

# Is callose a barrier for lead ions entering *Lemna minor* L. root cells?

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**Abstract** Plants have developed a range of strategies for resisting environmental stresses. One of the most common is the synthesis and deposition of callose, which functions as a barrier against stress factor penetration. The aim of our study was to examine whether callose forms an efficient barrier against Pb penetration in the roots of *Lemna minor* L. exposed to this metal. The obtained results showed that Pb induced callose synthesis in *L. minor* roots, but it was not deposited regularly in all tissues and cells. Callose occurred mainly in the protoderm and in the centre of the root tip (procambial central cylinder). Moreover, continuous callose bands, which could form an efficient barrier for Pb penetration, were formed only in the newly formed and anticlinal cell walls (CWs); while in other CWs, callose formed only small clusters or incomplete bands. Such an arrangement of callose within root CWs inefficiently protected the protoplast from Pb penetration. As a result,

Pb was commonly present inside the root cells. In the light of the results, the barrier role of callose against metal ion penetration appears to be less obvious than previously believed. It was indicated that induction of callose synthesis is not enough for a successful blockade of the stress factor penetration. Furthermore, it would appear that the pattern of callose distribution has an important role in this defence strategy.

**Keywords** Pb · Callose · Root · Duckweed · Tolerance · Cell wall

## Abbreviations

Pb	Lead ions $Pb^{2+}$
callose	1,3 $\beta$ -glucan
TEM	Transmission electron microscopy
IS	Intercellular spaces
CW	Cell wall
CWs	Cell walls

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## Introduction

According to the Environmental Protection Agency, Pb is one of the most common heavy metal contaminants in the environment (Pattee and Pain 2003). It is a non-essential element in metabolic processes and of critical concern to human and environmental health, because it is a persistent contaminant, has a low solubility, and is classified as carcinogenic and mutagenic (Diels et al. 2002; Prasad 2004). One of the most obvious symptoms of Pb toxicity in plants is the rapid inhibition of their growth. This has been attributed to several factors, among which is a decrease in

mitotic activity (Wierzbicka 1999; Samardakiewicz and Woźny 2005; Glińska et al. 2007). Plants have developed various resistance strategies against Pb, which include a range of avoidance mechanisms (Prasad 2004; Vorwerk et al. 2004; Vinocur and Altman 2005), for example formation of physical and/or chemical barriers which partly or completely limit the penetration of the organism by this stress factor. One of the most common strategies which protect plant cells from abiotic as well as biotic stress factors, e.g. infection by fungal pathogens or viruses, is the formation of the callose layer (Epel 2009; Hématy et al. 2009; Luna et al. 2011). Induction of this cell wall (CW) compound synthesis has also been observed under the influence of trace metals such as Al, Cd, Co, Cu, Ni, and Zn (Peterson and Rauser 1979; Llugany et al. 1994; Kartusch 2003; Ueki and Citovsky 2005; Poschenrieder et al. 2008; Stass and Horst 2009). The precise role of the induction of callose synthesis in plant cells exposed to toxic metal ions is still unclear. The newly produced callose may, for example, perform the function of a physical barrier which can inhibit the transport of metal ions from the apoplast to the symplast (Eticha et al. 2005; Krzesłowska 2011). However, in roots of *Lemna minor* exposed to Pb, despite the induction of callose synthesis and its deposition in the root apex (Samardakiewicz et al. 1996), Pb quickly appeared within the protoplasts (Samardakiewicz and Woźny 2000). Therefore, the present study was undertaken to attempt to explain the cause of this phenomenon.

