Metabolic And Biochemical Changes Associated With Root Growth Restriction Under Cd Stress During Maize Pre-Emergence

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Abstract

Cadmium (Cd) pollution of agricultural soils is a growing global problem. Plant growth restriction is the main visible symptom of Cd toxicity, and this metal may be particularly harmful to the preformed, seminal root during the pre-emergence stage. In the present study, we focused on Cd phytotoxicity on seminal root growth, nutrient composition, redox status, and hormone homeostasis during the pre-emergence stage, distinguishing between the root apex and the remaining root tissue. After 72 h of metal exposure (50 and 100 μM $CdCl_2$), root length and biomass was diminished, as well as Ca, Fe, Mg, and Mn contents. A redox imbalance was evidenced by changes in peroxidase activities and decreased ascorbate-dehydroascorbate ratio in both root parts. There was less accumulation of carbonylated proteins in both root fractions upon exposition to 50 μM Cd, compared to 100 μM Cd, and this was related to increased 20S proteasome activities. Cd incremented ABA, IAA, and SA contents, but drastically reduced the biologically active gibberellin GA4 and the conjugate jasmonoyl isoleucine (JA-Ile). We demonstrated that the whole root tissue is involved in maize response to Cd stress, which entails redox and hormonal rearrangements, probably directed to widen plant defense lines at the expense of root growth.

Highlights

-Cd reduced maize root growth during pre-emergence and altered its mineral composition.

-Cd disrupted ascorbate homeostasis in the root apex and the remaining root tissue.

-Cd induced CAT and GPX activities and reduced GA4 and JA-Ile contents.

- Less carbonylated proteins accumulated at 50 μM Cd compared to 100 μM Cd.

- 20S proteasome activity was induced 48 and 72 h after 50 μM Cd exposure (whole root).

Introduction

Cadmium is a transition metal ion released to the environment by industrial activities and urbanization. In cultivated soils, Cd derives mainly from P fertilizers (Sterckeman et al. 2018). Due to its relatively high mobility and high toxicity for living organisms–even at very low doses–, cadmium is considered a particularly dangerous pollutant (Vardhan et al. 2019). The increasing contamination of soil and food crops represents a serious global problem nowadays (Rehman et al. 2018; Dala-Paula et al. 2018; Cai et al. 2019). Plant growth restriction is one of the main visible symptoms related to Cd phytotoxicity (Gallego et al. 2012). Despite being a redox-inactive metal, Cd toxicity has been in part associated with oxidative stress production (Gallego y Benavides 2019).

It is known that plant growth regulation and plant responses to stress depend on the interplay between hormonal and redox balances (Santner y Estelle 2009; De Tullio et al. 2010; Bartoli et al. 2013). Plant

hormones comprise a series of natural compounds required in low concentrations to fulfill their function. While each plant hormone has its specific pathway that acts in a non-redundant way, their activities are interconnected by a complex network, and it is the interaction and cooperation between hormones that dynamically regulate plants' development and physiology (Vanstraelen y Benková 2012). It has been described that the exogenous application of phytohormones reduces the toxic effects of metals, in part through the improvement of the cell antioxidant potential (Singh et al. 2016).

On the other hand, it is known that plant cell redox homeostasis is controlled by a complex system known as the Foyer-Halliwell-Asada pathway, responsible for reactive oxygen species (ROS) scavenging (Foyer y Noctor 2011). This antioxidant defense machinery includes enzymes and low molecular weight compounds, like glutathione (GSH) and ascorbic acid (ASC) (Foyer y Noctor 2016). Nevertheless, an excessive ROS production that overwhelms the protective antioxidant mechanism can occur when plants are subjected to adverse environmental conditions (Gill y Tuteja 2010). A consequence of cell redox imbalance is protein oxidative damage, commonly expressed by carbonyl group increases (Møller et al. 2007). Carbonylated proteins can form high-molecular-weight aggregates that compromise several cellular functions (Nyström 2005). Because protein carbonylation is a covalent, non-reversible modification, oxidatively damaged proteins have to be rapidly degraded, mainly by the 20S proteasome activity in the cytoplasm and nucleus (Pena et al. 2007; Polge et al. 2009).

Maize (*Zea mays* L.) is one of the most important crops used for human and animal diet in the world (Godfray et al. 2010), and in some cases, maize-producing lands are at high risk of cadmium contamination (Chumbley y Unwin 1982; Dharma-Wardana 2018). It has been reported that Cd reduces growth, induces chlorosis, alters chloroplast ultrastructure, produces oxidative damage, modifies cell wall composition, and affects polyamine metabolism in maize plants (Anjum et al. 2015; Vatehová et al. 2016; Seifikalhor et al. 2020). Also, previous data indicate that maize plants tend to retain and accumulate cadmium at the root level (Anjum et al. 2015; Vatehová et al. 2016), where ROS production is induced soon after metal exposure (Liu et al. 2019).

The emergence of maize coleoptile from the soil surface delimits the onset of plant phototrophic lifestyle and takes place at 5 to 7 days after planting under favorable, natural conditions (Abendroth et al. 2011). The embryonically preformed root type dominates during this stage of development (Hochholdinger 2009). Apart from being vital for the vigor of young maize plants during the first weeks after germination, the embryonic root system is the first plant organ expected to interact with the underground environment and eventually suffer the toxic effects caused by Cd present in soils (Tai et al. 2016). Several reports indicate a higher Cd^{2+} influx in the root tip region, even when cadmium acquisition could be achieved through the entire root utilizing metal transporters. Direct xylem loading due to the lack of Caspary band and higher expression of transport systems associated with Cd uptake located close to the root tip have been related to this phenomenon (Piñeros et al. 1998; Laporte et al. 2013; Chen et al. 2018). Thus, the root apex could be the main site prone to suffer the toxic effects of metal ions.

