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Sandalwood Research Newsletter

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EDITOR'S NOTE

Welcome to the first edition of the SRN in 2002. I'd like to wish all our readers the best for the new year. Although we start in a new year, with new research to report on, the articles continue to reflect aims towards improved conservation and management of sandalwood worldwide.

Increasingly for many countries, the regeneration of sandal is problematic where illegal harvesting, overgrazing, uncontrolled burning and even statutory regulations have resulted in the decline of this species (see SRN Issue 10). In India, an additional burden on the resource is the incidence of Spike disease, a pathogen which has resulted in the death and decline of many sandal trees around Karnataka and Tamil Nadu. This disease was first discovered in Coorg in 1899 and has attracted a great deal of attention and research, especially since 1969 when its mycoplasma etiology was confirmed. At present there is no record of Spike disease outside of India.

Methods for mass propagation and genetic improvement have been investigated for some time to help reverse the decline in sandal. Somatic embryogenesis is identified as an important research tool with several potential advantages for mass production of superior genotypes, such as Spike resistant Santalum *or the production of high oil yielding varieties. Ilah, Abdin and Mujib (p2-3) report on somatic embryogenesis irregularities found in* in vitro *clonal reproduction experiments. Similar to the findings by other researches in this area, problems such as the occurrence of frequent developmental abnormalities in somatic embryogenesis in solid medium was found. For further information on somatic embryogenesis and clonal propagation published in the SRN refer to Issue 6 (Ramshankar & McComb), Issue 8 (Bapat & Rao), and Issue 13 (Dey).*

In Australia, regeneration of native sandalwood is frequently affected by native and exotic herbivores. Another factor that is thought to affect regeneration levels is the method of harvest and recruitment. Stem coppice and direct seedling experiments were investigated by Ryan and Brand (p4-7) on a sheep station in Western Australia. In this area natural recruitment is generally low, possibly due to grazing and/or poor seed dispersal. Improving natural recruitment and regeneration after harvesting is vital for the sustainable supply of all sandalwood species worldwide.

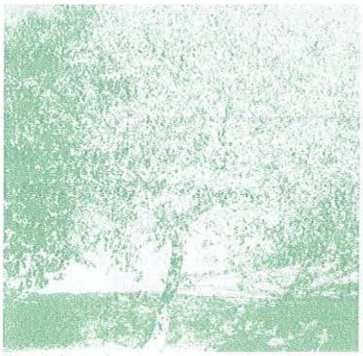
Conservation and sustainable management of sandal globally is supported by the efforts of collaboration between organizations and across countries, such as SPRIG Phase 2 - an AusAID-funded regional project in the South Pacific (see page 7 for details). The first SPRIG project focussed on the conservation and management of many species including two sandalwood species indigenous to Fiji, Tonga and Vanuatu. It is a credit to all those involved in the success of Phase 1, that the SPRIG project is extended to Phase 2, which will build on the knowledge gained and the resources and networks established to date.

The Forest Products Commission in Western Australia has also supplied information regarding Santalum album *seed supply and sales (p8) for northern Western Australia. Please refer to the back page for contact details.*

Articles for publication for the next edition should be received by 31st March 2002. Questions, comments and feedback are also welcome at any time. Thanks also for the positive feedback received from many subscribers to date.

I hope you enjoy this issue.

Tanya Vernes



Sandalwood

inside...

Somatic embryo irregularities in *in vitro* cloning of sandal (*Santalum album* L.)

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Plant tissue culture based propagation (multiple shoot/ somatic embryogenesis) has been employed with conventional methods for the reproduction of Sandlwood seedlings for some time. In somatic embryogenesis, we found an initial high potential of embryo induction was followed by a low, declining conversion frequency of plantlets. In this process of embryo formation, maturation and germination, large-scale abnormalities were noticed. Advanced stage embryos remained quiescent for varied periods before germination. This may be due to dormancy, and further exchange of ideas is needed for rapid synchronous seedling production.

Introduction

The Indian sandalwood is known worldwide because of its unique fragrance in oil and wood. Though native to peninsular India, the plant *Santalum album* L., is cultivated in other parts of India and abroad. Depletion by cutting and the incidence of spike disease are the two major problems in India. In India and Asia Pacific, the plant parts have been proudly used in several traditional incidences including marriage and death – the two crucial events of life.

