果 树 学 报 2021,38(6):957-966
Journal of Fruit Science

DOI:10.13925/j.cnki.gsxb.20200539

基于2个保守基因分析湘赣南部区域野生柑橘上衰退病毒分离株的种群特征

王长宁,易 龙*,周 俊,钟 可,陈毅群

(赣南师范大学生命科学学院,江西赣州 341000)

摘 要:【目的】比较分析湘赣南部地区野生柑橘上的CTV种群特征,为解析CTV种群的遗传进化提供重要依据。**【方法】**应用RT-PCR对江西崇义、湖南道县、湖南莽山和湖南江永4个不同地理区域的野生柑橘CTV分离株的*CP*基因和*p23*基因进行扩增、测序,所获得序列与从GenBank下载的国内外具有代表性的分离株相应基因序列进行碱基组成、分子变异(AMOVA)分析、系统发育和中介网状关系结构分析。**【结果】**碱基组成分析显示,4个地区的CTV分离株上2个基因的A+T碱基含量均高于G+C含量;*CP*基因和*p23*基因AMOVA分析结果显示,种群内分子变异分别占85.8%和82.38%,种群间分别占14.20%和17.62%,基因流*Nm*分别为6.749和1.475;碱基错配分析显示,2个基因均呈多峰分布曲线,表明4个地区CTV种群变化保持稳定;重组分析显示,2个基因均未检测出重组事件的发生,基于2个基因构建的系统发育树显示,相同地理来源的CTV种群大部分聚集在同一簇中,少部分分散在不同簇,表明研究分析的CTV种群间亲缘关系与其地理来源存在相关性,中介网状关系结构图得出类似分析结果。**【结论】**4个地区的野生柑橘上CTV种群间遗传变异主要来自种群内,各地区间基因交流频繁,遗传差异小,CTV种群保持稳定,*CP*基因和*p23*基因构建的系统发育分析和中介网状关系结构图表明,湘赣南部地区的野生柑橘上CTV种群间亲缘关系与其地理来源存在相关性。

关键词:野生柑橘;柑橘衰退病毒;种群特征;分子系统地理学

中图分类号:S666 S436.661 **文献标志码:**A **文章编号:**1009-9980(2021)06-0957-10

Population characteristics analysis of citrus tristeza virus isolates from wild citrus in southern Hunan and Jiangxi based on two conserved genes

WANG Changning, YI Long*, ZHOU Jun, ZHONG Ke, CHEN Yiqun

(*School of Life Science, Gannan Normal University, Ganzhou 341000, Jiangxi, China*)

Abstract:【Objective**】**The experiment aimed to clarify the population characteristics of *Citrus tristeza virus* (CTV) in wild citrus, and to provide an important basis for analyzing the genetic evolution of CTV population. **【**Methods**】**With 10 positive CTV samples from four different geographic regions of Chongyi (Jiangxi), Daoxian (Hunan), Mangshan (Hunan) and Jiangyong (Hunan), the *CP* gene and *p23* gene amplification were carried out. The samples were named Chongyi 1-10 (C-1 to C-10), Daoxian 1-10 (D-1 to D-10), Mangshan 1-10 (M-1 to M-10), and Jiangyong 1-10 (Y-1 to Y-10). The samples were preserved at −40 ℃ for later assay. After purification and recovery, the PCR products were sent to Shanghai Bioengineering Company for paired and sequencing. MEGA-X was used to calculate the conservative site, abbreviated information site, mutation site, descent locus and the ratio of the base transition rate *Ts* to the transversion rate *Tv*. The software Arlequin 3.1 was used for the analysis of molecular variance (AMOVA), and GenAIEx 6.5 was used for gene flow *Nm* calculation. DnaSP was used to perform three neutrality tests and mismatch analysis to infer population expansion and genetic evolution. RDP 5 was used to perform recombination event analysis. In order to clarify the genetic relationship

收稿日期:2020-12-29 **接受日期:**2021-03-03

基金项目:国家自然科学基金(31860488);赣州市科技创新人才计划([2018]50)

