Application of environmental scanning electron microscopy to study plant surfaces

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Studying plant surfaces is inconceivable without scanning electron microscopy (SEM). The standard preparation procedure is divided into several steps. Fixation (chemical fixation, cryofixation) is necessary to preserve sensitive structures, dehydration series and drying procedures (e.g., critical point drying) are required to get rid of the water and coating with metals or carbon is mandatory to produce a conductive specimen. These steps, however, are not artifact-free. Removal or changes of lipid substances (waxes), shrinkage, cracking, and distortion of the surface are some of the possible artifacts [1].

The environmental scanning electron microscope (ESEM) was introduced to view hydrated, non-conductive samples without further sample preparation [2], which are imaged in a partial pressure of gas, in most cases water vapor, and secondary electrons emitted from the sample are detected after gas cascade amplification [2, 3].

The microscope used in the author's lab (XL 30 ESEM, tungsten; FEI) offers two modes. In the "wet" mode a chamber pressure of up to 1300 Pa is possible and this enables in combination with a cooling stage up to 100% humidity at the sample surface. In the "low vacuum" mode (secondary electron detection) chamber pressure is limited to 133 Pa and the humidity at the sample surface is < 10% at room temperature.

Plant surfaces are well protected against excessive water loss by the presence of a nearly water impermeable layer covering all above-ground organs (cuticle) and plants often have thick, and lipid-impregnated outer cell walls. These characteristics cut them out to be investigated by ESEM. In the last years a lot of different plant samples and all kinds of outer plant surfaces (leaves, stems, roots, seeds, and flowers) were investigated using ESEM and an overview of this research will be given. Emphasis will be placed on the pros and cons of the "wet" and the "low vacuum" mode in comparison to standard (high vacuum) SEM.

One has to be aware that non-coated biological samples are very sensitive to beam damage, that the effective resolution is reduced compared to SEM of metal-coated samples, and that in many cases samples can only be studied once [1, 3-4].

Several application areas for ESEM turned out to be of special interest in plant sciences. It is used for fast screening of surface characteristics (epidermal cells, hairs, stomata, dust deposits), also as a "control" to detect preparation artifacts, and for studying the presence or absence of insects or pathogens on plant surfaces. It is a valuable tool in studying trichome and glandular morphology and function (Fig. 1). The unrivalled possibilities of ESEM, however, are the investigation of dynamic processes that can be experienced at first hand. The direct observation of anther opening processes [5, 6] (Fig. 2) or the studying of native pollen germination on stigmas (Fig. 3) are examples for these examinations.

ESEM opens a wide range of applications in plant sciences and shows the sample surface in its native state. It has become an indispensible tool in studying plant surfaces.

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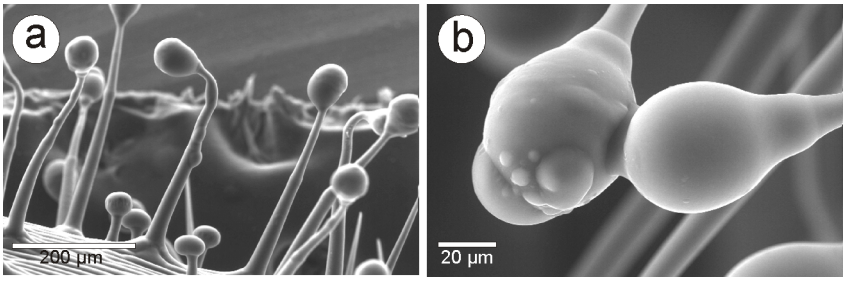

Figure 1. ESEM images of glandular hairs on leaves of *Pelargonium sp.* The low magnification image (a) shows the presence of short and long glandular hairs and some drops of secretion products on the stalks, which are seen in (b) on the head cells of the hairs (room temperature, chamber pressure: 120 Pa).

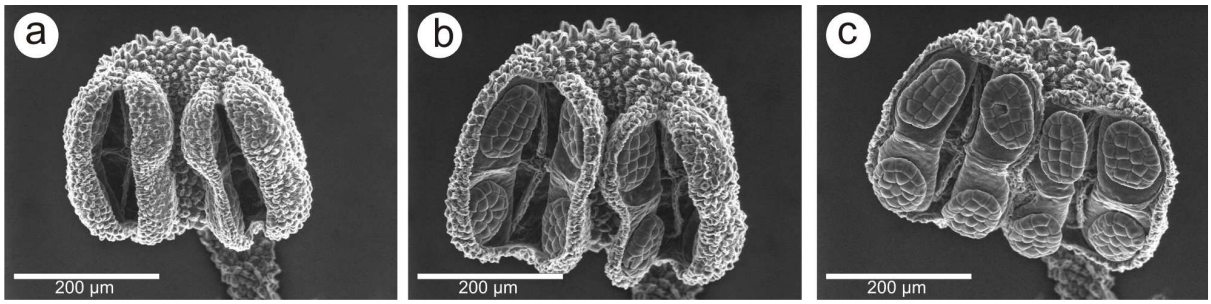

Figure 2. ESEM images of one anther of *Inga feuillei* showing the opening process (a-c) with a time span of 45 minutes between a) and c) (room temperature, chamber pressure: 120 Pa).

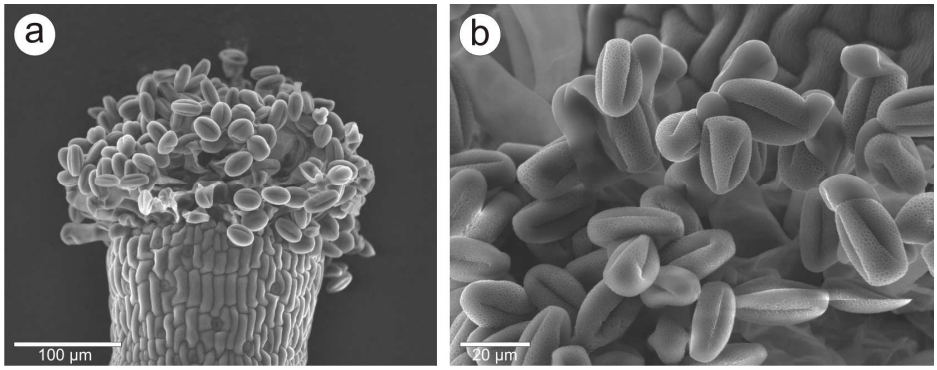

Figure 3. ESEM images of pollen of *Arabidopsis thalliana* on the stigma (a) and showing the contact zones between pollen and stigma hairs (b) (room temperature, chamber pressure: 120 Pa).

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