

川乌头F3' 5' H基因的cDNA克隆与表达分析

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Cloning and Expression Pattern Analysis of Flavonoid-3',5'-hydroxylase Gene from *Aconitum carmichaeli*

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摘要 利用RT-PCR结合RACE技术,从川乌头(*Aconitum carmichaeli* Debx.)花朵中克隆到1个类黄酮-3',5'-羟化酶基因的cDNA全长序列,全长1 720 bp,包含1个编码506个氨基酸的开放阅读框,命名为Ac-F3' 5' H(GenBank登录号:JN635708)。序列分析表明Ac-F3' 5' H编码的氨基酸序列中包含有已确定的保守基序,包括CYP基序、I螺旋区和血红素结合区等。氨基酸序列比对显示Ac-F3' 5' H与其它物种的F3' 5' H有很高的序列相似性。以川乌头18S rRNA基因(FJ748878)为内参,通过半定量RT-PCR对Ac-F3' 5' H的时空表达模式进行了分析,结果显示Ac-F3' 5' H随花朵发育,表达量呈递增趋势,并且在正在开放的花朵中达到最高,而在根、茎、叶中不表达,推测该基因可能在调节川乌头蓝色花朵形成中发挥作用。

关键词: 川乌头 类黄酮-3' ′ 5' ′ -羟化酶基因 克隆 基因表达

Abstract: A novel Flavonoid-3',5'-hydroxylase (F3' 5' H) gene, named Ac-F3' 5' H (The accession number in GenBank: JN635708) was cloned from the flower of *Aconitum carmichaeli* Debx. using RT-PCR and RACE approaches. The cDNA sequence was 1 720 bp and included a whole open reading frame, which encoded 506 amino acids. Sequence alignment results showed that the Ac-F3' 5' H had three known motifs such as "PPGP" motif, I-helix, and P450 haem-binding. The amino acid sequence of the Ac-F3' 5' H has higher similarity with other F3' 5' H. Using the 18S rRNA (FJ748878) transcripts as an internal control, semi-quantitative RT-PCR analysis showed that the Ac-F3' 5' H mRNA was highly transcribed in late phase of flowers development, which was concomitant with the appearance of anthocyanins in flower tissue. The transcript was abundant in the purple flowers but absent in leaves or roots. This indicated that the Ac-F3' 5' H might play a role in regulating the forming of blue flowers in *Aconitum carmichaeli* Debx.

Keywords: *Aconitum carmichaeli* Debx., flavonoid-3',5'-hydroxylase, cloning, gene expression

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