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VEGETATIVE PROPAGATION OF THE SANDALWOOD SPECIES:

S. yasi, S. album **and** ***F1 S.*** **hybrid** ***(yasi × album)***

MALONI HAVEA

VEGETATIVE PROPAGATION OF THE SANDALWOOD SPECIES

S. yasi, S. album and *F1 S.* hybrid *(yasi* × *album)*

By

MALONI HAVEA

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

M.Sc. thesis submitted to the

Division of Biological Sciences

School of Biological, Chemical & Environmental Sciences

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The University of the South Pacific

Suva

The Republic of the Fiji Islands

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DECLARATION OF ORIGINALITY

I, Maloni Havea declare that this thesis is my own work and that, to the best of my knowledge, assistance and work by other people has been properly acknowledged and cited.

SIGNED: ---------------------------------

Maloni Havea

Supervisor

Date: 30/04/08

You are encouraged to cite my thesis, or parts thereof, with proper citation and acknowledgements.

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DEDICATION

In memory of my Mother and mother-in-law Siaina Lauliva Halamehi Havea (1982) and Mele Pitisi Faivai'lo (2006)

Neongo kuo mo pulia ka ‘oku kei ongo mai pe ho’omo le’o ‘oku mau faka’amu ange pe ke tau fakataha ki ho tau api masani ‘o etau tamai I langi. Si’i faka’anaua ia mei ho’omo tama, makapuna Sofaia, Maloni, Siaina Lauliva Halamehi Havea mo Mesui Anaua He Feinga MSc Sione Fangufangu Havea.

ACRONYMS

ACIAR	Australia Centre for International Agricultural Research
AUD	Australia Dollar
Aus Aid	Australia Aid
CABI	Centre for Agriculture and Biosciences International
CSIRO	Commonwealth Scientific and Industrial Research Organisation, Australia
DBH	Diameter breast height (of a tree)
FAO	Food and Agricultural Organisation of the United Nations
FJD	Fiji Dollar
FFP	Forestry and Forest Products (Division of CSIRO)
FST	Faculty of Science and Technology, University of the South Pacific
GA	Gibberellic Acid
GTZ	German Agency for Technical Cooperation
IAA	Indole Acetic Acid (rooting hormone)
ICFRE	Indian Council of Forestry Research and Education
IBA	Indole Butyric Acid (rooting hormone)

MAFFF	Ministry of Agriculture, Food, Forestry and Fisheries
MSc	Master of Science
NAA	Naphthalene Acetic Acid (rooting hormone)
NE	North-east
NTFPs	Non-timber Forest Products
PIC's	Pacific Island Countries
PLR	Primary Lateral Root (big root)
PNGFRI	Papua New Guinea Forest Research Institute
R&D	Research & Development
SLR	Secondary Lateral Root (small root)
SPC	Secretariat of the Pacific Community
SPSS	Statistical Package for the Social Sciences
SPRIG	South Pacific Regional Initiative on Forest Genetic Resources
SRD	Siliviculture Research Division (in Fiji)
TOP	Tonga pa'anga
UNDP	United National Development Programme

URC	University Research Committee
USD	United States Dollar
USP	University of the South Pacific
VUV	Vanuatu Vatu

Comparable USD values for:	USD rates/1.00 for 2008	Average amount
AUD	0.5235	1.06
FJD	0.9351	1.50
TOP	0.666	1.91
VUV	0.01053	94.96

ABSTRACT

The genus *Santalum* belongs to the Santalaceae, a family in the Order Santales. *Santalum* comprises 16 species. *Santalum* taxa are hemi-parasites, and the availability of

appropriate host plants is crucial to their successful establishment. The economic and cultural values of *Santalum* species are attributed to the fragrant oils present in its heartwood. *Santalum* taxa have considerable cultural and economic importance to many communities in Pacific Island countries and territories.

This study investigated vegetative propagation of three sandalwood taxa, viz: *Santalum yasi*, *S. album* and their hybrid (*S. yasi x S. album*). Experiments were undertaken to induce root suckering in mature sandalwood plants growing in the field in two locations in southeast Viti Levu (Vunimaqo and Colo-i-Suva), Fiji the technique employed for root sucker induction was to partially or fully sever larger near-surface roots. Root suckers were produced on the segment of damaged or cut root, which is positioned away from the trunk, suggesting that chemicals from the tree inhibit the development of root suckers. Substantial differences in the number of shoots produced were observed between roots which had been partially cut (primary lateral root) and those that were fully cut through or severed (secondary lateral root). Induction of root suckering was most successful for the hybrid plants: on average 3.7 ±0.6 shoots were induced on fully cut secondary lateral roots, while 1.20 ±0.18 shoots were produced on partially cut primary lateral root roots. For *S. yasi*, averages of 0.61 ±0.18 shoots were induced on fully cut roots and 0.15 ±0.11 shoots on partially cut roots. For *S. album*, averages of 0.96 ±0.65 shoots were induced on fully cut roots and 1.2 ±0.34 shoots on partially cut roots.

The optimal root diameters of severed lateral roots for stimulating shoot production was 1.40 ±0.10 cm for hybrids, 1.98 ±0.11 cm for *S. album* and 1.60 ±0.11cm for *S. yasi*. The optimal root diameters for stimulating shoot production in partially cut larger roots was 5.03 ±0.90 cm for hybrids, 4.78 ±0.35 cm for *S. album* and 4.77 ±0.57 cm for *S. yasi*. The distance from the trunk at which the root is cut has a strong influence on shoot induction. The optimal distances from the trunk for stimulating shoot production were 56 ±4 cm for hybrids, 33 ±3 cm for *S. album* and 32 ±2 cm for *S. yasi* for completely severed or cut through secondary lateral roots. However, for half-cut through primary lateral roots the optimum distance from the trunk was 20 ±4 cm for hybrids, 19 ±5 cm for *S. album* and 9.1 ±2.5 cm for *S. yasi*. In addition, this investigation confirmed that complete severing of roots was more effective than partial severing of roots for stimulating shoot production for cuttings material of *Santalum* taxa.

An investigation was undertaken to identify effective treatments for propagating *Santalum* taxa through stem cuttings. Five factors were investigated for their effect on rooting success of the cutting. These factors were Indole Butyric Acid (±),root promoting hormone Naphthalene Acetic Acid, at concentrations of (0, 0.5 and 1.0 mg L^{-1}) seedling age (6-12 and 12-24 months), cutting type (root sucker shoots or seedlings) and cutting position (apical or basal). The rooting percentage and number of roots formed was increased by Indole Butyric Acid application in interaction with 30:70 sand:peatmoss, and apical shoots. Under these conditions, the average number of roots formed on *S. yasi* was 2.8 ±0.3: corresponding numbers of roots were 2.6 ±0.2 for *S. album* and 3.6 ±0.3 roots for hybrids. The average length of roots formed on *S. yasi* cuttings was 2.1 ±0.2 cm, 2.2 ±0.2 cm for *S. album* and 2.9 ±0.3 cm for hybrids. The application of 1.0 mg L^{-1} Naphthalene Acetic Acid (NAA) in 30:70 sand:peatmoss medium, and originating from the apical portion further enhances rooting success of root number and length for *S. yasi* 2.5 ±0.2 roots and 2.7 ±0.2 cm, *S. album* 2.5 ±0.3 roots and 1.8 ±0.3 cm and hybrid 3.1 ±0.2 roots and 2.9 ±0.2 cm. Younger seedling age (i.e. 6-12 months) in a 30:70 sand:peatmoss medium, and originating from the apical portion further enhances rooting success, both root number and length, for *S. yasi* 1.9 ±0.2 roots and 2.0 ±0.2 cm, *S. album* 1.5 ±0.2 roots and 1.3 ±0.2 cm and hybrid 2.8 ±0.3 roots and 2.2 ±0.2 cm.

The result revealed that cuttings taken from apical positions gave much higher rooting percentages than those taken from basal positions. Rooting of cuttings was improved (i.e. more and longer roots) in a 30:70 sand:peatmoss medium compared with mahogany compost. Application of rooting hormone (Naphthalene Acetic Acid 1.0 mg L^{-1} and with Indole Butyric Acid) increased the numbers of roots and root length compared to than two other concentrations of Naphthalene Acetic Acid. Cuttings from younger seedlings (aged 6-12 months) produced more and longer roots than did cuttings from older seedlings (aged 12-24 months). The results revealed that *Santalum* hybrid is the most suitable of the three investigated taxa for conventional vegetative propagation.

The final experiment was an investigation of the development and survival of treated cutting-derived plants following transplanting. The aim of this work was to investigate the survival of apical and basal stem cuttings following transplanting. Transplanted rooted cuttings derived from Naphthalene Acetic Acid (1.0 mg L^{-1}) treatment, younger seedlings (6 to 12 months old) in interaction with 50:50 sand:peatmoss media developed longer roots in all three taxa 1.4 ±0.1 cm in *S. yasi*, 1.3 ±0.1 cm for *S. album* and 1.5 ±0.1 cm for hybrid. Rooted cuttings that had been propagated in 30:70 sand:peatmoss with hormone (1.0 mg L^{-1} Naphthalene Acetic Acid) application produced longer roots after transplanting. The transplanted rooted cuttings from Indole Butyric Acid, apical position age 6 to 12 months in interaction with 30:70 sand:peatmoss media further enhanced root development (length) for *S. yasi* 1.5 ±0.1 cm, 1.5 ±0.1 for *S. album* and 1.1 ±0.0 cm for hybrid. Rooted cuttings that had been propagated in 30:70 sand:peatmoss with hormone Indole Butyric Acid application produced longer roots after transplanting.

Based on the experiments reported in this study, higher percentages of successful cutting-derived plants of *Santalum* species can be attained by taking apical cuttings from younger seedlings (up to age of 12 months) treating them with Indole Butyric Acid and Naphthalene Acetic Acid (1.0 mg L^{-1} concentration) and rooting them in a 30:70 sand:peatmoss medium (rather than in media derived from local compost).

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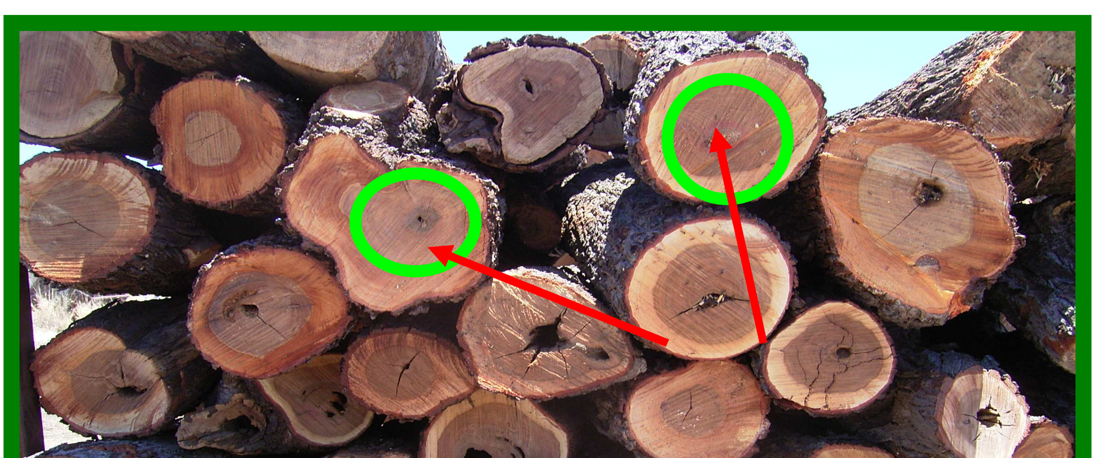
CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

Sandalwood have been used for thousands of years and is of great social, religious and economic importance in several countries (Weiss 1997) (Plate 1.0.). Presently the oil from the heartwood (Plate 1.1) of several sandalwood taxa including *Santalum yasi*, *Santalum album* and *Santalum* hybrid (*S.yasi x S. album*) is extracted for use in cosmetics, scenting of soaps, aromatherapy, perfumery and medicines, while the wood is powdered for incense sticks or used for carving ornamental (Plate 1.2) or ceremonial (Plate 1.3) objects, for which it is greatly prized (Srinivasan *et al.*, 1992).

Plate 1.0 Sandalwood seedlings in Colo-i-Suva nursery (Fiji) are of great social, religious and economic importance in several countries (photo: Havea, 2006).



Heartwood of sandalwood

Plate 1.1 Presently the oil from the heartwood of sandalwood is extracted for use in cosmetics, scenting of soaps, aromatherapy, perfumery and medicines (photo: Thomson *et al*., 2005).

Plate 1.2 Carvings for religious, cultural and tourism trade. Some carved items are even available in island nations such as Solomon Islands and Vanuatu (photo: Thomson *et al*., 2005).

Plate 1.3 Incense burning is integral to Indian and Chinese religious rites (photo: Thomson *et al*., 2005).

The availability of sandalwood on the world market is in decline due to a greatly diminished natural resource base. Sandalwood resources have declined as a result of over-exploitation associated with high prices and demand, and other human activities such as forest clearing for agricultural purposes and pests and diseases (J.C. Doran *et al.*, 2002). Sandalwood growing and harvesting is one of the world's oldest, and still one of its most valuable, forestry enterprises (Evans 2003).

Sandalwood has always been a prime natural resource for remote communities in developing countries. The multitude of functions and services provided by sandalwood over the years has met most of the subsistence and semi-subsistence commercial needs of rural communities (Jiko, 1993). It is economically and culturally important to many countries around the Pacific and Eastern Indian Ocean regions where it grows or is traded.

In the South Pacific region, sandalwood is very rarely used nowadays because of its scarcity and cash value. The grated wood was traditionally used to a limited extent to scent coconut oil and/or cultural artifacts such as tapa cloth. The historical significance of sandalwood to the people of the Pacific is well known and valued (Thaman and Whistler, 1996).

There are 16 species in the sandalwood genus *Santalum*, not all of which are harvested commercially (Chapter 2). They grow naturally and are distributed throughout the Pacific and Eastern Indian Ocean regions (Chapter 2) (Barret and Fox 1995). Sandalwood are evergreen trees ranging in size from tall shrubs to small to medium-sized trees. They grow in a variety of climates from the Australian desert to subtropical New Caledonia and at elevations from sea level to over 8000 feet (~2,460 m.). The most well-known and economically important species is *Santalum album* (Shea *et al.,* 1998), as it has the highest oil content (6-7%) and a desirable aroma profile. *Santalum austrocaledonicum* (New Caledonia and Vanuatu) and *S. yasi* (Fiji and Tonga) are also distilled to produce essential oils. *Santalum spicatum* from Western Australia has been valued for its wood for many years, and has recently also become a source for essential oils (McKinnell 1990).

Trees harvested for oils are selected by age and size because of the higher proportion of heartwood (and thus essential oil) in larger trees (Plate 1.4). Dead-standing or fallen trees are also harvested because the wood holds onto the essentials soils for many years. The whole tree is harvested and used, including the sawdust and the stump (which has the highest oil content) and the sapwood (which contains small amounts of oil). The lower grades of sandalwood, such as the sapwood, are used for incense and for chips and powder, while the better logs are used in carving (small objects to furniture).

Plate: 1.4 Trees harvested for oils are selected by age and size because of the higher proportion of heartwood (and thus essential oil) in larger trees (photo: Thomson *et al*., 2005).

The economic value of sandalwood, witnessed from a range of published studies, reveals that it makes a major contribution to the economies of countries in these areas. Evans (2003) also highlighted that the sandalwood in Tonga is a highly promising tree species for rural income generation. This is not surprising, given that sandalwood provides a non-perishable product (heartwood and essential oil) that has found good markets from the South Pacific Islands for more than 200 years.

In the Asia Pacific region, many sandalwood taxa are threatened with extinction either at the species or at the population level (Doran, *et al.*, 2002). With rather limited resources the research partners in Australia and Indonesia and also in the Pacific region have called for a greater awareness of the urgent need to conserve remaining forest. This lack of resources has allowed serious consideration to be given to growing sandalwood. It is accepted that efforts must be made now in order to preserve an adequate genetic resource base of the various species and varieties for future generations.

There is continued demand globally for sandalwood. It would appear that vegetative propagation of sandalwood by means of cuttings is playing an increasingly important role in retaining the specific characteristics of the selected *Santalum* taxa mother trees. This vegetative propagation could bring about considerable gains in terms of volume production, uniformity in wood quality and growth, and rapid return to investment, as well as being the foundation for a secure future resource. Overall, there has been a growing awareness about the importance of sandalwood, not only for the role it plays in subsistence economies, but also for its present and potential importance to the national economies of many developing countries.

1.1 Problem statement

The wide range of uses and the importance of the economic and cultural value derived from the tree to the subsistence and semi-subsistence livelihoods of people in many countries in the Asia-Pacific region, is what prompted my interest in sandalwood as a research topic. More specifically, the role of sandalwood in the subsistence and semi-subsistence commercial sector of the Pacific region is of special interest for rural development. The selection of sandalwood for this research because of the second most costly wood (Boland *et al.*, 1984).

To the author’s knowledge, sandalwood is one of the main underdeveloped resources which could help in the development of rural peoples in Pacific Island Countries (PICs). The questions that need to be asked are:

(a) What is the economic value of sandalwood for rural income generation in the Pacific?

(b) How can the ongoing community harvest of wild sandalwood be made sustainable?

(c) What are the most appropriate propagation techniques that need to be developed for sandalwood in order to provide larger numbers and better quality planting stock for replanting programs?

The availability of sandalwood on the world market is in decline due to a greatly diminished natural resource base. Sandalwood resources have declined as a result of over-exploitation associated with high prices and demand (Doran *et al.*, 2002). In the Pacific, for example, *Santalum yasi* was heavily exploited in the early 1800s and quickly became rare (Bulai, 1995). Over-exploitation of sandalwood in Tonga in the 1960s and 1970s has severely reduced the resource (Kaufusi *et al.*, 1999). It is now imperative that the remnant natural resources of sandalwood be protected and managed in a sustainable manner and that planting programs be instigated to ensure their contribution to rural livelihoods and continuity of supply.

Humans have caused damage to sandalwood stands through indiscriminate hacking and cutting of undersized trees, while fire has wiped out regenerations of seedling. The main cause of the reduction of sandalwood comes from overharvesting, whereby all larger individuals (i.e. the main fruit-bearing trees) are periodically cut down. Failure to retain an adequate number of seed trees for natural regeneration or for enrichment planting means that populations take a very long time to recover after such intensive exploitation (Wiser *et al.*, 1999).

Research on sandalwood in the Pacific region is quite difficult for several reasons including a specific harvesting season and difficulties in non-destructively collecting wood samples and obtaining planting materials. Some sandalwood species have been reported as difficult to propagate or else there is no information on whether they may be successfully rooted from stem cuttings (Walker *et al.,* 1999). However, some of the trials carried out have shown that vegetative cuttings root well (Bulai, 1995). Collins *et al.* (2000) have shown that *S. yasi* is amongst the easiest of the sandalwood species to root from cuttings of juvenile plants.

1.2 Objectives of the study

The overall aim of this research is to identify propagation techniques for sandalwood and to provide larger numbers and better quality planting stock for replanting programs.

1.2.1 The specific objectives of this research are:

- Generate a protocol for the vegetative propagation of *Santalum yasi, S. album* and *Santalum* hybrid (*S. yasi x S. album*).
- Ascertain whether the rate of shooting varies with partial or total cut in the underground root of the parent (stock) tree.
- Determine the conditions necessary for successful stem-cutting propagation of sandalwood, to standardize a technique for vegetative propagation of sandalwood and utilize that technique to facilitate the multiplication of sandalwood trees.
- Compare the degree of rooting relative to cutting position (i.e. apical and basal portion) of the cut lateral roots, rooting hormone (i.e., with and without Indole Butyric Acid (IBA) and different concentrations of Naphthalene Acetic Acid (NAA) and effect of rooting medium (i.e. peatmoss or mahogany compost), and seedling ages of the cutting (i.e. 6 to 12 and 12 to 24 months).
- Evaluate the survival of the transplanted rooted cuttings.

1.3 Significance of the study

Several studies have been published on the vegetative propagation of sandalwood. Most have focused on the effect of season when cuttings were collected, the effect of the origin (position on plant) of the cutting materials and the effect of different levels of IBA as root promoters. Teklehaimanot *et al.,* (2004) looked at the influence of the origin of stem cutting, season of collection and auxin application on the vegetative propagation of African sandalwood (*Osyris lanceolata*; family Santalaceae) in Tanzania. As was mentioned previously *Osyris lanceolata* was used as a source of sandalwood-type oils in the past but these are little traded nowadays (Coppen 1995; Weiss 1997). Their results revealed that stem cuttings collected from the sprouting stumps had potential to be used in propagating: stem cuttings collected in September, originating from the basal portion had the highest rooting percentage (43.8 ±3.9%), with application of IBA between 50 and 100 parts per million (ppm), further enhancing rooting success.

Balasundaran (1998) found that *Santalum album* had 56% rooting and sprouting in talcum-based IBA treatment and 14% in alcohol-based treatments. Srimathi and Nagaveni (1976) achieved vegetative propagation of mature *Santalum album* by inducing rooting around the shoot primordial, and then out-planting the shoot primordial with the original roots. As another option, they also cut off the original root along with the shoot primordial and induced rooting after transplanting. However, they did not succeed in propagation by stem cuttings. Uniyal *et al.,* (1985) researched the vegetative propagation of *Santalum album* by root cuttings and reported vegetative propagation through this means: secondary roots or thin superficial roots dug out, cut into 5-cm long pieces, and treated with seradix B sprouted and rooted in 30-40 days. Vijayakumar *et al.,* (1981) reported and observed that trenches dug 60-90 cm from the main trunk give maximum induction of adventitious shoots.

Walker *et al.,* (1999) reported that preliminary vegetative propagation experiments with *S. album, S. austrocaledonicum* and *S. yasi* met with only variable success. Substantial differences in survival and rooting success were observed between *S. album* and *S. austrocaledonicum,* with an average rooting success of 63.5% for *S. austrocaledonicum* compared to only 9.5% for *S. album*. For *S. austrocaledonicum,* the peat medium also resulted in better rooting success than the pure sand medium. However, in another study also by Walker *et al*., (1999) the rooting ability of five different *Santalum* species (*S. album, S. austrocaledonicum, S. yasi, S. macgregorii* and *S. lanceolatum*) was compared. All had a much lower strike rate, thought to be due to cooler weather conditions during the period of setting the cuttings. Despite this, results once again showed *S. austrocaledonicum* to be an easier sandalwood species for vegetative propagation (20.2%), and *S. yasi* achieved an excellent strike rate (46.1%).

In the Pacific Region, research on vegetative propagation of sandalwood has been limited. Bulai, (1995) the Siliviculture Forestry Division (SRD), Colo-i-Suva, Fiji, also reported that vegetative cuttings taken from young *S. yasi* seedlings at the 2-4-leaf stage rooted well, with a success rate of 90%. Bulai and Nataniela, (2002) showed that shoot cuttings taken from 18 month old plants produced a 41% strike rate under mist conditions. Collins *et al.,* (2000) and other studies conducted have shown that *S. yasi* is the easiest of the sandalwood species to root.

In compiling this review, no detailed studies on vegetative propagation of Pacific sandalwood were located. The present study will help fill this gap and also complement and build on previous work on *S. album*. Furthermore, the study is useful for three reasons. Firstly, it documents the voice of an indigenous scholar and resource owner (myself) in relation to the subject of vegetative propagation. Secondly, it provides background work for future research. Finally, it will produce information about vegetative propagation of sandalwood, which it is hoped, will be used by rural communities, government and resource developers in their quest to develop and apply more efficient vegetative propagation techniques for sandalwood resource development in the Pacific region.

My research aims to provide valuable information on the vegetative propagation of sandalwood for the Pacific region, especially the rural communities. More importantly, the results could be used to assist the government, private nurseries and the villagers in the genetic improvement of their sandalwood resource. It is hoped that the study will support the author's belief that the enhanced uses of vegetative propagation of sandalwood could serve as one of the most important foundations for better conserving and sustainably developing the genetic resources of this promising genus throughout the Pacific Islands.

1.4 Layout of the thesis

The thesis is divided into six chapters. This chapter provides a general overview of the whole study, by defining the topic and briefly highlighting relevant earlier studies on the subject. It states the main objective of the research and outlines the author's personal perception of why this research is different from other previous work conducted on induction of root-sucker formation, vegetative propagation and transplanting of rooted cuttings of three *Santalum* taxa (*S. yasi, S. album* and *Santalum* hybrid, *S.yasi x S. album*).

An overall review of the literature is presented in Chapter 2, mainly on the economic importance of sandalwood in the PICs, traditional uses, history of trade and current utilization using existing published texts from international and regional studies, including current studies in the PICs. Furthermore, the review highlights the botany, ecology, conservation status, and threats to *S. yasi* in Fiji and Tonga.

Chapter 3 presents the findings from the shoot production (induction of root-sucker formation). It describes the methodologies and techniques that the researcher used during the conduct of this research. The types of data that were collected are mainly from induction of root-sucker formation (circumference of roots, length of partial or total cut in the underground shoot from the trunk and number of shoots).

Chapter 4 presents the findings from the vegetative propagation of three *Santalum* taxon.

Chapter 5 is mainly a presentation of the author's results from the study of survival and subsequent root development of transplanted cuttings subjected to different treatments of three *Santalum* taxa (i.e. *S. yasi, S. album* and hybrid[1] *S. yasi x S. album*). The analyses in Chapters 3, 4 and 5 are supported and supplemented by data presented in tables and graphs.

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[1] The native Fijian species, *S. yasi* is able to naturally hybridise with *S. album* in mixed plantings. This natural hybridisation predominantly occurs with the maternal *S. yasi*. Seed collected from *S. yasi* trees in mixed planting with *S. album* will give up to 20% hybrid seed. Seed collected from *S. album* within the same planting produce much lower hybrid progeny. Germination trials from *S. yasi* seed parents that are located close to *S. album* trees, have up to 25% F1 hybrid germinates (Bulai and Nataniela, 2002).

Chapter 6 is the general discussion and conclusion. This chapter provides an overview of the findings of the current study. It highlights the main constraints of the study and suggests the implications for future research in this area. Also included in this chapter are recommendations that might be useful for the community of the Pacific Islands to further enhance the sustainable management of their sandalwood resource.

CHAPTER 2
INTRODUCTION TO *SANTALUM* TAXA

2.0 INTRODUCTION

The potential value of sandalwood to the national economies of Pacific Island States and to their rural peoples indicates that more attention should be paid to the development of sandalwood cultivation. One of the main objectives of the South Pacific Regional Initiative on Forest Genetic Resources (SPRIG) project throughout the Pacific Island Countries (PICs) is to help to conserve, improve and better promote the wise use of the genetic resources of regional priority tree species, including sandalwood. It is expected that this approach will promote economic and rural development (SPRIG, 2006). Sandalwood in the PICs region is an important tree because of its high economic value compared to other tree species. The heartwood has a good smell due to fragrant essential oils, which upon steam distillation have an exceptionally high commercial value (e.g. $F 1000 per litre) (Bulai, 1995).

Sandalwood is one of the most interesting plant groups to study due to its life history and difficulties in propagation and cultivation. The generic name is derived from the Greek *santalon* (meaning sandalwood) which in turn is derived from *Chandana*, a Sanskrit term meaning the tree as well as the wood and oil derived from it (Brummitt, 1992; Barrett and Fox, 1995a). Sandalwood is sometimes described as 'this beautiful tree' or 'royal tree' not only because of its economic and cultural values, but also because of its considerable cultural and economic importance to communities in several PICs and territories (Weiss, 1997).

Cultivation of sandalwood is, however, not an easy matter, as all taxa of *Santalum* are root hemi-parasites.This term describes a plant which photosynthesizes, but which derives water and some nutrients through attaching to roots of other species, or the tree absorbs soil nutrients through. Accordingly the availability of appropriate host plants is crucial to the plant's successful establishment (Srinivasan *et al*., 1992; Scott, 1871).

Sandalwood is one of the most promising genera for rural economies in the Pacific region. It is commonly known as 'sandalwood' (English name), '*sandalwud'* (Vanuatu: Bislama), *'ahi'* (Tonga), *yasi dina* (Fiji). Sandalwood is the name applied to any species of the genus *Santalum* of the sandalwood family (Santalaceae), a family in the order Santales (Brummitt, 1992). *Santalum* comprises 16 species. Only a few species in the genus are utilized commercially, for either wood or oil or both in most of the Asia and the Pacific region, including Australia (Appendix 1.0, 1.1, Table. 1.0 and 1.1.): Over-capacity of the sandalwood processing industries has been practised and is ongoing in the PICs as a result of overexploitation associated with illegal cutting activities. In addition, some harvesting methods (e.g. cutting for Christmas trees in Tonga), land use expansion, forest clearing and pests and diseases have resulted in a decline in sandalwood populations. With continuing exploitation and spiraling prices, it is likely that sandalwood resources in the PICs will come under extreme pressures over the next few years.

One of the objectives[2] of the research presented here has been to assess and identify improved propagation methods for sandalwood using cuttings.

This research has drawn on the rather limited previous research information as a guide. An aim is to develop vegetative propagation by macro-cuttings to the stage where it may be used routinely on an operational basis. It is intended that this study will contribute to providing additional information that will assist local authorities and organizations in planning future replanting and management of sandalwood. In addition to this investigation, information on root induction and transplanting of rooted cuttings using three *Santalum* taxa (i.e. *S. yasi*, *S. album* and *Santalum* hybrid) will be used to fill gaps in information on vegetative propagation of sandalwood in its native Pacific Islands countries.

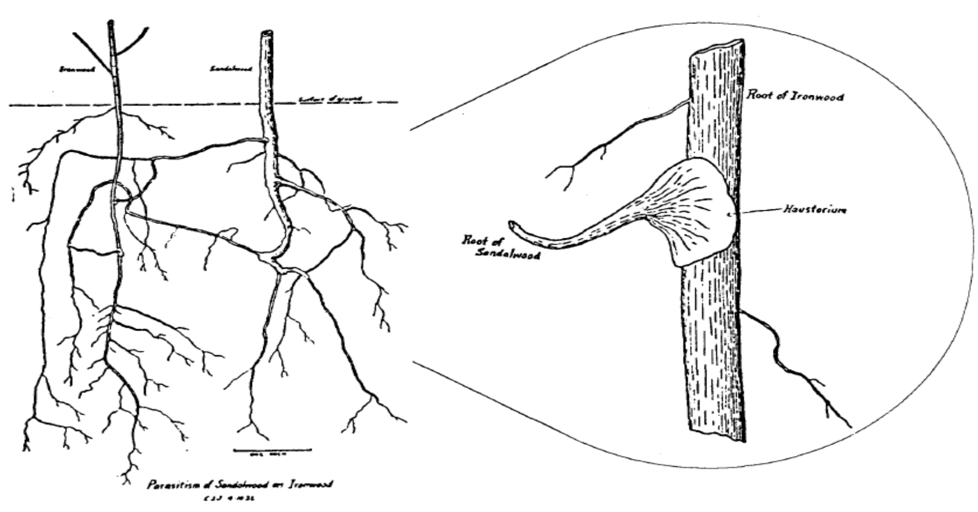


Figure 3—Haustoria of *Santalum* species is shown parasitizing ironwood tree (*Casuarina* sp.). Drawings by C.S. Judd, reproduced from: Cartwright (1935, p. 21).

[2] To determine the conditions necessary for successful stem-cutting propagation of sandalwood, to standardize a technique for vegetative propagation of sandalwood, and to utilize that technique to facilitate the multiplication of sandalwood trees.

Plate 2.0 Haustoria of sandalwood are shown parasitizing ironwood tree (*Casuarina* sp.) Drawing by C.S. Judd reproduced from Cartwright (1935,p.21), in Hamilton and Conrad, 1990.

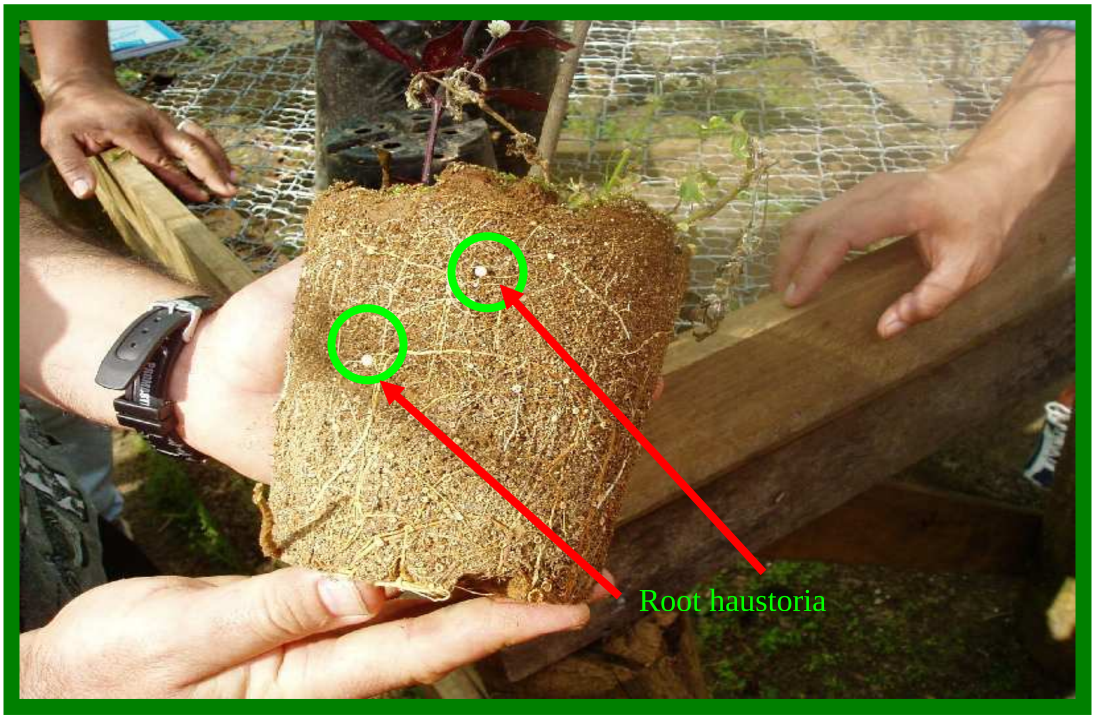


Plate 2.1 Haustoria of *Santalum yasi* seedling is shown parasitizing host plant at Colo-i-Suva Forestry Nursery (photo: Bulai, 2006).

