops. Belowground competition can mask or suppress many of the long-term benefits conferred by trees in simultaneous agroforestry systems as tree root development in the crop root zone will lead to competition for resources (Akinnifesi et al., 2004; van Noordwijk and Purnomosidhi, 1995).

Several studies have examined the distribution of structural primary roots (Akinnifesi et al., 1999a; Asaah et al., 2010; Coutts, 1983; Toky and Khosla, 1989). A few others have described and compared trees fine root distribution: on eight tree species in India (Chaturvedi and Das, 2003); on 13 multipurpose trees in Nigeria (Akinnifesi et al., 1999a); on *Grevillea robusta* and *Gliricidia sepium* in Kenya (Odhiambo et al., 2002), and on *Senna siamea* in three regions of Togo (Vanlauwe et al., 2002). Closely associated tree and crop roots often compete when the nutrient depletion zones around the roots overlap in soil mass (Akinnifesi et al., 1996; Schroth, 1998) although gains in profit from the mixtures can greatly exceed the loss in value of arising crop Yield Gap (the difference between potential yield of food crops and the actual yield achieved by farmers through mix agroforestry system) [Asaah et al., 2011, Leakey, 2010]. Moreover, as in the case of fruit trees, net benefits can be slow to materialize due to the long gestation period before tree maturation. However, vegetative propagation of mature tissues using marcotting, grafting and rooting of stem cuttings (from mature marcots and grafts) can speed up delivery of these benefits. Moreover, such trees propagated vegetatively, have an earlier capacity to flower and fruit with the possibility to extend the fruiting season.

Unfortunately, little is known of the belowground interactions of rooting systems of sexually mature indigenous fruit trees propagated by either seed or vegetative means. The present study investigated the effect of propagation methods on fine root distribution of fruiting *D. edulis* trees of both seed and vegetative origin (cuttings and marcots) in view of hypothesising their potential relative competiveness for belowground resources.

Specifically, the study tested the following key hypotheses:

- *D. edulis* trees of seed and vegetative origin have similar fine root density and root length density.
- *D. edulis* trees of seed and vegetative origin differ in fine root distribution.

4.2 Material and methods

4.2.1 Study site

The study site characteristics are reported in **Chapter 3, section 3.2.1.**

4.2.2 Sampling technique and fine root assessment

Five (05) *D. edulis* trees of the same age and of seed and vegetative (cutting and marcots) origins respectively were randomly selected and their fine root system assessed between June and July, 2007. A trench was dug perpendicular to the tree row, 5 cm away from the tree base and extending 1.2 m away from the tree base into the tree rows and 80 cm deep (Figure 4.1).

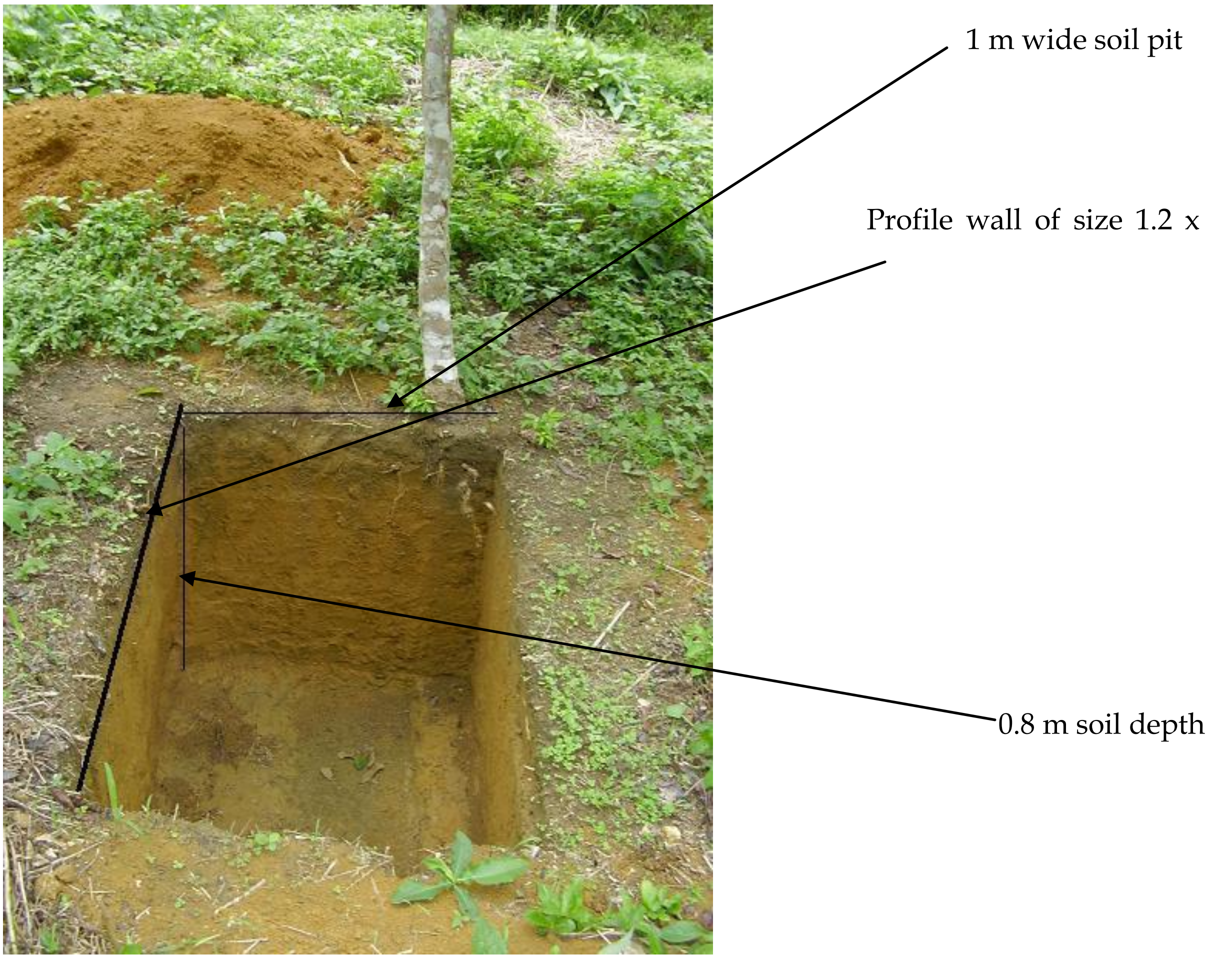


Figure 4.1. Typical trench of size 1.2 m x 1m x 0.8 m next to *D. edulis* tree from where fine root characteristics were determined (Photo by EK Asaah)

After smoothing the vertical wall plane, root abundance was determined using a 10 by 10 cm^2 grid by counting all fine roots (diameter (d) ≤ 2 mm) of *D. edulis* (Figure 4.2) following the method of Akinnifesi et al. (1999). *D. edulis* fine roots were identified by their white colour and separated from large roots with the aid of a calliper (Junior Roche France) graduated to 0.01mm before recording. Every cut-off root was recorded where it crossed the plane of observation. However, this technique counts roots of different orders of branching as individual roots. Roots lying parallel to the profile wall were recorded once per 10 x 10 cm grid.

After determining root abundance, soil samples were taken from the profile walls at 15, 45, 75 and 105 cm distances away from the tree bases. At each lateral distance, eight square monolith soil samples (10 x 10 x 10 cm^3) (Figure 4.3) were dug out beginning from the surface at increments of 10 cm to 80 cm below the ground. A total of 480 soil units were dug out from the trench dug perpendicular to the 15 selected trees that were used to assess root length and root weight density. Each soil block was washed over a 710 µm Endecotts laboratory soil sieve (BS 410/1986, serial number 609553) and fine roots of *D. edulis* (d≤2 mm) were separated from large roots with a calliper.

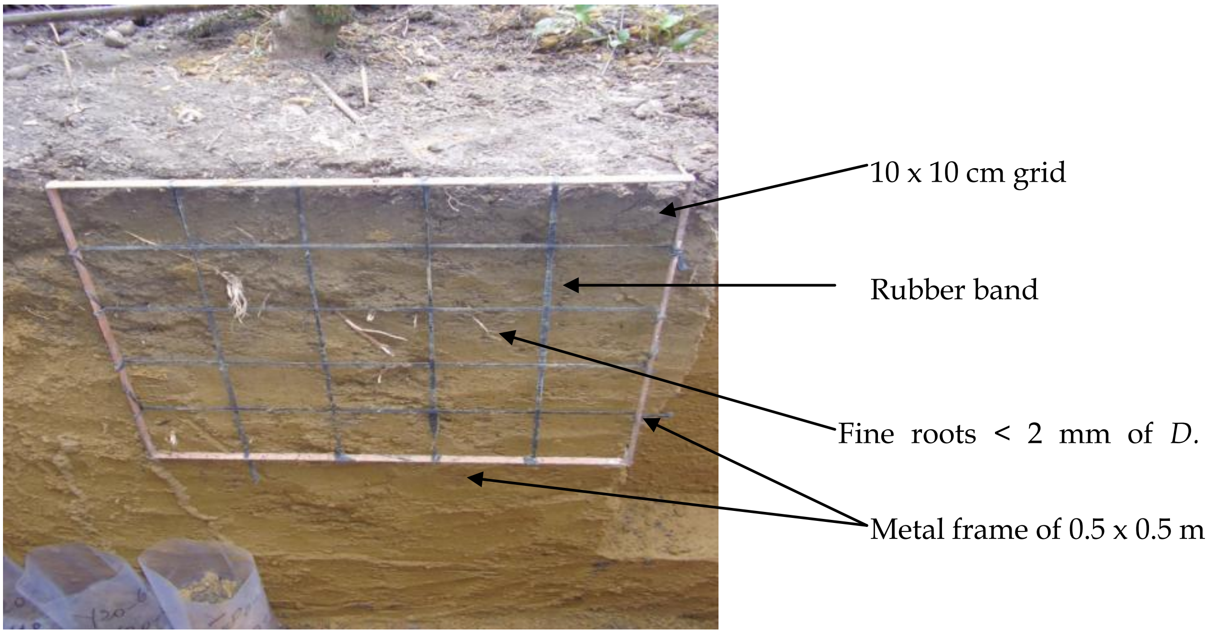


Figure 4.2. Typical soil blocks on profile wall from which *D. edulis* roots were counted (Photo by EK Asaah)

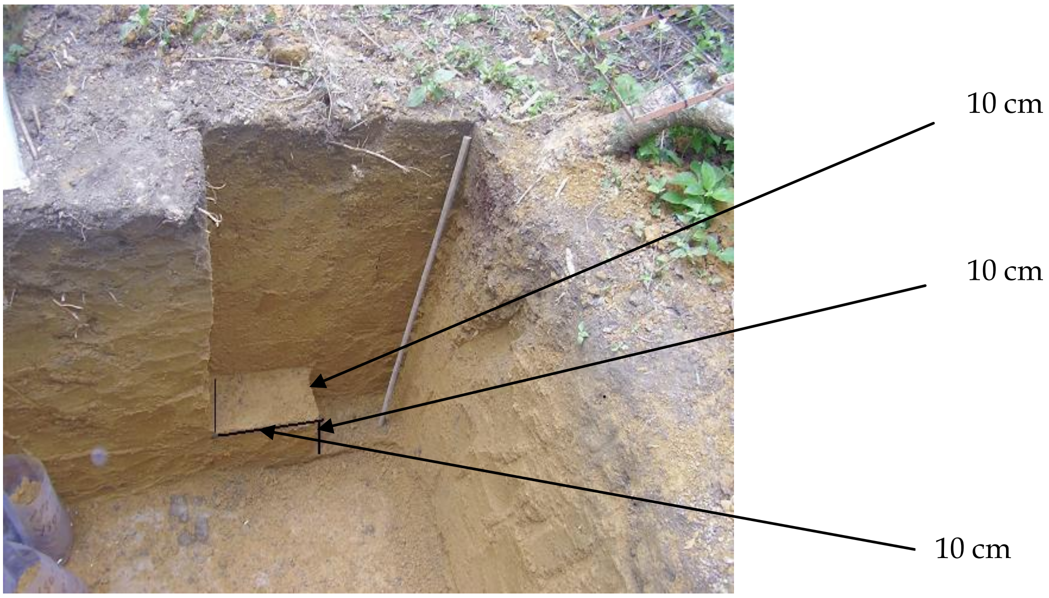


Figure 4.3. A typical soil monolith (10x10x10 cm) sample from where *D. edulis* fine roots were extracted (Photo by EK Asaah)

Root lengths of trees were estimated using the line intercept method (Eq. 4.1; Figure 4.4) described by Tenant (1975) and derived using the following formula:

$L = \pi \times N \times D / 4$……..Eq. 4.1,

where: L (mm) = root length, π = 3.14, N = number of counts, and D (mm) = graph paper grid size

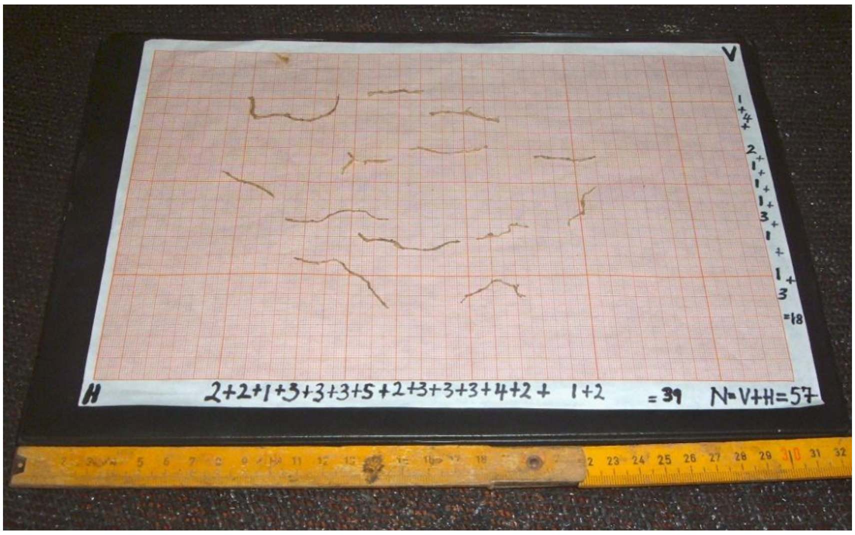


Figure 4.4. Assessment of root length on graph paper using the line intercept concept in Eq. 4.1 (Photo by N Wanduku)

Root length density (RLD) was obtained by dividing root length by the volume of soil from which roots were extracted (1000 cm^3). After measuring these lengths, root samples were dried in an oven at 80^o C for 24 hours (MacDicken et al., 1991) and weighed. Root weight density was obtained by dividing the root weight of each sample by its volume (1000 cm^3).

4.2.2 Data analysis

The numbers of fine roots counted from the vertical profile wall plane in grids of 10 x 10 cm^2 were grouped into four distance categories away from the tree bases (0-30, 30-60, 60-90 and 90-120 cm) while maintaining the eight depth classes (0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70 and 70-80 cm). Root density (RD) was computed as total number of roots inventoried divided by the area in dm^2. Correlations among the three variables RD, root length density (RLD) and root weight density (RWD) were computed to assess the relationship among them. However, for statistical analysis purposes, values for root density, root length and root weight densities were then transformed (Eq. 4.2) to meet normality and variance homogeneity requirements prior to statistical analysis (Gomez and Gomez 1984) using Genstat statistical software V.12 for windows.

Transformed Y = 1 + Square Root (observed value of Y); ………… Eq. 4.2,

Y representing root density, root length density or root weight density

Thereafter, square root-transformed data was subjected to a three-way analysis of variance (ANOVA) to assess the effects of factors (tree type or origin, soil depth, lateral distance from tree base and their interactions) on the measured parameters. Significantly different means were separated using the 5% Fisher least significant difference test. A similar decreasing fine root distribution pattern was observed from a 30 cm soil depth stratum for all trees types. Exponential curves were generated to conform to the biological responses of the variables (Eq. 4.3).

$Y = a + b (r^X)$…….. (Eq. 4.3)

where: = Y is the dependent variable (RD or RLD or RWD); X = the independent variable (soil depth or lateral distance): and a, b and r are coefficients that determine the shape and similarity of the responses among the curves per tree type. For each dependent variable, the significance of each term (F-probability of ≤ 0.05) in the accumulated analysis of deviance was used to select the best model.

4.3 Results

4.3.1 Tree type, soil depth and lateral distance interactions with *D. edulis* fine root characteristics

Relationships between root density (RD), root length density (RLD) and root weight density (RWD) were positive and highly significant ($p<0.01$) with correlation coefficients greater than 74% (Table 4.1). As a result of the strong correlation of the root characteristic (74%), and avoid redundancy, only the results of RD and RLD are presented and discussed.

Table 4.1. Strength of relationships of *D. edulis* fine root characteristics

Root density	-		
Root length density	0.82 ***	-	
Root weight density	0.74 ***	0.85 ***	-
	Root density	Root length density	Root weight density
***: Significant at 0.01%	Number of observations: 480		

The analysis of variance (Table 4.2) revealed a highly significant ($p<0.01$) two way interaction of tree type by soil depth and soil depth by lateral distance on *D. edulis* root characteristics (RD and RLD). A 2-way tree type by lateral distance interaction was not significant (p.>0.41). Similarly, a 3-way tree type, soil depth, by lateral distance interaction, was not found to affect significantly ($p>0.052$) *D. edulis* root characteristics respectively, (Table 4.2).

Patterns of fine root distribution were assessed in relation to RD and RLD against soil depth for *D. edulis* trees of seed and vegetative origins respectively. Soil depth was the only factor affecting very significantly ($P<0.01$) the drop in root density values with 42.5% of total variation accounted for by this factor (Table 4.3). Regression analysis of fine root characteristics (RD and RLD) at both upper (0-20 cm) and beyond 20 cm soil depth revealed a non significant ($P>0.55$) exponential decrease of fine root characteristics with soil depth for both trees of seed and vegetative (cutting and marcot) origins. The fine roots of trees of seed origin had a continuous exponential declining pattern for RD and RLD downwards from the upper soil stratum to a depth of 80 cm; while trees of vegetative origin (cuttings and marcots), showed a quadratic declining pattern with a point of inflexion at 20-30 cm soil depth where RD and RLD were at their maximum values beyond which they declined continuously approaching zero at a depth of 80 cm. (Figures 4.5 and 4.6).

