

# 我国柑橘近年新发生的病毒及类似病害研究进展

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**摘要:** 柑橘病毒类病害是由病毒、类病毒引起的严重危害世界柑橘产业的病害。随着柑橘产业的发展,新的柑橘病毒类病害不断出现,柑橘病毒类病害的危害也日趋严重。笔者对柑橘黄脉病、柑橘褪绿矮缩病、柑橘叶斑驳病、柑橘树皮裂纹类病毒、柑橘类病毒V和柑橘类病毒VI 6种我国近年来新发柑橘病毒类病害的发生分布、生物学特性和分子生物学特性进行总结,并对新发柑橘病毒类病害的检测和防治方法进行阐述,以期为我国柑橘产业的健康可持续发展提供借鉴。

**关键词:** 柑橘;新发病毒类病害;分子生物学特性

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## Progress in study of new *Citrus* viruses and viroids diseases in China

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**Abstract:** Citrus is one of the most popular fruit crops of the world from the equator to 41 degrees of latitude north and south. As a perennial plant, citrus is high value crop which is infected by a big number of viruses and viroids in the field due to vegetative propagation and grafting. Some of these pathogens cause severe crop losses and often reduce the productive life of the orchards. The most important cause for the worldwide spread and accumulation of viruses and viroids has been the international trading of infected nursery plant material. Subsequently, vector transmission might occasionally play a role in the spreading of these agents at the local level. In China, citrus was infected by more than 20 virus and viroids diseases, including Citrus tristeza, Citrus tatter leaf, Citrus satsuma dwarf, and Citrus exocortis etc, which severely affect its production as well as the quality. During recent years, there has been significant increase in the citrus production in China, and the citrus production of China stayed stably ca 33 million tonnes, which is of economical importance to farmers in the southern part. With the development of new detecting techniques, especially the next-generation sequencing, three new citrus virus and three viroids diseases were identified in China. These diseases are becoming more harmful to citrus production. Progress in studies on the new citrus virus and viroids diseases was summarized. The results obtained in recent years are as following: (1) *Citrus yellow vein clearing virus* (CYVCV), a member of genus *Mandarivirus*, family *Alphaflexiviridae*, is the causal agent of yellow vein clearing disease. CYVCV was first observed in Ruili, Yunnan province of China in 2009. Now this virus is widely distributed in China. CYVCV infects most citrus species, varieties and hybrids, and lemon (*Citrus limon*) is most sensitive to CYVCV. CYVCV is transmitted through vegetative propagation of infected buds, scion or rootstocks, and by mechanical inoculations of

