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Genome Organization of the Top Component of Citrus Psorosis Virus and Identification of the Coat Protein Gene

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ABSTRACT. Citrus psorosis virus (CPsV) is associated with a widespread and damaging disease of citrus in many regions of the world. In this study we show that an isolate of CPsV called citrus ringspot virus (CtRSV-4) contains at least three genomic RNAs; two of them (RNA 2 and RNA 3) are found in the top component and the largest (RNA 1) in the bottom component. RNA 3, the smallest RNA, was cloned and a partial sequence of 1,454 nucleotides was determined. One open reading frame (ORF), which encodes a 439 amino acid protein with a MW of 48,654 was found. This was identified as the coat protein by expressing part of the ORF in *Escherichia coli* and detecting the product in Western blots with an antiserum specific for the virions. The nucleotide sequence of RNA 2 was determined and found to consist of about 1,620 nucleotides. In the viral-complementary RNA, one open reading frame (ORF) is present coding a 476 amino acid protein of MW 53,694 with unknown function. Analyses by Northern blot show that both positive and negative strands of CPsV RNA 2 and 3 are encapsidated and the negative strand is the viral RNA, which is present much more abundantly. No subgenomic RNAs were found.

Citrus psorosis (CPsV) is associated with a serious disease causing the death of citrus trees in many countries (5). Due to low concentration of the virus and poor stability of the particles, its genome has not been fully characterized. It is known that two sedimenting components (top and bottom), separated by sucrose gradient centrifugation, are required for infectivity.

Derrick et al. (2, 3) and Barthe et al. (1) have reported that the citrus ringspot virus (CtRSV-4) isolate of CPsV from USA (4) contains one small RNA in the top component coding for the coat protein (CP) gene, and two larger RNAs in the bottom component, one single stranded, the other double stranded.

Here, we show that the top component of CPsV contains two genomic RNAs, RNA 2, which codes for a 54 kDa protein of unknown function, and RNA 3 coding for the CP. Both genes are present in the negative sense in the viral RNAs. For this study, the source of virus was *Chenopodium quinoa* leaves bearing local lesions, from which top component was purified. Complementary DNA (cDNA) of the RNA extracted from the top fraction was obtained using random primers. The fragments were inserted into pcDNAII vector and cloned in *Escherichia coli* DH5-α. The library was screened by colony-blotting using ^{32}P-labeled cDNA as probe (6), and a second screening was done with a probe made from a healthy plant preparation to discard clones containing cDNA inserts derived from host RNAs. To extend the sequences to the 3' and 5' ends of RNA 3 and RNA 2, another strategy was applied. The RNA was poly(A)-tailed, reverse transcribed and amplified by PCR using internal and oligo-dT primers. The PCR products of the expected size were cloned in linearized pGEM-T plasmid (Promega). The clones were then sequenced and analyzed.

The nucleotide sequence of RNA 2 was found to consist of about 1,620 nucleotides. Meanwhile 1,600 nt were determined by Northern blot (denatured), indicating that the consensus sequence is near to the complete RNA 2 sequence. In the viral-complementary RNA, one open reading frame (ORF) is present coding for a 476 amino acid protein of

MW 53,694 Da. All attempts to find a putative function gave no significant similarity with known polypeptide sequences from the data bases.

The partial sequence of RNA 3, the smallest RNA, consists of 1,454 nucleotides. This is close to the 1,500 nt value estimated from the Northern blot (denatured) indicating again that it is almost the complete sequence of RNA 3. One ORF codes for a 439 amino acid protein with a MW of 48,654 da. This polypeptide was identified as the CP by expressing part of the ORF in *E. coli*. A fragment of 19.5 kDa was cloned in pET19b vector and expressed in *E. coli* BL21(DE). After confirmation of the clones by its complete sequence, a Western blot using an antiserum specific for the virions (kindly provided by R. Milne) gave a clear result indicating that the gene is coded by RNA 3, thus corresponding to the CP protein.

Analyses by Northern blot showed that the strands coding the genes of RNA 2 and RNA 3 (e.i. the positive strands) are present in much smaller amounts than the negative strands. For this reason, we have defined viral RNAs 2 and 3 negative strands as viral (v), and viral complementary (vc) RNAs as the positive strands. We did not find subgenomic RNAs in RNA 2 and RNA 3, or double strand RNAs in native Northern blots.

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Note added in proof. Part of the work summarized here has been published in Sánchez de la Torre et al. (7).

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