Sandal and Its Products

Proceedings of an international seminar held on 18–19 December 1997 organised by the Institute of Wood Science and Technology (ICFRE) and Karnataka State Forest Department, Bangalore, India

Editors: **A.M. Radomiljac, H.S. Ananthapadmanabho, R.M. Welbourn and K. Satyanarayana Rao**

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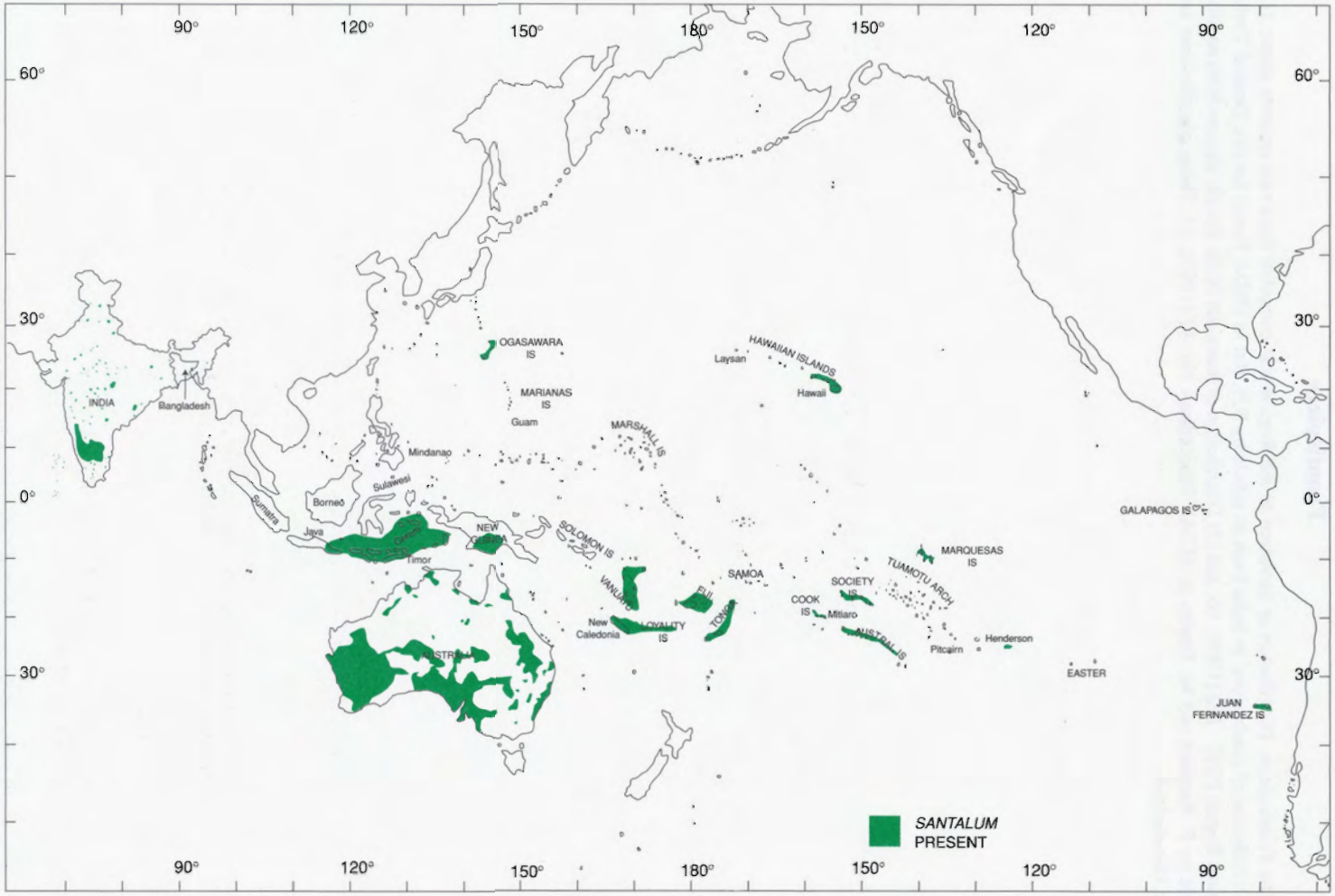
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SANTALUM PRESENT

Frontispiece

The Frontispiece, *Distribution of* Santalum *in the Region* was compiled from two separate maps: (1) *Distribution of sandalwood in India* from an article by S.N. Rai in USDA Forest Service General Technical Report PSW - 122 (1990): 66; and (2) *Distribution of* Santalum *in the Pacific Region* from an article by P. Brennan and M. Merlin in ACIAR Proceedings No. 49 (1993): 31. Those contributions are acknowledged.

Foreword

THE INSTITUTE OF Wood Science and Technology (IWST) is one of the institutes under the Indian Council of Forestry Research and Education (ICFRE) at Dehra Dun. It carries out research on wood science, tree improvement, and multifaceted research on sandal tree (*Santalum album* L.).

The genus *Santalum* includes some 16 species distributed in India, Indonesia, Australia, Papua New Guinea, New Caledonia, and throughout the South Pacific. *S. album* is a most important species, and is confined to India and Indonesia. It is highly valued for its fragrant scented heartwood and oil. The potential economic importance of sandal has generated much interest in the international market. India exports some 2000 tonnes of wood and 100 tonnes of oil annually to various countries. The prices of both the wood and the oil have been increasing for some time, and have reached a record high recently. The supply is dwindling, encouraging synthetics and substitutes, and this has obviously disturbed the Indian natural oil industry. Efforts are proposed to increase the area of cultivation and to improve productivity with the aim of sustainable supply.

In view of the importance of sandalwood oil in India, IWST organised this international seminar on Sandal and its Products from 18–20 December 1997 at Bangalore. The objectives were to: bring together current knowledge on the silviculture, genetics, biotechnology, propagation, management of pests and disease, and utilisation of scented heartwood and oil; identify the most important problems and constraints; identify research needs and priorities; and develop collaborative research projects.

Some 140 delegates from India, Australia, Sri Lanka, and New Caledonia participated in the seminar at which over 60 papers were presented. It is hoped that these Proceedings will be a source of valuable reference information for future scientists, practising foresters, and all those in the sandal-based industries.

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Director General
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Dehra Dun, INDIA

Contents

Preface

THE UPSURGE IN interest in *Santalum* species management and sandal utilisation is substantiated by the significant scientific contribution made to this seminar. The number and quality of the papers recorded here complement the work of earlier symposiums, such as *Sandalwood Seed Nursery and Plantation Technology* in 1994 and *Sandalwood in the Pacific Region* in 1991. The record of the latter, published as ACIAR Proceedings No. 49, noted that:

Sandalwood, produced from the timber of several species of Santalum, *has considerable cultural importance in many countries in the Asia-Pacific Region. For that reason alone, its conservation is an important issue and deserves more attention. Sandalwood also has high economic value, and if suitable methods of cultivation can be developed, it has the potential to make a significant contribution to the rural economies of several countries.*

Cultivation of sandalwood is, however, not an easy matter, as all species of Santalum *are [root hemi-] parasites. This means that natural regeneration or artificial establishment is dependent on the presence of suitable host plants, as well as suitable environmental conditions. In addition, sandalwood is vulnerable to fire and browsing, both common factors of the environment of all species. Nearly all species of* Santalum *have been heavily exploited in the past, to the point where there are grounds for concern for the survival of some species.*

These points are clearly endorsed and extended by the many contributors to this seminar. As a result, we not only have a good understanding of the distribution and importance of sandal in the region—India, Indonesia, Papua New Guinea, Australia, the South Pacific—but research is well under way on how best to conserve and use it.

The papers reproduced here are edited versions or abstracts of papers given or submitted at the international seminar *Sandal and its Products* held on 18–19 December 1997 at the Institute of Wood Science and Technology, Bangalore, India. Against a backdrop of conservation and utilisation, the objective of the seminar was to bring together the prolific current work on sandal in fields as diverse as economics and law, biotechnology and silviculture, chemistry and utilisation, tree improvement, and pests and disease. The result is a compendium of our knowledge of what several contributors described as 'this beautiful tree'.

Thanks are due to the organisers, Dr K.S. Rao and the staff of the Institute of Wood Science and Technology (ICFRE) and Karnataka State Forest Department. An enormous effort is needed to design a program, bring together contributors, and guide authors and presenters towards advancement in the diverse disciplines involved in an international seminar such as this. Particular credit must go to the seminar convenor, Dr H.S. Ananthapadmanabha, and the Australian coordinator, Dr A.M. Radomiljac. Without their cooperation, this publication could not have been brought to fruition.

Summary of Discussion and Recommendations

H.S. Ananthapadmanabha
Seminar Convenor

THESE SEMINAR PAPERS demonstrate that sandal tree will play a major role in the international market, contributing to trade in materials of great commercial importance such as timber (scented heartwood), oil, perfumes, and medicines.

Delegates considered various aspects of sandal, such as:

- economic and legal aspects,
- interspecific hybridisation,
- in-vitro and in-vivo regeneration and multiplication,
- cell and molecular approaches,
- improved clonal forestry techniques, and
- pest management utilisation.

They noted with serious concern the diminishing sandal population, and the resulting declining supply of oil for pharmaceutical industries, agarbathi, handicrafts, and other industries. The international market has been weakened by both diminishing and fluctuating production and supply in recent years.

Seminar delegates adopted the following ten recommendations.

1. ***Collaborative research*** should be undertaken to develop high-quality planting stock; modern techniques, such as plant tissue culture, molecular biology and genetic engineering, application of multimolecular markers for the early identification of promising clones, and selection of 'candidate plus trees' (CPT), should be used.
2. ***Gene banks*** to exploit genetic diversity, both within and between species, should be developed for future genetic improvement through breeding and other modern techniques.
3. ***Silvicultural practices*** should be developed, including application of biofertilizers like 'vesicular arbuscular mycorrhiza' (VAM), nitrogen-fixing bacteria, intermediate and long-term hosts, and integrated pest management, taking into consideration different eco-climatic and agro-chemical conditions and a package of practices to be made available to growers.
4. ***Sandal plantations*** should be encouraged as a profitable proposition, for which present laws need to be liberalised, as proposed by the Tamil Nadu Forest Department, in the direction of free trade.
5. ***Uniform extraction and transport policy*** should be formulated and followed in order to regulate the industry on a viable commercial basis.
6. ***Conventional breeding methods*** for genetic improvement of sandal should be given more emphasis.
7. ***Tissue culture*** techniques should be scaled up from the laboratory and linked with foresters for field evaluation in order to assess their commercial viability.
8. ***'Candidate plus trees'*** from different provenances need better identification, and a suitable methodology should also be evolved to delineate genetic and environmental factors.

9. ***Wide-ranging provenance studies*** should be undertaken.
10. ***Exchange of scholars*** between the sandal-growing countries should be encouraged, and Internet facilities on sandal made available.

Overall, it was agreed that research in countries where sandal grows, or has the potential to grow, should be strengthened in view of the considerable economic benefits that can flow from its intensive cultivation. In particular, high priority should be given to the exploration and critical evaluation of genetic resources, for which international support and collaboration will be needed.

Economic and Legal Aspects

The Status of Sandalwood in India

SANDAL (*Santalum album*) is indigenous to peninsular India. Its natural distribution is about 9600 km^2, mainly in Karnataka and Tamil Nadu; it now covers 8300 km^2 in those two States, and is regenerating well under natural conditions. It is an important forest timber in southern India, and its supply has been under threat. The status of its management and silviculture is outlined by forestry or forest research agencies in the six states most concerned: Karnataka, Tamil Nadu, Kerala, Andhra Pradesh, Orissa, and Madhya Pradesh.

Karnataka

M.H. Swaminathan, B.J. Hosmath and B.B. Mallesha

Due to large domestic and international demand coupled with inadequate state regulation and control, sandal is indiscriminately exploited illegally. Consequently the natural forests have few mature trees with a diameter (dbh) of more than 30 cm. Cyclic epidemics of spike disease have also contributed to the dwindling of the species, both in area and density. The Karnataka Forest Department has taken up various restoration measures to improve stocking in natural forests, as well as in plantations, by taking up extensive artificial regeneration programs.

The area of sandal is 5245 km^2, which is half the total area of the state. It is most plentiful in Shimoga, Chickamagalur, Coorg, Hassan, Mysore, Dharwad and Bangalore districts, and is also important in Kolar, Belgaum, Uttara Kannada, Dakshina Kannada, Bellary and Tumkur. It thrives best at 600–900 m altitude, with rainfall of 650–1600 mm. The tree requires good drainage and grows well in ferruginous loam with varying fertility. Sandal tree is not found in dense high forests of good growth, but occurs mainly in open scrub forests, hedgerows, bushes, and round the edges of the cultivated lands.

Most of the growing sandal populations are not dense. They are devoid of larger girth classes due not only to illicit felling, but also to grazing, browsing, hacking and other biotic interferences. No mature trees were left in the forest areas. Working Plan procedures are not followed due to the high incidence of smuggling; only stumps and dead, fallen, diseased, and mature trees were removed due to the smuggling threat.

The quantities of sandalwood being extracted or confiscated are shown in Table 1. A wide fluctuation of yield has been observed in the recent past. Recent figures show a trend of reduction in growing stock and yield. It has been established that the growing stock has been reduced to 25 per cent of its initial level in the last two decades. Another important cause for dwindling Sandal population and growing stock is spike disease, which has caused substantial damage and eliminated some good sandal-bearing areas.

Table 1. Recent sandalwood production in Karnataka (t)

Year	Extracted	Confiscated	Totals
1991–92	382	209	591
1992–93	317	127	444
1993–94	1216	257	1473
1994–95	649	475	1124
1995–96	354	155	509
1996–97	225	78	303
Totals	**3143**	**1301**	**4444**

Plantations

The number of sandal plantations has increased in Sirsi, Dharwad, Sagar, Shimoga, Mysore and Bangalore Divisions. Their performance is good, but again the problem lies in their protection from smuggling.

Sandal naturally regenerates profusely through seeds in the sandal tracts of open forests. These are to be protected from fire, grazing, browsing, hacking and encroachment. Artificially the dibbling of seeds on bushes, pits, mounds and other working areas are carried out regularly in our plantations programs, particularly in natural sandal-bearing areas. About 20 t of sandal seeds were collected, processed, tested, treated and distributed by the Seed Development Unit during 1997–98 for dibbling to enrich the

sandal wealth in Karnataka under an OECF project. Planting and distribution of container-raised seedlings was taken up in almost all the Divisions.

To encourage landowners to protect and preserve the sandal tree, the Government of Karnataka has run a Sandal Bonus Scheme since 1969. The sandal tree has to be supplied to Government only, and the owner is entitled to the bonus which is 75 per cent of the value of the tree.

Protection rules

Karnataka Forest Department has powerful sandal protection rules prescribing stringent punishments of smugglers under the Forest Act and Rules 71 A–F. The Rules provide for imprisonment or heavy fines or both, as well as confiscation of vehicles. The existing sandal protection rules are adequate, provided that they are implemented firmly. To ensure this, we propose that sandalwood offences should be:

- non-bailable,
- fully cognisable (as contemplated in the law), and
- tried by a First Class Magistrate.

Future management

Since much of the sandal wealth and natural sandal-bearing area have been lost, at least the remaining sandal trees are to be protected effectively and remaining natural sandal-bearing areas are to be preserved. Steps to be taken are as follows.

- Full protection against biotic interference such as fire, grazing, browsing, and hacking.
- Existing sandal-bearing areas are to be fenced and protected.
- Smuggling activities are to be curbed by intensive protection measures and laws, with the help of local people, NGOs and other local bodies.
- Smuggling and export of sandal oil will be rigidly controlled.
- Large-scale sandal plantations are to be raised in natural sandal-growing areas in the state.
- Dibbling of pelletised sandal seeds in and around bushes, advance work areas, ripping areas and other workings are to be carried out on a large scale every year.
- Private farmers and plantation owners are to be encouraged to plant more.
- Existing laws, rules and regulations are to be relaxed and modified to suit the public interest.
- Sufficient funds are to be provided for sandal regeneration and protection schemes.
- The artisans and Gudigards are to be encouraged to use other wood in place of sandalwood, e.g. shivani, beete, teak.
- Enterprises depending on sandalwood and its products are to be encouraged to establish large-scale captive plantations of their own.
- Private tree-growers are to be encouraged to introduce sandal as one of the progressive high-value tree species such as teak and mangium.
- People in urban and rural areas are to be given free saplings to plant in their home yards every year on a large scale, and are to be encouraged to grow and save such a highly valuable, sacred, endangered tree.
- A condition is to be imposed on every Range Forest Officer in the state to raise at least 10000 seedlings of sandal compulsorily, and to plant these on a large scale.
- Techniques for easy sandal regeneration through seeds are to be developed (seed technology); fast-growing, quality wood-yielding, superior varieties in the existing stock are to be identified.
- The Department should organise 'Sandal Week' (or Day) every year, and should supply free of cost sandal seeds and seedlings to families.

Intensive silvicultural and management practices based on sound ecological principles should be evolved and applied to sandal-bearing forests. Steps should be taken to protect these forests from problems such as grazing, browsing, hacking, fire, and encroachment. Improved cultural practices should be encouraged.

Sandal is distributed over an area of 3040 km^2, mostly in the northern part of the state. Small isolated populations are also found in the other areas. For example, dense populations still exist in Chitheri Hills and Javadi Plateau, while in other areas it has been depleted due to illegal felling, distress-felling by the Department, and spike disease.

Silviculture and management of sandal has been a priority in Tamil Nadu since the early 19th century. Natural regeneration has been greatly affected by grazing, fire and soil erosion. At this rate sandal will not only loose its position as top revenue earner, but the species itself may also face loss of genetic diversity and therefore require rehabilitation as an endangered species.

Natural regeneration

Conservation of moisture is necessary for natural regeneration and the water regime of the area can be improved by constructing a series of water-harvesting structures. These measures, when coupled with protection against grazing and fire, have brought the desired results in sandal areas in Kalrayan and Chitheri Hills.

Construction of gully plugs, percolation ponds and contour trenches in sandal estate areas of Salem circle has improved soil and moisture levels of the area, and this has supported natural regeneration of sandal.

By contrast, chainlink fencing of nucleus plots of sandal have failed completely due to the movement of wildlife and cattle. Non-maintenance of fencing in the subsequent years is the primary reason for the failure of fencing. The tribal watchers employed for protection of sandal areas are generally used for sundry works and therefore the regeneration suffers.

Artificial regeneration

Seeds are collected from known seed sources and stored in the godowns of Genetics Division at Coimbatore. Before dispatching the seeds to the indenting officers, viability and germination tests are carried out in a scientific manner. The seed is certified for viability and degree of germination. An average of 2900 kg of seeds is supplied to the various user agencies every year.

Methods of artificial regeneration in use are:

- dibbling of seeds in bushes, pits and mounds,
- trenching around the trees for obtaining root suckers, and
- raising plantations using container-grown seedlings.

A clonal seed orchard (CSO) with 26 clones has been established in Kurumbapatty. It is proposed to raise a half-sib progeny trial cum seedling seed orchard during 1998. It is also proposed to establish CSOs in Chitheri Hills and Melchengam during 1998.

Management of sandal on patta lands

The ownership of sandal trees vests in Government in respect of trees in:

- porambokes and land under sivojima cultivation, and
- private lands assigned after a notified date which differs from area to area.

Landholders own trees which have since appeared in pattalands assigned before 1907, and between 1907 and 1928 in certain areas of Coimbatore district.

Tamil Nadu

K. Chidambaram and K.K. Chaudhuri

Future strategies

The silviculture and management of sandal should involve:

- improvement in nutrient and moisture status of the soil by constructing a series of small-scale soil conservation structures;
- protection against grazing by fencing small areas with a stone wall or barbed wire supported from outside by a thorny impenetrable vegetative fence; maintenance of these fences is much more important than establishing additional areas;
- employing tribal watchers from local settlements for protection against grazing and fire;
- providing incentives to the people of forest villages for complete protection of specified sandal regeneration areas against grazing and fire; and
- planting of fruit-bearing species which attract birds for enhancing ornithodispersal of sandal seeds.

If the natural regeneration of sandal tracts carefully carried out, it may not be necessary to supplement it with artificial regeneration. Even so, artificial regeneration raised under agro-cultivation has been extremely successful.

Kerala

H. Nagesh Prabhu

The sandal-bearing forests of Kerala are mainly located in the Anjanad valley which is on the eastern side of Western Ghats falling in Marayoor Forest Range of Munar Forest Division. Sandal also occurs on a limited scale in Arienkavu range of Thenmala Forest Division. Heavy grazing hampers its regeneration, and spike disease has been reported at Marayoor and is spreading quickly.

Table 2. Recent sandal harvests in Kerala

Year	Quantity extracted (t)	Quantity sold (t)	Av. price (Rs/t)
1992	105	101	1.92
1993	136	108	2.40
1994	65	99	3.10
1995	68	54	3.26
1996	65	56	3.40

In major sandal-growing areas, tracts occur in both hills and valleys. Artificial regeneration by trenching to wound the roots around the mother plants, to initiate root suckers has been found successful. Only dead or diseased trees are uprooted every year; they are cleaned and sold at public auctions.

There are 21 factories distilling sandalwood oil in the Karnataka-Tamil Nadu border region. An average factory consumes 170 t of sandalwood per annum, from which it produces 6 t of sandalwood oil. Only a

small proportion of wood is harvested officially; the majority comes unofficially from Tamil Nadu and Karnataka. Similarly the official quantity of sandalwood oil sold is much less than the unofficial quantity of sandalwood oil that finds its way into the international market.

The best way to attack this illicit trade is to liberalise the states' monopoly over sandal, and to unify acts and rules for the entire nation.

Andhra Pradesh

N. Ramakrishna Rao

Andhra Pradesh is the fifth largest state in India, and the third largest in forest area. Of the 23 districts in the state, 15 districts support predominantly dry deciduous forests with thorny scrub growth extending over nearly 25 000 km^2. The drought-prone and drier southern region of 200 km^2 bears natural sandal. The districts of Anantapur, Chittoor and Cuddapah support natural stands of sandal in measurable quantities.

Sandal was one of the important timber trees in this tract which was silviculturally worked under successive working plans during 1930–67. Sandal sowing in the State dates back to 1914. It was artificially regenerated in certain select reserves of these districts following the working plans. Sandal seed, along with seed of certain shrubs like *Cajanus indicus*, *Cassia tora* and *Dodonaea viscosa*, was dampened by making shallow saucer pits with the break of monsoon. The areas were protected from browsing and direct exposure to sun.

In later years, pre-treated sandal seed was sown inside the bushes by using long hollow bamboo sticks. The bamboo sticks with one end sharpened were used for raking up the soil under the bushes, and the seed was covered after dumping through the hollow bamboo. These sown areas were serial numbered to monitor the germination and follow-up action for protection. The germination rate was 7–13 per cent. Introduction of sandal in this way was practised mostly in areas worked under coppice, where there was possibility of shade as well as protection from fire and grazing. Some 3000 ha of sandal plantations met with partial success.

Sandal occurs in sizeable amounts on private lands, institutional lands, village common lands where the status of regeneration is satisfactory. It is a rare sight to see a mature sandal tree as the farmers harvest the trees early for an economic return and to escape the risk of illicit cutting.

During the early 1970s most of the sandal trees to be found were scattered in the forest areas and were extracted because of indiscriminate illicit cutting. A sample survey conducted in Anantapur District during 1990–91 in 19 reserves revealed that the natural sandal distribution is 2–37 trees per ha. However, the natural regeneration of sandal is encouraging in degraded forest areas protected for only 2–3 years and managed under a participatory approach.

Orissa

M. Gourinath

Sandal occurs in limited patches of Jeypore and Rayagada Forest Divisions, and rarely in Kalahandi and Parlakhemundi Divisions. The occurrence of sandal has been recorded in 25 forest plots of Rayagada Division, and most of it occurs in seven plots. Similarly in Jeypore Division sandal occurs in five plots, four of which are more important.

There is little recorded evidence as to how and from where these sandal trees started growing, and why they are concentrated in a few forest blocks. There is also no authenticated record as to whether they are of plantation or natural origin. However the old Forest Department files show that they were planted by the ex-rajas of Jeypore, one of whom planted sandal trees in Naktidongar block near Jeypore and also in Ghatgumar and Nandapur block. Due to suitable edaphic and climatic conditions prevailing during that period, the plantations were successful. Later on the birds carried seeds from these trees to nearby areas and thereby the naturally-grown sandal forest occurred in the area.

Serious thought had not been given to regenerating these sandal-bearing forest blocks till 1982–83. Except for a few sporadic plantation activities undertaken as listed below, protection through forest department staff was the only management practice followed. The few plantations established as revealed from old records are listed in Table 3.

Table 3. Sandal plantations in Orissa

Division	Year planted	Area planted (ha)
Jeypore	1961–62	0.5
	1965–66	4.2
	1966–67	12.2
	1981–82	2.0
Rayagada	1981–82	2.0

The above plantations were not successful due to large-scale mortality from nursery bed to plantation site.

The climatic and edaphic conditions in the forest blocks (where sandal tree occurs naturally) are suitable for propagation. The only management required is protection from fire, injury and smuggling. In addition to that, areas having larger clearings need be identified and sandal plantation need be taken up in smaller patches of 5–10 ha. This should be planned through a Master Plan, and an action plan prepared to be implemented continuously for 7–10 years.

No systematic and continuous approach has as yet been taken up for the propagation of sandal forests of Koraput Districts. Moreover, intermittent smuggling has further deteriorated the sandal forest.

Madhya Pradesh

K.P. Tiwari and R.K. Pandey

Madhya Pradesh is the largest state in the Indian Union, is situated in the centre of the country, and one-third is forested. Sandal is not a naturally occurring associate of these forests. However, it has ecologically adapted as an associate in natural dry deciduous teak forest.

The earliest recorded sandal introduction to the natural forests of Madhya Pradesh is in 1880, and it has since spread throughout some 2360 ha in 21 compartments. The first plantings, 175 ha in 1979, were planted in Seoni District; subsequently, substantial plantings were made in revenue lands in Dewas, Indore, and Rajgarh Districts on the Malwa plateau at 250–600 m above sea-level.

Soil of the sandal-bearing forests of South Seoni Division, and other localities where the species has adapted as an associate of the prevailing forest ecosystem, ranges from ferruginous sandy loam, to clay-loam, to lateritic. Annual rainfall is 1200– 1600 mm in South Seoni, and 900–200 mm in Indore, Mandsour, Rewa, Raisan, and Sehore; rainfall below 90 mm has been recorded in Guna Forest Division. Evidently sandal grows in a wide range of soil and rainfall conditions.

Because of its high economic value, sandalwood is being given considerable attention by the State Forest Department and the State Forest Research Institute, Jabalpur.

Distribution

Sandal has acclimatised itself in various forest types ranging from moist to dry tropical deciduous forest, and hence occurs in scattered patches of natural forests in the Seoni, Sagar, Rewa, Raisan, Guna, Mandsour and Dewas Districts.

In the tropical dry deciduous forests it is found as an associate of teak in South Seoni and Sagar Divisions. It is also regenerating well in mixed deciduous forest without teak in Malwa. The current distribution of sandal in various forest divisions is shown in Table 4.

Table 4. Distribution of sandal in natural forests of Madhya Pradesh

Sl. No.	Division	District	Area (ha)
1	South Seoni	Seoni	2361
2	Sagar	Sagar	2300
3	Sehore	Raisan	100
4	Mandsour	Mandsour	800
5	Dewas	Dewas	**
6	Guna	Guna	5
7	Rewa	Rewa	2
8	Indore	Indore	**

Note: ** in revenue lands

The main associates of forests in the area where sandal has established itself are:

- in the upper canopy—*Tectona grandis, Lannea coromandelica, Boswellia serrata, Madhuca latifolia, Albizzia labback, Terminalia tomentosa, Diospyros melanoxylon, Anogeissus latifolia, Lagerstroemia parviflora,* and *Adina cordifolia*;
- in the lower canopy—*Cassia fistula, Aegle marmelose, Buchnania lanzan, Dendrocalamus strictus*, and *Saccopetalum tomentosum*; and
- in the understorey, there are several species along with ground associates—*Cassia spinarum, Gymnosporia montana, Zyzyphus xylopyra, Randia dumetotrum, Lantana camara, Cassia tora, Ageratum conyzoides* and several grass species and small herbs.

Regeneration

In the Chandan Bag area of the Seoni District sandal is spreading naturally and regenerating in adjoining forests. For example, in South Seoni Forest Division it has occupied about 4800 ha of forest area comprising 21 compartments.

Regeneration and establishment of sandal in Compartment 249 in South Seoni Forest Division clearly shows the pattern of successful association in the prevailing ecosystem. However in 1996 and 1997 this species was suffering from biotic pressures resulting in the trees of various girth classes drying and dying. Initially, spike disease was suspected, but no evi-

dence of it has been reported in the locality. The distribution and density of sandal trees observed in various compartments of the same locality in South Seoni Division also indicated that the area can support a young and middle-aged crop of sandal as an associate of dry deciduous teak forest. Maximum density of 3931 and 2007 trees per ha corresponded with girth classes 31 and 45 cm, respectively, in 14 compartments in 1996–97.

Phyto-sociological studies were carried out in and around various adjoining compartments of an old plantation site (Compartment 249) in South Seoni Forest Division in 1997. Sandal was growing better in sloping lands than on the plateau and plains of the locality. In Compartment 249 itself, the density of sandal trees was 4.2 per ha. By contrast, in Compartment P-75, situated on the plateau just adjacent to the old plantation site, sandal was absent, although there were 268 trees per ha of six other species. In this locality average density was 461 with 39 tree species growing as an associate of sandal. Thus the site carries a wide diversity of tree associations, but sandal occurrence was very low in the plateau area.

In Compartment 247, situated in the plain, sandal density was 24 trees out of a total of 508 per ha consisting of 18 species, of which most were in young and middle girth classes (21–90 cm). However observations on sloping and undulating areas in Compartments 247 and 248 suggested that sandal was contributing more density even though the total density of the site was less than that of the plain and plateau area of the locality. In Compartment 247 total density of 16 associate tree species was 504 trees per ha, of which sandal contributed about five per cent. Similarly in the sloping areas of Compartment 248 the total density of tree species was found to be 228 with 17 species, and sandal contributed four per cent. Both the localities are away from the old plantation sites, but they have good drainage and comparatively less biotic impact, and provide hospitable conditions for natural regeneration. The associate tree species of this area are in the 21–60 cm girth class.

Regeneration status and distribution of sandal was very poor in the plateau and plain localities. However in the sloping and undulating areas in Compartments 248 and 247, regeneration was satisfactory. In Compartment 248 the density and frequency were 33 saplings per ha and 22 per cent, and no seedlings were found. In Compartment 247 the corresponding figures were 23 and 60.

Regeneration in a protected experimental plantation site at SFRI

The State Forest Research Institute at Jabalpur has raised plantations of 20 species in the Regional Research Center at Seoni, which is completely protected from biotic factors. Four ha were planted in 1979 and 1980. After 17 years sandal was regenerating well and had covered most of the area of plantation sites.

Phyto-sociological observations on sandal regeneration sites suggested that the sandal had appeared with associate species on the sloped area as well as in the site of plantations. The high frequency of saplings and seedlings of sandal as recorded on different plantation sites, indicate that protected sites of experimental plantations are a congenial habitat for regeneration. For example, 27 saplings per ha were observed in the Sissoo plantation which contains most of the sloping terrain in the area. Moreover, 26 saplings per ha were recorded in mixed plantation sites of the same area.

Similarly 6260 sandal seedlings per ha were recorded in mixed plantation sites. *Leucaena* sp. and *Clestanthus collinus* plantation sites also provide congenial conditions for regeneration with 3330 and 1220 seedlings per ha respectively. However in other plantation sites on the plain, regeneration of sandal was not satisfactory.

Conclusion

The State Forest Research Institute has conducted experiments in different agro-climatic zones since 1979. Growth and increment data and the regeneration status of sandal plantations are encouraging for future plantation strategies in Madhya Pradesh. Climatic conditions in the sandal pockets are very congenial for its regeneration and growth, and it is free from spike disease. Ecologically, sandal has also proved its acceptability for in-situ regeneration in various soil and rainfall conditions.

An Overview of Sandalwood and the Development of Sandal in Farm Forestry in Western Australia

S.R. Shea*, A.M. Radomiljac†A, J. Brand§ and P. Jones¶

Abstract

Of the four species of *Santalum* that occur naturally in Western Australia (*S. spicatum, S. lanceolatum, S. murrayanum* and *S. acuminatum*), only *S. spicatum*, and to a much less extent *S. lanceolatum*, is commercially harvested for sandalwood. Western Australia has maintained an almost entirely export-oriented sandalwood industry for more than 150 years. *S. spicatum* is mostly used for the manufacture of joss sticks in Southeast Asia, although new uses have recently been developed. *S. spicatum*, which occurs naturally in the wheatbelt and rangeland regions of Western Australia, is the focus of a research program aimed at understanding its natural regeneration requirements and its establishment in tree farms on agricultural land. *S. album* (Indian, or East Indian sandal) has a higher santalol oil content, faster growth rate, and larger tree habit than other *Santalum* species. It is the centre of a research program aimed at the development of an irrigated sandal tree-farm resource in northern Western Australia. As with most *Santalum* species, *S. album* silviculture is complex due to its parasitic requirements. A reliable nursery and silvicultural system has been developed and is routinely used. A tree-farm resource of both *S. spicatum* and *S. album* will supplement the green-wood harvest of *S. spicatum* from natural stands in the arid rangelands. The need for a coordinated approach to marketing the world's remaining supplies of sandalwood is discussed. It is important that the maximum value of this important resource is realised, and that the future global sandalwood industry is sustained.

Key words: sandalwood, Western Australia, research, management, *S. album*, *S. spicatum*, tree farm

THE WIDELY DISTRIBUTED and economically important *Santalum* genus consists of 16 species (Hamilton and Conrad 1990; Barrett and Fox 1995), which are xylem-tapping root hemi-parasites with a highly valued aromatic heartwood. Four *Santalum* species are native to Western Australia: *S. spicatum* (R.Br.) A.DC., *S. acuminatum* (R.Br.) A.DC., *S. murrayanum* (Mitchell) C. Gardn., and *S. lanceolatum* R.Br. (Hewson and George 1984). Of these, only *S. spicatum* has commercial significance. As in most regions with natural *Santalum* stands, sandalwood exploitation in Western Australia has a long and coloured history. The development of a reliable sandalwood silvicultural system is intrinsically complex due to its parasitic habit. This paper gives a succinct overview of the status of management and current research on sandal in Western Australia.

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History of the Western Australian *Santalum spicatum* Industry

Western Australian *S. spicatum* has provided the basis of a small but important export industry since the early 1840s. The boom period of the sandalwood industry (1860s–1920s), which was associated with unregulated marketing procedures and harvesting controls, lasted until 1929 (Applegate and McKinnell 1993). Between 1892 and 1901, some 50 000 t of sandalwood were exported from Western Australia; 14355 t were exported in 1920—the highest annual export quantity recorded (Statham 1990). The bulk of this wood was exported to China (Shinberg 1967; Statham 1990). However over-harvesting during this period lead to the accumulation of huge sandalwood stockpiles and to fears that the level of exploitation was not sustainable (Underwood 1954).

In 1923, in reaction to these concerns, the first serious attempts to control the Western Australian sandalwood industry occurred (Underwood 1954; Statham 1990). Western Australian Forest Department regulations were created to protect the commercial interests of sandalwood harvesters, conserve natural stands, and ensure that the Crown collected a sandalwood royalty. The 1923 regulations incorporated:

- an overall quota on production which was determined by government, but not to exceed 6000 tonnes;
- introduction of export licences;
- an increase in royalty payments; and
- the appointment of forest staff to enforce minimum size restrictions on harvesting, and check illegal sandalwood harvesting operations.

It was thought that these regulations would restrict supply, leading to an upward trend in world price and a reduction in the immense stockpiles of sandalwood.

The 1929 Sandalwood Act legalised the 1923 regulations. This Act also imposed a government set quota, of which only 10 per cent could come from private property; the Act also led to the creation of the Australian Sandalwood Company in 1930 (Statham 1990). Sandalwood exports continued until 1943 at an average of 1460 t per annum. For a two-year period (1944–45) sandalwood was not exported. It recommenced in 1946 from a very low base of 143 t, and has risen steadily since then, peaking at 2040 t in 1993 (Fig. 1).

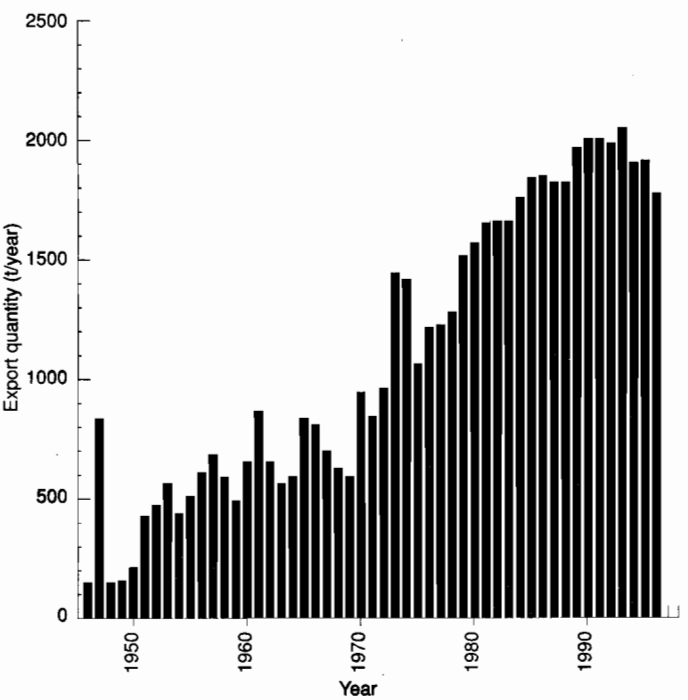

Figure 1. Western Australia sandalwood (*Santalum spicatum*) exports, 1946–96. (Sources: Statham 1990; CALM annual reports.)

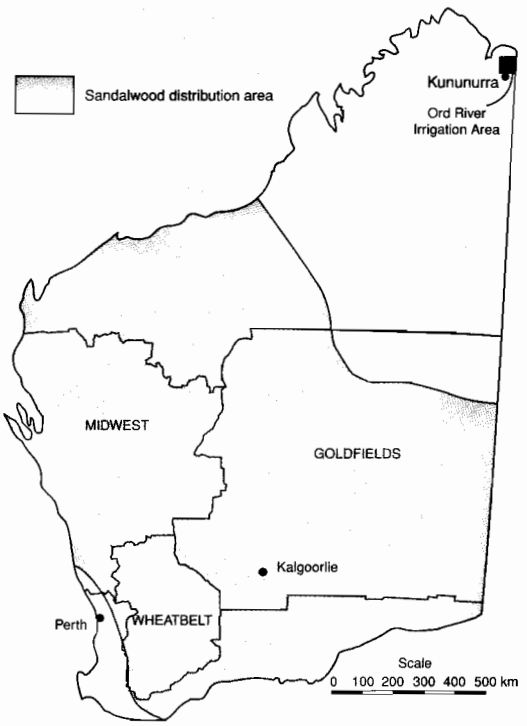

Figure 2. Distribution of *Santalum spicatum* in Western Australia. (Adapted from Hewson and George 1984; Loneragan 1990; Kealley 1991; Mitchell and Wilcox 1994; CALM data 1997).

Management and utilisation of *Santalum spicatum* in W.A.

The management of *S. spicatum* is controlled by the Department of Conservation and Land Management (CALM) in accordance with State legislation and departmental management plans (Kealley 1991). *S. spicatum* is distributed over 42 million hectares and there is an estimated sandalwood resource of 117000 t (CALM data 1996). *S. spicatum* is a shrub or small tree about eight metres high with a bushy growth habit (Hewson and George 1984; McKinnell 1990; Barrett and Fox 1995). It occurs naturally over a large area of Western Australia, from the edge of the high forest zone out to the desert interior (Fig. 2). It has virtually disappeared from the 300–600 mm rainfall zone due to widespread agricultural clearing (McKinnell 1990). A large proportion of the current *S. spicatum* population occurs on pastoral lease land where the predominant natural host species are *Acacia acuminata* Benth. and *A. aneura* F. Muell. ex Benth. (Loneragan 1990).

Revenue gained from sandalwood sales directly assists the conservation of *S. spicatum* and is used to fund the management and conservation of the population, which includes the purchase of pastoral leases for conservation reserves, silvicultural and ecological research, plantation establishment and resource inventories.

To ensure that *S. spicatum* is managed on a sustainable basis, harvesting is strictly controlled through a limited number of harvesting contracts issued to private sector sandalwood pullers. Harvesting contracts are issued for specific supply areas, and the quota for each contract is related to the sustained yield of that area. Minimum stem diameter limits for living trees are imposed at harvesting. These limits are calculated on the basis of the population distribution within defined landforms. Dead trees of any size are harvested, since fire or drought readily kills *S. spicatum* trees (Kealley 1987; McKinnell 1990). Harvesting live trees is strictly controlled to ensure that two-thirds of living trees on particular landforms are retained. Current harvesting procedures produce a 1:1 ratio of dead and green heartwood. Virtually the whole tree is utilised down to about 2 cm diameter pieces, including root and branch heartwood and bark material. The identification of alternative uses of *S. spicatum*, such as oil distillation for aromatherapy and medicinal purposes, to supplement the traditional Southeast Asia market use for joss stick manufacturing is ongoing. CALM retains full possession of the sandalwood resource from harvesting to sale, with harvesting, transport, processing and marketing conducted by the private sector on a contract basis.

Recent technological advances have allowed CALM to conduct a number of specific projects aimed to further improve CALM's management of *S. spicatum*. These include the utilisation of remote-sensing techniques to gather inventory data of the widely distributed population. Coupled with this inventory program is the phenotypic selection of candidate *S. spicatum* 'plus' trees. This initiates the first stage of a tree breeding program which will ultimately supply seed for direct-sowing regeneration in the rangelands, and tree-farm establishment in the higher rainfall agricultural zones. A *S. spicatum* tree-

farm resource may supplement the harvest from natural stands.

Santalum spicatum ecology in the W.A. rangelands

The majority of *S. spicatum* harvesting in Western Australia occurs in the arid pastoral regions of the Goldfields and Midwest: the rangelands (Fig. 2). Inventories have shown that regeneration is generally poor on developed and grazed pastoral leases (Kealley 1991). Also, it is heavily grazed by domestic and feral herbivores, such as sheep, goats and rabbits (Loneragan 1990). To conserve sandal and promote regeneration in the rangelands, CALM has purchased Jaurdi, Mt Elvire and Goongarrie and Burnerbinmah pastoral leases over the past ten years. These stations have been de-stocked of sheep, and goats are controlled through mustering and eradication programs. CALM is currently developing cooperative management schemes that provide incentive for other leaseholders to better manage the *S. spicatum* population on their leases.

Long-term trials have been established on these stations to monitor *S. spicatum* recovery after destocking. Besides grazing, the trials are also examining other factors affecting regeneration such as host species and soil type (Brand 1996). *S. spicatum* seeds have been sown beneath many potential host plants in the rangelands, mainly from the genera *Acacia*, *Senna* and *Eremophila*. The potential hosts grow on a variety of land types including granite outcrops, greenstone ridges, sandplains, washplains and saline soils. A better understanding of suitable host species and of soil types in which to grow *S. spicatum* will help future regeneration programs in the rangelands.

S. spicatum root biomass and oil content are being investigated to determine whether a higher proportion of root should be harvested. The commercial value of *S. spicatum* roots that remain after conventional harvesting is not known. If a significant proportion of the *S. spicatum* root is not extracted using conventional harvesting techniques, then a new technique could be used to increase tree utilisation. This harvesting procedure would have to be practical in terms of time efficiency and cost.

Santalum spicatum tree-farm systems for the W.A. wheatbelt region

Research into the establishment of commercial *S. spicatum* tree farms in the wheatbelt has also been conducted by CALM for more than ten years. The objective is to establish *S. spicatum* on farms with a medium to high annual rainfall (400–600 mm). Planting *S. spicatum* would also form part of the revegetation program to reduce salinity in the wheatbelt (Havel and McKinnell 1993; Bailey et al. 1997).

S. spicatum is commonly established by direct seeding beneath 1–2 year-old host trees, such as *A. acuminata*. This simple technique is a practical method of incorporating *S. spicatum* into wheatbelt farm forestry programs. Establishment success and initial growth rates have been encouraging, with a mean annual diameter increment of 7.5 mm (at 150 mm). This growth-rate is very fast compared with trees growing naturally in the rangelands, which normally have an annual diameter increment of 1–2 mm. Current research is examining the effects of host species, stocking rates and soil types on *S. spicatum* growth.

Irrigated *Santalum album* tree-farm systems for northern W.A.

Recent investigations into techniques for irrigated *S. album* plantations in northern Western Australia also have the objective of supplementing the *S. spicatum* green-wood harvest from natural stands (Kealley 1991). Indian sandalwood, due to its religious significance and high heartwood santalol oil content, is the most economically important of all *Santalum* species (Srinivasan et al. 1992; Radomiljac et al. 1998b). With very few exceptions, *S. album* has endured a level of exploitation unrivalled by other timber species.

S. album tree farm development in northern Western Australia has been made possible by an immense man-made dam near Kununurra which supplies water to flood-irrigate 13000 hectares of agricultural land known as the Ord River Irrigation Area (ORIA) (Fig. 2) (Radomiljac and Borough 1995). The establishment of *S. album* and other tropical timbers within the ORIA also has the objective of ameliorating the imminent problem of a rising ground-watertable Rising ground-watertables and ground-water salinisation have reduced the productivity and sustainability of irrigated agricultural schemes worldwide.

A series of recent silvicultural studies has identified a protocol for *S. album* silviculture; as for other *Santalum* species, this silvicultural system is more complex than traditional monocultural systems as it involves the establishment of a debilitating root hemi-parasite (Radomiljac 1998; Radomiljac et al. 1998a).

Global sandalwood production has declined markedly over the past 20–30 years (Srinivasan et al. 1992; Havel and McKinnell 1993; Radomiljac et al. 1998b), due to:

- unsustainable exploitation and spike disease (Rai 1990);
- uncontrolled fire and grazing (Havel and McKinnell 1993);
- illegal harvesting (Murthy 1985);
- inappropriate plantation silvicultural systems (Harisetijono and Suriamihardja 1993); and
- regulations that are a disincentive to *Santalum* conservation (Husain 1983).

The comparative advantage of *S. album* tree farms at ORIA is that there are no evident impediments to successful plantation development. There has been no report of major *S. album* pest and disease problems, a sound silvicultural system has been developed and is now routinely used, uncontrolled fire and grazing do not occur, and the ORIA is close to major Southeast Asian markets.

Nursery propagation commences in October and it usually takes about nine months to raise robust *S. album* seedlings. Seed propagated *S. album* is raised in large nursery containers. Three months after germination, cuttings of a herbaceous pot-host (*Alternanthera* spp.) are placed into each seedling container (Radomiljac 1998). This parasite-host combination continues throughout the nursery phase and into the early stages of field establishment. Intermediate and long-term host seedlings are raised simultaneously in the nursery, and are strategically placed in the field at establishment. Field establishment occurs in June and July. The long-term host, which must persist as the final host for the entire rotation-length, is planted up to four metres from the *S. album* seedling. The rotation-length for ORIA tree farms is still unclear, but is estimated to be 25–30 years. An intermediate host is planted between the *S. album* and long-term host seedlings. The intermediate host is parasitised for 4–5 years, until it dies (Radomiljac and McComb 1998). Superior intermediate hosts are usually fast-growing, short-lived leguminous trees (Fig. 3) (Radomiljac and McComb 1998).

Rather than considering *S. album*'s parasitic requirements an impediment to tree-farm development, the current research program is investigating the incorporation of one or more high-value timber species in the silvicultural system to act as long-term hosts. A biodiverse farm forestry system, producing two or more high-value timber products, appears possible.

International Sandalwood Management and Marketing Cooperation

A period of sandalwood scarcity is looming in both India and Indonesia, the main producers of *S. album*. In these countries, the decline in the sandalwood resource is due to heavy pressure for clearing forested land for food production, the destruction of sandal host trees for wood products such as fuelwood, and illegal harvesting (Srinivasan et al. 1992; Havel and McKinnell 1993). For example, in India the volume in the official sales of sandalwood from the two main producing States, Karnataka and Tamil Nadu, has declined. Also, whereas 25 years ago only 10 trees were harvested to produce a tonne of sandalwood, about 100 trees are required now (Karnataka and Tamil Nadu State Forest Department data), indicating that the average size of *S. album* trees being harvested has fallen significantly. This is strong evidence of serious resource decline.

There is interest in *Santalum* species plantation establishment in response to the pressures on sandalwood supplies. In many developing countries, a major incentive to plantation establishment is the increasing pressure for conversion of natural forest land to agriculture. This means that increasing demands for timber supplies must be met, at least in part, by producing more timber from intensively managed plantations. For *Santalum*, plantations are the only way to redress increasing problems of resource availability of this important genus (Hamilton and Conrad 1990).

It is important that the Indian supply of sandalwood be maintained: if it disappears, there are potentially serious downstream effects for Australia and South Pacific producers such as PNG, Vanuatu and Fiji. If the natural sandalwood market collapses due to supplies declining below a certain level, there could be three serious outcomes for those countries. First, there would be overwhelming pressure to overharvest to compensate for the loss of the Indian resource, thus destroying current management programs, and possibly leading to the loss of some species. Second, the opportunity to develop viable and sustainable industries based on *S. yasi*, *S. macgregori* and *S. austrocaledonicum* in the smaller South Pacific countries would be lost. Third, alternatives to sandalwood or sandalwood oil may be found, making it difficult to recapture the market when plantation sandalwood becomes available.

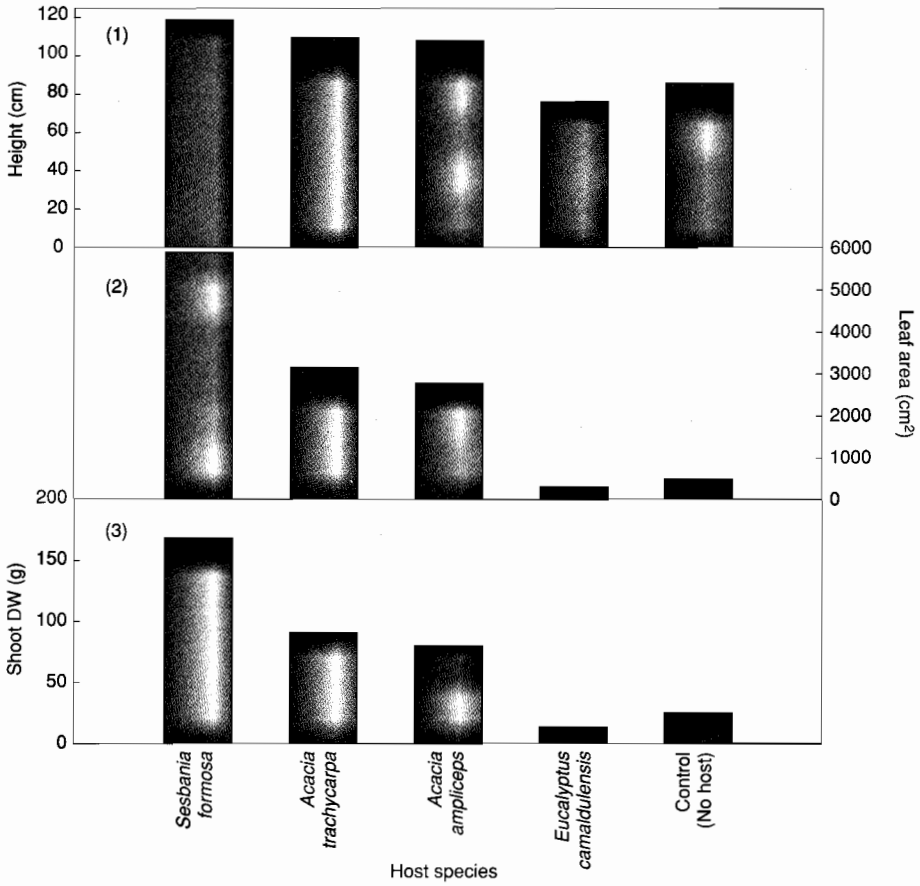

Figure 3. *Santalum album* (i) mean height, (ii) mean leaf area and (iii) mean shoot dry-weight (DW) whilst grown with *Sesbania formosa* (Papilionaceae), *Acacia trachycarpa* (Mimosaceae), *A. ampliceps*, *Eucalyptus camaldulensis* (Myrtaceae), and as a no-host control for 38 weeks as single-plant pairings in 25-litre pots under nursery conditions, near Kununurra.

The benefits of a coordinated sandalwood marketing strategy are clear in light of the looming global sandalwood supply crisis. A fragmented sales approach, such as in Indonesia, PNG, Vanuatu and Fiji, allows many buyers to purchase sandalwood from a position of strength, often offering low prices for premium sandalwood logs (Gjerum et al. 1995). This cheaply purchased wood is either on-sold at greatly inflated prices or stockpiled to wait for supply levels to fall further. The production and marketing of sandalwood is shrouded with mystique and secrecy which makes the task of gaining reliable information on global supply trends and prices very difficult. This creates large discrepancies in trade between large and small producers. For example, both India and Indonesia supply sandalwood from the same species, *S. album*, and therefore one would assume that the product quality is similar. However India receives about US$13 000 per tonne, whereas Indonesia prices are as low as US$4000–5000 (Radomiljac et al. 1998b). A further complication to sandalwood marketing is the large volume of traded illegal wood. This trade not only threatens the future supply of sandalwood from natural stands, but also severely undercuts the value of legally produced wood.

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Malady and Remedy of Sandal Cultivation in Farmlands and Private Lands—An Overview

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Abstract

Sandal is one of five scheduled trees in Tamil Nadu. The tree is called the 'Royal Tree' because its distribution is restricted, its oil is unique and of high value, and the santalol in the heartwood makes the wood impenetrable by termites. So far there are no private plantations of sandal in India; but, of late, due to rapidly increasing market demand and price for sandalwood oil, and possible relaxation of the rules by the State Government, farmers are beginning to show willingness to cultivate sandal. This paper highlights the difficulties faced by planters in protecting the trees, disposing of the wood, and conforming to the legislation. Suggestions are also made on how to motivate farmers to grow sandal on a large scale.

SANDAL IS ONE of five scheduled trees in Tamil Nadu. *Santalum album* L. is called the 'Royal Tree' because:

- the oil extracted from sandal is of high value in perfumery and medicine;
- the sandal heartwood is extensively used in carving works;
- the heartwood contains 'santalol', which makes the heartwood impenetrable to termites;
- the sandal plantation is nearing extinction due to over-exploitation and failure of artificial regeneration methods;
- there is no known artificial substitute for sandal oil; and
- the distribution of sandal is limited.

For these reasons, sandal is considered precious and is accorded 'scheduled tree' status.

Sandal is found all over India. The area covered in Karnataka is 5254 km^2; Tamil Nadu, 3045 km^2; Andra Pradesh, 175 km^2; Maharastra, 84 km^2; Madhya Pradesh, 33 km^2; and Kerala, 15 km^2. Extensive studies have indicated that *S. album* is the only species yielding high quality sandalwood and oil. A few other species of *Santalum* and four other genera also yield fairly scented wood oil, but the quality of *S. album* oil and wood is superior (Kaikini 1969; Srinivasan et al. 1992). The sandal area and the actual plantation area is declining drastically due to over-exploitation, poor germination, poor regeneration, and failure of artificial regeneration.

Problems of Sandal Cultivation on Private Lands

The difficulty in protecting sandal trees from smugglers is the main reason for not growing sandal on private farmlands. The assurance of the state governments paying 70–75 per cent of net sale proceeds to private sandal growers is affected by red tape and procedural delays in state extraction and disposal of sandal.

Marketing is done only by government. Pricing is done by the government at its discretion, and only a minimum percentage of the value is given to the farmer. Hence, there is no financial assurance for

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him. The farmer has very little control over sandal cultivation and marketing.

Smuggling

This is a major problem in all districts and states wherever sandal grows. Even the Government finds it difficult to protect its forest wealth from smuggling. In this context, it would be difficult for farmers to protect even stray sandal trees. A sandal plantation is bound to attract even armed smugglers. The general decline in the incidence of sandal in Government forests has boosted the high market price which makes the business of smuggling much more lucrative. Latest model lorries, cars and vans are used by the armed smugglers aided by cellular telephones.

Rotation

All the forestry species have a specific rotation age. In the case of sandal, a physical rotation is followed, where the extraction is done after the death of the tree. This long rotation period is another major problem for farmers' economic return.

Edaphic and related factors

Three particular concerns discourage sandal cultivation on private lands:

- sandal requires a host plant for nutrient uptake during the seedling stage, but the period for which a host is required is not known;
- the absence of control measures against 'sandal spike disease' (from which protection is essential) discourages sandal cultivation; and
- sandal plantations regenerate naturally, but there are very few artificially regenerated plantations in existence due to difficulty of artificial regeneration.

These issues are discussed in Thirawat (1955), Jain et al. (1988), and Srinivasan et al. (1992).

Effects of the various States' Forest Acts

Due to the high value of the wood and oil both in India and abroad, sandal had been declared a reserved tree in all states of South India during the times of British Raj and the Feudal States. The existence of sandal trees even on private lands had been recorded in the revenue records during the survey settlements. The Forest Act provided for:

- special rules for extraction and disposal of sandal trees in all states;
- monopoly extraction of sandal trees on both Government and private lands by the state forest department agencies;
- special protection for sandal on private lands;
- information on sandal thefts; and
- payment of fees to owners of private lands for sandal trees growing therein, after extraction and disposal.

The rules paved the way for control on smuggling; but they could not provide complete protection. Thefts were widespread due to weaknesses in the Act, and inadequate forest officials. The very high remuneration for sandalwood billets paid by the distillation units motivate the common people towards theft. These people manipulate through providing wrong information; and mix good quality sandalwood billets from the natural forest with poor quality sandalwood from private growers.

The Forest Acts provide for total control of sandal. Governments handle felling, extraction and marketing themselves, and thereby control the smuggling to a major extent. They also regulate the trade effectively, thereby keeping sandal prices stable. The Acts provide for rigid rules regarding sandal. For example, the Madras Forest Act of 1882 provides for the maintenance of accounts in respect of all classes of sandalwood stored on private lands, markets or factories, for industrial or commercial purposes.

The differences between the states in legislation controlling sandal is the main problem in controlling smugglers. For example, there are 19 distillation units in Kerala, of which 15 are located in the border region with Tamil Nadu; they use sandalwood which is mainly smuggled from Tamil Nadu. This is only possible because of differences in the Forest Act between the two states.

The procedure for extraction and disposal

The procedure for extraction, transport and conversion is long, rigid and time-consuming. Trees destined for extraction have to be marked, measured and uprooted by digging the nearby soil. Every piece below two cm in diameter must be numbered before transport. At the depot, cut sandal trees are transported, and the billets are restacked to form the tree. Sapwood removal is called 'dressing to heartwood'. The dressed wood is classified into 24 different classes for use and disposal.

Disposal from the depot is by periodical open auction. From there it goes to state-owned factories, state-owned corporations, and (under concessional quota) to artisans and religious institutions, and small-scale retail outlets for religious purposes. Payment to the owner of the trees is 75 per cent of net

sale value, after deducting costs of extraction and dressing and 10 per cent supervision charges. The payment of a bonus to farmers is based on past average sale prices revised by the government sales outlets. These prices are fixed and revised at 1–2-year intervals by the forest department. Market prices for illicit trade in sandal are controlled by the distilleries.

Pros and Cons of the Existing Rule

The existing rule is that the landholder is responsible for the preservation of the trees which are the exclusive property of the state government. Any injury or theft must be reported to the forest officers or police. No person shall possess sandal in the form of wood or oil in any form without a valid licence except for bona-fide domestic use. Permission must be obtained for felling or exploitation, and stock must be declared. These rules help tree growers and farmers prevent theft or injury to trees.

The sandal is being smuggled for its high price. Even with the existing stringent rules, the smuggling rate is very high. Many vehicles are seized from smugglers by the forest department officers. Evidently, smuggling is rife; but if the rules were removed, it would become even more so.

Conclusion

There has been increased interest among farmers in growing sandal in farmlands and private lands. Although there are some bottlenecks and administrative delays, the existing rules should be continued to protect the sandal trees in the farmers' fields as well.

Before any action is taken to renew or remove the rule, the pros and cons should be carefully considered. Farmers and private people are affected on one side by the lack of adequate knowledge on sandal cultivation, and on the other side by the rigid rules of the present forest legislation. We suggest that if the rules are better publicised by the Department and the share paid to farmers increased, many farmers would go into sandal cultivation and buying agencies could be arranged by the farmers themselves. Differences in the Forest Acts between the three states considered could be amicably settled to overcome the problems of smuggling and over-exploitation.

Acknowledgment

We are very grateful to our Director K. Subramaniyam, IFS, Institute of Forest Genetics and Tree Breeding, for his continuous encouragement. Thanks are also due to the Dr Kumaravelu, Group Coordinator (Research) for his valuable suggestions.

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The Effect of Sandalwood Availability on the Craftsman Community

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Abstract

Sandalwood (*Santalum album* Linn.) is supplied by the Karnataka Forest Department (KFD) to traditional and professional carvers on registering their names with the Karnataka State Handicraft Development Corporation. A survey was conducted on the utilisation of sandalwood around Sirsi. It was found that a decrease in the availability of sandalwood has resulted in the redesign of woodworking and carving tools in order to maximise utilisation. Further, there is partial substitution of sandalwood by sandalwood-like timbers in the concealed portions of the carved items. There is also a trend to work on other timber species, as the available sandalwood is insufficient to keep people fully employed.

SANDALWOOD HAS BEEN intimately associated with human civilisation since time immemorial. It has fascinated artists and craftsmen. This wood, 'nature's gift to mankind' has been known to Indians for some 23 centuries (Campbell 1883). Sandal is legally treated as a Government tree in Karnataka where, as in other states of India, it is sold to the public by auction sales.

The traditional community of professional sandalwood carvers is known as the Gudigars. The carvers are found in small numbers in Sirsi, and also in Siddapur, Kumta, and Ankola (small towns in Uttara Kannada district) and Sagar and Soraba (the neighbouring towns in Shimoga district). They came to settle in Uttara Kannada after the establishment of Portuguese rule in Goa, in the neighbouring state of Karnataka. They carve sandalwood, ivory and ebony with great skill. The range of their exquisite articles includes carved boxes, jewellery boxes, small utility boxes, chariots, elephants, camels, photo frames, fans and fan handles, letter-openers, pen-holders, combs, card cases, and a host of other fancy items.

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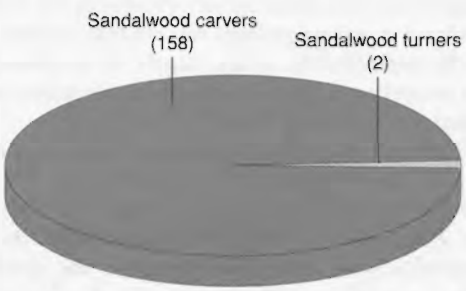

Figure 1. Number of craftsman families engaged in sandalwood work

A survey was conducted among the 160 members of the carving community in and around Sirsi to determine the pattern of usage of sandalwood, one of the finest carving woods for intricate work (Fig. 1). Each member of the carving community has registered his name with KFD to get his quota of sandalwood. The approximate requirement is around 18 tonnes per year for the whole community; this is calculated at a rate of 9.5 kg heartwood per month per carver, and 18 kg per month per turner. Earlier, they were getting sandalwood at regular intervals, but the supply is now erratic

and fluctuates from year to year. The availability of sandlewood from 1991–92 is shown in Fig. 2.

