DOMESTICATION OF INDIGENOUS FRUIT AND NUT TREES FOR AGROFORESTRY IN SOLOMON ISLANDS

Thesis submitted by

Richard Larry PAUKU BAgr (USP), MSc (Wye)

in October 2005

for the degree of Doctor of Philosophy
in Tropical Plant Sciences
within the School of Tropical Biology
James Cook University
Cairns, Qld, Australia.

STATEMENT OF ACCESS

I, the undersigned, the author of this thesis, understand that James Cook University will make this thesis available for use within the University Library and, via the Australian Digital Theses network (unless granted an exemption), for use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act.

Beyond this, I do not wish to place any further restriction on access to this work.

19th October 2005

Richard Larry Pauku

STATEMENT ON SOURCES

DECLARATION

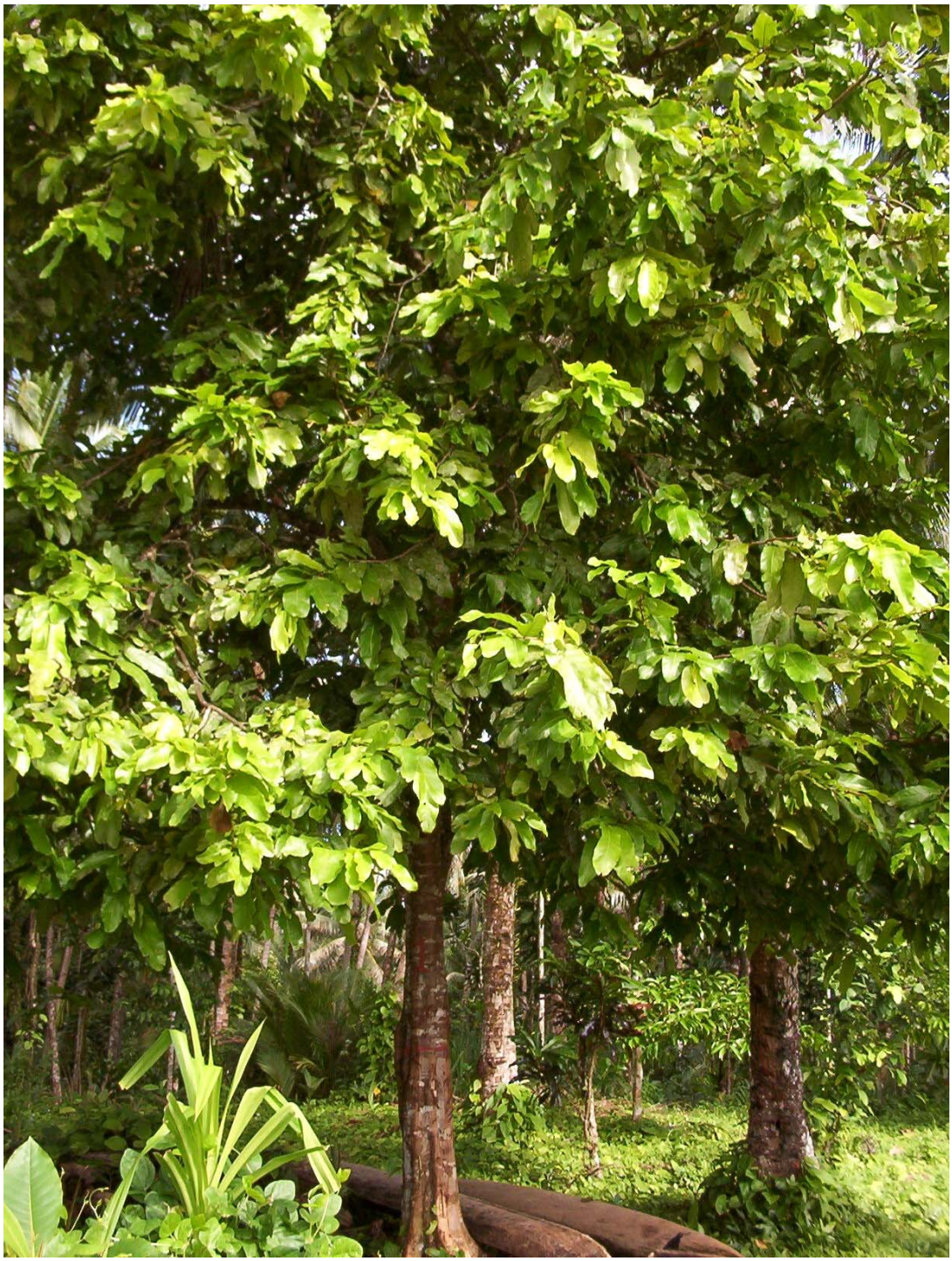
I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

I declare also that all research procedures reported in the thesis received the approval of the James Cook University Ethics/Safety Review Committee. Assistance received from others towards this thesis is duly acknowledged.

19th October 2005

Richard Larry Pauku

Mature trees of *Barringtonia procera* (Cutnut). Poporo village in Kolombangara, Solomon Islands.



Mature tree of *Inocarpus fagifer* (Tahitian chestnut). Tututi village in Kolombangara, Solomon Islands.

ACKNOWLEDGEMENTS

I thank my God for granting me knowledge, wisdom and strength to undertake this study. This thesis could not have been accomplished without the assistance and the support of others. Firstly, I acknowledge with sincere gratitude the people of Kolombangara Island, especially chiefs, elders, farmers and the general public of Vovohe, Ringgi, Tututi, Kolowoata, Seusepe, Rei, Poporo and Hunda villages, who willingly participated in the participatory survey I conducted in October 2002 and who allowed me to use their plant resources.

Funds committed and expended on this study came from various sources. Most importantly, this study would not have been possible without the John Allwright Fellowship awarded to me by the Australian Council for International Agriculture Research (ACIAR). I wholeheartedly thank ACIAR for the scholarship and their continuous support and understanding throughout my study. The operating costs of this thesis were co-funded by the Rainforest Cooperative Research Centre (CRC), James Cook University (JCU) and Kolombangara Forest Products Limited (KFPL). I acknowledge the financial support given by these organisations during the three and half years of my study. I especially thank KFPL for allowing me to use their nursery and medical facilities at Ringgi Cove.

I am greatly indebted to my principal supervisor, Professor Roger Leakey, whose professional advice and untiring guidance constantly inspired and helped me to maintain my focus on all aspects of this thesis. I thank him for his positive attitude towards my study. I also thank my co-supervisors, Professor Paul Gadek, Dr. Tony Page and Dr. Paul Reddell for their professional advices and guidance. Special thanks go to Dr. Paul Reddell who assisted me to secure the John Allwright Fellowship. My thanks also go to Dr. Mike Steele who guided me in the statistical analysis of my field data. The laboratory component of this thesis was undertaken at the University of Queensland under the supervision of Dr. Andrew Lowe, assisted by Dr Leon Scott and Mr. Trevor Wardill. I sincerely thank them for their professional advice and training in molecular techniques. I thank Leanne Verrall for proof-reading this thesis and Katherine Fowler for helping me with the

Endnote program. I also thank Alex Salvador and Larissa Siliezar of the JCU AusAid office (International Student Centre) in Townsville for their role in administering my scholarship.

The fieldwork component of this thesis was carried out in the Solomon Islands. During my absence in Australia the nursery and field sites were maintained by staff of KFPL. I especially thank Douglas Poa, Margaret Andresen, Derol Sikua, Jenty Isaac, Buka Palmer and Moses Pukabose for their support and Mary Qila, Hata Sione, Maremare Pita, Cynthia Luke, Nanette Hila, Peter Qalopui, Boki Qalopui, Habakkuk Qalopui, Jones Pauku, Grace Bingo, Ferguson Kili, Miriam Bingo, Caroline Alex and Veronica Wale for their assistance during my fieldwork.

Finally, I would like to acknowledge the support and encouragement from my parents, family, relatives and friends in the Solomon Islands and in Cairns. I thank Mum and Dad for their confidence in me and for their prayers for me in this undertaking. Last but not least, I dedicate this thesis to my dear wife, Doris Pauku and our three children, Bonnie, Ronarose and Ryan as an expression of my appreciation for their patience, understanding, commitment and love for me throughout this undertaking. Thank you and I love you all.

ABSTRACT

In the Solomon Islands subsistence agriculture, monoculture plantations, new settlements and commercial timber extraction have resulted in indiscriminate deforestation. Agroforestry is an approach to sustainable landuse aimed at reversing these land degradation processes worldwide. In recent years, the domestication of indigenous fruit and nut trees has been added to the package of techniques making agroforestry more effective. By improving the livelihood benefits derived from agroforestry, the domestication of agroforestry trees is becoming a tool for the alleviation of the severe ecological and socio-economic problems of many developing countries.

This thesis describes research to develop techniques for the domestication of indigenous nut tree species in the Solomon Islands. The first step was to determine which species the local communities considered to be their top priorities for domestication. Consequently, participatory surveys were undertaken in 155 households from five villages (Ringi, Seusepe, Rei, Poporo and Hunda) around Kolombangara Island. These surveys identified that *Barringtonia procera* (Cutnut) and *Inocarpus fagifer* (Tahitian chestnut) were the species that were most important as a source of food and income, while also filling in critical niches in the farming systems. A review of the literature found that very little is known about the biology of either species and that no previous studies had been done to domesticate these species. Farmers, however, confirmed that they were growing seeds from trees with desirable nut characters.

The next step was to quantitatively characterise the phenotypic variation in the dry matter partitioning between different components of fruits and nuts from the five target villages. Whenever possible, 24 fruits were collected from each of 119 trees of *B. procera* and separated into their components (pulp, nut and kernel) for measurement. Within each population, highly significant (P= 0.001) and continuous intraspecific variation was found in all the measured traits. However, site-to-site variability was not significant. This quantitative data was also used to:

(i) identify the market-oriented traits which could be combined to describe the 'ideal tree' or 'ideotype', in which 'Harvest Index' is maximised through the partitioning of dry matter to the commercially and domestically important kernel, (ii) identify the elite trees, which could be vegetatively propagated and (iii) ascertain through an anlaysis of the frequency distribution of the data, the degree to which farmers have already from their own actions initiated the domestication process.

This study was complemented by a molecular study of genetic variation in each population. This molecular study found significant genetic diversity within and between the five populations of *Barringtonia procera*. It was also used in parallel with the morphological data, to evaluate: (i) the relatedness of three edible species of *Barringtonia*, and (ii) the relatedness of elite trees within the five populations. The results imply that the field collections failed to accurately distinguish the different species because of overlapping morphological characteristics. There was no conclusive evidence of any hybridisation between these species, it was clear that elite trees were generally unrelated. Further studies are required to elucidate the taxonomy of the three species.

The final section of this thesis examined the factors which affect the rooting ability of both *B. procera* and *I. fagifer* stem cuttings. These results are then used to define the most appropriate material and techniques for the development of robust vegetative propagation protocols for village scale nurseries. Both species were found to be easily propagated by single-node, leafy, stem cuttings. Seventeen experiments tested the main factors known to affect the rooting of tropical tree cuttings. It was found that auxin (indole-3-butyric acid) did not significantly increase the rooting percentage, although there were significant differences in the numbers of roots formed, which in both species were maximal with 0.8% IBA. There were no consistent significant differences between cuttings from different nodes. However, the presence of a leaf was essential for rooting with 100% mortality in leafless cuttings of *I. fagifer* and 79 % mortality in *B. procera*. Both species, regardless of leaf area, leafy cuttings had 77-100% rooting success.

Having identified the optimal treatments for stem cuttings from juvenile trees, the study progressed to an examination of one of the major constraints to developing cultivars from mature trees of any species, namely how to root cuttings taken from the mature (ontogenetically-mature) crown. Three approaches were examined:- (i) a comparison of the rooting ability of juvenile seedlings and shoots from potted mature marcots; (ii) a study of the factors affecting the successfulness of marcotting (air-layering) and (iii) the separation of physiological and ontogenetic ageing in the intact tree crown. In *B. procera*, juvenile cuttings from seedlings rooted better than cuttings from mature potted marcots, because the latter suffered leaf abscission. In *I. fagifer* mature and juvenile cuttings both rooted well. Shading mature stockplants of *B. procera*, however, significantly improved rooting ability of mature cuttings. Marcots of both species rooted 100% and a few factors were found to reduce this, although survival of the marcots declined if they were not harvested within 3-4 months. Attempts to separate ontogenetic and physiological ageing within the mature crown were partially successful, resulting in shoots which were comparable morphologically. However, enhanced rooting percentages were not consistently achieved across all treated shoots. Nevertheless, the number of roots per rooted cutting was significantly increased in the treated mature shoots.

Marcotting resulted in establishment of mature stockplants in the nursery, which can be used in future as the source of mature cuttings for further work to develop cultivars from selected elite individuals.

In conclusion, this study has developed robust and simple techniques which are appropriate for the domestication of *B. procera* and *I. fagifer* in remote communities in the Pacific, like Kolombangara Island. This opens the way for a programme of participatory domestication for these indigenous nuts in the Solomon Islands. This should greatly enhance the opportunities to commercialise indigenous nuts and to use them as a means to enhance income generation and to improve the livelihoods of rural people, as well as to develop more sustainable agricultural production systems based on agroforestry.

TABLE OF CONTENTS

FIGURES

TABLES

PLATES

CHAPTER 1: INTRODUCTION

1.1 RATIONALE OF THE THESIS

The world's forests, including natural forests and forest plantations, have been estimated to cover about 25% of the land area but they are very unevenly distributed across continents (FAO 1996). By ecological zones, the distribution of forest area is: 47% in the tropics, 33% in boreal zone, 11% in temperate areas and 9% in the sub-tropics. The greater proportion (95%) of the world's forests is natural or semi-natural forests, and only 5% are forest plantations (FAO 2000). In recent years, countries in the tropics have lost more forest than countries in temperate areas which were deforested centuries ago. For example, between 1990 and 2000, about 12.3 million hectares of forests were lost in the tropics, while there was a net increase of 2.9 million hectares in non-tropical areas due to reafforestation (FAO 2000). The loss of forests in developing countries was mainly due to expansion of subsistence agriculture, although commercial timber extractions, creation of new settlements and infrastructure developments, and bush fires have all contributed to this effect.

Forests have been described as the most essential *biomes* of the planet earth because they play an important role in the earth's biophysical system, and support human well-being (Aplin *et al.,* 1999; WCFSD 1999). Across the globe, forests have been recognised for meeting the needs of the people for timber, food, medicines, fibre, etc. (FAO 1989; Leakey and Newton 1994a; WCFSD 1999). Traditionally, they have sustained the livelihoods of people, first through hunting and gathering and then through shifting cultivation. However, forests are often now cleared extensively to satisfy the needs and aspirations of the rapidly expanding population and their increasing materialism. Forests are also the domain of hundreds of thousands of plants and animals species, which live in complex and dynamic ecosystems, bound intricately together through food chains and life cycles.

Today, the world's population is 6.4 billion people and is growing at the rate of 76 million people per annum (UNPF 2004c). The United Nations has projected that about 2.5 billion more people will be added by 2050. Importantly, 96% of the projected growth will be in developing countries, whose populations are largely subsistence farmers with some dependence on forests for their livelihoods. Now, about 350 million of world's poorest people depend on forests for subsistence and survival, while 1 billion people depend on remnant woodlands, homestead tree gardens and agroforestry for their household needs such as food, fuelwood and fodder (WCFSD 1999). The growing urban population also has demands on forest products. It is expected to reach 50% of the world's population by 2007, and will rise to 5 billion in 2030 (UNPF 2004c). This shift to urban life in all regions of the world challenges the way we manage, conserve and utilise our forests and agricultural land, especially in developing countries.

Globally, population growth has led to land degradation, and the loss of natural resources to support life. In parallel with this, globalisation of the world's economy has meant that more people are dependent on money for everyday life. As a result, more people are now suffering from poverty and are often hungry and malnourished. In addition, one in every three people die premature or are born with disability (WHO 2005), 153 million children under 5 years in developing countries are under weight and about 11 million younger than 5 years died every year, more than half of which are due to malnutrition and hunger related causes (FAO Corporate Document Repository 2002). The United Nations statistics in 2002 indicated 2.8 billion people live on less than US$1 per day, of these 1.1 billion people did not have access to good drinking water and 2.4 billion were without basic sanitation. Together, these deficiencies resulted in the death of 1.7 million in 2000 (UNPF 2004a). Poverty and hunger are inextricably linked to environment degradation. In developing countries, where biodiversity is rich, poor people exploit their local environment to meet subsistence needs in ways that are destructive and unsustainable. Traditional practices, such as shifting agriculture on 20-30 years fallow cycle may have been ecologically sustainable when the population was small, but population growth has led to shortened periods of fallow which do not permit for environmental recovery. Environmental pollution

exacerbates these problems, affecting air and water quality, human health (UNPF 2004b) and causing climate change through an increase in global warming. Global deforestation threatens the genetic diversity of the world's plants and animals worldwide. Consequently, about 12.5% of the world's 270,000 plant species and 75% of the world's mammals are considered to be threatened (WCFSD 1999).

Confronted with these multifaceted problems, from extreme poverty and hunger to health, education and environmental disasters, the world leaders came together through the United Nations in September 2000 Millennium Summit to draw up the "Millennium Development Goals," which provide a framework for the whole United Nations system to function coherently together for a common cause. The eight MDGs and related targets as described by Garrity (2004) are:- *(i) Goal 1: Eradicate extreme poverty and hunger: Target for 2015: Halve the proportion of people living on less than a dollar a day and those who suffer from hunger, (ii) Goal 2. Achieve universal primary education. Target for 2005: Ensure that all boys and girls complete primary education, (iii) Goal 3. Promote gender equality and empower women. Target 2005 and 2015: Eliminate gender disparities in primary and secondary education preferably by 2005, and at all levels by 2015, (iv) Goal 4. Reduce child mortality. Target for 2015: Reduce by two thirds the mortality rate among children under five, (v) Goal 5. Improve maternal health. Target for 2015: Reduce by three-quarters the ratio of women dying in childbirth, (vi) Goal 6: Combat HIV/AIDS, malaria and other diseases. Target 2015: Halt and begin to reverse the spread of HIV/AIDS and the incidence of Malaria and other major diseases, (vii) Goal 7. Ensure environmental sustainability. Targets: Integrate the principles of sustainable development into country policies and programmes and reverse the loss of environmental resources. By 2015, reduce by half the proportion of people without access to safe drinking water; By 2020 achieve significant improvement in the lives of at least 100 million slum dwellers (viii) Goal 8. Develop a global partnership for development. Targets: Develop further an open trading and financial system that includes a commitment to good governance, development and poverty reduction – nationally and internationally; Address the least developed countries' special needs, and the special needs of landlocked and small island developing States; Deal comprehensively with*

developing countries' debt problems; Develop decent and productive work for youth; In cooperation with pharmaceutical companies, provide access to affordable essential drugs in developing countries; In cooperation with the private sector, make available the benefits of new technologies – especially information and communications technologies."

Globally, slow but promising progress is being made toward these goals. For example, percentage population living on less than US$1.00 a day declined between 1990 and 1999 in most regions of the world (United Nations 2002). The Consultative Group for International Agriculture Research (CGIAR) was inaugurated in 1971 to lead the "Green Revolution," a major international initiative to reduce hunger through the development of more productive varieties of the major staple food crops. The number of international agriculture research centres was expanded slowly and in the 1990's three natural resources centres were formed. One of these was the International Centre for Research in Agroforestry (ICRAF), now called the World Agroforestry Centre (WAC), which joined the CGIAR in 1991 to promote sustainable land use systems through agroforestry in rural tropics through tree-based farming and to improve peoples' livelihoods through food security, cash generation and the development of a more sustainable agricultural environment (World Agroforestry Centre 2005). The WAC, through agroforestry science and practice, is working towards the achievement of the Millenium Development Goal in the following seven areas (Garrity 2004):

i. Assist to eradicate hunger by ensuring food production methods through agroforestry, especially in soil fertility and land regeneration.

ii. Improve rural poor by promoting market driven tree domestication at village level that generate income and build assets.

iii. Enhance rural health and nutrition through the promotion of agroforestry systems, especially the expansion of fruit tree cultivation to include indigenous fruits and nuts.

iv. Conserve biodiversity by promoting agroforestry based technologies, innovations and policies, in areas of sustainability of the landscape, mitigation and adaptation to climate change and ways in which to harmonise environmental stewardship and rural development.

v. Protect watershed services through agroforestry based solutions, especially promoting suitable trees to farm, their configuration in the landscape and location so as to create an effective buffer against flooding and soil erosion in the watershed areas.

vi. Help poor people to adapt to climate change and make them understand and be motivated by benefits of emerging *carbon* markets through tree cultivation.

vii. Build and strengthen human and institutional capacity in agroforestry research and development, through initiatives as, "The Farmers of the Future" and other tertiary networking, e.g. African Network for Agroforestry Education (ANAFE) and Southeast Asia Network for Agroforestry Education (SEANAFE).

These global trends can also be seen at the national level. For example, in Solomon Islands, population has increased from 280,000 in 1976 to 400,000 in 1999. Annual income from employment per capita is estimated to be US$22.40, and the majority of the population (97%) are not in formal employment (MOF 1995). Forests have been cut extensively for timber export and to clear land for plantation crops. Consequently, 90.3% of land forested in 1990 was reduced to 88.8% by 2000 (United Nations 2002). Intensive cultivation has led to soil degradation and erosion, the siltation of rivers and streams. The prevailing tropical storms, wind and temperatures exacerbate this situation. Much of the fertile and biologically rich environment in which the people used to live is now degraded. Gone are the days when indigenous islanders can just enjoy hunting, fishing and the cultivation of crops and trees on their tribal lands. Now, they are increasingly under pressure to feed and sustain their families on a small area of land and pay for

health care and the education of their children. This has led to landscapes dominated by exotic species, mainly commercial monocultures such as: oil palm plantations (e.g. Guadalcanal Plains and Merusu in the Marovo lagoon), coconut plantations (e.g. Russell Islands) and plantation forestry (e.g. Kolombangara Island) (Plate 1.1).

Plate 1.1: Eight years old exotic teak plantation of KFPL in Kolombangara Island

Land suitable for small-scale agriculture by subsistence farmers is becoming scarce in most islands, as people change from traditional to modern lifestyles. For example, around Buma village in Solomon Islands, government-sponsored cattle, cocoa and copra projects pushed traditional farming farther inland onto steep, sloping and more marginal lands away from the coastal villages (Manner 1993) causing women to have to carry farm produce on their back for long distances. These changes also result in social change. For example, women are disadvantaged by remaining at home more than men to do most of the household activities. There is also a tendency for young people, in particular boys, to move to urban centres in search of income and more modern life styles. These changes also result in a loss

of traditional knowledge about the management of land and its resources including the use of indigenous plants for food, medicines and other daily needs. This knowledge is now retained predominantly in the older generation who reside mainly in rural areas.

In Solomon Islands, unsustainable logging and the establishment of forest plantations has had serious impacts on land availability. Pressure from foreign companies for logging concessions has intensified and become the main single source of disputes over customary rights on the land and its resources. There were 21 foreign and locally owned logging companies operating Solomon Islands in 1999 (Sirikolo and Gua 1999), as well as hundreds of small operators who are using mainly portable chainsaws. With this number of operators competing for just 598,500 ha of merchantable or loggable forest (MFEC 1995), problems of corruption over timber rights has increased, and there is confusion over who is the legitimate owner of the land and its resources. This occurs as a result of inadequate enforcement of the code of practice for timber harvesting and delays in the enactment of a revised Forest Act.

The Green Revolution promoted high input agriculture, instead of traditional farming systems which appeared to be unproductive. However, now it appears that these traditional systems did have some good qualities, in terms of nutritional security, arising from the wide range of species present in farmland and in terms of their environmental sustainability and risk aversion both nutritionally and environmentally. The Green Revolution model has now been recognised to have some negative impacts on the environment and that they are unsustainable and partly to blame for environmental degradation (Conway 1997). Consequently, there is now an urgent need to bring back some of the benefits of traditional land use systems. One approach which has been extensively researched over the last 25 years is agroforestry (Nair 1989).

In the small island states of the South Pacific, agroforestry has been part of traditional polycultural systems practiced by the people in which trees are protected or planted with root crops and vegetables for domestic requirements

(Clarke and Thaman 1993; Rogers and Thorpe 1999; Elevitch and Wilkinson 2000). Indigenous fruit and nut trees such as *Canarium* spp., *Barringtonia* spp. *Artocarpus altilis*, *Mango minor* and *Inocarpus fagifer* are important components in this system (Clarke and Thaman 1993). However, through colonial influence and an evolving world people often adopted a mono or duo cropping system of exotic species such as *Cocos nucifera, Theobroma cacao* and *Elaeis guineensis*. Consequently, in the face of increasing population densities, the people have sometimes lost their method of sustainable agriculture, whilst others have integrated them into their current farming systems.

Concerted efforts are needed to develop better practices that meet the needs of farmers in the Pacific and especially in Kolombangara Island. This requires that farmers are trained in sustainable land use practices and that Government addresses the policy needs of the people to preserve the environment, support the traditional rights of the people on their land resources. Meeting this need will require a multidisciplinary approach to problem solving and recognition that traditional values and knowledge are crucial to finding long-term 'development' options for these island nations.

Enhancing current agroforestry practices of farmers is one way of taking a step forward to alleviate the growing socio-economic and environment problems that are the focus of the Millennium Development Goals. Agroforestry practices come in many forms and seek to address many of the problems associated with land use degradation, declining livelihoods, poor nutrition and health. Over the last 10 years, one agroforestry initiative that has become significant is the move to domesticate indigenous food and medicinal plants – especially the trees that used to be important in traditional land use systems and culture. The domestication of indigenous trees, and in particular, fruit and nut species is seen as an incentive for farmers to adopt agroforestry (Leakey 2001). Unfortunately, useful indigenous tree species that can be domesticated are little known outside their natural range and have attracted little scientific interest internationally. Many common and traditionally important tree species in the developing countries fall into this category. These species have been collectively named the 'Cinderella' species

(Leakey and Newton 1994b). They are largely neglected yet provide many of the basic necessities of life for indigenous people such as food, fodder, fuelwood, medicine and construction materials. Increasingly, these traditionally important products have a market value, both in the rural and urban environment. These markets provide an opportunity for income generation in ways that are understood and adopted by local people.

Through its Tree Domestication Programme, WAC developed strategies and technologies to bring these Cinderella species into cultivation (Simons 1996). The approach that has been developed is Participatory Tree Domestication (Tchoundjeu *et al.,* 1998; Leakey *et al.,* 2003). This involves farmers in identifying the indigenous tree species to be subjected to scientific investigation. This 'Bottom-Up' approach in tree domestication was first developed in West Africa (Franzel *et al.,* 1996). However, it has become an international programme and there are now many other examples promoted by other organisations such as *Gnetum gnemon* in Indonesia (Suhardi 1999), *Artocarpus heterophyllus* in Sri Lanka (Pushpakumara *et al.,* 1999). In the Pacific farmers have selectively planted and grown indigenous trees in their home gardens for centuries and this is the start of the domestication process. However, tree domestication has not been scientifically developed in the Pacific, although there has been some preliminary work on *Canarium indicum* in Solomon Islands (Evans 1996, 1999) and Papua New Guinea (Akus 1996), and *Terminalia catappa* in Vanuatu (per. comm. Thomson 2002). This thesis takes this initiative forward and starts to domesticate two indigenous nut species of the Pacific on Kolombangara Island in the Solomon Islands.

1.2 SCOPE OF THE RESEARCH

1.2.1 Hypotheses tested in this thesis

This thesis examines the following three principal hypotheses that form the basis of a research strategy leading towards a solution to the problems of poverty, food insecurity and environmental degradation in Solomon Islands:-

i. Rural communities in Kolombangara Island are interested in the domestication and commercialization of indigenous tree species producing non-timber forest products through the application of agroforestry systems and practices.

ii. There is sufficient phenotypic and genetic variation in the chosen priority tree species to merit selection of superior trees for the creation of potential cultivars.

iii. Trees of the chosen priority species can be propagated vegetatively to produce cultivars.

1.2.2 Research questions of this thesis

To test these hypotheses, this thesis examines four important research questions:-

i. Which are the priority indigenous species for domestication and commercialisation?

ii. Which traits are the most important for selection if these species are to become new crop plants?

iii. How much phenotypic and genetic variation is there in these important traits?

iv. Which factors affecting the vegetative propagation of these species need to be optimised to develop robust techniques for development of cultivars of these species?

1.2.3 Research objectives of this thesis

i. To conduct a participatory survey in five villages around Kolombangara Island to identify farmers' priority fruit tree species for domestication and

their potential to contribute to enhancing their livelihoods through agroforestry.

ii. To develop appropriate nursery and vegetative propagation techniques and protocols to create cultivars for cultivation in agroforestry systems.

iii. To assess intra-specific variation of different fruit traits and to develop 'ideotypes' having desired traditional and market values.

1.3 OUTLINE OF THE THESIS

There are 10 Chapters of this thesis. Chapter 1 is an introduction and sets the study topic into context, in terms of the rationality, research questions, hypotheses and objectives. This is followed by a review of the literature on related topics in agroforestry and tree domestication (Chapter 2). General materials and methods applied in this study are described in Chapter 3. The three objectives of the thesis are addressed in the following chapters. Chapter 4 describes a farmers' participatory survey conducted in 5 sites in Kolombangara Island, leading to the identification of two indigenous fruit tree species as priority for investigation in the thesis. The review of literature about the two priority species is presented in Chapter 5. In Chapter 6, the focus is on determining important factors affecting vegetative propagation of these priority species. Studies on characterisation of genetic variation of the species using morphological and molecular techniques are described in Chapter 7 and 8 respectively. Chapter 9 contains a general discussion of the whole research and the conclusions drawn from the study are presented in Chapter 10.

CHAPTER 2: LITERATURE REVIEW OF AGROFORESTRY AND TREE DOMESTICATION

2.1 CURRENT STATE OF KNOWLEDGE ON AGROFORESTRY

2.1.1 History

Agroforestry has been developed as a science over the last 50 years, but it has been practiced by subsistence farmers in the tropics for a very long time (Nair 1989). Agroforestry is a land use system that is seen as having potential to resolve the emerging ecological and socio-economic problems arising from unsustainable land use (Huxley 1999).

Modern agroforestry is a fairly recent development, first mentioned by professional foresters in the 1940's and 50's. Agroforestry was institutionalised by John Bene and his colleagues, when in 1975 the Canadian International Development Research Centre (IDRC) commissioned John Bene and others to: a) review the interface between agriculture and forestry, b) assess the interdependence between these two disciplines, and c) determine the agroforestry research and development needs of developing countries in the tropics requiring support from international donors and agencies (Nair 1989; Huxley 1999). The review highlighted the need to improve the integration of trees with agricultural crops and/or animals (Nair 1989). The report also recommended the establishment of an internationally financed council for research in agroforestry. The report was well received and the International Council for Research in Agroforestry (ICRAF) was formed in 1977 (Nair 1989), later becoming the International Centre for Research in Agroforestry (1993) and the World Agroforestry Centre (2002). In its Medium Term Plan (ICRAF 1997), ICRAF set as its objective: to alleviate poverty, provide food and nutritional security, and create environmental resilience. Over the last 25 years the science of agroforestry has been recognised as an important component of sustainable land use (Sanchez 1995).

2.1.2 Concept and Development

In the early days of ICRAF many claims were made about the beneficial impacts and outcomes from the adoption of agroforestry. As recently as 1999, 17 of these hypotheses had not yet been properly substantiated (Huxley 1999). All of these hypotheses are based on the production and service functions of trees in association with agricultural crops in farm or rangeland. The present study in a small way contributes to trying to substantiate some of these hypotheses, particularly to determining techniques that make agroforestry attractive to farmers and, through income generation, to improving the livelihoods of subsistence farmers.

Since its early days, agroforestry has sought to help farmers with nurseries, tree planting and distribution (Huxley 1999). However, this was often implemented in a 'top-down' fashion in which farmers' participation was minimal. In spite of this, farmers benefited from a wide range of agroforestry practices based on planting trees with agriculture crops and/or animals, but more recently, participatory approaches have been implemented (Franzel *et al.,* 1996), and the potential of agroforestry to improve farmers livelihoods has improved.

2.1.2.1 Definition of Agroforestry

Agroforestry, which is a relatively complex form of productive land use involving the planting or protection of trees among agricultural crops, has been defined in many different ways (Nair 1989) and comes in many forms (Tejwani 1987; Young 1989; Nair 1993; Wojtkowski 1998). Nair (1993) recognised eighteen distinct practices (Appendix 2.1), although each of these has an infinite number of variations (Leakey and Simons 1998). Agroforestry practices fall into two groups – those that are sequential (e.g. fallows, taungya) and those that are simultaneous, such as alley cropping, contour hedges, boundary plantings and homegardens (Sanchez 1995; Cooper *et al.,* 1996). In the context of Pacific island states, agroforestry is a traditional land use practice (Raynor and Fownes 1991; Clarke and Thaman 1993; Elevitch and Wilkinson 2000). According to Nair (1989),

agroforestry involves two or more plant species and/or animal combinations and at least one of which is a woody perennial, invariably produce two or more outputs, has a cycle that is always more than one year and in contrast to monocropping system, it is ecologically (structurally and functionally) and economically complex. Nair (1989; 1993) indicates that agroforestry practices should be productive, sustainable and easy to adopt, and suggests that these are the key underlying characteristics or attributes that make agroforestry different from other land use systems.

Leakey (1996) has argued that the early definitions of agroforestry define agroforestry as stand-alone agronomic technologies and overlook the fact that species mixtures progress through an ecological succession in which trees (woody perennials) dominate in the mature phase. He therefore, suggested a broader, more ecological definition, which saw the integration of trees in farmland as the means to developing an agro-ecological succession that leads to mature agroecosystem:- an agroforest. In this way, a mature agroforest resembles the natural ecosystem as well as ensuring the maintenance of agroecosystem functions. However, by integrating trees that produce marketable products, mature agroecosystems can also generate income for the farmers. This ecological view of agroforestry has been recently accepted and agroforestry is defined as "*a dynamic, ecologically based, natural resources management system that, through the integration of trees in farms and in the landscape, diversifies and sustains production for increased social, economic and environmental benefits for land users at all levels*" (ICRAF 1997).

The above has many similarities with a concurrent definition in the Pacific in which Clarke and Thaman (1993) defined agroforestry as, '*the deliberate incorporation of trees into, or the protection of trees within an agroecosystem in an effort to enhance its short and long-term productiveness, its economic and cultural utility and its ecological stability*.' Their emphasis centred on the usefulness and the role of trees in a farming system, while Abel *et al.,* (1997) considered that in agroforestry it is the combination of productive utilization of trees planted on farms with their role in agroecosystem functions that is important.

Thus consensus has emerged building that agroforestry systems should be designed for productivity and sustainability of desirable components of the system.

2.1.2.2 Agroforestry systems and practices

2.1.2.2.1 Traditional agroforestry in the humid tropics

The current more ecological concepts of agroforestry have some similarities with traditional land use in many tropical countries. The four following case studies indicate the relationships between traditional land use and agroforestry:

a) Shifting cultivation is a traditional agroforestry practice widely adopted in the tropics worldwide usually where the population pressures are relatively low. In India, where it was practiced widely in the northeastern hill region, it was known there as Jhum (Tejwani 1987). However, it was also practiced in Andhra Pradesh and Orissa states where it was called Podu. In these examples, the areas include steep landscapes, with high rainfall and high relative humidity (rarely below 75%). With these characteristics, and the isolation of the hill tribes, shifting cultivation was an appropriate form of land use. In 1974 an estimate of 2.7 million ha was under shifting cultivation. In the past, the fallow cycle ranged from a 30-40 year cycle (Tejwani 1987) but by the 1970's had been reduced to 5-7 year because of the increased population and limited land available to the community. Average size of a garden plot per family (2 adults and 3-4 children) is 1-2.5 ha. With many varieties of crops (some 8-13 different crops are grown), the system insures farmers - when one or more crops fail some of the others may give good return under adverse climatic conditions.

b) The swidden fallow agroforestry in Bora Indians of the Yaguasyacu river in the Peruvian Amazon (Padoch and de Jong 1987). The practice begins with the cutting, drying and burning of new fields both in primary and secondary forest. During this stage, traditionally important trees including fruit trees are spared. Crops are planted between these trees in various ways but individual plots can include staple dietary annuals (e.g. *Manihot*

esculenta) and different perennials and woody plants (e.g. *Erythroxylon coca*). Cropping period may run for two years and then the land is left to fallow, meanwhile the farmer clears a new site for farming. In one particular plot, it was found nine years later that the field contains five cultivated species and numerous other naturally generated species useful for constructions, medicines, foods and handicrafts (e.g. *Cedrela odorata*). One example had three cultivated fruit trees and fifty-three other useful species.

c) The Kayabó indigenous farming practice in Brazil (Posey 1982) gives another good example of traditional agroforestry practices. The practice involves clearing of the forest first and then followed by planting of useful species of crops and trees. The field will eventually turn into a mature forest with a diversity of concentrated resources including animals and become known as the 'old field' forest. The 'old field' forest is also enriched by the naturally regenerated diversity of species that turn the field into a healthy, biodiverse mixture of species providing useful products and services for the indigenous people. The 'old field' of forest is then cleared again when the canopy gets too high, dense and inefficient in production. Such benefits attract the Kayabó people to consistently revisit their 'old field' forests.

   A particular important feature in this practice is that the agricultural plots are carefully designed so that they remain productive throughout this reforestation cycle. It also means that the fields are not deserted after initial cropping as in the case of slash-and-burn agriculture. In 'new fields' planted crops are in production for the first 2-3 years but continue to produce for many more years. For example, sweet potato can still be in production for another 4-5 years, yams and taro for a further 5-6 years and papaya and banana even longer.

d) The damar (*Shorea javanica*, Family: Dipterocarpaceae) forest system in Sumatra, Indonesia is another classical example of complex indigenous

agroforestry (de Foresta and Michon 1994), and extends the concepts of the previous case studies, as the damar trees are managed for resin production for 50-120 years. It is believed that damar trees have been grown since the late 19th century and subsequently established for the sale of resins. In this system, the damar trees form about 65% of the stand with a mean density of 245 trees per ha. Fruit trees, including durian (*Durio zibethinus*) and duku (*Lansium domesticum*) comprise almost a quarter, and the remaining area is covered by a wide range of other tree species with traditionally important attributes, such as fruits, vegetables, medicines, fuelwood and wood for construction. The villagers/smallholders manage this agroforest and even carry out enrichment planting to increase diversity of trees and improve environmental functions of the agroforests. Because, it takes 15-20 years before the damar tree to be ready for their first tapping, farmers introduce other agriculture perennial crops after a season of rainfed rice cultivation for income generation. These cash crops include coffee and pepper. They also plant leguminous species to enrich soil fertility. In some ways, this resembles the taungya practice, however, the difference is that in the damar system the farmers own the land and the trees, and make the choice of tree species to plant, as well as receiving all the benefits themselves. In the taungya system, the land and trees typically belong to the government, or a large company and the farmers are often forest workers who are allowed to produce food crops within young timber plantations. Throughout the succession of the damar agroforests, the farmers are able to continuously harvest commercial and domestic products from their managed damar forest for the full life of the damar trees.

The above case studies have demonstrated the ways in which people have in the past traditionally used agroforestry principles extensively to meet their livelihoods. They also demonstrated that land availability including biophysical characteristics of the area and population pressure are important considerations for developing appropriate modern agroforestry practices, and that there are ways in which agroforestry systems can become appropriate alternatives to shifting agriculture and the environmentally damaging slash-and-burn. Since the mid 1990's ICRAF

has had a major integrated natural resources management program called the "Alternatives to Slash and Burn" (ICRAF 1996).

2.1.2.2.2 Modern agroforestry systems and practices in the humid tropics

In contrast to the traditional agroforestry are the so-called 'modern' agroforestry or institutional agroforestry systems. The main difference between these two approaches to agroforestry is described by Clarke and Thaman (1993). The traditional systems are developed "*on the basis of empirical, non-quantitative experimentation by local practitioners,*" while the "modern" systems are "*promoted by government, quasi-government organisations, private agencies, companies, and aid donors and that involve external funding, formal training, agronomic research and extension services*."

Systems and practices in modern agroforestry are many and varied across the tropics depending on the prevailing biophysical and socio-economic factors. Tropical ecosystems are known to be fragile and prone to environmental degradation, and if unsustainable agricultural activities persist, will result in serious degradation and loss of productivity. Furthermore, abiotic tropical factors such as high temperature, humidity, heavy precipitation and intense solar radiation that favour rapid plant growth are also responsible for the deterioration of the ecosystem, for example, through soil erosion and nutrient leaching (Vergara 1987). In contrast, agroforestry practices that promote soil conservation and minimise nutrient losses, while at the same time providing food and generating income for the people can help to rehabilitate degraded lands (see Appendix 2.1).

2.1.3 Benefits and Limitations of Agroforestry

A review of agroforestry literature reveals a wide range of advantages that can be derived from agroforestry practices, while there are few disadvantages and these can be minimised by good management of the different combinations of trees, crops and livestock. Agroforestry has been recognised to have the potential to generate ecological and socio-economic benefits for farmers (Nair 1993; Rogers

and Thorpe 1999; Ratukalou *et al.,* 2000). The resource sharing between the tree component and the crops or animals is a typical feature of agroforestry, leading to high productivity and diversified farm output. However, competition between them for light, water and nutrients can be a disadvantage and this is more critical in the simultaneous system than the sequential one (Sanchez 1995). This situation implies that there is a need for a sound design and management of these components (Abel *et al.,* 1997; Wojtkowski 1998)

The role trees play in agroforestry systems (Fig 2.1) is a function of their deep and extensive rooting system, perennial, accumulates larger biomass, long life span, and relatively high level of tolerance to acidic or alkaline soils (Jordan 1991; Sanchez *et al.,* 1997). This allows them to stabilise soil structure and protect it against erosion. The fallen leaves and branches enrich soil fertility through increased organic matter, while protective shade improves microclimate, suppresses weed regrowth and maintains good moisture retention (Ratukalou *et al.,* 2000). The use of leguminous trees in agroforestry systems increases nitrogen fixation and enhances nutrient recycling within the system therefore maintains soil fertility and eliminates the need to apply inorganic fertilisers.

There are two components of biodiversity in agroecosystems: unplanned biodiversity – which refers to those organisms above and below ground that naturally occurred at different niches in the agroecosystems and planned biodiversity which involves human manipulation aimed at maximising ecosystem processes (e.g. nutrient cycling, production, light requirements) and structural complexity (Leakey and Simons 2000).

Consequently, agroforestry re-establishes a species-rich environment for birds and animals (e.g. bees for pollination, wasps or birds for biological control) to live, and creates an environment that can sustain the functioning of food chains (Thaman 1994; Elevitch and Wilkinson 2000; Nair 2001). A good example is the damar agroforest in Krui region, Lampung province, Sumatra in Indonesia, which exhibits a high level of plant and animal diversity – it contains over 50% of the regional pool of tropical birds and 70% of plants. Damar agroforests allow direct

production of useful forest species but also acts as a habitat for species which are not directly useful to man (de Foresta and Michon 1994), so playing a conservation function.

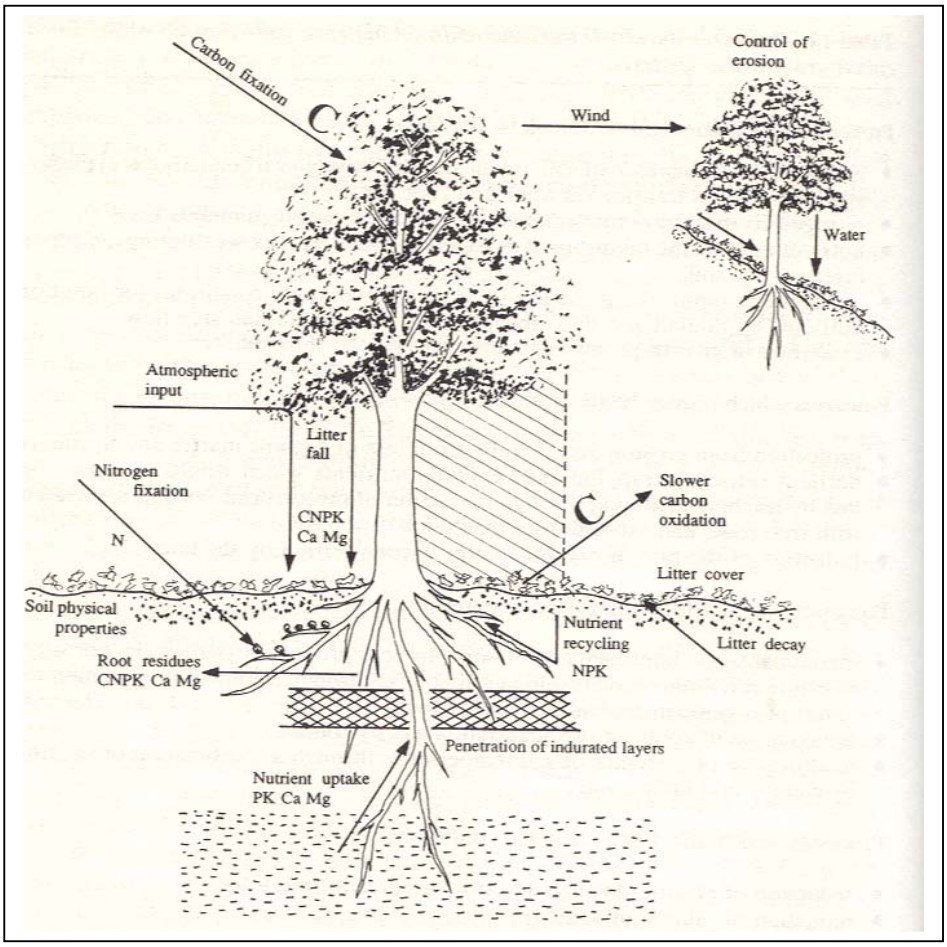


Fig 2.1: Schematic presentation of trees on soil improvement

Source: Adapted from Young (1989)

One criticism of agroforestry has been that it can be labour intensive. This is the case with alley cropping, which was developed by researchers, but not readily adopted by farmers. Other constraints can be the ownership the trees and land tenure. These problems need to be clarified prior to tree planting.

2.2 ADDING VALUE TO AGROFORESTRY

2.2.1 Concept of tree domestication

ICRAF has been promoting the domestication of a number of indigenous tree species for 10 years with programmes in each of the six eco-regions in which it operates: the humid lowlands of West Africa, the tropics of Latin America, the humid tropics of Southeast Asia, the sub-humid plateau of southern Africa, the highlands of East Africa and the semi-arid lowlands of West Africa (Leakey and Simons 1998). The domestication of agroforestry trees, one of the three pillars of ICRAF's program, aims to improve the genetic quality of agroforestry trees by collecting, evaluating and selecting germplasm for compatible production of food, fodder, fuelwood, timber and other products with companion crops and for providing environmental services such as soil conservation and the amelioration of soils (Leakey *et al.,* 1996).

Simply, tree domestication is aimed at enhancing the product and service functions derived from trees (Leakey and Tomich 1999). The concept of crop domestication has been defined differently over the years but each definition is really just expressing the same concept (Harlan 1975; Leakey and Simons 1998).

Putting these definitions in a broader context, Leakey and Newton (1994a) stated that a tree domestication process has 3 stages (Fig 2.2) namely: identification and characterization of its germplasm resources; the capture, selection and management of genetic resources and how the integration of these domesticates into farming systems for positive environment and socio-economic impacts.

Clement and Villachica (1994) have stated that the domestication of a crop passes through three stages: starting as a wild or managed species, becoming semi-domesticated and finally fully domesticated. Managed species refers to species that are purposely and deliberately protected during land clearing for swiddens, or left around dwellings or along paths. Semi-domesticates refers to crop species that have been manipulated and changed from the wild state but can survive if no appropriate attention is given to them and domesticates refers to cultivated crop

species which require human involvement to survive new conditions. However, it is also clear that some species have been in the process of domestication for thousands of years (e.g. rice, maize, wheat, oranges, cutnut, etc), but there is always the opportunity for further improvement, so the domestication process is going on.

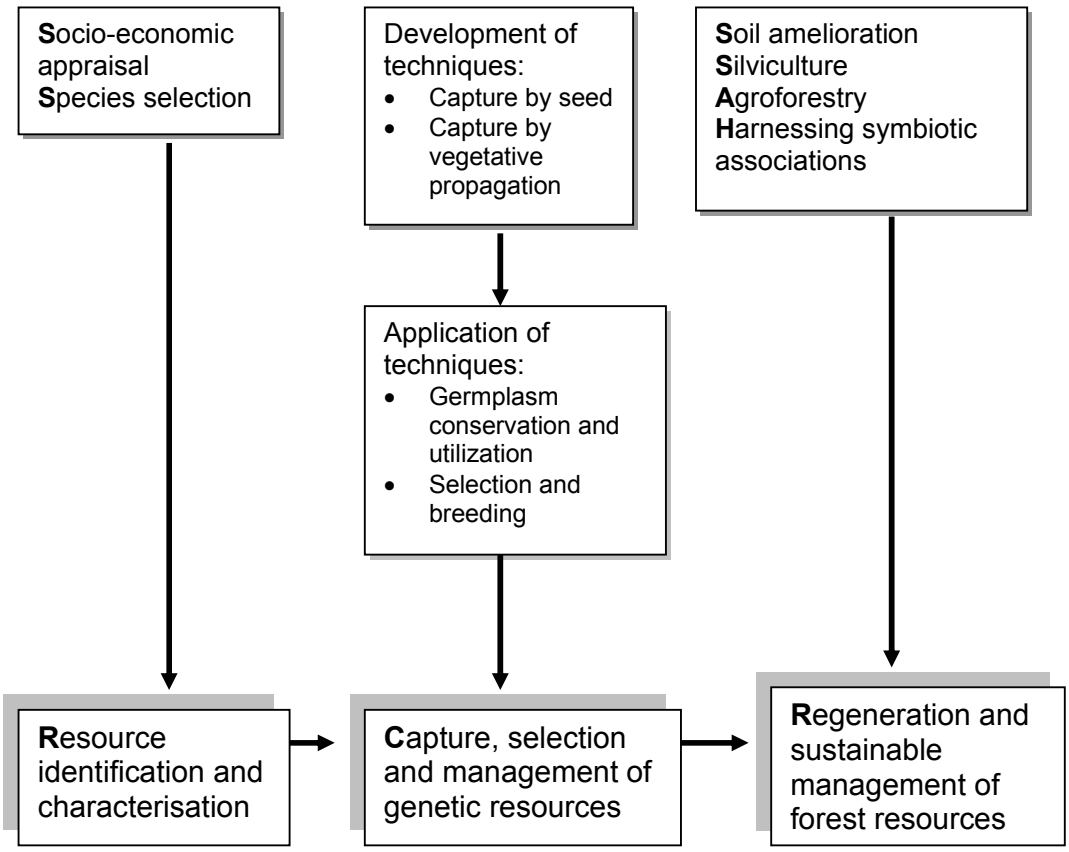


Fig 2.2: The process of domestication of tropical trees.

Source: Adapted from Leakey and Newton (1994).

To a considerable degree, the above view is similar to that of Wiersum (1996) who considers that tree domestication in agroforestry is a multifaceted process envolving progressively by close interaction occurring between people and plant resources. He pointed out three dimensions in the process: a) acculturalization – involving changes from wild uncontrolled utilisation to controlled exploitation of

tree products. This requires the need to define user rights and the formation of relevant management entities to formulate rules and regulations for resource management and utilisation; b) modification of the biophysical environment – involves the protection and cultivation of trees either in natural forests (*in situ*) or in agroforest gardens (*ex situ*) thus focussing on the manipulation of the growing conditions of tree and its morphological characteristics, and c) modification of a tree's biological characteristics – involves the manipulation of trees morphology and genetic characteristics to meet biological, ecological and socio-economic objectives.

Within the tree domestication process there are two extreme pathways (Leakey and Simons 1998), with many intermediate options, that can be followed (Fig 2.3). These are:

a) Incremental improvement of the species through their management on farms – a farmer-driven strategy and therefore typically a small-scale operation aimed at local consumption. Farmers in Cameroon and Nigeria have, for example improved fruit size by 66% and 44% respectively in *Dacryodes edulis* and *Irvingia gabonensis* (Leakey *et al.,* 2004), and

b) Major improvements of the species through genetic selection and breeding – typically a market-driven strategy that involves scientific approaches to crop development.

In the past, the science led pathway has tended to lead to monocultures. However, Leakey and Simons (1998) have suggested that it is possible to merge these two extremes and to involve farmers in genetic improvement programs. In this way, smallholder farmers can develop their own genetically improved cultivars from naturally available resources.

Tree domestication plays an important role in social development and livelihood strategies, as a means of creating a sustainable food supply and generating income. This is evident from the transition from hunting and gathering to farming as extractivism led to subsistence cultivation (Clement and Villachica 1994). For

example, in the Amazon many trees and shrubs, such as the Brazil nut (*Bertholletia excelsa*) and cupuaçu (*Theobroma grandiflora*), that are now being cultivated for products such as fruits, nuts and oils were originally extraction products (Prance 1994).

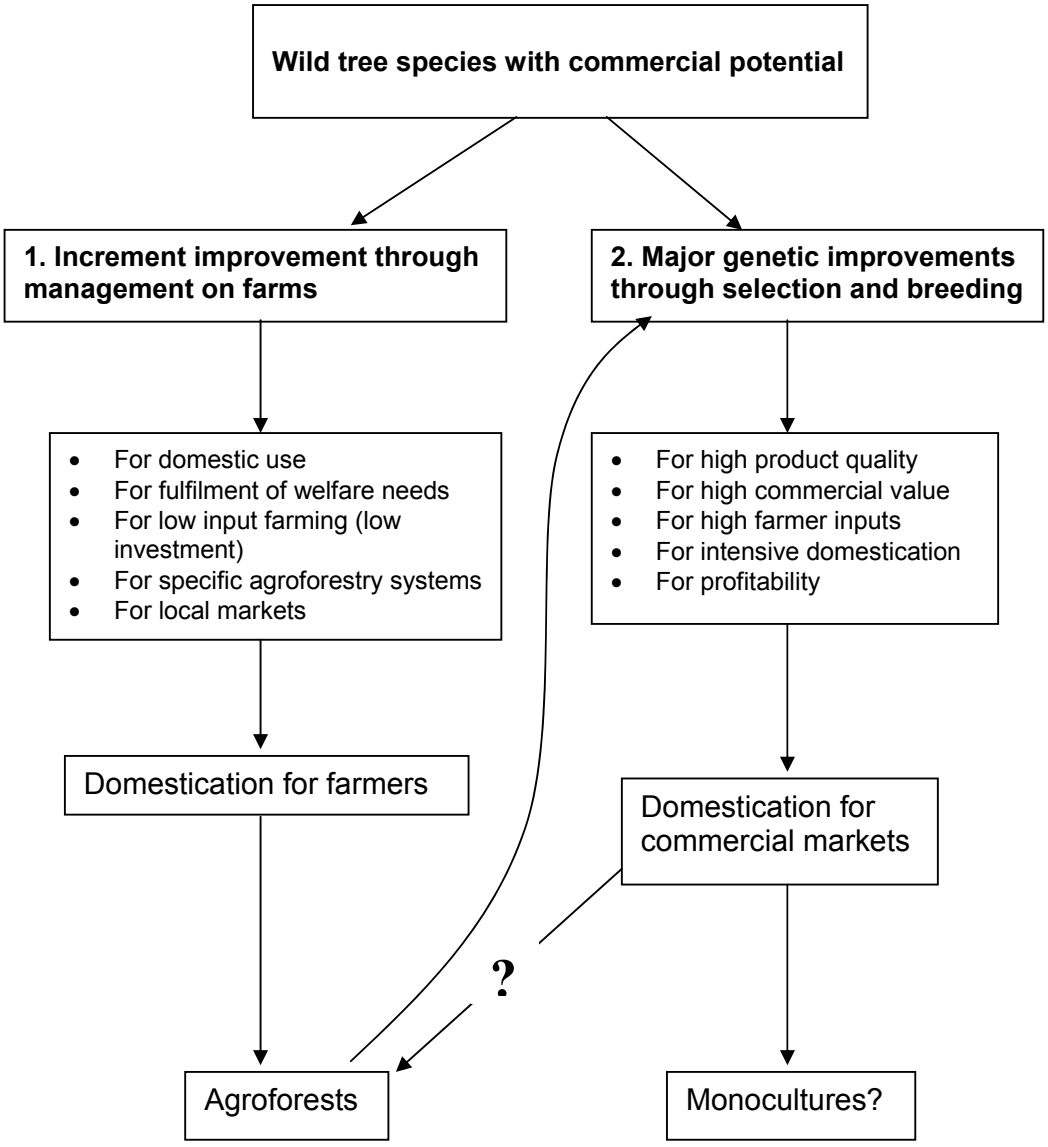


Fig 2.3: Two extreme pathways envisaged in the domestication and commercialisation of non-timber forest products

Source: Leakey and Simons (1998).

2.2.2 Tree domestication and Commercialisation

It is now recognized that the domestication of trees for agroforestry is a means to alleviating the ecological and socio-economic problems in the developing countries. This is seen by the number of international agroforestry tree domestication programs and initiatives (Leakey and Newton 1994a, b; Simons 1996; Christie and Nichols 1999; Harwood 1999; Moestrup 1999; Pottinger 1999). These programs and initiatives have different perspectives but all have one common purpose - that of bringing tree species into cultivation.

In Cameroon, the tree domestication programme has been closely linked to the Alternatives to Slash-and-Burn Programme, as the indigenous fruit and nut trees being domesticated, especially *Dacryodes edulis*, is grown as the shade tree in areas of cocoa production (Leakey and Tchoundjeu 2001). However, following the recognition that forests have many valuable biological resources than just timber (FAO 1995), interest in research to commercialise the products from indigenous trees for agroforestry is growing internationally. The term non-wood forest product (NWFP) has been adopted as the umbrella term for goods of biological origin other than wood, as well as services derived from forests and allied land uses (FAO 1995). However, it was recently recognised that farmers are cultivating a number of indigenous tree species and are not entirely harvesting tree products from the wild to support their livelihoods (Ruiz-Perez *et al.,* 2004). Thus, a new term – Agroforestry Tree Product (AFTP) was suggested to distinguish tree products collected from the wild from that being cultivated on farm (Simons and Leakey 2004).

Tree domestication without the development of the markets for the products will not succeed in bringing about the socio-economic or environmental benefits that could be achieved by agroforestry. In the same way, commercialisation without domestication is likely to fail. This failure could arise from unimproved tree resources, and consequently leading to the production and distribution of inferior planting materials to farmers. It is therefore essential to integrate domestication of trees and commercialisation of their products. In the Solomon Islands there has recently been an example of this. In the 1990's there were efforts to commercialise

Ngali nuts (*Canarium indicum*) without overcoming the supply-side issues of irregular production, variable quality and poor product processing. After initial success the project failed.

2.3 AGROFORESTRY: STATE-OF-ART IN THE PACIFIC

Island countries in the Pacific are geographically isolated and ecologically different from each other, and are often made of many heterogeneous islands. This means there are numerous ecological, cultural and socio-economic interaction, which need to be considered when examining and modifying traditional agroforestry.

Traditionally, the concept of agroforestry and its practices is not new in the Pacific region but the inclusion of modern science technologies is new. Within this scientific context, a number of initiatives, awareness workshops and publications on agroforestry have been developed (Thaman 1990; Raynor 1991; Bonie 1993; Clarke and Thaman 1993; Thaman and Whistler 1996; Wescom and Sairusi 1999; Elevitch and Wilkinson 2000; Thaman 2001). These initiatives are widely supported by the governments of the island states as well as in corporation with other regional and international bodies.

2.3.1 Traditional agroforestry practices in the Pacific

Traditional agroforestry practices in the Pacific vary from country to country, as well as within countries, as a consequence of their ecological and socio-economic differences. However, it is common to find similarities between geographically close island countries with similar geology. Shifting cultivation or slash-and-burn agriculture is practiced widely in the Pacific island states (Allen 1989; Bourke 1989; Clarke and Thaman 1993) and is the mainstay of subsistence farming. Akin to systems in Asia (Tejwani 1987) and South America (Padoch and de Jong 1987), this traditional practice, also known as the swidden cultivation, is a form of agroforestry (section 2.1.2.2.1).

The crops predominantly grown in the Pacific by shifting cultivators are root crops (*Ipomea batatas, Dioscorea* spp*, Manihot esculenta and Colocasia* spp.), cereals such as *Zea mays* and *Oriza sativa*, and vegetables (e.g. *Abelmoschus manihot, Fern* spp*., Phaseolus vulgaris, Cucumis sativus, Arachis hypogaea, Lycopersicon esculentum*). Common perennial crops grown include *Musa* cvs., *Carica papaya* and *Ananas comosus*. Indigenous trees such as *Barringtonia* spp*., Mangifer indica, Artocarpus altilis, Canarium* spp*., Spondius dulcis, Burkella obovata, Gnetum gnemon* are either planted or deliberately protected in the gardens.

In many cases, the swidden cultivation is often forced inland sometimes onto steep hills as land use systems (e.g. commercial monoculture) occupy the coastal lowlands. These sites are prone to landslides and soil erosion when cleared for intensive cultivation. Swidden cultivation can be labour intensive (usually provided by the whole family) but requires little capital. The work of men and women is well defined, with men doing forest clearing and crop protection, while women do burning, cleaning, sowing, weeding and harvesting. Now that population pressures have increased, the system becomes unsustainable as the fallow period has to be shortened. Also, people who were 'forced' to farm hillsides usually have no customary knowledge of sustainability, hence have no sustainable practices put in place (e.g. contouring of felled logs). Consequently, there is now a need to adopt this system and improved agroforestry techniques are seen to be a viable option.

2.3.2 Evolution of agroforestry in the Pacific

Agroforestry in the Pacific has a history dating back many thousands of years through different evolutionary stages (Thaman 1996). These stages include:

a) *Period of agriculturalisation of the forest:* The beginning of agriculture by first inhabitants of the Pacific forest occurred around 40,000 years ago in Papua New Guinea, about 4,000 years ago in Eastern Melanesia, Western Polynesia and Micronesia and around 1,000 years ago in parts of Eastern Polynesia. During this era, the people selectively cleared, protected and

used different indigenous plant species and either deliberately or accidentally introduced a number of plant and animal species they brought with them. Through time they established gardens of trees, crops and animals incorporated and integrated in the same land unit, akin to modern agroforestry albeit with different design and management styles.

b) *Period of indigenous agroforestry enrichment and deforestation:* After successful settlement, the people began to move around, voyaging from one island to another, introduced new plants (e.g. *Abelmoschus manihot* and *Saccharum edule*) and animals to their settlements on their return and also through inter-island trade. This is also a period when more land was cleared for the introduction of new species into polycultural agroforestry systems. During this period, population increased leading to increased deforestation and subsequent environmental degradation. This period lasted for tens of thousands of years in Papua New Guinea and about eight hundred to three thousand years for most of Melanesia, Polynesia and Micronesia.

c) *Period of colonial agroforestry enrichment and agrodeforestation:* European colonists arrived in the 19th century. During this time small-and large-scale monoculture cropping, as well as livestock for export were introduced and there was little emphasis on encouraging and maintaining existing indigenous polycultural systems. Colonial government initiatives led to increased deforestation, the conversion of the best land to monocropping and grazing. Even so, the islanders did incorporate exotic tropical American crops, such as cassava, pineapple, chili, papaya, avocado, soursop and leucaena into their polyculture systems.

d) *Post-World War II agroforestry enrichment and accelerated agrodeforestation:* World War II had great impact on the Pacific islands, with war bringing new infrastructures such as roads, wharves, bridges and airfields as well as new foods and drinks. At this time the islanders also increasingly gained access to the outside world in terms of markets,

agricultural technologies and transport throughout the world. Consequently, the promotion of export crops occurred such as coconut, cocoa, oil palm, banana, pineapple, citrus and sugarcane. Meanwhile, fruit trees and other traditionally useful trees were increasingly marginalised and under pressure to give way to monoculture cash crops. This has led to what Thaman (1996) termed 'agrodeforestation.'

e) *21st Century 'modern agroforestry':* The fundamental question at the present time is, "what should be the way forward for agroforestry in the Pacific island states?" Promotion of agroforestry practices to increase food production, generate income and protect the environment was seen as a useful option for farmers.

2.3.3 Modern agroforestry practices in the Pacific

Rogers and Thorpe (1999) have suggested the following five agroforestry practices that may be appropriate for the Pacific island states.

a) *Improved fallow in shifting cultivation:* this is practiced in Fiji, Papua New Guinea, Tonga and Samoa (Rogers and Thorpe 1999), using leguminous species from Central America such as *Calliandra calothypsus* and *Gliricidia sepium*. These species protect the soil from erosion as well as improve fertility. Similar plantings can also use indigenous species like *Casuarina oligodon* and *Paraserianthes falcataria* to restore soil fertility in old gardens as practised in highlands of Papua New Guinea (Clarke and Thaman 1993; Bourke 1989). These species also provide timber for fencing, housing and firewood. In Samoa, *Securinega flexuosa* is grown for poles after three years of agricultural cropping, while *Erythrina subumbrans* is planted in both Samoa and Tonga after 2-3 years of taro crop cycle, as the basis for improving the soil (Rogers and Thorpe 1999).

b) *Tree gardens and home gardens:* These practices are widespread in the Pacific and are established for multiple outputs. Success has been reported

from Kiribati (Thaman 1990; Ubaitoi 1999). Fruit trees such as breadfruit, dwarf coconut, pandanus and papaya are spaced out among root crops and vegetables. The mixture of trees and crops provides a wide range of products for the people as well as shade and protection from dust and salt spray. In the Pacific indigenous fruits and nuts are common components.

c) *Trees and shrubs on farmland:* In Fiji, a 600-acre beef and pig farmland has been rehabilitated by agroforestry, using species such as coconut and citrus for income and food for the pigs, a leguminous species (e.g. *Sesbania, Calliandra* and *Gliricidia sepium*) is used to protect soils on steeper slopes and provide mulch, fodder and shade for the animals (Radler 1999). They are also intercropped with Kava to improve soil fertility. Mahogany has been planted on the boundary of the farm as a source of timber, while being a live fence and a windbreak.

d) *Hedgerow or alley cropping*: has been adopted mainly in Tonga and Fiji (Nakalevu and Seru 1999; Rogers and Thorpe 1999). Although promoted worldwide the adoption has been poor due to high labour demand. *Calliandra* trees are planted at 4 m spacing between rows on sloping land with poor fertility. Dried wood from *Calliandra* is used as fuelwood. It burns well and is particularly favoured in places where indigenous firewood species are becoming scarce.

e) *Plantation crop combinations:* This involves the grazing of livestock under coconuts and the growth of other agriculture crops such as cocoa, citrus or coffee and timber species. For example, on a 60 ha farm in Vanuatu, 100 cattle were grazed in 40 ha of mixed improved native pasture and 3 ha of whitewood (*Endospermum medullosum*). Grazing begins when the trees are 2 years old and have generally attained 7-8m in height and are unlikely to be damaged by the animal (Macfarlane 1999).

2.3.4 Current state of Agroforestry in Solomon Islands

2.3.4.1 Background

In 1999, approximately, 84% of the population (409,000 people) live in the rural areas and support themselves by subsistence farming. Traditional agriculture is therefore the most vital occupation of the people. Unfortunately, government schemes have promoted cattle, coconut and cocoa smallholder projects on the accessible, fertile and flat coastal lands, leaving about 63% of the land area (28,000 km^2) for traditional agriculture land greater than 20% slope (Hansell and Wall 1975).

2.3.4.2 Traditional agroforestry practices in Solomon Islands

As elsewhere in the Pacific, shifting cultivation is the common traditional agroforestry practice in Solomon Islands but is only sustainable in areas of low population density. Where population density exceeds 10 people per km^2, the fallow period has to be shortened (Mackay 1988), causing soil erosion, loss of soil fertility and declining crop yields.

In some areas farmers have developed better practices. For example, in Kologhona village of Guadalcanal Island there are two methods of traditional farming locally called: ago-puka and ago-male (Clarke and Thaman 1993). In the ago-puka method, usually practiced in old forest a long way from the village, debris from land clearance is piled around the bottom of bigger trees and burnt. The stumps are not removed and the land is not levelled before planting, so that crops such as taro and yam are commonly planted beside the stump, which slowly rots away releasing nutrients for the crop. Ago-male is more common close to the villages and typically located in alluvial and colluvial soils. When secondary forest is cleared for new gardens, the debris is not burnt, but pushed into windrows and the area planted with crops such as sweet potato (*Ipomea batatas*), yams, taro, sugar cane, cassava, pumkin, pineapple, maize, chillie peppers and tobacco. The windrow is good for climbing crops and again as the windrow breaks down, nutrients are released. In addition, trees with edible fruits and nuts (e.g. *Artocarpus*

altilis, *Areca catechu*, *Inocarpus fagifer*, *Barringtonia edulis*, *Syzygium malaccense* and *Ficus copiosa)* are either protected during land clearance or deliberately planted amongst crops. Other traditionally important trees or tree-like species (e.g. *Pandanus tectorius*, *Ceiba pentandra*, *Metroxylon salomonense* and *Heliconia indica* are also maintained in the gardens. The latter two species are commonly found in poorly drained soils near rivers.

Another traditional farming practice is used in Buma, Malaita Province (Clarke and Thaman 1993). New gardens are normally located next to existing gardens for easy transfer of planting material. Men are normally engaged in more difficult tasks such as tree felling, log removal and emplacements as boundaries, while women do the planting, weeding and under-brushing. Traditionally important trees (e.g. *Areca catechu*, *Artocarpus altilis* and *Canarium indicum*) are protected. Debris and litter from land clearance is gathered into small piles and burnt when dry. The ashes are then spread in the garden.

Commonly grown crops in new gardens include taro, giant taro (*Alocasia macrohorrhiza*) and yam (*Dioscorea esculenta*), while older gardens contain *Alocasia macrorrhiza*, *Ananas comosus* and sweet potato, cassava, bananas. Gardens are left fallow after 2 years of cropping. Fallows commonly contain a wide range of important tree species for building materials, medicines, dyes and food and other crops like betel-nut (*Areca catechu*), ngali nut (*Canarium indicum*), papaya (*Carica papaya*) and hibiscus spinach (*Hibiscus manihot*). Thus, an area remains productive and useful for the people while the agroecosystem is restored.

2.3.4.3 Current agroforestry systems in Solomon Islands

In Solomon Islands, current agroforestry practices are similar to those described earlier for the Pacific island states (see section 2.3.3). The following represents variations on the general theme:

a) Improved fallows can include species like *Canarium* spp (Ngali nut) and *Securinega flexuosa* as an enrichment planting.

b) Homegardens: a widely spread agroforestry practice in Solomon Islands involving combinations of food crops (e.g. cassava, sweet potato, beans, cabbages) planted with fruit trees (e.g. banana, pawpaw, *Barringtonia* spp. *Canarium* spp. and coconuts) simultaneously. Homegardens are found close to villages and common in semi-urban and urban centres as well.

c) On Kolombangara Island, because of the importance of forestry industry based on the timber plantation company KFPL, farmers grow a number of exotic timbers species such as *Gmelina arborea, Eucalyptus deglupta, Tectona grandis* and *Ochroma lagopus*. KFPL provides farmers the seedlings at US$0.20 per seedling. Recognising the long time to realise income from the timber species, the forestry company has encouraged farmers to grow agricultural crops to support their subsistent requirements, as well as selling their surplus produce in local markets for income.

d) In Choiseul, farmers incorporate indigenous nut trees (e.g. *Canarium* spp.) with timber species (*Eucalyptus deglupta* and *Tectona grandis*) as a forest fallow. This involves under-brushing but no burning. Seedlings of timber trees are planted in well spaced rows among those already found in the fallow forest. The difference between this practice and the improved fallow is that in this system the farmer undertakes under-brushing to promote the seedling growth, which is not the case in the improved fallow practice.

e) Perhaps the most developed agroforestry system in Solomon Islands is found in Temotu Province. It is called the 'Improved Temotu Traditional Agriculture' or ITTA and has been formalised in a book for extension workers (Bonie 1993). This is a classical example of a complex agroforestry practice aimed for food production, soil improvement and income-generation. ITTA is a very intensive multi-cropping practice utilizing about twenty-three plants species including, fourteen tree species (e.g. *Magnifera indica, Inocarpus fagifer, Teminalia catappa, Barringtonia* spp*. Gnetum gnemon, Artocarpus altilis*), six root crops (e.g. *Dioscorea* spp*, Ipomea batatas, Manihot esculenta, Colocasia esculenta*) and three

vine species (e.g. *Vigna* spp*, Lycopersicum esculentum*). These are all grown together in a carefully formulated design aimed at establishing a sustainable agroecosystem, which minimises competition between components and optimises production.

ITTA is based on traditional practice and indigenous knowledge, but also involves modern scientific principles. It has a multi-storey structure composed of different fruit and leguminous trees as an overstorey above a diverse array of agricultural crops for food and nutritional security. *Inocarpus fagifer* trees between 5-10 years have been found to yield 10 kg fruits per tree. At maturity (26 years or more), yields rise to 75 kg fruits per tree. Root crops, such as yam (*Dioscorea alata*) for example, yield around 37 metric tonnes per hectare per year (Bonie 1993).

2.3.4.4 Tree domestication in Solomon Islands

The cultivation of trees is clearly an integral component of traditional agroforestry and the culture of Melanesian society. Throughout the Pacific, farmers have selected indigenous fruit and nut trees such as breadfruit, cutnut and *Canarium* nut for cultivation. However, domestication of indigenous fruit tree species for commercial purposes is a recent development in Solomon Islands, and is generally true in the South Pacific region. In 1988, the government realised the potential of Ngali nut (*Canarium* spp.) as a source of income for rural people in addition to copra, cocoa and chillies (Pelomo *et al.,* 1996). This led to work done on the commercialisation of nuts from *Canarium* spp., *Barringtonia* spp. and *Terminalia catappa* (Evans 1996, 1999). However, the initiative was only successful for a short time and then over-riding supply issues caused the collapse of the market. These issues were related to nut quality and regularity of supply. For example, *Canarium* spp. oil which has been commercially marketed to the United Kingdom failed to meet quality standards (Pelomo *et al.,* 1996). This was a consequence of great variability from trees in the wild (nuts are collected from trees in the wild) and poor village level processing. Thus, there is a need for tree domestication to select superior genotypes to meet the market demand and for better processing.

In terms of timber species, the Government Forestry Department concentrated mainly on exotic species such as *Eucalyptus deglupta, Gmelina arborea, Tectona grandis,* and *Swietenia macrophylla* rather than on indigenous timber species. However, in 1998 the government in collaboration with the South Pacific Regional Initiative on Forest Genetic Resources (SPRIG) have set goals to domesticate priority indigenous timber-producing species (e.g. *Pterocarpus indicus, Vitex copasus, Gmelina moluccana* and *Intsia bijuga*). Consequently, the provenance trials were established in Kolombangara Island in 1999.

In view of international developments in agroforestry and the needs of the people in the Solomon Islands, it seems clear that there is potential to domesticate indigenous tree species, especially edible fruit and nut species that have traditional value and commercial potential. Thus, this project seeks to develop techniques, using species identified through a participatory priority setting process with farmers conducted in October 2002 (Chapter 4).

CHAPTER 3: GENERAL MATERIALS & METHODS

3.1 THE SITE

3.1.1 Solomon Islands

3.1.1.1 Location

The Solomon Islands is a double chain of islands geographically located in the southwest Pacific between 155^{o} 30' and 170^{o} 30'W longitude and between 5^{o} 10' and 12^{o} 45'S latitude. It is made of different islands with rugged mountains and low-lying coral atolls stretching about 1,400km in a northwest to southeast direction. The eastern outer islands of Solomon Islands are located close to the northern end of Vanuatu and the western islands are located close to Bougainville in Papua New Guinea. There are 990 islands in total covering a land area of around 28,000 square kilometres. Six main islands are Choiseul, New Georgia, Santa Isabel, Guadalcanal, Malaita, and Makira (Fig 3.1). These islands are intersected by deep and narrow valleys and were mostly covered with vegetation type classified as tropical rainforest (Whitmore 1969).

In comparison to Papua New Guinea and New Caledonia, the Solomon Islands is considered geologically "young" (Clarke and Thaman 1993). Based on the islands geology, Falvey *et al.,* (1991) divided Solomon Islands into three major provinces (Pacific, Central and Volcanic) and two minor provinces (Oceanic Volcanic and Oceanic Atoll). The islands of Malaita, Ulawa and northeast Santa Isabel are grouped into the Pacific Provinces, whereas Makira, Guadalcanal, the Florida islands, the southeast Santa Isabel and Choiseul belong to the Central Province. The Volcanic Province consists of New Georgia group, Russell islands, the Shortlands, the northwest Guadalcanal and Savo. Temotu islands are grouped in Volcanic Province while Rennell, Bellona and Ontong Java islands are within the Oceanic Atoll Province.

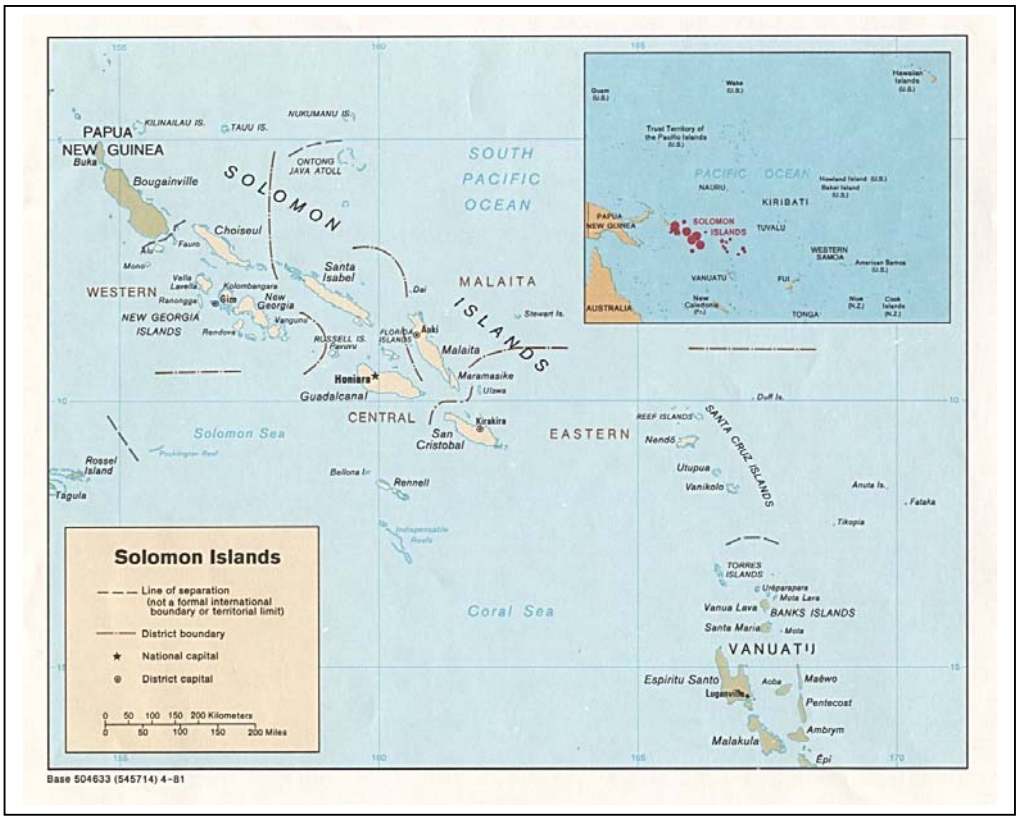


Fig 3.1: Geographical map of the Solomon Islands.
Source: http://www.lib.utexas.edu/maps/islands_oceans_poles/solomonislands.jpg

3.1.1.2 Climate

The climate in Solomon Islands is tropical and similar to other countries in the Pacific with plenty of sunshine, hot, humid and high annual rainfall and temperatures across the region. The mean daily temperature fluctuates between 25^{o}C and 32^{o}C throughout the year. Rainfall varies across the country, higher in the mountainous than in low-lying islands, but within the range of 3,000 to 5,500mm per annum. There are no marked wet and dry seasons but wet and dry periods do occur with heaviest rain between November and March. In contrast, two short dry periods occur in April to June and September to October (Solomon Islands Meteorological Service 2002). Heavy storms and tropical cyclones are not unusual in Solomon Islands, but occur more frequently in Guadalcanal, especially the Weather Coast area, Rennell and Bellona Islands and the eastern part of the country than the rest of the country. For example, the 1986 cyclone 'Namu' caused significant flooding and damaged to homegardens and properties and claimed

many lives in Guadalcanal Island compared to other islands in the west of the country. On average, the Solomon Islands tends to have 1-2 cyclones per year, although very intense cyclones are rare (Solomon Islands Meteorological Service 2002). However, strong winds and thunderstorms occur every year between November and April.

3.1.1.3 Population and Economy

The Solomon Islands' population of 409,000 people comprises 93% of Melanesian race, 4% Polynesian, 1.5% Micronesian, 0.8% European, 0.3% Chinese and 0.4% others (Government Population Statistics 1999). Currently, the population is growing at a rate of 2.6% per annum. Population density varies between provinces (Table 3.1) but the national average is 13 people per square kilometre. About 84% of the population live in rural communities.

Table 3.1: Summary of Solomon Islands population by provinces (adapted from the Solomon Islands Government Report on 1999 population and housing census).

Province	*Population*	*Households*	*Pop. density(sq. km)*	*Average Households*
Solomon Islands	**409,042**	**65,014**	**13**	**6.3**
Choiseul	20,008	3,142	5	6.4
Western	62,739	9,992	8	6.3
Isabel	20,421	3,556	5	6.7
Central	21,577	3,625	35	5.7
Rennell-Bellona	2,377	432	4	6.0
Guadalcanal	60,275	10,399	11	5.5
Malaita	122,620	18,606	29	5.8
Makira/Ulawa	31,006	4,926	10	6.6
Temotu	18,912	3,413	22	6.3
Honiara City	49,107	6,921	2,244	7.1

The economy of Solomon Islands is principally based on timber, fish, copra, cocoa, oil palm and minerals. Social unrest occurred in late 1998 and badly affected the trading in these commodities. Consequently, the closure of the Solomon Islands Plantations Limited (SIPL) which specialised in palm oil and kernel export and the Gold Ridge Mining Ltd (GRML) which was exporting gold and silver further constrained the economy. The logging sector was less affected

by the social unrest and was heavily dependent upon during this period. In 2000, the Central Bank of Solomon Islands (CBSI 2001) reported a record low in the total export of individual commodities: logs = 536,000 cubic meters, fish = 21,163 tonnes, copra = 19,004 tonnes, coconut oil = 8,553 tonnes, cocoa = 2,315 tonnes, palm oil and kernel = nil (closed), gold = 49,954 ounces and silver = 20,744 ounces (before closure). However, the economy since then has recovered, and according to a statement made by the Governor of the Central Bank of Solomon Islands during the launching of 2003 Annual Report (Houenipwela 2004), the economic turn around began in 2002. It was stated also that the productive sector (e.g. agriculture, fisheries and forestry) and others resumed activities well before the introduction of the regional assistance mission to Solomon Islands (RAMSI) in July 2003. Consequently, a 5.8% economic growth in real term was achieved, and was especially attributed to the dedication of ordinary Solomon Islanders in rural communities participating in small scale economic activities (e.g. farming and fishing).

3.1.1.4 Vegetation

Six vegetation or forest types are distinguished in Solomon Islands, which vary in magnitude from one province to another (Table 3.2), and reflect the geological formation, ranging from acidic volcanic origin in the bigger islands to alkaline limestones in low-lying atolls. According to Whitmore (1969), the range and type of plant species present is fairly similar between islands despite the geographical spread of the islands. However, they are affected by six factors: soil type (based on parent rock), climate (e.g. rainfall and temperature), topographical features, altitude, natural catastrophes (cyclone and earthquakes) and human activities.
The six vegetation types are:- the lowland rainforest, hill forest, montane forest, freshwater swamp and riverine forest, saline swamp forest and the grassland and other non-forest areas (MFEC 1995):

i. *Grassland and other non-forest areas:* compose of non-tree species, mainly herbaceous species. Predominant species include *Imperata cylindrical, Dicranoptera linearis and Themeda australis.* Examples of commonly occurring species are *Mimosa invisa, Morinda citrifolia,*

Saccharum spontaneum, Polygala paniculata and Timonius timon. Some of these species (e.g. *M. invisa*) are very common in disturbed areas.

ii. *Saline Swamp forest:* is subject to tidal influence as they are found in estuaries and foreshores. Examples of species composed of this vegetation include *Barringtonia asiatica, Calophyllum inophyllum, Casuarina equisetifolia, Terminalia catappa, Intsia bijuga, Inocarpus fagifer, Pandanus* spp*., Barringtonia racemosa* and species of mangroves. This group of species are also known as the 'Indo-Pacific Strand Flora' (Whitmore 1966).

iii. *Freshwater Swamp and Riverine forest:* are commonly found in poorly drained land at low altitudes with little micro-relief. Species such as *Inocarpus fagifer, Mextroxylon salomonense, M. sagu, Barringtonia racemosa* are found here, although some important timber species are also present (e.g. *Terminalia brassii* and *Dillenia salomonensis*).

iv. *Lowland rainforest:* includes forests at altitudes up to 5 - 70m, often with complex structure due to greater number of species from upper or hill forest and patches of freshwater swamp forest. Occasional cyclones and human activities often disturb this forest type as evident in high incidence of re-growth and secondary species. Species predominant in this vegetation include timber species such as *Campnopserma brevipetiolata, Dillenia salomonensis, Endospermum medullosum, Parinari salomonensis, Terminalia calamansanai, Schizomeria serrata, Maranthes corymbosa, Pometia pinnata, Gmelina moluccana, Elaeocarpus sphaericus* and *Vitex cofasus*. Most indigenous fruit trees are also found in this forest including *Canarium* spp*, Syzygium malaccensis, Magnifera minor, Spondius dulce, Barringtonia procera, B. edulis, Artocarpus altilis, Gnetum gnemon,* and *Burkella obovata.*

v. *Hill forest:* occurs at altitudes of 400 - 600m and on well-drained soils and exhibits complex structure with varying tree heights and canopy density.

Some species in the lowland forest are also present here as well as those species commonly found in the montane forest. Species forming this forest include *Pometia pinnata, Gmelina moluccana, Elaeocarpus sphaericus, Vitex cofasus, Campnosperma brevipetiolata, Dillenia salomonensis, Endospermum medullosum, Parinari salomonensis, Terminalia calamansanai, Schizomeria serrata, Maranthes corymbosa,* and *Vitex cofasus.* Fruit tree species such as *Canarium* spp., *Gnetum gnemon* and *Artocarpus altili*s are also present.

vi. *Montane forest:* refers to forests found generally above 600m contour, on ridge tops and mountain summits, but can be found in lower elevations under harsher conditions. It is characterised by dense and compact canopy with small lighter tree crowns. Species in this forest type include *Callophyllum kajewskii, Callophyllum pseudovitiense, Eugenia* spp*., Dacrydium* spp*., Pandanus* spp., *Racembambos scandens* and ferns*.*

It was estimated that there are about 5000 plant species in Solomon Islands. In 1966, only 1931 species had been described by Whitmore (1966). This rose to 3210 species in 1988 (Henderson and Hancock 1988). Of the described species about 500 to 600 are exotic, mainly ornamentals, and have been introduced into the country at different times and for different reasons e.g. commercial plantations of *Eucalyptus deglupta* in 1960s (MFEC 1995).

Table 3.2: General vegetation types in Solomon Islands.
The total area is not exactly agreeing with the gross area of country as not all islands are surveyed and the different method used (FRIS – ERM-S). *excludes Rob Roy and Vaghena islands (approximately 15500ha). Source: MFEC (1995).

	Guadalcanal		Central		Malaita	
Vegetation type	**Area (ha)**	**% land area**	**Area (ha)**	**% land area**	**Area (ha)**	**% land area**
Montane	51204	9.6	174	0.3	6612	1.6
Hill	401936	75.1	38765	61.3	354544	84.4
Lowland	58844	11.0	13546	21.4	20144	4.8
Freshwater and Riverine	10100	1.9	2700	4.3	10705	2.5
Saline swamp	1328	0.2	3112	4.9	9992	2.4
Grassland and other non-forest areas	10920	2.0	212	0.3	4016	1.0

	*Choiseul		Isabel		Western	
Vegetation type	**Area (ha)**	**% land area**	**Area (ha)**	**% land area**	**Area (ha)**	**% land area**
Montane	704	0.2	10164	2.5	22044	4.4
Hill	286868	87.0	325667	78.7	351436	69.9
Lowland	5932	1.8	17812	4.3	53312	10.6
Freshwater and Riverine	10760	3.3	25216	6.1	39888	7.9
Saline swamp	4144	1.3	17852	4.3	10544	2.1
Grassland and other non-forest areas	7128	2.2	8215	2.0	18756	3.7

Vegetation type	Makira		Renbell		Temotu	
	Area (ha)	**% land area**	**Area (ha)**	**% land area**	**Area (ha)**	**% land area**
Montane	11204	3.4	-	-	512	0.6
Hill	265466	80.4	23120	33.1	56500	65.3
Lowland	14996	4.5	2200	3.1	6076	7.0
Freshwater and Riverine	9096	2.8	280	0.4	200	0.2
Saline swamp	908	0.3	188	0.3	2504	2.9
Grassland and other non-forest areas	8610	2.6	528	0.8	8172	9.4

3.1.2 Kolombangara Island

3.1.2.1 Location

The Island of Kolombangara is located within the New Georgia group of islands and between 8^{o} S latitude and 157^{o} W longitude (Fig 3.1). The island has a total land area of 685 km^{2} and has been described as a classical example of a cone-shaped volcano (Hansell and Wall 1975). The island is almost circular and it rises from coastal plains through broad, flat-topped ridges to increasingly narrow ridges with steep slopes right up to a rugged crater rim. These steep and sloping ridges are intersected by a number of rivers. Vila river is the largest on the island and cuts through the ridges from the rugged crater rim down to the coastal plains in the

southeast direction. The highest peak of the island is Mt. Veve at 1760m (Hansell and Wall 1975).

Since 1989, two thirds of the island has been under commercial forest plantations of the Kolombangara Forest Products Limited (KFPL). Prior to KFPL occupation, this part of the island was subject to intensive logging activities of Levers Pacific Timbers Limited (LPT) for 10 years. The indigenous people occupy the remaining one third of the island, plus the coastal fringe within the KFPL lease areas (less than five kilometres across), which is still under traditional (customary) management (Fig 3.2). Various small commercial logging companies mainly from Asia have been involved in logging parts of the area occupied by the population.

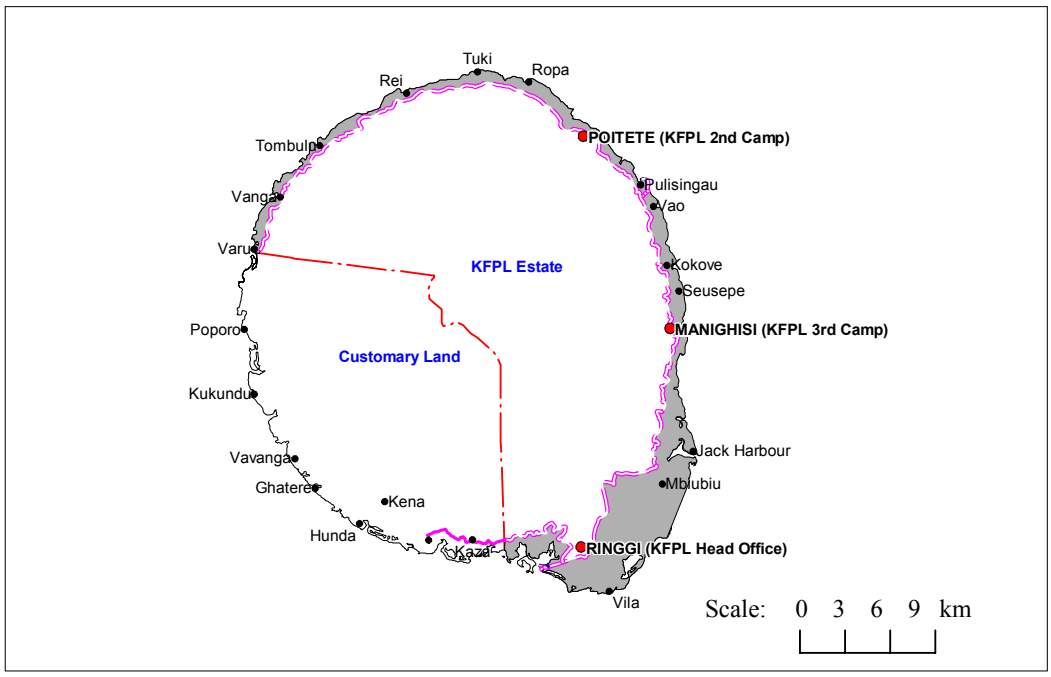


Fig 3.2: Map of Kolombangara Island, shaded grey is coastal strip of land below the road within KFPL estate inhibited by the native people.

3.1.2.2 Climate

The climate on Kolombangara Island is similar to other parts of the Solomon Islands described above. The annual rainfall is well distributed with a mean of 3155mm (KFPL 1998). The wettest period is between December and March (Fig

3.3). Mean monthly temperature is between 22°C and 23°C minimum and a maximum of 30°C and 34°C. On the central mountain the temperature falls to 13°C - 15 °C at night (Wairiu 2001). The relative humidity does not fall below 50%.

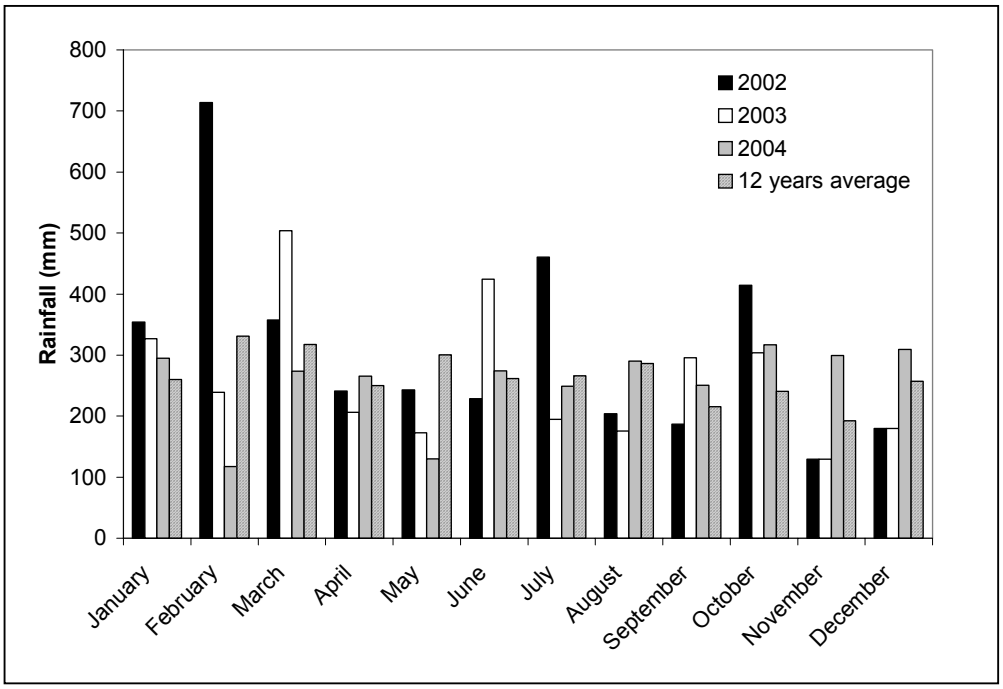


Fig 3.3: Total monthly rainfall of 2002-2004 and 12 years average (1993-2004) recorded in KFPL Ringgi weather station.

3.1.2.3 Population

In 1999, the total population of Kolombangara Island was 5,600 people in 943 households (Government Population Statistics 1999), an increase of 3,200 people (59%) since 1970. The southern part of the island is more populated (3,600 people) than the north (2,000 people). This includes the institutional sector population (e.g. KFPL, the National Forestry College and Government Forestry Station). The village population alone has also increased steadily from 43% to 63% of the total population during the same period. Assuming that two thirds of the island is under plantation forestry and not available for rural settlement, an estimate of population densities during this period indicates a rise from 10 to 25 people per square kilometre. This population density is high in the context of numerous existing biophysical and social problems (e.g. soil degradation resulted from logging and

coconut monocultures on coastal plains, and the issues arising from loss of land tenure). Consequently, land shortages leading to rural poverty are a growing problem in Kolombangara Island.

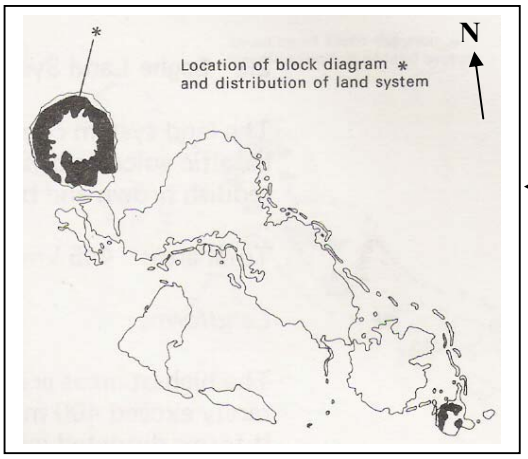
3.1.2.4 Vegetation

Human activities have affected the vegetation of Kolombangara Island which can consequently be grouped into four main types: montane, primary, secondary and coastal forests. The primary forest is found above the 400m contour and below the montane forest, which runs right up to the crater. Secondary forest is generally found within a belt between the 40 and 400m contours. This area of primary lowland forest has been selectively logged by LPT in the 1960s and 1970s. Coastal forest is the most heavily subject to human-influence and comprises large coconut monocultures, home gardens and brackish swamps. The species found in the secondary and coastal forests are mainly indigenous, however, a number of naturalised and recently introduced exotic species are also present. For example, in the secondary forest exotic species such as *Gmelina arborea, Eucalyptus deglupta, Tectona grandis, Swietenia macrophylla* and *Triplochiton scleroxylon* have been grown. The last species is mainly confined to an old experimental plot by the State Forest Department. The forest canopy is denser in some areas than in others. This is partly influenced by competition for light, nutrients, landform and other biotic factors (e.g. microorganisms and floristics), and from the impact of past logging activities by LPT and the current plantation forest of KFPL.

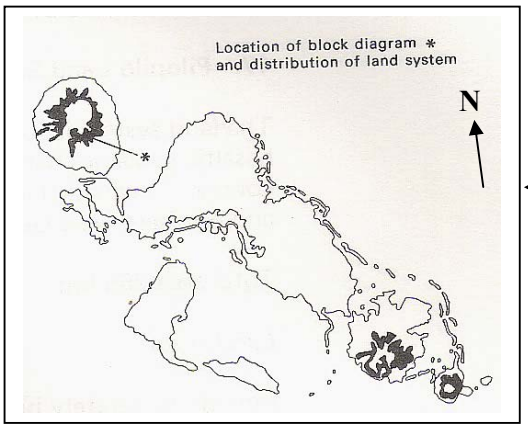
3.1.2.5 Soil

The soils on Kolombangara are well documented (Wairiu 2001) compared to other islands in the Solomons. Hansell and Wall (1975) classified the soils as Haplorthox of the soil order Oxisol in accordance with the US Soil taxonomy classification system. They have also identified nine land units or systems and propose corresponding local nomenclature as: Ringgi, Patupaele, Londumoe, Serambuni, Kotu, Lomuso, veve, and Pusaraghi land systems. The distribution of these land systems within the island varies (Fig 3.4 and Appendix 3.1).

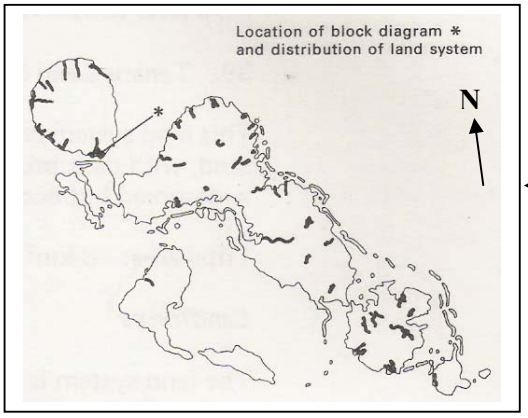
The Ringgi land system is by far the commonest and has been defined as a tract of land with similar or re-occurring parent material, landform, vegetation and rainfall. The soils have been developed over andesitic and basaltic lava and are highly weathered, typically with brownish to red clays, acidic with pH commonly below 5, very low available and reserve nutrients, low CEC and very low base saturation percentages (Hansell and Wall 1975; Chase *et al.,* 1986). Soil monitoring conducted by KFPL concurred to these results, although severe deficiency was pronounced in soils of the Patupaele land system (Table 3.3). Available phosphorus is the major limiting nutrients in soils on Kolombangara Island (Webb *et al.,* 1999), causing a 80% growth reduction in a nursery experiment, in teak (*Tectona grandis*) seedlings. Subsequent field experiments confirmed this. When phosphorus is added in the field through application of Triple Super Phosphate fertiliser, wood volume doubled relative to the control within 27 months of planting.



RINGGI LAND SYSTEM (RLS): Low to moderately high, lightly dissected volcanic debris slopes and lava flows from the land system with highly weathered, commonly humus-rich soils on the wide radiating ridges. RLS occupies land area of 22,710ha (63%), of which 53% is considered highly suitable for agriculture, 2.5% medium and 7.5% with low suitability. See Appendix 3.1 for further details of land facet, landform, soil types and original vegetation.

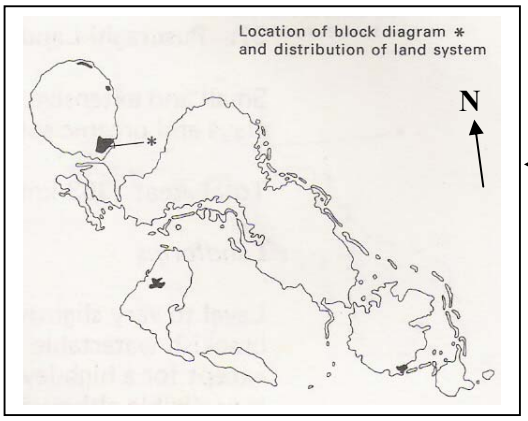


PATUPAELE LAND SYSTEM (PLS): Low to moderately high ridges radiating from basaltic volcanic centre on the island. The soils range from red to reddish brown and brown clay and dark loams, almost entirely under lowland forest. PLS occupies land area of 3,700ha (10%), of which 1% is considered highly suitable for agriculture, 7.5% medium and 1.5% with low suitability. See Appendix 3.1 for further details of land facet, landform, soil types and original vegetation.

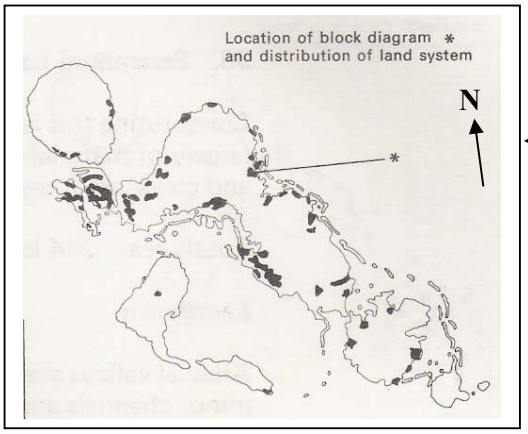


SERAMBUNI LAND SYSTEM (SLS): Recent alluvial valleys and low terraces formed largely of material eroded from volcanic parent materials. Soils are brownish loams and clays, and are forested and cultivated. SLS occupies land area of 4,730ha (13%), of which 6% is considered highly suitable for agriculture, 4.5% medium and 2.5% with low suitability. See Appendix 3.1 for further details of land facet, landform, soil types and original vegetation.

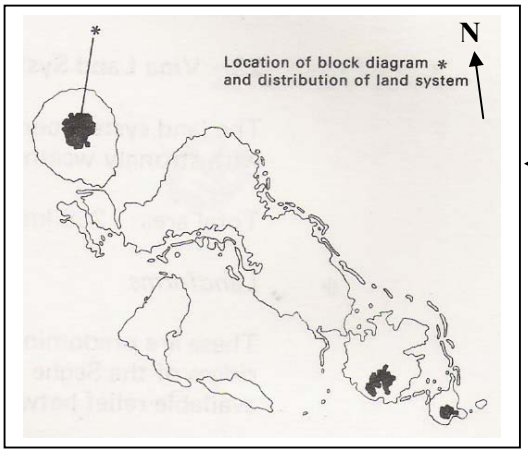
Fig 3.4: The distribution of the main land systems in Kolombangara Island (together with other islands in New Georgia group). Kolombangara Island is predominantly formed of the Ringgi land system, and followed by the Patupaele land system.
Source: Modified from Hansell and Wall (1975).



LONDUMOE LAND SYSTEM (LLS): Comprises coalescent fans formed mainly of fluvial deposits, largely forested. LLS occupies land area of 1,950ha (5%), of which 4% is considered highly suitable for agriculture, 1% medium and none with low suitability. See Appendix 3.1 for further details of land facet, landform, soil types and original vegetation.



PUSURAGHI LAND SYSTEM (PuLS): Small and extensive freshwater swamps with predominantly deep, very poor drained clays and organic soils. PuLS occupies land area of 1,290ha (4%), of which was considered highly suitable for agriculture. See Appendix 3.1 for further details of land facet, landform, soil types and original vegetation.



VEVE LAND SYSTEM (VLS): Comprises high altitude ridges forming the central core of the island. The soils have a thick surface humus layer and the vegetation consists mainly of medium-height and low, mossy forest. VLS was not considered suitable for agriculture. See Appendix 3.1 for further details of land facet, landform, soil types and original vegetation.

Cont. **Fig 3.4: The distribution of the main land systems in Kolombangara Island (together with other islands in the New Georgia group). Kolombangara Island is predominantly formed of the Ringgi land system, and followed by the Patupaele land system.**
Source: Modified from Hansell and Wall (1975).

Table 3.3: Some physical and chemical properties of soils under Ringgi and Patupaele land systems in Kolombangara Island. Source: KFPL Technical report (1998).

Variables	Ringgi land system - altitude			Patupaele land system - altitude		
	Low	*Mid*	*High*	*Low*	*Mid*	*High*
Bulk density (g/ml)	0.9	0.8	0.8	0.9	0.9	1.0
pH	5.1	5.3	4.4	5.2	5.2	5.3
Organic matter (%)	7.6	8.2	11.5	3.3	5.2	6.1
Total nitrogen (%)	0.4	0.4	0.5	0.1	0.2	0.2
P (ug/ml)	4.6	1.9	5.3	3.8	4.4	5.9
K (me/100g)	0.3	0.5	0.3	0.2	0.2	0.1
Ca (me/100g)	3.4	2.3	1.0	1.4	1.8	1.2
Mg (me/100g)	0.9	1.0	0.5	0.6	0.8	0.2
Na (me/100g)	0.1	0.1	0.7	0.1	0.1	0.1
Al (me/100g)	1.2	1.1	2.8	na	na	na
C.E.C. (me/100g)	15.4	20.7	19.3	11.5	13.8	12.0

At high altitude, and especially along the flat to narrow ridges, the soils are covered with a layer of undecomposed organic material intertwined with lateral roots of the trees on site, often referred to as "mattress." This organic layer varies in depth (0.5-1.0m) depending on the forest type. It was considered that clearing of the forest around the 400m contour would destroy this layer (mattress), and release locked nutrients in it, especially aluminium, thus leading to acidification of soils at lower altitude through leaching and surface runoff (pers. comm. Paul Reddell and Mike Webb 1998). This process would certainly impoverish land that is accessible to the people.

3.1.2.6 Land use and ownership

Land tenure is crucial to access and the development of land resources. It is one of the most sensitive topics in Solomon Islands culture including Kolombangara Island. People regard land as the basic necessity of life, the source of food, water, fodder, raw construction materials, firewood, etc. However, this strong indigenous concept has been challenged in Kolombangara Island during the colonial era. In 1903, the Government of the Solomon Islands (then British Solomon Island Protectorate) granted a large part of Kolombangara Island to the Pacific Islands Company through a certificate of occupation. Lever Brothers of the United Kingdom purchased the Certificate from the Pacific Islands Company in 1905 and occupied the island under a 999 year occupation license (Whitmore 1974). When Lever Brothers began clearing areas in the southeast of the island for copra

plantation, tribes from land in the southwest objected. A Lands Commission was established in 1919 to try to settle disputes (KFPL 1999). Following this some lands were given back to indigenous people but Lever Brothers occupied about 66% of the island. In 1972, this 'alienated' land was reverted to the Solomon Island Government but the logging rights over the natural forest were licensed to an associated Unilever Company, Lever's Pacific Timbers Limited (LPT). Since 1968, LPT exploited rainforests of Kolombangara Island, predominantly on timber extraction for log exports mainly to Japan. As part of this crisis, an influx of people into Kolombangara Island occurred from different parts of Solomon Islands to work for LPT. This has enhanced the negative social and environmental complexities on the island (e.g. trespass on customary land and pollution of rivers/streams).

The logging operation by LPT was very thorough and efficient, removing *Calophyllum* spp.; *Campnosperma brevipetiolata*; *Dillenia salomonensis*; *Endospermum moluccana*; *Gmelina moluccana*; *Parinari salomonensis*; *Pometia pinnata*; *Schizomeria serrata*; and *Terminalia* spp (KFPL 1999). Even trees with a diameter of 35-45cm (graded super smalls) were felled, leaving very few trees standing between the coast and the 400m contour, although substantial blocks of primary forest remain above this altitude towards the central core of the island.

After about 10 years of operation, LPT left Kolombangara Island in 1978. Instead of the land being returned to the indigenous people, the government of Solomon Islands (SIG) still retained the land title. In 1988 SIG initiated a joint venture with the Commonwealth Development Corporation (CDC) of the United Kingdom and formed the Kolombangara Forest Products Limited (KFPL). An Investment and Co-operation Agreement was signed in 1989 for a 75 year lease to KFPL.

When LPT finished operation, a substantial infrastructure, consisting of a township with schools, clinics, churches, airfield, deep sea port and wharf, an engineering workshop, small ship slipway and an extensive road system was left behind. To support its plantation operations, KFPL took possession of this infrastructure and facilities and made further improvements. The KFPL estate now covers a total land

area of around 39,000ha. Of this area, about 18,000ha is suitable for plantation while the remaining area is allocated for forest reserves including areas above 400 m contour and areas greater than 30% slope which are unsuitable for plantation forest (KFPL 1999).

Through these commercial transactions, there has been one clear message to the indigenous people - that the land is unavailable for them to use. Thus, from the beginning of the 20th century, there has been pressure from the indigenous people of Kolombangara on the Solomon Island Government to return all alienated land to customary control. In 1992, the SIG agreed to give the Perpetual Title to the people of Kolombangara, but the Commissioner of Lands currently holds this in trust. Even if they finally get the Perpetual Title, it is unlikely that the land will be available for small-scale activities unless commercial operations of KFPL are discontinued.

3.1.2.7 Impacts of commercial activities

The devastating impact of LPT's natural timber extraction on rivers, land and biodiversity resulted in loss of soil structure and fertility and increased ecological and socio-economic problems over 10 years until 1978. Remnants of heavy plants and vehicles and bulldozer tracks in the forest are still visible today. But since then, natural regeneration of indigenous species and the gradual return of ecosystem to natural status has begun, although recovery varies across the logging areas depending on the intensity of extraction and soil fertility (e.g. soils of Patupaele land system are poorer than those of the Ringgi land system). To date, however, there is no enrichment planting with indigenous species on these logged over lands.

KFPL began its operation about ten years after LPT left. There were marked differences between the operations of LPT and KFPL, in terms of their impacts on the ecology, environment, social and rural economy in Kolombangara Island. The former extracted natural timber resources, while the latter operates a forest plantation on the logged over lands by LPT. Judging purely from their activities, it

is clear that LPT caused greater environmental degradation, and to a limited extent, fewer socio-economic problems than KFPL.

KFPL is the largest plantation forestry in Solomon Islands. It is also the only plantation forestry company in the South Pacific region that has been certified by the Forest Stewardship Council (FSC) in recognition of its practice of sustainable management in its plantation forest since 1998. Within this scope, KFPL has contributed considerably towards the better management of its forests including reserves and is very conscious of issues of biodiversity and conservation and environmental degradation. As a priority, it has also sought solutions of mutual benefit. This has been evident through KFPL's proactive programs in tree growth monitoring, standard harvesting practices, water quality assessments, and collaborations with a number of international and regional projects (e.g. ACIAR and SPRIG) to seek solutions to improved tree growth as well as the protection and conservation of soils and the environment (Plate 3.1).

Plate 3.1: A log-landing site at KFPL plantation in Kolombangara Island rehabilitated with trial planting of *Acacia aulococarpa* and *Eucalyptus deglupta*, with cover crop growing understorey

To improve the local situation, KFPL has contributed by providing income and basic health services and education not only to its own employees and contractors but also to rural population of Kolombangara Island. KFPL provides contract jobs for communities and individuals throughout the Western Province, although priority was given to indigenous people of Kolombangara Island. Villagers are also earning income from selling coir to KFPL. Coir is the decomposed coconut husk used by KFPL as potting media to raise its seedlings. In addition, the farmers have also been provided with improved seedlings, which they purchase at nominal cost (US$0.25 per seedling). They also get free consultations and advice on many aspects of forestry including:- land preparation, planting, silviculture, maintenance and management of their trees. Women, who are mostly responsible for subsistence gardening to support their families, have also been assisted with alternative small-scale projects (e.g. honey-bee farming, poultry, etc.) in collaboration with government extension service and other non-government entities. Among the NGO's playing an important role in the Solomon Islands is the Kastom Gaden Association, a "grass-roots" organisation implementing the "Farmers First Programme," which helps farmers to produce and disseminate seeds and trains rural communities in nursery management.

Within the above context, and coupled with KFPL capacity, the introduction of agroforestry and tree domestication in local communities on Kolombangara Island has great potential to address the previously described socio-economic problems in rural communities: population pressure, land shortage and degradation, food security and income generation. It is hoped that the outputs of this thesis will help organisations like KFPL and the Kastom Gaden Association throughout Solomon Islands and the Pacific States in their efforts to enhance the livelihoods of rural people through a better understanding of agroforestry and the concept and practices of tree domestication.

3.1.3 Study areas

Five study sites (Ringgi Cove, Seusepe, Rei, Poporo and Hunda) on the coastal strip were identified for this study. These study sites differ in sizes and are located

within the two distinct segments of the Kolombangara Island – one segment is largely controlled by KFPL and the other is under customary land (Fig 3.5). The sites were chosen on the basis of five criteria: geographical location, composition of ethnic groups, religious affiliation, land tenure and ownership and easy contact.

Contrasting characteristics of these sites are presented in Table 3.4. Sites in the north of the island generally had more rainfall than in the south (Fig 3.6) (KFPL 1998). North-easterly winds seem to be more prevalent in the north due to open waters, in contrast to the south of the island facing the Vona vona lagoon. In terms of soil types, all sites were formed of soils classified under the Ringgi land system (Fig 3.4 and Appendix 3.1), although pockets of different soils from other land systems are present.

