ESEM Comparative Studies of Hop (*Humulus lupulus* L.) Peltate and Bulbous Glandular Trichomes Structure

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Summary

Hop glandular trichomes are developing on the epidermis of hop cones bracts. The anticline cross cut of hop peltate glandular trichomes in Environment Scanning Electron Microscopy (ESEM) observations confirmed spheroid shape of cells. In our research, length of internal peltate glandular trichomes cells varied from 16.00 to 21.47 μm, width from 4.21 to 4.70 μm and volume from 1.19×10^{-6} to 1.99×10^{-6} mm³. The diameter of cell walls varied from 2.11 to 2.94 μm. Volume of observed peltate glandular trichomes varied from 1.65×10^{-2} to 1.95×10^{-2} mm³. The cell structure of bulbous glandular trichomes in anticline cross cut was not observed. According to 121 observations, bulbs formation was visible on the surface of peltate glandular trichomes in the last phase of morphogenesis. Biosynthesis of hop secondary metabolites and activation of their genetic mechanisms are the most intensive at the end of technological maturity of hop cones of hop cultivar Aurora. It is possible that bulbous glandular trichomes represent the final stage of peltate glandular trichome morphogenesis, instead of being separate morphological formations. During biosynthesis of hops secondary metabolites partial pressures of liquids and gasses increase causing breakage of cell walls, which withdraw towards tunica and form papillary texture.

Key words

hop, *Humulus lupulus*, ESEM, peltate glandular trichomes, bulbous glandular trichomes, biosynthesis of hop secondary metabolites

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Introduction

Hop glandular trichomes are developing on the epidermis of hop cones bracts. Two types of hop glandular trichomes – peltate and bulbous were described by Oleveira and Pais (1988, 1990). Mentioned authors found that fully developed peltate trichomes are built of a glandular head consisting of 30 to 72 cells, four stalk cells and four basal cells. On the other hand, bulbous trichomes were also formed from a protodermal cell by an anticlinal division, followed by two periclinal ones which produce the initials of the glandular head cells, and the basal and stalk cells. Moreover, according to the same authors, fully developed bulbous trichomes consist of four (occasionally eight) head glandular cells, two stalk cells and two basal cells. The density of peltate trichomes decreased with the expansion of the leaves. However, both glandular trichomes, also referred to as secretory or peltate trichomes, are lypophilic glands comprising a group of secretory cells and a cuticle-enclosed cavity that fills with the secreted compounds (Oliveira and Pais, 1990; Saito et al., 1995; Kim and Mahlberg, 2008; Wang et al., 2008). The plastids in glandular trichomes have less-defined membrane structures than chloroplasts and may be associated with synthesis and/or secretion of secondary metabolites, such as terpenoids and flavonoids (Oliveira and Pais 1990; Wang et al., 2008). There are seven morphological development stages of peltate glandular trichomes and also the relationship between their morphogenesis and accumulation of secondary metabolites was well described (Saito et al., 1995; Hirosawa et al., 1995; Čeh et al., 2007; Nagel et al., 2008; Wang et al., 2008). However, development of lupulin glands is strictly divided into a growth phase and biosynthetic-secretory phase.

After slightly detaching of cuticle from the glandular head cells there was a strong activity of VPS gene, encoding valerophenone synthase that is involved in the first steps of bitter resins (α-acids) biosynthesis (Sugiyama et al., 2006). The biosynthesis of hop secondary metabolites is divided into three biosynthetic pathways A, B and C (Wang et al., 2008) (Fig.1). VPS gene is active in C pathway of biosyntesis of terpene derived hop natural compounds. It is important to point out that the final step of C pathway is biosynthesis of humulone or α-acids, which is the most important hop compound (Nagel et al., 2008; Wang et al., 2008). The accumulation of humulone or α-acids is most intensive in third and fourth week after hop flowering (Wang et al., 2008).

The aim of this research was to provide the comparative studies of hop peltate and bulbous glandular trichomes in order to find the changes in their morphology and structure from the beginning till the end of glandular trichomes morphogenesis.

