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Morphology of glandular trichomes of Japanese catnip (*Schizonepeta tenuifolia* Briquet) and developmental dynamics of their secretory activity

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ABSTRACT

Schizonepeta tenuifolia Briquet, commonly known as Japanese catnip, is used for the treatment of colds, headaches, fevers, and skin rashes in traditional Asian medicine (China, Japan and Korea). The volatile oil and its constituents have various demonstrated biological activities, but there is currently limited information regarding the site of biosynthesis. Light microscopy and scanning electron microscopy indicated the presence of three distinct glandular trichome types which, based on their morphological features, are referred to as peltate, capitate and digitiform glandular trichomes. Laser scanning microscopy and 3D reconstruction demonstrated that terpenoid-producing peltate glandular trichomes contain a disk of twelve secretory cells. The oil of peltate glandular trichomes, collected by laser microdissection or using custom-made micropipettes, was demonstrated to contain (−)-pulegone, (+)-menthone and (+)-limonene as major constituents. Digitiform and capitate glandular trichomes did not contain appreciable levels of terpenoid volatiles. The yield of distilled oil from spikes was significantly (44%) higher than that from leaves, while the composition of oils was very similar. Oils collected directly from leaf peltate glandular trichomes over the course of a growing season contained primarily (−)-pulegone (>80% at 32 days after germination) in young plants, while (+)-menthone began to accumulate later (>75% at 80 days after germination), at the expense of (−)-pulegone (the levels of (+)-limonene remained fairly stable at 3–5%). The current study establishes the morphological and chemical characteristics of glandular trichome types of *S. tenuifolia*, and also provides the basis for unraveling the biosynthesis of essential oil in this popular medicinal plant.

1. Introduction

Schizonepeta tenuifolia Briquet (Lamiaceae) is an annual plant employed in traditional medicine in China (jing jie), Japan (keigai) and Korea (hyonggae), primarily for the immunomodulatory and anti-inflammatory properties of extracts prepared from above-ground parts (Fung and Lau, 2002; Kang et al., 2008; Wang et al., 2012). While no in-depth clinical data are available for *S. tenuifolia* oil or its constituents, the herb is contained, among nine others, in Zemaphyte®, a Chinese herbal remedy for which clinical trials to treat atopic dermatitis have been completed (reviewed in Fung and Lau, 2002). Oils of *Schizonepeta* species have also been tested for their pesticidal activities against agricultural pests. For example, it was demonstrated that *S. tenuifolia* oil inhibits the growth of larvae of *Lycoriella ingenua* Dufour, a species of flies that negatively affects the artificial production of edible mushrooms (Park et al., 2006). *S. multifida* oil was shown to exert insecticidal activities against grain storage insects such as the maize weevil (*Sitophilus zeamais*) and the red flour beetle (*Tribolium castaneum*) Liu et al. (2011). Acaricidal activities of *S. tenuifolia* oil were

Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; FID, flame ionization detection; GC, gas chromatography; GT, glandular trichome; LMD, laser microdissection; LSM, laser scanning microscopy; MS, mass spectrometry; SEM, scanning electron microscopy.

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reported against several species of house dust and stored food mites (Yang and Lee, 2013).

Volatile monoterpenes are the most studied constituents of *S. tenuifolia* oil, but a range of other specialized metabolites have also been characterized, including flavonoids, monoterpene alcohols, monoterpene glycosides, sesquiterpenes, and organic acids (Sasaki et al., 1981; Oshima et al., 1989; Fung and Lau, 2002; Zhang et al., 2006; Lee et al., 2008; Zhao et al., 2017). Monoterpenes such as limonene and pulegone have been shown to impact the quality of *S. tenuifolia* oils, and analytical protocols have been developed to profile these and other monoterpenes (Maruyama et al., 2001; Lee et al., 2008; Xie et al., 2009; Chun et al., 2010, 2011). Oil analyses have been performed with leaf and flower material harvested at different levels of plant maturity (Yu et al., 2011), but an in-depth investigation of the developmental regulation of oil formation has not yet been published in the peer-reviewed literature.

The essential oils of members of the Lamiaceae are often synthesized and stored in specialized anatomical structures called glandular trichomes (GTs), and these protuberances may assume many different shapes and sizes, with the cellular composition varying substantially across species (Fahn, 2000). Depending on the structure of the secretory head, GTs are referred to as peltate (shield-shaped), capitate (ending in a distinct compact head) or digitiform (shaped like a finger) (Heinrich et al., 2002; Avato et al., 2005; Naidoo et al., 2012; Munien et al., 2015; Muravnik et al., 2016), and such GT characteristics are taxonomically important markers for differentiating species, including *Schizonepeta* (Moon et al., 2009; Eiji and Salmaki, 2016). In the present study, we establish the morphological and chemical characteristics of glandular trichome types on leaves, spikes and stems of *S. tenuifolia*. We also describe the developmental patterns of leaf monoterpene accumulation during a growing season, thereby providing vital information for follow-up studies to unravel the biosynthesis of essential oil constituents.

