果 树 学 报 2020,37(4):574-581
Journal of Fruit Science

DOI:10.13925/j.cnki.gsxb.20190392

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

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摘 要:【目的】了解柑橘衰退病毒(*Citrus tristeza virus*,CTV)侵染对‘赣南早’脐橙植株组织微形态结构变化及光合特性的影响,以便进一步探明CTV对寄主的侵染特性和致病机制,为生产上病害的防控提供参考依据。**【方法】**通过石蜡切片等技术观测感染CTV的‘赣南早’脐橙植株及其健康植株叶片、枝条和根部组织微形态结构,利用紫外可见分光光度计和光合仪分别测定叶片光合色素、丙二醛(MDA)含量、净光合速率和胞间CO_2浓度变化,结合叶片叶绿体超微结构电镜观察,分析CTV侵染对寄主组织结构、光合器和生理功能的影响。**【结果】**受CTV侵染后,‘赣南早’脐橙植株叶片栅栏组织细胞间隙增大、排列松散,枝条木质部出现凹陷点、结构皱缩,根部细胞萎缩、内含物减少,叶绿体结构发生畸变;叶绿素a、b以及叶绿素总含量分别下降23.9%、16.3%和21.4%,MDA含量增加了28.5%,叶片净光合速率下降25.7%,胞间CO_2浓度上升12.1%。**【结论】**通过分析发现CTV侵染后植株组织结构、光合器形态、叶片光合色素和MDA含量、净光合速率和胞间CO_2浓度都发生了显著变化,CTV侵染影响植株叶绿体结构和光和能力降低碳水化合物制造能力,影响木质部结构萎缩阻碍光合产物向地下运输,患病植株根部细胞萎缩、内含物减少,致根系吸收和向枝叶输送水分养分能力下降,进而引起枝叶生长衰弱。

关键词:‘赣南早’脐橙;柑橘衰退病毒;组织结构;叶绿体;光合特性

中图分类号:S666.4 **文献标志码:**A **文章编号:**1009-9980(2020)04-0574-08

Structural and photosynthetic changes in ‘Gannanzao’ navel orange plants infected with *Citrus tristeza virus*

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Abstract:【Objective】The objective of this study is to evaluate the effects of *Citrus tristeza virus* (CTV) infection on ‘Gannanzao’ navel orange plants from the perspective of its morphology and photosynthetic characteristics changes, which provides insights on how the pathogen causes damage to the host. The characteristics of CTV infection and the mechanism of the damage it caused in the host plant was analyzed, which could provide some useful information for the prevention and control of CTV. 【Methods】One-year-old healthy ‘Gannanzao’ navel orange seedlings were provided by the germplasm resource nursery of National Navel Orange Engineering Research Center. Seedlings were inoculated with CTV and cultured separately in an isolation net. The fresh leaves and roots from eighteen months old culture of ‘Gannanzao’ navel orange plants infected with CTV were collected with non-infected healthy leaves and roots served as a control. Preparing 40 samples per sample tissue and being embedded in paraffin, the leaves and the roots were cut into a thickness of 6 μm slices by using a Leica RM2245 slicing machine and the anatomical structure was observed under a Leica DMIL-LED fluorescence inverted microscope after being stained with saffron. The thickness of the phloem, xylem and medullary part, palisade tissue and spongy tissue of the leaves were measured by using the LAS V4.0

