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Cadmium induced ultrastructural changes in shoot apical meristem of *Elodea canadensis* Rich.

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Abstract

The ultrastructure of cells of ground meristem in the peripheral zone of shoot apical meristem in *Elodea canadensis* Rich. was studied after treatment with cadmium in concentrations 0.5, 1, 2, 3, 5, and 6 g(Cd^{2+}) m^{-3}. After 5 d treatment, changes in the structural organisation of the plastid apparatus were found, namely in proplastid, amyloplast, and amoeboid stages of plastid development.

Additional key words: plastid; plastoglobuli; starch; thylakoid.

Introduction

The organisation of apical meristem of the shoot is more complex than that of the root. Because of differences in meristem activity and structural characteristics of cells, a clearly pronounced cytohistological zonal distribution was observed in shoot apical meristem. This enabled structural studies on apical meristem in terrestrial plants, directed at clarifying the mechanisms of cell differentiation and morphogenesis. Important facts for better understanding of apical growth changes and their implications in morphogenesis were provided by quantitative anatomical studies of Seidlová (1988). A thorough structural study of shoot apical meristem was done with *Pyrus pyrifolia* Nakai (Peng and Iwahori 1994a,b, 1995). These studies supplied anatomical, ultrastructural, and cytochemical data on the meristem in the process of differentiation. Plastid development in apical meristem was often studied (Cran and Possingham 1974, Oross and Possingham 1991). Studies on the influence of external factors with toxic effect on plants were much more limited and focused on the root (Jásik 1986, Jasik *et al.* 1987, Vázquez *et al.* 1987, De Lima and Copeland 1994, Kupidlowska *et al.* 1994, Mostowska 1997).

Meristem tissues in higher aquatic plants were considerably less studied. Blazencic (1971) analysed the influence of ecological factors on the morphogenesis in genus *Trapa*. Bugnon and Mignotte (1984) proposed a model for the structural development of the shoot apical meristem in *E. canadensis*.

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Both the internal and external factors play an important role for the structure and the function of shoot apical meristem in submersed aquatic plants. The aim of the present study was to investigate the toxic effect of cadmium on meristematic cells in shoot apical meristem of *E. candensis* Rich.

Materials and methods

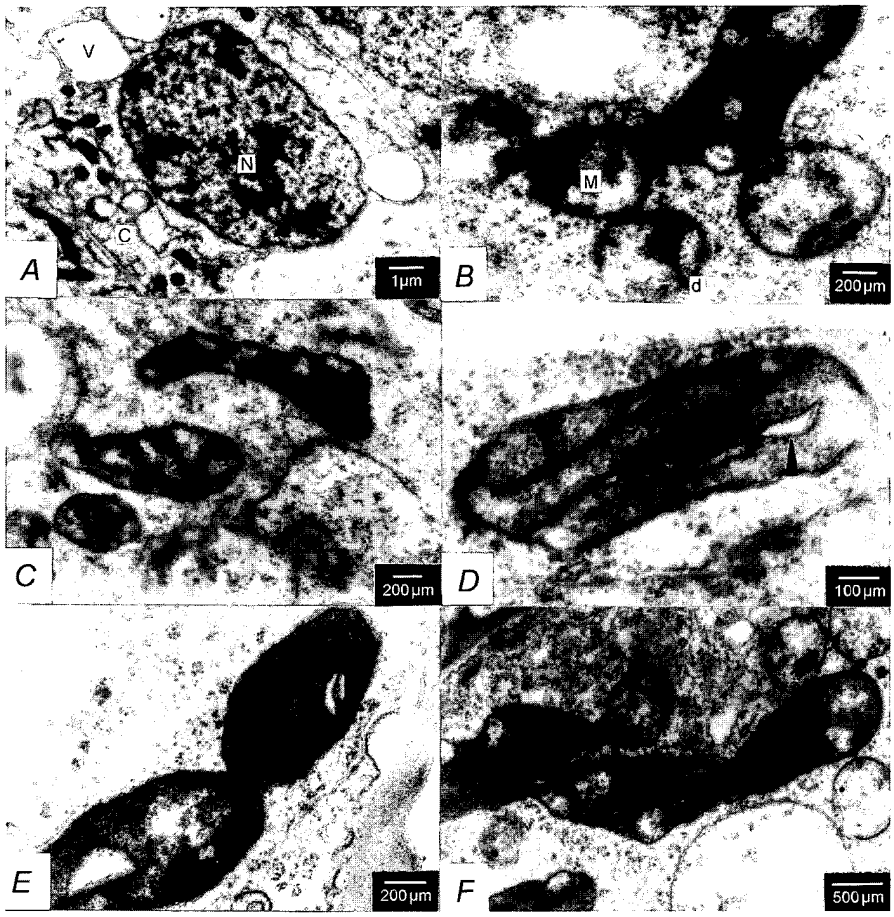
We used *E. canadensis* Rich. after 5 d of cultivation in a phytostat. The plants were grown in tap water, at a neutral pH (7.0-7.5), water temperature 18 °C, irradiance 20-25 W m^{-2}, and natural photoperiod (16/8 h day/night). Cd was introduced as cadmium sulphate, 0.5, 1, 2, 3, 5, and 6 g(Cd^{2+}) m^{-3}. Plants grown under conditions of the experiment but without Cd were used as the controls. Shoot tips (apices) for electron microscopy were collected from experimental plants fixed in phosphate buffered 3 % glutaraldehyde (pH 7.4) for 12 h at 4 °C, and postfixed in 2 % OsO_4 for 4 h at room temperature. After dehydration the material was embedded in *Durcupan* (*Fluka*, Switzerland). Ultrathin longitudinal sections in the shoot apex were cut using a *Reichert* (Austria) ultramicrotome. They were stained with lead citrate, and examined under a *JEOL 1200 EX* (Japan) electron microscope.

