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RESEARCH

BIOPHYSICAL CHARACTERISTICS OF SANDALWOOD (*SANTALUMALBUM* L.), AN ANALYSIS

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Abstract: Sandalwood (*Santalum album* L.) is an economically important versatile hardwood species in India and it is known to grow in varied regions and climatic conditions. Identifying the appropriate plant traits is crucial for ensuring sustained yield in plantation over the long term. The objective of this study was to explore the biophysical characteristics of sandalwood, uncovering variations in the examined traits. The investigation reveals diverse leaf colorations ranging from yellowish-green to dark green, exhibiting varying degrees of intensity. Seven distinct leaf shapes were identified, including Ovate, Obcordate, Elliptical, Lanceolate, Sickle-shaped, Oblanceolate, and Obovate. The leaf lamina displayed a surface area spanning from 7.6 cm² to 21.26 cm², while thickness along the midrib ranged from 498.92 µm to 877.13 µm. Additionally, laminal side thickness varied from 196.09 µm to 406.73 µm. Microscopic analysis unveiled stomatal indices on the abaxial leaf surface, ranging from 19.28% to 29.46%. Furthermore, the study assessed epicuticular wax content, revealing a maximum of 11.97 g and a minimum of 2.41 g per leaf. These findings provide valuable insights into the diverse leaf traits within the species, highlighting the significance of morphological and anatomical characteristics in plant taxonomy and ecology. Further exploration of these traits may unveil their significance in conferring resistance against pests and pathogens affecting sandalwood.

Keywords: Sandalwood, Biophysical, Leaf, variations

INTRODUCTION

Indian sandalwood (*Santalum album* L.), commonly known as Chandan, is a medium- to large-sized tree native to the Indian subcontinent that is prized for its heartwood and oil (Sundararaj and Sharma, 2010).The unscented white sapwood is used in handicrafts and furniture(Brandis, 1903)and India has maintained its position as the primary producer and exporter of sandalwood oil for over 5000 years(Hansda, 2009).In India it occurs in southern dry deciduous and thorn forest types either along with other species as an "associate" or along farm bunds and fence of private holdings (Sundararaj and Sharma, 2010). Poor management and over exploitation of sandalwood resources have depleted natural stands, which have seen dramatic global declines over the last 100 years (Barbour et al., 2010). In order to overcome this situation, government policies were amended in favour of promotion of sandalwood cultivation by farmers and private entrepreneurs. Since, sandalwood is naturally a hemiparasite and parasitize more 300 species of plants, it is emerging as one of the important agroforestry species (Sundararaj *et al*.,2018).The growth and yield of heartwood production depend on various factors such as climate, soil, seed selection, maintenance, pest and disease control measures, and protection from smuggling. However, one of the most overlooked factors is the selection of the right traits of seedlings for plantation.Better growth, quality and broad adaptability can be achieved through careful selection of the best species (Kjaer and Foster, 1996). Due to cross-breeding and the wide range of agroclimatic conditions prevailing in the habitats of sandalwood, tree growing will contribute to a high rate of diversity in the sandalwood, which has a complex genetic structure background. The present study was focused on the variation in the biophysical characteristics of sandalwood and the findings are presented in this communication.

The core sample from each tree was extracted using a Hagl of increment borer

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MATERIALS AND METHODS

Study Area

The variation in the biophysical characteristics of sandalwood was studied by undertaking survey and sampling in the Bangalore sandalwood provenance which is located in the campus of ICFRE- Institute of

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Wood Science and Technology, Bangalore with the latitude of 13.0055° N and longitude of 77.5692° E during July 2022, wherethe mean minimum and maximum temperatures ranged from 22 to 30°C and theannual rainfall measured102.9 cm.

Sampling for morphological traits

For the estimation of leaf morphological diversity and its biophysical properties,randomly ten sandalwood trees of 5–6-year-old were selected (Fig 1).Three sets of five leaves each from different branches and different heights were collected from the selected trees. The study was focused on four macroscopic (leaf color, leaf shape, thickness and leaf area) and two microscopic (stomatal index and epicuticular wax) biophysical traits.

