Cyto-Histological Features of Malformations in Mulberry Roots Induced by Heavy Metals

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(Received September 1, 1983)

Anatomical studies on the effects of heavy metals on the morphogenesis of mulberry roots were carried out, using malformed roots induced by heavy metals. Cadmium-induced imbalances in the growth of root tissue were observed, especially inhibition of cell division in the cortex, shrinkage of stelar cells by extraordinary expansion of cortical cells, cell crushing and malformations of the nuclei. Copper mainly induced histogenetic disorders of the root apex, chimeralization of the tissues, imbalance of cell growth within and between tissues, separation of the tissues from each other and condensation of nuclear matter by collapse of the membrane system. Zinc affected scarcely root morphogenesis and induced only local swelling of the cortex.

In soils containing excess heavy metals such as zinc and cadmium in area around zinc smelters, growth of mulberry has been observed to be inhibited and various malformed roots have appeared (Homma and Shirata, 1977). Similar growth inhibition and malformation of roots were induced experimentally by supplying heavy metals (Takagishi et al., 1976). As roots make contact with soils containing excess heavy metals which have been accumulating for a long time in mulberry fields, the most important and primary problems to consider on the effects of heavy metals on mulberry plants must be how they influence the function and morphogenesis of roots. However, few studies have been performed on the mechanism of growth inhibition by heavy metals from the point of view of the effects on mulberry morphogenesis. This experiment has therefore concentrated on the effects of heavy metals on the morphogenesis of mulberry roots, and anatomical research into malformations induced by heavy metals was carried out.

Materials and Methods

Mulberry seeds of the variety "Roso" were sown on 0.8% agar containing Knop's medium and grown for 15 days after germination at 28-30°C and 12 hrs L-12 hrs D regime in phytotron. Mulberry seedlings were then cultured for a week in Kasugai's nutrient solutions for land crops with and without heavy metals.

Two cm long root segments were collected from tips and fixed in FAA. Segments were dehydrated, embedded in paraffin and sections 8-10 μm in thickness cut with a microtome. Root sections were then stained using the PAS reactions and observed under a light microscope.

Additional heavy metals such as cadmium, copper and zinc were used as sulfate salts at concentrations of 10⁻⁵ M to 10⁻³ M and the pH of each nutrient solution was adjusted to within 6.4 to 6.6.

Results

Cyto-histological features of tissues from near
Figs. 1-9. Longitudinal sections of untreated controls and roots treated with heavy metals.
the root tips of control mulberry plants grown in solutions without supplied heavy metals are shown in Figs. 1, 6, 8, 10, 11, and 18. In longitudinal sections, cells of the epidermis, cortex, stele, and root cap were rectangular and arranged regularly soon after initiation from apical initial cells (Fig. 6). In transverse sections, tissues such as the epidermis, cortex, and endodermis were easily distinguishable from each other (Fig. 10 and 12). In the stele, protophloem (p) and proto-vessel (v) were differentiated as diarch types of radial vascular bundles (Fig. 13). Compared with these roots of control mulberry plants, many kinds of variations were observed in the histological features of plants treated with heavy metals.

**Morphological features of roots treated with cadmium.**

In roots treated with cadmium at a concentration of $10^{-5}$ M, the histological features were almost normal except extraordinary expansion of the cortical cells (Fig. 2). At a concentration of $10^{-4}$ M, one-sided cell growth was induced within the root (Fig. 14). At a concentration of $10^{-3}$ M, profiles of roots cut in transverse section took a half-moon shape (Fig. 15) or were polyhedra (Fig. 16). In such roots, phenomena such as shrinkage of the nuclei, malformation of the stelar cells, and enlargement of the cortex cells and enlargement of the cortex cells causing stelar crushing were observed. From the above-mentioned results, abnormal imbalances of cell growth between and within tissues were assumed to be induced in the roots by the cadmium treatment.