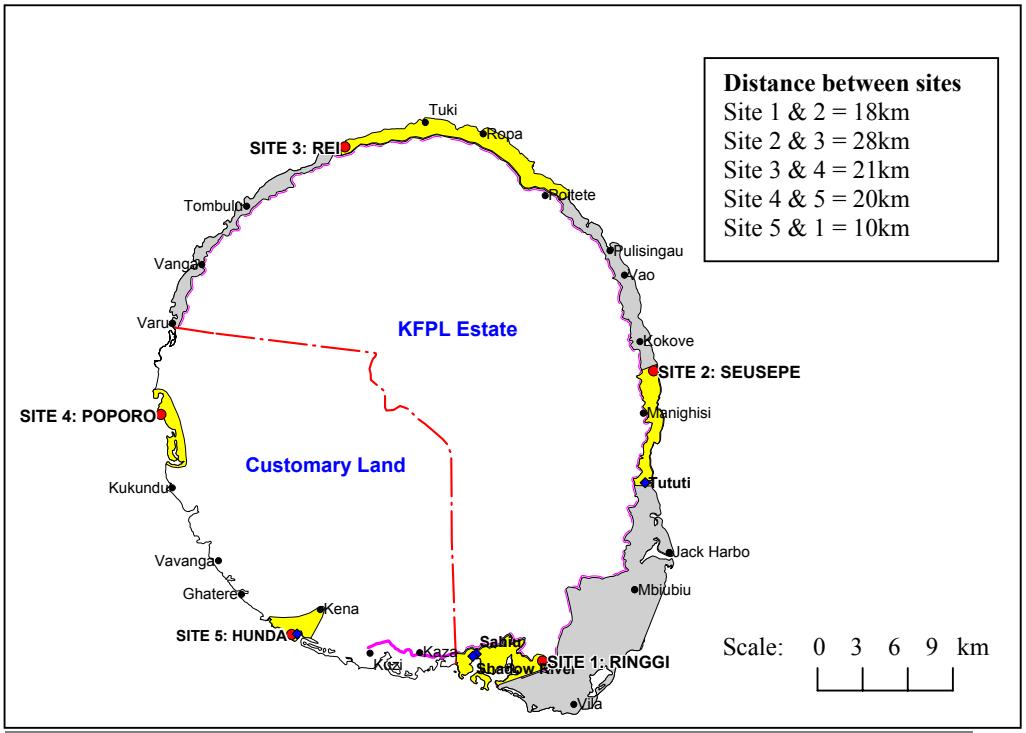


Fig 3.5: Five study sites (Ringgi, Seusepe, Rei, Poporo and Hunda) on Kolombangara Island

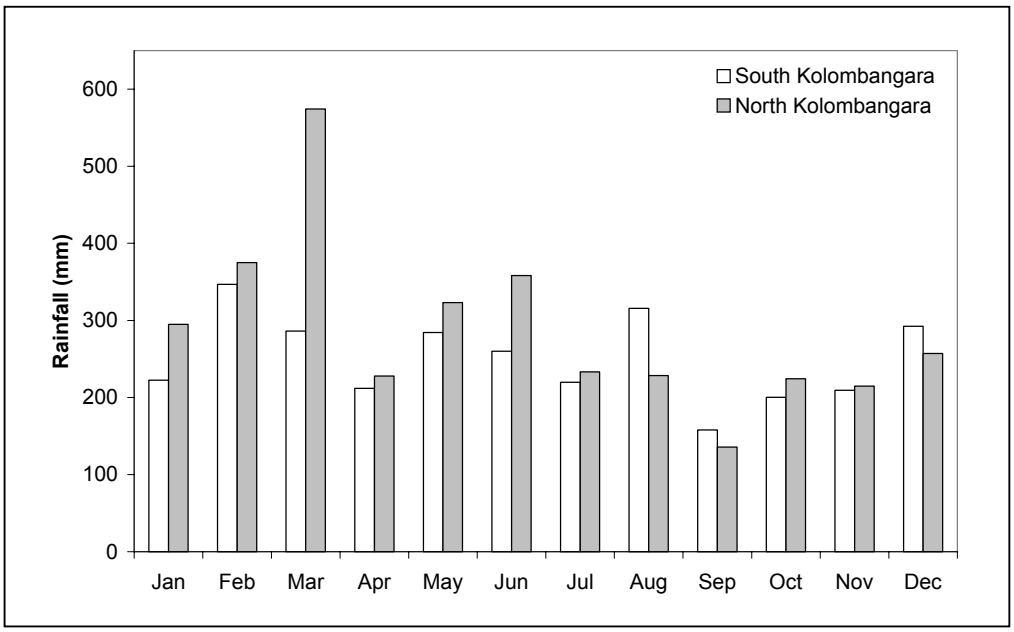


Fig 3.6: Five year average (1997-2001) rainfall (mm) recorded at Ringgi weather station (South) and Poitete weather station (North) in Kolombangara Island.

Table 3.4. Main physical characteristics of the five study sites on Kolombangara, Solomon Islands

Study site (n = no. of villages covered)	Altitude	Location of villages and hamlets	Population density estimate[+]	Ethnic and Religions *	Agroecology	Essential infrastructures (e.g. roads, markets, schools, electricity, communications)
Ringgi/ Vovohe (n = 11)	25 m asl (highest point) 5 m asl (lowland)	On coastal strip (<5 km wide) of customary land adjacent to KFPL estate. Some villages are located within coastal customary segment	High	Indigenous and immigrants from other ethnic groups including KFPL employees. Main religion groups: SDA, UC, SSEC, Catholic and Anglican	Low to high montane forest. KFPL plantation forestry of exotic species occupied land between coastal strip and 400 m contour	Local market at Ringgi, and villages connected with gravelled roads. Electricity and communication facilities and Primary and High schools only at Ringgi township. Some villages with piped water
Seusepe/ Tututi (n = 5)	6 m asl	On coastal strip (<5 km wide) of customary land adjacent to KFPL estate	Low	Indigenous and settlers from different ethnic through customary arrangements. Main religion groups: SDA, UC, SSEC, Catholic and Anglican	Coconut monoculture, Coastal secondary forest. KFPL plantation forestry of exotic species occupied land between coastal strip and 400 m contour	Villages connected with KFPL roads and have access to KFPL market venues. Main local market is at Noro (a separate island c. 20 km away). Children attend school at Poitete township. No electricity or piped water
Rei (n = 8)	8 m asl	On coastal strip (<5 km wide) of customary land adjacent to KFPL estate	High	Indigenous and immigrants from other ethnic groups including KFPL employees. Main religion groups: SDA, UC, SSEC, Catholic and Anglican	Coconut monoculture, Coastal secondary forest. KFPL plantation forestry of exotic species occupied land between coastal strip and 400 m contour	Villages connected with KFPL roads and have access to KFPL market venues. Electricity and communication facilities only at Poitete township Primary school at Tuki (near Rei). Some villages have piped water.
Poporo (n = 5)	6 m asl	Coastal customary lands	Medium	Indigenous. Main religion group is SDA	Low to High montane forest. Low forest zone deforested for food gardens and coconut and cocoa monocultures	Main local market is at Gizo (a separate island c. 30 km away). Piped water in some villages. Primary and High schools at Kukudu (c. 2-3 km away). No electricity, no communication facilities
Hunda/Ireke (n = 4)	10 m asl (Hunda) 7 m asl (Ireke)	Coastal customary lands	High	Indigenous. Main religion groups: UC and CFC	Low to high montane forest. Low forest zone deforested for food gardens and coconut and cocoa monocultures	Main local market is at Gizo (a separate island c. 40 km away). Primary school at Kena. Piped water in some villages. No electricity, no communication facilities

** UC = United Church, SDA = Seventh Day Adventist, SSES = South Seas Evangelical Church, CFC = Christian Fellowship Church*
+ based on observation as no census data available.

3.1.4 KFPL nursery

KFPL nursery at Ringgi Cove, south Kolombangara, is one of the two operational nurseries of KFPL; the second one is located at Poitete, in the north of the island. These nurseries have infrastructure (e.g. road, drainage, security fencing, weather station) and facilities (e.g. pipe water, mist sprayers, electricity, seedbeds, coir (medium), hand tools and growth sheds) which enabled them to each produce approximately 50,000 *Gmelina arborea,* and 20,000 *Eucalyptus deglupta, seedlings* every month, and they have the capacity to accommodate increased production if required. Other species including *Tectona grandis*, *Acacia* spp. and *Ochroma lagopus* are also produced but in smaller numbers.

The nurseries also provide basic facilities (e.g. greenhouse, mist sprayers) for research, and the company supports research initiatives that are compatible to its commercial objectives. Both nurseries are overseen, day-to-day, by an assistant supervisor with most nursery jobs such as coir preparation, setting cuttings, sowing seeds and maintenance and upkeep being undertaken by local villagers under agreed contract. Seedlings were also produced from these nurseries for local communities under KFPL outgrower extension programme. Both nurseries come under the responsibility of the respective Divisional Manager.

The author was allowed to use the infrastructure of the nursery, following approval by the Management and the Board of the company. In addition to the granting of access to its facilities, KFPL provided a work space, two growth sheds, five double seedbeds (three 3m long and two 15m long) and 0.3ha of land to establish a stockplant garden and a germplasm collection (Fig 3.7). Some of these facilities (e.g. seedbeds and nursery shed) needed repairs to bring them to an acceptable standard. These repair works were achieved with assistance from the nursery staff and paid casuals. The allocated land for the stockplant garden (section 3.2.1.1) was first surveyed by the company's surveyors to determine area allocation. This area of land was then prepared for use by assigning contractors to clear the bush and cutdown selected trees. When this was completed, the area was aligned according to the plan and was ready for planting.

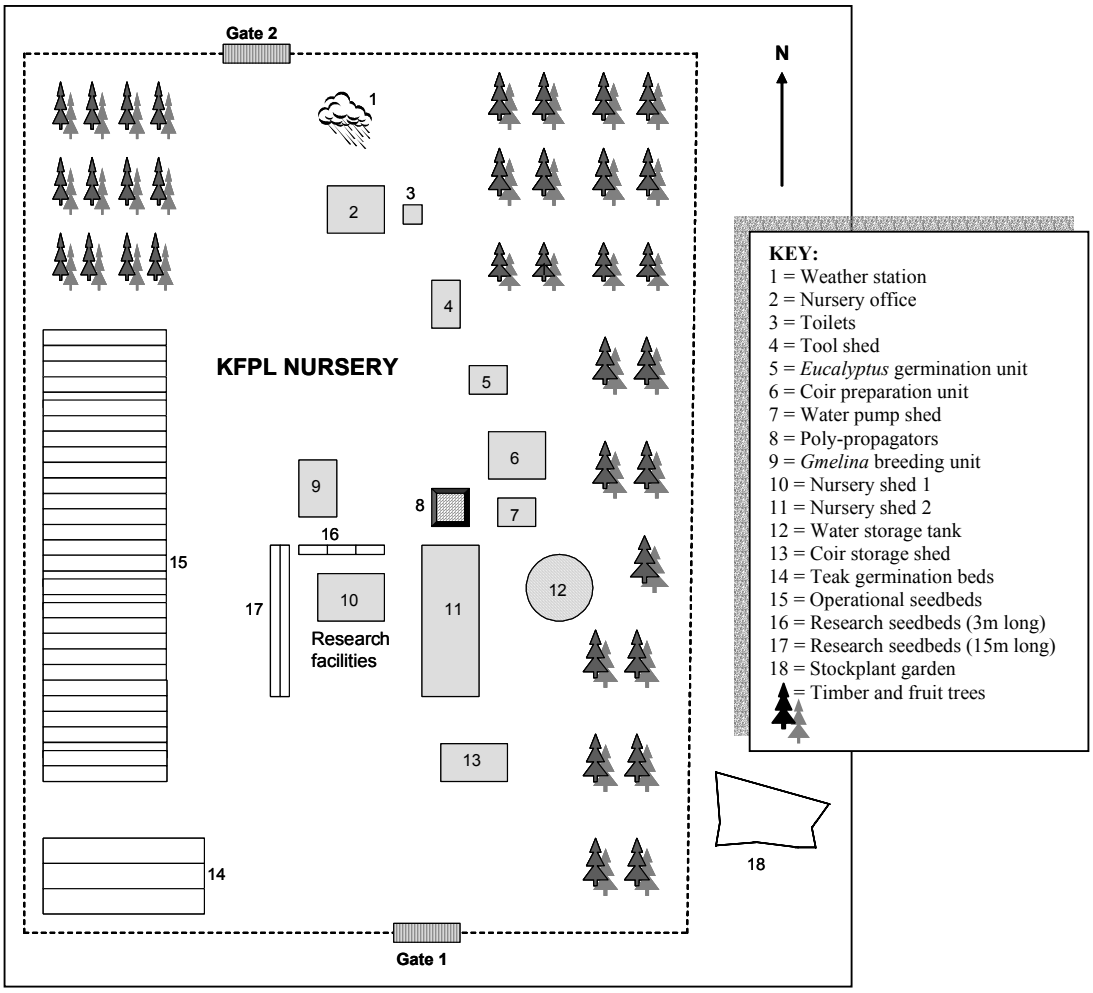


Fig 3.7: A sketch map of KFPL nursery at Ringgi. KFPL nursery facilities utilised exclusively for this study are number 10, 16 and 17. Facilities under sharing arrangement include number 1, 2, 3, 4, 6, 7, 11, 12 and 13. Number 8 and 18 are established by the author under this project.

3.2 GENERAL EXPERIMENTAL DETAILS

3.2.1 Plant material

3.2.1.1 Seeds and seedlings

For this thesis, over one hundred bare-rooted wildings (naturally germinated seedlings) of both *B. procera* and *I. fagifer* were collected from the villages of Tututi and Hunda. The wildings were collected from around selected trees which were also used in the studies to characterise morphological variation in fruits and

nuts. The planting materials were transported to KFPL nursery at Ringgi where they were planted out in lines at 1m x 1m spacing of different progenies to form a stockplant garden (Fig 3.8) for vegetative propagation studies (Chapter 6). As time was pressing, seedlings were planted when the weather was dry, and this reduced the survival rate to about 90%. Additional seeds and wildings of *I. fagifer* were brought in from Choiseul to supplement the collection in Kolombangara. The stockplant garden was established under the shade (approximately 70-75% light penetration) of scattered overstory mixed secondary forest species such as *Pometia pinnata*, *Cananga odorata* and *Macaranga* spp. in November 2002. Establishing a stockplant garden for both *B. procera* and *I. fagifer* and getting it ready to provide plant materials for the vegetative propagation studies involved waiting about six months. The routine management of this stockplant garden (section 3.2.2.1) was also heavily dependent on KFPL staff and workers because the author had to return to James Cook University.

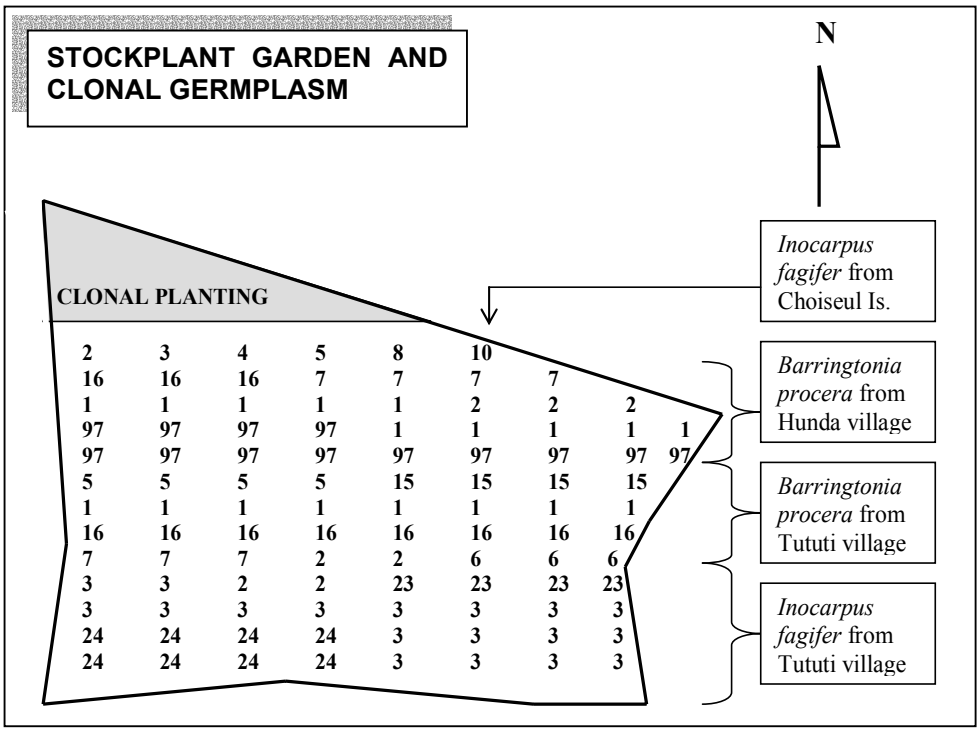


Fig 3.8: Sketch (not to scale) of a stockplant garden (0.3ha) of *B. procera* and *I. fagifer* at KFPL nursery in Ringgi, Kolombangara. Numbers represent parental trees of the seedlings. Over 200 seedlings at 1m x 1m spacing for stockplants and about 50 clones 5m x 3m in clonal planting.

Additional stockplants were developed from seeds. Fallen, ripe fruits of *B. procera* and *I. fagifer* were randomly collected from both villages as well. The seeds were sown in root trainers and/or in 1-2 litre polybags at Ringgi nursery, mainly for studies investigating fertiliser effects on the growth of seedlings at the nursery stage. Seedlings differed from wildings in that they appeared vigorous in form, partly because, while seedlings held their cotyledons, many of the wildings lost their cotyledons. The seedlings were transferred to a 5 litre polybag for experimentation as they began to develop roots.

3.2.1.2 Marcots

Marcotting or air layering is a technique that produces roots on the stem of plants, and is commonly used for difficult-to-root species from cuttings. It is a technique used to make a genetic copy of a desirable genotype from a mature tree. Several mature trees of both *B. procera* and *I. fagifer* in Ringgi, Tututi and Hunda were used for experiments to test the factors affecting the success of this technique (Chapter 6). Procedures involved in marcotting are described in Chapter 6 (section 6.3).

Successful marcots of both *B. procera* and *I. fagifer* were detached from their parent trees and potted in 5 litre black polythene bags in the KFPL nursery at Ringgi to provide propagules for later vegetative propagation studies. The marcots rooted earlier than anticipated, and occurred when the author had returned to James Cook University. So, collection of the marcots was done by KFPL staff and workers. This occurred later than was desirable and consequently there was about 90% mortality in both species, reducing the number of stockplants obtained from mature trees substantially.

3.2.2 Vegetative propagation

3.2.2.1 Stockplant management

Stockplants of *B. procera* and *I. fagifer* (Plate 3.2) were established as described in section 3.2.1.1 and used as the source of leafy stem cuttings for vegetative propagation experiments. During their initial establishment, the seedlings were

watered by hand using 15L knapsack sprayers when there was a period of prolonged drought. No inorganic fertilisers were given to the seedlings, and so their growth depended entirely on the soil fertility of the site. The soils of the site were classified as the Ringgi land system (section 3.1.2.5), thus of high fertility. The stockplants were maintained by regular weeding to remove invasive weeds such as *Merremia* and *Mikania* from climbing and suppressing the seedlings.

Plate 3.2: Two year old stockplants of *B. procera* (top) and *I. fagifer* (bottom) at Ringgi nursery. Cuttings collected after six months.

Seedling stockplants of both *B. procera* and *I. fagifer* were ready for the first harvest of cuttings within 6-12 months from initial planting. For *B. procera*, the seedlings were severed at 50-100mm above the ground, leaving 1-3 internodes

with or without leaves retained on the stump. These stumps re-sprouted within 2-3 weeks and a new crop of cuttings was ready within 12-16 weeks, although some seedlings grew faster than others. Overgrown (>10mm diameter) shoots were re-stumped at 5-10mm from the main stem to encourage regeneration of new shoots. In *I. fagifer*, the seedlings were managed as a hedge, by regularly pruning the branches, instead of stumping because they tended to produce multiple branches early. Initially, seedlings were trimmed back to a height of 200-500mm and side branches along the main stem were trimmed to encourage top shoot production. New shoots rapidly emerged from the axillary buds after severance and were generally ready for the next crop harvest after 4-6 weeks.

3.2.2.2 Propagation environment

A high humidity, non-mist, airtight, and watertight poly-propagator as described by Leakey *et al.* (1990), was constructed and used to raise cuttings. Enclosed in a polythene covered frame, the poly-propagator was made of successive layers of sand, small pebbles, stones and topped with a rooting media. Its timber frame was covered with a clear plastic (Plate 3.3). The propagator was divided into 5 equal compartments. The lower layers of the propagator were saturated up to the base of the rooting medium by manually pouring water in through a 30cm piece of cut PVC pipe in each of the growth compartments. The water in each of the growth compartments keeps the medium above it damp by capillary action and this moisture maintains the humidity inside the propagator. The air temperature inside the propagator ranged between 25 – 32^{o}C (daytime) and 19 - 22^{o}C (at night). Two poly-propagators used in this study were placed adjacent to each other under a mature guava (*Guava javanica*) tree for some shade to reduce air temperature, thus increasing relative humidity and reducing vapour pressure deficit inside the poly-propagator. In addition, these conditions were maintained by keeping the lid closed except when absolutely necessary. As a precautionary measure, the propagator was disinfected occasionally with Benlate ® at a rate of 5g per 15L water. Similarly, to avoid infection the rooting media (coir) was replaced after the termination of each experiment.

Unless otherwise stated, coir alone was used as the medium for rooting cuttings. Coir is decomposted coconut husk obtained from rural communities and has excellent physical properties. It is light and easy to handle with good water retention capacity, and holds the roots together very well. Coir has a neutral pH and is inert. The coir used in the experiments was sterilised following KFPL's standard commercial nursery practices: i.e. heated in 200L batches to 100^{o}C for 30-45 minutes and then left over night to cool down before use. During heating, the coir was stirred and turned over thoroughly 4-5 times.

Plate 3.3: High humidity, watertight and airtight poly-propagator.

(a) Frame. Shade cloth in background surrounds the propagator when established, (b) stone layers, which was then covered with water to form the water table, (c) range of different media on top of the water table, (d) propagation filled with cuttings. Spray gun used to keep leaves damp when lid is open. Lid closed to maintain airtightness.

3.2.2.3 Cutting production and preparation

Cuttings were produced from a stockplant garden (section 3.2.1.1) established at KFPL nursery in Ringgi. The first cuttings were ready for harvest within 6-12 months from initial planting. At this age, under good growing conditions, the stockplants of *B. procera* had attained 0.8-1.5m in height and produced shoots with 4-6 nodes. Stem diameters were 10-15mm and internode lengths of 2-15cm. Subsequently, after the first harvest of cuttings it took about 12-16 weeks for the next batch of shoots to be ready for the second harvest of cuttings. Typically 2-6 shoots were produced from individual stumps, and they were harvested when they were about 40-50cm in height, with 2-3 fully elongated internodes. By contrast, at this age the seedlings of *I. fagifer* had attained 0.5-1.5m in height and produced 6-9 single-node cuttings with diameters varying from 2-5mm and lengths of 2-10cm. A cropping cycle for *I. fagifer* cuttings of about 4-6 weeks was attained during the growing season. Leaving new shoots more than this time resulted in increased lignification which was considered unfavourable for rooting cuttings.

Single-node cuttings were collected in the morning or late afternoon when it was cool. Only shoots that were healthy and pest-free were collected. Shoots were severed with a clean cut using a sharp knife or hand pruners. Typically, there are two leaves per node. Unless otherwise stated, only one leaf was retained per node, which was trimmed to about half its length on the field to minimise water loss and reduce physiological stress. As the cuttings are being harvested at a distance from the nursery, a cool box and buckets containing some water was used to store them. Thus, the whole shoot was kept intact until arrival at the nursery. The shoots from each plant/clone were kept separate and their correct identity labelled inside and outside the plastic bag.

In the nursery, the shoots were sorted into groups in accordance with the experimental design. Shoots were then cut into single-node, leafy, stem cuttings using a surgical blade held by a removable handle. Cuttings were kept in node order, from the apex to the base. This routine practice avoided committing unnecessary errors and quickened the insertion of cuttings. Unless otherwise stated, the leaf lamina of all cuttings were trimmed to 50 cm^2, using a template

prepared by drawing around specimen leaf of the species. Cuttings were dipped into 0.8% IBA commercial rooting powder, Rotex ® manufactured by Bass Laboratories P/L, 9 Waldheim Rd, Bayswater, Vic 3153, before being placed in the poly-propagator according to the experimental design. The cuttings were then inspected daily for occurrences of pest and diseases but also to remove those that were dead. Water level inside each growth compartment of the propagator was also checked and refilled as required.

3.2.2.4 Assessment of rooting

Unless otherwise stated, rooting was assessed at intervals of about seven days. Key parameters measured include: the number of cuttings rooted, the number of roots (greater than 1 mm long) per rooted cutting, the length of roots on each cutting. The date of rooting or cutting death was recorded. Root length was measured using a metric ruler. Rooted cuttings were potted immediately after assessment and removed from the experiment.

3.2.3 Identification of trees for fruit and nut characterisation study

Identifying trees to use for this thesis depended on information from farmers and the author's observation on what represents a good tree. According to the farmers, a good tree produces a lot of fruits, and kernels that are sweet and without defects. Thus, tree yield and the taste and form of the kernel were the primary selection criteria. In addition to these criteria, trees are also identified for the study if they were in any way unique (e.g. in height/dwarfness, colour of the fruit or flower and the ease of removing the shell) and accessible. Some mature trees identified by farmers were not fruiting when this exercise took place, and the author had to rely on the farmers' knowledge of his/her trees. Some difficulty was experienced in differentiating between *B. procera* and *B. edulis*, which are phenotypically similar with overlapping intra-specific variation. Although the author was extremely careful to identify *B. procera*, it cannot be guaranteed that misidentification did not occur. To check on this, leaf samples were collected for molecular studies

using “amplified fragmented length polymorphism-polymerase chain reaction” (AFLP-PCR) technique (Chapter 8).

A total of one hundred and nineteen trees were selected for the fruit characterisation study. This was done by walking with the farmer and tagging the trees that met the selection criteria. As tags attract vandals, the tagged trees were later re-labelled using paint, to ensure correct identification of the trees in future visits. The location of these trees and the prevailing land use system was recorded on a field sheet. Changes in land use were also recorded. When insufficient numbers of trees were found on one farm, the survey was extended to neighbouring plots to form a contiguous population. Trees from each site (village) were identified as a population regardless of whether or not they had been planted by the farmer or had regenerated naturally.

The height and diameter of each tree was measured using a clinometer (SUNNTO TANDEM, 1380A TANDEM-360PC/360R) and a diameter tape. Farmers gave information about the age of each tree. The trees were visited 2 times per month to observe flowering and fruiting phenology. This process was interrupted by the author having to return to James Cook University. During the fruiting season, fruits of these marked trees were collected for morphological characterisation of variation. The leaves and the cambium were collected for molecular analysis of genetic variation.

3.2.4 Statistical analysis

3.2.4.1 Participatory survey

The survey data consisted mainly of a farmer’s response to the questions they were asked. Thus the data is discrete involving ranking variables, scoring attributes and calculating percentages of respondents responding to given variables. Data was recorded using Microsoft Excel 2003 spreadsheets. Differences in the responses in terms of percentage were tested for significance using Moore (2004) significance tests for comparing proportions and the standard error of the proportion calculated as:

$$SE = \sqrt{\frac{\hat{p}(1-\hat{p}\ )}{n}}$$

Where $\hat{p}$ = sample proportion of successes

n = sample size

This method is simply a count of successes in both samples combined divided by counts of observation in both samples combined – this allows comparison between choices in priotization and the determination of whether or not the percentage difference between the two choices is significant.

3.2.4.2 Vegetative propagation

Rooting data were subjected to analyses of variance (ANOVA) on mean root numbers, and results with significant differences between treatment means were subjected to the Least Significant Difference (LSD) test (or Fisher's LSD test) using Minitab 13.1. Analysis of percentage rooting and dead cuttings were carried out using significance test for comparing two proportions, and the standard errors (SE) of proportion calculated as described by Moore (2004). This method is simply a count of successes in both samples combined divided by counts of observation in both samples combined – this allows comparison between treatments and the determination of whether or not the percentage difference is significant. Interactions between treatment factors were included in the ANOVA, followed by regression tests where appropriate to determine the influence of different variables on rooting ability. Correlation between two variables was determined using Pearson's product-moment correlation using Minitab 13.1. Where distribution was not normal, the rooting data were square root transformed in Minitab 13.1. Unless otherwise stated, the statistical significance is given at the 5% level (P<0.05).

3.2.4.3 Phenotypic characterisation

A Kolmogorov-Smirnov (K-S) normality test was undertaken to evaluate each set of character measurements for normal distribution. This test was done for all dataset including data collected for organoleptic analysis. Principal Component Analysis (PCA) was carried out using mean of nine variables to determine the proportion of total variation represented by each tested trait. One way analysis of variance was used to determine significant differences of traits within and between populations, and where significant differences occur, the data was subjected to the Least Significant Difference (LSD) test (or Fisher's LSD test) using Minitab 13.1, for pair comparisons to identify variables that causes significance in the result. Linear associations between variables were tested using Pearson's product-moment correlation, and regression analysis undertaken to determine the relationships.

3.2.4.4 Genetic characterisation using molecular techniques

Procedures involved in the analysis of binary data matrix generated from AFLP molecular marker used in this study is discussed in Chapter 8.

3.3 TIME ALLOCATION TO OVERSEAS FIELDWORK

While registered at JCU, Cairns Campus, the fieldwork component of this study was undertaken abroad, 26.5 months were spent in Australia and 15.5 months spent in Solomon Islands (Fig 3.9). Field work in Solomon Islands was implemented during 4 visits to Kolombangara Island (October – December 2002; May – October 2003; January – June 2004; December 2005). When the author was away from the study sites, all routine field works in progress were overseen by staff and workers of KFPL (see Acknowledgement). These prolonged absences from the field site made it difficult to follow-up some experiments and restricted some activities to particular seasons.

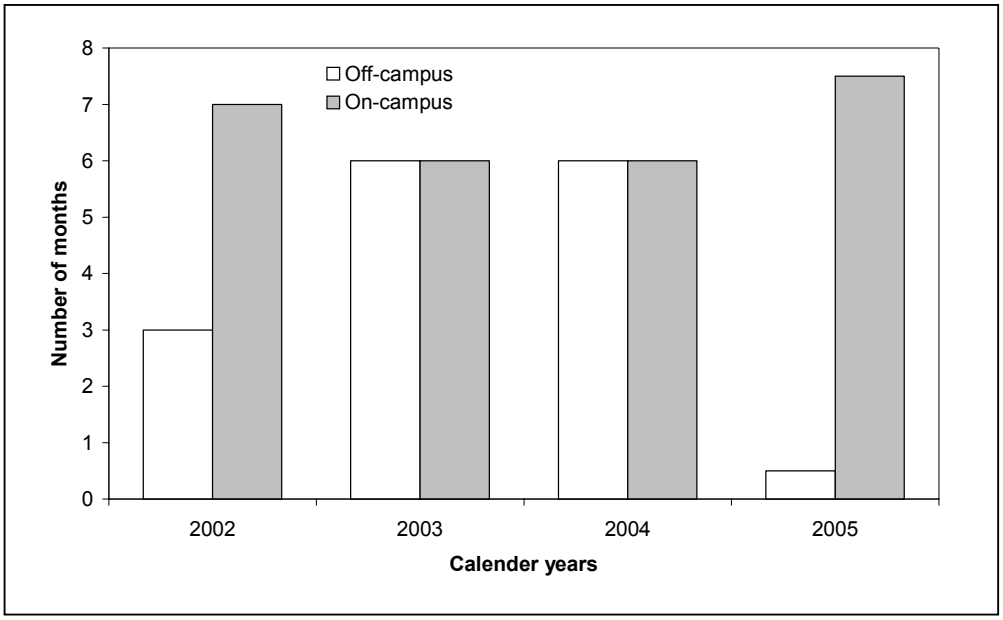


Fig 3.9: Time (month) allocation for on-campus and off-campus activities.

This thesis commenced in March 2002 and ends in August 2005.

CHAPTER 4: PARTICIPATORY PRIORITY SETTING

4.1 INTRODUCTION

4.1.1 Significance of participatory priority setting

Tree domestication process begins with the correct identification of the species to be involved (Leakey and Newton 1994a). The identification of a priority species is a process that involves the incorporation of indigenous knowledge and perspectives (Walker *et al.,* 1995). In the Melanesian context, indigenous knowledge forms the basis of identity, and is inextricably linked to the relationship between people, their land and the environment (Jansen and Tutua 2001). This means knowledge about land, its resources and the surrounding environment can be best acquired through the involvement of indigenous people themselves.

Rocheleau (1987) validated this concept when she pointed out that the development of effective external interventions could be best achieved "*once we know what they already know, and what else might be most useful to add to their store of knowledge and tools.*" Furthermore, Rocheleau (1987) recognised the important contribution by farmers and stated that, "a*groforestry has not entered the tropical research and development scene in a vacuum, but rather in a historical context that includes a wealth of recent experience and accumulated knowledge in several related fields such as agriculture, forestry, watershed management and rural development programs,*" implying the need to recognise farmers' role, needs and perspectives in priority setting. Other stakeholders involved with farmers such as relevant entities within the government and non-government organizations must also be consulted and involved, and the incorporation of their needs, experiences and contributions in priority setting is also crucial to the success of any tree domestication initiative. In addition, Franzel *et al.,* (1996) reported that rather than commencing with an inventory of research

alternatives, the procedure should begin with an inventory of the clientele of the research.

In the past, researchers often identified priority species based on scientific criteria and would ignore the needs of the farmers. Perhaps, this attitude arose from perceptions of colonial masters with negative views towards indigenous people, that their knowledge and ideas were superior. This top-down approach often led to suboptimal utilisation of natural resources and poor adoption of new technologies because it was done without the farmers being involved (Kadzere *et al.,* 1998). With increased awareness of the benefits of participatory rural appraisal techniques, agroforestry researchers now typically determine the priorities of the farmers, choosing species on the basis of farmers' interest in the products and service functions of familiar indigenous trees (Jaenicke *et al.,* 1995; Franzel *et al.,* 1996). Kadzere *et al.,* (1998) reported that farmers' input and technical knowledge is crucial to: -

a) Benefit from knowledge of the uses, derived products and market potentials of the species and their products
b) Achieve farmers' acceptance of the chosen species to plant, and so ease adoption of new technology developed to minimise constraints affecting fruit production at farmers' level.
c) Accelerate the process of tree domestication, especially with the initial documentation of the traditional values of the indigenous species.

Participatory household surveys are therefore, highly desirable for the determination of the priorities of a tree domestication program, involving new agrotechnologies (e.g. propagation techniques, genetic improvements and designing agroforestry practices) that meet the diversity of farmers' needs, including the provision of a sustainable food supply and the generation of income.

Participatory approaches to selecting priority species can also involve other forms of decision-making, such as review of relevant literature as well as conducting surveys and field studies (John and Brouard 1995; Walker *et al.,* 1995). However,

depending on the scale, the approach is often a lengthy and costly process (Sinclair and Walker 1999) because it involves surveying of villages for a fuller understanding of both peoples' socio-economic conditions and the environment within which they live. Whichever approach is taken, it has been suggested (Rocheleau 1987; Leakey and Newton 1994a; Jaenicke *et al.,* 1995; Ngungu *et al.,* 1995) that selection of priority species should involve an assessment of: -

a) Species that have the potential for improving farmers' livelihoods and that can be integrated into some form of agroforestry practices.
b) Whether or not the species is easy to handle, establish and maintain in the farmland.
c) Farmers' preferences, needs and interest in the species, its potential for tree improvement work and present and future socio-economic prospects and values.
d) Whether or not the species has the ability to provide services as a secondary function to the main products.
e) Whether or not the species has the ability to tolerate extreme growth conditions such as arid and humid environments, poor soil fertility, salinity, heavy clay and waterlogged soils and exposed coastal areas.
f) Which species to retain and which to ignore through a process of elimination of species with least potential to improve livelihoods of the people based on the data and information generated from the survey, literature review or experimentation.

The above suggestions were strongly supported by concerted efforts of ICRAF and the International Service for National Agriculture Research (ISNAR) through the development of Guidelines for "Selecting the Right Species" of multipurpose tree (MPT) species for improvement purposes in West Africa (Franzel *et al.,* 1996). The approach is principally multidisciplinary combining traditional knowledge of the farmers and the expertise of the scientists and knowledge of local markets.

These Guidelines developed in Humid West Africa have been used as the starting point of domestication activities throughout the tropics, particularly in the

Peruvian Amazonian Basin (Weber *et al.,* 1997) and Southern Africa (Maghembe *et al.,* 1995; Boffa 1999; Roshetko and Evans 1999). The application of this approach has not been formally tried in the Pacific Region, but is similar to traditional decision-making. Traditionally, indigenous people discuss and debate before agreeing to implement important village activities (e.g. Meaneaba system of Kiribati and Pulenu systems of Polynesia) (Tofinga 1996) and many other systems in Melanesia (e.g. Turituri kazi in Choiseul, Solomon Islands). This traditional approach differs from the modern guidelines only in its lack of formal analytical techniques used at the different stages in the decision making process.

4.1.2 Pre-priority setting considerations

The manner in which research priorities are set is crucial in the decision making process to choose a priority species. Four types of priority setting methods have been described by Collion *et al.* (1993), including checklists, scoring, cost-benefit analysis and mathematical programming. Checklists involve checking of alternatives against set criteria, while in scoring method, given scores in research priorities are weighted against set criteria and then ranked in order of priority. Cost-benefit analysis is based on comparison of the cost and return of investment, and the mathematical programming involves modelling to choose the optimal research priority. Of the four approaches, scoring method is widely used because it is simple, time-saving and very appropriate in dealing with differently broad types of objectives (e.g. economic impacts, equity and conservation of resources), although this method is prone to misuse, especially when criteria are overlapping (Jaenicke *et al.,* 1995). Consequently, for the decision process to be effective, one must understand the user needs and preferences, technological opportunities and systematic methods of ranking species (Fig 4.1) (Raintree 1991; Jaenicke *et al.,* 1995).

The approach developed by ICRAF/ISNAR represents a great advance in conducting priority setting and involves seven steps which consecutively reduce the number of species under consideration. The steps are: (i) team building and planning, (ii) assessment of clients need, (iii) assessment of species used by clients, (iv) ranking of products, (v) identification of priority species, (vi) valuation

and ranking of priority species and (vii) final choice. However, prior to these steps, important questions (Franzel *et al.,* 1996) such as those indicated below must be satisfied:

a) What is the objective of the domestication program?
b) Who are target clients?
c) What activities and opportunities are involved; magnitude and intensity?
d) What sources of information are available?
e) What are the expected benefits to be achieved?

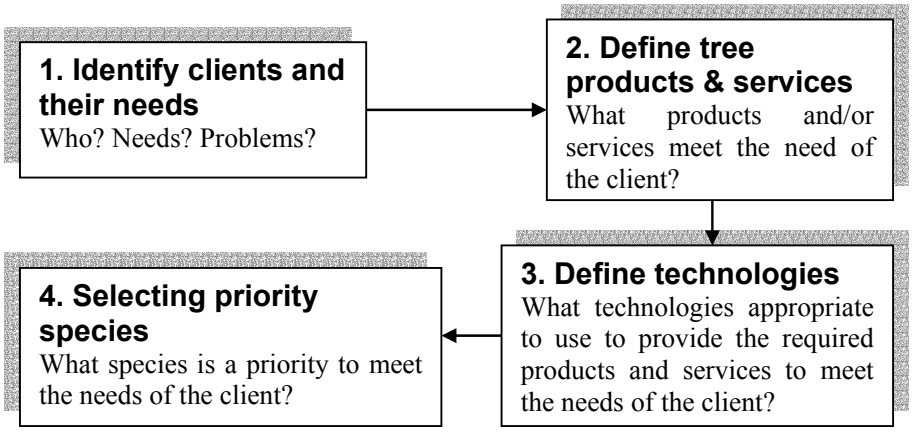


Fig 4.1: Decision making process in priority setting.
Source: Modified from Raintree (1991).

Thoughtful answers to these questions are crucial to the success of a tree domestication program. Achieving this partly involves the farmers who have intimate experience of their production practices (Sinclair and Walter 1999), but it is also important to remember that the research priorities could be considerably overshadowed by the lack of well-defined objectives of the domestication program (Franzel *et al.,* 1996). Thus, it is imperative to determine what direction is desired for the research in order to focus the research itself. For example, agroforestry in the past has focussed on timber trees and soil fertility enhancing leguminous trees. The improvement of these species was typically similar to that for pure forestry,

that is, provenance, progeny selection and the tree breeding. This is still a new aspect of agroforestry but the early results support that the adoption and impacts will be considerable (Tchoundjeu *et al.,* 2006). With the change to species for indigenous non-timber products (e.g. food, fodder, fuelwood, medicines and construction materials) and services (e.g. amelioration of soil fertility and shade/shelter), Leakey and Newton (1994) suggested that a more horticultural approach was relevant. Thus, ascertaining desirable goals (e.g. economic growth, income distribution, gender or increasing the value of products and services from local trees is essential to form the basis of the domestication objectives.

It is also important to consider the type of products and services a tree provides. These vary between species and occur either naturally or as a result of artificial processing (Franzel *et al.,* 1996). To evaluate the product and service, they can be scored against a set of criteria driven by economic as well as ecological benefits (Leakey and Newton 1994a). Service benefits of trees, although generally difficult to assess, may be gauged against criteria (Franzel *et al.,* 1996), for example, in the case of soil nutrient enrichment the opportunity cost of fertilizer could be calculated. In more difficult cases, the values can be estimated using proxies based on comparison with other relevant species. Thus, the expected benefits from an improvement program can be expressed as:

Value of expected benefits from improvement	=	Annual value of products per year	x	% increase in value expected from improvement	x	% expected adoption	x	Other non-financial factors (modifiers)
		(1)		(2)		(3)		(4)

The benefits are a function of (1), (2), (3) and (4) and may be expressed as a factor greater than 1 for beneficial factors and less than 1 for detrimental ones (Franzel *et al.,* 1996).

A potential complication in the evaluation of priorities is associated with benefit sharing between the farmers and the society at large (Franzel *et al.,* 1996). For instance, farmers may not value certain improvements because it takes time to realise the benefits (e.g. nutrient cycling from deeper soil or improved soil water

retention). Or they may have no interest in it at all (e.g. a cocoa farmer may choose not to support research on a new shade species, if he/she feels very strongly about the benefits he/she has derived from current practice). This can have negative implications to the priority setting process. The need to strike a balance between benefits to different groups of farmers (private perspective) and researchers (societal perspective) is thus a paramount consideration (Franzel *et al.,* 1996).

To prioritise the selection of species for this study in Kolombangara, Solomon Islands, the specific objectives were:

a) To gather traditional knowledge on locally-important indigenous fruit tree species, and on their biological and socio-economic attributes that could be of value to farmers, and that could be enhanced by domestication and cultivation in agroforestry practices.

b) To identify the top priority indigenous fruit tree species that are traditionally utilized by local people of Kolombangara Island and which have the potential to contribute to enhancing their livelihoods through agroforestry.

c) To determine the traditional agroforestry practices used by rural communities on Kolombangara Island, and identify areas requiring improvements and suggest ways in which agroforestry could improve farming systems on the Island.

4.2 MATERIALS AND METHODS

4.2.1 Sites and sample size

Five sites were identified in Kolombangara Island:- Ringgi, Seusepe, Rei, Poporo and Hunda (Chapter 3) for the farmers' participatory survey. These sites were selected on the basis of five criteria as described in Chapter 3. Originally, thirty households were assigned per site, however, the actual proportion of households

surveyed on each study site varied (Fig 4.2), the differences being due to the number of people who were available to be interviewed. Variation in the turn out of people for the interview across sites did not indicate their disapproval of the research but rather it was because of their time being committed to do other things at that time. This was evidenced in the high turn up for the public meeting that was conducted earlier. The final outcome was that 15-57 households were surveyed per study site. A total of one hundred and fifty-five households were interviewed.

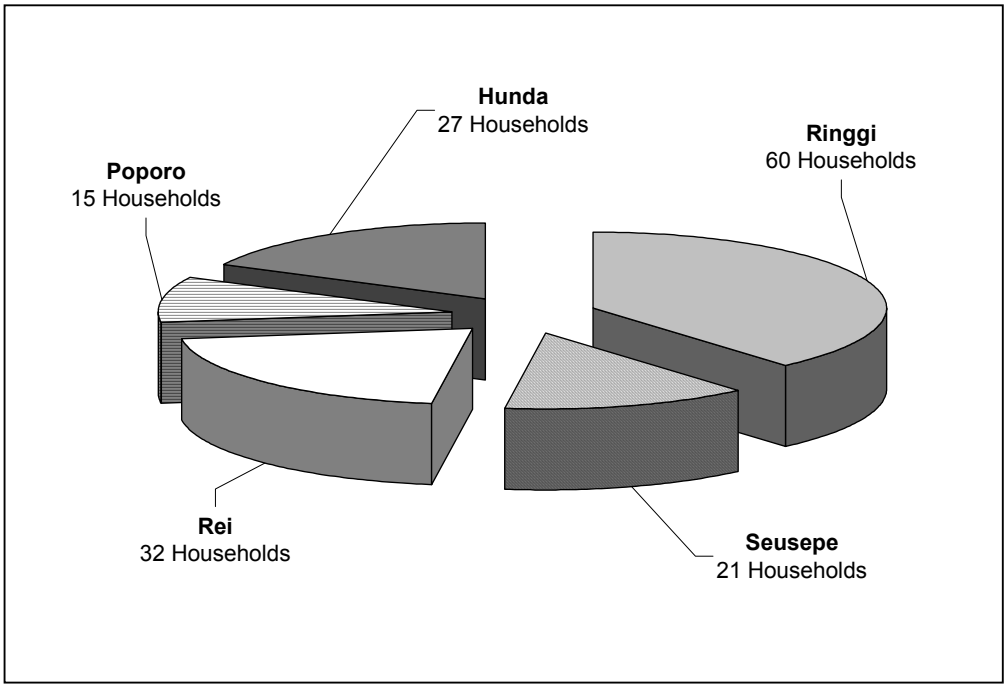


Fig 4.2: Number of Households surveyed in 2002 at each study site on Kolombangara, Solomon Islands.

4.2.2 Access to plant resources

Under customary ownership, tree planting or access to wild trees is a right of the tribe, clan and family. It is not normally an individual entitlement, thus consent has to be sought from other members of the tribe or clan should an individual wish to undertake commercially orientated activities, for example, tree (timber and fruit) planting or natural logging, as well as non-commercial research projects. Planting for domestic use does not normally require tribal consent if is to be done within

existing allocated land. However, if the land in question is a virgin forest land of the tribe, then a tribal consent must be sought. At present, there is no formal legislation that restricts the harvest of fruits and nuts in the wild. However, there is a Forestry Law (1999) that governs timber extraction in which operators must have a permit before harvesting timber trees, especially the harvesting of endangered forest species with traditional value (e.g. *Gmelina mollucana*).

4.2.3 Data collection

4.2.3.1 Public community meeting

At each study site, a public community meeting preceded the farmers' participatory survey. The public meeting was organised in consultation with the village chiefs and elders and was aimed at bringing the people and the author together to talk about issues pertaining to the research project. Specific agenda for such meetings included an overview of the research project highlighting the field components that would involve the people and their plant resources. Explicit explanation was also made about the objectives and the content including the expected outcomes of the survey. It was unequivocally emphasized at the meeting that the integrity and success of the survey would be dependent on the peoples' participation and the accuracy of information they provided. Accuracy is extremely important in a priority setting exercise. The meetings also provided an opportunity for the people to express their views in respect of the research project. The community in each village made the decision willingly to permit the author to conduct research at their village and to use their plant resources.

In addition to public community meetings, a number of consultative meetings were also held with those responsible for agriculture and forestry in both the national and the provincial governments as well as certain non-government organizations, such as the Kastom Gaden Association (KGA), Honiara Susup Gaden (HSG), the Development Bank of Solomon Islands (DBSI), Commodity Export Marketing Authority (CEMA) and the Kolombangara Forests Products Limited (KFPL), who were all engaged in activities to help rural farmers.

During the surveys, visits were made to rural home gardens to observe the types of agricultural practices being adopted by the farmers. During farm visits, the author independently took note of the types of crops found in the gardens. This is used to complement information collected during the survey.

4.2.3.2 Farmers participatory survey

The survey was conducted in October 2002 using pre-prepared and structured questionnaires[1], designed to serve two primary purposes:

a) To generate information on traditional knowledge on the phenology, utilisation, management and marketing of the popular indigenous fruit and nut species, as well as gathering information on the socio-economic status of the people. The information was used to identify two top priority fruit and nut species that people would like to see domesticated (Appendix 4.1).

b) To generate information related to farmers' traditional agricultural activities, such as the main staple food crop grown in their home gardens, length of fallow period, number of cropping cycles before land is left to fallow, major sources of cash income and the ways in which soil fertility is traditionally maintained (Appendix 4.1).

Members of farm households were interviewed at random as groups or individuals and data collected by age class and gender group based on who was available. One-to-one interviews were favoured when interviewing females to avoid male dominance. However, where cultural barriers exist group interviews were used for convenience and comfort of the interviewees. All interviews and discussions were conducted in Pidgin English, the common language of Solomon Islands. For correct identification and accurate understanding of the species, scientific names of most fruit and nut species used during the survey were translated into Nduke (Kolombangara vernacular) prior to the survey.

[1] Developed using information from Allen *et al* (1994), Maghembe *et al* (1998), Bourke (1999) and Lepping (2000)

4.2.4 Data Analysis

Data collected were sorted and analysed in Excel 2000, by scoring and ranking of farmers' responses from the survey. See Chapter 3 for details.

The intensity of land use, which is a powerful index of land pressure as it combines the fallow period and cropping cycle (Ruthenberg 1980) was calculated and is expressed as R value using the following formula:

$$R = c \times \frac{100}{(c + f)}$$

Where R = Intensity of land use (land pressure)
c = cropping cycle (years)
f = fallow period

The value of R can range from 1 to 100 (Table 4.1) and a value of 1 means that if the cropping systems were unaltered, the land would be used for only 1 year in every 100 years. A value of 100 shows continuos cropping. The R-value calculated here gives a temporal measure of land use intensity but not a spatial measure, and only serves as a measure of intensity of current land in use.

Table 4.1: The range of R values

R-value range	*Land use intensity*
1-9	Very low
10-32	Low
33-66	Medium
67-100	High

4.3 RESULTS

The responses to the questionnaires provided evidence of the depth of farmers' knowledge in Kolombangara Island about:

a) Social structure of rural communities
b) Socio-economic conditions in rural communities
c) Traditional agricultural practices
d) Indigenous fruit and nut species

4.3.1 Social structure of rural communities

About 80% of households interviewed during the survey lived in coastal villages. Each village has a community of people that have some form of common identity and some families are genealogically related in some way. Discussion with the communities suggested that blood relationships and common rights to customary land are the two main features distinguishing a community. Thus, people establish villages on land they inherit from their forefathers and which have passed from one generation to another. It is rare to purchase customary land to establish new settlements.

The impact of religion (Christianity) was also observed to be strong among the people, as community tends to represent a single denomination. Intermarriage was observed to have had a significant impact on community structure resulting in a population of mixed and different ethnic origins in most villages. Each community has tribal chiefs and a paramount chief, religious leaders and village elders take charge of different functions, so ensuring peace and harmony in their respective villages. Government workers especially teachers and medical nurses were present in the main villages of Ringgi, Poporo and Hunda. The Royal Solomon Islands Police were stationed at Ringgi.

The people surveyed from 155 households were 59% male and 41% female. For the purpose of analysis, the communities were divided in five age classes (10-19, 20-29, 30-39, 40-49 and 50+) (Fig 4.3). The majority of the interviewees came from age class 30-39, representing 17% male and 14% female. Interestingly, more girls than boys turned up for the interview in the 10-19 age class. By contrast, men were especially dominant in age classes over 40 years.

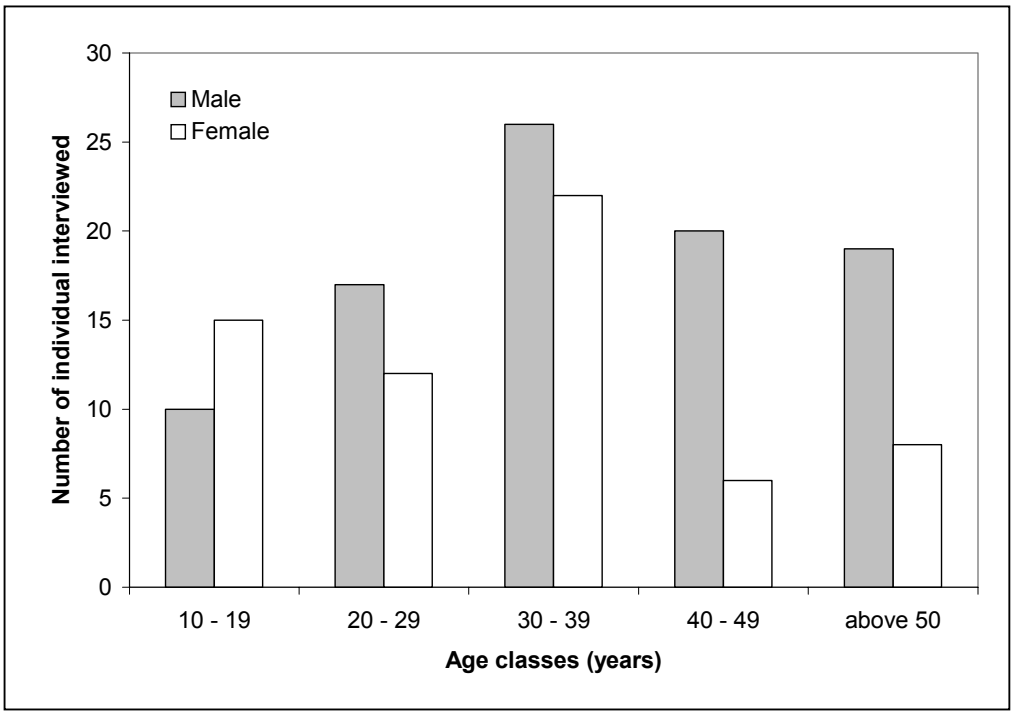


Fig 4.3: Comparative proportion of different age groups participated in the Farmers Participatory Survey conducted in 2002 in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara Island.

4.3.2 Socio-economic conditions in rural communities

Two socio-economic issues are highly relevant to this study of the rural people on Kolombangara Island. They are: "Household food security" and "Income generating activities," which are keys to the livelihoods of farmers and their interest in the domestication of indigenous fruit and nut species.

4.3.2.1 Household food security

Generally, people were satisfied with the level of food supply they had for household consumption. Having said this, however, 95% of farmers interviewed indicated that they occasionally experienced food shortages during the year. These shortages were attributed to a number of different reasons (Fig 4.4), of which pests and diseases and labour shortages were the most common - together accounted for more than 50% of the shortages.

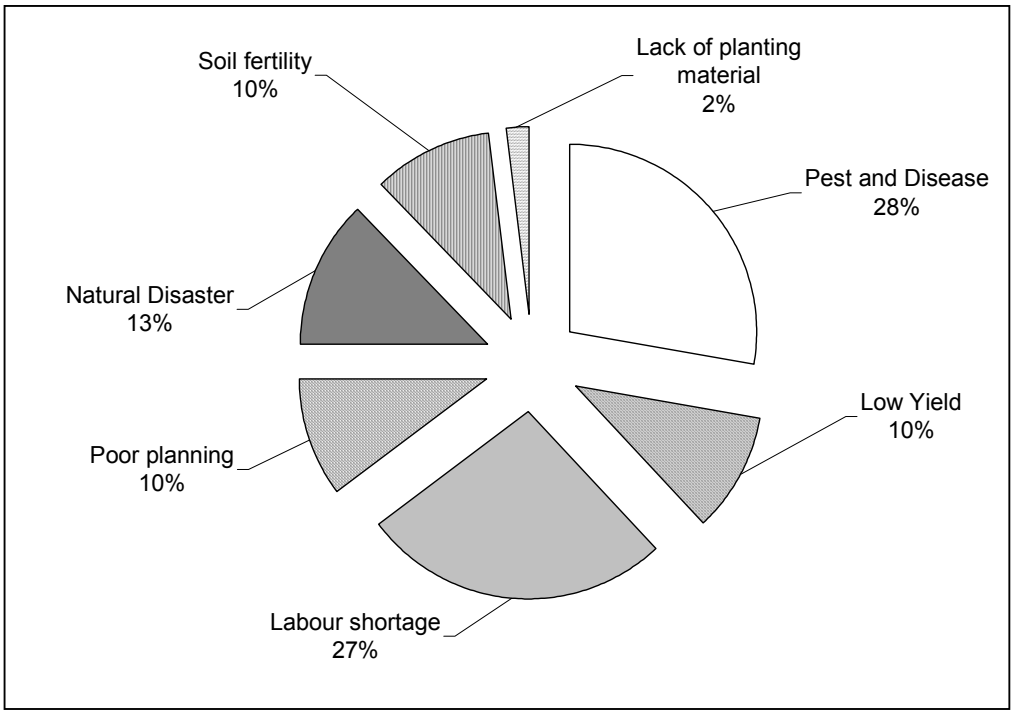


Fig 4.4: Reasons given for occasional food shortage from farmers' survey (2002) in 5 sites (Rinngi, Seusepe, Rei, Poporo, Hunda) on Kolombangara Island.

Labour shortages were particularly a problem for farmers who had leadership roles in their community or who had more or less continuous employment with KFPL. Only 2% of farmers indicated that the shortage of planting material was a contributing factor to the occasional food shortage, with poor planning accounting 10%. Natural disasters in the form of prolonged dry and wet spells account for 13% of farmers with food shortages. Occasional food shortages can be devastating but farmers indicated that they have measures to overcome them (Fig 4.5). For example, 30% of farmers indicated that at these times they return to their old gardens to harvest crops that have been abandoned in the fallows (e.g. cassava and banana) to support their livelihoods. These difficult times also prompt some farmers to clear bush for new gardens and to plant more food crops.

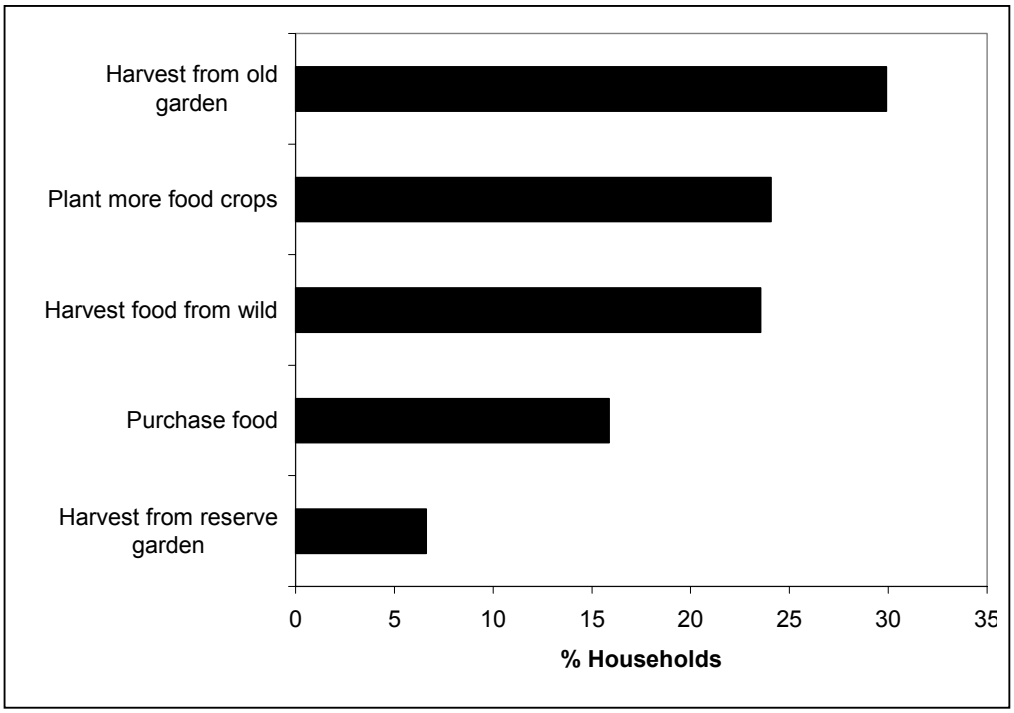


Fig 4.5: Immediate rescue measures undertaken by farmers during occasional food shortage in rural communities on Kolombangara Islands (from farmers' survey (2002) in 5 sites – Rinngi, Seusepe, Rei, Poporo, Hunda).

During food shortages, 25% of farmers resorted to wild foods (e.g. wild yam), while 16% depended largely on purchased food. Less than 10% of farmers claimed to have a reserve garden to use when their normal food supply was inadequate. These reserve gardens were mainly planted with swamp taro (*Cyrtosperma chamissonis* (Schott) Merr.).

In addition to the above emergency measures, all farmers indicated that they also supplemented their family diets with some indigenous fruit and nut trees. Ten indigenous fruit and nut species were identified as being commonly used (Fig 4.6). *Barringtonia* species (*Barringtonia procera*, *B. edulis*, *B. novae-hiberniae*) were most important followed by *Canarium indicum* and *C. salomonense*. However, other species were also important in supporting the livelihoods of the people. For example, *Gnetum gnemon* provides nuts and young leaves as vegetables in the household diet.

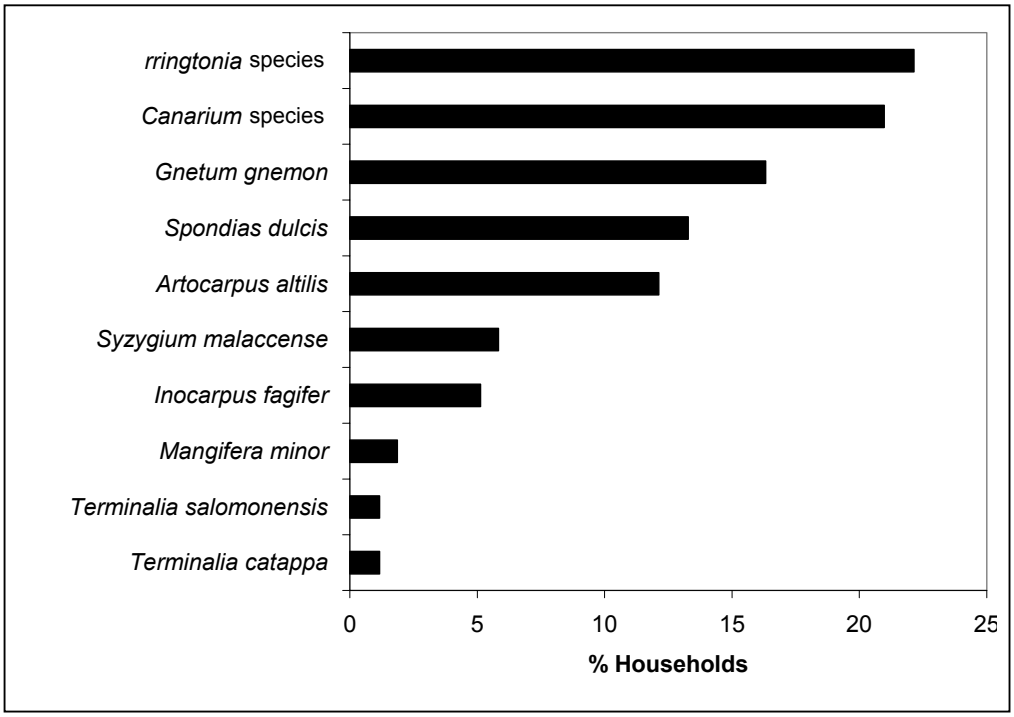


Fig 4.6: Some indigenous fruit and nut species used by farmers during occasional food shortage in rural communities on Kolombangara Island (from farmers' survey (2002) in 5 sites – Ringgi, Seusepe, Rei, Poporo, Hunda).

4.3.2.2 Household cash generating activities

Major cash generating activities in Kolombangara Island are farming (mainly the sale of root crops and vegetables), fishing, contractual work and employment with KFPL (Fig 4.7). The extent to which people are involved in each of these activities varied from one study site to another. However, in all sites, farming was the major income generating activity (33% - 49%) of the people interviewed. Second was fishing for people living within the segment of the island under customary management (e.g. Poporo and Hunda), but contractual work for those living within or adjacent to KFPL estate (e.g. Seusepe and Rei). The fifth site, Ringgi, spans villages both within and adjacent to KFPL estate as well as within the customary ownership segment. Despite the proximity with KFPL, fishing was the second major source of income, probably because at the time of the survey, KFPL operations were in the north of the island and those in the south are less involved in contractual work.

Cash income is also generated from selling fruits and nuts of both exotic and indigenous species to local markets as well as markets at Gizo and Noro in the neighbouring islands within the New Georgia group of islands. In all sites, income derived from exotic fruits and nuts was higher than income obtained from indigenous fruits and nuts (Fig 4.8). Exotic fruit and nut species commonly sold in local markets include: coconut, avocado, banana, citrus (sweet orange, mandarin, lime), guava, papaya, jackfruit and soursop. By contrast, the indigenous fruit and nut species commonly traded include: *B. procera*, *C. indicum*, *C. salomonense*, *S. malaccensis*, *M. minor*, *I. fagifer*, *S. dulcis*, *G. gnemon* and *A. altilis*. Income generated from indigenous fruits and nuts is low at sites within or adjacent to KFPL estate as compared to sites located within the customary segment (Fig 4.8). Nevertheless, farmers and their households are very dependent on indigenous species for traditional foods which are an important component of local life and culture, as well as providing nutritional security and medicines. Generally, farmers cultivate or collect from the forest the sixteen indigenous fruit and nut species for subsistence. However, surplus stocks are sold for cash, chiefly at the local markets, Gizo and Noro markets and visiting boats, at a price that varied with the selling unit used (e.g. a parcel or a bag or a heap or a fruit) and between species (Appendix 4.2).

The two top priority species identified later for domestication, *B. procera* and *I. fagifer* (see section 4.3.4.2), provide substantial income for the people as indicated by the number of farmers trading *B. procera* and *I. fagifer* at the domestic markets or with visiting boats (Table 4.2). In all sites, the number of farmers involved in trading *B. procera* fruits is greater than those trading *I. fagifer*. This difference was a reflection on the number of on-farm trees, as well as the natural population distribution of the two species. For example, *I. fagifer* was only plentiful in the wild at Ringgi, Seusepe and Hunda. In the survey, all farmers reported that they planted *B. procera*, while only 7% planted *I. fagifer*. The reasons for this include but not limited to generating little money and plenty in the wild (see Apendix 4.4).

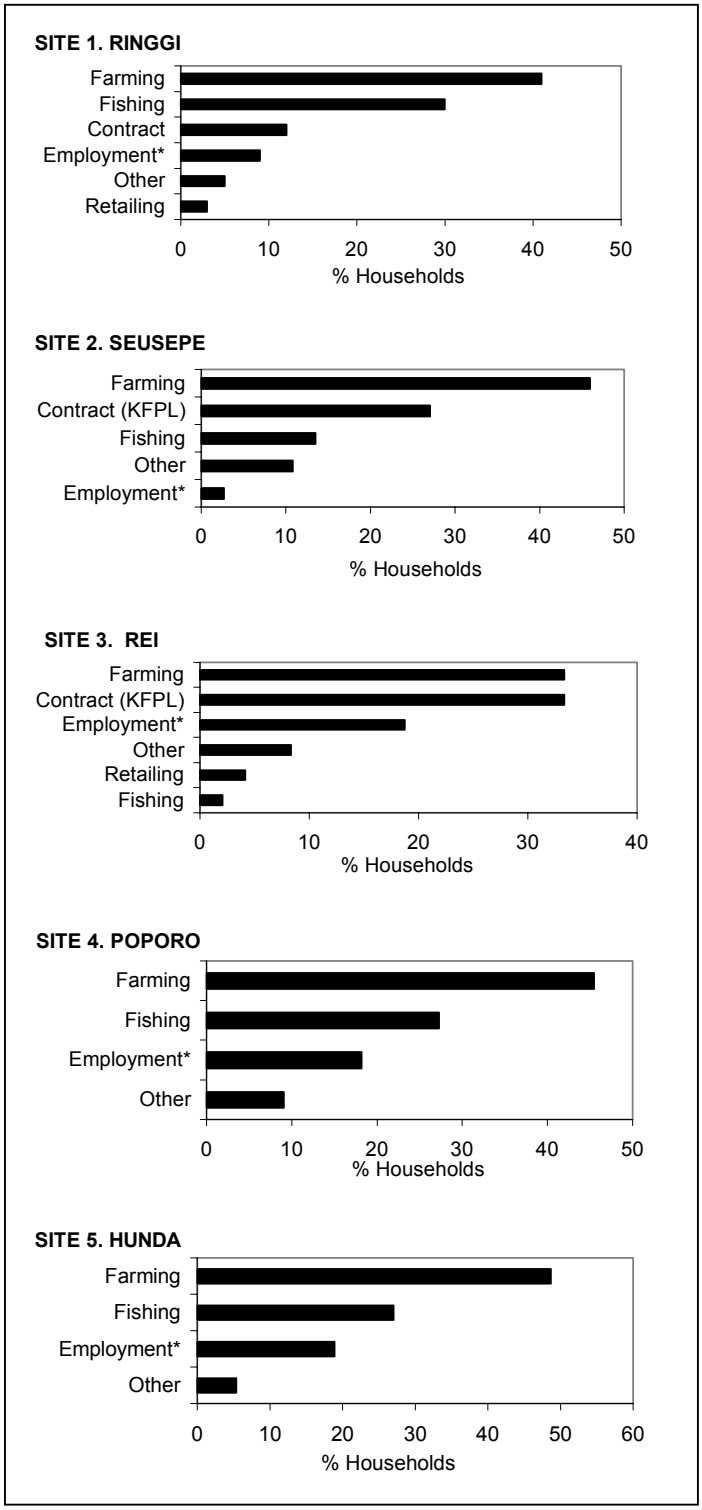


Fig 4.7: Major income at rural Households from farmers' survey (2002) in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara, Solomon Islands.

***only includes a member (s) of the household who was employed by KFPL.**

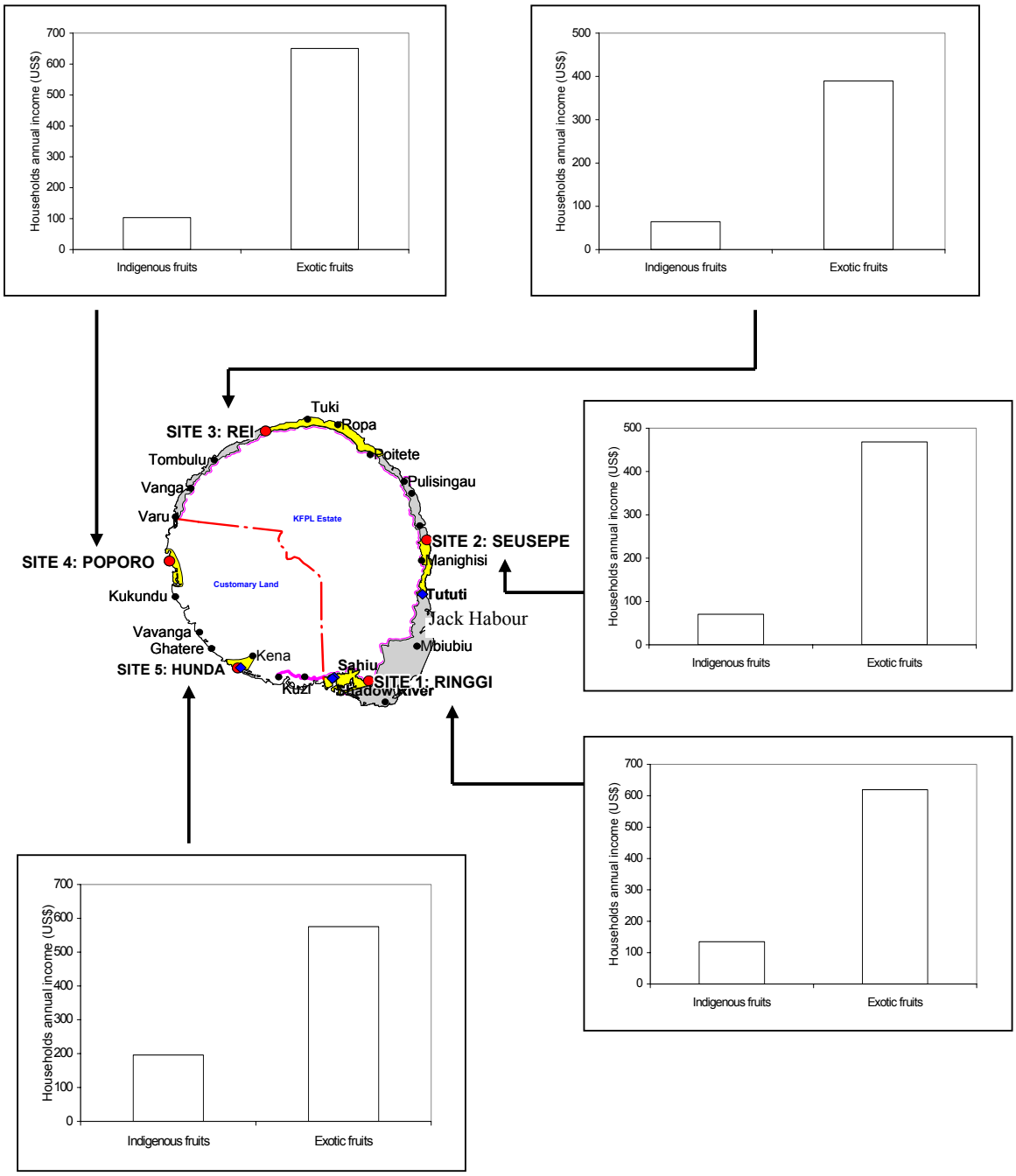


Fig 4.8: Comparative household annual income earnings from both exotic and indigenous fruit trees from farmers' survey (2002) in 5 study sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara Island.

Table 4.2: The number of farmers earning cash from the sale of nuts of *B. procera* and *I. fagifer* per annum in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara, Solomon Islands (from farmers' survey in 2002).

Study sites	Farmers selling edible *B. procera*		Farmers selling *I. fagifer*	
	No.	%	No.	%
Ringgi	26	43.3	1	1.2
Seusepe	9	42.8	2	9.5
Rei	3	9.4	0	0
Poporo	9	60.0	0	0
Hunda	17	62.9	2	9.5

The level of cash income generated from the two species varied significantly between sites (Fig 4.9). Earnings from *I. fagifer* were less than US$14 per annum at Ringgi, Seusepe Hunda and zero at Rei and Poporo. In contrast, the trading on *B. procera* nuts was common in all sites, although cash income was higher at sites outside KFPL estate where more farmers were involved in selling the nuts of this species – with income ranging from US$11 to US$50. These sales were restricted to local markets, although the nuts may have potential on national and regional trade if the infrastructure was developed.

Nuts for sale of both species were collected from both cultivated and wild trees, with those of *B. procera* being sold as a heap (10 -12 nuts) or parcel (20-24 halved kernels) for US$0.15 per unit. The nuts of *I. fagifer* are mainly sold in a heap of 10-15 nuts at US$0.30 per unit. The selling price of both species was consistent from one site to another. Any member of the household could collect and market the nuts.

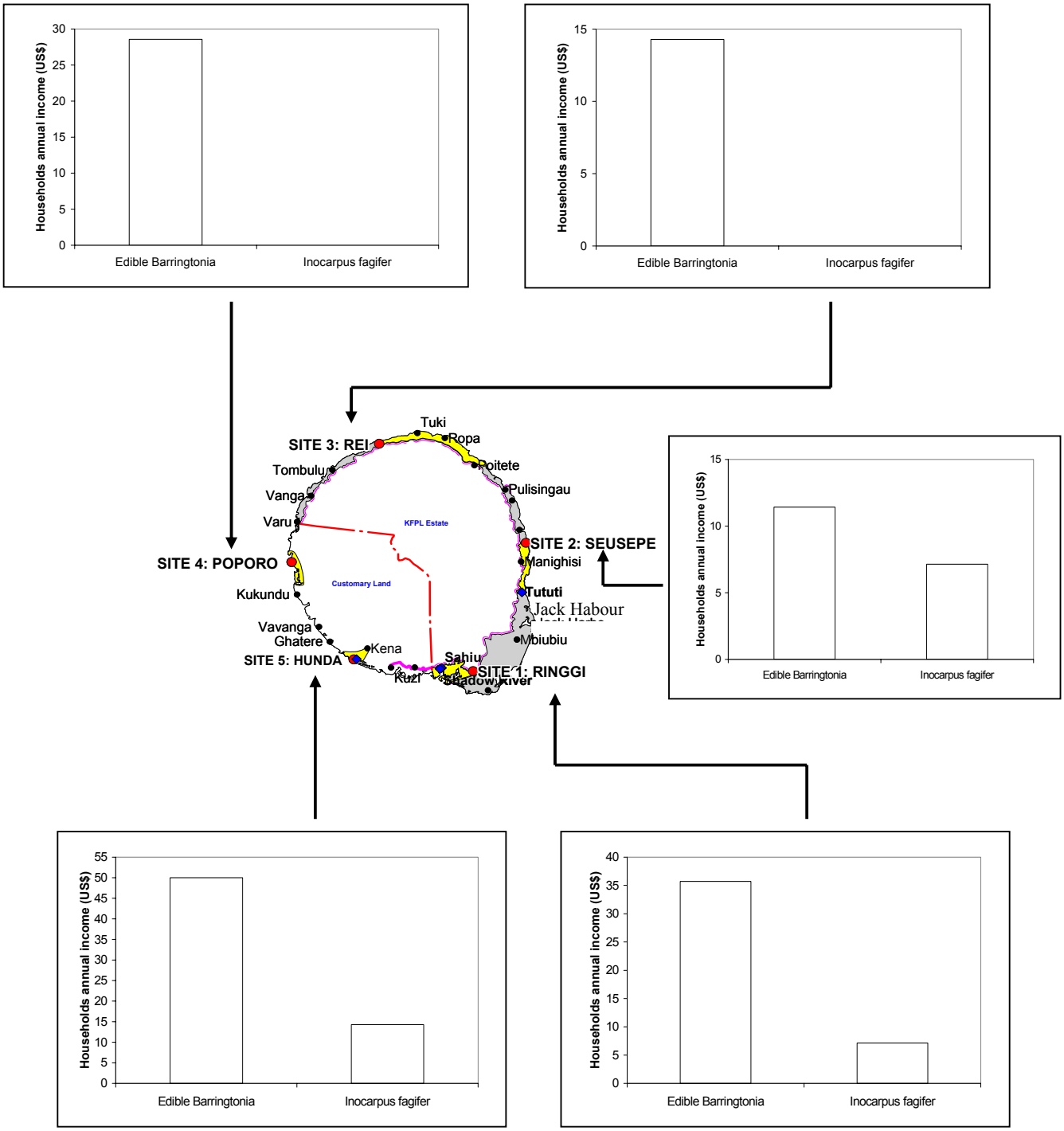


Fig 4.9: Comparative household income generated from the two top priority nut trees (*B. procera* and *I. fagifer*) from farmers' survey (2002) in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara Island.

4.3.3 Traditional agricultural practices

4.3.3.1 Important food crops

Important staple foods in Kolombangara Island were found to be sweet potato, cassava, banana, yam and taro (Fig 4.10). However, the two latter were less important being absent in some food gardens.

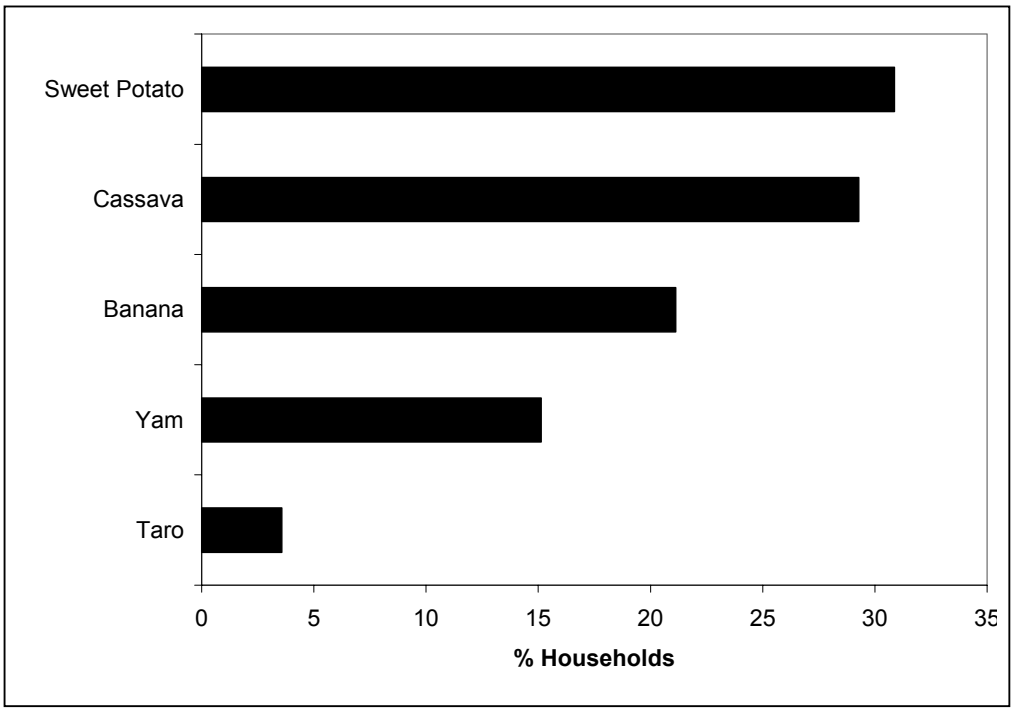


Fig 4.10: Main staples of rural people from farmers' survey (2002) in 5 sites – Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara Island.

The relative importance of these staples as well as combinations of crops grown in food gardens varied from one village to another. Sweet potato and cassava occupied more than 50% of the area in all village gardens visited. Farmers in most villages planted bananas in garden boundaries.

4.3.3.2 Traditional agriculture

Traditional agriculture in rural village communities of Kolombangara Island is typically a shifting cultivation or swidden fallow system described in Chapter 2.

Two types of agricultural activities are found on Kolombangara island: (i) homegardens typical of the Pacific region, primarily established to provide for and sustain a farmer's own family household, (ii) cash crops such as cocoa, coconut and timber trees grown as monocultural plots. A recent development has been the start of cash crops cultivation within homegardens, with farmers establishing crops such as sweet potato, cassava, banana, yam and taro, fruits and vegetables to generate cash to improve their livelihoods.

Farmers growing cash crops are practicing a number of planting configurations such as planting in rows, blocks, intercropping and mixed planting in their gardens (Fig 4.11). Mixed planting is the common arrangement throughout the island, with fruit trees being grown at random or in irregular patches either inside food gardens or along land boundaries. Some of the trees are protected during land clearing but planting is the normal process of establishing these trees. Timber trees are sometimes grown in woodlots, while others are in the mixed planting or in lines intercropped with food crops. Intercropping of food and tree crops is uncommon on Kolombangara Island, particularly within the five sites surveyed as people used to mix crop farming. However, in Temotu Province of Solomon Islands farmers are practicing intercropping (Bonie 1993) as they are advanced in tree selection.

The survey results found that farmers maintain soil fertility in two ways – by planting N-fixing legumes, mainly beans and peanuts, and by traditional fallows. There was no evidence of purposeful planting of leguminous trees (e.g. *Gliricidia*) to improve soil fertility. In rural communities of Kolombangara, about 86% of farmers had grown crops with 3-4 harvests before fallowing their land, while 9% claimed to have more than 6 harvests (2 crops per year) before fallowing. The remaining 5% of farmers practiced less than 3 harvests on their land before letting it go to fallow. On average, farmers had 4.1 harvests before fallowing their land. Fallow periods range between 3 months to 4 years, with majority of farmers (83%) fallowing their land within 1-4 years. The calculation of land use intensity for home gardens across five study sites, gave R-values of 44-62 (medium rating) and 71-92 (high rating). About 79% of farmers were in the medium rating and 21% are in the high rating of intensity in the use of their land.

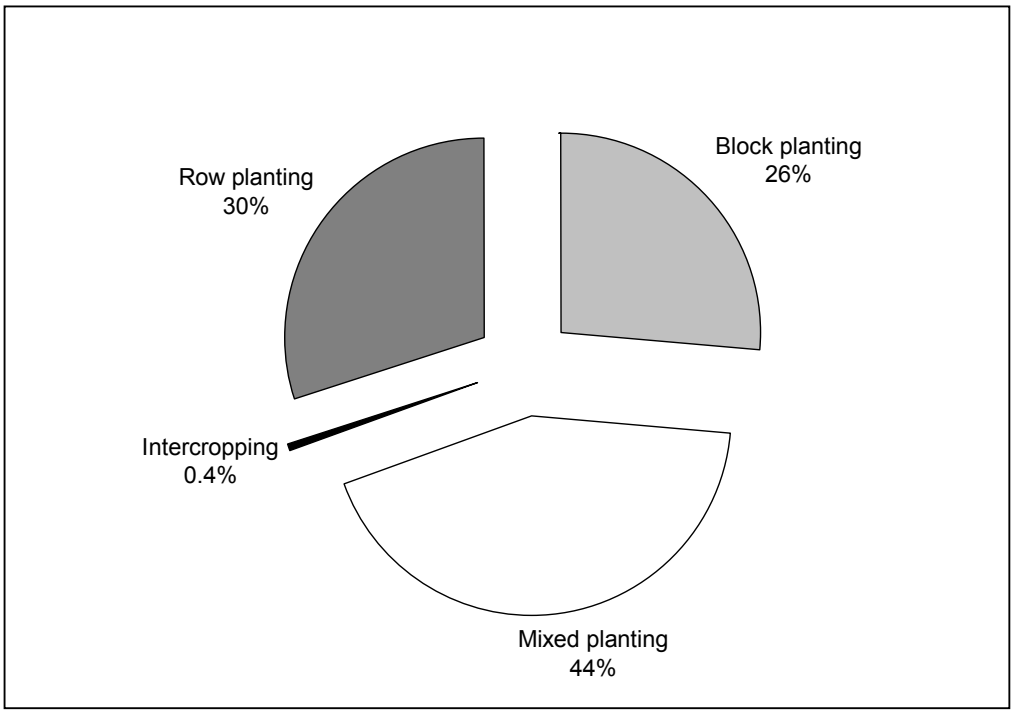


Fig 4.11: Planting configurations adopted by farmers in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) from farmers' survey (2002) on Kolombangara Island.

4.3.3.3 Labour availability

The current survey did not determine the size of household available labour but 27% of farmers indicated that labour shortages were responsible for occasional food shortages. Discussions with farmers suggested that increased commitments to children's education, rural health and religion in rural communities occupied the time of many farmers and that these social obligations often took priority over home gardens or other income generating activities.

4.3.4 Indigenous fruit and nut species

The survey discovered a range of uses and attributes of the indigenous fruit and nut species grown naturally and/or cultivated on peoples' land. The data also indicates the availability of land, and the ability of people to have access to or plant indigenous fruit trees. Analysis of this data ranked the indigenous fruit and

nut species (Fig 4.12) for domestication and provided evidence of how these species, and other popular indigenous fruit and nut species, are traditionally managed and utilised by the people to meet their needs and enhance their livelihoods.

4.3.4.2 Identification of the top priority fruit and nut species for domestication

Sixteen fruit and nut species of importance to farmers were identified on Kolombangara Island from the participatory survey. Aggregate ranking of species across priorities 1-16 determined the priority species. Eleven species (*Barringtonia procera, Canarium indicum, Artocarpus altilis, Mangifera minor, Syzygium malaccense, Inocarpus fagifer, Canarium salomonense, Spondias dulcis, Gnetum gnemon, Terminalia kaernbachii* and *Terminalia catappa*) were each identified as priority species by more than 50% of farmers interviewed (Fig 4.12).

Farmers' choice for *B. procera* was significantly ($P<0.05$) different from the other 14 species. The next top five species (*C. indicum, A. altilis, M. minor, S. malaccense, I. fagifer)* were very similar and the differences between them were not statistically significant in terms of farmers' rank order, while the next six species (*C. salomonense, S. dulcis, G. gnemon, T. kaernbachii, T. catappa* and *T. salomonensis*) were of steadily decreasing importance, but the differences at each step were not significant. However, significant differences occurred between every group of 2-3 species, for example, farmers' choice for *C. salomonense* was not significantly different to *S. dulcis*, but was significant to *G. gnemon*. The last 4 species (*Burckella obovata, Paratocarpus venenosa, Pometia pinnata* and *Gnetum latifolium*) were of low importance and again not significantly different (similar in rank order).

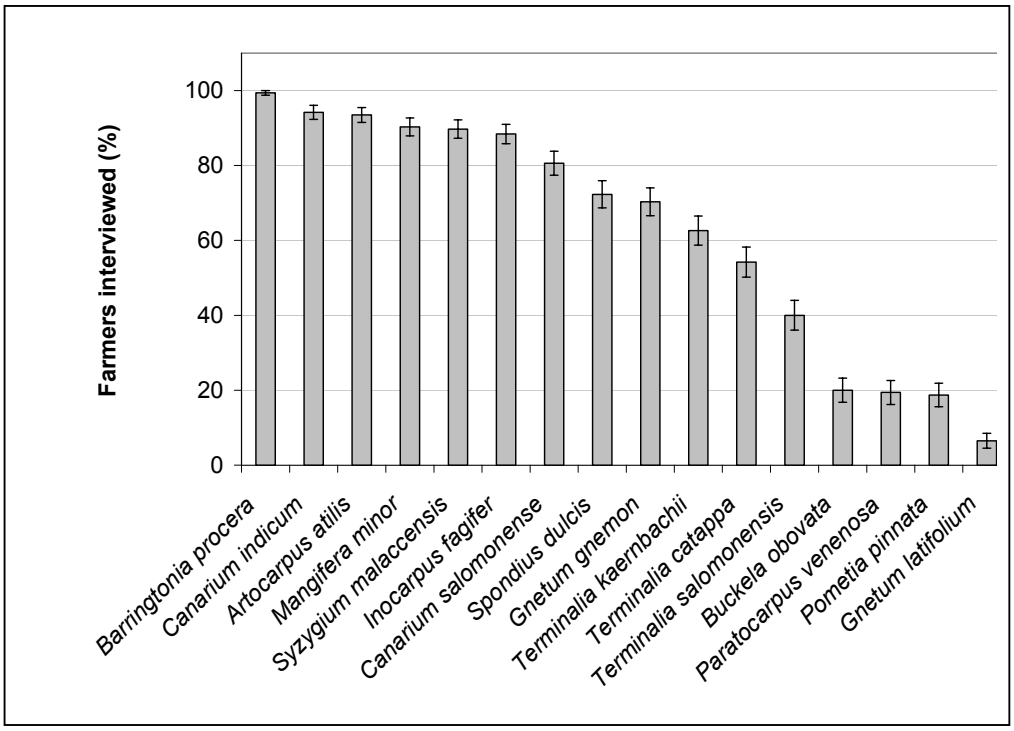


Fig 4.12: Farmers' priority ranking (±SE) on aggregate order of sixteen most popular fruit and nut trees for domestication, from farmer's survey (2002) in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara Island.

Farmers' choice of the top priority species for domestication was clearly *B. procera*, it being the first choice of 36% of farmers (Fig 4.13), and *C. indicum* was clearly the second choice (27% support). Only 0.6% - 10% of farmers chose *A. altilis, M. minor, S. malaccensis, I. fagifer, C. salomonense, T. salomonensis, G. gnemon, P. venenosa* and *S. dulcis* as their top priority species for domestication. No farmers rated *B. obovata, T. kaernbachii, T. catappa, P. pinnata* and *Gnetum latifolium* as their first choice as a candidate for domestication.

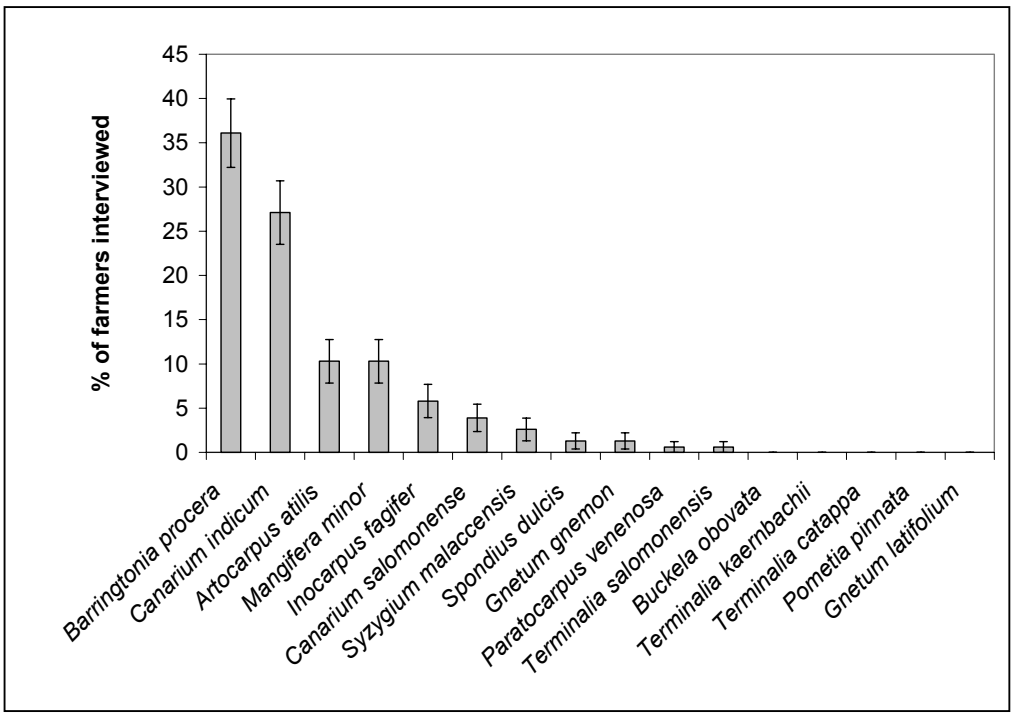


Fig 4.13: Farmers first choice rating (±SE) of 16 indigenous fruit and nut species for domestication from farmers' survey (2002) in 5 sites (Rinngi, Seusepe, Rei, Poporo, Hunda) on Kolombangara Island.

Farmers' rankings can also be examined for each species, as farmers individually ranked each species from 1-10 (1 = best). This approach confirms the top two species as *B. procera* and *C. indicum* (Fig 4.14), and the bottom species are *P. pinnata* and *B. obovata*, with other species being of intermediate ranking either being scored differently by many farmers (e.g. *M. minor*) or being consistently scored 4th -5th (e.g. *I. fagifer* and *S. malaccense*).

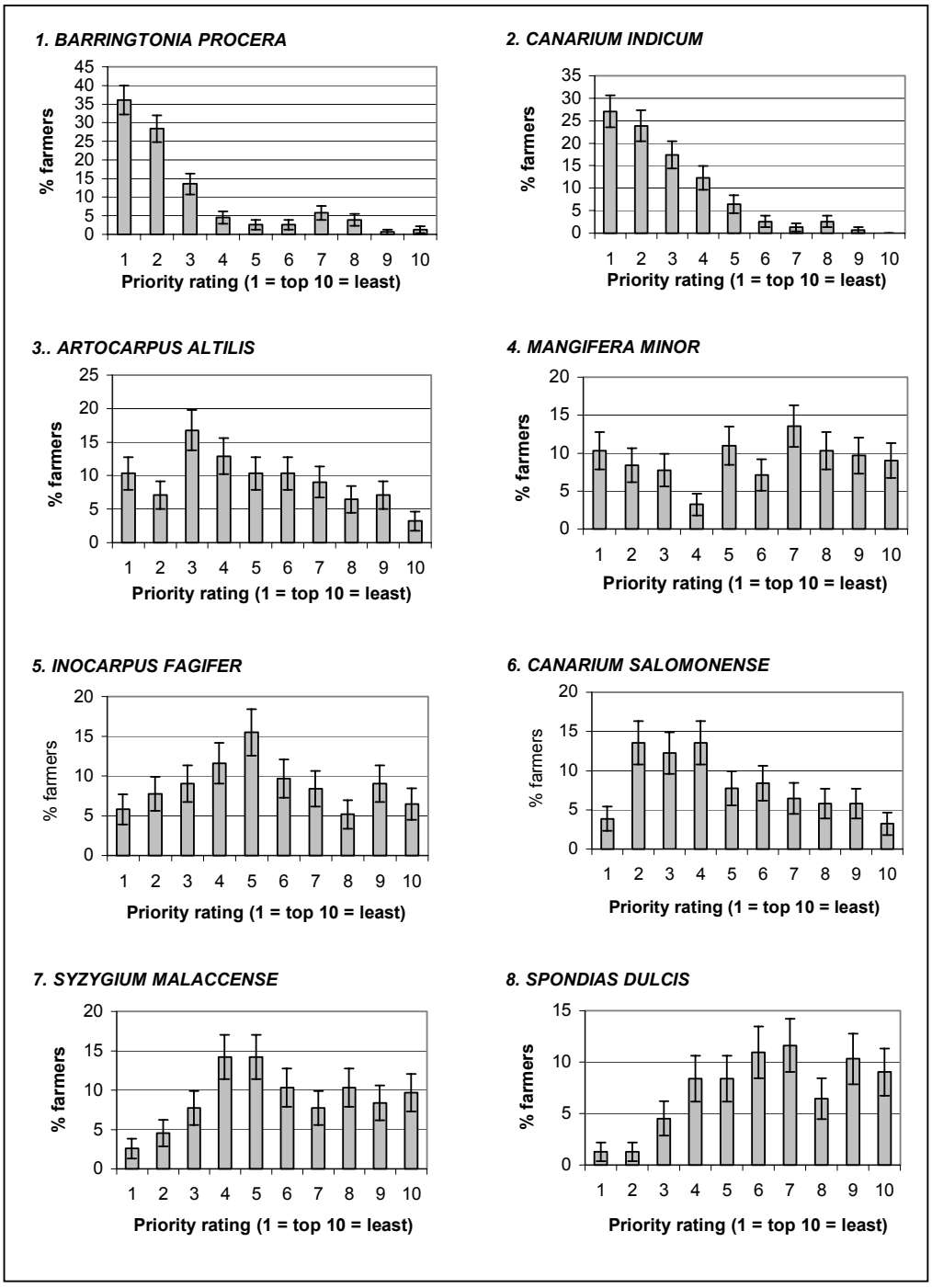
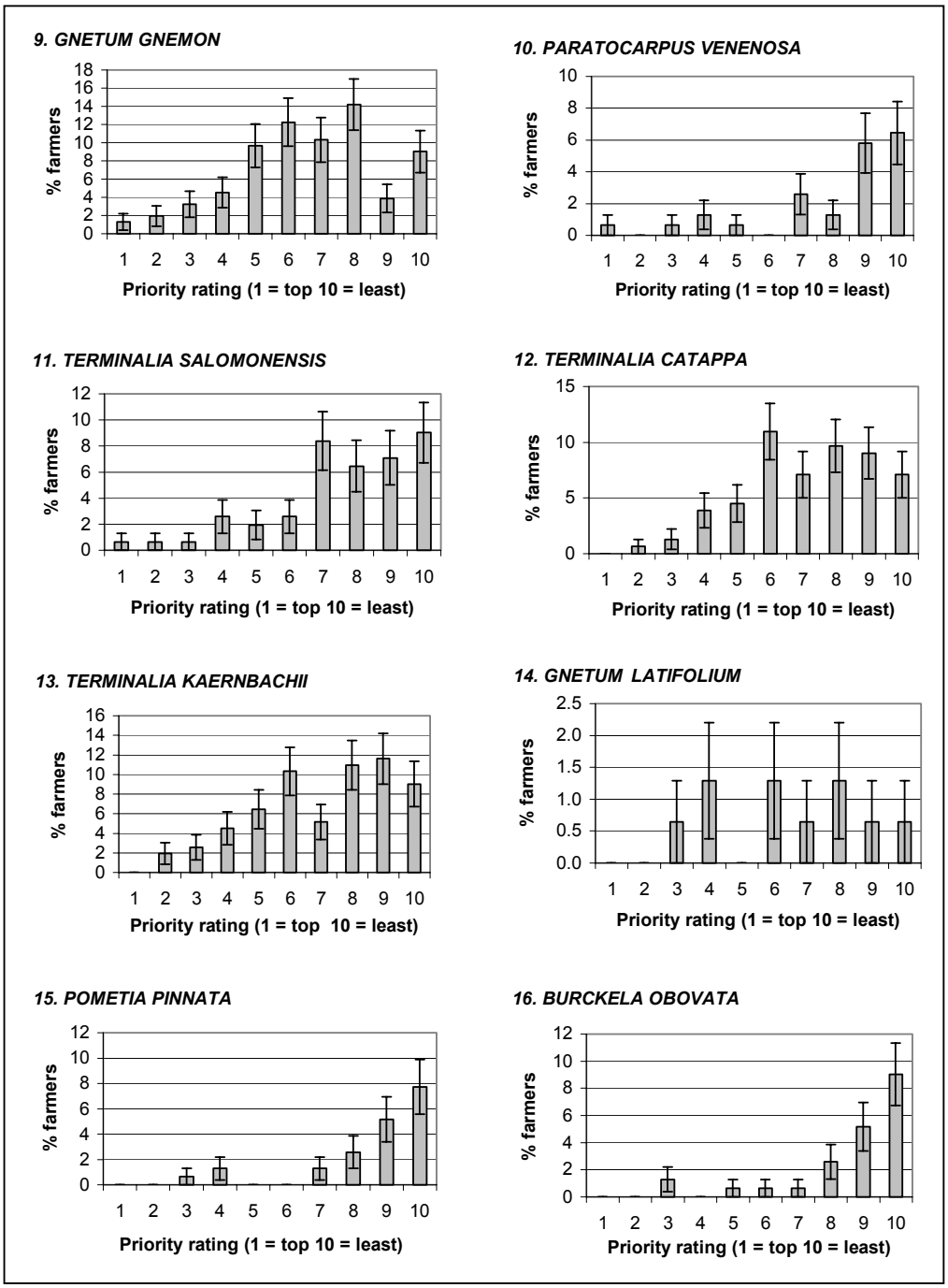


Fig 4.14: Percentage (±SE) of farmers ranking each species in order 1 to 10 in a survey (2002) at 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara Island.



Cont. **Fig 4.14: Percentage (±SE) of farmers ranking each species in order 1 to 10 in a survey (2002) at 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara Island.**

4.3.4.3 Traditional uses of tree products from priority species

Most importantly, these fruit and nut species are a food resource in rural communities of Kolombangara Island. Traditionally, these species provide an array of food products that has contributed to the sustained livelihoods of the native people for many decades. Fruits basically produce two products – the flesh and the nuts/seeds, which usually have edible kernels. The uses of the food products of the sixteen species identified from this survey are summarised in Table 4.3. As stated earlier, every member of the household takes part in collecting fruits and nuts for subsistence.

Table 4.3: Different food products from 16 popular indigenous species from farmers' survey (2002) in five sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara, Solomon Islands

Provider species	Tree product	Food product	Food preparation
Canarium indicum *Canarium salomonense* *Barringtonia* procera *Inocarpus fagifer* *Terminalia catappa* *Terminalia kaernbachii*	Nut	Kernel	With the exception of *I. fagifer*, the kernel of all other species is eaten fresh. The kernel of *I. fagifer* must be cooked (roasted or boiled) before eating. The kernels of *C. indicum*, *C. salomonense* and *B. procera* are also cooked (roasted).
Mangifera minor *Spondias dulcis* *Syzygium malaccense* *Pometia pinnata* *Paratocarpus venenosa* *Burckella obovata* *Terminalia salomonensis*	Fruit	Flesh	Except for *T. salomonensis*, the flesh of all other species is eaten fresh. The fruit (flesh) of *T. salomonensis* must be cooked (roasted or boiled) before eating.
Artocarpus altilis	Fruit Nut	Flesh Kernel	Both products must be cooked (roosted or boiled) before eating.
Gnetum gnemon *Gnetum latifolium*	Nut Leaves	Kernel, Leaves (young and tender)	Both products must be cooked before eating. The kernel is either boiled or roasted while the leaves are made into various dishes.

Medicinally, all sixteen of these fruit and nut trees were reported to be of cultural significance. Almost all parts of a tree (bark, leaf, fruit/nut, sap, shoot and root)

can be used by the native Kolombangarans as traditional medicines, although the bark and the leaves were the main parts commonly used by people as traditional medicines.

Farmers identified that there are many different products and services from the sixteen species of this survey (Appendix 4.3). All the fruit and nut tree species, except for *A. altilis*, *B. obovata*, *C. indicum* and *P. pinnata*, produce poor quality timber. In addition, wood of *I. fagifer*, *M. minor*, *S. dulcis*, *T. catappa*, *T. kaernbachii* and *T. salomonensis* are used for tools and items of every day use such as axe handles, spears, bowls and canoe making. These products are commonly produced for both domestic use and cash. Some species such as *P. pinnata* and *I. fagifer*, were considered better than others (e.g. *C. indicum*, *C. salomonense* and *T. catappa*) for making tools, although there was agreement among farmers that none of the species were highly suitable for making artefacts, although some species were regarded as useable. Despite the multiple uses, fruit and kernel are the most common product.

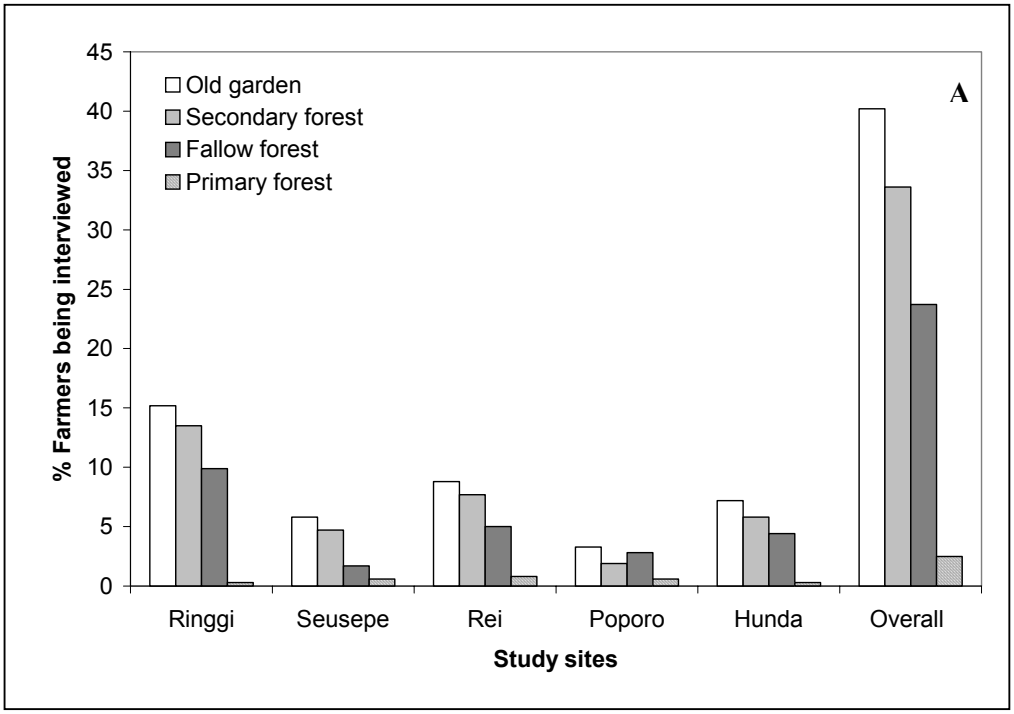
Environmental service functions provided by trees were clearly identified by the farmers' responses to the participatory survey. Farmers agreed that the following service functions have been recognised traditionally by the people:- the provision of shelter/shade, the improvement on soil fertility and structure, the reduction of erosion effects and the maintenance of biodiversity. Farmers' ranking of the species in providing such services varied considerably. In terms of providing shade and shelter functions, a high proportion (>60%) of farmers chose *I. fagifer*, *M. minor*, *S. malaccense* and *T. catappa* as the best species, while, *B. procera*, *I. fagifer*, *M. minor*, *P. venenosa*, *P. pinnata*, *S. dulcis*, *T. catappa* and *T. salomonensis* were highly ranked (>60% of farmers) in providing control over soil erosion and for the improvement of soil structure and fertility. Most species were highly regarded for maintaining biodiversity by attracting different birds and animal species.

4.3.4.4 Traditional tree management

The sixteen popular indigenous fruit and nut species identified during the farmers' participatory survey are managed traditionally in various ways (Appendix 4.4). Application of these management regimes varies from species to species, both in their natural environment and in cultivation. Most of these species are found to grow naturally in old gardens and primary, secondary and fallow forests as illustrated by the two top priority species (*B. procera* and *I. fagifer*) (Fig 4.15). *B. procera* was found mainly in old homegardens and are commonly planted by seeds within the surroundings of human settlements either in coastal or inland villages.

During bush clearing to establish food gardens on these contrasting ecological sites, not all farmers retain fruit and nut trees – the reasons for this being varied (Appendix 4.4). The extent to which trees are spared varied considerably between species. For instance, 30% - 40% of farmers interviewed said they retain *B. obovata, T. kaernbachii,* and *T. catappa* during any type of land clearing. In contrast, trees of eleven species (*A. altilis*, edible *Barringtonia* spp., *M. minor*, *S. malaccensis*, *I. fagifer*, *T. salomonensis*, *G. gnemon*, *Gnetum latifolium*, *P. venenose*, *P. pinnata* and *S. dulcis*) were reported to be retained more during bush clearing, by 59% - 97% of farmers interviewed. Interestingly, however, all farmers claimed to have retained trees of *C. indicum* and *C. salomonense* during bush clearing.

Most farmers claimed that trees they normally removed were biologically sterile and non-productive. Furthermore, some trees are cut down because they are incompatible with other food crops or are thought to be a weed on garden sites. Trees of some species (e.g. *I. fagifer*) were reported to be cut down for firewood as their wood can burn well even when in green form and is used for drying copra.



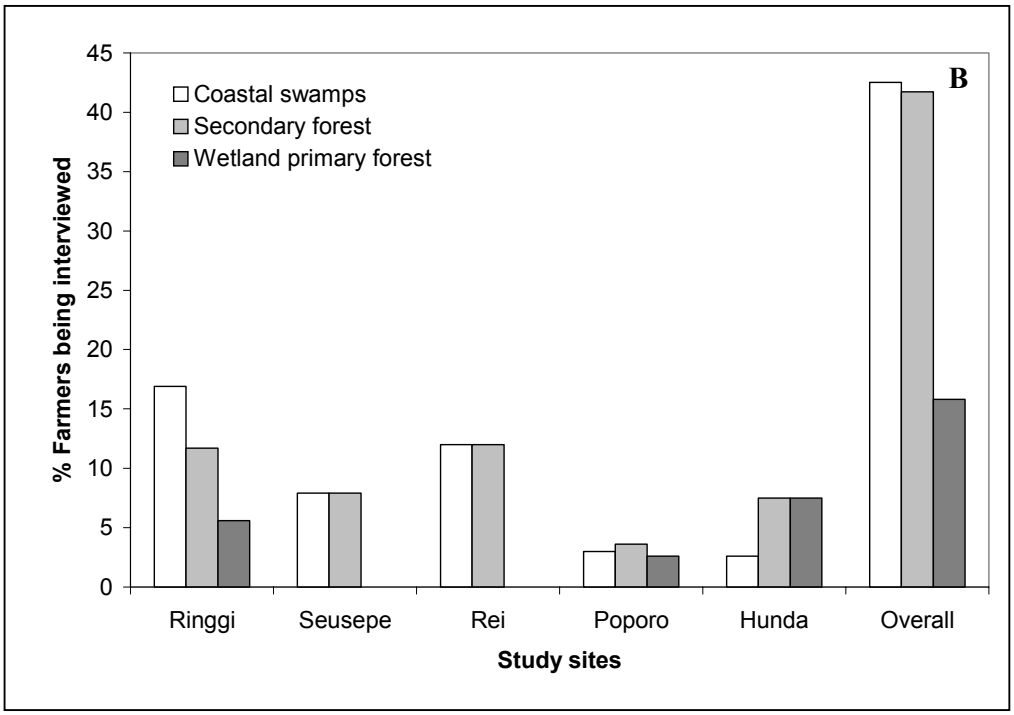


Fig 4.15: Natural ecological distribution of (A) *B. procera* and (B) *I. fagifer* from farmers' survey (2002) in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara Island.

However, scattered plantings of some species were evident in various agroecological sites and land use systems being visited, but this is variable between species. Most farmers preferred planting fruit and nut species around their dwellings, for example, *B. procera* (Fig 4.16).

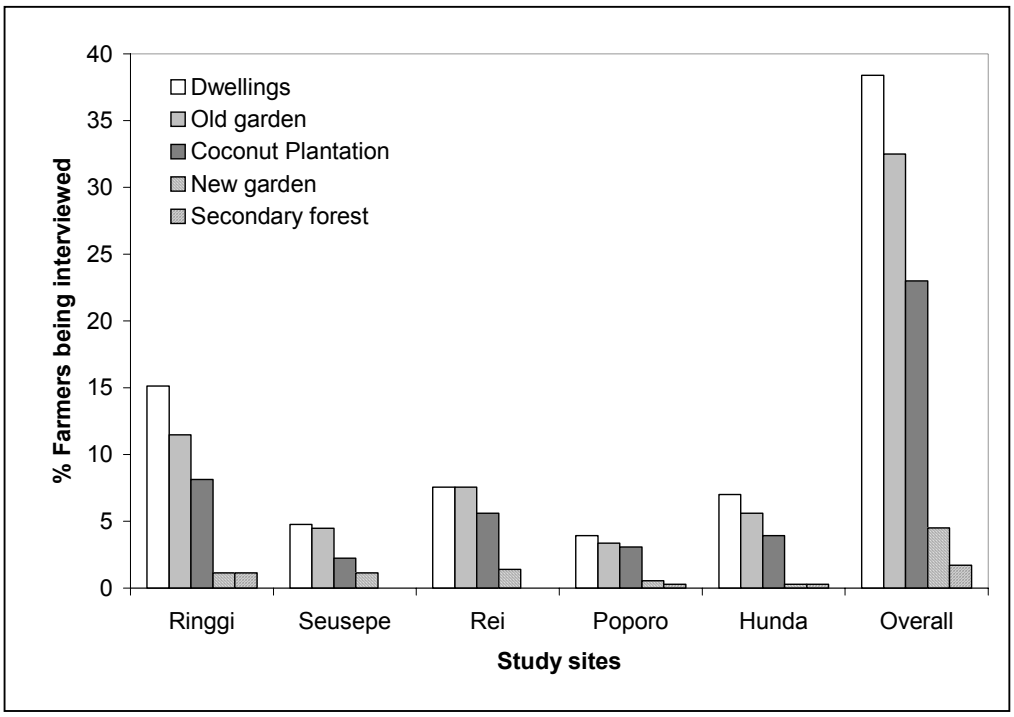


Fig 4.16: Farmers' choice of different ecological sites to grow edible *Barringtonia* species from farmers' survey (2002) in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara Island.

Five species (*B. obovata*, *G. gnemon*, *G. latifolium*, *T. catappa* and *T. salomonensis*) were never reported to be planted outside their natural habitat by farmers during the farmers' participatory survey. The other eleven species have been cultivated mainly using seeds, but the percentage of farmers engaged in planting these species is very low. For example, under 25% of farmers were actually planting the majority of the cultivated species (*A. altilis*, *M. minor*, *S. malaccense*, *I. fagifer*, *P. venenosa*, *P. pinnata* and *T. kaernbachii*). Although, a high proportion of farmers (86% - 100%) were cultivating four species (*B. procera*, *C. indicum*, *C. salomonense* and *S. dulcis*).

The reasons given for farmers not to plant indigenous fruit and nut species varied between species. All farmers (100%) voted against planting 12 out of 16 fruit and nut species because they did not generate sufficient income, while 11 species were voted by 100% of farmers to be abundant in the wild (Appendix 4.4). For example, farmers' main reason for not planting *I. fagifer* is its abundance in the wild (Fig 4.17).

