REVIEW PAPER

Root responses to cadmium in the rhizosphere: a review

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Abstract

This article reviews the responses of plant roots to elevated rhizosphere cadmium (Cd) concentrations. Cadmium enters plants from the soil solution. It traverses the root through symplasmic or apoplasmic pathways before entering the xylem and being translocated to the shoot. Leaf Cd concentrations in excess of 5–10 μg g⁻¹ dry matter are toxic to most plants, and plants have evolved mechanisms to limit Cd translocation to the shoot. Cadmium movement through the root symplasm is thought to be restricted by the production of phytochelatins and the sequestration of Cd-chelates in vacuoles. Apoplasmic movement of Cd to the xylem can be restricted by the development of the exodermis, endodermis, and other extracellular barriers. Increasing rhizosphere Cd concentrations increase Cd accumulation in the plant, especially in the root. The presence of Cd in the rhizosphere inhibits root elongation and influences root anatomy. Cadmium concentrations are greater in the root apoplasm than in the root symplasm, and tissue Cd concentrations decrease from peripheral to inner root tissues. This article reviews current knowledge of the proteins involved in the transport of Cd across root cell membranes and its detoxification through sequestration in root vacuoles. It describes the development of apoplastic barriers to Cd movement to the xylem and highlights recent experiments indicating that their maturation is accelerated by high Cd concentrations in their immediate locality. It concludes that accelerated maturation of the endodermis in response to local Cd availability is of functional significance in protecting the shoot from excessive Cd loads.

Key words: Accumulation, apoplasm, cadmium, endodermis, maize, root, suberin lamellae, symplasm, tissue asymmetry, transport.

Introduction

Cadmium (Cd) is toxic to plant cells, even at low concentrations. Leaf concentrations greater than 5–10 μg Cd g⁻¹ DM are toxic to most plants (White and Brown, 2010), although some ecotypes of a few plant species have adapted to grow on soils with high Cd concentrations and can tolerate leaf concentrations in excess of 100 μg Cd g⁻¹ DM (Reeves and Baker, 2000; Broadley et al., 2001; Verbruggen et al., 2009). These Cd-hyperaccumulator plants include ecotypes of Noccaea (formerly Thlaspi) caerulescens (J&C Presl.) FK Mey (Reeves et al., 2001), Arabidopsis (formerly Cardaminopsis) halleri (L.) O’Kane and Al-Shehbaz (Bert et al., 2002), Sedum alfredii Hance (Yang et al., 2004), Viola baoshanensis Shu, Liu et Lan. (Wei et al., 2004), Thlaspi praecox Wulf. (Vogel-Mikuš et al., 2005), Picris divaricata Vant. (Tang et al., 2009), and Phytolacca americana L. (Liu et al., 2010b). The roots of some of these exceptional plant species proliferate in Cd-enriched patches of soil, which contrasts with the behaviour of roots of most plant species that generally avoid such patches (Whiting et al., 2000; Liu et al., 2010a). To prevent Cd accumulation in shoot tissues, plants have evolved various mechanisms to restrict the entry of Cd to the xylem. This article reviews our current knowledge of these mechanisms, which include (i) the production of Cd-chelates in the cytoplasm of root cells and the sequestration of Cd-chelates in the vacuole to restrict Cd delivery to the xylem from the symplast, and (ii) the development of physical barriers to the extracellular movement of Cd to the xylem to restrict Cd delivery to the xylem from the apoplasm.
Cadmium in the soil

To limit Cd concentrations in edible produce, Cd concentrations lower than 3 μg g⁻¹ dry soil have been recommended for agriculture and horticulture (Mengel et al., 2001). Cadmium concentrations in non-polluted soils increase with clay concentration, but are generally lower than 1 μg g⁻¹ dry soil (Mengel et al., 2001). Cadmium is concentrated in the topsoil, where it is associated with organic matter. Solutions extracted from non-polluted soils generally have Cd concentrations less than 40–300 nM (Wagner, 1993). Cadmium availability to plants is greater in acid soils (Mengel et al., 2001; Tudoreanu and Phillips, 2004; Kirkham, 2006), and its solubility is increased by root exudates (Zhu et al., 1999). Cadmium occurs in the soil solution predominantly as Cd²⁺, but also as Cd-chelates (Tudoreanu and Phillips, 2004). Low Cd²⁺ concentrations in the soil solution, in combination with low diffusion coefficients for Cd²⁺ in aqueous solutions, suggest that transpiration-driven mass-flow of the soil solution will dominate the delivery of Cd²⁺ to plant roots (Sterckeman et al., 2004). This is consistent with reports that Cd accumulation by plants grown in soil is directly related to transpiration (Ingwersen and Streck, 2005). Higher soil Cd concentrations can occur either naturally or through anthropogenic activities (Mengel et al., 2001; He et al., 2005; Kirkby and Johnson, 2008). Natural mineral outcrops can be enriched in Cd through the weathering of Cd-rich rocks. Cadmium pollution of the environment has occurred through mining and refining of metal ores, and through the application of Cd-containing phosphate fertilizers, sewage sludge, and municipal composts to agricultural soils. The ability to grow on soils with high Cd concentrations is generally related to the ability of roots to exclude Cd from the plant and/or of plant tissues to chelate Cd as a non-toxic compound or sequester it in a non-vital cellular compartment. Plants tolerating high Cd concentrations, and especially plants accumulating this metal in their above-ground parts, have potential utility for the phytosequestering and phytochelation of contaminated soils (Raskin and Ensley, 2000; Schwitzguébel et al., 2009).

Cadmium uptake by plant roots

In nature, shoot Cd concentrations vary greatly. Although much of this variation can be attributed to environmental factors, there is appreciable phylogenetic variation in shoot Cd concentrations (Broadley et al., 2001; Watanabe et al., 2007). When grown in the same environment, shoot Cd concentrations are generally highest in species from the Caryophyllales and Lamiales, and lowest in monocot species (Broadley et al., 2001). Shoot Cd concentrations are determined largely by Cd entry to the root, sequestration within root vacuoles, translocation in the xylem and phloem, and dilution within the shoot through growth. Cadmium concentrations are often (but not always) greater in roots than in shoots, suggesting that Cd transport to the xylem is restricted in most plants, and lowest in seeds, fruits, and tubers, suggesting that Cd is not readily translocated in the phloem (Seregin and Kozhevnikova, 2008; Conn and Gillilham, 2010). Grafting experiments have suggested that shoot Cd concentrations in Nicotiana (N. tabacum L. and N. rustica L.; Wagner et al., 1988), Solanum (S. integrifolium, S. melongena, and S. torvum; Arao et al., 2008), and Noccaea Thlaspi (Noccaea caerulescens and Thlaspi perfoliatum; Guimarães et al., 2009) are controlled by root properties.