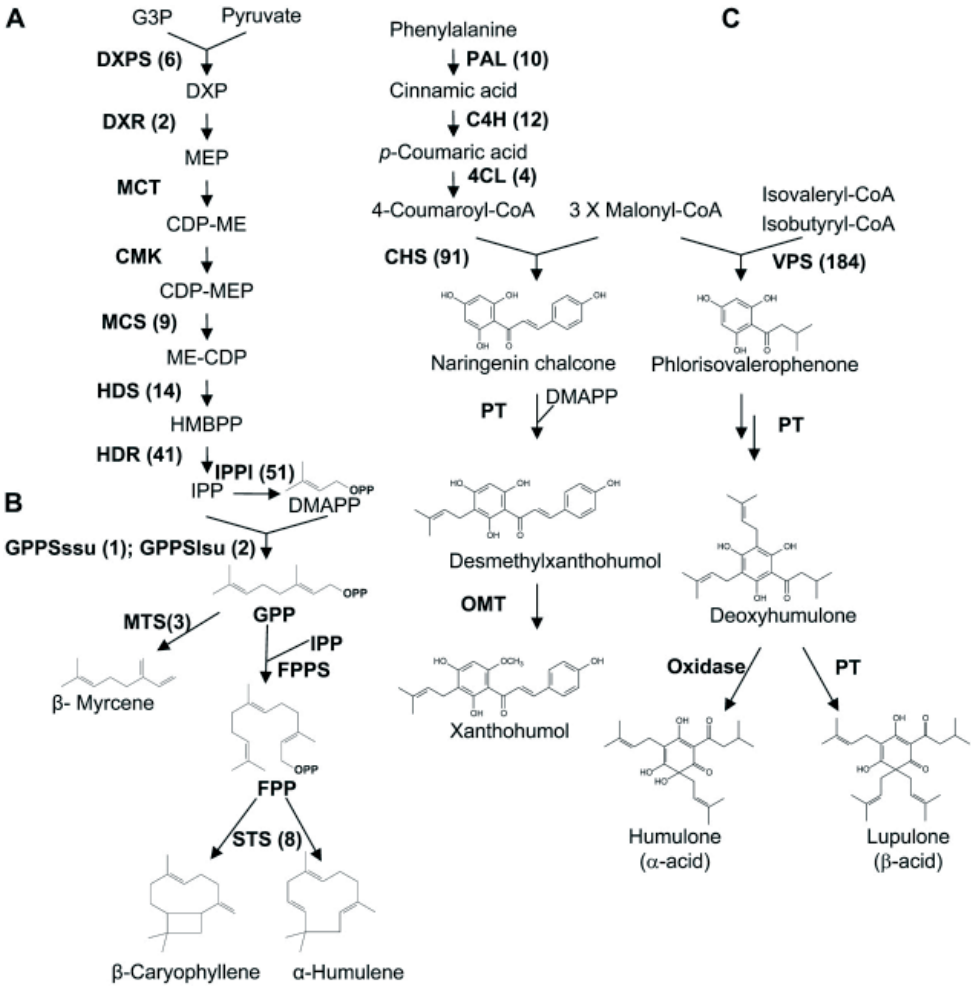

Figure 1.
Biosynthetic pathways for terpene-derived natural products found in hop trichomes, showing EST abundance in the hop glandular trichome cDNA library (Wang et al., 2008)

Materials and methods

Research was carried out in the hop garden of Hop Co-operative in Gregurovec (northwest part of Croatia), during vegetation year of 2009. Considering the weather conditions during the hop vegetation, the year of 2009 was completely normal in the comparison with data of multiyear climatological monitoring. From the beginning of technological maturity (August 10) till the end of hop harvest (September 15), five samples of hop cones were collected from hop plants of cultivar Aurora. The samples were collected from upper parts of the same plants. The 121 ESEM observations of hop glandular trichomes morphology and structure were provided on bracts separated from central string (*rachis*) of the chosen hop cones, which were of approximately equal length and diameter. The cross sections of peltate glandular trichomes were made with surgical scalpel (for neurosurgery operations). In ESEM studies, Philips XL 30 ESEM (detector: Edax, type PV 9760/68 ME, resolution 134.30 eV and BSE detector: Philips PW 6848/00) and software EDAX Genesis v.5.21 were used. Photographs were taken at an accelerating voltage of 25 kV under recording time of 5 seconds and diameter of observed area was 10 mm.

Volume of peltate glandular trichomes and their cells was calculated by following equation for volume calculation of spheroid bodies (1):

$$V = \frac{4}{3}\pi \cdot a^2 b \qquad (1)$$

a= width (distance between two points on x-axis in µm)
b= height (distance between two points on y-axis in µm)

Results and discussion

It was obvious that phases of morphogenesis (Fig. 2) in general correspond with the results of Saito et al. (1995) and Hirosawa et al. (1995).

Volume of observed peltate glandular trichomes varied from 1.65×10^{-2} mm³ to 1.95×10^{-2} mm³, depending on phase of morphogenesis. The cells of hop peltate glandular trichomes in anticline cross cut had spheroid shape. Volume of internal peltate glandular trichomes cells varied from 1.19×10^{-6} to 1.99×10^{-6}

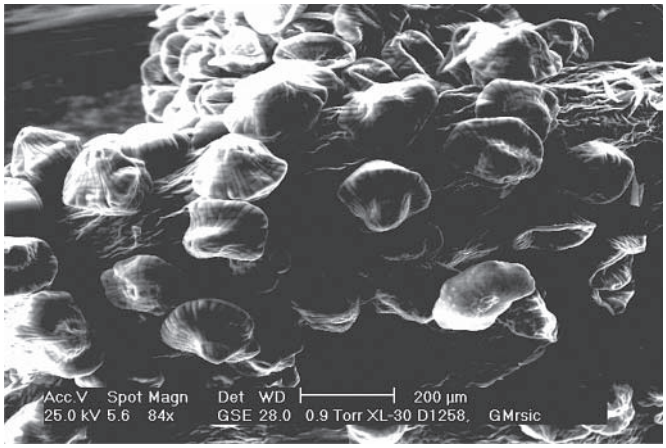

Figure 2. Hop peltate glandular trichomes in different phases of morphogenesis (magnification 84×, bar = 200 µm)

