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BRIEF COMMUNICATION

Lanthanum relieves salinity-induced oxidative stress in *Saussurea involucrata*

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Abstract

Changes in growth, physiological and biochemical characteristics under salt stress with or without La^{3+} treatment in *Saussurea involucrata* Kar. *et* Kir. were investigated. The results showed that La^{3+} relieved the plant growth inhibition, improved the leaf water potential and water content, increased the soluble protein and the proline contents and decreased malondialdehyde content under salt stress. Further, addition of La^{3+} significantly increased the activities of superoxide dismutase, ascorbate peroxidase, catalase, and glutathione reductase, decreased the photosynthetic pigment decomposition and increased the ratio of total chlorophyll to carotenoids under salt stress.

Additional key words: antioxidative enzymes, carotenoids, chlorophyll, growth, leaf water potential, proline, proteins.

Saussurea involucrata Kar. *et* Kir. is an endangered medicinal plant growing in the Tianshan and Kunlun mountain ranges of China, and is very sensitive to salinity stress. High salinity causes both water stress and ionic toxicity, which disputes the various enzymes, function of photosynthetic apparatus and many other processes and severely affects yield and quality of this medicinal plant (Chen *et al.* 1999). It is well known that rare-earth elements form a special group and have interesting effects on plant growth (He and Loh 2000, Ouyang *et al.* 2003). Lanthanum is a rare-earth element and can relieve oxidative damage in plants exposed to a variety of environmental stresses (Gao and Chen 2005, Shi and Chen 2005, Wei *et al.* 1997). Feng *et al.* (1999) reported that La^{3+} could alleviate the decrease of osmotic potential, increase the content of proline and reduce the accumulation of MDA under osmotic stress in the leaves of maize seedlings. There is salt stress affecting the growth of *S. involucrata* in saline area. Up to now, there is few reports on the role of La^{3+} in alleviating the oxidative damage in this plant under salt stress. So, study on this may improve the endangered plant growth and production. In the present study, the effect of La^{3+} on oxidative damage in *S. involucrata* under salt stress was investigated. The investigation also goes in some way towards studying the effects of La^{3+} on plant growth, soluble protein content and photosynthetic pigments in this species in response to salinity stress.

Seeds of *Saussurea involucrata* Kar. *et* Kir. were surface-disinfested and aseptically planted into *Vermiculite*, then they were irrigated with liquid NaCl solution at different concentrations (0, 20, 50 and 100 mM) on 0, 5th, 10th and 15th day. The 15-d-old germinated seedlings were divided into two groups. Previous reports indicated that 0.1 mM La^{3+} was more suitable concentration for alleviating environment stress (Pang *et al.* 2002, Zhang *et al.* 2003). So, in this experiment, treatment group seedlings under varying salinity stress were sprayed with 100 cm^3 0.1 mM $La(NO_3)_3 . 6\ H_2O$ solution once a day for five consecutive days. The control group seedlings under varying salinity stress were sprayed with the same volume water. The plants were cultivated under natural light in a greenhouse at 25 ºC. At the end of the treatments, plants were harvested, rinsed three times in sterile distilled water, and divided into shoots and roots for further analyse and determination.

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Abbreviations: APOX - ascorbate peroxidase; CAT - catalase; d.m. - dry mass; f.m. - fresh mass; EDTA - ethylenediaminetetraacetic acid; GR - glutathione reductase; MDA - malondialdehyde; NADP - nicotinamide adenine dinucleotide phosphate; ROS - reactive oxygen species; SOD - superoxide dismutase; WC - water content, ψ_w - leaf water potential.
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Length and fresh mass (f.m.) of the shoots and roots were recorded. Then fresh materials were incubated in an oven at 60 ºC for 48 h and dry mass (d.m.) was recorded. Water content (WC) was calculated as:

$WC = (f.m. - d.m.)/f.m. \times 100.$

Five leaves from each treatment were sampled to measure leaf water potential (ψ_w) using plant pressure chamber (*PMS Instrument Company, Model 1000*, Albany, USA) method (Gong *et al.* 2003).