Shoot Cd concentration often increases to a maximum value with increasing Cd concentration in the soil (Logan et al., 1997; Zhao et al., 2003; Sterckeman et al., 2004; Vogel-Mikusi et al., 2005; Chen et al., 2008; Peng et al., 2009; Liu et al., 2010b), bulk soil solution, or nutrient solution (Yang et al., 1995; Perriguey et al., 2008; Street et al., 2009), although non-saturating relationships have also been reported (Salt et al., 1995; Ingwersen and Streck, 2005). The concentration dependence of Cd uptake from hydroponic solutions measured over short periods into either excised roots or intact plants generally follows the sum of a single Michaelis–Menten component plus a linear component (Table 1). The linear component is often attributed to tight Cd binding to cell walls, but it could also represent an apoplastic Cd flux through the xylem (cf. White, 2001; White, 2005; Plaza et al., 2008; Pedas et al., 2008; Verbruggen et al., 2009). The presence of organic acid anions in hydroponic solutions increases the capacity of both the Michaelis–Menten component and the linear component of Cd uptake, but the reasons for this are unclear (Berkelaar and Hale, 2003a; Han et al., 2006; Zhao et al., 2006), but Cd uptake by the Cd-hyperaccumulating Ganges ecotype of N. caerulescens was not inhibited by divalent cations nor by La³⁺ (Lombi et al., 2001; Zhao et al., 2002). The presence of organic acid anions in hydroponic solutions increases the capacity of both the Michaelis–Menten component and the linear component of Cd uptake, but the reasons for this are unclear (Berkelaar and Hale, 2003a; Han et al., 2006).

Cadmium can enter root cells as Cd²⁺ through ZIP (Zinc-regulated transporter/Iron-regulated transporter-like Protein) transporters, such as orthologues of AtIRT1 and TcZNT1/TcZIP4, through orthologues of the wheat TaLCT1 transporter, or via cation channels, such as depolarization-activated calcium channels (DACC), hyperpolarization activated calcium channels (HACC), and voltage-insensitive cation channels (VICC), all of which are relatively non-selective between cations (Fig. 1; Clemens et al., 1998; Cohen et al., 1998; White and Broadley, 2003; White, 2005; Plaza et al., 2007; DalCorso et al., 2008; Pedas et al., 2008; Verbruggen et al., 2009). In addition, Cd might enter root cells as Cd-chelates through YSL (Yellow-Stripes 1-Like) proteins (Curie et al., 2009). Cadmium can then reach the xylem through a symplasmic pathway formed by the cytoplasm of individual root cells connected by plasmodesmata. The Cd species transported through the symplasm are unknown, but could include Cd²⁺ or Cd-chelates (Verbruggen et al., 2009). Cadmium is loaded from the symplasm into the xylem by heavy metal P₁B-ATPases, such as orthologues of AtHMA2 and AtHMA4, and possibly also by YSL proteins.
The Arabidopsis ABC transporter AtPDR8 has been implicated in Cd efflux across the plasma membrane of root hairs and epidermal cells (Kim et al., 2007).

In many plant species, Cd tolerance is related to Cd accumulation in the vacuole (Chardonnes et al., 1998; Cosio et al., 2005; Korenkov et al., 2007; Seregin and Kozhevenkova, 2008). Cadmium can be translocated across the tonoplast by H+/Cd2+-antiporters, such as orthologues of AtCAX2 and AtCAX4 (Korenkov et al., 2007, 2009), by heavy metal P1B-ATPases, such as orthologues of AtHMA3 (Morel et al., 2009), and as Cd-chelates by ABC transporters, such as orthologues of AtMRP3 (Tommasini et al., 1998; Table 1. Published relationships between cadmium (Cd) uptake (V) by excised roots or intact plants and Cd in the external solution ([Cd]_ext), described by Michaelis–Menten functions using the terms V_max (V, when [Cd]_ext = 0) and K_m ([Cd]_ext, when V = 0.5V_max), and a linear term k (V/[Cd]_ext).

