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柑橘黄龙病菌侵染对‘纽荷尔’脐橙组织结构的影响

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摘 要:**【目的】**了解‘纽荷尔’脐橙受柑橘黄龙病菌侵染后植株组织微形态结构的变化,以便进一步探明柑橘黄龙病对寄主的破坏特点及作用机制。**【方法】**通过电镜扫描、石蜡切片等技术观测感染柑橘黄龙病和健康脐橙的叶片、果蒂组织微形态结构,利用紫外可见分光光度计测定二者叶片光合色素、丙二醛(MDA)含量和过氧化物酶(POD)、过氧化氢酶(CAT)、超氧化物歧化酶(SOD)活性变化,分析柑橘黄龙病侵染对寄主组织结构和生理生化的影响。**【结果】**柑橘黄龙病菌侵染后的脐橙叶片厚度变薄、气孔萎缩,栅栏组织细胞由长条形变为椭圆形、细胞间隙增大、排列松散,果蒂的韧皮部部分维管束堵塞。患病叶片的叶绿素a、叶绿素b和类胡萝卜素含量分别是健康叶片的19.87%、22.13%和47.71%,MDA含量增加了120.39%,POD、CAT和SOD酶活性显著提升。**【结论】**柑橘黄龙病菌侵染后植株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,寄主抵御病原菌入侵和扩展能力减弱。

关键词:‘纽荷尔’脐橙;柑橘黄龙病;解剖结构;病理变化

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Structural changes in ‘Newhall’ navel oranges infected with *Candiatus* Liberibacter Asiaticus

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Abstract:【Objective】The objective of this study is to determine the effects of an infection of ‘Newhall’ navel oranges by Citrus Huanglongbing on the morphology of microtissues and physiological and biochemical activities in order to provide insights on how the pathogen causes damage to the host. The characteristics and mechanism of the damage to the host that is infected would be further analyzed, which could provide some useful information for the prevention and control research of Citrus Huanglongbing.【Methods】Infected leaves and fruit with typical symptoms of Citrus Huanglongbing were collected from an experimental orchard, the apparent healthy leaves and fruit were used as the control. After being wiped clean, the blade tissues 1 cm ×1 cm from both sides of the central leaf veins of the leaves were quickly cut. The pedicel was cut into 1-2 mm thickness from the sheet and put on a platform with conductive adhesive for spraying. Then, the FEI-450 type scanning electron microscope was used to observe and photograph the stomata and pedicel cell the morphology map. After being embedded in paraffin, the leaves and the pedicel were cut into a thickness of 6 μm sized slices by using a Leica RM2245 slicing machine and the anatomical structure was observed by 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 and Image- Pro Plus 6.0 software. Contents of the photosynthetic pigments and malondialdehyde (MDA) were measured, and the activities of peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) were recorded. The experiment was repeated three times, and results were analyzed using the SPSS12.0 software.【Results】Observations with a scanning electron microscopy showed that the surrounding tissues of the stomata of the healthy leaves were explanated and smooth, while the sto-

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mata of the infected leaves were cavated and atrophic, and the surrounding tissues were crinkled, rough and uneven. Moreover, the phloem, xylem and pulp in the diseased leaves became significantly thinner than that of the healthy ones, in which the thickness of the phloem in the diseased leaves was 72.3% of that in the healthy tissue and the thickness of the xylem and pulp in the diseased leaves was 81.2% of that in the healthy tissue. There was no significant change in the thickness of the palisade tissue and the sponge tissue. The cells of the palisade tissue in healthy leaves were long striped, regular and closely arranged, and the intercellular space was small, however, the cells of the palisade tissue in the diseased leaves were elliptical, loosely arranged and had large spaces between the cells. The cells of the healthy pedicel were smooth and their sizes were relatively similar, but the cells infected by Citrus Huanglongbing were uneven, folded, became distorted, swollen, and loose, leaving large intercellular spaces among the cells. The vascular bundles of the infected pedicel were blocked. The content of the photosynthetic pigments in the infected leaves decreased compared to that of the healthy leaves. The contents of the chlorophyll a, chlorophyll b and carotenoid were 19.87%, 22.13% and 47.71% of that in the healthy leaves, respectively. In contrast, the content of the malondialdehyde in the diseased leaves increased significantly, by 120.39%, compared to that of the healthy leaves. The enzymatic activity of POD, CAT and SOD in the leaves of the infected plants were significantly higher than those of the healthy plants, among which the activity of POD increased by 8.43 times.【Conclusion】Our results showed that the infection of leaves and fruit by Citrus Huanglongbing could cause damages to various tissues in the host, which decreased the content of the photosynthetic pigments while significantly increasing the enzymatic activities, which led to more cells being damaged and also weakened their ability to resist the invasion and expansion of pathogenic bacteria.

