

Effects of Lanthanum on Redox Systems in Plasma Membranes of *Casuarina equisetifolia* Seedlings Under Acid Rain Stress*

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Abstract: The effects of lanthanum on some redox system (PMRS) properties of the plasma membrane (PM) vesicles from *Casuarina equisetifolia* seedlings under artificial acid rain (pH 4.5) stress were studied. The results show that there are NADH oxidase and EDTA-Fe³⁺ reductase, and nitrate reductase in the seedling PM, and they have different responses to soaking seeds for 8 h in a series of LaCl₃ solution. The NADH oxidase activities and the Nitrate reductase activities can be stimulated when La³⁺ concentrations is in the range of 50 ~ 200 mg L⁻¹, but their activities are inhibited or fluctuate by the higher La³⁺ concentrations. The EDTA-Fe³⁺ reductase activities can be stimulated by La³⁺ concentrations in the range of 50 ~ 400 mg L⁻¹. The research also revealed that La³⁺ reduces the relative permeability of membranes and have the function in protecting membranes under acid rain stress by the way of inhibiting the leakage of electrolyte.

Key words: ecology; PM redox system; lanthanum; *Casuarina equisetifolia*; acid rain stress; rare earths

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The plasma membrane is a penetrable barrier, which can control the exchange of substances across membranes in cells, and also is the intermedium and receptor of energy or information transference between cells and environment. The plasma membrane redox system (PMRS) means the electron transfer chains on plasma membrane. Owing to have the possibility of ejecting protons, energizing plasma membrane and having the function of accelerating transportation of solute across membrane, the plasma membrane redox systems were paid much attention to^[1]. Acid rain is one of the most important environment problems worldwide. The plasma membrane is damaged firstly when the plant is exposed to acid rain. The rare earth elements can obviously protect the plants from being affected by acid rain^[2], but the mechanism of action needs further study.

Casuarina spp. with strong tolerance to drought, poor soil, soloncheks and other poor environmental conditions is the best choice to form shelter forest in coastal areas in China, which brings remarkable ecological benefits such as wind break and fixing sand, greening coast, protecting cropland. In recent years, the problems of forest stands declining and be-

ing difficult to its regeneration have appeared among the *Casuarina* forests in southeastern seashore in China. Some research indicated that improving the stress resistance is one of the important measures to comprehensively bring *Casuarina* forests declining under control^[3]. This study focuses on the effects of lanthanum on the relative permeability of membranes and some properties of the redox systems of plasma membranes with a view to seeking an approach to improving the stress resistance of *Casuarina* shelter forests as well as to providing some scientific data for it.

1 Materials and Methods

1.1 Materials

The chosen species was *Casuarina equisetifolia*, whose seeds were supplied by the *Casuarina* Seedlings Base of Chihu Forestry Center in Huian County, Fujian Province. The seeds used for this experiment after putting in cold storage for 6 months.

1.2 Methods

1.2.1 Methods of cultivating After being randomly selected with the method of quartation, *Casua-*

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rina equisetifolia seeds were surface sterilized in 0.5% KMnO_4 (5 min), then washed and filtered three times with sterile distilled water. The surface sterilized seeds were respectively soaked for 8 h in $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ with different concentrations as following: 0 (CK, sterile distilled water); 50, 100, 200, 300, 400 mg L^{-1} , and the weight of seeds in each treatment was 10 g (the average weight of one thousand grains was 1.44 g), followed by flushed and filtered La^{3+} from the surfaces. The seeds were equably planted in clean and thin river sands which were put in a 26 cm \times 11 cm plastic basketry covered with nylon net. The sands were 2~3 cm deep. Then the basketry was set in a suited plastic basin filled culture solution with quantitative artificial acid rain. The pH value of artificial acid rain was 4.5 as the same as that of acid rain happened higher frequently in Xiamen region^[4]. The ions concentration of artificial acid rain was according to Ref. [5] (Table 1). Every treatment repeated 9 times. The basins were kept in the glasshouse of the arboretum in Xiamen university. In the period of culture, the basins were take out of the solution to aerate for 1 h every nightfall, and the pH value of artificial acid rain in the basins was kept invariable. The culture temperature was (22 \pm 2). Two weeks later, the seedlings were taken to experiment when the height of plants was about 5~6 cm.

