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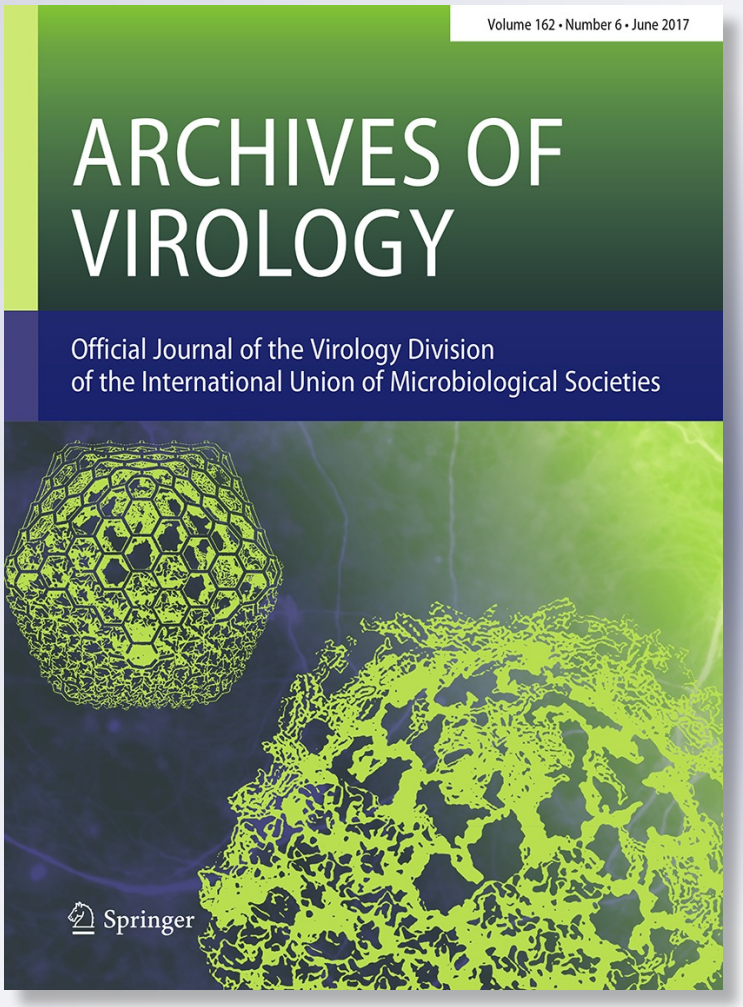

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ANNOTATED SEQUENCE RECORD

Complete genome sequence of sunflower ring blotch virus, a new potyvirus infecting sunflower in Argentina

Dariel Cabrera Mederos[1,2] · Nicolás Bejerman[1,2] · Verónica Trucco[1] · Soledad de Breuil[1,2] · Sergio Lenardon[1] · Fabián Giolitti[1]

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Abstract The complete genome sequence of sunflower ring blotch virus (SuRBV), a previously undescribed potyvirus infecting sunflower in Argentina, is reported. The SuRBV genome comprises 9555 nucleotides (nt) and encodes a polyprotein of 3061 amino acids, flanked by 5′ and 3′ untranslated regions of 117 and 255 nt, respectively. Phylogenetic analysis showed that SuRBV belongs to the potato virus Y (PVY) subgroup and clusters together with sunflower chlorotic mottle virus and bidens mosaic virus. Percentage nucleotide identity between the whole genomes of SuRBV and BiMV was 70.6%, suggesting SuRBV should be considered a distinct species in the genus *Potyvirus*.

Sunflower (*Helianthus annuus* L.) is the second most widely cultivated oilseed crop in Argentina, with more than 1,200,000 hectares planted annually [7]. Two potyviruses have been reported and characterized infecting sunflower in Argentina: sunflower chlorotic mottle virus (SuCMoV) [6], which belongs to the potato virus Y (PVY) subgroup [4], and sunflower mild mosaic virus (SuMMoV) [8]. In this study, the complete genome sequence of a new and distinct potyvirus species infecting sunflower plants in Argentina was determined.

In 2012, ring blotch symptoms (Supplementary Figure 1) were observed in sunflower fields located in the northeast region of Argentina. Potyvirus-like particles were observed under electron microscopy in a crude sap preparation taken from infected plants (data not shown). Sunflower leaf extracts from symptomatic plants were mechanically inoculated on to healthy sunflower seedlings, which then showed similar symptoms to those observed in the field samples. The presence of a potyvirus in symptomatic plants was tested and confirmed by enzyme-linked immunosorbent assay (ELISA), using potyvirus group antiserum (Agdia, Elkhart, IN). However, none of the symptomatic plants tested positive when analysed by ELISA using specific antiserum to SuCMoV or SuMMoV (data not shown).