Table 4.2. Tree type, soil depth and lateral distance interactions with *D. edulis* root characteristics

Source of variation	d.f.	**Roots density**		**Roots length density**		**Roots weight density**	
		S.S.	F pr.	S.S.	F pr.	S.S.	F pr.
Tree type	2	25.31	<.01	0.01	<.001	0.09	<.001
Soil depth	7	273.16	<.01	0.21	<.001	2.78	<.001
Lateral distance from the tree base (Width)	3	109.05	<.01	0.14	<.001	1.61	<.001
Tree type (origin) x Soil depth	14	96.27	<.01	0.03	<.001	0.42	<.001
Tree type (origin) x Lateral distance from tree base	6	1.713	0.41	0.01	0.63	0.02	0.74
Soil depth x Lateral distance from tree base	21	33.16	<.01	0.02	<.001	0.29	<.001
Tree type (origin) x Soil depth x Lateral distance	42	16.45	0.05	0.01	0.92	0.16	0.94
Residual	384	106.66		0.06		2.17	
Total	479	661.76		0.47		7.54	

Table 4.3. Cumulated analysis of deviance after regression analysis of *D. edulis* fine root characteristics

		Root density		Root length density		Roots weight density	
Change	df	S.S.	F pr.	S.S.	F pr.	S.S.	F pr.
+ Soil Depth	2	127.43	<.001	0.13	<.001	1.82	<.001
+ Tree type (origin)	2	2.31	0.09	0.02	<.001	0.22	<.001
+ Depth * Tree type (origin)	2	0.75	0.45	0	0.01	0.07	0.02
+ Separate nonlinear	2	0.58	0.55	0	0.47	0.01	0.61
Residual	351	166.57		0.17		3.04	
Total	359	297.65		0.31		5.15	
R-squared %		42.5		40.4		35	

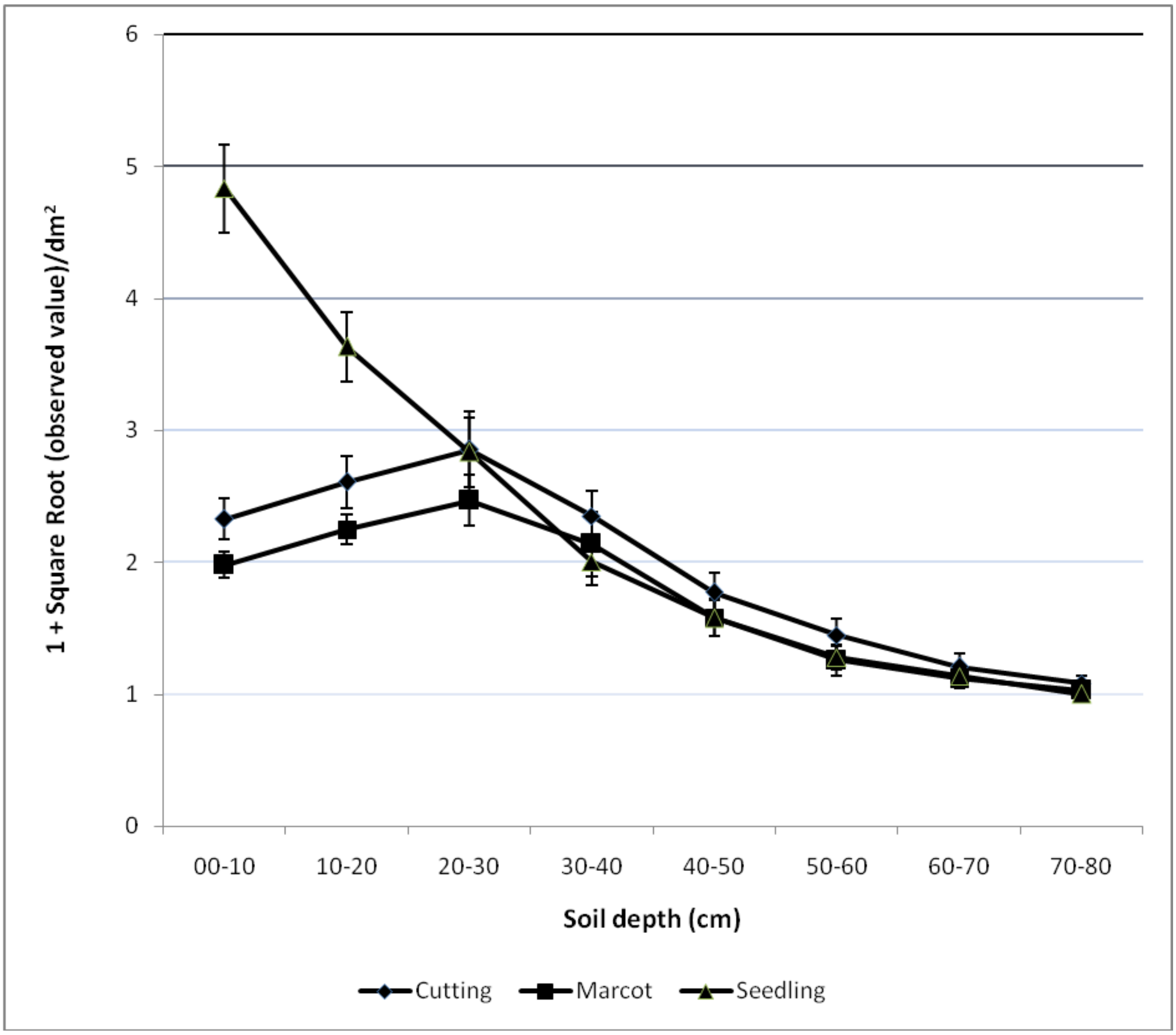


Figure 4.5. Fine root density of *D. edulis* trees of seed and vegetative (cutting and marcot) origins with soil depth (0-80 cm)

4.3.2 *D. edulis* fine root density variations against soil depth

The root densities in the upper soil layers (0-20 cm) amongst trees of seed and vegetative origins (cutting and marcot) at any given soil depth were significantly different ($P \leq 0.05$). Trees of seed origin recorded the highest RD values at soil depths of 0-10 cm and 10-20 cm (4.83 roots dm^{-2} and 3.63 roots dm^{-2}, respectively); followed by trees of cutting origin (2.33 roots dm^{-2} and 2.61 roots dm^{-2}, respectively) and lastly by trees of marcot origin (1.98 roots dm^{-2} and 2.25 roots dm^{-2}, respectively) [Figure 4.5].

4.3.3 *D. edulis* fine root length density variations against soil depth

Amongst trees of seed and vegetative origins, RLDs were significantly different ($p<0.01$) at the topmost soil layer (0-30 cm) whereas trees of seed origin were highest ($6.60.10^{-3}$ $cm.cm^{-3}$), followed by trees of cutting origin ($2.70.10^{-3}$ $cm.cm^{-3}$) and last by trees of marcot origin ($1.50.10^{-4}$ $cm.cm^{-3}$). However, at 10-20 cm depth, RLDs were similar for trees of seed ($4.10.10^{-3}cm.cm^{-3}$) and cutting ($4.10.10^{-3}cm.cm^{-3}$) origins but differed from those of trees of marcot origin ($2.50.10^{-3}.cm.cm^{-3)}$, [Figure 4.6].

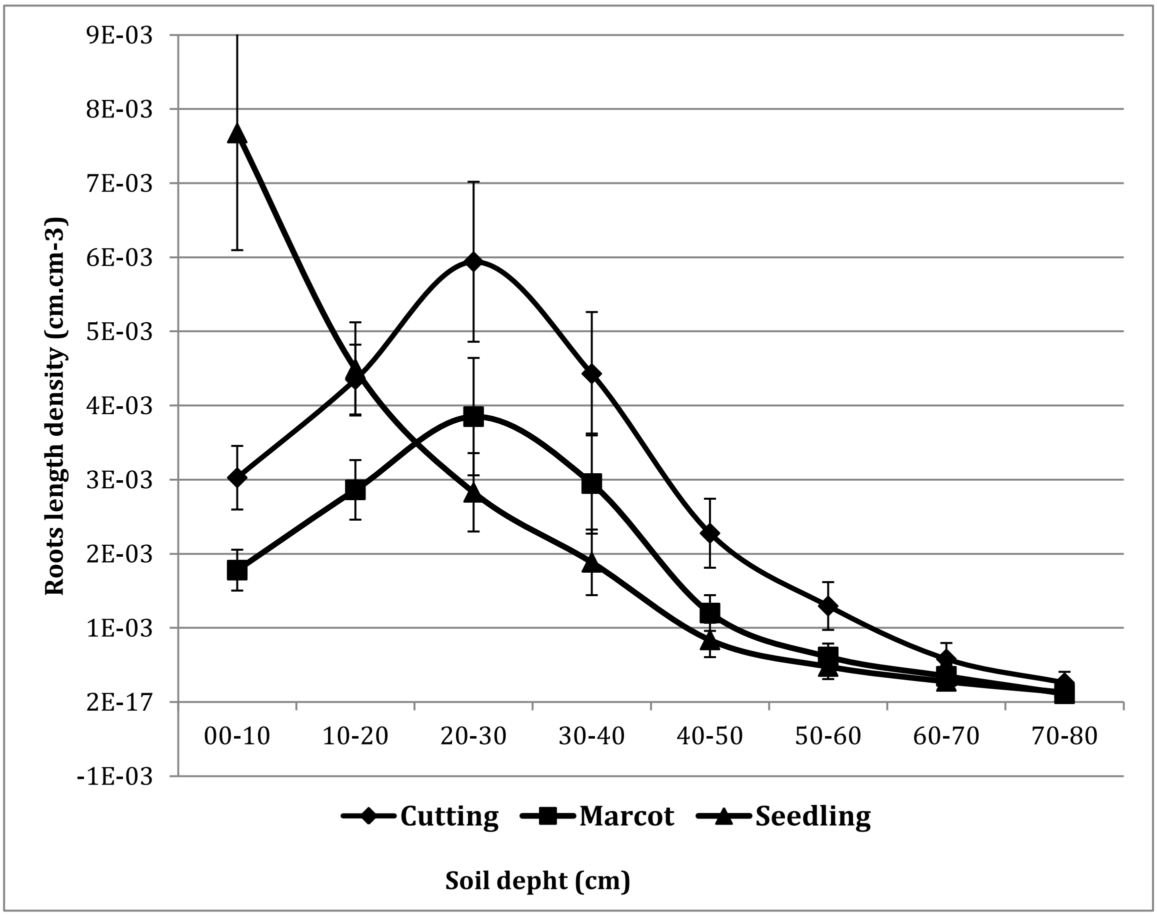


Figure 4.6. Fine root length density of *D. edulis* trees of seed and vegetative (cutting and marcot) origins with soil depth (0-80 cm)

4.3.4 Lateral root and crown spread in 5 years old *D. edulis* trees of seed and vegetative (cutting and marcot) origins

The lateral root spread in 5 years-old *D. edulis* trees of seed and vegetative origins (cuttings and marcots) did not vary significantly (p=0.065). The lateral root spread range from 5.11 m in trees of seed origin to 5.73 m in trees of cutting origin and 5.36 in trees of marcot origin. Crown spread on the contrary varied, with trees of marcot origin having a crown spread of 3.9 m, which differed significantly (p=0.024) with crown spread in trees of seed and cutting origins 5.83 and 6.1 m respectively (Table 4.4).

Table 4.4. Relative root and crown spread in 5 years old *D. edulis* trees of seed and vegetative (cutting and marcot) origins

Root characteristics	Seedlings	Cuttings	Marcots	P-value
Lateral root spread	5.11^{a}	5.73^{a}	5.36^{a}	0.65
Crown spread	5.82^{a}	6.1^{a}	3.9^{b}	0.024
Root to crown spread ratio	0.88	0.94	1.37	

Means with the same letter(s) in a row are not significantly different according to the P-values

4.4 Discussion

In this study, *D. edulis* trees of seed origin were found to have higher root density (RD) and root length density (RLD) within 0-20 cm soil depth than either of the trees of vegetative origin. Similar overall patterns were found in root length density (RLD) values in 0-10 cm soil stratum (Figures 4.5 and 4.6). This is probably due to the proliferation of fine roots from structural roots (d>2mm) located in this soil layer. Asaah et al. (2010), reported, that *D. edulis* trees of seed origin had significantly higher structural roots at soil depth of 0-20 cm compared to those of *D. edulis* trees of vegetative (cuttings and marcots) origin. *D. edulis* trees of seed origin are known to have a taproot system while trees of vegetative (cuttings and marcots) origin have adventitious roots (Asaah et al., 2010). According to Akinnifesi et al. (1999), tap root size is a superior estimator of fine root density. This may further explain the high RD and RLD observed in *D. edulis* trees of seed origin over trees of cutting and marcot origins within 0-20 cm soil depth.

Studies on root system of ten tree species (*Acacia nilotica*, *Albizia lebbeck*, *Azadirachta indica*, *Dalbergia sissoo*, *Melia azadarach*, *Morus alba*, *Zizyphus mauritiana*, *Populus deltoids*, *Eucalyptus tereticornis* and *Leucaena leucocephala*) of seed origin by Toky and Bisht (1992) reported high RD just like in D. edulis trees of seed origin within the top 30 cm soil stratum. Similar high RD have been reported in *Calliandra calothyrsus* trees raised from seeds at 0-20 cm soil depth (Jama et al., 1998), and in *Pinus sylvestris*, *Calluna vulgaris* and *Vaccinium vitis-idaea* at soil depths 0-10 cm Persson (1978).

At 20-30 cm soil depth stratum (Figures 4.5 and 4.6), in both *D. edulis* trees of cutting and marcot origins RD and RLD were increasing whereas the same root characteristics were declining in trees of seed origin. This could be due to the fact that at this soil depth, trees of vegetative origin had developed main adventitious roots similar to tap root diameter size in *D. edulis* trees of seed origin as reported by Asaah et al. (2010) (chapter 3). The maximum RD and RLD of *D. edulis* trees of vegetative origins occurred within the 20-50 cm soil depth (Figures 4.5 and 4.6). Similar results have been reported for *Leuceana leucocephala (*Akinnifesi et al., 1995), and *Acacia seyal* in Mali (Groot and Soumare, 1995).

The results of this study indicate the existence of a strong correlation between RD, RLD and RWD (Table 4.1), suggesting that as root number per soil volume increases, so does root length and root weight per soil volume, independent of *D. edulis* tree type (or origin). However, beyond 20 cm soil depth, RD values declined for both *D. edulis* trees of seed and vegetative (cuttings and marcot) origin. Similar RD declining situation with increase in soil depth had been reported to occur in several multipurpose trees species of seed origin: eight tree species in India (Chaturvedi and Das, 2003); *Prosopsis chilensis* (Jonsson et al., 1998); *Grevillea robusta* and *Gliricidia sepium* in Kenya (Odhiambo et al., 2002) and *Senna siamea* in three regions of Togo (Vanlauwe et al., 2002), e.g. *Prosopsis chilensis* (Jonsson et al., 1998).

Fine roots of trees of seed origin had a continuous exponential declining pattern for RD and RLD downwards from the upper soil stratum to a depth of 80 cm. Trees of vegetative origin (cuttings and marcots) on the other hand, showed a quadratic declining pattern with a point of inflexion at 20-30 cm soil depth stratum where RD (Figure 4.5) and RLD (Figure 4.6) were at their maximum values beyond which they declined continuously approaching zero at a depth of 80 cm. In a separate study of the primary structural roots of the same trees (Asaah et al., 2010); (Chapter 3), trees of vegetative origin produced vertical sinker roots, while trees of seed origin had tap roots. This suggests that the quadratic pattern of fine root distribution in these trees of vegetative origin could be associated with the points of origin of these sinker roots.

Depending on the environment trees have been reported to have extensive root spread. Chaturvedi and Das (2003) reported horizontal root spread that range from 1.1 to 1.6, greater than crown horizontal spread in *Acacia nilotica*, *Dalbergia sissoo*, *Pithecellobium dulce* and *Syzygium cumini*. Similar root to crown spread ratios were reported by Prasad and Mishra (1984) in five years old *Tectonia grandis* (1.1) and *Terminalia tomentosa* (2.0). Toky and Bisht (1992) reported a root to crown ratio spread that range from 1.23 to 1.26 in 6 years-old specimens of *Prosopis cineraria*, *Eucalyptus tereticornis* and *Propulus deltoids*. Horizontal root spread to crown spread values of *D. edulis* trees of seed and cutting origins range from 0.88 to 0.94 respectively, which differed significantly ($p=0.024$) in trees of marcot origin with horizontal root to crown spread of 1.37 (Table 4.4).

The result of this study suggests that *D. edulis* trees of seed origin had significantly ($P \leq 0.05$) higher RD and RLD in the 0-20 cm soil depth and lateral distance from tree base compared to trees of vegetative origin. Annual crops like maize and cowpea have been reported to have 96% and 98% respectively of their roots within the 0-30 cm soil depth (Akinnifesi et al., 1996). Fine roots according to Anderson and Ingram (1993) are the most important part of the root system responsible for water and nutrient uptake. Bayala et al. (2004) reported that pruning of tree crowns of karité (*Vitellaria paradoxa*) and néré (*Parkia biglobosa*) between 1999 to 2001, reduce fine root densities in 0-20 cm soil stratum from 59% (0.477 cm cm^{-3}) and 69% (0.447 cm cm^{-3}) to 32% (0.051 cm cm^{-3}) and 34% (0.078 cm cm^{-3}) in pruned trees of néré and karité respectively. The study concluded that reduce belowground competition for the benefit of companion crops could be achieve as a result of the observed temporary reduction in tree fine root densities within crop rooting zone. In the current study, it was observed that fine root density within 0-20 cm soil depth between *D. edulis* trees of seed and vegetative origins were reduced as a result of propagation methods. This provide new evidence that vegetative propagation does not only provide options to reduce the long juvenile phase to producing fruits of desirable traits in *D. edulis*, but also trees of vegetative origin (cuttings and marcots) with less fine root densities in crop root zone. Thus, with regard to the competitive interactions between trees and crops in agroforestry systems, the fine root system of *D. edulis* trees of vegetative origin appears to be less competitive than those of trees of seed origin. This could qualify vegetatively propagated trees of *D. edulis* as a desirable associative tree, based on its fine roots architecture in simultaneous agroforestry systems where limited fine root development in the crop root zone will minimize competition for belowground resources.

Although fine root density is considered an important parameter for nutrient uptake, the presence of tree roots do not necessarily imply root competition in agroforestry as decomposing and senesced tree roots are a nutrient source for other plants (Schroth, 1995; Akinnifesi et al., 1997). In addition to management factors, the efficiency of physiological uptake mechanisms, nitrogen fixation, root turnover, mychorrizal infections or allelopathy may influence belowground tree/crop interactions to an unknown extent in agroforestry (Akinnifesi et al., 1999b).

4.5 Conclusion

This study presents an original attempt to quantify the influence of sexual and asexual methods of propagation on the inherent variability of fine root distribution pattern of an indigenous fruit tree of the humid tropics of Africa. The study reveals that fine root distribution of *D. edulis* trees of seed and vegetative (cutting and marcot) origins vary with depth and lateral distance from the tree base. *D. edulis* trees of seed origin had roots that were exponentially distributed with depth while those of trees of vegetative (cuttings and marcots) origins had a quadratic distributed pattern with depth. Furthermore, *D. eduis* trees of seed origin showed significantly ($P \leq 0.05$) higher RD and RLD in the 0-20 cm soil stratum compared to *D. edulis* trees of vegetative (cuttings and marcots) origin with lower RD and RLD within the same soil stratum. These differences were thought to arise from the fine root origin. Fine roots in trees of seed origin, emanate from the tap root whereas in trees of vegetative origin they develop from the adventitious root system in trees of vegetative origin. High root density and root length of *D. edulis* trees of seed origin might have important implications on companion crops in terms of competition for water and nutrient within 0-30 cm soil stratum compared to trees of vegetative origin. The effect of such competition can be quantified by monitoring companion crop growth and development in association with *D. edulis* trees of seed and vegetative origins, which were beyond the scope of this study.

CHAPTER 5

5. EFFECT OF PROPAGATION METHODS ON TREE STATURE AND C STOCK IN 10 YEARS OLD *DACRYODES EDULIS* (G. DON) H. J. LAM TREES OF SEED AND VEGETATIVE (CUTTING AND MARCOT) ORIGINS

Abstract

Aboveground growth attributes of 10 years old trees of *Dacryodes edulis* (G. Don) H. J. Lam of seed and vegetative (cutting and marcot) origins were assessed in Mbalmayo near Yaoundé, Cameroon. Shoot density, defined as: number of shoots per tree, and height differed significantly (p=0.004 and p=0.005, respectively) amongst tree origins. Trees of seed and cutting origins had single-stem shoots whereas marcots had 6 shoots per tree on average. Mean height of trees of cutting origin measured 8.4 ± 2.2 m compared to 6.7 ± 0.9 m and 7.6 ± 1.9 m for trees of marcot or seed origins, respectively. However, diameter at breast height did not differ between different tree types. Mean carbon (C) sequestration values over 10 years, estimated using allometric models differed significantly (p= 0.014) between trees of vegetative origin and those of seed origin, with *D. edulis* trees of cutting and marcot origins having sequestrated averagely 26.8 ± 19.1 Mg C ha^{-1} and 21.74 ± 12.8 Mg C ha^{-1} over 10 years respectively, compared to 13.10 ± 9.4 Mg C ha^{-1} for trees of seed origin. Vegetative propagation should be promoted in *D. edulis* domestication due to this method's ability not only to reduce the long juvenile period to first fruiting but also to maintain trueness in the transfer of desirable (fruit) traits over generations, whereas it also produces bigger trees with higher carbon sink values. The superiority of *D. edulis* trees of vegetative origin in sequestrating significantly more C compared to trees of seed origin is a win-win situation. The findings of this study suggest that propagating *D. edulis* vegetatively, has resulted not only in the production of improved trees yielding fruits for food and income, but also in the development of trees with higher C sinks.

