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A method for staining sieve tubes in the bark of *Hevea brasiliensis*

Gopika Gopal and Vinoth Thomas*
Rubber Research Institute of India, Kottayam- 686 009, Kerala, India
**Correspondence author E-mail: vt@rubberboard.org.in*

INTRODUCTION

Hevea brasiliensis, the prime source of natural rubber, is exploited commercially by severing the latex vessels present in the bark of tree trunk. A number of anatomical and histochemical studies on *Hevea* bark were carried out for analysing bark characteristic in the evaluation process of newly developed clones (Dijkman, 1951). Sieve tubes in the soft bark are functionally active tissues concerned with the downward translocation of photo-assimilate that nourishes laticifers in the bark for the biosynthesis of latex. A simple and specific stain is used first time for sieve tubes in cross sections of the bark of *H. brasiliensis*.

MATERIALS AND METHODS

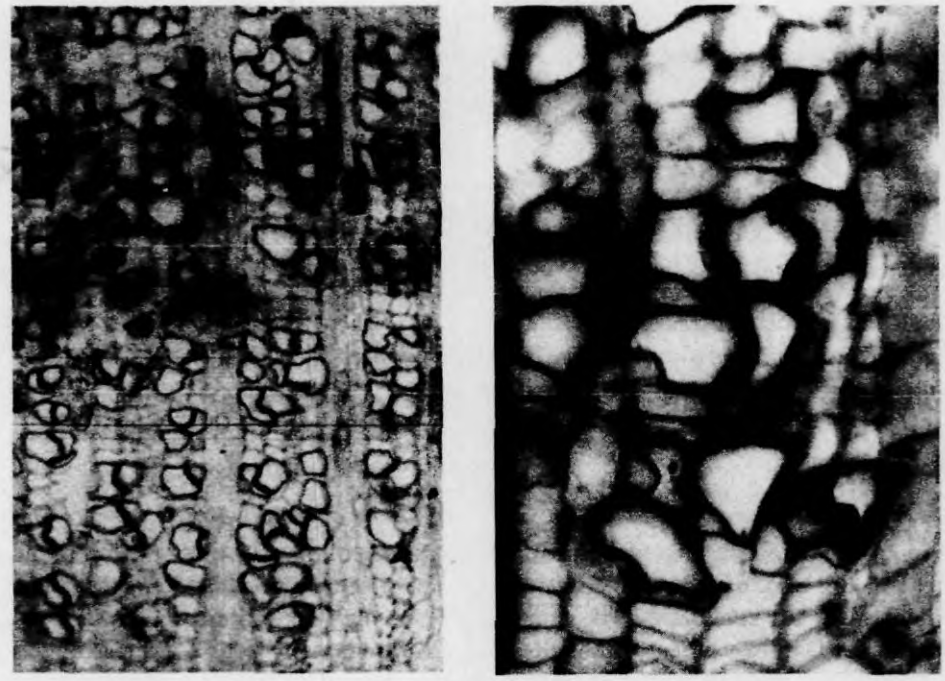
Bark samples from the trunk of ten mature trees of *Hevea brasiliensis* (clone RRII 105, 20 year old) were collected monthly for a period of one year from the experimental farm of Rubber Research Institute of India, Kottayam, India using a chisel and hammer.

Fresh bark samples were sectioned in transverse plane (20-30µm thickness) on a sledge microtome (Leica SM2000R) and fixed in 4% glutaraldehyde prepared in 0.1M phosphate buffer at pH 7.2 and stored under 4°C refrigeration. Sections were then treated with a mixture containing O-dianisidine and 1% Hydrogen peroxide in 0.1M phosphate buffer (pH 7), for about 0.5-1 minute. After treatment, sections were rinsed 2-3 times with buffer and mounted on a glass slide. Underlying sections from the bark were stained with Oil Red-O for laticifers. Observations and photographs were taken by using Leica QWin V3 Image Analyser attached to Leica DM 1000 Microscope.

RESULTS AND CONCLUSIONS

The bark of *H. brasiliensis* can be demarcated into inner soft and outer hard bark regions based on the structural organisation. In the bark, metabolically active tissues including sieve tubes, laticifers, *etc.*, are mostly confined to soft bark.

Transverse sections of the soft bark when treated with O-dianisidine, gave a deep brown coloration for the cell walls of sieve tubes (Figs. 1-3) in the inner soft bark, including the recently differentiated ones from the derivatives of cambium (Fig.1). The cell wall of companion cells, axial and radial parenchyma, and laticifers remained unstained so as to make the stained tissues prominent. The latex in the laticifers alone stained black (Fig.4) with O-dianisidine. As the sieve tubes and laticifers in the bark have thick cell walls and are more or less similar in size and shape in cross sectional view, the result was further confirmed through histochemical staining techniques.



Figs.1 & 2. Transverse section of the bark stained with o-dianisidine for sieve tubes.
1. Sieve tubes in the soft bark stained brown keeping the surrounding tissues unstained X85.
2. Recently differentiated sieve tubes (ST) occur adjacent to cambial zone (CZ) X 320.

O-dianisidine, also known as 3, 3'-dimethoxybenzidine ($C_{14}H_{16}N_2O_2$), is a colorimetric substrate used in ELISA procedures (Worthington Enzyme Manual, 1993), and for localizing or estimating peroxidase activity in plant tissue. In the presence of hydrogen peroxide, O-dianisidine gets oxidised to give a brown to purple colouration to the plant tissue. With the concentration specified for the localization of peroxidase activity in plant tissue, the sieve tubes in *Hevea* remain unstained. However, in the present study, sieve tubes and phloic rays in the soft bark gained a brown colour which may possibly be due to excessive oxidation that had taken place as a result of increased concentration of hydrogen peroxide.

A number of histochemical stains have been used to identify sieve tubes in the tangential longitudinal view. The tangential longitudinal sections stained with Aniline blue or Tannic acid-Ferric chloride-Lacmoid, and viewed under ultra-violet illumination gave fluorescence of callose deposited on the sieve plate. Ponceau S also stains sieve plate and not cell walls (Parker, 1965). None of these stains are found to be specific for sieve tubes, particularly in transverse plane as the plane of cutting always need not expose sieve plates.

The present procedure enables an easy identification of sieve tubes in the bark, which comprises an anatomical parameter for the evaluation of *Hevea* clones.

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