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Praseodymium enhanced the tolerance of maize seedlings subjected to cadmium stress by up-regulating the enzymes in the regeneration and biosynthetic pathways of ascorbate and glutathione

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Abstract: To test whether praseodymium (Pr) regulates cadmium (Cd) tolerance, we explored the effects of Pr on enzymatic activities in the regeneration and biosynthetic pathways of ascorbate and glutathione in maize seedlings under Cd stress. The findings demonstrated that Cd stress increased enzymatic activities in the regeneration pathway (ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR)) and in the biosynthetic pathway of ascorbate and glutathione (γ-ECS and GalLDH), as well as ascorbate (AsA) and glutathione (GSH) contents. However, Cd stress significantly decreased AsA/dehydroascorbic acid (DHA) ratio and GSH/oxidised glutathione (GSSG) ratio, net photosynthetic rate (P_n), chlorophylls (*Chl*) and carotenoids (Car) contents, maximum photochemical efficiency of PSII (F_v/F_m), photochemical quenching (qP) and quantum efficiency of PSII photochemistry (Φ_{PSII}), as well as plant height and biomass. Application of Pr to Cd-stressed seedlings enhanced above enzymatic activities, AsA and GSH contents, AsA/DHA and GSH/GSSG ratios, P_n, *Chl* and Car contents, F_v/F_m, qP and Φ_{PSII}, as well as plant height and biomass. Meanwhile, the application of Pr to Cd-stressed seedlings reduced malondialdehyde (MDA) content and electrolyte leakage. The above results indicated that Pr enhanced Cd tolerance of maize by up-regulating enzymatic activities in regeneration and biosynthetic pathways of ascorbate and glutathione.

Keywords: cadmium toxicity; rare earth element; redox state; antioxidant; *Zea mays* L.

As an environmental pollutant, cadmium (Cd) has been proved to be a toxic heavy metal. Excess Cd induces adverse effects on plant growth, physiological and biochemical processes (Lombardi and Sebastiani 2005, Yang et al. 2021). Furthermore, Cd stress usually induced peroxide damage to plants (Leng et al. 2021). To defend against Cd-induced peroxide damage, plants could enhance their antioxidant system, including antioxidases and antioxidants (Valentovicová et al. 2010).

As antioxidants in plants, ascorbate (AsA) and glutathione (GSH) played significant roles in fighting against peroxide damage. Increasing studies showed that the contents of ascorbate and glutathione could be regulated through their metabolic pathways, mainly including regeneration and biosynthetic pathways. In plants, the main biosynthetic pathway of ascorbate is the L-galactose pathway, in which L-galactono-1,4-lactone dehydrogenase (GalLDH) is an important key enzyme (Broad et al. 2020). Gamma-glutamylcysteine

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synthetase (γ-ECS) is an important key enzyme responsible for glutathione biosynthesis (Dringen 2000). The ascorbate-glutathione (AsA-GSH) cycle is the regeneration pathway of ascorbate and glutathione. Many studies have indicated that exogenous substances could regulate the contents of ascorbate and glutathione through the above regeneration and biosynthetic pathways (Shan and Ou 2018). Hence, we can use exogenous substances to adjust their contents in plants by regulating the regeneration and biosynthetic pathways.

It has been reported that biotic and abiotic factors could improve the Cd tolerance of plants. Awan et al. (2020) reported that *Bacillus siamensis* enhanced the Cd tolerance of wheat plants. Khanna et al. (2019) found that plant growth-promoting rhizobacteria alleviated Cd toxicity in *Solanum lycopersicum* L. seedlings. Li et al. (2021) showed that glycinebetaine could enhance the Cd tolerance of tobacco plants through genetic engineering. Increasing evidences showed that plant hormones could improve Cd tolerance of plants, including kinetin (KT), salicylic acid (SA) and abscisic acid (ABA), etc. (Gondor et al. 2016, Yan et al. 2016, Bashri et al. 2021). Several researches have also shown that rare earth elements (REEs) were important for plant growth and stress responses, such as cerium (Ce), lanthanum (La) and neodymium (Nd) (Liu et al. 2016, Lu et al. 2020, Zhong and Chen 2020). Praseodymium (Pr) is another important REE. Some researches also showed that Pr could improve the Cd tolerance of rice (Zhou et al. 2009, Ren et al. 2010). Ren et al. (2010) revealed that Pr promoted root growth and increased antioxidants activities of rice seedlings under Cd stress. Zhou et al. (2009) showed that Pr promoted seed germination of rice subjected to Cd stress. Under copper stress, Liu et al. (2016) reported that Ce enhanced the regeneration and biosynthesis of ascorbate and glutathione in the leaves of turf grass *Poa pratensis* L. Under chromium stress, Lu et al. (2020) indicated that neodymium (Nd) improved the regeneration of ascorbate and glutathionethe through the AsA-GSH cycle in the leaves of wheat seedlings. However, the knowledge for whether and how Pr regulates ascorbate and glutathione metabolism under Cd stress is still blank. Therefore, it is interesting to explore whether and how Pr affects ascorbate and glutathione metabolism in maize crops under Cd stress, which will provide a theoretical basis for the application of Pr in crop cultivation and management.

