

BRIEF COMMUNICATION

The influence of lead on callose formation in roots of *Lemna minor* L.

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Abstract

The treatment of *Lemna minor* L. plants with $\text{Pb}(\text{NO}_3)_2$ for 90 min, 8 and 24 h resulted in intensified deposition of (1,3)- β -glucan (callose) in plants roots. It was localized in the protodermis of the root tip, and in the center of the stele in the region at the proximal part of the root cap and slightly above.

Additional key words: cytochemistry, duckweed, resistance mechanisms.

Many trace elements, lead among them, contaminate the natural environment. In *Lemna minor* L. lead caused reduction of both root growth and chlorophyll synthesis (Woźny and Manikowska 1990). Plants are capable, to certain extent, to reduce the harmful impact of stress factors. The example of such mechanism may be the synthesis of callose in cells in response to plasmolysis, pathogen infection, mechanical injury, high and low temperature, ultrasounds, and many harmful chemical compounds including heavy metal salts (Fincher and Stone 1981). This work aimed at answering the question if callose is synthesized also under influence of lead.

Lemna minor L. plants were cultured on a liquid medium according to Wang (1990), of pH 5.7, in the temperature of 23 ± 1 °C, constant irradiance of $66 \mu\text{mol m}^{-2} \text{s}^{-1}$ and relative humidity of 75 %. To obtain stabilised stock culture every seven days six colonies, each consisting of three fronds, were transferred to 100 cm³ Erlenmeyer flasks. The mother frond root of each colony was 10 - 12 mm long. For the experiment individuals of the same feature characteristics were transferred to 60 cm³ of aqueous solution of $\text{Pb}(\text{NO}_3)_2$ at the concentration of 0.3 μM Pb^{2+} . They were incubated for 90 min, 8 and 24 h. Control plants were kept for the same period in 60 cm³ of bidistilled water. Lead taken up by plants was localized using the

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sodium rhodizonate method (Glater and Hernandez 1972). Vitality of the cells was estimated by observation of plasmolysis/deplasmolysis processes under the influence of 1 M KNO₃ and bidistilled water, respectively. Callose was detected after 5 min incubation with aniline blue (0.1 % water-soluble aniline blue in 0.15 % K₂HPO₄, pH 8.2 - Jensen 1962, modified). Observations were performed with the fluorescent microscope *Fluoval* (Carl Zeiss, Jena, Germany, mercury lamp *HB200*, excitation filters *B223g*, *B224g*, supression filter *G249*).

The first traceable fluorescence was observed in the apical end of the root as early as after 60 min of exposure to lead but its intensity was very small. It became very clear after 90 min of incubation. In the root tip callose was present in the cell walls of the protodermis (Fig. 1C). The strands of callose, placed lengthwise in the radial walls of the protodermis, formed a cylinder surrounding root meristematic region. Weaker callose fluorescence was observed sporadically in the root at the proximal end of the root cap, in the form of a single plug and a very subtle trail in the root center.

After prolonging the incubation time up to 8 and 24 h the callose strands became longer and their fluorescence stronger. Besides the presence of callose in the meristematic region, peculiar to this variants was also a well-marked streak visible along the length of the root in the region directly above and at the proximal region of the cap (Fig. 1E), in the site corresponding to the axial cell tract (xylem). Sometimes a characteristic plug occurred at the distal end of the streak. After 24 h of incubation no differences were found in comparison with the 8 h variant.

In the case of control plants, fluorescence was visible only in the root cap in the form of a ring in its proximal part (Fig. 1A), and also as rarely and irregularly distributed grains on its surface (Fig. 1B). Similar fluorescence sites could be also observed as additional to the described ones in the lead variants.

These results showed that lead intensified deposition and probably also production of callose in *Lemna minor* L. roots. This has prompted us to make use of polyacrylamide gel electrophoresis. This method showed an increase in the enrichment of two polypeptide bands in the plants treated with lead in comparison with the control ones. The molecular mass of one of the polypeptide (M_r 52 kD) suggests that it may be a components of callose synthase (β -1,3-D-glucan synthase) - Li *et al.* (1993). Further investigations on this issue are already underway.

In order to see if the sites of callose deposition corresponded to lead accumulating regions, the sodium rhodizonate method was used. After 90 min of incubation lead was localized in the root epidermis at the proximal region of the root cap and in the cap itself just at the apical meristem of the root (Fig. 1D). After 8 h the area in which lead was present as well as the intensity of the reaction increased.

In addition to that a red strip appeared in the stele slightly above and at the proximal part of the root cap. The cells in this region were the first to show the signs of necrosis after 24 h of exposure to lead.

