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Effects of La^{3+} on the Active Oxygen-Scavenging Enzyme Activities in Cucumber Seedling Leaves[1]

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Abstract—The effects of La^{3+} on the antioxidant enzyme activities and the relative indices of cellular damage in cucumber seedling leaves were studied. When cucumber seedlings were treated with low concentrations of $LaCl_3$ (0.002 and 0.02 mM), peroxidase (PO) activity increased, and catalase (CAT) activity was similar to that of control leaves at 0.002 mM La^{3+} and increased at 0.02 mM La^{3+}, whereas superoxide dismutase (SOD) activity did not change significantly. The increase in the contents of chlorophyll (including chlorophylls *a* and *b*), carotenoids in parallel with the decrease in the level of malondialdehyde (MDA) suggested that low concentration of La^{3+} promoted plant growth. However, except the increase in SOD activity at 2 mM La^{3+}, CAT and PO activities and the contents of pigments decreased at high concentrations of La^{3+} (0.2 and 2 mM), leading to the increase of MDA content and the inhibition of plant growth. It is suggested that lanthanum ion is involved in the regulation of active oxygen-scavenging enzyme activities during plant growth.

Key words: Cucumis sativus - lanthanum ion - antioxidant enzymes - cellular damage

INTRODUCTION

Though oxygen is fundamental for the survival of all aerobic organisms, it is subjected to be activated into toxic forms *in vivo*. As plants are immobile and perform oxygenic photosynthesis, they have the highest internal oxygen concentrations among other organisms. The concentration of molecular oxygen in plant leaf cells is 250 μM [1]. It has been estimated that 1% of the oxygen consumed by plant is diverted into active oxygen [2]. High concentration of these oxygen species can damage cellular constituents like lipids, proteins, and nucleic acids [3, 4]. As such, oxidative damage to these vital molecules can result in the perturbation of the cellular metabolism, the plant cells have developed an array of enzymatic and nonenzymatic mechanisms for scavenging these toxic components [3, 5]. Superoxide dismutase (SOD), catalase (CAT), and peroxidase (PO) have been viewed as primary enzymatic defense systems, whose combined purpose is to protect the cells from active oxygen damage [6, 7].

Among various heavy metals, rare-earth elements form a special group with interesting biological effects on plant cells [8–10]. Some recent studies have shown that lanthanum ion can enhance plant tolerance to environmental stresses [11, 12]. Pang *et al.* [12] reported that lanthanum could increase antioxidant enzyme activities of wheat seedlings to enhance their tolerance to lead stress. The studies of Zhang *et al.* [13] further indicated that lanthanum could elevate some antioxidant enzyme activities in wheat leaves. In order to investigate the involvement of lanthanum ion in primary enzymatic defense systems, we examined the effect of La^{3+} on several indices of cellular damage, as well as on the activities of antioxidant enzymes (SOD, CAT, and PO) in cucumber seedling leaves.

MATERIALS AND METHODS

Plant material and treatments. Cucumber (*Cucumis sativus* L.) seeds were surface-sterilized with 0.1% (w/v) aqueous $HgCl_2$ for 15 min, and then washed and imbibed in distilled water for 24 h. After germination, these seedlings were transferred to quartz sand and grown at a 18-h photoperiod, 25 ± 0.5°C temperature,

[1] This article was submitted by the authors in English.

Abbreviations: CAT—catalase; MDA—malondialdehyde; PO—peroxidase; SOD—superoxide dismutase.

and an irradiance of 28 μmol/(m^2 s). Fresh Hoagland nutrient solution was supplied regularly. When three true leaves appeared, the plants were divided into five groups and treated with $LaCl_3$ solutions (0, 0.002, 0.02, 0.2, and 2 mM, respectively) by leaf spraying twice daily for three days. After seven days, all leaves were collected.

Enzyme assays. Leaf tissues (0.5 g) were homogenized in 1 ml ice-cold 50 mM sodium phosphate buffer (pH 7.0) that contained 1% polyvinylpolypyrrolidone. The homogenate was centrifuged at 30000 *g* for 30 min, and the supernatant was used for assaying the enzyme activities. SOD activity was assayed by its capacity to inhibit the photochemical reduction of nitro blue tetrazolium. Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM nitro blue tetrazolium, 2 μM riboflavin, 100 μM EDTA, and 200 μl of the enzyme extract. The increase in absorbance at 560 nm because of the production of blue farmazan was monitored. CAT activity was determined by measuring the rate of H_2O_2 disappearance at 240 nm. Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 12.5 mM H_2O_2, and 200 μl of enzyme extract [14]. PO activity was measured according to Reuveni *et al.* [15] with slight modifications. Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 8 mM H_2O_2, 18 mM guaiacol, and 200 μl of enzyme extract. The absorbance was determined at 470 nm with an UV/Vis-120-02 spectrophotometer (Shimadzu, Japan).