1.2.2 Methods of determining Determination of the relative permeability of cell membranes: According to Ref. 6, the leakage of electrolyte was as the index of permeability of cell membranes, and which was determined by DDS-11 conductometer (The unit of electric conductivity was $\mu\text{s cm}^{-1}$).

Isolation and purity of plasma membrane vesicles: Plasma membrane vesicles were prepared referring to the method described by Zheng H L^[7] with some modification. Briefly, 20 g seedlings in each treatment were taken to be cut into sections (about 1 cm section), followed by homogenized with 100 ml cold extraction solution (pH 7.8, containing Tris-Mes 15 mmol L^{-1} , EDTA- Na_2 3 mmol L^{-1} , Sucrose 0.25 mmol L^{-1} , 0.6% (v/v) PVP K-30, PMSF 1 mmol L^{-1}), then filtered with two layers of gauze. The percolate was centrifuged for 15 min at 1000 r min^{-1} , and the supernatant was centrifuged for 30 min at 5000 r min^{-1} to yield a crude microsomal pellet. The supernatant was discarded and the pellets

were resuspended in 5 mmol L^{-1} potassium phosphate buffer (pH 7.8, containing DTT 0.1 mmol L^{-1} , KCl 1 mmol L^{-1}). The resuspended membranes then were loaded onto the two-phase system with a cold polymer mixture at a ration of 1/3 containing 6.2% (w/w) Dextran T500, 6.2% (w/w) PEG3350, 0.25 mol L^{-1} Sucrose, 3 mmol L^{-1} KCl, 5 mmol L^{-1} K_3PO_4 , pH 6.8. The mixed system was separated into two phases by centrifugation (1500 r min^{-1} , 15 min). By being washed 3 times, all upper phases were collected, diluted with Tris-Mes 5 mmol L^{-1} , DTT 0.1 mmol L^{-1} , Sucrose 0.25 mmol L^{-1} , pH 7.8, and centrifuged at 100000 $\times g$ for 30 min. The resulting pellet was resuspended in a medium containing the same as diluted solution to get purified plasma membrane vesicles. The purified plasma membrane-enriched fractions were used immediately or frozen at -70 for later use.

Determination of NADH oxidase activity (oxidation rate of NADH) in plasma membrane: The medium of reaction was referred to the method developed by Chen et al.^[8], took $\text{Fe}(\text{CN})_6^{3-}$ as electron acceptor, measured the rate of decreasing absorbance of reaction solution at 340 nm. The rates of NADH oxidation were calculated as consistent with extinction coefficient of 6.23 for a millimolar NADH.

Determination of EDTA- Fe^{3+} reductase activity (the reduction rate of EDTA- Fe^{3+}) in plasma membrane: According to the method of Jiao et al.^[9], taking NADH as electron donor, the change of extinction coefficient was measured at 535 nm. The reduction rate of EDTA- Fe^{3+} was calculated by contrasting the standard curve of Fe^{2+} .

Nitrate reductase activity was assayed as described by Hong et al.^[10] with some modification. A 1.0 ml reaction solution containing 25 mmol L^{-1} potassium phosphate buffer (pH 7.5), 5 mmol L^{-1} KNO_3 , 0.02% Triton X-100 (v/v), 0.5 mmol L^{-1} NADH, and 50 μl vesicles. The reactants were incubated in 30 for 15 min, 17 mmol L^{-1} P-Aminobenzenesulphonic acid anhydrous was put in to stop reaction, and 7 mmol L^{-1} -Naphthylamine was put in to coloration. Then the optical density was measured at 530 after stationary 15 min. One unit of enzyme activity was described as the amount which catalyzed the reduction of 1 $\text{nmol NO}_3^- \text{mg}^{-1} \text{min}^{-1}$. Protein content was determined by using Coomassie Brilliant Blue