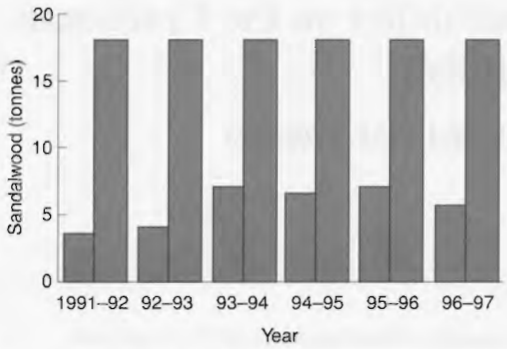

Figure 2. Availability of sandalwood

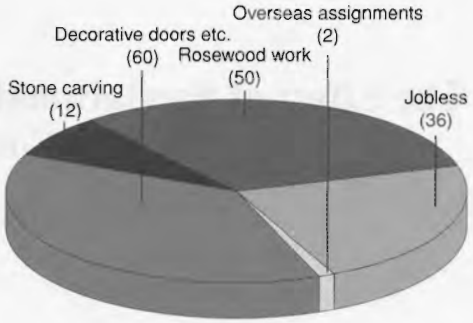

Figure 3. Craftsman families engaged in other activities during slack periods

Due to the shortage of raw material, they have tried to achieve maximum utilisation of available material by improving their equipment and methods in ways such as smaller gauge saws, smaller chisels, and thinner slices while planing the wood. They have also reduced the number of wooden members in the assembled carved items. The handicraft agency also has started buying waste sandalwood chips on a weight basis from these carvers. Previously the waste produced in making garlands was not fully utilised.

The unpredictable, erratic supply of sandalwood has compelled them to turn to either alternative raw material or alternative work.

Craftsmen started substituting sandalwood in some parts of carved articles with wood similar in colour, texture, lustre and grain without affecting the decorative aspects and quality of the carved items. These substituted wood pieces belong to the tree species *Adina cordifolia* and *Mitragyna parvifolia*. The substitute pieces are in hidden parts of the carved articles, such as the base of some boxes and partition plates. Joints and matchings between the sandalwood and other wooden pieces are so perfect that they cannot be distinguished from the original sandalwood. The small wooden pieces required for this purpose are generally selected by the professional carvers from the sawmill wastes; they are called 'junglewoods' and cost little. The amount of substitution depends on the size and pattern of the articles; it is generally 30–40 per cent by weight of the assembled articles.

The erratic availability of raw material causes uncertainty in continuity of work and livelihood (Fig. 3). Carvers diversify into other timbers which are more regularly available with reasonable and affordable rates (Pearson and Brown 1932).

Shivni (*Gmelina arborea*) is excellent for carving figures and statues. The wood is easy to work, has a good lustrous ivory finish, and fetches a good price for carved articles.

Rosewood (*Dalbergia latifolia*) is gaining in importance as it is readily available compared with sandalwood and Shivni. After the completion of work with sandalwood allotted to them, the artisans engage in carving rosewood cabinets and other fancy articles of interest. Only about one-third of sandalwood carvers have switched to rosewood.

Some have started carving on locally available stones during their slack periods. They also accept orders for carving main door and window frames and shutters and other cabinet work for temples. Teak (*Tectona grandis*), jack (*Artocarpus integrifolia*), and rosewood are mainly used for these purposes. As their work is superior to that of traditional carpenters, their wages are higher.

There is no immediate solution to their problem of raw material shortage. Effective steps are to be taken for establishing sandal plantations, as in the case of some fast growing hardwoods. The KFD may plan for annual planting of sandal in its natural distribution range with other economically important timber yielding species. This is a ray of hope for this professional carving community starved of raw material.

Acknowledgment

The authors are grateful to the officials of KFD, Sirsi Division for their cooperation in this survey.

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A Strategy for Sustainable Supply of Sandal

V.V. Srinivasan, H.S. Ananthapadmanabha and C.R. Rangaswamy*

Abstract

Tree improvement in sandal mainly aims to develop trees that can yield more heartwood and oil in minimum time. It is imperative that they are resistant to spike disease, and attack by heartwood borers and other pests and diseases. Our knowledge regarding the biology, bionomics, seed, nursery techniques, vegetative propagation, growth yield and tree improvement of sandal is extensive. The literature has been reviewed in detail, and new management practices by which yield and production could be improved have been suggested. In spite of availability of large areas where sandal can grow, and improved seeds and management practices, production has not increased to the desired extent. Hence the supply of wood and oil has been gradually decreasing over the last decade. To meet current and future demands on a sustainable basis at reasonable prices, it is imperative that immediate efforts be made to cultivate sandal both in forests and private lands. Current issues on cultivation of sandal, including conservation of germplasm for sustained yield, are discussed in this paper.

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Is Sandalwood Oil 'Forest Produce'? A Scientific and Legal Perspective

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Abstract

What is 'wood oil'? Is sandalwood oil, obtained from *Santalum album*, a wood oil? Or is only the gurjan balsam obtained from *Dipterocarpus* spp. the wood oil? The legal profession grappled with these questions. Resolving them was essential to establishing whether sandalwood oil is forest produce or not. This paper talks about how scientific input helped the legal profession to resolve this tangle.

Keywords: sandalwood oil, wood oil, gurjan balsam, forest produce

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Trade Liberalisation in Sandalwood

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Abstract

Sandal (*Santalum album*) occurs naturally in southern states of India such as Karnataka, Tamil Nadu and Kerala. It had an export market from the period of Tippu Sultan or earlier for European and Middle-Eastern countries; it was therefore declared as a 'Royal Tree' in Karnataka. Sandal is Government property wherever it is found in Karnataka, and a special chapter exists in the Karnataka Forest Act. There are strict rules and regulations for its protection in, and harvest from, private lands, and for its movement, trade and use. Such restrictions can also be counterproductive; for example, people destroy regeneration in their holdings. Even the declaration of a bonus up to 75 per cent of its value has not encouraged sandal growing, because of the inherent procedures and delays. There is a need for rethinking and liberalising the existing rules, regulations and restrictions. The payment of a bonus also needs simplification.

SANDAL GROWS NATURALLY and extensively in Karnataka, Tamil Nadu, and Kerala. It was also introduced into other areas including parts of Andhra Pradesh, Orissa and Madhya Pradesh, but with little success. It occurs in southern dry deciduous and thorn forest types, either along with other species as an 'associate' or along farm bunds and fences of private holdings. Natural regeneration is spreading faster through birds eating the fleshy fruits that are produced twice annually. *Santalum* sp. is also reported in Australia, Indonesia and New Zealand.

Scented heartwood of the sandal tree that yields the famous sandalwood oil was in great demand in European and Middle-Eastern countries for products such as perfumery, and hence it has been exported since the time of Tippu Sultan, or even earlier, from South India. Due to its great importance and value at that time, it was declared a 'Royal Tree' although a clear definition of that term is not forthcoming from any records, including the Indian Forest Act ('The IFA') and the Karnataka Forest Act ('The KFA') of 1963.

Wherever the sandal tree is found in Karnataka, it is the property of the State Government. No-one is allowed to buy, sell or trade except through the Forest Department. The major quantity of heartwood obtained from dead and dying trees has been harvested for Sandal Oil Factories at Mysore and Shimoga. Small quantities are being released on a retail sale basis and for temples and carving. Small consignments were also exported on a trial basis by the Karnataka State Forest Industries Corporation.

Restriction on Trade

Each state has its own rules and regulations on sandal trees. Indeed, the Sandalwood Advisory Committee formed by the Government of India could not make any positive suggestions to satisfy all the member states.

This itself can be a problem. For example, in Kerala, even though sandal trees are not available in appreciable quantities, several small-scale sandalwood distilleries have been established along the bor-

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der of Karnataka and Tamil Nadu and most of the smuggled billets reach there. The reduced intake by the sandal oil factories of Mysore and Shimoga has caused a large accumulation of sandal heartwood stock in the kotis of the Forest Department of Karnataka. This has caused anxiety about fire protection during storage, leading to payment of huge insurance premiums annually.

Sandalwood is defined as 'forest produce' under section 2(7)(a) of the KFA, whether found in or brought from a forest or not. Therefore the control of sandalwood in transit is vested in the Karnataka State Government (s. 50), like any other forest produce listed under section 7(a). It is not however listed as a 'reserved tree' in section 2(15), probably because of the special status already given to it in the KFA; for example, the KFA has a separate chapter (Ch. X) relating to sandalwood. The IFA does not have any special provisions for sandalwood.

Sandalwood is an exclusive property of the Karnataka State Government (s. 84), and under section 85, the landholders are responsible for the preservation of all sandal trees grown thereon. If they fail to report any theft of or injury to sandal trees, they are liable for a penalty and for compensation to the State Government. Conviction of an offence pertaining to sandal trees brings imprisonment up to seven years and fines up to Rs. 25000.

Such stringent rules have discouraged sandal trees in private landholdings. People uproot the sandal recruits and small seedlings to avoid responsibility and future penalty in the event of any offence. This has considerably reduced the population of sandal trees on private holdings. A licence to store, sell or disintegrate sandalwood or sandalwood oil is required under section 87. This has also discouraged entrepreneurs from taking up industries based on sandalwood as raw material such as soaps and perfumery products. The sandalwood definition under section 2(18) includes even bark and leaves which have no monetary value, yet the same restrictions and penalties are imposed, thereby further impeding the growing of sandal trees.

Under Karnataka Forest Rules (KFR) 1969, section 104, all owners are required to declare the presence of sandal trees on their land, and renew that record every five years to include all new recruits. The owner will have right to bonus on harvesting only if he has declared the trees to the local Division Forest Officer. This in fact binds him to protect the sandal trees, failing which he becomes liable for penalty and punishment as well as compensation. Therefore many people believe it is safer to remove and destroy sandal trees as they regenerate on their land.

There is also an elaborate procedure to obtain bonuses from the Forest Department. The bonus is 75 per cent of the value of the harvest, less the working and handling costs of the Forest Department. To speed up payment, owners help to uproot the trees and transport them to the nearest depot. Even so, it can take several months before the material is converted and assessed for the payment of the bonus. There are a lot of complaints that the amount one finally receives is far less than that estimated or assured, due to careless and bad conversion. Even after the bonus is declared, the payment from the Divisional Office comes only after sufficient allotment for the purpose is received from the Government, which sometimes takes 1–2 financial years. By this time, the owner might have paid several visits to the Divisional Office expecting payment and returned disappointed.

Even if half of the estimated bonus were to be paid on the spot, owners would still be unhappy with the system. Normally dead and dying trees are harvested by the Forest Department; but if one wants to remove a few living trees to make room for buildings, plantings, or other purposes, it may take many months before approval is granted. All these procedures, rules and delays have put a curse on the existence of sandal trees on private lands. Even if one or two mature sandal trees are available on any private lands, it is always under threat of illicit felling or smuggling, leading to harassment of owners by the Police or Forest Department.

Liberalised Trade

Sandal tree is the only tree species subject to such severe restrictions. Catechu tree (*Acacia catechu*) and rosewood tree (*Dalbergia latifolia*) also have some regulations under the Karnataka Forest Rules. The nationalised sale of rosewood in Karnataka has also been criticised due to elaborate sale procedures and delay. Many people have left the fallen or dead rosewood trees to decay on their lands rather than salvage them for money. Many have made furniture out of it, thus depriving its best utilisation and revenue to Government.

At present, any wood is valuable. For example, 1 m^3 of good quality rosewood will fetch Rs. one lakh

or more. Some tree companies have asserted that an investment of Rs. 1000 in teak (*Tectona grandis*) would return about Rs. five lakhs in 20 years, which is more than the expected value of sandalwood.

The smuggling or illicit cutting of sandal trees is still rampant in spite of all these restrictions, and has not in any way contributed to the preservation of sandal trees. Lopping of sandal trees by graziers continues on government lands. The rules and regulations are more frequently flouted than adhered to, due to laxity on the part of the controlling authorities. The collection of dry fuelwood on head loads and free grazing of cattle permitted by the Government in Reserve Forests, and the ongoing encroachments in government lands including Reserve Forests, leave little scope for any control and thus the provisions remain mainly 'on paper'.

Wood of many species has become more expensive, but we cannot put all of them under such rigid restrictions. The argument that once the rules are amended and relaxed for sandal, there will be large-scale exploitation of this species leading to wiping out of the entire population, is baseless. If that is so, it would have happened to teak and rosewood species already. On the contrary, many are investing in teak plantations offered by companies.

In this period of liberalisation in all sectors, it is necessary to review these restrictions and amend the legislation to bring in free trade in sandalwood also. People should be able to possess and sell sandalwood to anyone who offers a good price. The Forest Department should make arrangements to protect their own forest produce without imposing any restrictions on others' material. This will encourage people to grow more and more sandal trees because of the value it fetches at present. Moreover, people must also grow other tree species as host plants, and this itself will encourage tree-growing. This would indirectly help in bringing more trees onto farm lands thus improving the ecology and environment of the locality.

Sandal trees are not very specific to soil and climatic conditions and establish quickly and easily in many soil types and climates. Hence growing sandal trees will be attractive as a supplementary income. Once the population of sandal trees increases and the market rate stabilises, there will be no need to fear theft or smuggling because people themselves will protect their valuable property. Also, the price may ultimately come down making it less attractive for smugglers.

Conclusion

The restrictions imposed on the Royal Tree more than 200 years ago, when demand was greater and the then rulers wanted a monopoly, deserve to be relaxed and free trade introduced as for any other valuable tree species or forest product. Free trade will encourage people to grow sandal trees and even tree companies may come forward to raise sandal trees as they now do with teak, poplar and mangium.

Biotechnology and Silviculture

cDNA Cloning and Characterisation of a Proline- (or hydroxyproline-) rich Protein from *Santalum album* L.

G. Lakshmi Sita*[A] and Anirban Bhattacharya*

Abstract

A proline- (or hydroxyproline-) rich cDNA clone, *SaPRP,* was isolated from sandal *(Santalum album* L.), somatic embryos pre-treated with salicylic acid. The longest open-reading frame in *SaPRP* encodes a polypeptide of 326 amino acids. It reveals that 48 per cent identity in 233 amino acids overlap with proline-rich glycoprotein from maize. Southern hybridisation with sandalwood genomic DNA digests suggests that *SaPRP* possibly belongs to a small gene family. From 'northern blot analysis', this *SaPRP* is expressed predominantly in leaf tissues The induction of the *SaPRP* was observed in somatic embryos treated with salicylic acid.

Key words: cDNA cloning, cell-wall protein, salicylic acid (SA), plant defence, systemic acquired resistance (SAR)

COMMERCIALLY, SANDAL *(Santalum album* L.) is an important tree. Annual world requirement for sandalwood oil is about 200 t, which equates to 10 000 t of wood. Only ten per cent of this is met from natural resources. Sandalwood production has decreased considerably in the last decade. This is attributed to many factors, particularly disease. For example, spike disease caused by mycoplasma is a major threat to sandal populations. Tree improvement in sandal mainly aims to develop trees which can yield more heartwood and oil. It is imperative that they are resistant to diseases like spike, to heartwood borers, and to other pests and diseases. Several methodologies such as application of chemicals have already been tried, but they are neither adequate nor economically justified for field application.

Two options are available to the forester. One is to identify the phenotypically disease-free plants, and produce large numbers of them by biotechnological approaches such as tissue-culture. The second approach is to genetically produce disease-resistant plants by classical breeding. However, conventional breeding of forest trees including sandal has been difficult.

Another approach is to understand the biochemical and molecular basis of disease, clone the genes for disease-resistance, and transfer them into the chosen plants. Plants have evolved an array of biochemical defences against invading organisms such as fungi, bacteria, virus, and mycoplasma. The plant defence response is accomplished by activation of a plethora of defence-related genes such as pathogenesis-related (pR) genes; these are a group of inducible host-plant encoded proteins whose synthesis is associated with resistance to plant pathogens, as well as with various forms of physical and chemical stress (Carr and Klessig 1989). Plants also induce structural changes in cell-wall composition with the accumulation of cell-wall proteins as a result of infection. These include:

- extensins, which are hydroxyproline-rich glycoprotein (HRGP) (Chen and Varner 1985);
- glycine-rich protein (GRP) (Condit and Meagher 1986; Keller et al. 1988); and
- proline-rich protein (PRP), regarded as reactive biopolymers (Hong et al. 1987, 1990).

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These proteins are characterised by basic repeat motifs that vary among the different classes of cell-wall proteins: Ser-$(Hyp)_4$ for extensin, $(Gly\text{-}X)_n$ for GRP, and Pro-Pro-Val-X-Y for PRP. They are generally specified by plant gene families that are induced during infection, as well as by biotic and abiotic elicitors. In contrast to the situation in crop plants, information about tree species is somewhat limited (Bao et al. 1992).

Our laboratory has been involved in the application of biotechnology for the development of disease-resistant sandal trees. Using cell and tissue-culture technology, we have produced disease-free trees (Lakshmi Sita et al. 1979; Lakshmi Sita 1986, 1991). However one cannot be certain whether they are genetically-resistant trees. There are reports indicating that over-expression of pathogenesis-related genes like chitinase and glucanase in tobacco have resulted in transgenic plants with somewhat more tolerance against fungal diseases (Zhu et al. 1994). Hence we have undertaken the following study to clone pathogenesis-related genes and over-expressed these in transgenic sandal trees. We report here the successful cloning and characterisation of the proline-rich protein, cDNA (*SaPRP*) from sandal.

Materials and Methods

Plant material

Somatic embryos used for this study were obtained by direct somatic embryogenesis from internodal segments of young shoots. Briefly, explants were inoculated in MS medium supplemented with thidiazuran (TDZ) and benzyl amino purine (BAP) for direct somatic embryogenesis. Globular embryos thus obtained were transferred to MS medium supplemented with gibberellic acid (GA). Somatic embryos 3–4 weeks old were used for induction with salicylic acid (SA) in the subsequent experiments, Leaf and stem tissues used for expression studies were collected from matured trees.

Construction and screening of cDNA library

The somatic embryos were treated with l0 mM salicylic acid for 24 hours. Total RNA was prepared using the GITC-acid phenol extraction method (Condit and Meagher 1986). Poly A+ RNA was prepared using 'messenger affinity paper' (Amersham). Double-stranded cDNA was prepared, adaptor-ligated, and then cloned in λ*gt-11* using Amersham's cDNA synthesis and cloning module kit. The library thus prepared was amplified before screening. It was then screened by duplicate plaque hybridisation using soybean extensin cDNA clone as a probe. The plaques were transferred to nylon membrane (Hybond N, Amersham) and pre-hybridised for four hours at 55°C in 6XSSC, 5X Denhardt's solution, 0.1% (w/v) SDS and 250 µg/ml salmon sperm DNA (Condit and Meagher 1986). The labelling of soybean extensin cDNA insert with α-^{32}P dATP was done by the megaprime labelling kit (Amersham). Hybridisation was carried out for 24 hours under same conditions as the pre-hybridisation. Filters were finally washed under 'moderately stringent' conditions (0.5X SSC, 0.1% SDS at 50°C). Positive plaques were selected and subjected to another round of screening at low density to ensure purity and elimination of false positives. Positive plaques were then subjected to PCR analysis using λ-gt11 primers to check the size of the inserts. The authenticity of the biggest cDNA (1.8 Kb) was confirmed by 'southern hybridisation' using the same probe under the same conditions (Sambrook et al. 1989). Lambda DNA was isolated as described by Ausubel et al. (1992). The *EcoRI* insert was finally subcloned in pGem3Zf(-) to yield *pSaPRP*.

Sequencing of *pSaPRP*

Sequencing was carried out either by *Sequenase* (version 2.0) DNA sequencing kit (USB Biochemicals), or by the automated sequencing facility. The analysis of the amino acid sequence was carried out using a *Swiss Prot.* data base.

DNA isolation and blot hybridisation

Total genomic DNA was extracted from *S. album* tender leaves following Dellaporta et al. (1983). Aliquots of 10 µg DNA were digested with different restriction enzymes, separated in 0.8% agarose gel and transferred onto a nylon membrane, *Hybond N* (Amersham). Pre-hybridisation and hybridisation were carried out in 50% formamide, 6XSSC, 5X Denhardt's solution, 0.1% SDS and 250 µg/ml salmon sperm DNA at 42°C. 1.8 Kb *pSaPRP* insert was labelled with α-^{32}P dATP by the 'megaprime labelling kit' (Amersham) and used as probe. The blots were finally washed under 'high stringency conditions', 0.1XSSC, 0.1% SDS at 65°C for 30 minutes.

RNA isolation and northern blot hybridisation

Total RNA was isolated, following Chomczynski and Sacchi (1987), from sandal leaves, stem pieces, and somatic embryos either treated with SA or mock-

treated with water. Before electrophoresis, the RNA was suspended in 50% formamide, 20 mM 3-(N-morpholino)-propanesulphonic acid (MOPS), 5 mM sodium acetate, 1 mM EDTA, 6% formaldehyde, 6% glycerol, and incubated at 65°C for 5 minutes. 10 µg of RNA was applied per lane on a 1.2% agarose formaldehyde (40% v/v) gel. Ethydium bromide was added in the gel to confirm the quality of the samples. After electrophoresis, the RNA was transferred onto a nylon membrane, *Hybond-N* (Amersham). The hybridisation conditions were the same as those described in southern blotting experiments. The filters were finally washed at 55°C for 20 minutes in 1X SSC, 0.1% SDS. The same blots were re-probed with rice rRNA gene probe to confirm equal loading of the samples. All these hybridisation experiments were carried out at least three times to confirm the observations.

Results

Isolation and sequence analysis of the proline-rich protein cDNA clone

The cDNA library, constructed from SA-treated sandal somatic embryos was screened with soybean extensin cDNA as a probe under moderately stringent conditions. After multiple rounds of screening, 21 positive plaques were picked up for PCR analysis using λgt-11 primers. The size of the inserts was determined by agarose gel electrophoresis; the largest one, 1.8 Kb after subcloning in pGEM3Zf(-), was taken up for further characterisation. Authenticity of the insert at this stage was confirmed by southern hybridisation of the PCR fragment (data not shown) with a heterologous probe, using the same conditions as those used during screening. The complete sequence of the clone, *pSaPRP,* and the deduced amino acid sequence of the encoded protein was determined. The cDNA contained 1744 bp with a 978 nucleotide open-reading frame. The polypeptide contained repeat units of SPTPP and related sequences throughout (Fig. 1).

Sequence comparison

The sequence of the polypeptide was aligned with related sequences and compared. A low but significant identity (30–48%) is obtained with various extensin precursors as shown in Table 1.

```
gcttggctgccacaaccaagttatccctgtggtaacttttctgacacctctagcttcaaa

ttccgaagttctaaaggatcgtcgataggccacgctttcacggttcgtattcgtactgaa

aatcagaatcaaacgagcttttaccctttgttccacacccaatttgagttctcgttgag

ctcatcttaggacacctgcgttatcttttaacagatatgccgccccagccaaactcccca
                                        M  P  P  Q  P  N  S  P

ctgacaatgtcttcccgccggatcgatgcacgagcgtgcgtcttggttctaaaaagagg
P  D  N  V  F  P  P  D  R  C  T  S  V  R  L  G  S  K  K  R

ggcgagagccccgctcccgattcacggaataagaataacgttaaaagtagtggtatttca
G  E  S  P  A  P  D  S  R  N  K  N  N  V  K  S  S  G  I  S

cttttgccgccgtctgctcccacttatcccacacctctccaagtcatttcacaaagtcgg
L  L  P  P  S  A  P  T  Y  P  T  P  L  Q  V  I  S  Q  S  R

actaaaattccaagcccaacattgggtcttccttttcccccgctggatccaagaaaaccc
T  K  I  P  S  P  T  L  G  L  P  F  P  P  L  D  P  R  K  P

ccaagtgggccttttggtaatccaaattcaccggcgtcactaatacctttggcgacgctgg
P  S  G  P  F  G  N  P  N  S  P  A  S  L  I  P  W  R  R  W

gaccatactcccccgttgtttaaccctccatcggatcccccctactccgtacggcacccca
D  H  T  P  P  L  F  N  P  P  S  D  P  P  T  P  Y  G  T  P

cccactgttcgacgcagcccgagcccccccggaaagtgccagccccagtccgtcccccgga
P  T  V  R  R  S  P  S  P  P  E  S  A  S  P  S  P  S  P  G

ccgcccgcggcgaccccctccccgcccaagcagcccgacgagtaccccaccaacagat
P  P  A  A  T  P  T  P  R  P  S  S  P  T  S  T  P  P  T  D

ggggaaacacccaggccgccggggcctcgaagccctagcccctctccaggtccgcccagt
G  E  T  P  R  P  P  G  P  R  S  P  S  P  S  P  G  P  P  S

tgttcgcctagcccaaagagccctagcccgccggcaagcagtccaccacgctcccggcca
C  S  P  S  P  K  S  P  S  P  P  A  S  S  P  P  R  S  R  P

ggacccccctgactacacaacgtctcctagccccccaaccccctaggagcgtgcctccaact
G  P  P  D  Y  T  T  S  P  S  P  P  T  P  R  S  V  P  P  T

ccaccggctagtccttccccgccgaccgctaaaccctcccctccgtcacgggggtcttcc
P  P  A  S  P  S  P  P  T  A  K  P  S  P  P  S  R  G  S  S

ccgagcccacctacatcgccgacgcccacgccgcggccccccatcctactcaccaagcccg
P  S  P  P  T  S  P  T  P  T  P  R  P  P  S  Y  S  P  S  P

acgccaccaagcagccggccagccctcctctcaggagtcccatactaaccccgccttct
T  P  P  S  S  R  P  S  P  P  L  R  S  P  I  L  T  P  P  S

ccagcagccgttcctcctatcggacggagccctccgtcgcctatcgacccaccatgcagc
P  A  A  V  P  P  I  G  R  S  P  P  S  P  I  D  P  P  C  S

agccccgagccgtcgagcccgccaaccagtccacccactccgtcaagcccctactagcac
S  P  E  P  S  S  P  P  T  S  P  P  T  P  S  S  P  Y  *

ctggggagtgagcccgggcggagcgtccgtcggtgcggatcttggtggtagtagcaaata

ttcaaatgaaactttgaaggccgaaaggggaaaggttccatgtgaacggcacttgcacat

ggattagtcgatcctaagagacgggggaagcccgtccgataccgtgcaccacgcgagctt

cgaaagggaatcgggttaaaattcctcaaccgggatgtggcggctgacggcaaccgttag

ggagtccggagacgtctcgagggcctcgggaagagttatcttttctgtttaacagcctgc

ccaccctgaaacggtttaccggaggtagggtccacggctggaacagcaccgcacgtcgcg

tggtgtctggtgcgcccctcgcggcccttgaaaatccggaggaccgagtgccttccacgcc

tggtcgtactcataaccgcatcaggtctccaaggtgaacaacctctggtccatggaacat

ttac
```

Notes: The stop codon is marked by an asterisk. SPSPP/SPTPP repeat sequences are underlined.

Figure 1. Nucleotide and the deduced amino acid sequences of *SaPRP* cDNA

Table 1. Proportion of identity of aligned amino acid sequences of glycoprotein rich in proline (hydroxyproline).

Characteristic	SaPRP	Extensin type				
		Maize	Tobacco	Sorghum	*Arabidopsis*, anther-specific	Tobacco, pistil-specific
SaPRP identity (%)	100	48.1	34	33.3	37.6	30.6
SaPRP, amino acid overlap	326	233	314	207	149	216

Genomic organisation of *SaPRP*

Total DNA was digested to completion with *EcoRI, HindIII, BamHI, BstNI* and *XhoI* and subjected to southern blot hybridisation with SaPRP insert as probe (Fig. 2). *BamHI* has one internal site within the cDNA, while the others have none. Along with the major expected bands, there seem to be many weak bands. In view of earlier reports from other plants, this suggests that *SaPRP* possibly belongs to a small gene family.

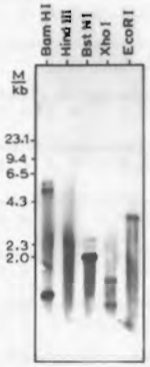

Figure 2. Southern hybridisation analysis
Notes: Genomic DNA (10 µg per lane) was digested with the indicated restriction enzymes. The migration of the molecular weight standards are indicated.

Induction of *SaPRP* transcript

Northern blot analysis with *SaPRP* was used to monitor the expression in somatic embryos treated with SA (Fig. 3). The results indicate the clear induction of *SaPRP* transcript in the embryos when treated with SA. The probe hybridises specifically to single mRNA species of the appropriate size. From a separate experiment we observe that longer exposure results in the appearance of weak signal in the uninduced RNA lane (Fig. 4). As SA has been implied as a major signalling molecule in local as well as systemic resistance, our data suggests that this *SaPRP* induction may also be a part of defence response (see Discussion, below).

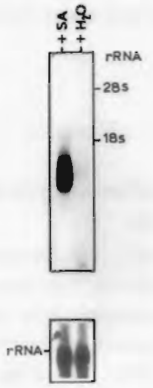

Figure 3. Expression of *SaPRP* transcript on SA-treated somatic embryos
Notes: 10 µg of total RNA was loaded in each lane. The upper panel shows the induction of *SaPRP* mRNA in somatic embryos of sandal when treated with salicylic acid. The same blot was then washed and rehybridised with *rRNA* gene probe, shown in the bottom panel.

Tissue-specificity of *SaPRP* expression

Since cell-wall proteins were implied in spatial and temporal expressions, northern blot analysis was further extended using leaf and stem tissues. As mentioned in the previous experiment RNA from uninduced embryos was loaded in one of the lanes. Evidently, *SaPRP* is predominantly expressed in leaf tissues (Fig. 4). The probe here also hybridises specifically to a single band; however the size of the transcript appears to be different from that in embryo samples, whether induced or not.

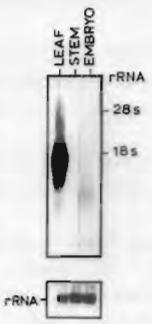

Figure 4. Tissue-specificity of *SaPRP* transcript
Notes: 10 µg of total RNA from leaf, stem and embryo was loaded on each lane as indicated. The upper panel shows the expression of *SaPRP* transcript in leaf tissue. Embryo RNA lane was overexposed. Faint appearance of transcript in the shorter length can be observed. The same blot was re-probed with *rRNA* gene as shown in the bottom panel.

Discussion

Tree improvement programs are well in progress in various countries. As a preliminary step towards developing disease-free plants, we have established the technology using cell-culture and tissue-culture (Lakshmi Sita et al. 1979; Lakshmi Sita 1986, 1991). Plants were produced by somatic embryogenesis from callus cultures derived from mature phenotypically disease-free plants. It was possible to produce and establish hundreds of plants. However we are not certain that they are genetically resistant. The advent of molecular techniques, and reports of transgenic plants expressing high levels of chitinase and glucanase with enhanced resistance (Broglie et al. 1991; Zhu et al. 1994), stimulated us to clone defence-related genes. Because its concentration rises dramatically after pathogen infection, salicylic acid is thought to have a central role as a signalling molecule leading to systemic acquired resistance (SAR) (Malamy et al. 1990; Metraux et al. 1990; Rasmussen et al. 1991; Yalpani et al. 1991; Enyedy et al. 1992; Uknes et al. 1993; Delaney et al. 1994).

Furthermore, exogenously applied SA leads to typical SAR responses such as increased resistance to viral infection (White 1979; Ye et al. 1989; Malamy and Klessig 1992). Salicylic acid being implied as a major signalling molecule in local as well as systemic defence response (Davis and Ausubel 1989; Ryals et al. 1996), we decided to pre-treat the somatic embryos with the same preparation as that for the cDNA library. Subsequently, we could isolate a proline- (or hydroxyproline-) rich protein clone from this induced cDNA library of sandal somatic embryos. From our genomic hybridisation studies, and also from other reports (Showalter and Varner 1989), we believe that *SaPRP* possibly belongs to a small gene family. Sequence analysis reveals its relatedness with other proteins isolated from different systems. Even though our *SaPRP* definitely shares certain features with other related proteins, it has some unique characteristics. For example, it has the repeat motif of SPTPP instead of SPPPP as described in extensins. Interestingly, it also looks similar to certain tissue-specific extensin precursors (Table. 1).

Tissue-specificity of these groups of proteins has been reported in many crop plants (Hong et al. 1989; Wyatt et al. 1992). This compelled us to check whether *SaPRP* also has some tissue-specificity in expression. From our northern blot analysis it was clear that *SaPRP* is expressed abundantly in leaf tissues compared with stems, yet barely in somatic embryos. Significant induction of it was observed in somatic embryos treated with salicylic acid. To the best of our knowledge, all the reported extensins are expressed rather less in leaf tissues. However, one of the proline-rich proteins from soybean, SbPRP3, was reported to be abundant in leaves (Hong et al. 1990). Thus, in functional terms, our *SaPRP* is closer to the proline- (or hydroxyproline-) rich protein group than to extensins. However, at present we cannot explain the functional significance of the absence of SaPRP transcript in stems. Our expression studies reveal that this SaPRP is highly inducible by SA, justifying our ability to clone it from the SA-induced embryos. This also reflects the fact that the induction of this gene may occur during wounding or infection through an SA-mediated signal transduction pathway.

We observed that the size of the transcript present in leaf tissue was different from that present in the induced or uninduced embryos. We do not know yet whether these two transcripts come from different genes or from the same gene. It is possible that they originate from the same gene, but are processed differently. Moreover, the gene seems to be developmentally regulated, while expression of it becomes significant only at the later stages of development in a tissue-specific manner. The expression as a consequence of SA treatment at the early embryo stage is probably a part

of a defence response. However, this does not explain the apparent difference in size of the transcripts. If these transcript populations are derived from different genes, one would expect to detect an additional, SA-induced, transcript of different size in the leaf or stem tissue when treated with elicitors. Experiments are now directed towards these questions.

The present report of cloning of a defence-related gene is one of the first of its kind in forest trees. Simultaneous studies of genetic transformation with marker genes are in progress, and the protocols developed will be used for the over-expression of defence-related genes. These experiments could mark the beginning of a new approach to disease control of mycoplasma and other diseases in sandal.

Acknowledgment

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The nucleotide sequence data reported will appear in GenBank under the accession number AF020261.

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Interspecific Hybridisation between *Santalum album* and *S. spicatum*

J.A. McComb and M.G.K. Jones*

Abstract

The possibility of producing inter-specific and intra-specific hybrids of *Santalum album* and *S. spicatum* was investigated. A study of the reproductive biology of the species showed that both *S. album* and *S. spicatum* are obligate out-crossing species, with pre-and post-fertilisation barriers preventing self-pollination and inter-specific pollination. Initial fruit-set was low with 70–100% fruit abscission. Reasons for failure of fruit-set included lack of fertilisation, lack of an embryo sac, and failure of endosperm development. An in-vitro culture technique was developed which could induce embryogenesis and thus 'rescue' embryos of intra-specific crosses at 3.5–6 months old, but not from putative inter-specific hybrid fruit, which abscised at 1–3 months old. Putrescine spray increased fruit-set and delayed fruit abscission for both intra-specific and inter-specific crosses. However only a few putative hybrid fruits were retained until four months after pollination; they were not harvested for ovary culture, but allowed to develop to maturity. These mature putative hybrid seeds did not germinate, but their endosperms were confirmed to be hybrid by random amplified polymorphic DNA (RAPD) analysis.

Key words: *Santalum album, Santalum spicatum*, inter-specific hybridisation

WEST AUSTRALIAN SANDAL, *Santalum spicatum*, is indigenous to Australia. Although it initially grew in more temperate areas, it is now restricted to arid and semi-arid areas in South Western Australia and South Australia (Applegate and McKinnell 1991).

It typically has an oil content of two per cent, and in its natural habitat is a slow-growing species. Some 2000 t of wood are harvested each year and utilised for incense. The better known Indian sandal, *S. album* L. has a higher oil content (6–7%) and more valuable oil, and grows in a wide range of temperatures and soil types in tropical and subtropical areas of India, Sri Lanka and Indonesia (Anon. 1990). We investigated the possibility of producing inter-specific hybrids between *S. album* and *S. spicatum* with the objective of combining the drought and stress tolerance of *S. spicatum* with the high oil content and faster growth of *S. album.*

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The breeding biology of *Santalum* species has not been extensively studied, and published information on *S. album* is contradictory. Bhaskar (1992) and Jyothi et al. (1991) found it to be an obligate outcrossing species, while Sindhuveerendra and Sujatha (1989) reported partial inbreeding. Less is known about *S. spicatum*, although the phenology of flowering has been described; in common with other *Santalum* species, it has a very low fruit-to-flower ratio (Barrett 1987).

The peculiarities of the *Santalum album* placenta, ovule and embryo sac structure and development of the endosperm and embryo were recognised early (Griffith 1843; Iyengar 1937; Rao 1942), and Rugkhla (1997) has described the *S. spicatum* ovary. In *Santalum* the unilocular ovary has 2–3 fused carpels, and is almost filled with an enlarged placenta (mamelon) which bears 2–3 'ovules'. The embryo sacs protrude from these ovules, but initially remain embedded in the placenta. As the flower matures, the embryo sacs emerge from the placenta. The 'micropylar' end bears the egg apparatus and is at first curved to point towards the base of the ovary, but

later turns and grows up to the base of the style. After fertilisation the endosperm from one embryo crushes the other embryo sacs and the mamelon, so at maturity there is a mass of endosperm directly inside the fruit wall. There is no seed coat. Inside the endosperm is embedded a small dicotyledonous embryo.

Intra-specific and inter-specific hybridisation has not been widely attempted in sandal, and to understand the problems of production of inter-specific hybrids it was first necessary to study intra-specific crosses in some detail.

Materials and Methods

S. spicatum (seed origin, Kalgoorlie and Bullock Holes, Western Australia), and *S. album* (seed origin, Bangalore and Marayoor, India) 7–12 years old, growing at Curtin University W.A., were utilised. Pollinations were carried out between February and August. One to two days before anthesis, spiral wire frames were placed over inflorescences and they were then covered with perforated polyethylene bags. Where necessary, flowers were emasculated before anther dehiscence. Pollen from freshly dehisced anthers was used. Preliminary analyses of pollen-tube growth in the style, and observations of stigma receptivity, showed the optimum time for pollination to be 2–3 days after anthesis. Success of pollination was scored as:

- failure—when flowers abscised before ovary enlargement;
- initial fruit-set—when ovaries enlarged to 1.5mm; and
- mature fruit-set.

Anatomical studies involved fixation of material in 3% gluteraldehyde (*pH* 7, 0.025M phosphate buffer), dehydration in an alcohol series, and embedding in Spurr's resin. Longitudinal sections were cut at 1-3μ and stained with 'toluidine blue O' (Feder and O'Brien 1968). Assessment of pollen-tube growth and developmental stages of embryo sacs were also observed after fixing material in Carnoy's solution, dehydration in an alcohol series, then softening in 0.8N NaOH at 60°C for up to two hours followed by staining in 0.1% aniline blue in phosphate buffer for 10 minutes squashing in 80% glycerol and observation under fluorescence microscopy (Martin 1959).

In some experiments aimed to extend the time of fruit retention, all flowers on an inflorescence were hand-pollinated; putrescine or arginine (in 0.1M 2-N-morpholino-ethan sulfonic acid (MES) and 0.01% *Tween-80*, pH 7) was sprayed onto inflorescences when 25 per cent of the flowers reached anthesis.

Embryos extracted from endosperm—or cut out with a piece of endosperm and pericarp attached to the basal end of the embryo—were cultured from fruits of different ages. The media were:

- K—Kao and Michayluk (1974) medium with 10 μM GA_3, 0.5 μM BAP, 0.5 μM kinetin, 1 μM zeatin and 1 μM IAA;
- 0—Murashige and Skoog (1962) medium with 5 μM 2,4-D and 1.8 μM kinetin; and
- T—Murashige and Skoog (1962) medium with 2–4 μM thidiazuron.

DNA from fully expanded leaves or endosperm of *S. spicatum* × *S. album* putative hybrids was extracted using the method of Doyle and Doyle (1987). After extraction it was found necessary to include a treatment with RNase A at 10μg mL and to incubate at 37°C for 30 minutes. PCR amplification was conducted using the methods of Williams et al. (1990) and eight different primers were used of which OPA-08 (Operon Technologies) was the best to discriminate between the species. The parameters of the reaction were optimised for each species. For *S. album* 1.9 mM $MgCl_2$ and 0.18 units of Taq polymerase was optimal, while for *S. spicatum* it was 2.4 mM Mg Cl_2 and 0.22 units Taq polymerase.

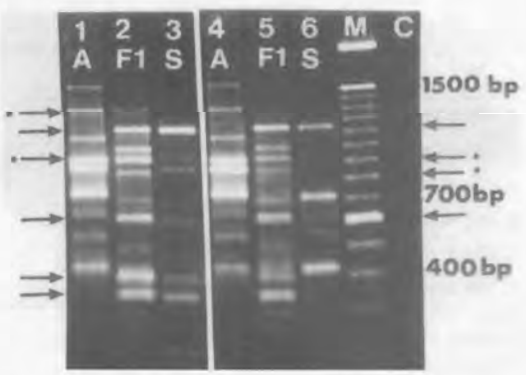

Figure 1. RAPD bands of *S. album* (lanes 1 and 4), *S. spicatum* (lanes 3 and 6), and endosperm of two putative hybrid seeds of *S. spicatum* × *S. album* (lanes 2 and 5)
Notes: Arrows with an asterisk indicate unique bands from the *S. album* parent (pollen parent) that are present in the hybrid. Arrows without an asterisk are unique bands from *S. spicatum* (female parent) present in the hybrid. Lane M is molecular marker 100 bp ladder, lane C the negative control without DNA. Lanes 1–3 were run under conditions optimised for *S. spicatum*, and lanes 4-6 conditions optimised for *S. album*.

Results

The number of pollen tubes observed in styles and ovaries was highest after intra-specific cross-pollination and lowest after self-pollination or inter-specific crossing (Table 1). Histological examination of ovaries showed that the frequency of fertilisation, based on the division of the endosperm nucleus, was higher in the *S. spicatum* × *S. album* cross than in the reciprocal cross. Variation in fruit-set between genotypes was observed, with one *S. album* tree setting no fruit despite using four other *S. album* pollen trees (Table 2). The average final fruit-set was low for both species (Table 3). The rare mature fruit formed after self-pollination of each species was seedless. Although there was an initial low level of fruit-set after inter-specific crossing, none reached maturity. Fruits of *S. album* reached maturity after 7–8 months, while those of *S. spicatum* required 9–11 months.

The female gametophytes were immature at anthesis, and did not reach full differentiation until three days after anthesis. The egg apparatus showed aniline blue induced fluorescence which became intense 2–4 days after anthesis and faded after fertilisation. In *S. album* up to 18 per cent of flowers lacked embryo sacs, and in the *S. album* tree which failed to set fruit, the embryo sacs were very slow to mature.

Histological studies showed that a coenocytic endosperm developed and began to become cellular after 1–2 months. We did not observe development of two compartments in the endosperm with the chalazal nucleus resting as reported by Iyengar (1937) and Rao (1942). The zygote remained quiescent for one month. In inter-specific crosses the endosperm formed only 6–10 nuclei, aggregated on one side of the embryo sac, then degenerated. The hybrid zygote did not divide.

Embryos from intra-specific crosses extracted from fruits 2–3 months old and cultured in vitro, produced only callus. Embryos of *S. spicatum* from fruits 3.5–6 months old, when cultured with a portion of endocarp at the basal end of the embryo, produced adventitious embryos on Kao and Michayluk (1974) medium with a combination of five growth regulators, or on a more simple medium (Murashige and Skoog (1962) with 5 μM 2,4-D and 1.8 μM Kinetin) (Table 4). Zygotic embryos of *S. spicatum* at six months or older did not require the attached endosperm and peri-

Table 1. Pollen tubes observed in the style, ovary and embryosac two days after hand-pollination (intra-specific) crosses, and three days after pollination (inter-specific) crosses

Cross	Pollen tubes (mean no.)		Ovaries with pollen tubes (%)	Fertilised ovules (%)
	Style	Ovary		
S. spicatum selfed	2.0 (0.2)	0.3 (0.3)	20	0
S. spicatum cross	23.5 (4.8)	1.3 (0.1)	50	40
S. album selfed	6.8 (5.0)	0	0	0
S. spicatum cross	14.0 (2.9)	6.3 (2.2)	65	10
S. album × *S. spicatum*	13.6 (5.1)	1.0 (0.6)	20	0
S. spicatum × *S. album*	3.0 (2.0)	0.5 (0.3)	20	0

Notes
- Data are means of 5–20 replicates with SE in parenthesis.
- Unpollinated control flowers showed no pollen tubes.

Table 2. Frequency of fertilisation as determined by the division of the endosperm nucleus (% ovaries)

Cross	Unfertilised	Fertilised	Unidentified
S. spicatum × *S. spicatum*	30	64	6
S. album × *S. album*	38	54	8
S. album × *S. spicatum*	70	5	10
S. spicatum × *S. album*	57	40	3

Note
- 20–24 ovaries were examined for each cross.

Table 3. Fruit development and seed germination after intra-specific or inter-specific pollination

Cross	Fowers pollinated (No.)	Initial fruit-set (%)	Mature fruit-set (%)	Germination (%)
S. spicatum × *S. spicatum*	2500	4.2	1.3	70.4
S. album × *S. album*	865	19.3	7.2	52.0
S. spicatum selfed	370	0.2	0.2[a]	0
S. album selfed	400	0.5	0.3[a]	0
S. album × *S. spicatum*	680	0.4	0	–
S. spicatum × *S. album*	1250	0.3	0	–
Bagged, unpollinated	150	0	0	–

[a] Seedless fruit

Note

• Data from crosses using 22 trees of *S. spicatum* and 7 trees of *S. album* are pooled.

carp, and could develop adventitious embryos on medium with thidiazuron. *S. album* immature embryos were less responsive than those of *S. spicatum* in culture.

Best results for *S. album* were obtained with embryos attached to a portion of endosperm and cultured on media with thidiazuron (Table 4).

When inflorescences were sprayed with putrescine or arginine, there was an increase in initial and final fruit-set in most treatments (Table 5). Putrescine spraying of unpollinated flowers did not induce ovary enlargement. Some inter-specific fruit-set was obtained after spraying; histological examination of such fruit showed that, by Day 60, the endosperm had grown to fill half the embryo sac and the embryo had developed to the octant stage. Those few *S. spicatum* fruit from inter-specific crossing which were retained to maturity did not contain an embryo.

Table 4. Effect of growth regulators and the presence of endocarp and endosperm on in-vitro development from immature zygotic embryos 3.5–6 months after pollination (% of explants)

Species	Medium	Attached tissues	Callus	Somatic embryos	Radicle elongation	Shoot development
S. spicatum	K	endocarp and endosperm	20	30	40	30
		none	100	0	0	0
	O	endocarp and endosperm	40	60	0	60
		none	100	0	0	0
	T	endosperm	0	0	0	0
		none	0	60	0	5
S. album	K	endocarp and endosperm	80	0	0	0
		none	100	0	0	0
	O	endocarp and endosperm	100	0	0	0
		none	100	0	0	0
	T	endosperm	0	40	60	40
		none	0	0	0	0

Notes

• There were 10–20 samples in each treatment.

• Media K, O and T are explained in the text.

The RAPD bands generated using different primers showed that 3–15 per cent were common to both species. DNA from the endosperm of two putative *S. spicatum* × *S. album* hybrid seeds showed most marker bands of both parents when tested under the different sets of PCR conditions optimal for each parent (Fig. 1). A few paternal bands were missing or weakly expressed in the putative hybrid, and there were also some non-parental bands present.

Discussion

From observations of pollen-tube growth and seed set, *S. spicatum* and *S. album* were shown to be outcrossing species in which pre-and post-fertilisation incompatibilities prevent inbreeding. The rare fruits which matured after selfing were seedless. For *S. album* we thus confirmed the evidence of Bhaskar (1992) and Jyothi et al. (1991) and contradicted the results of Sindhuveerenda and Sujatha (1989). For most genotypes, stigma receptivity and female gametophyte maturity were well coordinated, but in one *S. album* tree, embryo sacs were not receptive at the same time as the stigma. The placenta had no ovules in 18 per cent of *S. album* flowers. *S. acuminatum* has also been reported to develop ovaries without ovules in some flowers (Sedgley 1982).

Attempts to produce inter-specific hybrids between *S. album* × *S. spicatum* were unsuccessful. Pollen-tubes were able to grow down the styles in the reciprocal crosses but fertilisation rarely occurred, particularly when *S. album* was used as the maternal parent (Tables 1, 2). When ovules of the *S. spicatum* × *S. album* cross were successfully fertilised, abnormal endosperm development precluded zygote development. As in the fruit species apple and pear (Costa et al. 1986; Crisosto et al. 1988), putrescine spray improved fruit-set after hand pollination of *S. album* and *S. spicatum*. Increased fruit-set (0.2%), was also obtained after inter-specific crossing of the *Santalum* species. After putrescine spray the embryos in the hybrid seeds developed to at least the octant stage, but fruits at maturity did not contain an embryo. Specific RAPD markers for each species were used to confirm the hybrid nature of the endosperm of such seeds. Only 3–15 per cent of the RAPD bands were common between the species indicating that the relationship between *S. spicatum* and *S. album* is distant.

A successful method of rescuing immature embryos from intra-specific crosses was developed. As with the other species (Liedl and Anderson 1992), culturing very small embryos was either unsuccessful or resulted in production of callus only. Embryos from *S. spicatum* fruits 4–6 months old could be induced to produce somatic embryos in vitro. For *S. spicatum* it was found to be important to retain the attachment of the basal end of the embryo to the endosperm and pericarp. This suggests that the suspensor and the vascular connection between the ovule and the endocarp (Rao 1942) play an important role in embryo nutrition.

If hybrid embryos are to be raised successfully, different genotype combinations or a more effective putrescine treatment will need to be developed to allow embryos to grow to sufficient size for in-vitro rescue. In addition it may be possible to raise triploid embryos from hybrid endosperm as has been done for *S. album* (Lakshmi Sita et al. 1980). An alternative pathway for production of hybrids between *S. album* and *S. spicatum* may be to raise somatic hybrids through protoplast fusion. This has been shown to be possible, and fusion products have grown to the stage of small calli (Rughkla 1997).

Table 5. Effect of putrescine and arginine sprays on fruit-set of hand-pollinated flowers

Cross	Initial fruit-set				Mature fruit-set			
	Putrescine			Arginine	Putrescine			Arginine
	0	10 mM	100 mM	100 mM	0	10 mM	100 mM	100 mM
S. spicatum × *S. spicatum*	6.5	5.4	6.5	7.2	0.3	2.9	3.8	3.2
S. album × *S. album*	4.2	11.6	6.0	9.1	1.2	4.3	2.2	3.5
S. spicatum × *S. album*	0.1	2.0	2.1	0.4	0	0	0.2	0.1
S. album × *S. spicatum*	0	0	0	0	0	0	0	0

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Biotechnological Approaches for Propagation of Sandal (*Santalum album* L.)

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Abstract

The potential uses of plant biotechnology in forestry have been recognised. Sandal is a commercially important forest tree, and is the source of fragrant wood and oil. Conventionally, new trees are raised through seeds and vegetative propagation has not been successful in this species. Clonal multiplication of genetically superior trees using tissue-culture techniques would therefore be advantageous. Sandal tissue-culture systems have been extensively investigated in our laboratory, and various in-vitro approaches have been developed. Plant regeneration by shoot-bud differentiation has been achieved in hypocotyl segments obtained from seedlings as well as from nodal segments. Callus development, induction of somatic embryos in callus, and plantlet regeneration have been achieved in both hypocotyl explants and internodal stem segments from mature, elite trees. Embryogenic callus tissues have been maintained through repeated subcultures without loss of embryogenic potential. Various factors influencing somatic embryogenesis in callus, as well as in cell suspensions derived from callus, have been elucidated. Addition of cyanobacterium extract could induce somatic embryos in callus tissues in the absence of growth regulators. Maturation and desiccation processes of somatic embryos enhanced conversion of somatic embryos to plants. Cell cultures were successfully grown in a bioreactor, and harvesting of somatic embryos from the bioreactor was also attempted. Protoplasts were isolated from cell cultures; after successive divisions, they resulted in calli which differentiated into somatic embryos and plantlets. Somatic embryos obtained in vitro were encapsulated in sodium alginate and synthetic seeds were prepared. Retrivation of plants from the encapsulated embryos was achieved.

Key words: *Santalum*, in vitro, somatic embryo, protoplast, synthetic seed

AMONG INDIAN FOREST trees, sandal (*Santalum album* L.) has been rated as the most precious and valuable forest tree, and is well known for its fragrant wood and oil. The oil is present in the heartwood and root, and hence the tree is invariably harvested by uprooting for extraction of oil. Trees 30–60 years old with a girth of 40–60 cm generally have the best heartwood for furniture and related products as well as for oil extraction. Sandalwood oil is in great demand for cosmetics, soap and perfumes, and is also used in ayurvedic and allopathic systems of medicine. Constituents of the benzene extract of sandalwood bark have been reported to exhibit excellent insect growth-inhibiting properties and chemosterilant activity.

Sandal is a semi-root parasite, and during early stages of development attacks a wide range of host plants for its nutrition. The tree is evergreen and produces large quantities of seed. Normally seeds remain dormant for two months and viability gradually decreases after 9–10 months. Attack by microorganisms, heat, grazing by animals, and excessive water are other factors which considerably reduce the survival of seeds. Vegetative propagation of sandal by cutting or by grafting has not met with much success. The sandal tree is plagued by a range of pathogens, of which mycoplasma is very serious and has threatened this species with extinction. Biotechnological methods are therefore very important. We have extensively investigated and have defined various

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in-vitro approaches for multiplication of this important tree species, and this paper summarises the achievements of the work carried out so far (Bapat and Rao 1989; Rao and Bapat 1992a, b, 1995).

Development of Embryos and Plantlets

Regeneration of plants through induction of multiple shoots

Hypocotyl segments from aseptic seedlings, as well as stem segments from young shoots of mature trees have the potential to produce multiple shoots when cultured on a cytokinin medium. However less than ten per cent of shoots produce roots upon excision and culture on a rooting medium.

Induction of callus and somatic embryos

Somatic embryogenesis in sandal is one of the earliest of such occurrences reported in forest trees. Somatic embryogenesis in sandal is governed by a process influenced by several physical and chemical factors coupled with other parameters such as genotypes, age of the source tissue and location of the source material. Hypocotyl segments from aseptic seedlings, as well as stem segments from 30-year-old plants, produce actively growing callus tissue.

This callus on transfer to an appropriate medium produced highly regenerative embryogenic tissue with many embryos. These embryos through successive ontogenic development exhibited globular, heart and torpedo stages similar to seed embryos. Continuously regenerating callus cultures were maintained by subculturing the tissue on the fresh medium of the same composition. A sequential change in the media helped the development of embryos into viable plantlets. In many cultures the embryo, instead of developing into plantlets, de-differentiated and produced a callus mass which again showed intense secondary embryogenesis. The cyanobacterium *Plectonema boryanum* has been shown to promote embryogenesis in sandal. Induction of embryos and conversion of embryos to plantlets was possible on medium containing extracts of cyanobacteria.

Establishment of cell suspensions and somatic embryos

It is possible to produce good cell suspensions from sandal callus on transfer to agitated liquid medium. During the growth phase of suspension cells, thousands of cells per mL were developed. On alteration in the hormonal composition, they formed embryos. Mature, organised embryos developed into plantlets on transferring these embryos from liquid to solid medium.

Sandal embryos in a bioreactor

Sandal cells growing in flasks were used as the inoculum for cultivating the cells in larger vessels popularly called 'bioreactors'. A seven litre tank-bioreactor was used for the conversion of non embryogenic cells to embryogenic cells and secondly a bell jar bioreactor was used for the development of proglobular embryos to mature embryos. The significance of this study was that cells and embryos did not loose the ability to form plants even when the cells were cultivated in a special bioreactor environment.

Isolation of protoplast from callus and cell suspension

Protoplasts (i.e. cells without walls) could be isolated from various sources such as stem callus, cell suspensions and leaf mesophylls. Particular combinations of enzyme solutions yielded abundant protoplasts, a high proportion of which divided and developed into colonies. Embryos, which later developed into plantlets, regenerated from such colonies.

Maturation and desiccation of somatic embryos

The process of maturation and desiccation of zygotic embryos is controlled by a variety of external and internal factors. To ensure that most embryos convert to plants, it is necessary to study and apply these factors in somatic embryogenesis. Various studies of sandal revealed that desiccation of embryos encouraged them to develop into plants. This demonstrated both the ability of the desiccated embryos to survive, and the potential of desiccated embryos to regenerate into plants. The callus which was dried and kept in aseptic condition for four weeks rejuvenated and formed embryos when transferred to the fresh medium.

Preparation of Synthetic Seeds from Somatic Embryos

The embryos could be encapsulated in a nutrient gel to prepare synthetic seeds. Rapid and inexpensive delivery of tissue-cultured plants, storage of elite germplasm and easy transport of plant material are some of the advantages of synthetic seeds. Sandal

embryos have been encapsulated and plants have been obtained from these seeds. However prior to encapsulation, synchronisation of embryogenesis and development of well-developed embryos are important prerequisites. Significant success has been obtained in this direction.

The increasing accuracy and reliability of tissue-culture in forest trees with the advances being made in understanding plant responses, including growth and development, are leading towards a rational control of tissue-culture technology. However, there are certain potential difficulties. One is the conversion of somatic embryos to plantlets in a large numbers followed by successful acclimatisation. The other factors include genetic fidelity and economic viability of tissue-cultured plants for large-scale plantations. These limitations are also exacerbated by natural variability.

Conclusions

The work described above clearly demonstrates the feasibility of using somatic embryogenesis as the most appropriate mode for rapid micropropagation of sandal. The concept of 'synthetic seeds' is a new idea and could be an effective tool for micropropagation of sandal and several other forest trees. Besides, the versatile nature of cells of sandal under in-vitro conditions makes it an ideal system to solve some of the basic problems. Study of enzymatic pathways responsible for embryogenesis or for understanding host–parasite relationships are examples. Investigations on metabolic, physiological and genetic parameters determining oil content in plus trees by appropriate use of plant tissue-culture methods will lead to better manipulation and maintenance of high-oil-yielding trees. Successful regeneration of plants from protoplasts opens up many opportunities for genetic engineering experiments. Disease problems would be better addressed by integrating the latest techniques of tissue-culture with conventional methods.

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In-vitro Shoot Multiplication from the Mature Tree of *Santalum album* L.

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Abstract

The main incentive to clone mature trees is to capture specific, mostly non-additive, gene combinations that import superior characteristics. A protocol for direct organogenesis for shoot-tip or nodal segments of mature sandal trees has been studied. Adventitious buds were initiated on 'Murashige and Skoog' medium containing various combinations and concentrations of cytokinins. Repeated cycles of culture and regeneration produced more adventitious buds. Histological studies revealed that proliferation of buds originated directly from the superficial layers of the explants without an intermediate callus phase.

Key words: *Santalum album*, clonal propagation, direct organogenesis.

Abbreviations:
BAP = 6-benzylaminopurine
IAA = Indole-3-acetic acid
IBA = Indole-3-butyric acid
KN = Kinetin
NAA = 1-Naphthalene acetic acid
PVP = Polyvinyl pyrrolidone

INDIAN SANDAL IS valued for its fragrant heartwood which yields oil highly preferred for perfumeries. It's scented oil is a major ingredient in cosmetics, medicines and perfumes produced world-wide.

Depletion of existing stands and failure to establish new stock suggest that mature trees are likely to be in short supply over the next decade. Also, sandal is subjected to only a few diseases, of which spike disease is the most destructive. Since the natural regeneration of this tree is inadequate and trees are not being planted on a large scale, there is need to develop a reliable method of in-vitro propagation.

Micropropagation of tree species is being increasingly recognised as a tool with much potential for applications in the field of forestry. It offers a rapid means of afforestation, multiplying woody biomass, and conserving elite and rare germplasm (Winton 1978; Bajaj 1986; Bonga and Durzan 1987). There are several reports of indirect regeneration of *S. album* on juvenile or mature explants (e.g. Lakshmi Sita 1986; Rao and Bapat 1992). However, plantlet regeneration from mature trees of sandal is still rare (Rao and Bapat 1992).

Therefore the present study was undertaken to define optimal culture conditions for high-frequency regeneration from explants of sandal.

Materials and Methods

Plant material

Shoot tips and nodal segments were collected from 50–60-year-old trees. The explants were surface-sterilised by the following process:

- 30-40 minutes in running tap water; then

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- 5–10 minutes in 0.1% v/v *Tween-20 solution* followed by 20–30 minutes in running tap water; then
- 5–10 minutes in 0.1% fungicide (*Bavistin*) followed by 4–5 minutes in distilled water; then
- 10–15 minutes in 0.15% mercuric chloride; then
- washed with sterile distilled water.

Culture media

The following culture media were used.

- MS-1: MS semi-solid medium (Murashige and Skoog 1962) was used at full strength with various concentrations and combinations of auxins and cytokinins. The medium was supplemented with 2% sucrose and was gelled with 0.8% sugar.
- MS-2: Half-strength MS liquid medium with 3% sucrose and different concentrations and combinations of auxins.

The media used for shoot elongation were adjusted to pH 5.8 activated charcoal 0.3% (w/v) or PVP 800 mg/L. Cultures were incubated at 25±2°C under 12 hours per day of fluorescent light (40μ Em^2/sec).

For histological examination, samples were fixed in formalin or acetic acid or alcohol (FAA), dehydrated gradually in an alcoholic series, then embedded in paraffin wax. Sections were cut at 10–12 mm thickness and stained with alcoholic haematoxylin.

Regeneration studies

Shoot proliferation from shoot-tip and nodal bud explants were attempted in two sets of experiments. In the first set, BAP and KN, separately (1.0–5.0 mg/L) and in combination (0.5–2.0 mg/L), were incorporated into MS-1 medium to select the best cytokinin for shoot proliferation. In the second set, a cytokinin that showed a good response in the previous experiment was tested in combination with auxin to induce the synergistic effect and to determine the optimum growth-regulator treatment for shoot proliferation.

The individual shoots were isolated from nodal bud culture growing on optimum growth regulator treatment and transferred to MS-1 medium supplemented with lower level of cytokinin and PVP or 0.3% charcoal. For in-vitro rooting, elongated shoots were transferred to MS-2 medium supplemented with different concentration and combinations of auxins. Roots were not initiated in any of the combinations.

In this study, at least 12 proliferating shoots were used per treatment.

Results and Discussion

Shoot proliferation was obtained from the shoot tips and nodal stem segments of sandal. On medium containing cytokinins, both types of explants initiated shoots after 4–5 weeks of inoculation. The proportion of cultures showing multiple shoot formation, shoot number per explant and length varied according to the type and concentration of cytokinin used. Nodal explants with two axial meristems gave a better response (Fig. 1(1), (2)) than the shoot tip explant with single apical meristem.

Nodal segments grew better than shoot tip explants cultured on medium containing BAP 5 mg/L (Table 1). For both explant types, BAP appeared to be more effective in inducing shoot proliferation and growth (Fig. 1(3)). Similar results were also reported in some other forest trees like *Syzgium alternifolium* (Kahn et al. 1997) and *Madhuca longifolia* (Rout and Das 1993). In most cases, shoot tips were dead and shoots had developed from axillary buds (Fig. 1(4)). Similar results have been reported in *S. alternifolium* (Kahn et al. 1997) and *S. aromaticum* (Mathew and Hariharan 1990).

Within 4–5 weeks, 10–12 adventitious shoots were directly formed on the internodal region of shoot segments cultured on MS-1 medium supplemented with 2–5 mg/L BAP. The shoot-buds originated directly from the superficial layers of explants without an intermediate callus phase. Histological studies revealed that mitotic activity was localised in epidermal and sub-epidermal cell layers. The formation of the meristematic dome developed into buds and formed adventitious shoots. Most of the buds emerged near the distal part of the explant.

The shoot multiplication rate and length increased with the increasing number of subcultures, but declined after 4–5 subcultures. Similar results have been observed in *Pistachio* and *Guava*. Addition of vitamins like panthothenic acid, biotin, and riboflavin did not improve shoot initiation or shoot multiplication. When a low concentration of IAA or NAA was added to the cytokinin-enriched medium, it greatly inhibited bud-breaking and favoured the growth of loose callus. Since the aim was to induce shoots without a callus phase on the stem explants, auxins were excluded. Similar results were reported in *Artocarpus heterophyllus* (Amin and Jaiswal 1993).

