

## PEROXIDASE ISOENZYME ACTIVITY IN LIVING BARK TISSUE AS A MARKER FOR THE OIL-BEARING CAPACITY IN SANDAL

K. PARTHASARATHI, V. G. ANGADI, K. H. SHANKARANARAYANA and  
A. N. RAJEEVALOCHAN

*Sandal Research Centre, Bangalore 560 003, India.*

### ABSTRACT

The activity of a specific peroxidase isoenzyme in the living bark tissue was found to bear a strong negative correlation with the oil content in the heartwood in mature sandal (*Santalum album* L) plants. The utility of this relation in forecasting, even at a young age, the oil-bearing capacity of a sandal plant is indicated.

### INTRODUCTION

**S**ANDAL (*Santalum album* L) is a polymorphic species showing a considerable variation in leaf pattern<sup>1</sup>. Kulkarni and Srimathi<sup>2</sup> reported the occurrence of ovate, lanceolate, linear and elliptic patterns in sandal leaves—the ovate leaves occurring in normal, small and big sizes and recorded the following ranges of variations (in cm) in length (*L*) and breadth (*B*) and model class values (given in brackets, in cm) in the leaves of the different patterns: *Ovate*: Normal: *L*: 4.0–9.0 (5.4), *B*: 1.8–3.7 (2.7), Small: *L*: 2.5–4.5 (3.2), *B*: 1.0–1.9 (1.5), Big: *L*: 5.5–11.5 (8.0), *B*: 2.2–5.3 (3.7), *Lanceolate*: *L*: 4.0–8.5 (6.7), *B*: 1.9–3.4 (2.5); *Linear*: *L*: 5.0–9.5 (8.2), *B*: 1.1–2.1 (1.4); *Elliptic*: *L*: 4.0–9.0 (5.4), *B*: 1.8–3.7 (2.7). Further, in the sandal plants with ovate normal leaves, we see plants with leaves having yellowish green colour and leaves having a wavy/non-wavy margin. However, sandal plants having ovate leaves (normal and big) most predominantly occur in nature while those with lanceolate leaves occur to a small extent and those with elliptic or linear leaves occur at a low frequency. Based on leaf peroxidase isoenzyme pattern, Parthasarathi *et al*<sup>3</sup> showed further the occurrence of 3 sub-types in sandal plants with ovate big leaves; thus 10 well-defined types showing variations in leaf pattern are recorded. However, sandal plants with leaves ovate in pattern but intermediate to the normal and the big, in size, are common.

Our studies on the peroxidase isoenzyme pattern in *Bursera penicillata* have shown that while the pattern, in the leaves, varies from vegetative to reproductive stage in the plant, it remains constant in the living bark tissue unaffected by the stage of development of the plant<sup>4,5</sup>. In the present study, the peroxidase isoenzyme pattern in the living bark tissue has been

examined in the ten well-defined sandal types mentioned above, and it was seen that (i) the pattern, in any given type, remained constant, throughout, unaffected by the stage of development of the plant, and (ii) a major isoenzyme band, that appeared to be species-specific, was common to the patterns seen in the different types. Quantitative study of the activity of the specific peroxidase isoenzyme (SPI) in the common band showed that it remained at a stationary level throughout in any given plant. Further, the SPI activity shows a strong negative correlation to the oil content. Details of this study are presented and the utility of SPI activity as a marker for forecasting the oil-bearing capacity in a sandal plant is discussed.

### EXPERIMENTAL

For examining the peroxidase isoenzyme pattern in the living bark tissues of the ten sandal types, samples of twigs (0.5 to 0.8 cm dia) were taken separately from 4 plants in each type from among the sandal plants in the Experimental Forest of Sandal Research Centre. The isoenzyme pattern was studied during April, August and December. From the twig samples in each case, the thin bark layer was peeled off and the outer dead bark portion scrapped off to get the living bark tissues. These tissues (2 g in each case) were cut into small bits and macerated with acid-washed sand and cold water (8 ml) containing 0.1% ascorbic acid. The resulting solution was centrifuged (8000 rpm, 10 min). The supernatant was separated and 0.05 ml of this (corresponding to 12.5 mg of tissues) was used for electrophoresis on polyacrylamide gel as detailed earlier<sup>4</sup>. The isoenzyme pattern obtained in any given type remained constant, throughout, unaffected by the stage of development of the plant. The isoenzyme



stirred well. After colour development for 2 min, the optical density (O.D.) of the same was read in a Systronix colorimeter using yellow green filter (No. 625), control solution remaining as blank. Thus, the O.D. measured the activity of SPI/5 mg of living bark tissue, under the conditions of experiment. Cutting of the SPI band from the gel for estimating SPI activity, facilitated an accurate determination by the reading of O.D. after full colour development, avoiding band diffusion. The SPI activity was determined during April, August and December, and the value did not exhibit seasonal variation. From each of these mature plants, a sample of the heartwood at breast height (1.3 m) was taken, powdered and the air-dry powder was used for determining sandal oil content by steam distillation. The RF values of the isoenzyme bands, SPI activity (in terms of O.D.) and sandal oil per cent, in respect of the 24 plants are presented in table 2.

