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Effects of La^{3+} on ATPase Activities of Plasma Membrane Vesicles Isolated from *Casuarina Equisetifolia* Seedlings under Acid Rain Stress*

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Abstract: The effects of La^{3+} on the growth and the ATPases activities of plasma membrane (PM) vesicles isolated from *Casuarina equisetifolia* seedlings under artificial acid rain (pH 4.5) stress were studied. The results show that the height, length of roots, fresh weight and PM H^+-ATPase activites of *Casuarina equisetifolia* seedlings increase by the treatments of soaking seeds in $LaCl_3$ solutions with lower concentrations, and those can reach their peak values by treating with 200 mg·L^{-1} La^{3+}. However, in comparison with the CK, those are inhibited by the higher La^{3+} concentrations; PM Ca^{2+}-ATPase activity is inhibited with the treatments of La^{3+}. The results also reveal that the H^+-ATPase activity and the growth of cell enlarge have a remarkable positive correlation, and La^{3+} activating H^+-ATPase can facilitate plant growth. La^{3+} also can alleviate cytosolic acidification of plant under acid rain stress and indirectly maintain the stability of intracellular environment. In order to resistant to acid rain and accelerate the growth of *Casuarina equisetifolia*, the suitable range of La^{3+} concentrations to soak seeds for 8 h is 50～200 mg·L^{-1}.

Key words: ecology; PM ATPase; lanthanum; *Casuarina equisetifolia*; acid rain stress; rare earths
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As a main kind of membrane transporter, plasma membrane ATPases facilitate the transport of minerals, sugars, metabolites, and other compounds across the membrane of the cells, only by which could plant cells communicate with environment to transfer substance and information, which is essential for plant growth and cellular homeostasis[1]. As the experiment materials, the highly purified plasma membrane vesicles from *Casuarina equisetifolia* seedlings cultivated under artificial acid rain stress, were isolated by aqueous two-phase partitioning methods. The study mainly focused on the effects of La^{3+} on the PM ATPases activities, and was expected to show some inherence mechanisms and provide some scientific basis for seeking an approach of utilizing rare earth elements to improve the resistance of *Casuarina* shelter forests to resist acid rain stress.

1 Materials and Methods

1.1 Plant material

The *Casuarina equisetifolia* seeds were supplied by the *Casuarina* Seedling Base of ChiHu Forestry Center in HuiAn County, Fujian Province. The average weight of one thousand grains was 1.44 g. After being randomly selected, the seeds were surface sterilized in 0.5% $KMnO_4$ (5 min), then washed and filtered three times in sterile distilled water. The sterilized seeds were soaked for 8 h in $LaCl_3 \cdot 7H_2O$ with the concentrations of 0 (CK, sterile distilled water); 50, 100, 200, 300 and 400 mg·L^{-1} respectively, and 10 g seeds each treatment were weighed, and flushed, then filtered to remove La^{3+} from the surfaces. Then the seeds were cultured in the basin filled with sands, and the culture solution is the artificial acid rain with pH value of 4.5 as the same as that of naturally happened in higher frequency in Xiamen region[2]. The ions concentration of artificial acid rain was according to Ref. [3]. Every treatment repeated nine times. The basins were kept in the glasshouse. In the period of culture, the basins were taken out of the solution to aerate for 1 h every nightfall, and the pH value of artificial acid rain was kept invariable. The culture temperature was (22 ±2) ℃. Two weeks later, at that

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time the plants were about 5～6 cm height, the seedlings were taken to experiment.

1.2 Determination

Determination of the plant growth and biomass: 10 seedlings were randomly taken from each treatment group, then the plant height and the length of main root were measured. The fresh weight of each seedling was weighed by electron scale.

The technique method for preparing plasma membrane vesicles by the method of two - phase partition was referred to that described by Zheng[4].

The PM H^+-ATPase activity was determined by the method described by Zhang[5]: 20 μl vesicle membrane preparations was added into 0.5 ml reaction medium consisted of $MgSO_4$ 3 mmol·L^{-1}, K_2SO_4 25 mmol·L^{-1}, Triton X-100 0.02% (*V/V*), Tris-Mes 50 mmol·L^{-1}, pH 6.5, and ATP-Na_2 3 mmol·L^{-1}. The reactants were incubated in 37 ℃ for 30 min and the reaction was stopped by 20%(*W/V*) TCA 200 μl. The activities of the enzyme were calculated by the amounts of Pi formed by hydrolysis in the reactions.