Fresh shoots of seedlings were used for biochemical analyses. Soluble proteins were estimated by the method of Bradford (1976) using bovine serum albumin as a standard. The contents of photosynthetic pigments were determined according to Lichtenthaler and Wellburn (1983) in 80 % acetone extract. Proline was estimated according to Bates *et al.* (1973). Lipid peroxidation was determined by measuring malondialdehyde (MDA) formation using the thiobarbituric acid method of Ohkawa *et al.* (1979).

For enzyme assay, fresh shoots (0.2 g) were homogenized in 10 cm^3 chilled extraction buffer consisting of 0.1 M phosphate buffer, pH 7.5, 0.5 mM EDTA for superoxide dismutase (SOD; EC 1.15.1.1) and catalase (CAT; EC 1.11.1.6), or 0.1 M phosphate buffer, pH 7.5, 0.5 mM EDTA, 1 mM ascorbic acid in case of ascorbate peroxidase (APOX; EC 1.11.1.11). The homogenate was centrifuged at 15 000 *g* for 20 min and the supernatant fraction was used for the assays. All operations were carried out at 4 ºC. All spectrophotometric analyses were preformed with a *SP-2000* UV spectrophotometer (*Shanghai Precision and Scientific Instrument Co.*, Shanghai, China) at room temperature. SOD activity was estimated at 560 nm according to the method of Beauchamp and Fridovich (1971). APOX activity was determined by measuring the decrease in absorbance of the oxidized ascorbate at 290 nm, according to Nakano and Asada (1981). CAT was determined by measuring the decrease in absorbance of hydrogen peroxide at 240 nm (Aebi 1984). Glutathione reductase (GR; EC 1.6.4.2) activity was determined based on the decrease in absorbance at 340 nm due to the oxidation of NADPH to NADP (Fryer *et al.* 1998).

All the experiments were repeated three times. In each treatment, at least five replicates were used. Prior to statistical analyses, the percentage data were transformed into arc sine square roots. The statistical analysis was performed using the *PROC ANOVA* of *SAS* version *6.12*.

Salt treatment significantly retarded the growth of seedlings. The length of shoots and roots was remarkably reduced under different salt concentrations. In the case of 100 mM NaCl, the length of shoots and roots was 23.3 and 23.1 % of the lengths without NaCl treatment respectively. Lanthanum application considerably increased the length of shoots and roots under salt stress (Table 1). The mean f.m. and d.m. of a single shoot and root decreased under different salt treatment. La^{3+} application markedly alleviated the plant growth inhibition under salt stress (Table 1). The effects of salinity on ψ_w varied with the salt concentration to which seedlings exposed. Salt treatment, when the salt concentrations were in the range from 0 to 100 mM, decreased the ψ_w significantly (from -0.98 to -1.53 MPa), whereas La^{3+} application alleviated this trend (Table 1). Without La^{3+} treatment, the leaf WC was considerably reduced from 83.6 % at 0 mM NaCl treatment to 74.6 % at 100 mM NaCl treatment. La^{3+} application alleviated the effects of salt on water content, which reduced from 87.0 to 81.3 % (Table 1). Similar results were observed in the study of lanthanum effects on plantlet differentiation and growth of *Prunus pseudocerasus* Lindl (Li *et al.* 2005).

Soluble protein content declined significantly under salt stress. However, the decline in La^{3+} treated group was lower than that in non-treated group. Under salt stress, proline content significantly increased in many plants (Agarwal and Pandey 2004). The increase of proline with increasing NaCl concentration indicated that the seedlings had self-protective function from the damage by salt stress. At the same NaCl concentration, the addition of La^{3+} promoted proline formation, which indicated that La^{3+} can decrease the damage of salt stress. Lipid peroxidation often acted as an indicator of oxidative damage under salt stress and was determined by measuring MDA content, which was related to lipid membrane damage (Bandeoğlu *et al.* 2004). Compared to the control, La^{3+} application alleviated the increasing trend of MDA content (Table 1).

