果 树 学 报 2022,39(10):1903-1910
Journal of Fruit Science

DOI:10.13925/j.cnki.gsxb.20220261

重庆柑橘产区柑橘褪绿矮缩病毒的发生、分布和分子特性研究

王甲军[1],乔兴华[2],秦阳阳[1],陈 力[2],周 彦[1*]

([1]西南大学柑桔研究所国家柑桔工程技术研究中心,重庆北碚 400712;
[2]重庆市万州区植物保护与果树技术推广站,重庆万州 404199)

摘 要:【目的】明确柑橘褪绿矮缩病(*Citrus chlorotic dwarf-associated virus*, CCDaV)在重庆柑橘产区的发生、分布和分子特性,为制定有效防治措施提供理论基础。**【方法】**对采自重庆的361份疑似感染褪绿矮缩病的柚类和柠檬样品进行CCDaV检测鉴定,通过背靠背引物扩增获得CCDaV的全序列,进而分别使用RDP4和SDTv.1.2进行重组和相似性分析,并采用邻接法构建系统发育树。**【结果】**在3份红宝石柚(*Citrus paradisi*)样品中检测出了CCDaV,其全长分别为3642、3640和3641 bp。全基因组分析未发现重组事件,且上述3个CCDaV毒株与已知41个CCDaV毒株的核苷酸和氨基酸相似性分别为99.1%~99.7%和97.3%~99.7%。系统进化树分析显示,本研究中CCDaV毒株被分为了4个不同的类群,其中巫溪样品WX-4-6和WX-4-9,与广东沙田柚上的CCDaV毒株聚在一簇;而巫溪样品WX-2-5与中国柚类上获得的CCDaV毒株聚于另一个分支。**【结论】**在重庆首次发现了CCDaV,可能是随带病苗木进入。

关键词:柑橘褪绿矮缩病毒;检测;序列分析;进化树

中图分类号:S666 **文献标志码:**A **文章编号:**1009-9980(2022)10-1903-08

Occurrence, distribution and molecular characteristics of *Citrus chlorotic dwarf-associated virus* in Chongqing

WANG Jiajun[1], QIAO Xinghua[2], QIN Yangyang[1], CHEN Li[2], ZHOU Yan[1*]

([1]National Citrus Engineering Research Center, Citrus Research Institute, Southwest University, Beibei 400712, Chongqing, China;[2]Plant Protection and Fruit Tree Technology Popularization Station in Wanzhou District of Chongqing City, Wanzhou 404199, Chongqing, China)

Abstract:【Objective】In 2015, an emerging citrus viral disease caused by *Citrus chlorotic dwarf-associated virus* (CCDaV) was first reported in Yunnan province of China, followed by Guangxi and Guangdong provinces. The present study was undertaken to provide further insight into the distribution of CCDaV in Chongqing municipality, and get a more complete overview of the molecular variability of CCDaV.【Methods】The field survey and collection of samples were conducted from January 2020 to December 2021 in 8 counties of Chongqing municipality, including Beibei, Wanzhou, Jiangjin, etc. A total of 361 suspected CCDaV-infected pummelo and lemon samples were collected from 27 orchards. The samples were randomly evaluated both on normal appearance and on virus-like symptoms of a V-shaped notch and chlorotic freckling on leaves. During sampling, the symptoms on leaves were carefully observed. Total DNA extracts were obtained from each plant with DNA extraction kit and used for PCR detection with specific CCDaV primers (sense: 5'- GAGCATGTTGAGTGTGAGG -3' and antisense (5'- CAGACATAT CCATCAGCGC -3'), which were based on the conserved region of movement protein of CCDaV (Genbank accession No. JQ920490). For cloning, three CCDaV-positive samples were selected. The complete genome of CCDaV was amplified by using one pair of primers

收稿日期:2022-05-16 **接受日期:**2022-07-13

基金项目:国家重点研发计划(2019YFD1001802);财政部和农业农村部:国家现代农业产业技术体系(CARS-26-05B);重庆市自然科学基金项目(cstc2019jcyj-msxmX0557)

