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Lanthanum improves the cadmium tolerance of *Zea mays* seedlings by the regulation of ascorbate and glutathione metabolism

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Abstract

The effect of lanthanum on the metabolism of ascorbate (AsA) and glutathione (GSH) in the leaves of maize seedlings under cadmium stress was investigated. The findings showed that Cd remarkably increased electrolyte leakage (EL), the activities of ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase (MDHAR), glutathione reductase, L-galactono-1,4-lactone dehydrogenase, and γ-glutamylcysteine synthetase, and the content of reduced AsA, reduced GSH, total AsA, total GSH, malondialdehyde (MDA), and Cd, compared with control. However, Cd significantly decreased the dry biomass of roots and shoots. Treatment with La + Cd evidently increased the activities of above enzymes except MDHAR, the content of reduced AsA, reduced GSH, total AsA and total GSH, and the dry biomass of roots and shoots, compared with Cd stress alone. Meanwhile, treatment with La + Cd remarkably decreased EL and the content of Cd and MDA compared with Cd stress alone. Our results suggested that La could be used as a regulator to improve the Cd tolerance of maize for its role in the alleviation of Cd-induced oxidative damage by regulating the metabolism of AsA and GSH.

Additional key words: ascorbate peroxidase, electrolyte leakage, glutathione reductase, maize, malondialdehyde.

Introduction

Cadmium is a heavy metal with high toxicity and exists widely in environment (Naïja *et al.* 2016). It is absorbed by plants and its accumulation induces the overproduction of the reactive oxygen species (ROS) which can exceed the capacity of ROS-scavenging system and result in oxidative damage to plants (Wu *et al.* 2015). To protect against the oxidative damage, plants have a defense system including antioxidant enzymes and non-enzymatic antioxidants (Daud *et al.* 2016).

Ascorbate (AsA) and glutathione (GSH) are two important non-enzymatic antioxidants. Their content in plants can be regulated by phytohormones such as jasmonic acid (Shan and Liang 2010). Lanthanum is an important rare earth element. In plants, La has many roles in regulating the growth and development, root organogenesis (Guo *et al.* 2012), seed germination (Hong *et al*. 2003), and secondary metabolism (Zhou *et al.* 2012). Further, La can improve the NaCl and Cd tolerance of plants by enhancing the activities of antioxidant enzymes (Xu *et al.* 2007, Wang *et al.* 2012,

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Abbreviations: APX - ascorbate peroxidase; AsA - ascorbate; DHAR - dehydroascorbate reductase; γ-ECS - γ-glutamylcysteine synthetase; GalLDH - L-galactono-1,4-lactone dehydrogenase; GR - glutathione reductase, GSH - glutathione, MDHAR - monodehydroascorbate reductase; ROS - reactive oxygen species.
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Shan and Zhao 2014). However, whether La regulates the regeneration and biosynthesis of AsA and GSH in plants under Cd stress remains unclear.

The aim of this study was to elucidate whether La regulates the metabolism of AsA and GSH in maize seedlings under Cd stress and to provide new knowledge for the possible application of La in agriculture to alleviate the effect of Cd on the growth of maize.

Materials and methods

Seeds of maize (*Zea mays* L.) cv. Xindan29 were germinated and grown in distilled water in artificial climate chamber under day/night temperatures of 25/15 °C, a relative humidity of 65 %, and a 10-h photoperiod with a photosynthetic active radiation of 500 μmol m^{-2} s^{-1}. When the first leaf was fully expanded, the seedlings were transferred into plastic boxes filled with half-strength Hoagland's nutrient solution and kept their roots in the dark. The Hoagland's solution was exchanged every two days. When the third leaf was fully expanded, the roots were placed in beakers containing 100 cm^3 of 80 μM $CdCl_2$ solution for 48 h. The $CdCl_2$ solution was prepared by adding $CdCl_2$ into Hoagland's solution. To select a suitable concentration of lanthanum, the effects of 0, 10, 30, 60, and 90 μM $LaCl_3$ on the content of reduced AsA, total AsA, reduced GSH and total GSH in leaves of maize seedlings were investigated. The $LaCl_3$ solution was prepared by adding $LaCl_3$ into Hoagland's solution. To study the effect of La on the metabolism of AsA and GSH under Cd stress, the seedlings were pretreated with 30 μM $LaCl_3$ (a suitable concentration selected from above five concentrations) for 12 h and then exposed to Cd stress for 48 h. Control seedlings were treated with Hoagland's solution alone. After treatment for 24 and 48 h, the third leaf of maize seedlings was collected to analyze the electrolyte leakage (EL), the activities of enzymes in the metabolism of AsA and GSH, and the content of reduced AsA, total AsA, reduced GSH, total GSH, and malondialdehyde (MDA).

