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BRIEF COMMUNICATION

Morphology and glandular activity of unicellular trichomes of *Epilobium hirsutum*

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Abstract

The unusual glandular trichomes of willow-herbs (*Epilobium hirsutum* L.) were investigated. They are unicellular, without a specialized basal cell at the layer of the epidermis. They have a cutinized cell wall, a prominent and heavily cutinized wall thickening at the base, and a protruding pore on the top. Among other compounds they contain several flavonoids, *e.g*. quercitrin and myricitrin. Flavonoids were localized inside the upper part of the trichome cell and most likely were not excreted through the trichome pore.

Additional key words: epidermis, flavonoids, willow-herbs.

The aerial parts of many plants, including the willow-herb (*Epilobium* sp., family *Onagraceae*), are covered with non-glandular and glandular trichomes. Although the glandular trichomes associated with *Epilobium* species have been known for a long time (Haussknecht 1884), their glandular activity, though not the chemical composition of their exudates, has been shown only recently (Strgulc Krajšek *et al.* 2006). Glandular trichomes in different plants synthesize various chemical substances, including different phenolics, *e.g*. phenolic acids, flavonoids, tannins, lignans (reviewed in Spring 2000). Flavonoids such as 3-*O*-glycosides of quercetin, myricetin and kaempferol (Ducrey *et al.* 1995), and a macrocircular dimeric ellagitannin oenothein B (Ducrey *et al.* 1997) have been detected in dried fragments *Epilobium hirsutum, E. parviflorum, E. montanum, E. roseum* and *E. collinum* and used in folk medicine. However, it is not known in which willow-herb tissue those flavonoids are synthesized and/or stored (Yang *et al.* 2008). In this communication we report the structure of the unicellular glandular trichomes of *Epilobium hirsutum*. In addition, we examined if the flavonoid compounds are localized in the glandular trichomes and/or their secretory products.

Stems, leaves, inflorescences and fruits of *E. hirsutum*, whose epidermes were densely covered with short and blunt unicellular glandular trichomes, were collected in summer seasons in Ljubljana, Slovenia. Voucher specimens are deposited in the Herbarium LJU (ID 10020418). The morphology of their glandular trichomes was examined with an *AxioImager Z1* light microscope (*Carl Zeiss*, Jena, Germany) equipped with a system for the acquisition of optical sections *Apotome*, *DIC* optics, and an *AxioCam MRm* monochrome digital camera or an *AxioCam HRc* colour digital camera (*Carl Zeiss*), and with scanning electron microscopy (*JEOL 840A*, Tokyo, Japan). If not stated otherwise, plant material was fixed and stained as described elsewhere (Kladnik *et al*. 2004). For scanning electron microscopy, fresh pieces of leaf, stem and fruit tissue were fixed in 1 % glutaraldehyde and 0.4 % formaldehyde in 0.1 M cacodylate buffer for 2 h. Samples were washed with 0.1 M cacodylate buffer, dehydrated in an ethanol series, critical-point dried using a *Balzers CPD 030* (*BAL-TEG AG*, Germany) critical point dryer, and coated with gold.

The willow-herb glandular trichome consisted of a single cell, which was wedged among surrounding epi-

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dermal cells and protruded above the surface (Fig. 1*A*-*C*). The relatively large nucleus was positioned at the lower centre of the cell (Fig. 1*B*). At the epidermal level, the trichome cell was rounded (Fig. 1*D*). As shown by staining with a saturated solution of Sudan IV in 85 % ethanol and observing under blue radiation excitation (450 - 490 nm), the trichome has a prominent and heavily cutinized wall thickening (Fig. 1*C*). The trichome apex was also cutinized and had a protruding pore on the top (Fig. 1*C*,*E*). The glandular trichomes of *E. hirsutum* differed from the trichomes of most other plants in the simplicity of their structure. Among the glandular trichomes described in the literature, unicellular structures are rare. Moreover, when the secretory cell is unicellular, the surrounding epidermal cells form a stalk in which the lowermost part of the secretory cell is embedded, as in the stinging trichomes of *Urtica*, where the epidermal cells surrounding the emergence form a sheath around the base of the hair (Uphof 1962), or in the trichomes of *Fagonia* (Fahn and Shimony 1996).

