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REVIEW

What is new in the research on cadmium-induced stress in plants?

Ricardo A. Azevedo[1], Priscila L. Gratão[2], Carolina C. Monteiro[2] & Rogério F. Carvalho[2]

[1]Departamento de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo (USP), Piracicaba, São Paulo, Brazil
[2]Departamento de Biologia Aplicada à Agropecuária, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Jaboticabal, São Paulo, Brazil

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Correspondence
Ricardo A. Azevedo, Departamento de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Av. Pádua Dias 11, 13418-900, Piracicaba, São Paulo, Brazil. Tel: +55-19-3429-4475; Fax: +55-3447-8620; E-mail: raa@usp.br

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Abstract

Cadmium (Cd) is an important metal due to its industrial use but also one of the most dangerous metals because of its accumulation in the environment. This can eventually lead to entrance into the food chain if the Cd is taken up by crop plants used for feeding animals and humans. Thus, a large number of reviews have discussed the many aspects of stress induced by Cd and other metals in a wide range of species. In relation to plants, useful reviews have been published over the years regarding molecular and biochemical aspects of Cd stress. In this minireview, we have concentrated on promising and emerging topics of Cd-stress research in plants, such as hormonal control of the antioxidant system and interaction between organisms and plants.

Introduction

Among all the environmental stresses, the effect of metal accumulation has been considered one of the most disturbing factors arising in the late 19th and early 20th centuries. Some metals, such as zinc (Zn), mercury (Hg), copper (Cu), arsenic (As), lead (Pb), and cadmium (Cd), can be persistent and bioaccumulative elements, thus being potentially toxic to living organisms, from microorganisms to animals. These elements may be introduced into the environment by many anthropogenic activities, such as mining, fertilizer use, metal-based pesticides, and a wide range of industrial activities, which release metals into the environment (Zawoznik et al. 2007). In the case of plants, metals in the soil can enter the roots through symplastic or apoplastic pathways before entering the xylem and being translocated to the shoot (Lux et al. 2011), although transport through the phloem may also play a key role in delivering metals (Mendoza-Cózatl et al. 2011). Plants have a range of structural and biochemical barriers that can control the loading and unloading of elements, and these include the exodermis and endodermis, as well as the production of metal chelators. Metals can also trigger a series of changes that can lead to phytotoxicity (Gratão et al. 2009; Kopittke et al. 2010; Lux et al. 2011). Therefore, research into metal-induced plant stress is one of the most rapidly growing areas of agricultural research, which can be readily confirmed by the number of papers published weekly on this subject (Martinez-Penalver et al. 2012; Mihucz et al. 2012; Tian et al. 2012).

Cd is a toxic metal because of its relatively high mobility in the soil–plant system (Benavides et al. 2005; Groppa et al. 2012). Cd can affect cell biochemical mechanisms and structural aspects, for example, by lowering the control of the cell redox state, so inducing oxidative stress and disruption of membrane composition and function (Gratão et al. 2009; Gallego et al. 2012). Cd can induce severe disturbances in the physiological processes of a plant, such as photosynthesis, water relations, and mineral uptake (Lopez-Chuken and Young 2010; Gill et al. 2012). Hence, a complex biochemical pathway within the cell can be triggered concomitantly with transcription regulation of Cd-responsive genes, such as induction of antioxidant systems (Gratão et al. 2012) and increase in expression of transcription factors (Shim et al. 2009). Moreover, a cross talk between many molecules involved in the modulation of Cd-induced signaling pathway has been explored, such as the interaction of reactive oxygen species (ROS) or antioxidants with hormones. In fact, strong evidence has been presented to show that hormones are a major player in the signaling pathways of Cd-induced stress (Monteiro et al. 2011; Gratão et al. 2012; Noriega et al. 2012).

