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ORIGINAL ARTICLE

Overexpression of the iron transporter *NtPIC1* in tobacco mediates tolerance to cadmium

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Abstract

Key message **Overexpression of the iron transporter NtPIC1 increases iron concentration in shoots and reduces Cd uptake/accumulation in plants, mediating tolerance to cadmium.**

Abstract Cadmium (Cd) is toxic to plant cells and causes plants to display a typical iron (Fe) deficiency phenotype. *NtPIC1* (Permease In Chloroplast1) is an Fe transporter protein in tobacco, required for Fe homeostasis. Based on preliminary results in transformed *Saccharomyces cerevisiae* BY4741 cells, which showed that NtPIC1 expression increased Cd tolerance, this study evaluated Cd tolerance in tobacco plants overexpressing NtPIC1 (NtPIC1-OE). We show that these plants have longer roots and higher fresh weights than wild-type (WT) plants after Cd exposure. Under Cd stress, WT plants display more chlorosis, stronger growth inhibition, and lower chlorophyll concentrations than NtPIC1-OE plants. Importantly, NtPIC1-OE plants had higher Fe concentrations in shoots and lower Fe concentrations in roots, and Cd concentrations in NtPIC1-OE plants were significantly lower compared to those in WT plants. Moreover, Fe transport-related genes (*NtPIC1*, *NtNRAMP1*, and *NtFER1*) were upregulated in NtPIC1-OE plants, while Fe deficiency-related genes (*NtFRO1*, *NtIRT1*, and *NtZIP1*) that mediate Cd uptake were down-regulated. We also found that the activities of several antioxidative enzymes were significantly higher in NtPIC1-OE plants than in WT plants under Cd stress. Overall, our results demonstrate that overexpression of *NtPIC1* is an efficient way to increase shoot Fe concentrations and reduce Cd uptake/accumulation in plants.

Keywords NtPIC1 · Iron · Cadmium · Tobacco · Tolerance · Antioxidative enzyme

Introduction

Heavy metal pollution is a significant environmental problem that affects many physiological and biochemical processes in plants (Williams and Salt 2009; Clemens et al. 2013). Cadmium (Cd), a non-essential element in plants, is a major, widespread industrial and agricultural pollutant (Sanità di Toppi and Gabbrielli 1999). Environmental Cd is toxic to plants and animals, including humans (Raskin et al. 1997; Sanità di Toppi and Gabbrielli 1999).

In plants, Cd toxicity disrupts photosynthesis and slows thylakoid membrane development, as well as the synthesis of chloroplast–protein complexes (Das et al. 1997; Fagioni et al. 2008). It also disturbs plant water status (Perfus-Barbeoch et al. 2002) and injures cell components by producing reactive oxygen species (ROS) (Schützendübel and Polle 2002; Lee et al. 2003). As a result, visible symptoms occur in affected plants, including chlorosis, the browning of root tips, and growth retardation. The toxicity of Cd is due to binding competition with essential metals

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[e.g., iron (Fe), magnesium (Mn), and zinc (Zn)] for low specificity metal transporters such as iron-regulated transporter 1 (IRT1: Eide et al. 1996; Cohen et al. 1998; Vert et al. 2002; Yoshihara et al. 2006) and natural resistance-associated macrophage protein 1 (NRAMP1: Curie et al. 2000; Thomine et al. 2000; Williams et al. 2000; Thomine et al. 2003). This competition reduces the uptake of essential metals, especially Fe (Garty et al. 1992; Cohen et al. 1998; Clemens 2001), an essential micronutrient for all organisms.

Previous research has demonstrated the tight link between Fe homeostasis and Cd. For example, Cd induces Fe deficiency in tobacco plants by inhibiting the expression of Fe-uptake-related genes (Yoshihara et al. 2006). In addition, phytosiderophore-mediated Fe acquisition can protect plants from Cd toxicity (Meda et al. 2007). Furthermore, mutant OPT3 (essential to shoot–root Fe signaling) in *Arabidopsis* causes increased Cd accumulation in the seed and root, as well as redistribution of Fe and Cd (Mendoza-Cózatl et al. 2014; Zhai et al. 2014). These findings clearly emphasize the important relationship between Fe homeostasis and Cd partitioning.

Thus, a clear understanding of the components in Fe transport is necessary to combat the effects of Cd toxicity in plants. This study investigates NtPIC1, a chloroplast iron transporter in tobacco. When overexpressed, NtPIC1 was found to increase iron concentration in chloroplasts and overall chlorophyll concentrations in the plant (Gong et al. 2014). This study was initiated to investigate the mechanisms underlying decreased Cd uptake and translocation to shoots following overexpression of NtPIC1 in tobacco. Elucidation of these mechanisms will contribute to enhancing the efficiency of micronutrients and reducing the levels of non-essential toxic metals in plants.

Materials and methods

Cd tolerance assays in yeast expressing *NtPIC1*

Cells from the *Saccharomyces cerevisiae* BY4741 strain (*MATa his3 lue2 met15 ura3*), transformed either with a pYES2 empty vector (EV) or with a pYES2-NtPIC1 (Gong et al. 2014), were grown on synthetic-defined (SD) medium (0.67 % bacto-yeast nitrogen base, pH 5.8; Difco, Detroit, MI), supplemented with 2 % D-glucose. An amino acid mixture without uracil was used for transformant selection. Transformed cells were then grown on SD-ura medium. SD-ura liquid cultures, inoculated with the respective transformants, were adjusted to an optical density at 600 nm (OD_{600}) of 1.0 and diluted to an OD_{600} of 0.1–0.001. Subsequently, 10 µL each of the dilutions was spotted onto SD-ura agar medium (control) or onto plates containing different concentrations of $NiSO_4$, $CuSO_4$, $CoCl_2$, or $CdCl_2$. All cultures were incubated at 30 °C for 3 days. Growth of the yeast transformants in SD-ura liquid medium supplemented with 50 µM $CdCl_2$ was monitored at OD_{600}.