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sap extracts onto herbaceous indicator hosts. CYVCV can also be transmitted by *Aphis craccivora* and *A. spiraecola* from lemon to bean (*Phaseolus vulgaris*), and from bean to bean. No other transmission ways are known. Comparison of the whole genome sequences of CYVCV isolates indicated that there is a very low level of sequence heterogeneity among CYVCV isolates of different geographic origins and hosts. (2) Citrus chlorotic dwarf-associated virus (CCDaV), a recently described member of the family *Geminiviridae*, is considered the causal agent of citrus chlorotic dwarf disease (CCDD). CCDD is considered to be the most serious disease of citrus in Turkish, only sweet orange showed some tolerance to the disease. Until recently, CCDD had not been reported to be present in any area outside of Turkey. However, in 2009, the typical symptoms caused by CCDD were first observed at one Eureka lemon yard in Ruili, Yunnan province, and now CCDaV was still restricted in few orchards in China. CCDaV is transmitted through vegetative propagation of infected buds, scion or rootstocks, and by stem-slash inoculation of sap extracts onto sensitive citrus species, such as *C. macrophylla*, rough lemon (*C. jambhiri*) and sour orange (*C. aurantium*). CCDaV could also be transmitted by the Japanese bayberry whitefly (*Parabemisia myricae*) effectively. (3) *Citrus leaf blotch virus* (CLBV), a member of the family *Flexiviridae*, has a 9 kb single-stranded, positive-sense genomic RNA encapsidated by a 41 ku coat protein. CLBV dispersal occurs primarily by propagation of infected buds, but seed transmission at low rates has been detected in at least three citrus species or hybrids. CLBV was first detected and characterized in a Nagami kumquat (*Fortunella margarita*) in Spanish, showing bud union crease on Troyer citrange (*C. Sinensis* × *Poncirus.trifoliata*). In recent years, CLBV was found in *Prunus avium*, *Actinidia chinensis* and Citrus in China. (4) Three new viroids were detected, including *Citrus bark cracking viroid* (CBCVd), *Citrus viroid V* (CVd-V), and *Citrus viroid V* (CVd-VI). Three new viroids species have been identified in citrus and belong in two genera: *Cocadviroid* (CBCVd), and *Apscaviroid* (CVd-V; CVd-VI), all belonging to the family *Pospiviroidae*, and they are unencapsidated, small, circular, covalently closed, single-stranded RNAs of 246 to 401 nucleotides (nt). Three new viroids infect most citrus species, varieties, hybrid of genera citrus. They are transmitted through vegetative propagation of infected buds, scion or rootstocks, and by knife-cut inoculation of sap extracts onto sensitive citrus species. 42 samples of 33 cultivars with such viroid symptoms as stunting, bark scaling, and cracking on *P. trifoliata* rootstock, were collected from citrus orchards. CBCVd was detected in cv. Meishan No. 9 (*C. sinensis*) from Sichuan, cvs. Akemi (*C. reticulata*) and Nishirokaori (*C. reticulata*) from Zhejiang, while CVd-V was detected in cvs. Nishirokaori, Haruka (*C. tamuranua*), and Kiyomi (*C. unshiu* × *C. sinensis*) from Zhejiang, Hunan and Chongqing, respectively. CVd-VI was detected by RT-PCR in nine samples (20.9%) collected from four different growth regions (Hunan, Sichuan, Chongqing and Zhejiang). Selection of resistant cultivars is the most effective way of combating virus and viroids diseases of citrus, but so far after many years of research the success is rather limited. As a result, control strategies are mainly focused on the production and use of virus-free plant propagative material and control the vectors.

**Key words:** *Citrus*; New virus and viroids diseases; Molecular characteristics

柑橘作为多年生果树,因农事操作和媒介昆虫在田间极易感染一种或多种病毒类病害,造成植株生长发育受阻,产量降低,品质变劣,甚至植株死亡。我国柑橘生产上的病毒类病害主要包括柑橘衰退病(*Citrus tristeza virus*, CTV)、柑橘碎叶病(*Citrus tatter leaf virus*, CTLV)、温州蜜柑萎缩病(*Satsuma*

*dwarf virus*, SDV)和柑橘裂皮病(*Citrus exocortis viroid*, CEVd)等。近年来,伴随柑橘产业结构调整,柑橘繁殖材料交流加快,新的病毒类病害问题亦不断出现。笔者对柑橘黄脉病、柑橘褪绿矮缩病、柑橘叶斑驳病、柑橘树皮裂纹类病毒、柑橘类病毒 V 和柑橘类病毒 VI 6 种中国新发柑橘病毒类病害的发生分布

(表1)、危害、传播途径、分子生物学特性,以及检测和防治方法作一综述,以期有效防控柑橘新发病毒类病害、保障我国柑橘产业健康持续发展提供借鉴。

表1 中国新发6种病毒及类病毒的发生与分布情况

Table 1 The occurrence and distribution of six new viruses and viruses in China

病害种类 Disease type	时间 First occurrence year	分布 Distribution
柑橘黄脉病 <i>Citrus yellow vein clearing virus</i>	2009	云南、四川、广西、江西、湖南、广东、福建、贵州、重庆 Yunnan, Sichuan, Guangxi, Jiangxi, Hunan, Guangdong, Fujian, Guizhou, Chongqing
柑橘褪绿矮缩病 <i>Citrus chlorotic dwarf-associated virus</i>	2009	云南 Yunnan
柑橘叶斑驳病 <i>Citrus leaf blotch virus</i>	2017	江西、四川、湖北、云南 Jiangxi, Sichuan, Hubei, Yunnan
柑橘树皮裂纹类病毒 <i>Citrus bark cracking viroid</i>	2010	四川、浙江、江西、重庆、湖南、云南 Sichuan, Zhejiang, Jiangxi, Chongqing, Hunan, Yunnan
柑橘类病毒 V <i>Citrus viroid V</i>	2010	浙江、云南、重庆 Zhejiang, Yunnan, Chongqing
柑橘类病毒 VI <i>Citrus viroid VI</i>	2012	浙江、四川、湖南、重庆 Zhejiang, Sichuan, Hunan, Chongqing