收稿日期:2019-09-12 接受日期:2020-01-12

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

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and Image-Pro Plus 6.0 software. Taking 3rd to 4th leaves from top to bottom of healthy and CTV-infected plants, the blade tissues of about 1 mm ×1 mm on both sides of the central leaf veins of the leaves were quickly cut, fixed overnight in 2.5% glutaraldehyde at 4 ℃, phosphoric acid buffer (0.1 mol·L^{-1}, pH 7.0) was rinsed three times, 1% osmium tetroxide fixed at 4 ℃ for 2 hours, and penetrated in turn with acetone∶epoxy resin (2∶1), acetone∶epoxy resin (1∶1), epoxy resin overnight for 12 hours at 37 ℃. After being embeded by spur low viscosity resin, the sample was cut into slices by using a Leica EM UC7 slicing machine and the ultrastructure of chloroplast was observed under a FEI Tecnai G^2 20 TWIN transmission electron microscope after double staining with uranium acetate and lead citrate. The absorbance values at 665 649 470 nm and 450 532 600 nm were determined by using an ultraviolet-visible spectrophotometer, respectively, calculating the contents of photosynthetic pigments and malondialdehyde (MDA). The net photosynthetic rate (P_n) and intercellular CO_2 concentration (C_i) of fully expanded leaves from the top to bottom of control and infected plants were measured by LI-6400 portable photosynthetic apparatus from 9:00 to 11:00 a.m. The experiment was repeated in triplicate manner, and results were graphically plotted using the SPSS 12.0 software.【Results】Fluorescence microscopic images revealed that the phloem, xylem, and pulp in the diseased leaves became significantly thinner than in the healthy ones, in which the thickness of the phloem in diseased leaves was 82.7% of that in healthy tissue and the thickness of xylem and pulp in diseased leaves was 87.4% of that in healthy tissue. There were no significant changes in the thickness of the palisade tissue and the sponge tissue, but the diseased leaves showed a loose arrangement of palisade tissue cells and a larger gap between the cells compared to the healthy leaves. The xylem of the plants infected with CTV showed the symptom of stem pitting. Electron microscopic observations revealed that the ultra-structure of xylem tissue was prominently damaged by the CTV infection. The root cells were found to be atrophied in diseased plants, with inclusions reduced and attached to the intima tissues. The content of photosynthetic pigments in the infected leaves was considerably reduced compared to that of the healthy leaves. The contents of chlorophyll a, chlorophyll b and total chlorophyll decreased significantly by 23.9%, 16.3%, and 21.4%, respectively. The content of MDA increased by 28.5%, and the net photosynthetic rate of CTV infected leaves decreased by 25.7%, and the intercellular CO_2 concentration increased by 12.1%. The decrease in total chlorophyll content, net photosynthetic rate and the increase in intercellular CO_2 concentration indicated that the photosynthetic perception of host leaves decreased, followed by the disease resistance ability of the infected host plant, compared to the normal healthy plant. The chloroplast structure in the CTV infected leaves changed significantly, characterized by swollen chloroplast, enlarged matrix area, and loosened lamellar structure. Enlargement in the chloroplast matrix area led to the osmophilic pressure changes inside the cells. The decrease in the photosynthetic rate of infected plant leaves was due to distortion in chlorophyll structure and chlorophyll pigment reduction.【Conclusion】Our results revealed that CTV infection in ‘Gannanzao’ navel orange plants drastically altered the morphology and anatomical structure of the leaves and roots. The total chlorophyll content and chloroplast structural changes considerably reduced the overall photosynthetic rate in the infected plants. Ultrastructure of xylem and phloem in infected plants exhibited the tissue atrophy and thereby it affected the inherent ability of nutrient and water conductivity of the plants, and caused the growth of branches and leaves to weaken. In general, CTV infection in ‘Gannanzao’ navel orange plants is dreadful damage to the citrus industry.

Key words: ‘Gannanzao’ navel orange; *Citrus tristeza virus*; Anatomy; Chloroplast; Photosynthetic characteristics

柑橘衰退病毒(*Citrus tristeza virus*，CTV)引起的柑橘衰退病在全世界各柑橘产区均有发生，该病主要通过接穗和蚜虫进行扩散和传播，严重影响柑橘的树势、产量和品质，对柑橘产业造成严重的威胁[1]。在我国CTV分布也极为普遍，田间以CTV强毒株为主[2-3]，且近年柑橘衰退病在我国各产区危害日趋严重，成为了制约柑橘产业健康发展的重要因素。

病原物侵染植株后，会导致寄主组织微形态结构、细胞器和生理生化等发生变化。李燕宏等[4]研究发现蚕豆受萎蔫病毒2号分离物侵染后叶绿素含量减少，叶片净光合速率明显下降，光合器结构遭到不同程度的破坏；王伟英等[5]对薯瘟菌侵染甘薯后‘胜利百号’品种叶片细胞膜透性增大、叶绿素含量降低、胞间CO_2浓度和内源ABA含量急剧升高；易龙等[6]通过分析柑橘黄龙病侵染‘纽荷尔’脐橙植株后寄主叶片叶绿素含量降低，严重影响光合作用，同时细胞受损程度加大；Huseynova等[7]研究发现烟草病毒感染引起寄主丙二醛（MDA）含量增加，过氧化物酶活性改变，感病叶片光系统II活性和叶绿素（a和b）含量显著降低。而关于CTV侵染对寄主组织结构、光合器形态、叶绿素含量及光合特性等方面的影响研究较少。‘赣南早’是近年在赣南地区培育出的被认为是目前全世界成熟期最早的脐橙新品种，有望缓解当前品种成熟期集中、鲜果上市期短等问题，优化我国主栽脐橙品种产业结构[8]。进一步了解‘赣南早’脐橙品种受CTV侵染后组织结构、生理功能的变化，明确CTV对‘赣南早’品种的致病特点和危害性，可为今后在生产中推广‘赣南早’脐橙品种，防控柑橘衰退病提供科学依据。