For the current research, we investigated the effects of Pr on malondialdehyde (MDA) content, electrolyte leakage (EL), the activities of metabolic enzymes responsible for ascorbate and glutathione metabolism, reduced ascorbate and reduced glutathione contents, AsA/dehydroascorbic acid (DHA) and GSH/oxidised glutathione (GSSG) ratios, net photosynthetic rate (P_n), chlorophylls (*Chl*) and carotenoids (Car) contents, maximum photochemical efficiency of PSII (F_v/F_m), photochemical quenching (qP) and quantum efficiency of PSII photochemistry (Φ_{PSII}), as well as plant height and biomass under Cd stress. The hypotheses of our research regarding the effects of Pr on Cd tolerance were: (1) up-regulation of ascorbate and glutathione metabolism; (2) improvement of photosynthetic traits, and (3) an increase in antioxidant capacity and plant growth. The significance of current research is to show light on the influences of Pr on ascorbate and glutathione metabolism in maize crops subjected to Cd stress and give us more information for the role of Pr in improving Cd tolerance, which will provide a theoretical basis for the application of Pr in crop cultivation and management.

MATERIAL AND METHODS

Plant material and treatment. Seeds of maize (*Zea mays*) cv. Xindan29 were germinated and cultured in the artificial chamber. The growth conditions were set below: 25 °C at the day, 15 °C at night, 500 μmol/m^2/s photosynthetic active radiation and 12 h photoperiod. After the first leaves expanded enough, maize seedlings were transplanted to plastic boxes containing full-strength Hoagland's solution. In order to keep the roots in the dark, black plastics were used to wrap the outside of plastic boxes. The Hoagland's solution was replaced each day. After the third leaves expanded enough, we picked out maize seedlings with similar growth status for the whole experiment.

To choose the suitable Cd concentration, we explored the effects of 0, 50, 100, 150 and 200 mg/L $CdCl_2$ on AsA and GSH contents and AsA/DHA and GSH/GSSG ratios in leaves of maize seedlings. 150 and 200 mg/L $CdCl_2$ all induced obvious wilting status. 50 mg/L $CdCl_2$ induced no obvious wilting status. Under 100 mg/L $CdCl_2$, the leaves of maize plants were partially wilted, but plants could continue to grow. Thus, 100 mg/L $CdCl_2$ were selected as the suitable treatment concentration. To explore the functions of praseodymium nitrate ($Pr(NO_3)_3$) in regulating the ascorbate and glutathione metabolism, three groups

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of maize plants were firstly treated by 10, 30 and 90 μmol/L $Pr(NO_3)_3$ for 1 day, respectively. Then above Pr-treated seedlings were subjected to Cd stress for 7 days. Control seedlings were only treated by distilled water. Different treatments were all repeated four times. After 3 days of treatment, the third leaves were sampled and stored in an ultra-low temperature freezer until analyses. For the determination of *Chl* and Car contents, MDA and EL, fresh third leaves were collected and then used for the analyses of the above four indicators. After 7 days of treatment, we determined plant height and biomass.

Measurement of APX, GR, DHAR and MDHAR activities. Ascorbate peroxidase (APX) activity was measured by recording the decrease in absorbance at 290 nm in 3 min (Nakano and Asada 1981). Glutathione reductase (GR) activity was measured by recording the decrease in absorbance at 340 nm in 3 min (Grace and Logan 1996). Monodehydroascorbate reductase (MDHAR) activity was determined by recording the decrease in absorbance at 340 nm in 2 min (Miyake and Asada 1992). Dehydroascorbate reductase (DHAR) activity was analysed by recording the decrease in absorbance at 265 nm in 3 min (Dalton et al. 1986). The unit for the above enzymatic activities was expressed as units/mg protein. Protein content was analysed according to Bradford (1976). Four repetitions were done for each treatment.

Measurement of GalLDH and γ-ECS activities. L-galactono-1,4-lactone dehydrogenase (GalLDH) was extracted and determined according to Tabata et al. (2001). The increase in absorbance at 550 nm was measured by a spectrophotometer (Beijing, China). Gamma-glutamylcysteine synthetase (γ-ECS) was extracted and determined according to Rüegsegger and Brunold (1992). The absorbance at 660 nm was measured by a spectrophotometer. The unit for GalLDH and γ-ECS activities were expressed as units/mg protein. Protein content was analysed according to Bradford (1976). Four repetitions were done for each treatment.

Measurement of AsA and GSH contents, AsA/DHA and GSH/GSSG ratios. AsA and total ascorbate contents were measured according to Hodges et al. (1997). DHA content was calculated from the difference between total ascorbate and AsA. The ratio of AsA content to DHA content was expressed as AsA/DHA. Total glutathione and oxidised glutathione (GSSG) contents were measured according to Griffith (1980). glutathione (GSH) content was calculated from the difference between total glutathione and GSSG. The ratio of GSH content to GSSG content was expressed as GSH/GSSG. Four repetitions were done for each treatment.

