Preliminary research on lead absorption and translocation in root tip cells of *Populus nigra* "Italica" Moench.

KRYSTYNA IDZIKOWSKA

Laboratory of Electron Microscopy, Adam Mickiewicz University, Grunwaldzka 6, 60-780
Poznań, Poland

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Abstract

Observations were carried out to define the place of lead absorption within three regions of the poplar adventitious root tip (*Populus nigra* "Italica" Moench.) after 24-hour exposure to a solution of $\text{Pb}(\text{NO}_3)_2$ ($25 \text{ mg dm}^{-3} \text{ Pb}$). Deposits of lead were not observed in certain cells of the apical part of the meristem. In other cells, lead deposits were first observed in the lumen of several endomembrane compartments — the endoplasmic reticulum, dictyosomal stacks and nuclear envelope. Certain differences were noted in the amount of deposits in protoplasts with varied electron density. In the cells with greater deposits, lead was also observed in the cytoplasm, the mitochondria and the paramural bodies. Practically no lead precipitated in the cell wall. The presence of lead in the form of small deposit granules in the plasmodesmata may suggest transfer from cell to cell.

Key words: root tip, lead treatment, ultrastructure, *Populus nigra* "Italica"

INTRODUCTION

Many studies have shown that plants accumulate lead and stated precisely with what cell structures lead precipitation and accumulation is associated (eg. Wilkins 1957, Cannon and Bowles 1962, Malone et al. 1974, Lane and Martin 1977, Simola 1977, Garland and Wilkins 1981, Lane and Martin 1982, Woźny et al. 1982, Książek et al. 1984, Wierzbicka 1984, Hübner et al. 1985, Przymusiński and Woźny 1985). However, little has been done to examine lead absorption and translocation within the root apical tip meristem. This work is an attempt to define the place of lead absorption and translocation within the poplar adventitious root tip.

MATERIAL AND METHODS

Cuttings of *Populus nigra* "Italica" Moench. were rooted in a solution of $\text{Ca}(\text{NO}_3)_2$, (0.7 g dm^{-3}). After the rooting process (about 15 days) the cuttings (on the average 6.4 cm long) were exposed to 24-hour incubation in a solution of $\text{Pb}(\text{NO}_3)_2$ at a concentration of 25 mg dm^{-3} Pb. The root tips were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, at 4°C . Postfixation was conducted in 2% OsO_4 , buffered as above and finally stained en bloc with 1% uranyl acetate for 2 hours. Following dehydration, the specimens were embedded, sectioned and poststained with uranyl acetate or were left unstained. Ultrathin sections were examined in a JEM 7A electron microscope.

RESULTS

For purposes of simplification the observations of the root tip were limited to three regions: a) the apical part of the meristem (Fig. 1-I), b) central cap cells (columella) (Fig. 1-II) and c) secretory cells (Fig. 1-III).

THE CONTROL VARIANT

Region I — Fig. 2. The cytoplasm of the cells contained numerous free ribosomes. Only a few profiles of endoplasmic reticulum with attached ribosomes were found in the cytoplasm. Golgi bodies, mitochondria, and undifferentiated plastids were the other cytoplasmic organelles to be usually observed in the cells. The nuclei of these cells were spherical and contained dark-stained nucleoli with small nucleolar vacuoles. Plasmodesmata were present in both the longitudinal and transverse walls of these cells.

Region II — Fig. 3. The central part of this region was occupied by cells of varying electron density. The cytoplasm stained less intensely and was less finely granular than that of cells in region I. Mitochondria, Golgi bodies and strands of endoplasmic reticulum were found in the cytoplasm along with abundant ribosomes. The most conspicuous organelles were plastids packed full of starch grains. These cells contained several small vacuoles. Plasmodesmata were numerous on the transverse walls.

PLATE I

Figs. 1-4. Control variant. Fig. 1. Longitudinal section of the adventitious root tip of *Populus nigra* "Italica" Moench. Region I — the apical part of meristem. Region II — central cap cells (columella). Region III — secretory cells. $\times 400$. Fig. 2. Fragments of cells from region I. D — dictyosome, ER — rough endoplasmic reticulum, M — mitochondrion, N — nucleus, P — plastid. $\times 19000$. Fig. 3. Fragments of two cells from region II (columella). CW — cell wall, Nu — nucleolus, S — starch grain. Other abbreviations as in Fig. 2. $\times 14000$. Fig. 4. Fragments of high vacuolated cells from the secretory region (III). Abbreviations as in Figs. 2 and 3. $\times 18000$

