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STAINING PROCEDURE FOR LATICIFEROUS SYSTEM OF *HEVEA BRASILIENSIS* USING OIL RED O

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A staining procedure using 1 per cent Oil red O prepared in 90 per cent isopropyl alcohol was tested for its ability to stain laticifers in *H. brasiliensis*. Laticifers were stained a deep-red maroon with Oil red O, whereas a red colour was developed with Sudan III and Sudan IV. Oil red O took the least time for stain preparation and gave maximum stainability when compared to Sudan III/IV preparations. It was found to be a suitable stain for laticifers of *Hevea brasiliensis*.

Key words: *Hevea brasiliensis*, Laticifers, Oil red O, Staining procedure

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INTRODUCTION

Sudan III and IV are non-polar lipid stains (Pearse, 1968; Wigglesworth, 1988) widely used for the histochemical staining of laticiferous tissues in *Hevea brasiliensis* (Gomez *et al.*, 1972; Panikkar, 1974; Abraham *et al.*, 1992; Premakumari *et al.*, 1992; Reghu *et al.*, 1996). Staining methods reported earlier for laticiferous tissue of *H. brasiliensis* are given in Table 1. Hamzah *et al.* (1988) compared conventional staining of bark samples of *H. brasiliensis* using Sudan III, Sudan blue and Oil red O and discussed the colouration of stained laticifers. But the staining protocol was time consuming. The solvents usually used for Sudan stains are ethyl alcohol, acetic acid and glycerol combinations, which take a minimum period of 48 h for preparation. Moreover, overnight incubation of tissue sections in the staining medium is required for adequate staining of laticiferous system. The formation of precipitate during the preparation of Sudan stain is another drawback, which in turn adversely affects the clarity of stained sections.

Oil red O is a hydrophobic, non-polar,

Table 1. **Staining methods for latex vessels of *H. brasiliensis***

Stain	Solvent used	Embedding medium	Duration of staining	Colour developed	Reference
Aquous safaranin and malachite green	Water	Araldite-polybed	2h to overnight	Latex vessel wall carpo blud; latex cyclamen purple	Hamzah *et al.* (1988)
Sudan III & IV	Methyl alcohol (75%)	Nil Latex vessel rings dissected from KOH (10%) treated bark samples	10-15 min	Red	Zhao Xin-Qian (1987)
Oil red 4B	Ethyl alcohol (95%)	Paraffin wax	90 min	Red	Wimalaratna (1973)

solvent dye commonly used for staining lipids in plant tissues (Lillie, 1965; Pearse, 1968; Jayabalan and Shah, 1986) and for hydrophobic compounds like unsaturated triglycerides, sterol esters and fatty acids in animal tissues (Lillie and Ashburn, 1943). The use of Oil red O for staining rubber globules in guayule (*Parthenium argentatum*) has been reported earlier (Addicott, 1944; Mehta and Hanson, 1980; Jayabalan and Shah, 1986). Oil red O has been widely used in plant systems for bright field and fluorescence microscopy. Hence an attempt was made to develop an easy quick alternate staining procedure for laticiferous tissue of *H. brasiliensis* using Oil red O.

MATERIALS AND METHODS

One gram of Oil red O (C.I.No.26125. Hi Media) powder was dissolved in 100 ml of isopropyl alcohol (90%) in a tightly capped bottle, mixed well, decanted and stored at 4°C.

Bark samples fixed in FAA were washed thoroughly in distilled water. Bark sections in cross sectional (CS), tangential longitudinal (TLS) and radial longitudinal (RLS) planes were cut at 40-50 mm thickness using a sledge microtome. Sections were incubated in isopropyl alcohol (25%) for 30-45 seconds, stained with Oil red O for 15 to 20 minutes and washed 3 to 4 times in distilled water. After the last wash the sections were kept in distilled water for about 15 minutes and mounted in glycerine (50%) for observation.

Sections were also stained in Sudan III and IV (1%) for the same time duration (15-20 min), as well as overnight, for comparison. The stained sections were observed under Leitz Aristoplan research microscope in bright field using neutral density filter and analysed using image analysis system (Leica Q500 IW). Photomicrographs were prepared (with MP 46 Photoautomat) on 35 mm colour film (Konica).

RESULTS AND DISCUSSION

Latex vessels were stained with maximum intensity, contrast and specificity in Oil red O. Compared with Sudan stains, the latex vessels showed intense colouration and clarity in Oil red O (Table 2).

