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木麻黄质膜转运系统对酸雨胁迫的响应及调控

Responses of Plasma Membrane Transport Systems of *Casuarina equisetifolia* to Acid Rain Stress and Regulation

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摘　要

酸雨是全球性大气污染环境问题之一, 由于酸雨对生态系统影响的复杂性，酸雨污染的危害及机制还远未得到充分认识。木麻黄防护林是东南沿海最重要的防风固沙林，在改善当地生态环境和促进经济发展上发挥了难以取代的作用。通过水双相分配法提取高纯度质膜，首次从木麻黄幼苗质膜转运系统的活性响应角度研究酸雨胁迫以及酸雨和NaCl双重胁迫对木麻黄的危害及调控机制，探索植物对酸雨及盐胁迫的信息感受与传递机制，并分析酸雨胁迫对木麻黄－土壤体系的有机溶质和微量元素代谢影响特点。主要研究结果如下：

酸雨对盆栽普通木麻黄（*Casuarina equisetifolia*）幼苗的生长有影响。在系列酸度（pH4.5～2.5）酸雨处理三个月后，与对照相比，木麻黄幼苗地上部的表观生长和鲜重并没有显著变化，但其根系伸长显著受抑制，单株干物质积累明显变少，而且木麻黄的结瘤水平显著降低。

木麻黄幼苗嫩枝质膜 H^+-ATPase 和 Ca^{2+}-ATPase 对酸雨非常敏感，常随酸雨强度的加大其活性受愈来愈强的抑制。pH4.5～2.5 酸雨处理使质膜 H^+-ATPase 活性被抑制 76.64%～85.88%，而 Ca^{2+}-ATPase 活性仅为对照的 14.12%～4.7%。在 pH3.0 酸雨处理组，质膜 H^+-ATPase 和 Ca^{2+}-ATPase 活性却出现分别高于对照 175.32%和 91.28%的反弹升高，这可能是木麻黄－盆土系统经一定强度和一定时间的酸雨累积胁迫后，木麻黄表现出的一种抗逆性应激生理反应。

酸雨严重影响盆栽木麻黄幼苗嫩枝质膜上氧化还原系统的活性。pH4.5～2.5 酸雨胁迫下，NADH 氧化酶、$Fe(CN)_6^{3-}$ 还原酶和 EDTA -Fe^{3+}还原酶以及质膜硝酸还原酶活性随酸雨 pH 值的变化呈相似的变化趋势，一般随酸雨胁迫强度（酸度）的增大，首先表现为活性降低，随着酸雨胁迫强

度的累积，逐渐产生活性应激性反弹回升现象，但在高强度酸雨胁迫后活性又还是下跌。酸雨胁迫三个月后木麻黄幼苗小枝质膜氧化还原酶的活性峰值均在 pH3.0 处，而活性最低一般位于 pH2.5 处（除硝酸还原酶活性最低谷位于 pH4.5 处外）。质膜氧化还原酶对酸雨的应激性反馈行为略早于质膜离子泵对酸雨的响应。