Higher levels of BAP after 4–5 subcultures induced transient leaves. So shoot clusters were subcultured on the same medium supplemented with a

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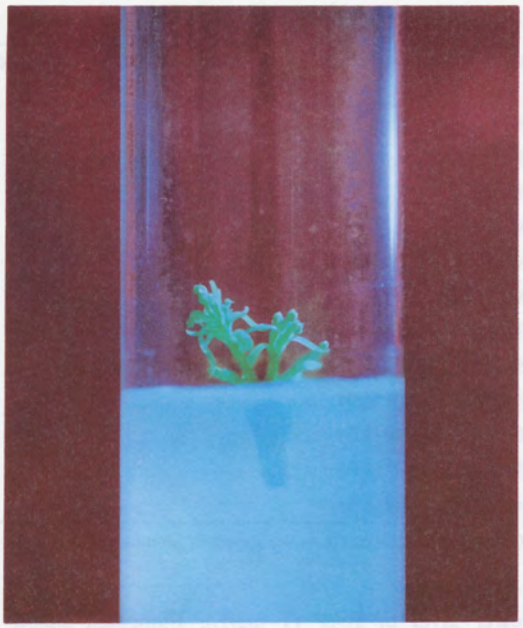

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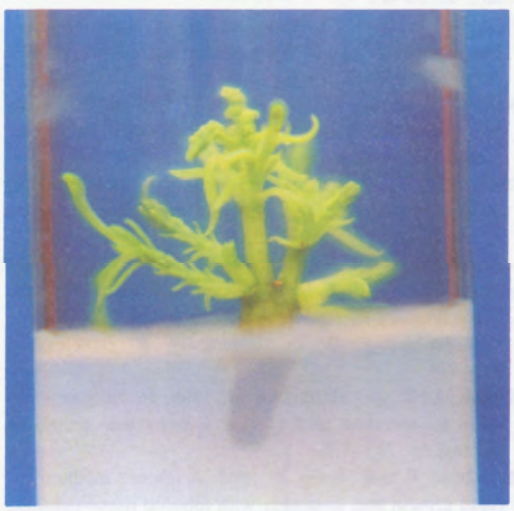

(3)

(4)

Figure 1. Lateral or axillary bud multiplication on MS-1 basal medium with BAP 5 mg/L.
(1) and (2): Proliferation of shoots at early stages.
(3) and (4): Well-developed shoots after third and fourth subcultures.

Table 1. Response of nodal stem segments of *Santalum album* to various cytokinins after 4–5 weeks (each treatment consists of 12 replicates

MS-1 medium average[a]	Cultures with multiple shoots (%)	Number of shoots per culture ± sd	Length of longest shoot ± sd (mm)
BAP (1.0)	30	4.7 ± 1.7	28 ± 6.5
BAP (2.0)	40	4.8 ± 1.4	70 ± 5.7
BAP (5.0)	50	10.9 ± 2.5	89 ± 7.0
KN (1.0)	5	2.1 ± 1.0	22 ± 1.7
KN (2.0)	5	2.1 ± 1.0	22 ± 1.7
KN (5.0)	10	4.0 ± 1.5	25 ± 2.8
BAP (0.5) + KN (0.5)	25	4.6 ± 2.1	39 ± 4.5
BAP (0.5) + KN (1.0)	25	4.6 ± 2.1	39 ± 4.5
BAP (1.0) + KN (1.0)	28	4.0 ± 1.8	40 ± 2.5
BAP (2.0) + KN (2.0)	30	4.5 ± 2.7	48 ± 1.5
BAP (2.0) + KN (2.0)	30	4.3 ± 1.5	27 ± 7.7
BAP (2.0) + KN (1.0)	32	4.6 ± 2.3	28 ± 4.5

[a]Concentration of cytokinins are shown in parentheses in mg/L.

lower level of BAP (1 mg/L). Elongated shoots (3–4 cm) were transferred to MS-2 medium supplemented with different concentrations and combinations of IBA, IAA and NAA.

In all experiments roots were not initiated, but browning of the cut ends of shoots was observed. Further rooting experiments are in progress.

Conclusions

The present work demonstrates a simple successful procedure for rapid shoot multiplication. The establishment of a stabilised shoot-culture system for sandal is a major step in the utilisation of biotech-nology. Shoot cultures can be used as explant tissue for protoplast isolation and culture (Smith and McCown 1983; Russell and McCown 1986). Such protoplast research may eventually lead to the production of interspecific hybrids which cannot be obtained by conventional methods.

This study will also be valuable in *Agrobacterium*-mediated transformation in-vitro micrografting, cryopreservation, and in research into woody plant growth and development.

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Alternanthera nana R.Br. Nursery Sowing-time Influences *Santalum album* L. Growth Following Field Planting

A.M. Radomiljac and J.A. McComb*

Abstract

In past pot-host selection experiments the benefit of pot-hosts for *Santalum* has usually been assessed during and at the end of the period of growth under nursery conditions. Our results with *S. album* and the pot-host *Alternanthera nana* show that, compared with the carry-over effects after field establishment, effects on growth during this period are insignificant. The benefit of the presence of the pot-host was increased survival and growth of *S. album* in the field. Further, the timing of the introduction of *A. nana* to the *S. album* pot during the nursery phase was critical. Under the climatic conditions at Kununurra, Western Australia, *S. album* grown together with *A. nana* for 109 days before field planting showed maximum survival (98%), and those in combination for 134 days showed maximum growth six months after being planted into the field in June.

THERE IS INTEREST in cultivating *Santalum album* L. as a plantation species in the Ord River Irrigation Area (ORIA), northern Western Australia (McKinnell 1993). *S. album* silviculture is more complex than traditional monocultural plantations due to the need to provide a range of host plants for *S. album*. The establishment of *Santalum* species in plantations involves initial culture under nursery conditions, during which time a pot-host is essential (Srinivasan et al. 1992; Surata 1992; Nasi 1995; Barrett and Fox 1995; Fox et al. 1996; Radomiljac 1998). The pot-host functions both in the nutrition and water relations of *S. album*, and it reduces outplanting stress when transferred to the field. *Alternanthera nana* R. Br. has been shown to be a superior pot-host for *S. album* (Radomiljac 1998).

In this study we report that the time at which *A. nana* is introduced into the nursery container with *S. album* directly affects survival and growth of *S. album* in the field.

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Methods

S. album seedlings were grown in 1.4-litre pots following Radomiljac (1998). Uniform seven-week-old seedlings were selected and *A. nana* cuttings introduced, so that at the time of field planting *S. album* seedlings had variously 134, 109, 84, 60 and 35 days' growth in association with *A. nana*. There were 60 seedlings for each time period and 60 control pots without *A. nana*.

At the time of field establishment *S. album* height and diameter at 20 mm above ground were recorded. Seedlings were harvested from each treatment and *S. album* leaf, stem, root and *A. nana* shoot dry weight was measured. Seedlings were then planted in the field in a fully randomised complete block design using standard establishment procedures and irrigation regimes (Radomiljac 1998). Each treatment plot consisted of a single row of ten seedlings planted at three-metre spacings along the row, replicated five times with a 1.8m buffer between rows.

Plants were assessed up to 23 weeks after planting for *S. album* survival, height and diameter at 100 mm above ground and also for *A. nana* survival. At each assessment a sample of three representative *S. album*

seedlings was taken from each treatment and *S. album* stem, leaf, root and *A. nana* shoot dry weight was measured after the plant material had been oven-dried at 80°C for 48 hours.

Results and Discussion

A. nana did not enhance growth of *S. album* seedlings in the nursery prior to field establishment. The growth of *A. nana* was dependent on the time at which it was introduced into the pot during the nursery phase (Table 1).

Virtually all *S. album* plants survived for the first 16 weeks in the field. After that, survival of *S. album* seedlings without *A. nana* as a pot-host decreased. *S. album* survival rate was proportional to the length of time that seedlings had been grown with *A. nana* before field establishment, except that survival for seedlings in the 134-day treatment was slightly less than for the 109-day treatment (Fig. 1(i)). The growth in height and diameter of the seedlings that were grown with *A. nana* for 134 and 109 days before field establishment was almost double that of seedlings grown without *A. nana* (Fig. 1(ii)). Data is not shown for diameter growth.

The beneficial effect of 134 and 109 days of *A. nana* is also seen in the dry-weight of the plants (only data for plants after 11 weeks in the field are shown) (Fig. 1(iii)). Unattached *S. album* showed an increase in root:shoot ratio, while the root:shoot ratio for those seedlings grown with *A. nana* was lower and remained relatively stable after field establishment (Fig. 1(iv)).

Several studies have evaluated pot-host species for *S. album*, but have dealt only with parasite and host performance during the nursery phase (Surata 1992; Fox and Doronila 1993). In this study, the growth of seedlings grown without *A. nana* was similar to that of seedlings grown with *A. nana* during the nursery phase. This indicates that seedlings may grow satisfactorily in the absence of a pot-host when conditions are favourable. This experiment showed that the influence of the pot-host was most apparent following transplanting to the field and shows the importance in evaluating pot-host species performance after field establishment.

We found that the length of time that there was a parasitic relationship between *S. album* and *A. nana* significantly affected growth and survival after transfer to the field. Highest survival and growth of *S. album* in the field was after 109–134 days of association with *A. nana* in the nursery. *S. album* growth was highest given 134 days' nursery association with *A. nana*, but survival was slightly less than for the 109 days' treatment, suggesting that growth of the pot-host may have been too vigorous for the small *S. album* seedling.

Other pot-host species, such as *Cajanus cajun* Huth., *Desmanthus virgatus* (L.) Willd., *Alternanthera* spp. Forsskal cv., and *Acacia villosa* Willd., are reported to be utilised for periods much longer than 134 days in India and Indonesia (Srinivasan et al. 1992; Surata et al. 1995; Fox et al. 1996).

The differences in root:shoot ratio in this study suggest that partitioning of resources changes from root to shoot once effective haustorial connections have been made. This means that the parasite would be very vulnerable to water and nutrient deficits if the host plant were to die.

Table 1. The effect of the number of days in which *Santalum album* and *Alternanthera nana* are grown together, on *S. album* height, stem diameter at 20 mm, plant dry-weight (DW), and *A. nana* shoot DW immediately prior to field establishment. (Control is no *A. nana* pot-host.)

Age of *S. album* at the time of *A. nana* introduction (days)	Length of *S. album*/*A. nana* association in a pot before field establishment (days)	*S. album* height (mm)	*S. album* diameter (mm)	*S. album* plant DW (g)	*A. nana* shoot DW (g)
54	134	374.1	3.9	59.1	221.5
79	109	398.2	4.4	56.0	52.6
104	84	390.0	4.2	65.3	11.6
128	60	329.5	4.0	53.1	10.2
153	35	379.5	4.1	64.3	4.4
Control	0	394.5	4.2	67.4	–

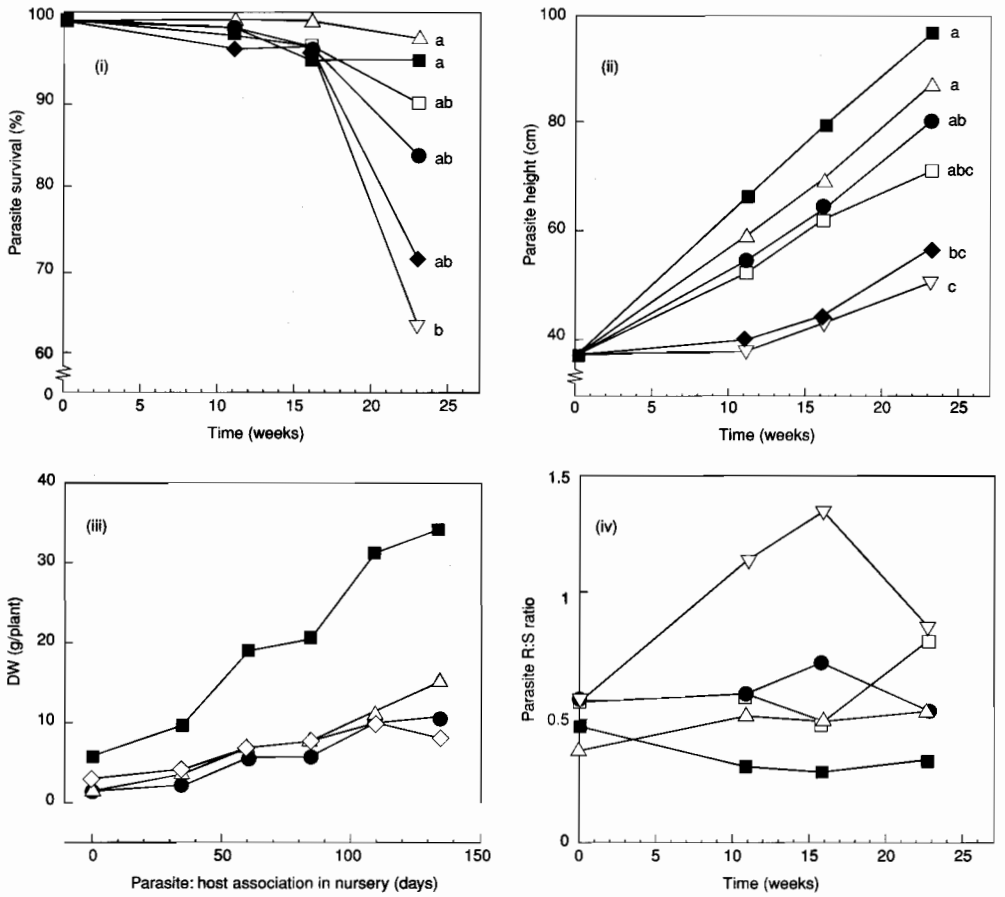

Figure 1. Growth of *Santalum album* following field establishment when attached to *Alternanthera nana* as a pot-host for (■) 134, (△) 109, (●) 84, (□) 60, (◆) 35 days, or had no pot-host (▽) whilst in a nursery container before field establishment. (Means followed by the same letter are not significantly different (P>0.05) using Tukey's pairwise t-test.)
(i) Survival of *S. album* (data from 5 replicates)
(ii) Height growth of *S. album* (5 replicates)
(iii) *S. album* plant dry-weight (3 replicates) ■ = Plant DW; △ = Leaf DW; ● = Stem DW; ◇ = Root DW
(iv) *S. album* root:shoot ratio (3 replicates)

Conclusion

This nursery and field experiment showed that early survival and growth of *S. album* plantations can be markedly improved by precise timing of utilisation of *A. nana* as a pot-host.

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Nitrogen-fixing and Non-nitrogen-fixing Woody Host Influences on the Growth of the Root Hemi-parasite *Santalum album* L.

A.M. Radomiljac and J.A. McComb*

Abstract

The growth responses of *S. album* grown with nitrogen-fixing and non-nitrogen-fixing woody hosts as single plant pairings in large nursery containers were compared over time at Kununurra, Western Australia. *S. album* growth was greater, and the root:shoot ratio lower, when it was attached to three nitrogen-fixing woody hosts (*Sesbania formosa, Acacia trachycarpa*, and *A. ampliceps*) compared with both unattached *S. album* seedlings and *S. album* grown with *Eucalyptus camaldulensis*. *S. album* shoot dry-weight increment per unit dry-weight of host shoot was greatest when attached to *S. formosa.* The growth of parasitised hosts was lower than that of unparasitised hosts. For *Santalum,* host quality is the single most important silvicultural component influencing early growth. Therefore, heartwood yield may be dependent on host species associated with *Santalum.*

SANDAL HOSTS CAN be divided into three categories—pot-hosts, intermediate hosts, and long-term hosts—the function of the former (*Alternanthera nana* R.Br.) having been described elsewhere (Radomiljac, 1998; Radomiljac et al., 1998a,b; Radomiljac and McComb, these proceedings). The intermediate host acts as a 'bridging agent' between the pot-host and long-term host, and by stimulating early growth may be an important determining factor in the future heartwood production of *S. album.*

Early growth in softwood species has been shown to be positively correlated with the extent of heartwood production in *Pinus radiata* D.Don (Hillis and Ditchburne 1974; Hillis 1987; Wilkes 1991; Climent et al. 1993). In this paper we examine the effect of various leguminous and non-leguminous woody hosts on the growth of *S. album* seedlings.

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Methods

The experiment was conducted at the Department of Conservation and Land Management's nursery at Kununurra (lat. 15° 46' S., long. 128° 44' E), Western Australia and examined the effect *S. album* growth of four intermediate host species (*Sesbania formosa* (F. Muell.) N. Burb. (Papilionaceae), *Acacia trachycarpa* E. Pritzel (Mimosaceae), *Acacia ampliceps* Maslin, *Eucalyptus camaldulensis* Dehnh. (Myrtaceae)) and a no-host control.

Six-month-old *S. album* seedlings propagated following Radomiljac (1998) were transferred to 25-litre pots, with a mix of sand:peat:perlite (3:2:2) and 10 g of slow-release fertilizer. At the time of transplanting, the *S. album* seedlings were 40.5 (± 2.99) cm high and 3.9 (± 0.58) mm in diameter. Seedlings of the intermediate hosts inoculated with appropriate *Rhizobium* strains were positioned in the 25- litre pots about 15 cm from the *S. album* seedlings. Four replicate pots of unparasitised intermediate hosts were also established. At the time of transplanting, *A. nana* pot-hosts were cut to soil level; four weeks later they were completely removed.

The experiment comprised eight complete randomised replicates with five pots per *S. album* : host association in each replicate. Plants were assessed and a sample of three pots from each treatment was harvested at five intervals: immediately before setting up the association, and 13, 24, 33 and 38 weeks afterwards.

At each harvest *S. album* height and diameter at 20 mm were measured, and (for the plants that were harvested) host and *S. album* shoot, root and leaf dry-weight and leaf area were assessed.

Results

The growth of *S. album* (height and diameter) in 25-litre pots ceased after Week 33, except for those plants grown in combination with *A. trachycarpa* which continued to grow (Fig. 1(i)). (Data is not shown for diameter growth.)

Growth of *S. album* attached to leguminous hosts was markedly better than for those grown with *E. camaldulensis*. When *S. album* was attached to

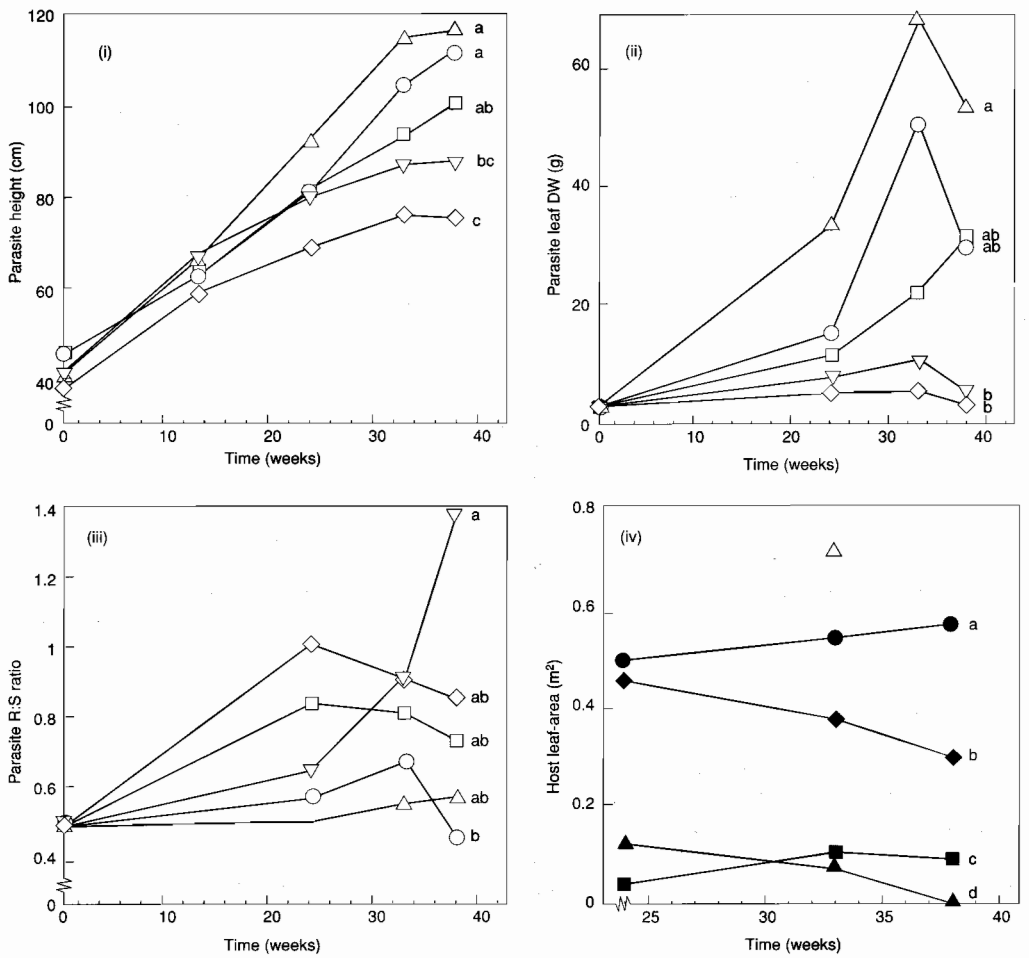

Figure 1. Growth of *Santalum album* when attached to: (△) *Sesbania formosa*, (○) *Acacia ampliceps*, (□) *A. trachycarpa*, and (◇) *Eucalyptus camaldulensis*; or grown as a no-host control (▽) cultured in 25-litre pots. (Means followed by the same letter are not significantly different ($p>0.05$) using Tukey's pairwise t-test.)

(i) Height growth of *S. album* (data from 8 replicates)

(ii) Leaf dry-weight of *S. album* (3 replicates)

(iii) *S. album* root:shoot ratio (3 replicates)

(iv) Leaf area of the host plants parasitised by *S. album* (solid symbols); and Week 33 leaf area of unparasitised *S. formosa* (open symbol) (3 replicates)

E. camaldulensis, its growth was less than when grown without a host.

The dry-weight of leaves and leaf area of *S. album* began to decline after Week 33, except for plants attached to *A. trachycarpa* (Fig. 1(ii)). As with height and diameter growth, the leaf area and dry weight of *S. album* attached to *E. camaldulensis* was less than the unattached controls. (Data is not shown for *S. album* leaf area.)

When *S. album* was grown without a host, the root:shoot ratio increased exponentially during the 38-week study. The root:shoot ratio of *S. album* attached to the poorest host, *E. camaldulensis*, was higher than those attached to the leguminous hosts (Fig. 1(iii)). At the end of the study the root:shoot ratio declined for those seedlings attached to *A. trachycarpa* and *A. ampliceps*.

After Week 33, leaf dry-weight and leaf area of parasitised *Sesbania formosa* and *E. camaldulensis* declined, while that of *A. ampliceps* continued to increase and that of *A. trachycarpa* remained constant (Fig. 1(iv)). The leaf dry-weight and leaf area of unparasitised *S. formosa* was far greater than that of the parasitised *S. formosa*. (Data are not shown for host leaf dry-weight.)

Discussion

This study showed that leguminous species promoted higher *S. album* biomass growth than the non-nitrogen-fixing *E. camaldulensis*. This is consistent with work by Rai (1990) and Taide et al. (1994) who reported *Casuarina equisetifolia* L. (Casurinaceae) to be a good *S. album* host, although in these experiments several non-nitrogen-fixing species were better hosts than the leguminous *Albizia lebbeck* (L.) Benth., *A. auriculiformis* Cunn. Ex Benth., *Leucaena leucocephala* (Lam.) De Wit (all Mimosaceae) and *Cassia fistula* L. (Caesalpinaceae).

S. album grew most when attached to *Sesbania formosa* as an intermediate host; but after 33–38 weeks *S. album* growth on this host declined in comparison with those seedlings attached to *A. trachycarpa*. It is possible that *A. trachycarpa* is a more durable host than *S. formosa*, which may be too short-lived.

The observation that *S. album* growth is poorer when attached *to E. camaldulensis* than when grown without a host suggests either that within the pot environment *E. camaldulensis* roots are better competitors for mineral nutrients than the *S. album* roots or that *E. camaldulensis* has allelopathic properties. There are other reports of root hemi-parasites performing particularly poorly on non-leguminous hosts, such as *Olax phyllanthi* (Labill) R. Br. attached to *Amaranthus caudatus* L. and *Portulaca oleracea* L. (Tennakoon and Pate 1996).

The changes in root:shoot ratio confirm the observation that unattached *S. album* directs dry-matter to the root system at the expense of its shoot (Radomiljac, McComb and Shea 1998a). After attachment, dry-weight partitioning then shifts from root to shoot, as a result of which the root:shoot ratio falls.

This study shows that *S. album* is a debilitating parasite. For *S. formosa* there was a 96 per cent decrease in leaf area as a result of parasitism. Other root hemiparasites have also been shown to have deleterious affects on their hosts (Graves et al. 1990; Graves 1995; Tennakoon and Pate 1996).

Conclusion

A nitrogen-fixing intermediate host increased early *S. album* growth. *S. formosa* was the best host of the species tested over a 38-week period, but *A. trachycarpa* may be a more sustainable host. *E. camaldulensis* was a poor host, resulting in lower *S. album* growth than that of unattached *S. album*. The longer-term performance of *S. album* on these intermediate hosts is being tested under field conditions.

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Biological and Physiological Aspects of the Woody Root Hemi-parasite *Santalum acuminatum* (Quandong) and Its Common Hosts

K.U. Tennakoon* **and J.S. Pate†**

Abstract

Our study examined a range of biological and functional aspects of the quandong (*Santalum acuminatum*) and its principal hosts in pristine bushland of the northern coastal sand plains of south Western Australia. Preliminary studies showed that the quandong spreads clonally by prolific root-suckering. Resprouting after fire occurred from bases of burnt shoots, and from new suckers forming at intervals along the lateral root-stocks of a parent clone. Systemic death of whole clones was observed after particularly intense fire. Recruitment from seed occurred very rarely, and mortality rates of seedlings in nature were high.

Key words: *Santalum acuminatum*, root hemi-parasite, haustoria, water relations, xylem transport, ^{15}N-labelled substrate

ASSESSMENTS BASED ON densities of above-ground biomass, ground-cover, and frequencies of haustoria on host roots indicated that woody nitrogen-fixers (legumes and *Allocasuarina*) were principal hosts for *S. acuminatum*.

Formation and functional life of haustoria were closely coordinated with seasonal growth of hosts, with haustoria surviving summer and overlapping functionally with new ones establishing in the next autumn. This strategy ensures effective continuity with hosts in terms of abstraction of water and nutrients across and between seasons. Structural studies of haustoria show that the interface with host xylem is almost entirely parenchymatous tissue. No luminal continuities were observed between xylem conducting elements of the partners.

Daytime transpiration rates and photosynthetic rates of *S. acuminatum* were consistently less than the principal host, *Acacia rostellifera*, and water-use efficiencies were similar. This suggested that quandong might depend heavily on its hosts for carbon. From subsequent studies examining C:N ratios of xylem sap and dry-matter of a range of *A. rostellifera* hosts and *S. acuminatum* (Tennakoon and Pate 1996), it was estimated *S. acuminatum* obtains more than one third of its carbon requirements heterotrophically (H) (Table 1).

Table 1. Heterotrophic input of carbon (H) to *Santalum*

Mean concentration in xylem sap (μg/mL)	Source of carbon		
	Amino acids	Sugars	Organic acids
C	128 ± 28	834 ± 140	46.6 ± 10.0
N	35.5 ± 7.6	–	–

Notes:
- H = (xylem-sap C:N) × 100 / (dry-matter C:N)
- *Santalum* xylem-sap C:N ratio = 28.4 ± 5.6
- *Santalum* dry-matter C:N ratio = 71.6 ± 9.7
- C% in *Santalum* dry-matter = 48.0 ± 6.6
- N% in *Santalum* dry-matter = 0.67 ± 0.12
- H to *Santalum* from the commonly parasitising host *Acacia rostellifera* is 39.6%.

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Gas-exchange data obtained for *S. acuminatum* and *A. rostellifera* at a series of intervals in each season of a year correlated well with carbon isotope discrimination values ($\delta^{13}C$) for dry-matter of the canopy leaves of the same two species. The specific hydraulic conductivity values for water-flow through roots of quandong were consistently lower than those recorded for *Acacia*. This was viewed as evidence of conservatism in water-use by the parasite, just as suggested above from the gas-exchange studies.

Solute composition of root xylem sap of common native hosts of quandong was compared with that of corresponding xylem sap and ethanolic extracts of endophytic tissues of haustoria of the hemi-parasite. The data indicated only limited direct flow of amino compounds between xylem streams of host and parasite, a finding consistent with the above-mentioned absence of lumen-to-lumen xylem vessel continuity. Proline predominated in the haustorium and xylem of quandong, but was at negligible levels in the xylem of most hosts. Sucrose, fructose, glucose malate and citrate were at high levels in all saps, and fructose was especially prominent in *Santalum*. Infiltration of xylem of haustoria-bearing root segments of the major host (*A. rostellifera*) with a range of ^{15}N-labelled substrates resulted in 40–80 per cent of the ^{15}N of endophytes of the attached haustoria being recovered as proline. The study concluded that haustoria of *Santalum* function as major sites of synthesis and export of proline, and might therefore play an important role in osmotic adjustment of quandong in acquiring water from hosts under differing levels of stress.

While *S. acuminatum* has so far not been significant to the sandalwood industry, it is hoped that the approaches employed and findings gained in the present study provide insight on how other more significant species of the genus might behave in natural or plantation situations.

Part of the work summarised here has been published elsewhere as a pair of papers (Tennakoon, Pate and Arthur 1997; Tennakoon, Pate and Stewart 1997).

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In-vitro and In-vivo Micrografting of *Santalum album* L. Shoot-tips

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Abstract

In-vitro and in-vivo micrografting of *Santalum album* was achieved by placing 1–2 cm scion explants derived from in-vitro and field-grown shoot apices on the hypocotyls of decapitated 40–45-day-old seedlings. Under favourable conditions, the scion and hypocotyl united to form a complete plant which produced 2–4 leaves after 6–8 weeks. These plants were transplanted to the greenhouse having been conditioned to ambient humidity for two weeks.

Abbreviations:
BAP = 6-benzylaminopurine
DIECA = sodium diethyl-dithiocarbamate
GA = gibberellic acid

VEGETATIVE PROPAGATION IS an important method of propagating clones of tree species. Several methods of vegetative propagation, for example through leaves, stem-cuttings, root-suckers, air-layering, and grafting, are possible (Mahalstal and Haler 1959). Mature trees are often preferred for cloning because of their long survival in the field and proven superior value.

Clonal propagation of sandal is achieved by cleft grafting on seedlings at least one year old, and by propagating root-suckers. However, propagation through shoot-tips or branch-cuttings have not been successful. Sandal plants have been successfully micropropagated by in-vitro methods either using juvenile or mature plants (Lakshmi Sita 1986; Rao and Bapat 1992). Difficulties have been encountered in rooting of shoot apex in sandal (Rao and Bapat 1992). The process of shoot apex grafting has been reviewed (Jonard 1986). In-vitro or in-vivo shoot-apex grafting in sandal helps in propagating disease-resistant trees and trees with high oil content. This paper describes a novel method for micropropagation of sandal plants by micrografting.

Preparation of Rootstocks

Freshly collected *Santalum album* L. seeds were de-coated, washed 5–10 times in water and treated with GA (500 mg/L) overnight; these seeds were used for germinating seedlings for root stock.

In-vitro

GA-treated seeds were thoroughly washed with distilled water (5–10 times), surface-sterilised by immersing in 1.5% $HgCl_2$ for ten minutes, and washed with sterile water (8–10 times) under aseptic conditions. These seeds were inoculated on MS basal medium (Murashige and Skoog 1962) supplemented with 3% sucrose and 0.6% agar. They germinated in eight-hours (in the dark) and 16 hours (under cool white fluorescent light of 40 μE m^2/sec) at 25±2°C with 70% relative humidity.

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In-vivo

GA-treated seeds were sown in 300 mL root-trainers filled with *Soilrite* and watered regularly.

Preparation of Scions

Lateral branches (10–12 cm length) containing preformed apical and axillary buds were collected from 50–60-year-old trees. Shoots 8–10 cm long were washed with running tap water (30–40 minutes), then treated with detergent (0.1 v/v Linbro®) for 5–10 minutes, followed by 0.1% fungicide (Bavestin®) treatment for 3–5 minutes, then washed thoroughly with distilled water. Shoots 2–3 cm long were used for in-vivo micrografting.

For the grafting itself, shoots were sterilised with 0.05% $HgCl_2$ for ten minutes and washed with sterile water (3–4 times). Shoots 1–2 cm long were excised and kept ready in a petriplate containing sterilised wet cotton. A few shoots were also inoculated on MS medium with 5 mg/L BAP for multiple shoot production. Shoots derived in-vitro were used as scions.

Micrografting Procedure

In-vitro

Under aseptic conditions a 0.5–1 cm vertical split was made on the top of the decapitated root stock using a sharp surgical blade. A shoot-apex scion developed either in vitro or in vivo was inserted into the stock incision. After grafting, the young plants were transferred to tubes containing liquid MS medium (half-strength) with 3% sucrose. The radical of the plant was pushed through the hole in the filter paper bridge as a support (Figs 1–4).

In-vivo

An incision of 0.5–1 cm was made on the decapitated greenhouse-grown seedling (about 40–45-days-old) using a surgical blade (Fig. 5). A drop of DIECA (1.5 g/L) and Zeatin® or BAP (0.1 mg/L) was placed on the wounded zone. A shoot-apex 1–2 cm long derived from the tree was inserted into the incision, and an elastic strip or paper bandage was applied to cover the grafted zone (Figs 5–8).

Figure 1. In-vitro micrografting, development of scion on root-stock graft

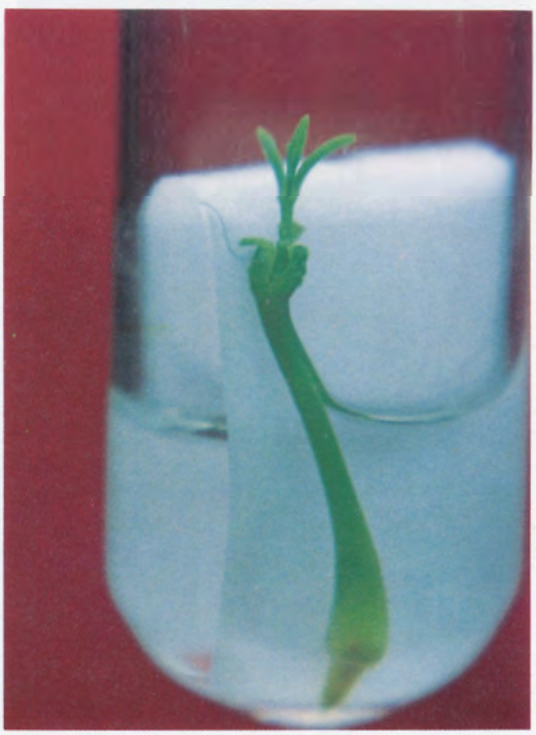

Figure 2. In-vitro micrografting, development of scion on root-stock graft

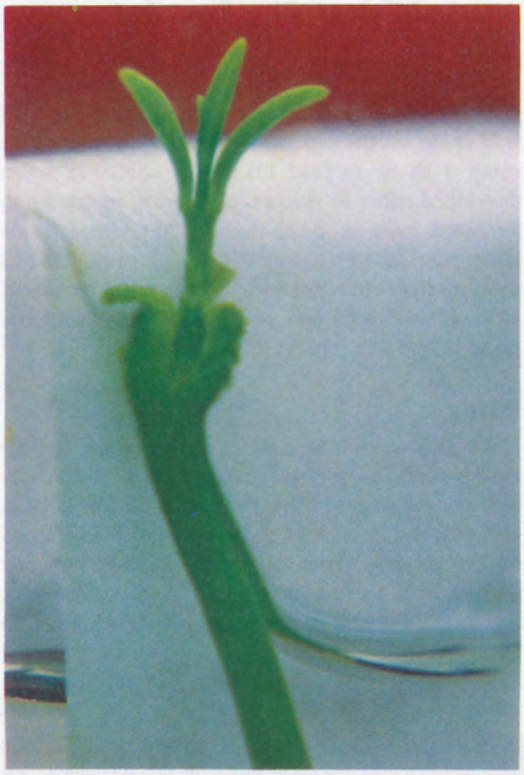

Figure 3. In-vitro micrografting, established plant

Figure 4. In-vitro micrografting, established plant

Figure 5. In-vivo micrografting, incision on root-stock

Figure 6. In-vivo micrografting, positioning of the pre-treated scion

Figure 7. In-vivo micrografting, applying elastic strip to protect the grafted zone

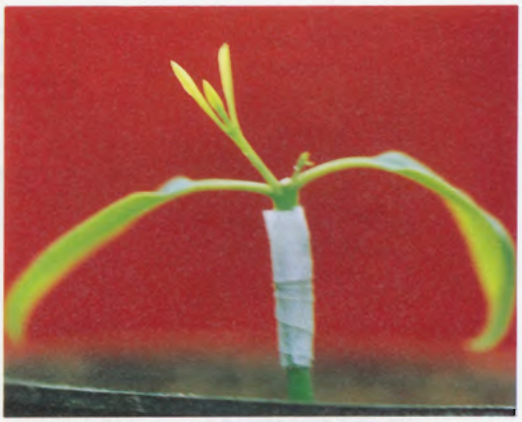

Figure 8. In-vivo micrografting, graft-established plant

Maintenance of Grafted Plantlets

Culture tubes with the shoot-apices / root-stock graft were incubated in a culture chamber 21±1°C with continuous illumination (40 µE/m2/sec), and observed periodically to evaluate survival of scion and root-stocks. The proportions of survival and rooting of these plants were recorded four weeks after grafting, while number of roots per shoots and elongation of scion were determined 6–8 weeks after grafting. Adventitious shoots arising from root-stocks were removed aseptically with surgical scissors.

In-vivo grafts were covered with plastic to minimise moisture loss, kept at 22±1°C under continuous light (40 µE/m2/sec), and observed periodically to evaluate survival of scion (Fig. 8). Plants were watered by syringe, and grafted shoots and elongation of scion were determined 4–6 weeks after grafting.

Hardening and Transplanting

After about 6–8 weeks of in-vitro shoot apex grafting, plants with at least 2–4 expanded leaves grown from scion, were transplanted to root-trainers containing sterilised Soilrite®. The top portion of the root-trainers was covered with plastic and maintained at 22±1°C under continuous light. After two weeks, transplanted seedlings were moved to the greenhouse. Well-established in-vivo shoot-apex grafted plants with 2–4 expanded leaves with axillary buds were transferred to the nursery via the greenhouse.

Results and Conclusions

The frequency of success in shoot-apex grafting of *Santalum album* was highest in root-stock and shoots derived in vitro (Table 1). The probability of successful graft unions, both in vitro and in vivo, increased with length of scion material (Tables 1 and 2). The likelihood of union between stock and scion was increased when the scion material had more leaf primordia with the apical dome. Shoot-tips (scion) grafted on to the top of the lower hypocotyl region of root-stocks (40–45 days old), gave better growth by 4–6 weeks than those grafted on to the top of the hypocotyl region as root-stock. This may be due to the fleshier and thicker region nearer the food reserve of the developing seedling. However, success of grafting

Table 1. Influence of length of explant on in-vitro micrografting

Component	Success indicators		
	No. of explants	Length of scion (cm)	No. of successful grafts
Apical dome	20	0.4–0.5	2
Apical dome and two leaf primordia	20	1.0–1.5	7
Apical dome and 4–6 leaf primordia	20	1.0–2.0	12

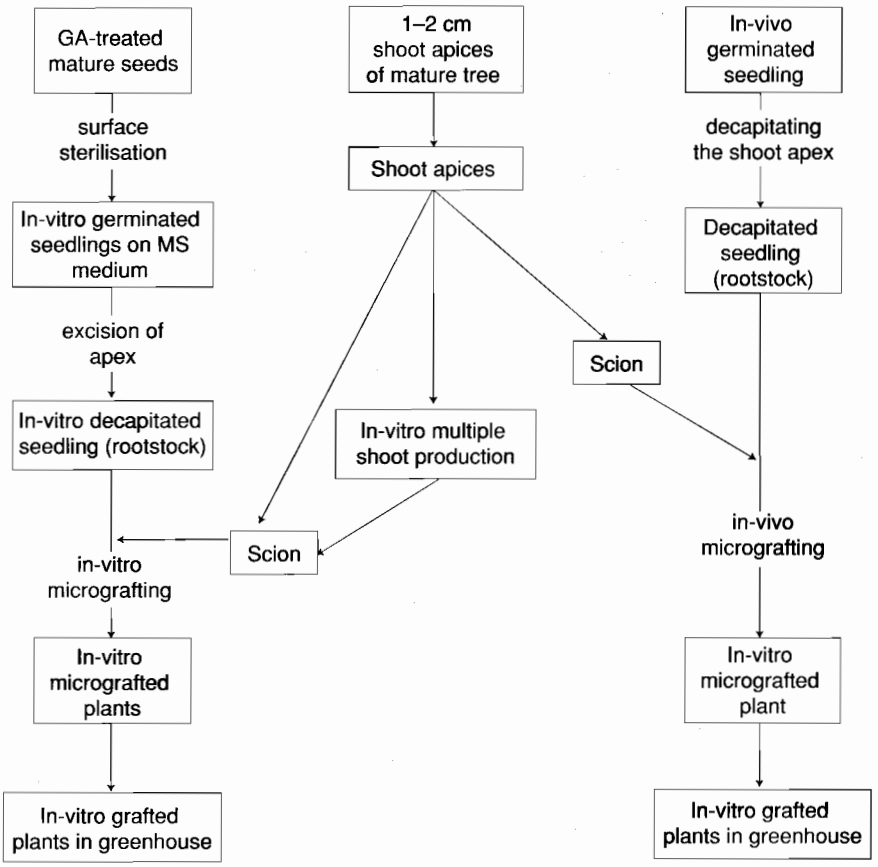

Figure 9. A schematic representation of in-vivo and in-vitro micrografting in sandalwood

declined as age of the seedling increased, since it has been observed that the hypocotyl region becomes narrower and harder with age.

In-vitro and in-vivo micrografting is simple and efficient (Fig. 9). Many desired propagules or clones can be produced quickly. However, further investigation on the standardisation of micrografting under different conditions is needed and is in progress.

Table 2. Influence of length of explant on in-vivo micrografting.

Component	Success indicators		
	No. of explants	Length of scion (cm)	No. of successful grafts
Apical dome	20	0.8–1.0	0
Apical dome and two leaf primordia	20	1.0–1.5	0
Apical dome and 4–6 leaf primordia	20	1.0–2.0	6
Apical dome and > 6 leaf primordia	20	2.5–3.0	8

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Influence of Carbon Source and pH on Rapid Mass Propagation of *Santalum album* by Somatic Embryogenesis: the Application of Biotechnology in Agroforestry

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Abstract

Effective in-vitro regeneration of *Santalum album* through somatic embryogenesis has been widely reported. It may be ten times faster than multiple-shoot induction multiplication by somatic embryogenesis. In the present investigation the type and concentration of carbon source requirement were optimised for embryo production. The influence of media pH has also been studied. Among the three carbon sources tested, 4% sucrose was best. Constant pH at 6.0 promoted maximum embryo production with minimum abnormalities. The relevance of the work in relation to agroforestry is discussed.

AS IN OTHER plants, the importance of in-vitro propagation of the aromatic tree *Santalum album* has been realised (Rangaswamy and Rao 1963; Lakshmi Sita et al. 1979). Apart from heterogeneity with regard to the oil-content of the field grown plants, this important genetic resource is also prone to severe pathogenic attack and is the victim of human greed (Srinivasan et al. 1992).

In addition to state-level efforts, agroforestry on wasteland with private participation may help to conserve this useful germplasm in an effective manner. In-vitro selection and genetic manipulation through biotechnological routes are other attractive propositions. Development of rapid mass propagation methods will facilitate the achievement of these aims.

The micropropagation of sandal plants has been known for some time (e.g. Lakshmi Sita et al. 1980). Somatic embryogenesis has been observed to be more effective in this regard. Further, somatic embryo production in a bioreactor and their encapsulation to form 'synthetic seeds' have also been researched (Bapat and Rao 1988; Bapat et al. 1990). The detailed investigations on the influence of various physiochemical parameters in relation to the maximisation of normal somatic seedling production are, however, not well documented even though these have a significant impact in improving the process efficiency.

In the present article the influence of carbon sources and pH on mature somatic embryo production are reported.

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Materials and Methods

Slant media were used in 38 × 200 mm borosilicate culture tubes in 25 replicates for each treatment.

Plant material

To establish the culture, seed of the 'Elite Kerala' clone was initially obtained from the Institute of Wood Science and Technology, Bangalore.

Medium

Modified MS solid medium (MS minerals + B5 vitamins) was used with various sugar supplements, phytohormones/regulators and buffers. Carbon sources at 0–10% (w/v) levels and pH 4.5–7.0 were also used.

Illumination

The cultures were exposed to cool, white fluorescent light at 1500 lux. The culture room temperature and humidity were 26±2°C and 60–70 %.

Explants

Nodal and hypocotyl explants of in-vitro germinated seedlings were initially used.

Table 1. Regeneration frequency through multiple-shoot induction and somatic embryogenesis

Explant	Response	No. of shoots or normal torpedo-stage embryo/ explants	Incubation time (weeks)
Nodal	Multiple-shoot formation	3–5	6–8
Hypocotyl (through calli)	Somatic embryogenesis	25–60	3–4

Phytohormones:
- for shoot induction: BA (0.5 ppm)
- for somatic embryogenesis: BA (0.5 ppm) + IAA (0.5 ppm)

Results and Discussion

Regeneration through multiple-shoot induction and somatic embryogenesis

The comparative regeneration frequency data is presented in Table 1. It is clear that regeneration through somatic embryogenesis is nearly ten times faster as a method of propagule production. Moreover, in a multiple-shoot formation pathway, much time is required for induction.

Effects of carbon sources on somatic embryogenesis

Sucrose, glucose and fructose were tested at 0–10% levels (Table 2). Maximum embryogenesis occurs in glucose up to the 2% level, whereas the best carbon source at higher than 2% levels is sucrose. Sucrose at the 4% level was optimum (Table 3). Embryo abnormalities increased greatly above 4% sugar levels.

Influence of media pH on embryogenesis

Media pH were adjusted separately in the range of 4.5–7.0. Medium pH of 6.0 was optimum with regard to embryo production level and minimum abnormalities.

The data presented in Tables 1–4 indicate that sucrose at the 4% level and medium pH of 6.0 were optimum for maximising the production of normal mature embryos. With lower sucrose concentrations the embryos appeared more swollen and resulted in brittle somatic seedlings after germination.

Table 2. Effects of carbon source on somatic embryogenesis

Sugar concentration (% w/v)	Mature embryos (No./g fresh wt)			Normal embryos (%)		
	Sucrose	Glucose	Fructose	Sucrose	Glucose	Fructose
0	–	–	–	–	–	–
1	121	168	152	66.8	63.8	59.8
2	222	243	231	62.1	53.1	51.3
4	301	207	173	54.4	39.2	33.2
6	312	162	112	31.6	25.3	20.5
8	129	21	–	8.2	6.3	–
10	55	15	–	–	–	–

Culture conditions:
- hormones—BA (0.5 ppm) + IAA (0.5 ppm)
- pH: 5.8
- incubation period: 4 weeks

Table 3. Effects of sucrose concentration on the growth and development of somatic embryos

Sugar concentration (% w/v)	Embryos at different stages of development (Number)				Normal mature embryos (%)
	Globular	Heart	Torpedo	Mature	
0	–	–	–	–	–
1	275	185	212	121	65.8
2	410	231	383	222	62.1
4	655	237	625	301	55.5
6	673	287	516	290	31.6
8	675	350	323	129	8.2
10	337	372	75	55	0

Culture conditions:
- hormones—BA (0.5 ppm) + IAA (0.5 ppm)
- pH: 5.8
- incubation period: 4 weeks

Such embryos had increased tendencies of callusing during germination. One of the major problems of somatic embryogenesis on solid media was the formation of cell-mass aggregates which made the separation of normal mature embryos difficult. The use of agitated liquid media helped to overcome the problem partially. Further research on these aspects are necessary. Rooting of the multiple shoots was difficult to induce and performance was non-reproducible in batches.

Table 4. Effects of pH on somatic embryogenesis

Media pH	Mature embryos (No./g fresh wt)	Normal embryos (%)
4.5	269	33.9
5.0	307	37.1
5.5	371	57.2
6.0	385	61.3
6.5	353	33.2
7.0	326	22.0

Culture conditions:
- hormones: BA (0.5 ppm) + IAA (0.5 ppm)
- pH: 5.8
- incubation period: 4 weeks
- Sugar: 4% w/v
- MES buffer: 2 g/L
- inoculum: embryogenic callus

Conclusion

Somatic embryogenesis is a more effective propagation means than multiplication by multiple-shoot induction. This was further complicated by poor root induction. Proper optimisation of carbon sources and pH improved somatic embryogenesis. Optimisation of other parameters may improve performance.

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Silvicultural Strategies for Augmentation of Sandal Regeneration

C. Surendran, K.T. Parthiban, C. Bhuvaneswaran and M. Murugesh*

Abstract

The various silvicultural approaches to encourage regeneration of sandal and its subsequent establishment are described. The potential for augmenting sandal regeneration, and various problems that limit it, are also discussed. Early germination was achieved after treating the seeds with GA (0.05%). Among the various host species introduced, *Albizia saman* improved the growth and development of sandal seedlings best. Tending-plot technique and soil-working also increased the germination capacity of self-sown seeds and their growth and development.

THE FRAGRANT HEARTWOOD of *Santalum album* is sandalwood, most of which is obtained from natural forests. *S. album* is indigenous to peninsular India and its natural distribution is about 9600 km^2. This limited distribution, and the complexities involved both in germination and in host-specificity, imperils the existence of this species in the near future.

Failure of regeneration efforts is one of the main causes of sandal depletion, and although natural regeneration of sandal seedlings is prolific, mortality of seedlings due to natural calamities is equally high. Moreover, in natural forests sandal occurs in association with other species. Though the knowledge of sandal as a root parasite dates back to 1871, its host-specificity is still in dispute.

Artificial regeneration of the species has not been preferred because of poor understanding of the host–parasite relationship and edaphic factors. Studies were carried out at Forest College and the Research Institute, Mettupalayam, on germination, host–parasite relationships, and soil-working with the aim of encouraging artificial regeneration.

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Materials and Methods

Germination studies

Seeds were collected from identified superior trees. After depulping the fruits, the seeds were shade-dried and different pre-sowing treatments imposed. Germination rate was observed four weeks after sowing.

Host species evaluation

These experiments were carried out on two bases:

- initial growth of sandal seedling, and
- host species' growth attributes.

Firstly, 30-day-old sandal seedlings were transplanted with host plants of different species. The trial was replicated three times, with ten plants per replication. Host plants were pruned periodically once in 30 days. Observation on shoot growth of sandal was made at 60-day intervals.

Secondly, different host species were evaluated for growth attributes. Root volume, root and shoot biomass, and root:shoot ratio were assessed. The trial used destructive sampling, and was replicated twice.

Host species manipulation

Albizia saman was manipulated in two ways for better sandal establishment. One set of hosts was

manipulated for 120 days, and then leading shoots were cut and partially pruned; another set was severely and periodically pruned once per month. Mortality, shoot length and growth rate of sandal was evaluated at 30-day intervals.

Rhizosphere soil influence

Soil under the sandal tree crown was raked to encourage regeneration. The raked soil was protected from biotic influence by erecting chain-link fence. The sandal regeneration, growth and establishment were studied in both tended and untended plots at monthly intervals. The regeneration abundance was worked out using a quadrate method. No cultural operation took place on the control plot.

Statistical analysis

The data obtained in the present investigations were subjected to analysis of variance, and treatment differences were tested for significance following Panse and Sukatme (1967).

Table 1. Effect of pre-sowing seed treatments on germination

Treatments	Mean germination (%)				
	Control	Acid scarification (minutes)			
		5	20	25	30
Control	–	7	18	15	10
GA 0.05%	5	6	22	28	10

Results and Discussion

Germination studies

Germination rates of 80 per cent under laboratory conditions, and 60 per cent (in the field) have been reported elsewhere (ICFRE 1997). In this investigation, maximum 30-day germination (28%) was achieved when fresh seeds were scarified with H_2SO_4 for 25–30 minutes and soaked overnight with GA (0.05%). By contrast, untreated seeds recorded nil germination at 30 days (Table 1), and other treatments had no germination. The poor germination recorded in the present investigation may be due to genotype.

Host species evaluation

On the basis of initial growth of sandal seedling

The shoot growth rate of sandal differed significantly with various host species. This variation manifested only after six months.

Among nine host species evaluated for their influence on the initial growth of sandal, four species (*Acacia leucophloea, Albizia saman, Casuarina equisetifolia* and *Cajanus cajan*) enhanced the shoot growth of sandal more than other species (40 cm, 360 days after transplanting—see Table 2). The four nodulating plants have also been reported as host plants for sandal by many authors (e.g. Subbarao et al. 1990; Bagchi and Veerendra 1991; Taide et al. 1994).

Table 2. Evaluation of host species based on initial growth of sandal seedlings

Host species	Mean shoot length of sandal (cm)			
	180 DAT[a]	240 DAT	300 DAT	360 DAT
Acacia leucophloea	18.0	26.0	54.0	69.0
Albizia lebbeck	15.0	17.5	21.5	29.5
Albizia saman	25.0	31.0	39.5	49.0
Cajanus cajan	14.0	18.0	32.0	41.5
Cassia fistula	12.0	14.0	21.0	28.0
Casuarina equisetifolia	22.0	30.0	39.5	45.0
Eucalyptus tereticornis	17.5	19.5	21.0	22.0
Leucaena leucocephala	16.5	17.5	20.5	26.0
Peltophorum pterocarpum	13.0	15.0	23.5	30.0
Control	10.0	15.7	18.0	25.0
S.E. (d)	0.157	0.490	0.441	0.504
C.D. (5%)	0.33	1.03	0.93	1.06

[a] DAT = Days after transplanting

Sandal cannot grow normally without a host plant (Rangaswamy et al. 1986; Ananthapadmanabha et al. 1988). It depends on its host for phosphorus, potassium, and magnesium. Sandals with hosts have a higher survival rate (Nagarajaiah and Rao 1993) and increased plant height and volume of the main stem (Shinde et al. 1993) than those without hosts.

On the basis of growth attributes of host plants

Among the species evaluated, *Albizia saman* registered the highest root volume (12 cm^3), root biomass (3.31 g) and root:shoot ratio (2.4) associated with higher initial establishment rate.

In the other host species, root volume was 2.0–8.6 cm^3, root biomass was 0.8–1.87 g, and root:shoot ratio was 0.4–1.6 (Table 3). This study revealed that host species having higher root:shoot ratio and root biomass may have greater potential in nature for infecting sandal. For long-term field establishment, it was reported by Fox et al. (1990) that the root growth and haustorial development with the pot host is an essential requirement.

Host species manipulation

Observation at 30-day intervals revealed that besides little difference in the mean shoot length, greater mortality rate (27–30%) was observed from the sandal seedlings associated with unpruned host plants compared with that of the pruned host plants (11–24%) in the early stages of establishment (120 days-after-transplanting (DAT)). This clearly shows that for better establishment of sandal seedlings, particularly at the early stages, pruning of the host is warranted.

At 150 DAT, there were significant differences in the rate of height growth between the sandal seedlings associated with severely pruned hosts and those with partially pruned hosts (Table 4). Sandal seedlings associated with severely pruned host showed height growth of 1.5 cm/month, compared with up to 4.5 cm/month with hosts not so pruned. Pruning the host severely significantly reduced shoot-growth rate, particularly from 150 DAT. Sivaramakrishnan et al. (1984) reported that the height of the host should be kept below the sandal seedlings by clipping off the terminals. But pruning the host to a severe degree as practised by forest departments will reduce sandal growth as the continued growth of the seedlings depends on attachment to a suitable vigorous host (Crossland 1982).

Rhizosphere soil influence

Soil-working improved the germination of self-sown seeds. The intensity of seedlings in the sandal plot was up to 46.0 per square metre during September, compared with 13 in the control plot. A steady decline in sandal establishment was noticed, both in the tended and untended plots. This is mainly attributed to the decrease in moisture content of the soil and other climatic changes.

Table 3. Evaluation of host species based on their growth attributes.

Host species	Growth attribute			
	Root volume (cm^3)	OD weight (g)		Root:shoot ratio
		Shoot	Root	
Acacia leucophloea	4.0	0.70	1.12	1.6
Albizia lebbeck	3.0	1.28	1.16	0.9
Albizia saman	12.0	1.36	3.31	2.4
Cajanus cajan	8.5	1.75	1.14	1.3
Cassia fistula	2.0	0.83	1.04	1.3
Casuarina equisetifolia	4.5	1.81	0.80	0.4
Eucalyptus tereticornis	6.5	1.42	1.64	1.2
Leucaena leucocephala	6.0	1.40	1.52	1.1
Peltophorum pterocarpum	8.0	1.80	1.87	1.0
S.E. (d)	0.183	0.015	0.027	0.021
C.D. (5%)	0.40	0.03	0.06	0.04

Table 4. Effect of pruning of host on sandal establishment and growth rate

Period	Mortality rate (%)		Shoot length (cm)		Shoot growth-rate (cm/month)	
DAT[a]	Pruned	Unpruned	Pruned	Unpruned	Pruned	Unpruned
30	11.10	26.69	12.00	11.18	0.30	0.56
60	23.60	29.00	12.26	11.76	0.27	0.58
90	22.30	30.00	12.92	14.10	0.65	2.34
120	15.00	18.00	18.42	20.60	5.51	6.51
150	12.00	11.50	20.48	27.63	2.06	7.02
180	4.60	5.20	21.00	34.14	0.54	6.51
210	0.00	0.00	21.80	40.15	0.80	6.00
240	0.00	0.00	23.53	64.35	1.70	6.20
Mean	11.07	15.04	17.81	25.74	1.48	4.47
	Treat	Interaction	Treat	Interaction	Treat	Interaction
S.E. (d)	0.810	2.064	0.124	0.350	0.032	0.091
C.D. (5%)	1.67	4.25	0.25	0.72	0.07	0.19

[a] DAT = days after transplanting

Table 5. Effect of tending operation on regeneration dynamics and growth rate of sandal

Plots	Seedling regeneration (no./ m^2)							Shoot growth (cm)	
	Sep. 95	Nov. 95	Jan. 96	Mar. 96	May 96	July 96	Sep. 96	Average	Maximum
Tended	46.0	35.5	28.5	26.0	22.0	21.5	19.0	3.3	47.0
Untended	13.0	12.0	10.5	9.0	6.0	5.5	5.0	1.2	21.0

At the end of one year, 19 of the 46 naturally-regenerated seedlings became established in the tended plot, compared with five in the untended plot. Further shoot growth was also higher in the tended plot compared with the untended plot (Table 5).

Future Strategies

Selection of 'plus' trees

For future improvement programs, more plus trees need to be identified using the selection index method. Seeds and clonal materials may be collected from these trees, and may be used for further development programs.

Promotion of natural regeneration

Regeneration can be promoted by maintaining the association of species. Sandal seeds usually germinate well in bushy areas. So by providing a suitable environment by maintaining the various host species, the retention of natural vegetation will promote regeneration.

Propagation by root-sucker

Sandal is a good coppicer at early stages and also produces root suckers. Root-suckers can also be induced by digging a 45 cm deep circular trench around the trunk of the tree at a radius of up to 30 cm. This exposes the primary roots of sandal in the pre-monsoon season, and these root-suckers will establish without host species.

Establishing sandal estates

More sandal estates are to be established using both seed and clonal propagation methods. They will form the basis for future breeding programs.

Clonal approaches

Research on both micro- and macro-propagation methods should be increased. Although protocols are available for in-vitro multiplication of sandal, its field

establishment is still in its infancy. Macro-propagation using cuttings should also be developed for mature trees so that true-to-type progenies can be produced for re-introduction into their original habitats.

Conclusions

Investigations were carried out to encourage the regeneration of sandal through soil-working, tended-plot techniques and host species evaluation.

We found that natural regeneration of sandal was better in tended plots (40.2 seedlings/m^2) than in untended plots (9.1 seedlings/m^2). In the same way, soil-working practice improved the germination of self-sown seeds. Artificial regeneration of sandal was improved by treating the seeds with H_2SO_4 (commercial grade) for 25 minutes, followed by GA application (0.05%) overnight.

Studies were also carried out to evaluate different host species on the basis of sandal growth, growth attributes of host species, and host species manipulation. On the basis of the initial growth of sandal, four species (*Acacia leucophloea, Albizia saman, Casuarina equisetifolia* and *Cajanus cajan*) were screened for better sandal seedling growth. On the basis of growth attributes and amenability for pruning, *Albizia saman* was found to be the best host.

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In-vitro Strategies for the Mass Multiplication of Sandal

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Abstract

The sandal tree (*Santalum album* L.) is indigenous to peninsular India and is prized for its globally-acclaimed sandalwood oil. This tree is facing extinction owing to wanton felling by smugglers, despite stringent anti-smuggling measures taken by the Government. In-vitro strategies for restoring it to its pristine glory are suggested in this paper. In-vitro regeneration has the advantage of surmounting the problem of long gestation periods and the heterozygous nature of the trees; so it was tried for large-scale multiplication of elite sandal phenotypes. Superior phenotypes were identified and explants, e.g. nodal segments, shoot tips, inflorescence, and seeds, were collected for the investigation. Very good axillary shoot multiplication was achieved less than four weeks after inoculation on MS medium supplemented with a wide range of treatments with BAP and Kinetin. These shoots were further separated and subcultured for further shoot multiplication, and also for root induction for which different host species were also introduced into the medium. Callus cultures were established using inflorescence and hypocotyl segments after 3–4 weeks from inoculation. The calli were pale yellow to yellow in colour. These callus cultures were periodically subcultured for shoot bud induction (organogenesis) and somatic embryo development (embryogenesis). Shoot bud organogenesis was achieved in callus cultures using BAP and Kinetin as growth supplements. These shoot-buds were further elongated and separated for root induction. Single-rooted plantlets were established in some cultures. However most of the treatments for rooting trials were unsuccessful. The potential of other tissue culture techniques is also discussed.

SANDAL GROWS NATURALLY in the states of Tamil Nadu and Karnataka of India. It occupies a prime position in the country's forestry, earning valuable foreign exchange. In view of their intrinsic worth, all trees, whether grown on private or public land, belong to the state, and their management is governed by stringent regulations.

Owing to the dysgenic selection practised by smugglers, even the existing population comprises mostly genetically inferior trees. Added to this, sandal tree suffers from a number of diseases of which spike disease is the most destructive (Muthana 1995). Concerted efforts in the preservation and protection of existing sandal plantations, and the development of alternate techniques for rapid multiplication of resistant trees, is required. Tissue culture studies have already been reported using juvenile and mature explants (Rao and Bapat 1978; Bapat and Rao 1979, 1984, 1989; Lakshmi Sita and Raghava Ram 1995). Artificial seeding using somatic embryo encapsulation and its recovery has also been successful (Bapat and Rao 1989). However attempts at in-vitro rooting of mature trees have not been successful so far. Hence the present investigation was carried out to multiply superior sandal trees using direct and indirect organogenesis.

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Materials and Methods

Selection of superior phenotypes

A reconnaissance survey was made in the 1977 sandal plantation in Kavalur of Alangayam range in the Tirupattur, Haur and Coimbatore divisions of Tamil Nadu. A total 17 candidate trees were selected for their straight and cylindrical bole, narrow crown, small branch and freedom from pests and disease. The morphometric traits total height, bole height and gbh were measured. The details of 'plus' trees selected are presented in Table 1. Only those candidate trees which met the minimum standards of superiority of each character were selected as plus trees following the approach of Pitcher and Doru (1967): superiority (S%) = (C/A × 100) – 100, where C = candidate trees, and A = average of comparison trees.