Estimation of cellular damage. Pigments were extracted with cold 80% acetone in darkness. The amount of chlorophylls in the extracts was measured according to Arnon [16]. The carotenoid content was estimated spectrophotometrically in the same extract [17]. Lipid peroxidation was determined by the concentration of malondialdehyde (MDA) using the thiobarbituric acid reaction as described in [18], but with the introduction of a butanol extraction step for the removal of interfering compounds.

Experimental results are represented as the means from three experiments and their standard errors.

RESULTS AND DISCUSSION

Effects of La^{3+} on antioxidant enzyme activities. SOD activity did not show significant changes in the leaves of plants treated with 0.002, 0.02, and 0.2 mM La^{3+} in comparison with the control leaves; however, it increased in the presence of 2 mM La^{3+} (Fig. 1a). Generally, CAT and PO activities in the cucumber leaves showed similar trends of changes with increasing concentrations of La^{3+} (Fig. 1b), except that they differed at 0.002 mM La^{3+}. That is, CAT activity remained unchanged and PO activity significantly increased. Activities of CAT and PO were both higher in the presence of 0.02 mM La^{3+} than those in the control leaves,

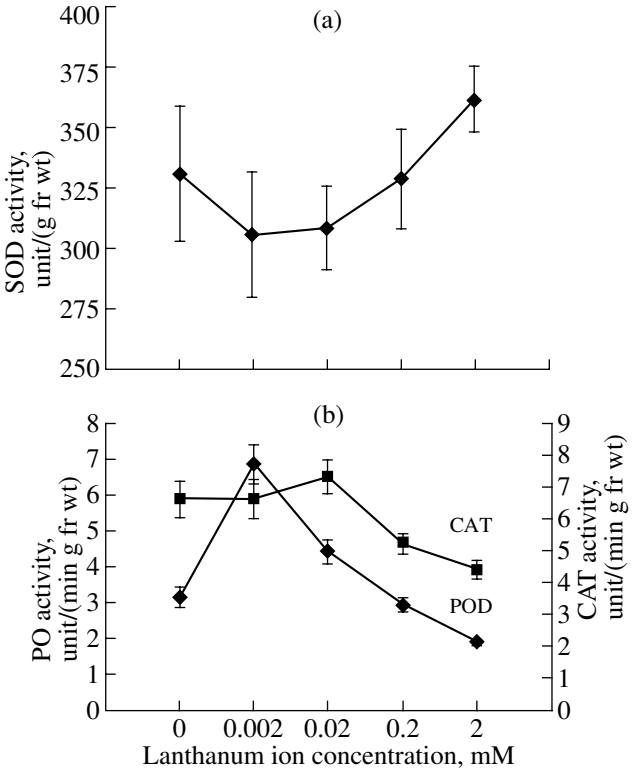

Fig. 1. Effects of various La^{3+} concentrations on antioxidant enzyme activities: (a) SOD activity; (b) CAT and PO activities.

whereas they declined at the La^{3+} concentrations of 0.2 and 2 mM. Especially at 2 mM, they decreased sharply to 33% and 40% of the control test, respectively.

There is considerable evidence that plant and animal tissues can generate superoxide radical ($O_2^{\bullet -}$), which is converted to hydrogen peroxide (H_2O_2) by SOD [19, 20]. An interaction between $O_2^{\bullet -}$ and H_2O_2 may also generate singlet oxygen ($^*O_2$) and hydroxyl free radical ($OH^\bullet$). The ability of $O_2^{\bullet -}$, $^*O_2$, and $OH^\bullet$ to initiate lipid peroxidation has been documented [18]. Thus, the availability of $O_2^{\bullet -}$ and the production $^*O_2$ and $OH^\bullet$ would be diminished by operation of SOD, CAT, and PO to protect the cell from lipid peroxidation. Our results suggest that optimum La^{3+} concentration may contribute to suppressing the accumulation of H_2O_2.

The most relevant function of SOD seems to be the scavenging of $O_2^{\bullet -}$. However, it has been reported that overexpression of a gene that encodes SOD in transfected mouse L cells and human HeLa cells enhanced the formation of lipid hydroperoxides [21], suggesting that an increase in SOD activity can result in an imbalance between the rate of formation of H_2O_2 and its removal. Both CAT and PO are important antioxidant systems that catabolize H_2O_2 [22], and the inactivation

The effects of La^{3+} treatments on the content of chlorophylls in cucumber seedling leaves, mg/g fr wt

Concentration of La^{3+}, mM	Chlorophyll *a*	Chlorophyll *b*	*a*/*b*
0	0.89 ± 0.01	0.64 ± 0.03	1.38
0.002	0.91 ± 0.03	0.67 ± 0.02	1.37
0.02	0.94 ± 0.02	0.74 ± 0.01	1.27
0.2	0.93 ± 0.03	0.71 ± 0.01	1.31
2	0.72 ± 0.02	0.64 ± 0.02	1.14

of these enzymes could result in an increase of H_2O_2 levels inside leaf cells [23]. Therefore, it was of interest to compare the ratios of SOD to CAT and SOD to PO activities. Much lower ratios were obtained under 0.002 and 0.02 mM La^{3+} treatments (Fig. 1). Thus, lower levels of $O_2^{\cdot -}$ and H_2O_2 could result from the cell capacity to maintain the high activity not only of SOD but also of CAT and PO.

