Arch Virol (2009) 154:723–725
DOI 10.1007/s00705-009-0356-2

ANNOTATED SEQUENCE RECORD

Full-length sequence analysis of a distinct isolate of *Bidens mottle virus* infecting sunflower in Taiwan

J. Y. Liao · Chung-Chi Hu · C. C. Chen · C. H. Chang · T. C. Deng

Received: 24 November 2008 / Accepted: 2 March 2009 / Published online: 24 March 2009

Abstract The full-length genome of a potyvirus, previously known as sunflower chlorotic spot virus isolate SF-1 (SCSV-SF-1) which causes novel symptoms on sunflowers (*Helianthus annuus*), was sequenced and analyzed. The genome of SCSV-SF-1 is 9,741 nucleotides long, encoding a polyprotein of 3,071 amino acids containing the consensus motifs of potyviruses. Sequence comparison revealed that the 3′-terminus of SCSV-SF-1 shared over 96% similarities with isolates of *Bidens mottle virus* (BiMoV). However, SCSV-SF-1 has a very narrow host range, excluding the diagnostic host species for BiMoV, *Bidens pilosa* and *Zinnia elegans*. Therefore, SCSV-SF-1 is a distinct isolate of BiMoV. This is the first report of the full-length nucleotide sequence of BiMoV infecting sunflower in Taiwan.

Introduction

Sunflowers (*Helianthus annuus*) showing symptoms of chlorotic ringspot, enation, yellowing and stunting were found in the field in Puli, Taiwan, in 2000. The causal agent was identified to be a new potyvirus, provisionally designated sunflower chlorotic spot virus isolate SF-1 (SCSV-SF-1), based on electron microscopy examination of the morphology of virions and inclusion bodies, electrophoresis analysis of the coat protein, and immunological detection by genus- or species-specific mono- or polyclonal antibodies [6]. To elucidate the taxonomy of SCSV-SF-1, the 3′-terminal 1,445 nt of the SCSV-SF-1 genome was cloned and sequenced, and subsequently deposited in GenBank under the accession number AF538686 in 2002. Initial database searches performed from 2002 to 2006 using the partial SCSV-SF-1 sequence as the query revealed no known sequences with nucleotide sequence identity higher than 80%. Based on the criteria used for demarcating species in the family *Potyviridae* [1, 9], the result suggested that SCSV-SF-1 should be a member of a distinct species in the genus *Potyvirus.*

However, recent studies implied that SCSV-SF-1 should be regarded as an isolate of *Bidens mottle virus* (BiMoV) [2, 10], a currently accepted species of the genus *Potyvirus* [3], according to comparisons of partial genomic sequences. Nevertheless, there were still concerns over the taxonomic status of SCSV-SF-1: (i) only the 3′-terminal sequences of SCSV-SF-1 and two isolates of BiMoV, 620 nt and 1,800 nt for EF467235 [2] and EU078960 [10], respectively, were available for comparison; (ii) the identities of the BiMoV isolates used by Baker et al. [2] or Yousssef et al. [10] were only verified by serological assays, host ranges, and symptomatolgy; and (iii) the phylogenetic analysis raised the possibility that intramolecular recombination might have occurred in the SCSV-SF-1 genome (unpublished).

To address the question concerning the taxonomy status of SCSV-SF-1, the complete genome of SCSV-SF-1 was sequenced in this study, and the coat protein (CP) coding region was used to compare with that of the standard isolate of BiMoV (ATCC-PV165 isolate), which was purchased from the American Type Culture Collection

J. Y. Liao · C. C. Chen · C. H. Chang · T. C. Deng
Division of Plant Pathology, Agricultural Research Institute, Council of Agriculture, Wufeng, Taichung 413, Taiwan, ROC

C.-C. Hu (✉)
Graduate Institute of Biotechnology, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung 402, Taiwan, ROC
e-mail: cchu@dragon.nchu.edu.tw

(ATCC). The results of sequence analysis confirmed that SCSV-SF-1 is a unique isolate of BiMoV.