Micropropagation of select trees

Explants (shoot tips and nodal segments) were collected from the identified plus trees for in-vitro use. They were washed thoroughly with tap water, rinsed with 95% ethanol and disinfected sodium hypochlorite at 1% (v/v) for 3–5 minutes. They were then washed thoroughly with sterile water and inoculated on to the MS medium (Murashige and Skoog 1962) supplemented with various concentrations and combinations of growth regulators. In each 24-hour cycle period the cultures were kept for 16 hours at 1500 lux light intensity and 22–26°C.

Nodal explants were inoculated either singly or jointly onto the multiplication medium containing various concentrations and combinations of BAP and Kinetin. Four weeks after inoculation, cultures were measured for multiple shoot induction and number of shoots per explant.

In-vitro rooting of shoots

The multiple shoots produced were separated out individually and in groups of 2–3 shoots, and inoculated on to various rooting media with different concentrations and combinations of IAA and IBA. Different host species exudates were also added into the medium. Host species—*Eucalyptus tereticornis, Casuarina equisetifolia* and *Acacia senegal*—were introduced in vitro.

Indirect organogenesis

Mature and immature inflorescences and hypocotyl segments were collected from the selected superior trees. After disinfection they were inoculated onto auxin-rich media for callus induction. The calli induced in this way were subcultured periodically for organogenesis and embryogenesis.

Table 1. Growth attributes of candidate trees in natural stands

Tree No.	GBH (cm)	Total height (m)	Bole height (m)	Location (Division)
1	67.0	8.5	2.1	Kavalur, Tirupattur
2	83.0	10.0	2.5	"
3	73.0	10.0	3.0	"
4	81.0	7.5	1.8	"
5	81.0	11.0	3.5	"
6	100.0	9.0	1.8	"
7	75.0	12.0	1.3	"
8	88.0	10.0	3.0	"
9	79.0	9.5	1.8	"
10	85.0	10.0	2.5	"
11	63.5	11.5	4.0	Mumbarai, Chitteri, Harur
12	71.0	11.0	3.5	"
13	39.0	8.0	4.0	"
14	86.0	9.5	4.0	"
15	82.0	9.0	2.5	"
16	45.0	6.5	1.8	Mettupalayam, Coimbatore
17	40.0	8.0	2.0	"

Results and Discussion

Multiple shoot production

Cultured nodal segments differentiated into shoot buds (Table 2). Maximum shoot differentiation was obtained on MS medium supplemented with Kinetin (2.0 mg/L) and BAP (1.0 mg/L) with 80 per cent of the cultures showing shoot induction. The same treatment also proved superior in terms of average number of shoots per explant (2.8). Addition of Cytokinin enhanced shoot bud induction and further development from hypocotyl segments of sandal seedlings, confirming the results of Bapat and Rao (1989) who also reported that 90 per cent of the hypocotyl explants excised from the basal region showed potential for producing buds. Axillary shoot multiplication was achieved on MS+BAP (1.0 mg).

Bapat and Rao did not induce such response from the axillary buds with other Cytokinins such as Kn and Zn. But in the present study treatments with BAP and Kinetin, either singly or in combination, expressed axillary shoot multiplication from the nodal segments.

Table 2. Effect of different media on shoot induction of sandal

Sl. no.	Treatments (mg/L)	Cultures with shoot induction (%)	Mean shoot no.
1	MS + 2.0 BAP	40	0.7
2	Woody plant medium	40	1.1
3	MS + 2.0 Kin	70	2.3
4	MS + 2.0 BAP + 1.0 GA	10	0.7
5	MS + 1.0 BAP + 2.0 Kin	80	2.8**
6	MS + 2.00 BAP + 1.0 Kin	8	0.7
7	MS Basal	40	1.1
8	MS + 2.0 Kin + 1.0 GA	30	1.8
9	MS + 1.5 Kin	30	1.1
10	Half MS + 2.0 Kin	20	1.1
SED			0.44
CD			0.74

** Significant at p<0.01

Root induction

To develop complete plantlets, the regenerated shoot buds were excised and individually placed on a variety of root induction media (Table 3). They were also placed in combination with the host species *Casuarina equisetifolia, Acacia senegal* and *Eucalyptus tereticornis*.

Table 3. Effect of auxins on rooting of shoots

S.no.	Treatment	Morphogenic response
1	MS + 1.0 IAA	Formation of callus clump
2	MS + 2.0 IAA	Callus initiation
3	MS + 3.0 IAA	Callus plus shoot induction
4	MS + 1.0 IBA	Callus initiation
5	MS + 2.0 IBA	Callus initiation/root initiation
6	MS + 2.0 IBA	Callus initiation/root initiation
7	MS + 1.0 IAA + 1.0 IBA	Shoot elongation
8	MS + 1.0 IAA + 2.0 IBA	Shoot induction and elongation
9	MS + 3.0 IAA + 3.0 IBA	Shoot induction and elongation
10	MS + Seed extract (*Cajanus cajan*)	Callus initiation
11	MS + Root Extract (*Casuarina*)	Callus initiation

Almost all the treatments produced no result, except for a few cultures which after 15 weeks of inoculation developed single roots on MS medium supplemented with IBA at 2.0 mg/L. In other cultures with host species, *C. equisetifolia* and *E. tereticornis* did not show any positive result for sandal. However, when sandal shoots were associated with *Acacia senegal*, they had a healthy appearance with elongated shoots. Profuse rooting of *Acacia senegal* was observed on MS medium containing 2.0 mg/L IBA, but the sandal explant produced only a small callus at the base which just touched the roots of the host.

Such failure in rooting of isolated shoot buds was also reported earlier (Bapat and Rao 1989). This effect is still under investigation.

Indirect organogenesis

Callus induction was discernible in 11 treatments using inflorescence as explants. Among the various explants tried, immature inflorescence performed

Table 4. Effect of growth regulators on callus induction

S. no.	Treatment (mg/L)	Cultures with callus induction (%)	Callus rooting growth
1	MS	16.6	+
2	MS + 1.0 2,4-D	66.6	++
3	MS + 1.5 2,4-D	91.6	+++
4	MS + 2.0 2,4-D	66.6	+++
5	2.5 2,4-D	75.0	++
6	3.0 2,4-D	66.6	++
7	1.0 NAA	41.6	++
8	1.5 NAA	33.3	+
9	2.0 NAA	58.3	++
10	2.5 NAA	58.3	++
11	3.0 NAA	50.0	++

Note:
Callus growth is assessed by observation of size and colour.
+++ = Good
++ = Medium
+ = Poor

Table 5. Effect of cytokinins on multiple-shoot induction from callus cultures

S.no.	Treatment	Cultures with shoot induction (%)	Average no.of shoots/callus
1	MS	20	1.0
2	MS + 1.0 mg/L BAP	40	1.0
3	MS + 2.0 mg/L BAP	60	2.0
4	MS + 3.0 mg/L BAP	40	1.7
5	MS + 1.0 mg/L Kin	60	1.8
6	MS + 2.0 mg/L Kin	70	2.7
7	MS + 3.0 mg/L Kin	60	2.1
8	MS + 1.0 mg/L each of BAP & Kin	60	2.0
9.	MS + 2.0 mg/L each of BAP & Kin	60	2.5
10.	MS + 3.0 mg/L each of BAP & Kin	70	2.0

best in terms of percent cultures with callus induction and their organogenic potential. Among the responded treatments, MS medium containing 2,4-D (1.5 mg/L) and 2,4-D (2.0 mg/L) produced good callus (Table 4). The calli were both friable and compact and mostly yellow. With long incubation periods these calli turned brown and lost their organogenic potential.When transferred to the induction medium (MS plus Cytokinins), the calli produced shoot-buds 3–4 weeks after inoculation. Maximum shoot-bud differentiation of 70 per cent was observed in MS medium supplemented with 2.0 mg/L Kinetin and in MS medium supplemented with 3.0 mg/L each of BAP and Kinetin. However the highest average number of shoots per callus occurred on MS medium supplemented with 2.0 mg/L Kinetin (Table 5). Such an induction of callus and shoot-bud differentiation in somatic embryogenesis through callus-mediated cultures from mature explants was reported by Bapat and Rao (1992). Complete plantlet production through callus-mediated somatic embryogenesis was reported by Lakshmi Sita and Raghava Ram (1995).

Conclusions

In the present investigation shoot bud organogenesis was achieved in a wide range of treatments. But these callus cultures when transferred to root induction media failed to produce roots, other than a few which produced single roots.

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Effect of Composition of Media and Seed Density on Germination of Sandal (*Santalum album* L.)

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Abstract

Conventional sowing density is 500 g/m^2 of seed in a seedbed containing a mixture of sand and Red earth. However, this prescription is arbitrary and needs substantiation. Experiments conducted using a soil mixture of uniform grade and reduced seed density have shown a significant increase in both germination and the proportion of plantable seedlings.

SEEDS OF SANDAL (*Santalum album* L.) are viable for about 12 months. Seeds stored for four months give maximum germination of 60 per cent when sown at 500 g/m^2 in sand and Red earth. This equates to 15000 seedlings from a standard bed of 1 × 10 metres (Srinivasan et al. 1992). Each square metre contains about 3000 seeds which lie close to each other and compete for existence. The ungerminated seeds present in the bed may be a source of contamination and may increase mortality of the developing seedlings.

In order to get maximum germination with minimum loss of seed, it is necessary to:

- standardise the density of seed required for the bed, and
- optimise the soil mixture.

This study seeks to do that.

Materials and Methods

Two experiments have been undertaken: (1) using soil mixture of uniform size after sieving; and (2) using different seed density rates per bed. Having obtained favourable results in the first experiment, the second experiment was undertaken.

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Experiment 1

Soil mixtures used in this experiment contain sand and Red earth sieved to a uniform size (5 × 2.5 mm mesh). Seed beds prepared without sieving were used as control. Different treatments were:

- T_1—sand : Red earth, 2:1 (with sieving)
- T_2—sand : Red earth, 3:1 (with sieving)
- T_3—sand : Red earth, 2:1 (without sieving).

The one-square-metre raised beds were arranged in a randomised block design with five replications, and 500 g of seed were sown in each bed. Beds were sprayed with fungicide and nematicide before the start of the experiment.

Germination was recorded from the first day it started until the final count at 45 days (Table 1).

Table 1. Germination % in sieved and unsieved soil mixtures

Treatments			Sig.	C.D.
T_1	T_2	T_3		
72.4	70.4	60.4	***	4.29

Notes:
T_1—Sand : red earth 2 : 1 (with sieving)
T_2—Sand : red earth 3 : 1 (with sieving)
T_3—Sand : red earth 2 : 1 (without sieving)
***—significant at 1 % level
C.D.—critical difference

Table 2. Influence of seed density on seedling production

Characteristic	Mean values					Sig.	C.D.
	T_1	T_2	T_3	T_4	T_5		
Total germination (%)	68.87	61.93	69.46	71.17	72.63	NS	—
Plant %	60.62	56.2	68.8	68.3	70.38	***	6.96
Root length (cm)	7.1	7.0	8.6	7.6	7.8	NS	—
Shoot length (cm)	13.0	12.2	12.9	12.7	12.3	NS	—

Notes
- T_1 = 600 g; T_2 = 500 g; T_3 = 400 g; T_4 = 300 g; T_5 = 200 g.
- NS = not significant
- *** = significant at 1% level
- C.D. = critical difference

Experiment 2

After demonstrating the benefit of the sieved uniform soil mixture in Experiment 1, a further experiment was laid with varying seed densities in each bed with sieved soil mixture. Five beds were sown with 600 g (T_1), 500 g (T_2), 400 g (T_3), 300 g (T_4), and 200 g (T_5) of seeds. The beds were arranged in randomised block design with three replications. Germination count and seedling survival were assessed after 45 and 60 days, respectively (Table 2).

Results and Discussion

There were significant differences between treatments. Of the three treatments in Experiment 1, T_1 (sieved soil mixture of uniform size, sand : Red earth = 2:1) gave the highest percentage of germination (Table 1). This may be because of the presence of uniform moisture, and aeration in the soil mixture used.

In Experiment 2, germination percentage decreased as the density was increased.

Seedling survival was lower in beds sown with 500 and 600 g of seed. This may be because of competition for survival in the denser seed zone. By contrast, the seed-beds sown with 200–400 g showed significant production of plantable seedlings (Table 2).

Conclusion

In the bed stage, uniform sieved soil mixture is better for germination. The optimum seed rate is 400 g/m^2 which produces 16500 plantable seedlings per standard 1 × 10 m bed. In this way, one kilogram of seed per bed can be saved.

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Chemistry and Utilisation

Extraction of Oil from *Santalum spicatum* by Supercritical Fluid Extraction

P. Moretta, E.L. Ghisalberti, M.J. Piggott and R.D. Trengove*

Abstract

Santalum spicatum is native to Western Australia. The essential oil from the heartwood is used for perfumes and many cultural preparations. Unprocessed wood exports are valued at $10 million pa. The quality of the oil is determined by its santalol content. East Indian sandalwood oil contains over 90% santalol, and has therefore been the focus of much research. *S. spicatum* contains only 15% santalol, and the major component is *trans, trans*-farnesol. Extraction of essential oils from natural products has typically been performed using traditional techniques such as steam distillation and solvent extraction. These techniques are time-consuming, and the elevated temperatures involved with steam distillation leads to the degradation of some components of the essential oil. Comparative studies using supercritical fluid extraction (SFE) have shown similar yields, cleaner extracts and less thermal degradation of volatile components. A supercritical fluid is defined as a substance that is above both its critical temperature and pressure. Supercritical fluids possess gas-like diffusivity and viscosity with liquid-like densities. These properties allow fast penetration and high mass transfer from the wood matrix, resulting in shorter extraction times. Carbon dioxide is the most popular solvent used for SFE, having mild critical parameters of 31.1°C and 72.8 atm. The use of a low-extraction temperature has enabled the efficient collection of volatile components, and the effect of thermal degradation is minimised. It has been reported that the aroma of extracts using supercritical carbon dioxide better resemble the aroma of the natural plant than do steam-distilled extracts; this is a consequence of less thermal degradation.

Key words: *Santalum spicatum*, supercritical fluid extraction, steam distillation

THE UNPROCESSED WOOD of *Santalum spicatum*, a small tree native to Western Australia, produces annual exports of around $10 million (Loneragan 1990).

The essential oil from the heartwood is used for perfumes and many cultural preparations. The quality of the oil is determined by its santalol content. The oil from East Indian sandal (*S. album*) contains over 90 per cent santalol, and therefore has been the focus of much research (Demole et al. 1976). By contrast, the common varieties of *S. spicatum* contain only 15 per cent santalol with the major component being *trans,trans*-farnesol (35%) (Penfold 1928), although there are indications of varieties much richer in santalol (Penfold 1932).

Extraction of essential oils from natural products have typically been performed using techniques such as steam distillation and solvent extraction (Shankaranarayana and Parthasarathi 1987; Brophy et al. 1991). These techniques are time-consuming, and the elevated temperatures involved with steam distillation can lead to the degradation of some components of the essential oil. Comparative studies using supercritical fluid extraction (SFE) have shown similar yields, cleaner extracts and less thermal degradation of volatile components (Pickett et al. 1975; Reverchon and Senatore 1992).

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A supercritical fluid is defined as a substance that is above both its critical temperature and pressure. Supercritical fluids possess gas-like diffusivity and viscosity with liquid-like densities. These properties allow fast penetration and high mass transfer from the wood matrix, resulting in shorter extraction times. Carbon dioxide is the most popular solvent used for SFE, having mild critical parameters of 31.1°C and 72.8 atm. The use of a low-extraction temperature has enabled the efficient collection of volatile components, and the effect of thermal degradation is minimised. It has been reported that the aroma of extracts using supercritical carbon dioxide better resemble the aroma of the natural plant than do steam-distilled extracts; this is a consequence of less thermal degradation (Moyler 1993).

In SFE extraction, the solvating power of the supercritical fluid increases with density; therefore the composition of a supercritical carbon dioxide extract depends on the density of the SFE solvent used (Moyler 1993). The aim of this investigation was to examine the effect of different carbon dioxide densities and extraction times on the yield of oil obtained by SFE.

Experimental

Sample preparation

All sandalwood samples were initially ground up using a wood-carving tool fitted to an angle-grinder, and then blended in a *Warning* commercial blender in the presence of solid carbon dioxide.

Supercritical fluid extraction

Supercritical fluid extractions were performed using a *Hewlett-Packard 7680T* SFE module. *Air Liquide* carbon dioxide N45 grade and food grade were used for the extraction fluid and cryogenic cooling respectively. Ground samples of wood (c. 0.5 g) were packed between filter flocs (Machery-Nagel MN2101) in 7mL extraction thimbles.

Extraction time dependence tests

Successive extractions of ten minutes were performed to a total of 100 minutes. All extractions were performed under the following conditions:

- fluid—CO_2;
- density—0.90 g/mL;
- pressure—357 bar;
- chamber temperature—40°C;
- flow-rate—1.0 mL/min.

The trapping conditions were:

- nozzle temperature—45°C;
- trap temperature—0°C;
- trap packing—octadecylsilane (*Hypersil* 30 mm).

The solid-bed trap rinsing conditions were:

- volume—1.5 mL;
- rinse rate—1.0 mL/minute;
- nozzle temperature—45°C;
- trap temperature—40°C;
- 1st and 2nd rinse—light petrol;
- 3rd rinse—ethanol.

Extraction density dependence tests

All conditions, apart from the extraction density and time, were the same as for those for the extraction time dependence tests, listed above. The extraction time was 100 minutes, and extraction fluid densities of 0.5, 0.6, 0.7, 0.8 and 0.9 g/mL were examined.

Analysis

Analysis was performed using a *Hewlett-Packard 5890 Series II* gas chromatograph with a Flame Ionisation Detector (FID) equipped with an *HP-Innowax* column (30m × 0.25 mm i.d., 0.25 µm film thickness) using ultra-high purity hydrogen (BOC) as the carrier gas (79 k Pa constant flow). The injector temperature was 200°C, and detector temperatures 250°C. The temperature program was:

- 60°C, held for two minutes, then
- heating at 4°C per minute to a temperature of 140°C held for 60 minutes, then
- ramped at 4°C per minute to a final temperature of 240°C held for 10 minutes.

Results and Discussion

The yield of oil extracted from 10–60 minutes at a carbon dioxide density of 0.9 g/mL is shown in Figure 1. The percentage yield of total extract was calculated by the weight of extract per weight of wood extracted. The percentage yield of volatiles refers to the proportion of the total extract that was amenable to GC. This was calculated by recording the ratio of masses of neat extract to octanol, which was added to each extract as an internal standard. By comparing this value to the ratio of the areas detected by the FID, it was possible to estimate the proportion of the extract amenable to the GC.

The results show that, after the first 10 minutes, 86 per cent of the total volatiles has been extracted while only 72 per cent of the total extract has been

recovered. After 20 minutes, this increases to 96 per cent and 90 per cent respectively. This illustrates that the volatile components are extracted more efficiently than the involatile components of the oil. After 20 minutes, only the involatile compounds contribute to the increase in total extract.

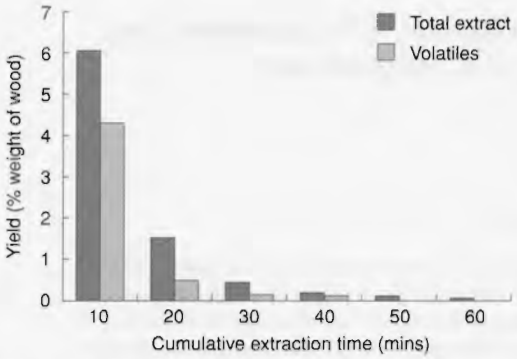

Figure 1. Yield of total extract and volatiles vs cumulative extraction time

It has been well documented that the solvating power of supercritical carbon dioxide increases as the density is increased (Moyler 1993). Figure 2 shows the effect of the extraction density on the yield of total extract and volatiles. The total yield of extract increases from 2.4 to 8.4% almost linearly as the extraction fluid density is increased from 0.5 to 0.9 g/mL. The yield of volatiles initially increases, then remains relatively constant after 0.7 g/mL. The increase in overall yield above 0.7 g/mL was therefore due to the extraction of involatile material as the density increases.

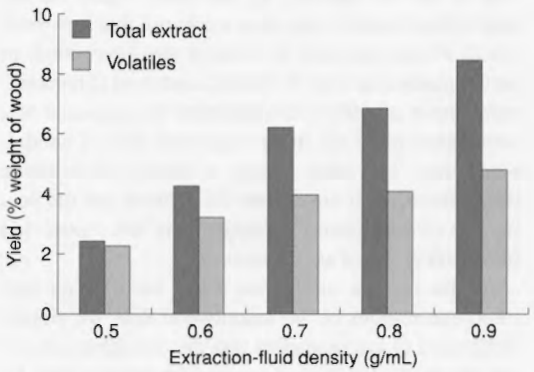

Figure 2. Yield of total extract and volatiles vs extraction-fluid density

Conclusion

At an extraction-fluid density of 0.9 g/mL, much of the involatile material was removed from the sandalwood. An extraction time of 20 minutes was required at this density to extract most of the oil. Using lower densities (0.5–0.6 g/mL) of the extraction fluid, most of the fraction recovered was volatile compounds.

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Content and Composition of Oil from the Central and Transition Zones of the Sandalwood Disc

K.H. Shankaranarayana, G. Ravikumar, A.N. Rajeevalochan, K.S. Theagarajan and C.R. Rangaswamy*

Abstract

In sandalwood disc (*Santalum album* L.) oil content decreases across the diameter from central heartwood to 'transition zone' (i.e. heartwood-sapwood boundary) by about 70 per cent. The chemical composition of oil obtained from central and transition zones of sandalwood disc differed significantly. While the level of oxygenated constituents (santalols and santalyl acetate) was about three per cent higher in the transition zone, the level of hydrocarbons (santalenes) was 50 per cent lower.

Abbreviations:
GLC = gas liquid chromatography
2m ss = 2 metre stainless steel
DEGS = diethylglycolsuccinate
FID = flame ionisation detector

THE SCENTED HEARTWOOD of sandal, for which the tree is mainly exploited, yields fragrant sandalwood oil by steam distillation. Yield is 2.5–6.2 per cent depending on age of the tree, and soil, climatic and genetic factors. The fixative properties and tenacious aroma of the oil are due to its major odoriferous sesquiterpenic constituents, α and β santalols, which constitute about 90 per cent of the oil. However, α and β santalenes and santalyl acetate, which form about six per cent of the oil, also contribute to its overall characteristic odour.

Earlier, we have reported on the:

- compositional differences in oils obtained from young and mature sandal trees (Shankaranarayana and Parthasarathi 1984);
- oil content variation in different coloured heartwood of sandal (Shankaranarayana et al. 1985); and
- content and composition of oil from heartwood at different levels in sandal. (Shankaranarayana and Parthasarathi 1987).

In the latter, studies on the oil content and composition of heartwood samples separately taken at the core (central region of 2 cm diameter) and periphery (the region up to 1 cm width near the perimeter) of the disc were also made. It was found that from core to periphery of only heartwood where no sapwood was included, there was an average decrease of 20 per cent in the oil content. In our latest study on the depot-based sandal sapwood, we found that sapwood, which is not supposed to contain any heartwood, in fact contained around 0.15% of sandal oil (Shankaranarayana et al. 1997); this indicates the presence of a small amount of oil in the sapwood part of sandalwood disc. Therefore, it was of interest to examine the differences, if any, in the oil content and the proportion of odoriferous constituents in the central and transition zones of sandal discs.

In the present study, we have focused on two important regions of the sandalwood disc: the central heartwood (3 cm diameter) and the transition zone (1 cm around the heartwood-sapwood boundary (Fig. 1).

From ten different undressed sandal logs available in sandal depots in Karnataka and Tamil Nadu, discs

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10 cm long and 15–20 cm diameter were prepared. Heartwood samples were taken from the central heartwood and the transition zone which also contained a portion of sapwood. Samples were powdered and steam-distilled to estimate the oil content. Composition of oil was studied by GLC using an *AIMIL-NUCON Model 5580* instrument with a packed 2m ss column of DEGS, at 165°C using FID. Nitrogen was the carrier gas with a flow rate of 40 mL per minute. Major peaks were identified by analysing authentic sandalwood oil under identical experimental conditions, using a chromatographic data station attached to the instrument. The results are shown in Table 1.

From the central to transition zone there was an average decrease of 70 per cent in the content of oil and about three per cent increase in the oxygenated constituents (santalols and santalyl acetate), while the content of hydrocarbons (santalenes) decreased by about half. These studies show variation in the composition of oil, particularly the slight increase in the level of oxygenated constituents at the transition zone with a considerable fall of hydrocarbon level, and the amount of oil in the sandalwood disc from the central portion of heartwood to transition zone.

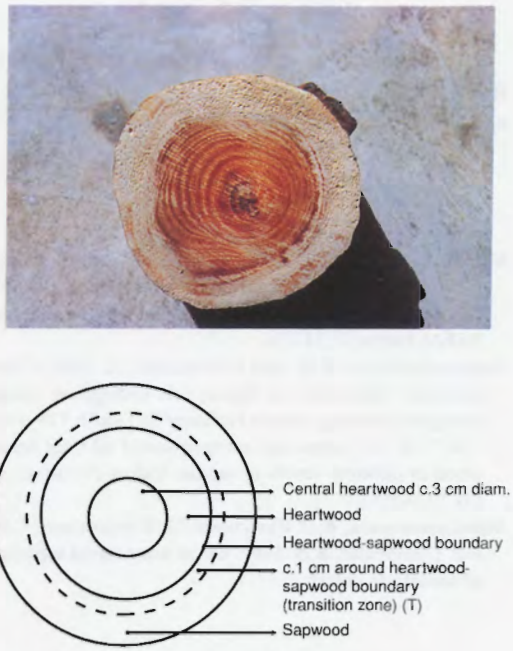

Figure 1. A typical sandalwood disc

Table 1. Content and composition of oil from central heartwood (C) and transition zone (T) of sandalwood disc

Sample #	Oil content (%)	Oxygenated constituents			Hydrocarbons (Santalenes) (%)
		Santalol (%)	Santalyl acetate (%)	Total (%)	
1C	2.0	86.5	8.5	95.0	2.8
1T	0.6	89.7	8.9	98.6	1.4
2C	3.0	84.5	5.9	90.4	5.9
2T	0.9	89.8	6.4	96.2	2.8
3C	3.6	86.4	7.2	93.6	3.2
3T	1.4	92.4	4.5	96.9	1.1
4C	2.4	79.7	7.4	87.0	1.5
4T	0.3	86.4	8.0	94.4	0.1
5C	4.2	90.8	5.1	95.9	3.2
5T	0.4	90.1	7.1	97.2	1.0
6C	3.8	92.3	4.8	97.1	1.7
6T	1.6	91.8	6.7	98.5	1.5
7C	4.1	90.2	5.5	95.7	2.9
7T	1.2	88.5	5.8	94.3	1.3
8C	3.6	89.0	6.8	95.8	2.7
8T	0.9	89.0	7.5	96.5	1.2
9C	5.5	87.0	4.9	91.9	1.5
9T	1.5	90.0	5.7	95.7	0.9
10C	5.2	85.0	3.1	88.1	4.2
10T	1.7	87.0	5.4	92.4	2.9

Acknowledgment

The authors wish to thank Dr K. Satyanarayana Rao, Director of the Institute, for his help and encouragement.

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Sandalwood, HESP and ESPO Oils from the Heartwood of *Santalum album* L.

K.H. Shankaranarayana, G. Ravikumar, R. Rangaswamy and K.S. Theagarajan*

Abstract

Heartwood of sandal (*Santalum album* L.) on steam distillation yields 2.5–6.2 per cent fragrant sandalwood oil of high perfumery value. Devoid of sandal oil, the spent powder of sandalwood does not have any commercial value, but it may be processed by using MEOH-HCl followed by steam distillation to get a new essential oil, HESP (1.2% yield), which has pharmacological properties. Spent sandalwood powder can also be processed by dichromate oxidation followed by steam distillation to get ESPO oil (1.2% yield) which is quite different from sandalwood and HESP oils. Infrared spectral and gas chromatographic profiles of sandalwood, HESP and ESPO oils are presented here.

SANDAL IS MAINLY exploited for its scented heartwood which yields fragrant sandalwood oil with steam distillation. The yield of oil is 2.5–6.2%, depending on the age of the tree, and soil, climate and genetic factors. The fixative properties and tenacious aroma of the oil are due to its major odoriferous sesquiterpenic constituents—alpha and beta santalols, which make up about 90 per cent of the oil. Alpha and beta santalenes and santalyl acetate, which form almost all the rest of the oil, also contribute to the overall characteristic odour of the oil.

Sandal heartwood powder becomes unscented after thorough steam distillation of the fragrant sandalwood oil. It is sold cheaply as spent sandalwood powder for use as a binder in the manufacture of agarbathis (incense or joss sticks). Earlier we reported that the hydrolysis of the non-steam volatile matter of the spent powder with methanol and hydrochloric acid provides a new essential oil, 'hydrolysed exhausted sandalwood powder' (HESP), the yield of which is 1.2% (Shankaranarayana and Parthasarathi 1986). On pharmacological screening HESP demonstrated antit-remorogenic, anti-inflammatory, anti-mitotic, anti-viral, anti-cancerous, hypertensive, anti-pyretic, and sedative properties (Desai et al. 1991). It is also a chemo-sterilant against female moths of *Atteva fabriciella* (Sivaramakrishnan and Shankaranarayana 1990).

In the current study, further utilisation of the spent sandalwood powder as a source of a dichromate-oxidised oil is examined. Oxidation of spent sandalwood powder with potassium dichromate with sulphuric acid, followed by steam distillation, gave rise to another essential oil, 'exhausted sandalwood powder oxidised' (ESPO) (1.2% yield).

To 200g of spent (exhausted) sandalwood powder, 100 mL of 5% potassium dichromate solution was added, followed by 10 mL of sulfuric acid in 50 mL of water. Sufficient water was further added and steam distilled for 18 hours to get 1.2% yield of brownish-yellow ESPO oil with a pleasant, strong, heavy, fragrant odour.

Infrared spectral and gas chromatographic details of ESPO oil compared with the oils of sandalwood and HESP are shown in Figures 1 and 2. The marked differences observed suggest that ESPO oil presently obtained from spent sandalwood powder is quite different from sandalwood and HESP oils. Further studies on ESPO oil, particularly its perfumery and pharmacological evaluation, are currently under way.

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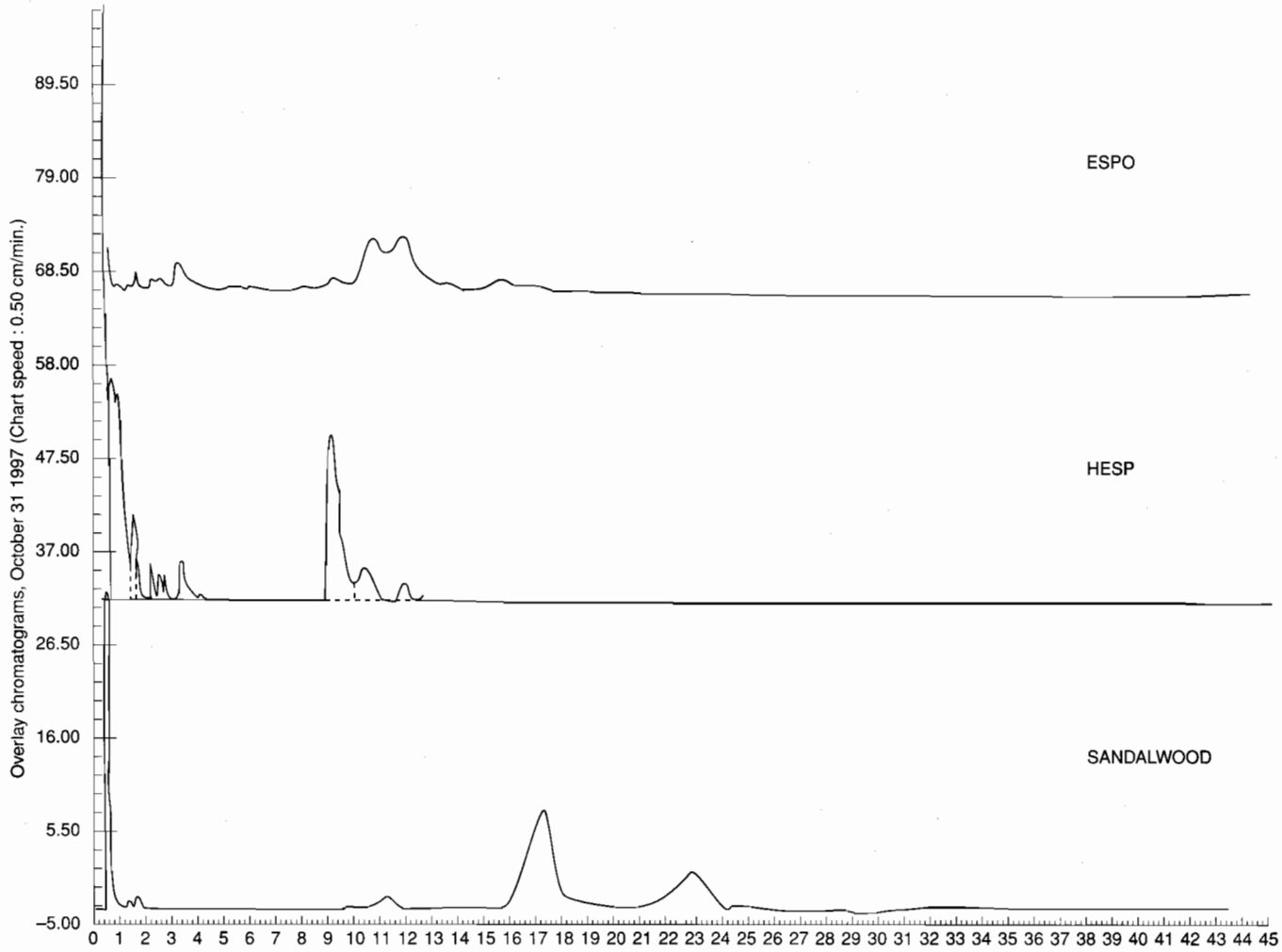

Figure 1. Chromatographic comparison of ESPO, HESP and sandalwood oils.

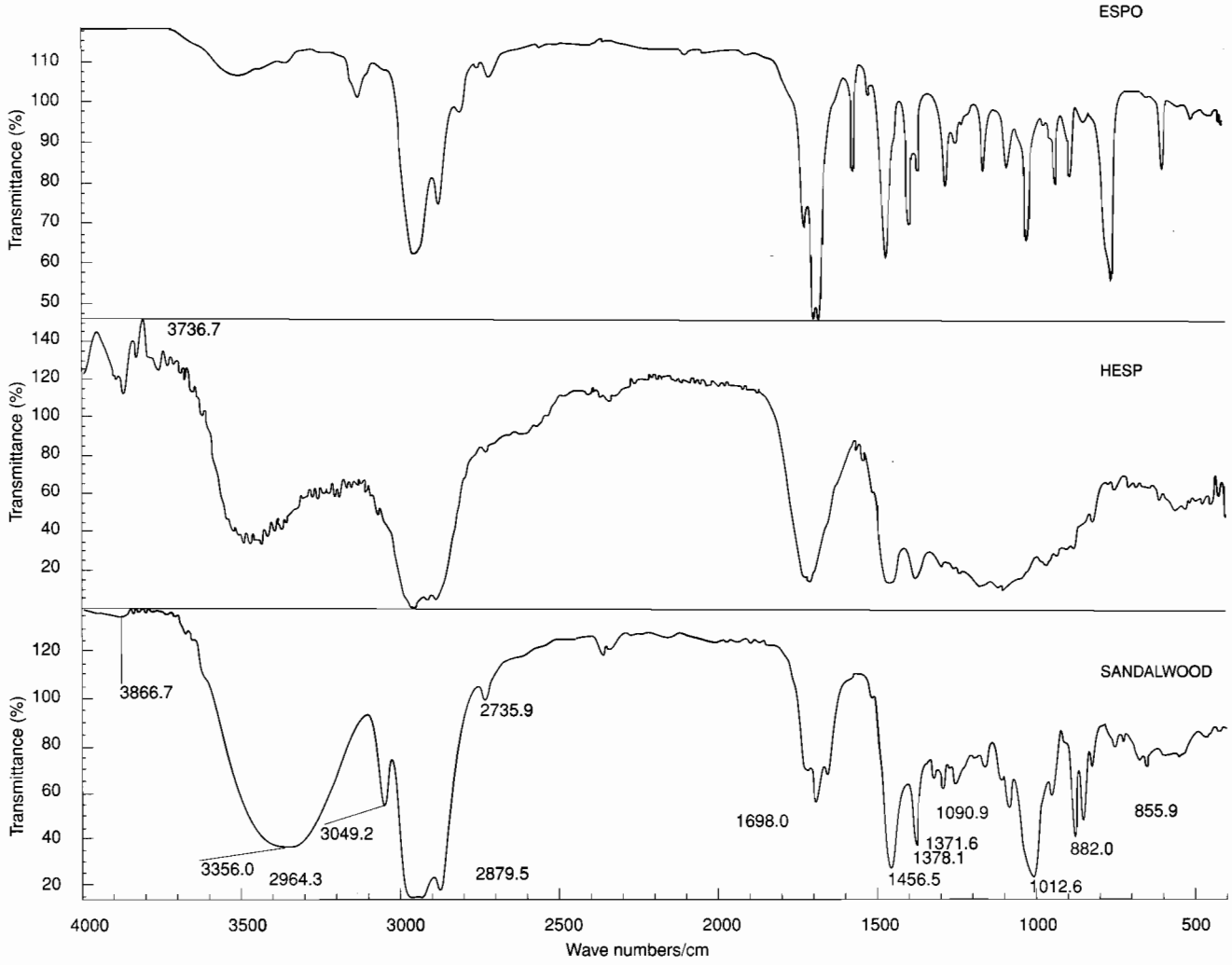

Figure 2. Infrared comparison of ESPO, HESP and sandalwood oils. (Note different vertical scales.)

The work brings out again the value of spent sandalwood powder as a source of aroma oil of different types by chemical modification.

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Stemwood and Rootwood Anatomy of *Santalum album* L. and the Problem of Wood Adulteration

R.V. Rao*, T.R. Hemavathi*, M. Sujatha*, Luxmi Chauhan† and R. Raturi†

Abstract

Identification of timber is an essential component of timber utilisation. This gains extra importance when timbers having high commercial value are either felled illicitly or adulterated with less important ones. *Santalum album* L. is one such important tree. The economic product of the plant is its coloured heartwood yielding essential oil and the wood for carving. Both rootwood and stemwood yield essential oil. Because of its value in trade, often the trees are cut illegally, converted into various forms and smuggled out. Since this plant is State property, the Forest Departments seize it and refer it for authentification. Also, certain timbers resembling sandal are adulterated with it and sold as sandal. In the present paper, the details of rootwood and stemwood anatomy are described for identification along with woods of *Adina cordifolia* (Roxb.) Hook.F. ex. Brand., *Buxus wallichiana* Baill., *Mitragyna parvifolia* (Roxb.) Korth., *Gardenia latifolia* Ait., *Drypetes porteri* (Gamble) Pax and Hoffm., and *Mansonia gagei* Drumm. which are used as adulterants. Based on anatomical differences, sandalwood can be distinguished from these species.

Key words: *Santalum album*, stemwood, rootwood, adulteration, identification

IN A BIG country like India, with very diverse timber resources, the problem of identification of timber has great significance. Besides its use in construction, furniture and other structural and non-structural purposes, certain timbers are also sources of by-products having significant commercial and economic implications. The heartwood (both stemwood and rootwood) of *Santalum album* is prized in the market for its oil and the wood is also used for carvings, trinket boxes and other utility items. Because of its economic value, the trees are often felled illegally, converted into various forms and smuggled out. Many timbers externally similar to sandalwood, are passed off by the temporary application of sandal oil. The consumer cannot know, so often gets cheated. Because of these problems, the Wood Anatomy Branch is regularly asked to determine the identity of timber in order to settle legal disputes.

Some of the timbers which are adulterated with sandalwood because of their fine texture are: *Adina cordifolia*, *Buxus sempervirens*, *Drypetes porteri*, *Gardenia latifolia*, *Mansonia gagei*, and *Mitragyna parvifolia* (Ghosh 1958; Ghosh and Shahi 1961; Purkayastha 1985).

The basis of timber identification is its structure as seen at macroscopic and microscopic level along with their general features. While some timbers can be identified at macro-level, most timbers can be identified using a microscope where detailed anatomical structure can be seen. Although stemwood anatomy of *Santalum album* has been studied (Pearson and Brown 1932; Purkayastha 1985; Nair 1987; Agarwal and Pande 1992), no detailed information is available on its rootwood. In the present paper, the detail of sandal stemwood and rootwood anatomy is described, along with that of other timber species

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which are adulterated. A dichotomous key to identification based on anatomical features is presented.

Materials and Methods

General properties and gross structure of the wood were described based on samples available at FRI, Dehra Dun, and IWST, Bangalore, xylariums. Microscopic descriptions were based on the permanent slides already available in the collections.

Results and Discussion

Comparative anatomical characters along with their general properties are given in Table 1. Structural details are shown in Figures 1–16. Species details follow.

Key for separation of sandalwood from other timbers

1	Wood scented	2
1	Wood not scented	3
2	Rays, vessels, parenchyma and fibres storied	*Mansonia gagei*
2	Rays, vessels, parenchyma and fibres not storied	*Santalum album*
3	Vessel perforation scalariform and simple; intervascular pits non-vestured	4
3	Vessel perforation exclusively simple; intervascular pits vestured	5
4	Vessels very small, very numerous; crystals in parenchyma; rays absent	*Buxus sempervirens*
4	Vessels small, very numerous; crystals in parenchyma; rays present	*Drypetes porteri*
5	Silica in rays frequent	*Mitragyna parvifolia*
5	Silica in rays absent	6
6	Vessel frequency less than 40 per mm^2; perforated ray cells common	*Adina cordifolia*
6	Vessel frequency more than 60 per mm^2; perforated ray cells absent	*Gardenia latifolia*

***Santalum album* L. (Santalaceae)**

Trade name: Sandalwood. Common names: Chandana, Safed–chandan (Hindi), gandha, sandhanam, srigandam (Kannada), Chandana (Sanskrit).

Sapwood is white to pale yellow, sharply demarcated from the yellowish-brown to dark brown heartwood, hard, heavy (SG=0.87–0.91 air-dry), lustrous, straight-grained to slightly wavy, even and very fine-textured; heartwood with very pleasant characteristic scent.

Stemwood generally is diffuse-porous. Growth-rings are inconspicuous, delimited by thick–walled latewood fibres. Vessels are very small to extremely small, 50–80 mm in diameter (mean = 65 mm), 40–50/ mm^2, mostly solitary, perforation simple, intervascular pitting small 4–5 mm, pits to ray cells similar in size and shape, vessel members 100–500 mm in length. Parenchyma diffuse and also diffuse-in-aggregates, strands of 2–4 cells, crystals occasional solitary or in 4–6 locules, infrequent. Fibres libriform, non-septate, 500–1500 mm in length, 15–20 mm in diameter, interfibre pits numerous, bordered. Rays fine to very fine, 7–10 per mm, heterogeneous type II and 1–3 seriate, mostly 1–2, composed of procumbent cells, upright and square cells, 20–30 mm in width and up to 350 mm in height, crystals absent.The rootwood is diffuse-porous. Growth rings are inconspicuous, delimited by thick walled latewood fibres. Vessels very small to extremely small, 36–72 mm in diameter (mean 28 mm), 66–71/ mm^2, mostly solitary, round to oval, tyloses absent, no deposits, perforation simple, intervascular pitting small 4 mm, pits to ray cells 5 mm, vessel members 180–450 mm in length. Parenchyma diffuse and also diffuse-in-aggregates, strands of 2–4 cells, crystals present. Fibres thin to thick walled, non-septate, 990–1512 mm in length and 13–23 mm in diameter, interfibre pits numerous, bordered. Rays 9–11 per mm, heterogeneous type II, 1–3 seriate mostly 1–2 seriate, composed of upright and procumbent cells, 36 mm in width and up to 21 cells or 324 mm in height, crystals present.

***Adina cordifolia* (Roxb.) Hook.F. ex.Brand. (Rubiaceae)**

Trade name: Haldu. Common names: Lampatia tarak chapa (Assamese), Keli-kadam (Bengali), Sandang (Garo), haladwan, holdarvo (Gujarati), hardu, haldu, karam (Hindi), Bagting-phang (Kachari), avanu, kadambe (Kannada), manjakadamba (Tamil), bandaru, dadduga (Telugu), manjakadamba (Malayalam).

Sapwood yellowish-white; heartwood yellow ageing to yellowish-brown or reddish-brown, moderately hard, moderately heavy (SG = 0.58–0.73 air-dry), straight-grained to sometimes broadly interlocked, fine-textured, odourless.

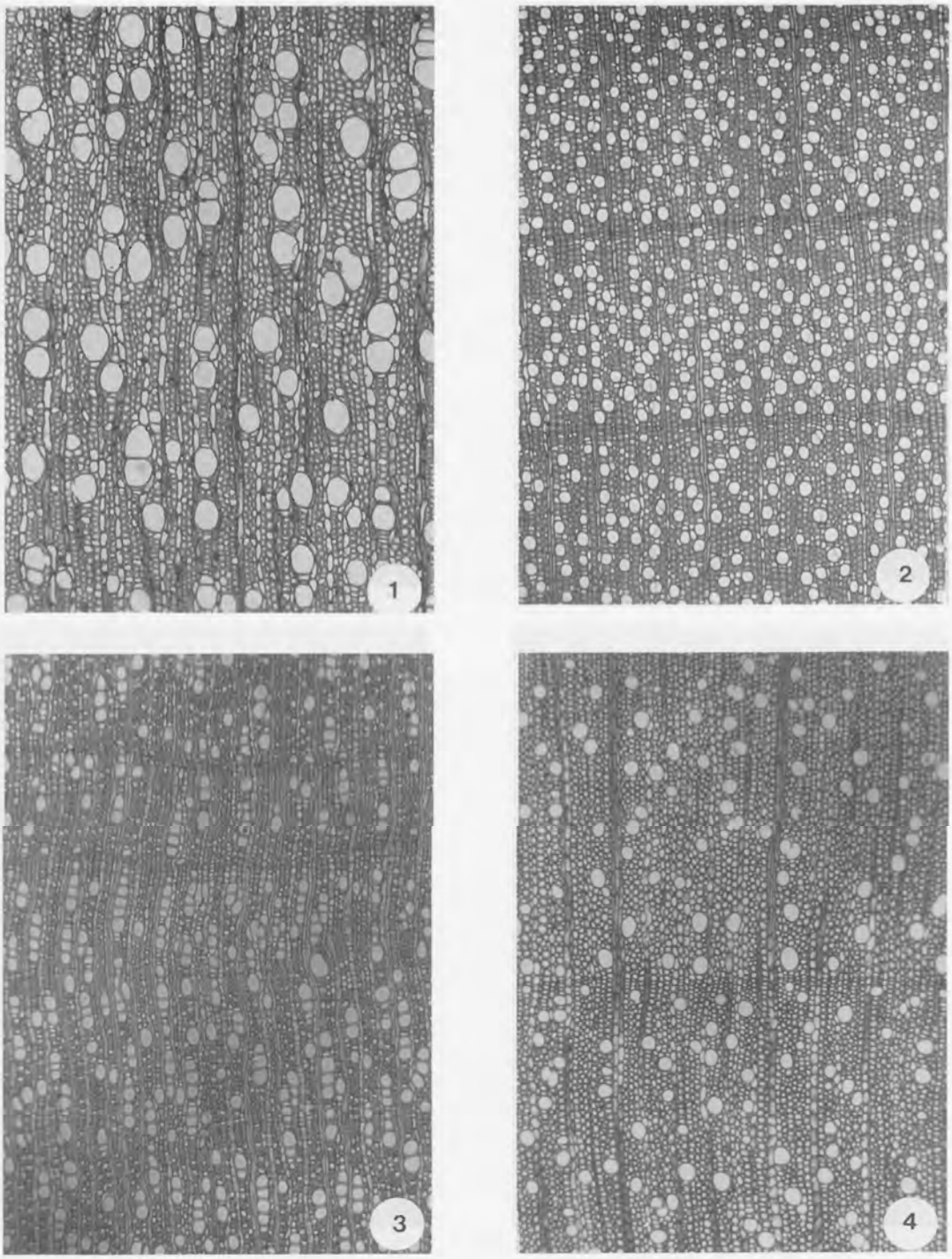
Table 1. Comparative general characteristics and anatomical features of sandalwood and timbers used as adulterants

Characteristics or features	*Santalum album* (Sandal) stemwood	*Santalum album* (Sandal) rootwood	*Adina cordifolia* (Haldu)	*Buxus wallichiana* (Boxwood)	*Drypetes porteri* (Cegilwood)	*Gardenia latifolia* (Gardenia)	*Mitragyna parvifolia* (Kaim)	*Mansonia gagei* (Kalamet)
General								
1. Colour of heartwood	Yellowish-brown turning dark brown or dark reddish-brown	Yellowish-brown turning dark brown or dark reddish-brown	Yellowish to brownish, ageing to reddish-brown	Yellowish-white or yellowish-brown	Pale yellowish-white to yellowish-brown	Creamy white to yellowish-brown	Pale yellowish-brown to light brown	Deep reddish- or olive-brown with dark streaks
2. Hardness	Hard	Hard	Moderately hard	Hard	Hard to very hard	Moderately hard to hard	Moderately hard	Very hard
3. Weight (SG air-dry value)	0.87–0.91	–	0.58–0.73	0. 82	0.83–0.89	0.73–0.83	0.56–0.73	0.82–0.97
4. Odour	Scented	Scented	Odourless	Odourless	Odourless	Odourless	Odourless	Sweet scented
Vessels								
1.Tangential diameter (μm)	50–80 (65)	36–72 (28)	60–110 (80)	45–50 (35)	30–55 (40)	30–60 (50)	60–120 (100)	35–65 (45)
2. Frequency (per mm^2)	40–50	66–71	25– 40	130– 180	100–180	60–90	20–25	85–96
3. IVP vestured	–	–	+	–	–	+	+	–
4. IVP size (μm)	4–5	4	4–6	3	3–4	3–4	4–6	3–4
5. Scalariform perforation	–	–	–	+	+	–	–	–
6. Simple perforation	+	+	+	_	+	+	+	+
7. Storied	–	–	–	–	–	–	–	+
Parenchyma								
1. Type	Diffuse & diffuse-in-aggregates	Diffuse & diffuse-in-aggregates	Diffuse & diffuse-in-aggregates	Diffuse to rarely diffuse-in-aggregates	Diffuse to rarely diffuse-in-aggregates	Diffuse-in-aggregates	Diffuse to diffuse-in-aggregates	Diffuse to diffuse-in-aggregates terminal
2. Crystals	+	+	–	–	rare	–	–	–
3. Silica	–	–	–	–.	–	–	+	–
4. Storied	–	–	–	–	–	–	–	+

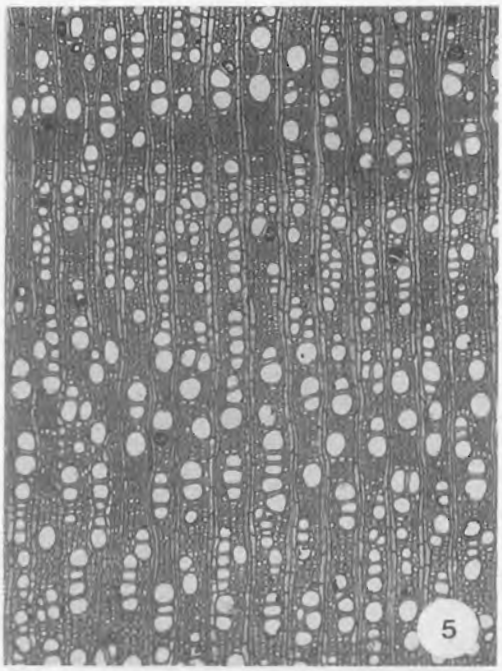
Table 1. (Continued) Comparative general characteristics and anatomical features of sandalwood and timbers used as adulterants

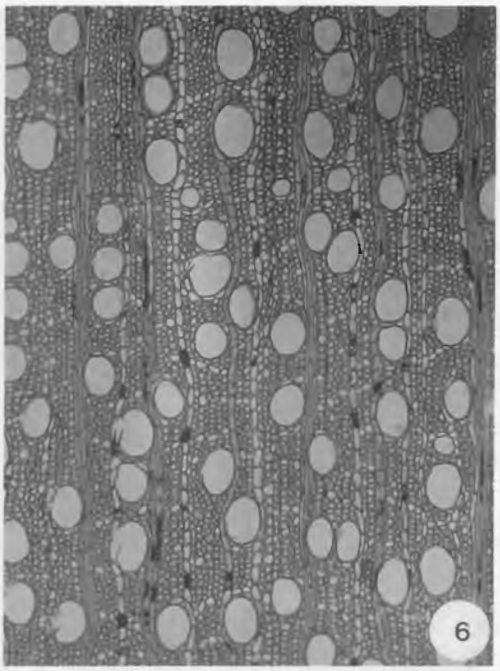
Characteristics or features	*Santalum album* (Sandal) stemwood	*Santalum album* (Sandal) rootwood	*Adina cordifolia* (Haldu)	*Buxus wallichiana* (Boxwood)	*Drypetes porteri* (Cegilwood)	*Gardenia latifolia* (Gardenia)	*Mitragyna parvifolia* (Kaim)	*Mansonia gagei* (Kalamet)
Rays								
1. Frequency (per mm)	7–10	9–11	13–16	9–14	16–18	12–16	12–17	9–13
2. Type	Heterogenous	Heterogenous	Heterogenous	Heterogenous	Heterogenous	Heterogenous	Heterogenous	Heterogenous
3. Width (μm/cells)	20–30 1–3 seriate	36 1–2 seriate	30–40 1–3 seriate	27–30 1–3 seriate	33 1–3 seriate	30–40 uniseriate	20–50 1–4 seriate	30 biseriate
4. Height (μm)	350	324	300–670	450	na	400	450– 650	210
5. Crystals	–	+	–	–	+	–	–	+
6. Silica	–	–	–	–	–	–	+	–
7. Storied	–	–	–	–	–	–	–	+
8. Perforated ray cells	–	–	+	–	–	–	+	–
Fibres								
1. Libriform	+	+	–	+	+	+	+	+
2. Storied	–	–	–	–	–	–	–	+
Growth rings	Inconspicuous, delimited by thick-walled latewood fibres	Inconspicuous, delimited by thick-walled latewood fibres	Indistinct	Distinct, delimited by narrow darker line of denser fibres	Conspicuous, delimited by latewood thick-walled fibres	Inconspicuous, when conspicous demarcated by thick-walled latewood fibres	Distinct to indistinct, demarcated by thick-walled latewood fibres	Indistinct, delimited by thick-walled latewood fibres sometimes by marginal parenchyma

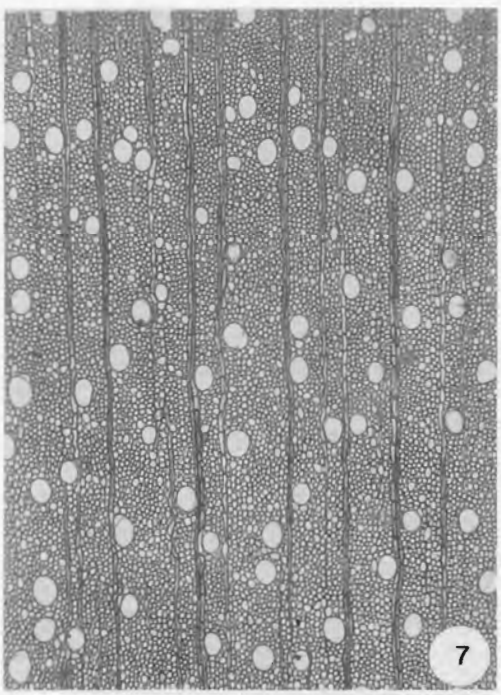
Legend
IVP = Intervascular Pitting present (+) or absent (–).
na = not available.
SG = specific gravity.

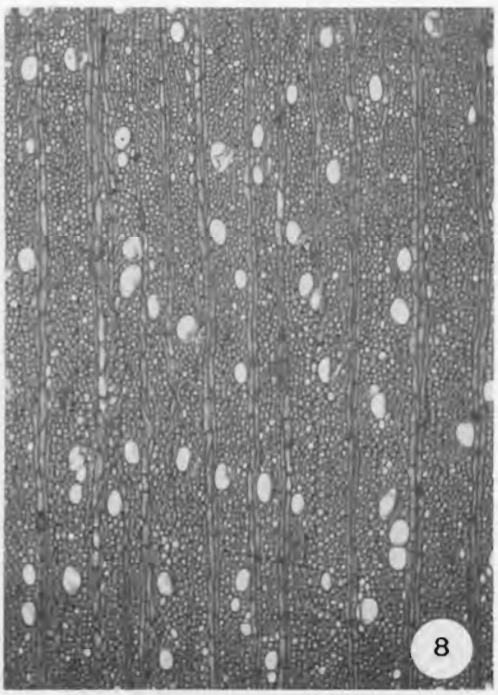


Figures 1–4: Cross-sections of *Adina cordifolia, Buxus wallichiana, Drypetes porteri,* and *Gardenia latifolia* (all at 40× magnification)

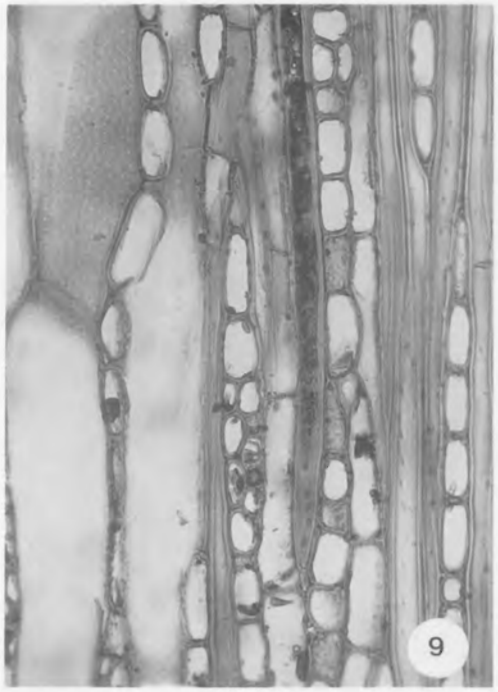


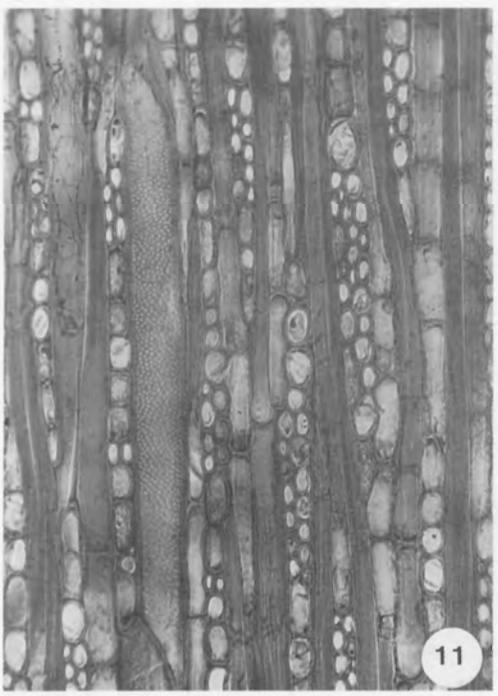


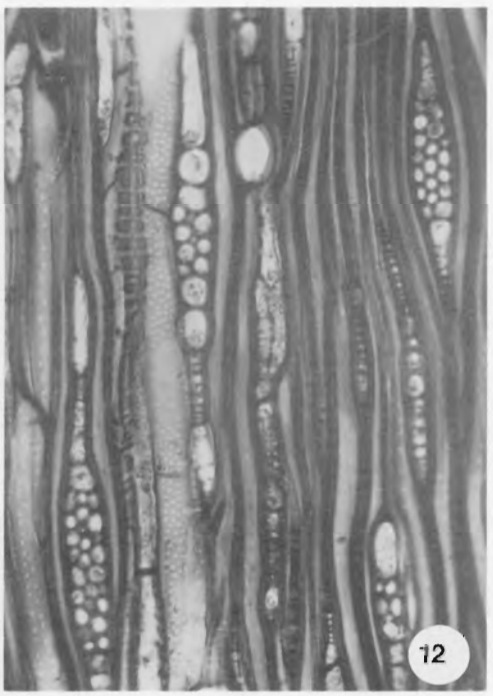




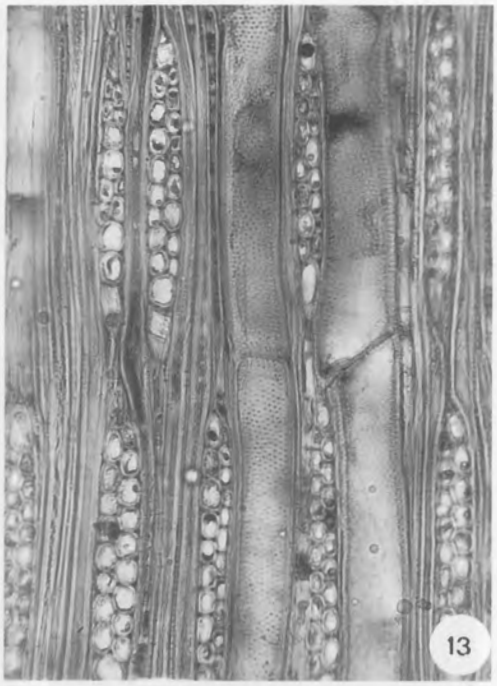
Figures 5–8: Cross–sections of *Mansonia gagei, Mitragyna parvifolia, Santalum album* (stemwood), and *Santalum album* (rootwood) (all at 40× magnification)

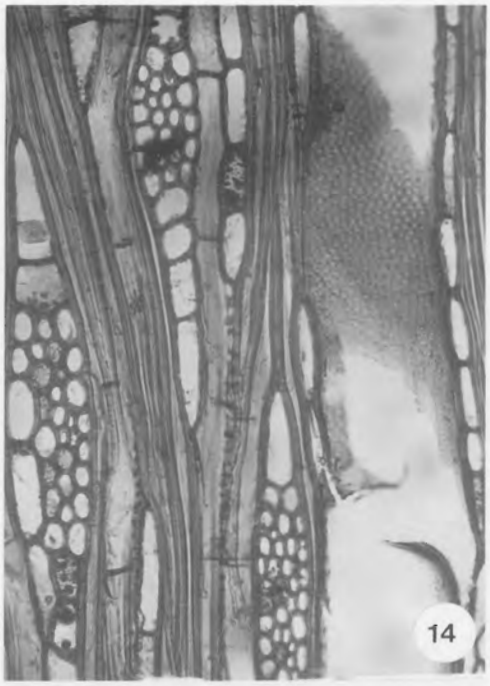


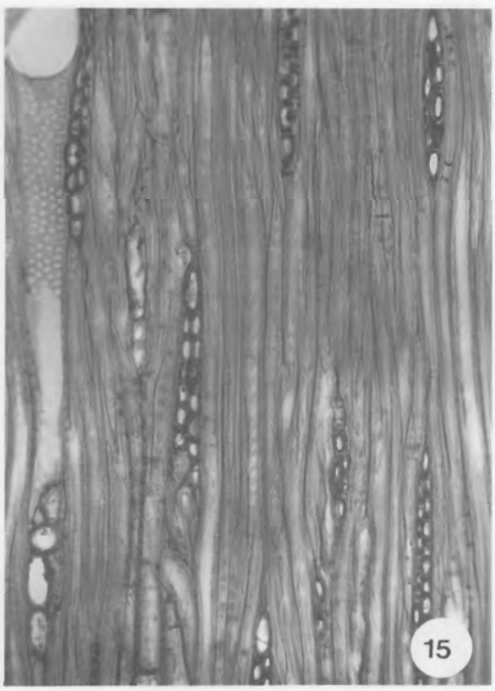


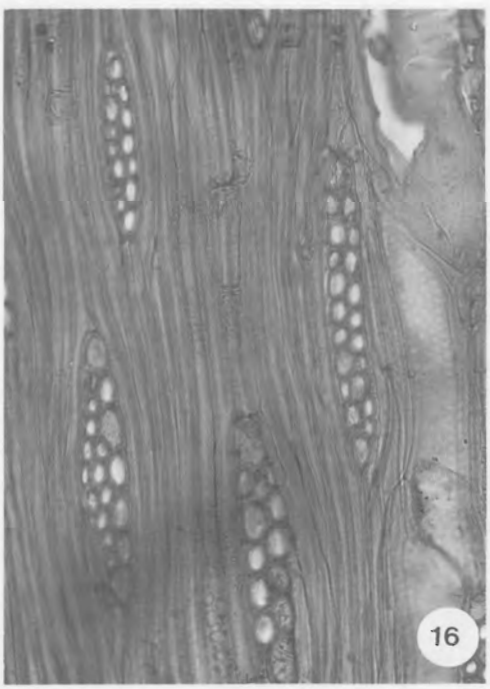


Figures 9–12: T.L.S. of *Adina cordifolia*, *Buxus wallichiana*, *Drypetes porteri*, and *Gardenia latifolia* (all at 120× magnification)









Figures 13–16: T.L.S. of *Mansonia gagei*, *Mitragyna parvifolia*, *Santalum album* (stemwood), and *Santalum album* (rootwood) (all at 120× magnification)

The wood is diffuse-porous. Growth rings are usually indistinct. Vessels are small to very small, 60–110 mm in diameter (mean 80 mm), 25–40/mm^2, mostly solitary and radial multiples of 2–3, perforation simple. Intervascular pitting, small to medium, 4–6 mm, vestured, pits to ray similar in shape and size, vessel members 400–1200 mm in length. Parenchyma diffuse to diffuse-in-aggregates. Strands of 7–9 cells, crystals absent. Fibres non-libriform, non-septate, 800–2300 mm in length and 32–40 mm in diameter, interfibre pits numerous, bordered. Rays fine to very fine, 13–16 per mm, heterogeneous type I, 1–3 seriate, composed of upright and procumbent cells, multiseriate portion composed of only procumbent cells, uniseriate is up to 18 cells in height and composed of upright and square cells and rays are fused to form long rays, 30–40 mm in width and 300–670 mm in height, uniseriate extensions longer than multiseriate part, crystals absent. Perforated ray cells common.

