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BRIEF COMMUNICATION

Lanthanum improves salt tolerance of maize seedlings

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Abstract

In this study, the effects of lanthanum were investigated on contents of pigments, chlorophyll (Chl) fluorescence, antioxidative enzymes, and biomass of maize seedlings under salt stress. The results showed that salt stress significantly decreased the contents of Chl and carotenoids, maximum photochemical efficiency of PSII (F_v/F_m), photochemical quenching (q_P), and quantum efficiency of PSII photochemistry (Φ_{PSII}), net photosynthetic rate (P_N), and biomass. Salt stress increased nonphotochemical quenching (q_N), the activities of ascorbate peroxidase, catalase, superoxide dismutase, glutathione peroxidase, and the contents of malondialdehyde and hydrogen peroxide compared with control. Pretreatment with lanthanum prior to salt stress significantly enhanced the contents of Chl and carotenoids, F_v/F_m, q_P, q_N, Φ_{PSII}, P_N, biomass, and activities of the above antioxidant enzymes compared with the salt-stressed plants. Pretreatment with lanthanum also significantly reduced the contents of malondialdehyde and hydrogen peroxide induced by salt stress. Our results suggested that lanthanum can improve salt tolerance of maize seedlings by enhancing the function of photosynthetic apparatus and antioxidant capacity.

Additional key words: antioxidant enzyme; lanthanum chloride; photosynthesis; salinity; *Zea mays*.

Salt stress adversely affects the growth and survival of plants (Ferreira-Silva *et al.* 2012). Particularly, PSII is vulnerable to salt stress, which results in the reduction in photosynthesis and plant production (Mehta *et al.* 2010). This is mainly because salt stress induces the over-production of reactive oxygen species (ROS), which induces oxidative damage to plants (Rubio *et al.* 2009). Fortunately, plants can protect themselves against oxidative damage through the antioxidant defense system, including superoxide dismutase (SOD) and ascorbate peroxidase (APX), *etc*.

Lanthanum (La) is an important rare Earth's element. In plants, it has been documented that La can promote root organogenesis (Guo *et al.* 2012), mediate secondary metabolite synthesis (Zhou *et al.* 2012), promote nitrogen metabolism (Huang *et al.* 2013), *etc*. Increasing evidence has demonstrated that La can improve salt tolerance of plants by enhancing antioxidant metabolism (Xu *et al.* 2007). In addition, the effects of salt stress on P_N, Chl fluorescence, and the contents of photosynthetic pigments have been well documented in many crops (Zheng *et al.* 2009, Sarkar *et al.* 2013). However, little is known how La influences the above mentioned parameters. Thus, the aim of this study was to investigate the effects of La on the contents of Chl and Car, Chl fluorescence, antioxidant metabolism, and biomass of maize seedlings under salt stress, and provide information for its application in order to promote salt tolerance of maize.

Seeds of maize (*Zea mays* L., cv. Xindan 29) were germinated in Petri dishes with filter paper moistened by distilled water and grown in an artificial climate chamber under a day/night temperature of 25/15°C,