The macroscopic features of leaf like color, shape, length, width, area and thickness were measured. Freshly collected leaves were initially classified based on colouras yellow-green, light green, medium green or dark green.The leaf length then measured from the apex to the beginning of the petiole along the midrib and the leaf width was measured across the widest part of the leaf. Then the sample leaves were spread out and dried overnight by pressing under blotting paper and theleaf shapes werecategorized into ovate, obovate, oblanceolate, elliptical and lanceolate by visual observation. Also, the area of leaves were measured by placing leaves on graph sheet and the margin of the leaves were traced out. Using an appropriate scale of 1 cell is equal to 1cm^2 on the graph sheet.The thickness of the leaf measured using a transverse section of the leaf taken through the midrib and stained with safranin. These sections were visualized under phase contrast microscope (NIKON ECLIPSE) and diameter of the leaf is measured using the software NIS elements DR 4.30.

For the microscopic trait, the stomatal index, abaxial surface of leaf were considered. Three leaves were collected from each of the ten sandalwood trees, from different branches of the tree. The epidermal peel of abaxial surface of leaves considered for calculation of stomatal index. Also alternatively, clear nail polish smeared imprint is also used for the study. Images were captured using the software NIS elements DR 4.30.The number of stomata and the epidermal cells in the microscopic field were counted.

Stomatal Index was calculated using the formula –

$$\text{Stomatal Index (SI)} = \left[\frac{\text{Number of stomata}}{\text{Number of epidermal cells} + \text{Number of stomata}}\right] * 100$$

The epicuticular wax was extracted by immersion method of using chloroform (Hujon and Saral, 2022). The leaf bits of 4 cmsquares were cut out from the centre of the leaves. Five clean dry petri-dishes (15mm × 70mm) were pre-weighed and10 ml of chloroform was poured into each of the petri-dishes. The square cuttings from the five leaves of one plant were placed in the petri-dishes. After 1 minute the leaf pieces were removed and the setup was left undisturbed in a hot air oven until all the chloroform evaporated. The final weight of each petri-plate was taken with the condensed epicuticular wax smeared in and around the petri plate surfaces. The wax concentration (mg/leaf) was calculated using the formula of Yin et al. (2011), where Wax concentration = (W_1 – W_0), given W_1 is the final weight of the petri-plate with condensed epicuticular wax (mg), and W_0 is the initial weight of the fresh, dried petri-plate (mg).

RESULTS AND DISCUSSION

The sandalwood plant was mainly planted and exploited for its aromatic oil. It is a small evergreen hemi-parasitic glabrous tree with slender drooping branches (Sindhu et al., 2010). The present study on the biophysical characteristics of sandalwood gives essential link in varietal selection to identify individuals that are in the interests of economic value and domestication of the species. The descriptive statistics were made from 10 morphological trait taken from *S.album* L. revealed an important variability among the tree species.

Leaves are the major part of the plant which are involved in photosynthetic carbon fixation, respiration and transpiration and also are the most sensitive parts to climatic changes (Chen et al., 2012; Carlson et al., 2016). Leaf colour varied from yellowish green, light, medium todark green with varying degree of colour intensity. Then seven different shapes were observed viz., Ovate, Obcordate, Elliptical, lanceolate, Sickle shape, OblanceolateandObovate (Fig 2.). Among these most plants shared ovate type of leaf morphology. The average length of the leaf varied from highest of 8.4 cm to a lowest of 4.9 cm and width varied with highest value of 3.4 cm to a lowest value of 2.1 cm. This resultcorroborates the report of Karthik et al. (2023) in terms of the measurements of leaf size and shapes (Fig. 3).