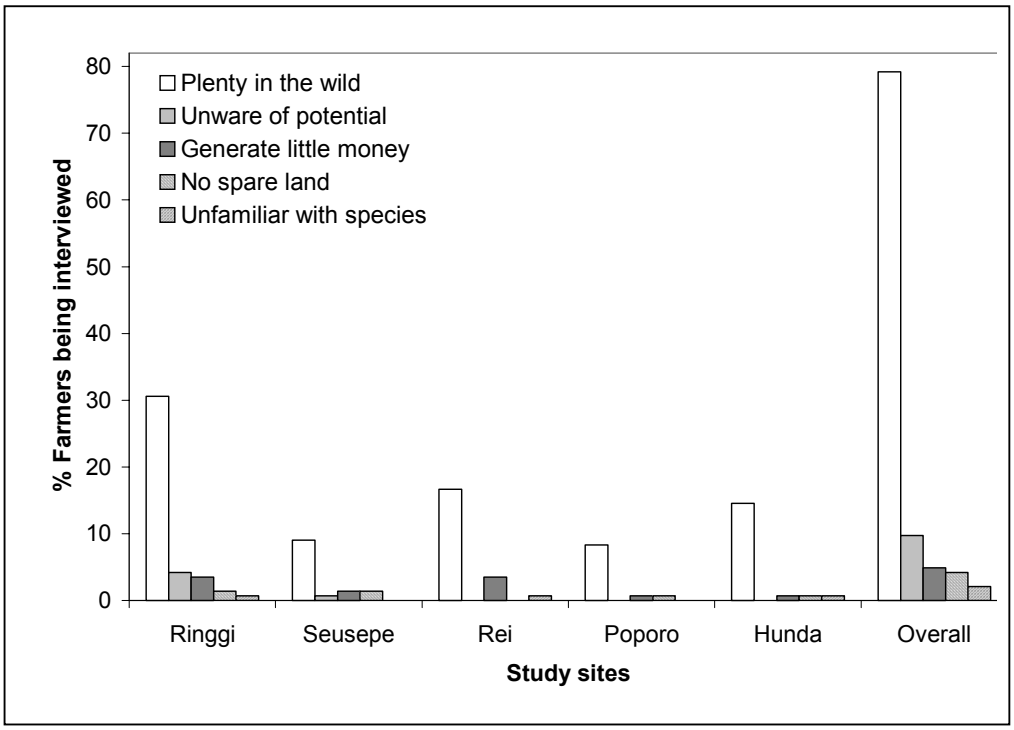


Fig 4.17: Reasons given by farmers not interested in planting *I. fagifer* from farmers' survey (2002) in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara Island.

Farmers who have cultivated indigenous fruit and nut species identified the problems, which have reduced the productivity of their tree crops, and thus restricted their interest in expanding cultivation (Appendix 4.4). Of the 11 species cultivated, 10 species were considered by farmers (100 %) to have low yield, 6 species were considered to have limited planting materials and 5 species were recognised as having pest and disease problems. The specific problems encountered by farmers growing *B. procera* and *I. fagifer*, were quite common from one study site to another (Fig 4.18 and Fig 4.19). Low yield was identified as

a common problem in both species, as indicated by 31% of farmers for *B. procera* and 36% for *I. fagifer*.

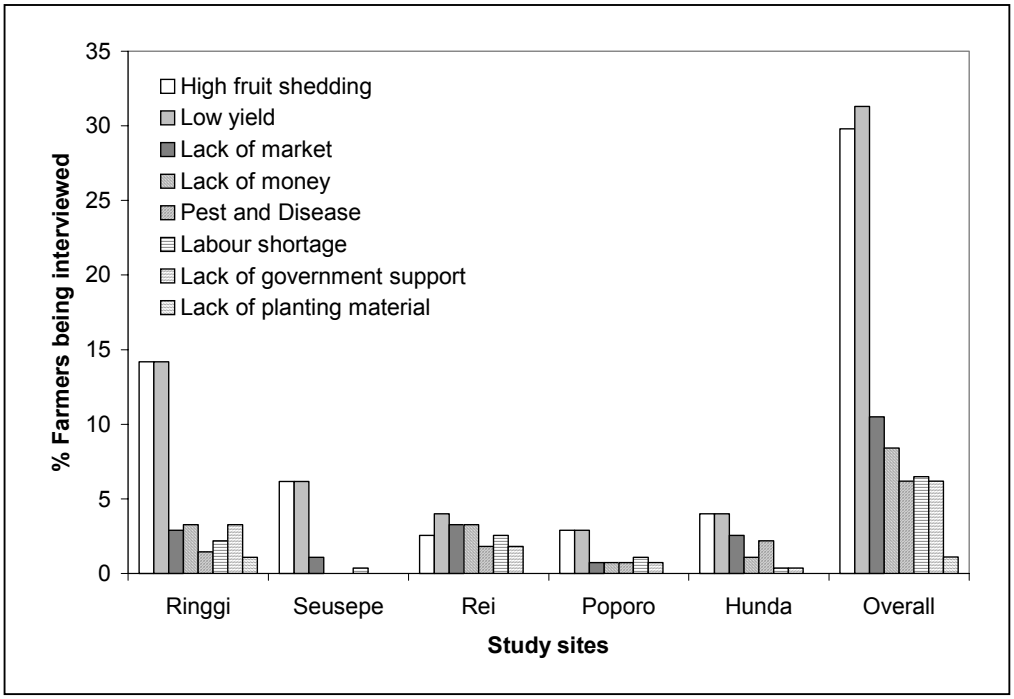


Fig 4.18: Problems farmers claimed to have encountered when planting *B. procera* from farmers' survey (2002) in 5 sites (Ringgi, Seusepe, Rei Poporo, Hunda) on Kolombangara Island

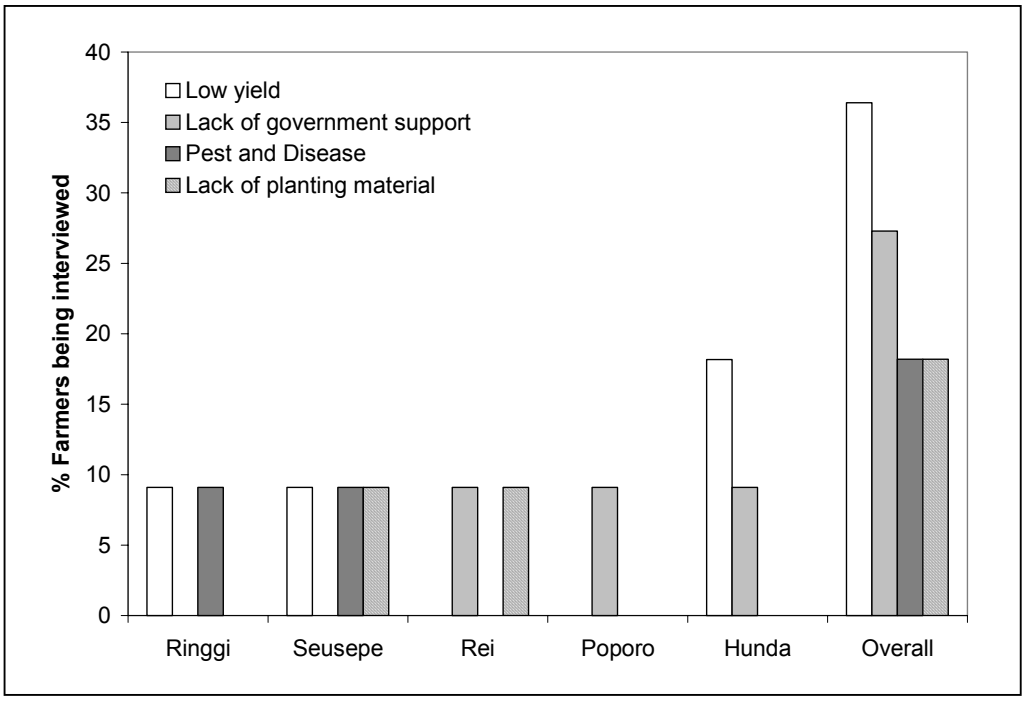


Fig 4.19: Problems farmers claimed to have encountered when planting *I. fagifer* from farmers' survey (2002) in 5 sites (Ringgi, Seusepe, Rei Poporo, Hunda) on Kolombangara Island

4.3.4.5 Tree phenology and potential improvements

With the exception of *B. procera*, only a small proportion of farmers (less than 35%) confidently knew the different seasons of flowering, fruit setting and harvesting of the other fifteen indigenous fruit and nut species (appendix 4.5). Clearly, the majority of farmers were not as aware of these species as they were of *B. procera*, which is commonly grown around the dwellings and thus is observed by most people. Interestingly, this species does not have a well-defined fruiting season, as different trees fruit almost throughout the year although with peaks in March and September.

Based on their knowledge and association with these species, farmers identified the following as traits for improvement: a) kernel extraction, b) the fruit and kernel shelf life, c) size and taste of fruit and kernel, d) tree yield, e) tree height, f) early fruiting and g) resistance to pests and diseases.

4.4 DISCUSSION

The results of the present study represent a major advance in understanding of the traditional life in rural communities of Kolombangara Island, especially in relation to the indigenous fruit and nut tree resources. This study has for the first time also determined the peoples' priority species for participatory domestication both to increase food security and to diversify income generation.

There was no evidence that the approval to domestication of *B. procera* and *I. fagifer* should be different in sites that vary in their ethnic group, religion affiliation, geographical location and land tenure and ownership. However, there was evidence that in Hunda, the people have progressed further in their own efforts to domestic *B. procera* as exemplified by ease of cracking.

- **Benefits of indigenous fruit and nut trees**

Farming has been the backbone of many rural subsistence economies in developing countries, supporting the livelihoods of the rural majority. A number of indigenous tree species, either planted or protected have been a part of traditional farming practices for many hundreds of years. The benefits derived from indigenous fruit and nut trees are threefold: food and nutritional security, a source of income and the basis of farming systems protecting the environment, conserving soils and maintaining biodiversity (Sanchez *et al.,* 1997; Leakey and Simons 1998). Trees produce desirable products such as, timber, fruits, medicines, fibre and fuelwood, and environmental services such as shade, shelter and soil restoration (Leakey *et al.,* 1996; Leakey and Simons 1998).

The present study revealed that up to 50% of farmers interviewed in Kolombangara Island are mainly dependent on farming as the source of income to support their family, principally from sales of garden produce such as root crops and vegetables. This finding agrees with a socio-economic survey conducted more than a decade ago by Mackey (1989). It was reported then that 65% of sampled households (40 households) were earning income from food crops. The fact that,

farmers remain dependent on food crops for cash, suggests that there has been little change in the importance of food crops in sustaining rural livelihoods.

This study has shown that in Kolombangara Island exotic fruits are dominant in the sales of farm produce. However, indigenous fruits and nuts remain important crops, generating income of up to US$200 per annum per household, especially on farms in customary land. The difference in income between the exotic and indigenous fruit and nut trees is mainly attributed to the greater income from green coconuts and copra (60.4% of households). Coconut is one of the main cash crops in rural Solomon Islands - about 68% of households earn income from copra (Mackey 1989). In contrast, this is about threefold more than the national figure (22.9% of rural households, (MOF 1995). There appeared to be imbalance in income between indigenous and exotic fruit and nut species – one reason is that the government support for indigenous species is lacking and that there is a general lack of awareness about the importance of indigenous species. This is also evident from the lack of market outlets and lack of planting materials. Agroforestry tree domestication is a means to address this situation and would promote the sustainable cultivation of indigenous fruits and nuts.

Although the sale of indigenous fruits and nuts was not found to be the principal income earning activity of farmers on Kolombangara Island, they do supplement farmers' income. Some of the indigenous fruit and nut species, for example *Barringtonia* species, bear fruit almost all year around and are less labour intensive than, for example, copra. This ease of harvesting and processing makes them attractive to farmers, especially women. Similar benefits to farmers' have been reported for *D. edulis* in Africa, which generate income during the off-seasons of cocoa and coffee crops (Schreckenberg *et al.,* 2002).

Sixteen traditionally important fruit and nut species were identified in the present study (Fig 2.15). Eleven of these were protected by the majority (59%-97%) of farmers during bush clearing, while only 4 species were cultivated (86% - 100% farmers). Mixed and scattered plantings of some of these species were evident in various agroecological sites and land use systems. Throughout the tropics there are

many tree species which are undomesticated and relatively un-studied by science (Leakey and Newton 1994a). This lack of scientific understanding constraints their use in agriculture and, hence has denied farmers of the opportunity to cultivate them for their products and services. Scientific studies are needed if these species are to become available for sustainable food security, income generation and effective environmental services.

- **Household food security**

The Food and Agriculture Organisation (FAO) of the United Nations, defines food security as "*when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life*" (FAO 1998). One of the specific foci of this chapter is an assessment of the role of indigenous fruits and nuts in household food security in Kolombangara Island, and specifically the availability of these products. The nutritional aspects of food security are outside the scope of this study, as laboratory facilities were not available in Solomon Islands. The study thus focussed on assessing people's access to and use of indigenous fruit and nut species to support their livelihoods.

While there may be issues of nutritional insecurity in diets of the people of Kolombangara Island, households were generally self-sufficient and people had enough to eat. However, food shortages were reported to occur in Kolombangara Island occasionally, mainly due to pests and diseases affecting agricultural crops. According to the farmers, these outbreaks of pests and diseases were associated with the alternation of dry and wet seasons. Clearly, this problem prompts the need to implement an integrated pests and diseases program in farmers' fields. As chemicals are expensive, environmentally unfriendly, and can potentially harm human lives if incorrectly applied, non-chemical methods are desirable. To do this requires scientific input to understand the biology of the crop species and their interactions with pest and disease organisms.

During occasional food shortages, farmers reported that people resorted to visiting old gardens and harvesting food from the wild. This emphasises the dependence of

people on their land resources for survival. This practice is common in many developing countries of the tropics, for example, by Bora Indians of the Yaguasyacu river in Peru (Padoch and de Jong 1987), Kayapo people in Brazil (Posey 1982), damar owners in Sumatra, Indonesia (de Foresta and Michon 1994) and the Southern Highlanders of Papua New Guinea (Haley 2001; Robinson 2001). This sense of dependency on forest resources to sustain peoples' livelihoods indicates the need to be pragmatic about the way we manage our forests and the environment.

- **Subsistence agriculture in Kolombangara Island**

Millions of farmers in developing countries in the tropics rely heavily on subsistence agriculture for their livelihoods, such as slash-and-burn practices. The present study found that farmers in Kolombangara Island are not a typical, and that they either protect and/or plant trees such as *Barringtonia procera*, *B. edulis*, *B. novae-hiberniae*, *Canarium* spp., *Areca catechu*, and *Cocos nucifera* in their homegardens, under a mixed cropping system.

With regard to the understory crops grown in homegardens, the present study found that rural farmers in Kolombangara Island are mainly planting sweet potato, cassava, banana, yam and taro as for main staple foods, and complemented them with different varieties of vegetables of both native (e.g. *Abelmoschus manihot*) and exotic (e.g. beans, green capsicum, Chinese pak choi cabbage) origin. There are similarities with staples in the Pacific island countries, although *Colocasia esculenta*, *Xanthosoma sagittifolium*, *Cyrtosperma chamissonis* and the giant taro (*Alocasia macrorrhiza*) are the main staple food species supporting household diets in Polynesia and Micronesia (Clarke and Thaman 1993). By contrast cereal crops such as rice and maize are the main staples in Asia and Africa.

Based on observations of a number of food gardens visited, the size of holding (= total area cultivated by a household) per household was less than one hectare, but this varied considerably between households and villages. Mackey (1989) has reported that an average holding in Kolombangara Island is 0.95ha, and that this increases to 2.3ha (with 0.1ha under food crops) when farmers have tree crops.

Conversely, farmers with no tree crops have only 0.13ha which is under food crops. Despite the smallness of holdings, farmers in Kolombangara Island planted a wide range of different crops, including root, perennial and vegetable crops.

Worldwide, social values are changing and people are migrating to urban centres seeking more rewarding life styles and income, thereby creating greater urban demand for agricultural produce and forest products, and leading to changes in the pattern of village agriculture. This way of life is new in Solomon Islands and people in Kolombangara Island are starting to adjust themselves to accommodate the impact of a cash-driven society, as they now need money to buy clothes and send their children to school. The KFPL forest estate has forced the population of Kolombangara Island onto one third of the land and employs less than 5% of the people. Like other tropical areas, increasing population size is also creating population pressure. Together these two things are causing farmers to shorten the fallow cycle on their land with consequent soil degradation. For example, in past years (1980s), the mean length of fallow was 4.7 years and crop harvests were 5.0 times before the land was fallowed (Mackey 1989). This contrasts with the average of 1.2 years fallow and 4.1 crop cycles (harvests) observed in the present study. Land pressure, as expressed by R-value is medium to high in Kolombangara Island, with the majority (79%) of farmers fall under medium rating in the intensity of land use. This result is comparable to studies in many developing countries of the tropics. For example, in Andra Pradesh and Orissa in India, fallow cycle has dropped from 30-40 years to 5-7 years in 1974 (Tejwani 1987), as a consequence of increasing population and limited land availability.

From the community meetings and household interviews conducted in Kolombangara Island, it was clear that there have been substantial changes over recent years in rural communities of Kolombangara Island. To avoid negative impacts on peoples' lives, particularly on the use of land and its limited resources, it is apparent that the rural community sees agroforestry as a desirable way forwards and that there is a role for increased growth and use of indigenous fruits and nuts. Other innovations may include the adoption of improved fallow. This can be done through selective planting of fast-growing trees that fix atmospheric

nitrogen that enriches the soil. This might include the intercropping of fallow species with indigenous fruits and nuts. Improved fallow has been practiced in the highlands and small islands of Papua New Guinea (Bourke 1989; Clarke and Thaman 1993; Bourke 1999), Samoa, Tonga and Fiji (Rogers and Thorpe 1999). Some farmers on Kolombangara Island have also planted commercially important timber trees but it is clear that there are opportunities for species including medicinal species which could be used in order to maximise social, economic and environmental benefits from the system.

- **Selection of two species for domestication**

Choosing the species for domestication based on farmers' traditional knowledge is an important event that recognises cultural value and traditional uses of indigenous species in rural communities of developing countries. It also builds on the experience in West Africa (Franzel *et al.,* 1996), Southern Africa (Maghembe *et al.,* 1998) and other areas. In essence, this is the first step into tree domestication. Increasingly, indigenous tree species are prioritised for domestication following local demands for traditional products in urban areas as well as mitigation against impacts of HIV/AIDS on peoples' living standard (Barany *et al.,* 2003; Leakey *et al.,* 2005c).

In accordance with Franzel *et al.,* (1996), the instigators of participatory tree domestication, which this study took into account: (i) farmers' priorities, (ii) market priority and (iii) the existing research activities and researchability. *B. procera* was first for (i) and (ii) and there is no research in progress. *C. indicum*, the second priority of farmers is also important in the market (Nevenimo *et al.,* in press; Leakey and Bunt in press), but is currently the subject of domestication research in PNG (Leakey *et al.,* in press) and has been already extensively researched (Evans, 1991, 1994 and 1999). Artocapus altilis is mainly grown for domestic consumption and not widely marketed, and is the subject of intensive research led by the Breadfruit Institute (Hawaii) (www.traditionaltree.org). *M. minor* is purely domestic consumption and basically unknown species in trade and has problems of researchability on Kolombangara Island due to its failure to produce fruits in the wet climate. Consequently, *I. fagifer*, the fifth choice of

farmer was selected for this study because it is important in regional market (study in Fiji by McGregor and McGregor 1997) and is amenable to research in Kolombangara Island.

Prior to this thesis, perhaps the most comprehensive studies of these species are those of Evans (1999) and Walter and Sam (2002), although they were limited to morphological descriptions of the species. According to Evans (1996), *B. procera* was more fecund than *B. edulis* or *B. novae-hiberniae*, and has a higher kernel : nut ratio, resulting in greater kernel production per tree. In contrast, *I. fagifer* has never been previously studied, although it was reported by Bonie (1983) to be a good candidate for boundary planting. The fact remains, however, that little is known about the biology or propagation of either species.

The important product from both species is the edible kernel, which is extracted by cutting open the fibrous shells, in contrast to the brittle shell of *Canarium* spp. which needs to be cracked using a stone (Evans 1999). Kernels of *B. procera* are eaten fresh, unlike that of *I. fagifer* which because of toxicity, must be cooked (boiled or roasted) before eating. Fresh kernels have a limited shelf life, with the kernels of *I. fagifer* deteriorating much more quickly than *B. procera*. Perishability of the kernels has been recognised to limit commercial prospect and potential of these species (Bourke 1996; Evans 1996). On the other hand, this constraint suggests the need to develop appropriate preservative methods that will prolong the shelf-life of the kernels and open opportunities for export markets (McGregor and McGregor 1997).

4.5 SUMMARY

This study generated information about the social structure of the rural communities, socio-economic conditions of the people, traditional farming practices and indigenous fruit and nut species in Kolombangara Island, which are useful for decision making in the agroforestry tree domestication process. In the subsequent chapters of this thesis, the two species chosen as candidates for domestication by farmers in Kolombangara Island are studied to seek techniques

that can be used to bring them into cultivation. Firstly, in the next chapter, a literature review identifies what is known about the biology of these species, their agroecological distribution and potential for domestication.

CHAPTER 5: LITERATURE REVIEW OF PRIORITY SPECIES

This review of the literature about the two priority species (*Barringtonia procera* and *Inocarpus fagifer*) is extracted from (Pauku 2005a, b).

5.1 *BARRINGTONIA PROCERA* (CUTNUT)

5.1.1 Introduction

Barringtonia procera is a medium size evergreen tropical tree found in secondary forests of Solomon Islands, Vanuatu, and Papua New Guinea, commonly grown in homegardens and coconut plantations, in lowland and coastal rural villages (Henderson and Hancock 1988; Bourke 1996; Evans 1999; Walter and Sam 2002). *B. procera* is commonly called cutnut (English) but known by different names in other countries in the Pacific. It is called *navele* in Vanuatu and in Papua New Guinea it is *pao*. In Solomon Islands pidgin it is called *katnat*, but has names in many local dialects, including: *fala/aikenu* in Kwara'ae (Malaita Is.), *kenu* in To'oabaita (Malaita Is.), *vele* in Varisi (Choiseul Is.), *fara* in Santa Ana (Santa Ana Is.), *kino* in Nduke (Kolombangara Is.), *tinghe* in Roviana (New Georgia Is.), *oneve* in Marovo (New Georgia Is.), *fala* in Maringe (Isabel Is.), *nofe* in Zabana (Isabel Is.) (Henderson and Hancock 1988). The tree is associated with human settlements and is unlikely to occur in a truly wild form. Throughout Melanesian countries *B. procera* is well known as a nut tree, and the people have both planted and protected it on their land. *B. procera* prefers light shade, making it a good companion tree to overstory tree species such as vi (*Spondias cyathera*), canarium nut (*Canarium* spp.), and breadfruit (*Artocarpus altilis*). Its open canopy structure allows sufficient light penetration to the ground level for other crops to be interplanted under it. For instance, farmers in Temotu province of the Solomon Islands have used *B. procera* as companion and interline tree crop in an improved traditional agroforestry system (Bonie 1993). On Kolombangara Island, the Solomon Islands, trees have been used as a trellis tree for the cash crop betel leaf (*Piper betle*), as well as for marking land boundaries and creating windbreaks.

B. procera is a medium-size tree which can reach a height of 24 m (see front page photo). The typical tree height is thought to be in the range of 8–12 m, with a crown diameter of 0.8–6 m. The diameter of the trunk at breast height of mature fruiting trees ranges from 2 to 45 cm (mean = 18 cm).

5.1.2 Distribution

B. procera is indigenous to the Solomon Islands, Vanuatu, and Papua New Guinea (Henderson and Hancock 1988; Bourke 1996; Walter and Sam 2002) in areas characterized as wet tropical lowland rainforest (Whitmore 1969). *B. procera* or a closely related species has been introduced into Australia (Jebb 1992) and Fiji (McGregor and McGregor 1997).

5.1.2.1 Climate

B. procera is commonly found in areas with warm to hot temperatures (20 – 35 ^{o}C) throughout the year (Pauku 2005a). In its natural range *B. procera* does not normally experience a dry season of more than a few months. It is adapted to high rainfall up to 4300 mm per annum. The species tolerates the tropical cyclones which usually occur during the wet season from November to March in the Solomon Islands, Papua New Guinea, and Vanuatu (Bourke 1996; Evans 1996; Walter and Sam 2002).

5.1.2.2 Soils

B. procera generally grows in coastal coral (alkaline) soils with light to heavy texture (sands, sandy loams, loams, sandy clay loams, clays, clay loams, and sandy clays). It occurs in soils with medium to high fertility, and tolerates shallow, rocky, saline, and infertile soils. It prefers soils that have free drainage.

5.1.2.3 Tolerances

B. procera is intolerant to prolonged droughts. It grows well in full sunlight, but is usually found as a sub-canopy species in low-density mixed species environments. *B. procera* tolerates 20–70% shade. Mature trees are more tolerant than young seedlings. In Kolombangara, the Solomon Islands, five-month-old seedlings grown

under 30% shade and in the full sunlight grew equally well (stem heights were about 34 cm for both). *B. procera* is likely to be intolerant to fire and drought, and is likely to be sensitive to temperatures below 15–20°C (59–68°F). *B. procera* has medium to high tolerance to steady and strong winds including cyclones. The tree grows in mildly acid to neutral/mildly alkaline soils (pH 5.1–8.5), but does not tolerate waterlogged soils.

5.1.3 Botanical description

The genus *Barringtonia* belongs to the family Lecythidaceae, mainly nut-bearing trees including Brazil nut (*Bertholettia excelsa*) and monkey pot trees (*Lecythis* spp) (Evans 1999). Previously, this genus has been classified into the family Barringtoniaceae (Clifford and Ludlow 1978), mainly characterised by their bisexual flowers, ovoid tube calyx, limb close in bud and splitting into 2-4 valvate segments or rarely with 3-4 lobes and imbricate in bud. It usually possesses 4-5 petals attached at the base to staminal cup, and has many stamens in different series fused at the base into a ring or cup. Fruits are hard and indehiscent enclosing a single seed without endosperm. Leaves are alternate, simple and exstipulate.

5.1.3.1 Leaves

The large, simple, lanceolate leaves of *B. procera* are arranged in a whorl at each node. Leaf size varies, typically measuring 215–660 mm long and 50–205 mm wide. The upper surface of the leaves is dark green and glossy and is slightly paler beneath. Typically, the leaves have a truncated base and an acuminated apex, with margins undulated. Veins are reticulated and vary in number according to leaf size, but up to 34 on each side. The short thick petiole is up to 6 mm in length and a mean width of 10 mm at the basal end.

5.1.3.2 Flowers

B. procera has a racemose inflorescence with a 30–110 cm long pendulous spike containing up to 150 densely packed flower buds, arranged in a spirally alternate pattern, and varies in color, typically from green to white or red (Plate 5.1). Flowering is terminal on the shoots. Flower buds are semi-sessile to sessile and are protected by a calyx closed in the bud, which ruptures into 2–4 lobes, forming

pseudo-lobes. The calyx apical pore varies in diameter, depending on the stage of development of the flower. It is completely closed at the very early stage but later opens, making way for fully developed flower buds. *B. procera* flowers are bisexual, with male and female reproductive parts occurring on the same flower. Flowering occurs irregularly 2–3 times per year (Bourke 1996; Evans 1999). In Kolombangara Island (Solomon Islands), two peak seasons occur in May/June and October/November each year, although some off-season fruiting occurs.

Plate 5.1: Flowers in white, yellow, and red. Tiny bees can be seen foraging on the flowers. Hunda, Kolombangara, Solomon Islands.

5.1.3.3 Fruits

Fruits are multiple, sessile, and borne on a pendulous rachis (Plate 5.2). At maturity they are indehiscent, but the skin can be easily peeled off when ripe. The elongated, oblong to obovoid fruits taper towards apex and base. The shape of the fruit at apex is emarginate-rounded and the base is truncaterounded. Typical length of a mature fruit is 25–95 mm. Width at apex, mid-section, and base is, respectively, 14–45 mm, 22–59 mm, and 15–50 mm. Fruits in Vanuatu are longer

and more cylindrical than those in the Solomon Islands. Fruit color is variable from grayish green to purplish red.

Plate 5.2: Variation in fruits of *B. procera.* When ripe, the skin peels off (bottom left). Vovohe in Kolombangara, Solomon Islands.

5.1.3.4 Seeds

The seed or kernel is contained in a fibrous, white to purplish, cylindrical and eight-sided endocarp shell (prominent when exocarp and mesocarp are removed) (Plate 5.3). The fleshy mesocarp is food for animals such as cockatoos and flying foxes, and they disperse the seeds. The testa of green fruits in certain variety can have a reddish/purplish color.

Plate 5.3: Variation in kernels of *B. procera*. Hunda, Kolombangara, Solomon Islands. Colour of testa and shell vary from white to reddish purple. Kernels (right) are whole, from a variety that can be cracked open instead of cutting fruit into half to extract the kernel.

5.1.3.5 Bark description

The bark is smooth in the early stages of growth but becomes fissured as the trees grow older. Large lenticels up to 5 mm (0.2 in) across are present (Jebb 1992).

5.1.3.6 Other species

Thirty-nine species of *Barringtonia* have been recorded around the world of which fifteen are found in the Pacific. Seven of these species (Table 5.1) are indigenous to the Solomon Islands (Payens 1967; Henderson and Hancock 1988).

The three edible species of *Barringtonia* found in the Pacific region (all called cutnut) are: *B. procera, B. edulis* and *B. novae-hiberniae*. Typically, the latter species is distinguished by its simple, near entire leaves. The distinction between *B. procera* and *B.edulis* is more difficult because of their great variability and overlapping morphological characteristics (Table 5.2) (Plate 5.4). Typically, however, *B. procera* is recognized as having glossy leaves, very short to sub-sessile petioles and short to no pedicel.

Table 5.1: Seven *Barringtonia* species in Solomon Islands Source: Payens (1967); Henderson and Hancock (1988).

Species	Common synonym	Status
Barringtonia procera (Miers) Knuth	*B. guppyana* Knuth *B. magnifica* Laut. *B. schuchardtian* K. Sch.	Edible nut
Barringtonia edulis Seem.	*B. seaturae* Guppy	Edible nut
Barringtonia asiatica (L.) Kurtz	*B. littorea* Oken	Inedible
Barringtonia novae-hyberniae Laut.	*B. brosimos* Merr. & Perry *B. excelsa* (non Bl.) Guill. *B. oblongifolia* Kunth	Edible nut
Barringtonia racemosa (L.) Spreng	*B. salomonensis* Rech.	Inedible
Barringtonia niedenzuana (K. Schum.) Knuth	*B. araiorhachis* Merr. & Perry *B. bougainvilleana* Kunth *B. quadrigbbosa* Laut.	Inedible
Barringtonia samoensis A. Gray	*B. rubra* Miq.	Inedible

Table 5.2: Comparative morphological characteristics of edible *Barringtonia* species. Source: Payens 1967 and Evans 1999.

Species	Leaf length (mm)		Leaf width (mm)		Petiole Length (mm)	Pendicel Length (mm)	Fruit shape	Calyx in bud
	min	max	min	max				
Barringtonia procera	45 - 60	48 - 60	15 - 24	17-24	Sub-sessile	Sessile	8-gonous	Closed/Open
Barringtonia edulis	38-45	55-48	15-16	17-23	short	Pedicelled	Ovoid	Closed
Barringtonia novae-hiberniae	20-25	23-35	7-10	8-15	long	Pedicelled	Broad ovoid	Large apical pore

Plate 5.4: Left to right: Leaves and fruits of *B. procera* (dwarf tree), *B. edulis, B. procera*, and *B. novae-hiberniae*. (photo: Barry Evans, reproduced with permission from Evans (1999).

Geographically *B.novae-hiberniae* and *B. procera* occupy overlapping geographic areas (sympatric). *B. edulis* and *B. procera* often have overlapping ranges, but *B. edulis* is absent from New Britain Province in Papua New Guinea and is present in Fiji. *B. novae-hiberniae* is largely undomesticated and thus is commonly found in secondary forests, fallow forests and under coconut plantations, but is less abundant around and within village surroundings.

5.1.4 Variability

In the Solomon Islands, four varieties of *B. procera* have been recorded varying in fruit color, leaf size, and tree height (Table 5.3). One of the varieties has a purplish testa and inner shell (Plate 5.3). However, there has been little formal research on germplasm conservation or tree improvement in this pool of genetic resources. A provenance trial was set up in 1989 at Avuavu on the south coast of Guadalcanal in the Solomon Islands.

Table 5.3: Characteristics of different types of *Barringtonia* within species. Source: Evans 1999

Species	Type	Description
Barringtonia procera	1	Purple/grey fruit
	2	Green fruit
	3	Green fruit + purple endocarp + large leaf
	4	Green fruit and dwarf tree
Barringtonia edulis	1	Purple fruit
	2	Green fruit + purple endocarp
Barringtonia novae-hiberniae	1	Purple fruit
	2	Green fruit
	3	Green fruit + long fruit

5.1.5 Associated plant species

Associated species within the natural range of *B. procera* include: - canarium nut (*Canarium* spp.), breadfruit (*Artocarpus altilis*), coconut (*Cocos nucifera*), Tahitian chestnut (*Inocarpus fagifer*), poumuli (*Flueggea flexuosa*), sago palm (*Metroxylon salomonense*), Malay apple (*Syzygium malaccense*), *Mangifera minor*, *Ficus* spp., *Macaranga* spp.,*Terminalia* spp., and tava (*Pometia pinnata*) (Whitmore 1969; Henderson and Hancock 1988; Clarke and Thaman 1993).

5.1.6 Growth and development

Mean annual height increment (MAI) for trees up to five years is 62 cm, thereafter the MAI is about 1 m. Diameter at breast height appears to be relatively uniform with age. Trees aged at 5 to 20 years old have attained MAI for diameter at breast height in the order of 1.4–1.6 cm.

5.1.7 Propagation

The most common method of propagating *B. procera* is by direct planting of fruits into the field or raising the seedlings in the nursery before transplanting into the field. Procedures for seed collection, processing, storage, pre-planting treatments, germination and nursery management have been described (Pauku 2005a).

Seeds are recalcitrant and lose viability after 3-4 weeks. Lack of appropriate post-harvest extraction, drying, and storage of kernels at village level can be a production constraint.

5.1.8 Pests and Diseases

B. procera is relatively free of major pests and diseases but Leaf miners can be a problem at the seedling stage in the nursery. At maturity, foliar damage appears to be minimal, but developing flowers and fruits are susceptible to attack from pests and diseases. Cockatoo and flying foxes feed on the fruits, and parrots feed on the flowers.

5.1.9 Production systems

B. procera is a component of traditional agroforestry practices in Melanesia (Walter and Sam 2002; Stevens et al 1996; Hancock and Henderson 1988). The species has been planted or protected in homegardens, along boundaries, and in secondary forests. Like canarium nut (*Canarium* spp.), it traditionally indicated occupation and ownership of tribal lands. The species is also used for services such as mulching, soil stabilization, crop shade/overstory, living fences, and windbreaks (Pauku 2005a).

The species is interplanted with other tree species and agriculture crops to maximize farm output (Bonie 1993). In this respect, *B. procera* provides good environmental services such as soil amelioration, shade, and shelter. It is a good middle story companion tree species that provides easy access to the top of clear bole species such as canarium nut, breadfruit (*Artocarpus altilis*), and sago palm (*Metroxylon salomonense*).

Suggested planting spacing for small scale commercial planting is 5–6 x 5–6 m, which gives 278–400 trees/ha (Pauku 2005a). Yields of *B. procera* have been estimated to be 10–50 kg of fruits per tree per year (Bonie 1993; Evans 1999). Yields begin as early as 2–3 years in dwarf cultivars but fruiting generally occurs on the fifth year from planting.

5.1.9.1 Uses and products

Almost every part of the *B. procera* plant is used traditionally, with the kernels being an important food. Leaves and bark are largely important for medicinal purposes. Leaves were used to treat inflammation of the ear and headaches, while sap from the bark has been used for treating ciguatera poisoning, coughs, and urinary infections, and the red leafed form is used as a contraceptive and for abortion (Walter and Sam 2002). Leaves are also traditionally used for wrapping and parceling nuts and kernels. Fallen branches are used for firewood. Despite its poor quality, the wood is also used for crafts and temporary light construction. It is sometimes used for making paddles in the Reef Islands of Temotu Province (Henderson and Hancock 1988).

Fruits are harvested for their edible kernels either at maturity or are collected after they have fallen to the ground when ripe. The kernel inside the fruit is edible, tasty, and highly nutritious and is eaten as a snack or prepared into dishes for a main meal (Walter and Sam 2002). For example, in the western Solomon Islands kernels are roasted and baked into puddings together with edible hibiscus (*Abelmoschus manihot*) and coconut cream. The nutritious raw kernels contain 10% protein and 25% carbohydrate (Table 5.4).

Kernels are mostly grown for domestic use, but are also sold locally. A small export market is emerging. The outer flesh (mesocarp) is inedible by humans, but ripe fruits are attractive and aromatic and can be used as feed for free range chickens. The tree is good for bee forage. The kernel oil has potential for cooking and in body care products.

The edible kernel is the primary commercial product. In the domestic market kernels are sold in fresh, dried, boiled, roasted or in *masimasi*/*lap-lap*, a traditional pudding with edible hibiscus leaves (Henderson and Hancock 1988; Walter and Sam 2002). In the Solomon Islands, a parcel of fresh kernels (extracted from 10–12 fruits) is worth about US$0.15. In terms of international trade, Vanuatu is the only country in the Pacific that exports kernels, in sealed jars (Plate 5.5). *B. procera* has potential to become an export commodity, but currently the supply is

inadequate and market chains underdeveloped. Kernels are extracted by cutting through the fruit with a sharp knife for immediate use or they can be dried or smoked to allow storage for several months (Walter and Sam 2002).

Table 5.4: Chemical composition of *Barringtonia* spp. kernel (100 g). Source: Institute of Applied Science University of the South Pacific cited in McGregor and McGregor (1997).

	+ Raw kernel (Fiji)	+ Raw kernel (Vanuatu)	* Dried kernel (North Qld)
Moisture	38.9	53.8	8.9
Energy (kJ)	1017	929	-
Energy (kcal)	243	222	-
Protein (g)	9.7	8.7	12.2
Total fat (g)	11.8	18.1	-
Carbohydrate (g)	25.1	7.1	14.4
Dietary fibre (g)	10.2	7.7	8.3 (testa removed) 12.2 (testa intact)
FAT (%)			
Saturated			30.0
Monounsaturated			32.8
Polyunsaturated			37.2
MINERALS (mg/100g)			
Sodium	10	16	2.2
Potassium	410	555	760
Calcium	11	11	-
Magnesium	121	115	-
Iron	2.4	2.0	2.8
Zinc	2.3	1.4	4.2
VITAMIN			
B carotene (equiv.)	36 µg	193	-
Thiamin (mg)	0.15	0.10	trace
Riboflavin (mg)	0.02	0.02	0.12
Niacin (mg)	2.6	3.4	4.2
Vit C (mg)	7.0	11	-

+No indication of which species of *Barringtonia*, **Barringtonia procera*

Plate 5.5: From left to right. Bottled kernels (dried) of *B. procera*, *Terminalia catappa*, and *Canarium indicum* for export in Vanuatu. Photo by Roger Leakey.

5.2 *INOCARPUS FAGIFER* (TAHITIAN CHESTNUT)

5.2.1 Introduction

Inocarpus fagifer is a medium size, evergreen tropical tree found in secondary forests, homegardens, coconut plantations, along riverbanks, in swamps and marshes, and within coastal shorelines. *I. fagifer* is commonly called Tahitian chestnut (English) but known by different names in the Pacific. In Vanuatu Bislama it is called *namambe* (Walter and Sam 2002), *aila* in Papua New Guinea (Bourke 1996), *chataignier de Tahiti* (French), *ifi* in Samoa, Tonga, Niue, Horne Islands and 'Uvea and *te ibi* in Kiribati (Thaman and Whistler 1996), and *mworopw* in Pohnpei (Raynor 1991). Some local names of *I. fagifer* in the Solomon Islands include: *ailali* in Kwara'ae (Malaita Is.), *dulafa* in To'oabaita (Malaita Is.), *dola* in Varisi (Choiseul Is.), *mwaqe* in Santa Ana (Santa Ana Is), *Naqi* in Nduke (Kolombangara Is.), *ivi* in Roviana (New Georgia Is.) and Marovo (New Georgia Is.), *julapa* in Bugotu (Isabel Is.), *Zulapa* in Zabana (Isabel Is.) (Henderson and Hancock 1988). It appears that *I. fagifer* was cultivated more

intensively in the past. Today the species is found mostly in wild form. *I. fagifer* is a leguminous, evergreen tree that produces a seed that is edible when cooked, and is among the most important nut species in the Pacific.

I. fagifer is a medium size tree reaching a typical height of 20 m (see front page photo). Some trees in Santa Cruz and Vanuatu, grow to less than 10 m tall (Walter and Sam 2002) and trees in Choiseul and Kolombangara in the Solomon Islands reach 30 m tall. Mature fruiting trees have a typical crown diameter of 4–6 m. The trunk diameter at breast height (dbh) of mature trees ranges from 7 to 90 cm and is typically 30 cm. The trees have a distinctive, short, thick, irregular, and very fluted bole. Branches have a spirally alternate arrangement. Secondary branching creates a network of branches within the dense canopy. In its native range, mature trees of *I. fagifer* are found scattered with varying density. In Veratalevu, Fiji, for example, 206 trees/ha have been found (McGregor and McGregor 1997), compared with an estimated density of 10–20 trees/ha in Kolombangara, Solomon Islands.

5.2.2 Distribution

I. fagifer is indigenous to many South Pacific countries (from Java in the west to the Marquesas in the east). It is found in Melanesian countries (the Solomon Islands, Vanuatu, Fiji and Papua New Guinea) where it is believed to be indigenous (Henderson and Hancock 1988; Bourke 1996; McGregor and McGregor 1997; Evans 1999; Walter and Sam 2002). In parts of Polynesia (Samoa, Tonga, Cook Islands and French Polynesia) and Micronesia (Pohnpei, Marshall Islands and Kiribati), the species is believed to be an aboriginal introduction (Clarke and Thaman 1993). It has been introduced to the Philippines, although traditionally not very much was cultivated (Walter and Sam 2002).

5.2.2.1 Climate

I. fagifer grows in the lowland humid tropics, with warm to hot temperature (20 – 35^{o}C) throughout the year (Pauku 2005b). In its natural range *I. fagifer* does not normally experience a dry season of more than a few months. It has adapted to high rainfall of up to 4300mm per annum. The species tolerates the tropical cyclones which usually occur during the wet season from November to March in

the Solomon Islands, Papua New Guinea, and Vanuatu (Bourke 1996; Evans 1996; Walter and Sam 2002).

5.2.2.2 Soils

I. fagifer has been classified as a beach forest species (Whitmore 1969) and generally grows in poorly drained seasonal to permanently waterlogged soils. It also occurs in soils with medium to very low fertility with mildly acid to alkaline soils (pH 5–14) (Pauku 2005).

5.2.2.3 Tolerances

I. fagifer may become intolerable to prolonged drought of more than several months duration. It commonly grows in areas with full sunlight, although seedlings can grow up through the understory, i.e., in partial shade. It can tolerate up to 20–80% shade. Heavy shading appears to slow down growth of seedlings more than mature trees. As a swamp species, *I. fagifer* rarely experiences fire, and is likely to be intolerant. *I. fagifer* tolerates salt as it naturally grows close to the sea. The trees are windfirm due to their height and a strong lateral root system including buttresses.

5.2.3 Botanical description

The species *I. fagifer* (Parkinson) Fosberg belongs to the family Fabaceae. There are about 480 genera and 12,000 species within this family worldwide (Pedley *et al.,* 1995). This list was earlier on thought to be around 650 genera and about 18,000 species (Wagner *et al.,* 1990). The family Fabaceae consists of a diversity of plant types including trees, shrubs, lianas, vines and herbs, often bearing root nodules that harbour nitrogen-fixing bacteria. These plants usually have alternate or spirally arranged leaves which are rarely simple, flowers with generally 5-toothed sepals (calyx), five petals variously modified – upper petal the largest overlapping the others, outermost in bud, the lowest 2 petals fused to form a keel, and with stamens generally joined into bundle hidden in the keel. Fruits are either dehiscent or indehiscent with 1-seeded segment (Whitmore 1966; Clifford and Ludlow 1978; Wagner *et al.,* 1990; Pedley *et al.,* 1995).

5.2.3.1 Leaves

The leaves are simple, oblong, alternately arranged, dark green and leathery to the touch (Plate 5.6) (Walter and Sam 2002). They are 16–39 cm in length and 7–13 cm in width, and the petiole is 0.5–2.5 cm long. The leaf apex is slightly pointed, the base lobed and the margin entire. Leaf veins are opposite, yellow and conspicuously arranged along the mid-vein.

5.2.3.2 Flowers

The flowers are fragrant and formed at the apex of branches, stems, and twigs clustered along a short rachis (Plate 5.6) (Henderson and Hancock 1988; Walter and Sam 2002). The flowers are about 1 cm in length and have five petals that vary in color from white to yellowish (Growers 1976). Trees begin flowering at an age of 3–5 years in the Solomon Islands. Flowering is seasonal and in most cases occurs in November–December, with fruiting in January–February of the following year. A similar pattern is found in PNG and Vanuatu (Bourke 1996; Walter and Sam 2002).

5.2.3.3 Fruit

The fruits are ovoid but irregular, slightly flattened, and rounded or oblong with a flange down one end (Plate 5.7) (Gowers 1976; Walter and Sam 2002). They are produced either singly or in clusters. Fruits weigh 50–110 g and measure 46–130 mm in length, 34–120 mm in width, and 40 mm in thickness. The skin is smooth and covers a fibrous shell encasing the kernel. Young fruits usually are green but as they ripen the color usually changes from green to orange-brown. However, in some varieties or cultivars the fruits remain green even when ripe. At maturity the fruits are usually indehiscent, although there are some dehiscent varieties. The division of the shell is visible when the mesocarp is removed. *I. fagifer* generally fruits once a year. In Vanuatu, fruits reach maturity between January and April (Walter and Sam 2002). In the Milne Bay region of PNG (Bourke 1996) and parts of the Solomon Islands, especially in Choiseul and Kolombangara Island, fruiting occurs from November to February. In Fiji, two seasons per year have been reported (January–March and May–July), although fruiting is more pronounced in the former (McGregor and McGregor 1997). Considerable year-to-year variation

in the fruiting season has been reported in Fiji (McGregor and McGregor 1997) and the Solomon Islands.

Plate 5.6: Typical flowers and leaves of *I. fagifer*. Hunda (left), and Ringgi (right), Kolombangara, Solomon Islands.

5.2.3.4 Seeds

The white, kidney-shaped seed or kernel is contained in a fibrous, brownish, relatively thin (about 2–3 mm) to thick shell (Plate 5.7). Kernels (seeds) are large, each weighing 5–50 g, and measuring 20–70 mm in length by 16–40 mm in width. The kernel is edible when cooked, but is highly perishable and has a short shelf life. It rapidly changes color from white to reddish brown after being extracted from the shell. The fleshy mesocarp, or pulp, is eaten by flying foxes and cockatoos. These animals bite off fruits and fly with them to other trees, dispersing the seeds. The kernel (seed) must remain encased inside the shell to be viable.

Plate 5.7: Typical fruits (top left), kernels (top right) and fibrous shells (bottom) of *I. fagifer*. Babarego village, Choiseul, Solomon Islands.

5.2.3.5 Bark description

The bark is rough and flaky and varies in color from brown to grayish (Walter and Sam 2002). The grayish color is more common in older trees. Other bark characteristics appear relatively constant with age.

5.2.3.6 Rooting habit

At the base of the trunk are 3–4 thin buttresses that extend up the trunk up to a height of 1 m and reach laterally, snake-like, for a long distance. Sometimes lateral

roots extending from the buttresses are exposed on the soil surface (not buried in the soil); this could well be due to soil erosion.

5.2.4 Variability

I. fagifer displays a variety of forms, and there is great diversity in leaf and fruit size, shape, and color. In Vanuatu, four morphotypes can be distinguished mainly by fruit shape and color—the most common morphotype bears broadly rounded or quadrangular fruits which have a green or brown color at maturity (Walter and Sam 2002). Significant intraspecific variation was observed in fruit shape and color in the Solomon Islands, but requires a quantitative characterization study in order to accurately determine the extent to which this occurs elsewhere. Typically, the species has buttresses at the base of the trunk, but a variety found in the east of Johore, Sarawak and Sabah does not form these (Walter and Sam 2002).

Given the great diversity in size, shape, color, and form of the tree and its leaves, flowers, and fruits and the long history of cultivation, it is highly likely that *I. fagifer* has a number of farmer selected varieties that have not been formally recognized or described. Currently, *I. fagifer* is the only edible and culturally important species in the genus *Inocarpus*.

5.2.5 Associated planted species

Associated species within the natural range of *I. fagifer* include: - canarium nut (*Canarium* spp.), breadfruit (*Artocarpus altilis*), coconut (*Cocos nucifera*), cutnut (*Barringtonia* spp.), *Flueggea flexuosa*, sago palm (*Metroxylon salomonense*), Malay apple (*Syzygium malaccense*), *Mangifera minor*, *Ficus* spp., beach hibiscus (*Hibiscus tiliaceus*), beach she-oak (*Casuarina equisetifolia*), *Intsia bijuga*, *Terminalia* spp., and narra (*Pterocarpus indicus*). In Choiseul, Solomon Islands, *I. fagifer* is commonly naturalized together with coconuts in coastal locations and in woody secondary regrowth, but it occurs with mangroves on muddy shorelines in Kolombangara, Solomon Islands.

5.2.6 Growth and development

Generally, *I. fagifer* growth is moderate but this varies significantly between trees. Seedlings can reach 1–2 m in the first year in ideal conditions. The tree is reported as a fast growing tree in Fiji and the Cook Islands (Thaman 1999). At early stages of growth, *I. fagifer* can be smothered by rapidly growing vines such as *Mikania* and *Merremia*, but mature trees do compete well with other tree species within their native range. Generally, reduced vegetation is found beneath the canopy of the mature trees, although seedlings are usually abundant under the canopy.

5.2.7 Propagation

The common method of propagating *I. fagifer* is by direct seeding into the field or by raising the seedlings in the nursery before transplanting into the field. Procedures for seed collection, processing, storage, pre-planting treatments, germination and nursery management have been described (Pauku 2005b).

Seeds of *I. fagifer* are similar to that of *B. procera* in that they are recalcitrant, therefore can lose viability after 2-3 weeks. Similarly, the lack of appropriate post-harvest extraction, drying, and storage of kernels at village level can be a production constraint.

5.2.8 Pest and Diseases

I. fagifer is relatively free of major pests and diseases, but Leaf miners can attack seedlings in the nursery. Developing flowers and fruits are susceptible to fruit flies. The fruit flies lay eggs on the skin of immature fruits. As the eggs hatch the larvae burrow into the fleshy mesocarp and feed on the kernel, which deteriorates the eating quality. Severe fruit fly infestation may result in 100% loss of the edible kernel. Some types are more resistant to fruit fly infestation than others. Cockatoos and flying foxes feed on the mesocarp of fruits.

5.2.9 Production systems

I. fagifer is a component of traditional agroforestry practices in Melanesia, Micronesia, and Polynesia (Bonie 1993; Clarke and Thaman 1993; Bourke 1996; Walter and Sam 2002). The tree grows well amongst other trees such as canarium nut (*Canarium* spp.), cutnut (*Barringtonia* spp.), oceanic lychee (*Pometia pinnata*), sea almond (*Terminalia catappa*), *Burckella obovata*, and Malay apple (*Syzygium malaccense*) and other multipurpose trees that are either planted or protected in land boundaries, secondary forests, homegardens, and within the surroundings of human settlements. Although yet to be confirmed, it is probably a nitrogen fixing tree that makes atmospheric nitrogen available within agroecosystems. The species has been used for services such as mulching, soil stabilization, crop shade/overstory, living fences, boundary marking and windbreaks (Pauku 2005b).

Suggested planting spacing for small scale commercial planting is 5 x 5 m or 400 trees/ha (Pauku 2005b). Yields of *I. fagifer* have been estimated to be 10–50 kg of fruits per tree per year (Bonie 1993; Evans 1999). Based on limited data, the potential yield for trees in the Solomon Islands is 4–30 mt/ha fresh fruit annually at a density of 400 stems/ha (Pauku 2005b). Annual yields are estimated to increase with age. For example, a 5–10 year old tree is estimated to produce 10 kg fresh fruits per tree increasing to 75 kg fruits per tree older than 25 years (Bonie 1993). Usually, fruiting begins after five years but some plants bear fruits on the third year from planting.

5.2.9.1 Uses and products

Almost every part of the plant has been used traditionally, with the kernels being an important food. Leaves and bark are important for medicinal purposes. The bark was grated and mixed with coconut milk or bark sap to treat urinary infections in the Solomon Islands, while the juice from the mesocarp of green fruits was used in Tonga to treat insect bites and burns (Walter and Sam 2002). Leaves were also traditionally used for wrapping and parceling throughout the Pacific islands. In Fiji, cooked kernels were wrapped with the leaves when sold in

the market (McGregor and McGregor 1997), and in Tonga, the leaves were used for making belts (Walter and Sam 2002) and were once used to cover the ground beneath mats.

Fallen branches are used for firewood, and even green wood is burned to dry copra in Choiseul, Solomon Islands. The wood is also used for crafts, tool handles, canoes, and light construction in Fiji, the Solomon Islands, Vanuatu, and Tonga (Henderson and Hancock 1988; McGregor and McGregor 1997; Walter and Sam 2002). It is used for flooring in Temotu and for making canoes in Renell and Bellona, the Solomon Islands (Henderson and Hancock 1988). Treating the wood with appropriate preservatives may provide protection against wood borers, and increase suitability for light construction purposes. The buttress is used in the Reef Islands (the Solomon Islands) as a platform for dancing; when placed over a hole it provides a resounding tone (Henderson and Hancock 1988). In Wallis, the leaves were sewn together to make sails for boats (Walter and Sam 2002).

Fruits are harvested for their edible kernels either directly from the tree at maturity or are collected after they have fallen to the ground. It is available in Vanuatu between the two yam seasons (Walter and Sam 2002). The edible kernel is an important indigenous food in many island countries in the Pacific. It is an important traditional supplemental staple in Fiji although today its importance has declined in favor of cassava and imported rice (McGregor and McGregor 1997). The kernel must be cooked to make it edible, and is prepared in many different ways, including roasting, grilling, boiling, baking, and mashed in pudding in PNG, Fiji, the Solomon Islands, Vanuatu, and Polynesia (Henderson and Hancock 1988; Walter and Sam 2002). Well known dishes include *lap lap* (Vanuatu) (Walter and Sam 2002), *koko* (Fiji) (McGregor and McGregor 1997), and *masimasi* or *robe* (the western Solomon Islands) (Pauku 2005b). The nutritious raw kernels contain 5% protein and 22% carbohydrate (Table 5.5).

Table 5.5: Chemical composition of *I. fagifer* kernel (100g). Source: Institute of Applied Science University of the South Pacific cited in McGregor and McGregor (1997).

	Boiled kernel (Fiji)	Boiled kernel (Vanuatu)	Raw kernel (Vanuatu)
Moisture	63.3	61.1	76.4
Energy (kJ)	549	507	337
Energy (kcal)	131	121	81
Protein (g)	3.4	4.5	4.0
Total fat (g)	0.8	1.9	0.9
Carbohydrate (g)	27.4	21.5	14.4
Dietary fibre (g)	4.7	6.3	3.0
MINERALS (mg/100g)			
Sodium	7	10	10
Potassium	361	499	338
Calcium	38	21	-
Magnesium	37	46	27
Iron	1.4	1.4	2.7
Zinc	0.8	1.1	3.0
VITAMIN			
B carotene (equiv.)	trace	trace	trace
Thiamin (mg)	0.08	0.12	0.19
Riboflavin (mg)	trace	0.03	0.08
Niacin (mg)	1.8	2.4	1.3
Vit C (mg)	11	4.0	4.0

The kernel is the primary commercial product, and is extracted from the fruit by cutting through the fruit with a sharp knife. In Fiji it is estimated that around 35 tons are sold in domestic markets annually, fetching about US$28,000 or US$0.80/kg (McGregor and McGregor 1997). In the Solomon Islands, kernels are sold fresh for US$0.15 to US$0.30 per kg during peak seasons. The domestic market for the product of this species can be increased if processing technology to improve the shelf life of the kernel is developed. A market study in Fiji revealed export opportunities to Polynesian communities in Australia, New Zealand, and the US (McGregor and McGregor 1997).

CHAPTER 6: VEGETATIVE PROPAGATION

6.1 INTRODUCTION

6.1.1 What is vegetative propagation?

The goal of vegetative propagation is to produce plants that are genetically identical to their parent plant (Hartmann *et al.,* 1997). In simple terms, it involves the creation of new plants from vegetative organs (shoots or roots) with meristematic activity that can initiate new shoots and roots. The new plants produced from a single plant are called a clone. Vegetative propagation is a very powerful tool with which to capture genetic variation in wild populations and mass produce it within domestication programmes (Leakey 1998). The technology is being increasingly used in tree domestication (Leakey and Simons 2000).

Naturally, vegetative regeneration occurs in some plants through specialised structures such as bulbs, corms, tubers or stolons and rhizomes (Hartmann *et al.,* 1997). Good examples of agricultural and horticultural crops that regenerates this way include; *Solanum tuberrosum, Dioscorea* spp*, Ipomea batata, Musa* spp*., Manihot esculenta* and *Saccharum* spp. Vegetative propagation of plants is also an artificial process widely used nowadays in agriculture, horticulture and forestry (Leakey 1985). However, the practice of artificial vegetative propagation is not new as Theophrastus discussed propagation of trees by cuttings and grafts in 300 BC (Janick 1979). Another ancient example is the cloning of Chinese fir (*Cunninghamia lanceolata*) and sugi (*Cryptomeria japonica*) in China and Japan respectively which have a history going back 800 years (Minghe and Ritchie 1999).

There are a number of different techniques of vegetative propagation including grafting, budding, marcotting, rooting stem cuttings, and tissue culture or

micropropagation (Hartmann *et al.,* 1997; Leakey 1998). These techniques are possible because:

a) Plant cells are totipotent and possess the genetic information to regenerate and form new plant tissues to produce plants from undifferentiated meristematic cells.
b) There is continuous cell division during normal growth in most plants.

6.1.2 Vegetative Propagation Techniques

6.1.2.1 Grafting and Budding

Grafting is the art of fusing a shoot scion from one plant onto a rootstock from another to create a new plant. The scion is the upper portion of the union containing several dormant buds which, when united with the rootstock will in time become active and form into new shoots. Scions are usually collected from small branches of a desirable parent plant. Rootstocks are the lower portion of the graft and usually grown from seedlings or can be clonally propagated plants whose origin is often well adapted to local soil conditions. The rootstock grows to form the root system of the grafted plant. When only a single axillary bud is the scion, the technique is called budding.

Grafting is an art that has a long history back to ancient times and was known to the Chinese around 1560 B.C. (Hartmann *et al.,* 1997). The Apostle Paul in his letter to the Romans discussed grafting between the 'good' and the 'wild' olive trees (Romans 11:17-24). Today, the importance of grafting in horticulture and forestry has been particularly realised where fruit and nut trees are difficult to propagate by cuttings or layering (Hartmann *et al.,* 1997), but also for its major advantage in the ability to propagate already sexually mature crops, hence inducing early crop production (Leakey and Simons 2000).

The capacity for compatibility in graft union depends on the close juxtaposition of the cambium across the graft, which gives direct and functional connections within

xylem and phloem (Leakey 1985). However, graft incompatibility can occur as a consequence of anatomical abnormalities of vascular tissue in the callus bridge (e.g. vascular discontinuity in the union area) (Hartmann *et al.,* 1997). In graft formation, the cells from each component of the union must be held firmly in contact, form callus, unite and differentiate to fuse the union (Leakey 1985; Hartmann *et al.,* 1997).

The success of grafting is a composite function of many genetic, environmental, anatomical and physiological factors (Leakey 1985). Genetically, it is important to note that success is greatest between closely related plants (Hartmann *et al.,* 1997) because the tissue rejects any foreign proteins. Successful grafting between plants of different families and genera (heteroplastic grafting) is rare because they have incompatibilities (Leakey 1985), but over 800 combinations are known (Sziklai 1967 cited in Leakey 1985). Homeoplastic grafting between clones of the same species is often easier and with greater union compatibility akin to autoplastic grafts within a clone, although occasionally some species (e.g. peaches) will graft better on to other species than on to themselves (Leakey 1985).

The environmental factors are also important to the success of grafting. The graft union must be protected from loss of cell turgidity and desiccation, attack from pests and diseases (e.g. virus infection), and fuses best under an optimal temperature that enhances rapid cell (callus tissue) growth (Leakey 1985). Vascular tissues (e.g. cambium) can be damaged during insertion of the scion into the stock, thus due care must be taken to prevent this happening. In temperate regions, grafting is most suitable in spring when temperature is favourable and plant tissues are active because winter dormancy has been broken. Under such conditions, for example, around 90% grafting success was achieved in *Pseudotsuga menziesii* (Copes 1970). In the tropics similar conditions occur in the transition from dry to wet seasons (Okoro 1976).

In terms of physiological factors, the scion orientation is important for the growth of the graft union because of the polarity of plant tissues. Therefore, for example, in stem grafts, the proximal end of scion is inserted into the distal end of the stock,

but when shoots are grafted directly on to roots, their proximal ends are joined together (Hartmann *et al.,* 1997). It has been recognised that the physiological conditions of the scion and stock are important for the successful development of grafted union, however, there is not as much physiological data available to indicate which conditions are important as there is for the successful rooting of cuttings (Leakey 1985). Huang and Millikan (1980) working on apple *in vitro* compared seasonal variation using *in vivo* and *in vitro* micro-grafted scions. They found consistent success year-round when scions were grown *in vitro*. The success in the graft involving *in vivo* grown scions is usually best when the scion is dormant, but has been chilled, while the rootstock is in active growth or just beginning (Holst *et al.,* 1956). There may also be some factors associated with endogenous hormonal activities.

Graft incompatibility is also attributed to biochemical events and has been classified as 'translocated' incompatibility and 'localised' incompatibility (Jeffree and Yeoman 1983). The latter includes graft combinations in which a mutually compatible interstock overcomes the incompatibility of scion and stock, while incompatibility in the former cannot be overcome by the insertion of a compatible interstock (Hartmann *et al.,* 1997). In translocated incompatibilities, the phloem degenerates and suffers necrosis. By contrast, the localised incompatibilities often occur due to translocation difficulties, such as the abnormally early termination of xylem growth (Copes 1975).

6.1.2.2 Marcotting

Air layering or marcotting is a technique of rooting a stem while it remains part of the parent plant (Hartmann *et al.,* 1997). The physical attachment of stem to the plant during rooting permits continuing translocation of water, mineral, carbohydrates and hormones through the vascular tissues. Layering involves the wounding or girdling of the chosen stem and subsequently wrapping the wounded portion with some soil or rooting media enclosed in polythene plastic. Over time the stem develops roots and is later detached from the parent plant and grown separately to become a new plant with its own roots. The technique was popular in Europe during 18th to early 20th centuries for propagating woody shrubs and tree

species (McDonald and Lassoie 1996). This is a useful technique for capturing the mature stage of a selected phenotype for domestication.

6.1.2.3 Rooting stem cuttings

Plants can also be propagated by taking stem cuttings (a portion of a stem with an axillary bud) and inducing it to root and develop into a new plant. Up to 90% of tropical forest tree species experimented so far have been successfully rooted from leafy stem cuttings (Longman 1993). Two distinct types of stem cutting used are: leafless stem cuttings and leafy stem cuttings. The leafless stem cuttings are often large (10 – 50 cm) woody shoots containing reserves that can be mobilised to develop roots. Good examples of species having potential for this type of propagation include; *Manihot esculenta*, *Gliricidia sepium*, and *Pterocarpus indicus*. By contrast, leafy stem cuttings are vulnerable to water loss because their tissues are young and succulent and relatively unlignified. They require high humidity or intermittent misting to survive. The important difference between the two types of cuttings is that the former rely on carbohydrates stored within the stem tissues and the hydrolysis of starch reserves to allow root growth, while the latter is dependent on current photosynthates produced while in the propagation bed (Leakey 1985, 2004b).

Vegetative propagation by stem cuttings is used on a commercial scale for the production of clonal materials in many places. For example, large-scale reforestation programmes with clonal *Eucalyptus* hybrids occurred in the Congo Republic and in Brazil, while clonal improvement program on *Gmelina arborea* was undertaken at Sabah Softwoods, near Tawau Sabah and on *Triplochiton scleroxylon* in Nigeria and Ivory Coast (Leakey 2004a). In Solomon Islands, the Kolombangara Forest Products Limited (KFPL) has been planting clonal *Gmelina arborea* using stem cuttings since 1990s. However, care must be taken when using lateral shoots to avoid plagiotropic growth. This non-erect growth is commonly found in genera such as *Araucaria* and *Agathis* and in agricultural crops (e.g. cocoa and coffee) when using lateral shoots (Tchoundjeu 1989).

6.1.2.4 *In vitro* propagation

Not all plant tissues are suitable for *in vitro* propagation (Leakey 1985), especially long lived perennials, because they do not respond to tissue culture manipulations; a condition in the plant tissue being referred to as "recalcitrance" (Benson 2000). This is a big challenge in the biotechnological approaches to exploit economically important plant species. McCown (2000) reported that "recalcitrance" is genetically driven and is therefore difficult to control by environmental and nutritional manipulations in microculture. However, this approach to plant propagation continues to be rapidly developed and often involves costly facilities and special skills. Living plant parts are taken from a parent plant and cultured under controlled environments. They are then grown in small containers with the nutrients and specific plant hormones needed to control the growth and subsequent development of the new plant.

Essentially, the capacity for *in vitro* propagation extends to and within the limit of the establishment and maintenance of aseptic and conducive growth environment for speedy division and subsequent differentiation of cells (Benson 2000). The explants must be free from external contaminants which thrive in culture, and thus must be kept in sterile conditions, while being provided with, a) macro- and micro-nutrients, b) a source of energy, normally sucrose, c) vitamins such as amino acids, etc, and d) a balance and sequence of plant growth hormones and co-factors to regulate and control the subcellular processes of cell division and differentiation of shoot, root or embryo (Taji *et al.,* 1993). Factors such as pH of growth medium (either as solid or liquid), osmotic pressure and the physical environment also influence the success of tissues cultured under *in vitro* propagation.

There are three types of *in vitro* propagation: organogenesis (callus culture), embryogenesis (i.e. production of somatic embryos) and meristem proliferation or micro-propagation (Hartmann *et al.,* 1997). A problem common to these systems is the exudation of toxic phenolic compounds into the medium. However, corrective techniques have been used to minimise this problem, including the use of sodium hypochlorite rather than alcohol. Explant sterilization can be achieved

by soaking the explants for 1 hour in hot-water at 42.5^{o}C prior to culturing them (Gamborg and Phillips 1995; Langens-Gerrits *et al.,* 1998). Culturing in the dark with activated charcoal was essential to prevent phenol exudation which otherwise can lead to the loss of cultures (Birmeta and Welander 2004).

With organogensis, the adventitious shoots and or roots are differentiated from cultured callus (Hartmann *et al.,* 1997). This approach is important for its capacity in attaining a high multiplication rate of individual cells, and was successful in *Kigelia pinnata* (Family: Bignoniaceae), a fast growing multipurpose tree used traditionally for ornamental and medicines by aboriginal people and traditional healers in India (Thomas and Puthur 2004). However, undesirable genetic changes can occur during organogensis (Leakey 1985). In forest trees, organogenesis most commonly occurs in callus cultures obtained from embryos or from hypocotyls and cotyledon explants, as found by Giovannelli *et al.,* (2004) in chestnut (*Castanea sativa* Mill.). They also found that growth regulators did not significantly influence root regeneration, but differentiation of shoot primordia from callus requires cytokinins.

With embryogenesis, the development of embryos occurs from the vegetative cells instead of the union of male and female gametes (Hartmann *et al.,* 1997). This approach to *in vitro* propagation has been reported to be successful with different plant parts in a number of forest and agricultural trees. For example, in *Coffea arabica* and *C. canephora,* leaf and stem explants were successful in the medium treated with triacontanol (TRIA) Giridhar *et al.,* (2004). In *Quercus suber* L., Pinto *et al.,* (2002) cultivated somatic embryos from leaf explants, with on average, 7.5% of the initial explants formed embryogenic calluses, and 10% of the somatic embryos germinated, of which 40% converted into plants. Silva and Debergh (2001) found staminoide explants more successful than the petals in *Theobroma cacao*, while Steger and Preece (2005) found significant interaction between genotype and media when using explants from cotyledons of immature seeds of *Juglans nigra* L. Depending on species and medium used, the embryo development can be affected by certain growth regulators such as auxins, cytokinins and gibberellins as well. For example, in *Eucalyptus nitens* Maiden

(shining gum), Gomes and Canhoto (2003) found the best multiplication rate (2.25 somatic embryos per culture) when seedling shoots were cultured on a medium containing growth regulators (e.g. IAA and IBA) in combination with benzyladenine. By comparison, growth regulators were found to inhibit embryogensis of *Citrus* on medium containing galactose (Kochba *et al.,* 1982). The addition of benzyladenine was required later to germinate the embryo.

Micro-propagation is the third form of *in vitro* propagation. It involves the establishment of a new plant from small pieces of meristematic tissue. This approach has been applied successfully to a number of tree species and tropical plantation food crops (Taji and Williams 1996). Micro-propagation involves four basic steps: establishment of explants, multiplication of microshoots, rooting of new plants and progressive acclimatization of young plants (Gamborg and Phillips 1995). These steps are to a certain extent interdependent so the importance of getting each step done correctly is the key to successful propagation of a desirable genotype.

In the establishment stage, ensuring that tissues from stockplants are disease free is crucial. Explants are therefore sterilized (e.g. sodium hypoclorite) to get rid of potential diseases and other contaminants. Activated charcoal, ascorbic acid or citric acid are added to the medium to control exudation of undesirable substances, such as phenolics from the cut surface, which could inhibit development (Taji *et al.,* 1993). The choice of explant to use – its origin, size and type is important, for example, explants must be collected from shoot tips of plants with relatively active growth.

Stage two in micro-propagation involves shoot multiplication from the loss of apical dominance, so that the cultured explant grows into a cluster of microshoots arising from the original single bud. These microshoot clusters need to be frequently subcultured or split up onto new culture medium to bring about the multiplication of the clones. The medium usually contains higher levels of cytokinin and minerals than in stage one - depending on the species, cultivars and type of culture (Hartmann *et al.,* 1997).

The third stage in micropropagation involves the rooting of shoots that have been formed *in vitro*. Individual shoots are usually removed and placed on new growth medium modified to induce rooting. This also prepares the microplants for the subsequent transfer from artificial heterotropic environment of the test tube to an autotropic free-living condition (Hartmann *et al.,* 1997), and the final stage of micro-propagation in greenhouse or nursery environments. This stage involves the removal of rooted plants from the culture vessel, the removal of agar by washing plantlets carefully with water to get rid of potential agar related contamination and finally the transplanting of planlets into suitable potting medium, which initially must be retained under shade with high relative humidity (Hartmann *et al.,* 1997). Once the functional roots are formed within several days the plantlets are then gradually exposed to lower relative humidity and higher radiation.

6.1.3 Benefits and Limitations of Vegetative Propagation

Most tropical tree species are out-breeding in the wild and so possess extensive genetic diversity which is normally distributed. This occurs by random mating between trees within and between populations. During meiosis, which is a cell division process that segregates sex cells, the diploid chromosome number was halved (haploid), but it was later restored during the fertilization process, resulting in the creation of new individuals containing chromosomes from both parents (Mauseth 2003). The progenies inherit a particular combination from their parents (Hartmann *et al.,* 1997). Thus, sexual propagation results in siblings which are different from each other. Consequently, within wild populations there are individuals, which are both considerably worse and better than the population mean. Vegetative propagation allows the rapid multiplication of individual plants that have some particular value to mankind for food, medicines, construction products, etc. It is therefore a powerful tool for domestication.

6.1.3.1 Advantages

The advantages of vegetative propagation are: -

i. the ability to capture superior genotypes or "plus" trees in the wild or planted populations, leading to improved planting material (Leakey 1991; Leakey and Simons 2000).

ii. the ability to multiply desirable and rare genotypes arising from breeding and genetic selection (hybridization or genetic engineering) in large quantities, so enhancing genetic improvements available for future production populations (Leakey 1991; Mudge and Brennan 1999).

iii. the ability to overcome shortages in supply of seeds by producing alternative planting stock therefore provide a sustainable supply of best and high quality planting materials (Leakey 1991). This is especially crucial when propagating tree species, hybrid or selection of biologically seedless individuals (Mudge and Brennan 1999).

iv. vegetative propagation areas are easier to manage and maintain compared to a seed orchard. Stockplants management is crucial to the successful rooting of cuttings and so factors such as light, water and nutrition known to influence rooting (Leakey 1991) can be easily manipulated within a relatively small garden rather than a large seed orchard.

v. ability to capture the mature reproductive condition of trees and so to form new plants with the ability to flower and fruit early (Leakey 2004a). For example, in Lychee (*Litchi chinensis*) early fruiting achieved through marcotting (Mudge and Brennan 1999).

6.1.3.2 Disadvantages

There are fewer disadvantages of vegetative propagation, they include:

i. the expense of the techniques that are applied. For example tissue culture involves environmentally controlled buildings and a skilled work force. Grafting too can be more expensive, similarly budding is three times more

costly than cuttings (Maynard and Bassuk 1990). The need for expensive facilities can be overcome by low cost technology such as poly-propagator (Leakey *et al.,* 1990) developed for raising stem cuttings, appropriate in rural tropics where electricity and pipe water are often lacking.

ii. the risk of reducing the genetic diversity of the propagated population by multiplying a few genetically identical genotypes, that may have originated from a narrow genetic pool. This can be overcome or minimised by using clones from unrelated populations (Leakey and Simons 2000).

6.1.4 Factors influencing rooting ability of stem cuttings

The factors affecting the rooting ability of stem cuttings are numerous and complex but can be classified as:- Pre-severance and Post-severance. Pre-severance, rooting ability is dictated by physiological and morphological differences arising between cuttings from:- within the shoot, between shoots, between plants, between clones, between species and between environments. Post-severance, however, rooting ability of cuttings is affected by the interactions between pre-severance differences in physiology and morphology and the propagation environment and post-severance treatments (Leakey 1985, 2004b). These factors can be addressed and manipulated by:- propagation environment, post-severance treatments and stockplant factors such as the cutting origin, and the pre-severance stockplant environment.

6.1.4.1 The propagation environment

A conducive propagation environment will promote physiological activity through active photosynthesis and transpiration occurring in the leaf, and by reducing physiological stress in the tissues of the cuttings resulting from loss of turgor or respiration. This will enhance meristematic activity (mitosis and cell differentiation) in the stem (Leakey 2004b). Mesén *et al.,* (1997a) suggest that the physiological shock experienced by cuttings as a result of severance from the stockplant, is minimised by optimising the propagation environment.

Cuttings can be raised in different types of propagating systems. One of the systems involves the use of an 'airtight, watertight, high humidity, non-mist propagator' (Leakey *et al.,* 1990). The other two common systems for leafy cuttings include: intermittent mist and fogging. The non-mist poly-propagator (Fig. 6.1) is appropriate for the rural tropics where electricity and piped water are often lacking. This is because of its low cost, lack of functioning parts and effectiveness (Leakey *et al.,* 1990). The lid of the propagator must only be opened when absolutely required, because vapour pressure deficit (VPD) increases when the lid is opened (Newton and Jones 1993). When closed, the system creates a good microenvironment for rooting with low temperature, high relative humidity and a low vapour pressure deficit (VPD), which together minimise water stress in cuttings. The system also provides sufficient light essential for photosynthesis. Interestingly, however, maximum photosynthesis in cuttings occurs at relatively low irradiance (Leakey 2004b), when physiological stresses are minimised.

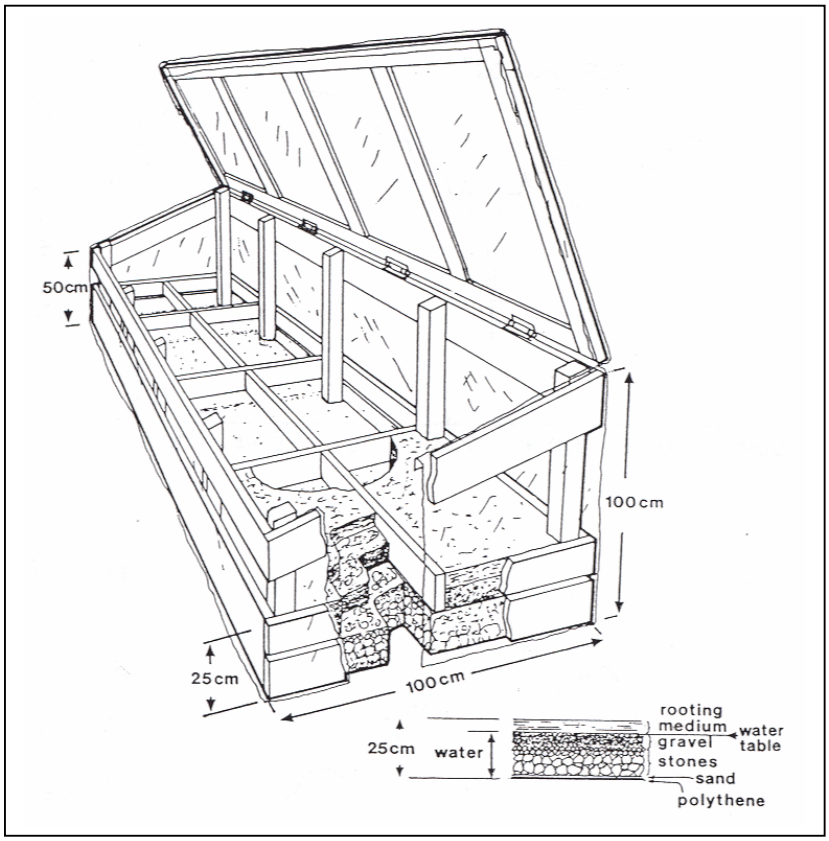


Fig 6.1: A diagram of non-mist poly-propagator (Source: Leakey *et al.,* 1990).

Another important aspect of the propagating environment is the rooting media. The rooting media must provide physical stability, moisture and allow aeration and the respiration from tissues to occur efficiently at cutting base (Dick *et al.,* 1994). To create a good growth environment, filling the poly-propagator with layers of different size particles such as gravel, coarse sand and a good water retention medium (e.g. sand, peat or coir - decomposed coconut husks) keeps the medium damp to achieve different air: water ratios as needed for different species and maintains high relative humidity inside the poly-propagator (Leakey *et al.,* 1990).

A positive carbon balance occurs when cuttings are producing assimilates faster than they are lost through respiration (Mesén *et al.,* 1997a), and this is critical to the formation of adventitious roots in cuttings. Discussion about the importance of carbon content in rooting cuttings has dated back to the early 20th century (Kraus and Kraybill 1918 cited in Veierskov (1988)) and the hypothesis that a high C/N ratio in cuttings was desirable for adventitious root formation. This hypothesis has been widely accepted, although a few studies have questioned its validity. Das *et al.,* (1997) studying the metabolic changes during rooting in pre-girdled stem cuttings and air-layers of *Heritiera fomes* and *H. littoralis* found that a low C/N ratio promoted root initiation.

In further pursuit of understanding about such relationships, Dick *et al.,* (1994) used an oxygen electrode to study stem respiration in leafy cuttings of *Prosopis juliflora* during the rooting process. Their findings indicate that the rate of respiration per gram of dry matter decreased linearly towards the cutting base, with increasing stem diameter. They suggested that this could be attributed to an increase in non-respiring lignified tissues. They also suggested that carbon losses are probably more than compensated for by greater mass of tissues in cuttings with larger diameters, and so the total respiration was greatest in cuttings with larger diameters. They found that the respiration is greater in the 1 cm long segment at the cutting base than in the stem above, creating a concentration gradient which increases transport of assimilates to the base and enhances rooting. These findings have been used to validate a model of the rooting processes developed by (Dick and Dewar 1992) on carbohydrate dynamics during adventitious root development

in leafy cuttings. It uses the rate of respiration at the base of the cutting as the driver for the translocation of assimilates to root initials.

6.1.4.2 Post-severance treatments

There is a considerable body of literature on the effects of post-severance treatments on the rooting of stem cuttings, although most have failed to produce a consistent result on the effects of post-severance treatments between and within species. This is because the studies have largely presenting situation-specific results, rather than improving the physiological understanding of the important post-severance factors such as the application of auxin, leaf area, and cutting length and diameter (Leakey 2004b).

a) Role of auxin

Auxin, the Greek word "to increase or grow" (Saupe 2004), is one of many plant growth substances that exist naturally in plants (Hartmann *et al.,* 1997), and which play crucial roles in root initiation and development (Gaspar and Hofinger 1988) by promoting the transport of carbohydrates basipetally in stem cuttings (Saupe 2004). Auxins originate in actively growing tissue such as young shoot apex, leaves and fruits, and they move from the shoot tip towards the roots (Saupe 2004). They are responsible for the polarity of shoots (Hartmann *et al.* 1997).

The main aims in the application of auxin to cuttings are to:

- ⇒ Hasten root initiation
- ⇒ Increase percentage of rooted cuttings
- ⇒ Increase the number and quality of roots
- ⇒ Increase uniformity of rooting

Such beneficial effects have been recorded in many species including *Cordia alliodora* in which 70% rooting of cuttings treated with 1.6% Indole-3-butyric acid (IBA) compared to 10% rooting in control cuttings (Mesén *et al.,* 1997b). Similarly, Brennan and Mudge (1998) found in *Inga feuillei* that 0.8% IBA enhances percentage rooting twofold success, and on the number of roots per rooted cutting threefold in large diameter (8-20mm) cuttings.

Synthetic auxins have been formulated and widely used to promote rooting in horticulture and forestry, either alone or in combination, with other growth regulators (Leakey 1985). IBA is considered the most effective (Leakey 1985, 2004b), however, α-napthalene acetic acid (NAA) has also been found to be effective in certain species – e.g. *Parkia biglobosa* (Teklehaimanot *et al.,* 1996). Generally, IBA has been recommended in rooting stem cuttings for a number of plant species including many woody species (Leakey *et al.,* 1982b).

b) Importance of leaf area

The leaf is responsible for a number of functions including light interception and gas exchange (Atwell *et al.,* 1999). The presence of leaves on cuttings exerts a strong stimulating influence on rooting (Hartmann *et al.,* 1997). This is because the leaves play a vital role in photosynthesis which supplies the cutting with the assimilates that hasten root formation. Consequently, cuttings that shed leaves or have necrotic, bleached, diseased and rotting leaves typically fail to root. According to Leakey (2004b), leaf abscissions on cuttings can be due to (a) the tissues in the cuttings being too old (senescent) and are being photosynthetically inactive as a consequence of passing their compensation point, water stress and a high concentration of starch in the leaves, and (b) an unfavourable growth environment that is either too hot, too cold or too dry.

Optimising the leaf area of cuttings is necessary to achieve a balance between photosynthesis and transpiration (Newton *et al.,* 1992) and is particularly important in difficult-to-root species (Leakey 1985). The optimal leaf area to achieve this balance varies (Tchoundjeu 1989; Aminah *et al.,* 1997) depending on species and clone-specific factors, like leaf area, stomatal density, leaf morphology (waxiness) and the age of the leaf (Leakey 2004b). Leaf areas of 50 cm^2 were optimal to achieve optimal rooting in cuttings of *T. scleroxylon* (Leakey *et al.,* 1982a) compared to 100 cm^2 in *Khaya ivorensis* and 200 cm^2 in *Lovoa trichilioides* under a non-mist propagator (Tchoundjeu 1989). In *Terminalia spinosa* Engl., Newton *et al.,* (1992) found that the leafless cuttings did not root in contrast to 80% rooting in cuttings with leaf area of 7.5cm^2, 15cm^2 and 30cm^2 after 3 weeks.

c) Length of cutting

Leafy stem cuttings can be cut to any length that is desirable for rooting. One option is to have a constant length which may then be formed from a different number of nodes, while another is to select and use certain number of nodes present on the stem (i.e. 1, 2, or 3 node cuttings) which may then differ in length depending on the internode. The decision about which option to use may depend on the purpose. For practical purposes, long cuttings usually root best, and give a uniform planting depth and height above the medium and hence, are easy to manage (Leakey 2004b). In addition, long cuttings may supply greater reserves of carbohydrates, assimilates and other essential substances for root development and an early and faster cutting growth (Komissarov 1969). For research purposes, it may be useful to use cuttings of different length. For example, Leakey and Mohammed (1985) in *Triplochiton scleroxylon* used different cutting lengths with basipetal and acropetal gradient to develop greater understanding of the within-shoot factors affecting rooting.

6.1.4.3 Stockplant factors

Two sources of variation in rooting ability are attributable to stockplant factors associated with the origin of cuttings (within shoot and between shoot) and to the stockplant environment (Leakey 2004b). These are discussed below:

a) Origin of Cutting: Within shoot and between shoots

There are numerous gradients of variation within any shoot (Leakey 2004b). Variation by age is one which affects the leaf size, leaf water potential, leaf carbon balance, leaf senescence, internode length, internode diameter, stem lignification, nutrient and stem carbohydrate content and respiration (Dick *et al.,* 1999; Tchoundjeu and Leakey 2000). These gradients of variation suggest that no two cuttings are physiologically and morphological identical, thus, the capacity of any two cuttings to root are different (Leakey 2004b).

Furthermore, the prior management of the stockplant affects the rooting of cuttings. In *Triplochiton scleroxylon* the ability of auxin-treated cuttings to root is

affected by treatments imposed on the stockplant (Leakey 1983). For example, a greater proportion of cuttings rooted from upper than lower nodes of stems on undecapitated (control) stockplants. This is in accordance with the gradients of factors in the stem at the time of severance. However, the rooting percentage of cuttings from unpruned stockplants was lower (15-43%) than cuttings from the lateral stems of pruned stockplants of the same size and origin (40-83%).

Trees grow from a juvenile to a reproductively mature state and eventually reach a threshold above which any newly developing shoots have the capacity to produce flowers and fruits. This is the process of ontogenetic ageing and is called "Phase Change" (Leakey 2004b). The rooting ability of cuttings is said to be affected by this gradient towards reproductive maturity, because cuttings from mature shoots are much more difficult to root compared to cuttings from juvenile shoots (seedling or coppice) (Leakey 2004b; Dick and Leakey *in press*). For example, early studies found auxin to be ineffective on difficult-to-root cuttings from mature phase *versus* juvenile phase plants of the same species (Hess (1959); Porlingis and Therios (1976) cited in Hackett (1988)). This finding suggests that endogenous auxin level is not the major factor limiting rooting in mature plants and that other factors such as the physiological condition of the shoot, and environmental variables may be important (Brennan and Mudge 1998; Fett-Neto *et al.,* 2001). Nevertheless, others have reported reasonable success in rooting cuttings from old mature trees (Schneck 1997). These contrasting findings necessitate further investigation. One possibility is that physiological aging is critical, rather than ontogenetic aging (Leakey 2004b).

b) Environment

The most important environmental factors affecting rooting include light and nutrients (Leakey 2004b). Light has three properties that are important for plant growth and development: quality (colour or wavelength), quantity (light intensity, total radiation or photoflux density) and duration (day length) (Mauseth 2003). The quality and quantity of light change as light passes through green leaves in the forest canopy (Fig 6.2). About 40-50% of the solar energy received falls within the spectral region of 390-760 nm (wavelengths perceived as the visible

light)(Gardner *et al.,* 1985). Two extremes of the light spectrum are the short-wavelength of ultraviolet radiations (100-380nm) and the long-wavelength of infrared radiations (780-3000nm). In the infrared region, leaves reflect 70% of radiation, but they reflect no more than 3% of Ultraviolet radiation (Larcher 1980). The visible light, which coincides well with what plants use for photosynthesis (= photosynthetically active radiation (PAR), wavelengths between 400 - 700 nm), is reflected by leaves at an average of 6-12%, albeit, more in green (10-20%) than orange and red lights (3-10%). Larcher (1980) reported that the capacity to reflect light depends on the nature of the leaf surface (e.g. wax, etc.) and hence, between species and possibly cultivars.

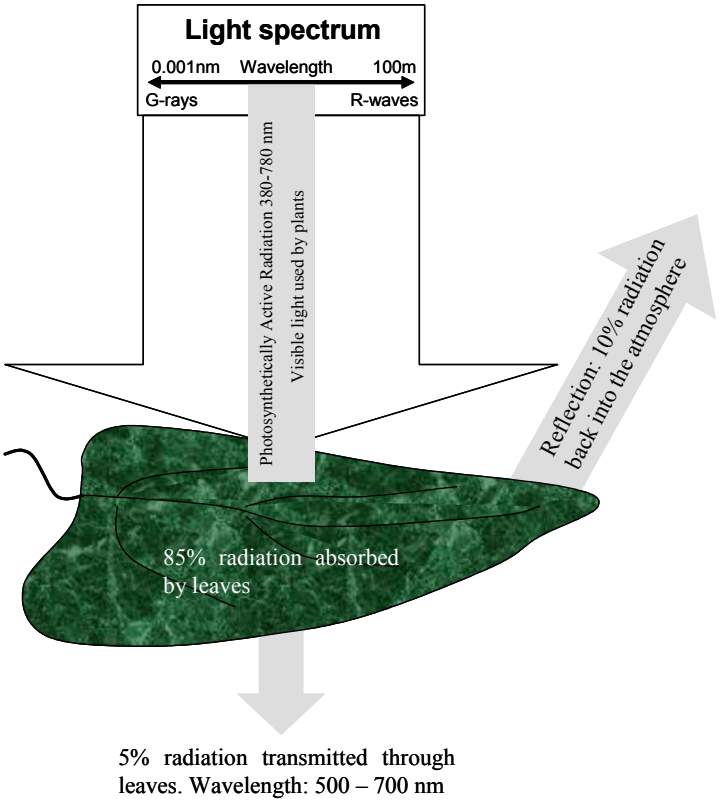


Fig 6.2: A simple schematic representation of light interception by forest canopy

Absorption of light by plant leaves varies for different wavelengths of light (Mauseth 2003). For example, most UV is retained by the outerlayer of the epidermis and the phenolic compounds in the cell sap in this layer, so only 2-5%

enters the deeper cells of the leaf. Effectively the epidermis acts as filter protecting the parenchyma in which the photosynthesis takes place. Larcher (1980) has reported that plant leaves do not absorb much infrared in the region to 2000 nm. In contrast, leaves absorb 85% of photosynthetically utilizable visible light in their chloroplast pigments (chlorophylls and carotenoids) (Atwell 1999). As light passes through a leaf it is attenuated, hence the increase in the amount of light captured by successive cell layers is almost exponential (Larcher 1980; Atwell *et al.,* 1999).

The quality of light transmitted through the leaves depends on leaf structure and thickness (Larcher 1980), which vary according to genotype and can also be influenced by environmental factors (Atwell *et al.,* 1999), especially adaptation of plant species to different climatic conditions. Thick and leathery leaves may not transmit any light at all Larcher (1980), but about 5% sun's irradiance is transmitted through leaves (Atwell *et al.,* 1999). According to Larcher (1980), reflection is high at wavelengths which have great transmission like the green and near infrared wavelengths. Radiation filtered through the leaves is therefore around 500 – 800 nm wavelengths.

Light is required as a source of energy for photosynthesis, and optimal photon flux density and duration will ensure adequate assimilate production for growth, in excess of their use in respiration. The effects of stockplant irradiance and subsequent rooting of cuttings have been controversial (Andersen 1986). Thus findings have suggested that increased irradiance may inhibit or delay, promote or have no effect on rooting (Moe and Andersen 1988). Various studies have found that cuttings of *Pinus sylvestris* (Hansen *et al.,* 1978), *Populus* spp. and *Salix* spp (Eliasson and Brunes 1980) and *Malus* spp (Christensen *et al.,* 1980) rooted readily when stockplants were exposed to irradiance below photosynthetic saturation point. However, it is obvious that both the stockplant and cuttings require a certain minimal level of light to photosynthesise. The optimal level of light required for stockplant may vary between species and cultivars (Moe and Andersen 1988).

Studies on *Triplochiton scleroxylon* cuttings under artificial lighting have had high rooting percentage at lower red to far-red ratio (R:FR) light (1.6) than cuttings exposed to high R:FR ratio of 6.3 (Leakey and Storeton-West 1992). Similar studies in *Eucalyptus grandis,* involving pruned stockplants (7-10 cm height) which were subjected to a photon flux density (PFD) of 200 μmol m^2s^1 and red to far-red ratios (0.4, 0.7, 1.3, 3.5 and 6.5) showed significant effects on shoot development and partitioning of dry matter (Hoad and Leakey 1994). For example, shoot length was greatest at R:FR ratios of 0.4 and 0.7, while in the partitioning of dry weight between leaves and stems, these low R:FR ratios of radiation resulted in greater stem dry weight than leaf dry weight. Additionally, the partitioning of dry weight and leaf area between the most dominant shoot and all other shoots was greatest at low R:FR ratios. Hoad and Leakey (1994) also found that the photosynthetic rate per unit leaf area and the level of leaf chlorophyll increased with increasing R:FR ratios, however, photosynthesis per unit chlorophyll concentration is greatest at low R:FR ratios. This implies that photosynthesis is enhanced by low R:FR, although factors such as rate of transmission, stomatal conductance and water use efficiency do have effects on the rate of photosynthesis as well, because they increase with rising R:FR ratio (Hoad and Leakey 1994, 1996). Furthermore, these responses are due to the regulatory activity of phytochrome - pigments or photoreceptors that monitor the light environment (stimulated by red light or the length of a dark period) and regulate different photomorphogenic responses to optimise growth and development of plants (Whitelam *et al.,* 1993; Nakasako *et al.,* 2005), as they absorb red (Pr) and far-red (Pfr) lights, and ultimately affecting vegetative phenotypes of the plant (Weinig 2002).

The growth regulatory effects of R: FR ratios were subsequently found to be related to differences in rooting capacity (Hoad and Leakey 1996). For example, in *Eucalyptus grandis*, (Hoad and Leakey 1996) found that a high percentage of rooting was achieved by cuttings with longer stems and greater volume from stockplants exposed to low R:FR ratio (0.4 and 0.7). This relates to low pre-severance starch and water-soluble sugar concentrations, and a greater total water-soluble carbohydrate content (TWSC) in cuttings. In addition, they found that

there is a difference in concentration of TWSC and starch in leaf and stem. Cuttings originating from high R:RF ratios (3.5 and 6.5) have a greater concentration of TWCS and starch (2-3 and 3-4 fold respectively) in their leaves than in stems.

In *Albizia guachapele* low irradiance (200 µmol $m^{-2}s^{-1}$) favours rooting with 53.8% rooting compared to 11.2% at high irradiance (500µmol $m^{-2}s^{-1}$) (Mesén *et al.,* 2001). Low irradiance also encouraged shoot elongation in the stockplants, but more of NPK fertiliser reduced shoot elongation and lowered rooting percentage. The authors suggested that this difference in response to nutrient by cuttings might be the result of a diversion of nutrients in the high nutrient treatments to leaf biomass production rather than root formation. Additionally, the result could be due to the inhibitory effect of excess nutrients accumulated in soils in the high nutrient treatments, as evident in the reduction in the photosynthetic rates in stockplants grown under the high nutrient and irradiance treatments as explained by Leakey (1983) when observing differences in rooting ability in shaded shoots.

Mineral nutrition is among many factors affecting formation of adventitious roots in cuttings. In order to enhance a physiological condition that promotes rooting, stockplants must be maintained under optimal nutrition before harvesting propagules (Hartmann *et al.,* 1997). Interaction between mineral nutrients and adventitious rooting is complex, although they are intimately related (Blazich 1988). As rooting is a multi-stage process rather than a single event, the influence of mineral nutrients can have different impacts at different stages in rooting, namely; root initiation and root growth and development (Blazich 1988; Hartmann *et al.,* 1997). However, response to mineral nutrients by cuttings can be unpredictable as was evident from experimentation with *Triplochton scleroxylon* (Leakey 1983). It was found that the application of complete fertilisers on the stockplants of *T. scleroxylon* enhances the rooting ability of shaded suppressed basal shoots but not cuttings from the apical shoots. This has been demonstrated as a result of the interactions between photon flux density and nutrients (Leakey and Storeton-West 1992). On the other hand, under other circumstances, low nutrient

treatments have been found to produce the greatest number of rooted cuttings (Garton *et al.,* 1983).

Studies have been carried out to determine the movement of nutrients into the base of cuttings during root initiation (Hartmann *et al.,* 1997). For example, in plum (*Prunus domestica* L. cv. Marianna 2624), nitrogen (N) was redistributed in stem cuttings following auxin treatment (Strydom and Hartmann 1960). In contrast to this, all major nutrients – N, P, K, Mg or Ca were found immobile and were not redistributed in stem cuttings during root initiation of Japanese holly (*IIex crenata*) (Blazich and Wright 1979; Blazich *et al.,* 1983). In another study with nutrients, *Geranium* stockplants raised at low, medium and high levels of N, P and K using a factorial arrangement of treatments, indicated strong positive influences of N on root initiation, especially at low and medium N levels (Haun and Cornell 1951). Overall, N had a greater influence on rooting responses of the cuttings than P or K, nevertheless, some treatment combinations of both these nutrients had positive response on rooting of *Geranium* cuttings (Blazich 1988). These results showed the importance of N in root initiation, and in nucleic acid and protein synthesis (Blazich 1988). These results may also relate to carbohydrate availability, C/N ratio and hormonal interactions (Hartmann *et al.,* 1997).

Other minerals may also have an influence on plant growth and development, thus affecting rooting of cuttings. Phosphorus (P) is involved in most metabolic processes, and is required for the storage of energy, photosynthesis, transport of electrons and carbohydrates and regulates functions of some enzymes (Dell *et al.,* 1995). Potassium (K) is required for the maintenance of turgor in plant tissues, stomatal control, pH stabilisation and osmoregulation of cells, protein synthesis, carbohydrate and lipid metabolism (Dell *et al.,* 1995). K deficiency will affect protein synthesis, photosynthesis and cell extension.

Calcium (Ca) is required for membrane integrity and function, largely responsible for cell division and growth (Dell *et al.,* 1995). Its deficiency significantly affects the growth of shoot and root tips. Magnesium is required for the co-ordination of chlorophyll functions, protein synthesis, activation of many enzymes and

regulatory functions in cellular pH and cation-anion balance (Dell *et al.,* 1995). There are few studies investigating Mg during root growth and development (Blazich 1988). However, it has been found that Mg deficiency inhibits protein synthesis (Mengel and Kirkby 1982), hence it is crucial for rooting.

The influence of minor nutrients (e.g. zinc (Zn), manganese (Mn) and boron (B)) on root initiation and the subsequent plant growth and development is also important. For example, Zn and Mn were reported to affect the level of endogenous auxin (Blazich 1988). Zn is required for the activity of many other enzymes, photosynthesis and synthesis of the endogenous auxin and the production of precursor tryptophan (Thimann 1985). Manganese is required for the evolution of oxygen from the splitting of water in photosynthesis, redox reactions and electron transport in chloroplasts, and acting as an activator of IAA oxidase, which breaks down endogenous auxin (Thomaszewski and Thimann 1966; Dell *et al.,* 1995). Boron (B) is required for cell division, cell growth and maybe membrane function (Dell *et al.,* 1995). Jarvis *et al.,* (1984) cited in Blazich (1988) suggested that B influences rooting by controlling endogenous auxin levels through promoting IAA oxidase activity. B deficiency in plants can seriously cause death of shoot and root tips, and the reduction of wood lignification (Dell *et al.,* 1995).

6.1.5 Factors influencing rooting ability of marcots

Factors affecting rooting ability of marcots are quite similar to those described for stem cuttings above, typically involves complex interactions of plant growth hormones (e.g. auxin), the ontogenetic and physiological aging of the tree that varies with species and clones, seasons, the type and size of the marcotted branch, rooting media and the environmental factors such as light, temperature and humidity. Because these factors have been discussed in lengths above, this review is very brief.

According to Menzel (2002), many authors recommended marcotting during warm humid spells because the roots are less likely to dry out. In addition, upright, 2cm

diameter and 80cm long branches under full sunlight from a mature tree free from pests or diseases were recommended (Menzel 2002). However, these factors vary with different species. In *Dacryodes edulis*, Mialoundama *et al.,* (2002), found large horizontal branches with thick bark rooting best, although test between different rooting media was not significant. The effect of auxin on rooting of marcot varies between species. For example, rooting of marcots performed on 1-2.5cm diameter branch of *Inga feuillei* was 100% successful with and without auxin (Brennan and Mudge 1998). In contrast marcotting in *Irvingia gabonesis* had low success rate (30%) with even lower survival rate (10%) after severance (Tchoundjeu *et al.,* 1999). It was considered that treating marcots with auxin and better management of rooted propagules should have improved rooting. Marcots of two mangrove species (*Sonnetatia apetala* B. Ham and *Xylocarpus granatum* Koen) only rooted during monsoon and post monsoon months in auxin treated shoots (Kathiresan and Ravikumar 1995), indicating the effect of seasons and auxin in rooting marcots.

Literature on the effect of branch height on rooting ability of marcots is limited. However, studies have shown that branches of different height have different hydraulic conductance due to differences in flow path length of the conductance, from soil to leaf in the branch and the branch growth (Pothier *et al.,* 1989; Schafer *et al.,* 2000), suggesting the potential of this to affect rooting ability of marcots. In *Pinus ponderosa*, Hubbard *et al.,* (2002) found that there is no difference between lower and higher branches in terms of leaf gas exchange or leaf specific hydraulic conductance. They also found that branches positioned at 25m canopy had lower sapwood area ratios ($0.17m^2$ per cm^2) than those branches positioned at 10m canopy ($0.27m^2$ per $2cm^2$). Thus, suggesting that height alone may not be entirely responsible for changes in hydraulic conductance of a tree, hence rooting ability of marcots.

Consequently, there is a need to test a range of all these pre-severance and post-severance factors, in order to develop robust protocols of vegetative propagation by the rooting of cuttings. However, from what is known from other species it is possible to predict the optimum range of the factors, and so just to refine the

techniques when starting work with new species (Tchoundjeu 1989; Tchoundjeu and Leakey 2000). The present study investigates some of the key factors affecting rooting including auxin concentration, leaf area, rooting media, cutting size and length, stockplant treatments (light and fertiliser) and ontogenetic aging of cuttings in *B. procera* and *I. fagifer*. The studies focused on the determination of rooting ability of single-node leafy stem cuttings. In addition, the present study also investigates appropriate techniques for propagating mature trees of *B. procera* and *I. fagifer* using cuttings and marcots.

6.2 EXPERIMENTAL SECTION: PROPAGATING JUVENILE CUTTINGS

The general Materials and Methods for all experiments have been described in Chapter 3.

6.2.1 Effects of different concentrations of rooting hormone (indole-3-butyric acid, IBA) on rooting of single-node leafy stem cuttings from seedling stockplants of *Barringtonia procera* and *Inocarpus fagifer*

6.2.1.1 Experiment 1: Experimental details

Experiment 1a: B. procera: Cuttings of *B. procera* used in this experiment were obtained from six month old stockplants (Chapter 3). One hundred and ninety-two single-node leafy stem cuttings were collected from seedlings originating from Tree numbers 1, 2 and 97 of Hunda and Tree number 16 from Tututi. As stockplants are new, only 4 cuttings can be obtained from a seedling, thus a total of 48 seedlings were used. At the nursery, the cuttings were prepared and leaf area treated as described in Chapter 3 (section 3.2.2.3). Cuttings were then dipped into one of two different IBA powders (0.3% and 0.8% IBA) or left as an untreated control (0% IBA). Excess powder was removed by gently tapping the base of the cutting. Immediately after IBA treatment, cuttings were inserted into soil (from riverbank) medium contained within a non-mist propagator as described in Chapter 3, in the order of their node position starting from the apex. Sixteen single-node cuttings were allocated to each of the three IBA treatments. Each treatment was replicated four times in a randomised complete block design.

Rooting was assessed after 4 and 6 weeks by counting the number of cuttings that had rooted and the number of roots (greater than 1mm long) produced per cutting. Data analysis is described in Chapter 3.

Experiment 1b: I. fagifer: This experiment is a repeat of the above Experiment 1a, but done with *I. fagifer* instead of *B. procera*. The following details are, however, different:

i. Single-node leafy stem cuttings were collected from 6 month old seedlings, originating from trees numbers 1, 3 and 24 from Tututi and established as stockplants (Chapter 3).
ii. Experimental design is 25 cuttings x 3 IBA concentrations x 4 replicates, giving a total of 300 cuttings. Thirty-four seedlings were used for this experiment, and each seedling produced about nine cuttings.
iii. Cuttings were assessed for rooting after 4, 5 and 6 weeks

6.2.1.2 Results

- *Effects of auxin (IBA)*

Experiment 1a: B. procera: After four weeks in the propagator, no significant (P>0.05) differences in rooting percentage (control = 34%, 0.3% IBA = 44% and 0.8% IBA = 49%) were found between treatments (Fig. 6.3). Similar non-significant trends were seen for cuttings in all treatments two weeks later. Overall, there was little effect of increased levels of IBA concentration on the percentage rooting (control = 62%, 0.3% IBA = 66% and, 0.8% IBA = 72% by week 6).

Cutting mortality was between 28-38%. The cuttings in the control had the highest mortality, although differences in percentage mortality between IBA treatments were not significant. By week six, all cuttings had either rooted or died (Fig 6.4). Mortality was greatest at basal nodes.

The effect of IBA concentration on the number of roots per rooted cutting was significant ($F_{2, 78}$ = 8.97, P = 0.001) (Plate 6.1), with the number of roots per rooted cutting increased with successive increases in IBA concentration on week 4 (Fig 6.5). By contrast, new roots developing between weeks 4 and 6 did not differ significantly between treatments (Table 6.1).

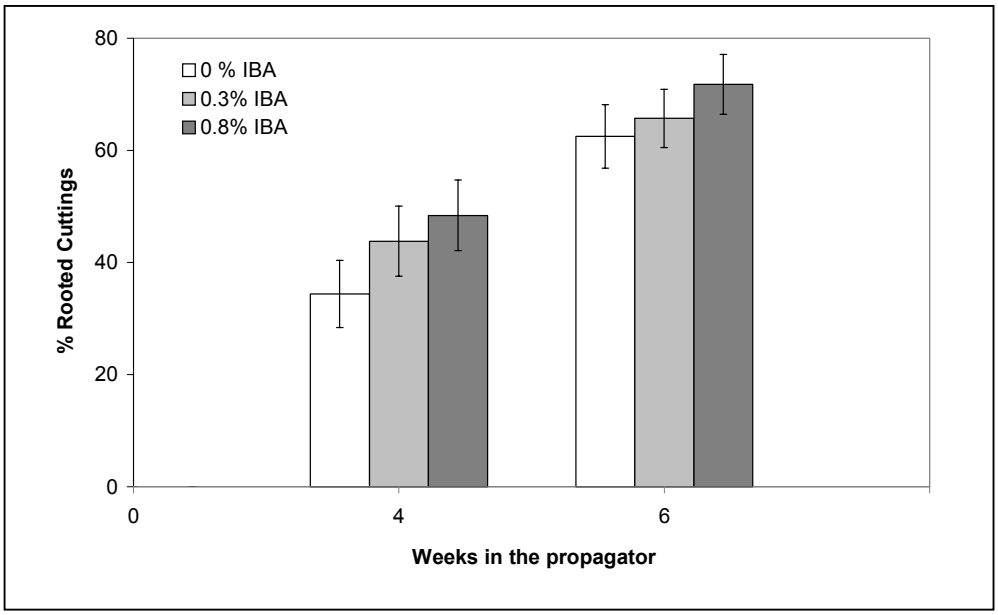


Fig 6.3: Effects of three different IBA concentrations (0% IBA, 0.3% IBA and 0.8% IBA) on the rooting percentage (±SE) of cuttings of *B. procera* over time.

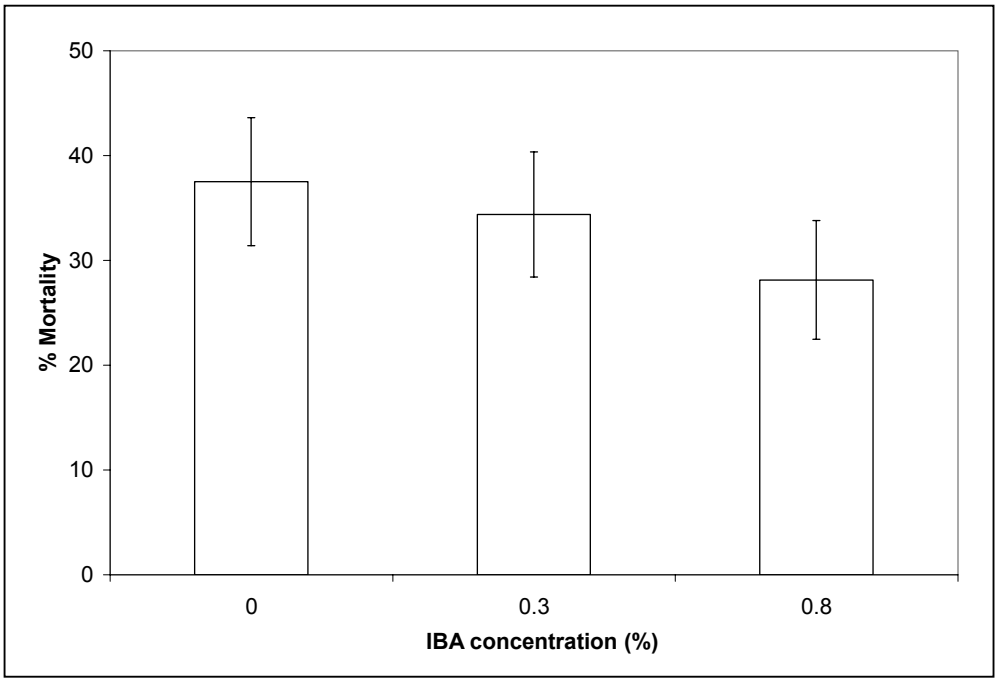


Fig 6.4: Overall percentage (±SE) mortality on cuttings of *B. procera* treated with 3 different IBA concentrations (0, 0.3 and 0.8%).

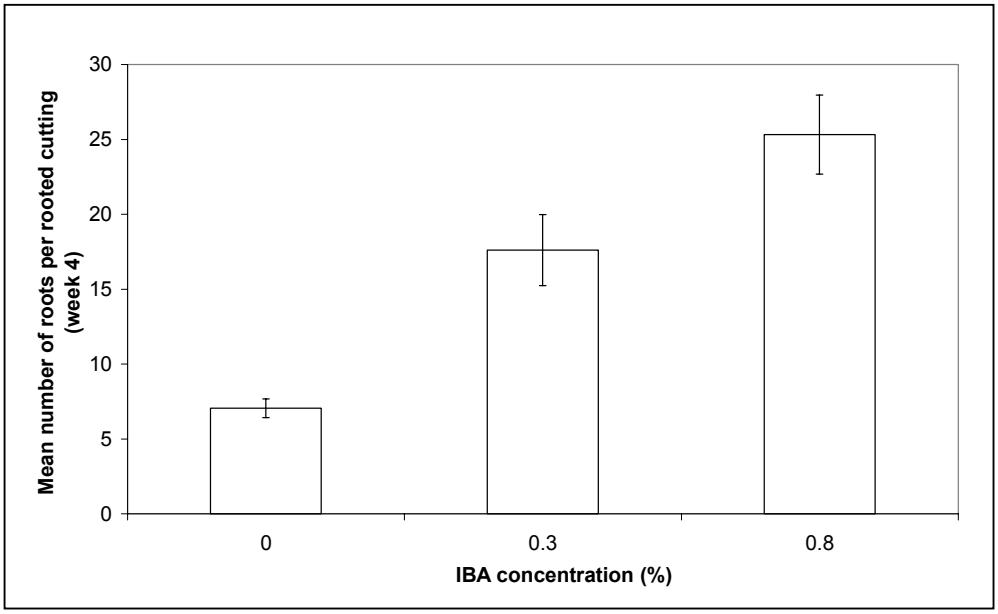


Fig 6.5: Effects of three different concentrations of IBA (0, 0.3 and 0.8%) on the number (±SE) of roots per rooted cutting of *B. procera.*

Table 6.1: Effects of auxin on the mean number of roots (±SE) per rooted cutting from single-node leafy (50cm^2) stem cuttings of *B. procera.*

IBA concentration (%)	Mean number of roots per rooted cutting	
	Week 0-4 (n = 81)	*Week 4-6 (n = 47)*
0	7.1 ± 0.87	5.3 ± 0.87
0.3	17.6 ± 3.21	4.3 ± 1.13
0.8	25.3 ± 3.37	6.1 ± 0.97
Significance	**	NS

NS = non-significant, ** significant P = 0.001

Plate 6.1. Cuttings of *B. procera* (L) and *I. fagifer* (R) showing rooting after 3 weeks in the poly-propagator

The percentage rooting of cuttings from successive nodes varied between IBA treatments. With IBA, apical nodes had the highest rooting, while without IBA, node 3 was best (Fig 6.6). Difference between with and without IBA were significant only (P<0.05) for node 1. The number of roots per rooted cutting was significantly different between treatments for node 3 (Table 6.2).

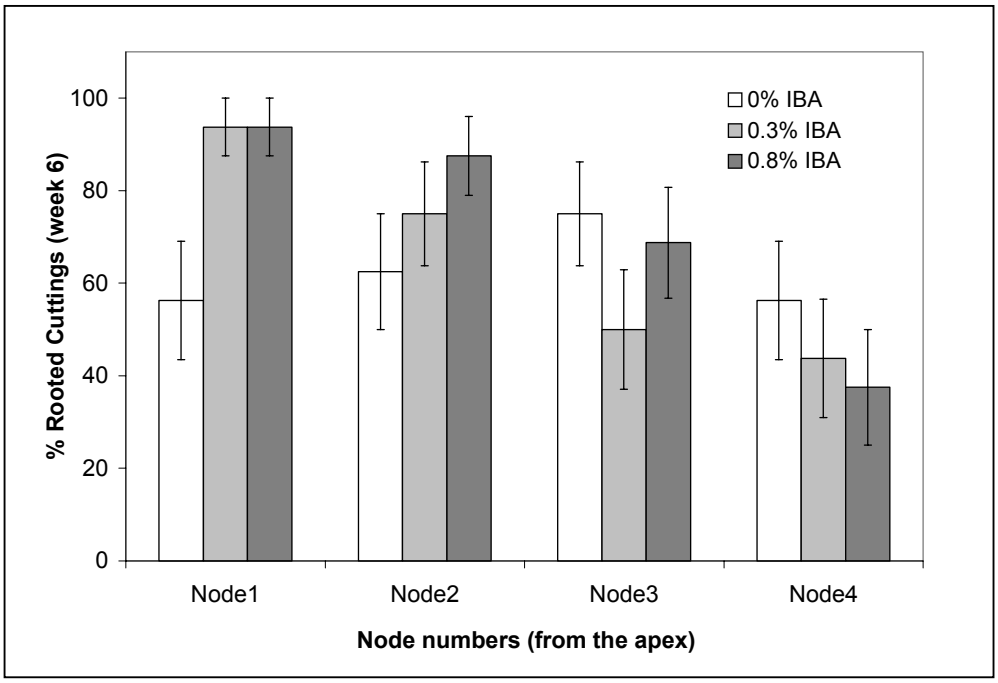


Fig 6.6: Effects of node numbers and three different concentrations of IBA (0%, 0.3% and 0.8%) on percentage (±SE) rooting of cuttings of *B. procera* after 6 weeks.

Table 6.2: Effects of auxin concentration and node position on the mean (±SE) number of roots per rooted cutting from single-node leafy (50cm^2) stem cuttings of *B. procera* on week 4.