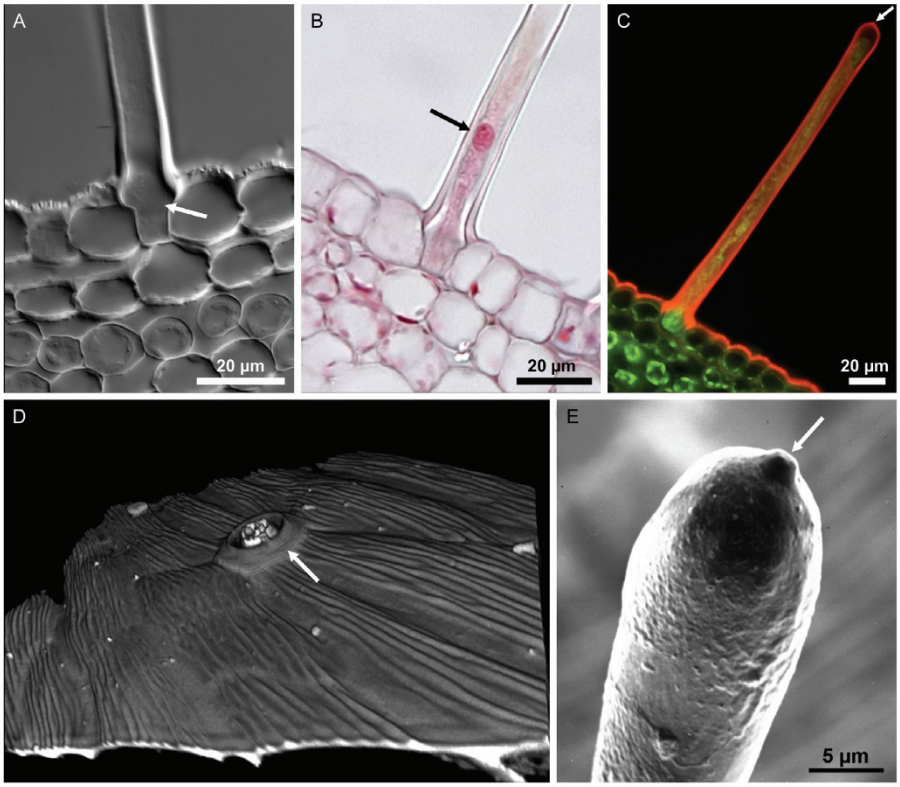

Fig. 1. Morphology of glandular trichomes of *E. hirsutum*. *A*,*B*,*C* - Transversal section of a stem with glandular trichomes. *A* - The base of a unicellular glandular trichome is wedged among surrounding epidermal cells (*arrow*) and protruded above the surface; DIC optics. *B* - Feulgen stained section; nucleus is indicated by *arrow*. *C* - The cutinized trichome wall with a pore on the apex (*arrow*) and heavily cutinized wall thickening at the base; Sudan IV stained section observed with blue light excitation. D - Surface of the stem epidermis and base of the trichome cell with a prominent and heavily cutinized wall thickening; reconstruction from the optical sections. *E* - Protruded pore on the glandular trichome apex (*arrow*); SEM micrograph.

The secretion of glandular trichomes was observed with a stereomicroscope (*Stemi SV 11*, *Carl Zeiss*) equipped with a colour digital camera (*AxioCam MRc*) on fresh cuts of leaf, stem and fruit material. The small droplets were visible on the surface of the trichome apices (Fig. 2*A*). To test if the glandular trichomes secrete flavonoic compounds, plant material was sprayed with 1 % aminoethyldiphenylborinate in methanol (*Naturstoff-reagenz A*, *Sigma-Aldrich*, St. Louis, USA) (Jork *et al.* 1989) and observed under excitation by blue radiation (450 - 490 nm) (Agati *et al.* 2002). In the trichome exudates no fluorescence that could be related to the flavonoids was detected (Fig. 2*A*). However, staining with the *Naturstoffreagenz A* of the fixed material revealed flavonoids inside the upper part of the trichomes as a yellow-orange emission (Fig. 2*B*).