作者简介:王甲军,男,硕士,研究方向为分子植物病理学。Tel:13251317657,E-mail:571934151@qq.com

*通信作者 Author for correspondence. Tel:13320292375,E-mail:zhouyan@cric.cn

(3202F: 5’- GTTCTGTGTTTCGACCCGTTTCTTGCACTGG -3’and 3202R (5’-CCCCGGTTCTTC-GACCTCCTCTCCGTA-3’), designed based on the CCDaV genome (No. JQ920490), as previously described. PCR amplified matters were purified with EasyPure quick gel Extraction kit and cloned into pCE2 TA/Blunt-Zero vector. Twelve randomly selected positive clones were custom sequenced. The genomic sequences were assembled by using SeqMan software and submitted to GenBank under accession numbers ON063221-ON063223. Multiple nucleotide sequence alignments of the complete genome of the 3 CCDaV isolates from this study, and 41 CCDaV isolates available in GenBank were conducted separately with Megalign, and the sequence identities were calculated using SDTv.1.2. MEGA 11 was used to construct neighbor-joining phylogenetic trees with 1000 bootstrap replicates. Furthermore, complete genomes of the 44 CCDaV isolates were used for recombination analysis, which was performed with the RDP v4.97 software package.【Results】CCDaV was detected from 0.83% of the collected samples in Chongqing municipality (3 samples were positive out of the 361 samples surveyed) and all of them were originated from Ruby Green pomelo (*Citrus grandis*) in Wuxi. CCDaV was not found on other pomelo or lemon species. The results showed that CCDaV was not widely distributed in Chongqing municipality. In this study, the young shoots of CCDaV-infected Ruby Green pomelo showed severe leaf twist, shrink and yellowing symptoms. Mild inverted cupping and shrink symptoms were observed on some of Ruby Green pomelo plants, in which CCDaV was not detected, but no variegation symptoms were found in CCDaV-negative plants. The complete genomes of the WX-2-5 had the lengths of 3642 nucleotides (nt) and the base composition was 27.6% A, 16.8% C, 27.3% G and 28.4% T. The complete genomes of the WX-4-6 had the lengths of 3640 nt and the base composition was 27.6% A, 16.7% C, 27.5% G and 28.3% T. The complete genomes of the WX-4-9 had the lengths of 3641 nt and the base composition was 27.6% A, 16.7% C, 27.4% G and 28.3% T. The 5’ and 3’ untranslated regions (UTRs) of the three CCDaV isolates were 194 nt and 220 nt, respectively. The genome organization of all these CCDaV isolates was consistent with that published before. Compared with the CCDaV isolate WX-4-6, WX-4-9 had additional base (T) at position 1203, and WX-2-5 had two more bases (TT) at 1203 and 1204 nt. The amino acid sequence alignment showed that there were 4 point mutations at amino acid sequences between WX-2-5 and 41 known CCDaV isolates at positions 303 (C→L), 304 (I→H), 341 (S→N) and 1163 (F→S). There were 3 same point mutations in the amino acid sequences between WX-4-6 and 41 CCDaV isolates at 43 (L →W), 567 (H→R) and 722 (R→C). In addition to the same mutation as WX-4-6, WX-4-9 also had mutations at positions 633 (S→N) and 1137 (R→M). Comparisons of the whole genome sequences of the 3 CCDaV isolates from this study as well as 41 isolates previously reported from around the world revealed that the sequence identity ranged from 99.1% to 99.7% at nucleotide level and 97.3% to 99.7% at amino acid level, respectively, indicating that there was a very low level of sequence heterogeneity among CCDaV isolates. No recombination event was found among these 44 CCDaV strains. Phylogenetic tree analysis showed that these 44 CCDaV isolates belonged to four different groups based on geographical origins and host species. CCDaV isolates from China were phylogenetically distinct from the isolates from Turkey, and the CCDaV isolates from Turkey were clustered together in the same branch. CCDaV isolates from Thailand, except CCDaV isolate Tha 30, were all clustered with Chinese CCDaV isolates from pomelo. CCDaV isolates WX-4-6 and WX-4-9 were clustered together with CCDaV isolates on Shatian pomelo from Guangdong province, and CCDaV isolate WX-2-5 strains were clustered in another branch. CCDaV isolates from China and Thailand were more closely related than those from Turkey.【Conclusion】To our knowledge, this is the first report of CCDaV in Chongqing, and the movement of CCDaV-infected planting materials was probably one of the important routes of

CCDaV transmission in Chongqing.

Key words: *Citrus chlorotic dwarf-associated virus*; Detection; Sequence alignments; Phylogenetic tree analysis

柑橘褪绿矮缩病是一种危害柑橘的新型病毒病，最早发现于20世纪80年代末土耳其的地中海地区[1-2]，随后在泰国也有报道[3]。已有研究表明，柑橘褪绿矮缩病可侵染几乎所有的柑橘品种，仅甜橙具有一定抗性，柠檬、葡萄柚等最为敏感。植株发病后嫩叶边缘一侧或两侧有“V”形缺口和褪绿色斑点，成熟叶片皱缩、反卷、黄化，后期产量逐渐减低[4-5]。目前柑橘褪绿矮缩病已成为土耳其柑橘产业面临的头号病害[6]。

