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研究报告

石蒜黄烷酮3－羟化酶基因*LrF3H* 的克隆及表达分析

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Cloning and Expression Analysis of Flavanone 3-hydroxylase Gene *LrF3H* from *Lycoris radiate*

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摘要 采用RT-PCR 和RACE 技术相结合的方法，从石蒜花瓣中克隆到1 个黄烷酮3－羟化酶（F3H）基因的cDNA 全长序列，全长1 293 bp，包含1 098 bp 的完整开放阅读框，编码365 个氨基酸；氨基酸序列比对显示该序列编码的氨基酸与水仙的F3H 具有高达91%的同源性，将其命名为*LrF3H*。二级结构预测表明，随机卷曲是LrF3H 蛋白最大量的结构元件，而α−螺旋和延伸链散布于整个蛋白中。保守结构域预测表明该基因编码的蛋白具有典型的F3H 蛋白功能结构域，其保守结构域中含有铁离子及2－O－酮戊二酸结合位点，属于2OG-FeⅡ_Oxy 双加氧酶超家族。实时荧光定量PCR 分析表明，*LrF3H* 在整个花发育过程中均表达，而且从初蕾期到盛开期随着花瓣着色加深表达量逐渐增加，盛开期表达量最高，之后随着花朵萎蔫表达量下降；在不同器官中，*LrF3H* 的表达量在花瓣和花葶中最高，而在根、鳞茎和叶片中都很低，推测该基因在石蒜花色形成过程中起着关键作用。

关键词： 石蒜 黄烷酮3－羟化酶 基因克隆 表达

Abstract: Flavanone 3-hydroxylase（F3H）is a crucial enzyme in the early stage of anthocyanins biosynthesis which is important in regulating the formation of flower color. In the study, a full-length cDNA sequence of *F3H* gene was cloned from petals of Lycoris radiate using RT-PCR and RACE approaches. The cDNA sequence was 1 293 bp and included a whole open reading frame of 1098 bp encoding 365 amino acids. The amino acid sequence of this gene shared up to 91% homology with F3H from *Narcissus tazetta*, and then was named *LrF3H*. The predicted secondary structure indicated that random coil was the most important structural element. However, alpha helix and extended strand distributed in the whole protein. The conserved structural domain analysis revealed that *LrF3H* had the typical functional domains of F3H protein, containing 2-oxoglutarate and iron ion combination sites and belonging to the 2OG-Fe(Ⅱ)-dependent dioxygenase superfamily. Quantitative RT-PCR analysis showed that *LrF3H* was expressed in the whole phases of flower development, and the expression level was increasing concomitant with the growth of flower bud and pigmentation until the phase of blooming flower. The transcript level was highest in anthocyanin-pigmented petals, moderate in cape and lower in root, corm and leaf. Those indicated that *LrF3H* gene might play a role in flower pigmentation in *Lycoris radiate*.

Keywords: *Lycoris radiate*, flavanone 3-hydroxylase（F3H）, gene cloning, expression

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