<table>
<thead>
<tr>
<th>Species (Genus)</th>
<th>Conditions</th>
<th>[Cd]_ext (µM)</th>
<th>K_m (nM)</th>
<th>V_max (nmol g⁻¹ FW h⁻¹)</th>
<th>K_m (µM)</th>
<th>V_max (nmol g⁻¹ FW h⁻¹)</th>
<th>k (nmol g⁻¹ FW h⁻¹ µM⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean (Glycine max (L.) Merr.)</td>
<td>Intact plants</td>
<td>0.000002.5–0.5</td>
<td>76</td>
<td>22.9</td>
<td>1.2</td>
<td>232</td>
<td>ND</td>
<td>Cataldo et al., 1983</td>
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<tr>
<td>Maize (Zea mays L.)</td>
<td>Intact plants</td>
<td>0.063–0.164</td>
<td>30–100</td>
<td>*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Mullins and Sommers, 1986</td>
</tr>
<tr>
<td>Maize (Zea mays L.)</td>
<td>Intact plants</td>
<td>0.25–5.0</td>
<td>200</td>
<td>20.31</td>
<td>ND</td>
<td>ND</td>
<td>16</td>
<td>Han et al., 2006</td>
</tr>
<tr>
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<td>Intact plants</td>
<td>0.00001–100</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Yes</td>
<td>Perriguey et al., 2008</td>
</tr>
<tr>
<td>Maize (Zea mays L.)</td>
<td>Excised roots</td>
<td>0.05–50</td>
<td>260</td>
<td>23.6**</td>
<td>ND</td>
<td>ND</td>
<td>3.6**</td>
<td>Redjala et al., 2009</td>
</tr>
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<td>Lupin (Lupinus albus L.)</td>
<td>Intact plants</td>
<td>0.00005–5.0</td>
<td>42</td>
<td>11.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Costa and Morel, 1993</td>
</tr>
<tr>
<td>Lettuce (Lactuca sativa L.)</td>
<td>Intact plants</td>
<td>0.00005–5.0</td>
<td>8–30</td>
<td>32–43**</td>
<td>0.011–0.054</td>
<td>2420–7740**</td>
<td>ND</td>
<td>Costa and Morel, 1994</td>
</tr>
<tr>
<td>Pea (Pisum sativum L.) [Fe-replete]</td>
<td>Excised roots</td>
<td>1–100</td>
<td>600</td>
<td>34</td>
<td>ND</td>
<td>ND</td>
<td>5.3</td>
<td>Cohen et al., 1998</td>
</tr>
<tr>
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<td>Intact plants</td>
<td>0.05–1.25</td>
<td>20</td>
<td>26</td>
<td>ND</td>
<td>ND</td>
<td>28</td>
<td>Hart et al., 1998</td>
</tr>
<tr>
<td>Bread wheat (Triticum aestivum L.)</td>
<td>Intact plants</td>
<td>0.05–1.5</td>
<td>59</td>
<td>33</td>
<td>ND</td>
<td>ND</td>
<td>Yes</td>
<td>Hart et al., 2002</td>
</tr>
<tr>
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<td>Intact plants</td>
<td>0.05–1.25</td>
<td>40</td>
<td>29</td>
<td>ND</td>
<td>ND</td>
<td>22</td>
<td>Hart et al., 1998</td>
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<tr>
<td>Durum wheat (Triticum turgidum L. var. durum)</td>
<td>Intact plants</td>
<td>0.05–1.5</td>
<td>67</td>
<td>38</td>
<td>ND</td>
<td>ND</td>
<td>Yes</td>
<td>Hart et al., 2002</td>
</tr>
<tr>
<td>Durum wheat (Triticum turgidum L. var. durum)</td>
<td>Intact plants</td>
<td>0.05–1.8</td>
<td>166</td>
<td>0.87</td>
<td>ND</td>
<td>ND</td>
<td>1.69</td>
<td>Harris and Taylor, 2004</td>
</tr>
<tr>
<td>Noccaea caerulescens (Prayon)</td>
<td>Intact plants</td>
<td>0.2–60</td>
<td>260</td>
<td>33</td>
<td>ND</td>
<td>ND</td>
<td>6.0</td>
<td>Lombi et al., 2001</td>
</tr>
<tr>
<td>Noccaea caerulescens (Prayon)</td>
<td>Intact plants</td>
<td>0.2–60</td>
<td>930</td>
<td>21.8</td>
<td>ND</td>
<td>ND</td>
<td>4.2</td>
<td>Lombi et al., 2002</td>
</tr>
<tr>
<td>Noccaea caerulescens (Ganges)</td>
<td>Intact plants</td>
<td>0.2–50</td>
<td>180</td>
<td>160</td>
<td>ND</td>
<td>ND</td>
<td>11.2</td>
<td>Lombi et al., 2001</td>
</tr>
<tr>
<td>Noccaea caerulescens (Ganges)</td>
<td>Intact plants</td>
<td>0.2–50</td>
<td>1000</td>
<td>187.6</td>
<td>ND</td>
<td>ND</td>
<td>3.6</td>
<td>Lombi et al., 2002</td>
</tr>
<tr>
<td>Noccaea caerulescens (Ganges)</td>
<td>Intact plants</td>
<td>&lt;5</td>
<td>450</td>
<td>143</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Zhao et al., 2002</td>
</tr>
<tr>
<td>Noccaea caerulescens (Nc-H)</td>
<td>Excised roots</td>
<td>0.05–50</td>
<td>390</td>
<td>130**</td>
<td>ND</td>
<td>ND</td>
<td>1.38**</td>
<td>Redjala et al., 2009</td>
</tr>
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<td>Excised roots</td>
<td>0.05–50</td>
<td>70</td>
<td>23.4**</td>
<td>ND</td>
<td>ND</td>
<td>2.22**</td>
<td>Redjala et al., 2009</td>
</tr>
<tr>
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<td>Excised roots</td>
<td>0.05–50</td>
<td>350</td>
<td>39.7</td>
<td>ND</td>
<td>ND</td>
<td>15</td>
<td>Zhao et al., 2006</td>
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<tr>
<td>Noccaea caerulescens (Nc-L)</td>
<td>Excised roots</td>
<td>0.05–50</td>
<td>2540</td>
<td>44</td>
<td>ND</td>
<td>ND</td>
<td>0.8</td>
<td>He et al., 2007</td>
</tr>
<tr>
<td>Noccaea caerulescens (Nc-L)</td>
<td>Excised roots</td>
<td>0.05–1.2</td>
<td>380</td>
<td>270**</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Mori et al., 2009</td>
</tr>
<tr>
<td>Noccaea caerulescens (Nc-L)</td>
<td>Excised roots</td>
<td>0.05–1.2</td>
<td>353</td>
<td>**</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Mori et al., 2009</td>
</tr>
</tbody>
</table>
Cobbett, 2000; DalCorso et al., 2008; Verbruggen et al., 2009). Within plant cells, Cd is bound to S-containing ligands, such as those present in glutathione, metallothioneins, and phytochelatins (DalCorso et al., 2008). In many plant species, the synthesis of phytochelatins is induced by Cd exposure (see Salt et al., 1995; Schat et al., 2002; Clemens, 2006; Ernst et al., 2008; Seregin and Kozhevnikova, 2008; Persson et al., 2006) and Cd is often sequestered in the vacuole as Cd-phytochelatin complexes (Cobbett, 2000; Clemens, 2006; Ernst et al., 2008). The involvement of phytochelatins in Cd detoxification is consistent with observations that mutants deficient in phytochelatin synthesis show lower Cd tolerance than wild-type plants and mutants with enhanced phytochelatin synthesis can show higher Cd tolerance than wild-type plants (Cobbett, 2000; Clemens, 2006). However, differences in Cd tolerance among natural ecotypes of several plant species appear to be unrelated to differences in phytochelatin synthesis, suggesting additional mechanisms of Cd-detoxification (Schat et al., 2002). In some plant species, metallothioneins play an important role in Cd detoxification (Ernst et al., 2008; Shim et al., 2009). The production of metallothioneins is also induced by exposure to Cd, and plants genetically engineered to produce metallothioneins in greater concentrations show increased Cd tolerance (see DalCorso et al., 2008; Korenkov et al., 2009). Cadmium is thought to be exported from the vacuole by NRAMP (Natural Resistance-Associated Macrophage Protein) transporters, such as orthologues of AtNRAMP3 and AtNRAMP4 (Thomine et al., 2003; Verbruggen et al., 2009).

It is also possible for Cd$^{2+}$ and Cd-chelates to reach the xylem solely via an extracellular, apoplasmic pathway in regions of the root lacking a Casparian band (Berkelaar and Hale, 2003b; Lux et al., 2004). The uptake of cationic elements reaching the xylem via an apoplasmic pathway is generally restricted to the extreme root tip and to regions in which lateral roots are being initiated (White, 2001; Moore et al., 2002). Although the relative contributions of the symplastic and apoplasmic pathways to the delivery of Cd to the xylem are unknown, it is likely that the relative contribution of the apoplasmic pathway will increase as the Cd concentration in the rhizosphere solution increases, as has been proposed for both Zn and Na (White et al., 2002; Plett and Möller, 2010).

