

A note on Peroxidase reagents to distinguish between high and low yielders of Sandal (*Santalum album*) in the field

Angadi V.G., S.H. Jain, A.N. Rajeevalochan, G. Ravikumar and K.H. Shankaranarayana

Institute of Wood and Technology, Malleswaram, Bangalore, 560003, India

A simple, less expensive peroxidase colour reaction has been developed to distinguish high and low yielders of Sandal in the field.

Peroxidase (donor: H_2O_2 -Oxidoreductase, EC 1.11.1.7) enzyme which catalyses dehydrogenation of a large number of organic compounds such as Phenols, aromatic amines and hydroquinones has often been used as a marker enzyme by Plant Scientists (Van Huystee and Cairns 1980). In Sandal (*Santalum album* L), the enzyme peroxidase present in living bark tissue was used as a marker for oil bearing capacity (Parthasarathi *et al.* 1986) of the progeny.

Sandal (*Santalum album* L) is a small evergreen tree popularly known for its scented heartwood and oil content. In breeding studies or in selection of Candidate Plus Trees (CPTs) for Clonal Seed Orchards (CSOs), oil is one of the important criteria. Earlier methods of estimating oil content in sandalwood by steam distillation in Clevenger's apparatus and by rapid method (Shankaranarayana *et al.* 1997) were time consuming and non-field oriented. In view of this, an attempt was made to develop a simple field method to distinguish high and low yielders of sandal by modifying and simplifying the earlier methods (Parthasarathi *et al.* 1986).

For this purpose, twelve sandal plants (six high yielders - containing > 3% oil; and six low yielders - containing < 3% oil) were selected from among the experimental forest Institute of Wood Science and Technology, Bangalore. Twig samples (0.5 to 0.8 cm diameter) were taken from each plant and thin bark layers were peeled off and outer dead bark portions scraped off to get the living bark tissue. In each case, the living bark of 5 x 0.2 cm (1 x b) were selected: cut into small bits and placed into 50 ml conical flasks containing peroxidase reagents to study the colour reaction.

The following two peroxidase reagents were used to develop colour reaction:

a. Guaiacol peroxidase Reagent (GPR)

2ml of 1% Guaiacol in 50% ethanol + 2ml of 1% Hydrogen Peroxide + 6ml of distilled water – shaken well occasionally and allowed to develop for 15 to 20 minutes. Light brownish yellow developed for high yielders and reddish brown developed for low yielders.

b. Benzidine Peroxidase Reagent (BPR)

2ml of 1% Benzidine in 25% acetic acid + 2ml of 1% Hydrogen Peroxide + 6ml of distilled water – shaken well occasionally and allowed to develop for 30 to 45 minutes. Light brownish pink developed for high yielders and dark brownish pink developed for low yielders.

“ This method is simple, user friendly, less expensive and field oriented which could be useful for rapid screening of sandal plants in the field for selection of high yielders ”

The above observations were further confirmed by diluting 1 ml of each coloured solution with 5 ml of distilled water. In case of GPR, an almost colourless solution developed for high yielders, while the colour persisted for low yielders. For BPR, a similar pattern occurred for high and low yielders.

This method is simple, user friendly, less expensive and field oriented which could be useful for rapid screening of sandal plants in the field for selection of high yielders in breeding studies or in general use.

Acknowledgements

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

References

- Van Huystee R.B, and Cairns, W.L. (1980). Appraisal of studies on induction of peroxidase and associated porphyrin metabolism. *The Botanical Review*: **46**: 429-46.
- Parthasarathi K., Angadi, V.G., Shankaranarayana, K.H. and Rajeevalochan, A.N. (1986). Peroxidase isoenzyme activity in the living bark tissue as a marker for the oil-bearing capacity in Sandal. *Current Science* **55**: 831-34.
- Shankaranarayana K.H., Angadi, V.G., Rajeevalochan, A.N., Theagarajan, K.S., Sarma, C.R. and Rangaswamy C.R. (1997). A rapid method of estimating essential oil content in heartwood of *Santalum album* Linn. *Current Science* **72**: 241-24.