Results and discussion

The peripheral meristem of the shoot apex was built by basic meristematic cells with high meristem activity. They possessed dense cytoplasm rich in ribosomes, with transparent prevacuole bodies and large nucleus with evenly distributed heterochromatic areas (Fig. *A*). A large number of uniformly structured mitochondria and plastids were submerged in the cytoplasm (Fig. *B*). The mitochondria were oval with long perpendicular cristae and a free central zone of a low electronic density. The plastid apparatus was represented by proplastids with a small number of thylakoids shaped as narrow tubular structures (Fig. *C*). The tubules were characterised by peripheral location in the plastid, points of contact with the envelope, and a nearly perpendicular orientation at the point of contact with these membranes. Their functional significance for the plastids in morphogenesis was reviewed by Oross and Possingham (1991). A small number of plastoglobuli was found in the plastids. Despite of their clarified relation with thylakoid formation, no lipid or protein bodies were found in the stroma (Cran and Possingham 1974).

High structural stability of meristematic cells was demonstrated after 5 d Cd-treatment of plants. All protoplasmic components excluding plastids preserved structure similar to the one in the control plants. Only the reaction of the plastid apparatus could be considered an indicator of the effect of Cd on the protoplast. Its structural organisation changed in direct correlation with Cd concentrations. 0.5 g(Cd^{2+}) m^{-3} caused swelling in some of the peripheral thylakoids (Fig. *D*, *arrow*). This reaction of the membrane system to toxicity is universal for the differentiated

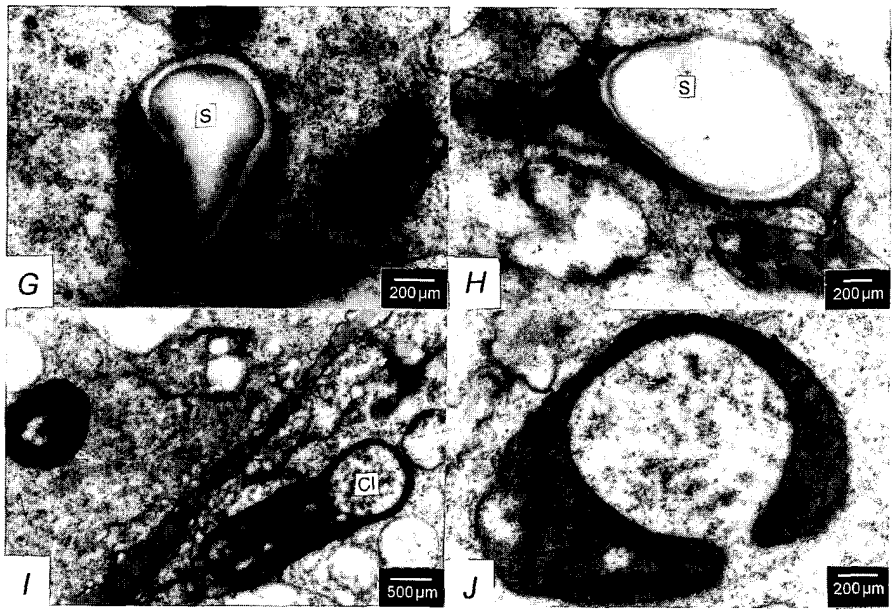
similar experimental conditions (Stoyanova and Chakalova 1990). Evidently the plastid apparatus reacts uniformly to low Cd concentrations independently of the stage of plastid development or the different structural and functional characteristics of the cells. Increase in density of the stroma and density of plastoglobuli in plastids with single swollen thylakoids was observed after treatment with 1 g(Cd^{2+}) m^{-3} (Fig. *E*). Similar morphological deviations from the control in differentiated chloroplasts may be a product of destructive processes (cf. Stoyanova and Tchakalova 1997).