In this work, the impact of Cd on the nutrient composition, redox balance, and phytohormone profile of embryonic maize roots was analyzed, distinguishing between the first 5 mm from the root tip, considered the root apex (Ap), and the remaining root tissue (Rt). Because plants are still under a chemoheterotrophic lifestyle at the pre-emergence stage, our analysis lets aside the well-known effects of cadmium on photosynthesis.

Materials And Methods

2.1. Plant material and growing conditions

Seeds of maize (*Zea mays* L. cv 2741MGRR2 kindly provided by DON MARIO Semillas, Buenos Aires, Argentina) were imbibed and germinated on filter paper in a plastic box containing deionized water for 72 h. Then, uniformly developed seedlings with primary roots of approximately 1.5 cm length were carefully transferred to a hydroponic system containing 250 mL of diluted (1/10) Hoagland's nutrient solution (Hoagland y Aron 1950) without (control, C) or with 50 or 100 µM $CdCl_2$; 30 seedlings were distributed in each container. Cd speciation was calculated with Visual MINTEQ ver. 3.1 (J P Gustafssons, KTH, Sweden). In the nutrient solution containing 100 µM of Cd, about 88% was in Cd^{2+} form, the most phytoavailable. Plants were grown in a controlled climate room at 24 ± 2°C. All the experiments were carried out in the darkness to mimic soil conditions during germination and post-germinative growth. After 72 h of treatment, roots were gently washed with distilled water. The tissue collected from each container was considered a biological replicate. Root length, fresh weight (FW), and dry weight (DW, determined after drying the roots at 80 ºC until constant weight) were measured. Also, dried root powder was used to determine Cd and nutrient content. Determinations were performed in parallel using root apical segments obtained from the first 5 mm from the tip (Ap) or all the remaining root tissue (Rt).

2.2. Nutrient composition of maize roots

Elemental analysis was performed at the Spectrometry Core Facility INQUISAL, Universidad Nacional de San Luis (UNSL-CONICET). Briefly, dried root powder (50 mg) was homogenized in 1 mL of 65% (v/v) HNO_3 in an ultrasonic bath during 30 s. Then, 0.5 mL of H_2O_2 was added, and the mixture was incubated for 1 h at 60°C in a thermostatic bath. After diluting the samples with ultrapure water, inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer Elan DRC) was applied to estimate Cd, Cu, Ca, Fe, K, Mg, Mn, P, S, and Zn content.

2.3. Enzymatic and non-enzymatic antioxidants

Protein extracts were prepared from 0.1 g of fresh tissue homogenized in 1 mL of 50 mM phosphate buffer (pH 7.4) containing 1 mM EDTA and 0.5% (v/v) Triton X-100, at 4°C. The homogenates obtained centrifuged at 13,000×g for 30 min at 4°C, and the supernatants were used for the assays. Protein content was estimated according to Bradford (1976).

The activity of catalase (CAT, EC 1.11.1.6), guaiacol peroxidase (GPX, EC 1.11.1.7), and ascorbate peroxidase (APX, EC 1.11.1.11) were measured as described previously (Nakano y Asada 1981; Aebi 1984). CAT content was expressed in pmol mg^{-1} and calculated using $k = 4.7 \times 10^{-7}$ M^{-1} s^{-1}. One unit of GPOX was defined as the amount of tetraguaiacol formed (mmol) per min, and one unit of APOX was defined as mmoles of oxidized ascorbate per min.

Ascorbate (ASC) and dehydroascorbate (DHAs) were determined as described by Law et al. (1983). Extracts were obtained by homogenizing 0.1 g of root tissue in 1 mL of 0.1 N HCl. After centrifugation (13,000×g, 30 min, at 4°C), the supernatants were used for the assays. A standard curve of commercial ASC was used for calibration.

2.4. Quantitative dot blot analysis of carbonylated proteins

Protein extracts were prepared by homogenizing 0.1 g of root tissue in 0.5 mL of loading buffer (60 mM Tris-HCl (pH 6.8), 5% (v/v) β-mercaptoethanol). After centrifugation at 26,000*x*g for 15 min at 4 ºC and protein derivatization with 2,4-dinitrophenylhydrazine (2,4-DNPH) dot blot analysis was performed as described Weher and Levine (2012). Membranes were photographed and then analyzed with Gel-Pro software, and the amount of oxidized proteins was expressed as arbitrary units (assuming control value equal to 100 units), based on the absolute integrated optical density of each dot.

2.5. Proteasome activities

Proteasome activity in root tissue was determined as described by Kim et al. (2003). Protein extracts were prepared in 135 mM Tris-acetate buffer (pH 7.5) containing 12.5 mM KCl, 80 μM EGTA, 6.25 mM 2-mercaptoethanol, and 0.17% (w/v) octyl-β-D-glucopyranoside. After homogenizing 100 mg of root tissue (Ap or Rd) in 0.5 mL buffer, the extracts were centrifuged at 6,400 g for 30 min at 4°C, and the supernatants were further used to determine chymotrypsin-like (Q), trypsin-like (T), and peptidylglutamylpeptide hydrolase (PGPH) activities (Matayoshi et al. 2020). Due to the extraction buffer interference with Bradford reaction, protein content was determined by the method of Lowry et al. (1951).

2.6. Plant hormone analysis

Hormone extraction and analysis were carried out as described in Durgbanshi et al. (2005), with few modifications (Matayoshi et al. 2020). Absolute levels of hormones (indole-3-acetic acid, IAA; abscisic acid, ABA; salicylic acid, SA; jasmonic acid, JA; jasmonoyl isoleucine, JA-Ile, and gibberellins, GA3, 4, 7, 20) were determined using an ultra-performance liquid chromatography system (Acquity SDS, Waters Corp., Milford, MA, USA or Waters Alliance 2695, Waters Corp.) coupled to a triple quadrupole mass spectrometer (TQS, Micromass Ltd., UK). All data were acquired in negative electrospray mode and processed using MassLynx v4.1 software. Quantitation was achieved after external calibration with standards of known amount and referenced to actual sample weight.

