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An isopentenyl transferase transgenic wheat isoline exhibits less seminal root growth impairment and a differential metabolite profile under Cd stress

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Abstract

Cadmium is one of the most important contaminants and it induces severe plant growth restriction. In this study, we analyzed the metabolic changes associated with root growth restriction caused by cadmium in the early seminal root apex of wheat. Our study included two genotypes: the commercial variety ProINTA Federal (WT) and the P_{SARK}::*IPT* (IPT) line which exhibit high-grade yield performance under water deficit. Root tips of seedlings grown for 72 h without or with 10 μM $CdCl_2$ (Cd-WT and Cd-IPT) were compared. Root length reduction was more severe in Cd-WT than Cd-IPT. Cd decreased superoxide dismutase activity in both lines and increased catalase activity only in the WT. In Cd-IPT, ascorbate and guaiacol peroxidase activities raised compared to Cd-WT. The hormonal homeostasis was altered by the metal, with significant decreases in abscisic acid, jasmonic acid, 12-oxophytodienoic acid, gibberellins GA20, and GA7 levels. Increases in flavonoids and phenylamides were also found. Root growth impairment was not associated with a decrease in expansin (EXP) transcripts. On the contrary, *TaEXPB8* expression increased in the WT treated by Cd. Our findings suggest that the line expressing the P_{SARK}::*IPT* construction increased the homeostatic range to cope with Cd stress, which is visible by a lesser reduction of the root elongation compared to WT plants. The decline of root growth produced by Cd was associated with hormonal imbalance at the root apex level. We hypothesize that activation of phenolic secondary metabolism could enhance antioxidant defenses and contribute to cell wall reinforcement to deal with Cd toxicity.

1 | INTRODUCTION

Cadmium (Cd) is a nonessential element harmful to all living organisms. It is present in the environment from natural and soil anthropogenic sources (Mahmood et al., 2019). Cd uptake can occur through the entire root, but the apex zone was described as the most active region for Cd^{2+} influx (Piñeros et al., 1998). A previous study demonstrated that Cd induced the production of reactive oxygen species (ROS) and protein-oxidative damage in wheat (*Triticum aestivum* L.) root tip (Pena et al., 2012). In particular, oxidative modification of cell cycle proteins in the root apical meristem (RAM) would be, in part, responsible for the root growth arrest under metal stress (Pena et al., 2012, 2015).

Along with cell proliferation, primary root growth depends on cell expansion in the meristem (Ivanov & Dubrovsky, 2013). In the RAM, different regions are defined according to the cell developmental

phase. Nearest the quiescent center (QC) is the proximal meristematic zone (MZ), where cells divide several times. Then, cells move through the transition zone (TZ) to the elongation zone (EZ), where they lose the capacity to proliferate but increase their length. Finally, cells reach the differentiation zone (DZ), acquiring their specific characteristics and functions (Baluska et al., 2010). Expansins are cell wall proteins implicated in acid-induced wall extension (Cosgrove, 2015). The members of the expansins superfamily are classified into four subfamilies: α-EXPANSIN (EXPA), β-EXPANSIN (EXPB), EXPANSIN-like A (EXLA), and EXPANSIN-like B (EXLB) (Kende et al., 2004). Together with their function during normal growth, expansins also play a critical role in plant adaptation to environmental stress via relaxation and extension of plant cell walls (Tenhaken, 2015).

The functionality of a complex antioxidant defense system developed by plant cells is essential to keep up cell redox homeostasis during cadmium stress (Gallego et al., 2012). Plants have developed an efficient radical scavenging system as a strategy to avoid the imbalance between the ROS and the antioxidants formed in response to metal stress. ROS scavengers include enzymes like superoxide dismutase (SOD, EC 1.15.1.1), which converts superoxide anion (O_2^-) to H_2O_2, catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), and a variety of general peroxidases (GPOX, EC 1.11.1.7) responsible for H_2O_2 detoxification (Halliwell, 2006). The non-enzymatic antioxidant system comprises low molecular weight compounds like glutathione and ascorbic acid (Foyer & Noctor, 2016).

However, adjustments in the redox and hormonal homeostasis are necessary for plants to cope with abiotic stress (Bartoli et al., 2013; Raja et al., 2017). Exogenous application of hormones usually alleviates metal toxicity, the modulation of redox balance being one of the mechanisms associated with cell protection (Sytar et al., 2019). The modification in the endogenous cytokinin levels (CKs) during abiotic stress suggests the involvement of this hormone in plant stress response (O'Brien & Benkova, 2013). In this context, engineering CKs metabolism was considered a promising way to improve crop plants to cope with adverse growth conditions (Wani et al., 2016; Zalabák et al., 2013).

The enzyme isopentenyl transferase (IPT) catalyzes the first and rate-limiting step in the biosynthesis of CKs (Sakakibara, 2006). Plants overexpressing *IPT* under the control of different promoters have been developed the last years to improve the level of CKs. One of the promoter used is the maturation- and stress-inducible *SENESCENCE ASSOCIATED RECEPTOR PROTEIN KINASE* promoter (P_{SARK}) isolated from *Phaseolus vulgaris*. Consequently, transgenic plants expressing the *IPT* gene under the control of P_{SARK} have been regarded as useful to maintain higher CKs concentration during stress, but not high enough to impinge on developmental processes (Delatorre et al., 2012; Peleg et al., 2011).

The root system of wheat comprises two root types: (1) the seminal roots, which emerge when seeds germinate, and (2) the nodal roots (adventitious or crown roots), which arise from the lower nodes of the shoot. The early seminal roots support the plant growth until the nodal roots emerge and are the primary organ that eventually comes in contact with pollutants present in soils. Thus, in wheat, as in other cereals, control and reprogramming early seminal root growth during abiotic stress is essential for plant emergence and survival.

Previous research conducted by our group indicated that wheat root apex was particularly affected by metal stress, which resulted in increased ROS levels and impaired cell cycle and RAM development, leading to severe root growth decrease (Pena et al., 2012, 2015). However, information regarding primary metabolic responses in the RAM due to toxic Cd levels is still scanty. Herein, we studied the metabolic response induced by cadmium in the root apex and analyzed the antioxidant enzyme activities, the hormonal balance, and root apex's metabolite profile. Since RAM development depends on auxin and cytokinin crosstalk (Su et al., 2011), and considering that Cd stress affects water relations and water limitation restricts plant growth, we decided to use a wheat line expressing the P_{SARK}::*IPT* construction. This line was selected because of its high-grade yield performance under water deficit and is a good tool to investigate possible metabolic and biochemical differences in response to Cd stress.

2 | MATERIALS AND METHODS

2.1 | Plant material and growing conditions

Seeds of *Triticum aestivum* L. cultivar ProINTA Federal (WT) and a transgenic isoline expressing P_{SARK}::*IPT* (IPT) were used for the experiments. The P_{SARK}::*IPT* isoline contains the cassette *SARK:IPT:NOS*, which carries the *IPT* gene from *Agrobacterium tumefaciens* under the regulation of the SARK promoter from bean and the nopaline synthase (NOS) terminator (Rivero et al., 2007). Based on the fact that Cd induces dehydration in plants (Rucińska-Sobkowiak, 2016), the IPT line used in this work—among the six events obtained—was selected because of its better yield performance under water deficit in greenhouse experiments (Beznec, 2016). Seeds were placed in flasks containing 30 mL of demineralized water (WT or IPT) or 10 μM $CdCl_2$ (Cd-WT or Cd-IPT), 20 seeds per flask. Flasks were randomly distributed in a rotary shaker (100 rpm) and incubated at 24 ± 2°C in darkness. At least eight flasks per treatment were prepared for each experiment and three independent experiments were performed. The Cd concentration used was selected according to our previous experience (Pena et al., 2012) and corresponds to the lowest dose that caused growth differences between lines. It is a realistic concentration that can be found in soils used to cultivate edible crops (Mahmood et al., 2019) and it is also within the range commonly used to study the effect of this metal in wheat plants. To let aside cadmium effects on photosynthesis, we evaluated Cd effect on root apex before the time to which, theoretically, wheat coleoptiles sprout through the soil surface and phototrophic lifestyle begins. After 72 h of incubation, the seedlings contained in each flask were gently washed with distilled water and used for the assays. Germination percentage was always above 80%, and it was unaffected by the experimental conditions. As plant growth parameters, the longest root's length and root fresh (FW) and dry weight (DW) (oven-dried at 75°C

until constant weight) were recorded. For analytical determinations, apical root segments (first 10 mm from the root tips) obtained from all seedlings in different flasks were pooled and used to prepare one extract.