IBA concentration (%)	Mean number of roots per rooted cutting			
	Node1	*Node2*	*Node3*	*Node4*
0	8.2 ± 1.4 (n=5)	6.3 ± 1.9 (n=4)	5.2 ± 1.1 (n=6)	8.3 ± 2.1 (n=7)
0.3	14.9 ± 5.0 (n=11)	18.0 ± 7.8 (n=6)	20.7 ± 7.3 (n=6)	19.4 ± 8.3 (n=5)
0.8	22.0 ± 4.8 (n=12)	29.2 ± 6.9 (n=10)	37.6 ± 6.4 (5=5)	10.3 ± 6.4 (n=4)
Significance	NS	NS	*	NS

NS = non-significant, *significant P<0.05

Percentage rooting of cuttings originating from four trees in Hunda and Tututi varied. Significant differences (P<0.05) between IBA treatments occurred in cuttings from Hunda2 and Tututi16 (Fig 6.7) on week 6. In the same week, significant effects of IBA on the number of roots per rooted cuttings occurred in Hunda1 (Table 6.3).

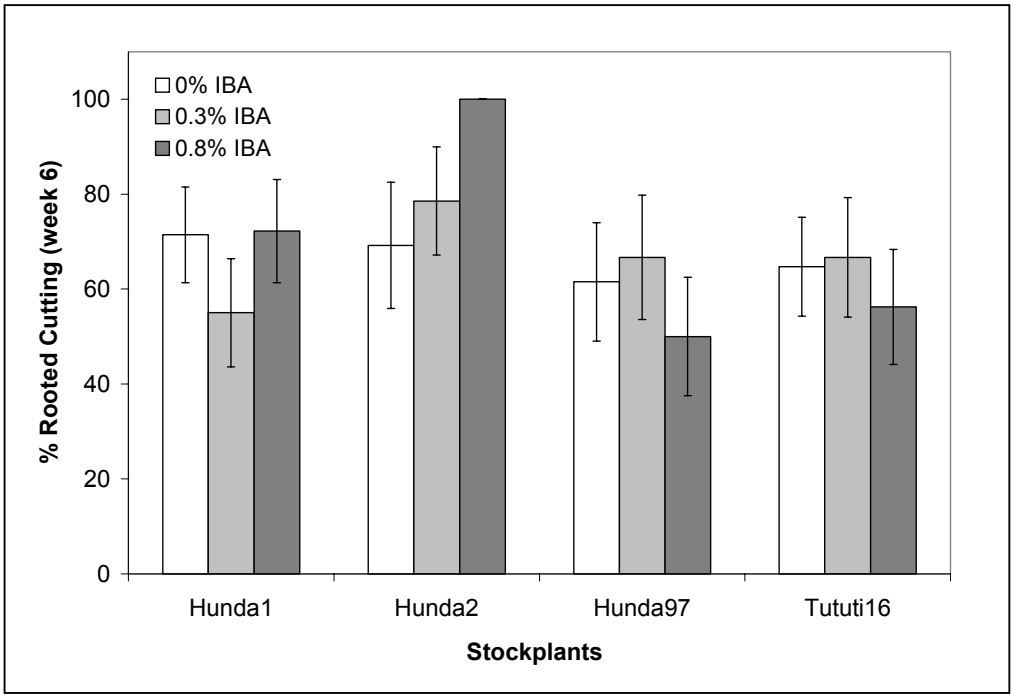


Fig 6.7: Effects of stockplants and three different concentrations of IBA (0% IBA, 0.3% IBA and 0.8% IBA) on percentage (±SE) rooting of cuttings of *B. procera.*

Table 6.3: Effects of IBA concentration on the number (±SE) of roots per rooted cutting from single-node leafy (50cm^2) stem cuttings originating from different trees of *B. procera* on week 6.

IBA concentration (%)	Mean number of roots per rooted cutting (Week 6)			
	Hunda1	*Hunda2*	*Hunda97*	*Tututi16*
0	5.1 ± 1.0 (n=15)	7.8 ± 1.7 (n=9)	12.0 ± 4.4 (n=8)	5.2 ± 0.9 (n=11)
0.3	13.6 ± 4.5 (n=11)	15.2 ± 5.7 (n=11)	6.7 ± 1.6 (n=10)	16.9 ± 5.9 (n=10)
0.8	20.2 ± 4.5 (n=13)	19.5 ± 4.0 (n=12)	17.0 ± 5.3 (n=9)	19.8 ± 5.4 (n=9)
Significance	*	NS	NS	NS

NS = non-significant, *significant P<0.05

Experiment 1b: I. fagifer: There were no effects of auxin on the percentage rooting of cuttings of *I. fagifer*. Variations between IBA treatments in successive weeks were not significant (P>0.05). The rate of rooting was greatest between the insertion (week 0) and week 4 (0% IBA = 78%, 0.3% IBA = 74% and 0.8% IBA = 72%) (Fig 6.8). By week 6 all cuttings had rooted and no mortality occurred.

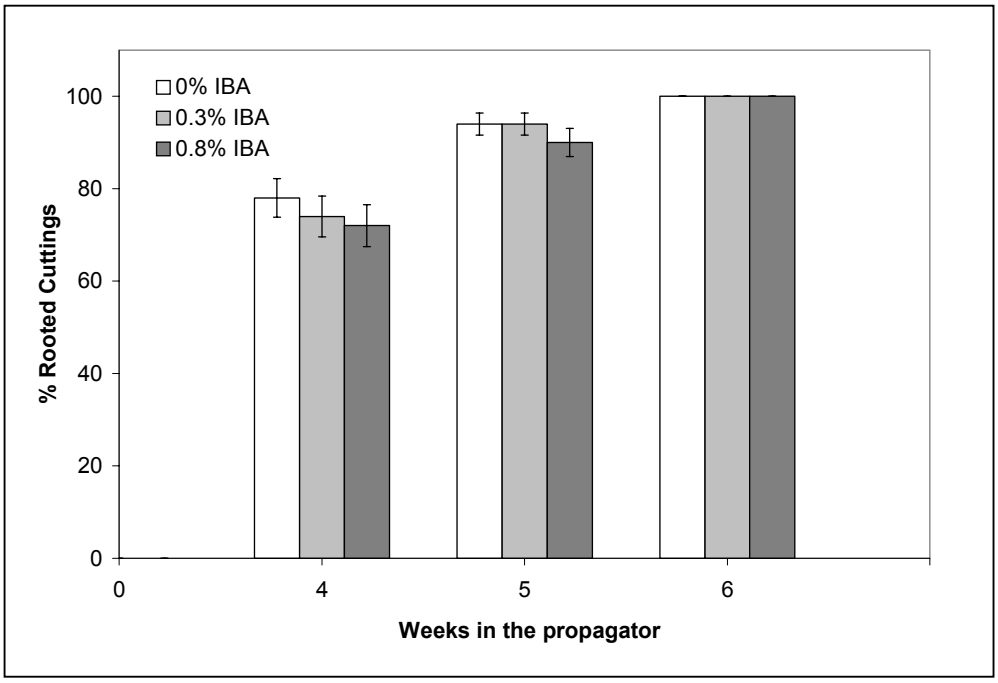


Fig 6.8: Effects of three different concentrations of IBA (0% IBA, 0.3%IBA and 0.8% IBA) on the rooting percentage (±SE) of cuttings of *I. fagifer* over time.

The effect of IBA treatments on the number of roots per rooted cutting was significant on week 4 ($F_{2, 221} = 5.96$, P<0.05) and 6 ($F_{2, 19} = 4.98$, P<0.05) but not on week 5, with control cuttings having significantly fewer roots than those treated with 0.3%. Differences between control and 0.8% IBA were not significant on week 4 but significantly more roots in 0.8% IBA on week 6 (Table 6.4). Cuttings produced more roots with successive increase in IBA concentration on week 6 (Fig 6.9).

Table 6.4: Effects of auxin concentration on the mean number (±SE) of roots per rooted cutting from single-node leafy (50cm^2) stem cuttings of *I. fagifer.*

IBA concentration (%)	Mean number of roots per rooted cutting		
	Week 0-4 (n=224)	*Week 4-5 (n=54)*	*Week 5-6 (n=22)*
0	3.0 ± 0.21	2.1 ± 0.26	1.2 ± 0.17
0.3	4.3 ± 0.41	2.1 ± 0.26	2.0 ± 0.47
0.8	1.6 ± 0.19	2.2 ± 0.26	2.7 ± 0.33
Significance	*	NS	*

NS = non-significant, * significant P<0.05

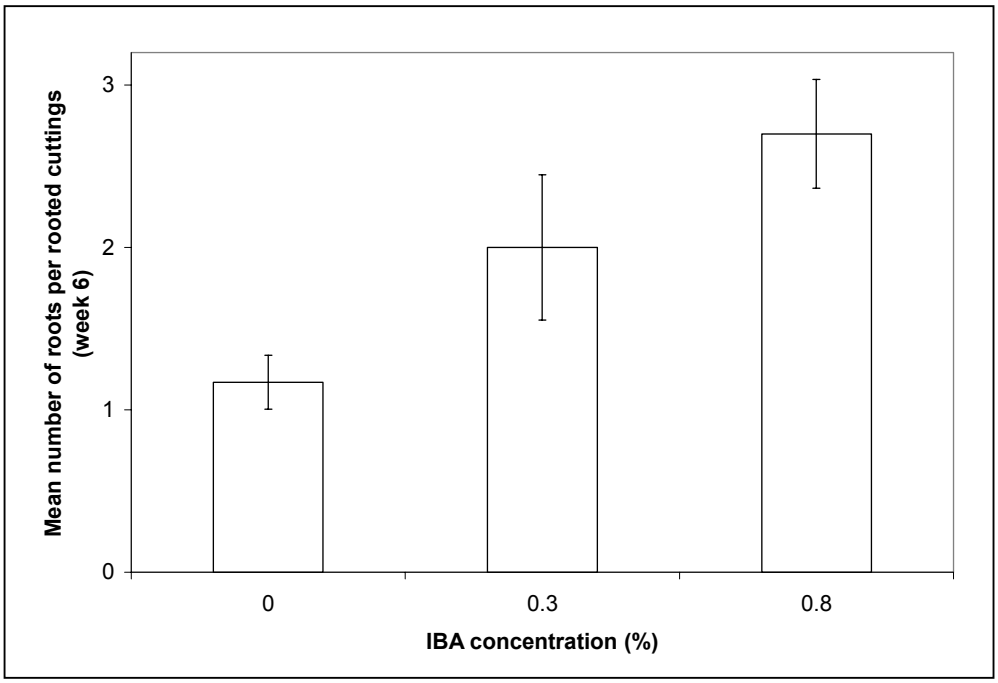


Fig 6.9: Effects of three different concentrations of IBA (0, 0.3 and 0.8%) on the number (±SE) of roots per rooted cutting of *I. fagifer.*

There were no significant (P>0.05) interactions in percentage rooting between IBA treatments and node numbers (Fig 6.10). Number of roots per rooted cutting on week 4 was significant between IBA treatments for node 4 ($F_{2, 26}$ = 4.91, P<0.05) and node 9 ($F_{2, 15}$ = 3.74, P<0.05) (Table 5.5). Cuttings from nodes 4 and 9 produce more roots when treated with 0.3% IBA than the control and 0.8% IBA.

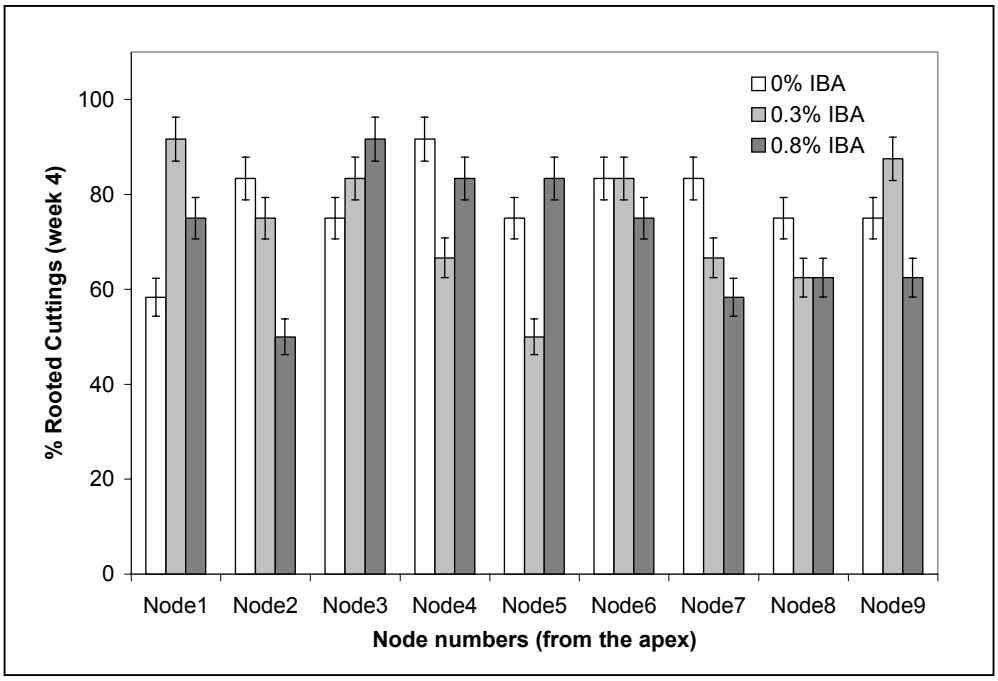


Fig 6.10: Effects of node numbers (±SE) and three different concentrations of IBA (0% IBA, 0.3% IBA and 0.8% IBA) on percentage rooting of cuttings of *I. fagifer.*

Table 6.5: Effects of auxin concentration and node position on the mean (±SE) number of roots per rooted cutting from single-node leafy (50cm^2) stem cuttings of *I. fagifer* on week 4.

Node position	Mean number of roots per rooted cutting			
	No IBA	*0.3% IBA*	*0.8% IBA*	*Significance*
Node1 (n=27)	2.6 ± 0.57	3.5 ± 0.68	3.1 ± 0.59	NS
Node2 (n=25)	3.0 ± 0.66	4.1 ± 0.89	3.0 ± 0.68	NS
Node3 (n=30)	3.8 ± 0.70	3.3 ± 0.65	3.8 ± 0.62	NS
Node4 (n=29)	2.0 ± 0.38	5.3 ± 1.26	3.3 ± 0.50	*
Node5 (n=25)	2.9 ± 0.51	3.5 ± 0.85	2.5 ± 0.79	NS
Node6 (n=29)	3.4 ± 0.82	4.3 ± 1.08	3.1 ± 0.35	NS
Node7 (n=25)	3.8 ± 0.74	2.8 ± 0.92	2.9 ± 0.40	NS
Node8 (n=16)	2.3 ± 0.49	3.4 ± 1.12	3.2 ± 0.58	NS
Node9 (n=18)	2.8 ± 0.40	8.9 ± 2.51	3.4 ± 0.93	*

NS = Non-significant, * significant P<0.05

There were no significant differences (P<0.05) at week 4 in percentage rooting between cuttings originating from Hunda and Tututi. Cuttings from Tututi 3 rooted better than those from Tututi 1 and 24 across all treatments (Fig 6.11). By week 4,

the number of roots formed per cutting of Tututi 1 was significantly ($P<0.05$) greater with 0.3% than the control (Table 6.6).

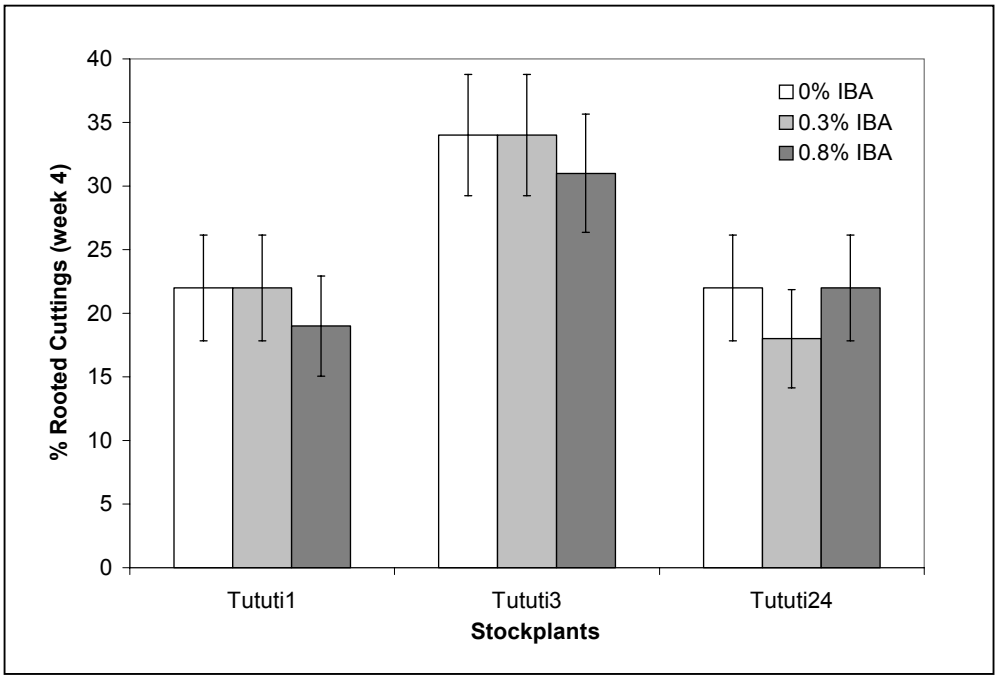


Fig 6.11: Effects of stockplants from Tututi and three different concentrations of IBA (0% IBA, 0.3% IBA and 0.8% IBA) on percentage (±SE) rooting of cuttings of *I. fagifer*.

Table 6.6: Effects of auxin (IBA) concentration on the mean (±SE) number of roots per rooted cutting from single-node leafy (50cm^2) stem cuttings of 3 trees of *I. fagifer* on week 4.

IBA concentration (%)	Mean number of roots per rooted cuttings (Week 4)		
	Tututi1 (n=63)	*Tututi3 (n=99)*	*Tututi24 (n=62)*
0	2.3 ± 0.32	3.5 ± 0.36	2.9 ± 0.38
0.3	4.8 ± 1.01	4.4 ± 0.52	3.4 ± 0.58
0.8	3.2 ± 0.38	3.4 ± 0.30	2.8 ± 0.31
Significance	*	NS	NS

NS = Non-significant, * significant $P<0.05$

6.2.2 Effects of different lamina area of single-node leafy stem cuttings from seedling stockplants of *Barringtonia procera* and *Inocarpus fagifer*

6.2.2.1 Experiment 2: Experimental details

Experiment 2a: B. procera: A total of 288 single-node leafy stem cuttings of *B. procera* were collected from six months old seedlings, originating from trees in Hunda and Tututi and established as stockplants (Chapter 3). At the nursery, the single-node cuttings were prepared as described in Chapter 3 (section 3.2.2.3). The single leaf was then trimmed to one of the three leaf areas (0 cm^2, 30 cm^2 and 50 cm^2), guided by a template cut from graph paper. Prior to insertion into the rooting media (river soil), the cuttings were dipped into 0.8% IBA powder, and excess powder was removed by gently tapping the base of the cutting. Cuttings were then inserted in node position starting from the apex. The design for this experiment was 24 cuttings x 3 treatments x 4 replicates, laid out in a randomised complete block. Assessment of rooting and data analysis is described in Chapter 3.

Experiment 2b: I. fagifer: This is a repeat of Experiment 2a, except that it used *I. fagifer*. Differences include:

i. Single-node leafy stem cuttings were collected from 6 month old seedlings, originating from tree number 3 from Tututi and established as stockplants (Chapter 3).

ii. Experimental design was 12 cuttings x 4 levels of leaf area (0cm^2, 20cm^2, 50cm^2 and 80cm^2) x 4 replicates, for a total of 192 cuttings. Thirty-two seedlings were used for this experiment, and each seedling produced six cuttings.

6.2.2.2 Results

- *Effects of leaf area on rooting*

Experiment 2a: B. procera: Leaf area affected rooting ability of cuttings differently, with leaf areas 0cm^2, 30cm^2 and 50cm^2 having 21%, 77% and 83% rooting respectively by week 5. Significant differences (P = 0.001) on percentage rooting were found between leafy and leafless cuttings (Fig 6.12). By contrast, percentage rooting did not differ significantly between cuttings with leaf areas of 30cm^2 and 50cm^2.

A significantly greater level of mortality was recorded in leafless than in leafy cuttings (Fig 6.13), with most (35%) occurring by week 2. Cuttings with leaf area of 50cm^2 recorded the least percentage mortality (17%), but did not differ significantly from that of 30cm^2.

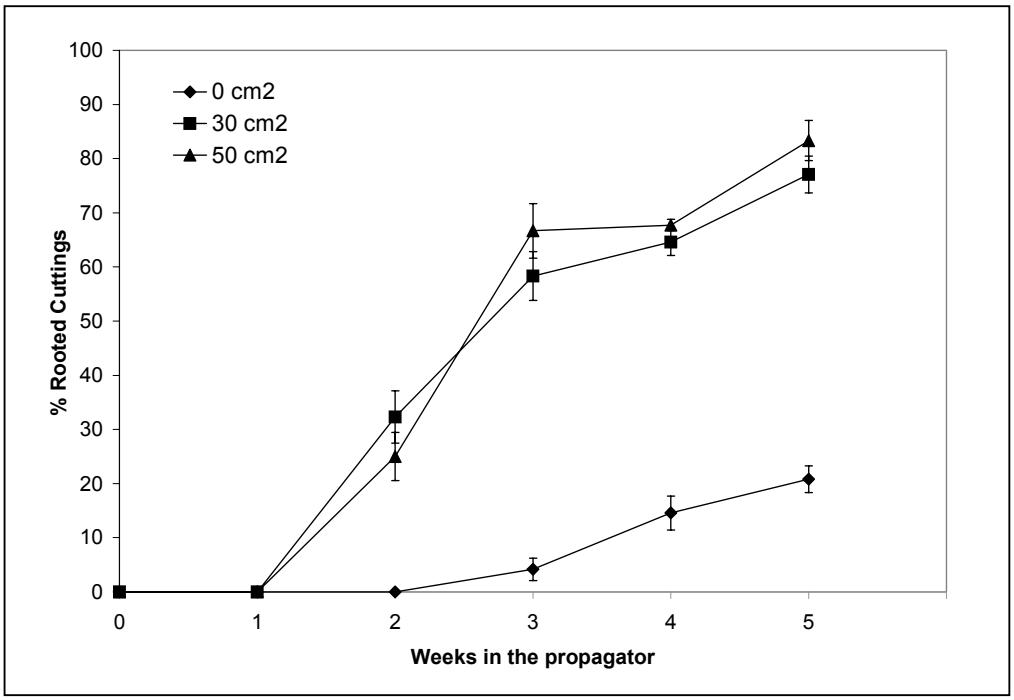


Fig 6.12: Effects of leaf areas (0cm^2, 30cm^2, and 50cm^2) on the rooting ability (±SE) of cuttings of *B. procera* over time.

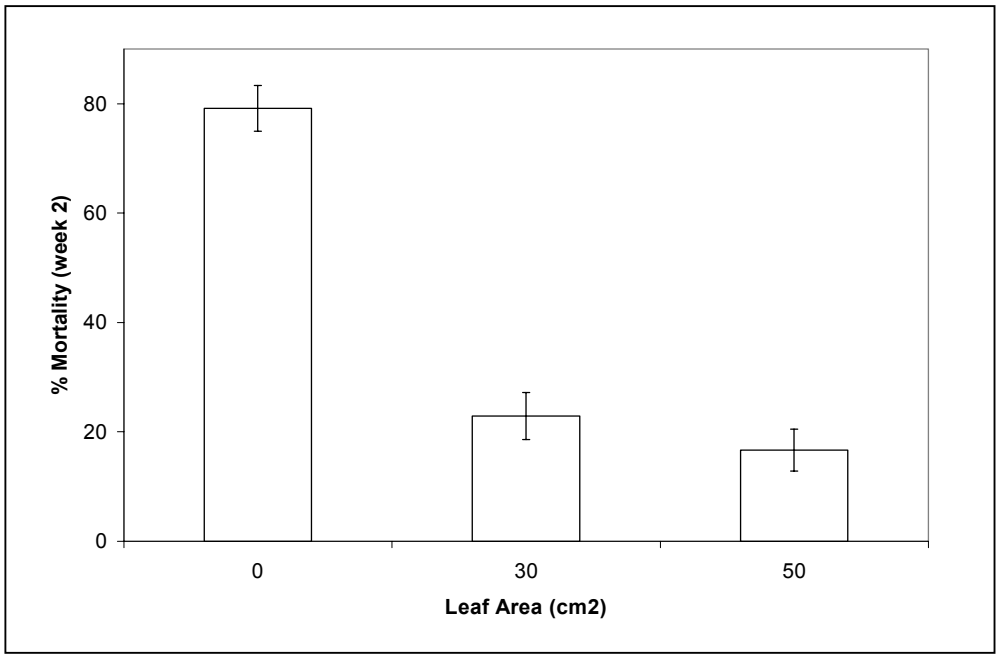


Fig 6.13: Effects of leaf areas (0cm^2, 30cm^2, and 50cm^2) on the percentage (±SE) mortality of cuttings of *B. procera*

Leaf area also affected root production. Cuttings with their leaf reduced to 30cm^2 had significantly fewer roots per root cutting than those with a leaf reduced to 50cm^2 (Fig 6.14).

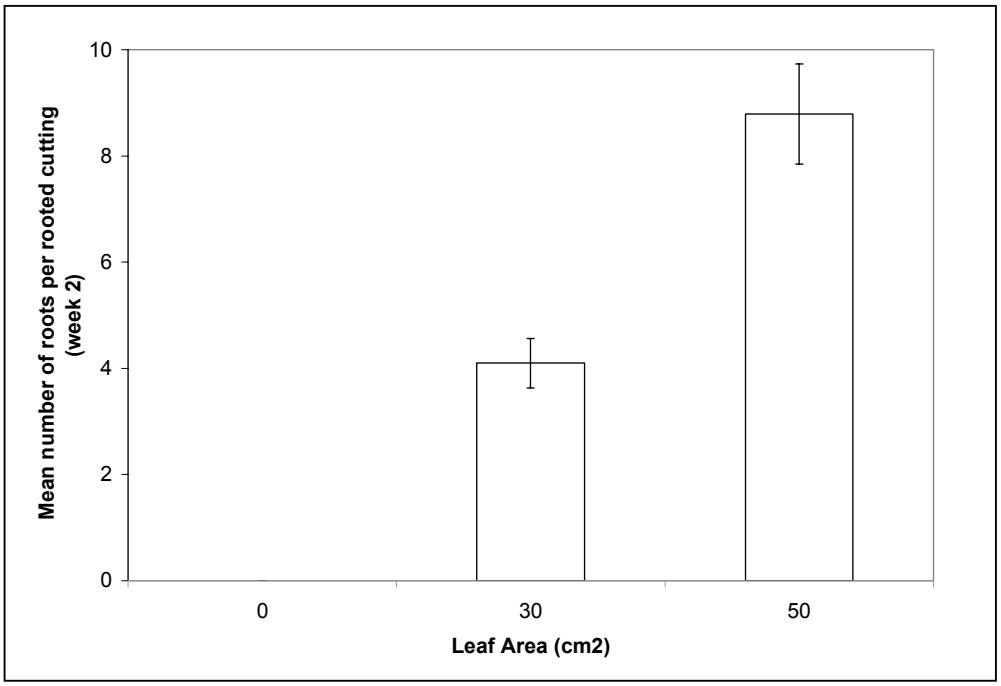


Fig 6.14: Effects of leaf areas (0, 30 and 50 cm^2) on the number (±SE) of roots formed per cuttings of *B. procera.*

Leafless cuttings were slower to start rooting (week 3), while leafy cuttings started to root by week 2. Leafless cuttings produced significantly fewer roots than those cuttings with a leaf (Table 6.7). Cuttings from nodes 1-4 responded differently to different leaf areas. Significant differences occurred between leafy and leafless cuttings in all nodes (Fig 6.15).

Table 6.7: Effects of leaf areas (0, 30 and 50 cm^2) on the number (±SE) of roots formed per single-node leafy cuttings of *B. procera* over time.

Leaf Area (cm^2)	Mean number of roots per rooted cutting	
	Week 3 (n = 69)	*Week 5 (n = 50)*
0	2.5 ± 0.29	2.3 ± 0.36
30	3.8 ± 0.67	5.4 ± 0.95
50	7.2 ± 0.74	4.6 ± 0.82
Significance	*	*

NS = non-significance, * significance P<0.05

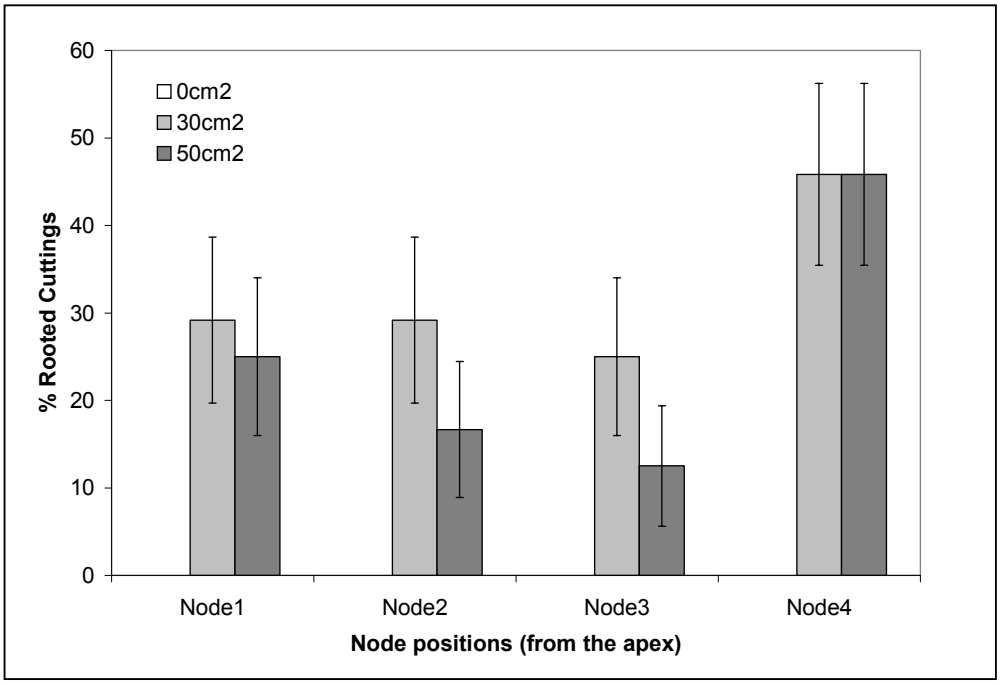


Fig 6.15: Effects of leaf areas (0cm^2, 30cm^2 and 50cm^2) node position on percentage (±SE) rooting of cutting from single-node leafy stem cuttings of *B. procera.*

Experiment 2b: I. fagifer: Leaf area affected rooting ability of cuttings of *I. fagifer*. After 3 weeks cuttings with 50cm^2 leaves had rooted significantly (P<0.05) better than those with 20cm^2 leaves but not those with 80cm^2 leaves. However, percentage rooting was not significantly different between treatments on week 5 (Fig. 6.16). The rate of rooting between different leaf area treatments was greatest between weeks 1-3. Leafless cuttings all died; approximately 90% died on week 3. Leafy cuttings were all alive except for those with 20cm^2 leaves (2% mortality).

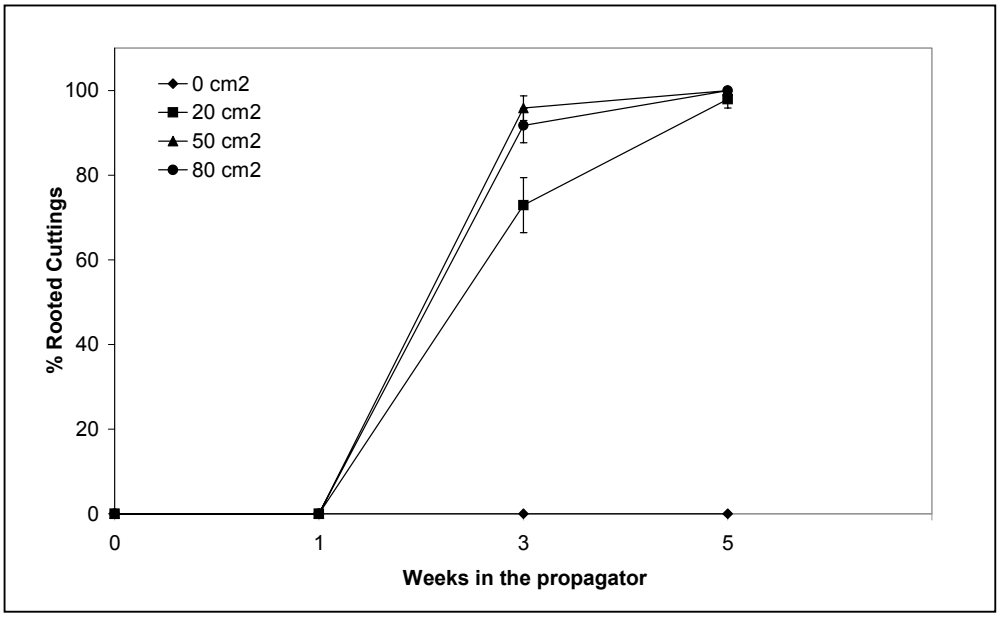


Fig 6.16: Effects of leaf areas (0cm^2, 20cm^2, 50cm^2 and 80cm^2) on the rooting ability (±SE) of cuttings of *I. fagifer* over time.

The effect of leaf area on the number of roots per rooted cutting was significant ($F_{2, 122}$ = 4.58, P<0.05) on week 3. Cuttings with 80cm^2 leaves had significantly more roots than those with 20cm^2 or 50cm^2 leaves (Fig 6.17). The number of newly formed roots per rooted cutting varies over time, being greatest on week 3 for 50 and 80 cm^2 leaves and greatest on week 5 for 20cm^2 leaves (Table 6.8).

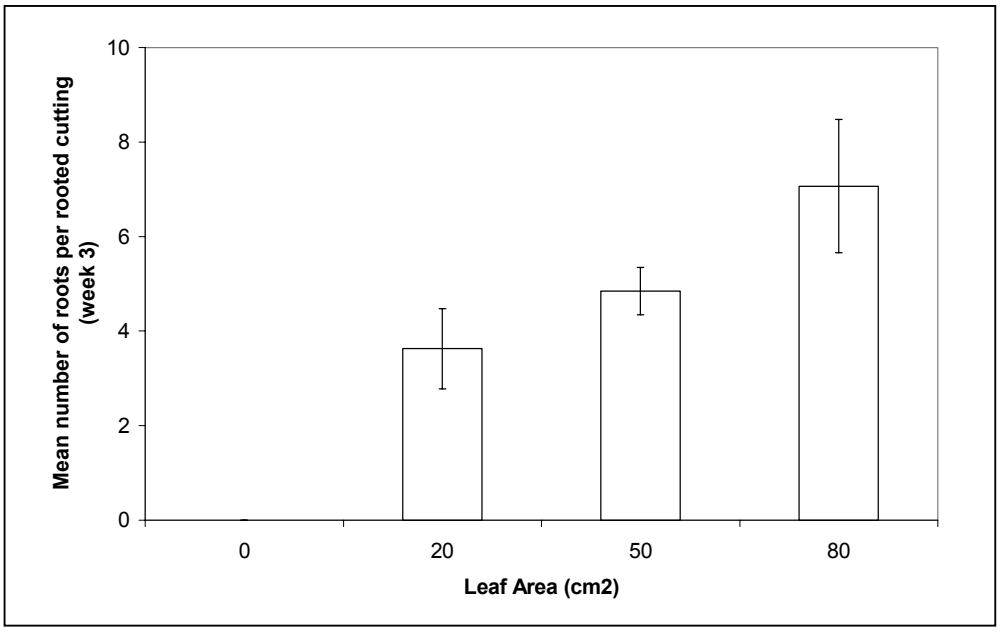


Fig 6.17: Effects of leaf areas (0, 20, 50 and 80 cm^2) on the number (±SE) of roots formed per cutting of *I. fagifer.*

Table 6.8: Effects of leaf areas (0, 20, 50 and 80 cm^2) on the number (±SE) of roots formed per single-node leafy cutting of *I. fagifer* over time.

Leaf Area (cm^2)	Mean number of roots per newly rooted cutting		
	Week 0-1 (n=192)	*Week 1-3 (n=125)*	*Week 3-5 (n=18)*
0	0	0	0
20	0	3.6 ± 0.38	4.7 ± 0.85
50	0	4.8 ± 0.49	1.5 ± 0.50
80	0	7.1 ± 1.17	5.0 ± 1.41
Significance	-	*	NS

NS = non significance, * Significant at P<0.05

Cuttings from nodes 1-6 responded differently to different leaf areas. Significant differences occurred between leafy and leafless cuttings in all nodes (Fig 6.18). Furthermore, cuttings from lower (basal) nodes produced more roots than those from the apical end in all leaf areas except 80cm^2 leaves (Table 6.9), but the differences were only significant ($F_{2, 18} = 4.45$, $P<0.05$) for node 5.

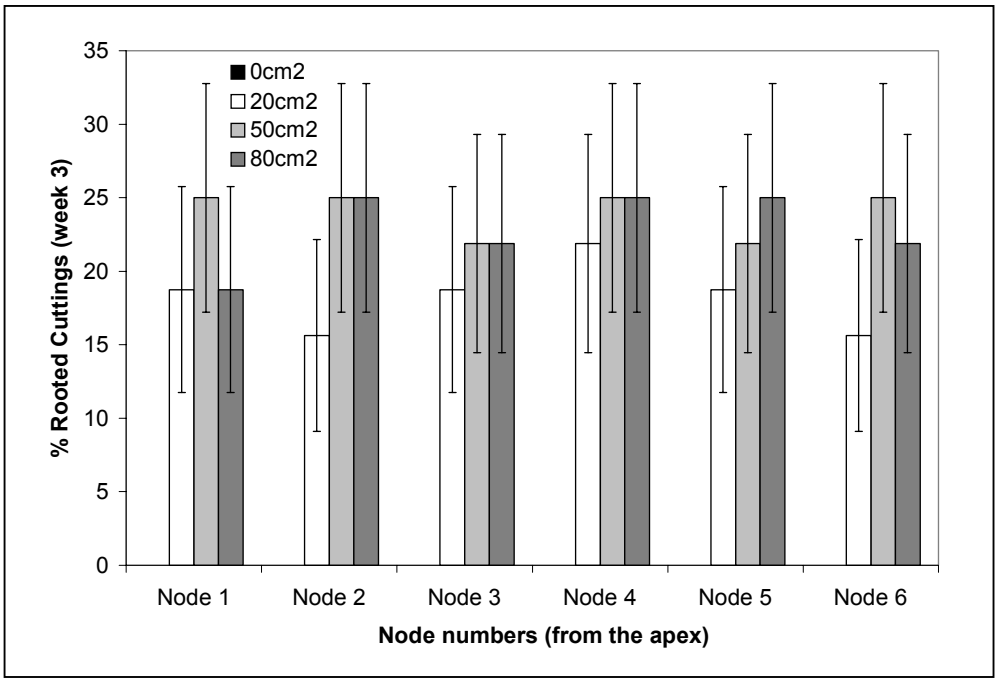


Fig 6.18: Effects of leaf areas (0cm2, 20cm2, 50cm2 and 80cm2) and node position on the mean (±SE) number of roots per rooted cutting from single-node leafy stem cuttings of *I. fagifer*.

Table 6.9: Effects of leaf area (0, 20, 50, and 80 cm2) and node position on the mean (±SE) number of roots per rooted cutting from single-node leafy stem cuttings of *I. fagifer* on week 3.

Leaf Area (cm^2)	Mean number of roots per rooted cutting					
	Node1 (n=8)	*Node2 (n=8)*	*Node3 (n=8)*	*Node4 (n=8)*	*Node5 (n=8)*	*Node6 (n=8)*
0	0	0	0	0	0	0
20	2.7 ± 0.21	3.0 ± 0.55	3.8 ± 1.01	3.9 ± 0.59	4.2 ± 0.54	4.2 ± 2.27
50	2.9 ± 0.52	2.6 ± 0.38	3.9 ± 0.67	5.8 ± 1.11	7.0 ± 1.29	7.1 ± 1.72
80	2.2 ± 0.40	3.4 ± 0.78	5.1 ± 1.24	4.5 ± 0.78	15.3 ± 3.99	11.0 ± 3.71
Significance	NS	NS	NS	NS	*	NS

NS = Non-significant, * significant P<0.05

6.2.3 Impact of rooting media on the rooting of single-node leafy stem cuttings of *Barringtonia procera* and *Inocarpus fagifer*

6.2.3.1 Experiment 3: Experimental details

Experiment 3a: B. procera: A total of one hundred and eighty single-node leafy stem cuttings of *B. procera* were collected from six month old seedlings originating from different trees in Hunda and Tututi and established as stockplants (Chapter 3). A non-mist propagator was set up (Chapter 3) to test five different rooting media (forest soil, river soil, coir, coastal coral and coastal gravel). These media all had different chemical and physical properties (Table 6.10). Except for coir, all other media were unsterilised. Coir was sterilised by heat treatment following KFPL standard nursery practice. This involves heating the coir for 30-45 minutes at about 100^{o}C using firewood. Coir was contained in a halved 200-litre barrel and was turned over thoroughly 4-5 times during heating.

The bulk density and porosity of the media were calculated based on the formulae described below. Except for coir, all the media were sampled at topsoil using a cylindrical sampling core with known volume. The samples were oven dried at 70^{o}C for 48 hours, and then weighed using an electronic balance. Because coir is different from soil particles, porosity was determined using water displacement method – i.e. the amount of water displaced was divided by the total volume of water absorbed in the air pores.

$$\text{Bulky density} = \frac{\text{Oven dry weight of medium}}{\text{Total volume of medium}}$$

$$\text{Porosity}\,(\%) = 1 - \left(\frac{\text{Bulk Density}}{\text{Particle Density}} \right) \text{x}\,100$$

NB: The particle density of 2.65g^{-1}cm^{3} was used as it was being considered to be adequate average for mineral specific gravity for sand fraction since the mineral grains in many soils are quartz and feldspar (Indiana University Soil Geomorphology Laboratory 2005).

Table 6.10: Physical and chemical properties of rooting media investigated for effects on rooting of single-node leafy stem cuttings of *B. procera* and *I. fagifer.*

Rooting media	Physical and Chemical Properties			
	Bulk density (g/cm3)	*Porosity (%)*	*Texture*	*pH*
Forest soil	1.20	54.8	Loamy clay	4.5 - 5.7
River soil	0.97	63.3	Clayish, silty loam	5.5 - 5.7
Coir	0.09	58.0	Soft rough, distinct organic aggregate	7.0
Coastal coral	1.23	53.6	Sandy gravel	>8
Coastal gravel	1.37	48.2	Clayish, sandy gravel	>8
1:1 coir to river sand mix	0.68	74.3	Sandy, soft rough, distinct organic aggregate	6.5

At the nursery, the cuttings were prepared and treated as described in Chapter 3 (section 3.2.2.3). Limited by planting materials, only twelve cuttings were randomly allocated to each rooting media, and each treatment replicated three times, totalling thirty-six cuttings per media treatment. Rooting was assessed at weeks 1, 3 and 4. Rooting assessment and data analysis is described in Chapter 3.

Experiment 3b: I. fagifer: This is a repeat of Experiment 3a above, but done with *I. fagifer* instead of *B. procera*. The following details are different:

i. Single-node leafy stem cuttings were collected from six month old seedlings, originating from trees number 3, 24 and 5 from Tututi and established as stockplants (Chapter 3).

ii. Experimental design is 24 cuttings x 5 media treatments (coir, river soil, coir/sand 1:1 mixture, forest soil and coastal coral) x 3 replicates, giving a total of 360 cuttings. Sixty seedlings were used for this experiment, and each seedling produced six cuttings.

6.2.3.2 Results

- *Impacts of different media on rooting*

Experiment 3a: B. procera: The percentage rooting of *B. procera* cuttings differed between media, being best in forest soil and coir and worst in coastal coral and gravel (Fig 6.19). On week 3, cuttings set in the forest soil rooted significantly

better (P<0.05) than those from coastal gravel. Percentage rooting between forest soil, river soil and coir were not significantly different from each other. The rate of rooting was greatest between 1-3 weeks (77%) for all media. Cutting mortality was 11-39% (Fig 6.19), and highest mortality occurred within the first 3 weeks. By week 4 all cuttings had either rooted or died.

The effect of rooting media on the number of roots per rooted cutting was significant ($F_{4, 110} = 4.73$, P<0.05) on week 3 (Fig 6.20). Forest soil, coir and river soil, all had significantly greater numbers of roots than coastal gravel in cuttings rooted by week 3, but not in cuttings that rooted between weeks 3-4 (Table 6.11).

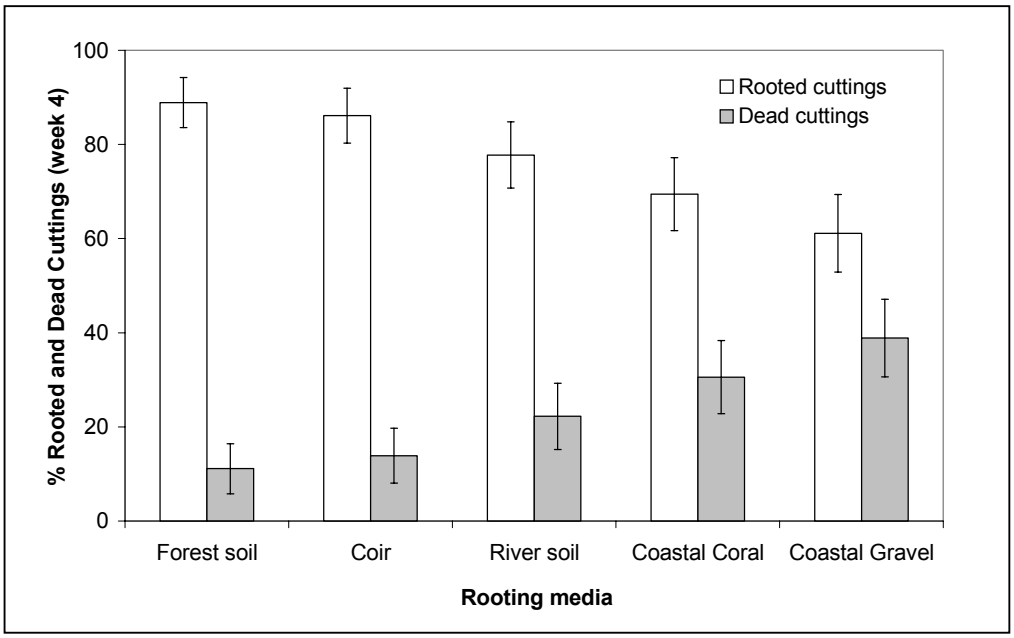


Fig 6.19: Effects of 5 different media on the percentage (±SE) rooting and mortality of cuttings of *B. procera.*

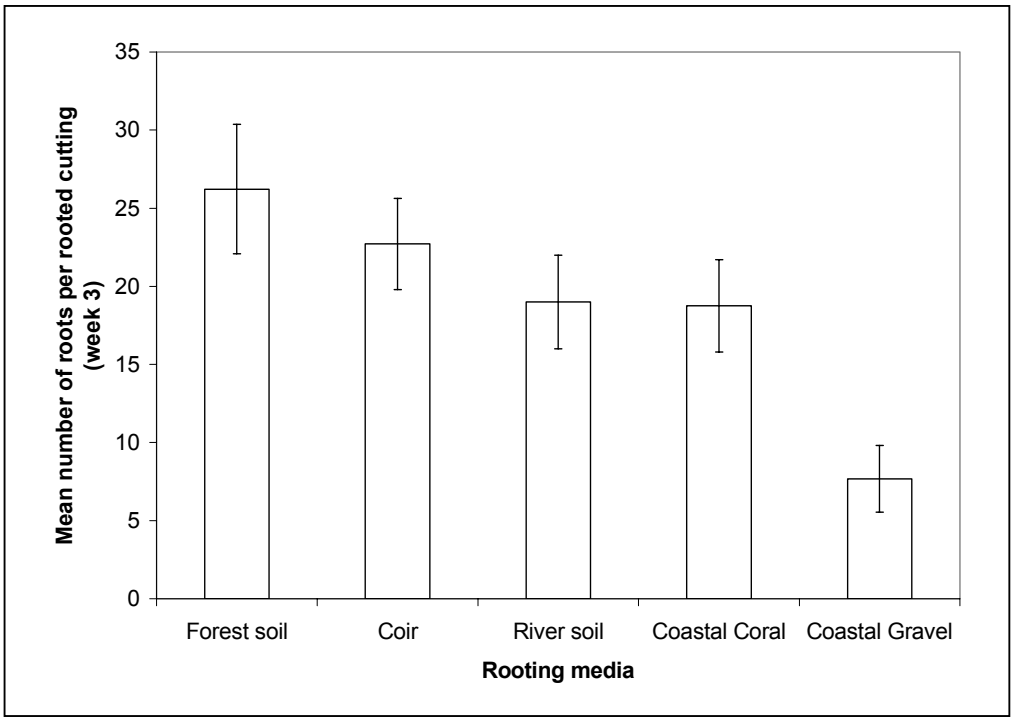


Fig 6.20: Effects of 5 different media on the number (±SE) of roots per rooted cutting of *B. procera.*

Table 6.11: Effects of rooting media on the mean number (±SE) of roots per rooted cutting from single-node leafy (50cm^2) stem cuttings of *B. procera.*

Rooting media	Mean number of roots per newly rooted cutting		
	Week 0-1 (n=180)	*Week1-3 (n=115)*	*Week3-4 (n=23)*
Forest soil	0	26.2 ± 3.38	4.8 ± 0.86
Coir	0	22.7 ± 2.53	5.0 ± 0.58
River soil	0	19.0 ± 2.69	3.7 ± 1.20
Coastal Coral	0	18.8 ± 2.65	4.2 ± 0.37
Coastal Gravel	0	7.7 ± 2.21	3.1 ± 0.51
Significance	-	*	NS

NS = non-significant, *significant P<0.05

Across all media, percentage rooting in cuttings from successive nodes was not significantly different. Generally, better rooting occurred in cuttings at lower nodes, with lower percentage mortality (Fig 6.21). Number of roots per rooted cutting was not significant between nodes at week 3, although cuttings from node 3 in Forest soil produced more roots (Fig 6.22). Rooting at lower nodes was poor in costal gravel.

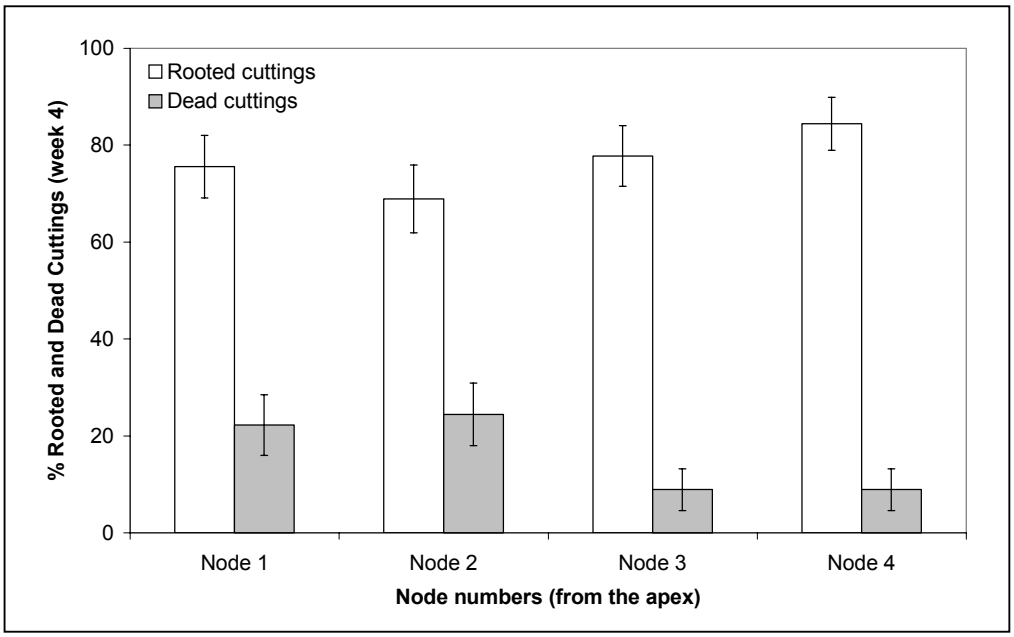


Fig 6.21: Effects of node numbers and 5 different rooting media on percentage (±SE) rooting of cuttings of *B. procera.*

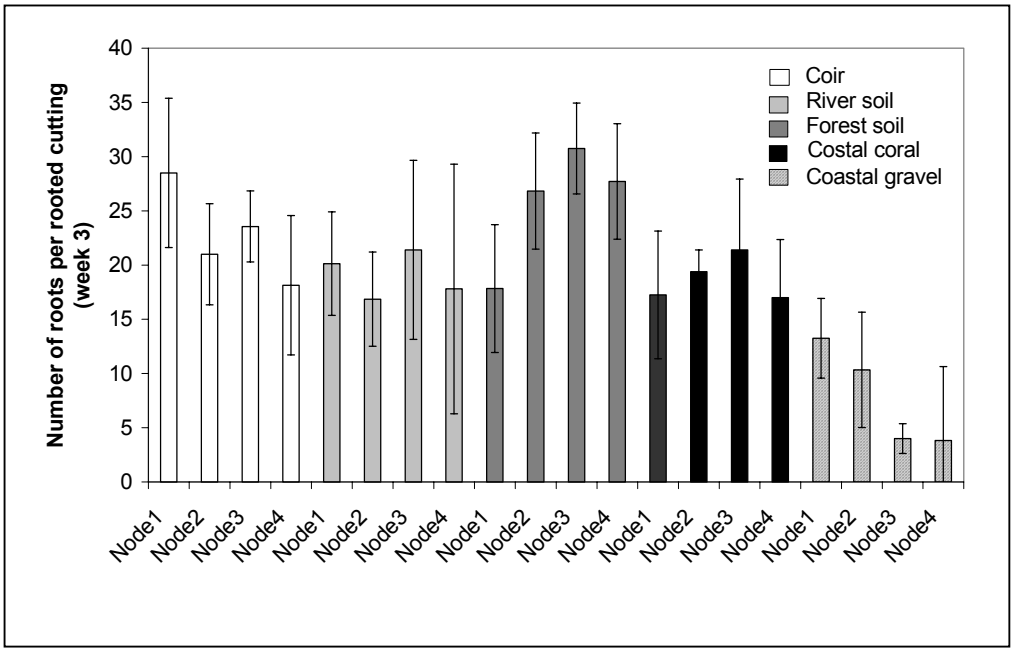


Fig 6.22: Effects of node numbers on the number (±SE) of roots per rooted cutting of *B. procera* across all 5 media.

Percentage rooting declined with an increase in the bulk density of the media in a fairly weak relationship (r^2 = 0.21, P>0.05) (Fig 6.23). In contrast, rooting

significantly increased with an increase in percentage porosity, with a moderately strong correlation ($r^2 = 0.34$, $P>0.05$) (Fig 6.24).

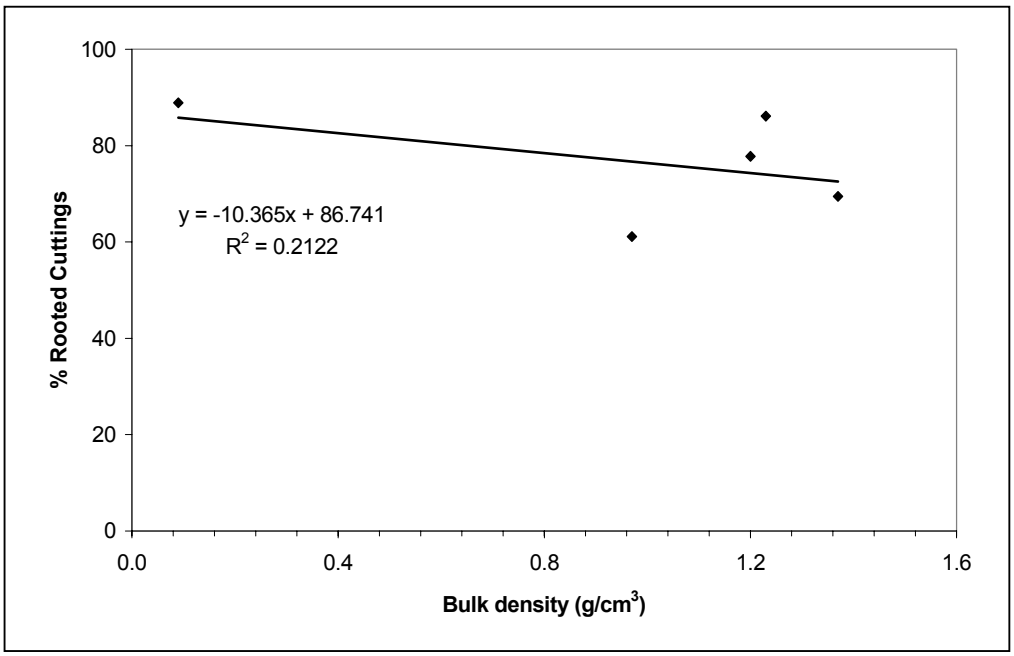


Fig 6.23: Relationship between percentage rooting of cuttings and the bulk density of rooting media in *B. procera*.

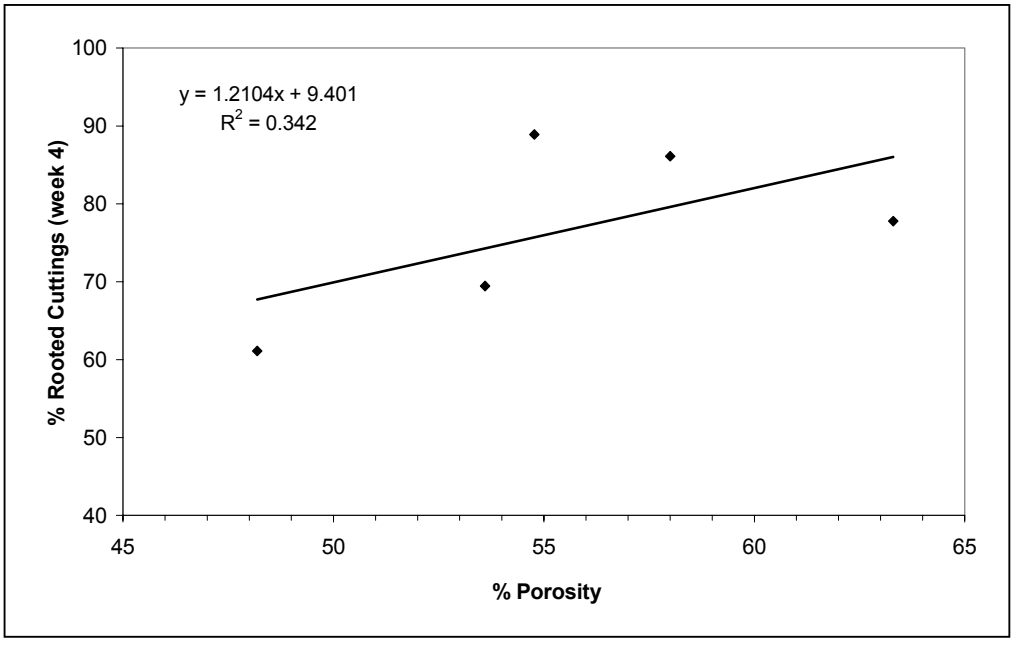


Fig 6.24: Relationship between percentage rooting of cuttings and percentage porosity of rooting media in *B. procera*.

Experiment 3b: I. fagifer: Cuttings of *I. fagifer* rooted well (80.6% - 95.8%) in all the tested media. The only differences which were significant (P<0.05) were between coir and coastal (Fig 6.25). The speed of rooting was greatest in the first 3 weeks. Cutting mortality was less than 20% for all treatments (Fig 6.25). Mortality in coir was significantly lower (P<0.05) than coir/sand mixture (10%) or coastal coral (11%), but differences in percentage mortality were not significant for other media. Mortality mainly occurred in the first 3 weeks.

The number of roots per rooted cutting was significant ($F_{4, 275} = 5.90$, $P = 0.001$) between media treatments at week 3 (Fig 6.26 and Table 6.12). Cuttings rooted in coir had significantly more roots than those in the coastal coral, forest soil or river soil, while cuttings in coir/sand mixture had significantly more roots than coastal coral and forest soil.

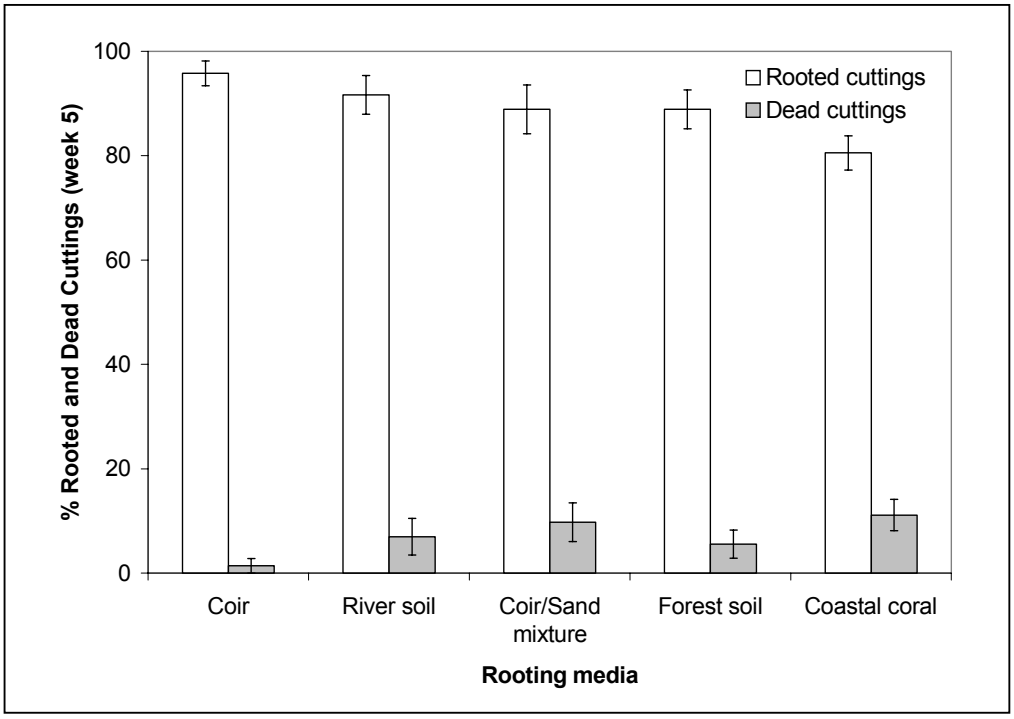


Fig 6.25: Effects of 5 different media on the percentage (±SE) rooting and mortality of cuttings of *I. fagifer.*

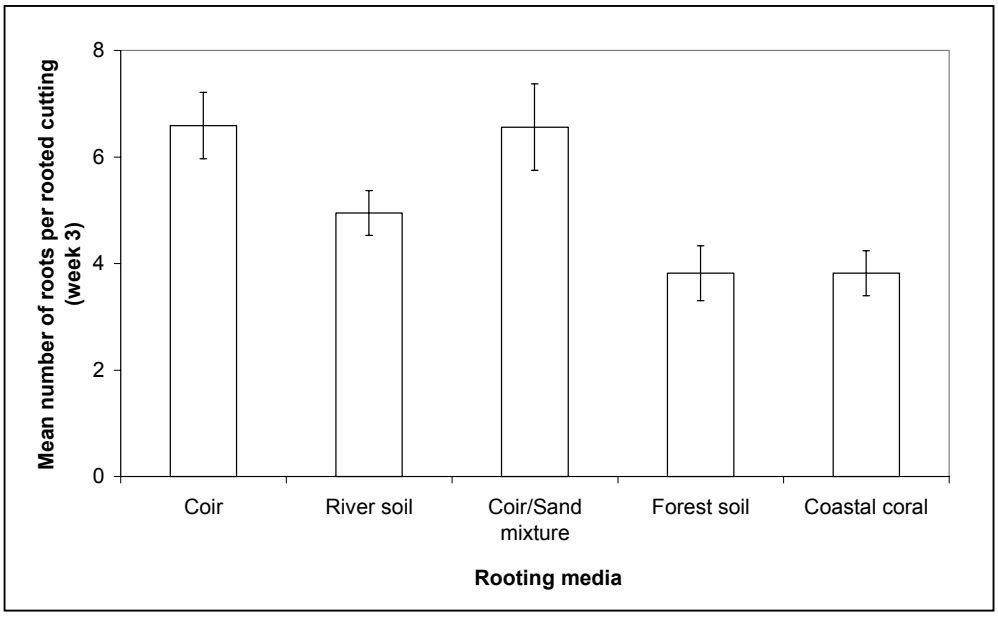


Fig 6.26: Effects of 5 different media on the number (±SE) of roots per rooted cutting of *I. fagifer.*

Table 6.12: Effects of rooting media on the mean (±SE) number of roots per rooted cutting from single-node leafy (50cm^2) stem cuttings of *I. fagifer.*

Rooting media	Mean number of roots per newly rooted cutting		
	Week 0-1 (n=360)	*Week 1-3 (n=280)*	*Week 3-4 (n=41)*
Coir	0	6.6 ± 0.62	4.1 ± 0.79
River soil	0	4.9 ± 0.42	3.9 ± 0.52
Coir/Sand mixture	0	6.6 ± 0.81	3.3 ± 0.78
Forest soil	0	3.8 ± 0.38	2.4 ± 0.60
Coastal coral	0	3.8 ± 0.42	2.6 ± 0.69
Significance		**	NS

NS = non-significant, **significant P = 0.001

Over all media, cuttings from node 4 rooted the best and with no mortality (Fig 6.27). Number of roots per rooted cutting increased exponentially in cuttings from nodes 1-6 at week 3. Generally, lower nodes produce more roots than apical ones (Fig 6.28). However, differences in number of roots per rooted cuttings between nodes were not significant.

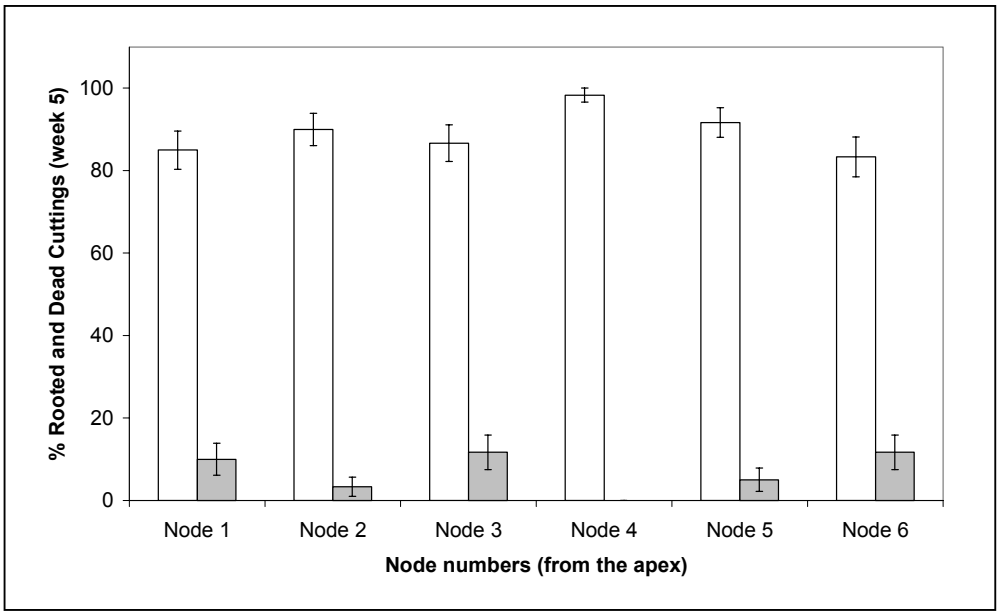


Fig 6.27: Effects of node numbers and 5 different rooting media on percentage (±SE) rooting of cuttings of *I. fagifer.*

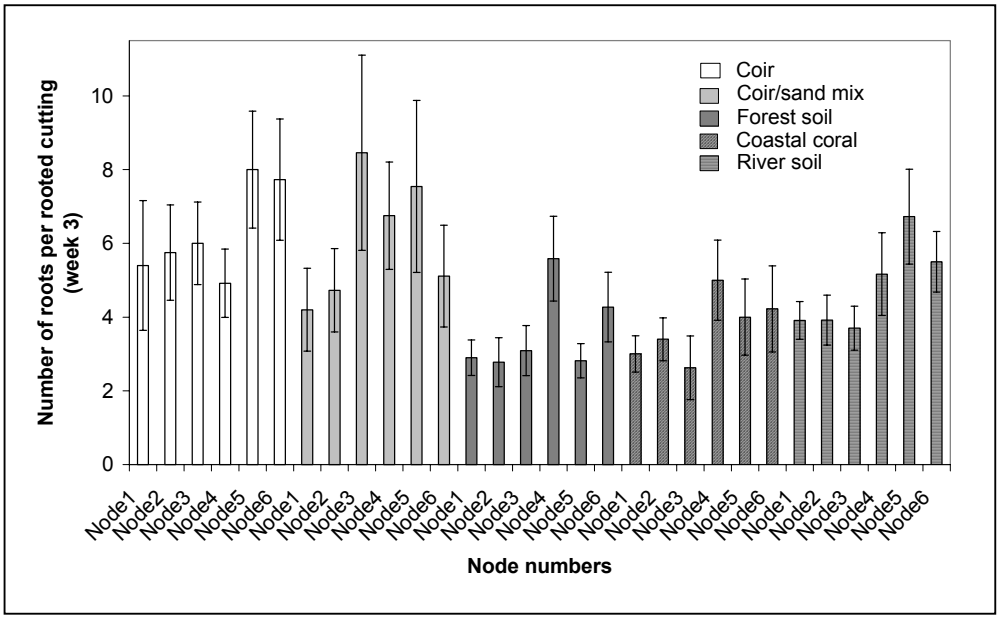


Fig 6.28: Effects of node numbers on the number (±SE) of roots per rooted cutting of *I. fagifer* across all 5 media.

Percentage rooting declined with an increase in the bulk density of the media, in a significant and moderately strong relationship (r^2 = 0.59, P>0.05) (Fig 6.29).

Rooting increased with an increase in percentage porosity, but the regression analysis showed they are unrelated ($r^2 = 0.06$, $P>0.05$).

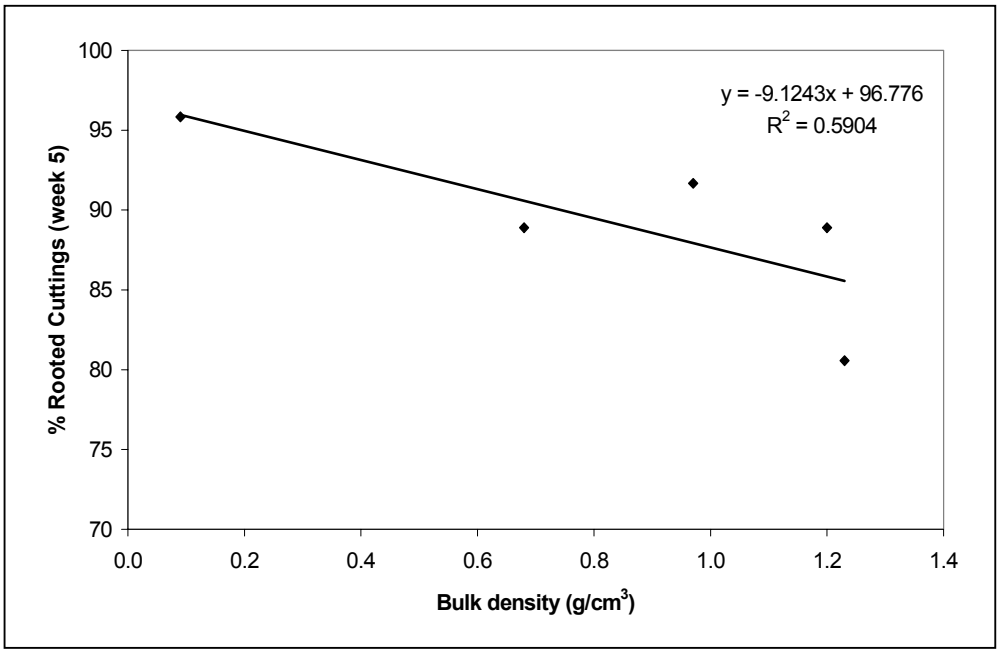


Fig 6.29: Relationship between percentage rooting of cuttings and the bulk density of rooting media in *I. fagifer*.

6.2.4 Effects of stem diameter and length on rooting of single-node leafy stem cuttings of seedling *Barringtonia procera*

6.2.4.1 Experiment 4: Experimental details

Experiment 4: B. procera: A total of 120 single-node leafy stem cuttings of *B. procera* were collected from six month old seedlings originating from trees numbers 1 and 97 of Hunda and 1, 5 and 7 of Tututi, and established as stockplants (Chapter 3). At the nursery, the single-node cuttings were prepared as described in Chapter 3 (section 3.2.2.3). The single leaf was then trimmed to 30 cm^2, guided by a template cut from graph paper.

Cuttings were then dipped into 0.8% IBA powder before they were inserted into the media contained within a non-mist propagator as described in Chapter 3, in node position starting from the apex. Excess powder was removed by gently tapping the base of the cutting. Six stem size treatment combinations (small-short;

small-medium; small-long; large-short; large-medium and large-long) were experimented. Stem length is defined as: short = 4 mm; medium = 8 mm and long = 10 mm, and stem diameter is as: small = 5-10 mm and large = 10-15 mm. The design for this experiment is 4 cuttings x 6 treatment combinations x 5 replicates, laid out in a randomised complete block. Rooting was assessed at 1, 2, 3 and 5 weeks analysed as described in Chapter 3.

6.2.4.2 Results

- *Effects of cutting size and length*

Experiment 4: B. procera: Small diameter cuttings rooted better than large diameter cuttings, while long cuttings rooted better than short cuttings (Fig 6.30). Cuttings with small-medium stems rooted the least (8%) on week 2, but the success rate increased to 50% by week 5. Percentage rooting of cuttings that are small-short, small-medium, large-short, large-medium and large-long were not significantly different. Greater proportion of cuttings rooted on week 3. Mortality also varied between treatments, with death in large-short cuttings being more common than others (Fig 6.31). Small-long cuttings had the lowest percentage mortality.

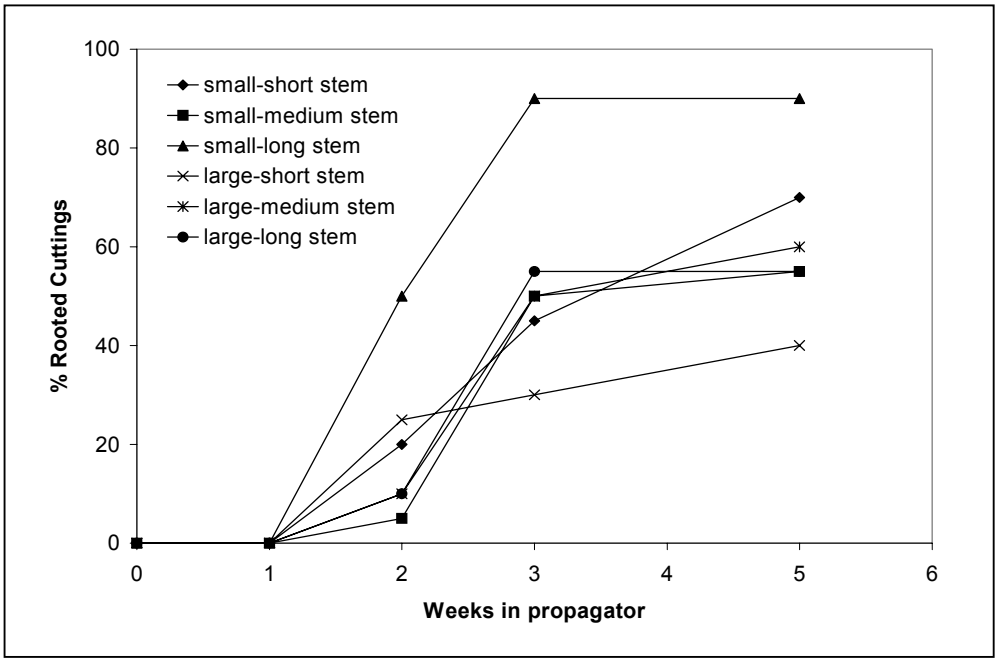


Fig 6.30: Effects of stem diameter and length on percentage rooting of single-node leafy cuttings of *B. procera* over time.

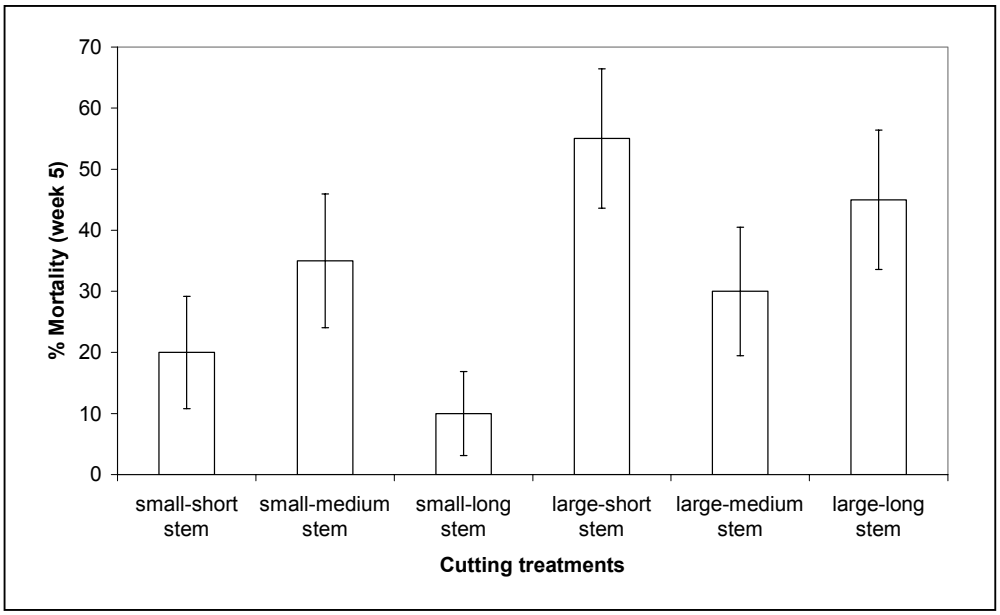


Fig 6.31: Effects of stem size and length on percentage mortality (±SE) of single-node leafy cuttings of *B. procera.*

The effect of stem size and length on the number of roots per rooted cutting varied, with small-long cuttings consistently producing more roots than the other treatments on weeks 2 and 3 (Table 6.13), but the differences in root number were not significant. The number of cuttings rooted from individual treatments was very low on week 5.

Table 6.13: Effects of stem size and length on the mean (±SE) number of roots per rooted cutting from single-node leafy (50cm^2) stem cuttings of *B. procera.*

Stem length treatments	Mean number of roots per newly rooted cutting		
	Week1 (n=120)	*Week2 (n=23)*	*Week3 (n=40)*
Small-short	0	9.5 ± 2.36	4.6 ± 0.93
Small-medium	0	2.0 ± 0.00	5.9 ± 0.95
Small-long	0	10.7 ± 2.09	13.6 ± 4.45
Large-short	0	6.3 ± 2.29	4.0 ± 0.00
Large-medium	0	7.0 ± 2.00	6.6 ± 2.09
Large-long	0	8.0 ± 4.00	7.2 ± 1.67
Significance	-	NS	NS

NS = non-significance

Relationships between percentage rooting and stem volume were positive in both small and large diameter cuttings (Fig 6.32), with correlation only being strong in the latter relationship ($r^2 = 0.820$, $P>0.05$). Relationships between number of roots per rooted cutting and stem volume were negatively weak in large diameter

cuttings and positively weak in small diameter cuttings (Fig 6.33). The interaction between stem length and diameter on the number of roots produced per rooted cutting was not significant. However, individually, stem length is strongly correlated with number of roots ($r^2 = 0.84$, $P<0.05$), while stem diameter isn't ($r^2 = 0.15$, $P>0.05$).

Cuttings from nodes 1-4 responded differently to different stem treatments, but with few consistent trends (Fig 6.34), some being significant at P<0.05. Small-long cuttings on nodes 3 and 4 rooted better than those with large-short, large-medium and large-long stems. Across all treatments, the relationships between percentage rooting and stem volume was positively strong ($r^2 = 0.754$, $P>0.05$) (Fig 6.35).

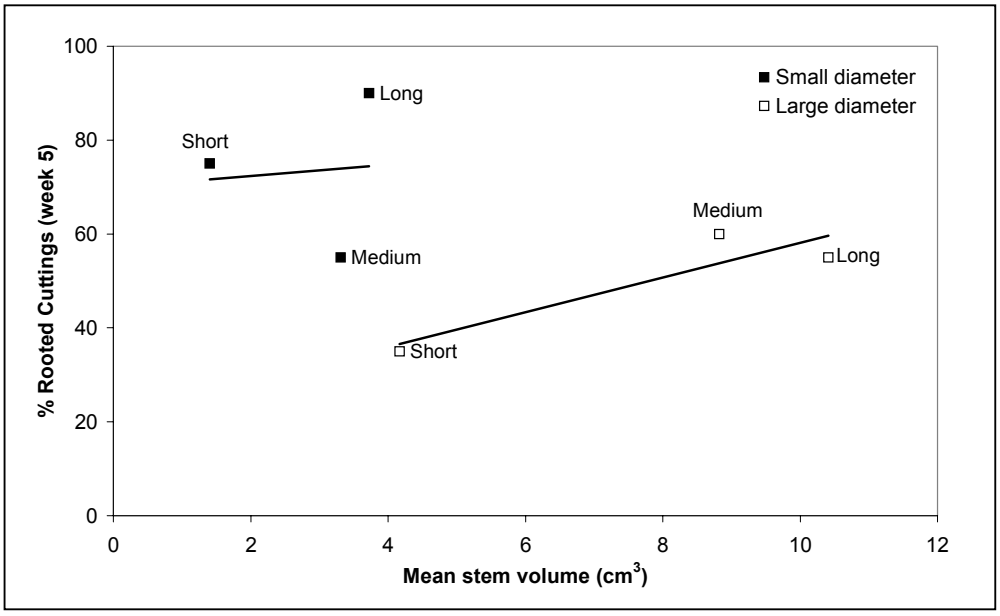


Fig 6.32: Relationship between stem length (4, 8 and 10 cm) and the number of roots produced from cuttings of *B. procera.*

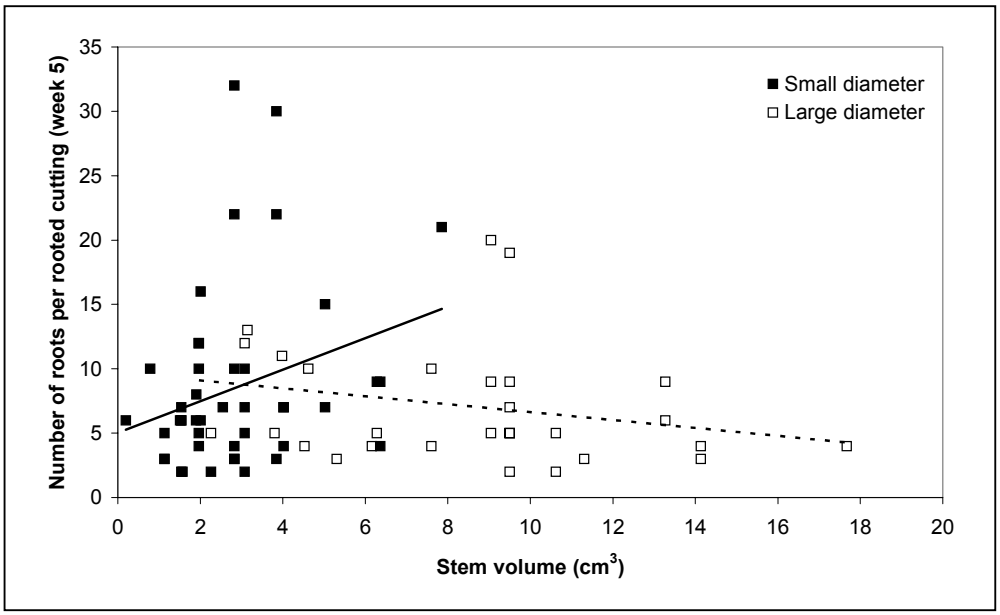


Fig 6.33: Relationship between stem diameter and the number of roots produced from cuttings of *B. procera.*

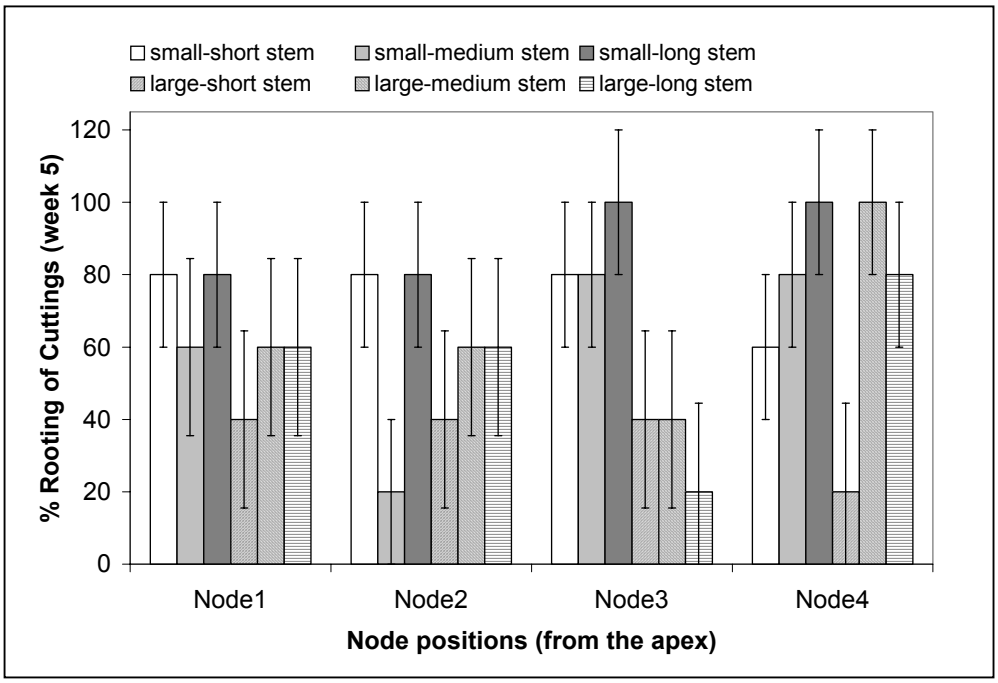


Fig 6.34: Effects of stem size and length and node position on percentage (±SE) rooting of *B. procera* cuttings.

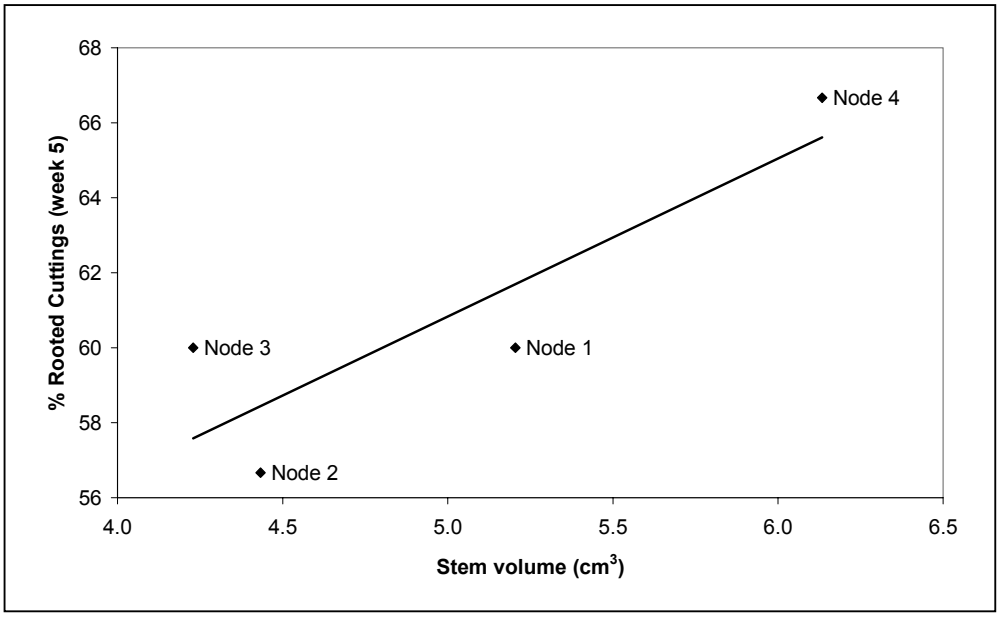


Fig 6.35: Relationship between percentage rooting and stem volume of *B. procera* cuttings across all treatments.

6.2.5 Effects of stem length per node on root initiation in sequential single-node cuttings of *Barringtonia procera* and *Inocarpus fagifer*

6.2.5.1 Experiment 5: Experimental details

Experiment 5a: B. procera: A total of forty-eight shoots were collected for this experiment from six month old seedlings stockplants (Chapter 3) originating from trees numbers 1, 2 and 97 of Hunda and trees numbers 6, 7 and 16 of Tututi. Four single-node leafy stem cuttings were obtained from each shoot, adding up to one hundred and ninety-two cuttings in total. In the nursery, cuttings were cut into single nodes according to 3 stem lengths *viz* increasing length acropetally (i.e longest at the apical end and shortest at the basal end), increasing length basipetally (shortest at the apical end and longest at the basal end) and constant stem length. In acropetal length, the cuttings were sized by node (N) position as: N1 = 87-90 mm; N2 = 68-70 mm; N3 = 53-55 and N4 = 33-35 mm. By contrast in basipetal length, cuttings were sized by node position as: N1 = 33-35 mm; N2 = 43-45 mm; N3 = 57-60 and N4 = 73-75 mm. Cuttings with constant length were

sized to 52-55 mm long for all node positions. Cuttings were cut to these lengths from shoots with internodes (Fig 6.36).

The single leaf on each cutting was trimmed to 30 cm^2. Immediately after severance, cuttings were dipped into 0.8% IBA concentration before insertion into coir medium in node order starting from the apex. The design for the experiment is 16 cuttings x 3 stem treatments x 4 replicates, in a randomised complete block. Rooting was assessed weekly up to 5 weeks and analysed as described in Chapter 3.

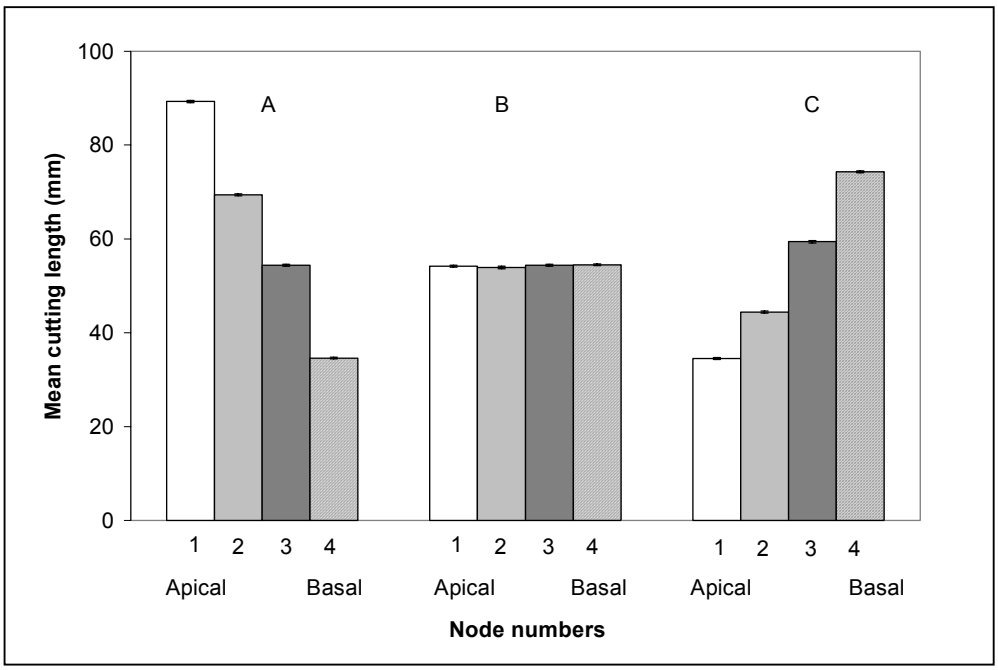


Fig 6.36: Mean (±SE) cutting lengths of single-node leafy cuttings of *B. procera* determined into three treatments as: acropetally and basipetally increasing length and those constant lengths.

Experiment 5b: I. fagifer: This is a repeat of Experiment 5a above, but on *I. fagifer*. The following details are different:

i. Single-node leafy stem cuttings were collected from six month old seedlings originating from tree number 3 of Tututi and established as stockplants (Chapter 3).

ii. In acropetal length, the cuttings were sized by node (N) position as: N1 = 33-35 mm; N2 = 27-30 mm; N3 = 22-25; N4 = 18-20 mm N5 = 13-15 and N6 = 7-10 mm. By contrast in basipetal length, cuttings were sized by node position as: N1 = 7-10 mm; N2 = 12-15 mm; N3 = 18-20; N4 = 23-25 mm N5 = 28-30 and N6 = 33-35 mm. Cuttings with constant length were sized to 22-25 mm long for all node positions (Fig 6.37).

iii. Experimental design is 12 cuttings x 3 stem treatments x 4 replicates, giving a total of 144 cuttings. Twenty-four seedlings were allocated from this experiment, and each seedling produced 6 cuttings.

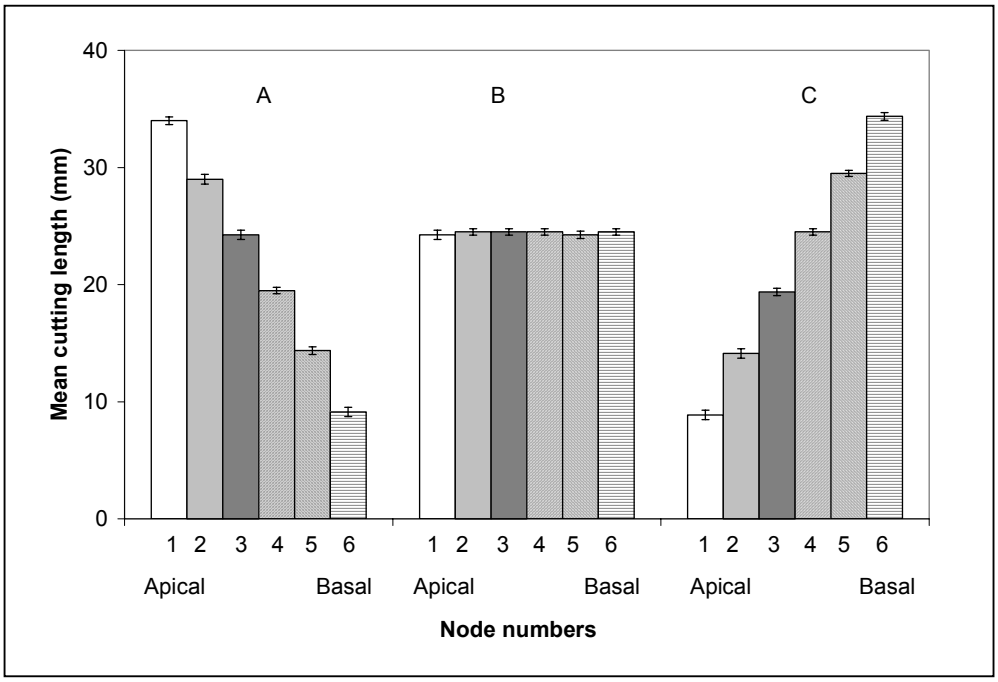


Fig 6.37: Mean (±SE) cutting lengths of single-node leafy cuttings of *I. fagifer* determined into three treatments as: acropetally and basipetally increasing length and those constant lengths.

6.2.5.2 Results

- *Effects of length and position of cuttings on the shoot*

Experiment 5a: B. procera: Cuttings cut with an acropetal gradient (A) rooted less well than those with a basipetal gradient (C). Constant length cuttings (B) were rooted the best, with cuttings from node 4 rooting best in all treatments (Fig 6.38).

Cutting mortality was 19-41% in this experiment, being significantly greater in cuttings with an acropetal length gradient (41%) and least in those of constant length (19%). In all treatments, percentage mortality tends to decline towards basal nodes (Fig. 6.39). Number of roots per rooted cuttings also differs with node position in all treatments (Table 6.14), but the differences were not significant.

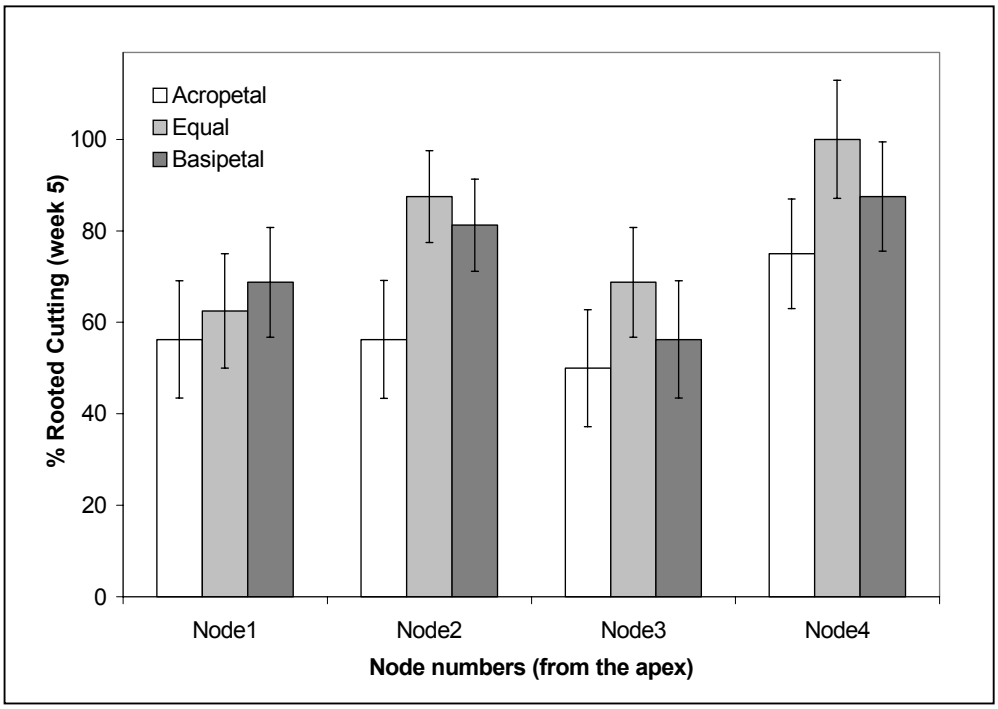


Fig 6.38: Effects of three different cutting lengths on the percentage (±SE) rooting on different node positions of cuttings of *B. procera.*

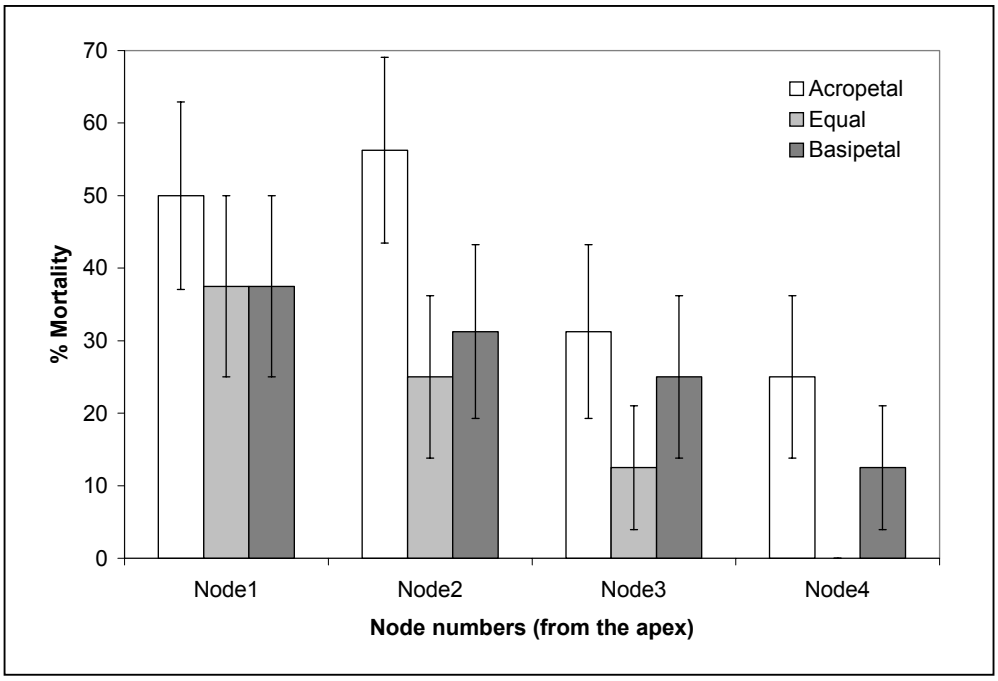


Fig 6.39: Effects of three different cutting lengths on the percentage (±SE) mortality on different node positions of cuttings of *B. procera* after week 5.

Table 6.14: Effects of cutting length on the number (±SE) of roots per rooted cutting of single-node cuttings of *B. procera.*

Treatments	Mean number of roots per rooted cutting (week 2-5)				
	Node1	*Node2*	*Node3*	*Node4*	*Significance*
Acropetal length	8.3 ± 2.64	5.6 ± 0.91	6.0 ± 0.73	5.1 ± 0.53	NS
Constant length	8.4 ± 1.89	12.1 ± 1.33	13.8 ± 3.03	7.8 ± 1.38	NS
Basipetal length	9.0 ± 1.37	7.5 ± 2.32	5.5 ± 1.39	7.6 ± 1.51	NS
Significance	NS	*	*	NS	

NS = non-significant, *significant P<0.05

The relationships between percentage rooting and cutting lengths varied in strength with time (Table 6.15). When cuttings had an acropetal length gradient, the correlation was weak on week 2 (Fig 6.40), and further weakened on week 5 (Fig 6.41). Similarly, the relationship weakened in cuttings with a basipetal length gradient. By contrast, when cuttings were cut in the same lengths, negative correlation on week 2 (Fig 6.40) was strengthened on week 5 (Fig. 6.41).

Table 6.15: Correlation in relationships between percentage rooting, number of roots per rooted cutting and different cutting lengths.

% rooting	*Correlation (r^2)*		
	Acropetal length	*Constant length*	*Basipetal length*
Week 0-2	0.180, *P*>0.05	0.165, *P*>0.05	0.984, *P*<0.05
Week 2-5	0.049, *P*>0.05	0.857, *P*>0.05	0.635, *P*>0.05

No. of roots	*Correlation (r^2)*		
	Acropetal length	*Constant length*	*Basipetal length*
Week 0-2	0.377, *P*>0.05	0.119, *P*>0.05	0.373, *P*>0.05
Week 2-5	0.713, *P*>0.05	0.137, *P*>0.05	0.766, *P*>0.05

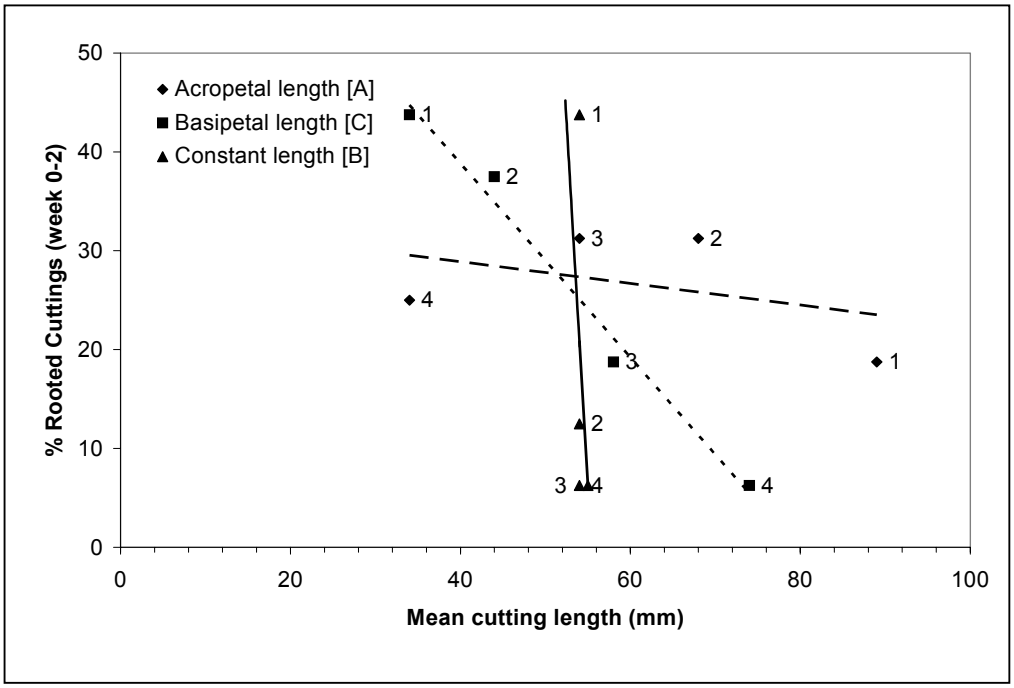


Fig 6.40: Relationship in *B. procera* between cutting length and percentage rooted cuttings on weeks 0-2.

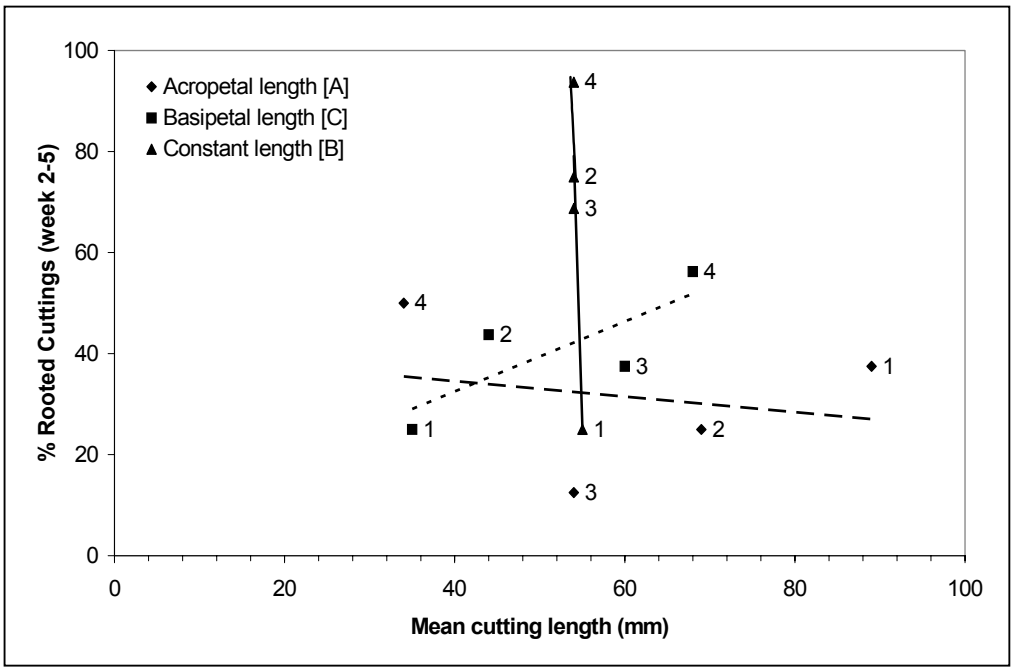


Fig 6.41: Relationship in *B. procera* between cutting length and percentage rooted cuttings on weeks 2-5.

The relationships between cuttings length and the numbers of roots per rooted cutting were a direct contrast to that of percentage rooting (Fig 6.42 and Fig 6.43). Weak positive correlations in acropetally and basipetally cutting lengths on week 2 were strengthened on week 5. However, cuttings with similar lengths had a weak positive correlation ($r^2 = 0.12$, $P>0.05$) on week 2 but was negative and slightly strengthened ($r^2 = 0.14$, $P>0.05$) on week 5.

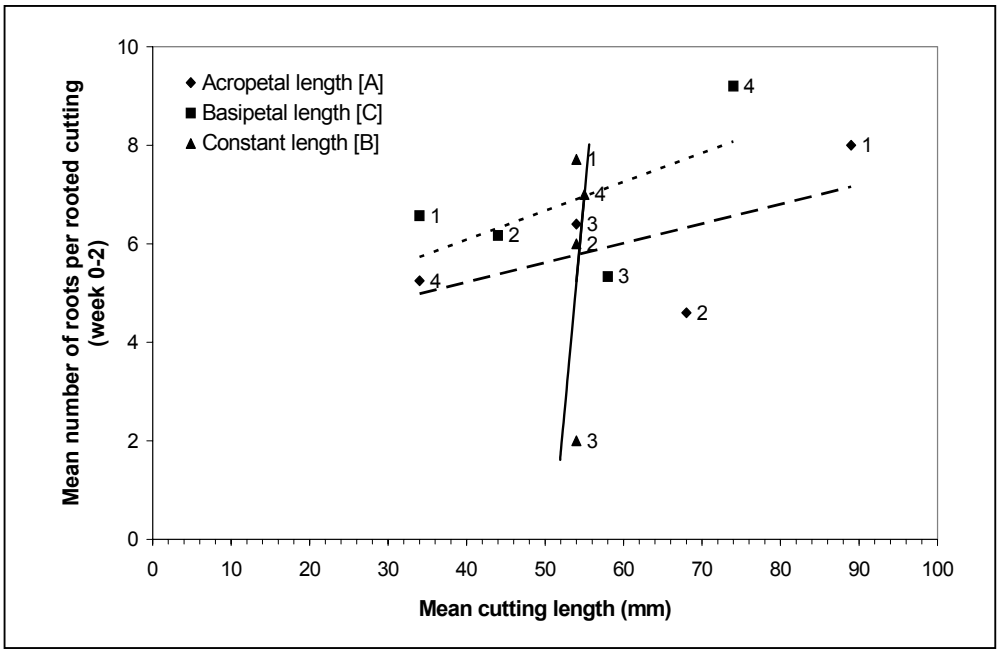


Fig 6.42: Relationship in *B. procera* between cutting length and the number of roots per rooted cutting on weeks 0-2.

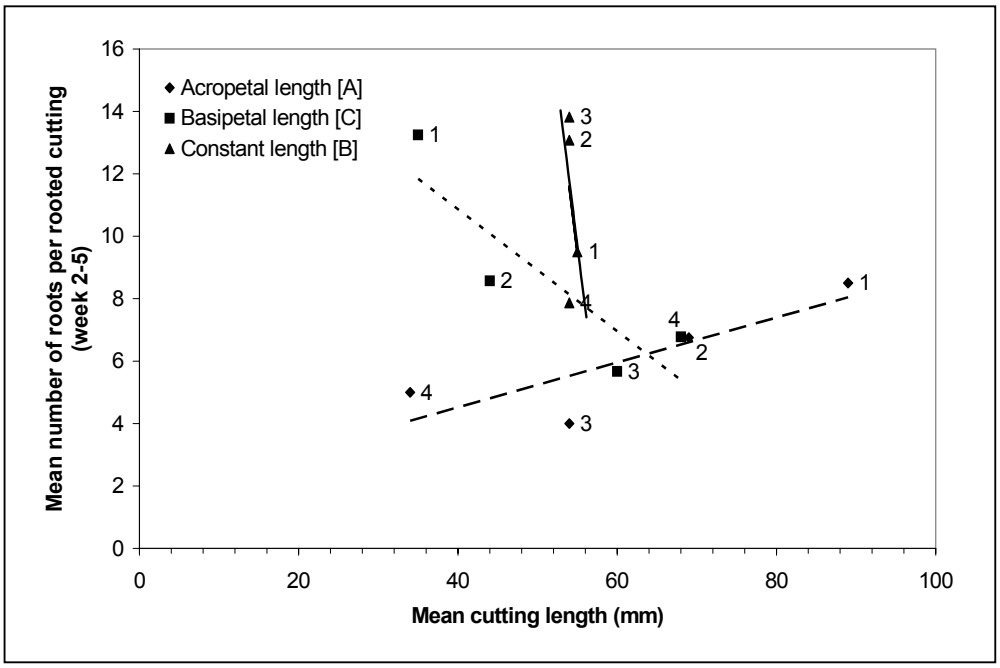


Fig 6.43: Relationship in *B. procera* between cutting length and the number of roots per rooted cutting on weeks 2-5.

Experiment 5b: I. fagifer: By week 5, 100% of all *I. fagifer* cuttings from the three treatments had rooted. However, earlier in the experiment, there had been variation in the rooting ability of cuttings from the different treatments. Cuttings with both acropetal and basipetal gradient in cutting length did not have node-to-node variation in rooting ability at either week 3 or week 4 (Table 6.16). However, in contrast, cuttings of constant length had significantly different node-to-node variation in rooting percentage (Table 6.16) having a trend for rooting best in cuttings from apical nodes in the acropetal gradient and from basal nodes – cuttings with a basipetal gradient (Table 6.16). However, there are some inconsistencies between specific nodes in these overall trends.

Number of roots per rooted cuttings also differed in a similar way between treatments at week 3, but by week 4 all cuttings types and all node positions, were not significantly different (Table 6.17).

Table 6.16: Effects of cutting length on percentage (±SE) rooting of single-node cuttings of *I. fagifer.*

Node numbers	% Rooting by cutting treatments		
	Acropetal lengths	*Similar lengths*	*Basipetal lengths*
Week 0-3			
Node1	37.5 ± 18.3	50.0 ± 18.9	37.5 ± 18.3
Node2	37.5 ± 18.3	87.5 ± 12.8	37.5 ± 18.3
Node3	25.0 ± 16.4	62.5 ± 12.5	25.0 ± 16.4
Node4	12.5 ± 12.5	37.5 ± 18.3	37.5 ± 18.3
Node5	12.5 ± 12.5	37.5 ± 18.3	25.0 ± 16.4
Node6	25.0 ± 16.4	37.5 ± 18.3	37.5 ± 18.3
Significance	NS	*	NS
Week 3-4			
Node1	50.0 ± 18.9	37.5 ± 18.3	37.5 ± 18.3
Node2	62.5 ± 18.3	12.5 ± 12.5	62.5 ± 18.3
Node3	75.0 ± 16.4	37.5 ± 18.3	37.5 ± 18.3
Node4	62.5 ± 18.3	50.0 ± 18.9	62.5 ± 18.3
Node5	50.0 ± 18.9	62.5 ± 18.3	50.0 ± 18.9
Node6	50.0 ± 18.9	25.0 ± 16.4	25.0 ± 16.4
Significance	NS	*	NS

NS = non-significant, *significant P<0.05

Table 6.17: Effects of cutting length on the number (±SE) of roots per rooted cutting of single-node cuttings of *I. fagifer.*

Node numbers	Mean number of roots per rooted cutting		
	Acropetal lengths	*Similar lengths*	*Basipetal lengths*
Week 0-3			
Node1	1.7 ± 0.3	1.3 ± 0.3	2.3 ± 0.3
Node2	3.0 ± 0.6	3.1 ± 0.4	2.3 ± 0.7
Node3	2.0 ± 1.0	2.6 ± 0.5	2.5 ± 0.5
Node4	3.0 ± 0.0	2.7 ± 0.3	3.3 ± 0.3
Node5	2.0 ± 0.0	2.7 ± 0.3	1.5 ± 0.5
Node6	2.0 ± 1.0	5.0 ± 1.2	1.7 ± 0.3
Significance	NS	*	NS
Week4			
Node1	3.5 ± 1.2	2.7 ± 0.7	2.6 ± 0.7
Node2	2.4 ± 0.4	3.0 ± 0.0	2.2 ± 0.5
Node3	3.8 ± 0.3	2.0 ± 0.6	3.3 ± 1.3
Node4	3.4 ± 1.0	2.0 ± 1.0	2.8 ± 0.6
Node5	3.0 ± 0.4	2.2 ± 0.4	2.8 ± 0.5
Node6	4.3 ± 0.3	2.5 ± 0.5	3.5 ± 0.5
Significance	NS	NS	NS

NS = non-significant, *significant P<0.05

Positive correlations between cutting length and the percentage rooting were stronger on week 3 than week 4 for cuttings with acropetal gradient (Table 6.18). Relationships between cutting length and the percentage rooting were very weak and negative (Fig 6.44 and Fig 6.45) for cuttings with basipetal length gradient on both weeks 3 and 4. In contrast, when cutting lengths were constant, the correlations were relatively strong (Table 6.18).