pH4.5 酸雨和 NaCl(2~8g/kg 土)双重胁迫促使盆栽木麻黄幼苗质膜嫩枝 H^+－ATPase 和 Ca^{2+}-ATPase 活性均高于其在 pH4.5 酸雨单因子胁迫下的活性，这显示出轻度酸雨和 NaCl 双重胁迫可以部分缓解酸雨对木麻黄幼苗生理的胁迫作用。pH3.0 高酸度酸雨和 NaCl 胁迫却对木麻黄质膜 H^+－ATPase 和 Ca^{2+}-ATPase 表现出协同作用效应，使 H^+－ATPase 和 Ca^{2+}-ATPase 活性均低于其在 pH3.0 酸雨单因子胁迫下的活性。在 pH4.5 酸雨胁迫和 NaCl(2~8g/kg 土)双重胁迫下，木麻黄小枝 N、P、K、Ca 含量均随 NaCl 胁迫强度的增大而呈上升趋势，其中 K、Ca 含量显著高于酸雨单因子胁迫，N、P 含量与 NaCl 胁迫浓度呈显著正相关关系；而在 pH3.0 酸雨和 NaCl 双重胁迫下，木麻黄小枝 N 含量随 NaCl 胁迫浓度的加大而减少，二者呈显著负相关，K 含量随 NaCl 胁迫强度的增大而上升，P、Ca、Mg 的含量无显著变化。pH4.5 酸雨胁迫和 NaCl(2~8g/kg 土)双重胁迫下，质膜 H^+－ATPase 和 Ca^{2+}-ATPase 活性与小枝 K 和 Mg 含量存在显著正相关关系，与小枝 N 含量存在正相关，与 Ca 含量存在较显著负相关，与 P 存在不显著负相关，其中质膜 H^+－ATPase 活性与小枝 K 含量的相关性达到极显著水平。pH3.0 酸雨和 NaCl 双重胁迫下，质膜 H^+－ATPase 和质膜 Ca^{2+}-ATPase 活性与 N、P、K、Ca 含量存在正相关，与 Mg 含量存在显著负相关关系。可见植物体吸收、运输以及转化累积营养元素是复杂的生理生化过程。

对盆栽木麻黄喷施 5~250mmol·L^{-1} 的 Ca^{2+}可以部分解除酸雨胁迫对质膜 H^+-ATPase 和 Ca^{2+}-ATPase 活性的抑制作用。推测外源 Ca^{2+}调控质膜 H^+-ATPase 和 Ca^{2+}-ATPase 对逆境的积极应答行为的原因包括：1）Ca^{2+}通过

减轻酸雨胁迫下膜脂的伤害来减缓酸雨胁迫对质膜 H^+-ATPase 和 Ca^{2+}-ATPase 活性的抑制作用；2）由于外源钙处理可以提高植物组织和细胞的游离钙水平，促进 Ca^{2+}发挥胞内第二信使功能。而且胞内游离 Ca^{2+}与 Ca^{2+}-ATPase 的相互作用，使钙循环得以维持，从而介导持久性的细胞反应。因此，虽然喷施 Ca^{2+}只是短暂的行为却可以有持久延续的效果；3）促使质膜 H^+-ATPase 和 Ca^{2+}-ATPase 的自抑制区解除自抑制功能，增强 H^+-ATPase 和 Ca^{2+}-ATPase 的活力表达；4）增加水解底物，提高对底物的水解能力；5）胞外钙离子亦可作为第一信使(胞外信使)，直接调控离子泵活性。Ca^{2+}还可能影响膜电位的变化以及通过调节有关基因的表达而实现其对质膜离子泵的调控作用。质膜 Ca^{2+}-ATPase 活性受激后，又将引发信号转导反应后的过量 Ca^{2+}运出细胞质，以完成刺激信号的灭活。

在 La^{3+}浓度为 50～200 mg•L^{-1} 范围浸种后,于 pH4.5 酸雨条件下沙培，木麻黄幼苗的株高、根长以及鲜重随 La^{3+}浓度的加大而明显增强，在 200 mg•L^{-1} 处理达到峰值，在 400 mg•L^{-1}[La^{3+}] 浸种处理后相对于对照表现出抑制效应;幼苗干重对 La^{3+}的响应与此相似,但其增长的峰值后移于 300mg•L^{-1} 处理点。PM H^+-ATPase 活性在 50～200 mg•L^{-1}[La^{3+}] 浸种后逐渐增强，在[La^{3+}] 达到 300～400 mg•L^{-1} 时，其活性受到抑制。实验结果还体现 H^+-ATPase 活性与细胞的伸长生长呈显著正相关关系，La^{3+}对 H^+-ATPase 的活化作用有助于促进植物的生长，且适量的 La^{3+}具有缓解酸雨胁迫下生长的植物细胞质酸化，维持细胞内环境稳定的间接作用。La^{3+}抑制幼苗 PM Ca^{2+}-ATPase 的活性，且随着 La^{3+}浓度的增加，抑制作用越来越强，推测主要是由于 La^{3+}对质膜上 Ca^{2+} 具竞争性替代作用所致。在 La^{3+}浓度为 50～200 mg•L^{-1} 范围浸种可以明显提高幼苗质膜 NADH 氧化酶和质膜硝酸还原酶活性，但较高浓度 La^{3+}浸种（300～400 mg•L^{-1}）会对它们产生抑制或波动上升等负效应；而在 La^{3+}浓度为 50～400 mg•L^{-1} 范围浸种后其幼苗质膜 EDTA -Fe^{3+}还原酶活性均显著提高。La^{3+}同时能降低植物细胞膜的透性，

