ANATOMY AND ULTRASTRUCTURE OF SPUR NECTARY OF *GYMNADENIA CONOPSEA* (L.) ORCHIDACEAE

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ABSTRACT

The anatomy and the ultrastructure of spur nectaries of *Gymnadenia conopsea* at different developmental stages were investigated. The secretory epidermis surrounded the inside of spur and formed many unicellular papillae, which significantly enlarged the secretory surface. At the activity stage the epidermal cells contained characteristic plastids with well developed intraplastidal membrane system and numerous osmiophilic globules. The contact of plastids and endoplasmic reticulum indicates a possibility of the involvement of these structures in the secretory processes. The cell wall and the cuticle did not form a barrier for the secreted nectar and no pores or cracks were visible in the cuticle covering secretory papillae.

KEY WORDS: anatomy, ultrastructure, nectary, *Gymnadenia conopsea*, Orchidaceae.

INTRODUCTION

The fragrant orchid is a species visited by moths as well as butterflies (Faegri, van der Pijl 1971). The insects are attracted by the intensive scent of the flowers and the presence of nectar filling the nectary spur. Such spur-shaped nectaries, formed by the fusion of the proximal part of labellum, appear also in another European orchids such as *Anacamptis pyramidalis* (Proctor, Yeo 1975), *Limodorum abortivum* (Figueiredo, Pais 1992), *Platanthera bifolia* (Stpiczyńska 1997), which are also pollinated by Lepidoptera. In cantharophilous (beetle-pollinated) flowers of *Coeloglossum viride* nectar is secreted in the short spur and additionally, there are two small nectar glands on the basal part of labellum. Surprisingly, the spur of *Orchis mascula* does not contain nectar at all (Proctor, Yeo 1975).

In spite of the similar origin of spur nectaries, the studies conducted so far revealed some differences in their anatomy and ultrastructure. These are probably connected with a degree of specialisation of nectary tissue and secretory processes operating there (Figueiredo, Pais 1992; Pais, Figueiredo 1994; Stpiczyńska 1997).

The aim of this study was the examination of the anatomy and ultrastructure of *Gymnadenia conopsea* nectary, with special attention paid to the changes occurring in secretory cells during different stages of their activity.

MATERIAL AND METHODS

The flowers of the fragrant orchid were collected in the first decade of July 1995-1996, from plants growing in a natural stand near Chełm.

The flowers representing four developmental stages were analysed: stage I – 2 mm bud with 1 mm spur (about 5 days before anthesis), stage II – 3 mm bud with spur of 4 mm (2 days before anthesis), stage III – flowers at the first day of anthesis, stage IV – flowers at the fourth flowering day with first symptoms of senescence.

The observations were conducted using light (LM) and electron microscope (TEM and SEM). For LM and TEM, dissected nectaries were cut with a razorblade into the fragments of about 1 mm and fixed in buffered 3% glutaraldehyde with paraformaldehyde, at room temperature, for 5 h (Karnovsky 1965). After several rinses in phosphate buffer (pH 7.0) nectaries were postfixed in 2% osmium tetroxide for 2 h at 0°C. Dehydrated material was embedded in epoxy resin (Spurr 1969). Ultrathin sections for TEM were stained with uranyl acetate followed by lead citrate (Reynolds 1963) and observed in TESLA electron microscope at 60 kV. Semithin sections for LM were stained with methylene blue – azure B and 0.05% toluidine blue O, which stains lignified walls bluish green (O'Brien et al. 1964, Hayat 1975). Some free hand sections from fresh material were treated with JKI to ascertain starch

Figs 1-4. Anatomy of nectary spur, LM.

Fig. 1. Cross section of nectary spur. Papillae of epidermal cells and numerous vascular bundles in parenchyma, $\times 85$.

Fig. 2. Fragment of spur section in distal part (plastids in secretory cell marked by an arrow), $\times 350$.

Fig. 3. Transverse section of secretory epidermis forming unicellular papillae, $\times 950$.

Fig. 4. Section of spur's proximal part, epidermis without papillae, $\times 400$.

Abbreviations to Figs 1-13

SE – secretory epidermis, CW – cell wall, SV – secretory vesicle, G – Golgi apparatus, M – mitochondrion, P – plastid, L – lipid droplet, ER – endoplasmic reticulum, V – vacuole, VB – vascular bundle

Figs 5-6. Secretory papillae at the activity stage, TEM.

Fig. 5. Plastid with osmiophilic material inside, secretory vesicles and ER membranes near cell wall, $\times 17\,000$.

