

樟树钙调蛋白基因 CAM 的克隆与表达

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摘要: 当今, 干旱现象频发, 影响的范围极广, 持续的时间很长。干旱胁迫对植物的危害很大。钙调蛋白 (calmodulin, CAM), 是一种耐热的小分子蛋白质, 钙调蛋白本身是没有酶活性, 但是与钙离子结合后, 便可作为第二信使去调节植物的各种生理活动。CAM 对植物生长发育有重要作用, 它能应对包括干旱在内的许多生物和非生物胁迫, 在干旱胁迫下高度表达。本课题通过从樟树中克隆 CAM 基因, 分析它与其他植物 CAM 基因的同源性, 对该基因进行序列分析, 以及研究该基因在干旱胁迫下的表达模式, 为樟树的干旱生理研究及耐旱樟树新品种选育奠定基础。研究成果如下:

(1) *CoCAM1* 与 *CoCAM2* 基因序列总长都为 454bp, 都编码 149 个氨基酸。*CoCAM1* 与华盖木、无油樟、罂粟、油棕的同源性 80% 以上, 其中与华盖木同源性尤为显著, 达到了 90.60% 的高水平。*CoCAM2* 与华盖木、澳洲坚果、蒂罗花、莲的同源性 80% 以上, 其中与华盖木同源性尤为显著, 达到了 91.28% 的高水平。

(2) 预测出 *CoCAM1* 的氨基酸分子式为 $C_{727}H_{1144}N_{190}O_{255}S_{10}$, 分子量为 16946.80, 等电点 4.14, 不稳定系数 27.39。*CoCAM2* 的氨基酸分子式为 $C_{728}H_{1146}N_{190}O_{255}S_{10}$, 分子量为 16960.83, 等电点 4.15, 不稳定系数 29.58。预测出 *CoCAM1* 和 *CoCAM2* 均定位于细胞核, 均无信号肽, 均为亲水蛋白质;

(3) *CoCAM1* 蛋白质的二级结构: α 螺旋占 61.74%, β 折叠占 5.37%, β 转角占 9.40% 和无规则卷曲占 23.49%。*CoCAM2* 蛋白质的二级结构: α 螺旋占 64.43%, β 折叠占 3.36%, β 转角占 8.72% 和无规则卷曲结构占 23.49%;

(4) *CoCAM1* 和 *CoCAM2* 蛋白质都具有哑铃型的三级结构, N 端和 C 端有两个相似的球型结构域。并且每个球型结构域中存在两个 EF-Hand 结构域。

(5) 荧光实时定量 PCR 试验显示, 在干旱胁迫下, *CoCAM1* 和 *CoCAM2* 的表达量均增加。

关键词: 樟树; 干旱胁迫; CAM 基因; 基因克隆; qPCR; 基因表达

Cloning and expression of *CoCAM* gene in *Cinnamomum Camphora*

Abstract: Nowadays, drought phenomena occur frequently, with a wide range of impacts and a long duration. Drought stress poses great harm to plants. Calmodulin (CAM) is a heat-resistant small molecule protein that does

not have enzymatic activity. However, when it binds to calcium ions, it can act as a second messenger to regulate various physiological activities in plants. CAM plays an important role in plant growth and development, as it can cope with many biotic and abiotic stresses, including drought, and is highly expressed under drought stress. This project aimed to clone CAM gene from *Cinnamomum Camphora*, analyze its homology with other plant CAM genes, perform sequence analysis on the gene, and study its expression pattern under drought stress, laying the foundation for the study of drought physiology in *Cinnamomum Camphora* and the breeding of drought tolerant new varieties. The research results were as follows:

(1) The total length of the *CoCAM1* and *CoCAM2* gene sequences was 454 bp, both encoding 149 amino acids. *CoCAM1* had over 80% homology with *Manglietiastrum sinicum*, *Amborella trichopoda*, *meconium*, and oil palm, with a particularly significant homology of 90.60% with *Manglietiastrum sinicum*. *CoCAM2* had a homology of over 80% with *Manglietiastrum sinicum*, *Macadamia*, *Tilapia flowers*, and *lotus*, with a particularly significant homology of 91.28% with *Manglietiastrum sinicum*.

(2) The predicted amino acid molecular formula of *CoCAM1* was $C_{727}H_{1144}N_{190}O_{255}S_{10}$, with a molecular weight of 16946.80, an isoelectric point of 4.14, and an instability coefficient of 27.39. The amino acid formula of *CoCAM2* was $C_{728}H_{1146}N_{190}O_{255}S_{10}$, with a molecular weight of 16960.83, an isoelectric point of 4.15, and an instability coefficient of 29.58. It was predicted that *CoCAM1* and *CoCAM2* were both located in the nucleus, without signal peptides, and are hydrophilic proteins;

(3) The secondary structure of *CoCAM1* protein consisted of 61.74% alpha helices, 5.37% beta folds, 9.40% beta turns, and 23.49% irregular curls. The secondary structure of *CoCAM2* protein consisted of 64.43% alpha helices, 3.36% beta folds, 8.72% beta turns, and 23.49% irregular curled structures;

(4) Both *CoCAM1* and *CoCAM2* proteins had dumbbell shaped tertiary structures, with two similar spherical domains at the N-terminus and C-terminus. And there were two EF-Hand domains in each spherical structural domain.

(5) Fluorescence real-time quantitative PCR assay showed that under drought stress, the expression levels of *CoCAM1* and *CoCAM2* increased.

Keywords: *Cinnamomum Camphora*; Drought stress; CAM gene; Gene cloning; qPCR; gene expression