对处于酸雨胁迫下的细胞膜有抑制电解质外渗的保护功能。为抗酸雨和促生长，木麻黄经 La^{3+}浸种 8h 的最适宜 [La^{3+}]为 50～200 mg•L^{-1}。

在系列 pH 值模拟酸雨处理后，盆栽木麻黄幼苗小枝可溶性糖在低酸度酸雨胁迫后略低于对照，在中度和重酸度酸雨胁迫后，含量显著高于对照；脯氨酸和总氨基酸含量增加，各酸雨处理组的含量均高于对照，而且在中度和强度酸雨胁迫下，与对照相比，脯氨酸含量占总氨基酸含量的比值明显增大；谷胱甘肽和可溶性蛋白质含量亦明显增加。逆境因子作用于植物时，都会直接或间接地引起植物水分胁迫，而水分胁迫的主要危害是渗透胁迫。结果表明，在酸雨胁迫下，木麻黄幼苗具有积累较高浓度的有机溶质，改善渗透调节的生理应答能力。

轻度酸雨（pH4.5~4.0）胁迫下，木麻黄小枝含硫量随酸雨强度的增大而增加，比对照增加了 8.15~11.10%，但随酸雨强度的继续加大，其含硫量明显下降，比对照降低 4.85~14.13%。酸雨虽然增加了外源硫的输入量，但中强度酸雨胁迫阻碍了植物体内硫的正常代谢，对外源硫的利用效率降低。在系列酸度的酸雨胁迫条件下，木麻黄小枝中 Cr、Cd、Pb 含量有出现在轻酸度（pH4.5）或重酸度（pH2.5）酸雨胁迫时比对照含量显著升高现象，而微量金属元素 Cu、Zn、Fe、Mn、Al 在木麻黄小枝中的含量通常都低于对照组含量。

三个月的酸雨使木麻黄－土壤体系的土壤 pH 值随酸雨强度的增大而下降，土壤电导率升高。各酸雨处理组土壤的 Cu、Zn 含量均比略低于对照，而各处理组土壤的 Pb 含量均高于对照组 Pb 含量，Fe、Mn、Al、Cr、Cd 含量随酸雨酸度的加大或升或降，无一定规律性。在所有 pH4.5~pH2.5 酸雨处理组土壤的有效铁含量均低于对照组的有效铁含量，而且受酸雨胁迫后土壤有效铁在总铁中的含量均低于无酸雨胁迫组。酸雨胁迫后，土壤活性锰含量比对照组活性锰含量增加 80.83～163.23%，而且加大了土壤活性锰占总锰含量的比率。对所测定的土壤微量金属含量与木麻黄小枝中相应

的微量金属含量进行相关分析，发现二者呈正相关关系，但相关性未达显著水平。总之，三个月酸雨胁迫后，已明显体现出土体中可供给植物利用的铁含量降低，植物受活性锰及有毒重金属危害的风险性加大。