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Abbreviations: APX – ascorbate peroxidase; Car – carotenoids; CAT – catalase; Chl – chlorophyll; F_v/F_m – maximum photochemical efficiency of PSII; GPX – glutathione peroxidase; JA – jasmonic acid; MDA – malondialdehyde; P_N – net photosynthetic rate; Φ_{PSII} – effective quantum yield of PSII; q_N – nonphotochemical quenching; q_P – photochemical quenching; ROS – reactive oxygen species; SOD – superoxide dismutase.
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Table 1. The effects of different concentrations of $LaCl_3$ on the contents of MDA, Chl, and biomass under salt stress. The plants were treated as follows: CK – half-strength Hoagland's solution; CK + 10 μM La – half-strength Hoagland's solution + 10 μM $LaCl_3$; CK + 30 μM La – half-strength Hoagland's solution + 30 μM $LaCl_3$; CK + 60 μM La – half-strength Hoagland's solution + 60 μM $LaCl_3$; CK + 90 μM La – half-strength Hoagland's solution + 90 μM $LaCl_3$; NaCl – 100 mM NaCl; NaCl + 10 μM La – 100 mM NaCl + 10 μM $LaCl_3$; NaCl + 30 μM La – 100 mM NaCl + 30 μM $LaCl_3$; NaCl + 60 μM La – 100 mM NaCl + 60 μM $LaCl_3$; NaCl + 90 μM La – 100 mM NaCl + 90 μM $LaCl_3$. The plants were pretreated with $LaCl_3$ for 24 h, and then exposed to half-strength Hoagland's solution or salt stress for 6 d. Values represent mean ± SE, *different letters* indicate statistical difference at $P<0.05$ in the same column. Chl – chlorophyll; DM – dry mass; FM – fresh mass; La – lanthanum; MDA – malondialdehyde.

Treatment	MDA [nmol g^{-1}(FM)]	Chl [mg g^{-1}(FM)]	Biomass [g(DM) per plant]
CK	5.5 ± 0.39^{d}	1.83 ± 0.15^{b}	1.92 ± 0.17^{b}
CK + 10 μM La	5.3 ± 0.41^{d}	1.91 ± 0.18^{b}	1.96 ± 0.17^{b}
CK + 30 μM La	4.5 ± 0.38^{e}	2.15 ± 0.21^{a}	2.17 ± 0.20^{a}
CK + 60 μM La	5.4 ± 0.46^{d}	1.85 ± 0.19^{b}	2.00 ± 0.18^{b}
CK + 90 μM La	5.9 ± 0.48^{d}	1.77 ± 0.16^{b}	1.88 ± 0.19^{b}
NaCl	19.3 ± 1.75^{a}	1.16 ± 0.12^{d}	1.40 ± 0.12^{d}
NaCl + 10 μM La	17.8 ± 1.53^{b}	1.25 ± 0.13^{d}	1.46 ± 0.11^{d}
NaCl + 30 μM La	13.9 ± 1.50^{c}	1.44 ± 0.14^{c}	1.70 ± 0.15^{c}
NaCl + 60 μM La	15.8 ± 1.26^{c}	1.20 ± 0.12^{d}	1.52 ± 0.13^{d}
NaCl + 90 μM La	20.9 ± 1.98^{a}	1.03 ± 0.10^{e}	1.33 ± 0.13^{e}

500 μmol(photon) m^{-2} s^{-1} PAR, and a 10-h photoperiod. When the first leaf was fully expanded, the seedlings were transferred into plastic boxes filled with half-strength Hoagland's solution. The roots of seedlings were kept in the half-strength Hoagland's solution and plastic boxes were wrapped by black plastic cloth. The pH of Hoagland's solution was maintained close to 6.5 every day. The half-strength Hoagland's solution was changed every two days. When the third leaf was fully expanded, the seedlings of uniform height and growth were selected for experiments. The roots of plants were placed in beakers containing 100 mL of 100 mM NaCl solution and wrapped with aluminium foil for 6 d under above conditions. The NaCl solution was prepared by adding NaCl into half-strength Hoagland's solution. In order to study the effect of La, a group of plants was pretreated with 30 μM $LaCl_3$ for 24 h and then exposed to half-strength Hoagland's solution (CK+La) or salt stress (NaCl+La) for 6 d. The $LaCl_3$ solution was prepared by adding $LaCl_3$ into half-strength Hoagland's solution; 30 μM $LaCl_3$ used in this study was selected from different concentrations (10, 30, 60, and 90 μM) by preliminary experiments (Table 1). Control plants were treated with half-strength Hoagland's solution alone (CK).

