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THE STRUCTURE OF THE OVARY EPIDERMIS EMITTING ODOROUS COMPOUNDS IN *Allium karataviense* REGEL

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A b s t r a c t

Many plants develop in the petal epidermis papillae emitting essential oils. In species of the genus *Allium*, papillae occur in the ovary epidermis. The aim of this study was to determine the structure of papillae in *Allium karataviense* Regel and to perform histochemical tests that would allow the components of their secretion to be investigated, mainly the presence of essential oils. Examination was performed using light and scanning electron microscopy. It was found that in conical papillae, with a height of about 83 μm, there were large cell nuclei, a thick outer wall with a layer of striated cuticle, and a varying degree of cell vacuolation, depending on the age of the ovary. Using histochemical reactions, we demonstrated neutral lipids, acid lipids and pectins to occur in the papillae. Neutral lipids are characteristic of essential oils.

Key words: *Allium* L., ovary, epidermis, micromorphology, structure, histochemistry

INTRODUCTION

The genus *Allium* includes 700 plant species. Many ornamental onions that are currently cultivated belong to the subgenus *Melanocrommyum* [1].

Allium karataviense, Turkestan onion, originates from the Turkestan region. It is a small plant reaching a height of 20–25 cm. The stem produces only two wide leaves with a bluish tinge and a narrow red margin. They are arranged almost horizontally and their length corresponds to the height of the inflorescence peduncle [2]. Due to the ornamental features of their leaves, these plants are valued in flower beds also before the flowering period. This species flowers at the end of April and in May. Its silver-pink flowers are borne in a globular umbel with a diameter up to 10 cm. The inflorescence consists of 400–500 flowers [3]. Because this species is also characterized by beautiful fruits, it is an ornament in gardens also after senescence. The bulb diameter can reach even 15 cm [4, 5]. *Allium karataviense* is recommended for rock gardens and container culture [2]. It is also used as a component of dry bouquets [6].

Many species of the genus *Allium* are a source of pollen and nectar for insects [7, 8]. Our observations also show that *Allium karataviense* flowers are visited by insects gathering pollen and nectar. Odorous substances, which are attractants for insects [9], are most frequently emitted in the flowers by the petals [10, 11]. The petal surface releasing a scent is often characterized by protrusions in the external walls of the epidermal cells or by the formation of globular or conical papillae [10, 12, 13].

Our earlier analysis of the micromorphology of the flowers of two *Allium* species (*A. aflatunense* and *A. giganteum*) showed that papillae did not occur on the tepals, but they were present on the ovary of the pistil [14, 15].

The aim of the present study was to analyse the structure of the papillae occurring on the surface of the ovary in *Allium karataviense* Regel and to evaluate their secretory properties using several histochemical tests.

MATERIALS AND METHODS

Flowering stems of *Allium karataviense* Regel (Alliaceae) were obtained from the ornamental plant

collection of the University of Life Sciences in Lublin (51°13'22''N; 22°37,56''E).

Flowers were collected for investigation at the beginning of anthesis and at full anthesis. Examination was performed by light microscopy (LM) and scanning electron microscopy (SEM). Fresh and fixed specimens (70% ethanol) were analysed by light microscopy. Cross sections of the ovary were hand made using a blade. To make the structure of the epidermal cells better visible, the specimens were stained with acidic fuchsine, aniline blue or safranin O. The following histochemical tests were also used: neutral red [16], Sudan III [16], Sudan red B [17] and Sudan black B [18] for lipids, Nile blue sulphate [19] for acid and neutral lipids, Ruthenium red [20] for acid polysaccharides, such as pectins, ferric trichloride [20] for phenolic compounds, and IKI [16] for starch.

Specimens for scanning electron microscopy were prepared following the procedure described in the paper by Żuraw et al. 2010 [15].

RESULTS

The flowers of *Allium karataviense* attract insects with their colour and fragrance. The following stand out among the colourful parts of the flower: the white-pink perianth (Fig. 1), dark yellow anthers, and the pink-green ovary (Figs 2,3). In the ovary epidermis, our attention is drawn to large-sized papillae (Figs 3,4) which reach a height of 70–90 μm (on average 82,8 μm), while the diameter of the subepidermal parenchyma cells is 39–52 μm (on average 46,1 μm).