1 材料和方法

1.1 供试材料

‘赣南早’脐橙试验苗由国家脐橙工程技术研究中心种质资源圃提供，为网室中栽培的以枳壳作砧木嫁接‘赣南早’脐橙接穗后生长1年的健康无病苗木。2015年12月取保存CTV毒源YC-3分离株的‘锦橙’实生苗上5 mm × 10 mm的树皮嫁接接种到‘赣南早’植株上，每处理5株，每株嫁接3块皮，5次重复，以相同数量和处理的健康皮接种的植株为对照，嫁接后将接种的苗木置于隔离网中单独培养，室温控制在25 ℃左右，18个月后取接种的植株进行CTV检测。

1.2 叶片、枝条和根部组织结构特征分析

参考王艳芳等[9]的方法对‘赣南早’脐橙植株叶片和根部进行石蜡包埋切片，每个样本组织制备40个样品。用Leica RM2245切片机将样品切成厚度为6 μm大小的薄片，经番红染色后在Leica DMIL－LED荧光倒置显微镜下观察叶片和根部解剖结构，并利用LAS V4.0和Image-Pro Plus 6.0软件测量叶片的韧皮部、木质部和髓部、栅栏组织、海绵组织的厚度。取接种CTV后的‘赣南早’和对照样品的枝条撕去表皮观察木质部症状，并取枝条茎段制成约1~2 mm厚度的薄片，置于贴了导电胶的载台上进行喷金处理，利用FEI-450型扫描电镜观察并拍摄枝条木质部组织结构。

1.3 叶绿体超微结构特征观察

将健康和CTV侵染的‘赣南早’脐橙植株顶部向下第3~4枚完全展开叶片，切取主脉两侧大小1 mm × 1 mm的小片，4 ℃于2.5%戊二醛中固定过夜，磷酸缓冲液（0.1 mol·L^{-1}，pH 7.0）漂洗3次，1%四氧化锇4 ℃固定2 h，磷酸缓冲液浸洗3次，乙醇梯度脱水后，依次以丙酮∶环氧树脂（体积比2∶1）、丙酮∶环氧树脂（体积比1∶1）、环氧树脂于37 ℃温箱渗透过夜12 h，用Spurr低黏度树脂包埋，Leica EM UC7超薄切片机切片，醋酸双氧铀和柠檬酸铅双染色后置于FEI Tecnai G^2 20 TWIN透射电镜观察叶绿体超微结构。

1.4 光合色素、净光合速率、胞间CO_2浓度测定

光合色素含量参照李合生[10]的方法，测定在波长665、649、470 nm下的吸光度值。MDA含量参照邹琦[11]的方法，测定在450、532和600 nm处的吸光度值。参照于力等[12]的方法在上午9:00—11:00采用LI-6400型便携式光合仪测定对照组和感病植株顶部向下3~4枚完全展开叶片的净光合速率（P_n）和胞间CO_2浓度（C_i）。以上数据测定试验均3次重复，采用SPSS 12.0软件进行统计分析。