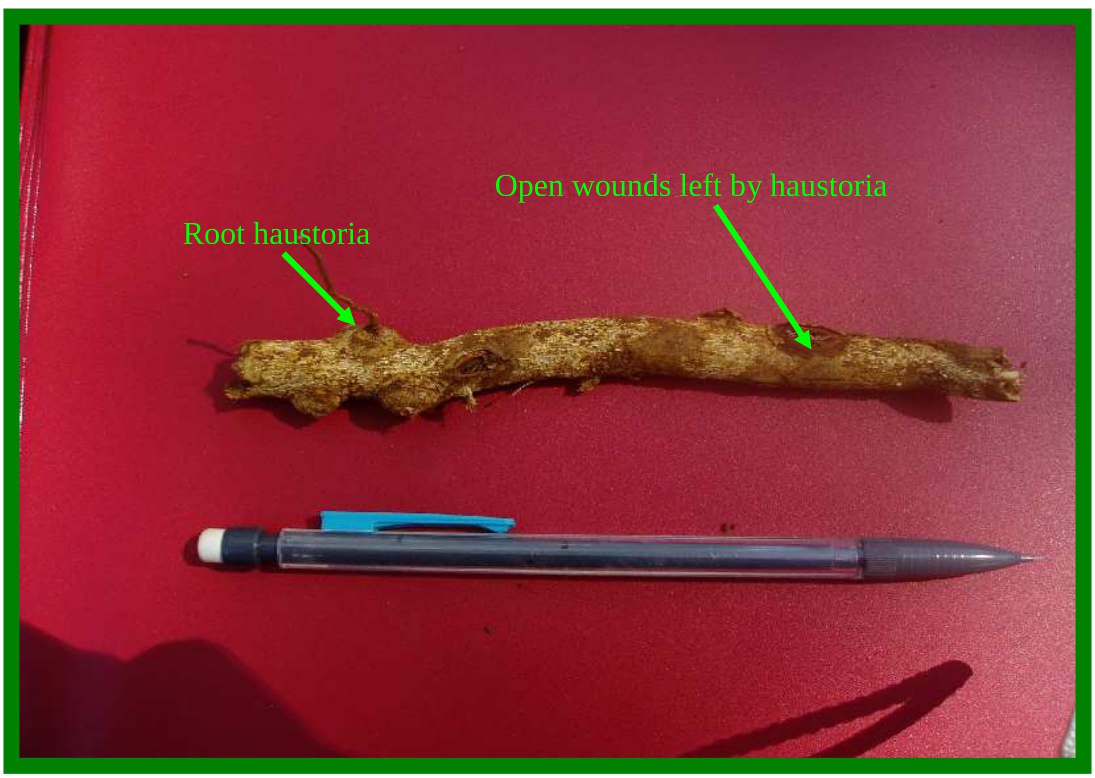


Plate 2.2 Orange trees haustorised by *Santalum* hybrid (*S. yasi x S. album*) at Colo-i-Suva Forestry demonstration plot, (photo: Bulai, 2006).

2.1 Economic importance of *Santalum* taxa in the PICs

Sandalwood is an important commercial industry in the South-western Pacific (Robson, 2004). A number of sandalwood species occur across the South-western Pacific; including *S. austrocaledonicum* in New Caledonia and Vanuatu, and *S. yasi* in Fiji and Tonga (Appendix 1.0). Management and harvesting of existing sandalwood stands along with new sandalwood plantings is mainly undertaken by villagers. There is a growing interest among villagers, other small-scale growers and governments to expand the scale of planting in those four countries. The most common type of planting is garden plantings (agroforestry system - a farming system that integrates crops and/or livestock with trees) of sandalwood by villagers.

In Fiji *Santalum yasi (yasi dina)* was especially heavily exploited in the early 1800s and quickly became rare (Bulai, 1995). In New Caledonia, the landowners sell the unprocessed wood to the distillers for AUD 4 /kg. Approximately 10 litres of oil can be extracted from 350 kg of heartwood chip and shrubs. Commercial utilization has only recently started again. The markets in Southeast Asia received over 100 tonnes of sandalwood a year from Fiji, It was Exploitation started again only recently with 1115 tonnes harvested between 1993 and 1994, earning in excess of FDJ 7 million in foreign exchange (Bulai, 1995). Between 1984 and 1993, the prices fluctuated over that 10-year period, from AUD 2,780 /tonne in 1984, to AUD 11, 000 /tonne in 1991, and down to AUD 2, 200 /tonne in 1992 (Bulai, 1995). Ehrhart (1996, 1997) reported that the sandalwood resources on Tonga had been depleted of harvestable trees between 1960 and

1970. A total ban on harvesting in Tonga existed until the late 1990s. A small harvest in 1996-97 netted timber owners AUD 3,000 / tonne (Kaufusi *et al.*, 1999).

Traditional farmers in the PICs are increasingly interested in growing and cultivating sandalwood as a means of earning income in the future. Whilst it is a long-term activity, the returns are expected to be high. Leaving gardens fallow and enabling sandalwood to mature until the land is needed for farming again enhances the returns from subsistence farming practices. There are potentially major economic, environmental and cultural benefits from planting sandalwood for the landowners in the countries of the Pacific. In Vanuatu, larger investors are now realizing this and establishing more extensive plantations using elite genetic material.

2.1.1 Traditional uses of sandalwood in the PICs

Traditional uses of sandalwood in the PICs provided economic, social, and ecological benefits to local communities (Applegate and Davis, 1990), as mentioned at the beginning of Chapter One. It is rarely used nowadays for local uses because of its scarcity and high cash value. The oil was traditionally used to a limited extent to scent coconut oil and cultural artifacts such as tapa (Thaman and Whistler, 1996).

2.1.2 History of *Santalum* trade

The most important use of sandalwood is still as a raw material for the incense burnt during worship in Asian countries and India (Plate 2.5). It was around 1760 that explorers, traders and whalers found sandalwood on a number of PICs and, according to Shineberg (1967), it was in 1786 that a company was set up in Boston (America) for the purposes of whaling and sandalwood harvesting in the PICs. However, at 50 pounds

(British pounds) a tonne sandalwood trading yielded an excellent profit to suppliers. This sandalwood was therefore sought around the PICs by fortune hunters. Fiji was the first island group to experience the “sandalwood rush” in 1816, but, thirty years on, the trade had spread to most of the islands of southern Melanesia including Erromango in 1830 and Isle of Pines in 1840 (Shineberg, 1967).

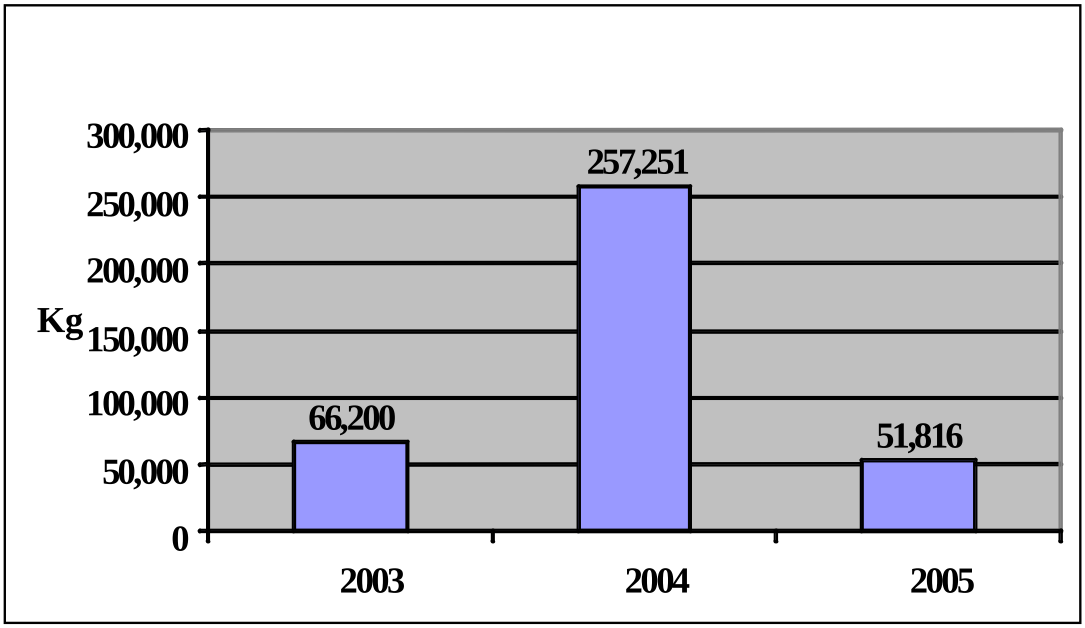
The first connecting link between western trading systems and the PICs was the whaling industry (Hughes, 1977). Expansion of whaling in the 1760s meant that whaling ships from England and France penetrated the PICs region. Although they were primarily interested in whalers, these whaless were exposed to commodities such as sandalwood. Pacific Island peoples were also hired as labourers, and Tongans, for example, were contracted to cut the sandalwood in Erromango (Vanuatu) (Shineberg, 1967).

When the British become more active in Asia in search of products that would redress their trade imbalance in China there was a boom in the sandalwood trade in the PICs. The consequences were enormous: sandalwood became commercially extinct in Fiji and the Marquesas by 1816, sandalwood trees were destroyed in Hawaii by the 1830s, and the sandalwood traders/cutters moved to New Caledonia, the Loyalty Islands, the New Hebrides and Vanuatu by the 1830s (Shineberg, 1967).

Plate 2.3 Sandalwood is still a raw material of the incense burnt during worship in Asian countries and India (photo: Thomson *et al*., 2005).

2.1.3 Marketing and Current utilization of sandalwood

Santalum yasi, the only sandalwood in Fiji, earned F$4.74 million in foreign exchange over the period 1987–90 (Jiko, 1993, Usumaki, 1981). The marketing of sandalwood from the PICs was very high during the first exploitation period (1800 to 1805). Bulai (1995) highlighted that between 1984 and 1993, 1,115 tonnes was harvested in Fiji for export, and the markets for the produce were in Singapore, Hong Kong, Taiwan, Korea and Japan. Prices have fluctuated markedly from year to year, e.g., $F 3,383/tonne in 1984 to $F13, 452/tonne in 1991, and down to $F 2,700/tonne in 1992. Ehrhart (1996) and Ehrhart (1997) reported that the sandalwood resources on Tonga were essentially depleted of harvestable trees. In Tonga the price for the heartwood of *S. yasi* has increased substantially in recent times from TOP 5/kg in 2003 to TOP 8/kg in 2005 (Figure 2.0). The main export market for sandalwood is Taiwan. The highest export was about 257,251 kg of sandalwood was in 2004 contributing TOP 1,672,133 to the country’s economy.



TOP 430,300 TOP 1,672,133 TOP $336,804

Figure 2.0 Sandalwood exports for the years 2003-05 in Tonga. (Source: Likiafu and Robson, 2005)

In Vanuatu in 1993, sandalwood was sold at VUV 250 per kg; in 2003 the price increased from VUV 400 to VUV 600 per kg of heartwood harvested from natural forest; and in 2005 sandalwood heartwood were purchased at VUV 800 per kg. The increases in price shows that sandalwood is an important commercial industry and represents a small but vitally important industry for Vanuatu. Sandalwood in Vanuatu is normally exported in the form of oil, chips, and logs (Plate 2.4 and 2.5). Research into sandalwood in Vanuatu is one of the priority programs so that Vanuatu can compete in the high value global markets (VDoF, 2000).

Plate 2.4 Sandalwood in Vanuatu ready to send to Efate for wood chip (photo: Thomson *et al*., 2005).

Plate 2.5 Sandalwood in Vanuatu sent from the outer Island to Efate (photo: Thomson *et al*., 2005).

International market for sandalwood

To date, sandalwood harvested to meet local demand and export purposes has been derived from only natural stands. The major buyers of sandalwood logs for carving are from Hong Kong and Taiwan. They in turn distribute to China, Japan, and Singapore.

India produces the best sandalwood logs for carving due to the fine grain of its *S. album*, but utilizes it all domestically.

2.2 Botany and ecology of *Santalum* taxa in Fiji and Tonga

2.1 Botanical features of the genus *Santalum*

- The botanical features of the genus were summarised by Barrett and Fox (1995a) as follows:
- Root parasitic shrubs or small trees. Leaves opposite or whorled, well developed, penniveined. Flowers bisexual, in terminal or axillary panicles or racemes, rarely in small umbels with caducous bracts. Sepals 4 (rarely 5 or 6) usually with hair tufts at base behind stamens. Stamens 4 (or same number as sepals), disc prominently or slightly lobed. Ovary inferior; ovules 2-4, without differentiated integument; style short; stigma small. Fruit a spherical or ovoid drupe with succulent or firm mesocarp and woody, often rugose endocarp'.

The range in mature heights of each of the principal commercial species of sandalwood is given in Appendix 1.0. They are generally erect, small to medium sized, single stemmed trees under better growing conditions, but in harsh growing conditions may be reduced to gnarled, multi-stemmed shrubs.

- **The ecology of *Santalum* taxa in Fiji and Tonga**

The climatic ranges of these species are highlighted in CABI (2001) and Thomson and Uwamariya (2001) (Appendix 1.2) listed the ranges of important climatic factors for different taxon including the two taxa, *S. album* and *S. yasi*, which can be grown commercially in Tonga and Fiji. *Santalum* taxa cover a wide variety of climatic types. In Fiji the sandalwood is usually found on stony ridge soils, and shallow degraded soils with rock outcrops, coastal sands and infrequently on the edges of swamps. In Tonga, the sandalwood has a broad edaphic range and is found growing in coralline soils (sand over raised limestone terraces) and brackish soils near the coast; it has also been found on volcanic soils in mountainous regions (Yuncker, 1959).

CABI (2001) and Thomson and Uwamariya (2001) provide further information on edaphic requirements for three *Santalum* taxa; e.g they can re-use alkaline (pH up to 9.0) and slightly saline soil but are unable to tolerate waterlogged sites.

- **Environmental factors of sandalwood**

All sandalwood are sensitive to fire and grazing, particularly in the first few years of growth. These factors, combined with over-exploitation, have contributed to the current perilous position of many sandalwood populations (CABI 2001; Gunn *et al*., 2002). Plants of some species will regrow from coppice following fire e.g., young *S. album* (Applegate and Davis, 1990), *S. austrocaledonicum* (Nasi, 1995), *S. spicatum* from milder locations (Applegate and Davis, 1990) and *S. lanceolatum*. Many *Santalum* taxa are capable of root suckering so long as not too much of the root system is removed during harvest. 'Spike disease', a mycoplasma-like disease spread by insects, is a very serious pest of *S. album* in India (Yusuf 1999, CABI 2001). In New Caledonia, *S. austrocaledonicum* is sometimes attacked by insect pests belonging to the genera

Ceroplastes and *Coccus*, but damage is usually minor (Nasi and Ehrhart, 1996). Fungal pathogens can result in damage to leaves and young seedlings in the nursery.

2.2 Botanical description of *Santalum yasi*

The specific epithet *yasi dina* or *'ahi*, is the name by which Fijian and Tongan *Santalum yasi* (Plate 2.6), and several unrelated myrtaceous species, are commonly known in each country respectively.

Plate 2.6 *Santalum yasi* seedling at Colo-i-Suva Forestry nursery (photo: Bulai, 2006).

- **Botanical features of *Santalum yasi***

In Fiji and Tonga, *S. yasi* grows naturally in open forest types, including secondary forests that have developed from old garden sites (Jiko, 1993). Botanical descriptions are provided in Smith (1985) and Jiko (1993), and the species is illustrated in Parham (1972) and Anon. (1996). Mature specimens commonly grow to about 10 m tall and a maximum height of 13-15 m, and attain stem diameters of 40 cm or more after 40-50 years' growth. The bark is smooth to slightly fissured and greyish-brown in colour. The young branches are slender and often pendulous, and some plants display a characteristic semi-weeping habit.

- **Foliage**

Leaves are simple, opposite, narrow or broadly lanceolate, typically 6-7 cm long and 1.5-2 cm wide and very slender in seedlings. There is considerable variation in foliage size: leaves of adjacent plants in Kadavu (Fiji) have been observed to range from 5cm long and 1cm wide to 8cm long and 2.5 cm wide (Thomson and Uwamariya, 2001). The foliage is light green and shiny (Jiko, 1993), but plants growing in the open with few host trees have a yellowish-green appearance (Bulai, 1995).

- **Inflorescences, flowers and fruits**

Flowers are small, clustered in panicles, 4- or 5-parted, at first greenish white but turning pink then red. The flowers have cream-coloured perianth segments (tepals) that turn to rich pink, and purplish red (Plate 2.8); the anthers are yellow and red-tinged; the disk lobes are dark yellow; the style and stigma are pale yellow (Smith, 1985). The fruit is a one-seeded drupe, ellipsoidal in shape (9-11 x 6-8 mm) with a small, round, calyx scar (2 mm in diameter) at the apex, enclosing a rather stout, cone-shaped point. Immature fruits are light green in colour, turning reddish-purple (Plate 2.9), and finally dark purple or black at full maturity (Smith, 1985; Bulai, 1995; Jiko, 1993). Fruits and seed characteristics are useful for distinguishing *S. yasi* from related tropical sandalwood.

- **Phenology**

There is considerable variation in flowering and fruiting of *Santalum yasi*. Trees flower and fruit throughout the year (Smith, 1985; Bulai, 1995) with two peaks. Bulai

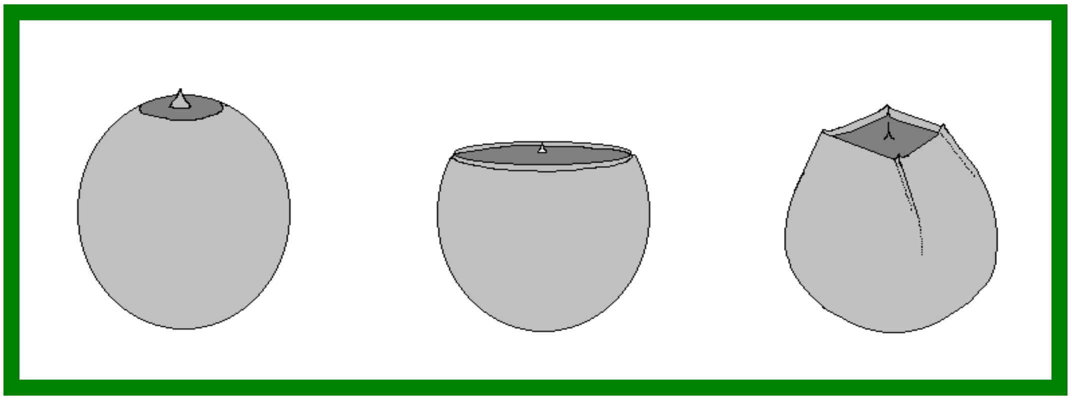
(1995) reported that the two main flowering periods are February and October-November. The main fruiting season corresponds to the wet season, January-March, with light fruiting in the cooler, dry season (June-August). Birds are the principal method of dispersal of the seed. Plants begin fruiting from an early age, about 3-4 years (Jiko, 1993).

- **Stem and bark**

Mature specimens typically grow to 8–10 m in height and crown spread of 8–12 m (maximum 15 m heigh and 13 m with). Maximum bole diameter at breast height is 40–50 cm. *S. yasi* typically has a short, crooked bole and spreading crown in open situations. In forest and sheltered situations, the bole may be straight for more than half of the tree's height. The bark is smooth to rough, slightly longitudinally fissured or reticulated, the markings and fissures more pronounced with age, (greyish or reddish brown, mottled with patches of lichen).

Plate 2.7 Fruiting may occur throughout the year (photo: Bulai, 2006).

Plate 2.8 Flowering may occur throughout the year (photo: Bulai, 2006).



S. yasi *S. album* *S. austrocaledonicum*

Plate 2.9 Fruits of the three *Santalum* taxa planted in the South-west Pacific (diagram: from Thomson and Uwamariya 1998).

2.2 Botanical description of *Santalum album*

Santalum album or Indian sandalwood (Plate 2.12) is the most commercially important sandalwood as it has the highest oil content (6-7%) of all sandalwood species with excellent santalol[3] content, large tree habit and faster growth rate than other *Santalum* species. It also has a thousand year history of trade and use encompassing major Asian cultures (Srinivasan *et al*., 1992).

- **Botanical features**

A tree of straight form to 15 m (maximum 18 m) in height, typically with a high bushy crown when grown in dense shade; evergreen, glabrous, with slender drooping branchlets. Bark reddish-brown to dark brown, smooth in young trees, rough and fissured in older trees (Luna, 1996).

[3] Santalol is a perfume component useful in perfume composition. It is was isolated from sandalwood oil by distillation under vacuum and is characterized by nuclear magnetic resonance and gas chromatography mass.

Even though *S. album* seedlings may grow for up to 12 months without hosts, it is widely accepted that *S. album* growth is greater in association with host plants (Ananthapadmanabha *et al*., 1984). Under natural conditions *S. album* preferentially parasitises leguminous species (Rai, 1990). *Santalum album* is regarded as a slow growing tree in natural stands, increasing in stem diameter at 1-1.5 cm per year (Srinivasan *et al.*, 1992). Under favourable conditions stem diameter growth can be as high as 3- 4 cm per year. Growth in natural stands is not uniform (Barrett, 1988) and heartwood formation in individual trees can be negligible (Sen-Sarma, 1977).

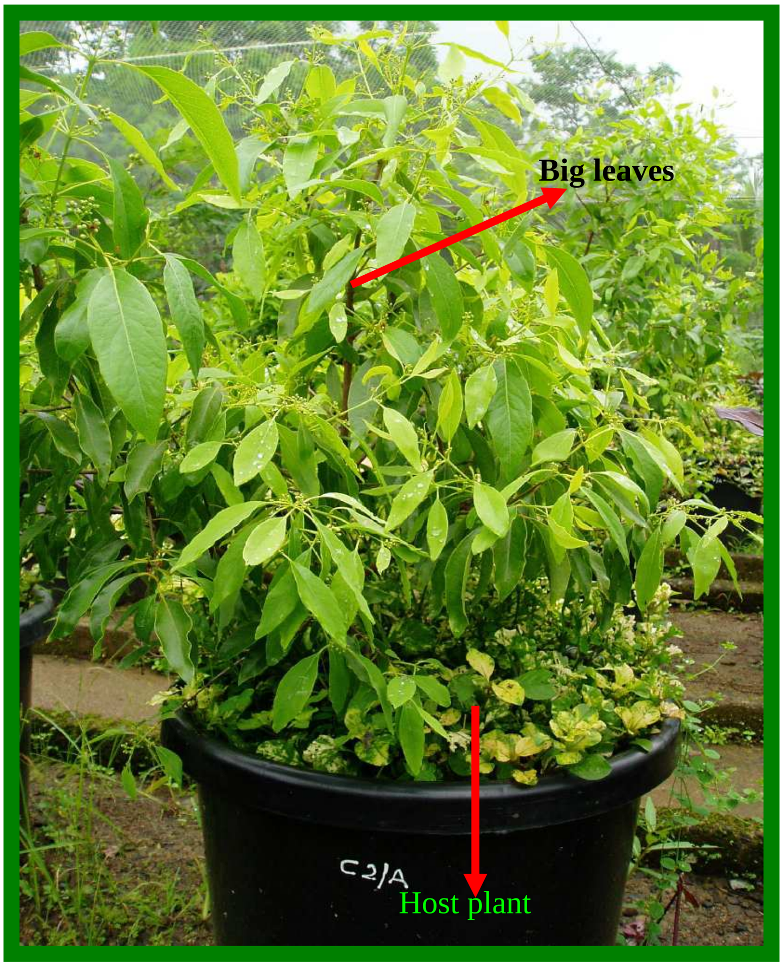


Plate 2.10 *Santalum album* Colo-i-Suva Forestry (photo: Bulai, 2006).

Luna (1996) discusses the rate of growth and heartwood production in *S. album* from different localities in India. When grown in natural stands under favourable conditions heartwood formation normally commences at age 10 years when trunk diameter is approximately 24 cm and tree height is 3 m. Heartwood formation is rapid from about 20 years onwards when stem girth is between approximately 40 to 60 cm (Lakshmi- Sita, 1991). An 8- year- old tree, growing near Mysore, Karnataka, having a diameter of 14.3 cm and height of 8.5 m, yielded approximately 15 kg of heartwood (Barrett, 1988).

- **Foliage**

Leaves are ovate-elliptic, acute at base, acute or rarely obtuse at the apex; 37.5 - 62.5 mm long, pale below, membranous, veined, of varying form on a branch and ovate or ovate-elliptic or ovate-lanceolate (de Candolle, 1857). Several morphological forms based on leaf pattern are reported from India (Barrett and Fox, 1995b).

- **Inflorescence, flowers and fruits**

The flowers are pale yellow at first before turning red or purple. They are not overly showy, but attractive. They bloom February through April. The fruit is purple black when ripe, contains a single seed, and is highly desired by birds and other animals.

The flowers had four perianth lobes and four stamens whose anthers dehisced by longitudinal slits. The pollen became caught in long unicellular hairs adjacent to the anthers (De Candolle, 1857). The central disc secreted nectar through raised stomata. The stigma papilla cells had a cuticle with rough surface overlying thick walls. The half-inferior ovary normally contained two ovules. The embryo sac extended beyond the ovule at the micropylar end and into the placenta at the chalazal end (*ibid*). Half of the ovaries observed at both anthesis and four days following anthesis had no embryo sacs and the other half had one embryo sac. Occasional ovaries had two embryo sacs and some underdeveloped embryo sacs were observed that did not extend beyond the ovule or into the placenta. Pollen tubes had reached the ovary by one day following pollination and the stigma was receptive for eight days following anthesis. Only half of the pistils had pollen tubes in the ovary. Unpollinated flowers had no pollen tube growth in the pistil (Barrett and Fox, 1995a).

Fruit is a globose drupe the size of a small cherry (Plate 2.11), eventually becoming black (De Candolle, 1857). Under favourable conditions fruiting begins around two to three years of age. The drupe is about 7 - 8 mm long. The mesocarp is creamy yellow and endocarp more or less spherical, up to 6-8 mm diameter, and smooth. The kernel is firm and white (Barrett and Fox, 1995a).

Plate 2.11 Mature fruits of *Santalum album* on‘Eua (photo: Lex Thomson, 2004).

- **Phenology**

Flowering and fruiting seasons vary depending on locality. In India, flowering begins in May (end of the dry season) with fruit maturity commencing in September (end of the wet season) (Srimathi and Nagaveni, 1995). A second flowering commences in November with fruit beginning to mature in February. Most trees usually flower and fruit in two periods each year; however some trees have been observed to flower and fruit only once a year while others may flower and fruit intermittently throughout the year. *Santalum yasi* appears to freely hybridize naturally with *S. album*, but *S. yasi* also is apparently self pollinating as isolated plants may set heavy fruit crops.

Flowers, buds and mature fruit can be found on a single tree at the same time (Barrett, 1988; Luna, 1996). Natural spread and regeneration is via bird dispersal of seeds, and root suckers.

2.3 Distribution of *S. yasi* (native and planted)

The range extends from ‘Eua, a southern island in the Tongan group, west and northwards to the Udu Peninsula, north-eastern Vanua Levu, Fiji (Jiko, 1993; Ehrhart, 1996). *Santalum yasi* grows throughout Tonga, but its abundance varies between islands (Ehrhart, 1996; Ehrhart, 1997). *Santalum yasi* is very common on ‘Eua, especially in secondary forest but rare to very rare on Tongatapu, in coastal forest and tropical lowland forest (Map 1.1).

In Fiji, the species occurs in five principal areas (Smith, 1985; Jiko, 1993; Bulai, 1995). It grows abundantly in the Bua and Macuata provinces, coastal and grassland areas of Fiji. Although it also occurs in south-western Vanua Levu, northern Lau, Kadavu and Viti Levu, the species is believed to be native to Bua. (Jiko, 1993; Bulai, 1995; Ehrhart, 1997) (Map 1.0).

2.4 Distribution of *S. album* (planted, naturalized and hybrids)

The hybrid (*S. yasi x S. album*) is capable of rapid growth on Rotuma (and other parts of Fiji) and heartwood formation can be initiated from a young age less than seven years (Bulai and Nataniela, 2002). On Rotuma the fastest growing trees have reached a height of 8-9 m and near-ground diameter or 20-26 cm after 6-7 years. The average size was 6.2 m height and 14 cm diameter (near ground level) (Bulai *et al.*, 2002). Under fertile conditions, a suitable rotation age for hybrid (*S. yasi x S. album*) sandalwood is anticipated to be 20 to 25 years. However, given good silviculture, including regular weeding in early years and interplanting with a mixture of compatible long-term hosts, the rotation age for a hybrid (*S. yasi x S. album*) might be reduced to 15 to 20 years at which time each tree might have produced 40-80 kg of heartwood (Bulai and Nataniela, 2002).

The natural distribution of *S. album* is in the tropical belt of the Indian Peninsula, up to an elevation of 1200 m above sea level (Rai, 1990) and in the highland regions of eastern Indonesia, primarily on the islands of Nusa Tenggara Timur, up to an elevation of 2000 m (Harisetijono and Suriamihardja, 1993). *Santalum album* is considered indigenous along the southern islands of the Indonesian archipelago to eastern Java (*ibid*).

The origin of *S. album* is disputed, and it has been hypothesized that *S. album* was introduced to India from West Timor (Brand, 1994). Recently concluded, unpublished phylogeographic studies of *Santalum* taxa have shown that the ancestral sandalwood species originated in Australia (Harbaugh, 2007). Therefore, it seems more probable that *S. album* originated in Indonesia (or even northern Australia) and was an ancient introduction to India, spread by birds or humans (Map 1.2). Introduced populations occur in Central and Northern India (Srinivasan *et al.*, 1992), Java, Bali, and the coastal areas of northern Australia, near Darwin (Barrett and Fox, 1995b). Experimental introductions have been performed in China (Li and Yu, 1984), Fiji (Bulai, 1995), New Caledonia (Chauvin, 1988), Hawaii (Merlin and VanRavenswaay, 1990), Tonga (Kaufusi *et al*, 1999), Papua New Guinea (Paul, 1990), Nepal (Neil, 1990), and eastern Indonesia (Harisetijono and Suriamihardja, 1993) (Appendix 1.1; Table 1.0 and Table 1.1).

2.5 Conservation status and threats to *S.yasi*

Conservation of germplasm of all commercial *Santalum* taxa has been a major concern in many *Santalum* growing countries (Havel and McKinnell, 1993).

Plate 2.12 Family trial/gene conservation seed stand at Vunimaqo (photo: Bulai, 2004).

With very little information available on *Santalum yasi*, it was thought the species' survival was in doubt and very much at risk of complete depletion by over-harvesting. However, since the reawakening of interest in the species and the recent distribution studies undertaken under the SPRIG project, it has been discovered that there are isolated pockets of *Santalum yasi* in villages and often individual trees are owned by a landowner (Plate 2.12). Research, development and tree improvement for sandalwood in the Pacific Islands has made significant progress in the last three years. A large amount of germplasm material has been vegetatively propagated and managed centrally in very accessible areas (Fox and Barrett, 1995b).

The SPRIG project established plots of sandalwood in a collaborative effort with national partners to collect germplasm from these stands to establish both *in situ* and *ex situ* conservation seed production orchards. So far two seed stands of *Santalum* in Tonga, totalling 1.1 ha, have been successfully established on Tongatapu (Sia'atoutai) and 'Eua, and were reported in 2004 (Likiafu and Robson, 2005).

In Fiji, reported by Bulai and Nataniela (2002) the Forestry Department has established four seed stands of *S. yasi:* Vunimaqo (Serua Province, south-eastern Viti Levu) - 0.15 ha (Plate.2.12), Lololo (Western Viti Levu), Lekutu (Bua, Vanua Levu) – 0.29 ha, and Nawailevu (Bua, Vanua Levu) – 0.31 ha. A key objective of these stands is to provide seed for propagation and sale as well as serve as *ex situ* gene conservation stands (Bulai and Nataniela, 2002).

2.5.1 Threats

In 2000, Thomson *et al*., reported that the two most damaging agents to *Santalum* taxa in Bua province (Fiji Island) were humans and fire. Humans cause damage through indiscriminate hacking and cutting of undersize trees, while fire wipes out any young regeneration. Suggested reasons for the decline of *S. yasi* ('*ahi*) in Tonga include over-harvesting without natural regeneration or replanting, the latter being associated with a low importance attached to planting. *Santalum yasi* is a long-term crop so only a registered owner would grow and/or protect plants, and furthermore seedlings may not be readily available (Wiser *et al*., 1999). During garden development, plants (especially seedlings and young saplings) may be either inadvertently or deliberately cut or otherwise destroyed. The young saplings are often cut for Christmas trees.

2.5.2 Fire and grazing:

All sandalwood are sensitive to fire and grazing, particularly in the first few years of growth. These factors combined with over-exploitation have contributed to the current perilous position of many sandalwood populations (CABI 2001, Gunn *et al*., 2002). Plants of some species will regrow from coppice following fire.

2.6 Seed germination

Thomson *et al*., (2000) reported that nicked *S. austrocaledonicum* seeds commence germination after two weeks and germination is complete by eight weeks; most germination occurs between 30 and 40 days. Whole seeds are much slower to germinate, commencing after about 40 days. Germination rates of greater than 50% are expected for fresh seeds that have been collected from mature fruits still on the tree and appropriately handled and cleaned. Germination of nicked *Santalum yasi* seeds occurs rather slowly over a long period, e.g. from 40 to120 days after sowing.

As they appear, germinants are transplanted into pots, preferably before the seed coat falls from the germinating shoot. Seedlings are picked out at the 2- or 4-leaf stage and plated into 16 x 6.5 cm polypots. At this time a host plant is usually planted with each seedling.

2.6.1 Germinating sandalwood seed

Seeds can be sown without any physical or chemical treatments but the recommended seed pre-treatment method for optimum germination is as follows: firsly, nick seed coat at pointed end of seed using a sharp knife; Secondly, soak seeds overnight in a solution of gibberellic acid (GA3) at rate of 0.1 to 0.25 g/l; and thirdly, drench seed in fungicidal solution (e.g. benlate) for a few minutes before sowing (optional).