柑橘褪绿矮缩病的病原为柑橘褪绿矮缩病毒（*Citrus chlorotic dwarf-associated virus*，CCDaV），属于双生病毒科（*Geminiviridae*）的*Citlodavirus*，为单链环状DNA病毒[7]。其基因组大约有3.6 kb，含有5个开放阅读框。CCDaV的正义链编码外壳蛋白（V1）和运动蛋白（V2和V3），互补链编码复制相关蛋白（C1和C2）[8-9]。CCDaV主要通过嫁接进行传播，纯化后的病毒粒子也具有侵染能力[10]。有报道，实验条件下CCDaV还可通过杨梅类白粉虱（*Parabemisia myricae*）进行传播[8,11]。

自2015年首次在中国云南瑞丽的尤力克柠檬上发现CCDaV以来[4]，已陆续在中国广西、广东等地的泰国红宝石柚、三红蜜柚和墨西哥莱檬等多个柑橘品种检测到了CCDaV，且对广西等地的柚类生产造成了严重损失[12-13]。重庆尚未有该病发生的报道。重庆作为长江中上游柑橘优势区，以及国内最大的柑橘无毒苗木繁育基地，苗木交流频繁，柠檬、红宝石柚等感病品种都有引种、繁育和种植。因此本研究针对重庆目前种植柠檬、柚类的主要区县开展系统调查，以期掌握CCDaV在重庆柑橘产区的发生、分布及其分子特性，从而为明确CCDaV传入重庆的主要途径，以及防控柑橘褪绿矮化病提供重要的理论依据。

1　材料和方法

1.1　田间调查

2020年1月—2021年12月从重庆北碚区、万州区、江津区等8个重要柠檬、柚类产区采集了11个品种的361份表现叶片皱缩、扭曲、黄化等疑似柑橘褪绿矮缩病症状的样品（表1）。取样时从每个植株的5个不同方向上总共选取30 mg老熟或展平的叶片组织。混合后使用植物基因组DNA抽提试剂盒（Biospin）提取总DNA，−20 ℃保存备用。

参照Zhou等[15]的方法采用特性引物sense（5’-ACAAGACTATCATAGCACG AGACG-3’）和anti-

表1　采样信息

Table 1　Specie and collection area of citrus samples used in this study

采样地 Source	品种 Cultivar	样品数 Sample number	采样地 Source	品种 Cultivar	样品数 Sample-number
北碚区 Beibei	2号暹罗红 Xianluohong 2	2	万州区 Wanzhou	红心柚 Hongxin pomelo	5
	泰国青柚 Thailand Green pomelo	14		三红柚 Sanhong pomelo	13
	红宝石柚 Ruby Green pomelo	5		尤力克柠檬 Eureka lemon	24
	越南青柚 Vietnam green pomelo	7	永川区 Yong-chuan	三红柚 Sanhong pomelo	4
	琯溪蜜柚 Guanximiyou pomelo	11		红宝石柚 Ruby Green pomelo	5
	尤力克柠檬 Eureka lemon	7		尤力克柠檬 Eureka lemon	14
江津区 Jiangjin	三红柚 Sanhong pomelo	14	綦江区 Qijiang	垫江晚柚 Dianjiang late pomelo	21
	红心柚 Hongxin pomelo	3		酸柚 Sour orange	5
	泰国青柚 Thailand Green pomelo	5		金橙柚 Jincheng pomelo	16
	红宝石柚 Ruby Green pomelo	5		尤力克柠檬 Eureka lemon	11
巫溪县 Wuxi	红宝石柚 Ruby Green pomelo	34	丰都县 Fendu	酸柚 Sour orange	3
	金橙柚 Jincheng pomelo	7		红心柚 Hongxin pomelo	32
	琯溪蜜柚 Guanxi pomelo	37		三红柚 Sanhong pomelo	10
	泰国青柚 Thailand Green pomelo	11		金橙柚 Jincheng pomelo	14
	三红柚 Sanhong pomelo	15	垫江区 Dianjiang	晚白柚 Wanbai pomelo	7

sens（5’- TTTGAACTGTTTAAGTCCATCCC-3’）进行CCDaV检测。20 μL反应体系包含Green taq mix（vazyme）10 μL，10 mmol·L^{-1}正反向引物各0.2 μL，总DNA模板2.0 μL，RNase-free ddH_2O 7.6 μL。扩增条件为：95 ℃预变性3 min；95 ℃变性30 s；55 ℃退火30 s；72 ℃延伸50 s，35个循环；72 ℃终延伸5 min。PCR产物进行1.2%琼脂糖凝胶电泳观察结果。