2.7. Statistical analysis

Each container had 30 seeds from which 0.1 g of tissue was collected and considered as a biological replicate. Tables and figures show means ± SEM of three or five independent experiments, with three biological replicates per treatment. Differences among treatments were analyzed by 1-way ANOVA, taking $P < 0.05$ as significant, followed by Tukey's multiple comparisons test

Results And Discussion

3.1. Cadmium accumulation reduced maize root growth and modified root nutrient composition

The presence of Cd in the hydroponic solution reduced maize root growth by about 70% in length and 45% in biomass (Table 1), in line with previous reports (Xu et al. 2014; Anjum et al. 2016b; Li et al. 2020a), and Cd accumulation in maize root was clearly dose-dependent (Fig. 1). However, a similar degree of growth impairment was observed under both Cd concentrations tested. Laboratory soil-less systems abolish the complex physicochemical interactions that take place under natural field conditions and may alter nutrients' and pollutants' bioavailability. Among the soil properties that govern Cd diffusion flux towards the root surface, soil pH, clay content, metal oxides, cation exchange capacity, organic matter content, and Ca^{2+} concentration have been reported, and also total Cd content impacts on Cd uptake (Liu et al. 2015a; Lin et al. 2016; Yi et al. 2020).

Table 1

Effect of Cd on root length and biomass. Maize seedlings were grown in a hydroponic system containing diluted (1/10) Hoagland's nutrient solution without (control, C) or with 50 and 100 µM of $CdCl_2$, and root length, fresh weight (FW), and dry weight (DW) were determined 72 h later.

	Control	µM $CdCl_2$	
		50	100
Length (cm)	8.0 ± 1.2^a	2.7 ± 0.3^b	2.4 ± 0.7^b
FW	558 ± 79^a	269 ± 65^b	337 ± 52^b
DW	38 ± 12^a	17 ± 6^b	17 ± 6^b

Data are expressed in mg/10 seedlings; means ± SEM of five independent experiments, with three biological replicates per treatment, are shown. Different letters within rows indicate significant differences ($P < 0.05$), according to Tukey's multiple range test.

Plants have not developed any specialized uptake system for cadmium because this element has no biological function. Nevertheless, this metal can be easily taken up by plant roots through membrane transporters of essential nutrients (Sterckeman y Thomine 2020). Current evidence indicates that Cd root symplastic influx in maize is controlled by high- and low-affinity transport systems (Redjala et al. 2009, 2010). Furthermore, cadmium can be strongly adsorbed on the maize cell wall, resulting in a large amount of Cd^{2+} being retained in the root apoplast (Redjala et al. 2009).

As Table 2 shows, Cd accumulation in emerging maize roots was accompanied by significant decreases in Ca, Fe, Mg, and Mn contents. A reduction of 48% and 68% in Ca level was determined for 50 and 100 µM Cd, respectively. For both Cd concentrations assayed, the reduction in Mg level was close to 60%, and for Fe and Mn, similar decreases of about 38% were detected. On the other hand, Zn was incremented by 16% over the control only under 50 µM Cd, and Cu content doubled that of the control in the roots of seedlings subjected to 100 µM Cd.

Table 2

Effect of Cd on root chemical composition. Maize seedlings were grown in a hydroponic system containing diluted (1/10) Hoagland's nutrient solution without (control, C) or with 50 and 100 µM of $CdCl_2$. After 72 h of treatment, roots were harvested and used for analytical determinations. Element concentrations are expressed in mg kg^{-1} of dry weight.

	Cu	Ca	Fe	K	Mg	Mn	P	S	Zn
C	30 ± 10[b]	2135 ± 326[a]	79 ± 09[a]	15367 ± 1436[a]	1372 ± 460[a]	8 ± 1[a]	9871 ± 420[a]	1688 ± 398[a]	59 ± 1[b]
50 µM	35 ± 4[b]	1103 ± 94[b]	49 ± 3[b]	19474 ± 1611[a]	525 ± 9[b]	5 ± 1[b]	10868 ± 165[a]	1723 ± 124[a]	69 ± 4[a]
100 µM	73 ± 9[a]	700 ± 49[b]	50 ± 2[b]	17927 ± 178[a]	654 ± 12[b]	5 ± 1[b]	9299 ± 268[a]	1644 ± 196[a]	55 ± 2[b]

Data are mean ± SEM of three independent experiments, with three biological replicates per treatment. Different letters within columns indicate significant differences ($P < 0.05$), according to Tukey's multiple range test.

Change in nutrient absorption/distribution patterns is one of the most recognized cadmium harmful effects and has been mainly attributed to competition with divalent cation transporters (Huang et al. 2020). Ca and Mg (typically the most abundant divalent cations in plants) reductions could have affected normal growth and development. In this sense, it has been pointed out that growth restriction under Cd stress would be a nutrient deficiency symptom and the result of homeostatic balance loss between these cations (Tang y Luan 2017; Thor 2019; Kleczkowski y Igamberdiev 2021). Similarly, Cd reduced Fe and Mn contents in maize root. According to several reports, Cd shares similar plant entry routes with these relevant nutrients, so that the decreases found can be the outcome of Cd competition with Fe and Mn transporters (Thomine et al. 2000; Wu et al. 2016; Chen et al. 2017b; Chang et al. 2020). On the other hand, it has been demonstrated that the external addition of Ca, Mg, Fe, or Mn to the nutrient solution restricted Cd uptake and translocation, resulting in alleviation of Cd stress (Pal'ove-Balang et al. 2006; Sterckeman et al. 2011; Liu et al. 2013; Kudo et al. 2015; Rahman et al. 2016; Huang et al. 2017; Chen et al. 2017a; Hussain et al. 2020).

A complex interaction between Cd and Zn has been documented before, and it was proposed that uptake/translocation of Zn would increase in the presence of Cd (Nan et al. 2002). Moreover, it was demonstrated that induction of several genes belonging to the ZIP family–a group of proteins that

mediate Zn and Cd transport–depends on the Zn:Cd ratio in the growing medium (Barabasz et al. 2016; Palusińska et al. 2020).

