Sandalwood Research Newsletter

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The Need for Increased *Santalum* Species Research Collaboration for Germplasm Conservation

Editor: Andrew Radomiljac

From every indication the first issue of the Sandalwood Research Newsletter (SRN) has been very well received throughout South East Asia and Pacific island nations currently involved in *Santalum* species management and research. This highlights the strong interest generated in this important genus. Sandalwood timber products have considerable cultural importance and its high economic value highlights the importance for continual research and collaboration to develop suitable silvicultural techniques for plantation establishment and species conservation. *Santalum* species have the potential to make a considerable contribution to the rural economies of several countries (McKinnell 1993).

Sandalwood oil, from *S. album* in particular, is a perfumer's jewel. It possesses unique fragrance characteristics and has a special position in the perfume industry. Sandalwood oil has been utilised for thousands of years as a fragrance source (Muller 1984). This essential oil is one of the oldest, most valuable and expensive of the raw materials available to the perfumer (Hall 1985).

Demand for *Santalum* species oil and various forms of timber products - wood and joss sticks is very strong with no indication of assuagement. Strong demand for this high valued resource increases harvesting pressures on decreasing *Santalum* species populations. Hence, the need for research collaboration on *Santalum* species throughout the South East Asian and Pacific region is critical. Species conservation through the establishment of germplasm reserves is of high priority. Researchers at the Balai Penelitian Kehutanan (Forestry Research Institute) Kupang, Indonesia acknowledge the importance of *S. album* conservation through germplasm reserves. Harisetijono and Surimihardja (1993) indicate the commencement, with the Australian Centre for International Agricultural Research (ACIAR), of establishing representative ranges of *S. album* genetic resources in Nusa Tenggara Timur (NTT) to enable the selection of high yielding genotypes for future reforestation programs. Selection of plus trees and the establishment of seed orchards is of high priority.

A short paper described within this SRN issue summarises the ongoing efforts of establishing germplasm reverses. However, efforts are not always fruitful and increased collaboration between researchers, managers and organisations involved with *Santalum* species will increase the effectiveness of attempts to achieve satisfactory germplasm conservation.

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Genotypic Variation in *Santalum album*

Jonathan E Brand

Jonathan Brand has completed his M(Sc) thesis at Curtin University, Western Australia.
M(Sc) Thesis: Phenotypic and genotypic variation within Santalum album in West Timor.
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Through allozymic studies it has become clear that most species are not single panmictic units. Instead, limited gene flow and genetic drift have resulted in genetic differentiation between and within populations (Schwaegerle, Garbutt and Bazzaz, 1986). Isozyme activity in *Santalum album* was investigated by Egerton-Warburton (1990), but genetic variation within the species has not been studied. The genetic association between sandalwood growing in India and Indonesia is also unknown (Rai, 199). Indian's settled in Indonesia over a thousand years ago and it has been hypothesised that *Santalum album* was introduced to India from West Timor (Rajagopal Shetty, 1977).

The aim of this study was to examine the level of genetic diversity within and between populations of *Santalum album* in West Timor. *Santalum album* populations from West Timor and India were also compared to determine the level of genetic differentiation between the two countries.

Materials and methods

Santalum album seeds were obtained from 10 populations in West Timor and two populations in India.

The West Timor populations sampled were Ajaubaki, Buat, Netpalen, Niki Niki, Oenutnanen, Oinlasi and Siso. From each population, seed was collected from 12-20 trees, except Netpalen where seed was collected from four separate populations of 20 trees. Within India, bulk seed collections were obtained from Bangalore and Rayalpad. Seeds were germinated at Curtin University, Western Australia, and juvenile leaf tissue from 18-23 seedlings per population were used in isozyme analysis. Twenty three different isozyme systems were tested and of these, eight were sufficiently active in *Santalum album*. The active isozyme systems were: *Acp, Gdh, Lap, Mdh, Mdr, Pgi, Pgm* and *Sdh*. From these isozyme systems a total of 12 different isozyme loci was obtained.

Genetic variation measures were calculated using BIOSIS-1 (Swofford and Selander, 1989) and GENESTAT-PC (Lewis and Whitkus, 1989) computer programs.