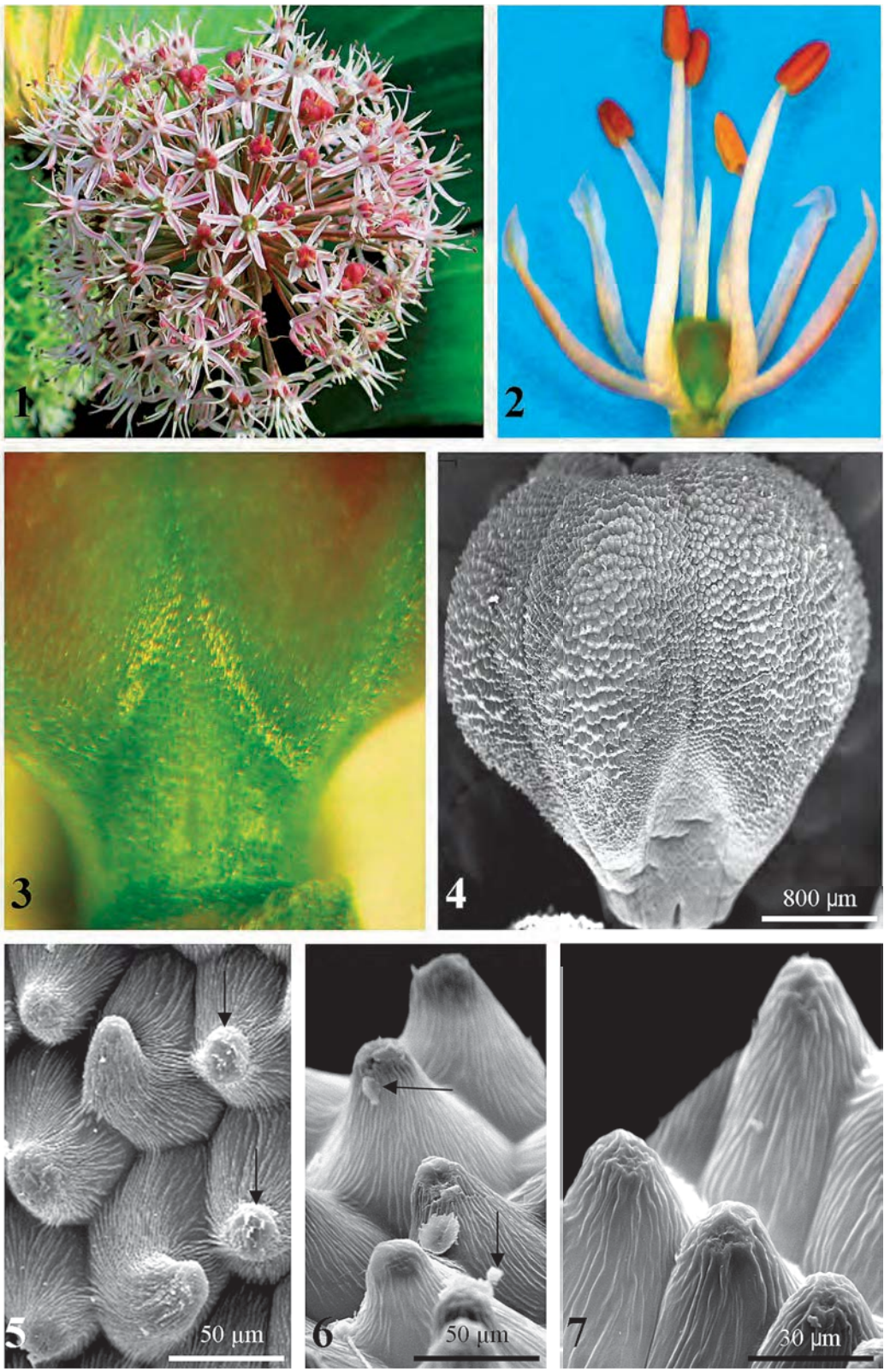
The papillae are conically shaped. Their outer walls are covered with striated cuticle (Figs 5–7) and secretion residues can be seen in many places on the surface of the apical parts of the papillae, which was recorded by scanning electron microscopy (Figs 5,6). Under a light microscope, droplets of secreted substance were observed both at the tips of the papillae and between the papillae (Figs 15, 17). The outer cell walls of the papillae had a significant thickness (Figs 8–13), with the largest dimensions in the apical part (13 μm – 17 μm). In some papillae in this region, there was a semicircular projection on the wall on the cytoplasm side (Fig. 11). The radial walls and the walls adjacent to the parenchyma cells were much thinner (Fig. 12).