Plant growth conditions and heavy metal treatments

Experiments were performed using three transgenic tobacco lines (*Nicotiana tabacum* cv. 'NtPIC1-OE1', 'NtPIC1-OE13', 'NtPIC1-OE33') that overexpressed NtPIC1 (Gong et al. 2014), and wild-type (WT) tobacco plants (*N. tabacum* cv. SR1). NtPIC1-OE and WT seedlings were germinated on half-strength Murashige and Skoog (1/2 MS) basal agar medium (pH 5.8) (Murashige and Skoog 1962) in a growth chamber under a 16 h/8 h light/dark cycle at 25 °C. After 4 days, the seedlings were transferred to 1/2 MS basal medium supplemented with 2 % sucrose, 0.8 % agar, and 0, 75, or 100 µM $CdCl_2$. Seedlings were weighed and root lengths were measured; changes in root length were determined after 7 days of further vertical growth. Ten seedlings of each line were measured and three replicate experiments were performed.

Fe and Cd concentrations were compared across NtPIC1-OE and WT plants grown on either MS medium (control) or medium plus 100 µM Cd (Cd). Two-week-old NtPIC1-OE and WT seedlings were transferred to MS agar plates with 100 µM $CdCl_2$. After 10 days in a growth chamber under a 16-h light period, the shoots and roots of the plants were separately harvested. Part of each was used for total RNA extraction to determine gene expression profiles, as well as for measuring Fe and Cd concentrations.

Measurement of Fe and Cd concentrations

The concentrations of Fe and Cd were assessed using roots and shoots of NtPIC1-OE and WT plants grown as described above. At harvest, intact roots of NtPIC1-OE and WT plants were soaked in 20 mM EDTA and washed three times with deionized water (Liu et al. 2011a, b). The tissues were then oven-dried at 65 °C for 3 days. Dried samples were placed into a solution of 65 % HNO_3 and 30 % H_2O_2 (9:1) at 140 °C for 10 h in a digestion stove (DongYe, China). Concentrations were determined using an ICP-OES (Perkin Elmer) as described by Yuan et al. (2005).

Measurement of chlorophyll concentration

Chlorophyll was extracted from NtPIC1-OE and WT leaves following the methods of Johnston et al. (1984). Weighed tobacco leaves were ground in a mortar and pestle with 20 mL of 80 % acetone and 0.4 g $CaCO_3$ per gram of

fresh leaves. Homogenates were centrifuged (10,000×*g* for 10 min), and the pellets were again extracted with 10 mL of 80 % acetone. Extractions were performed 4–5 times until the leaf samples were colorless. All extraction procedures were carried out in the dark at 0–5 °C. Absorbance of the combined supernatants was measured at 663 and 645 nm using a spectrophotometer (UV-5100, Shanghai, China). The reference sample for these measurements was 80 % acetone. Chlorophyll extractions were performed for at least three plants grown under each condition, and the experiment was repeated three times. Results are expressed as milligrams of chlorophyll per gram fresh weight.

Quantitative real-time PCR analysis of gene expression

Total RNA was extracted from root and shoot tissues of NtPIC1-OE and WT plants, using an RNAprep Pure Plant kit (TIANGEN, Beijing, China). Each RNA sample was isolated from three seedlings, grown and harvested at the same time. Single-strand cDNA was synthesized from total RNA using a ReverTra Ace kit (Toyobo Life Science, Shanghai, China). Quantitative RT-PCR (qRT-PCR) was performed using an Applied Biosystems 7300 real-time cycler. The thermocycling protocol was as follows: 5 min at 95 °C, followed by 40 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 20 s. PCR products were then subjected to a dissociation protocol of 95 °C for 15 s, 60 °C for 20 s, and 97 °C for 15 s. Primers used for these reactions are listed in Supplemental Table 1. *NtACT* served as the internal control and all data were normalized against *NtACT* levels (Hodoshima et al. 2007). Each 20 μL reaction contained 10 μL of SYBR Premix Ex Taq II (TaKaRa), 2 μL of sample, 1.6 μL of 10 μM primer pairs, 6.0 μL of water, and 0.4 μL of ROX reference dye (50×). Gene expression levels were determined using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen 2001). Three replicate PCR amplifications were performed for each sample.

Measurement of antioxidant enzyme activities and MDA content

NtPIC1-OE and WT plants were grown in MS medium (control) for 4 weeks and transferred to MS with 100 μM $CdCl_2$ (Cd) for 10 days. We determined superoxide dismutase (SOD) activity by monitoring the inhibition of nitroblue tetrazolium (NBT) reduction (Beauchamp and Fridovich 1971). Peroxidase (POD) activity was measured via a standard method (Moerschbacher et al. 1988). Catalase (CAT) activity was monitored using the decomposition of H_2O_2 at 240 nm (Aebi 1984). Malondialdehyde (MDA) was determined by measuring the concentration of thiobarbituric acid-reacting substances (Buege and Aust 1978). Three plants were analyzed from each line. All of the above experiments were carried out three times.