2.2 | Root meristem-size measure

Roots were fixed overnight in FAA (paraformaldehyde:ethanol:glacial acetic acid 1:4:1) dehydrated in an ethanol series, transferred to xylene, and processed by the inclusion in paraffin. Longitudinal apex sections of 10 μm were cut following standard botanical microtechniques. The slides were stained with a safranin-fast green combination and mounted on a glass slide with dibutylphthalate polystyrene xylene (DPX) and then observed using a Zeiss optic light microscope equipped with a digital photo camera. The distance between the QC and the first cortical elongated cell in the TZ was measured using the software analysis package ImageJ (http://rsb.info.nih.gov/ij). For three independent experiments, at least 5 roots were analyzed, and the mean and SEM were calculated (n = 15).

2.3 | Expansin transcripts profile

Transcript profiles of expansins were determined by reverse transcriptase polymerase chain reaction (RT-PCR) in root tip tissues, according to the method described by Marone et al. (2001). Total RNA was extracted from root apical segments using a TRIzol (Quick- ZOL, Kalium Technologies) procedure as described by the manufacturer. RNA was treated with DNase Turbo (Life technologies) and converted to cDNA with oligo (dT)18 using the RevertAid Reverse Transcriptase (Thermo Scientific). Primers' sequences and PCR conditions are described in Table S1. PCR reactions were performed using a Termocycler T 18 (Ivema). PCR products were separated by electrophoresis on 1% (w/v) agarose gels and visualized with DSView Nucleic Acid Stain (Dongssheng biotech). Wheat *TUBULIN* was used as the housekeeping gene. Gels were photographed and analyzed with Image J software. Data are expressed as arbitrary units (a.u.) based on the ratio of the absolute integrated optical density of each *EXPANSIN* band and the corresponding *TUBULIN* band. Each expression profile shown is representative of three independent experiments with two replicates.

2.4 | Hydrogen peroxide determination

Hydrogen peroxide content was determined by homogenizing 200 mg of root apex tissue in 0.5 mL 0.1% (w/v) trichloroacetic acid (TCA). After centrifuging at 12,000*g* for 15 min, 0.2 mL of supernatant was mixed with 0.2 mL of 10 mM phosphate buffer (pH 7.0) and 0.4 mL of 1 M potassium iodide (KI), and the absorbance at 390 nm was recorded (Singh et al., 2006). H_2O_2 content was calculated from a standard curve of H_2O_2.

2.5 | Antioxidant enzyme activity assays and peroxidase zymogram

Protein extracts were prepared by homogenizing apical root segments (from at least 15 seedlings of each flask) in 1 mL of extraction buffer containing 50 mM potassium phosphate buffer (pH 7.4), 1 mM ethylenediaminetetraacetic acid (EDTA), 1% (w/v) polyvinylpyrrolidone (PVP) and 0.5% (v/v) Triton X-100 at 4°C. Homogenates were centrifuged at 26,000*g* for 30 min, and the supernatant fraction was used for assays. Measurements represent the maximal extractable activities of antioxidant enzymes (Noctor et al., 2016). Superoxide dismutase and guaiacol peroxidase activities were quantified in a final volume of 0.2 mL in 96-well UV-microplates using a FlexStation 3 microplate reader, equipped with an internal shaker and temperature incubator (Pena et al., 2020). Superoxide dismutase activity was assayed by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) (Giannopolitis & Ries, 1977), and SOD-specific activity was expressed as units per mg of protein. Catalase activity was determined in apex homogenates, according to Aebi (1984). The pseudo-first-order reaction constant ($k' = k \times [CAT]$) of the decrease in H_2O_2 absorption was determined, and catalase content (pmol mg^{-1}) protein was calculated using $k = 4.7 \times 10^{-7}\ M^{-1}\ s^{-1}$. Ascorbate peroxidase activity was measured as described by Nakano and Asada (1981). One unit of APX was defined as the number of nmoles of oxidized ascorbate formed per min and mg of protein. Guaiacol peroxidase activity was determined in the homogenates by measuring the increase in absorption at 470 nm due to the formation of tetraguaiacol ($\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$), in a reaction mixture containing 50 mM potassium phosphate buffer (pH 7), 0.1 mM EDTA, 10 mM guaiacol, and 10 mM H_2O_2. One unit of GPOX was defined as the amount of tetraguaiacol formed (μmol) per minute. Data are representative of three independent experiments with three replicates. At least three technical replicates of each protein extract were used for each enzyme activity determination.

To determine the peroxidase (POX) isoforms' pattern, equal amounts of protein (50 μg) were subjected to discontinuous 4% stacking and 10% running native PAGE (Laemmli, 1970). After electrophoretic separation, gels were soaked for 10 min in 0.25 M Na-acetate buffer (pH 5.3) and then transferred to 0.2% benzidine in methanol: Na-acetate buffer (75:25) and 0.5 mM H_2O_2 (Moore et al., 1978). Gels were fixed in ethanol:acetic acid:water (40:10:60) and photographed.

Protein concentration was determined according to Bradford (1976), using bovine serum albumin as standard.

2.6 | Endogenous phytohormone determination

Hormone extraction from the root apex was carried out as described in Durgbanshi et al. (2005) with modifications (Matayoshi et al., 2020). For hormone analysis, samples were injected into an ultra-performance liquid chromatography system (Acquity SDS or Waters Alliance 2695, Waters Corp.). Chromatographic separations were carried out on a reversed-phase C18 column (Gravity, 50 × 2.1 mm 1.8-μm particle size, Macherey-Nagel GmbH), using a methanol:water

(both supplemented with 0.1% acetic acid) gradient at a flow rate of 300 μL min^{-1}. Gibberellins (GA_3, GA_4, GA_7, GA_{20}), abscisic acid (ABA), jasmonic acid (JA), 12-oxophytodienoic acid (OPDA), JA-isoleucine conjugate (JA-Ile), indole-3-acetic acid (IAA), and salicylic acid (SA) were quantified with a triple quadrupole mass spectrometer (Micromass) connected online to the output of the column through an orthogonal Z-spray electrospray ion source. The spectrometer was operated in negative ionization electrospray mode, and plant hormones were detected according to their specific transitions using a multi-residue mass spectrometric method.

2.7 | Analysis of metabolites using liquid chromatography-mass spectrometry (UPLC-QqTOF-MS)

For LC/MS analyses, 10 mg of lyophilized root tips were extracted in 500 μL of 70% methanol (LC/MS grade, Panreac) spiked with biochanin (1 mg L^{-1}) by ultrasonication for 10 min at room temperature. Sample preparation was carried out as described by Zandalinas et al. (2017). Chromatographic separations were performed on a 100 mm × 2.1 mm internal diameter, 1.6 μm, Luna Omega Polar C18 (Phemomenex) column, using acetonitrile and ultrapure water, both supplemented with formic acid at a concentration of 0.1% (v/v), as solvents with an Acquity SDS system (Waters Corp. Ltd.) interfaced to a QTOF Premier from Micromass Ltd. through an ESI source (Zandalinas et al., 2017). Samples were analyzed in both negative and positive ionization modes in the 50–1000 amu scan range using a capillary and cone voltages of 3.5 KV and 30 V, respectively. Mass chromatograms were centroided using the accurate mass of the molecular ion of leucine enkephalin (ESI+ 556.2771 and ESI− 554.2625). After acquisition, files were converted to NetCDF for subsequent xcms processing. Chromatographic peak detection was performed using the matchedFilter algorithm, and retention time correction was achieved in three consecutive iterations reducing bandwidth. Ion type identification, adduct annotation, and grouping of related mass chromatographic features were achieved with the CAMERA package (Kuhl et al., 2012). Normalization of peak areas was achieved by taking into account the internal standard biochanin area and the actual sample weight. Significantly-altered mass chromatographic features were identified after ANOVA using genotype and treatment as factors.

Annotation of metabolites was achieved by comparison of mass spectra and retention time with an in-house-built database. Additionally, unknown metabolites were identified by manual curation of mass pseudospectra (generated by CAMERA) and comparison of actual pseudomolecular ions and fragmentation patterns with those available in public databases (Metlin, MassBank, and HMDB).

2.8 | Statistics

Values are expressed as means ± SEM. Differences among treatments were analyzed using InfoStat software (Di Rienzo et al., 2019) by two-way ANOVA, taking $P < 0.05$ as significant according to Tukey–Kramer's multiple range test.

To investigate any underlying data structure in metabolomics data, raw xcms output matrices were normalized as above and subjected to principal component analysis (PCA). Moreover, following ANOVA and annotation of mass chromatographic features as individual metabolites, results were row-wise standardized and represented as heatmap for a better visualization after row-wise standardization. Annotated metabolites in rows were subjected to hierarchical cluster analysis (HCA) to investigate potential co-regulations, setting 'Euclidean distance' as the distance metrics and 'complete' as the clustering method.

