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Differential absorption of cadmium and zinc by tobacco plants: Role of apoplastic pathway

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ABSTRACT

Cadmium (Cd) contamination presents a significant challenge in global agriculture. This study explores the efficacy of chemical induction, specifically using sodium chloride (NaCl), to limit Cd uptake in tobacco (*Nicotiana tabacum*) and assesses its impact on essential divalent metal ions (DMIs). We conducted a comprehensive analysis encompassing ion absorption, root histology, and biochemistry to understand the influence of this method. Our results revealed that NaCl induction led to a notable 30 % decrease in Cd absorption, while maintaining minimal impact on zinc (Zn) uptake. Intriguingly, the absence of essential DMIs, such as calcium (Ca), magnesium (Mg), and Zn, was found to diminish the plant's capacity to absorb Cd. Furthermore, moderate NaCl induction resulted in an increased diameter of the root stele and enhanced lignin content, indicating a restriction of Cd absorption through the apoplastic pathway. Conversely, a compensatory absorption mechanism via the symplastic pathway appeared to be activated in the absence of essential elements. These findings highlight the potential of chemical induction as a strategy to mitigate agricultural Cd risks, offering insights into the complex interplay between plant ion transport pathways and metal uptake regulation.

1. Introduction

Excessive cadmium (Cd) intake is a major concern for agricultural safety worldwide due to its harmful effects on the human body, particularly the kidneys and respiratory system [1]. Furthermore, Cd is prevalent in farmlands and accumulates easily in certain crops [2]. While progress has been made in breeding low-Cd-absorptive varieties of major grain crops, manipulation of Cd uptake often results in disorders of element absorption, particularly for zinc (Zn), manganese (Mn), and iron (Fe) [3–5]. It is widely accepted that most plant species do not have specific approaches for Cd absorption and instead use the gateways of other divalent metal ions (DMIs) that have similar chemical properties [4,6]. Thus, any regulations targeting these transporters or related genes would also impact the absorption of other DMIs.

In plant ion transport, the apoplastic pathway is crucial, facilitating diverse physiological processes. Campbell et al. [7] highlighted its role in ion translocation to salt glands. Expanding this understanding, Duan et al. [8] demonstrated the pathway's significance in nitrogen allocation to shoots under high ammonium conditions. Additionally, Sattelmacher et al. [9] emphasized the apoplast's impact on plant mineral nutrition, particularly in nutrient efficiency and ion tolerance. These studies collectively underscore the apoplastic pathway's integral role in plant ion transport, crucial for understanding the regulation of both harmful and beneficial ion uptake.

Zinc, a plant-essential heavy metal [10], shares transporters with Cd and exhibits synergistic absorption patterns [4,10–12]. Some members of the P1B-type ATPase family (heavy metal ATPase, HMAs) are known to simultaneously manage both Zn and Cd transport in tobacco [4], Arabidopsis [13], and rice [14]. Recent studies have shown that reinforcing root lignification, achieved through ectopic expression [15] or chemical induction [16], can reduce Cd accumulation in plants, possibly by limiting the apoplastic transport efficiency of Cd, as seen in wheat [17], soybean [18], and tobacco [16]. However, it remains unclear whether the intensified root lignification would hinder the absorption of

Abbreviations: DMIs, divalent metal ions.

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plant-essential DMIs, such as Zn.

As a widely cultivated cash crop and model plant, tobacco (*Nicotiana tabacum*) can accumulate high levels of Cd in its shoots [19]. Furthermore, the K326 cultivar of tobacco has been found to have a higher capacity for extracting Cd from severely contaminated soils compared to other leading cultivars grown in China [20]. Therefore, the present study aims to investigate the impacts of intensified root lignification on Cd and Zn uptakes in tobacco cultivar K326. To achieve reinforced root lignification in tobacco, we selected sodium chloride (NaCl) as the chemical induction agent, based primarily on our previous screening results among nine candidates, including NaCl, $Cd(CH_3COO)_2$, $Cd(NO_3)_2$, $CdCl_2$, $KHNO_3$, polyethylene glycol 6000 (PEG-6000), indole-3-acetic acid (IAA), β-aminobutyric acid (BABA), and glutathione (GSH) [16]. Additionally, we chose NaCl for its documented effects on Cd accumulation in tobacco [21] and *Arabidopsis* [22], as well as for its practical relevance in agricultural applications.

2. Materials and methods

2.1. Plant material and hydroponic culture

Seeds of tobacco cultivar K326 were obtained from the National Tobacco Germplasm Resources, Qingdao, China. The seeds were germinated and grown in sterilized quartz sand for seven weeks under controlled conditions typical for tobacco cultivation. Subsequently, the tobacco seedlings were transplanted to three different concentrations of a modified Hoagland solution (50 %, 75 %, and 100 % of the original concentration) [16] consisting of 1 M K_2SO_4, 250 mM NaH_2PO_4, 1.88 M $Ca(NO_3)_2$, 250 mM $MgSO_4$, 20 mM $FeSO_4$, 10 mM Na_2-EDTA, 36 mM $MnCl_2$, 184 mM H_3BO_3, 3.2 mM $ZnSO_4$, 1.2 mM $CuSO_4$, and 300 μM $(NH_4)_6Mo_7O_{24}$ with a pH of 6.0. The seedlings were exposed to each solution concentration for three days, in ascending order, ensuring the solution was well aerated and renewed every three days. Hydroponic culture was conducted in a growth chamber with constant temperature of 25 °C and irradiance of 280 μmol m^{-2} s^{-1}, under a 12-h light and 12-h dark photoperiod. After nine days of hydroponic culture adaptation, healthy seedlings were selected for the chemical induction procedure.

2.2. Chemical induction

To intensify root lignification, two concentrations of NaCl, 50 mM and 100 mM, were added to the hydroponic system, with each treatment lasting for one week [16].

2.3. Pot experiment

Tobacco plants with and without NaCl pretreatment were transplanted into sterilized fine brown soil collected from Jimo Test Base, Qingdao, China. The soil contained available nitrogen (N), phosphorus (P), and potassium (K) at concentrations of 49.58, 18.07, and 178.57 mg/kg respectively. It also had an organic matter concentration of 1.15 % and a pseudo-total Cd concentration of 0.21 mg/kg (digested by HNO_3–HF–H_2O_2, analyzed by inductively coupled plasma mass spectroscopy (ICP-MS)), and a pH of 7.05. To construct Cd-contaminated soil, $Cd(CH_3COO)_2$ was added to a final concentration of 3.35 mg Cd/kg. The soil was throughly blended and allowed to equilibrate for a month before use. Each polystyrene pot contained 8 kg of soil and one tobacco plant. Compound fertilizer (50 g each of $N:P_2O_5:K_2O$ = 15:15:15; total nutrient content = 45.0 %, Xilaifeng Fertilizantes, China) was applied twice to each pot, and soil moisture was maintained at around 80 % of holding capacity. The pot experiment was conducted in a glasshouse with natural lighting and a 16-h (24 °C)/8-h (18 °C) light/dark cycle. On the fifth week after transplanting, tobacco plants were sampled for analysis of shoot biomass, Cd, and Zn contents.