PLATE II

Figs. 5-11. Lead variant — Region I. Figs. 5 and 6. Fragments of cells from the apical part of meristem. None of the structures demonstrate any signs of the presence of lead deposits. Plastid (P) with phytoferritin. D — dictyosome, N — nucleus, M — mitochondrion. $\times 18000$ — Fig. 5 and $\times 69000$ — Fig. 6. Fig. 7. Small lead deposits inside the lumen of the nuclear envelope (NE), N — nucleus. $\times 78000$. Fig. 8. Lead deposits in the cytoplasm of the left cell, and very small grain deposits inside the lumen of the endoplasmic reticulum in the right cell. ER — endoplasmic reticulum, CW — cell wall. $\times 43200$. Fig. 9. Lead deposits inside trans- and cis-pole cisternae and in trans-pole dictyosomal vesicles. Abbreviations as in Figs. 5, 6. $\times 67000$. Fig. 10. Fragments of two cells in which lead deposits are observed in the right cell only. Small grains of lead are inside the plasmodesmata (arrow). CW — cell wall. $\times 81000$. Fig. 11. Lead deposits inside the paramural body. Abbreviations as in Figs. 5, 6 $\times 48500$

PLATE III

Figs. 12-16. Lead variant — Region II. Fig. 12. Lead in the lumen of the nuclear envelope (NE). N — nucleus, S — starch grain. $\times 51300$. Figs. 13-15. Directly connected tubular (tER) and rough endoplasmic reticulum (ER) and plasmodesmata. CW — cell wall. $\times 42000$ — Fig. 13, $\times 41000$ — Fig. 14 and $\times 41500$ — Fig. 15. Fig. 16. A few lead deposits in trans- and cis-pole cisternae of dictyosomes and in trans-pole dictyosome vesicles. M — mitochondrion, D — dictyosome. $\times 61000$

PLATE IV

Figs. 17-18. Lead variant — Region II. Fig. 17. Lead deposits close to or inside the plasmodesmata. CW — cell wall. $\times 72000$. Fig. 18. Small vesicles with fine deposits of lead between the plasma membrane and cell wall (arrows). ER — endoplasmic reticulum. $\times 42000$

Figs. 19-21. Lead variant — Region III. Fig. 19. Protoplasts of the cells from the outer edge of the root cap revealed very large differentiation in lead absorption. M — mitochondrion. Other abbreviations as in Fig. 18. $\times 35000$. Fig. 20. Large amount of lead deposits in the dictyosome structures (D). $\times 63000$. Fig. 21. Cup-shaped dictyosome containing lead. $\times 50000$

Region III — Fig. 4. The cells of this region were generally quite highly vacuolated. The granularity of the cytoplasm in these cells indicated that they were rich in free ribosomes. Mitochondria, Golgi bodies, and endoplasmic reticulum were found in the cytoplasm. Their nuclei often appeared lobed.

THE LEAD VARIANT

Region I — Figs. 5-12. The cells of this area showed relatively few deposits of lead. Some cells did not demonstrate any traces of the presence of lead (Figs. 5 and 6). In other cells of this area with less dense protoplasts, granular deposits were observed in the cytoplasm (Fig. 8 — left cell, Fig. 11 — right cell), in the lumen of the nuclear envelope (Fig. 7) and in trans- and cis-pole cisternae of dictyosomal vesicles (Fig. 9). Very fine lead deposits were observed inside the plasmodesmata (Fig. 10). In some cells lead deposits were observed inside the paramural bodies (Fig. 11).

Region II — Figs. 12-18. The protoplasts of this region indicated the presence of lead deposits in the same structures as those of region I. Also the

amount of lead deposits was not equal in all cells. In the protoplasts of certain cells very distinct lead deposits were noticed in the lumen of the nuclear envelope and in the rough- and tubular-endoplasmic reticulum (Fig. 12 — NE and Fig. 13 — tER). The reticulum was occasionally closely connected with the plasma membrane and with plasmodesmata (Figs. 14 and 15 — tER). Sometimes the tubular endoplasmic reticulum was directly connected with the rough endoplasmic reticulum (Fig. 13). Individual lead deposits were observed in trans- and cis-pole cisternae of dictyosomes and in trans-pole dictysomal vesicles (Fig. 16). In the cells with high lead content in the cytoplasm, the grains could be observed near or inside the plasmodesmata (Fig. 17). Sometimes fine deposits of lead enclosed in small vesicles were observed between the plasma membrane and the cell wall (Fig. 18 — arrows).