Provenance of virus material

The standard isolate of BiMoV (ATCC-PV165 isolate) was purchased from the American Type Culture Collection (ATCC). The SCSV-SF-1 isolate, found in the Pu–Li area in central Taiwan [6], was propagated and maintained on *Chenopodium quinoa* in an insect-proof greenhouse. We prepared antisera by immunizing rabbits with purified SCSV-SF-1 virions. The antiserum was used for the serological identification of SCSV-SF-1 and the ATCC-PV165 isolate of BiMoV. Viral RNA was extracted from infected leaves using a QIAamp Viral RNA Mini Kit (Qiagen, Valencia, California, USA) and used as the templates for reverse transcription. After cloning and sequencing, the fragments with overlapping regions were assembled. The 5′ RACE system (Invitrogen, Carlsbad, California, USA) was used for amplification of the 5′ cDNA end of SCSV-SF-1.

Sequence properties

The full-length genome of SCSV-SF-1 was found to be 9,741 nucleotides in length, containing a single open reading frame from nt 134 to nt 9,349, which encodes a polyprotein of 3,071 amino acid residues with a predicted molecular weight of 348 kDa (Fig. 1). The complete genomic sequences of SCSV-SF-1 was submitted to GenBank as an update to the original SCSV-SF-1 record (GenBank accession no. AF538686), which will be released upon the publication of the present manuscript. Using the genomic sequence of SCSV-SF-1 as the query, the similarity searches in GenBank revealed that this region of SCSV-SF-1 shared highest similarities (96–98%) with two published partial genomic sequences of BiMoV (GenBank accession numbers EF467235 and EU078960), as has also been reported by Baker et al. [2] and Youssef et al. [10], and an unpublished partial sequence representing the 3′-terminal 1,800 nts of BiMoV isolate Kunming (GenBank accession no. EU925833). The second closest relatives of SCSV-SF-1 are vanilla distortion mosaic virus isolate VMM1 (AY943945), Amaranthus leaf mottle virus (AJ580095), Altroemeria mosaic virus (AB158522), Bidens mosaic virus isolate BiMV-p (AY960150), and pepper mottle virus (AF227728), with local nucleotide sequence similarities ranging from 78% to 84%. Viruses of the other known potyvirus species shared lower than 75% sequence similarities with SCSV-SF-1.

The conserved and consensus amino acid sequences for both NIb and CP coding regions were analyzed further with those of the other potyviruses [4, 5, 7]. The residues glutamine (Q_{82}) and alanine (A_{83}), constituting the putative cleavage site between the NIb and the CP proteins, are common within the family *Potyviridae* and are similar to PVY-NsNr (X68224) as well as its isolates. The expected DAG motif related to aphid transmission was found in the N-terminal region of CP, in accordance with the fact that this virus is aphid-transmissible. Consensus sequence motifs of potyviruses, MIE, WGY, LEQ, GNAPY and ALR, were found in the putative NIb protein sequence at 1, 5, 22, 35, and 43 amino acids the upstream of the cleavage site (Q_{82}/A_{83}) of the putative NIb and CP. The predicted amino acid sequences of CP showed the following consensus motifs of potyviruses: DAG, MNGLMVW, CIENGTSP, ING and RQIMAHFSD at 8, 114, 120, 129, and 158 amino acids downstream of the Q_{82}/A_{83} cleavage site. Thus, the complete amino acid sequences of NIb and CP support that the virus is a member of the potyviruses.