Statistical analysis

Statistical analyses were performed using the Statistical Product and Service Solutions software package, version 16.0 (SPSS Inc.). Data were evaluated for normality and homogeneity of variance. A one-way analysis of variance (ANOVA) was used to test for significant differences between treatments. The ANOVA was followed by a Duncan's Multiple Range test to compare individual means. Differences were considered significant at $p < 0.05$. Data represent mean values of three replicates.

Results

Overexpression of *NtPIC1* results in increased tolerance to Cd

The BY4741-pYES2-NtPIC1-transformed *S. cerevisiae* yeast *fet3fet4* cells were more sensitive to Ni, Cu, and Co than EV cells. However, the NtPIC1-transformed cells showed greater growth than EV cells in the Cd-supplemented SD-ura medium (Supplemental Fig. 1). We hypothesized that NtPIC1 expression was related to increased Cd tolerance in yeast cells.

To test this hypothesis, we compared the growth of NtPIC1-OE1, -OE13, and -OE33 with WT plants, under a series of Cd treatments. We used fresh weight and primary root length as measures of growth.

Four-day-old OE1, OE13, OE33, and WT seedlings were grown vertically on medium containing 0, 75, or 100 μM $CdCl_2$ for 7 days. Figure 1a shows representative OE1 plants compared with WT plants; the latter exhibited significant growth inhibition under all Cd concentrations, but the effect was especially pronounced under 100 μM Cd. Specifically, compared with OE1 plants, WT plants were much smaller and showed signs of chlorosis. Next, the fresh weights of the three OE plants were significantly higher (Fig. 1b) and their roots significantly longer (Fig. 1c) than those of WT plants. Even in the absence of Cd, OE plants were likely able to accumulate fresh weight faster than WT plants because higher NtPIC1 levels result in the transport of more Fe to the chloroplast. Finally, the relative growth inhibition rates for roots of OE1, OE13, and OE33 plants were lower than WT plants under 100 μM Cd stress (Fig. 1d).

