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Biological and molecular characterisation of Bidens mosaic virus supports its assignment as a member of a distinct species in the genus *Potyvirus*

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Abstract The complete nucleotide (nt) sequence of Bidens mosaic virus (BiMV) isolate SP01 was determined and shown to consist of 9,557 nt. Since it shared highest identities in the nt sequence of the whole genome (66–73 %) and in the aa sequence of the polyprotein (60–76 %) with viruses of the potato virus Y subgroup, it was compared with them genetically and biologically. Phylogenetic analysis showed that the closest relative of BiMV is sunflower chlorotic mottle virus, from which it, however, differed significantly in various respects. These results indicate that BiMV should represent a distinct species in the genus *Potyvirus*.

Bidens mosaic virus (BiMV) was described in 1961 [1] as a virus causing mosaic symptoms in *Bidens pilosa* L. and belonging to the genus *Potyvirus.* This virus is apparently restricted to Brazil and was reported to naturally infect *B. pilosa*, *Helianthus annuus* [2], *Coreopsis lanceolata* [3], *Pisum sativum* [4], *Lactuca sativa* [5], and *Galingsoga parviflora* [6]. Although BiMV was initially considered a separate potyvirus [1–5], it is currently classified as a strain of potato virus Y (PVY) [7] based on its coat protein (CP) gene sequence [8]. In order to clarify the taxonomic status of BiMV, we sequenced the complete genome of an isolate of the virus.

The isolate SP01 of BiMV was collected from infected *B. pilosa* plants found in Botucatu, São Paulo State, Brazil, in 2008. It was then sap transmitted in a host range experiment. Total RNA was extracted from infected *Chenopodium quinoa* leaves using a Total RNA Purification Kit (Norgen Biotek). The cDNA was prepared with a poly(T) primer and with the primers CIrev and HPrev [9], using AMV reverse transcriptase (Promega) according to the manufacturer's instructions. The CI and HC-Pro genes were amplified using previously described universal primers for members of the family *Potyviridae* [9]. The CP and nuclear B inclusion (NIb) genes were amplified using the universal primer WCIEN [10] with the primer 7587 [11]. The gaps between these regions were amplified using specific primers designed from the previously obtained sequences. Polymerase chain reactions (PCRs) were performed using Platinum high-fidelity Taq DNA polymerase (Invitrogen). The 5'-proximal region of the genomic sequence was determined using the 5' Rapid Amplification of cDNA Ends (RACE) (Clontech) following the manufacturer's instructions. The amplified fragments were cloned using the pGEM-T Easy Vector system (Promega). For each fragment, three independent clones were sequenced in both directions at Macrogen Inc. (Republic of Korea). A consensus sequence was assembled using DNAMAN 4.0 (Lynnon Corporation), and the final genome sequence was deposited in the DDBJ/EMBL/GenBank database under the accession number KF649336. Comparisons were made between the BiMV-SP01 sequence and the available complete sequence of the most closely related potyviruses. Also, the CP gene sequences of two BiMV isolates available from GenBank were compared to BiMV-SP01. The sequences were aligned using ClustalW [12]

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with default parameters. Maximum-likelihood phylogenetic trees were prepared using MEGA 5.0 [13] with 1,000 bootstrap replicates. Recombination analysis was performed with the programs GENECONV, RDP, Bootscan, MaxChi, Chimaera, 3Seq, and SiScan packaged in the software RDP3 [14] using default settings.

The host range data for BiMV-SP01 were consistent with those reported for other BiMV isolates that cause local symptoms in *Chenopodium amaranticolor* and *C. quinoa* and systemic infection in *Helianthus annus* "Catissol 1", *Lactuca sativa* "Trocadero", *Nicotiana benthamiana*, *N. clevelandii*, *N. occidentalis*, *N. rustica*, *N. tabacum, Pisum sativum* and *Zinnia elegans* [2–4, 15]. BiMV-SP01 also systemically infected *Gomphrena globosa* (symptomless) and *Cucurbita pepo*, which had not previously been reported as BiMV hosts. The virus did not infect *Amaranthus viridis*, *Capsicum annum* "Magda", *Chenopodium murale, Datura metel, D. stramonium, Solanum lycopersicum* "Santa Clara", *Nicandra physaloides, Nicotiana glutinosa, Petunia hybrida, Physalis floridana* and *Sonchus oleraceus.* From symptomatic and symptomless plants, total RNA was extracted as described previously, and the presence of BiMV was verified by RT-PCR [6].

The complete genome of BiMV-SP01 consisted of 9,557 nucleotides, excluding the 3' terminal poly(A) tail. The AUG codon located at nt position 118–121 is likely to be the translation initiation codon, since it was in a context (CAAAUGGCA) that is similar to that observed in other potyviruses [9]. The 5' non-coding region (NCR) was 117 nt in length, AU-rich, and terminated in several A residues. The 3' NCR was 256 nt in length and AU-rich. The stop codon (UGA) was at position 9,301–9,303. The putative translation product contained 3,066 amino acids (aa) long, with a calculated molecular mass of 348.2 kDa (http://web.expasy.org/cgi-bin/protparam/protparam). According to the conserved cleavage sites predicted by Adams et al. [16], a total of 10 proteins are expected: P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa, NIb, and CP. The observed cleavage sites of BiMV-SP01 were perfectly consistent with the known sites of potyviruses (Supplementary Fig. 1). The putative open reading frame (ORF—PIPO) with the $G_{1\text{-}2}A_{6\text{-}7}$ motif [17] was found at nucleotides 2847 to 3074 and contained 76 aa. The calculated mass of PIPO was 9.1 kDa.

