Role of root functions on cadmium uptake by plants – structural aspects of root organization

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Abstract: Plant root is the main organ of water and nutrient entry to the plant body. It is also the main organ for uptake of pollutants including heavy metals like cadmium. Structural adaptations of roots for cadmium transfer from water or soil solution to the plant are reviewed in this paper. Differences between the plants differing in uptake of cadmium and tolerance to this toxic metal and the effect of cadmium on root structure are summarized.

Keywords: cadmium, root, endodermis, exodermis, peri-endodermal layer, apoplast, symplast

1. Introduction

The structure of root is closely related with its function. Various aspects of plant root response are studied in reaction to cadmium (Cd), one of the most important inorganic pollutants. Cadmium disturbs cellular redox environment of the root causing oxidative stress [1]. Various parts of root metabolism are affected as a consequence of Cd, including water and nutrient uptake and inhibition of several enzyme activities [2]. In vast majority of the species the large proportion of Cd is deposited in roots, and only a small amount is translocated to the shoot. Practical consequences of this phenomenon are health danger of edible roots like carrot or radish cultivated even in slightly contaminated soils [3] and the possibility to use some species for phytostabilization of Cd in soils by their roots [4]. In a few Cd hyperaccumulator species the concentration of Cd in shoots is higher than in roots. The limit value for hyperaccumulation was set to 100 $\mu g.g^{-1}$ DW in above-ground plant parts [5]. Hyperaccumulation and high tolerance of Cd by plants is a complex phenomenon. Reactions of plants and specifically roots to Cd treatment are intensively studied. Numerous results document metabolical and physiological changes induced by toxic effect of this metal as well as adaptation of tolerant species. Fewer results are available about the structural background of metal tolerant plants and the differences between closely related species or clones of the same species. The aim of the present overview is focused on some structural aspects of plants in their reaction to Cd stress and the differences between species and clones with different accumulation and translocation of Cd and sensitivity to this toxic metal.

2. Material and Methods

Plants of various species including model plants of Cd hyperaccumulators (*Thlaspi caerulescens* syn. *Noccaea caerulescens*), accumulators (*Silene vulgaris*) and non-accumulating sensitive species (*Thlaspi arvense*, *Arabidopsis thaliana*), crop species maize (*Zea mays*), radish (*Raphanus sativus*), wheat (*Triticum durum*) and some woody plants (*Salix spp.*, *Karwinskia spp.*, tea plant *Camellia sinensis*) and some South African bulb species were studied in their reaction to Cd treatment. Plants were grown mostly hydroponically in controlled conditions of growth chambers, or on agar media and treated with Cd in the form of $Cd(NO_3)_2 .4H_2O$ in several concentrations from 5 µM up to 100 µM.

For structural observations the root samples were prepared as hand sections stained by berberine or Fluorol Yellow 088 for visualization of suberin in exo- and endodermis [6]. For lignin histological determination phloroglucinol reaction was used. Other samples were embedded in Spurr resin and semi-thin sections were compared and proportions of tissues were quantified in light microscope using image analysis by Lucia (v.4.8, Laboratory Imaging Praha). For detail observations TEM and SEM or cryo-scanning electron microscopy were used. The Cd concentrations were determined by flame atomic absorption spectroscopy (AAS) (PerkinElmer #1100), or ICP-MS (Elan 6000, Pe Sciex, Canada).

3. Results and Discussion

Root apoplastic barriers and the effect of cadmium

Specific role in the root function is attributed to apoplastic barriers. The innermost cortical layer of all vascular plants (with few exceptions) is developed as endodermis with characteristic cell wall modifications. Endodermal ontogenesis may proceed in three states: formation of Casparian bands occurs in the first developmental state, deposition of suberin lamella in the second. In the third state, development of thick secondary walls (sometimes called tertiary walls, if suberin lamella is considered as secondary wall layer) takes place. At this state the function of endodermis is mostly mechanical and it protects the vascular cylinder in older root parts. Casparian bands represent local wall impregnation by lignin and suberin and they form, together with the tight junction between the cell wall and plasma membrane in the area of Casparian bands, an apoplastic barrier for transport of water and ions. However, it was shown, that this barrier is not completely “safe”, and at least some water can pass through these regions [7]. Development of endodermis is only rarely finished in this first state (e.g. in some aquatic species). The role of “safer” apoplastic barrier is attributed to the second state, when the whole inner surface of endodermal cell walls is covered by layers of lamellar deposits of suberin. In the third state, sometimes several micrometers thick layers of secondary wall material are deposited internally and they are often impregnated by lignin, in some cases even by silicon.