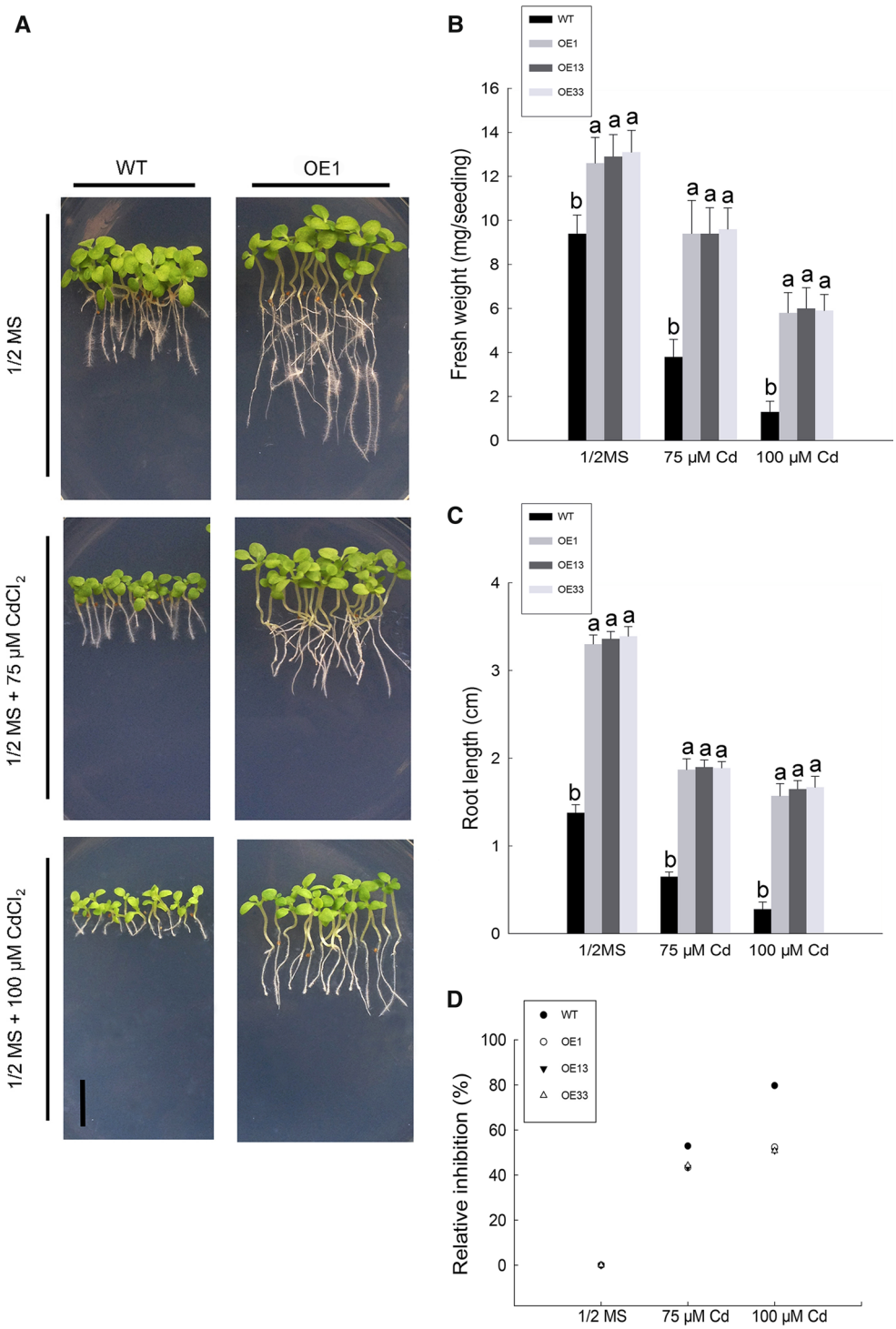

Fig. 1 Fresh weights and root lengths of NtPIC1-OE and WT plants exposed to different Cd concentrations. **a** Phenotypes of representative plant OE1 grown on half-strength MS agar plates supplemented with 0, 75, or 100 μM $CdCl_2$ were photographed 7 days after transfer to the Cd treatments. *Scale bars* 1 cm. **b** Fresh weights were measured after 7 days. **c** Root lengths were measured after 7 days. **d** The relative inhibitions of root growth in NtPIC1-OE and WT plants. WT seedlings show severe root growth inhibition at 100 μM $CdCl_2$. Fresh weight and root length values correspond to mean ± SE ($n = 10$). *Letters above* the *error bars* (SD) indicate significant differences ($p < 0.05$)

NtPIC1-OE and chlorophyll, Fe and Cd concentrations

To examine the basis for the increased Cd tolerance of NtPIC1-OE plants, we measured chlorophyll levels in leaves and Cd and Fe concentrations in roots and shoots of OE1, OE13, OE33, and WT plants. The phenotypes of OE1, OE13, OE33, and WT plants treated with 100 μM Cd are shown in Fig. 2a; the WT plants were more chlorotic than the OE1, OE13, OE33 plants. The chlorophyll concentrations in the leaves of OE1, OE13, OE33 plants were significantly higher ($p < 0.05$) than in WT leaves (Fig. 2b–d). Compared with WT plants under both control and Cd stress, the three OE plants had lower Fe concentrations in

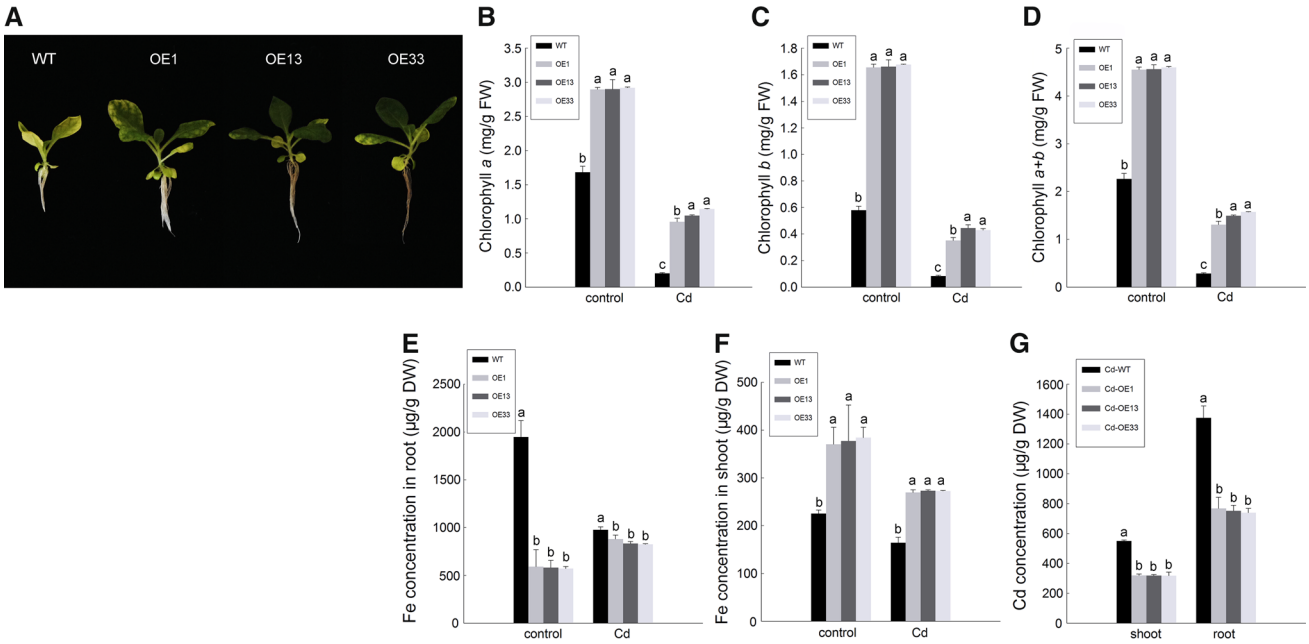

Fig. 2 The concentrations of Fe, Cd, and chlorophyll in NtPIC1-OE and WT plants. **a** Phenotypes of NtPIC1-OE and WT plants grown on MS medium containing 100 μM $CdCl_2$ for 10 days. **b–d** Chlorophyll concentrations in leaves of NtPIC1-OE and WT plants. **e–g** Fe and Cd concentrations in root and shoot tissues of NtPIC1-OE and WT plants with or without Cd treatment. *Letters above* the *error bars* (SD) indicate significant differences ($p < 0.05$). *Scale bars* 1 cm

the roots and higher Fe concentrations in the shoots (Fig. 2e, f). In addition, when under Cd stress, compared with WT plants, the shoots and roots of OE plants had significantly lower Cd concentrations ($p < 0.05$; Fig. 2g). These results support the interpretation that higher Fe levels in plants overexpressing NtPIC1 contribute to a reduction in both Cd uptake and Cd transport from the roots to the shoots.

