Diagnosis of Citrus Greening Disease by qPCR Analysis

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Introduction

Citrus greening, also known as Huanglongbing disease, is caused by the gram-negative bacterium, *Candidatus Liberibacter asiaticus*. CLas is transmitted by the insect, Asian psyllid *Diaphorina citri*. This disease mainly affects orange trees but it can also affect other closely related species such as lime trees. The disease causes trees to have asymmetrical blotchy molting of leaves (Figure 1), the tree produces small lopsided green oranges that are very bitter (Figure 2), and because of this, they lose their economic value [1]. There is currently no cure for citrus greening. The best way to manage citrus greening is by implementing the use of healthy planting material, a prompt removal of infected trees and branches as well as controlling the psyllid population [1].

Quantitative real time PCR (qPCR) is a sensitive laboratory technique that monitors the amplification of targeted DNA in real time (Figure 3). qPCR can be used for a variety of purposes such as viral quantification, pathogen detection and somatic mutation analysis [2,3,4]. The purpose of this experiment was to determine if qPCR could be used as a way to detect the presence of the CLas bacterium that causes the citrus greening.

Methods and Materials

-Leaf samples from eight different trees were obtained from a citrus farmer in Sarasota, FL. Trees number six, seven and eight were not sick and were used as the control.
-DNA was extracted from four different leaves by using the Qiagen DNeasy Plant mini kit.
-After extraction the purity and concentration of the DNA was measured with a nanophotometer.
DNA sequences from two citrus plant genes (EF and COX) as references and four CLas bacterium genes [5] were used as primers for qPCR.
-20 nanograms of DNA were used for each qPCR sample. qPCR was performed using Step-One-Plus real time qPCR system and data analyzed.
-This process was repeated multiples times in order to determine fidelity

Data

Figure 1: A side by side comparison of citrus leaves affected by citrus greening (left) and healthy leaves

Figure 2: Five oranges from a tree affected by citrus greening disease.

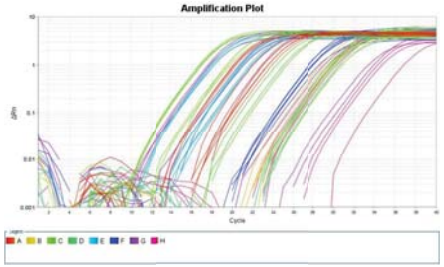

Figure 3: qPCR amplification plot showing different patterns for the different genes in our leaf samples

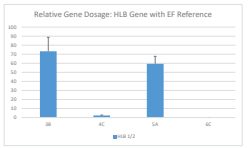

Figure 4: Relative gene dosage of HLB gene with EF reference

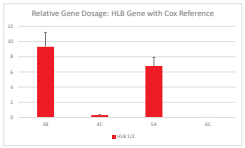

Figure 5: Relative gene dosage of HLB gene with COX reference

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Results and Discussion

Our results showed that using the citrus EF gene and COX gene as reference, Tree 3B and Tree 5A both had higher dosages of the CLas DNA, followed by Tree 4C (Figures 4 and 5), suggesting these trees have been infected by the CLas bacterium that causes the citrus greening disease. Tree 6C had no detectable CLas DNA (Figures 4 and 5). Based on the results of the qPCR data obtained from the various trails performed it can be concluded that qPCR can be used to accurately diagnose citrus greening. The qPCR analysis produced consistent enough results to be a reliable way to determine if a tree is inflicted with the disease. This method is a way to genetically prove whether the tree is sick or not. This analysis was done with extracted DNA from the leaves, but it could also be done by extracting DNA from the roots of the tree. While there is yet a cure for citrus greening, being able to diagnose a sick tree is a vital part of restricting the spread of the disease

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