2 结果与分析

2.1 寄主组织结构的变化

接种18个月后进行CTV感染情况检测，结果发现接种CTV毒源YC-3分离株的‘赣南早’植株均感染了CTV，而对照组均未检测到CTV（表1）。

表1　接种CTV后‘赣南早’植株感病检测结果

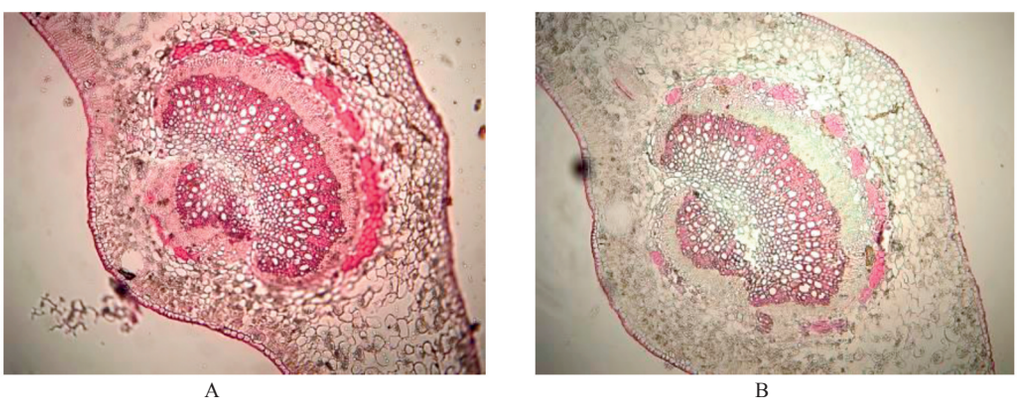
Table 1　Result of detection of citrus tristeza of‘Gannanzao’plants infected with CTV

处理 Treatment	重复1 Repetition 1	重复2 Repetition 2	重复3 Repetition 3	重复4 Repetition 4	重复5 Repetition 5
对照组 Control	0/5	0/5	0/5	0/5	0/5
接种CTV组 CTV inoculation	5/5	5/5	5/5	5/5	5/5

注：表中／前面的数字表示发病株数，后面的数字表示接种株数。

Note: The number in front of / indicates the number of diseased plants，the following number indicates the number of inoculated plants in the table.

‘赣南早’植株受CTV侵染后，经测定发现其叶片的韧皮部、木质部和髓部的厚度明显低于健康叶片（图1），韧皮部厚度为健康组织的82.7%，木质部和髓部的厚度为健康组织的87.4%，栅栏组织和海绵组织厚度无明显变化（表2），但患病叶片与健康叶片相比栅栏组织细胞排列松散、细胞之间间隙增大（图2-A）；感染CTV的‘赣南早’植株枝条木质部出现凹陷点、茎干内部组织结构皱缩（图2-B图箭头所指部位），受CTV侵染的植株根部与健康植株相比，健康植株根部筛管细胞富含内含物，离散程度高，而患病植株根部细胞萎缩、内含物减少且附着在内膜上（图2-C）。



A　　B

A. 健康叶片；B. 患病叶片。

A. Healthy leaf; B. Diseased leaf.

图1　感染CTV的‘赣南早’植株叶片韧皮部、木质部和髓部结构

Fig. 1　The phloem, xylem and medullary part structure of ‘Gannanzao’ plants infected with CTV

表2　感染CTV的‘赣南早’植株叶片各组织层厚度

Table 2　The thickness of each tissue layer of leaf of ‘Gannanzao’plants infected with CTV　　μm