**Morphological features of roots treated with copper.**

By treating plants with copper at a concentration of $10^{-5}$ M, the terminal part of the root became thickened and the cortex stained less densely in a similar way to roots treated with cadmium (Fig. 3). At a concentration of $10^{-4}$ M, malformed roots were frequently observed as shown in Fig. 5, becoming swollen in a region 1000 µm behind tip but extremely slender the base. Observing these roots in detail, histological zonaion of the tissues became undistinguishable and the cell arrangement of tissues was disordered (Fig. 7). Other phenomena such as separation of the exodermis and inhibition of cell division in the cortex (Figs. 9 and 20) were also observed. At a concentration of $10^{-3}$ M, shrinkage of cortical cells was induced and nuclei were appressed against the cell wall. In addition, the membrane system of stelar cells was seen to be collapsed and nuclear matter had begun to be condensed (Figs. 17, 21, and 22).

**Morphological features of roots treated with zinc.**

The terminal root was sometimes thickened

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**Fig. 1.** Histological features of normal control root. Tissue organization such as cortex (c) and stele (s) are clearly recognized.

**Fig. 2.** Root treated with Cd at a concentration of $10^{-5}$ M. Cortex swollen about 700 µm behind the tip was observed.

**Fig. 3.** Root treated with Cu at a concentration of $10^{-5}$ M. Swollen tip caused by extraordinary expansion of cortical cells has appeared.

**Fig. 4.** Root treated with Zn at a concentration of $10^{-4}$ M.

**Fig. 5.** Root treated with Cu at a concentration of $10^{-4}$ M. Remarkably swollen tip is shown.

**Fig. 6.** Cytohistological features of control root apex (a). Distinction between stelar initials (s. i.) and cortical initials (c. i.) is apparent.

**Fig. 7.** Root treated with Cu at a concentration of $10^{-4}$ M. Histological organization of root apex was not easily recognized. Starch grains (s. g.) of root cap cells were fewer and smaller in comparison with control roots.

**Fig. 8.** Root cap of control plant. It was rich in large starch grains.

**Fig. 9.** Cortex and stele of roots treated with Cu at a concentration of $10^{-4}$ M. Inner cortical cells near the tip are locally swollen (s. c.).
Figs. 10-22. Transverse sections of control and treated roots.
Malformed roots of mulberry by heavy metals

The most terminal root cap cells of control plants were rich in starch (Fig. 8) but those of plants treated with copper and cadmium were smaller and poorly developed (Fig. 7).

When roots were exposed to excess heavy metals, many kinds of malformations were induced. Copper has been reported to induce roots like barbed wire or a lion's tail in rice plants (Chino and Kitagishi, 1966) and onion roots swollen near the tips (Ichikura et al., 1970). In Akita prefecture, roots shaped like scrubbing brushes appeared in paddy fields polluted by local mine workings (Tsukamoto, 1957). Furthermore, histological research on malformed roots induced experimentally supplying copper or copper pollutants from a factory producing precision machines was carried out by the author (1972). This work revealed that histological abnormalities such as extraordinary expansion of cells, cell death by collapse, delay of organization and tissue chimeralization characterised by difference of growth between cell layers occurred in the region near the apical meristem.

Of malformations induced by cadmium, curved roots in green cow pea plants (Imai and Siegel, 1973) have been found. In this experiment cadmium induced unbalanced growth of root tissues especially by inhibition of cell division in the cortex, shrinkage of stelar cells by extraordinary expansion of the cortical cells and malformation of the cortical cells and malformation of the nuclei.

Copper mainly induced histogenetic disorder in the root apex, chimeralization and separation of the tissues and condensation of nuclear matter caused by collapse of the membrane system. Zinc, however, showed only a slight effect on root morphogenesis except for swelling of the cortex.