## Material and methods

*Lemna minor* L. (duckweed) plants were cultured on a liquid medium according to Wang (1990): NaNO<sub>3</sub> (225 mg l<sup>-1</sup>), NaHCO<sub>3</sub> (150 mg l<sup>-1</sup>), MgSO<sub>4</sub>·7H<sub>2</sub>O (147 mg l<sup>-1</sup>), MgCl<sub>2</sub>·6H<sub>2</sub>O (121.6 mg l<sup>-1</sup>), CaCl<sub>2</sub>·2H<sub>2</sub>O (44.1 mg l<sup>-1</sup>), K<sub>2</sub>HPO<sub>4</sub> (10.4 mg l<sup>-1</sup>), MnCl<sub>2</sub>·4H<sub>2</sub>O (4.15 mg l<sup>-1</sup>), Na<sub>2</sub>EDTA·2H<sub>2</sub>O (3 mg l<sup>-1</sup>), FeCl<sub>3</sub> (0.96 mg l<sup>-1</sup>), H<sub>3</sub>BO<sub>3</sub> (1.85 mg l<sup>-1</sup>), Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O (72.6 μg l<sup>-1</sup>), ZnCl<sub>2</sub> (32.7 μg l<sup>-1</sup>), CoCl<sub>2</sub> (7.8 μg l<sup>-1</sup>), CuCl<sub>2</sub> (0.11 μg l<sup>-1</sup>). The plants, which were to be the object of the study, were obtained from the Prof. E. Landolt collection (Geobotanical Department of ETH, Zürich) and have been cultivated for many years in *in vitro* conditions in our laboratory. Stock cultures were incubated on Wang medium under a constant illumination of 70 μmol m<sup>-2</sup> s<sup>-2</sup> at 23±1°C. For the experiments, morphologically similar individuals were transferred for 6 h to a 50-fold diluted Wang medium with lead nitrate, containing 15 μM Pb. After such exposure conditions, Pb occurred in all layers of the root section. Moreover, what was important for the current experiments, in these conditions the amount of Pb within CW was markedly higher (Samardakiewicz and Woźny 2000). In addition, the 6-h plant exposure to Pb was

also the period of time exposure to Pb for callose induction (Samardakiewicz et al. 1996).

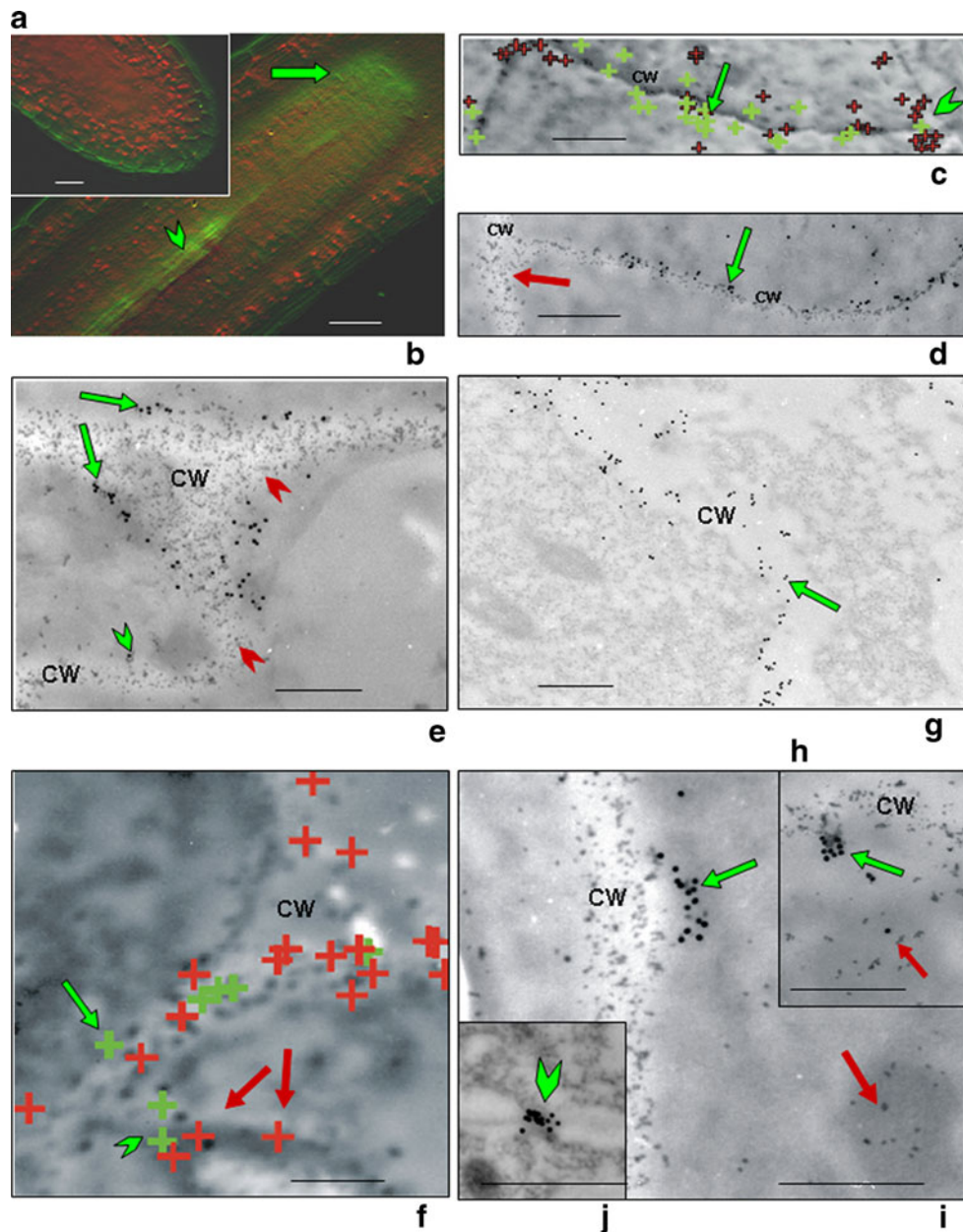
Callose was detected with aniline blue and observations were performed with a fluorescent microscope Axiovert 2000 M (Carl Zeiss, mercury lamp HB200) and in a transmission electron microscopy (TEM) (JEOL JEM 1200 EX II) by using immunogold detection with the anti-β-1,3-glucan antibody (Biosupply) according to the method described by Zadworny et al. (2007).