The composition of the trichome flavonoids were analyzed with a thin-layer chromatographic (TLC) system. The TLC plates (10 × 10 cm) covered with 0.2 mm silica gel were developed using mobile phase for flavonoid glycosides (ethylacetate : glacial acetic acid : formic acid : water, 100:11:11:27) (Wagner *et al.* 1983). Plates were air dried, sprayed with 1 % *Naturstoffreagenz A* and observed under UV radiation at 365 nm. In order to obtain only those drops which oozed from the trichome pores, twenty 1.5 cm long stem and fruit pieces were subsequently very gently pressed to the starting position of lane 1 at the TLC plate (Fig. 2*C*). The results

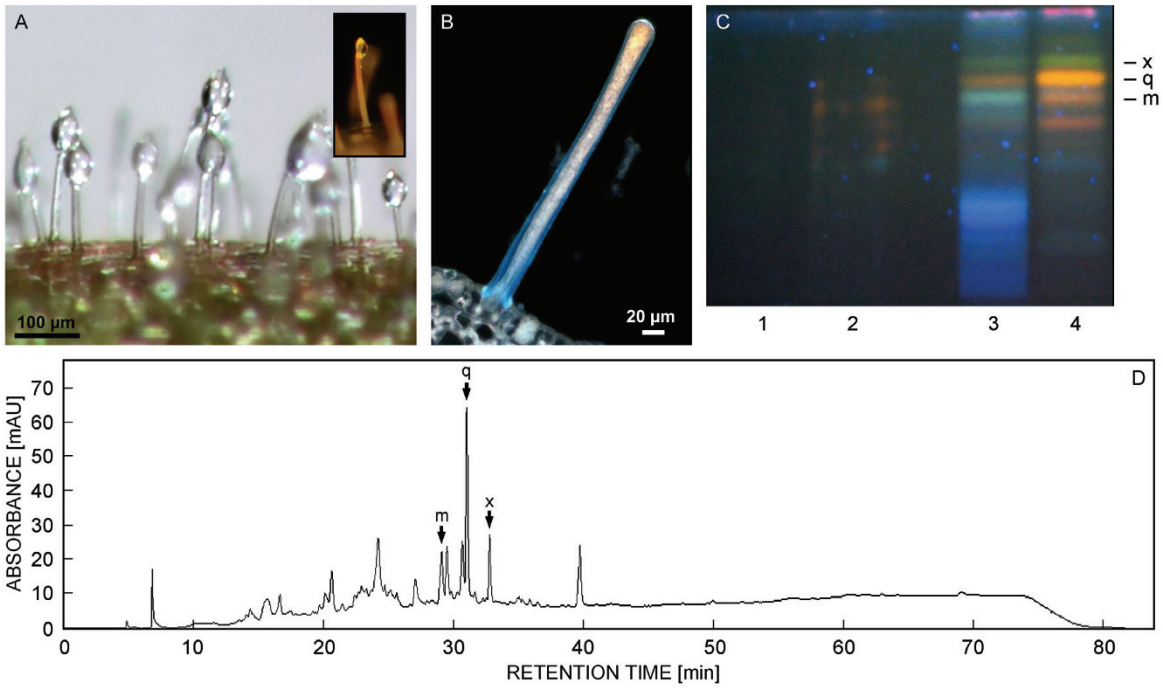

Fig. 2. Flavonoid compounds of the glandular trichomes of *E. hirsutum*. *A* - Fresh stem epidermis with glandular trichomes and secreted droplets; stereomicroscopy. *B* - Transversal section of a glandular trichome on the fruit epidermis after reaction with *Naturstoffreagenz A* observed under blue radiation excitation. *C* - *Naturstoffreagenz A* sprayed TLC plate; *lane* 1: trichome exudates, l*ane* 2: trichome exudates mixed with the trichome content, *lane* 3: surface tissue extract A, *lane* 4: surface tissue extract B. *D* - HPLC spectra of sample B; q - quercitrin, m - myricitrin and x - a compound associated with authentic quercitrin.