3 | RESULTS AND DISCUSSION

3.1 | Cadmium decreased root elongation and affected root apical meristem size

The decrease in root growth induced by heavy metal has been reported to be related to a decrease in cytokinins production (De Smet et al., 2015). Also, it is well known that heavy metal stress affects plant water relations, reducing plant growth and affecting various biochemical and metabolic processes (Rucińska-Sobkowiak, 2016). Thus, to add information regarding root metabolic responses to Cd stress, a comparative analysis was performed using a transgenic wheat line expressing P_{SARK}::*IPT*.

Under no stress germination conditions, the IPT line showed similar root length but higher root biomass (20% FW and 14% of DW) compared to the WT line (Table 1). This slight increment in root biomass could represent a better carbon (C) and nitrogen (N) remobilization from seed reserves to the root in the IPT line. Improvement in C and N metabolism has been described in different P_{SARK}:*IPT* transgenic plant species, especially during stress conditions (Gujjar & Supaibulwatana, 2019; Peleg et al., 2011; Reguera et al., 2013).

Cadmium treatment negatively affected root biomass in the same proportion in both lines. However, root length reduction was higher in WT than in IPT plants (30 and 20%, respectively, compared to the respective control; Table 1). Li et al. (2011a) reported that root length was the most sensitive to cadmium toxicity among various wheat growth parameters assessed. To evaluate if the cadmium-induced reduction of root elongation could be attributed, at least in part, to differences in cell expansion, the distance from the QC to the TZ was determined in longitudinal root apex cross-sections. Interestingly, the distance in IPT lines was diminished compared to that in the WT under no stress condition (Figure 1). It is well known that an antagonistic interaction between cytokinins and auxins in the TZ regulates RAM development (Perilli et al., 2012; Schaller et al., 2015; Su et al., 2011).

On the one hand, auxins support meristem activity by promoting cell division. On the other hand, cytokinins promote cell differentiation by repressing auxin signaling and transport. Thus, the RAM size

TABLE 1 Effect of cadmium on seminal root biomass

Growth parameter	WT	Cd-WT	IPT	Cd-IPT
Length (cm)	3.9 ± 0.1^{a}	2.7 ± 0.1^{c}	4.0 ± 0.1^{a}	3.1 ± 0.1^{b}
Fresh weight	308 ± 8^{b}	252 ± 18^{c}	369 ± 4^{a}	265 ± 4^{c}
Dry weight	19.6 ± 0.4^{b}	18.8 ± 0.2^{b}	22.3 ± 0.9^{a}	20.0 ± 0.5^{ab}

Note: Wheat wild type (WT) and transgenic plants expressing P_{SARK}::*IPT* (IPT) seeds were germinated and grown in flasks containing 30 mL of water or 10 μM of $CdCl_2$ for 72 h. Fresh and dry weight (FW and DW, respectively) are expressed in mg per 25 seedlings. Values represent means ± SEM. Different letters indicate significant differences at $P < 0.05$ according to Tukey–Kramer's multiple range test.

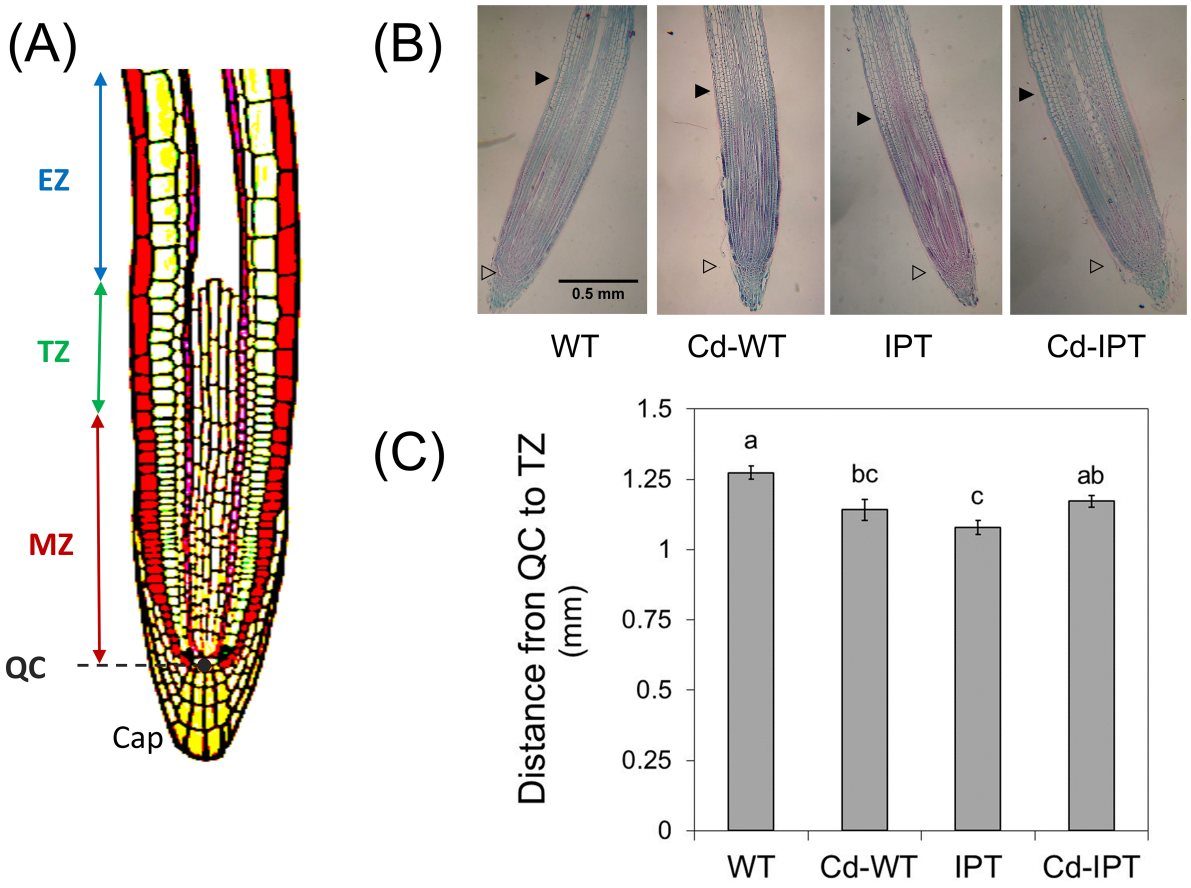

FIGURE 1 (A) Schematic longitudinal view of the root apical meristem (RAM) structure: Quiescent center (QC), meristem zone (MZ), transition zone (TZ) and elongation zone (EZ). (B) Representative images of longitudinal cross-sections of the wheat root apex. Wheat seeds from WT and IPT line (20 per flask) were germinated in 30 mL of water or 10 μM $CdCl_2$ for 72 h. empty and filled black arrowheads indicate the QC and the cortex TZ in the RAM, respectively. (C) Distance from the QC to TZ. Values are mean ± SEM of three independent experiments with five roots analyzed (n = 15). Different letters indicate significant differences ($P < 0.05$) according to Tukey–Kramer's multiple range test

decrease is consistent with cytokinins' effect in IPT lines (Dello Ioio et al., 2007). Cadmium decreased RAM size in WT wheat seedlings but increased QC-to-TZ distance in the IPT line (Figure 1). This data reinforces the idea proposed by Bruno et al. (2017), who suggested that Cd differentially affected cell expansion by altering auxin/cytokinin signaling homeostasis.

In this regard, the expression of four wheat *EXPANSIN* genes with higher expression in the roots than other organs (Han et al., 2019; Zhang et al., 2018) was evaluated in this study. As a general trend, the expression level of the expansin genes analyzed was unaffected by Cd addition in the IPT line but augmented in the WT. The *TaEXPA6* expression was similar in both lines and unaffected by the metal, whereas *TaEXPB8* expression was higher in the IPT line and rose upon Cd treatment only in the WT line. Among the four analyzed expansins, three of them were upregulated in the IPT line, suggesting that they were CKs-dependent, while Cd increased their expression only in the WT line (Figure 2). Consequently, an enhanced expression of *EXPs* in the root growth zone could be contributing to the maintenance of primary growth during stress (Cosgrove, 2015). It is important to highlight that 241 expansin genes were recently identified in the wheat genome (Han et al., 2019). However, the specific physiological role of each expansin under normal or stressful conditions is still unknown. In