Supporting the presence of an apoplasmic pathway, are observations that the root tip is the most active region of the root for Cd$^{2+}$ influx (Piñeros et al., 1998), and that Cd accumulation by wheat cultivars is positively correlated with their number of root apices (Berkelaar and Hale, 2000). A linear component to the concentration dependence of Cd uptake on Cd concentration in the nutrient solution is almost universally observed in short duration studies on hydroponically-grown plants (Table 1). This phenomenon can be interpreted as the result of an apoplasmic Cd flux to the xylem. In challenging this view, Xing et al. (2008) showed that, among accessions of N. caerulescens with contrasting abilities to take up Cd and Zn, the amount of Cd taken up by roots over a 24 h period and translocated to the shoot was inversely related to apoplasmic water flows assayed using a membrane-impermeable fluorescent dye.

![Fig. 1. Proteins thought to be responsible for Cd influx to the symplasm, sequestration in the vacuoles of root cells, and efflux to the rhizosphere and to the xylem (see text for details).](image_url)
However, this is inconsistent with studies of the effects of transpiration on Cd accumulation by other plant species (Ingwersen and Streck, 2005). Reducing transpiration by the addition of ABA to the nutrient solution has also been shown to reduce both xylem sap Cd concentration and shoot Cd accumulation of hydroponically grown plants (Salt et al., 1995; Hsu and Kao, 2003; Zhao et al., 2006; Liu et al., 2010b), but this has been interpreted as an effect of ABA on symplastic loading of Cd into the xylem since Cd uptake by roots themselves was unaffected by the presence of ABA (Salt et al., 1995; Zhao et al., 2006). More persuasive arguments for symplastic delivery of Cd to the xylem are (i) that there is competition between Cd$^{2+}$ and other cations for uptake by roots (Hart et al., 2002) and (ii) the contrasting abilities of $N$. caerulescens accesses to accumulate Cd and Zn independently in the shoot provides evidence of transport selectivity (Xing et al., 2008), both of which can be taken as evidence for protein-mediated symplasmic transport (White, 2001).

**Barriers to apoplastic movement of solutes to the xylem**

The isolation of the stele from the peripheral cell layers of the root is critical for the control of solute transport to the shoot. It is effected by cell wall impregnations and the most important impregnating substance in this context is suberin (Franke and Schreiber, 2007).

Cadmium uptake by plant roots is generally restricted to young subapical regions of actively growing roots (Piñeros et al., 1998). The peripheral cell layers in these regions are specialized for solute uptake. The epidermal layer, which constitutes the outermost cell layer and differs from the epidermal layer of above-ground plant parts both in lacking cutin and through the development of root hairs, is termed the rhizodermis (von Guttenberg, 1968). The rest of the peripheral tissues are termed the cortex. The endodermis separates the cortex from the stele and extracellular movement of solutes to the xylem is restricted by suberin deposited in endodermal cell walls. Suberin, together with lignins, form the impregnation material of Casparian bands developed in radial and transverse endodermal cell walls (Schreiber et al., 1999; White, 2001). This impregnation, the lack of intercellular spaces between endodermal cells, and a tight junction between cell walls and the plasma membrane form the apoplastic barrier of the root (White, 2001). The endodermis with its Casparian bands represents a considerable, but not impenetrable, barrier to solute movement through the apoplasms (Steudle et al., 1993; White, 2001; Ranathunge et al., 2005).

Casparian bands represent the first endodermal ontogenic stage (Stage I), which is only rarely (e.g. in some aquatic species; Seago, 2002) the final stage. The deposition of lamellar suberin, in this case on the whole inner cell wall surface, is the commonly occurring second endodermal ontogenic stage (White, 2001). Stage II endodermis presents a more complete apoplastic barrier to the radial flow of water and solutes to the xylem in more mature parts of the root (Melchior and Steudle, 1993; Peterson et al., 1993; Steudle and Peterson, 1998; White, 2001). Nevertheless, the absolute amount of suberin deposited in root cell walls affects the radial transport of water and ions, as demonstrated by the enhanced suberin1 (esb1) mutant of Arabidopsis thaliana Heynh. (Baxter et al., 2009). This mutant has elevated amounts of suberin in the root, most likely in the endodermis, reduced water flow to the xylem, and a decrease in shoot Ca, Mn, and Zn accumulation (Baxter et al., 2009).

However, quantitative differences in root suberin concentrations may not be the only factor influencing the apoplastic movement of water and solutes to the xylem, and both the chemical nature of the suberins, together with the microstructure of deposits, must also be taken into consideration (Schreiber et al., 2005). Differences in suberin composition along the root axis during the development of the endodermis are indicated by an increase in fatty acid ω-hydroxylation (Thomas et al., 2007; Höfer et al., 2008). However, association of these changes with functional properties of suberized barriers in the endodermis remains to be demonstrated.

In some plant species, more distant from the root apex, in even older parts of the root, the endodermis may pass to the third stage (von Guttenberg, 1968; White, 2001). Stage III endodermis is characterized by thick cellulose secondary walls (sometimes classified as tertiary walls) deposited over the suberin lamellae. This cell wall layer, together with the original primary wall, is often lignified, and in some species impregnation of walls with silicon may occur (Sangster and Parry, 1976; Lux et al., 1999). In Stage III, the function of the endodermis is already mostly mechanical and radial transport of water and solutes is limited (Melchior and Steudle, 1993; White, 2001).

The gradual changes in cell wall composition of the endodermis can extend for a considerable distance along the root. The first stage, characterized by Casparian bands, often starts very close to the root apex. The zone of gradual development of the second stage, the deposition of suberin lamellae, is usually very long. It may vary from several millimetres to several hundred millimetres from the root apex. Throughout this distance the number of endodermal cells without suberin lamellae decreases, and the cells not covered by suberin lamellae are called passage cells. Passage cells can be present even when the majority of endodermal cells have entered the third stage of development. Indeed, passage cells can remain in the first state permanently, although in the majority of the plant species this does not occur.

Hydropodermal layers, characterized by a gradual thickening of cell walls, are often present in older roots, especially in monocotyledons. A suberized periderm is developed in older roots of dicotyledons and gymnosperms. Periderm has been shown to act as a barrier preventing the movement of water and ions (Vogt et al., 1983), gases (De Simone et al., 2003), and pathogen incursion (Lulai and Corsini, 1998). From a functional viewpoint, these older regions of the root contribute little to water uptake (Melchior and Steudle, 1993).
and are mostly engaged in the long-distance transport of water and solutes. This function is concentrated in inner root tissues, within the vascular cylinder, or in the secondary vascular tissues.

