Technical Note

Preparation of Epidermal Tissues for Light Microscope Studies of *Vitis* — leaves

J. J. SWANEPOEL AND A. C. DE LA HARPE

Viticultural and Oenological Research Institute, Private Bag X5026, Stellenbosch, 7600, Republic of South Africa.

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A method for preparing epidermal tissues of leaves of *Vitis* spp. for the study of surface characteristics with the aid of a light microscope is described. Certain morphological characteristics such as trichrome length and frequency, stomata frequency, diameter and index of two cultivars were determined by means of a light microscope which compared very favourably with the results obtained when using a scanning electron microscope.

Various methods of preparing epidermal tissues of leaf surfaces for light microscope studies have been described. For the clearance of leaf surface Arnot (1959) and Rodin & Davis (1967) used NaOH and chloral hydrate, Mickel & Lersten (1967) used chlorine bleach, while Pohl (1967) used 40 - 80% HNO_3 and 1 - 2% $KC10_4$ solutions. Some methods for separating and clearing the epidermal tissue from the mesophyll even included the use of micro-organisms (Whittenberger & Naghski, 1948) but the availability and/or host specificity of the organisms are often problematic.

All these procedures are time consuming. Bevege (1968) used an autoclave to clear vesicular-arbuscular mycorrhiza infected roots within 30 min. Using the same principle O'Brien & von Teichman (1974) achieved similar results for soft flowers, grass blades and clover leaves. According to these authors delicate specimens such as grass blades should be autoclaved for 15 min at 100 kPa and then soaked in glycerine, Farrant's medium, lactic acid or lacto-phenol, respectively, until clear, while normal specimens (e.g. clover leaves) should be autoclaved in 80% ethanol and 1% KOH for 15 min at 100 kPa. For difficult specimens such as the leaves of most ferns and Myrtaceae, the use of 5% KOH is recommended after which the tissue could be bleached with a household bleach containing 5% sodiumhypochloride. Ram & Nayyar (1974) used cupric sulphate and hydrochloric acid to isolate both the abaxial and adaxial epidermis of leaves of several plants. Getliffe-Norris (1982) introduced a method in which the tissue is bleached while the epidermal cells are removed.

In the present study damage of epidermal cells occurred when the methods of O'Brien & von Teichman (1974) or that of Ram & Nayyar (1974) were used. The Getcliffe-Norris (1982) method could not effect perfect removal of chlorophyll, resulting in difficulty in identifying single cells. Therefore, a method for preparing epidermal tissues for light microscopy was developed and is subsequently described.

MATERIALS AND METHOD

Vine leaves are cut into small pieces (1 - 2 cm^2) and the side which is not needed for the study (either ad — or abaxial) is damaged by scraping with a steel blade. This facilitates the penetration of bleaching solutions in order to obtain fast removal of chlorophyll and colour pigments during autoclaving. The tissue is then vacuum impregnated with 80% ethanol in 25 ml screw cap bottles until sinking occurs. Containers are capped tightly, autoclaved for 30 min at 100 kPa. The content is subsequently transferred to a 50% sodiumhypochloride solution till nearly colourless (20 min). The tissue is then washed in distilled water, stained in 1% safranine solution for 10 min and mounted in a drop of distilled water. If permanent specimens are required, the tissue can be placed on a glass slide covered with Haupt's fixative (Haupt, 1930), dehydrated with an ethanol series (10%, 30%, 50%, 70%, 100%), cleared in 100% xylene and mounted in Canada Balsam.

RESULTS AND DISCUSSION

Basal leaves of the rootstocks Jacquez (*Vitis aestivalis* M. × *V. cinerea* E. × *V. vinifera* L.) and 420A (*V. berlandieri* P. × *V. riparia* M.) were collected 16 and 24 weeks after budbreak and prepared according to the

TABLE 1
Morphological characteristics of *Vitis* leaves as determined with light- and scanning electron microscope

	Jacquez (*V. aestivalis* × *V. cinerea* × *V. vinifera*)					420A (*V. berlandieri* × *V. riparia*)				
Features / Microscope	Trichrome length (μm)	Trichrome frequency (per mm²)	Stomata frequency (per mm²)	Stomata diameter (μm)	Stomata index	Trichrome length (μm)	Trichrome frequency (per mm²)	Stomata frequency (per mm²)	Stomata diameter (μm)	Stomata index
LM_1	543,9	10	174,8	20,9	11,3	404,4	3,3	225,9	22,8	8,7
SEM_2	547,4	25	269,9	19,6	11,5	414,2	13,2	223,5	21,5	8,7
LSD_3	17,2	0,7	9,5	2,1	0,5	22,7	0,8	9,3	1,5	0,5