Expression of genes associated with iron transport

The above analysis indicated that root-to-shoot transport activity was reduced in NtPIC1-OE plants exposed to Cd. To determine whether this effect involves genes responsible for Fe uptake and transport, we analyzed the expression of the Fe transporter-related genes *NtPIC1, NtFER1* (ferritin), *NtNRAMP1, NtFRO1* (ferric-chelate reductase oxidase), *NtIRT1,* and *NtZIP1* (zinc-regulated transporter/Fe-regulated transporter) in NtPIC1-OE and WT plants, under both control and Cd stress conditions. Representative results concerning the OE1 line are shown in Figs. 3 and 4. We used both *NtACT* and *NtGAPDH* as reference genes for qRT-PCR and performed parallel experiments with the two standards. We obtained equivalent results from both standards, and so we have chosen to show the data obtained from *NtACT* here and included the data from *NtGAPDH* as online supplemental material (Supplemental Fig. 2, 3). Our analysis showed that Cd stress had little effect on *NtPIC1* expression in WT roots (Fig. 3a). In contrast, the Fe storage protein *NtFER1* and the Fe transporter *NtNRAMP1* were both significantly downregulated ($p < 0.05$; Fig. 3b, c), while the Fe deficiency-response genes *NtFRO1, NtIRT1*, and *NtZIP1* were all significantly upregulated ($p < 0.05$; Fig. 3d–f). In NtPIC1-OE plants, NtPIC1 expression was higher than in WT plants regardless of the experimental conditions, because the OE plants were ectopically overexpressing NtPIC1 (Figs. 3g, 4g). However, in comparison to WT plants, *NtFER1* and *NtNRAMP1* expression was significantly upregulated in OE1 roots ($p < 0.05$; Fig. 3h, i), while *NtFRO1* and *NtIRT1* expression was significantly downregulated ($p < 0.05$; Fig. 3j, k). *NtZIP1* expression was significantly upregulated in control conditions, but significantly downregulated under Cd treatment in the roots of OE1 plants compared with WT plants ($p < 0.05$; Fig. 3l).

A similar analysis of gene expression was performed using shoot tissues. In WT plants under Cd treatment, *NtPIC1* and *NtFER1* expressions were significantly downregulated ($p < 0.05$) (Fig. 4a, b), while *NtNRAMP1*, *NtFRO1*, and *NtIRT1* expressions were significantly higher than under control conditions ($p < 0.05$; Fig. 4d–f). Unlike that in WT plants, *NtFER1* and *NtNRAMP1* expression levels were significantly upregulated in the shoots of OE1 plant under control conditions ($p < 0.05$; Fig. 4h, i). Again, unlike that in WT, *NtFRO1* and *NtIRT1* expression levels were significantly downregulated in the shoots of