Ascorbate peroxidase (APX; EC 1.11.1.11), glutathione reductase (GR, EC 1.6.4.2), dehydroascorbate reductase (DHAR, EC 1.8.5.1), and monodehydro-ascorbate reductase (MDHAR, EC 1.6.5.4) were extracted according to the method of Shan and Liang (2010). The activities of APX, GR, MDHAR, and DHAR were measured according to Nakano and Asada (1981), Grace and Logan (1996), Miyake and Asada (1992), and Dalton *et al*. (1986), respectively. One unit of APX activity was defined as the amount of APX catalyzing the oxidation of 1 μmol of ascorbate per minute. One unit of GR activity was defined as the amount of GR catalyzing the reduction of 1 μmol of NADPH per minute. One unit of MDHAR activity was defined as the amount of enzyme that oxidizes 1 μmol of NADH per minute. One unit of DHAR activity was defined as the amount of enzyme that produces 1 μmol of AsA per minute. L-galactono-1,4-lactone dehydrogenase (GalLDH, EC 1.3.2.3) was extracted and measured by the method of Tabata *et al.* (2001). One unit of GalLDH activity was defined as the amount of extract required to oxidize 1 nmol of L-Gal per minute. Gamma-glutamylcysteine synthetase (γ-ECS, EC 6.3.2.2) was extracted and measured by the method of Rüegsegger and Brunold (1992). One unit of γ-ECS activity was defined as 1 mmol of cysteine-dependently generated PO_4^{3-} per minute. The specific activities of above enzymes were expressed as units mg^{-1}(protein) and protein content was measured according to Bradford (1976).

Total ascorbate (AsA + dehydroascorbate, DHA) was measured according to Kampfenkel *et al.* (1995). Samples (0.5 g) were extracted by 10 cm^3 of 6 % (m/v) trichloroacetic acid. AsA content was measured by using a method based on the reduction of ferric ion to ferrous ion with AsA in acidic solution followed by formation of red chelate between ferrous ion and bathophenanthroline. The absorbance of the red chelate was recorded at 525 nm. Total ascorbate content was measured through a reduction of DHA to AsA by dithiothreitol. A standard curve prepared by using AsA and DHA was used in the calculation of the content of these metabolites.

Total glutathione (GSH + GSSG) was measured according to Griffith (1980). Samples (0.5 g) were extracted with 10 cm^3 of 5 % (m/v) sulfosalicylic acid. Total glutathione was determined by an enzymatic cycling assay method. GSSG was measured by removing GSH by 2-vinylpyridine derivatization. GSH content was then estimated from the difference between total glutathione and GSSG. A standard curve prepared by using GSH and GSSG was used in the calculation of the content of these metabolites. Malondialdehyde (MDA) content was measured by thiobarbituric acid (TBA) reaction as described by Hodges *et al.* (1999). Electrolyte leakage (EL) was determined according to Anjum *et al.* (2015).

After treatment for 7 d, the seedlings of each treatment were harvested. Then, these seedlings were divided into shoots and roots. Fresh masses of shoots and roots were recorded and then oven dried at 80 °C for 72 h, and dry masses were recorded. All dry samples of each treatment were ground and mixed thoroughly. To analyze the content of La and Cd in the roots and shoots, fine powder (0.5 g) was digested in a mixture of 7 cm^3 of concentrated HNO^3 + 1 cm^3 of concentrated $HClO_4$ at 170 °C by the method of Dai *et al.*(2013). Subsequently, the content of La and Cd in extracts were determined by flame atomic absorbance spectrometry (*Hitachi 180-80*,

Kyoto, Japan). Standard curves were prepared by using a series of diluted solutions of commercially available standards (*Shenzhen City Billiton Tylenol Technology*, Shenzhen, China).

The results presented are the means of six replications. Means were compared by one-way analysis of variance (*ANOVA*) and Duncan's multiple range test at the 5 % level of significance.

Results

Compared with Cd stress alone, only application of 30 μM $LaCl_3$ evidently enhanced the content of reduced AsA, total AsA, reduced GSH, and total GSH under Cd stress among different concentrations of $LaCl_3$ (Table 1). Compared with Cd stress alone, 30 μM $LaCl_3$ increased the content of reduced AsA, total AsA, reduced GSH and total GSH by 23.6, 20.5, 19.6, and 19.7 % under Cd stress, respectively. These results indicated that 30 μM $LaCl_3$ was a suitable concentration for this study.