The surface area of the leaf lamina ranged from a maximum of 21.26 cm^{2}to minimum of 7.6 cm^2 (Table 1).The younger leaves are greener in colour than the mature leaves, it corresponds to nutrients distributed in the leaf and amount of chlorophyll content. The deeper green colour of older leaf is due to more nutrients, thus having more chlorophyll (Nurdin *et al*., 2009). The data reflected a huge difference in leaf blade shape within same genotype of sandalwood. Larger leaf area in plants usually corresponds to more surface area for transpiration and hence more water loss from the plant. However, it is observed from the data that plants with larger leaf area also exhibit greater thickness and hence higher epicuticular wax content. The wax layer probably curbs rapid water loss through the leaves. In plants, gaseous exchange and regulation of water levels in the body is essential for physiological processes such as respiration and photosynthesis. Leaf thickness along midrib ranged from the highest value of 877.13 µm with lowest value of 498.92 µm, similarly laminal side thickness ranged from 406. 73 µm to 196.09 µm (Table 2; Fig. 3).

The microscopic characters like stomatal index of abaxial surface of leaf ranges from maximum of 29.46 % to a minimum of 19.28% (Table 3; Fig. 4). They are paracytic and are irregularly oriented. Venal regions are without stomata. Exchange of water, carbon dioxide, oxygen, other gases and compounds in plant takes place through the stomatal aperture which consists of the two large dumb-bell shaped or kidney bean shaped guard cells surrounding the central opening and 4 to 5 subsidiary cells. Stomatal movement (opening and closing) is predominantly controlled by water availability and CO_2 concentration and also influenced by various endogenous factors along with environmental factors. Stomata are most commonly present on the surfaces of leaves, but they can also be present on inflorescences, fruits, herbaceous stems, petioles, tendrils and other parts of plant (Paul et. al., 2017). The highest amount of epicuticular wax of the leaf is 11.97 g and the least amount is 2.41g per leaf(Table 4).Leaf epicuticular waxes are the interface between leaves and the atmosphere, acting in the reduction of water loss (Medeiros et al., 2017). As per the observation, there is direct correlation between the area of leaf and distribution of stomata with higher stomatal index in larger leaves. The hypostomatic nature of sandalwood, presence of stomata only on the lower epidermis of leaves might be an adaptation to minimize water loss through transpiration aiding the plant to tolerate high temperatures and maintains its evergreen nature. The host plant's morphological characters serve as a non-preference mechanism for feeding and oviposition by insects (Painter, 1951). Among the biophysical characteristics studied leaf lamina thickness is known to impart resistance against jassids (Khalil *et al*., 2017) and mired bugs (Song *et al*., 2021) and higher wax content in the leaf are known to offer resistance against leafhoppers (Laxman *et al*., 2017).

Table 1. Leaf measurement, colour and shape of *S. album*

Tree sample	Avg. Length (cm)	Avg. Width (cm)	Avg. Area (cm^2)	Colour	Shape
1	4.97	2.61	7.66	Yellow green	Ovate
2	6.69	3.31	14.26	Medium green	Ovate
3	6.91	3.47	15.46	Dark green	Ovate
4	5.36	2.25	7.97	Light green	Ovate
5	6.48	2.65	11.23	Light green	Ovate, obovate and oblanceolate
6	6.87	2.81	13.80	Light green	Elliptical
7	6.8	2.69	12.44	Medium green	Elliptical
8	8.45	3.7	21.26	Dark green	Ovate
9	8.39	2.77	15.98	Medium and dark green	Lanceolate
10	6.7	2.13	9.89	Medium green	Elliptical

Table 2. Leaf thickness (in µm) of *S. album*

Tree sample	Region of leaf	Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5	Average
1	**MR**	447.64	450.04	426.46	445.75	452.52	**444.48**
	LS	357.57	352.04	346.54	354.36	352.55	**352.61**
2	**MR**	430.16	422.92	422.14	421.6	447.84	**428.93**
	LS	343.38	334.42	335.25	344.27	342.6	**339.98**
3	**MR**	657.36	798.42	784.8	669.06	867.92	**755.51**
	LS	220.1	225.53	217.43	224.92	287.93	**235.18**
4	**MR**	437.75	438.72	435.95	428.21	438.46	**435.82**
	LS	344.15	344.07	343.81	341.17	343.76	**343.39**
5	**MR**	650.86	463.79	411.57	520.68	551.64	**519.71**
	LS	259.63	246.22	216.47	239.05	231.79	**238.63**
6	**MR**	802.61	629.61	516.08	553.78	700.31	**640.48**

