

## Ultrastructural responses of leaf mesophyll and trap wall cells of *Utricularia vulgaris* to cadmium

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### Abstract

Ultrastructure of leaf mesophyll and two-layer trap wall of *Utricularia vulgaris* L. was studied after 24-h and 48-h treatment with different concentrations of cadmium. The treatment of plants with  $0.25 \text{ g(Cd}^{2+}) \text{ m}^{-3}$  caused different changes in the chloroplasts of the two organs. The thylakoids swelled in chloroplasts of leaf and the starch content increased in chloroplasts of traps. Higher concentrations of Cd ( $0.5$  and  $1.5 \text{ g m}^{-3}$ ) caused osmiophilisation of thylakoids in the chloroplasts of both studied organs. The secretory cells in the trap wall was resistant to Cd.

*Additional key words:* aquatic carnivorous plants, granal and intergranal membranes, thylakoids, chloroplasts.

### Introduction

Scientific attention to carnivorous plants was attracted by the presence of highly specialized secretory organs which are involved in the capture and digestion of small animal prey (Fineran and Lee 1974, 1975, Muravnik 1988, Vassilyev and Muravnik 1988a,b, Taylor 1989). Most workers have concentrated on the species of *Utricularia* (*Lentibulariaceae*). The extraordinary developmental morphology of seven non-aquatic species of *Utricularia* was described by Brugger and Rutishauser (1989) who also provided rich anatomic data on the leaves and roots of the studied species. The traps (modified foliage) of the carnivorous plant *Utricularia* are among the most specialized structures in the plant kingdom. More profound morphological studies including seasonal and individual variation in trap number and biomass, size and shape of traps were carried out with *Utricularia vulgaris* L. (Friday 1991, 1992). Few studies on *U. vulgaris* provide data on the uptake and translocation of nutrients and the role of traps in these processes (Friday and Quarmby 1994).

The aim in this work is to provide structural information on the leaves and traps of *Utricularia vulgaris* L. after treatment with different Cd concentrations.

## Materials and methods

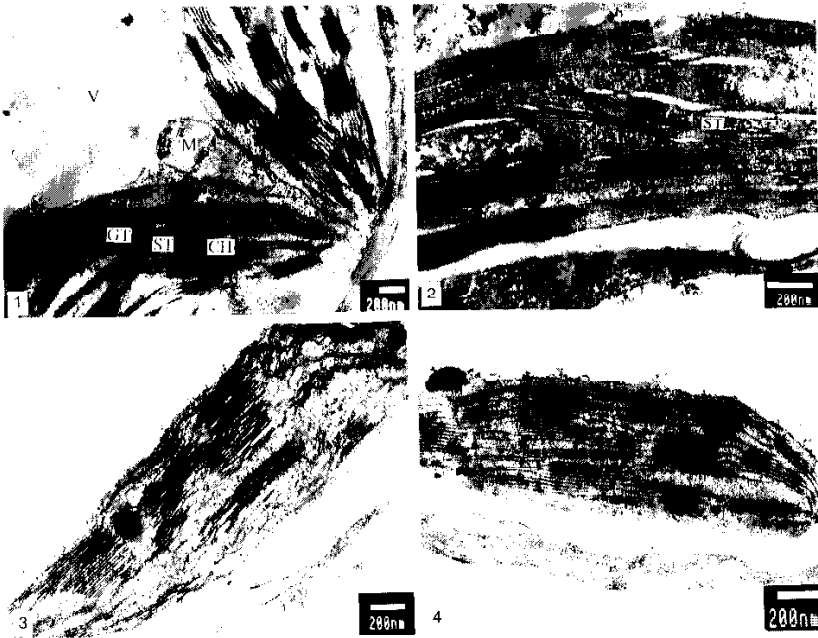
*Utricularia vulgaris* L. is rootless and free-floating plant, growing at the apex while dying away behind. The main stolon gives rise to side shoots at intervals and each of these lobes is equivalent to a "leaf" in the nomenclature used by Friday (1989). Each lobe bears a series of large traps along the mid-line, smaller traps towards the periphery and between one and five traps at the base of the leaf on the stolon. The investigations were carried out with primary leaf segments and the mid-line traps. *U. vulgaris* was collected during summer in an artificial basin in the Southern Park (Sofia). The plants were transferred to a climatic chamber (12-h photoperiod, photon flux density of  $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and grown in tap water at neutral pH (7.0 - 7.5) and water temperature  $18^\circ\text{C}$ . After 5 d, Cd was applied as cadmium sulfate at concentrations 0.25, 0.5 and  $1.5 \text{ g}(\text{Cd}^{2+}) \text{ m}^{-3}$ . The highest concentration applied was the threshold for survival of the species established by plasmolysis (Repp 1963). The plants were studied after 24-h and 48-h treatment with the above mentioned Cd concentrations. Plants cultivated under the same conditions but without Cd were used as controls.

