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白花丹参顺式还原酮加双氧酶基因的克隆、 分子特征和表达调控分析

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中文摘要:目的: 获得白花丹参参与乙烯和多胺合成的顺式还原酮加双氧酶(acireductone dioxygenase,*ARD*)基因(命名为*SmARD*)序列,并进行生物信息学分析和初步的表达特性研究。 方法 :利用全长cDNA文库技术,从两年生白花丹参根中获得目的基因*SmARD*序列。利用BLAST进行序列比对,ORF Finder寻找该基因的开放读码框,prosite分析蛋白质的基本结构域。用半定量RT-PCR检测其在白花丹参幼苗根、茎、叶和成花中的表达情况。 结果 :得到688 bp的*SmARD*基因全长序列。具有一个591 bp的开放读码框,编码196个氨基酸,预测蛋白质相对分子质量23.27 kDa。使用预测的*SmARD*蛋白序列在NCBI中进行蛋白保守域分析,发现*SmARD*与*ARD*/*ARD*'家族具有很高的同源性。半定量RT-PCR表明,*SmARD*在白花丹参幼苗根、茎、叶和成花等组织中均有转录水平的表达,但是在根部表达最强。水分亏缺处理3 d,150 mmol • L^{-1} NaCl处理1 d,4 ℃低温处理1 d和100 μmol • L^{-1} ABA处理1 d均抑制*SmARD*的表达,100 μmol • L^{-1} 茉莉酸甲脂(MJ)和10 μmol • L^{-1} 乙烯利(ETH)处理1 d诱导*SmARD*的表达。 结论 :首次得到白花丹参的顺式还原酮加双氧酶(*ARD*)基因序列,为其参与白花丹参响应逆境和次生代谢产物合成的信号调节功能研究奠定了基础。

中文关键词:白花丹参 顺式还原酮加双氧酶(*ARD*)基因 序列分析 表达分析

Cloning,molecular characterization and expression of acireductone dioxygenase (*ARD*) gene from *Salvia miltiorrhiza*

Abstract: Objective : To study the acireductone dioxygenase (designated as Sm*ARD*) gene of *Salvia miltiorrhiza* through bioinformatics and characterization of its tissue expression and response expression on stress in shoot. Method : *SmARD* gene was obtained by sequencing cDNA library constructed by us. BLAST was used for alignment, ORF finder software was applied to find open reading frame, prosite was used to analyze the protein characterization. Semi-quantitative RT-PCR was used to detect the gene expression level. Result : The full-length cDNA of *SmRAD* was 688 bp long with a 591 bp ORF (open reading frame) that putatively encoded a polypeptide of 196 amino acids, with a predicted molecular mass of 23.27 kDa. The deduced amino acid sequence of *SmRAD* of gene shared high homology with other known RADs. Semi-quantitative RT-PCR analysis indicated that *SmRAD* was constitutively expressed in roots, stems, flower and leaves of *S. miltiorrhiza*, with the high expression in roots. In addition, *SmRAD* expression level under different stress condition was also analyzed in root, including signaling components for plant defence responses, such as methyl jasmonate, salicylic acid and ABA, as well as drought, cold and salt abiotic stress. The expression of *SmRAD* was suppressed by water deficit treatment for 3 d, 150 mmol • L^{-1} NaCl, 4 ℃ cold and 100 mmol • L^{-1} ABA treatment for 1 d, but induced by 100 mmol • L^{-1} MJ and 10 mmol • L^{-1} ETH. Conclusion : A novel *SmARD* gene was cloned from *S. miltiorrhiza*. This study will enable us to further understand the role of *SmARD* in the defense response under different abiotic stress and in synthesis of active cmpounds in *S. miltiorrhiza* at molecular level.

keywords:*Salvia miltiorrhiza* acireductone dioxygenase (*ARD*) gene sequence analysis expression analysis

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