作者简介:王长宁,男,在读硕士研究生,主要从事柑橘病毒病害检测与防控研究工作。Tel:15779743368,E-mail:2281135935@qq.com

*通信作者 Author for correspondence. Tel:15216176601,E-mail:yilongswu@163.com

among the CTV isolates of wild citrus from different regions of the same gene, this study combined the 40 *CP* gene sequences and *p23* gene sequences obtained by sequencing and the *CP* gene sequences and *p23* genes of eight CTV isolates downloaded from GenBank and built phylogenetic trees. In the process, it used maximum likelihood (ML) and the Kimura 2-parameter model respectively, and each branch node confidence in the phylogenetic tree underwent repetitive verification for 1000 times based on bootstrap. The isolates data downloaded from GenBank included those of US isolates T36 (U16034) and T30 (AF260651), Spanish isolates T318A (DQ151548) and T385 (Y18420), Israeli isolates VT (U56902), Egyptian isolates Qaha (AY340974), the Mexican isolate Mexico-ctv (DQ272579) and the Japanese isolate NuagA (AB046398). Through Network 10.0 and the Median-joining method, we constructed the haplotype network based on *CP* gene and *p23* gene respectively, and analyzed the evolutionary relationship among those isolates.【Results】The base composition analysis showed that the *CP* genes base A, base T, base C, and base G nucleotide base contents of CTV isolates from wild citrus in four regions were 28.32%, 26.99%, 18.49% and 26.20%, respectively. The ratio of transition to transversion was 5.049, with 555 conservative sites, 80 abbreviated information sites, 117 mutation sites, and 37 descent loci. The nucleotide content of base A, base T, base C, and base G in *p23* gene were 30.48%, 24.74%, 19.60%, and 25.18%, respectively. The ratio of transition to transversion was 4.415, of which the transition was higher than the transversion, with 485 conservative sites, 97 abbreviated information sites, 145 mutation sites, 48 descent loci, and AT base content was higher than that of GC. According to the AMOVA results of the *CP* gene and *p23* gene, it showed that the molecular variation within the population accounted for 85.8% and 82.38%, and the molecular variation between the populations accounted for 14.20% and 17.62%, respectively, which indicated that the genetic variation mainly came within the population. The gene flow Nm was 6.749 and 1.475, respectively, indicating that there was a high level of gene exchange among the four regions. The result of the neutrality test showed that the CTV population may remain in a stable state or experienced population expansion, but the P value was not significant. Through base mismatches, it was further found that the two genes both formed the multimodal distribution curve, indicating that the CTV population from four regions remained stable. Through recombination analysis, it showed that there was no recombination event detected for both genes. The phylogenetic trees constructed based on the two genes showed that most of the CTV population of the same geographic origin were aggregated in the same cluster, and a few were scattered in different clusters. For example, in the phylogenetic tree constructed based on *p23* gene, the first group included 8 Chongyi isolates; the second group included 5 Daoxian isolates, 8 Jiangyong isolates and 2 Mangshan isolates; the third group included 2 Jiangyong isolates, 5 Daoxian isolates, 7 Mangshan isolates, 2 Chongyi isolates and VT, NuagA, T318A, T385 isolates; the fourth group included 1 Mangshan isolate and Qaha, T36, Mexico-ctv isolates. It indicated that the genetic relationship among CTV populations on wild citrus from different geographic sources was related to geographic distance, and the haplotype network structure yielded similar analyzing results.【Conclusion】The genetic variation among CTV populations on wild citrus in the four regions mainly came within the population. Although gene exchanges were frequent among regions, genetic differences were small, and CTV populations remained stable. Moreover, phylogenetic analysis and haplotype network structure showed that the genetic relationship among CTV populations on wild citrus from different geographical sources was correlated with geographical distance.

Key words: Wild citrus; *Citrus tristeza virus*; Population characteristics; Molecular phylogeography

柑橘衰退病是世界范围内危害柑橘最严重的果树病害之一，该病害由柑橘衰退病毒（*Citrus tristeza virus*，CTV）引起。CTV属于长线型病毒科（Closteroviridae）长线型病毒属（*Closterovirus*）的正义单链RNA病毒，病毒颗粒大小约12 nm×2000 nm[1]。CTV基因组中含有12个开放阅读框（open reading frames, ORFs）[2]，分别为ORF 1a、ORF 1b、ORF 2~ORF 11；其中，*CP*基因属于OFR 7，*CP*基因大小为672 bp，编码97%的外壳蛋白，是CTV病毒颗粒的主要组装成分[3]；*p23*基因属于3’端的ORF 11，*p23*基因大小约为630 bp，属于CTV沉默抑制子之一，可短暂抑制局部沉默[4]，有研究表明，*p23*基因表达的蛋白在寄主与病毒互作中具有关键性作用[5]。

明确CTV分离株的遗传多样性，对了解CTV进化历史、各基因功能及变异情况具有重要意义。吴官维[6]将中国不同地区的栽培柑橘CTV分离株上的*CP*基因和*CPm*基因与国外CTV分离株进行系统发育比较分析，结果显示，各分离株间无明显的地域相关性，且中国与其他国家和地区的CTV分离株存在明显的基因交流；刘志芳等[7]对赣南地区栽培柑橘CTV分离株上的*CP*基因聚类分析，发现各分离株间亲缘性和同源性较高，但与已知CTV分离株序列的亲缘性和同源性较低，推测CTV基因组中可能存在不对称的序列变异；易龙等[8]对野生柑橘和栽培柑橘CTV分离株上的*p23*基因进行比较分析，发现野生柑橘和栽培柑橘CTV分离株的*p23*基因在碱基组成、非同义突变与同义突变比值等遗传特征方面具有较高相似性。

分子系统地理学是一门通过生物信息学研究生物种群内和种群间演化过程[9]的新兴学科，目前主要用于分析不同地理种群动植物间的相关性[10-13]，在病毒上主要集中在种群结构、遗传特征及致病机制相关方面的研究[14-16]，分子系统地理学分析较少。笔者主要对不同生境野生柑橘上CTV基因组的*CP*基因和*p23*基因进行遗传多样性和分子系统地理学分析，明确不同地区野生柑橘上CTV分离株间的分子遗传差异，为解析CTV种群的遗传进化提供重要依据。

1　材料和方法

1.1　试验材料

供试材料分别采自江西崇义、湖南道县、湖南莽山和湖南江永野生柑橘主群落，将采集的样品通过反转录-聚合酶链式反应（RT-PCR）的方法进行柑橘衰退病检测。随后从4个地区的CTV阳性样品中分别随机选取10份进行后续试验，选取样品分别命名为崇义1~10（C-1~C-10）、道县1~10（D-1~D-10）、莽山1~10（M-1~M-10）和江永1~10（Y-1~Y-10）。样品于-40 ℃保存备用。

1.2　试验方法

按照TRIzol试剂（TaKaRa，Beijing，China）说明书提取40份野生柑橘总RNA，并参照Gillings等[17]的方法对*CP*基因（CP1:ATGGACGACGAAACAAAG，CP3:TCAACGTGTGTTGAATTT）和*p23*基因（p23-F:ATGGATAATACTAGCGGACA，p23-R:TCAGATGAAGTGGTGTTCAC）进行扩增，将纯化后的PCR产物送至上海生物工程有限公司进行双向测序。