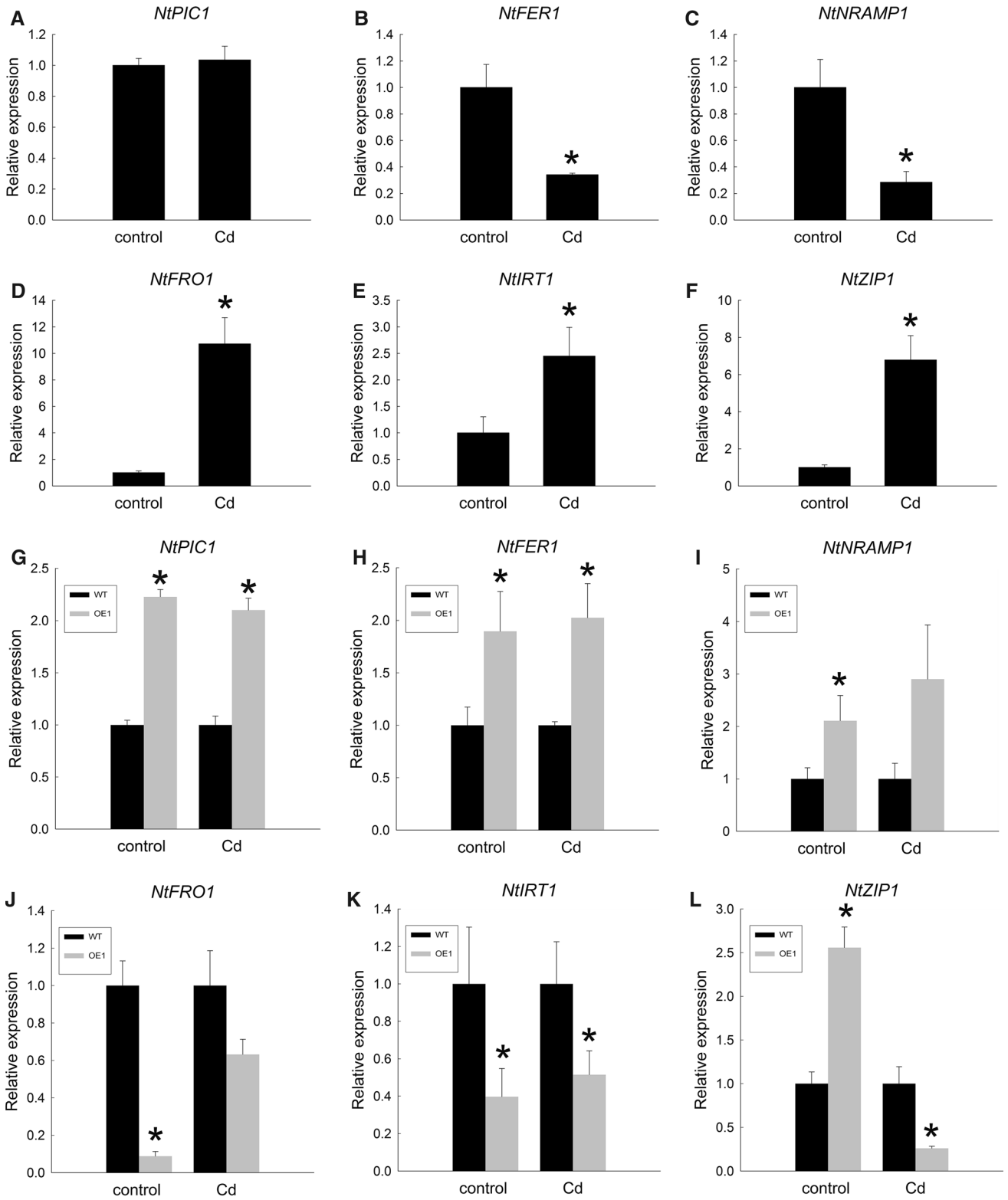

Fig. 3 Quantitative RT-PCR analysis of iron metabolism-related genes in NtPIC1-OE1 and WT plant roots. Levels of *NtPIC1*, *NtFER1*, *NtNRAMP1*, *NtFRO1*, *NtIRT1,* and *NtZIP1* transcripts were normalized against *NtACT*. Data represent the means of three technical replicates. **a**–**f** WT plants in control or Cd treatments. **g**–**l** NtPIC1-OE1 plants and WT plants in control and Cd treatments. **a**–**f** *Asterisks* significant differences from control ($p < 0.05$; Student's *t* test). **g**–**l** *Asterisks* significant differences from WT ($p < 0.05$; Student's *t* test)

