

Structural Features of Glandular and Non-glandular Trichomes in Three Species of *Mentha*

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The trichomes on leaves of three species of Mentha such as M. spicata, M. suaveolens, and M. piperita var were examined by scanning electron microscopy. Simple non-glandular trichomes and peltate glandular trichomes were distinctively occurred on these leaves. In M. spicata, short and sharp non-glandular trichomes were dominantly appeared on adaxial surface, whereas capitate glandular trichomes were commonly localized on abaxial surface. In M. suaveolens, non-glandular trichomes were identified with simple unbranched and branched. Unbranched trichomes which were sharp and pointed in shape occurred on adaxial surface, however, branched and v-shaped trichomes appeared abundantly on abaxial surface. Peltate trichomes consisted of a large eight-celled head. Small capitates trichomes consisted of a cylindrical head with an one-celled uniseriate stalk. In M. piperita var, single non-glandular trichomes and peltate trichomes were present on adaxial surface. Small capitate trichomes consisted of a globose unicellular head with a two- or three-celled uniseriate stalk. Peltate trichomes were distinctly present on abaxial surface, whereas they were not observed on adaxial surface. The trichomes were less dense in this species. Peltate trichomes consisted of a large eight-celled head, with an enlarged secretory cavity, attached to an one-celled short stalk.

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INTRODUCTION

The surfaces of plant organs are often covered by various non-glandular and glandular trichomes. Scientific interest in trichomes is established on their functional and toxonomic importance and on the economic usefulness of some trichome produced substances. Trichomes are epidermal appendages of various form, structure and function. They are derived from the various epidermal cells on vegetative organs like stem or leaf. Trichomes are considered as the physical barriers against external factors, such as animals and pathogens. They also serve to restrict loss of water or to give protection against extreme temperature and ultraviolet radiation (Werker, 2000). The characteristics of trichomes and the composition of secretory substances secreted by them,

vary greatly among species and are used in plant classification to distinguish between the related genera or species (Xiang et al., 2010).

Some of the epidermal cells grow out to a considerable length, giving rise to trichomes. Trichomes are often classified as being glandular or non-glandular, based on whether or not they function as secretory structures (Peterson & Vermeer, 1984). The unique characteristics of trichomes have traditionally been used as a taxonomic key in plant classification (Wagner, 1991; Xiang et al., 2010). The most of non-glandular trichomes are simple, branched or starshaped. In many cases, such trichomes are living cells whereas in others they are dead and the protoplasm is replaced by air spaces (Fahn, 1988).

The trichomes are glandular and secrete essential oils, which

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give those leaves their distinctive fragrance. The natural essential oils have great commercial value. Many species of Lamiaceae are aromatic, and are used as spices, herbs, medicines, and a source of fragrance. Most of aromatic species of this family such as *Origanum dictamunus*, *Perilla ocymoides*, *Nepeta cataria*, *Dracocephalum moldavica*, bear glandular trichomes on their surfaces (Kolalite, 1998).

Glandular trichomes are generally accepted as the functionally specialized tissues that produce various secondary metabolites, which are then stored or volatilized at the plant surface (Kim & Mahlberg, 1997; Mahlberg & Kim, 2004). Many plants use glandular trichomes to produce secondary compounds that are used for pollination, defense, and protection. Several plant genera have been studied specifically for their potential uses in industry. Lamiaceae have been focused on the essential oils secreted by glandular trichomes. A lot of these plants are cultivated for ornamental and other economical purpose which are used in medicines and cosmetics (Huang et al., 1994; Richheimer et al., 1999; Sancheti & Goyal, 2007; Geuenich et al., 2008; Pokorny, 2008).

Glandular trichomes were often classified morphologically into two main types—peltate and capitate—and could be distinguished by head size and stalk length (Ko et al., 2007; Baran et al., 2010). Peltate trichomes secrete only lipophilic substances, while capitate trichomes mainly secrete polysaccharide products (Huang et al., 2008). Peltate trichomes generally have a short stalk and a large head, containing 4 to 18 cells; capitate trichomes have a stalk that is twice as long as their head (Ascensao & Pais, 1998). Peltate trichomes have broad secretory cavities 40~60 µm in diameter, while capitate trichomes have globular secretory cavities 10~30 µm in diameter (Luo et al., 2010). This work presents the observations on the secretory trichomes of three *Mentha* species and, aims to provide the relationship between the volatilization of essential oil and glandular structures.

MATERIALS AND METHODS

The mature leaves of *Mentha spicata*, *M. suaveolens*, and *M. piperita var* were collected from the plants grown at Konkuk University. For scanning electron microscopic analysis of trichomes, leaf segments were prefixed with 2% (v/v) glutaraldehyde in a 0.1 M sodium phosphate buffer (pH 7.2), and postfixed with 1% unbuffered osmium tetroxide at room temperature. The fixed specimens were dehydrated with a graded ethanol series, and followed by critical point drying with solvent-substituted liquid carbon dioxide (EMS 850, Hatfield, PA, USA). After coating with a thin layer of gold in an ion sputter coater (Cressington 108 AUTO, Watford, UK), the specimens were observed with a field emission-scanning electron microscope (JSM-6390LV, JEOL, Tokyo, Japan).