Casparian bands are often developed very close to the root apex; the distance varies according to the species, root type and the speed of root growth. Generally it is between 1 and 10 mm from the root apex. The zone of gradual development of the second state (suberin lamellae deposition) along the root axis is usually very long. It may vary from several millimeters from the root apex to several hundred millimeters. Along this distance the number of endodermal cells with suberin lamellae increases. The number of cells not covered by suberin lamellae is decreasing and cells remaining in the first state are called passage cells. They may become delayed in ontogenesis even in the case when majority of endodermal cells is in the third state. Eventually they may remain in the first state permanently, however in the majority of the plant species it is not so.

A special type of hypodermis that develops Casparian bands is defined as the exodermis [8] and its presence was shown in majority of angiosperm species. That layer can be developed in the same three states as endodermis. The exodermis can be uniseriate or multiseriate, contrary to (almost always) uniseriate endodermis. The exodermis usually develops in a greater distance from the root apex than the endodermis.

The development of endodermis under the effect of Cd is accelerated and Casparian bands and suberin lamellae are developed closer to the root apex [9, 10]. Earlier maturation, closer to the root apex may be partially attributed to the reduced speed of the growth. However, even if the root length is expressed proportionally to the reduced growth, the onset of individual developmental states starts earlier. This was observed in maize roots after Cd treatment [11, 12]. Schreiber et al. [11] found that in maize roots also composition of endodermal suberin and lignin was modified. Cadmium stress resulted in approximately three-fold increase of endodermal suberin and two fold increase of endodermal lignin. Earlier maturation of endodermis induced by Cd was observed in several other species. In *A. thaliana* the width of Casparian bands and thickness of suberin lamellae in endodermal cells increased under Cd influence (Martinka unpublished). All these endodermal changes may be interpreted as a reaction of root reducing radial transport of Cd to roots and its translocation to the shoot.

Interesting results were obtained when willow clones with different characteristics of Cd tolerance, accumulation and translocation were compared [13]. The most prominent difference was found between high and low translocators. In high translocating clones suberin lamellae were deposited more distant from the root apex compared with those with low translocation (Fig. 1).

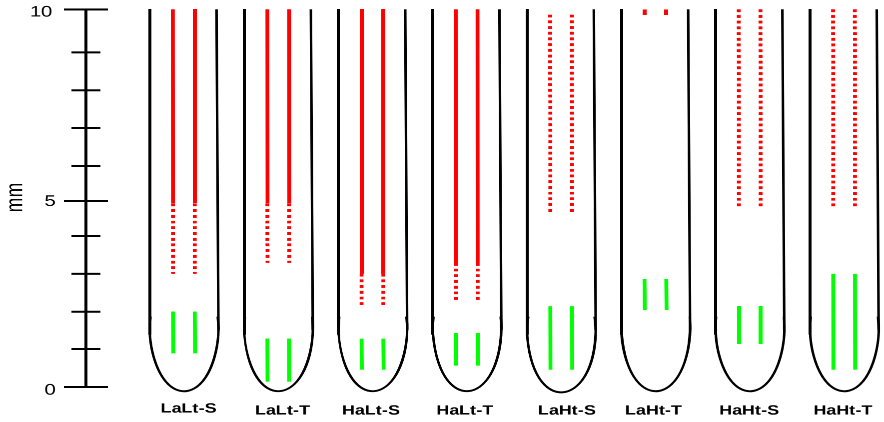

Fig. 1 Appearance of Casparian bands (state 1 of endodermal development; solid line close to the root apex) and suberin lamellae (state 2 of endodermal development; dotted line) in roots of different *Salix* clones with various properties of Cd accumulation and Cd tolerance. Clone names refer to the property to accumulate, translocate and tolerate Cd. La, low accumulation; Ha, high accumulation; Lt, low translocation; Ht, high translocation; T, tolerant; S, sensitive. Note the development of suberin lamellae close to the root apex in clones with low Cd translocation contrary to the clones with high Cd translocation. Reproduced with permission from Physiologia Plantarum (modified after Lux et al., 2004).

Root reaction to cadmium stress

Reaction of root exposed to Cd stress results in decrease of growth, necrosis and death. Various species react differently, according to their sensitivity to this toxic metal. It is well known, that in many species even a low concentration of Cd in soil or water reduces growth and several other structural changes can occur. Premature lignification of xylem elements induced by Cd was reported in several species, e.g. in pine roots in the distance from the root apex which usually represents the elongation zone [14]. Similarly in cereals the premature xylogenesis occurs in roots (in barley [15], in various hybrids of maize; Lukáčová unpublished). Abnormal premature lignification was observed also in other root tissues as induced by Cd treatment.