In Fiji the seeds (hard nuts) are dried and cracked or nicked at the sharp end (the hard shell is cut without damaging the embryo) to speed germination.

2.7 Vegetative propagation

While seed remains the main source of improved genetic material for planting, vegetative propagation is able to capture non-additive genetic gains (Shelbourne *et al*., 1986).

A more comprehensive trial was established in Queensland in late 1998 to compare/contrast the rooting performance of *S. austrocaledonicum* and *S. album.* Seeds were set under a mist propagation environment in both sand and peatmoss potting media. Substantial differences in survival and rooting success were observed between both species, with an average rooting success of 63.5% for *S. austrocaledonicum,* compared to only 9.5% for *S. album*. For *S. austrocaledonicum,* the peatmoss medium also resulted in improved rooting success over the pure sand media. Substantial variation was also observed between the 15 different *S. austrocaledonicum* famil, with rooting successes ranging from 25% up to 88.9%.

2.8 Propagation system

Cuttings are usually set in greenhouses often with misting and bottom heat. These provide protection from the outside environmental conditions such as wind and temperature. Mist propagation is such an efficient and economical system of producing large quantities of rooted cuttings and is one of the most widely used means of propagating cuttings in nurseries. This method has aided in the rooting of difficult species and helps to decrease the rooting time of more "friendly" propagated species (Hartmann *et al* 1990).

CHAPTER 3

INDUCTION OF ROOT-SUCKERING FORMATION IN THREE SPECIES (*S. YASI, S. ALBUM* AND HYBRID *S. YASI X S. ALBUM).*

3.0 INTRODUCTION

Commercial nursery production of *Santalum* seedlings is expensive and inefficient, even though sandalwood is propagated mostly by seeds. Sandalwood fruits are attractive to birds, and fallen fruits are also ingested by rats and other ground dwelling animals. Rats may also eat the seeds (P.Bulai, pers.comm. 2006), so reducing the number of viable seeds for planting. Accordingly collection of seeds from wild stands is difficult, costly and unreliable. Furthermore the seed requires pretreatment for uniform germination within a reasonable time frame. The genetic quality of nursery stock from seed is low compared to stock produced from selected superior phenotypes by vegetative/clonal propagation.

Sandalwood shows wide variation in growth, heartwood formation, and oil content within populations. Outstanding phenotypes or candidate trees showing the desirable characteristics of fast growth, early formation of heartwood and higher oil content have been detected in reserve forests. However, production of 'true-to-type' planting stock has been inhibited because there is no effective method of vegetative propagation other than formation of root-suckers around the mother trees.

Root-suckers typically develop as shoots on injured or severed roots. Deliberately inducing them is one method for sandalwood regeneration in forests. However, the regenerated plants are confined to an area around the mother tree, and this means that the trees become too crowded to develop large stems and also may be prone to wind throw during cyclones. The additional seed produced on the root suckers can aid in natural regeneration through bird dispersal of the seeds. Rao and Srimathi (1976) achieved vegetative propagation of mature sandalwood by inducing rooting around shoots, and then out-planting the shoot with the original roots. As another option, they also cut off the original roots along with the shoot and induced rooting after transplanting.

However, they did not succeed in propagation by stem cuttings. This is because juvenility[4] in sandalwood (an important factor for inducing rooting of stem cuttings) is confined to seedlings and root suckers. Vijayakumar *et al*., (1981) attempted vegetative propagation of 2-12 month seedlings to study the role of juvenility in rooting of stem

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[4] Juvenility in sandalwood actually refers to how much growth a sandalwood plant needs to develop before it can produce flowers or become mature.

cuttings. They found that maximum root development (96%) occurred in three-month old seedlings. As the seedling aged, the rooting ability of the cutting decreased; in 11-12 month seedlings, there was no rooting. Root-sucker formation induced by trenching around a tree has been 60% successful (Vijayakumar *et al.*,1981).

Clonal forestry[5] using rooted cuttings is presently the primary means of reforesting many tropical forest tree species such as *Acacia* hybrid, *Eucalyptus* and *Gmelina*. This practice is facilitated by the overall good rooting of the species concerned and by basal sprouting characteristics (Walker *et al*., 1999). Although ageing phenomena obviously occur in these taxa, restoration and maintenance of a satisfactory level of juvenility is achievable by stimulation of basal coppicing (Walker *et al*., 1999). Sandalwood cuttings are generally difficult to root (Balasundaran, 1998), and high rates of root development are mainly or exclusively confined to juvenile material.

However, techniques to rejuvenate mature tissues have seldom been studied in sandalwood. In Balasundaran's experiment, research was undertaken to develop an efficient method for large-scale production of ramets (clonal propagules) from mature trees. In the present study, a preliminary investigation was made of the ability to initiate shoots from severed and injured roots of mature trees.

3.1 Materials and methods

Roots of mature sandalwood trees were subjected to different types of root damage in order to investigate the development of root sucker shoots. Root sucker

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[5] Clonal forestry is the propagation of superior tested individual clones by vegetative multiplication: foresters can select clones with higher growth rates, increased disease resistance, increased wind resistance, and improved wood quality these clones provides more uniform and predictable products.

induction experiments were undertaken on 24 trees (nine of *S. yasi*, 10 of *S. album* and five of the hybrid *S. yasi x S. album*) aged 5-10 years old, with 11-20 cm DBH and between six and 10 m height. The trees were selected from sandalwood plantings located within forestry lease areas at Colo-i-Suva (Silviculture Research Division (SRD) Fiji Forestry Department) and Vunimaqo (Serua Province, SE Viti Levu Fiji). For the root suckering experiment, different sized roots were cut through (Plate 3.0) or partially cut (Plate 3.1). In addition, 10 seedlings ranging from 6-12 and 12- 24 months old of from each *Santalum* taxon (10 seedlings of *S. yasi*, 10 seedlings of *S. album*, 10 seedlings the *S. yasi x S.album* hybrid) were selected from the SRD nursery for planting materials (total 30 seedlings).

Plate 3.0 Secondary lateral roots (SLR) of sandalwood cut through (photo: Havea, 2005).

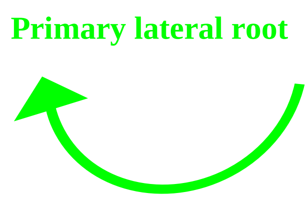

Plate 3.1 The selected stock plants were excavated both sides and a Primary Lateral Root was partially cut through (photo: Havea, 2005).
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The average diameter of primary lateral roots (PLR) selected for treatment was 4.7 cm; these roots were injured by making a diagonally half cut about 2 cm ± deep (Plate 3.1). The average diameter of treated secondary lateral roots (SLR) was 1.6 cm; these were completely cut through to induce sprouting (Plate 3.2). Shoots arising from severed and injured roots were harvested at five to six months after treatment.

Trenches 14 x 40 x 14 cm were made on either two or four sides and 30- 100 cm away from the base of the stock trees depending upon the size of each tree. The trenches were made during September 2005. The exposed roots were covered with mulch (Plate 3.2) and kept damp until the shoots started to sprout from the cut end (Plate 3.3), after which time the roots were exposed again.

Plate 3.2 The exposed roots of hybrid (*S.yasi x S. album*) were covered with mulch. Photo of a severed and injured of sandalwood root (photo: Havea, 2005).

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Plate 3.3 The shoots sprouting from the severed roots of hybrid (*S. yasi x S. album*) at Colo-i-Suva (photo: Havea, 2005).

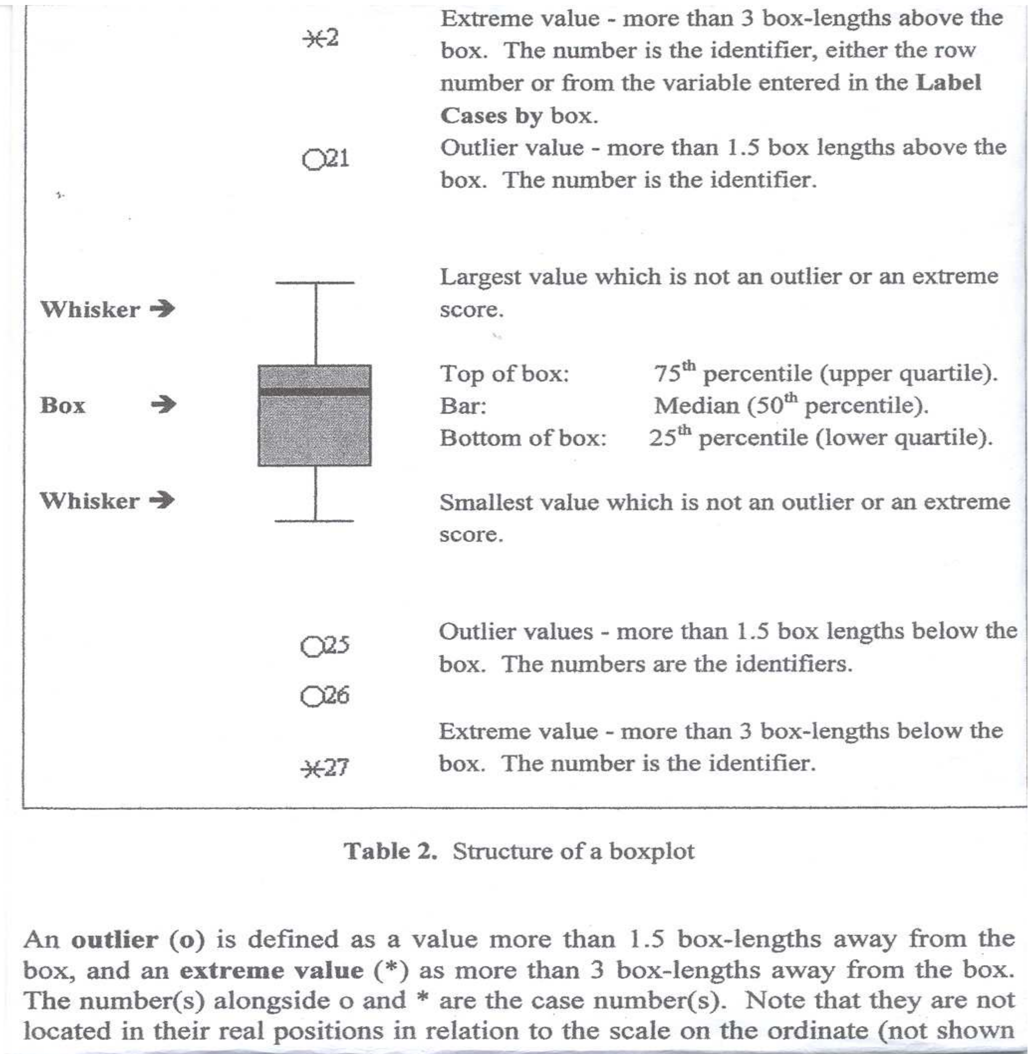
3.2 Data collection and analysis

The data gathered in relation to shoot initiation for each *Santalum* species and treatment was as follows.

- Firstly, the number of severed and injured roots that produced shoots, and the number of shoots at various distances from the trunk were recorded (Plate 3.4).
- Secondary, mean root length and mean diameter of root were recorded.
- Summary statistics were calculated separately for each sandalwood plant. A preliminary analysis indicated that these data were not amenable to a parametric approach.
- Accordingly box and whisker plots (Table 3.0) and a Kruskal-Wallis test were used to analyse the data.

Plate 3.4 recording the number of severed SLRs and PLRs (photo: Bulai, 2005).

Table 3.0 The structure of a box plot (Paul and Gray, 2000).



	*2	Extreme value - more than 3 box-lengths above the box. The number is the identifier, either the row number or from the variable entered in the **Label Cases by** box.
	O21	Outlier value - more than 1.5 box lengths above the box. The number is the identifier.
		Largest value which is not an outlier or an extreme score.
Whisker →		
Box →		Top of box: 75th percentile (upper quartile). Bar: Median (50th percentile). Bottom of box: 25th percentile (lower quartile).
Whisker →		Smallest value which is not an outlier or an extreme score.
	O25 O26	Outlier values - more than 1.5 box lengths below the box. The numbers are the identifiers.
	*27	Extreme value - more than 3 box-lengths below the box. The number is the identifier.

Table 2. Structure of a boxplot

An **outlier (o)** is defined as a value more than 1.5 box-lengths away from the box, and an **extreme value** (*) as more than 3 box-lengths away from the box. The number(s) alongside o and * are the case number(s). Note that they are not located in their real positions in relation to the scale on the ordinate (not shown

3.3 Results

The experiment was designed to investigate factors that might affect shoot production from damaged roots of *S. yasi* and *S. album* and the hybrid (*S. yasi x S. album*). Results showed that some of the severed SLR and injured PLR treatments failed to develop a useful number of root suckers (Table 3.1). Characteristically, a cluster of sprouts appeared from the edge of the severed roots at 1-2 months; observation of the location of the sprouts indicated that all of them arose close to the cut edge and 5% were at the front of the area on the root close to the severed and injured point. No sprouts emerged from the exposed root portion which remained connected with the tree (Digram 3.1).

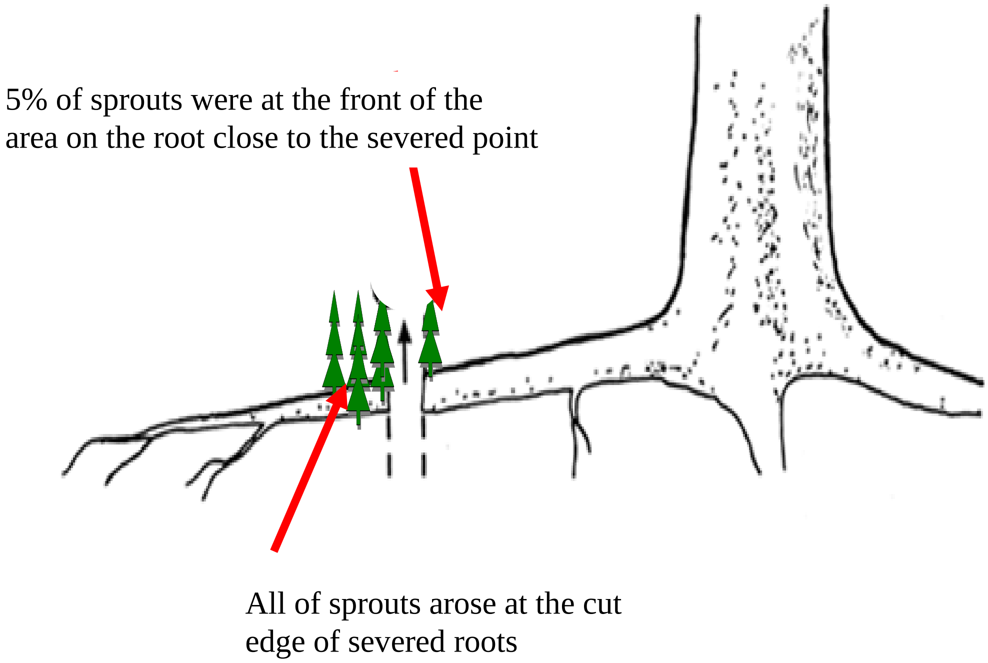


Diagram 3.1 The diagram showed a cluster of sprouts appeared from the edge of the severed roots.

Table 3.1 Shoot formation from three *Santalum* taxa in trenches

Tree No.	Diameter DBH (cm) of stock tree	No. of exposed roots		No.of sprouts developing on root portion located away from stem (in relation to damage)		No. of sprouts developing on root remaining connected or closest to trunk	
		SLR Severed	PLR Injured	SLR Severed	PLR Injured	SLR Severed	PLR Injured
Hybrid (*S. yasi x S. album*)	**Colo-i-Suva**						
1	10.0	17	4	3	1	-	-
2	9.0	15	2	4	4	-	-
3	10.0	14	3	3	2	-	-
4	12.0	16	3	9	2	-	-
5	11.0	18	3	8	1	-	-
S. yasi	**Colo-i-Suva**						
1	10.5	6	3	3	1	-	-
2	15.0	4	3	2	-	-	-
3	9.0	2	2	1	-	-	-
4	9.0	4	3	1	1	-	-
S. yasi	**Vunimaqo**						
1	6.5	5	3	1	-	-	-
2	7.0	4	4	1	-	-	-
3	7.0	8	3	1	-	-	-
4	20.0	2	2	1	-	-	-
5	6.0	6	3	-	-	-	-
S. album	**Colo-i-Suva**						
1	9.0	1	1	-	-	-	-
2	10.5	1	1	-	1	-	-
3	12.0	2	1	-	-	-	-
4	9.0	3	3	-	-	-	-
5	11.0	2	2	-	-	-	-
S. album	**Vunimaqo**						
1	14.0	6	5	-	3	-	-
2	13.5	2	6	-	3	-	-
3	14.5	2	4	3	1	-	-
4	14.0	3	4	1	1	-	-
5	10.5	5	3	-	3	-	-

Some of the severed SLR of the three sandalwood taxa failed to produce shoots and in some cases, the brush cutter damaged the shoots (Plate 3.5). The persistence of shoots was variable: although clusters of up to 16 shoots arose from a severed root (Plate 3.6), most of these perished within a few days due to tip die back (and in some cases were damaged by a brush cutter despite the plant being roped off). Most of shoots from the hybrid (*S. yasi x S. album*), both on PLR and SLR, showed comparatively more vigour, survival and establishment than did shoots from *S. yasi* and *S. album*. Such healthy root-suckers (shoots) attained a height of 20-40 cm within 60 days. The half-cut PLRs produced fewer shoots (1-4 shoots). However, one or two among them also established into healthy root-suckers (shoots).

Plate 3.5 The shoots from the secondary lateral roots were damage by the brush cutter (photo: Havea, 2005).

Plate 3.6 A cluster of sprouts appeared from the end of the severed roots of *S. album;* Vunimago area (photo: Havea, 2005).

In the current study, 34% of the hybrid (*S. yasi x S. album*), 27% of *S. yasi* and 15% of *S. album* produced shoots on completely severed SLRs whilst on partly injured PLRs, 67% of the hybrid (*S. yasi x S. album*) produced shoots, 8% of *S. yasi* and 40% of *S. album* (Table 3.2).

Table 3.2 The proportion of the total shoots produced by severed SLRs and injured PLRs of three *Santalum* taxa.

Secondary lateral root (SLR) severed

Sandalwood	**number**	**No. producing shoots**	**%**
Santalum hybrid	80	27	34
S. yasi	41	11	27
S. album	27	4	15
Primary lateral root (PLR)			
	number	**No.producing shoots**	**%**
Santalum hybrid	15	10	67
S. yasi	26	2	8
S. album	30	12	40

In the hybrid (*S. yasi x S. album*), the PLR producing a higher number of shoots were the PLR of 3-5 cm in diameter. The root length (variable distances from the trunk) at which the cut was made had a major effect on the number of shoots. In the three *Santalum* taxa, the number of shoots induced differed significantly (Table 3.3). For the SLR, the number of shoots on the hybrid (*S. yasi x S. album*), *S. album* and *S. yasi* differed very significantly (P<0.00) compared to the diameter and length of the SLR while for the PLR, the diameter and length of roots did not exhibit any significant effect on the number of shoots. Shoot production was species dependent. In the hybrid (*S. yasi x S. album*) shoots were most evident on PLRs and SLRs. The highest number of shoots were at 50- 60 cm along the root from the trunk for the hybrid (*S. yasi x S. album*) and 30 to 40 cm along the root for S. *yasi* and *S. album* (Table 3.3).

Some of the injured roots (PLR) failed to develop a useful number of root suckers. Shoot production on injured PLR's of *S. yasi* was very low either closer than 15 cm length to the trunk or more than 40 cm away from the trunk, but increased within or closer to 9 cm and up to 19 cm in length from the trunk (Table 3.3). The shooting ability of *Santalum* taxa was sensitive to root diameter and distance of the root from the trunk. The *Santalum* hybrid (*S. yasi x S. album*) produced more shoots per severed and injured roots.

Table 3.3 Summary statistics (Kruskal-Wallis Test) for root diameter, root length (distance of cut from the trunk) and number of shoots for three *Santalum* taxa.

Secondary Lateral Root (SLR)

Species	Root diameter	Mean rank	Variables	Root length	Mean rank	Variables	Shoots number	Mean rank	Variables
Santalum hybrid	36	42.5		36	71.1		36	72.6	
S. yasi	41	50.5		41	42.1		41	43.3	
S. album	27	68.8		27	43.5		27	39.7	
			$12.7 \times\sigma^2$			$21.1 \times\sigma^2$			$31.4 \times\sigma^2$
			2 Df			2 Df			2 Df
			0.002 Significan			0.000 Significan			0.000 Significan
PLR's									
Santalum hybrid	15	33.9		15	49.43		15	45.4	
S. yasi	26	34		26	30.17		26	26.65	
S. album	30	38.78		30	34.33		30	39.4	
			$0.95 \times\sigma^2$			$8.70 \times\sigma^2$			$13.09 \times\sigma^2$
			2 Df			2 Df			2 Df
			0.621 Significan			0.13 Significan			0.001 Significan

Plate 3.7 Harvesting of shoots production from secondary lateral roots of hybrid (photo: Havea, 2005).

In summary, results showed that the PLRs produced more shoots than the SLRs. A higher percentage of shoots was recorded on the hybrid (*S.yasi x S. album*) both on severed SLRs and injured PLRs. In contrast, *S. yasi* and *S. album* produced low percentage of shoots on severed SLRs and injured PLRs (Figure 3.4 and 3.5). This experiment also indicated that secondary lateral roots of hybrid (*S. yasi x S. album*) produce new shoots for up to 3 months.

Figure 3.0 Boxplots summarizing root diameters of SLRs of three *Santalum* taxa the bar represents median, interquartile range and whisker. 75% were extreme value also scoring. The circle 71 for *S.yasi* and circle 86 for *S. album* stand for the outlier value more than 1.5 box lengths above the box. This number is the identifier.

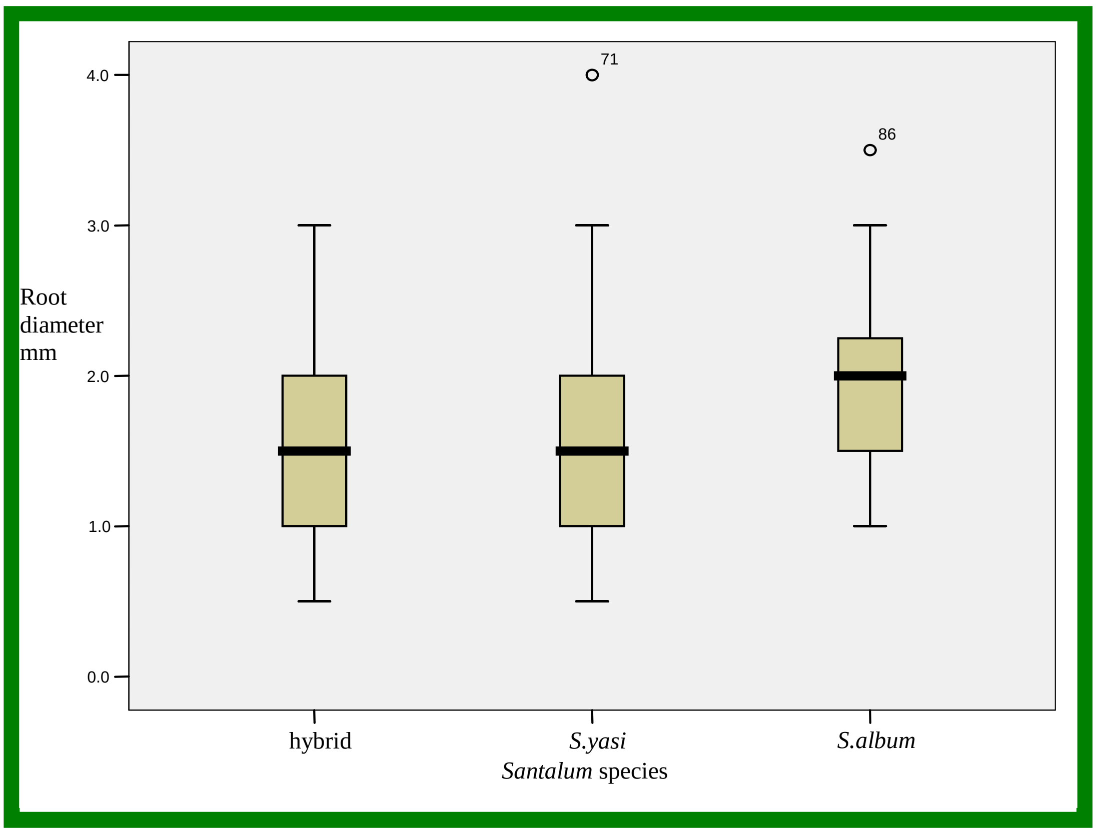


Figure 3.1 Boxplots summarizing lengths of SLRs of three *Santalum* taxa. The bar represents median, interquartile range and whisker. 75% were extreme values also scoring. The circle 71 for *S.yasi* and circle 86 for *S. album* stand for the outlier value more than 1.5 box lengths above the box. This number is the identifier.

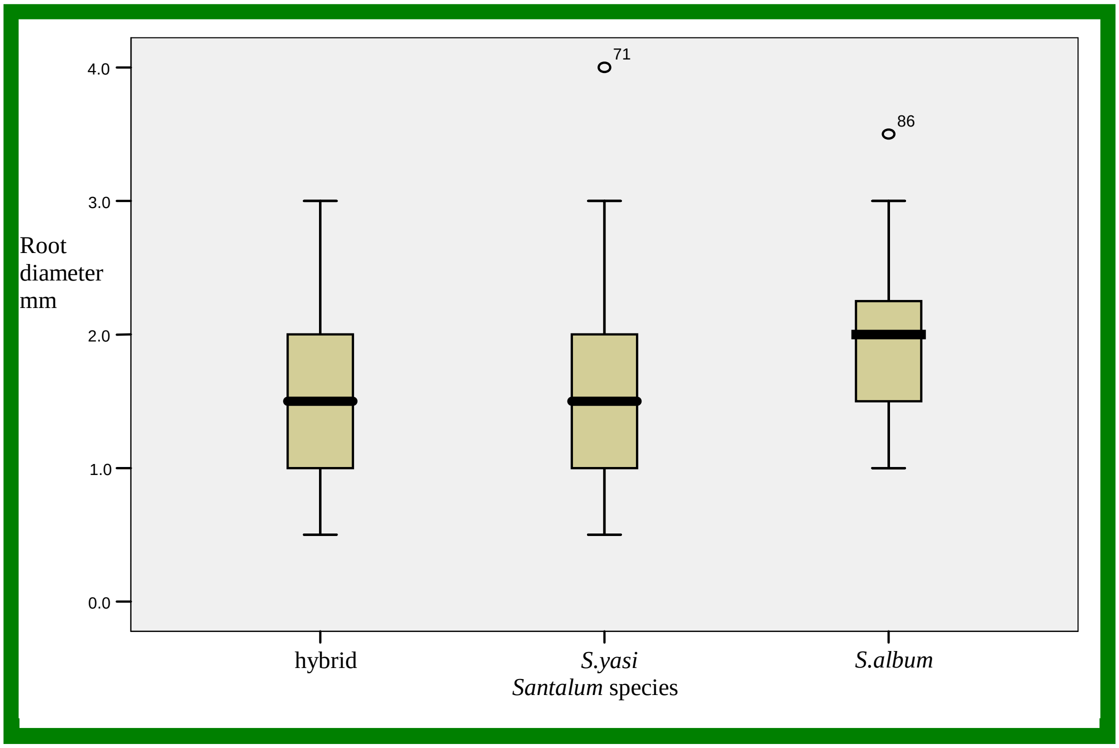


Figure 3.2 Boxplots summarizing shoot numbers of SLRs of three *Santalum* taxa. The bar represents median, interquartile range and whisker. 75% were extreme values also scoring. The circle stand for the outlier value more than 1.5 box lengths above the box. This number is the identifier.The stars stand for the extreme value as more than three box-lengths above the box. The number is the identifier, either the row number or from the variable entered in the label cases by box.

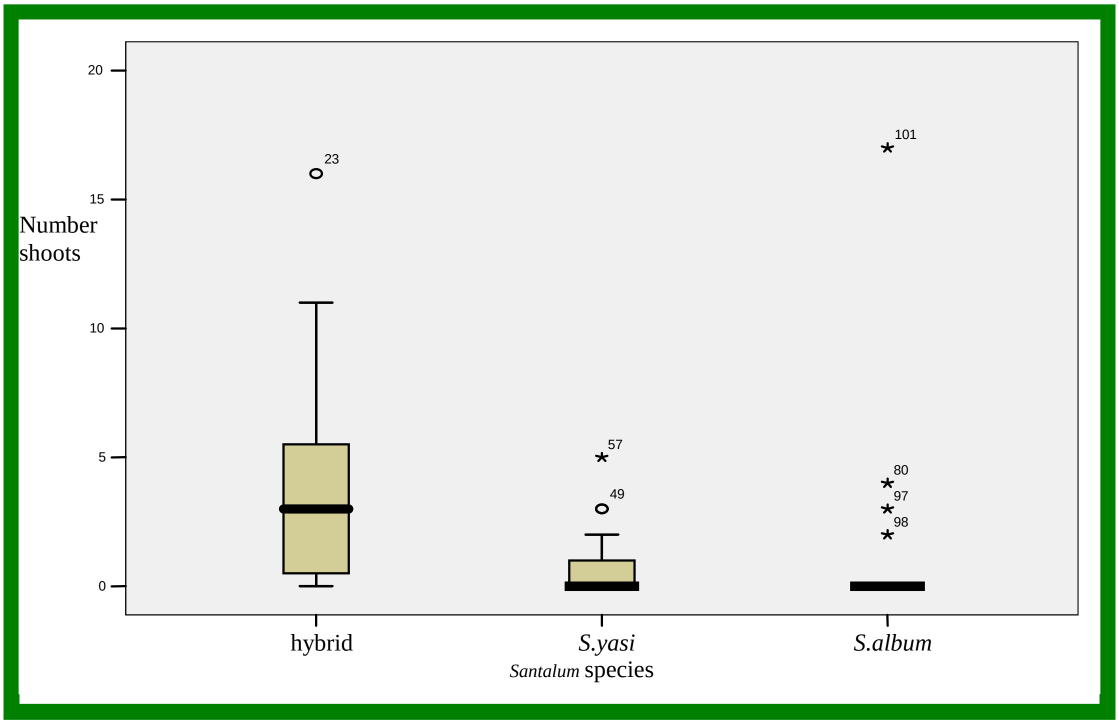


Figure 3.3 Boxplots summarizing root diameters of PLRs of three *Santalum* taxa the bar represents median, interquartile range and whisker. 75% were extreme values also scoring. The circle stand for the outlier value more than 1.5 box lengths above the box. This number is the identifier.The stars stand for the extreme value as more than three box-lengths above the box. The number is the identifier, either the row number or from the variable entered in the label cases by box.

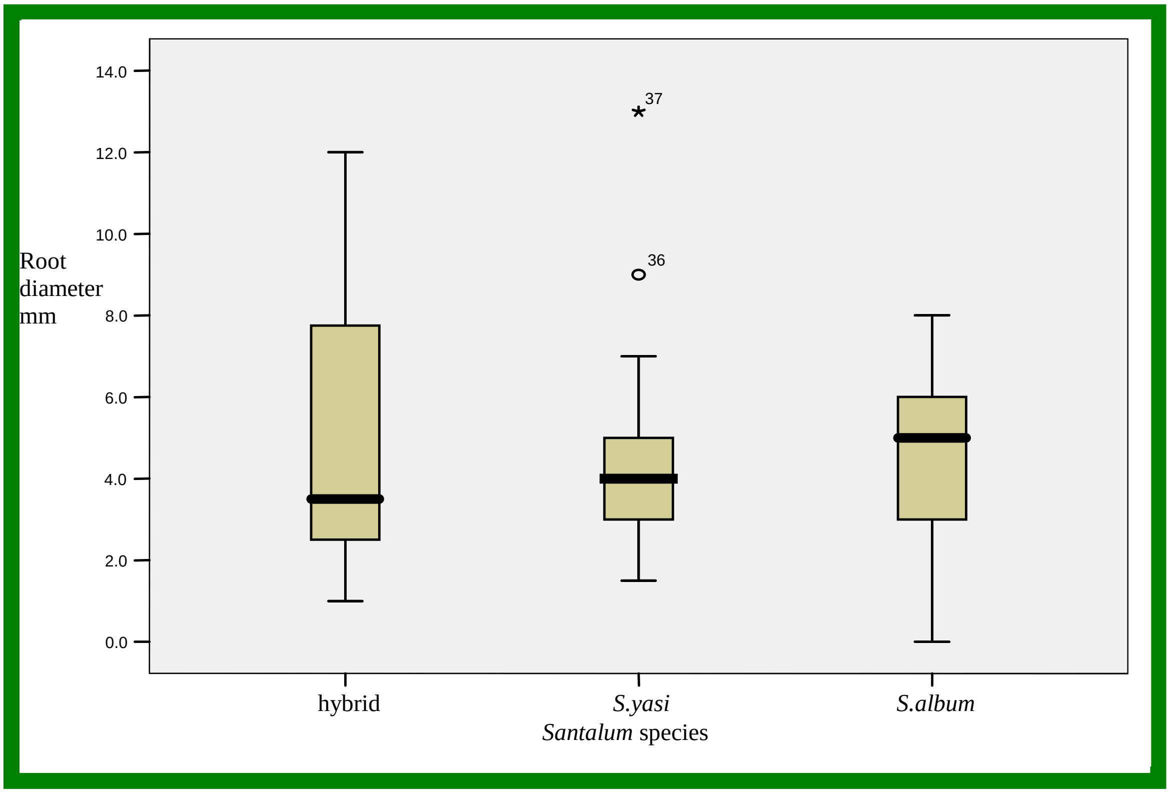


Figure 3.4 Boxplot plots summarizing roots length of primary lateral roots (PLR) of three *Santalum* taxa the bar represents median, interquartile range and whisker 75% were extreme values also scoring. The circle stand for the outlier value more than 1.5 box lengths above the box. This number is the identifier.The stars stand for the extreme value as more than three box-lengths above the box. The number is the identifier, either the row number or from the variable entered in the label cases by box.