1.2 全序列测定

以CCDaV阳性样品的总DNA为模板，参照Yu等[9]的方法，用背靠背引物3202F（5’-GTTCTGTGTTTCGACCCGTTTCTTGCACTGG-3’）和3202R（5’-CCCCGGTTCTTCGACCTCCTCTCCGTA-3’）扩增CCDaV的基因组。PCR反应体系包括Prime STAR max（TaKaRa）12.5 μL，10 mmol·L^{-1}正反引物各0.4 μL，模板DNA 2 μL，RNase-free ddH_2O 4.7 μL。反应条件：98 ℃预变性3 min；98 ℃变性30 s；60 ℃退火15 s；72 ℃延伸3 min，35个循环；72 ℃终延伸5 min。

PCR产物纯化后连接于pCE2 TA/Blunt-Zero载体（vazyme），并转化感受态细胞DH5α，每个毒株挑取12个阳性克隆送上海生工进行测序。经SeqMan软件拼接后得到CCDaV的全基因组序列上传GenBank（ON063221-ON063223）。

1.3 序列分析

将获得的CCDaV毒株WX-2-5、WX-4-6和WX-4-9与GenBank中41个CCDaV毒株的基因组序列用Megalign法进行序列比对分析，分别采用SDTv.1.2和RDP4软件分析毒株间基因组的相似性和重组分析，并用MEGA 11软件采用邻接法（Neighbor-Joining，NJ）构建系统进化树，自展值（bootstrap）设为1 000。所用CCDaV参照毒株的信息见表2。

表2 本研究中用于分析的柑橘褪绿矮缩病毒毒株信息

Table 2 Accession numbers and collection area of *Citrus chlorotic dwarf-associated virus* (CCDaV) isolates used in this study for analysis

来源 Origin	毒株 Isolate	寄主 Host	长度 Length/nt	登录号 Accession number	来源 Origin	毒株 Isolate	寄主 Host	长度 Length/nt	登录号 Accession number
中国瑞丽 Ruili, China	YN-EL-9	尤力克柠檬 Eureka lemon	3642	MT680004	中国 China	PL8	柚 Pomelo	3641	MT655993
	YN-EL-19		3642	MT683769		PL7		3642	MT655992
	YN-EL-26		3641	MT680008	中国南宁 Nanning, China	GX-RG-1	红宝石柚 Ruby Green pomelo	3642	MT680006
	YN-EL-71		3641	MT683766		GX-RG-2		3643	MT680011
	YN-EL-72		3642	MT680012		GX-RG-3		3642	MT683767
	YN-EL-77		3641	MT683770	中国西双版纳 Xishuangbanna, China	YN-RG-1	红宝石柚 Ruby Green pomelo	3642	MT680007
	YN-EL-93		3641	MT683768	中国红河 Honghe, China	YN-RG-2	泰国青柚 Thailand Green pomelo	3642	MT680002
	YN-EL-94		3641	MT680003	中国广州 Guangzhou, China	GD-sh-1	三红柚 Sanhong pomelo	3640	MT680001
	YN-EL-131		3641	MT680010	中国江西 Jinagxi, China	JX	柚 pomelo	3642	MW160707
	CN001		3642	KF561253	泰国 Thailand	Tha1-17	红宝石柚 Ruby Green pomelo	3642	MN509440
	YN-EL1		3640	KX840467		Tha1-19		3639	MN509441
	YN-EL2		3640	KX840468		Tha30		3640	MN509442
	YN-EL3		3640	KX840469	土耳其 Turkey	MER46	柠檬 Lemon	3640	MG566050
	YN-EL3		3640	KX840470		MER50	酸橙 Sour orange	3641	MG566051
	YN-ML-66	墨西哥莱檬 Mexico lime	3641	MT680009		MER52	葡萄柚 Grapefruit	3640	MG566052
	YN-Th-168	塔西提莱檬 Tahiti lime	3641	MT680005		HAT62	宽皮柑橘 Mandarin	3640	MG566053
中国 China	PL1	柚 Pomelo	3642	MT655986		ADA74	明尼奥拉橘 Minneola tangelo	3642	MG566054
	PL2		3643	MT655987		ANT80	酸橙 Sour orange	3641	MG566055
	PL3		3642	MT655988		TK4	柑橘 Citrus	3640	JQ920490
	PL4		3642	MT655989	中国重庆 Chongqing, China	WX-2-5	红宝石柚 Ruby Green pomelo	3642	ON063221
	PL5		3642	MT655990		WX-4-6		3640	ON063222
	PL6		3642	MT655991		WX-4-9		3641	ON063223