## DISCUSSION

From figure 1, it is seen that all the ten different types of sandal do not show characteristic differences in the peroxidase isoenzyme pattern in the living bark tissue. Plants with ovate normal wavy, ovate normal non-wavy and ovate small leaves on the one hand and plants having ovate big leaves, sub-types II and III on the other, show a common pattern. It is possible that the types showing a common pattern are genetically related. It is further seen that the SPI band (RF 0.36), which appeared to be species-specific, remained common to all the sandal types. In the case of sandal types, ovate big sub-types II and III and lanceolate, the major isoenzyme band (RF 0.36) seems to have split into two bands (RF 0.36 and RF 0.40). The cause of occurrence of this split needs further study.

The RF values of the peroxidase isoenzyme bands

**Table 2** Levels of activity of the specific peroxidase isoenzyme (SPI), sandal oil content and RF values of peroxidase isoenzyme bands in mature sandal plants

Sl. No.	Plant type	RF values of peroxidase isoenzyme bands										Activity of SPI** (O.D.)	Sandal oil content***	
		1	2	3	4	5	6	7	8	9	10			
1	*		0.14	0.18			0.36			0.52			0.14	4.7
2	*		0.14	0.18			0.36			0.52			0.15	4.0
3	*		0.14	0.18			0.36			0.52			0.16	4.4
4	*		0.14	0.18			0.36			0.52			0.17	3.7
5	*		0.14	0.18			0.36			0.52			0.17	3.4
6	*		0.14	0.18			0.36			0.52			0.20	3.6
7	*		0.14	0.18			0.36			0.52			0.21	3.1
8	*		0.14	0.18			0.36			0.52			0.21	3.1
9	*		0.14	0.18			0.36	0.40			0.56		0.21	2.4
10	Lanceolate		0.14	0.18		0.26	0.36	0.40		0.52	0.56		0.24	2.4
11	OB-1		0.14		0.20		0.36			0.52	0.56		0.20	2.9
12	OB-1		0.14		0.20		0.36			0.52	0.56		0.21	3.4
13	ON-W	0.12	0.14	0.18			0.36			0.52	0.56		0.21	2.7
14	ON-W	0.12	0.14	0.18			0.36			0.52	0.56		0.23	2.7
15	OS	0.12	0.14	0.18			0.36			0.52	0.56		0.21	2.3
16	ON-NW	0.12	0.14	0.18			0.36			0.52	0.56		0.24	2.3
17	Thindlu	0.12					0.36		0.48				0.15	4.0
18	*	0.12					0.36			0.52			0.27	1.8
19	*	0.12					0.36			0.52			0.27	1.6
20	*	0.12					0.36			0.52			0.28	1.8
21	*	0.12					0.36			0.52			0.30	1.4
22	*	0.12					0.36			0.52			0.33	1.1
23	*	0.12					0.36			0.52			0.34	0.7
24	OB-3			0.18			0.36	0.40		0.52			0.16	4.1

Note – (i) OB-1 : ovate big, sub-type 1; OB-3 : ovate big, sub-type 3; ON-W : ovate normal wavy; ON-NW : ovate normal non-wavy; OS : ovate small, (ii) Thindlu type is characterized by ovate big leaves and lean stem with a thin layer of sapwood and maximum per cent of heartwood, (iii) Mature sandal plants with linear/elliptic type leaves were not available.

\* – Plants which do not conform to the types already characterized.

\*\* – Activity of SPI in terms of O.D./5 mg living bark tissue under the conditions of the experiment.

\*\*\* – Percentage on the basis of air-dry heartwood powder.

obtained in respect of the 24 mature plants (table 2) show that, while 8 of them correspond to one or the other of the already well-characterized types, 16 of them do not correspond to any one of these types. The leaves of these 16 plants were ovate, lying between the ovate normal and ovate big leaves in size. Even here, the plants under Serial Nos 1 to 8 form one group and those under Serial Nos 18 to 23 form another group while the plant at Serial No 9 shows a pattern different from either of these two groups. Further, a negative correlation is seen between the SPI activity and sandal oil per cent in the mature sandal plants. Statistical analysis of the data showed strong negative correlation and the two variables can be correlated by the regression equation:

$$y = 6.89 - 18.50 x,$$

where  $y$  represents the sandal oil per cent and  $x$  represents SPI activity in terms of O.D.

Further, it is noted that the variation in the sandal oil per cent, even in the plants falling under the same type (based on their peroxidase isoenzyme pattern), is quantitatively correlated with SPI activity. Thus, the SPI activity seems to form a reliable basis for indicating the oil per cent in any individual mature plant.

In order to correlate SPI activity in a young plant, otherwise identical in characters with a mature plant, 2 year old sandal plants were raised through root suckers<sup>7</sup> from six mature sandal plants conforming to one or the other of the types mentioned in table 2. Interestingly it was found that the SPI activity in the living bark tissue of the young plant did not differ from that of the mature mother plant (table 3).

It is thus seen that the SPI activity in a young sandal plant, irrespective of its age (subject to the fact that it should at least be of 2 to 3 years age to give twigs of the type needed for the study) can be used in forecasting the sandal oil per cent at maturity. This will facilitate in rapid screening of sandal plants, raised by hybridization, for their oil-bearing potential. In general, the oil-bearing capacity of sandal plants having SPI activity (O.D.) less than 0.2 are superior and those having the activity higher than 0.28 are inferior.

**Table 3** SPI activity in living bark tissues of the mother plants and the root-suckers

Serial Nos in table 2 to which the mother plant belonged	SPI activity in living bark tissue (O.D./5 mg tissue)	
	Mother plant	Root sucker
1 to 8	0.15	0.15
1 to 8	0.17	0.17
14	0.21	0.22
15	0.23	0.23
18 to 23	0.27	0.26
18 to 23	0.27	0.27

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