Owing to the huge importance in domestic and foreign market, rapid deforestation and pilferage has been taking place in India and around. The incidence of spike disease has also made things more complicated. Along with conventional methods, plant tissue culture propagation has also been integrated for some time, in which the somatic embryogenesis pathway has been proven to be very effective for rapid production of propagules (Bapat *et al.* 1990; Mujib *et al.* 1998). However, even after four decades of intensive research, conversion frequency to mature seedlings from embryo is not very high, thus, proper utilization of this technique is not fully enjoyed. The authors have focused on some of these aspects in detail.

Materials and Methods

Locally collected seeds were germinated *in vitro*. The hypocotyl and nodal stem were used as explants for the establishment of callus. Media supplemented with 2.26 µM 2,4-D and 2.68: M CPA separately proved to be very effective. Two weeks old callus was immediately transferred to solid media supplemented with 2.70 µM NAA and 2.22 µM BAP to produce embryogenic mass (Figure 1a).

The embryogenic callus was then cultured on both solid and liquid Murashige & Skoong (MS) and McCown media for maturation and development of embryo. All the cultures were incubated in a culture room at a temperature of 24±20C, 70-80% relative humidity and illumination at 20-µM $m^{-2}S^{-1}$.

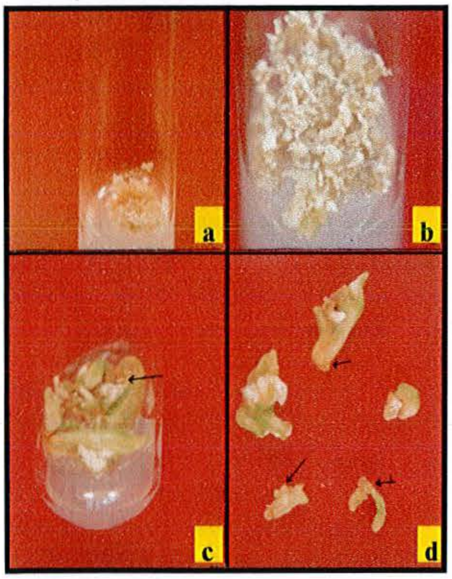

Figure 1
a) Embryogenic callus mass on solid medium, b) somatic embryos with different stages of development, c) advanced stages of embryos, arrowhead shows adventive embryo, d) irregularities on somatic embryos; top arrowhead: less developed root, left arrowhead: secondary callusing from embryo, right arrowhead: fused somatic embryos at the base.

Results and Discussion

The induced callus has a strong tendency towards forming somatic embryos, as a large number were immediately induced upon transfer to embryogenic media (Figure 1b). Both the solid and liquid media were equally effective, however, liquid medium at the initial stage was more responsive (Table 1). Better oxygen availability and uptake to growing cell masses in liquid medium may trigger ready induction of proembryo and embryo (Huang *et al* 1992; Jay *et al* 1992).

For embryo maturation, woody plant medium (WPM) which contains inorganic compounds of lesser amounts, performed better in comparison to MS. The maturation and growth of embryo was virtually arrested unless the produced embryos were separated and plated on agar solidified media. This reduced frequency of transformation towards the mature stage is quite obvious as these bipolar somatic embryos received little polar effect when cultivated in agitated liquid medium. All the mature stage embryos were not transformed into truly developed seedlings (Figure 2c).

Abnormality was very high on both solid and liquid media and was manifested by the appearance of several irregularities (Figure 1c, 1d) on embryo which includes aggregation of pro-embryos and embryos, growth arrestation, browning of embryos, embryo with no or ill developed

Growth Medium (MS/MC)	G	H	T	C	Abnormality (%)
Solid					
MS	22.10±3.19	18.10±1.89	14.20±1.56	12.80±0.81	68.40
MC	------	------	16.20±1.28	15.20±2.31	54.10
Liquid					
MS	27.50±2.12	24.20±2.22	12.10±2.43	10.20±1.81	56.20
MC	------	------	13.10±2.36	9.20±1.01	52.80

G= Globular, H= Heart, T= Torpedo, C= Cotyledonary, MS=Murashige & Skoong, MC=McCown,
------ = data not scored due to insignificant deviation.
All the values are expressed as mean ± SD. At least 3 replica per treatment.