1.4　致病性分析

将获得的CCDaV毒株WX-2-5、WX-4-6和WX-4-9分别嫁接接种于一年生无病毒尤力克柠檬、费米耐劳柠檬、沃柑、纽荷尔脐橙、鸡尾葡萄柚、琯溪蜜柚和三红柚上。每个品种重复5株，并各设置3株无病植株作为负对照。嫁接后所有植株放置于25~28 ℃的温室中备用。每月采用1.1中的方法进行检测，直至检测出病毒，同时观察植株症状。

2　结果与分析

2.1　田间调查结果

对采集的361份疑似感染柑橘褪绿矮缩病样品进行检测发现，3份采自巫溪县同一个果园的红宝石柚样品感染了CCDaV，检出率为0.83%。其余柚类样品和柠檬样品都未能检测出CCDaV。所有感染了CCDaV的红宝石柚嫩梢都出现了严重的叶片扭曲、皱缩症状。部分未感染CCDaV的红宝石柚也出现了叶片轻微或部分扭曲和皱缩，但所有阴性植株都没有发现退绿症状（图1）。

2.2　CCDaV基因组分析

图1　田间红宝石柚健株（左）和感染柑橘褪绿矮缩病的病株（右）

Fig. 1　Healthy (left) and *Citrus chlorotic dwarf-associated*-infected (right) Ruby Green pomelo plants in the field

对来自重庆地区的3株CCDaV毒株进行全序列分析，结果显示，WX-2-5分离株含有3642 nt，碱基组成为27.6% A、16.8% C、27.3% G、28.4% T；WX-4-6毒株含有3640 nt，碱基组成为27.6% A、16.7% C、27.5% G、28.3% T；WX-4-9毒株含有3641 nt，碱基组成为27.6% A、16.7% C、27.4% G、28.3% T。3个分离毒株的5’和3’非翻译区（UTR）都为194 nt和220 nt。与WX-4-6毒株相比，WX-4-9在1203 nt处多了个“T”，WX-2-5在1203和1204 nt处都多出一个“T”。3个毒株与已报道的CCDaV基因组结构特征一致。

氨基酸序列比对显示，WX-2-5毒株与41个已知CCDaV全序列的氨基酸序列之间有4个氨基酸位点发生了变异，分别对应氨基酸序列的第303（C→L）、304（I→H）、341（S→N）、1163（F→S）位；WX-4-6与41个CCDaV毒株的氨基酸序列之间有3个共同氨基酸位点发生了变异，分别在第43（L→W）、567（H→R）和722（R→C）位。WX-4-9除与WX-4-6有相同变异外，在第633（S→N）和1137（R→M）位还发生了突变。

WX-2-5、WX-4-6和WX-4-9毒株间的核苷酸和氨基酸相似性分别为99.3%~99.9%和97.7%~99.7%；这3个毒株与之前报道的41个CCDaV毒株间核苷酸和氨基酸相似性较高，分别为99.1%~99.7%和97.3%~99.7%。此外，在44个CCDaV全基因组序列间均未发现重组事件（图2）。

2.3　系统发育分析

根据CCDaV基因组构建的系统发育进化树（图3）显示：来源于土耳其的CCDaV毒株聚于相同的分支；泰国样品除Tha30外，都和中国柚类上获得的CCDaV毒株聚在一起。此外，巫溪样品WX-4-6和WX-4-9，与广东沙田柚上的CCDaV毒株聚在一簇；

