A Method for Localizing Embryonal Laticifers by Combined Conventional and Fluorescence Microscopy¹

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Summary

The authors describe a simple method based on malachite green and acid fuchsin for the detection of laticifers during the embryogenesis of some Euphorbiaceae plants by conventional and fluorescence microscopy. The strong sensitivity and specificity of the method make it suitable for the ontogenetic studies of laticifers. The results obtained are discussed in the context of the reactive mechanism of the staining and of the chemical composition of the embryonal laticifers.

Keywords: Double staining; Euphorbiaceae; Fluorescent staining; Laticifers.

1. Introduction

Recently, great attention has been devoted to the study of the latex composition of Euphorbiaceae plants; first, for medical purposes due to the vesicant, irritant, and co-carcinogenic properties in animal and human skin and mucous membranes (Watt and Breyer-Brandwijk 1962, Morton 1971, Kinghorn and Evans 1975, Keeler et al. 1978), and second, for purposes pertaining to market technology such as fish poison, insecticide and future resources of gasoline (Morton 1971, Buchanan et al. 1976). Despite this, the studies on the genesis and morphological organization of the latex system represent a much neglected field of research (Mahlberg and Sabharwal 1968, Bruni et al. 1978). The genesis of non-articulated laticifers, the formation of the latex system, and the correlation with the other tissues during growth and differentiation show very intricate problems, partially due to the difficulty of localizing the laticifers in the context of the embryonal tissues.

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We have devised a simple method, based on malachite green-acid fuchsin dyes, which has proved most efficient, both in conventional and fluorescence microscopy.

2. Materials and Methods

The plant material used was mature embryos of *Euphorbia marginata*, *E. lucida*, *E. amygdaloide*, and *E. characias*. The embryos, obtained by dissecting dry seeds, were fixed in a paraformaldehyde solution (10% w/v) in 0.2 M phosphate buffer at pH 7.4 for 1 hour, or in FAA (ethanol 75-glacial acetic acid-formalin, 9 : 5 : 5) for 24 hours. In both cases, the material was thoroughly washed after fixation. The embryos were dehydrated in graded ethanol and embedded in a mixture consisting of butyl-methyl methacrylates 7 : 3 (Merck, Darmstadt). After polymerization in a 50 °C oven, the specimens were cut at 2-3 μm by an LKB Pyramitome with glass knives. Transversal and longitudinal sections were individually expanded in a drop of water on a clean slide. The plastic was removed by benzene, and the sections were immersed for 5 minutes each in absolute, 95%, 70%, and 50% alcohol.

The specimens were stained by the following procedure, modified from PIANESI (1896): a) staining with a solution composed of malachite green 1% aqueous-acid fuchsin 1% aqueous-distilled water 5 : 1 : 9 for at least 2 hours; b) rinsing of specimens by pouring a few drops of 95% alcohol over each slide, to wash away the excess dye; c) immersion for two minutes in 95% alcohol and then treatment with absolute alcohol and xilene to clean; d) mounting in a rapid synthetic nonfluorescent medium (UV-inert, Serva, Heidelberg). As a control, specimens were observed unstained, or after only malachite green or acid fuchsin staining. All the preparations were examined and photographed with a Zeiss Photomicroscope II, equipped with an incident fluorescence condenser and a 75 Watt Xenon arc source. The band pass filter BP 546/10 was employed as a primary filter to give “green excitation”, while a chromatic beam splitter FT 580 and longwave pass filter LP 590 proved to be optimal for these studies.

3. Results

Results indicate that the proposed method offers several advantages, both for the facility in execution and for the inexpensive cost of the materials employed, when compared to the fluorescamine method (BRUNI et al. 1977). By conventional microscopy, the embryonal laticifers are easily localizable by malachite green-acid fuchsin double stain. The cytoplasm matrix is coloured in a pale purple-red, while the small protein bodies, typical of the embryonal laticifers, and nuclei are dark green in colour (Fig. 1 a). The chromaticity of the dyes is good; malachite green, however, permits an easier detection of the laticifers since acid fuchsin only furnishes a weak purple contrast. The cells surrounding the laticifers react very poorly to the double staining, and only nuclei and some protein bodies appear weakly stained.

With fluorescence microscopy, embryonal laticifers are easily detectable because of the strong red fluorescence emitted by the cytoplasmic matrix (Fig. 1 b). The nuclei are weakly fluorescent and difficult to localize. The protein bodies of laticifers, which are smaller (1/4-1/8) than those of other embryonal cells, are quite evident, but less fluorescent than the cytoplasmic matrix. Generally, in comparison with observations in “white light”, the