PM Ca^{2+}-ATPase hydrolysis activity was determined by the method of Li X M[6]: 1.1 ml reaction system included imidazole 10 mmol·L^{-1}, $MgCl_2$ 5 mmol·L^{-1}, $CaCl_2$ 50 μmol·L^{-1}, ATP-Na_2 3 mmol·L^{-1}, pH 7.0, and 20 μl membrane preparations. The enzyme reaction followed the steps of measuring H^+-ATPase activity. Determination of Pi was according to Ohnishi[7]; Protein content was determined by using Coomassie Brilliant Blue G-250 referred to Bradford[8]. All the determinations were repeated six times.

2 Results and Discussion

2.1 Effects of lanthanum on seedling growth

The experiment results (Table 1) indicate that the effects of lanthanum on the growth of *Casuarina equisetifolia* seedlings under acid rain stress with pH value 4.5 is obvious, and the growth including the height, length of roots, and fresh weight of *Casuarina equisetifolia* seedlings is promoted gradually by soaking seeds for 8 h in $LaCl_3 \cdot 7H_2O$ solution with the increasing concentrations from 50 to 200 mg·L^{-1}, and in the treatment of 200 mg·L^{-1} La^{3+}, all those values reach the highest points. But in the treatment of 300 mg·L^{-1} La^{3+}, the effects descend. As to treating with 400 mg·L^{-1} La^{3+}, the plant growth is inhibited. The experiment results indicate that rare earth elements have obvious function in alleviating the effects of acid rain on plant biomass; It also suggests that rare earth elements with suitable concentration can promote the growth of plant. Therefore it is feasible that La^{3+} could enhance the ability of *Casuarina equisetifolia* to resistant to acid rain stress.

Table 1 Effect of La^{3+} on seeding growth of *Casuarina equisetifolia* **under acid rain stress($\bar{X}$ ±SD)**

La^{3+}/(mg·L^{-1})	0	50	100	200	300	400
Height of plant/cm	5.43±0.49	5.66±0.46	5.76±0.39	6.32±0.79	5.54±0.38	5.31±0.24
Length of roots/cm	3.09±0.36	3.17±0.39	3.22±0.32	3.54±0.34	3.28±0.41	2.85±0.26
Fresh weight/mg	8.04±0.1	8.36±0.15	9.70±0.36	9.90±0.65	9.30±0.15	7.82±0.26

2.2 Effects of La^{3+} on PM H^+-ATPase activity

After treating with La^{3+} of different concentrations, the changes of PM H^+-ATPase activity of *Casuarina equisetifolia* seedlings show that lower concentration of La^{3+} can stimulate the activity, but higher concentration will inhibit it. The seedling PM H^+-ATPase activity increases gradually with the increase of La^{3+} from 50 to 200 mg·L^{-1}. While in the treatment of 200 mg·L^{-1}, PM H^+-ATPase activity reaches its peak value, and increases 35.85% compared with CK; However, when the concentration is over 200 mg·L^{-1}, the activity of PM H^+-ATPase is inhibited and lower than that of CK. In the treatments of 300 and 400 mg·L^{-1} La^{3+}, the activities are 72.29% and 90.88% of CK respectively.

H^+-ATPase of the plasma membrane is considered as a master enzyme. The improvement of PM H^+-ATPase activity would facilitate building up the gradient of proton electrochmical potentials[9], which can provide stronger driving force for inorganic ions, or organic solutes such as sugars and amino acids, thereby the transport of substance and growth of plant are accelerated. Acid rain in China belongs to sulfate type. The transport of sulfate on plant plasma membrane is powered by an electrochemical proton gradient generated by proton pump of ATPase that extrudes protons to the cell exterior. Sulfate is actively cotransported into plant cells along with protons at a stoichiometry of one sulfate to three protons, thus sulfate transporter is able to couple the influx of net protons to the transport of sulfate into the cell[1]. Which would influence the stabilization of cytosolic pH (cytosolic pH is remark-

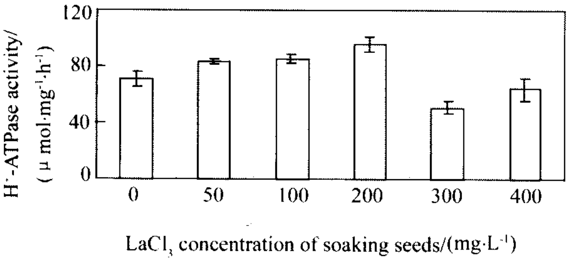

Fig. 1 Effects of La^{3+} on PM H^+-ATPase activity of *Casuarina equisetifolia* seedlings under acid rain stress (pH 4.5)

ably stable, generally remaining at 7.3～7.5). H^+-ATPase has a function in extruding protons to the cell exterior, and the promotion of H^+-ATPase activity facilitates transporting proton out of cell to prevent acidification of the cytoplasma, which keeps inner cellular environment in normal and stable state when plant is under acid rain stress.

