

江苏省番茄黄化曲叶病的病原分子诊断

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摘要: 2007年冬季在江苏省兴化市和南京市一些日光温室大棚中发生了一种新的番茄病毒病, 主要表现为植株矮缩, 叶片上卷, 叶缘黄化等, 危害十分严重。为明确其病原, 对田间表现典型症状的 20份病样进行了分子检测, 结果全部检测到粉虱传双生病毒; 对其中 12份进行序列同源性分析, 发现其同源性超过 98%, 说明其极可能是同一个分离物; 将该序列与 GenBank 进行 BLAST 分析, 发现其与番茄黄化曲叶病的同源性最高, 达 99%。

关键词: 番茄; 番茄黄化曲叶病; 粉虱传双生病毒; 分子诊断

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Molecular Diagnosis of Tomato Yellow Leaf Curl Disease in Jiangsu Province

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Abstract: In winter of 2007, a new virus disease on tomato broke out in Jiangsu and caused great losses to local tomato production. The infected tomato showed severely stunted, upward curling and prominent yellowing along margins and interveinal regions. Based on its symptoms and the occurrence of *Bemisia tabaci* in the fields, whitefly-transmitted geminivirus (WTG) was suspected to be the pathogen, and WTG was identified in all the 20 samples. The nucleotide sequence identities between these isolates were extremely high (over 98%), which means no complex infection was found. The result of BLAST revealed they shared high sequence identities (over 99%) with a member of *Begomovirus*: Tomato yellow leaf curl virus (TYLCV).

Key words: tomato; tomato yellow leaf curl disease; whitefly-transmitted geminivirus; molecular diagnosis

双生病毒是一类具有双联体结构的植物单链环状 DNA 病毒, 包括 4 个属, 200 多个种 (Fauquet et al., 2005), 其中对作物造成危害的大多属于菜豆金色花叶病毒属 (*Begomovirus*), 其传播介体为烟粉虱 (*Bemisia tabaci*), 也称为粉虱传双生病毒 (whitefly-transmitted geminivirus, WTG)。目前此类病害在亚洲、美洲中部、欧洲、南非中北部及地中海等地区广泛分布和流行 (Moriones & Navas-Castillo, 2000; Morales & Anderson, 2001; Crescenzi et al., 2004), 据初步统计, 至少已有 39 个国家的棉花、木薯、番茄等作物遭受此类病毒毁灭性的危害 (Moffat, 1999)。

在我国, 龚祖隍等 (1982) 通过电镜观察到烟草曲叶病毒孳生粒体, 证实了双生病毒在国内的发生。20 世纪 90 年代后, 该类病毒在我国台湾、广西、云南、海南、广东、福建等地发生, 对番茄、烟草、番木瓜等作物造成了严重的危害 (刘玉乐等, 1998; Zhou et al., 2001; Wang et al.,

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2004; Xiong et al, 2005; Mugiira, 2007)。2006年底与江苏毗邻的上海和浙江两地此类病害爆发成灾(王东生等, 2006; 张穗等, 2006; 吴永汉等, 2007; 张纯胄, 2007; Mugiira et al, 2008), 致使当地的番茄生产蒙受了惨重的损失。

2007年11月江苏省部分地区一些番茄日光温室也发生了一种新的病害, 症状表现极似双生病毒病。该病在部分地区已造成严重的危害。笔者对江苏省兴化市和南京市保护地番茄上采集的20份疑似病样进行了分子诊断, 从中均检测到粉虱传双生病毒。

1 材料与方法

20份番茄病样于2007年冬季采自江苏省兴化市昭阳镇、南京市江宁区玄武区日光温室大棚。根据双生病毒共同区及外壳蛋白基因保守序列设计简并引物 PA/PB (Deng et al, 1994)。

PA: 5'-TAA TATTACCKGW KGVCCSC-3',
PB: 5'-TGGACYTTRCAWJJBCCGCACA-3', 由上海英骏生物技术有限公司合成。

采用CTAB法提取病样总DNA (Harrison et al, 1997)。PCR反应条件: 94 预变性 2 min; 94 45 s, 53 45 s, 72 1 min; 35个循环; 72 继续延伸 10 min。反应完毕后于1%琼脂糖凝胶电泳中检测扩增产物。

