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# TRIPLOID LIME IS MORE TOLERANT TO HLB THAN DIPLOID LIME BECAUSE SPECIFIC PHYSIOLOGICAL AND ANATOMICAL TRAITS ASSOCIATED TO BETTER DETOXIFICATION PROCESSES

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## Abstract

Huanglongbing (HLB), also known as citrus greening is a major issue for the citrus industry. It is caused by the bacteria *Candidatus Liberibacter* spp that is spread by *Diaphorina citri*, also called citrus psyllid. HLB is responsible of the synthesis of callose at sieve plate of the phloem leading to the obstruction of the pores that provide connection between adjacent sieve elements, thus limiting the symplastic transport of sugars and starch synthesized in leaves to the other organs of the plants.

Triploid (3x, *Citrus latifolia*) and diploid (2x, *Citrus aurantiifolia*) limes were investigated when infected by HLB. Analysis by Scanning Electron Microscopy (SEM), as well as physiological and biochemical analysis were performed to decipher the potential role of polyploidy in the tolerance to HLB. Physiological results showed that triploid performed better than diploid. Analysis by SEM showed that pores were larger in 3x than in 2x. Important deposition of callose onto 2x and 3x pores were observed in symptomatic leaves of 2x and 3x. Our results provide the first insights regarding the better tolerance of citrus polyploid to HLB.

## Introduction

Infection by HLB will cause a rapid decline and the death of the trees. In Brazil and Florida, the disease already affected several million trees and thousands of hectares were removed. Nowadays, this disease affects the entire Caribbean basin including Guadeloupe and Martinique.

After the injection of the bacteria by the psyllid leading to HLB infection, the reaction of the tree will result in the synthesis of callose in the pores of the phloem sieve cells [1] thus leading to a stop of elaborated sap flow. As the elaborated sap flow cannot be transmitted to various organs of the tree, the products of photosynthesis such as starch are not translocated. This will lead to apparition of asymmetric mottling that can be observed in leaves. The fruits cannot be consumed leading to the loss of production. Today there is no strict resistance to HLB within the *Citrus* genus. However, polyploids such as Tahiti lime, which is a natural triploid variety (3x), is much less affected by the disease [2] than other diploid varieties (2x) that are very sensitive.

## Materials and Methods

### Plant Material

Diploid (2x, *Citrus aurantiifolia*) and triploid (3x, *Citrus latifolia*) limes grafted onto diploid citrumelo 4475 (*Citrus paradisi* × *Poncirus trifoliata*) rootstocks were investigated when naturally infected by HLB in the field. Trees were 3 years old.

### Methods

#### Leaf, photosynthesis and transpiration within light-adapted

Photosynthesis and transpiration were measured with LCpro+ Portable Photosynthesis Systems (ADC BioScientific Ltda., Hoddeston, England). Measurements were done using photosynthetic photon flux density varying from 200, 900 & 2000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ .

#### Scanning Electron Microscopy

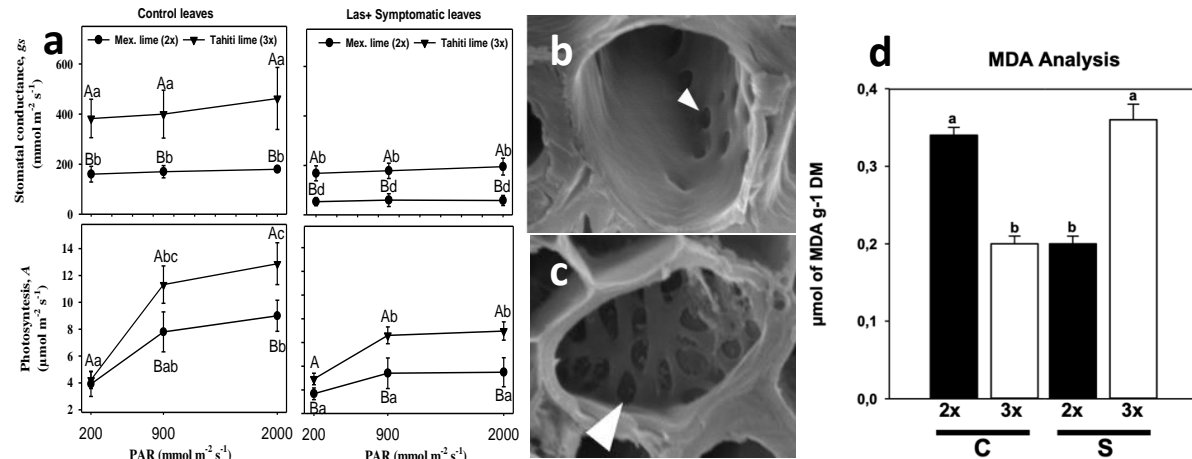
The anatomy of leaf petiole was analyzed using Scanning Electron Microscope (SEM) to observe callose deposition at sieve plate of the phloem. Analysis were performed at the C3MAG microscopy platform of the University of Antilles, Guadeloupe.

#### Malondialdehyde assay

This stress biomarker was determined according to the method previously described [3].

## Results

In the field, triploid variety present less symptoms of chlorosis due to HLB. Results of stomatal conductance and photosynthesis, showed that the more the plant is infected by HLB, the more there is a decrease of those activities compared to control plants (Fig. 1a). However, values remained greater in 3x than in 2x at any stage of the disease.



**Figure 1:** Stomatal conductance and photosynthesis measured in 2x and 3x limes (a). Analyze by SEM showed that the pores in the phloem cells of 2x (b) were smaller than in 3x (c). Analyze of MDA in 2x and 3x in control and symptomatic leaves (d).

Analysis by SEM of the petiole sieve plate in control samples shown that pores were about 1/3 smaller in 2x (Fig. 1b) than in 3x (Fig. 1c). Thus, it is possible that polyploid can maintain a phloem sieve flux for a longer time when infected HLB. SEM analysis in infected petiole samples of leaf presenting HLB symptoms showed important deposition of callose onto 2x and 3x pores.

HLB induces oxidative stress. We thus investigated MDA stress biomarker (Fig. 1d). The results showed that in control leaves MDA is lower in 3x than in 2x. In symptomatic leaves, an opposite figure is observed. This last result could be explained by the fact that leaves in 3x do not fall compare to the 2x. Thus, 3x leaves have been probably be infected for a longer time and experienced more stress. Indeed, the analysis of detoxification enzymes, like catalase or ascorbate peroxidase, of reactive oxygen species, that are toxic for the organisms [4, 5], revealed a much better behavior in 3x than in 2x.

## Conclusion

Taking together, our physiological, anatomical and biochemical results could explain why 3x behaves much better than 2x and suggest that polyploidy may present a great interest to improve tolerance to HLB. Further investigations are needed, especially in asymptomatic leaves to determine the detriment of the tolerance of polyploid to HLB.

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