Two aspects are known to the growth of plants. The first is an increase in the number of cells in limited parts in plant body; the second is the enlargement in the size of each cell, and this kind of growth promotion is also caused by the plant hormone, auxin[9]. The proton pump is activated by auxin. The inner surface of the cell wall is acidified by the H^+ formed. The acidification of the cell wall activates the proteins of loosening H-bonds within the cell wall and allows turgorgenerated growth[1]. Finally, elongation of the plant body is accelerated.

Therefore the activation of H^+-ATPase directly contributes to the growth of plant. The growth promotion by lanthanum is just due to a similar machanism to that described above. Table 1 and Fig. 1 indicate that there are coherent trend of responding to lanthanum between plant height and H^+-ATPase activation. Analyzing the correlation using the two group average data of plant height and H^+-ATPase activity shows that they have a remarkable positive correlation. It could be supposed that La^{3+} activating PM H^+-ATPase is one of the mechanisms that La^{3+} could accelerate plant enlarging growth.

The C terminal of H^+-ATPase polypeptide in cytoplasma forms an autoinhibitory domain, and the removal of the autoinhibitory domain activates the enzyme considerably[1]. The modulation mechanism of autoinhibitory domain in the C-terminal of PM H^+-ATPase is possibly by the way of binding an effector, thus the C-terminal part of the H^+-ATPase loses the autoinhibitory function[10]. The mechanism of La^{3+} modulating H^+-ATPase activity might be as follows: first, La^{3+} as an effector binding the C terminus region of H^+-ATPase relieves autoinhibition function, which could increase the express of H^+-ATPase activation; second, La^{3+} predominantly combines phospholipid of plasma membrane[11], and the combination is as an effector to relieve the autoinhibition function of H^+-ATPase C terminus, therefore the enzyme activation is affected. As to PM H^+-ATPase activity decreases with La^{3+} treatment in higher concentration (300～400 mg·L^{-1}), it might attribute to that when the La^{3+} concentration is over the concentration limit and become a kind of ion stress, it would inhibit the gene expression of part of H^+-ATPase.

2.3 Effects of La^{3+} on PM Ca^{2+}-ATPase activity

The effects of La^{3+} on the plasma membrane Ca^{2+}-ATPase activities show as Fig. 2 that the activities of PM Ca^{2+}-ATPase are inhibited and the activities decrease with La^{3+} concentration increasing from 50 to 400 mg·L^{-1}.

As a second messenger, Ca^{2+} participates in many intracellular actions, which is essential for conveying intracellular information and modulating cell metastasis. The transport of Ca^{2+} across the plasma membrane is finished by three kinds of transport proteins: Ca^{2+}-ATPase, Ca^{2+}/H^+ antiporter and Ca^{2+} channel. Ca^{2+}-ATPase and Ca^{2+}/H^+ antiporter are in charge of efflux of Ca^{2+} across the plasma membrane, and Ca^{2+} channel is in charge of influx of Ca^{2+}[12]. It is known that, in biology systems, rare earth elements can represent similar physiological action as Ca^{2+}, and replace Ca^{2+} binding on the plasma membrane surface, and block the efflux and influx of Ca^{2+}[13]. Several studies indicate that La^{3+} inhibits Ca^{2+}-ATPase activity[14], and rare earth elements are Ca^{2+} antagonist and Ca^{2+} channel blocker of cell membrane surface[15]. However, the mechanism of action is not clear yet.

PM Ca^{2+}-ATPase, acting as specific active transport system of Ca^{2+} on plasma membrane of higher plant, can directly hydrolyze ATP and actively transport Ca^{2+}, therefore it plays an important role in low-

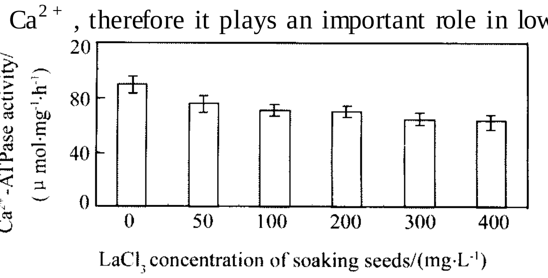

Fig. 2 Effects of La^{3+} on PM Ca^{2+}-ATPase activity of *Casuarina equisetifolia* seedlings under acid rain stress (pH 4.5)

ing the Ca^{2+} concentration of plasma and maintaining Ca^{2+} concentration at a stable level (the cytosolic concentration of free Ca^{2+} is about 0.2 μmol·L^{-1}). In plasma membrane, Ca^{2+}-ATPase activity can be enhanced by calmodulin[1].