1.3　数据分析

测序获得的核苷酸序列经CExpress 6.0软件进行序列拼接与比对，获得40条*CP*基因和*p23*基因序列，将序列上传至GenBank（表1）。利用MEGA-X进行碱基组成分析，计算保守位点（conservative site）、简约信息位点（abbreviated information site）、变异位点（mutation site）、自裔位点（descent locus）、碱基转换率（*Ts*）和颠换率（*Tv*）。利用软件Arlequin 3.1进行分子变异（AMOVA）分析，计算自由度（degree of freedom）、平方和（sum of squares）、变异组分（variance components）和变异百分比（pecentage of variation）。

利用RDP 5软件对测序获得的*CP*基因和*p23*基因序列进行重组事件分析，并与从GenBank中下载的8个CTV分离株[美国速衰型强毒株T36（U16034）和弱毒株T30（AF260651）、西班牙茎陷点型强毒株T318A（DQ151548）和弱毒株T385（Y18420）、以色列茎陷点型强毒株VT（U56902）、埃及的强毒株Qaha（AY340974）、墨西哥茎陷点型和速衰型强毒株Mexico-ctv（DQ272579）以及日本苗黄型强毒株NuagA（AB046398）]的*CP*基因和*p23*基因序列，利用软件MEGA-X中最大似然法（Maximum likelihood，ML）的Kimura 2-parameter模型，构建系统发育树，自展值检验重复1000次。

利用软件Network 10.0基于中点连接法（median-joining）分别构建基于*CP*基因和*p23*基因的中介

表1 各序列上传至GenBank获得的登录号

Table 1 Each sequence is uploaded to the login number obtained by GenBank

基因 Gene	地区 Region	编号 Serial number	登录号 Accession number
CP	江西崇义 Chongyi, Jiangxi	C-CP-1~C-CP-10	MT954976~MT954985
	湖南江永 Jiangyong, Hunan	J-CP-1~J-CP-10	MT955006~MT955015
	湖南道县 Daoxian, Hunan	D-CP-1~D-CP-10	MT954986~MT954995
	湖南莽山 Mangshan, Hunan	M-CP-1~M-CP-10	MT954996~MT955005
p23	江西崇义 Chongyi, Hunan	C-p23-1~C-p23-10	MT955056~MT955065
	湖南江永 Jiangyong, Hunan	J-p23-1~J-p23-10	MT955086~MT955095
	湖南道县 Daoxian, Hunan	D-p23-1~D-p23-10	MT955066~MT955075
	湖南莽山 Mangshan, Hunan	M-p23-1~M-p23-10	MT955076~MT955085

网状关系图，分析各分离株之间的进化关系。利用GenAIEx 6.5进行基因流（*Nm*）计算。利用DnaSP软件进行Tajima's *D*、Fu and Li's *D*及Fu and Li's *F* 3种中性检验，并进行碱基错配分析，推断种群扩张及遗传进化情况。

2 结果与分析

2.1 碱基组成及序列变异

经双向测序校对后获得40条672 bp的*CP*基因序列和630 bp的*p23*基因序列，无碱基缺失和插入，序列比对分析发现，*CP*基因序列保守位点555个（占82.59%），简约信息位点80个，变异位点117个，自裔位点37个；*p23*基因序列保守位点485个，简约信息位点97个，变异位点145个（占23.02%），自裔位点48个。*CP*基因A、T、C、G核苷酸碱基含量分别为28.32%、26.99%、18.49%、26.20%，转换与颠换比值为5.049，转换高于颠换；*p23*基因A、T、C、G碱基含量分别为30.48%、24.74%、19.60%、25.18%，转换与颠换比值为4.415，转换高于颠换。2个基因的（A+T）含量均大于（C+G）含量，碱基C的含量最少，表现出AT偏向性。

2.2 分子变异与基因流

4个地区野生柑橘上CTV分离株的*CP*基因和*p23*基因分子变异分析（AMOVA）结果（表2）显示，分离株间的遗传变异主要来自于种群内，分别占85.8%和82.38%，种群间分子变异分别占14.20%和17.62%。基因流分别为6.749和1.475，表明CTV分离株的*CP*基因和*p23*基因在各地间基因交流较为频繁，遗传差异较小。

2.3 种群动态分析

中性检验结果（表3）显示，道县、莽山和江永野生柑橘上CTV分离株的*CP*基因Fu and Li's *D*、Fu

表2 湘赣南部区域野生柑橘上CTV分离株*CP*基因和*p23*基因分子变异分析

Table 2 Molecular variation analysis of *CP* gene and *p23* gene in CTV isolates from wild citrus in southern Hunan and Jiangxi

基因 Gene	变异来源 Source of variation	自由度 *df*	平方和 Sum of squares	变异组分 Variance components	变异百分比 Pecentage of variation
CP	种群间 Among populations	3	199.053	1.842 68 Va	14.20
	种群内 Within population	124	1 381.080	11.137 74 Vb	85.80
	总数 Total	127	1 580.133	12.980 42	100.00
p23	种群间 Among populations	3	282.578	2.719 34 Va	17.62
	种群内 Within population	124	1 576.243	12.711 63 Vb	82.38
	总数 Total	127	1 858.820	15.430 97	100.00

注：Va、Vb表示方差组分的数量。

Note: Va and Vb represent the number of variance components.