Compared with control, the activities of APX, GR, DHAR, MDHAR, γ-ECS, and GalLDH and the content of reduced AsA, reduced GSH, total AsA and total GSH were all increased by Cd application (Tables 2 and 3). Compared with Cd stress alone, pre-treatment with La

Table 1. Effects of Cd and different La concentrations on the content of reduced AsA, total AsA, reduced GSH, and total GSH in leaves of maize seedlings. The plants were pre-treated with $LaCl_3$ for 12 h and then exposed to 80 μM $CdCl_2$ for 48 h. Means ± SEs, $n = 6$; different letters indicate significant differences at $P \leq 0.05$.

Treatments	Reduced AsA [μmol g^{-1}(f.m.)]	Total AsA [μmol g^{-1}(f.m.)]	Reduced GSH [μmol g^{-1}(f.m.)]	Total GSH [μmol g^{-1}(f.m.)]
Control	2.71 ± 0.31d	2.81 ± 0.25d	0.48 ± 0.04c	0.50 ± 0.06c
80 μM Cd	3.48 ± 0.37b	3.76 ± 0.33b	0.56 ± 0.06b	0.61 ± 0.06b
10 μM La + 80 μM Cd	3.70 ± 0.37b	3.93 ± 0.41b	0.59 ± 0.05b	0.65 ± 0.07b
30 μM La + 80 μM Cd	4.30 ± 0.40a	4.53 ± 0.36a	0.67 ± 0.06a	0.73 ± 0.08a
60 μM La + 80 μM Cd	3.79 ± 0.35b	4.04 ± 0.36b	0.60 ± 0.07b	0.66 ± 0.08b
90 μM La + 80 μMCd	3.17 ± 0.30c	3.40 ± 0.32c	0.56 ± 0.05b	0.63 ± 0.06b

Table 2. Effects of Cd and La on the specific activities of enzymes [U mg^{-1} (protein)] involved in the metabolism of AsA and GSH. The plants were pre-treated with 30 μM $LaCl_3$ for 12 h and then exposed to 80 μM $CdCl_2$ for 24 and 48 h. Means ± SEs, $n = 6$; different letters indicate significant differences at $P \leq 0.05$.

Treatments	Time [h]	APX	GR	DHAR	MDHAR	γ-ECS	GalLDH
Control	24	1.7 ± 0.17c	1.1 ± 0.10c	1.2 ± 0.14c	1.1 ± 0.12b	1.7 ± 0.14c	1.8 ± 0.16c
	48	1.8 ± 0.20c	1.0 ± 0.11c	1.0 ± 0.11c	1.3 ± 0.15b	1.5 ± 0.16c	2.0 ± 0.21c
80 μM Cd	24	2.2 ± 0.20b	1.5 ± 0.13b	1.6 ± 0.15b	1.5 ± 0.14a	2.6 ± 0.26b	2.5 ± 0.22b
	48	2.4 ± 0.25b	1.7 ± 0.16b	1.5 ± 0.17b	1.7 ± 0.19a	2.7 ± 0.24b	2.6 ± 0.26b
30 μM La + Cd	24	2.9 ± 0.33a	2.2 ± 0.19a	2.0 ± 0.19a	1.6 ± 0.15a	3.3 ± 0.29a	3.5 ± 0.37a
	48	2.8 ± 0.25a	2.2 ± 0.24a	2.1 ± 0.23a	1.6 ± 0.18a	3.5 ± 0.33a	3.3 ± 0.32a

Table 3. Effects of Cd and La on the content of reduced AsA, total AsA, reduced GSH, and total GSH [μmol g^{-1} (f.m.)] in maize leaves. The plants were pre-treated with 30 μM $LaCl_3$ for 12 h and then exposed to 80 μM $CdCl_2$ for 24 and 48 h. Means ± SEs, $n = 6$; different letters indicate significant differences at $P \leq 0.05$.