Table 1: No. of embryos/10mg or ml of embryogenic callus mass.

root, and root with less developed shoot axis (Figure 2a).

Many irregulaties were common but in the solid medium, embryo development was further plagued by swelling of embryos, callusing with further embryogenesis, large-scale adventive or secondary embryo formation and root degeneration.

Induction of woody plant medium, which was very active on embryo induction, was found to be less efficient. Root formation was sometimes complemented with excised *in virto* shoots (Figure 2b) however, frequency, number and growth of roots were limited (Table 2). Although the use of bioreactor was useful in some sense (Das *et al.* 1999), a sizable abnormality frequency still exists. Mujib *et al.* (1998) earlier indicated that *in vitro* raised somatic embryo of direct and indirect origin exhibited dormancy of varied nature and required time for germination.

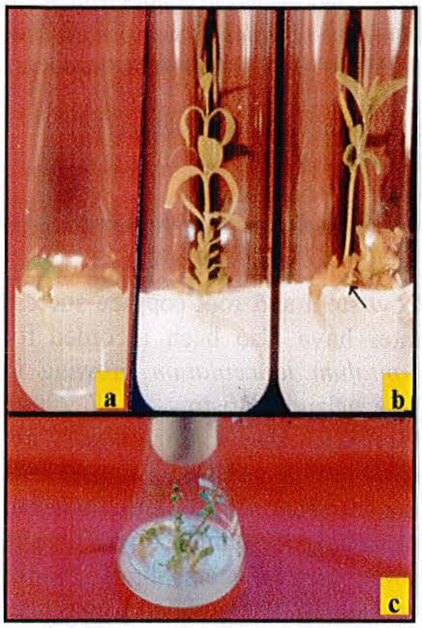

Figure 2.

a) Somatic embryo with root, less developed shoot.
b) Root regeneration from excised shoot tips, secondary embryo (arrowhead)
c) Non-synchronous somatic seedlings, germinated *in vitro*.

Plant Growth Regulator (PGR)	Concentration. (µM)	Number of Roots	Percentage of Root Frequency (%)	Length of Roots (cm)
NAA	5.4	2.5±0.81	60	0.75±0.21
NAA	10.8	3.1±1.51	66.6	0.9±0.32
NAA	21.6	2.1±0.83	44.4	0.92±0.41
IAA	5.71	2.75±1.78	83.3	1.25±1.01
IAA	11.42	3.6±1.81	33.3	1.8±0.36
IAA	22.84	2.3±0.81	42.8	0.75±0.23
IBA	4.90	2.2±1.94	37.4	0.45±0.21
IBA	9.80	2.61±1.98	32.1	0.85±0.23
IBA	19.60	------	------	------

Table 2: Root induction at various hormonal treatments. Data scored after 45 days of incubation. All values are expressed as Mean ± SD.

As the *in vitro* embryos do not have any seed coat the inhibitor(s) or physiological barrier(s) may lie within the embryo itself.

The authors call for participation from a range of scientists, including plant physiologists, to take up this challenge for rapid uniform, maturation and conversion of all or nearly every somatic embryo into seedlings to be useful in future planting programme.

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Techniques to improve sandalwood (*Santalum spicatum*) regeneration at Shark Bay, Western Australia: stem coppice and direct seeding.

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Two separate trials were established to determine the effectiveness of stem coppice and direct seeding on Santalum spicatum *regeneration levels at Nanga station, near Shark Bay. In August-December 1988, 200 naturally occurring* S. spicatum *trees were harvested by cutting the stem at 150 mm above the base. These cut stems were divided into four protection treatments: control, brambles, ring-lock and rabbit netting. Twelve years after harvesting, 40.8 % of the trees were alive. At this age, mean height of the coppice was 1.7 m and mean stem diameter was 28 mm. The mean number of coppice stems per tree was nine. There was no evidence of grazing and the protection treatments were not significantly different. Coppice stems began flowering at age four years, and 15 % of coppice stems produced mature fruit at age 12 years. In May 1990, 900* S. spicatum *seeds were planted in natural vegetation on four dune face slopes: west-facing, valley, east-facing and ridge top. In October 1990, 40.0 % of the seeds had germinated. At age 10 years,* S. spicatum *mean seedling survival was 30.4 % and was highest on the west-facing slope (41.5 %). However, dune position had no significant influence on seedling survival. Growth was similar between treatments, with mean height of 1.9 m and a mean stem diameter of 32 mm. Mature fruit was present on 24 % of the seedlings at age 10 years. Both stem coppice and direct seeding are effective regenerating techniques for S. spicatum at Nanga Station.*