Cu increase and Mn decrease could account for cell redox homeostasis disruption under Cd stress. Cu is a redox-active metal and Mn, apart from having free radical scavenging capacity (Coassin et al. 1992), acts as a cofactor of an important enzymatic antioxidant, superoxide dismutase (Mn-SOD); Ca is also a signaling messenger intimately interconnected with ROS (Mazars et al. 2010; Steinhorst y Kudla 2013). Thus, the nutrient imbalance could be part of the indirect mechanisms by which Cd induces oxidative stress in maize roots.

3.2. Cadmium differentially affected peroxidase activities along the root and disrupted ascorbate homeostasis

In maize seminal root, CAT and APX activities were mostly localized in the root tip (Ap), while GPX activity was predominantly in the remaining tissue (Rt) (Fig. 2). Among peroxidases, CAT catalyzes the dismutation of H_2O_2 in the absence of electron donors. Its activity is largely found in subcellular compartments with H_2O_2 generation, such as peroxisomes, and also in mitochondria, chloroplasts, and the cytosol (Sharma y Ahmad 2014). CAT activity increased in the Ap under 100 μM Cd^{2+} (130% over the control), but in the Rt, CAT activity increased by 67% under 50 μM Cd^{2+} and decreased by 42% under 100 μM Cd^{2+} compared to the control. An increase in CAT activity may be interpreted as a cell-protective strategy against the detrimental effect of H_2O_2. On the contrary, a decrease in CAT activity deprives cells of their normal antioxidant capacity and results in oxidative stress. Catalase inactivation by metals has been associated with the oxidation of the protein structure (Pena et al. 2011) and the suppression of CAT gene expression (Ye et al. 2014).

To counteract an excessive H_2O_2 formation in plant tissues, non-specific peroxidases acting on one- or two-electron donors (including phenolic compounds such as guaiacol) are usually induced. In plants, GPX activity is mainly located in vacuoles and cell walls but not in organelles (Asada 1992). Under both concentrations, Cd increased GPX activity by about 70% in the Ap, while in the Rt, increases over the control of 47% and 72% for 50 and 100 μM Cd^{2+}, respectively, were recorded (Fig. 2). GPX activity rise during Cd stress would be involved not only in the control of H_2O_2 levels but also in the modulation of plant growth and development through the control of hormonal and cell wall metabolism (Jouili et al. 2011).

Ascorbate peroxidase reduces H_2O_2 to H_2O using ascorbate as the specific electron donor. Different APX isoforms are located in chloroplasts, cytosol, mitochondria, and peroxisomes, as well as in the apoplastic space (Gill y Tuteja 2010; Hasanuzzaman et al. 2019). In maize root apex, APX activity was not affected by Cd treatment, in line with previous observations in barley root tips (Bocova et al. 2012), but the activity of this enzyme was particularly impaired in the Rt, dropping to near to half under both Cd concentrations (Fig. 2). Because of a higher APX affinity for H_2O_2 than CAT and GPX, it has been suggested that this

enzyme has a more crucial role in the scavenging of ROS during abiotic stress (Sofo et al. 2015; Anjum et al. 2016a).

In both root portions, total ASC (ASC plus DHAs) levels augmented under Cd treatment due to a pronounced rise in DHAs content, resulting, at the same time, in the reduction of ASC/DHAs ratio (Table 3). This finding suggests that Cd altered the adequate functioning of the ASC-GSH cycle.

Table 3

Effect of Cd on ascorbate (ASC) and dehydroascorbate (DHAs) content. Maize seedlings were grown in a hydroponic system containing diluted (1/10) Hoagland's nutrient solution without (control, C) or with 50 and 100 µM of $CdCl_2$ for 72 h. Concentrations are expressed in nmol g^{-1} of fresh weight.

	Ap			Rt		
	ASC	DHAs	ASC / DHAs	ASC	DHAs	ASC / DHAs
C	218 ± 10^{a}	475 ± 31^{c}	0.5	915 ± 50^{A}	310 ± 60^{B}	2.9
50 µM	248 ± 5^{a}	2265 ± 81^{a}	0.1	1090 ± 20^{A}	590 ± 40^{B}	1.8
100 µM	151 ± 5^{b}	1600 ± 69^{b}	0.1	1010 ± 60^{A}	1030 ± 120^{A}	1.0

Data are means ± SEM of five independent experiments, with three biological replicates per treatment. Different letters within columns indicate significant differences ($P < 0.05$), according to Tukey's multiple range test.

3.3 Cadmium-induced accumulation of oxidatively damaged proteins was prevented by 20S proteasome increased activity

Protein carbonylation is considered a reliable parameter of oxidative stress (Shulaev y Oliver 2006). Also, the accumulation of oxidized proteins reflects the balance between their production and degradation, mainly by the 20S proteasome activity. Under our experimental conditions, only 100 µM Cd incremented protein carbonyl group content along the whole root (Fig. 3).

A time-dependent analysis of three peptidase activities was assayed for 50 µM Cd treatment. As it is shown in Fig. 4, the metal incremented 20S peptide hydrolyzing activities. At 72 h, all of them were significantly increased in the Ap, and also T and Q in the Rt. Thus, the lack of carbonylated protein accumulation in maize roots subjected to 50 µM treatment could be attributed to the increase in the activity of the 20S proteasome, in a similar way to that previously described (Pena et al. 2007).

3.4. Cadmium altered hormonal root homeostasis

Cadmium enhanced IAA and ABA levels in the entire root tissue, whereas SA content increased only in the Rt portion (Table 4). IAA increments by Cd in rice roots were related to the overexpression of the biosynthetic genes OsASA2 and OsYUCCA1 (Ronzan et al. 2019). Also, it has been described that Cd affects not only IAA content but also its distribution, metabolism, and transport (Chmielowska-Bak et al. 2014), suggesting an eventual switch to an alternative morphogenic root program to counteract metal

stress (Hu et al. 2013; Fattorini et al. 2017; Piacentini et al. 2020). Also, numerous reports indicate that exogenous application of IAA, as well as the IAA precursor indole-3-butyric acid (IBA), reduced Cd toxicity in plants (Agami y Mohamed 2013; Li et al. 2020b; Zhang et al. 2020; Zhou et al. 2020; Piacentini et al. 2020; Demecsová et al. 2020). However, more information is needed to know if endogenous IAA levels reached in maize root during Cd stress can induce a similar effect compared to that observed when IAA is exogenously added.