In the majority of angiosperms another apoplasmic barrier, the exodermis, can develop in parallel with the endodermis (Perumala et al., 1990; Peterson and Perumala, 1990; Hose et al., 2001; Ma and Peterson, 2003). The exodermis develops in the same three stages as the endodermis. The exodermis can be uniseriate or multiseriate, in contrast to the uniseriate endodermis. The exodermis usually develops at a greater distance from the root apex than the endodermis (Ma and Peterson, 2003). However, environmental conditions can modify the rate of development of the exodermis (Zimmermann and Steudle, 1998) and accelerated development of the exodermis has been associated with reduced Cd uptake by roots (T Redjala and I Zelko, personal communication). In some plant species, and under specific environmental conditions, the exodermis may differentiate earlier than the endodermis. This has been observed in some wetland plants (Seago et al., 1999; Soukup et al., 2002), in tea (Homma et al., 2000; Tanimoto et al., 2004), and in maize (Zea mays L.) grown in soil (T Redjala and I Zelko, personal communication). The importance of the exodermis as an environmentally variable barrier to the uptake of water and ions was recognized by Peterson et al. (1993), and several subsequent studies have confirmed this conclusion (Peterson, 1997; Meyer et al., 2009). Under some conditions, such as in hydroponics, the exodermis can be absent, even in species in which it is normally present (Zimmerman and Steudle, 1998).

The rhizodermis, exodermis, and endodermis have all been shown to act as barriers to the apoplasmic movement of toxic elements, including Cd (Gierth et al., 1999; White, 2001; Enstone et al., 2003; Seregin et al., 2004; Seregin and Kozhevnikova, 2008). The additional peri-endodermal layer of cells with lignified cell walls present in N. caerulescens may function similarly (Zelko et al., 2008). These apoplasmic barriers develop closer to the root apex when roots are exposed to high concentrations of potentially toxic elements. Accelerated development of both the endodermis and exodermis have been observed in various plant species in response to salinity (Reinhardt and Rost, 1995; Karahara et al., 2004), and the multiple environmental stresses caused by cultivation in municipal solid waste slag with high salt and heavy metal content have been shown to induce extensive thickening of the inner tangential walls of maize endodermal cells (Degenhardt and Gimmler, 2000). Exposure to Cd has been found to result in the formation of Casparian bands and suberin lamellae closer to the root apex in several plant species, including A. thaliana (Schreiber et al., 1999), Silene dioica (Martinka and Lux, 2004), woody shrub species such as Karwinskia humboldtiana (Zelko and Lux, 2004), and maize (Fig. 2; Vaculì¬k et al., 2009). Maturation of the endodermis closer to the root apex can be attributed partly to a reduction in the rate of root

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**Fig. 2.** The development of endodermal suberin lamellae in the roots of maize plants after 10 d growth in Hoagland’s solution containing no cadmium (Cd0) or 5 μM Cd(NO₃)₂·4H₂O (Cd5). Three regions of the root can be distinguished: A, a region in which endodermal suberin lamellae are fully developed (solid green lines), B, a region in which the suberin lamellae are partially developed (broken green lines), and C, a region lacking endodermal suberin lamellae. Because the length of roots grown in the absence and presence of Cd differed, the distance from the root tip is expressed as percentage of the total root length. Endodermal suberin lamellae developed closer to the root apex in roots exposed to Cd, when expressed in either absolute or percentage terms. White arrows indicate suberin lamellae in the endodermis.
extension, but it is also a consequence of accelerated development of the endodermis. Exposure of maize roots to Cd resulted in an approximately 3-fold increase of endodermal suberin and a 2-fold increase of endodermal lignin (Schreiber et al., 1999). It also altered the chemical composition of endodermal suberin and lignin (Schreiber et al., 1999). All these changes can be interpreted as acclimatory responses that reduce the apoplastic movement of Cd to the xylem and its translocation to the shoot. This hypothesis is supported by the observation that low Cd translocation to the shoot among willow clones with contrasting Cd tolerance, accumulation and translocation traits was correlated with the deposition of suberin lamellae closer to the root apex (Fig. 3; Lux et al., 2004).

Effects of cadmium on root anatomy

In addition to the accelerated development of the endodermis and exodermis, many other aspects of root anatomy are altered when roots are exposed to elevated Cd concentrations in the rhizosphere (Seregin et al., 2004; Seregin and Kozhevnikova, 2008). Elevated rhizosphere Cd concentrations often result in changes in the relative proportion and size of root tissues and cell types. Although many of these changes depend upon the rhizosphere Cd concentration, and appear to be species- and tissue-specific, they can be found both in plants permanently exposed to elevated Cd concentrations in the natural environment as well as in plants growing in artificial media in the laboratory.

Cadmium concentrations in the rhizosphere that inhibit root growth by about 50% have been shown to increase the production of root hairs close to the root apex in maize (Seregin and Ivanov, 2001), radish (Raphanus sativus L.; Vitória et al., 2003), barley (Hordeum vulgare L.; Durčeková et al., 2007), sorghum (Sorghum bicolor L.; Kuriakose and Prasad, 2008), and rhode grass (Chloris gayana Kunth.; Kopittke et al., 2010), suggesting that Cd accelerates the maturation of these cells. However, higher Cd concentrations in the rhizosphere can result in reduced root hair production and the disintegration of the rhizodermis and outer cortical cell layers (Seregin et al., 2004; Kuriakose and Prasad, 2008; Gratão et al., 2009). The disintegration of cortical cells, together with a general loss of cell turgor, results in the occurrence of conspicuous intercellular air spaces and irregular-shaped epidermal and cortical cells in roots of willow (Salix alba L.), poplar (Populus × euramericana) and radish exposed to high rhizosphere Cd concentrations (Lunácková et al., 2003; Vitória et al., 2003).

Several studies have indicated that root diameter is increased by rhizosphere Cd concentrations that do not cause significant necrosis. For example, roots of willow, poplar, Miscanthus sinensis L. and maize plants grown in media containing Cd were shorter and thicker than plants grown in media lacking Cd, although their apices turned brownish (Lunácková et al., 2003; Scebbba et al., 2006; Maksimović et al., 2007). Maksimović et al. (2007) attributed the greater diameters of maize roots exposed to Cd to an increase in the size of parenchyma cells, and proposed that the enlargement of cortical tissues had a functional role by increasing resistance to radial flows of water and solutes. The size of cells in the exodermis of maize roots, as well as cells in the central cylinder and vascular tissues, were unaffected by Cd exposure (Maksimović et al., 2007). By contrast, willow clones characterized by high Cd tolerance had a greater proportion of epidermal, exodermal, and endodermal tissues than Cd-sensitive clones, which had a greater proportion of mid-cortical tissues (Lux et al., 2004). Other studies have observed no effect of Cd exposure on diameters of bean (Phaseolus vulgaris L.) roots (Vázquez et al., 1992), nor the diameters, lengths or specific surface areas of maize roots (Florijn et al., 1993), although Vázquez et al. (1992) did observe an increase in the size of parenchyma cells in the cortex of bean roots exposed to Cd.

