Supplemental Methods and Legend for Supplemental Figure 1

Methods

Total RNA was purified using NucleoSpin Plant Kit (Clontech) from leaves 7-9 of CaLCuV-infected and mock inoculated plants at 12 dpi. Total RNA (5 µg) was used as template in reverse transcription reactions (20 µL total) containing an oligo (dT)15 primer (Promega) and PowerScript reverse transcriptase (Clontech), according to the Clontech cDNA reverse transcription protocol (PT3396-2). The cDNA reactions were performed in duplicate for both tissue types.

For semi-quantitative RT-PCR assays, 1 µL of a cDNA dilution (1 in 20) was amplified in 22 µL of PCR Supermix (Invitrogen) in the following conditions: initial hot start of 94ºC for 3 min, then a variable number of cycles of 94ºC for 30 sec, 60ºC for 30 sec and 72ºC for 30 sec, followed by a 7 min incubation at 72ºC. To ensure linearity, the number of PCR cycles varied between 25-30 dependent on template abundance. The oligonucleotide primer pairs for the different mRNAs are listed in Supplemental Table 7 online.

For quantitative RT-PCR assays, 20 ng of cDNA template was diluted to 25 µL in a reaction containing 100 nM each primer and 1x SYBR green PCR master mix (Applied Biosystems). Samples were analyzed in duplicate in a MX3000P qPCR thermocycler (Stratagene). Selected primer pairs from the Primer Library for Arabidopsis Pathogen-Inducible Genes (PR0100, Sigma) were used in the analysis. Analysis of data was performed as described by Livak and Schmittgen (2001), using ubiquitin conjugating enzyme (UCE) as the normalization control (Livak and Schmittgen, 2001).

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-(Delta Delta C(T)) Method. Methods **25:** 402-408.

Supplemental Figure 1. Validation of microarray results

(A) The levels of RNAs corresponding to selected pathogen response genes were compared in mock-inoculated (M) and CaLCuV-infected (I) leaves at 12 dpi using semi-quantitative RT-PCR. Microarray results are shown on the right – (↑) up in infected leaves, (↓) down in infected leaves, (nc) no change, (†) discrepancy between microarray and RT-PCR, and (*) not on the

ATH1 chip. GAPDH was used as an internal reference control. The *q-value* for the microarray analysis of each gene is given on the right.

(B) The levels of RNA corresponding to selected DNA replication and core cell cycle genes were compared in mock-inoculated (M) and CaLCuV-infected (I) leaves using semi-quantitative RT-PCR. Microarray results are shown on the right – (↑) up in infected leaves, (↓) down in infected leaves and (nc) no change. The q-value for the microarray analysis of each gene is given on the right. RBR was used as an internal reference control.

(C) Comparison of quantitative RT-PCR and microarray analyses of the relative levels of RNAs corresponding to selected pathogen response genes in mock-inoculated and CaLCuV-infected leaves. The black bars represent ATH1 microarray results while the white bars correspond to quantitative RT-PCR analysis. Several genes included in the Sigma kit were not represented on the ATH1 array and do not have corresponding black bars. Ubiquitin conjugating enzyme was used as an internal reference control. The Arabidopsis gene numbers for the genes in B and C are in Supplemental Table 7 online.Semi-quantitative and quantitative RT-PCR.