

Sandalwood Research Newsletter

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EDITOR'S NOTE

This edition of the SRN examines host trials with quandong (*Santalum acuminatum*) and genetic variation within Indian sandalwood (*Santalum album*). In South Australia, *S. acuminatum* is being grown in plantations for its edible red fruit. Matthew Lethbridge is examining *S. acuminatum* performance with different *Acacia* species that also have the potential for commercial use in the wattle seed industry. In previous trials, *Acacia* species have proven to be very good hosts for *S. spicatum* and *S. album*.

Studies on *S. album* from India and West Timor indicate that there is a high degree of variation within the species, and there may in fact be separate varieties or races. Within India, Angadi, Jain and Shankaranarayana examine the levels of genetic diversity within and between eight populations of *S. album*. Jain, Angadi and Shankaranarayana also discuss environmental, morphometric and genetic characteristics of nine *S. album* populations growing within India.

Recently I changed Departments, and I now work for the Forest Products Commission, Western Australia. However I am still researching sandalwood and I will continue to be editor of the SRN. My new mailing address is shown at the end of this newsletter.

Jon Brand

Progress report: Integrated wattle and quandong orchard

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Abstract

Eight species of semi arid acacia species with potential for wattle seed production in the native food industry were tested for their suitability as hosts for quandong (*Santalum acuminatum*) in an irrigated area at the Australian Arid Lands Botanic Gardens, Port Augusta, South Australia. Preliminary results indicate that *Acacia victoriae* is superior to the seven other species tested.

Introduction

The quandong fruit (*Santalum acuminatum*) and wattle seed from acacia species occupy significant niches in the fledgling native food industry (Graham and Hart 1996, Ahmed and Johnson 2000). *Acacia* species are showing significant promise in the sandalwood industry as host plants, (Brand *et al.*, 2001, Radomiljac *et al.*, 1999a) with the expectation that leguminous species could also provide valuable timber products.

A survey by Maslin *et al* (1998) identified 47 species of acacia that have potential for both seed production for human consumption and cultivation in the southern semi arid regions of Southern Australia. There has been a small but increasing demand for wattle seed in the native food industry and is part of investigations (all be it minor) into the commercial output in the broad scale planting of acacias to combat dryland salinity (Simpson and Chudleigh 2001). The current study is extending the theme of utilising the multipurpose nature of leguminous

acacias as host plants for quandong fruit production.

Materials and Methods

Within the research area of the Australian Arid Land Botanic Gardens is a chenopod heathland (predominantly *Atriplex* and *Maireana* species, these were retained) of deep alkaline (pH 9.5) loam of zero inclination. The area had been previously deep ripped (1992). Eight species of semi-arid acacia (Table 1) were planted in a grid pattern of 6 m by 4 m. Each species was planted as two rows of 11 plants from tube stock grown on site. They were irrigated with single drippers (8 litres per hour) at the base of each acacia for 2hrs, twice a week (32 litres per week). *Acacia papyrocarpa*, *A. victoriae* and *Santalum acuminatum* occur naturally on the site (outside the irrigated area).

Procedure

In September 1999, the acacia seeds were germinated and transplanted to large forestry tubes. At age seven

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months, the acacia seedlings were planted with a handful of slow release native Osmocote and mulching. There was no further addition of fertiliser to the site. In April 2001, two germinated kernels of quandong (orchard derived seed, Whyalla South Australia) were direct seeded within ten centimeters of each dripper. Quandong heights were recorded in April 2002 and October 2002 (Figures 1 and 2).

Statistics

Quandong mean heights were compared using one-way ANOVA, and Least Significant Difference (LSD) tests.