1 = Light microscope. 2 = Scanning electron microscope. 3 = Least significant difference (P = 0,01)

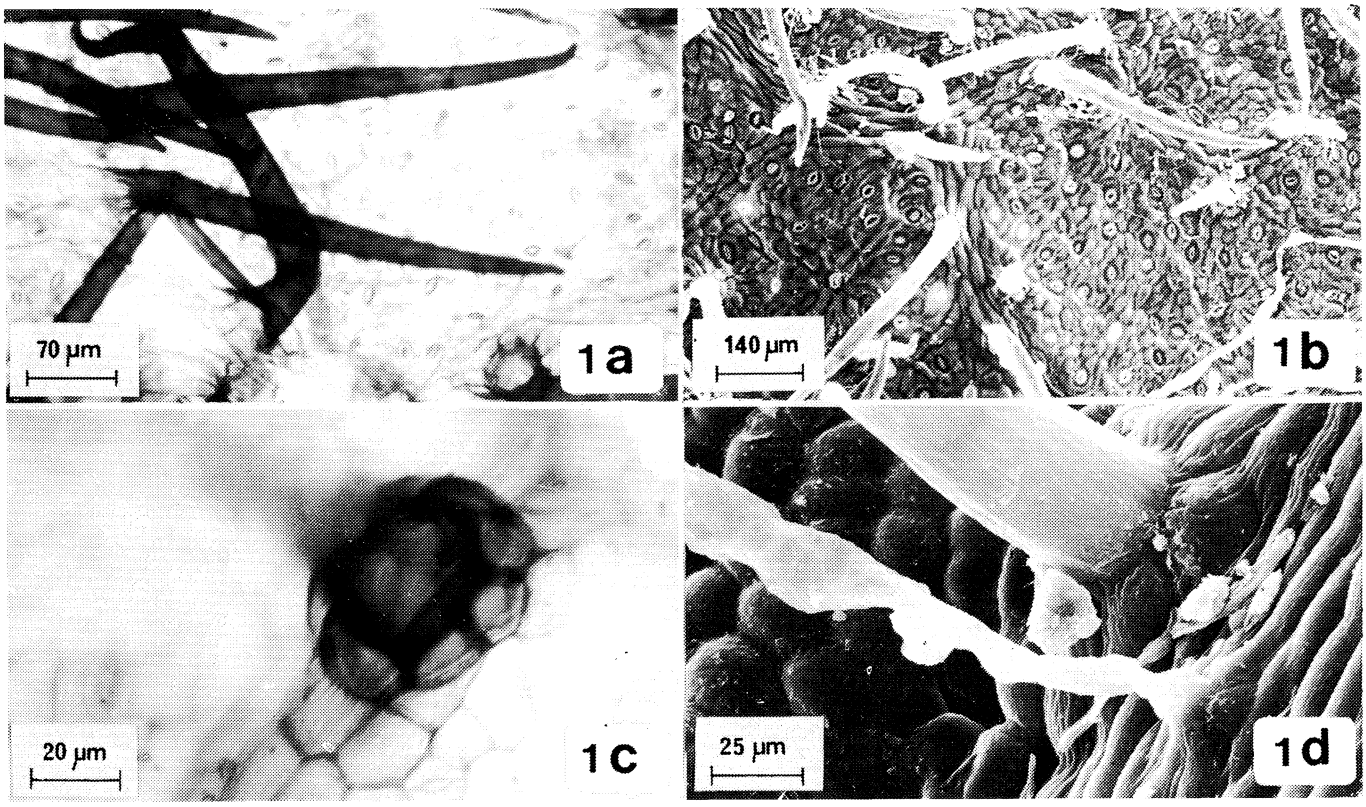

Fig. 1
Light (a,c) and scanning electron micrographs (b,d) illustrating trichrome frequency (a,b) and podiumtypes (c,d) of the leaves of 420A (a,b) and Jacquez (c,d)