Lead taken up by plants was localized by using the TEM supplied with a scanning attachment and a Link AN 10 000 Energy-Dispersive Spectrometry system for X-ray mapping microanalysis of chemical elements (Samardakiewicz and Woźny 2000). This method was also used for co-localization of lead and signals from gold particles, reflecting the location of callose. The observations were performed in the three replications on the basis of approximately a dozen cross sections of nine roots.

## Results

The treatment of *L. minor* L. plants with Pb for 6 h resulted in the induction of the synthesis and deposition of callose in their roots. This was not present in the control roots, with the exception of the root cap cells, and rarely detected in such CW regions of *L. minor* roots exposed to this metal where no Pb occurred (Fig. 1a, b). Thanks to immunodetection of callose in TEM it was possible to show precisely the regions of callose occurrence in plant tissues and within individual cells. Thus, it was revealed that although callose was rather commonly synthesized in roots of *L. minor* treated with Pb, it was not evenly distributed in root tissues and within the cells. Callose was predominantly detected in the protoderm and in the centre of the root tip (procambial central cylinder) (Fig. 1b). This corresponded to the callose distribution detected by means of the fluorescent microscope (Fig. 1b).

Callose was identified in a relatively high amount in the newly formed CW (Fig. 1c, d). It primarily occupied their central parts, sometimes accompanied by irregular CW thickening formation or near the border between the newly formed CW and the CW of the mother cell (Fig. 1c, d). Moreover, callose was often observed in the anticlinal CW. In such cases, it was evenly distributed throughout their length and formed rather continuous bands (Fig. 1g). In the other CWs of *Lemna* roots, e.g. tangential and radial ones, the immunodetection in TEM showed that callose formed only small clusters or broken bands (Fig. 1e, f, h, i). In tangential CWs gold particles, reflecting the location of callose was visible in the outer CW of the protoderm in the region of the junction of two cells (Fig. 1e, f). In the radial CWs, most of the gold particles identifying callose were



**Fig. 1** Occurrence and distribution of lead (Pb) and callose in *Lemna minor* L. cells of the root tip; CW cell wall; **a, b** detection of callose by aniline blue staining (green colour) and autofluorescence of chlorophyll (red colour). Root cells of *L. minor* contain chloroplasts in the root (like other water plants), even in the root tip. The occurrence of chloroplasts and the absence of proplastids in meristematic cells—unusual for vascular plants (Landolt 1986). **a** control cells containing callose only in the CW of the root cap, **b** callose in the protoderm (green arrow) and in the procambial central cylinder (green arrowhead) in lead-treated plant. **c–j** Detection of callose and Pb by TEM in plant exposed to lead, callose indicated by immunogold (black particles), Pb visible as the electron-dense, greyish deposits, **c, e, f** callose and Pb detected by the X-ray mapping microanalysis (callose-green cross, Pb-red cross), **c, d** callose which forms continuous

bands localized in newly formed CW in its central part (green arrow and green cross) and near the border between the newly formed CW and the mother CW (green arrowhead). Pb deposits in CW (red arrow and red cross), **e** callose visible in the outer tangential CW of the protoderm (green arrow) and **f** in the radial CW (green arrowhead) as small clusters or incomplete band. Pb deposits localized mostly in the CW (red arrowhead), much lower amount of Pb detected in the protoplast than in the CW (red arrow), **g** callose which forms a continuous band in anticlinal CW of root tip (green arrow)—Pb not visible in this case, **h–j** localization and distribution of callose and Pb in radial CW. Callose indicated in the area of plasmodesmata (green arrowhead) and in the CW thickening (green arrow), Pb localized mostly in the CW, much lower amount of Pb in the protoplast than in the CW (red arrow), **a, b** Bars 25  $\mu\text{m}$ , **c–j** Bars 200 nm

localized within the plasmodesmata (Fig. 1j) and sometimes in the local CW thickenings (Fig. 1f, h, i).