Introduction

In Western Australia, approximately 2000 tonne of *Santalum spicatum* is harvested per annum (Jones 2001). Harvesting occurs mainly from natural stands in the semi-arid regions of the Goldfields and Midwest. Natural recruitment in these regions is generally low (Kealley 1991 Brand 1999), perhaps due to poor seed dispersal and grazing (Brand 2000). Moving *S. spicatum* seeds away from parent trees and planting them near suitable host plants protected from grazing has shown to dramatically improve regeneration in semi-arid regions (Brand 2000). This planting technique is now incorporated in the *S. spicatum* harvesting guidelines to improve regeneration and promote sustainable harvesting.

Besides seed enriching host plants, stem coppice may also be a suitable method to improve regeneration. In Western Australia, the entire *S. spicatum* tree is normally harvested using a four-wheel-drive vehicle because the roots contain valuable oils, and stem coppice survival is generally low in places such as the Goldfields (Loneragan 1990). Low stem and root coppice success rates have also been recorded for *Santalum lanceolatum* growing in Queensland (Bristow *et al* 2000). However, stem coppice occurs frequently in *S. spicatum* after harvesting at Shark Bay (Garth Pinnegar, personal communications), and some coppice stems have reached commercial size. *S. spicatum* trees growing at Shark Bay used to be harvested by cutting the stem at ground level because the vegetation is too dense and the soil is too sandy to harvest with vehicles. In this region, the *S. spicatum* grow on deep red sand dunes that support a dense covering of *Acacia* shrubs (Payne *et al* 1987). In 1988, 71 mature *S. spicatum* growing naturally at Nanga were cut near the base and 58 % of these had live coppice three years later (Doronila and Fox 1991).

At Nanga, the *S. spicatum* trees also appear to be more common on south and west-facing dune slopes than north and east-facing slopes (Garth Pinnegar, personal communications), which may be due to the cool sea breeze. The *S. spicatum* are growing within 10 km of the ocean and the south-westerly sea breeze in the summer may reduce heat stress and promote survival on the south and west sides of sand dunes. The influence of dune position may affect seedling survival of *S. spicatum* in these environments.

The aim of this study was to determine the effectiveness of regenerating *S. spicatum* after harvesting using (i) stem coppice and (ii) direct seeding. This study examined the effect of grazing on stem coppice survival. The effect of dune position on *S. spicatum* seedling survival was also investigated.

Methods

On Nanga station (26°13'S, 113°52'E), *S. spicatum* coppice and seedling trials were established in natural vegetation on deep red sand dunes. Nanga was managed for sheep grazing and was stocked during the trial (1988-2000). Besides sheep, there was also the potential of grazing by goats and rabbits. The trial sites consisted of a dense covering of potential host species including *Acacia ramulosa, A. sclerosperma* and *A. tetragonophylla*. The mean annual rainfall at Denham (25°56'S, 113°32'E) is 227 mm, and between 1989 and 2000 the annual rainfall ranged from 171 to 375 mm.

Stem coppice

In 1988, 200 mature *S. spicatum* trees growing naturally at the site were selected within an area of approximately 30 ha. All trees were commercial size, with stem diameters at 150 mm above the ground between 151 and 364 mm. In August-December 1988, each *S. spicatum* tree was harvested by cutting the stem (at 150 mm) with a chainsaw.

The harvested trees were randomly allocated to four protection treatments: control, brambles, ring-lock and rabbit netting. No protection was given for the control stems. Discarded branches (or 'brambles') were placed on the cut stems as a cheap form of protection. Standard ring-lock was fenced around cut stems in a radius of approximately 1 m by a height of 1 m to prevent grazing by sheep and goats. Rabbit netting was fenced around the other stems in a radius of approximately 1 m and a height of 1 m to prevent grazing by all herbivores. Each treatment contained 10-15 stems and was replicated four times. Coppice survival, height, stem number and diameter (at 150 mm) were measured at age four, eight and 12 years.