Results

It is evident from the results (Table 2) that *Acacia victoriae* appears to be a superior host to the seven other selected native food acacias. None of the quandongs showed any obvious nutrient deficiency symptoms by visual leaf inspection. (see Barrett *et al* 1993). It was also noted that where two germinants survived, both plants usually showed similar heights, perhaps indicating the vital role of the host acacia's root system in sustaining growth of the quandong. It should also be noted that the quandong survival from germinated seed was $53 \pm 4.5\%$ across the eight species, and for *Acacia victoriae* 50% of the planted germinated seed survived. It is often reported that transplantation

Table 1. The Eight acacia species and their seed source, selected as hosts for *S. acuminatum*. The species were selected from the list prepared by Maslin (1998) and suitability for the site.

Species	Source
<i>A. murrayana</i>	70 km. West of Cobar (AALBG collection)
<i>A. victoriae</i>	Kenmore Park SW (thornless) (AALBG)
<i>A. rivalis</i>	RG 554 K (Australian Bush products)
<i>A. brumalis</i>	CALM D1001
<i>A. calamifolia</i>	44 Km. West of Yunta (AALBG)
<i>A. hakeoides</i>	Quorn (AALBG)
<i>A. hemiteles</i>	CALM N98177
<i>A. argyrophylla</i>	Carrieton (AALBG)

of quandong seedlings can be problematical. The most economic and efficient method for establishing *S. spicatum* is by direct seeding. (Brand and Jones 1999). This methodology may have practical use in the quandong industry.

“*Acacia victoriae* is currently the most important wattle used in the Australian Native Food Industry “

Discussion

Quandong planted near *Acacia victoriae* showed significantly better growth characteristics for the first 18 months of growth when compared to the other seven host species at this site. From a commercial point of view this is a good result as *Acacia*

victoriae is currently the most important wattle used in the Australian Native Food Industry (Maslin 1998), and is likely to continue so, given its potential dual role. Native food growers have used this combination because of its native food association and probably more as a legume of convenience than from scientific rational (Schwarz, 2001).

A significant feature of *Acacia victoriae* is its high salt tolerance (Maslin 1998). *Santalum acuminatum* is classified as a salt tolerant plant (Walker 1989). Hence this combination may have value in salt land reclamation. Neither species has yet been recognised for this purpose (Marcar 1995). It is also reasonable to assume that studies with other combinations of acacia/ santalum may have some pre-



(a).



(b).

Figure 1ab. Quandong, age 18 months, growing with 30 month old (a) *Acacia victoriae*—quandong ht: 1.24 m; and (b) *Acacia argyrophylla*—quandong ht: 1.1 m.

Table 2. Growth parameters of quandong (18 month old) associated with indicated acacia species (30 months old). Same letter following score indicates that they are not significantly different (LSD 0.05).

Species	Average Quandong ht. (cm.), October 2002
<i>A. victoriae</i>	98.2 a
<i>A. hemiteles</i>	81.0 b
<i>A. argyrophylla</i>	74.7 bc
<i>A. murrayana</i>	68.6 bc
<i>A. calamifolia</i>	66.2 cd
<i>A. rivalis</i>	62.0 cd
<i>A. brumalis</i>	58.2 cd
<i>A. hakeoides</i>	52.6 d

dictive value in species selection. A *S. album* host study by Radomiljac (1999a) included *Acacia ampliceps*, which has high salt tolerance (Marcar 1995) and out performed *Acacia trachycarpa* as a host for tropical sandalwood. Another pot study by Byrne (1998) used the highly salt tolerant *A. cyclops* (Marcar 1995) but could not recommend this species as a good host.

Acacia brumalis has sometimes been found growing in moderately saline soils (Maslin 1998), but in this study it did not stand out as a host for quandong. Similarly *A. saligna* is often found on moderately saline soils (Marcar 1995, Maslin 1998). In a similar study (although non irrigated) in WA, Brand *et al* (2001) found *A. saligna* to be a very good primary host for *Santalum spicatum*, when compared to *A. acuminata*, *A. hemiteles* and *A. microbotrya*. However, this may be attributable to the fast growth rate of this species, nevertheless, combinations of host and quandong with revegetation value in saline areas can only be considered a useful addition to farm management practices. The attribute of salt tolerance in plants is often associated with water conservation strategies.