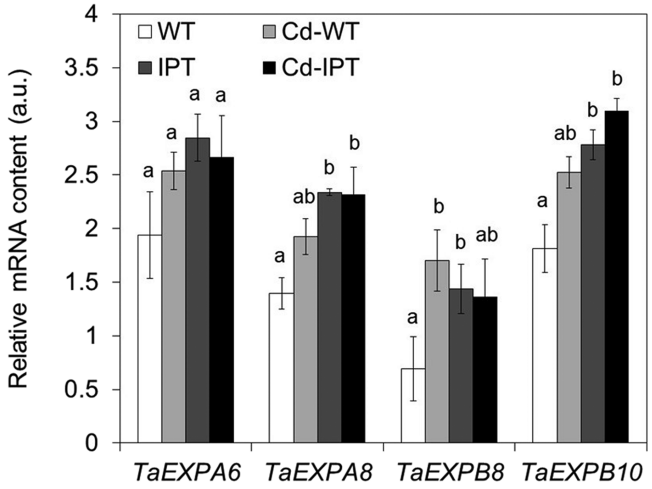

FIGURE 2 Expansin genes transcript accumulation in root apex. Semi-quantitative RT-PCR analysis of *TaEXPA6*, *TaEXPA8*, *TaEXPB8*, and *TaEXPB10* genes was preformed using specific primers for wheat expansins. *TUBULIN4* transcript was used as housekeeping gene. Values are mean ± SEM of three independent experiments with two replicates (n = 6). Different letters indicate significant differences ($P < 0.05$) according to Tukey–Kramer's multiple range test

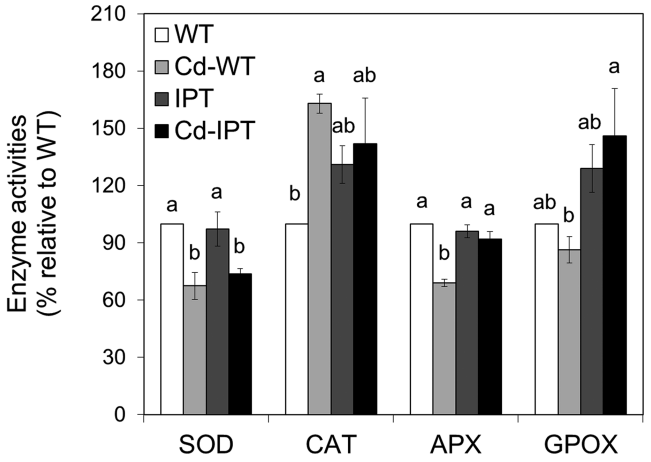

FIGURE 3 Effect of cadmium on superoxide dismutase and peroxidase activities. Enzymatic activities were assayed in root tip extracts, as indicated in materials and methods, and expressed as a percentage of the WT activity. Values of specific activities in control plants: SOD 0.024 ± 0.009 U mg^{-1} protein, CAT 2.1 ± 0.2 pmol mg^{-1} protein, APX 23.4 ± 2.1 U mg^{-1} protein, GPOX 187 ± 20 U mg^{-1} protein. Values are mean ± SEM of three independent experiments with three replicates (n = 9). Different letters indicate significant differences ($P < 0.05$) according to Tukey–Kramer's multiple range test

this regard, it has been demonstrated that overexpression of *TaEXPB7-B* in Arabidopsis enhanced plant tolerance to low-temperature stress (Feng et al., 2019). Furthermore, the ectopic expression of *TaEXPB23* was associated with phosphorus availability (Han et al., 2014) and drought (Li, Xing, et al., 2011b), as well as in oxidative stress tolerance (Han et al., 2015; Li et al., 2015). Also, the overexpression of the *TaEXPA2* gene in tobacco was involved in drought stress tolerance and improved salt tolerance by regulating Na^+/K^+ interchange (Chen et al., 2016, 2017). In tobacco, *TaEXA2* induced oxidative stress tolerance by upregulating the expression and activity of cell wall peroxidases (Chen et al., 2018); besides, this expansin modulated the absorption and transportation of the metal to cope with Cd stress (Ren et al., 2018). Interestingly, in these studies, it was determined that expansins overexpression was related to hormone response and improved antioxidant capacity during stress.

3.2 | Cadmium altered redox status in the root apex

In various wheat cultivars, cadmium tolerance has been related to the antioxidant capacity (Afzal et al., 2019; Guo et al., 2019; Wu et al., 2003). Our results showed that Cd decreased SOD activity by 33 and 24% in Cd-WT and Cd-IPT, respectively, compared to untreated controls (Figure 3). SOD is considered the first line of antioxidant defense to control the relative amounts of O_2^- and H_2O_2. The SOD activity decline could generate a disruption in the balance between O_2^- and H_2O_2, and thus affect the normal RAM development (Tsukagoshi et al., 2010).

A slight increment in CAT activity was measured in the IPT line compared to the WT, but a significant increase was observed in Cd-WT (63% over WT control) (Figure 3). Catalase is a peroxisomal antioxidant enzyme involved in regulating H_2O_2 levels not only under physiological but also during stress conditions (Sandalio & Romero-Puertas, 2015). The role of CAT in overcoming Cd-induced toxicity has been well documented in different plant species, developmental stages, and plant tissues, but its involvement in the root apex is reported here for the first time. Guo et al. (2019) reported CAT activity increases in the leaves of two wheat varieties exposed to Cd with different tolerance to this metal, but no information related to the root apex was reported before.

APX and GPOX significantly increased in Cd-IPT lines compared to Cd-WT (Figure 3), indicating an adjustment of the cell redox balance through the induction or activation of peroxidases. Also, the decrease in peroxidase activities in Cd-WT correlated with the rise in H_2O_2 (Figure 4), consistently with the ROS accumulation generated by Cd in wheat root tips already communicated (Pena et al., 2012). The variable responses of peroxidase activity to Cd can be partly associated with the subcellular distribution of the metal, but also to Cd-susceptibility of the antioxidant enzymes located in subcellular compartments (Li, Zhou, et al., 2011a). An elevated activity of phenolic-oxidizing enzymes, such as GPOX, in root tips was associated with increased phenolics contents and lignification in response to Cd (Schützendübel et al., 2001). Likewise, an improvement in antioxidant defenses due to the increased peroxidase activity was described in P_{SARK}::*IPT* tobacco plants under nitrogen deficiency (Rubio-Wilhelmi et al., 2011). This response was consistent with the

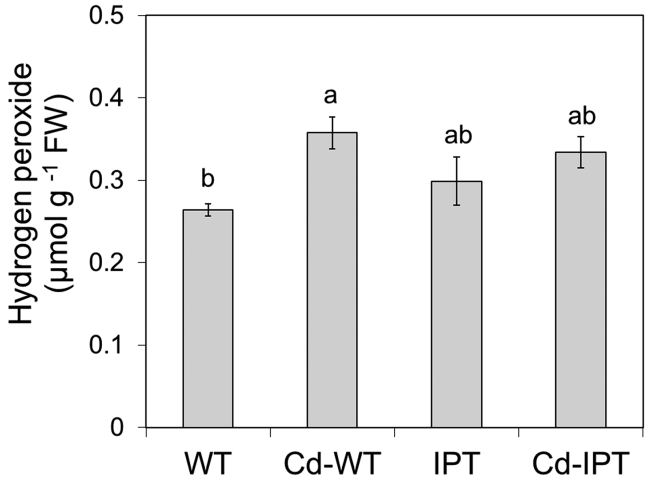

FIGURE 4 Hydrogen peroxide content in the root apex. Wheat seeds from WT and IPT line (20 per flask) were germinated in 30 mL of water or 10 µM $CdCl_2$ for 72 h. values are mean ± SEM of three independent experiments with three replicates (n = 9). Different letters indicate significant differences ($P < 0.05$) according to Tukey–Kramer's multiple range test

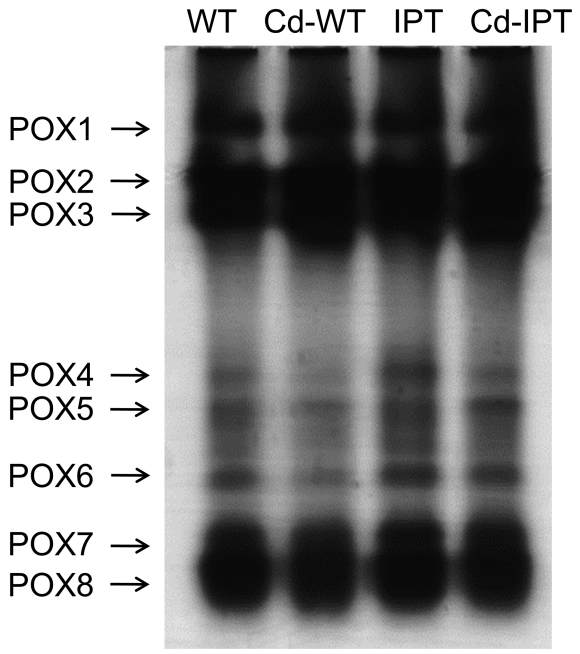

FIGURE 5 Peroxidase (POX) isoform profile. Proteins (50 µg protein per well) were separated by native-PAGE and stained for peroxidase activity, as described in materials and methods

already documented CKs' protective role and the antioxidant system adjustment under stress (Ahanger et al., 2018; Hönig et al., 2018; Synkova et al., 2006).

