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生物技术—研究报告

TMV、CMV双价抗性RNA沉默表达载体的构建

陈瑜欣[1],高玉龙[2],徐照丽[2],丁灿[3]

1. 云南农业大学；云南省烟草农业科学研究院
2.
3. 云南农业大学烟草学院

摘要：

本研究以在植物体内转录出RNA沉默的高效诱导因子双链RNA（dsRNA）为目标，根据已报道的TMV ΔMP和CMV ΔRep的核苷酸序列设计特异性引物，分别从质粒pBIN-TMV ΔMP(i/r)、pBIN-CMV ΔRep(i/r)扩增MP基因、Rep基因，并在pGEM-T Easy 载体上将2片段串联起来，再用PCR扩增串联好的正反向MP-Rep基因。正向MP-Rep基因用BamHI和Kpn I切下，将所得基因片段与用同样酶切开的含内含子的pKAN-In相连，取名+pKAN；用同样的方法，将反向MP-Rep基因用Xba I切下，将所得基因片段与用同样酶切开的+pKAN相连，取名pKAN-In-MP-Rep，再用Not I将包括Intron和正反向MP-Rep基因在内的片段切下，将其定向插入同样酶切开的pART27上，获得重组质粒pART27-In-MP-Rep。获得的载体可以应用于植物转基因抗病毒育种工程中。

关键词： CMV

Construction of TMV and CMV Binary Virus Resistant RNA Silencing Vector

Abstract:

In order to effectively transcribe dsRNA in plants, an inducer of RNA silencing, the specific primers was designed according to the published sequence of TMV ΔMP gene and CMV ΔRep gene. MP and Rep fragment was obtained by PCR amplification respectively using pBIN-TMV ΔMP (i/r) and pBIN-CMV ΔRep(i/r) as template, then MP and Rep were concatenated and cloned into pGEM-T Easy Vector. Sense MP-Rep fragment was obtained by digesting with BamHI and Kpn I and inserted into vector pKAN-In, then called the recombinant plasmid +pKAN. Antisence MP-Rep gene cut with Xba I was linked with +pKAN to construct hairpin structure. The fragment including Intron, the sense and antisence ΔMP-Rep gene was cut by Not I, and was inserted into pART27 to construct plant transformation vector. The vector could be applied in transgenic breeding engineering for plant viral resistance.

Keywords: Cucumber Mosaic Virus

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通讯作者: 陈瑜欣

作者简介:

作者Email: yaodanzhu@163.com

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