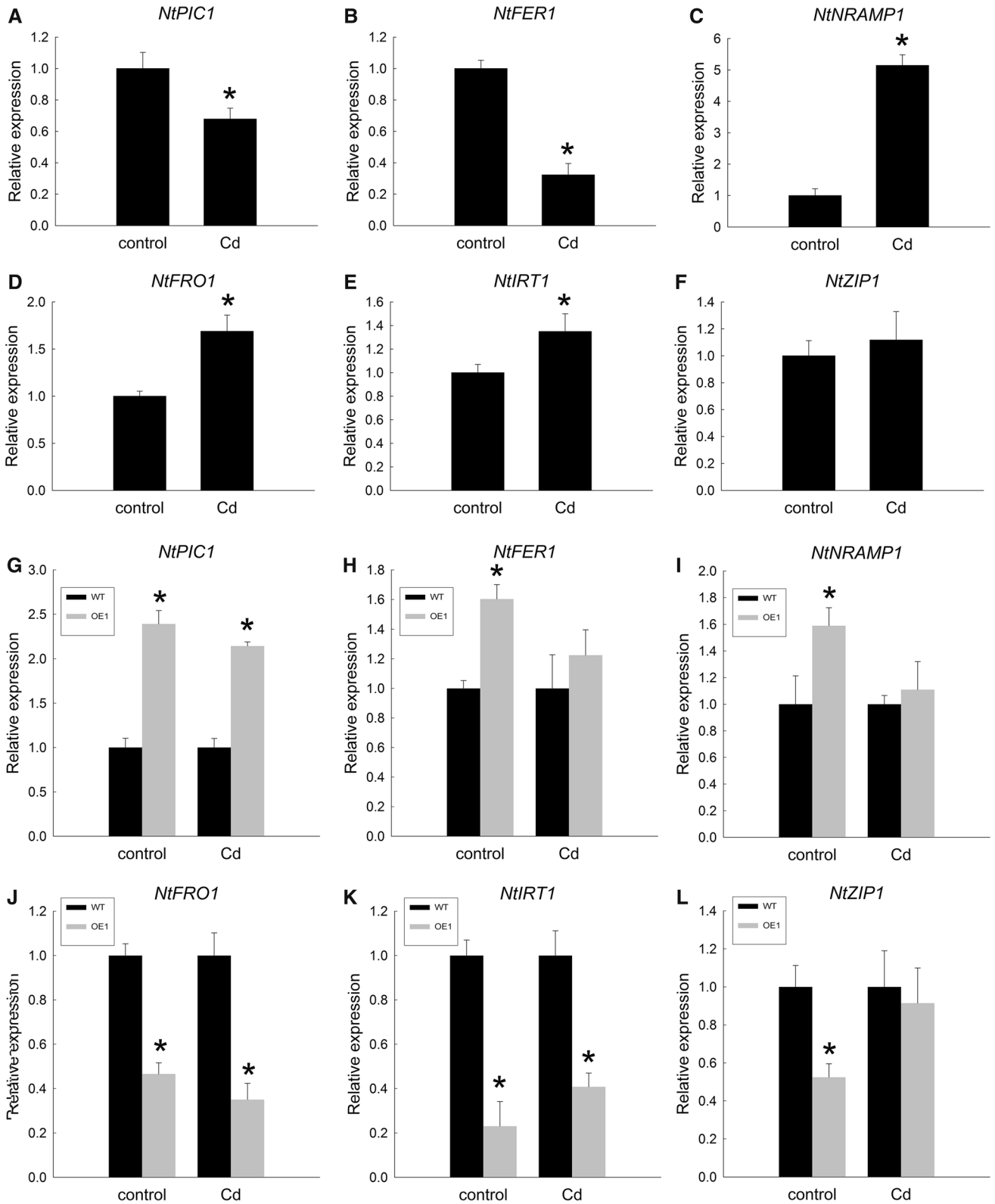

Fig. 4 Quantitative RT-PCR analysis of iron transporter-related genes in NtPIC1-OE1 and WT plant shoots. Levels of *NtPIC1*, *NtFER1*, *NtNRAMP1*, *NtFRO1*, *NtIRT1*, and *NtZIP1* transcripts were normalized against *NtACT*. Data represent the mean of three technical replicates. **a–f** WT plants in control and Cd treatments. **g–l** NtPIC1-OE1 plants and WT plants in control or Cd treatments. **a–f** *Asterisks* significant differences from control ($p < 0.05$; Student's t test). **g–l** *Asterisks* significant differences from WT ($p < 0.05$; Student's t test)

OE1 plants under both control and Cd treatment conditions (Fig. 4j, k); *NtZIP1* expression (Siemianowski et al. 2014) was also downregulated in control conditions (Fig. 4l). Based on these results, we suggest that reduction of Cd uptake in NtPIC1-OE plants might be related to the decreased expression of the *NtIRT1*, *NtFRO1*, and *NtZIP1* genes.

Antioxidant index analysis

Finally, we investigated antioxidant enzymes in NtPIC1-OE plants by screening the activities of SOD, POD, and CAT (Fig. 5a–c) and assessing MDA contents (Fig. 5d). Compared to WT plants, OE1, OE13, and OE33 plants showed significantly higher SOD, POD, and CAT activities under both control and 100 μM $CdCl_2$ stress conditions ($p < 0.05$). The MDA contents of WT plants did not differ significantly from those of OE1, OE13, OE33 plants in control conditions, but after Cd exposure, MDA levels in WT plants were significantly higher than in the three OE plants ($p < 0.05$; Fig. 5d). This result indicates that Cd-induced membrane damage was more severe in WT plants than in NtPIC1-OE plants.

Discussion

Cd toxicity is mainly due to the disruption of essential metal uptake and homeostasis (Clemens et al. 2013). This disruption is caused by binding competition between Cd and other essential metals, especially Fe (Schützendübel and Polle 2002). Thus, we hypothesized that NtPIC1 overexpression should lead to higher cell Fe levels, reducing Cd ion accumulation, and thereby alleviating the impact of Cd toxicity.

In higher plants, Cd phytotoxicity inhibits root elongation (Schützendübel and Polle 2002). Our results demonstrated that under Cd stress, NtPIC1-OE plants had significantly longer roots, higher fresh weights, and lower growth suppression than WT plants (Fig. 1). Moreover,