## 1 柑橘黄脉病

1988年首次在巴基斯坦的柠檬(*Citrus limon*)和酸橙(*C. aurantium*)上发现了一种新的柑橘病害,根据其产生的黄化脉明症状将其命名为柑橘黄脉病<sup>[1]</sup>。随后在印度、土耳其的‘香檬’(*C. medica*)、‘尤力克’柠檬(*C. limonia*)和多种柠檬品种上也观察到类似症状,柠檬发病后减产可超过50%<sup>[2-3]</sup>。2009年我国首次在云南瑞丽的‘尤力克’柠檬上观察到典型的柑橘黄脉病症状<sup>[4]</sup>,随后在四川安岳地区也检测出柑橘黄脉病<sup>[5]</sup>。目前柑橘黄脉病在我国分布较广,除云南、四川外,在广西、广东、江西和福建等多个柑橘主产区都有分布<sup>[6]</sup>。

柑橘黄脉病的病原为 $\alpha$ 线性病毒科(*Alphaflexiviridae*),印度柑橘病毒属(*Mandarinivirus*)的柑橘黄化脉明病毒(*Citrus yellow vein clearing virus*, CYVCV)<sup>[7]</sup>。CYVCV粒子呈弯曲线状,大小约为(13~14)nm $\times$ 685 nm<sup>[8-9]</sup>。CYVCV基因组是由7 529个核苷酸组成的正单链RNA,具有5'、3'端非编码区(UTR)和Poly(A)结构<sup>[7]</sup>。部分CYVCV毒株5'UTR的第28和29位核苷酸会出现2个碱基(“-CA-”)的插入,尚不清楚该结构具有何种功能<sup>[6]</sup>。CYVCV的基因组含有6个以ATG作为起始密码子,并分别以TAA和TGA为终止密码子的开放读码框(ORFs)。其中ORF1编码病毒的复制酶蛋白,ORF2、ORF3、ORF4部分重叠组成一个三基因区域,分别合成25 ku、12 ku和6.4 ku的蛋白质,其功能可能与病毒在细胞间的运动有关。ORF5编码病毒的

外壳蛋白。ORF6与ORF5部分重叠,编码一个23 ku的细胞核酸结合蛋白<sup>[7,10]</sup>。

对23个CYVCV毒株全序列分析发现,该病毒较为保守,其基因组不因地域或柑橘品种差异而发生显著变异。此外,来自中国、巴基斯坦和土耳其的CYVCV毒株分别位于进化树上的不同分支,意味着CYVCV可能存在不同的起源地<sup>[6]</sup>。

CYVCV寄主范围广泛,除可侵染柑橘属的大多数种及其杂种外<sup>[4,11-12]</sup>,还可以侵染豇豆(*Vigna unguiculata*)、菜豆(*Phaseolus vulgaris*)、辣椒(*Capsicum annuum*)、藜麦(*Chenopodium quinoa*)等草本植物<sup>[4,12-13]</sup>。近年来在锦葵(*Malva sylvestris*)、龙葵(*Solanum nigrum*)、野芥(*Sinapis arvensis*)和田野毛茛(*Ranunculus arvensis*)上也检测出了CYVCV<sup>[14]</sup>。

CYVCV在不同柑橘品种上引起的症状差异明显,其中以在柠檬、酸橙和‘德威特’橘橙(*C. reticulata* $\times$ *C. sinensis*)上的症状最为显著<sup>[11,15]</sup>。病株表现出叶脉黄化、脉明、叶背水浸状、叶片反卷和皱缩。叶片老熟后黄化症状消退并转绿,但叶片皱缩症状不会恢复,且对光看脉明明显。此外,葡萄柚(*C. paradisi*)、琯溪蜜柚(*C. grandis*)和部分甜橙、宽皮柑橘(*C. reticulata*)品种感病后仅春梢嫩叶表现出轻微脉明,且枝梢老熟后症状消失<sup>[6]</sup>。近来的研究还显示,CYVCV在不同寄主上引起的症状差异与植株中的病毒含量无显著关联<sup>[16]</sup>。