Figs. *A-C*. Structure of meristematic cells in shoot apical meristem of control plants of *Elodea canadensis*. C = cytoplasm; CI = cytoplasmic inclusion; M = mitochondrium; N = nucleus; P = plastid; S = starch grain; V = vacuole.
Fig. *D*. Changes of thylakoids after treatment with 0.5 g(Cd^{2+}) m^{-3}.
Fig. *E*. Changes of stroma and plastoglobuli after treatment with 1 g(Cd^{2+}) m^{-3}.
Fig. *F*. Elongated plastids in meristematic cells after treatment with 1 g(Cd^{2+}) m^{-3}.

Proplastids with similar structure were registered in normally developed apical meristem (Danilova and Kozubova 1980) which indicates that their condition in similarly treated material could not be interpreted as a stress reaction to cadmium. Elongated plastids were found in the same meristematic cells (Fig. *F*). Whatley (1980) relates this shape of the plastids to the process of division and change in their number. Probably the irregular shape of plastids correlated with the processes of differentiation of cells which are carried out irrespective of the exogenous factors in the experiment.

Independent of their shape, all plastids accumulated a considerable amount of starch after treatment with 2g(Cd^{2+}) m^{-3} (Figs. *G* and *H*). They possessed the structural characteristics of amyloplasts. If they represented the amyloplast stage of chloroplast development, the accumulation of starch could not be a direct product of photosynthesis and the plastid was in this respect comparable to a root amyloplast. Disturbed flow of assimilates (cf. Vassilev *et al.* 1997) could not be a valid explanation in this case. Studies on roots of wheat seedlings treated with aluminum indicate a possibility that the mobilization of starch is linked to a coincident increase in fermentative metabolism in all Al-stressed wheat roots. 3, 5, and 6 g(Cd^{2+}) m^{-3} were concentrations affecting the shape of plastids in total lack of starch (Figs. *I* and *J*). After 5 d treatment with the above concentrations, the plastid shape became irregular. In some meristematic cells the plastid apparatus was cup-shaped (with cytoplasmic inclusions) (Fig. *I*). In other cells typical amoeboid plastids were found (Fig. *J*). Such plastid shapes may be defined as ontogenic stages (amoeboid stage)



Figs. *G, H.* Increase in starch after treatment with 2 g(Cd^{2+}) m^{-3}.
Figs. *I, J.* Changes in plastid shape after treatment with 5 g(Cd^{2+}) m^{-3} (9) and 6 g(Cd^{2+}) m^{-3} (10).

of the basic pathway (Whatley 1974, 1977, Brangeon and Nato 1981, Jásik and Hudák 1987, Olmos and Hellin 1996). On the other hand, in the root meristem amoeboid-shaped plastids occur frequently under stress (Jásik *et al.* 1987, Vázquez *et al.* 1987). According to Jásik *et al.* (1987) the appearance of amoeboid plastids was the reaction of cell organelles to the stress caused by the increased content of vanadium in the cultivation solutions. Cadmium concentrations above 3 $g(Cd^{2+})$ m^{-3} may similarly affect the plastid apparatus of the studied meristem tissue in *E. canadensis*.

In general, the structural organisation of plastid apparatus in the peripheral zone of apical meristem in *E. canadensis* after gradient Cd treatment was identified with part of the stages of plastid development, *i.e.*, proplastid, amyloplast, and amoeboid stages. Thus in this meristem region the Cd treatment induced plastid differentiation controlled by the gradient of concentration. The used treatment induced a moderate Cd stress for the meristem in which plastid morphology may be involved as an adaptive process. Our results demonstrate that changes in plastids may be the necessary components of adaptation to external factors that depend on internal factors.

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