Nowadays, most of the knowledge about the components of Cd-stress signaling has been acquired through the use of advanced analytical systems such as transcriptome, metabolome, and proteome studies (Arruda and Azevedo 2009), as well as by the use of mutants and transgenic species, which have altered sensitivity to Cd (Clemens et al. 1998; He et al. 2007; Daud et al. 2009; Zhao et al. 2009; Shen et al. 2012). For example, through such techniques, it has been possible to identify numerous putative genes involved in the response to Cd stress (Fusco et al. 2005; Kovalchuk et al. 2005) and further to identify and understand the role of the components involved in the signaling pathway of antioxidant systems during Cd stress (Masood et al. 2012). Moreover, more detailed investigations into the interaction of bacteria or mycorrhizae with plants revealed a reduced harmful effect of Cd, indicating other important biochemical and molecular mechanisms involved in Cd-stress tolerance (Aloui et al. 2011; Farinati et al. 2011; Garg and Bhandari 2012).

In this minireview, we do not intend to present a full literature review of Cd stress in plants; there are already good comprehensive reviews available (Benavides et al. 2005; Gratão et al. 2005; Cuypers et al. 2011), so we have focused on what we feel may be key important topics that should received more attention. We have also concentrated on the more recent literature, particularly after the year 2009.

Biochemical and Molecular Approaches to Study the Antioxidant System Responses to Cd-Induced Stress

One of most common responses to environmental stress in plants is the induction of an oxidative stress condition. A large number of reports has shown that Cd induces several ROS, such as the superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH•) (Benavides et al. 2005). These ROS can accelerate lipid peroxidation, thus affecting cell membrane fluidity and permeability due to an alteration in the composition of membrane lipids (Tian et al. 2012). Consequently, these responses trigger plant ROS-scavenging mechanisms. In fact, during Cd stress, systems capable of preventing uncontrolled oxidation are induced or stimulated, and these include some key enzymes, such as superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX), and other peroxidases, which detoxify H_2O_2 (Gratão et al. 2008; Roychoudhury et al. 2012). Nonenzymatic mechanisms of ROS detoxification can also operate, and the main nonenzymatic antioxidants include ascorbate and glutathione (GSH), as well as vitamins, flavonoids, alkaloids, and carotenoids (Foyer and Noctor 2009, 2012; Ahmad et al. 2010).

Along with these important biochemical aspects, further molecular and genomic studies are required to elucidate how plants respond to Cd stress, and several researchers have already reported encouraging results using plants bioengineered with antioxidant enzymes. For example, transgenic *Arabidopsis thaliana* plants constitutively overexpressing a peroxisomal *APX* gene (*HvAPX1*) from barley were shown to be more tolerant to Cd stress, a process that also resulted in a higher accumulation of Cd in the shoots (Xu et al. 2008). Furthermore, the expression of genes encoding antioxidant enzymes has been shown to be differentially regulated by exposure to Cd (Smeets et al. 2009). Pawlak et al. (2009) demonstrated that Cd induced copper/zinc superoxide dismutase (Cu/Zn-SOD) mRNA accumulation in the roots of soybean (*Glycine max*) seedlings. More recently, Cd stress was shown to induce the expression of *FeSOD*, *MnSOD*, *Cu/ZnSOD*, *APX*, *GPX*, and *GR* genes in perennial ryegrass (*Lolium perenne* L.) (Luo et al. 2011). However, in the roots of *A. thaliana* seedlings, a reduction in *Cu/ZnSOD* gene expression after exposure to Cd was observed (Cuypers et al. 2011). This indicates that the molecular control of antioxidant systems induced by Cd depends on the plant species. Certainly, there are many other factors, including the time length of Cd exposure and its magnitude, together with external environmental conditions, which can contribute to the modulation of the enzymatic antioxidant system (DalCorso et al. 2008).

Nonenzymatic antioxidants in plants may also be involved in the internal detoxification of the stress induced by Cd. For instance, anthocyanins, which are important flavonoid molecules, clearly show powerful antioxidant capacity against various free radicals during Cd stress (Dai et al. 2012). Interestingly, following analysis of two key enzymes in the anthocyanin synthesis pathway, chalcone synthase (CHS) and dihydroflavonol reductase (DFR), Dai et al. (2012) verified that the expression of *AiDFR* gene, and to a lesser extent of *AiCHS* gene, was significantly induced in Cd-grown plants in comparison with the Cd-free control.