Key words:‘Newhall’navel oranges; Citrus huanglongbing; Anatomy; Pathological change

柑橘黄龙病(Citrus Huanglongbing,HLB)由一种迄今尚不能人工培养的韧皮部限制性难培养细菌(*Candiatus* Liberibacter spp.)引起,广泛分布于亚洲、非洲、美洲及中国南方柑橘主产区,严重威胁着各产区柑橘产业,是柑橘生产上最为严重的病害。根据病原物的热敏感性、发生区域和传播虫媒类型,柑橘黄龙病病原存在亚洲种(*Ca.* L asiaticus)、非洲种(*Ca.* L africanus)和美洲种(*Ca.* L amercanus)[1-2]。随着全球气候持续变暖,扩大了柑橘黄龙病菌传播媒介柑橘木虱的活动范围,柑橘黄龙病危害日趋严重,该病已造成上亿株柑橘树染病或死亡,给部分柑橘产区造成了毁灭性的危害[3]。柑橘黄龙病菌侵染后树体长势严重削弱,植株表现出叶片斑驳黄化、果实畸形且着色不均等症状,同时植株体内代谢出现紊乱,果实的产量和品质显著下降[4]。到目前为止没有抗病品种,柑橘黄龙病的防控主要通过砍除病树、种植无病苗木和杀灭传播媒介来进行,生产中尚缺有效的治疗措施,主要因为柑橘黄龙病菌至今无法进行人工培养,对于病菌的研究和防治造成了严重阻碍[5-6],因此制约了病害的防控技术和抗病品种的选育工作。

植物受到病原菌侵染后,其形态、结构和生理生化特征常常发生变化,导致叶片细胞膜透性明显增大,叶绿素含量下降,植株体内丙二醛、木质素含量显著提高,出现组织结构变形、细胞形态异常、细胞间隙增大等现象,破坏植株组织结构从而影响寄主的抗病能力[7-8],同时还会引起寄主体内与抗性相关的POD、SOD和CAT等防御酶活性变化[9]。国外研究发现柑橘黄龙病菌侵染植株后寄主筛管分子细胞壁间层肿大,韧皮部部分细胞塌陷,筛管阻塞、淀粉积累[10-12],国内戴泽翰等[13]利用光学显微镜观察发现被黄龙病菌侵染的柑橘叶片最明显的病理变化是淀粉的累积和韧皮部组织的异常。而关于柑橘黄龙病对寄主组织微形态结构、叶片光合色素和丙二醛含量及防御酶活性的影响等方面研究较少,因此笔者通过分析柑橘黄龙病菌侵染后寄主组织结构和生理生化发生的变化,明确柑橘黄龙病菌对寄主的破坏特点及作用机理,为今后柑橘黄龙病抗病品种的选育和病害的防控研究提供理论基础和科学依据。

1　材料和方法

1.1　供试材料

试验于2015年3月在崇义县关刀坪脐橙病害防控基地开展，选取在苗木繁育圃中培育2 a（年）的健康‘纽荷尔’脐橙植株栽种在试验基地果园，置于隔离网中单独培养，分别嫁接由国家脐橙工程技术研究中心病害防控研究室提供的健康和携带柑橘黄龙病的‘纽荷尔’脐橙接穗，2 a后等嫁接的携带柑橘黄龙病的接穗长成的枝条开始挂果，叶片、果实表现出黄龙病症状时，采集典型症状的叶片和带枝条的果实及健康样本在4 ℃低温条件下立即带回实验室检测，用于酶活性测定的叶片采集后经液氮冷冻，在-70 ℃超低温冰箱保存备用。

1.2　叶片气孔及果蒂形态电镜观察

将供试的叶片擦拭干净后，快速切取叶片中央叶脉两侧约1 cm ×1 cm大小的叶片组织，取果蒂中段并制成约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叶片气孔和果蒂细胞形态图。

