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园艺—研究报告

齿兰环斑病毒CP基因的原核表达及其产物抗血清制备

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摘要:

本研究在于探讨侵染兰花的重要病毒之一齿兰环斑病毒（Odontoglossum ringspot virus，ORSV）的检测技术。从福建省漳州市采集感染齿兰环斑病毒的建兰病样，设计一对特异性引物扩增并克隆得到该病毒的外壳蛋白基因，该基因开放阅读框长477 bp，编码158 aa约合18.0 kDa的蛋白质，随后将目的基因插入pET-29a（+）中构建相应的原核表达载体进行诱导表达，目的蛋白经纯化后免疫家兔获得了特异性抗血清。Western blot检测结果表明，抗血清与诱导表达的ORSV外壳蛋白发生特异性反应。间接酶联免疫吸附检测结果表明，抗血清可检测病叶的最低稀释度达1:25600（v/v），最佳工作浓度为1:12800（v/v），病汁液灵敏度为0.195 mg/mL（w/v），而与TMV等11种同源或异源病毒均无明显的血清学交叉反应。

关键词: 抗血清

Prokaryotic Expression and Antiserum Preparation of the CP gene of Odontoglossum Ringspot Virus

[2], [2]

Abstract:

The aim was to study the technology which detected Odontoglossum ringspot virus (ORSV), which was one of the most important and worldwide viruses attacking orchids. Chinese Cymbidium plants which showed disease symptoms were collected from Zhangzhou, Fujian. One pair of specific primers was designed for amplification of the coat protein (CP) gene from the samples infected with ORSV. The open reading frame encoding CP of ORSV isolate obtained from Zhangzhou, Fujian is 477 bp, encoding a 18.0 kD protein with 158 aa. The expected CP gene was then inserted into the pET-29a (+) vector for the prokaryotic expression. And the aimed protein was purified and used to immune the rabbit for antiserum preparation. According to the result of ID-ELISA analysis, specific rabbit anti-ORSV serum was prepared with a high titre of 1:25600, a working concentration of 1:12800, and a sap sensitivity of 0.195 mg/mL. Western blot analysis confirmed that the antiserum reacted strongly and specifically to the CP of ORSV. There were no obvious cross reactions between the antiserum and 11 kinds of homologous or heterologous viruses such as Tobacco Mosaic Virus (TMV).

Keywords: antiserum

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参考文献:

[1] Jensen DD,Gold HA..A virus ring spot of Odontoglossum orchid:symptoms, transmission and electron microscopy[J].Phytopathology .1951,41:648-65 [2]Kado CI.nullvan Regenmortel MHV,Knight CA ..Studies on some strains of tobacco mosaic virus in orchids I.Biological,chemical,and serological

studies[J]..Virology,1968,:- [3]Chng CG,Wong SM,Mahtani PH, et al.The complete sequence of a Singapore isolate of odontoglossum ringspot virus and comparison with other tobamoviruses [J].Gene,1996,171(2):155-161 [4]Ikegami M,Isomura Y,Matumoto Y,Chatani M, et al.The complete nucleotide sequence of odontoglossum ringspot virus (Cy-1 strain) genomic RNA[J].Microbiol Immunol,1995,39(12):995-1001 [5]Ryu KH,Park WMA..The complete nucleotide sequence and genome organization of odontoglossum ringspot tobamovirus RNA[J].Arch Virol,1995,140(9):1577-1587 [6]朱常香,宋云枝,王玫玫,等.烟草环斑病毒外壳蛋白基因的原核表达及抗血清的制备[J].中国病毒学,2005,20(4):434-437 [7] 陈文华,韦传宝,贾玉成,等.大豆花叶病毒CP基因原核表达与抗血清制备[J].生物技术通报2009,10:98-10 [8]吴兴泉，陈士华，吴祖建，等.马铃薯X病毒CP基因的原核表达及特异性抗血清的制备[J].郑州工程学院学报,2003,24(2):26-28 [9]罗金水.建兰花叶病毒CP基因的原核表达及抗血清制备[J].热带作物学报,2009,30(8):1113-1119 [10]明艳林，郑国华，李梅.齿兰环斑病毒的鉴定及其抗血清的制备与应用[J][J].中国病毒学,2006,21(1):64-67 [11]潘俊松,刘志昕,康由发,等.齿兰环斑病毒外壳蛋白基因的表达及其表达产物抗血清的制备[J].热带作物学报,1999,20(2):62-66

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