Results

The mean genetic diversity measures for the *Santalum album* populations from West Timor and India are shown in Table 1. Within West Timor, the mean number of alleles per locus *(A)* ranged from 1.7 at Niki Niki to 2.0 at Oinlasi. The mean percentage of polymorphic loci per population was 52.5% and ranged from 50.0 to 58.3%. Populations with the highest expected heterozygosity (H_e) were Ajaubaki (0.20), Oinlasi (0.20) and Siso (0.20). The lowest H_e values came from Oenutnanen (0.13), Netpalen C (0.14) and Niki Niki (0.15). The mean observed heterozygosity (H_o) averaged over all populations was 0.14, which was

Table 1 Mean genetic diversity estimates (Nei, 1973) based on 12 loci in 12 populations of *Santalum album*

Population	*N*	*A*	*P*	H_e	H_o
West Timor					
1. Ajaubaki	21	1.9	58.3	0.20 (0.06)	0.17 (0.05)
2. Buat	23	1.8	50.0	0.18 (0.06)	0.13 (0.04)
3. Niki Niki	18	1.7	50.0	0.15 (0.05)	0.11 (0.04)
4. Oenutnanen	22	1.8	50.0	0.13 (0.05)	0.11 (0.05)
5. Oinlasi	22	2.0	58.3	0.20 (0.06)	0.15 (0.05)
6. Siso	23	1.9	50.0	0.20 (0.07)	0.15 (0.05)
7. Netpalen A	20	1.8	50.0	0.18 (0.06)	0.14 (0.04)
8. Netpalen B	19	1.9	50.0	0.19 (0.06)	0.16 (0.05)
9. Netpalen C	20	1.8	58.3	0.14 (0.04)	0.11 (0.03)
10. Netpalen D	20	1.9	50.0	0.16 (0.05)	0.13 (0.04)
Mean		1.8	52.5	0.17	0.14
India					
1. Bangalore	22	1.8	41.7	0.18 (0.07)	0.16 (0.06)
2. Rayalpad	21	1.7	41.7	0.13 (0.05)	0.10 (0.04)
Mean		1.8	41.7	0.16	0.13

N - sample size per locus; *A* - mean number of alleles per locus; *P* - percentage of polymorphic loci per population (0.99 criterion); H_e - expected heterozygosity; H_o - observed heterozygosity; standard errors in parentheses.

lower than the mean H_e (0.17). The two Indian populations had mean genetic diversity measures as follows: *A* (means 1.8, range 1.7-1.8); *P* (mean 41.7%); H_e (mean 0.16, range 0.13-0.18); and H_o (mean 0.13, range 0.10-0.17). Mean *P*, H_e and H_o values from the Indian populations were lower than the West Timor populations.

Within West Timor, the genetic distance between populations was generally low, ranging from 0.000 to 0.074 (Table 2). The genetic distance between the two Indian populations was 0.076. The mean genetic distance between the West Timor and Indian populations was 0.447, and ranged from 0.394 to 0.527. This high genetic distance clearly shows that the West Timor populations and the Indian populations are well differentiated.

Discussion

Within West Timor, the mean expected heterozygosity (H_e) from all *Santalum album* populations was 0.17, which is lower than that reported from other long-lived woody perennials (H_e = 0.267; Hamrick, Linhart and Mitton, 1979).The low level of genetic diversity within *Santalum album* may be related to its localised distribution. Plant species growing over a widespread range generally have significantly higher levels of genetic diversity than more narrowly distributed or localised species. Widespread plant species often have large continuous populations that are less susceptible to losses of genetic variation due to genetic drift (Hamrick and Godt, 1990). The *Santalum album* populations examined were small and isolated, which may have made them more prone to genetic drift. The relatively low H_e level found in *Santalum album* is comparable to other plant species growing over a similar geographic region. Two examples are *Eucalyptus diversicolor* (H_e = 0.15; Coates and Sokolowski, 1989) and *Eucalyptus crucis* (H_e = 0.19; Sampson, Hopper and James, 1988).

West Timor and Indian *Santalum album* populations had a mean genetic distance *(D)* of 0.447, which is very large compared with intra-specific distances in other species. Yacine and Lumaret (1989) examined 16 populations of *Quercus ilex* from around the Mediterranean and found a mean *D* of 0.023. Joly *et al.* (1992) found that the average genetic distance between western and eastern populations of *Acacia albida* in Africa was 0.273, and hypothesised that they were different races. The large genetic distance between the West Timor and Indian *Santalum album* populations indicates that they should be considered, at least, as separate varieties or races.

If sandalwood was introduced to India from West Timor over a thousand years ago (Rajagopal Shetty, 1977), a bottleneck effect may have caused genetic differentiation. Rapid speciation can occur if a population is founded by a small number of individuals. When population size is very small, random genetic drift affects gene frequencies (Nei, Maruyama and Chakraborty, 1975). Sandalwood descendants within India could be derived from a small portion of the species gene pool in Timor.

