INTRODUCTION

Scanning electron microscopy (SEM) has become an indispensable tool for visualizing the plant surface. A fundamental requirement of conventional SEM (CSEM) is the need for high-vacuum conditions throughout the column, typically around \(10^{-3} \text{~to~} 10^{-5} \text{~Pa}\), to minimize primary electron scattering (Stokes, 2008). This requirement necessitates a series of complex specimen preparation steps, including fixation, dehydration, drying, and metal coating, because specimens should be vacuum stable, vacuum friendly, and electrically conductive (Stabentheiner et al., 2010). The specimen preparations themselves can change the structural or chemical nature of the specimens, leading to the imaging of unwanted artifacts (Stokes, 2008).

Variable pressure SEM (VP-SEM) is often called environmental or low-vacuum SEM. It allows observations to be carried out in the presence of gas at pressures of ca. 2 Torr (300 Pa) in the specimen chamber (Mohan et al., 1998). With a differential pumping system, VP-SEM maintains a gas (typically water vapor) pressure in the specimen chamber, while the electron gun remains at high vacuum (McGregor & Donald, 2010). The gas ions that are produced by the collisions with secondary electrons (SE) compensate for the negative charge on the surface of nonconductive specimens (Stabentheiner et al., 2010). These features suggest that VP-SEM is well suited to biological imaging, by offering topographic images with a remarkable depth of field, without
the need for specimen preparation or unwanted artifacts (McGregor & Donald, 2010). Furthermore, the modern modality of field emission SEM (FESEM) equipped with VP abilities ensures the superb resolution and more versatile imagery than CSEM and FESEM. The genus *Plectranthus* (Lamiales: Lamiaceae), also referred to as spurflowers, consists of ca. 300 species of evergreen perennials and subshrubs distributed through the tropical regions (Ascensão et al., 1999; Rice et al., 2011). With a diversity of ethnobotanical uses, the species of *Plectranthus* have been reported to produce monoterpenoids, sesquiterpenoids, diterpenoids, and phenolics (Lukhoba et al., 2006). As *Plectranthus* was the focused material of research activity at the Royal Botanical Gardens (Kew, UK), the genus from eastern and southern Africa was used as a model for defining a role for herbarium data in Red List assessments (Willis et al., 2003). In particular, the Vick’s plant *Plectranthus tomentosa* is one of the most popular succulent herb plants. Although many *Plectranthus* species have been investigated because of their high content of essential oils, the leaf surface features have been reported only for a few species (Ascensão et al., 1999). As a plant surface sculpture, trichomes represent unicellular or multicellular appendages that develop outwards on the surface of various plant organs (Evert, 2006; Kim et al., 2011). Trichomes of *Plectranthus* and other genera have been observed by (i) CSEM after preparation process (Ascensão et al., 1998 and 1999; Bhatt et al., 2010) and (ii) VP-SEM without preparation (Kolb & Müller, 2004; Stabentheiner et al., 2010). Backscattered electrons are usually detected for imaging in VP-SEM (Kim, 2012) and compositional contrast and reduced charging in CSEM (Kim et al., 2008), whereas SE have topographic relief for surface imaging. Recently, the trichomes of chili pepper were observed by detecting SE in VP-FESEM (Kim et al., 2012); however, there are very few reports of SE imaging (SEI) by VP-FESEM. Detailed microscopic studies of the trichome profiles of *P. tomentosa* are demanded to further exploit the ornamental and pharmaceutical usability of the plant. Here, I use VP-FESEM to provide rapid and high-resolution morphological profiling of the foliar trichomes of fresh *P. tomentosa*.

**MATERIALS AND METHODS**

**Plant Material**

A 10-cm-tall *P. tomentosa* plant was grown indoors in a pot under natural light. The plant had numerous trichomes on the leaf surface (Fig. 1). A peculiar aroma emanating from the plant could be detected even with a light touch. Fresh and fully developed leaves were collected from the plant for VP-FESEM examination.

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**Variable Pressure Secondary Electron Imaging**

Leaf segments (ca. 5×5 mm²) were excised from the detached leaves using scissors. The specimens were readily mounted on a metal stub using the double-sided carbon tape (Kim, 2012). Without metal coating and cooling, they were directly examined with a Schottky type FESEM instrument (Supra 55VP, Carl Zeiss, Oberkochen, Germany) operated at an accelerating voltage of 15 kV. The SE signals were detected using a lateral below-lens VP SE detector under ca. 15 Pa. Both the adaxial (upper) and abaxial (lower) leaf surfaces were observed with the electron microscope.

**RESULTS AND DISCUSSION**

VP-FESEM revealed that the leaf specimens were well preserved without severe collapse or charging effects (Fig. 2A). The adaxial surface of *P. tomentosa* leaf was covered with several types of trichome. Based on their shape, size, and secretion mode, foliar trichomes could be categorized into the following different groups. *Nonglandular trichomes* (NGT) were single, uniseriate, sharply pointed, and straight or curved in shape (Fig. 2B). NGT consisted of a basal cell and a long (up to ca. 300 μm) multicellular (five to seven cells) stalk. In some cases, minute globes were found on the terminal portion of the stalks (Fig. 2C). Approximately four epidermal cells were arranged around the base, forming a cellular pedestal to support the NGT.