Direct seeding

The effect of dune face on *S. spicatum* seedling survival and growth was examined on four different dune faces: east-facing slope, valley floor, west-facing slope and ridge top. On each dune face, three seeds were planted 2.5 m apart in a row (or plot) containing ten planting spots. Plots were established 4 m apart in a grid pattern and pseudoreplicated 20-30 times, giving a total of 200-300 planting spots on each dune face. Each dune face treatment was 0.2-0.3 ha and within 1 km of the other treatments.

The *S. spicatum* seeds were collected from Nanga and were sown 2-3 cm below the surface in May 1990. Seedling survival, height and stem diameter (at 150 mm) were measured at age one, two, three, four, six and 10 years.

Statistics

Mean *S. spicatum* survival and growth measurements (height, stem diameter and number of stems) were compared between treatments using one-way Analysis of Variance (ANOVA). *S. spicatum* survival was angular transformed and Systat® was used to analyse the data.

Results

Stem coppice

After harvesting, the proportion of *S. spicatum* trees with live coppice was 53.1± 4.7 % at age four years, 44.4± 4.6 % at age eight years, and 40.8± 4.6 % at age 12 years (Figure 1). At age 12 years, survival was highest within rabbit netting (56.7 ± 8.4 %, Figure 2) and lowest in the control (31.7 ± 5.7 %) but there were no significant differences between treatments (p = 0.276). There was no evidence of grazing within each protection treatment.

Growth was similar between treatments, with mean heights of 0.8 ± 0.05 m at age four years, 1.3 ± 0.05 m at age eight years and 1.7 ± 0.1 m at age 12 years (Figure 3).

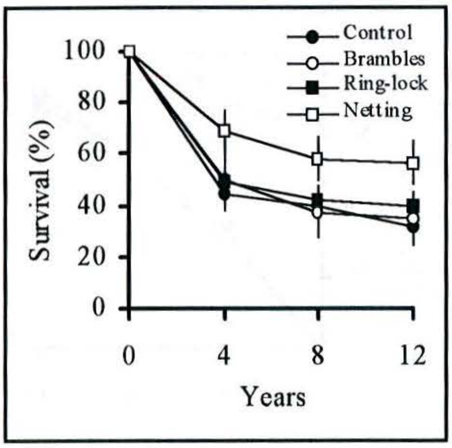

Figure 2. Mean survival (± standard error) of *S. spicatum* coppice treatments at Nanga over 12 years.

At age 12 years, mean height was the same between coppice treatments (p = 0.123). The mean number of coppice stems per tree was 9.1 ± 0.6 and was not significantly different between treatments (p = 0.066). The mean diameter of the largest stem from each tree was 28 ± 1 mm and was similar between treatments (p = 0.630). Flowers were observed on one of the coppice stems at age four years. At age 12 years, 15 % of the trees were producing fully developed seeds.

Direct seeding

In October 1990, *S. spicatum* germination was 40.0 ± 2.1 % and was similar between treatments. In November 2000 (age 10 years), mean seedling survival was 30.4 ± 3.2 % (Figure 4) and was not significantly different between dune face treatments (p = 0.108).

Figure 1. *S. spicatum* stem coppice at age 12 years. This individual had over 20 stems.

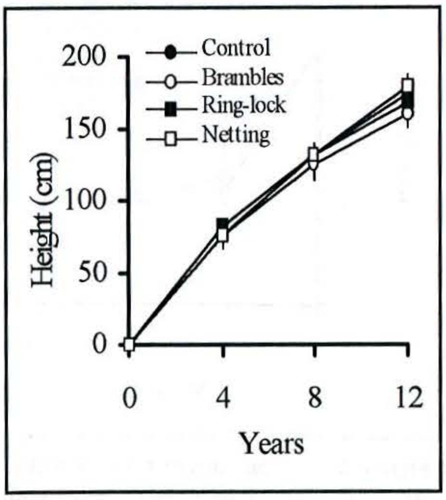

Figure 3. Mean height (± standard error) of *S. spicatum* coppice treatments at Nanga over 12 years.