The papillose epidermal cells had nuclei several times larger than the subepidermal parenchyma cells (Figs 8, 12). The nuclei intensely stained with aniline blue and basic fuchsine. At the presecretion stage and at the beginning of secretion in the papillae, the nuclei were located closer to the apical part of the cells (Figs 8 – 10, 15, 19–21), whereas after the end of secretion they were observed in the basal parts of the papillae (Figs 12, 13, 24). At the beginning of secretory activity, the epidermal cells were poorly vacuolated and were distinguished by a cytoplasm with numerous granular and vesicular structures (Figs 10, 14, 16, 17), while at the end of their secretory activity the degree of cell vacuolation significantly increased and large centrally located vacuoles were observed in them (Figs 13, 19).

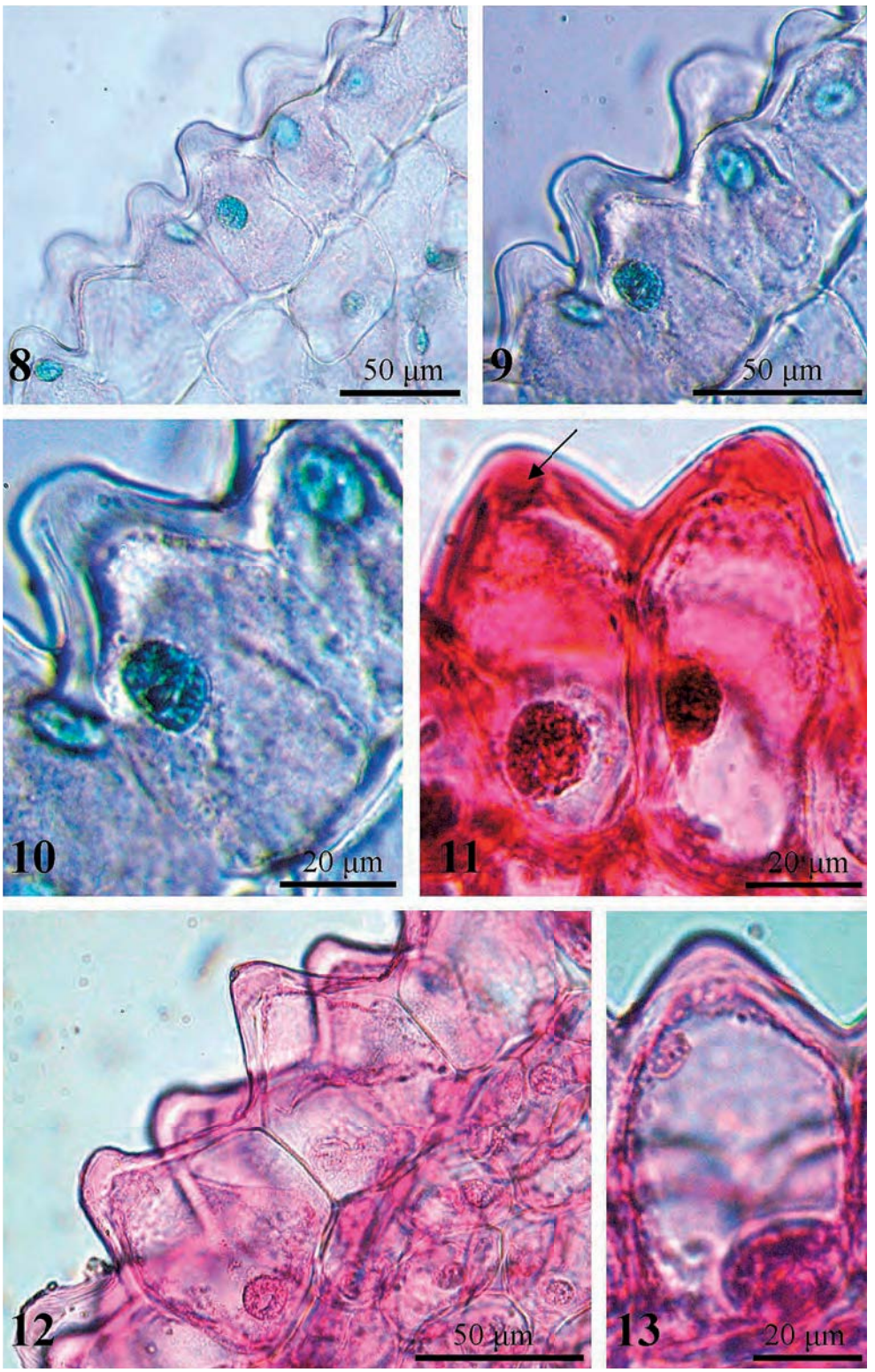
The parenchyma cells situated underneath the secretory epidermis were characterized by a quite dense arrangement (Figs 8, 10). Before the emission of the secretion, no starch was detected in them after treatment with IKI.