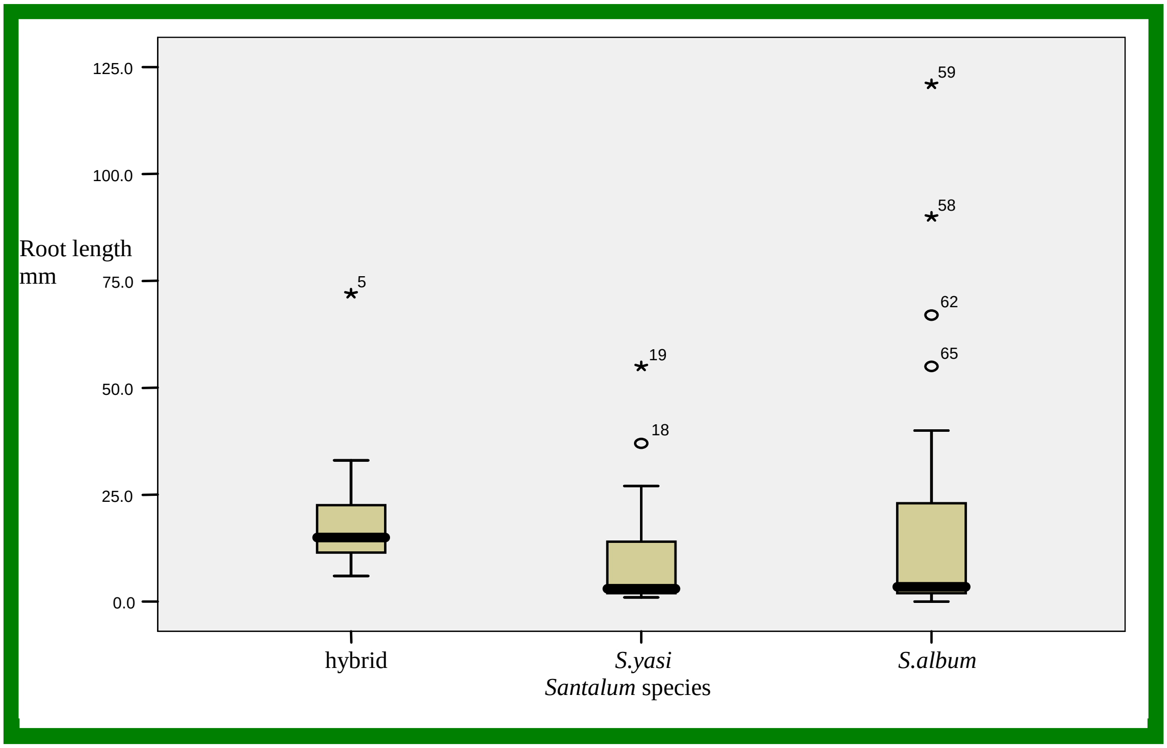
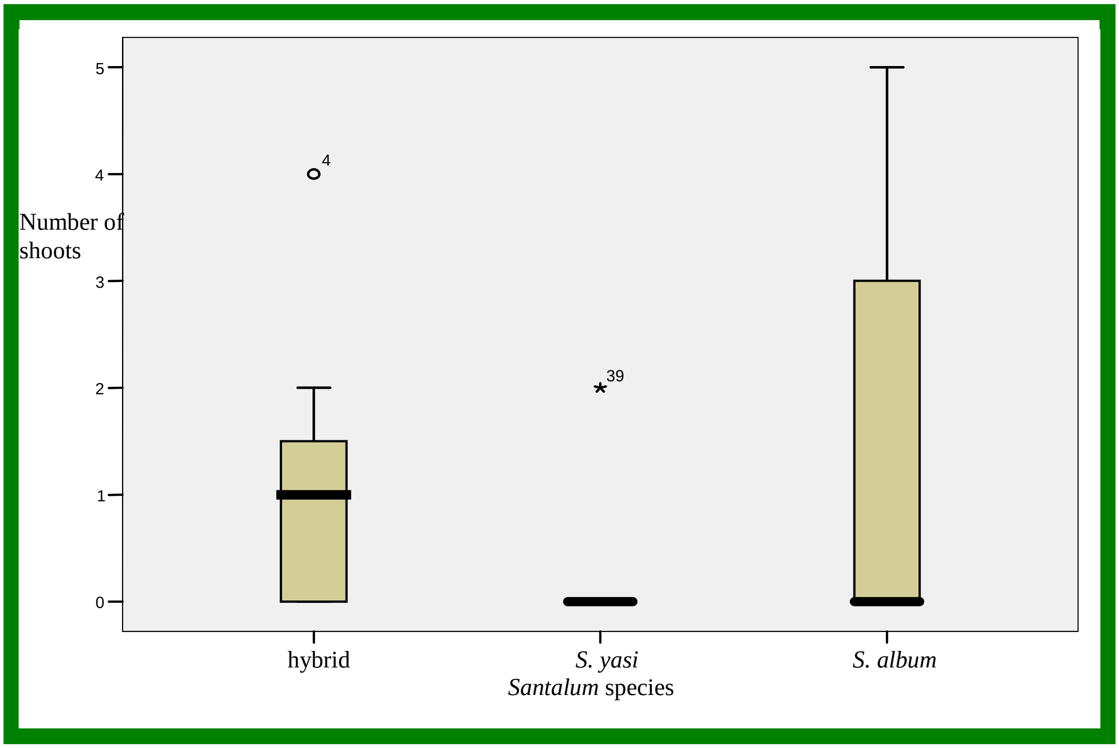


Figure 3.5 Boxplot plots summarizing shoots number of primary lateral roots (PLR) of three *Santalum* taxa the bar represents median, interquartile range and whisker 75% were extreme values also scoring. The circle stand for the outlier value more than 1.5 box lengths above the box. This number is the identifier.The stars stand for the extreme value as more than three box-lengths above the box. The number is the identifier, either the row number or from the variable entered in the lable cases by box.



3.4 Discussion

This research showed that secondary lateral roots of the *Santalum* hybrid (*S. yasi x S. album*) produced new shoots for up to three months after severing the roots. The better shoot producing ability of SLRs may be due to their having higher food/carbohydrate reserves or it could have been due to the accumulation of natural auxins in the root bases (Al-Saqri & Alderson, 1996). It could also be due to juvenility factors (Jawanda *et al.*, 1991) that occur along the root of a plant (Hartmann *et al.*, 1990) and Nautiyal et *al*., (1992).

Other studies have also found differences among species in the number of shoots forced from dormant stem segments (Henry *et al*., 1992). On northern red oak shoot production varied by tree and section and increased with decreasing distance from the tree trunk and where trees tend to be mostly juveniles (Hackett, 1985). The difference in shoot production among three *Santalum* taxa may be influenced by genetic variation in sprouting capability or by microsite conditions[6]. However, the trees were grown in close proximity and site conditions were relatively uniform, suggesting that probably shoot production was more related to genetic differences among the taxa than to microsite variation.

Shoot induction appears to be geopositionally or perhaps phototropically based, at least in part, as shoots never appeared on the undersides of any of the root sections (Table 3.1).

The cut was vertical and might be expected to allow the formation of more shoots because more latent buds would be in a position where growth could occur (Plate 3.8). However, horizontal (sides face up) cut root sections did not produce significantly more shoots.

However, the relationships between length (section) of root and shoot production varied with species. Greater shoot production has been reported on the largest diameter (therefore the greatest surface area) stem sections of red maple (*Acer rubrum*), but with Japanese maple (*Acer palmatum*), moderate-sized stems tended to produce the greatest number of shoots (Henry *et al*., 1992).

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[6] microsite is a term used in ecology to describe a pocket within an environment with unique features and characteristics. Classifying different microsites may depend on temperature, humidity, amount of sunlight, vegetative cover

Plate 3.8 The cut was vertical and might be expected to allow the formation of more shoots (photo: Havea, 2006).

If only *Santalum* hybrid (*S. yasi x S. album*) is considered, the gradient of decreasing numbers of shoots from the base of the trunk outwards may be a function of exposed surface area or an ontogenetic gradient within the tree. The reasons for variation in shoot induction at different distances from the trunk are unclear. It is consistent with other tree species in which different amounts of food reserves are available to various parts of the tree at different seasons (Hartmann *et al*., 1997). That may explain the variation in shooting success relative to distance from the trunk.

In general, the different *Santalum* taxa displayed similar responses to cutting type, root diameter and distance of the cut from the trunk in term of stimulating shoot production. Uniyal *et al*., (1985) also indicated that vegetative propagation through root cuttings of SLRs produce more shoots (Figure. 3.2) than the cuts in big (PLR) roots.

Working with *S. album,* Vijayakumar *et al.,* (1981) observed that trenches dug 60-90 cm from the main trunk give maximum induction of shoots, but that numbers of shoots varied proportionately to the thickness of exposed root.

Further research is needed to determine whether this result is due to differences in cutting severity, or differences in frequency of shoot primordia and/or their ability to be liberated. The difference in shoot production between the root lengths of the three *Santalum* taxa could be related to differences in the nutritional status (Hansen, 1986). Hansen found that the root affected subsequent shoot growth in *Hedera canariensis*, although shoot growth differed depending on the surrounding environment in *Hedera helix*.

3.5 Conclusion and summary

To conclude this experiment, root cutting has the potential to serve as an alternative shoot production technique for planting material to propagate *Santalum* taxa. For fully severed SLRs the best diameter for stimulating shoot production appears to be 1.4 cm in the *Santalum* hybrid (*S. yasi x S. album*), 1.9 cm in *S. album* and 1.6 cm in *S. yasi* (Table 3.2: Figure 3.0). However, the best root diameter for stimulating shoot production in half-cut PLRs is 5.0 cm in the *Santalum* hybrid (*S. yasi x S. album*), 4.7 cm in *S. album* and 4.7 cm in *S. yasi* (Figure 3.3). The distances from the trunk at which the cut is made has a major effect on the number of shoots. The best distances from the trunk to be used for stimulating shoot production should be around 50-60 cm for the *Santalum* hybrid (*S. yasi x S.album*) and 30-35 cm for *S. album* and *S. yasi* on severed roots (SLR) (Figure3.1). While for injured roots (PLR) the cut should be made about 20 cm from the trunk for *Santalum* hybrid (*S.yasi x S. album*) and *S. album* and 10 cm away from the trunk for *S. yasi*. In addition, this investigation confirmed that severed SLRs and injured PLRs of the *Santalum* hybrid (*S. yasi x S. album*) stimulate more shoot production that can be used as planting material of sandalwood.

was to investigate the shoot production from injured PLRs The aim of this experiment and severed SLRs. Induction of shoots through root suckering is the first step from mature sandalwood plants that are more likely to be amenable to vegetative propagation as rooted cuttings.

Similar studies could be undertaken for root suckering species where a demand for shoot multiplication exists: a table of predicted shoot percentages could be developed as in Table 3.2 to assist in decisions about what diameter and distance from the trunk would be most efficient for a source of shoots for propagation. The gradient of ontogeny (or the progress from juvenility to maturity) for the *Santalum* hybrid (*S. yasi x S. album*) was shown to increase with increasing distance from the trunk of the tree (rather than closer to the trunk) and smaller root diameter. Stimulating shoot production from dormant root segments could be a practical way to produce shoots for vegetative propagation.

CHAPTER 4

VEGETATIVE PROPAGATION OF *S. YASI, S. ALBUM* AND *SANTALUM* HYBRID (*S. YASI X S. ALBUM*) STEM CUTTINGS

4.0 INTRODUCTION

Planting using vegetative cuttings is an extensively practised method of propagating plants. It has many advantages, since it is economical, requires little space and is rapid and generally technically simple. Cuttings can be made from the stem, modified stem, roots or leaves. Stem cuttings have been used to propagate a wide variety of plant species. In vegetative propagation through stem materials, cuttings are taken from the shoots of the plants with terminal or lateral buds that are capable of developing adventitious roots and subsequently forming a complete plant (Hartmann *et al.*, 1997). Successful rooting of the cuttings is dependent on factors such as position of the cuttings on the plant, rooting medium and hormone concentration, season when the cuttings are made as well as physical and environmental factors (Wilson, 1993). The effects of these factors on the rooting of sandalwood stem cuttings is either limited or unknown.

4.1 Materials and Methods

The study was carried out between July 2005 and December 2006 in south-eastern Viti Levu, Fiji. Field research was undertaken in the Forestry Department's sandalwood research plots at Colo-i-Suva and Vunimaqo. The study was conducted in the glasshouse (Colo-i-Suva) to determine the effects of different treatments on rooting of cuttings in the three sandalwood taxa. Stock plants were sandalwood seedlings of two different ages (6-

12 months and 12-24 months) provided by the Forestry Department. Two sources of cuttings were used: one from the seedlings provided by the Forestry Department and the other from induced root suckers produced in the experiments describes in Chapter 3. For the purpose of this study, stem segments from the two sources were treated the same.

The experiment was conducted at the end of March 2006 after the shoots from induced roots were well-established. Experiments on vegetative propagation of cuttings of 2cm to 4 cm in length were set on April 20, 2006. The cuttings were taken from the 'apical' (toward the apex) and 'basal' (towards the base) positions. Half of cuttings were 'quick-dip' treated with Indole Butyric Acid (IBA) and different levels of Naphthalene Acetic Acid (NAA) and planted in either peatmoss or mahogany compost in Vic Pots (Plate 4.0) as appropriate. Misting was regulated using a leaf balance sensor. During the day the misting typically came on for about 15 seconds every 15-20 minutes. Observations recorded included planting whether rooting had taken place following root number and root length.

The objective of this investigation was to study the effect of cutting position, rooting medium, cutting age and hormone on the rooting of stem cuttings of sandalwood. The main variables studied were:

- Cutting position of the suckers or seedlings (apical or basal);
- Rooting media (peatmoss vs. mahogany compost);
- Rooting hormone (with or without IBA; and NAA)
- Different concentralions of NAA 0 mg L^{-1}, 0.5 mg L^{-1} and 1.0 mg L^{-1}.
- Age of the seedling from which the cutting was made (either 6-12 months or 12-24 months).

Thirteen apical portions and 13 basal portions were planted in individual tubes of 30:70 sand:peatmoss (total 26) and 12 apical portions and 12 basal portions were planted in individual tubes of sand:mahogany compost. The other variable (IBA, NAA and seedling age) were varied utilizing different trays and considered as separate experiments (Figure 4.0, 4.1, and 4.2).

At intervals throughout the experimental period, cuttings were assessed for rooting, root number and length of the longest root at 24 weeks after setting and transplanting the best rooted cuttings from the last experiment (Chapter 5). Data collection was done by carefully separating the rooted cuttings from the seedling trays followed by washing away the rooting media with water. Rooting was monitored through regular visual inspection of roots protruding through the bottom of the pots. After it was evident that a reasonable proportion of cuttings had rooted cuttings were assessed and number of cuttings rooted, number of roots per cutting, and individual root lengths were recorded. Unrooted cuttings were re-set and rooting re-assessed after an appropriate interval (3-4 weeks), when more cuttings were observed to have struck roots.

4.1.1 Experimental layout

The experimental layout consisted of three experiments run simultaneously within the experimental area

- **Experiment A.** Cuttings from either apical or basal sections were treated with or without rooting hormone IBA, and set in two different media (i.e., 30:70 sand:peatmoss and 30:70 sand:mahogany compost) three hundred cuttings from root suckers were used (Figure 4.1), and young seedlings (6-12 months) were used.

- **Experiment B**. Different concentrations of NAA (0, 0.5 and 1.0 mg L^{-1}) were used to treat the apical and basal portions from seedlings and juvenile shoots from root suckers and set in two different media (30:70 sand: peatmoss and 30:70 sand:mahogany compost). Four hundred and fifty cuttings from root suckers and 450 from seedlings were used (Figure 4.2). Young seedlings (6-12 months) were used.
- **Experiment C.** Apical or basal sections from seedlings of two different ages (6-12 and 12-24 months old) were used for cuttings. Half of the seedlings were dipped in IBA rooting hormone and set in two different media (30:70 sand:peatmoss and 30:70 sand:mahogany compost). In total, 300 cuttings from 6-12 month old seedlings and 300 cuttings from 12-24 month old seedlings were used (Figure 4.0).

Figure 4.0 Experiment A: Cuttings of three sandalwood taxa from apical and basal portions were treated with or without IBA and set in two different media (i.e. 30:70 sand: peatmoss and 30:70 sand:mahogany compost).

***Santalum yasi* with or without IBA**

1. With IBA

sand:peatmoss					sand:maho. compost				
Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12
2	9	12	6	9	3	6	1	4	11
3	8	13	5	10	2	7	12	5	10
4	7	1	4	11	1	8	11	6	9
5	6	2	3	12	13	9	10	7	8

2. without IBA

sand:peatmoss					sand:mahogany				
Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12
2	9	12	6	9	3	6	1	4	11
3	8	13	5	10	2	7	12	5	10
4	7	1	4	11	1	8	11	6	9
5	6	2	3	12	13	9	10	7	8

13 apical portion were treated with IBA and 13 basal portion also treated with IBA set in 30:70 sand:peatmoss while 12 of apical portion were treated with IBA and 12 basal portion were treated with IBA and set in 30:70 sand:mahogany compost each Vict Pots consist 50 cuttings.

Santalum album with or without IBA

1. With IBA

sand:peatmoss					sand:maho. compost				
Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12
2	9	12	6	9	3	6	1	4	11
3	8	13	5	10	2	7	12	5	10
4	7	1	4	11	1	8	11	6	9
5	6	2	3	12	13	9	10	7	8

2. without IBA

sand:peatmoss					sand:mahogany				
Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12
2	9	12	6	9	3	6	1	4	11
3	8	13	5	10	2	7	12	5	10
4	7	1	4	11	1	8	11	6	9
5	6	2	3	12	13	9	10	7	8

Santalum hybrid (*S. yasi x S. album*) with or without IBA

1. With IBA

sand:peatmoss					sand:maho. compost				
Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12
2	9	12	6	9	3	6	1	4	11
3	8	13	5	10	2	7	12	5	10
4	7	1	4	11	1	8	11	6	9
5	6	2	3	12	13	9	10	7	8

2. without IBA

sand:peatmoss					sand:mahogany				
Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12
2	9	12	6	9	3	6	1	4	11
3	8	13	5	10	2	7	12	5	10
4	7	1	4	11	1	8	11	6	9
5	6	2	3	12	13	9	10	7	8

Figure 4.1 Experiment B. The layout of cuttings of three sandalwood taxa from apical and basal portions of seedlings and juvenile shoots were treated with different concentrations of NAA but no IBA and set in two different media (30:70 sand:peatmoss and 30:70 sand: mahogany compost).

Santalum yasi shoots

1. nil NAA mg L^{-1}

sand:peatmoss sand:maho. compost

Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12
2	9	12	6	9	3	6	1	4	11
3	8	13	5	10	2	7	12	5	10
4	7	1	4	11	1	8	11	6	9
5	6	2	3	12	13	9	10	7	8

2. 0.5 NAA mg L^{-1}

sand:peatmoss sand:mahogany

Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12
2	9	12	6	9	3	6	1	4	11
3	8	13	5	10	2	7	12	5	10
4	7	1	4	11	1	8	11	6	9
5	6	2	3	12	13	9	10	7	8

3. 1.0 NAA mg L^{-1}

sand:peatmoss sand:maho. compost

Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12
2	9	12	6	9	3	6	1	4	11
3	8	13	5	10	2	7	12	5	10
4	7	1	4	11	1	8	11	6	9
5	6	2	3	12	13	9	10	7	8

***Santalum album* shoots**

1. nil NAA mg L^{-1}

sand:peatmoss sand:maho. compost

Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12
2	9	12	6	9	3	6	1	4	11
3	8	13	5	10	2	7	12	5	10
4	7	1	4	11	1	8	11	6	9
5	6	2	3	12	13	9	10	7	8

2. 0.5 NAA mg L^{-1}

sand:peatmoss sand:mahogany

Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12
2	9	12	6	9	3	6	1	4	11
3	8	13	5	10	2	7	12	5	10
4	7	1	4	11	1	8	11	6	9
5	6	2	3	12	13	9	10	7	8

3. 1.0 NAA mg L^{-1}

sand:peatmoss sand:maho. compost

Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12
2	9	12	6	9	3	6	1	4	11
3	8	13	5	10	2	7	12	5	10
4	7	1	4	11	1	8	11	6	9
5	6	2	3	12	13	9	10	7	8

***Santalum* hybrid (*S. yasi x S. album*) shoots**

1. nil NAA mg L^{-1}

sand:peatmoss sand:maho. compost

Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12

2. 0.5 NAA mg L^{-1}

sand:peatmoss sand:mahogany

Apical			Basal		Apical		Basal		
1	10	11	7	8	4	5	2	3	12

2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

3. 1.0 NAA mg L^{-1}

sand:peatmoss   sand:maho. compost

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*
2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

***Santalum yasi* seedlings**

1. nil NAA mg L^{-1}

sand:peatmoss   sand:maho. compost

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*
2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

2. 0.5 NAA mg L^{-1}

sand:peatmoss   sand:mahogany

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*
2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

3. 1.0 NAA mg L^{-1}

sand:peatmoss   sand:maho. compost

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*
2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

***Santalum album* seedlings**

1. nil NAA mg L^{-1}

sand:peatmoss   sand:maho. compost

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*

2. 0.5 NAA mg L^{-1}

sand:peatmoss   sand:mahogany

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*

2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

3. 1.0 NAA mg L^{-1}

sand:peatmoss sand:maho. compost

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*
2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

***Santalum* hybrid (*S. yasi x S. album*) seedlings**

1. nil NAA mg L^{-1}

sand:peatmoss sand:maho. compost

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*
2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

2. 0.5 NAA mg L^{-1}

sand:peatmoss sand:mahogany

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*
2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

3. 1.0 NAA mg L^{-1}

sand:peatmoss sand:maho. compost

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*
2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

Figure 4.2 Experiment C. The layout of cuttings of three sandalwood taxa from apical and basal portion of two different age seedling (6-12 and 12 -24 months) were treated with or without IBA and set in two different media 30:70 sand:peatmoss and 30:70 sand:mahogany compost.

***Santalum yasi* age seedling 6-12**

1. with IBA 6-12 months

sand:peatmoss sand:maho. compost

Apical			Basal		Apical		Basal		
1	***10***	***11***	***7***	***8***	***4***	***5***	***2***	***3***	***12***
2	***9***	***12***	***6***	***9***	***3***	***6***	***1***	***4***	***11***
3	***8***	***13***	***5***	***10***	***2***	***7***	***12***	***5***	***10***
4	***7***	***1***	***4***	***11***	***1***	***8***	***11***	***6***	***9***
5	***6***	***2***	***3***	***12***	***13***	***9***	***10***	***7***	***8***

2. without IBA 6-12 months

sand:peatmoss sand:mahogany

Apical			Basal		Apical		Basal		
1	***10***	***11***	***7***	***8***	***4***	***5***	***2***	***3***	***12***
2	***9***	***12***	***6***	***9***	***3***	***6***	***1***	***4***	***11***
3	***8***	***13***	***5***	***10***	***2***	***7***	***12***	***5***	***10***
4	***7***	***1***	***4***	***11***	***1***	***8***	***11***	***6***	***9***
5	***6***	***2***	***3***	***12***	***13***	***9***	***10***	***7***	***8***

Santalum album age seedling 6-12

1. with IBA 6-12 months

sand:peatmoss sand:maho. compost

Apical			Basal		Apical		Basal		
1	***10***	***11***	***7***	***8***	***4***	***5***	***2***	***3***	***12***
2	***9***	***12***	***6***	***9***	***3***	***6***	***1***	***4***	***11***
3	***8***	***13***	***5***	***10***	***2***	***7***	***12***	***5***	***10***
4	***7***	***1***	***4***	***11***	***1***	***8***	***11***	***6***	***9***
5	***6***	***2***	***3***	***12***	***13***	***9***	***10***	***7***	***8***

2. without IBA 6-12 months

sand:peatmoss sand:mahogany

Apical			Basal		Apical		Basal		
1	***10***	***11***	***7***	***8***	***4***	***5***	***2***	***3***	***12***
2	***9***	***12***	***6***	***9***	***3***	***6***	***1***	***4***	***11***
3	***8***	***13***	***5***	***10***	***2***	***7***	***12***	***5***	***10***
4	***7***	***1***	***4***	***11***	***1***	***8***	***11***	***6***	***9***
5	***6***	***2***	***3***	***12***	***13***	***9***	***10***	***7***	***8***

Santalum hybrid (_S. yasi x S. album_) age seedling 6-12

1. with IBA 6-12 months

sand:peatmoss sand:maho. compost

Apical			Basal		Apical		Basal		
1	***10***	***11***	***7***	***8***	***4***	***5***	***2***	***3***	***12***
2	***9***	***12***	***6***	***9***	***3***	***6***	***1***	***4***	***11***
3	***8***	***13***	***5***	***10***	***2***	***7***	***12***	***5***	***10***
4	***7***	***1***	***4***	***11***	***1***	***8***	***11***	***6***	***9***
5	***6***	***2***	***3***	***12***	***13***	***9***	***10***	***7***	***8***

2. without IBA 6-12 months

sand:peatmoss sand:mahogany

Apical			Basal		Apical		Basal		
1	***10***	***11***	***7***	***8***	***4***	***5***	***2***	***3***	***12***
2	***9***	***12***	***6***	***9***	***3***	***6***	***1***	***4***	***11***
3	***8***	***13***	***5***	***10***	***2***	***7***	***12***	***5***	***10***
4	***7***	***1***	***4***	***11***	***1***	***8***	***11***	***6***	***9***
5	***6***	***2***	***3***	***12***	***13***	***9***	***10***	***7***	***8***

Santalum yasi age seedling 12-24

1. with IBA 12-24 months

sand:peatmoss sand:maho. compost

Apical			Basal		Apical		Basal		
1	***10***	***11***	***7***	***8***	***4***	***5***	***2***	***3***	***12***
2	***9***	***12***	***6***	***9***	***3***	***6***	***1***	***4***	***11***

2. without IBA 12-24 months

sand:peatmoss sand:mahogany

Apical			Basal		Apical		Basal		
1	***10***	***11***	***7***	***8***	***4***	***5***	***2***	***3***	***12***
2	***9***	***12***	***6***	***9***	***3***	***6***	***1***	***4***	***11***

3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

Santalum album age seedling 12-24

1. with IBA 12-24 months

sand:peatmoss | sand:maho. compost

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*
2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

2. without IBA 12-24 months

sand:peatmoss | sand:mahogany

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*
2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

Santalum hybrid (*S. yasi x S. album*) age seedling 12-24

1. with IBA 12-24 months

sand:peatmoss | sand:maho. compost

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*
2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

2. without IBA 12-24 months

sand:peatmoss | sand:mahogany

Apical			Basal		Apical		Basal		
1	*10*	*11*	*7*	*8*	*4*	*5*	*2*	*3*	*12*
2	*9*	*12*	*6*	*9*	*3*	*6*	*1*	*4*	*11*
3	*8*	*13*	*5*	*10*	*2*	*7*	*12*	*5*	*10*
4	*7*	*1*	*4*	*11*	*1*	*8*	*11*	*6*	*9*
5	*6*	*2*	*3*	*12*	*13*	*9*	*10*	*7*	*8*

4.1.2 Statistical tests

Data for vegetative propagation of three sandalwood taxa were analysed using logistic regression. The analyes were used to determine whether the treatment applied for the respective experiments (i.e. apical or shoots basal) presence or absences and concentration of rooting hormone, compost or peatmoss, and age) caused the outcome of rooting. Logistic regression may be used when predictor variables are being investigated for their effect on a binary outcome (i.e. ‘rooted’ or ‘not rooted’). All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) by Dr. Linton Winder (Statistician, USP).

4.1.3 Greenhouse experiment layout

The humidity level in the propagation unit was maintained through a misting regulated by a leaf balance sensor (Plate 4.1). During the day the misting typically came on for about 15 seconds every 15-20 minutes. Throughout the experimental period, the humidity of the greenhouse was measured using a hygrometer. The minimum and maximum humidity ranged from 23% to 72%. The measured mean minimum and maximum temperatures during the study period were, respectively, 21.9º C and 26.6º C.

Plate 4.1 Sandalwood cutting were set out under a mist propagator in a greenhouse located at the Forestry Division Colo-i-Suva (photo: Havea, 2005).

Plate 4.2 The cutting of the three sandalwood taxa were planted in two media, i.e. 30:70 sand:peatmoss and 30:70 sand:mahogany compost (photo: Havea, 2005).

Plate 4.0 Two node-cuttings made from the cuttings were quickly dipped in rooting hormone IBA and or NAA powder. The cuttings were planted in 30:70 sand:peatmoss and 30:70 sand:mahogany compost medium in Vic Pots (photo: Havea, 2005).

4.2 Results

Experiment A.Cuttings of three sandalwood taxa from apical and basal portions with and without IBA (Indole Butyric Acid) treatment.

The results of Experiment A showed that stem cuttings originating from the terminal portions (i.e. apical) had better rooting performance than did those from the basal portions in the three sandalwood taxa (Table 4.0; Plate 4.3). Lower strike rates were achieved in mahogany compost for all sandalwood taxa (Table 4.0). *Santalum* hybrid (*S. yasi x S. album*) cuttings rooted more easily (Plate 4.4).

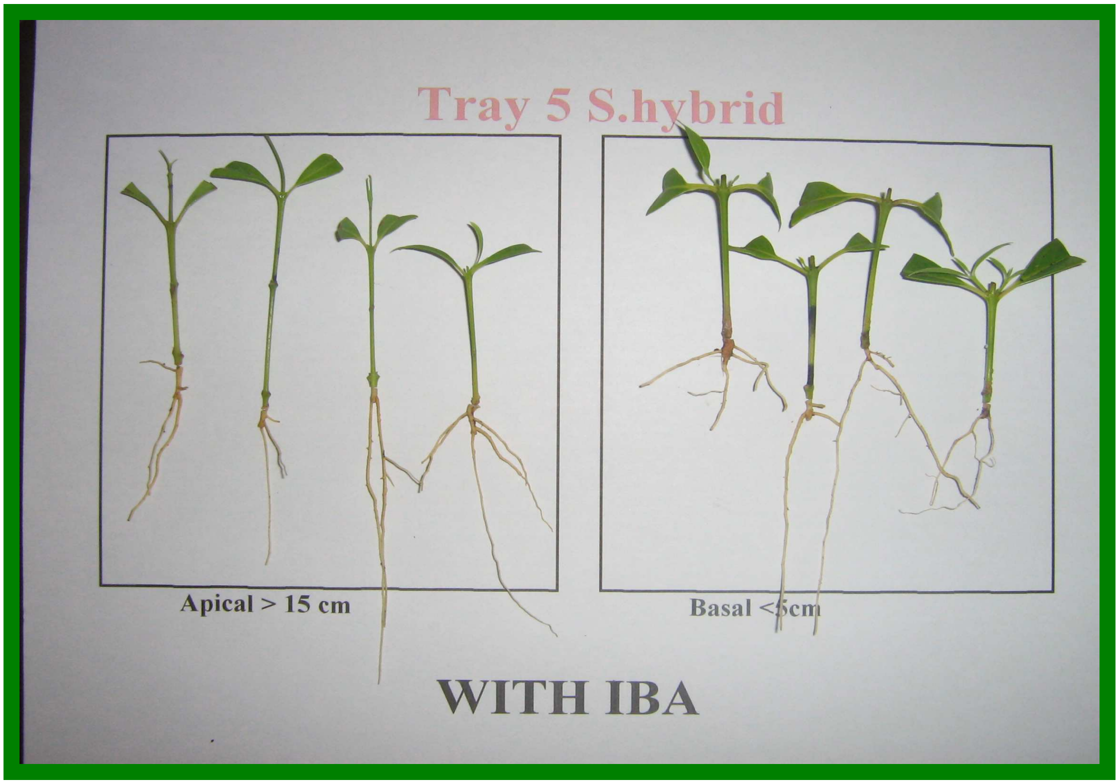


Plate 4.3 Rooted cuttings from hybrid show that stem cuttings originating from the terminal portions (i.e. apical) had better rooting performance with IBA (rooting hormone) than did those from the basal portions (photo: Havea, 2005).

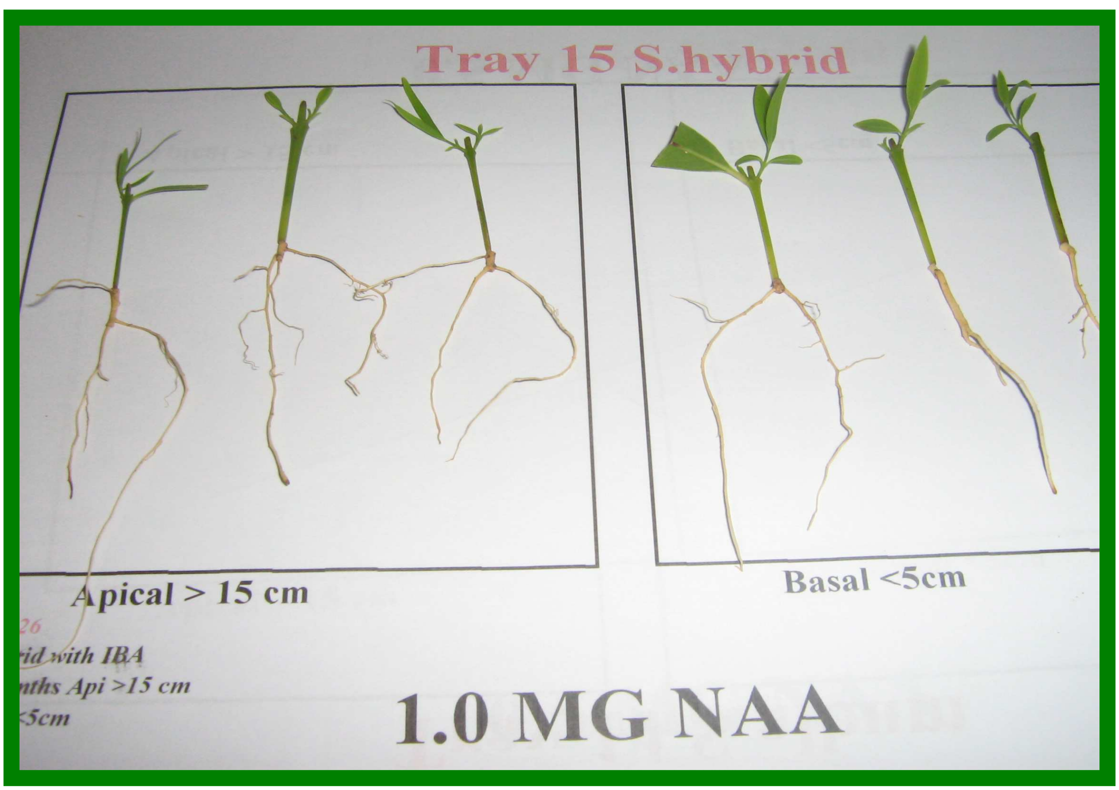


Plate 4.4 *Santalum* hybrid (*S. yasi x S. album*) cuttings rooted more easily with rooting hormone (photo: Havea, 2005).

