研究论文

毛白杨乙酰-乙酰载体蛋白硫脂酶基因(PtFATB)的克 隆与表达分析

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植物脂肪酸合成的主要部位是叶绿体,叶绿体向外运输脂肪酸的种类和数量受到乙 酰-乙酰载体蛋白硫脂酶(FATB)控制。FATB 基因在植物生长过程起着非常关键的作 用。本研究以毛白杨为材料,将生物信息学知识和分子生物学手段相结合,首先利用 现有的杨树基因组 EST 序列库资源,通过同源序列搜索,经过多次拼接合并获得了 理论的杨树脂肪酸去饱和酶基因 PtFATB 序列全长,利用 RT-PCR 手段成功克隆得到 ▶浏览反馈信息 了毛白杨 FATB 基因全长编码序列 cDNA, 该 cDNA 全长 1,450 bp,包括起始密码子 ATG 和 144 bp 的5' 末端非编码区,终止密码子 TGA 和 40 bp 的 3' 末端非编码 区,开放阅读框编码 421 个氨基酸。通过 RT-PCR 半定量研究了PtFATB 在叶片组织 中的表达量最高,茎、根中的表达量依次降低。在低温、干旱、NaC1、ABA四种条件 下诱导生长 24 h,只有在低温的条件下发现 PtFATB 表达量略微降低,其他几种情 况未有变化,该结果表明PtFATB 呈组成型表达。上述结果为植物脂肪酸的基因工程 提供了基础。

关键词 毛白杨; 乙酰-乙酰载体蛋白硫脂酶; 电子和分子克隆; 反转录PCR; 表达 分析

分类号

Cloning and Expression Analysis of PtFATB Gene **Encoding the Acyl-acyl Carrier Protein Thioesterase in** Populus tomentosa Carr.

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Abstract

<P>Acyl-ACP thioesterases (FATs) terminates the fatty acid synthesis and allow the transport of fatty acids out of the plastids, which are the important determinants of cellular metabolism. FATB is a member of FAT enzymes that has been described previously in most of the plants. In silico cloning is a new method that utilizes the bioinformatics on the complete genome and available EST database. In this study, a fulllength cDNA clone of PtFATB gene was isolated from Populus tomentosa using this approach. It is 1,450 bp in length and the open reading frame encodes a peptide of 421 amino acids. The predicted amino acid sequence shows significant homology with those from other plant species, which contain typical domains owned by FATB proteins. The transcripts of PtFATB were abundant in leaves, and less in roots detected by using semiguantitative RT-PCR. When the shoots were subjected to the stress treatments (cold, dry, NaCl) and ABA (Abscisic acid), the expression of PtFATB was only slightly

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reduced under the treatment of low temperature. This suggests that the expression of PtFATB is in a constitutive fashion. This study provides the basis not only for the identification and characterization of this gene but also for the improvement of cold tolerance by controlling the expression of the PtFATB gene in trees in near future.

Key words Populus tomentosa Carr.; Acyl-acyl carrier protein thioesterase (PtFATB); in silico and molecular cloning; RT-PCR; expression analysis

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