Table 1 Ion composition and concentration of artificial acid rain

Ion concentration	K^+	Na^+	Ca^{2+}	Mg^{2+}	NH_4^+	H^+	SO_4^{2-}	NO_3^-	Cl^-	F^-
Content/ $(\mu\text{mol L}^{-1})$	15.0	18.0	50.0	15.5	40.5	36.5	140.0	21.0	7.5	7.0

G250 referring to Bradford^[11]. All the determinations were repeated three times. In the experiments, UNF-CAM UV310 Thermo Spectrophotometer was used, and all reagents were in AR degree.

2 Results and Discussion

2.1 Effects of lanthanum on membrane permeability

Membrane permeability is related to plant resistance^[6], electric conductivity can be used to indicate the changes of cell membrane permeability. When plants are stressed by acid rain, cell membranes respond firstly with the increase of their permeability, the leakage of endocellular electrolyte and the loss of nutritious elements, so that electric conductivity of plant cell extraction is increased. The more severe the plant cell is impaired, the more the intracellular electrolyte leaks. This experiment results (Table 2) indicate that lanthanum can apparently reduce membrane relative permeability of *Casuarina equisetifolia* seedlings under simulated acid rain stress. Membrane permeability decreases remarkably with La^{3+} concentration increasing in the range of 50 to 200 $\text{mg} \cdot \text{L}^{-1}$, while in the treatment of 200 $\text{mg} \cdot \text{L}^{-1}$, and the permeability reaches the lowest value in all six treatments. After treating with La^{3+} from 200 to 400 $\text{mg} \cdot \text{L}^{-1}$, the membrane permeability gradually increases. Contrasting to the treatments of La^{3+} in the range of 50 to 200 $\text{mg} \cdot \text{L}^{-1}$, and the effects of La^{3+} on the decrease of the membrane permeability are descended gradually with the increase of La^{3+} concentration from 300 to 400 $\text{mg} \cdot \text{L}^{-1}$. The experiments above mentioned suggest that suitable concentrations of La^{3+} can inhibit the electrolyte leakage of seedlings cell membrane under acid rain stress and alleviate the loss of nutrition ion. The effects that soaking seeds with La^{3+} solution reduces the cell membrane permeability of plant seedlings might be caused by that La^{3+} , which is absorbed by seeds, can be in close combination with the compositions of organ cell membrane when the organ cell dif-

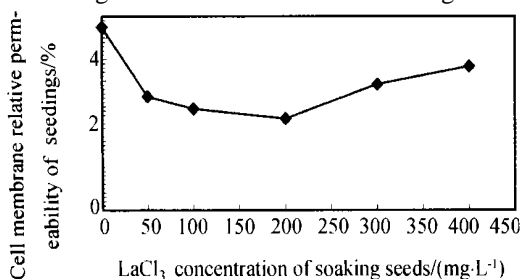


Fig. 1 Effects of lanthanum on cell membrane permeability of *Casuarina equisetifolia* seedlings under acid rain stress (pH 4.5)

ferentiate and mitosis at their early stage of growth so as to reduce the leakage of electrolyte and the permeability of cell membrane.

2.2 Effects of lanthanum on activity of PM NADH oxidase

The results (Fig. 2) indicate that there are NADH oxidase in the plasma membranes of *Casuarina equisetifolia* seedlings under acid rain stress, and the oxidation rate of NADH can be promoted by lanthanum of suitable concentrations. With $\text{Fe}(\text{CN})_6^{3-}$ as electron receptor, the oxidation rate of NADH increases owing to the treatments with increasing La^{3+} concentration from 50 to 200 $\text{mg} \cdot \text{L}^{-1}$, and it has its peak value when La^{3+} concentration is 200 $\text{mg} \cdot \text{L}^{-1}$. The oxidation rate of NADH decreases when La^{3+} exceeds 200 $\text{mg} \cdot \text{L}^{-1}$. In comparison with the CK, when La^{3+} is in the range of 300 ~ 400 $\text{mg} \cdot \text{L}^{-1}$, the oxidation rate of NADH decreases with the concentration of La^{3+} increasing and is even lower than that of the check test. This reveals that the activity of NADH oxidase is inhibited by higher La^{3+} concentrations (300 ~ 400 $\text{mg} \cdot \text{L}^{-1}$). From Fig. 1, it also can be seen that NADH oxidase is more sensitive to La^{3+} with high concentrations than plasma permeability is.