The fact that callose is synthesized in response to Mn (Wissemeier and Horst 1987, Wissemeier *et al.* 1993), Co, Ni, Zn (Peterson and Rauser 1979), Al (Wissemeier *et al.* 1987, Barcelo and Poschenrieder 1990, Horst *et al.* 1992, Staß and Horst 1995) and B (McNairn and Currier 1965) is quite well known. Recently,

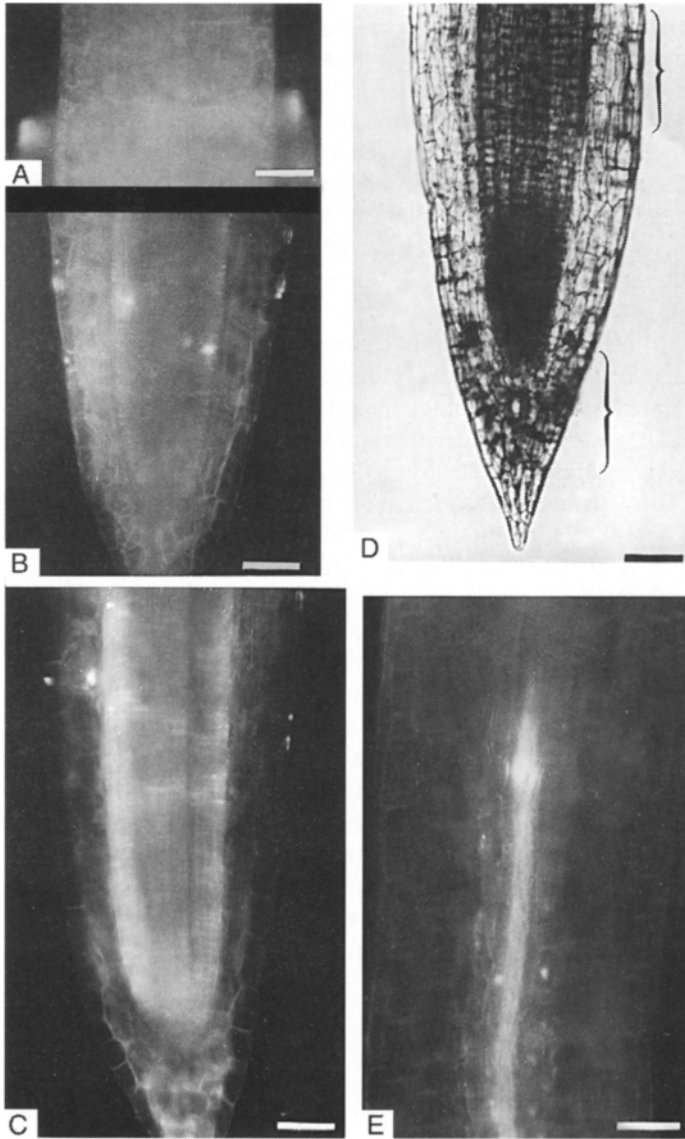


Fig. 1. Fluorescence micrographs, stained with aniline blue for (1,3)- β -glucan (fluorescent material - callose), of *Lemna minor* L. incubated in aqueous solution without and with Pb supply (scale bar = 110 μ m): *A* and *B* - control without Pb supply; *A* - the root at the proximal end of the root cap, *B* - the apical part of the root; *C* - the apical part of the root after 90 min of incubation in the aqueous solution of $\text{Pb}(\text{NO}_3)_2$ at the concentration of 0.3 μM Pb^{2+} ; *E* - the root at the proximal end of the root cap, after 24 h incubation in the aqueous solution $\text{Pb}(\text{NO}_3)_2$ at the concentration of 0.3 μM Pb^{2+} ; *D* - bright field micrograph, stained with sodium rhodizonate method (lead accumulating regions - braces), of the apical part of the root, after 90 min of incubation in the aqueous solution of $\text{Pb}(\text{NO}_3)_2$ at the concentration of 0.3 μM Pb^{2+} .

Lummerzheim *et al.* (1995) also identified lead as a causal factor in callose formation in *Arabidopsis thaliana*. The results obtained in this work showed that lead induced synthesis of callose in *Lemna minor* L. roots. Callose was deposited mainly in the cells of the meristematic region, in the protodermis walls. On the other hand, lead was shown to be present outside the meristematic region, mainly in the cells of the root cap. It is known that the meristematic region is particularly sensitive to stress factors, heavy metals included (Przymusiński and Woźny 1985, Wierzbicka 1988, Baker and Walker 1989, Woźny and Jerczyńska 1991). Therefore, it would be advantageous for the plant to use the lead induced callose synthesis in the stress avoidance strategy, *i.e.* in excluding lead from the uptake or considerably reducing it (Woźny 1993). Callose as well as other noncellulose cell wall polysaccharides (Fincher and Stone 1981) may modify physical and chemical properties of the cell wall, which can cause the metal exclusion from the symplast and protect the plasma membrane. The plasma membrane is the primary site of phytotoxic activity of many metal ions, lead among them (Christie and Costa 1984, Meharg 1993). Callose may function as a mechanical barrier (Skou 1982) and so protect the plasma membrane from metal ions (Cumming and Taylor 1990). The fact that the cells in the meristematic region were still alive after 24 h of exposure to lead while the rest of the root was dead, seems to confirm the protective role of callose.

Intensified callose accumulation in response to Co, Ni and Zn was observed in sieve plates of bean seedlings (Peterson and Rauser 1979). In *Lemna minor* L. root callose deposits were observed in the region corresponding to the central trachea of the stele (at the proximal region of the cap and slightly above). It was also the site of the most intensive lead accumulation after 24 h, and of the earliest necrosis symptoms. Not all of the described callose localization can be, though, easily explained. The fluorescent ring observed in the proximal part of the cap could be a result of callose deposition following mechanical injury, namely, separation of the cap during early stages of root development, since the cap of *Lemna minor* L. originates from the subepidermal layer of the frond (Landolt 1986). Although callose deposition on the exoplasmic side of the plasma membrane may be viewed as a resistance reaction of the cell, exceeding certain time of exposure to stress factor causes death of the plant.

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