表3　湘赣南部区域野生柑橘上CTV分离株不同基因核苷酸多样性及中性检验

Table 3　Nucleotide diversity and neutral test of different genes in CTV isolates of wild citrus from southern Hunan and Jiangxi

基因 Gene	地区 Region	核苷酸多样性 P_i	中性检验 Neutralily test		
			Fu and Li's *D*	Fu and Li's *F*	Tajima's *D*
p23	江西崇义 Chongyi, Jiangxi	0.032 92	−0.397 31 ($p > 0.1$)	−0.514 08 ($p > 0.1$)	−0.483 11 ($p > 0.1$)
	湖南道县 Daoxian, Hunan	0.050 34	−2.157 25 ($0.05 < p < 0.1$)	−2.083 20 ($0.05 < p < 0.1$)	−0.986 86 ($p > 0.1$)
	湖南莽山 Mangshan, Hunan	0.040 03	−0.970 68 ($p > 0.1$)	−0.520 56 ($p > 0.1$)	0.647 40 ($p > 0.1$)
	湖南江永 Jiangyong, Hunan	0.045 33	−0.394 42 ($p > 0.1$)	−0.495 44 ($p > 0.1$)	−0.527 57 ($p > 0.1$)
CP	江西崇义 Chongyi, Hunan	0.032 36	−1.514 75 ($p > 0.1$)	−1.550 08 ($p > 0.1$)	−0.937 54 ($p > 0.1$)
	湖南道县 Daoxian, Hunan	0.032 24	0.358 91 ($p > 0.1$)	0.296 98 ($p > 0.1$)	0.040 26 ($p > 0.1$)
	湖南莽山 Mangshan, Hunan	0.034 40	0.191 84 ($p > 0.1$)	0.655 68 ($p > 0.1$)	1.308 65 ($p > 0.1$)
	湖南江永 Jiangyong, Hunan	0.035 93	0.049 85 ($p > 0.1$)	0.176 84 ($p > 0.1$)	0.428 77 ($p > 0.1$)

and Li's *D*和Tajima's *D*检验值均大于0，结果不显著（$p > 0.1$），表明3个地区CTV种群的*CP*基因可能经历种群扩张；崇义、道县和江永野生柑橘上CTV分离株的*p23*基因和崇义野生柑橘上CTV分离株的*CP*基因中性检验值均小于0（$p > 0.1$，$0.05 < p < 0.1$），表明这3个地区CTV种群的*p23*基因和崇义地区CTV种群的*CP*基因可能处于种群稳定或收缩状态。碱基错配分析结果显示，错配分析曲线均呈多峰曲线分布（图1），表明4个地区的野生柑橘上CTV分离株的2个基因种群大小均保持稳定。核苷酸多样性分析显示，道县地区野生柑橘上CTV分离株的*p23*基因群体P_i值明显高于其他地区，说明该地区的*p23*基因具有较高的核苷酸多样性，*CP*基因上各地区核苷酸多样性无明显差异（表3）。

2.4　重组分析与系统发育分析

对获得的序列进行重组事件分析，结果显示，7种检测方法（RDP、Chimaera、Maxchi、Bootscan、GENECONV、Siscan和3Seq）均未检测到重组事件，说明*CP*基因和*p23*基因序列并未发生重组事件。

基于CTV *CP*基因构建的系统发育树（图2-A）显示，CTV分离株可分为3个类群。第1类群包括6个崇义分离株、5个道县分离株、5个莽山分离株和9个江永分离株；第2类群包括1个江永分离株、4个崇义分离株、3个道县分离株、5个莽山分离株和T36、VT、Mexico-ctv、NuagA分离株；第3类群包括2个道县分离株和T30、T385、T318A、Qaha分离株（图2-A）。

基于CTV *p23*基因构建的系统发育树显示，CTV分离株可分为4个类群。第1类群包括8个崇义分离株；第2类群包括5个道县分离株、8个江永分离株和2个莽山分离株；第3类群包括2个江永分离株、5个道县分离株、7个莽山分离株、2个崇义分离株和VT、NuagA、T318A、T385分离株；第4类群包括1个莽山分离株和Qaha、T36、Mexico-ctv分离株（图2-B）。

*CP*和*p23*基因构建的系统发育树均显示，地理来源相同的CTV分离株大部分聚集在同一簇中，少部分分散在其他簇中。

2.5　中介网状关系图分析

基于*CP*基因构建的中介网状关系图（图3-A）显示，CTV分离株可分为2个类群。类群A包括5个莽山分离株、7个道县分离株、6个崇义分离株和9个江永分离株；类群B包括5个莽山分离株、3个道县分离株、4个崇义分离株和1个江永分离株。

基于*p23*基因构建的中介网状关系图（图3-B）显示，CTV分离株可分为2个类群。类群A包括8个莽山分离株、5个道县分离株、2个崇义分离株和3个江永分离株；类群B包括2个莽山分离株、5个道县分离株、8个崇义分离株和7个江永分离株。