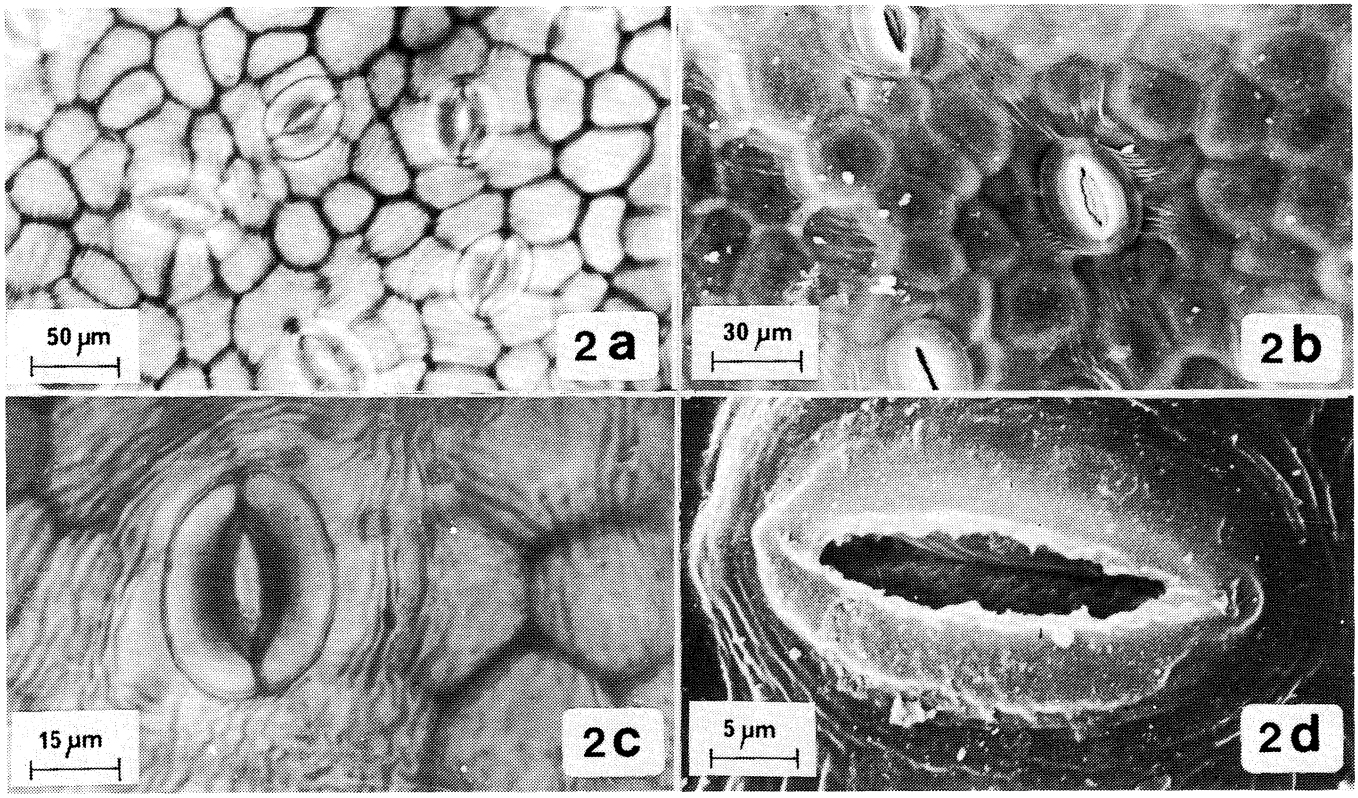

Fig. 2
Light (a,c) and scanning electron micrographs (b,d) showing stomata characteristics of 420A (a,b) and Jacquez (c,d)

method described above. A Zeiss photomicroscope III was used, and for comparison and evaluation of the procedure the same specimens were prepared for scanning electron microscopy (SEM) on a ISI-100A using the method of De La Harpe & Archer (1981). The morphological features investigated were trichrome length (μm), trichrome frequency (number/mm^2), stomata frequency (number/mm^2), stomata diameter (μm) and stomata index (number of stomata + epidermal cells) $\times$ 100. The results obtained with the light microscope were compared by studying the same morphological features with the SEM. Four vines of each rootstock were randomly selected and 20 leaves per vine were studied. Significance of differences between results obtained by light microscope and SEM were shown by means of a factorial analysis based on Turkey's formula (Snedecor & Cochran), 1967) and executed on a Burroughs 7800 computer of the Department of Agriculture.

Only in the case of trichrome frequency was a significant difference ($P = 0{,}01$) found between the results obtained with the light microscope and SEM (Table 1, Fig. 1a, b, c, d). The trichrome frequency (10 per mm^2 Jacquez and 3,3 per mm^2 for 420A) obtained by using the light microscope was much lower than that obtained by using the SEM (25 per mm^2 for Jacquez and 13,2 per mm^2 for 420A). No significant differences in the trichrome length or the stomata parameters were found, and the clear definition of these structures under the light microscope resulted in easy counting and measuring procedures (Fig. 2a, b, c, d).

Although the described procedure caused a loss of trichromes, a sufficient number of these structures remained so that accurate measurements could be obtained. The proposed method enables preparation of epidermal tissues of *Vitis*- leaves of various ages for light microscope studies in laboratories where access to a scanning electron microscope is not readily available. Other advantages are the ease with which the material can be prepared and the low artifact frequency.

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