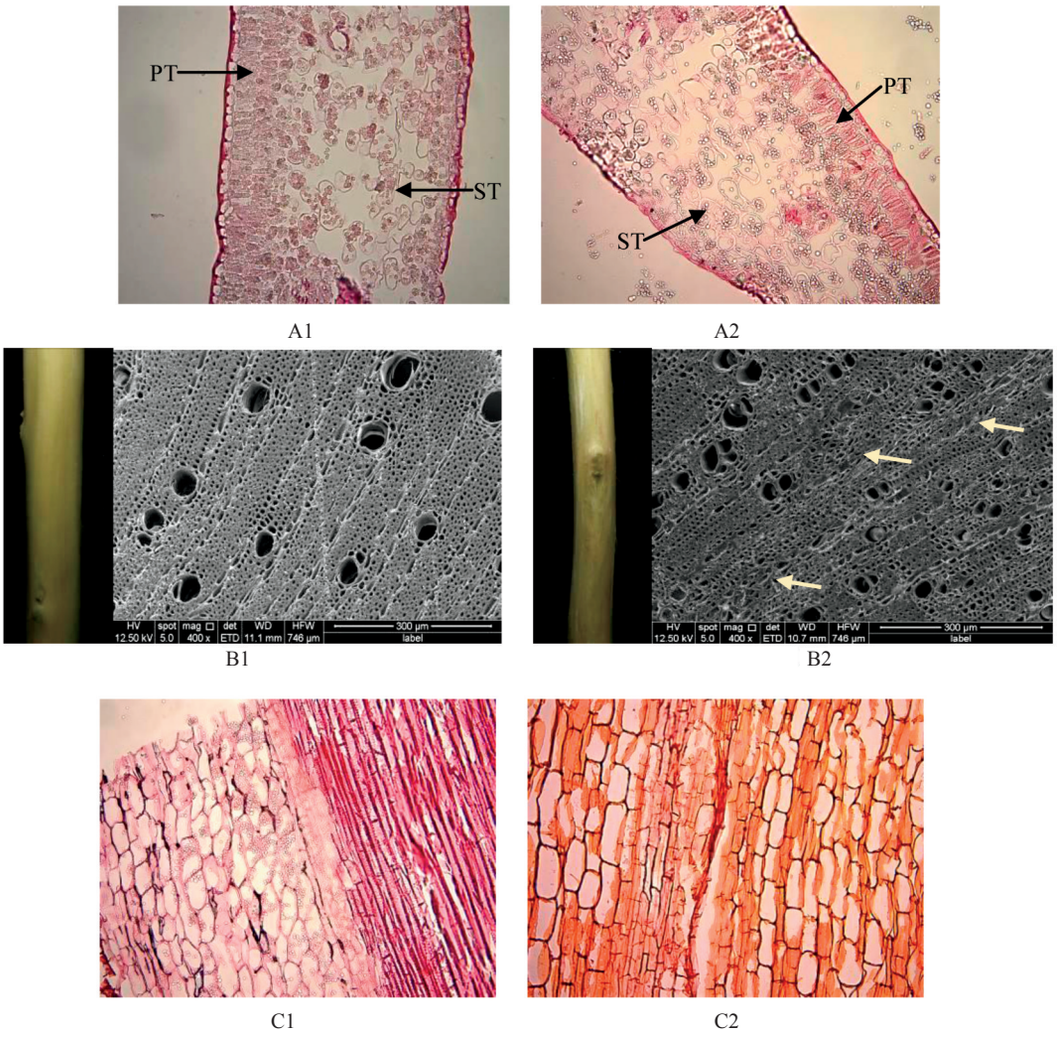
处理 Treatment	韧皮部 Phloem	木质部和髓部 Xylem and medullary part	栅栏组织 Palisade tissue	海绵组织 Spongy tissue
健康叶片 Healthy leaf	127.4±6.5 a	312.8±13.1 a	87.3±5.5 a	235.8±10.7 a
患病叶片 Diseased leaf	105.3±4.6 b	273.5±11.7 b	83.8±6.9 a	227.4±11.2 a

注：同一列数据后不同小写字母表示差异显著（$\alpha = 0.05$, SNK-q 检验法）。下同。

Note: Values followed by different small letters in the same column are significantly different ($\alpha = 0.05$, SNK-q test). The same below.

2.2　光合色素和MDA含量、净光合速率及胞间CO_2浓度测定结果

感染CTV的‘赣南早’植株叶片光合色素、MDA含量、净光合速率（P_n）和胞间CO_2浓度（C_i）变化情况见表3。其中，感染CTV的植株叶片光合色素含量显著低于健康叶片，叶绿素a、b以及叶绿素总含量明显下降，分别下降23.9%、16.3%和21.4%，而受CTV侵染后的‘赣南早’植株MDA含量增加了28.5%，显著高于健康叶片。测定的叶片净光合速率和细胞间CO_2浓度与对照相比有明显的变化，净光合速率下降25.7%，细胞间CO_2浓度上升12.1%。



A1. 健康叶片；A2. 患病叶片；B1. 健康枝条；B2. 患病枝条；C1. 健康根；C2. 患病根。PT. 栅栏组织; ST. 海绵组织。

A1. Healthy leaf; A2. Diseased leaf; B1. Healthy branch; B2. Diseased branch; C1. Healthy roots; C2. Diseased roots. PT. Palisade tissue; ST. Spongy tissue.