In the three sandalwood taxa longer roots were produced in the 30:70 sand:peatmoss medium than in the 30:70 sand:mahogany compost (Table 4.1). Nevertheless, after more than 24 weeks the roots produced in the sand:peatmoss medium were about the same as those in the sand:mahogany compost. Based on the collected data, root length of the basal cuttings was influenced by IBA rooting hormone. Very few roots were produced in the absence of rooting hormone IBA (Appendix 1.4, Plates 1.4.1, 1.4.3, 1.4.5, 1.4.16, 1.4.18, 1.4.20, 1.4.22, 1.4.24, 1.4.26) and cuttings from basal positions did not show a good response to applied hormone.

Table 4.0 Summary statistics for mean root number (mean ± standard error of the mean) of rooted cutting of three *Santalum* taxa in two different media.

Medium & species	Hormone	Rooted apical cutting	Mean root number	Rooted basal cutting	Mean root number
S. yasi-peatmoss	IBA	9	2.78±0.32	10	1.70±0.15
	No IBA	5	1.00±0.00	3	1.33±0.33
S. yasi- m.compost	IBA	10	2.60±0.31	5	2.20±0.37
	No IBA	2	1.50±0.50	2	1.50±0.50
S. album-peatmoss	IBA	9	2.56±0.24	8	1.88±0.13
	No IBA	3	1.00±0.00	3	1.00±0.00
S. album-mahogany compost	IBA	4	1.75±0.25	3	1.00±0.00
	No IBA	-	-	-	-
Santalum hybrid-peatmoss	IBA	11	3.64±0.28	8	2.50±0.19
	No IBA	5	1.00±0.00	6	1.00±0.00
Santalum hybrid-m.compost	IBA	9	2.78±0.28	9	2.44±0.24
	No IBA	3	1.33±0.33	2	1.00±0.00

Table 4.1 Summary statistics for mean root length (mean ± standard error of the mean) of rooted cutting of three *Santalum* taxa in two different media.

Media	Hormone	Rooted apical cutting	Mean root number	Rooted basal cutting	Mean root number
S. yasi-peatmoss	IBA	9	2.11±0.20	10	1.50±0.17
	No IBA	5	1.00±0.00	3	1.00±0.00
S. yasi-M.compost	IBA	10	1.70±0.26	5	2.00±0.32
	No IBA	2	1.00±0.00	2	1.00±0.00
S. album-peatmoss	IBA	9	2.22±0.22	8	2.25±0.37
	No IBA	3	1.00±0.00	3	1.00±0.00
S. album-M.compost	IBA	4	2.25±0.48	3	2.67±0.33
	No IBA	-	-	-	-
Santalum hybrid-peatmoss	IBA	11	2.91±0.28	8	2.00±0.19
	No IBA	5	1.00±0.00	6	1.00±0.00
Santalum hybrid-M.compost	IBA	9	2.67±0.17	9	2.44±0.18
	No IBA	3	1.00±0.00	2	1.00±0.00

The major influencing factor for rooting success in cuttings of the three sandalwood taxa was rooting hormone which shows a highly significant effect ($P<0.001$) (Table 4.2). Rooting hormone affected the root development, root number and root length of the cuttings. Throughout the experimental period apical cuttings performed better than did basal cuttings in terms of rooted cutting percentage (Appendix 1.4, Plate. 1.4.0, 1.4.2, 1.4.4). Thus, compared with stem cuttings, apical cuttings are better planting materials for successful vegetative propagation of sandalwood.

Table 4.2 Summary statistics for logistic regression to determine factors influencing the outcome of the occurrence of roots. Effects of medium, cutting position and rooting hormone on root number and length of rooted cutting in three *Santalum* taxa are shown.

Source of variation	Wald statistic[7]	DF	Significance
Santalum yasi			
Medium	1.909	1	0.167
Cutting position	1.812	1	0.178
Rooting hormone	18.267	1	0.000
Santalum album			
Medium	11.156	1	0.001
Cutting position	0.264	1	0.607
Rooting hormone	14.877	1	0.000
Santalum hybrid			
Medium	1.160	1	0.281
Cutting position	0.443	1	0.506
Rooting hormone	16.649	1	0.000

The results show that rooting of stem cuttings of three sandalwood taxa can be best achieved with apical cuttings and that suitable levels of IBA treatment are needed (Table 4.3). The best results were achieved in the *Santalum* hybrid (*S. yasi x S. album*) with cuttings propagated in peatmoss and treated with IBA (Appendix 4.1, Plate 1.4.1).

The percentage of plants rooting and the development of roots from basal cuttings took place much more slowly when compared to the development of apical cuttings. Exceptions were *S. yasi* in 30:70 sand:peatmoss, where 76% of basal cuttings rooted compared to 69% of apical cuttings, and the *Santalum* hybrid (*S. yasi x S. album*) in 30:70 sand:mahogany compost where 75% of both basal and apical cuttings rooted. The response of both apical and basal cuttings to the rooting hormone was sufficient to enhance rooting in cuttings of *Santalum* hybrid (*S. yasi x S. album*) (Table 4.3 and Appendix 1.4 (Plate 1.4.4, 1.4.5, 1.4.12, 1.4.13, 1.4.14, 1.4.19, 1.4.20, 1.4.25 and 1.4.26).

Table 4.3 The effect of IBA rooting hormone on root initiation in three *Santalum* taxa from stem cuttings. The percentage shows the proportion of the total rooted cuttings attributed to that treatment.

[7] Significance of individual regressors. SPSS prints out the value of what it calls the Wald statistic for each regressor in each model, together with a corresponding significance level. The Wald statistic has a chi-squared distribution, but apart from that it is used in just the same way as the *t* values for individual regressors in linears regression.

	Hormone	No. rooted cutting		No. planted cuttings		%	
		Apical	Basal	Apical	Basal	Apical	Basal
S. yasi-peatmoss	IBA	9	10	13	13	69	76
	No IBA	5	3	13	13	38	23
S. yasi-m.compost	IBA	10	5	12	12	83	41
	No IBA	2	2	12	12	16	16
S. album-peatmoss	IBA	9	8	13	13	69	61
	No IBA	3	3	13	13	23	23
S. album-m.compost	IBA	4	3	12	12	33	25
	No IBA						
Santalum hybrid-peatmoss	IBA	11	8	13	13	84	61
	No IBA	5	6	13	13	38	46
Santalum hybrid-m.compost	IBA	9	9	12	12	75	75
	No IBA	3	2	12	12	25	16

Experiment B. Different concentration of NAA treating apical and basal portions from seedlings and root sucker shoots in two different media.

The experiment showed that NAA strongly influenced rooting, with an increase in rooting shown with increased concentrations (Table 4.4). The 30:70 sand:peatmoss medium produced a beneficial effect on rooting with NAA (concentrations 0.5 and 1.0 mg L^{-1}, Appendix 1.4: Plates 1.4.7, 1.4.8 for *S. yasi*, 1.4.10 and 1.4.11 for *S. album*, and 1.4.13, 1.4.14 for hybrid (*S. yasi x S. album*). Cuttings rooted well at lower levels of NAA (e.g. 0.5 mg L^{-1}) when peatmoss was used as a medium (Appendix 1.4: Plates 1.4.7, 1.4.10, and 1.4.13).

The higher concentration of NAA (1.0 mg L^{-1}) increased root length in both types of medium (Appendix 1.4: Plates 1.4.8, 1.4.9, 1.4.11). Roots produced from apical cuttings were longer than the roots produced from basal cuttings in the three *Santalum* taxa (Table 4.5).

Table 4.4 Summary statistics for mean root number (mean ± standard error) of rooted cutting of three *Santalum* taxa treated with different concentrations of NAA.

Media	NAA concentration	Rooted apical cutting	Mean apical root number (+/-S.E)	Rooted basal cutting	Mean basal root number (+/-S.E)
S. yasi-peatmoss	Nil	8	1.00±0.00	6	1.00±0.00
	0.5 mg/ L^{-1}	8	1.50±0.19	6	1.00±0.00
	1.0 mg/ L^{-1}	19	2.53±0.22	18	2.00±0.11
S. yasi-m.compost	Nil	5	1.00±0.00	3	1.00±0.00
	0.5 mg/ L^{-1}	6	1.33±0.21	5	1.00±0.00
	1.0 mg/ L^{-1}	11	2.18±0.26	7	1.71±0.18
S. album-peatmoss	Nil	4	1.00±0.00	2	1.00±0.00
	0.5 mg/ L^{-1}	5	1.40±0.24	6	1.00±0.00
	1.0 mg/ L^{-1}	11	2.45±0.25	9	1.44±0.18
S. album-m.compost	Nil	3	1.00±0.00	1	1.00±0.00
	0.5 mg/ L^{-1}	3	1.00±0.00	1	1.00±0.00
	1.0 mg/ L^{-1}	7	2.00±0.31	6	1.67±0.21
Santalum hybrid-peatmoss	Nil	8	1.13±0.13	6	1.17±0.17
	0.5 mg/ L^{-1}	8	1.63±0.18	8	1.50±0.19
	1.0 mg/ L^{-1}	19	3.11±0.24	14	1.86±0.18
Santalum hybrid-m.com	Nil	7	1.57±0.20	3	1.00±0.00
	0.5 mg/ L^{-1}	6	1.33±0.21	5	2.20±0.20
	1.0 mg/ L^{-1}	12	2.67±0.26	10	1.70±0.21

Table 4.5 Summary statistics for mean root length mm (mean ± standard error) of rooted cutting of three sandalwood taxa.

Medium and taxon	NAA concentration	Rooted apical cuttings	Mean apical root number (+/-S.E)	Rooted basal cuttings	Mean basal root number (+/-S.E)
S. yasi-peatmoss	Nil	8	0.69±0.09	6	0.50±0.00
	0.5 mg/ L^{-1}	8	1.13±0.16	6	1.00±0.18
	1.0 mg/ L^{-1}	19	2.74±0.19	18	1.89±0.15

S. yasi-m.compost	Nil	5	1.50±0.00	3	0.50±0.00
	0.5 mg/ L^{-1}	6	1.33±0.17	5	0.90±0.19
	1.0 mg/ L^{-1}	11	2.41±0.26	7	2.00±0.31
S. album-peatmoss	Nil	4	0.63±0.13	2	0.50±0.00
	0.5 mg/ L^{-1}	5	1.30±0.30	6	1.33±0.28
	1.0 mg/ L^{-1}	11	1.82±0.28	9	2.28±0.30
S. album-m.compost	Nil	3	0.83±0.33	1	0.50±0.00
	0.5 mg/ L^{-1}	3	1.33±0.17	1	1.00±0.00
	1.0 mg/ L^{-1}	7	2.07±0.28	6	1.75±0.17
Santalum hybrid-peat	Nil	8	0.06±0.24	6	1.42±0.44
	0.5 mg/ L^{-1}	8	2.06±0.41	8	1.88±0.21
	1.0 mg/ L^{-1}	19	2.92±0.21	14	1.89±0.14
Santalum hybrid- m.com	Nil	7	1.07±0.23	3	0.83±0.33
	0.5 mg/ L^{-1}	6	2.25±0.17	5	1.70±0.30
	1.0 mg/ L^{-1}	12	2.63±0.31	10	1.65±0.21

Out of all three *Santalum* taxa, the concentration of rooting hormone (NAA) and medium in *S. yasi* had a highly significant effect ($P<0.001$) on rooting parameters (Table 4.6) on the likelihood of rooting. Apical cuttings also showed greater uniformity in the number of roots produced compared to basal cuttings after setting in three *Santalum* taxa. Overtime however, with increases in the concentration of rooting hormone, cuttings showed positive responses to each level and more cuttings developed roots. The level of NAA had a delayed effect on *S. yasi* basal cuttings in enhancing root initiation as well as increasing number of roots developed.

A high percentage (34-73%) of rooted cuttings was achieved in all three *Santalum* taxa in 30:70 sand:peatmoss rooting medium when treated with NAA 1.0 mg L^{-1} (Table 4.7).

Table 4.6 Summary statistics for logistic regression to determine factors influencing the occurrence of roots. Effect of medium, cutting position and rooting hormone on root number and length of rooted cutting of three *Santalum* taxa are shown.

Source of variation	Wald statistic	DF	Significance
Santalum yasi			
Medium	9.163	1	0.002
Cutting position	2.394	1	0.122
NAA	23.704	1	0.000
Santalum album			
Medium	4.203	1	0.040
Cutting position	1.467	1	0.226
NAA	16.090	1	0.000
Santalum hybrid			
Medium	3.916	1	0.048
Cutting position	3.099	1	0.078
NAA	20.496	1	0.000

Table 4.7 The effect of different concentrations of NAA rooting hormone on root initiation. The percentage of the cuttings that rooted for that treatment is shown.

S. yasi	**Hormone**	**No. Rooted**		**No. Planted**		**%**	
		Apical	Basal	Apical	Basal	Apical	Basal
S. yasi-peatmoss	Nil	8	6	26	26	30	23
	0.5 mg/ L^{-1}	8	6	26	26	30	23
	1.0 mg/ L^{-1}	19	18	26	26	73	69
S. yasi-m.compost	Nil	5	3	24	24	20	12
	0.5 mg/ L^{-1}	6	5	24	24	25	20
	1.0 mg/ L^{-1}	11	7	24	24	45	29

Total		57	45	150	150		
S. album-peatmoss	Nil	4	2	26	26	15	7
	0.5 mg/ L^{-1}	5	6	26	26	19	23
	1.0 mg/ L^{-1}	11	9	26	26	42	34
S. album-m.compost	Nil	3	1	24	24	12	4
	0.5 mg/ L^{-1}	3	1	24	24	12	4
	1.0 mg/ L^{-1}	7	6	24	24	29	25
Total		33	25	150	150		
Santalum hybrid-peatmoss	Nil	8	6	26	26	30	23
	0.5 mg/ L^{-1}	8	8	26	26	30	30
	1.0 mg/ L^{-1}	19	14	26	26	73	53
Santalum hybrid-m.compost	Nil	7	3	24	24	29	12
	0.5 mg/ L^{-1}	6	5	24	24	25	20
	1.0 mg/ L^{-1}	12	10	24	24	50	41
Total		60	46	150	150		

Experiment C. Apical or basal cuttings from seedlings of ages - 6-12 months old and 12-24 months old

This experiment showed that most rooting took place on cuttings of age 6-12 month on the apical cutting portion (Table 4.8) (Appendix 1.4: Plates 1.4.15, 1.4.17 and 1.4.19). The number of roots produced per rooted cutting from both apical and basal cuttings was influenced by seedling age. Cuttings derived from older seedlings (aged 12-24 months) produced fewer roots (Table 4.8).

Table 4.8 Summary statistics for mean root number (mean ± standard error) of rooted cutting of *S. yasi*, *S. album* and *Santalum* hybrid (*S. yasi x S. album*) in different media.

Peatmoss					
Portion	Ages (months)	With IBA	Number of rooted cuttings	without IBA	Number of rooted cuttings
S. yasi- peatmoss					
Apical	6 to 12	1.90±0.18	10	1.00±0.00	5
	12 to 24	1.71±0.18	7	1.00±0.00	4
Basal	6 to 12	1.20±0.13	10	1.00±0.00	4
	12 to 24	1.67±0.21	6	1.00±0.00	3
S. yasi-mahogany compost					

Apical	6 to 12	1.75±0.48	4	1.00±0.00	3
	12 to 24	1.67±0.33	3	1.00±0.00	2
Basal	6 to 12	1.33±0.33	3	1.00±0.00	2
	12 to 24	1.33±0.33	3	1.00±0.00	2
S. album-peatmoss					
Apical	6 to 12	1.50±0.22	6	1.00±0.00	3
	12 to 24	1.50±0.29	4	1.00±0.00	3
Basal	6 to 12	1.80±0.37	5	1.00±0.00	2
	12 to 24	1.33±0.33	3	1.00±0.00	3
S. album-mahogany compost					
Apical	6 to 12	1.75±0.25	4	1.00±0.00	2
	12 to 24	1.25±0.25	4	-	
Basal	6 to 12	1.67±0.33	3	1.00±0.00	2
	12 to 24	1.67±0.33	3	-	
Santalum hybrid-peatmoss					
Apical	6 to 12	2.75±0.30	12	1.17±0.17	6
	12 to 24	1.75±0.22	12	1.00±0.00	4
Basal	6 to 12	2.18±0.26	11	1.00±0.00	4
	12 to 24	1.38±0.18	8	1.00±0.00	3
Santalum hybrid-mahogany compost					
Apical	6 to 12	2.71±0.52	7	1.00±0.00	3
	12 to 24	2.25±0.25	4	1.00±0.00	2
Basal	6 to 12	2.00±0.26	6	1.00±0.00	2
	12 to 24	1.67±0.33	3	1.00±0.00	2

The summary results reveal that the 6 to 12 months old seedlings in the three *Santalum* taxa produced longer roots in 30:70 sand:peatmoss than in 30:70 sand:mahogany compost (Table 4.9). Nevertheless, beyond 24 weeks after setting the roots produced were about the same in both media. The 6 to 12 months old seedling show longer roots in *Santalum* hybrid (*S. yasi x S. album*) than the other two sandalwood. Very few roots were produced in the cuttings from 12 to 24 month old seedlings. Cuttings from basal cutting positions from 12 to 24 month old seedlings did not show good response (Table 4.9) (Appendix 1.4: Plates 1.4.22, 1.4.23, 1.4.24, 1.4.25, 1.4.26).

Overall, the results reveal that cuttings treated with rooting hormone (NAA) and derived from younger seedling ages (6-12 months) form roots more quickly than did those derived from older seedlings (aged 12-24 months).

Table 4.9 Summary statistics for mean root length (mean ± standard error) of rooted cuttings of three *Santalum* taxa.

Portion	Ages	Root length (mm) with IBA	Number of rooted cutting	Root length (mm) without IBA	Number of rooted cutting
S. yasi –peatmoss					
Apical	6 to 12	2.00±0.21	10	0.60±0.10	5
	12 to 24	1.29±0.31	7	0.50±0.00	4
Basal	6 to 12	1.40±0.19	10	0.63±0.13	4
	12 to 24	1.83±0.28	6	0.50±0.00	3
S. yasi-mahogany compost					
Apical	6 to 12	2.13±0.13	4	0.50±0.00	3
	12 to 24	1.50±0.29	3	0.50±0.00	2

Basal	6 to 12	183±0.17	3	0.50±0.00	2
	12 to 24	2.00±0.29	3	0.50±0.00	2
S. album- peatmoss					
Apical	6 to 12	1.25±0.17	6	0.67±0.17	3
	12 to 24	2.50±0.29	4	0.50±0.00	3
Basal	6 to 12	1.90±0.24	5	0.75±0.25	2
	12 to 24	1.83±0.17	3	0.50±0.00	3
S. album-mahogany compost					
Apical	6 to 12	1.38±0.24	4	0.50±0.00	2
	12 to 24	1.88±0.31	4		
Basal	6 to 12	1.83±0.17	3	0.50±0.00	2
	12 to 24	1.33±0.33	3		
Santalum hybrid-peatmoss					
Apical	6 to 12	2.21±0.18	12	0.83±0.17	6
	12 to 24	1.58±0.15	12	0.75±0.14	4
Basal	6 to 12	1.45±0.14	11	1.00±0.20	4
	12 to 24	1.44±0.15	8	0.67±0.17	3
Santalum hybrid-mahogany compost					
Apical	6 to 12	1.79±0.18	7	1.00±0.29	3
	12 to 24	1.50±0.29	4	0.50±0.00	2
Basal	6 to 12	1.67±0.25	6	1.00±0.50	2
	12 to 24	1.83±0.17	3	0.75±0.25	2

The seedling age of sandalwood had no major influence on the number of roots produced per cutting and length of roots formed. This shows that the type of media used and application of IBA hormone is not significantly affected by the age of seedlings and cuttings. Differences in the rooting structure were observed between the two media. Cuttings propagated in 30:70 sand:peatmoss produced fine and highly branched root systems, which would likely have a high transplanting success rate whereas mahogany compost produced long and coarse roots. The type of medium also affected the number of roots produced. Compared with 30:70 sand:mahogany compost, cuttings propagated in 30:70 sand:peatmoss produced a higher number of roots. Nevertheless, both medium and rooting hormone influenced root length in the three sandalwood taxa (Table 4.10).

Table 4.10 Summary statistics for logistic regression to determine factors influencing the outcome of the occurrence of roots. Effect of medium, cutting position and rooting hormone on root number and length of rooted cutting of three *Santalum* taxa are shown.

Source of variation	**Wald statistic**	**DF**	**Significance**
Santalum yasi			
Medium	13.098	1	0.000
Cutting position	0.624	1	0.429
IBA	10.153	1	0.001
Age	2.963	1	0.085
Santalum album			
Medium	2.415	1	0.120
Cutting position	0.737	1	0.391
IBA	7.901	1	0.005
Age	1.435	1	0.231
Santalum hybrid			
Medium	17.228	1	0.000
Cutting position	3.164	1	0.075
IBA	28.174	1	0.000
Age	4.371	1	0.037

This increase in rooting percentage with an increase in the number of days after setting followed generally the same pattern throughout the experimental period except in seedlings to which no rooting hormone was applied (Table 4.11). This low percentage of rooted *S. yasi*, *S. album* and *Santalum* hybrid *(S. yasi x S. album)* cuttings was associated with high cuttings mortality, 33% to 43% from seedlings aged 6-12 and 12-24 months, respectively (Appendix 1.4).

Table 4.11 The effect of different seedling age (i.e., 6 to 12 and 12 to 24 months) on root initiation of *S. yasi*, *S. album* and *Santalum* hybrid from stem cuttings. The percentage shows the proportion of the total rooted cutting attributed to that treatment.

Santalum taxa	seedling age (months)	number planted		number rooted		%	
S. yasi-peatmoss							
		IBA	No IBA	IBA	No IBA	IBA	No IBA
Apical	6 to 12	13	13	10	5	76	38
	12 to 24	13	13	7	4	53	30
Basal	6 to 12	13	13	10	4	76	30
	12 to 24	13	13	6	3	46	23
Total		52	52	36	16		
S. yasi-mahogany compost							
Apical	6 to 12	12	12	4	3	33	25
	12 to 24	12	12	3	2	25	16
Basal	6 to 12	12	12	3	2	25	16
	12 to 24	12	12	3	2	25	16
Total		48	48	13	9		

S. album-peatmoss							
Apical	6 to 12	13	13	6	3	46	23
	12 to 24	13	13	4	3	30	23
Basal	6 to 12	13	13	5	2	38	15
	12 to 24	13	13	3	3	23	23
Total		52	52	18	11		
S. album-mahogany compost							
Apical	6 to 12	12	12	4	2	33	16
	12 to 24	12	12	4	0	33	0
Basal	6 to 12	12	12	3	2	25	16
	12 to 24	12	12	3	0	25	0
Total		48	48	14	4		
Santalum hybrid-peatmoss							
Apical	6 to 12	13	13	12	6	92	46
	12 to 24	13	13	12	4	92	30
Basal	6 to 12	13	13	11	4	84	30
	12 to 24	13	13	8	3	61	23
Total		52	52	43	17		
Santalum hybrid-mahogany compost							
Apical	6 to 12	12	12	7	3	58	25
	12 to 24	12	12	4	2	33	16
Basal	6 to 12	12	12	6	2	50	16
	12 to 24	12	12	3	2	25	16
Total		48	48	20	9		

4.3 Discussion

4.3.1 Effect of IBA rooting hormone

The results of these experiments show that sandalwood stem cuttings can root successfully under misting. High rooting percentage of cuttings were recorded in certain treatment, which implies that vegetative propagation of these species is feasible. An understanding of these findings is hampered because the amounts of endogenous hormones and amounts of root co-promoters in the cutting of sandalwood are unknown. Howard, (1996) indicated the need for application of higher levels of rooting hormone when the concentrations of these compounds in a plant are low.

Rooting hormones play an important role in root development of the cuttings (Wiessman-Ben & Tchoundjeu, 2000). The results reported here are consistent with many other studies on different plant species and has confirmed their beneficial effect in adventitious root development (Arya *et al.*, 1994; Al- Saqri & Alderson, 1996; Aminah *et al.*, 1997; Hartmann *et al.*, 1997). Cuttings of three *Santalum* taxa that had been treated with the rooting hormone IBA showed an increased percentage of rooted cuttings, root number, root length and uniformity of rooting on cuttings in all three *Santalum* taxa (Figure 4.3 A, B and C).

Though their roles are to promote rooting, it is necessary to apply an appropriate concentration of rooting hormones since application of unsuitable concentrations can inhibit rooting or it can act as a growth retardant when applied at higher concentrations (Hartmann *et al.*, 1990; Wiessman-Ben & Tchoundjeu, 2000).

It is expected that rooting percentage could probably be further increased by manipulating various environmental conditions, especially better optimizing the misting regime.

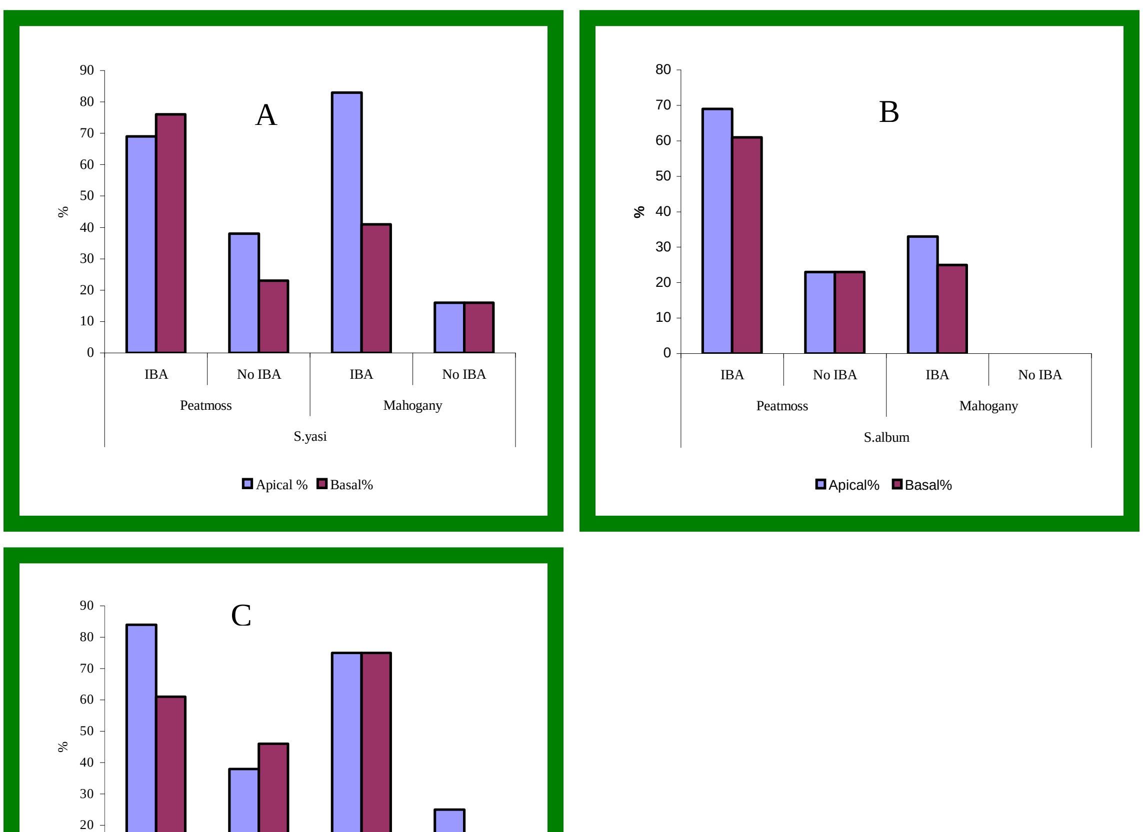


Figure 4.3 The percentage of three sandalwood taxa showing the proportion of total rooted cutting attributed to that treatment (i.e. IBA rooting hormone, cutting position and media).

According to Wiessman-Ben & Tchoundjeu (2000) hormones such as IBA, Indole Acetic Acid (IAA), and NAA play an important role in root growth. The current result shows that the application of IBA to cuttings from the apical position and planted in sand:peatmoss medium also increase percentage of rooting in cuttings, root number and root length in sandalwood. Among the exogenous rooting hormones, Indole Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) are two synthetic chemicals that have been found to be reliable in promoting roots in cuttings. IBA is widely applied for general use because it can remain non-toxic within a wide range of concentrations and improves root initiation of cuttings for most plants species (Al-Barazi & Schwabe, 1982; Hartmann *et al*., 1997).

IBA also accelerates the translocation of nutrients from the upper part of the cuttings to their basal ends by increasing the activity of enzymes. This rapid translocation increases hydrolysis of carbohydrates by providing enough energy for the rooting response of the cells (Arya *et al*., 1994). As recorded by Al-Barazi & Schwabe (1982), occasionally IBA treatment seems to stimulate cell division in the ray cells between the primary bundles to improve root initiation and to increase uniformity of rooting. Jawanda *et al*. (1991)'s study supported their view, when plum cuttings treated with 0.01% IBA followed by 0.005% IBA recorded a higher rooting percentage than did the control. Ofori *et al*. (1996) also reported that 0.002% IBA treatment increased the final rooting percentage of *Milicia excelsa* by 9% above that of the control. Similarly, Puri & Vermat (1996) described rooting of *Dalbergia sissoo* cuttings triggered and enhanced by rooting hormone application.

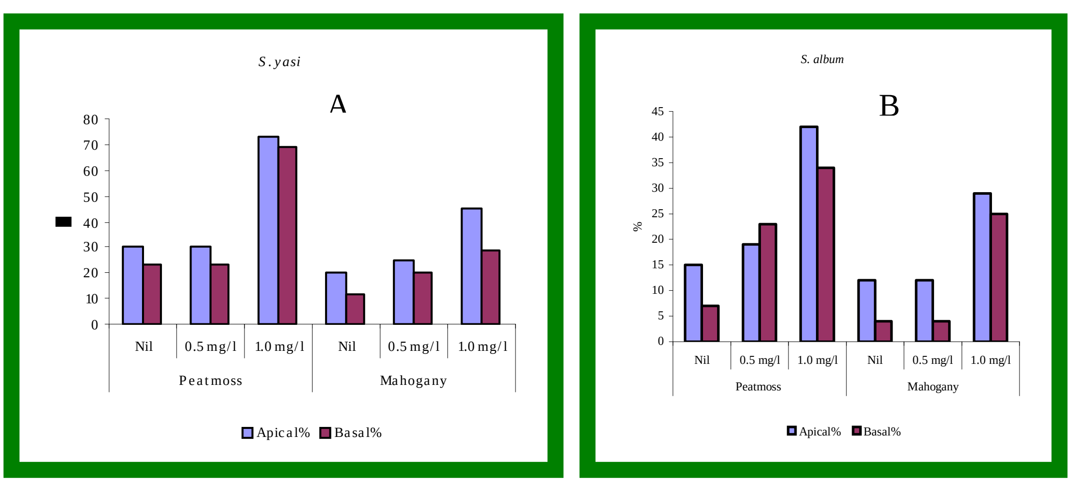
4.3.2 Effect of different concentration of NAA

The result for sandalwood cuttings are consistent with the above reports. Even though IBA rooting hormone promotes adventitious root development of stem cuttings, the use of rooting hormone at the appropriate concentration is essential (Hartmann *et al*., 1990; Wiessman-Ben & Tchoundjeu, 2000). It is evident that the cuttings of the three *Santalum* taxa have different optimum concentrations of rooting hormone for rooting.

As indicated in Figure 4.4 A, B and C, better rooting required concentrations of 0.5 mg L^{-1} NAA and 1.0 mg L^{-1} NAA. Similar result were indicated in *Cordia alliodora* by Mesen *et al*., (1997), who found that a concentration of 1.6% NAA was required for a 0.8% optimum rooting percentage. Similar results where reported by Leakey & Mohammed (1985) in rooting of *Triplochiton scleroxylon*. On the other hand, successful rooting without applied rooting hormone has been reported in a number of other tropical tree species, such as *Nauclea diderrichii* and *Vochsia hondurensis* (Leakey, 1990), *Shorea macrophylla* (Lo, 1985) and *Milicia excelsa* (Ofori *et al*., 1996). This may indicate that these plants are already well-supplied with endogenous rooting hormone (Ofori *et al*., 1996).

However, root number as well as root length was higher in the 30:70 sand:peatmoss medium for all three *Santalum* taxa (Figure 4.4, A, B and C). This is probably due to the differences in moisture holding capacity of the two media compared. On the other hand, when cuttings were grown in peatmoss, they showed beneficial results to a low level of NAA (0.5 mg L^{-1}) and more roots were produced than with NAA (1.0 mg L^{-1}). This shows that cuttings respond well to a lower level of NAA when sand:peatmoss was used as a medium. On the contrary, with sand:mahogany compost, more roots were produced with higher levels of NAA (1.0 mg L^{-1}). According to Hartmann *et al.*, (1997), these act as co-factors and interact with endogenous rooting hormone to promote rooting and to increase root number and length. These authors also reported that these compounds reduce the destruction of rooting hormone by indoleacetic acid oxidase enzyme.

Data obtained in this experiment suggest that rooting ability of sandalwood basal cuttings can be improved by using an appropriate level of NAA. The cuttings were found to be sensitive to NAA 1.0 mg L^{-1}. Higher rooting percentage and more and longer roots can be obtained with rooting hormone in the three *Santalum* taxa. Since root number is important in successful establishment of cuttings, it is important to identify the most suitable rooting media.