***Buxus wallichiana* Baill., syn. *Buxus sempervirens* L. (in part) (Buxaceae)**

Trade name: Boxwood. Common names: Chickri, Chickdi (Kashmiri) Shamshad, Samsad (Punjabi) Papri, Sansadu (Hindi).

Wood light yellow to whitish-yellow ageing to brownish yellow, hard and heavy (SG = 0.82 air-dry) straight grained, fine to very fine textured with silky lustre and odourless.

The wood is diffuse porous. Growth rings are distinct, delimited by narrow darker line of denser fibres, 8–12 per cm. Vessels very small, occasionally in tangential pairs, 45–50 mm in diameter (mean 35 mm), 130–180/ mm^2, predominantly solitary, perforation scalariform, 10–20 bars, intervascular pitting scarce, very small, 3 mm, vessel ray pitting similar in size and shape, vessel member length 335–700 mm, yellow gummy deposits common. Parenchyma diffuse to rarely diffuse-in-aggregates and also scanty paratracheal without crystals. Fibres, libriform, non-septate, 680–1075 mm in length and 20–23 mm in diameter with abundant small bordered pits. Rays fine to very fine, 9–14 per mm, heterogeneous type II frequent, 1–3 seriate, multiseriate rays 2–3 seriate composed of procumbent and upright cells, 27–30 mm in width and up to 25 cells or 450 mm in height. Occasionally uniseriate extensions of two rays join up to form high rays. Crystals absent.

***Dryptes porteri* (Gamble) Pax and Hoffm. syn. *Hemicyclia porteri* (Euphorbiaceae)**

Trade name: Cegil wood

Sapwood and heartwood indistinct, wood pale yellowish-white to light yellowish-brown, hard to very hard, heavy to very heavy (SG = 0.83–0.89 air-dry), straight grained and fine-textured, odourless.

The wood is diffuse porous. Growth rings are not conspicuous, delimited by latewood thick walled fibres. Vessels are small to extremely small, 30–55 mm in tangential diameter (mean 40 mm), numerous, numerous, 100–180/mm^2, solitary and radial multiples of 2–4, round, a few filled with gum, perforation mostly simple, but few scalariform, intervascular pitting very small 3–4 mm, pits to ray cells similar in shape and size. Parenchyma diffuse to diffuse-in-aggregates, strands of 4–10 cells, crystals present rarely in chambered locules. Fibres libriform, thick walled, non–septate average fibre diameter 18 mm with reduced border pits. Rays fine to very fine, 16–18 per mm, heterogeneous type I, 1–3 seriate, mostly biseriate composed of upright, square and procumbent cells, up to 33 mm in width, uniseriate extensions often join to form high rays, solitary crystals abundant in upright and procumbent cells.

***Gardenia latifolia* Ait. (Rubiaceae)**

Trade name: Gardenia. Common names: Donga, kuru, gogal paria (Gondi), papra (Hindi), kambi, kalkamb, advibikke (Kannada), papro (Koli santali), pempri (Mal Pahari), ghogar (Marati), dam-kota ranga, jantia (Oriya) kambil, kottumari–kalan (Tamil), karinguva, peddabikki (Telugu).

Sapwood and heartwood not distinct. Wood creamy white to yellowish-brown, moderately hard to hard, moderately heavy to heavy (SG = 0.73–0.83 air-dry), straight-grained to somewhat interlocked, very fine-textured, odourless.

The wood is diffuse-porous. Growth rings are inconspicuous, when distinct, demarcated by thick-walled latewood fibres. Vessels are very small to extremely small, 30–60 mm in diameter (mean 50 mm), 60–90/mm^2, mostly solitary or in short radial multiples of 2–3, perforation simple, intervascular pitting vestured, small, 3–4 mm, ray-vessel pitting similar in shape and size, vessel members 150–700 μm in length. Parenchyma diffuse-in-aggregates. Strands of 2–4 cells, crystals absent. Fibres thick-walled, semi-libriform to libriform with small bordered pits, non-septate, 300–1500 mm in length and 20–25 mm in diameter. Rays fine to very fine, 12–16 per mm, heterogeneous type I and type II, 1–3

seriate, composed of upright and procumbent cells, 30–40 mm in width and up to 400 mm in height, uniseriate extensions 2–8 cells, crystals absent.

***Mansonia gagei* Drumm. (Sterculiaceae)**

Trade name: Kalamet

Sapwood yellowish or brownish white, heartwood deep reddish or olive brown, with darker streaks, very hard, heavy to very heavy, (SG = 0.82–0.97 air-dry), sometimes sweet-scented, straight-grained to interlocked, very fine textured.

The wood is diffuse porous. Growth rings are usually indistinct, delimited by thick-walled latewood fibres and sometimes with marginal parenchyma. Vessels are small to extremely small, 35–65 µm in diameter (mean 45 µm), 85–96/mm^2 mostly solitary and in short radial multiples, open, a few plugged with gum, perforation simple, intervascular pitting very small 3–4 mm, pits to rays similar in shape and size, vessel members storied. Parenchyma diffuse to diffuse-in-aggregates, and also marginal as discontinuous line, strands of 3–5 cells, storied. Crystals absent. Fibres angular in cross section, non-septate, storied, thick walled with simple to small bordered pits, average fibre diameter 15 mm. Rays fine to very fine, 9–13 per mm, heterogeneous type II, 1–3 seriate, mostly biseriate, storied, composed of upright and procumbent cells, up to 30 mm in width and up to 20 cells or 210 mm in height, crystals present in upright cells, solitary or in locules of 2–3.

***Mitragyna parvifolia* (Roxb.) Korth. (Rubiaceae)**

Trade name : Kaim. Common names: Gulikadam, kali–kudur, mitkunia (Bengali) phaladu (Garhwal, Kumaon), mundi (Gondi), kaem, keim (Hindi), naye kadambe (Kannada), gore (Santali), nirkadambai (Tamil), battaganapu, botrugua (Telugu).

Sapwood and heartwood indistinct. Wood pale-yellowish brown ageing to light brown to reddish-greyish-brown, moderately hard, moderately heavy (SG = 0.56–0.73 air-dry) straight to shallowly interlocked grain, fine textured, odourless.

The wood is diffuse porous. Growth rings are distinct to indistinct, demarcated by thick-walled latewood fibres. Vessels are moderately large to small, 60–120 mm in diameter (mean 100 mm), 20–25/mm^2, mostly solitary and in short radial multiples of 2–3, perforation simple, intervascular pitting vestured, small 4–6 mm, pits to ray similar in shape and size, vessel members 700–1000 µm long. Parenchyma diffuse to diffuse-in-aggregates, strands of 4–6 cells, silica present. Fibres non-libriform to libriform, non-septate, 450–2100 mm in length and 25–30 mm in diameter, interfibre pits distinct, bordered. Rays fine, 12–17 per mm, heterogeneous both type I and II, 1–4 seriate, composed of square, upright and procumbent cells, 20–50 mm in width and 450–650 mm in height, crystals absent. Silica inclusions present. Perforated ray cells common.

Conclusions

Anatomically both stemwood and rootwood in sandal look alike, except that in rootwood the vessels appear to be thinner and more frequent; however, very many rootwood samples of varying diameters have to be studied for this proposition to be confirmed. The wood of sandal can be differentiated when it gets adulterated with other timbers. It can usually be distinguished from:

- *Buxus sempervirens* and *Drypetes porteri* by the absence of scalariform perforations in vessels;
- *Adina cordifolia, Gardenia latifolia, and Mitragyna parvifolia* by the absence vestured intervascular pitting; and
- *Mansonia gagei* by the absence of storied rays, fibres and parenchyma.

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Tree Improvement

Descriptions of some Sandal Tree Populations in the South West Pacific: Consequences for the Silviculture of these Species and Provenances

Y. Ehrhart*

Abstract

Many of the islands of the South West Pacific that bear sandal have been visited and the stands described. Mostly the population is depleted, but some stands still exist. Depending on the status of the existing population, several possible management strategies are feasible. The aim is to rebuild stands which are as diverse as possible which will be able to be managed sustainably in a few decades. Some are presently managed with the objective of regular annual heartwood production with an increase of the stock. The observations reported here, especially those regarding shade intensity, can be used to improve the silviculture of the various provenances which differ markedly. Even aspects of seed storage differ, and this demands further investigation. New techniques, which differ significantly from those previously identified for the Ile des Pins provenance, are proposed.

THE DISTRIBUTION OF sandal species in the South Pacific is particular. They are located in two 'poles', separated by a large 'hole'—the Central Pacific—where sandal has never been found. In the east, one widespread species, *Santalum insulare*, still remains; it is spread from the Gambier and Austral Islands to the Marquesses Islands in French Polynesia. In the west side of the ocean, two species are described, *S. austrocaledonicum* located in the Melanesian islands (Vanuatu and New Caledonia), and *S. yasi* on the Polynesian islands of Tonga and Fiji. The latitudinal extent of each species is pertinent:

- *S. insulare*—12–23°S,
- *S. austrocaledonicum*—15–22°S,
- *S. yasi*—16–22°S.

In the frame of South Pacific Forestry Development Project, with the financial support of the French Fond Regional de Cooperation, several consultancies consisting of training workshops for sandal seed technology, nursery practices and first silviculture, as well as stand visits and descriptions, were carried out in 1996 and 1997. These observations are reported here.

* CIRAD Forêt, NEW CALEDONIA

Description of the Stands

Two main species are described in that part of the Pacific Ocean covered by this study. We shall not comment on the botanical description of the species, since that can be easily found elsewhere (e.g. Barrett and Fox 1995). The species are *Santalum austrocaledonicum* and *S. yasi*.

S. austrocaledonicum

This species occurs in south Melanesia, New Caledonia (NC) and Vanuatu (V). It is common on the Loyalty Islands (NC), Ile des Pins (NC), Aniwa (V), Erromango (V) and Espiritu Santo. It is scattered on Tanna (V). It has been seen on all the TAFEA group

islands (South of Efate). The other islands of Vanuatu have not been recently surveyed, but Sandal is known on Malekula as well as at least one location on Efate. Its latitudinal range is 15°S (Espiritu Santo) to 22°20'S (Ile des Pins).

There are substantial, botanically consistent stands on Ile des Pins, Loyalty Islands, and Aniwa, and scattered ones on the Grande Terre, Tanna, and Erromango.

Habitat

S. austrocaledonicum grows on soils developed from several bases:

- coralline rock—either very shallow (Aniwa), or quite deep (Ile des Pins);
- volcanic ash with influence of coralline rock (Loyalty islands);
- mixed volcanic and coralline sedimentary substrate (Erromango); and
- metamorphic rocks, schists, and phtanites (NC, main islands).

It grows mainly in secondary forests and agricultural fallow or clear dry forests with *Acacia spirorbis* on non-coralline soils. A few big or tall trees can be found along the edge of the dense forest or scattered in it, where they were able to grow in a temporary opening following the fall of a big old tree. In Aniwa, the vegetation is more bushy and sparse, not higher than six metres on the upper terrace.

Habit

S. austrocaledonicum has quite different habits depending on the provenance; for example, on Ile des Pins (NC), the provenance is light-demanding and many trees are found isolated in good sanitary conditions. The seedlings grow quickly, with one straight leader and thin perpendicular branches in open areas which are not too dry or windy. However, the best conditions are a lateral shade and protection, and a full vertical opening over the seedling. This is because if conditions are too shady, the seedlings grow very weak and thin. The tall, straight trees found in closed secondary forests—or rarely in dense forests—are isolated because they have grown in a cleared area, mainly due to old agricultural clearings or damage following the fall of a big tree which has been blown down; therefore they are very scattered. The behaviour of the variety in the south of the main island of New Caledonia, *S. austrocaledonicum* var. *pilosulum*, is similar.

The provenances from the Loyalty islands although considered to be the same botanical variety as that on Ile des Pins, present a very different habit. The seedlings have major leaf and stem morphological differences and look more like *S. yasi*. The shape of light-exposed seedlings is always very branchy, and a leader is hardly found. The branches are inserted in the bole at 40–50°, and tend to curve upward. They are thick and often stronger than the leader which will quickly disappear. Only the trees that have grown inside a shady forest stand (strong lateral and light vertical shade) can show an interesting bole shape. The difference is not only obvious morphologically, but also physiologically. The seed conservation of the Ile des Pins provenance is well known, and the germination rate stays acceptable for 3–4 years. By contrast, the germination rate of the Loyalty provenance stored and treated exactly like the other provenances, decreases very quickly to a rate close to zero after only 18 months storage (Chauvin and Ehrhart 1997). Heartwood rate and oil content rate are also significantly lower than the Ile des Pins provenance.

In Vanuatu, sandal is quite shade-tolerant, and its behaviour is intermediate between the two New Caledonian provenances described above.

On Erromango, the trees are scattered in the clear *Acacia spirorbis* forest where they reach heights of 13 metres, with sometimes eight metres of merchantable bole. These trees are often well shaped with a long and straight clear bole. Unfortunately, presently the high pressure of cattle in these forests stops any regeneration, and there are no saplings.

In the dense forest, beautiful trees are sometimes located but they are very scarce. Trees are more frequent in agricultural areas where they can establish just after the culture periods; but they are small, and often poorly shaped due to the strong light. The few trees seen in the open areas were bushy and crooked.

On Aniwa, the trees are very different. The general shape is bad, mainly small, thin, leaning, and crooked with many branches. The fruits are big, almost twice the volume of the Erromango provenance. A few big trees surrounded by tall bushes were seen on the lower, more fertile terraces of the island. Almost all the trees found on the upper terrace are leaning and crooked. In fact, nearly all of them come from suckers, which has two main consequences. First, since the root system of a sucker stays superficial rather than making a new tap root, the tree does not stand firmly to the wind. Secondly, the sucker does not develop the juvenile form of the seedling or the stump sprout; instead it immediately shows the morphology of a mature tree (broad leaves and no leader dominance), so it is very crooked and does not develop an strong leader.

Even though this species on Aniwa is taxonomically consistent with the description of *S. austrocaledonicum*, its morphological characteristics are quite different from the Ile des Pins provenance. Hence the provenances of *S. austrocaledonicum* should be studied again with a view to separating the species into several varieties. This work is already in process in New Caledonia for the Loyalty provenances.

S. yasi

This species occurs from Eua (20°30' S), South of Tonga to Vanua Levu (16°30' S), North of Fiji. It is very common on EUA (T), rare on Tongatapu, quite rare in the Ha'apai and common in the Vava'u groups (T). In Fiji it has been described on several islands (e.g. the Lau group, Viti Levu, Vanua Levu) but the stands are depleted and have not yet been visited (Bulai 1995).

Habitat

In Tonga the visited stands were growing on soils developed on volcanic ash influenced by the coralline rock below. On Eua the volcanic ash is deep; on Vava'u, less so. On the Ha'apai group, the soils are typical coralline soils developed on raised coral and sand.

S. yasi grows mainly in secondary forests, open areas, and agriculture fallow. Some trees were found in young dense forest in Vava'u (T), but they established themselves when the surrounding trees were smaller and the stand was open for agriculture, notably vanilla. Sandal occurs frequently in the forest covering the small, untouched coralline lime islands of Vava'u; here the forest has many openings due to the severe topographic conditions.

Habit

The shape of *S. yasi* trees and the colour of the leaves are highly dependant on the surrounding vegetation. If the canopy is light, Sandal grows very bushy with many leaders. The lateral branches always take over the previous leader, and so on, giving a strongly ramified crown without a single leader. The foliage is yellowish to yellow. Usually, fruiting is abundant. Such a tree has little economic value due to the small size of the numerous leaders.

In several places in Eua or Ha'apai, consistent sandal stands grow from completely open land to close dense forest through all the intermediate situations: open bushes, close bushes, bushes and trees, and dense tree stands. These areas were old agriculture fallow where trees have slowly taken over the bush and the grass. In open areas, the sandal trees are bushy like the ones described above; but when they grow with the lateral shade of bushes or under the vertical shade of tall trees, their shape improves. Under these conditions, they usually present one single leader, sometimes with big branches that rarely compete with the leader. The foliage is glossy green, and the trees look healthy.

However, when the shade becomes too dense, the crown is thin and the leader and branches are weak, even though the leaves are still bright green. This Sandal species has a photosynthetic physiology that is well adapted to shady conditions. The best shade:light ratio is 40–60 per cent. In these conditions, the trees are growing fast in height with a single leader that is quite straight and a crown that is still well developed. However, the crowns are very prone to deformation by branches from the upper-storey trees.

Tree origin also influences shape:

- seedling—a single straight leader in good conditions;
- sucker—crooked and heavily branched, with no leader; and
- stump shoot—often similar to seedlings initially, due to their very fast initial growth.

This observation is the same for *S. austrocaledonicum* (Ile des Pins), except that its seedlings are generally straight with perpendicular branches or—rarely—big branches growing upward and competing with the main stem.

Status of the Stands

The status of *Santalum austrocaledonicum* and *S. yasi* varies considerably from one island to the other. Many different situations are found in the South West Pacific. They can conveniently be considered in four categories:

- Ile des Pins and Loyalty Islands (New Caledonia);
- Eua (Tonga) and Aniwa (Vanuatu);
- Erromango; and
- Tanna (Vanuatu), Ha'apai (Tonga), and Fiji.

Ile des Pins, Loyalty Islands (New Caledonia)

These stands are consistent, well spread over the islands, and continuous. Many beautiful trees are still living. The original sandal stands were strongly depleted, but since the environment has encouraged regeneration and seedling growth, the present population is substantial with a balanced distribution of diameters.

The early felling was done in very short periods, removing the major part of the existing trees. The regeneration and vegetative multiplication were efficient, particularly in open or cleared forests or in agricultural fallow. In these conditions the genetic pool should not have lost much of its variability. A heavy felling allows regeneration to occur and to develop well, and the short period of logging hinders natural selection by regularly removing the best trees, keeping the worst or smaller ones in the stands. However, since the most beautiful trees were located in the densest stands, they were usually not fruiting so few seedlings would have grown after the exploitation. Thus, if the suckers and the stump shoots were not strong enough to survive in the surrounding forest, their genetic information could have been lost.

Management plans with annual yields have been drawn for two of the islands, but since the transition was not properly organised, no felling was done for years. The stock must still increase and the annual yield be maintained at half of the annual volume increment observed during the last inventories. Natural regeneration is important, but plantations should be encouraged too.

Eua (Tonga), Aniwa (Vanuatu)

These stands are continuous and well stocked in number, but depleted in volume since the trees are young and small and often badly shaped. Very few big trees still exist. Even if the valuable stands were completely destroyed, plentiful regeneration and vegetative multiplication have kept a large distribution of the trees on the islands. Genetic diversity should not have decreased too much, even if it is 'hidden' in a general bad shape due to the vegetative origin. The continuity of the stands allows easy gene exchange. However, the cases are not the same and different strategies should be followed on Eua and Aniwa.

On Eua, where many beautiful seedlings are found in the bushy areas, a complete ban on logging should be enforced for at least 20 years in order to allow the present trees to reach maturity. The ways to implement such a ban are many and depending much on local customs The marketable stock will be then consistent and a sustainable management plan can be followed. Natural regeneration is plentiful but often located in open areas where it will not give beautiful trees. Plantations should complement it as soon as possible, and villager awareness be raised in order to protect and improve the shape of existing trees. Seed collection on the best trees of the island is not difficult; these trees should be selected mainly on their shape and registered.

On Aniwa, the present stand will hardly give any merchantable trees because of the poor shape mainly due to their sucker origin. We assume that the genetic stock is not too much depleted owing to the number of trees found and their distribution continuity. Thus, an important improvement of the general shape of the stand is possible by seedling planting. Natural regeneration is rare.

Large-scale plantations would enrich the stand. Since Aniwa sandal seems quite different of that of Tanna and Erromango (e.g. very big fruits), in the absence of reliable information on variability between the islands, only local seeds should be used. Since the present shape of the trees does not reflect their potential shape, seeds should be collected from as many trees as possible without regard to their shape.

Exploitation of dying and damaged sandal in the present stand can be done; but the return will be very low. For example, in 1997, the average heartwood weight per logged tree was 18 kg with a dbh of 12.5 cm (Hook 1997).

Erromango

These stands are located around the villages and settlements or very scattered in forest clearings. The former are depleted but still in significant number, with a poor shape due to the open area where they have grown. The latter are often beautiful, well-shaped and straight, but are under pressure from the villagers. They represent an important genetic resource, but are very difficult to mobilise because of geographical dispersion, their unknown location, the size of trees, the low fruit production, and the high pressure of cattle and pigs that destroy the new seedlings. In this case, the best way—and almost only way—to preserve a large genetic pool is ex-situ conservation.

So far vegetative multiplication by the usual means (e.g. cutting and marcote) has not been successful with this species. Studies of mobilisation techniques are therefore needed.

Ex-situ seed production stands should contain as many mother trees as possible. Fencing of the located stands, thinning of the surrounding trees to give full light to the crown as well as better germination of the fallen seeds, should allow the regeneration to develop. However, any plantation will face wild animal damage, which restricts them to small areas. In this island, villagers are transplanting young wildlings in their fields, and providing that the fencing is well maintained they already have worthwhile experience.

Vava'u in Tonga has some similarities. There is one consistent stand located South West of the main island where the trees are small but well shaped. Few other small and isolated trees were seen elsewhere on this island. It seems that many trees are still growing on the small islands scattered around the main island. Owing to the steep cliffs that surround them, they are often very difficult to reach so some beautiful trees should be found. Routine in-situ seed collection is not possible in these conditions, but a base ex-situ seed production stand could be built up that would provide seeds for large plantations on the main islands.

Tanna (Vanuatu), Ha'apai (Tonga), Fiji

These trees are rare, scattered in the bush on the islands. Sometimes very localised stands can be found (e.g. Ha'apai), but they are mainly young trees and the genetic base is narrow. Reconstitution of new populations from the few existing trees would be risky, so input from other provenances should be planned for wood production.

Supporting this last option for Tonga, the results of a recent analysis of sandalwood oil composition shows that the variation between the provenances of *S. yasi* in Tonga are quite low; and in terms of oil composition, the tested samples are homogeneous (Alpha 1997). Importation of other Tongan provenances on Ha'apai will not lead to disappearance of a particular characteristic that should be kept pure, but further genetic research should clarify this point.

Silvicultural Consequences

Some general rules can be developed from the above observations. They cover matters like shade management, and can be linked to the species and even the provenances inside the species. In fact, at the present stage of knowledge, a distinction can be drawn between two groups:

- *S. yasi* and the *S. austrocaledonicum* provenances of the Loyalty Islands and Vanuatu; and
- the Ile des Pins and Grande Terre provenances of *S. austrocaledonicum.*

Since our usual seed origin in New Caledonia is Ile des Pins, we have specifically worked on it and the proposed silviculture as well as the seed technology were elaborated for it (Ehrhart 1996c; Ehrhart and Nasi 1996a, b; Ehrhart and Fox 1995). In fact, recent observations and studies (Chauvin and Ehrhart 1997) show that we must reconsider our techniques for the Loyalty provenances, and probably for the Vanuatu provenances and the *S. yasi* varieties as well.

Soil

Sandal species are mostly versatile concerning their soil requirements. The general rules are:

- must be well-drained;
- both species grow easily in coralline soils on raised coral or sand;
- heavy clayey soils should be avoided, or trees only planted on slopes;
- temporarily water-logged soil must be avoided; and
- acidic, depleted soils (e.g. fernland) must be avoided.

Climate

Sandal species require a tropical climate. The main characteristics to be taken into account for their silviculture are rainfall and wind. Both are linked since the terminal leaves and bud of sandal are very prone to wilting and can easily be killed; that causes the development of lateral branches and a subsequent bushy shape.

S. austrocaledonicum is drought-resistant, but it prefers a sufficient water supply. We have not enough experience with *S. yasi* for this factor, but it should be less drought-resistant than *S. austrocaledonicum.* This is because *S. yasi* usually grows in well-watered areas, although it is found on the dry side of Viti Levu, Fiji, where precipitation is around 1400 mm/year (Bulai 1995).

The general rules are:

- on well-drained soils there is no major problem, although low precipitation could be detrimental to shape;
- on heavy soils, high precipitation would cause water accumulation; such soils must be avoided particularly on flat areas; and
- during severe drought clay soils are unsuitable sandal, although they could be selected, provided that they present a slope and an existing vegetation provides lateral protection against wind and sun.

Light

This is the major factor to be considered in sandal silviculture. It causes important differences between provenances.

S. yasi	*S. austrocaledonicum* (Ile des Pins)
	
Needs shade at the young stage.	Tolerates full sun during the first years, but prefers lateral shade with vertical light.
More light-demanding once the crown begins to develop, but always needs lateral shade.	Full light is not detrimental to adult trees, provided that bushes and small trees are surrounding the tree for sufficient parasitism.
Often, the lateral branches take over the previous leader, which causes a very branchy form without a straight and permanent leader stem; this characteristic is invariable in full sun, but far less in shade where usually there is one single leader. Shape pruning of a dense pure stand of branchy trees (fully exposed to sun) has induced the natural death of many small healthy branches; the active crown is now located on the top of the trees, which leads to self-pruning.	The stem is dominant while vertical growth is strong; lateral shade helps to keep this shape. The development of lateral branches as multiple leaders happens when the growth is difficult. This characteristic stresses the importance of fertility and water supply on the tree form; it is exacerbated by wilting due to wind and strong sun during dry periods.
Diameter growth is stronger.	Height growth is better in the first years.
Wind resistant.	Less wind resistant when young (but at the same age the trees are taller, and the stem is more rigid).

Both types present an easy healing of the small wounds caused by pruning. Both are prone to major stem deformation when disturbed by branches from the overstorey trees.

Main Silvicultural Recommendations

Planting on bare land

S. yasi	*S. austrocaledonicum* (Ile des Pins)
(1) Not recommended (except for seed production); or (2) planting of the host first then sandal planting at least one year after (but depending on host's growth). If the host provides a heavy shade, then pure rows of each are recommended. They will be oriented across the prevailing wind. If the host provides only a light shade, alternate planting in the row. The distance between the sandal and the host will then depend on the growth of the host.	(1) Possible with simultaneous planting of sandal and host (provided that the host is fast-growing for the first year, particularly on dry areas); or (2) if large spacing is planned between host and sandal, the host should be planted first.

Both types are strongly prone to deformations when disturbed by lateral or over hanging branches from the host plant. They must be pruned regularly.

Planting inside existing vegetation

Several cases are found. Either the shade is provided by bushes, or it is provided tall trees in which case it must be light (light:shade = 50%).

S. yasi	*S. austrocaledonicum* (Ile des Pins)
Tall trees: very appropriate. All the existing small bushes are kept around the sandal. (Experience has still to be gained additional host planting in this case.)	Tall trees: too heavy; vertical shade is not recommended.
Bushes: rows opened across the prevailing wind, one metre wide.	Bushes: rows opened across the prevailing winds, 1–2 metres wide depending on the bushes' height. If the rows are too wide, they will not provide enough shade to the seedlings, and will not inhibit weeds.
Trees and bushes: in this case the shade ratio from the upper storey trees should not exceed 30%. Rows are opened as above, but the orientation depends less on wind direction.	Trees and bushes: only light shade from the upper storey trees is recommended (*Casuarina* trees gave good results in this design).

Shape management

Wherever the sandal does not grow quickly in height (e.g. low fertility or dry areas), or if the shade ratio is low (e.g. at the beginning of a plantation on clear land), shape pruning is needed.

This removes all the branches that are growing upward and that could take over the leader. The aim is a straight and clear bole 2–4 metres long (depending on the fertility of the soil). This operation is easy to carry out and very fast when it is done early, but becomes longer and more difficult as soon as the trees are taller than about two metres. Also, during an early operation the choice of the branches to cut is easy and does not demand much experience and skill. Therefore, it is recommended that pruning begins in the second year, and is repeated regularly every two years until the stem has reached the targeted length. Above this height, in the best cases, it would eventually happen naturally.

Advantages of planting in existing vegetation

This design has several advantages that recommends it for all the cases where an existent technical structure is not available. In the South Pacific this is almost always the situation. The benefits are:

- low initial investment (seedlings and the opening of the row in the existing bushes);
- tree density can be low by opening the rows at large spacings without the problem of shade ratio;
- only few seedlings are planted (sandal alone, rather than sandal plus hosts);
- further maintainence works are easy to implement (bush trimming in the rows, and pruning of the sandal);
- workers (either hired or on their own land) prefer to work standing and under shade, instead of being bent and exposed to the sun in a new plantation; and
- tree shape will be far better in the event of the plantation not being maintained.

However, it is better to avoid the areas where the existing vegetation is strong and dynamic (strong *Hibiscus tiliaceus* bush, for example), because the sandal could be very quickly eliminated in the absence of maintenanc.e In less vigorous vegetation, like that found in dry forest, the sandal will easily survive and grow even without any maintenance work.

Improvement of existing natural or artificial stands

Shape pruning is only done on newly planted seedlings and the most beautiful existing sandal. Three categories are discussed.

Natural young trees grown in good shade conditions

The stems are quite straight, there are few branches that have become leaders, the diameter of which is still less than five cm. Pruning of the most disturbing branches (up 60% of the foliage can be removed), in which case, all the plagiotropic branches are kept, even when they are located on the bottom of the stem. If needed, one or two years later, a second pruning will be done in order to remove more leaders and, depending on the size of the crown, to lop the low branches.

This technique should be also used when the trees are poorly shaped but already too big to be treated with the next technique; the objective is to reach a two-metre-clear stem length, whatever the fertility of the soil. Sometimes, in case of too strong pruning and too much lateral light, some shoots come out at the major wounds. They must be removed as soon as possible (within 6–12 months).

Beautiful 6–10 year old stands in Eua have been improved with this technique.

Poorly-shaped young trees from seedlings (up to 5–7 cm of diameter at ground level)

Stems are cut a few cm above the ground. Less than one year later, only the best shoot will be kept and the others removed. Since the shoots are growing fast at the beginning, they always have a straight stem shape.

Very badly shaped older trees from seedlings (diameter >7cm) or from suckers

With the previous techniques, either the size of the stump or the existing root system of a sucker would not allow the future tree to develop a sound and balanced root system, or the trees are too poorly shaped to be improved. The previous operation could be detrimental to the stand value (big wound, difficult recovery). The badly-shaped trees are left growing, and a new plantation of seedlings is established at a large spacing. Only the disturbing badly-shaped sandal are eliminated. The others are kept to be cut at maturity. They will provide oil-grade logs.

Conclusions

Currently, only the New Caledonian populations are able to support regular sustainable logging. Surveys on Ile des Pins and the Loyalty Islands have given information on available sandalwood volume. Successive inventories gave us the annual volume increment of the stand on Ile des Pins and potential annual heartwood yield. They are the basis of long-term manage-

ment aiming to increase the present stock (Ehrhart and Nasi 1996a). For the moment there is no exploitation.

In the other countries, complete protection should be organised in order to stop the present depletion of the rare reserves of immature trees. In the case of some Vanuatu islands (e.g. Erromango and Espiritu Santo), the stands could possibly support sustainable management. However the present ignorance of the exact available stock and its value, and the difficulty of checking whether logging operations comply with annual cut authorisations, demand a long-term ban until the full knowledge of these elements is available. There is still strong logging in Espiritu Santo (more than 100 tonnes were exported in 1997 by Chinese traders) and there is no figure at all of the stands there.

Germplasm banks should be build up in several places (e.g. Erromango, Tanna, and Vavu'u) in order to provide seeds for large-scale planting while keeping genetic diversity as high as possible. Pure provenance-conservation plantations are also an option. In New Caledonia, separate conservation is compulsory because provenances differ markedly. When morphologic differences are not obvious, further studies should be done to identify if separate conservation is appropriate. In Tonga for example, oil composition is similar throughout the country (Alpha 1997). Therefore, separate provenance conservation is not appropriate for this factor; it would be better to gather all the vegetal material in one ex-situ seed production stand to give a larger variability to the pool, instead of working with separate small populations.

In the South Pacific, sandal populations are mostly depleted except on a few islands where the stock is still consistent. In these latter, if the population were surveyed sustainable logging management is possible (e.g. New Caledonia). When no figure is known for the existing resource, sandal should be protected until the knowledge of the stock allows sustainable management (e.g. Vanuatu). In all the other cases, a sound recovery requires complete protection of the last few remaining trees.

Reconstitution of valuable stands would need plantations in many cases. Depending on the sandal status on the considered islands, the seed collection strategy will be different and in many cases, ex-situ seed production stands are recommended.

Observations of natural stands and current plantations have identified several technical itineraries adapted to the variable provenance behaviour depending on light. In particular, *S. yasi* and *S. austrocaledonicum* provenances from Loyalty Islands and Vanuatu need shade (shade:light ratio = 50%) to grow properly and produce high value timber; that conclusion is contrary to previous technical practices adapted to Ile des Pins provenance. Therefore (except for Ile des Pins provenance), the plantation design generally recommended is planting in rows opened in existing vegetation of bushes and trees (clear forests). Plantations on open land must be reserved for seed orchards.

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Germination of Two Provenances of *Santalum austrocaledonicum* var. *austrocaledonicum*

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Abstract

During the germination phase of a *Santalum austrocaledonicum* progeny trial, important and unexpected differences occurred in the germination rates between two provenances. The 'Ile des Pins' provenance conformed with expectations, previous experimentations and routine germination (55% after two years' storage). By contrast, the unstudied 'Mare' provenance failed completely (one lot had a 19% germination rate; the others, nothing after 15 months' storage in identical conditions). Germination rate seems to correlate with mean seed-size of a provenance. This may be significant since the seeds of other *Santalum* species and varieties of the South Pacific look more like the Mare, even though the only large experiment was conducted on the Ile des Pins provenance. Present projects of genetic diversity conservation or species/provenance trials in South Pacific countries should be aware of that result, and test their seed lots regularly in order to minimise loss of costly material.

AN IMPORTANT PROGENY trial of *Santalum austrocaledonicum* has been initiated in New Caledonia in order to study some genetic characteristics of this species. Collections were conducted on 10 trees on the Ile des Pins (IdP), located 80 km southeast of the main island and 13 trees on Maré Island located 130 km east of the main island, in the Loyalty Islands archipelago.

All previous studies were carried out with the easiest provenance to collect: 'Nouméa'. Much information was also gathered with the most-used provenance for plantations: IdP, that behave like Nouméa in terms of conservation, germination and juvenile growth. The Loyalty provenances, including Maré, should be another variety due to major morphological differences with IdP; they have not been used for plantations, so their characteristics are unknown.

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Methodology

Fruits were collected ripe on the trees under the direct supervision of the officer in charge of the Forestry Seed Centre of New Caledonia, managed by CIRAD-Forêt. The fruits were immediately depulped with a potato peeler. Seeds were first tested by flotation, then dried under shade until transport to the Seed Centre where they were treated against fungus and stored in the dry cold-storage room at 1–3°C. All lots were stored together; they were:

- *Santalum austrocaledonicum* var. *austrocaledonicum* (doubtful denomination);
- 10 lots of more than 300 seeds each, collected 7–10 March 1995, each on a single tree on Ile des Pins; and
- 13 lots of more than 300 seeds each, collected 10 October 1995, each on a single tree on Maré.

After two years, on 3 March 1997, the seeds were withdrawn from the cold-storage room, scarified by removing the extremity of the hard coat, partially soaked overnight inside a fridge in a 0.1 g/L of a giberellic acid solution. The solution levelled in

order to have the top of each seed outside the solution. On 4 March 1997 they were sown on disinfected perlite (thoroughly watered with chloride, rinsed with treated tap water, then dried under the sun). The dried perlite was laid in boxes, then treated with a strong fungicide, *Terachlore*. One third to a half of each seed was left outside the germination medium. Bed heaters were not used because the germination was done during the hot season. Watering was by sprinkler at the rate of 1 mm every 10 minutes in order to keep the seeds humid but not wet. Every week, the seeds were treated with a fungicide (*Benlate*) solution. As soon as a seed germinates, it is withdrawn from the germination bed and sown in a second germination box (peat and sand) where it will grow until pricking out to the pots takes place.

Results

The differences between the two provenances are shown clearly in Table 1 and Figure 1.

Seed size

Unfortunately, since the Maré lots were unsuccessful, the individual germination record cards for each lot were not kept. We do not have the exact weight of the seeds for those lots, except for the lot that germinated poorly (35.5 g/100 nicked seeds). However, previous studies on the New Caledonian seeds (Bailly 1986; Kagy 1987; Nasi 1995) show that the average dry-seed weight of the lots vary around 40±0.6 g/100 whole seeds for Maré and 16.4±0.4 g for IdP. These values conform to the values measured here, considering that the latter were nicked seeds, which are slightly lighter than entire seeds. The Maré seeds are 2.4 times heavier than the IdP seeds.

Germination rates

The average germination rate of the IdP provenance is 55%, all 10 lots are between 38% and 70%, and the coefficient of variation is 19%; individual variation between progenies is important. By contrast, the 12 lots from Maré were completely unsuccessful (that is, virtually no germination), with the only one to germinate having a rate of 19%, half that of the worst IdP rate.

First germination

The IdP provenance has little variation and all the lots started to germinate the eighth or ninth day after sowing. The only Maré lot that germinated started on the 24th day after sowing.

Table 1. Germination results of two provenances of *S. austrocaledonicum*

Provenance	Characteristic	Provenance													
		1	2	3	4	5	6	7	8	9	10	11	12	13	Mean
Ille des Pins	Germination rate (%)	49	38	52	54	60	67	44	57	70	45	–	–	–	55
	100 seed weight (g)	12.3	17	14.8	14.6	12.9	15	11.8	14.6	12.4	14.8	–	–	–	14.02
	Germination start (days)	9	8	9	9	8	9	8	9	9	9	–	–	–	8.7
Maré	Germination rate (%)	0	19	0	0	0	0	0	0	0	0	0	0	0	19
	100 seed weight (g)	?	35.5	?	?	?	?	?	?	?	?	?	?	?	35.5
	Germination start (days)	~	27	~	~	~	~	~	~	~	~	~	~	~	27

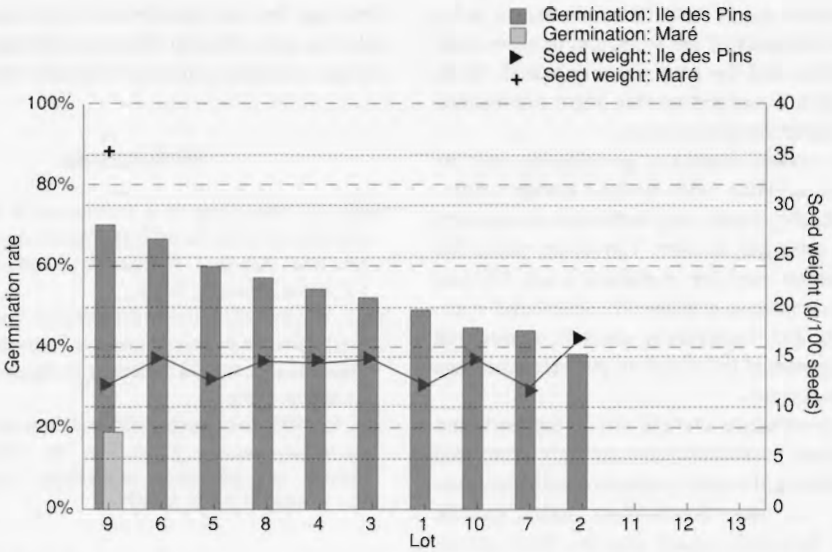

FIgure 1. Seed germination rates of progenies. Comparison between Ile des Pins and Maré.

Discussion

The behaviour of the two provenances concerning seed conservation and germination start is very different.

IdP

The germination rate of the IdP provenance was 54% after two years of dry cold storage conservation. This value is slightly under the germination rate (70–80%) in the large study in 1986 (Bailly 1986; Nasi 1995), but this latter was realised in laboratory conditions with the Main Island provenance, whose seeds are even smaller. Moreover, even if the cold storage room is dry, the experimental conditions of the 1986 study were air-tight boxes with silicagel that ensured a permanent and very low humidity level that is not reached in usual storage conditions. Given these remarks, we estimate that the observed germination rate of IdP seeds conforms to that expected.

However, the variation between progenies is high and must be further investigated. If the progeny trial shows sufficient genetic heritability, seed orchards of tested plus trees will be established. In order to ensure a regular and homogeneous quality seed supply, we will need to know the germination rate value and its evolution during storage. If these characteristics are linked to the seed bearer (either genetic or depending on physiological characteristics, their prediction will be possible and the management of the seed-lots collected will be far easier.

Maré

The Maré seeds were kept in the same conditions as the IdP ones. The storage length was only 15 months. Average germination rate was zero. (Only one lot among the 13 germinated, and the rate was low—19%.)

The conservation period for these Maré seeds is significantly shorter than the one for IdP, and we went beyond the limit of viability; so conservation for more than one year should not be considered.

Another factor that reinforced the low germination rate was the length of germination associated with the big size of the seeds. Until the beginning of the germination, the nicked seeds were exposed for longer. Even if sanitary precautions are enforced, they cannot completely protect the seeds from pathologic agents, mainly rot and fungus. The loss is therefore higher than for the IdP seeds.

Conclusion

Seed conservation of the Maré provenance seems to be very short. The present experience shows that the conservation period cannot exceed one year. In fact,

this conservation should only be conducted in order to break the dormancy of the seeds and to allow time between fruiting and the next planting period. More experimentation is needed on this Maré provenance in order to clarify that proposition.

We have already started a germination test vs length of conservation with several storage conditions. In fact, this factor may influence the present sandalwood program in New Caledonia since the main provenance used for plantation is the IdP one that has proved to have a better oil content and vegetative growth. But biodiversity must be considered too, so it is important to be able to produce seedlings of other provenances.

Since the provenance average size of the seeds and the conservation possibility seem strongly correlated in New Caledonia, the seed production and conservation strategy for other South-West Pacific species (e.g. *S. yasi* that looks much like the Maré provenance) or varieties (e.g. Vanuatu, the seed size of which is more like the Maré provenance), must be addressed and the viability evolution of the seeds identified in order to avoid any loss of seed. This is essential in case of an expected extended international program of species/provenance comparison or a genetic conservation one like that presently in process in some South Pacific Countries. Since the major part of the stands are depleted and the collection of seeds are few in quantity and very costly, the rare ones that are collected should not be kept too long in storage without a permanent viability test.

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Identification of Provenances of Sandal in India for Genetic Conservation

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Abstract

Important sandal-bearing areas in the states of Karnataka, Andhra Pradesh, Tamil Nadu, Kerala, Orissa and Madhya Pradesh were covered by reconnaissance survey. Eight potential provenances in different ecoclimatic zones have been identified. A comparison of the data from the present survey with that of 1970, indicates that the sandal population has declined substantially due to biotic and abiotic factors. This situation demands fresh initiatives for protection and propagation of this valuable tree species.

PROVENANCE IN FORESTRY refers either to places where trees are growing or the place of origin of seeds or trees. Langlet (1962) regards the investigation of provenances as the study of ecological variability within species, the relationship between this variability and the influence of environment, and reaction of different populations to transfer to an environment foreign to them.

Distribution

Sandal is found from the southern-most part of India in Kerala to Uttar Pradesh in the north. The spread is extensive and ecoclimatic conditions and edaphic factors vary. It is possible that the sandal trees could have adapted to conditions prevailing in those areas. Variation in morphology, heartwood formation and phenology have been reported.

Nearly 90 per cent (8300 km^2) of the total area of sandal in India is in Karnataka and Tamil Nadu. The rest is distributed in most other states: Andhra Pradesh, Kerala, Madhya Pradesh, Orissa, Maharashtra, Rajasthan, Uttar Pradesh, Bihar and Manipur (Venkatesan and Srimathi 1981; Srinivasan et al. 1992).

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In Karnataka, sandal is spread over 5245 km^2, mainly in the southern parts of the state and sparsely in the north. The important sandal-growing areas are Shimoga, Chickmagalur, Mysore, Kodagu, Uttara Kannada and Dakshina Kannada. The sandal is mainly spread in natural forests along with other species, and a few plantations have been raised by dibbling seeds in bushes and planting container seedlings in small areas.

The sandal population in Tamil Nadu is distributed over some 3000 km^2, mainly in North Arcot (Javadi's and Yelagiri hills), Harur, Salem and Vellore districts.

In Andhra Pradesh sandal is restricted to about 170 km^2, mainly in Chittoor, Kadapa, Tirumala hills, Hyderabad and Arakku valley. In Kerala it is distributed over 15 km^2 in Marayoor, Wynad and Thenmalai; the population in Marayoor reserve of Anjanad valley is dense. The sandal tracts of Orissa occur in an area of 35 km^2 in Rayagada and Jeypore forest divisions, mostly concentrated in Koraput district. In Madhya Pradesh sandal trees are scattered in the forest divisions of Sehora, Sagar and Seoni covering 33 km^2.

These figures were confirmed by a reconnaissance survey carried out in collaboration with the state forest departments, and are summarised in Table 1.

Table 1. The main sandal-bearing areas and population densities

State	Forest Division	Sandal-bearing area (ha)	Density
Karnataka	Shimoga	62 529	medium
	Sagar	87 350	sparse
	Chickmagalur	47 121	medium
	Mysore	48 712	sparse
Tamil Nadu	Harur	60 000	dense
	Tirupattur	16 517	dense
Kerala	Munnar	1 497	dense
Andhra Pradesh	Paderu	2 638	sparse
Orissa	Rayagada	2 541	medium
Madhya Pradesh	Seoni	3 000	medium

Karnataka

In Karnataka sandal is scattered and growing with other forestry species in natural forests. Densities are 20–30 trees per ha (> 50 cm girth class) and 200–300 (< 50 cm girth class). Many areas are devoid of trees of higher girth classes mainly because of rampant smuggling activity, extraction by the forest department, and biotic factors.

Mandagadde range of Shimoga division has a few patches of larger-girth trees. The population is free from major pests and diseases, except for sooty fungus in high rainfall areas. Regeneration is fairly good, but survival is poor due to fire and grazing. This area has potential to grow more sandal.

The sandal population in all the ranges of Sagar division has declined and larger-girth trees are missing. Chandrakala hills, known as high-density area earlier, is now reduced and only regenerated sandal (< 15–20 cm girth class) occurs. Regeneration is good, in spite of reported smuggling.

In Chickmagalur division the sandal population has dwindled in most areas, with only trees less than 20 cm girth found in few patches. Of the five ranges in the division, Kadur range has a good sandal population. The 60 ha Thangli sandal reserve of this range has 3000 trees 40–105 cm in girth and 5–12 m high. The area is well protected, and regeneration is good.

The sandal stock in the natural forests of Mysore division, which was well known for its scented heartwood, has declined considerably. The 30 ha Arabithittu plantation has trees 20–30 cm in girth.

Tamil Nadu

Sandal occurs mainly in Harur forest division; in other divisions, it is scattered and higher girth classes are absent. It is associated with other plants like lantana, bamboo, tamarind, *Carisa caranda*, and *Hardwickia*. The 5164 ha Chitteri section of Harur range consist of thickly-populated sandal 20–110 cm in girth and 10–15m in height. Of the three ranges in Tirupathur forest division, Alangayam range has 35–50 trees per ha of girth class 20–110 cm; Tiruvannamalai contains only a thin, scattered population.

Andhra Pradesh

The total area is 175 km^2, mainly in Araku valley of Paderu forest division which is a part of Araku forest range consisting of thin patches of sandal along the edges of the valley. Chittoor forest division, Tirumala hills and Nehru Zoological park, Hyderabad are the other areas of sandal growth.

Kerala

Sandal is restricted to 15 km^2 in Marayoor, Wynad and Thenmalai. In Marayoor range it occurs in four sections, mostly confined to Marayoor section. The population is dense, 500–1000 trees per ha, with 20–110 cm average girth. This is considered to be one of the best populations, and has been declared as a seed stand.

Orissa

Sandal is confined to Koraput range of Rayagada forest division and Jeypore of Jeypore forest division. Important sandal-bearing areas of Koraput are Janiguda, Pittaguda, Subai A and B, Nandapura, Bidaghatti, and Thuba. Sandal in this region occurs in forests mixed mainly with herbs and shrubs and very few trees of Sandal, *Terminalia* spp., *Cassia siamia* and *Pongamia*. The density is 40–300 trees per ha of 15–30 cm girth. Illicit felling and smuggling activity is very high and trees are not able to reach large girths. Natural regeneration is very good and there is good potential to grow more sandal.

Madhya Pradesh

Sandal is spread over 33 km^2 in Seoni, Sehore, Sagar and Indore divisions. Moderate density is found in South Seoni. However, they are more concentrated in compartments 75, 245, 246, 247, 248 and 249, which lie on either side of the Seoni Katangi road to the extent of over 15 km. According to records of enumeration carried out during 1987, there exist 16561

trees of over 20 cm girth. Many trees are also found in agricultural land, and in and around farmers' houses. Smuggling in this region is reported to be rampant, since there are many connecting routes to major cities.

Results

Eight sandal-bearing areas have been identified as potential provenances on the basis of population density, phenotypic characteristics, latitude, longitude and ecoclimate (Table 2).

These potential provenances vary in climatic and edaphic factors. They are maintained and protected in collaboration with the respective forest divisions for in-situ conservation to develop a gene base. Studies are being carried out on soil properties, morphology, phenology, heartwood formation, and oil content; and seeds are being collected and tested for seed characteristics, viability and germination. Isoenzyme study is being used to confirm the provenances and to find out genetic distance between them.

Discussion

The current survey and reports from respective forest divisions reveal that the sandal population has declined substantially in most of the major sandal-growing areas. This is mainly due to rampant smuggling activity, extraction by departments, and biotic factors.

Karnataka

Although no state-wide enumeration has been done during the past two decades, an enumeration carried out during the 1970s reported that 1000000 trees above 15 cm girth over more than 5000 km^2, i.e. less than two trees per ha (Venkatesan 1980; Venkatesan et al. 1995). Today this figure is reduced to less than one tree per ha, although some areas have a higher-density population:

- Mandagadde of Shimoga forest division—50 trees per ha;
- Thangli sandal reserve of Chickmagalur forest division—50 trees per ha; and
- IWST campus of Bangalore forest division—1000 trees per ha above 20 cm girth.

These three areas are potential provenances.

Tamil Nadu

No state-wide enumeration has been done, but higher-density populations found particularly in Javadis of Tirupattur forest division and Chitteri hills of Harur forest division have been selected as potential provenances.

Table 2. Potential sandal provenances

Potential provenance	Forest Division & State	Latitude & longitude	Altitude (m)	Mean annual rainfall (mm)	Temp—Max /Min (°C)	Soil type	pH	TSS EC Mhos/cm
Bangalore	Bangalore, Karnataka	12°58' N, 77°38' E	1000	850	36.8/12.2	Red loam	6.3–6.5 Acidic	251.2 μ mhos
Thangli	Chickmagalur, Karnataka	13°40' N, 76°00' E	766	1500	44.0/10.5	Red loam & alluvium	7.5–7.8 Alkaline	2.3 μ mhos
Mandagadde	Shimoga, Karnataka	13°9' N, 75°40' E	650	2000	38.1/13.0	Red loam	5.5–5.8 Acidic	317.0 μ mhos
Chitteri	Harur, Tamil Nadu	12°0' N, 78°6' E	1050	1000	35.2/8.2	Red sandy-loam	6.0–6.3 Acidic	327.3 μ mhos
Javadis	Tirupattur, Tamil Nadu	12°3' N, 78°7' E	930	1200	38.0/12.4	Red loam	6.6-6.7 Acidic	432.5 μ mhos
Marayoor	Munnar, Kerala	10°1' N, 77°1' E	1000	1450	36.0/10.0	Black clay	6.2-6.7 Acidic	362.0 μ mhos
Koraput	Rayagada, Orissa	19°55' N, 82°35'	859	1525	38.0/4.5	Red sandy-loam	6.2–6.6 Acidic	310.0 μ mhos
Seoni	Seoni, Madhya Pradesh	22°1' N, 79°5' E	900	1600	40.0/5.0	Laterite	–	–

Kerala

A density of 500 trees per ha of trees above 40 cm girth occurs in 15 km^2 of Marayoor range, Nachivyal 1 and 2 areas. Illicit felling and smuggling activities are negligible and department is extracting only dead and dried trees. This area is selected as potential provenance.

Orissa

Sandal is concentrated in patches in Koraput district. Indiscriminate felling and lack of protection in the area has led to absence of higher girth class trees. Natural regeneration is high, with soil and climate very conducive to growing sandal. This can be supplemented by dibbling seeds as well as planting container-grown seedlings. This area is a potential provenance.

Madhya Pradesh

A few trees of more than 50 cm girth with clear bole height of 4–5 m having heartwood slightly yellow and scented were noticed in Seoni. in some of the areas there are 25–40 trees per ha. Overall regeneration is poor, but there is potential to grow more sandal here.

Conclusion

The survey indicates that sandal populations in most of the states have declined due to biotic and abiotic factors. Eight potential sandal-bearing areas in different ecoclimatic zones have been identified as potential provenances. Over-exploitation and distress felling of higher girth classes is to be avoided to prevent genetic erosion and to aid preservation of germplasm. Natural regeneration can be supplemented by dibbling of seeds or planting container-grown seedlings.

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Physiological Variation in Seeds of Provenances of Sandal (*Santalum album* L.)

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Abstract

Physiological variation exists in seeds, as well as morphological variation. The germination test, in which seeds are actually induced to germinate and commence growth is by far the most dependable method of assessing the viability of a sample of seeds. Moisture content of seeds during storage is the most influential factor affecting their longevity. This paper describes the variation in physiological aspects of seeds of *Santalum album* L. from various provenances: Mandagadde, Thangli (Karnataka), Marayoor (Kerala) and Chitteri (Tamil Nadu).

SANDAL IS A small evergreen tree popularly known for its scented heartwood and oil. It is distributed mostly in the southern part of Karnataka, the northern part of Tamil Nadu, and parts of Andhra Pradesh, Kerala, Maharashtra, Madhya Pradesh, Orissa and Rajasthan. These states vary in their ecoclimatic and edaphic factors. Variation in sandal leaf, fruits and colour of heartwood was reported by Srinivasan et al. (1992). From a reconnaissance survey of sandal-bearing areas in these states, eight potential provenances were identified: Mandagadde, Thangli and Bangalore in Karnataka, Chitteri and Inner Javadis in Tamil Nadu, Marayoor in Kerala, Koraput in Orissa, and Seoni in Madhya Pradesh (Jain et al. these proceedings).

Sandal is propagated mostly by seeds. Though information in seed polymorphism is available (Nagaveni and Ananthapadmanabha 1986), information on seeds of different provenances is scant. Hence a study of different parameters of seeds collected from different provenances was carried out.

Materials and Methods

Ripened fruits of sandal from the different provenances mentioned above were collected during the fruiting season. Fruits were depulped by soaking them in water overnight and drying them in shade; they were then stored in polybags. Different seed characteristics such as weight, moisture content, viability and germination percentage were determined in three replications.

The seed weight study used 100 seeds. For moisture content determination, a known weight of seeds was kept at 103±2°C for about 17 hours. Viability was determined by using the 'Tetrazolium Test' (TTC method). After decortication the seeds were soaked in water for about 20 hours, and a transverse cut made. Then they were treated with 1% TTC solution in dark for about eight hours. For germination studies, seeds after a dormancy period of two months were sown in plastic germination trays filled with pure sterilised sand. Watering was done on alternate days.

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Table 1. Seed characteristics in Mandagadde, Marayoor, Thangli and Chitteri provenances.

Provenance	Seed weight (g/100 seeds)	Moisture content (%)	Viability (%)	Germination (%)
Mandagadde	19.77	10.58	81	65
Marayoor	19.37	10.08	74	50
Thangli	19.73	10.38	58	52
Chitteri	15.27	9.86	50	45

Results and Discussion

The review of the results indicates that positive correlation exists between seed weight, viability and germination percentage. This conforms with earlier work on tropical hardwoods (e.g. Calinawan and Halos 1983; Gupta et al. 1983).

Of all the provenances, seeds collected from Mandagadde provenance weigh more, are more viable, and germinate better (Table 1). The seed quality of other provenances, in order of merit, is: Thangli > Marayoor > Chitteri.

Our study indicates that seed weight is a determining factor for assessing seed quality. This will be useful in screening seeds at the time of gene conservation and cultivation.

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We wish to thank Dr K.S. Rao, Director, Institute of Wood Science and Technology, for his encouragement.

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Variation in Seed Characteristics in Provenances of Sandal (*Santalum album* L.)

H.C. Sindhuveerendra, S. Ramalakshmi, B.B. Mallesha, H.S. Ananthpadmanbha and C.R. Rangaswamy*

Abstract

Seeds of *Santalum album* (Sandal) from various seed sources show significant variation morphologically and physiologically. Data on seed parameters showed significant variation between different seed sources. Highest range of seed length is noted in seeds from Mulbagal provenance (10.27–6.25 mm with a mean of 7.87, SE ± 0.1), whereas the lowest has been observed in Mysore provenance (8.92–7.45 mm with a mean of 8.0, SE ± 0.08). Similarly, the highest seed width was observed in Shimoga provenance (9.29–6.19 mm with a mean of 7.12, SE ± 0.09), and the lowest has been observed in Mandagadde provenance (7.79–6.09 mm with a mean of 7.16, SE ± 0.03). For seed weight, the highest was in Honagal provenance (0.34–0.11 mm with a mean of 0.12, SE ± 0.005), and the lowest was in Marayoor provenance (0.22–0.08 mm with a mean of 0.16, SE ± 0.003). The coefficient of variation (CV), which helps in comparing variability among different provenances, has been calculated. The highest CV for seed length was recorded in Mandagadde provenance (15.0%) followed by Mulbagal provenance (12.2%). CV for seed width is highest in Mysore provenance (14.0%) followed by Shimoga (11.9%). The same has been observed for seed weight in Honagal provenance (29.3%) which is highest, followed by Bangalore provenance (27.7%). The distribution patterns of CV among different provenances and in seed characteristics indicate the presence of natural variability in those provenances. Some outstanding trees may be selected from those provenances which showed highest CV for different seed characteristics, and may be incorporated as base population while establishing second generation seed orchards of sandal at different places. This is a prerequisite for formulating a multiple population breeding strategy.

Key words: *Santalum album*, genetic variation

SANDAL IS A tree species in the family Santalaceae. In earlier studies much emphasis was given to improvement of quantitative traits such as heartwood and oil content. Under that multiple tree-breeding strategy, there is an immediate need to infuse fresh gene pools into n-2 generation (Meskimen 1983; Reddy and Rockwood 1989) at one or two sites. Hence, a preliminary survey has been undertaken to evaluate the existing natural populations. Seeds from nine provenances were collected, and variation in seed characteristics is reported here.

Materials and Methods

Experimental material comprised seeds from nine provenances which were spread over Karnataka and Kerala where sandal grows profusely. These provenances were Mandagadde, Bangalore, Shimoga, Chamarajnagar, Mysore, Mulubagal, Thangli and Honagal in Karnataka and Marayoor in Kerala. All these areas receive moderate rainfall. In each provenance, data were recorded from 15–20 trees. Fallen

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fruits were collected from under each tree, depulped, washed in water and air dried. From each provenance, 100 seeds were taken randomly for seed weight, seed width and seed length. The data was analysed statistically.

Results and Discussion

The results of statistical analysis of three seed characteristics are presented in Table 1 and Figure 1. A large amount of variability exists among different provenances in all seed characteristics studied. Maximum range of variability in seed length was observed in two provenances. They are Mulabagal provenance (10.27–6.25 mm, with a mean of 7.87±0.1) followed by Mandagadde provenance (10.07–7.22 mm, with a mean of 8.02±0.12). For seed width, maximum range was observed in Shimoga provenance (9.29–6.19 mm, with a mean of 7.12±0.09). Maximum range for seed weight was observed in Honagal provenance (0.34–0.11 mg, with a mean of 0.12± 0.005).

The minimum range for seed length was observed in Mysore provenance (8.92–7.45 mm, with a mean of 8.00±0.08). Similarly, the same was observed for seed width in Mandagadde provenance (7.79–6.09 mm, with a mean of 7.16±0.03). The range was minimum for seed weight in provenances of Marayoor (0.22–0.08 mm, with a mean of 0.16±0.003).

Table 1. Seed characteristics in *Santalum album*

Provenance	Seed length (mm)				Seed width (mm)				Seed weight (mg)			
	Range	Mean	SE±	CV%	Range	Mean	SE±	CV%	Range	Mean	SE±	CV%
Mandagadde	10.07–7.22	8.02	0.12	15.0	7.79–6.09	7.16	0.03	3.90	0.32–0.11	0.21	0.004	20.2
Bangalore	9.11–6.62	7.50	0.05	6.8	8.57–5.14	7.03	0.04	6.40	0.30–0.05	0.18	0.005	27.7
Shimoga	9.44–6.78	7.76	0.06	7.4	9.29–6.19	7.12	0.09	11.9	0.27–0.06	0.19	0.005	26.8
Chamara-janagar	9.62–6.71	7.87	0.06	7.4	9.07–6.42	7.08	0.04	6.10	0.26–0.07	0.17	0.003	22.3
Mysore	8.92–7.45	8.00	0.08	10.4	8.30–7.28	7.20	0.10	14.0	0.33–0.15	0.12	0.005	27.0
Mulbagal	10.27–6.25	7.87	0.10	12.2	8.97–6.11	7.11	0.06	8.03	0.24–0.05	0.16	0.004	26.4
Marayoor	9.25–6.84	7.88	0.04	6.1	8.25–6.44	7.30	0.04	5.09	0.22–0.08	0.16	0.003	19.8
Thangli	9.84–6.99	8.00	0.06	7.0	8.46–6.35	7.20	0.04	5.60	0.31–0.11	0.21	0.003	18.0
Honagal	9.38–6.86	7.84	0.05	6.6	8.33–6.25	7.14	0.04	5.70	0.34–0.11	0.12	0.005	29.3

Note: CV = coefficient of variation; SE = standard error.