Little or no research has been done at this stage into the rate of water use of acacia species (Simpson and Chudleigh 2001). Species with high water use rates, will perpetuate a less favourable water potential gradient (Radomiljac 1999b, Byrne 1998) from host to parasite with consequent re-

ductions in growth. A study of the water use rates of the eight species used in this study and others may yield some useful correlations with the hemi parasites growth rate. A host study by Brand *et al* (2000) concluded that the faster growth rate of *S. spicatum* near *A. acuminata* did not appear to be due to greater access to water, especially during the summer period but this study compared *Acacia*, *Allocasuarina* and *Eucalyptus* species, where nutritional differences may be more significant. Host : parasite interactions are clearly complex and empirical data will always be a valuable means of assessing species as host plants.

Acknowledgements.

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used for isoenzyme analysis and results obtained (Angadi *et al*) were used to study the genetic variation. A method of Nei (1972 and 1973) which is based on identity of genes between populations was used for the purpose. Of the eight enzymes tested, four enzymes namely POD, MDH, GDH, GLUDH were active in the seed tissue.

Genetic diversity between sandal populations of different provenances in India

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Abstract

Genetic distance between Sandal (*Santalum album* L.) populations of different provenances has been studied, using isoenzyme data. Results show that there is a gap in genetic distance between eight identified provenances of Sandal which indicates that they are of separate varieties or races, thus further confirming their status.

Introduction

Sandal, which is represented in the genus *Santalum*, is indigenous to peninsular India. Earlier it was of the opinion that Sandal was introduced to India from Timor, in Indonesia (Fischer, 1928, 1938). A recent study by Brand (1994) concluded that Sandal in West Timor and India are of different varieties or races, because of large genetic distance between the two countries.

In India, Sandal is distributed all over the country and more than 90% of it lies in Karnataka and Tamil Nadu. Based on a recent survey undertaken in India, eight potential Sandal bearing areas in different eco-climatic zones have been identified as potential provenances (Jain *et al* 1997) and their status was confirmed using isoenzyme study (Angadi *et al*, in press). In spite of their known genetic identity with reference to various parameters, information on their genetic diversity between sandal populations of different provenances is lacking. In this present work, attempt has been made to study the genetic distance between Sandal populations of different provenances using isoenzyme study. The concept of genetic distance used here is based on Nei's (1971) theory which means

normalized identity of genes between populations is equivalent to protein identity. The term genetic distance is then defined as number of nucleotide or codon differences per unit length of DNA molecule.

“Thus the gap in genetic distance between different provenances of Sandal indicates that they are separate varieties or races “

Materials and Methods

Santalum album L. seeds were collected from eight different provenance areas during fruiting season. Twenty individual seeds per population were

Results and Discussion

The mean genetic diversity measures for *Santalum album* L. populations of different provenances are presented in Table 1. The mean number of alleles per locus ranges from 1.4 (Bangalore and Seoni) to 3.6 (Mandagadde). The percentage of polymorphic loci per population varies from 33.0% (Seoni) to 69.2% (Marayoor). The maximum heterozygosity (0.50) was found in Bangalore provenance and minimum (0.18) in Mandagadde provenance. Other provenances show medium heterozygosity (0.26 to 0.44).

Table 2 indicates estimates of Nei's (1972) genetic distance (D) between Sandal populations of different provenances. Normalized genetic identity between Koraput and Mandagadde and between Koraput and Javadi were both close to zero, hence calculation of genetic distance between them were omitted (Nei, 1972). Genetic distance between Mandagadde and the Bangalore provenance is found to be minimum (0.125), where as genetic distance between Thangli and Seoni-provenance is found to be maximum (0.585).

Table 1. Mean genetic diversity estimates (Nei's 1972) of different provenances of sandal.