Key words: agroforestry, carbon sequestration, domestication, growth attributes, vegetative propagation

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5.1 Introduction

Strategies to reduce the amount of atmospheric carbon (C) by storing the latter in the terrestrial biosphere are among the various options proposed to compensate greenhouse gas (GHG) emissions (Albrecht and Kandji, 2003; Nair et al., 2009; Roshetko et al., 2007; Soto-Pinto et al., 2010; Takimoto et al., 2008a). Trees within the terrestrial biosphere are apparently the oldest, largest, and best-provisioning living organisms for home use and income generation, in addition to their environmental regulatory services. However, there is an increasing conflict for land between tree plantations, natural forests and trees on farms. According to a study conducted by Gockowski et al. (2001) in Cameroon on the trade-off between carbon storage and profitability, the authors concluded that tropical deforestation is profitable and may sometimes contribute to poverty reduction. The authors maintain that there are trade-offs between carbon storage and profitability and that there was no win-win land use system (i.e. high carbon storage and high profitability). Nonetheless, they identified some 'no regrets' situations with medium carbon storage and medium profitability like in agroforestry systems. Agricultural landscapes constitute major potential C sinks capable of absorbing large quantities of C if trees are integrated into farming systems and judiciously managed together with crops and/or animals (Albrecht and Kandji, 2003; Mutuo et al., 2005; Roshetko et al., 2007).

Agroforestry has emerged as a mechanism to promote multifunctional agriculture by providing intermediate land use alternatives akin to natural forests with a potential to generate good economic returns and acceptable levels of critical environmental services (Leakey, 2010; Nair, 2007; Roshetko et al., 2007; Takimoto et al., 2008b). Nair et al. (2009), in a review on agroforestry as a strategy for carbon sequestration, advanced challenges involved in quantifying and/or demonstrating the contributions of agroforestry in maintaining or increasing the supply of ecosystem services - water, soil health, biodiversity and products - under changing climates in order to continue supporting both human needs and the functioning of natural ecosystems. That notwithstanding, agroforestry is considered as 'a dynamic, ecologically-based, natural resources management system that, through the integration of trees on farms and in the agricultural landscape, diversifies and sustains production for increased social, economic and environmental benefits for land users at all levels' (www.icraf.cgiar.org). According to Nair et al. (2009), the recent recognition of agroforestry as a greenhouse gas mitigation strategy under the Kyoto Protocol has given it new impetus as a strategy for biological C sequestration. Watson et al. (2000) had long reported that C sequestration rates ranging from 1.5 to 3.5 Mg C ha^{-1} y^{-1} and a tripling of C stocks over a 20-year period, to 70 Mg C ha^{-1}, was achievable in mature agroforestry systems. Albrecht and Kandji (2003) also projected that the C sequestration potential in agroforestry systems could range between 29 and 53 Mg ha^{-1} in agro-silivicutral systems in the humid tropics of Africa.

Agroforestry providing a dual function of strengthening food security and C sequestration to fight climate change is still little understood. Lack of reliable estimates on the extent of total area under agroforestry systems in different ecological zones is a serious problem in projecting the extent to which agroforestry practices could counter C emission from deforestation and other land uses. This difficulty is compounded by the fact that C sequestered in agroforestry systems may vary with sites and has system-specific characters, including climate, soil type, tree planting densities, and tree management (Albrecht and Kandji, 2003; Nair et al., 2009). Consequently, the productivity and role of the different tree species in agroforestry systems and their provision of products and environmental services needs to be realistically assessed so that appropriate incentives can be put in place to increase the number of trees integrated into agricultural landscapes. The question thus becomes whether tree cultivation should be promoted to improve environmental services or livelihood options, or both? The integration of highly valued indigenous fruit and medicinal plants in agroforestry landscape mosaics in the humid tropics of Africa is aimed at enhancing the availability of traditional fruits, nuts and medicinal products important for livelihood (Leakey, 2001b; Schreckenberg et al., 2006; Takimoto et al., 2008a; Tchoundjeu et al., 2008). Literature on the contribution of indigenous trees species to C sequestration is scarce. However, farming systems that produce a variety of tree products (both wood and non-wood) are preferred by farmers as a means to securing tree products for home use and income generation in addition to minimizing the impact of crop failure (Roshetko et al., 2001; 2007; Soto-Pinto et al., 2010; Takimoto et al., 2008b). This could be an incentive for farmers to protect existing trees while integrating more trees into agricultural landscapes. Generally, in these tree-based systems, the greater part of the aboveground C stock is found in the main stem(s) and branches of the trees. Most agroforestry tree products (fruits, nuts, vegetables, spices, bark, oils, resin, etc.) can be harvested with negligible impact on the C stock of that system. In parallel, farmers can also gain extra revenue from the C stock in their tree-based systems through various environmental service reward mechanisms.

One of such trees widely cultivated in West and Central Africa for its food and income value is *Dacryodes edulis* (G. Don) H. J. Lam. *D. edulis* is an evergreen tree, indigenous to central Africa and the Gulf of Guinea regions (Silou, 1994; Auberville, 1962). Its preferred habitat is in a shady humid tropical forest environment. However, it adapts well to various soil types, humidity levels, temperatures and day lengths. Its natural range extends from Angola in the south, Nigeria in the north, Sierra Leone in the west to Uganda in the east. It is also cultivated in Malaysia (Kengue, 2002). *D. edulis* is cultivated mainly for its fruit (safou) which is rich in lipids, essential oils, vitamins and minerals. *D. edulis* fruits are highly commercialized with transactions known to occur both at national and international levels (Awono et al., 2002; Tabuna, 1999). It is common to find *D. edulis* trees randomly planted in agroforestry systems, home gardens, food crop fields and coffee/cocoa plantations as a shade crop.

The tree is propagated both sexually via seeds and vegetatively through stem cuttings (Mialoundama et al., 2002), marcotting (Kengue and Tchio 1994; Mampouya et al., 1994) and to a lesser extent grafting (Damasse et al. (2001) cited by Kengue, 2002) and tissue culture (Youmbi and Benbadis, 2001). Vegetative propagation techniques are promoted due to their faithfulness in the transfer of desirable (fruit) traits over generations, and the reduction of the long juvenile phase to flowering and fruiting typical of sexually-propagated trees. As a consequence, there is growing interest in the multiplication of agroforestry trees like *D. edulis* through vegetative propagation. The effect of propagation methods on structural roots (≥ 2mm) of mature, fruiting *D. edulis* trees of seed and vegetative origins (cutting and marcot) has been reported by Asaah et al. (2010) (Chapter 3). The latter established that *D. edulis* trees of vegetative origin have a well-developed, adventitious primary root system and deep sinker roots which confer stability against wind damage just like it is with tap roots in trees of seed origin. In a separate study on fine root distribution in *D. edulis* trees of seed and vegetative origins, it was established that trees of vegetative origin had lower density of fine roots distributed within the top 30 cm layer of soil compared to trees of seed origin, therefore rendering them less competitive for belowground resources (Asaah et al., In press) (Chapter 4).

Interestingly, we are not aware of any published studies that have rigorously quantified the effect of propagation methods on aboveground growth attributes and C storage potential of trees of seed and vegetative origins of any indigenous fruit tree in Africa under domestication. Meanwhile, a clear understanding of the aboveground growth attributes as a result of propagation methods could be vital in designing and managing agroforestry systems and their inherent agro-ecosystems services. Consequently, the current study was conducted with the primary objective to identify (these) distinctive aboveground tree growth attributes in *D. edulis* trees of seed and vegetative origins. A secondary objective was to quantify the mean amount of carbon stored (C stock) in ten years old fruiting *D. edulis* trees of seed and vegetative origins (cutting and marcot) in Cameroon.

5.1.1 Specific objectives

- Identify the variability in aboveground tree growth attributes in both *D. edulis* trees of seed and vegetative (cutting and marcot) origins.
- Assess the variability in tree biomass yield, C stock and CO_2 equivalent (CO_2e) sequestrated by both *D. edulis* trees of seed and vegetative (cutting and marcot) origins.

5.1.2 Research questions

- Do propagation methods affect aboveground tree growth attributes of *D. edulis* trees of seed and vegetative (cutting and marcot) origins?
- Do propagation methods affect both tree C stock and CO_2e sequestrated by *D. edulis* trees of seed and vegetative (cutting and marcot) origins?

5.1.3 Hypotheses

- *D. edulis* trees of seed and vegetative origins have the same growth attributes and architecture.
- *D. edulis* trees of seed and vegetative origins have the same biomass yield, C stock and CO_2e sequestration potential.

5.2 Material and methods

The study site characteristics are reported in **Chapter 3, section 3.2.1.**

Thirty *D. edulis* trees each of seed, cutting and marcot origins used were randomly selected from an orchard set up as a comparative growth trial of *D. edulis* trees of both seed and vegetative origins. Trees were from different mother plants. Cuttings where from juvenile stockplants of *D. edulis* while marcots were from mature trees and had at most three months difference in age. Trees of both seed and vegetative origins had been raised in black, perforated polybags carrying about 3 kg of rich top soil mixed with river sand at a ratio of 2:1 and put on a raised platform to facilitate air pruning of roots at ICRAF's nursery in Mbalmayo Cameroon for 6 months before field planting in 2001.

Tree growth parameters (e.g. plant height, tree bole, diameter at breast height (DBH), crown diameter, flowering and fruit production were assessed annually. The current study is based on four aboveground tree growth attributes (plant height, tree bole, diameter at breast height (DBH) and crown diameter) measured on each of the *D. edulis* trees between June and July 2011. Flowering and fruiting performance of *D. edulis* trees are not reported in the current study.

5.2.1 Tree assessment

Tree growth can be influenced by several factors among which are site quality, season, age, shade, and companion crops. Parameters such as plant height, tree bole length, diameter at breast height (DBH), and crown diameter are adequate indicators to express differences in tree growth attributes (MacDicken et al., 1991).

Tree height is equal to total stem length measured from relatively level ground to the highest (apical) growing point (MacDicken et al., 1991). Tree height (H) was measured using a SUUNTO clinometer. The latter height was obtained by measuring angels from the eye to the tree apex (plus angle = a) and to the tree base (eye to ground angle= b). The sum of both angels was divided by 100 then multiplied by the horizontal distance from the observer to the tree (distance from observer to the tree measured to the nearest centimetre = c) (Eq. 5.1) (Figure 5.1).

Thus, $H= (a+b/100)*C$ ….. *Eq. 5.1,*

where:

H = tree height in meters,

a = angle from eye of observer to tree apex ('plus angle'),

b = angle from the eye of the observer to tree base ('eye to ground angle'),

c = distance from observer to tree base measured to the nearest centimetre),

100 = is slope in precent.

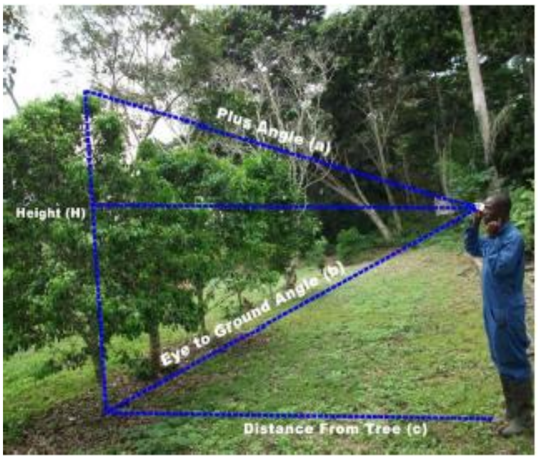


Figure 5.1. Measuring *D. edulis* tree height and bole length with a SUUNTO clinometer, Mbalmayo, Cameroon

Tree bole is equal to the part of the stem of a tree from the base or roots to the first significant branch (MacDicken et al., 1991). It is the exploitable part of a tree that can be used as timber or fuel wood. Tree bole length was measured with a SUUNTO clinometer exactly as tree height with a difference that the plus or upper angel was projected on the first significant branch of the tree. All other measurements were similar as when measuring tree height. The formula for estimating tree bole height was also the same as for tree height (*Eq. 5.1*).

Stem diameter: is a measure of lateral growth expressed by the main truck diameter. Traditionally, foresters measure diameter at breast height (dbh), i.e. 1.3 m. However, this standard may not give useful information for agroforestry trees that often have short multiple stems (Hairiah et al., 2010; MacDicken et al., 1991). Most of our *D. edulis* trees had single and sometimes multiple stems especially with trees of vegetative origin. It was therefore necessary to have a measure of diameter that was adequate for both stem configurations. The latter authors are unanimous that the diameter of multiple stems can be estimated using the formula:

$$D = \sqrt{(D1^2 + D2^2 + D3^2 + D4^2 + \cdots Dn^2)}. \ldots..Eq.\ 5.2,$$

where:

$D1^2$ to Dn^2 are diameters at 1.37 m of multiple stems on the same tree.

DBH of all sampled *D. edulis* trees of seed and vegetative origins (cuttings and marcots) were measured with the aid of a diameter tape to the nearest centimetre. These values were computed in Eq. 5 2, to obtain the stem diameter of individual trees.

Crown diameter: tree crowns are usually irregular and because of their size and position they present problems such as dead portions or broken branches. Crown diameters (CD) of all sampled *D. edulis* trees of seed and vegetative origins were measured by projecting the edges of the respective crowns on to the ground and measuring the length along one axis from edge to edge through the crown centre. To make up for irregular crown shapes, diameters were measured along any two axes at 90 degrees (d_1 and d_2) to each other (MacDicken et al., 1991). CD values were averaged using an arithmetic mean to obtain any individual tree's crown diameter. CD was calculated as:

$$\text{CD arithmetic mean} = \frac{d1+d2}{2} \ldots\ldots\ldots Eq.\ 5.3,$$

where:

CD = crown diameter,

d_1 = diameter of the maximum axis of the projected crown in (m), and

d_2 = diameter of the minimum axis of the crown in (m).

5.2.2 Tree biomass assessment

Tree biomass can be assessed using destructive and non-destructive methods (Djomo et al., 2010; 2011; Hairiah et al., 2010; Ketterings et al., 2001; Ter-Mikaelian and Korzukhin 1997). In the destructive method, tree biomass is determined directly by felling the tree, and actually weighing and analysing its different components (e.g. roots, stem(s), branches, and/or foliage) (Chave et al., 2004; 2005; Djomo et al., 2010; Hairiah et al., 2010; Parresol, 1999). It is a straightforward method to estimate fresh biomass in the field. It could also be used to determine dry biomass but the procedure is not very practical as the whole tree or parts have to be oven-dried first (Stewart et al., 1992) to estimate dry biomass of the tree.

Meanwhile, in the non-destructive method, fresh tree biomass can be estimated without felling the tree (Montes et al., 2000). This method is mainly applied when the species is of interest (e.g. fruit/nut/medicinal species), rare or protected. In non-destructive biomass estimation, easily obtainable tree component parameters such as diameter at breast height (DBH), total tree height, etc. are computed in allometric regression models that convert individual tree inventory data to estimates of aboveground biomass (Brown, 1997; Hairiah et al., 2010; Ketterings et al., 2001, van Breugel et al., 2011). The non-destructive method was used in assessing the aboveground biomass of *D. edulis* trees of seed and vegetative origin. Tree growth parameters: height (H) and Diameter at Breast Height (DBH) were inventoried. This data was subsequently computed into allometric regression models to estimate aboveground biomass.

The most common method to estimate individual tree biomass is through mathematical models calculated by regression analysis (Ketterings et al., 2001; Hairiah et al., 2010; van Breugel et al., 2011). In almost all published work in relation to the development of allometric regression models for the estimation of aboveground biomass, authors are unanimous to the fact that generic equations should be stratified by ecological zone. Therefore, it is necessary to choose the right equation adapted to the region and context of any given study, or conduct research towards deriving site-specific allometric regression models.

Djomo et al. (2010), in a recent study on deriving allometric regression models for biomass estimation in Cameroon, concluded that mixed-species allometric regression equations provided reliable total aboveground biomass estimates for Campo-Ma'an (south region in Cameroon) forest when only using tree stem diameter as input variable with an average error of only 7.4%. The latter authors maintain that including height in the model did not improve its precision. However, including the three variables of diameter, tree height and wood density improved the estimate's precision to 3.4%. Unfortunately, Campo-Ma'an is situated in the Atlantic coastal forest ecosystem of Cameroon whereas our own study site (Mbalmayo) is in the humid equatorial forest ecosystem (central Cameroon). Therefore, in the absence of a site-specific allometric regression model for Mbalmayo, the current study adopted the pan moist tropical regression model of Chave et al. (2005) (Eq. 5.4) as an appropriate alternative with an average error of 20.3%, compared to an average error of 29.5% in the general allometric regression model developed by Djomo et al. (2010; 2011):

AGB= exp(-2977 + In(ρD^2H) ....Eq. 5.4,

where:

AGB = aboveground biomass in Mg ha^{-1},

exp = exponential,

ln = natural logarithm,

ρ = wood density (g m^{-3}),

D = DBH = Diameter at Breast Height (cm), and

H = tree height (m).

Chave et al.'s (2005) regression model (Eq. 5.4) was derived using datasets from 1505 trees through destructive sampling. The latter authors maintain that the trees' diameters at breast height range from 5 to 156 cm and were measured from 27 study sites in both secondary and old-growth dry, moist, wet, lowland, montane and mangrove forests. Chave et al. (2005) concluded that Eq. 5.4 was robust, and can be used reliably to predict aboveground tree biomass across a broad range of tropical forests.

The wood of *D. edulis* is yellowish-pink, moderately heavy, and has a density of about 0.6g/m^3 at 12% moisture content (Anonymous, 2011b). Thus, measured growth parameters of each *D. edulis* tree, and wood density values were computed into the allometric regression model (Eq. 5.4) to derive its aboveground biomass expressed in kg per tree. As trees were planted at 100 trees per hectare, aboveground biomass was extrapolated to kg ha^{-1}.

5.2.3 Root biomass estimation

The knowledge body on belowground biomass (BGB) estimation is limited as compared to that on aboveground biomass estimates. However, regression models, which can predict root biomass based on aboveground measurement data, have been developed (Cairns et al., 1997; Kraenzel et al., 2003; Mokany et al., 2006).

After reviewing 160 studies, across tropical, temperate and boreal forests, Cairns et al. (1997) suggested a default mean root-to-shoot ratio (RS) of 0.26 with a range from 0.18 to 0.30. Kraenzel et al. (2003) reported a mean RS ratio of 0.16 with a range from 0.11 to 0.23 on 20 years old *Tectona grandis* trees in Panama. According to Mokany et al. (2006), RS ratios provide a general description of the relationship between roots and shoot biomass in forests and woodlands, whereas individual RS ratios for each specific forest and woodland type would provide even more accurate root biomass estimates. However, Cairns et al. (1997) maintain that RS ratios are constant between latitude (tropical, temperate and boreal), soil texture (fine, medium and coarse), and tree type (angiosperm and gymnosperm). The latter authors went ahead to develop an allometric model through which the root biomass of a forest can be predicted from the shoot biomass that was subsequently used in many studies to estimate root biomass. The equation that fits best our current objective to estimate belowground biomass in *D. edulis* trees of seed and vegetative origins, is that recommended for use in all forest types with known aboveground biomass and tree age (AGE) as variables (Eq. 5.5).

$$\mathrm{BGB} = exp[-1.3267+0.8877*\ln(\mathrm{AGB}) + 0.1045\bullet\ln(\mathrm{AGE}) \text{.........Eq. 5.5,}$$

where:

BGB = belowground biomass in Mg ha^{-1},

exp = exponential,

ln = natural logarithm,

ABG = aboveground biomass Mg ha^{-1}, and

AGE = age of trees in years.