Treatment of the ovary tissues with Sudan III caused the cuticle (Figs 19, 22) and droplets of lipids/essential oils in the papillae and on the surface of their cell walls (Figs 20–22) to stain orange. The use of neutral red also revealed the presence of blue-green coloured droplets in the cytoplasm, which may indicate the content of lipids (Figs 16, 17). They were also visible within the cell walls and on their surface. The cell walls and the contents of the vacuole, in which irregularly shaped structures were detected, were intensely stained in one series of neutral red (Fig. 18).

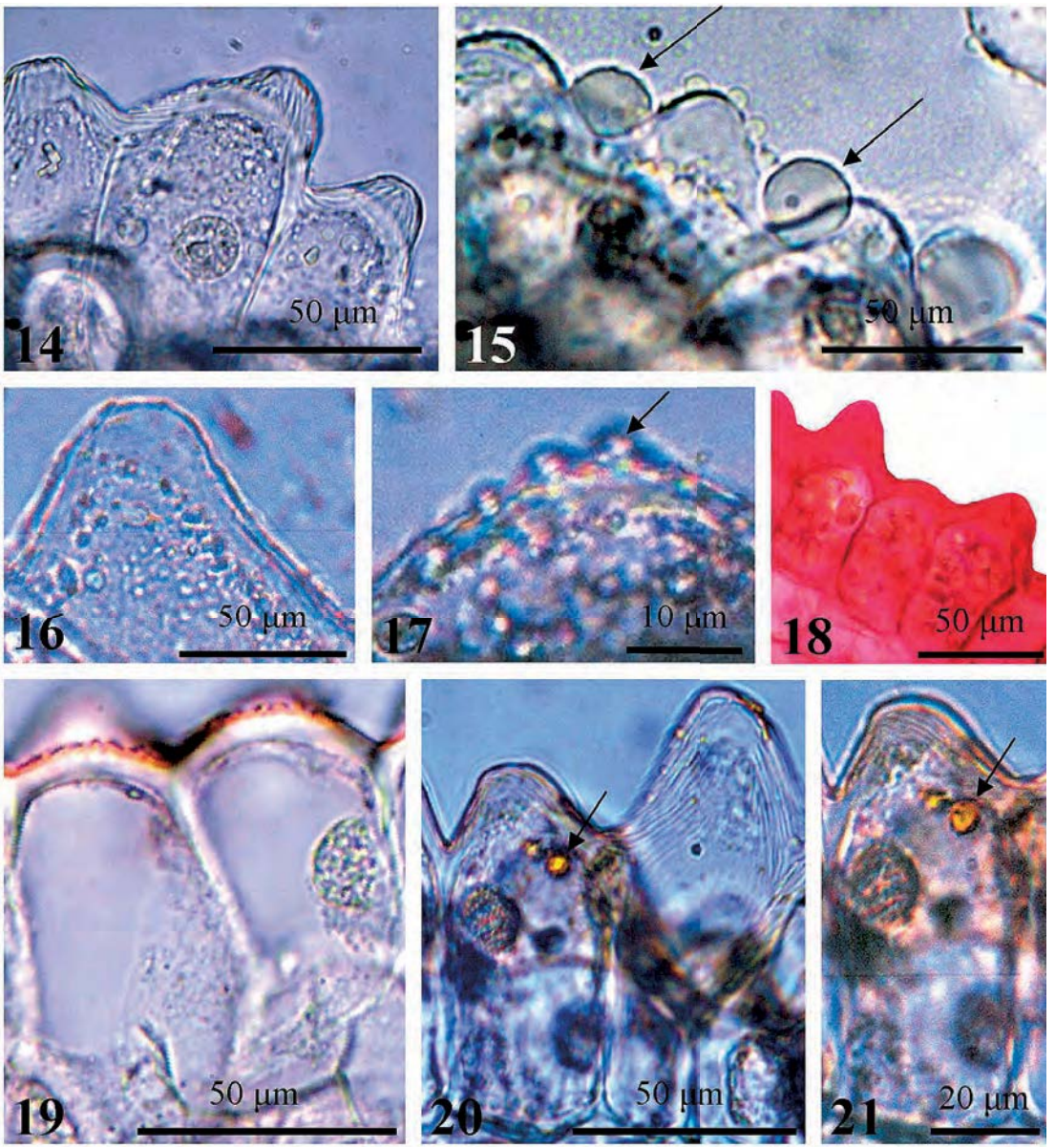
The presence of lipid substances on the cell wall surface was also shown when Sudan red B and Sudan black B were used. After treatment with Sudan red B, the cuticle and lipid droplets stained red (Fig. 22), whereas Sudan black B produced a bright blue colour in the cuticle and a dark blue colour in the lipid secretion (Figs 24, 25). The occurrence of lipids in the epidermal cells is also confirmed by the reaction of these compounds with Nile blue (Fig. 23). After this reagent was used, a pink stain was observed in the apical part of the papillae, which shows the location of neutral lipids, whereas in the basal part of the papillae and in the parenchyma cells a blue colour appeared, which is evidence of the presence of acid lipids. Treatment of the examined tissues with ruthenium red produced a red colour of the cell walls and a pink colour in the protoplasts of the papillae, which confirmed the presence of pectins and possibly gums and mucilages in them (Fig. 26). The use of iron chloride allowed us to demonstrate that in the papillae there were no phenolic compounds since these cells were not found to stain black (Fig. 27).