After 3 and 6 d of treatment, the top fully expanded leaves were collected and immediately used to measure the contents of Chl and Car according to the method of Lichtenthaler and Wellburn (1983). A *Yaxin-1161G* fluorometer (*Yaxin*, China) was used to measure Chl fluorescence parameters from 10:00 to 12:00 h after 3 and 6 d of treatment. For dark adaptation, leaves were covered for 30 min. Then F_v/F_m, q_P, q_N, and Φ_{PSII} were measured by the fluorometer and calculated according to Hanachi *et al.* (2014).

For the assays of antioxidative enzymes, the top fully expanded leaves after 3 and 6 d of treatment were collected and frozen in liquid nitrogen, and then kept at –80°C. SOD (EC 1.15.1.1) activity was assayed according to Giannopolitis and Ries (1977) by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. One unit of SOD was defined as a 50% inhibition of the reduction of NBT. Glutathione peroxidase (GPX, EC 1.11.1.9) activity was measured according to He *et al.* (2006) at 470 nm. One unit of GPX activity was defined as 1 μmol(GSH) min^{-1}. APX (EC 1.11.1.11) activity was measured according to Nakano and Asada (1981) by monitoring the decrease in absorbance at 290 nm for 1 min. One unit of APX activity was defined as the oxidation of 1 μmol(ascorbate) min^{-1}. Catalase (CAT, EC 1.11.1.6) activity was measured according to the decomposition of H_2O_2 at 240 nm for 1 min. All the enzyme activities were calculated per protein content. The specific activity for above enzymes was expressed as U mg^{-1}(protein). Protein concentration was measured according to Bradford (1976). Malondialdehyde (MDA) content was measured according to Hodges *et al.* (1996). Hydrogen peroxide content was estimated by the method of Prochazkova *et al.* (2001). P_N was determined by photosynthesis measuring system (*Licor-600*, USA) from 10:00 to 12:00 h after 3 and 6 d of treatment. For the measurement of biomass, the whole plants were dried in an air oven at 80°C until a constant mass to obtain dry mass (DM).

Statistical analysis of the data were performed by using the statistical program *SPSS 13.0* (*SPSS*, Chicago, USA). The results presented were the means of five replications and the standard errors (SE). Means were compared by one-way analysis of variance and *Duncan*'s multiple range test at the 5% level of significance.

Compared with CK, pretreatment with 30 μM $LaCl_3$ alone significantly decreased the MDA content and increased biomass and Chl content at different $LaCl_3$

Table 2. The effects of $LaCl_3$ on the contents of Chl, Car, MDA, and H_2O_2, the values of F_v/F_m, q_P, q_N, Φ_{PSII}, P_N, biomass, and the activities of APX, CAT, SOD, and GPX in leaves under salt stress. The plants were treated as follows: CK – half-strength Hoagland's solution; CK + La – half-strength Hoagland's solution + 30 μM $LaCl_3$; NaCl – 100 mM NaCl; NaCl + La – 100 mM NaCl + 30 μM $LaCl_3$. The plants were pretreated with $LaCl_3$ for 24 h, and then exposed to half-strength Hoagland's solution or salt stress for 6 d. Values represent mean ± SE, *different letters* indicate statistical difference at $P<0.05$ in the same row. APX – ascorbate peroxidase; Car –carotenoids; CAT – catalase; Chl – chlorophyll; DM – dry mass; FM – fresh mass; F_v/F_m – maximum photochemical efficiency of PSII; GPX – glutathione peroxidase; H_2O_2 – hydrogen peroxide; La – lanthanum; MDA – malondialdehyde; P_N – net photosynthetic rate; Φ_{PSII} – effective quantum yield of PSII; q_N – nonphotochemical quenching; q_P – photochemical quenching; SOD – superoxide dismutase.