Table 6.18: Correlation in relationships between percentage rooting and different cutting lengths

% rooting	*Correlation (r^2)*		
	Acropetal length	*Constant length*	*Basipetal length*
Week 0-3	0.508, *P*>0.05	0.487, *P*>0.05	0.062, *P*>0.05
Week 3-4	0.052, *P*>0.05	0.313, *P*>0.05	0.070, *P*>0.05

Relationships between cutting length and the number of roots per rooted cutting was positive but very weak on week 3 in cuttings with acropetal gradient in shoot length (Fig 6.46), and remain weak but negative by week 4 (Fig 6.47). Cuttings with a basipetal gradient in cutting lengths strengthened between weeks 3 and 4 (Table 6.19). There was no marked change in these correlations in cuttings with constant lengths (Table 6.19).

Table 6.19: Correlation in relationships between number of roots per rooted cutting and different cutting lengths.

No. of roots	*Correlation (r^2)*		
	Acropetal length	*Constant length*	*Basipetal length*
Week 0-3	0.010, *P*>0.05	0.113, *P*>0.05	0.181, *P*>0.05
Week 3-4	0.194, *P*>0.05	0.146, *P*>0.05	0.356, *P*>0.05

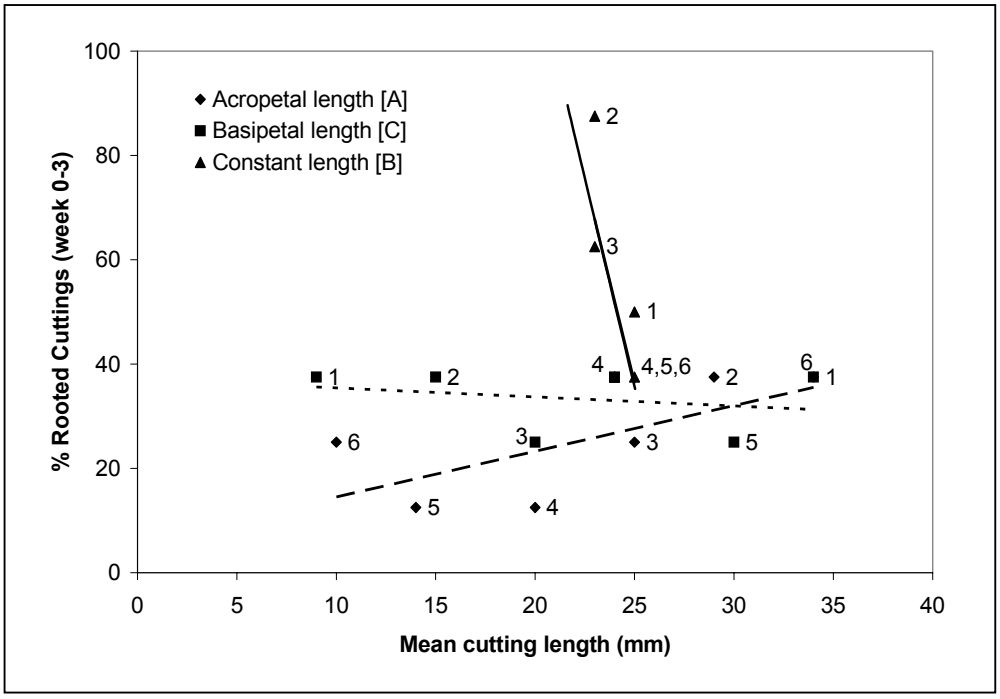


Fig 6.44: Relationship in *I. fagifer* between cutting length and percentage rooted cuttings on weeks 0-3.

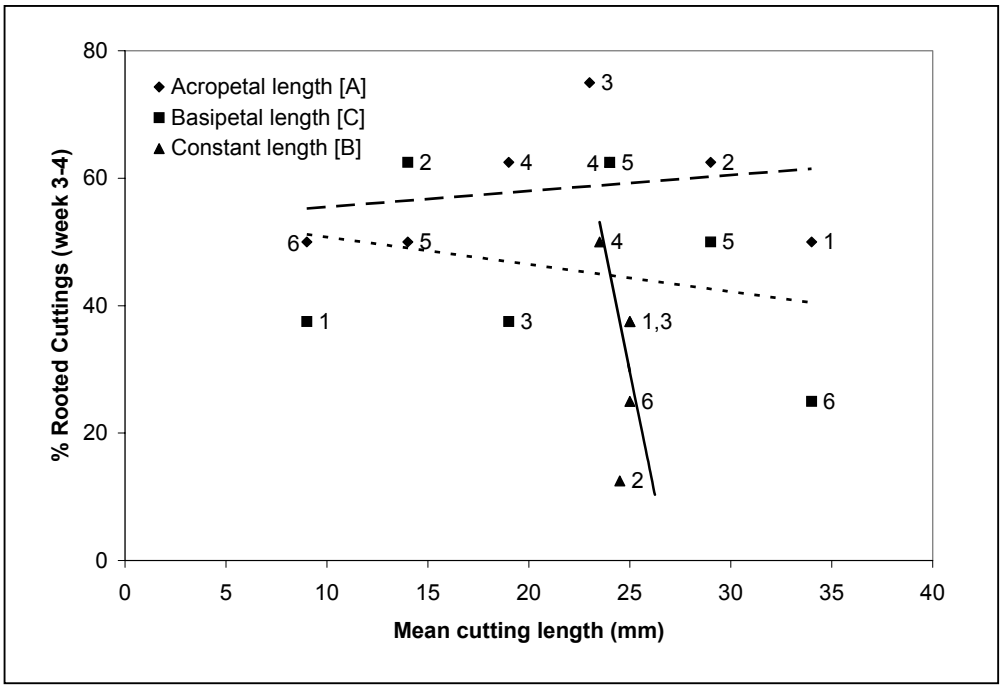


Fig 6.45: Relationship in *I. fagifer* between cutting length and percentage rooted cuttings on weeks 3-4.

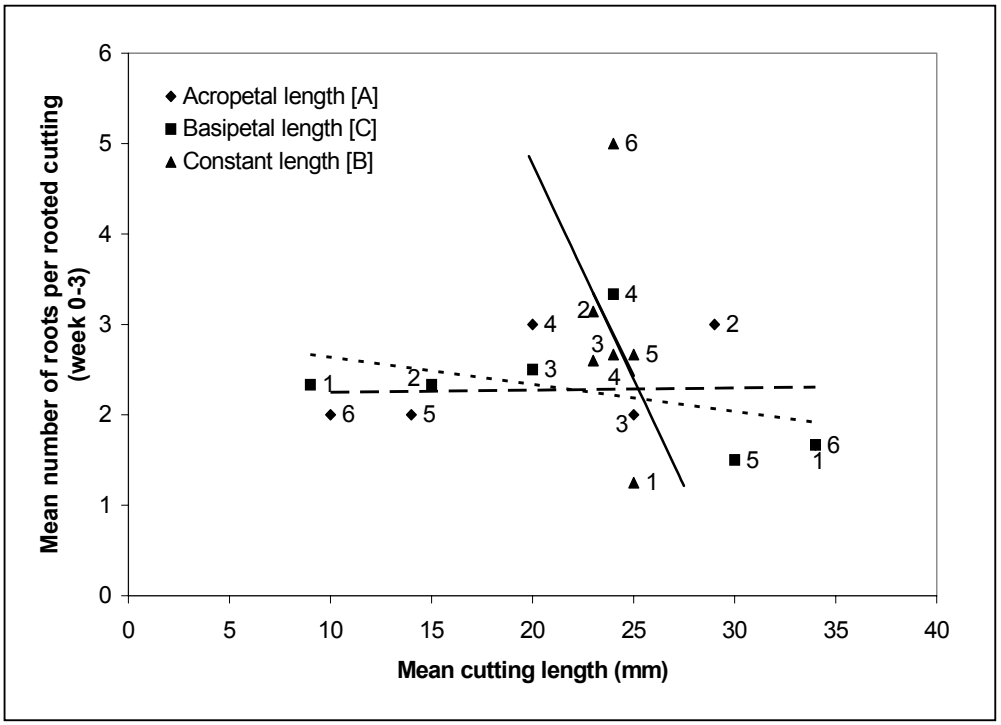


Fig 6.46: Relationship in *I. fagifer* between cutting length and the number of roots per rooted cutting on weeks 0-3.

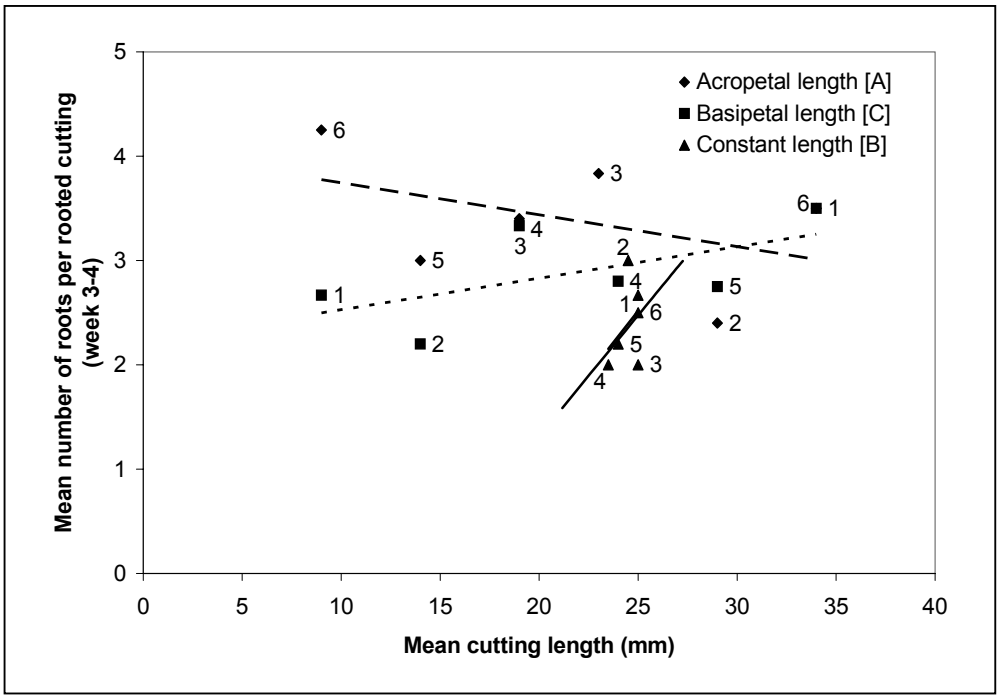


Fig 6.47: Relationship in *I. fagifer* between cutting length and the number of roots per rooted cutting on week 3-4.

6.3 PROPAGATING MATURE TREES

6.3.1 Comparison of ontogenetically mature and juvenile cuttings on the rooting ability of single-node leafy stem cuttings from juvenile (seedlings) and mature (marcotts) stockplants of *Barringtonia procera* and *Inocarpus fagifer*

6.3.1.1 Experiment 6: Experiment details

Experiment 6a: B. procera: Cuttings of *B. procera* were collected from two different sources: seedlings and marcotts. Both sets of plants were potted in a five litre black polythene bag filled with coir inoculated with slow release fertiliser (N = 16%, P = 4.4% and K =8.3%), Nutricote® following KFPL's standard nursery practice at 10g per litre coir, and were raised under 70% light at Ringgi nursery in Kolombangara Island. The seedlings were six months old, while the marcots were

eighteen months old, when cuttings were taken from them. Marcots were presumed to be ontogenetically mature.

A total of eighty cuttings were collected (forty from each of the two sources). In the nursery, single-node cuttings were prepared and the leaf trimmed to 30 cm^2. Before insertion into the coir, the cuttings were dipped into 0.8% IBA powder. Excess rooting powders on cuttings were removed by gently tapping the stem base. Cuttings were then inserted in node position starting from the apex. The design for this experiment is 10 cuttings x 2 treatments x 4 replicates, laid out in a randomised complete block. Rooting was assessed and analysed as described in Chapter 3.

Experiment 6b: I. fagifer: This is a repeat of Experiment 6a above with *I. fagifer*. The basic set up and protocols applied are similar. Differences include:

i. Experimental design is 16 cuttings x 2 treatments x 4 replicates, giving a total of 128 cuttings.

ii. Cuttings were assessed for rooting at weekly interval up to week 5 and data analysed as for experiment 6a.

6.3.2.2 Results

- *Effects of the status of stockplants*

Experiment 6a: B. procera: Juvenile cuttings of B. procera from seedlings rooted significantly better (85%) than mature cuttings from marcots (65%) on week 3 (Fig 6.48). Percentage mortality was greater in mature cuttings (38%) than juvenile ones (20%).

The ontogenetic age of cuttings affected the number of roots produced by cuttings. Cuttings originated from seedlings (juvenile) produced significantly ($F_{1, 39} = 5.64$, $P<0.05$) more roots per rooted cutting on week 2 than those from the mature (marcots) cuttings. This trend of superior rooting by juvenile cuttings was still evident ($F_{1, 17} = 7.78$, $P<0.05$) in newly rooted cuttings at week 3 (Fig 6.49).

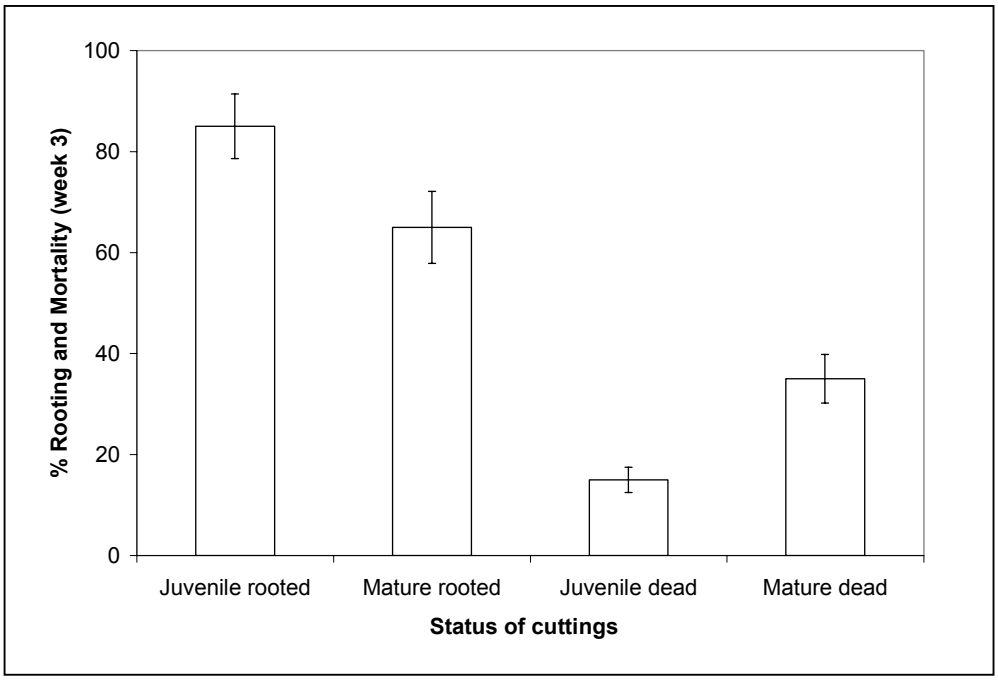


Fig 6.48: Comparative percentage (±SE) rooting and mortality of juvenile and mature cuttings of *B. procera* over time.

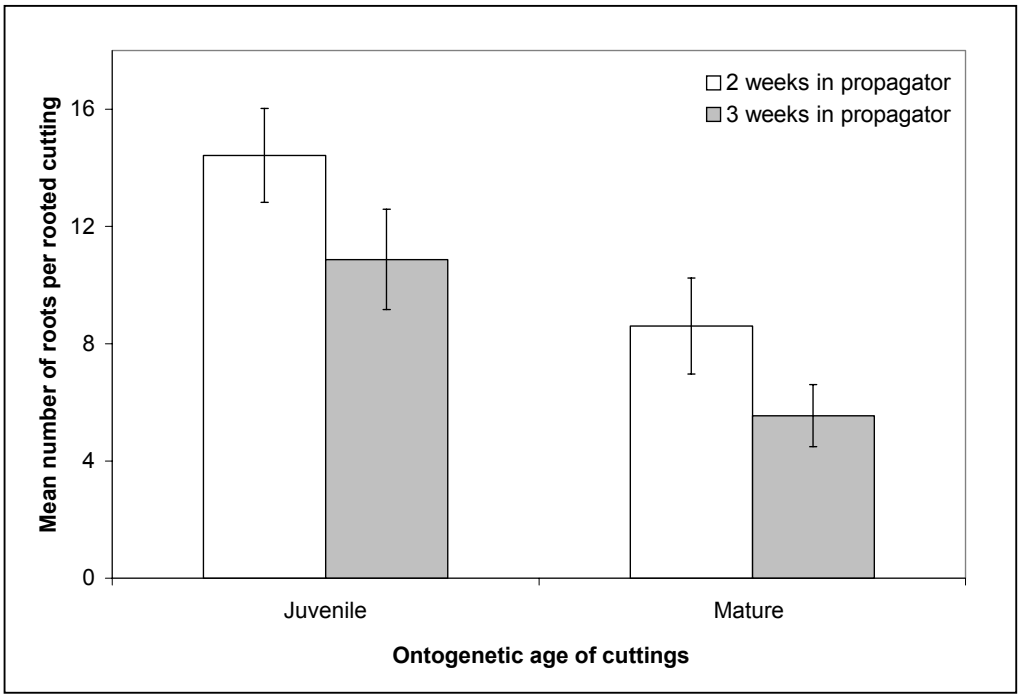


Fig 6.49: Effects of ontogenetic age of cuttings on the number (±SE) of roots per rooted cutting of *B. procera.*

Overall, percentage rooting between treatments did not differ significantly with node positions of cuttings, although cuttings from node 2 of seedlings had higher percentage rooting ($P<0.05$) than those from marcots (Fig 6.50).

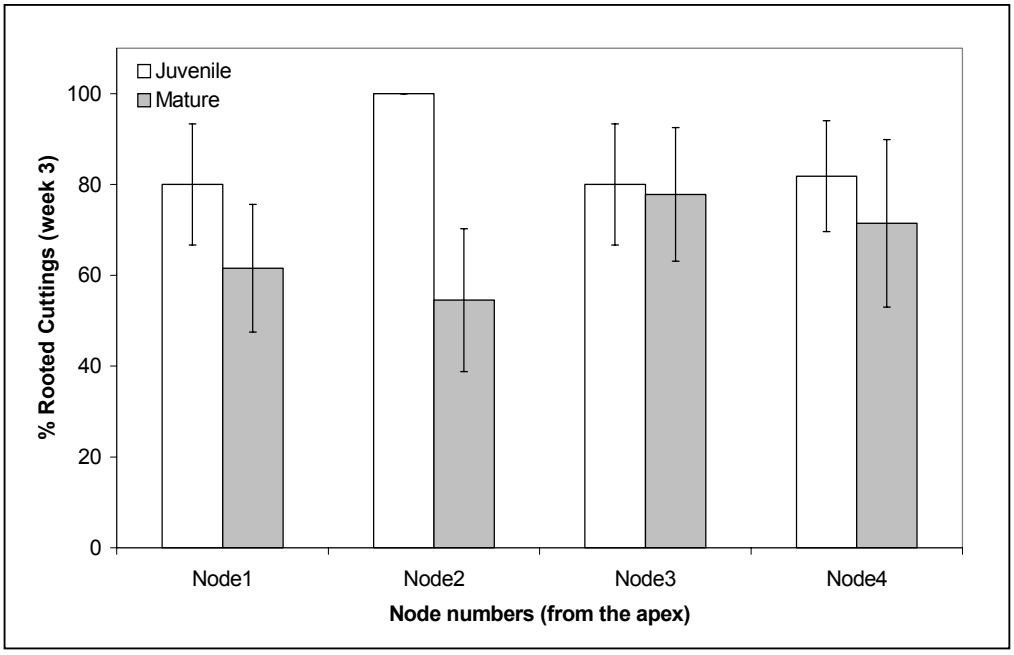


Fig 6.50: Effects of node position on percentage (±SE) rooting between juvenile and mature cuttings of *B. procera.*

Experiment 6b: I. fagifer: On week 2, juvenile cuttings of I. fagifer from seedlings rooted significantly ($P<0.05$) better (38%) than mature (marcot) cuttings (4%) (Fig 6.51). However, by week 4, mature cuttings had rooted significantly better (77%) than those from the seedlings (56%). By this time, percentage mortality was greater in juvenile cuttings (31%) than in mature ones (15%). Juvenile cuttings seemed to loose their leaves (6.3%) more than the mature cuttings (4.7%) but the difference was not significant.

The effect of ontogenetic age of cuttings on the number of roots produced was not significant (Table 6.20). Overall, percentage rooting between treatments did not differ significantly with node positions of cuttings, although Node 1 and Node 6

from the marcots rooted significantly ($P<0.05$) better than those from seedlings (juvenile) (Fig 6.52).

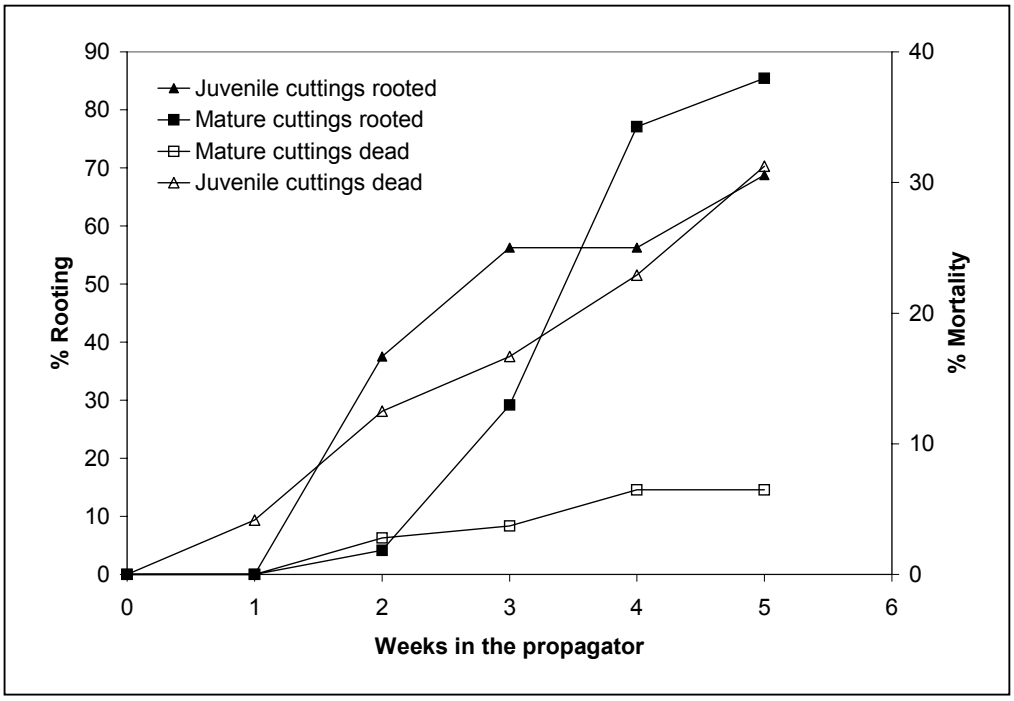


Fig 6.51: Comparative percentage rooting and mortality of juvenile and mature cuttings of *I. fagifer* over time.

Table 6.20: Effects of ontogenetic age of cuttings on the number (±SE) of roots per rooted cutting of *I. fagifer* over time.

Weeks	Mean number of roots per rooted cutting		
	Juvenile	*Mature*	*Significance*
1 (n=96)	0.00	0.00	-
2 (n=20)	4.6 ± 0.57	1.5 ± 0.50	NS
3 (n=21)	3.4 ± 0.44	2.6 ± 0.40	NS
4(n=23)	None	3.1 ± 0.36	NS
5 (n=10)	2.7 ± 0.61	2.0 ± 1.0	NS

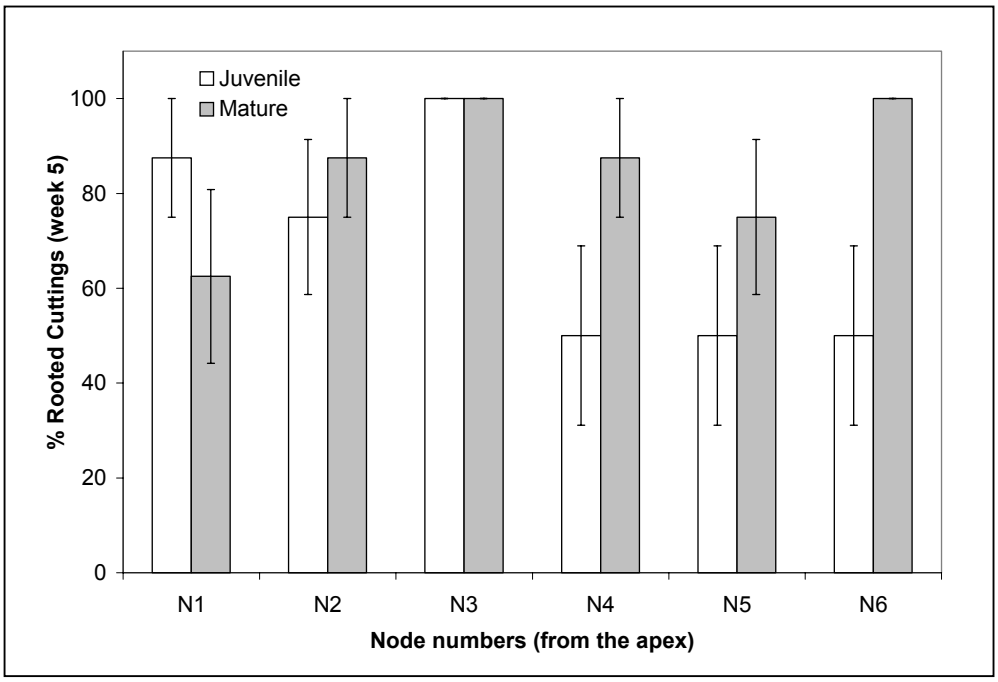


Fig 6.52: Effects of node position on percentage (±SE) rooting between juvenile and mature cuttings of *I. fagifer*.

6.3.2 Separation of ontogenetic and physiological aging in *Barringtonia procera*

6.3.2.1 Experiment 7: Experiment details

Three mature trees (Trees 53, 54 and 55) of *B. procera* were identified in Vovohe village (Chapter 3). The trees had been pollarded in December 2002 and over a period of about six months the pollarded stumps had resprouted, producing 3-5 new shoots per stump (Plate 6.2). On 7th July 2003, a plastic plant pot with 2-4 holes cut in the base was fixed to the top of each stump, with screws. About 2-3 shoots were allocated to each of the treatments (control and rejuvenation). Shoots allocated to the rejuvenation treatment were inserted through the holes in the base of each pot, while those allocated to the control were untreated and left outside the pot. All shoots were defoliated and cut back to about 20cm.

On 5th September 2003, after the shoots had resprouted, a marcot was performed on each of the shoots following procedures described in Chapter 3. All marcots were created at a point about 10cm above the base of the pot. The debarked portion of stem was treated with commercial rooting hormone ("Rootex-PD-08" Bass laboratories P/L) containing indole-butyric acid (IBA) in talc at 0.8%. The pots containing shoots were then filled with garden soil. After 5 months (10th February 2004) between one and four single-node (only shoots of 'rejuvenated' shoots produced 4 nodes), leafy stem cuttings were taken from the 1-6 leafy new shoots and set in an experiment comprising 12 cuttings x 4 replicates x 2 treatments, in a complete randomised block design.

In the field, the leaves on these cuttings were trimmed by half and then transferred to KFPL nursery at Ringgi Cove in moistened and sealed polythene bags for propagation. In the nursery, the leaves were reduced to 30 cm^2. Cuttings were treated with 0.4% IBA dissolved in alcohol, as described by Leakey *et al*. (1994) and placed in a high humidity, poly-propagator (Leakey *et al*., 1990). The diameter and length of each cutting was measured and cuttings were subsequently assessed weekly for leaf abscission, sprouting, rooting or death. Data was analysed as described in Chapter 3.

Plate 6.2. Rejuvenation of ontogenetically mature branches in the tree crown. L-R (follow the arrows): Pollarding (week 0), Sprouting (week 24), Marcotting (week 32) and Shoots producing cuttings (week 52)

6.3.1.2 Results

- *Effects of the rejuvenation of shoot*

Experiment 7: B. procera: The stem diameter and length of the cuttings were not significantly different between treatments or node positions (Fig 6.53 and Fig 6.54). Percentage rooting in cuttings from 'rejuvenated' shoots was consistently greater than those from 'natural shoots during the 5 weeks of propagation, but the difference between the two shoot treatments was not significant (Fig 6.55). Mortality in cuttings was greater in 'natural' (52%) than in 'rejuvenated' shoots (42%). By week 5, nearly all cuttings had either rooted or died. In respect to the number of roots per rooted cutting, cuttings from rejuvenated shoots had more roots than those cuttings obtained from the natural shoots, which was significant on week 4 (Fig 6.56).

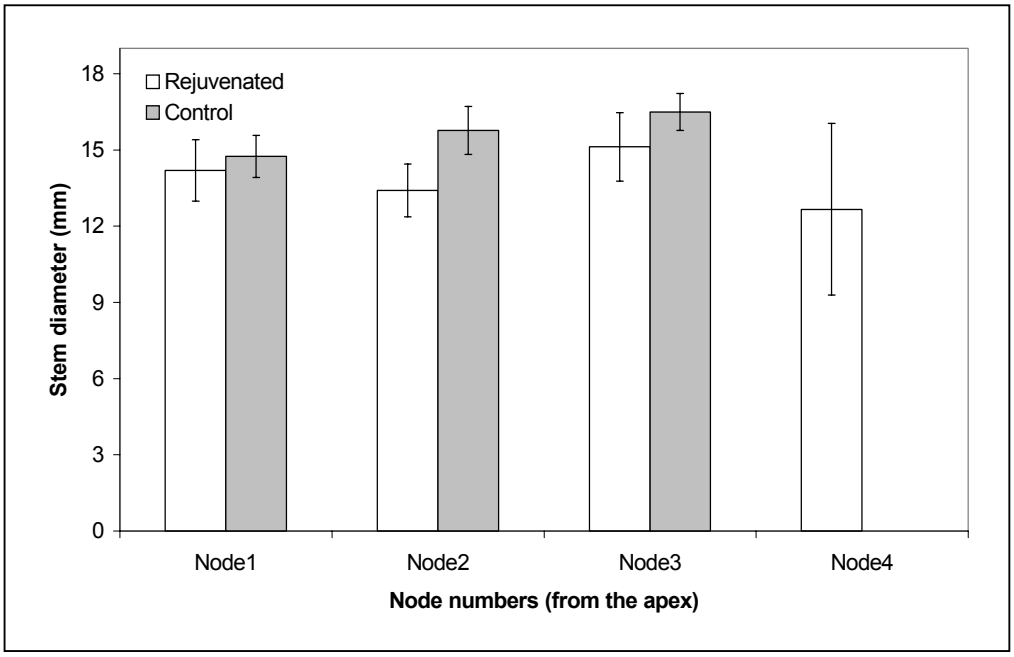


Fig 6.53: Effects of physiological rejuvenation on stem diameter (±SE) of ontogenetically mature cuttings of *B. procera* from different node positions.

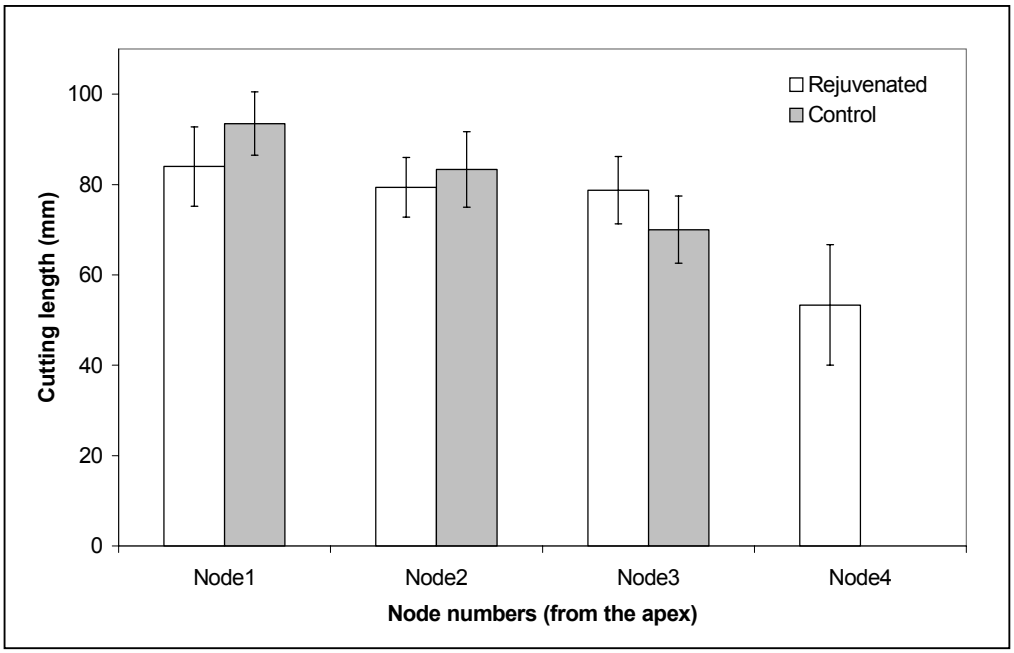


Fig 6.54: Effects of physiological rejuvenation on stem diameter (±SE) of ontogenetically mature cuttings of *B. procera* from different node positions.

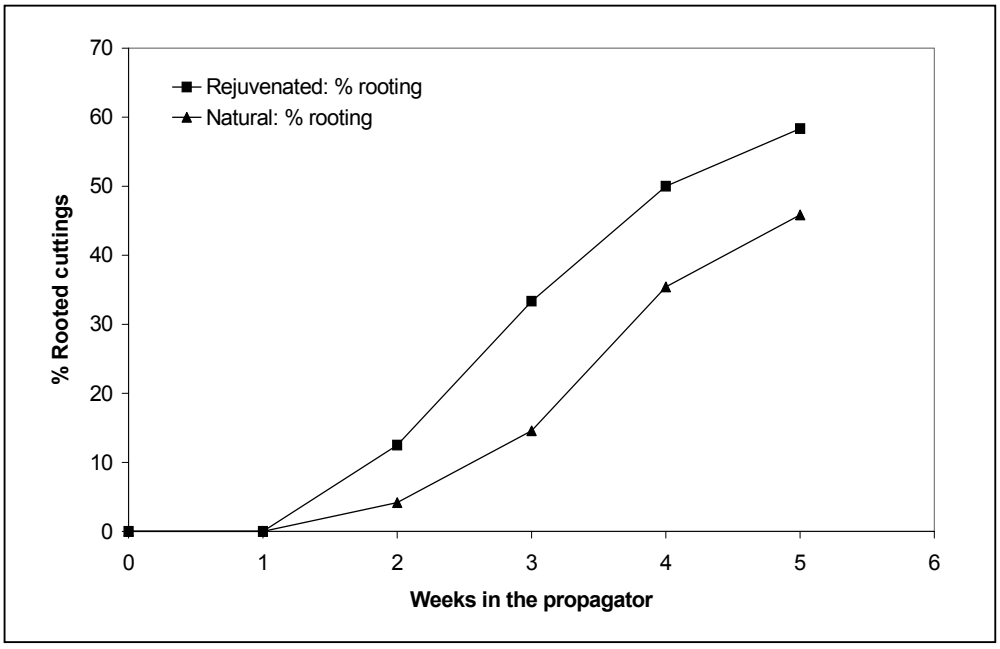


Fig 6.55: Effects over time of 'physiological rejuvenation' on cutting mortality and rooting capacity of 'ontogenetically mature' cuttings of *B. procera.*

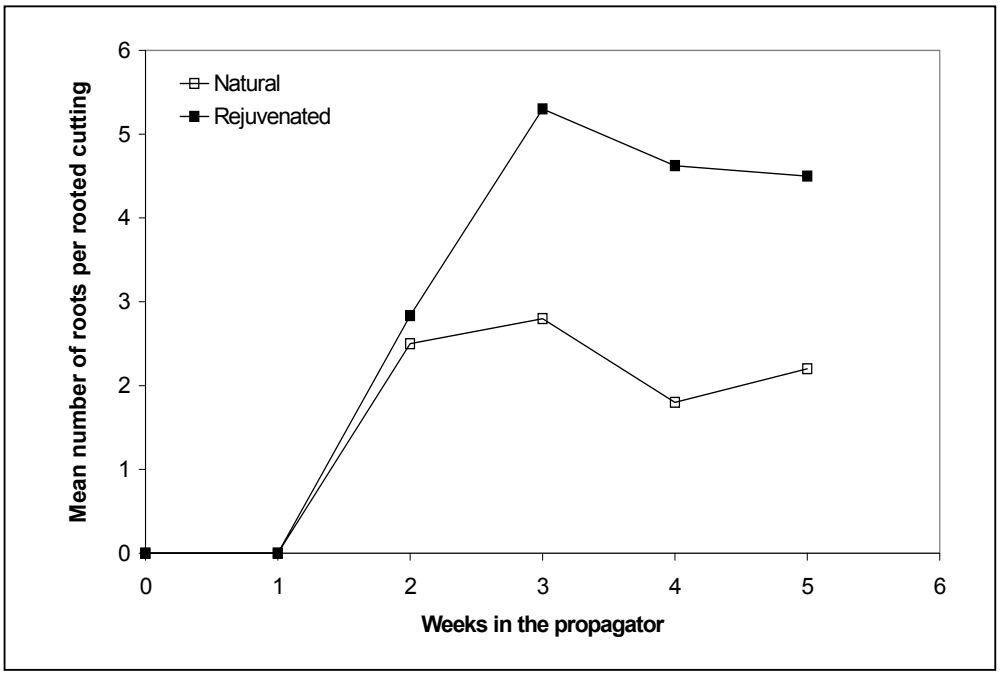


Fig 6.56: Effects over time of 'physiological rejuvenation' on the number of roots per rooted cutting of 'ontogenetically mature' cuttings of *B. procera.*

The majority of cuttings lost their leaves through abscission after one week in the propagator (79% each). By week 5, cuttings from the 'natural' shoot lost more leaves (90%) than cuttings from rejuvenated shoots (85%). Cuttings that were rooted also lost their leaves, with about 17% abscission in natural shoots and 23% in cuttings from rejuvenated shoots (Fig 6.57). However, greater percentage of rooting occurred in cuttings that retained their leaves. The overall effect of treatment on the physiology of these cuttings is illustrated by a very strong relationship ($r^2 = 0.99$) between leaf abscission and rooting (Fig 6.58).

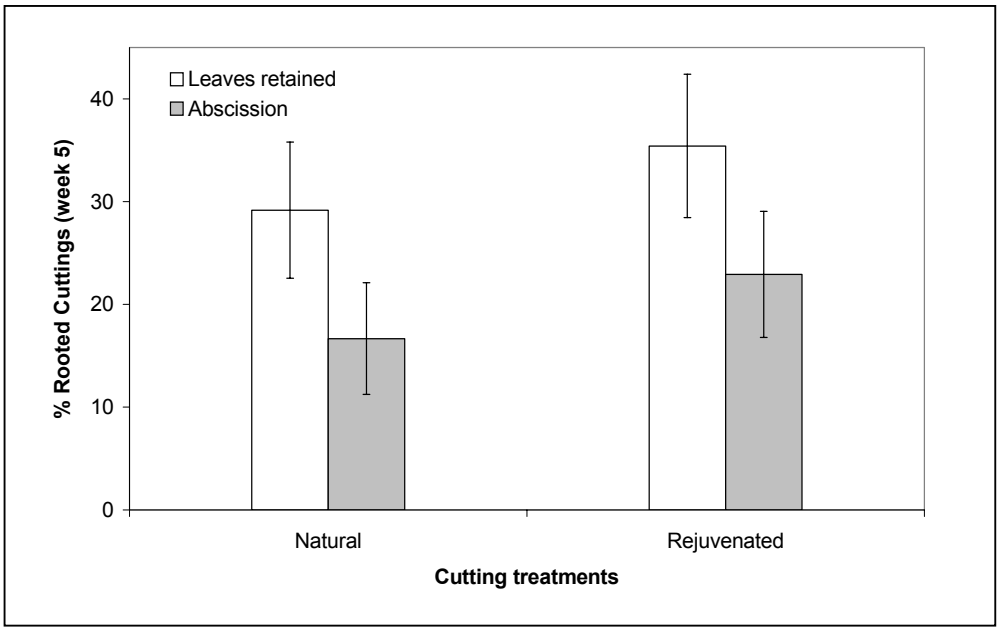


Fig 6.57: Effects of 'physiological rejuvenation' on leaf abscission and rooting (±SE) capacity of 'ontogenetically mature' cuttings of *B.procera.*

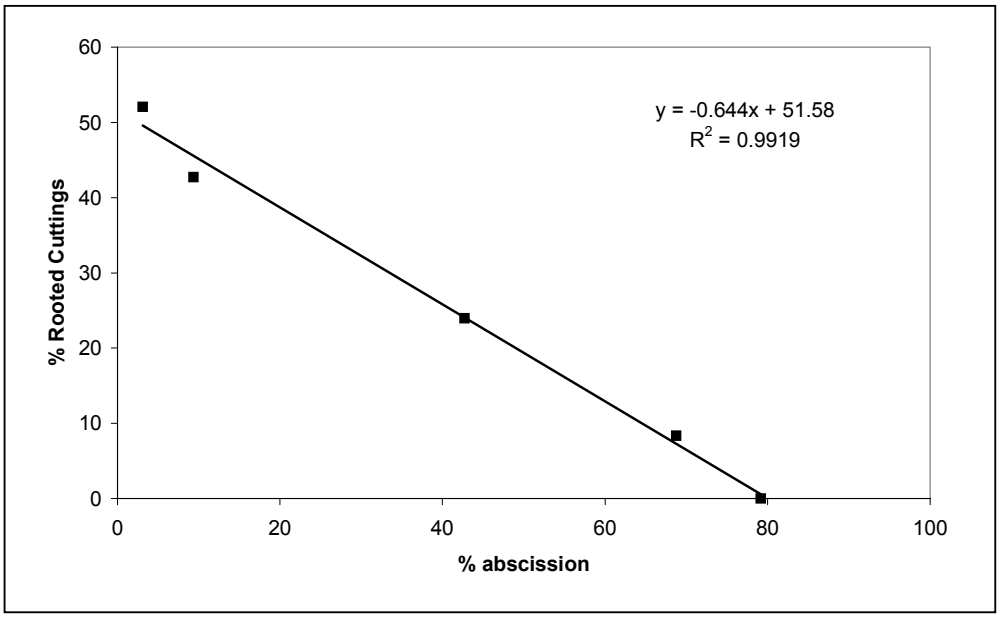


Fig 6.58: Effects of 'physiological rejuvenation' on leaf abscission and rooting capacity of 'ontogenetically mature' cuttings of *B. procera*

6.3.3 Effects of auxin, rooting media and branch orientation on the rooting of marcotted stems of *Barringtonia procera* and *Inocarpus fagifer*

6.3.3.1 Experiment 8: Experimental details

Experiment 8a: B. procera: Five vigorously growing mature trees of *B. procera* (numbers 8, 20, 49, 53 and 59) were identified and marked at Tututi village. On each tree, 12 relatively similar (4-6cm) size (diameter) branches at 2-4m height were identified for marcotting. Marcots were made by ring barking the selected stem, making two cuts through the bark about 10 mm apart around the stem close to an adjacent shoot. The bark and conductive tissues were then gently peeled off, and then the wound was treated either with or without 0.8% IBA. The marcots were covered immediately with the appropriate medium (soil or coir) wrapped with a clear plastic (Plate 6.3). Both ends of the plastic were secured by tying firmly with a strip of rubber. There were 12 treatment combinations (2 media x 2 IBA concentrations x 3 branch orientations (diagonal, horizontal and vertical). Individual trees represented replicates of the treatments.

The marcotts were harvested after six months and assessed for rooting (percentage and the number of roots per rooted marcots) and survival. Due to considerable rooting in some marcots, it was quite difficult to accurately count the number of roots. Thus, the roots were estimated into three rooting classes: light (<50 roots), moderate (>50<100 roots) and heavy (>100 roots). Data were analysed as described in Chapter 3.

Experiment 8b: I. fagifer: This is a repeat of Experiment 8a above on *I. fagifer*. The differences include:

i. Five mature naturally regenerated trees (number 1, 22, 23, 24 and 25) of Tututi village. It was estimated that they were between 10 to 30 years old by their owners.

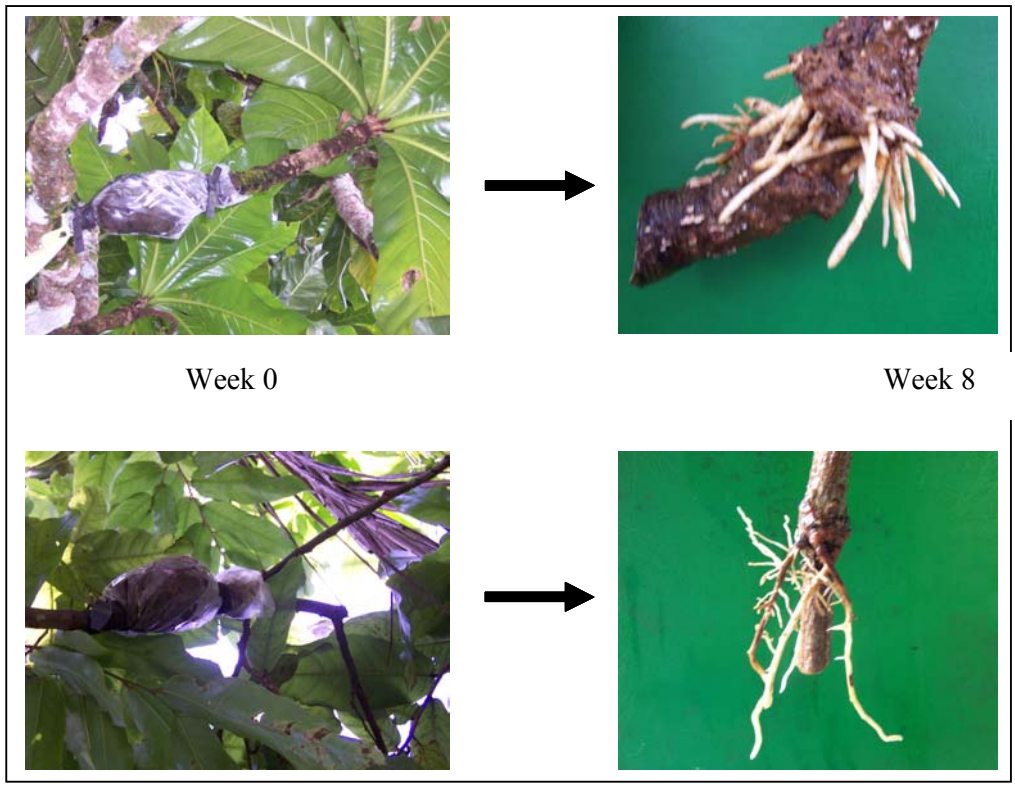


Plate 6.3. Rooting marcots in *B. procera* (Top) and *I. fagifer* (Bottom) at Tututi, Kolombangara, Solomon Islands.

ii. There were six marcots per tree in combinations of 2 x media, 2 x branch orientation and 2 x IBA treatments. The branch diameter was about 2-3cm at 2-3m height.

6.3.1.2 Results

- *Effects of auxin, media and branch orientation*

Experiment 8a: B. procera: In mature trees of *B. procera*, marcots of all treatments rooted in all treatments with a high percentage (40-80%) of IBA treated marcots producing more than 100 roots. The proportion is greater in soil than in coir, especially in diagonal branches (Fig 6.59). Marcots without IBA produced fewer roots, but 20-80% still had more than 100 roots (Fig 6.60). Interactions between auxin, media and branch orientation were not significant.

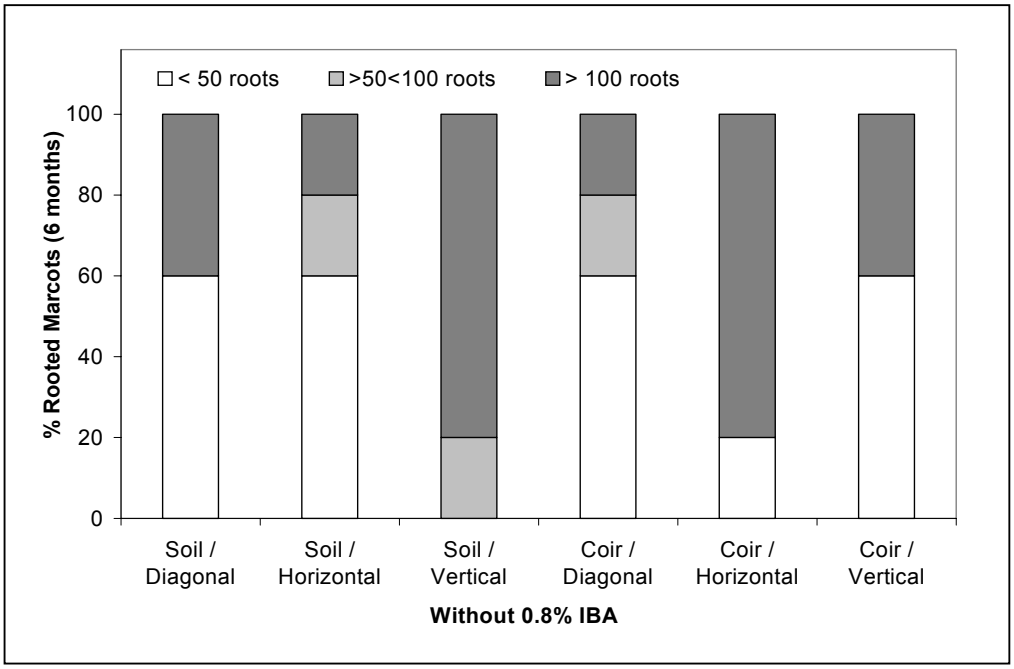


Fig 6.59: Effects of media and branch orientation on percentage rooting and the number of roots produced on IBA treated marcots of *B. procera.*

After six months, marcots were severed from the trees and potted up in the nursery. Mortality was high, and significantly different between treatments, with lowest survival (40%) in non-IBA treated marcots made on vertical branches and

covered in coir (Fig 6.61). It appeared that marcots with the most roots had the greatest mortality – this is because rooting occurred prior to severance and that mortality occurred after severance.

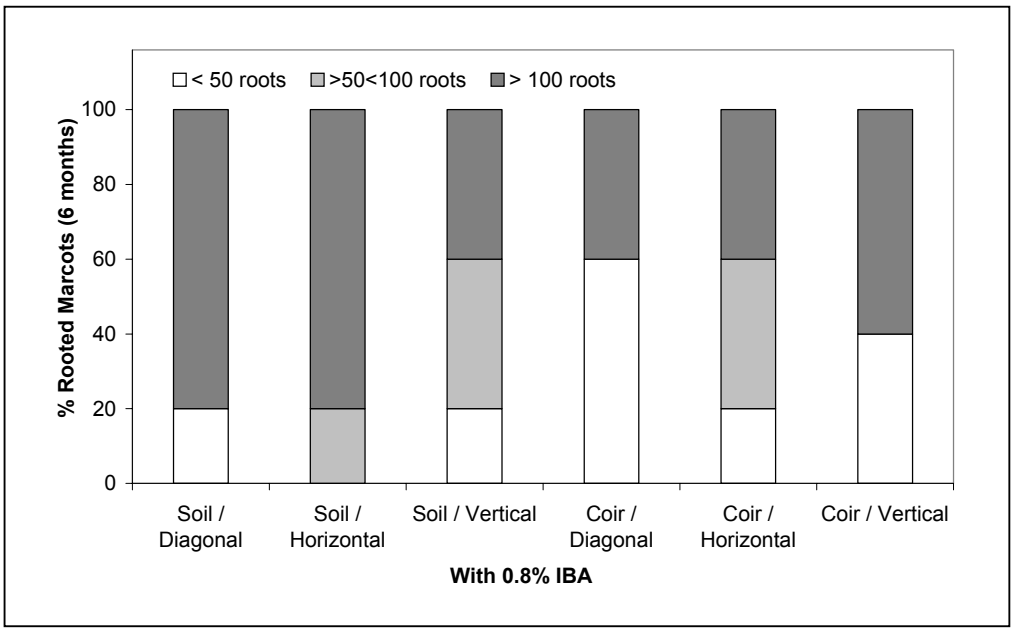


Fig 6.60: Effects of media and branch orientation on percentage rooting and the number of roots produced on non-IBA treated marcots of *B. procera.*

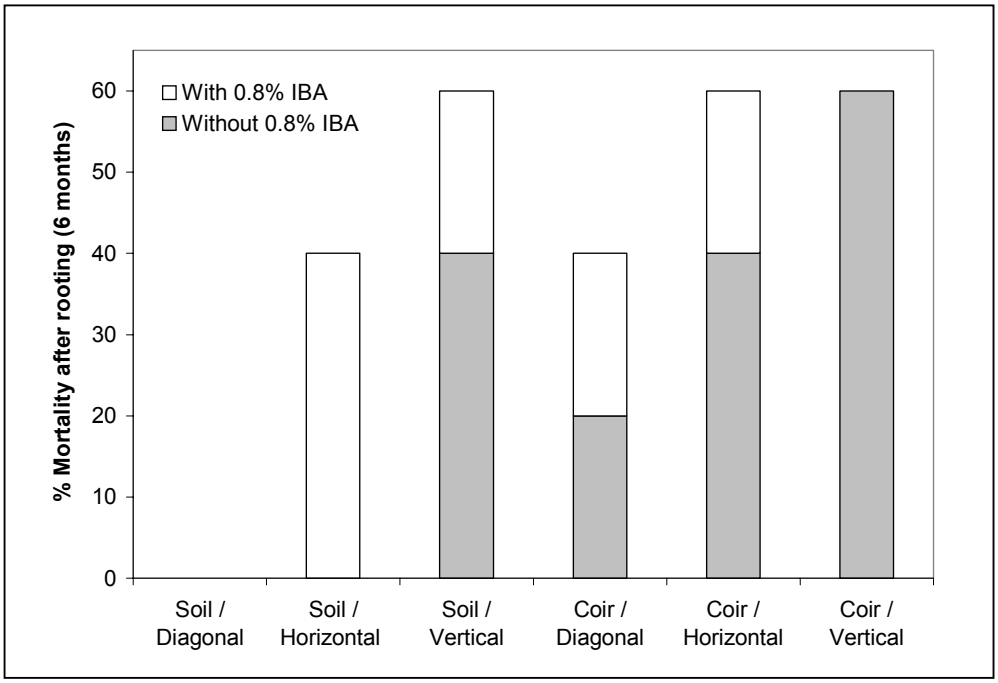


Fig 6.61: Percentage mortality after marcot had rooted in *B. procera.*

Experiment 8b: I. fagifer: All the marcots set on *I. fagifer* rooted. With IBA, the greatest number of roots was produced in soil media and in vertical shoots (Fig 6.62). A pattern was less clear in marcots without IBA (Fig 6.63). Interaction between auxin, media and branch orientation was not significant.

Marcot mortality after six months was greater in soil than in coir, and with IBA than without (Fig 6.64). Similar thing appeared here as in *B. procera*, that the marcots with the most roots had greatest mortality – this is because rooting occurred prior to severance and that mortality occurred after severance.

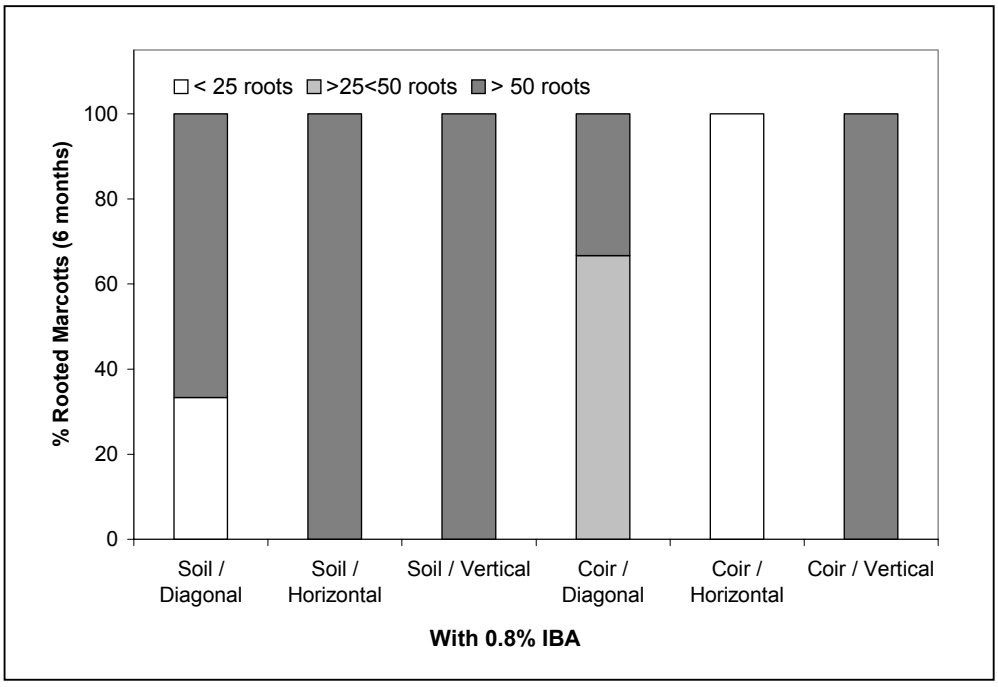


Fig 6.62: Effects of media and branch orientation on percentage rooting and the number of roots produced on IBA treated marcots of *I. fagifer.*

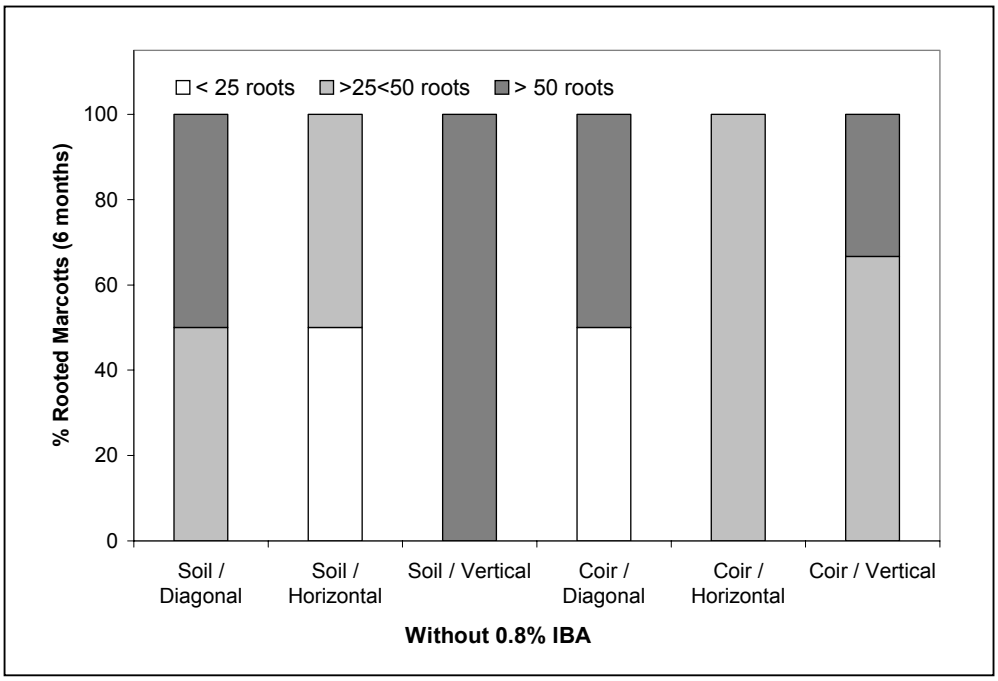


Fig 6.63: Effects of media and branch orientation on percentage rooting and the number of roots produced on non-IBA treated marcots of *I. fagifer.*

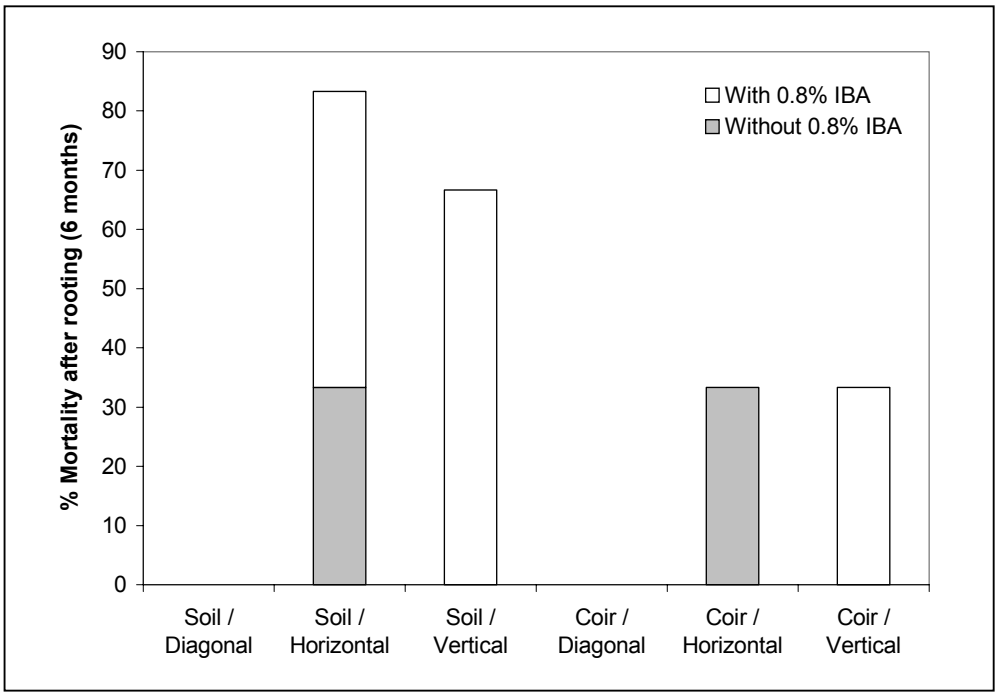


Fig 6.64: Percentage mortality after marcots had rooted in *I. fagifer.*

6.3.4 Effects of branch height and diameter on the rooting of marcots from pruned branches of *Barringtonia procera* and *Inocarpus fagifer* prior to severance.

6.3.4.1 Experiment 9: Experimental details

Experiment 9a: B. procera: Nine mature, naturally regenerated trees (numbers 53, 54, 57, 59, 65, 66, 72, 73 and 94) of *B. procera* were randomly selected at Hunda village. The owners estimated the trees to be 15-30 years old. The height of the trees ranged from 7-19m, while diameter at breast height was from 14-49cm. Tree height was measured using a clinometer (SUNNTO TANDEM, 1380ATANDEM-360PC/360R) and diameter at breast height was measured with a diameter tape. In February 2004, four healthy and vigorously growing branches from each tree were randomly selected for marcotting. The selected branches differed in size (diameter) and locations (height) within the tree.

The marcots were set as described in Experiment 8 (section 6.3.3.1). The marcots were monitored for rooting twice a month by looking through the clear plastic wrapper. Marcots had rooted after 5 weeks, although rooting was not visually detected through the plastic wrapper in some marcots. At this time (week 5), the marcotted branch was then cut back, approximately 30cm distal to the position of the marcot (see diagram in Fig 6.65). Following the severance, the marcotted branches remained attached to the parental tree until they either died or produced new shoots. After 15 weeks, the marcots were assessed by counting the number of surviving marcots and the number of roots per rooted marcots and measuring the length of the longest root. Data was analysed as described in Chapter 3.

Experiment 9: I. fagifer: This is a repeat of the Experiment 9a above on *I. fagifer*. The differences include:

i. Seven mature naturally regenerated trees (numbers 1, 13, 17, 22, 23, 24, and 25) of Tututi village. The trees were estimated by their owners to be 10-30 years old

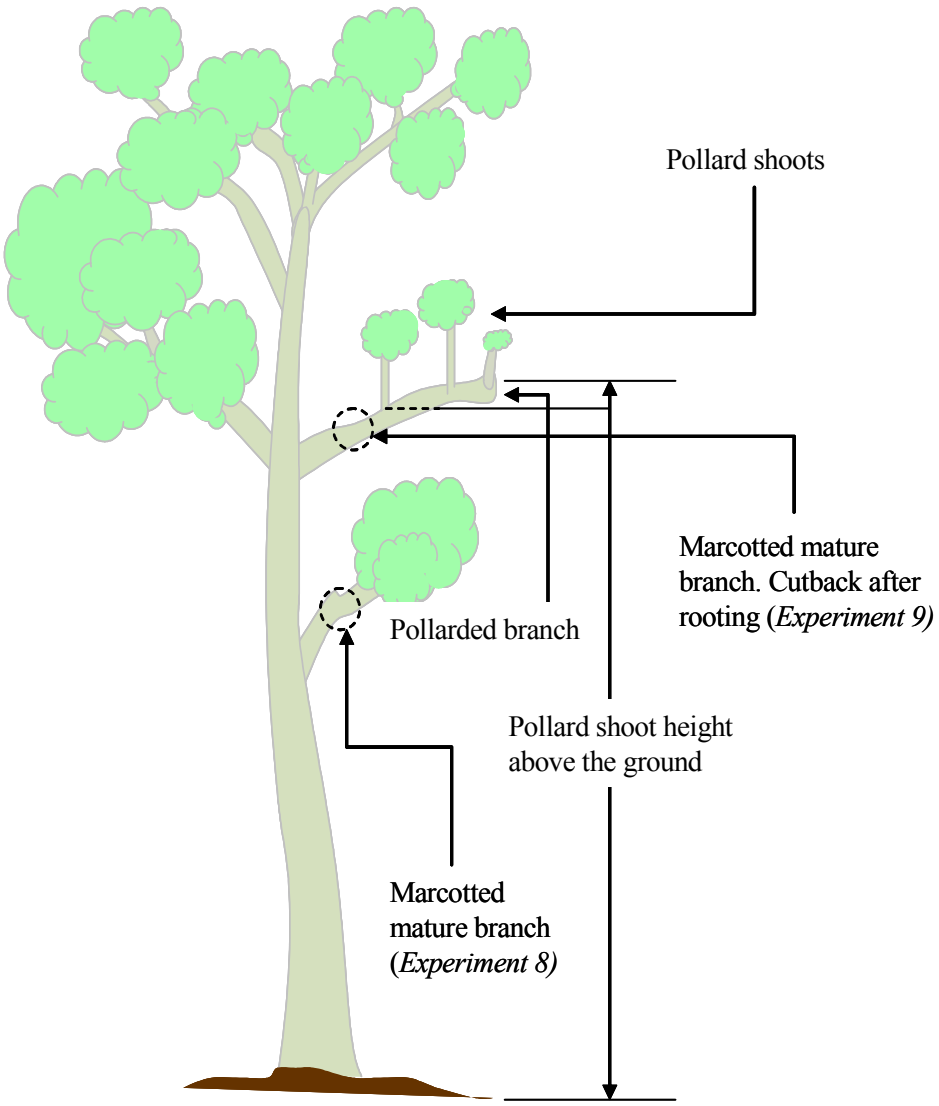


Fig 6.65: Diagram illustrating different marcotting experiments performed on trees of *B. procera* and *I. fagifer*.

In Experiment 8, mature branches at different orientation (vertical, horizontal, diagonal) were marcotted. In Experiment 9, the marcots on mature branches in both species were later cutback after they had rooted, shown as pollarded branch.

ii. Experimental design is 7 trees x 4 marcots per tree, giving a total of 28 marcots.

iii. Marcots were evaluated after 11 weeks and data analysed as for experiment 9a.

6.3.4.2 Results

- *Effects of stem cutback*

Experiment 9a: B. procera: Pruning the marcotted stem after 5 weeks did not cause any mortality. Number of roots per rooted marcot was significantly different ($F_{8, 27} = 2.58$, $P<0.05$) between trees after 15 weeks (Fig 6.66). Marcots from tree 73 had the most roots, and those from trees 57 and 65 had the least. The root length, but not the number of roots per marcot was significantly different between branches from different heights in the tree crown ($F_{2, 33} = 4.49$, $P<0.05$) after 15 weeks (Table 6.21). There was no significant effect of branch diameter on rooting of the marcots.

Table 6.21: Effect of height and diameter of the marcotted branch on root number and length on marcots on *B. procera* trees after 15 weeks. (±SE)

Variables	Root number	Root length (mm)
Height = 2m	42.6 ± 16.1	118.6 ± 6.1
= 3m	44.2 ± 6.4	96.1 ± 5.8
= 4m	53.0 ± 11.5	90.3 ± 5.4
Significance	NS	*
Diameter = 10-15mm	40.0 ± 4.6	101.6 ± 20.0
= 16-20mm	68.1 ± 16.2	101.5 ± 7.5
= 21-25mm	41.8 ± 7.9	95.3 ± 5.2
= 26-30mm	37.3 ± 9.4	101.5 ± 10.8
= 31-35mm	40.0 ± 29.9	111.7 ± 8.8
Significance	NS	NS

NS = Non-significance, * significance $P<0.05$

The number of newly formed sprouts per marcotted shoot after pruning was significantly ($F_{8, 27}$ = 2.71, P<0.05) different between trees after 10 weeks. Marcots on Tree 72 produced the most sprouts (Fig 6.67). Trees 65, 57 and 66 were slow to sprout, while other trees had started to lose shoots. After 15 weeks, the total number of sprouts per marcot was not statistically significant between trees. Number of roots per rooted marcot seemed to have decreased with an increase in number of sprouts per marcotted shoot, however, this relationship was very weak (r^2 = 0.157, *P*>0.05) and not significant.

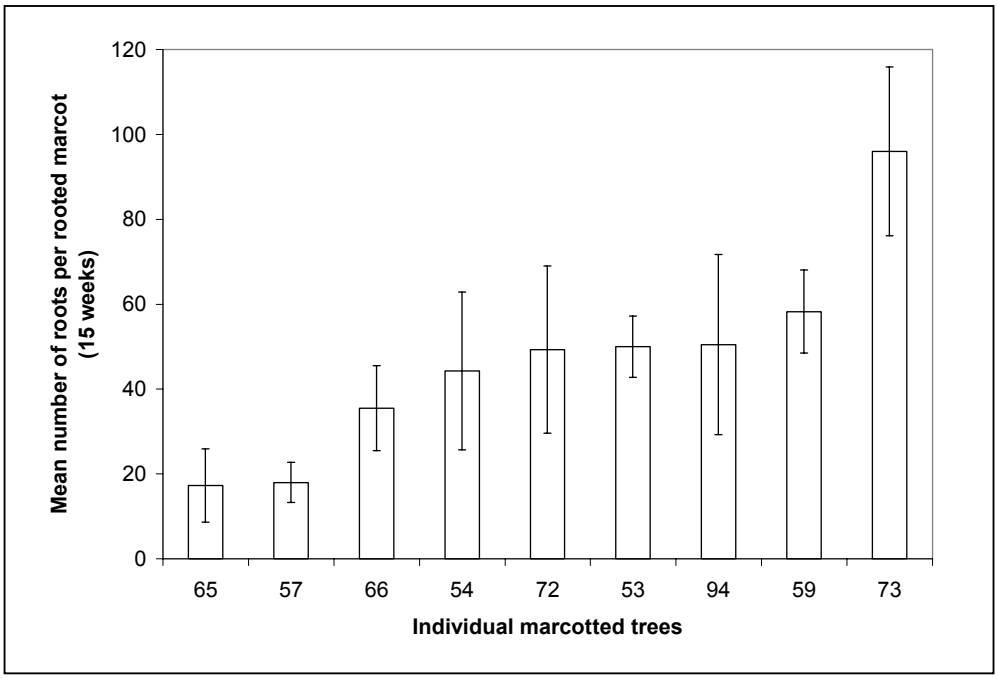


Fig 6.66: Rooting ability of different *B. procera* trees marcotted, in the ascending order of the mean (±SE) number of roots per rooted marcot.

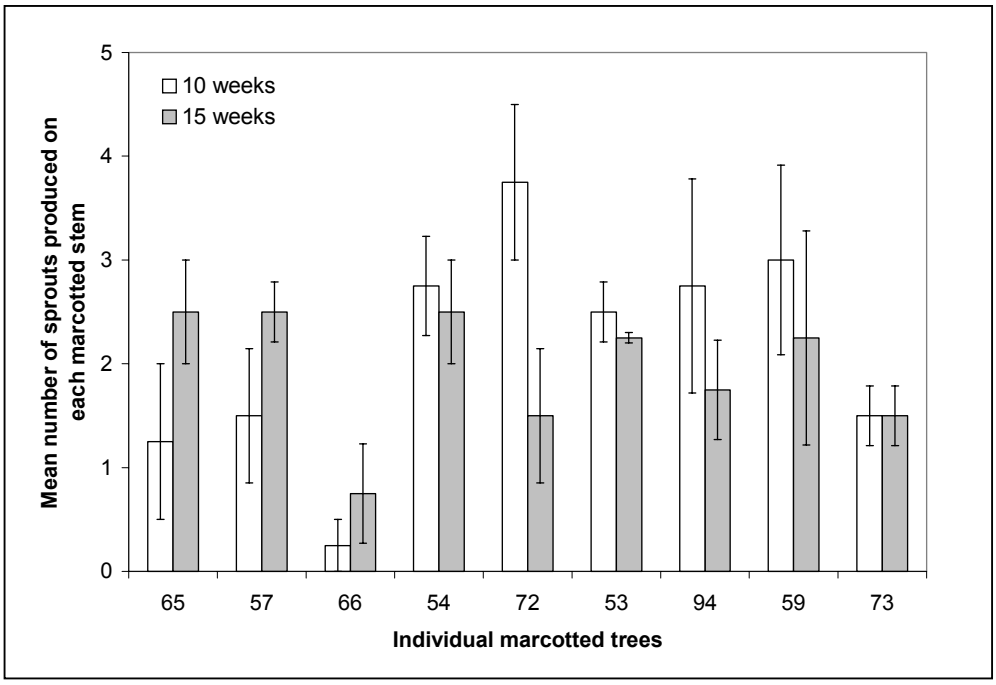


Fig 6.67: Sprouting at 10 and 15 weeks on stems of different *B. procera* trees marcotted, in the ascending order of the mean (±SE) number of roots per rooted marcot.

Experiment 9b: I. fagifer: In *I. fagifer*, 14% of marcots died following pruning. The number of roots per rooted marcot was not significantly different between trees after 11 weeks (Fig 6.68). The height and diameter of the marcotted branch had no significant effect on the length and the number of roots on the marcots after 11 weeks (Table 6.22).

Table 6.22: Effect of height and diameter on rooting ability of the marcots on *I. fagifer* trees after 11 weeks. (±SE)

Variable	Root number (n = 22)	Root length (mm) (n = 22)
Height = 2 m	14.3 ± 6.4	146.7 ± 18.6
= 3 m	9.0 ± 2.7	145.0 ± 49.1
= 4 m	10.7 ± 2.6	156.7 ± 12.3
= 5 m	13.7 ± 2.3	163.3 ± 17.6
= 6 m	9.3 ± 3.8	101.0 ± 50.3
= 9 m	7.7 ± 1.2	206.7 ± 29.1
Significance	NS	NS
Diameter = 5-10mm	8.1 ± 1.5	134.9 ± 15.1
= 11-15mm	11.5 ± 1.5	150.5 ± 16.8
Significance	NS	NS

NS = Non-significance

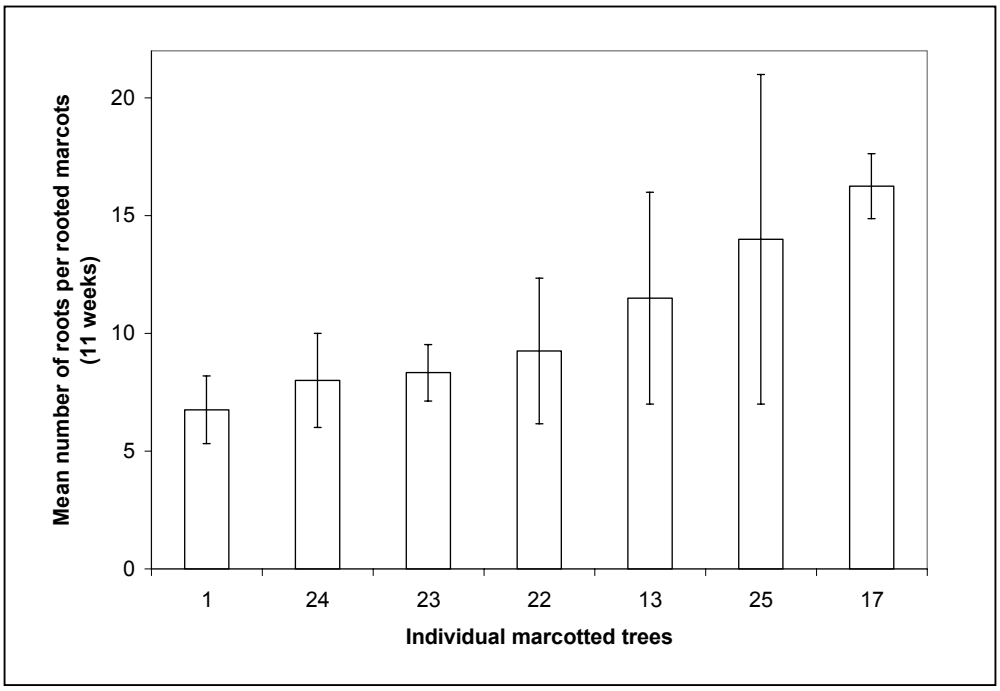


Fig 6.68: Rooting ability of different *I. fagifer* trees marcotted, in the ascending order of the mean number (±SE) of roots per rooted marcot.

Sprouts were slow to emerge and only the marcotted stem of tree 1 had new sprouts by week 9. However, by week 11 other trees had sprouted, and the differences in the number of sprouts was not significantly different (Fig 6.69). There was no relationship between the number of roots and number of sprouts per rooted marcot ($r^2 = 0.008$, $P>0.05$).

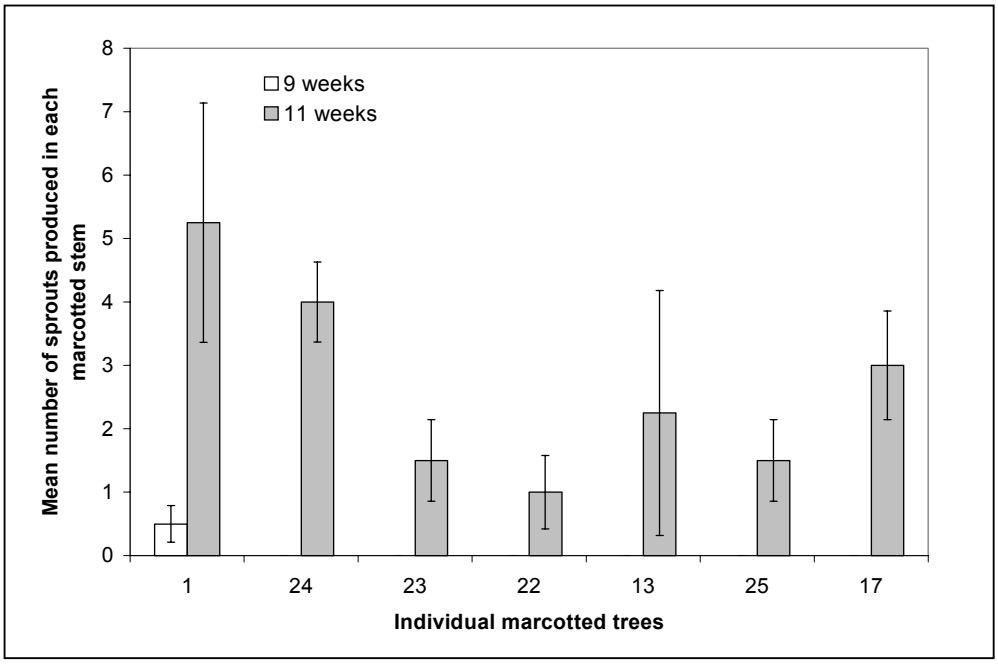


Fig 6.69: Sprouting at 9 and 11 weeks on marcotted stems of different *I. fagifer* trees, in the ascending order of the mean (±SE) number of roots per rooted marcot.

6.3.5 Effects of light on rooting of single-node leafy stem cuttings taken from established marcots of *Inocarpus fagifer*

6.3.5.1 Experiment 10: Experimental details

Experiment 10: I. fagifer: Cuttings were collected from twenty 6-month old marcots of *I. fagifer* raised in a five litre poly-bag filled with forest soils and placed in the nursery under two different light environments (70% light and full sunlight). The 70% light was achieved by using nursery shade cloth which can provide 30% shade. The marcots were obtained from Experiment 8 (section 6.3.1.1). Three months later, two hundred single-node, leafy stem cuttings were collected from 10 marcots (5 marcots from each light environment). The design for this experiment is 25 cuttings x 2 light treatments x 4 replicates in a randomised complete block. Each cutting had a leaf trimmed to 50cm^2. The cuttings were dipped into 0.8% IBA powder before insertion into the rooting media (1:1 coir/river soil ratio). Rooting was assessed weekly for 5 weeks and analysed as described in Chapter 3.

6.3.5.2 Results

- *Effects of light on rooting of cuttings from established marcots*

Experiment 11: I. fagifer: Cuttings from stockplants under 70% light rooted significantly better than those under full sunlight (Fig 6.70) and cutting mortality was significantly greater in cuttings from full sunlight. After 5 weeks all cuttings had either rooted or died.

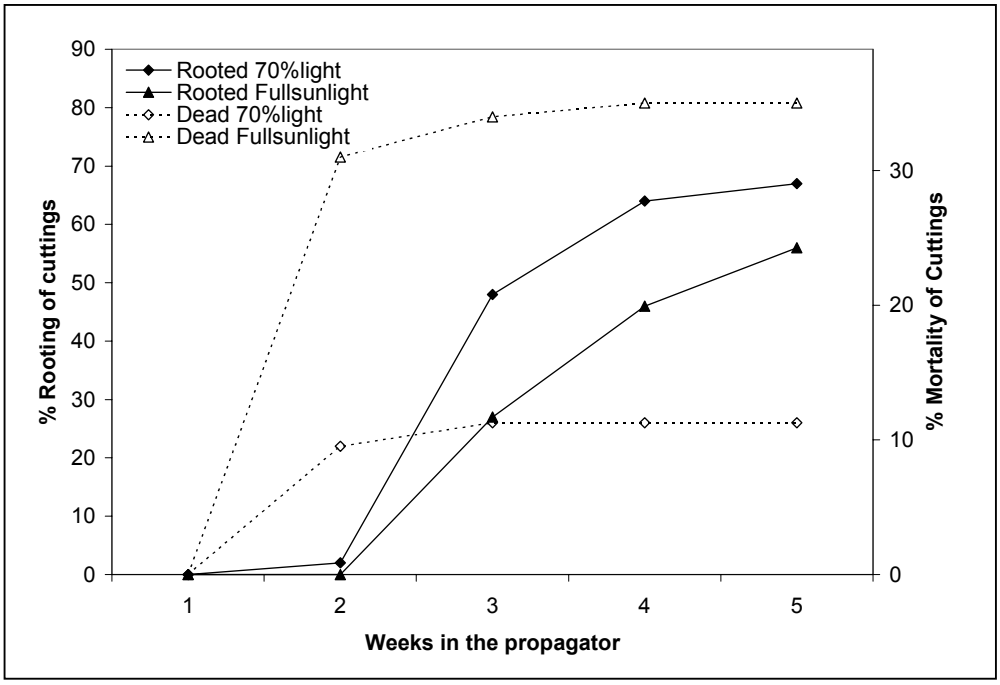


Fig 6.70: Effects of light on the rooting and mortality of cuttings from marcotted stockplants of *I. fagifer.*

However, despite a relatively poor rooting percentage, cuttings under full sunlight produced more and longer roots per rooted cutting but did not differ significantly between treatments. Percentage rooting was varied and inconsistent (Fig 6.72). However, significant differences between treatments with respect to light levels occurred at node positions 2, 4 and 6.

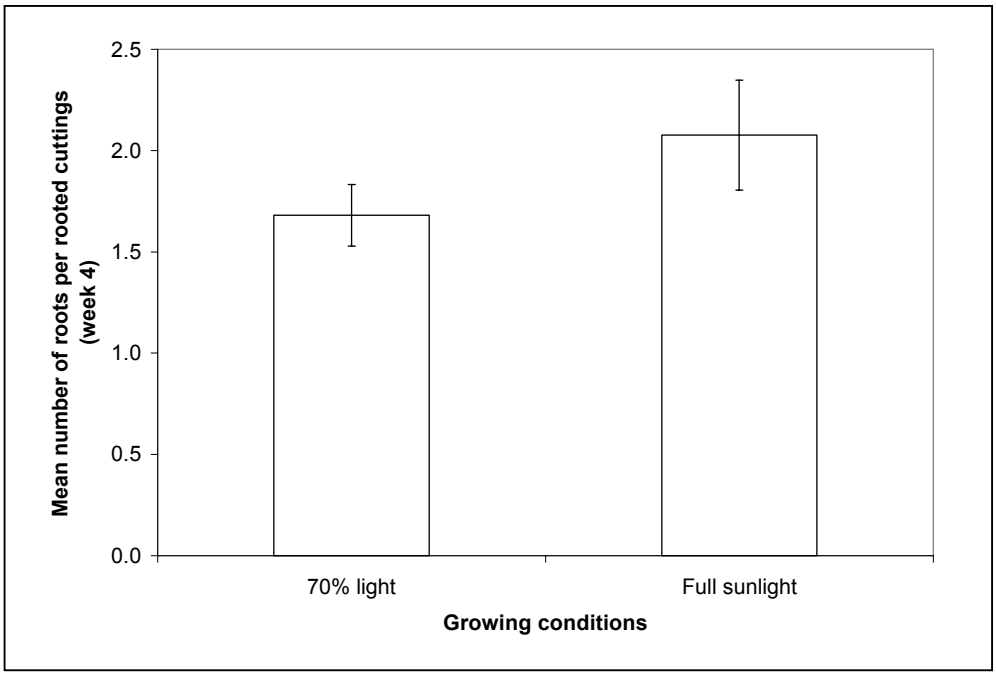


Fig 6.71: Effects of physiological conditions of cuttings on the number (±SE) of roots per rooted cutting of *I. fagifer.*

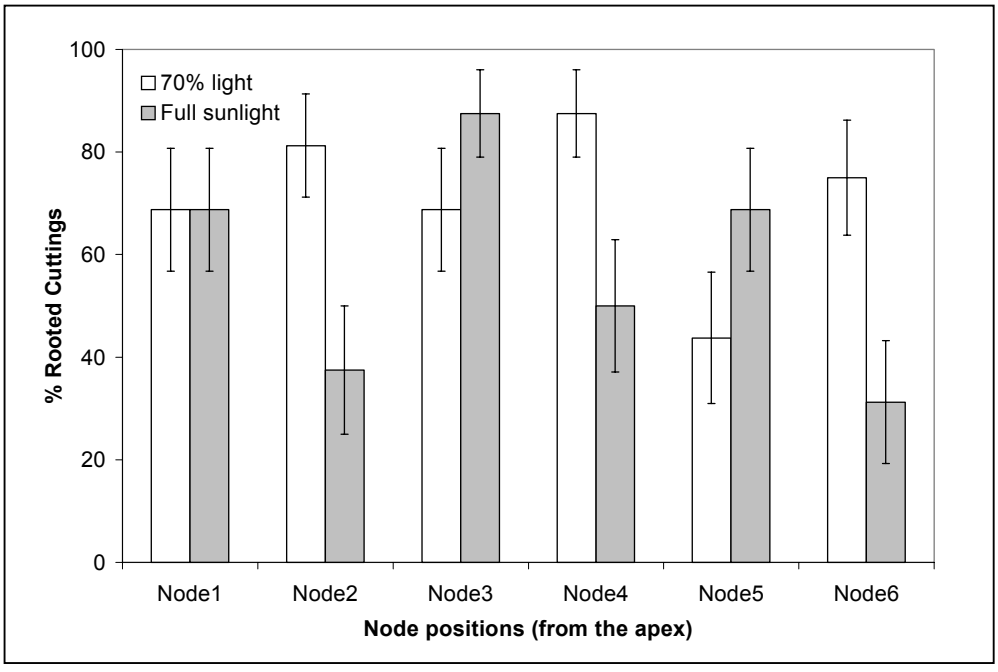


Fig 6.72: Effects of node position on percentage (±SE) rooting in cuttings of *I. fagifer* under 70% and full sunlight.

6.4 DISCUSSION

This study has identified that both *Barringtonia procera* and *Inocarpus fagifer* are easily rooted species and that high rooting success can be achieved by optimal combinations of factors that are commonly important in the rooting process. This is very fortuitous as almost nothing is known about rooting capacity of these species before this study was initiated. Single-node leafy, juvenile cuttings of *B. procera*, with a leaf area of 50cm^2 and treated with 0.8% IBA, root within 2-3 weeks. Similarly, *I. fagifer* cuttings root best with a leaf area of 50cm^2 and 0.8% IBA with no cutting mortality. In both species there are relationships between rooting and cutting length and diameter, and rooting is also affected by the rooting environment, especially the rooting medium, with forest soil and coir promoting the greatest success. This study then uses this information to test factors affecting the more difficult situation of rooting cuttings of mature trees.

- **Juvenile cuttings**

⇒ ***Effects of auxin***

Auxins have the ability to enhance rooting by facilitating transport of carbohydrate down the stem base (Hartmann *et al.,* 1997). Typically, the highest percentage rooting in *Triplochiton scleroxylon* was achieved at 0.4% IBA (Leakey *et al.,* 1982a), higher than 0.2% IBA in *Shorea leprosula* (Aminah *et al.,* 1995), but lower than the optimal concentration (1.6% IBA) found for *Cordia alliodora* (Mesén *et al.,* 1997b). In a mechanistic model of carbohydrate dynamics during the development of adventitious roots in leafy cutting of *T. scleroxylon*, Dick and Dewar (1992), found that sugar levels in the leaf and internode initially peaked between 14-17 days due to photosynthesis, but then decreased as the sugar is converted to an immobilised form (starch) or translocated to the root. They suggest that auxins enhance the translocation of sugar to the cutting base so promoting rooting. However, the response to applied exogenous auxin varies between species, and in a few species (e.g. *Shorea macrophylla* (Lo 1985) and in *Nauclea diderrichii* (Leakey 1990)) there is no proven evidence of auxin enhancing rooting.

In the present study, increasing IBA concentration from 0% to 0.8% did not affect percentage rooting significantly in either *B. procera* or *I. fagifer*. This is unusual and suggests that these two species are in the small group of unresponsive species. However, in the present study the higher IBA concentration promoted the greatest number and length of roots per rooted cutting in both species which then raises doubts about what is meant by 'unresponsive'. In addition, there was an indication that cuttings from plants originating from different mother trees (*B. procera* Hunda 2 *versus* 97 and *I. fagifer* Tututi 3 *versus* 1) responded differently to a range of IBA concentrations.

⇒ *Effects of leaf area*

Leaf area influences rooting of single-node cuttings in many tropical trees (Hartmann *et al.,* 1997). Good rooting success can be obtained by optimising leaf area because it is important for photosynthesis of cuttings while in the propagator. In softwood stem cuttings, unlike hardwood cuttings which are often taken in the winter or "resting stage," the presence of a leaf is usually essential for successful rooting and survival, for example, in *Vitis vinifera* (Thomas and Schiefelbein 2004).

In the current study of *B. procera* and *I. fagifer*, as expected, there is a significant difference between the rooting ability of leafy and leafless cuttings. In *B. procera*, 20% of the leafless cuttings rooted, but no leafless cuttings had either rooted or survived in *I. fagifer*, affirming the requirement of leaves as essential for rooting cuttings of these species. However, the occurrence of rooting in some leafless cuttings of *B. procera*, in spite of being significantly lower than the percentage rooting in the leafy cuttings, indicates that even without current assimilates from photosynthesis these cuttings were able to produce roots. This implies that they used stored reserves. This ability explains why the effects of leaf area were minimised. By comparison, the leafless cuttings of *I. fagifer* apparently had none or insufficient reserve to initiate or support rooting. Furthermore, the final percentage rooting is not significantly different between higher leaf areas in *B. procera* and *I. fagifer,* except for the number of roots per rooted cutting. This result is akin to *Terminalia spinosa* (Newton *et al.,* 1992) and *Nauclea diderrichii*

(Leakey 1990), indicating that *B. procera* and *I. fagifer* species are easy to root, and that the leaf is not a major factor determining rooting. Essentially in both species, regardless of the leaf areas, the photosyntheses from leafy cuttings seem to increase the number of roots per rooted cutting.

This study found that 50cm^2 leaves were optimal in *B. procera* and *I. fagifer*. This is typical of many species – e.g. *T. scleroxylon* (Leakey *et al.,* 1982a; Leakey and Coutts 1989), a species in which there has been detailed physiological studies which have indicated that there is a need for a balance between the loss of water through transpiration and assimilate production through photosynthesis. By comparison, the optimum leaf area for *B. procera* and *I. fagifer* is five times larger than the optimal leaf area for *Khaya ivorensis* (Tchoundjeu and Leakey 1996) and about three times larger than that of *S. leprosula* (Aminah *et al.,* 1997).

⇒ *Rooting medium*

The importance of rooting media for rooting vegetatively propagated species is widely recognised (Hartmann *et al.,* 1997). Success of rooting media is dependent upon good drainage and proper aeration, which are important for gas exchanges and respiration and moisture to replace water losses through transpiration, and so prevent wilting of cuttings and stomatal closure (Leakey 2004b), which is critical for photosynthesis for the production of assimilates for rooting cuttings. Different types of media provide different physical properties and tree species vary in their response to different media due to their ability to adapt to different environment and habitats (Leakey *et al.,* 1990; Leakey 2004b). For example, sawdust was the best media to root *Irvingia gabonensis* (Shiembo *et al.,* 1996) and *Milicia excelsa* (Ofori *et al.,* 1996), but was poor in rooting *Cordia alliodora*, while gravel was preferred medium for *Cordia alliodora* (Mesén *et al.,* 1997b), *Albizia guachapele* (Mesén 1993) and *Lovoa trichiliodes* (Tchoundjeu and Leakey 2001). Coir/sand mixture is successful in rooting *Swietenia macrophylla* in Fiji (Bevu 1999).

There were significant differences between some media in this study, and a strong ($r^2 = 0.71$) and positive correlation between percentage rooting and the porosity of the media was found in *B. procera*. Furthermore, both species exhibited negative

correlations between percentage rooting and the bulk density of the media, which were weaker *in B. procera* (r^2 = 0.21) than in *I. fagifer* (r^2 = 0.59). These correlations are consistent with *I. fagifer*'s tolerance to water logging, but suggest that *B. procera* is rooted best in an open medium with good drainage and sufficient air space to prevent water logging and subsequent rotting of the cutting. Taking these differences into account, forest soil and coir are the suggested medium for rooting cuttings of *B. procera* and *I. fagifer* respectively. However, for practical purposes, coir is recommended for both species as it is easy to use and less damaging to roots during transplantation.

In coastal gravel and coral media, many cuttings died before rooting. The reasons are not clear but perhaps indicate that the media was over saturated as the test for porosity indicated a low air content and high bulk density. Excess water in the media creates a barrier to diffusion of oxygen, resulting in anoxia at the cutting base (Loach 1986; Hartmann *et al.,* 1997).

⇒ *Stockplant factors – Effects of cutting diameter, length and position*

The node position of cuttings is usually very important in rooting because it represents a wide range of morphological and physiological conditions (Leakey 1985; Leakey and Mohammed 1985). However, in this study the effects of node position were not clear, perhaps because the partitioning of data to different node positions severely reduced the replication. Typically, as in *T. scleroxylon*, the apical nodes of an individual shoot root better than the basal nodes (Leakey 1983). However, in a few species, e.g. *Khaya ivorensis*, basal cuttings root best (Tchoundjeu and Leakey 1996).

In *B. procera*, long and small diameter cuttings rooted better than large diameter and short cuttings and there was a strong positive correlation between the number of roots per cutting and the cutting length. This agrees with Dick *et al.,* (1991) for *P. juliflora* and Tchoundjeu and Leakey (1996) for *K. ivorensis*. In contrast, cutting diameter was weakly related to the number of roots per rooted cutting, implying that cutting diameter is a less important factor than length in the

determination of *B. procera* rooting capacity. However, the absence of a strong relationship between number of roots per rooted cutting and stem volume suggests that the determining factors may be more to do with the availability of stored reserves rather than their overall quantity. In *T. scleroxylon*, rooting ability was found to be influenced by both the amount of stored reserves and their availability (Leakey and Storeton-West 1992), with starch-filled cuttings having very poor rooting capacity.

⇒ *Cutting length versus node position*

The interactions occurred in the above experiment were further examined by separating the confounding effects of node position or cutting length and cutting diameter. Typically, internode length decreases and cutting diameter increases sequentially down the stem. Thus cuttings from basal nodes are usually shorter and have a large diameter than apical nodes. Rooting capacity is also commonly found to decline sequentially down a shoot, but so too do many other things such as water potential, leaf age, lignification and wood structure.

This study therefore repeated one done in *T. scleroxylon* in which three treatments were imposed on sequentially harvested cuttings (Leakey and Mohammed 1985). The first treatment maintained the natural gradient of the shoots with longer internodes at the apical end. The second treatment was the converse, with internodes cut to be progressively shorter towards the apical end, while the third treatment made all cuttings the same length, although they differed in diameter, according to the natural gradient. This experiment found a strong correlation between cutting length and rooting ability, regardless of node position, so determining that cutting length *per se* is more important than node position and all the morphological variables associated with it (Leakey and Mohammed 1985). However, in cuttings of constant length, there was still a gradient in percentage rooting by node position, illustrating the importance of stem diameter, and this was explained as probably an effect of the amount of stored reserves proportional to stem volume (Leakey *et al.,* 1992).

In the present study with *B. procera* and *I. fagifer*, similar treatments tested reciprocal gradients in cutting length on percentage rooting, but added the relationship between sequential node positions and the number of roots per cutting. In addition, the study examined how these relationships changed with time. It was clear that, in agreement with the study in *T. scleroxylon*, cutting length *per se* substantially influenced rooting. Interestingly, however, this relationship was stronger in terms of the number of roots per rooted cutting ($r^2 = 0.71$) than the percentage rooting ($r^2 = 0.05$) for cuttings increasing in length acropetally and in cuttings increasing in length basipetally ($r^2 = 0.77$ and $r^2 = 0.64$ respectively) on week 2-5. When cuttings were cut to a constant length, the relationships were $r^2 = 0.86$ (% rooting) and $r^2 = 0.14$ (no. of roots). In *I. fagifer* these relationships, although weak, were stronger for the number of roots per rooted cuttings than the percentage of cuttings rooted. In both these species, the results were less conclusive. Analysis of the relationships between gradient of cutting length, stem diameter and node position and rooting as percentage and number of roots per rooted cutting, illustrated the complexity of these relationships which changed from week 0-2 and 2-5 in *B. procera*. This suggests that the source of assimilates and reserves changes with time across the rooting period, again with possible effects induced by the availability of reserves. In *I. fagifer* the results were similar although the effects of node position were less marked. Further work is required to gain a deeper understanding of these dynamic factors in the rooting process.

Leakey (1990) in a study of the effects of leaf area in easily rooted *Nauclea diderrichii* and difficult-to-root *Clestophilus glauca* concluded that the more difficult a species is to root the more important it is to optimise leaf area. This study suggests that the same principle is also true with regard to auxin concentration, and that the easier a species is to root the less important it is to provide optimal auxin concentration. Nevertheless, despite being relatively unresponsive to standard treatments, this study also identified some factors that were not optimal for rooting. Thus, even in these species it is important to acquire a detail understanding of the factors affecting rooting as only in this way will it be possible to develop robust protocols for mass propagation.

- **Mature cutting and marcotting**

Building on what is known from juvenile cuttings, the study went on to test the factors affecting the rooting of cuttings of mature trees. Typically, this is a more difficult problem as rooting ability is said to decline as trees become ontogenetically mature (phase change) (Hartmann *et al.,* 1997). However, Leakey (2004b) has hypothesised that this loss of rooting ability may be more to do with physiological ageing than ontogenetic ageing. Recently some evidence supporting this hypothesis has been reported by Dick and Leakey (*in press*).

This study further examines the relative importance of ontogenetic and physiological ageing using marcotting techniques to confer physiological youth on ontogenetically mature shoots for comparison between cuttings from seedlings and cuttings from potted marcots. When marcots are severed from the mother plant, they are established on their own roots and can either be grown to be a productive plant, or can be managed as a stockplant for regular supply of ontogenetically mature stem cuttings. The first experiment seeks to determine if these two sets of cuttings have similar rooting ability. To test this, cuttings were collected from juvenile seedlings and mature shoots originating from rooted marcots of *B. procera* and *I. fagifer* established as stockplants in the nursery.

In *B. procera*, juvenile cuttings obtained from the seedling stockplants rooted (percentage and number) significantly better than the mature cuttings from the marcotted stockplants, and mature cuttings suffered greater mortality due to loss of leaves. The importance of retaining leaves on cuttings has been discussed above, and in this study a strong correlation ($r^2 = 0.99$) was found between percentage rooting and leaf abscission. Similar results have been observed in other studies with *Triplochiton scleroxylon* (Nketiah *et al.,* 1999) and in *Prunus avium* (Dick and Leakey *in press*). Thus it appears that mature cuttings are more susceptible to water stress. However, in *I. fagifer*, the opposite was found and overall percentage rooting was better in mature than juvenile cuttings. Again leaf loss and mortality was associated with poor rooting performance, apparently due to water stress. Thus, this experiment does not resolve the question about the relative importance

of ontogenetic and physiological ageing but highlights the need for further studies on the factors involved.

⇒ *Effects of light on rooting of cuttings from marcots established post-severance as stockplants*

In juvenile stockplants, the pre-severance environment is an important factor determining the success of vegetative propagation. The light environment, in particular, strongly affects rooting ability affecting the partitioning of assimilates and nutrients within the stockplant, the storage of carbohydrate reserves and their availability for the growth of roots. Light quality and quantity both seem to be independently important (Hoad and Leakey 1996). Light also interacts with nutrients in *Albizia guachapele* (Mesén *et al.,* 2001), *T. scleroxylon* (Leakey and Storeton-West 1992), and *Eucalyptus grandis* (Hoad and Leakey 1996). The quality and quantity of light affects the morphology (leaf area and stem length), the levels of carbohydrates and gas exchange through stomatal conductance in plants (Atwell *et al.,* 1999; Mauseth 2003). As a consequence of poor rooting of cuttings from mature marcots in the previous experiment, this experiment sought to determine whether the rooting of cuttings from mature stockplants derived from marcots could be improved by shading (70% v. 100% full light) the stockplants.

Unfortunately, the experiment was only done with *I. fagifer*, but it is likely that the results are of relevance to *B. procera*, the species that performed least well in the previous experiment. The results confirmed that as in juvenile stockplants, shade enhanced rooting ability of cuttings which also had longer internodes. In this study only PAR was changed, but based on the findings of Leakey and Storeton-West (1992), it is likely that light quality with lower R:FR ratio would provide further improvement. A future comparison of rooting between cuttings from juvenile seedlings and mature marcots under optimal light environments might find that they have similar rooting capacity. This would open the way to mass propagation of selected cultivars from mature stockplants.

- **Factors affecting the propagation of mature shoots by marcotting**

Marcotting is currently the best option for capturing mature phenotype as it provides the opportunity to capture the genotype and to create mature stockplants which may be, at least partially rejuvenated physiologically. The next set of experiments sought to identify the factors (*e.g.* auxin, media and orientation and size of the branch) determining successful propagation of mature shoots and their survival.

⇒ *Effects of auxin, rooting media and branch orientation on marcotting*

Surprisingly, there is much less knowledge about the factors affecting the success of air-layering, than there is about the rooting of stem cuttings. Typically, studies have examined branch size, orientation and different rooting media. The first experiment in this series examined these factors, and as with the rooting of cuttings of *B. procera* and *I. fagifer* 100% success was achieved regardless of treatment. Unfortunately due to my absence from Solomon Islands at this time, it was not possible to make a time series of observations that might have identified differences between the treatments. Consequently the only possible approach to examining this data is to use the number of roots formed as a surrogate for rooting capacity. However, the genuine effect of branch orientation, auxin and media is not possible to determine. Nevertheless, in *B. procera*, when the medium was soil, vertical branches rooted well without auxin, while horizontal branches rooted well with auxin. By comparison, in *I. fagifer*, when the medium was soil, vertical branches rooted well both with and without auxin. The present study in some ways agrees with Mialoundama *et al*., (2002), especially in *B. procera* with best rooting of horizontal branches with auxin when the medium was soil.

When the marcotted branches were cutback after rooting to allow for manageable length, some marcots died. It was hypothesised that mortality may have set in quicker in the marcots with a large root biomass because there is an unmet demand for water, nutrients and space for root development and expansion for an extended period of time.

⇒ *Effects of height and diameter of marcotted branches*

As phase change in trees is thought to occur gradually as a tree grows larger, until a threshold is reached when thereafter all shoots are ontogenetically mature and capable of flowering and fruiting (Hartmann *et al.,* 1997), it can be hypothesised that the capacity to root marcots on intact shoots might also be affected by the position in the tree (height within the crown). Thus, the present study examined the effects of height and diameter of the mature branch in a number of different trees. Again there was 100% rooting of *B. procera* and *I. fagifer* after 15 and 11 weeks respectively and so no differences between treatments in the percentage rooting. This time there was no mortality. This supports the earlier suggestion that mortality may be due to the marcots of experiment 8 being constrained in the marcot package too long.

Again, the number of roots on the marcots was not significantly different between various heights in *B. procera* or *I. fagifer*, however, longer roots are found in *B. procera* at lower heights. This result implies that the rooting of intact shoots, as opposed to detached shoots, is not affected by "phase change." Similarly, it suggests that physiological factors, such as water stress are not implicated. This concurs with Hubbard *et al.,* (2002), who reported that height does not have any effects on gas exchange and response of stomata to leaf hydraulic conductance of the flow path from soil to leaf in open tall trees. In terms of the diameter of the marcotted branch, there was no significant effect on the number and length of roots in either species. However, there was significant tree-to-tree variation in these two species.

⇒ *Separation of ontogenetic and physiological age*

Building on the concept and results of Leakey (2004b) and Dick and Leakey (*in press*), this study went on to investigate "phase change" by trying to separate ontogenetic and physiological age within the mature crown shoots of *B. procera*. This was done by comparing cuttings collected from rooted and unrooted pollard shoots within the mature tree crown. The rooted shoots were achieved by marcotting and potting new shoots within the tree crown. This was done on the

assumption that the presence of roots would invigorate these shoots, providing nutrients, water and perhaps growth regulators. This assumption tests the hypothesis proposed by Wareing and Frydman (1976) that the proximity of roots is associated with the juvenile state. The results partially supported the hypothesis, as cuttings from the 'rejuvenated' shoots, which were morphologically similar to the untreated shoots, produced more roots, and had a reduced incidence of leaf abscission. However, they did not have a significantly higher percentage rooting, although shoots from 3 of the 6 potted systems did root better than the unpotted controls. Unfortunately, data was not collected to determine whether the variation in the rooting capacity of cuttings from these "potted" shoots was related to the root biomass in the pots. Further studies are required to determine whether rooted shoots within the tree crown can be fully rejuvenated physiologically so that the rooting is like that of seedlings or coppice shoots.

6.5 SUMMARY

It is clear that *B. procera* and *I. fagifer* are easy rooting species. Generally, research is targeted at difficult problems, but it is possible that easy-to-root species like *B. procera* and *I. fagifer* actually offer big opportunities to develop a better understanding of some of the complexities of the rooting process in tropical tree cuttings. Thus, further investigation of pre-severance environmental factors in *B. procera* and *I fagifer* is required to develop a better understanding of the species biology and physiology, so enhancing the robustness of vegetative propagation techniques for these two species, by either single-node leafy cutting or marcotting techniques. This is important to allow the capture of the phenotypic variation in fruit and nut traits in elite cultivars, as outlined in the next chapter.

CHAPTER 7: PHENOTYPIC STUDY OF VARIATION

7.1 INTRODUCTION

7.1.1 Concept and rationale

Traditionally, throughout the tropics, indigenous fruits and nuts are eaten as an important source of food and nutritional security. Within the domestication process, for horticultural species, the selection of plus-trees is an important step towards the development of improved planting material, as cultivars can be developed by vegetatively propagating plus-trees. This step involves the capture and characterisation of genetic variation (Leakey and Newton 1994a). In the domestication of timber species for forestry, plus-trees would be selected as breeding stock for seed orchards (Harwood 1999).

To be able to make selections of fruit and nut trees from the wild population, it is necessary to know the extent of variation available, through an assessment of the phenotypic expression of quantitative traits that are culturally and commercially important. This approach has been successfully applied in West and Central Africa on *Dacryodes edulis* (Waruhiu 1999; Leakey *et al.,* 2002; Anegbeh *et al.,* 2005) and *Irvingia gabonensis* (Leakey *et al.,* 2000; Atangana *et al.,* 2001, 2002; Anegbeh *et al.,* 2003; Leakey *et al.,* 2004), and in *Sclerocarya birrea* (Leakey 2005; Leakey *et al.,* 2005b; Leakey *et al.,* 2005a). In these species, studies have sought the superior phenotypes in wild populations with a combination of desirable traits. This has involved the characterisation of intraspecific (tree-to-tree) variation in fruits, nuts and kernels, identification of multi-trait ideotypes, including analysis of organoleptic attributes, which are important for selection and potential cultivar development. In addition, it is desirable to assess the relationship between fruit mass and market price (Leakey *et al.,* 2002).

7.1.2 Quantitative descriptors for intra-specific variation

The characteristics of fruits are very diverse and heterogeneous. Because trees are out-breeding (i.e. allele segregation occurs during meiosis), even trees derived from seeds collected from the same mother trees will vary greatly. The characterisation of fruit, flesh, nut and kernel traits has been found to be a useful tool to quantify this variation. This then allows researchers and extension workers to demonstrate to farmers the ways in which fruit and nut trees vary between individuals. This is information which can also be used to determine the best combination of traits to produce a particular marketable product – an ideotype (Leakey *et al.,* 2002; Leakey and Page *in press*). The ideotype is also a simple way of explaining to policy makers the level of improvement that can be achieved without opting for costly, time-consuming and highly complicated tree breeding and biotechnology (Atangana *et al.,* 2001; Leakey *et al.,* 2004).

In a study of *Dacryodes edulis* in West and Central Africa, fruits from one hundred trees of *D. edulis* from five villages (four in Cameroon and one in Nigeria) were characterised (Leakey *et al.,* 2002). Thirteen fruit characteristics (fruit length, width, flesh depth, fruit mass, kernel mass, shell mass, skin colour, kernel colour, kernel taste, fiborosity, and oiliness) were measured (Waruhiu 1999) to provide understanding of the genetic variability in the fruit within and between trees. In contrast to earlier reports based on non-quantitative descriptions of tree-to-tree variation in this species (Okafor 1983), significant and continuous tree-to-tree variation was found in all fruit traits except kernel mass (Waruhiu *et al.,* 2004; Anegbeh *et al.,* 2005). This study also found greater within village variation in important traits, than between villages, and that only a few trees produced large fruits (Waruhiu *et al.,* 2004; Anegbeh *et al.,* 2005). For example, fruit length, width, flesh depth, fruit mass, flesh mass and kernel mass varied by 80, 86, 88, 84, 84 and 97% respectively. Nevertheless there was significant variation between the mean values of all these traits per village, except for kernel mass (Leakey *et al.,* 2002). This suggests that kernel mass is one of the least variable traits, both between and within village populations.

In addition to morphological traits, this study of *D. edulis* found that skin and flesh colour also differed between trees, with the identification of 25 skin colours and 23 flesh colour: the most common being 18D6 (greyish violet) and 29A7 (yellowish) (described using the Methuen Colour Code) (Waruhiu *et al.,* 2004; Anegbeh *et al.,* 2005). These different morphological and product quality traits of fruits are very important selection criteria for the domestication of *D. edulis*, as colour and fruit size affect the market price.

In the above study, the frequency of *D. edulis* planted in different land use systems varied significantly (Waruhiu *et al.,* 2004). In Cameroon (Chop Farm, Elig Nkouma, Makénéné and Nko'ovos II) more *D. edulis* trees were found in cocoa fields (65.5%) than in other land use systems (e.g. crop fields -16.5%, home garden - 9.5% and fallow fields - 7.5%), while in Nigeria (Ilile) the trees were mostly found in large homegardens (57%) – only 4% of trees were from cocoa farms. No correlations were found between the fruit traits of *D. edulis* and the different land use systems (Waruhiu 1999).

Similar results were obtained in a parallel study in *I. gabonensis* in West and Central Africa (Atangana *et al.,* 2001, 2002; Anegbeh *et al.,* 2003), which confirmed the general patterns of phenotypic variation in fruit traits between trees, such as fruit length, width, flesh depth, kernel mass, flesh taste and fibrosity found in *D. edulis*. However, some traits were more variable than others, for instance, fruit mass varied 439% in comparison to 67% and 61% variation in fruit length and fruit width respectively. The colour of skin and flesh also vary considerably with yellow (Methuen Colour Code 4A8) being observed most frequently (Atangana *et al.,* 2001).

One important finding in these studies of fruit traits was that some traits are clearly related, while others are not (e.g. fruit size and taste) (Atangana *et al.,* 2001, 2002). This is especially important in species like *I. gabonensis* which are important for both their flesh and their kernels, as it allows plus-tree selection to be directed towards two different markets – fresh fruit pulp and extracted kernels. Indeed, even the kernel traits can vary in importance for different markets – i.e. kernels for

food thickening compounds and kernels for extractable vegetable oils (Leakey 1999; Leakey *et al.,* 2005d; Leakey and Page *in press*).

The fruit ideotype in *I.gabonensis* combines characteristics such as fruit size, good taste and low fibrosity, while the kernels ideotype combines characteristics such as drawability, kernel mass and shell brittleness. In *I.gabonensis*, it was also possible to sub-divide the kernel ideotype into oil and food-thickening ideotypes, as opportunity to develop different markets was recognised (Leakey *et al.,* 2005d). Furthermore, the food-thickening ideotype had the option for further subdivision, after two unrelated traits (drawability and viscosity) were identified requiring further research to determine the consumers preference (Leakey *et al.,* 2005d).

7.1.3 Nutritional characterisation of fruit traits

Multi-trait selection for superior indigenous fruit and nut trees for domestication should not only be based on the morphological characteristics of the tree such as height, fruiting and flowering phenology, colour, size or taste of the fruit and kernel. Instead, in developing countries, where malnutrition is a problem, the nutritional value of the fruit and kernel should be added as a vital criterion (Thiong'o *et al.,* 2002). To determine nutritional value of fruit or kernel, these products must be chemically analysed for a number of useful elements and compounds they have such as moisture, dietary fibre, protein, carbohydrate, fat, vitamins (e.g. B carotene, thiamin, riboflavin, niacin, vit C) and minerals (e.g. Sodium, Potassium, Calcium, Magnesium, Iron, Zinc).