CYVCV除通过感病苗木、接穗和嫁接传播外,还可通过豆蚜(*Aphis craccivora*)和绣线菊蚜(*A. spiraeicola*)在菜豆之间,以及柠檬到菜豆间进行传播,

其传毒率均高于 40%<sup>[13]</sup>。虽然从 CYVCV 病株中获得的粗提液只能侵染菜豆和藜麦等草本寄主,但通过蔗糖超速离心法纯化的病毒颗粒可有效侵染多个柑橘品种<sup>[17]</sup>。目前尚未发现该病毒可进行种传的证据<sup>[18]</sup>。

## 2 柑橘褪绿矮缩病

自上世纪 80 年代中期首次在土耳其发现柑橘褪绿矮缩病以来,该病迅速扩散。目前,在土耳其各主要柑橘产区均有分布,已成为在土耳其危害最严重的柑橘病毒类病害,葡萄柚等敏感品种感病后减产超过 50%<sup>[19]</sup>。柑橘褪绿矮缩病长期以来只分布于土耳其,但 2009 年在中国云南省德宏州的‘尤力克’柠檬上首次鉴定出该病<sup>[20]</sup>。目前柑橘褪绿矮缩病仍零星分布于我国云南的少数地区<sup>[21-22]</sup>。

早期橄榄隐症病毒(*Olive latent virus*, OLV-1)被认为可能是引起柑橘褪绿矮缩病的主要原因<sup>[23]</sup>,但随后研究显示二者间不存在直接的关联<sup>[24]</sup>。近年来,Loconsole 等<sup>[25]</sup>通过深度测序技术证明柑橘褪绿矮化病的病原为一种新的双生病毒,并将其暂命名为柑橘褪绿矮缩相关病毒(*Citrus chlorotic dwarf-associated virus*, CCDaV)。CCDaV 基因组约 3.64 kb,具有双生病毒特有的 TAATATTAC 序列,以及因包含回文序列而形成发夹结构的基因间隔区。CCDaV 基因组含有 5 个 ORFs,其中 ORF1 编码 1 个 15.3 ku 双生病毒特有的蛋白保守结构域 V2,ORF2 与 ORF1 部分重叠,编码 1 个 27.9 ku 的外壳蛋白,ORF3 和 ORF4 可能与病毒在细胞间的移动与复制有关<sup>[26]</sup>。

CCDaV 可侵染大多数柑橘品种,其中以葡萄柚、酸橙、粗柠檬(*C. jambhirilush*)等柑橘类型最为敏感,温州蜜柑(*C. unshiu*)和甜橙对 CCDaV 具有一定的抗性<sup>[27-28]</sup>。敏感品种感染 CCDaV 后,表现出严重的叶片皱缩,反转,并伴随褪绿现象。此外,病树枝条畸形,节间缩短,植株矮小<sup>[19,29]</sup>。CCDaV 除通过感病苗木、接穗和嫁接传播外<sup>[30]</sup>,还可通过杨梅粉虱(*Parabemisia myricae*)进行传播<sup>[29]</sup>。由于杨梅粉虱在中国以及意大利和土耳其等地中海沿岸都有广泛分布,因此上述国家和地区也面临 CCDaV 入侵和爆发的风险<sup>[25]</sup>。此外,CCDaV 还可以通过刀割的方式感染酸橙、粗柠檬等敏感柑橘类型<sup>[29]</sup>。

## 3 柑橘叶斑驳病

柑橘叶斑驳病最早在西班牙的金橘(*Fortunella*

*margarita*)上被发现<sup>[31]</sup>。随后在日本、澳大利亚、西班牙、美国、意大利、新西兰和古巴等国也相继有柑橘叶斑驳病发生的报道<sup>[32-36]</sup>。近年来,中国在甜樱桃(*Prunus avium*)和猕猴桃(*Actinidia chinensis*)上陆续检测出柑橘叶斑驳病的病原<sup>[37-38]</sup>。项周等<sup>[39]</sup>在我国云南、四川、江西和湖北柑橘主产区检测到该病。