Although gene expression profiling has increased our understanding of Cd-stress signaling, it is still important to know how the transcriptional changes are reflected at the translational level. Thus, proteomics studies are also an important tool that can be used to gain a comprehensive understanding of plant responses to Cd. For instance, changes in the protein profile in response to Cd treatment in a number of studies have revealed a wide range of antioxidant molecules in different species, such as in poplar (*Populus nigra*) (Visioli et al. 2010), rice (*Oryza sativa*) (Lee et al. 2010; Nwugo and Huerta 2011), and wheat (*Triticum aestivum* L.) (Wang et al. 2011). Nevertheless, much more has to be done to elucidate the molecular mechanisms involved in posttranscriptional regulation during the induction of antioxidant systems in response to Cd.

Hormonal Modulation of Cd Stress

In plants, one of the most new and fascinating question related to signaling pathway responses to environment stress is the role of hormones (Monteiro et al. 2012). Literature reports have shown that virtually all hormonal classes can act as components of Cd-stress signaling: cytokinin (Munzuroglu and Zengin 2006), abscisic acid (Hsu and Kao 2008), gibberellin (Celik et al. 2008), auxin (Zhao et al. 2011), brassinosteroid (Hayat et al. 2007; Villiers et al. 2012), and jasmonic acid (Noriega et al. 2012). However, more recent investigations using hormonal mutants have shown that ethylene may have a fundamental role in the signaling pathway response to Cd stress. Monteiro et al. (2011) and Gratão et al. (2012) verified that the ethylene-insensitive *Never ripe* (*Nr*) mutant of tomato is able to withstand or avoid stress imposed by Cd, through an enhanced antioxidant system. Additionally, the *Nr* mutant treated with Cd exhibited an increase in the intercellular spaces and decrease in the size of the mesophyll, as well as alterations in diameter and disintegration of the epidermis and the external layers of the cortex of roots (Gratão et al. 2009). This indicates that at least ethylene is required during the morphological alterations induced by Cd (see the review paper by Lux et al. 2011). However, similar accumulation of Cd in root, leaf, and fruit between the wild type and *Nr* mutants (Monteiro et al. 2011) obviously raises many questions regarding the function of ethylene in the biochemical and morphological mechanisms related to the Cd-stress response. Thus, ethylene signaling can act through mechanisms, which appears to be stressor and tissue dependent such as observed for salt stress (Monteiro et al. 2011), besides the fact that ethylene can interact with a wide range of factors during stress, such as other hormones (Divi et al. 2010). Recently, Masood et al. (2012) verified in mustard (*Brassica juncea*) that ethylene plays an important role in sulfur-induced alleviation of Cd stress. Even though ethylene can act as an important signaling molecule in regulating plant responses to Cd stress, further analysis is still needed, and there is plenty of room for more research to be carried out in order to gain a better understanding of the mechanisms connecting ethylene and Cd stress, and perhaps, the same is also true for other plant hormones.

The Role of Plants in Cleaning up Cd-Contaminated Soils

It is possible that the damage caused by metals on the agricultural ecosystem could be solved by using tolerant plants to clean up the metal-contaminated areas (Singh and Prasad 2011). However, it is essential to investigate and understand how plants work and what metabolic pathways and genes are involved in such a process (Verbruggen et al. 2009). The mechanisms of Cd detoxification, accumulation, and tolerance have become the basis for using plants for the remediation of Cd-contaminated soil (Mendoza-Cózatl et al. 2011).

In this context, high tolerance to heavy metal toxicity can be related to the ability of roots to tolerate Cd, as the toxic metal enters the roots first. For instance, roots of Cd hyperaccumulator plants have been shown to possess mechanisms for metal tolerance, such as the direct activation of GSH synthesis and induction of additional sinks for reduced sulfur, suggesting that some other SH compounds are possibly involved in Cd detoxification (Tian et al. 2012).

Current research has reported encouraging results about the important role of phytochelatins (PC) in Cd detoxification. Cd-tolerant plants induce the synthesis of phytochelatins, which can in turn combine with Cd to form a 9-kDa complex corresponding in apparent molecular mass to stable high-molecular-weight (HMW) CdS–PC complexes, thus reducing the translocation of Cd to shoots (Liu et al. 2012; Najmanova et al. 2012).