Mean seedling survival was 41.5 ± 6.5 % on the west, 36.3 ± 7.7 % on the hill, 24.1 ± 5.9 % in the valley, and 22.3 ± 6.3 % on the east. At age 10 years, mean height was 1.9 ± 0.1m, and was not significantly different between treatments ($p = 0.393$). Mean stem diameter was 32 ± 2mm, and was also similar between treatments ($p = 0.273$). At the same age, 24 % of the seedlings were producing fully developed seeds.

Discussion

Stem coppice

At Nanga, stem coppice was an effective method of regenerating *S. spicatum*, with a mean survival of 40.8 % at age 12 years. Protection treatments had no significant effect on coppice survival or growth. It appears that grazing was not a factor affecting coppice survival during the trial. Growth was similar between treatments, with stem diameters (at 150 mm) increasing only 2-3 mm yr^{-1}. These slow growth rates are typical of *S. spicatum* growing in semi-arid regions (Loneragan 1990). At age 12 years, 15 % of the coppice stems were producing mature fruit, which has the potential to promote further regeneration of the species.

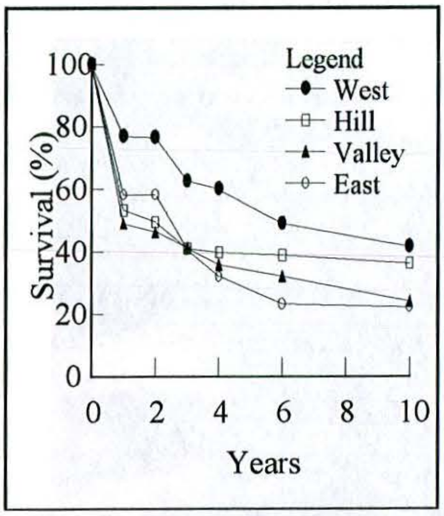

Figure 4. *S. spicatum* survival on four different dune faces: west, hill, valley and east.

The relatively high stem coppice survival was not due to rainfall, because the mean annual rainfall near Nanga was only 229 mm during the trial. Instead, the high coppice rate at Nanga may be related to the trees growing on deep red sands. The soft soils may have enabled a relatively large root system to develop, thus providing a large resource base to successfully coppice.

> *"It appears that stem coppice may be a useful method of sustaining S. spicatum after harvesting, especially on deep red sands."*

It appears that stem coppice may be a useful method of sustaining *S. spicatum* after harvesting, especially on deep red sands. Regenerating *S. spicatum* using stem coppice might also be a useful method of obtaining a second rotation of commercial timber from a plantation, especially in the higher rainfall regions. *S. spicatum* plantations are currently being established on farmland in the 400-600 mm annual rainfall regions of the Wheatbelt and Midwest (Shea *et al* 1998). The effectiveness of stem coppice on these plantations should be investigated.

Direct seeding

Planting *S. spicatum* seeds in dense vegetation was an effective regeneration technique at Nanga station, with a mean germination of 40% and a mean survival of 30.4 % at age 10 years. Brand (2000) also recorded *S. spicatum* survival rates of 20-30 % at age three years when planted near *Acacia burkittii*, *A. ramulosa*, *A. tetragonophylla* and *Hakea recurva*, at Ninghan station. These results further show that direct seeding *S. spicatum* near suitable host species is an effective method to promote regeneration within natural stands.

Although *S. spicatum* seedling survival was highest on the west face (41.5 %), dune face direction had no significant impact on seedling survival. Survival rates were highly variable within treatments, which was probably due to the availability of suitable host roots. The *S. spicatum* seeds were planted in a grid pattern in vegetation that contained a range of species. Therefore, suitable host roots may not have been available to all *S. spicatum* seedlings.

Seedling growth was similar between treatments, with a mean seedling height of 1.9 m and a mean stem diameter of 32 mm at age 10 years. At this age, 24 % of the seedlings were producing mature fruits. Therefore, the seeds from these seedlings also have the potential to further increase *S. spicatum* regeneration at Nanga station.

Acknowledgements

Special thanks go to Garth Pinnegar for his assistance with harvesting and establishing the trial plots. This study was funded by the Department of Conservation and Land Management, and the Forest Products Commission, Western Australia.