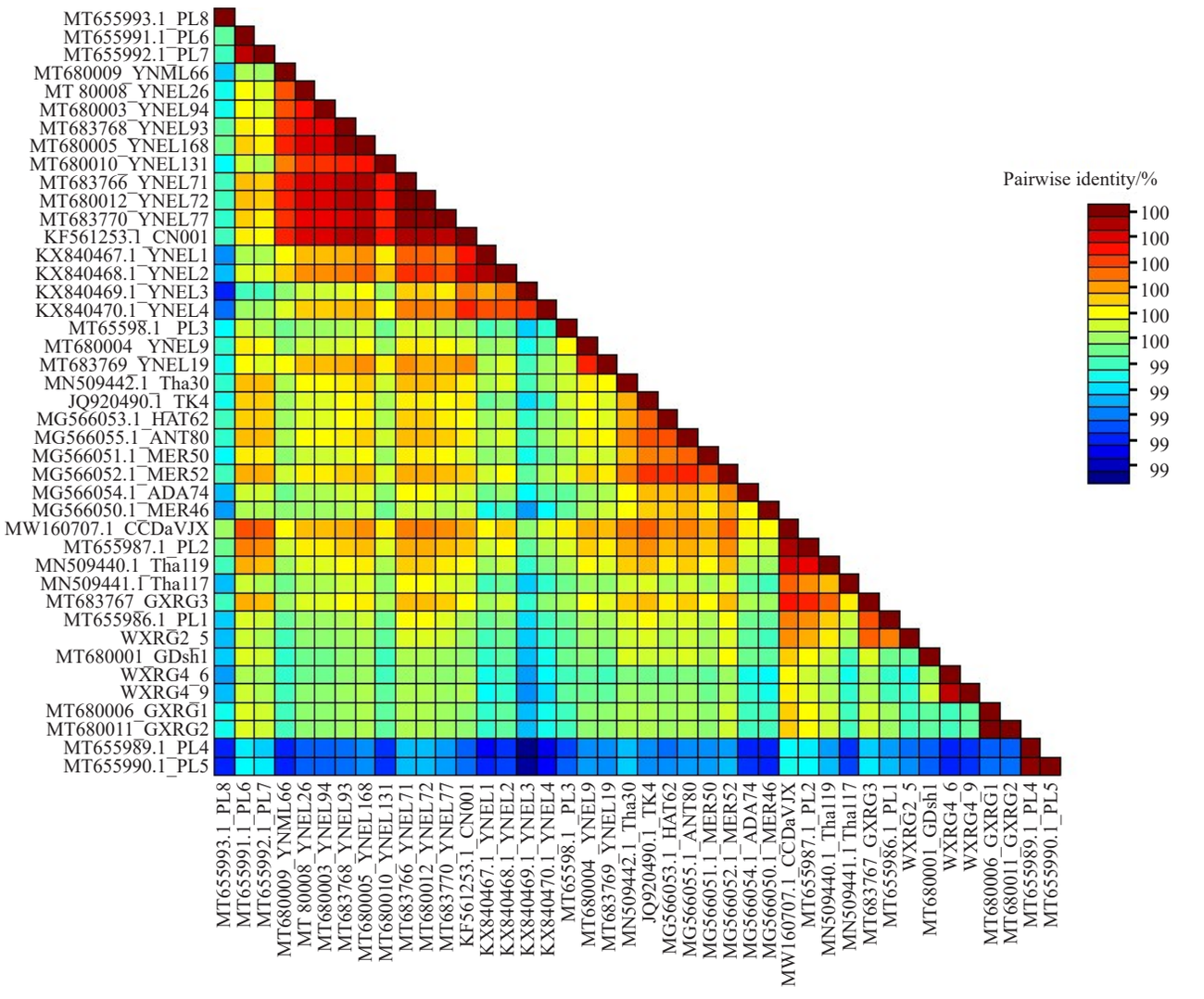
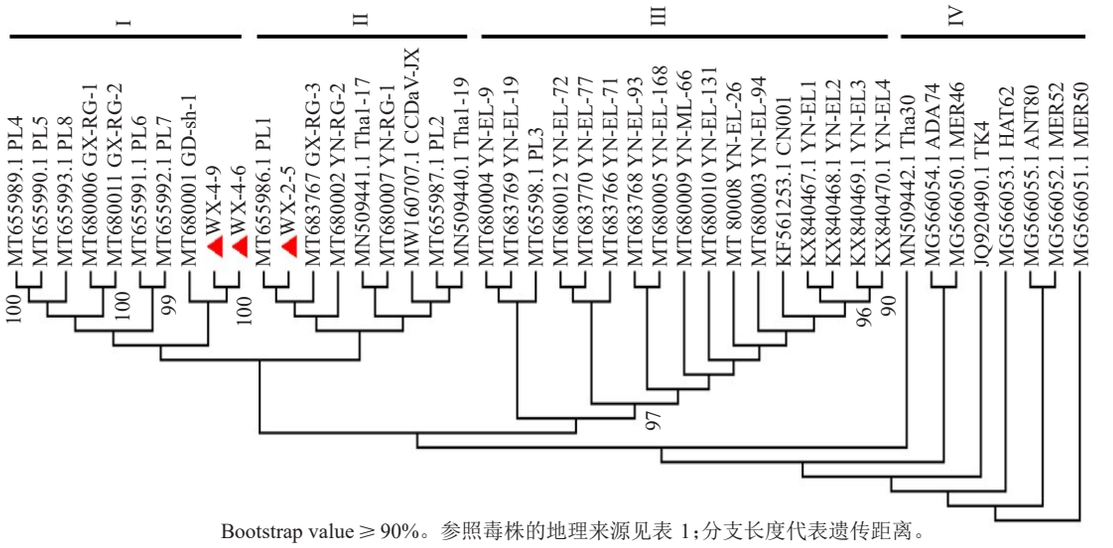


图 2 柑橘褪绿矮缩病毒不同毒株基因组相似性分析

Fig. 2 Similarity analysis of full-length nucleotide sequences of different *Citrus chlorotic dwarf-associated virus* isolates



Bootstrap value ≥ 90%。参照毒株的地理来源见表 1；分支长度代表遗传距离。

Complete gene Bootstrap value ≥ 90%. Geographic origin of referred isolates are listed in Table 1；Branch length represents the genetic distance.