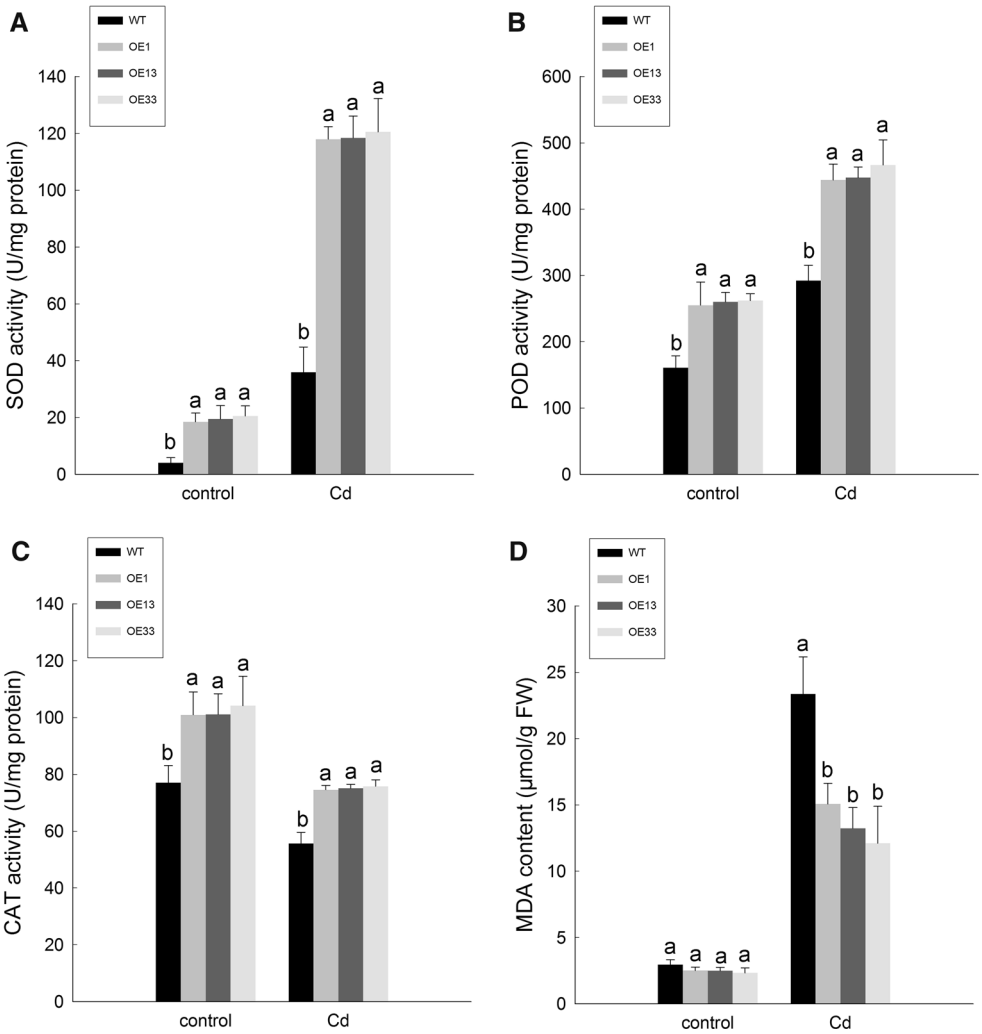

Fig. 5 Effect of *NtPIC1* overexpression on antioxidant enzymes and MDA amounts in NtPIC1-OE and WT plants treated with Cd. **a** Superoxide dismutase (SOD) activity. **b** Peroxidase (POD) activity. **c** Catalase (CAT) activity. **d** Malondialdehyde (MDA) content in leaves of transgenic and WT plants grown in 100 μM $CdCl_2$ for 10 days. Values shown are mean ± SE. Three plants were measured in each line. *Letters above* the *error bars* (SD) indicate significant differences ($p < 0.05$)

NtPIC1-OE plants had higher chlorophyll levels and remained green, while WT plants exhibited severe chlorosis (Fig. 2a–d). These results indicate that NtPIC1-OE plants were more Cd tolerant than WT plants. In contrast to Fe, the Cd concentrations in the roots and shoots of NtPIC1-OE plants were significantly lower than in WT plants (Fig. 2e–g). This pattern suggests that *NtPIC1* overexpression increased Fe transport from roots to shoots and decreased Cd accumulation in shoots and roots. At the same time, Cd interferes with Fe homeostasis, causing Fe deficiency in the upper plant parts. Low Fe concentrations then induce two processes in the roots: the upregulation of Fe deficiency-response genes *NtFRO1* and *NtIRT1* (Fig. 3d, e) (Yoshihara et al. 2006) and the downregulation of the Fe storage protein NtFER1 (Figs. 3b, 4b). A recent study found that upregulation of IRT1 resulted in Cd accumulation because of a wide range of substrate transport properties, leading to Cd accumulation (Korshunova et al. 1999; Mendoza-Cózatl et al. 2014). In this study, changes to transport components were also observed: the potential metal uptake-related genes *NtFRO1*, *NtIRT1*, and *NtZIP1* were expressed at lower levels in Cd-exposed NtPIC1-OE plants (Figs. 3j–l, 4j–l), leading to lower Cd accumulation. Additionally, the iron transporter *NtNRAMP1* was upregulated in NtPIC1-OE roots, possibly facilitating Fe accumulation under Cd stress. These results indicate that Cd exposure interferes with the Fe homeostasis mechanism (Bovet et al. 2006; Yoshihara et al. 2006; Hodoshima et al. 2007), but the resultant downregulation of *NtFRO1*, *NtIRT1*, and *NtZIP1* may prevent toxic levels of Cd accumulation.

Plant tolerance to Cd involves numerous physiological and molecular mechanisms, such as cell wall binding, chelation with phytochelatins, and compartmentalization in vacuoles (Wu et al. 2012). According to the results presented here, higher Cd tolerance in NtPIC1-OE plants is a consequence of increasing Fe entry into plants, particularly root-to-shoot partitioning, which results in the restriction of Cd uptake (Wu et al. 2012). Although Cd does not participate in the Fenton and Haber–Weiss reactions, exposure to the metal can still cause oxidative stress (Clemens 2006). Cd increases ROS in plants (Cuypers et al. 2011; Liu et al. 2011a, b), injuring cell components (Romero-Puertas et al. 2004; Heyno et al. 2008). Following previous research (Halliwell 2006; Møller et al. 2007; Gill and Tuteja 2010), we used MDA levels and antioxidant enzyme activity as diagnostics for oxidative stress (ROS presence). We found the NtPIC1-OE plants exhibited lower levels of MDA content and higher levels of antioxidant enzyme activity (Fig. 5), which is in accord with the decrease in Cd accumulation. Our results thus indicate that NtPIC1-OE plants experience less oxidative stress than WT plants when exposed to Cd (Fig. 5).

In summary, this work has shown clearly that under Cd stress, NtPIC1-OE plants were more efficient than WT in Fe translocation from roots to shoots. Further, NtPIC1-mediated Fe acquisition effectively mitigated Cd toxicity by reducing the risk of Cd oxidative damage at the cellular level. Higher Fe concentrations in NtPIC1-OE plants also appeared to suppress the expression of iron deficiency genes (*NtFRO1*, *NtIRT1*, and *NtZIP1*) (Zhao and Eide 1996; Connolly et al. 2002; Enomoto et al. 2007), which are putatively involved in Cd uptake. Thus, their downregulation likely resulted in the reduction of root and shoot Cd concentrations. These findings indicate that Fe plays a considerable role in the alleviation of Cd toxicity in plants, which should contribute greatly to biofortification efforts aimed at reducing heavy metal contamination of crops.

Author contribution statement Changhong Guo conceived and designed the experiments. Xun Gong and Lin-Wei Yin performed the experiments. Xun Gong wrote the manuscript. Jiaqi Chen analyzed data.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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