confirmed the stereomicroscopic observations, since no flavonoid compounds were detected in the samples. At lane 2 (Fig. 2*C*) an additional twenty 1.5 cm long stem pieces were pressed firmly. The resulted faint flavonoid signal might be associated with the trichome contents released after damage caused by pressing the tissue on the TLC plate. For flavonoid extraction, 10 fresh shoots (approx. 20 g) were immersed in 150 cm^3 of 96 % ethanol for a few seconds (designated as sample A1), the excess alcohol was shaken off, re-immersed in a second 150 cm^3 portion of 96 % ethanol for a few seconds (designated as sample A2) and the ethanol was shaken off again. The shoots were then immersed in a third 150 cm^3 portion of 96 % ethanol for 1 min (designated as sample B). Samples A1 and A2 were combined and designated as sample A. Ethanol extracts were filtered through filter paper prior to further analysis. 90 cm^3 of sample A and 60 cm^3 of sample B were separately dried by a rotary evaporator and each diluted in 1 cm^3 of 96 % ethanol. The samples were centrifuged for 5 min at 9 000 *g* to remove insoluble components. 0.05 cm^3 of samples A and B were loaded at the TLC plate (Fig. 2*C*). The fractional ethanol extraction resulted in the different chemical composition of samples A and B. In sample A different phenolic acids were detected than in sample B. Comparison with authentic standards showed the occurrence of quercitrin, myricitrin and a small amount of an unidentified compound that was also present as an impurity in standard quercitrin (Fig. 2*C*). Both, quercitrin and the unidentified compound were also present in sample A. The sample at lane 3 contained some flavonoids that were identical to those in sample B, including myricitrin. Myricitrin, quercitrin and the unidentified compound that was also present as an impurity in standard quercitrin were additionally confirmed by the HPLC analyses of sample B (Fig. 2*D*). The applied HPLC system (*Knauer*, Berlin, Germany) consisted of a *K501* pump and a *K2500* detector. The column was *Kromasil 100 C8* (15 cm × 4.6 mm) (*Bia Separations*, Ljubljana, Slovenia) and the eluents were 0.1 % trifloracetic acid in water (A) and 0.5 % trifloracetic acid in methanol : acetonitrile (1:9) (B). The gradient used was 0 - 100 % B over 50 min at a flow rate of 0.7 cm^3 min^{-1}.

The obtained results were in accordance with our hypothesis that the flavonol glycosides previously reported in *Epilobii herba* drug and *E. hirsutum* extracts (Ducrey *et al.* 1995, Toth *et al.* 2006) are the product of trichome activity. The frequent localization of phenolic compounds in the trichomes (Heinrich *et al.* 2002, Tattini *et al.* 2000, 2005, Fico *et al.* 2007) suggests their roles as feeding deterrents or defensive products, shielding plants from the damaging effects of UV radiation (Croteau *et. al.* 2000, Tattini *et al.* 2000, 2005, Kostina *et al.* 2001, Rautio *et al.* 2002). The natural habitats of *E. hirsutum* are open, sunny sites. Accordingly, the distribution of trichomes on the upper, most exposed parts of *E. hirsutum* and their flavonoid content may indicate the

protective roles of these structures. Additionally, although it has not been shown that the antimicrobial and antifungal activities of *Epilobium* extracts are directly linked to the flavonoids occuring in the *Epilobii herba* drug (Ducrey *et al.* 1995), the possibility of a defensive function against putative pathogens of trichome flavonoids, localized mainly in the vulnerable floral parts, could not be ignored.

References

Agati, G., Galardi, C., Gravano, E., Romani, A., Tattini, M.: Flavonoid distribution in tissues of *Phillyrea latifolia* L. leaves as estimated by microspectrofluorometry and multispectral fluorescence microimaging. - Photochem. Photobiol. **76**: 350-360, 2002.

Croteau, R., Kutchan, T.M., Lewis, N.G.: Natural products. - In: Buchanan, B.B., Gruissem, W., Jones, R.L. (ed.): Biochemistry and Molecular Biology of Plants. Pp. 1250-1318. American Society of Plant Physiologists, Rockville 2000.

Ducrey, B., Marston, A., Göhring, S., Hartmann, R.W., Hostettmann, K.: Inhibition of 5α-reductase and aromatase by the ellagitannins oenotein A and oenothein B from *Epilobium* species. - Planta med. **63**: 111-114, 1997.

