Sequence Analysis of Citrus Psorosis Virus Bottom Component RNA

G. Naum, M. L. García, M. E. Sánchez de La Torre, and O. Grau

ABSTRACT. Citrus psorosis virus (CPsV) isolate CPV-4, type member of the *Ophiovirus* genus, contains three different RNAs called RNA 1, RNA 2 and RNA 3, that are encapsidated in independent particles. To study the nucleotide sequence of RNA 1 present in the bottom component, a library of complementary DNA (cDNA) was prepared and the clones sequenced. A consensus sequence of 8,184 nucleotides was obtained, from which two open reading frames (ORFs) were evident. One large ORF located close to the 3' end of the positive sense strand codes for a 2,416 amino acid protein (280 kDa). This ORF showed a limited homology with an RNA polymerase of Rhabdovirus. Therefore, these data suggest that RNA 1 would contain the information to code the viral RNA polymerase which might perform as replicase and/or transcriptase. The other ORF, close to the 5' end of the positive sense RNA codes for a 206 amino acid protein (24 kDa). Preliminary Northern blot analysis indicate that viral RNA 1 is negative sense.

Partial characterization of several citrus psorosis *Ophiovirus* (CPsV) isolates in USA (2, 3), Spain (8, 9, 10), Israel (1), and Argentina (4, 5) indicates that it can be separated into at least two sedimenting components, called top and bottom. In both components, circular filamentous particles are observed, with similar morphology, although the bottom particles are about five times larger than the top particles (6). Both particles share a single coat protein (CP) (2, 5).

Derrick et al. (3) found two bands of viral RNA present in the bottom fraction. One RNA, 10,000 nt seemed to be dsRNA according to its stability to RNAse treatment, and other faster migrating RNA was described as ssRNA since it did not resist the RNAse treatment. Due to the native conditions of the electrophoresis, the dsRNA was described as a genomic RNA or a replicative form of a ssRNA. Later, Garcia et al., (7), purified viral RNA from the bottom fraction, corresponding to the dsRNA described by Derrick et al. (3), and prepared a cDNA library using random primers. The virions were obtained from *Chenopodium quinoa* local lesions and partially purified through a sucrose gradient. The bottom component was phenol-extracted and the viral RNAs were separated from host nucleic acids by agarose gel electrophoresis. The RNA was recovered from the gel, and reverse transcribed. The cDNA fragments were cloned in *Escherichia coli* DH5-α using pcDNAII as vector. The clones were sequenced using the Taq Dye Deoxy™ Terminator Cycler Sequencing Kit (Applied Biosystems Biosystems Model 373A) DNA Sequencing equipment. Using one large clone called 224m, a large RNA was detected by Northern blot (7) confirming that the 224m clone belongs to the RNA. Due to the native conditions of the Northern blot, the size of the RNA was estimated about 9,000 nt. In this paper, we describe the analysis of RNA 1 by Northern blot under denaturing conditions using the clone 224m as probe. Under these conditions, RNA 1 behaves as if it were 8,300 nt long compared with RNA molecular weight markers.

We have sequenced both ends of 118 clones and constructed a consensus sequence using two different programs, the Wisconsin Genetic Computer Group (GCG) software Package Version 9.0, and the Phrap and Phrapview programs.

In agreement with the 8,300 nt size of the RNA 1 estimated by Northern blot, the 118 clones give a consensus sequence of 8,184 nucle-

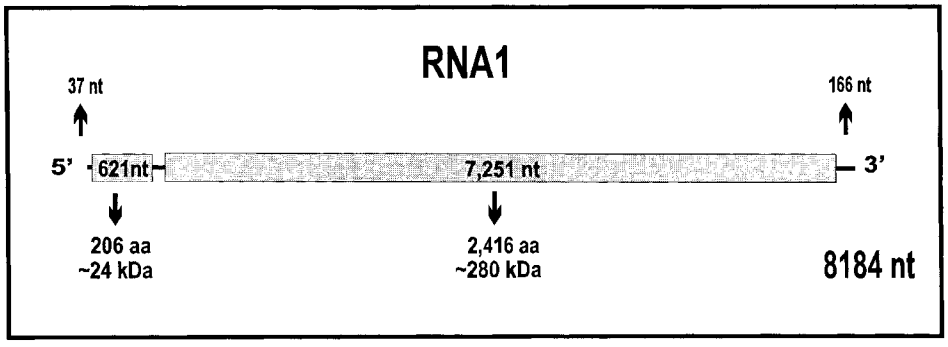

Fig. 1. Scheme representing the genomic organization of the citrus psorosis virus (CPsV) RNA 1.

