

Machado-contract-132

Proposal Title: Analysis of transcriptome of citrus infected with *Ca. Liberibacter asiaticus* and *Ca. L. americanus*.

Main goal

Analysis of transcriptome of sweet orange during the infection of *Candidatus Liberibacter* spp.

Narrative

Experiments – From February to June 60 plants of sweet orange Hamlin were grafted into Rangpur lime and established at screen house. After reached 30 cm tall, they were grafted with budwoods infected with *Ca. Liberibacter americanus* (CLam) and budwoods infected with *Ca. Liberibacter asiaticus* (CLas). All budwoods were checked by conventional PCR and RT-qPCR by the presence of the bacteria. Healthy plants are the negative control. After the plants were confirmed bacteria positive (RT-qPCR) they were pruned and transferred to a growth chamber at 22 to 24 oC and photoperiod of 16/8 hours. Approximately 40 days after pruning 15 cm branches were collected. Leaves and barks were separately grinding in liquid nitrogen. Concentration and integrity of total RNA were evaluated before hybridization experiments in microarray platform (Roche Nimblegen).

Hybridization experiments - The RNA samples were sent to Roche NimbleGen Systems, where cDNA synthesis and Cy3 labeling was performed. Hybridization, scanning, and image analysis of the arrays were performed according to the manufacturer's recommendations. The oligo-array includes 32,000 genes of sweet orange with six replicas of each one with density of 340,000 spots. Preliminary results were presented in the previous report.

Next steps – Array hybridizations with plants infected with *Ca. L. asiaticus*, but symptomless. Array experiments with symptomatic plants infected with *Ca. L. asiaticus* and plants infected with *Ca. L. americanus*. Statistical analysis of the global gene expression experiments. Primers design for specific gene expression during the infection with both bacteria. First draft of the manuscript for publication.

Expected results and practical application

The main expected result is the knowledge on the gene expression pattern in sweet orange during the process of infection by *Candidatus Liberibacter* spp. Such knowledge will enlarge our understanding on the basic aspects of the disease and may be help to approach new technologies for control, especially technology based on gene silencing or gene over expression.

Revised Budget for the Second Year

The last part (15 %) of the approved budget will be used for the payment of the temporary Post doctor working in the project. 10 % will to be deducted for the Department, according with the contract (Considerations 6.2).