图 3 柑橘褪绿矮缩病毒全基因核苷酸遗传进化分析

Fig. 3 Phylogenetic analysis of CCDaV isolates basing on nucleotide sequences

而巫溪样品WX-2-5与中国柚类上获得的CCDaV毒株聚于另一个分支。与来自土耳其的CCDaV毒株相比，中国和泰国的CCDaV毒株间的亲缘关系更近。

2.4　致病性分析

嫁接接种1个月后，除对照植株外，所有接种植株都检测出了CCDaV。接种半年后，尤力克柠檬、费米耐劳柠檬和鸡尾葡萄柚表现出叶片皱缩。琯溪蜜柚和三红柚除叶片皱缩外，还出现了退绿症状。沃柑的新梢脉明、退绿。纽荷尔脐橙没发生明显的症状（图4）。

A. 尤力克柠檬；B. 沃柑。

A. Eureka lemon; B. Orah.

图4　柑橘褪绿矮缩病毒致病性分析

Fig. 4　Pathogenicity analysis of *Citrus chlorotic dwarf-associated* virus

3　讨　论

重庆作为中国重要的柑橘产区和苗木繁育基地，不仅柠檬和柚类等特色品种的栽培面积较大，而且与国内外的柑橘种质资源交流频繁。前期研究显示，除葡萄柚外，柑橘褪绿矮缩病对柠檬、红宝石柚、泰国青柚、三红柚等多个柑橘品种的危害也较为严重[13]。本研究首次在重庆柑橘产区检测出CCDaV。虽然检出率较低，但已对当地柑橘生产造成了潜在威胁。此外，致病性分析还显示CCDaV可能会对中国栽培面积最大的杂柑品种沃柑造成影响。因此本研究结果对于防治柑橘褪绿矮缩病具有重要意义。在调查的11个柑橘品种中只有红宝石柚感染了CCDaV，由于随后在病株附近进行的大范围检测都未检测出病毒，也未发现其传播媒介杨梅类白粉虱（数据未显示），且该红宝石柚病株引自有CCDaV发生记录的广西南宁。由此推测，该病可能主要是通过苗木引入重庆。因此，今后需进一步规范苗木市场，通过使用无病毒苗木，进而有效阻止柑橘褪绿矮化病发生范围的进一步扩大。此外，鉴于除杨梅类白粉虱外，CCDaV可能还存在其他的媒介昆虫[11,13]，今后还需监测果园中昆虫的带毒情况，从而鉴定出可能的传播媒介。

前期研究显示，不同地理来源植株上的CCDaV毒株分子变异较小[14-15]。本研究对采自重庆的3个CCDaV毒株进行全序列分析的结果进一步证实，CCDaV毒株间的相似性很高，达99.3%，且基因组间未发生重组[13]。造成CCDaV基因组变异较小的原因可能是其基因组较为稳定，不易因地理或寄主差异而发生显著的变异[13]。进化树分析显示，根据寄主类型和采样地的差异，CCDaV毒株被分为了4个不同的类群，其中来源于柚类和柠檬的CCDaV毒株分别位于独立的分支。此外，在重庆巫溪获得的3个CCDaV毒株分别聚于两个不同的簇，暗示其可能具有不同的来源。

4 结 论

研究首次在重庆柑橘产区检测出CCDaV。全基因组分析的结果暗示，其可能是随带病的红宝石柚苗木传入重庆。研究结果为进一步研究中国CCDaV的流行病学和分子特征提供了重要的数据参考。

参考文献 References：

[1] ÇINAR A，KORKMAZ S，KERSTING U. Outbreaks and new records. Turkey. Presence of a new whitefly-borne citrus disease of possible viral aetiology in Turkey[J]. FAO Plant Protection Bulletin，1994，42(1)：73-74.

[2] GARNSEY S M. *Citrus* chlorotic dwarf，a new whitefly-transmitted disease in the eastern mediterranean region of Turkey[C]. International Organization of Citrus Virologists Conference Proceedings，1996，3(13)：2313-5123.

[3] YANG Z，ZHANG L，ZHAO J F，LI T S，LIU Q Y，CAO M J，ZHOU Y. First report of *Citrus chlorotic dwarf-associated virus* on pomelo in Nakhon，Thailand[J/OL]. Plant Disease，2020，104(4)：1262. DOI：10.1094/PDIS-10-19-2093-PDN.