Despite GPOX activity was differentially affected by Cd in IPT and WT plants, a similar profile in POX isoenzymes was observed in the zymogram (Figure 5). The presence of at least eight bands (POX1 to POX8) was visualized in both genotypes. In particular, the diversity of isoforms, distribution, and the variety of substrates used by these enzymes place peroxidases as essential proteins regarding plant plasticity and response to abiotic stress (Jovanović et al., 2018). In the root apex, peroxidases are involved in the control of RAM size by modulating ROS balance in the TZ (Tsukagoshi et al., 2010). Also, in the root apex of wheat genotypes, peroxidases have been associated with different tolerance degrees to stress (Csiszár et al., 2012).

3.3 | Cadmium-induced endogenous phytohormone imbalance in the root apex

Plant responses to Cd involve multiple signaling pathways that regulate many aspects of plant growth and development (Gallego et al., 2012). Plant hormones integrate a complex network of interactive pathways involved in plant growth and development and efficient stress responses (Verma et al., 2016). Herein, plant hormone profiles in response to cadmium were analyzed in the root tip. Two-way ANOVA showed that Cd influenced ABA, OPDA, JA, and GA20 contents ($P < 0.05$). Indole acetic acid was the only hormone that was slightly incremented by Cd exposure in both lines (Table 2), in association with shorter RAM (Figure 1). In line with this finding, the accumulation of IAA and the root growth reduction were previously described in barley root apex after Cd treatment (Tamás et al., 2015). Our results using an IPT line support the idea that not only the amount but also the IAA and CKs homeostasis would determine the size of the RAM during Cd stress. Early studies suggested that perturbation of auxin distribution, more than its content, is the most relevant effect of Cd in the root tip (Bruno et al., 2017).

The presence of Cd decreased the ABA content in the root tip (Table 2). This could be attributed to metal-triggered water imbalance in the root tip (Kuromori et al., 2018). Previous findings demonstrated that root length and seedling fresh weight were comparable in the wild type and ABA-deficient and ABA-insensitive *Arabidopsis thaliana* mutants during Cd exposure (Sharma & Kumar, 2002), suggesting the lack of involvement of this hormone in Cd-induced growth restrictions.

Cd caused decreases in JA and OPDA (JA-precursor) levels in both lines (Table 2), indicating metal interference with the JA biosynthetic pathway. Other researchers documented JA and ABA reductions in citrus roots subjected to Cd^{2+} (López-Climent et al., 2011). Previous reports correlated the deficiency in endogenous JA level with the enhanced sensitivity of tomato seedlings to Cd stress (Zhao et al., 2016). Conversely, exogenous JA application minimized Cd accumulation in roots and mitigated the negative impact of Cd stress (Ahmad et al., 2017). Among the jasmonates derivatives, JA conjugated with isoleucine (JA-Ile) represents the most active form of this plant growth regulator and triggers defense responses (Fonseca et al., 2009). Interestingly, the IPT line showed increased JA-Ile content compared with the WT, but Cd treatment reduced JA-Ile content in the root apex. Given that JASMONOYL-ISOLEUCINE SYNTHASE (JAR1) is involved in JA-Ile synthesis, further studies are necessary to decipher the factors that regulate this enzyme activity in the RAM.

TABLE 2 Phytohormone contents in root apex segments

DW (ng g^{-1})	WT	Cd-WT	IPT	Cd-IPT
IAA	166.6 ± 22.5^{a}	207.1 ± 54.9^{a}	193.2 ± 20.1^{a}	227.9 ± 10.1^{a}
ABA	69.4 ± 0.5^{a}	57.8 ± 2.7^{b}	64.8 ± 2.7^{a}	58.8 ± 2.3^{b}
SA	157.4 ± 25.7^{a}	120.6 ± 6.4^{a}	150.6 ± 10.4^{a}	124.1 ± 22.4^{a}
OPDA	327.7 ± 14.0^{a}	237.0 ± 8.1^{b}	297. 6 ± 10.0^{a}	245.5 ± 12.6^{b}
JA	625.9 ± 55.1^{a}	461.8 ± 65.7^{ab}	612.7 ± 67.2^{a}	407.4 ± 28.6^{b}
JA-Ile	215.0 ± 10.5^{b}	231.4 ± 32.7^{b}	343.4 ± 25.7^{a}	202.1 ± 21.5^{b}
GA20	123.9 ± 4.2^{a}	86.6 ± 7.0^{b}	122.7 ± 4.2^{a}	78.3 ± 3.8^{b}
GA1	2.1 ± 0.2^{ab}	1.9 ± 0.2^{b}	2.7 ± 0.3^{a}	2.0 ± 0.1^{b}
GA3	51.0 ± 4.4^{a}	43.7 ± 2.9^{a}	53.3 ± 11.8^{a}	51.8 ± 5.8^{a}
GA7	2243 ± 136^{a}	1718 ± 83^{b}	2143 ± 175^{a}	1942 ± 105^{ab}
GA4	41.7 ± 12.3^{ab}	47.4 ± 14.7^{ab}	34.1 ± 3.7^{b}	58.3 ± 4.2^{a}

Note: Wheat wild type (WT) and transgenic plants expressing P_{SARK}::*IPT* (IPT) seeds were germinated and grown in flasks containing 30 mL of water or 10 μM of $CdCl_2$ for 72 h. Mean values ± SEM are shown. Different letters indicate significant differences ($P < 0.05$) according to Tukey–Kramer's multiple range test.

Cd treatment resulted in a decreased level of GA20, the precursor of the bioactive 13-hydroxylated forms GA1 and GA3 (Table 2). Since GA intermediates are involved in the long-distance transport of active GAs (Binenbaum et al., 2018), it is possible to assume that Cd interfered with GA20 movement toward the root tip. Nevertheless, the GA20 transformation into the bioactive forms GA1 and GA3 was unaffected by the metal. The most abundant bioactive GA in the root tip was the non-13-hydroxylated GA7. Cd produced an opposite effect in the non-13-hydroxylated GAs by decreasing GA7 and increasing GA4 amount (Table 2). Interestingly, since bioactive GAs synthesis occurs at the same site of action, Cd could have differentially impacted each cell line at the RAM level. Thus, although RAM morphology changes may have been caused mainly by a hormonal imbalance governed by the auxin/cytokinin ratio, gibberellins involvement cannot be excluded.

3.4 | Cadmium led to metabolite adjustments in the root apex

During germination and seedling emergence, a metabolic adjustment in primary root tips could be essential to cope with metal stress and support seedling growth and development. Herein, ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) was applied to explore metabolic alterations in the root apex of wheat seedlings after Cd stress. The data of all the mass chromatographic features were preliminary analyzed using PCA of positive and negative ion modes were constructed. Their score plots based on the first two principal components were presented in Figures S1 and S2. The first principal components (PC) explained almost 69% of the total variation, 51.5% for PC1 and 17.1% for PC2 for the positive ion mode. Similarly, the first PC explained almost 77% of the total variation, 54.5% for PC1 and 21.9% for PC2 for the negative ion mode. Thus, from the PCA score plots of both ion modes, it may be deduced that differentiation among groups occurred mainly along PC1, and the largest source of variation was Cd treatment. This result suggests that metal addition is the main driver of the changes observed. After multivariate and univariate analyses, the selected parent ions were retrieved using online databases to obtain the corresponding candidate compounds (Table S2).

An unsupervised multivariate HCA was applied to the hydrophilic metabolites that showed statistical differences among the samples to visualize general grouping information (Figure 6). In general, the dendrograms grouped compounds into two clusters: metabolites that were reduced (*up cluster*) or increased (*down cluster*) by Cd in both lines.

Fatty acid, phospholipids, and sphingolipids are among the metabolites decreased by the metal (Figure 6). Taken into account that these compounds comprise a metabolic group that is not long-distance transported in the plant, this result could indicate that Cd induced modification or redistribution of lipid metabolism at the apex level. Remodeling membrane lipid composition has been described as a potential mechanism to enhance plant abiotic stress tolerance (Liu et al., 2019). Also, the decrease in JA content would be in agreement with the observed alterations in the metabolism of unsaturated fatty acids (Table 2).