关键词：酸雨；普通木麻黄（*Casuarina equisetifolia*）；质膜 H^+-ATPase；质膜 Ca^{2+}-ATPase；质膜氧化还原酶；Ca^{2+}调控；La^{3+}调控

Abstract

Acid rain is one of serious environmental problems in the world. However, its dangers and pollution mechanisms haven't been completely understood yet, because of the complexity of the influences of acid rain on eco-system. Casuarina shelter forests are very important to breaking wind and fixing sand in China's southeastern seashore. They play a remarkable role in improving the ecological environment and promoting the economic development. Obtained the highly purified plasma membrane vesicles from *Casuarina equisetifolia* seedlings cultivated under artificial acid rain stress as experiment materials, which isolated by aqueous two-phase partitioning methods, the study first focused on the responses of plasma membrane transport system activities to acid rain stress or the double stresses of acid rain and NaCl, to study the harm to *Casuarina equisetifolia* done by those stresses and the chemical regulation of the impair, and to probe into the mechanism of receiving and transferring the stress signals. The study also analysed the effects of acid rain to the organic solutes and trace elements metabolize in *Casuarin equisetifolia* –soil system. The mainly results of the study are as following:

There were some effects of acid rain on the growth of *casuarina equisetifolia* seedlings based on pot experiments. In comparison with control, there isn't significant change of the apparent growth of above ground seedling and fresh weight of the seedling, but the elongation of root system and the dry matter accumulation of the individual seedling were obviously reduced, and the levels of producing nodule also markedly fell under the acid rain stress with a series of acidity（pH4.5～2.5）for 3 months.

The plasma membrane H^+-ATPase and Ca^{2+}-ATPase of *Casuarina equisetifolia* seedling were both sensitive to acid rain, and the inhibition of their activities were more and more strongly with the acidity increase of acid rain generally. pH4.5～2.5 acid rain led to the reduce of PM H^+-ATPase activities to 76.64%～85.88% and made the PM Ca^{2+}-ATPase activities 14.12%～4.7% of CK. But pH3.0 acid rain enhanced H^+-ATPase and Ca^{2+}-ATPase activities 175.32%and 91.28% separately. The rebound was probably due to a kind of physiological stimulated respond to resist stress of the *Casuarina equisetifolia* seedlings when the intension of acid rain stress accumulation to a certainty in the system of *Casuarina equisetifolia* and pot soil after period of time.

Acid rain brought obvious effects on the plasma membrane redox system of potted *Casuarina equisetifolia* seedling. The activities of NADH oxidase, $Fe(CN)_6^{3-}$ reductase, EDTA -Fe^{3+} reductase, and nitrate reductase have similar responses to pH4.5～2.5 acid rain. In generally, with the acidity of acid rain increased, it showed the activities decreased at first, then with the accumulation of acid rain stress intension, the activities were stimulated to rebound higher than CK, while the activities decreased severely under the acid rain stress with high acidity. The peak values of the plasma membrane redox enzymes activities all appeared at pH3.0 acid rain treatment, and the lowest values all represented at pH2.5 acid rain treatment except for nitrate reductase (the lowest value of nitrate reductase activities represented at pH4.5 acid rain treatment). The results also showed that the feedback actions with stimulation of the plasma membrane redox enzymes to acid rain are earlier than those responses of plasma membrane ion pumping to acid rain.

The PM H^+-ATPase and Ca^{2+}-ATPase activities of *Casuarina equisetifolia* seedling under the double stresses of pH4.5 acid rain and NaCl