Ducrey, B., Wolfender, J.L., Marston, A., Hostettmann, K.: Analysis of flavonol glycosides of thirteen *Epilobium* species (Onagraceae) by LC-UV and thermospray LC-MS. - Phytochemistry **38**: 129-137, 1995.

Fahn, A., Shimony, C.: Glandular trichomes of *Fagonia* L. (Zygophyllaceae) species: structure, development and secreted materials. - Ann. Bot. **77**: 25-34, 1996.

Fico, G., Rodondi, G., Flamini, G., Passarella, D., Tome, F.: Comparative phytochemical and morphological analyses of three Italian *Primula* species. - Phytochemistry **68**: 1683-1691, 2007.

Haussknecht, C.: Monographie der Gattung *Epilobium*. - Gustav Fischer Verlag, Jena 1884.

Heinrich, G., Pfeifhofer, H.W., Stabentheiner, E., Sawidis, T.: Glandular hairs of *Sigesbeckia jorullensis* Kunth (Asteraceae): morphology, histochemistry and composition of essential oil. - Ann. Bot. **89**: 459-469, 2002.

Jork, H., Funk W., Fischer W., Wimmer H.: Dünnschicht-Chromatographie, Reagenzien und Nachweismethoden. Band 1a. - VCH Verlagsgesellschaft, Weinheim 1989.

Kladnik, A., Chamusco, K., Dermastia, M., Chourey, P.: Evidence of programmed cell death in post-phloem transport cells of the maternal pedicel tissue in developing caryopsis of maize. - Plant Physiol. **136**: 3572-3581, 2004.

Kostina, E., Wulff, A., Julkunen-Tiitto, R.: Growth, structure, stomatal responses and secondary metabolites of birch seedlings (*Betula pendula*) under elevated UV-B radiation in the field. - Trees **15**: 483-491, 2001.

Rautio, P., Markkola, A., Martel, J., Tuomi, J., Härmä, E., Kuikka, K., Siitonen, A., Riesco, I.L., Roitto, M.: Developmental plasticity in birch leaves: defoliation causes a shift from glandular to nonglandular trichomes. - Oikos **98**: 437-446, 2002.

Spring, O.: Chemotaxonomy based on metabolites from glandular trichomes. - Adv. Bot. Res. **31**: 153-174, 2000.

Strgulc Krajšek, S., Dermastia, M., Jogan, N.: Determination key for Central European *Epilobium* species based on trichome morphology. - Bot. helvet. **116**: 169-178, 2006.

Tattini, M., Gravano, E., Pinelli, P., Mulinacci, N., Romani, A.: Flavonoids accumulate in leaves and glandular trichomes of *Phillyrea latifolia* exposed to excess solar radiation. - New Phytol. **148**: 69-77, 2000.

Tattini, M., Guidi, L., Morassi-Bonsi, L., Pinelli, P., Remorini, D., Degl'Innocenti, E., Giordano, C., Massai, R., Agati, G.: On the role of flavonoids in the integrated mechanisms of response of *Ligustrum vulgare* and *Phillyrea latifolia* to high solar radiation. - New Phytol. **167**: 457-470, 2005.

Toth, H.B., Balazs, A., Vukics, V., Szoeke, E., Kery, A.: Identification of *Epilobium* species and willow-herbs (Onagraceae) by HPLC analysis of flavonoids as chemotaxonomic markers. - Chromatography **63** (Suppl.): S119-S123, 2006.

Uphof, J.C.T.: Plant hairs. - In: Zimmermann, W., Ozenda, P.G. (ed.): Encyclopedia of Plant Anatomy. Pp. 1-206. Bornträger, Berlin 1962.

Wagner, H., Bladt, S., Zgainski, E.M.: Drogenanalyse. Dünnschichtchromatographische Analyse von Arzneidrogen. Pp. 299-305. Springer-Verlag, Berlin 1983.

Yang, Y., He, F., Yu, L.J.: Dynamics analyses of nutrients consumption and flavonoids accumulation in cell suspension culture of *Glycyrrhiza inflate*. - Biol. Plant. **52**: 732-734, 2008.