Parameter	Time [d]	CK	CK + La	NaCl	NaCl + La
Car [mg g^{-1}(FM)]	3	0.71 ± 0.08^b	0.85 ± 0.07^a	0.42 ± 0.04^d	0.56 ± 0.06^c
	6	0.63 ± 0.06^b	0.73 ± 0.08^a	0.31 ± 0.02^d	0.45 ± 0.04^c
Chl [mg g^{-1}(FM)]	3	1.94 ± 0.19^b	2.25 ± 0.21^a	1.30 ± 0.16^d	1.69 ± 0.16^c
	6	1.90 ± 0.16^b	2.11 ± 0.18^a	1.15 ± 0.10^d	1.59 ± 0.13^c
F_v/F_m	3	0.74 ± 0.08^b	0.81 ± 0.09^a	0.61 ± 0.06^d	0.67 ± 0.08^c
	6	0.82 ± 0.07^a	0.82 ± 0.08^a	0.60 ± 0.05^c	0.70 ± 0.10^b
q_P	3	0.50 ± 0.06^b	0.58 ± 0.06^a	0.28 ± 0.06^d	0.41 ± 0.06^c
	6	0.48 ± 0.05^b	0.55 ± 0.06^a	0.24 ± 0.02^d	0.36 ± 0.04^c
q_N	3	0.30 ± 0.02^c	0.32 ± 0.03^c	0.40 ± 0.04^b	0.49 ± 0.05^a
	6	0.33 ± 0.03^c	0.36 ± 0.04^c	0.45 ± 0.05^b	0.55 ± 0.05^a
Φ_{PSII}	3	0.37 ± 0.03^b	0.47 ± 0.04^a	0.17 ± 0.02^d	0.27 ± 0.03^c
	6	0.39 ± 0.03^b	0.45 ± 0.05^a	0.14 ± 0.02^d	0.25 ± 0.03^c
P_N [μmol(CO_2) m^{-2} s^{-1}]	3	7.14 ± 0.65^b	8.72 ± 0.81^a	4.39 ± 0.37^d	5.67 ± 0.48^c
	6	6.55 ± 0.61^b	7.93 ± 0.73^a	3.25 ± 0.33^d	4.94 ± 0.44^c
Biomass [g(DM) per plant]	6	2.00 ± 0.19^b	2.27 ± 0.21^a	1.45 ± 0.13^d	1.76 ± 0.18^c
APX [U mg^{-1}(protein)]	3	1.11 ± 0.12^d	1.74 ± 0.15^c	2.22 ± 0.20^b	3.26 ± 0.31^a
	6	1.26 ± 0.11^d	1.59 ± 0.13^c	2.51 ± 0.28^b	3.14 ± 0.26^a
SOD [U mg^{-1}(protein)]	3	1.23 ± 0.11^d	1.55 ± 0.12^c	1.93 ± 0.19^b	2.72 ± 0.25^a
	6	1.05 ± 0.09^d	1.38 ± 0.14^c	1.96 ± 0.21^b	2.55 ± 0.22^a
CAT [U mg^{-1}(protein)]	3	0.92 ± 0.10^d	1.20 ± 0.13^c	1.44 ± 0.13^b	2.10 ± 0.22^a
	6	0.87 ± 0.10^d	1.09 ± 0.11^c	1.67 ± 0.17^b	2.13 ± 0.20^a
GPX [U mg^{-1}(protein)]	3	0.15 ± 0.02^d	0.23 ± 0.02^c	0.22 ± 0.02^b	0.30 ± 0.03^a
	6	0.13 ± 0.02^d	0.18 ± 0.02^c	0.25 ± 0.03^b	0.35 ± 0.03^a
MDA [nmol g^{-1}(FM)]	3	5.12 ± 0.61^c	4.92 ± 0.43^c	15.63 ± 1.44^a	10.51 ± 1.10^b
	6	6.05 ± 0.55^c	5.00 ± 0.47^d	17.72 ± 1.59^a	11.74 ± 1.26^b
H_2O_2 [μmol g^{-1}(FM)]	3	0.52 ± 0.06^c	0.45 ± 0.05^c	2.35 ± 0.28^a	1.37 ± 0.17^b
	6	0.61 ± 0.07^c	0.40 ± 0.04^d	2.52 ± 0.24^a	1.57 ± 0.15^b

concentrations (Table 1). Compared with salt stress alone, application of 30 μM $LaCl_3$ significantly decreased the MDA content and increased biomass and the Chl content under salt stress. These results suggested that 30 μM $LaCl_3$ was a suitable concentration to study the effect of La on salt tolerance of maize seedlings.