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Table 2 Estimates of Nei's (1972) genetic distance *(D)* among the 12 populations of *Santalum album.*

Population		*West Timor*										*India*	
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*1*	*2*
West Timor	1	-											
	2	0.060	-										
	3	0.064	0.004	-									
	4	0.031	0.137	0.139	-								
	5	0.073	0.066	0.065	0.116	-							
	6	0.004	0.051	0.052	0.047	0.047	-						
	7	0.005	0.068	0.070	0.056	0.051	0.001	-					
	8	0.000	0.064	0.067	0.043	0.060	0.000	0.000	-				
	9	0.004	0.073	0.072	0.058	0.067	0.003	0.001	0.000	-			
	10	0.002	0.074	0.074	0.051	0.062	0.002	0.000	0.000	0.000	-		
India	1	0.460	0.432	0.472	0.527	0.403	0.465	0.436	0.480	0.476	0.484	-	
	2	0.404	0.427	0.437	0.551	0.422	0.429	0.394	0.412	0.398	0.428	0.076	-

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Germplasm Conservation of Sandalwood

Peter Burgess

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The Australian International Development Assistance Bureau (AIDAB) funded a cooperative project, with the Australian Tree Seed Centre (ATSC), CSIRO Division of Forestry and the Western Australian Department of Conservation and Land Management (CALM), aimed to help collect and conserve germplasm of the rare and often endangered Pacific species of *Santalum.* The genus comprises 16 species and 17 varieties, many of which are geographically and probably reproductively isolated (Applegate *et al.* 1990).

The aims of the program are:

- Coordinate seed collections of *Santalum* species from the Pacific region. Store the seed in Canberra for the later establishment of a gene conservation bank/seed orchard, by CALM in Kununurra, Western Australia.
- Literature survey to cover growing conditions and cultural requirements of the various species.

Contact was established with ten countries in the Pacific region but seed was only available from four countries (Table 1). The Cook Island's *S. insulare* has not carried a crop for the last few years, while the Japanese *S. boninense* is reproduced only vegetatively.

Further attempts to obtain seed from other areas will be made. Personal contact has proven to be far more rewarding than mailed requests.

Table 1: Origin of *Santalum* species seed collection

Species	*Origin*	*Results*
album	Australia	0.3 kg
austrocaledonicum	New Caledonia	10 tree collection 200 seed bulk
lanceolatum	Australia	0.05 kg
yasi	Tonga	10 tree collection

Literature survey

A search, concentrating on species other than *S. album* was conducted. This together with DFR library accessions was compiled into a list of 46 references. Copies of all references are held by ATSC.

As a result of a recent visit by Stephen Midgley to India, ATSC expects to receive a collection of *S. album* in the near future. Although the Indian populations were not targeted in the program this will be a valuable addition to the project.

Gene pool and species/ provenance trials

The Department of CALM has commenced establishing *Santalum* species gene pool/family trials. In 1993 a *S. austrocaledonicum* gene pool/ family trial was established under flood irrigation, Kununurra. This trial consists of 10 *S. austrocaledon-icum* families collected from the New Caledonian island of Ile des Pins by the Cirad Foret.

Nursery propagation of this *S. austrocaledonicum* and *S. yasi* seed from New Caledonia and Tonga commenced in late 1993 and trials will be planted in 1994.

Collection of *Santalum* species seed for gene pool conservation is an important component of the Australian Centre for International Agricultural Research (ACIAR) *Santalum* species research program. Consequently, it is actively ongoing.

Germination tests

S. lanceolatum, after surface sterilisation, nicking the endocarp and using a medium of vermiculite, incubated at 25°C resulted in 90 percent germination. *S. austrocaledonicum* also responded well to the nick treatment with 80 percent germination. *S. yasi,* on the other hand, was more recalcitrant. The nick treatment or soaking in 30 percent hydrogen peroxide gave no germination. Overnight soaking in 0.25g/L GA3 has resulted in 10 percent germiantion after three weeks. Seedlings have grown quite well, in pots, in a heated glasshouse in Canberra for over a year. *Casuarina* has been a suitable host as it does not grow too vigorously.

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Determining Heartwood Oil Content within *Santalum album* and *S. spicatum*

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M(ForSc) Thesis: The Quantity and Quality of Heartwood in two species of sandalwood.
Supervisor: Dr W E Hillis

Sandalwood oil is produced commercially by steam distillation. The average commercial oil yield from *S. album* varies between four and six percent (Hughes and Richmond, 1980; Rai, 1990) but individual tree estimates have varied from 0.2 to 7.25 percent (Iyengar, 1968; Parthasarathi *et al.*, 1986). The reported average oil content of *S. spicatum* is two percent (Applegate *et al.* 1990) but individual oil content estimates have varied between 0.5 and 6.1 percent (Fleming and Uren, 1981).