Region III — Figs. 19-21. The protoplasts of cells from the outer root cap revealed very large differences in lead absorption. There were some cells in which very small grains of lead could be observed in the endoplasmic reticulum (Fig. 19 — right cell). However, there were also certain cells in which very large amounts of lead deposits in all structures was noticed (Fig. 19 — left cell and Fig. 20). Only in the cells of this region were cup-shaped dictyosomes with lead deposits found (Fig. 21).

The presence of lead was not detected in cell walls and in large vacuoles.

DISCUSSION

Following 24-hour exposure of *Populus nigra* "Italica" adventitious roots to a solution of $\text{Pb}(\text{NO}_3)_2$ (25 mg dm^{-3} Pb), electron-dense lead deposits were found in all root tip cells with the exception of some cells of the apical part of the meristem. The relatively small amount of lead deposits was more or less similar in the meristem and columella regions. The deposits took the form of fine granules of varying size. Most of them were observed in the cells of the secretory region, which was directly exposed to lead. Distinct differences in respect to lead content between the cells of the central region of the meristem and secretory cells were observed earlier in *Raphanus sativus* (Lane and Martin 1977) and in *Lupinus luteus* roots (Przymusiński and Woźny 1985). The latter indicated that the central region of the meristem was especially "protected" against the presence of lead and that this protection of the meristem is due to its peculiar localization. However, they observed a large amount of lead deposits in the vacuoles and in the cell walls of this region. This seems to indicate that the mechanism of lead detoxication is identical or at least similar in different kinds of plant cells.

In this research no traces of lead deposits were found in the cell walls and the vacuoles of both the meristem and secretory areas. Instead, very fine lead deposits were found inside the channels of the plasmodesmata (desmotubules).

It seems plausible to suggest that one of the ways of lead transport may be the translocation of the element in the form of electron-dense grains by way of plasmodesmata. This hypothesis is supported by the existence of direct contact between the tubular endoplasmic reticulum and both the plasmodesmata and the rough cisternal endoplasmic reticulum. The retention of structural continuity between the two structures — the endoplasmic reticulum and desmotubules — has been confirmed by earlier observations (Phillips and Torrey 1974, a, b, Hawes et al. 1981, Overall et al. 1982, Stephenson and Hawes 1986). Stephenson and Hawes (1986) argue that "the close apposition of endoplasmic reticulum and the cell wall implies a potential for the transfer of material between those compartments". The existence of direct links between the tubular and rough endoplasmic reticulum was observed earlier in developing seeds of *Vicia faba* during the protein deposition phase of seed maturation by Harris (1979).

Dictyosomal stacks were the other endomembrane compartments in which electron-dense deposits were observed. Fine, granular deposits were found in the lumen of trans- and cis-pole cisternae, as well as in trans-pole dictyosome vesicles. The presence of deposits in analogous elements of the Golgi apparatus was attested by Hübner et al. (1985) who used Pb as an electron-dense marker in their investigation of endocytosis in maize root cap cells. However, those authors rarely observed dictyosomes with deposits in more than one cisterna.

Lead deposits were also observed in the nuclear envelopes and in the small vesicles between the plasma membrane and the cell wall. The presence of this element in certain membrane compartments (tubular and cisternal endoplasmic reticulum, nuclear envelope, small vesicles and dictyosomes) indicates the great role these structures play in the complex process of lead translocation.

Lead translocation is an intensely complex process and its conclusive explanation still requires further research.

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Wstępne badania nad absorpcją i przemieszczaniem się ołowiu w komórkach wierzchołka wzrostu korzenia Populus nigra "Italica" Moench.

Streszczenie

Przeprowadzono obserwacje miejsc absorpcji ołowiu w komórkach trzech stref wierzchołka wzrostu korzeni przybyszowych topoli *Populus nigra* "Italica" po 24 godzinach działania azotanu ołowiawego ($25 \text{ mg dm}^{-3} \text{ Pb}$). Nie stwierdzono obecności ołowiu tylko w niektórych komórkach apikalnej części merystemu. W pozostałych komórkach ołów absorbowany był w takich strukturach komórkowych, jak: retikulum endoplazmatyczne, otoczka jądrowa i diktiosomy. Obserwowano pewne różnice w ilości strąków w protoplastach o różnej gęstości elektronowej. W komórkach o większej zawartości ołowiu spotykano go dodatkowo w cytoplazmie, mitochondriach oraz w strukturach granicznych. Nigdy nie spotykano strąków ołowiu w samej ścianie komórkowej. Obecność ołowiu w formie drobnych ziaren stwierdzano natomiast w plasmodesmach, co może w pewnym stopniu sugerować przemieszczanie się tego pierwiastka z komórki do komórki, również poprzez te struktury.