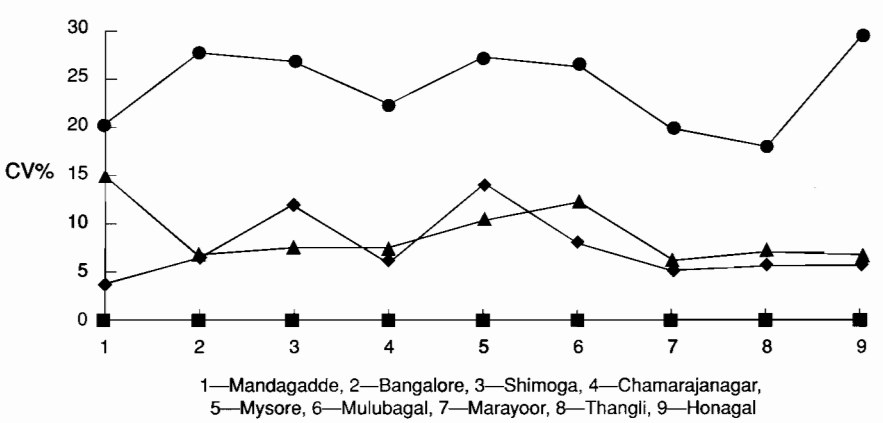

Figure 1. Coefficient of variation among different provenances for different seed characters in sandal. (■ = source, ▲ = seed length, ◆ = seed width, and ● = seed weight).

From the above observations, it can be concluded that a significant amount of geographic variation exists among the provenances that were studied here. No single provenance showed significant variation for the other two seed characteristics studied. This spread of variability across the geographical range through which the species grows indicate a fair degree of genetic dislocation among provenances. It may also be concluded that such linkage disequilibrium among populations indicates that they have a disjunct population structure (Namkoong 1984).

Coefficient of variation (standard deviation/mean) (CV), which helps in comparing relative magnitude of variability present among unrelated populations for different characteristics, was also used in this study. It can be observed that a large amount of variability was present in all the populations for all the characteristics studied. For seed length the amount of variability varies from 6.1% (Marayoor provenance) to 15% (Mandagadde provenance). For seed width the minimum CV was observed in Mandagadde provenance (3.9%) with maximum in Mysore provenance (14%). Similarly, for seed weight the minimum CV was recorded in Thangli provenance (18%) with a maximum of 29.3% in Honagal provenance.

Conclusions

From the above observations, it can be inferred that Mandagadde provenance may be selected for seed length variability. For variability in seed width and seed weight, Mysore provenance and Honagal provenances may be selected respectively for further infusion into base populations for a sandal breeding program.

Patterns of divergence reflect steady-state behaviour of natural populations. Within-stand divergence may actually reflect very recent contact between formerly disjunct stands which may be of localised mating patterns. This pattern of mating ultimately generates local allelic associations. It is particularly revealing when patterns of stand structure are not consistent within the same species. It is significant that no single provenance showed a significant amount of variation for any two characteristics. Although we have not studied intra-provenance variation here, it may be assumed that, due to high CVs observed in these provenances, there may be less intra-provenancial variation. Absence of such localised variation in those provenances indicates spread of variability across the natural populations which may be additive. This observation suggests selecting some phenotypically superior trees for inclusion into the base population of the n-2 generation sandal breeding population.

Acknowledgments

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A Method for Clonal Propagation of Sandal

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Abstract

Vegetative clonal propagation of sandal could be an important technique for producing disease-resistant clones and true-to-type seedlings of plus trees. However, sandal is a difficult material to root, mainly because we do not know how to induce the juvenility which is necessary to initiate root formation. In this paper, an efficient method for large-scale production of ramets (clonally produced propagules) from mature trees is described.

Key words: clonal propagation, juvenility, rooting, vegetative propagation

SANDAL SHOWS WIDE variation in growth, heartwood formation, and oil content within populations. 'Candidate plus' trees showing the desirable characteristics of fast growth, early formation of heartwood, increased oil content, and resistance to spike disease have been detected in reserve forests. However, production of true-to-type seedlings has been inhibited because there is currently no effective method of vegetative propagation other than formation of root-suckers around the mother trees.

Root-suckers can start as shoot primordia from injured or severed roots. Inducing them is one method for sandal regeneration in forests. However, the regenerated plants are confined to an area around the mother tree, and this means that the trees are too crowded. Rao and Srimathi (1976) achieved vegetative propagation of mature sandal by inducing rooting around shoot primordia, and then outplanting the shoot primordia with the original roots. As another option, they also cut off the original root along with the shoot primordia and induced rooting after transplanting.

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However, they did not succeed in propagation by stem cuttings. This is because, juvenility in sandal, an important factor for inducing rooting of stem cuttings, is confined to seedlings only. Vijayakumar et al. (1995) attempted vegetative propagation of 2–12 month seedlings to study the role of juvenility in rooting of stem cuttings. They found that maximum rooting (96 per cent) occurred at the third month. 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 the tree has been 60 percent successful (Vijayakumar et al. 1981).

In the present study, a preliminary investigation is made on the ability of the cuttings from shoots arising as root-suckers to root and sprout.

Materials and Methods

Induction of root-sucker formation

Root-sucker formation was induced on 14 trees 5–15 years old and 10–63 cm in girth. Trenches 15 × 45 × 15 cm were made on 2–4 sides, 30–100cm away from the base of the trees depending upon the size of the trees. The trenches were made during

November 1996, the exposed roots were covered with mulch and kept damp for a week, after which the roots were exposed again.

The circumference of the exposed roots was 2.5–18 cm, but only those up to 12 cm were cut to induce sprouting. On roots more than 12 cm in circumference, a superficial 'V' cut about one cm deep was made to inflict injury.

Rooting of cuttings

Shoots arising from severed and injured roots were harvested at 45–60 days. Two node-cuttings made from them were momentarily dipped in talcum powder or in 4000 ppm IBA in 70% alcohol. Five cuttings were taken from each of ten shoots; seven trees were used altogether. Two node-cuttings were also taken from three different positions on branches of the seven selected trees:

- tip of twigs showing fresh sprouting,
- the green portion of branches behind the apical region, and
- the brown portion of the twigs just behind the green portion.

Ten cuttings were taken from each position, so 210 cuttings were available in all from various positions. The cuttings were planted in vermiculite medium in root trainers, and they were transferred to a mist chamber with intermittent misting to keep the leaf surface damp. The humidity was kept above 85 per cent and temperature at 30±5°C. Hoagland solution was supplied to the cuttings every week as the nutrient.

Results and Discussion

A cluster of sprouts appeared from the edge of the severed roots at 20–45 days; 92 percent of them arose close to the cut edge, and eight percent were at the front of the area on the root close to the injured point. No sprouts emerged from the exposed root portion which remained connected with the tree. Details are in Table 1.

Uniyal et al. (1985) also reported vegetative propagation through root cuttings: secondary roots or thin superficial roots dug out, cut into five cm long pieces, and treated with Seradix B® sprouted and rooted in 30–40 days.

Table 1. Root-sucker formation from sandal trees in trenches

Tree No.	Girth (cm)	No. of exposed roots		No. of sprouts from severed roots		No. of sprouts from injured root	No. of root-suckers surviving > 45 days
		Severed root	Injured root	Tree side	Severed end		
1	42.2	3	1	–	15	–	1
2	49.0	1	1	–	–	–	–
3	24.1	1	1	–	–	–	–
4	23.0	3	1	–	–	–	–
5	53.3	2	1	–	12	–	2
6	44.0	5	2	–	6	–	–
7	43.0	6	3	–	–	–	–
8	16.9	1	1	–	–	–	–
9	19.2	3	1	–	–	1	–
10	20.1	5	2	–	–	2	2
11	32.3	3	1	–	38	3	1
12	16.0	3	2	–	11	–	1
13	12.5	5	2	–	41	2	4
14	10.2	3	4	–	15	4	3
Totals		44	23	–	138	12	14

Survival of shoot primordia

Although clusters of up to 12 shoot primordia arose from a severed root, most of them perished within a few days. One or two primordia which showed comparatively more vigour survived and established. Such healthy root-suckers attained a height of 20–40 cm within 60 days. Less shoot primordia were produced by the injured roots. However, one or two among them also established into healthy root-suckers. Vijayakumar et al. (1981) observed that trenches dug 60–90cm from the main trunk give maximum induction of adventitious shoots. The number of adventitious roots produced varied proportionately to the thickness of root exposed.

In this experiment, it was not possible to monitor the growth of the root-suckers beyond 15–60 days because most of them were harvested to test for rooting ability.

Sprouting of stem cuttings

Stem cuttings from both root-suckers and branches showed sprouting initials after two weeks. However, these initials from branches were temporary, and all the cuttings with brown stems defoliated and decayed after two weeks. After one month, the surviving cuttings were gently uprooted from the vermiculite to see whether rooting had occurred. None of the cuttings from branches rooted, but all the surviving cuttings from root-suckers developed adventitious roots (Fig. 1). Rooting and sprouting in talcum-based IBA

Figure 1. Shooting and sprouting induced on shoot cuttings from root-suckers

treatments was 56 per cent, and 14 per cent in alcohol-based treatments. A few of the cuttings from branches retained their green colour even after four months, but they did not root.

Uniyal et al. (1985) reported that the cuttings with shoots and roots transplanted to pots have established successfully. The rooted cuttings were transferred to earthen pots with soil and host plants for further observation.

As with eucalypts, elite trees of sandal with desirable characteristics can be identified in forests and multiplied vegetatively by rooting cuttings from root-suckers. KFRI has adopted this technique to multiply sandal trees resistant to spike disease growing in the heavily diseased area at Marayoor. However, unlike eucalypts, the technique of clonal propagation of sandal is hampered by two bottlenecks. One is that, in order to produce a large number of juvenile shoots, coppicing of elite sandal trees cannot be practised. From root-suckers, only limited numbers of ramets are available. Moreover, deliberate injury to the roots in order to induce root-sucker formation may result in fungus infection causing butt rot and heart rot.

Acknowledgment

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Isoenzyme Technique—A Powerful Tool in Research on Sandal

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Abstract

Variation in isoenzyme pattern of seed tissue of different provenances in sandal (*Santalum album* L.) is reported. Isoenzyme techniques used in sandal research for characterisation of phenotypes, development of biochemical marker for oil-bearing capacity and usefulness of technique are discussed.

ISOENZYMES ARE MULTIPLE molecular forms of enzyme occurring within the same organism having identical catalytic activity and separable in the field of electric charge. The isoenzyme technique was developed in the late 1950s and used by scientists of various disciplines (e.g. forestry, agriculture and medicine). The first reports of the use of isoenzyme technique in the field of forestry research were in 1970 (van Lear and Smith 1970; McMullan and Ebell 1970). It was utilised all over the world, mainly in these areas:

- identification of clones, hybrids and varieties of forest species;
- early prediction of quality characteristics of forest species;
- sex differentiation (Parthasarathi et al. 1982; Parthasarathi and Angadi 1984); and
- mating patterns and genetic analysis of forest trees in breeding studies.

Isoenzyme work on sandal was carried out in 1977 at the erstwhile Sandal Research Centre. The 'PAGE method' (Davis 1964) with some modifications (Parthasarathi et al. 1985) was used to study the isoenzymes. Leaf and living bark tissues were used as sources of enzyme. Peroxidase (POD), malate dehydrogenase (MDH), and esterase (EST) isoenzymes were studied in leaf tissue; POD was also studied in living bark tissue.

In this paper, variation in POD isoenzyme pattern of seed tissue of various provenances in sandal (*Santalum album* L.) and the usefulness of isoenzyme technique in forestry research in general, and sandal research in particular, is reported.

Materials and Methods

In the present study, the following eight potential sandal-bearing areas were deemed to be provenances:

- Mandagadde, Thangli and Bangalore (in Karnataka state);
- Chitteri and Inner Javadi (Tamil Nadu);
- Marayoor (Kerala);
- Koraput (Orissa); and
- Seoni (Madhya Pradesh).

Seeds were collected from these provenances during the fruiting season. Seed and foliar tissue were studied to confirm provenance status and to find the genetic distance between them.

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The procedure used for isoenzyme study was similar to that already described earlier (Parthasarathi et al. 1985) except that 0.1% ascorbic acid containing 0.03% hydrogen peroxide solution was used as the enzyme-extracting medium for POD. POD activity was either nil or very weak when extraction was carried out with ascorbic acid alone or other media as reported earlier (Egerton-Warburton 1990). Twenty individual seeds were used for each provenance. Each seed was macerated with 1.0 mL of extracting medium and 0.1 mL of enzyme solution was used for electrophoresis. POD and MDH isoenzymes in the seed tissue of Marayoor and Mandagadde provenances were studied.

Results and Discussion

In case of POD, 13 maximum and three minimum number of bands were recorded in Mandagadde provenance; nine maximum and two minimum number of bands were recorded in Marayoor provenance.

POD activity was observed at seven and eight loci in Marayoor (Figs 1(a) to 1(g)) and Mandagadde (Figs 2(a) to 2(g)) provenances, respectively.

All the seven loci in the POD activity of seed tissue of Marayoor provenance were found to be polymorphic. The number of alleles at each locus are as follows.

Locus	Number of alleles
POD-1	3
POD-2	2
POD-3	5
POD-4	7
POD-5	2
POD-6	6
POD-7	2

Of the eight loci observed in Mandagadde provenance, POD-1 was found to be monomorphic. Activity of POD at other loci is as follows.

Locus	Number of alleles
POD-2	5
POD-3	3
POD-4	3
POD-5	6
POD-6	10
POD-7	7
POD-8	8

In case of MDH, only three bands with monomorphic pattern were recorded in all the samples both in Marayoor and Mandagadde provenances (Fig. 3).

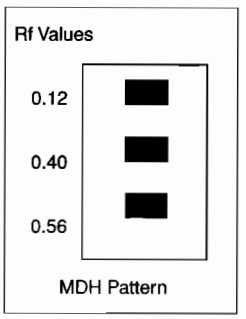


Figure 3. Zymograms with schematic representation of phenotypes for malate dehydrogenase isoenzyme (MDH) in the seed tissue of sandal (*Santalum album* L.) of Marayoor and Mandagadde provenances.

Application of Isoenzyme Techniques in Other Areas of Sandal Research

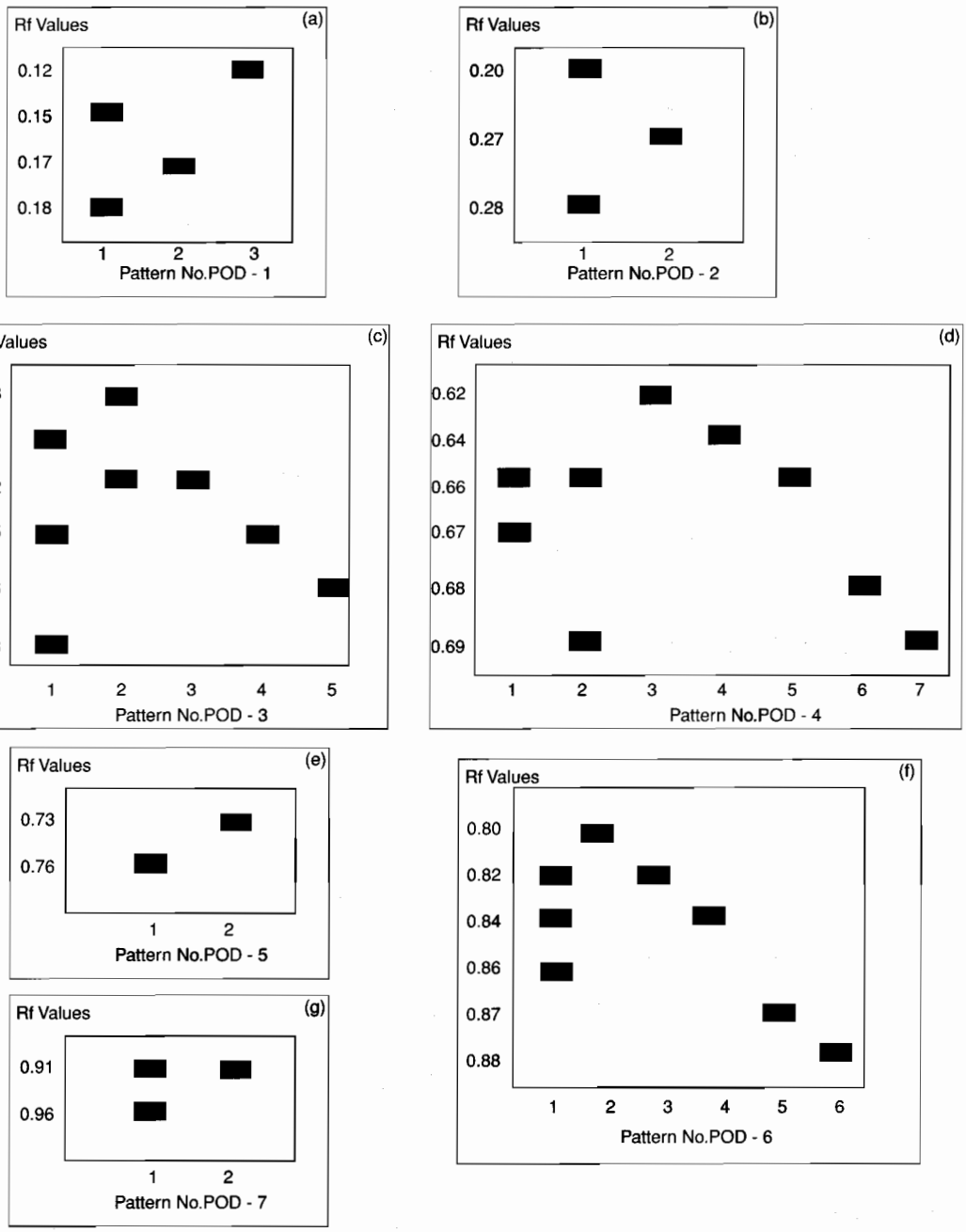
Characterisation of phenotypes of sandal trees at cell level

Kulkarni and Srimathi (1980) reported the occurrence of morphological variations such as ovate, elliptic, lanceolate and linear patterns in sandal leaves. Studies by Parthasarathi et al. (1985) showed that:

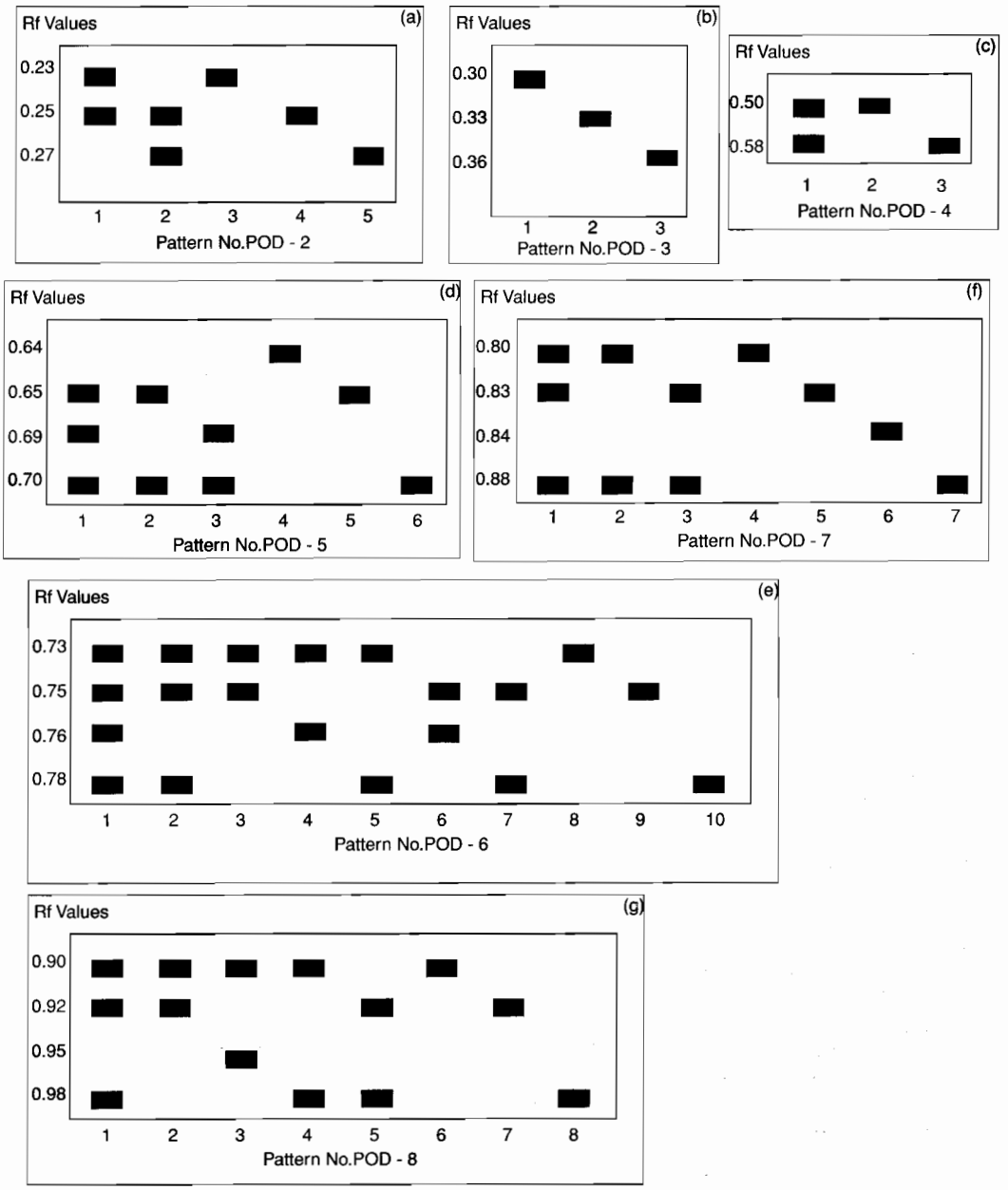
- there are characteristic differences between the sandal leaf types in their pattern of isoenzymes of POD and MDH, both at vegetative and flowering stages;
- three sub-types exist in the sandal plants with big ovate leaves; and
- the sandal plants with normal ovate wavy and normal ovate non-wavy leaves are genetically very close.

Development of biochemical markers for oil-bearing capacity in sandal

Unlike leaf tissue, living bark tissue showed no variation in isoenzyme pattern between vegetative and flowering stages. Parthasarathi et al. (1986) reported that the specific POD enzyme in living bark tissue has shown strong negative correlation with the oil content in the heartwood of mature sandal plants. This relationship can be put to use in forecasting oil-bearing capacity of a sandal plant, even at an young age.



Figures 1 a–g. Zymograms with schematic representation of phenotypes for peroxidase isoenzyme in the seed tissue of sandal (*Santalum album* L.) of Marayoor provenance



Figures 2 a–g. Zymograms with schematic representation of phenotypes for peroxidase isoenzyme in the seed tissue of sandal (*Santalum album* L.) of Mandagadde provenance

Diagnosis of spike disease at an early stage

Angadi and Ananthapadmanabha (1988) observed variations in isoenzyme pattern in the sandal plants affected with spike disease. The change in the pattern of POD and MDH isoenzymes in the diseased plant could be used to confirm the presence of spike disease in doubtful cases, and even at an early stage of onset of disease.

Diagnosis of deficiency symptoms of trace elements in seedlings in the nursery

Kamala et al. (1986) reported changes in the multimolecular forms of POD associated with deficiency of trace elements in sandal seedlings. The change in the pattern of POD isoenzyme due to deficiency of particular trace elements precedes the visible symptoms of disease in the seedlings. It is helpful in diagnosing the symptom of a deficiency of a particular element and restoring normalcy by providing that trace element at the proper time.

Identification of provenances and estimation of genetic distance

The analysis of isoenzyme results is of great use in studying provenances (Egerton-Warburton 1990) and estimating genetic distance (Brand 1994) between sandal plants within the same population and in different populations.

Conclusion

Isoenzyme studies help in:

- identifying provenances,
- characterising phenotypes,
- developing a biochemical marker for oil-bearing capacity, and
- diagnosing early-stage spike disease and trace-element deficiency.

In spite of its usefulness, isoenzyme technique is very much dependent on proper extraction of enzyme, its nature and zymogram segregation. However by carefully following the procedure, and with experience, these problems can be surmounted.

Acknowledgments

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The authors are thankful to Dr K.S. Rao, Director, Institute of Wood Science and Technology, for the interest evinced in the study, and to Sri G. Ravikumar for his help in preparing zymogram sheets.

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Floral Biology and Breeding Systems in Sandal, *Santalum album* L.

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Abstract

The shape and size of sandal flowers vary. Most flowers are campanulate, and some are cylindrical and obconical. Size and shape of fruit and seed also vary markedly. Globose seeds predominate, and ovoid seeds also occur. The average time from bud initiation to complete flower development is 34 days. The reproductive cycle from bud initiation to fruit ripening requires 110–140 days. The anthesis occurs at 7.00 am with a peak period at 8.00 am. The apparent pollen fertility was 80–94%. Pollen germination in 20% sucrose solution is 52%, and the pollen-tube is 357μ long. The breeding system studies showed that sandal, like many other tree species, has an ambivalent reproductive system, and can be designated as an 'often cross-pollinated' species. Natural selfing at the 5% level (range 0–12%) occurs in this species, and the inbreeding coefficient is estimated at 0.025. The maximum seed output was 7586, and reproductive capacity was 5370 in this study.

IN ANY TREE improvement program, knowledge of floral biology and breeding systems are essential to raise seed orchards.

Considerable literature on floral biology is available on forest trees like willow, pine, poplar, spruce, douglas fir, and teak (Faulkner 1975; Wright 1976); *Bombax cieba* (Khosla et al. 1982); *Butea monosperma* (Srivastava 1983); *Moqhania chappar* (Srivastava et al. 1987); and *Eucalyptus* spp. (Potts et al. 1995). However, there are few such studies on sandal; the only data available are descriptions of floral parts, flowering seasons and embryology (Troupe 1921; Iyengar 1937; Bhatnagar 1965). Hence, a detailed study on floral biology in *Santalum album* is recorded here.

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Methods and Materials

Various aspects of floral biology on which observations were recorded are detailed as follows.

Morphology of reproductive parts

Fifty bud, flower, fruit and seed dimensions were measured for each sandal type, including length and breadth of perigonal tube, anther and filament, ovary, style and stigma, fruit and seed.

Flower bud and fruit development

The visible emerging buds on inflorescences of ten different twigs were tagged. The time taken for various developmental stages from initiation of bud to ripening of fruit were recorded. For each stage, the length and diameter of ten floral buds and fruits were measured.

Anthesis and anther dehiscence

Anthesis was observed by tagging ten fully developed floral buds due to open next day. These floral buds were observed at hourly intervals from 5.00 am

one day to 7.00 pm the next day. The opening of the floral bud is manifested by four longitudinal splits on the mid-portion of the perianth tube, and widening towards the apex. Temperature and relative humidity were also recorded. The observations were carried out for five days. Anther dehiscence was observed immediately after the anthesis with the help of a hand lens (10×).

Stigmatic receptivity

Receptivity was studied by visual observation of the stigma for five days. In order to determine the duration of stigmatic receptivity, observations on ten floral buds and opened flowers were made for:

- 'One day before opening of flower' (OBOF),
- 'On the day of opening of the flower' (ODOF),
- 'One day after opening of the flower' (OAOF),
- 'Two days after opening of the flower' (TAOF),
- 'Three days after opening of the flower' (THOF), and
- 'Four days after opening of the flower' (FAOF).

Pollen studies

Studies on pollen morphology, apparent pollen fertility and viability were made under laboratory conditions. The pollen shape and size was determined for six sandal types following Radford, et al. (1974). Apparent pollen fertility was studied by staining the pollen grains in 'Muntzing's solution'. Pollen germination studies were conducted following Kirby and Stanley (1976).

Breeding systems (incompatibility and compatibility studies)

Five trees were marked for studies on breeding systems. Some 360–1283 flowers were examined for open pollination, selfing, cross pollination, and apomictic mode of reproduction following Radford et al. (1974).

Estimation of natural selfing

Estimation of the frequency of self-pollinated seeds and seedlings was made for 18 sandal trees. Four-hundred seeds in four replications of 100 each were tested by decoating the seedcoat of filled and unfilled seeds. The seeds with full endosperm were considered to be full seeds, and those without endosperm or shrivelled endosperm were considered to be empty seeds. Following germination of seeds, detection of achlorophyllous mutant seedlings per tree was recorded. Estimation of the natural selfing and inbreeding coefficient was made following Sorenson (1973) and Wright (1976).

Seed output and reproductive capacity

Seed output and reproductive capacity were estimated following the procedure outlined by Misra (1968).

Results

Morphology of flower, fruit and seed

The results of studying the morphology of seven sandal types are shown in Table 1 and Figure 1.

The flowers were purplish-brown, minute, odourless tetramerous, actinomorphic, bisexual, perigynous and regular; they were borne in auxiliary or terminal cymose panicles. The plants were also characterised by bearing tri-, penta-, and hexamerous flowers in the same tree.

The shape of the perianth tube, which is either campanulate, obconical or cylindrical (Fig. 1), is more pronounced in the bud than the flower. The cylindrical flower was 5.5 mm long and 3.0 mm wide; the obconical flowers were small, 3.5 mm long and 2.5 mm wide. However, the campanulate flowers were larger, up to 4.5 mm long and 3.5 mm wide. Stamen and pistil size varied according to perianth tube dimensions. There were as many epiphyllous stamens as perianth lobes; however, stamen number and position differed in penta- and hexamerous flowers. The capitate stigma was usually trilobed, but 4–5-lobed stigmas were also observed. In heterostylous flowers the style length is 1.65 mm.

The fruit size and shape also varied considerably. The fruits derived from campanulate flowers were mostly sub-globose to globose. The fusiform shape of fruit was associated with cylindrical or campanulate-cylindrical flowers. The big globose fruit with a round base measured 1.4 cm in length and 1.3 cm in breadth, while the small globose fruit measured 1.0 cm in length and 0.9 cm in width. The length of globose or fusiform fruits with an elongated base is greater than that of fruits with a round base.

The fusiform fruit with a round base was 1.1 cm long and 0.9 cm wide; that with an elongated base was 1.6 cm long and 1.1 cm wide. The perigonal scar was 2–6 mm in diameter. It was observed that the compact or wide perianth base was always related to an inferior ovary (2 mm diameter, narrow perigonal scar) or a semi-superior one (4–6 mm, wide perigonal scar).

The globose fruits invariably produced globose seeds, while the fusiform fruits produced ovoid seeds with a pointed apex. The ovoid seeds were 0.78–9.0 cm long, and 0.50–0.53 cm wide. The globose seeds were 0.4–0.9 cm long and 0.4–0.85cm wide.

Table 1. Mean values of floral parts, fruits and seeds

Plant Code (Sample)	Bud Size		Flower Size		Stamen size (mm)				Ovary	Pistil size (mm)				Stigma	Shape of Perianth tube	Fruit shape	Fruit size (cm)		Perigonal ring diameter (mm)		Seed Shape	Seed size (cm)	
	L	B	L	B	Filament		Anther		L	Stylar (L)				L									
	(mm)	(mm)	(mm)	(mm)	L	B	L	B	(mm)	Long	Medium	Short		(mm)			L	B	Range	Average		L	B
A	3.50	2.00	5.50	3.00	1.20	0.20	0.40	0.40	1.60	1.60	1.50	1.45		0.30	Cylindrical	Fusiform, round base	1.1	0.9	2–3	2.00	Ovoid, pointed apex	0.78	0.50
B	2.50	2.00	4.00	3.00	1.10	0.20	0.40	0.40	1.50	1.60	1.50	1.35		0.35	Cylindrical Campanulate	Fusiform, elongated base	1.6	1.1	2–3	2.50	Ovoid, pointed apex	0.90	0.53
C	2.00	1.50	3.50	2.50	0.50	0.20	0.40	0.30	1.40	1.50	1.40	1.30		0.20	Obconical	Globose	1.0	0.9	2–3	2.20	Globose	0.40	0.40
D	2.50	2.00	4.00	3.00	1.10	0.20	0.40	0.45	1.40	1.65	1.50	1.30		0.30	Campanulate	Globose, elongated base	1.4	0.9	2–3	2.50	Globose	0.60	0.60
E	2.50	2.50	4.50	3.50	1.00	0.20	0.40	0.45	1.40	1.65	1.50	1.35		0.35	Campanulate	Globose, round base	1.2	0.9	3–6	4.50	Globose	0.75	0.75
F**	2.00	1.50	2.00	1.50	–	–	–	–	–	–	–	–		–	Campanulate*	–	–	–	–	–	–	–	–
G	2.50	2.50	4.50	3.50	1.30	0.30	0.50	0.45	1.50	1.60	1.50	1.40		0.40	Campanulate	Globose roundbase	1.4	1.3	2–4	4.00	Globose	0.90	0.85

* = Malformed Flower
** = Completely sterile plant
L = Length
B = Breadth

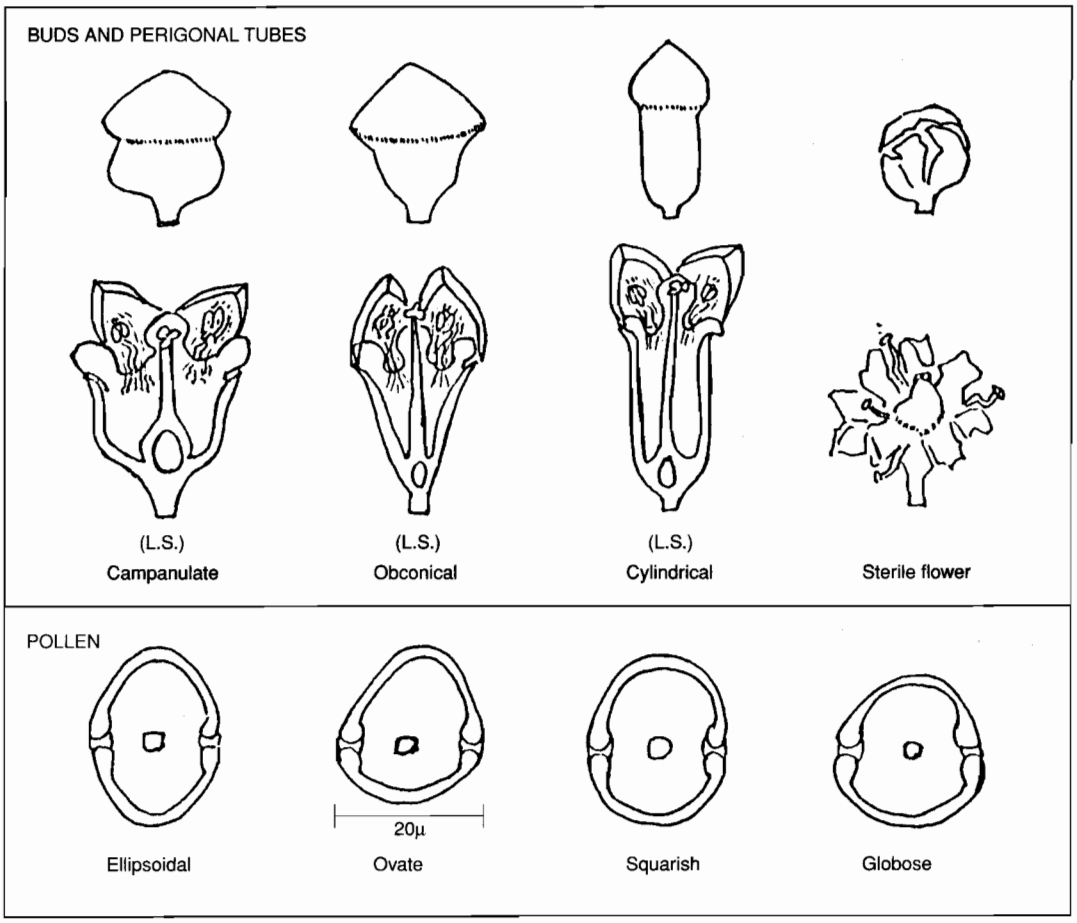

Figure 1. Floral variation in *Santalum album* L.

In one tree an abundance of complete sterile flowers in both seasons was observed. Further, the malformed flowers were observed opened in the bud stage itself. The mature minute flowers remained pale green and showed flat bulged pistil having no demarcation of ovary, style and stigma. The stamens were malformed with wiry filaments having pointed anthers at the tip. This tree did not produce any fruits and was completely sterile.

Flower and fruit development

The total time required for the inflorescence to complete blooming was 20–45 days depending on the length of the inflorescence and the number of cyme bunches per inflorescence. The flowering pattern was asynchronous and the duration required for completion of blooming for each inflorescence varied. Each day, only 2–8 flowers per bunch opened in the branchlet of the inflorescence.

The developmental phases from bud-initiation to fruit-ripening are expressed as 16 stages as follows. (Details are shown in Table 2.)

1. Minute, globular, greenish-white bud initials appear in 10–14 days.
2. Bud elongates longitudinally, becomes pale green in 10–14 days.
3. Fully developed buds show constriction in the mid-portion of the perianth tube in 5–6 days.
4. The pale green buds open and anthesis starts in 1–2 days with a slit in the upper portion of the perianth.
5. Completely opened flowers show erect perianth lobes, and become white or pale green. Anther

dehiscence is completed. The anther, style and stigma are greenish-white.

6. Perianth colour changes to purple as flower becomes old.
7. Flower colour turns to brown. The upper perianth is star-shaped with reflexed lobes. This is the fertilisation and maturation stage. Anther and stigma become yellowish-brown, and style reddish.
8. Flower turns dark brown and perianth lobes are completely reflexed. It is an old flower.
9. The dried upper portion of the perianth starts separating from the mid-portion of the ovary along with anthers, while the lower perianth is intact.
10. Young fruit starts developing with persistent perianth.
11. The developing fruit is green with persistent style. The perianth falls off.
12. The ovary is encased by lower perianth with a visible perigonal ring at the top portion of the fruit formed by the fall of the upper perianth. The fruit is cup shaped and green.
13. Fruit is bigger and still green.
14. Fully developed fruit is globose to sub-globose and green.
15. Fruit matures and changes from green to red.
16. The ripe fruit is fleshy, and changes from red to purplish-black during abscission.

The average period required from the initiation of the bud to the complete development of flower and anthesis was 34 days. The complete development of fruit took 80–100 days. The reproductive cycle from bud-initiation to fruit-ripening required 110–140 days.

Time of anthesis and anther dehiscence

Anthesis started at 7.00 am and continued until 12 noon (Table 3). Rarely, 2–6% anthesis was also recorded between 3.00 and 5.00 pm. Maximum anthesis (54%) occurred at 8.00 am; minimum (2%) at 11.00 am.

Anther dehiscence occurred either at the fully opened or partially opened bud stage (Table 3). During the process of dehiscence, all four anthers longitudinally split and the pollen appeared as a fine yellowish wet sticky powdery mass. Numerous hairs were observed near the base of the filaments which were characterised by dense cytoplasm and large nucleus.

Stigmatic receptivity

After 4–6 hours of anthesis, the stigma became slightly receptive exhibiting a shiny sugary drop on the stigmatic surface. The proportion of stigmatic receptivity before and after flower opening is shown in Table 4. Normally about half of the stigmatic receptivity had been observed one day after the opening of the flower.

Table 2. Chronology of flower and fruit development.

Stage	Time taken in days (Range)	Size (mm)	
		Length	Breadth
Bud			
01 st	10–12	1.0	1.0
02 nd	12–14	2.0	1.2
03 rd	5–6	2.5	2.0
04 th	1–2	3.5	3.0
Flower			
05 th	0.5–1	4.0	3.0
06 th	1–0	4.0	3.0
07 th	1–0	4.0	3.0
08 th	1–2	4.0	3.0
09 th	2–3	4.0	3.0
Fruit			
10 th	2–3	0.5	1.0
11 th	6–8	0.5	1.0
12 th	12–14	4.5	3.5
13 th	15–20	7.0	6.0
14 th	18–20	11.0	10.0
15 th	10–12	11.0	10.0
16 th	15–20	11.5	10.5

Pollen studies

The pollen grains were ellipsoidal, ovoid and globose (Figs 1 & 2 and Table 5). The pollen appear triangular in cross section. The pollen grains were uninucleate and three-zonoporate with thin intine and smooth thick exine. Pollen length was 25.9–27.7 μ; breadth was 22.2–23.5 μ. Apparent pollen fertility was 80.72–94.82 per cent (Fig. 2). Pollen germination studies under different concentrations of sucrose solution after 12 hours of incubation revealed that the maximum germination (52%) occurred in 20% sucrose solution, and the minimum germination (6.6%) in one percent solution (Table 6). The pollen grains were invariably monosiphonous. A maximum pollen tube length of 357 μ was recorded in 20% sucrose. In lower concentrations, however, only 3.2–6.4 μ pollen tube outgrowth is recorded (Fig. 2). Wide variation in pollen size, viability and germination is observed in seven sandal types (Tables 5 and 6).

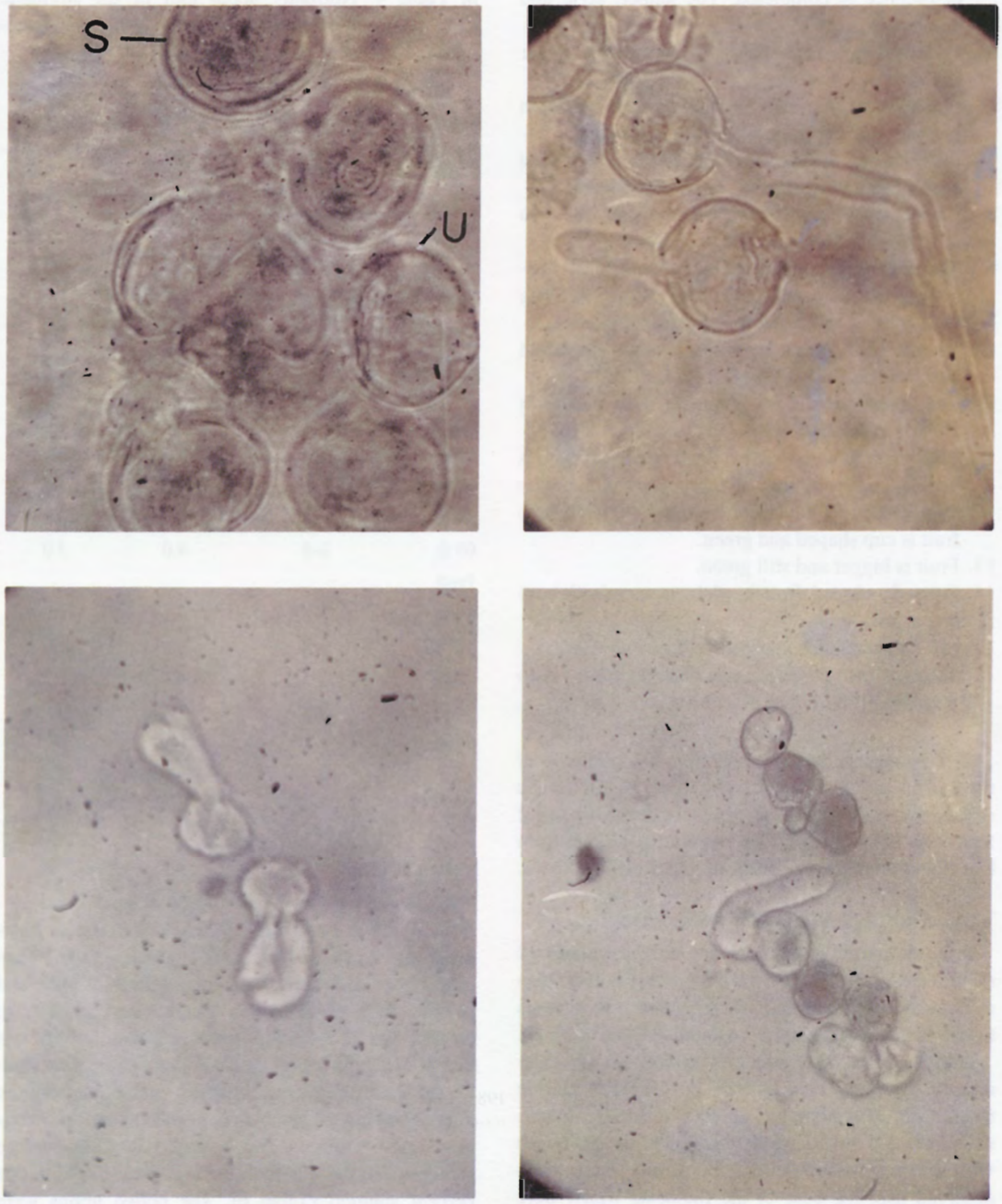

Figure 2. Top left: Stained (S) and unstained (U) pollen grains (× 450)
Top right: Germinated pollen grains (× 450)
Bottom: Germinated pollen grains in 20% sucrose solution (× 225).

Table 3. Anthesis and anther dehiscence

Temperature (°C)		Relative humidity (%)		Morning (hours)							Noon (hours)					Evening (hours)		
Min	Max	A	B	05.00	06.00	07.00	08.00	09.00	10.00	11.00	12.00	13.00	14.00	15.00	16.00	17.00	18.00	19.00
20.30 **	28.0	91	72	–	–	10	40	10	10	10	10	–	–	–	10	–	–	–
19.30 **	27.9	89	69	–	–	10	40	30	10	–	–	–	–	–	10	–	–	–
20.50 *	28.6	80	64	–	–	10	80	10	–	–	–	–	–	–	–	–	–	–
19.50 **	27.8	88	68	–	–	10	50	10	10	–	–	–	–	–	10	10	–	–
20.80 *	28.6	78	68	–	–	10	60	20	10	–	–	–	–	–	–	–	–	–
Average																		
20.08 **	28.16	85.5	68.2	–	–	10	54	16	8	2	2	–	–	–	6	2	–	–

A = Relative humidity at 08–30 hours.
B = Relative humidity at 17–30 hours.
* = Clear day and sunny morning
** = Cloudy day and foggy morning

Table 4. Stigmatic receptivity

Temperature (°C)		Relative humidity (%)		Proportion of stigma receptive from 10.00 am to 11.00 am (%)					
Min	Max	A	B	OBOF	ODOF	OAOF	TAOF	THOF	FAOF
20.30	28.0	91	72	–	20	70	10	–	–
19.30	27.9	89	69	–	10	60	20	10	–
20.50	28.6	80	64	–	30	50	20	–	–
19.50	27.8	88	68	–	30	30	30	10	–
20.80	28.6	78	68	–	30	40	30	–	–
Average									
20.08	28.16	85.5	68.2	–	24	50	22	4	–

OBOF = One day before opening of the flower (before anthesis)
ODOF = On the day of opening of the flower (at anthesis)
OAOF = One day after opening of the flower (24 hours)
TAOF = Two days after opening of the flower (48 hours)
THOF = Three days after opening of the flower (72 hours)
FAOF = Four days after opening of the flower (96 hours)

Table 5. Morphology, viability and germination of pollen

Plant code Sample	Pollen size (m)		Pollen shape	Pollen fertility (%)	
	Length	Breadth		Staining	Germination**
A	26.6 ± 1.5	22.9 ± 1.5	Ellipsoidal, ovoid, globose	84.45	45.14
B	27.1 ± 4.1	23.1 ± 1.0	Ellipsoidal, ovoid, globose	93.95	58.00
C	26.8 ± 3.9	22.4 ± 1.7	Ellipsoidal, ovoid, globose	80.72	40.89
D	26.7 ± 1.8	22.2 ± 1.3	Ellipsoidal, ovoid, globose	90.51	54.90
E	27.7 ± 1.4	22.6 ± 2.7	Ellipsoidal, ovoid, globose	88.98	31.00
F*	–	–	–	–	–
G	25.9 ± 2.3	23.5 ± 1.7	Ellipsoidal, ovoid, globose	94.82	60.43

* = Sterile plant
** = Solution (standardized) in 20% sucrose

Table 6. Pollen germination

	Concentration of sucrose solution (%)							
	1	2	5	10	15	20	25	30
Germination (%)	6.6	12.3	15.9	30.5	38.6	52.0	44.6	18.4

Breeding systems

The data on fruit set under different modes of pollination are presented in Table 7. Observations on natural open pollination showed that the flowers were pollinated by many diverse pollinators such as bees, flies, ants, butterflies and beetles. In natural open pollination, 18.8 percent fruit set was recorded. Cross pollination showed nearly nine percent fruit set. Selfing by bagging resulted in the lowest fruit set of 0.45 percent. Pollination by hand for selfing gave moderate results with 4.3 percent fruit set. Apomixis is found to be totally absent as flowers emasculated and bagged did not yield any fruit.

Estimation of natural selfing

Out of the eighteen trees, four trees segregated into normal seedlings and achlorophyllous seedlings. The estimation of natural selfing was 0–12 per cent, with a mean of five. The occurrence of empty seeds was 2–8 percent with a mean of four. For 18 half-sib families, an 0.025 inbreeding coefficient (F) was estimated.

Seed output and reproductive capacity

Marked variation in annual seed output and reproductive capacity was recorded for seven sandal types (Table 8). Seed output of 1306, 4238, 144, 982, and 8429, and reproductive capacity of 718, 2346, 378, 333, and 5370 were recorded for six sandal types: linear, lanceolate, ovate (small), ovate (big) and elliptic. The highest seed output of 1.73 kg (10 557 seeds) and reproductive capacity of 596.3 was recorded for one tree which flowered throughout the year.

Discussion

Floral biology of any tree species is variable depending on variety, habitat, nutrition and environmental conditions.

The present findings revealed that maximum blooming occurred at the top branches facing the sunlight. Anthesis is influenced by atmospheric temperature and humidity, normally occurring between 8.00 am and 9.00 am. Similar results are also reported for *Butea monosperma* (Srivastava 1983).

In sandal the pollen grains shed at the two-celled stage (Bhatnagar, 1965) and were uninucleate (Iyengar 1937). Our studies confirm the above observations. Palliwal (1956) observed bi-celled pollen grains germinating frequently in situ due to the shiny secretions of the unicellular glandular hairs before opening of the flower. However, the germination of pollen grains on the stigma does not take place readily (Iyengar 1937), which is an indication to check self pollination, and this is also confirmed by our studies.

Table 7. Breeding system data.

Mode of pollination	Number of flowers observed	Flowers emasculated	Flowers pollinated	Fruit set (%)	Fruit maturation (%)
Open pollination:					
a) natural—open pollination	424	–	–	22.40	18.80
b) open—natural pollination after emasculation	1113	1113	–	17.87	12.90
Self-pollination:					
a) selfing (bagging)	1283	–	–	0.93	0.45
b) selfing (hand-pollination)	625	625	484	8.67	4.33
Cross-pollination	625	625	417	12.70	9.40
Apomixis	360	360	–	–	–

Wide variations in pollen size have been recorded in the present study. Our studies confirm the pollen measurements of 29 × 23 μ made by Bhatnagar (1965). It is interesting to note that the pollen size of Hawaiian species of *Santalum* (Stemmermann 1977) differ from *S. album*. Pollen germination studies revealed a high amount of sterility in the seven sandal types studied. The maximum length of pollen tube growth recorded in *S. album* in the present investigation is 357 μ. In cucurbits such pollen tube growth has also been recorded by Vasil (1960). Germination of pollen in different concentrations of sucrose exhibited marked variation, which may be due to nutritional variation, genetic constitution, or both. The results corroborate those reported by Singh (1962) in peaches.

Breeding systems

There is no unanimity as far as the pollination type is concerned in sandal. Badami and Venkata Rao (1930) and Muniyappa et al. (1980) reported that sandal is highly cross-pollinated crop. On the other hand, Sindhuveerendra and Sarma (1991) reported it as a self-pollinated crop. The present studies revealed that sandal adopts an ambivalent reproductive strategy of both self- and cross-pollination.

Radford et al. (1974) listed characteristics of autogamous and xenogamous plants of which 14 and 13, respectively, tally with *S. album*. The tendency for outbreeding in sandal is reinforced by asynchronous flowering, insect pollination, heterostyly, and self-incompatibility. However, inbreeding is aided by floral adoption where the floral structure is more suited for autogamy. Nevertheless, from the bagging and emasculation experiments, it is shown to be a cross-pollinated species.

Further staggered and abundant flowering also seem to support outcrossing by eliminating the competition for the pollinators. Bawa (1977) postulated that without this heterogeneity in the flowering pattern and long duration of flowering, there would be little interplant movement of pollinators, and gene flow would be reduced. Hence, it is not surprising that, like many other tree species, sandal has an ambivalent reproductive system that allows effective operation of both breeding mechanisms; therefore, *S. album* now can be regarded as an 'often cross-pollinated' species. Similarly, species of *Eucalyptus* (Davis 1969; Eldridge 1970), *Tectona* (Bryndum and Hedegart 1969), *Alnus* and *Betula* (Hagman 1975) were cross-pollinated, despite sporadic selfing. In fact, the capacity for selfing is an evolutionary advantage in the survival of populations especially when out-crossing fails. We concur with the above observations.

Asexual reproduction in *S. album* occurs through root suckers. However, when sexual reproduction fails, asexual reproduction is the only method by which the species can survive. Radford et al. (1974) opined that although the mechanism of sexual reproduction ensures variability and evolutionary potential through recombinations, it is the asexual reproduction which has a strong survival value. This is confirmed in the present study.

Estimation of natural selfing

In the present study the occurrence of five percent achlorophyllous seedlings and empty seeds in 18 half-sib progeny might be due to the recessive homozygosity of the deleterious genes. Achlorophyllous seedlings and empty seeds have also been reported in *Pinus caribaea* (Venator 1976), *Pinus sylvestris* (Johnson 1976), *Gmelina arborea* (Bolstad and Bawa 1982) and *Azadirachta indica* (Kulkarni 1987). Further, the authors postulated that the homozygous recessive action of deleterious genes following selfing (inbreeding) results in achlorophyllous seedlings and empty seeds.

Table 8. Seed output and reproductive capacity data

Plant code	Average number of:			Flowers in an inflorescence (Y×Z)	Proportion of flowers producing fruits (W%)	Seed output (estimated)		Seed output (by collection)		Germination (%)	Reproductive capacity	
(sample)	Flowering shoots per tree (X)	Cyme branches per inflorescence (Y)	Flowers per cyme (Z)			No.	kg	No.	kg		Estimated	Observed
A	2005	7	4.14	29 F = 58145 S = 9 kg	4.6	2672.8	0.44	1306	0.21	55	1471.7	718.3
B	2436	7	3.71	26 F = 39353 S = 11 kg	6.7	4238.6	0.7	3128	0.52	75	3178.9	2346
C	1711	6	3.83	23 F = 39353 S = 7 kg	3.7	1443.4	0.24	1052	0.17	36	519.6	378.7
D	2896	10	2.10	21 F = 60816 S = 10 kg	7.2	4378.8	0.73	4062	0.67	80	3503	3249.6
E	3390	9	6.44	58 F = 196620 S = 32 kg	0.5	982.4	0.16	666	0.11	50	491.2	333
F *	1560	2	5.00	10 F = 15600 S = 2.6 kg	–	–	–	–	–	–	–	–
G	3090	9	4.33	39 F = 120510 S = 20 kg	7.0	8429.4	1.4	7586	1.26	70	5900	5370

* = Sterile Plant
F = Total estimated flowers per tree (X.Y.Z)
S = Hypothetical seed yield (kg) (6000 seeds weigh 1 kg)

It is of interest to note that Sorenson (1973) estimated seven percent natural selfing in Douglas Fir and opined that an inbreeding coefficient (F) of 0.1 resulted in a depression of five percent in height and six percent in volume. We recorded an inbreeding coefficient of 0.025 in *S. album*, and further studies on inbreeding depression will be rewarding.

Seed output and reproductive capacity

Despite the hypothetical seeds yield of 32 kg per tree per year, only 1.26 kg has been recorded in the present study. The detailed studies on flower and fruit development and fruit set showed that only a small proportion of flowers eventually mature to fruits; this may be due to factors such as lack of pollination and fertilisation, pollen sterility, and incompatibility. Further, many flowers and fruits abort. In terms of reproductive strategy, these wastes are the 'biological costs'.

Reproductive capacity of plant species is very variable. For example, Kamaluddin (1987) reported a seed output of 54 364 and reproductive capacity of 33 251 in *Bombax cieba*. Maximum seed output of 7586 and reproductive capacity 5370 is reported for *S. album* in the present study; variation is observed in seven biotypes, so may be due to genetic differences. Seed output and reproductive capacity have a direct bearing on clonal seed orchards. Selection of clones with high seed output coupled with other economic traits is therefore suggested for production of 'quality seeds' in large quantity.

There is no record of sterility in *S. album* except for spike-affected plants where the reproductive phase changes to vegetative phase due to the pathological condition. In the present investigation, one case of genetic sterility was observed and is a new record for sandal. This sterility could be due to natural hybridisation between extremely unrelated parents.

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Leaf Development Studies in *Santalum album* L. (Sandal)

H.D. Kulkarni* and M. Muniyamma†

Abstract

Leaf development in *Santalum album* is homoblastic. The time required for completion of leaf development is about three months, and it follows a sigmoid growth curve pattern. There is a gradual increase in dry-weight associated with chlorophyll N, P, K, Ca and S content.

THE PHENOMENON OF leaf-shedding and flushing enables the plant to grow continuously until senescence. Several changes take place during the development of leaf. In order to record such changes in *Santalum album* L., a study was undertaken to determine the time required for leaf development and associated characteristics like chlorophyll and major nutrients.

Materials and Method

Leaf margins on 25 sprouting leaves on 20 different trees were traced on graph paper at regular intervals, and length, breadth and area were recorded. More leaves of the same node and similar age and dimension were used for determination of: green and dry weight, chlorophyll, nitrogen, phosphorous, potassium, calcium and sulfur. One gram of leaf was taken and analysed for estimation of various components.

For chlorophyll content, the method described by Harborne (1973) was used. Nitrogen estimation was done by the 'Kjeldahl wet-digestion method' (Piper 1967). Phosphorous content of leaf extract was determined by the 'vandomolybdophosphoric acid yellow colour method' using a blue filter (Koening and Johnson 1942). The potassium content of the leaf sample was determined by the 'EEL flame photometer method'. Sulfur content was estimated by the 'turbidometric method' using a blue filter (Bardsley and Lancaster 1960). Calcium content of leaf was determined by the 'versinate method' (Piper 1967).

Results

Maturation of the leaf from its inception took three months (Fig. 1 and Table 1). In the initial stages, the leaf was delicate and yellowish or pink in colour. As the development stages advanced, the leaf colour changed from yellowish-green to dark green. During these three months of development, the leaf length increased from 1.24 to 7.46 cm, and the breadth from 0.38 to 3.30 cm. The length-to-breadth ratio was initially high (3.26) due to higher leaf length, but later stabilised at 2.0–2.24. The leaf area gradually increased from 27 to 1250 mm^2.

Associated with the increase in leaf lamina, other parameters have also increased: green weight (12.13 to 263 mg); dry weight (2.22 to 110.9 mg); internodal distance (2 to 23 mm); chlorophyll 'a' (1.47 to 8.3 mg/g); chlorophyll 'b' (1.65 to 4.8 mg/g); nitrogen (1.26 to 2.3%); phosphorous (0.173 to 0.424%); potassium (0.94 to 2.8%); calcium (0.18 to 2.86%); and sulfur (0.312 to 0.95%).

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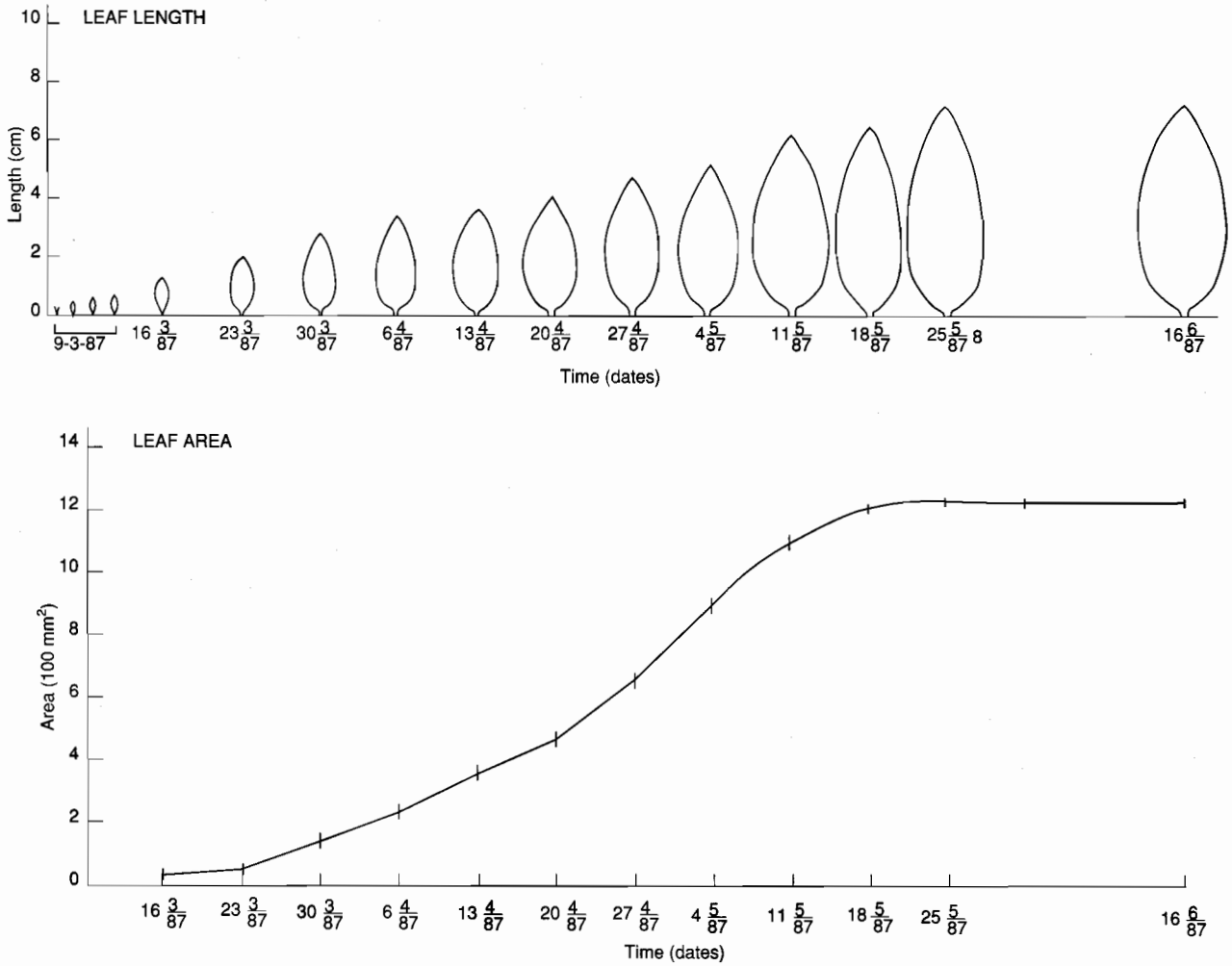

Figure 1. Growth of developing leaf in sandal

Table 1. Leaf development in sandal

Characteristic	Time (week # - day/month in 1987)											
	1 - 16/03[a]	2 - 23/03	3 - 30/03	4 - 6/04	5 - 13/04	6 - 20/04	7 - 27/04	8 - 04/05	9 - 11/05	10 - 18/05	11 - 25/05	14 - 16/06[b]
Length (cm)	1.24± 3.18	1.93± 0.32	2.30± 0.30	2.89± 0.31	3.20± 0.44	3.72± 0.44	4.42± 0.57	5.10± 1.18	5.30± 1.27	7.00± 0.45	7.18± 0.54	7.46± 0.56
Breadth (cm)	0.38± 0.10	0.71± 0.20	1.19± 0.27	1.40± 0.28	1.60± 0.24	1.77± 0.37	2.06± 0.39	2.38± 0.40	2.22± 0.59	2.84± 0.46	3.29± 0.58	3.30± 0.55
Length/breadth ratio	3.26	2.71	1.93	2.06	2.0	2.10	2.20	2.17	2.36	2.50	2.25	2.24
Area (mm^2)	26.73± 3.28	56.93± 5.94	147.73± 8.74	244.0± 7.82	381.00± 9.20	479.80± 20.69	686.00± 31.40	922.00± 49.18	1156.86± 21.74	1239.30± 17.58	1249.70± 19.74	1250.00± 13.40
Green weight (mg)	12.13± 2.82	24.00± 2.60	46.80± 2.83	66.90 ± 4.26	76.00± 4.90	96.40± 14.24	133.00± 8.80	132.00± 14.00	166.65± 15.55	273.60± 19.57	274.50± 20.55	263.00± 13.55
Dry weight (mg)	2.22± 0.64	4.30± 0.60	10.84± 3.35	14.80± 0.81	25.60± 3.00	34.33± 5.65	39.40± 3.35	50.70± 3.28	66.60± 4.75	104.78± 6.70	110.30± 8.20	110.90± 7.35
Internodal distance (mm)	2.00± 0.48	9.00± 1.65	13.00± 1.53	16.24± 2.40	16.60± 2.00	17.00± 3.10	17.34± 0.40	20.00± 0.60	21.60± 0.32	22.00± 0.30	23.00± 0.30	23.00± 0.20
Chlorophyll 'a' (mg/g)	1.478	3.068	3.402	4.532	5.761	5.784	5.831	6.446	6.562	8.260	8.260	8.300
Chlorophyll 'b' (mg/g)	1.165	1.968	2.000	2.759	3.370	3.381	3.402	3.649	3.783	4.772	4.800	4.880
Total chlorophyll (mg/g)	2.643	5.036	5.402	7.291	9.131	9.165	9.233	10.095	10.345	13.032	13.060	13.180
Chlorophyll a/b ratio	1.27	1.56	1.70	1.64	1.71	1.71	1.71	1.77	1.73	1.73	1.72	1.70
Nitrogen (%)	1.26	1.29	1.52	1.69	1.71	1.78	1.83	2.04	2.19	2.22	2.22	2.30
Phosphorus (%)	0.173	0.177	0.183	0.211	0.212	0.250	0.272	0.293	0.424	0.496	0.470	0.424
Potassium (%)	0.940	1.070	1.200	1.200	1.350	1.650	1.950	2.100	2.250	3.000	3.000	2.800
Calcium (%)	0.180	1.102	1.380	1.632	1.956	2.350	2.400	2.650	2.800	3.030	3.220	2.860
sulfur (%)	0.312	0.313	0.313	0.350	0.388	0.450	0.650	0.750	0.775	0.912	0.910	0.950

Notes:
[a] Initial growth (7 days)
[b] Maximum growth (week 14)

Leaf initiation starts in most of the trees between February and March. In a few trees leaf formation was also seen in April–May following summer rains. Between May and June, most of the trees are full of leaves. Formation of leaves continues until June–July. Shedding of leaves starts in November and continues until April–May. In summer, a few sandal trees in dry scrubby forests are observed practically leafless.