Fig. 6. Close contact of ER with plastid (arrow) and secretory vesicles near plasmalemma, $\times 17\,000$.

storing and with Sudan III – for the presence of lipids or cutinized walls. Additionally, the presence of lignin was tested using of phloroglucinol with HCL (Gerlach 1972).

For SEM intact spurs fixed as above were cut each along its axis. After dehydration in ethanol series and acetone the surface of nectaries was examined with TESLA scanning electron microscope at 25 kV. Fresh, unfixed material was also investigated in SEM – the observations were conducted at the voltage 5 kV.

RESULTS

The nectary spur of *G. conopsea* was 25-30 mm long and 0.7 mm in diameter. The traces of nectar appeared inside the day before anthesis. The nectar production reached the maximum on the second day of flowering and the secretory activity was extremely high in the afternoon.

The LM observations revealed the occurrence of distinct secretory epithelium. It was formed of adaxial epidermal cells surrounding the inside of the spur (Fig. 1). Secretory cells contained a large nucleus, numerous globoid plastids,

2-3 μm in diameter, dense, intensively stained cytoplasm and usually a few small vacuoles. Significant feature of secretory epidermal cells was the formation of numerous short papillae, 20-30 μm long (Figs 2, 3). They were formed in a bud (II stage), with frequency of 190 papillae on 1 mm^2 . Papillae occurred only in the distal part of the spur – to the half of its length. Proximal nectary part was covered by smooth epidermis built of cells, which contained also large nuclei and numerous plastids (Fig. 4). Two subepidermal layers of small parenchyma cells were almost free of plastids. Some of these cells were characterized by large nucleus and dense cytoplasm and the others by thin, partially located cytoplasm and a very large vacuole. Numerous (10-12) vascular bundles were found in the subepidermal parenchyma (Figs 1, 4). In cell walls of secretory epidermis and of parenchyma lignin and cutinized layers were not detected. Parenchyma cells adjoining to the external (abaxial) epidermis did not reveal a secretory character: they were rather large cells with a few typical chloroplasts.

Plastids in secretory cells did not contain starch at any of the investigated stages. TEM observations of cells at the

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Figs 7-9. Ultrastructure of nectary cells at the activity stage, TEM.

Fig. 7. Mitochondria, short tubules of ER and secretory vesicles near cell wall, $\times 25\,500$.

Fig. 8. Secretory cells with numerous secretory vesicles and Golgi apparatus, $\times 16\,000$.

Fig. 9. Mitochondria, tubules of ER and secretory vesicles along the cell wall between neighbouring secretory epidermal cells. Plasmodesmata marked by an arrow, $\times 18\,000$.

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bud stage showed almost homogenous, electron dense stroma with a few membranes in plastids, whereas at the secretory stage, system of many membranes associated with osmiophilic globules frequently appeared (Figs 5, 6). Large portions of plastid surfaces were attached to the endoplasmic reticulum (ER). Short tubules of ER and mitochondria were present especially near the cell walls of secretory papillae (Fig. 7). The walls in these places irregularly protruded, but typical ingrowths did not occur. Numerous dictyosomes were present in the basal part of secretory cells (Fig. 8). Plasmodesmata were observed exclusively in the walls connecting neighbouring epidermal cells (Fig. 9). Secretory vesicles, fusing with plasmalemma, were found in a close proximity to the uppermost part of papillae cell walls and between the wall and the cuticle the material in the structure similar to the content of secretory vesicles was visible (Fig. 11). Accumulation of the secretion caused forming cuticle protrusions on the papillae surface, however the presence of pores or cracks in the cuticle was not detected (Fig. 10).

At the end of the secretion (stage IV) vacuoles, in some cells very large, stained blue-green with toluidine blue, indicating the presence of phenolic material. Frequently, the nucleus was larger and showed an irregular, lobed shape (Fig. 12). Significant feature of the nectary cells was also an increase in a number of lipid droplets in cytoplasm and osmiophilic globules in plastids. Generally, cytoplasm was brighter and the Golgi apparatus was absent (Fig. 13).

DISCUSSION

The nectary spur of *Gymnadenia conopsea* revealed some features common with orchid spur nectaries previously described in the literature such as of *Limodorum abortivum* (Figueiredo, Pais 1992; Pais, Figueiredo 1994) or *Platanthera bifolia* (Stpiczynska 1997). It is characterized by the presence of secretory epidermis surrounding the inside of the spur, and several layers of parenchyma involved in the transport of pre-nectar. Some differences

Figs 10-11. Surface of secretory papillae.