图2 感染CTV的‘赣南早’植株叶、枝条和根组织结构

Fig. 2 The tissue structure of leaves, branches and roots of ‘Gannanzao’ plants infected with CTV

表3 患病植株叶片光合色素、MDA含量及净光合速率和胞间CO_2浓度测定结果

Table 3 The content of photosynthetic pigments, MDA and the net photosynthetic rate, intercellular CO_2 concentration

处理 Treatment	*w*(叶绿素a) Chlorophyll a content/(mg·g^{-1})	*w*(叶绿素b) Chlorophyll b content/(mg·g^{-1})	*w*(总叶绿素) Total chlorophyll content /(mg·g^{-1})	*b*(MDA) MDA content/(μmol·g^{-1})	净光合速率(P_n) Net photosynthetic rate/(μmol·m^{-2}·s^{-1})	胞间CO_2浓度(C_i) Intercellular CO_2 concentration/(μmol·mol^{-1})
健康植株 Healthy plant	2.236±0.167 a	0.853±0.054 a	3.762±0.052 a	18.33±1.79 a	2.572±0.209 a	186.73±9.33 a
患病植株 Infected plant	1.701±0.083 b	0.714±0.068 b	2.957±0.021 b	23.56±2.08 b	1.912±0.235 b	209.31±9.47 b

2.3 CTV侵染对叶绿体超微结构的影响

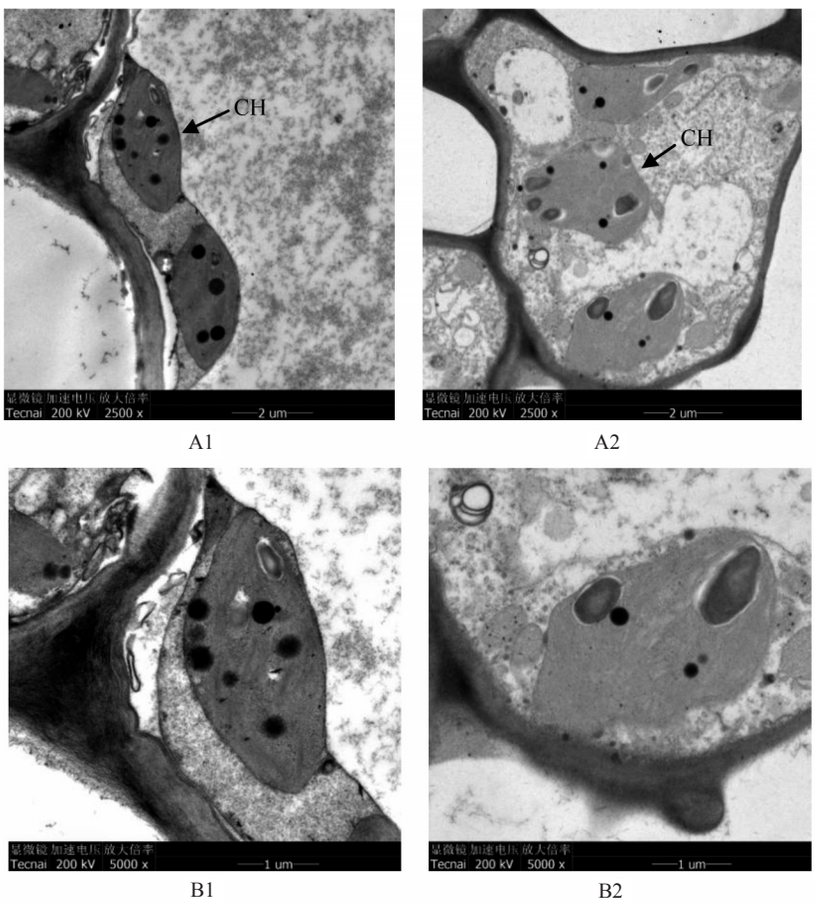
被CTV侵染的‘赣南早’植株叶片叶绿体结构与健康植株相比发生了明显变化，叶绿体变得膨大肿胀畸形，长轴变短，短轴变长，片层结构疏松、间隙变大，淀粉粒变大（表4），在细胞中排列松散（图3-A2、B2），健康叶片的叶绿体呈梭形，基粒片层和基质片层垛叠整齐，紧贴细胞质膜沿细胞周缘排列整齐有序（图3-A1、B1）。

表4　CTV侵染对‘赣南早’植株叶绿体超微结构的影响

Table 4　Effects of chloroplast ultrastructure in ‘Gannanzao’ plants infected with CTV