In addition, two kinds of *glandular trichomes* (GT) were found on the leaf surface. (i) *Capitate GT* (CGT) had a secretory head and a short or long stalk (Fig. 2D). The secretory head was globose (ca. 50 μm in diameter) and often possessed a spherical projection at its apex. (ii) *Peltate GT* (PGT) was characterized by a globose (ca. 20 μm in diameter) secretory head and a very short stalk (almost sessile) on a basal cell.

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**Fig. 1.** A leaf of *Plectranthus tomentosa*. Trichomes (arrows) are discernible and prevalently distributed on the epidermis.
Collapsed PGT exhibited a slightly wrinkled surface on the secretory head. Some CGT consisted of a conelike secretory head and a short stalk (Fig. 2F). They possessed a minute spherical projection at their apex. No stomata were present on the adaxial leaf surface.

The abaxial leaf surface bore numerous NGT and GT on the adaxial leaf surface.
VP-FESEM of Plectranthus tomentosa Trichomes

leaf vein and the leaf lamina (Fig. 3A). NGT was characterized by a pointed, long, and slightly curved appearance (Fig. 3B). There was no morphological difference between NGT on the abaxial leaf surface and that on the adaxial leaf surface. CGT was also commonly found on the abaxial surface (Fig. 3C). As shown on the adaxial surface, a minute spherical

Fig. 3. Field emission scanning electron micrographs of the abaxial leaf surface of Plectranthus tomentosa. (A) Overall leaf surface. Both glandular trichomes (arrows) and nonglandular trichomes (NGT; arrowheads) are present on the leaf surface. Bar=100 μm. (B) Higher magnification of an NGT. It comprises a basal cell (arrow) and a stalk (arrowhead). Bar=20 μm. (C) Capitate glandular trichomes (CGT; arrows). They have a spherical projection at their apex. Bar=50 μm. (D) CGT. Long-stalked CGT (arrows) have a bigger secretory head and a longer stalk than short-stalked ones (arrowheads). Bar=50 μm. (E) CGT (arrows). They have a conelike secretory head and a short stalk. Bar=100 μm. (F) Digitiform trichomes (arrows). They consist of several cells in line. Stomata are present on the abaxial leaf surface. Bar=50 μm.
projection was present at the apex of CGT. A tilted view revealed that CGT varied in stalk length (Fig. 3D). Longstalked CGT possessed a relatively large globose (ca. 50 μm in diameter) secretory head and a relatively long (ca. 50 μm) stalk, whereas short-stalked CGT had a small globose (ca. 20 μm in diameter) secretory head and a short (less than ca. 20 μm) stalk. CGT with a conelike secretory head and a short stalk was commonly observed (Fig. 3E). The secretory heads were ca. 50 μm in diameter, and had a spherical projection at their apex. As another type, (iii) digitiform trichomes (DT) comprised several cells in line on the abaxial leaf surface (Fig. 3F). The glandular apical cells were rarely distinguished from the stalks. Numerous stomata were present on the abaxial leaf surface.

This study demonstrated the trichome profiling of *P. tomentosa* by VP-FESEM. Four types of trichome could be determined on the leaf surface: NGT, CGT, PGT, and DT (Fig. 4). Although DT was found only on the abaxial leaf surface, the other types of trichome occurred on both adaxial and abaxial leaf surfaces. It was evident that NGT with a long stalk was mostly responsible for the hairy surface of the leaf (Fig. 4A). The most striking difference between PGT and CGT was the stalk height (Fig. 4B to 4D). As it is almost sessile, PGT is likely to have a collapsed secretion head.

An intriguing finding was that several distinct secretion stages could be determined by VP-FESEM based on the morphology of the secretion droplets on CGT. The spherical projections on CGT were strikingly similar to the secretion droplets on CGT of *Cucurbita* under VP conditions (Fig. 4E and F) (Kolb & Müller, 2004). This implies that the CGT of *P. tomentosa* was under the early stage of secretory process. It is possible that the degenerated secretion head represents the active stage of the secretory process (Fig. 2B and 4G). Given the unrivaled capabilities of VP-FESEM as described above, the minute globules on NGT were assumed to be secretion droplets derived from neighboring CGT, as suggested from the CGT of other plants observed by VP-SEM (Kolb & Müller, 2004; Sarria et al., 2010). DT could not be reasonably considered GT, in the absence of any report on the secretion process from the apical cell (Fig. 4H) (Ascensão et al., 1999).

**CONCLUSIONS**

VP-FESEM enabled the observation of dehydration-sensitive trichomes in their near-native state by SEI. Due to the direct specimen observations at the FESEM resolution, this approach facilitates the rapid and detailed morphological analysis of a variety of trichomes in diverse plant taxa with reduced labor and preparation.

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**REFERENCES**