In a study of variation in the nutritional quality of *Sclerocarya birrea* fruits, Thiong'o *et al.,* (2002) found significant variation between trees in a number of nutritional properties. For example, vitamin C, proteins, moisture, phosphorus differed significantly between trees in fruit skin and pulp, while copper differed between trees in endocarp (nut = kernel). They also found a significantly higher vitamin C content (161 mg^{-1} 100 g) in the skin and flesh of *S. birrea* in contrast to the dietary allowable recommendation of 30-60 mg^{-1} 100 g for children 1-10 years old. This information about the nutritional value of different products of *S. birrea*

is important for multiple trait selection. In the study of morphological characterisation in *S. birrea*, there was a strong and significant relationship between fruit mass and flesh mass, but not kernel mass (Leakey 2005). These differences in relationships provide an opportunity for selecting trees with big fruits for pulp production with high vitamin C, or trees that produce relatively small fruits but large kernels for oil production or high levels of protein and minerals for edible nuts.

7.1.4 Organoleptic characterisation of fruits

Organoleptic fruit characterisation is a technique for the selection of taste (sweetness, acidity, odour, sourness, oiliness, etc.) and odour. These can be used to expand the traits included in ideotype, and can perhaps be related to other traits such as protein, fibre and carbohydrate content of the fruits or kernels (Kengni *et al.,* 2001; Leakey *et al.,* 2002).

The results of an organoleptic assessment of *D. edulis* in Cameroon (Kengni *et al.,* 2001) examined characteristics of aromatic, oiliness, acidity, astringency, bitterness, fibrosity, sourness, saltiness and wateriness in the selection criteria for cultivar development. These are organoleptic characteristics that influence price and industrial importance. Such an approach helps to ensure the quality of the improved product and its ability to satisfy consumers at different market levels.

In other studies, it has been reported that there are interactions between some of these components. For example, in *Dacryodes edulis* lipid content is higher in the mesocarp than it is in the seed of large fruits (Youmbi *et al.,* 1989 cited in Leakey *et al.,* 2002) while regardless of fruit size, non-structural carbohydrates content is higher in the seed than in the mesocarp. With the same species, Kapseu and Tchiegang (1996 cited in Leakey *et al.,* 2002) noted that fatty acid content differs very little between fruits of different sizes. Tree-to-tree assessment of organoleptic qualities of indigenous fruit and nut is a new development in the South Pacific, but currently there is a study in progress on *Canarium indicum* in Papua New Guinea.

7.1.5 Fruit traits and market price relationship

If farmers are to have an incentive to grow selected cultivars, they need to receive a premium price for the product. To determine if different markets pay a premium for superior quality fruits, a study with *Dacryodes edulis* investigated the relationship between fruits, nuts and kernel characteristics and the market prices (Leakey and Ladipo 1996). They found that the price paid for a fruit was influenced by the fruit size and pulp yield. However, they also noted that market price is determined by other factors such as cooking quality and flavour. On the other hand, Waruhiu (1999) and Leakey *et al.,* (2002) found no relationship between fruit size and price in *Dacryodes edulis* fruits in wholesale markets, although there was a strong relationship in retail city markets. This situation probably arises because wholesale traders are unable to obtain large quantities of genetically uniform fruits. It is anticipated that the development of cultivars will change this situation.

The above African experiences demonstrate the complexity of factors affecting the pricing of fruits. Understanding these fruit characteristics in relation to market price is therefore crucial to successful tree domestication for agroforestry. This chapter examines the tree-to-tree phenotypic variation within and between populations of *B. procera* in Kolombangara Island, in terms of both fruit, nut and kernel morphology, as well as the assessment of kernel taste.

7.2 MATERIALS AND METHODS

7.2.1 Fruit characterisation

Wherever possible twenty-four mature fruits of *B. procera* were harvested from each of one hundred and ninety trees, in five populations (Vovohe, Tututi, Rei, Poporo and Hunda). Locations of these populations are described in Chapter 3. Tree age and the measurements of tree height and diameter at breast height were also determined, as described in Chapter 3.

Fruits were harvested during the peak of the season in 2002, 2003 and 2004. The intention was to collect fruits randomly from the 4 quadrants of the crown at two third of tree height, and to collect 24 fruits x 50 trees x 5 populations; a total of 4,800 fruits. However, this plan was not always possible to achieve as trees varied in the number of fruits maturing at any one time. In addition, despite being asked not to collect the fruits, some fruits were harvested by people, especially the children. Consequently, the number of fruits collected varied by tree, and by year (Table 7.1). A total of 3,267 fruits were collected and characterized. After collection, the fruits were transported to the nursery in plastic bags, labelled with the tree number, and collection date.

Table 7.1: Year-to-year variation in fruit collection for characterisation study in Kolombangara Island.

Population	**2002**		**2003**		*2004*	
	No. of trees	*No. of fruits*	*No. of trees*	*No. of fruits*	*No. of trees*	*No. of fruits*
Vovohe	3	38	8	155	4	55
Tututi	0	0	15	390	5	81
Rei	0	0	9	200	4	57
Poporo	0	0	15	438	3	68
Hunda	18	312	46	1273	11	200

At the nursery, individual fruits were characterised following the method described by Leakey *et al.,* (2000) (Fig 7.1). Ten fruit traits were quantitatively assessed including: fruit length, width and mass, nut mass, shell mass, flesh mass and depth, and kernel length, width and depth. Fruit, flesh and shell mass were determined using portable kitchen scales graduated to 2g, while the length, width and depth of both the fruit and the kernel were measured with callipers graduated to 0.1 mm. Nut (fruit mass – flesh mass) and kernel (nut – shell mass) mass were obtained by difference. The fruit: kernel ratio was calculated using mass of these two variables. Fruit skin colour was assessed by comparing it with a colour chart (Kornerup and Wanscher 1967). The Methuen Code of Colours is a 3-dimensional code for hue, tone and colour intensity.

Plate 7.1: Characterisation of *B. procera* fruits to determine tree-to-tree variation in different fruit and kernel traits in Kolombangara, Solomon Islands.

To assess the level of domestication achieved by farmers, frequency distribution curves are developed according to the hypothesis of Leakey *et al.,* (2004) that this can distinguish five stages of domestication.

7.2.2 Organoleptic fruit characterisation

Three fruits were collected from thirty *B. procera* trees at each of three sites (Vovohe, Poporo and Hunda) in 2004 for a preliminary assessment of variation in organoleptic traits of the kernels. These trees were amongst the 119 trees described earlier for the morphological characterisation study. The fruits were collected at random from different quadrants at two thirds of tree height. After collection, the fruits were transported in labelled plastic bags, denoting tree identity and date.

At the nursery, individual fruits were characterized following the method described above (section 7.2.1). The fresh fruits were then cut in half and the edible kernel extracted from the fibrous shell using a kitchen knife. The extracted kernel was then cleaned up, by removing the thin layer of testa covering it. Following this, the kernels were individually placed on pieces of white paper and anonymously served to the panel of judges for assessment. A panel of 3 judges composed of rural farmers who had been trained by the author for at least a month to recognise the relative intensity of organoleptic characteristics based on taste, odour and visual appearances of the kernel. The training simply involved tasting of kernels from different fruits and recognising different taste attributes. A list of organoleptic attributes (sweetness, aroma, bitterness, oiliness, consistency and wateriness) for kernels, modified from Kengni *et al.,* (2001) was given to the judges and they evaluated the relative intensity of each variable using a scoring system described by Kengni *et al.,* (2001); that is a continuous scale of 0-5 (0 = absent; 0-1 = barely; 1-2 = perception; 2-3 = delicate; 3-4 = moderate; 4-5 = strong). Four sessions were organized in total and the judges had 5-10 minutes recess in between each session. Each judge evaluated a maximum of 8 samples on each session, and between samples the judges rinsed their mouths with tap water to neutralize previous perception or odour.

7.3 RESULTS

7.3.1 Extent and quantitative descriptors of continuous intraspecific variation in fruit and kernel traits

7.3.1.1 Fruit, flesh, nut, shell and kernel mass

Tree-to-tree variation in fruit, nut, shell and kernel mass were highly significant (P = 0.001) within populations, but only kernel mass was significant (P>0.05) between populations. Tree number 1 of Hunda had the greatest mean fruit and kernel mass. The Poporo population had the greatest mean fruit mass (68g), while the Rei population had trees with the lowest mean fruit mass (60g) (Appendix 7.1). Within population, the trees number 5, 12, 2, 3 and 1 of Vovohe, Tututi, Rei, Poporo and Hunda respectively had the greatest mean kernel mass (Table 7.2). Mean fruit, flesh, nut, shell and kernel mass all varied from tree-to-tree (Fig 7.2 and Fig 7.3). These two figures illustrate that variation is primarily at village level, therefore it is an appropriate unit for domestication. Significant tree-to-tree variation also occurred in the fruit to kernel ratio (Fig 7.4).

Table 7.2: Morphological characteristics of 5 top trees of *B. procera* selected from 119 trees, based on kernel mass. Mass measured in grams and length, width and depth in millimetres. (±SE)

Traits	Vovohe Tree 5	Tututi Tree 12	Rei Tree 2	Poporo Tree 3	*Hunda Tree 1*
Fruit mass	89.7 ± 4.2	102.4 ± 2.8	86.3 ± 2.0	67.9 ± 3.5	110.6 ± 2.4
Nut mass	52.3 ± 2.1	50.8 ± 1.4	50.6 ± 1.3	35.2 ± 2.1	57.2 ± 1.7
Flesh mass	37.3 ± 3.0	51.7 ± 1.8	35.6 ± 1.3	32.7 ± 1.6	53.3 ± 0.8
Shell mass	34.0 ± 1.8	32.8 ± 0.8	30.6 ± 1.1	19.4 ± 1.4	32.8 ± 0.9
Kernel mass	18.3 ± 0.8	17.9 ± 0.8	20.0 ± 0.7	15.8 ± 1.2	20.6 ± 0.6
Fruit length	81.6 ± 1.4	80.9 ± 0.7	69.2 ± 0.6	69.2 ± 2.1	74.4 ± 0.9
Kernel length	42.5 ± 0.8	37.6 ± 0.7	34.4 ± 0.5	35.1 ± 1.0	45.2 ± 0.5
Fruit width (apex)	32.1 ± 1.0	31.6 ± 0.4	29.0 ± 0.5	24.4 ± 0.6	38.9 ± 0.6
Fruit width (middle)	46.5 ± 1.0	46.4 ± 0.5	44.0 ± 0.5	37.2 ± 1.1	51.1 ± 0.6
Fruit width (base)	34.1 ± 1.0	38.5 ± 0.8	32.7 ± 0.6	25.8 ± 0.4	41.7 ± 0.6
Kernel width (apex)	21.4 ± 0.5	18.2 ± 0.5	17.6 ± 0.8	16.8 ± 0.5	29.2 ± 0.6
Kernel width (middle)	27.4 ± 0.7	21.8 ± 0.5	24.0 ± 0.3	21.3 ± 0.6	41.2 ± 0.6
Kernel width (base)	20.4 ± 0.7	18.8 ± 0.5	20.1 ± 0.4	17.5 ± 0.3	31.7 ± 0.4
Kernel depth	27.5 ± 0.6	22.3 ± 0.8	24.3 ± 0.3	24.0 ± 0.5	29.4 ± 0.8

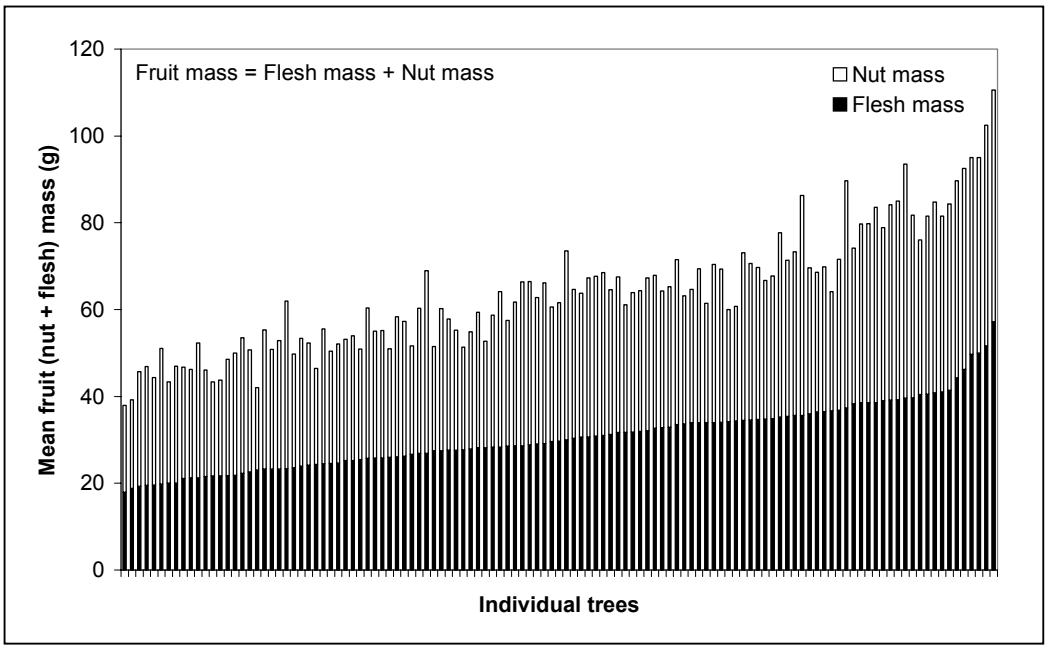


Fig 7.2: Intraspecific variation in mass of fruit, flesh and nut across 5 populations of *B. procera* in Kolombangara, Solomon Islands, in ascending order of flesh mass

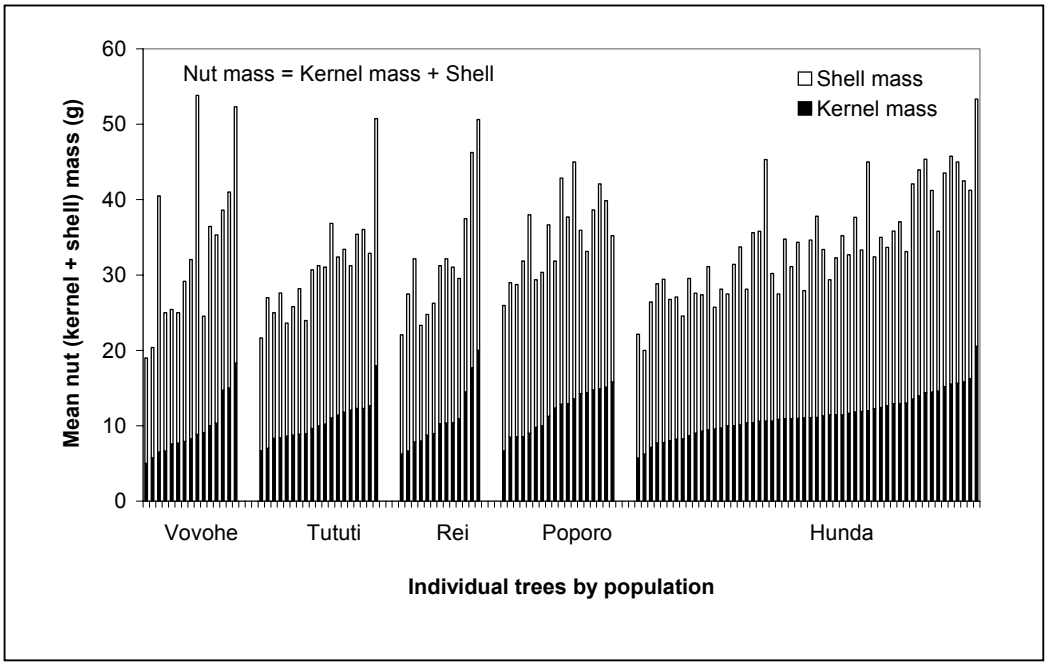


Fig 7.3: Intraspecific variation in mass of nut, kernel and shell by different populations of *B. procera* in Kolombangara, Solomon Islands, in ascending order of kernel mass

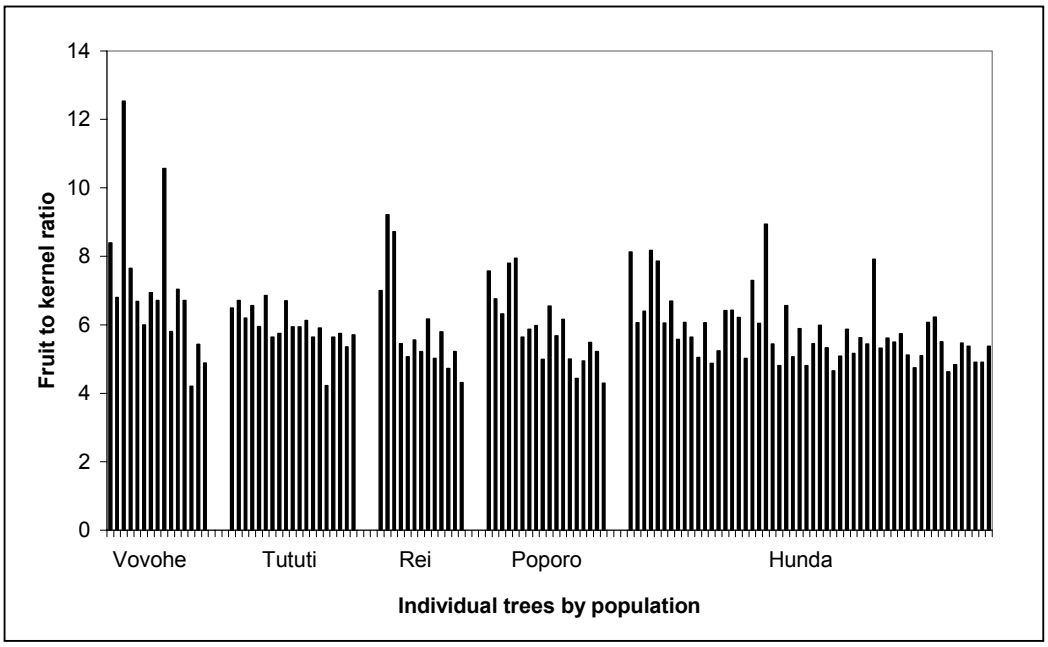


Fig 7.4: Intraspecific variation in fruit to kernel ratio by different populations of *B. procera* in Kolombangara, Solomon Islands, in ascending order of kernel mass (compare with Fig 7.2).

7.3.1.2 Fruit and kernel length and width

Tree-to-tree variation in fruit and kernel length were highly significant (P = 0.001) within but not between populations. Fruit lengths vary continuously (Fig 7.5), with fruit from 51.0 mm (Tree 78 of Hunda) to 85.4 mm (Tree 8 of Rei). Kernel length varies from 14.3 mm (Tree 48 of Hunda) to 47.4 mm (Tree 21 of Poporo). Similarly, fruit and kernel width vary significantly (P = 0.001) from tree-to-tree (Fig 7.6), but are not significantly different from each other between populations. Tree 1 in Hunda had fruit (51.1 mm) and kernel (41.2 mm) that is widest, in contrast to narrow fruits (33.4 mm) found in Tree 40 of Hunda and kernels (13.6 mm) in Tree 8 of Rei. Overall, mean fruit and kernel lengths were 67.6 mm ± 0.40 and 32.5 mm ± 0.31 and mean fruit and kernel widths were 39.6 mm ± 0.26 and 21.8 mm ± 0.23.

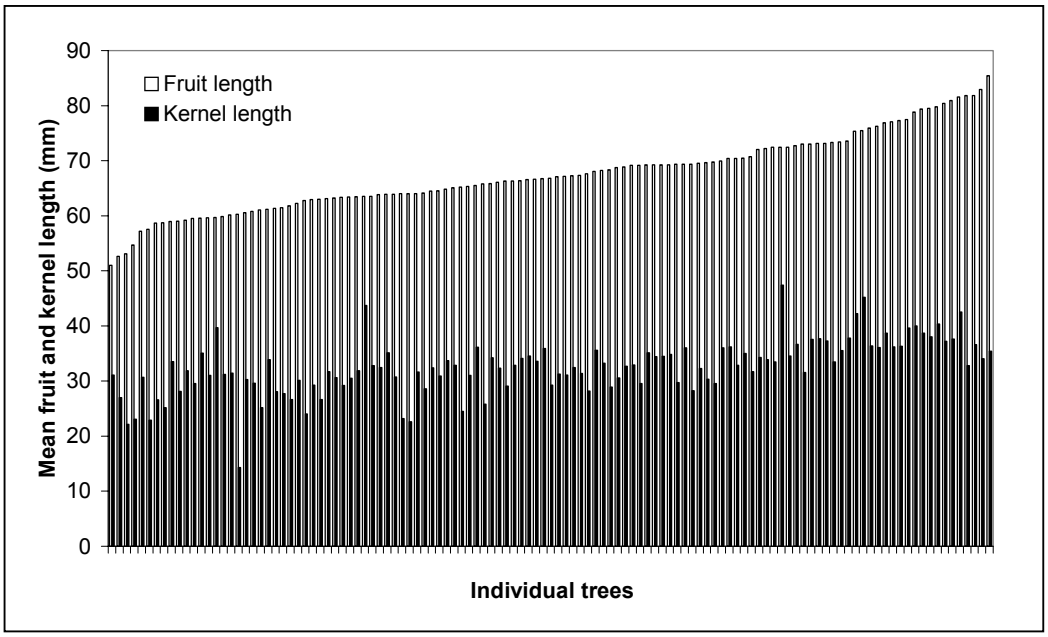


Fig 7.5: Intraspecific variation in fruit length across 5 populations of *B. procera* in Kolombangara, Solomon Islands, in order of ascending fruit length.

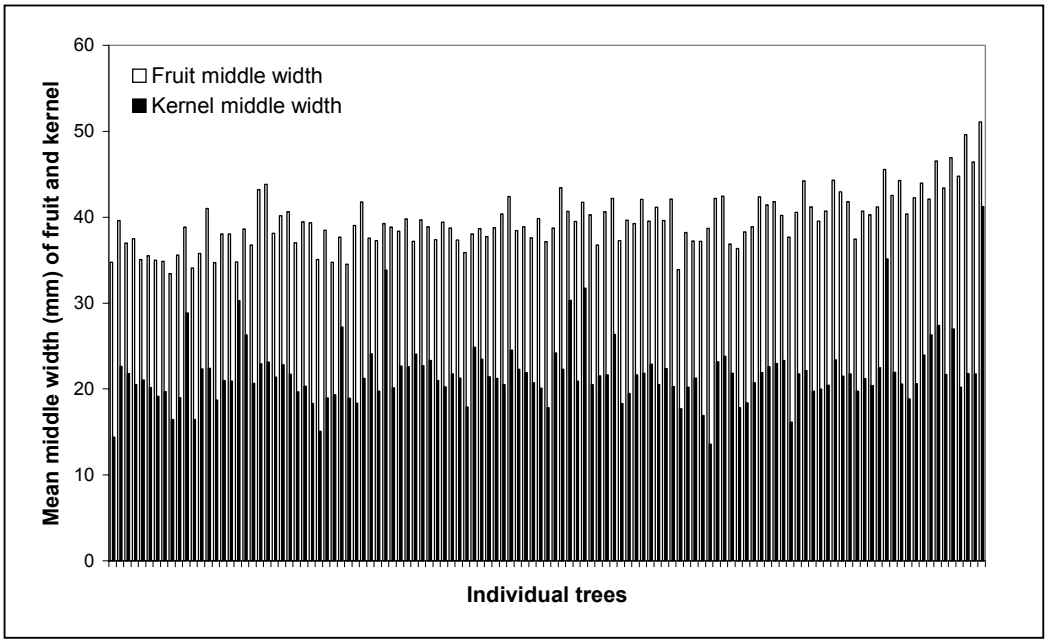


Fig 7.6: Intraspecific variation in fruit and kernel width across 5 populations of *B. procera* in Kolombangara, Solomon Islands, in order of ascending mean fruit mass.

7.3.1.3 Fruit skin colour

Skin colour of mature fruit was variable between five populations. Visually, fruits fell into colour categories *green* (67.2%) and *purple* (32.8%), although this was further defined into 23 colours (Fig 7.7) using Methuen Colour Code from Kornerup and Wanscher (1967), and the most frequent colour were patina green (28C6) (27%) and greyish green (28D7) (24%).

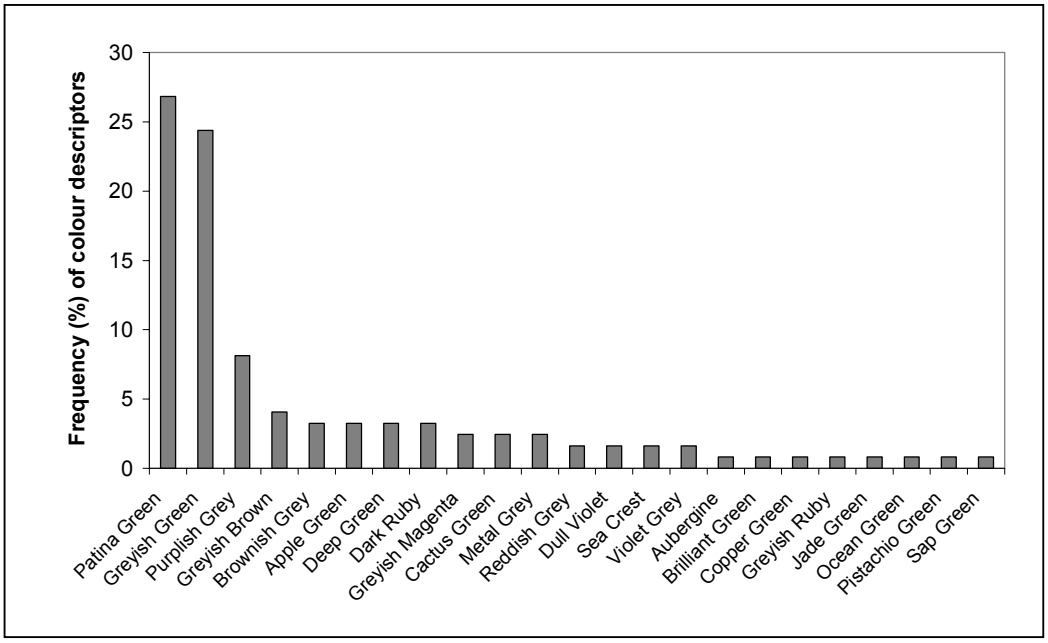


Fig 7.7: Percentage frequency of different fruit colours in *B. procera* across 5 populations (Vovohe, Tututi, Rei, Poporo, Hunda) in Kolombangara, Solomon Islands.

7.3.2 Relationships between characteristics

7.3.2.1 Tree height versus diameter

The fruiting trees sampled ranged in height and diameter at breast height, from 2-24m in height and 3-49 cm in diameter. Relationship between tree height and diameter across population (sites) was significant (P = 0.001), positive, and moderately strong (r^2 = 0.29) (Table 7.3). Similar significant relationship was found by individual population but varied in strength (Fig 7.8 and Table 7.3). Tree

size increased with age (Fig 7.9), with varied coefficient of correlation (Table 7.3) (Appendix 7.10). Trees in Hunda were relatively large and short due to pollarding of trees to safe height around village surroundings.

Table 7.3: Relationships between tree diameter and either tree height or tree age of *B. procera* in 5 populations in Kolombangara Island.

	Vovohe	***Tututi***	***Rei***	***Poporo***	***Hunda***
Diameter *v* Height	$r^2 = 0.415$ P = 0.001 y = 1.00x + 4.47	$r^2 = 0.347$ P = 0.001 y = 0.98x + 7.88	$r^2 = 0.919$ P = 0.001 y = 1.19x + 3.14	$r^2 = 0.455$ P = 0.001 y = 1.11x + 2.09	$r^2 = 0.318$ P = 0.001 y = 1.71x + 8.74
Age* *v* Diameter	$r^2 = 0.528$ P = 0.001 y = 0.27x + 3.65	$r^2 = 0.451$ P = 0.001 y = 0.28x + 5.27	$r^2 = 0.911$ P = 0.001 y = 0.91x – 2.85	$r^2 = 0.730$ P = 0.001 y = 0.73x + 2.04	$r^2 = 0.291$ P = 0.001 y = 0.24x + 6.98

**Tree age provided by farmers*

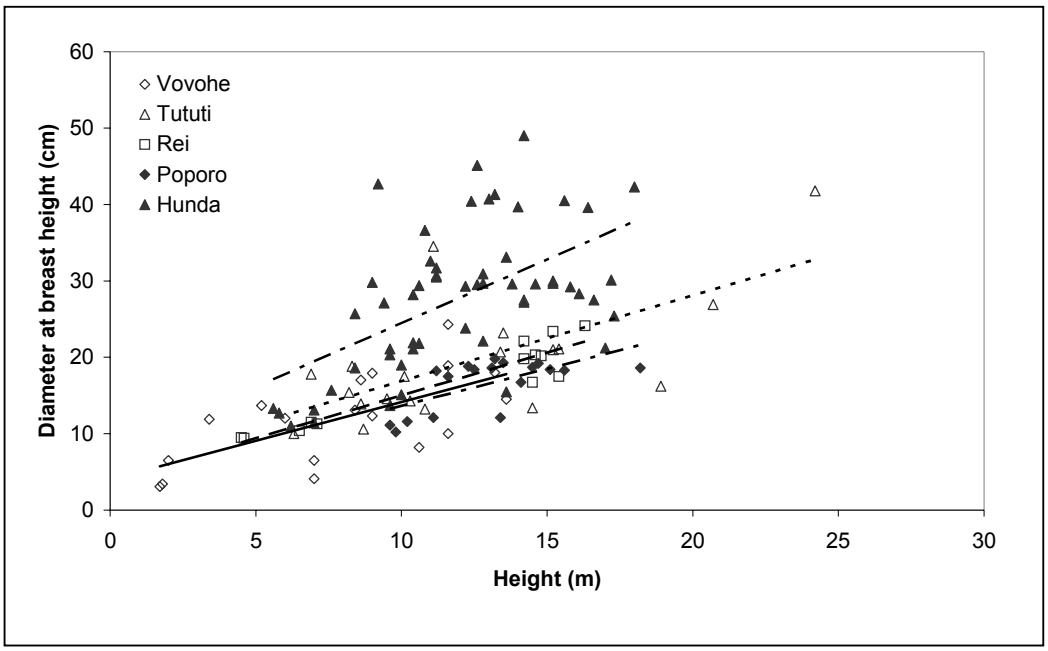


Fig 7.8: Relationship between height and diameter of 119 *B. procera* trees in five different populations in Kolombangara Island.

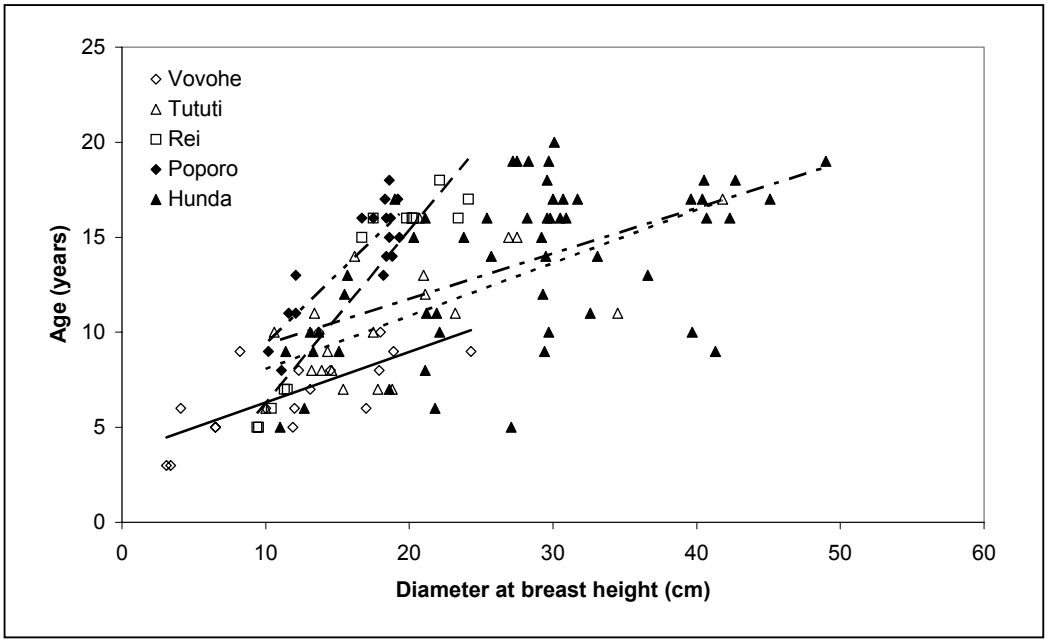


Fig 7.9: Relationship between age and diameter of 119 *B. procera* trees across 5 different populations in Kolombangara Island.

7.3.2.2 Fruit and kernel characteristics

The relationship between the main fruit and kernel characteristics (mass, length, width and fruit to kernel mass ratio) varied from tree-to-tree as well as among populations. Kernel mass increased with increasing fruit mass, length and width (Fig 7.10, Fig 7.11 and Fig 7.12). The overall fruit and kernel mass relationship ($y = 0.165x + 0.435$) is significant ($P = 0.001$), positive and strong, with $r^2 = 0.57$. Calculated fruit to kernel mass ratio for individual trees per population (Fig 7.4) showed that trees with larger fruits are not necessarily producing large kernels (i.e. with lower fruit to kernel ratio), and this was common in all five populations. Overall, the fruit to kernel mass ratio is 6.0, although this varies within and between populations. The ratio of fruit to kernel mass across all trees in five populations was in the order of 7.1, 6.0, 5.9 and 5.9 for Vovohe, Poporo, Tututi and Hunda respectively.

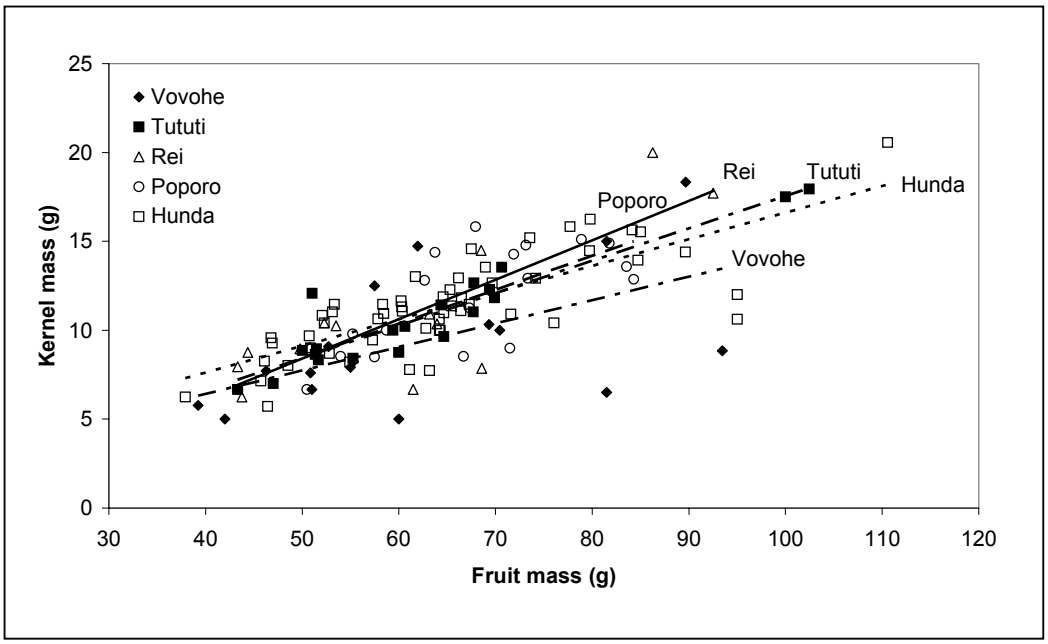


Fig 7.10: Relationship between kernel and fruit mass of 119 *B. procera* trees in five different populations in Kolombangara Island.

Vovohe y = 0.1x + 3.37, r^2 = 0.31; Tututi y = 0.2x – 1.46, r^2 = 0.93; Rei y = 0.2x – 3.10, r^2 = 0.80; Poporo y = 0.2x – 0.72, r^2 = 0.51; Hunda y = 0.1x + 1.96, r^2 = 0.61). Overall r^2 = 0.57

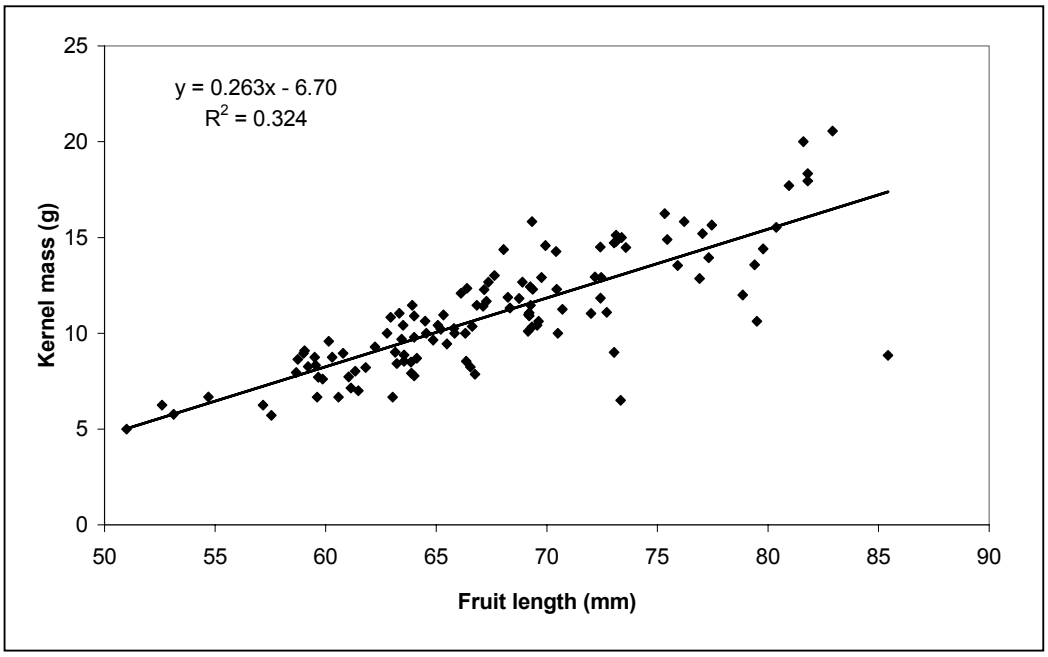


Fig 7.11: Relationship between kernel mass and fruit length of 119 *B. procera* trees across five different populations in Kolombangara Island.

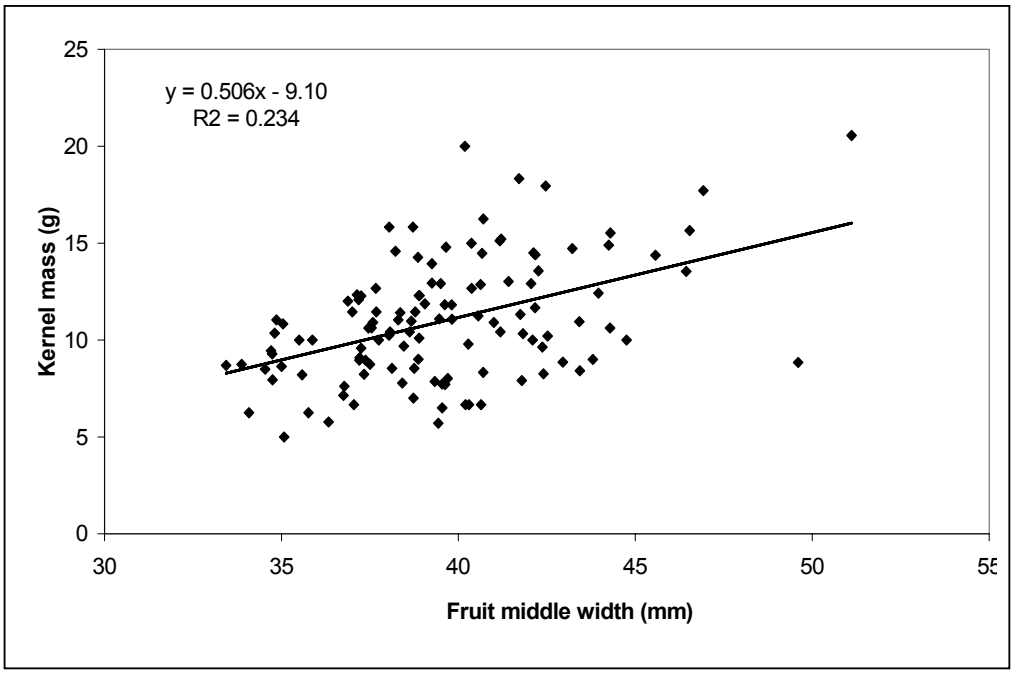


Fig 7.12: Relationship between kernel mass and fruit middle width of 119 *B. procera* trees across five different populations in Kolombangara Island.

7.3.3 Variation in the frequency distribution of fruit and kernel traits

The frequency distribution of variation of fruit and kernel traits across all five populations was calculated, and the extent of normality in fruit and kernel mass, length, width (middle) is demonstrated in Fig 7.13 and Fig 7.14. Deviation from normality was greatest in fruit mass ($\sigma = 16.6$) and least in fruit width (middle) ($\sigma = 4.2$). Deviation from normality in kernel width (middle) was also low ($\sigma = 4.4$) compared to its mass ($\sigma = 4.6$) and length ($\sigma = 5.9$).

Frequency distribution (%) of fruit and kernel traits in individual populations showed different degrees of skewness and the kurtosis (Fig 7.15). The estimated $^{g}_{1}$ and $^{g}_{2}$ of the frequency distribution was generally less than one for all traits, although this varied between populations (Appendix 7.1). For example, the distribution of fruit mass was greatly negative or positive skewed in Tututi and least skewed in Poporo. For kernel mass, negative or positive skewness was greatest in Rei. A leptokurtic distribution of kernel mass was found in all populations except in Poporo, which had a platykurtic distribution.

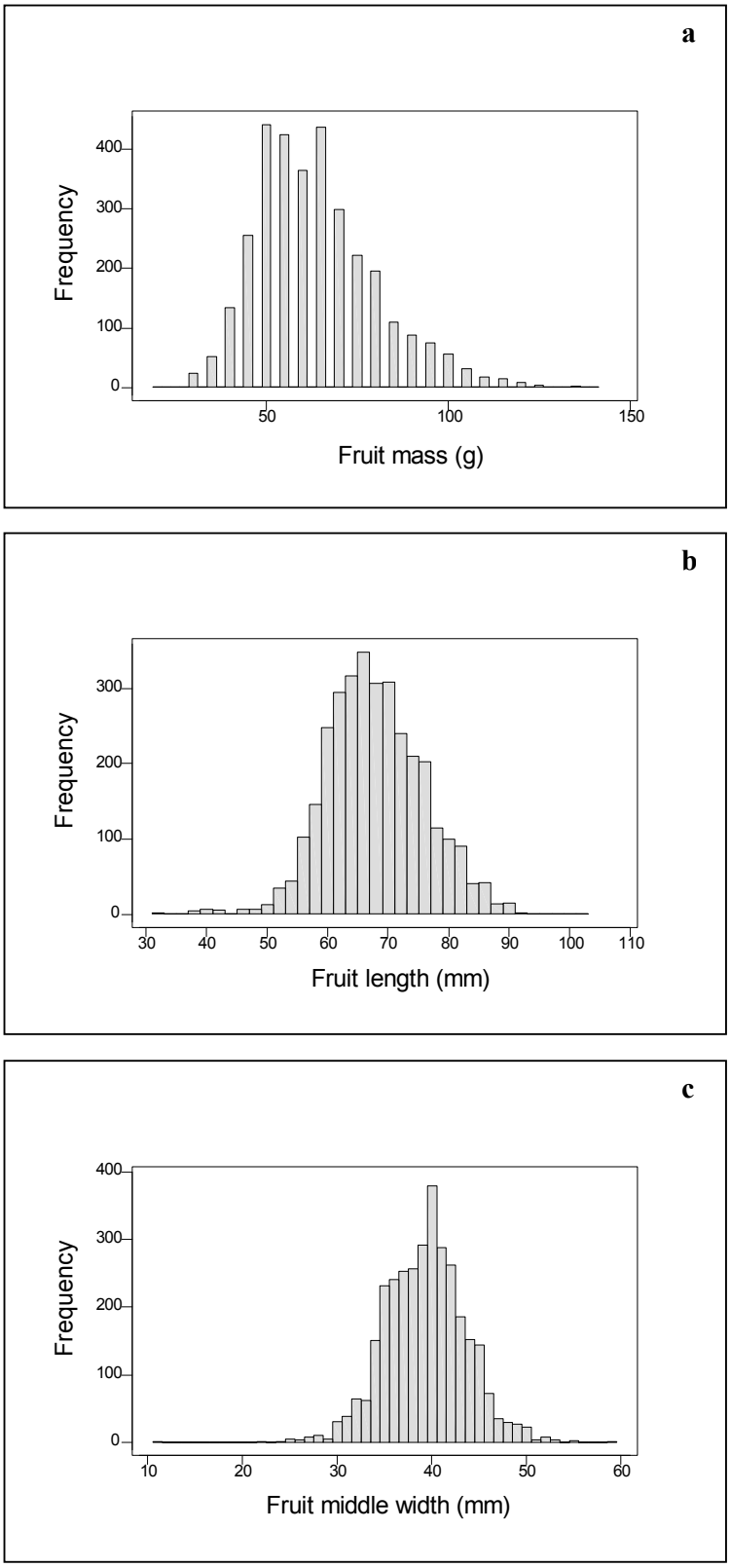


Fig 7.13: Frequency distribution of variation in important fruit traits of *B. procera* across 5 populations (Vovohe, Tutuli, Rei, Poporo, Hunda): a = fruit mass, b = fruit length and c = fruit width (middle)

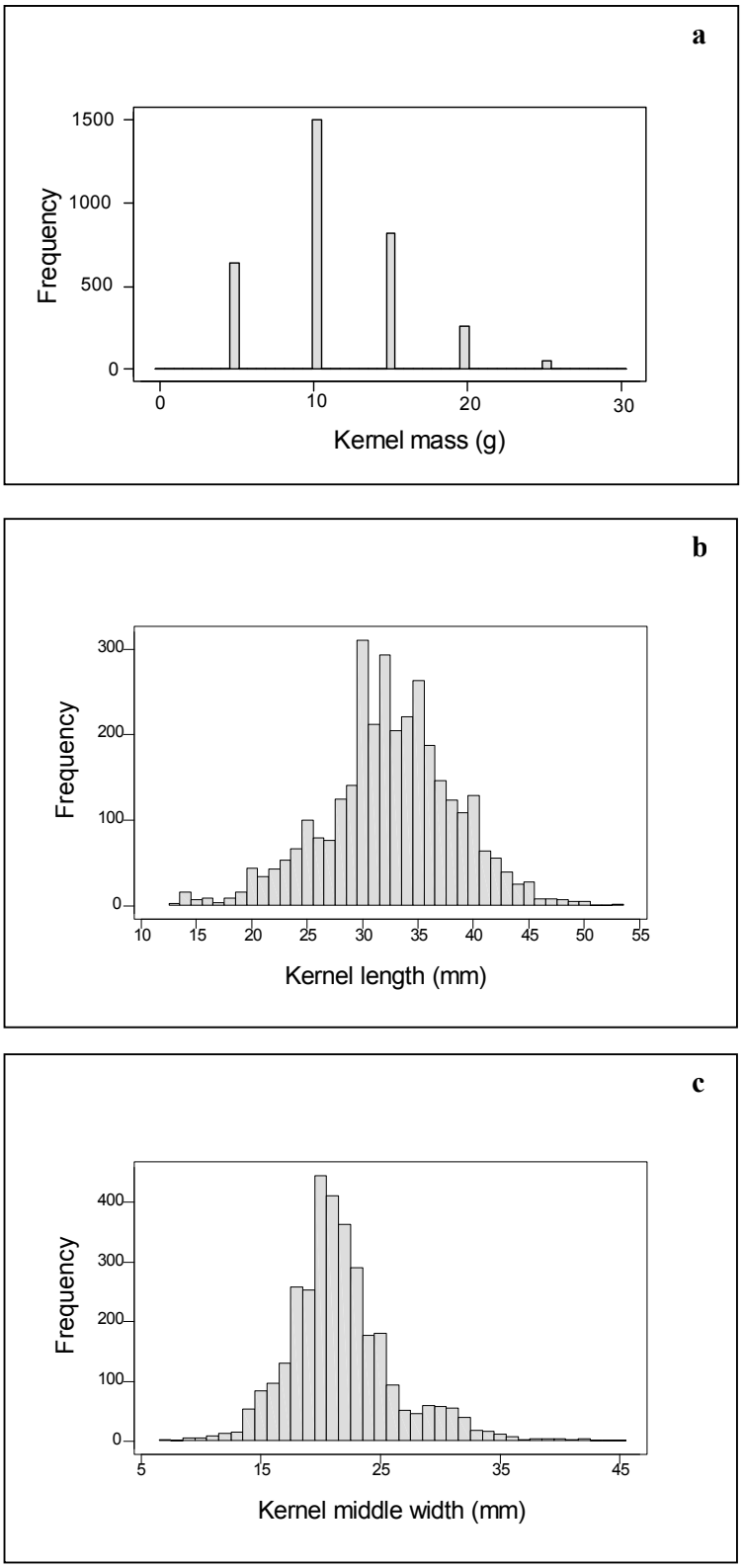


Fig 7.14: Frequency distribution of variation in important kernel traits of *B. procera* across 5 populations (Vovohe, Tutuli, Rei, Poporo, Hunda): a = kernel mass, b = kernel length and c = kernel width (middle)

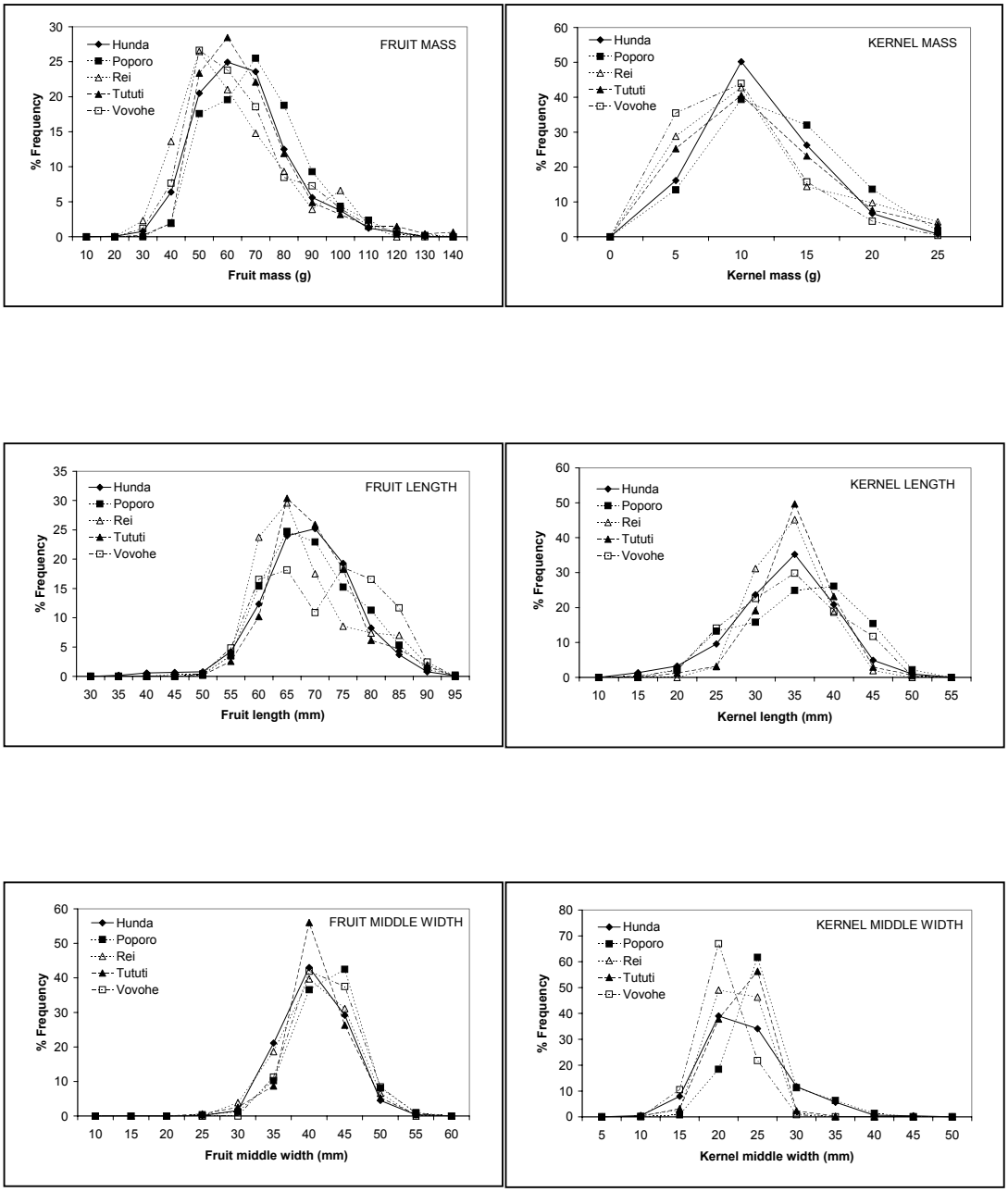


Fig 7.15: Comparative frequency distribution (%) of fruit and kernel characteristics in 5 populations (Vovohe, Tututi, Rei, Poporo, Hunda) of *B. procera* in Kolombangara, Solomon Islands.

7.3.4 Principal component analysis

Variation across nine fruit and kernel traits (fruit, flesh, nut, shell, kernel mass and length and width (middle) of fruit and kernel) was graphically represented by plotting the first two components of PCA (Fig 7.16). This analysis showed that the first principal component accounted for 63.4% of the total variation, constituting mainly of variation in mass (fruit, nut, flesh and shell). However, five populations were not significantly different from each other as indicated in a plot of PC1 and PC2 (Fig 7.16) and its ANOVA. The second principal component explained only 13.4% of the total variation, which was weighted heavily towards variation in kernel length and middle width. The ANOVA of the second principal component was also not significant.

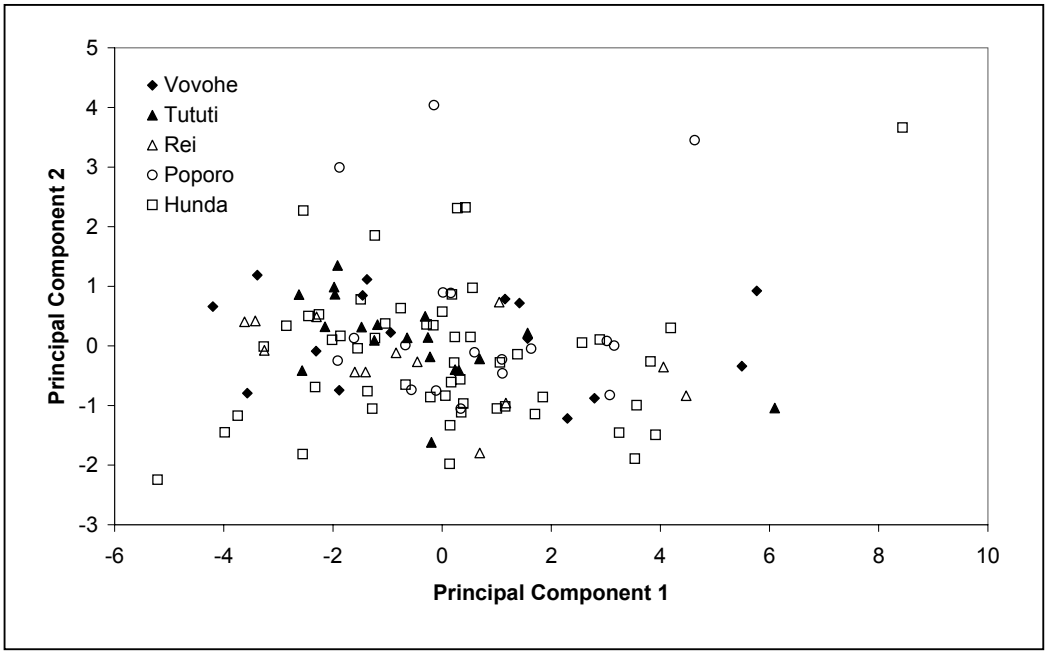


Fig 7.16: Principal component analysis using nine fruit and kernel traits in 5 populations of *B. procera* in Kolombangara Island. PC1 and PC2 are indices of variation across all traits and explained 76.8% of the total variation.

7.3.5 Organoleptic variation in kernel taste

Tree-to-tree variation in morphological characteristics of 30 trees of *B. procera* used for this study was highly significant (P = 0.001). The average value of individual fruit and kernel traits across all 30 trees were calculated (Table 7.4). Fruit mass ranged 45g to 120g, while kernel mass was 5g to 20g. These traits also varied significantly between populations, except kernel mass and fruit width (Table 7.5). Large and long fruits were obtained from Poporo population. The fruit mass to kernel mass ratio, which varied due to considerable variation in fruit and kernel mass was significant (P<0.05) within and between populations.

Table 7.4: Means of different fruit and kernel traits of 30 *B. procera* trees represented in organoleptic assessments.

Traits	***Mean*** ± *SE*	***Range***
Fruit mass	68.4 ± 1.5	45 - 120
Fruit length	69.6 ± 0.8	53 - 90
Fruit width	40.7 ± 0.4	22 - 52
Flesh mass	32.9 ± 0.8	20 - 50
Nut mass	34.6 ± 0.7	20 - 50
Shell mass	22.8 ± 0.5	10 - 35
Kernel mass	11.8 ± 0.4	5 - 20
Kernel length	34.7 ± 0.6	21 - 48
Kernel depth	24.2 ± 0.5	15 - 38
Fruit to kernel ratio	6.0 ± 0.2	4.4 - 10

Table 7.5: Means of different fruit and kernel traits of 30 *B. procera* trees by individual population.

Traits	***Mean ± SE***		
	Vovohe	**Poporo**	**Hunda**
Fruit mass	64.5 ± 1.9 a	76.8 ± 3.0 b	64.0 ± 2.4 a
Fruit length	67.7 ± 1.5 a	72.5 ± 1.4 b	68.6 ± 1.2 a
Fruit width	40.5 ± 0.6 a	41.9 ± 1.0 a	39.5 ± 0.6 a
Flesh mass	31.7 ± 1.1 a	37.3 ± 1.4 b	29.8 ± 1.5 c
Nut mass	32.5 ± 1.1 a	37.5 ± 1.2 b	33.8 ± 1.2 a
Shell mass	22.0 ± 0.8 a	24.8 ± 1.0 b	21.5 ± 0.8 a
Kernel mass	10.5 ± 0.8 a	12.7 ± 0.7 a	12.3 ± 0.7 a
Kernel length	33.2 ± 0.8 a	37.3 ± 1.1 b	33.7 ± 1.1 a
Kernel depth	22.0 ± 0.5 a	27.7 ± 0.9 b	23.1 ± 0.8 a
Fruit to Kernel ratio	7.0 ± 0.6 a	6.3 ± 0.3 b	5.4 ± 0.3 b

NB: Comparison between populations: Data followed by same letter is not significant and data followed by different letters is significant at 5% confidence interval.

Variation in the judgement of the three tasters for different attributes (sweetness, bitterness, aroma, oiliness, consistency, wateriness) of the kernel was not significant (P>0.05). The effect of interaction between the tasters and all kernel attributes was also not significant (Table 7.6). Tree-to-tree variation in all kernel organoleptic attributes was not significant. Similarly, variation in kernel organoleptic attributes between the three populations was not significant (Fig 7.17). There was no significant relationship between kernel mass and kernel sweetness or kernel oil content.

Table 7.6: ANOVA for scoring by tasters as index of agreement. KM = Kernel mass.

Source of variance	***d.f***	***s.s***	***m.s***	***F-value***	P-value
Sweetness					
Tasters	2	3.712	2.516	0.950	0.390
Kernel mass	3	11.659	10.259	2.590	0.059
Tasters * KM	6	4.197	4.197	0.530	0.784
Aroma					
Tasters	2	0.392	0.236	0.880	0.419
Kernel mass	3	0.239	0.071	0.270	0.850
Tasters * KM	6	2.181	0.363	1.350	0.245
Oiliness					
Tasters	2	0.168	0.298	0.310	0.732
Kernel mass	3	3.928	1.320	1.390	0.252
Tasters * KM	6	4.452	0.742	0.780	0.587
Consistency					
Tasters	2	0.067	0.237	0.510	0.604
Kernel mass	3	2.851	0.979	2.10	0.107
Tasters * KM	6	1.904	0.317	0.680	0.665
Bitterness					
Tasters	2	0.974	0.227	0.210	0.811
Kernel mass	3	5.230	1.282	1.190	0.320
Tasters * KM	6	4.645	0.774	0.720	0.637
Wateriness					
Tasters	2	0.698	0.126	0.100	0.909
Kernel mass	3	3.909	1.083	0.860	0.463
Tasters * KM	6	2.995	0.499	0.400	0.878

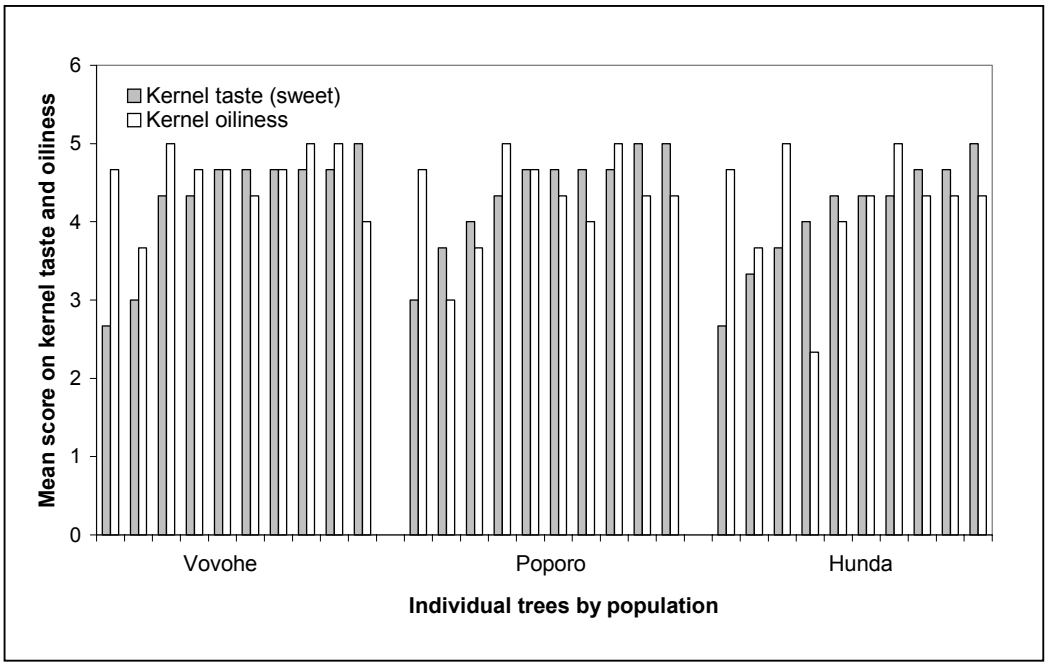


Fig 7.17: Tree-to-tree variation in kernel taste (sweetness) and oiliness of 30 *B. procera* trees in three different populations in Kolombangara Island, in the ascending order of taste score.

7.3.6 Multi-trait assessment to select a kernel ideotype of *B. procera*

The 'ideal tree' for cultivar development is not necessarily the one with the largest kernels as other characteristics are also important – i.e. taste and ease of kernel extraction. The ideal tree will partition its dry matter to kernel rather than to fruit, flesh or shell and have easily extracted, tasty kernels with oil characteristics meeting market specifications. Web-diagrams were formulated in order to visualise the multi-trait characteristic of different trees. Variations in the relative superiority and inferiority among the fruit and kernel traits between populations were considerable (Fig 7.18 to Fig 7.22), with Trees 5, 12, 2, 3 and 1 of Vovohe, Tututi, Rei, Poporo and Hunda respectively having the greatest kernel mass (Table 7.2). These web diagrams can be compared with an ideotype (Fig 7.23) that has the best combination of different traits.

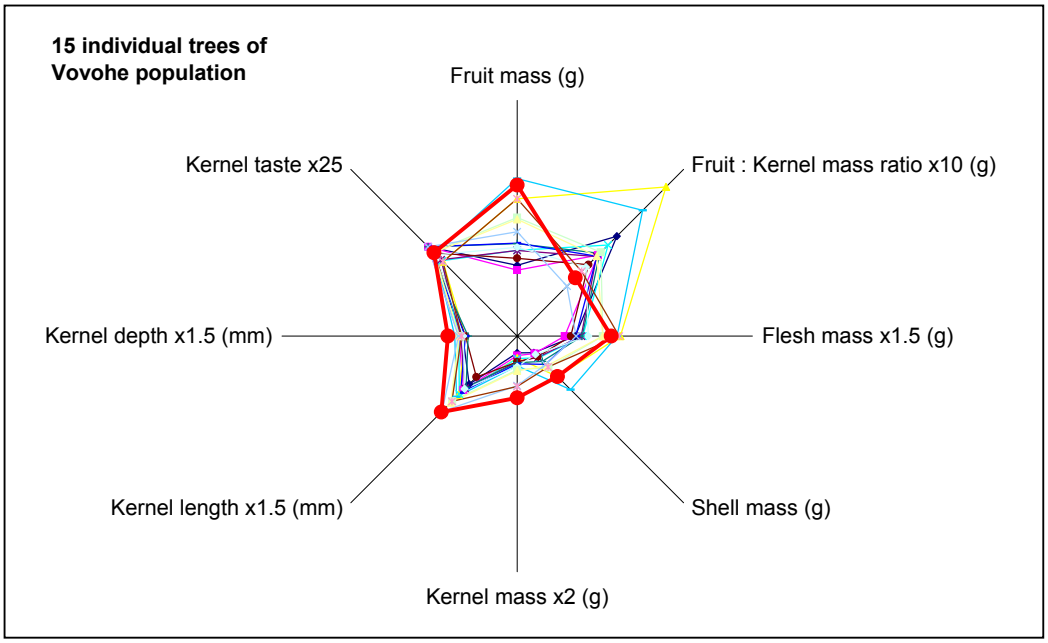


Fig 7.18: Web diagram showing tree-to-tree variation in fruit and kernel traits of the 15 trees of *B. procera* from Vovohe population. Tree 5 (bold red) is the best based on kernel mass.

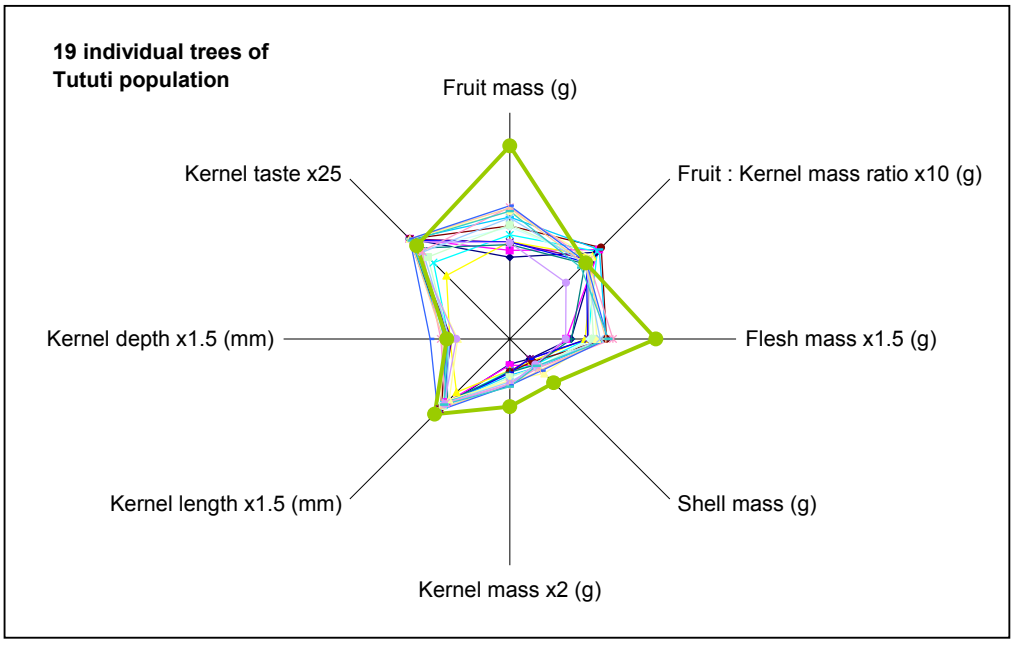


Fig 7.19: Web diagram showing tree-to-tree variation in fruit and kernel traits of the 19 trees of *B. procera* from Tututi population. Tree 12 (bold light green) is the best based on kernel mass.

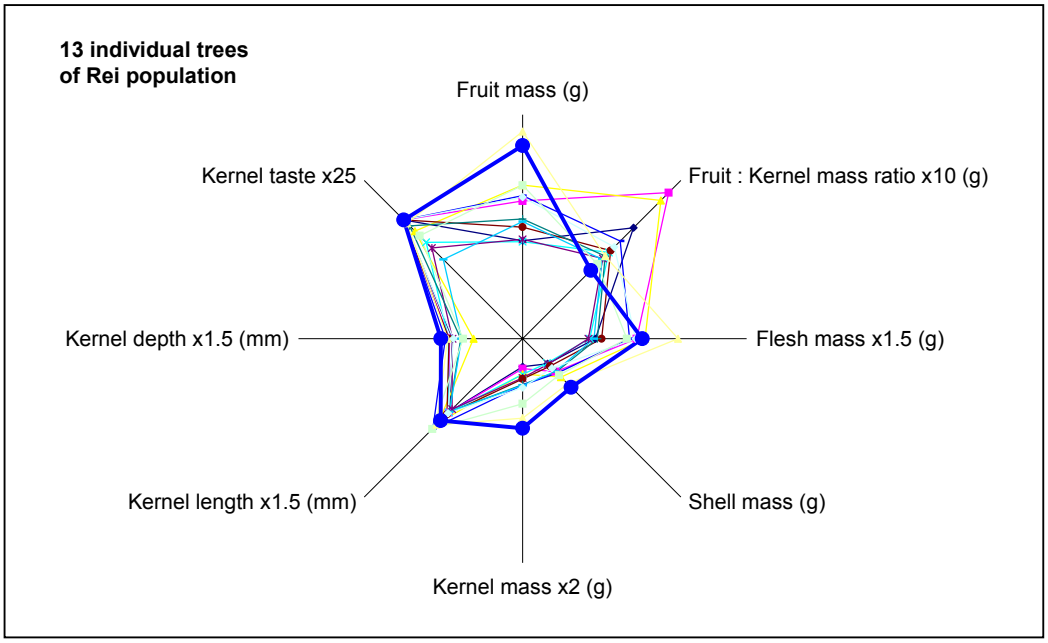


Fig 7.20: Web diagram showing tree-to-tree variation in fruit and kernel traits of the 13 trees of *B. procera* from Rei population. Tree 2 (bold blue) is the best based on kernel mass.

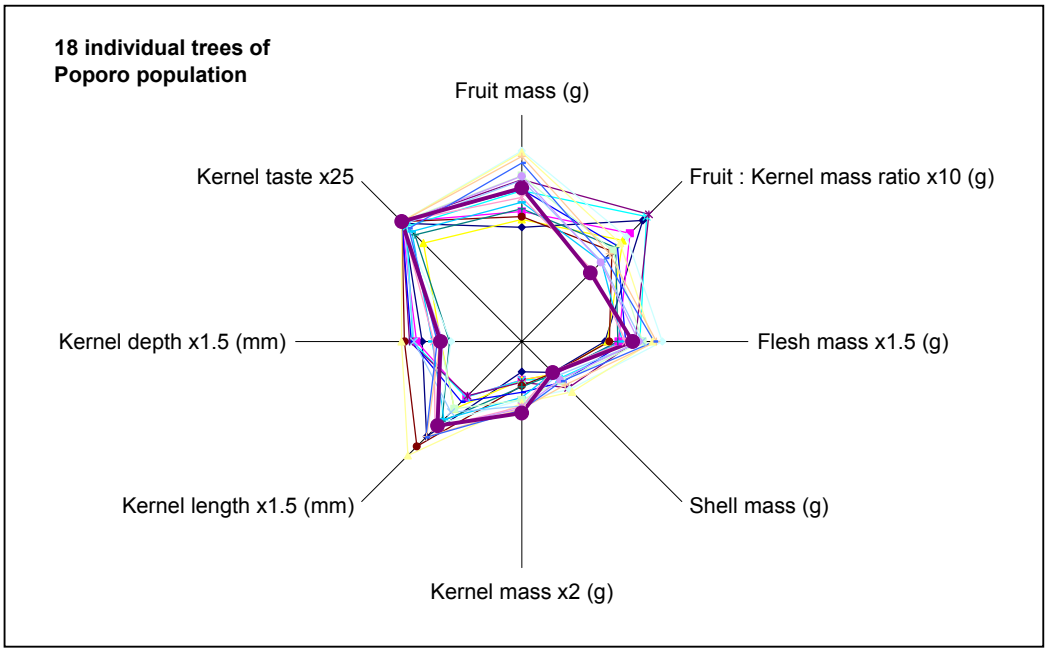


Fig 7.21: Web diagram showing tree-to-tree variation in fruit and kernel traits of the 18 trees of *B. procera* from Poporo population. Tree 3 (bold purple) is the best based on kernel mass.

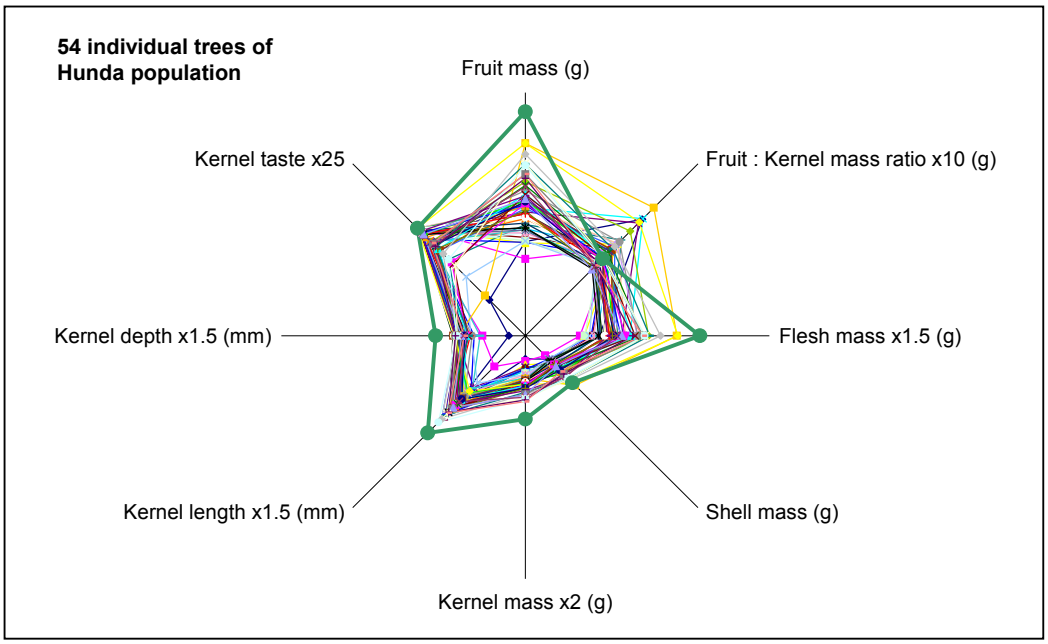


Fig 7. 22: Web diagram showing tree-to-tree variation in fruit and kernel traits of the 54 trees of *B. procera* from Hunda population. Tree 1 (bold green) is the best based on kernel mass.

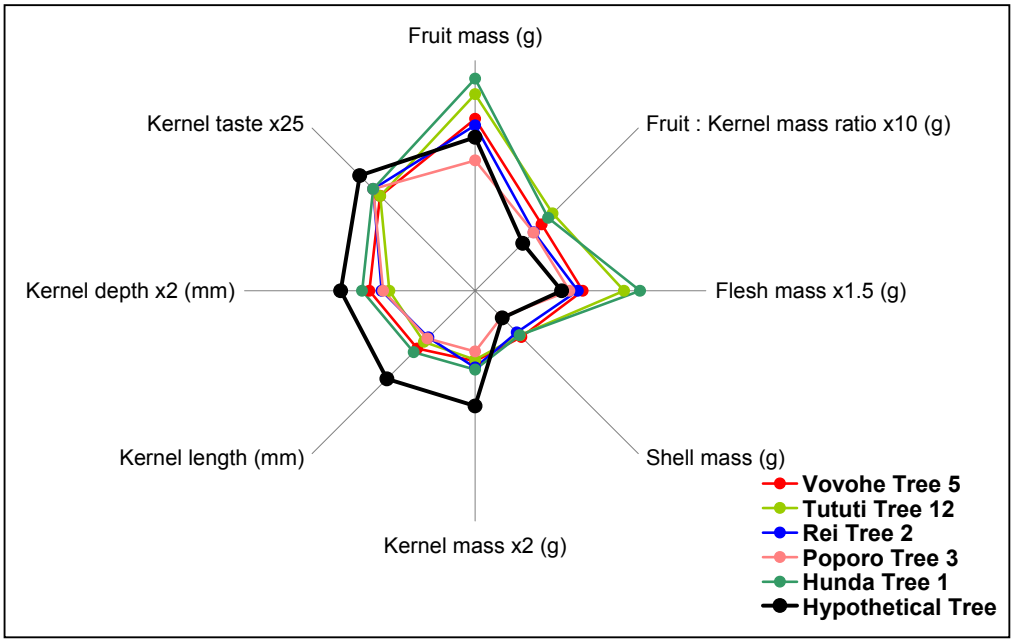


Fig 7.23: Web diagram showing kernel ideotype from the best 5 trees in each population compared to hypothetical tree for improved kernel ideotype (bold black).

7.4 DISCUSSION

Prior to this study, it was recognised that the kernel (seed) is the important product of *B. procera* for subsistence and sale, and thus the characterisation of kernel traits are essential for the selection and domestication of *B. procera* as an agroforestry tree. However, apart from Evans (1999) and Walter and Sam (2002), there is very limited literature describing morphological differences in fruit and kernel of *B. procera*. Moreover, there has never been any quantitative description of tree-to-tree variation of any of the important traits (e.g. mass, length, width, depth, colour and taste) of the fruit and the kernel. The present study is the first to provide such information. The concept of quantitative analysis of important traits of interest for domestication was based on (Leakey *et al.,* 2000) for *Irvingia gabonensis* and Waruhiu (1999) for *Dacryodes edulis*.

The present study showed considerable phenotypic variation in *B. procera*, in all the traits measured. This is typical for an outbreeding tree species and agrees with recent findings in other indigenous fruit and nut trees from Africa: *I. gabonensis* (Atangana *et al.,* 2001, 2002; Anegbeh *et al.,* 2003; Leakey *et al.,* 2005d) and *D. edulis* (Leakey *et al.,* 2002; Leakey *et al.,* 2004; Waruhiu *et al.,* 2004; Anegbeh *et al.,* 2005). The present study in *B. procera* found fruit mass and length ranged from 30-126g and 45-91mm respectively, while kernel mass and length ranged from 5-25g and 18-48mm respectively. This 2-5 fold variation is similar to that reported for *I. gabonensis* (Atangana *et al.,* 2001) and suggests that there is opportunity to select elite trees to multiply as cultivars (Leakey *et al.,* 2002). Essentially, the continuous variation found in the fruit and kernel traits of *B. procera* questions the validity of the recognition of "varieties" by farmer, suggesting that it is just their description of particular traits within the normal range of phenotypic variation. This study found that true distinct "varieties" based on genetic selection do not exit, and this is further supported by molecular data (Chapter 8).

This study, like that with *D.edulis*, *I. gabonesis* and *S. birrea* has indicated that in indigenous fruits and nuts the main sources of variation are between trees and

within a population. This is very beneficial to the development of participatory domestication as it means that villages are not in competition with each other to develop the best cultivars and that this strategy to domesticate will not lead (at least in the short to medium term) to a narrowing of genetic basis (the latter point is confirmed by the molecular study (Fig 8.11 and Fig 8.12).

The range and frequency of variation in tree sizes (height and diameter) is considerable. One reason contributing to this variation is farmers' practice of pollarding trees to a convenient height, mainly for safety around homes although some farmers have expressed gains in yield by doing it. The oldest and largest trees were found in Hunda, however, the integrity of tree age is subject to the information given by farmers.

The present study found close relationships between certain traits and not between others, as found by Atangana *et al.,* (2001; 2002) in *I. gabonensis*. For example, in *B. procera* the relationship between kernel mass and nut mass is strongly correlated ($r^2 = 0.75$), while kernel mass and fruit mass are more weakly related ($r^2 = 57$) indicating that kernel mass can be more accurately predicted by nut mass than by fruit mass, of 10.9%. It is clear therefore that to select trees with the best kernels it will be necessary to extract the kernels.

To develop the kernel "ideotype" for *B. procera* it is necessary to determine which are the important traits. Kernel weight (size) is obviously the most important traits for consideration, but the ease of kernel extraction has implications on the cost of extraction and the willingness of labourers to do the job. One measure of this may be shell mass, as thin shelled nuts are probably more brittle and less fibrous. Fruit : Kernel ratio assesses the best allocation of dry matter to the kernel. Maximising the partitioning of the dry matter to the harvestable product can be used to derive a "Harvest Index" (Cannell 1989). In addition, qualitative attributes of the kernel such as taste (sweetness, aroma, oiliness, consistency and wateriness) are also important considerations in developing a cultivar (Kengni *et al.,* 2001).

In *B. procera* the development of the ideotype is at an early stage and not yet involving a hierarchy of traits as described by Leakey and Page (2004) for African species. Future work should examine market preferences and economic value of different fruit characteristics – this will involve collecting data and linking between morphological traits and economic value of different traits. Nonetheless, this study has given the farmers the knowledge of what product they are looking for and on what criteria to base their selection.

The lack of significant differences between trees in the organoleptic assessment of kernel taste suggests that there is little variation in the organoleptic properties of the kernel both within and between populations, or that more precise methods are needed to assess kernel taste of *B. procera*. Farmers had the perception that some trees produce kernels tastier than others. Thus it appears that taste has already been a selection criterion over many years and has become a dominant trait, and so did not vary significantly between trees or populations. Nevertheless, in the future it may be necessary to re-evaluate taste in selected cultivars. The present study did not carry out chemical analysis of *B. procera* kernels, thus there is no evaluation of its potential as an ingredient in manufactured food. However, it would be important to undertake such research in the future, as experience in *Dacryodes edulis* (Mbofung *et al.,* 2002) indicates the commercial importance of expanding market opportunities for farmers. Variation in kernel oil content and composition may also become important in the future. Thus, further research is needed to ensure that the quality of improved product is meeting the needs of the consumers at all levels (Kengni *et al.,* 2001). This is an area in which the international food industry can contribute to the domestication process by indicating its priorities for genetic improvement (Leakey 1999), and especially outlining quality attributes required by the food industry for new products, which can influence the selection criteria for desirable ideotypes (Leakey *et al.,* 2005d).

Farmers have indicated that the value of kernels of *B. procera* sold in local markets within Kolombangara Islands and in Gizo, the capital centre of Western Province range from US$0.15 - US$0.30 per parcel of 20-24 halved kernels (Chapter 4). The present study did not quantitatively assess the relationship

between fruit or kernel traits and the market price. However, from observations in local markets, *B. procera* is sold in different units, commonly as heaps (of fruits) or parcels (of kernels). Heaps typically include ten to twelve fruits for about US$0.15, but this can vary depending on size, while parcels contain 20-24 halved kernels. The number of fruits sold in each selling unit is therefore similar, and differences in the selling price between fruit and kernel form represent the labour of extracting the kernels. So price of fruits and kernels is fixed per unit, but the quantity of fruits and kernels per unit can vary. In this species price per unit varied depending on quality and size.

The evidence that farmers have initiated their own domestication process by bringing wild nut species into cultivation illustrates the hypothesis proposed by Homma (1994) that man's utilization of natural resources follows a progression for exploitation to domestication. Leakey *et al.,* (2004) have taken this forward by using data from *D. edulis* and *I. gabonensis* and tested five statistically identifiable stages of domestication arising from truncated selection - these can be recognized by changes in the frequency distribution of a given trait from normality to positively skewed, back to normal with platykurtosis, to negatively skewed and back to normality. This study with *D. edulis* and *I. gabonensis* seemed to support the hypothesis. The results of the current study identified that fruit mass and fruit length were positively skewed at all five sites and that kernel mass was more strongly positively skewed at Rei and Tututi than at the other three sites. On the assumption that the Leakey *et al.,* (2004) hypothesis is valid, it appears that *B. procera* can be said to be in the second stage of domestication.

7.5 SUMMARY

The fact that significant intraspecific phenotypic variation was found both within and between populations in this study, suggests that considerable tree-to-tree variation (genetic diversity) is a feature of each population. This is important for the maintenance of genetic diversity in the domesticated population. Participatory domestication at the village level will therefore maintain considerable diversity at the national level, as each village will have a set of unrelated cultivars. However,

as phenotypic variation can be influenced by other environmental factors, it is necessary to validate this level of genetic diversity by a molecular method. The population differences of *B. procera* in Kolombangara Island is interesting as geographical distances (see Chapter 3) between populations are short. Future research should broaden to other islands within the country, with different geology, rainfall and agroecosystems. The next Chapter (Chapter 8) examines genetic diversity of *B. procera* in Kolombangara Island using molecular techniques.

CHAPTER 8: MOLECULAR STUDY OF VARIATION

8.1 INTRODUCTION

8.1.1 Rationale

Molecular techniques generate data that underpin the understanding of the genetic variation in species and populations. There are several reasons why molecular genetic markers are popular in ecological and evolutionary studies (Avise 1994). One of them is especially relevant to this study:- it is that these markers reveal specific genetic information about genetic diversity and the relatedness of individuals and genealogical relationships with morphological traits. For example, the level and distribution of genetic variation in 449 species from 165 genera were classified into eight ecological and life-history traits based on: taxonomic status, geographical range, regional distribution, life form, mode of reproduction, breeding system, seed dispersal and stage of succession (Table 8.1) (Hamrick *et al.,* 1991).

Genetic diversity describes inheritable variation found within populations. It is expressed in many different forms in an organism – e.g. a range of colours, sizes, height, etc. Genetic diversity is quantitatively expressed as the richness (measure of abundance) and the evenness (distribution of variation) of these different traits (Lowe *et al.,* 2004). Genetic diversity allows populations to adapt to environmental changes (Frankham *et al.,* 2004), and can be evaluated at the genomic (DNA), proteome (Protein), metabolomic (Metabolite), transcriptomic (RNA) or phenomic (Phenotype) levels (Henry 2005). The assessment of genotypic variation provides an understanding of the genetic basis of the phenotypic variation in plants (Henry 2005), quantifies the amount and distribution of genetic variation in populations and provides knowledge of processes that influence the patterns of genetic variation (Coates and Byrne 2005).

Variation in one or a small number of genes can be expressed as considerable morphological differences in plants (Henry 2005). Genes, which are a sequence of four nucleotides, code for different traits at different sites (loci) on a DNA molecule. Any slight differences in the nucleotide sequences result in genetic diversity, and are expressed as levels of polymorphism, heterozygosity and allelic diversity (Frankham *et al.,* 2004; Lowe *et al.,* 2004).

Table 8.1: Relationship between the characteristics of species and genetic diversity (Source: Hamrick *et al.*,1991).

A. At the species level

Characteristics	***Low genetic diversity***	High genetic diversity
Taxonomy status	Dicots	Monocots and gymmosperms
Life form	Short-lived perennials and annuals	Long-lived perennials
Geographical range	Endemic species	Widespread species
Regional distribution	Not significant	Not significant
Breeding system	Selfing, mixed mating, animal-pollinated species	Outcrossing species
Seed dispersal	Explosively dispersed seed	Animal-attached seed
Mode of reproduction	Not significant	Not significant
Successional status	Not significant	Not significant

B. At the population level

Characteristics	***Low genetic diversity***	High genetic diversity
Taxonomy status	Dicots	Monocots and gymmosperms
Life form	Short-lived perennials and annuals	Long-lived woody perennials
Geographical range	Endemic species	Widespread species
Regional distribution	Boreal-temperate species	Temperate and tropical species
Breeding system	Selfing species	Wind-pollinated species
Seed dispersal	Explosively dispersed seed	Animal-attached and wind-dispersed seed
Mode of reproduction	Not significant	Not significant
Successional status	Early successional species	Late successional species

C. Among populations

Characteristics	***Low genetic diversity***	High genetic diversity
Taxonomy status	Gymmosperms	Angiosperms
Life form	Long-lived woody perennials	Annuals
Geographical range	Not significant	Not significant
Regional distribution	Boreal-temperate species	Temperate and tropical species
Breeding system	Outcrossed wind-pollinated species	Selfing
Seed dispersal	Gravity-dispersed and animal attached seed	Gravity-dispersed seed
Mode of reproduction	Not significant	Not significant
Successional status	Late successional species	Early and midsuccessional species

8.1.2 Species concepts in *Barringtonia*

In this study of the domestication of *Barringtonia procera*, one serious problem identified in the field work was the possible misidentification of the species due to the overlapping variation in the morphological traits that were used to differentiate *B. procera* from the other two related species (*B. novae-hiberniae* and *B. edulis*) (Fig 8.1). Consequently, this study uses molecular techniques to test the integrity of *B. procera* and assess the validity of field identifications. In addition, this study examines the level of genetic diversity within and between the sampled *Barringtonia* populations. This study goes on to compare the molecular analysis with the intra- and inter-population genetic variation found in different morphological traits of fruits and kernels reported in Chapter 7.

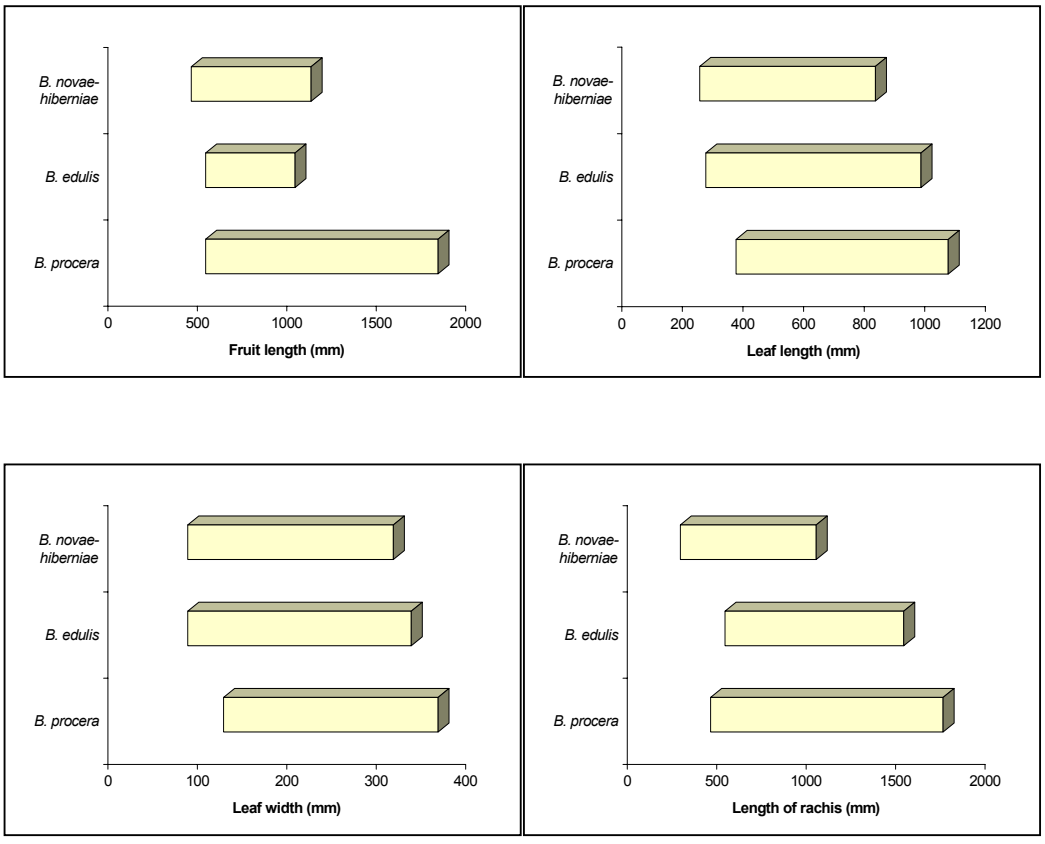


Fig 8.1: Overlap of variation of systematically important morphological traits between three *Barringtonia* species as described by Walter and Sam (2001).

8.1.2. DNA analysis

8.1.2.1 Sampling

It is important to match the sampling strategy to the purpose of the analysis because populations undergo various evolutionary processes and differ in many ways, such as population size, rate of migration, gene flow and heterogeneity (Marshall and Brown 1975; Mariette *et al.,* 2002; Cavers *et al.,* 2005). When sampling natural populations for their genetic variation, the sampling strategy should fit the question. In the present study, the purpose was to relate genetic information to morphological characteristics and test species concepts, therefore, the same trees as were sampled for the morphological study were used for DNA sampling.

In this study leaf tissues were collected from 176 trees, of which 76 trees were the same as were sampled for morphological study in Chapter 7. Typically, leaf tissue is used when sampling plants for molecular analysis (Cavers *et al., unpublished*) because they are abundant. However, collecting leaf tissue can be difficult in trees due to their height. In addition, the abundance of defensive chemicals and the secondary metabolites in leaf tissue can be high because of the need in long-lived perennials for protection from insect attack. These metabolites can inhibit DNA extraction and subsequent PCR applications. Therefore, extracting DNA from cambium tissue has been suggested as another option (Cavers *et al., unpublished*). Silica gel is used to dehydrate leaf samples collected in the field and needs to be replaced regularly for complete drying, because ill dried leaf samples can result in DNA being fragmented and difficult to quantify.

8.1.2.2 Molecular techniques for measuring genetic variation

The use of molecular markers to assess the extent of genetic variation in plant populations is a relatively recent development (Parker *et al.,* 1998). In plant domestication, molecular markers have been used in a number of ways, for example, (i) to confirm the mode of reproduction of *Hypericum perforatum* between apomixis, self-pollination, haploid parthenogenesis and cross-fertilisation (Arnholdt-Schmitt 2000), (ii) to identify contrasting ecotypes (wet and dry

populations) of *Cedrela odorata,* as the basis of a conservation strategy to protect loss of genetic diversity (Cavers *et al.,* 2003), and (iii) to assess the impact of tree domestication on genetic diversity in *Inga edulis* in the Peruvian Amazon (Hollingsworth *et al.,* 2005). These examples demonstrate the range of uses for molecular techniques in the study of genetic variation in plants, for the purpose of conserving and developing techniques for the sustainable use of plant genetic resources.

There are a number of molecular techniques available to measure genetic diversity:- protein immunology, micro satellites, protein electrophoresis, DNA-DNA hybridisation, restriction analyses and polymerase chain reaction (Avise 2004). These techniques each have their advantages and disadvantages, depending on the research question, the genetic resolution required, funding constraints and technical expertise available (Marshall and Brown 1975; Mueller and Wolfenbarger 1999).

The use of DNA fingerprinting and genotyping techniques are generally based on the two latter techniques named above – i.e. involves "classical, hybridisation-based fingerprinting," and "Polymerase Chain Reaction" (PCR) based fingerprinting strategies. In the former, genomic DNA is cut into fragments with restriction endonucleases and the DNA fragments are then electrophoretically separated (Botstein *et al.,* 1980; Tanksley *et al.,* 1989; Jeffreys *et al.,* 1991). The Restriction Fragment Length Polymorphisms (RFLPs) technique is in this category. By comparison, the PCR strategy involves *in vitro* amplification of particular DNA sequences using specific or arbitrary primers and a thermostable polymerase. Techniques in this category are RAPD and Arbitrarily Primed-PCR. The amplified fragments length polymorphism (AFLP) technique combines both these strategies (see section 8.1.2.3). When amplifying genes in the PCR reaction, it is necessary to have sufficient high quality DNA starting template for sequencing or genotyping as insufficient high quality genome would produce fewer fragments and simple banding pattern (Invitrogen Life Technologies 2003).

PCR involves three steps: denaturation, annealing and extension. The double helix DNA is denatured by heating to 94^{o}C. The heat melts open the double helix DNA into single strands, and stops all enzymatic reactions. During annealing or binding process at 54^{o}C, the primers (short, usually 16-25 nucleotides, single-stranded sequence) move around by Brownian motion, and form bonds with the single stranded template. Some bonds are stronger than others and those that are stable remain bound and the polymerase can attach and begin copying the template. In the extension phase of the PCR reaction, usually around 72^{o}C, bases (Adenosine (A), Guanosine (G), Thymidine (T), Cytidine (C)) are coupled onto the end of the primer and strands are synthesised using a thermostable DNA polymerase (taq). These three steps are programmed in an automated thermocycler and repeated 20 or more times consecutively (Avise 1994). The amplification products are distinguished by electrophoresis (Invitrogen Life Technologies 2003).

PCR is being used increasingly to generate molecular data because PCR derived markers generally yield higher levels of polymorphism than allozyme techniques (Nybom 2004), which is a protein based electrophoresis, that separates protein variants according to their mobility in an electric field (Beebee and Rowe 2004). Moreover, the popularity of the PCR-based markers such as random amplified polymorphic DNA (RAPD) (Williams *et al.,* 1990), inter-simple sequence repeat (ISSR) (Zietkiewicz *et al.,* 1994) and amplified fragment-length polymorphism (AFLP) (Vos *et al.,* 1995) is due to the lack of species specificity in the primers. Consequently, these methods are suitable for studying a broad range of species, including *Barringtonia procera* that have had not been previously subjected to molecular genetics research (Nybom and Bartish 2000; Nybom 2004; Bussell *et al.,* 2005). Differences between these molecular markers and their particular advantages have been reviewed by Bussell *et al.,* (2005). For example, in terms of the potential size of bands: RAPD = 200-2000bp, ISSR = 500-4000bp and AFLP = 50-500bp. This large range of band size provides greater capacity for matching bases per primer binding site.

8.1.2.3 Application of AFLP marker to evaluate genetic variation

During genotyping using AFLP, samples are compared to identify the common and different bands – these differences reflect DNA polymorphisms. AFLP markers are useful for assessing genetic differences among individuals, populations and independently evolving lineages, such as a species. AFLP is also useful to study systematics, pathotyping and biodiversity surveys, population conservation genetics, fingerprinting and kinship and mapping of genes affecting quantitative variation (QTL mapping) (Mueller and Wolfenbarger 1999). The technique is relatively cheap, easy, and reliable (Vos *et al.,* 1995; Mueller and Wolfenbarger 1999). However, the drawback of this technique is the inability to distinguish heterozygote genotypes from homozygote genotypes. This limits its use in population genetic studies (Mueller and Wolfenbarger 1999), but is considered not critical to achieve the objectives of this study in *B. procera*. AFLP is more reproducible than RAPD but is also more expensive. Automated techniques for the scoring of markers have been developed for AFLP (Vos *et al.,* 1995; Newton *et al.,* 1999).

While the AFLP technique is relatively new, the above mentioned advantages make its application in molecular studies increasingly popular. AFLP has been applied widely to a range of agricultural (Kashkush *et al.,* 2001; Giancola *et al.,* 2002) and tropical timber species (Krauss 2000; Ribeiro *et al.,* 2002; Cavers *et al.,* 2003; Lowe *et al.,* 2003). For these reasons, AFLP was chosen in this study to determine the genetic diversity within and among populations of *Barringtonia* species in Kolombangara Island.

AFLP is a technique for fingerprinting or genotyping DNA, by rapidly generating hundreds of highly reproducible markers from DNA with a high genetic resolution. It has the capacity to simultaneously screen many different DNA regions that are randomly spread across the entire genome (Mueller and Wolfenbarger 1999). AFLP aims to selectively amplify a subset of restriction fragments from the main fragments of genomic DNA (Avise 1994) using 4 main steps (Fig 8.2). Total genomic DNA is digested with two restriction enzymes

(endonucleases): an infrequent cutter (e.g. *Eco*R 1 with a 6bp recognition sites) and a frequent cutter (e.g. *Mse* 1 a with 4bp sites) (Bussell *et al.,* 2005). DNA adaptors are fitted to ligate the ends (site) of the DNA fragments (Vos *et al.,* 1995).

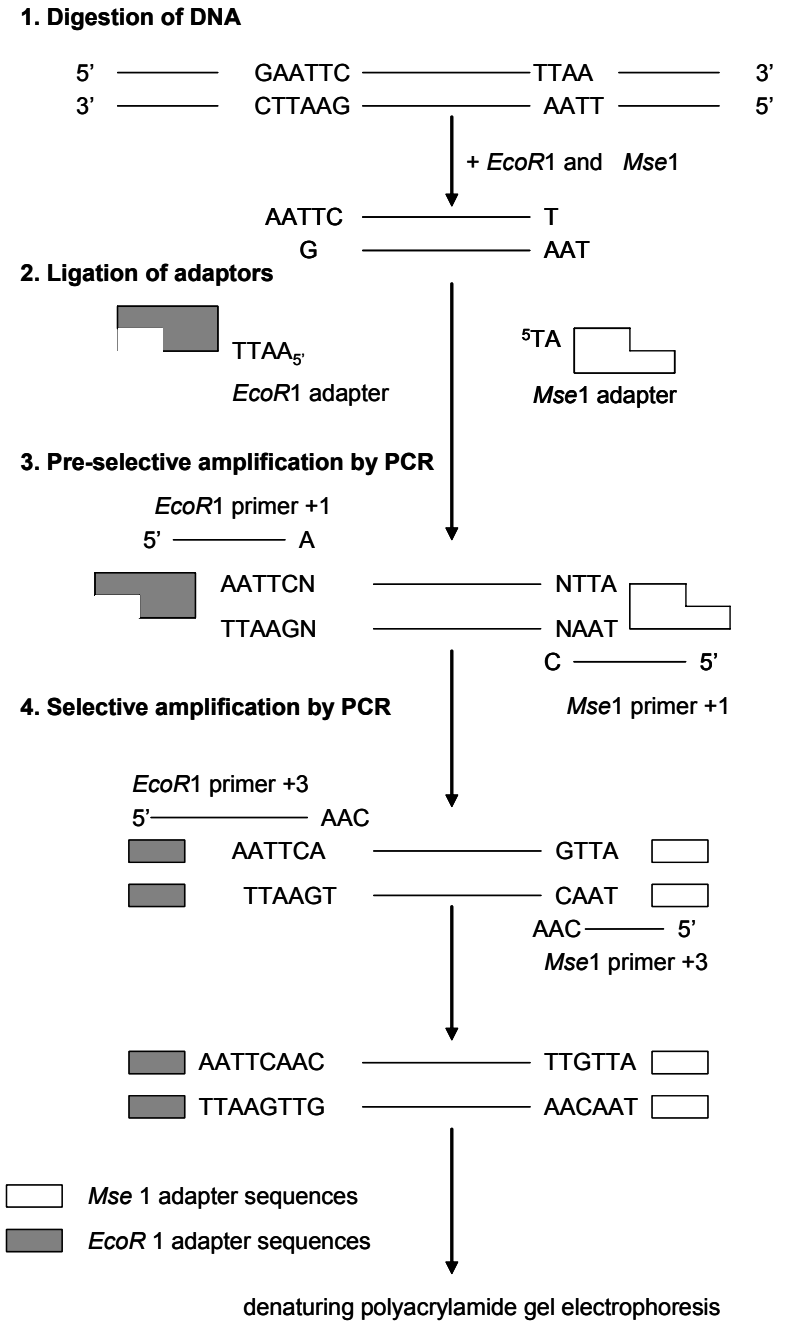


Fig 8.2: Procedures of the AFLP using one primer pair. Source: Invitrogen™ life technologies, Instruction Manual Version B (2003).

Use of the primers *Eco* R1 and *Mse* 1 for each primer pair combination (+3/+3) in AFLP usually produces 50 products (fragments), with a size range of 50-500bp (Hedren *et al.,* 2001; Vekemans *et al.,* 2002). The products are selectively amplified using PCR primers (Vos *et al.,* 1995; Newton *et al.,* 1999). In particular, the advantage of AFLP over RAPD is that it is highly reproducible and is robust to variation in DNA concentration (Lin and Kuo 1995; Jones *et al.,* 1998). It uses longer primers, accurate anchoring sequences and requires higher annealing temperature (touchdown at 70^{o}C-60^{o}C). The occurrence in AFLP of primer template mismatching (Okano *et al.,* 1998) is rare (O'Hanlon and Peakall 2000) and errors are generally the result of misinterpretation of the data and not errors inherited from the reaction or resolution of the product (Hansen *et al.,* 1999).

The specific objectives of the current study are:-

- To test the validity of field identifications and determine whether *Barringtonia procera* is genetically different from *B. novae-hiberniae* and *B. edulis*.
- To determine the level of genetic diversity and population structure of *Barringtonia procera (sensu latissimo).*
- To evaluate the genetic basis of fruit and kernel traits of *Barringtonia* populations.

8.2 MATERIALS AND METHODS

8.2.1 Plant materials

In December 2004, leaf samples for molecular analysis were collected from 176 *Barringtonia* trees, selected at random from 5 populations (Vovohe, Tutuni, Rei, Poporo and Hunda) on Kolombangara Island. Of these, 26 were identified in the field as *B. edulis*, 21 as *B. novae-hiberniae* and 129 as *B. procera*. Seventy-six of these trees, mainly *B. procera* were among the trees that had also been previously chosen and used for the characterization study (Chapter 7). The other 100 individuals were randomly chosen to represent each species and to increase the

sampling size. Because of the identification difficulties – the classification of these trees is tentative and they will be termed as 'notional species' in this study. These species identifications were made recognising that there is overlapping variation of morphological traits between these 3 'notional species' (Fig 8.1), and that this makes identification difficult. There was an unequal number of trees in each population (Table 8.2) depending on the abundance and access to the trees.

Table 8.2: Number of trees sampled from each 'notional species' in each population

Population	*B. procera*	*B. edulis*	*B. novae-hiberniae*
Vovohe	25	1	7
Tututi	33	6	3
Rei	15	8	8
Poporo	20	5	1
Hunda	36	6	2
Total number of trees	**129**	**26**	**21**

8.2.2 Sampling and storage methodology

Leaf samples were collected from 1-2 young leaves on lower branches of mature trees. The leaves were torn into small pieces and then stored in sealed plastic bags containing a small quantity of 100% silica gel to dry the leaves. The silica gel was replaced every 12 hours for the first 48 hours and, thereafter, replaced as and when it changed colour from blue to pinkish white, indicating that the gel is saturated. These dried leaf samples were brought back to James Cook University, and were stored for 3 months in a refrigerator at $4^{o}C$ before laboratory analysis. The analysis of these foliar samples was undertaken at the molecular laboratory of the School of Integrative Biology of the University of Queensland in Brisbane, Australia.

8.2.3 DNA extraction

DNA was extracted using a CTAB based protocol modified from Scott and Playford (1996) (see Appendix 8.1). The leaf material (about $1cm^2$) was ground using a Retsch Mill MM300. One ml of an extraction buffer (see Appendix 8.2) was added and mixed for 15 minutes using Retsch Mill MM300 at 30Hz. Extracts

were centrifuged at 6000 rpm for 10 minutes at room temperature. The supernatant was transferred to a new plate and then 150µl of a wash buffer (see Appendix 8.2) was added to the mixed solution and spined for 15 seconds at 30Hz. Then, 40µl of 5% Sarkosyl was added and the plates were inverted several times to mix the solutions. The mixture were then agitated at 600rpm at room temperature for 15 minutes and centrifuged for 1 minute at 2500rpm at room temperature. 400µl of CTAB buffer (see Appendix 8.2) was then added. The mixture was shaken at 600rpm for 30 minutes at 55^{o}C using an "environshaker". This was followed by centrifugation at 6000rpm for 10 minutes at room temperature. The supernatant was then transferred to a new plate and mixed with 800µl of Chloroform: Iso-Amyl (24:1) and then centrifuged at 4000rpm for 5 minutes at room temperature. Approximately 400µl of supernatant was then carefully transferred to a new plate, using the Corbett Research Robot and mixed with 40µl of ammonium acetate (7.5M) and 400µl of ice cold 100% ethanol. The solution was then manually mixed by gently shaking the plates sideways. It was then allowed to precipitate in the freezer at -20^{o}C for 15 minutes. Following precipitation, the solution was then centrifuged at 4000rpm for 15 minutes at room temperature. The supernatant was then emptied and the DNA dried in Speedivac at 65^{o}C for 15 minutes before being re-suspended in 35µl MQ H_2O.

8.2.4 AFLP procedures

The extracted *Barringtonia* DNA concentration of 100ng was used for the AFPL analysis, which was performed as per Invitrogen Instruction Manual (2003) modified by Scott *et al.,* (see Appendix 8.3). Selective amplification was achieved using primers that annealed to either the *Eco*R1 or *Mse*1 end of a restriction fragment and had three additional 3-4' nucleotides. The *Eco*R1 primers are referred to as Eco+2 and the *Mse*1 as Mse+3-4. The sequences of the *Eco*+2 primers were 5'- GACTGCGTACCAATTCXX-3' (where XX is either AC or CC). The sequences of the *Mse*+3-4 primers were 5'-GATGAGTCCTGAGTAAXXXX-3' (where XXXX is CAG, ACAA, ACAG or GACC). The list of the four primer combinations used in this study is given in Table 8.3.

Table 8.3: Primer-enzyme combinations used for selective amplification

Primer-enzyme combination	Eco+3 primer (5'-3')	*Mse+3 primer (5'-3')*
1	GACTGCGTACCAATT/AC	GATGAGTCCTGAGTAA/CAG
2	GACTGCGTACCAATT/CC	GATGAGTCCTGAGTAA/ACAA
3	GACTGCGTACCAATT/CC	GATGAGTCCTGAGTAA/ACAG
4	GACTGCGTACCAATT/CC	GATGAGTCCTGAGTAA/GACC

Procedures used in the AFLP analysis are:-

Step 1: Restriction and ligation of DNA template: Total genomic DNA was digested with two restriction endonucleases (enzymes): *Eco*RI (Ammersham) and *Mse*I (NEB #R0525S) (Fig 8.2). First a restriction master mix was set-up (total volume = 1070µl) (see Appendix 8.3 for chemicals and reagents). 100ng of high purity *Barringtonia* DNA was then added to the master mix, and then incubated at 37^{o}C for 60 minutes to inactivate the restricted enzyme and the genomic DNA fragments. The *Eco*RI and *Mse*I adaptors were denatured at 94^{o}C for 2 minutes and transferred to ice to stabilise. The second step in this reaction involved setting up a ligation master mix (total volume = 555.9µl) (see Appendix 8.3 for chemicals and reagents). 2.5µl of ligation mixture was then added to the restriction reactions and incubated at room temperature for 3 hours. The restriction ligation (R/L) reaction was checked for restriction by visualising in agarose gel. The reaction was then diluted to produce a working R/L stock at concentration: 15µl R/L + 5µl H_2O for 100ng DNA.

Step 2: Amplification of the restricted DNA fragments: This is done through PCR in two consecutive reactions. The first reaction is called pre-selective amplification, and it involves amplification of genomic DNA with AFLP primers with one selective nucleotide. The second reaction, called selective amplification, uses diluted PCR products of the pre-selective amplification reaction as a template for the selective amplification using two AFLP primers, each consisting of three selective nucleotides.

For pre-selective amplification: First, a pre-selective amplification master mix (total volume = 173.8µl) was set up (see Appendix 8.3 for chemicals and reagents). 3µl of the R/L mix was then added to the pre-selective amplification master mix before the mixture was amplified through PCR reaction at different temperatures and times for 31 cycles (see Appendix 8.3). Four primer-enzyme combinations were used (Table 8.3). 5µl of the pre-selective amplification product was loaded with 3µl loading dye in the agarose gel to visualise the level of polymorphism in the product. The pre-selective amplification product (i.e. remaining 15µl) was diluted by adding 210µl of $TE_{0.1}$ for the next step.

For selective amplification: The final selective amplification master mix (total volume = 136µl) was first set-up (see Appendix 8.3 for chemicals and reagents). 5µl of the diluted pre-selective amplification DNA was added to the final selective amplification master mix. The mixture was then amplified through PCR reaction at different temperatures, and times in 39 cycles (see Appendix 8.3). Four primer-enzyme combinations were used (Table 8.3). The amplification product was visualised for polymorphism in GS2000.

Step 3: Gel analysis of the amplified fragments (Electrophoresis): In electrophoresis, the amplification products migrated through the gel by electric conductivity. Products with high molecular weight are slower than those with lower molecular weight. The movement of the products is shown as bands at different positions in the gel. Two types of gels were prepared and used for electrophoresis process in AFLP and were known as DNA grade Agarose and polyacrylamide gels.

In AFLP, the DNA grade Agarose was run to determine the presence of DNA in the extraction sample. 5g of the DNA grade Agarose (Omnigel from Edwards Instruments) was dissolved in 250ml of 1 x TBE (10.8g Tris, 5.5g boric acid, 4ml 500mM EDTA in 1.0l of distilled water) and heated in a microwave for about 3 minutes. Immediately after dissolving, the solution was then mixed with 2µl of ethidium bromide (EtBr) (for DNA quantification) under airflow cabinet, before it was cast into a gel tray mounted with 18-wells comb, and kept under the airflow

cabinet to solidify for about 15 minutes. The 18-wells comb was then dismounted and the gel was cast in an electrophoresis tank (BioRad SubCellGT), and immersed in 1x TBE solution. The DNA extract was then loaded into individual wells with loading dye at concentration ratio of 3µl (dye): 5µl (DNA). The electrophoresis tank was connected to 200V (BioRad Powerpac 300) and was run for 20 minutes to separate the amplified fragments by molecular weight. The presence of DNA in the extract was viewed in the gel, after electrophoresis, on an ultraviolet transilluminator (Vilber Lourmat), and recorded as a digital TIFF image.

The purpose of polyacrylamide gel in AFLP analysis is to determine the amplification fragments of PCR reactions, and the gel was run in GS2000. Procedures for gel preparation being followed in this study were adapted from Corbett Research's Gel-Scan 2000 DNA Fragment Analysis Operators Manual (Version 2) (appendix 8.4 and 8.5). The gel was prepared by adding 15ml of 5% DeNature polyacrylamide gel mix (42g Urea, 6ml 10 x TBE (Amresco), 12.5ml 40% acryl.BIS-acryl (19:1) and 81.5ml MQH_2O) to 10% APS (0.1g ammonium persulphate and 1.0ml deionised water) and 6-10µl Temed. The mix resulted in Polymerising polyacrylamide gel which was then applied onto plates. Fifty-two wells comb was then firmly inserted into the gel to create loading channels or wells. The gel was placed into GS2000, and bolted firmly with the indented frosted glass plate at the front. The bottom of the buffer tank was filled with 1.0l of 0.6 x TBE. The gel was run in GS2000 for 2-3 hours depending on the variability of the primer enzyme combinations (PECs) used.

The resultant banding pattern (genotyping) was viewed in the GS2000 for amplification products measured for their molecular weight. The bands were scored manually as either presence (1) or absence (0) and recorded in a binary data matrix for statistical analyses (Section 8.3.5). The concentration of DNA per individual sample (extract) was estimated through comparison with a known genomic DNA standard (MegaBACE ET900-R SIZE Standard) ranging in length from 60 to 900 base-pairs.

8.2.5 Data analysis

8.2.5.1 AFLP analysis

The samples collected were identified as 3 'notional species' of edible *Barringtonia* (*B. procera*, *B. edulis* and *B. novae-hiberniae*). Because identification errors may have occurred, two approaches were taken when analysing the data:-

a) To maintain the field identification as "notional" species for comparative purposes in order to test for the validity of field identification and determine the integrity of the three species.

b) To treat the whole dataset as *B. procera* (*sensu latissimo*)

A binary data matrix was generated by scoring the bands as 'present' or 'absent'. This is the basis of the analysis to determine the degree of differentiation and genetic diversity within and between populations of the species. Only fragments that were unambiguous and polymorphic were scored.