Measurement of MDA and EL. MDA was measured by thiobarbituric acid (TBA) reaction according to Hodges et al. (1999) with some modifications. Leaf samples were homogenised in 0.1% trichloroacetic acid (TCA) and then centrifuged. The supernatant was mixed with 20% TCA containing 0.5% TBA and then heated at 100 °C for 20 min. After cooling, the absorption values at 450, 532 and 600 nm were measured by spectrophotometer. EL was analysed according to Naeem et al. (2020). Leaf discs were incubated in distilled water in test tubes at 25 °C for 6 h, and then electrical conductivity (EC) was measured and recorded as EC_1. Samples were then heated at 100 °C for 20 min, and EC was measured after cooling and recorded as EC_2. EL was calculated according to the below equation:

$$EL = (EC_1/EC_2) \times 100\%$$

Four repetitions were done for each treatment.

Measurement of *Chl* and Car contents. *Chl* and Car were analysed according to Lichtenthaler and Wellburn (1983) with some modifications. The samples of leaves were cut into small pieces and then homogenised in 80% acetone. After filtration, the absorption values at 665, 649 and 470 nm were measured by spectrophotometer. Four repetitions were done for each treatment.

Measurement of P_n and chlorophyll fluorescence parameters. We measured P_n by using the photosynthesis system (Licor-6800, Lincoln, USA) on a sunny day. The radiation for the measurement was 1 500 μmol photon/m^2/s, and the CO_2 flux was adjusted to maintain a concentration of 400 μmol/mol from 9:00 to 11:00 h. We determined chlorophyll fluorescence parameters (F_v/F_m, q_P, q_N (non-photochemical quenching) and Φ_{PSII}) using a Yaxin-1161G fluorometer (Yaxin, China). The intensity of saturation flash for the measurements of F_v/F_m was 2 400 μmol photon/m^2/s. Four repetitions were done for each treatment.

Measurement of plant height and biomass. The ruler was used to measure plant height. Fresh weights of plants were recorded and dried in the oven for 96 h at 80 °C. Then dry weights were recorded. Four repetitions were done for each treatment.

Data analysis. Data presented in figures were means of four replications. The difference between means was compared by one-way analysis of variance and Duncan's test at $\alpha = 0.05$ level.

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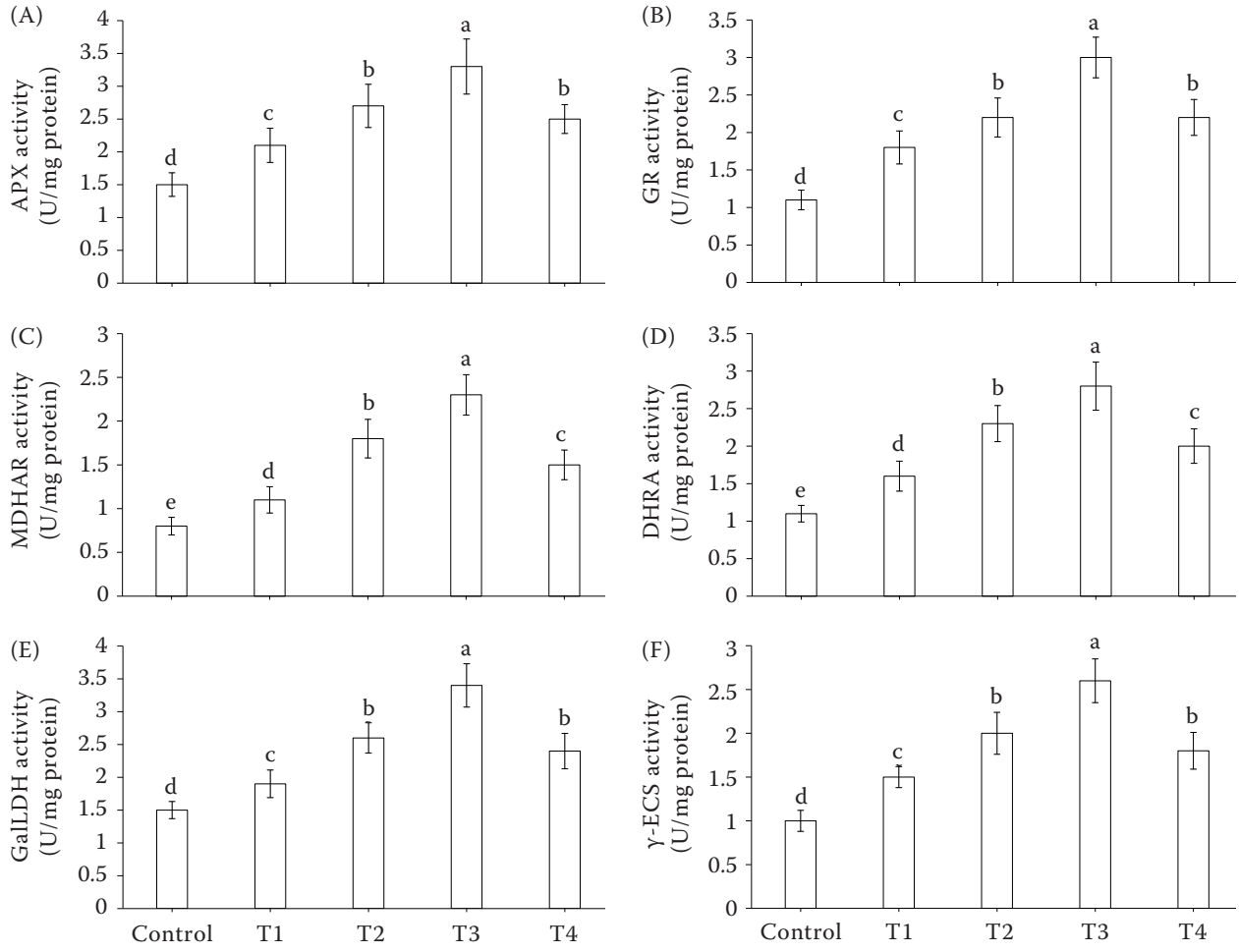