Treatments	Time [h]	Reduced AsA	Total AsA	Reduced GSH	Total GSH
Control	24	2.75 ± 0.30c	2.90 ± 0.27c	0.45 ± 0.04c	0.47 ± 0.05c
	48	2.97 ± 0.28c	3.10 ± 0.33c	0.42 ± 0.05c	0.44 ± 0.04c
80 μM Cd	24	3.53 ± 0.36b	3.80 ± 0.40b	0.55 ± 0.05b	0.61 ± 0.06b
	48	3.49 ± 0.31b	3.77 ± 0.35b	0.53 ± 0.06b	0.60 ± 0.07b
30 μM La + Cd	24	4.13 ± 0.38a	4.02 ± 0.39a	0.66 ± 0.08a	0.71 ± 0.08a
	48	4.40 ± 0.42a	4.31 ± 0.37a	0.68 ± 0.06a	0.73 ± 0.07a

significantly increased the activities of above enzymes except MDHAR and the content of above metabolites in stressed leaves. After treatment of 48 h, La + Cd increased the activities of APX, GR, DHAR, γ-ECS, and GalLDH by 16.7, 29.4, 40.0, 29.6, and 26.9 %, respectively. Meanwhile, La + Cd increased the content of reduced AsA, reduced GSH, total AsA, and total GSH by 26.1, 28.3, 14.3, and 21.7 %, respectively. These results indicated that La could regulate the metabolism of AsA and GSH in the leaves of maize seedlings under Cd stress.

Compared with control, Cd evidently increased MDA content and EL by 120.3 and 153.9 %, respectively. Compared with Cd stress alone, La + Cd decreased MDA content and EL by 30 and 29.6 %, respectively (Table 4). These results indicated that La had important role in the acquisition of the Cd tolerance of maize seedlings.

The application of Cd significantly increased Cd content in both roots and shoots. Compared with control, Cd stress increased Cd content in roots and shoots by 53.3- and 62-fold, respectively. Compared with Cd stress alone, La + Cd significantly decreased Cd content in roots and shoots by 36.5 and 28.6 %, respectively. These results indicated that La can decrease the accumulation of Cd in maize seedlings.

Table 4. Effects of Cd and La on MDA content and EL of maize leaves. The plants were pre-treated with 30 μM $LaCl_3$ for 12 h and then exposed to 80 μM $CdCl_2$ for 48 h. Means ± SEs, $n = 6$; different letters indicate significant differences at $P \leq 0.05$.

Treatments	MDA [nmol g^{-1}(f.m.)]	EL [%]
Control	5.9 ± 0.72c	10.2 ± 1.27c
80 μM Cd	13.0 ± 1.13a	25.9 ± 2.25a
30 μM La + Cd	9.1 ± 1.03b	18.2 ± 1.64b

To further investigate whether La can alleviate the Cd toxicity, the effect of La on the dry biomass of roots and shoots under Cd stress was studied. Compared with control, Cd decreased the dry mass of roots and shoots by 35.1 and 28.2 %, respectively (Table 5). Compared with Cd stress alone, La + Cd evidently increased the dry biomass of roots and shoots by 29.2 and 17.9 %, respectively. These results indicated that La can alleviate the Cd toxicity of maize seedlings.

Table 5. Effects of Cd and La application on the content of La and Cd, and the dry mass of roots and shoots of maize seedlings. The plants were pre-treated with 30 μM $LaCl_3$ for 12 h and then exposed to 80 μM $CdCl_2$ for 7 d. Means ± SEs, $n = 6$; different letters indicate significant differences at $P \leq 0.05$.

Treatments	La [mg·kg^{-1}(d.m.)]		Cd [mg·kg^{-1}(d.m.)]		DM [g·$plant^{-1}$)]	
	shoot	root	shoot	root	shoot	root
Control	0.01 ± 0.00b	0.05 ± 0.00b	0.1 ± 0.00c	0.3 ± 0.00c	1.56 ± 0.17a	0.37 ± 0.04a
80 μM Cd	0.02 ± 0.00b	0.08 ± 0.00b	6.3 ± 0.61a	16.4 ± 0.20a	1.12 ± 0.11c	0.24 ± 0.02c
30 μM La + Cd	42.50 ± 5.51a	401.90 ± 25.99a	4.0 ± 0.35b	11.7 ± 0.13b	1.32 ± 0.15b	0.31 ± 0.04b

Discussion

Increasing concentrations of Cd could induce oxidative damage in plants (Tamas *et al.* 2015). In our study, an enhanced lipid peroxidation indicated by MDA content and EL was observed in maize leaves under Cd stress, which suggested that Cd stress also induced oxidative stress to maize seedlings. Wu *et al.* (2015) documented that plants could protect themselves against Cd stress by increasing the activities of APX, GR, and DHAR, and the content of reduced GSH and reduced AsA. Guan *et al.* (2015) reported that Cd induced the expression of *GSH1* and *GSH2* genes, and the content of reduced GSH in tobacco. In the present study, our results showed that Cd stress could increase the activities of APX, GR, and DHAR, and the content of reduced AsA and reduced GSH in leaves of maize seedlings. Besides, we found that Cd stress enhanced the activities of MDHAR, GalLDH, and γ-ECS involved in AsA and GSH metabolism and the content of total AsA and total GSH in maize leaves.