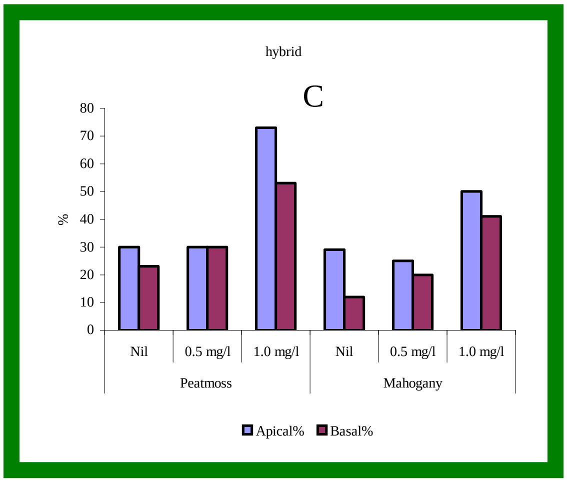


Figure 4.4 The percentage of *S. yasi* 'A', *S. album* 'B' and the *Santalum* hybrid 'C' shows the proportion of the total rooted cutting attributed to different treatments (i.e. NAA rooting hormone, cutting position and medium).

A combination of rooting hormones probably promoted root induction in sandalwood cuttings. The efficiency of rooting hormone was also reported to vary when used in combination with each other (Smalley *et al*., 1991; Puri & Vermat, 1996). Arya *et al*. (1994) found that a combination of NAA + IBA+ thiamine and IAA + IBA were the best rooting hormone treatments stimulating rooting in 50-60% of *Prosopis cineraria* cuttings than when the hormones were used singly. The effect of rooting hormones also varies with timing of application (Hartmann *et al.*, 1997; Boeing *et al*., 1999). Dipping the base of softwood olive cuttings in solutions containing IBA in the range of 0.4 to 0.5% was as effective in stimulating root production as a 24-hour soaking treatment of 0.005%. Kromwijk & Van Mourik (1992) also reported that dipping of *Ficus benjamina* cuttings in a 0.025% solution for four hours was more effective than using higher IBA concentrations or a rooting powder.

4.3.3 Effect of rooting media

Temperature of the rooting medium also affects the rooting of cuttings (Hartmann *et al*., 1997). In propagation, the air content in the media should be between 20% and 45% of the volume to promote root formation and growth (*ibid*.). Most media contain combinations of sand, peat, sphagnum moss, vermiculite, perlite, compost, and shredded bark or sawdust (*ibid*).

A thermo-chemical reactions, i.e. the speed of plant activity, is directly related to temperature, and is one of the most important aspects of successful propagation (Hartmann, 1997). As temperature rises, the respiration of a plant also rises. In common terms, higher temperatures, to some point, will generate higher activity levels in the plant. Lebrun *et al*., (1998) confirmed this when cuttings of *Syzygium paniculatum* grown on a non-heated substrate (average temperature 16º C) failed to root. However, when the substrate was heated (average temperature 22 ±0.5º C), the mean rooting rate exceeded 75% on the best media or substrates.

In this sandalwood study, there were some differences in the rooting habit in the two media. A slightly higher rooting success occurred in 30:70 sand:peatmoss in all three *Santalum* taxa. This result is consistent with studies by Walker *et al*., (1999) who showed that both sand and peat potting media are suitable for the propagation of *Toona ciliata* cuttings under a mist propagation environment. Substantial differences in survival and rooting success were observed between the three sandalwood taxa in peatmoss and mahogany compost with the average rooting success of *Santalum* hybrid greater than those of *S. yasi* and *S. album.*

The results suggest that the 30:70 sand:composted mahogany medium is probably not suitable for propagation but can be used as a low-cost and effective substitute for imported peatmoss. Once again, the *Santalum* hybrid, with acceptable strike rates proved to be a more suitable candidate for vegetative propagation. Higher root zone temperatures may also help in developing root in cuttings of *Santalum* taxa.

Light is probably one of the factors that could influence root development. Strong light has an inhibitory effect on the initiation of lateralroot primordia on stem cuttings of Lombardy poplar, *Populus nigra* var. *italica* Muenchh. (Shapiro 1958). It also reduced lateral-root elongation and primaryroot growth in some studies (e.g., Golaz and Pilet 1985). In the study by Stromquist and Eliasson (1979) (using *Picea abies*) Karst, the rooting of cuttings was almost totally inhibited by light when the bases of the stem cuttings were irradiated. The molecular and biochemical background of the inhibitory effect of light is not yet clear, but there are suggestions that light may destroy rooting hormone, change the ratio of promoting and inhibiting phenolics, or activate rooting hormone oxidases (Maynard 1995). However, sprouting of root cuttings was at the same level in both light and dark conditions (Stromquist and Eliasson, 1979).

Since better root development, including greater numbers of roots, is most important in the establishment of cuttings, it is recommended that propagation of sandalwood be done by using apical cuttings in 30:70 sand:peatmoss medium with the application of rooting hormone (IBA).

4.3.4 Effect of cutting position

In addition to cutting position, recent studies show the importance of internode length on the rooting ability of cuttings. The optimal length of cuttings will vary depending on the species. The relationship between cutting length and percentage of rooting was stronger when cuttings increased in length apical than basal (Leakey, 1983; Leakey & Mohammed, 1985). Leakey & Mohammed (1985, usiing *Triplochiton scleroxylon*) and Wilson (1993, using *Eucalyptus globules*) found that when cutting length was greatest at apical nodes, rooting percentages and the number of roots per rooted cutting were higher from apical nodes than from basal nodes. Palanisamy & Kumar (1997) reported similar results on rooting of neem (*Azadirachta indica*).

On the other hand, when cutting lengths of *Triplochiton scleroxylon* were greater at basal nodes, rooting percentages and number of roots per rooted cutting was also significantly greater from basal nodes. When cuttings from all nodes were similar in length, rooting percentages and numbers of roots per rooted cutting were generally greatest from basal nodes (Leakey, 1983; Leakey & Mohammed, 1985; Wilson, 1993). The effect of cutting length is related to carbohydrate accumulation at the base of the cutting; and the carbohydrate amount was more optimal for root formation in cutting with long basal nodes compared to short ones (Hartmann *et al*., 1990).

The size of the plant stem cuttings probably also contributes to variation in rooting ability, since diameter of the stem varies along the shoot (Wilson, 1993). These differences reflect variations in the types and variability of carbohydrate and other reserves between cuttings from different positions (Hartmann *et al*., 1990; Leakey, 1990). The experiments reported here indicate that the percentage rooting of basal cuttings in three *Santalum* taxa declined. Leakey & Mohammed, (1985) reported that in *Triplochiton scleroxylon* rooting percentage was also found to decline in basal cutting.

Leakey & Mohammed (1985) reported a correlation between cutting diameter and cutting length, where thicker and longer cuttings rooted better than did shorter and thinner ones, perhaps because larger cuttings contain more starch in the stem than do thin cuttings. The lack of sufficient starch may cause the mortality of thin cuttings before they get a chance to root (Hartmann *et al*., 2002). Nevertheless, sometimes thinner-stemmed cuttings also root better (Howard & Ridout, 1992).

Nutrients and metabolites are required for optimal growth for both the mother plant and the cuttings. The metabolic process in stem cuttings takes place in the retained leaves (Wiessman-Ben & Tchoundjeu, 2000). In addition to this, their presence also has a considerable influence on rooting of cuttings, because of their ability to produce endogenous auxins, carbohydrates by means of photosynthesis and their influence on water status of the cuttings (Leakey & Coutts, 1989; Hartmann *et al*., 1990; Newton *et al*., 1992; Ofori *et al*., 1996).

4.3.5 Effect of ages of seedling

Central to this theme is the physiology of ontogenetic maturity or the progress from juvenility to maturity. Rooting capability, a juvenile trait, decreases with increasing maturity (Hackett 1985, 1988). Cuttings from mature stock are often difficult to root and, if rooted, may exhibit slow, plagiotropic growth (Hartmann *et al.*, 1997). In this experiment with sandalwood, the rooting percentage of the three *Santalum* taxa was influenced by the two different seedling ages (i.e. 6-12 and 12-24 months) from which the cuttings were taken. A slightly higher rooting percentage was recorded in seedlings aged 6 -12 months than in those aged 12-24 months (figure 4.5 A, B and C).

Ontogenetic maturity, the adult phase of development capable of reproduction, is attained with increasing distance from the base of the tree (Hartmann *et al*., 1997).

Therefore, vegetative propagation problems associated with mature stock can be eliminated or significantly reduced if cuttings are obtained from juvenile (typically basal) tissues within the tree (Hackett 1985).

Decreasing rooting potential with increasing distance from the ground was found in rooting trials of cuttings collected from varying positions in the crown of Douglasfir (*Pseudotsuga menziesii* (Black 1972), teak (*Tectona grandis*) (Nautiyal *et al.* 1992), and sheoak (*Casuarina equisetifolia*) (Venkataramanan *et al.* 1998) but it is dependent on genotype (Peer and Greenwood 2001).

In this study, the cuttings produced from Experiment 1 Chapter 3 produced better rooting when cuttings were taken from juvenile parts of the plant. When taking shoot cuttings from the same stock plant at the same time some cuttings may have different rooting ability. While a cutting may be young in growth age it may be old relative to the stem from which the cutting is taken.

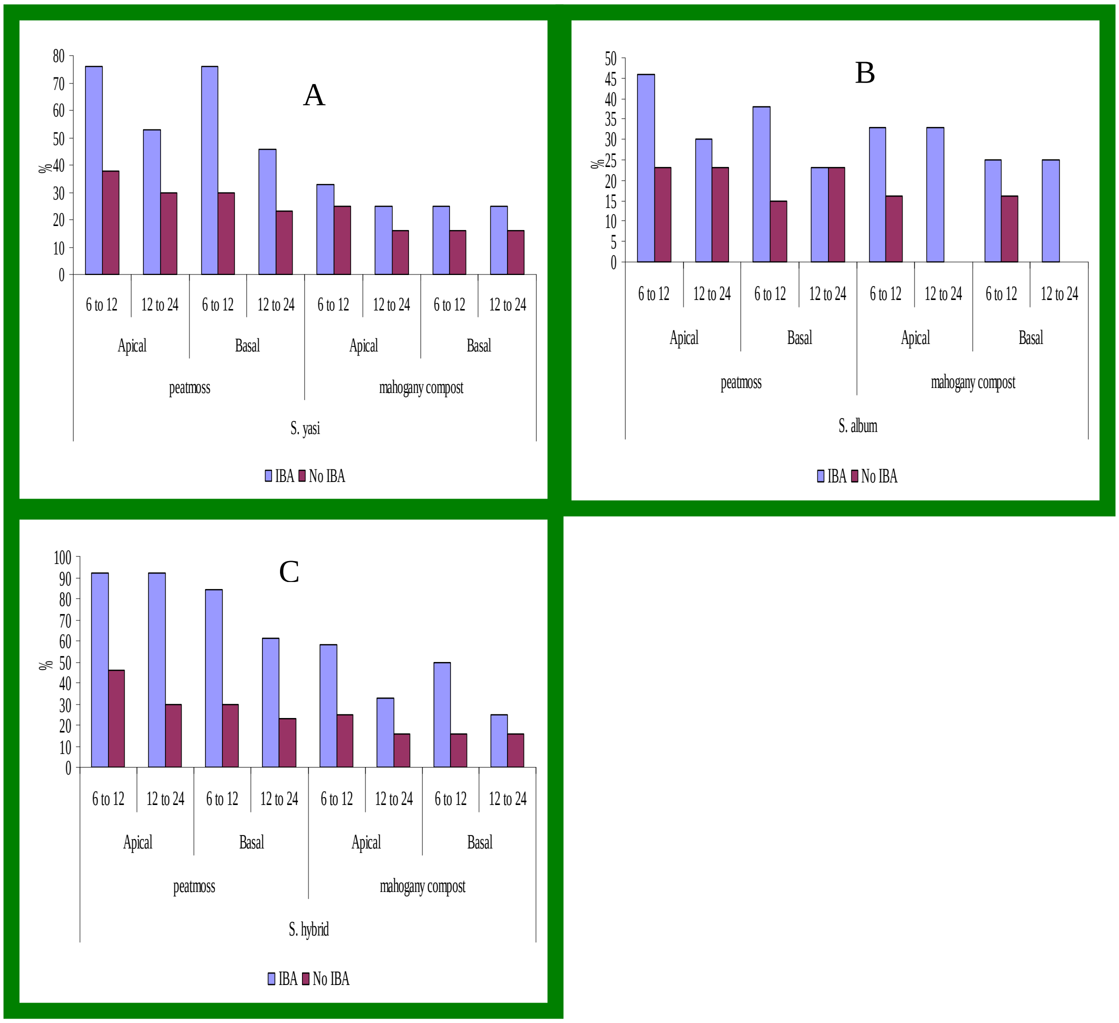


Figure 4.5 The different percentage of three sandalwood taxa showing the proportions of total rooted cuttings attributed to each treatment (i.e. cutting position, medium and seedling age).

4.4 Conclusion and summary

The results of these experiments indicated that stem cuttings on a mist bed could be used to propagate sandalwood successfully. Vegetative propagation of sandalwood was affected by factors such as the cutting position, rooting medium, rooting hormone and age of the seedling from which the cutting was taken. Cuttings started to develop roots after 24 weeks and rooting increased up to 27 weeks after setting. This result shows the importance of allowing cuttings sufficient time for rooting in the mist bed for successful propagation.

The results suggest that apical cuttings of sandalwood propagated in peatmoss with rooting hormone could attain vegetative propagation in 24 weeks since root number was the most important factor for successful establishment of the cutting.

CHAPTER 5

SURVIVAL AND SUBSEQUENT ROOT DEVELOPMENT OF TRANSPLANTED CUTTINGS SUBJECTED TO DIFFERENT TREATMENTS

5.0 INTRODUCTION

It is important to record the survival of rooted cuttings after the development of adventitious roots (Hartmann & Kester, 1983). Cuttings of many plant species can develop roots but some do not survive after rooting. The reason for this could be due to attack by microorganisms after rooting, or due to the roots' failure to adapt to the field environment (Hartmann & Kester, 1983; Wassner & Ravetta, 2000). The survival ability of apical and basal rooted stem cuttings of the three *Santalum* taxa was investigated following transplanting from the second experiment (Chapter 4).

5.1 Materials and methods

The study was carried out at the Colo-i-Suva nursery of the Fiji Forestry Department during 2006. An experiment was conducted to evaluate the success of rooting, transplanting survival and subsequent root development of rooted cuttings. In this experiment rooted cutting of the three *Santalum* taxa were taken from uniform, healthy and vigorous rooted cuttings produced during Experiment 2 (Chapter 4). The experiment was divided into two, and arranged according to the husbandry requirements (Plate 1.0). For experiment 'A', the transplanted rooted cuttings treated with NAA (age 6 to 12 months) were used. For experiment 'B', rooted cuttings treated with or without IBA (age 6 to 12 months) were used.

All cultural practices were the same during the entire period of study, and data on the number of roots was collected by using standardized procedures. Between 10 and 25 rooted cuttings of best appearance from each treatment of Experiment 2 were selected and transplanted into black polyethylene bags filled with 50:50 sand/peatmoss mix or 50:50 sand/mahogany compost mixes. The transplanted cuttings were left in the mist house site for one week before they were moved to an open area in order to allow them time to recover from the shock of transplanting. The experiment on *Santalum* taxa rooting with respect to the time when the cuttings were taken (Chapter 4) showed that successful extended rooting started 30 days after transplanting. During this period, fungicide was applied as foliar spray to prevent cuttings from becoming fungally infection.

5.1.0 Statistical tests

At six weeks after transplanting, the cuttings were re-assessed to determine the length of new roots on the cuttings. Growth and development indices were made by carefully separating the cuttings from the potting medium. Summary statistics were calculated, and the data were subjected to logistic regression to determine whether root length had extended or not. All analyses were performed using Statistical Package for the Social Sciences (SPSS).

5.2 Results

Experiment 'A'- The transplanted rooted cuttings from NAA (i.e nil mg/ L^{-1},0.5 mg/ $L^{-}$and 1.0 mg/ L^{-}) and set in two different media (50:50 sand:peatmoss and 50:50 sand:mahogany compost).

The rooting ability of *Santalum* taxa was sensitive to cutting position from the stock plant. Rooted cuttings of three *Santalum* taxa treated with NAA at concentration of 1.0 mg/ L^{-1} generally recorded longer roots than did those rooted cuttings treated with NAA concentrations of 0 mg/ L^{-1} and 0.5 mg/ L^{-1} (Table 5.0). The summarised statistic test on Table 5.0 reveal three sets of similar results. This could be due to changes in the proportions of media from experiment two (where 30:70 sand:peatmoss and 30:70 sand:mahogany compost were used) to this experiment three were 50:50 sand:peatmoss and 50:50 sand:mahogany compost were used. This change could have affected the mean root length. On the other hand the NAA concentrations higher then 1 mg/ L^{-1} blocked root elongation (Leakey & Mohammed, 1985).

Table 5.0 Summary statistic test (logistic regression) of mean root length (mean ± standard error of the mean) of transplanted rooted cutting of three sandalwood treated with different levels of NAA and set in two different media.

Media	**NAA concentration**	**Mean root length (+/-S.E)**
S. yasi - peatmoss	0 mg/ L^{-1}	1.00±0.00
	0.5 mg/ L^{-1}	1.00±0.00

	1.0 mg/ L^{-1}	1.40±0.10
S. yasi -mahogany compost	0 mg/ L^{-1}	1.30±0.10
	0.5 mg/ L^{-1}	1.00±0.00
	1.0 mg/ L^{-1}	1.30±0.10
S. album - peatmoss	0 mg/ L^{-1}	1.00±0.00
	0.5 mg/ L^{-1}	1.00±0.00
	1.0 mg/ L^{-1}	1.30±0.10
S. album- mahogany compost	0 mg/ L^{-1}	0.80±1.00
	0.5 mg/ L^{-1}	1.30±0.30
	1.0 mg/ L^{-1}	1.40±0.10
Santalum hybrid - peatmoss	0 mg/ L^{-1}	1.30±0.10
	0.5 mg/ L^{-1}	1.50±0.20
	1.0 mg/ L^{-1}	1.50±0.10
Santalum hybrid - mahogany compost	0 mg/ L^{-1}	1.40±0.10
	0.5 mg/ L^{-1}	1.10±0.10
	1.0 mg/ L^{-1}	1.40±0.10

Even though there were no significant differences in root development between the media, growth differences in the systems were observed due to the prior use of NAA for *S. album* (Table 5.1). There was an indication that cuttings planted or propagated in mahogany compost tended to grow coarser and longer roots, whereas cuttings from the peatmoss grow fine and highly branched roots.

Table 5.1 Results of logistic regression of medium and concentration of NAA on length of rooted cutting of the three *Santalum* taxa.

Source of variation	**Wald statistic**	**DF**	**Significance**
S. yasi			
Medium	0.296	1	0.586
Rooting hormone (NAA)	2.854	1	0.091
S. album			
Medium	2.653	1	0.103

Rooting hormone (NAA)	6.643	1	0.010
Santalum hybrid			
Medium	1.223	1	0.269
Rooting hormone (NAA)	0.243	1	0.622

Experiment 'B'. The transplanted rooted cuttings treated with or without IBA and set in two different media (50:50 sand:peatmoss and 50:50 sand:mahogany compost).

Results showed that the prior use of rooting hormone influenced further rooting in *S. yasi* and the *Santalum* hybrid (*S. yasi x S. album*) (Table 5.2 and 5.3) whilst no effect due to the rooting medium was observed. However, when mahogany compost and river sand was used as a medium, the cuttings had well branched, slender and flexible root that were much more suitable for transplanting into the field (Table 5.2). This difference in rooting system or in the growth of the adventitious roots was related to the moisture holding capacity of the media.

This experiment indicated that apical cuttings of three *Santalum* taxa could be propagated successfully using mahogany compost, and few roots are longer in the absence of IBA. This result could be probably because the wrong concentration can inhibit rooting or it can act as a growth retardant when applied in high concentrations (Table 5.2).

Table 5.2 Summary statistics of mean root length (mean ± standard error of the mean) of transplanting rooted cutting for apical cuttings with and without IBA for seedlings aged 6 to 12 months.

Media	**Hormone**	**mean root length (+/-S.E)**
S. yasi - peatmoss	Without IBA	1.10±0.10
	With IBA	1.50±0.10
S. yasi - mahogany compost	Without IBA	1.10±0.10
	With IBA	1.50±0.20

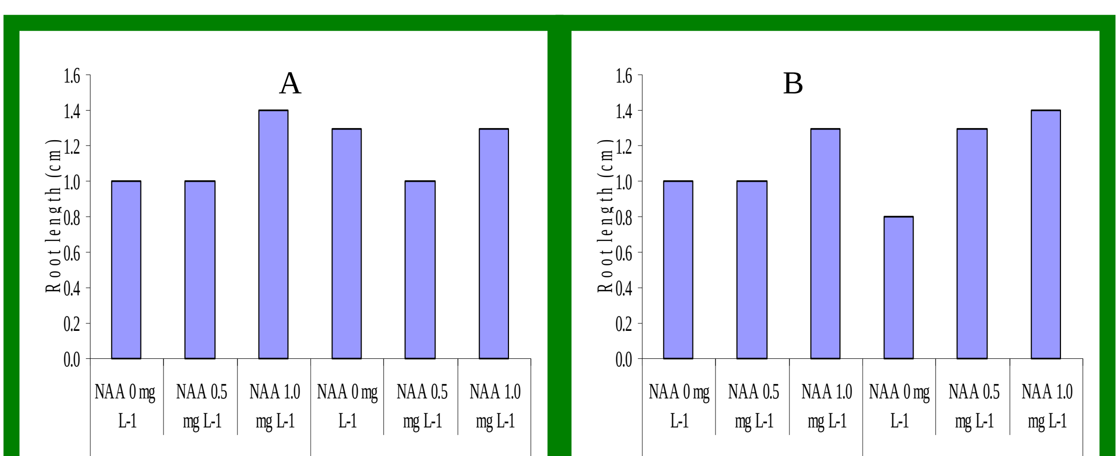
S. album - peatmoss	Without IBA	1.20±0.10
	With IBA	1.50±0.10
S. album - mahogany compost	Without IBA	0.50±0.00
	With IBA	1.40±1.00
Santalum hybrid - peatmoss	Without IBA	1.50±0.10
	With IBA	1.10±0.00
Santalum hybrid - mahogany compost	Without IBA	1.10±0.10
	With IBA	1.50±0.10

Table 5.3 Summary statistics test (logistic regression) of medium, apical position and without and with IBA on length only of rooted cutting of the three *Santalum* taxa.

Source of variation	Wald statistic	DF	Significance
S. yasi			
Medium	0.274	1	0.600
Rooting hormone (IBA)	4.858	1	0.028
S. album			
Medium	0.006	1	0.936
Rooting hormone (IBA)	0.459	1	0.498
Santalum hybrid			
Medium	0.001	1	0.977
Rooting hormone (IBA)	16.937	1	0.000

5.3 Discussion

Transplant survival of rooted cuttings is the second step after the development of adventitious roots by cuttings. The survival ability of rooted cutting of three *Santalum* taxa stem cuttings is not known. Therefore, the aim of this study was to investigate the survival ability of only apical stem cuttings that were propagated in peat moss:river sand and in mahogany compost:river sand with or without IBA rooting hormone and different level of NAA (i.e., 0 mg L^{-1} , 0.5 mg L^{-1} and 1.0 mg L^{-1} (Figure 5.0).



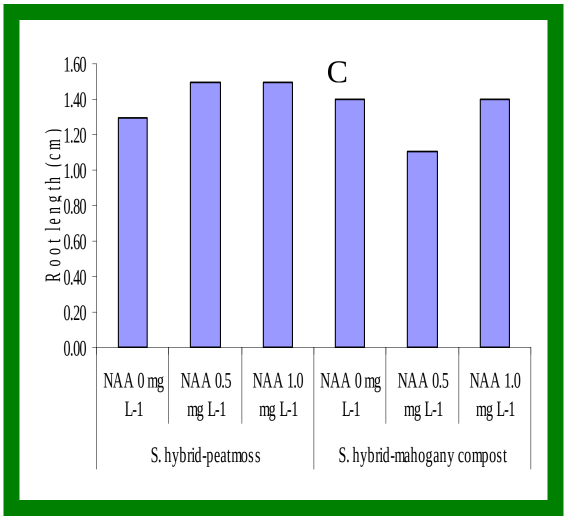


Figure 5.0 The mean root length of transplanted rooted cuttings treated with different level of NAA and set in two different media.

Survival of rooted sandalwood stem cuttings followed a similar pattern to that of rooting percentage of the cuttings. Like rooting percentage, survival ability of rooted apical and basal cuttings was also influenced by the source of the cuttings in Experiment 2. In that experiment, more apical cuttings survived than did the basal cuttings. However the use of different media (peatmoss or mahogany compost) during the propagation period did not influence the survival of cuttings.

As far as mortality is concerned, it was minimum after transplanting. The use of rooting hormones positively influenced the survival rate. Except in a few cases (tables 5.0, 5.2; fig 5.0) the results pertaining to root length revealed significant superiority. Root length of *S. album* showed highly significant differences ($P<0.001$) on rooting hormone and seedlings (i.e. 6 to 12 month old).

In woody plants this difference in rooting due to cutting position can be related to the difference in the chemical composition of the shoots (position where the cuttings were taken: apical or basal), age of the stem, to carbohydrate accumulation, or to bud growth (basal cuttings especially may have root-promoting substances from buds and leaves). If this is the case the best rooting material in such plants is the basal cuttings. In deciduous species where no carbohydrate or root promoting substances is present, cuttings from the soft shoot of the plant root best (Hartmann *et al.*, 1997). Many authors (Leakey, 1983; Hansen, 1986, 1988; Jawanda *et al.*, 1991; Hartmann *et al.*, 1990; Al-Saqri & Alderson, 1996; Hartmann *et al.*, 1997) also reported results in agreement with the general statement of Hartmann & Kester (1983) that the best rooting of cuttings usually comes from the basal portions of shoots.

On the other hand, successful transplanting of rooted cuttings of three *Santalum* taxa was affected by cutting position. This difference between apical and basal cuttings could be due to higher concentrations of endogenous root promoting substances in the apical cuttings which arise from the terminal buds and also “more cells” which are capable of becoming meristematic (Hartmann & Kester, 1983). These factors helped the apical cuttings to produce more and longer roots as compared to basal cutting, which in turn resulted in higher survival percentage of the apical portion.

Cuttings treated with hormone during the propagation (or initial rooting) period showed a continuing beneficiary effect ($P<0.001$) in terms of increased length of roots after transplanting. Cuttings propagated with IBA rooting hormone produced longer roots than did cuttings with no hormone. Similarly, longer roots were developed in the period after transplanting from cuttings propagated with hormone treatment than did those not treated with hormones. On the other hand, via visual observation, cuttings transplanted with already well-developed root systems established more successfully and grew vigorously compared to those that had fewer adventitious roots. Many of the cuttings transplanted with fewer adventitious roots failed to survive.

Differences in media and hormone treatment did not directly affect survival of the transplanted cuttings.Although the medium affected root length in mahogany compost, the difference in root length due to media was not significant. As stated in Chapter 3 peatmoss had a higher moisture holding capacity than did mahogany compost and according to Grange & Loach (1983) and Loach (1992) the water uptake of cuttings is directly related to the water content of the medium and moisture is one of the most important factors in rooting success of cuttings.

Application of hormone (IBA or NAA) resulted in longer roots before and after transplanting. Palakill & Feldman (1993) also reported increased root lengths after transplanting because of the successful growth of the rooted cuttings. Berhe & Negash (1998) reported similar results in *Podocarpus falcatus* when plants propagated by cuttings survived well due to their greater root number. The same was also true for *Juniperus procera* where cuttings with more roots showed better growth and establishment than did those with fewer adventitious roots (Berhe & Negash, 1998).

5.4 Conclusions and Summary

This experiment showed that transplanting survival of three *Santalum* taxa was affected by original cutting position, seedling age, presence or absence of IBA (Figure 5.1) and NAA (Figure 5.0) and medium. Rooted apical cuttings had a higher survival percentage following transplanting in all three *Santalum* taxa. Similarly, cuttings with well-developed adventitious root systems established more easily and successfully than did those with few roots. Therefore, successful transplanting survival of rooted cutting of sandalwood can be attained with apical cuttings position, irrespective of medium, seedling age, different levels of NAA and use of IBA. Transplanting cuttings with a higher number of roots will ensure more successful establishment. In addition, this investigation confirmed that basal cuttings taken from younger seedlings (6-12 months age) with longer roots could be obtained by propagating them in mahogany compost and treating them with IBA and NAA hormone.

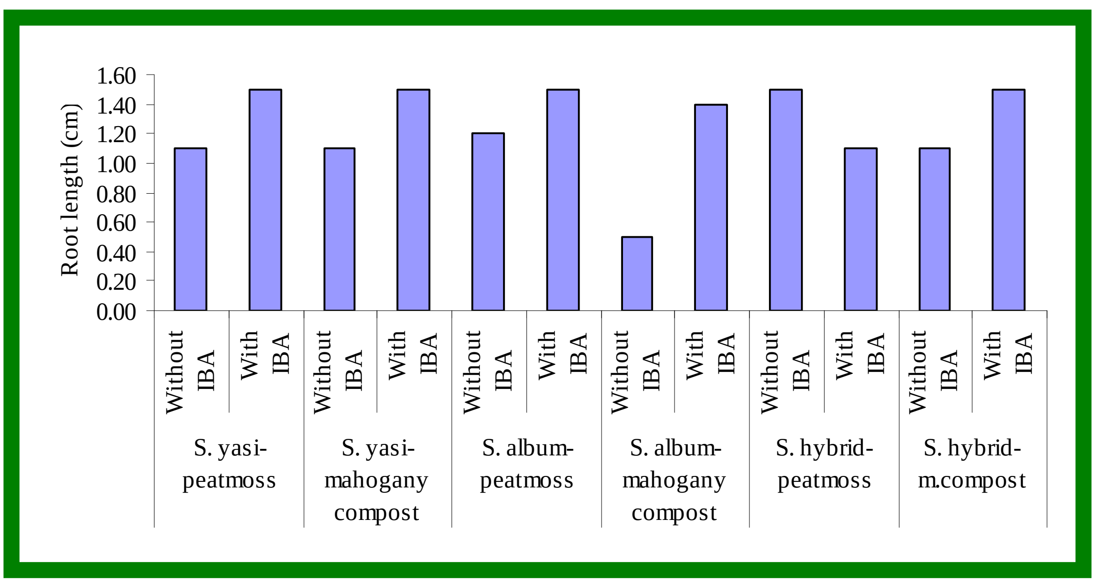


Figure 5.1 The mean root length of transplanting rooted cuttings treated with and without IBA and set in two different media.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

6.0. General discussion and conclusion

This study investigated vegetative propagation of three *Santalum* taxa, including treatment of major roots to induce shoots for cuttings, effects of different treatments on rooting in vegetative cuttings and effect of treatments on survival of transplanted rooted cuttings. The experiments were conducted at the Siliviculture Forestry Division (SFD) mist house, and *Santalum* experimental plots at Colo-i-Suva and Vunimaqo (Viti Levu, Fiji).

The treatments investigated included:

- Effect of diameter and length of severed and injured root from the trunk on shoot induction,
- Effect of cutting position (apical vs. basal), media (30:70 sand:peatmoss vs. 30:70 sand:mahogany compost), response of basal and apical cuttings to IBA hormone and different levels of NAA on root induction in cuttings from seedlings of different ages; and
- Effect of treatments on survival of rooted cuttings following transplanting.

Induction of root suckering offers much potential for the large-scale production of clonal shoots for cutting material of *Santalum* taxa. Root sucker induction is a useful feature in sandalwood (and especially mature trees), and potentially enables phenotypically superior sandalwood trees to be cloned (e.g. producing disease-resistant clones, rapid growth, early formation of heartwood and increased oil and santalol contents). *Santalum album* has been shown to produce shoots from roots of mature trees, with a 92 % success rate achieved in a trial conducted at Kerala Forest Research Institute, India, with 25% success for subsequent striking cuttings from these sprouting shoots (Balasundaran, 1998). In the current study, which included three *Santalum* taxa, shoots developed on 9-64% of severed lateral roots (SLR) while 8-50% of injured primary lateral roots (PLR) formed shoots which formed an average? of 39% were able to strike roots in subsequent cuttings experiments

Vegetative propagation of *Santalum* taxa by stem cuttings is therefore feasible, at least for juvenile material. High strike rates may be able to be attained if the main factors that affect rooting are understood and optimised. Based on findings with other plant genera and limited observations on sandalwood, the most important factors affecting strike rate are likely to be cutting position, media, use of rooting hormone and seedling age. Differences in growing media generally did not show an effect on rooting percentage and root length but more roots were produced in a medium of 30:70 sand:peatmoss. However, fine highly branched roots were observed when cuttings were struck and grown in 30:70 sand:peatmoss but were coarser and longer when grow in 30:70 sand:mahogany compost .

The cutting type (apical or basal), media and hormone treatment (NAA and IBA) also affected survival of rooted cuttings following transplanting. Higher survival percentage was recorded with apical cuttings. This was due to their ability to produce more roots as well as longer roots, compared to basal cuttings. As might be expected, cuttings with a well-developed adventitious root system established more easily and successfully than did those with a poor root system. Therefore, successful field and transplanting survival of *Santalum* taxa can be achieved with apical cuttings and it is important to transplant cuttings with well developed root systems for successful establishment.

Rooting ability of basal cuttings can be improved when a suitable hormone concentration is applied. Cuttings of *Santalum* taxa responded to higher NAA concentrations in terms of root initiation and root number, perhaps because they contain high endogenous levels of polyphenols. Higher rooting percentage and more roots were obtained with NAA (1.0 mg L^{-1}) and with IBA. It is also important to consider the type of rooting and growing medium used.

As a result of the financial benefits realised from the recent exploitation of sandalwood in the Pacific Islands and better technologies for cultivating sandalwood, there is a renewed interest in the cultivation of *Santalum* taxa. The challenge now for the Forestry departments of Pacific Island nations and others in the private sector is to provide the technical support to keep this impetus going. This will require the development of simple and successful propagation and establishment techniques that can be implemented. Despite recent advances (including from results reported in this study) a considerable amount of research is still required to provide the information needed. Provided research is continued and the regeneration work is carried out to a good standard, the stocks of sandalwood in Pacific Island nations should increase markedly over the next few years. The main area of concern now is the continuing inadequate supply of good quality nursery stock and seed for the regeneration program. As there are few mother trees remaining there is an urgent need to establish special seed production areas. It may also be necessary to place a ban on the sale of *Santalum*, except for traditional purposes, in order to conserve the severely depleted genetic resources of the species.