RESULTS

The leaves of *M. spicata*, *M. suaveolens*, and *M. piperita* var were characterized by an abundance of non-glandular trichomes and two glandular trichomes-small capitate and large peltate glandular trichomes. But they differed from each other in morphology, density and location on the leaf more or less. The two types of trichomes occurred on *Mentha* species, non-glandular, simple trichomes; capitate glandular trichomes with an one-celled apical head. High density of glandular trichomes was observed on the leaves of *M. piperita* var, and low density on *M. spicata*. However, the different pattern of density was showed on adaxial and abaxial surfaces in *M. suaveolens*.

Mentha spicata

Long and short non-glandular trichomes were dominantly present on adaxial surface (Fig. 1A). The former was 490 μm in height, the latter was 10~20 μm . The long trichomes were often bended horizontally toward the surface (Fig. 1B). The capitate glandular trichomes were commonly observed on abaxial surface (Fig. 1C). Although short non-glandular trichomes stood straight, however, long non-glandular trichomes were perpendicularly bent to the epidermal cells (Fig. 1D). The developed capitate trichomes are about 44 μm in height and 26 μm in diameter at the spherical head (Fig. 1E). They consist of a short stalk and a large globose head (Fig. 1F).

Mentha suaveolens

Two types of non-glandular trichomes were observed. The first one was sharp, pointed trichomes on adaxial surface, and the second one was branched, v-shaped trichomes appeared abundantly on abaxial surface (Fig. 2A-D). The length was about 380 µm and the diameter of distal region was 52 µm (Fig. 2B). Non-glandular trichomes were often bent horizontally to the epidermis (Fig. 2C). Two types of glandular trichomes, sessile peltate and stalked capitate, were present on abaxial surface. The peltate glandular trichomes each consisted of a large eight-celled head, with an enlarged secretory cavity, attached to a two-celled short stalk. A head of peltate trichome had a diameter of 54 µm, and a stalk showed a length of 8~10 µm (Fig. 2E). A small capitate glandular trichome consisted of a cylindrical head attached to leaf with an one-celled uniseriate stalk. A stalk had a length of 24 µm, and a head had a diameter of 13 µm (Fig. 2F).

Mentha piperita var

Single non-glandular trichomes and peltate glandular trichomes were present on adaxial surface. A small capitate glandular trichome consisted of a globose unicellular head attached to leaf with a two- or three-celled uniseriate stalk.



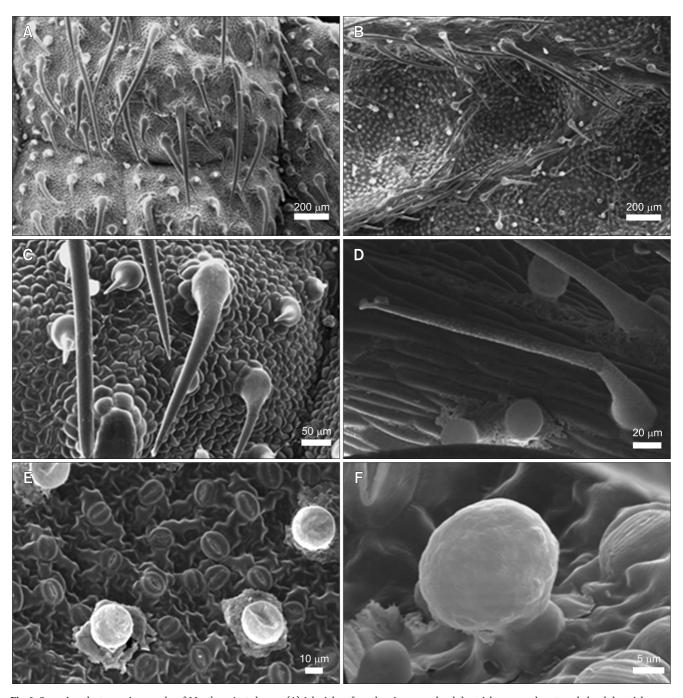


Fig. 1. Scanning electron micrographs of *Mentha spicata* leaves. (A) Adaxial surface showing non-glandular trichomes and scattered glandular trichomes. (B) Abaxial surface showing lots of capitate trichomes in intervenous area. Note non-glandular trichomes are mostly restricted in the leaf vein. (C) Non-glandular trichomes on the adaxial surface bend to the epidermal cells. (D) High magnified non-glandular trichomes showing perpendicularly bent. (E) A large number of stomata and some capitate glandular trichomes are shown. (F) Magnified capitate glandular trichome is shown.