Advances using the “omics” techniques have potential in developing this green technology, identifying markers for Cd tolerance and accumulation capacities to improve the development of efficient phytoextraction strategies. For instance, special attention should be directed to the role of glutathione (GSH) as a precursor for PCs in the binding of metals (Seth et al. 2012). In addition, the overexpression of phytochelatin synthase, a key enzyme catalyzing the final step of PC biosynthesis in rice, can be used as a potential mechanism to enhance the tolerance of plants to Cd stress (Venkataramaiah et al. 2011). Furthermore, in a recent research by Lima et al. (2012), phytochelatin synthase activity was shown to be possibly modulated by protein phosphorylation, which may lead to new ways to target phytochelatin synthase activity and enhance Cd uptake and tolerance.

Many proteins and signaling pathways may be involved in Cd-stress responses, and the identification of the key ones within the stress response network is essential. Approaches allowing the identification of plant genes encoding metal-ion transporters implicated in phytochelatin-dependent detoxification (Park et al. 2012) and vacuolar sequestration of Cd (Khoudi et al. 2012) have aided plant breeding efforts.

As previously mentioned, it is important to understand how plants work in the case of using them to remediate metal-contaminated areas. Recent work by Ganeshan et al. (2012) has showed that immature spike culture can be used to study Cd accumulation trend in grains and help delineating hyperaccumulating durum wheat compared with bread wheat at Cd concentrations of 2 mg/L and above (Ganeshan et al. 2012). According to these authors, contrary to bread wheat, the expression patterns of the Cd-related genes glutathione reductase, phytochelatin synthase, and metallothionein were downregulated or remained unchanged.

For effective phytoextraction, it is also essential to investigate and take into consideration the long timescale required when studies concern more than one environmental contaminant (Zhang and Liu 2011) and involve a long-term field experiment (Shahid et al. 2012). An example of this is the transgenic alfalfa plants coexpressing human *CYP2E1* (cytochrome P450 that has been related to the metabolism of several xenobiotic contaminants in mammalian systems) (Zhang and Liu 2011) and glutathione *S*-transferase, an important enzyme that play a key role in stress tolerance and cellular detoxification. Such plants may have a great potential for phytoremediation because of the cross-tolerance showed between heavy metals and organic pollutants.

From the preceding discussion, it remains clear that in order to breed plants that are good phytoremediators, both fundamental and applied research must be carried out in association. This should lead to a clearer view of the molecular, biochemical, and genetical aspects related to sequestration and improve the ability of a plant to accumulate the target metal in the upper parts, for rapid harvesting and removal.

The Magnitude of the Interaction Between Plants and Microorganisms in Cd-Stress Tolerance

High tolerance to metal toxicity can be related to reduced metal uptake or increased internal sequestration in plants that have acquired specialized mechanisms to detoxify and partition metals between roots and shoots (Mendoza-Cózatl et al. 2011). In this context, the establishment of a symbiotic association between roots and microorganisms can benefit plants by adaptation and alleviation of a variety of environmental stresses including toxic metals (Schutzendubel and Polle 2002; Andrews et al. 2010; Garg and Chandel 2010; Sousa et al. 2012). Such type of studies are gaining importance and may shed light on the improvement of major adaptive functions and productivity in crop plants by manipulating the microbial cohabitants (Tikhonovich and Provorov 2011; Andrews et al. 2012).

Although recent studies have indicated the importance of arbuscular mycorrhizal (AM) fungi in the alleviation of the toxic effects of metals (Andrade et al. 2010), very little is known regarding the molecular and biochemical mechanisms of such interactions. The presence of the AM fungi *Glomus mosseae* of *Cajanus cajan* (L.) Mill sp revealed a correlation between the intensity of mycorrhizal infection and increased stimulation of glutathione production and levels of PCs (Garg and Aggarwal 2011). Additionally, shoots of mycorrhizal legumes can avoid Cd toxicity through the glycolysis-mediated mobilization of defense mechanisms at the expense of the photosynthesis-dependent symbiotic sucrose sink (Aloui et al. 2011).