However, it has been shown that SCSV-SF-1 does not infect *Bidens pilosa* or *Zinnia elegans* [6], the diagnostic host species for BiMoV [8]. In fact, SCSV-SF-1 has a very narrow host range, infecting only *Helianthus annuus*, *Nicotiana benthamiana*, *Chenopodium quinoa*, and *C. amaranticolor* among the 26 plant species tested [6], whereas BiMoV has a much broader host range, spanning several plant families [2, 8]. The significant difference in this biological property distinguishes SCSV-SF-1 from other BiMoV isolates. Therefore, it is concluded that SCSV-SF-1 previously reported in Taiwan is a distinct isolate of BiMoV that has a very narrow host range. This is

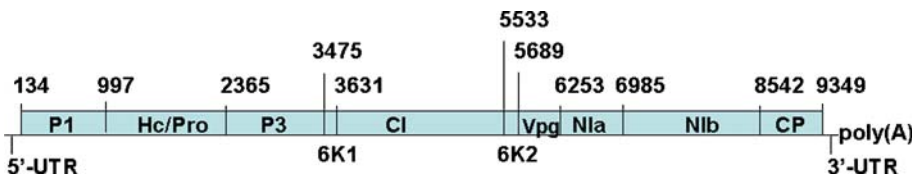

Fig. 1 Annotated diagram of the complete genome of the BiMoV (SCSV) SF1 isolate. The genome of BiMoV contains a single open reading frame (boxed area), flanked by a 5′ untranslated region (5′-UTR) and a 3′-UTR followed by a polyadenylated tail [poly(A)]. The predicted proteolytic cleavage sites (nucleotide positions) are indicated at the top of the diagram. The relative position of each functional protein, denoted as P1, helper component (HC), P3, 6K1, cylindrical inclusion (CI), 6K2, viral protein genome-linked (Vpg), nuclear inclusion A (NIa), nuclear inclusion B (NIb), and capsid protein (CP), is indicated

the first report of the full-length nucleotide sequence of BiMoV infecting sunflower in Taiwan.

References

1. Adams MJ, Antoniw JF, Fauquet CM (2005) Molecular criteria for genus and species discrimination within the family *Potyviridae*. Arch Virol 150:459–479
2. Baker CA, Kamenova I, Raid R, Adkins S (2007) Bidens mottle virus identified in tropical soda apple in Florida. Plant Dis 91:905
3. Berger PH, Adams MJ, Brunt AA, Hammond J, Hill JH, Jordan RL, Kashiwazalci S, Rybicki E, Spence N, Stenger DC (2005) Family *Potyviridae*. Taxonomy of viruses. In: Fauquet CM, Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds) The international committee on the taxonomy of viruses, 8th Report. Elsevier, London, pp 819–841
4. Dujovny G, Usugi T, Shohara K, Lenardon SL (1998) Characterization of a potyvirus infecting sunflower in Argentina. Plant Dis 82:470–474
5. Gibbs AJ, Ohshima K, Phillips MJ, Gibbs MJ (2008) The prehistory of potyviruses: their initial radiation was during the dawn of agriculture. PLoS ONE 3(6):e2523. doi:10.1371/journal.pone.0002523
6. Liao JY, Chang CA, Chen CC, Deng TC (2001) Isolation and identification of a virus causing sunflower chlorotic spots in Taiwan. Plant Path Bull 10:173–180
7. Ohshima K, Tomitaka Y, Wood JT, Minematsu Y, Kajiyama H, Tomimura K, Gibbs AJ (2007) Patterns of recombination in Turnip mosaic virus genomic sequence indicate hotspots of recombination. J Gen Virol 88:298–315
8. Purcifull DE, Christie SR, Zitter TA (1976) *Bidens mottle virus*. In: Jones AT, Robinson DJ, Boonham N, Mumford R (eds) CMI/AAB description of plant viruses, vol 161. Association of Applied Biologists, Wellesbourne, UK, http://www.dpvweb.net/dpv/showdpv.php?dpvno=161
9. Ward CW, McKern NM, Frenkel MJ, Sbukla DD (1992) Sequence data as the major criterion for potyvirus classification. In: Barnett OW (ed) Potyvirus taxonomy. Springer, New York, pp 283–297 (Arch Virol [Supp] 5)
10. Youssef F, Marais A, Canresse T (2008) Partial genome sequence of Bidens mottle virus sheds light on its taxonomy. Arch Virol 153:227–228