Figure 1. Effects of different praseodymium (Pr) concentrations on the activities of enzymes involved in ascorbate and glutathione metabolism in leaves of maize seedlings under cadmium (Cd) stress. The plants were treated as follows: control – distilled water; T1 – 100 mg/L $CdCl_2$; T2 – 10 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$; T3 – 30 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$; T4 – 90 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$. The plants were pre-treated with $Pr(NO_3)_3$ for 12 h, and then exposed to 100 mg/L $CdCl_2$ for 48 h; APX – ascorbate peroxidase; GR – glutathione reductase; MDHAR – monodehydroascorbate reductase; DHRA – dehydroascorbate reductase; GalLDH – glutathione; γ-ECS – ascorbate

RESULTS

Effects of Pr on the activities of GalLDH and γ-ECS. Cd stress significantly increased the activities of key biosynthesis GalLDH and γ-ECS (Figure 1), compared with control. Application of $Pr(NO_3)_3$ significantly further enhanced the activities of the above two key biosynthesis compared to Cd stress. In comparison with Cd stress, 10, 30 and 90 μmol/L $Pr(NO_3)_3$ respectively improved GalLDH activity by 36.8, 78.9 and 26.3%, improved γ-ECS activity by 33.3, 73.3 and 20.0%. These findings implied that $Pr(NO_3)_3$ enhanced the biosynthetic ability of ascorbate through GalLDH and the biosynthetic ability of glutathione through γ-ECS in maize seedlings under Cd stress.

Effects of Pr on the activities of APX, GR, DHAR and MDHAR. Cd stress significantly enhanced the activities of APX, GR, DHAR and MDHAR, compared to control (Figure 1). Application of $Pr(NO_3)_3$ significantly further enhanced the activities of the above enzymes compared to Cd stress. In comparison with Cd stress, 10, 30 and 90 μmol/L $Pr(NO_3)_3$ respectively improved APX activity by 28.6, 57.1 and 19.0%, improved GR activity by 22.2, 66.7 and 22.2%, improved MDHAR activity by 63.6, 109.1 and 36.4%, and improved DHAR activity by 43.8, 75.0 and 25.0%. Current findings indicated that $Pr(NO_3)_3$

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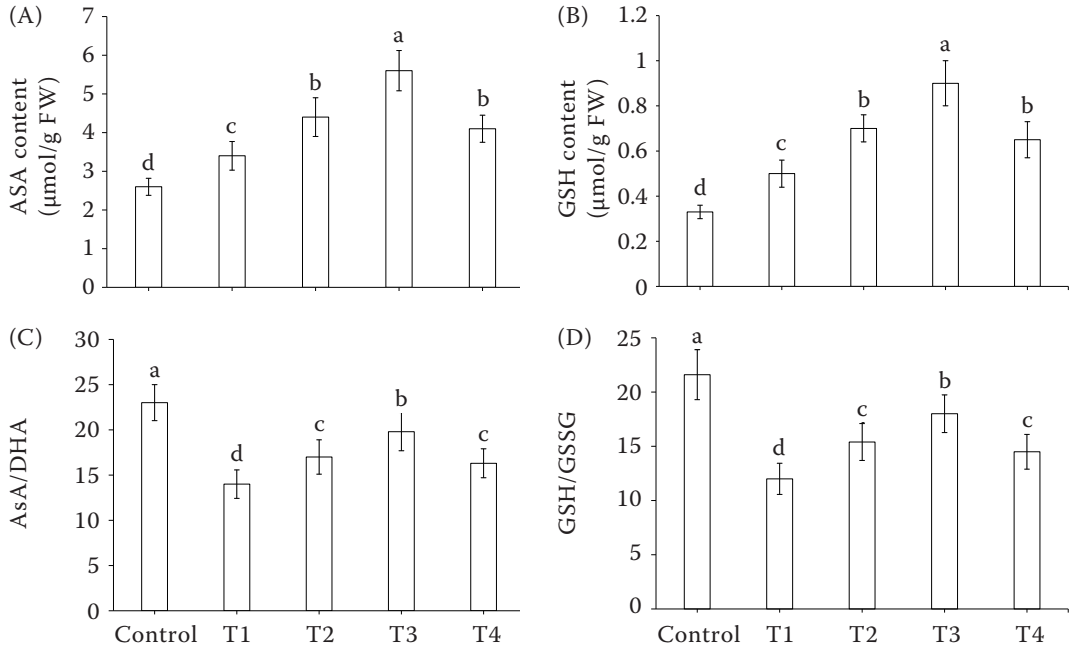