Table 4

Effect of Cd on hormone content. Extracts were obtained from root apex (Ap) and the remaining root tissue (Rt) of maize seedlings subjected to hydroponic culture without (control, C) or with 50 and 100 µM of $CdCl_2$ for 72 h.

Hormone	Ap			Rt		
content	C	$CdCl_2$ (µM)		C	$CdCl_2$ (µM)	
(ng g^{-1} FW)		50	100		50	100
IAA	10.8 ± 1.7^{a}	13.9 ± 0.6^{b}	20.5 ± 0.7^{c}	8.1 ± 2.3^{A}	23.6 ± 2.6^{B}	36.3 ± 5.7^{C}
ABA	1.94 ± 0.15^{a}	5.46 ± 0.61^{b}	3.49 ± 0.12^{b}	1.67 ± 0.49^{A}	8.65 ± 0.34^{B}	12.51 ± 0.63^{B}
SA	9.3 ± 0.2^{a}	17.3 ± 1.6^{b}	5.6 ± 0.5^{c}	16.6 ± 1.7^{A}	25.6 ± 1.6^{B}	25.0 ± 1.0^{B}
GA20	3.1 ± 0.3^{a}	3.0 ± 0.3^{a}	4.9 ± 0.1^{b}	2.7 ± 0.2^{A}	3.7 ± 0.6^{A}	6.4 ± 0.2^{B}
GA7	146 ± 9^{a}	123 ± 4^{b}	125 ± 6^{b}	146 ± 4^{A}	148 ± 8^{A}	156 ± 3^{A}
GA3	5.1 ± 0.8^{a}	3.7 ± 0.3^{b}	5.3 ± 0.3^{a}	3.3 ± 0.6^{B}	5.8 ± 0.7^{A}	3.9 ± 0.7^{B}
GA4	58.6 ± 14.3^{a}	25 ± 2^{b}	18.2 ± 2^{c}	65.7 ± 10.0^{A}	38 ± 10^{B}	35 ± 1.8^{B}
JA	61.2 ± 10.7^{a}	64.2 ± 5.8^{a}	38.4 ± 2.5^{b}	82.8 ± 12.1^{A}	71.8 ± 1.9^{A}	89.3 ± 8.5^{A}
JA-Ile	47.6 ± 5.7^{a}	9.5 ± 2.8^{b}	3.7 ± 0.2^{b}	57.9 ± 9.6^{A}	14.8 ± 0.3^{B}	20.9 ± 3.1^{B}

Data are means ± SEM of three independent experiments, with three biological replicates per treatment. Values represent means ± SEM. Different letters within rows indicate significant differences ($P < 0.05$), according to Dunnett's multiple comparisons test.

In plants, ABA is recognized as a modulator of the adaptive abiotic stress responses (Cutler et al. 2010) and a key player in alleviating heavy metal stress (Hu et al. 2020). Hsu and Kao (2003) reported a close relationship between endogenous ABA content and Cd tolerance in rice seedlings. Also, it was described that exogenous ABA application would partially relieve Cd toxic effects by increasing GSH and

phytochelatins biosynthesis (Chen et al. 2016; Song et al. 2016), as well as restrict Cd uptake and distribution (Han et al. 2016; Shen et al. 2017; Tang et al. 2020).

SA increase in the Rt may be involved as a mechanism to counteract oxidative stress induced by Cd. It has been well established that SA application improves plant acclimation to Cd excess by reducing the metal uptake and/or promoting plant antioxidant capacity (Popova et al. 2009; Hayat et al. 2010; Agami y Mohamed 2013; Shakirova et al. 2016; Guo 2019). In accordance, an Arabidopsis SA-deficient mutant resulted in negative effects on Cd tolerance, mainly due to the lowered GSH status (Guo et al. 2016).

Cadmium increased the root concentration of GA20, the precursor of the active form 13-hydroxylated GA3. Interestingly, the total root content of GA3 remained similar to the control at 100 µM Cd but decreased in the Ap and increased in the Rt at 50 µM Cd. On the other hand, the contents of non-13-hydroxylated GA7 and GA4 were reduced under both Cd treatments. A similar drastic decrease in GA4 content was reported during copper stress (Matayoshi et al. 2020).

The mechanism by which metals affect GA4 homeostasis could involve interference with hormone biosynthesis but also with subsequent gibberellin transformations. Liu et al. (2015b) reported up-regulation of two genes encoding GA2-oxidase, a major enzyme for deactivating bioactive gibberellins, in response to Cd stress.

The presence of Cd had a dramatic consequence on the active form JA-Ile, whose concentration was strongly diminished under the metal treatment. JA-Ile is considered the most metabolically active jasmonate (Fonseca et al. 2009), and, although the exogenous application of JA or methyl jasmonate (MJ) has been shown to alleviate Cd-toxic effects in plants (Singh y Shah 2014; Siddiqi y Husen 2019; Lei et al. 2020), little attention has been paid to Ile-JA regarding cadmium stress. Kurotani et al. (2015) suggested that deactivation of JA-Ile results in enhanced salt tolerance in rice. It would be of special interest to evaluate the turnover of JA-Ile in the context of Cd stress in future studies.