2.4. Ion-interaction study

In hydroponic conditions, six plant-essential DMIs (Zn^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+} and Fe^{2+}) were individually added to a simplified Hoagland solution, which contained only regular concentrations of K^+, Na^+, NH^{4+}, SO_4^{2-}, H_2PO^{4-}, H_3BO_3 and Mo^{6+}, along with 10 μM $Cd(CH_3COO)_2$. Two concentrations for each DMI treatment were used, with one equal to and the other five times the concentration in the regular Hoagland solution. After 24 h, the shoot DMI contents were analyzed.

2.5. Assessment of element contents

Soil samples were air-dried and ground to pass through a 2 mm sieve. Acid digestion of soil samples was performed in a mixture of HNO_3:H_2O_2:HF (5:2:2) at 120 °C for 2 h in a closed microwave digestion system (MDS-6G, SINEO, China). Plant samples were dried in an oven at 105 °C for 30 min and then at 80 °C until reaching a constant weight, followed by adequate grinding. Acid digestion of plant samples was performed in a mixture of HNO_3:H_2O_2 (9:1) at 120 °C for 2 h in the same digestion system. DMIs (Cd^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+} and Fe^{2+}) contents in the digested solution were analyzed using inductively coupled plasma mass spectroscopy/atomic emission spectrometry (ICP-MS/AES, X Series 2, Thermo Fisher Scientific, USA). All DMIs contents were calculated based on the dry weight of the plants.

2.6. Histological observation

To observe the primary lateral roots of 60-70-day-old hydroponic tobacco plants, sections 2 cm from the root tips were selected for histological observation. Root transverse and longitudinal slices were obtained using paraffin and free-hand section techniques, respectively (Lux et al., 2004). Visualization was performed using confocal microscopy (Leica Microsystems TCS SP8, Leica, Germany), and image processing and data capture were achieved using software Leica LAS AF 1.00.

2.7. Enzymatic assay

To determine peroxidase (POD, EC 1.11.1.7) activity, fresh primary roots (0.5 g) of tobacco were homogenized in 5 ml of 67 mM phosphate buffer solution (PBS, pH = 7.0), as referred to Neves et al. [18]. The extract was centrifuged at 2200×*g* (4 °C) for 5 min, and the supernatant was collected for soluble POD activity determination. The deposition was then incubated in 2 ml of 1 M NaCl (4 °C, prepared in 50 mM PBS, pH 7.0) for 1 h, and the homogenate was centrifuged at 2200×*g* (4 °C) for 5 min, with the supernatant collected for the determination of cell wall-bound POD activity. The reaction mixture (3 ml) contained 25 mM PBS (pH 6.8), 2.58 mM guaiacol, and 10 mM H_2O_2, and the reaction was initiated by adding 1 ml of enzyme extract. The oxidation of guaiacol was observed for 5 min at 470 nm, and the enzyme activity was calculated from the extinction coefficient. Activities of POD were expressed as nmol/μmol of tetraguaiacol per minute per gram of fresh weight.

2.8. H_2O_2 quantification

H_2O_2 content in plants was determined according to Neves et al. [18]. Fresh primary roots (1 g) of tobacco were homogenized in 3 ml of 0.1 % trichloroacetic acid, and the homogenate was centrifuged at 2200×*g* for 20 min. An aliquot (0.5 ml) of the supernatant was mixed with 0.5 ml of 10 mM PBS (pH 7.0) and 0.2 ml of 5 mM potassium iodide. Absorbance was measured for 1 min at 390 nm. For the blank, potassium iodide was replaced by 0.2 ml of water. A standard curve was created using known concentrations of H_2O_2 to calculate the H_2O_2 content in each sample. Results were expressed as nmol of H_2O_2 per gram of fresh weight.

2.9. Statistical analyses

Pearson correlation analysis, Student's *T*-test, and ANOVA were performed using IBM SPSS v21.0 software.

3. Results

3.1. Effect of chemical induction on Cd and Zn uptakes by tobacco plants

In our study, tobacco plants subjected to different Cd conditions, with or without NaCl induction, exhibited notable variations in growth and metal uptake. Plants grown in Cd-enriched soil (+Cd) displayed a significant reduction in leaf number (Fig. 1a) and shoot biomass (Fig. 1b) compared to those in low-Cd soil (-Cd). Specifically, in the +Cd condition, shoot Cd concentration increased fivefold, while shoot Zn concentration decreased by 57 % (Fig. 1d and e). Conversely, plants induced with 100 mM NaCl (−/+Cd) showed a marked alleviation in these adverse effects. Notably, in NaCl-induced plants, shoot Cd uptake was reduced by 34.5 %, and shoot Zn uptake increased by 63.2 % compared to non-induced plants (Fig. 1d and e). Furthermore, we observed dynamic changes in Cd and Zn absorption in tobacco plants following NaCl pretreatment in a hydroponic system under Cd-contaminated conditions. Within 6 h, NaCl-pretreated plants (100 mM NaCl + Cd) consistently exhibited a 33 % lower shoot Cd concentration than untreated plants (+Cd) (Fig. 2c). Initially, the shoot Zn concentration in NaCl-pretreated plants was significantly lower than in untreated plants, but it gradually approached the control (-Cd) level (Fig. 2b). Over 6 h, the disparity in shoot Zn concentration between NaCl-pretreated plants and the control narrowed, while the difference between untreated plants and the control remained stable (Fig. 2d).

