Identification and Sequence Analysis of *Potato yellow vein virus* Capsid Protein Minor Gene*

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Received June 11, 2002; Revised July 3, 2002; Accepted July 25, 2002

Abstract. *Potato yellow vein virus* (PYVV) is a whitefly-transmitted (*Trialeurodes vaporariorum*) clustervirus (WTC) with an as yet unidentified genome composition. PYVV dsRNA preparations consist of three high molecular weight dsRNA species (dsRNAs 1, 2 and 3) 8.0, 5.5 and 4.0 kbp in size respectively, as well as two low molecular weight dsRNA species of 2.0 and 1.8 kbp (denoted x and y). The PYVV capsid protein minor (CPm) gene was identified on the dsRNA 3 species, and was subsequently cloned and sequenced. The PYVV CPm gene is 2022 nucleotides long and putatively encodes a protein with estimated size 77.5 kDa. The PYVV CPm gene product is considerably larger than the equivalent proteins encoded by the bipartite criniviruses, *Lettuce infectious yellows virus* (LIYV) and *Cucurbit yellow stunting disorder virus* (CYSDV) (52 and 53 kDa, respectively). The PYVV CPm possesses a centralized domain which is absent from both the LIYV and CYSDV CPm counterparts. Pairwise comparisons as well as phylogenetic analysis based on the available amino acid sequences of the CPm of various WTCs, showed that PYVV is closely related to LIYV, CYSDV and also *Beet pseudo-yellows virus*.

Key words: *Crinivirus*, dsRNA, nucleotide sequence, *Potato yellow vein virus*, phylogeny, PYVV capsid protein minor gene, taxonomy

Introduction

The family *Closteroviridae* contains positive-stranded RNA plant viruses with long, flexuous elongated virions containing genomes of up to 20 kbp [1]. The members of this family are transmitted in a semi-persistent manner by specific *Homopteran* vectors [1]. The family *Closteroviridae* is currently divided into two genera, based on the number of genomic RNAs: the genus *Closterovirus*, with *Beet yellows virus* (BYV) as the type member, includes viruses with unipartite genomes; whilst the genus *Crinivirus*, with *Lettuce infectious yellows virus* (LIYV) as the type member includes whitefly-transmitted viruses (WTCs) with bipartite genomes [2].

To date, most of the crinivirus genomes are partially characterized and only the nucleotide sequence and gene organization of LIYV RNAs 1 and 2 and *Cucurbit yellow stunting disorder virus* (CYSDV) RNA 2 have been fully determined [3,4]. LIYV RNA 1 is approximately 8.1 kb and encodes proteins associated with virus replication [3,5]. LIYV and CYSDV RNA 2 are similar in size (approximately 7.2 kb) and contain gene arrays, which both encode a small hydrophobic protein (P5), a heat shock
protein 70 homolog (HSP70h), two proteins (P59 and P9) of unknown function, the capsid protein (CP), the minor capsid protein (CPm) and another protein (P26) of unknown function [3,4].

Potato yellow vein virus [PYVV; 6] is a WTC and a tentative species in the genus Crinivirus [7]. PYVV has sporadically caused problems to potato crops in South America for over 50 years and is now spreading within that continent [6]. PYVV has a limited host range, is transmitted by Trialeurodes vaporariorum (Westwood) and is not mechanically transmissible. Little is known about the organization of PYVV genome and thus far only a sort stretch of sequence from the HSP70h gene has been reported [6].

We have identified, cloned and sequenced the complete PYVV CPm gene. Interestingly, the PYVV CPm gene is significantly larger than the LIYV and CYSV counterpart genes and is located on the third largest dsRNA 3 species of the virus. In pairwise comparisons and phylogenetic analysis, PYVV was related to LIYV, CYSV and Beet pseudo-yellows virus (BPYV) [8].

Materials and Methods

Virus Source and Purification of dsRNAs

The PYVV isolate used in this study was maintained by graft-inoculation of healthy potato seedlings as described previously [6]. PYVV dsRNA was isolated from virus-infected potato leaves [9] and subjected to DNase I treatment as described before [10]. Individual dsRNA species were purified from agarose gels using the RNAid kit (BIO 101, La Jolla, CA, USA).

Identification of PYVV CPm Gene and Reverse Transcription Polymerase Chain Reaction

A cDNA library was generated from PYVV dsRNA 3 [11]. Based on the nucleotide sequences of the cloned and sequenced cDNAs, oligonucleotide primers were designed to prime upstream or downstream of the known sequence in a reverse transcription polymerase chain reaction (RT-PCR) genome walking procedure as described before [4,12]. The complete PYVV CPm gene was RT-PCR amplified using virus specific oligonucleotide primers CPm1 (5’-ATGGATAAATCTGTTTATAG-3’) and CPm2 (5’-TCAAAAAATTGATTCCATTC-3’) from total RNA extracts or purified dsRNA species [10]. Amplification products were cloned into pGEM-T Easy vector (Promega, Madison WI, USA) according to the manufacturers instructions, transformed into DHz Escherichia coli cells and sequenced.

Northern Hybridization Analysis

PYVV dsRNA preparations were analyzed by Northern hybridization using 32P-labelled riboprobes [13] produced with a Riboprobe kit (Promega, Madison, WI, USA) from cloned RT-PCR amplification products for hybridization with viral dsRNA negative-strands.

Nucleotide Sequencing and Phylogenetic Analysis

Both strands of plasmid DNA were sequenced by the Sanger chain-termination method, using dye-terminator cycle sequencing with AmpliTaq DNA polymerase FS (ABI PRISM 377) and M13 universal and internal oligonucleotide primers. Sequence data were assembled and compared with databases using the BLAST server on the NCBI Web server [14, http://www.ncbi.nlm.nih.gov/BLAST]. Phylogenetic trees were constructed using the CLUSTAL W program [15] and drawn using TREEVIEW version 1.6.1. freeware [16].

Results and Discussion

Isolation and Analysis of PYVV dsRNAs

Yields of PYVV dsRNA isolated from virus-infected potato plants were reasonably high (ca. 100 μg kg⁻¹ tissue). Electrophoretic analysis consistently revealed the presence of at least five dsRNA species (Fig. 1A, lane 4), absent from healthy plant extracts (data not shown). The sizes of these dsRNAs (denoted dsRNA 1, dsRNA 2, dsRNA 3, x and y) were estimated to be approximately 8, 5.5, 4.0, 2.0 and 1.8kb respectively, by comparison with CYSV dsRNAs (Fig. 1A, lane 2) and molecular weight markers (Fig. 1A, lanes 1 and 3).