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海岛棉转录因子EREB5基因的克隆及特征研究

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Molecular Cloning and Characterization of *EREB*5, a Novel Transcription Factor Gene of ERF Family from *Gossypium barbadense* L.

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摘要

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Supporting Info

摘要 近年来,ERF(Ethylene-responsive element binding factor)家族转录因子已成为植物抗逆、抗病的分子机制和作物分子育种研究的热点。本研究以电子克隆、同源扩增和RACE(Rapid-amplification of cDNA ends)技术相结合的方法从海岛棉中分离出了一条新的ERF族转录因子基因,命名为*EREB*5。该基因包含一个573 bp长的开放阅读框,预期编码190 aa长的蛋白质分子。序列分析表明该蛋白除含有一个ERF保守域外,还含有一段核定位信号序列、一段富丝氨酸的激活域、多种磷酸化位点和一个土豆抑制子 I 型家族基序等。构建系统发育树分析表明该因子属于ERF亚家族B3亚组。瞬时表达实验证明该因子定位于细胞核内,同时,凝胶阻滞实验结果说明EREB5蛋白和GCC盒具有较强的结合能力。再者,荧光定量PCR结果表明乙烯和黄萎病菌处理可以诱导该基因的表达。这些结果暗示EREB5蛋白很可能在黄萎病抗性机制中扮演者重要角色。

关键词: 海岛棉 ERF 黄萎病 EREB5 基因克隆

Abstract: In recent years, ERF(Ethylene-responsive element binding factor) family transcription factors have become the research hot spot of crop molecular breeding and molecular mechanisms of resistance or tolerance of plant to bio-or abiotic stresses. In this study, one novel gene, designated as *EREB*5, was firstly isolated from *Gossypium barbadense* treated with *Verticillum dahliae* by cloning in silico, homologic amplification and RACE (Rapid-amplification of cDNA ends) techniques. The bioinformatics analyses demonstrated that the full-length cDNA contained a 573 bp open reading frame which encoded a deduced protein which included 190 amino acid residues, contained an ERF conserved domain, and belonged to the B3 subgroup of ERF subfamily. It also contained one nucleus localization signal, one serine-riched domain, some sites of phospholation and one motif of potato inhibitor I family. Transient expression analysis showed that EREB5 protein was localized in nucleus, meanwhile, the results of Electrophoretic mobility shift assay revealed that EREB5 protein had the ability of binding specifically to the GCC-box. Moreover, the results of expression analysis indicated that EREB5 was able to be induced in *Gossypium barbadense* seedlings, by inoculating with *Verticillum dahliae* or stimulation of ethylene, respectively. These results imply that *EREB*5 should play an important role in resistance of *Gossypium barbadense* to Verticillum wilt.

Keywords: Gossypium barbadense ERF Verticillum wilt EREB5 gene cloning

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