It was indicated that root meristem of *Lemna*, in contrast to root meristems of land plants, contained evenly spaced

intercellular spaces (IS). In the CWs adjacent to those IS, small clusters of gold particles reflecting the location of callose were also detected.

Distribution of callose in *Lemna* CWs only partly corresponded to Pb localization. Pb deposits were commonly observed within the protoplasts (Fig. 1f, h, i). However, using TEM X-ray microanalysis, we showed that in newly formed CW and in the anticlinal CW, where callose often formed continuous bands, a lower number of Pb signals were detected or Pb was completely absent (Fig. 1c, d, g). Contrary to this, in the lateral parts of the newly formed CW, where lower number of callose signals were detected, higher number of Pb signals occurred (Fig. 1c). Thus, the presence of callose in the newly formed CW limited the amount of Pb in those CWs and protoplasts nearby, while in the other CWs, where callose occupied only small CW regions (Fig. 1f, h, i), Pb deposits occurred in high amounts and only rarely were surrounded by callose clusters (Fig. 1h, i).

## Discussion

Callose may function as a mechanical barrier (Skou 1982; Maor and Shirasu 2005; Krzesłowska 2011) and protect the plasma membrane from metal ion penetration (Cumming and Taylor 1990). Moreover, this polysaccharide, located in the adjacent CW, could also effectively block symplastic transport of metal ions through plasmodesmata (Sivaguru et al. 2000; Levy et al. 2007; Zavaliev et al. 2011). It may protect the plant from a wide spread of toxic metal ions.

However, in the light of the results included in this paper, the barrier role of callose against the penetration of metal ions is not as obvious as previously believed. The obtained results showed that Pb commonly induced callose synthesis in *L. minor* roots. When callose formed continuous bands as, e.g. in the newly formed CW and in the anticlinal CW, then it formed an efficient blockade to prevent Pb entering the protoplasts. However, in the other CWs of *L. minor* roots, where callose was deposited locally as small clusters or incomplete bands, Pb was frequently observed within the protoplasts. These facts strongly suggest that the barrier role of callose against Pb penetration depends on its deposition pattern in the cell.

The induction of callose synthesis has also been observed in other plant species exposed to this metal, e.g. *Arabidopsis thaliana* (Lummerzheim et al. 1995) or *Funaria hygrometrica* (Krzesłowska et al. 2009; Krzesłowska et al. 2010). In *F. hygrometrica* protonema for example, which elongates thanks to the tip-growing apical cell, rich in callose CW thickenings were formed at the cell apex (Krzesłowska and Woźny 2000; Krzesłowska et al. 2009; Krzesłowska et al. 2010). This was the main region where Pb entered the protonema cell (Krzesłowska and Woźny 1996). Deposited in

this site, callose could effectively limit the amount of Pb entering the protoplast. In fact, a slight amount of Pb was detected within the protoplast (Krzesłowska and Woźny 2000; Krzesłowska et al. 2009; Krzesłowska et al. 2010). Moreover, it has been shown recently that after transferring the protonemata from the medium containing Pb to the medium lacking this metal, all Pb deposits removed from the protoplast to CW and sequestered there were separated from the protoplast by a callose layer. It undoubtedly protected the cell from Pb returning to the protoplast. Indeed, almost no Pb was detected within the protoplast of the transferred protonemata, excluding Pb accumulated in the vacuole (Krzesłowska et al. 2010). Thus, in *F. hygrometrica*, callose played an important role in the defence response of the protonemata cell to this metal as an effective barrier markedly limiting the amount of Pb which entered the protoplasts.

The above appears to show that the effectiveness of callose as a barrier for Pb penetration might be different in different plants. In *L. minor* roots, unlike those of *F. hygrometrica*, although callose synthesis was commonly induced by Pb, its local distribution in most of the CW did not result in the formation of an efficient barrier against Pb penetration into the protoplasts.

Hence, it is not a rule that the appearance of callose always effectively protects the plant symplast from the entrance of Pb. It depends markedly on its deposition pattern. To our knowledge, this is the first report of an insufficient barrier being formed by callose in response to Pb, and this may shed a new light on this defence strategy, which can play such a different role in various plants despite being a reaction to the same stress factor.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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