Relatively little information is available about Cd-induced changes in the development of cells and tissues localized within the central part of roots. This topic requires more attention, especially considering the importance of xylem loading in regulating Cd fluxes to the shoot (Papoyan et al., 2007; Ueno et al., 2008; Lu et al., 2009; Uraguchi et al., 2009b; Verbruggen et al., 2009). Vitória et al. (2003) observed the proliferation of cambial cells followed by a loss of organization of the cambial region in radish roots exposed to 0.5 mM Cd, suggesting that Cd accelerated root maturation, including the development of xylem elements in the central cylinder. Consistent with this interpretation, Schützendübel et al. (2001) found that exposure to 50 μM Cd caused accelerated lignification of protoxylem elements.

Fig. 3. Development of Casparian bands (stage 1 of endodermal development; solid green lines close to the root apex) and suberin lamellae (stage 2 of endodermal development; red lines) 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 high translocation and distant from the root apex in clones with low translocation of Cd. Modified from Lux et al. (2004) with permission from Physiologia Plantarum.
closer to the root apex in Scots pine (Pinus sylvestris L.), and Důrcová et al. (2007) reported premature xylogenesis in barley roots exposed to Cd. Lunáčková et al. (2003) observed that cambial activity and the formation of lateral primordia occurred closer to the root apex in plants exposed to Cd, suggesting Cd accelerated the production of lateral roots. By contrast, although Vázquez et al. (1992) found an increase in the number of pericycle cells when roots were exposed to Cd, they observed a reduction in cell differentiation and lignification in the vascular cylinder of Cd-treated bean plants. Similarly, Ederli et al. (2004) found no significant changes in the structure of reed (Phragmites australis (Cav.) Trin. ex Steud.) root exposed to 100 μM Cd.

Root anatomy responds to local cadmium concentrations in the rhizosphere

To elucidate responses in root anatomy to local high rhizosphere Cd concentrations, as might occur in natural soils, a novel experiment was designed. The aim of this experiment was to determine whether unilateral exposure of roots to Cd could induce asymmetrical development of the endodermis or other root tissues. This would test whether individual root cells responded directly to Cd in their immediate vicinity to restrict Cd movement to the xylem through the development of appropriate apoplastic barriers.

Maize (Zea mays L. hybrid Josefina) seedlings with seminal roots 5–6 cm in length were placed between two layers of agar (Fig. 4A). In the control–control treatment (Cd0–Cd0) neither agar layer contained Cd. In the control–Cd treatment (Cd0–Cd100), one agar layer contained no Cd whilst the other agar layer contained either 50 μmol Cd(NO₃)₂·4H₂O or 100 μmol Cd(NO₃)₂·4H₂O. Agar layers were placed in the two halves of square Petri dishes (245×245 mm, Corning Incorporated, USA) fixed vertically in a growth chamber with controlled environmental conditions (temperature, 24±1 °C; relative air humidity, 70%; light intensity, 150 μmol m⁻² s⁻¹). To avoid the diffusion of Cd²⁺ from agar layers containing Cd, plastic spacers were placed between the agar layers to maintain a distance of 1 mm, which corresponded to the diameter of the growing maize roots. This design ensured that only the root surface touching the agar layer containing Cd was exposed directly to Cd in the environment. Roots exposed to the Cd0–Cd0 treatment grew gravitropically and after 4 d they had elongated by 4–5 cm. By contrast, roots exposed to the Cd0–Cd100 treatment exhibited limited growth and after 4 d had elongated by only 1.0–1.5 cm. The apices of roots exposed to the Cd0–Cd100 treatment bent into the agar layer containing Cd and their growth then stopped (Fig. 4B). Bending was caused by the cessation of cell elongation on the side of the root exposed to the Cd-containing agar, whilst cell elongation continued on the other side of the root.

Two days after placing plants in Petri dishes, several roots were removed and transverse sections were cut at regular 0.5 cm distances from the root apex to the base. Transverse sections were stained with Fluorol Yellow 088 to identify suberin lamellae (Brundrett et al., 1991; Lux et al., 2005) and with phloroglucinol–HCl to identify lignin.

Fig. 4. Effects of unilateral exposure of maize roots to 100 μM Cd(NO₃)₂·4 H₂O. (A) Maize seedlings with 5–6 cm long seminal roots were placed between two layers of agar containing zero or 100 μM Cd. (B) Photograph taken 4 d after unilateral exposure of maize roots to Cd showing them bending in the direction of the Cd layer. (C) Longitudinal section of the apex of a root bending toward the Cd agar layer stained by phloroglucinol–HCl to detect lignin. A lateral root primordium has been initiated on the side opposite the Cd layer. Abbreviations: lrp, lateral root primordium; asterisk, ectopic deposition of lignin. Scale bar: 500 μm.
In the Cd0–Cd0 treatment, endodermal cells with suberin lamellae first started to appear at 8 cm from the root tip. At 9 cm from the tip approximately 50% of endodermal cells developed suberin lamellae and in 10 cm from the root tip 80–100% of endodermal cells were covered by suberin lamellae (Fig. 5). In the Cd0–Cd100 treatment, the effect of unilateral Cd exposure resulted in accelerated and irregular maturation of the endodermis. The results were similar for both Cd concentrations. Rhizodermal, cortical, and, to some extent, cells in the vascular cylinder were all affected on the side of the root directly exposed to Cd. Direct exposure to Cd resulted in the collapse of peripheral root tissues close to the apex. Endodermal cells reacted by accelerated production of suberin lamellae, which were already present at a distance of 0.5 cm from the root apex in tissues adjacent to agar containing Cd (Fig. 5). At this distance no suberin lamellae were observed in root tissues adjacent to the Cd-free agar, which developed at a greater distance from the root apex (Fig. 5). About 10% of endodermal cells adjacent to Cd-free agar contained suberin lamellae at 1 cm from the root apex, and almost 100% of these endodermal cells had suberin lamellae at 2 cm from the root apex. In the endodermal cells exposed directly to Cd, suberin lamellae were no longer detected at these distances from the root apex and lignification of cells had occurred in the inner cortical tissues and in the pericycle (Fig. 5). In addition, ectopic lignification of protoxylem elements occurred in the half of the root directly exposed to Cd, whereas no, or only very weak, lignification of cells occurred in the half of the root exposed to Cd-free agar (Figs 4C, 6). Primordia of lateral roots also started to develop in roots exposed to the Cd0–Cd100 treatment (Fig. 4C; 6A). These only appeared in the half of the root exposed to Cd-free agar. The production of lateral roots in the Cd-free agar could be a response to root bending, in a manner analogous to the initiation of lateral roots at the convex side of roots subjected to gravitropic or mechanical stimuli (Richter et al., 2009), and would constitute an adaptive avoidance response to patches of high Cd in the rhizosphere.

