STUDIES ON THE PEROXIDASE ISOENZYME PATTERN IN RELATION TO THE CINEOLE CONTENT OF *EUCALYPTUS* HYBRID LEAF

V. V. PRABHU and K. S. THEAGARAJAN
Forest Research Laboratory, Bangalore 560 003, India.

ABSTRACT

Cineole-rich oil yielding trees exist in *Eucalyptus* hybrid, which cannot be distinguished from others morphologically. Refractive index of the oil and stomatal characters of the leaf were found to help in distinguishing these trees from others. Slab-gel electrophoresis can be used as another useful technique to distinguish them from others in view of the characteristic differences observed in the peroxidase isoenzyme pattern of the leaves. The 'cineole-rich' ones (above 60% cineole content) give only two bands while others give nine bands, including these two.

INTRODUCTION

PLANTATIONS of *Eucalyptus* hybrid have been raised on a large scale in India to meet the growing demand for fuel and pulpwood in view of its tremendous adaptability to various eco-climatic conditions.

Earlier investigations showed that leaves of some of the *E.* hybrid trees give medicinal oil similar to that of *E. globulus*[1]. As they are morphologically indistinguishable from others a simple and rapid method to know the cineole content of the oil was developed by finding a correlation between refractive index and cineole content of the oil, which could be helpful in the rapid screening of plantations to identify 'cineole-rich' trees[2]. Stomatal characters of the leaves studied by ordinary and scanning electron microscopes also indicated distinct differences in the case of 'cineole-rich' and 'cineole-poor' ones[3].

Considerable information is available on variation in the isoenzyme pattern in plants in relation to their development and among 'strains' within the species[4]. The difference in the isoenzyme pattern can thus be used as a laboratory tool in the biochemical characterization of plants. Hence, it was considered worthwhile to examine the differences, if any, in the peroxidase isoenzyme pattern of *E.* hybrid leaf and find out the utility of this technique in distinguishing the 'cineole-rich' strain or variety from others.

In this study only a few leaves are required for the work unlike the refractive-index method developed earlier, which requires at least a handful of leaves to distil a few drops of oil[2]. About 30 trees of almost identical age were selected at random, from among those growing in the campus of Forest Research Laboratory and their leaves of almost identical maturity were taken for this study.

MATERIALS AND METHODS

For the experiment two grams of freshly plucked leaves from each tree were taken and washed. The leaf sample was ground to a paste with 5 ml of ascorbic acid (0.1%) and sucrose (0.4 g) solution using a pre-cooled mortar and pestle. The paste was strained through a clean muslin cloth. The solution thus obtained was centrifuged (15000 r.p.m.) for 10 minutes. The supernatent liquid was used for the electrophoresis (4 hr) on a polyacrylamide slab-gel as described in the manual[5]. The isoenzyme solution (5 μ l) was loaded in the 'wells' of already set gel, followed by bromophenol blue (5 μ l, 0.04%) and sucrose (5 μ l, 20%). The remaining part of the 'well' was filled with the tank buffer.

Initially the electrical input was set at 100 volts and 4 mA and run till the marker dye crossed the tracer gel, followed by an increase to 180 volts and 6 mA. The gel was run till the marker dye almost touched the surface of the buffer solution in the lower tank, which took approximately 4 hr. The gel was then carefully removed from the plate and the peroxidase isoenzyme pattern was studied by treating the gel first with H_2O_2(0.03%) solution in water for 2 min followed by 1% benzidine solution in 25% acetic acid in water, to develop the coloured bands. In view of the fugitive nature of the coloured bands, they were photographed soon after development and the distances where the bands appear in the gel were measured from the 'well'. The peroxidase

* For Correspondence.

isoenzyme pattern obtained in respect of 'cineole-rich' and 'cineole-poor' oil yielding varieties of *E.* hybrid and the R_f value of the bands obtained are shown in the figure.

Simultaneously, a handful of leaves of each sample was steam-distilled and the refractive index of the oil obtained thereof, was found out using an Abbe refractometer. Cineole content of the oil was calculated using the regression equation in each case[2] (table 1).