1.3　组织解剖结构特征分析

参考王艳芳等[14]的方法对叶片、果蒂进行石蜡包埋切片，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。

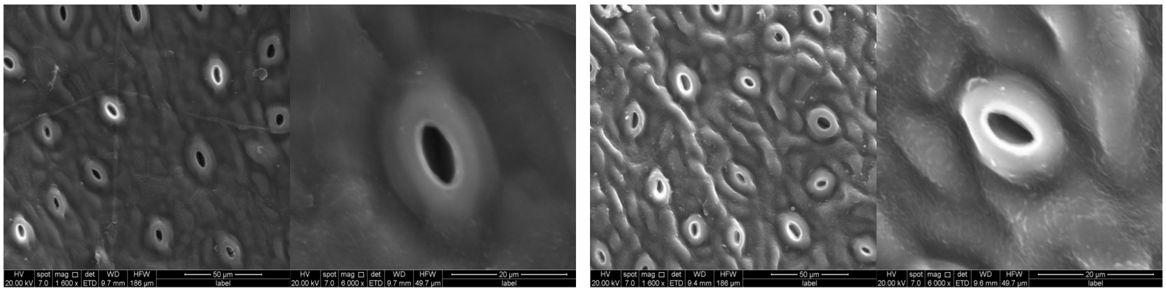
1.4　光合色素、丙二醛含量及酶活性的测定

光合色素含量参照李合生[15]的方法，测定在波长665 nm、649 nm、470 nm下的吸光度值。丙二醛（MDA）含量参照邹琦[16]的方法，测定在450 nm、532 nm和600 nm处的吸光度值。过氧化物酶（POD）活性参考Qin等[9]的方法，CAT和SOD活性测定参照Dória等[17]的方法进行。以上数据在TU-1901双光束型紫外可见分光光度计上进行测定，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　叶片气孔形态及组织结构

电镜扫描观察发现健康叶片的气孔状态正常，气孔周围组织平展、光滑，感染柑橘黄龙病症状叶片的气孔凹陷、萎缩，气孔周围组织皱缩、粗燥、不平整（图1）。



A　　B

A. 健康叶片气孔形态；B. 患病叶片气孔形态。

A. The stomatal morphology of a healthy leaf; B. The stomatal morphology of a diseased leaf.

图1　感染柑橘黄龙病的植株叶片气孔形态电镜扫描

Fig. 1 The stomatal morphology of a leaf infected by *Citrus* Huanglongbing

经测定感染柑橘黄龙病叶片的韧皮部、木质部和髓部厚度明显低于健康叶片，韧皮部厚度为健康组织的72.3%，木质部和髓部的厚度为健康组织的81.2%（表1）；虽然患病和健康叶片栅栏组织、海绵组织厚度相比差异不明显，但健康叶片栅栏组织细胞呈长条形，排列整齐紧密，细胞间隙小，患病叶片

表1　感染柑橘黄龙病叶片各组织层厚度

Table 1 The thickness of each tissue layer of a leaf infected by *Citrus* Huanglongbing

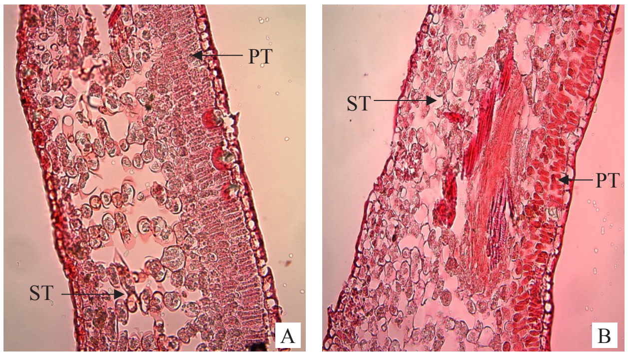
μm

	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	101.3±5.1 a	298.6±11.2 a	83.4±9.6 a	250.6±15.4 a
患病叶片Diseased leaf	73.2±3.5 b	242.5±12.3 b	71.8±8.2 a	231.3±12.5 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.

