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Short communication

Lanthanum improves the antioxidant capacity in chloroplast of tomato seedlings through ascorbate-glutathione cycle under salt stress

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ABSTRACT

This paper investigated the effects of $LaCl_3$ on the ascorbate-glutathione (AsA-GSH) cycle in chloroplast of tomato seedlings under salt stress. Our findings displayed that salt stress significantly enhanced the activities of ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR), and the contents of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), but significantly decreased AsA/DHA and GSH/GSSG, the net photosynthetic rate (P_N), the contents of Chl and Car, plant height, stem diameter and dry weight, in contrast with the control. In contrast with salt stress alone, salt stress plus $LaCl_3$ significantly increased the activities of above enzymes, AsA/DHA, GSH/GSSG, P_N, Chl content and Car content, La accumulation, plant height, stem diameter and dry weight, and markedly cut down MDA content and H_2O_2 content in chloroplast. Meanwhile, pre-treatment with $LaCl_3$ alone also markedly improved above indicators except MDA content and H_2O_2 content in chloroplast, in contrast with the control. These findings indicated that $LaCl_3$ absorbed by plants up-regulated the antioxidant capacity in the chloroplast through AsA-GSH cycle, which improved the salt tolerance of tomato seedlings.

1. Introduction

Salt stress has serious effects on the growth and development of plants (Ryu and Cho, 2015). Many studies showed that salt stress enhanced the excessive accumulation of reactive oxygen species (ROS) (Guo et al., 2017), which, in turn, induced serious oxidative damage to plants. Chloroplast is an important organelle which is responsible for the photosynthesis of plants. However, Chloroplast is easily affected by oxidative damage induced by stresses. To fight against stresses-induced oxidative damage, chloroplast has an complex antioxidant defense system. In chloroplast, the ascorbate–glutathione (AsA-GSH) cycle is an important part of the antioxidant defense system. In this cycle, APX can remove hydrogen peroxide (H_2O_2). DHAR and MDHAR can realize the regeneration of reduced ascorbate (AsA). GR is responsible for the regeneration of reduced glutathione (GSH) (Asrar et al., 2014). Thus, the AsA-GSH cycle has important roles in defending oxidative damage and balancing the redox status of ascorbate and glutathione.

Rare earth elements (REEs) can regulate plant growth and development and the responses of plants to stresses (Ouyang et al., 2003). As a rare earth element, lanthanum (La) has important roles in regulating plant growth and development and metabolic processes (Guo et al., 2012; Huang et al., 2013). It has been reported that La could alleviate the stress-induced oxidative damage to plants, including salt stress (Xu et al., 2007). Whereas, there is still no report about La-induced responses of AsA-GSH cycle in the chloroplast of plants exposed to salt stress. Thus, it is interesting to clarify the role of La in regulating AsA-GSH cycle in the chloroplast under salt stress.

1 This study investigated the roles of lanthanum (III) chloride ($LaCl_3$) in regulating the activities of enzymes in AsA-GSH cycle, AsA/DHA, GSH/GSSG, MDA content and H_2O_2 content in chloroplast, and net photosynthetic rate (P_N), chlorophyll (Chl) content and carotenoids (Car) content in leaves of tomato seedlings exposed to salt stress. Meanwhile, the effects of $LaCl_3$ on plant height, stem diameter, plant dry weight and La content of seedlings under salt stress were investigated. The objective of our study was to clarify the roles of $LaCl_3$ in regulating AsA-GSH cycle in chloroplast of tomato seedlings exposed to salt stress, which, in turn, provide more knowledge for the function of $LaCl_3$ in enhancing salt tolerance and theoretical foundation for its use in the salt-resistant cultivation and production of tomato crops.

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2. Materials and methods

2.1. Plant growth conditions and treatments

Seeds of tomato (cultivar Fuyou NO.1) were germinated in petri dishes with filter paper moistened by distilled water (dH_2O) and grown in artificial climate chamber. The culture temperature is 25/15 °C (day/night). The light intensity is 600 μmol $m^{-2} s^{-1}$ photosynthetic active radiation. The photoperiod is 16 h. After the first leaves were fully expanded, seedlings were moved into boxes containing 500 ml 1/2 Hoagland's solution by keeping the roots in dark. The Hoagland's solution was replaced every day. After the third leaves were fully expanded, seedlings with similar plant height and stem diameter were chosen for the next experiment.

The suitable treatment concentration of NaCl (80 mM NaCl) was selected from 40, 80, 120 and 160 mM NaCl. After 48 h of treatment, the apparent wilting symptom was found in seedlings exposed to 120 and 160 mM NaCl. However, no apparent wilting symptom was found in seedlings exposed to 40 and 80 mM NaCl. Therefore, we selected 80 mM NaCl as the suitable treatment concentration. The roots of seedlings were placed in beakers containing 100 ml 80 mM NaCl for 48 h under above conditions. To choose an appropriate $LaCl_3$ concentration, the effects of 10, 30, 50 and 100 μM $LaCl_3$ on the contents of MDA and H_2O_2 were investigated. 30 μM $LaCl_3$ was selected as the appropriate concentration for next experiment. To study the effect of $LaCl_3$, seedlings were firstly treated by 30 μM $LaCl_3$ for 12 h and then treated by 80 mM NaCl or 1/2 Hoagland's solution for 48 h. Control seedlings were only treated by 1/2 Hoagland's solution or 30 μM $LaCl_3$. The whole experiment was repeated six times with four seedlings per treatment each time.