RESULTS AND DISCUSSION

From table 1, it is clear that there were only two characteristic isoenzyme patterns, one giving only *two* bands and the other giving *nine* bands. However, the first *two* bands were common only in both the patterns while the other seven bands were missing in leaf samples giving 'cineole-rich' oil (figure 1).

Table 1 *Cineole content vs No. of bands*

Refractive index	Cineole content (%)	No. of bands
1.462	67.42	2*
1.471	51.59	9
1.497	5.81	9
1.471	51.59	9
1.464	63.90	2*
1.475	44.53	9
1.489	19.89	9
1.462	67.47	2*
1.495	9.30	9
1.470	53.34	9
1.485	26.93	9
1.484	28.69	9
1.494	11.09	9
1.478	39.25	9
1.462	67.42	2*
1.476	42.77	9
1.472	49.82	9
1.477	41.01	9
1.476	42.77	9
1.491	16.37	9
1.493	12.85	9
1.477	41.01	9
1.471	51.59	9
1.470	53.34	9
1.468	56.86	9
1.461	69.18	2*
1.460	70.94	2*
1.472	49.82	9
1.472	49.82	9
1.459	72.70	2*

* Cineole-rich

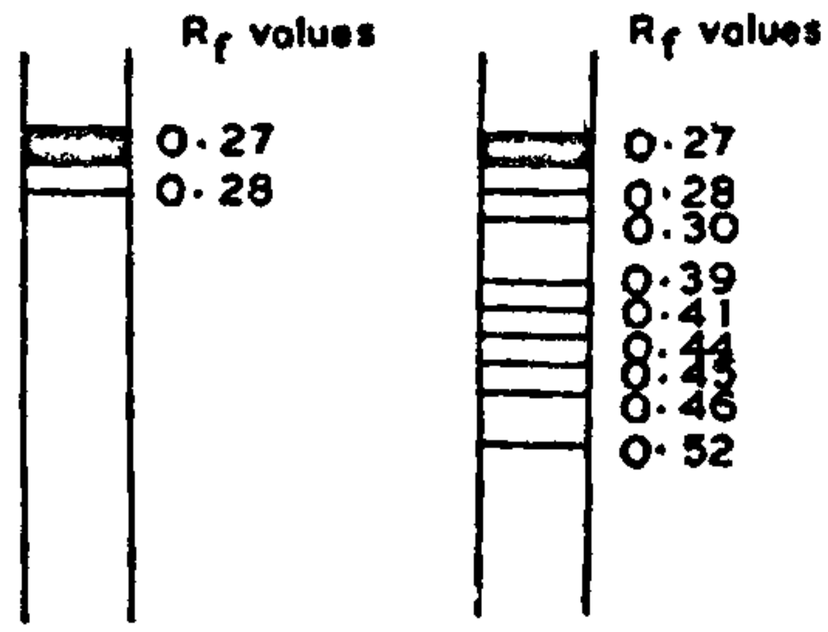

Figure 1. Peroxidase isoenzyme pattern with R_f values (diagrammatic). **A.** rich in cineole (> 60%); **B.** not rich in cineole.

It was also observed that the peroxidase isoenzyme patterns in the leaves remained the same both during vegetative and flowering periods during one year. The characteristic differences occurring in peroxidase isoenzyme pattern of the leaves could thus be useful in classifying the trees as 'cineole-rich' and 'cineole-poor' strains or varieties.

ACKNOWLEDGEMENT

The authors are highly grateful to Sri S. N. Sharma, for his constant encouragement and keen interest. They are also thankful to Sri G. Ravi Kumar for technical assistance.

17 September 1986; Revised 31 December 1986

1. Theagarajan, K. S. and Rao, P. S., *Indian For.*, 1970, **96**, 347.
2. Theagarajan, K. S., Prabhu, V. V., Subramani, M. and Ananthanarayana, A. K., *Curr. Sci.*, 1977, **46**, 733.
3. Bisen, S. P., Prabhu, V. V. and Theagarajan, K. S., *Indian For.*, 1984, **110**, 577.
4. Feret, P. P. and Bergmann, F. *Modern methods in forest genetics*, (ed.) J. P. Miksche, Springer-Verlag, Berlin, 1976, p. 49.
5. Polyacrylamide gel electrophoresis, Laboratory technique by Pharmacia Fine Chemicals, Uppsala, Sweden.