Metabolic adjustment included the increment of some phenolic secondary metabolites such as flavonoids and phenylamides. Flavonoids are mainly biosynthesized by the phenylpropanoid pathway from shikimate (Nabavi et al., 2020) and are recognized for their key role in plant defense against both biotic and abiotic stress and also for their involvement in plant-microorganism interactions (Falcone Ferreyra et al., 2012). Among their various molecular properties, antioxidant and ion-chelating capacities were described (Cherrak et al., 2016). Phenylamides (PhA)-conjugates of biogenic aliphatic polyamines or arylmonoamines and hydroxycinnamic acids are a

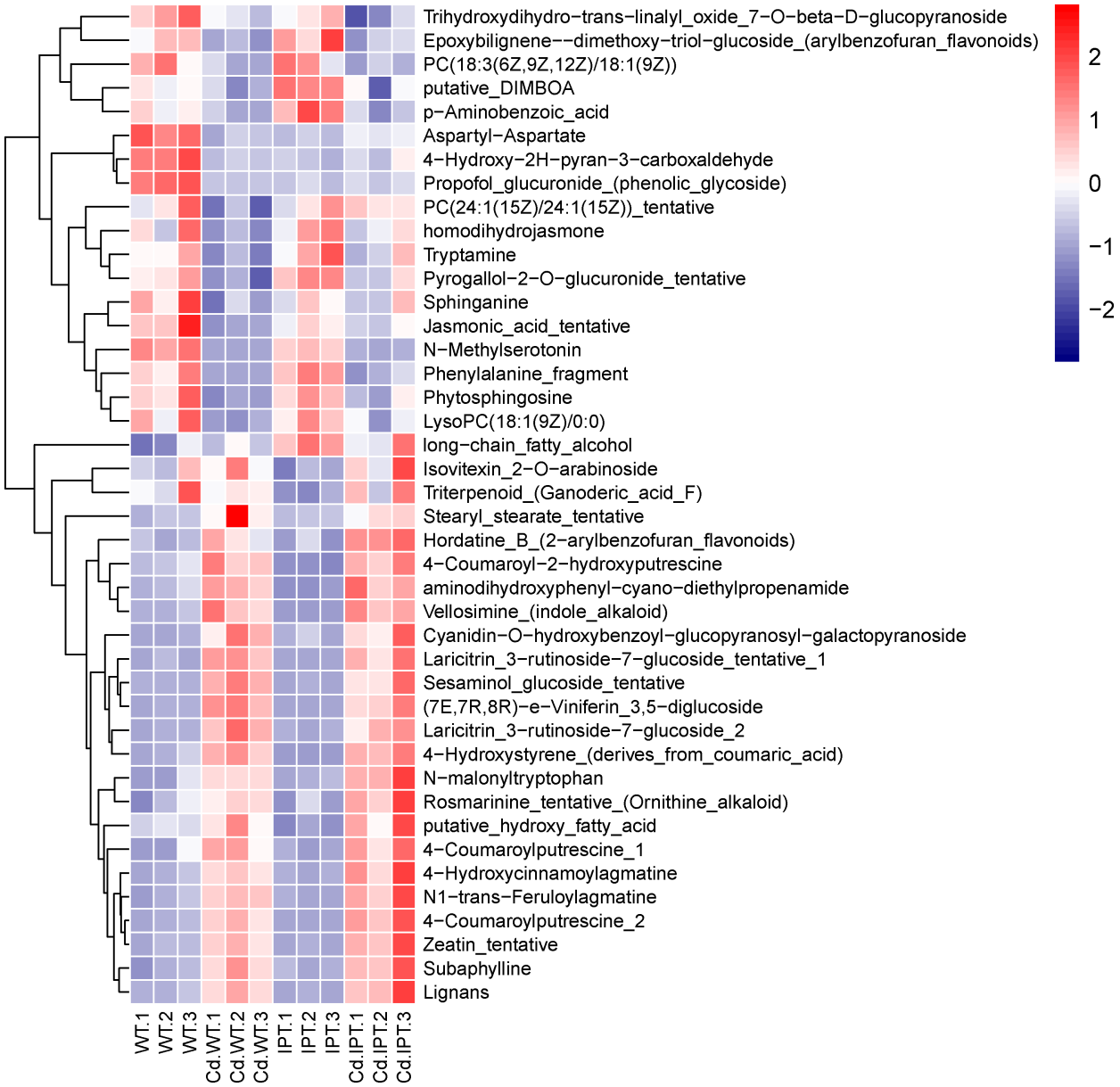

FIGURE 6 Metabolites heatmaps combined with hierarchical clustering analysis. Wheat seeds from WT and IPT line (20 per flask) were germinated in 30 mL of water without or containing 10 μM $CdCl_2$ for 72 h. the column represents the samples and the row displays metabolite. The blue color represents a decreasing trend and red a rising trend

widespread class of secondary metabolites distributed in plants (Bassard et al., 2010). Cadmium increased the PhA level, particularly those formed from putrescine and agmatine with hydroxycinnamoyl, coumaroyl, or feruloyl substitutions. During abiotic stress, PhA may act as antioxidant agents and ROS scavengers (Edreva et al., 2007). As substrates for peroxidases, they may have a role in eliminating H_2O_2 and participating in the cell-wall reinforcement by cross-linking (Bassard et al., 2010). The participation of PhA in the crosstalk between phenolic and nitrogen metabolism has been suggested previously (Bassard et al., 2010). Further studies specifically designed to gain a deeper knowledge about PhA metabolism in wheat root apex under cadmium stress are certainly needed.

4 | CONCLUSION

In the present work, we added valuable information regarding redox, hormonal, and metabolite adjustments occurring in the wheat root apex in response to Cd stress, using transgenic wheat plants expressing P_{SARK}::*IPT* construction, taking advantage of the water deficit tolerance of this line to study its behavior under Cd stress.

Our results suggest that the P_{SARK}::*IPT* construction could add an adaptive advantage to the normal cell homeostasis, enabling better performance of young seedlings to cope with Cd stress. Among these features, hormonal and metabolic adjustments in the root apex and upregulation of several expansin genes at the root apex may be

mentioned. Changes in phenolic secondary metabolites could lead to an improved antioxidant ability and cell wall reinforcement, resulting in less Cd toxicity inside root apex cells.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: Nabila M. Gomez Mansur, Liliana B. Pena, María P. Benavides, Susana M. Gallego. Performed the experiments: Nabila M. Gomez Mansur, Liliana B. Pena, Dalia M. Lewi, Vicent Arbona. Analyzed the data: Nabila M. Gomez Mansur, Liliana B. Pena, Adrián E. Bossio, Dalia M. Lewi, Ailin Y. Beznec, Eduardo Blumwald, Vicent Arbona, Aurelio Gómez-Cadenas. Supervised the experimental procedure and wrote the paper: María P. Benavides, Susana M. Gallego.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Aebi, H. (1984) Catalase *in vitro*. *Methods in Enzymol*, 105, 121–126.

Afzal, J., Hu, C., Imtiaz, M., Elyamine, A.M., Rana, M.S., Imran, M. et al. (2019) Cadmium tolerance in rice cultivars associated with antioxidant enzymes activities and Fe/Zn concentrations. *International journal of Environmental Science and Technology*, 16, 4241–4252.

Ahanger, M.A., Alyemeni, M.N., Wijaya, L., Alamri, S.A., Alam, P., Ashraf, M. et al. (2018) Potential of exogenously sourced kinetin in protecting *Solanum lycopersicum* from NaCl-induced oxidative stress through up-regulation of the antioxidant system, ascorbate-glutathione cycle and glyoxalase system. *PLoS One*, 13, e0202175.

Ahmad, P., Alyemeni, M.N., Wijaya, L., Alam, P., Ahanger, M.A. & Alamri, S. A. (2017) Jasmonic acid alleviates negative impacts of cadmium stress by modifying osmolytes and antioxidants in faba bean (*Vicia faba* L.). *Archives of Agronomy and Soil Science*, 63, 1889–1899.

Baluska, F., Mancuso, S., Volkmann, D. & Barlow, P.W. (2010) Root apex transition zone: a signalling-response nexus in the root. *Trends in Plant Science*, 15, 402–408.

Bartoli, C.G., Casalongué, C.A., Simontacchi, M., Marquez-Garcia, B. & Foyer, C.H. (2013) Interactions between hormone and redox signalling pathways in the control of growth and cross tolerance to stress. *Environmental and Experimental Botany*, 94, 73–88.

Bassard, J.E., Ullmann, P., Bernier, F. & Werck-Reichhart, D. (2010) Phenolamides: bridging polyamines to the phenolic metabolism. *Phytochemistry*, 71, 1808–1824.

Beznec, A.Y. (2016) *Evaluación de la tolerancia a estrés hídrico en plantas de trigo (Triticum aestivum L.) con niveles de citoquininas modificados mediante transformación genética*. DPhil Thesis. Universidad Nacional de Luján.

Binenbaum, J., Weinstain, R. & Shani, E. (2018) Gibberellin localization and transport in plants. *Trends in Plant Science*, 23, 410–421.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248–254.