2. Results and discussion

2.1. Three types of glandular trichomes are present on aerial surfaces

Scanning electron microscopy (SEM) was employed to survey *S. tenuifolia* aerial surfaces for the occurrence of trichomes. Non-glandular trichomes and three types of glandular trichomes (GTs) were distinguishable. Peltate GTs assumed a globular dome shape at maturity (Fig. 1A) and contained highly fluorescent material (Fig. 1B and C). In this context it should be noted that fluorescence was reported by Bergau et al. (2016) to be a characteristic feature of GTs containing secretions. At post-secretory stage, peltate GTs of *S. tenuifolia* leaves were approximately 15 μm in height and 50 μm in diameter. Following histochemical staining of nuclei with 4′,6-diamidino-2-phenylindole (DAPI), the peltate GTs contained a disk of twelve secretory cells, with four smaller cells in the center and eight larger cells on the outside, an arrangement that was confirmed in a 3-dimensional reconstruction (Supplementary Fig. 1). Capitate GTs, which were characterized by a short stalk and relatively large, two-celled head (Fig. 1D), fluoresced very weakly (Fig. 1E and F). Mature capitate GTs were about 25 μm in height and 20 μm in diameter. Digitiform GTs had a longer stalk and a single cylindrical head cell (Fig. 1G). A yellowish secretion, which was also fluorescent, was detected near the apex (Fig. 1H and I). Mature digitiform GTs were approximately 30 μm in height and 10 μm in diameter.

We then assessed the abundance of trichome types on aerial surfaces. Non-glandular trichomes were the most prevalent feature on all above-ground organs (Fig. 2). On the adaxial leaf surface,

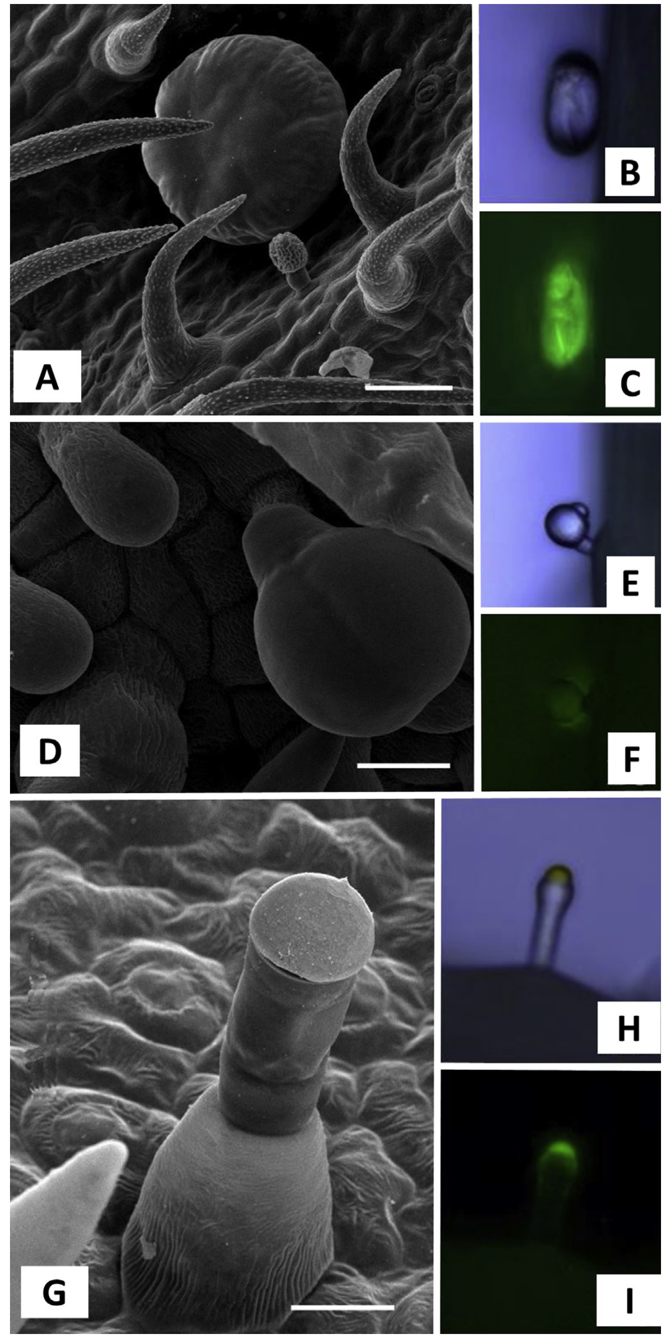

Fig. 1. Morphology of *S. tenuifolia* GTs. Peltate GTs on leaves as visualized by SEM (**A**), light microscopy (**B**), and fluorescence microscopy (indicating strongly fluorescent material) (**C**). Capitate GTs on leaves as visualized by SEM (**D**), light microscopy (**E**), and fluorescence microscopy (indicating lack of fluorescent material) (**F**). Digitiform GTs as visualized by SEM (**G**), light microscopy (note yellowish secretion) (**H**), and fluorescence microscopy (indicating strongly fluorescent material below the apex) (**I**). Fluorescence microscopy employed a 460–500 nm band pass excitation filter.

peltate GTs were fairly abundant in the area between the margin and the major vein (Fig. 2A), while on the abaxial side, peltate GTs clustered along the margin and beside the major vein, with a smaller number distributed throughout the remaining surface area (Fig. 2B). Capitate GTs were distributed evenly across both the adaxial and abaxial leaf surface (Fig. 2 A, B). Digitiform GTs were a rare appearance on most surfaces, with the exception of a high abundance on the oldest stem internode (Fig. 2C). The surface of the young calyx had a few capitate GTs, but peltate GTs were much