In this experiment, the reasons that La^{3+} inhibits the activities of PM Ca^{2+}-ATPase may be: Firstly, the rise of PM Ca^{2+}-ATPase activities increases its resistance to acid rain stress. One of our experiments showed that compare to *Casuarina equisetifolia* seedlings without the acid rain stress, the seedlings treated with acid rain stress with pH 4.5 have a higher value of PM Ca^{2+}-ATPase activities. Owing to using rare earth elements, plant's sensitivity to the acid rain is reduced[3], the sensitivity of PM Ca^{2+}-ATPase to the acid rain can be reduced, and keep the activities of PM Ca^{2+}-ATPase at a relatively low level by substituting Ca^{2+} with La^{3+}. Secondly, as La^{3+} is the antagonist of Ca^{2+} on PM surface, the position of Ca^{2+} can be occupied by La^{3+} competively, blocking the efflux and influx of Ca^{2+} in the cell, and influencing Ca^{2+} signal transmission, which hinders the activation of Ca^{2+} binding proteins such as calmodulin, and inhibits Ca^{2+}-ATPase activity. Thirdly, Ca^{2+} has a relatively higher dissociation constant with PM than that of La^{3+}, and La^{3+} can replace the bond position of Ca^{2+} in PM. The electricity of La^{3+} is higher than that of Ca^{2+}, so this kind of replace may result in the changes of PM electric potential and then indirectly influence the activities of PM Ca^{2+}-ATPase. Fourthly, acid rain can bring some damage to the PM lipid, and the stress can affect the structure and function of plasma membrane lipid. Some studies have showed that the Ca^{2+}-ATPase activities of the purified plasma membrane without lipid would be inhibited more and more strongly by La^{3+} with increasing concentrations[6]. It can be supposed that, the decrease of PM Ca^{2+}-ATPase activities by La^{3+} might be owing to the abnormal change of plasma membrane lipid environment brought by acid rain, and the inhibition of the activities are strengthened with the increasing La^{3+} concentrations.

3 Conclusions

1. The growth including height, length of roots, fresh weight and PM H^+-ATPase activities of *Casuarina equisetifolia* seedlings are stimulated and increase by the treatments of soaking seeds for 8 h in $LaCl_3$ with lower concentrations, and their peak values all appear with 200 mg·L^{-1} La^{3+}. However, those are inhibited by higher La^{3+} concentrations. There is remarkable positive correlation between H^+-ATPase activity and plant height. La^{3+} activates PM H^+-ATPase is one of the reasons that La^{3+} can accelerate the growth of cell enlarging. La^{3+} can enhance H^+-ATPase activity, alleviate cytosolic acidification of plant under acid rain stress and indirectly maintain the stability of intracellular environment.

2. La^{3+} inhibits PM Ca^{2+}-ATPase activity, and the inhibition is strengthened with the increasing La^{3+} concentrations from 50 to 400 mg·L^{-1}. The replacing of Ca^{2+} with La^{3+} in PM could weaken the sensitivity of Ca^{2+}-ATPase to acid rain stress, and hinder calmodulin activation, and change membrane voltage, which may lower Ca^{2+}-ATPase activity. And the decreased activity also is due to the abnormal membrane lipid environment brought by acid rain.

3. In order to resistant to acid rain stress and accelerate the growth of *Casuarina equisetifolia*, the suitable concentration range of $LaCl_3$ treatment is 50～200 mg·L^{-1}.

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Influence of RE on Cohesive Force of Spray Coating from One-shot Self-felt Iron-Based Composite Powder

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Abstract : The strength of coating layer obtained by one-shot self-felt powder with subsonic oxygen-acetylene spraying technique was discussed. Due to self-heating effect among elements in powders and micro-alloying , the highest strength is gotten when RE contents in the one-shot self-felt powder are 0.9 %. The experimental results show that coating layer could be purified by adding proper amounts of RE , which make the inclusion change from reticulate pattern to spherical. RE-compound could be formed when the RE acted with other elements , which make the melting point of powders fall down and the wetting ability could be increased , which should be beneficial to density coating layer and the wear-ability could be increased.

Key words : inorganic materials ; self-felt ; composite powder ; wear-ability ; rare earths

(See J. Chin. RE. Soc. (in Chin.) , 2003 , 21(6) :733 for full text)

Effects of Rare Earth Elements La and Ce on Ti-Ni Alloy

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Abstract : Rare earth elements Ce and La were added into the near-equal atomic titanium-nickel alloy. Differential scanning calorimetry and X-ray diffraction were applied to study the effect of the Ce and La. The results show that adding a small amount of Ce into TiNi alloy can improve the phase transformation obviously , but the phases in TiNi alloy do not change. The results show that adding a small amount of Ce or La can improve the properties of titanium-nickel alloy.

Key words : metal materials ; TiNi alloy ; phase transformation ; rare earths

(See J. Chin. RE. Soc. (in Chin.) , 2003 , 21(6) :736 for full text)