It has been proved that low concentrations of La can relieve oxidative damage in plants exposed to Cd stress, but higher La concentrations may enhance oxidative damage in plants exposed to Cd stress (Wang *et al.* 2012). However, there is still very little knowledge about the regulation of the AsA and GSH metabolism by La under Cd stress. AsA has important role in stress defense (Arafa *et al.* 2009, Khafagy *et al.* 2009). The cellular content of AsA can be determined by GalLDH, DHAR, MDHAR, and APX activities. It has been documented that La increased the activity of APX in *Saussurea involucrata* under salt stress and in *Vicia faba* seedlings under Cd stress (Xu *et al.* 2007, Wang *et al.* 2012). Zhang *et al.* (2003) reported that La could enhance the activities of APX and DHAR in the leaves of wheat. In the present study, we found that La also increased the activities of APX and DHAR in maize seedlings under Cd stress. Besides, our study also showed that La increased the

activity of GalLDH, and the content of reduced AsA and total AsA under Cd stress. However, our results showed that La did not affect the MDHAR activity in maize seedlings under Cd stress, which was consistent with the results of Zhang *et al.* (2003). So, La could regulate the regeneration of AsA through APX and DHAR and the synthesis of AsA through GalLDH in maize seedlings under Cd stress.

GSH is another important compound of plant antioxidant system. The cellular content of GSH can be determined by γ-ECS and GR. It has been documented that La increased the activity of GR in *Saussurea involucrata* under salt stress (Xu *et al.* 2007). Our previous study showed that La increased the activity of γ-ECS and GR in *Vigna radiata* seedlings under salt stress (Shan and Zhao 2014). However, Zhang *et al*. (2003) reported that La did not affect the activity of GR in wheat. Above difference may be due to the difference in species. The results of our present study also showed that La could regulate the glutathione metabolism by increasing the activity of γ-ECS and GR, which was consistent with previous studies. Besides, our study also indicated that La increased the content of reduced GSH and total GSH under Cd stress.

It has been reported that up to 5 μM La had no effect on maize biomass but decreased mungbean biomass (Diatloff *et al.* 2008). In other study, La had also no effect on the growth of maize seedlings (Hu *et al*. 2006). However, Liu *et al*. (2013) reported that low concentrations of La could enhance the growth of rice seedlings, while high concentrations of La inhibited the growth of rice seedlings. Under Cd stress, Babula *et al*. (2015) reported that La decreased the biomass of *Hypericum perforatum*. However, our findings showed that 30 μM La could increase the dry biomass of maize seedlings under Cd stress. Further, La decreased the content of reduced AsA and increased the content of reduced GSH in *H. perforatum.* However, we found that La increased the content of reduced AsA and reduced GSH content in maize. In the ascorbate-glutathione cycle, the decrease in the content of reduced AsA induced by La inhibited the activity of this cycle in *H. perforatum*. In order to defend against oxidative stress, *H. perforatum* used reduced GSH as an alternative to scavenge ROS through glutathione peroxidase. So, reduced GSH could not be used to chelate Cd any longer in *H. perforatum.* La increased the content of reduced AsA, enhanced the activity of the ascorbate-glutathione cycle, and the ability to defend against oxidative stress induced by Cd stress. So, reduced GSH increased by La could be used to chelate Cd in maize seedlings. For above reason, La enhanced the growth of maize seedlings in this study but inhibited the growth of *H. perforatum* under Cd stress. This interpretation can be also proved by Cd content in *H. perforatum* and maize seedlings. For *H. perforatum*, La significantly increased Cd content in shoots under Cd stress. However, La significantly decreased Cd content in both roots and shoots of maize seedlings under Cd stress in our study. As the Cd toxicity to plants is due to its over-accumulation in tissues. So, in the present study, La increased the biomass of maize seedlings under Cd stress.

Our results clearly imply that La can improve the Cd tolerance of maize seedlings by regulating the metabolism of AsA and GSH, which provide new knowledge for the role of La in regulating the Cd tolerance of plants.

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