S. No	Population	Sample Size	Mean No of Alleles per locus	% of Polymorphic Loci	Heterozygosity (figures in brackets indicate stand. error)
1	Mandagadde	20	3.6	57.1	0.18 (0.14)
2	Bangalore	20	1.4	37.5	0.50 (0.23)
3	Thangli	20	1.6	33.5	0.44 (0.15)
4	Marayoor	20	2.7	69.2	0.26 (0.25)
5	Chitteri	20	1.8	55.6	0.42 (0.20)
6	Javadi	20	1.5	40.0	0.38 (0.28)
7	Seoni	20	1.4	33.0	0.43 (0.21)
8	Koraput	20	1.8	50.0	0.40 (0.22)

Table 2. Genetic Distance (D) between sandal populations of different provenances (using all loci).

Population	Man	Ban	Mar	Seo	Tha	Chi	Jav	Kor
Man	-----							
Ban	0.125	-----						
Mar	0.131	0.161	-----					
Seo	0.161	0.260	0.174	-----				
Tha	0.252	0.260	0.357	0.585	-----			
Chi	0.292	0.168	0.337	0.276	0.328	-----		
Jav	0.292	0.357	0.456	0.420	0.482	0.432	-----	
Kor	-	0.482	0.561	0.469	0.509	0.328	-	-----

Table 3. Mean Genetic Distance (D) between individual provenance and other identified provenances.

Sl. No.	Provenance	Mean Genetic Distance (D) from other Provenances
1	Mandagadde	0.55
2	Bangalore	0.26
3	Thangli	0.31
4	Marayoor	0.34
5	Chitteri	0.40
6	Javadi	0.31
7	Seoni	0.49
8	Koraput	0.85

Data in Table 2 were used to calculate mean genetic distance between individual provenances and other identified seven provenances, and are represented in Table 3. From this, it is found that Bangalore and other provenances had a minimum mean genetic distance of 0.26; Koraput and other provenances had a maximum mean genetic distance of 0.85. The mean genetic distance between other provenances ranges from 0.31 (Marayoor / Chitteri and other provenances) to 0.55 (Mandagadde and other provenances). Thus the gap in genetic distance between different provenances of Sandal indicates that they are separate varieties or races which further confirms their status.

Conclusion

The genetic diversity measures for *Santalum album* L. population of different provenances very well indicate

that they are of separate varieties or races. The larger genetic distance between them further confirms their status. And it is also concluded that the provenance of Bangalore was found to be the best in terms of heterozygosity and hence suitable for breeding and propagation work on Sandal.

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Edaphic, environmental and genetic factors associated with growth and adaptability of Sandal (*Santalum album* L.) in provenances

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Abstract

Sandal tree grows under different edaphic and eco climatic conditions. Considering large genetic distance between provenances, it is concluded that under diverse locality factors sandal adapts very well in terms of growth, heartwood and oil content.

Introduction

Nearly 90% (8100 sq.km) of the total area of Sandal (*Santalum album* L.) 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.

Sandal tree is mainly exploited for its heartwood which yields the renowned East Indian Sandalwood oil, rated very high for its sweet fragrant, persistent, spicy, warm, woody note, tenacious aroma and fixative property. Sandal heartwood currently priced at Rs.12 lakhs per ton and its oil Rs, 22,000/-per kg.

At present, India produces 1000 tons of heartwood and 40 tons of oil per annum. For many years till now, sandalwood oil alone contributed nearly 25% of the revenue earned from the export of various essential oils.

The aim of this provenance investigation is to gather improved knowledge of genetic variation of the species. It is therefore, necessary that the purpose of provenance testing should combine both genetic variability and environmental factors.

Material and Methods

Nine sandal bearing areas within India were identified as potential provenances on the basis of population density, phenotypic character, latitude,

and longitude and eco-climatic (Jain *et al* 1998). Status of these provenances were confirmed by isoenzyme studies (Angadi *et al* 1999), which also found that the girth of sandal tree influences the percentage of oil. Heartwood from young trees (around 10 years of age, height <10m, girth <50 cms) contains 0.2 to 2% of oil and that from the mature trees (30 to 50 years of age, height 20 m, girth 100 cms) contains 2.8 to 6.2% of oil.

