ANATOMY AND ULTRASTRUCTURE OF OSMOPHORES OF *CYMBIDIUM TRACYANUM* ROLFE (ORCHIDACEAE)

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(Received: May 9, 1992. Accepted: August 16, 1993)

ABSTRACT

The intense smell secreted by flowers of *Cymbidium tracyanum* Rolfe (Orchidaceae) derives from osmophores situated on the axipetal surface, mainly at the petals' base and the margin of labellum. The epiderm in those places created vesicular or somewhat elongated glandular cells, particularly on the labellum. In the production of smell 2-3 layers of subepidermal cells also take part. Submicroscopic examinations showed that those cells were characterized by the presence of a big nucleus. There were also numerous granules of starch and plastoglobules in plastids, a great amount of mitochondria and smooth-surfaced endoplasmic reticula. The traces of secretion products are visible on the surface of glandular cells. The above mentioned features are typical for osmophore cells.

KEY WORDS: osmophore, anatomy, ultrastructure, *Orchidaceae*, *Cymbidium tracyanum*

INTRODUCTION

The fragrant surfaces of flowers covered by specific tissue were called osmophores for the first time by Arcangeli in 1883 (cit. after Stern et al. 1987), who described the surfaces of spadix secreting smell in some species of the *Araceae* family (*Dracunculus vulgaris*, *Amorphophallus rivieri*). The presence of osmophores was stated also in many other plants, among others in *Narcissus* - on corolla appendage (Essau 1973), in *Ceropegia* - on perianth (Vogel 1960). But more detailed examinations concerning osmophores were led on various species of the orchids. In spite of variety of the morphology of the perianth in the *Orchidaceae* the osmophores are usually found on the labellum, for example in *Gongora* (Curry and Stern 1990), in *Coryanthes* and *Catasetum* (Proctor and Yeo 1975), in *Maxillaria*, *Dendrobium*, *Satyrium* (Vogel 1954), in *Stanhopea* (Stern et al. 1987), in *Kegeliella* (Curry and Stern 1991). The osmophores on elongated sepals were described in *Restrepia* (Pridgeon and Stern 1983). Curry et al. (1991) report that the differences in the morphology, the anatomy and the ultrastructure of the osmophores are essential taxonomic features in closely related species of the genera *Stanhopea* and *Seivekingia*.

The odoriferous substances secreted by orchid flowers are, in general, a mixture of various terpenoids and aromatic compounds (Dodson et al. 1969, Williams and Dodson 1972, Curry 1987). They attract a special kind of insects, allowing an optimal choice of the pollinator. This is of great importance in pollination ecology of many orchid species (Faegri and Van der Pijl 1971, Proctor and Yeo 1975). In literature no information was found concerning the structure of the osmophores in *Cymbidium*. The aim of this study was to examine the morphology, anatomy and ultrastructure of the osmophores in *Cymbidium tracyanum*.

MATERIAL AND METHODS

For the present study flowers of *Cymbidium tracyanum*, cultivated in a glass house of RZD Felin near Lublin, were used.

For the purpose of localization of the osmophores, the whole, fresh flowers from the first and the second day of anthesis were immersed in 0.1% neutral red. This is the simplest test for determination of the presence of osmophore tissue (Vogel 1962 cit. from Essau 1973), the cells of which intensely retain the stain.

Hand-cut slides of the perianth were prepared and treated with Sudan III and Lugol solution in order to detect lipids and starch granules in the cells.

The fragments of petals and labellum tissue of ca 1 mm thickness for the transmission electron microscope were fixed in a buffered 3% glutaric aldehyde, then repeatedly washed in 0.1 M phosphate buffer (pH 7.0) and again fixed in 2% osmium tetroxide. After washing in buffer the slides were dehydrated in succession in alcohol series and acetone and mounted in epoxy resin (Spurr 1969). Next the preparates were cut into semi-thin slides of 0.75 µm with an ultramicrotome Reichert OmU3. For the light microscope the slides were stained with azur B (Hayat 1975) and toluidine blue. Ultra-thin slides of 60 nm were also prepared, stained with uranyl acetate and lead citrate and examined with a transmission electron microscope BS 500 TESLA.

The surface of the labellum was observed by means of a scanning electron microscope BS 300 TESLA. For this purpose the similarly fixed and dehydrated in acetone fragments of labellum were Critical-point dried with CO₂ in a CPD 020 drier (BALZERS UNION) and coated in vacuo with gold using a Sputter Coater SC 100.

Fig. 1. Flower habit of *Cymbidium tracyanum* (slightly reduced)

Fig. 2. Flower with exhibited labellum, arrows show the osmophore localization on labellum and at the petal base

RESULTS

In result of staining with neutral red, the osmophore tissue was localized on axipetal surface, next to the petals' base (tepala) of the external and internal whorl and on the marginal surface of labellum (Figs. 1, 2).