The redox system of plant plasma membrane is closely related to the H^+ -ATPase. The oxidation of NADH makes e^- and/or H^+ out of plasma membrane, this results in changes of the membrane electric potential, further some of H^+ -ATPases are activated, and more protons are pumped out of the plasma. Therefore, NADH oxidase and H^+ -ATPase act on membrane electrochemical potential jointly, they prompt the active transport of solution across plasma membrane, which affects plant's growth and metabolism.

However, in our experiment we also revealed that when *Casuarina equisetifolia* seedlings grows under the acid rain stress after being treated with La^{3+} , the elongation rate of seedlings and the PM H^+ -ATPase activi-

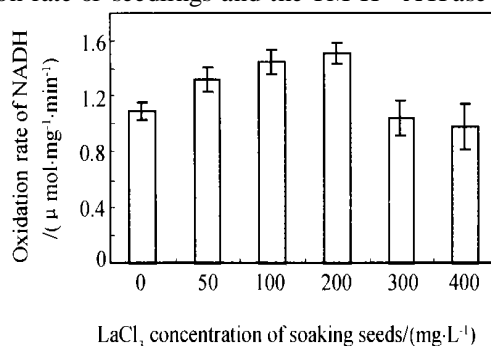


Fig. 2 Effects of lanthanum on PM NADH oxidase activity of *Casuarina equisetifolia* seedlings under acid rain stress (pH 4.5)

ty, and the PM NADH oxidase activity have the same response tendency to the treatments of La^{3+} with increasing concentrations showed a " " type. In this study, La^{3+} may act as an allosterism effector of enzyme, by the action the NADH oxidase and H^+ -ATPase activated, the secretion of H^+ from the cytoplasm to the cell wall is prompted, which finally result in pH value of ground substance in cell wall decreasing. Under this acid condition, enzymes, which can degrade cell wall, are activated. The bonds, which connect xyloglucan and microfibril, are cut off. Finally the cell wall became loose and soft, cell expansion pressure lessened, more water absorbed, and the cell volume is expanded. The identical effects of La^{3+} on PM NADH oxidase, PM H^+ -ATPase and plant macro-morphogenesis are consistent with acid growth hypothesis of auxin reaction^[12].

2.3 Effects of La^{3+} on PM EDTA- Fe^{3+} reductase

To prompt the reduction and absorption of iron is one of the most evident physiological actions of plant PM redox system. Plant can not directly make use of Fe^{3+} . Only when Fe^{3+} is reduced into Fe^{2+} or combined with chelator to form chelate, can plant cell utilize Fe^{3+} through PM^[11].

The effects of La^{3+} on the reduction rate of PM EDTA- Fe^{3+} of seedlings (Fig. 3) show that, in comparison with the check test, the reduction rate of PM EDTA- Fe^{3+} increases obviously after the seeds have been treated with La^{3+} ranging from 50 to 400 mg L^{-1} . This show that La^{3+} can enhance the PM EDTA- Fe^{3+} reductase activity, and the mechanism of action needs further study.