As mentioned above, the apical meristem see-

Fig. 10. Control root, 800 µm behind tip. Exodermis (e'), cortex (c), endodermis (en) and stele (s) are clearly recognized.
Fig. 11. Control root, 1000 µm behind tip. Cortical cells are generally slightly expanded.
Fig. 12. Control root, 1500 µm behind tip. In the stele, protophloem (p) and protovessels (v) are differentiated as a diarch type of radial vascular bundle.
Fig. 13. Stele of control root. Protophloem (p) was clearly recognized by the formation of sieve tubes (outlined in black).
Fig. 14. Root treated with Cd at a concentration of 10^{-4} M. Growth pattern of one half differs from the other and in the right side of this figure remarkable shrinkage of each tissue is observed.
Fig. 15. Root treated with Cd at a concentration of 10^{-3} M. The cut profile of a transverse section was half-moon shaped. In the cortex, the outer cells shrank but the inner cells expanded.
Fig. 16. Root treated with Cd at a concentration of 10^{-3} M. Cortical cells shrank as a whole and their nuclei were appressed against the cell walls and lens-shaped.
Fig. 17. Root treated with Cu at a concentration of 10^{-3} M. Membrane system of stelar cells (s) has collapsed and nuclear matter has begun to be condensed. Also, the nuclei of the inner cortical cells are appressed against the cell wall. No vascular tissues can be observed in stelar cells.
Fig. 18. Control root, 500 µm behind tip. Exodermis cells are actively dividing.
Fig. 19. Root treated with Cd at a concentration of 10^{-4} M. Histological features resemble those of control roots.
Fig. 20. Root treated with Cu at a concentration of 10^{-4} M. Separation of the exodermis from the cortex has been induced.
Fig. 21. Root treated with Cd at a concentration of 10^{-3} M. Abnormally swollen cortical cells are seen to crush stelar cells.
Fig. 22. Longitudinal section of root like that shown in Fig. 21. A curved tip and a stele (s) crushed by swollen cortex (c) can be observed.
med to be most susceptible of root tissues to excess heavy metals. With copper, development of the apical meristem was suppressed, due partially or wholly to inhibition of cell division (Kuno, 1983). Histogenetic disorders of the apical meristem and other abnormalities of the root apex were more marked at higher concentrations.

In roots damaged by excess copper, Kamata and Chino (1977) reported abnormalities such as lack of apex and appearance of primordia of lateral roots initiated close to the terminal region of the root together with unequal growth of the root apex between the left and right sides.

However, cadmium or zinc showed rather stronger influences on the development of formed tissues or tissue initials such as the cortex or stele. Cadmium and zinc induced unbalanced growth of tissues or irregularity of mitotic division within a tissue. As a result, malformations such as curved roots and swollen cortex appeared near the tip.

With reference to cytological abnormalities, delay of cell division and deterioration of the membrane system were observed in roots of *Alnus rubra* due to higher concentrations of cadmium (Wickliff and Evans, 1980). In cytological research on the toxicities of heavy metals in animal cells, abnormal phenomena were observed such as vacuolation of mitochondria, deterioration of the membrane system, disappearance of chromatin and the increment of lysosomes (Mizuhira *et al.*, 1975). Differences clearly exist between animals and plants in the effects of heavy metals, but deterioration of the nuclear and membrane system appear to be common to both plant and animal cells. Such abnormalities induced by heavy metals seem to be a serious problem for cell survival.

Chino (1979) studied transport and accumulation of heavy metals in plant tissues by X-ray micro-analyzer and observed that copper accumulated in the epidermis, exodermis and pericycle around the stele whereas zinc existed in the epidermis at lower concentrations. At higher concentrations, it was found that both heavy metals tended to be heavily accumulated in the stele.

From the report of Chino and the results obtained in this experiment, the regions where inhibition of cell growth and malformation of tissues occurred were considered to coincide with areas where heavy metals tend to accumulate.

Acknowledgement: I am grateful to professor Dr. S. Homma, Tokyo University of Agriculture and Technology, for his help in reviewing the manuscript.

References


久野勝治：重金属で生じた奇形根の細胞・組織的性状

カドミウム、銅および亜鉛のそれぞれ 10⁻³〜10⁻² M で処理して出現した奇形根の細胞組織的性状を観察した。カドミウム処理根では先端部の肥大などのほか皮層細胞の収縮。異常肥大、中心柱細胞の圧縮、壊死などが高濃度処理区でみとめられた。銅処理根では各組織の細胞分裂が多方向で阻害された。すなわち、組織形成の乱れ、組織の異常化、組織間または組織内の生長の不均衡、核の変形、組織間の剥離、さらに進んで組織の崩壊による核質の凝集が引き起こされていいた。亜鉛処理根では皮層の局所的な肥大現象以外奇形現象は高濃度処理区でもほとんど認められなかった。異常現象の発生を根の区域別にみると根端付近に集中していた。