Conclusion

Maize seedlings exposed to Cd arrested root growth, and the entire primary root was found to be involved in redox and hormonal adjustments to trigger and/or to support defense mechanisms to cope with Cd stress. The integrated analysis of our experimental data shows that Cd addition decreases the root content of several essential nutrients, disrupts ASC homeostasis, and causes a strong decline in GA4 and JA-Ile levels, along with root growth inhibition. Faced with the incapacity of maintaining ASC homeostasis, CAT and GPX would be alternative enzymatic defense lines for seminal roots to remove ROS excess during Cd stress. Finally, the 20S proteasome seems to be a relevant defense component to cope with the oxidative damage generated by cadmium during this early stage of plant development.

Declarations

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Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Figures

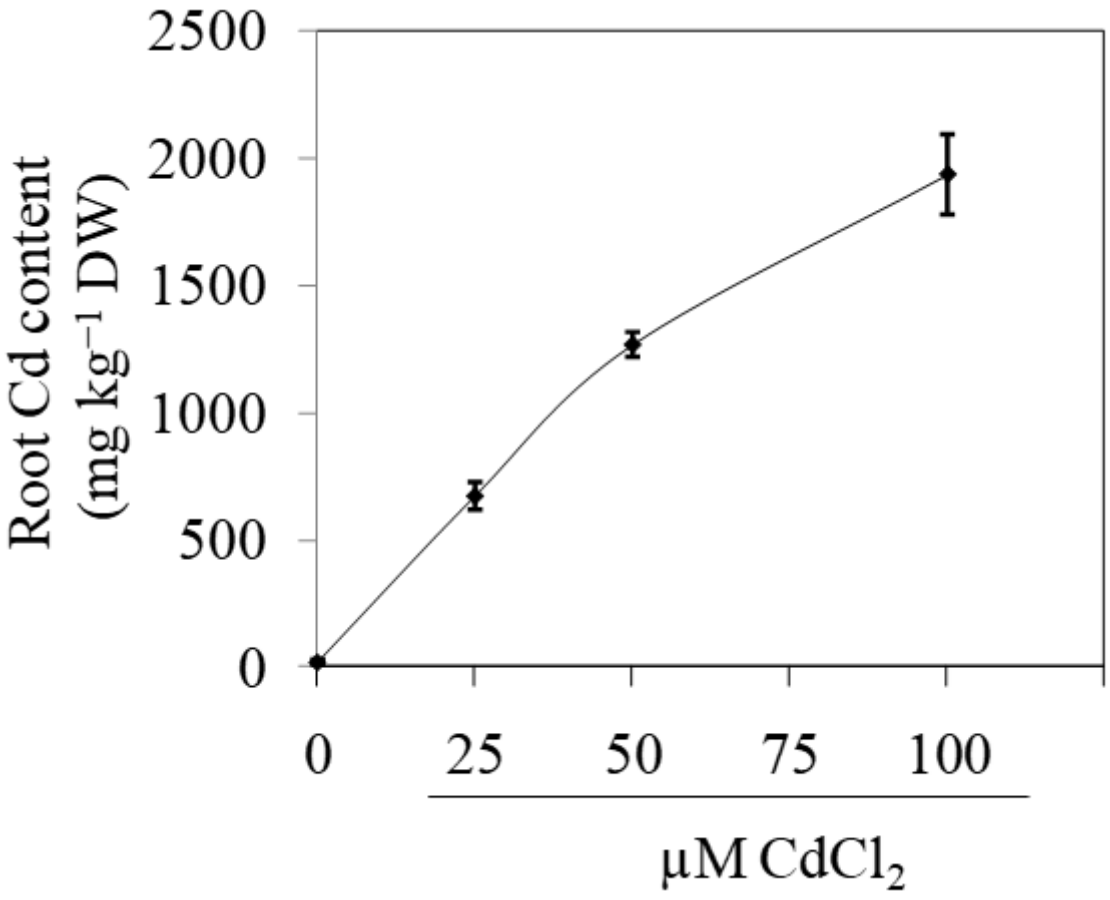

Figure 1

Root cadmium content. Maize seedlings were subjected to hydroponic culture without (control, C) or containing 50 and 100 μM of CdCl2. After 72 h of treatment, roots were harvested and used for Cd

determination. Data are means ± SEM of five independent experiments, with three biological replicates per treatment. Different letters indicate significant differences (P < 0.05), according to Tukey's multiple range test.