In reactive oxygen species (ROS) scavenging system, the primary scavenger is SOD. SOD activities increased under different NaCl concentrations, but at the same NaCl concentration, La^{3+} application significantly increased the SOD activities, especially at 50 mM NaCl. In the ascorbate-glutathione cycle, APOX reduces H_2O_2 using ascorbate as an electron donor. APOX activities increased significantly with increasing NaCl concentration. The results of the present study were in conformity with those obtained by Sairam *et al.* (2006). Compared with non-lanthanum treatment, APOX activities increased 60.9 and 52.7 % at 50 mM and 100 mM NaCl with La^{3+} treatment, respectively. CAT is another enzyme which is also involved in the detoxification of H_2O_2 by converting H_2O_2 into water and oxygen (Mandhania *et al.* 2006). CAT activities increased under salt stress. Moreover, there was significant difference between with and without La^{3+} application. Compared with non-lanthanum treatment group, GR activities increased 57.9 and 51.6 % at 50 mM and 100 mM NaCl with La^{3+} treatment, respectively (Table 1). Similar results were obtained in different plants such as cucumber (Zhu *et al.* 2004), pea (Hernández *et al.* 2000) and cotton (Gossett *et al.* 1994). So, addition of La^{3+} promoted the activities of the four enzymes, especially at 50 and 100 mM NaCl, increase the antioxidant defence abilities, and alleviate the oxidative damage of proteins and lipids of *S. involucrata* under salt stress.

Salinity greatly decreased chlorophyll content (Table 1) similarly as reported *e.g.* Gadallah and Ramadan (1997). Also the ratio of total chlorophyll to carotenoid content was negatively correlated with NaCl concentration. The

ratio was 7.30 and 7.10 in with and without La^{3+} group at 0 mM NaCl, however, the reduction of the ratio were 11.1 and 22.5 % at 100 mM NaCl concentration, respectively (Table 1). The change may be due to the formation of proteolytic enzymes such as chlorophyllase which is responsible for chlorophyll degradation (Sabater and Rodriguez 1978). Compared to the non-lanthanum treatment, La^{3+} application led to higher ratio of total chlorophyll to carotenoid content and decreased the photosynthetic pigment decomposition.

It can be drawn a conclusion from above results that lanthanum application promoted the growth of *S. involucrata*, alleviated the oxidative damage and protected the photosynthetic pigments from damage under salt stress.

Table 1. Effects of La^{3+} treatment on growth characteristics, water potential, water content, protein content, proline content, antioxidant enzyme activities and pigment content of *Saussurea involucrata* under different NaCl concentrations. Means ± SE of 5 replicates.