Figs. 3-6. Cells of the secreting surface of the petal and labellum (scanning microscope)

Fig. 3. Cells of the lower epiderm at petal base, 980x

Fig. 4. Fragment of petal surface near base covered with unicellular, spheric, secreting hairs, 60x

Fig. 5. Setting of secreting cell of labellum with visible cuticle (arrow), 700x

Fig. 6. Fragment of secreting cell of labellum with irregular cuticle surface, 1000x

OBSERVATIONS UNDER THE SCANNING ELECTRON MICROSCOPE

The tiny, unicellular, a little globular secreting hairs were found on the axipetal surface of the petals (on a surface ca 2 cm wide, near base). The remaining surface of petals, both axipetal (upper) and abaxial (lower), was covered with considerably bigger, flat or a little convex cells (Figs. 3, 4).

The marginal, a little wavy surface of the labellum was covered with vesicular cells, similar to those situated near the petals' bases (Fig. 4). These were of different size; at the edge there were big, pear-shaped unicellular secreting hairs, whereas others, localized somewhat deeper, were considerably smaller, almost spheric (Figs. 5, 7). The cuticle on the walls of glandular cells, both petals and labellum, was irregular with visible protuberances, whereas on the surface of walls it was possible to observe extracellular matter - probably secretion (Fig. 8).

Fig. 7. Edge of labellum with secreting cells on surface of osmophore, 126x

Fig. 8. Traces of secretion on the surface of secretory cells, 1800x

Figs. 7-8. Secreting surface of labellum (scanning microscope)

Figs. 9-14. Fragments of transversal sections of petal and labellum (light microscope)

Fig. 9. Cells of secretory epidermis of the petal; lipid droplets visible in the cytoplasm and on the wall surface, 50x

Fig. 10. Fragment of section near top of petal; lack of unicellular secreting hairs and distinct differences between lower and upper epidermis, 25x

Fig. 11. Enlarged fragment of epidermis and subepidermal layers from Fig. 10., numerous lipid droplets in cytoplasm, 100x

Fig. 12. Cells of secretory epidermis and subepidermal layers of labellum, 50x

Fig. 13. Thin-walled, vesicular cells at the edge of labellum, 100x

Fig. 14. Fragment of secretory epidermis of labellum, the pits are visible in the walls of cells of subepidermal layers (polarized light), 150x

OBSERVATIONS UNDER THE LIGHT MICROSCOPE

Thin-walled cells of the upper epidermis and 2-3 cellular subepidermal layers of the petals differed considerably from the deeper located basic ground tissue. They were tinier and possessed smaller vacuoles and big nuclei. Numerous lipid droplets were found in the intensively stained cytoplasm, especially in the place of localization of the unicellular hairs (Fig. 9). Small, not numerous granules of starch and lipid drops were visible in the basic ground tissue near the lower epidermis, built up from cells with thick external walls. The difference between the upper and lower epidermis and subepidermal layers contained a great amount of lipid droplets in cytoplasm, gradually disappearing with distance off the petal's base (Figs. 10, 11).

Secretory epidermal cells of labellum had thin walls. They were considerably vacuolized and possessed a big nucleus whereas numerous granules of starch and lipid drops were present in the cytoplasm (Figs. 12, 13). Big nuclei and dense cytoplasm with great amount of starch but a smaller number of lipids characterized the 2-3 layers of thin, subepidermal cells. There were numerous, oval pits in the walls of these cells (Fig. 14).

Figs. 15-18. Fragments of secretory cells of labellum (transmission electron microscope)

Fig. 15. Aggregations of osmophilic substance along plasmalemma and among fibrils of cell wall of the secretory epidermis (arrows) 10000x

Fig. 16. Cytoplasm with endoplasmic reticulum. Aggregations of osmophilic substance in vacuole along tonoplast (arrow), 4000x

Fig. 17. Plastids with starch granules and plastoglobules in subepidermal cells, 16000x

Fig. 18. Mitochondria near plasmalemma, small vesicles existing in cytoplasm and between plasmalemma and cell wall (arrows), 18000x

Abbreviations: W - cell wall; M - mitochondrion; P - plastid; S - starch

OBSERVATIONS UNDER THE TRANSMISSION
ELECTRON MICROSCOPE

Agglomerations of osmophilic substances were found between the fibrils of walls of glandular cells of labellum and petal epidermis, however it was difficult to observe them on the surface of the cuticle (Fig. 15). Those cells had usually a few small vacuoles. The intensely stained material sedimented sometimes along the tonoplast (Fig. 16).

Tiny vesicles, full of dark grey, probably lipid substances were visible near plasmalemma and between plasmalemma and the cell wall. Numerous mitochondria were found particularly near the plasmalemma, similarly like plastids from subepidermal cells of the labellum which contained one big nucleus or a few tiny granules of starch and great amounts of plastoglobules (Figs. 17, 18). It was possible to observe also plastids without starch, with great amounts of plastoglobules. Canals and cisterns of smooth surfaced endoplasmic reticulum were present frequently in a considerably granular cytoplasm (Fig. 16), however, very seldom rough surfaced endoplasmic reticulum and dictyosomes were visible. Irregularly-shaped lipid droplets were observed sporadically.