Cell wall thickening is associated with high heavy metal and salt stresses [16]. In some maize hybrids and in radish seminal roots early thickening of endodermal cell walls was observed after severe Cd stress (Fig. 2). In radish lateral roots lignification of cortical cell walls adjacent to endodermis and lignification of pericycle occurred. In sorghum occasionally multi-layered endodermis was formed after influence of Cd treatment. In certain bulbous monocotyledon species unusual formation of wound periderm was observed as a reaction to Cd stress with suberin and lignin deposition to the cell walls internally adjacent to exodermal layer. Resulting additional layer of impermeable cell walls close to the root surface may decrease the radial transport of Cd ions to the vascular cylinder. Additionally to these unpublished results several other cases of abnormal lignification and suberinization of epidermal and endodermal cell walls were described in literature. In reed (*Phragmites australis*) these processes occurred in absorption and elongation zones of root apex [17]. Another structural feature of some wetland plant species is connected with metal exclusion ability of tolerant species. These species show “tight barrier”, formed by lignin and suberin deposition, and densely packed sclerenchymatous fibers with thick secondary walls in the peripheral layers of cortex. The barriers conferred to exclusion ability of tolerant species [18]. All these observations demonstrate the formation of protective additional barriers preventing or at least decreasing the radial transport of toxic Cd ions to the vascular cylinder and to the xylem. Xylem is the major route for root-to shoot translocation, as it was shown in several species [19, 20]. Higher Cd xylem loading and rapid root to shoot translocation are the major processes determining the high accumulation in shoots and grains of rice plants [19].

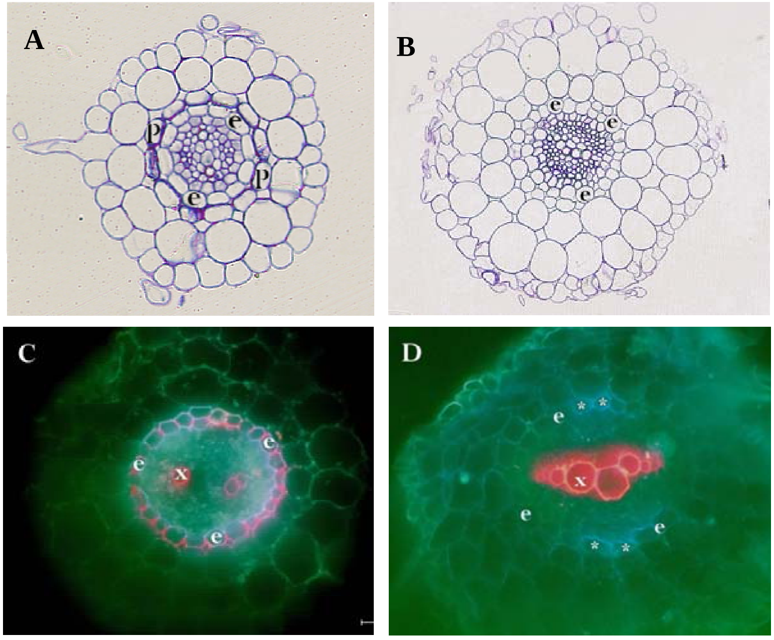

Fig. 2 Transversal sections of roots showing structural modifications related with cadmium hyperaccumulation and cadmium treatment. A/ root of hyperaccumulating species *Thlaspi caerulescens*. Externally adjacent to endodermis (e) the additional layer of lignified peri-endodermal cells (p) is formed. B/ root of sensitive non-accumulating species *Thlaspi arvense* do not form peri-endodermal cell wall thickenings. C/ root of radish (*Raphanus sativus*) forms premature lignified endodermis (e) after cadmium treatment. D/ root of radish (even in more advanced ontogenic stage as in C) in control conditions; endodermis is starting to form suberin lamellae in the cells opposite to phloem poles (*), no lignification occurs contrary to cadmium treated roots. (x – xylem vessels)

The mechanisms that control Cd absorption by roots and the role of root apoplast and symplast in accumulation and transport of Cd to vascular elements is still not completely understood [21]. Retention of Cd in root apoplast may form a part of a defense reaction that limits the entry of toxic metal. However, the differences between the species exist. Dynamics of distribution of Cd among its different forms in apoplast and symplast were investigated in various species. In roots of soybean the largest portion of Cd was found in the intercellular spaces at the beginning of its uptake, the content started to decrease after one hour and between 13 and 24 h treatment the equilibrium was reached in which about 70 % of Cd was found inside cells [22]. On the other hand, several authors [23, 24] reported that Cd is stored mainly in the apoplast of hyperaccumulator *Thlaspi caerulescens* roots. Exclusion from the symplast may play a role in Cd tolerance in this species. Similarly in the hairy roots of *T. caerulescens* Cd accumulated particularly at the root apices and in the root hairs. High Cd concentration in root hairs was observed soon after exposure to the metal, however, Cd accumulation in the apices increased from 7th day to 21th day, when Cd staining was intensive in the apex. In contrast to *T. caerulescens* the root apices of *Nicotiana tabacum* were essentially Cd-free. However, Cd was toxic to *N. tabacum* hairy roots even though about 75% of the metal remained with the cell walls [25]. These results together with several other published findings about Cd uptake and tolerance by plants support previously reported suggestion that, rather than greater metal uptake levels relative to non-hyperaccumulators, the distinguishing feature of hyperaccumulator roots is their ability to tolerate heavy metals and continue growth while assimilating high concentrations of metal ions [26].