3.2. Cd uptake by tobacco plants is highly dependent on the presence of essential divalent metal ions

To determine the specificity of Cd absorption in tobacco plants, we supplemented the simplified Hoagland solution with a single divalent metal ion (DMI)-Zn, Cu, Ca, Mg, Mn, or Fe (II)-in addition to 10 μM Cd $(CH_3COO)_2$. After 24 h, we analyzed the ion concentrations in tobacco shoots from the hydroponic culture. Both ion-replenished (1 ×) and enriched (5 ×) treatments significantly increased the concentration of the corresponding added ion in tobacco shoots (Fig. 3a–f). Interestingly, tobacco plants without DMI supply absorbed a negligible amount of Cd (Fig. 3g). However, when DMIs were replenished (1 ×), tobacco plants' Cd absorption capacity was restored, especially with Ca, Mg, and Zn (Fig. 3g). On the other hand, DMI enrichment (5 ×) did not promote but rather suppressed Cd uptake (Fig. 3g). These results demonstrate typical ion competition between the absorption of Cd and other DMIs.

3.3. Sodium chloride pretreatment reduces Cd uptake by tobacco plants by inducing root lignification

Histological observations of primary lateral roots of tobacco plants with (Fig. 4a and c) and without (Fig. 4b and d) NaCl pretreatment showed no apparent differences in root structures, except for the size of the root stele (Fig. 4a–d). The root stele diameter of NaCl-pretreated plants was 32 % larger than that of untreated plants (Fig. 4e). Additionally, assays revealed that the root lignin content increased by 34 % and 74 % for the 50 mM and 100 mM NaCl treatments, respectively (Fig. 4f). NaCl treatment at concentrations of 50 mM and 100 mM significantly increased cell wall-bound peroxidase (POD) activity by 26.8 % and 40.9 %, respectively (Fig. 4g). Soluble POD activity also increased by 10.7 % and 44.8 %, respectively (Fig. 4h). These increased POD activities were associated with decreased H_2O_2 levels (Fig. 4i), with root H_2O_2 levels of tobacco plants treated with 50 and 100 mM NaCl decreasing by 21.7 % and 27.9 %, respectively (Fig. 4i). Overall, these

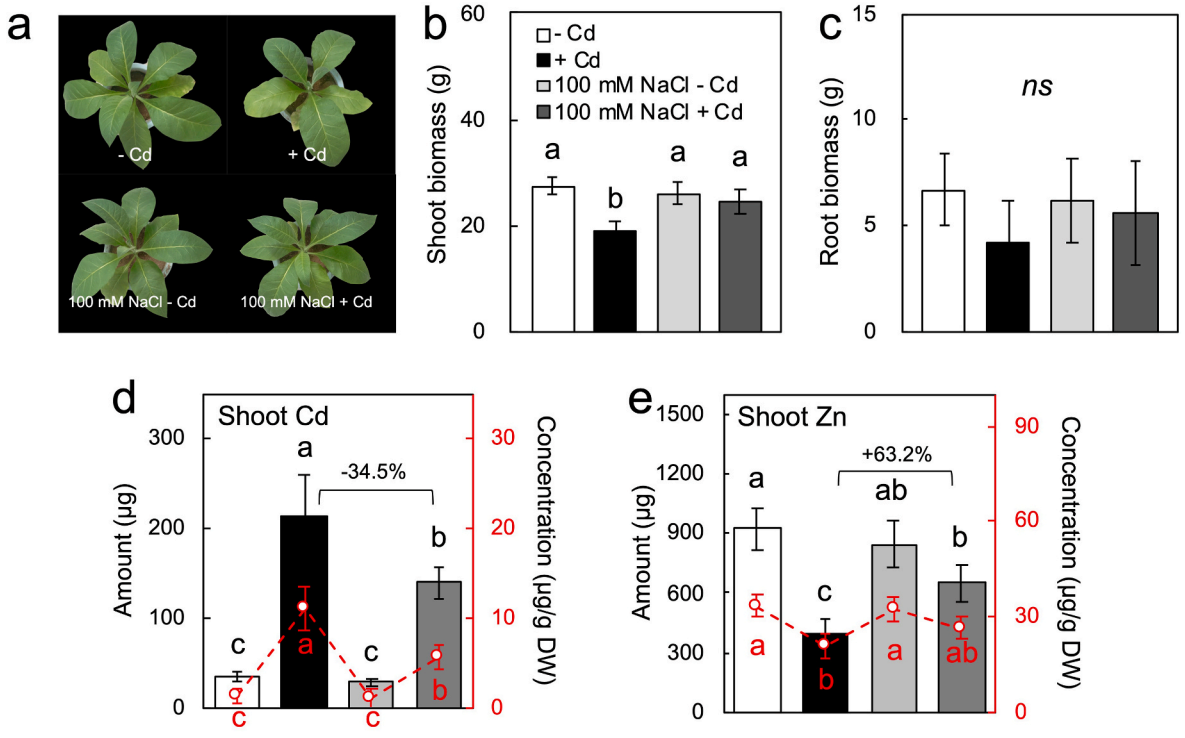

Fig. 1. Differences in phenotype (**a**), shoot biomass (**b**), root biomass (**c**), Cd amount and concentration (**d**), and Zn amount and concentration (**e**) of tobacco plants with or without 7 days induction by 100 mM NaCl, and followed by 5-weeks grown in low-Cd (available Cd = 0.21 mg/kg, denoted as "-Cd") or Cd-enriched (available Cd = 3.35 mg/kg, denoted as "+Cd") soils. The data are presented as means ± SD (n = 5 biological replicates). Different lowercases letters indicate significant difference at the $p < 0.05$ level (ANOVA).