For transmission electron microscopy (TEM)  $1 \text{ mm}^2$  pieces were collected from primary leaf segments and the wall of the mid-line traps from the same plants, and fixed in 3 % glutaraldehyde in phosphate-buffer (pH 7.4) for 12 h at  $4^\circ \text{C}$ . The samples were post-fixed in 2 %  $\text{OsO}_4$  in the same buffer for 4 h at room temperature. After dehydration the material was embedded in *Durcupan* (Fluka). Ultrathin sections were cut using *Reichert* ultramicrotome. They were stained with uranyl acetate and basic lead citrate. Examination was performed with *JEOL 1200EX* electron microscope.

## Results and discussion

**Leaves:** The mesophyll of *Utricularia vulgaris* L. leaves possesses all morphological characteristics of a completely differentiated photosynthesizing tissue. Each mesophyll cell is filled with one large vacuole so chloroplasts and other organelles are limited to a relatively thin layer adjacent to the cell wall (Fig. 1). The chloroplasts possess a well developed inner membrane system. It is built of regularly arranged grana stacks and intergranal membranes. After 24-h treatment with  $0.25 \text{ g}(\text{Cd}^{2+}) \text{ m}^{-3}$  slightly pronounced swelling of stromal thylakoids was observed (Fig. 2, *arrow*). After 48-h treatment with the same Cd concentration, thylakoids were more swelled and changed their spacial orientation to wavelike (Fig. 3). This structural organisation of the chloroplasts was preserved also in plants treated 24-h with  $0.5 \text{ g}(\text{Cd}^{2+}) \text{ m}^{-3}$ . Similar changes were observed in lead-exposed leaves of *Ceratophyllum demersum* (Rebechini and Hanzely 1974). Under certain conditions the chloroplasts of some terrestrial plants exhibit similar changes after treatment with Cd (Barcelo *et al.* 1988). A different type of change in membrane system of chloroplasts was registered after 48-h treatment with  $0.5 \text{ g}(\text{Cd}^{2+}) \text{ m}^{-3}$  and 24-h and 48-h treatment with  $1.5 \text{ g}(\text{Cd}^{2+}) \text{ m}^{-3}$ . The chloroplasts had stained thylakoid

membranes and dark thylakoid loculi (Fig. 4). Under these concentrations partial fragmentation of stromal thylakoids was established. In a previous study chloroplasts possessing similar characteristics were found in stem mesophyll cells of *Myriophyllum spicatum* control plants (Stoyanova and Tchakalova 1997). Some



Figs. 1 - 4. Ultrastructure of leaf mesophyll in control plants of *Utricularia vulgaris* L. and after treatment with Cd (CH - chloroplast, GT - granum thylakoid, ST - stroma thylakoid, V - vacuole, SG - starch grain, M - mitochondrion, D - dictyosome, ER - endoplasmic reticulum, P - plasmodesm).

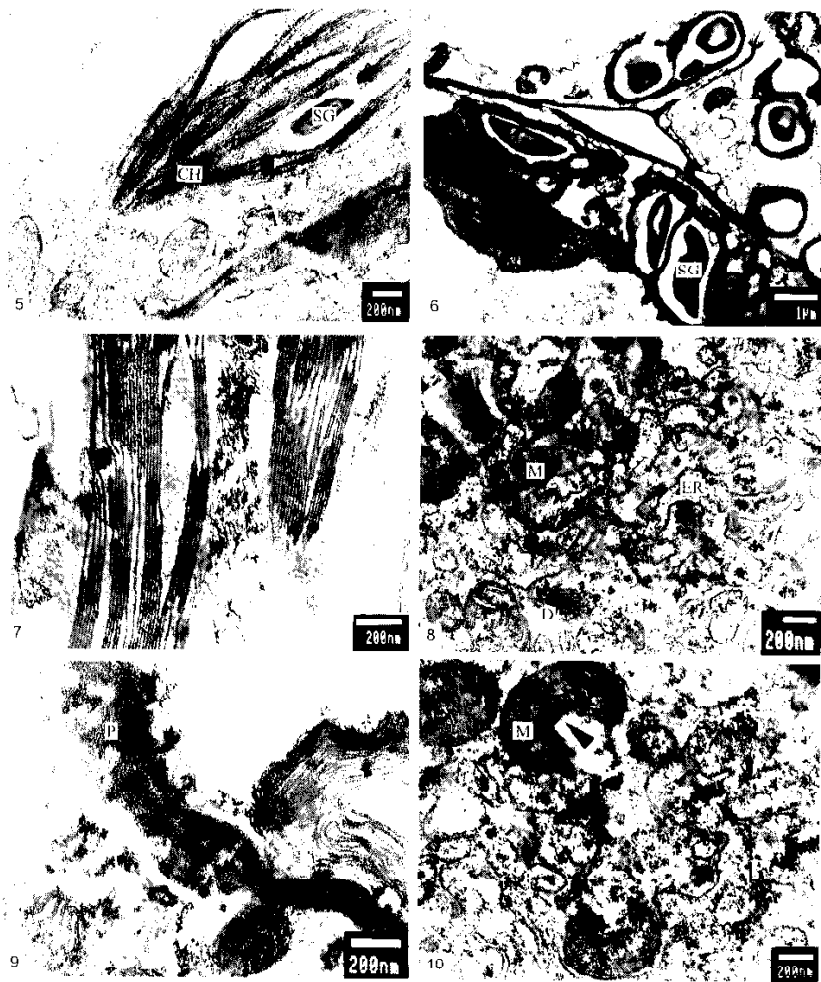
Fig. 1. Part of mesophyll cell in control plant.