otides (Fig. 1). It is supported in all regions by at least three clones. The size of the consensus sequence indicates that we are very close to the complete sequence of the RNA 1. The 8,184 nt were translated and confronted with the Swissprot data base. Two different open reading frames (ORFs) with the same polarity were evident. One large ORF is located close to the 3' end of the positive sense strand and codes for a 2,416 amino acid polypeptide (280 kDa). This ORF showed a limited homology with an RNA polymerase of Rhabdovirus. We suggest that RNA 1 contains the information to code for the viral RNA polymerase which could function as replicase and/or transcriptase.

The small ORF, close to the 5' end of the positive sense, codes for a 206 amino acid (24 kDa) protein. No homology with any other sequence has been obtained from the data bank.

Preliminary Northern blot analysis using the 224m transcript indicates that the viral RNA 1 is of negative sense.

RNA 1 thus appears to be a bicistronic RNA, which is uncommon in plant virus translation strategies.

ACKNOWLEDGMENTS

This work was supported by grants of SECYT-CONICET-BID802-OC/AR PID 332 and the European Union Contract EBBICI8CT960044. O. Grau is recipient of a Research Career award from Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Argentina. M. L. G. is also from CONICET. G. Naum is a fellow of CONICET, Argentina.

LITERATURE CITED

1. da Graça, J. V., M. Bar-Joseph, and K. S. Derrick
1993. Immunoblot detection of citrus psorosis in Israel using citrus ringspot antiserum. In: *Proc. 12th Conf. IOCV*, 432-434. IOCV, Riverside, CA.
2. Derrick, K. S., R. H. Brlansky, J. V. da Graça, R. F. Lee, L. W. Timmer, and T. K. Nguyen
1988. Partial characterization of a virus associated with citrus ringspot. Phytopathology 78: 1298-1301.
3. Derrick, K. S., R. F. Lee, B. G. Hewitt, G. A. Barthe, and J. V. da Graça
1991. Characterization of citrus ringspot virus. In: *Proc. 11th Conf. IOCV*, 386-390. IOCV, Riverside, CA.
4. García, M. L., E. L. Arrese, O. Grau, and A. N. Sarachu
1991. Citrus psorosis disease agent behaves as a two-component ssRNA virus. In: *Proc. 11th Conf. IOCV*, 337-344. IOCV, Riverside, CA.
5. García, M. L., O. Grau, and A. N. Sarachu
1991. Citrus psorosis is probably caused by a bipartite ssRNA virus. Res. Virol. 142: 303-11.

6. García, M. L., E. Dal Bo, O. Grau, and R. G. Milne
1994. The closely related citrus ringspot and citrus psorosis viruses have particles of novel filamentous morphology. J. Gen. Virol. 75: 3585-3590.
7. García, M. L., M. E. Sánchez de la Torre, E. Dal Bó, K. Djelouah, E. Luisoni, R. G. Milne, and O. Grau
1997. Detection of Citrus Psorosis-Ringspot Virus using RT-PCR and DAS-ELISA. Plant Pathol. 46: 830-836.
8. Navas-Castillo, J. and P. Moreno
1993. Biological diversity of citrus ringspot isolates in Spain. Plant Pathol. 42: 347-57.
9. Navas-Castillo, J. and P. Moreno
1995. Filamentous flexuous particles and serologically related proteins of variable size associated with citrus psorosis and ringspot diseases. Eur. J. Plant Pathol. 101: 343-48.
10. Navas-Castillo, J., P. Moreno, M. Cambra, and K. S. Derrick
1993. Partial purification of a virus associated with a Spanish isolate of citrus ringspot. Plant Pathol. 42: 339-46.