“The existence of different provenances within species may be taken into consideration while making a choice of planting material for further multiplication and mass propagation”

In Table 1, data in respect of area, density, latitude, longitude, altitude, rainfall, temperature, soil type, pH, percentage of oil and genetic distance (Angadi *et al.* 2003), from all nine sandal provenances is shown.

Results and Discussion

The provenances Bangalore, Marayoor and Thangli (Table 1) are growing well at an altitude of 1000m, average annual rainfall 850-1450 mm, max. temperature 33-38°C, minimum temperature 8-12°C, on soil red sandy loam or black clay having slightly acidic to slightly basic pH (6.5 to 7.5) and moderate nutrient status. The genetic distance (D) existing between populations of provenances was found

to vary from 0.052 to 0.292. Thus the larger genetic distance between different provenances concludes that they are of separate varieties or races.

The observed variation in trees from widely different parts of the species range could be referred to as geographical or racial variation. From the history, it is known that the sandal seeds are originally either spread through natural selection or by means of propagation from central provenance of Mysore State. Over a period of years these trees might have adjusted to local environmental and edaphic factors leading to the establishment of geographical races or provenances in this species, which has a large genetic distance. The existence of different provenances within species may be taken into consideration while making a choice of planting material for further multiplication and mass propagation. It is seen from Table 1 that, sandal adapts very well under diverse locality factors in terms of growth, heartwood, oil content and biochemical characteristics.

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The authors are thankful to Dr. K.S. Rao, Director of the Institute for his encouragement during the course of this study.

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Table 1. Edaphic, environmental and genetic factors of Sandal provenances.

Provenance	Forest Division State	Sandal bearing area ha.	Density trees/ha in area	Latitude Longitude	Altitude	Mean Annual Rainfall mm	Temp. Max. Min. °C	Soil Type	pH	% of oil	Girth range (cm)	Genetic identity	Genetic distance
Bangalore	Bangalore Karnataka	24648.00	500	12° 58' N 77° 38' E	1000 m	850	36.8 12.2	Red loam	6.3-6.5 Acidic	3.19	30-140	0.75	0.125
Thangli	Chickamagalur Karnataka	47121.00	300	13° 40' N 76° 00' E	766 m	1500	44.0 10.5	Red loam & Alluvium	7.5-7.8 Alkaline	2.76	30-100	0.56	.052
Mandagadde	Shimoga Karnataka	65529.00	100	13° 9' N 75° 40' E	650 m	2000	38.1 13.0	Red loam	5.5-5.8 Acidic	1.55	30-70	*	*
Chitteri	Harur Tamil Nadu	60000.00	70	12° 0' N 78° 6' E	1050 m	1000	35.2 8.2	Red sandy loam	6.0-6.3 Acidic	2.06	30-60	0.51	0.292
Javadis	Tirupattur Tamil Nadu	16517.00	90	12° 3' N 78° 7' E	930 m	1200	38.0 12.4	Red loam	6.6-6.7 Acidic	1.59	30-70	0.51	0.292
Marayoor	Munnar Kerala	1496.67	500	10° 1' N 77° 1' E	1000 m	1450	36.0 10.0	Black clay	6.2-6.7 Acidic	3.15	30-100	0.74	0.131
Koraput	Rayagad Orissa	2540.00	200	19° 55' N 82° 35' E	859 m	1525	38.0 4.5	Red sandy loam	6.2-6.6 Acidic	0.8	30-50	0.0025	-
Seoni	Seoni Madhya Pradesh	3000.00	60	22° 1' N 79° 5' E	900 m	1600	40.0 5.0	Laterite	6.4-6.9 Acidic	2.06	30-80	0.69	0.161
Horsely Hills	Chittoor East	3050.00	70	13° 5' N 78° 8' E	4312 m	900	30.0 5.0	Red loam	6.5-6.8 Acidic	3.34	30-80	#	#

* Genetic identity / Genetic distance measured from Mandagadde provenance

Newly identified provenance work is in progress

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