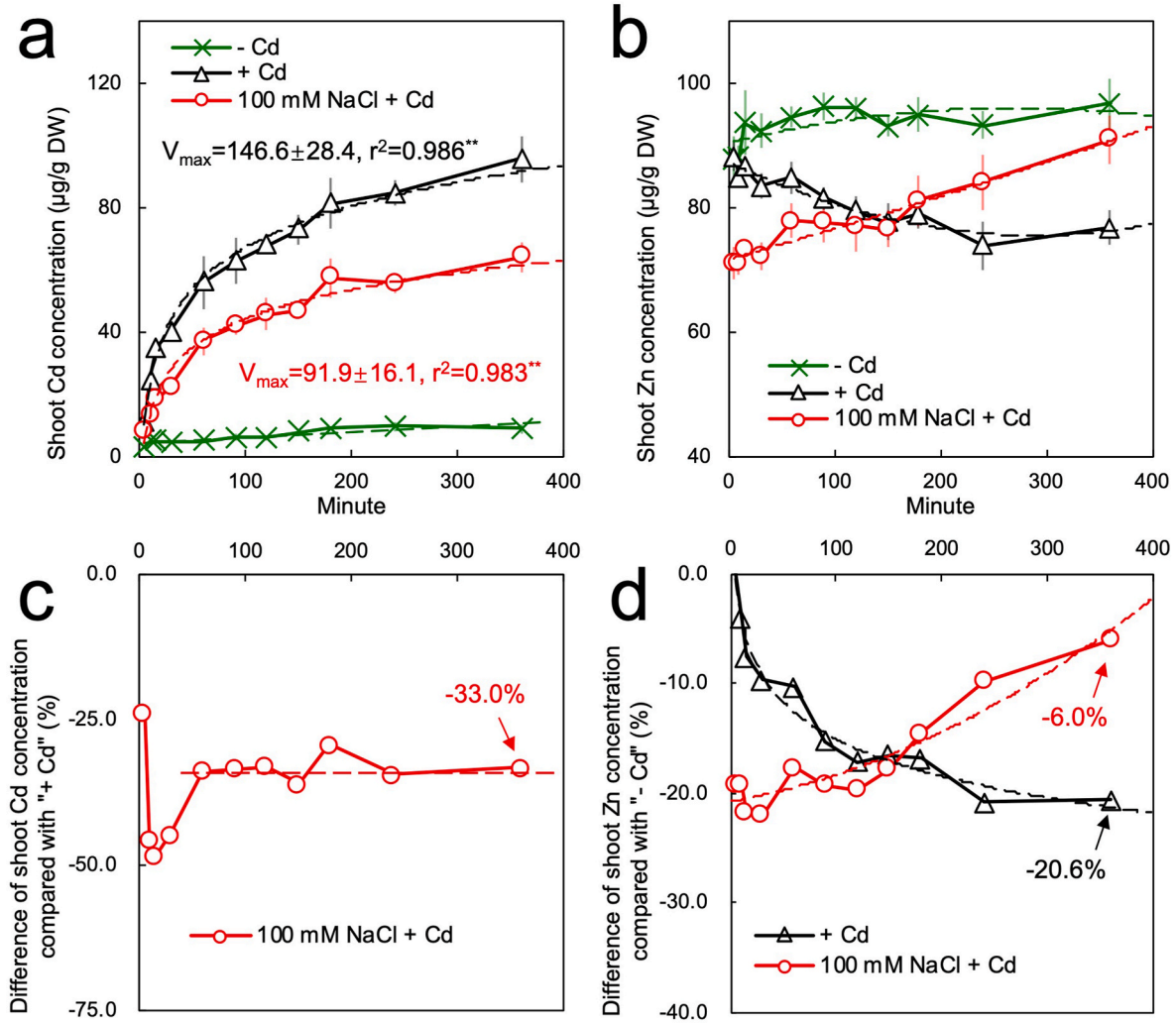

Fig. 2. Time-dependent uptakes of Cd (**a**) and Zn (**b**) by tobacco plants after NaCl pretreatment in hydroponic condition. Panel (**c**) shows the difference in Cd concentration compared to the "+Cd" treatment. Panel (**d**) shows the difference in Zn concentration compared to the "-Cd" treatment. The "+Cd" and "-Cd" treatments refer to the addition or absence of 10 μM $Cd(CH_3COO)_2$ into the regular Hoagland solution, respectively. The V_{max} in panel (**a**) was calculated by Sigmoidal fitting (Hill, $y = V_{max}*x^n/(k^n + x^n)$). The data are presented as means ± SD (n = 3 biological replicates).

results demonstrate that NaCl pretreatment induces root lignification, which potentially led to decreased Cd uptake by tobacco plants.

To investigate the relationship between NaCl-induced root lignification and Cd and Zn uptake, tobacco plants were pretreated with 0, 50, and 100 mM NaCl and transferred to Hoagland solution containing 10 μM $Cd(CH_3COO)_2$ and 3.2 mM $ZnSO_4$. After 3 days of hydroponic culture, only the higher NaCl dose (100 mM) significantly reduced Cd uptake (Fig. 5a), while neither 50 nor 100 mM NaCl treatments significantly affected Zn uptake (Fig. 5b). Moreover, there was a significant negative correlation between shoot Cd concentrations and both root stele diameter (Fig. 5c) and lignin content (Fig. 5d) at the 0.01 level (Pearson's correlation, two-tailed, n = 45).

4. Discussion

To date, approximately 400 plant species have been identified as Cd hyperaccumulators [10,12,19]. However, there is limited evidence to suggest that plants have a specific pathway for Cd absorption, with the exception of *Thlaspi caerulescens*, Ganges ecotype [11]. The criterion for defining a plant as a Cd hyperaccumulator is that the Cd enrichment factor (plant/soil or leaf/soil) should be above 1.0 [23]. Tobacco (*N. tabacum*) has been shown to achieve an enrichment factor above 10.0 under controlled conditions [19]. Although the results of the current study suggest that there may be no specific absorption pathway for Cd in tobacco plants (Fig. 3), several proteins involved in the transport of essential divalent metal ions (DMIs) in plants, such as AtHMA2, AtHMA4 [13], NtHMA4.1, NtHMA4.2 [4], AtIRT2 (the iron transporter, IRT) [5], AtNramp1 (the natural resistance-associated macrophage protein, NRAMP) [3], and NtNramp5 [24], have been identified that may also play a role in the transport of Cd. Thus, it may be difficult to specifically limit Cd absorption to a transmembrane process in roots.

In vascular plants, the root apoplast acts as a natural barrier, limiting the absorption of heavy metal ions like Cd [18]. A highly lignified or compacted apoplast in roots can significantly restrict the entry of heavy metals like Cd into the vascular cylinder [25]. After all, the main flow of heavy metals from the rhizosphere to the root stele is first achieved by apoplastic transport [25]. In tobacco plants, the overexpression of 35S::*AtHMA4* induces intensified root lignification, which obstructs a considerable amount of Cd uptake in tobacco shoots [15]. Similarly, the strengthening of root lignification achieved by salinity stimulation could also significantly decrease the absorption of Cd in tobacco plant shoots [16].