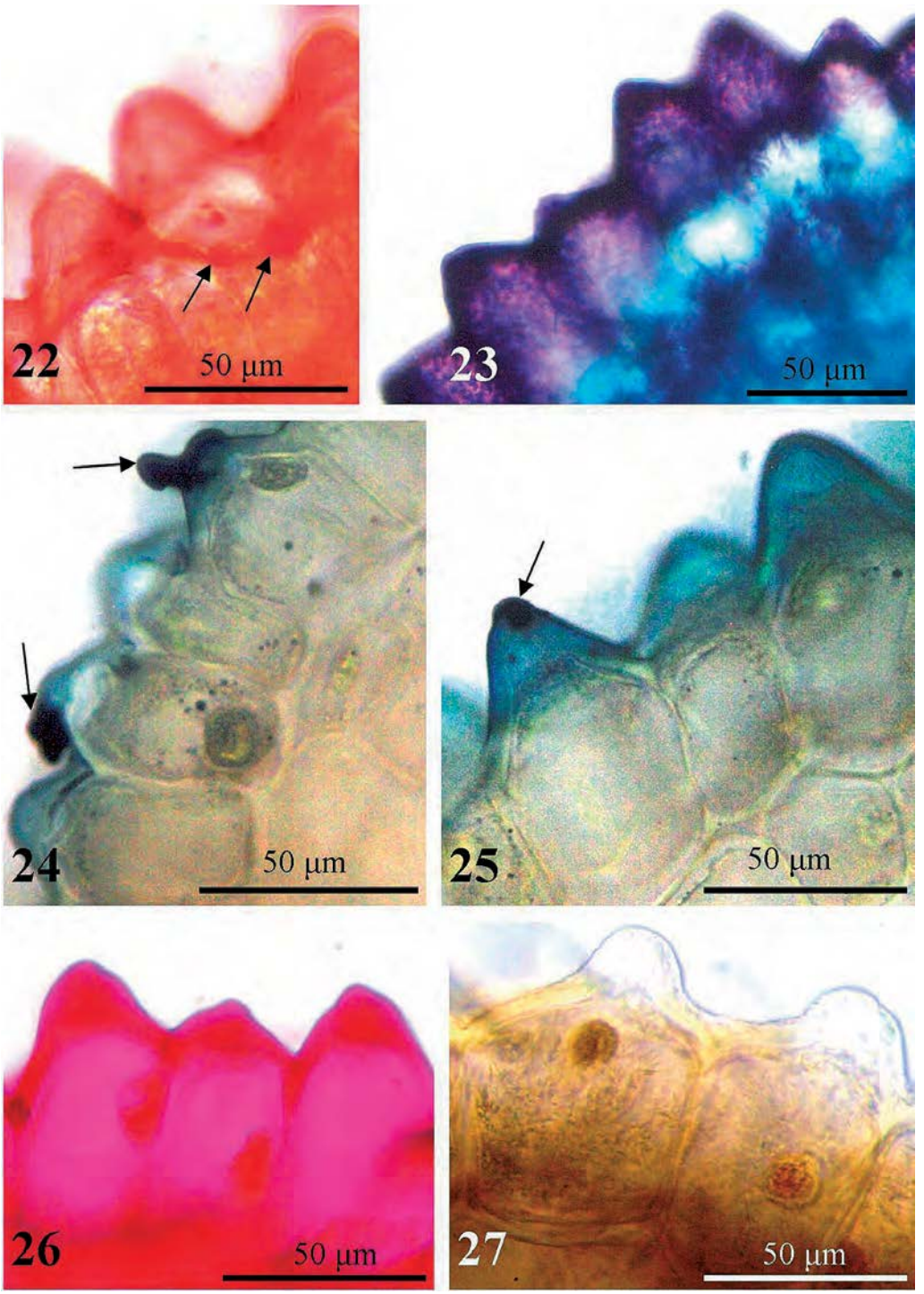
Figs 1–7. Flowers and portions of the ovary of *Allium karataviense*.
1 – Inflorescence with flowers at different age.
2 – Flower habit after removal of one tepal and stamen.
3 – Lower part of the ovary with the gynophore (LM).
4 – Ovary with visible papillae (SEM).
5, 6, 7 – Papillae in the ovary epidermis with secretion visible on the surface (arrows) (SEM).



Figs 8–13. Papillae from the ovary surface after different staining methods were used (LM).
8–10 – Specimens stained with aniline blue.
11 – Stained with safranin.
12, 13 – Stained with fuchsine.



Figs 14–21. Papillae with secretion in the ovary epidermis from fresh specimens and treated with reagents (LM).
14, 15 – Papillae in a fresh specimen, visible droplets of essential oils (arrows).
16–18 – Papillae treated with neutral red, secretion droplets marked with an arrow.
19–21 – Cells after treatment with Sudan III, orange-stained cuticle (19) and droplets of lipid/essential oil are visible (20, 21).



Figs 22–27. Papillae after histochemical tests were applied (LM).
22 – Sudan red B.
23 – Nile blue.
24–25 – Sudan black B.
26 – Ruthenium red.
27 – Ferric trichloride.
The stained secretion is marked with arrows.