6.1 Future work

Santalum taxon produces fragrant santalol-rich heartwood, which is amongst the most expensive in the world on a per kg basis. Large economic benefits of planting *Santalum* taxa are likely to be realized for Pacific island nations and their peoples if appropriate replanting management plans are put into place. These programs need to be supported by continuing research on *Santalum* propagation and improvement such as this vegetative propagation study. It is clear from the findings that propagation of *Santalum* taxa by vegetative cuttings is difficult, and more research is needed on the factors that affect root initiation. For the time being a suitable approach is to fine tune and improve on the new methods of vegetative propagation of *Santalum* taxa. In future it may prove more efficient to develop tissue culture techniques and somatic embryogenesis, practices which are currently being investigated by the private sector in Vanuatu. Recommendations are given below which will hopefully lead to a more appropriate plan for further research and development and extension of conventional vegetative propagation practices of *Santalum* taxa, the latter of which is particularly important for prospective sandalwood farmers on outer islands.

The recommendations given below are brief and not intended to be exhaustive. They are based on the findings and conclusions from this study and from other studies such as Thomson and Uwamariya (2001), Rao and Srimathi (1976), Uniyal *et al.*, (1985), Vijatakumar *et al.*, (1981), Lakshmi Sita (1986), Frank McKinnell, Andrew Rado and co-workers in Western Australian and Indonesia during the early 1990s, Walker (1998- 99), Walker *et al*., (1999). Doran *et al*., (2002) revealed that over the past 10-15 years there has been considerable research into aspects of propagation and plantation silviculture of *Santalum* taxa, notably in Australia, New Caledonia, Indonesia and India, but much remains unknown. For certain Pacific *Santalum* taxa such as *S. macgregorii*, *S. yasi* and *S. insulare* research and development work has only recently commenced and there is little information about their silviculture and ecology both in native and planted stands. Intensive study of the resources, nursery techniques, plantation and silviculture of *Santalum insulare* (var. *marchionense* and var. *deckeri*) in French Polynesia commenced in 1998. The Forestry Departments in Vanuatu and Fiji have indicated a high priority for

Santalum work in phase 2 of SPRIG, while the PNGFRI/CSIRO have recently begun various Research and Development (R&D) activities on *S. macgregorii*.

For most *Santalum* taxa research is urgently needed on vegetative propagation by stem cutting techniques, including experiments on the effect of season at which cuttings are collected (in different Pacific Islands) and different misting propagator/bottom heat systems. There is a need to improve knowledge of the ecology of *Santalum* taxa in natural stands, and especially regeneration of *Santalum* taxa following harvesting and at other times-e.g. identify factors critical to successful regeneration. In root-suckering species there is a need to investigate *Santalum* taxa coppicing versus extraction of the whole tree including root systems, to determine if the conservation benefits of the former approach might compensate for lost income (due to incomplete harvesting). Villagers would also benefit from research to identify simple features or methods that can be used to indicate when a sandalwood tree is ready for harvesting; i.e. when substantial heartwood development has taken place. This is because heartwood formation can vary considerably between individual plants, even of the same size/diameter growing in the same area.

In undertaking the current study previous research on vegetative propagation of *Santalum* taxa was found that appeared to be based on a few observations rather than from detailed, appropriately designed studies. Accordingly, there is a need to reassess the status of this species and knowledge of appropriate cultural practices more accurately. Clearly, future work is needed to maximize the rooting success of cuttings.

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APPENDIX 1.0

Table 1.0. *Santalum* species of commerce (after Coppen 1995)

Species	Common name	Country	Product[1]
S. album L.	East Indian sandalwood, chandal	India, Indonesia, Australia	W,O
S. austrocaledonicum Vieillard	sandalwood	Vanuatu, New Caledonia	W,O
S. ellipticum Gaudichaud	Hawaiian sandalwood	Hawaii (USA)	W
S. insulare Bertero	sandalwood	French Polynesia, Pitcairn Islands, Cook Islands	W
S. lanceolatum R. Br.	northern sandalwood, plum bush	Australia	W,O
S. macgregorii F. Muell.	PNG sandalwood	Papua New Guinea	W,O
S. spicatum (R. Br.) A. DC.	Australian sandalwood	Australia	W,O
S. yasi Seeman	Fijian sandalwood	Fiji, Tonga	W,O

[1] W = wood, O = essential oil (Source: Coppen, 1995).

APPENDIX 1.1

Table 1.0 List of countries in Asia with natural populations of *Santalum album*

India: (Andhra Pradesh, Goa, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu, Uttar Pradesh) and Indonesia: (Lesser Sunda Islands).
(Source: Thomson and Uwamariya, 2001).

<u>Location of introductions</u>

Introduced populations occur in Central and Northern India (Srinivasan *et al*., 1992), Java, Bali, and the coastal areas of northern Australia, near Darwin (Barrett and Fox, 1995).

Experimental introductions have been performed in China (Li and Yu, 1984), Fiji (Bulai, 1995), New Caledonia (Chauvin, 1988), Hawaii (Merlin and VanRavenswaay, 1990), Tonga (Kaufusi, 1995), Papua New Guinea (Paul, 1990), Nepal (Neil, 1990), Sri Lanka (Tennakoon, personal communication), East Indonesia (Harisetijono and Suriamihardja, 1993) and North Queensland, Australia (Keenan, personal communication). Commercial irrigated plantations are currently being established near Kununurra, northern Western Australia (Radomiljac *et al*.,).

It is reported that *S. album* was introduced to Kenya, Nigeria, Zimbabwe, Tanzania and Uganda with varied success (Streets, 1962). There is no recent published literature on these introductions.

<u>Table 1.1 List of countries where *S. album* has been planted</u>

Asia
Bangladesh
China: (Guangdong)
India: (Delhi, Himachal Pradesh and West Bengal)
Indonesia: (Java, Kalimantan, Lesser Sunda Islands, Moluccas and Sulawesi)
Myanmar
Nepal
Pakistan
Sri Lanka
Africa: (Kenya, Nigeria, Tanzania, Uganda and Zimbabwe)
Western Hemisphere
USA: (Hawaii)
Oceania
Australia: (Australian Northern Territory, Queensland, Western Australia
Cook Islands
Fiji, New Caledonia, Papua New Guinea,Tonga andVanuatu (Source: Thomson and Uwamariya, 2001).

APPENDIX 1.3 Table 1.0 Total mortality rate of cuttings of three *Santalum* taxa.

Summary data of roots produced from apical and basal cutting and the motality number.

		Summary data of rooted cutting					
Species	Number of treatment	Tray No.	Apical	Basal	Total cutting	without root	Dead cuttings (mortality)
S.yasi	With IBA juvenil shoot/root	1	19	15	34	6	10
	Without IBA juvenil shoot/root	2	7	5	12	10	28
S.album	With IBA juvenil shoot/root	3	13	11	24	9	13
	Without IBA juvenil shoot/root	4	6	6	12	7	31
S.hybrid	With IBA juvenil shoot/root	5	20	17	37	6	7
	Without IBA juvenil shoot/root	6	8	8	16	10	24
S.yasi	nil mg NAA juvenil shoot/root	7	6	3	9	8	33
	0.5 mg NAA juvenil shoot/root	8	9	7	16	11	23
	1.0 mg NAA juvenil shoot/root	9	18	14	32	10	8
S.album	nil mg NAA juvenil shoot/root	10	4	2	6	9	35
	0.5 mg NAA juvenil shoot/root	11	5	3	8	5	37
	1.0 mg NAA juvenil shoot/root	12	11	9	20	12	18
S.hybrid	nil mg NAA juvenil shoot/root	13	7	6	13	12	25
	0.5 mg NAA juvenil shoot/root	14	9	8	17	13	20
	1.0 mg NAA juvenil shoot/root	15	20	16	36	6	8
S.yasi	6-12 mths seedling with IBA	16	14	13	27	10	13
	6-12 mths seedling without IBA	17	8	6	14	18	18
S.album	6-12 mths seedling with IBA	18	10	8	18	22	10
	6-12 mths seedling without IBA	19	5	4	9	15	26
S.hybrid	6-12 mths seedling with IBA	20	19	17	36	7	7
	6-12 mths seedling without IBA	21	9	6	15	10	25
S.yasi	12-24 mths seedling with IBA	22	10	9	19	16	15
	12-24 mths seedling without IBA	23	6	5	11	13	26
S.album	12-24 mths seedling with IBA	24	8	6	14	16	20
	12-24 mths seedling without IBA	25	3	3	6	16	28
S.hybrid	12-24 mths seedling with IBA	26	16	11	27	11	12
	12-24 mths seedling without IBA	27	6	5	11	10	29
S.yasi	nil mg NAA seedling	28	7	6	13	13	24
	0.5 mg NAA seedling	29	5	4	9	14	27
	1.0 mg NAA seedling	30	12	11	23	18	9
S.album	nil mg NAA seedling	31	3	3	6	11	33
	0.5 mg NAA seedling	32	2	1	3	14	33
	1.0 mg NAA seedling	33	7	6	13	19	18
S.hybrid	nil mg NAA seedling	34	8	4	12	10	28
	0.5 mg NAA seedling	35	5	5	10	17	23
	1.0 mg NAA seedling	36	11	9	20	18	13
	Total cutting rooted		336	272	608	432	757