8.2.5.2 Testing the validity of field identification and integrity of the species

To validate field identification and the integrity of the species, non-metric multidimensional scaling (NMDS) was used, because it ordinates individuals based on their genetic similarity (see below). Thus, if field identification is accurate, 3 discrete groups or clusters are expected. The raw binary data matrices were analyzed using NTSYS-pc 2.02i computer software (Rohlf 1998). First, binary data was calculated for similarity coefficients between all pairs of individuals using the Jaccard coefficient (Sneath and Sokal 1973):

$$Sij = \frac{a}{(a+b+c)}$$

Where; S = the measure of similarity between individuals i and j
a = the number of DNA fragments common to both i and j

b = the number of bands present in i but absent in j
c = the number of bands absent in i but present in j.

If S = 0, means samples have no fragments in common, and
S = 1, means identical AFLP profiles exist between two samples.

The Jaccard coefficient was defined as the number of (matched) bands common to both individuals, divided by the total number of comparisons, excluding (0,0) matches (Clifford and Williams 1976). This study used the Jaccard coefficient because it was considered appropriate for dominant markers such as AFLP, which do not provide information about the number of alleles at a single locus (see expected Heterozygosity below). Thus, the absence of a band from any two genotypes does not necessarily imply that there is similarity between them at this locus.

The ordination of individuals is visualized graphically through a non-metric multidimensional scaling (NMDS). NMDS is an ordination procedure that finds the positions of all individuals in a reduced space, while minimizing the effect on the similarity coefficients between each individual that are provided in the input Jaccard similarity matrix. This procedure generated a random configuration of points. Consequently, the difference between the random, and the original distances between all pairs can be calculated. The process is iterative using a steepest descent algorithm to minimize stress. Stress is a measure of the departure from a fit, and it is the difference between the distances in the arbitrary configuration space and those in the original multi-dimensional space. Kruskal (1964) and Rohlf (1998) reported that a stress value of less than 0.1 indicates a good representation of the true distances in the original data set.

$$Stress1 = \sqrt{\frac{\sum (\dot{d}ij - \hat{d}ij)^2}{\sum \dot{d}ij^2}}$$

Where d^*ij = the initial configuration distances between all pairs of ij points, which are computed and compared with the original distances, d ij, to generate a monotone function d ij between the two variables.

8.2.5.3 Evaluating the population genetic structure of the species

Two measures of genetic diversity used were: percentage of polymorphic loci and expected heterozygosity.

a) *Percentage of polymorphic loci:* When the frequency of the most common allele is more than 0.95, then a locus is said to be polymorphic (Lowe *et al.,* 2004).

b) *Expected heterozygosity:* AFLP is a dominant marker therefore cannot differentiate heterozygous alleles from homozygous ones present in one locus, therefore is not possible to estimate allele frequencies or heterozygosities directly. Thus, a number of assumptions are made to calculate expected heterozygosity (H_e) in dominant binary data. Nei's (1987) unbiased measure of H_e is one of them and is used in this study because it gives a good estimate of genetic diversity, and considers the systematic bias that a small sample size can cause. The statistical software package Arlequin version 2.0 (Schneider *et al.,* 2000) was used to calculate percentage of polymorphic loci and Nei's (1987) unbiased measure of expected heterozygosity for each *B. procera* population.

To examine the extent of genetic differentiation of populations, this study uses classification and ordination of AFLP fragments, the analysis of molecular variation (AMOVA), and the analysis of principal coordinates. The relationship between the genetic and geographical distances was also examined. These are described below:

a) *Classification and ordination:* The raw binary data matrices were analysed using NTSYS-pc 2.02i computer software (Rohlf 1998). Procedures involved are described above (see section 8.3.5.2). Two techniques of pattern analysis were used: hierarchical classification and ordination, both of which can be visualized graphically through a cluster analysis and non-metric multidimensional scaling. To classify individuals, the similarity coefficients were subjected to the UPGMA (unweighted pair-group method

of arithmetic means). This method is used widely as it minimises differences between the input and output distances unless evolutionary rates along branches are significantly different (Hartl and Clark 1989). A dendrogram of the clustering was constructed using the SAHN procedure, and based on the individual's similarity coefficients, in which the accuracy of the fit of data to the dendrogram was determined by calculating the correlation between the phenetic matrix and the original similarity matrix. The ordination of individuals using the non-metric multidimensional scaling (NMDS) was described above (see section 8.3.5.2).

b) *Analysis of molecular variation (AMOVA):* The level of differentiation within and between 5 populations was determined through the analysis of molecular variance (AMOVA) using multi-platform version of Arlequin (Alequin ver. 2.000) (Schneider *et al.,* 2000), which is based on Excoffier *et al.,* (1992). The analysis is based on the frequency of genes, but also taking into account the number of mutations between molecular haplotypes. The analysis recognises that as the populations studied are defined, a particular genetic structure require for testing is also defined. Pairwise comparisons between each of the 5 populations for the levels of genetic divergence were also undertaken. The significance of each variance component was tested using non-parametric permutations (Excoffier *et al.,* 1992).

c) *Principal coordinates analysis:* The genetic affinity of individual trees was also determined by using principal coordinates analysis (PCA). This is a multivariate technique which permits plotting of major variation patterns within a multivariate dataset (e.g. multiple loci), such as produced by AFLP. PCA has axes of variation that are located within multidimensional dataset, and each axis explains proportionately less of the total variation. The greater proportion of variation was shown by the first 2-3 axes. The analysis was done using GenAlEx6 (Peakall and Smouse 2005).

d) *Test for isolation-by-distance:* The Mantel test was undertaken, using GenAlEx6 (Peakall and Smouse 2005) to determine correlation between Jacaard similarity genetic distances and geographical distances of the studied populations. The *p*-value cannot be used to determine the significance of the relationship because the N x (N-1) (where N = individuals) elements within each matrix are not independent (Peakall and Smouse 2005). So, the test for significance was assessed through a correlation coefficient (ranging from -1 to +1) for the two data matrices – the closer the observed value is to -1 or +1 than the generated value by random permutation, the significant is the relationship (Peakall and Smouse 2005). The distances between sites were short and sites are generally geographically similar, thus it was expected that the relationship would not be significant. This analysis was conducted to test this hypothesis.

8.2.5.4 Assessing genetic implications in fruit and kernel traits of the species

Seventy six of the 176 trees across five populations (Vovohe = 11, Tututi = 12, Rei = 10, Poporo = 17 and Hunda = 26) were identified as *B. procera* (*sensu lat.*) in the field were sampled for molecular analysis. These same trees had been previously used in the fruit and kernel characterisation study (Chapter 7). Consequently, the two techniques can be compared in order to understand the genetic implications of fruit and kernel traits in this species. The raw binary data matrices were analysed using NTSYS-pc 2.02i computer software (Rohlf 1998). Procedures involved are similar to that described above (see section 8.3.5.2). The classification of individuals is visualized graphically through a cluster analysis, also similar to that described above (see section 8.3.5.3(a)).

8.3 RESULTS

8.3.1 AFLP analysis

The four-primer enzyme combinations that were screened produced a total of 282 bands in 171 individuals (Fig 8.3). Five individuals did not produce bands or were

not reproducible in all primer-enzyme combinations, thus were omitted from the analysis.

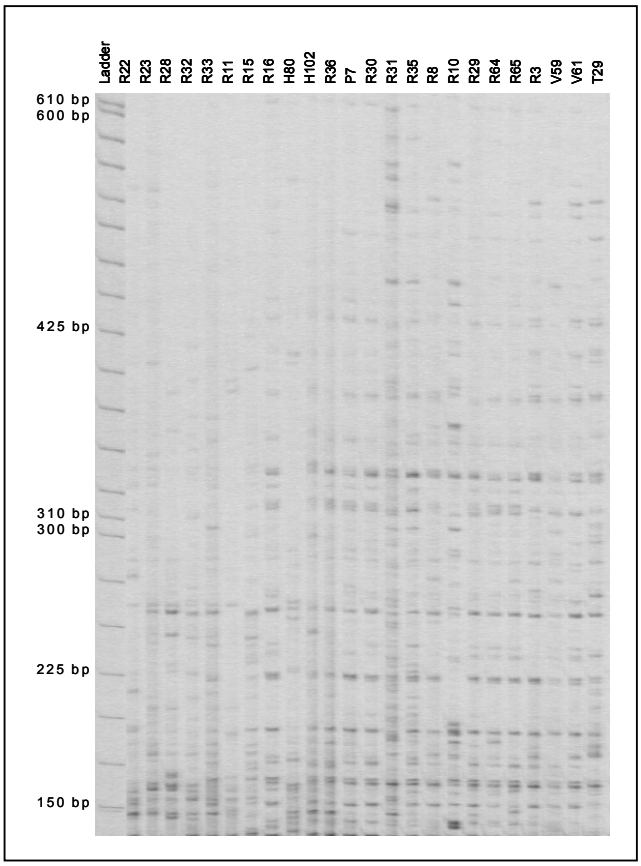


Fig 8.3: AFLP fragments (bands) derived by one of the primer-enzyme combinations used: *Eco*R1+CC/*Mse*1+ ACAA. Each lane represents individuals (only 12 shown here from 171 individuals in 5 populations). Lane 1 = Ladder of genomic DNA standard from 60 to 900 base-pairs.

When advancing to perform various statistical analyses, it was found that certain analysis (e.g. the test for isolation-by-distance) intended to be carried out using GenAlex6 depended on input of data using Microsoft Excel 2003 spreadsheet, which has a limit of 256 columns. Other software programmes, e.g. NTSYS-pc 2.02i used would also require a Microsoft Excel 2003 spreadsheet to prepare the data before they can be exported to these programmes. Sorting the data in rows (in Microsoft Excel 2003 spreadsheet) to accommodate 282 loci was again a problem when using NTSYS-pc 2.02i and GenAlex6 programmes. This difficulty, arising

from the need to use more than one statistical programme to analyse different variables in order to achieve the desired output, led to the decision to first compare the two sets of data (i.e. 282 *versus* 254 loci) using just a few variables that can be analysed using only Arlequin ver. 2.000 programme with both data sets. The data was reduced by deleting 28 loci with lower base pairs from the least polymorphic primer.

The result showed that reducing the number of loci from 282 to 254 did not alter the results (Table 8.4), and was unlikely to significantly impact on the focus of subsequent discussions. Consequently, 254 bands were used for subsequent analysis because this is compatible with the Microsoft Excel 2003 spreadsheet and the statistical programmes (e.g. NTSYS-pc 2.02i and GenAlex6) used in this study.

Table 8.4: Comparative analysis between 282 (A) and 254 (B) loci (b ands) from 171 individuals of *Barringtonia procera (sensu. lat.)* across 5 populations in Kolombangara, Solomon Islands.

A. 282 loci (bands)

Average % polymorphism loci	AMOVA (% variation)	ΦST value	Mean He	Mean He std. devn.	P-value
72.9%	16.6 (among pop.) 83.4 (within pop.)	0.166	0.969	0.0002	P>0.001

B. 254 loci (bands)

Average % polymorphism loci	AMOVA (% variation)	ΦST value	Mean He	Mean He std. devn.	P-value
71.2%	16.5 (among pop.) 83.5 (within pop.)	0.165	0.969	0.0003	P>0.001

In the subsequent analysis using 254 loci, each primer-enzyme pair generated a different pattern of bands and the number of scorable polymorphism (Table 8.5). From a total of 254 fragments, 181 (71.2%) were polymorphic. Primer-enzyme combination 2 had the highest number of polymorphic bands, whilst primer-enzyme combination 1 had the least.

Table 8.5: Summary of polymorphism achieved by each primer-enzyme combination in *Barringtonia procera sensu. lat.* across 5 populations in Kolombangara, Solomon Islands.

Primer-enzyme combination	No. scorable bands	No. polymorphic bands	% polymorphism
1	17	15	88.2
2	113	81	71.7
3	80	53	66.3
4	44	32	72.7

8.3.2 Testing the validity of field identification and the integrity of the 3 'notional species' of *Barringtonia*.

8.3.2.1 Classification and Ordination

Differentiation of the 'notional species' as identified in the field in Kolombangara Island was determined following non-metric multi-dimensional scaling (NMDS) procedures. In two dimensions the randomly generated points converge after 88 iterations with a Stress One value of 0.22637, and when re-run in three dimensions the Stress One value was reduced to 0.15598 after 59 iterations. The Stress One value in three dimensional representation is lower than 2-dimension, indicating a better representation of the individuals in the analysis. The NMDS revealed that there was clustering of the *B. novae-hiberniae* and *B. procera*, with the *B. edulis* cluster occuring within the *B. procera* cluster (Fig 8.4). The mixing of individuals may have resulted from misidentification in the field. A few individuals identified as *B. procera* (e.g. P-H68, P-T36, P-T32, P-T63) are outliers.

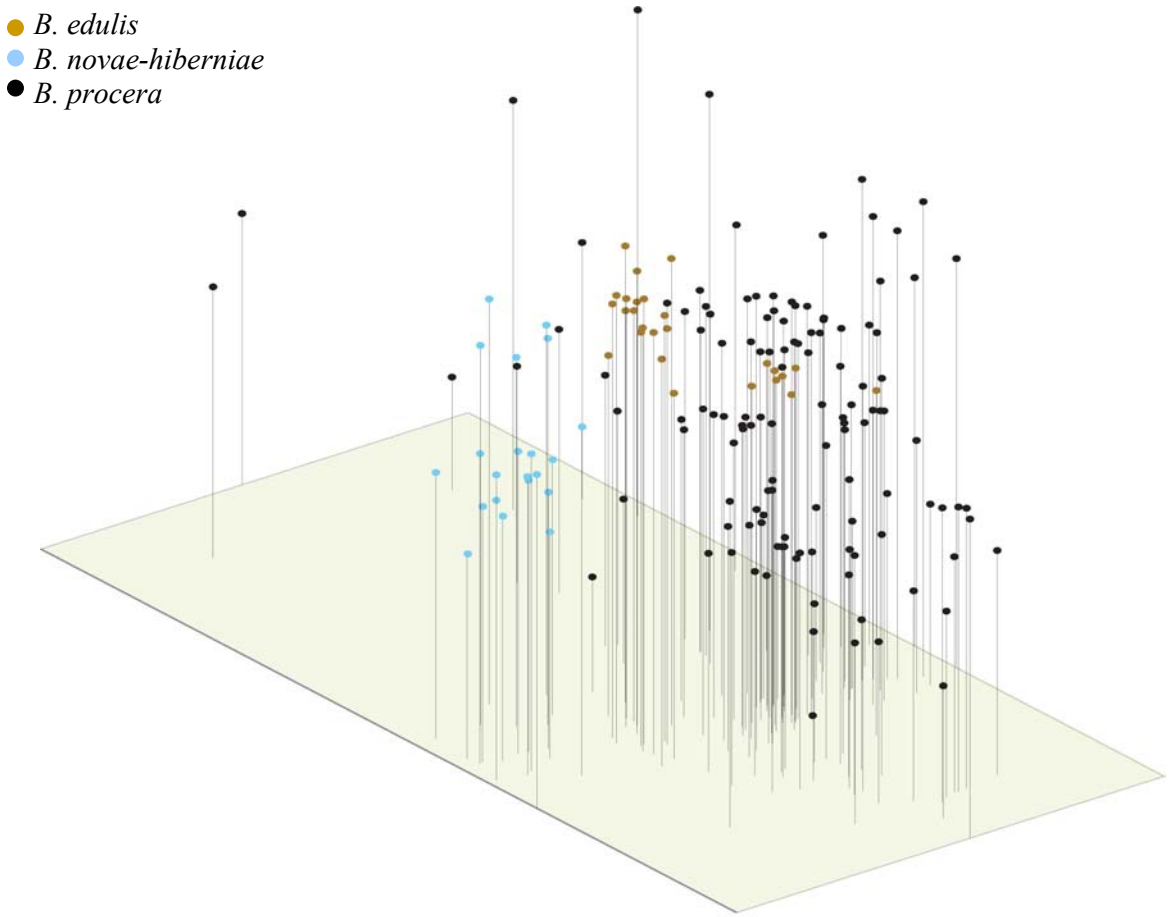


Fig 8.4. Three dimensional non-metric multidimensional scaling (NMDS) representation of genetic diversity of 171 individual trees of the 3 'notional species' of *Barringtonia* (*B. edulis, B. novae-hiberniae, B. procera*) across 5 populations in Kolombangara, Solomon Islands, generated from a Jaccard similarity matrix using AFLP fragments.

8.3.3 Evaluating population genetic structure of *B. procera* (*sensu lat.*)

8.3.3.1 Genetic diversity

The relative level of genetic diversity within and between five populations, as measured by the percentage polymorphic loci (bands) and Nei's (1987) unbiased measure of expected average heterozygosity was significant (Table 8.7). The average expected heterozygosity is almost identical in all populations. Mean polymorphism of 72.6% was generated from 254 fragments across 5 populations. The percentage of polymorphic bands was high in all five populations. Vovohe, Tututi and Rei populations exhibited a higher level of genetic diversity (79.9%, 81.5% and 70.5% respectively) than the lower genetic diversity of Poporo (59.8%) and Hunda (64.2%) populations.

Table 8.6: Genetic diversity estimates for *Barringtonia procera (sensu lat.)* populations in Kolombangara, Solomon Islands, analyzed for 254 AFLP fragments. Standard error in parenthesis.

Population >>	Vovohe	Tututi	Rei	Poporo	Hunda
Sample size	33	40	31	26	41
No. polymorphic loci	203	207	179	152	163
% polymorphism loci	79.9	81.5	70.5	59.8	64.2
Mean H_e	0.968	0.973	0.967	0.962	0.975
H_e std. deviation	0.0003	0.0003	0.0003	0.0002	0.0002

8.3.3.2 Classification and Ordination

Cluster analysis of AFLP data by UPGMA resulted in a dendrogram that separates the five different populations: Vovohe, Tututi, Rei, Poporo and Hunda (Fig 8.5), but not into completely discrete blocks. The co-phenetic correlation coefficient between similarity matrix and the dendrogram was 0.843, suggesting that the information used to construct the dendrogram is subjectively a good representation of the original similarity data (Rohlf 1998).

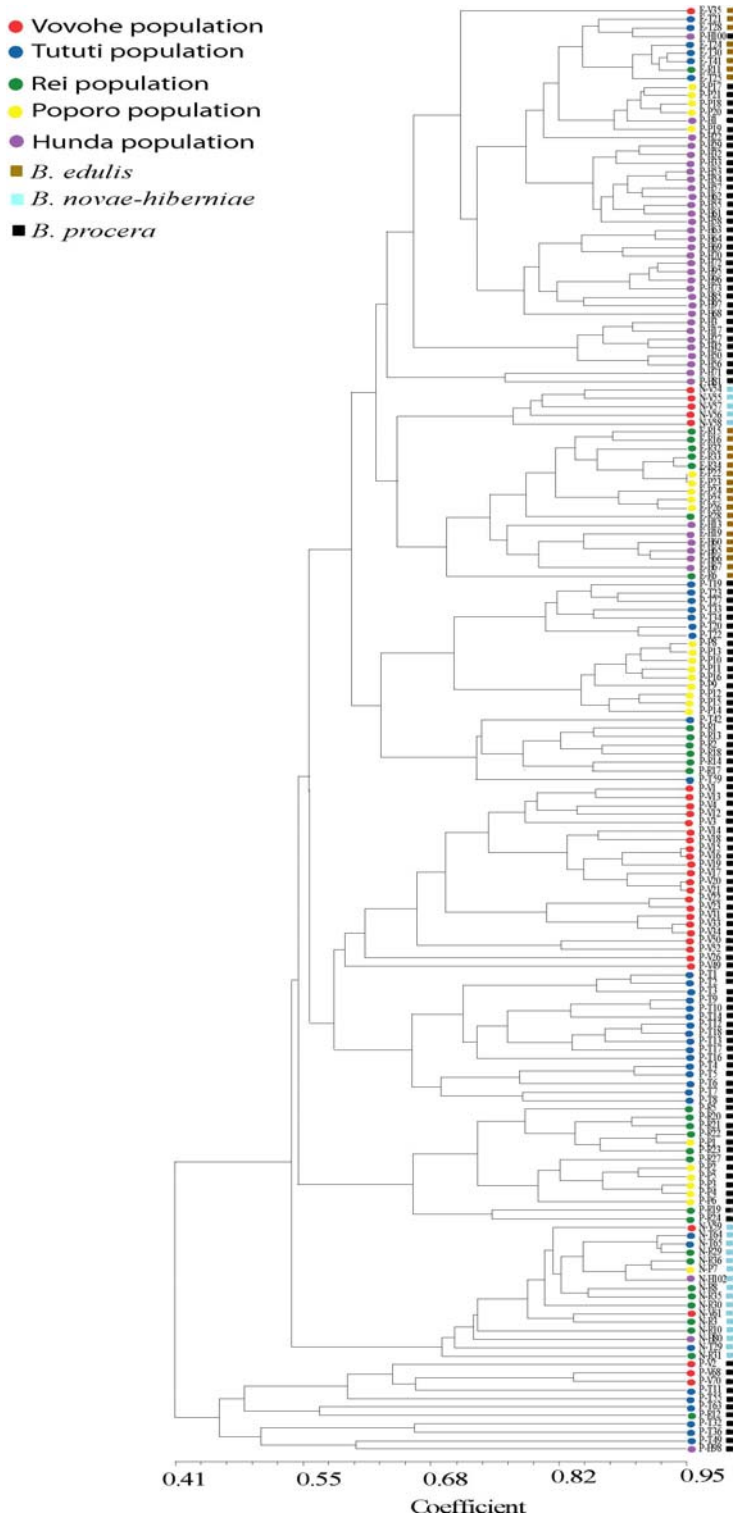


Fig 8.5. Neighbour-joining dendrogram constructed from Jaccard's estimate based on a shared presence of 254 AFLP fragments from 171 individual trees of 3 "notional species" of *Barringtonia* from 5 populations on Kolombangara Island, Solomon Islands.

There are a number of clusters consisting of individuals from the same population. For example, nine individuals from Poporo population (P-P8, P-P13, P-P10, P-P11, P-P16, P-P9, P-P12, P-P15, P-P14); seven from Tututi population (P-T19, P-T23, P-T27, P-T33, P-T34, P-T20, P-T22); six each from Hunda and Rei populations (P-H3, P-H17, P-H27, P-H42, P-H50, P-H56 and P-R1, P-R13, P-R2, P-R18, P-R14, P-R17 respectively) and five from Vovohe population (N-V54, N-V55, N-V57, N-V56, N-V58). Branch length discriminating the clusters was long and there was also a high degree of overlap between site clusters. Two individuals (E-P22 and E-P23) from Poporo population are identical.

The 3 'notional species' of *Barringtonia* (*B. edulis, B. novae-hiberniae* and *B. procera*), as identified by the differences in their morphological characters on the field, are not discrete clusters on the neighbour-joining dendrogram, with several sub-clusters for each 'notional' species. There was some mixing of individuals from different populations, and site clustering was also not discrete. There were eleven individuals of *B. procera* from 4 different populations (Vovohe, Tututi, Rei and Hunda) which were outliers.

Non-metric multi-dimensional scaling (NMDS) in two dimensions converged after 60 iterations with a Stress One value of 0.1955, and when re-run in three dimensions the Stress One value was reduced to 0.14128 after 53 iterations. The Stress One value in both two and three dimensional representation is low, indicating a good representation of the individuals from the original dataset in the analysis. Clustering of individuals in each population is observed again here and closely agrees with the results obtained in the neighbour-joining dendrogram. However, there are individuals in each population that were isolated from the main clustering, but were closely clustering with individuals from another population (Fig 8.6). For example, P-T11 of Tututi population was closely clustered with P-R12 and P-V70 of Rei and Vovohe populations than with individuals (P-T55, P-32 and P-T63) from the same population.

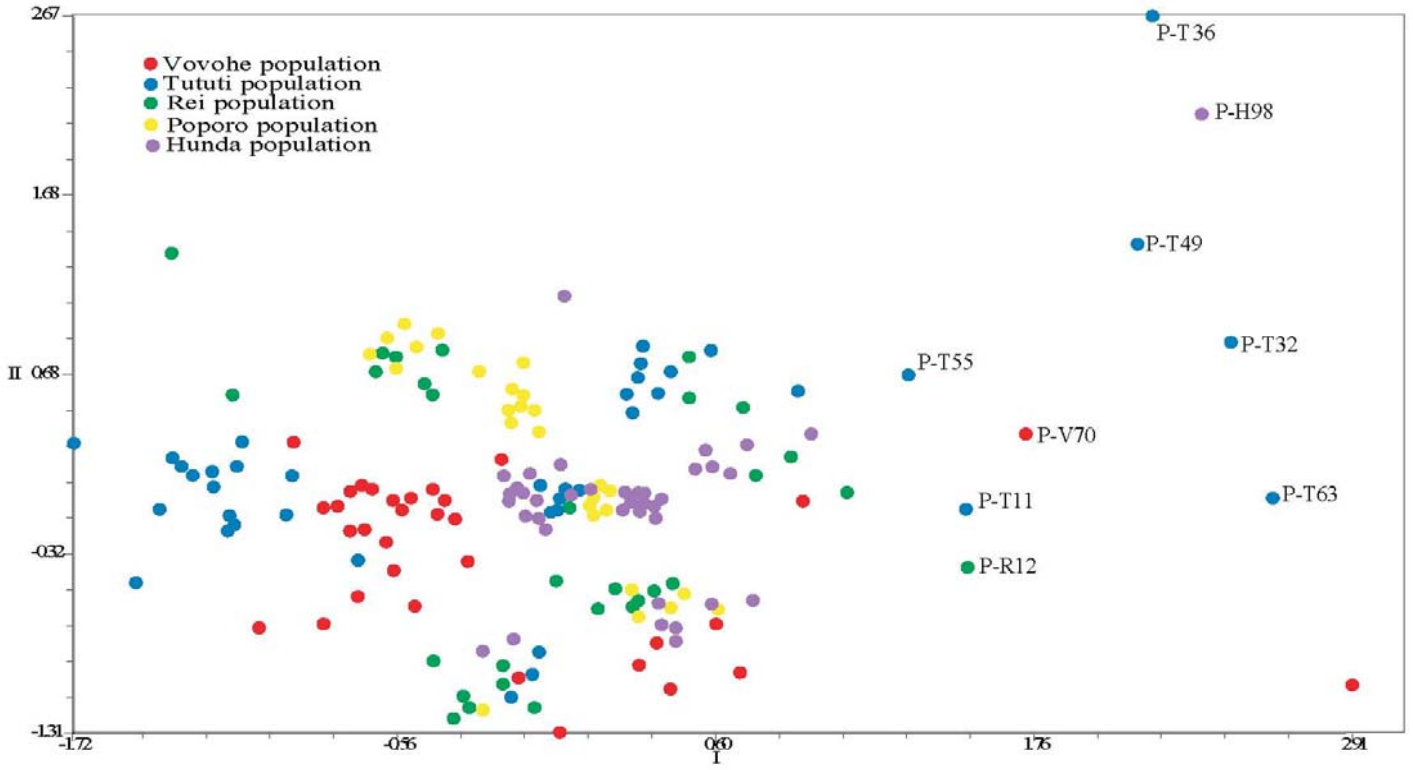


Fig 8.6. Two dimensional non-metric multidimensional scaling (NMDS) representation of genetic diversity of 171 individual trees of *Barringtonia procera* (*Sensu lat.*) from 5 populations in Kolombangara, Solomon Islands, generated from a Jaccard similarity matrix using AFLP fragments.

8.3.3.3 Analysis of molecular variance (AMOVA)

The level of partitioning of variation was different within and between populations. Differentiation of the 5 populations, as measured by the analysis of molecular variation (AMOVA) (Table 8.9) was significant (P = 0.001) and was greater within than among populations, with a high fixation index, Φ_{ST} value of 0.165 in 1023 permutations. When comparisons were made, using Φ_{ST} values for individual populations obtained from AMOVA, the differences between populations was highly significant (P<0.001, tested using about 110 PW permutation) (Table 8.10).

Table 8.7: AMOVA results portioning variation and population differentiation in *Barringtonia procera (sensu lat.)* (FST = 0.165).

Source of variation	*d.f.*	*Sum of squares*	*Variance component*	*P-value*	*% of variation*
Among populations	4	45678.3	4.967	<0.001	16.5
Within populations	11544	289316.1	25.07	<0.001	83.5

Table 8.8: FST values between pairs of populations in *Barringtonia procera (sensu lat.)*. (below diagonal), and probability values based on 110 permutations (above diagonal).

	Vovohe	Tututi	Rei	Poporo	Hunda
Vovohe	-	0.0000	0.0000	0.0000	0.0000
Tututi	0.13076	-	0.0000	0.0000	0.0000
Rei	0.15008	0.13166	-	0.0000	0.0000
Poporo	0.20930	0.15782	0.12794	-	0.0000
Hunda	0.22548	0.17997	0.16091	0.15952	-

8.3.3.4 Principal coordinates analysis

The level of genetic variation of individuals of *B. procera (sensu latissimo)*, examined by the principal coordinates' analysis is similar to the output of NMDS ordination. The clustering of trees, as an indication of genetic affinity was observed (Fig 8.19) but there was no discrete segregation of populations. However, five populations were significantly (P = 0.001) differentiated, although more pronounced in Tututi and Poporo populations than Vovohe, Rei or Hunda.

Significant variation between populations was explained by 16.6% in the PC1 and 13.2% in the PC2.

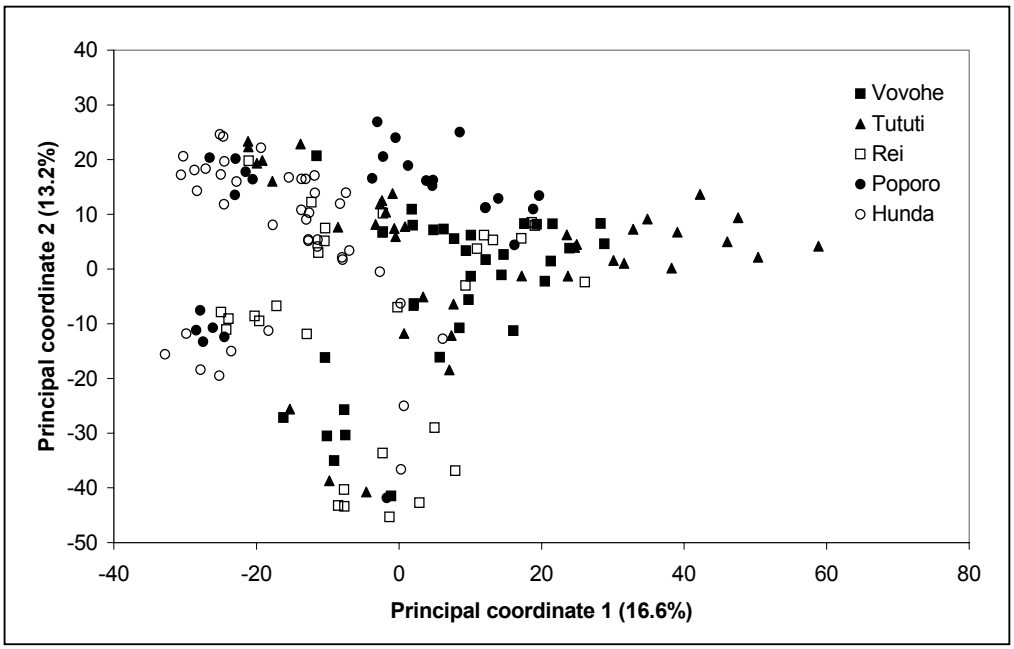


Fig 8.4: Principal coordinates analysis (PCA) via genetic distance matrix. PC1and PC2 are Eigen values of individual trees of *Barringtonia procera (sensu lat.)* in 5 populations in Kolombangara Island.

8.3.3.5 Test for isolation-by-distance

There was no relationship between the genetic and geographical distances of the 5 populations of *B. procera (sensu lat.)*. Correlation between the two variables was negative and weak and not significant ($r^2 = 0.015$, $P>0.05$).

8.3.4 Assessing genetic implications in fruit and kernel traits

Morphological analysis of variation based on eleven fruit and kernel traits (mass of fruit, flesh, nut, shell and kernel, length and width of fruit and kernel, kernel taste and depth and fruit: kernel ratio) has led to the identification of five best-fit individuals to kernel ideotype (Chapter 7). When each of these traits was superimposed on the neighbour-joining dendrogram constructed from Jaccard's genetic similarity estimates (Fig 8.8), it was found that the individuals producing

these traits were not clustered together. In addition, there were 30 individuals in total producing one or more of these traits. Six of them have more than 3 desirable traits and they are well dispersed across the dendrogram. However 2 of these elite trees (P-R1 and P-R2) were closely related and come from the Rei population. Two other elite trees (P-P21 and P-H1) from Poporo and Hunda populations respectively, were also closely related.

In addition, five of these eleven traits (fruit mass, shell mass, kernel mass, kernel length, kernel depth, and fruit to kernel mass ratio) that were considered most important to the development of the kernel ideotype were again superimposed on the neighbour-joining dendrogram constructed from Jaccard's genetic similarity estimate (Fig 8.9 to Fig 8. 14). Kernel taste was excluded because it did not differ significantly between trees or populations (see Chapter 7). For each trait, the trees were ranked (coloured-coded) in seven groups of 10's and one of 6 trees, from the best to the worst trees. No pattern can be discerned between the relatedness of the individual trees and their ranking for any of important fruit or kernel traits.

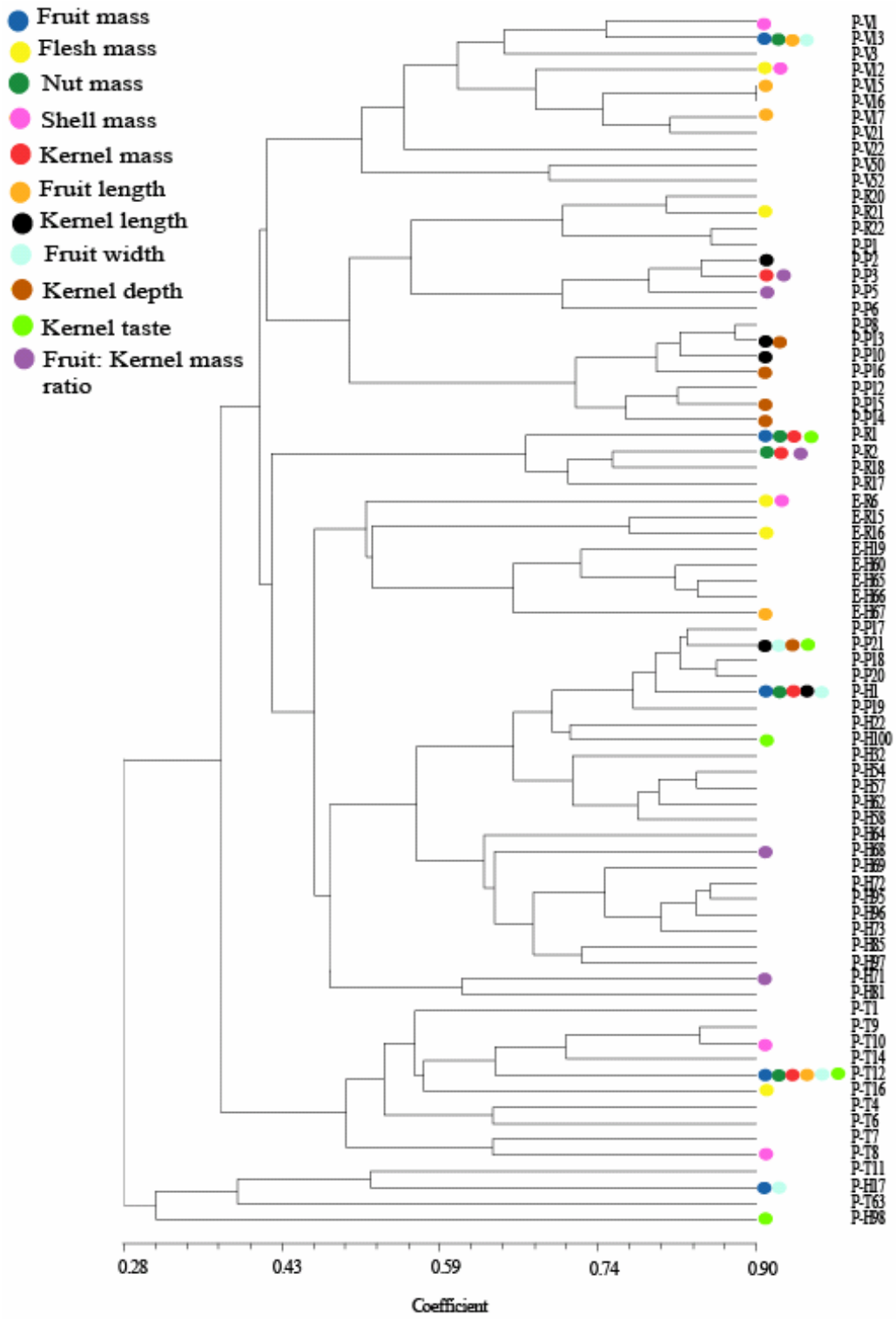


Fig 8.8. Neighbour-joining dendrogram constructed from Jaccard's estimate based on a shared presence of 254 AFLP fragments from 76 individual trees of *Barringtonia procera* (*sensu lat.*) in Kolombangara, Solomon Islands. Thirty best-fit individuals to kernel ideotype based on 11 traits across 5 populations selected in morphological study.

FRUIT MASS ranking from the best (1-10) to the worst (71-76) trees

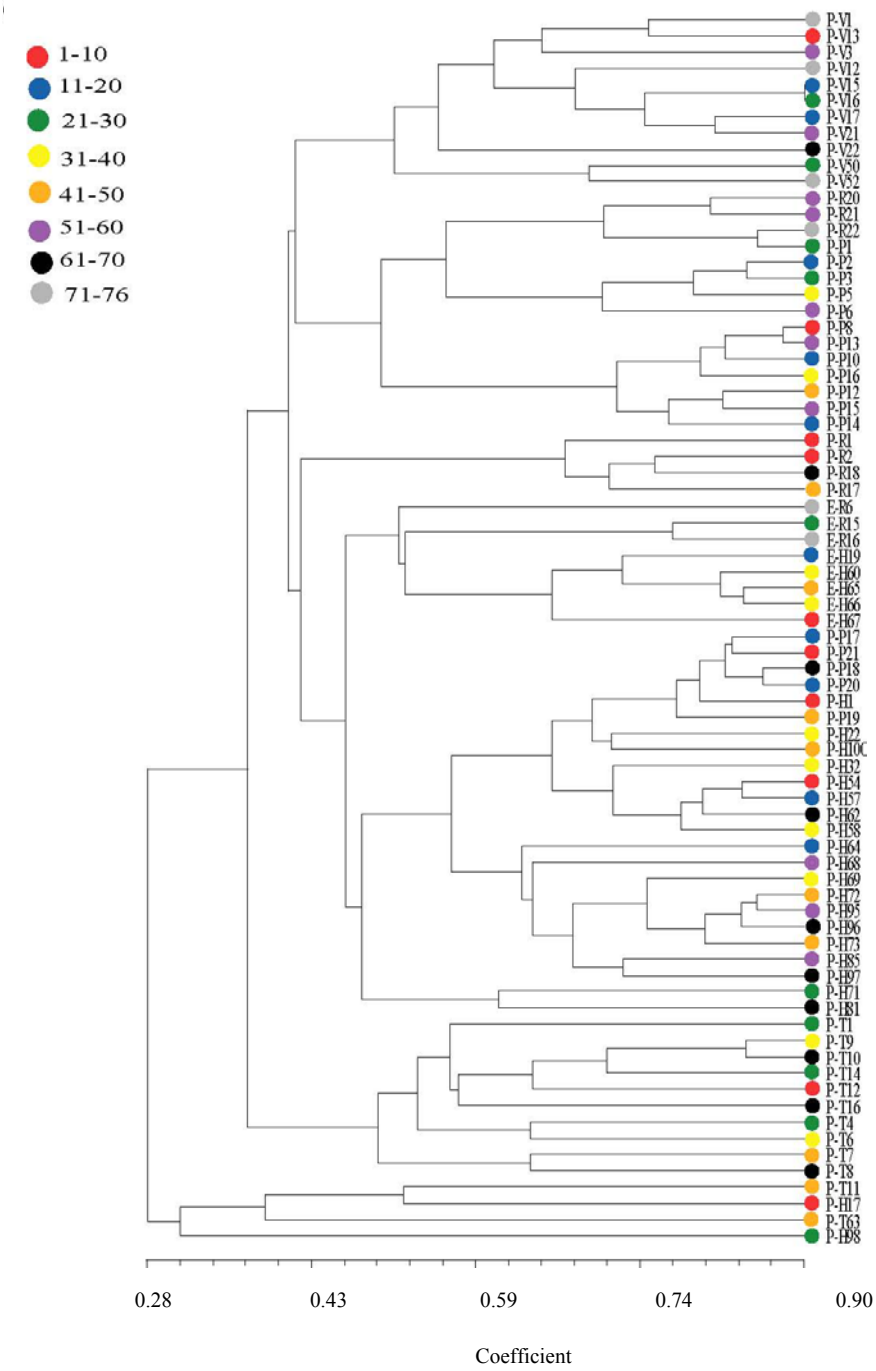


Fig 8.9. Neighbour-joining dendrogram constructed from Jaccard's estimate based on shared presence of 254 fragments of 76 individual trees of *Barringtonia procera* (s*ensu lat.*) in Kolombangara, Solomon Islands. Ranking of best-fit individuals to kernel ideotype based on fruit mass across 5 populations selected in morphological study.

SHELL MASS ranking from the best (1-10) to the worst (71-76) trees

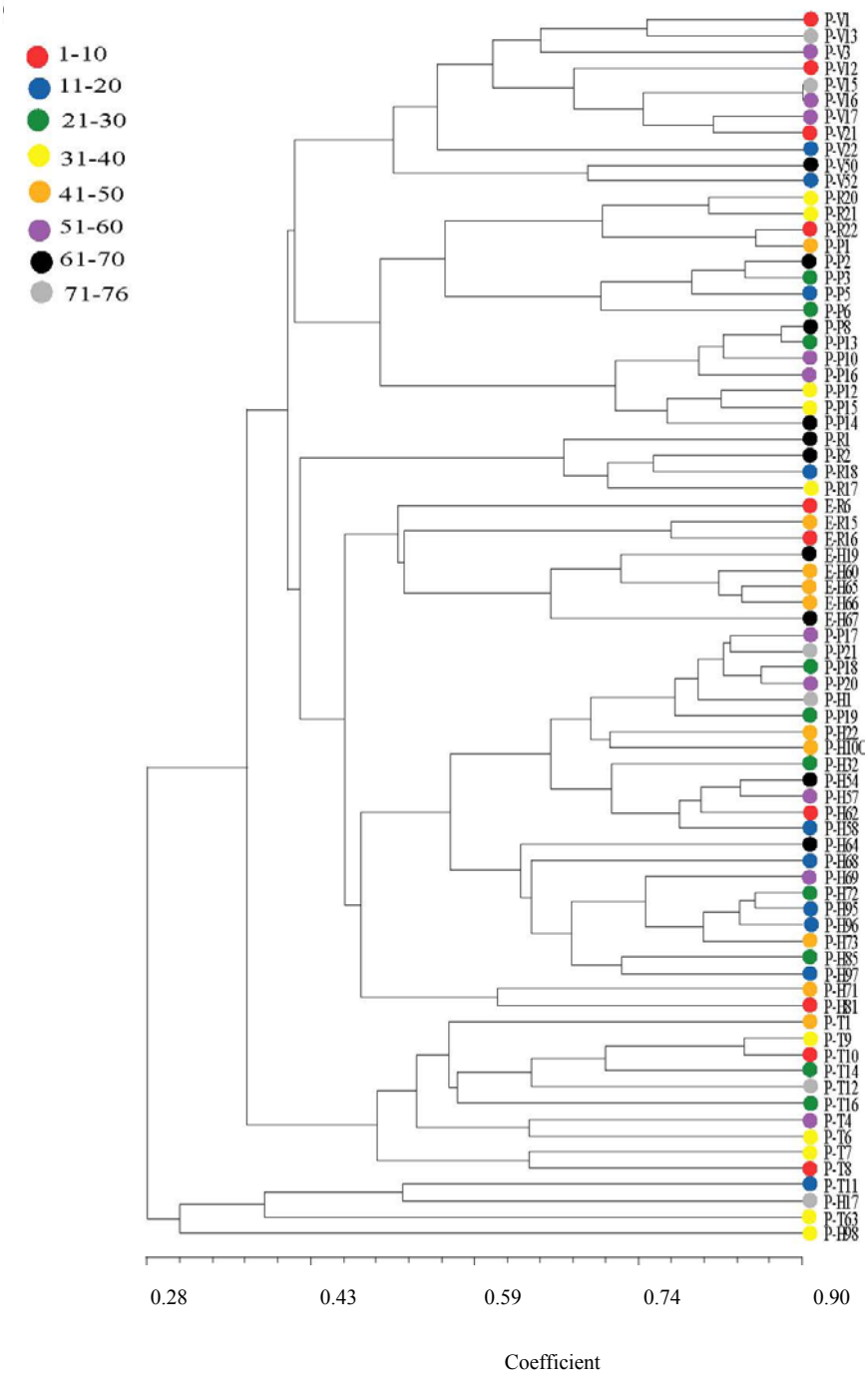


Fig 8.10. Neighbour-joining dendrogram constructed from Jaccard's estimate based on shared presence of 254 fragments of 76 individual trees of *Barringtonia procera* (*sensu lat.*) in Kolombangara, Solomon Islands. Ranking of best-fit individuals to kernel ideotype based on shell mass across 5 populations selected in morphological study.

KERNEL MASS ranking from the best (1-10) to the worst (71-76) trees

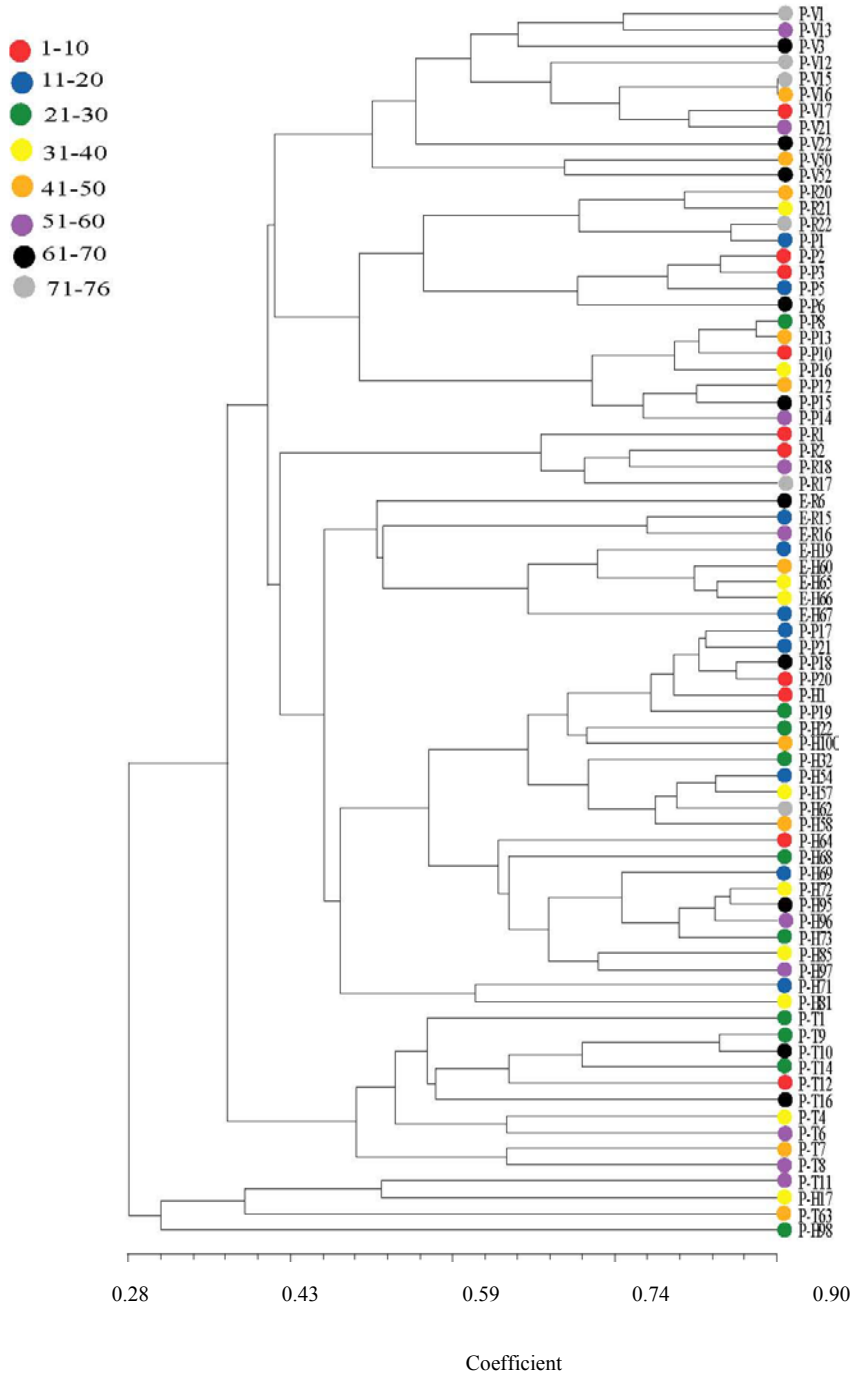


Fig 8.11. Neighbour-joining dendrogram constructed from Jaccard's estimate based on shared presence of 254 fragments of 76 individual trees of *Barringtonia procera* (*sensu lat.*) in Kolombangara, Solomon Islands. Ranking of best-fit individuals to kernel ideotype based on kernel mass across 5 populations selected in morphological study.

KERNEL LENGTH ranking from the best (1-10) to the worst (71-76) trees

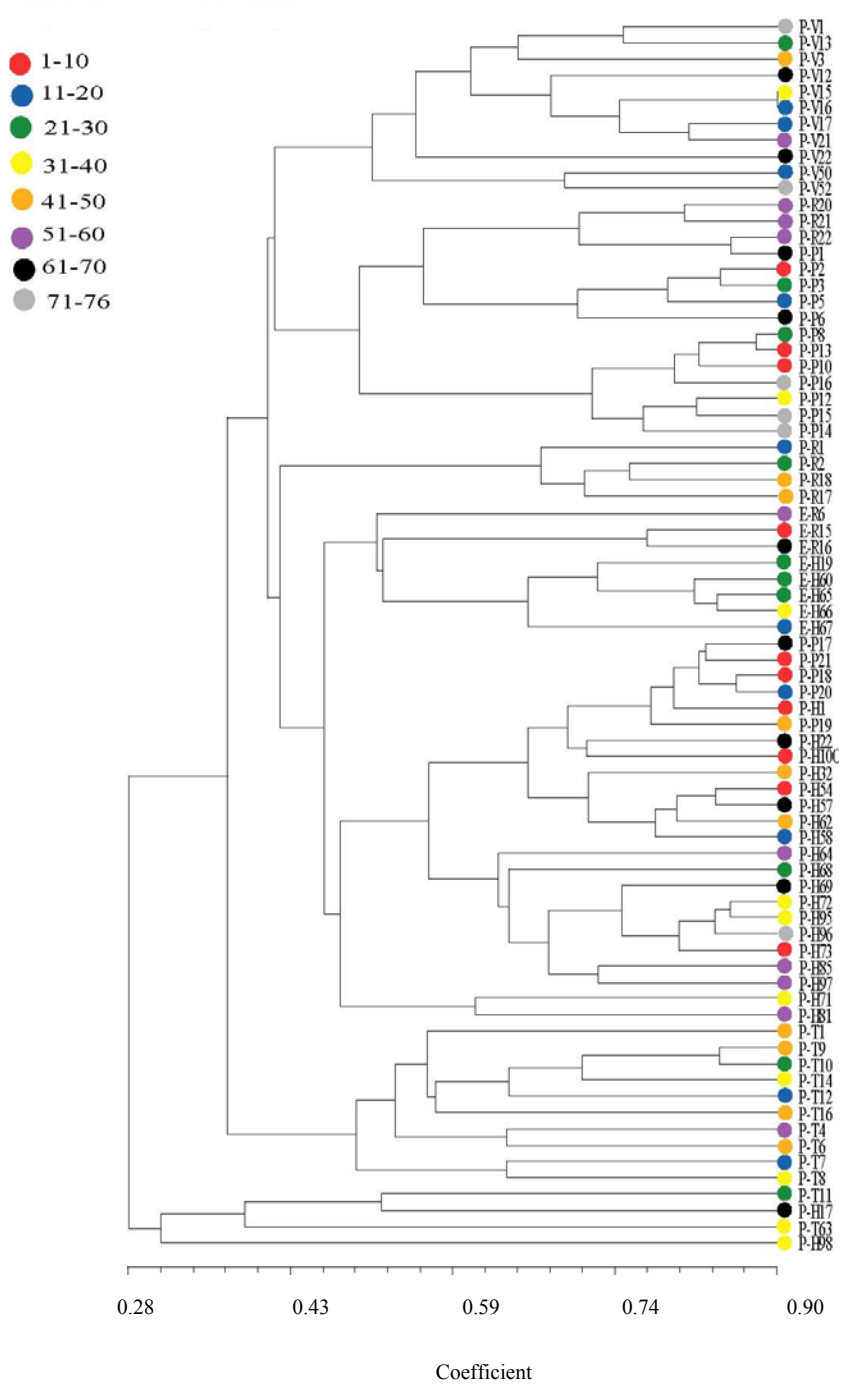


Fig 8.12. Neighbour-joining dendrogram constructed from Jaccard's estimate based on shared presence of 254 fragments of 76 individual trees of *Barringtonia procera* (*sensu lat.*) in Kolombangara, Solomon Islands. Ranking of best-fit individuals to kernel ideotype based on kernel length across 5 populations selected in morphological study.

KERNEL DEPTH ranking from the best (1-10) to the worst (71-76) trees

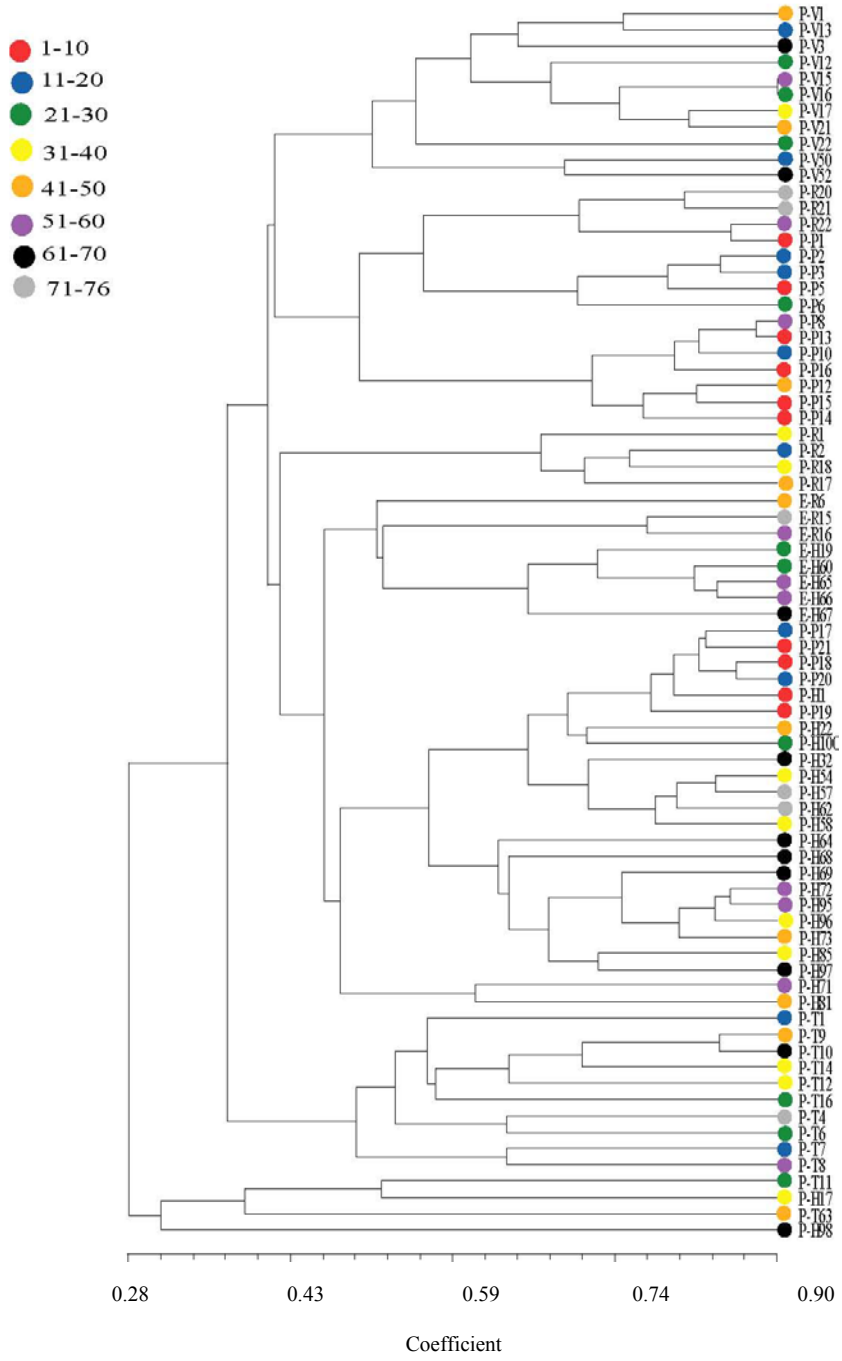


Fig 8.13. Neighbour-joining dendrogram constructed from Jaccard's estimate based on shared presence of 254 fragments of 76 individual trees of *Barringtonia procera* (*sensu lat.*) in Kolombangara, Solomon Islands. Ranking of best-fit individuals to kernel ideotype based on kernel depth across 5 populations selected in morphological study.

FRUIT: KERNEL MASS RATIO ranking from the best (1-10) to the worst (71-76) trees

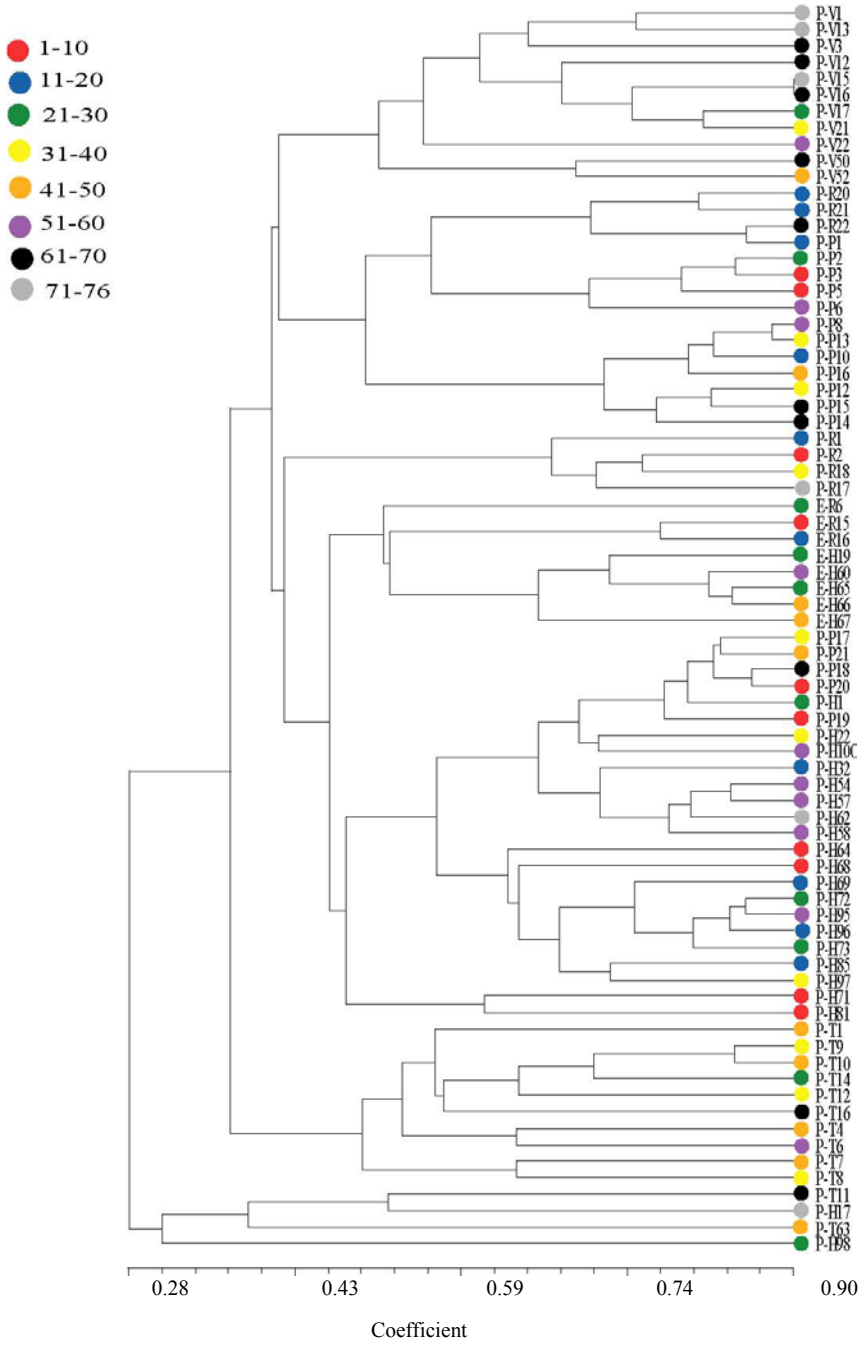


Fig 8.14. Neighbour-joining dendrogram constructed from Jaccard's estimate based on shared presence of 254 fragments of 76 individual trees of *Barringtonia procera* (*sensu lat.*) in Kolombangara, Solomon Islands. Ranking of best-fit individuals to kernel ideotype based on fruit to kernel mass ratio across 5 populations selected in morphological study.

8.4 DISCUSSION

This study has investigated the genetic diversity of *Barringtonia* species for the first time, and provides information useful to their domestication. It is clear that this molecular study also highlights the difficulty of making definitive species identifications between these 3 edible species of *Barringtonia* because of the overlapping variation of morphological traits as defined by Walter and Sam (2002) (Fig 8.1).

- **Population structure and genetic diversity of *Barringtonia procera***

⇒ **Between population genetic diversity**

Species that are wind-pollinated and outcrossing maintain higher levels of genetic variation at the population level than species that are self-pollinated (Hamrick *et al.,* 1993). The present study agrees with this statement, as evident in the statistically significant (Φ_{ST} = 0.17, P<0.001) genetic differentiation between populations of *Barringtonia* for this small island of Kolombangara. However, compared with species from continental land masses, the variability was small – for example, as in mahogany species in Central America; *Swietenia macrophylla* (Φ_{ST} = 0.38) (Lowe *et al.,* 2003) and *Cedrela odorata* (Φ_{ST} = 0.89) (Cavers *et al.,* 2003). Nevertheless, the degree of partitioning of genetic diversity in the present study, calculated in the AMOVA, shows higher levels of variation within (83.5%) rather than between (16.5%) populations. This level of within population variation is similar to that in other mainland outbreeding species – for example, 80% in *S. macrophylla* (Gillies *et al.,* 1999) and 81.5% in South American conifer (*Pilgerodendron uviferum*) (Allnutt *et al.,* 2003). Genetic variability between different parts of a species range is also recognised, especially in forestry in which provenance selection is often the first step in tree improvement (Pinyopusarerk and Williams 2000; Leakey 2004a). This can also lead to ecotypic variation, as for example, in *Cedrela odorata* populations on two sides of the central dividing range of Central America – were ecotypes with different genetic variability:- 79.8% (dry zone) and 52.6% (wet zone) maintained within populations (Cavers *et al.,* 2003).

In the present study, genetic differentiation was most pronounced between Vovohe and Poporo (F_{ST} = 0.209) and Vovohe and Hunda (F_{ST} = 0.225) populations. Interestingly, these villages are quite close to each other (Fig 3.6 in Chapter 3). All other village combinations ranged from 0.128 to 0.180, indicating significant gene flow between populations. A test for genetic isolation-by-distance, using the Mantel test, found no significant relationships, implying that the level of genetic diversity between these five populations is independent of their geographical distances. This is consistent with the proximity of sites on a small island with similar climate, geology and ecology. Again, this result is in contrast, not surprisingly, with the genetic structure of *Swietenia macrophylla*, which found significant correlation between geographical distance and all pairwise measures of genetic divergence in eight naturally established populations of *S. macrophylla* from six Mesoamerican countries (Novick *et al.,* 2003). Similarly, Lowe *et al.,* (2000), found significant effect of geographical distance on the genetic structure of the indigenous nuts *Irvingia gabonensis* and *I. wombulu* in West and Central Africa, where genetic similarity of individuals was reduced with an increase in geographical distance at the regional level, suggesting limited gene flow and subsequent genetic isolation between populations. The low level of genetic diversity between populations may to some extent also be a consequence of the high genetic diversity within populations as found in the current study and that of *Irvingia gabonensis* and *I. wombulu* in West and Central Africa (Lowe *et al.,* 2000). Similar findings have been reported from Southern Africa in the indigenous fruits *Scerocarya birrea* and *Uapaca kirkiana* (Agufa 2002).

⇒ **Within population genetic diversity**

The five populations had a high level of within population genetic diversity. This is demonstrated by the significantly high level of heterogeneity found within populations, which is almost identical in all five populations. This is important as it would show that the wild population is not inbred and thus that each village would have sufficient broad genetic diversity to be able to initiate participatory domestication without threat to the genetic base of this species. In addition, the total genetic diversity level across 5 populations of *Barringtonia procera* (*sens.*

lat.) was very high (H_T = 0.97) compared to other tropical woody species, for example, *Cedrela odorata* (H_T = 0.27) (Cavers *et al.,* 2003), a species which has been subjected to heavy and unsustainable logging. The high genetic diversity with populations of *B. procera* concurs with the high morphological tree-to-tree variation found in Chapter 7. This combination of genetic and morphological diversity was also found in *Irvingia* species (Atangana *et al.,* 2001, 2002; Anegbeh *et al.,* 2003). This may reflect the differences between species which are destructively harvested (timber) and non-destructively harvested (fruits and nuts). This may also reflect the fact that farmer's collect different genotypes of food species and usually propagate them by seed. In Africa, farmers have made genetic gains in fruit size of *Irvingia gabonensis* up to 44% through selection over the years (Leakey *et al.,* 2004; Leakey *et al.,* 2005d), consequently contributing to the increase in genetic diversity within population.

As typical of outcrossing species, the high within population genetic variability found in *Barringtonia procera* (*sens. lat.*) may be ascribed to random mating between individuals. Little is known about the reproductive biology of *Barringtonia procera* (*sens. lat.*) but *Barringtonia procera* (*sens. lat.*) is monoecious, with male and female reproductive parts occurring in the same flower.

- **Genetic implications in fruit and kernel traits**

This study identified 30 individual trees that produced one or more fruit and kernel traits considered to fit the kernel ideotype. Interestingly, only two of these individuals were closely related and from the same population. Even when all 76 trees were ranked from best to worst, there was no distinct pattern or clustering of 'superior' or 'inferior' trees when superimposed on the dendrograms for genetic relatedness. This suggests that despite the fact that farmers may be making genetic selections when sowing seeds of *Barringtonia*, there is within a population, a random mixture of 'good' and 'bad' trees. This illustrates that the identification of elite trees in different village populations is likely to still maintain a high level of genetic diversity in other traits across the population of cultivars formed by participatory domestication at the village level.

- **Integrity of *Barringtonia procera***

The molecular analysis (Fig 8.4) showed that the three species clustered together. However, as illustrated in the non-metric multi-dimensional scaling, there were no distinct clusters of individuals of each species. There are two possible explanations for this lack of discrete species groupings from this molecular data:-

i. Misidentification – however, the clustering of the 'notional species' in Fig 8.5 suggests that this misidentification was based on the recognition of some consistency of recognised visual differences.

ii. Hybridization – however, it would be expected that the two parent species would be distinct and that the mixed progeny would fall between them, perhaps *B. edulis* falls between *B. procera* and *B. novae-hiberniae* in Fig 8.4, but it falls at one of the extreme ends in Fig 8.5. Thus, hybridization is not evident in the dendrogram.

Interestingly, there is a general clustering and not a scattering of individuals between the 3 'notional species' of *Barringtonia*, indicating that the field identifications were recognising some tangible morphological traits between the three 'notional' species. A number of individuals were outliers and unrelated to the main cluster, and there are 1-2 quite separate clusters within *B. procera*. These individuals might not be a different species, but rather could be a new introduction of the same species from a different island (i.e. a distinct provenance).

It is likely that in recent times there has been movement or anthropogenic dispersal of germplasm between population sites, which has resulted in greater than natural introgression of genetic materials. There is some evidence of this in Fig 8.6. The non-metric multi-dimensional scaling analysis also provides some support for 'notional' *B. edulis* falling between *B. novae-hiberniae* and *B. procera*, possibly as a hybrid between them (Fig 8.4). However, the neighbour-joining dendrogram do not support this interpretation (Fig 8.5). Perhaps, the current data set is too limited in its geographic range to address this question, as it is likely that the isolation of

discrete populations on different islands in the Solomon Islands, as in *Santalum austrocaledonicum* in New Caledonia (Bottin *et al.,* 2005), would lead towards speciation and associated morphological changes. Then, over time the situation may be reversed by anthropogenic transfer of germplasm between islands.

Seeds of *Barringtonia* species can only be effectively dispersed long distances by humans, as seed dispersal by bats or cockatoos is limited to short distances and so is unlikely between islands. This is contrary to the situation in sandalwood (*Santalum autrocaledonicum*) where it is unlikely that anthropogenetic germplasm dissemination has occurred, as is evident in a strong genetic differentiation between islands (Bottin *et al.,* 2005). This mode of dispersal has important implications in *Barringtonia* species, in terms of their use and distribution in a tree domestication program, in which a strategy of germplasm exchange may be desirable to diversify the genetic base and introduce desirable traits.

If the current classification of 3 edible *Barringtonia* species (Jebb 1992) is valid, an interpretation of the molecular analysis would be that eight individuals (E-V35, E-T21, E-T28, E-T24, E-T30, E-T41, E-R11 and E-T25) were misidentified during sampling and should be *B. procera* and not *B. edulis* as recorded during the field work (Fig 8.4 and Fig 8.5).

8.5 SUMMARY

This study has initiated molecular investigations to measure the level of genetic diversity and population differentiation of *Barringtonia procera* (*sens. lat.*), and opens the way forward for further research. Unequivocally, the results suggest the need for more detailed use of molecular techniques to examine the species concepts in the genus and possible re-classification of the species within the genus, as well as examining the potential for hybridization and introgression between species. Future molecular investigations in *B. procera* and its relative species (*B. novae-hiberniae* and *B. edulis*) should examine the variation in other islands with and without different ecology, climate and geology. Thus, would also test

suggestion that the outliers found in the current study may originate from other islands/ provenances.

The high level of genetic diversity found in *Barringtonia procera* (*sens. lat.*) in the current study is an important attribute of the species, of value for participatory domestication because it provides opportunity for selection of superior genotypes in the wild populations for cultivation without seriously depleting the intraspecific genetic diversity. The high level of heterozygosity within populations also suggests that each village can independently carryout a community-based participatory domestication without the need for germplasm exchange, at least in the early phases of domestication. This has important practical implications for maintaining a simple domestication strategy until the communities have gained substantial experience in these techniques.

CHAPTER 9: GENERAL DISCUSSION

This study which was implemeneted in the Solomon Islands, especially with rural communities in Kolombangara Island has considered three principal hypotheses and subsequently tested each one following research questions being developed. The research questions have formed the basis of the research objectives of the thesis. The following discussion provides a summary of findings in relation to the hypotheses and the research questions.

Hypothesis 1: Rural communities in Kolombangara Island are interested in the domestication and commercialization of indigenous tree species producing non-timber forest products through the application of agroforestry systems and practices. This hypothesis prompted the question of whether or not the farmers are indeed keen to participate in domestication and commercialisation of indigenous tree species. A particpatory survey in five sites in Kolombnagara Island to determine farmers' interest found farmers top priority was *B. procera* followed by *C. indicum*, *A. altilis*, *M. minor* and *I. fagifer*. This study selected *B. procera* and *I. fagifer* on the basis of the combination of farmers' priority, market and researchability.

The results of the study are directly applicable to agroforestry tree domestication as a farmer-driven and a market-led process which has been promoted in partnership with farmers (Raintree 1991; Franzel *et al.,* 1996). This participatory approach to tree domestication ensures that the farmers are the beneficiaries of the venture. Particularly in West and Central Africa, agroforestry tree domestication has been successful in fruit and nut species, for example, *Dacryodes edulis* (Anegbeh *et al.,* 2005), *Irvingia gabonensis* (Atangana *et al.,* 2001), with a number of indigenous fruits also being selected in Southern Africa (e.g. *Sclerocarya birrea*, Leakey 2005).

Farmers' interest in the priortized tree species was deomonstrated by their responses to questionnaires about the relevance of indigenous fruit and nut species to their farming practices, particularly a focus to enrich homegardens and mixed cropping systems. This finding is in accordance with similar project in Africa with *D. edulis* and *I. gabonensis* which has illustrated the importance of indigenous fruit and nut trees for farmer livelihoods and the diversification and improvement of traditional mix cropping systems (Schreckenberg *et al.,* 2002). The present study with *B. procera* and *I. fagifer* has developed important knowledge for use by farmers to practice sustainable agriculture for food security and cash generation, and thus enhancing their interest and livelihoods.

Hypothesis 2: There is sufficient phenotypic and genetic variation in the chosen priority tree species to merit selection of superior trees for the creation of potential cultivars. This study characterised the phenotypic variation in different fruit traits within and between populations of *B. procera* from five sites in Kolombangara Island.

Tree-to-tree variation within the wild populations tested found the greatest variation (fourfold between the lightest and the heaviest kernel) to be at village level. This variation was continuous offering very considerable opportunities for elite tree selection across this and a number of other different traits. This knowledge provides the opportunity for multi-trait selection of cultivars based on ideotypes developed for their market opportunities.

Again the present result concur with those in West and Central Africa (Leakey *et al.,* 2004), where participatory domestication is being vigorously adopted by communities over the last decades (Tchoundjeu *et al.,* 2006). This study also found that farmers have already initiated their own processes of domestication for *B. procera* as in West Africa (Leakey *et al.,* 2004) and have achieved the second stage of domestication in Kolombangara Island.

The results of a molecular study on the same trees as used for morphological characterization have provided insights into the relationships between genetic

diversity and the variability of these semi-domesticated populations. The examination of the relatedness of the tree identified as elite for kernel mass was found to be minimal, indicating that there is only a low risk of narrowing the genetic base of the population through this kind of village level domestication. Together with the development of cultivars from many unrelated populations from different villages, this also means that the "production population" will maintain high genetic diversity. Taking all these results into account it is clear that this species is appropriate for participatory domestication to enhance livelihoods and to meet the Millennium Development Goals of the United Nations.

The molecular study furthermore indicated that farmers have been exchanging planting materials for cultivation from one site to another. This again indicates both farmers' interests and opportunity for considerable benefits for domestication and cultivation of this species.

Hypothesis 3: Trees of the chosen priority species can be propagated vegetatively to produce cultivars. The question of which factors affecting the vegetative propagation of the priority species need to be optimised in order to develop robust techniques for development of cultivars of these species was raised in relation to this hypothesis. In the agroforestry tree domestication process, the elite trees that have been identified must be cloned in order to make copies of their specific traits. Vegetative propagation is the means to do this because it produces plants that are genetically identical to their parent plant (Hartmann *et al.,* 1997).

The present study found that these two priority species were easily rooted in a non-mist propagator using single-node leafy stem cuttings. This makes it easy to develop cultivars which capture the tree-to-tree variation. This study has found that robust propagation occurs when the factors that determine the rooting ability of other tropical tree species are applied to *B. procera* and *I. fagifer*. Thus, the use of a non-mist propagator, the application of IBA at 0.8% and the trimming of the leaves of single-node, leafy juvenile stem cuttings to 50cm^2 resulted in very high rooting percentages and well-developed root systems. When this protocol was applied to cuttings from the mature crown or to potted, mature marcots reasonable

rooting was also obtained although further study is required. Importantly, the present study also made some progress towards attaining a better understanding of the factors constraining the rooting ability of mature cuttings. Two systems were developed. First, a sequential system which roots cuttings from marcotted stem potted as stockplants, and secondly a direct system, which is partially successful, roots cuttings direct from crown. The latter system needs further research to understand better factors controlling rooting in cuttings when ontogenetic and physiological age of plants are separated. Further resolution of this problem, will be a major advance in horticultural science.

In conclusion, this study has extended the global initiatives to domesticate indigenous fruit and nut species as producers of AFTPs for enhancement of farmer livelihood through agroforestry, from Africa to the Pacific. The many similarities between the findings of the present study and those in West and Central Africa provide confidence that the overall approach and strategies have widespread applicability.

CHAPTER 10: CONCLUSIONS

This study has successfully achieved its objectives and opens the way to full scale participatory domestication of these two indigenous nut species. The key outcomes of the present study were:-

1. *B. procera* and *I. fagifer* are indigenous nuts of Solomon Islands and other Pacific countries which are important to rural communities for food and nutritional security and which have the potential through domestication to generate income and so enhance rural livelihoods.

2. These same species have important roles in traditional agriculture on Kolombangara Island, meeting the needs of the people and contributing to the development of permanent mixed cropping systems to replace shifting cultivation through the enrichment of homegardens and cash crop systems such as cocoa, coconut and timber trees. In this respect, domestication of these species should help the displaced population of Kolombangara Island to achieve greater self-sufficiency in the communal area which is about one third of Kolombangara Island.

3. Significant tree-to-tree variation between and within populations of *B. procera* indicates opportunities for development of cultivars based on kernel 'ideotype' to meet peoples' needs and market potential, and to progress beyond stage two of domestication.

4. The molecular study highlights the difficulty of making definitive species identifications in the genus *Barringtonia* based on the overlapping variation of morphological traits, suggesting the need for better taxonomic classification of edible *Barringtonia* at species level. Significant genetic diversity was identified within populations indicating the opportunity to utilize participatory

domestication at the village level without severe negative impacts on genetic diversity. It was clear that trees with superior traits were unrelated.

5. *B. procera* and *I. fagifer* are easily rooted species, using single-node juvenile, leafy stem cuttings. Optimum IBA concentration was 0.8%, optimum leaf area was 50cm^2 and the most appropriate rooting medium in a non-mist poly-propagator was coir.

6. Propagation of mature cuttings to capture superior phenotypes (elite trees) was activated using two approaches – marcots, followed by rooting cuttings and rooting cuttings direct from crown. Both these approaches separate ontogenetic and physiological ageing so paving the way to overcome phase change, a common constraint to progress in tree improvement programs.

7. This package of techniques and results provides the information and skills required for the implementation of the participatory domestication of these indigenous nuts.

REFERENCES

Abel, N., Baxter, J., Cambell, A., Cleugh, H., Fargher, J., Lambeck, R., Prinsley, R., Prosser, M., Reid, R., Revell, G., Schmidt, C., Stirzaker, R. and Thorburn, P. (1997). *Design Principles for Farm Forestry: A Guide to Assist Farmers to Decide Where to Place Trees and Farm Plantations on Farms.* Rural Industries Research and Development Corporation, Canberra, Australia.

Agufa, C. A. C. (2002). Genetic variation in *Scerocarya birrea* and *Uapaca kirkiana* - indigenous fruit trees of the Miombo Woodland. *MSc thesis*, *Jomo Kenyatta University of Agriculture and Technology*, Nairobi, Kenya.

Akus, W. L. (1996). The *Canarium* nut: research and development at the Lowland Agriculture Experiment Station, Keravat, Papua New Guinea. *In* M. L. Stevens, R. M. Bourke and B. R. Evans (eds), *Overview of Resource Potential for Indigenous nut Production in the South Pacific. South Pacific Indigenous Nuts.* 110-112. ACIAR Proceedings No. 69, Canberra, Australia.

Allen, B. J. (1989). Dynamics of fallow successions and introduction of robusta coffee in shifting cultivation areas in the lowlands of Papua New Guinea. *In* P. K. R. Nair (ed.), *Agroforestry Systems in the Tropics*. 277-290. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Allnutt, T. R., Newton, A. C., Premoli, A. and Lara, A. (2003). Genetic variation in the threatened South American conifer *Pilgerodendron uviferum* (Cupressaceae), detected using RAPD markers. *Biological Conservation*. **114**: 245-253.

Aminah, H., Dick, J. M. and Grace, J. (1997). Rooting of *Shorea leprosula* stem cuttings decreases with increasing leaf area. *Forest Ecology and Management*. **91**: 247-254.

Aminah, H., Dick, J. M., Leakey, R. R. B., Grace, J. and Smith, R. I. (1995). Effect of indole butyric acid (IBA) on stem cuttings of *Shorea leprosula*. *Forest Ecology and Management*. **72**: 199-206.

Andersen, A. S. (1986). Environmental influences on adventitious rooting in cutting of non-woody species. *In* M. B. Jackson (ed.), *New Root Formation in Plants and Cuttings*. 223-254. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

Anegbeh, P. O., Usoro, C., Ukafor, V., Tchoundjeu, Z. and Leakey, R. R. B. (2003). Domestication of *Irvingia gabonensis*: 3. Phenotypic variation of fruits and kernels in a Nigerian village. *Agroforestry Systems*. **58**: 213-218.

Anegbeh, P. O., Ukafor, V., Usoro, C., Tchoundjeu, Z., Leakey, R. R. B. and Schreckenberg, K. (2005). Domestication of *Dacryodes edulis*: 1. Phenotypic variation of fruit traits from 100 trees in southeast Nigeria. *New Forests*. **29**: 149-160.

Aplin, G., Beggs, P., Brierley, G., Cleugh, H., Curson, P., Mitchell, P., Pitman, A. and Rich, D. (1999). *Global Environmental Crises: An Australian Perspective.* Second edn, Oxford University Press, Melbourne.

Arnholdt-Schmitt, B. (2000). RAPD analysis: a method to investigate aspects of the reproductive biology of *Hypericum perforatum* L. *Theoretical and Applied Genetics*. **100**: 906-911.

Atangana, A. R., Ukafor, V., Anegbeh, P., Asaah, E., Fondoun, J.-M., Ndoumbe, M. and Leakey, R. R. B. (2001). Domestication of *Irvingia gabonensis*: 1. Phenotypic variation in fruits and kernels in two populations from Cameroon. *Agroforestry Systems*. **53**: 55-64.

Atangana, A. R., Ukafor, V., Anegbeh, P., Asaah, E., Fondoun, J.-M., Ndoumbe, M. and Leakey, R. R. B. (2002). Domestication of *Irvingia gabonensis*: 2. The selection of multi traits for potential cultivars from Cameroon and Nigeria. *Agroforestry Systems*. **55**: 221-229.

Atwell, B. J., Kriedemann, P. and Turnbull, C. (eds) (1999). *Plants in Action: Adaptation in Nature, Performance in Cultivation.* MacMillian Publishers Australia Pty Ltd.

Avise, J. C. (1994). *Molecular Markers, Natural History and Evolution.* Chapman & Hall, New York.

Barany, M., Hammett, A. L. and Leakey, R. R. B. (2003). Potential Income Generating Opportunities for Smallholders Affected by HIV/AIDS: Linking Agro-Ecological Change and Non-Timber Forest Product Markets. *Journal of Management Studies*. **39**: 26-39.

Beebee, T. J. C. and Rowe, G. (2004). *An Introduction to Molecular Ecology.* Oxford University Press Inc., New York, USA.

Benson, E. E. (2000). *In Vitro* plant reclacitrance: An Introduction. *In Vitro Cellular and Development Biology - Plant*. **36**: 141-148.

Bevu, T. (1999). Cutting propagation of Mahogany (*Swietenia macrophylla* King). *Pacific Islands Forest and Trees*. **4**: 13-14.

Birmeta, G. and Welander, M. (2004). Efficient micropropagation of Ensete ventricosum applying meristem wounding: a three-step protocol. *Plant Cell Report*. **23**: 277-283.

Blazich, F. A. (1988). Mineral nutrition and adventitious rooting. *In* T. D. Davis, B. E. Haissig and N. Sankhla (eds), *Adventitious Root Formation in Cuttings*. 61-69. Dioscorides, Portland.

Blazich, F. A. and Wright, R. D. (1979). Non-mobilization of nutrients during rooting of *Ilex crenata* cv. Convexa stem cutiings. *Horticultural Science*. **14**: 242.

Blazich, F. A., Wright, R. D. and Schaffer, H. E. (1983). Mineral nutrient status of '*Convexa*' holly cuttings during intermittent mist propagation as influenced by exogenous auxin application. *Journal America Society Horticultural Science*. **108**: 425-429.

Boffa, J.-M. (1999). Agroforestry parklands in Sub-Saharan Africa:. *FAO Conservation Guide*. 34, Food and Agriculture Organization of the United Nations, Rome, Italy.

Bonie, J. M. (1993). *Improved Temotu Traditional Agriculture.* Provincial Press Ltd., Honiara, Solomon Islands.

Botstein, D., White, R. L., Skolnick, M. and Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*. **32**: 314–331.

Bottin, L., Verhaegen, D., Tassin, J., Olivieri, I., Vaillant, A. and Bouvet, J. M. (2005). Genetic diversity and population structure of an insular tree, *Santalum austrocaledonicum* in New Caledonian archipelago. *Molecular Ecology*. **14**: 1979-1989.

Bourke, R. M. (1989). Food, coffee and casuarina: an agroforestry system from Papua New Guinea Highlands. *In* P. K. R. Nair (ed.), *Agroforestry Systems in the Tropics*. 269-275. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Bourke, R. M. (1996). Edible Indigenous Nuts in Papua New Guinea. *In* M. L. Stevens, R. M. Bourke and B. R. Evans (eds), *South Pacific Indigenous Nuts*. 45-55. ACIAR Proceedings No. 69, Canberra, Australia.

Bourke, R. M. (1999). *Vanuatu Agriculture System Survey.* Tropical Agriculture Consultants, Pty Ltd, Canberra, Australia.

Brennan, E. B. and Mudge, K. W. (1998). Vegetative propagation of *Inga feuillei* from shoot cuttings and air layering. *New Forests*. **15**: 37-51.

Bunt, C. and Leakey, R. (*in press*). Domestication potential and marketing of *Canarium indicum* nuts in the Pacific: 4. Commercialisation and market development. *Agroforestry Systems*. **0:** 000-000.

Bussell, J. D., Waycott, M. and Chappill, J. A. (2005). Arbitrarily amplified DNA markers as characters for phylogenetic. *Perspectives in Plant Ecology, Evolution and Systematics*. **7**: 3-26.

Cannell, M. G. R. (1989). Food Crop Potential of Tropical Trees. *Experimental Agriculture*. **25**: 313-326.

Cavers, S., Navarro, C. and Lowe, A. J. (2003). A combination of molecular markers identifies evolutionarily signifcant units in *Cedrela odorata* L. (Meliaceae) in Costa Rica. *Conservation Genetics*. **4**: 571-580.

Cavers, S., Bandou, E., Caron, H., Colpaert, N., Gheysen, G. and Lowe, A. J. (*unpublished*). A technique for sampling for DNA analysis of trees: trunk cambium as an alternative to canopy leaves.

Cavers, S., Degen, B., Caron, H., Lemes, M. R., Margis, R., Salgueiro, F. and Lowe, A. J. (2005). Optimal sampling strategy for estimation of spatial genetic structure in tree populations. *Heredity*. **95**: 281-289.

CBSI (2001). *Annual Report 2000.* Honiara, Solomon Islands.

Chase, L. D. C., Prasad, R. A. and Morrison, R. J. (1986). Classification of some benchmark soils from Solomon Islands. *Environmental Studies Report no. 29*, Institute of Natural Resources, University of the South Pacific, Suva, Fiji.

Christensen, M. V., Eriksen, E. N. and Andersen, A. S. (1980). Interactions of stockplant irradiance and auxin in the propagation of apple rootstocks by cuttings. *Science Horticulture*. **12**: 11-17.

Christie, B. and Nichols, M. (1999). The contributions that Massey University and horticulture can make to the domestication of agroforestry trees. *In* J. M. Roshetko and D. O. Evans (eds), *Domestication of Agroforestry Trees in Southeast Asia*. 77-82. Winrock International in collaboration with ICRAF, Yogyakarta, Indonesia.

Clarke, W. C. and Thaman, R. R. (1993). *Agroforestry in the Pacific Islands : Systems for Sustainability.* United Nations University Press, Tokyo.

Clement, C. R. and Villachica, H. (1994). Amazonian fruits and nuts: potential for domestication in various agroecosystems. *In* R. R. B. Leakey and A. C. Newton (eds), *Tropical Trees: the Potential for Domestication and the Rebuilding of Forest Resources*. 230-238. HMSO, London, UK.

Clifford, H. T. and Williams, W. T. (1976). Similarity measures. *In* W. T. Williams (ed.), *Pattern Analysis in Agricultural Science*. 37-46. CSIRO, Melbourne.

Clifford, H. T. and Ludlow, G. (1978). *Keys to the Families and Genera of Queensland Flowering Plants (Magnoliophyta).* University of Queensland Press, St, Lucia, Queensland, Australia.

Coates, D. J. and Byrne, M. (2005). Genetic variation in plant populations: assessing cause and pattern. *In* R. J. Henry (ed.), *Plant Diversity and Evolution: Genotypic and Phenotypic Variation in Higher Plants*. 139-164. CABI Publishing, Wallingford, UK.

Collion, M. H., Kissi, A. and Reed, M. (1993). *A Trainer's Guide to Program Planning and Priority Setting.* The Hague, The Netherlands.

Conway, G. (1997). *The Doubly Green Revolution: Food for all in the 21st Century.* Penguin Books Ltd, London, UK.

Cooper, P. J. M., Leakey, R. R. B. and Rao, M. R. (1996). Agroforestry and the mitigation of land degradation in the humid and sub-humid tropics of Africa. *Experimental Agriculture*. **32**: 235-290.

Copes, D. L. (1970). *Effect of date of grafting on survival in Douglas-fir.* Experimental Station. PNW, USA.

Copes, D. L. (1975). *Graft incompatibility in Pinus contorta.* NW Forest Range Experimental Station. PNW, USA.

Das, P., Basak, U. C. and Das, A. B. (1997). Metabolic changes during rooting in pre-girdled stem cuttings and air-layers of *Heritiera*. *Botanical Bulletin of Academia Sinica*. **38**: 91-95.

de Foresta, H. and Michon, G. (1994). Agroforests in Sumatra: Where ecology meets economy. *Agroforestry today*. **6**: 12-13.

Dell, B., Malajczuk, N. and Grove, T. S. (1995). Nutrient Disorders in Plantation *Eucalyptus. ACIAR Monograph*. 31, ACIAR, Canberra, Australia.

Dick, J., Magingo, F., Smith, R. I. and McBeath, C. (1999). Rooting ability of *Leucaena leucocephala* stem cuttings. *Agroforestry Systems*. **42**: 149-157.

Dick, J. M. and Dewar, R. C. (1992). A mechanistic model of carbohydrate dynamics during adventitious root development in leafy cuttings. *Annals of Botany*. **70**: 371-377.

Dick, J. M. and Leakey, R. R. B. (*in press*). Differentiation of the dynamic variables affecting rooting ability in juvenile and mature cuttings of cherry (*Prunus avium*). *Journal of Horticultural Science*.

Dick, J. M., Blackburn, D. G. and McBeath, C. (1994). Stem respiration in leafy cuttings of *Prospis juliflora* during the rooting process. *New Forests*. **8**: 179-184.

Elevitch, R. C. and Wilkinson, K. M. (eds) (2000). *Agroforestry Guides for Pacific Islands.* Permanent Agriculture Resources, Holualoa, Hawaii, USA.

Eliasson, L. and Brunes, L. (1980). Light effects on root formation in *Pisum sativum* cuttings. *Physiologia Plantarum*. **48**: 261-265.

Evans, B. (1996). Overview of Resource Potential for Indigenous nut Production in the South Pacific. *In* M. L. Stevens, Bourke, R.M., and Evans, B.R. (ed.), *South Pacific Indigenous Nuts.* 10-28. ACIAR Proceedings No. 69, Canberra, Australia.

Evans, B. (1999). *Edible Nut Trees in Solomon Islands: A variety collection of Canarium, Terminalia and Barringtonia.* ACIAR Technical Report No. 44, Canberra, Australia.

Excoffier, L., Smouse, P. E. and Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitocondrial DNA restriction sites. *Genetics*. **131**: 479-491.

Falvey, D. A., Colwell, J. B., Coleman, P. J., Greene, H. G., Vedder, J. G. and Bruns, T. R. (1991). Petroleum Prospectivity of Pacific Island Arc: Solomon Islands and Vanuatu. *The APEA Journal*. **31**: 191-212.

FAO (1989). *Forestry and Food Security.* Food and Agriculture Organization of the United Nations, Rome.

FAO (1995). *Non-wood Forest Products for Rural Income and Sustainable Forestry.* Food and Agriculture Organisation, Rome, Italy.

FAO (1996). Forest Resources Assessment 1990. Survey of tropical forest cover and study of change processes. *FAO Forestry Papers No. 130*. Rome, Italy.

FAO (1998). *Committee on world food security. Twenty-fourth session. Guidelines for national food insecurity and vulnerability information and mapping systems (FIVIMS).* FAO of United Nations, Rome, Italy.

FAO (2000). *Global Forest Resources Assessment*. [webpage], Retrieved from http://www.fao.org/forestry/fo/fra/index.jsp.

FAO Corporate Document Repository (2002). *The state of food insecurity in the world 2002*. [webpage], Retrieved from http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/005/y7352e/y7352e00.htm.

Fett-Neto, A. G., Fett, J. P., Goulart, L. W. V., Pasquali, G., Termignoni, R. R. and Ferreira, A. G. (2001). Distinct effects of auxin and light on adventitious root development in *Eucalyptus saligna* and *Eucalyptus globulus*. *Tree Physiology*. **21**: 457-464.

Frankham, R., Ballou, J. D. and Briscoe, D. A. (2004). *A Primer of Conservation Genetics.* Press Syndicate of the University of Cambridge, Cambridge, UK.

Franzel, S., Jaenicke, H. and Janssen, W. (1996). Choosing the right trees: setting priorities for multipurpose tree improvement. *ISNAR Research Report*. 8, The Hague: International Services for National Agricultural Research.

Gamborg, O. L. and Phillips, G. C. (eds) (1995). *Plant Cell, Tissue and Organ Culture: Fundamental Methods.* Springer-Verlag Berlin Heidelberg, Berlin, Germany.

Gardner, F. P., Pearce, R. B. and Mitchell, R. L. (1985). *Physiology of Crop Plants.* The Iowa State University Press, Iowa, USA.

Garrity, D. P. (2004). Agroforestry and the achievement of the Millennium Development Goals. *Agroforestry Systems*. **61**: 5-17.

Garton, S., Read, P. E. and Farnham, R. S. (1983). Effect of stockplant nutrition on macro-and micro-propagatability of *Salix*. *Acta Horticulturae*. **131**: 141-151.

Gaspar, T. and Hofinger, M. (1988). Auxin metabolism during adventitious rooting. *In* F. T. J. Davies, B. E. Haissig and N. Sankhla (eds), *Adventitious Root Formation in Cuttings*. 345-351. Dioscorides Press, Portland.

Giancola, S., Marcucci, P., Lacaze, P. and Hopp, H. E. (2002). Feasibility of integration of molecular markers and morphological descriptors in a real case study of a plant variety protection system for soybean. *Euphytica*. **127**: 95-113.

Gillies, A. C. M., Navarro, C., Lowe, A. J., Newton, A. C., Hernandez, M., Wilson, J. and Cornelius, J. P. (1999). Genetic diversity in Mesoamerican populations of mahogany (*Swietenia macrophylla*), assessed using RAPDs. *Heredity*. **83**: 722-732.

Giovannelli, A., Gianninig, R., Bennici, A. and Mori, B. (2004). *In Vitro* organogenesis of chestnut (*Castanea sativa* Mill.) cotyledon explants: responses to growth regulators and developmental aspects. *In Vitro Cellular and Development Biology - Plant,*. **40**: 509-514.

Giridhar, P., Indu, E. P., Ravishankar, G. A. and Chandrasekar, A. (2004). Influence of Triacontanol on somatic embryogenesis in *Coffea arabica* L. and *Coffea canephorap*. Ex FR. I. *In Vitro Cellular and Developmental Biology - Plant*. **40**: 200-203.

Gomes, F. and Canhoto, J. M. (2003). Micropropagation of *Eucalyptus nitens* Maiden (Shining Gum). *In Vitro Cellular and Development Biology - Plant,*. **39**: 316-321.

Government Population Statistics (1999). *Population and Housing Census.* Honiara, Solomon Islands.

Gowers, S. (1976). *Some Common Trees of the New Herbrides and their Vernacular Names.* Education Department, British Residency, Port Vila, Vanuatu.

Hackett, W. P. (1988). Donor Plant Maturation and Adventitious Root Formation. *In* T. D. Davis, B. E. Haissig and N. Sankhla (eds), *Adventitious Root Formation in Cuttings*. 11-28. Dioscorides Press, Portland.

Haley, N. C. 2001. Impact of the 1997 drought in the Hewa area of Southern Highlands Province. Paper presented to *Food Security for Papua New Guinea. Proceedings of the Papua New Guinea Food and Nutrition 2000 Conference, PNG University of Technology, Lae, 26-30 June 2000.*

Hamrick, J. L., Godt, M. J. W. and Murawski, D. A. (1991). Correlations between species traits and allozyme diversity: Implications for conservation biology. *In* D. Falk and K. Holsinger (eds), *Genetics and Conservation of rare plants*. 75-119. Oxford University Press, Inc., Oxford, UK.

Hamrick, J. L., Murawski, D. A. and Nason, J. D. (1993). The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. *Vegetatio*. **107/108**: 281-297.

Hansell, J. R. F. and Wall, J. R. D. (1975). *Land Resources of Solomon Islands, Vol. 4: New Georgia Group and the Russell Islands. Land Resources Study 18.* Land Resources Division, Surrey, England.

Hansen, J., Stromquist, L. H. and Ericsson, A. (1978). Influence of the irrandiance on carbohydrate content and rooting of cuttings of pine seedlings (*Pinus sylvestris*). *Plant Physiology*. **61**: 975-979.

Hansen, M., Kraft, T., Christiansen, M. and Nilsson, N. O. (1999). Evaluation of AFLP in *Beta*. *Theoretical and Applied Genetics*. **98**: 845-852.

Harlan, J. R. (1975). *Crops and Man.* American Society of Agronomy/Crop Science Society of America, Wisconsin, USA.

Hartl, D. L. and Clark, A. G. (1989). *Principles of Population Genetics.* Sinauer Associates, Sunderland, MA.

Hartmann, H. T., Kester, D. E., Davies, F. T. J. and Geneve, R. L. (1997). *Plant Propagation: Principles and Practices.* 6th edn, Prentice Hall, Englewood Cliffs, NJ.

Harwood, C. (1999). Domestication of Australian tree species for agroforestry. *In* J. M. Roshetko and D. O. Evans (eds), *Domestication of Agroforestry Trees in Southeast Pacific*. 64-72. Winrock International in collaboration with ICRAF, Yogyakarta, Indonesia.

Haun, R. and Cornell, W. (1951). Rooting response of geranum (*Pelargonium hortorum*) cuttings as influenced by nitrogen, phosphorus and potassium nutrition of stockplants. *American Social and Horticultural Science*. **58**: 317-323.

Hedren, M., Fay, M. F. and Chase, M. W. (2001). Amplified fragment length polymorphisms (AFLP) reveal details of polyploid evolution in *Dactylorhiza* (Orchidaceae). *American Journal of Botany*. **88**: 1868-1880.

Henderson, C. P. and Hancock, I. R. (1988). *A Guide to the Useful Plants of Solomon Islands.* Research Department, MAL, Honiara, Solomon Islands.

Henry, R. J. (2005). Importance of plant diversity. Genotypic and phenotypic variation in higher plants. *In* R. J. Henry (ed.), *Plant Diversity and Evolution*. 1-5. CABI Publishing, Wallingford, UK.

Hoad, S. P. and Leakey, R. R. B. (1994). Effects of light quality on gas exchange and dry matter partioning in *Eucalyptus grandis* W. Hill ex Maiden. *Forest Ecology and Management*. **70**: 265-273.

Hoad, S. P. and Leakey, R. R. B. (1996). Effects of pre-severance light quality on the vegetative propagation of *Eucalyptus grandis* W. Hill ex Maiden. *Trees*. **10**: 317-324.

Hollingsworth, P. M., Dawson, I. K., Goodall-Copestake, W. P., Richardson, J. E., Weber, J. C., Montes, S. C. and Pennington, R. T. (2005). Do farmers reduce genetic diversity when they domesticate tropical trees? A case study from Amazonia. *Molecular Ecology*. **14**: 497-501.

Holst, J. J., Santon, J. A. and Yeatman, C. W. (1956). Greenhouse grafting of spruce and hard pine. *Technical Notes, Canadian Department of Forestry, Forest Research Division, Petawawa Forest Experiment Station, Chalk River*. **33**: 170-175.

Homma, A. K.O. (1994). Plant extractivisim in the Amazon. Limitations and possibilities *In* M. Clusener-Godt and I. Sachs (ed.), *Extractivism in the Brazilian Amazon: Perspectives on Regional Development, MAB Digest 18*. MAn and the Biosphere, UNESCO, Paris, France.

Houenipwela, R. N. (2004). *CBSI 2003 Annual Report.* Honiara, Solomon Islands.

Huang, S.-C. and Millikan, D. F. (1980). *In vitro* micrografting of apple shoot tips. *Horticultural Science*. **15**: 741-743.

Hubbard, R. M., Bond, B. J., Senock, R. S. and Ryan, M. G. (2002). Effects of branch height on leaf gas exchange, branch hydraulic conductance and branch sap flux in open-grown ponderosa pine. *Tree Physiology*. **22**: 575-581.

Huxley, P. (1999). *Tropical Agroforestry.* Blackwell Science, Cambridge, UK.

ICRAF (1996). *1995 Annual report.* International Centre for Research in Agroforestry, Nairobi, Kenya.

ICRAF (1997). *ICRAF Medium Term Plan 1998-2000.* Niarobi: ICRAF.

Indiana University Soil Geomorphology Laboratory (2005). *Bulk density determination procedures*. [webpage], Retrieved from http://www.geology.iupui.edu/research/SoilsLab/procedures/bulk/Index.htm.

Invitrogen Life Technologies (2003). *AFLP® Analysis System I: AFLP® Starter Primer Kit version B*. [pdf], Retrieved from http://www.invitrogen.com/content/sfs/manuals/aflpi_man.pdf.

Jaenicke, H., Franzel, S. and Boland, D. J. (1995). Towards a method to set priorities amongst species for tree improvement research - a case study from west Africa. *Journal of Tropical Forest Science*. **7**: 490-506.

Janick, J. (1979). Horticulture's ancient roots. *Horticultural Science*. **14**: 299-313.

Jansen, T. and Tutua, J. (2001). Indigenous knowledge of forest food plants: a component of food security in the Solomon Islands. *In* R. M. Bourke, M. G. Allen and J. G. Salisbury (eds), *Food Security for Papua New Guinea: Proceedings of the Papua New Guinea Food and Nutrition 2000 Conference*. 112-123. ACIAR Proceedings No.99, PNG University of Technology, Lae, Papua New Guinea.

Jebb, M. (1992). Edible Barringtonias. *Kew Magazine*. **15**: 164-180.

Jeffree, C. E. and Yeoman, M. M. (1983). Development of intercellular connections between opposing cells in a graft union. *New Phytologist*. **93**: 481-509.

Jeffreys, A. J., McLeod, A., Tamaki, K., Neil, D. L. and Monckton, D. G. (1991). Minisatellite repeat coding as a digital approach to DNA typing. *Nature*. **354**: 204-209.

John, S. E. T. and Brouard, J. S. (1995). Genetic improvement of indigenous fruit trees in the SADC region. *In* A. J. Maghembe, Y. Ntupanyama and P. W. Chirwa (eds), *Improvement of Indigenous Fruit trees of the Miombo Woodlands of Southern Africa*. 12-24. ICRAF, Nairobi, Keyna.

Jones, C. J., Edwards, K. J., Castaglione, M. O., Winfield, M. O., Sala, F., van de Wiel, C., Vosman, B., Matthes, M., Daly, A., Brettschneider, R., Maestri, E., Marmiroli, N., Aert, R., Volckaert, G. and Karp, A. (1998). Reproducibility testing of AFLPs by a network of European laboratories. *In* A. Karp, P. G. Isaac and D. S. Ingram (eds), *Molecular Tools for Screening Biodiversity*. 191-192. Chapman and Hall, London, UK.

Jordan, C. F. (1991). Nutrient cycling processes and tropical forest management. *In* A. Gomez-Pompa, T. C. Whitmore and M. Hadley (eds), *Rain Forest Regeneration and Management*. 159-180. Parthenon Publishing Group, Paris.

Kadzere, I., Chilanga, G. T., Ramadhani, T., Lungu, S., Malembo, N. L., Rukuni, D., Simwanza, P. P., Rarieya, M. and Maghembe, A. J. (1998). Choice of priority indigenous fruits for domestication in southern Africa. *In* A. J. Maghembe, A. J. Simons, F. Kwesiga and M. Rarieya (eds), *Selecting indigenous trees for domestication in southern Africa: Priority setting with farmers in Malawi, Tanzania, Zambia and Zimbabwe*. 1-15. ICRAF, Nairobi, Kenya.

Kashkush, K., Jinggui, F., Tomer, E., Hillel, J. and Lavi, U. (2001). Cultivar identification and genetic map of mango (*Mangifera indica*). *Euphytica*. **122**: 129-136.

Kathiresan, K. and Ravikumar, S. (1995). Vegetative propagation through air-layering in two species of mangroves. *Aquatic Botany*. **50**: 107-110.

Kengni, E., Tchoundjeu, Z., Tchouanguep, F. M. and Mbofung, C. M. F. (2001). Sensory evaluation on *Dacryodes edulis* fruit types. *Forest Trees and Livelihoods*. **11**: 57-66.

KFPL (1998). *Kolombangara Forest Products Limited Year Book.* Ringgi, Kolombangara Island.

KFPL (1999). *KFPL Management Plan (1999 - 2003).* Ringgi, Kolombangara Island.

Kochba, J., Spiegel-Roy, P., Neumann, H. and Saad, S. (1982). Effect of carbohydrates on somatic embryogenesis in subcultured nucellar callus of *Citrus* cultivars. *Zeitschrift fur Pflanzenphysiologie*. **105**: 359-368.

Komissarov, D. A. (1969). *Biological Basis for the Propagation of Woody Plants by Cuttings.* Edited by M Kohn, translated by Z. Shapiro, Israel Program for Scientific Translations, IPST Press, Jerusalem, IS.

Kornerup, A. and Wanscher, J. H. (1967). *Methuen Handbook of Colour.* Methuen & Co. Ltd, London, UK.

Krauss, S. L. (2000). Patterns of mating in *Persoonia mollis* (Proteaceae) revealed an analysis of paternity using AFLP: implications for conservation. *Australian Journal of Botany*. **48**: 349-356.

Kruskal, J. B. (1964). Nonmetric multidimensional scaling: a numerical method. *Psychometrika*. **29**: 115-129.

Langens-Gerrits, M., Albers, M. and DeKlerk, G.-J. (1998). Hot-Water Treatment before tissue culture reduces initial contamination in *Lilium* and *Acer*. *Plant Cell Tissue and Organ Culture*. **52**: 75-77.

Larcher, W. (1980). *Physiological Plant Ecology.* Springer-Verlag, Berlin, Germany.

Leakey, R. R. B. (1983). Stockplant factors affectng root initiation in cuttings of *Triplochiton scleroxylon* K. Schum., an indigenous hardwood of West Africa. *Journal of Horticultural Science*. **58**: 277-290.

Leakey, R. R. B. (1985). The capacity for vegetative propagation in trees. *In* M. G. R. Cannell and J. E. Jackson (eds), *Attributes of Trees as Crop Plants*. 110-133. Institute of Terrestrial Ecology, Midlothian, Scotland.

Leakey, R. R. B. (1990). *Nauclea diderrichii*: rooting of cuttings, clonal varation in shoot dominance, and branch plagiotropism. *Trees*. **4**: 164-169.

Leakey, R. R. B. (1991). Clonal Forestry: towards a strategy. Some guidelines based on experience with tropical trees. *In* J. E. Jackson (ed.), *Tree Breeding and Improvement*. Royal Forestry Society of England, Wales and Northern Ireland, Tring, England.

Leakey, R. R. B. (1996). Definition of agroforestry revisited. *Agroforestry today*. **8**: 5-7.

Leakey, R. R. B. (1998). The use of biodiversity and implications for agroforestry. *In* D. E. Leihner and T. A. Mitschein (eds), *A Third Millennium for Humanity? The Search for Paths of Sustainable Development*. 43-58. Peter Lang, Frankfurt, Germany.

Leakey, R. R. B. (1999). Potential for novel food products from agroforestry trees: a review. *Food Chemistry*. **66**: 1-14.

Leakey, R. R. B. (2001). Win:Win landuse strategies for Africa: 1. Building on experience with agroforestry in Asia and Latin America. *International Forestry Review*. **3**: 1-10.

Leakey, R. R. B. 2004a. Clonal approaches to hardwood forestry in the tropics. Paper presented to *Proceedings of a workshop presented by Private Forestry North Queensland at the Centre for Tropical Agriculture, Mareeba, North Queensland, 19th – 21st October 2004*, Published as a CD-ROM by Private Forestry North Queensland Association Inc., Kairi, Qld.: 1-13.

Leakey, R. R. B. (2004b). Physiology of vegetative reproduction. *In* J. Burley, J. Evans and J. A. Youngquist (eds), *Encyclopaedia of Forest Sciences*. 1655-1668. Academic Press, London, UK.

Leakey, R. R. B. (2005). Domestication potential of Marula (*Sclerocarya birrea* subsp. *caffra*) in South Africa and Namibia: 3. Multiple trait selection. *Agroforestry Systems*. **64**: 51-59.

Leakey, R. R. B. and Mohammed, H. R. S. (1985). Effects of stem length or root initiation in sequential single-node cuttings of *Triplochiton scleroxylon*, K. Schum. *Journal Horticultural Science*. **60**: 431-437.

Leakey, R. R. B. and Coutts, M. P. (1989). The dynamcs of rooting in *Triplochiton scleroxylon* cuttings: their relation to leaf area, node position, dry weight accumulation, leaf water potental and carbohydrate composition. *Tree Physiology*. **5**: 135-146.

Leakey, R. R. B. and Storeton-West, R. (1992). The rooting ability *Triplochiton scleroxylon* K. cuttings: interactions between stockplant irradiance, light quality and nutrients. *Forest Ecology and Management*. **49**: 133-150.

Leakey, R. R. B. and Ladipo, D. O. (1996). Trading on genetic variation - fruits of *Dacryodes edulis*. *Agroforestry today*. **8**: 16-17.

Leakey, R. R. B. and Simons, A. J. (1998). The domestication and commercialisation of indigenous trees in agroforestry for the alleviation of poverty. *Agroforestry Systems*. **38**: 165-176.

Leakey, R. R. B. and Tomich, T. P. (1999). Domestication of tropical trees: from biology to economics and policy. *In* L. E. Buck, Lassoie, J.P., and Fernandes, E.C.M. (ed.), *Agroforestry in Sustainable Agricultural Systems*. 319-338. Lewis Publishers, Boca Raton, Florida, USA.

Leakey, R. R. B. and Simons, A. J. (2000). When does vegetative propagation provide a viable alternative to propagation by seed in forestry and agroforestry in the tropics and sub-tropics. *In* H. Wolf and J. Albrecht (eds), *Problem of Forestry in Tropical and Subtropical Countries - The Procurement of Forestry Seed - The Example of Kenya*. 67-81. Ulmer Verlag, Germany.

Leakey, R. R. B. and Page, T. (*in press*). The 'ideotype concept' and its application to the selection of cultivars of trees providing agroforestry tree products. *Forest Trees and Livelihoods*. **0**: 000-000.

Leakey, R. R. B., Chapman, V. R. and Longman, K. A. (1982a). Physiological studies for tropical tree improvement and conservation. Some factors affecting root initiation in cuttings of *Triplochiton scleroxylon* (K. Schum). *Forest Ecology Management*. **4**: 53-66.

Leakey, R. R. B., Last, F. T. and Longman, K. A. (1982b). Domestication of tropical trees: An approach securing future productivity and diversity in managed ecosystems. *Commonwealth Forest Review*. **61**: 33-41.

Leakey, R. R. B., Schreckenberg, K. and Tchoundjeu, Z. (2003). The participatory domestication of West African indigenous fruits. *International Forestry Review*. **5**: 338-347.

Leakey, R. R. B., Shackleton, S. and du Plessis, P. (2005a). Domestication potential of Marula (*Sclerocarya birrea* subsp. *caffra*) in South Africa and Namibia: 1. Phenotypic variation in fruit traits. *Agroforestry Systems*. **64**: 25-35.

Leakey, R. R. B., Pate, K. and Lombard, C. (2005b). Domestication potential of Marula (*Sclerocarya birrea* subsp *caffra*) in South Africa and Namibia: 2. Phenotypic variation in nut and kernel traits. *Agroforestry Systems*. **64**: 37-49.

Leakey, R. R. B., Fondoun, J.-M., Atangana, A. and Tchoundjeu, Z. (2000). Quantitative descriptors of variation in the fruits and seeds of *Irvingia gabonensis*. *Agroforestry Systems*. **50**: 47-58.

Leakey, R. R. B., Atangana, A. R., Kengni, E., Waruhiu, A. N. and Usoro, C. (2002). Domestication of *Dacryodes edulis* in West and Central Africa: characterisation of genetic variation. *Forests, Trees and Livelihoods*. **12**: 57-71.

Leakey, R. R. B., Tchoundjeu, Z., Schreckenberg, K., Schackleton, S. E. and Schackleton, C. M. (2005c). Agroforestry Tree Products (AFTPs): Targeting Poverty Reduction and Enhanced Livelihoods. *International Journal for Agricultural Sustainability*. **0**: 000-000.

Leakey, R. R. B., Greenwell, P., Hall, M. N., Atangana, A. R., Usoro, C., Anegbeh, P. O., Fondoun, J.-M. and Tchoundjeu, Z. (2005d). Domestication of *Irvingia gabonensis*: 4. Tree-to-tree variation in food-thickening properties and in fat and protein contents of dika nut. *Food Chemistry*. **90**: 365-378.

Leakey, R. R. B., Mesen, J. F., Tchoundjeu, Z., Longman, K. A., Dick, J. M., Newton, A. C., Matin, A., Grace, J., Munro, R. C. and Muthoka, P. N. (1990). Low-technology techniques for the vegetative propagation of tropical trees. *Commonwealth Forest Review*. **69**: 247-257.

Leakey, R. R. B., Tchoundjeu, Z., Smith, R. I., Munro, R. C., Fondoun, J.-M., Kengue, J., Anegbeh, P. O., Atangana, A. R., Waruhiu, A. N., Asaah, E., Usoro, C. and Ukafor, V. (2004). Evidence that subsistence farmers have domesticated indigenous fruits (*Dacryodes edulis* and *Irvingia gabonensis*) in Cameroon and Nigeria. *Agroforestry Systems*. **60**: 101-111.

Leakey, R. R. B. and Newton, A. C. (eds) (1994a). *Domestication of Tropical Trees for Timber and Non-Timber Products.* MAB Digest/UNESCO17, UNESCO, Paris.