Fig. 10. Cuticle protruded on the surface of secretory papilla (arrow), SEM, $\times 6000$.

Fig. 11. Protrusions of cuticle, affected by secreted nectar (arrow) and fusion of secretory vesicles with plasmalemma, TEM, $\times 19\,700$.

Figs 12-13. Epidermal cells at the end of secretory activity.

Fig. 12. Epidermis and parenchyma cells with large, lobed nuclei, LM, $\times 1067$.

Fig. 13. The cell with plastid containing osmiophilic globules inside and with numerous lipids droplets in cytoplasm, TEM, $\times 15\,000$.

however, were also ascertained. Internal (adaxial) epidermis of spur formed numerous papillae, which significantly enlarged secretory surface like in *Platanthera* nectaries, but in *Gymnadenia*, they were about ten times shorter. Secreting nectar caused numerous protrusions of a cuticle covering the surface of papillae walls, but the rupturing of the cuticle was not observed, contrary to *Platanthera* (Stpiczyńska 1997) or *Limodorum* (Figueiredo, Pais 1992) nectaries, where distinct pores were noted.

The significant feature reported in orchids nectaries was the presence of plastids with intraplastidal membranes and with globular, osmiophilic contents. Through the successive stages of the secretory activity, the quantity of lipophilic substances increased. Plastids in *G. conopsea* and *P. bifolia* occurred mainly in secretory epidermis, whereas in *L. abortivum* also in parenchyma. Unusually, the plastids of *G. conopsea* did not contain starch, always present at presecretory stages in previously investigated orchid species. Starch stored in plastids of nectary cells may be used as a source of energy for intensive metabolic processes or – indirectly – as the source of secreted sugars (Durkee et al. 1981; Belmonte et al. 1994). Because starch

was absent in nectary cells of *G. conopsea*, sugars secreted there were probably delivered by the phloem sap, and decisively modified in secretory cells. Presumably, that was the reason of an abundance of vascular bundles in such a small nectary in comparison with much larger *Platanthera* spur (Stpiczyńska 1997), which had only two vascular bundles.

Plasmodesmatal contact of secretory cells in a fragrant orchid spur was scant and the transport of pre-nectar along the cell walls probably played a significant role, especially as an apoplastic barrier in the cell walls was not detected. Nectary cells of many other plants were connected by numerous plasmodesmata and symplastic pre-nectar flow was suggested by the authors (Gunning, Hughes 1976; Sawidis et al. 1987; Robards, Stark 1988; Kronstedt-Robards, Robards 1991; Figueiredo, Pais 1992).

Numerous secretory vesicles appearing near plasmalemma and fusing with it might indicate granulocrine secretion operating in *Gymnadenia* nectaries. They probably originated from the membranes of ER frequently occurring in the cytoplasm beneath the cell wall. The close contacts of ER with plastids may indicate the involvement of these structures in the secretory processes. Pais and Figueiredo

(1994) paid also attention to the engagement of plastids in the nectar secretion, although plastids with intraplastidal membranes filled with lipophilic substances were mainly noted in other types of secretory tissues – in osmophores, (Curry 1987), or in resin ducts (Fahn 1988) as a site of terpenoids synthesis.

Because the plastids did not contain starch, their involvement in sugar metabolism and secretory processes in *Gymnadenia* nectaries need more detailed, cytochemical studies.

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ANATOMIA I ULTRASTRUKTURA NEKTARNIKA OSTROGOWEGO *GYMNADENIA CONOPSEA* (L.) ORCHIDACEAE

STRESZCZENIE

Anatomię i ultrastrukturę nektarnika ostrogowego gółki długoostrogowej *Gymnadenia conopsea* (L.) badano w różnych stadiach aktywności sekrecyjnej. Epiderma okalająca wnętrze ostrogi tworzyła liczne brodawki znacznie powiększające powierzchnię wydzielniczą. Komórki epidermy charakteryzowała również obecność plastydów z osmofilnymi globulami w fazie sekrecyjnej. Plastyny te nie gromadziły skrobi. Kontakt plastydów z retikulum endoplazmatycznym występującym licznie w cytoplazmie wskazuje na możliwy udział tych struktur w procesie wydzielniczym. Kutykula pokrywająca ściany brodawek nie stanowiła bariery dla wydzielającego się nektaru i tworzyła ciągłą warstwę na powierzchni komórek.

SŁOWA KLUCZOWE: anatomia, ultrastruktura, nektarnik, *Gymnadenia conopsea*, Orchidaceae.