When plants face salinity stress, the activities of antioxidant enzymes may increase in several crop species, such as maize [26], cotton [27], sunflower [28], and tomato [29]. Hence, the antioxidant system could protect plants against the reactive oxygen species generated during

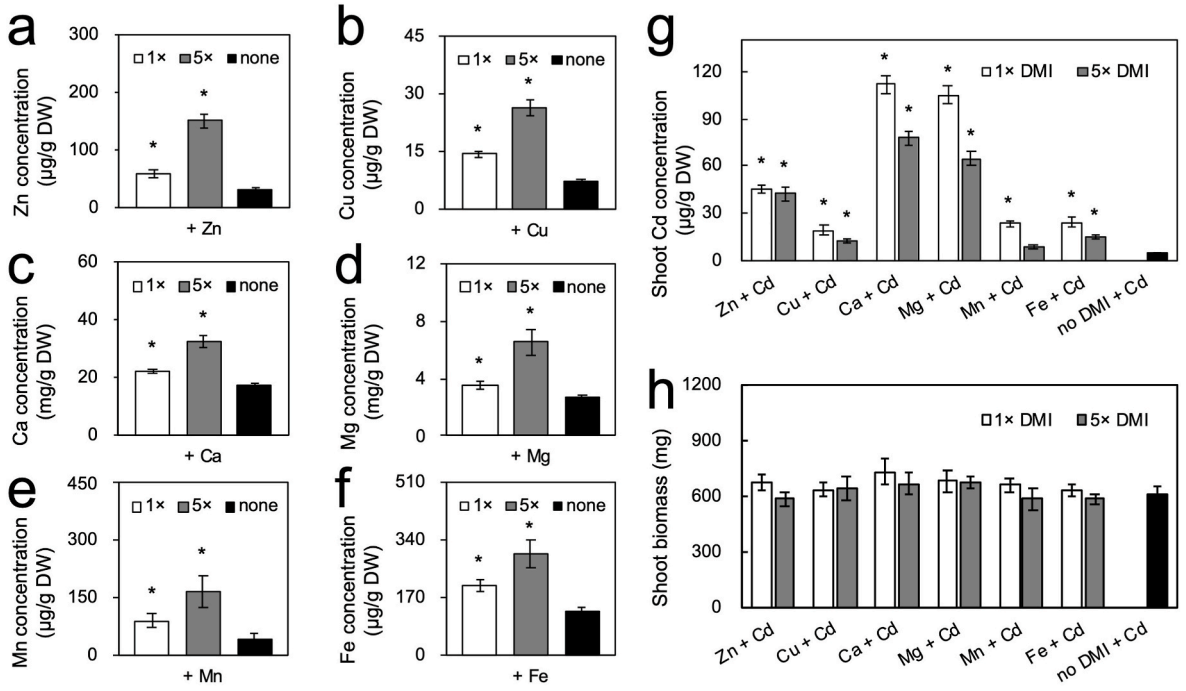

Fig. 3. Ionomic profile of tobacco shoot (**a**–**g**) and shoot biomass (**h**) after 24-h hydroponic culture with single DMI and 10 μM $Cd(CH_3COO)_2$. The abscissae under subgraphs indicate only the indicated DMI was added into the simplified Hoagland solution. "1 × " and "5 × " represent the indicated DMI in use was "equal to" and "five times of" the corresponding concentration in the regular Hoagland solution. The data represents means ± SD ($n = 5$ biological replicates). An asterisk in **a** - **g** indicates significant differences from the "no DMI + Cd" treatment at the $p < 0.05$ level (Student's *T*-test). DW represents dry weight.

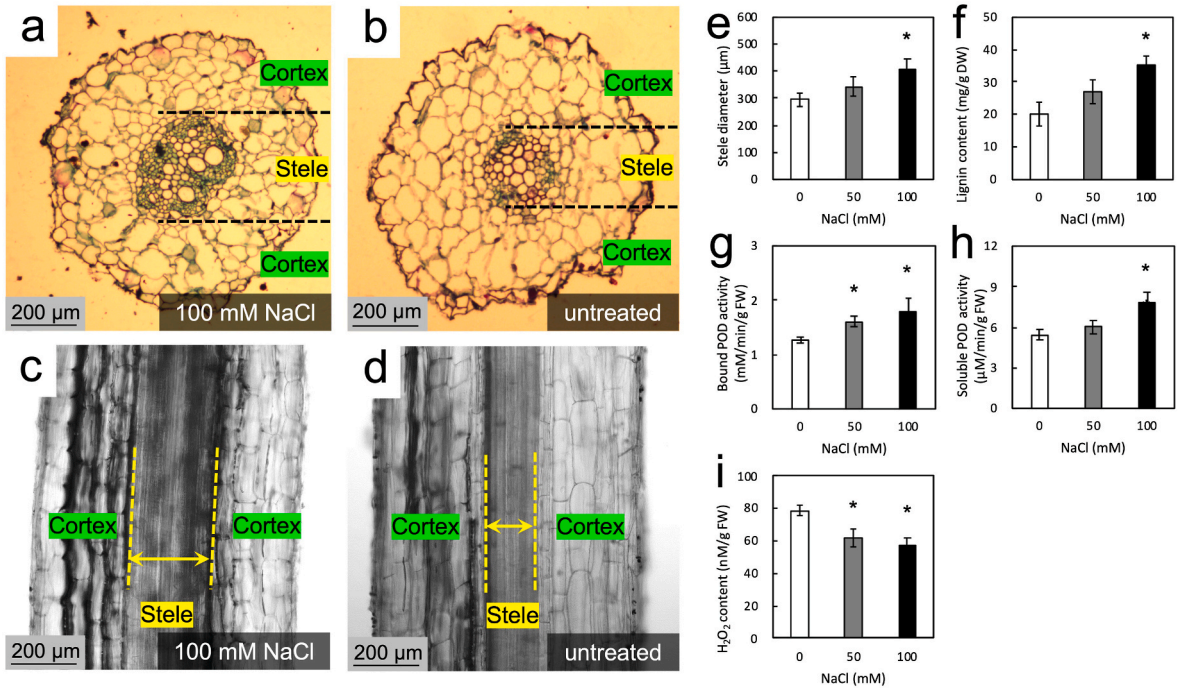

Fig. 4. Histological observations and analyses of primary lateral roots located 2 cm from the apex. Transverse sections (**a** and **b**) and longitudinal sections (**c** and **d**) were performed, and the stele diameter (**e**) and lignin content (**f**) were measured. Bound and soluble peroxidase activities (**g** and **h**) and hydrogen peroxide content (**i**) were also analyzed. Data for stele diameter, lignin content, peroxidase activities, and hydrogen peroxide content represent means ± SD ($n = 15$, with five plant replicates and three measurements for each plant). Asterisks in **e**-**i** indicate significant differences compared to the "none NaCl" treatment at the $p < 0.05$ level (according to Student's *T*-test).