μm

处理 Treatment	叶绿体长轴 Long axis of chloroplast	叶绿体短轴 Short axis of chloroplast	淀粉粒长轴 Long axis of starch grain	淀粉粒短轴 Short axis of starch grain
健康植株 Healthy plant	3.741±0.032 a	1.698±0.027 a	0.539±0.016 a	0.240±0.009 a
患病植株 Infected plant	3.507±0.027 b	2.458±0.019 b	1.175±0.021 b	0.641±0.011 b



A1　　A2

B1　　B2

A1. 健康叶片的叶绿体(2 500×)；A2. 患病叶片的叶绿体(2 500×)；B1. 健康叶片的叶绿体(5 000×)；B2. 患病叶片的叶绿体(5 000×)；CH. 叶绿体。

A1. Chloroplast of healthy leaf(2 500×)；A2. Chloroplast of diseased leaf(2 500×)；B1. Chloroplast of healthy leaf(5 000×)；B2. Chloroplast of diseased leaf(5 000×). CH. Chloroplast.

图3　感染CTV的‘赣南早’植株叶绿体超微结构

Fig. 3　The ultrastructure of chloroplasts in ‘Gannanzao’ navel orange infected with CTV

3　讨　论

前人众多研究发现植物叶片栅栏组织、海绵组织排列紧实度与寄主抗病性呈正相关[13-14]，在本研究中发现感染CTV的‘赣南早’植株叶片栅栏组织细胞间隙增大、排列松散，从组织形态结构变化看，叶片光合作用营养功能可能减弱；观察发现感染CTV的‘赣南早’植株枝条木质部出现茎陷点症状，电镜扫描发现木质部内部组织结构皱缩，可能导致地面营养向根系输送能力下降，不利于根系发育和吸收能力发挥；同时研究还发现患病植株根部细胞相对健康植株呈现萎缩状态、内含物减少，可能影响植株根部生长活力和吸收功能，成为柑橘感染CTV后树势衰弱、果实变小、畸形的重要成因之一。

叶绿素含量的高低直接影响叶片光合作用，与寄主碳水化合物养分制造能力呈正相关[15-16]，据报道植物病毒侵染寄主后会阻碍寄主正常的生理代谢，破坏寄主叶绿体结构，叶绿素分解酶活性增加，

甚者叶绿体发生降解，使叶绿素含量下降，导致寄主光合磷酸化作用减弱，从而影响寄主的生理功能[17-18]。受病原菌侵染的寄主植物经检测还发现感病后病斑大小与病情严重程度和叶片MDA含量呈正相关[19]。本研究测定接种CTV后‘赣南早’植株叶片叶绿素a、叶绿素b以及叶绿素总含量、净光合速率与健康植株相比均下降，胞间CO_2浓度上升，说明寄主叶片的光合作用受到了抑制，影响寄主的生理功能和生长发育；感染CTV的‘赣南早’植株与健康植株相比MDA含量增加了近1/4，研究结果与Perez-Clemente等[20]研究发现墨西哥棘檬被CTV侵染后体内MDA含量提升加大了对寄主氧化损伤的结果相一致。电镜观察发现健康‘赣南早’植株叶绿体结构完整，而受CTV侵染的植株叶绿体超微结构发生了明显变化，叶绿体结构发生畸变致使寄主叶绿素含量的降低和光合作用的减弱。

研究植物病毒与寄主的互作关系来解析病毒的致病机制，有望为病毒病害的预防和应对策略的制定提供理论和技术支持，但关于植物病毒病害致病机制方面研究进展缓慢，后续作者将在本研究的基础上进一步对‘赣南早’植株受CTV侵染后寄主细胞组织出现一系列生理、病理变化进行分析，探明CTV对寄主生理功能的影响机制，为选育和推广抗CTV的品种、优化我国脐橙品种结构提供参考依据。

4 结 论

感染CTV的‘赣南早’植株组织微形态结构特征、光合器形态、叶片光合色素和MDA含量、净光合速率和胞间CO_2浓度都发生了显著变化，发现CTV侵染影响植株叶绿体结构和光和能力，降低碳水化合物制造能力，致使木质部结构萎缩，阻碍光合产物向地下运输，患病植株根部细胞萎缩、内含物减少，造成根系吸收和向枝叶输送水分养分能力下降，进而引起枝叶生长衰弱。

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