Figure 2. Effects of praseodymium nitrate ($Pr(NO_3)_3$) on the contents of ascorbate (AsA) and glutathione (GSH), and the ratios of AsA/DHA and GSH/oxidised glutathione (GSSG) in leaves. The plants were treated as follows: control – distilled water; T1 – 100 mg/L $CdCl_2$; T2 – 10 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$; T3 – 30 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$; T4 – 90 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$. The plants were pre-treated with $Pr(NO_3)_3$ for 12 h, and then exposed to 100 mg/L $CdCl_2$ for 48 h; FW – fresh weight

strengthened the regeneration ability of ascorbate and glutathione through their recycling enzymes in maize seedlings under Cd stress.

Effects of Pr on AsA and GSH contents and AsA/DHA and GSH/GSSG ratios. Compared to control, Cd stress significantly reduced AsA and GSH contents and AsA/DHA and GSH/GSSG ratios (Figure 2). Compared to Cd stress, $Pr(NO_3)_3$ significantly further improved AsA and GSH contents and AsA/DHA and GSH/GSSG ratios. In comparison with Cd stress, 10, 30 and 90 μmol/L $Pr(NO_3)_3$ respectively improved AsA content by 29.4, 64.7 and 20.6%, improved GSH content by 40.0, 80.0 and 30.0%, improved AsA/DHA by 21.4, 41.4 and 16.4%, and improved GSH/GSSG by 28.3, 50.0 and 20.8%. The above results suggested that Pr improved AsA and GSH contents and AsA/DHA and GSH/GSSG ratios by enhancing the ascorbate and glutathione metabolism in maize seedlings under Cd stress.

Effects of Pr on photosynthetic physiological indexes. Compared to the control, Cd stress significantly reduced *Chl* and Car contents, P_n, F_v/F_m, qP and Φ_{PSII}, but increased qN (Figure 3). Compared to Cd stress, $Pr(NO_3)_3$ significantly further increased above photosynthetic physiological indexes. In comparison with Cd stress, 10, 30 and 90 μmol/L $Pr(NO_3)_3$ respectively improved *Chl* content by 17.3, 30.0 and 13.3%, improved Car content by 28.2, 53.8 and 17.9%, improved P_n by 17.1, 32.9 and 10.4%, improved F_v/F_m by 20.0, 37.7 and 11.1%, improved qP by 26.9, 46.2 and 15.4%, improved qN by 22.2, 41.7 and 13.9%, improved Φ_{PSII} by 28.6, 52.4 and 19.0%, respectively. The above results suggested that Pr improved the function of the photosystem of maize seedlings under Cd stress.

Effects of Pr on plant growth, MDA content and EL. To further explore the roles of $Pr(NO_3)_3$ in improving Cd tolerance of maize crops, the effects of $Pr(NO_3)_3$ on plant growth, MDA and EL under Cd stress were investigated. Cd stress significantly decreased plant height and biomass and promoted MDA content and EL (Figure 4). $Pr(NO_3)_3$ significantly increased plant height and biomass and decreased MDA content and EL under Cd stress. In comparison with Cd stress, 10, 30 and 90 μmol/L $Pr(NO_3)_3$ respectively improved plant height by 13.6, 27.3 and 11.4%, improved plant biomass by 16.3, 29.3 and 12.0%, decreased MDA content by 26.8, 43.1 and 22.7%, and decreased EL by 21.2, 35.9 and 16.3%. The above findings indicated that $Pr(NO_3)_3$ improved Cd tolerance of maize seedlings.