Effects of La^{3+} leading to cellular damage. In contrast to control leaves, both chlorophylls *a* and *b* showed an increase in the presence of 0.002–0.2 mM La^{3+} (table). However, the percentage of chlorophyll *b* increase was higher than that of chlorophyll *a*. The table also shows that there was a significant decrease in the level of chlorophyll *a*, but not in chlorophyll *b* after 2 mM La^{3+} treatment. The change in carotenoid contents was consistent with that of chlorophyll in the presence of 0.002–2 mM La^{3+} (Fig. 2). Carotenoid and chlorophyll levels were higher at 0.002–0.2 mM La^{3+} than control values, whereas both pigments declined sharply at 2 mM La^{3+}.

Lipid peroxidation in cucumber leaves was estimated by the content of MDA (Fig. 3). Its levels decreased dramatically at 0.002 and 0.02 mM La^{3+}; especially at 0.02 mM, it sharply declined by 48%. At high La^{3+} concentrations (0.2 and 2 mM), it increased in comparison with the effects of lower concentrations (0.002 and 0.02 mM). However, it should be noted that the level of lipid peroxidation remained lower at 0.2 mM La^{3+} than the level found in the control leaves.

It is known that chloroplasts are potentially the most powerful source of active oxygen species generated there during photosynthesis [5]. The previous works have shown that lanthanum ion can affect the photosystems [24]. We revealed that the contents of chlorophylls and carotenoids changed after the treatments with La^{3+}. Apparently, the optimum treatments with La^{3+} may contribute to the favorable role of antioxidant enzymes, which protect the chloroplasts against oxidative damage.

Many previous studies on the effects of rare-earth elements on plant growth indicate that appropriate concentration of lanthanide ions can promote growth and development of plants. However, excessive amounts become toxic and cause physiological constraints [9, 10]. We took into account the changes in MDA level and concluded that the promotion of cucumber seedling growth at the optimum La^{3+} concentration (data not shown) appeared to be due to the elevation of CAT and PO activities that can limit lipid peroxidation. However, at high La^{3+} concentration, CAT and PO activities became lower, although SOD activity increased significantly, that has led to the accumulation of H_2O_2 in seedling leaves. As a result, the leaves turned yellowish.

In conclusion, the present study demonstrated a La^{3+}-dependent change in antioxidant activities in cucumber seedling leaves as a response to various La^{3+} concentrations. Further research is required to clarify the mechanism of regulation of the antioxidant enzyme activities by La^{3+}.

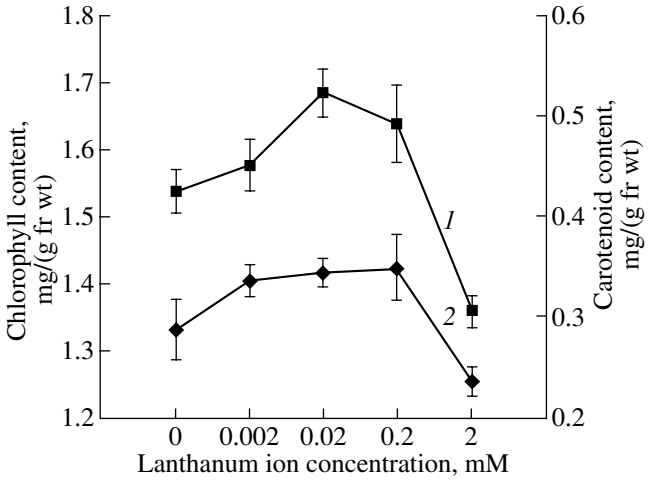

Fig. 2. Effects of La^{3+} concentrations on (*1*) total chlorophyll and (*2*) carotenoid contents.

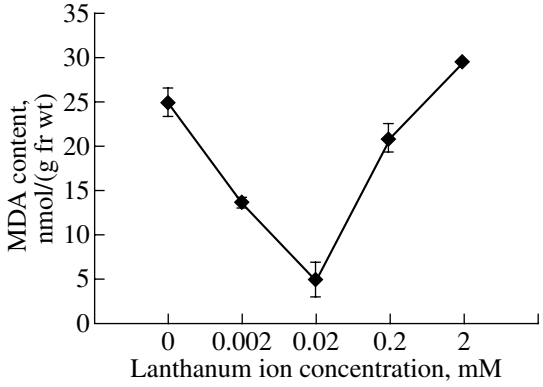

Fig. 3. Effect of La^{3+} concentrations on levels of lipid peroxidation.

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