从20份样品中选取12个有代表性的分离物对其PCR产物进行序列测定, 详细信息如表1。

测序由上海生工生物工程技术有限公司完成。采用DNA star软件及NCBI网站上的BLAST程序进行比对分析。

表1 测序样品信息

Table 1 Information of 12 isolates sequenced			
采样地点 Sampling place	采样时间 Sampling time	编号 No.	分离物 Isolation
南京市玄武区 Xuanwu, Nanjing	2007 - 12 - 18	1	JS-NJ-JAAS1
		2	JS-NJ-JAAS2
		3	JS-NJ-JAAS3
		4	JS-NJ-JAAS4
南京市江宁区 Jiangning, Nanjing	2007 - 12 - 07	5	JS-NJ-JN1
		6	JS-NJ-JN2
		7	JS-NJ-JN3
		8	JS-NJ-JN4
兴化市昭阳镇 Shaoyang, Xinghua	2007 - 11 - 19	9	JS-XH1
		10	JS-XH2
		11	JS-XH3
		12	JS-XH4

2 结果与分析

2.1 田间病株症状

20份病样均表现为植株矮化, 叶片上卷, 变小, 叶缘黄化, 症状类似于番茄黄化曲叶病(图1)。



图1 病样的田间症状

A: 对照; B: 病株。

Fig. 1 Symptoms of the virus-infected tomato compared with healthy plant

A: Healthy plant; B: Virus-infected plant

2.2 PCR检测

20份样品抽提 DNA 后, 根据双生病毒基因间隔区和外壳蛋白保守序列设计的 PA /PB 引物进行分子检测 (以采自上海确认含有粉虱传双生病毒的番茄病样作为阳性对照, 以健康番茄样品作为阴性对照), 结果全部检测到 500 bp 左右的目的条带, 初步证明其中都含有粉虱传双生病毒。

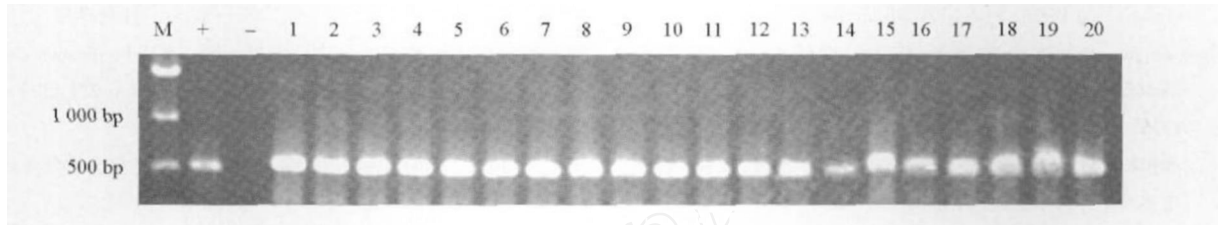


图 2 PCR检测结果

M: Marker; +: 阳性对照; -: 阴性对照; 1~20: 番茄病样。

Fig. 2 Results of PCR

M: Marker; +: Positive control; -: Negative control; 1 - 20: Samples from Jiangsu

2.3 序列分析

将 12 个分离物的序列进行同源性分析, 发现其同源性非常高, 高于 98%, 说明其极有可能是一个分离物。将序列与 GenBank 进行 BLAST 分析, 显示序列与番茄黄化曲叶病毒 (EF523478) 的同源性最高, 超过 99%, 从而初步证明作者所检测到的是番茄黄化曲叶病毒。

表 2 12 个样品序列之间的同源性

Table 2 Percentage of similarity of 12 samples

	1	2	3	4	5	6	7	8	9	10	11	12
1	* * *	99.5	99.3	99.3	99.8	99.5	100	100	100	100	99.8	100
2		* * *	98.9	99.3	99.3	99.1	99.5	99.5	99.5	99.5	99.3	99.5
3			* * *	98.6	99.1	99.1	99.3	99.3	99.3	99.3	99.1	99.3
4				* * *	99.1	98.9	99.3	99.3	99.3	99.3	99.1	99.3
5					* * *	99.3	99.8	99.8	99.8	99.8	99.5	99.8
6						* * *	99.5	99.5	99.5	99.5	99.3	99.5
7							* * *	100	100	100	99.8	100
8								* * *	100	100	99.8	100
9									* * *	100	99.8	100
10										* * *	99.8	100
11											* * *	99.8
12												* * *

番茄黄化曲叶病毒 (tomato yellow leaf curl virus, TYLCV) 属双生病毒科 (Geminiviridae) 中的菜豆金色花叶病毒属, 传播介体为烟粉虱, 能在番茄上造成毁灭性危害。该病毒自 1964 年报道以来 (Cohen & Hapaz, 1964), 许多国家和我国的许多地区都已有相关报道。2007 年底该病毒在江苏省首次被检测到, 并且在局部地区已造成了较严重的危害。鉴于此类病毒扩散蔓延非常迅速, 危害异常严重, 生产上应当密切关注其发生危害情况, 及时发现, 尽早防治。

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