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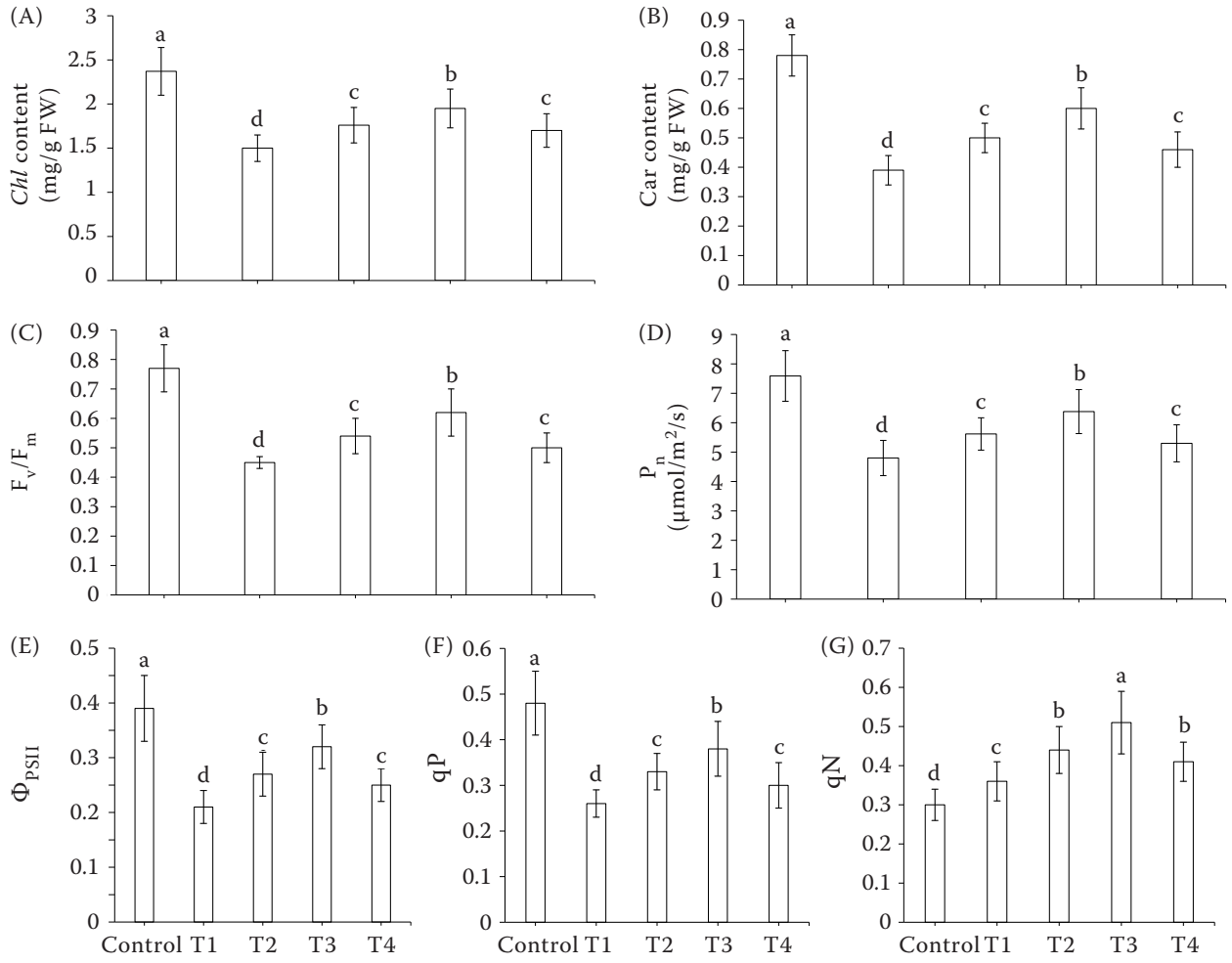

Figure 3. Effects of praseodymium nitrate ($Pr(NO_3)_3$) on the contents of photosynthetic pigments, photosyntheticrate and chlorophyll fluorescence parameters. The plants were treated as follows: control – distilled water; T1 – 100 mg/L $CdCl_2$; T2 – 10 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$; T3 – 30 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$; T4 – 90 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$. The plants were pre-treated with $Pr(NO_3)_3$ for 12 h, and then exposed to 100 mg/L $CdCl_2$ for 48 h. *Chl* – chlorophylls; Car – carotenoids; F_v/F_m – maximum photochemical efficiency of PSII; P_n – net photosynthetic rate; Φ_{PSII} – quantum efficiency of PSII photochemistry; qP – photochemical quenching; qN – non-photochemical quenching; FW – fresh weight

Effects of Pr on Pr contents in leaves and roots. Compared with control and Cd stress, $Pr(NO_3)_3$ significantly increased Pr contents in leaves and roots of maize plants under Cd stress (Figure 5). In comparison with Cd stress, 10, 30 and 90 μmol/L $Pr(NO_3)_3$ increased Pr contents in leaves by 3.0, 6.8 and 17.0 fold, respectively. 10, 30 and 90 μmol/L $Pr(NO_3)_3$ increased Pr contents in roots by 3.4, 7.6 and 18.44, respectively. The above findings indicated that Pr contents in leaves and roots were increased with the increase in the concentration of $Pr(NO_3)_3$.