A stalk had a length of 22 μ m and a head had a diameter of 15 μ m. Peltate trichomes were distinctly present on abaxial surface, whereas they were not entirely observed on adaxial surface (Fig. 3A). Very low density of trichomes was detected in *M. piperita var* (Fig. 3A and B). Prominent non-glandular trichomes were examined on leaves (Fig. 3C). Peltate

glandular trichomes consisted of a large eight-celled head, with an enlarged secretory cavity, attached to an one-celled short stalk. A head of peltate trichome had a diameter of 73 μm (Fig. 3D-F).



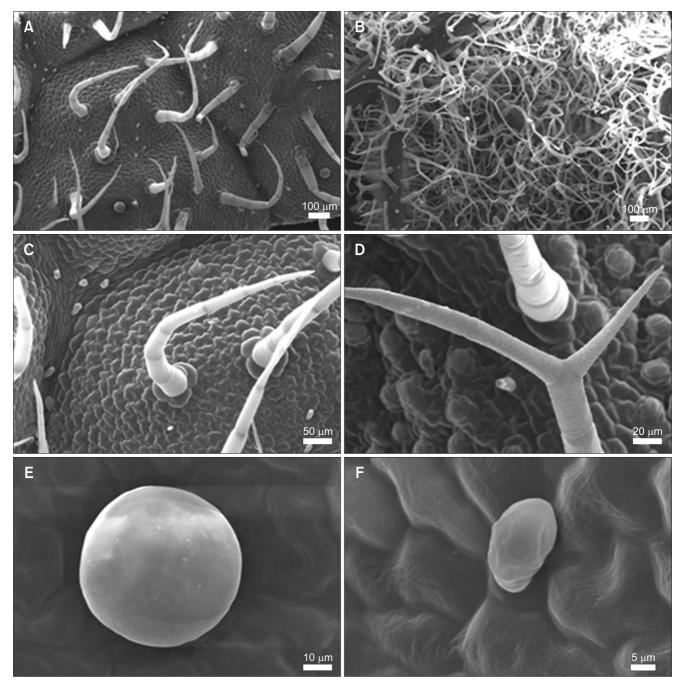


Fig. 2. Scanning electron micrographs of *Mentha suaveolens* leaves. (A) Large peltate trichomes and small capitate trichomes are shown on adaxial surface. (B) Huge number of non-glandular trichomes are present on abaxial surface. (C) Some non-glandular trichomes composed of seven stalk cells on adaxial surface. (D) A branched non-glandular trichome is visible on abaxial surface. (E) Close-up of a peltate trichome showing its bicellular head. (F) A young capitate trichome having the slightly wrinkled head.

DISCUSSION

Trichomes distribution is uniformly not distributed over the leaf surface. There are approximately twice as many glandular trichomes produced on abaxial surface than adaxial surface, and more glandular trichomes are produced within the basal and middle regions of leaf surface than the apical region (Turner et al., 2000). Leaves of *M. spicata*, *M. suaveolens*, and *M. piperita var* were commonly characterized by an abundance of non-glandular trichomes and two glandular trichomes-small capitate and large peltate glandular trichomes. However, they differed from each other in



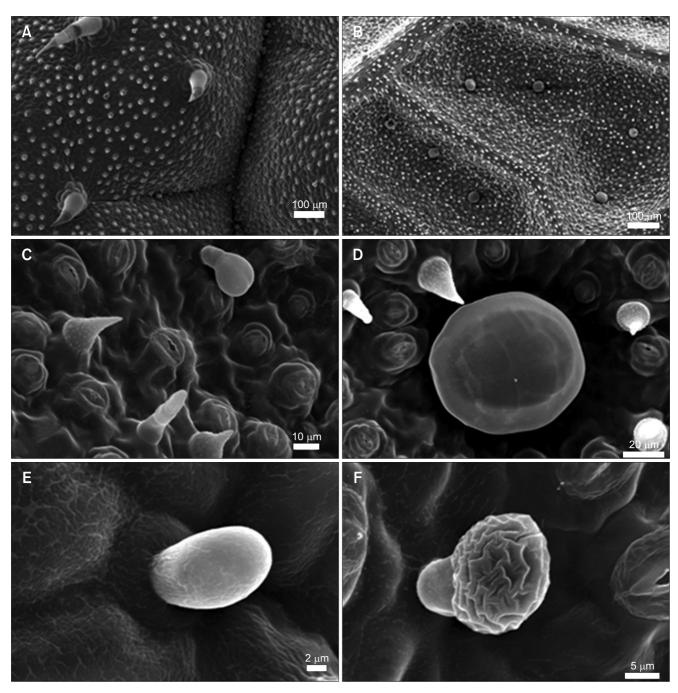


Fig. 3. Scanning electron micrographs of *Mentha piperita var* leaves. (A) Adaxial surface showing long and short non-glandular trichomes and scattered glandular trichomes. (B) Abaxial surface showing glandular trichomes and non-glandular trichomes. (C) Small capitate trichomes and short non-glandular trichomes are observed between prominent stomata. (D) A big peltate trichome is transparently shown the secretory cells in the cavity. (E) An early developmental stage of capitate trichomes is shown without distinctive head. (F) A capitate trichome showing its wrinkled surface of head.

morphology, density and location on leaves. Two types of non-glandular trichomes, simple unbranched and v-shaped branched trichomes; capitate trichomes, with an one-celled apical head of secretory cells.