Parameters	La^{3+} [mM]	NaCl [mM] 0	20	50	100
Shoot length	0	11.42 ± 0.55	6.31 ± 0.35	4.77 ± 0.77	2.66 ± 0.51
[cm]	0.1	14.65 ± 0.42	8.11 ± 0.39	7.26 ± 0.31	5.71 ± 0.17
Root length	0	9.36 ± 0.24	6.15 ± 0.32	4.53 ± 0.34	2.16 ± 0.21
[cm]	0.1	12.11 ± 0.42	7.82 ± 0.27	6.97 ± 0.26	5.46 ± 0.32
Shoot f.m.	0	1.33 ± 0.02	0.98 ± 0.05	0.79 ± 0.04	0.69 ± 0.03
[g]	0.1	1.68 ± 0.05	1.31 ± 0.08	1.06 ± 0.04	0.96 ± 0.02
Shoot d.m.	0	0.19 ± 0.02	0.17 ± 0.03	0.14 ± 0.01	0.11 ± 0.01
[g]	0.1	0.23 ± 0.03	0.20 ± 0.02	0.17 ± 0.01	0.15 ± 0.01
Root f.m.	0	0.48 ± 0.03	0.35 ± 0.01	0.31 ± 0.02	0.24 ± 0.01
[g]	0.1	0.57 ± 0.03	0.44 ± 0.03	0.35 ± 0.01	0.33 ± 0.02
Root d.m.	0	0.05 ± 0.002	0.04 ± 0.001	0.04 ± 0.001	0.03 ± 0.002
[g]	0.1	0.06 ± 0.004	0.06 ± 0.003	0.05 ± 0.001	0.05 ± 0.002
ψ_w	0	0.98 ± 0.05	1.22 ± 0.05	1.37 ± 0.07	1.53 ± 0.05
[-MPa]	0.1	0.87 ± 0.05	1.06 ± 0.05	1.18 ± 0.06	1.35 ± 0.03
WC	0	83.60 ± 3.50	81.60 ± 2.90	76.40 ± 2.80	74.60 ± 3.30
[%]	0.1	87.00 ± 3.50	84.20 ± 0.40	81.70 ± 2.90	81.30 ± 3.50
Protein	0	0.97 ± 0.03	0.76 ± 0.01	0.73 ± 0.02	0.64 ± 0.04
[mg g^{-1}(d.m.)]	0.1	1.13 ± 0.04	0.97 ± 0.02	0.90 ± 0.03	0.77 ± 0.03
Proline	0	3.17 ± 0.21	3.98 ± 0.12	4.41 ± 0.17	5.48 ± 0.13
[nmol g^{-1}(d.m.)]	0.1	4.67 ± 0.22	5.36 ± 0.20	6.02 ± 0.26	7.93 ± 0.19
MDA	0	70.40 ± 5.18	112.68 ± 4.73	134.17 ± 4.01	180.19 ± 8.00
[nmol g^{-1}(d.m.)]	0.1	67.52 ± 3.86	103.21 ± 4.57	111.46 ± 5.57	123.57 ± 3.99
SOD	0	104.90 ± 5.18	127.30 ± 6.07	179.50 ± 7.38	315.60 ± 8.88
[U g^{-1}(d.m.)]	0.1	158.00 ± 10.16	212.40 ± 11.84	344.00 ± 9.61	410.70 ± 10.42
APOX	0	2.78 ± 0.84	5.45 ± 0.76	8.89 ± 0.60	12.25 ± 0.67
[U g^{-1}(d.m.)]	0.1	5.11 ± 0.69	10.37 ± 0.97	14.30 ± 1.10	18.70 ± 1.05
CAT	0	314.70 ± 10.35	331.60 ± 12.14	396.70 ± 14.75	481.30 ± 16.76
[pmol g^{-1}(d.m.)]	0.1	507.50 ± 14.54	535.90 ± 13.76	553.50 ± 13.52	719.00 ± 15.85
GR	0	297.50 ± 15.25	322.90 ± 14.25	364.80 ± 21.96	482.00 ± 20.60
[U g^{-1}(d.m.)]	0.1	472.60 ± 16.40	464.50 ± 18.86	575.90 ± 15.63	730.80 ± 22.22
Chlorophylls	0	9.02 ± 0.62	5.82 ± 0.47	5.12 ± 0.51	4.92 ± 0.37
[mg g^{-1}(d.m.)]	0.1	11.98 ± 0.82	8.43 ± 0.36	7.34 ± 0.45	6.89 ± 0.43
Carotenoids	0	1.27 ± 0.05	0.96 ± 0.06	0.91 ± 0.05	0.89 ± 0.38
[mg g^{-1}(d.m.)]	0.1	1.64 ± 0.04	1.27 ± 0.04	1.17 ± 0.05	1.06 ± 0.04
Chl/Car	0	7.10	6.04	5.61	5.50
	0.1	7.30	6.64	6.29	6.49

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