柑橘叶斑驳病的病原为  $\beta$  线性病毒科(*Betaflexiviridae*),柑橘病毒属(*Citriovirus*)的代表种柑橘叶斑驳病毒(*Citrus leaf blotch virus*, CLBV),病毒粒子呈弯曲丝状,大小为 960 nm  $\times$  14 nm<sup>[33]</sup>。CLBV 基因组为 8 747 个核苷酸构成的正单链 RNA,含有 3 个 ORFs<sup>[40]</sup>。其中,ORF1 编码 1 个 227 ku 与复制相关的多聚蛋白,ORF2 编码 1 个 40 ku 与病毒在细胞间运动相关的蛋白,ORF3 编码 40.7 ku 的外壳蛋白<sup>[41]</sup>。CLBV 基因组中的 5' 末端具有甲基化帽子结构,3' 末端具有 poly(A)结构<sup>[42]</sup>。通过分析 CLBV 的 R 结构域和 C 结构域,以及采自甜樱桃、猕猴桃、柑橘及其近缘种上 CLBV 毒株外壳蛋白基因(CPG)发现,CLBV 的序列较为保守,遗传变异性差<sup>[38-39,43]</sup>。

CLBV 可以侵染大多数柑橘品种,其中以‘枳柚’(*C. paradisi*  $\times$  *Poncirus trifoliata*)、‘特洛依’枳橙(*C. sinensis*  $\times$  *P. trifoliata*)、‘香椽’、‘四季橘’(*C. mitis*)等柑橘品种最为敏感<sup>[44-45]</sup>。CLBV 也可以感染甜樱桃和猕猴桃<sup>[38-39]</sup>,并在试验条件下侵染本生烟(*Nicotiana benthamiana*)<sup>[46]</sup>。CLBV 在大多数柑橘品种上不表现明显的症状,但可在‘德威特’橘橙、‘香椽’和‘甜橙’上分别引起叶片褪绿、斑驳,茎陷点和脉明症状<sup>[32-33]</sup>。此外,CLBV 还可以导致枳柚和特洛依枳橙砧木上嫁接口开裂<sup>[47]</sup>。CLBV 除通过嫁接传播外,还可以借助沾染病毒的工具或汁液进行传播<sup>[48]</sup>。此外,CLBV 还可通过种子进行传毒,但传毒率较低<sup>[49]</sup>。

## 4 类病毒病害

近年来我国在柑橘上发现了柑橘树皮裂纹类病毒(*Citrus bark cracking viroid*, CBCVd)、柑橘类病毒 V(*Citrus viroid V*, CVd-V)和柑橘类病毒 VI(*Citrus viroid VI*, CVd-VI)3 种通过嫁接、苗木和汁液进行传播的类病毒,且均属于马铃薯纺锤形块茎类病毒科(*Pospiviroidae*)成员<sup>[50-52]</sup>。

CBCVd 最早于 1988 年在美国加利福尼亚州的枳橙砧木上被发现<sup>[53]</sup>,随后在以色列、土耳其和斯洛文尼亚也有报道<sup>[54-56]</sup>。我国自 2010 年首次发现 CB-

CVd以来,目前在重庆、四川、浙江、江西、湖南和云南等多个主要柑橘产区都检测出了CBCVd<sup>[51]</sup>。CBCVd原名柑橘类病毒IV,2011年国际病毒分类委员会(ICTV)将其更名为CBCVd。CBCVd是椰子死亡类病毒属(*Cocadviroid*)的成员<sup>[57]</sup>,含有284个核苷酸,具有棒状二级结构,在其结构中71%的核苷酸存在碱基配对<sup>[58]</sup>,具有苹果锈果类病毒属(*Apscaviroid*)特有的中央保守区(CCR)、末端保守发卡末端(CCR)和末端保守区(TCR)<sup>[59]</sup>。CBCVd的V结构域和TR结构域有80~90 nt与CEVd相同,其TL结构域与啤酒花叶类病毒(*Hop stunt viroid*, HSVd)的相似性高于76%,由此推测该类病毒是CEVd和HSVd的嵌合体<sup>[58,60]</sup>。CBCVd寄主范围广泛,可侵染大多数的柑橘种及其杂种。其中枳橙最为敏感,引起树皮开裂,树皮下对应木质部出现绿色条纹和突起<sup>[61]</sup>。香橼感病后嫩梢叶片出现轻微和短时间的反卷<sup>[58]</sup>。除柑橘外,CBCVd还可以侵染冬瓜(*Benincasa hispida*)、啤酒花(*Humulus lupulus*)、菊花(*Chrysanthemum morifolium*)、黄瓜(*Cucumis sativus*)、曼陀罗(*Datura stramonium*)、西红柿(*Lycopersicon esculentum*)和秘鲁番茄(*L. peruvianum*)<sup>[52,58]</sup>。