Discussion

In *Santalum album* L. the rate of development of leaf seems to follow a sigmoid curve pattern. The length and breadth ratio signifies that leaf development is homoblastic. The chlorophyll content is less in younger leaves, and increases considerably as the leaf develops. The chlorophyll 'a' increase is more than that of chlorophyll 'b'. There is a gradual increase in dry weight, nitrogen, phosphorous, potassium, calcium and sulfur. The growth-rate of the developing leaf is slow at first, then speeds up, and finally slows down almost to cessation. The other associated characters also increase until the leaf attains normal size. It is also contended that the variation in dry weight coupled with other N, P, K, Ca and S content could be due to translocation of photosynthate and mobile elements from the leaf to other permanent parts of the plant.

In spite of *Santalum album* L. being an evergreen tree, it showed seasonality in respect of leaf flushing. However, there was no complete cessation of flushing activity. This tree being hemiroot parasite, even in summer periods under normal conditions, the foliage is full on the tree as it derives nutrition from the host plant.

Acknowledgment

We thank Mr B. Sachidananda, Soil Scientist, JNKVV, Jabalpur (MP) for his help rendered during the nutrient analysis.

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Tree Improvement Efforts in Sandal: the Need to Employ Novel Strategies

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Abstract

Tree improvement in sandal aims at evolving trees with more heartwood and oil in a short period. Although the diploid chromosome number of sandal is 20, a two- to five-fold increase in chromosomes owing to endopolyploidy has been reported. Except in a few plus trees, the length and width of leaf is largely influenced by environment, causing interclonal variability. Germination and survival of seedlings are under genetic control, and governed by separate genetic mechanisms. Foliar variations are important taxonomic characters; they have been subjected to severe biometrical analysis, indicating the existence of six biotypes. For genetic resource conservation, both in-situ and ex-situ methods have been adopted. Characters such as clear bole, height, good crown and heartwood depth were found to be important in selecting plus trees. In progeny trials the existence of segregation, pleiocotyly, variation, twin and triplet seedlings, and albinism have been reported. Apart from both somatic and protoplast culture techniques to get desired somaclones and to manipulate genomes, 'mutation breeding' technique has also been attempted. Tetraploid sandal is reported to exhibit greater spectral variation over diploids. Sandal has not had the comprehensive genetic work and concerted breeding effort which its importance would seem to warrant. However, biotechnology is a promising avenue for tree improvement.

Key words: genetics, breeding, strategies, variation, biometrics

SANDAL IS HETEROZYGOUS and thus adaptable to different eco-geographical conditions. The evidence for this is:

- different tree-forms,
- varied leaf types,
- both compact and spreading branching habits, and
- highly variable oil content.

There have been few genetic analyses of these traits. Tree improvement in sandal mainly aims at evolving trees that can yield more heartwood and oil in a short period of time, coupled with spike disease resistance.

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Natural Variability

Root-tip cytological analysis by the 'Darlington and Wylie method' indicated the diploid chromosome number of sandal as 20. However, drift in chromosomal number in various tissues and parts of the tree has been reported. In the haustorium up to 40 chromosomes have been reported. In many reports a two- to five-fold increase in size of the chromosomes was observed. This has been attributed to the phenomenon of endopolyploidy and chromosomal degeneracy (Srimathi and Srinivasayya 1962).

Mather (1953) found that intra-organism variability exists, and that it is governed genetically. A study of intra-tree and inter-tree variation in the leaves of sandal showed maximum variability in the middle and lower portions of the crown; it increased in the later phase of growth (Kushalappa 1983). Inter-tree studies

showed that the trees vary significantly for leaf length and leaf width (Bagchi and Veerendra 1985).

The standard error of leaf traits varied considerably from plant to plant, which suggests that they are governed by genetic factors. From the studies of Bagchi and Veerendra (1985), it is clear that average germination and survival are mostly less than 45 percent and governed by independent systems.

Since the highest accumulation of oil is in the heartwood region, genotypes may differ in heartwood-size or in other related characters. Anatomical characteristics are not only used by taxonomists and anatomists for identification (Veerendra and Bagchi 1986), but also to study variability both within and between trees. A study of six anatomical characters (section diameter, xylem cell diameter, epidermal thickness, cortex width, and number of vascular bundles of apical stem portion) has shown that there are genotypic differences in the population. Appropriate mating designs could be employed to understand the nature of gene action.

Related species of *Santalum* include, *S. austrocaledonicum*, *S. lanceolatum*, *S. spicatum*, *S. paniculatum*, *S. murrayanum*, and *S. acuminatum*. By contrast with *S. album*, these related species are eco- and niche-specific. Floral and foliar variations are important taxonomic characters (Srinivasan et al. 1992). Biometric analysis of floral-related parameters shows that sandal has six biotypes. Bagchi and Veerendra (1985) suggested that biometrical analysis of leaf morphology could be used to define sandal types. Srimathi and Kulkarni (1983) reported variations in leaves, flowering behaviour, germination, and morphotypes.

Several State Forestry Departments have established germplasm banks and clonal orchards (Bajaj 1986).

Vulnerability to Spike Disease

There is no natural resistance to spike disease, apparent resistance being due to the absence of adequate spike inoculum and other congenial conditions for the pathogen (Venkatesh and Kedarnath 1963). Although complete recovery of infected plants is usually not achieved after chemical treatment, remission of symptoms for up to two years has been achieved in some tree species. Hence this technique must become more economical before it can be used over a large forest area (Srimathi et al. 1979). Once the vector is identified, studies on the management of spike disease through vector control using chemical or biological agents should also get priority.

For a long-term solution of spike disease, production of disease-resistant sandal trees through breeding could be the best method in a heavily diseased area. The existence of apparently healthy sandal trees in areas of otherwise diseased trees is a promising starting point. Such trees might have escaped the disease by genetic resistance or in some other way (Venkatesh 1978). Such trees should be preserved and utilised for the production of seedlings through vegetative means for planting afresh in the endemic area. However, before any large-scale breeding work is initiated, such plants should be subjected to vigorous tests to confirming the genetics of resistance.

Poor knowledge of vector biology and inability to prove Koch's postulates could be impediments in both breeding for resistance and biological control of insects. Hence, we should try to cultivate pathogen and vectors in-vitro. Systematic studies should also be carried out on matters such as:

- the life-cycle of the vector;
- the vector–pathogen relationship, including minimum acquisition period;
- incubation period of the pathogen in the vector;
- feeding behaviour; and
- proportion of viruliferous insects in the vector population.

Conventional Tree Improvement Techniques

The techniques used in tree improvement to find and select superior trees depend on the type of stands in which selection is to be made. Numerous suggestions have been made for selecting hardwoods. A determination of the best selection technique depends on several factors, including:

- species characteristics,
- history,
- the present condition of the forest,
- variability and inheritance pattern of important characteristics, and
- the objectives of a particular tree improvement program.

Selection methods suggested for sandal are:

- mass selection,
- family selection,
- a combination of family and within-family selection, and
- selection based on progeny testing.

Mass selection depends on the phenotype of the characteristic. If the characteristic has high heritability, then maximum gain is achieved by this method. Further, depending on age of the sandal population, the number of traits to be improved, and the number of traits considered for selection, one can follow a regression selection system, a multi-trait selection index (both tandem and independent culling), and a selection index.

Family selection involves selecting an entire family, based on average phenotypic value; within-family selection involves selecting the superior member of the family. Both family and within-family approaches may be followed, depending on the traits under consideration.

While selecting a plus tree, one should consider all those traits which are directly or indirectly correlated to fast growth and oil content. Different characteristics to be considered are clear bole height, good crown, higher heartwood oil content, fragrance, and heart colour. The oil content of heartwood cannot be readily assessed from standing trees. Emphasis should be laid on selection based on progeny trials.

Novel Techniques

Apart from conventional breeding systems, mutation and biotechnology are very useful in creating novel and superior desired variability. Biotechnology is a powerful adjunct to conventional breeding programs, and it is the process by which we can manipulate critical monogenic or polygenic traits that can not be physically or economically introduced by conventional breeding (Lakshmi Sita et al. 1982). The major areas in which cell and tissue culture and related techniques are of potential value are:

- shoot-tip culture, which allows rapid clonal multiplication since propagation by traditional method is time-consuming;
- embryo culture of hybrids of desired sandal crosses that cannot develop normally due to incompatibility between the embryo and the maternal tissue;
- manipulation at cellular level in order to complement conventional sexual breeding programs with asexual plant improvement (Bapat and Rao 1979).

There are several approaches at cellular level which can help to increase genetic diversity, such as:

- variation in tissue culture, leading to polyploidy;
- haploid plant production from pollen and anther culture, leading to homozygous diploid production;
- fusion of cells by protoplast cultures, enabling interspecific and intergeneric crosses; and
- transgenesis, leading to transfer of desirable genes from one species to the other.

We will not be able to use the potential of biotechnology until we know more about the cell and molecular biology of forest species (Bajaj 1986). Further, fundamental knowledge about the biological processes that affect the growth, metabolism, reproduction and interactions of important species is a prerequisite.

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Development of Allozyme Markers in *Santalum album* L., and their Application in Population Genetic Studies

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Abstract

Among the electrophoretic techniques for assessing plant proteins, isozyme/allozyme analysis is widely used for its efficiency and cost effectiveness in intra-specific variability studies. Here we report the allozyme markers screened and developed using adult leaves of *Santalum album* L. Thirty different enzymes covering major metabolic pathways were screened with different tissues of *S. album*, extraction buffers and electrophoretic running conditions in a starch gel system. Fourteen enzymes covering 25 loci showed good resolution. Ten loci, covering the enzymes GPI, G6Pdh, Mdh, ME, MR, PGM, 6PGdh, showed mono- and di-allelic polymorphism. The results point to the usefulness isozyme/allozyme analysis in genetic resource mapping of highly disturbed *S. album* populations.

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Association of Sandal with Vesicular Arbuscular Mycorrhiza (VAM) Fungi

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Abstract

Santalum album, L. is a root parasite, the many primary and secondary host plants of which have been studied in detail. During our survey it was observed that the root region of sandal had more nitrogen-fixing bacteria and VAM fungi than those of certain hosts, despite its parasitic habit. However, addition of VAM to the rhizosphere of both sandal and host boosted growth and biomass, especially in the root system. The VAM association and its effect on growth has been detailed in this paper.

Key words: root parasite, rhizosphere, vesicular arbuscular mycorrhizal fungi, VAM

THE PARASITIC NATURE of sandal has been known for over a century. Barber (1905, 1906, 1907) made detailed investigations on the formation of haustoria, structure, development and mode of attachment to the root of host plants. Most of the work was concentrated on the host–parasite relationship and behaviour of sandal with different host plants. Notwithstanding sandal's parasitic habit, a symbiotic association with nitrogen-fixing bacteria and VAM fungi has been reported (Subba Rao et al. 1990).

The vesicular arbuscular mycorrhiza is a ubiquitous symbiont in the world's ecosystems, probably occurring in over 90 per cent of vascular plant species. Most tropical trees established mycorrhizal associations for better establishment, additional support for nutrient uptake, and to utilise otherwise unavailable forms of nutrient. A detailed survey was undertaken to observe the symbiotic relationship of VAM in addition to the sandal host association. It was found that different species of VAM were associating in the rhizosphere of sandal. A detailed experiment was conducted to see the behaviour of sandal plants associated with different species of VAM, and the results are reported here.

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Materials and Methods

In each experiment, there were 100 plants for each treatment including control. *Cajanus cajan* was given as host for all the plants except in Experiment No. 3. Initial height of seedlings was recorded just after transplanting and thereafter every month, and survival percentage was also noted.

At the end of experiment, a few plants were taken out and washed carefully to remove adhering soil; seedlings were cut from the collar region, and fresh weight of shoots and roots was recorded separately. Dry weight was determined after oven-drying at 80°C for two days. Soil samples from each treatment were collected for spore count using a wet-sieving and decanting technique following Gerdemann and Nicolson (1963). Fine feeder roots from all treatments were cut into one cm segments and randomly selected for estimation of degree of VAM following Phillips and Hayman (1970).

Experiment No. 1

Uniformly grown healthy 6–8 leaved sandal seedlings (from seeds from a known source) were transferred to polythene bags containing a potting mixture of sand, red earth and FYM (2:1:1). The following species were used for inoculation as a separate treatment, in addition to a control where seedlings were maintained without VAM inoculum:

- *Glomus fasciculatum*
- *G. aggregatum*
- *G. caledonicum*
- Composite spores.

All species of VAM inoculum were collected from known sources, and composite spores were extracted from the soil collected from the rhizosphere zone of sandal and multiplied by pot culture technique. Inoculum contained 300–350 spores per 100 gm of soil. 100 gm of VAM soil was used for inoculation of the root zone of each plant.

Experiment No. 2

Sandal plants were planted in a root trainer, and 30 gm of inoculum of composite VAM spores was fortified in the root zone of sandal.

Experiment No. 3

Composite spores of VAM were introduced into the root zone of sandal plants without any hosts. A control was also maintained without VAM inoculation and host plants.

Results and Discussion

Figure 1 shows the height increment in sandal seedlings with the inoculation of different species of *Glomus* (VAM) at the nursery stage. For the first month, the growth of treated and untreated seedlings was almost the same. But from the third month onwards, the difference in growth between treated and untreated seedlings was distinct. Composite spore treatment gave a higher increment than that of any individual species of inoculation, as well as untreated seedlings.

Even though there is species variation in survival percentage of sandal seedlings, inoculated seedlings showed more than 80% survival compared with 55% in the control (Fig. 2). As the seedlings treated with composite spore performed better than individual species of VAM, further work was carried out by treating plants with the composite spore. The growth of VAM-associated seedlings showed clearly in biomass increment (Fig. 3). Both fresh weight and dry weight were significantly higher in the inoculated seedlings compared with the control. Sandal seedlings with VAM inoculation showed 60–70% infection compared with less than 15% in untreated ones. The number of spores was significantly higher (around 500 spores/100 g) in VAM-treated soil compared with uninoculated soil (around 50–60). *Glomus* and *Gigaspora* species predominated.

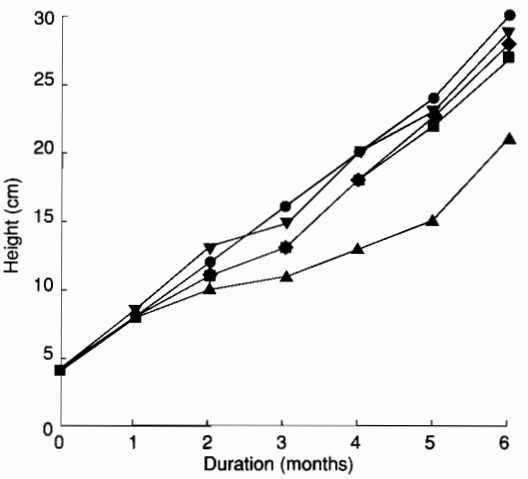

Figure 1. Effect of VAM inoculation on growth of sandal seedlings. Inoculum: ◆ = *G. fasciculatum*, ■ = *G. aggregatum*, ▼ = *G. caledonicum*, ● = Composite spores, ▲ = Control

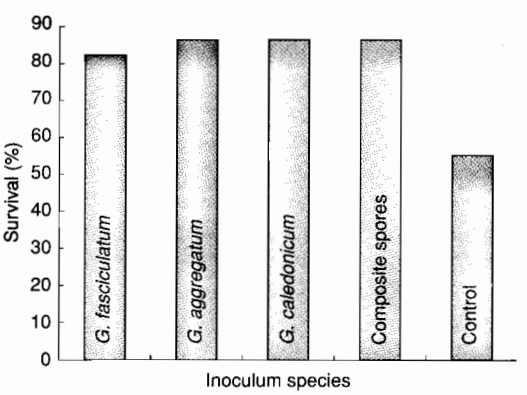

Figure 2. Survival of seedlings with different species of VAM inoculation

In the second experiment, where the composite spores were fortified in the rhizosphere zone of sandal plants in root trainers, the performance of the treated plants was better than that of the uninoculated ones. The degree of infection was higher in this experiment than with a 'polybag' container. As the root trainer is conical in shape, being narrow at the root zone, all the inoculum will contact the feeder roots of plants, and the chance of infection is greater than in a wider container such as a polybags. Also, the quantum of inoculum can be minimised (30–50 g) in the root trainer. This is the best technique with which to inoculate the plants with VAM to reliably give an adequate level of infection.

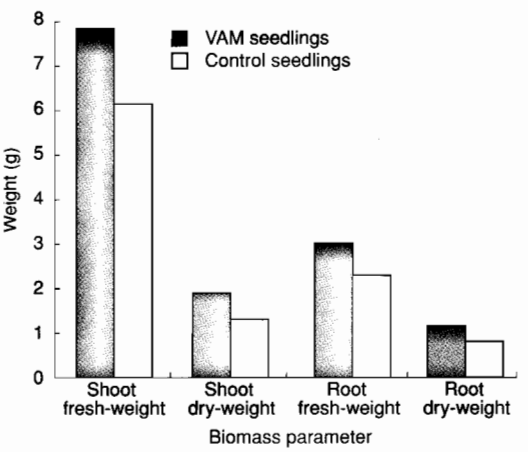

Figure 3. Effect of VAM inoculation on biomass of sandal seedlings

In view of the better performance and establishment of sandal seedlings with VAM inoculum, an experiment with VAM inoculation, but without host plant, was conducted to see whether sandal plants can perform better without help of the host plant in the presence of VAM. The sandal plants did not survive well without a host plant even though the plants were fortified with VAM inoculum.

This shows that sandal can only do well with a host plant because the major nutrients to sandal plants are taken through the host roots by haustorial connection (e.g. Sreenivasa Rao 1933; Parthasarathi et al. 1974; Ananthapadmanabha et al. 1984). However, Ramaiah et al. (1962) revealed that occasional diffusion of some nutrients into surrounding cells could take place through sandal roots. The possibility of absorption of these nutrients directly from soil also was very much in evidence as sandal roots possess cation exchange capacity at a level comparable with those occurring in many of its hosts (Parthasarathi et al. 1974). This may be due to mycorrhiza present in the root zone. Hyphae can take up nutrients such as phosphorous and translocate them to the host plants (Jakobsen 1992). This hyphal absorption might have increased nutrient acquisition in sandal, which might have resulted in most spectacular increase in growth and biomass of plants.

Composite VAM faired better than individual species in sandal plants in growth, biomass and percentage of infection. Host specificity (VAM) in sandal plants is not the criterion for the association of plants. The colonisation of different VAM fungi on a single host seems to be a common and widespread phenomenon. Indeed, the dual and multiple mycorrhizal infection was a consistent feature in most of the species. Various host plants associate simultaneously with more than one species of VAM fungi, and Narayanan and Manokaran (1996) observed that plants inoculated with a combination of *Glomus fasciculatum* and *G. mossae* had good vigour and increased shoot height compared with individual VAM in teak plants. Composite spore extracted from the rhizosphere of sandal showed facultative mycotropy with several *Glomus* and *Gigaspora* species (Subba Rao et al. 1990).

Conclusions

It is known that garden soil has VAM inoculation which may help symbiotic associations, but the quantity of inoculum may not be sufficient to reach an adequate mycotropy level to have a positive effect on the plants. On a commercial scale, soil cannot be used under sterilised conditions, so it is not advisable to use individual species. This is especially so in sandal, as it did not show obligatory dependency on one species.

Sandal plants have a symbiotic association with VAM fungi in addition to their parasitic habit. This might have helped in the absorption of more nutrition, and resulted in growth and biomass. Overall, fortification of the root zone with VAM has yielded improved growth, biomass and survival of seedlings which may further help in establishment during planting in the field.

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Status of Vesicular Arbuscular Mycorrhizal (VAM) Association of *Santalum Album* L. (Sandal) in Black Cotton Soils

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Abstract

The Vesicular Arbuscular Mycorrhizal (VAM) fungi are widely distributed in varied ecosystems and associated with many plant species. Among the different tree species, sandal (*Santalum album* L.) is an economically important tree generally accepted as indigenous to Peninsular India. Considering the economic value of this tree species, an intensive survey was made to investigate the status of VAM fungal associations in *S. album* in forestry plantations. The extent of mycorrhizal colonisation in roots and fungal spore populations in rhizosphere soils was investigated. VAM colonisation in roots of sandal was low, although more VAM spores were isolated from the rhizosphere soils. Spores isolated from the rhizosphere soils were identified as different species of *Glomus, Acaulospora* and *Sclerocystis* endomycorrhizal fungi. Among these genera, *Glomus* and *Acaulospora* are dominant and commonly occurring in the rhizosphere soils. Selection of the predominant VAM fungi for further studies is in progress. The significance of these findings is discussed.

MOST PLANT SPECIES are colonised by mycorrhizal fungi in order to form symbiotic associations. These fungi play a key role in plant nutrition and growth improvement. The study of endomycorrhizal fungi in tropical tree species is gaining importance because limited work has been done on endomycorrhizal fungi in association with different trees. Among these fungi, Vesicular Arbuscular Mycorrhizal (VAM) fungi are widely distributed in varied ecosystems and associated with many plant species. VAM fungi in tree species differ in their ecological requirements and adaptability.

Among the different tree species, *Santalum album* L. is an economically important evergreen tree. Information on the status of endomycorrhizal fungi in association with sandal tree is lacking. Hence, an attempt was made to investigate the type of mycorrhizal association and scan out the VAM fungal population from the rhizosphere of sandal tree in black cotton soils in Tamil Nadu.

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Materials and Methods

Root and rhizosphere soil samples of both seedling and adult trees of *S. album* were collected from the experimental fields of IFGTB, Forest Campus, Coimbatore. The root samples were washed thoroughly in tap water and fixed in FAA (13 mL Formalin + 5 mL glacial acetic acid + 200 mL 75% alcohol) for further studies. The 'Phillips and Hayman method' (1970) was adopted for clearing and staining the roots for rapid assay of endomycorrhizal association. The VAM fungal spores were isolated from the rhizosphere soil samples by using wet sieving and decanting (Gerdemann and Nicolson 1963). The species-level identification of different VAM fungi was done following the keys provided by Trappe (1982) and Schenck and Perez (1987).

Table 1. Status of VAM fungi in roots and rhizosphere soil samples of sandal in black cotton soils (mean value of three replications)

	Site 1		Site 2	
	Root colonisation (%)	Soil spore population/ 100 g soil	Root colonisation (%)	Soil spore population/ 100 g soil
Seedling	23	431	31	506
Adult tree	42	1152	47	1224

Results and Discussion

The root and rhizosphere soil samples of sandal plants occurring in the experimental fields of IFGTB, Forest Campus, Coimbatore were examined for the presence of VAM fungi. The study reveals the extent of root colonisation and soil-spore population of VAM fungi from samples of both seedlings and adult trees (Table 1). VAM colonisation was moderate in the roots, but the spore population was greater in soil samples. Among the different samples screened, those collected under the root-zone of young seedlings (<1 year) show lower colonisation and spore number than samples collected under the root-zone of adult trees (3–15 years). The variation in root colonisation and soil-spore population of VAM fungi in different samples of sandal plants could be due to soil and other site factors.

Rhizosphere soil samples were processed for isolation and identification of different VAM fungi. Spores of three different VAM fungal genera were recorded, showing *Glomus* (6 species), *Acaulospora* (3), and *Sclerocystis* (1). Among them, two endomycorrhizal fungi *Glomus fasciculatum and G. microcarpum* were most commonly distributed in the rhizosphere soils, and their spore numbers were maximum in both seedling and adult trees (Table 2). Mass multiplication of these VAM fungi is being undertaken for further studies on sandal in nursery and plantation conditions. The role of VAM fungi in soil fertility of sandal plantations in different parts of the State is yet to be ascertained.

Table 2. Distribution of VAM fungi in the rhizosphere of sandal in black cotton soils

VAM fungi	Seedling stage	Adult stage
Glomus citricolum	–	+
G. fasciculatum	+	+
G. microcarpum	+	+
G. occultum	+	–
Glomus sp.	–	+
Glomus sp.	+	+
Acaulospora scrobiculata	–	+
Acaulospora sp.	+	–
Acaulospora sp.	–	+
Sclerocystis sp.	–	+

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A Further Tree Improvement Strategy in Sandal (*Santalum album* L.)

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Abstract

Sandal (*Santalum album*) is a heterozygous tree species. Although the predominant method of pollination is out-crossing, selfing was also reported as an alternative. Hence, a multiple population breeding program has been proposed which helps to overcome most of the deficiencies of a conventional breeding strategy. Multiple population breeding involves different options for the structure and management of population. Although this strategy would substantially increase the size and cost of the program, it is essential considering the length of the gestation period and therefore evaluation of one generation in sandal. This strategy involves the use of multiple populations in more than one field location for a generation and for the management and testing of the breeding population. It must be assured that this strategy will work effectively long-term, because each location may be used for improvement of a single trait. This approach reduces inherent difficulties and risks associated with a tree-breeding program with a long rotation age by reducing the time period. It also is an insurance against pests, diseases and natural disasters. Here, a number of populations dedicated to producing heartwood and oil are kept separate so as to produce trees with different gene complexes. The objective of maintaining separate sublines or multiple populations at different sites is to maintain and/or create differences between populations while practising intensive breeding within them. Diversity will occur between sub-populations, even if the selection pressure, selection criteria and environment are the same; this is because major economic traits are under multiple gene control and different permutations and combinations of genes will form, and currently neutral alleles will not be lost in all populations. At any time, variation can be reinstated and new populations with specific attributes can be created by crossing between populations.

Key words: multiple population, tree breeding, variation

IN INDIA, Santalum album grows profusely in Karnataka, parts of Tamil Nadu, Andhra Pradesh and Kerala with a satellite distribution in central and eastern India. The total area of natural distribution is about 5100 km^2. There is morphological variation in the populations, but no specific varieties or ecotypes have been identified so far. However, there are some genetic differences in the isoenzyme pattern (Parthasarathi et al. 1985). The flowering pattern in *S. album* is annual, six-monthly or continuous (Sindhuveerendra and Ananthapadmanabha 1996).

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Variations occur in vegetative and reproductive parts such as leaf structure (cutinised to non-cutinised), shape (ovate, lacerate, big and small), bark structure and seed polymorphism (Sindhuveerendra et al. 1991). Amount of heartwood present varies from tree to tree in proportion to sapwood. Oil content varies from 0.5% to 5% in heartwood. Flower structure varies from conical to ovate. Stamen number varies with number of tepals. The flower is greenish-yellow, less showy, and small; the anther produces small amounts of sticky pollen which is entomophilous. Ants, bees and butterflies are regular visitors. Obligatory self-pollination was observed to a small extent and is genotypic. Fruit set was observed, but the fruit do not mature due to gametophytic self-

incompatibility. Heterostyly was observed. Natural out-crossing is the predominant method of pollination (53–75%, with a mean of 60%) (Sindhuveerendra and Ananthapadmanabha 1996). Parthenocarpy was not observed. Since the species is endemic to a small area, inbreeding was high (estimated at 7%), and natural hybrids were observed.

The pressure on the existing population is high due to low availability of seed. Hence, immediate domestication efforts began through genetic improvement of existing populations. Tree improvement plans are either short-term or long-term strategies. In the short-term strategy, emphasis was on utilisation of existing natural populations for seed production. Hence, as a first step under the tree-breeding program, seed production areas were selected based on above-average populations in natural stands. The long-term strategy includes selection of 'candidate plus' trees (CPTs) and grafting of all CPTs into a clone bank, followed by raising of CSOs and progeny trials. A sandal breeding program and a breeding strategy for the next generation are reported here for the first time.

Breeding Strategy

Previous work

A serious sandal tree improvement effort began in the 1980s. The major features of the program were as follows:

- Selection of superior provenances followed by raising provenance trial plots. Provenances selected were Marayoor (Kerala), Chitteri Hills and Javadi Hills (Tamil Nadu), Araku Valley (Andhra Pradesh) and Thindlu (Karnataka). Accordingly, the provenance trial plots were established in these states, except Kerala. Significant differences between provenances have been observed.
- Selection of seed production areas (SPAs) from those provenances for immediate use in an afforestation program. This involved distribution of improved seed from SPAs to various state forest departments. Such SPAs are at Chitteri Hills and Javadi Hills (Tamil Nadu), Marayoor (Kerala), and Arabithittu and Thindlu (Karnataka).
- The selection of SPAs was followed by selection of superior phenotypes. The improvement program and 77 CPTs were selected from four southern states: 37 from Karnataka, 31 from Tamil Nadu, 3 from Kerala and 6 from Andhra Pradesh. They were grafted and collected in a germplasm bank. From these CPTs, progeny trial plots were established along with CSOs. Three CSOs are established so far to meet the immediate requirement for seeds. The design adopted in these CSOs is a computer-generated 'permutated neighbourhood' with single-ring isolation which facilitates maximum panmixis.
- Progenies from 76 CPTs were raised during 1982, 1984 and 1986 at the Institute of Wood Science and Technology. The evaluation of these progenies is in progress. The design used is randomised block design. These progeny trial plots were established on a site which has an identical environment to its natural habitat.
- Two progeny trial plots with 25 families with 25 seedlings per family were established at Karnataka and Tamil Nadu. These two areas represent different environments.

New approaches

Three progeny trials, one provenance trial plot, and three CSOs will be available for seed production to meet the immediate requirement of seed for afforestation. The total requirement of 5 tonnes of seed may be met from these sources. Although a major portion of seed requirement was met from seed collection from SPAs, a significant quantity of seed comes from CSOs and progeny trials, irrespective of their genetic makeup. After two years, seed collection from SPAs may be stopped; that from provenances has already stopped. This completes one cycle of first-generation breeding and seed production. At any time, seven field locations are available simultaneously. Each progeny trial plot may serve as a seed production area, a base population, and a progeny test.

There are two field locations containing approximately 42 half-sib families arranged in CRBD with 25 tree plots per family with non-contiguous plots. The two other progeny plots established at Karnataka and Tamil Nadu states in four field locations may be treated as individual populations. They may fulfil four functions in the following temporal sequence:

- a genetic base population;
- a progeny test of previous generation;
- a partial seed source of the next generation's base population; and
- a seedling seed orchard.

Open-pollinated management of the breeding population is carried out in order to meet a requirement for any controlled pollination experiment as well as to give freedom to the breeder to select best genotypes. Since all the families are open-pollinated and managed accordingly, the inherent risk of the

inbreeding among next generation populations is obviated.

Future Programs

Multiple populations

The next step in breeding strategy involves different options for the structure and management of populations. Although such strategies would increase the size and cost of programs dramatically, this is essential after considering the gestation period for evaluation of one generation. Hence, it is wise to use multiple population sublines (Carson et al. 1990; White et al. 1992), because each location may be used for the improvement of a single trait. This approach reduces the inherent difficulties and risks associated with a tree-breeding program with a long rotation age by reducing the time period; it also serves as an insurance against pests, diseases and natural disasters.

The future breeding strategy may be formulated with access to all 77 CPTs for heritability of economic characters, and thus retain at least 60 CPTs for each field location. From these CPTs, the five best-performing offsprings are selected. Thus there will be 300 progenies from each field station. Out of six provenances, at least another 100 phenotypically superior trees will be identified. Thus 400 trees will form the base population for the next generation for each field location.

From these field locations, it is possible to quantify the amount and type of genotype–environment interaction (GEI) that exists across the four locations. Since they are established at different climatic and edaphic conditions. GEI is very important and it is possible to manage each of the four field locations for its own special environment. Then, over a few generations, the four populations would begin to diverge genetically to form four land races.

The spatial organisation of local populations and concomitant patterns of gene flow are important determinants of whether a species becomes genetically differentiated over its geographic range. The extent to which the species occur as ecologically and geographically differentiated populations over their geographic range is a matter of practical interest to any tree breeder, particularly when tree-improvement programs are initiated. If regional populations cannot be distinguished genetically, then there might be less need to take population structure into account. Conversely, the existence of distinct genetic populations would establish the need for subdivision of breeding populations during improvement programs.

The pattern and amount of pollen and seed dispersal also affect the spatial arrangement of genotypes. Limited gene flow promotes the formation of families and family clusters among which allele frequencies differ.

The physiology of parasitism, unique features of nursery practice, breeding systems, rate of inbreeding, and possible isoenzyme variations in relation to seed characteristics in sandal are described elsewhere.

The ramets in all provenance and progeny trials of sandal are measured frequently at 1-year, 2-year and 6-year intervals and evaluation of growth rate is in progress. Since economic emphasis is on production of heartwood with oil-bearing capacity, the existing data is being processed to give meaningful rankings of families in future evaluations.

The main traits selected in sandal improvement are two qualitative traits: heartwood, and oil content in heartwood.

Here, a number of populations are kept separate so as to produce trees with different gene complexes (Namkoong 1986; Namkoong et al. 1989). The object of maintaining separate sublines or multiple populations at different sites is to maintain and/or create differences between populations while practising intensive breeding within them. Diversity will occur between sub-populations, even if selection pressure, selection criteria and the environment are the same. This is because major economic traits are under multiple gene control, and different permutations and combinations of genes will form, and currently neutral alleles will not be lost in all populations. At any time variation can be reinstated and new populations with specific attributes may be created by crossing between populations.

In sandal, adoption of a multiple-population breeding strategy for a tree-breeding program could help overcome most of the deficiencies of a conventional breeding strategy. Some examples follow.

1. Long-term pedigree can be controlled by judicious mixing of individual trees, progeny trials and populations (provenance trials).
2. The breeder could maximise the intensity of selection within a population and be able to overcome inbreeding or regenerate variation at any point.
3. Sandal is a minor species as it grows in limited areas, and there are chances of some degree of neighbourhood-relatedness resulting in neighbourhood inbreeding. But the proposed strategy has the

flexibility of adding new material as sub-populations, and simple breeding techniques may be used until commercial seed is produced.

4. Spacio-temporal variations can be passed on to the native forests by preserving and enhancing the existing variations indefinitely in a sub-population. The GEI can be developed and used to increase production by planting diverse populations on multiple locations instead of planting all genotypes over all sites.

Thus the strategy is flexible and at any point of time new variations can be introduced to allow breeder freedom without taking any unacceptable risks (Barnes 1981, 1984, 1986).

Genetic checks

Since considerable time has already been spent on genetic improvement in sandal, there is a need to establish genetic checks in the breeding program to monitor the performance of genotypes. The aim is to assess the rate of gain and to compare performances between genotypes for operational use or for infusion into the next breeding program. To ensure that their performance is as consistent as possible over time and site, only half-sib families which are performing genotypically should be selected for their stability over environments.

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Pests and Diseases

In-vitro Comparative Morphogenetic Studies of Normal and Spike-diseased Tissues of Sandal (*Santalum album* L.)

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Abstract

Initiation of callus from the exposed cut surfaces of normal sandal took place 3–4 weeks after inoculation on MS basal medium fortified with 2,4-D (0.1 mg/L) and BA/Kinetin(1.0 mg/L). Gibberellic acid (GA_3) at 2–4 mg/L was found to be an obligate adjuvant along with 2,4-D and BA/Kinetic for initiation of callus from spike-diseased segments. Callus appeared as pinheads at the exposed cut ends 5–6 weeks after inoculation. These calli from normal and spiked tissues were further subcultured at monthly intervals and maintained on similar media. The rates of growth were very slow, taking approximately six weeks in its spiked counterparts. Inclusion of GA_3 in the media did not have any additional effect on the initiation or yields of callus from healthy segments. The differentiation of callus into shoot-buds and embryoids occurred after 5–6 months from normal tissue, and nearly eight months from spiked tissue. Some of the spiked callus cultures also produced small plantlets. This difference in morphogenetic effects is attributed to the altered metabolic activities and reduced endogenous growth substances (particularly GA_3) in the spike-diseased tissues.

SANDAL POSSESSES A highly valuable wood, known for its scented oil and carvings. Spike disease of sandal has attracted world wide attention due to its destructive nature. The disease is characterised by extreme reduction in size of leaves and internodes, accompanied by stiffening of leaves. In advanced stages, the whole shoot looks like a 'spike' inflorescence. Spiked plants do not bear flowers, although occasionally phylloid or abortive flowers are developed. Mycoplasma aetiology of spike disease was confirmed by Dijkstra and Ie (1969), Hull et al. (1969) and Varma et al. (1969) by electron microscopic studies.

Application of tissue culture techniques to forest species is well known, but very little information is available on its use for in-vitro forest pathology. The in-vitro approach may find its best expression in forestry where the study of pathology is hampered by tree size, rugged terrain and variable environment over the long forest life.

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Methodology

The present study was undertaken with the following objectives:

- to establish continuous cultures of healthy and spike-diseased tissues of sandal; and
- to determine the nutritional and growth regulatory requirements of these tissues.

Both White's and Murashige and Skoog's (MS) basal media were used (Table 1); at later stages only MS medium was employed (Murashige and Skoog 1962; White 1963)). For the initiation, growth, maintenance and/or differentiation of callus, the basal media have been supplemented with growth regulators, auxins (2,4-D IAA, NAA), cytokinins (BA and Kinetin) and gibberellic acid (GA_3) at varied concentrations. Corrections as suggested by Singh and Krikorian (1980) were taken care of in the original MS medium.

Young twigs of about 3–4 mm diameter with a new flush of leaves were collected from healthy and spike-diseased trees from Bhanneraghatta National Park, which is about 20 km from Bangalore. After

Table 1. Composition of the organic constituents and supplements of White's and MS basal media

Substance	Concentration (mg/L)	Group
Glycine	2.0	-
Thiamine HCl	0.1	W-I
Pyridoxine HCl	0.5	-
Nicotinic acid	0.5	-

defoliation, they were washed in dilute liquid carbolic soap solution and tap water.

The washed twigs were first rinsed with 0.1–0.2% $HgCl_2$ for few seconds followed by 2–3 washings in double-distilled sterile water. The explants were than transferred to 50% (v/v) of sodium hypochlorite with few drops of *Tween 20* detergent for 20 minutes. Finally they were thoroughly washed in double-distilled sterile water (6–8 times) to remove any traces of sterilants.

The surface-sterilised explants were aseptically cut into segments approximately one cm long with an oblique cut on each side. They were transferred to the medium at the rate of four segments per flask.

Results

Initiation of callus on White's basal medium

We tried to induce callus formation on internodal segments from both healthy and spiked sandal trees on White's basal medium supplemented with different growth regulators: IAA (indole-3 acetic acid) (at 0, 0.1, 1 and 10 mg/L), in combination with kinetin (at 0, 1, 2, 3 and 4 mg/L).

In most of these treatments, the healthy segments remained green for about two months; the slight callus on a few segments did not continue growing. IAA at 10 mg/L was toxic, and the healthy segments turned brown and died. Spiked tissue turned brown and died one week after inoculation with all treatments (Table 2(a)).

Increasing the kinetin levels to 2–4 mg/L with IAA 0–10 mg/L did not help the segments to induce more callus (Table 2(b)). As in the initial experiment, the spiked segments turned brown one week after inoculation and died later.

Substituting benzyl adenine (BA) for kinetin (at 0, 1, 2, and 4 mg/L) and IAA (0–10 mg/L) also resulted in keeping the segments from healthy trees green for up to two months without any callus initiation; later, they turned brown and died. The internodal segments from spiked tissues turned brown with all treatments and died later. Napthalene acetic acid (NAA) (0–20 mg/L) in combination with BA or kinetin (0–4 mg/L) did not elicit any callusing.

In another experiment, 2,4-Dichlorophenoxy acetic acid (2,4-D) was included in place of NAA or IAA along with benzyl adenine, and the results are presented in Table 3.

The 2,4-D at 0, 0.1, 1 and 10 mg/L, both with and without BA (1 mg/L) was applied. The 2,4-D at 0.1 mg/L with BA resulted in the most intense callusing. There was slight callus initiation with other treatments both with and without BA, but they were much less intense; also, the callus did not continue to grow with these treatments. The 2,4-D at 10 mg/L was found to be toxic, and segments quickly turned brown. Spiked segments failed to induce callus with any of these treatments.

The callus initiation took place from the cut surfaces exposed outside the medium 3–4 weeks after inoculation. These callus-initiated segments were further subcultured on similar media where the callus grew further, but the rate of growth was very slow. It took 3–4 months to attain about one cm^3 of callus, although it gradually covered all the segments.

When the stem pieces were placed on the medium, it became dark brown below and around the segments. Similar browning of the agar medium is reported in the literature, and it has been attributed to tannins and polyphenols. When the stem pieces were transferred to a fresh medium at weekly intervals, the browning diminished in successive transfers. The relatively slow growth rate of the callus necessitated a long-term experiment, especially in tests of organ formation, and hence other new media had to be found.

Callus initiation and growth on MS basal media

As the initiation and further growth of the callus was very slow on White's basal medium, all the treatments were repeated with Murashige and Skoog's basal medium (MS). On this medium neither NAA nor IAA (0–10 mg/L), with either BA or kinetin, induced callus from normal healthy stem segments. However, in some cases they remained green even after two months. A few other treatments showed initial signs of callus, but without further growth; in still others, the segment merely turned brown and died. None of these treatments was favourable for the induction of callus from spiked segments. However, 2,4-D in combination with BA again resulted in the initiation of callus. Here also, as with White's basal medium, the initiation took 3–4 weeks from inoculation.

Table 2. Effect of IAA and certain concentrations of kinetin on induction of callus from healthy and spiked segments on White's basal medium

(a) Lower kinetin

IAA (mg/L)	Kinetin (mg/L)	Healthy segments		Spiked segments	
		Colour	Callus induction	Colour	Callus induction
0	0	brown	none	brown	none
0.1	0	green	slight	brown	none
1	0	green	slight	brown	none
10	0	brown	none	brown	none
0	1	green	slight	brown	none
0.1	1	green	slight	brown	none
1	1	green	slight	brown	none
10	1	brown	none	brown	none

(b) Higher kinetin

IAA (mg/L)	Kinetin (mg/L)	Healthy segments		Spiked segments	
		Colour	Callus induction	Colour	Callus induction
0	2	green	none	brown	none
0.1	2	green	none	brown	none
1	2	green	none	brown	none
10	2	brown	none	brown	none
0	4	green	none	brown	none
0.1	4	green	none	brown	none
1	4	green	none	brown	none
10	4	brown	none	brown	none

Table 3. Effect of 2,4-D and BA on callus initiation from healthy and spiked segments on White's basal medium

2,4-D (mg/L)	BA (mg/L)	Healthy segments		Spiked segments	
		Colour	Callus induction	Colour	Callus induction
0	0	brown	none	brown	none
0.1	0	green	+	brown	none
1	0	green	+	brown	none
10	0	brown	none	brown	none
0	1	brown	none	brown	none
0.1	1	green	++++	brown	none
1	1	green	++	brown	none
10	1	brown	none	brown	none

Notes:
- Intensity: + (low); ++ (moderate); +++ (high); ++++ (very high)
- Callus was routinely further subcultured on similar medium with 2,4-D (0.1 mg/L) and BA (1mg/L)

Healthy shoot-segments growing on MS basal medium and supplemented with 2,4–D (0.1 mg/L) and BA (1 mg/L) showed initiation of callus at 3–4 weeks. White callus appeared all over the cut surfaces of the internodal segments exposed outside the medium. By contrast with other treatments, all growing segments showed uniform callusing. These callus-initiated segments were further subcultured on to a similar medium, and different stages of growth up to the fifth passage were recorded. The growth of the callus could be perpetuated by transferring the sliced tissue to a fresh medium every four weeks.

MS basal medium supplemented with 2,4-D (0.1 mg/L) and BA (1 mg/L) was conducive for callus initiation and growth from stem internodal segments of normal healthy sandal trees.

Comparison of White's and MS basal media for growth of callus from normal healthy tree tissues

Approximately equal amounts of callus from healthy segments were subcultured on the White's and MS basal media; both were supplemented with 2,4-D (0.1 mg/L) and BA (1 mg/L). The callus tissue growth was better on MS than White's medium; that on MS was pure white, while on it was slightly brown. For later experiments this isolated callus was routinely subcultured and maintained only on MS basal medium with the above concentrations of auxin and cytokinin.

Callus initiation and growth from spiked segments

Spiked segments did not survive to initiate callus in any of the treatments with auxins and cytokinins in different concentrations and combinations of different media.

In an experiment with 2,4-D (0.1 mg/L), BA (1 mg/L) and gibberellic acid (GA_3) (0–4 mg/L) in MS basal medium, the spiked stem segments remained green and showed callus initiation; relatively healthy callus resulted from 2–4 mg/L of GA_3. The healthy stem segments showed callus production uniformly from all the segments with or without the GA_3. The effects of GA_3 on callus initiation from healthy and spiked segments are shown in Table 4. When the gibberellic acid concentrations were increased to 10 mg/L, the segments remained green for few days but failed to grow further; at still higher concentrations of GA_3 (20–30 mg/L) both the spiked and healthy segments turned brown and died within few days after inoculation because of its toxicity.

Therefore, only when gibberellic acid was included in the growth medium with 2,4-D and BA, did the spiked segments remain green and show signs of callus initiation. Among different concentrations of GA_3, a range of 2–4 mg/L with 0.1 mg/L of 2,4-D and 1 mg/L of BA or kinetin was conducive to the continuous growth of the callus.

The segments in the flasks showed a pin-head type of callus initiation and took nearly five weeks to initiate. After eight weeks the callus grew further and covered the entire segment, but the rate of growth was very slow. It was white, and was further subcultured at monthly intervals on new media. Thus, it was found that the GA_3 was a critical requirement for initiation and growth of callus from spiked segments. The isolated callus was continuously maintained on a similar medium.

Table 4. Effect of 2,4-D, benzyl adenine (BA) and gibberellic acid (GA_3) on induction of callus from healthy and spiked tissues of sandal on MS basal medium

2,4-D (mg/L)	BA (mg/L)	GA_3 (mg/L)	Healthy segments		Spiked segments	
			Colour	Callus induction	Colour	Callus induction
0.1	1	0	green	++++	brown	none
0.1	1	0	green	++++	green	++
0.1	1	2	green	++++	green	++++
0.1	1	4	green	++++	green	++++
0.1	1	10	green	++	pale green	none
0.1	1	20	brown	none	brown	none
0.1	1	30	brown	none	brown	none

Notes
• Intensity: + (low); ++ (moderate); +++ (high); ++++ (very high)

Differentiation of callus

The callus from healthy segments growing on MS basal medium with 2,4-D (0.1 mg/L) and BA (1 mg/L) after the sixth or seventh passage (i.e. 6 or 7 months) gradually turned green, and on further subculturing started giving rise to shoot-buds and embryoids. Similarly the callus from spiked trees routinely subcultured on MS basal medium supplemented with 2,4-D (0.1 mg/L), BA (1.0 mg/L) and GA_3 (4.0 mg/L) also turned green; after the eighth passage (about 8 months) it gradually gave rise to shoot-buds and embryoids. Some of the cultures also produced small plantlets, which had all the phenotypic characters of the sandal plant.

Discussion

Callus initiated and maintained on nutrient media in-vitro is dual-purpose: it can be studied to help understand plant growth and development; and it can be exploited for plant products and propagation. Its induction requires three equally important considerations:

- selection of an explant;
- provision of a suitable nutrient medium and culture conditions; and
- isolation and maintenance of callus for subsequent generations.

With these points in mind, the internodal segments were selected as explants from both healthy and spike-diseased trees and were aseptically transferred to the White's basal medium supplemented with different growth regulators:

- auxins (IAA, NAA and 2,4-D),
- cytokinins (BA and Kinetin), and
- gibberellic acid.

IAA (0–10 mg/L) with kinetin (0 and 1 mg/L) was not congenial for callus initiation in both normal healthy segments and spike-diseased segments. However, segments from the normal healthy trees remained green up to two months with IAA (0.1 and 1.0 mg/L) and 1 mg/L of kinetin, or both. Few of them showed initial callus initiation, without any further growth. The spiked segments turned brown immediately one week after inoculation with all treatments. Srimathi and Sreenivasaiah (1963) attempted to culture root tips, haustoria and lateral buds from normal healthy trees on White's basal medium and reported that only 10 per cent of the root tips showed an increase in their length from 4–5 mm to 7–8 mm without any callus initiation. However, Rangaswamy and Rao (1963), using endosperm as explants, reported callus initiation from healthy sandal trees on White's medium supplemented with two mg/L of 2,4-D, five mg/L of kinetin and 0.25 per cent yeast extract. Hence, the mature endosperm tissue did not proliferate in the absence of an embryo.

Even when kinetin levels were increased to 2–4 mg/L with IAA, neither healthy nor spiked segments induced any callus initiation. Here also the healthy stem segments remained green up to two months at 0.1 and 1 mg/L of IAA and 2–4 mg/L of kinetin, and the spiked segments gradually turned brown and died.

Substituting BA (1, 2 and 4 mg/L) for kinetin has resulted only in keeping the healthy tree segments green up to two months without any callus initiation. NAA (0–20 mg/L) in place of IAA and BA or kinetin (0–4 mg/L) did not help induce callus initiation and growth. Again, the spiked segments could not survive on any of these supplements in the medium.

Healthy segments with 2,4-D and BA produced callus; a combination of 2,4-D (0.1 mg/L) and BA (1.0 mg/L) was optimal. Diseased segments remained brown and without callus. Thus, 2,4-D combination with BA was effective for induction of callus and for further subculture of healthy tissues. Similar results have been reported by many others, but with using growth adjuvants of undefined organic nature, such as yeast extract and coconut milk, in the medium (e.g. Rangaswamy and Rao 1963; Rao 1965; Rao and Rangaswamy 1971).

Initiation of callus from the exposed cut surfaces of healthy sandal took place 3–4 weeks after inoculation, and further growth was also very slow. To obtain a callus mass of about one cm^3 took 3–4 months.

With a view to increasing the rate of callus growth, all treatments of growth regulator combinations were repeated on Murashige and Skoog's (MS) basal medium. It is known that MS medium is richer in most of the inorganic and organic constituents than White's medium. MS basal medium supplemented with NAA or IAA, and BA or kinetin (1–4 mg/L) did not induce callus formation, either from healthy segments or spiked segment.

However, when 2,4-D was substituted for IAA or NAA in the presence of BA (1 mg/L) it resulted in fairly uniform, compact, white callus initiation at 3–4 weeks; it could also be subcultured at monthly intervals on a similar medium. Spiked segments could not survive on 2,4-D with BA on MS medium.

Even though Rao and Rangaswamy (1971) obtained callus from embryos from fruits of sandal,

Rao and Bapat (1978) regenerated shoot-buds on hypocotyl segments but failed to get any callus from excised stem segments of mature sandal plants. Lakshmi Sita et al. (1979) also reported that callus initiation was very slow at first on shoot segments, and that it took two months for a good callus to develop. Only MS medium supplemented with 1 mg/L of 2,4-D and 0.2–0.5 mg/L of kinetin or 1 mg/L of NAA or IAA with 0.5–1.0 mg/L of BA developed good callus.

Lakshmi Sita et al. (1982), Rao and Raghavaram (1983), and Bapat and Rao (1984) demonstrated callus induction from healthy sandal segments on media with either auxin alone or an auxin and a cytokinin in the presence of complex organic nutrient factors like coconut water, malt extract or yeast extract. As already pointed out, in the present study it was intended to avoid organic growth factors in order to standardise the growth of callus cultures on purely synthetic medium to study the disease-causing organisms, and this objective was achieved.

Different combinations of growth regulators, particularly auxins and cytokinins, that were tried for standardisation of callus initiation and its further growth from normal healthy segments, were not favourable for the spiked segments. In addition to an auxin (2,4-D) and a cytokinin (BA), gibberellic acid was necessary in MS basal medium for initiation and further subculturing of spiked tissue cultures. At concentrations of GA_3, 2–4 mg/L, 2,4-D (0.1 mg/L) and BA (1 mg/L) the spiked segments remained green and the callus was initiated with uniform growth on all the segments.

From these results it was also clear that gibberellic acid is an obligate adjuvant along with 2,4-D and BA for the successful induction of callus and for its further growth from spiked segments. Callus induction was not only very slow, but the quantity was much less; and it appeared like a pin-head at the exposed ends after about five weeks of inoculation, as against 3–4 weeks on healthy segments. This might be because of altered metabolic activities and reduced endogenous growth substances in the spike-diseased tissues, a question which is discussed below later.

The salient findings of these investigations are that the spike-diseased stem segments were cultured for the first time and further, gibberellic acid in the medium is critical with 2,4-D and BA for the initiation of the callus and for further maintenance of axenic culture of spiked tissue. Literature survey has revealed that so far spiked tissues have not been cultured in vitro, even though the disease was discovered a century ago, and that this is possible now on a synthetic medium.

The routinely subcultured callus from healthy trees maintained on MS basal medium fortified with 2,4-D (0.1 mg/L) and BA (1 mg/L) after the 6th or 7th passage (6–7 months) gradually showed greenish tinges on the callus. Further, these portions turned greener with protuberances, eventually giving rise to shoot-buds and embryoids.

Also the callus pieces from spiked segments periodically subcultured and maintained on MS basal medium with 2,4-D (0.1 mg/L), BA (1 mg/L) and GA_3 (4.0 mg/L) showed signs of differentiation after the 8th passage (about eight months). Here again in the beginning green islands appeared on the growth callus and gradually differentiated into shoot-buds and embryoids. Some of the cultures also produced small plantlets from the growing callus pieces, and these could grow into independent sandal plants in soil.

Similar studies on differentiation of callus from normal healthy trees into embryoids and plantlets were reported by Rao (1965) and Rao and Rangaswamy (1971) who obtained callus from embryos on White's basal medium supplemented with yeast extract, kinetin and 2,4-D. Shoot-buds were also regenerated from hypocotyl segments of healthy sandal on basal media supplemented with auxins alone (Rao and Bapat 1978), but excised stem segments of mature plants failed to respond (Bapat and Rao 1984). Similarly, shoot-bud regeneration from hypocotyl explants was achieved on all auxin media (Rao and Ranghavaram 1983). In some instances, they proliferated into a callus tissue from which many globular embryos differentiated.

From the point of view of a tree improvement program, regeneration of plants from sections of embryos, endosperm and young seedlings has one considerable disadvantage. Although these embryos and seedlings might have been obtained from seeds (or fruits) of selected superior varieties, their genotypes might be different from that of their parents because segregation and superior characteristics in a parent are not necessarily retained by propagules. Therefore Lakshmi Sita et al. (1979) took shoot-segments of 20-year-old trees of proven quality and induced shoots directly from shoot-tip callus and embryoids in shoot-segment callus on a medium supplemented with GA_3. Further, Lakshmi Sita et al. (1980a) induced embryogenesis in suspension cultures, and Bapat et al. (1985) regenerated somatic embryos from protoplasts isolated from stem callus.

Conclusions

At present we cannot define the conditions necessary for embryogenesis. Lakshmi Sita et al. (1980b) differentiated embryoids occurring on a medium supplemented with GA_3. But in our experiments the differentiation of the callus into embryoids occurred without any GA_3 in normal healthy segments, whereas GA_3 was necessary for callus induction, maintenance and differentiation into somatic embryos of spiked tissue.

We found that GA_3 was not inhibitory, despite earlier reports to the contrary (e.g. Lakshmi Sita 1986). Describing the advantages of somatic embryogenesis, Lakshmi Sita reported that plantlets arising from embryoids have a taproot system which is superior to adventitious roots. By inducing somatic embryogenesis, numerous embryoids can be obtained. For practical application of somatic embryogenesis from callus cultures, the source of cells to be propagated should be of vegetative origin, such as shoot-pieces (Lakshmi Sita 1982).

In-vitro culture of spiked tissue was accomplished here. Its advantages are:

- difficulty in isolating the organism can be overcome;
- maintenance of dual cultures of host-parasite is facilitated;
- correlative phenomena of the host can be overcome;
- toxins produced and metabolic changes at tissue and cellular level can be readily studied; and
- obligate parasites like mycoplasma can be isolated in vitro.

So far no work, other than the present findings, has been reported on tissue-culturing of spike-diseased tissues of sandalwood. However similar studies are reported in other plant mycoplasma diseases. Petru et al. (1971) cultured tomato plants infected with potato witches' broom and found that callus was easier to obtain from diseased plants than from healthy ones on relatively simple media, and that callus isolated from diseased plants grew much faster. This may be because of the herbaceous nature of tomato plants, but callus tissue cultures established by Ulrychova and Petru (1975) from stems of both healthy and mycoplasma-infected tobacco plants observed no differences in the growth and development of either tissue; further, callus derived from diseased plants differentiated after three months, whereas callus from healthy controls showed differentiation only after six months.

The difference in callus formation, sustained growth and differentiation between diseased and healthy stem segments is attributed to the reduced amounts of endogenous GA_3 in the spiked tissues.

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Spike Disease of Sandal (*Santalum album* L.): A Patho-physiological Study

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Abstract

Intact leaves, callus and differentiated tissues of normal sandal trees contain higher amounts of chlorophyll pigments, and lower amounts of starch and reducing sugars, than those from spike-diseased trees. The presence of mycoplasma-like organisms (MLO) only in the spike-diseased trees, demonstrated by electron microscopic studies, confirmed the mycoplasmic nature of the disease. Further, these bodies showed signs of gradual degeneration in cultured callus tissues after the first subculture; in subsequent subcultures they were not observed at all, even in differentiating tissues. Thus co-cultures of host and pathogen could only be maintained in vitro for a short time. As spiked callus differentiates into plantlets, there is also a possibility of disease-free clones. We hypothesise that the pathogen (MLO) is diverting the host metabolites for its own benefit towards abscissic acid and sterols (specifically required by mycoplasmas for their growth in vitro), at the expense of $GAv3v_3$ and chlorophyll pigments in the stunted, chlorotic and leaf-abscissed, spike-diseased trees.

FOR A LONG time, spike disease was thought to be of viral nature because of the disease syndrome and its graft-transmissibility (Coleman 1917). It is now shown that spike disease of sandal is due to a mycoplasma-like organism (MLO) (e.g. Hull et al. 1969; Dijsktra and Ie 1969). Mycoplasma diseases have increased in recent years all over the world (Ulrychova et al. 1983). Owing to the limitations for the isolation and cultivation in vitro of the causal agent, the technique of tissue culture and axenic host-pathogen co-cultures are an important tool for the study of the metabolism of the disease.

Much biochemical work has been done from 1940 to 1982. It emphasises the physiological characteristics that differentiate spiked and healthy sandal plants, without deciphering the actual biochemical targets and mechanisms of the disease itself. It is only recently that it has been reported to be caused by mycoplasma (Hull et al. 1969), although the true nature of the causal organism awaits further confirmation in in-vitro studies. Technological advances in the past 10–15 years have permitted the cultivation of many biotrophic parasites in axenic and monoxenic cultures on host calluses. The mechanisms of the action of MLO and RLO (Rickettsia-like organism) agents of plant diseases are likely to be elucidated when these agents are grown in culture media. So far, *Spiroplasma citri* has yielded some data, but nothing is known about the mechanism of action of all other microorganisms of yellows, big bud and witches' broom diseases caused by MLOs.

When a microorganism successfully colonises a host cell, the metabolism of the cell is altered to the advantage of the parasite. Although most of the metabolic pathways are common between the parasite and host, the parasite also has some distinct metabolic pathways. Therefore a study of host–parasite interaction at cellular and tissue-culture level helps understand shifts in host metabolism. Alterations in growth regulators are characteristic of diseased plants. Observations on the involvement of growth regulators in the development

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of symptoms of infected plants have been made, but not clearly understood in many diseases, including: yellows, downy mildews, dwarfs, grassy stunt, leaf curling and malformation.

Investigation of phytopathogenic MLOs in plant tissue culture is both theoretically and practically important. Tissue culture provides homogenous controlled conditions for studying the characteristics of reproduction and development of MLOs in the plant cell. The practical significance of investigations on mycoplasmas of man and animals performed in animal tissue cultures, lies in the possibility of using the same methods to obtain disease-resistant plants. The currently employed method of antibiotic therapy often does not completely rid the plants of mycoplasma infection.

Methods

The aims of the present work were to:

- demonstrate the MLO nature of the pathogen in spike disease by observing penetration of an MLO into tissue culture obtained from sandal spike explants containing large accumulations of MLO in the phloem tissue;
- eliminate the mycoplasmic infection using tissue culture with subsequent organogenesis and formation of plant regenerates.

It has been reported that spike-diseased stem segments could be cultured on MS basal media supplemented with 2,4-D, BA and gibberellic acid (Gowda and Narayana 1986). Intact leaves, callus tissues and differentiated (regenerated) tissues from healthy and spike-diseased trees were analysed for physiological parameters such as chlorophyll, reducing sugars and starch content. Also, the axenic cultures were examined by electron microscopy.

Electron microscopy

Callus tissues derived from diseased and normal sandal plants were cut into small sections of about 2.0 mm^3 and fixed in three per cent gluteraldehyde (prepared in cacodelyte buffer, pH 7.3) for 24 hours. They were vacuum-infiltrated for 30 minutes, with frequent agitation to make the segments sink in the fixative solutions, before they were transferred to the refrigerator and stored overnight in the same buffer.