(2~8g/kg soil) were both higher than those under the single stress of pH4.5 acid rain, which indicated the double stresses of acid rain with low acidity and NaCl might partly alleviate the single acid rain stress on the physiological process of *Casuarina equisetifolia*. While acid rain stress with high acidity(pH3.0) might cooperate with the NaCl stress, which brought severe effects on PM H^+-ATPase and Ca^{2+}-ATPase activities of *Casuarina equisetifolia* seedling, and the ATPases represented lower activities than those under the single stress of pH3.0 acid rain. The contents of N, P, K, Ca in *Casuarina equisetifolia* seedling twigs increased with the treatment concentration of NaCl increased under the double stresses of pH4.5 acid rain and NaCl, the contents of K and Ca under the double stresses were significantly higher than those under the single acid rain stress, and the contents of N and P have significant positive correlation with the content of NaCl. While under the double stresses of pH3.0 acid rain and NaCl, the content of N in *Casuarina equisetifolia* seedling twigs decreased with the content of NaCl increasing, and there is a negative correlation between them. With the content of NaCl increasing, the content of K rose, but the contents of P, Ca and Mg have no significant change under the double stresses. Both of the PM H^+-ATPase and the PM Ca^{2+}-ATPase activities of *Casuarina equisetifolia* seedling twigs had significant positive correlation with the contents of K and Mg, and had positive correlation with the contents of N, and had comparatively negative correlation with Ca, and had negative correlation with no significance with P in *Casuarina equisetifolia* seedling twigs under the double stresses of pH4.5 acid rain and NaCl. In which the correlation between the PM H^+-ATPase activities and the content of K reached the highest significant level. While under the double stresses of pH3.0 acid rain and NaCl, both of the H^+-ATPase and the Ca^{2+}-ATPase activities had positive correlation with the contents of N, P, K, and

Ca respectively, and had significant negative correlation with the content of Mg. The analyses indicated that the physical and physiochemical process of absorbing, transporting and transforming the nutrition elements in plant is a complicated process.

Applying 5~250mmol • $L^{-1}Ca^{2+}$ to spray the potted *Casuarina equisetifolia* seedlings could partly relieve the inhibition to the H^+-ATPase and Ca^{2+}-ATPase activities from acid rain stress. The reasons that the positive response of the H^+-ATPase and Ca^{2+}-ATPase by outside Ca^{2+} modulating were supposed including that as following: 1）Ca^{2+} could alleviate the hurt brought to plasma membrane lipid by acid rain , which would relieve the inhibition of the H^+-ATPase and Ca^{2+}-ATPase activities. 2）The content levels of free Ca^{2+} in the tissues and in the cells were enhanced by outside Ca^{2+} treatments. The cellular Ca^{2+}recycle could be maintained by the reciprocity of Ca^{2+} and Ca^{2+}-ATPase, which lead to the durative reaction in the cells. Therefore, there was a continued impact on the plant, although the seedlings were sprayed with Ca^{2+} only for a short time. 3）Ca^{2+} might relieve the autoinhibition function of the H^+-ATPase and Ca^{2+}-ATPase autoinhibitory domain, which could increase the express of H^+-ATPase and Ca^{2+}-ATPase activation. 4）The contents of substance hydrolyzed might increase by the treatment with Ca^{2+}, and also the ability to hydrolyze substance might enhance. 5）As a first messenger of transporting the signal(extracellular messenger), the outside Ca^{2+} also can directly modulate the ATPases activities. Ca^{2+} can also modulated the ATPases activities by changing the plasma membrane potential and regulating the express of related gene. The activation of plasma membrane activities might pump the excessive Ca^{2+} out of the cytoplasm after Ca^{2+} stimulated the signal transduction reaction, which helped to make the stimulating signals lessened.