Bienfait et al.^[13] believed that: there are two type of iron-cyanide reductase systems, and one is Turbo system, which can be induced only when iron is deficient, many kinds of Fe^{3+} chelate and iron-cyanide can be reduced by this system. Another is standard

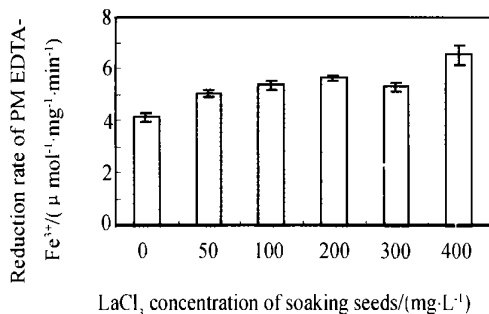


Fig. 3 Effects of La^{3+} on PM EDTA- Fe^{3+} reductase activity of *Casuarina equisetifolia* seedlings under acid rain stress (pH 4.5)

system, only iron-cyanide can be reduced by this way, the other Fe^{3+} chelate can not. Iron in the plant do not affect the activity of standard system. This study also indicated that EDTA- Fe^{3+} can receive electron from NADH to be reduced if the seedlings are cultivated without Iron deficiency. Under acid rain stress, lanthanum may cause the seedlings PM switch on a new reduction system of Fe^{3+} chelate, which is different from the systems mentioned above. The results of this study is identical with that of Qiu et al.^[14], who has one new redox system started under water stress and which is different from the standard system.

2.4 Effects of lanthanum on PM nitrate reductase

Nitrate reductase is a kind of key enzyme for nitrogen metabolism of plant. PM nitrate reductase not only can absorb nitrate from the outside of PM, reduce nitrate to nitrite and release nitrite to inside of PM, but also may reduce the electron receptor which is in competition with nitrate and located outside of PM, and simultaneously pump H^+ out of PM^[10]. The result (Fig. 4) show that there is nitrate reductase in the purified PM of *Casuarina equisetifolia* seedlings, whose electron donor is NADH, and lanthanum significantly affects the PM nitrate reductase activity. In comparison to the check test, the PM nitrate reductase activity increased with La^{3+} from 50 to 200 mg L^{-1} . But the nitrate reductase activity of seedlings was significantly inhibited when La^{3+} concentration was 300 mg L^{-1} , which was lower than that of the check test. In the case of 400 mg L^{-1} La^{3+} treatment, the PM nitrate reductase activity was higher than that of 300 mg L^{-1} La^{3+} treatment. This rebound might be due to that some La^{3+} survived the cells of seedlings during the process of germinating after the seeds were soaked in the high concentration of La^{3+} solution, and the detained La^{3+} may act as ion stress and induce nitrate reductase activity increasing.

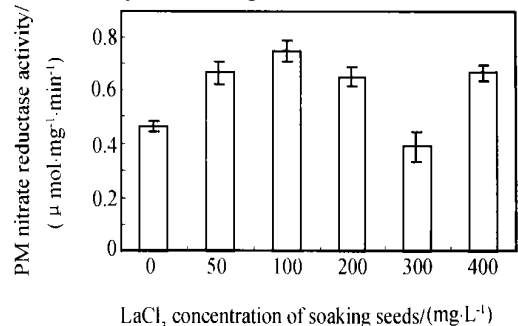


Fig. 4 Effects of lanthanum on the PM nitrate reductase activity of *Casuarina equisetifolia* seedlings under acid rain stress (pH 4.5)

La^{3+} usually has larger association constant and max. ion combination quantity with PM surface than Ca^{2+} , Mg^{2+} , etc. does. These ions have similar membrane surface physical and chemic properties with La^{3+} . La^{3+} closely combines with the composition of membrane in competition with other kinds of ions, and which cause charge density and potential of PM surface change greatly. La^{3+} adjusts the oxidization rate of NADH, which results in the change of the redox potential, and the redox potential and energy charge transmitting are in charge of nitrate reductase reaction, and adjust the activation and passivation of nitrate reductase. All these may be main reasons for that La^{3+} affects PM nitrate reductase.

3 Conclusions

1. By treating with $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ in the range of 50 to 400 mg L^{-1} to soak *Casuarina equisetifolia* seeds for 8 h, the cell membrane relative permeability of the earlier seedlings, which are cultivating under artificial acid rain (pH 4.5) stress, can be reduced.