As in the aboveground biomass estimation, the application of RS ratios represents a trade-off between costs of time, resources and accuracy as belowground biomass can also be assessed *in situ*, by taking soil cores below the trees from where roots can be extracted. The oven-dry weight of these roots can then be related to the cross-sectional area of the sample tree, and also to the belowground biomass on a per area basis (Djomo et al., 2010; 2011; Hairiah et al., 2010; Kraenzel et al., 2003; MacDicken, 1997).

5.2.4 C stock assessment

The knowledge of C content in live wood has been reported by various authors to be an essential element for quantifying forest C stocks (Clark et al. 2001; Lewis et al 2009; Martin and Thomas, 2011), yet generic assumptions (such as biomass consisting of 50% carbon on a weight/weight basis) remain widely used despite being supported by little chemical analysis. Carbon content in vegetation was reported to be constant across a wide variety of tissue types and species, and was always found to vary between 45 and 50% (by oven-dry mass) (Schlesinger, 1991). IPCC (2003), estimated the C content in dry biomass at 47%.

In the current study, the LULUCF value of 0.47 C fraction approved by IPCC (2006) was adopted. The aboveground biomass of *D. edulis* trees of seed and vegetative origins obtained from Chave et al.'s regression model (Eq. 5.4) was multiplied by the C fraction (0.47) to obtain aboveground C stock. Similarly, belowground C stock was estimated from belowground biomass (BGB) obtained from Cairns et al.'s regression model (Eq. 5.5). Total C stored in 10 years old *D. edulis* trees was obtained through the multiplication of the sum of AGB and BGB, by the C fraction (Eq. 5.6):

Total C stock = 47% * (AGB+BGB) ……….Eq. 5.6,

where:

AGB = aboveground biomass in kg ha^{-1}, and

BGB = belowground biomass in kg ha^{-1}.

5.2.5 Estimating CO_2 sequestrated by trees of *D. edulis* of seed and vegetative origins

Carbon dioxide (CO_2) sequestrated by *D. edulis* trees of seed and vegetative origins was estimated from the combination ratio derived from the atomic weights of the elements making up the CO_2 molecule to that of C, i.e. 3.7. The ratio was then multiplied by both AGB and BGB in *D. edulis* trees of seed and vegetative origins to estimate CO_2 sequestrated, defined as CO_2 equivalent (e) (CO_2 e) (IPCC guidelines).

5.3 Data analysis

All data on tree growth parameters were subjected to a two-way analysis of variance using GenStat Version 12 statistical package to test for differences in *D. edulis* tree parameters (height, bole length, diameter at breast height, crown diameter) as affected by propagation method. The same tree growth parameter values were also ran through appropriate allometric regression models to derive total tree biomass, tree carbon storage, root biomass and root carbon storage in *D. edulis* trees of seed and vegetative origins. Biomass and carbon storage data was further subjected to analysis of variance using GenStat Version 12 statistical package to test for differences in biomass yield and carbon storage capacity between different types of *D. edulis* trees.

5.3 Results

5.3.1 Variability in *D. edulis* tree growth attributes with propagation method

Mean tree heights and bole lengths of *D. edulis* trees of vegetative origin (cuttings and marcots) and those of seed origin varied considerably amongst each other. The tallest tree was 15 m while the shortest was 5 m. Trees of cutting origin where significantly (p=0.005) taller (8.4 ± 2.2 m) compared to trees of either marcot or seed origins (6.7 ± 0.9 m, and 7.6 ± 1.9 m respectively) (Table 5.1). Mean bole length varied very significantly (p =0.0001) between *D. edulis* trees of seed and vegetative origins. The greatest bole length was recorded in trees of seed origin (1.6 ± 0.6 m) followed by trees of cutting origin (1.4 ± 0.5 m) which both differed significantly (p=0.0001) from trees of marcot origin (0.3 ± 0.1 m) (Table 5.1).

Considerable variation was observed in shoot density per *D. edulis* tree type. Mean number of shoots per tree ranged from 1 to 11. Trees of marcot origin had averagely 6.2 ± 2.6 shoots which differed significantly (p=0.004) from the average number of shoots recorded from trees of cutting and seed origins (1.9 ± 1.1 and 1.1 ± 0.6, respectively) (Table 5.2).

Table 5.1. Mean tree height (m) and bole length (m) of 10 years old *D. edulis* trees of seed and vegetative (cutting and marcot) origins

Parameters/origin	Cutting	Marcot	Seed	P- value
Tree height (m)	8.4 ± 2.2 ^{a}	6.7 ± 0.9 ^{b}	7.6 ± 1.9 ^{b}	0.005
Bole length (m)	1.4 ± 0.5 ^{a}	0.3 ± 0.1 ^{b}	1.63 ± 0.5 ^{a}	0.000

NB: The values assigned to the same letter in the same row are not significantly different at 5%.

Mean stem diameter at breast height (DBH) did not vary between *D. edulis* trees of either origin type. The largest tree had a DBH measure of 36.3 cm while the smallest tree measured 10.9 cm. Mean DBH values recorded from *D. edulis* trees of vegetative origin (marcots 23.5 ± 6.5 cm and cuttings 23.4 ± 6.7 cm) did not differ significantly (p= 0.936) from stem diameters of trees of seed origin (16.2 ± 6.5 cm) (Table 5.2). Similarly, mean crown diameter of *D. edulis* trees of both seed and vegetative origins did not differ significantly (p=0.069) between each other (Table 5.2).

Table 5.2. Mean shoot density (number/tree), stem (cm) and crown diameters (m) of 10 years old *D. edulis* trees of seed and vegetative origins

Parameters /Origin	Cutting	Marcot	Seed	P- value
DBH (cm)	23.4 ± 6.7^a	23.5 ± 6.5^a	16.2 ± 6.5^a	0.936
Crown diameter (m)	9.0 ± 1.9^a	8.0 ± 2.1^a	6.6 ± 2.8^a	0.069
Shoot density	1.9 ± 1.1^b	6.2 ± 2.6^a	1.1 ± 0.6^b	0.004

NB: The values assigned to the same letter in the same row are not significantly different at 5%.

5.3.2 Aboveground biomass and C stock in 10 years old *D. edulis* trees of seed and vegetative (cutting and marcot) origins

Mean aboveground biomass recorded in 10 years old *D. edulis* trees of vegetative origin (cuttings 13.0 ± 9.5 Mg ha $^{-1}$; marcots 10.5 ± 6.3 Mg ha $^{-1}$) was significantly different (p = 0.022) from that obtained for trees of seed origin (6.3 ± 5.1 Mg ha $^{-1}$), (Table 5.3). Similarly, aboveground C stocks differed considerably between *D. edulis* trees of seed and vegetative origins. C stocks ranged from 6.1 ± 4.5 Mg ha $^{-1}$ to 4.9 ± 2.9 Mg ha $^{-1}$ in trees of cutting and marcot origins which differed significantly from C stocks in trees of seed origin (2.9 ± 2.4 Mg ha $^{-1}$) (Table 5.3).

Table 5.3. Aboveground biomass and C stock in *D. edulis* trees of seed and vegetative (cutting and marcot) origins (mean ± sd Mg ha $^{-1}$)

Carbon parameter		Tree origin		
		Cutting	Marcot	Seed
Shoot	Aboveground biomass	13.0 ± 9.5 ^{a}	10.5 ± 6.3 ^{a}	6.3 ± 5.1^{b}
	C stock	6.1 ± 4.5 ^{a}	4.9 ± 2.9 ^{a}	2.9 ± 2.4^{b}

NB: The values assigned to the same letter in the same row are not significantly different at 5%.

5.3.3 Belowground biomass and C stock in 10 years old *D. edulis* trees of seed and vegetative (cutting and marcot) origins

Mean belowground biomass in 10 years old *D. edulis* trees varied from 1.3 ± 0.9 Mg ha^{-1} in trees of seed origin to 2.5 ± 1.6 Mg ha^{-1} and 2.1 ± 1.1 Mg ha^{-1} in trees of cutting and marcot origins, respectively (Table 5.4). A strong positive and significant correlation ($r = 0.89$, $p = 0.014$) was also observed between aboveground biomass and root biomass.

Mean belowground C in *D. edulis* trees of seed and vegetative origins had a similar trend to aboveground C stock. Belowground C values in the roots of trees of vegetative origin (cuttings 1.2 ± 0.8 Mg ha^{-1} and marcots 1.0 ± 0.5 Mg ha^{-1}) were significantly ($p = 0.039$) higher than in trees of seed origin (0.6 ± 0.5 Mg ha^{-1}), (Table 5.4).

Table 5.4 Belowground biomass and C stock in 10 years old *D. edulis* trees of seed and vegetative (cutting and marcot) origins (mean ± sd Mg ha $^{-1}$)

Carbon parameter		Tree origin		
		Cutting	Marcot	Seed
Root	Belowground biomass	2.5 ± 1.6 ^{a}	2.1 ± 1.1 ^{a}	1.3 ± 0.9 ^{b}
	C stock	1.2 ± 0.8 ^{a}	1.0 ± 0.5 ^{a}	0.6 ± 0.5 ^{b}

NB: The values assigned to the same letter in the same row are not significantly different at 5%.

5.3.4 CO_2e sequestrated by 10 years old *D. edulis* trees of seed and vegetative (cutting and marcot) origins

The amount of CO_2e sequestered aboveground in 10 years old *D. edulis* trees of seed and vegetative origins differed significantly. Cuttings sequestrated 22.5 ± 16.3 Mg ha^{-1} and marcots 18.1 ± 10.8 Mg ha^{-1}, which differed significantly ($p = 0.014$) from CO_2e sequestrated by trees of seed origin (10.9 ± 8.7 Mg ha^{-1}) (Table 5.5). CO_2e sequestrated belowground followed a similar trend to that of aboveground CO_2 sequestrated, with trees of vegetative (cutting and marcot) origins sequestrating 4.3 ± 2.8 Mg ha^{-1} and 3.6 ± 1.9 Mg ha^{-1} respectively which differed significantly ($p = 0.038$) from 2.2 ± 1.7 Mg ha^{-1} sequestrated by trees of seed origin (Table 5.5).

Table 5.5. Total CO_2e sequestrated in 10 years old *D. edulis* trees of seed and vegetative (cutting and marcot) origins (mean ± sd Mg ha^{-1})

CO_2 sequestration	Tree origin		
	Cutting	Marcot	Seed
Shoots	22.5 ± 16.3 ^{a}	18.1 ± 10.8 ^{a}	10.9 ± 8.7 ^{b}
Roots	4.3 ± 2.8 ^{a}	3.6 ± 1.9 ^{a}	2.2 ± 1.7 ^{b}
Total	26.8 ± 19.1 ^{a}	21.7 ± 12.8 ^{a}	13.1 ± 9.4 ^{b}

NB: The values assigned to the same letter in the same row are not significantly different at 5%.

5.4 Discussion

Agroforestry systems have the potential to sequester C. With adequate management of trees in agricultural landscapes, significant fractions of atmospheric C could be captured and stored in plant biomass and soils. However, there are some challenges to tree biomass estimation in general, and in tropical Africa in particular. The challenges are two-fold. First, there are those related to errors at the level of field data collection, and secondly, those related to the choice of allometric regression models used.

Chave et al. (2004) distinguished four sources from which errors could occur during field data collection, which could bring about significant uncertainties in subsequent aboveground biomass estimates from such data. Error sources include:

i. errors due to tree growth parameter measurements,

ii. errors due to the choice of the allometric model relating AGB to other tree parameters,

iii. sampling uncertainty, and

iv. the representativeness of sample size across the vast forest landscape.

In choosing allometric regression models to estimate and report on forest C stocks, tropical countries and in particular Congo Basin countries face two main challenges. First, there are few allometric regression models to choose from as only a small number of site-specific allometric regression models have been developed for the region to estimate aboveground biomass (e.g. Brown et al., 1992; Chave et al., 2001; DeWalt and Chave et al., 2004; 2005; Djomo et al., 2010; 2011; Saatchi et al., 2007) and second, very limited data or allometric regression models have been developed for Cameroon (Djomo et al., 2010; 2011). The knowledge body on belowground biomass estimates in tropical regions is even much lower (e.g. Cairns et al., 1997; Fox et al., 2010; Green et al., 2005), whereas we are aware of just one study that has attempted to develop site-specific allometric regression models, in this case for Cameroon’s Atlantic forest ecosystem (Djomo et al., 2010). This raises the difficulty of choosing the most suitable allometric regression model for estimating C pools for a tropical humid ecosystem within Cameroon. The risk is that an inappropriate allometric regression model may be chosen that could introduce a strong bias in biomass estimates (Chave et al., 2004; Green et al., 2005).

In a recent study to derive allometric regression models for estimation of biomass in Cameroon and pan moist tropical forests with data from Africa, Djomo et al. (2010) concluded that the aboveground biomass equations of Chave et al. (2005) are the best estimator across continents and sites in the absence of site-specific allometric equations even though they still have an average error of 20.3%.

The results of our study indicate that tree bole length was proportionate to tree height. *D. edulis* trees of seed and cutting origins recorded the greatest tree heights and longest bole lengths. Reduced bole length in trees of marcot origin could be the result of the fact that shoots in marcots grow from sprouted dormant buds after the rooted branch has been cut from the mother tree. Cutting back has been reported to typically induce growth redistribution towards traumatic reiterations and enhanced growth of unpruned axes (branches) on (mother) trees (Fumey et al., 2011). In many woody species, multiple growth flushes occur if trees are cut back under favourable conditions. This is often followed by more flushes of the terminal shoot if growing conditions remain favourable. In the case of *D. edulis* trees of marcot origin, the first growth flushes often consist of dormant bud breakage and the beginning of outgrowth of the newly-burst buds on the first flushing shoot. According to Cline et al. (2007), this response often involves the release of apical dominance which is significantly influenced by the auxin:cytokinin ratio as well as by other signals including nutrients and water. The first growth phase is often immediately followed by a second growth phase, if conditions remain favourable. This second phase consists of another bud outgrowth under the influence of apical control. Apical dominance refers to the strong genetically based preference for vertical growth of the terminal sprout relative to side branches (Buchholz, 2010) which may significantly affect crown form and structure. Top working through grafting of apple (*Malus domestica*) trees in Western Himalaya was observed to have a highly significant and positive correlation with shoot density (Das et al., 2011). High shoot density in *D. edulis* marcots is a desirable characteristic as high shoot density is often proportionate to productivity in apples (Das et al., 2011), and in *Uapaca kirkiana* (Akinnifesi et al., 2009). However, crown diameter was not proportionate to shoot density in *D. edulis* trees of seed and vegetative origins as no considerably statistical differences ($p=0.069$) were observed between different tree types. Similarly, no considerable statistical differences ($p=0.936$) were observed in stem diameters at breast height in either *D. edulis* trees of seed and vegetative (cutting and marcot) origins. Root collar diameter and crown spread in 8 years old *Uapaca kirkiana* trees of seed and vegetative (marcot and graft) origins in southern Africa have also been reported to be similar (Akinnifesi et al., 2009), which could confirm our findings.

In perennial crops, the selection of individuals for domestication is focused on reproductive structures (seeds, shell thickness, fruit characteristics, oil content, etc.), although changes in vegetative traits have been reported to occur during the domestication process (Miller and

Gross, 2011). A common vegetative feature of domesticated tree crops is dwarfism and/or a more compact growth stature characterised by yielding fewer and shorter internodes and branches as opposed to tall single stem wild relatives. For example, in apple (*M. domestica*) trees high shoot density is developed by grafting on dwarf rootstock (Gradziel and Beres, 1993; Seleznyova et al., 2003; 2008). Some of these traits selected for (dwarf rootstocks, reduced internode distances, etc.) in apple, can also be conferred through vegetative propagation (Seleznyova et al., 2008). Dwarfing has been reported to reduce root hydraulic conductance (or belowground water uptake) in olive (*Olea europea*) (Nardini et al., 2006). Dwarfism is a common vegetative feature of domesticated tree crops which has been documented in avocado (*Persea americana*), castor (*Ricinus communis*) and coconut (*Cocos nucifera*) (Miller and Gross, 2011). Dwarfing has been successfully conferred in *D. edulis* trees through marcotting (Kengue 2002). Changes in plant growth attributes have been reported to have a positive impact on harvest index (ratio of the harvested part to overall aboveground biomass) and size of the fruit or grain (Donald (1968) cited by Gepts, 2004; Seleznyova et al., 2008). For example, fruit load in *U. kirkiana* significantly differed between trees of vegetative origin (grafts and marcots) compared to trees of seed origin in southern Africa (Akinnifesi et al., 2009).

Among the common traits that best describe the domestication syndrome documented on almost all crops under domestication, are changes in plant stature, in this case dwarfness and bigger trees, all aimed at increased productivity. Through vegetative propagation, *D. edulis* trees under domestication are bigger in stature (especially marcots) compared to trees of seed origin. These trees also have a reduced juvenile phase of less than 4 years to first fruit production (Kengue, 2002). Therefore, besides the trueness in the transfer of desirable traits over generations through vegetative propagation techniques like grafting in mangoes (*Mangifera indica*; Bretell et al., 2004), micro-propagation in olive (Bati et al., 2006), or grafting and marcotting in *Uapaca kirkiana* (Akinnifesi et al., 2009*)*, tree stature is also significantly modified.

Agroforestry systems have been reported by various authors to have a higher potential to sequester C than pastures or field crops (Kirby and Potvin, 2007; Roshetko et al., 2007; Sharrow and Ismail, 2004). This supposition is based on the notion that tree incorporation in agricultural landscapes and pastures would result in greater net aboveground as well as belowground C sequestration as trees probably sequestrate C better than non-woody plants (Kiyono et al., 2007; Mutuo et al., 2005; Palm et al., 2004). Hence, the establishment of trees in agricultural landscapes like taunya systems, or coffee/cocoa plantations as diverse agroforestry systems, could also be viewed in the context of increasing the amount of C accumulated in both the above and below ground growth structures of the system (Soto-Pinto et al., 2010; Kiyono, 2007). Our study's results suggest that propagation methods significantly influence the carbon content in 10 years old *D. edulis* trees of either seed or vegetative origin. C stocks in trees of cutting and marcot origins were 2 and 1.5-fold higher respectively, than in trees of seed origin (Tables 5.3 and 5.4). This trend was also evidenced through CO_2e sequestrated from the atmosphere (Tables 5.4 and 5.5). 10 years old *D. edulis* trees of vegetative (cutting and maroct) origins sequestered respectively 26.8 ± 19.1 Mg C ha^{-1} and 21 ± 12.8 Mg C ha^{-1}, against 13.9 ± 9.4 Mg C ha^{-1} for trees of seed origin (Table 5.5). Albrecht and Kandji (2003) reported that carbon content in any vegetation type depends on the structure of the system among other factors. Genard et al. (2008), maintain that carbon allocation within a plant depends on complex rules linking source organs (mainly shoots) and sink organs (mainly roots and fruits). However, most agroforestry tree products (fruits, nuts, vegetables, spices, bark, oils, resin, etc.) can be harvested with negligible impact on the C stock of that system. It is most probably therefore that the well-developed and bushy stature of *D. edulis* trees of vegetative origin, explains the observed variation in C stock between trees of different types.

