

## STAINING PROCEDURE FOR SIEVE TUBES IN THE BARK OF *HEVEA BRASILIENSIS* USING O-DIANISIDINE

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A specific stain for the identification of sieve tubes in the bark of *Hevea* particularly in transverse plane is lacking, hence a new staining procedure has been developed. Fresh sections of bark preserved in 4 per cent glutaraldehyde were treated with a mixture containing O-dianisidine 1mg mL<sup>-1</sup> and 1 per cent hydrogen peroxide in 0.1 M phosphate buffer (pH 7), for about 0.5-1 minute. Observations and photomicrographs were taken by using Leica QWin V3 image analysis system attached to Leica DM 1000 microscope. Transverse sections of the soft bark when stained with O-dianisidine gave deep brown coloration for the cell walls of sieve tubes present in the inner soft bark, as well as the recently differentiated ones from the cambium. The cell wall of companion cells, axial and radial parenchyma, and laticifers remained unstained. The study revealed that the staining method using O-dianisidine is suitable for identifying sieve tubes in cross sectional plane of *Hevea* bark.

**Keywords:** Glutaraldehyde, O-dianisidine, Sieve tubes, Staining procedure

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### INTRODUCTION

*Hevea brasiliensis*, the prime source of natural rubber, is exploited commercially by severing the latex vessels present in the bark of the tree trunk (Dijkman, 1951). A number of anatomical and histochemical studies on *Hevea* bark have been carried out earlier using various stains viz., Sudan IV (Premakumari *et al.*, 1996) and Oil red O (Omman and Reghu, 2003) for laticifers, Tannic acid-ferric chloride - lacmoid (Cheadle *et al.*, 1953; Pramod *et al.*, 2011) and Aniline blue (Johansen, 1940) for definitive callose, mercuric bromophenol (Mazia *et al.*, 1953; Pramod *et al.*, 2008) for P-protein, phloroglucinol-HCl (Jensen, 1962; Thomas

*et al.*, 1995) for lignin, silver nitrite (Johansen, 1940; Thomas *et al.*, 2002) for crystals and amido black 10B (Weine, 1957; Thomas *et al.*, 2010) for protein storing cells and Iodine-Potassium iodide (Johansen, 1940; Hebant and Fay, 1980; Thomas *et al.*, 2002) for starch. Some of the above methods are commonly used for bark characteristic studies in the evaluation process of the newly developed clones of *H. brasiliensis* (Premakumari *et al.*, 1996; Pramod *et al.*, 2008; Thomas *et al.*, 2010).

Sieve tubes of phloem tissues in the soft bark are functionally active for the downward translocation of photo-assimilates for the biosynthesis of latex in the laticiferous system. The survey of