Such variation in oil content has been attributed to the age of the tree (Shankaranarayana and Parthasarathi, 1984), the site or provenance (Sinha, 1961, Quemin, 1988) or the grade of the heartwood (Shankaranarayana and Parthasarathi, 1987). To examine this variation in more detail, a simple method of determining the content of sandalwood oil is required.

In this paper I have presented a method of estimating the content of sandalwood oil and have suggested a way in which the oil content in *S. album* can be predicted by a simple Soxhlet extraction.

Method

Two cross-sections, each 5 mm thick, were cut at 150 mm aboveground level and at crown break (*S. spicatum*) or below the first fork (*S. album*), from 39 *S. spicatum* trees from Western Australia and 30 *S. album* trees from West Timor, Indonesia. The bark and sapwood were removed and the heartwood was chipped into 5 x 5 x 20 mm pieces and ground in a Wiley mill until it passed through a size 20 mesh.

Two 10g samples of heartwood sawdust from each cross-section were placed into 25 x 80 mm cotton extraction thimbles, plugged with cotton wool and "conditioned" over super-saturated $CaCl_2$ at 25°C to a constant weight (about one week). This ensured that all samples had a similar moisture content.

Simultaneous Distillation and Extraction (SDE)

One 10g sample from each cross-section was extracted by SDE in a Likens Nickerson apparatus (Koedam, 1987). The sawdust sample and 100 ml distilled water were stirred in a 2L Erlenmeyer flask immersed in a 140°C silicon oil bath. Ten ml hexane and two angular bumping granules were added to a 25 ml pear-shaped flask which was immersed in a 90°C water bath. SDE proceeded for 24 hours. The oil/hexane mix was transferred to a clean weighed flask, the hexane evaporated and the oil dried over $CaCl_2$ in an evacuated desiccator for two days before weighing. The empty thimble was conditioned for one day and weighed.

The content of sandalwood oil in the sawdust sample was expressed as a percentage of the "air-dry" or conditioned wood mass using the formula:

$$\text{oil \%} = \frac{\text{oil}}{\text{wood}_{\text{COND}}} \times 100$$

where:
oil = mass of flask and oil - mass of flask
woodCOND = mass of conditioned thimble and sawdust - mass of conditioned thimble

Estimation of the Total Ethanol Soluble Extractives (TEE)

The other 10g sawdust sample was extracted in a Soxhlet with 100 ml ethanol for 24 hours, oven-dried at 103°C for two days and weighed. The sawdust was discarded and the empty thimble oven-dried at 103°C for one day, weighed, conditioned for one day and weighed. The TEE% was calculated using the formula:

$$\text{TEE \%} = \frac{\text{wood}_{\text{COND}} - \text{wood}_{\text{EXT}}}{\text{wood}_{\text{COND}}} \times 100$$

where:
woodCOND = mass of conditioned thimble and sawdust - mass of conditioned thimble
woodEXT = mass of oven-dried thimble and extracted sawdust - mass of oven-dried thimble

This estimate of TEE percentage includes the extractives (such as polyphenols, tannins, etc.), the sandalwood oil and water.

Results

The mean heartwood oil content was significantly lower in *S. spicatum* than *S. album,* whereas the mean TEE was significantly higher in *S. spicatum* (Table 1). The mean sandalwood oil content of *S. spicatum* was nearly twice that reported by Applegate *et al.* (1990), whereas the mean oil content of *S. album* was similar to that reported by Hughes and

Richmond (1980) and Rai (1990).

The TEE estimates is a combination of oil, water and other extraneous compounds. If the oil and water (about seven percent moisture content) are subtracted, *S. spicatum* heartwood contains about 10.4 percent extraneous compounds compared with only 4.5 percent in *S. album* heartwood. Therefore the proportion of oil in the TEE estimate is lower in *S. spicatum* (17 percent) than *S. album* (34 percent). This last statistic is very important for the accuracy with which the oil content of sandalwood may be predicted from TEE. A poor relationship was found between the TEE and the oil content of *S. spicatum* (Figure 1a), whereas a much stronger relationship was found between these variables in *S. album* (Figure 1b). Linear regression produced the model.