DISCUSSION

Kaya et al. (2020) reported that pepper fought against Cd stress by increasing the activities of APX and GR. For maize plants, our findings also demonstrated that Cd stress increased the activities of APX and GR. However, Kaya et al. (2020) revealed that Cd stress decreased the activities of DHAR and MDHAR in pepper plants. Our findings showed that Cd stress increased the activities of DHAR and MDHAR in maize plants, which was not in agreement with Kaya et al. (2020). This discrepancy is most likely due to

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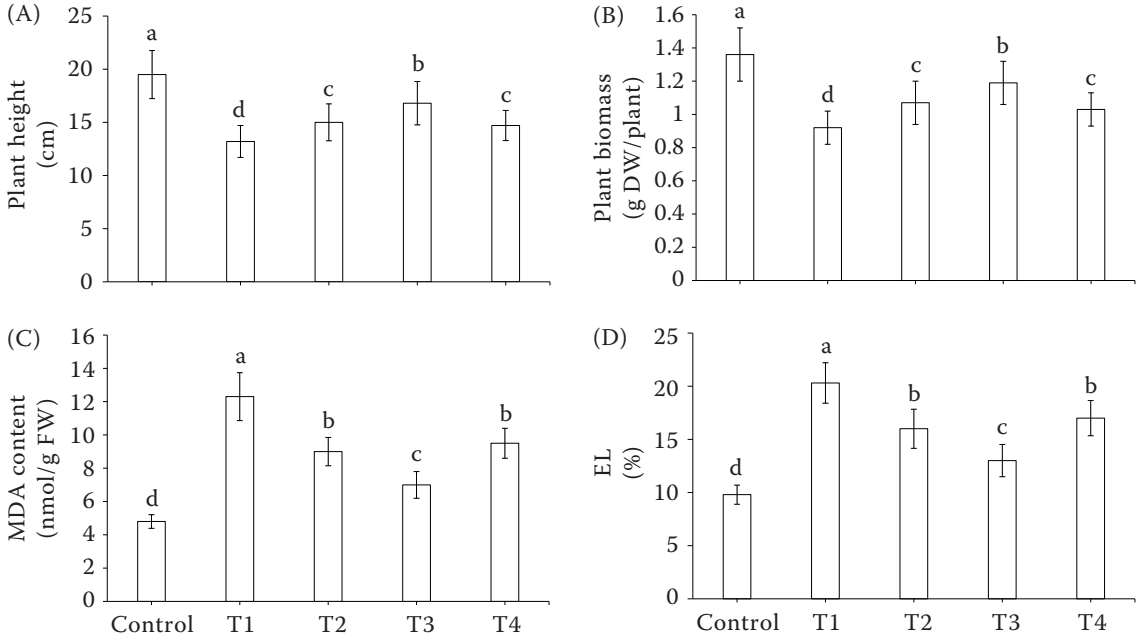

Figure 4. Effects of praseodymium nitrate ($Pr(NO_3)_3$) on plant height, plant biomass, malondialdehyde (MDA) content and electrolyte leakage (EL) of maize seedlings. The plants were treated as follows: control – distilled water; T1 – 100 mg/L $CdCl_2$; T2 – 10 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$; T3 – 30 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$; T4 – 90 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$. The plants were pre-treated with $Pr(NO_3)_3$ for 12 h, and then exposed to 100 mg/L $CdCl_2$ for 48 h. DW – dry weight; FW – fresh weight

the difference in species between pepper plants and maize plants. Furthermore, our current study showed that Cd stress increased the activities of GalLDH and γ-ECS in maize plants. Several more recent studies revealed that plants increased the contents of GSH and AsA through their metabolic pathways. For the current research, the findings also indicated that Cd stress improved the contents of GSH and AsA by enhancing the activities of enzymes in their metabolic pathways, including APX, GR, DHAR, MDHAR, GalLDH and γ-ECS. However, we found that Cd stress decreased AsA/DHA and GSH/GSSG ratios, which was closely associated with Cd-induced oxidative damage.

Zhou et al. (2009) and Ren et al. (2010) indicated that Pr increased APX activity in rice seedlings under Cd stress. For this study, our findings also demonstrated that Pr promoted APX activity in

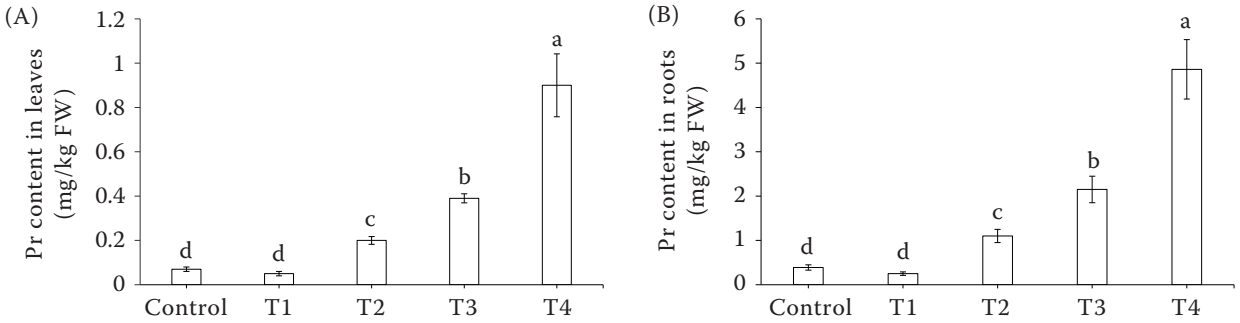

Figure 5. Effects of praseodymium nitrate ($Pr(NO_3)_3$) on praseodymium (Pr) contents in the leaves and roots of maize seedlings. The plants were treated as follows: control – distilled water; T1 – 100 mg/L $CdCl_2$; T2 – 10 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$; T3 – 30 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$; T4 – 90 μmol/L $Pr(NO_3)_3$ + 100 mg/L $CdCl_2$. The plants were pre-treated with $Pr(NO_3)_3$ for 12 h, and then exposed to 100 mg/L $CdCl_2$ for 48 h. FW – fresh weight

