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CITRUS TRISTEZA VIRUS RESISTANCE GENE LOCUS: SMALL RNA PROFILE AND PRELIMINARY EPIGENETIC STUDIES

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Resistance gene, tristeza, siRNA, DNA methylation

Small interfering RNAs (siRNAs), play a vital role in epigenetics of plant virus-host plant interactions. It has been extensively studied at both the transcriptional and post-transcriptional levels. In plants, siRNAs initiate and manage gene silencing by directing DNA methylation and/or histone methylation. In Arabidopsis, the ~24 nt siRNAs directs DNA methylation (RNA-directed DNA methylation, RdDM) and chromatin remodeling at their target loci. Recent advances in high-throughput sequencing techniques has enabled thorough exploration of small RNAs populations and allow rapid analysis of massive datasets to assemble complete full-length genome sequence for different plant species. This large database of sequence information also allows identification of genome regions specifically matched by siRNAs that likely differ among tolerant, resistant or susceptible hosts and advance epigenetic studies on diseased plants.

Resistance to *Citrus tristeza virus* (CTV), the most severe virus affecting *Citrus* spp., associated with a single dominant gene locus *Ctv* occurring in *Poncirus trifoliata* while all *Citrus* spp. are considered susceptible. This locus contains 22 putative genes, but their regulation and mechanism for resistance remains unknown.

In our study, CTV was graft-inoculated on Carrizo citrange (*Poncirus trifoliata* x *C. sinensis (I think)*) and *C. aurantium* (sour orange) seedlings, and the population of siRNA characterized by high-throughput sequencing using an ILLUMINA platform. The *Ctv*-derived siRNA (~2% of the total short reads) were dominated in both hosts by the 24-nt. However, CTV infection caused an increase in accumulation of 24-nt siRNA sequences homologous to the *Ctv* gene in Carrizo but it decreased in sour orange. Distribution of the 24nt along the *Ctv* gene locus (282Kb) had a clearly different distribution between the two host. The predominant hot spot of siRNA in Carrizo mapped in the putative gene *Ctv-20*, whereas in sour orange it associated to the intergenic region between the putative genes *Ctv-11* and *Ctv-12*, where a Copia-like retrotransposon C is located. This distribution profile was conserved for each species between CTV-infected and uninfected plants but, as previously mentioned, the frequency of the 24nt siRNAs was altered by the presence of the virus.

We supposed that the different profile of 24nt between the two host in the locus *ctv* is due to RdDM mechanisms. To demonstrate the methylation status of the resistance locus we performed a bisulfite treatment of DNA. in which unmethylated cytosine was converted to uracile, while methylated cytosine did not react. A methylcytosines mapping was carried out on *Ctv-11* and *Ctv-12* sequences. By specific software were found 5 different CpG islands in the Copia-like

retrotransposon sequence and 42 primer pair were designed. The PCR analyses have been carried out using MSP and BSP primers followed by combined bisulfite restriction analysis (COBRA).