D. edulis is a perennial crop from West and Central Africa. The species grow in mixed agroforestry systems with species choices often determined by household needs and markets. *D. edulis* has been reported by Awono et al. (2002), Ayuk et al. (1991), and Schreckenberg et al. (2006) to provide tangible socio-economic benefits. The management of these agroforestry systems should be flexible to limit risks and enable farmers to respond rapidly to changing marketing opportunities.

Quantification of C stocks of individual components of an agroforestry system is very relevant in the design of improved agroforestry systems capable of reducing the vulnerability of small-scale farmers to crop failures as more diversified farming systems would suffer less from shocks of projected climate variations and maintain the agility of farmers to adapt to changing conditions. According to Roshetko et al. (2007), carbon constitutes a new 'mysterious' product to farmers, which is even less tangible than other more easily perceived environmental services such as watershed management benefits or biodiversity conservation. The latter authors maintain that any reward from C storage in agroforestry systems should come as an addition to services rendered.

D. edulis is a long cycle perennial crop, and the woody component of the tree represents an important part of the trees' total biomass. Hence, tons of C can be sequestrated and stored in the bole, branches, roots and soil of this species over several decades. The results of our study provide new evidence that tree domestication does not only provide options for diversifying food and tree crop production systems but could also increase the sustainagility *sic* (Verchot et al., 2007) of such system against projected climate variability.

5.5 Conclusion

This is a pioneer study on the effect of propagation methods on aboveground growth attributes and carbon storage potential of an indigenous fruit tree species under domestication in the humid tropics of Africa. Research studies published on the contribution of individual perennial components within an agroforestry system in relation to buffering against climate variability are not numerous. In the fight against climate change through C sequestration, the end product of concern is CO_2.

In the current study, we looked at an individual species' (*D. edulis*) contribution to carbon sequestration. Results suggest that propagation methods significantly influence *D. edulis* tree growth attributes, together with C stocks and CO_2e sequestrated. *D. edulis* trees of vegetative origin (cuttings and marcots) were bigger and had 2 to 1.5 times more C stocks, respectively, compared to trees of seed origin.

D. edulis is cultivated in the humid tropics of West and Central Africa in homegardens, food crop fields, cocoa and coffee plantations all with the aim of diversifying farm products and to exploit the benefits of tree-crop interactions. The tree plays various roles including shading of crops to reduce evapotranspiration, erosion control and nutrient cycling besides producing nutritious fruits rich in lipids and vitamins.

Agroforestry systems are reputed for their high plant diversity. However, there is a need to quantify individual species growth attributes and C storage potential in order to better design new or enrich existing agroforestry systems with species that will enable the system to fulfil its diverse functions of providing food, medicine, fodder, timber and income to approximately 1.2 billion people the world over, estimated to be using agroforestry practices in 2009.

PART TWO

CHAPTER 6

6. DOMESTICATION STATUS OF *ALLANBLACKIA FLORIBUNDA* OLIV.

6.1 Taxonomy and nomenclature of *Allanblackia floribunda*

Genus *Allanblackia* belongs to the Clusiaceae. Until now, differentiation between species in the genus is not clear. A taxonomic revision of the genus by Bamps (1969) recognized 9 species, plus probably a tenth imperfectly known species from Fernando Po (now Equatorial Guinea). This view was maintained by Lebrun and Stork (1991-1997). The species, including their synonyms and their distribution in Africa are presented below (Table 6.1).

All nine species of *Allanblackia* are indigenous to the humid tropics of Africa (Van Rompaey, 2003). Three species (*A. floribunda, A. parviflora and A. stuhlmannii*) have been noted for their potential importance as safe food within the European Union market (EU) (Hermann, 2009) and cosmetic (soap and detergent) (Foma and Abdala, 1985).

A. floribunda has been reported to be the most widespread *Allanblackia* species in tropical Africa (van Rompaey, 2003). Bamps (1969) published an Africa-wide distribution map and Letouzey (1978) a detailed one for Cameroon. The latter generalized from Vivien and Faure (1985) for *A. floribunda* (Figure 5.1). It occurs from the extreme southeast of Benin through Nigeria, Cameroon, and Gabon to Congo. *A. floribunda* is a lowland forest species growing up to 800 m altitude. In West Africa the species is most abundant in the Atlantic forests in the west, less abundant in the Congolese forests in the south, and rare or absent from the semi-deciduous forests in (Letouzey, 1978).

Table 6.1. *Allanblackia* species and their distribution in Africa (based upon Van Rompaey, 2003)

Species names	Synonyms	Distribution
Allanblackia floribunda Oliver (1869)	*A. klainei*, *A. stanerana* Auct. non Exell & Mendonca, Spirlet (1966)	Nigeria to Democratic Republic of Congo (DRC) (grows up to 800 m a.s.l)
Allanblackia gabonensis (Pellegr.) Bamps (1969)	*A. floribunda* var. gabonensis	Cameroon and Gabon (grows in upland forest between 500 and 1,750 m a.s.l)
Allanblackia kimbiliensis Spirl. (1959)	none	DRC (Kivu), Uganda (grows between 1250 and 1800 m a.s.l)
Allanblackia kisonghi Vermoesen (1923)	*A. floribunda* var. *kisonghi* (Vermoesen) Pieraerts, *A. monticola* Auct. non Mildbr.ex Engl. Staner (1934) ; Spirlet (1966)	DRC (gallery forest of Kasai and Bas-Congo)
Allanblackia marienii Staner (1934)	none	DRC (in gallery and swamp forests)
Allanblackia parviflora A.Chevalier (1909)	*A. floribunda* Auct. non Oliver Keay(1954)	The only species in West Africa, Sierra Leone to Ghana
Allanblackia stanerana Exell & Mendonça (1936)	none	south est of Cameroon, DRC, Angola
Allanblackia stuhlmannii Engl. (1897)	*Stearodendron stuhlmannii* Engl. (1895)	Tanzania (540 - 1600 m a.s.l)
Allanblackia ulugurensis Engl. (1900)	none	Tanzania (700 - 2050 m a.s.l)

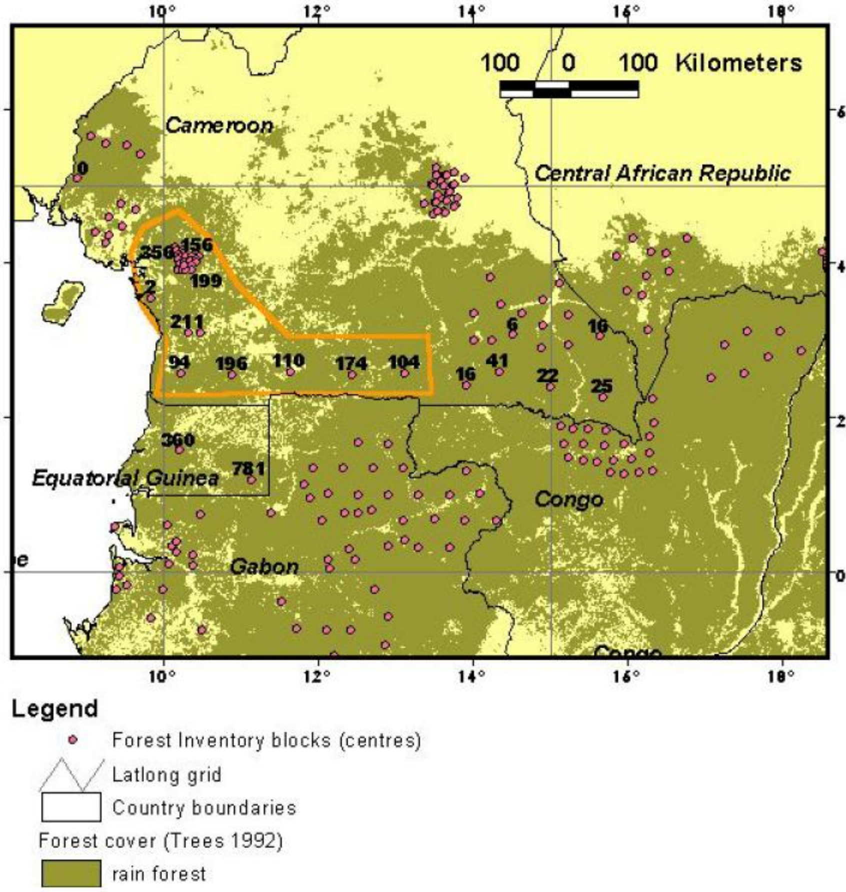


Figure 6.1. Estimated *Allanblackia floribunda* tree density in Central Africa

Forest cover from TREES (1992) map. Trees above 10 cm diameter per km^2 as found in forest inventories in Central Africa. Plots without label have no *Allanblackia* in the inventory report, either because it was not recorded or because it was absent. Orange line indicates the suggested area for potential 'wild harvesting' of Cameroon. For NW-Cameroon more data are needed

Source: Van Rompaey, 2003

As a result of their potential value, *Allanblackia* species have been described as a new oil cash tree crop for farmers in Africa. Market supply chains for *Allanblackia* oil are progressively been developed since 2002 by Novella Africa in Ghana, Tanzania and Nigeria (Jamnadass et al., 2010). Hence, research on the domestication of *Allanblackia* spp is needed for the wide-scale cultivation of the species. This has been initiated and the results are being scaled out through a private public partnership network of Unilever, World Agroforestry Centre, National Research Systems and local companies in Cameroon and Nigeria (*A. floribunda)*, Ghana (*A. parviflora*) and Tanzania (*A. stuhlmannii*).

This thesis focuses on *A. floribunda.* This species is abundant in Cameroon and grows naturally from Nigeria to the Democratic Republic of Congo.

6.2 Botanical description, ecology and distribution

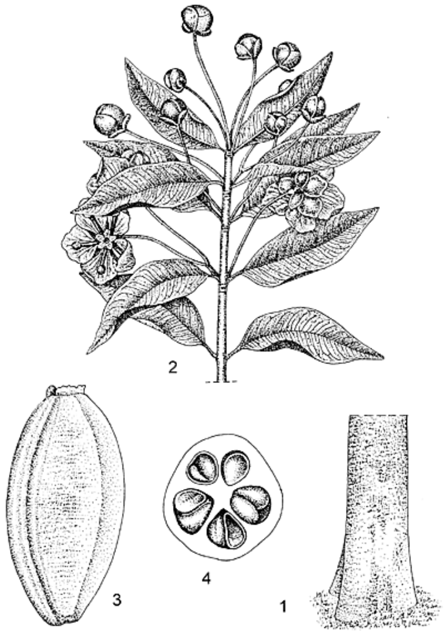
Allanblackia is a dioecious, tall evergreen rain forest tree with strongly plagiotropic weeping branches. It can grow more than 30 m high. Leaves are simple and opposite with white, pink or red, very fragrant flowers. Mature fruits are large ovoid, brown berry-like drupes containing several brown seeds (Figure 6.2). *A. floribunda* is a common understory tree of lowland closed evergreen rainforest and riverine forest, and also in secondary and swamp forest, up to 1000 m altitude and prefers areas with mean annual rainfall ranging from 1,200–2,400 mm.

6.3 Uses of *A. floribunda*

A. floribunda nuts have been certified by the European Union for safe usage in food products (Hermann, 2009). Nuts can also be used in cosmetics (soap and detergent) (Foma and Abdala, 1985). Consequently, its expected demand will soon rise. The oil from the nuts containing 52-58% stearic acid and 39-45% oleic acid [Hildicth (1958) cited in Atangana et al. 2006] is of high commercial importance, with considerable tree-to-tree variation in fatty acid profiles (Atangana et al., 2011). The fatty acid profile of the oil has been reported to lower plasma cholesterol levels (Bonanome and Grundy, 1988), thereby reducing the risk of heart attacks.

In Cameroon the bark is used against coughs, dysentery, toothache and as an aphrodisiac and pain reliever (Laird, 1996). Other possible uses include the treatment of HIV/AIDS as guttiferone F, an HIV-inhibitor, has been reported from bark extracts of *A. stuhlmannii* (Fuller et al., 2003).

Unilever is engaged in the process of building an *Allanblackia* oil production industry in Africa based on smallholder oil production and local supply chain development, which could give fair returns to farmers, collectors and local processors (Egyir, 2007).



1. base of bole
2. flowering twig
3. fruit
4. fruit in cross section showing seeds

Redrawn and adapted by Achmad Satiri Nurhaman

Figure 6.2. Botanical features of *A. floribunda*

Source: http://www.prota4u.org/searchresults.asp accessed 02/09/2011

6.4 Progress in the domestication of *A. floribunda*

Domestication studies have been in progress for *A. floribunda* since 2004 with the first study on vegetative propagation published in 2006 (Atangana et al., 2006). As an agroforestry species, the desire has been to rapidly acquire higher yields, early fruiting, better quality nuts and oil while also producing fruit bearing female trees due to dioecy in *A. floribunda.* Through cloning, there is the possibility to developed clones or cultivars that could offer more uniform products that would better meet market demands. Several vegetative propagation techniques can be utilized to achieve this, including the use of stem cuttings, marcots and grafting (Table 6.2).

Table 6.2. Summary of propagation methods applicable to *A. floribunda*

Species name	**Propagation techniques**	**Success rate**	**Reference**
A. floribunda	Germination	75%	Ofori et al., 2011*
		30%	ICRAF unpublished data
	Rooting of stem cuttings	68%	Atangana et al., 2006
	Marcotting (or air layering)	<30%	Asaah unpublished data
	Grafting	80%	Ofori et al., 2008*

Legend: * = Tested only on *A. parviflora*

The decision to pursue clonal propagation options in the domestication of an underutilized species such as *A. floribunda* would necessitate making a decision with regards to: (i) what propagation methods to adopt; (ii) what level of technology would be appropriate to achieve desirable genetic improvement; and (iii) whether to propagate using juvenile or mature tissues, or both.

6.4.1 Methods of propagation and the cloning process in *A. floribunda*

Propagation of *A. floribunda* is fundamentally by seed. However, research has also focused on the development of vegetative propagation techniques for these species.

6.4.1.1 Sexual propagation

Germination is very difficult in *Allanblackia* genus. This has been a constraint to the domestication process. *A. floribunda* seed takes 6-18 months to germinate (Vivien and Faure, 1996). Atwater (1980), postulated that some seed germinate less with time (seed deterioration) while others germinate more with storage (dormancy release). *Allanblackia,* seeds have been observed to germinate more with increase duration in seedbeds. According to Nikolaeva (1977), three types of physiological dormancy are distinguished based on physiological control of dormancy: non-deep, intermediate and deep. Non-deep physiological dormancy is broken by relatively short (1-8 weeks) periods of warm or cold stratification, depending on the species. Intermediate physiological dormancy is broken by long (8-14 week) periods of cold stratification. Deep physiological dormancy is broken by long periods of cold stratification. From personal observations, it could be suggested that endogenous morphological dormancy is imposed on *Allanblackia* seed in which case the embryo is not fully developed when the fruit is shed from the tree. However, it is not clear whether the seed coat is impervious or contains inhibitors and thus imposes exo-endodormancy (a combination of exogenous and endogenous dormancy conditions). For instance, physical (hard seed coat of *Allanblackia*) and intermediate physiological dormancy of a not fully developed embryo. According to Hartmann et al. (2002), such dormancy condition can be broken by scarification, followed by stratification. Scarification and stratification have been tested in *A. parviflora* seeds in polythene bags under shade conditions in a recent study by Ofori et al. (2011) in Ghana. Seeds with seed coat removed and kept in polythene bags started germinating from 2 weeks after and within 10 months, 75 and 68% germination were obtained for scarified seeds in transparent and black polythene bags, respectively.

The major inconvenience associated with seed propagation is that seedlings are genetically heterogeneous, each seed having inherited different parts of the genetic codes of its parent trees, with segregation of genes among the progeny. This is particular true for species that are out breeders (allogamy) and dioecious. In addition, sexual propagated trees of *A. floribunda* have a long juvenile phase of more than 12 years to fruiting (Vivien and Faure, 1996).

6.4.1.2 Vegetative propagation

Vegetative propagation can be considered as a phenomenon of regeneration of differentiated, somatic cells of plants to produce a new organ and restore body parts that have been lost by injury or autotomy (self amputation of body parts) (De Klerk, 2000).

6.4.1.3 Propagation by stem cuttings

Atangana et al. (2006), in a study on the rooting of stem cuttings of *A. floribunda* reported 68.7% rooting success at 25 weeks in a non-mist propagator after important factors (rooting substrate – river sand; clone and cutting leaf area – 25 cm^2) had been optimised. These stems cuttings were obtained from coppiced juvenile shoots and this rooting percentage did not change till the end of the experiment at 38 weeks. However, most of the cuttings developed single roots and this could compromise their tree stability. There is therefore a need to further fine tune the rooting protocol of *A. floribunda* by for example, optimizing the pre and post-severance stockplant factors, rooting environment etc. These factors have been reported by Leakey (2004) in his review on the physiology of vegetative propagation to impact the speed of rooting and consequently the number of roots developed per rooted cutting.

6.4.1.4 Propagation by marcotting (air layering)

This technique is being developed and tested on *Allanblackia.* It is hoped that the necessary conditions for successful rooting and management of *Allanblackia* marcots could be established, using similar approaches described earlier (Chapter two) for *D. edulis.*

6.4.1.5 Propagation by grafting

Grafting has proven to be very successful in *Allanblackia* species. For example, Ofori et al. (2008) reported grafting success of 80% using cleft grafting as opposed to 50% for side veneer grafting on *A. parviflora* in Ghana.

6.4.1.6 *In vitro* propagation

No work has been published on the *in vitro* propagation of *Allanblackia* species.

6.5 Managing genetic variability in *A. floribunda*

Domestication is generally considered to reduce the genetic diversity of the species being domesticated, as only the best 'individuals' are brought into domestication programmes (Doebley et al., 2006). The reduced number of individuals from the population brought into the domestication programme results in a genetic bottleneck (a reduction in genetic variation across the genome, including neutral variation) in cultivated populations relative to their wild progenitors (Olsen and Gross, 2008).

According to Miller and Gross (2011), through comparative analyses of perennial fruit crops and their wild progenitors, it has been demonstrated that cultivated perennial fruit crops retained averagely 94.8% of the neutral variation found in wild populations. Genetic studies using microsatellites revealed that cultivated perennial populations could retain at least 64.8% and up to 126.9% of the variation found in wild populations of *Inga edulis (*Hollingsworth et al., 2005); apple (Coart et al., 2003) ; and olive (Lumaret et al., 2004). In similar molecular studies using AFLP and ISSR techniques, cultivated perennials have been reported to retained at least 62.5% and at most 117.8% of the variation found in wild populations in chestnut, *Castanea sativa* (Mattioni et al., 2008) ; apple (Coart et al., 2003) ; and pistachio (Shanjani et al., 2009).

More recently, evidence from phenotypic characterization studies on *A. floribunda* fruit/nuts characters (fruit mass, fruit diameter number of nuts, nut mass) from 70 trees distributed among four sites in wild stands in Cameroon revealed highly significant between-and within-tree variation in fruit and seed characters while between-site phenotypic variation was not significant (Atangana et al., 2011). In the same study, stearic and oleic acid content from seeds had a strong positive relationship with the seed fat. However, seed fat profiles did not vary with fruit characters. According to the latter author, the strong and positive relationships identified between fruit mass and fruit diameter, and between fruit mass and total nut mass are indicative that an improvement in one character might cause a simultaneous increase in the other. The same postulation could also be advanced for the strong and positive phenotypic relationship between stearic and oleic acid contents suggesting that improvement in one of these traits might lead to improvement in the other. In contrast, evidence from *I. gabonensis* (Atangana et al., 2002), *S. birrea* (Leakey, 2005c) and *D. edulis* (Waruhiu et al., 2004), illustrates that this outcome is not common between unrelated traits. However, fruit characters (fruit mass, fruit width, fruit length and flesh) have been reported to be heritable in *Olea europa* (Zeinanloo et al., 2009) while fruit mass, shape, flavour and colour are reported to be highly heritable in *Mangifera indica* (Brettell et al., 2004).