Conclusions

The oil content of *S. album* is well correlated with the TEE and this model could be used to predict the oil content (Figure 1b). The advantages with this method are that 1) Soxhlet extraction is a very simple, cheap and rigorous laboratory procedure compared with SDE or other methods of distillation; and 2) the method only requires 10g of sawdust, which could be bored from the side of a tree with little effect on tree health.

Since no relationship was found between the oil content and TEE in *S. spicatum*, the oil content must be determined by SDE.

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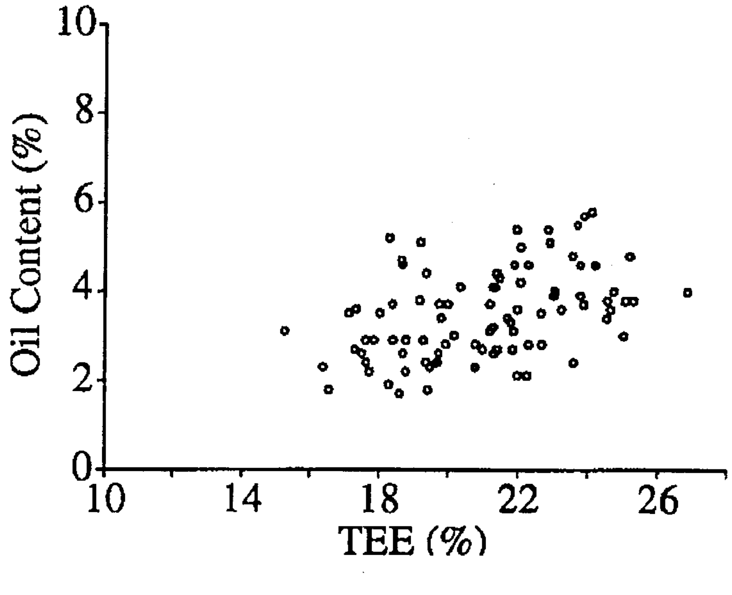
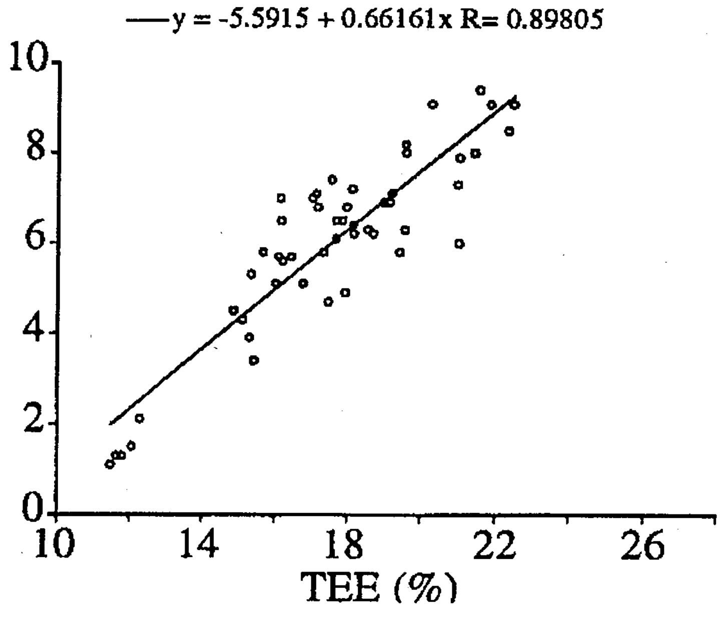
Table 1:
The mean oil content (oil%) and the amount of total ethanol soluble extractives (TEE%), expressed as a percentage of the "air-dry" wood weight at 150 mm aboveground level in *S. spicatum* and *S. album*. The standard deviation is given in parentheses.

Species	*Oil %*	*TEE %*
S. spicatum	3.69 (1.15)	21.1 (2.3)
S. album	6.07 (1.96)	17.6 (2.6)

Figure 1:
Relationship between the oil content and TEE in (a) *S. spicatum* and (b) *S. album*.



(a)



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The Sandalwood Research Newsletter

The SRN aims to promote the awareness of *Santalum* species and increase the exposure of *Santalum* species literature, the SRN is an avenue through which researchers and managers of *Santalum* species can achieve this. It is hoped articles will be received on a range of *Santalum* species research and management issues throughout the South East Asian and Pacific region. If you wish to contribute an article or wish to be included on the SRN mailing list, please write to the Editor giving your name, organisation and mailing address. All articles on relevant topics are welcomed.

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The Sandalwood Research Newsletter Editor invites all readers to write to the article authors to acquire additional information concerning *Santalum* species research and/or management work outlined in this SRN issue. An aim of the SRN is to promote collaboration between researchers and managers of *Santalum* species.

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