Fig. 2. 24-h treatment with  $0.25 \text{ g(Cd}^{2+}) \text{ m}^{-3}$ .

Fig. 3. 48-h treatment with  $0.25 \text{ g(Cd}^{2+}) \text{ m}^{-3}$ .

Fig. 4. 48-h treatment with  $1.50 \text{ g(Cd}^{2+}) \text{ m}^{-3}$ .

studies on plastid development in *Nymphoides indica* suggest that the presence of regular and modified type of thylakoids is related to the tissue specialisation (Van Steveninck and Van Steveninck 1980a,b). In terrestrial plants the thylakoid dimorphism is of functional significance (Danilova and Kashina 1995).



Figs. 5 - 10. Ultrastructure of trap wall in control plants of *Utricularia vulgaris* L. and after treatment with Cd.

Fig. 5. Chloroplast of trap wall in control plants.

Fig. 6. Changes in chloroplasts after 48-h treatment with  $0.25 \text{ g(Cd}^{2+}) \text{ m}^{-3}$ .

Fig. 7. Changes in chloroplast after 48-h treatment with  $1.5 \text{ g(Cd}^{2+}) \text{ m}^{-3}$ .

Figs. 8 - 9. Parts of basal epidermal cells in trap wall of control plants.

Fig. 10. Changes in mitochondria in the basal epidermal cells after 24-h treatment with  $1.5 \text{ g(Cd}^{2+}) \text{ m}^{-3}$ .

**Traps:** The traps of *U. vulgaris* are biconcave spheroidal structures with an inward-opening trap-door at the end furthest from the stalk. The trap wall consist of two epidermal layers with structural and functional characteristics typical for the epidermis of free-floating aquatic plants. The cells contained chloroplasts with a well developed inner membrane system (Fig. 5). Little starch grains were observed in the stroma. The autotrophic character of the cells indicated that in functional aspect the two epidermises as photosynthesizing tissue did not directly relate to the digestion taking place in the traps. After 24-h and 48-h treatment with  $0.25 \text{ g(Cd}^{2+}) \text{ m}^{-3}$  the chloroplasts generated big starch grains which pushed the inner membrane system close to the envelope (Fig. 6). The increase of starch indicated disturbed outflow of assimilates. The chloroplasts in the trap wall of plants treated with 0.5 and  $1.5 \text{ g(Cd}^{2+}) \text{ m}^{-3}$  did not possess starch grains. They were with stained thylakoid membranes and dark thylakoid loculi (Fig. 7). This structure of the inner membrane system was registered in the same concentrations of Cd as for the chloroplasts of leaf mesophyll.

Internal glandular hairs producing digestive enzymes were situated in the traps of *U. vulgaris*. The basal epidermal cells of the internal glandular hairs were part of the inner epidermal layer of the trap wall. Structurally they were completely different from the rest of the epidermal cells. The basal epidermal cells possess secretory structure (Fig. 8). The cytoplasm was characterised with very well developed rough endoplasmic reticulum represented by numerous, different in length, wide cisternae. It was a dominant component of cytosol and gave the cells a typical protein synthesizing cell appearance. The protoplasts of these cells are characterised by not many but highly active dictyosomes and a great number of condensed mitochondria (Muravnic 1988). In the contact surfaces between the basal epidermal cells and the rest of the epidermal cells a lot of plasmodesmata could be found which indicated good symplast connection between structurally and functionally different tissues (Fig. 9). The basal epidermal cells of the internal glandular hairs proved to be highly resistant to Cd. Regardless of duration, the treatment with  $0.25$  and  $0.5 \text{ g(Cd}^{2+}) \text{ m}^{-3}$  did not bring to significant structural changes. The highest ( $1.5 \text{ g m}^{-3}$ ) concentration caused changes in the shape of mitochondria. They were almost crescent-shaped with precipitated osmiophilic material in the form of aggregates on the envelope (Fig. 10). Regardless of the change in shape the mitochondria preserved their inner organisation and probably their functional activity remained uninfluenced.

**Conclusions:** TEM analysis of the plastid apparatus in the leaves and traps of *U. vulgaris* after treatment with Cd indicated that the structural reaction of chloroplasts are dependent on the concentration of the metal. The chloroplasts in the leaf mesophyll and the trap wall responded differently to  $0.25 \text{ g(Cd}^{2+}) \text{ m}^{-3}$ . The secretory structure of the basal epidermal cells in trap wall more inert to Cd. The chloroplasts are a structural indicator for toxic influences while the rest of the protoplasmic components are much more resistant.

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