

园艺—研究报告

黄瓜芽黄突变相关基因ClpP克隆及植物表达载体构建

朱伟伟¹,陈远良^{2,2},陈芳^{2,2},高剑峰^{2,2}

1. 石河子大学生命科学学院

2.

摘要:

为了获得黄瓜中ClpP基因的cDNA全序列,研究其与黄瓜芽黄突变现象的关系,本研究采用TRNzol法提取芽黄突变的黄瓜叶片总RNA,并以其为模板反转录合成cDNA第一链。根据NCBI预测的黄瓜ClpP基因序列设计并合成1对特异引物,通过PCR扩增得到目的条带后测序,并构建植物表达载体;成功获得黄瓜中ClpP基因的cDNA全序列,并提交到NCBI。该基因编码区全长495 bp,共编码氨基酸158个。预测其理论等电点为5.22,理论蛋白质分子量为41.00356 KDa,编码的蛋白包含S14_ClpP_2保守结构域,无信号肽。通过与其他植物Clp蛋白酶氨基酸序列比对发现与毛茛属植物的Clp蛋白酶同源性较高。并成功构建了以CaMV35S为启动子的植物表达载体pBI121-ClpP。黄瓜中ClpP基因的cDNA全序列的获得说明该基因在黄瓜中确实存在,这是首次克隆得到了黄瓜中的ClpP基因的cDNA全序列。

关键词: 载体构建

The Clone of ClpP Relating to Virescent Mutant in Cucumber and the Construction of Plant Expression Vector

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Abstract:

In order to obtain the cDNA of ClpP gene and analyze the relationships between ClpP gene and virescent mutation of cucumber, the total RNA was extracted from virescent mutational buds of cucumber by TRNzol method. The total RNA was used as template to synthesis the first chain of cDNA by reverse transcription approach. One pair of primers was designed and synthesized to amplify an aim fragment according to relatively ClpP gene sequence. The aim fragment was inserted to a plant expression vector after sequenced. The complete cDNA sequence was gain and submitted to NCBI database, which length is 495 bp and encode 158 aa. The predicted protein is about 41.00356 Kda, and its isoelectric point is about 5.22. It without signal peptide but included a S14_ClpP_2 conservative structure domain. The amino acids sequence of Clp protease and the amino acids sequence of Clp protease of buttercup family have a high homology. A plant expression vector pBI121-ClpP which has a CaMV 35S prompt was also successfully constructed in this essay. The results demonstrated the ClpP gene existed in cucumber, and ClpP gene was cloned in cucumber firstly and its whole sequence was got.

Keywords: vector construction

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通讯作者: 朱伟伟

作者简介:

作者Email: zww198627@sina.com

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