Next day the segments were post-fixed in one per cent osmium tetroxide (OsO_4) prepared in 0.1 M phosphate buffer (pH 7.3) for two hours. The tissue was washed twice in the same buffer, followed by dehydration in a graded ethyl alcohol series. First they were transferred to 70% ethanol for 60 minutes; then to 80% for the same time, followed by two changes in 90% ethanol at 30 minute intervals; then two changes in absolute methanol at 30 minute intervals. Enblock staining was done with a mixture of uranyl acetate and lead citrate for 60 minutes. Finally the tissues were washed in absolute methanol/methyl alcohol for 30 minutes, and transferred to propylene oxide with two changes at 15 minute intervals. The tissues were immersed in a mixture of propylene oxide and Spurr's embedding medium (1:1 w/w), and left on a rotator at 200 rpm overnight. Polymerisation was carried out in a dry oven at 70°C for 24 hours.

Ultra-thin sections were collected on copper grids and subsequently stained with uranyl acetate followed by lead citrate (Reynolds 1963). Then, they were examined under a transmission electron microscope for the presence of MLO bodies.

Estimation of reducing sugars by the 'Somogyi-Nelson micro-copper method'

A known amount (500 mg) of each dry tissue was fixed in boiling ethanol (96%), and boiled under reflux; the evaporating alcohol was condensed. This was followed by extraction in 80% ethanol, boiling for 10 minutes and decanting. These fractions were then grouped and evaporated to dryness. The residue was re-dissolved in 10 ml of distilled water. These extracts were preserved for the estimation of reducing sugars by 'Nelson's method'.

Estimation of chlorophylls

Five g of fresh tissue was ground in a pestle and mortar using 40 mL of 80 per cent acetone. This was filtered through a Whatman No. 1 filter paper in a Buchner funnel under vacuum. The volume of the filtrate was made up to 40 mL using 80 per cent acetone. This solution was diluted with 80 per cent acetone depending on the amount of pigment present in the solution. Absorbance was recorded at 663 and 645 nm wavelengths using a UV double-beam spectrophotometer.

Chlorophyll (mg/g) was computed by the formulas (Arnon 1949):

- chlorophyll 'a' = $12.7^{A}663 - 2.69^{A}645$ V/ (d × 1000 × W)
- chlorophyll 'b' = $22.0^{A} - 4.69^{A}663$ V / (d × 1000 × W)
- total chlorophyll = $20.2^{A}645 - 8.02^{A}663$ V/ (d × 1000 × W)

(where: V = final volume (mL); d = length of light path (cm); w = fresh-weight of the tissue (g)).

Estimation of starch

Starch content was estimated colorimetrically following Allen et al. (1974), and calculated using the formula: starch (%) = C × Solution volume (ml)/ (10 × Aliquot (ml) × Sample weight (g)) (where: C (mg) = starch obtained from the standard graph).

Results

Intact leaves, isolated callus and regenerating tissues from both healthy and spike-diseased trees were analysed for chlorophyll pigments, starch content and reducing sugars.

Chlorophyll

The results pertaining to chlorophyll pigments are presented in Tables 1–4. Total chlorophyll and chlorophyll 'a' were higher in all three types of tissue from normal healthy trees than in spiked trees. Chlorophyll 'b' was also higher in leaves and regenerated tissues of healthy plants, but the callus of healthy tissues has slightly less than that of spiked tissues. Chlorophyll contents of in healthy leaves were 2–3 times those of spiked leaves. Further, the chlorophyll 'a:b' ratio was 2.06 in healthy leaves compared with 1.84 in diseased leaves.

Pigment contents were relatively very low in callus and regenerated tissues compared with those in leaves (Tables 1–3). Total chlorophyll and chlorophyll 'a' levels were a little more in healthy callus than in spiked callus, and chlorophyll 'b' is the converse. Chlorophyll 'b' contents were more than chlorophyll 'a' in regenerated tissue derived from both healthy and spiked material. The chlorophyll 'a:b' ratios were 0.82 and 0.90 in healthy and spike differentiated material respectively.

Thus total chlorophyll and chlorophyll 'a' were higher in all three types of tissue (leaf, callus, and differentiating tissue) from healthy trees than spiked trees. The reduced amounts of pigment in spiked leaves is the result of yellowing of leaves, which is one of the conspicuous symptoms of spike-diseased trees. Parthasarathi (1979) also found reduced pigment contents in spiked leaves. The reduced chlorophyll content, in spiked leaves indicates reduced photosynthetic activity. Iyengar (1928) reported decreased photosynthetic activity of spiked leaves, because they fixed lower amounts of CO_2 per unit leaf area.

The pigments were very low in callus and regenerating tissues compared with leaves. Within these two, the pigment contents were slightly higher in differentiated and regenerating tissues than in undifferentiated callus. This is because of the differentiated and regenerated tissue consisted of shoot-buds and embryoids, which were green in colour, and more chlorophyll synthesis must have taken place as differentiation occurred. As the callus from healthy tissue was slightly greener than that from spiked callus, the former obviously contained relatively higher chlorophyll contents.

The spiked callus, regenerated healthy tissue and regenerated spiked tissue had slightly higher chlorophyll 'b' than chlorophyll 'a'. This is be the probable reason for the faster regeneration from healthy callus into shoot-buds and embryoids compared with spiked callus differentiation. None of the tissue-culture studies of sandal has analysed for pigment content; this is even more pertinent for the spiked tissues, as they have not been cultured hitherto.

Starch

Starch contents were estimated in intact leaves, callus tissue and differentiated tissues of both healthy and spike diseased trees (Table 2). The starch contents were relatively higher in spiked leaves, 'spiked

Table 1. Chlorophyll 'a' and chlorophyll 'b' content in leaves, callus and differentiated tissues from healthy and diseased trees (mg/g fresh weight)

Tissue	Chlorophyll 'a' CD = 0.1998 (5%)		Chlorophyll 'b' CD = 0.1087 (5%)		Chlorophyll 'a:b'	
	Healthy	Spiked	Healthy	Spiked	Healthy	Spiked
Leaves	1.130	0.350	0.550	0.190	2.06	1.84
Callus	0.007	0.005	0.006	0.007	1.14	0.72
Regenerated	0.015	0.014	0.018	0.016	0.82	0.90

callus' and 'spiked regenerated tissues'. Abnormal accumulation of starch in diseased leaves was also reported by Iyengar (1928) and Parthasarathi (1979).

Table 2. Starch content in leaves, callus and differentiated tissue of normal and spiked material (%)

Tissue	Healthy	Spiked	Difference
Leaves	6.4	8.2	1.8
Callus	5.8	6.5	0.7
Regenerated tissue	5.6	6.2	0.6

CD = 1.79 (5%)

Reducing sugars

Levels of reducing sugars were very high in all the spiked tissues—leaves, callus, and regenerated tissues (Table 3). There were significant differences between in leaves, callus and regenerated tissues of healthy and spiked trees. Similar results were reported by Iyengar (1928) and Parthasarathi (1979) in the intact leaves. According to them, the sugar accumulation presumably occurring in the chlorotic spiked leaves, (as a result of impaired translocation due to necrosis of phloem elements) naturally led to increased starch formation to prevent an abnormal rise in the osmotic pressure of the tissue. However, the enzymes related to starch breakdown also determine starch balance in the tissue.

Table 3. Reducing sugars in leaves, callus and differentiated tissues of normal healthy and spiked material (mg/g dry weight)

Tissue	Healthy	Spiked	Difference
Leaves	1250	1700	450
Callus	1200	1600	400
Regenerated tissue	1240	1680	440

CD = 204.4 (5%)

The spiked tissues had to be maintained on a medium supplemented with gibberellic acid in addition to other growth regulators. One of the formative effects of gibberellic acid is de-novo synthesis of α-amylase which hydrolyses starch; this might have resulted in reduced starch contents and increased reducing sugars in callus and regenerated tissues of spiked trees as well as the in-vitro tissues.

Discussion

The symptoms of spike disease are similar to those of so-called 'yellow diseases'. Hull et al.(1969) reported for the first time that electron microscopic examination of thin sections of infected leaves and stems of spiked sandal trees revealed the presence of MLO bodies in sieve tubes of phloem cells.

In the present investigations, electron microscopic examination of the ultra-thin sections of the internodal segments, leaf petioles and leaf midribs of spiked trees also showed MLOs in phloem cells. They were comparable with those described by Hull et al.—slightly irregularly shaped, round 100–600 nm in diameter. They occurred mostly as oval or spherical forms with a trilaminar unit membrane visible as two black lines separated by an electron-lucent space; they contained ribosome-like material and fibrillar strands of DNA. Such MLO bodies could be found in neither healthy sandal material nor parenchyma tissue of diseased trees.

Aseptically growing tree explants were also subjected to electron microscopic examination. Here also MLO bodies were observed, but their frequency and size were much less than their original tree explants. Further, these bodies showed signs of gradual degeneration in cultured callus tissues after the first subculture; in subsequent subcultures they were not observed at all, even in differentiating tissues. Thus co-cultures of host and pathogen could only be maintained in vitro for a short time.

In a similar study reported by Jacoli and Ronald (1974), plant tissue cultures were maintained in an effort to preserve and propagate the disease agent of aster yellows. As the tissues aged, the MLO bodies lost their distinct outlines, and diminished. According to Fedotina and Krylova (1976), MLOs survived in explants of the stems of tobaccos that serve as the starting material for tissue culture production after five weeks of residence on MS medium. However, they were absent in cells of the primary callus. Jacoli (1978a) reported that MLOs were seen in the phloem of the primary cultures of carrot explants, but during subsequent transfers they gradually degenerated and ultimately disappeared within 80 days; in differentiated tissues the new phloem did not contain MLOs. In yet another study by Jacoli (1978b), several forms of MLO were observed in electron micrographs of phloem cells from explants infected with aster yellows disease. The structures were found only in explants 11–25 days old, when the degenerating MLOs were first observed.

The internodal spiked segments, unlike healthy segments, required GA_3 in their growth medium in addition to 2,4-D and BA for the induction and sustained growth of callus and its differentiation into embryoids. This prompts us to compare the symptoms of the sandal spike disease with the formative effects of GA_3; this is shown in Table 4. It appears that symptoms of sandal spike contrast with the GA_3 effects, and would therefore be similar to GA_3 deficiency symptoms.

Hence, the differences in callus formation and further differentiation of diseased and healthy segments can be attributed to deficiency in endogenous levels of gibberellic acid in the spiked tissues.

Once exogenously supplied GA_3 reaches the threshold levels in the medium, the response of the callus induction and sustained growth is similar to that of the healthy segments. Reduced amounts of endogenous GA_3 in some viral diseases (Russel and Kimmins 1971; Maramorasch 1957) and fungal diseases (Bailiss and Wilson 1967; Evans and Wilson 1971) is reported. These hypotheses are further strengthened with the presence of MLO bodies by interrelating the metabolic pathways of the host and the pathogen. It is well known that mycoplasmas have a specific requirement for sterols for their growth in vitro.

In the biosynthetic pathway of terpenoids by the mevalonate route, the active isoprene, Isopentinyl pyrophosphate, gives rise on condensation to a series of terpenes—hemiterpenes, monoterpenes, sesquiterpenes, triterpenes, diterpenes, tetraterpenes, and polyterpenes—the configuration depending on the number of isoprene units involved in the condensation. By condensation of two units it gives rise to Geranyl pyrophosphate, which in turn gives rise to monoterpenes. With the addition of one more unit of IPPi to Geranyl PPi we get Farnesyl PPi which is the source material for sesquiterpenes (ABA) and triterpenes (sterols). If one more unit of IPPi is condensed with Farnesyl PPi, it gives rise to Geranyl geranyl PPi from which diterpenes (phytol, gibberellins) are synthesised.

Thus, both gibberellic acid (a diterpene) and sterols (triterpenes) are synthesised from the condensation of isoprene units.

Conclusions

It is reasonable to hypothesise that mycoplasm in the diseased spike tissue may divert the metabolites towards sterols and abscissic acid (ABA) at the cost of GA_3 and the phytol chain of chlorophyll pigment. (Leaf abscission is one of the functions of ABA.) It is possible that in the diseased tissue GA_3 and chlorophyll biosynthesis might have been inhibited, and this accounts for the low chlorophyll content. However, this hypothesis needs further confirmation by detailed studies on the enzyme systems which are likely to trigger these pathways in mycoplasma or chlorotic spike-diseased tissues, or both.

Regulation of carbon flow through the many branches of the isoprenoid formation in higher plants, and regulation of the synthesis of this vast array of compounds with their equally wide range of functions, offers a potentially fruitful area of research.

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Table 4. Comparison between sandal spike-disease symptoms and formative effects of GA_3

Characteristic	Sandal spike symptom	GA_3 effect
Internode growth	Shortening	Elongation
Senescence	Leaf yellowing/chlorosis	Prevention of senescence
Flowering	Inhibition	Induction
Vigour	Leaf abscission	Reversal of dwarfism
Sugars	Starch accumulation	De novo α-amylase synthesis

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Fluorescence Microscopy of Sandal Affected with Spike Disease

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Abstract

Fluorescent microscopic technique using a *Hoechst 33258* can diagnose the presence of spike phytoplasma in fresh, as well as stored, plant tissues. Phytoplasma was detected in plant materials stored for more than one month under different storage conditions. This technique was suitable for studying the distribution of spike phytoplasma at different stages of disease development. The 'compression method' suggested for soft tissues was also good for detecting phytoplasma in spiked trees.

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Detection of Phytoplasma in Spiked Sandal Using DAPI Stain

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Abstract

Spike disease caused by phytoplasma is the most serious disease affecting sandal (*Santalum album* L). It is characterised by extreme reduction in size of leaves and internodes, accompanied by stiffening of leaves. Spiked trees usually die within 12–36 months. The unicellular, non-culturable phytoplasma is seen exclusively in the phloem tissues of diseased plants. A DNA-specific fluorochrome, 4,6-diamidino-2-phenyl indole (DAPI), binds to the DNA of phytoplasma, especially that in phloem sieve tubes. Freehand sections of diseased and healthy sandal tissues were stained with 0.001% DAPI and viewed under a UV fluorescent microscope. Phloem tissues of diseased plants showed characteristic fluorescence. The concentration of the pathogen was high in the inner bark, and it decreased towards the root and shoot-tip; this was assessed by observing relative intensity of fluorescence and the number of fluorescent spots. No fluorescence was detected in the sieve tissues of healthy plants.

THE MOST SERIOUS disease of sandal, spike disease, is characterised by extreme reduction in size of leaves, accompanied by stiffening and reduction of internodes; in the advanced stage, the whole shoot looks like a chimney brush. Diseased trees die within 12–36 months after the appearance of symptoms (Rangaswamy and Griffith 1941). The causative agent of the disease, a non-culturable phytoplasma in the phloem was first detected by electron microscopy three decades ago (Dijkstra 1968; Hull et al. 1969; Dijkstra and van der Want 1970).

Light microscopic detection of phytoplasma has been reported using specific stains like Mann's stain (Parthasarathi et al. 1966), Giemsa and dienes (Ananthapadmanabha et al. 1973), and aniline blue (Ghosh et al. 1985).

As a tool in microscopy, fluorescence provides many possibilities in addition to absorption methods for studying this disease. It is possible to observe a very small number of fluorescent molecules; approximately 50 molecules can be detected in 1 μm^3 volume of a cell (Lansing Taylor et al. 1986). The DNA-binding fluorochrome, 4,6-diamidino-2-phenyl indole (DAPI), was first used to detect mycoplasma in tissue culture two decades ago; since then it has been employed by phytopathologists to detect phytoplasma in several plants, for example *Rubus fruticosus* (Marcone et al. 1994a) and *Lactuca sativa* (Marcone et al. 1994b). This paper reports the results of studies on detection of phytoplasma in spiked sandal using DAPI stain.

Materials and Methods

Twigs from sandal (scion) affected by spike disease collected from the reserve forests of Marayoor were wedge-grafted on to one-year-old healthy sandal in the glasshouse of Kerala Forest Research Institute, Thrissur.

Healthy and spike-diseased tissues of sandal collected from the field and maintained in the glasshouse were screened for phytoplasma. Stem, leaf, petiole, root and root-tip tissues were fixed in five per cent formaldehyde in 0.2 M phosphate buffer, pH 7.0, for 30 minutes. They were then washed in 0.01 M phosphate buffer, pH 7.0, for 3 minutes. Freehand sections of

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about 20 µm thickness were stained with 0.001% DAPI in 0.01 M phosphate buffered saline, pH 7.45, for one hour, mounted in water and viewed under a Leitz Dialux fluorescent microscope with a HBO 50W bulb.

Results

Grafts established on 75 percent of the plants. Spike disease symptoms appeared within 60 days after grafting. All the tissues of diseased sandal showed the characteristic yellow-green fluorescence in the phloem region after staining with DAPI. The intensity of fluorescence was high in the stem, inner bark and petiole compared with that in the leaf and root. No fluorescence was observed in healthy sandal tissues, nor ion the root-tips of diseased plants. In highly diseased trees the intensity of fluorescence was less towards the terminal portion of the stem tissues, while phloem of inner bark showed intense fluorescence as evident from large number of fluorescent spots in different layers of phloem tissues (Fig. 1).

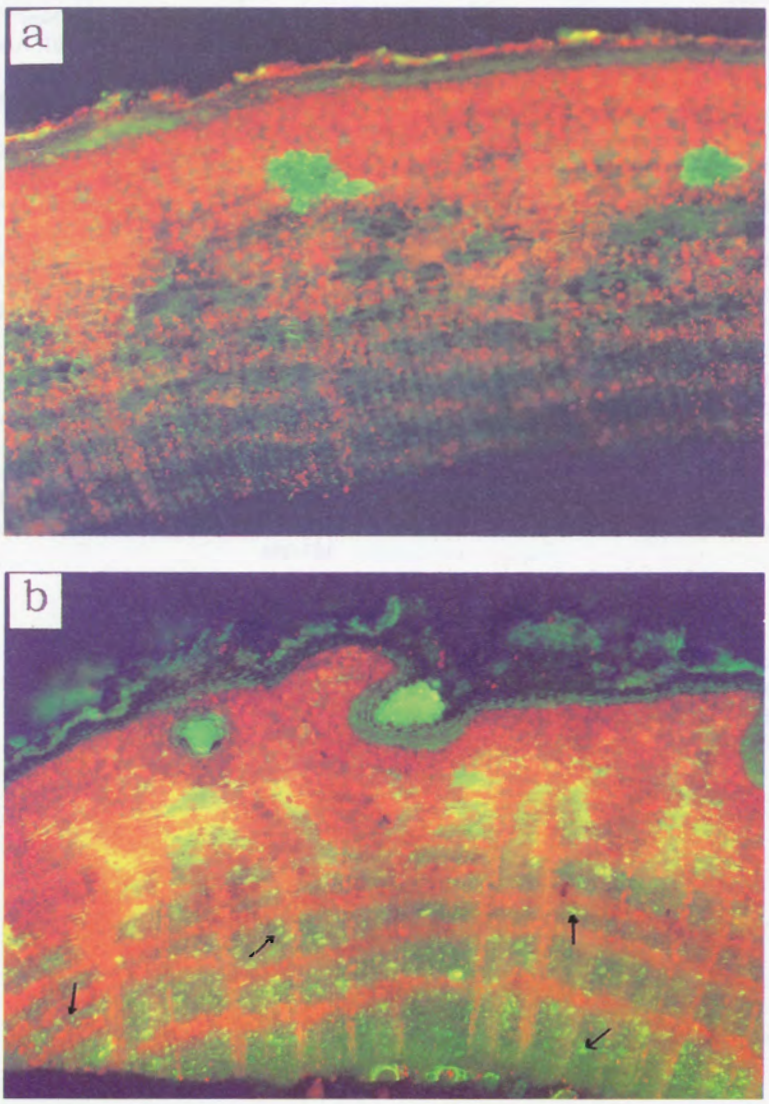

Figure 1. Fluorescent photomicrograph of (a) healthy, and (b) diseased sandal inner bark, showing fluorescent spots in the phloem of diseased tissue (cross-section ×70; DAPI stain)

Discussion

Compounds exhibiting fluorescence are called fluorochromes. When a fluorochrome absorbs light, energy is taken up for the excitation of electrons to higher energy states. The process of absorption is rapid and is immediately followed by a return to lower energy states, which can be accompanied by emission of light. According to Stokes's law, the wavelength of emission is almost always longer than the wavelength of excitation. It is this shift in wavelength that makes the observation of the emitted light in a fluorescence microscope possible.

The advantage of fixation with formaldehyde is that proteins are bound together by crosslinks and bridges, autolysis is prevented, and most lipids and some enzyme systems are preserved. Optimum fixation time was 30 minutes; increasing the fixation time resulted in high background fluorescence.

DAPI staining is reported to be more sensitive than electron microscopy, but it is limited when the MLO population is very low, as is often true for woody hosts (Lederer and Seemuller 1991). DAPI binds to the AT-specific double-stranded DNA. In our observations using DAPI, the intensity of florescence was high in inner bark, stem and petiole and less in root and leaf. No fluorescence was observed in the root-tip region. In severely diseased trees the fluorescent spots were fewer towards the terminal portion of the stem. This may due to the degeneration of newly-formed sieve tissues in the stem.

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Spike-like Disease in Sandal

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Abstract

Sandal trees are distributed in South Seoni District, Madhya Pradesh in an area of about 3000 ha. They contain a significant amount of scented heartwood and oil. Trees exhibited symptoms similar to spike disease occurring in South India. Symptoms observed were reduction in leaf size, occurrence of short dead branches, yellowing of leaves, and occasional occurrence of tufts of axillary shoots arising from the main branches. On closer observation of the trees, and based on different tests conducted for spike disease, it was evident that the disease-like symptoms were due to physiological and abiotic factors in the area, and there was no incidence of spike disease in the sandal population.

Key words: spike disease

AS EARLY AS 1980 sandal trees were introduced into South Seoni RF, Madhya Pradesh, by Sri Narayan Prasad in Compartment No 249. Now they have spread to an area of over 3000 ha. in 13 compartments; at the last count, in 1987, 16581 trees over 20 cm girth were marked. During 1995 there were more trees over 60 cm girth with a clear bole of over four metres. The density was 100–125 per ha. Fruit-setting and regeneration was poor. Many trees had dry branches, and from a distance the whole tree looked like spike disease.

Hence a thorough investigation was conducted to find the cause of death, and to suggest remedial measures to improve the sandal population in the area.

Methods

The entire area was surveyed during May 1995, and deformed trees were thoroughly investigated.

Nearly 80 per cent of trees were unhealthy; the leaves were yellow and small, and there were very few of them. Some of the branches showed leaf and tip drying (Fig. 1). In most trees, tufts of leaf arose directly from the bigger branches and looked just like spike disease. On closer observation it was found that these small leaves were a new flush of leaves. Also, some of the older leaves did not expand to their full length and breadth; they were yellowish, and instead of being firm were very soft to the touch. The twigs were dry and brittle. In many trees, the bark was black and did not adhere to the main stem.

A comprehensive suite of experiments was set up, and changes in sandal trees and surrounding vegetation were noted every three months until June 1996.

Disease

Even though the symptoms looked like spike disease from a distance, there were no typical morphological spike symptoms (McCarthy 1899). Evidently, the deaths were due to something else. Even so, the experiments were set up to rule out the possibility of spike disease.

Biometrical studies

Samples of small leaves were collected, and length and breadth recorded following Iyengar (1931).

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Figure 1. Sandal trees showing deceptive appearances of spike-like disease

Pollarding experiments

Six trees were selected in Chandanbagh (Seoni RF) in the midst of sandal population showing spike-like appearance when seen from a distance. Their branches were pruned, leaving the tree in the form of a pole. Soil working was done to a radius of 50 cm, to conserve moisture and to encourage shoot production.

Tetracycline therapy

Remission of symptoms by tetracycline injection or spray is an indirect test of whether the symptoms are caused by an MLO. Six sandal trees with yellow and small leaves were selected in Compartment 249, and sprayed and injected tetracycline hydrochloride 500 ppm. Four sprays and injections were given over 20 days from August to November 1995. The trees were monitored for recovery or remission of apparent disease symptoms.

Pests

Borer holes were often seen on the stem, and tunnels noticed by piercing with sharp sticks. Grubs were noticed when the stem was split vertically. Some 20 per cent of trees were attacked by *Aristobia* heartwood borers.

Termites

These were noticed in almost all the trees, either at the base of the stem or along it. The attack was mostly on the bark and sapwood, but also occasionally in the heartwood.

Abiotic factors

There was a continuous dry spell during 1994–95, and frequent fire occurred in the area damaging sandal trees and other vegetation. Understorey shrubs and herbs were absent. To try and control such groundfire, leaf litter was allowed to decompose, and trenches were dug 10 m apart to conserve moisture.

Results and Discussion

Eliminating spike disease

The following studies conclusively proved that the malformation of the trees being studied was due to factors other than spike disease.

- Biometrical studies revealed that the length:breadth ratio of leaves (L:B) was less than 2.5; it should have been above four if they were diseased (Iyengar 1931).
- The new flush of leaves and shoots produced after pollarding was normal; fresh twigs developed, and tended to produce normal branches (Fig. 2).
- Spike disease caused by MLO-like organisms are sensitive to tetracycline. Partially affected spike trees will produce normal and healthy leaves about three months after injecting tetracycline; this is a remission of disease symptoms. Treated trees in the field did not show any change in morphological symptoms, indicating that there was no disease inoculum in the trees.

These studies ruled out the possibility of spike in this area.

Figure 2. Pollarded trees showing normal fresh twig development

Influence of abiotic factors

Sandal is very sensitive to fire. Frequent groundfire increases the temperature on the ground surface. Shallow roots which run parallel to and just below the ground surface, particularly smaller roots, will be damaged by the heat. Consequently haustorial connection with the host roots dries up, with the result that absorption of water and mineral salts is affected. Fire also damages foliage and branches, and transpiration of water reduced accordingly.

The substantial reduction in transpiration and absorption of water from the soil caused tip and shoot drying. New leaves formed only in the major branches, and these did not attain normal size probably because adequate water and mineral salts were not available. The groups of leaf are too small to produce enough photosynthetic material to translocate to the developing region. In many trees, the bark was completely damaged at ground level, exposing the sapwood. The bark peeled, and the resulting discontinuation of the food-translocation tissue ringbarked the tree.

Conclusion

The cause of drying and death was not disease caused by any pathogen, but the cumulative effects of:

- constant groundfire,
- heartwood borer attack, and
- termite attack.

Stopping groundfire has increased leaf litter decomposition; conservation of moisture in the ground for one year significantly improved the growth of sandal trees. They looked much healthier and produced new flushes of normal green leaves. The smaller branches tended to develop into major branches and looked like a normal tree with a good crown. These progressive changes in the tree were mainly due to adequate soil moisture, no groundfire, and protection from grazing and browsing.

Regeneration by seed is likely in the future, and will conserve the germplasm resource.

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The Role of Trace Elements on the Growth of Sandal Seedlings at the Nursery Stage

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Abstract

Deficiency of the trace elements copper, zinc, manganese, molybdenum and boron was induced in three-month-old sandal seedlings grown in sand culture in controlled conditions. Deficiency symptoms such as stunted growth, chlorosis and curling of the leaves were observed. Boron, even in traces, was toxic to seedlings. Characteristic differences in the banding pattern of peroxidase isoenzyme in the affected leaves of seedlings compared with control can be taken as a diagnostic test, even before the visible manifestation of disease in the seedlings. There was a reduction in leaf area, decrease in chlorophyll activity and photosynthetic efficiency of the leaves suffering deficiency of trace elements. Application of individual trace elements to the sandal seedlings otherwise growing normally in a medium of sand, red earth and compost manure has increased growth and uptake of nitrogen, phosphorus, and potassium.

Key words: sandal seedling, trace element deficiency, peroxidase isoenzyme, chlorosis, photosynthetic efficiency, leaf area, chlorophyll

THE IMPORTANCE OF trace elements for the normal life processes of plants are well known, as are the disease symptoms under conditions of deficiency. Leaves seem to be particularly sensitive indicators of trace element deficiency, typical symptoms being size reductions, shape and structural abnormalities, and pale colour. The most commonly observed symptom of many deficiencies is chlorosis caused by interference with chlorophyll synthesis. A knowledge of the effects of deficiency of various trace on the growth of seedlings is necessary to understand the disease symptoms and to restore the plants to health.

Induction and Detection of Deficiency

In order to understand the type of deficiency symptoms that develop in a plant due to the absence of an individual trace element, it is necessary to induce deficiency. The study can be initiated by treating three-month-old sandal seedlings grown in sand culture at the nursery stage under controlled conditions with 'Arnon & Hoagland nutrient solution' (Arnon and Hoagland 1940), omitting one trace element at a time. Deficiency symptoms induced with the absence of boron need not be studied in the case of sandal, as it has already been recorded that boron is toxic to sandal seedlings (Kamala et al. 1986).

Deficiency symptoms can be detected by different methods, which are now discussed:

- morphological visual symptoms,
- isoenzyme studies,
- leaf-area index, and
- physiological studies.

Morphological visual symptoms

Visual disease symptoms (Table 1) develop in treated seedlings one month after the commencement of treatment, and some 60–70 percent of the treated seedlings die by ten months.

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Table 1. Symptoms observed in sandal seedlings under conditions of deficiency of various trace elements

Trace element	Deficiency symptoms
Copper	• White patches appear at the tip of the older leaves, gradually spreading to the entire part of the leaves, and ultimately resulting in the death of the seedlings. • Formation of white patches is accompanied by stunted growth in most of the seedlings.
Zinc	• Younger leaves turn yellow and become brittle as the leaves grow. • Unequal growth is seen in the leaf at some of the internodes. • The seedlings remain stunted in growth and gradually die.
Manganese	• Younger leaves turn yellow and become brittle as the leaves grow. • Irregular white patches appear on the leaves. • Unequal growth is seen in the leaf pair at some of the internodes. • Gradually the leaf dies.
Molybdenum	• Curling of the leaf occurs in some of the seedlings. • Older leaves show cupping. • Brown patches form, and subsequently turn white. • Gradually the seedlings die.

Isoenzyme studies

The study of peroxidase isoenzyme using the 'disc gel electrophoretic technique' in the control as well as in the diseased leaves shows characteristic differences in the pattern of the enzyme under the deficiency of different trace elements. The pattern under zinc and manganese deficiencies will be similar (Fig. 1). The banding pattern exhibited in the seedlings showing disease symptoms under the deficiency of the four trace elements studied could be seen in the seedlings even in the latent stage. Thus the biochemical changes in the seedlings occur under conditions of deficiency before visible manifestation of the disease symptoms in seedlings.

The characteristic banding patterns of leaf peroxidase isoenzyme associated with deficiencies of different trace elements can be used for quick detection of deficiency of particular trace elements. This technique can also be used to differentiate whether the disease symptom is due to trace element deficiency or viral infection. Once it is known that a particular trace element is deficient, application of that element brings the seedlings back to normalcy assuming that the disease has not progressed too far.

Leaf-area index

A plant which is deficient in trace elements will not produce normal leaves. It is likely that there will be a reduction in size due to impaired metabolic activity. In the study made on sandal seedlings, zinc deficiency had the greatest effect on leaf size, reducing the area to 3 cm^2, an 84 percent reduction compared with control; the reduction due to deficiency in copper, manganese, and molybdenum was 70, 72 and 67 percent, respectively (Table 2).

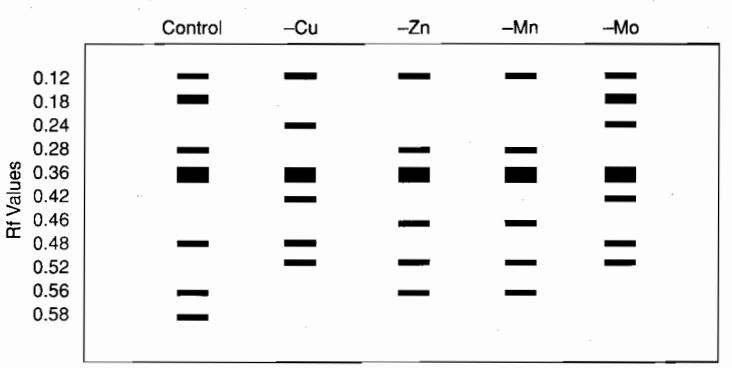

Figure 1. Pattern of peroxidase isoenzyme in the seedlings under the deficiency of trace elements and control seedlings

Table 2. Leaf-area of seedlings under deficiency of various trace elements

SI. No.	Trace element	Leaf area (cm^2)	Decrease in area (%)
1	*Control*	*22*	–
2	Copper	6.6	70
3	Zinc	3.5	84
4	Manganese	6.1	72
5	Molybdenum	7.3	67

Table 3. Chlorophyll activity and photosynthetic efficiency of leaves under deficiency of various trace elements

SI. No.	Trace element	Chlorophyll (mg/100 g of leaf tissue)	Photosynthetic efficiency ($mg/cm^2/sec.$)
1	*Control*	*142*	*0.49*
2	Copper	75	0.26
3	Zinc	57	0.20
4	Manganese	52	0.19
5	Molybdenum	110	0.38

Physiological studies

Trace element deficiency causes characteristic discolouration and considerable reduction in leaf area. These effects are due to metabolic changes in the seedlings.

The first symptom of expression depicted on the leaf is chlorosis. The chlorotic deficiency is due to reduction in the chlorophyll content, which in turn has caused the decrease in photosynthetic activity. Hence, photosynthetic efficiency is directly related to amount of chlorophyll present in the leaves. Loustalot et al. (1945) reported that deficiency of trace elements caused reduction in tung leaf area and decrease in photosynthetic activity. Keller et al. (1963) have reported the same phenomenon in Scotch and Norway Spruce. Sandal seedlings grown with deficiency of manganese showed least chlorophyll content and photosynthetic activity (Table 3).

Effect of the Application of Trace Elements

Application of individual trace elements copper, zinc, manganese and molybdenum at 'Arnon & Hoagland concentrations' to seedlings growing normally in pot culture in a soil medium increased uptake of N, P, and K and increased growth. Different elements have different effects, and molybdenum was most beneficial (Table 4). This study can be used in the nursery to boost the growth of sandal seedlings in a short time. Kolpikova (1973), Pudova (1974), Poggiani (1974), and Fessenden and Sutherland (1979) have also reported that application of certain individual trace elements to seedlings of forestry species, otherwise growing normally in a soil medium, showed much beneficial effect on their growth.

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Table 4. Growth increment and uptake of N, P & K by sandal seedlings under the influence of various trace elements

SI. No.	Trace element	Average growth increment (cm)	Mean uptake (dry-weight, %)		
			N	P	K
1	*Control*	*2.57*	*2.12*	*Traces*	*0.17*
2	Copper	3.41	3.39	0.09	0.22
3	Zinc	3.59	3.21	0.10	0.23
4	Manganese	3.56	3.36	0.10	0.19
5	Molybdenum	3.98	3.48	0.08	0.20

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Incidence, Damage Potential and Biology of Wood-borers of *Santalum album* L.

O.K. Remadevi and Raja Muthukrishnan*

Abstract

The wood of *Santalum album* is damaged by the borers *Indarbela quadrinotata* (Lepidoptera: Indarbelidae), *Zeuzera coffeae* (Lepidoptera: Cossidae) and *Aristobia octofasciculata* (Coleoptera: Cerambycidae). The infestation by these borers, especially the latter two, leads to die-back and mortality in smaller trees. *Indarbela quadrinotata*, the caterpillars of which feed on the bark, damage the sapwood by living in the bored cavities of the wood. The occurrence, nature of damage, symptoms and brief biology of the different borers are given in this paper. Survey of the sandal depots was conducted to study the extent of damage and loss of heartwood due to the attack of borers and termites. It was observed that 10–50 percent (average 25%) of sandal timber in the depots had hollowed heartwood. An average of 198.6 kg of heartwood is lost for every tonne of wood produced by sandal trees.

Key words: sandal; wood-borers; Cossidae; Cerambycidae.

SANDALWOOD IS A part of Indian culture and heritage, and is acclaimed worldwide because of its fragrant heartwood. A tree growing well can put on an increment of 1 kg per year and can attain a girth of over 1.5 metres (Rai 1990). But in practice this is impossible because of a host of insects, which can be termed 'wood-borers'. They attack the bark, sapwood and heartwood resulting in reduction of growth, dieback and even mortality of the trees. The trees affected by these borers neither grow properly nor have a healthy heartwood.

The main wood-borers of sandal are *Indarbela quadrinotata* Walker, *Zeuzera coffeae* Nietner and *Aristobia octofasciculata* Aurivillius. Surveys of sandal plantations, forests and depots during 1992–97 revealed that these insects are widely distributed. *A. octofasciculata*, for example, causes both mortality in younger trees, and substantial loss of heartwood in older trees. The observations on the occurrence, nature of damage and biology of the borers, and the assessment of loss of heartwood due to attack of heartwood borer, are presented in this paper.

Materials and Methods

Regular surveys were conducted in the sandal nurseries, plantations and natural forests in the southern states of India to identify the pest problems on the stem of sandal trees. The symptoms included holes on stems, galleries made of silk and frass, and pellets of excreta ejected or fallen from the stem holes. The drying of branches without any external symptoms also pointed to the presence of living borers inside the stem. The attacked parts were inspected and at times larval, pupal and adult stages were collected and studied in the laboratory. Sandal depots (sandal koti) were visited to assess the extent of damage of heartwood of timber stored in the open. Randomly selected lots were observed in detail to study the number of logs with bore holes. The dimensions (length, diameter, bore-hole diameter) of the individual logs

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with bore-holes were measured. The percentage loss in volume and weight of heartwood was calculated.

Observations and Discussion

Indarbela quadrinotata Walker (Lepidoptera: Indarbelidae)

Known as 'bark-eating caterpillar', this is a polyphagous pest reported on many trees (Beeson 1941). Survey of the sandal plantations at Hoskote revealed the presence of this insect in many trees, both young and old. Attack was mostly in the junction of branches. Young saplings show symptoms of dieback and develop epicormic shoots. The number of infestations indicated by the silken galleries on bark varied from 1–20. The frequency distribution of trees with varying number of galleries is given in Figure 1. Most trees had one gallery, and no trees died due to the attack.

The larvae bore tunnels downward into the sapwood and heartwood. At night they emerge to feed on the bark, excavating long patches which are roofed with galleries made of silk, bark and excreta. Full-grown larvae are 4–5 cm long. Pupation takes place in the shelter tunnel. The life cycle is annual and the moths emerge during May–July.

Zeuzera coffeae Nietner (Lepidoptera: Cossidae)

The red borer is a polyphagous pest which bores into the soft sapwood of saplings and young trees. It makes the stem so weak that it can be broken down by a gentle push or by wind.

During survey of plantations the occurrence of this borer was observed randomly. The occurrence was 2–5 percent in infested areas. Young saplings are sometimes killed by the attack. The larvae enter through the axil of leaf or stem and branch. The tunnels made by young larvae are cylindrical, and those by older larvae are wide with irregular cavities. The tunnels are kept clean by throwing down the white pellets of excreta which fall at the foot of the tree in a heap.

The larva is stout, up to 4 cm long, with a small hood-like, yellowish pronotal shield and abdominal segments, pinkish above and yellowish below.

The life-cycle takes about 4–5 months with a larval period of about 3–4 months. The larvae do not attack heartwood. Hence the attack is restricted to branches of older trees. Cutting off and burning the branches prevents the spread of the pest.

Aristobia octofasciculata Aurivillius (Coleoptera: Cerambyiidae)

This was reported as a pest boring the small branches and stem of saplings of *Santalum album* in North Salem Division (Beeson and Bhatia 1939). This monophagous pest is prevalent in all the sandal areas. Saplings and younger trees showed drying of branches and sometimes mortality due to the attack of this pest. Many living plantation trees contained several bore-holes leading into long cavities in the heartwood; and in older trees, the heartwood was found to be hollow to varying degrees. The survey of the sandal koties revealed that many logs had hollow heartwood. Evidently, this borer is able to both live in, and damage the heartwood.

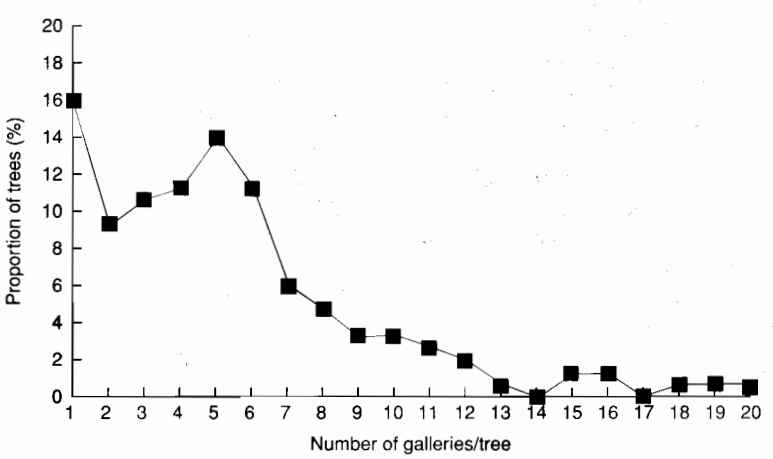

Figure 1. Distribution of galleries

The beetle is brick-red in colour with black antennae with muffs, and is 1.5–2.5 cm long. It attacks younger and older trees. The adult gnaws the bark and even girdles it. Eggs are deposited either on the main stem or branches. The larva feeds its way into the sapwood, bores down into the main stem, and excavates long tunnels in the heartwood which are packed with excreta. The tunnel goes to the root. Pupation occurs near the sapwood and the adult makes a circular hole to exit from during April–May. The exposure of heartwood through the bore-holes leads to the invasion of wood-rotting fungi and termites. Gradually the tunnels coalesce and increase in size leading to hollowness.

A study was undertaken to ascertain the reason for the die-back and mortality of sandal plants in one patch of the plantation of Yelwala, Mysore. The symptoms were drying of the twigs followed by death of the trees. Spike disease was not found. On closer observation, it was evident that most of the trees showed the presence of holes on the main stem and branches. The number of holes ranged from 1–5. The percentage of trees with 1, 2, 3, 4 and 5 holes were 57%, 12.96%, 12.96%, 5.55% and 11.11% respectively. Out of 100 trees, 73 trees had top drying (4 trees dead, 15 with most branches dried). Of the 73 trees, 59 had borer holes, but 14 did not show any holes. Out of the healthy-looking 27 trees, 4 had holes. It appeared that the trees have been attacked by *Z. coffeae* or *A. octofasiculata* or both. Most of the trees were 10–15 years old. The dead trees were examined after uprooting and splitting. Galleries of *A. octofasiculata* were visible with larvae inside, and the tunnels reached to the root.

Survey of the sandal depots (sandal koties) revealed that 10–50% of the timber in the different stacks had hollow heartwood. In the sandal koties, the wood extracted from the forest (usually when the tree is dead), and also that confiscated from smugglers, is stacked in the open on bare ground for years before final cleaning. The logs were hollow when they were brought to the depot. Out of 3517 logs inspected, 883 (25%) had hollow heartwood, usually varying in diameter from 1–12 cm. In the sandal koties, the final cleaned sandalwood with defects and hollowness is classified into 'Ghat Badla' (> 5 kg) and 'Bagradad' (< 5 kg).

Though it is known that the sandal under storage is attacked by powder-post beetles and wood-wasps, this was not apparent in the present survey. Termite attack was observed in only a few cases. The damage by the wood-rotting fungi appeared to be high in some depots.

The sandal logs in the depots showed great variation in size. Most (28%) were in the diameter class 16–20 cm; logs with diameter <10 cm and >40 cm were rare (Fig. 2). The highest number of logs (31.16%) had bore-holes measuring 7–9 cm (Fig. 3). The volume of sandal heartwood lost due to the presence of bore tunnels and hollow cavities was estimated and represented in Figure 4.

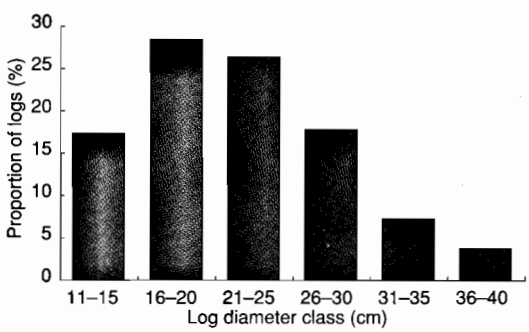

Figure 2. Frequency distribution of log diameter

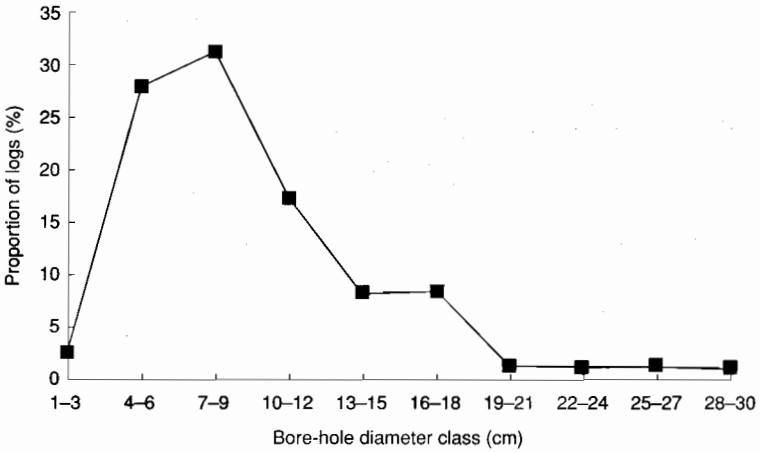

Figure 3. Distribution of bore-holes

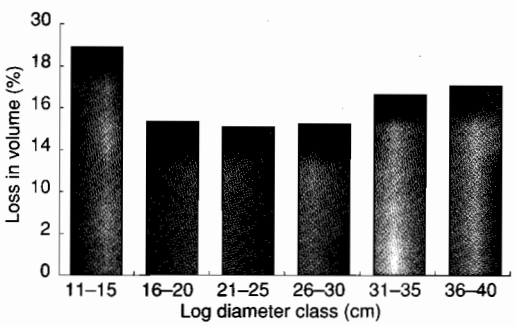

Figure 4. Volume loss due to hollows

Conclusions

The volume/weight loss observed in the diameter classes from 11–15 cm to 36–40 cm, did not show significant variation from each other. The overall loss in volume/weight was 19.86%. Hence it is clear that 198.6 kg of sandal heartwood is lost per tonne of wood produced by the trees. Estimating the cost of 1 kg heartwood as Rs. 500 ($US15), the loss is around Rs. 100000 ($US3000)/tonne. The loss was generally more in the lower diameter class. This shows that, as the tree grows in diameter, the hollowness also increases due to the continued deterioration of heartwood by the action of termites and fungi. Due to years of stacking in the open areas of the depots, the wood deteriorates further, especially in the bark and sapwood.

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Control of Arboreal Termites on *Santalum album* L. in Plantations

O.K. Remadevi, V.R. Sivaramakrishnan and C.R. Sarma*

Abstract

Arboreal termites, *Odontotermes* spp., are often observed in sandal plantations. The attack leads to loss of bark, poor health, and infestation of stem-boring insects. The infestation is highest (up to 50%) in winter. An experiment was conducted at Yelwala sandal plantation, maintained by the Institute of Wood Science and Technology, to study the comparative effectiveness of different insecticides (Chlorpyriphos, BHC, Phorate, Quinalphos and Lime) in various combinations and doses. Observations on termite incidence were taken periodically for one year. It was observed that use of Chlorpyriphos® 20 EC (1.5%) gave adequate protection for up to four months, with decreasing protection thereafter. The effectiveness of all the treatments were compared and are reported here.

Key words: termites, *Odontotermes*, Chlorpyriphos, insecticide

ARBOREAL TERMITES ARE common at the basal parts of *Santalum album* trees, especially in winter and summer months. Sometimes they also extend up to the growing tips. The bark is eaten off and earthen galleries cover the stem, reducing the vigour, health and growth of the trees (Fig. 1). Sandal is known to be attacked by three species of termites. *Microcerotermes fletcheri* Holmgren & Holmgren attacks both the bark of standing trees and the dead wood in storage (Srinivasan et al. 1992). *Odontotermes brunneus* (Hagen) and *Coptotermes heimi* (Wasmann) attack standing trees and dead logs of *S. album* (Sen Sarma et al. 1975). Field studies on the control of *Odontotermes* spp., the arboreal termites on sandal, are presented in this paper.

Materials and Methods

Studies using different insecticides were conducted at Yelwala sandal plantation in Mysore from February 1994 to January 1995. The trees, 10 and 15 years old, were been planted and maintained by Institute of Wood Science and Technology, Bangalore. The ten treatments were:

- T-1. Chlorpyriphos® 20 EC (1.5%)—3 litres/tree (poured around the bottom)
- T-2. Chlorpyriphos® 20 EC (1.5%)—2 litres/tree (poured around the bottom)
- T-3. Chlorpyriphos® 20 EC (1.5%)—1 litre/tree (poured around the bottom)
- T-4. Chlorpyriphos® 20 EC (1.5%)—Paste applied up to 1 metre
- T-5. BHC with lime—Paste applied up to 1 metre
- T-6. Phorate crystals—25g per tree
- T-7. Quinalphos (0.5%)—1 litre/tree
- T-8. BHC + lime + Chlorpyriphos—Paste applied up to 1 metre
- T-9. Water—3 litres/tree (poured around the bottom)
- T-10. Control (i.e. no treatment).

The experimental plot was selected in a termite-prone area. The experiment was laid out in a completely randomised block design (RBD) with four replications for each treatment. Each treatment was

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applied to a group of nine sandal trees. Before the start of the experiment, the number of trees with termite attack was recorded. Those earthen galleries with live insects were scraped off before the treatment. Observations were continued periodically for one year. Each time, the galleries were scraped off. In each treatment, the percentage of trees with attack was calculated. The percentage values were transformed and analysed by a covariance technique.

Figure 1. Arboreal termite attack on sandal tree

Results and Discussion

Initial observations on the incidence of termites on sandal revealed that 8–55% of the trees (average = 26%) were affected to some extent. The first observation was taken after one month of treatment, and periodically thereafter. The different treatments varied significantly in their efficacy. Three litres of Chlorpyriphos® 20 EC (1.5%) applied to the soil (i.e. T-1) in controlled the termites best. This was followed by Phorate application (T-6), then BHC+lime+Chlorpyriphos (T-8). Lower quantities of Chlorpyriphos applied to soil were next, followed by BHC with lime paste. Water treatment, Quinalphos (0.5%), and pasting Chlorpyriphos on trees were ineffective in controlling termite attack on sandal trees. The results of applying Duncan's multiple-range test to the data are shown in Table 1.

Table 1. Performance of treatments (in descending order)

Treatment number			
(high)	1 6 8 2 3 5	9 7 4 10	(low)

Notes:
1. Significance level = 5%.
2. Figures over the same bar do not differ significantly.

Analysis of individual periods of recording revealed the following.

- The trees for which soil treatment was given with three litres of Chlorpyriphos (1.5%), were highly infected by termites (36%) in the initial count (i.e. before treatment). In spite of that, this proved to be the best treatment in controlling the termite attack.
- Recording of data after one month showed that all the treatments, except water, controlled the incidence of termite equally effectively.
- Data collected after four months indicated that Chlorpyriphos in all doses is more effective than other treatments in controlling termite attack.
- The effect of the treatments faded out after 6–9 months, and after 11 months the incidence exceeded the initial attack.

These data are set out in Figures 2 and 3 and Table 2.

Termite attack of newly transplanted trees causes mortality as high as 80–100%, especially to exotic trees like *Eucalyptus* and *Pinus*. *S. album* is also known to be damaged by termites in the seedling stages, and to be inhabited by bark-eating termites in the tree stage (Roonwal 1979). The genera responsible for the attack on young trees are usually *Coptotermes*, *Eurytermes*, *Microtermes* and *Odontotermes*, which attack the taproot of transplants a few centimetres below the ground. Nair et al. (1986) have given the methods of controlling termites on *Eucalyptus*. Thakur et al. (1989) established that Chlorpyriphos is an effective soil insecticide for controlling termites on *Eucalyptus*. Nair and Varma (1985) observed that in addition to the root feeding, the live and dead bark of *Eucalyptus* stem was nibbled by termites under cover of mud plaster, particularly during the dry season. They found that 12 out of 17 root-feeding species were *Odontotermes* spp.

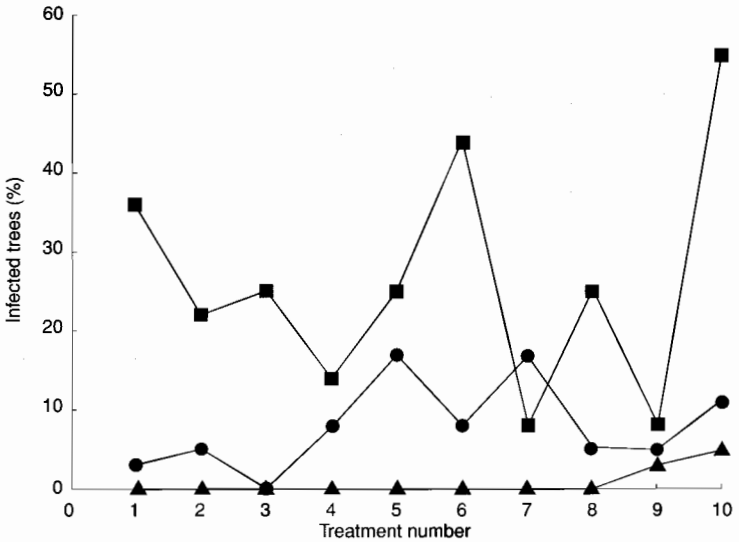

Figure 2. Efficacy of insecticides in controlling termite attack. (■ = Initial, ● = 1 month, ▲ = 4 months)

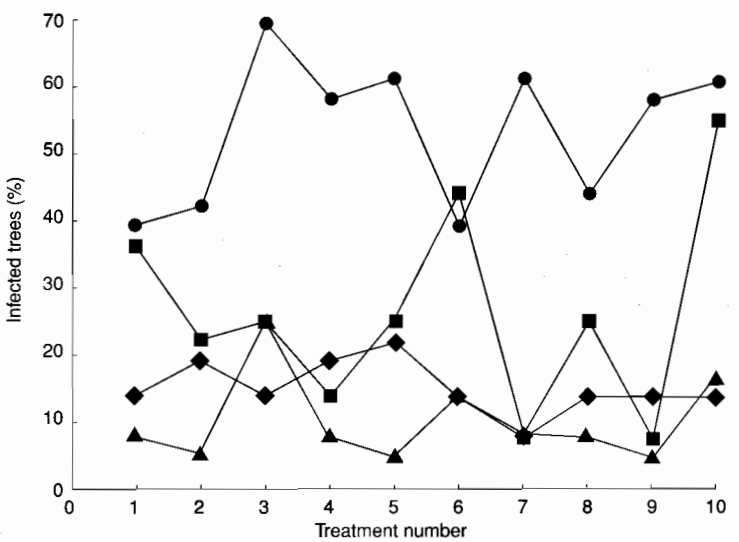

Figure 3. Efficacy of insecticides in controlling termite attack. (■ = Initial, ◆ = 6 months, ▲ = 9 months, ● = 11 months)

Field studies have shown that *Santalum album* is damaged to a very great extent by termites when trees are under stress due to attacks of wood-borers, especially the heartwood borer, *Aristobia octofasciculata*. In that case, the attack is not visible from outside. In the case of arboreal bark-eating termites, the earthen galleries are visible even as far as the terminal branches, but the trees are not killed. Usually, the bark of the tree forms an effective defence against termites foraging in earthen tunnels up the trunks. In the case of sandal, the arboreal termites are found to eat parts of bark which may in due course weaken the tree predisposing it to attack by wood-borers and fungi.

Table 2. Proportion of affected trees under different treatments over different periods, in ascending order

				Count before treatment						
Treatment no.:	7	9	4	2	4	5	8	1	6	10
Trees affected (%):	8	8	14	22	25	25	25	36	44	55
				After 1 month						
Treatment no.:	1	2	3	4	5	6	7	8	9	10
Trees affected (%):	0	0	0	0	0	0	0	0	3	5
				After 4 months						
Treatment no.:	3	1	2	8	9	4	6	10	5	7
Trees affected (%):	0	3	5	5	5	8	8	11	17	17
				After 6 months						
Treatment no.:	7	1	3	6	8	9	10	2	4	5
Trees affected (%):	8	14	14	14	14	14	14	19	19	22
				After 9 months						
Treatment no.:	2	5	9	1	4	7	8	6	10	3
Trees affected (%):	5	5	5	8	8	8	8	14	17	25
				After 11 months						
Treatment no.:	1	6	2	8	4	9	5	7	10	3
Trees affected (%):	39	39	42	44	48	48	61	61	61	69

Conclusions

Chlorpyriphos formulations are in use in many countries for protection of wood against termites. It kills termites quickly on contact. In the present studies, application of Chlorpyriphos solution has been found to protect the sandal trees for up to four months. The attack of *Odontotermes* on sandal, can lead to mortality of seedlings, and poor health and growth of trees.

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Studies on the Sap-sucking Pests of *Santalum album* L. in Nurseries and Plantations

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Abstract

Among the different insect pests including defoliators, stem borers and termites, the role of sap-sucking insects belonging mainly to the family Coccidae, is very crucial being deleterious to the normal health, growth and reproduction of the sandal plants. The main coccids which cause dieback and lessening of fruit setting are *Saissetia* sp., *Inglisia bivalvata* Green, *Ceroplastes ceriferus* and *Kerria lacca* Kerr. all belonging to the family Coccidae. The details of the habits, damage potential, nature of damage and control measures are given in this paper.

Key words: sap-suckers, Coccidae, dieback

MANY INSECT SPECIES are known to inhabit sandal, *Santalum album* L. (Santalaceae). However only very few of the some 150 species are serious pests which adversely affect the growth of the plants. These include defoliators, sap-suckers, stem-borers, and termites. A five-year survey was recently undertaken in south Indian states to study the prevalence and nature of damage by the important pests. These studies revealed that sap-suckers belonging to the family Coccidae (Order Hemiptera) cause dieback, poor growth, lessening of flowering and fruiting, and sometimes even mortality, to plants in nurseries and plantations. The occurrence, habitats, bionomics, nature of damage and control measures of the most prevalent sap-suckers are given in this paper.

Materials and Methods

Forest nurseries, plantations and natural forests in Karnataka, Tamil Nadu and Andhra Pradesh were surveyed during 1992–97 to study the occurrence and distribution of insect pests. Randomly selected trees were observed and the pest stages collected. The sample size was 10–50 percent of the population in an area, depending on accessibility to trees. The intensity of attack and nature of damage were assessed visually. Twigs with different developmental stages of various coccid species were caged in the laboratory and regularly observed under the microscope to study the morphological features, nymphal stages, and the presence of parasites or predators. Experiments using different insecticides were conducted to evolve suitable control measures for the major pests.

Observation and Discussion

The coccids were found to be major pests on sandal from seedling to tree stages. Chatterjee and Ayyar (1936) reported as many as 12 coccids from sandal. The important coccids were *Saissetia* sp., *Inglisia bivalvata*, *Kerria lacca* and *Ceroplastes ceriferus*. The habits and habitats of all these coccids were the same.

***Saissetia* sp.**

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Field surveys revealed the occurrence of two species of *Saissetia* throughout the sandal-growing areas. *Saissetia coffeae* and *S. nigra* were recorded by Ayyar (1929) on *S. album* at Coimbatore. About 50 plant species are known to be attacked by these coccids (Mathur and Singh 1960–61). The adult female of *S. coffeae* is elliptical in outline, convex, brown, and shining; *S. nigra* females are black and larger (Fig. 1) The fecundity is 500–1000 eggs in *S. coffeae*, whereas *S. nigra* lays an average of 2000 eggs. The nymphs move and settle on different parts of the plant. These scales feed on the sap of leaves, tender shoots, flowers, and fruits. The honeydew secreted by the insects leads to the development of black sooty mould on the surface of leaves and branches. When infestation is heavy, the branches start drying up. The affected flowers wither, and fruits dry up and fall off prematurely.

Figure 1. *Saissetia nigra* on sandal twig

Spraying 0.5% Quinalphos, followed by 0.5% BHC after two days, is recommended for the control of these insects (Sivaramakrishnan et al. 1987).

Inglisia bivalvata Green

This coccid was recently reported on *S. album* (Srinivasan et al. 1992). A survey of sandal nurseries and plantations revealed the occurrence of the pest only in Bangalore and Hoskote districts; 10–80 percent of branches were infected by different stages. Other trees such as *Thespesia populnea*, *Cajanus cajan* and *Pongamia* are also infected by this coccid (Ayyar 1929). Mild attack of the insect on *Polyalthia* sp. and *Acacia mangium* was observed during our study.

Creamy-brown, bivalved scales of the females encrust the twigs up to leaves and fruits (Fig. 2). As in other coccids, the leaves develop black sooty moulds on their surface due to honeydew secreted by active coccids. Leaves show browning, flowers wither, fruit formation reduces, and the branches show dieback later. When the attack is severe, saplings and small trees succumb to the infestation.

Figure 2. *Inglisia bivalvata* on sandal twig

Nymphs move about and settle on tender shoots and develop pinkish waxy encrustations. Life cycle of males is shorter. The females are stationary, big and with bivalved scales. Male puparia are covered by ornamental waxy laminae.

I. bivalvata is found parasitised by hymenopteran parasites belonging to Eulophidae, Encyrtidae and Aphelinidae. Many predators belonging to Coleoptera and Lepidoptera were also collected. Spraying of 0.2–0.3% Chlorpyriphos or Quinalphos was effective in controlling the pest.

Kerria lacca (Kerr.)

Kerria lacca is an economically important insect which produces the resin, lac, used in many industries. Though sandal is known as one of some 100 host species, the infestation in an epidemic and severe form was observed recently in the sandal plantations at Gotipura and Nallal (Remadevi et al. 1997).

The infestation was found in pockets in which all the adjacent trees were infected. The severity of infestation varied from one tree to other. The host plant, *Pongamia pinnata* was also severely infected. The early stages appear as brownish spots on the tender stems (Fig. 3). The mature colony develops as dark brownish-black encrustations and cover the entire twigs; due to the sap drainage, the branches dry off. In severe cases, the whole tree dies (Fig. 4). The black

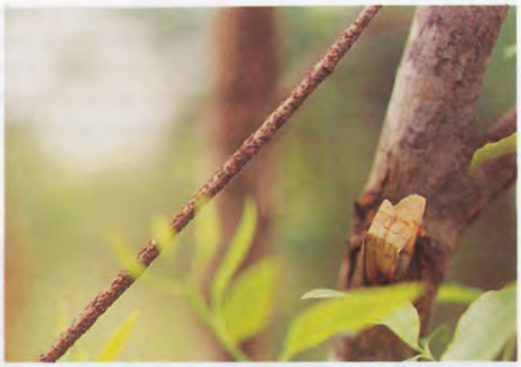

Figure 3. Early stages of lac insect attack

Figure 4. *Kerria lacca* attack

sooty appearance of the leaves and the twigs is one of the notable symptoms of attack.

The heavily affected branches were lopped and burnt to kill all live stages. A solution of 0.5% Ekalux (20 AF) along with 0.05% sticker sprayed thoroughly on the affected trees checked the infestation. The initial stages could be controlled by spraying 0.1% Rogor or 0.04% Cypermethrin.

***Ceroplastes ceriferus* (Fabr.)**

Ceroplastes ceriferus is one of the potential pests of sandal. It occurs either singly or in groups on isolated trees. It is known as a minor pest of *Boswellia serrata*, *Buchanania latifolia* and *Melia indica* (Beeson 1941), on citrus (Brown 1968), and on many other trees. The insects on the stem appear as irregular masses of white wax which fuse together in cases of heavy infestation, in which case, the leaves drop and the twigs dry. Spraying of Monocrotophos (0.02–0.05%) kills the scales.

Conclusion

The general symptoms caused by all the above coccids are:

- browning and falling of leaves, and twig drying (die-back);
- reduction in flowering and fruiting; and
- formation of black sooty mould on the leaves and branches.

Due to sap drainage, the growth of the plant is retarded. The sooty mould formation hinders photosynthetic activities and hence productivity. Mortality of the plants due to attack of *Saissetia* sp. and *C. ceriferus* was not observed. Saplings and small trees are killed by the attack of *I. bivalvata* and *K. lacca*. Hence coccid attack should be treated as a serious problem, and timely control measures should be employed to check its growth and spread.

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