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Review

Sample preparation for scanning electron microscopy of plant surfaces—Horses for courses

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ABSTRACT

Plant tissues must be dehydrated for observation in most electron microscopes. Although a number of sample processing techniques have been developed for preserving plant tissues in their original form and structure, none of them are guaranteed artefact-free. The current paper reviews common scanning electron microscopy techniques and the sample preparation methods employed for visualisation of leaves under specific types of electron microscopes. Common artefacts introduced by specific techniques on different leaf types are discussed. Comparative examples are depicted from our lab using similar techniques; the pros and cons for specific techniques are discussed. New promising techniques and microscopes, which can alleviate some of the problems encountered in conventional methods of leaf sample processing and visualisation, are also discussed. It is concluded that the choice of technique for a specific leaf sample is dictated by the surface features that need to be preserved (such as trichomes, epidermal cells or wax microstructure), the resolution to be achieved, availability of the appropriate processing equipment and the technical capabilities of the available electron microscope.

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1. Introduction

Scanning electron microscopy (SEM) is an ideal technique for examining plant surfaces at high resolution. Plant tissues must be preserved by dehydration for observation in an electron microscope because the coating system and the microscopes operate under high vacuum and most specimens cannot withstand water removal by the vacuum system without distortion (Holloway and Baker, 1974).

In order to examine the native structure of the sample, some microscopes are designed to image frozen hydrated samples and more recently environmental SEM microscopes have been devel-

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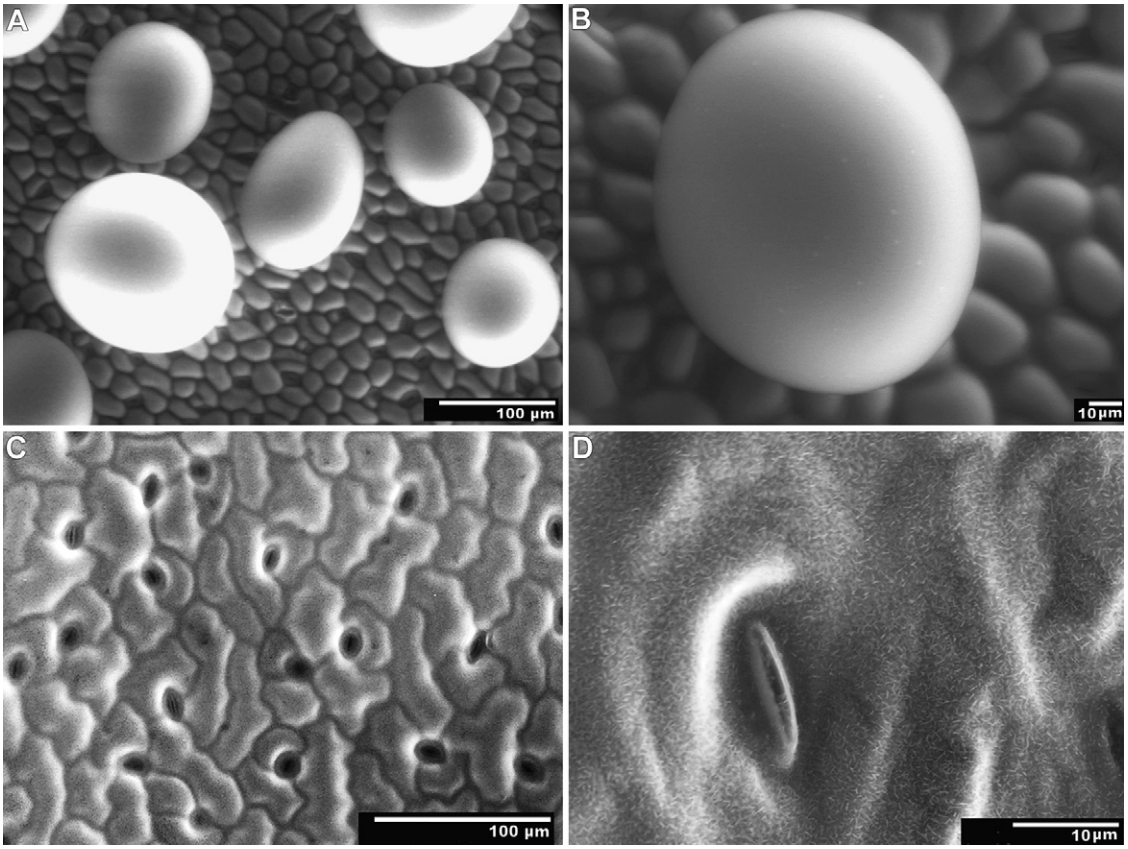

Fig. 1. Leaf surfaces from unprepared and uncoated specimen visualised under an environmental scanning electron microscope: (A) chenopodium leaf surface showing intact epidermal cells and salt glands; (B) chenopodium salt gland at high magnification, note that waxes are not visible using this technique; (C) pea leaf surface showing intact epidermal cells, but waxes are not clearly visible; (D) epidermal cell collapse in pea leaf surface at high magnification. Waxes are not clearly visible.

oped which can image the sample in their native-hydrated state. These microscopes are specialised equipments and may not be available in many labs. Hence, sample preparation by dehydration is still an important consideration for observation in conventional microscopes.

For samples that necessitate dehydration, many techniques other than just air-drying have been developed to remove water from the sample, all aiming at minimal distortion of the cell and maximal preservation of the original form and structure. These techniques include freeze-drying, critical point drying, and various types of chemical fixation treatments prior to dehydration of samples. However, acceptable methods offer less than ideal preservation for some plant species and may be inconsistent. The inconsistency is largely due to diversity in tissue types, form, structure and composition of plants. Inconsistencies also arise from variation in individual skills and equipment used across different labs. Hence, new/modified techniques are continually being tested and developed for the preparation of specific plant tissues for visualisation under electron microscopes.

The paper reviews common techniques/methods used in the past for leaf sample preparation for scanning electron microscopy. Selected examples from our own work on common plant species (monocots and dicots) of interest in pesticide research are presented and compared with those from previous studies that have used similar techniques for electron microscopy of plant tissues. Emphasis has been given to a simple, but robust leaf sample preparation technique (simple air-drying), which has proved highly effective for visualisation of plant waxes under a field emission scanning electron microscope (FESEM) at low kV.

2. Approaches for sample preparation and visualisation

Samples can be visualised in their native-hydrated state without pre-treatment, frozen hydrated state or after removing liquids from the samples using a variety of techniques. The choice of technique will depend on the sample, the equipment available and the surface features and structures that need to be visualised.

2.1. Hydrated samples

Rapid observations of fresh hydrated samples can be made by using an environmental scanning electron microscope (ESEM). The technique has the potential to provide excellent low magnification images of plant surfaces in their native-hydrated state. In addition, it allows the flexibility to alter stage temperature and vapour pressure in the specimen chamber. For example, leaf tissues can be examined at high humidity in the chamber and minimise sample dehydration during the imaging process. This technique can also be effectively used to perform 'dynamic' experiments in wet mode to examine biological events in developmental processes such as fungal growth on leaf surfaces.

A FEI Quanta ESEM[1] (FEI Company, USA) was used at an accelerating voltage of 10–20 kV, a stage temperature of 2 °C and a chamber pressure of 6 Torr to visualise unprocessed chenopodium and pea leaf surface in their native-hydrated state (Fig. 1A–D). Although the 'true-to-life' low magnification images of chenopodium leaves were successfully obtained, the wax microstructure

[1] Equipment used at Research Centre for Surface and Materials Science, University of Auckland, New Zealand.