From the forgoing, it is evident that perennial fruit crops maintain a greater proportion of total genetic variation in cultivated populations. However, Miller and Gross (2011) remarked that in some more recently domesticated perennial fruit crops, reduction in genetic variation could be as a result of selective propagation of some individuals in a cultivated setting, rather than to many generations of selective breeding that could more appropriately be termed a "domestication bottleneck". The authors concluded by making reference to older perennial fruit crops such as apple, olive, grapevine, and pistachio.— that have retained averagely 94.6% of the genetic diversity present in their wild relatives - (Aradhya et al., 2003; Coart et al., 2003; Lumaret et al., 2004; Shanjani et al., 2009). Thus if the extensive diversity of fruit/nut characters in *A. floribunda* reported by Atangana (2011) are under genetic control, then potential genetic gains can be achieved through vegetative propagation of the best individual trees in the wild.

Modern molecular techniques are useful in the development of a more informed strategy for the maintenance of genetic diversity. Within the geographic range of a particular species they can be used to identify 'hotspots' of intraspecific diversity e.g. Assogbadjo et al. (2006), suggested *in* or *ex situ* conservation options for *Adansonia digitata*, and Lowe et al. (2000) for *Irvingia* species. In a study on the genetic diversity of *Allanblackia* genus Russell et al. (2009), recognized the opportunities and challenges for the domestication and conservation of *A. floribunda*, *A. gabonensis* and *A. stanerana* in Cameroon with high overall levels of genetic variation in natural populations which could be exploited in the development of *Allanblackia* cultivars. In the same study, the authors maintained that *A. floribunda* and *A. stanerana* were genetically similar but well-separated and unrelated to *A. gabonensis.* Interestingly, *A. gabonensis*, and *A. parviflora* were genetically similar in spite of distinct known distribution of the two species, which neither overlap nor border geographically (*A. gabonensis* in Cameroon and Gabon only). *A. stuhlmannii* was clearly genetically different from all the above mentioned species corresponding to the significant geographical distance (>2,500 km) between the Eastern Arc Mountains of Tanzania and the locations in Cameroon and Ghana from which other taxa were sampled.

Thus, with a good understanding of intraspecific variation in *A. floribunda* fruit and oil characteristics of importance for selection and improvement to meet different market opportunities, clonal approaches can then be used for improvements in yield and nut quality traits (Atangana et al., 2011; Leakey et al., 2005a; Miller and Gross, 2011).

Attempts to root stem cuttings of *A. floribunda* have found the species to be both slow to root and also to produce too few roots for good stability. Consequently, it was decided to examine alternative approaches to clonal multiplication, in *A. floribunda* such as grafting.

6.6 Research question

- Can the long juvenile phase of *A. floribunda* to fruiting be reduced to less than 5 years through vegetative propagation (grafting)?

6.6.1 Objective

- To determine the amenability of *A. floribunda* to vegetative propagation through grafting

6.6.2 Hypothesis

- Grafting is an appropriate method for propagating *A. floribunda* trees vegetatively.

CHAPTER 7

7. DOMESTICATION OF *ALLANBLACKIA FLORIBUNDA* OLIV. (CLUSIACEAE): AMENABILITY TO GRAFTING

Abstract

Three *Allanblackia* species (*A. floribunda, A. stuhlmanniiand A. parviflora)* (Clusiaceae) with high nutritive, medicinal, cosmetic and economic values are currently being domesticated as new oil tree crops. *Allanblackia* seeds contain a white fat, solid at room temperature consisting mostly of stearic (52–58%) and oleic (39-49%) acids. This unusual fatty acid profile has the properties appropriate for many different food and cosmetic applications making them commercially interesting. Vegetative propagation studies on *A. floribunda*, which grows naturally in the moist forest of Cameroon and Nigeria, were initiated aimed at evaluating its amenability to grafting. Scions taken from fruiting female trees were grafted onto eighteen month old rootstocks of *A. floribunda* using side tongue, top cleft, side veneer, and whip-and-tongue methods under nursery conditions in Cameroon. In parallel, side tongue and inverted 'T' budding methods were also tested *in situ* on young *A. floribunda* wildings growing under semi-deciduous and evergreen tree covers. In addition, the effects of protecting side tongue grafts with non-perforated, translucent plastic, perforated translucent plastic and aluminium foil were assessed. Under nursery conditions, side tongue grafts were significantly more successful (80.0% ± 6.3), than grafts of side veneer (52.5% ± 7.9), top cleft (55.0% ± 7.9) and whip–and-tongue (50.0% ± 7.9). The success of side tongue grafts was further increased (86.7% ± 6.2) under the shade of evergreen trees when protected by non-perforated translucent plastic. These results indicate the potential for *in situ* grafting, 'top working' to graft pollinator branches on female *Allanblackia* trees. In addition, grafting is strongly recommended in the production of good-quality planting materials of *Allanblackia* for cultivation within multifunctional agricultural systems because the technique successfully reduced the long juvenile phase to fruiting in *A. floribunda* to 4 years.

Key words: *in situ* grafting, oil crop, underutilized species, vegetative propagation

This chapter is drawn from the following publication: Asaah E.K., Tchoundjeu Z., Ngahane W., Tsobeng A.C., Kouodiekong L., Jamnadass R., Simons A. (2010). *Allanblackia floribunda* – a new oil tree crop for Africa: amenability to grafting. *New Forest* 41:389- 398 DOI: 10.1007/s11056-010-9230-z.

7.1 Introduction

Nine species of *Allanblackia* (Clusiaceae) are indigenous to the African tropics (Van Rompaey, 2003). *Allanblackia* is dioecious and three species (*A. stuhlmannii*, *A. floribunda and A. parviflora*) are important in food (margarine) and cosmetic (soap and detergent) industries (Foma and Abdala, 1985). The oil from the seeds containing 52-58% stearic acid and 39-45% oleic acid (Hildicth 1958) and is of high commercial importance (Atangana et al., 2011; Jamnadass et al., 2010). *Allanblackia* oil is also interest because it requires less chemical processing and refraction than palm oil. The fatty acid profile of the oil has been reported to lower plasma cholesterol levels (Bonanome and Grundy, 1988). In Ghana, its bark is used to relieve toothache (Abbiw, 1990) while in Cameroon it is used against coughs, dysentery, toothache and as an aphrodisiac and pain reliever (Laird, 1996). Other possible uses include: the treatment of HIV-AIDS as Guttiferone F, an HIV-inhibitor, has been reported in bark extracts of *Allanblackia stuhlmannii* (Fuller et al., 2003), antimicrobial activity against a range of *Gram*-negative and *Gram*-positive bacteria and antileishmanial activity against *Leishmania amazonensis* reported in bark extracts of *Allanblackia gabonensis (*Azebaze et al., 2008) and anit-inflammatory action from methanolic extracts from fruits of *A. floribunda* on carrageenan induced paw oedema in rats (Ayoola et al., 2009).

Allanblackia in the last decade has become a subject of international interest to Unilever and other commercial enterprises as the seeds contain edible oil that can be used in food products. Their particular interest lies in the oil obtainable seed which has significant potential in the global food market as a ‘raw material’ for the production of healthy spreads that are low in trans-fats (Ochieng, 2007).

Unilever estimates that the potential market for *Allanblackia* oil at more than 100,000ts annually, provided the right quality standards are met. Together, Unilever, the World Agroforestry Centre (ICRAF) and the World Conservation Union (IUCN), along with national research institutions, farming communities, market traders and other parties, have formed a private-public partnership (PPP) known as ‘Novella Africa’ to develop a sustainable *Allanblackia* oil business (Attipoe et al., 2006). The partners involved in market development of *Allanblackia* seeds include Novel Development Companies (known collectively as Novel International) in Ghana, Nigeria and Tanzania, TechnoServe, the Institute of Cultural Affairs (Ghana), The Netherlands Development Organization (SNV), Faida Market Link (Tanzania) and INADES Formation. Those involved in the promotion of cultivation include Novel International, the forestry research institutes of Ghana, Nigeria and Tanzania, the International Tree Seed Centre (Ghana), the Tanzania Forest Conservation Group and the Amani Nature Reserve (Tanzania) (Jamnadass et al., 2010).

Already, *Allanblackia* oil has received approval of the European Union (EU) Novel Food Regulations that certify safe usage as a foodstuff (Hermann, 2009), opening up a high future demand in EU markets. In response to this development, supply chains for *Allanblackia* seed collected from natural stands of the tree have been developed in Ghana, Nigeria and Tanzania, and are under consideration in Cameroon and Liberia (Attipoe et al., 2006). According to Egyir (2007), economic projections suggest that by 2020, about 100,000 farmers could earn an extra income of on average 200 Euros per annum from planting *Allanblackia* trees, with greater returns possible in later years.

Although the potential for *Allanblackia* as a new crop is evident, prior experience in domesticating and commercializing indigenous fruits, nuts and other tree products indicates that promotion must be handled carefully if farmers are to benefit substantially (as suggested by Leakey et al., 2005b, Marshall et al., 2006). Harvest and cultivation of a new crop also has critical implications for biodiversity – for the particular species in question, as well as for other plants and animals – and careful management is required if outcomes are to be positive (Dawson and Jamnadass, 2007). Conservation considerations are especially relevant for the regions where *Allanblackia* grows, which are global hotspots of biodiversity and are subject to significant habitat fragmentation (Mittermeier et al., 2004). Approaches such as agroforestry – in which a range of trees, annual crops and other plants and animals is combined in productive and sustainable farming systems – have an important role in landscape restoration in these regions (Scherr and McNeely, 2008). Trees in farmland are not only important reservoirs of biodiversity in themselves, but also help maintain connectivity between remaining fragments of natural forest (Bhagwat et al., 2008). These issues are of particular concern to IUCN and explain their involvement in the current initiative.

From the forgoing, if current limitations (development of productive and quality planting materials) to cultivation are addressed, the foreseen returns from planting will compare well with other perennial crops such as cocoa, oil palm and tea (Shrestha et al., 2007). To this end, a tree domestication initiative supported by research led by ICRAF is underway. In this chapter, we describe how tree domestication techniques that offer an opportunity to improve the productivity and overall quality of mature trees, is achievable through simple genetic selection and vegetative propagation of 'elite' *Allanblackia floribunda* mature trees with marketable fruit/nut and oil traits.

Allanblackia floribunda Oliv. (Clusiaceae) is found in the humid forests of Cameroon (Letouzey, 1985). Trees grow to a height of 30m. The large elongated, five-sided fruits (1 – 2.5kg) are light brown with chestnut spots. Seeds are contained in a gelatinous pulp. *Allanblackia* species are among the underutilized species, which traditionally provide nutritional, economic or environmental benefits but have been neglected by mainstream domestication (Leakey and Simons, 1998). Farmers have always viewed these species as nature's gifts within their farming systems, but they are now disappearing through forest clearance for modern agriculture (Leakey et al., 1999). However in the last decade, such high-value agroforestry trees, especially those for fruit/nut production, have been the subject of domestication and studies on improvement of yield and quality (Tchoundjeu et al., 2006) for the alleviation of poverty, hunger, malnutrition and environmental degradation (Leakey et al., 2005b).

Germination of *A. floribunda* is very slow with an overall germination rate less than 5% (Vivien and Faure, 1996). Consequently, the cultivation of *Allanblackia* species on farm is constrained by propagation success (both sexual and vegetative propagation techniques) (Munjuga et al., 2008). Recently, however new techniques developed in Ghana and Tanzania with *A. parviflora* and *A.stuhlmannii* have greatly improved nursery germination success (Munjuga unpublished; Ofori et al., 2011).

However, *Allanblackia,* as an allogamous species, vegetative propagation techniques are needed to capture certain desirable fruit or tree traits so as to produce planting materials having the same genetic characteristics as the mother trees. Vegetative propagation (grafting, budding and marcotting) have also been used to achieve early fruiting and tree dwarfing (Akinnifesi et al., 2008). In this respect, there exist a number of vegetative propagation techniques including the rooting of stem cuttings, marcotting and grafting. Recent studies using leafy stem cuttings have shown this approach to vegetative propagation to be successful when juvenile shoots are used, although the rooting process is slow and only a few roots are produced per cutting. For example, 68.7% of cuttings obtained from juvenile shoots of a coppiced *A. floribunda* (Atangana et al., 2006). It is clear that, further work is needed to speed up rooting and increase root number (Leakey, 2009).

Vegetative propagation is a powerful tool to capture existing genetic traits identified through 'plus tree selection' and fixing them over generations so that they can be used as the basis for a genetic 'variety' or 'cultivar' development and building up mother stocks (Leakey, In press). For species like *Allanblackia*, the advantage of using clonal propagules would outweigh those of seedlings especially as the oil is of high nutritional or income value and the tree has a long juvenile phase to flowering and fruiting. Vegetative propagation techniques like grafting, and marcotting, can be used to multiply elite *Allanblackia* tree destine as mother stock. The mother stock can then be subsequently multiplied by stem cuttings, marcotting and grafting (Hartmann et al., 2002; Mng'omba et al., 2008).

Grafting with scions from ontogenetically mature trees identified through 'plus tree' selection (Atangana et al., 2002) on seedlings or wildings would greatly shortened or avoided the long juvenile phase (Hartmann et al., 2002) to flowering and fruiting. For example, grafted *Uapaca kirkiana* began to produce fruits only after 2-3 years, while those derived from seedlings took 12-15 years before fruiting (Akinnifesi et al., 2008).

Although considerable information is available on vegetative propagation of tropical African underutilized species using stem cuttings (Leakey et al., 1990; Leakey, 2004b; Tchoundjeu et al., 2006; Mng'omba et al., 2008) as a simple and inexpensive technology, much less is known about the potential graft success. This study therefore aimed at improving grafting techniques in *A. floridunda.* The objectives were to (i) determine the effectiveness of four grafting methods (side tongue, side veneer, top cleft and whip–and-tongue) on graft success, (ii) determine if protecting fresh *in situ* grafts improve graft success and (iii) determine if shade under semi-deciduous and evergreen tree cover affects *in situ* graft success.

7.2 Material and methods

7.2.1 Study site

The trials were carried out on station and on farm. On station trials were conducted at ICRAF experimental nursery – Nkolbisson Yaoundé (altitude: 700 m a.s.l, latitude 3^{o}52′ to 3^{o}53′ North, and longitude 11^{o}25′ to 11^{o}27′ East) and *in situ* trails carried out on farms at Essang dominated by semi-deciduous trees and Ngoumou (altitude: 695m a.s.l, latitude 2.1^{0} to 4.9 North and longitude 10.5^{0} to 16.2^{0} East) by evergreen tropical trees. The rainfall pattern for both Essang and Ngoumou is bimodal with an annual average of 1,672 mm. The relative humidity varies between 73 and 84 % while the average annual temperature is 25^0C (Ambassa-Kiki, 2000).

7.2.2 Experiment 1: nursery trial to compare grafting methods on *A. floribunda* rootstocks

The effectiveness of four grafting methods was tested on eighteen-month-old seedlings of *A. floribunda* in polybags that were at the vegetative growth phase. Scions were taken from twigs of the previous year's growth on branches in the crown of mature *A. floribunda* trees with buds which were about to sprout. Four grafting methods (side tongue, side veneer, top cleft and whip–and-tongue) were tested in a completely randomized design during the rainy season. Each method was applied to 40 plants selected at random, giving a total of 160 plants for the experiment. Standard grafting procedures, as described by Hartmann et al. (2002) and Crasweller (2005), were followed and grafts were assessed weekly for graft success or mortality from the third to the twelfth week. Success was assessed by the healing of the graft union, sprouting of scion buds and leaf formation, while mortality was determined by the lack of sprouting from green scions or by the dehydration and browning or drying of scions. Data were collected in binary form: graft success: '1' and graft mortality: '0'. Owing to the binomial distribution of the data collected, an analysis of deviance (ANODE) using Generalized Liner Model (GLM) procedures of Genstat 12th Edition with the logit function as the link function was carried out to assess the effect of grafting methods on graft success. Below is an equation of the fitted model for any specific week:

Logit $(\mathbf{p_i}) = \mathbf{log}\left(\mathbf{p_i}/(\mathbf{1} - \mathbf{p_i})\right)$ = constant and grafting method effect,

where:

p_i, is the probability of success of a branch grafted with method,

i, which is computed as the ratio of successful grafts over the total number of grafts per method (n=40).

Then chi-square (X^2) test associated with the analysis of deviance table was used to access whether graft success rate differed among graft methods. Thereafter, estimates of the grafting methods' success rate (P_1) along with their respective standard errors were obtained and back-transformed to the probability scale via the predict options of the dialog box.

Finally, treatment means were compared using Tukey's Honestly Significant Difference Test. Unless otherwise stated, statistical significance is given at the 5% level.

7.2.3 Experiment 2: *in situ* grafting of young wildings of *A. floribunda* under evergreen and deciduous forests

Assessment of *in situ* graft success of *A. floribunda* was carried out in two experimental sites, Essang (with semi-deciduous trees as the dominant vegetation) and Ngoumou (with evergreen trees as the dominant vegetation). Type of tree cover, assumed to affect light penetration, was used to investigate the effect of shade in combination with graft protection methods on graft success and survival in both sites. Two grafting methods [side tongue (ST) and budding] were tested in both sites. For side tongue grafts, three graft protection methods were applied: non-perforated translucent plastic (ST-NPP), perforated translucent plastic (ST-PP) and aluminium foil (ST-Al) making a total of four experimental treatments evaluated. Aluminium foil was wound around the plastic to increase light reflection and reduce heat absorption by the scion. In each site, 120 young wildings of pencil diameter of *A. floribunda* at the vegetative growth phase were identified, and 30 of the 120 were randomly assigned to each treatment. Side tongue and budding methods were chosen because side tongue graft success has been highest in experiment 1 while the budding method is widely used for *in situ* grafting in *Citrus* spp and *Hevea brasiliensis.* Grafting success was assessed as described in experiment 1 and success rate among graft protection methods compared within and between sites.

7.3 Results

7.3.1 Nursery trial: effect of grafting methods on *A. floribunda* graft success

The evolution of *A. floribunda* graft success per grafting methods tested over twelve weeks is presented in Figures 7.1 and 7.2. No graft unions were formed up to three weeks after grafting. However, mortality was significantly greater ($p < 0.001$) in top cleft grafts (17.5%) than in side veneer (5%) or side tongue and whip-and-tongue (2.5%) [Figure 7.1]. By week six some grafts had successfully formed unions with side tongue being significantly ($p < 0.001$) better than the others (Figure 7.2: side tongue = 50.0 ± 7.9%; whip-and-tongue = 27.5 ± 7.1%; top cleft = 17.5 ± 6.0%; side veneer = 25.0 ± 6.8%). Beyond nine weeks till the end of the experiment, the results did not change.(success = 80% for side tongue and 50-55% for others).