When the complete BiMV-SP01 genome and predicted polyprotein sequences were compared with other sequences of the most closely related potyviruses, it shared the highest nt (73 %) and aa sequence identities (76 %) with the C and CRS isolates of sunflower chlorotic mottle virus (SCMoV) (Supplementary Table 1). Nucleotide sequence identities of 69–71 % were obtained for the whole genome when compared with the sequences of PVY isolates. These identities were below the threshold value that discriminates between potyvirus species and strains according to the ninth ICTV report [7]. BiMV-SP01 shared identities of 96 and 98 % in the CP nt and aa sequences, respectively, with the BiMV-p and BiMV-b isolates (accession nos. AY960150 and AY960151).

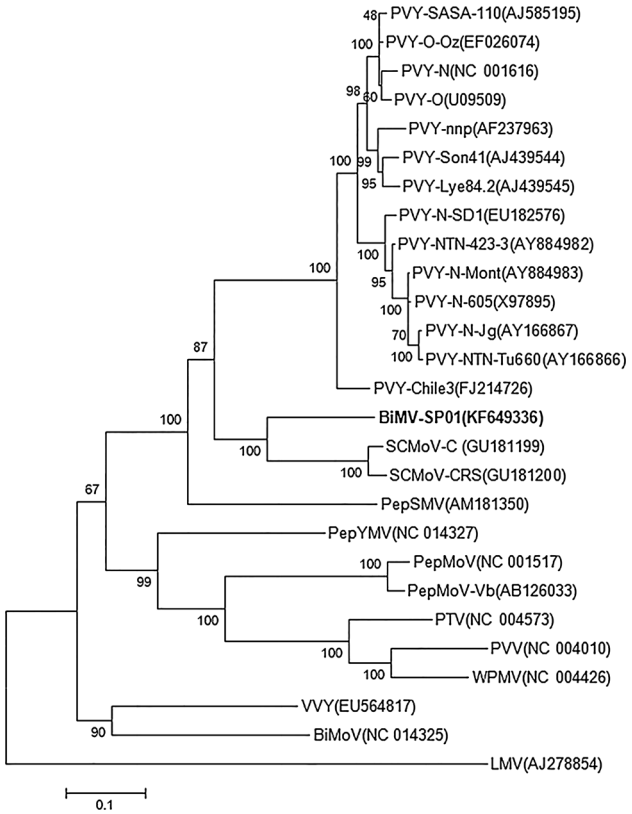

Fig. 1 Maximum-likelihood tree constructed from the entire polyprotein amino acid sequences of Bidens mosaic virus (BiMV) and the most closely related potyviruses: potato virus Y (PVY), sunflower chlorotic mottle virus (SCMoV), pepper severe mosaic virus (PepSMV), pepper yellow mosaic virus (PepYMV), pepper mottle virus (PepMoV), Peru tomato mosaic virus (PTV), potato virus V (PVV), verbena virus Y (VVY), wild potato mosaic virus (WPMV) and Bidens mottle virus (BiMoV). Lettuce mosaic virus (LMV) was used as an outgroup sequence. The values at the forks indicate the percentages of 1,000 bootstrap replicates. The corresponding GenBank accession numbers of each virus sequence are given in parentheses. The BiMV isolate is indicated in bold type

The phylogenetic tree based on multiple alignments of the deduced aa sequence of the entire polyprotein (Fig. 1) shows BiMV-SP01 in a separate branch together with the two SCMoV isolates. They formed a separate cluster with isolates of potato virus Y (PVY) and pepper severe mosaic virus (PepSMV), with a bootstrap value of 100 supporting the existence of an ancestral common origin to the PVY, PepSMV, BiMV, and SCMoV isolates. The Ninth Report of the International Committee on Taxonomy of Viruses (ICTV) lists BiMV as a strain of PVY and SCMoV as a member of a tentative species in the genus *Potyvirus* [7]; the latter was proposed after the whole genomes of two

isolates of SCMoV were sequenced [18]. The recombination analysis of the BiMV-SP01 sequence did not find any clear signal of a recombination event when aligned to the sequences of viruses belonging to the PVY subgroup.

Historically, the taxonomic position of BiMV has been questioned. Symptom similarities with another potyvirus, such as Bidens mottle virus (BiMoV), which was found infecting lettuce and *B. pilosa* in the USA [19], and with SCMoV, which was found infecting sunflower and *Z. elegans* in Argentina and Brazil [20, 21], led to proposals that they belong to the same species. BiMoV and BiMV infect at least 10 common hosts, and a serological relationship exists between BiMV and SCMoV [21]. However, when their entire genomes were compared, the nt sequence identity between BiMV and BiMoV was 67%. Despite the fact that cleavage sites for HC-Pro/P3, 6K1/CI, 6K2/VPg, Vpg/NIa, and NIa/NIb are the same in SCMoV and BiMV, the biological differences in experimental host range [20, 21] and the ability of BiMV to naturally infect pea and lettuce [4, 5], including the emergence of a strain with distinct biological characteristics that causes severe symptoms in the latter host, support the inclusion of BiMV in a distinct species in the genus.

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Conflict of interest The authors declare that they have no conflict of interest.

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