salinity stress. In rice roots, NaCl treatment increases the cell wall-bound POD activity [30], which directly participates in root lignification [18, 30–32]. In the current study, NaCl pretreatment induced a greater deposition of lignin (Fig. 4f) and enlarged stele (Fig. 4a and c) in tobacco roots, along with increased activities of the cell wall-bound (Fig. 4g) and soluble PODs (Fig. 4h), and decreased H_2O_2 contents (Fig. 4i). These

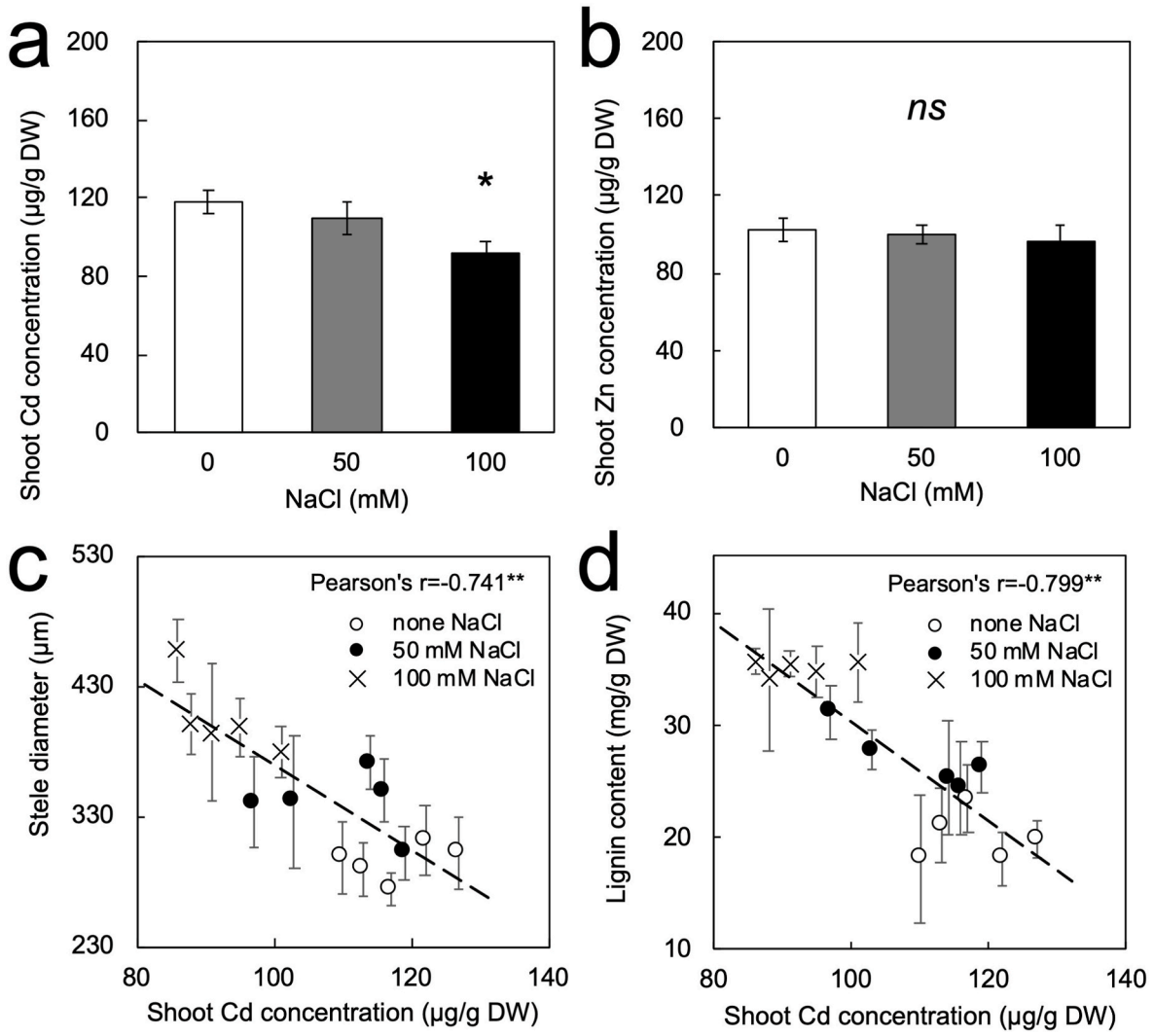

Fig. 5. Shoot Cd (**a**) and Zn (**b**) concentrations after 3 days of hydroponic culture with 10 μM $Cd(CH_3COO)_2$ and 3.2 mM $ZnSO_4$. Correlation analyses between shoot Cd concentration and root stele diameter (**c**), and lignin content (**d**). Data in (**a**) and (**b**) represent means ± SD ($n = 5$ biological replicates). Asterisk indicates significant differences compared with the "none NaCl" treatment at the $p < 0.05$ level (Student's *T*-test). "*ns*" indicates no significant difference. Data in (**c**) and (**d**) represent means ± SD ($n = 3$ root samples from each plant). Double asterisks indicate that the correlation is significant at 0.01 levels (two-tailed Pearson's correlation coefficient, $n = 45$).

physio-morphological variations cause rapid cross-linking of cell wall polymers [31,33] and consequently reduce the efficiency of apoplastic transport for macromolecules and heavy metal ions [18], which reasonably explains the reduced absorption of Cd (Fig. 1d).

There was some uncertainty regarding whether the NaCl-induced root lignification could limit Zn absorption, despite the fact that transgenic tobacco plants with reinforced root apoplastic barriers did not show any impact on shoot Zn concentration [15]. In the current study, no significant differences in shoot Zn concentration were observed between NaCl-pretreated and untreated tobacco plants grown under normal conditions (Fig. 1e). This could be attributed to the ability of plants to balance the absorption of essential elements to meet their physiological requirements [34]. To further investigate this possibility, we examined the dynamic uptake profiles of Cd and Zn by tobacco plants after NaCl treatment (Fig. 2). After a short period of adaptation, Zn uptake returned to control levels (Fig. 2b and d). Two potential explanations for the transient reduction in Zn uptake at the beginning are as follows: First, there could have been competition for absorption between Cd and Zn, which could scramble the Cd/Zn cotransporters, such as HMA4 [4], Nramp1 [35], and the YSL transporter [36]. This competition was likely eased by the suppression of Cd by the intensified root lignification (Fig. 2). Second, the temporary effects of NaCl treatment on tobacco plants, which can be viewed as a transient stress, could have also played a role.