CVd-V最早是由Serra等<sup>[62]</sup>在西班牙的柑橘近缘种 *Atalantia citroides* 上分离出一种新的类病毒。目前CVd-V主要分布于西班牙、尼泊尔、阿曼、伊朗、日本、巴基斯坦、土耳其和北非等地<sup>[52,63-67]</sup>。我国于2010年首次在浙江、云南和重庆等地引种的‘西之香’‘春香’和‘清见’等杂柑上检测出CVd-V<sup>[51]</sup>。CVd-V为苹果锈果类病毒属成员,具有293~294个核苷酸,富含GC(大约60%)<sup>[62]</sup>,最低自由能下预测二级结构为棒状结构,在其结构中68.7%的核苷酸存在碱基配对,具有苹果锈果类病毒属典型的CCR和马铃薯纺锤块茎类病毒科典型的TCR<sup>[68]</sup>。Serra等<sup>[69]</sup>通过CVd-V和柑橘矮化类病毒(*Citrus dwarfing viroid*, CDVd)的嵌合体侵染性克隆试验,证实了CVd-V的致病性决定区域是TL结构域。CVd-V寄主范围广泛,能够侵染大多数的柑橘种及其近缘种,但在多数情况下都不引起显著的症状,仅在香橼引起轻度矮化,并在枝条上产生细小的充胶坏死和裂口症状<sup>[68]</sup>。尚未发现CVd-V的草本寄主。

CVd-VI最早于2000年发现于日本的‘不知火’<sup>[70]</sup>。到目前为止,仅日本发现报道CVd-VI,但近年在采自中国浙江、四川、湖南和重庆等地区的多个

柑橘品种中都检测到了CVd-VI<sup>[71]</sup>。CVd-VI原暂定名为 *Citrus viroid original sample* (CVd-OS),国际柑桔病毒学家组织(IOC)在第9次会议报告中将其更名为CVd-VI。CVd-VI为苹果锈果类病毒属成员,含有330~331个核苷酸,具有苹果锈果类病毒属典型的CCR和TCR<sup>[70]</sup>。此外,序列分析显示, CVd-VI的C和TL结构域,V和TR结构域分别与柑橘矮化类病毒(*Citrus dwarfing viroid*, CDVd)、苹果凹果类病毒(*Apple dimple fruit viroid*, ADFVd)、CBCVd和CEVd的相似性超过65%,由此推测CVd-VI可能也是一种嵌合体类病毒<sup>[70,72]</sup>。CVd-VI可侵染大多数的柑橘种及其杂种,其中以在‘Etrog’香橼上症状最为明显,引起微弱的叶脉坏死和叶片曲折<sup>[72]</sup>。除柑橘外,CVd-VI还可以侵染柿子(*Diospyros kaki*)<sup>[73]</sup>。

虽然CDVd与CVd-V单独侵染柑橘时往往不引起明显的症状,但是CVd-V与CDVd复合侵染‘Arizona 861-S-1’香橼后会造造成严重矮化,卷叶,叶片中脉坏死,同时枝条出现裂纹、流胶症状<sup>[68,74]</sup>。柑橘类病毒间产生的协生现象可能与类病毒诱导的基因沉默抑制了植物的代谢途径有关<sup>[75]</sup>。除协生现象外,柑橘类病毒之间还会产生拮抗作用,CEVd会引起枳橙砧木上严重的裂皮及树皮表面坏死症状,但CBCVd与CEVd复合侵染枳橙时,则会减轻枳橙砧木上裂皮症状的发生程度<sup>[76]</sup>。