DISCUSSION

The positive test with neutral red indicates the secretory character of the marginal axipetal surface of petals and labellum in *Cymbidium tracyanum*. The cells in this place show features of secretory cells, described by Swanson et al. (1980), Pridgeon and Stern (1983), Stern et al. (1987), Curry and Stern (1990). These authors pay attention to the characteristic for the osmophores presence of great amount of starch, especially in the bud stage. During the anthesis the amount of starch rapidly decreases, and its quick decomposition delivers energy for the intensively occurring biochemical processes. The osmophores in *Cymbidium* were examined only during the first and the second day of anthesis, and that is probably why in their cells small amounts of starch were observed.

Except of starch there were many plastoglobules in plastids, similarly like in osmophore cells of the other orchid species. It was not observed, however, that membranes of endoplasmic reticulum were surrounded or connected with external plastid membranes, what was emphasized by Pridgeon and Stern (1983), Stern et al. (1987), Curry et al. (1991) and Schnepf (1969), Swanson et al. (1980) and Sevinete-Pinto and Antunes (1991) as a characteristic feature of oil glands. The lack of close relations of ER membranes with plastids resulted probably from the age of examined flowers (the first and the second day of anthesis, when processes of synthesis of odoriferous substances are not so intense). Among ER membranes the smooth reticulum was most often observed, whereas the rough reticulum only sporadically. Dictyosomes were seldom present. Similar observations were noticed in the studies of Curry (1987), Stern et al. (1987). As results from Curry's (1987), cytochemical experiments, terpenoids, the main element of orchid smell, are a composition of isoprene units synthesized in the metabolic pathway of mevalonic acid. The enzymes of this pathway were found mainly in smooth ER membranes and between external membranes of the mitochondria, indicating that they take active part in the terpenoid biosynthesis. The mitochondria of osmophore cells of *Cymbidium* occurred in great number, particularly along the cell wall, what was observed also in *Restrepia* (Prid-

geon and Stern 1983). In osmophore cells after anthesis the degree of vacuolisation increases. The storage of osmophilic substances, in the beginning along the tonoplast, and then in the whole vacuole, visible in the cells of *C. tracyanum* was noticed also in *Cypripedium* (Swanson et al. 1980) and *Restrepia* (Pridgeon and Stern 1983).

The vesicles containing intensely stained material were visible in cytoplasm near the plasmalemma and between plasmalemma and cell wall. The accumulation of secretions in a similar form at the plasmalemma was observed by Pridgeon and Stern (1983). In the examined species osmophilic substances /probably secretion / were present also among fibrils of the cell wall. Transmigration of secretion through the cell wall accompanied with rupture of the cuticle was observed in osmophores of *Restrepia* (Pridgeon and Stern 1983). In consequence the surface of the epiderm was distinctly perforated. Similar perforations in glandular cells of the epiderm were not observed in *Cymbidium*, however it was possible to notice the irregular, sometimes swollen surface in the cuticle and agglomerations of secretion. The accumulation of extracuticular matter was observed on the surface of osmophore cells in *Restrepia* (Pridgeon and Stern 1983) and *Stanhopea* (Stern et al. 1987) and on secreting hairs in *Cypripedium* (Swanson et al. 1980).

In many orchids the surfaces of osmophores are covered by vesicular trichomes, similar to those observed in *Cymbidium* among others in *Restrepia* (Pridgeon and Stern 1983) or various multicellular papilli like in *Stanhopea* (Stern et al. 1987, Curry et al. 1991).

The maximal increase of osmophore surface is connected with a more intense emission of smell.

The carried out observations confirmed the presence of structures described as typical for osmophores by the mentioned authors, like the intensely stained cytoplasm with numerous endoplasmic reticulum membranes, lipid drops and starch granules in plastids and the secretion on the surface of osmophore epiderm cells.

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ANATOMIA I ULTRASTRUKTURA OSMOFORÓW W *CYMBIDIUM TRACYANUM* ROLFE (ORCHIDACEAE)

STRESZCZENIE

Intensywny zapach wydzielany przez kwiaty *Cymbidium tracyanum* Rolfe (Orchidaceae) pochodzi z osmoforów położonych na doosiowej powierzchni, głównie u nasady płatków oraz brzegu warzki.

Epiderma w tym miejscu wytworzyła pęcherzykowate lub nieco wydłużone komórki gruczołowe, zwłaszcza na warzce. W produkowaniu zapachu biorą także udział 2-3 warstwy komórek subepidermalnych. Badania mikroskopowe wykazały, że komórki te charakteryzują się obecnością dużych jąder komórkowych, występują też liczne ziarna skrobi i plastoglobule w plastydach, duża liczba mitochondriów i błon gładkiej siateczki śródplazmatycznej. Na powierzchni komórek gruczołowych widoczne są ślady wydzieliny. Są to typowe cechy komórek osmoforowych.

SŁOWA KLUCZOWE: osmofory, anatomia, ultrastruktura, *Orchidaceae*, *Cymbidium tracyanum*