The practical implications of our findings for agricultural practices, particularly in managing Cd risks in crop cultivation, are significant. Our study demonstrates that NaCl pretreatment can effectively reduce Cd uptake in tobacco plants, suggesting a potential strategy for mitigating Cd accumulation in crops, especially in regions with high Cd contamination in soils. However, it's important to consider the variability in response across different crop species and specific objectives. For instance, Ozkutlu et al. [37] found that leaf-applied sodium chloride promotes Cd accumulation in durum wheat grain. Although, it is distinct from our root-applied strategy in tobacco plant, it suggests that the application of NaCl, possibly in combination with other agents, could be tailored to specific crops and productive organs to optimize Cd risk management in agricultural practices. One major limitation of the our study is the focus on a single crop species. Future research should explore the long-term effects of NaCl pretreatment on Cd and Zn uptake across a broader range of crop species. Additionally, it is important to acknowledge that NaCl is not the only chemical agent capable of inducing such responses in plants. Other agents, such as silicon (Si),

selenium (Se), and various phytohormones, have also been reported to influence heavy metal uptake and stress responses in plants [38,39]. Future studies could explore the efficacy of these alternative agents in modulating Cd uptake, providing a comparative understanding of different chemical inducers in managing Cd risks in agriculture.

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CRediT authorship contribution statement

Jia-Shuo Yang: Writing – original draft. **Rana Imtiaz Ahmed:** Writing – original draft. **Haiwei Liu:** Supervision, Methodology. **Song Sheng:** Supervision, Software. **Wenfeng Xiao:** Investigation. **Risheng Hu:** Resources, Project administration. **Yanjiao Dai:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] M. Tellez-Plaza, E. Guallar, B.V. Howard, J.G. Umans, K.A. Francesconi, W. Goessler, et al., Cadmium exposure and incident cardiovascular disease, Epidemiology 24 (2013) 421–429, https://doi.org/10.1097/EDE.0b013e31828b0631.

[2] T. Shi, J. Ma, X. Wu, T. Ju, X. Lin, Y. Zhang, et al., Inventories of heavy metal inputs and outputs to and from agricultural soils: a review, Ecotoxicol. Environ. Saf. (2018), https://doi.org/10.1016/j.ecoenv.2018.08.016.

[3] R. Cailliatte, A. Schikora, J.F. Briat, S. Mari, C. Curie, High-affinity manganese uptake by the metal transporter nramp1 is essential for Arabidopsis growth in low manganese conditions, Plant Cell 22 (2010) 904–917, https://doi.org/10.1105/tpc.109.073023.

[4] V. Liedschulte, H. Laparra, J.N.D. Battey, J.D. Schwaar, H. Broye, R. Mark, et al., Impairing both HMA4 homeologs is required for cadmium reduction in tobacco, Plant Cell Environ. 40 (2017) 364–377, https://doi.org/10.1111/pce.12870.

[5] G. Vert, M. Barberon, E. Zelazny, M. Séguéla, J.F. Briat, C. Curie, Arabidopsis IRT2 cooperates with the high-affinity iron uptake system to maintain iron homeostasis in root epidermal cells, Planta 229 (2009) 1171–1179, https://doi.org/10.1007/s00425-009-0904-8.

[6] R.M. Welch, W.A. Norvell, Mechanisms of cadmium uptake, translocation and deposition in plants, in: Cadmium in Soils and Plants, 1999, https://doi.org/10.1007/978-94-011-4473-5_6.

[7] N. Campbell, W. Thomson, K. Platt, The apoplastic pathway of transport to salt glands, J. Exp. Bot. 25 (1) (1974) 61–69, https://doi.org/10.1093/JXB/25.1.61.

[8] F. Duan, R. Giehl, N. Geldner, D. Salt, N. von Wirén, Root zone–specific localization of AMTs determines ammonium transport pathways and nitrogen allocation to shoots, PLoS Biol. 16 (2018), https://doi.org/10.1371/journal.pbio.2006024.

[9] B. Sattelmacher, K.H. Mühling, K. Pennewiß, The apoplast — its significance for the nutrition of higher plants, J. Plant Nutr. Soil Sci. 161 (1998) 485–498, https://doi.org/10.1002/JPLN.1998.3581610502.

[10] H. Küpper, E. Lombi, F.J. Zhao, S.P. McGrath, Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator Arabidopsis halleri, Planta 212 (2000) 75–84, https://doi.org/10.1007/s004250000366.

[11] E. Lombi, F.J. Zhao, S.P. McGrath, S.D. Young, G.A. Sacchi, Physiological evidence for a high-affinity cadmium transporter highly expressed in a thlaspi caerulescens ecotype, New Phytol. 149 (2001) 53–60, https://doi.org/10.1046/j.1469-8137.2001.00003.x.

[12] F.J. Zhao, R.E. Hamon, E. Lombi, M.J. McLaughlin, S.P. McGrath, Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator Thlaspi caerulescens, J. Exp. Bot. 53 (2002) 535–543, https://doi.org/10.1093/jexbot/53.368.535.

[13] C.K.E. Wong, C.S. Cobbett, HMA P-type ATPases are the major mechanism for root-to-shoot Cd translocation in Arabidopsis thaliana, New Phytol. (2009), https://doi.org/10.1111/j.1469-8137.2008.02638.x.

[14] R. Takahashi, Y. Ishimaru, H. Shimo, Y. Ogo, T. Senoura, N.K. Nishizawa, et al., The OsHMA2 transporter is involved in root-to-shoot translocation of Zn and Cd in rice, Plant Cell Environ. (2012), https://doi.org/10.1111/j.1365-3040.2012.02527.x.

[15] O. Siemianowski, A. Barabasz, M. Kendziorek, A. Ruszczyńska, E. Bulska, L. E. Williams, et al., HMA4 expression in tobacco reduces Cd accumulation due to the induction of the apoplastic barrier, J. Exp. Bot. 65 (2014) 1125–1139, https://doi.org/10.1093/jxb/ert471.

[16] J. Yang, Y. Dai, Y. Liu, S. Duan, Y. Li, R. Hu, et al., Reduced cadmium accumulation in tobacco by sodium chloride priming, Environ. Sci. Pollut. Res. (2020), https://doi.org/10.1007/s11356-020-09134-z.

[17] N. Jbir, W. Chaïbi, S. Ammar, A. Jemmali, A. Ayadi, Root growth and lignification of two wheat species differing in their sensitivity to NaCl, in response to salt stress, Comptes Rendus l'Academie des Sci.-Ser. III 324 (2001) 863–868, https://doi.org/10.1016/S0764-4469(01)01355-5.