的栅栏组织细胞呈椭圆状，排列松散、细胞间出现较大的间隙（图2）。



A. 健康叶片；B. 患病叶片；PT. 栅栏组织；ST. 海绵组织。

A. The healthy leaf; B. The diseased leaf; PT. Palisade tissue; ST. Spongy tissue.

图2 感染柑橘黄龙病的植株叶片组织结构解剖

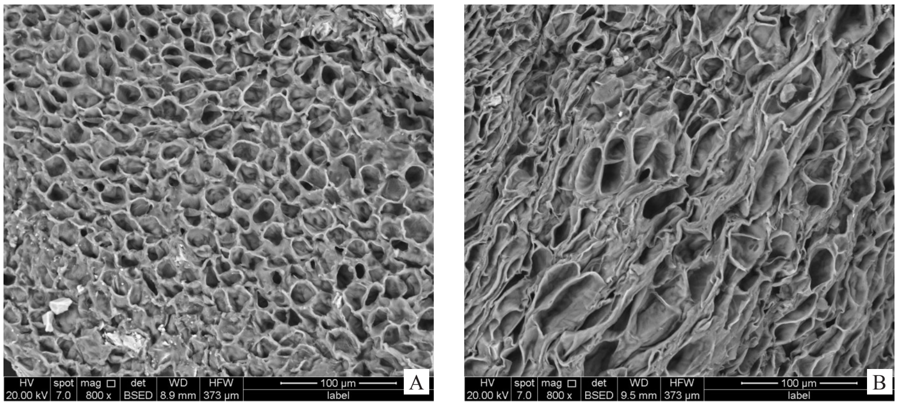
Fig. 2 The tissue anatomical structure of a leaf infected by *Citrus* Huanglongbing

2.2 果蒂细胞形态及组织结构

从图3可见健康果蒂组织细胞正常，大小一致，感染柑橘黄龙病的果蒂细胞大小不均，有的细胞褶皱，个别细胞扭曲变形、膨大松散，具有大的细胞间隙和胞间腔。同时果蒂的部分维管束堵塞（图4-B），导致营养物质、水分等运输途径受阻，无法输送到植物的果实部位。

2.3 光合色素、丙二醛含量及酶活性的测定结果

感染柑橘黄龙病的叶片光合色素、丙二醛（MDA）含量及过氧化物酶（POD）、过氧化氢酶（CAT）和超氧化物歧化酶（SOD）活性变化见表2。患病叶片光合色素含量显著低于健康叶片，叶绿素a、叶绿素b和类胡萝卜素的含量分别是健康叶片的19.87%、22.13%和47.71%；而患病叶片的丙二醛含量增加了120.39%，显著高于健康叶片；患病植株叶片POD、CAT和SOD酶活性均有不同程度的变化，

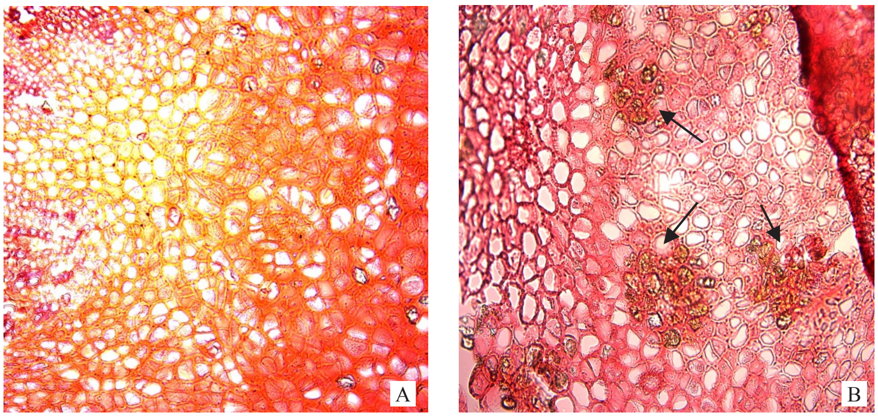


A. 健康果蒂；B. 患病果蒂。

A. The healthy pedicel; B. The diseased pedicel.

图3 感染柑橘黄龙病的果蒂细胞形态电镜扫描

Fig. 3 The pedicel cell morphology of infected *Citrus* Huanglongbing



A. 健康果蒂维管束；B. 患病果蒂维管束。

A. Vascular bundle of the healthy pedicel; B. Vascular bundle of the diseased pedicel.

图4 感染柑橘黄龙病的果蒂维管束形态解剖

Fig. 4 The vascular bundle morphology of a pedicel infected by *Citrus* Huanglongbing