It is evident from the results of this study that endodermal development is accelerated in parts of the root exposed directly to Cd in the rhizosphere. One can hypothesize that the accelerated production of a suberized endodermis, and

![Fig. 5. Schematic illustration of maize roots cultivated for 2 d between layers of agar containing no cadmium (Cd0–Cd0) or exposed unilaterally to 100 μM Cd (Cd0–Cd100; Fig. 4A). (A–F) Micrographs show the gradual development of suberin lamellae in the endodermis along the root axis visualized by Fluorol yellow 088 and fluorescence microscopy. In roots that were not exposed to Cd (Cd0–Cd0) all endodermal cells had developed suberin lamellae 95–100 mm from the root apex (A). Suberin lamellae were first deposited at a distance of about 80 mm from the root apex, and 90 mm from the apex approximately 50% of endodermal cells developed suberin lamellae (B). In roots exposed unilaterally to Cd (Cd0–Cd100) the development of the endodermis was accelerated and asymmetrical (D–F). Suberin lamellae had already developed on the side of the root exposed to Cd in endodermal cells 5 mm from the root apex (F). At this distance from the root apex no suberin lamellae were present on the side not exposed to Cd. At distances greater than 10–15 mm from the root apex, suberin was no longer detected in endodermal cells on the side exposed to Cd. However, some unidentified material exhibiting red fluorescence (excitation filter TBP 400+495+570, beamsplitter TFT 410+505+585, emission filter TBP 460+530+610, wavelengths are in nm) was deposited on the cell walls of endodermis, pericycle, in some xylem elements, and also in parenchyma cells of the vascular cylinder (D, E). At a distance of 20 mm from the root apex, suberin lamellae gradually developed on the side of the root not exposed to Cd (E) and at a distance of 25–30 mm from the root apex the entire half of the root which was not exposed to Cd had deposited suberin lamellae in the endodermis (D). White arrows indicate suberin lamellae in endodermis. Scale bars: 100 μm.]
The lignification of cell walls of inner cortical tissues and peripheral tissues of the vascular cylinder, in root tissues adjacent to a local Cd source could restrict the radial apoplasmic movement of Cd, and Cd loading to the xylem, thereby, protecting both unexposed root tissues and the shoot from Cd exposure. This phenomenon can be interpreted, therefore, as an adaptive response to protect plants from Cd toxicity. The bending of seminal roots towards a Cd source appears to initiate the production of lateral roots on the side opposite to the Cd source, which can also be interpreted as an adaptive response of roots to avoid patches of high Cd in the rhizosphere.

It is possible that the responses to local Cd exposure are initiated through Cd-induced oxidative stress, which has been implicated in the inhibition of root initiation and elongation in various plant species (Xiong et al., 2009). This hypothesis is consistent with a large subset of significant transcriptional responses to acute Cd exposure in roots of Arabidopsis thaliana being genes encoding reactive oxygen species (ROS)-scavenging enzymes and genes involved in the signal transduction pathway for ROS responses (Herbette et al., 2006; DalCorso et al., 2008; van de Mortel et al., 2008; Smeets et al., 2008; Zhao et al., 2009). Other genes with significant transcriptional responses to acute Cd exposure in Arabidopsis roots include genes involved in sulphur assimilation/reduction and glutathione metabolism, and genes with the gene ontology (GO) classifications ‘response to abiotic or biotic stimulus’, ‘response to stress’, and ‘signal transduction’ (Herbette et al., 2006; Weber et al., 2006; van de Mortel et al., 2008; Zhao et al., 2009). Intriguingly, the expression of genes involved in lignin biosynthesis is also up-regulated in roots of Arabidopsis thaliana and Noccaea caerulescens upon Cd exposure (Herbette et al., 2006; van de Mortel et al., 2008), suggesting candidate genes for the ectopic lignification of cell walls. Recently, increased expression of genes encoding heat shock transcription factors of class A4 (HsfA4a) have been associated with the up-regulation of genes encoding metallothioneins and Cd tolerance in both wheat and rice (Shim et al., 2009). It has been suggested that these transcription factors co-ordinate a concerted cellular response to Cd exposure (Shim et al., 2009), but this hypothesis has not been tested.

**Cadmium localization in root tissues**

In most plant species, roots have higher tissue Cd concentrations than shoots, although the opposite has been observed in many Cd-hyperaccumulating plants and in some non-hyperaccumulators, mostly from Compositae (e.g. Cichorium intybus, Bidens frondosa, Lactuca indica; Abe et al., 2008). Depending on the rhizosphere Cd concentration, Cd concentrations in the root can be up to 10 times higher than those in the shoot (Poleć-Pawlak et al., 2005; Wójcik and Tukiendorf, 2004, 2005; Solís-Domínguez

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**Fig. 6.** Cross-sections of maize roots exposed unilaterally to Cd (Cd0-Cd100; Fig. 4). Sections were cut at a distance of 20 mm from the root apex and stained by phloroglucinol–HCl to detect lignin. Lignification was asymmetrical, occurring primarily in the half of the root exposed to Cd (A, B). Weak lignification was observed in endodermal and xylem cells in the half of the root not exposed to Cd (C). There was a gradual increase in lignification from the unexposed side of the root to the side of the root directly exposed to Cd (D). Direct exposure to Cd induced strong lignification in the pericycle and inner cortical cells (D, E). Ectopic deposition of lignin was also observed in the lumen of protoxylem cells (D, E). The side of the root not exposed to Cd formed lateral root primordia (A), which were never observed on the side exposed to Cd. Abbreviations: e, endodermis; pc, pericycle; px, protoxylem; emx, early metaxylem; lmx, late metaxylem; lrp, lateral root primordium; asterisk, ectopic deposition of lignin. Scale bars: 100 μm (A, B); 40 μm (C, D, E).
et al., 2007, Wang et al., 2009; Lukačová and Lux, 2010). At low rhizosphere Cd concentrations, the root retains most of the Cd entering the plant, but the fraction retained by roots decreases as rhizosphere Cd concentrations increases. The Cd concentration in the root is influenced by the Cd concentration in the substrate (Wójcik and Tukiendorf, 2004, 2005; Wu et al., 2005; Van Belleghem et al., 2007), the phytoavailability of Cd (Wójcik and Tukiendorf, 2004; Abe et al., 2008), and the duration of exposure to Cd (Vázquez et al., 1992; Wójcik and Tukiendorf, 2004; Isaure et al., 2006). Root Cd concentrations increase rapidly upon exposure to Cd until steady-state tissue concentrations are achieved, generally after several days exposure, and root Cd concentrations are higher when plants are grown in substrates with greater Cd concentrations or Cd phytoavailabilities.