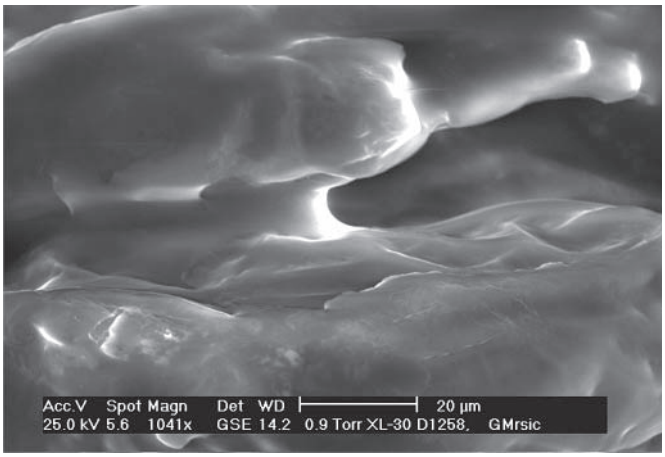

Figure 3. Anticlinal cross cut of peltate glandular trichome (magnification 1041×, bar = 20 µm)

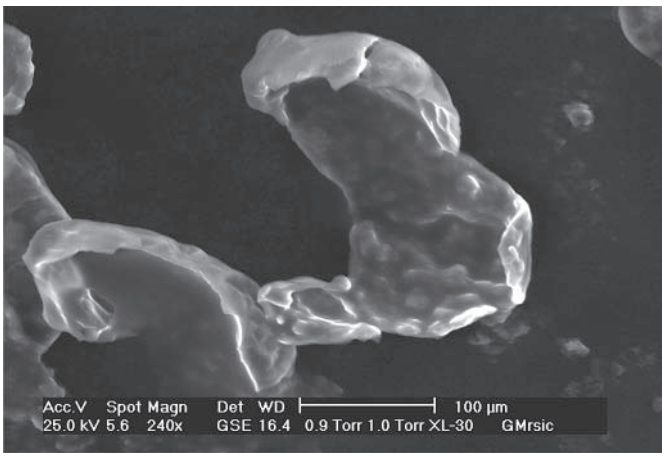

Figure 4. Anticlinal cross cut of bulbous glandular trichome (magnification 240×, bar = 100 µm)

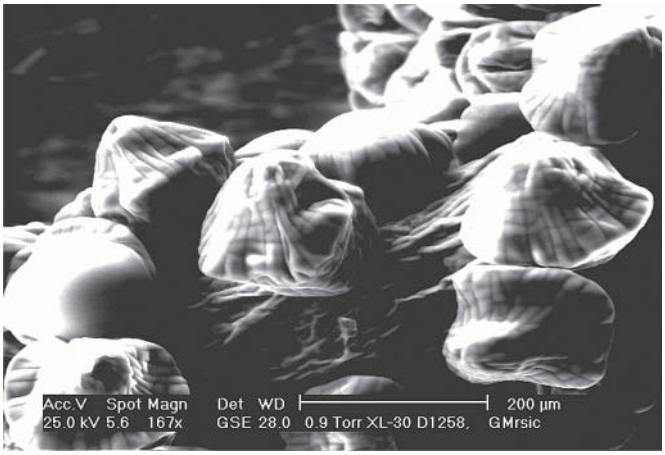

Figure 5. Formation of bulb on peltate glandular trichome (on left side of microphotography) in the last phase of morphogenesis (magnification 167×, bar = 200 µm)

mm³. Length of peltate glandular trichomes cell lumen varied from 16.0 to 21.47 μm, width varied from 4.21 to 4.70 μm and diameter of cell walls varied from 2.105 to 2.94 μm (Fig. 3).

On the other hand, in the comparison with peltate glandular trichomes, bulbous glandular trichomes in their anticline cross cut were empty, i.e. there was no visible cell structure of their interior (Fig. 4).

However, after 121 observations formations of bulbs on the surface of peltate glandular trichomes in the last phase of morphogenesis were visible (Fig. 5).

Considering biosynthesis pathways of hop secondary metabolites (Fig. 1) as well as genetic mechanisms that cause it, it is well known that biosynthesis of secondary metabolites is most intensive on the end of technological maturity of hop cones (Sugiyama et al., 2006; Čeh et al., 2007; Nagel et al., 2008; Wang et al., 2008). So it is possible that bulbous glandular trichomes are not separated morphological formations, but they represent the final stage of peltate glandular trichome morphogenesis.

The increase of partial pressure of liquids and gasses during the biosyntheses of hop secondary metabolites caused the breakage of cell walls, which withdrew to the internal side of tunica making the papillary texture of bulbous glandular trichomes interior visible. However, this hypothesis has to be confirmed by the future researches.

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