Table 2. **Staining of laticifers in *H. brasiliensis***

Stain	Duration of staining	Colouration of latex vessels
Oil red O*	15-20 min	Deep red – maroon
Sudan III** Nice Chemicals & Sudan III***	15-20 min	Faint red colour
Sudan III/IV	Overnight staining	Red colour

* C.I.No.26125. Hi Media; ** B.No.508168;
*** B.No.403141 Nice Chemicals

In the present study, isopropanol (90%) used as the solvent for Oil red O performed well. This is in accordance with the observation of McKinney and Riley (1967), Catalano and Lillie (1975), Jayabalan and Shah (1986) and Stotz *et al.* (1986) for lipid staining.

Pearse (1968) reported reddish colouration for guayule rubber (*Parthenium argentatum*) within 20 to 30 min using Oil red O. Jayabalan and Shah (1986) reported deep-red staining of the rubber particles of guayule in 3 to 5 min using formic acid as the solvent system. The different time durations tried for laticifer staining revealed that the maximum staining intensity was attained within 15 to 20 min for bright field microscopy. For detailed anatomical investigations with respect to latex vessels inclination, diameter, density and frequency of interconnections, Oil red O staining was found to be highly suitable. Staining of latex vessels in Oil red O for less than 15 min is sufficient for simple and routine anatomical observations.

Jayabalan and Shah (1986) used a combination Oil red O and dansychloride for epifluorescence microscopy to quantify the bright red coloured rubber particles in sec-

tions of guayule rubber and suggested that this microflurometric method could be used for the identification of high / low yielding varieties. They further concluded that the staining mechanism for rubber globules with a dye like Oil red O is due to the dissolution of dye molecules in the hydrophobic matrix of the rubber.

When the efficacy of Oil red O (Figs. 1&2) was compared with that of Sudan III and IV, for the same time duration (15-20 min), the latex vessels showed a very faint red colouration and lack of uniform staining with the Sudan stains (Fig. 3). Though overnight staining of latex vessels with Sudan III and IV gave red colouration (Fig. 4) these stains were not as efficient as Oil red O in terms of intensity and clarity. Stotz *et al.* (1986) evaluated the performance and staining quality of Oil red O and Sudan dyes through thin layer chromatography using different samples collected from the market. They separated three principal components, F_1, F_2 and F_3 and concluded that F_1 was the effective component in Oil red O while all the Sudan dyes (I to IV) contained only F_2 fraction. The better staining performance with Oil red O and poor stainability with Sudan dyes observed in the present investigation may be related to the dominance of F_1 fraction in the former. The starting material for preparation of Oil red O is a commercial mixture of aminotoluenes and aminoxylenes (Stotz *et al.*, 1986). After diazotization, coupling of two dimethyl units yields Oil red O. McKinney and Riley (1967) attempted Orcien-Oil red O stain and Sudan IV to demonstrate lipids in animal tissues and did not obtain a satisfactory result with the latter. Other Sudan dyes *viz.* I, II and III also could not discriminate lipids as effectively as Oil red O stain. Considering the advantages of Oil red O over Sudan III and IV with respect to stain preparation and staining efficiency observed in the present study, the former is more suitable for staining the laticiferous tissue in *Hevea brasiliensis*.

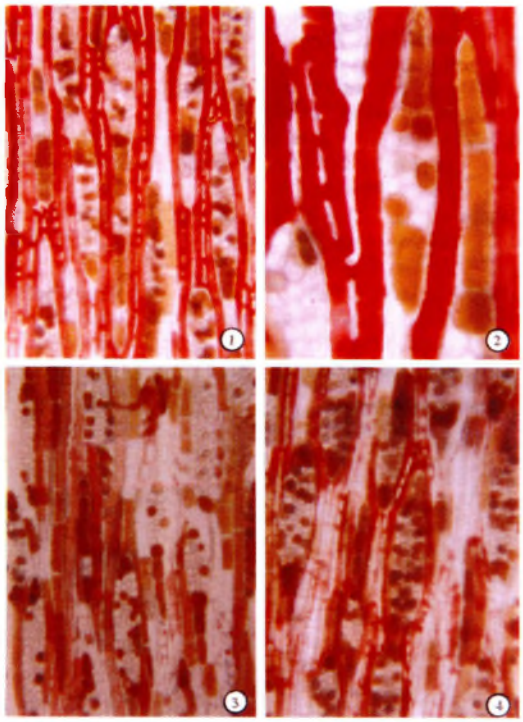

Fig. 1-4 Staining of transverse longitudinal sections of bark (*Hevea brasiliensis*): 1. Staining in Oil red O, X120; 2. Staining in Oil red O, X300; 3. Staining in Sudan dyes (10-15 min staining) X120; 4. Staining in Sudan dyes (Overnight staining) X120

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