The spatial patterns of Cd accumulation within the root have been studied in various plants, including Cd-tolerant, non-tolerant, and Cd-hyperaccumulating species (for reviews see Seregin and Ivanov, 2001; Seregin and Kožhevnikova, 2008). At low rhizosphere Cd concentrations, Cd accumulates mostly in the apical part of the root (Arduini et al., 1996; Seregin et al., 2004) or in the proximal subapical region (e.g. 3 cm from the tip of maize root; Seregin et al., 2007). Cadmium penetrates into the root cap, rhizodermis, and cortex (Seregin and Ivanov, 1997). In the same plant species, a well-developed root cap appears to prevent Cd incursion at the root apex (e.g. Pinus pinea and Pinus pinaster; Arduini et al., 1996). Relatively high Cd concentrations are observed at the surface of the rhizodermis (Liu et al., 2007). Tissue Cd concentrations gradually decrease from the outer cortical parenchyma to the endodermis (Vázquez et al., 1992, 2007; Küpper et al., 2000). The pericycle accumulates little Cd, which may account for the continued production of lateral roots in the presence of Cd (Seregin et al., 2004). Cadmium can accumulate in the vascular cylinder, mostly in conductive elements and their adjacent parenchyma cells, presumably as a consequence of long-distance Cd transport (Seregin and Ivanov, 1997; Seregin et al., 2004; Liu et al., 2007; Vázquez et al., 2007). Significantly higher Cd concentrations occur in the parenchyma cells located between the endodermis and xylem poles compared with the adjacent parenchyma cells. This patterning is probably related to the occurrence of passage cells in the endodermis, whose cell walls are likely to be more permeable to Cd than those of endodermal cells with suberin lamellae (Van Belleghem et al., 2007). High Cd concentrations occur in both the pericycle and vascular tissues in the basal part of the root or throughout the whole root when plants are exposed to high rhizosphere Cd concentrations in the medium (Wójcik and Tukiendorf, 2004; Isaure et al., 2006; Solis-Dominquez et al., 2007). This can result in a dramatic inhibition of root growth and branching (Seregin et al., 2004).

The highest Cd concentrations in root tissues are observed in the apoplas, mainly on the outer surface of the rhizodermis and in the cell walls of the rhizodermis and cortical cells, whilst much lower Cd concentrations are found within root cells (Liu et al., 2007; Seregin et al., 2004, 2007; Vázquez et al., 2007; Wang et al., 2009). Within root cells, Cd is mainly concentrated in vacuoles and nuclei, with lower Cd concentrations being present in the cytoplasm and plastids (Vázquez et al., 1992; Liu and Kottke, 2004; Liu et al., 2007). Cell fractionation studies suggest that Cd-sensitive plants have lower Cd concentrations in cell walls, and higher vacuolar Cd concentrations, than Cd-tolerant plants (Uraguchi et al., 2009a). In barley roots, 36% of the Cd is present in cell walls and 51% is present in a soluble fraction (Wu et al., 2005), whereas in metal-tolerant species, such as S. alfredii and N. caerulescens, more Cd is present in the cell wall/apoplasm than in the soluble fraction/vacuole (Vázquez et al., 1992; Ni and Wei, 2003; Redjala et al., 2009). In barley roots, Cd-phytochelatin complexes account for 34–50% of the soluble fraction (Persson et al., 2006). Three types of Cd-phytochelatin complexes have been reported in barley roots, the ligands being (Glu-Cys)n-Gly (PCn), (Glu-Cys)n-Ser (iso-PCn), and Cys-(Glu-Cys)n-Gly (des-γ-Glu-PCn), and a correlation between Cd tolerance and the accumulation of Cd-PC3 has been observed (Persson et al., 2006). Some Cd-tolerant plant species are able to increase the cation exchange capacity of their cell walls following exposure to Cd (Nyquist and Greger, 2009). According to transmission electron microscopy, Cd occurs in granules visualized as electron-dense aggregates. Electron-dense granules appear between the cell wall and the plasmalemma of cells in outermost root tissues, whilst relatively few are found in the vascular cylinder (Liu and Kottke, 2004; Liu et al., 2007; Van Belleghem et al., 2007; Daud et al., 2009). These distinctive granular deposits occur in all plant species studied, including A. thaliana (Van Belleghem et al., 2007), Iris pseudacorus (Zhou et al., 2010), and N. caerulescens (Wójcik et al., 2005). In the vascular cylinder, a significantly higher amount of granular precipitated Cd occurs in the apoplasm surrounding the parenchyma cells located between the endodermis and xylem poles compared with the adjacent parenchyma cells (Wójcik et al., 2005; Van Belleghem et al., 2007). Cadmium-containing granular deposits are also found in the middle lamellae between the endodermis and pericycle cells (Khan et al., 1984; Wójcik and Tukiendorf, 2004). In vacuoles, electron-dense granules are aggregated and formed into larger precipitates, which increase in number and size with increasing Cd exposure (Liu and Kottke, 2004; Solis-Dominquez et al., 2007). Cadmium is accumulated in the vacuoles of meristematic or cortical parenchyma cells of differentiating and mature roots, but little Cd is found in the vacuoles of cells within the vascular cylinder (Liu and Kottke, 2004; Liu et al., 2007; Van Belleghem et al., 2007). In the endodermis, Cd is sequestered as very fine and uniformly distributed granular deposits in the vacuole and as large granular deposits in the cytoplasm located near the cell wall. In the vascular cylinder, significantly more granular precipitated Cd occurs in the cytoplasm of parenchyma cells located between the endodermis and xylem poles compared with the adjacent parenchyma cells (Wójcik et al., 2005; Van Belleghem et al., 2007). The accumulation of Cd in the cytoplasm of the phloem and its companion cells (Khan et al., 1984; Wójcik and Tukiendorf, 2004) suggests the
retranslocation of Cd from the shoot to the root in plants that restrict Cd accumulation in the shoot (Van Belleghem et al., 2007).

Conclusions and perspective

Cadmium is exceedingly toxic to plant cells. Most plants limit shoot Cd accumulation by restricting Cd movement to the xylem through both the symplasmic and the apoplastic pathways. When plant roots are exposed to high Cd concentrations, they increase the production of phytochelatins and sequester Cd entering root cells as Cd-chelates in the vacuole. This is likely to reduce symplasmic Cd concentrations and, thereby, symplasmic movement of Cd to the xylem. In tandem, they restrict apoplastic Cd fluxes to the xylem by accelerating the maturation of the endodermis, and produce Casparian bands, suberin lamellae, and lignification closer to the root apex. The maturation of the endodermis appears to respond to the local Cd concentration in the environment, and will develop asymmetrically in response to Cd gradients in the rhizosphere. We hypothesize that the accelerated maturation of the endodermis in response to local Cd availability has functional significance in protecting the shoot from excessive Cd loads by reducing the entry of Cd to the xylem. The diffusion of Cd in aqueous media is slow, and endodermal suberization presents an additional barrier to Cd movement in the extracellular space.

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