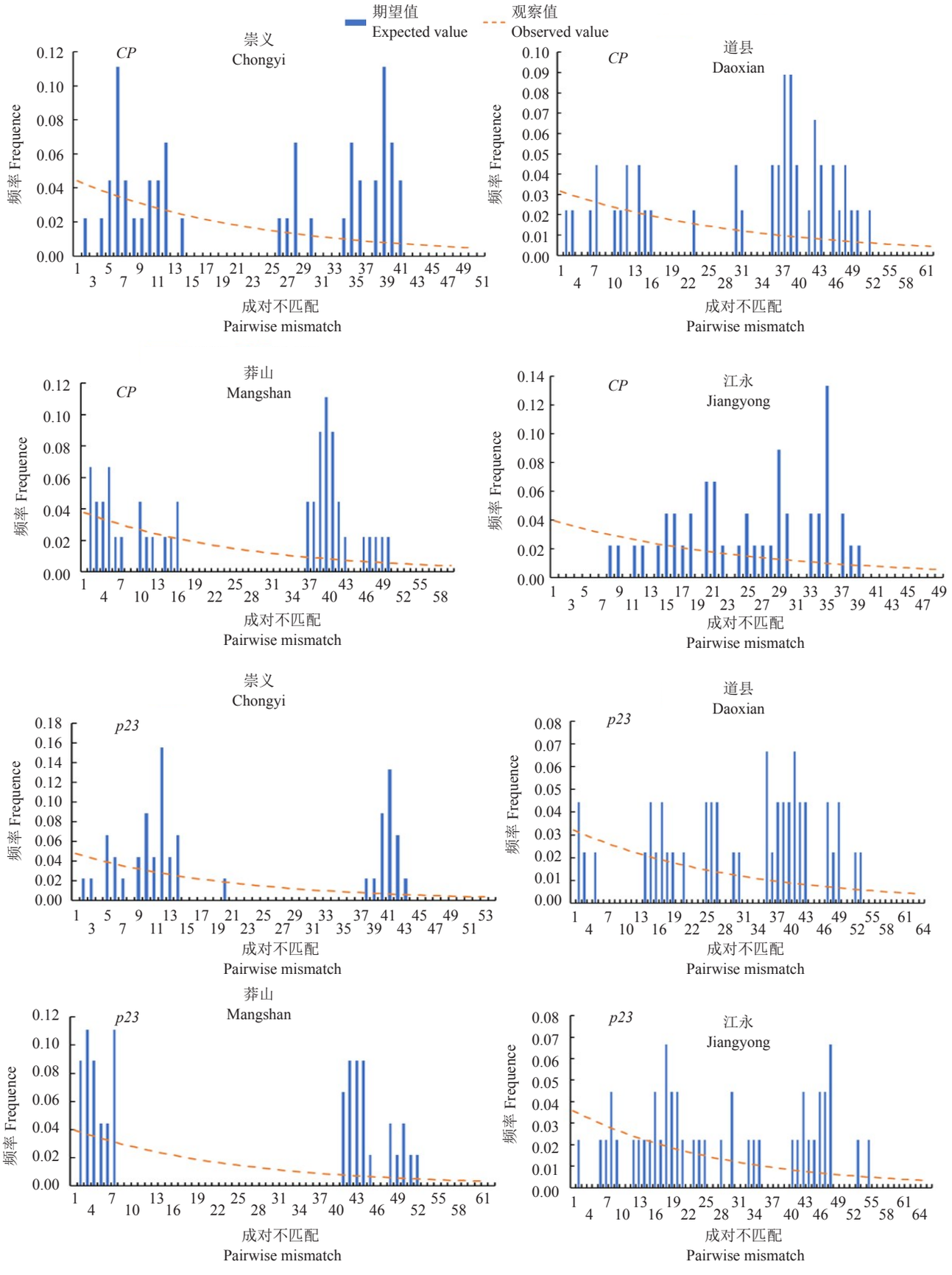
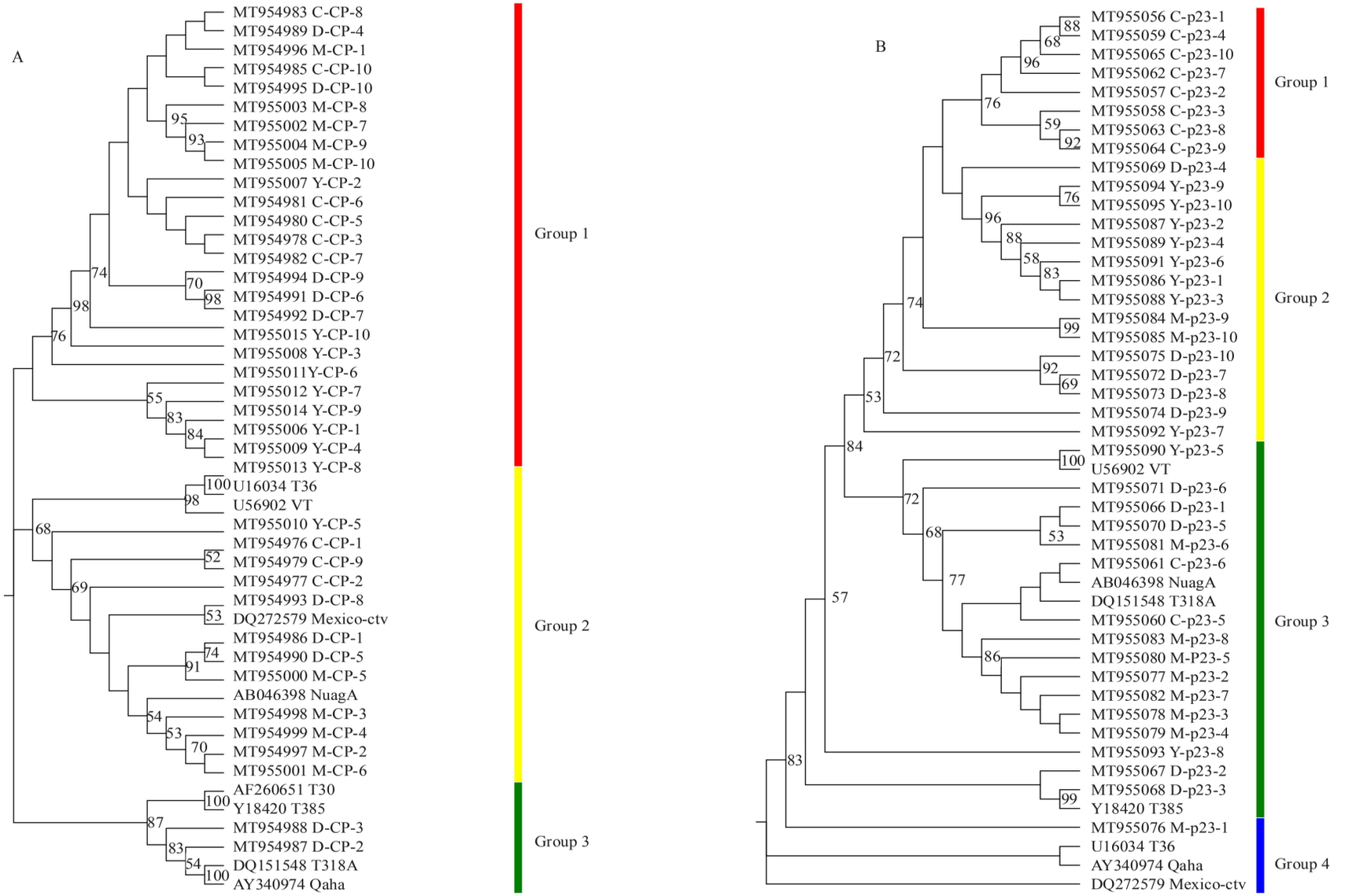


图 1　湘赣南部区域野生柑橘上 CTV 分离株 *CP* 和 *p23* 基因碱基错配分析

Fig. 1　Base mismatch analysis of *CP* and *p23* gene of CTV isolates from wild citrus from southern Hunan and Jiangxi



自展值检验重复 1000 次，自展值高于 50%显示在节点上。

The self-developing value test is repeated 1000 times, and the self-developing value above 50% is displayed on the node.