APPENDIX 1.4 The rooted cutting of three *Santalum* taxa in experiment 2

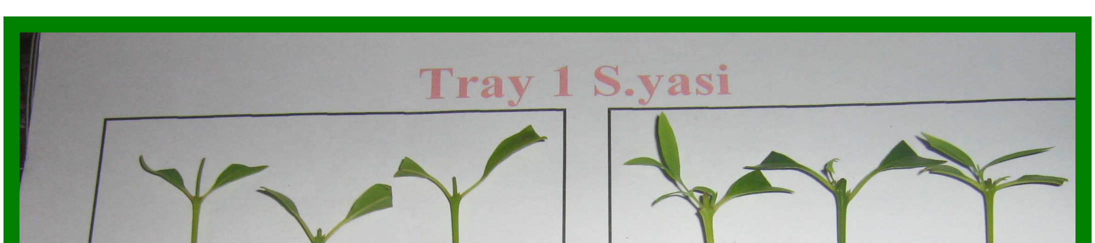


Plate 1.4.0 ***Santalum yasi*** **with IBA**

Plate 1.4.1 ***Santalum yasi*** **without IBA**

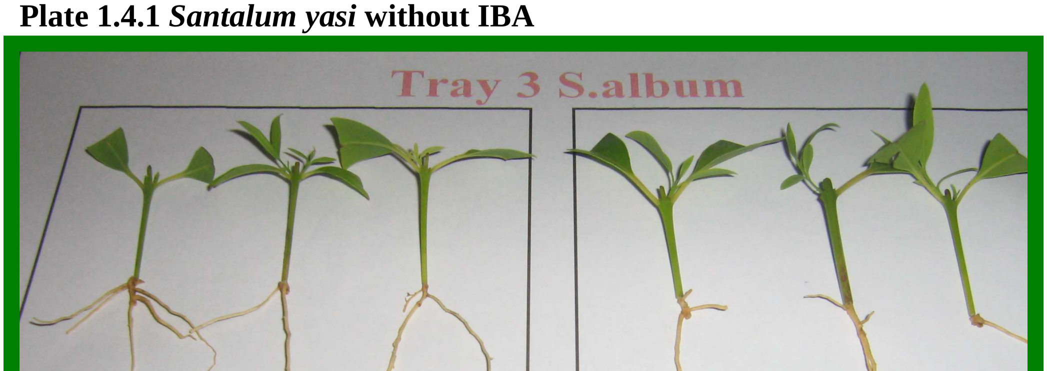


Plate 1.4.2 ***Santalum album*** **with IBA**

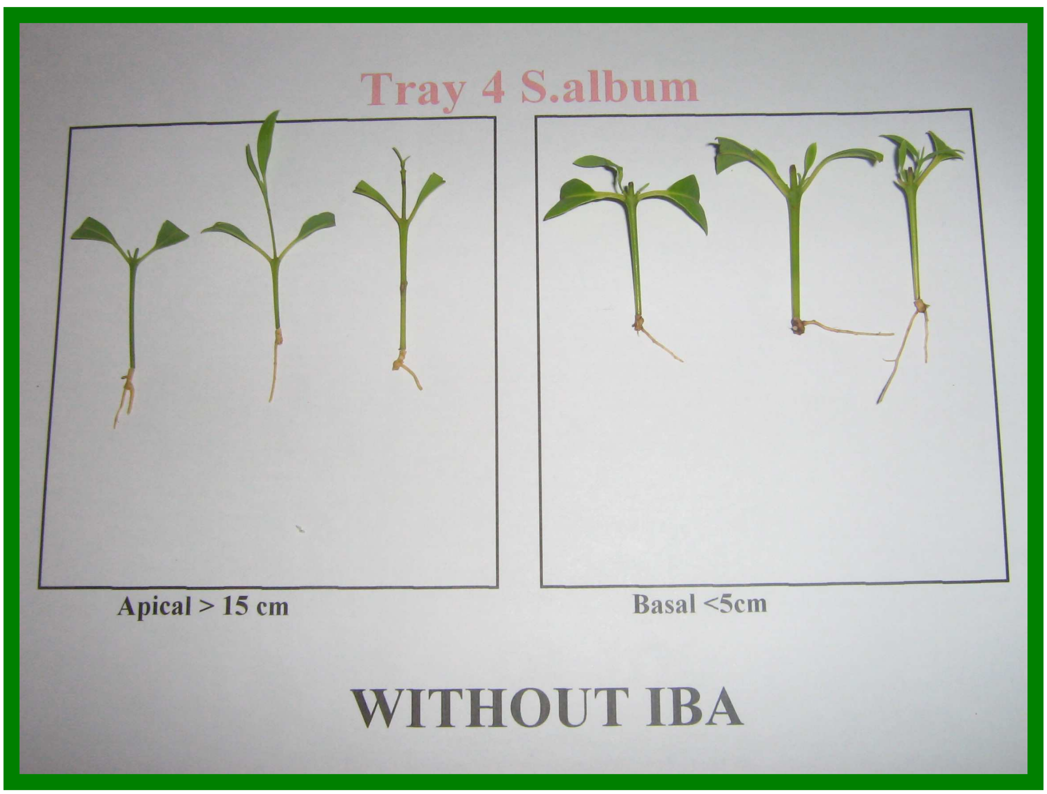


Plate 1.4.3 ***Santalum album*** **without IBA**

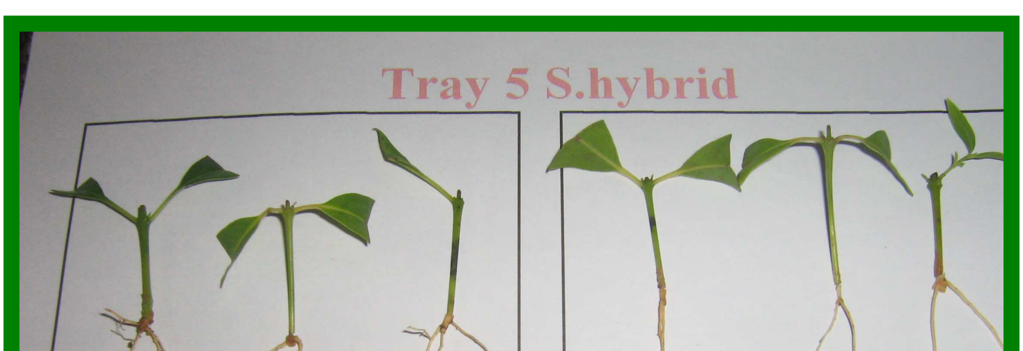


Plate 1.4.4 *Santalum* hybrid with IBA

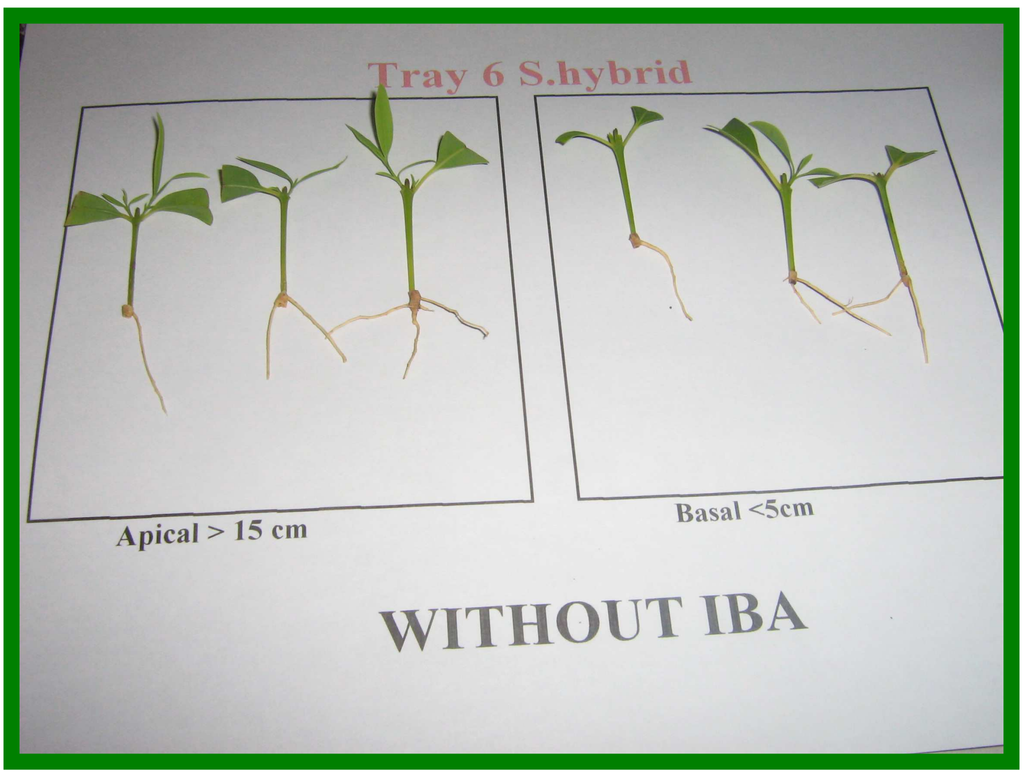


Plate 1.4.5 *Santalum* hybrid without IBA

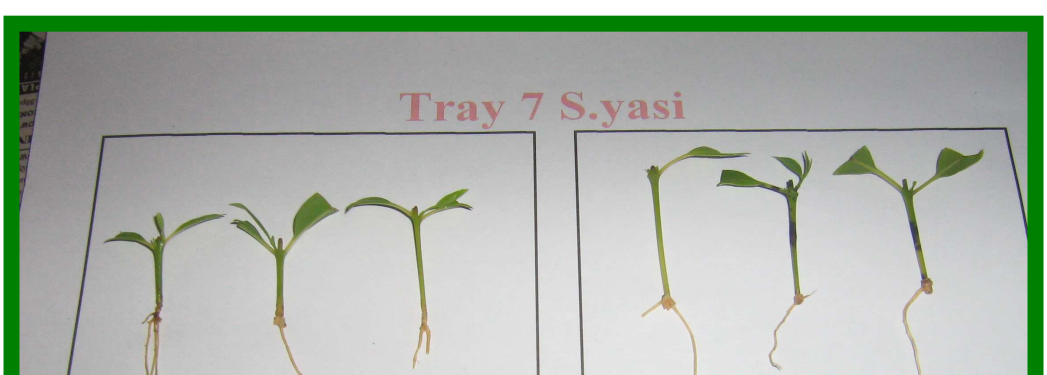


Plate 1.4.6 *Santalum yasi* NAA nil mg L^{-1}

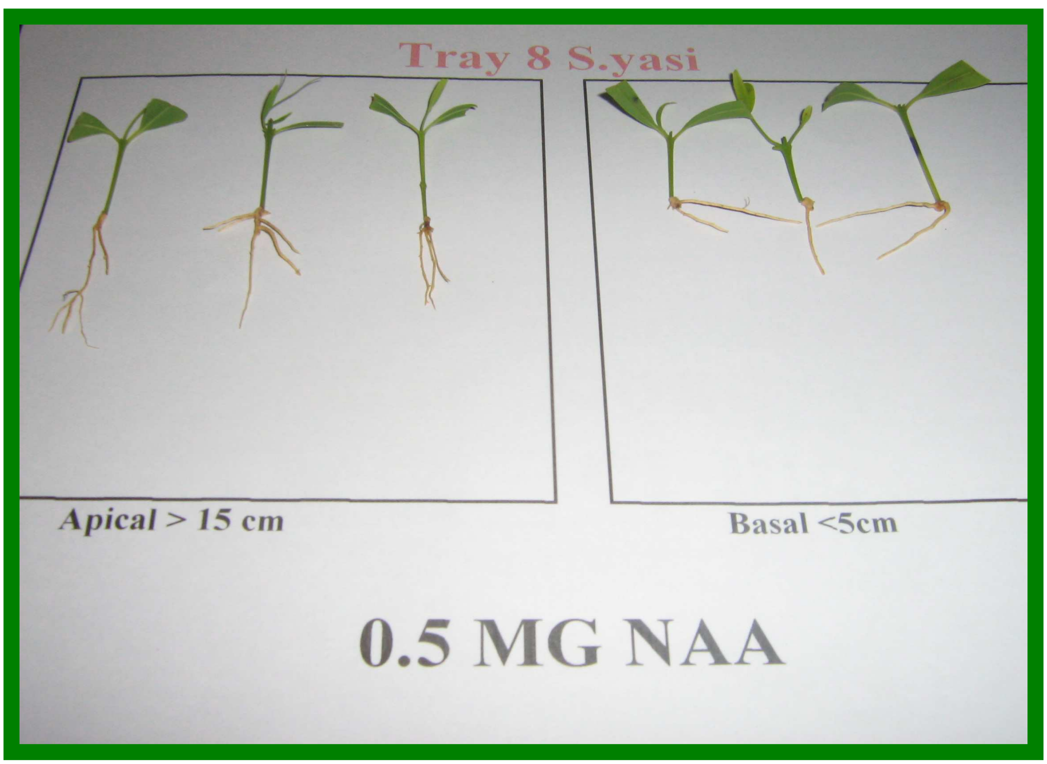


Plate 1.4.7 *Santalum yasi* NAA 0.5 mg L^{-1}

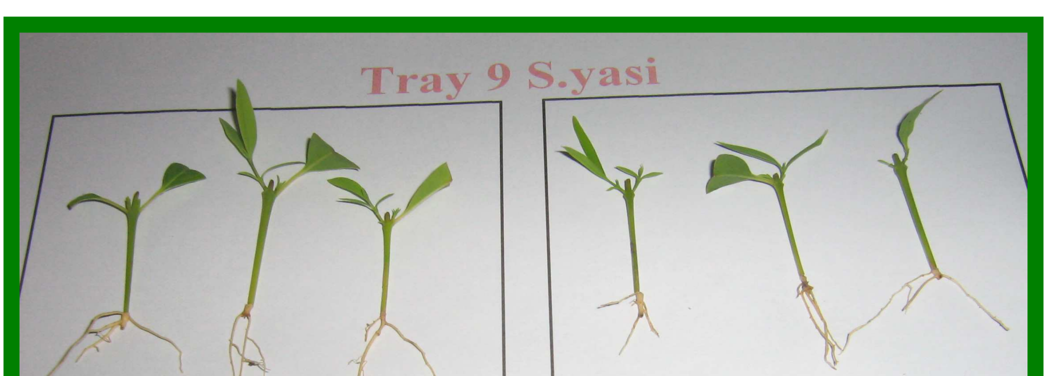


Plate 1.4.8 ***Santalum yasi*** **NAA 1.0 mg L^{-1}**

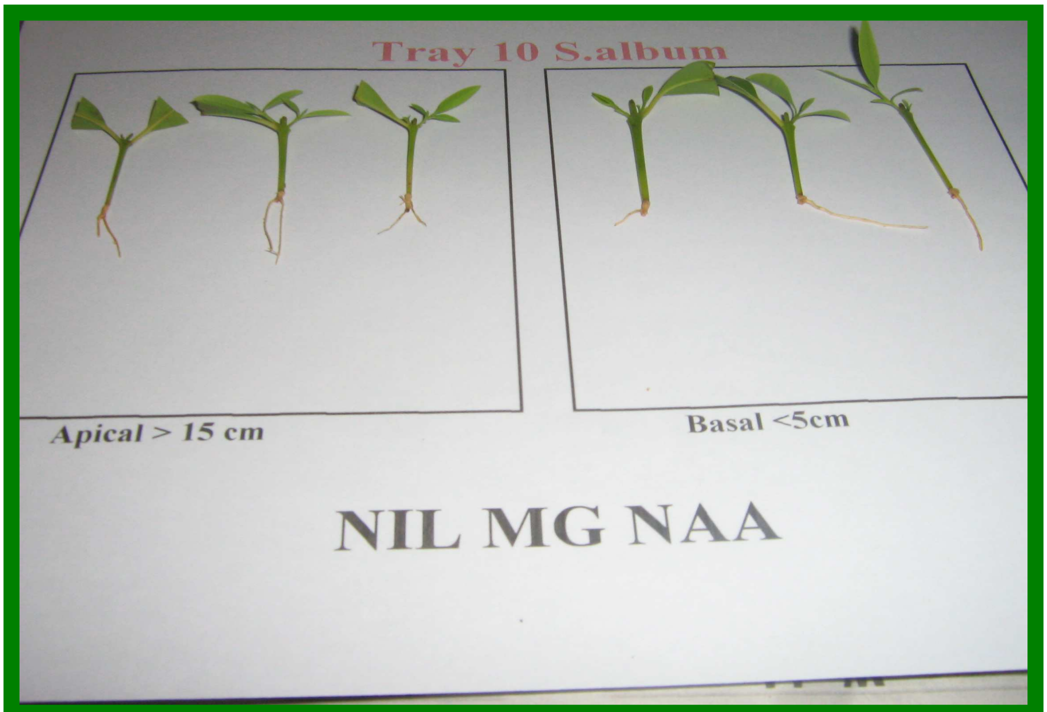


Plate 1.4.9 ***Santalum album*** **NAA 1.0 mg L^{-1}**

Plate 1.4.10 *Santalum album* NAA 0.5 mg L^{-1}

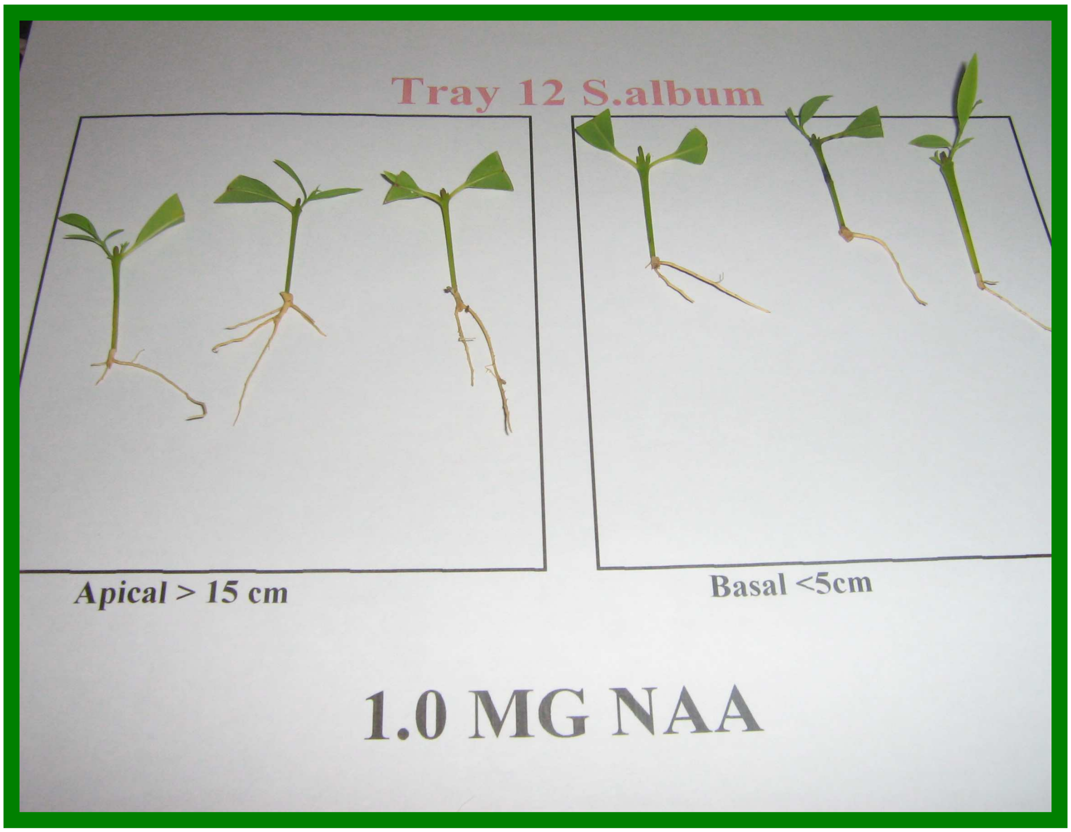


Plate 1.4.11 *Santalum album* NAA 1.0 mg L^{-1}

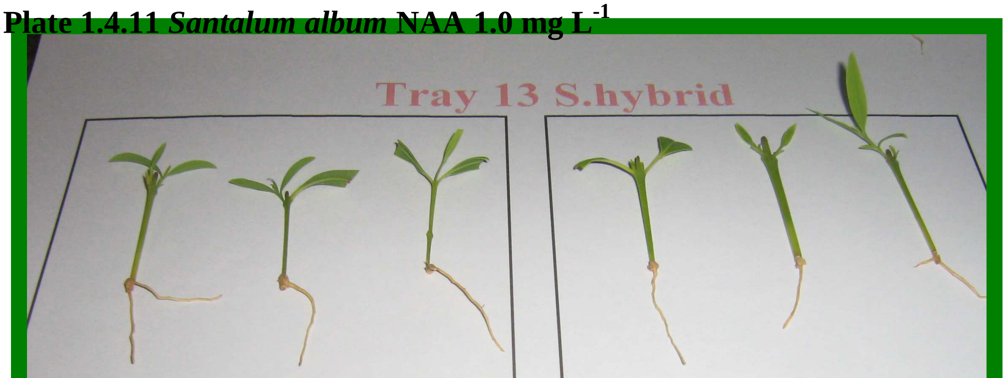


Plate 1.4.12 ***Santalum*** **hybrid NAA nil mg L^{-1}**

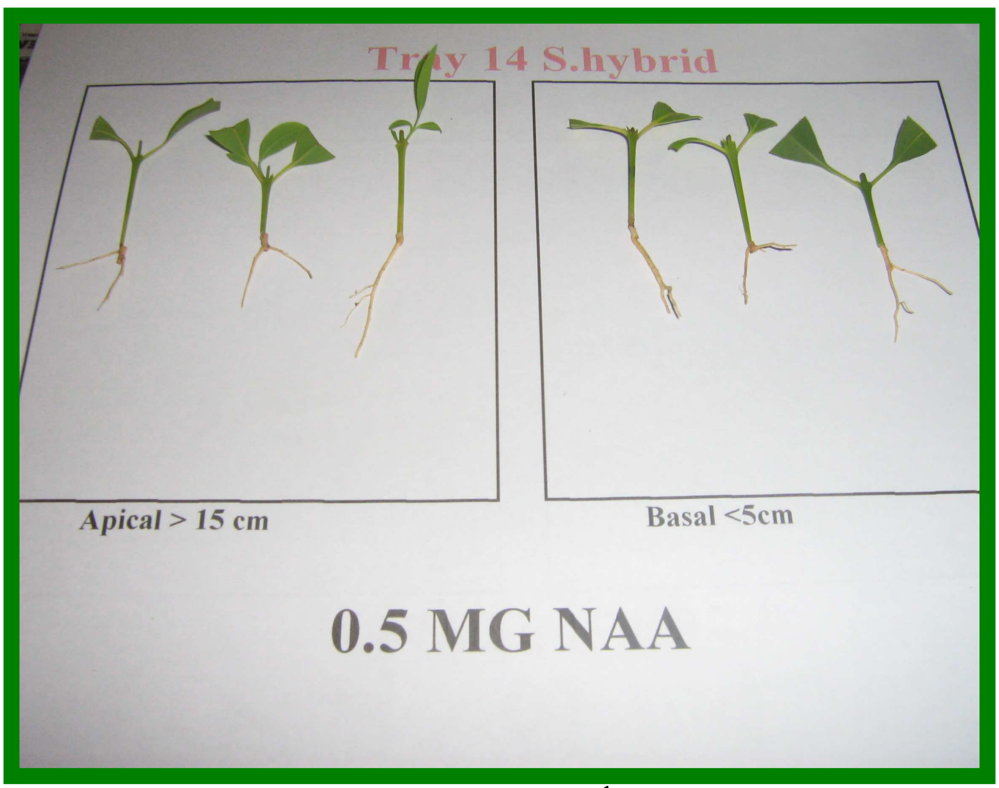


Plate 1.4.13 ***Santalum*** **hybrid NAA 0.5 mg L^{-1}**

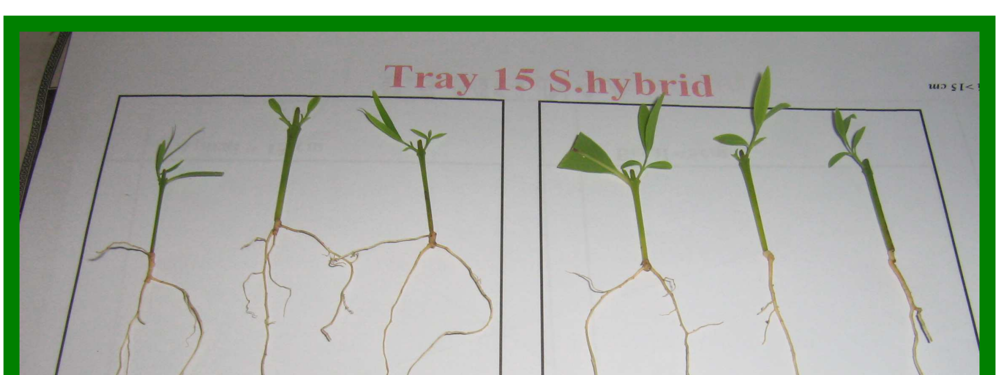


Plate 1.4.14 ***Santalum*** **hybrid NAA 1.0 mg L^{-1}**

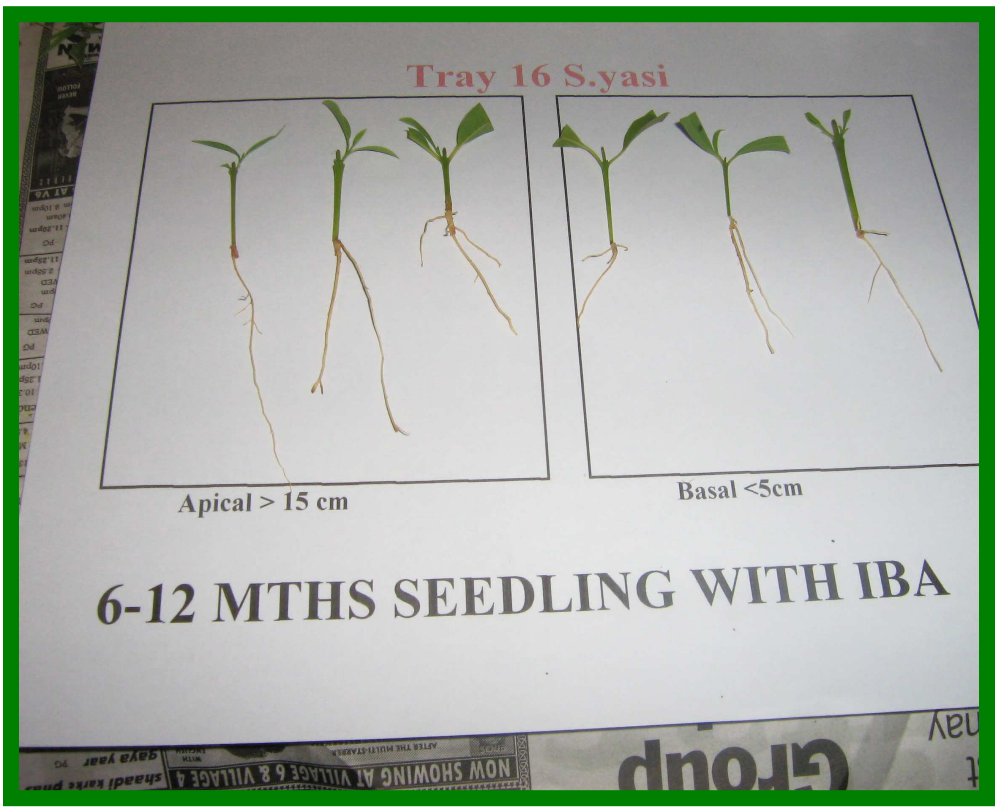


Plate 1.4.15 ***Santalum*** **yasi 6-12 month seedling with IBA**

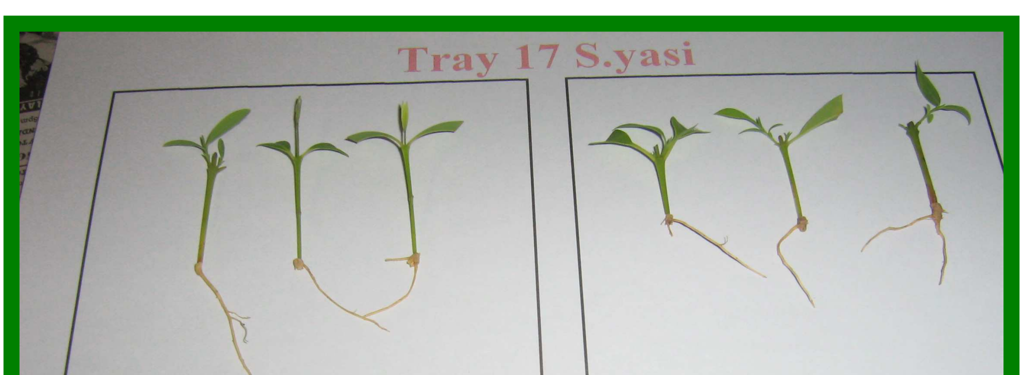


Plate 1.4.16 *Santalum* yasi 6-12 month seedling without IBA

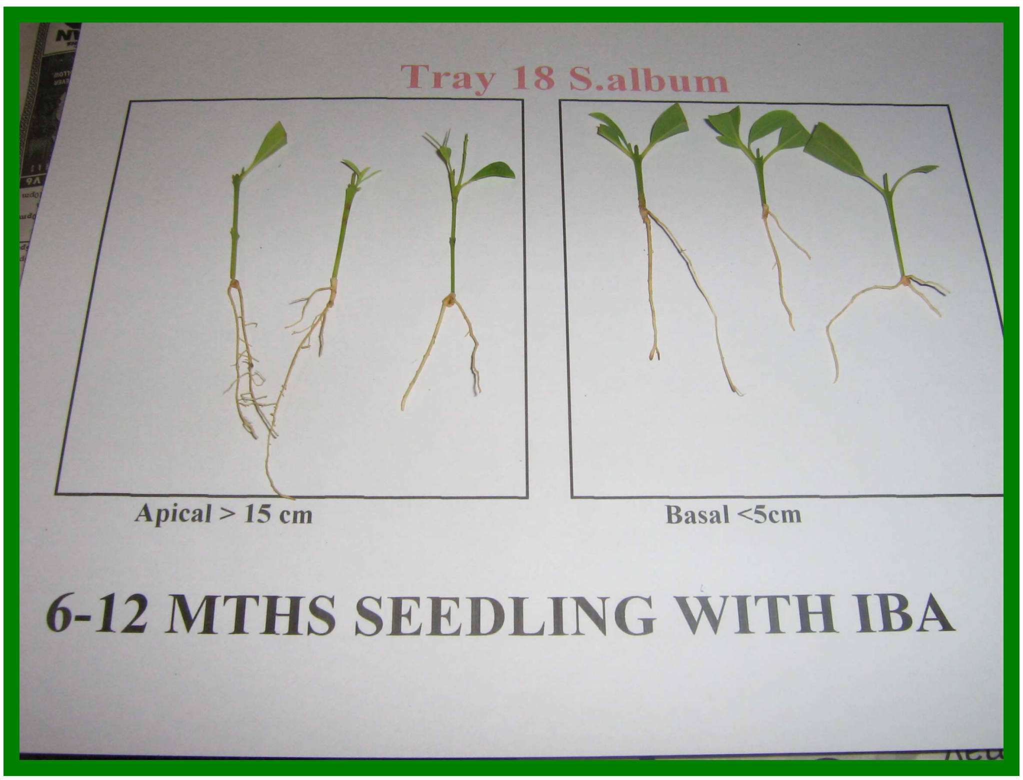


Plate 1.4.17 *Santalum album* 6-12 month seedling with IBA

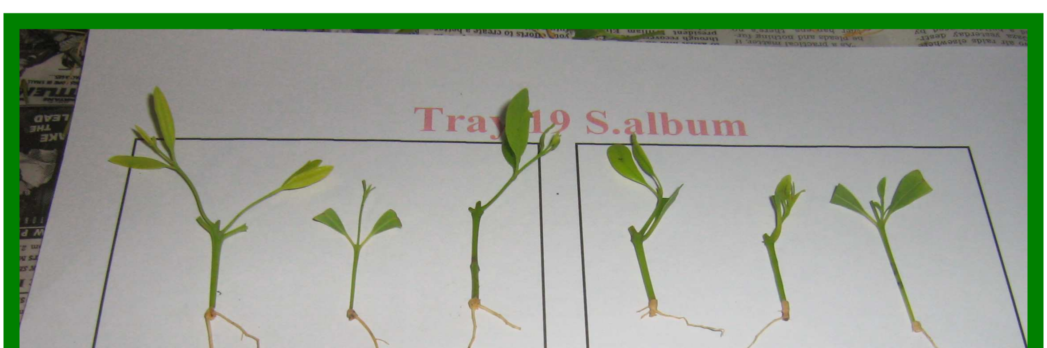


Plate 1.4.18 *Santalum album 6*-12 month seedling without IBA

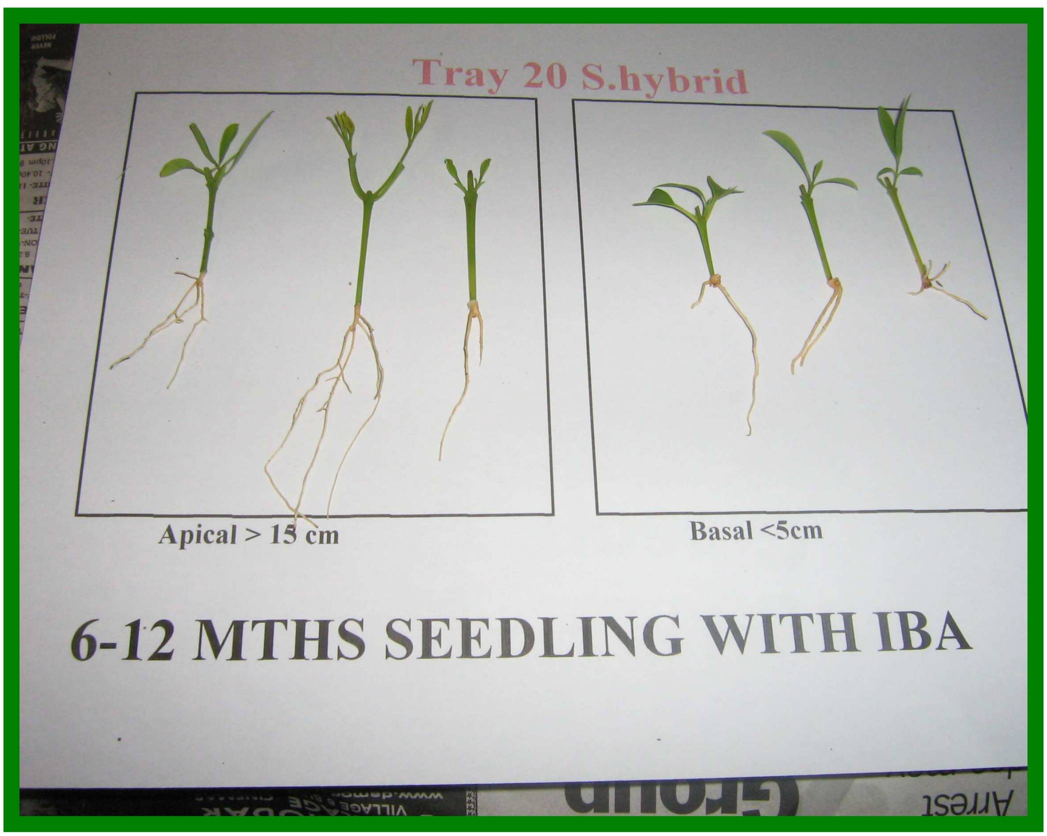


Plate 1.4.19 *Santalum* hybrid *6*-12 month seedling with IBA

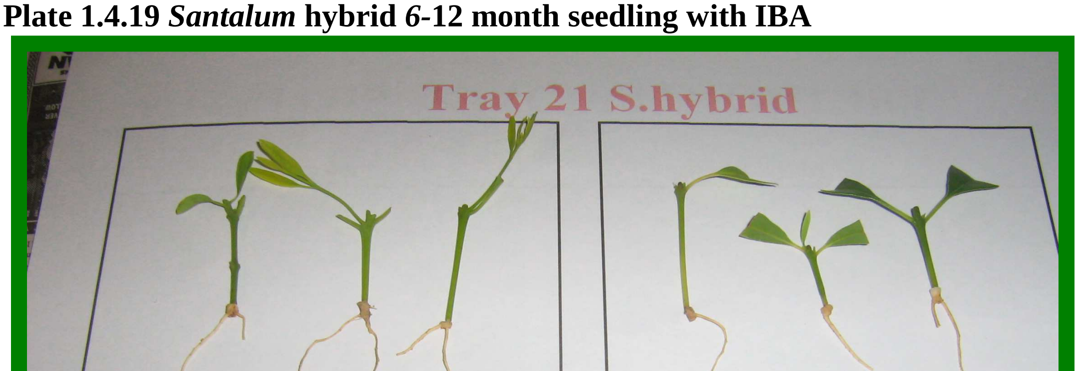


Plate 1.4.20 ***Santalum*** **hybrid** ***6*****-12 month seedling without IBA**

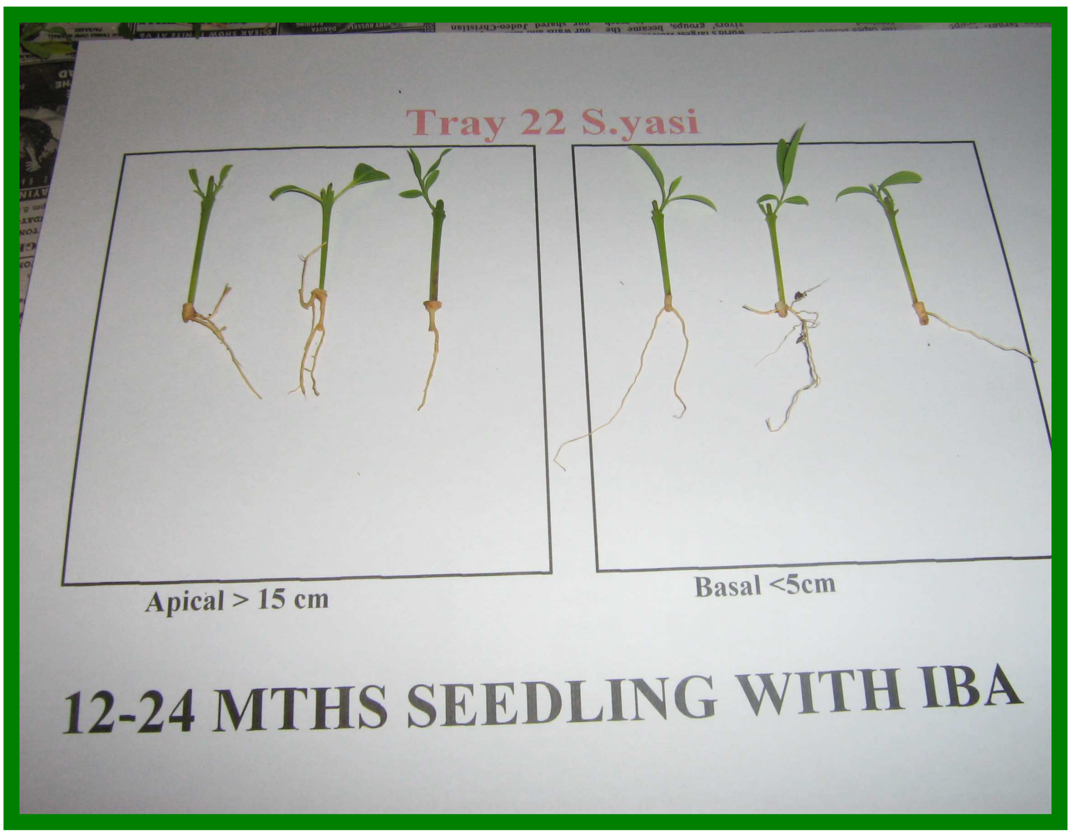


Plate 1.4.21 ***Santalum yasi*** **12-24 month seedling with IBA**

Plate 1.4.22 ***Santalum yasi*** **12-24 month seedling without IBA**

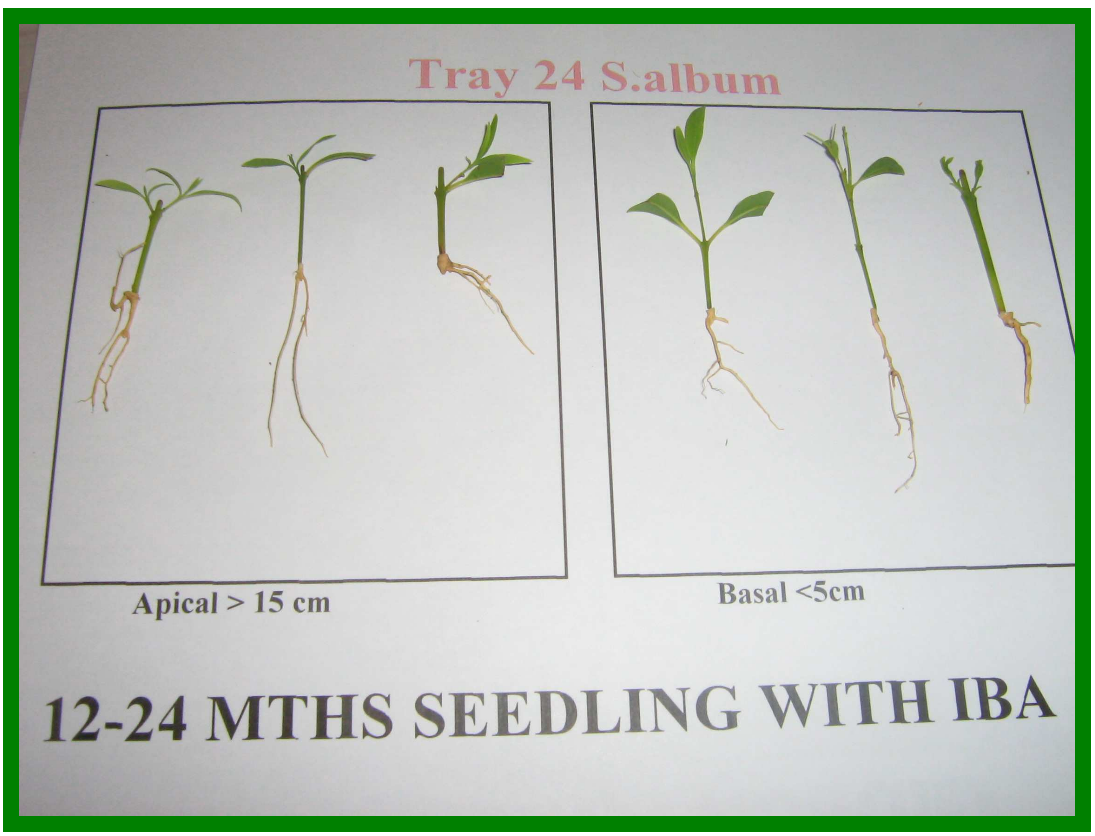


Plate 1.4.23 ***Santalum album*** **12-24 month seedling with IBA**

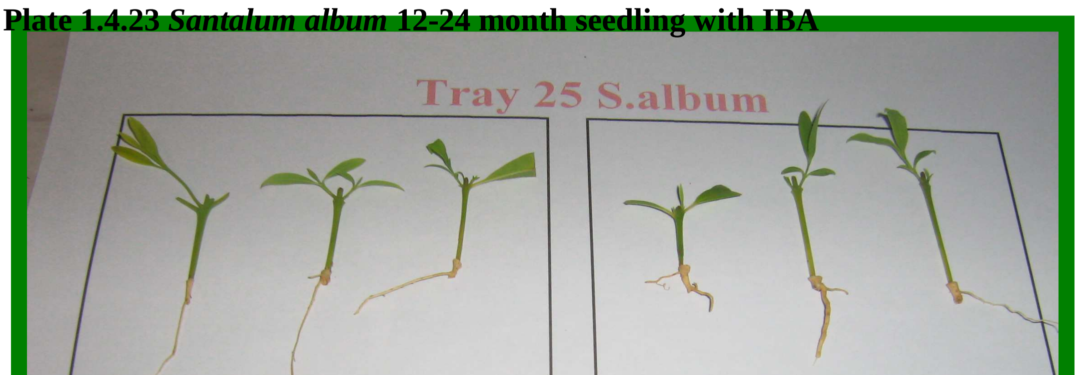


Plate 1.4.24 *Santalum album* 12-24 month seedling without IBA

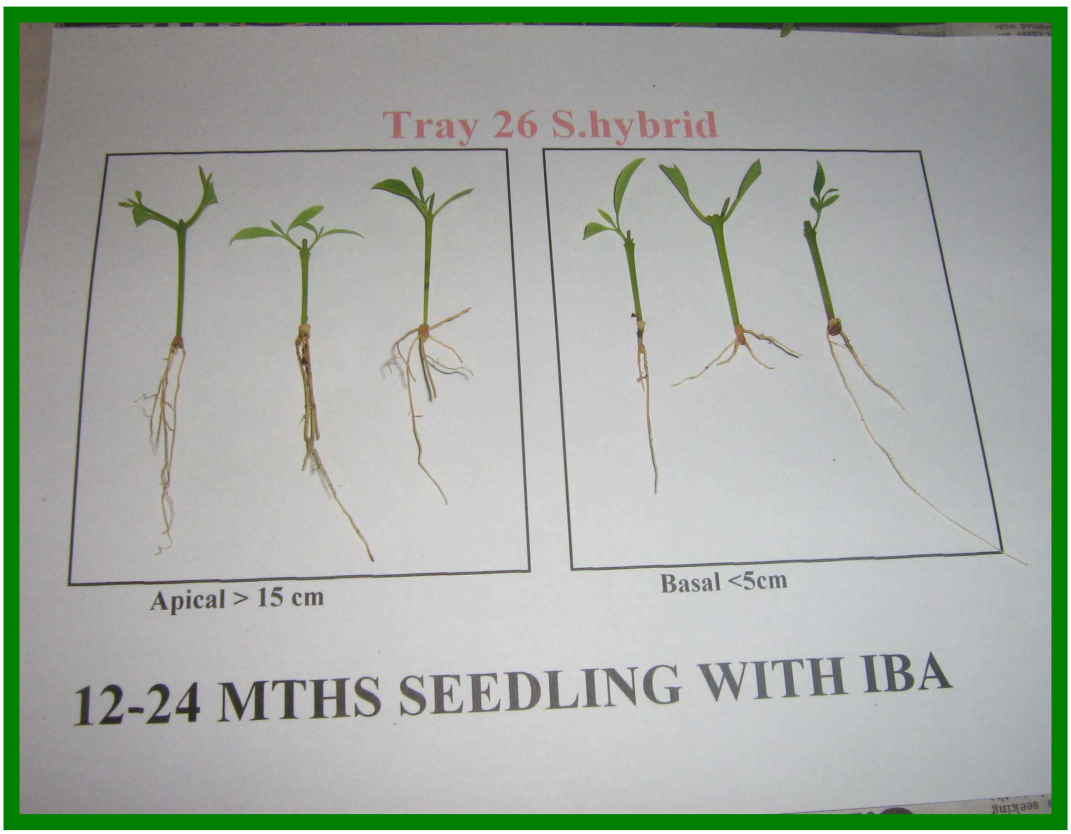
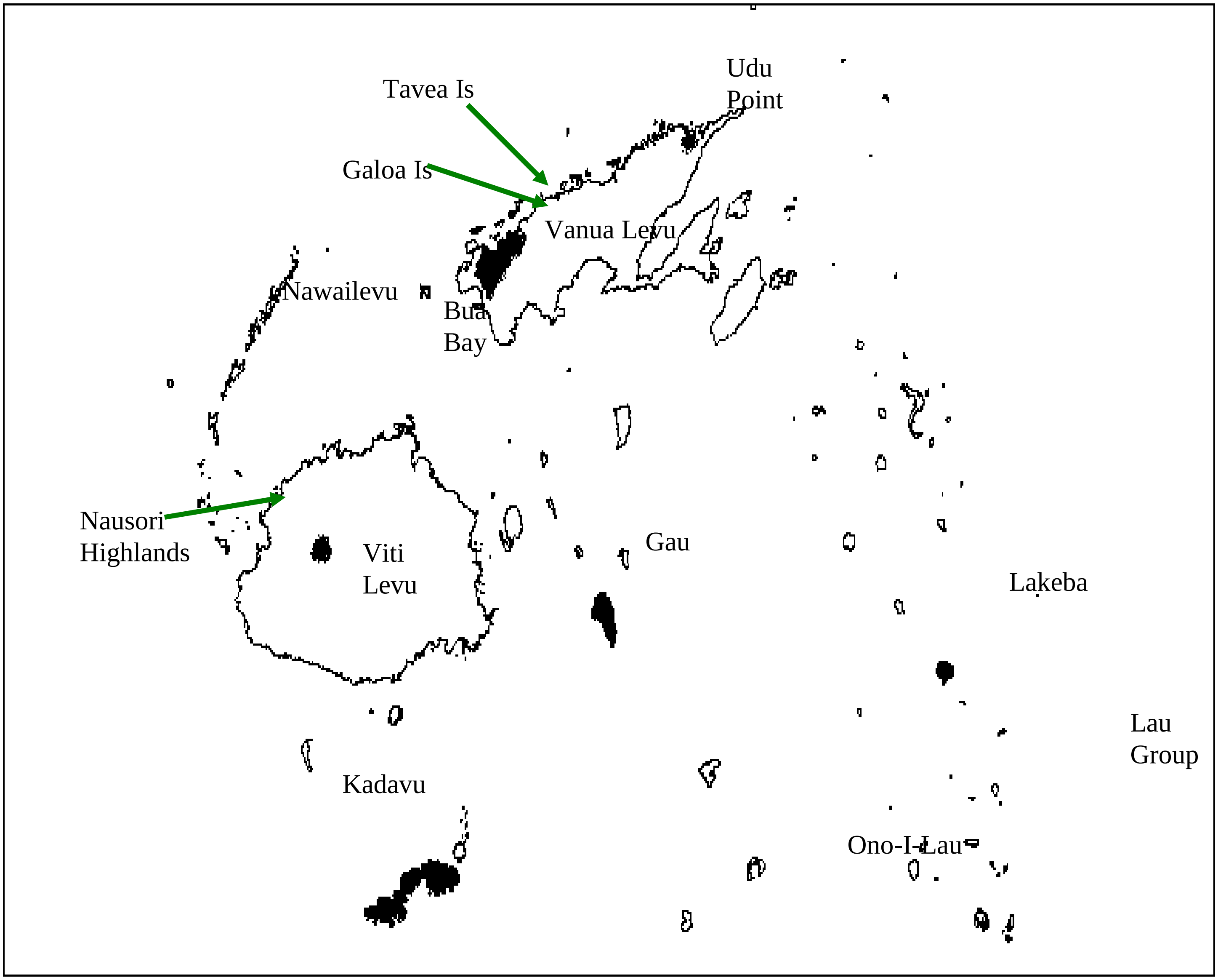


Plate 1.4.25 *Santalum* hybrid 12-24 month seedling with IBA

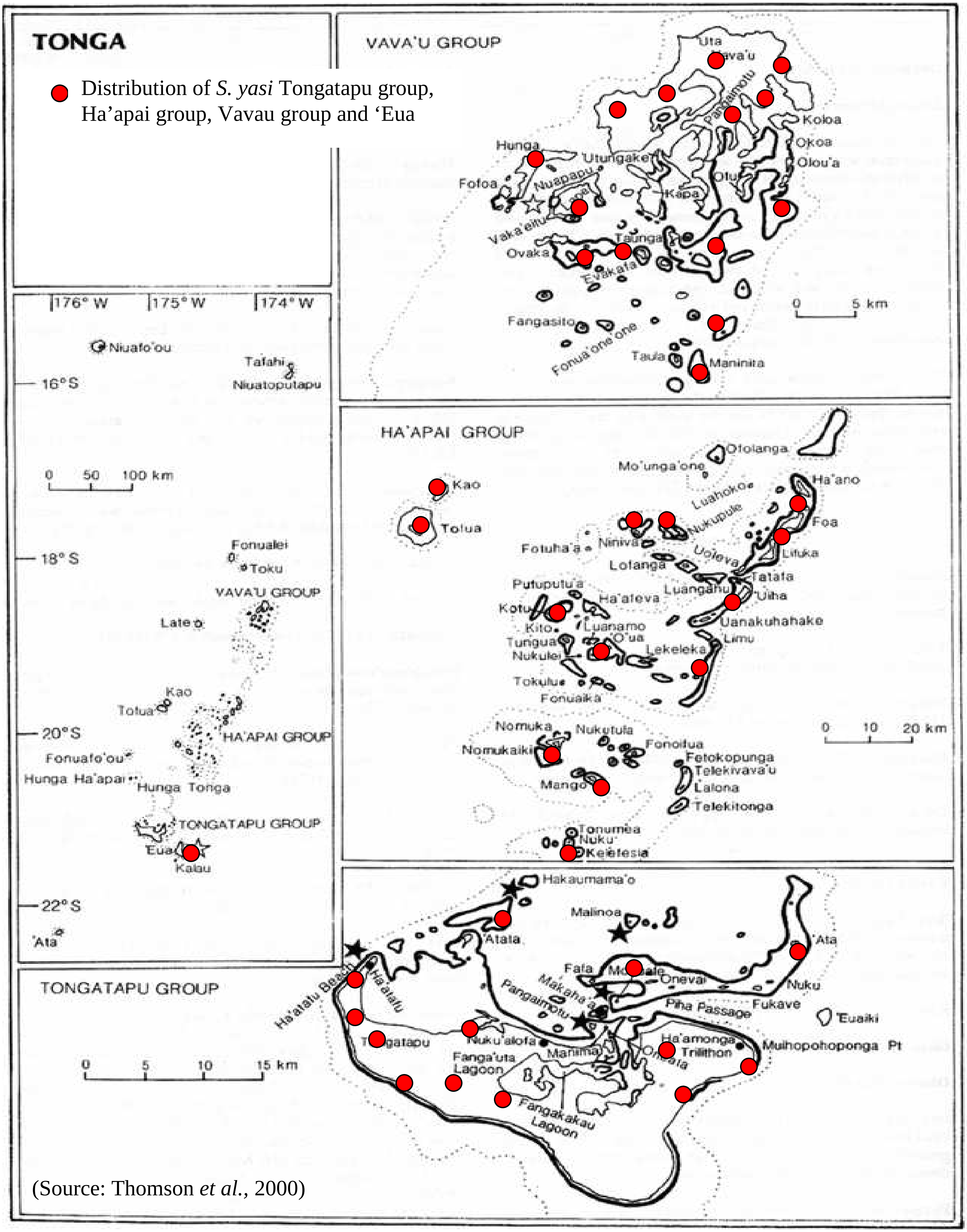
Plate 1.4.26 *Santalum* hybrid 12-24 month seedling without IBA

MAP 1.0 Natural distribution of *Santalum yasi* in Fiji and Lau group of Fiji



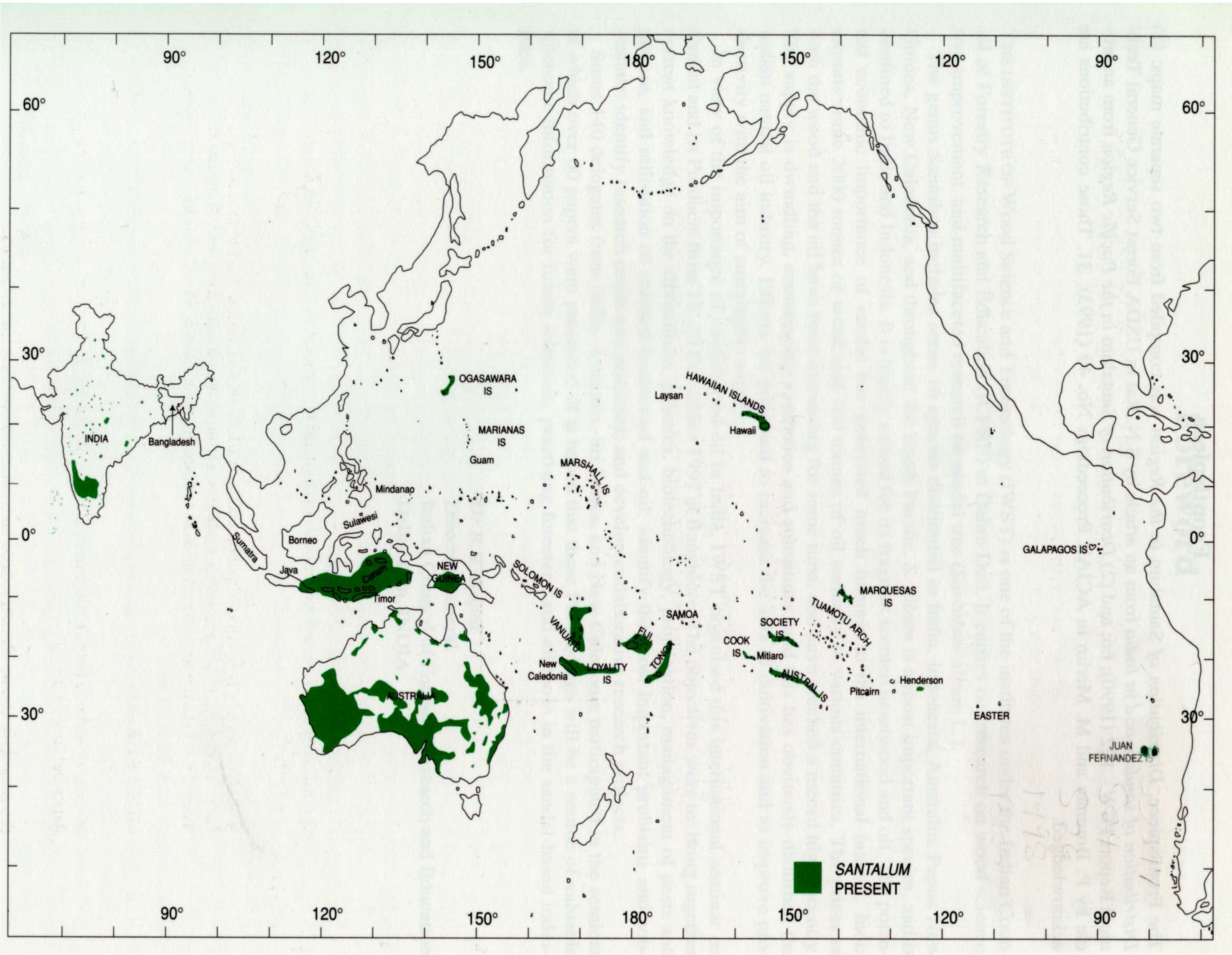
(Source: Thomson *et al.*, 2000).

MAP 1.1 Natural distribution of *Santalum yasi* in Tonga



(Source: Thomson *et al.*, 2000)

MAP 1.2 *Santalum* species distribution



(Source: Radomiljac, 1998)