In connection with the different retention of Cd in apoplast of *T. caerulescens* roots in comparison with non-tolerant species it is tempting to speculate about the role of recently reported specific structural feature of *T. caerulescens* roots [27, 28]. A cell layer termed “peri-endodermal layer” is developed in roots of this hyperaccumulator species adjacent to the outer side of the endodermis (Fig. 2). The cells of this layer develop irregular secondary wall thickenings impregnated by suberin/lignin on the inner tangential walls and surround endodermis externally. This layer was found in some closely related hyperaccumulator species (*T. goesingense*), but it is not present in closely related non-accumulating species (*T. arvense* or *A. thaliana*). The peri-endodermal layer is developed close to the root apex, in the distance less than 1 mm from the root apex. This distance corresponds to the zone where protoxylem elements are differentiating. The thickened walls of peri-endodermal layer might provide a deposition site of Cd ions and may increase the delay in Cd transport through plasma membrane into the endodermal cells and internally to the vascular cylinder. The temporary retention of Cd ions in the root apoplast is considered an important defence mechanism against Cd toxicity, enabling activation of intracellular detoxification mechanisms of Cd in *T. caerulescens* roots [28].

Other structural features which should be taken into consideration in transport processes across the root are proportions of individual tissues, proportion of intercellular spaces and/or formation of aerenchyma. Few data are available in literature about this topic. Previously we have found a relationship between the root structure and sensitivity to Cd ions in *Salix* clones with contrasting characteristics of Cd sensitivity. Clones characterized by high tolerance had statistically highly significantly higher proportion of epidermal, exodermal and endodermal tissues when compared with sensitive clones ([13] Fig. 3).

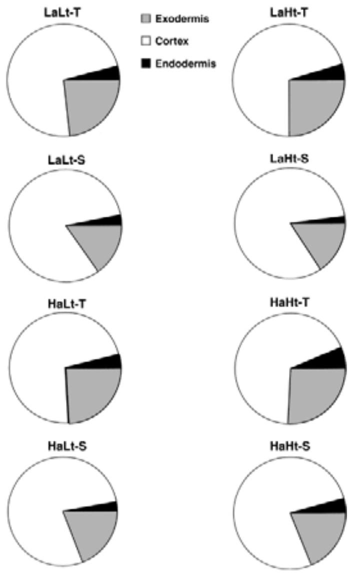

Fig. 3 Area proportions of exodermis, mid-cortex and endodermis of the total cortex area in transversal sections 2 mm distant from the root apex of different *Salix* clones with various properties of Cd accumulation and Cd tolerance. Clone names refer to the property to accumulate, translocate and tolerate Cd. La, low accumulation; Ha, high accumulation; Lt, low translocation; Ht, high translocation; T, tolerant; S, sensitive. Note higher proportions of endodermis and exodermis in tolerant clones. Reproduced with permission from Physiologia Plantarum (Lux et al., 2004).

Higher proportion of epidermal tissues was found also in *T. caerulescens* roots when compared with sensitive non/accumulating species *T. arvense* [28].

One aspect of root structural studies in relation with toxic metal uptake has been almost completely neglected. It is the role of lateral roots. Majority of our knowledge about the root structure in relation with the transport processes is based on the study of main roots (primary seminal roots, or adventitious roots). These long, fast growing roots represent only one part of the plant root strategy, reaching remote areas for colonization of large volume of soil or reaching deep sources of water. They presumably sacrifice some of the selective capacity [29]. The other role is attributed to fine, slowly growing roots with better control of ion transport. This problem was recently raised by Watt et al. [30], when roots of small grain cereals were investigated and five distinct types of lateral roots based on xylem anatomy and not on root diameter or branching order were found. This raised question of their functional significance, especially because the very small diameters of xylem tracheary elements in the most frequent type of laterals suggest low axial water flow. This may “slow down” drying of surrounding soil, conserve valuable deep water, and increase the time to absorb nutrients and also ions of toxic metals.

4. Conclusions

The plant root structure in connection with Cd uptake, translocation and sensitivity to this metal is still insufficiently investigated. In accordance with Markert et al. [31] we may agree that “More anatomical and cytological studies on plant structure (especially the root cortex) should be combined with detailed physiological studies on ion uptake and transport. These two aspects are generally studied individually. However, the modifications in the structure and function of root tissues may help us to explain differences in root system functioning and ion uptake between plant species.”

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