图 2　最大似然法基于 *CP* 基因（A）和 *p23* 基因（B）核苷酸序列构建的系统发育树

Fig. 2　Maximum likelihood method is based on phylogenetic tree constructed by nucleotide sequences of *CP* (A) and *p23* gene (B)

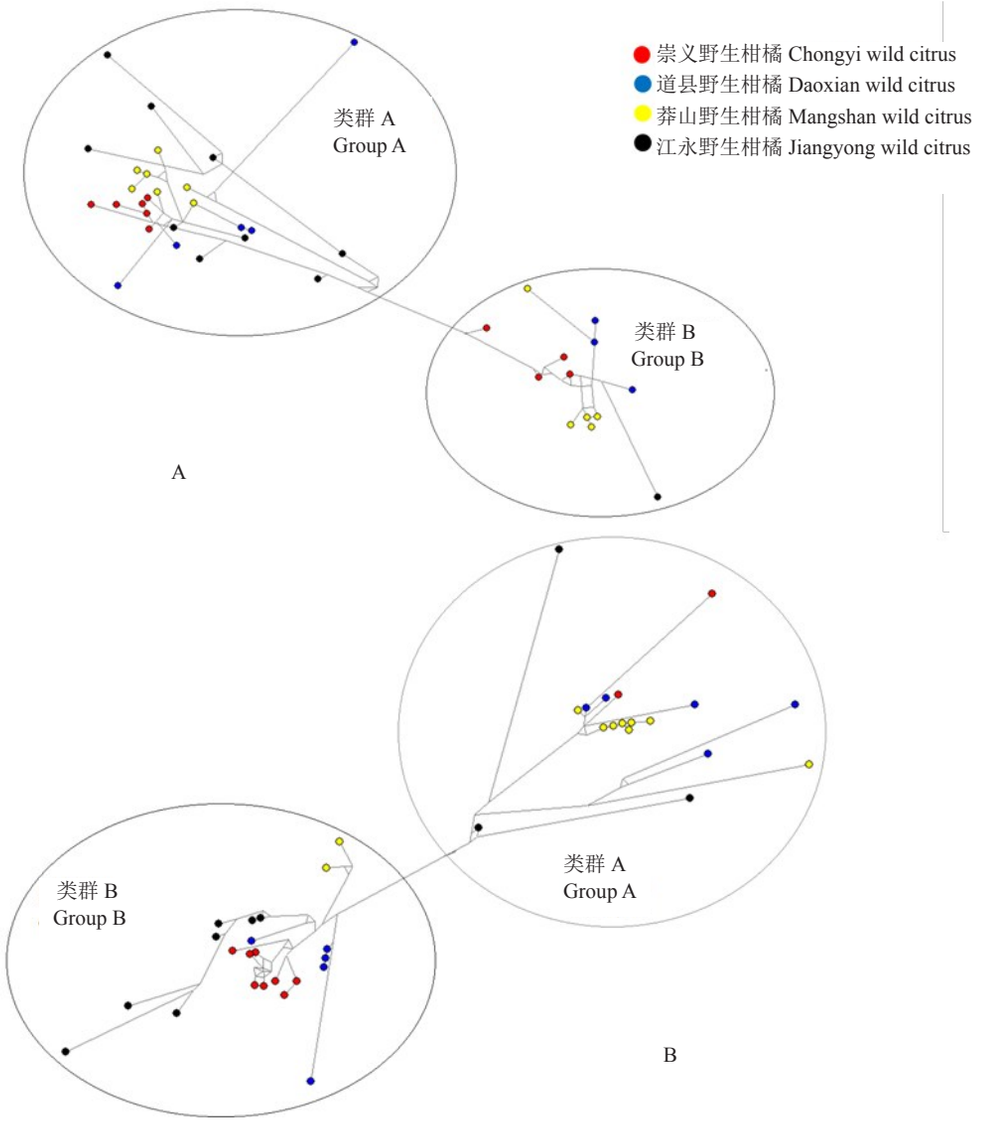


图 3　中点连接法基于 *CP* 基因（A）和 *p23* 基因（B）构建的湘赣南部区域中介网状关系

Fig. 3　The midpoint connection method is based on the haplotype network map of different regions constructed by *CP* gene (A) and *p23* gene (B)

3　讨　论

湖南和江西具有丰富的野生柑橘资源[18]，湖南江永、湖南道县、湖南莽山和江西崇义4个地区为中国群落主要分布区，各地域环境有所差异，如湖南道县地区气候温和、莽山地区年平均气温较低且全年气候差异变化较大、江永地区年日照及降雨差异较大，江西崇义全年热量丰富，日照偏少，雨量充沛，气候差异十分明显等，野生柑橘生长在此深山野林生境中，受人为影响更少，处于相对独立环境，其携带的CTV分离株种群特征尚不明晰，特别是不同地区间的野生柑橘上CTV种群特征的比对将为进一步解析CTV种群遗传进化提供重要参考。

