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厦门大学

硕士学位论文

钙及模拟酸雨对酸雨敏感及抗性树种四种钙相关基因表达的影响

Effects of Calcium and Simulated Acid Rain on Four Calcium-related Genes Expression of Sensitive and Tolerant Trees

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摘 要

对外界环境的适应是植物生长和发育过程中非常重要的特征。现今，酸雨已经是世界范围的严重环境问题，中国亦不例外。研究植物对酸雨响应的分子机制是酸雨研究的重要内容。本实验中，我们选取了在我国南方广泛分布的木本植物作为研究对象，分别是枫香（*Liquidambar formosana* Hance）、木荷（*Schima superba* Gardn et Champ）、马尾松（*Pinus massoniana* Lamb）以及南方红豆杉（*Taxus wallichiana* var. *mairei* (Lemée & H. Léveillé) L. K. Fu & Nan Li ）。应用实时荧光定量 PCR 技术分别研究上述四个物种的四个钙相关基因表达水平在不同钙浓度下对酸雨胁迫的响应，这四个基因分别是钙调素基因（*calmodulin9*，*CAM9*），类钙调磷酸酶 B 互作蛋白激酶基因（*Calcineurin B-Like protein interacting protein kinase1*，*CIPK1*），内质网型钙离子转运 ATP 酶基因（*endoplasmic reticulum-type calcium-transporting ATPase1*，*ECA1*）和钙网蛋白基因（*calreticulin1*，*CRT1*）。实验中钙水平设置为低钙 2.23 mmol/kg、正常钙 19.98 mmol/kg 和高钙 102.23 mmol/kg 三个水平；以 pH5.6 为对照，pH3.0 为模拟酸雨，植物幼苗经过酸雨处理后，在第一、三和七天收集植物叶片，用于基因表达水平的分析。

实验结果表明在中钙条件下，每个物种的 *CAM9*、*CIPK1*、*ECA1*、和 *CRT1* 都受酸雨的显著影响。同时这四个基因也受到钙浓度的影响。模拟酸雨处理下四种木本植物叶片中 *CAM9* 和 *CIPK1* 这两个基因的表达水平都随着钙浓度的增加而明显降低，*ECA1* 和 *CRT1* 的表达则随着钙浓度的增加而提高。相反，*CAM9* 和 *CIPK1* 的表达会随着钙浓度的降低而升高，*ECA1* 和 *CRT1* 的表达则出现减少的现象。与抗性树种木荷和红豆杉相比，敏感树种枫香和马尾松更易受到酸雨和钙浓度的影响，其基因表达变化幅度大；在高钙情况下，敏感树种四个钙相关基因表达在时间上比抗性树种早；而低钙时，敏感树种四个钙相关基因表达在时间上则比抗性树种慢。

以上结果表明植物可以通过对钙相关基因表达的调控响应酸雨的伤害，补钙可以明显增强植物对酸雨的耐受能力。与抗性树种相比而言敏感树种比较容易受酸雨的伤害，同时也比较容易受到钙水平的影响。

关键词：酸雨；钙；钙相关基因；敏感物种；抗性物种

Abstract

In plants, adaptation to stressful habitats is considered as a tactic to growth and development. Nowadays, acid rain has been a serious environmental problem in China. Therefore, studying and predicting the responses of plants to acid rain and improving the growth of plants are of important topics. We used quantitative real time-PCR to study relative expression of four calcium-related genes, including *calmodulin9* (*CAM9*), *CBL-interacting protein kinase1* (*CIPK1*), *endoplasmic reticulum-type calcium-transporting ATPase1* (*ECA1*), *calreticulin1* (*CRT1*). The plant species are the acid rain sensitive species including *Liquidambar formosana* Hance and *Schima superba* Gardn et Champ and the tolerant species including *Pinus massoniana* Lamb and *Taxus wallichiana* var. *mairei* (Lemée & H. Léveillé) L. K. Fu & Nan Li. All plant materials were treated by the simulated acid rain with pH=3.0 (pH=5.6 was used as control), in which three calcium levels including low calcium with 2.23mmol/kg, medium calcium with 19.98mmol/kg and high calcium with 102.23mmol/kg were set for LCa, MCa and HCa. Samples were collected on the first, third and seventh day after SiAR treatment. And then quantitative real time-PCR was used to study the relative expression of four calcium-related genes in the selected four species under SiAR at three calcium levels.

In medium calcium level, *CAM9*, *CIPK1*, *ECA1* and *CRT1* changed significantly under acid rain. Also, the four genes were affected by different calcium levels. Treated by simulated acid rain, the expression of the *CAM9* and *CIPK1* in four trees decreased significantly. Meanwhile, *CRT1* and *ECA1* performed higher levels with increasing concentration of the calcium. On the contrary, reducing the calcium concentration, the expression of *CAM9* and *CIPK1* revealed higher levels, *CRT1* and *ECA1* decreased significantly. Compared with the calcium-related gene expression change of tolerant species, *S. superba* and *T. wallichinana* var. *mairei*, the gene expression change of *L. formosana* and *P. massoniana* were more significant. We also

found calcium-related genes of most the sensitive species showed earlier response to simulated acid rain than that of the tolerant species under enough calcium condition. Meanwhile under calcium deficiency condition the calcium-related genes of sensitive species were later than that of the tolerant species. The results showed that plants could utilize calcium to alleviate the damage of acid rain by the regulation of calcium-related genes and calcium supplement enhanced the plant resistance to rain acid significantly. The sensitive species were more vulnerable to acid rain, also were affected significantly by calcium concentration than the tolerant species.

Keywords: sensitive species, tolerant species, acid rain, calcium, calcium-related genes