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广州地区番石榴根结线虫鉴定与*14-3-3*基因的克隆

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摘 要:【目的】摸清广州地区番石榴根结线虫的种类、分布及致病机制。**【方法】**观察番石榴根结线虫的雄虫、二龄幼虫、雌虫及其会阴花纹的形态学特征,根据根结线虫的通用引物#C2F3和#1108对线虫mtDNA的COⅡ和lrRNA基因间序列进行PCR扩增,获得750 bp的特异扩增产物,将扩增片段在GenBank上进行Blast比对。通过南方根结线虫与北方根结线虫的14-3-3序列设计简并引物,进一步扩增已鉴定的象耳豆根结线虫的14-3-3基因。**【结果】**广州地区番石榴根结线虫的形态与象耳豆根结线虫(*Meloidogyne enterolobii*)相似;其扩增片段与象耳豆根结线虫(*M. enterolobii*)比对,序列相似性在99%以上。扩增了象耳豆根结线虫的*14-3-3*基因,结果显示该基因的开放阅读框包含783 bp的片段,编码261个氨基酸,命名为*Me-14-3-3*。**【结论】**广州地区番石榴根结线虫为象耳豆根结线虫(*M. enterolobii*),14-3-3蛋白基因被成功克隆,扩增结果为进一步研究14-3-3蛋白基因在象耳豆根结线虫生长发育中的功能、摸清致病机制等方面奠定良好的基础。

关键词: 番石榴;根结线虫;种类鉴定;*Me-14-3-3*;克隆

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Identification of guava root-knot nematodes in Guangzhou and the cloning of *14-3-3* gene

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Abstract:【Objective】To make sure what species the root knot nematode was that affected the guava in Guangzhou, six guava cultivation areas affected by root knot nematodes were investigated. In addition, to study the pathogenic factor, the *14-3-3* gene was cloned from the identified *Meloidogyne enterolobii*. 【Methods】 The females were separated from the diseased roots of guava, and the second instar larvae were incubated from the eggs separated from the roots. The perineal were cut manually under an anatomical microscope, and then the perineal patterns were observed under microscope. The males were separated from the soils of diseased guava using the Baermann method. Then, the female, male and the second instar larvae were observed under optical microscope using the morphological method to identify the root knot nematode species they belonged to. Molecular biology method was also applied for further verification of the molecular results. The primer set #C2F3 and #1108 were used for mtDNA PCR amplification. Then, the degenerate primers were designed according to the *14-3-3* gene of *M. incongnita* and *M. hapla*. RT-PCR was performed to amplify the *14-3-3* gene using the designed primers. The PCR product was run with electrophoresis. The fragment was cloned and sequenced. The sequence was analysis by bioinformatics method. Sequence analysis of genes and proteins was carried out using Blast for sequence similarity analysis at NCBI. Open reading frame (ORF) was analyzed with ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The physicochemical properties were predicted by EXPASY (http://expasy.org/tools/

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