	LS	272.36	340.16	257.22	341.75	238.93	**290.08**
7	**MR**	709.05	932.39	664.65	736.85	548.53	**718.29**
	LS	306.09	368.1	352.38	268.69	262.38	**311.53**
8	**MR**	825.93	871.23	921.06	901.89	865.53	**877.13**
	LS	218.97	188.32	203.14	177.56	195.89	**196.78**
9	**MR**	747.72	501.48	869.77	840.28	606.18	**713.09**
	LS	501.8	437.54	536.09	299.61	258.6	**406.73**
10	**MR**	688.81	564.24	720.26	697.74	566.42	**647.49**
	LS	178.23	159.16	211.67	256.04	175.37	**196.09**

Table 3. Stomatal Index of leaf in percentage.

Tree sample	Sample Leaf	S	E	E+S	Stomatal Index (%)	Average
1	Leaf 1	50	139	189	26.46	
	Leaf 2	39	136	175	22.29	**25.80**
	Leaf 3	45	112	157	28.66	
2	Leaf 1	46	135	181	25.41	
	Leaf 2	33	110	143	23.08	**22.83**
	Leaf 3	28	112	140	20.00	
3	Leaf 1	35	133	168	20.83	
	Leaf 2	38	123	161	23.60	**23.57**
	Leaf 3	36	101	137	26.28	
4	Leaf 1	39	101	140	27.86	
	Leaf 2	53	117	170	31.18	**29.47**
	Leaf 3	37	89	126	29.37	
5	Leaf 1	41	116	157	26.11	
	Leaf 2	43	130	173	24.86	**27.12**
	Leaf 3	55	126	181	30.39	
6	Leaf 1	34	126	160	21.25	
	Leaf 2	38	129	167	22.75	**20.06**
	Leaf 3	27	140	167	16.17	
7	Leaf 1	33	123	156	21.15	
	Leaf 2	42	135	177	23.73	**21.72**
	Leaf 3	28	110	138	20.29	
8	Leaf 1	49	136	185	26.49	
	Leaf 2	54	113	167	32.34	**27.57**
	Leaf 3	38	121	159	23.90	
9	Leaf 1	40	120	160	25.00	
	Leaf 2	51	157	208	24.52	**26.49**
	Leaf 3	59	138	197	29.95	
10	Leaf 1	36	144	180	20.00	
	Leaf 2	37	129	166	22.29	**19.28**
	Leaf 3	21	114	135	15.56	

S= No of Stomata in the microscopic filed; E= No of epidermal cells in the same microscopic field; Stomatal Index (%) = S/(E+S)* 100

Table 4. Epicuticular wax of leaf in grams

Tree	Sample Leaves	W_1 (g)	W_0 (g)	W_1-W_0 (g per4cm^2)	Area of leaf	Total wax content (g)	Average(g)
	Leaf 1	31.02	31.02	0.001	11.225	2.81	
	Leaf 2	32.00	31.99	0.001	12.43	3.11	
1	**Leaf 3**	29.51	29.51	0	20.92	0.00	**2.72125**
	Leaf 4	31.74	31.74	0.001	14.68	3.67	
	Leaf 5	30.49	30.49	0.001	16.09	4.02	