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SANDALWOOD WORK IN SPRIG

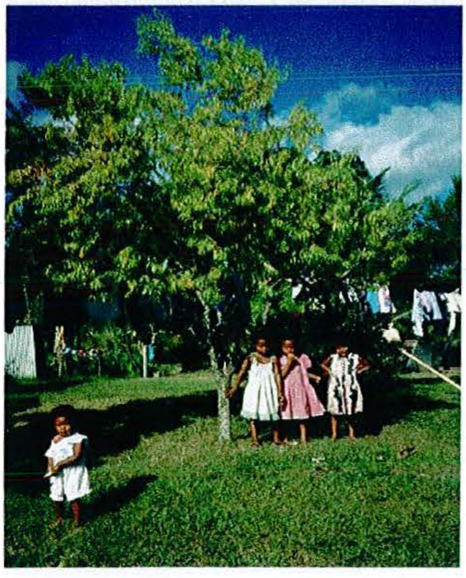
A pilot phase of SPRIG (South Pacific Regional Initiative on Forest Genetic Resources) ran for three years from 1996 – 2000. This project was funded by AusAID and implemented by the Forestry Departments in Fiji, Samoa, Solomon Islands, Tonga and Vanuatu and an Australian consortium comprising CSIRO Forestry & Forest Products, Queensland Forest Research Institute and Fortech. Sandalwood is an economically important, but threatened, indigenous tree in Fiji, Tonga and Vanuatu. During Phase 1, conservation and management strategies were developed for the two local species, viz. *Santalum yasi* in Fiji & Tonga and *S. austrocaledonicum* in Vanuatu. These strategies will continue to develop over time and be progressively implemented as resources allow. Copies of these strategies are available from the Australian SPRIG Project Office (see contact details).

SPRIG Phase 2 is a five-year AusAID-funded regional project in the South Pacific which commenced in May 2001. The project goal is "*to help Pacific Island countries conserve, improve and better promote the wise use of the genetic resources of priority regional tree species to enhance environmental protection and to promote economic and rural development*". The purpose of SPRIG Phase 2 is "*to strengthen the capacity of the participating Departments and Regional Organisations to conserve, improve and better promote the wise use of priority genetic resources in order to promote sustainable rural development*".

The five components of SPRIG 2 are:

1. Institutional strengthening and regional networking,
2. Conservation and sustainable management of priority species,
3. Tree improvement,
4. Demonstrating linkages between conservation, tree improvement and enhanced rural incomes, and
5. Project Management.

The main work on sandalwood in SPRIG Phase 2 will be in Components 2 and 4. As part of Component 2, key elements of the sandalwood conservation strategies will be implemented, while in Component 4 three ha of sandalwood seed stands/gene conservation stands will be established in each of Fiji, Tonga and Vanuatu. Training will include a short course in conservation of forest genetic resources, including sandalwood, and forest biodiversity in Vanuatu in June 2002.



Sandalwood: a vital tree genetic resource for sustainable rural development in the South Pacific (*Santalum yasi*, Vanua Levu, Fiji).

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Forest
Products
Commission
WESTERN AUSTRALIA

Sandalwood Seed

A number of changes are occurring with Sandalwood (*Santalum album*) seed supply from the Forest Products Commissionin Western Australia. The seed will still be sourced from the Kununurra plots in northern Western Australia, but the majority of the processing and storage will occur at the Forest Products Commission's Seed Centre in Manjimup. This change allows for improved cleaning and storage systems as well as supplying a viability test with each seed batch sold. This viability test is useful knowledge to nurseries so that they can determine their sowing rate and can calculate the expected seedling establishment.

Seed viability is only one aspect of quality seed supply. To maintain genetic vigour, the seed must be sourced from plots that contain diversity and maintain a high level of out-crossing between the trees. The Forest Products Commission is presently reviewing the genetic value of each of the Kununurra plots and a research plan to invigorate the population is being finalised.

If you would like to know more about the Seed Centre and its products, please look at our web site: www.fpc.wa.gov.au/seed/ or contact Dr. Liz Barbour (phone +61-8-9334-0302) for any queries.

The Sandalwood Research Newsletter (SRN) is published triennially by the Department of Conservation and Land Management and distributed free of charge. It is intended as a forum for information exchange on Santalum species worldwide. Articles on a range of Santalum species research and management issues are welcomed by the Sandalwood Research Newsletter.

If you would like to contribute an article to the SRN or wish to be included on the SRN mailing list, please send details to the Editor stating your name, title, position, organisation, postal address, telephone, fax and email address.

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