Many studies have demonstrated that the function of photosynthetic apparatus is sensitive to salt stress, as indicated by Chl fluorescence parameters (Aremu *et al.* 2014). Our results showed that salt stress significantly decreased F_v/F_m, q_P, and Φ_{PSII} (Table 2). This indicated that salt stress also reduced the PSII activity and PSII reaction centres were damaged, which had been proven by other study (Hanachi *et al.* 2014). However, we found that salt stress increased q_N (Table 2), which indicated that maize seedlings could protect the photosynthetic apparatus against salt stress by enhancing thermal dissipation (Aremu *et al.* 2014). Zheng *et al*. (2009) reported that salt stress decreased F_v/F_m, q_P, and Φ_{PSII}, and increased q_N in the leaves of wheat, which was consistent with our results in maize. Compared with salt stress alone, pretreatment with $LaCl_3$ followed by salt stress significantly increased the values of F_v/F_m, q_P, q_N, and Φ_{PSII} (Table 2). These results suggested that $LaCl_3$ could alleviate the negative effects of salt stress on the function of photosynthetic apparatus of maize seedlings by increasing the PSII activity and enhancing thermal dissipation.

Salt stress significantly reduced the contents of Chl and Car, which, in turn, decreased P_N and biomass (Table 2). Compared with salt stress alone, pretreatment with $LaCl_3$ followed by salt stress significantly increased the contents of Chl and Car, which, in turn, increased P_N and biomass (Table 2). Increasing evidence showed that La could significantly increase the contents of Chl and Car in plants, which was consistent with our results (Hong *et al.* 2001, Xu *et al*. 2007). Hong *et al.* (2001) reported that La participated in the biosynthesis of Chl by improving the assimilation of Mg. Jiang *et al.* (2008) suggested that La was an activator of enzymes involved

in the synthesis of Chl and indirectly improved the synthesis of Chl. Besides, Xu *et al.* (2007) reported that La could protect Chl from damage induced by salt stress. Therefore, we proved that La can increase the content of Chl by improving its biosynthesis and reducing its damage under salt stress.

Many studies reported that the damage of photosynthetic apparatus was mainly because of oxidative stress induced by various stresses (Zhang *et al.* 2010). In our study, salt stress enhanced the production of MDA and H_2O_2, which suggested that the reduction in the function of photosynthetic apparatus occurred also mainly due to the oxidative damage induced by salt stress (Table 2). In addition, salt stress also significantly increased the activities of APX, CAT, SOD, and GPX, which indicated that seedlings could protect themselves against oxidative damage by enhancing the activities of antioxidant enzymes (Table 2). Pretreatment with $LaCl_3$ followed by salt stress significantly increased the activities of the above enzymes and decreased the contents of MDA and H_2O_2 (Table 2). Combined with the results of pigments, Chl fluorescence, P_N, and biomass, above findings indicated that La could improve the function of photosynthetic apparatus by increasing the antioxidant capacity in maize leaves under salt stress. Xu *et al.* (2007) also reported that La could alleviate salinity-induced oxidative stress, which was consistent with our results. However, they did not investigate the effects of La on Chl fluorescence and P_N. Thus, the results of our study provide new insight into the role of La in improving salt tolerance of crops.

In the present study, the pretreatment with $LaCl_3$ showed the same effects both in the control or salt-treated plants as compared with nontreated controls (Table 2). This indicated that above mentioned effects had no correlation with salt stress. This point has been also proven by other studies (Zhang *et al.* 2003, Xu *et al.* 2007).

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