

论文

斑茅cDNA中抗病基因同源序列的分离和表达特性分析

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摘要:

植物抗病基因具有一些特定的保守结构域。本研究根据已知植物同源抗病基因(RGAs)保守序列设计简并引物, 从甘蔗近缘植物斑茅的cDNA中扩增出6条抗病基因同源序列, 它们在NCBI上登录号分别为EU685835、EU685836、EU685837、EU685838、EU685839和EU685840。序列分析表明, 这些RGAs均含有典型的NBS-LRR类抗病基因保守结构域P-loop、Kinase-2a、Kinase-3a和疏水结构域(Hydrophobic domain, HD)。氨基酸序列的同源性比对表明, 6条RGAs序列同11条参试的抗病基因之间的同源性为8.3%~93%, 而6条RGAs之间的氨基酸序列同源为30.5%~45.6%。另外, 本实验所克隆的6条斑茅抗病基因同源序列中, kinase-2(LLVLDDVW/D)最后一个氨基酸皆为色氨酸, 推测所克隆的NBS-LRR类抗病基因都属于non-TIR-NBS-LRR类。定量PCR分析表明, 6条斑茅抗病基因同源序列在根、茎和叶片中组成型表达, 同时这些抗病基因同源序列的表达会受外源信号分子水杨酸和过氧化氢的上调作用, 可能在斑茅的抗病性中具有一定的作用。

关键词: 斑茅 抗病基因同源序列 简并引物 定量PCR

Isolation and Characterization of Disease Resistance Gene Analogs from *Erianthus arundinaceus* cDNA

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Abstract:

Plant disease resistance genes (R-genes) encode some conserved motifs. According to the conserved motifs present in the known NBS-LRR R-gene sequences and R gene analogs (RGAs), several degenerate primers were designed and applied in the RGA isolation from *Erianthus arundinaceus* using PCR approach. In total, 6 RGAs were successfully obtained, with GenBank Accession numbers of EU685835, EU685836, EU685837, EU685838, EU685839, and EU685840. Multiple alignments showed that the encoding sequences of the six clones were highly conserved and strikingly similar to the eleven most typical NBS-LRR type R-gene peptide sequences, especially at the four NBS motifs of P-loop, kinase-2, kinase-3a, and HD. The identity percentage at the amino-acid level ranged from 8.3% to 93.0% among all 17 sequences tested and from 30.5% to 45.6% among the six cloned RGAs in this study. The results of cluster analysis and the existence of an aspartic acid residue (D) at the final residue position of the kinase-2 motif also indicated that all of *E. arundinaceus* RGAs might belong to non-TIR group. Finally, Real-time PCR results showed that all of the RGAs were constitutively expressed in roots, stalks and leaves of *E. arundinaceus*, and their expression could be up-regulated in leaves by the exogenous signal molecules SA and H₂O₂. Therefore, it suggested that *E. arundinaceus* RGAs might play important roles in disease resistance in an SA- and H₂O₂-dependent defense response pathway. Further studies should aim to clone full-length R-genes in *E. arundinaceus* and characterize their functions in defense responses.

Keywords: *Erianthus arundinaceus* Resistance gene analogs (RGAs) Degenerate primers Real-time PCR

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