DISCUSSION

Essential oils and other secondary metabolites secreted by different plant structures can perform the function of attractants that lure pollinating insects or repellents that play a major role in chemical defence against insects [9, 21].

Volatile lipids contained in oils affect insects at large distances, while non-volatile substances are important during immediate contact with an insect, which can be reflected in their development cycle [21, 22].

Papillae producing essential oils have been observed in the epidermis of petals or tepals of many plant species [10, 23, 24, 25]. Papillae have been found on the tepal epidermis in *Galanthus nivalis* [26] and *Crocus vernus* [27] as well as on the petal epidermis in *Viola ×wittrockiana* [28], *Rosa rugosa* [29], *Rosa ×hybrida* [30], and two *Petasites* species [31].

We also observed epidermal tissue emitting odorous compounds on the filaments of the stamens in *Asphodelus aestivus* Brot. [32, 33]. In this species, the epidermal cells produced different sized papillae on the staminal filaments expanded at their basal part, forming a surface characteristic for osmophores.

In the present study, we demonstrated that the epidermal cells producing papillae in the ovary of *Allium karataviense* performed secretory functions. We found the presence of lipid substances both in the protoplasts of these cells and in the cell walls and on their surface. We confirmed the lipid nature of these compounds by using histochemical tests (neutral red, Sudan III, Sudan red B, Sudan black B, Nile blue).

Our study showed that the papillae occurring in the ovary epidermis of *Allium karataviense* contained acid and neutral lipids (Nile blue treatment) as well as pectins (ruthenium red treatment). Neutral lipids, which also include essential oils, were found in the apical parts of the epidermal cells next to the papillae, which was associated with the location of their emission. On the other hand, acid lipids were concentrated in the cytoplasm of the basal part of the epidermal cells. In the studies of other authors, neutral lipids have also been recorded in the secretion of capitate trichomes emitting essential oils in different plants [34, 35].

After treatment with Ruthenium red, the papillae in *Allium* showed a colour indicating the presence of acid polysaccharides, such as pectins, both in the cytoplasm and in the cell wall. In our earlier studies, pectins were also detected in the hairs of many plants after using histochemical tests [34, 35, 36].

In the studied species, we did not find papillae on the tepals. Whitney et al. [37, 38] mention a number of functions that can be performed by the papillae on the perianth segments. Some of these functions can relate to the papillae found on the ovary of the pistil; they can facilitate pollinator grip on the flower, produce a scent and have an effect on the maintenance of proper moisture in the flower.

Essential oils, belonging to plant secondary metabolites, can show various biological activity. Insect antifeedants, feromones, defensive agents, allelochemicals, phytoalexins or signalling substances have been distinguished among them [11].

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Authors' contributions

The following declarations about authors' contributions to the research have been made: concept of the study: EW-C; crops research: HL, EP; microscopical analysis: BŻ, EW-C; writing of the manuscript: EW-C, BŻ; photographs: BŻ, EW-C.

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Struktura epidermy zalążni *Allium karataviense* Regel emitującej związki zapachowe

S t r e s z c z e n i e

Wiele roślin wytwarza w epidermie płatków papille emitujące olejki eteryczne. U gatunków z rodzaju *Allium* papille występują w epidermie zalążni. Celem pracy było określenie struktury papilli *Allium karataviense* Regel oraz przeprowadzenie testów histochemicznych w celu wykazania w ich zawartości rodzaju wtórnych metabolitów. Badania prowadzono w mikroskopie świetlnym i skaningowym elektronowym. Stwierdzono, że w stożkowatych papillach (o wysokości ok. 83 μm) występują duże jądra komórkowe, bardzo gruba zewnętrzna ściana z warstwą prążkowanej kutykuli oraz w zależności od wieku zalążni, różny stopień wakuolizacji komórek. Przy zastosowaniu reakcji histochemicznych wykazałyśmy, że w papillach występują lipidy obojętne, lipidy kwaśne i pektyny. Lipidy obojętne są charakterystyczne dla olejków eterycznych.

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