**09 - Detection of coffee ringspot virus (CoRSV) in the mite vector by RT-qPCR**

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The mite *Brevipalpus phoenicis* (Geijskes, 1939) (Acari: Tenuipalpidae) is responsible for the transmission of coffee ringspot. There is a great interest in the study of *Brevipalpus* transmitted viruses (BrTVs), and the interaction between them, their vectors and hosts. Electron microscopy analyses suggest that CoRSV replicates in the mite tissue, what would characterize a persistent propagative virus-vector relationship. This study aimed to establish a transcriptase quantitative polymerase chain reaction (RT-qPCR) method to evaluate and compare the replication capacity of the virus in the vector. Total RNA was extracted from a sample of 100 mites reared in symptomatic plants for CoRSV using two different methods: CTAB and Nucleo Spin RNA XS Kit (Macherey-Nagel) and quantified in a NanoDrop 8000 (Thermo Scientific). The RNA extracted with the kit was more concentrated and presented less variation between samples, and hence, this protocol was chosen as the standard for further analyses. The cDNA was synthesized from 5uL of RNA using random primers. Five different dilutions of the cDNA were tested (1:1, 1:10, 1:100, 1:1000 and 1:10000) with two primer pairs (CoRSV1 and CoRSV2) that amplify regions of the viral replicase in five different concentrations (10 nM; 30 nM; 60 nM; 90 nM and 120 nM). Two 2 uL of cDNA were used for the reaction with Sybr Green (ABI) in a 7500 Fast Real Time PCR System (Applied Biosystems). These tests allowed the optimization of the procedure, with 30 nM of the primers and 1:10 dilution of cDNA. The efficiency of the primers CoRSV1 and CoRSV 2 is of 88.8% and 83.98%, respectively, and hence, CoRSV1 will be used in the experiment. Based on these results, the experiments will be conducted to determine whether or not the virus replicate in the mite. This research was financially