The effects of lanthanum on the growth of sand-cultured *Casuarina equisetifolia* seedlings under acid rain stress with pH value 4.5 was obvious, and the growth including the height, length of roots, and fresh weight of *Casuarina equisetifolia* seedlings was promoted gradually by soaking seeds for 8h in La^{3+} solution with the increasing concentrations from 50 to 200 mg•L^{-1} , and in the treatment of 200mg•L^{-1} La^{3+}, all those values reach the highest points. But in the treatment of 300mg•L^{-1} La^{3+} , the effects descend. As to treating with 400 mg•L^{-1} La^{3+} , the plant growth were inhibited. and the dry weight had similar response, but its peak value of increase was backward in treating with 300 mg•L^{-1} La^{3+}. The PM H^{+}-ATPase activites were stimulated by treatment with 50～200 mg•L^{-1} La^{3+},and strongly inhibited by 300～400 mg•L^{-1} La^{3+}. The results also revealed that the H^{+}-ATPase activity and the growth of cell enlarge have a remarkable positive correlativity，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. PM Ca^{2+}-ATPase activity decreased with the treatments of La^{3+} in the range of 50-400mg•L^{-1}, and the inhibition was strengthen with the increasing La^{3+} concentrations. The main reason was supposed that La^{3+} can competively substituted for Ca^{2+} on the plasma membrane. The NADH oxidase activities and the Nitrate reductase activities of *Casuarina equisetifolia* seedlings can be stimulated with the treatment by soaking seeds for 8 hours in a series of La^{3+} solution when La^{3+} concentrations is in the range of 50～200 mg•L^{-1}, but their activities are inhibited or fluctuate by the higher La^{3+} concentrations; The EDTA -Fe^{3+} reductase activities can be stimulated by La^{3+} concentrations in the range of 50～400 mg•L^{-1}. The research also revealed that La^{3+} can reduce 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. 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}.

After the potted *Casuarin equisetifolia* seedlings were treated with simulated acid rain and different pH gradients for three months, the soluble sugar contents in seedling twigs under acid rain stress with low acidity were lower than that of control, however, the soluble sugar contents under moderate and intensive acid rain stress were significantly higher than that of control. Proline and total amino contents of the twigs increased with the treatment by acid rain and the values of every group under the stress was higher than that of control. In comparison with control, the Proline and total amino contents were intensively increased under moderate and intensive acid rain stress, and soluble protein and GSH contents increased as well. Unfavorable factors will inevitably lead to water stress direct or indirectly, and osmosis stress is the main harm to plant. In this thesis, the results indicated that high concentration organic matter in *Casuarin equisetifolia* seedlings is accumulated to improve the physiological responses of osmoregulatants.

The sulfur content of seedling twigs of *Casuarin equisetifolia* under mile acid rain stress(pH4.5~4.0) increased by 8.15-11.10%, while it decreased by 4.85~14.13% as the further increase of acid rain stress. Acid rain adds extraneous sulfur, but intensive acid rain stress for three months impeded the normal metabolism of sulfur and reduced the use efficiency of sulfur. In comparison with control, Cr, Cd and Pb contents of the seedling twigs increased under mild stress(pH4.5) and intensive stress(pH2.5), while the trace element contents like Cu, Zn, Fe, Mn and Al were normally lower than those of controls.

The acid rain reduced the pH of soil and enhanced the conductance efficiency of soil in *Casuarin equisetifolia* –soil system. Cu and Zn contents of

the soil under acid rain stress were a little lower than those of controls, while Pb contents were higher than that of controls, and Fe, Mn, Al, Cr, and Cd contents increased or decreased with acid rain stress. Among all the groups treated with acid rain of pH4.5~pH2.5, the available Fe contents were lower than those of controls. Furthermore, the ratios of available Fe contents/total Fe were lower than those of controls. Acid rain led to the increase of available Mn contents by 80.83～163.23％ in comparison with controls and enhanced the ratio of available Mn/total Mn. There was a positive correlation between the measured trace metal contents in soil and the corresponding trace metal contents in seedling twigs, but this correlation was not significant. Above all, after three months' acid rain stress, the available Fe contents in soil decreased significantly, and the hazard of available Mn and noxious heavy metal to plants enhanced.

Key words: acid rain; *Casuarina equisetifolia*; plasma membrane H^+-ATPase; plasma membrane Ca^{2+}-ATPase; plasma membrane redox enzymes; Ca^{2+} modulation; La^{3+} modulation

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