Bruno, L., Pacenza, M., Forgione, I., Lamerton, L.R., Greco, M., Chiappetta, A. et al. (2017) In *Arabidopsis thaliana* cadmium impact on the growth of primary root by altering SCR expression and auxin-cytokinin cross-talk. *Frontiers in Plant Science*, 27, 1323.

Chen, Y., Han, Y., Zhang, M., Zhou, S., Kong, X. & Wang, W. (2016) Over-expression of the wheat expansin gene *TaEXPA2* improved seed production and drought tolerance in transgenic tobacco plants. *PLoS One*, 11, e0153494.

Chen, Y., Han, Y., Kong, X., Kang, H., Ren, Y. & Wang, W. (2017) Ectopic expression of wheat expansin gene *TaEXPA2* improved the salt tolerance of transgenic tobacco by regulating Na+/K+ and antioxidant competence. *Physiologia Plantarum*, 159, 161–177.

Chen, Y., Ren, Y., Zhang, G., An, J., Yang, J., Wang, Y. et al. (2018) Over-expression of the wheat expansin gene *TaEXPA2* improves oxidative stress tolerance in transgenic Arabidopsis plants. *Plant Physiology and Biochemistry*, 124, 190–198.

Cherrak, S.A., Mokhtari-Soulimane, N., Berroukeche, F., Bensenane, B., Cherbonnel, A., Merzouk, H. et al. (2016) *In vitro* antioxidant versus metal ion chelating properties of flavonoids: a structure-activity investigation. *PLoS One*, 11, e0165575.

Cosgrove, D.J. (2015) Plant expansins: diversity and interactions with plant cell walls. *Current Opinion in Plant Biology*, 25, 162–172.

Csiszár, J., Gallé, A., Horváth, E., Dancsó, P., Gombos, M., Váry, Z. et al. (2012) Different peroxidase activities and expression of abiotic stress-related peroxidases in apical root segments of wheat genotypes with different drought stress tolerance under osmotic stress. *Plant Physiology and Biochemistry*, 52, 119–129.

De Smet, S., Cuypers, A., Vangronsveld, J. & Remans, T. (2015) Gene networks involved in hormonal control of root development in *Arabidopsis thaliana*: a framework for studying its disturbance by metal stress. *International Journal of Molecular Sciences*, 16, 19195–19224.

Delatorre, C.A., Cohen, Y., Liu, L., Peleg, Z. & Blumwald, E. (2012) The regulation of the SARK promoter activity by hormones and environmental signals. *Plant Science*, 193-194, 39–47.

Dello Ioio, R., Scaglia Linhares, F., Scacchi, E., Casamitjana-Martinez, E., Heidstra, R., Costantino, P. et al. (2007) Cytokinins determine arabidopsis root-meristem size by controlling cell differentiation. *Current Biology*, 17, 678–682.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W. InfoStat versión (2019) Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar

Durgbanshi, A., Arbona, V., Pozo, O., Miersch, O., Sancho, J.V. & Gómez-Cadenas, A. (2005) Simultaneous determination of multiple phytohormones in plant extracts by liquid chromatography-electrospray tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, 53, 8437–8442.

Edreva, A.M., Velikova, V. & Tsonev, T.D. (2007) Phenylamides in plants. *Russian Journal of Plant Physiology*, 54, 287–301.

Falcone Ferreyra, M.L., Rius, S.P. & Casati, P. (2012) Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Frontiers in Plant Science*, 3, 222.

Feng, X., XuY, P.L., Yu, X., Zhao, Q., Feng, S., Zhao, Z. et al. (2019) *TaEXPB7-B*, a β-expansin gene involved in low-temperature stress and abscisic acid responses, promotes growth and cold resistance in *Arabidopsis thaliana*. *Journal of Plant Physiology*, 240, 153004.

Fonseca, S., Chini, A., Hamberg, M., Adie, B., Porzel, A., Kramell, R. et al. (2009) (+)-7-iso-jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. *Nature Chemical Biology*, 5, 344–350.

Foyer, C.H. & Noctor, G. (2016) Stress-triggered redox signalling: what's in pROSpect? *Plant, Cell & Environment*, 39, 951–964.

Gallego, S.M., Pena, L.B., Barcia, R.A., Azpilicueta, C.E., Iannone, M.F., Rosales, E.P. et al. (2012) Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. *Environmental and Experimental Botany*, 83, 33–46.

Giannopolitis, C.N. & Ries, S.K. (1977) Superoxide dismutases.1. Occurrence in higher plants. *Plant Physiology*, 59, 309–314.

Gujjar, R.S. & Supaibulwatana, K. (2019) The mode of cytokinin functions assisting plant adaptations to osmotic stresses. *Plants*, 8, 542.

Guo, J., Qin, S., Rengel, Z., Gao, W., Nie, Z., Liu, H. et al. (2019) Cadmium stress increases antioxidant enzyme activities and decreases endogenous hormone concentrations more in Cd-tolerant than Cd-sensitive wheat varieties. *Ecotoxicology and Environmental Safety*, 172, 380–387.

Halliwell, B. (2006) Reactive species and antioxidants: redox biology is a fundamental theme of aerobic life. *Plant Physiology*, 141, 312–322.

Han, Y.Y., Zhou, S., Chen, Y.H., Kong, X., Xu, Y. & Wang, W. (2014) The involvement of expansins in responses to phosphorus availability in wheat, and its potentials in improving phosphorus efficiency of plants. *Plant Physiology and Biochemistry*, 78, 53–62.

Han, Y., Chen, Y., Yin, S., Zhang, M. & Wang, W. (2015) Over-expression of *TaEXPB23*, a wheat expansin gene, improves oxidative stress tolerance in transgenic tobacco plants. *Journal of Plant Physiology*, 173, 62–71.

Han, Z., Liu, Y., Deng, X., Liu, D., Liu, Y., Hu, Y. et al. (2019) Genome-wide identification and expression analysis of expansin gene family in common wheat (*Triticum aestivum* L.). *BMC Genomics*, 20, 101.

Hönig, M., Plíhalová, L., Husičková, A., Nisler, J. & Doležal, K. (2018) Role of cytokinins in senescence, antioxidant defence and photosynthesis. *International Journal of Molecular Sciences*, 19, 4045.

Ivanov, V.B. & Dubrovsky, J.G. (2013) Longitudinal zonation pattern in plant roots: conflicts and solutions. *Trends in Plant Science*, 18, 237–243.

Jovanović, S.V., Kukavica, B., Vidović, M., Morina, F. & Menckhoff, L. (2018) Class III peroxidases: functions, localization and redox regulation of isoenzymes. In: *Antioxidants and Antioxidant Enzymes in Higher Plants*. Cham: Springer, pp. 269–300.

Kende, H., Bradford, K., Brummell, D., Cho, H.T., Cosgrove, D.J., Fleming, A.J. et al. (2004) Nomenclature for members of the expansin superfamily of genes and proteins. *Plant Molecular Biology*, 55, 311–314.

Kuhl, C., Tautenhahn, R., Böttcher, C., Larson, R. & Neumann, S. (2012) CAMERA: an integrated strategy for compound spectra extraction and annotation of liquid chromatography/mass spectrometry data sets. *Analytical Chemistry*, 84, 283–289.

Kuromori, T., Seo, M. & Shinozak, K. (2018) ABA transport and plant water stress responses. *Trends in Plant Science*, 23, 513–522.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature*, 227, 680–685.

Li, D., Zhou, D., Wang, P. & Li, L. (2011a) Temperature affects cadmium-induced phytotoxicity involved in subcellular cadmium distribution and oxidative stress in wheat roots. *Ecotoxicology and Environmental Safety*, 74, 2029–2035.

Li, F., Xing, S., Guo, Q., Zhao, M., Zhang, J., Gao, Q. et al. (2011b) Drought tolerance through over-expression of the expansin gene *TaEXPB23* in transgenic tobacco. *Journal of Plant Physiology*, 168, 960–966.

Li, A.X., Han, Y.Y., Wang, X., Chen, Y.H., Zhao, M.R., Zhou, S.M. et al. (2015) Root-specific expression of wheat expansin gene *TaEXPB23* enhances root growth and water stress tolerance in tobacco. *Environmental and Experimental Botany*, 110, 73–84.

Liu, X., Ma, D., Zhang, Z., Wang, S., Du, S., Deng, X. et al. (2019) Plant lipid remodeling in response to abiotic stresses. *Environmental and Experimental Botany*, 165, 174–184.

López-Climent, M.F., Arbona, V., Pérez-Clemente, R.M. & Gómez-Cadenas, A. (2011) Effects of cadmium on gas exchange and phytohormone contents in citrus. *Biologia Plantarum*, 55, 187–190.