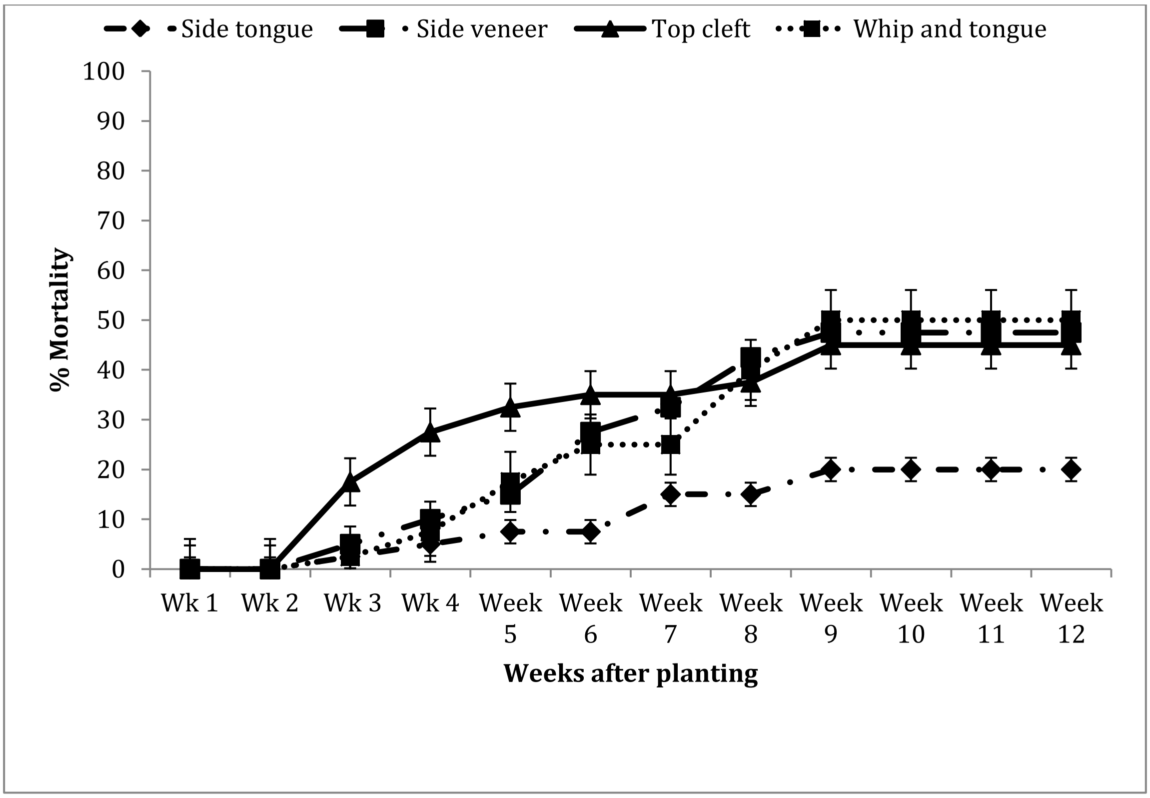


Figure 7.1. Mortality rate (%) in four grafting methods tested on, nursery-grown *A. floribunda* seedlings

7.3.2 *In situ* trial: effect of shade and graft protection on grafting success in *A. floribunda*

The number of successfully grafted plants reached maximum figures between the 3rd and 7th week after grafting, irrespective of sites (semi-deciduous and evergreen tree covers). Under semi-deciduous tree cover (Figure 7.3), graft success on wildings after five weeks was similar for side tongue grafts covered by non-perforated translucent plastic (46.7 ± 9.1%) and perforated translucent plastic (40.0 ± 9.8%) and both were significantly ($P<0.001$) better than those under aluminium foil (10.0 ± 5.5%). Budding was the least successful (3.3 ± 3.3%). Success rates did not improve after week nine.

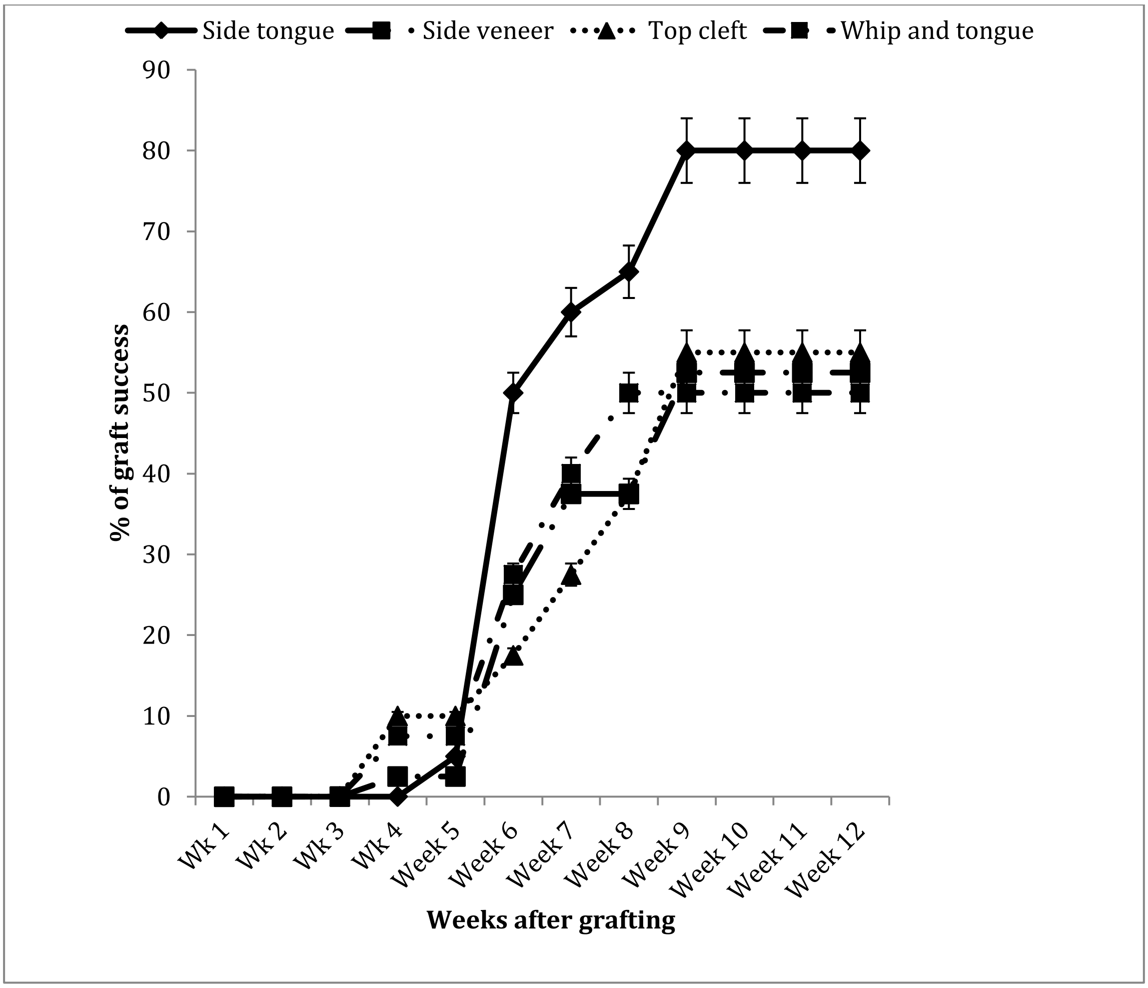
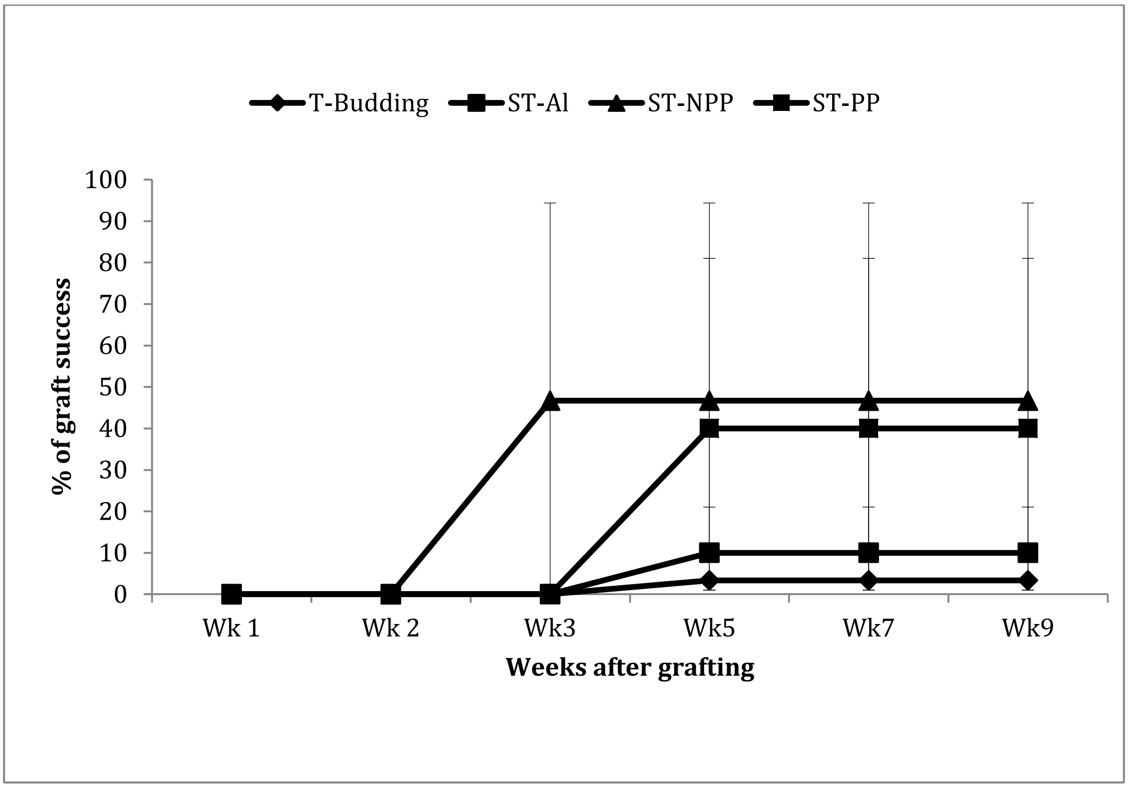


Figure 7.2. Grafting success (%) in four grafting methods tested on nursery-grown *A. floribunda* seedling

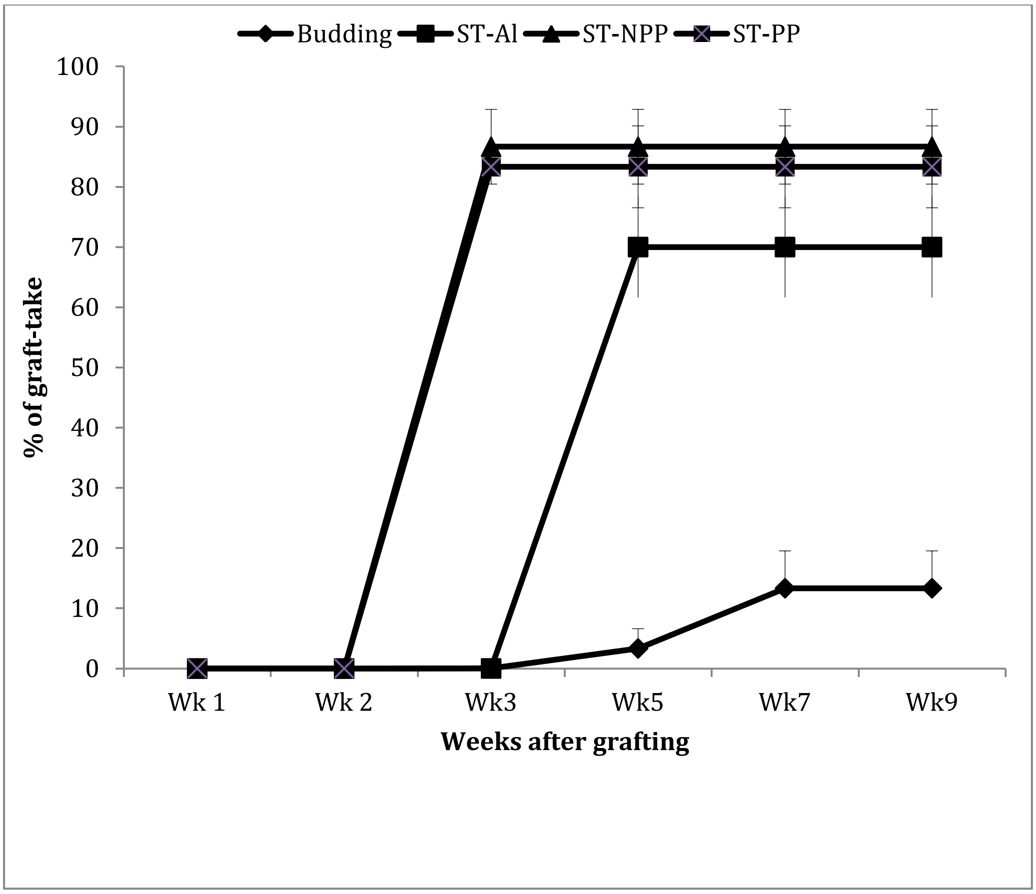
Under the evergreen tree cover (Figure 7.4), only side tongue grafts covered with non-perforated and perforated translucent plastic were successful by week three (86.7 ± 6.2% and 83.3 ± 6.8%, respectively). By week five, aluminium-covered scions and budded plants achieved 70.0 ± 8.5% and 3.3 ± 3.3% success, respectively. By the seventh week, success in budded plants had risen to 13.3 ± 6.2% and remained constant during the remaining weeks of the experiment.

Overall, the difference in graft success rate was highly significant (P<0.0001) among the graft protection methods tested and between the two sites. The most successful grafts were observed under evergreen tree cover in Ngoumou compared to semi-deciduous forest in Essang (Figures 7.3 and 7.4). Side tongue grafts protected with non-perforated translucent plastic had the highest graft success rate under evergreen and semi-deciduous tree covers (86.7 ± 6.2% and 46.7 ± 9.1%, respectively). This was followed by grafts protected with perforated translucent plastic under evergreen and semi-deciduous tree covers (83.3 ± 6.8% and 40.0 ± 9.8%, respectively), and grafts protected with aluminium foil under evergreen and semi-deciduous tree covers (70.0 ± 8.4% and 10.0 ± 5.5%, respectively). In contrast, budding success under evergreen and semi-deciduous tree covers was only 13.3 ± 6.2% and 3.6 ± 3.1%, respectively by week nine (Figures 7.3 and 7.4).



T-Budding= T-budded graft; ST-Al = side tongue graft protected with aluminium foil; ST-NPP = side tongue graft protected with non perforated plastic; ST-PP = side tongue graft protected with perforated plastic

Figure 7.3. Graft success (%) of *A. floribunda* wildings under semi-deciduous tree cover, Essang, Cameroon



T-Budding= T-budded graft; ST-Al = side tongue graft protected with aluminium foil; ST-NPP = side tongue graft protected with non perforated plastic; ST-PP = side tongue graft protected with perforated plastic

Figure 7.4. Graft success of *A. floribunda* under evergreen tree cover, Ngoumou, Cameroon

7.4 Discussion

This study was aimed at investigating the potential success of different grafting methods on both nursery-grown seedlings and wildings of *A. floribunda* under semi-deciduous and evergreen tropical tree cover. The results of the study suggest that, *A. floribunda* is amenable to grafting techniques. Other indigenous dioecious fruit trees such as *Uapaca kirkiana, Sclerocarya birrea* (Mng'omba et al., 2007; 2008), and *Vitellaria paradoxa* (Sanou et al., 2004) have been reported to be similarly amenable to grafting techniques. The level of success reported here for side tongue grafts (80%) is greater than those reported for other indigenous fruit and nut trees of Africa, such as *U. kirkiana* (65% graft success for *in situ* top wedge grafts, according to Mkonda et al., 2001).

It is well-known that grafting is more successful under conditions which minimize the risks of dehydration in the adjoining cut tissues, and that the maintenance of reduced dehydration is beneficial to successful graft fusion (Hartmann et al., 2002), as found in apples when air moisture falls below saturation point. This is said to be because the cambium and parenchyma cells of callus tissues are thin-walled and tender. The results of the present study substantiate these findings in two ways. Firstly, protecting the graft with translucent plastic increased grafting success, and this success was marginally reduced by perforating the plastic (increased dehydration). Secondly, success was greater under evergreen tree cover with presumably lower irradiance, hence higher humidity, lower temperature and consequently lower water stress. Shade (reduced irradiance and lower red:far red ratios) was reported by Leakey (2004b) to precondition shoots morphologically and physiologically so that the rooting ability of stem cuttings is enhanced. The present study suggests that shade has similar effects on the ability of shoots to produce callus and successfully form graft unions. This possibility requires further investigation to determine both the mechanism and the optimum conditions.

This study also found that under all the conditions of these experiments side tongue grafting technique registered the highest graft success on both *A. floribunda* nursery-grown seedlings and wildings. Next was top cleft grafting, while side veneer, whip-and-tongue and budding were increasingly less successful. The amenability of *A. floribunda* to *in situ* grafting implies that 'top-working' techniques can be used to rejuvenate an undesirable cultivar, such as old and unproductive plant or cultivar (Mng'omba et al., 2008). Topworking is a form of grafting usually done in the field where a scion from desired tree is connected by tissues to a growing tree as a rootstock. It can also be used to extend the production cycle, improve the resilience or cultivar value in a wild stand or an orchard (Akinnifesi et al., 2008, Hartmann et al., 2002). Top working has been used to enhance pollination in apples (*Malus domestica*) with highly significant positive correlation of shoot numbers, spur density and bloom density of pollinizers with fruit set and yield in North Western Himalayan region (Das et al., 2011).

For species that are dioecious, such as *A. floribunda*, top-working can be used to introduce male tree (staminate flowers) or branches into an existing male-deficient orchard or wild stand with predominantly female (pistillate) stand and vice versa has been reported to be necessary for *Allanblackia* species (Amanor et al., 2008). According to Hartmann et al. (2002), through topworking, singe citrus tree would grow oranges, lemons, grapefruit, mandarins, and limes; or plum, almond, apricot, and nectarine can be grafted on peach rootstocks.

Walnut (*Juglans regia*) and pistachio (*Pistacia vera*) are difficult to transplant, so producers plant seedling rootstock in orchards and graft *in situ* two years later (Hartman et al., *2002*). Similarly, farmers in Ghana, Tanzania, Nigeria, and Cameroon who have planted thousands of unimproved *Allanblackia* seedlings, (ICRAF unpublished data) could improved these plants through *in situ* grafting as reported in this study for *A. floribunda.* Grafted plants of *A. floribunda* produced in the cause of this study and transplanted in the field flowered and fruited with one tree carrying its fruit to maturity at 4 years (Figures 7.5 and 7.6).

Figure 7.5. Shorten juvenile phase to flowering and fruiting via grafting in *A. floribunda,* Mbalmayo Cameroon (Photo by EK Asaah)

Fruit/Nut characters	Measured values
Fruit length	157 mm
Fruit diameter	58.6 mm
Fruit weight	240 grams
Number of nuts	09
Total nut weight	72 grams (n=9)
Mean nut weight	08 grams (n=9)
Mean nut length	29.6 mm (n=9)

Figure 7.6. *A. floribunda* fruit and nut characteristics from 4 years old graft Mbalmayo Cameroon

This provides new evidence that through vegetative propagation (grafting), the long juvenile period to first fruit production of 10-12 years in *A. floribunda* according to literature, can be reduced to less than 5 years. This opens the way for farmers to start growing selected 'plus trees' of *A. floribunda* which will fruit less than 5 years. In this way, farmers can through grafting techniques develop superior planting materials with greater potential for fruit/nut production, as well as with desirable oil traits.

7.5 Conclusion

A major challenge for fruit/nut trees propagated through seeds in that there is a long juvenile phase (long waiting period). Investments in establishing a fruit/nut tree orchard from seedlings are likely to be high due to a long waiting period and also the cost of tree management during fruit bearing periods. Propagation of fruit/nut trees through vegetative methods is profitable since the break-even point occurs only at least after two to three years. For example, grafted *U. kirkiana* begin to produce fruits only after two to three years, while those derived from seedlings took 12-15 years before fruiting. Similarly, the long juvenile period to first fruit production in *A. floribunda* has also been reduced to less than 5 years through grafting. This result (on *A. floribunda* graft) is the first reported prove of concept on this species according to the authors knowledge.