通过对湘赣南部区域的野生柑橘上CTV分离株进行AMOVA分析、系统发育分析、中介网状关系分析和种群动态分析，可明确野生柑橘上CTV种群间遗传特征。笔者发现野生柑橘上CTV分离株*CP*基因和*p23*基因序列中碱基含量（A+T）>（G+C），与易龙等[8]研究结果一致。AMOVA分析显示，*CP*基因和*p23*基因种群内分子变异分别占85.8%和82.38%，表明遗传变异主要来自种群内。本研究中*CP*基因和*p23*基因的基因流分别为6.749和1.475，均大于1，表明4个地区间野生柑橘上CTV分离株存在基因交流，遗传差异较小[19]。基因重组对病毒的遗传演化和平衡有害突变具有重要作用，也可能产生更多的遗传多样性，帮助病毒适应新环境[20-21]，

本研究并未检测出重组事件的发生，表明重组事件不是引起4个地区野生柑橘上CTV分离株*CP*基因和*p23*基因遗传分化的主要原因。系统发育分析显示，地理来源相同的野生柑橘上CTV种群大部分聚集在同一簇中，少部分分散在不同簇，表明湘赣南部区域的野生柑橘上CTV种群间亲缘关系与地理来源存在相关性[22-23]，但也有研究显示，不同地区的栽培柑橘[24-25]和野生柑橘[8, 26]上CTV种群间亲缘关系与地理来源未有相关性，原因有待进一步分析。

病毒主要通过基因突变、遗传信息的交换和自然选择进行进化[27]，笔者本研究中通过中性检验和碱基错配分析2种方法分析4个地区的野生柑橘上CTV种群扩张事件，结果显示，4个地区的野生柑橘上CTV分离株均处于种群稳定状态，研究结果与吴官维[6]对我国湖北和江西两地栽培柑橘上CTV分离株的中性检测结果相一致，说明该CTV群体正处于种群扩张状态，推测种群扩张和基因交流是推动柑橘衰退病毒进化的重要因素。

基于对湘赣南部区域的野生柑橘上CTV分离株种群特征的研究结果，并结合前人对栽培柑橘上CTV的种群特征分析结果，可从不同的生境分析CTV种群的进化特征，将对解析CTV的遗传进化提供重要参考依据。

参考文献 References：

[1] KARASEV A V, BOYKO V P, GOWDA S, NIKOLAEVA O V, HILF M E, KOONIN E V, NIBLETT C L, CLINE K, GUMPF D J, LEE R F, GOWDA S. Complete sequence of the citrus tristeza virus RNA genome[J]. Virology, 1995, 208(2): 511-520.

[2] SATYANARAYANA T, BOYKO V P. An engineered closterovirus RNA replicon and analysis of heterologous terminal sequences for replication[J]. Proceedings of the National Academy of ences of the United States of America, 1999, 96(13): 7433-7438.

[3] FEBRES V J, ASHOULIN L, MAWASSI M, FRANK A, NIBLETT C L. The *p27* protein is present at one end of citrus tristeza virus particles[J]. Phytopathology, 1996, 86(12): 1331-1335.

[4] FEBRES V J, ASHOULIL N, MAWASSI M, FRANK A, NIBLETT C L . Citrus tristeza virus *p23* may suppress systemic silencing but is not related to the kind of viral syndrome[J]. Physiological and Molecular Plant Pathology, 2014, 87: 69-75.

[5] RICARDO F, SUSANA R R, NURIA S, JESÚS S N, CARMEN F, CARMELO L, LUIS N, PEDRO M, LEANDRO P. Citrus tristeza virus *p23*: a unique protein mediating key virus-host interactions[J]. Frontiers in Microbiology, 2013, 4: 98.

[6] 吴官维 . 我国柑橘衰退病毒的遗传多样性分析及其外壳蛋白的抗原表位鉴定[D]. 武汉：华中农业大学，2014.
WU Guanwei. Genetic diversity analysis of citrus decaying virus in China and epitopes identification of its coat protein[D]. Wuhan: Huazhong Agricultural University, 2014.

[7] 刘志芳，易龙，卢占军，高玉霞 . 赣南柑橘产区衰退病毒外壳蛋白基因初步分析[J]. 南方农业学报，2015，46（1）：15-20.
LIU Zhifang, YI Long, LU Zhanjun, GAO Yuxia. Gene analysis of the coat protein gene of fading virus in citrus growing areas in southern Jiangxi[J]. Journal of Southern Agriculture, 2015, 46 (1): 15-20.

[8] 易龙，周常勇 . 基于 *p23* 基因的野生柑橘和栽培柑橘上衰退病毒分离株间的遗传特征分析[J]. 果树学报，2017，34（7）：828-834.
YI Long, ZHOU Changyong. Analysis of genetic characteristics of fading virus isolates from wild citrus and cultivated citrus based on *p23* gene[J]. Journal of Fruit Science, 2017, 34(7): 828-834.

[9] AVISE J C. Toward a regional conservation genetics perspective: Phylogeography of faunas in the southeastern United States [M]. Springer US: Conservation Genetics, 1996: 431-470.

[10] 刘铸，蒋纹静，王奥男，杨茜，金志民，田新民，李殿伟 . 东北地区中鼩鼱遗传多样性与分子系统地理学[J]. 兽类学报，2020，40（3）：249-260.
LIU Zhu, JIANG Weijing, WANG Aonan, YANG Qian, JIN Zhimin, TIAN Xinmin, LI Dianwei. Genetic diversity and molecular systematic geography of middle shrews in northeast China [J]. Acta Theriologica Sinica, 2020, 40(3): 249-260.

[11] KOH H S, JANG K H, IN S T, EUI D H, JO J E, HAM E J, JEONG S K, LEE J H, KIM K S, KWEON G H. Genetic distinctness of *Sorex caecutiens hallamontanus* (Soricomorpha: Mammalia) from Jeju Island in Korea: cytochrome oxidase I and cytochrome b sequence analyses[J]. Animal Systematics, Evolution and Diversity, 2012, 28(3) : 251-219.