2	**Leaf 1**	31.02	31.02	0.002	30.71	15.36	**5.101**
	Leaf 2	32.00	31.99	0.001	20.67	5.17	
	Leaf 3	29.51	29.51	0	22.36	0.00	
	Leaf 4	31.74	31.74	0.001	19.93	4.98	
	Leaf 5	30.49	30.49	0	22.01	0.00	
3	**Leaf 1**	31.02	31.02	0.001	18.73	4.68	**4.3815**
	Leaf 2	32.00	31.99	0.002	18.6	9.30	
	Leaf 3	29.51	29.51	0	26.89	0.00	
	Leaf 4	31.74	31.74	0.001	17.33	4.33	
	Leaf 5	30.49	30.49	0.001	14.37	3.59	
4	**Leaf 1**	31.02	31.02	0.002	20.01	10.01	**8.0975**
	Leaf 2	32.00	31.99	0.003	17.67	13.25	
	Leaf 3	29.52	29.51	0.001	17.22	4.31	
	Leaf 4	31.74	31.74	0.002	17.47	8.74	
	Leaf 5	30.49	30.49	0.001	16.76	4.19	
5	**Leaf 1**	31.02	31.02	0.002	13.79	6.90	**5.1545**
	Leaf 2	32.00	31.99	0.001	16.67	4.17	
	Leaf 3	29.52	29.51	0.001	22.23	5.56	
	Leaf 4	31.74	31.74	0.002	13.45	6.73	
	Leaf 5	30.49	30.49	0.001	9.71	2.43	
6	**Leaf 1**	31.02	31.02	0.001	13.33	3.33	**2.724**
	Leaf 2	32.00	31.99	0.002	15.12	7.56	
	Leaf 3	29.51	29.51	0	12.69	0.00	
	Leaf 4	31.74	31.74	0.001	10.91	2.73	
	Leaf 5	30.49	30.49	0	13.215	0.00	
7	**Leaf 1**	31.02	31.02	0.002	12.23	6.12	**4.0905**
	Leaf 2	32.00	31.99	0.002	11.47	5.74	
	Leaf 3	29.52	29.51	0.001	12.45	3.11	
	Leaf 4	31.74	31.74	0.002	10.98	5.49	
	Leaf 5	30.49	30.49	0	9.51	0.00	
8	**Leaf 1**	31.02	31.02	0.003	24.28	18.21	**11.965**
	Leaf 2	32.00	31.99	0.002	24.32	12.16	
	Leaf 3	29.52	29.51	0.001	19.61	4.90	
	Leaf 4	31.74	31.74	0.003	24.3	18.23	
	Leaf 5	30.49	30.49	0.001	25.31	6.33	
9	**Leaf 1**	31.02	31.02	0.003	13.84	10.38	**8.501**
	Leaf 2	32.00	31.99	0.002	13.65	6.83	
	Leaf 3	29.52	29.51	0.002	18.51	9.26	
	Leaf 4	31.74	31.74	0.002	17	8.50	
	Leaf 5	30.49	30.49	0.002	15.09	7.55	
10	**Leaf 1**	31.24	31.24	0.001	10.14	2.54	**2.4135**
	Leaf 2	27.55	27.55	0	13.92	0.00	
	Leaf 3	26.95	26.94	0.002	9.78	4.89	
	Leaf 4	27.17	27.17	0.001	9.34	2.34	
	Leaf 5	25.91	25.91	0.001	9.23	2.31	

Figure 1. Sample trees of *S. album* surveyedfor the biophysicaltraits.

Figure2. Varied leaf shapes of *S.album* – Ovate, Obcordate, Elliptical, lanceolate, Sickle shape, Oblanceolate, Obovate (From left to right)