Peltate and capitate trichomes appear with a variable number of head cells, ranging from four in *Leonotis leonurus* (Ascensao

& Pais, 1998), to eight in *Mentha piperita* (Turner et al., 2000) and *Zeyheria montana* (Machado et al., 2006), to eighteen in *Micromeria fruticosa* (Werker et al., 1985). Peltate trichomes of Lamiaceae have common characteristics in structure and morphology across genera. They usually consist of a vacuolate basal cell with a short stalk and a cytochemically dense head



with secretory cells (Turner et al., 2000; Machado et al., 2006). In Lamiaceae species, most of plants have the glandular trichomes with 8~14 secretory cells. The arrangement of head cells are often classified into two types, according to circle number. In *Leonotis* and *Ocimum*, a small number of head cells are arranged in a single circle (Ascensao & Pais, 1998; Werker et al., 1993). In other species of Lamiaceae such as *Origanum* (Bosabalidis & Tsekos, 1984), *Satureja* (Bosabalidis, 1990), and *Salvia* (Baran et al., 2010), however, the secretory cells are typically arranged in two concentric circles.

Peltate and capitate glandular trichomes on *Mentha* leaves expectedly showed the similar structural features with those of other plants. In *M. spicata*, however, peltate trichomes were absent on both surfaces. A head of peltate trichomes was made up of 8~10 secretory cells, which were arranged in a single circle. Furthermore, they had common characteristics at subcellular level including dense cytoplasm, numerous mitochondria, plastids with plastoglobuli, and well-developed endoplasmic reticulum.

Leaves of M. suaveolens, M. piperita var were characterized by abundance of two glandular trichomes-small capitate and large peltate glandular trichomes. M. spicata, however, it mainly possessed capitate glandular trichomes on their leaf surface. Capitate glandular trichomes were presented on these species. M. spicata, M. suaveolens, M. piperita var, were consisted of one-celled head and attached to one-celled long stalk. Capitate glandular trichomes were distributed mostly on abaxial more than abaxial surfaces. Peltate glandular trichomes were observerd on two species except M. spicata. M. piperita var consisted of a large eight-celled head, with an enlarged secretory cavity and attached to a one-celled short stalk. M. suaveolens were consisted of a large six-celled head, with an enlarged secretory cavity, attached to a one-celled short stalk. Distribution of peltate glandular trichomes were found more in abaxial surfaces than in adaxial surfaces like

capitate glandular trichomes.

CONCLUSIONS

The trichomes on leaves of *M. spicata*, *M. suaveolens*, and *M.* piperita var were studied by scanning electron microscopy. Simple non-glandular trichomes and peltate glandular trichomes are distinctively occurred on these leaves of Mentha species. Long and short non-glandular trichomes were abundantly located on the abaxial surface of M. spicata which were ranged 10~490 µm in height. Most of capitate glandular trichomes were present on abaxial surfaces. The mature capitate trichomes were 44 μm in height and 27 μm in diameter at a globose head. However, any peltate glandular trichomes were not observed on adaxial or abaxial surface. Unbranched non-glandular trichomes were sharp, pointed trichomes, however, branched, v-shaped non-glandular trichomes occurred on leaves of M. suaveolens. Non-glandular trichomes were identified with simple unbranched and branched. The length was 380 µm and the diameter of distal region was 52 μm. Two types of glandular trichomes, sessile peltate and stalked capitate, were appeared on abaxial surface. Peltate glandular trichomes each consisted of a large eightcelled head with an enlarged secretory cavity, attached to a two-celled short stalk. The stalks had a length of 10 μm . Small capitate glandular trichomes consisted of a cylindrical head attached to the leaf with a one-celled uniseriate stalk. They were measured 24 μm in height and 13 μm in diameter. Single non-glandular trichomes and peltate glandular trichomes were observed on adaxial surface. A small capitate glandular trichome consisted of a globose unicellular head attached to leaf with a two- or three-celled uniseriate stalk. A stalk had a length of 22 µm and a head had a diameter of 15 μm. Peltate trichomes were distinctly present on abaxial surface, whereas they were not observed on adaxial surface.

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