[4] GUO J，LAI X P，LI J X，YUE J Q，ZHANG S Y，LI Y Y，GAO J Y，WANG Z R，DUAN H F，YANG J D. First report on *Citrus chlorotic dwarf-associated virus* on Lemon in Dehong Prefecture，Yunnan，China[J/OL]. Plant Disease，2015，99(9)：1287. DOI：10.1094/PDIS-01-15-0011-PDN.

[5] KARANFIL A，KORKMAZ S. Genetic diversity of Turkey *Citrus chlorotic dwarf-associated virus* isolates based on coat protein gene[J]. Fresenius Environmental Bulletin，2018，27(9)：6403-6407.

[6] CATARA A F，BAR-JOSEPH M，LICCIARDELLO G. Exotic and emergent citrus viruses relevant to the Mediterranean region[J]. Agriculture，2021，11(9)：1-27.

[7] ROUMAGNAC P，LETT J M，FIALLO-OLIVE E，NAVAS-CASTILLO J，ZERBINI F M，MARTIN D P，VARSANI A. Establishment of five new genera in the family *Geminiviridae*：*Citlodavirus*，*Maldovirus*，*Mulcrilevirus*，*Opunvirus*，and *Topilevirus*[J]. Archives of Virology，2022，167(2)：695-710.

[8] LOCONSOLE G，SALDARELLI P，DODDAPANENI H，SAVINO V，MARTELLI G P，SAPONARI M. Identification of a single-stranded DNA virus associated with citrus chlorotic dwarf disease，a new member in the family *Geminiviridae*[J]. Virology，2012，432(1)：162-172.

[9] YU Y Q，WU Q，SU H N，CHEN H M，WANG X F，CAO M J，ZHOU C Y. Small RNA deep sequencing reveals full-length genome of *Citrus yellow vein clearing virus* in Chongqing，China[J]. Journal of Integrative Agriculture，2017，16(2)：503-508.

[10] KORKMAZ S，ÇINAR A，BOZAN O，KERSTING U. Distribution and natural transmission of a new whitefly-borne citrus virus disease in the Eastern Mediterranean region of Turkey[C]. Proceedings of the 9th Congress of the Mediterranean Phytopathological Union，Granada，Spain，1994：437-439.

[11] SENGONCA Ç，UYGUN N，KERSTING U，ULUSOY M R. Successful colonization of *Eretmocerus debachi* (*Hym.*：*Aphelinidae*) in the eastern mediterranean citrus region of Turkey[J]. Entomophaga，1993，38(3)：383-390.

[12] 李战彪，刘丽辉，崔丽贤，陈锦清，朱英芝，李雯轩，谢慧婷，秦碧霞，蔡健和 . 侵染广西红宝石青柚的柑橘褪绿矮化相关病毒遗传进化分析[J]. 南方农业学报，2021，52(10)：2758-2764.
LI Zhanbiao，LIU Lihui，CUI Lixian，CHEN Jinqing，ZHU Yingzhi，LI Jixuan，XIE Huiting，QIN Bixia，CAI Jianhe. Genetic and evolutionary analysis of *Citrus chlorotic dwarf- associated virus* infecting Ruby green pomelo in Guangxi[J]. Journal of Southern Agriculture，2021，52(10)：2758-2764.

[13] YANG Z，ZHANG L，ZHAO J F，ZHANG X K，WANG Y，LI T S，ZHANG W，ZHOU Y. New geographic distribution and molecular diversity of *Citrus chlorotic dwarf-associated virus* in China[J]. Journal of Integrative Agriculture，2022，21(1)：293-298.

[14] BAKER R，CAFFIER D，CHOISEUL J W，CLERCQ P D，DORMANNSNE S E，GEROWITT B，KARADIOVA O E，LOVEI G，LANSINK A O，MAKOWSKI D，MANCEAU C，MANICI L，PERDIKIS D，PUGLIA A P，SCHANS J，SCHRADER G，STEFFEK R，STROMBERG A，TIILIKKALA K，VAN LENTEREN J C. Pest risk assessment made by France on *Citrus chlorotic dwarf virus* considered by France as harmful in the French overseas departments of French Guiana，Guadeloupe，Martinique and Réunion[J]. The European Food Safe Authority Journal，2008，6(5)：1-17.

[15] ZHOU Y，ZHANG Y H，LIU Y J，CHEN H M，LI T S，ZHOU C Y. Distribution and molecular characterization of *Citrus chlorotic dwarf-associated virus* in China[J]. Australasian Plant Pathology，2017，46(3)：227-229.