Mahmood, Q., Asif, M., Shaheen, S., Hayat, M.T. & Ali, S. (2019) Cadmium contamination in water and soil. In: Hasanuzzaman, M., Prasad, M.N.V. & Fujita, M. (Eds.) *Cadmium toxicity and tolerance in plants. From physiology to remediation* (pp. 141–161). London: Academic Press. Elsevier Inc.

Marone, M., Mozzetti, S., Ritis, D., Pierelli, L. & Scambia, G. (2001) Semiquantitative RT-PCR analysis to assess the expression levels of multiple transcripts from the same sample. *Biological Procedures Online*, 3, 19–25.

Matayoshi, C.L., Pena, L.B., Arbona, V., Gómez-Cadenas, A. & Gallego, S.M. (2020) Early responses of maize seedlings to Cu stress include sharp decreases in gibberellins and jasmonates in the root apex. *Protoplasma*, 257, 1243–1256.

Moore, R.W., Welton, A.F. & Aust, S.D. (1978) Detection of hemoproteins in SDS-polyacrylamide gels. *Methods in Enzymology*, 52, 324–331.

Nabavi, S.M., Šamec, D., Tomczyk, M., Milella, L., Russo, D., Habtemariam, S. et al. (2020) Flavonoid biosynthetic pathways in plants: versatile targets for metabolic engineering. *Biotechnology Advances*, 38, 107316.

Nakano, Y. & Asada, K. (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. *Plant & Cell Physiology*, 22, 867–880.

Noctor, G., Mhamdi, A. & Foyer, C.H. (2016) Oxidative stress and antioxidative systems: recipes for successful data collection and interpretation. *Plant, Cell & Environment*, 39, 1140–1160.

O'Brien, J.A. & Benkova, E. (2013) Cytokinin cross-talking during biotic and abiotic stress responses. *Frontiers in Plant Science*, 4, 451.

Peleg, Z., Reguera, M., Tumimbang, E., Walia, H. & Blumwald, E. (2011) Cytokinin-mediated source/sink modifications improve drought tolerance and increase grain yield in rice under water-stress. *Plant Biotechnology Journal*, 9, 747–758.

Pena, L.B., Barcia, R.A., Azpilicueta, C.E., Méndez, A.A. & Gallego, S.M. (2012) Oxidative post translational modifications of proteins related to cell cycle are involved in cadmium toxicity in wheat seedlings. *Plant Science*, 196, 1–7.

Pena, L.B., Méndez, A.A.E., Matayoshi, C.L., Zawoznik, M.S. & Gallego, S. M. (2015) Early response of wheat seminal roots growing under copper excess. *Plant Physiology and Biochemistry*, 59, 154–162.

Pena, L.B., Matayoshi, C.L., Méndez, A.A.E., Arán, M., Moratto, C.J., Vazquez-Ramos, J.M. et al. (2020) Metabolic rearrangements in imbibed maize (*Zea mays* L) embryos in the presence of oxidative stressors. *Plant Physiology and Biochemistry*, 155, 560–569.

Perilli, S., Di Mambro, R. & Sabatini, S. (2012) Growth and development of the root apical meristem. *Current Opinion in Plant Biology*, 15, 17–23.

Piñeros, M.A., Shaff, J.E. & Kochian, V. (1998) Development, characterization, and application of a cadmium-selective microelectrode for the measurement of cadmium fluxes in roots of Thlaspi species and wheat. *Plant Physiology*, 116, 1393–1401.

Raja, V., Majeed, U., Kang, H., Andrabi, K.I. & John, R. (2017) Abiotic stress: interplay between ROS, hormones and MAPKs. *Environmental and Experimental Botany*, 137, 142–157.

Reguera, M., Peleg, Z., Abdel-Tawab, Y.M., Tumimbang, E.B., Delatorre, C. A. & Blumwald, E. (2013) Stress-induced cytokinin synthesis increases drought tolerance through the coordinated regulation of carbon and nitrogen assimilation in rice. *Plant Physiology*, 163, 1609–1622.

Ren, Y., Chen, Y., An, J., Zhao, Z., Zhang, G., Wang, Y. et al. (2018) Wheat expansin gene *TaEXPA2* is involved in conferring plant tolerance to cd toxicity. *Plant Science*, 270, 245–256.

Rivero, R.M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S. et al. (2007) Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 19631–19636.

Rubio-Wilhelmi, M.M., Sanchez-Rodriguez, E., Rosales, M.A., Blasco, B., Rios, J.J., Romero, L. et al. (2011) Effect of cytokinins on oxidative stress in tobacco plants under nitrogen deficiency. *Environmental and Experimental Botany*, 72, 167–173.

Rucińska-Sobkowiak, R. (2016) Water relations in plants subjected to heavy metal stresses. *Acta Physiologiae Plantarum*, 38, 257.

Sakakibara, H. (2006) Cytokinins: activity, biosynthesis, and translocation. *Annual Review of Plant Biology*, 57, 431–449.

Sandalio, L.M. & Romero-Puertas, M.C. (2015) Peroxisomes sense and respond to environmental cues by regulating ROS and RNS signalling networks. *Annals of Botany*, 116, 475–485.

Schaller, G.E., Bishopp, A. & Kieber, J.J. (2015) The yin–yang of hormones: cytokinin and auxin interactions in plant development. *Plant Cell*, 27, 44–63.

Schützendübel, A., Schwanz, P., Teichmann, T., Gross, K., Langenfeld-Heyser, R., Godbold, D.L. et al. (2001) Cadmium induced changes in antioxidative systems, H_2O_2 content and differentiation in pine (*Pinus sylvestris*) roots. *Plant Physiology*, 127, 887–892.

Sharma, S.S. & Kumar, V. (2002) Responses of wild type and abscisic acid mutants of *Arabidopsis thaliana* to cadmium. *Journal of Plant Physiology*, 159, 1323–1327.

Singh, H.P., Batish, D.R., Kaur, S., Arora, K. & Kohli, R.K. (2006) Alpha-Pinene inhibits growth and induces oxidative stress in roots. *Annals of Botany*, 98, 1261–1269.

Su, Y.H., Liu, Y.B. & Zhang, X.S. (2011) Auxin–cytokinin interaction regulates meristem development. *Molecular Plant*, 4, 616–625.

Synkova, H., Semoradova, S., Schnablova, R., Witters, E., Husak, M. & Valcke, R. (2006) Cytokinin-induced activity of antioxidant enzymes in transgenic Pssu-ipt tobacco during plant ontogeny. *Biologia Plantarum*, 50, 31–41.

Sytar, O., Kumari, P., Yadav, S., Brestic, M. & Rastogi, A. (2019) Phytohormone priming: regulator for heavy metal stress in plants. *Journal of Plant Growth Regulation*, 38, 739–752.

Tamás, L., Mistrík, I., Alemayehu, A., Zelinová, V., Bočová, B. & Huttová, J. (2015) Salicylic acid alleviates cadmium-induced stress responses through the inhibition of cd-induced auxin-mediated reactive oxygen species production in barley root tips. *Journal of Plant Physiology*, 173, 1–8.

Tenhaken, R. (2015) Cell wall remodeling under abiotic stress. *Frontiers in Plant Science*, 5, 771.

Tsukagoshi, H., Busch, W. & Benfey, P.N. (2010) Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. *Cell*, 143, 606–616.

Verma, V., Ravindran, P. & Kumar, P.P. (2016) Plant hormone-mediated regulation of stress responses. *BMC Plant Biology*, 16, 86.

Wani, S.H., Kumar, V., Shriram, V. & Sah, S.K. (2016) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *The Crop Journal*, 4, 162–176.

Wu, F., Zhang, G. & Dominy, P. (2003) Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. *Environmental and Experimental Botany*, 50, 67–78.

Zalabák, D., Pospíšilová, H., Šmehilová, M., Mrízová, K., Frébort, I. & Galuszka, P. (2013) Genetic engineering of cytokinin metabolism: prospective way to improve agricultural traits of crop plants. *Biotechnology Advances*, 31, 97–117.

Zandalinas, S.I., Sales, C., Beltrán, J., Gómez-Cadenas, A. & Arbona, V. (2017) Activation of secondary metabolism in citrus plants is associated to sensitivity to combined drought and high temperatures. *Frontiers in Plant Science*, 7, 1954.

Zhang, J.F., Xu, Y.Q., Dong, J.M., Peng, L.N., Feng, X., Wang, X. et al. (2018) Genome-wide identification of wheat (*Triticum aestivum*) expansins and expansin expression analysis in cold tolerant and cold-sensitive wheat cultivars. *PLoS One*, 13, e0195138.

Zhao, S., Ma, Q., Xu, X., Li, G. & Hao, L. (2016) Tomato jasmonic acid-deficient mutant spr2 seedling response to cadmium stress. *Journal of Plant Growth Regulation*, 35, 603–610.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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