Propagation of indigenous trees such as *Allanblackia* is drawing increased attention in cultivation for commercial use as well as ecosystem restoration. Grafting is therefore, a promising technique for the propagation of improved *A. floribunda* planting materials starting from young seedlings and wildings both in nursery and shaded *in situ* conditions. Five grafting methods were tested in two experiments and *A. floribunda* graft success was found to decline in the following order: side tongue 80%, side veneer 53%, top cleft 50%, whip-and-tongue 50% and budding 13%. Protecting scions from dehydration with non-perforated translucent plastic was found to enhance success rates (86%). These results have important practical implications for the domestication of *Allanblackia* species as a new crop for edible oil production using agroforestry as a means to create multifunctional agricultural systems.

CHAPTER 8

8. GENERAL DISCUSSIONS, CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

8.1 General discussions

Plant domestication is an evolutionary process operating under the influence of human activities (Harlan, 1992). Overtime, this purposeful man-led selection has cause cultivated populations to diverge morphologically and genetically from their wild progenitors (Emshwiller, 2006; Pickersgill, 2007). The domestication process therefore produces a continuum of plant populations, ranging from exploited wild plants to incipient domesticates to cultivated populations that cannot survive without human intervention (Pickersgill, 2007; Clement et al., 2010). This suggests that cultivated populations exhibiting any morphological or genetic divergence from their wild ancestors be treated as part of the domestication continuum.

It is as a result of these morphological and genetic dynamics within the domestication continuum that tree crop production systems in which indigenous fruit trees like *D. edulis* and *A. floribunda* are being grown have emerged in the humid tropics of Africa. Thus, *D. edulis* and *A. floribunda* are part of the domestication continuum though *D. edulis* could be considered to be at an intermediate phase in the domestication continuum whereas *A. floribunda* is still at its early phases.

The net benefits emanating from tree crop cultivation can generally be slow to materialize due to a long juvenile phase prior to first fruit production. Long juvenile phases in tree crops impose severe limits on traditional breeding efforts because farmers are required to wait multiple years (in some cases, decades) before fruits can be evaluated, selected, and cultivated (Miller and Gross, 2011). However, early farmers sidestepped challenges associated with juvenile phase length by adopting vegetative (clonal) propagation, as the primary form of reproduction in fruit/nut tree crops and as a key component of the domestication syndrome in long-lived plants species (Zohary and Spiegel-Roy, 1975). The shift from sexual to clonal reproduction allowed for the faithful reproduction of individuals with superior features (traits) by eliminating uncertainty in the transmission of favoured traits over reproductive cycles associated with sexual reproduction (Bhojwani and Razdan, 1996). Clonal reproduction (rooting of stem cuttings, air layering, grafting and *in vitro* techniques) can result in rapid rates of change in domesticated systems because individuals with desirable traits, once identified, can be reproduced exactly and extensively (Miller and Gross, 2011).

Changes in the reproductive and vegetative growth have been established and reported for some species under domestication such as, *D. edulis* using stem cuttings (Mialoundama et al., 2002), marcotting (or air layering) (Kengue and Tchio, 1994) and to a limited extent grafting (Damesse et al., 2001) cited by Kengue (2002) and *in vitro* propagation (Youmbi and Benbadis, 2001). However, little is known of the belowground and aboveground growth attributes of mature *D. edulis* trees of vegetative and sexual origins. Similarly, vegetative propagation methods involving stem cuttings had been developed of *A. floridunda* (Atangana et al., 2006; 2007) and grafting has been successful on *A. parviflora* (Ofori et al., 2008). That notwithstanding, there is need to extend the grafting studies to *in situ* conditions in order to improve wild progenitors of *Allanblackia* in the field and also to assess the effective reduction in the long juvenile phase to first fruiting via grafting techniques. In response to the knowledge gap on belowground and aboveground growth attributes for *D. edulis* trees of vegetative and sexual origin and on *in situ* grafting conditions for *A. floribunda*, investigations were initiated.

Although the primary focus of selection in fruit/nut tree crops has been reproductive structures (seeds, shell thickness, fruit characteristics, oil content, etc.), changes in vegetative traits have been reported to occur during the domestication process. A common vegetative feature of domesticated tree crops is dwarfism, which has been documented in avocado (*Persea americana*), castor (*Ricinus communis*), coconut (*Cocos nucifera*), *D. edulis, A. floridunda,* among others (Asaah et al., 2011a; Chapter 5; Kengue, 2002; Miller and Gross, 2011). No mention is made on the belowground growth attributes of the roots or relative carbon storage of the tree.

The present research had as its main objective to establish the effect of propagation methods on rooting pattern/distribution and tree architecture of mature *D. edulis* trees of seed and vegetative origins. Concomitantly, we investigated how the long juvenile phase to first fruiting in *A. floribunda* could be reduced through grafting. *D. edulis* and *A. floribunda* are two African indigenous fruit/nuts trees with high food, income and agro-ecological values. This was done through specific studies on: (i) the effect of propagation methods on structural root architecture of fruiting trees of *D. edulis* of seed and vegetative (cuttings and marcots) origins; (ii) the fine root distribution of fruiting *D. edulis* trees of seed and vegetative (cutting and marcot) origins as affected by propagation methods; (iii) the effect of propagation methods on aboveground tree growth attributes, biomass yield, and C storage in *D. edulis* trees of seed and vegetative (cutting and marcot) origins and (iv) the potential of grafting in the effective reduction of the long juvenile phase to first fruiting in *A. floribunda.*

Various experiments were designed to assess the belowground (rooting system) and aboveground (tree architecture) growth attributes of fruiting *D. edulis* trees of vegetative and seed origins in the field. In the same vein, experiments were setup both under nursery and *in situ* conditions to assess the amenability of *A. floribunda* to grafting. The results of these investigations are reported in chapters 3, 4, 5 and 7 of this thesis.

D. edulis, in 1995, was chosen by farmers after a farmers species preference survey as a priority for more intensive domestication (Tchoundjeu et al., 2002) through a programme of genetic selection (Leakey et al., 2002, 2005) and vegetative propagation using stem cuttings and marcotting (Kengue and Tchio 1994; Mampouya et al., 1994; Mialoundama et al., 2002). While the clonal propagation methods of stem cuttings and marcotting have been well-researched and developed, it is commonly thought that trees of vegetative origin have an undesirable root system and are prone to wind-blow due to the absence of a tap root. However, through this work; (Asaah et al., 2010, Chapter 3), it was established that *D. edulis* trees of vegetative origin have well-developed adventitious primary root system and deep sinker roots which confer stability against wind damage just like it is with tap roots in trees of seed origin. According to a review of tree root architecture by Akinnifesi et al., (2004), root systems vary widely both within and between species and a wide variation in branching forms can be found within a species, depending on age, genotype and site conditions (Vanlauwe et al., 2002). Thus, the observed development of dormant vertical roots in *D. edulis* trees of vegetative origin could be an important adaptation to the absence of a tap root so as not to compromise the acquisition of soil-based resources (principally, water and dissolved ions) and anchorage. Stem cuttings of *Triplochiton scleroxylon* have been reported to develop good sinker roots (Ladipo unpublished data). The anchorage potential of deeply located lateral roots has been reported by Coutts (1983a), to result from a reinforcing and stiffening effect when the tree is being blown by wind.

The assessment of the rooting structure and distribution in *D. edulis* trees of seed and vegetative origins was further extended with a focus on its fine roots (with diameter ≤ 2 mm) distribution [Asaah et al., In press; Chapter 4]. *D. edulis* trees of seed origin were found to have a higher root density (RD) within 0-30 cm soil depth than either of the trees of vegetative origin. Similar overall patterns were found in root length density (RLD) in the 0-10 cm soil stratum. Thus, it could be hypothesised that with regards to competitive interactions between trees and potential annuals in agroforestry systems, the fine root system of *D. edulis* trees of vegetative origin are more complementary than those of trees of seed origin. This could qualify vegetatively propagated trees of *D. edulis* as desirable associative trees for intercropping based on their fine roots architecture. This would be especially true in simultaneous agroforestry systems where limited fine root development in the crop root zone will minimize competition for belowground resources.

The aboveground tree growth attributes, biomass yield, and C storage in *D. edulis* trees of seed and vegetative (cutting and marcot) origins were also assessed. The results suggest that propagation methods significantly influence *D. edulis* tree growth attributes and relative C storage. In addition, *D. edulis* trees of vegetative origin (cuttings and marcots) had more gigantic statures and had 2 and 1.5 folds more C stocks respectively compared to trees of seed origin.

Thus, *D. edulis* trees of vegetative origin can be promoted not only for increased uniform productivity of good quality fruits, marching market specifications, but also for their complementary belowground root attributes and for their high C storage capacity compared to *D. edulis* trees of seed origin.

Many perennial species are highly heterozygous (Petit and Hampe, 2006). Clonal propagation functions to maintain heterozygosity at the individual level (Zohary and Spiegel-Roy, 1975), but promotes genetic homogeneity at the population level (Miller and Gross, 2011). The latter authors maintain that approximately 75% of domesticated trees are primarily clonally propagated through cuttings, layering, grafting, or nuclear embryony (seeds are genetically identical to the parent). Clonal propagation has been reported to restrict the number of sexual cycles separating domesticated populations from their wild ancestors (McKey et al., 2010). According to Miller and Gross (2011), in the simplest domestication scenario, seeds or cuttings of one or a few individuals are taken from wild populations and transferred to a cultivated habitat, where they are maintained through clonal propagation. In this case, selection has occurred only once on a single sexual cycle, effectively isolating a favoured variant that will increase in frequency with clonal reproduction.

Similarly, individual heterozygous *A. floribunda* clones of favoured oil profile can be produced through grafting (Asaah et al., 2011a, Chapter 7) and promoted for cultivation as a homogenous genetic population in diversified agroforestry systems with other crops. Thus, selection of this *A. floribunda* variant with favoured oil profile for instance, would occur only once on a single sexual cycle of the 'plus tree', while the isolated and favoured variant (plus tree) could be increase in number through clonal multiplication techniques (grafting, rooting of stem cuttings and grafting). According to Miller and Gross (2011), this will led to the promotion of genetic homogeneity at the population level. Thus, diversification of such a clonal population is crucial to averse pest/disease risk and to avoid poor performance of individual trees due to inbreeding depression. However, through agroforestry, risk aversion may be achieved by diversifying the agro-ecosystems through the introduction of other species that could provide food, income and other agro-ecological system functions (nutrient recycling, erosion control, habit for flora and fauna, carbon sequestration etc.) (Leakey, 1999; 2010).

Agricultural lands are believed to be a major potential sink and could absorb large quantities of carbon (C) if trees are reintroduced to these systems and judiciously managed together with crops and/or animals. Thus, the importance of agroforestry as a land-use system is receiving wider recognition not only in terms of agricultural sustainability but also in issues related to climate change (Kirby and Potvin, 2007; Nair et al. (2009); Roshetko et al., 2007; Sharrow and Ismail, 2004; Verchot et al., 2007). According to Albrecht and Kandji (2003), the C sequestration potential of agroforestry systems are estimated between 12 and 228 Mg ha^{-1} with a median value of 95 Mg ha^{-1}. Therefore, based on the earth's area that is suitable for the practice (585–1215 × 10^6 ha), 1.1–2.2 Pg C could be stored in the terrestrial ecosystems over the next 50 years (Albrecht and Kandji, 2003). Tree components in agroforestry systems, constitute significant sinks of atmospheric C as a result of their fast growth and high productivity (Montagnini and Nair, 2004). By integrating trees in agricultural landscapes, agroforestry can, arguably, increase the amount of carbon stored in lands devoted to agriculture, while still allowing for the growing of food crops (Kursten 2000). Long rotation systems such as agroforests, homegardens and boundary plantings with species like *D. edulis* and *A. floribunda* among others, can sequester sizeable quantities of C in plant biomass and in long-lasting wood products. From the results of this work, the mean C estimated using allometric models to have been sequestrated by 10 years old *D. edulis* trees of vegetative origin (cuttings and marcots) was 26.8 ± 19.1 Mg C ha^{-1} and 21.7 ± 12.8 Mg C ha^{-1} respectively compared to 13.10 ± 9.4 Mg C ha^{-1} for trees of seed origin. It is therefore important to develop appropriate strategies to tap into this underexploited carbon sequestration benefits from indigenous fruit trees under domestication and cultivated in agricultural landscapes within current efforts to fight and adapt to climate variability.

The greatest impact of climate change on smallholder farmers is the variability in interannual rain falls and temperatures which have enormous influence on the overall productivity of their farming systems. Tree-based systems have been demonstrated through various studies to be more advantageous admits variable climatic situations. For example, the deep root systems of trees could provide synergical ecological processes as circulation of water and nutrients which could help during droughts, as a result of root filters thereby minimizing nutrient leaching. Trees in farming systems will also increased soil porosity, reduced runoff and increased soil cover leading to increased water infiltration and retention in the soil profile which can reduce moisture stress during low rainfall years (Buresh et al., 2004; van Noordwijk et al., 2004). Tree-based systems in addition, have higher evapotranspiration rates than crops or pastures and can thus modify the water budgets of cropping systems to increase rainfall utilization by pumping excess water out of the soil profile thereby trading non productive use of water for productive uptake by plants (Smith et al., 2004). Finally, tree-based production systems often produce crops of higher value than row crops. Thus, diversifying farmer production systems to include a significant tree component may buffer against income risks. For example, Ong et al. (1999), in a study on the profitability of a *Melia vokensi* (Meliaceae) mix cropping system in Kitui district in Kenya (semi arid zone), concluded that tree based systems were more profitable financially and produced diverse range of products even over drought periods. Farmers in the Sahelian parklands of West Africa have been reported to manage climate variability risks through the integration of scattered trees of dense shade like shea butter (*Vitellaria paradoxa*) and ne´re´ (*Parkia biglobosa*) which often reduces millet yield by 50–80% (Kater et al., 1992). Nevertheless, the trees are highly valued by farmers because economic yields from marketable tree products compensate for the loss of crop yield. In the humid forest zone of Cameroon Schreckenberg et al. (2006), reported evidence of indigenous fruit trees like *D. edulis* and *Irvingia gabonensis* in poverty reduction in Cameroon and Nigeria.

8.2 General Conclusions

The importance of domesticating and integrating high value fruit trees in agricultural landscapes by farmers is increasingly being recognized in Africa and beyond. Domesticated perennials are an important component of agricultural economies around the globe. Perennial crops produce an abundance of useful products including fleshy fruits [e.g., apples, (*Malus domestica)*; avocados, (*Persea americana)*; sweet cherries, (*Prunus avium)*; oranges, (*Citrus sinensis);* bush mango *(Irvingia gabonensis)*, safou (*Dacryodes edulis)* and dry fruits [e.g., almonds, (*Prunus dulcis)*; pecans, (*Carya illinoinensis);* walnuts, (*Juglans regia), cashew (Anacardium occidentale); Macadamia (Macadamia integrifolia),* bitter kola *(Garcina kola),* Kola *(Cola* spp).*; Allanblackia* spp etc. and interest in perennial grains is on the rise.

Tree crops cultivation is attracting increasing attention as important components of sustainable agriculture and as carbon sequestration strategy because of their carbon storage potential in diverse plant species and soil as well as its applicability in agricultural landscapes. Species like *Allanblackia*, offer promising options for novel food sources in Africa. Through domestication, it is possible to reduce the long juvenile phases to fruiting and develop mechanisms to avoid selfing, high rates of inter and intra specific hybridization, extensive population genetic variation, and limited population structure.

Under domestication, and in response to purposeful man-led selection, species like *D. edulis* have reduced juvenile phase, larger fruit size, extended fruiting season, more complementary rooting system if intercropped in agroforestry context with companion crops and are significant C sinks within the production system. Thus, establishing long-lived perennials like *D. edulis* and *A. floribunda* of vegetative origin will provide food, generate income through sells of tree products (fruits/nuts) and could enhance the provision of ecological services (erosion control, habitat for flora and fauna, nutrient recycling, carbon sequestration and help to counter climate change). Furthermore, filling the niches under the trees through intercropping with useful and marketable annuals – herbs, cereals and grain legumes would be important for the further improvement and diversification of the livelihoods options of the rural population, and the overall profitability and value of the system. This could lead to higher yields, increase rural incomes, greater food security and avoided emissions through the integration and retention of useful trees supplying diverse products in agricultural landscapes in the humid tropics of Africa.

8.3 Recommendations and directions for further research

D. edulis fruits are almost a staple food during its maturity season in most of the countries in West and Central Africa where it grows naturally. The benefits derived from fruits range from nutritional to increased income and employment across various actors in the supply chain. *A. floribunda* on the other hand is still a new oil tree crop for Africa. The oil extracted from the nut has been approved by the European Union for safe usage in food preparation within European Union. It has a well-developed supply chain in Ghana, Nigeria and Tanzania where it is exploited commercially and has been reported to have positive livelihood impacts on farmers. However, all collections there are from wild trees. Research efforts should be intensified to domesticate the species and promote its wide scale cultivation in agricultural landscapes or in association with other cash crops.

From the results of our investigations, *D. edulis* trees of vegetative origin have deep sinker roots that can confer stability to the trees against wind damage. Similarly, their fine roots are less competitive in the annual crop root zone of 0-30 cm compared to *D. edulis* trees of seed origin. *D. edulis* trees of vegetative origin (cuttings and marcots) were more gigantic in tree stature and had 2 and 1.5 folds more C stocks respectively compared to trees of seed origin.

It is therefore recommended that *D. edulis* trees of vegetative origin are suitable candidates for intercropping and sequestrating C in simultaneous agroforestry systems. Similarly, it is also recommended that grafting be used to reduce the long juvenile phase to first fruiting in *A. floribunda.* In addition, grafting would be very relevant to producing trees with known sexual characteristics (for example, female trees with desirable traits (nut size, oil profile etc.). In parallel, we also recommend the cultivation of superior heterozygous individual clonal trees of diverse species within homogenous clonal populations as pest and disease risk aversions measures in addition to minimising inbreeding depression.

However, there are still some elements in the production system of these economical and nutritional important species (*D. edulis* and *A. floribunda*) that require further research. These include and should not be limited to:

1. repeat the study on structural and fine root distribution in *D. edulis* trees of seed and vegetative origin in older trees of about 15 years to assess if the trends shown in the current study are maintained.

2. investigate water use efficiency and mineral uptake in vegetative propagated trees compared to tree of seed origin;
3. develop species specific allometric regression models to estimate with more precision, the carbon stored in *D. edulis* trees of seed and vegetative origins;
4. assess the effect of propagation methods on fruit/nut yield in trees of seed and vegetative origins (cuttings, marcots, grafts);
5. quantify genetic gains that could be derived from multiplying *D. edulis* and *A. floribunda* vegetatively;
6. develop a marker-assisted selection protocol for *D. edulis* and *A. floribunda* to facilitate development of distinct cultivars of desirable traits that can be multiplied vegetatively.

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In June 1995, he was recruited and joined the research team of World Agroforestry Centre (ICRAF) regional programme for west and central Africa, with regional office in Cameroon. He started in the programme as a research assistant and has risen to an associated scientist grade and also been supervisor for about 18 'Diplôme d'Ingénieur des Eaux, Forêts et Chasses 'dissertations. Within the framework of his PhD, he conducted his research (between 2005 and 2009) at ICRAF experimental nurseries and plots in Yaounde and Mbalmayo Cameroon. In February 2012 he successfully completed the Doctoral Training Program at the Faculty of Bioscience Engineering, Ghent University. He participated in many national and international scientific conferences, seminars and workshops with oral and poster contributions. In addition he is author and co-author of various international peer-reviewed publications.

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