[18] G.Y.S. Neves, R. Marchiosi, M.L.L. Ferrarese, R.C. Siqueira-Soares, O. Ferrarese-Filho, Root growth inhibition and lignification induced by salt stress in soybean, J. Agron. Crop Sci. 196 (2010) 467–473, https://doi.org/10.1111/j.1439-037X.2010.00432.x.

[19] H. Liu, H. Wang, Y. Ma, H. Wang, Y. Shi, Role of transpiration and metabolism in translocation and accumulation of cadmium in tobacco plants (*Nicotiana tabacum* L.), Chemosphere 144 (2016) 1960–1965, https://doi.org/10.1016/j.chemosphere.2015.10.093.

[20] H. Liu, H. Wang, Y. Zhang, H. Wang, J. Yang, J. Liu, et al., Comparison of heavy metal accumulation and cadmium phytoextraction rates among ten leading tobacco (*Nicotiana tabacum* L.) cultivars in China, Int. J. Phytoremediation 21 (2019) 699–706, https://doi.org/10.1080/15226514.2018.1556589.

[21] B. Zhang, S. Shang, H. Zhang, Z. Jabeen, G. Zhang, Sodium chloride enhances cadmium tolerance through reducing cadmium accumulation and increasing anti-oxidative enzyme activity in tobacco, Environ. Toxicol. Chem. (2013), https://doi.org/10.1002/etc.2183.

[22] M. León-Romero, P. Soto-Ríos, M. Fujibayashi, O. Nishimura, Impact of NaCl solution pretreatment on plant growth and the uptake of multi-heavy metal by the model plant Arabidopsis thaliana, Water, Air, Soil Pollut. (2017), https://doi.org/10.1007/s11270-017-3241-8.

[23] R.R. Brooks, in: R.R. Brooks (Ed.), Plants that Hyperaccumulate Heavy Metals: Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining, CAB International, New York, 1998, https://doi.org/10.2134/jeq1999.00472425002800030047x.

[24] Z. Tang, H. Cai, J. Li, Y. Lv, W. Zhang, F.J. Zhao, Allelic variation of NtNramp5 associated with cultivar variation in cadmium accumulation in Tobacco, Plant Cell Physiol. 58 (2017) 1583–1593, https://doi.org/10.1093/pcp/pcx087.

[25] I.V. Seregin, A.D. Kozhevnikova, Roles of root and shoot tissues in transport and accumulation of cadmium, lead, nickel, and strontium, Russ. J. Plant Physiol. 55 (2008) 1–22, https://doi.org/10.1134/s1021443708010019.

[26] A.D. De Azevedo Neto, J.T. Prisco, J. Enéas-Filho, C. E. B. De Abreu, E. Gomes-Filho, Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes, Environ. Exp. Bot. 56 (2006) 87–94, https://doi.org/10.1016/j.envexpbot.2005.01.008.

[27] D.A. Meloni, M.A. Oliva, C.A. Martinez, J. Cambraia, Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress, Environ. Exp. Bot. 49 (2003) 69–76, https://doi.org/10.1016/S0098-8472(02)00058-8.

[28] K. Rios-Gonzalez, L. Erdei, S.H. Lips, The activity of antioxidant enzymes in maize and sunflower seedlings as affected by salinity and different nitrogen sources, Plant Sci. 162 (2002) 923–930, https://doi.org/10.1016/S0168-9452(02)00040-7.

[29] M. Gapińska, M. Skłodowska, B. Gabara, Effect of short- and long-term salinity on the activities of antioxidative enzymes and lipid peroxidation in tomato roots, Acta Physiol. Plant. 30 (2008) 11–18, https://doi.org/10.1007/s11738-007-0072-z.

[30] C.C. Lin, C.H. Kao, Cell wall peroxidase activity, hydrogen peroxide level and NaCl-inhibited root growth of rice seedlings, Plant Soil 230 (2001) 135–143, https://doi.org/10.1023/A:1004876712476.

[31] L. Almagro, L.V. Gómez Ros, S. Belchi-Navarro, R. Bru, A. Ros Barceló, M. A. Pedreño, Class III peroxidases in plant defence reactions, J. Exp. Bot. 60 (2009) 377–390, https://doi.org/10.1093/jxb/ern277.

[32] M.A. Talano, E. Agostini, M.I. Medina, H. Reinoso, M. del Carmen Tordable, H. A. Tigier, et al., Changes in ligno-suberization of cell walls of tomato hairy roots produced by salt treatment: the relationship with the release of a basic peroxidase, J. Plant Physiol. 163 (2006) 740–749, https://doi.org/10.1016/j.jplph.2005.06.009.

[33] W. Boerjan, J. Ralph, M. Baucher, L ignin B iosynthesis, Annu. Rev. Plant Biol. 54 (2003) 519–546, https://doi.org/10.1146/annurev.arplant.54.031902.134938.

[34] C. Palmer, M. Lou Guerinot, A question of balance: facing the challenges of Cu, Fe and Zn homeostasis, Nat. Chem. Biol. (2009), https://doi.org/10.1038/nchembio.166.A.

[35] J.G. Meng, X.D. Zhang, S.K. Tan, K.X. Zhao, Z.M. Yang, Genome-wide identification of Cd-responsive NRAMP transporter genes and analyzing expression of NRAMP 1 mediated by miR167 in Brassica napus, Biometals (2017), https://doi.org/10.1007/s10534-017-0057-3.

[36] C. Curie, G. Cassin, D. Couch, F. Divol, K. Higuchi, M. Le Jean, et al., Metal movement within the plant: contribution of nicotianamine and yellow stripe 1-like transporters, Ann. Bot. 103 (2009) 1–11, https://doi.org/10.1093/aob/mcn207.
[37] F. Ozkutlu, L. Ozturk, H. Erdem, M. McLaughlin, I. Cakmak, Leaf-applied sodium chloride promotes cadmium accumulation in durum wheat grain, Plant Soil 290 (2007) 323–331, https://doi.org/10.1007/s11104-006-9164-6.
[38] M. Rizwan, S. Ali, A. Hussain, Q. Ali, M.B. Shakoor, M. Zia-ur-Rehman, M. Farid, M. Asma, Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (Triticum aestivum L.) and health risk assessment, Chemosphere (2017), https://doi.org/10.1016/j.chemosphere.2017.08.071.
[39] M. Hasanuzzaman, M.H.M.B. Bhuyan, F. Zulfiqar, A. Raza, S.M. Mohsin, J. Al Mahmud, M. Fujita, V. Fotopoulos, Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator, Antioxidants (2019), https://doi.org/10.3390/antiox8080287.