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maize seedlings under Cd stress. Moreover, current results indicated that Pr promoted the activities of other enzymes responsible for the AsA-GSH cycle. Meanwhile, we found that Pr enhanced the biosynthesis of AsA and GSH through GalLDH and γ-ECS, respectively. Furthermore, we found that Pr improved AsA and GSH contents and AsA/DHA and GSH/GSSG ratios in the leaves of maize plants under Cd stress. Therefore, Pr could improve Cd tolerance of maize crops by regulating the regeneration and biosynthesis of AsA and GSH, as well as AsA/DHA and GSH/GSSG ratios. Some studies showed that Pr alleviated Cd-induced peroxide damage in plants (Zhou et al. 2009, Ren et al. 2010). Zhou et al. (2009) and Ren et al. (2010) indicated that Pr reduced MDA content and EL in rice under Cd stress. For maize plants, we also revealed that Pr reduced Cd-induced MDA content and EL. These findings implied that Pr alleviated Cd-induced peroxide damage to maize plants by enhancing the ascorbate and glutathione metabolism.

More and more studies demonstrated that Cd showed negative effects on photosynthesis, which further inhibited plant growth. Akhtar et al. (2017) showed that Cd reduced *Chl* content and P_n of different maize cultivars, which further inhibited the growth of roots and shoot. Karalija and Selović (2018) reported that Cd stress reduced *Chl* content, biomass, shoot length, leaf length and width of maize plants. In the current study, the findings showed that Cd stress also reduced *Chl* content, P_n, plant height and biomass, which was in agreement with previous researches (Akhtar et al. 2017, Karalija and Selović 2018). In addition, we found that Cd stress obviously reduced Car content in maize plants. Previous studies have reported that Cd stress reduced chlorophyll fluorescence parameters F_v/F_m and qP but increased qN of maize plants (Zhao et al. 2018). In this study, we also showed the same results as Zhao et al. (2018). Besides, we also showed that Cd reduced chlorophyll fluorescence parameter Φ_{PSII} of maize plants. It has been documented that Pr could reverse the inhibitory influences of Cd stress on roots growth of rice (Zhou et al. 2009, Ren et al. 2010). However, there is still no report for the effects of Pr on the photosynthetic physiology and growth of maize plants. Current findings showed that Pr improved *Chl* and Car contents, P_n, F_v/F_m, qP, qN and Φ_{PSII} of maize plants, which enhanced the function of photosystem and improved plant growth of maize plants under Cd stress. Thus, current results indicated that Pr improved the growth of maize plants by enhancing the function of the photosystem.

In this study, we found that Pr contents in leaves and roots were increased with the increase in the concentration of $Pr(NO_3)_3$. However, the highest concentration of $Pr(NO_3)_3$ (90 μmol/L) did not show a better effect on Cd tolerance of maize plants. The medium concentration of $Pr(NO_3)_3$ (30 μmol/L) showed a better effect on Cd tolerance of maize plants. This finding indicated that Pr played a better effect on Cd tolerance at a suitable concentration. Thus, we should select the suitable concentration of Pr before its application in crop cultivation and management.

It has been reported that Cd stress altered hormone balance in plants (Pérez Chaca et al. 2014, Yan et al. 2016). Other studies showed that hormones KT and SA could enhance the Cd tolerance of plants by regulating the metabolism of ascorbate and glutathione, which further maintained redox balance in plant cells (Yan et al. 2016, Bashri et al. 2021). Ahammed et al. (2021) found that tomato RING E3 ubiquitin ligase gene SlRING1 played an important role in fighting against Cd toxicity by regulating the metabolism of ascorbate and glutathione. However, under Cd stress, whether Pr can regulate the metabolism of ascorbate and glutathione through the above hormones and RING E3 ubiquitin ligase gene SlRING1 is still unclear. Therefore, it will be interesting to further investigate the effects of Pr on the contents of plant hormones and the expression of SlRING1 in plants under Cd stress, which will further elucidate the regulatory metabolism of Pr in improving the Cd tolerance of plants.

In brief, our findings indicated that Pr regulated the contents of antioxidants AsA and GSH and the redox of ascorbate and glutathione by increasing the activities of enzymes responsible for their metabolism, which further improved the antioxidant ability of maize seedlings, the function of the photosystem and plant growth under Cd stress. These results provided more information for us to understand the roles of Pr in regulating the antioxidant metabolism in maize crops under Cd stress, which further provided a theoretical basis for the application of Pr in crop cultivation and management.

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