Figure 3. Various leaf colors and size of leaf samples of *S. album*

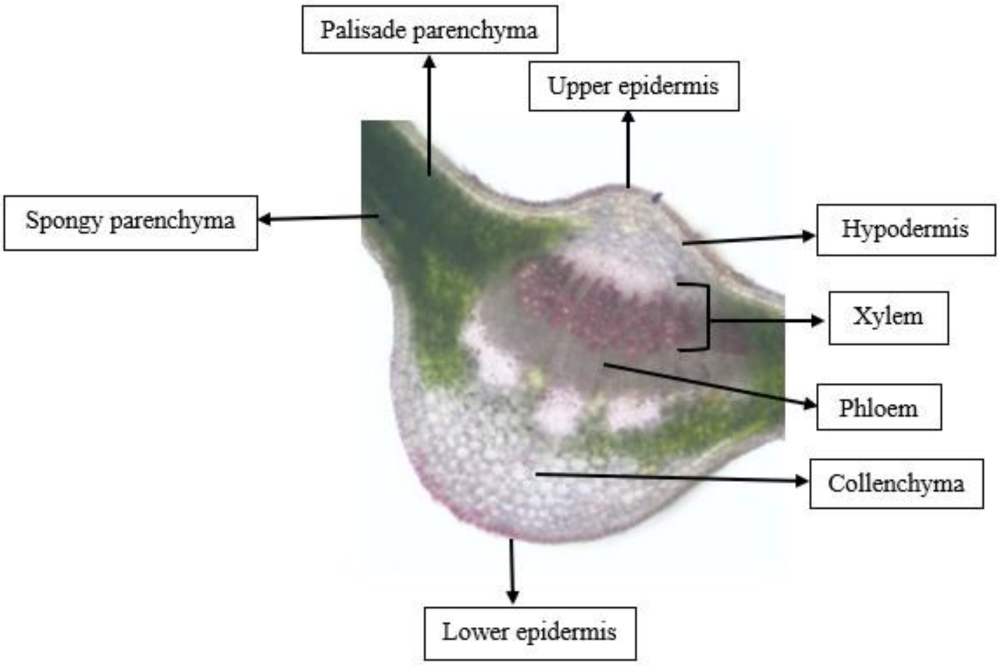

Figure 4. Description of transverse section of leaf of *S. album*

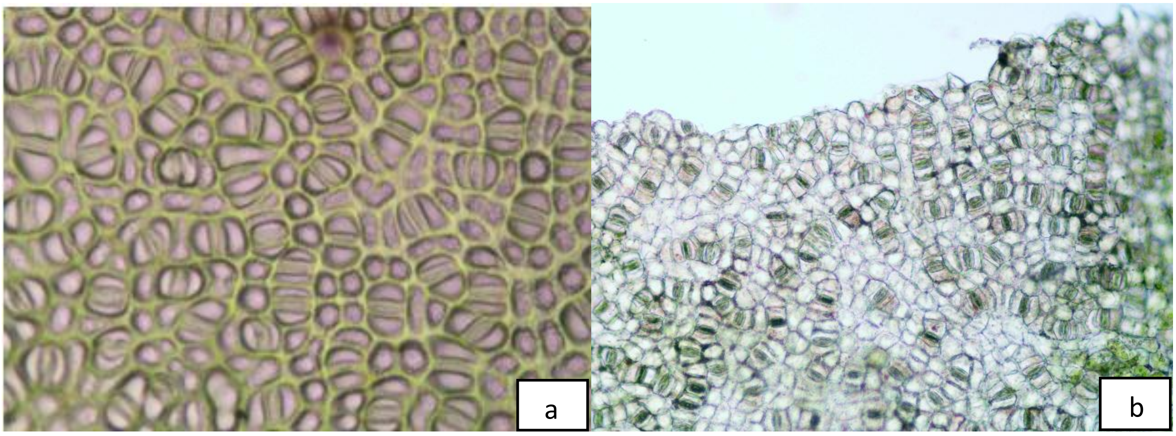

Figure 5. Image showing stomata fromabaxial surface of *S. album* leaf taken from (a) clear nail polish imprint and (b) epidermal peel

CONCLUSION

This study revealed the prevalence of variation in the biophysical traits of sandalwood, and hence further study will shed light on the resistance nature of these characters against various pests and pathogens. As epicuticular wax is known to impart resistance against leafhoppers, which are the potential vectors of sandalwood spike disease, identification of populations of sandalwood trees with higher epicuticular wax will play a prominent role in the management of spike disease.

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