According to Belimov et al. (2005), the presence of 11 Cd-tolerant bacterial strains stimulated root elongation in *Brassica juncea* L. Czern that was highly contaminated with Cd. This positive correlation with root elongation could be related to the presence of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity in the bacteria, which hydrolyzes ACC (the immediate precursor of the plant hormone ethylene) and thus promotes root growth (Belimov et al. 2005).

The inoculation of plants with microorganisms could be used to improve the growth of the plants in the presence of toxic Cd concentrations. For instance, Madhaiyan et al. (2007) showed that the plant growth promoting bacteria *Methylobacterium oryzae* strain CBMB20 and Burkholderia sp. strain CBMB40 isolated from rice reduced the toxicity of Cd in tomato and promoted plant growth by reducing Cd uptake and further translocation to the shoots. Moreover, the use of a bacterium-containing biofertilizer as an alternative nutrient supply has been shown to alleviate the toxic effect of Cd on sunflower (Eva et al. 2012).

Although the beneficial effects of plant root and microorganism association are well known, little information is available about the interactions among macronutrients and trace elements. A recent report indicated a possible synergistic effect between Cd and K, suggesting the interference by Cd of nutrient uptake by affecting ion transport processes through the alteration of plasma membrane permeability (De Maria et al. 2011). Cd and K concentrations were mostly increased in the shoots of *Salix caprea* after inoculation with ectomycorrhizal fungi and soil bacterial strains. This positive interaction could be related to the availability of nutrients in the soil increased by bacteria plus the increase of absorptive area around roots caused by the presence of fungi (De Maria et al. 2011).

Interdisciplinary studies into the association between plant roots and microorganisms have contributed to a more integrated understanding of the complex Cd tolerance of plants, but as in other fields, more research is needed. As recently investigated by Melo et al. (2011), the soil type is important. Soybean plants exhibited less oxidative stress when grown in Cd containing clay Oxisol than when grown in sandy Entisol. It is therefore important to consider how the metal, metal chemical form, plant species, microorganism, and soil physical and chemical properties interact. In this way, the onset of stressful conditions will become better understood and the knowledge generated used when designing strategies for plant stress improvement.

Epilog

This minireview is a follow-up to a series of publications, which have focused on some of the most important and recent questions regarding plant stress induced by Cd. We feel that some of the most promising and emerging topics of Cd-stress research are related to the antioxidant system, along with hormonal modulation and phytoremediation. The latter could act as a tool to improve our understanding of the entire process, placing together Cd, plant, soil, and microorganisms. Although activation of the antioxidant system is the most evident response induced by Cd, there is still a need for answers to many questions about the system. For example, what changes are there in the transcription and translation of components involved in antioxidant signaling? Which hormones are involved in these changes and how do they act? Are there unexplored factors that modulate the signaling pathway responses to Cd stress? Most likely the answer is yes, as recent evidence shows that photoreceptors can modulate metal stress (Carvalho et al. 2011; Shen et al. 2011).

Although genomics technology and the use of transgenic, mutant, and hyperaccumulator plant lines have helped to elucidate some of the questions presented above, many other alternatives do exist, such as the use of grafting techniques as previously suggested (Arruda and Azevedo 2009; Azevedo and Lea 2011). Such techniques, largely used for other purposes, could also contribute to our understanding of stress signaling among plant organs and tissues and open up a new perspective to study these grafted plants at the biochemical and molecular levels. For instance, grafting techniques have shown that the seed Cd concentration can be influenced by the difference in translocation of Cd to the seed and in the Cd accumulation capacity of roots among soybean cultivars (Sugiyama et al. 2007). Additionally, due to the conflict between the demand for sustainable food production and availability of arable land, the use of grafting of tolerant varieties could allow researchers to choose the best root system for coping with the stressful environment through the isolation of a commercial cultivar, helping crop breeders to develop staple food crops in contaminated soils. The use of the grafting technique to study toxic metals in plants has been subjected to relatively few molecular and biochemical studies involving the relationship between rootstock and scion (Tomaz et al. 2006). In other words, this is just the beginning and such a technique can be used to several other types of environmental stresses.

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Conflict of Interest

None declared.

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