[12] LEE S J, LEE M Y, LIN L K, LIN Y K, LI Y, SHIN E H, HAN S H, MIN M S, LEE H, KIM K S. Phylogeography of the Asian lesser white- toothed shrew, *Crocidura shantungensis*, in East Asia: role of the Korean Peninsula as refugium for small mammals[J]. Genetica, 2018, 146(2): 211-226.

[13] 段义忠 . 窄叶鲜卑花分子系统地理学研究[D]. 北京：中国科学院研究生院，2010.
DUAN Yizhong. Geographical study on the sub-system of Xianbei flower of narrow leaf [D]. Beijing: Graduate University of the Chinese Academy of Sciences, 2010.

[14] GAO F L, ZOU W C, XIE L H, ZHAN J S. Adaptive evolution and demographic history contribute to the divergent population genetic structure of Potato virus Y between China and Japan[J]. Evolutionary Applications, 2017, 10(4): 379-390.

[15] 易龙，陈毅群，李双花，黄爱军 . 柑橘衰退病毒侵染对‘赣南早’脐橙植株组织结构及光合作用的影响[J]. 果树学报，2020，

37(4):128-135.
YI Long, CHEN Yiqun, LI Shuanghua, HUANG Aijun. Effects of citrus decay virus infection on the plant structure and photosynthesis of 'Gannanzao' navel orange[J]. Journal of Fruit Science, 2020, 37(4): 128-135.

[16] 鄢明峰,贺哲,黄婷,崔朝宇,李俊科,徐世凤,蒋军喜 . 瑞昌山药花叶型病毒病毒原的分子鉴定及其遗传变异性分析[J]. 江西农业大学学报,2017,39(6):1126-1130.
YAN Mingfeng, HE Zhe, HUANG Ting, CUI Chaoyu, LI Junke, XU Shifeng, JIANG Junxi. Molecular identification and genetic variation analysis of the virulence of *Ruichang yam mosaic virus*[J]. Acta Agriculturae Universitis Jiangxiensis, 2017, 39(6): 1126-1130.

[17] GILLINGS M, BROADBENT P, INDSTO J, LEE R. Characterisation of isolates and strains of citrus tristeza closterovirus using restriction analysis of the coat protein gene amplified by the polymerase chain reaction[J]. Journal of Virological Methods, 1993, 44(2/3): 305-317.

[18] 廖振坤 . 湖南柑橘种质资源评价及主要病害分子鉴定[D]. 长沙:湖南农业大学,2007.
LIAO Zhenkun. Evaluation of citrus germplasm resources and molecular identification of major citrus diseases in Hunan[D]. Changsha: Hunan Agricultural University, 2007.

[19] MILLAR C I, LIBBY W J, FALK D A, HOLSINGER K E. Strategies for conserving clinal, ecotypic, and disjunct population diversity in widespread species[M]. Genetics and Conservation of Rare Plants, 1991.

[20] SIMON A E, BUJARSKI J J. RNA-RNA recombination and evolution in virus-infected plants[J]. Annual Review of Phytopathology, 1994, 32(1): 337-362.

[21] BENÍTEZ-GALEANO M J, RUBIO L, BERTALMÍO A, MAESO D, RIVAS F, COLINA R. Phylogenetic studies of the three RNA silencing suppressor genes of south American CTV isolates reveal the circulation of a novel genetic lineage[J]. Viruses, 2015, 7(7): 4152-4168.

[22] 李战彪,徐鹏超,秦碧霞,苏琴,谢慧婷,崔丽贤,蔡健和 . 广西番茄烟粉虱传双生病毒的分布及遗传多样性分析[J]. 南方农业学报,2017,48(1):72-81.
LI Zhanbiao, XU Pengchao, QIN Bixia, SU Qin, XIE Huiting, CUI Lixian, CAI Jianhe. Distribution and genetic diversity of tomato-infecting begomoviruses in Guangxi, China[J]. Journal of Southern Agriculture, 2017, 48(1): 72-81.

[23] 常佳迎,刘树森,石洁,郭宁,张海剑,马红霞,杨春凤 . 海南三亚和黄淮海地区玉米小斑病菌致病性及遗传多样性分析[J]. 中国农业科学,2020,53(6):1154-1165.
CHANG Jiaying, LIU Shusen, SHI Jie, GUO Ning, ZHANG Haijian, MA Hongxia, YANG Chunfeng. Analysis of pathogenicity and genetic diversity of maize speckle from Sanya, Hainan and Huang-Huai-Hai regions[J]. Scientia Agricultura Sinica, 2020, 53(6): 1154-1165.

[24] ERKIŞ-GÜNGÖR G, ÇEVIK B. Genetic diversity and phylogenetic analysis of citrus tristeza virus isolates from Turkey[J]. Advances in Virology, 2019, 1: 1-11.

[25] MARTIN S, SAMBADE A, RUBIO L, VIVES M C, MOYA P, GUERRI J, ELENA S F, MORENO P. Contribution of recombination and selection to molecular evolution of citrus tristeza virus[J]. Journal of General Virology, 2009, 90(6): 1527-1538.

[26] 易龙,周常勇,周彦,王志刚,唐科志 . 中国野生柑橘上衰退病毒分离株分子特征分析[J]. 中国农业科学,2007,40(5):932-939.
YI Long, ZHOU Changyong, ZHOU Yan, WANG Zhigang, TANG Kezhi. Molecular characterization of fading virus isolates from Chinese wild citrus[J]. Scientia Agricultura Sinica, 2007, 40(5): 932-939.

[27] 丁铲 . 病毒的进化与传播[J]. 中国动物传染病学报,2010,32(11):35-37.
DING Chan. Evolution and transmission of virus[J]. Chinese Journal of Animal Infectious Diseases, 2010, 32(11): 35-37.