2.2. Assays of the activities of enzymes in AsA-GSH cycle in chloroplast

Intact chloroplasts were obtained from fresh tomato leaves by density-gradient centrifugation in Percoll gradients according to Wang et al. (2009). The intact chloroplast samples were diluted 10 folds with ice-cold 50 mM potassium phosphate buffer (pH 7.0). The activities of APX, MDHAR, GR and DHAR were done according to Nakano and Asada (1981); Miyake and Asada (1992); Grace and Logan (1996) and Dalton et al. (1986), respectively. One unit of APX, GR, DHAR and MDHAR was defined as the oxidation of 1 μmol AsA per minute, the reduction of 1 μmol NADPH per minute, the production of 1 μmol AsA per minute and the oxidation of 1 μmol NADH per minute, respectively. The specific activities of above enzymes were expressed as Units mg^{-1} protein. The content of protein was estimated according to Bradford (1976).

2.3. Analysis of AsA/DHA and GSH/GSSG

The intact chloroplast samples purified from 2 g leaves were homogenized with 5 ml 5% ice-cold *meta*-phosphoric acid. The homogenates were centrifuged at 4 °C and 12,000*g* for 20 min. The supernatants were then used for next assay. AsA and DHA were determined by the method of Hodges et al. (1996). The ratio between AsA content and DHA content was expressed as AsA/DHA. GSSG and GSH were determined by the method of Griffith (1980). The ratio between GSH content and GSSG content was expressed as GSH/GSSG.

2.4. Measurement of MDA and H_2O_2 contents

MDA content was determined by the method of Heath and Packer (1968). H_2O_2 content was measured by the method of Brennan and Frenkel (1977).

2.5. Measurement of Chl and Car contents

The top fresh fully expanded leaves were selected to measure the contents of Chl and Car according to Lichtenthaler and Wellburn (1983).

2.6. Measurement of net photosynthetic rate (P_N)

After 48 h of treatment, P_N was measured by using the photosynthesis measuring system (Licor-600, USA) at 10:00 AM to 12:00 AM

2.7. Measurement of plant growth parameters

After 7 d of treatment, plant height was measured by a ruler, stem diameter was measured by a vernier caliper. Fresh weights of the seedlings of each treatment were recorded and then oven dried for 72 h at 80 °C. Dry weights of the seedlings of each treatment were then recorded.

2.8. Analysis of La content

After 7 d of treatment, all dry samples of whole seedlings in each treatment were ground and mixed thoroughly. To analyze La content, fine powder (0.5 g) was digested in a mixture (7 ml concentrated HNO_3 and 1 ml concentrated $HClO_4$) at 170 °C by the method of Dai et al. (2017). Subsequently, the content of La in extracts were determined by flame atomic absorbance spectrometry (Hitachi 180-80, Japan). Standard curves were prepared by using a series of diluted solutions of commercially available standards.

2.9. Statistical analysis

The data presented were the means of six times with four seedlings per treatment each time. Means were compared by one-way analysis of variance (ANOVA) and Duncan's multiple range test at the 5% level of significance.

3. Results

3.1. Effects of different $LaCl_3$ concentrations on MDA content and H_2O_2 content in chloroplast under salt stress

In contrast with salt stress alone, pre-treatments with 10, 30 and 50 μM $LaCl_3$ all markedly reduced the contents of MDA and H_2O_2 in chloroplast under salt stress (Table 1). However, pre-treatment with 100 μM $LaCl_3$ had no obvious effects on the contents of MDA and H_2O_2

Table 1
Effects of different concentrations of La on the contents of MDA and H_2O_2. The plants were treated as follows: Control, 1/2 Hoagland's solution; NaCl, 80 mM NaCl; 10 μM La, 10 μM $LaCl_3$; 30 μM La, 30 μM $LaCl_3$; 50 μM La, 50 μM $LaCl_3$; 100 μM La, 100 μM $LaCl_3$; 10 μM La + NaCl, 10 μM $LaCl_3$ + 80 mM NaCl; 30 μM La + NaCl, 30 μM $LaCl_3$ + 80 mM NaCl; 50 μM La + NaCl, 50 μM $LaCl_3$ + 80 mM NaCl; 100 μM La + NaCl, 100 μM $LaCl_3$ + 80 mM NaCl. The plants were pre-treated with $LaCl_3$ for 12 h, and then exposed to salt stress for 48 h.

Treatment	MDA	H_2O_2
Control	4.5 ± 0.51d	0.50 ± 0.08d
NaCl	14.3 ± 1.6a	2.01 ± 0.31a
10 μM La	4.2 ± 0.39d	0.45 ± 0.06d
30 μM La	4.4 ± 0.48d	0.48 ± 0.05d
50 μM La	4.5 ± 0.42d	0.52 ± 0.09d
100 μM La	4.8 ± 0.55d	0.55 ± 0.07d
10 μM La + NaCl	11.5 ± 1.1b	1.63 ± 0.15b
30 μM La + NaCl	9.0 ± 0.9c	1.20 ± 0.14c
50 μM La + NaCl	12.1 ± 1.4b	1.56 ± 0.14b
100 μM La + NaCl	15.0 ± 1.3a	1.81 ± 0.19a