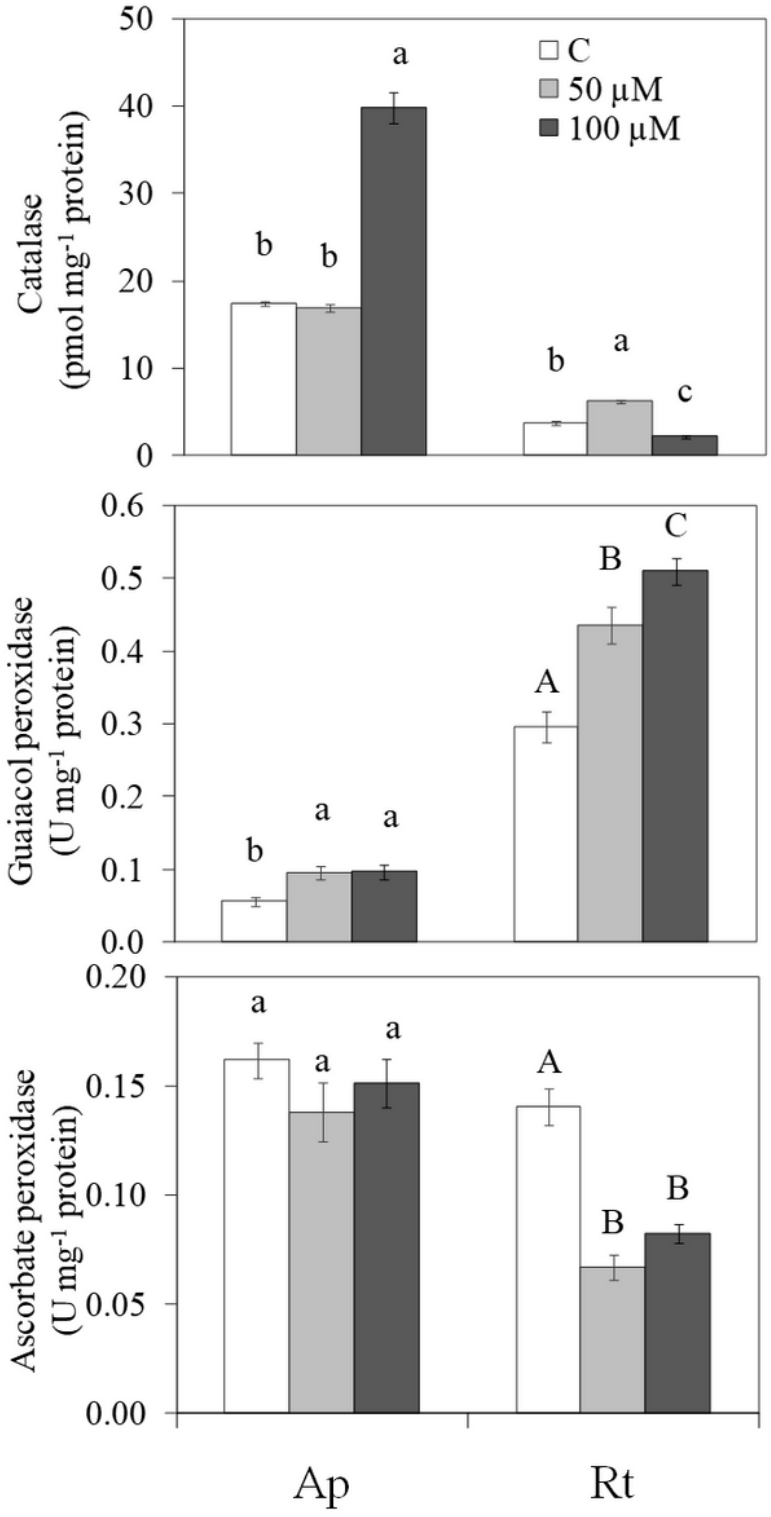

Figure 2

Effect of Cd on peroxidase activities. Maize seedlings were subjected to hydroponic culture without (control, C) or containing 50 and 100 µM of CdCl2 for 72 h. Determinations were performed on extracts

obtained from the root apex (Ap) and the remaining root tissue (Rt). Data are representative of five independent experiments with three replicates. At least three technical replicates of each protein extract were used for these determinations. Bars represent means ± SEM of five independent experiments, with three biological replicates per treatment. Different letters indicate significant differences ($P < 0.05$), according to Tukey's multiple range test.