## 5 检测技术

目前,柠檬、酸橙、‘尤力克’柠檬、墨西哥莱檬、‘Arizona 861-S-1’香橼等柑橘品种被用于CYVCV、CCDaV以及柑橘类病毒鉴定<sup>[7,27,77]</sup>。同时,豇豆、菜豆等草本植物也可用于CYVCV的检测<sup>[13]</sup>。

电子显微镜技术是早期研究中检测CYVCV、CCDaV和CLBV的重要手段<sup>[34,78]</sup>。通过电镜与荧光标记技术相结合发现CLBV在柑橘和本生烟的根茎叶中分布不均匀<sup>[79-80]</sup>。

宾羽等<sup>[81]</sup>已建立了CYVCV的血清学检测方法,在此基础上研发的直接组织点免疫(DTBIA)和免疫胶体金检测技术进一步简化了检测步骤,尤其适用于基层植保人员对田间样品的检测。

核酸杂交技术已应用于CLBV、CBCVd、CVd-V和CVd-VI的检测,其最少可检测出0.1 mg的新鲜组织中的病原,灵敏度可达pg级<sup>[47,82-83]</sup>。Galipienso等<sup>[47]</sup>为缩短CLBV的检测时间,发展出了基于组织印记

杂交(tissue print hybridization, TPH)和斑点杂交的核酸杂交技术(dot-blot hybridization, DBH)。这2种技术虽可用于检测‘尤力克’柠檬、马叙葡萄柚(*C. paradisi*)和金柑(*C. fortunella*)等多种柑橘品种中的CLBV,但无法检测出凤梨甜橙(*C. sinensis*)中的CLBV。

CYVCV、CCDaV、CLBV、CVd-V和CVd-VI都已建立了相应的(RT-)PCR和实时荧光定量RT-PCR检测体系,用于田间样品的快速检测<sup>[15,24,40,62]</sup>。为提高检测的灵敏度和特异性,周彦等<sup>[5]</sup>建立了CYVCV的巢式RT-PCR检测方法,其灵敏度较常规RT-PCR提高了100倍。此外,Takao等<sup>[84]</sup>建立的多重RT-PCR体系能同时检测CEVd、CVd-I-LSS、HSVd、CDVd、CVd-VI和柑橘类病毒IV(*Citrus viroid IV*, CVd-IV)6种类病毒,提高了检测效率。

刘科宏等<sup>[85]</sup>已针对CYVCV建立了相应的逆转录环介导等温扩增(LAMP)检测方法。该方法不需要PCR仪,70 min内即可完成反应,且灵敏度是普通(RT-)PCR的10倍,根据染料与dsDNA结合时由橘黄色变为绿色的原理,通过在反应时加入SYBR Green I荧光染料,不需要电泳,避免了电泳时可能产生的气溶胶污染。此外,Loconsole等<sup>[25]</sup>通过Solexa测序平台首次发现了危害柑橘的双生病毒CCDaV,并明确了印度柑橘病毒属一个新种CYVCV的全序列。

## 6 防治方法

防治病毒病最有效的方法是培育和种植抗病品种。由于柑橘病毒类病害主要通过嫁接传播,且柑橘育种的周期较长,因此短期防治的关键是加强苗木的市场管理和产地检疫,通过使用无病毒苗木、接穗、种子,确保种源无毒化,阻断病害随繁殖材料调运进行远距离传播。同时进行枝剪消毒,防止病害经农事操作在田间传播扩散。由于CYVCV和CCDaV可通过绣线菊蚜、豆蚜、杨梅粉虱等媒介昆虫进行传播,因此还需重视田间虫害的防控。此外,做好水肥管理,增强树势可在一定程度上减轻危害。

## 7 展 望

目前我国柑橘产业发展迅速,同时随着全球柑橘种质资源交流的增多,必然会有新的柑橘病毒类病害涌入,从而对我国柑橘产业造成严重的损失。因此在保证产地检疫的同时,还需研究和掌握柑橘

新发病毒类病害的种类、发生特点、流行规律,以及检测和防治技术,同时运用深度测序等新型检测技术对出现的未知病害进行鉴定,以期为我国柑橘新发病毒类病害的有效防控和抗病毒育种工作提供一些信息,从而为保障我国柑橘产业的健康发展提供必要的技术支持。

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