表2　感染柑橘黄龙病植株叶片光合色素、MDA含量及POD、CAT、SOD活性变化

Table 2 The content of photosynthetic pigments、MDA and the changes in activities of POD，CAT，SOD of a navel orange infected by *Citrus* Huanglongbing

材料 Material	ω(叶绿素a) Chlorophyll a content/(mg·g⁻¹)	ω(叶绿素b) Chlorophyll b b content/(mg·g⁻¹)	ω(类胡萝卜素) Carotenoid content/(mg·g⁻¹)	MDA含量 MDA content/(μmol·g⁻¹)	POD活性 POD activity/(U·mg⁻¹·min⁻¹)	CAT活性 CAT activity/(U·mg⁻¹·min⁻¹)	SOD活性 SOD activity/(U·mg⁻¹·min⁻¹)
健康植株 Healthy plant	1.978±0.152 a	0.759±0.094 a	0.677±0.047 a	16.72±2.82 a	2120.15±143.33 a	11.83±0.88 a	1658.24±29.04 a
患病植株 Infected plant	0.393±0.031 b	0.168±0.011 b	0.323±0.015 b	36.85±3.31 b	17881.34±131.38 b	24.32±1.89 b	2218.61±40.72 b

均显著高于健康植株，其中POD活性增加了8.43倍。

3　讨　论

植物叶片的表皮结构是抵御外界病菌入侵的第一道天然屏障，气孔是植物同外界进行气体和水分交换的重要门户[18]，栅栏组织、海绵组织排列紧实度与寄主抗病性呈正相关[19-20]。本研究发现感染柑橘黄龙病的‘纽荷尔’脐橙叶片厚度变薄，气孔萎缩、气孔周围组织皱缩，影响叶片的正常呼吸功能；栅栏组织细胞间隙增大排列松散，果蒂部分细胞变形呈膨大松散状，表明寄主组织结构抵御病原菌入侵和扩展能力减弱。还发现寄主韧皮部部分维管束堵塞，植物体内物质运输途径受阻，此研究结果同Achor等[12]对伏令夏橙、Madam vinous甜橙及葡萄柚的解剖结构分析结果相一致。

叶绿素含量是叶片光合能力的重要指标，与寄主的抗病性密切相关，当寄主植株受病菌侵染后，叶绿素分解酶被激活，致使叶绿素含量降低，从而影响叶片光合作用，降低了寄主的抗病性[21-22]。多项研究发现植株体内MDA与寄主抗病性呈负相关性，其含量的增加会加大对寄主细胞的损伤[8, 23]，POD、CAT和SOD酶活性的提升有助于寄主体内抗性的增加[24-25]，因此了解寄主体内此类物质的变化有助于分析病原菌对寄主生理生化造成的影响。本研究测定柑橘黄龙病侵染的寄主叶片叶绿素a、叶绿素b和类胡萝卜素的含量均低于健康柑橘叶片；MDA含量是健康叶片的2.2倍，POD、CAT和SOD酶活性与对照相比存在明显提升，表明柑橘黄龙病侵染植株后造成了寄主叶片叶绿素含量的降低，影响了光合作用，MDA含量的提升加大了对寄主细胞的破坏作用，相关防御酶活性的增加表明柑橘黄龙病入侵激发了寄主体内的抗性反应。

由于柑橘黄龙病菌至今无法人工培养，因此对其的作用机理方面研究进展缓慢，给生产中黄龙病的防控和抗病育种工作带来了很大的制约。而通过对患病寄主组织微形态和组织解剖结构的分析可了解病原菌对寄主造成的破坏特点，解析此类无法人工培养的病原菌对寄主造成的致病机制，为后续病害的防控研究工作提供理论依据。本研究对柑橘黄龙病侵染的寄主微形态结构及生理生化分析，发现病菌入侵后会导致寄主细胞组织出现一系列生理、病理变化，表现在影响叶绿素含量、破坏寄主细胞、组织结构变形、维管束堵塞等作用机制方面。关于柑橘黄龙病菌对寄主光合特性以及叶绿体、线粒体、类囊体等细胞器的超微结构影响，作者将作进一步的研究报道。

4　结　论

感染柑橘黄龙病的‘纽荷尔’脐橙组织微形态结构特征、叶片光合色素和丙二醛含量及防御酶活性与健康植株比较，发现受柑橘黄龙病菌侵染后植株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，寄主抵御病原菌入侵和扩展能力减弱。

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