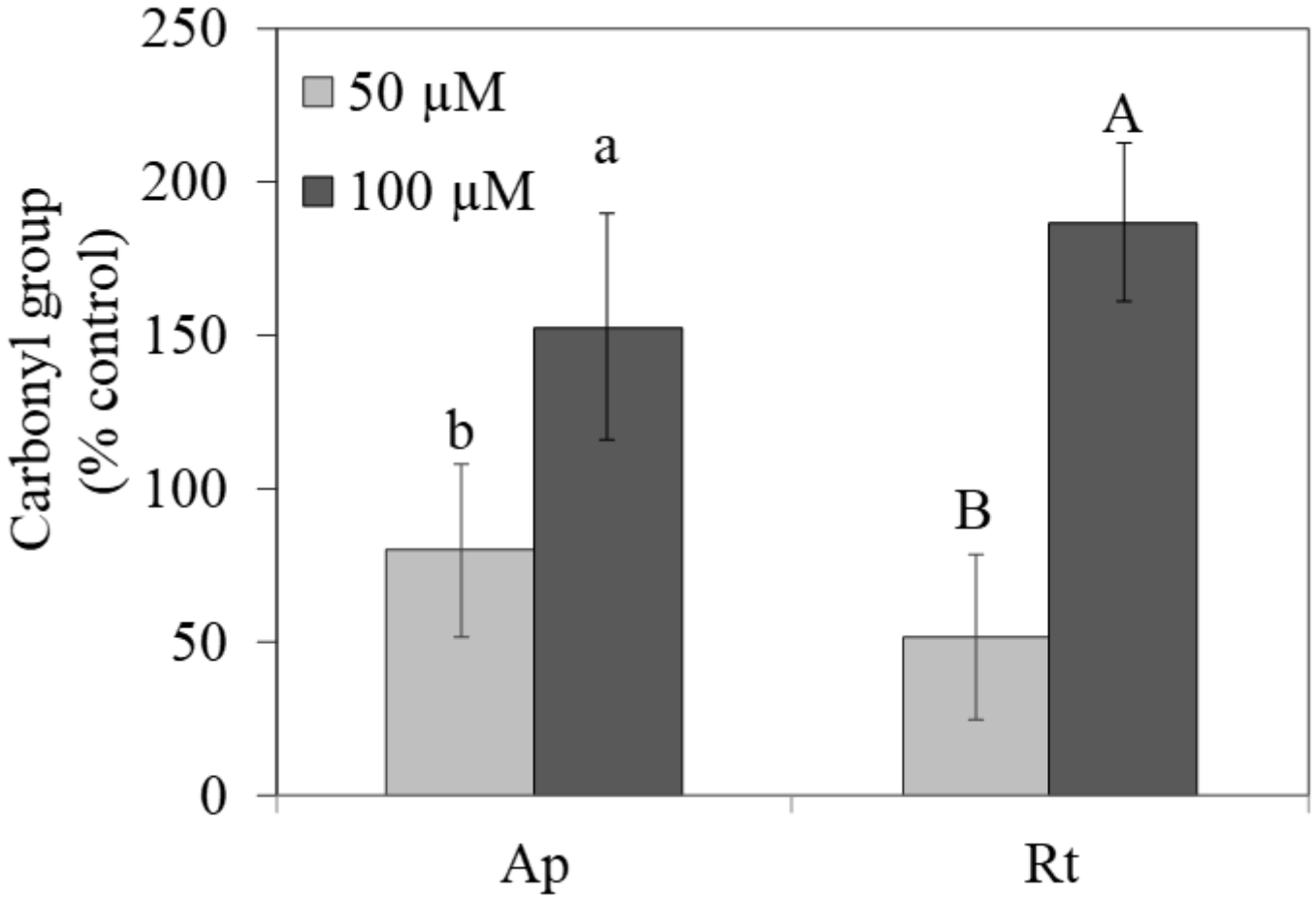

Figure 3

Effect of Cd on oxidative-damaged proteins. Maize seedlings were subjected to hydroponic culture without (control, C) or containing 50 and 100 µM of $CdCl_2$ for 72 h. Root protein extracts obtained from root tips (At) and the remaining root tissue (Rt) were used to determine DNPH-derivatized proteins by dot blot. Membranes were photographed and analyzed with Gel-Pro software. Quantification of oxidized proteins is expressed in arbitrary units (assuming control value equal to 100), based on the absolute integrated optical density (IOD) of each dot. Bars represent mean ± SEM. Different letters indicate significant differences respect to control at $P < 0.05$, according to Tukey's multiple range test.

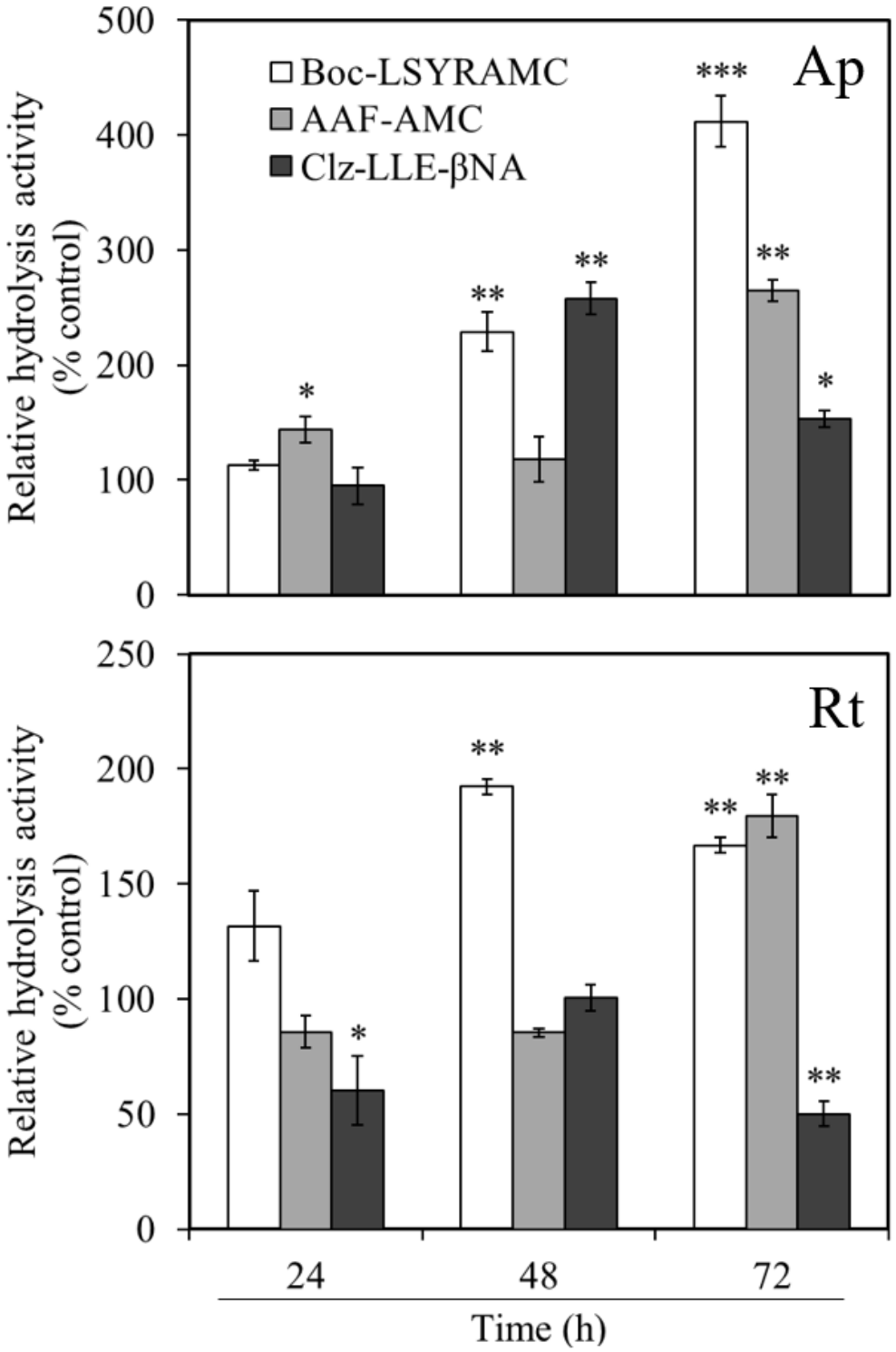

Figure 4

Effect of Cd on root proteasome proteolytic activities. Maize seedlings were subjected to hydroponic culture without (control, C) or with 50 CdCl2 up to 72 h. Trypsin-like, chymotrypsin-like, and peptidyl glutamyl peptide hydrolase (PGPH) proteasome activities were measured in roots extracts using three peptide substrates (Boc-LSYRAMC, AAF-AMC, and Clz-LLE-bNA, respectively) of the 20S proteasome in the absence or presence of the proteasome inhibitor MG132. Enzymatic activities were normalized for

protein concentrations and expressed as percentages of activity present in the controls. Data are representative of five independent experiments with three replicates. At least three technical replicates of each protein extract were used for each enzyme activity determination. Bars represent mean values ± SEM. Significant differences *P < 0.05, **P < 0.01 and ***P < 0.001 were observed between control and treated plants at each time, according to Tukey's multiple range test.