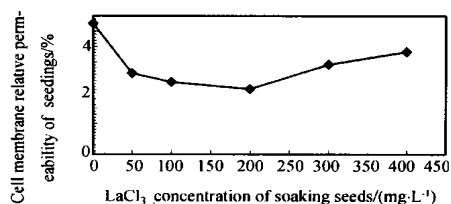
2. There are NADH oxidase and EDTA-Fe^{3+} reductase and nitrate reductase in the plasma membrane of *Casuarina equisetifolia* seedling. They have different responses to La^{3+} . Soaking seeds with La^{3+} concentration in the range of 50 ~ 200 mg L^{-1} can stimulate the NADH oxidase activities and the nitrate reductase activities of the seedlings, but La^{3+} with higher concentration in the range of 300 to 400 mg L^{-1} cause some negative effects on the activities. The activity of EDTA-Fe^{3+} reductase can be stimulated by treating with La^{3+} from 50 to 400 mg L^{-1} .

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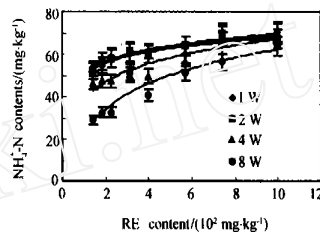
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Effects of lanthanum on cell membrane permeability of *Casuarina equisetifolia* seedlings under acid rain stress (pH 4.5)

- 582 Effect of Rare Earths on Fractionation and Transformation of Soil Available Nitrogen

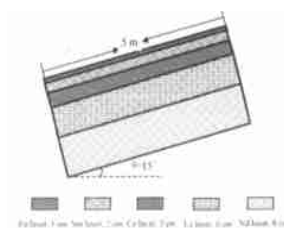
Ding Shiming, Liang Tao, Zhang Zili, Sun Qin, Zhang Chaosheng



Effect of RE on concentration of solid $\text{NH}_4^+\text{-N}$ under dry condition

- 587 Using REE Tracers to Measure Sheet Erosion Changing to Rill Erosion

Song Wei, Liu Puling, Yang Mingyi, Xue Yazhou



Vertical discharging depth of REE

- 591 Rare Earths of Magmatic Rocks of Yanshanian Stage in Adjacent Region of Anhui and Jiangxi Provinces, Jiangnan Uplift

Yuan Feng, Zhou Taofa, Yue Shucang, Zhu Guang, Hou Mingjin



Sketch map of magmatic rocks in adjacent region of Anhui and Jiangxi Provinces, Jiangnan Uplift

Notes

- 595 Synthesis, Characterization and Antioxidative Activity of Lanthanide Complexes with 3,5-Dibenzoyloxybenzoyl-2,4-Dihydroxybenzaldehyde-Hydrazone

Zhang Ling, Tang Ning*, Fang Jianguo, Tan Minyu

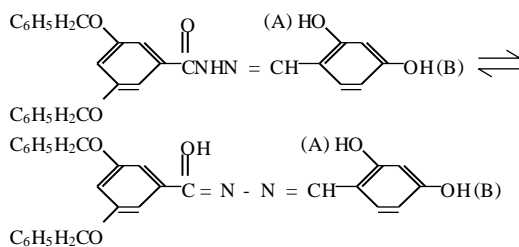
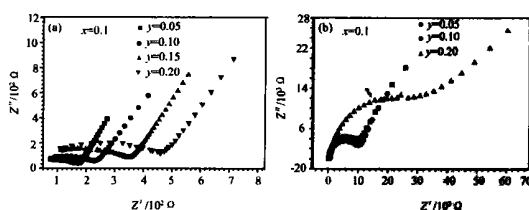


Fig. 1 Structure of the ligand (H_2L)

- 598 Study on Lithium Fast Ion Conductors of $\text{Li}_{3x}\text{La}_{0.67-x}\text{In}_y\text{Ti}_{1-2y}\text{P}_3\text{O}_3$ System

Li Ronghua, Zhang Ning, Wang Wenji



Complex impedance plots of composition $\text{Li}_{3x}\text{La}_{0.67-x}\text{In}_y\text{Ti}_{1-2y}\text{P}_3\text{O}_3$ at high (a) and low (b) frequency