Leakey, R. R. B. and Newton, A. C. (eds) (1994b). *Domestication of "Cinderella" species as the start of a woody-plant revolution. Tropical Trees: the Potential for Domestication and the Rebuilding of Forest Resources*. HMSO, London, UK.

Leakey, R. R. B., Temu, A. B., Melnyk, M. and Vantomme, P. (eds) (1996). *Domestication and Commercialization of Non-Wood Forest Products in Agroforestry Systems*. Food and Agriculture Organization of the United Nations No. 9, Rome, Italy.

Leakey, R. R. B., Fuller, S., Treloar, T., Steveson, L., Hunter, D., Nevenimo, T., Binifa, J. and Moxon, J. (*in press*). E otential and marketing of *Canarium indicum* nuts in the pacific: 3. characterization of tree-to-tree variation in morphological, nutritional and medicinal properties. *Agroforestry Systems*. **0**: 000-000.

Lepping, G. (2000). *A Report on SPRIG Rapid Rural Appraisal Survey of Priority Species in the Solomon Islands.* South Pacific Regional Initiative on Forest Genetic Resources, Honiara, Solomon Islands.

Lin, J.-J. and Kuo, J. (1995). AFLP: A novel PCR-based assay for plant and bacterial DNA fingerprinting. *Focus*. **17**: 66-70.

Lo, Y. N. (1985). Root initiation of *Shorea macrophylla* cuttings: effects of node position, growth regulators and misting regime. *Forest Ecology and Management*. **12**: 43-52.

Loach, K. (1986). Rooting of cuttings in relation to the propagation medium. *Proceedings of the International Plant Propagators' Society*. **35**: 472-485.

Longman, K. A. (1993). *Rooting Cuttings of Tropical Trees.* Commonwealth Science Council, ECTF, Penicuik EH26 0PH, Scotland.

Lowe, A. J., Harris, S. A. and Ashton, P. (2004). *Ecological Genetics: Design, Analysis and Application.* Blackwells, Oxford.

Lowe, A. J., Gillies, A. C. M., Wilson, J. and Dawson, I. K. (2000). Conservation genetics of bush mango from central/west Africa: implications from random amplified polymorphic DNA analysis. *Molecular Ecology*. **9**: 831-841.

Lowe, A. J., Jourde, B., Breyne, P., Colpaert, N., Navarro, C., Wilson, J. and Cavers, S. (2003). Fine-scale genetic structure and gene flow within Costa Rican populations of mahogany (*Swietenia macrophylla*). *Heredity*. **90**: 268-275.

Macfarlane, D. (1999). Smallholder grazing under whitewood. *In* S. Rogers and P. Thorpe (eds), *Pacific Agroforestry: An information kit*. 4-15. Pacific Regional Agricultural Programme, Suva, Fiji.

Mackay, E. (1988). *Report on Farming Systems Survey in Solomon Islands.* Unpublished report, Honiara, Solomon Islands.

Maghembe, A. J., Ntupanyama, Y. and Chirwa, P. W. (eds) (1995). *Improvement of Indigenous Fruit Trees of the Miombo Woodlands of Southern Africa.* Proceedings of a conference held on 23-27 January 1994 at Club Makokola, Mangochi, Malawi, International Centre for Research in Agroforestry, Nairobi, Kenya.

Maghembe, A. J., Simons, A. J., Kwesiga, F. and Rarieya, M. (eds) (1998). *Selecting Indigenous Trees for Domestication in Southern Africa: Priority Setting with Farmers in Malawi, Tanzania, Zambia and Zimbabwe.* ICRAF, Nairobi, Kenya.

Manner, H. I. (1993). Traditional agriculture in Buma. *In* W. C. Clarke and R. R. Thaman (eds), *Agroforestry in the Pacific Islands. Systems for sustainability*. 12-15. United Nations University Press, Tokyo.

Mariette, S., Corre, L. E., Austerlitz, F. and Kremer, A. (2002). Sampling within the genome for measuring within population diversity: trade-offs between markers. *Molecular Ecology*. **11**: 1145-1156.

Marshall, D. R. and Brown, H. D. (1975). Optimum sampling strategies in genetic conservation. *In* O. H. Frankel and J. G. Hawkes (eds), *Crop Genetic Resources for Today and Tomorrow*. 53-80. Cambridge University Press, Cambridge, UK.

Mauseth, J. D. (2003). *Botany: An Introduction to Plant Biology.* Jones and Barlett Publishers, Inc., Boston, USA.

Maynard, B. K. and Bassuk, N. L. (1990). Comparisons of stock plant etiolation with traditional propagation methods. *Combined Proceedings of the International Plant Propagation Society*. **40**: 517-523.

Mbofung, C. M. F., Silou, T. and Mouragadja, I. (2002). Chemical characterisation of Safou (*Dacryodes edulis*) and evaluation of its potential as an ingredient in nutritious biscuits. *Forests, Trees and Livelihoods*. **12**: 105-118.

McCown, B. H. (2000). *In Vitro* plant recalcitrance. Recalcitrance of Woody and Herbaceous Perennial Plants: Dealing with Genetic Predeterminism. *In Vitro Cellular and Development Biology - Plant*. **36**: 149-154.

McDonald, P. and Lassoie, J. P. (eds) (1996). *The Literature of Forestry and Agroforestry.* Cornell University Press, Ithaca, NY.

McGregor, A. M. and McGregor, I. K. (1997). *Establishing a commercial indigenous nut industry in Fiji: Opportunities and Requirements.* United Nations ESCAP/POC.

Mengel, K. and Kirkby, E. A. (1982). *Principles of Plant Nutrition.* 3rd edn, International Potash Institute, Berne.

Menzel, C. (2002). *Propagation and Establishment.* http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/005/ac681e/ac681e07.htm.

Mesén, F., Newton, A. C. and Leakey, R. R. B. (1997a). The effects of propagation environment and foliar areas on the rooting physiology of *Cordia alliodora* (Ruiz & Pavon) Oken cuttings. *Trees*. **11**: 401-411.

Mesén, F., Newton, A. C. and Leakey, R. R. B. (1997b). Vegetative Propagation of *Cordia alliodora* (Ruiz & Pavon) Oken: the effects of IBA concentration, propagation medium and cutting origin. *Forest Ecology and Management*. **92**: 45-54.

Mesén, F., Leakey, R. R. B. and Newton, A. C. (2001). The influence of stockplant environment on morphology, physiology and rooting of leafy stem cuttings of *Albizia guachapele*. *New Forests*. **22**: 213-227.

Mesén, J. F. (1993). Vegetative Propagation of Central American Hardwoods. *PhD thesis*, *University of Edinburgh*, Edinburgh, Scotland.

MFEC (1995). *The Forest of the Solomon Islands, Volume One: National Overview & Methods.* Solomon Islands National Forest Resources Inventory, ACIL Australia Pty Ltd. International Forest Environment Research and Management Pty Ltd, ERSIS Australia Pty Ltd.

Minghe, L. and Ritchie, G. A. (1999). Eight hundred years of clonal forestry in China: 1 Traditional afforestation with Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.). *New Forests*. **18**: 131-142.

Moe, R. and Andersen, A. S. (1988). Stockplant environment and subsequent adventitious rooting. *In* T. D. Davis, B. E. Haissig and N. Sankhla (eds), *Adventitious Root Formation in Cuttings*. 214-234. Dioscorides Press, Portland.

Moestrup, S. (1999). Introduction to DANIDA Forest Seed Centre. *In* J. M. Roshetko and D. O. Evans (eds), *Domestication of Agrofrestry Trees in Southeast Asia*. 61-63. Winrock International in collaboration with ICRAF, Yogyakarta, Indonesia.

MOF (1995). Rural Areas of Solomon Islands: Income and Expenditure Survey 1993. *Statistical Bulletin No. 18/95*. Honiara, Solomon Islands.

Moore, D. S. (2004). *The Basic Practice of Statistics.* 3rd edn, W.H. Freeman and Company, New York.

Mudge, K. W. and Brennan, E. B. (1999). Clonal propagation of multipurpose and fruit trees used in agroforestry. *In* L. E. Buck, J. P. Lassoie and E. C. M.

Fernandes (eds), *Agroforestry in Sustainable Agricultural Systems*. 157-190. CRC and Lewis Publishers, New York, USA.

Mueller, U. G. and Wolfenbarger, L. L. (1999). AFLP genotyping and fingerprinting. *Trees*. **14**: 389-394.

Nair, P. K. R. (1993). *An Introduction to Agroforestry.* Kluwer Academic Publishers, Dordrecht, The Netherlands.

Nair, P. K. R. (2001). Do tropical homegardens elude science, or is it the other way around? *Agroforestry Systems*. **53**: 239-245.

Nair, P. K. R. (ed.) (1989). *Agroforestry Systems in the Tropics.* Forestry sciences 31, Kluwer Academic Publishers in co-operation with ICRAF, Dordrecht, The Netherlands.

Nakalevu, T. and Seru, V. (1999). *Calliandra* in Fiji. *In* S. Rogers and P. Thorpe (eds), *Pacific Agroforestry: An information kit*. 3-81. Pacific Regional Agricultural Programme, Suva, Fiji.

Nakasako, M., Iwata, T., Inoue, K. and Tokutomi, S. (2005). Light-induced global structural change in phytochrome A regulating photomorhogenesis in plants. *The FEBS Journal*. **272**: 603-612.

Nei, M. (1987). *Molecular Evolutionary Genetics.* New York, USA.

Nevenimo, T., Johnson, M., Binifa, J., Gwabu, C., Angen, J. and Leakey, R. (*in press*). Domestication potential and marketing of *Canarium indicum* nuts in the Pacific: 2. Producer and Consumer surveys in Papua New Guinea (East New Briatain). *Agroforestry Systems*. **0**: 000-000.

Nevenimo, T., Moxon, J, Wemin, J., Johnson, M., Bunt, C. and Leakey, R. (*in press*). Domestication potential and marketing of *Canarium indicum* nuts in the Pacific:1. A literature review. *Agroforestry Systems*. **0**: 000-000.

Newton, A. C. and Jones, A. C. (1993). The water status of leafy cuttings of four tropical tree species in mist and non-mist propagation system. *Journal of Horticultural Science*. **68**: 653-663.

Newton, A. C., Muthoka, P. N. and Dick, J. M. (1992). The influence of leaf area on the rooting physiology of leafy stem cuttings of *Terminalia spinosa* Engl. *Trees*. **6**: 210-215.

Newton, A. C., Allnutt, T. R., Gillies, A. C. M., Lowe, A. J. and Ennos, R. A. (1999). Molecular phylogeography, intraspecific variation and the conservation of tree species. *Trees*. **14**: 140-145.

Ngungu, J., Jaenicke, H. and Boland, D. (1995). Considerations for germplasm collections of indigenous fruit trees in the miombo. *In* A. J. Maghembe, Y. Ntupanyama and P. W. Chirwa (eds), *Improvement of Indigenous Fruit*

Trees of the Miombo Woodlands of Southern Africa. 1-11. ICRAF, Nairobi, Keyna.

Nketiah, T., Newton, A. C. and Leakey, R. R. B. (1999). Vegetative propagation of *Triplochiton scleroxylon* in Ghana: effect of cutting origin. *Journal of Tropical Forest Science*. **11**: 512-515.

Novick, R. R., Dick, C. W., Lemes, M. R., Navarro, C., Caccone, A. and Bermingham, E. (2003). Genetic structure of Mesoamerican populations of big-leaf mahogany (*Swietenia macrophylla*) inferred from microsatellite analysis. *Molecular Ecology*. **12**: 2885-2893.

Nybom, H. (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*. **13**: 1143-1155.

Nybom, H. and Bartish, V. (2000). Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution and Systematics*. **3**: 93-114.

Ofori, D. A., Newton, A. C., Leakey, R. R. B. and Grace, J. (1996). Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of auxin concentration, leaf area and rooting medium. *Forest Ecology and Management*. **84**: 39-48.

O'Hanlon, P. C. and Peakall, R. (2000). A simple method for the detection of size homoplasy among amplified fragment length polymorphism fragments. *Molecular Ecology*. **9**: 815-816.

Okafor, J. C. (1983). Varietal delimitation in *Dacryodes edulis* (G. Don) H.J. Lam. (Burseraceae). *International Tree Crops Journal*. **2**: 255-265.

Okano, K., Uematsu, C., Matsunaga, H. and Kambara, H. (1998). Characteristics of selective polymerase chain reaction (PCR) using two-based anchored primers and improvement of its specificity. *Electrophoresis*. **19**: 3071-3078.

Okoro, O. O. (1976). Some factors affecting successful "take" of *Pinus caribaea* grafts. *Nigerian Journal of Forestry*. **6**: 20-23.

Padoch, C. and de Jong, W. (1987). Traditional agroforestry practices of native and ribereno farmers in the lowland Peruvian Amazon. *In* H. L. Gholz (ed.), *Agroforestry: Realities, Possibilities and Potentials*. 178-194. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

Parker, P. G., Snow, A. A., Schug, M. D., Booton, G. C. and Fuerst, P. A. (1998). What molecules can tell us about populations: Choosing and using a molecular markers. *Ecology and Society*. **79**: 361-382.

Pauku, R. L. (2005a). *Barringtonia procera (Cutnut)*. Retrieved from http//:www.traditionaltree.org.

Pauku, R. L. (2005b). *Inocarpus fagifer (Tahitian chestnut)*. Retrieved from http//:www.traditionaltree.org.

Payens, J. P. D. W. (1967). A Monograph of Genus *Barringtonia* (Lecythidaceae). *Blumea*. **15**: 157-263.

Peakall, R. and Smouse, P. E. (2005). *GenAlEx v6: Genetic Analysis in Excel. Population genetics software for teaching and research.* Canberra, Australia.

Pedley, L., Henderson, R. J. F. and Reynolds, S. T. (1995). *Flora of South-east Queensland, Volume 1*. Department of Primary Industries, State of Queensland, Australia.

Pelomo, M. P., Barasi, R. N., Liloqula, R. and Roposi, N. (1996). *Canarium* nut and oil marketing in Solomon Islands. *In* M. L. Stevens, R. M. Bourke and B. R. Evans (eds), *South Pacific Indigenous Nuts*. 76-78. ACIAR Proceedings No. 69, Canberra, Australia.

Pinto, G., Valentim, H., Costa, A., Castro, S. and Santos, C. (2002). Somatic embryogenesis in leaf callus from a mature *Quercus suber* L. tree. *In Vitro Cellular and Development Biology - Plant*. **38**: 569-572.

Pinyopusarerk, K. and Williams, E. R. (2000). Range-wide provenance variation in growth and morphological characteristics of *Casuarina equisetifolia* grown in Northern Australia. *Forest Ecology and Management*. **134**: 219-232.

Posey, D. (1982). Forest Islands (Kayapo Example). *In* C. R. Elevitch and K. M. Wilkinson (eds), *The Overstorey book. Cultivating Connections with Trees*. 14-20. Permanent Agriculture Resources, Holualoa, USA.

Pothier, D., Margolis, H. A., Poliquin, J. and Waring, R. H. (1989). Relationship between the permeability and the antomy of jack pine sapwood with stand development. *Canadian Journal of Forest Reserach*. **19**: 1564-1570.

Pottinger, A. (1999). Domestication of agroforestry trees: experiences of the Oxford Forestry Institute. *In* J. M. Roshetko and D. O. Evans (eds), *Domestication of Agroforestry Trees in Southeast Asia*. 73-76. Winrock International in collaboration with ICRAF, Yogyakarta, Indonesia.

Prance, G. T. (1994). Amazonian tree diversity and the potential for supply of non-timber forest products. *In* R. R. B. Leakey and A. C. Newton (eds), *Tropical Trees: the Potential for Domestication and the Rebuilding of Forest Resources*. 7-15. HMSO, London, UK.

Pushpakumara, D. K. N. G., Simons, A. J. and Gunasena, H. P. M. (1999). Reproductive biology and improvement of *Artocarpus heterophyllus* in Sri Lanka. *In* J. M. Roshetko and D. O. Evans (eds), *Domestication of Agroforestry Trees in Southeast Asia*. 203-207. Winrock International in collaboration with ICRAF, Yogyakart, Indonesia.

Radler, H. (1999). Multiplurpose trees and shrubs on farmland in Fiji. *In* S. Rogers and P. Thorpe (eds), *Pacific Agroforestry: An Information Kit*. 2-8. Pacific Regional Agricultural Programme, Suva, Fiji.

Raintree, J. B. (1991). *Social-economic Attributes of Trees and Tree Planting Practices.* Community Forestry Note No. 9, Rome.

Ratukalou, I., Nakalevu, T., Waradi, J., Hertel, H. and Reigber, E. (2000). *Agroforestry: A way to Better Farming - A Manual for Trainers, Teachers and Extension Workers.* Model 1, Fiji Ministry of Agriculture, Fisheries and Forestry (MAFF) and SPC/GTZ German Regional Forestry Project (PGRFP), Suva, Fiji.

Raynor, B. (1991). *Agroforestry Systems in Pohnpei: Practices and Strategies for Development.* UNDP/FAO South Pacific Forestry Development Programme, Suva, Fiji.

Raynor, W. C. and Fownes, J. H. (1991). Indigenous agroforestry of Pohnpei, 1. Plant species and cultivars. *Agroforestry Systems*. **16**: 139-157.

Ribeiro, M. M., Mariette, S., Vendramin, G. G., Szmidt, A. E. and Plomion, C. (2002). Comparison of genetic diversity estimates within and among populations of maritime pine using chloroplast length polymorphism data. *Molecular Ecology*. **11**: 869-877.

Robinson, R. 2001. Subsistence at Lake Kopiago, Southern Highlands Province, during and following the 1997-1998 drought. Paper presented to *Food Security for Papua New Guinea. Proceedings of the Papua New Guinea Food and Nutrition 2000 Conference*, PNG University of Technology, Lae, 26-30 June 2000

Rocheleau, M. D. E. (1987). The user perspective and the agroforestry research and action agenda. *In* H. L. Gholz (ed.), *Agroforestry: Realities, Possibilities and Potentials*. 59-87. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

Rogers, S. and Thorpe, P. (eds) (1999). *Pacific Agroforestry: An Information Kit.* Pacific Regional Agricultural Programme, Suva, Fiji.

Rohlf, F. J. (1998). *Numerical Taxonomy and Multivariate Analysis System 2.02i.* State University of New York, Exeter Software, Setauket, NY.

Roshetko, J. M. and Evans, B. R. (eds) (1999). *Domestication of agroforestry trees in Southeast Asia.* Forest, Farm and Community Tree Research Reports, Winrock International in collaboration with ICRAF, Yogyakart, Indonesia.

Ruiz-Perez, M., Belcher, B., Achdiawan, R., Alexiades, M., Aubertin, C., Caballero, J., Campbell, B., Clement, C., Cunningham, T., Fantini, A., de Foresta, H., Garcia Fernandez, C., Gautam, K. H., Hersch Martinez, P., de Jong, W., Kusters, K., Kutty, M. G., Lopez, C., Fu, M., Martinez Alfaro, M. A., Nair, T. R., Ndoye, O., Ocampo, R., Rai, N., Ricker, M., Schreckenberg, K., Schackleton, S., Shanley, P., Sunderland, T. and Youn, Y. (2004). Markets drive the specialisation strategies of forest peoples. *Ecology and Society*. **9**: 4.

Ruthenberg, H. (1980). *Farming Systems in the Tropics.* Claredon, Oxford, UK.

Sanchez, P. A. (1995). Science in agroforestry. *In* F. L. Sinclair (ed.), *Agroforestry: Science, Policy and Practice*. 5-55. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Sanchez, P. A., Buresh, R. J. and Leakey, R. R. B. (1997). Trees, Soils and Food Security. *In* D. J. Greenland, P. J. Gregory and P. H. Nye (eds), *Land Resources: on the Edge of the Malthusian Precipice*, 352. 949-961. CAB International and the Royal Society of London, Wallingford and London.

Saupe, S. G. (2004). *Plant Hormones - Auxin*. [webpage], Retrieved from http://employees.csbsju.edu/ssaupe/biol327/Lecture/hormone-auxin.htm.

Schafer, K. V. R., Oren, R. and Tenhunen, J. D. (2000). The effects of tree height on crown level stomatal conductance. *Plant Cell and Environment*. **23**: 365-377.

Schneck, V. (1997). Studies on the influence of clone on rooting ability and quality in the propagation of cuttings from 40 to 350-year old yews (*Taxus baccata* L.). *Silvae Genetica*. **45**: 246-249.

Schneider, S., Roessli, D. and Excoffier, L. (2000). *Arlequin ver 2.000. A Software for Population Genetics Data Analysis.* University of Geneva, Geneva, Switzerland.

Schreckenberg, K., Degrande, A., Mbosso, C., Baboule, B., Boyd, C., Enyoung, L., Kanmegne, J. and Ngong, C. (2002). The social and economic importance of *Dacryodes edulis* (G.Don) H.J. Lam. in Southern Cameroon. *Forests, Trees and Livelihoods*. **12**: 15-40.

Scott, K. and Playford, J. (1996). DNA extraction technique for PCR in rainforest plant species. *Biotechniques*. **20**: 974-975.

Scott, L. J., Shepherd, M., Henry, R. J., Dieters, M. and Nikles, D. G. (*in press*). Low efficiency of pseduotestcross mapping design was consistent with

limited genetic diversity and low heterozygosity in hoop pine (*Araucaria cunninghamii*, Araucariaceae). *Tree genetics and genomes*. **0**: 000-000.

Shiembo, P. N., Newton, A. C. and Leakey, R. R. B. (1996). Vegetative propagation of *Irvingia gabonensis*, a West African fruit tree. *Forest Ecology and Management*. **87**: 185-192.

Silva, J. J. and Debergh, P. (2001). Somatic embryogenesis from flower explants of cocoa (Theobroma cacao L.). *Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet.* **66**: 31-34.

Simons, A. J. (1996). ICRAF's strategy for domestication of indigenous tree species. *In* R. R. B. Leakey, A. B. Temu, M. Melnyk and P. Vantomme (eds), *Domestication and Commercialisation of Non-Timber Forest Products in Agroforestry Systems*. 8-22. FAO, Rome, Italy.

Simons, A. J. and Leakey, R. R. B. (2004). Tree domestication in tropical agroforestry. *Agroforestry Systems*. **61**: 167-181.

Sinclair, F. L. and Walker, D. H. (1999). A utilitarian approach to the incorporation of local knowledge in agroforestry research and exension. *In* L. E. Buck, J. P. Lassoie and E. C. M. Fernandes (eds), *Agroforestry in Sustainable Agricultural Systems*. 245-275. Lewis Publishers, Boca Raton, Florida, USA.

Sirikolo, M. Q. and Gua, B. (1999). *A Report on the State of the Forest Genetic Resources of Priority Species in the Solomon Islands*. Honiara, Solomon Islands.

Sneath, P. and Sokal, R. (1973). *Numerical Taxonomy: The Principles and Practices of Numerical Classification.* San Francisco, W.H. Freeman and Co.

Solomon Islands Meteorological Service (2002). *Climate Information*. [webpage], Retrieved from http://www.met.gov.sb.

Steger, M. M. and Preece, J. E. 2005. The influence of source tree on somatic embryogenesis from easter balck walnut (*Juglans nigra*) immature cotyledons. Paper presented to *ISHS Acta Horticulturae 625: XXVI International Horticultural Congress: Biotechnology in Horticultural Crop Improvement: Achievements, Opportunities and Limitations*: Retrieved from http://www.actahort.org/members/showpdf?booknrarnr=625_628.

Strydom, D. K. and Hartmann, H. T. (1960). Absorption, distribution, and destruction of indoleacetic acid in plum stem cuttings. *Plant Physiology*. **35**: 435-442.

Suhardi (1999). *Gnetum gnemon* and its prospects in agroforestry. *In* J. M. Roshetko and D. O. Evans (eds), *Domestication of Agroforestry Trees in*

Southeast Asia. 171-174. Winrock International in collaboration with ICRAF, Yogyakarta, Indonesia.

Taji, A. and Williams, R. (1996). Overview of plant tissue culture. *In* A. Taji and R. Williams (eds), *Tissue Culture of Australia*. 312. University of New England, Armidale, N.S.W.

Taji, A. M., Dodd, W. A. and Williams, R. R. (1993). *Plant Tissue Culture Practice.* 2nd edn, The University of New England, Armidale, N.S.W.

Tanksley, S. D., Young, N. D., Paterson, A. H. and Bonierbale, M. W. (1989). RFLP Mapping in Plant Breeding: New Tools for an Old Science. *Bio-Technology*. **7**: 257-264.

Tchoundjeu, Z. (1989). Vegetative Propagation of the Tropical Hardwoods: *Khaya ivorensis* A. Chev. and *Lovoa trichilioides* Harms. *PhD thesis*, *University of Edinburgh*, Edinburgh, Scotland.

Tchoundjeu, Z. and Leakey, R. R. B. (1996). Vegetative propagation of African Mahogany: effects of auxin, node position, leaf area and cutting length. *New Forests*. **11**: 125-136.

Tchoundjeu, Z. and Leakey, R. R. B. (2000). Vegetative propagation of *Khaya ivorensis* (African Mahogany): Effects of stockplant flushing cycle, auxin and leaf area on carbohydrate and nutrient dynamics of cuttings. *Journal of Tropical Forest Science*. **12**: 77-91.

Tchoundjeu, Z. and Leakey, R. R. B. (2001). Vegetative propagation of *Lovoa trichilioides*: Effects of provenance, substrate, auxins and leaf area. *Journal of Tropical Forest Science*. **13**: 116-129.

Tchoundjeu, Z., Duguma, B., Fondoun, J.-M. and Kengue, J. (1998). Strategy for the domestication of indigenous fruit trees of West Africa: case of *Irvingia gabonesis* in sourthern Cameroon. *Cameroon Journal of Biology and Biochemical Sciences*. **4**: 21-28.

Tchoundjeu, Z., Duguma, B., Anegbeh, P., Degrande, A., Mbile, P., Facheux, C., Tsobeng, A., Atangana, A., and Ngo-Mpeck, M-L. (2006). AFTPs. Putting participatory domestication into practice in West and Central Africa. *Forest, Trees and Livelihoods.* **16**: 53-69.

Tchoundjeu, Z., Asaach, E., Fondoun, J.-M. and Kengue, J. (1998). Strategy for the domestication of indigenous fruit trees of West Africa: case of *Irvingia gabonesis* in sourthern Cameroon. *Cameroon Journal of Biology and Biochemical Sciences*. **4**: 21-28.

Tejwani, K. G. (1987). Agroforestry practices and research in India. *In* H. L. Gholz (ed.), *Agroforestry: Realities, Possibilities and Potentials*. 109-136. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

Teklehaimanot, Z., Tomlinson, H., Lemma, T. and Reeves, K. (1996). Vegetative Propagation of *Parkia biglobosa* (Jacq.) Benth., an undomesticated fruit tree from West Africa. *Journal of Horticultural Science*. **72**: 205-215.

Thaman, R. R. (1990). *Kiribati Agroforestry: Trees, People and the Atoll Environment.* Smithsonian Institute, National Museum of National History, Washington, D.C.

Thaman, R. R. (1994). Land, Plant, Animals and People: Community-Based Biodiversity Conservation (CBBC) as a Basis for Ecological, Cultural and Economic Survival in the Pacific Islands. *Pacific Science Association Information Bulletin*. **46**: 1-16.

Thaman, R. R. (1999). Concepts and information related to the protection and development of atoll agroforestry systems in the Pacific Islands. *In* R. Wescom and S. Bulai (eds), *Report on Sub-regional Training Workshop on Agroforestry/Drum Ovens for Atoll Islands, 03-16 August 1999. With an Annotated Checklist of Important Pacific Atoll Agroforestry Species.* SPC/UNDP/AusAID/FAO Pacific Islands Forests and Trees Support Programme, Suva, Fiji.

Thaman, R. R. 2001. Trees of Life: Trees outside forests and agroforestry as a foundation for biodiversity conservation and sustainable development in the small island states of the Pacific islands. Paper presented to *FAO Regional Forestry Workshop on Trees Outside Forests*, Nadi, Fiji.

Thaman, R. R. and Whistler, W. A. (1996). A review of uses and status of trees and forests in landuse systems in Samoa, Tonga, Kiribati and Tuvalu with Recommendations for Future Action. RAS/92/361. *Working Paper*. 5, UNDP/FAO South Pacific Forestry Development Programme, Suva, Fiji.

Thimann, K. V. (1985). Plant growth hormone produced by *Rhizopus sinuis*. *Journal of Biological Chemistry*. **109**: 279-291.

Thiong'o, M. K., Kingori, S. and Jaenicke, H. (2002). The taste of the wild: Variation in the nutritional quality of marula fruits and opportunities for domestication. *Acta Horticulturae*. **575**: 237-244.

Thomas, P. and Schiefelbein, J. W. (2004). Roles of leaf in regulation of root and shoot growth from single node softwood cuttings of grape (Vitis vinifera). *Annals of Applied Biology*. **144**: 27-37.

Thomas, T. D. and Puthur, J. T. (2004). Thidiazuron induced high frequency shoot organogenesis in callus from Kigelia pinnata L. *Botanical Bulletin of Academia Sinica*. **45**: 307-313.

Thomaszewski, M. and Thimann, K. V. (1966). Interactions of phenolic acids, metallic ions, and chelating agents on auxin induced growth. *Plant Physiology*. **41**: 1443-1454.

Tofinga, M. 1996. Overview of Farming Systems and Development for the South Pacific: History, Players, and Issues. Paper presented to *Proceedings of an FAO/IRETA Workshop on The Farming Systems Approach To Sustainable Agriculture Development in the South Pacific*, Suva, Fiji: 30-32.

Tribe, D. (1994). IFeeding and Greening the World. The role of International Agricultural Research, CABI International, Wallingford, UK 274p.

Ubaitoi, I. (1999). Home gardens as an atoll agroforestry system in Kiribati. *In* S. Rogers and P. Thorpe (eds), *Pacific Agroforestry: An Information Kit*. 2-6. Pacific Regional Agricultural Programme, Suva, Fiji.

United Nations (2002). *Report of the Secretary General on Implementation of the Millenium Declaration*. [webpage], Retrieved from http://www.un.org/millenniumgoals/MDG-Page1.pdf.

UNPF (2004a). *State of World Population: Population and the Environment*. [webpage], Retrieved from http://www.unfpa.org/swp/2004/english/ch3/index.htm.

UNPF (2004b). *State of World Population: Population and the Environment [page 2]*. [webpage], Retrieved from http://www.unfpa.org/swp/2004/english/ch3/page2.htm.

UNPF (2004c). *State of World Population*. Retrieved from http://www.unfpa.org/swp/2004/english/ch1/page7.htm#1.

Veierskov, B. (1988). Relations between carbohydrates and adventitious root formation. *In* T. D. Davis, B. E. Haissig and N. Sankhla (eds), *Adventitious Root Formation in Cuttngs*. 70-78. Dioscorides Press, Oregon, USA.

Vekemans, X., Beauwens, T., Lemaire, M. and Roldan-Ruiz, I. (2002). Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology*. **11**: 139-151.

Vergara, N. T. (1987). Agroforestry: A sustainable land use for fragile ecosystems in the humid tropics. *In* H. L. Gholz (ed.), *Agroforestry: Realities, Possibilities and Potentials*. 7-19. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*. **23**: 4407-4414.

Wagner, W. L., Herbst, D. R. and Sohmer, S. H. (1990). *A Manual on the Flowering Plants of Hawai'i. Volume 1.* University of Hawai'i Press, Honolulu, USA.

Wairiu, M. M. (2001). Erosion and Land Use Effects on Soils Quality and Crop Yield on Sloping Lands in Solomon Islands. *PhD thesis*, *The Ohio State University*, Ohio, USA.

Walker, D. H., Sinclair, F. L. and Thapa, B. (1995). Incorporation of indigenous knowledge and persepctives in agroforestry development. Part 1: Review of methods and their application. *In* F. L. Sinclair (ed.), *Agroforestry: Science, Policy and Practice*. 235-248. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Walter, A. and Sam, C. (2002). *Fruits of Oceania.* [translated by P. Ferra from Fruits d'Océanie], ACIAR Monograph 85, ACIAR, Canberra, Australia.

Wareing, P. F. and Frydman, V. M. (1976). General aspects of phase change, with special reference to *Hedera helix* L. *Acta Horticulturae*. **56**: 75-69.

Waruhiu, A. N. (1999). Characterization of fruit traits towards domestication of an indigenous fruit tree of west and central Africa: A case study of *Dacryodes edulis* in Cameroon. *MSc thesis*, *University of Edinburgh*, Edinburgh, UK.

Waruhiu, A. N., Kengue, J., Atangana, A. R., Tchoundjeu, Z. and Leakey, R. R. B. (2004). Domestication of *Dacryodes edulis*. 2. Phenotypic variation of fruit traits in 200 trees from four populations in the humid lowlands of Cameroon. *Food, Agriculture and Environment*. **2**: 340-346.

WCFSD (1999). *Our Forests - Our future.* Summary Report: World Commission on Forests and Sustainable Development, WCFSD. Winnipeg.

Webb, M., Reddell, P. and Pauku, R. L. (1999). Significance of Soil Nutrient Limitations to the Growth of Forest Plantations. *Pacific Islands Forests and Trees*, 2/99, pp. 11-12.

Weber, J., Sotelo-Montes, C. and Labarta-Chavarri (1997). Tree domestication in the Peruvian Amazon Basin - Working with farmers for community development. *Agroforestry today*. **9**: 4-8.

Weinig, C. (2002). Phytochromes photoreceptors mediate plasticity to light quality in flowers of Brassicaceae. *American Journal of Botany*. **89**: 230-235.

Wescom, R. W. and Sairusi, B. (1999). *Report on Sub-Regional Training Workshop on Agroforestry/Drum Ovens for Atoll Islands*. With an Annotated Checklist of Important Pacific Island Atoll Agroforestry Species. SPC/UNDP/AusAID/FAO Pacific Islands Forests and Trees Support Programme, Suva, Fiji.

Whitelam, G. C., Johnson, E., Peng, J., Carol, P., Anderson, M. L., Cowl, J. S. and Harberd, N. P. (1993). Phytochrome A null mutants of *Arabidopsis* display a wild-type phenotype in white light. *The Plant Cell*. **5**: 757-768.

Whitmore, T. C. (1966). *Guide to the Forests of the British Solomon Islands.* Oxford University Press, London.

Whitmore, T. C. (1969). The vegetation of the Solomon Islands. *Philosophical Transactions of the Royal Society of London B*. **255**: 259-270.

Whitmore, T. C. (1974). *Changes with Time and the Role of Cyclones in Tropical Rainforest on Kolombangara, Solomon Islands.* Commonwealth Forestry Institute, University of Oxford, Oxford, UK.

WHO (2005). *Premature Death*. [webpage], Retrieved from http://www.who.int/en/.

Wiersum, K. F. (1996). Domestication of valuable tree species in agroforestry systems: evolutionary stages from gathering to breeding. *In* R. R. B. Leakey, A. B. Temu and M. Melnyk (eds), *Domestication and Commercialisation of Non-timber Forest Products in Agroforestry Systems*. 147-159. FAO, Rome, Italy.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*. **18**: 6531-6535.

Wojtkowski, P. A. (1998). *The Theory and Practice of Agroforestry Design : A comprehensive study of the theories, concepts and conventions that underlie the successful use of agroforestry.* Science Publishers, Inc., Enfield, NH.

World Agroforestry Centre (2005). *Our History*. [webpage], Retrieved from http://www.worldagroforestry.org/.

Young, A. (1989). *Agroforestry for Soil Conservation.* 2nd edn, CAB International, Wallingford, UK.

Zietkiewicz, E., Rafalski, A. and Labuda, D. (1994). Genomic fingerprinting by simaple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*. **20**: 176-183.

Appendices

Appendix 2.1: Major agroforestry practices and their characteristics (Legend: w = woody; h = herbaceous; f = fodder for grazing; and a = animals). Source: Adapted from Nair (1991) cited in Nair (1993)

Agroforestry practices	Brief description (of arrangement of components	Major groups of component	Agroecological adaptability
Agrisilviculture systems (crops including shrub/vine/tree crops and trees)			
(1) Improved fallow	Woody species planted and left to grow during the 'fallow phase'	w: fast-growing preferably leguminous h: common agriculture crops	In shifting cultivation areas
(2) Taungya	Combined stand of woody and agricultural species during early stages of establishment of plantations	w: usually plantation forestry spp. h: common agricultural crops	All ecological regions (where taungya is practiced); several improvements possible
(3) Alley cropping (hedgerow intercropping)	Woody species in hedges; agricultural species in alleys in between hedges; microzonal or strip arrangements	w: fast-growing, leguminous, that coppice vigourously h: common agricultural crops	Subhumid to humid areas with high human population pressure and fragile (productive but easily degradable) soils
(4) Multilayer trees on crop lands	Multispecies, multilayer dense plant associations with no organised planting arrangements	w: different woody components of varying form and growth habits h: usually absent; shade tolerant ones sometimes present	Areas with fertile soils, good availability of labour, and high human population pressure
(5) Multipurpose trees on crop land	Trees scattered haphardly or according to some systematic patterns on bunds, terraces or plot/field boundaries	w: multipurpose trees and other fruit trees h: common agricultural crops	In all ecological regions esp. in subsistence farming; also commonly integrated with animals.
(6) Plantation crop combinations	(i) Integrated multi-storey (mixed, dense) mixtures of plantation crops (ii) Mixture of plantation crops in alternate or other regular arrangement (iii) Shade trees for plantation crops; shade trees scattered (iv) Intercropping with agricultural crops	w: plantation crops like coffee, cacao, coconut, etc. and fruit trees, esp. in (i); fuelwood/fodder spp., esp. in (iii) h: usually present in (iv), and to some extent in (i); shade-tolerant species	In humid lowlands or tropical humid/subhumid highlands (depending on the plantation crops concerned); usually in smallholder subsistence system
(7) Homegardens	Intimate, multi-storey combination of various trees and crops around homesteads	w: fruit trees predominate; also other woody species, vines, etc. h: shade tolerant agricultural species	In all ecological regions, esp. in areas of high population density
(8) Trees in soil conservation and reclamation	Trees on bunds, terraces, raisers, etc. with or without grass strips; trees fro soil reclamation	w: multipurpose and/or fruit trees h: common agricultural crops	In sloping areas, esp. in highlands, reclamation of degraded, acid, alkali soils, and sand-dune stabilisation
(9) Shelterbelts and windbreaks, live hedges	Trees around farmland/plots	w: combination of tall-growing spreading types	In wind-prone areas

		h: agricultural crops of the locality	
(10) Fuelwood production	Interplanting firewood species on or around agricultural lands	w: firewood species h: agricultural crops of the locality	In all ecological regions
Silvopastoral systems (trees + pasture and/or animal)			
(11) Trees on rangeland or pastures	Trees scattered irregularly or arranged according to some systematic pattern	w: multipurpose; of fodder value f: present a: present	Extensive grazing areas
(12) Protein banks	Production of protein-rich tree fodder on farm/rangelands for cut-and-carry fodder production	w: leguminous fodder trees h: present f: present	Usually in areas with high person : land ratio
(13) Plantation crops with pastures and animals	Example: cattle under coconuts in south-east Asia and the south Pacific	w: plantation crops f: present a: present	In areas with less pressure on plantation crop lands
Agrosilvopastoral systems (trees + crops + pasture/animals)			
(14) Homegardens involving animals	Intimate, multi-storey combination of various trees and crops, and animals, around homesteads	w: fruit trees predominate; also other woody species a: present	In all ecological regions with high density of human population.
(15) Multipurpose woody hedgerows	Woody hedge for browse, mulch, green manure, soil conservation, etc.	w: fast-growing and coppicing fodder shrubs and trees h: (similar to alley cropping and soil conservation)	Humid to subhumid areas with hilly and sloping terrain
(16) Apiculture with trees	Trees for honey production	w: honey producing (other components may be present)	Depending on the feasibility of apiculture
(17) Aquaforestry	Trees lining fish ponds, tree leaves being used as 'forage' for fish	w: trees and shrubs preferred by fish (other components may be present)	Lowlands
(18) Multipurpose woodlots	For various purpose (wood, fodder, soil protection, soil reclamation, etc.)	w: multipurpose species; special location-specific species (other components may be present)	Various

Appendix 3.1. Land Systems in Kolombangara Island. The area (km^2 and %) described includes also areas surveyed on other islands in the New Georgia group. (Source: Wall and Hansell (1975))

1. Ringgi Land System

Land facet	*Area (km^2.%)*	*Landform*	*Soil*	**Vegetation and land use**
1	149 56%	Ridge crests: narrow to very broad, crestal slope almost flat to gently sloping	Deep, yellowish red or reddish brown clay with a deep, dark brown or dark reddish brown topsoil (Haplorthox) Deep, brown to dark brown clay	Lowland Forest now being logged extensively. Dominant species in the canopy include *Pometia pinnata*, *Calophyllum kajewskii*, *Calophyllum vitiense*, *Campnosperma brevipetiola*, *Terminalia calamansanai* and *Gmelina moluccana*. *Dillenia salomonensis* occurs in western and southern area of Kolombangara only. *Alangium javanicum* and *Horsefieldia irya* are common small trees. Subsistence cultivation in a few coastal areas
2	74 28%	Hill slopes: very short to short, straight or convex, moderate to moderately steep (10-45^o)		
3	27 10%	Gullies and lower hill slopes: ultra-short to very short slopes, straight to concave, steep (25-45^o)	Moderately deep, strong brown clay with few weathered rock fragments	
4	10 4%	Coastal margins: gentle, even crestal slope with moderate to moderately steep valley sides; limestone outcrops in places	Moderately deep to deep, yellowish red clay overlying coral (Tropudalfs) Shallow, brown to dark brown clay overlying coral (Lithic Rendolls)	Lowland Forest containing many *Pometia pinnata*, *Celtis* sp. and *Calophyllum kajewskii* Some well-established coconut estates
5	5 2%	Valleys: commonly incised, less than 30 m wide with intermittent small streams	Shallow to deep, dark sand and loams with stony subsoils (Tropepts, Fluvents)	Lowland Forest with large *Terminalia brassii* emergents

2. Patupaele Land System

Land facet	*Area (km^2.%)*	*Landform*	*Soil*	**Vegetation and land use**
1	28 9%	Broad ridges: less than 30m wide with gentle to moderate undulating crestal slopes	Deep, yellowish red clay, commonly beneath deep dark topsoil and thick surface organic matter (Haplorthox, Haplohumox)	Lowland Forest with tall, irregular canopy, containing common Dillenia spp., *Calophyllum kajewskii*, *Calophyllum vitiense*, *Neoscortechinia forbesii*, and *Campnosperma brevipetiolata*. Smaller trees and shrubs include many *Aglaia* sp? *Myristica fatua* and palms such as *Heterospathe woodfordiana*, *Gulubia niniu* and *Caryota rumphiana*. At ground level are *Pandanus* sp. and at high altitudes, *Freycinetia* sp. There are scattered small area under shifting cultivation in Kolombangara
2	177 57%	Slopes: medium to long, moderate to steep and straight in gross form. Mainly stable where less than 20^o	Deep, strong brown clay, commonly with deep dark topsoil (Haplorthox) Shallow to deep, dark loams and clays, commonly with mottled stony subsoil (Tropepts, Tropohumults)	
3	59 19%	Narrow ridges: less than 15 m wide with steep adjacent slopes	Deep, reddish brown clay, commonly with deep dark topsoil (Haplorthox)	
4	46 15%	Valleys: less than 100 m wide with boulder deposits	Not seen	*Terminalia brassii* forest seen in places from air

3. Serambuni Land System

Land facet	*Area (km^2.%)*	*Landform*	*Soil*	**Vegetation and land use**
1	98 48%	Terraces: slopes<20°, narrow, sinuous, maximum width 500 m, height 1-4 m	Deep, brown to dark brown clay loam to clay (Eutropepts)	Commonly used for shifting agriculture and for coconut groves and plantations Disturbed forest with *Pometia pinnata*, *Vitex cofassus*, *Celtis iatifolia*, *Ficus* spp. and *Alangium javanicum* common. The canopy is commonly broken and secondary regrowth is common
2	85 35%	Alluvial plains: almost flat slopes, maximum width 1 km, slightly hummocky microrelief, levees absent	Deep brown to yellowish brown, well to imperfectly or poor drained, interstratified clays and loams, locally mottled (Tropofluvents)	
3	49 20%	Channels and depressions: channels mostly sinuous, maximum width 80 m, depressions irregular, slightly concave	Shallow to deep, poorly drained,greyish brown to grey, commonly mottled clay loam or clay, rarely with organic-rich horizons (Aquents)	Lowland Forest similar to above but including *Terminalia brassii*, *Eugenia tierneyana*, *Campnosperma brevi petiolata* and *Intsia bijuga* Unvegetated river channels
4	12 5%	Colluvial fans: slopes 2-13°, slightly convex, maximum length 50-150 m irregular microrelief	Moderately deep to shallow clay to sandy loam intermixed with rocks and boulders	Disturbed Lowland Forest with many palms, gingers, heliconias and *Calamus* sp.

4. Lomousa Land System

Land facet	***Area (km^2.%)***	***Landform***	***Soil***	**Vegetation and Land use**
1	50 30% (est)	Coral platforms: slightly elevated above sea level, irregular micro-relief from coral outcrops	Shallow stony sands over coral, poorly drained in places (Lithic Troporthents)	In areas remote from habitation, particularly on New Georgia and Kolombangara, much beach forest remains. The canopy is irregular with common emergents dominated (68 spp. identified from 57 sites) by *Intsia bijuga*,*Pometia pinnata*, *Calophyllum kajewskii*, *Heritiera littoralis* and *Ficus* spp. In the undergrowth are common *Buchananian arborescens*, *Diospuros* sp., *Alangium. javanicum* and *H. littoralis* Many areas have been cleared for coconuts. The undergrowth in these gardens comprises *Ficus septica*,*Timonius timon*, *Morinda citrifolia*, *Premna corymbosa* and *Antidesma* sp. gingers and several types of fern
2	100 60% (est)	Beaches: linear, low and gently undulating or level	Moderately shallow to moderately deep, poorly excessively drained, dark sands or sandy loam over coral (Troporthents) Moderately shallow to deep, poorly to excessively drained, commonly stony pale sands, over coral (Troporthents, Tropopsamments)	
3	13 10% (est)	Inland margins: colluvial footslopes and floodplain fringes	Moderately shallow to moderately deep, well drained, yellowish brown sands or sandy loam (Tropopsamments) Moderately shallow to moderately deep, well to imperfectly drained, dark loams over sands (Eutropepts)	

5. Pururaghi Land System.

Land facet	*Area (km^2.%)*	*Landform*	*Soil*	**Vegetation and land use**
1	72 20% (est)	Coastal margin swamps: discontinuous low-level zones behind the littoral deposits, up to 1.5 km wide; watertable above or close to ground level	Moderately deep to shallow, peaty gleyed clay loam or clay over coal (Tropaquents) Shallow, sandy loam or sand over coral (Lithic Troporthents)	Tall mixed stands of *Calophyllum kajewski*i, *Eugenia tierneyana*, *Campnosperma brevipetiolata*, *Vitex cofassus* and scattered *Terminalia brassii*. Common small trees include *Alangium javani*, *Calophyllum cerasiferum*,*Horsefieldia spicata* with *Pandanus* spp. very common Areas of disturbed forest with much planted Metroxylon salomonense
2	110 30% (est)	Swampy river tracts: sinuous valleys up to 1.2 km wide along low-gradient river valleys; water at or close to ground level	Deep, poorly drained, pale brown or gleyed clay (Tropaquents)	
			Deep, imperfectly drained, geyish brown clay or clay loam (Tropofluvents)	Tall stands of *Terminalia brassii*
3	145 40% (est)	Deltas and estuaries: low-lying inerriverine areas up to3.5 km wide; hummocky microrelief, common small channels subject to frequent flooding	Deep, poorly drained, pale brown or gleyed clay (Tropaquents) Deep waterlogged peat>40cm (Tropohemists)	Tall *Campnosperma brevipetiola*-dominated forest with *Eugenia effusa*, *C. cerasiferum*, *Calophyllum paludosum*, *Neoscortechinia. forbesii*, *T. brassii* and *Inocarpus. fagifer*. Small trees include *H. spicata* and *Fagraea racemosa* Low, 12-20 m- tall *Pandanus* sp. dominated vegetation with occasional large-crowned trees such as E. effuse or *C. cerasiferum*
4	36 10% (est)	Inland topogenic swamps: closed depressions with a maximum width of 2.5 km with a watertable permanently above ground level	Moderately deep to shallow peat overlying limestone loams or clay (Tropaquents)	

6. Veve Land System

Land facet	*Area (km^2.%)*	*Landform*	*Soil*	**Vegetation and land use**
1	42 30%	Crests: narrow to very broad, uneven profiles; crestal slope, moderate to moderately steep	Deep, yellowish red to red clay commonly overlain by humus horizon of varied thickness, 5-40 cm (Haplohumox) Reddish brown or yellowish brown clay with abundant weathered rock fragments	Lowland Forest of low altitudes becoming smaller with finer crowns above 600 m where mossy-aspect forest dominates. Trees at higher altitudes include *Calophyllum vitiense*, *Eugenia* spp., *Homalium tatambense* and *Ascarina maheshwarii*
2	88 62%	Hill slopes: very short to long, moderately steep to precipitous, straight to irregular; common small spurs and gullies	Brown clay loam with common rock outcrops, unstable and subject to mass movement (Dystropepts)	
3	11 8%	Valley floors: high gradient, narrow, no floodplain development	Poorly sorted stream deposits and large boulders	Not recorded

7. Londumoe Land System

Land facet	*Area (km^2.%)*	*Landform*	*Soil*	**Vegetation and land use**
1	19 56%	Plain: almost flat to gently sloping, maximum width 4.5 km, maximum length 6 km	Dark brown, dark yellowish brown or strong brown clay (Haplorthox) As above with gravels and concretions throughout the profile	Lowland Forest now being widely logged. Common large trees include *Aglaia* sp., *Calophyllum kajewskii*, Alangium javanicum, *Pometia pinnata* and *Vitex cofassus* Small areas under shifting cultivation
2	8 25%	Colluvial/alluvial fans: width 500- 700m, gently sloping, merging inland with volcanic debris slopes	Dark reddish brown, reddish brown or yellowish red clay, mainly colluvial in origin	
3	6 19%	Valley slopes: moderate to moderately steep, rarely steep at gully sides; slope length 70-120m, straight		

8. Kumotu Land System

Land facet	*Area (km^2.%)*	*Landform*	*Soil*	**Vegetation and land use**
1	59 50% (est)	Coral platforms: intertidal, flat, except for irregular coral protrusions; flooded frequently by up to 1.5m seawater	Shallow, very poorly drained, greyish or brownish coral debris (Tropaquents)	Mangrove Forest occurs throughout with a low, even canopy dominated by *Rhizophora* spp. Locally there are concentrations of *Lumnitzera littorea* and species such as *Heritiera littoralis*, *Dolichandrone spathacea*, *Xylocarpus granatum* and *Bruguiera* sp. *Pandanus* spp. and small seedlings form the thin undergrowth.
2	59 50% (est)	Lower reaches of river and inner lagoon flats: intertidal, flat	Moderately shallow, very poorly drained, dark sandy loam or sandy clay loam (Tropaquents) Moderately shallow, very poorly drained, thin dark peat over peal sandy loam to sandy clay loam (Tropaquents, Sulfaquents)	
			Moderately deep to deep, very poorly drained, dark peat and muck, commonly overlying coral (Tropohemists, Sulfihemists)	

9. Tenaru Land System

Land facet	*Area (km^2.%)*	*Landform*	*Soil*	**Vegetation and land use**
1	6 80% (est)	Highest parts of beaches: gently convex, broad	Deep, well to excessively drained, dark to dark grayish brown, loose sand (Tropopsamments, Troporthents)	Widely used for coconuts, villages and tracks. Natural vegetation includes *Casuarina equisetifolia*, *Barringtonia asiatica*, *Terminalia catappa* and *Pandanus* spp.
2	2 20% (est)	Lowest parts of inland beaches: in swales or adjacent to inland swamps	Deep, imperfectly to poorly drained, dark sand overlying pale to dark, mottled or gleyed sand (Aquic Tropopsamments)	Mostly under mixed Lowland Forest and swamp Forest. Canopy species include *Heritiera littoralis*, *Calophyllum inophyllum*, *Inocarpus fagiferus* and *Barringtonia racemosa*. At ground level many *Pandanus* spp.

10. Ndsila Land System

Land facet	*Area (km^2.%)*	*Landform*	*Soil*	**Vegetation and Land use**
1	71 47%	Middle and upper slopes: moderate, medium, convex, locally stony but mostly stable	Deep, red or yellowish red clay over mottled weathering rock (Haplorthox) Deep, dark red or dark reddish brown clay (Orthox, Humitropepts)	Lowland Forest with irregular, broken canopy dominated by *Canarium indicum*, *Canarium salomonense*, *Dillenia* sp. And other species indicating former disturbance, such as *Kleinhovia hospita* and *Campnosperma brevipetiolata*. No dominant small trees but palms in shrub layer and *Selaginella* sp., *Calamus* sp. and *Pandanus* spp. in herb layer Much old regrowth from former cultivation or cyclone damage Small coconut gardens in coastal areas
2	27 18%	Lower and gully slopes: short to medium, straight to irregular, steep to precipitous and stony, mostly unsuitable	Moderately deep to moderately shallow, brownish loams over mottled, soft weathering rock (Eutropepts, Tropudalfs)	
3	36 24%	Ridges: knife- edged to narrow, broad in small areas, uneven profile		
4	9 6%	Rocky knolls: protrude by 3-60m from slopes or ridge lines, irregular, patchily bare surface, cliffed flanks	Shallow dark loams and clays over rock (Troporthents, Eutropepts)	
5	8 5%	Valleys: narrow, stony and bouldery, irregular gradient	No records	

11. Nonoi Land System

Land facet	*Area (km^2.%)*	*Landform*	*Soil*	**Vegetation and land use**
1	8 12%	Ridge summits: broad, rounded even profiles	Deep, dark reddish brown to weak red clay (Haplorthox) Deep, dark brownish, humus-rich clay loam over yellowish red clay (Haplohumox)	Mostly Lowland Forest having a canopy dominated by *Pometia pinnata*, *Dillenia* sp., *Calophyllum vitiense* and *Calophyllum kajewskii* on Vella La Vella and *P. pinnata* and *Celtis latifolia* on Kolombangara. There is a wide variety of smaller trees such as *Gomphandra* sp., several palms. *Selaginella* sp. is the dominant ground cover. Used in the past for shifting cultivation, now rarely used.
2	30 40%	Slopes: moderate to moderately steep ridge slopes, convex, short to long, stable	Shallow to deep, brownish stony loams and clay (Tropepts)	
3	10 13%	Ridges: narrow to knife-edged, even profile; common rounded rock outcrops		
4	15 20%	Slopes: steep to precipitous, ultrs-short, straight to concave, unstable		
5	11 15%	Valleys: narrow with minimal floodplain development, steep gradient	Weakly sorted sands and gravels with stony brown loams	

Appendix 4.1. FARMERS PARTICIPATORY SURVEY QUESTIONNAIRES

4.1.1. To identify the priority indigenous fruit and nut trees in Kolombangara, Solomon Islands

Respondent name:_____

Respondent reference code:_____ Sex: Male_____ Female_____

Age:_____ Village:_____ Location:_____

Respondent title/or role in the village: _____

A. SOCIO-ECONOMIC INFORMATION

1. Household features

(a) What is your marital status? 1 = never married, 2 = married, 3 = divorced, 4 = separated, 5 = widowed, 6 = other (specify):_____

(b) How many children you have?_____ Boys:_____ Girls:_____

(c) Who is the head of the household? 1 = male 2 = female

(d) How many people in your household? _____

(e) What is your highest education level? _____

(f) What did you do for living? 1 = farming, 2 = fishing, 3 = carving, 4 = weaving, 5 = catering, 6 = other (specify):_____

(g) Where do you come from originally? _____

B. INDIGENOUS FRUITS AND NUT SPECIES

1. Species and Utilization

Answers to the following questions must be entered into appropriate spaces provided in the attached table. Details for questions with 'specify' may be entered on this sheet.

(a) [*From the species list*]. Which fruit and nut species are most important to you? Order of priority, 1 = top priority. ……10 = lowest priority.

(b) What part of the plant (fruit and nut species) is eaten? 1 = leaves, 2 = fruit, 3 = seed (kernel), 4 = bark (skin), 5 = none, 6 = other (specify):_____

(c) [*From the species list*]. Are any of these fruit and nut species have been traditionally domesticated? Yes = tick (√), No = cross (x)

(d) If 'yes' how are they domesticated (propagated)? 1 = from root, 2 = from branch, 3 = from fruit (seed), 4 = wood, and 5 = other (specify):_____

(e) [*From the species list*]. Are any of these fruit and nut species traditionally known or used for medicinal purposes? Yes = tick (√), No = cross (x)

(f) If 'yes' which part of the tree is used for medicine? 1 = root, 2 = bark, 3 = leaves, 4 = sap, 5 = flower, 6 = fruit, 7 = wood and 8 = other (specify): _____

(g) [*From the species list*]. Are any of these fruit and nut species traditionally known or used for animal feed (fodder)? Yes = tick (√), No = cross (x)

(h) If 'yes' which parts of the tree is used to feed animal? 1 = root, 2 = bark, 3 = leaves, 4 = sap, 5 = flower, 6 = fruit, 7 = wood and 8 = other (specify): _____

(i) [*From the species list*]. Are any of these fruit and nut species traditionally known or used for shade and shelter (barrier against wind)? Yes = tick (√), No = cross (x)

(j) If 'yes' which fruit and nut species is most effective for shade and shelter? Order of effectiveness, 1 = very high, 2 = high, 3 = moderate, 4 = low and 5 = very low.

(k) [*From the species list*]. Are any of these fruit and nut species traditionally known or used for soil conservation and improvement? Yes = tick (√), No = cross (x)

(l) If 'yes' which fruit and nut species is most effective at this? Order of effectiveness, 1 = very high, 2 = high, 3 = moderate, 4 = low and 5 = very low.

(m) How does it (the species) improve or conserve soil? 1 = enrich soil fertility, 2 = hold soils firmly, 3 = reduces erosion, 4 = dries wetland, 5 = other (specify): _____

(n) [*From the species list*]. Are any of these fruit and nut species traditionally known or used for handicrafts (women for weaning string bags, rope, etc. and men for axe-handle, paddle, etc.)? Yes = tick (√), No = cross (x)

(o) If 'yes' which fruit and nut species is most desirable? Order of desirability, 1 = very high, 2 = high, 3 = moderate, 4 = low and 5 = very low.

(p) [*From the species list*]. Are any of these fruit and nut species traditionally known or used for house construction (as underground post)? Yes = tick (√), No = cross (x)

(q) If 'yes' which fruit and nut species is most desirable? Order of desirability, 1 = very high, 2 = high, 3 = moderate, 4 = low and 5 = very low.

(r) [*From the species list*]. Are any of these fruit and nut species traditionally known or used for house construction (as poles, etc.)? Yes = tick (√), No = cross (x)

(s) If 'yes' which fruit and nut species is most desirable of this? Order of desirability, 1 = very high, 2 = high, 3 = moderate, 4 = low and 5 = very low.

(t) [*From the species list*]. Are any of these fruit and nut species traditionally known or used for carving? Yes = tick (√), No = cross (x)

(u) If 'yes' which fruit and nut species is most desirable? Order of desirability, 1 = very high, 2 = high, 3 = moderate, 4 = low and 5 = very low.

(v) [*From the species list*]. Are any of these fruit and nut species traditionally known or used for timber or log for sale (domestic or export)? Yes = tick (√), No = cross (x)

(w) If 'yes' which fruit and nut species is most in demand? Order of demand, 1 = very high, 2 = high, 3 = moderate, 4 = low and 5 = very low.
(x) [*From the species list*]. Are any of these fruit and nut species known to you that can cause health problems? Yes = tick (√), No = cross (x)

(y) If "yes" what are these health problems? 1 = diarrhoea, 2 = headache, 3 = stomach ache, 4 = boil or swell, 5 = skin rush and 6 = other (specify): _____

2. Tree phenology and improvements

Answers to the following questions must be entered into appropriate spaces provided in the attached table. Details for questions with 'specify' may be entered on this sheet.

(a) [*From the species list*]. When do you notice these fruit and nut species flower? 1 = Jan, 2 = Feb, 3 = Mar, 4 = Apr, 5 = May, 6 = Jun, 7 = Jul, 8 = Aug, 9 = Sep, 10 = Oct, 11 = Nov, 12 = Dec

(b) [*From the species list*]. When do you notice these fruit and nut species set fruit? 1 = Jan, 2 = Feb, 3 = Mar, 4 = Apr, 5 = May, 6 = Jun, 7 = Jul, 8 = Aug, 9 = Sep, 10 = Oct, 11 = Nov, 12 = Dec

(c) [*From the species list*]. When do you notice fruits or nuts from these species mature? 1 = Jan, 2 = Feb, 3 = Mar, 4 = Apr, 5 = May, 6 = Jun, 7 = Jul, 8 = Aug, 9 = Sep, 10 = Oct, 11 = Nov, 12 = Dec

(d) [*From the species list*]. When do you notice fruits or nuts from these species ripens on the tree? 1 = Jan, 2 = Feb, 3 = Mar, 4 = Apr, 5 = May, 6 = Jun, 7 = Jul, 8 = Aug, 9 = Sep, 10 = Oct, 11 = Nov, 12 = Dec

(e) [*From the species list*]. When do you notice fruits or nuts from these species harvested? 1 = Jan, 2 = Feb, 3 = Mar, 4 = Apr, 5 = May, 6 = Jun, 7 = Jul, 8 = Aug, 9 = Sep, 10 = Oct, 11 = Nov, 12 = Dec

(f) [*From the species list*]. Which of these fruit and nut species would you like to improve? Picked = tick (√), Rejected = cross (x)

(g) [*From the species list*]. What improvement would you like to see done on the fruit and nut of these species? 1 = size, 2 = taste (sweet or sour), 3 = shelf life, 4 = stoniness, 5 = kernel extraction, 6 = other (specify):_____

(h) What improvement would you like to see done on the tree of these species? 1 = height, 2 = fruit production (yield), 3 = crown cover, 4 = thorniness, 5 = disease resistance, 6 = pest tolerance, 7 = tree strength, 8 = early fruiting, 9 = other (specify): _____

3. Tree management and Marketing

Answers to the following questions must be entered into appropriate spaces provided in the attached table 3. Details for questions with 'specify' may be entered on this sheet.

(a) [*From the species list*]. Do you normally retain any of these indigenous fruit and nut trees when clearing bush for a new garden? Yes = tick (√), No = cross (x)

(b) If 'yes' which of these fruit and nut species do you retain and where would you likely to find them most? Order of likely garden places to occur, 1 = primary forest, 2 = secondary forest, 3 = fallow forest, 4 = old garden, 5 = other (specify):_____

(c) If 'no' why not? Is it because, 1= not needed, 2 = a weed, 3 = harbours pests, 4 = incompatible with garden crops (shady, large roots, compete for plant food and water), 5 = against custom ritual, 6 = other (specify):_____

(d) Do you plant any of these fruit and nut species? Yes = tick (√), No = cross (x)

(e) [*From the species list*]. If 'yes' where would you most prefer to plant these fruit and nut species? 1 = old garden, 2 = new garden, 3 = secondary forest, 4 = fallow forest, 5 = primary forest, 6 = around dwellings, 7 = along paths, 8 = intercrop with tree cash crops (e.g. coconut, cocoa), 9 = coastal forest, 10 = all

(f) [*From the species list*]. Which of these fruit and nut species is easy to plant? Order of easiness, 1 = easy, 2 = moderately easy, 3 = difficult, 4 = unsure, 5 = impossible

(g) [*From the species list – only the ones being planted*]. How are they planted? 1 = root, 2 = bark, 3 = leaves, 4 = branch, 5 = flower, 6 = fruit (seed), 7 = wood, 8 = other (specify):_____

(h) [*If 'no' to (d)*] why not? Is it because, 1 = not interested, 2 = plenty around in the wild, 3 = difficult to plant, 4 = no spare land, 5 = generate no or little money, 6 = other (specify):_____

(i) Do you experience any problem or difficulty in growing your fruit and nut species? Yes = tick (√), No = cross (x)

(j) If 'yes' what are these problems? 1 = shortage of planting material, 2 = low seed germination, 3 = poor seedling growth, 4 = low yield, 5 = high flower shedding, 6 = no market outlet, 7 = no adequate labour, 8 = no government support and incentive, 9 = no starting capital (money), 6 = other (specify): _____

(k) How do you manage your fruit and nut species? 1 = weeding, 2 = singling, 3 = pruning, 4 = thinning, 5 = all, 6 = other (specify):_____

(l) [*From the species list*]. Which of these species produce fruits or nuts are? 1 = consumed at home, 2 = for sale (domestic market), 3 = export.

(m) Who usually collects the fruits and nuts for home consumption? 1 = women, 2 = men, 3 = girls, 4 = boys, 5 = all, 6 = nil

(n) Who usually collects the fruits and nuts for sale? 1 = women, 2 = men, 3 = girls, 4 = boys, 5 = all, 6 = nil

(o) When you sell your fruits and nuts at the market what unit of measure do you use? 1 = heap, 2 = bag, 3 = weight (kg or lbs), 4 = tin, 5 = parcel, 6 = bunch, 7 = other (specify):_____

(p) What is your selling price per unit of measure you use? 1 = $0.20, 2 = $0.50, 3 = $1.00, 4 = $1.50, 5 = $2.00, 6 = $5.00, 7 = $10.00, 8 = other (specify):_____

(q) Does your price vary with fruit or nut quality? 1 = yes 2 = no

(r) If 'yes' how do you make the decision? _____

(s) If 'no' why not?_____

(t) How much did you earn from your fruit and nut sale last year (2001)? 1 = >$100, 2 = >$200, 3 = >$300, 4 = >$400, 5 = >$500, 6 = other (specify): _____

(u) Where did you sell your produce (fruits and nuts)? 1 = local market, 2 = door-to-door, 3 = Honiara market, 4 = visiting ships/boats, 5 = schools and institutions, 6 = hotels and motels, 7 = other (specify):_____

(v) Do you trade your fruits and nuts in exchange of other goods or services? Yes = tick (√), No = cross (x)

(w) If 'yes' give details: 1 = basic goods (cloth, soap, rice, kerosene, fish, root crops, vegetables), 2 = luxury goods (petrol, tobacco, beer, furniture), 3 = common services (land clearing, weeding, repairing house, pulling canoe), 4 = occasional services (wedding, funeral, birthday), 5 = other (specify):_____

Associated with Questionnaire 4.1.1 (1) on Species and Utilization

Ref.	Latin names	Common names	Kolombangara venecular names	Priority	Eating	Domesticate		Medicinal		Animal feed		Shade/Shelter		Environ. Service		Handicraft		Undergrd post		Structure		Carving		Timber or log		Health risk	
				Qa	Qb	Qc	Qd	Qe	Qf	Qg	Qh	Qi	Qj	Qk	Ql	Qm	Qn	Qo	Qp	Qq	Qr	Qs	Qt	Qu	Qv	Qw	Qx	Qy
1	*Artocarpus altilis*	Breadfruit	Egolo																								
2	*Barringtonia* spp	Cut-nut	Kino																								
3	*Burckella obovata*	Burckella	Natu																								
4	*Canarium indicum*	Ngali nut	Koke																								
5	*Canarium salomonense*	Ngali nut	Haoro																								
6	*Gnetum gnemon*	Kingtree	Bia																								
7	*Gnetum latifolium*	-	Bia																								
8	*Inocarpus fagifer*	Tahitian chestnut	Nagi																								
9	*Mangifera minor*	Mango	Rekeu																								
10	*Paratocarpus venenosa*	-	Boe																								
11	*Pometia pinnata*	Pacific lychee	Gema																								
12	*Spondias dulcis*	Golden apple	Opiti																								
13	*Syzygium malaccense*	Malayan apple	Kalkipa																								
14	*Terminalia catappa*	Beach almond	Tatalise																								
15	*Terminalia kaernbachii*	Okari nut	Tatalise hololo																								
16	*Terminalia salomonensis*	-	Popoli																								

NOTE: Questions, Qa….Qy corresponds to questions on the questionnaire sheet

Associated with Questionnaire 4.1.1 (2) on Tree phenology and Improvements

Ref.	Latin names	Common names	Kolombangara venecular names	Phenological stages					Desired improvement		
				Qa	Qb	Qc	Qd	Qe	Qf	Qg	Qh
1	*Artocarpus altilis*	Breadfruit	Egolo								
2	*Barringtonia* spp	Cut-nut	Kino								
3	*Burckella obovata*	Burckella	Natu								
4	*Canarium indicum*	Ngali nut	Koke								
5	*Canarium salomonense*	Ngali nut	Haoro								
6	*Gnetum gnemon*	Kingtree	Bia								
7	*Gnetum* spp.	-	Bia								
8	*Inocarpus fagifer*	Tahitian chestnut	Nagi								
9	*Mangifera minor*	Mango	Rekeu								
10	*Paratocarpus venenosa*	-	Boe								
11	*Pometia pinnata*	Pacific lychee	Gema								
12	*Spondias dulcis*	Golden apple	Opiti								
13	*Syzygium malaccense*	Malayan apple	Kalkipa								
14	*Terminalia catappa*	Beach almond	Tatalise								
15	*Terminalia kaernbachii*	Okari nut	Tatalise hololo								
16	*Terminalia salomonensis*	-	Popoli								

NOTE: Questions, Qa….Qh corresponds to questions on the questionnaire sheet

Associated with Questionnaire 4.1.1 (3) on Tree management and marketing

Ref.	Latin names	Common names	Kolombangara venecular names	Retain		Remove	Planting				No planting	Problems/Solutions			Fruit/Nut	Home	Sale	Product sales details*					Surplus tradeoff	
				Qa	Qb	Qc	Qd	Qe	Qf	Qg	Qh	Qi	Qj	Qk	Ql	Qm	Qn	Qo	Qp	Qq	Qt	Qu	Qv	Qw
1	*Artocarpus altilis*	Breadfruit	Egolo																					
2	*Barringtonia* spp	Cut-nut	Kino																					
3	*Burckella obovata*	Burckella	Natu																					
4	*Canarium indicum*	Ngali nut	Koke																					
5	*Canarium salomonense*	Ngali nut	Haoro																					
6	*Gnetum gnemon*	Kingtree	Bia																					
7	*Gnetum* spp.	-	Bia																					
8	*Inocarpus fagifer*	Tahitian chestnut	Nagi																					
9	*Mangifera minor*	Mango	Rekeu																					
10	*Paratocarpus venenosa*	-	Boe																					
11	*Pometia pinnata*	Pacific lychee	Gema																					
12	*Spondias dulcis*	Golden apple	Opiti																					
13	*Syzygium malaccense*	Malayan apple	Kalkipa																					
14	*Terminalia catappa*	Beach almond	Tatalise																					
15	*Terminalia kaernbachii*	Okari nut	Tatalise hololo																					
16	*Terminalia salomonensis*	-	Popoli																					

* Responses to questions Qr and Qs should be written on the questionnaire sheet

NOTE: Questions, Qa….Qw corresponds to questions on the

4.1. To determine the traditional agroforestry practices in Kolombangara, Solomon islands

Respondent name:_____

Village name:_____ Village Code: _____

System Code:_____ Sub-system Code:_____ Altitude: _____

A. Household Food Security

(a) What is your main reason for gardening? 1 = home consumption, 2 = sale,
3 = both, 4 = other (specify):_____

(b) What staple food crops do you plant in your garden? 1 = taro, 2 = sweet potato, 3 = banana, 4 = cassava, 5 = yam/or pana, 6 = other (specify): _____

(c) Do you have any problems with insufficient supply of food for home consumption? 1 = yes 2 = no

(c) If 'yes' why? 1 = low yield due to poor fertility soil, 2 = cropping cycle short, 3 = shortage of planting material, 4 = shortage of labour, 5 = pest & disease, 6 = natural disaster (cyclone, flood, drought), 7 = other (specify):_____

(d) How do you overcome food shortage problem? 1 = plant more food crops, 2 = purchase more food, 3 = government relief supply, 4 = Harvest from wild, 5 = other (specify):_____

(e) Did you supplement your food supply with indigenous fruits and/or nuts during the period you are not having enough food? 1 = yes 2 = no

If 'yes' what indigenous fruit or nut species did you have and how do you use or eat them:

Species name	Usage
(i) ----------	----------
(ii) ----------	----------
(iii) ----------	----------
(iv) ----------	----------
(v) ----------	----------

B. Crops and Farming systems

(a) Sketch a generalised land use profile

(b) How long is fallow period (estimate from observation and village discussion)? 1 = long fallow not used, 2 = 1-4 years, 3 = 5-9 years, 4 = 10-15 years, 5 = >15 years

(c) How many times do you plant staple crops in your garden before rendering it to fallow? 1 = one planting only, 2 = two planting, 3 = three to five plantings, 4 = continuous cultivation (≥6 planting)
(d) [*From the crop list*]. Which of these fruit crops is the most important fruit grown in your food garden or collected from wild (select only first 10 common types)?

Ref	Latin name	Common name	Ref	*Latin name*	*Common name*
1	*Persea americana*	Avocado	14	Citrus paradisi	*Grapefruit*
2	*Musa cvs*	Banana	15	*Citrus limon*	Lemon
3	*Burckella obovata*	Burckella	16	*Citrus aurantifolia*	Lime
4	*Syzygium malaccense*	Malay apple	17	*Citrus maxima*	Pomelo
5	Mangifera minor	Mango	18	*Annona muricata*	Soursop
6	*Citrus reticulata*	Mandarin	19	*Spondias dulcis*	
7	*Ananas comosus*	Pineapple	20	*Pometia pinnata*	
8	*Carica papaya*	Pawpaw	21	Paratocarpus venenosa	
9	*Citus sinensis*	Orange	22	*Nephelium lappaceum*	Rambutan
10	Passiflora mollissima	Passionfruit	23	*Terminalia salomonensis*	
11	Psidium guajava	Guava	24	Other (specify)	
12	*Citrullus lanatus*	Watermelon			
13	*Artocarpus heterophyllus*	Jackfruit			

(e) [*From the crop list*]. Which of these nut crops is the most important nut grown in your food garden or collected from wild (select only first 5 common types)?

Ref	Latin name	Common name	Ref	*Latin name*	*Common name*
1	Artoccarpus altilis	Breadfruit	6	*Barringtonia* spp.	Cutnut
2	*Terminalia catappa*	Beach almond	7	*Gnetum gnemon*	King nut
3	*Terminalia kaernbachii*	-	8	*Canarium indicum*	Ngali nut
4	Canarium salomonense	Ngali nut	9	*Cocos nucifera*	Coconut
5	*Inocarpus fagifer*	Tahitian chestnut	10	Other (specify)	

(f) Is a cash crop planted with food crops in a sequence in the garden – that is one food crop (taro) followed by a cash crop (coconut)? 1 = none, 2 = minor (insignificant), 3 = significant, 4 = highly significant

(g) Describe crop sequence noted in (f) and how they are arranged within the same garden:_____

(h) Is a cash crop intercropped with food crops in the garden – that is one cash crop (coconut) in food garden (taro)? 1 = none, 2 = minor (insignificant), 3 = significant, 4 = highly significant

(i) Describe intercropping pattern noted in (h) and what planting space and density used and how they are appreciated:_____

C. Maintenance of soil fertility

(a) Is a legume crop (e.g. peanut) inter-planted with crops of a staple? 1 = none, 2 = minor (insignificant), 3 = significant, 4 = highly significant
(b) *Describe the crop-legume-crop sequence noted in (a)*:_____

(c) *Are trees planted purposely to be part of the fallow at the end of cropping period or not?* 1 = none, 2 = minor (insignificant), 3 = significant, 4 = highly significant

(d) *Describe tree species planted in (c)*:_____

D. Cash generating activities

Fruit and Nut Species

Ref	Activity	Rank Qa	*Income estimate* Qb
1	*Persea americana*		
2	*Musa cvs*		
3	*Burckella obovata*		
4	*Syzygium malaccense*		
5	Mangifera minor		
6	*Citrus reticulata*		
7	*Ananas comosus*		
8	*Carica papaya*		
9	*Citus sinensis*		
10	Passiflora mollissima		
11	Psidium guajava		
12	*Citrullus lanatus*		
13	*Artocarpus heterophyllus*		
14	Citrus paradisi		
15	*Citrus limon*		
16	*Citrus aurantifolia*		
17	*Citrus maxima*		
18	*Annona muricata*		
19	*Spondias dulcis*		
20	*Pometia pinnata*		
21	Paratocarpus venenosa		
22	*Nephelium lappaceum*		
23	*Terminalia salomonensis*		
24	Artoccarpus altilis		
25	*Terminalia catappa*		
26	*Terminalia kaernbachii*		
27	Canarium salomonense		
28	*Inocarpus fagifer*		
29	*Barringtonia* spp.		
30	*Gnetum gnemon*		
31	*Canarium indicum*		
32	*Cocos nucifera*		
33	*Theobroma cacao*		
34	*Coffea robusta*		
35	*Areca catechu*		

(a) [*From the list*]. Which of these crops and their products are you involved with to generate income for

(b) [*From the list*]. From your choice in (a) how much earnings do you make in a year (estimate)?

Other (specify): _____
(c) *Notes on income generating activities:* _____

Non-fruit and non-nut Species

Ref	Activity	Rank Qa	*Income estimate* Qb
1	*Animal skin/Plumes*		
2	Artifacts/mats/string bags		
3	Canoe		
4	*Cattle*		
5	Piggery		
6	Poultry		
7	Chillies		
8	Firewood		
9	Carving		
10	Fish		
11	*Fresh food*		
12	Marine product		
13	Timber		
14	Pepper betel		
15	Vegetables		
16	Tobacco		
17	Remittances		
18	Employment		

(a) [*From the list*]. Which of these non-fruit-and nut crops or animals and their products are you involved with to generate income

(b) [*From the list*]. From your choice in (a) how much earnings do you make in a year (estimate)?

Other (specify): _____

(c) *Notes on income generating activities:* _____

SOURCE OF FINDING:

Date: _____ (day) of _____ (month) of _____ (year)

Surveyor: _____ assisted by: _____

FURTHER NOTES:_____

_____

Appendix 4.2. Summary of some market aspects of 16 popular indigenous fruit and nut species from farmers' survey (2002) in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara, Solomon Islands

Tree species	Primary reasons for planting of the species (% of farmers)		Selling unit of the product	Selling price per unit of the product (US$0.00)	Product quality for sale in local markets	Domestic Market outlet
	Consumption	Sale				
Barringtonia procera	51%	49%	Heap Parcel	$0.15 $0.15 - $0.30	Big, sweet and well-formed kernel	Local market Visiting boats
Canarium indicum	81%	19%	Heap Parcel Tin Bag	$0.15 $0.15 - $1.50 $1.50 - $3.60 $1.50 - $3.00	Big, sweet and well-formed kernel	Local market Visiting boats
Artocarpus altilis	87%	13%	Single fruit	$0.30 - $0.70	Big, ripe and good form fruit	Local market Visiting boats
Magnifera minor	76%	24%	Parcel Single fruit	$0.15 - $0.30 $0.15 - $0.30	nut and sweet kernel Big and well-formed fruit	Visiting boats Local market
Inocarpus fagifer	79%	21%	Heap	$0.15 - $0.30	Size and freshness of	Local market
Canarium salomonense	81%	19%	Heap Parcel Tin Bag	$0.15 $0.15 - $1.50 $1.50 - $3.60 $1.50 - $3.00	Big, sweet and well-formed kernel	Local market Visiting boats
Syzygium malaccense	52%	48%	Heap	$0.05 - $0.15	Big and well-formed sweet fruit	Local market Visiting boats
Spondias dulcis	86%	14%	Single fruit	$0.05 - $0.10	Big and well-formed sweet fruit	Local market Visiting boats
Gnetum gnemon	Not applicable	Not applicable	Heap Parcel	$0.15 - $0.50 $0.15 - $0.30	Big nut, and good and tender young leaves	Local market
Paratocarpus venenosa	100%	0%	Not applicable	Not applicable	Not applicable	Not applicable
Terminalia salomonensis	100%	0%	Not applicable	Not applicable	Not applicable	Not applicable
Terminalia catappa	Not applicable		Not applicable	Not applicable	Not applicable	Not applicable
Terminalia kaernbachii	100%	0%	Not applicable	Not applicable	Not applicable	Not applicable
Gnemon latifolium	Not applicable	Not applicable	Heap Parcel	$0.15 - $0.30 $0.15 - $0.30	Big nut, and good and tender young leaves	Local market
Pometia pinnata	100%	0%	Not applicable	Not applicable	Not applicable	Not applicable
Burckella obovata	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable

Appendix 4.3. Farmers' rating of different traditional uses of various parts of a tree of 16 popular indigenous fruit and nut species from farmers' survey (2002) in five sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara, Solomon Islands. Rating: High = above 60% of farmers, Medium = 40-60% of farmers, Low = below 40% of farmers, NU = Never been used (0% of farmers).

Tree products	*Barringtonia procera*	*Canarium indicum*	*Artocarpus atilis*	*Magnifera minor*	*Inocarpus fagifer*
WOOD:					
Timber	Low	High	High	Low	Low
Underground post	Low	Low	Low	NU	High
Aboveground poles	Low	Low	Low	Low	Medium
Handicraft	Low	Medium	NU	Low	High
Carving	NU	Low	Low	NU	Low
Fuel	Low	High	Low	Low	High
BARK:					
Medicine	Medium	High	Low	Low	Medium
Weaving	NU	NU	Low	NU	NU
LEAVES:					
Medicine	Low	NU	Low	Low	Low
Food	NU	NU	NU	NU	NU
Spice	NU	NU	NU	NU	NU
FRUIT:					
Food (Flesh)	NU	Low	High	High	NU
Feed	Low	High	Low	Medium	Low
Medicine	NU	NU	Low	Low	NU
Oil	NU	NU	NU	NU	NU
Spice	NU	NU	NU	NU	NU
NUT:					
Food (Kernel)	High	High	High	NU	High
Oil	NU	NU	NU	NU	NU
Spice	NU	NU	NU		NU
OTHER: (Roots, Resin and Sap, Shell, etc)					
Medicine	Low	Low	Low	Low	Low
Other (candles, fuel, mulch)	Medium	High	Low	NU	Medium
SERVICE FUNCTIONS:					
Shade/Shelter					
Soil improvement	Medium	Low	Medium	High	High
Biodiversity	High	Medium	Medium	High	High
	High	High	High	High	High

Cont. **Appendix 4.3. Farmers' rating of different traditional uses of various parts of a tree of 16 popular indigenous fruit and nut species from farmers' survey (2002) in five sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara, Solomon Islands. Rating: High = above 60% of farmers, Medium = 40-60% of farmers, Low = below 40% of farmers, NU = Never been used (0% of farmers).**

Tree products	*Canarium salomonense*	*Syzygium malaccense*	*Spondias dulcis*	*Gnetum gnemon*	*Paratocarpus venenosa*
WOOD:					
Timber	Medium	Low	Low	NU	NU
Underground post	Low	Low	Low	NU	NU
Aboveground poles	Low	Low	Low	NU	Low
Handicraft	Medium	Low	Low	NU	NU
Carving	Low	Low	NU	NU	NU
Fuel	High	Low	Low	Low	Low
BARK:					
Medicine	Low	Low	Low	Low	Low
Weaving	NU	NU	NU	Medium	NU
LEAVES:					
Medicine	Low	Low	Low	Low	Low
Food	NU	NU	NU	High	NU
Spice	NU	NU	NU	NU	NU
FRUIT:					
Food (Flesh)	Low	High	High	NU	High
Feed	High	Low	Low	Low	Medium
Medicine	NU	NU	Low	Low	NU
Oil	NU	NU	NU	NU	NU
Spice	NU	NU	NU	NU	NU
NUT:					
Food (Kernel)	High	NU	NU	High	NU
Oil	NU	NU	NU	NU	NU
Spice	NU	NU	NU	NU	NU
OTHER: (Roots, Resin and Sap, Shell, etc)					
Medicine	Low	Low	Low	Low	Low
Other (candles, fuel, mulch)	High	NU	NU	Low	NU
SERVICE FUNCTIONS:					
Shade/Shelter					
Soil improvement	Low	High	Low	Low	Low
Biodiversity	Medium	Medium	High	Medium	High
	High	High	Medium	Low	High

Cont. **Appendix 4.3. Farmers' rating of different traditional uses of various parts of a tree of 16 popular indigenous fruit and nut species from farmers' survey (2002) in five sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara, Solomon Islands. Rating: High = above 60% of farmers, Medium = 40-60% of farmers, Low = below 40% of farmers, NU = Never been used (0% of farmers).**

Tree products	*Terminalia salomonensis*	*Terminalia catappa*	*Terminalia kaernbachii*	*Gnetum latifolium*	*Pometia pinnata*	*Burckella obovata*
WOOD:						
Timber	Low	Low	Low	NU	High	High
Underground post	NU	Low	Low	NU	Medium	Low
Aboveground poles	Low	Low	Low	NU	High	Low
Handicraft	Low	Medium	Low	NU	High	Low
Carving	NU	Low	Low	NU	NU	Low
Fuel	Medium	Medium	Medium	Low	High	Low
BARK:						
Medicine	Low	Medium	Low	Low	Low	Low
Weaving	NU	NU	NU	Medium	NU	NU
LEAVES:						
Medicine	Low	Medium	Low	Low	Low	Low
Food	NU	NU	NU	High	NU	NU
Spice	NU	NU	NU	NU	NU	NU
FRUIT:						
Food (Flesh)	High	NU	NU	NU	High	High
Feed	Low	Low	Low	Low	NU	Low
Medicine	NU	NU	NU	Low	NU	NU
Oil	NU	NU	NU	NU	NU	NU
Spice	NU	NU	NU	NU	NU	NU
NUT:						
Food (Kernel)	NU	High	High	High	NU	NU
Oil	NU	NU	NU	NU	NU	NU
Spice	NU	NU	NU	NU	NU	NU
OTHER: (Roots, Resin and Sap, Shell, etc)						
Medicine	Low	Low	Low	Low	Low	Low
Other (candles, fuel, mulch)	NU	Medium	Medium	Low	NU	Low
SERVICE FUNCTIONS:						
Shade/Shelter						
Soil improvement	Medium	High	Medium	Low	Medium	Low
Biodiversity	High	High	Medium	Medium	High	Medium
	Medium	Low	Low	Low	Medium	Medium

Appendix 4.4. Various traditional tree crop treatments and ecological distribution of 16 popular indigenous fruit and nut species, and associate problems affecting farmers in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara, Solomon Islands (from farmers survey 2002) * during bush clearing for food gardens and other eventualities

Tree species	***Artocarpus atilis***	***Barringtonia procera***	***Burckella obovata***	***Canarium indicum***	***Canarium salomonense***
Natural ecological distribution	Secondary forest Old garden Primary forest Fallow forest	Old garden Primary forest Secondary forest Fallow forest	Secondary forest Fallow forest Old garden Primary forest	Primary forest Old garden Secondary forest Fallow forest	Primary forest Old garden Secondary forest Fallow forest
Farmers retaining trees (%)*	92%	96%	38%	100%	100%
Reasons for cutting down trees	Sterile Low yield Incompatible Weed	Sterile Weed Incompatibility	Weed Incompatible Not interested Sterile	Not applicable	Not applicable
Farmers grow the species (%)	25%	100%	0%	97%	97%
Preferred sites to grow the species	Old garden Secondary forest Fallow forest	Around dwellings Old garden Coconut Plantation New gardens Secondary forest	Not applicable	Old garden Secondary forest Fallow forest Around dwellings New garden	Old garden Secondary forest Fallow forest Around dwellings New garden
Whether the species is easy to grow	Fairly easy	Very easy	Not applicable	Very easy	Very easy
Common planting method	Seed	Seed	Not applicable	Seed	Seed
Maintenance/Silviculture operations	Weeding Cleaning	Weeding Cleaning Pruning	Not applicable	Weeding Cleaning	Weeding Cleaning
Problems faced by farmers growing the species	Pest and Disease Low yield Poor seedling growth	Low yield High fruit shedding Lack of market Lack of money Shortage of labour Pest and Disease No Government support Lack planting material	Not applicable	Low yield Shortage labour Lack planting material Poor seedling growth	Low yield Shortage labour Lack planting material Poor seedling growth
Reasons for not growing the species	Plenty in the wild Generate little money No market outlet No spare land	Not application	Plenty in the wild No market outlet Generate little money No Government support	Plenty in the wild	Plenty in the wild

Cont. **Appendix 4.4. Various traditional tree crop treatments and ecological distribution of 16 popular indigenous fruit and nut species, and associate problems affecting farmers in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara, Solomon Islands (from farmers survey 2002) *during bush clearing for food gardens and other eventualities**

Tree species	***Gnetum gnemon***	***Gnetum latifolium***	***Inocarpus fagifer***	***Magnifera minor***	***Paratocarpus venenosa***
Natural ecological distribution	Secondary forest Fallow forest Primary forest Old garden	Secondary forest Fallow forest Primary forest Old garden	Coastal swamps Secondary forest Wetland Primary	Secondary forest Old garden Primary forest Fallow forest	Primary forest Secondary forest
Farmers retaining trees (%)*	84%	84%	59%	72%	92%
Reasons for cutting down trees	Plenty in wild Weed	Plenty in wild Weed	Weed Sterile Firewood Incompatible	Sterile Low yield Weed	Plenty in wild Not interested
Farmers grow the species (%)	0%	0%	7%	19%	1%
Preferred sites to grow the species	Not applicable	Not applicable	Coastal wetlands	Old garden Around dwellings Secondary forest	Fallow forest Old garden Around dwellings
Whether the species is easy to grow	Not applicable	Not applicable	Easy	Easy	Easy
Common planting method	Not applicable	Not applicable	Seed	Seed	Seed
Maintenance/Silviculture operations	Not applicable	Not applicable	Weeding Cleaning	Weeding Cleaning	Weeding Cleaning
Problems faced by farmers growing the species	Not applicable	Not applicable	Low yield No Government support Pest and Disease Lack planting material	Low yield High fruit shedding	Pest and Disease
Reasons for not growing the species	Plenty in the wild Generate little money	Plenty in the wild Generate little money	Plenty in the wild Generate little money Unware of potential No spare land Unfamiliar with species	High fruit shedding Generate little money	Generate little money Unfamiliar with species

Cont. **Appendix 4.4. Various traditional tree crop treatments and ecological distribution of 16 popular indigenous fruit and nut species, and associate problems affecting farmers in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara, Solomon Islands (from farmers survey 2002) *during bush clearing for food gardens and other eventualities**

Tree species	***Pometia pinnata***	***Spondias dulcis***	***Syzygium malaccense***	***Terminalia catappa***	***Terminalia kaernbachii***	***Terminalia salomonensis***
Natural ecological distribution	Primary forest Secondary forest	Secondary forest Primary forest Old garden Fallow forest	Secondary forest Old garden Fallow forest Primary forest	Coastal forest Shore lines	Secondary forest Fallow forest Primary forest Old garden	Secondary forest Primary forest Fallow forest
Farmers retaining trees (%)*	79%	97%	90%	30%	40%	84%
Reasons for cutting down trees	Plenty in wild Weed Incompatible	Sterile Incompatible Sour taste Weed	Weed Sterile Low yield Incompatible	Plenty in wild	Weed Sterile Incompatible Low yield	Plenty in wild Weed Incompatible
Farmers grow the species (%)	1%	86%	25%	0%	5%	0%
Preferred sites to grow the species	Around dwellings Old garden Fallow forest	Around dwellings Old garden Secondary forest Fallow forest	Around dwellings Old garden Secondary forest Fallow forest New garden	Not applicable	Fallow forest Secondary forest Old garden	Not applicable
Whether the species is easy to grow	Fairly easy	Fairly easy	Easy	Not applicable	Fairly easy	Not applicable
Common planting method	Seed	Seed	Seed	Not applicable	Seed	Not applicable
Maintenance/ silviculture operations	Weeding Cleaning	Weeding Cleaning	Weeding Cleaning	Not applicable	Weeding Cleaning	Not applicable
Problems faced by farmers growing the species	Low yield	No market outlet Low yield Lack planting material Labour shortage Poor seed germination Poor seedling growth No Government support	Pest and Disease Low yield High fruit shedding Lack planting material	Not applicable	Low yield Poor plant growth	Not applicable
Reasons for not growing the species	Generate little money Unfamiliar with species	Plenty in the wild Generate little money	Plenty in the wild Generate little money No Government support	Plenty in the wild Lack market outlet	Plenty in the wild Lack market outlet Lack planting material Generate little money Lack of labour Lack of capital No spare land	Plenty in the wild Generate little money

Appendix 4.5. The phenology and most desirable improvements for individual indigenous fruit and nut species from farmers survey (2002) in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara, Solomon Islands. +percentage (%) of farmers interviewed for each individual species *based on majority opinion of farmers interviewed who had some knowledge on the phenology of the species.

Tree species	+Farmers who know phenology of the species	* Flowering times	* Fruit maturity and harvesting times	Potential improvements based on farmers wish list	
				Product	Tree
Barringtonia procera	100%	January April July October	March June September December	Shelf-life Kernel extraction Kernel taste Nut size	Height Early fruiting Yield Pest/Disease resistance
Canarium indicum	32%	July August	November December	Kernel extraction Nut size	Height Early fruiting
Artocarpus atilis	16%	January June October	March August December	Shelf-life	Height Early fruiting Yield
Magnifera minor	18%			Shelf-life Fruit taste	Height Early fruiting Yield
Inocarpus fagifer	9%	January February	May June	Shelf-life Kernel taste Kernel size	Height Early fruiting Yield
Canarium salomonense	30%	January	April	Kernel extraction Nut size	Yield Height Early fruiting Yield
Syzygium malaccense	35%	May September	August November	Shelf-life Fruit taste	Yield Pest/Disease resistance
Spondias dulcis	19%	June	August	Fruit taste Fruit size	Early fruiting Yield Pest/Disease resistance
Gnetum gnemon	6%	June July	September October	Shelf-life Kernel taste	Early fruiting Yield
Paratocarpus venenosa	3%	August	November December	Shelf-life Fruit size	Height Yield
Terminalia salomonensis	5%	August	October November	Shelf-life Fruit size	Height Yield
Terminalia catappa	7%	January June	December March August September	Fruit size Shelf-life Nut size	Height Early fruiting Yield

Cont. **Appendix 4.5. The phenology and most desirable improvements for individual indigenous fruit and nut species from farmers survey (2002) in 5 sites (Ringgi, Seusepe, Rei, Poporo, Hunda) on Kolombangara, Solomon Islands. +percentage (%) of farmers interviewed for each individual species *based on majority opinion of farmers interviewed who had some knowledge on the phenology of the species.**

Tree species	+Farmers who know phenology of the species	* Flowering times	* Fruit maturity and harvesting times	Potential improvements based on farmers wish list	
				Product	Tree
Terminalia kaernbachii	8%	July September	October December January	Shelf-life	Height Yield
Gnemon latifolium.	6%	June July	September October	Shelf-life Kernel taste	Early fruiting Yield
Pometia pinnata	1%	October	December January	Shelf-life Fruit size	Height Yield
Burckella obovata	3%	April	June July	Fruit taste Fruit size	Height Yield

Appendix 7.1. Mean, range, skewness and kurtosis of different fruit and kernel traits *of Barringtonia procera* from 5 populations in Kolombangara, Solomon Islands.

Trait	Population	Mean +/-	Range	Skewness	Kurtosis
Fruit mass	Vovohe	61.4 +/- 1.1	30-115	0.787	0.199
	Tututi	64.5 +/- 0.8	30-140	1.472	2.936
	Rei	59.6 +/- 1.2	30-125	0.878	0.273
	Poporo	67.9 +/- 0.7	40-130	0.591	0.176
	Hunda	63.0 +/- 0.4	30-120	0.692	0.505
Fruit length	ovohe	69.6 +/- 0.6	47-89	0.003	-0.095
	Tututi	67.8 +/- 0.3	50-90	0.534	0.190
	ReiV	66.2 +/- 0.5	45-90	0.662	-0.006
	Poporo	67.7 +/- 0.4	50-92	0.399	-0.038
	Hunda	67.0 +/- 0.2	31-95	-0.032	1.179
Fruit width (apex)	Vovohe	28.7 +/- 0.2	20-41	0.402	0.752
	Tututi	28.6 +/- 0.2	19-39	-0.003	-0.016
	Rei	26.8 +/- 0.2	19-36	-0.015	-0.013
	Poporo	27.1 +/- 0.2	19-40	0.488	0.451
	Hunda	28.6 +/- 0.1	14-45	0.133	0.302
Fruit width (middle)	Vovohe	40.6 +/- 0.3	31-52	0.219	-0.007
	Tututi	39.2 +/- 0.2	24-55	-0.006	1.956
	Rei	29.7 +/- 0.2	26-49	0.220	-0.014
	Poporo	40.6 +/- 0.2	25-53	-0.032	0.892
	Hunda	38.8 +/- 0.1	11-59	0.002	1.325
Fruit width (base)	Vovohe	31.6 +/- 0.2	21-44	0.301	1.132
	Tututi	32.3 +/- 0.2	20-49	0.291	0.546
	Rei	29.7 +/- 0.2	22-39	0.220	-0.014
	Poporo	30.0 +/- 0.2	22-46	0.640	0.416
	Hunda	31.0 +/- 0.1	15-50	0.007	0.366
Flesh mass	Vovohe	28.7 +/- 0.5	15-55	0.919	0.416
	Tututi	32.0 +/- 0.4	15-70	1.292	2.857
	Rei	28.0 +/- 0.6	10-60	0.815	0.126
	Poporo	32.8 +/- 0.4	20-65	0.541	0.009
	Hunda	29.4 +/- 0.2	10-65	0.740	0.368
Nut mass	Vovohe	32.7 +/- 0.7	10-65	0.753	0.377
	Tututi	32.5 +/- 0.4	15-70	0.966	1.106
	Rei	31.6 +/- 0.7	10-65	0.937	0.544
	Poporo	35.1 +/- 0.4	20-65	0.658	0.268
	Hunda	33.5 +/- 0.2	10-65	0.524	0.238
Shell mass	Vovohe	23.2 +/- 0.6	5-60	1.039	1.582
	Tututi	21.3 +/- 0.3	10-45	0.931	1.200
	Rei	20.4 +/- 0.4	5-40	0.661	0.492
	Poporo	22.5 +/- 0.3	10-45	0.595	0.322
	Hunda	22.3 +/- 0.2	5-45	0.590	0.488
Kernel mass	Vovohe	9.5 +/- 0.3	5-25	0.788	0.327
	Tututi	11.2 +/- 0.2	5-25	0.722	0.152
	Rei	10.9 +/- 0.3	5-25	0.912	0.221
	Poporo	12.5 +/- 0.2	5-25	0.275	-0.041
	Hunda	11.3 +/- 0.1	5-25	0.483	0.146
Kernel length	Vovohe	32.6 +/- 0.4	15-48	-0.011	-0.018
	Tututi	33.2 +/- 0.2	20-47	-0.033	1.030
	Rei	32.5 +/- 0.2	21-44	0.281	0.206
	Poporo	33.8 +/- 0.3	19-50	-0.017	-0.072
	Hunda	31.9 +/- 0.1	13-53	-0.032	1.179
Kernel width (apex)	Vovohe	17.2 +/- 0.2	11-32	0.631	1.265
	Tututi	16.8 +/- 0.1	7-28	-0.001	0.347
	Rei	15.0 +/- 0.2	5-28	0.909	4.623
	Poporo	17.4 +/- 0.2	7-33	0.852	1.708
	Hunda	17.0 +/- 0.1	6-32	0.178	0.282
Kernel width (middle)	Vovohe	22.0 +/- 0.2	13-33	0.500	0.450
	Tututi	20.9 +/- 0.1	10-31	-0.036	1.993
	Rei	20.3 +/- 0.2	9-26	-0.044	0.867
	Poporo	23.4 +/- 0.2	10-39	1.143	1.935
	Hunda	21.6 +/- 0.1	7-45	0.816	1.259
Kernel width (base)	Vovohe	18.7 +/- 0.2	11-26	0.114	0.009
	Tututi	18.0 +/- 0.1	10-29	-0.004	0.646
	Rei	16.5 +/- 0.2	7-25	0.146	1.069
	Poporo	19.2 +/- 0.2	9-37	1.017	2.408
	Hunda	18.1 +/- 0.1	6-33	0.276	0.675
Kernel taste	Vovohe	2.8 +/- 0.04	0-3	-3.359	9.95
	Tututi	2.7 +/- 0.03	0-3	-2.744	8.687
	Rei	2.7 +/- 0.04	1-3	-1.961	2.519
	Poporo	2.9 +/- 0.02	0-3	-4.688	24.256
	Hunda	2.6 +/- 0.02	0-3	-2.274	4.309

Appendix 8.1. Procedures for 96 Well Plant DNA Extraction (Fresh/Frozen/Dried) using CTAB based protocol modified from Scott and Playford (1996). © 2005 Trevor Wardill – School of Integrative Biology/University of Queensland

1. Add one ball bearing to each well of a ***Qiagen 96 well 1.2 ml plate*** & place up to 50 mg of ***finely chopped*** leaf material or root material into each well and cap with strip caps.
2. Place clear cover over rack and knock the rack upside down against the bench 5 times to ensure that all ball bearings are free.
3. Clearly mark plate on one long side and place the plate into Retsch Mill MM300 (without clear cover) with the marked side facing outwards and secure with torque wrench (remember to balance the Retsch Mill with another plate)
4. Disrupt the plant material for 1-10 min (or until well ground) @ 30 Hz.
5. Place clear cover over rack and knock the rack upside down against the bench 5 times to ensure that all ball bearings are free.
6. Repeat Steps 4-5 with the unmarked long side facing outwards
7. Place clear cover over rack and knock the rack upside down against the bench 5 times to free the ball bearings.
8. Remove and discard old strip caps, add 1.0 ml of extraction buffer with Matrix Impact® autopipettor, one row at a time and seal with strip caps, then repeat Steps 4-6 for 15 s per disruption (or until pellet is free).
9. Centrifuge for 10 min. at max. speed (***6000 rpm for Qiagen***) @ RT & remove the supernatant with multichannel P200 or P300 with barrier wide bore or cut-off tips.
10. Add 150 µl of wash buffer using Matrix Impact® autopipettor & then repeat Steps 4-6 for 15 s per disruption (or until pellet is free)
11. Add 40 µl of 5% Sarkosyl using Matrix Impact® autopipettor and seal with strip caps, and invert several times to mix.
12. Plate shake @ 600 rpm @ RT for 15 mins.
13. Centrifuge for 1 minute at 2500 rpm @ RT.
14. Add 400 µl of CTAB buffer using Matrix Impact® autopipettor
15. Seal with strip caps, & shake @ 600 rpm @ 55 °C for 30 mins in enviroshaker.
16. Centrifuge for 10 minutes at max. speed @ RT.
17. Transfer supernatant to a new ***Axygen 96 deep well 2.0 ml plate***
18. Add 800 µl Chloro: Iso-Amyl (24:1) using Eppendorf Multipette® plus and pipette with multichannel P200 to mix.
19. Centrifuge for 5 minutes at ***4000 rcf for Axygen***) @ RT.
20. Carefully transfer the supernatant (~400 µl) using the Corbett Research Robot, making sure not to include any of the interface layer or chloroform to a NEW labelled ***Axygen 96 deep well 2.0 ml (or 1.0 ml) plate***.
21. Add 40 µl 7.5M ammonium acetate and 400 µl of ice cold 100 % ethanol using Matrix Impact® autopipettor.
22. Gently shake sideways to mix & put in freezer –20 °C for 15 minutes to precipitate.
23. Centrifuge for 15 minutes at max. speed @ RT.
24. Empty out supernatant and dry on absorbent paper towel.
25. Add 300 µl of ice cold 70 % ethanol using Matrix Impact® autopipettor
26. Put in freezer –20 °C for 5 minutes to precipitate.
27. Centrifuge for 15 minutes at max. speed @ RT.
28. Empty out supernatant and dry on absorbent paper towel.
29. Dry in Speedivac for 15 mins @ 65 °C and resuspend in 35 µl MQ H_2O.

Appendix 8.2. Chemicals for 96 Well Plant DNA Extraction for difficult species

Reagents	Stock concentration.	Chemical name	*Concentration (g) used*
Extraction buffer (200 mL)	50mM	Tris pH 8.0	1.20
	5mM	EDTA	0.36
	0.35M	Sorbitol	20.00
	0.1%	BSA	0.20
	10%	PEG 6000	20.00
CTAB (200 mL)	0.05M	CTAB	4.00
(Cetyl Trimethyl Ammonium	1M	Tris pH 8.0	2.42
Bromide)	0.5M	EDTA	1.48
	5M	NaCl	16.35
Wash Buffer (100 mL)	50mM	Tris pH 8.0	0.60
	25mM	EDTA	0.90
	0.35M	Sorbitol	10.00
5% Sarkosyl (N-Lauroylsarcosine sodium salt)			
24:1 Chloroform :Iso-Amyl Alcohol			
Ammonium acetate (100 ml)	7.5M	Amm. Ac.	58.00

Appendix 8.3. AFLP analysis based on Do-It-Yourself AFLP Protocol by Leon Scott (unpublished). There are 3 steps in this AFLP protocol: Restriction Ligation, Pre-amplification and Selective Amplification.

1. Restriction Ligation Reactions. List of chemicals, reagents, materials and equipment used:

- High quality purified genomic *Barringtonia* spp. DNA
- OPA^+ (one-pho-al) Buffer (Ammersham #27-0901-02
- EcoRI (Ammersham)
- MseI (10mg/mL)
- BSA (10mg/mL0
- MilliQ Water
- T4 DNA Ligase (Ammersham/USB E70005X)
- EcoRI Adapter (equal amount on two oligos CTCGTAGACTGCGTACC and AATTGGTACGCAGTCTAC)
- MseI Adapter (equal amounts on two oligos GACGATGAGTCCTGAG and TACTCAGGACTCAT)
- ATP (lithium salt)
- PCR machine
- Hot water bath
- Pipettes
- Miscellaneous plastic ware

Procedures:-

a. Setup a restriction master mix as follows:

Reagent	Source	Stock Concentration	Amount per reaction	1 x (µL) 100ng DNA	*1 x (µL) 220ng DNA*
OPA^+	Ammersham	10x	1.2x	2.10	46.2
EcoRI	Ammersham (USB)	12x	2U	0.17	37.5
MseI	NEB	10	2U	0.20	44.0
BSA	NEB	10	100µL/mL	0.18	38.4
High purity DNA			100-500ng	100ng	1070
H_2O					
Total volume (µL)				**17.5**	**17.5**

NB: Ammersham is unable to provide Ligase, so Invitrogen and Promega lieges were used.

b. Incubate at 37oC for 60 minutes, do NOT heat inactivate.
c. Denature EcoRI and MseI adaptors at 94oC for 2 minutes and transfer to ice.
d. Prepare ligation master mix as follows:

Reagent	Source	Stock Concentration	Amount per reaction	1 x (µL) 100ng DNA	*1 x (µL) 220ng DNA*
OPA^+	Ammersham	10x	1.2x	0.30	66
EcoRI adapter		5	2.5	0.50	110
MseI Adapter		50	2.5	0.50	110

T4 DNA Ligase	Ammersham	1	0.5U	0.50	110
BAS	NEB	10mg/mL	100 µg/mL	0.025	5.5
ATP	Lithium salt	100	See below*	0.02	4.4
H_2O				0.06	150
Total volume (µL)				**2.5**	**5.0**

** the final concentration in the restriction ligation mix is 100µM, irrespective of amount of DNA.*

e. Add 2.5 µL of ligation mixture to the restriction reactions (5µL for 500ng DNA)
f. Incubate at room temperature for 3 hours
g. Check restriction by visualising 5µL of the restriction ligation (R/L) agarose gel.
h. Dilute the remaining reaction to produce a working R/L stock (15µL R/L + 5µL H_2O for 100ng DNA, 1µL R/L + 19µL H_2O for 500ng DNA).

2. Pre-selective Amplification. List of chemicals, reagents, materials and equipment used:

- *Eco* Pre-selective primer: e.g. *E + A (GACTGCGTACCAATTCA)*
- *Mse* Pre-selective primer: *e.g. M + C (GATGAGTCCTGAGTAAC)*
- 10x PCR Buffer (Fisher TFI)
- dNTP mix (Fisher 10mM)
- $MgCl_2$ (Fisher 25mM)
- Taq DNA polymerase (Fisher TFI)
- MilliQ Water
- $TE_{0.1}$
- PCR machine
- Pipettes
- Miscellaneous plastic ware

a. Set up pre-selective PCR master mix a follows:-

Reagent	Source	Stock Concentration	1 x (µL)	*x10 rxns*
10x PCR Buffer TF1	Fisher	10x	2.00	20
dNTPs	Fisher	10mM	0.40	4
$MgCl_2$	Fisher	25mM	1.20	1.2
Eco Pre-selective Primer	e.g. E + A	10mM	0.54	5.4
Mse Pre-selective Primer	e.g. M+ C	10mM	0.54	5.4
Taq	Fisher TF1	5U/µL	0.20	2.0
Restriction / Ligation mix			3.00	
H_2O			12.12	125
Total volume (µL)			**20.0**	

b. Amplify with the following conditions:

Temperature	Time	*PCR Cycle*
94°C	4 minutes	1 x cycle
94°C	30 seconds	28 x cycle
60°C	1minute	
72°C	1 minute	
72°C	5 minutes	1 x cycle
10°C	2 minutes	1 x cycle

c. Run 5 µL of PCR on agarose gel, expected result is a smear from 100-700+bp
d. Dilute pre-selective amplification by adding 210µL of $TE_{0.1}$ to the remaining 15µL (Adjust volume depending on smear brightness).

3. Selective Amplification. List of chemicals, reagents, materials and equipment used:

- *Eco* Pre-selective primer: e.g. *E + ** (GACTGCGTACCAATTC**)*
- *Mse* Pre-selective primer: *e.g. M + *** (GATGAGTCCTGAGTAA***)*
- 10x PCR Buffer (Fisher TFI)
- dNTP mix (Fisher 10mM)
- $MgCl_2$ (Fisher 25mM)
- Taq DNA polymerase (Fisher TFI)
- MilliQ Water
- PCR machine
- Pipettes
- Miscellaneous plastic ware

a. Make up master mix according to the follow:-

Reagent	Source	Stock Concentration	1 x (µL)	*x10 rxns*
10x PCR Buffer TF1	Fisher	10x	2.00	2.0
dNTPs	Fisher	10mM	0.40	4.0
$MgCl_2$	Fisher	25mM	1.20	12

Eco Pre-selective Primer	e.g. E + **	10mM	0.10	1.0
Mse Pre-selective Primer	e.g. M+ ***	10mM	0.50	5.0
Taq	Fisher TF1	5U/µL	0.20	2.0
Diluted Pre-selective Amp		100	5.00	
H_2O			10.0	110
Total volume (µL)			**20.0**	

b. Amplify with the following conditions (Bilbo Cycle 90):

Temperature	Time	*PCR Cycle*
94°C	4 minutes	1 x cycle
94°C	30 seconds	
65°C	30 seconds	1 x cycle
72°C	1 minute	
94°C	30 seconds	
65°C	30 seconds	9 x cycle Touchdown 1°C/cycle
72°C	1 minute	
94°C	30 seconds	
58°C	30 seconds	26 x cycle
72°C	1 minute	
72°C	5 minutes	1 x cycle
10°C	2 minutes	1 x cycle

c. Run 5 µL of PCR on GS2000

Appendix 8.4. GS2000 procedures for Shark's tooth denature gels (for pouring 1 gel) adapted from Corbett Research's Gel-Scan 2000 DNA fragment analysis operators manual (Version 2) and further described by Corinna Lange & Trevor Wardill (unpublished).

1. Ensure plates are cleaned with Windex & Kim wipes with no residues remaining.
2. Place plastic spacers along edge of clean glass plates.
3. Place the bottom plate onto the gel-pouring rig.
4. Apply Bind Silane @ RT to the **back plate** in the approximate position of the comb (approx. 2cm from the top of the plate).
5. Dry bind silane for 1 min , remove excess with a clean Kim wipe & replace gloves.
6. Place top plate, frosted side down, onto the gel rig, ensuring it is square!
7. Ready 4 clamps and comb for the gel pouring rig and gel plates.
8. Using a 25ml syringe, take up ***15ml*** of 5% Acrylamide Denature Gel mix.
9. To degas Acrylamide, Insert plug (sealed yellow pipette tip) into syringe tip & pull plunger back to create a vacuum inside the syringe. Gently tap on side of sink and while under vacuum remove plug to allow air to escape from solution. ***Repeat several times***.
10. Insert plug final time and pull plunger out leaving polyacrylamide mix in syringe. Place plugged syringe into rack.
11. Ensure fume extraction is on & add Temed and 10% APS (stored in fridge) to acryl.
12. Carefully & quickly insert plunger into syringe, gently mix Acrylamide, APS and Temed. Remove plug and apply the polymerising polyacrylamide onto plates.
13. VERY CAREFULLY, push the top plate down and back so that it slides into place between the black pins and the base of the gel pouring rig (sandwiching the gel)– this will be quite tight and possibly slippery (also be aware that the base of the plates might slide out from the base of the rig when doing this).
14. Clip both bottom clamps simultaneously onto sandwiched glass plates. Then carefully, but firmly, insert comb into top of gel to the base of the frosted glass. Lastly, clip final two clamps simultaneously to the top of the glass plates.
15. Allow gel to polymerise for 1 hour. Once gel has set, remove clamps and the comb. With the comb, gently cut excess polyacrylamide away from the top of the wells – being carefully not to jam excess into the wells. Scrape excess gel off into ***path-waste bin*** not SINK!
16. Rinse plates under RO water; remove excess polyacrylamide, dry with Kimwipes.
17. Place plates into GS2000, with the indented frosted glass plate at the front and bolt in. ***Do not over tighten bolts*** – this will crack and chip the plates.
18. Fill bottom buffer tank with 1 litre of 0.6 x TBE (Amresco) up to the red mark.
19. Clamp top buffer tank to plates and GS2000. Fill top tank with 0.6xTBE.
20. Put lid of tank on and prepare to Pre-run gel.

Appendix 8.5. GS2000 procedures for Shark's tooth denature gels (for pouring 1 gel). Adapted from Corbett Research's Gel-Scan 2000 DNA Fragment Analysis Operators Manual (Version 2) and further described by Corinna Lange & Trevor Wardill (unpublished).

5% DeNature Polyacrylamide Gel Mix in 0.6xTBE:

Urea	42 grams	
*10x TBE (**Amresco**)*	6 ml	
40% acryl. BIS-acryl. (19:1)	12.5ml	
MQ water	81.5ml	100ml

Gel mix will have to be heated to dissolve urea (DO NOT HEAT IN MICROWAVE). Only add 40 ml of H2O as the dissolving urea will significantly increase the volume.

Syringe filter gel mix before putting into Schott bottle. Wrap in alfoil.
Do not store at 4°C – the urea will crystallise.

Shark's tooth gels (0.20mm, 18cm length)

5% Polyacryl. Gel Mix 15ml (degassed)	
10% APS	*60 µl to 100 µl*
Temed	*6 µl to 10 µl*

Bind Silane
4 µl Bind Silane (stock in main lab @ 4°C)
10 ml Absolute ethanol

10% APS (Ammonium persulphate)
Keep frozen fresh
0.1 g ammonium persulphate
1.0 ml of deionised water
It's good to make no more than 10ml at a time.

0.6x TBE (to a litre)
*60 ml 10xTBE (**Amresco**)*
940 ml deionised water

Loading Dye – Denature Gels
Blue dextran/formamide loading dye:
Blue dextran add to suit
Deionised formamide 20 ml

Add 20µl of Loading Dye to PCR product.

MegaBACE™ ET-4000-R SIZE Ladder

- Mix as required, keep cool!
- Load 2 *µl* per lane
- *5 µl of stock + 10 µl of loading dye*
- *Ladder sizes are: 60, 90 100, 120, 150, 160, 170, 190, 200, 220, 250, 270, 290, 300, 310, 330, 350, 360 ... 90*