A pool of leaves from five symptomatic sunflower plants inoculated with sap from field-infected plants was used for total RNA extraction using Trizol reagent (Life Technologies, USA), according to the manufacturer's instructions. Total RNA (5 µg) was sent to INDEAR (Genomics and Bioinformatics Platform, INDEAR Inc., Rosario, Argentina) for cDNA synthesis from polyadenylated RNA, followed by deep sequencing using Illumina HiSeq 1500.

The reads of the full-length genome were *de novo* assembled using the Mira v 4 assembling method with a coverage of 14,984 reads/nt. Maximum-likelihood (ML) phylogenetic trees were obtained by using PhyML software v3.0 (Guindon and Gascuel 2003) [9]. A ML bootstrap analysis (1000 replicates) was used to evaluate the robustness of the phylogenetic grouping. Recombination

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✉ Dariel Cabrera Mederos
cabrera.dariel@inta.gob.ar

✉ Fabián Giolitti
giolitti.fabian@inta.gob.ar

[1] Instituto de Patología Vegetal (IPAVE), Centro de Investigaciones Agropecuarias (CIAP), Instituto Nacional de Tecnología Agropecuaria (INTA), Av. 11 de Septiembre 4755, X5020ICA Córdoba, Argentina

[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290, C1425FBQ Buenos Aires, Argentina

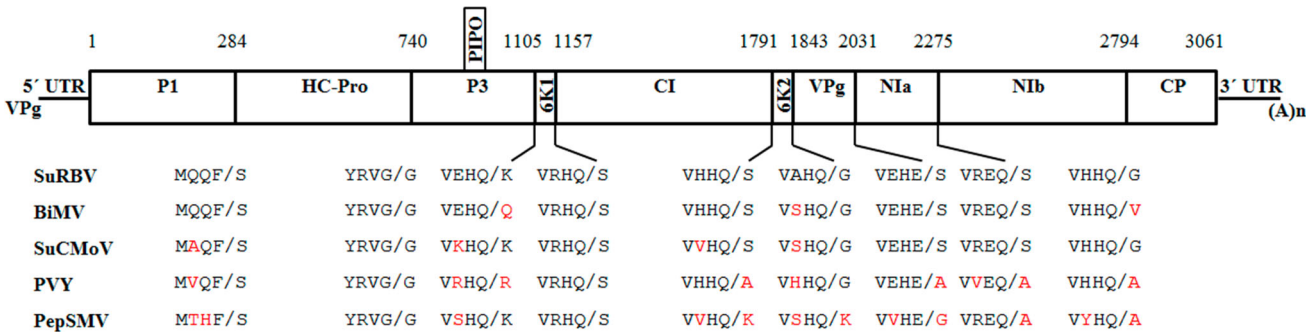

Fig. 1 Schematic representation of the genome organization of SuRBV. Untranslated regions (UTR) are represented by lines, and the large open reading frame (ORF) is depicted by an open box. The putative cleavage sites in the polyprotein are indicated below the genome, as well as the cleavage sites of other closely related viruses (see Fig. 2 legend for virus acronyms). Those amino acids which differ from the SuRBV cleavage sites are marked in *red*. The numbers indicate the predicted amino acid position for each mature protein in the polyprotein. The putative protein PIPO is indicated by the small box above the P3 protein (PIPO-encoding ORF not shown) (color figure online)

analysis was performed using the programs GENECONV, RDP, Bootscan, MaxChi, Chimaera, 3Seq, and SiScan packaged in the software RDP4 [11] using default settings.

The complete genome sequence of sunflower ring blotch virus (SuRBV) was found to consist of 9555 nucleotides (nt), excluding the 3′ poly(A) tail, with a 5′ UTR of 117 nt and a 3′ UTR of 255 nt (GenBank accession number KX856009). The major putative ORF starts at nt 118 and ends at nt 9300, encoding a large polyprotein of 3061 amino acid (aa) residues (Fig. 1). The putative cleavage sites in this polyprotein, targets for the viral-encoded proteinases, were identified according to the conserved cleavage sites predicted by Adams et al. [1] and bioinformatically yielded all 10 of the characteristic potyviral proteins with estimated sizes of 284, 456, 365, 52, 634, 52, 188, 244, 519 and 267 aa (for P1, HC-Pro, P3, 6 K1, CI, 6 K2, VPg, NIa, NIb and CP, respectively). Several conserved potyvirus motifs were identified in the SuRBV polyprotein, i.e. ^{256}FIVRG^{260} was present in P1, ^{463}FRNK^{466} and ^{592}PTK^{594} motifs were found in HC-Pro [10, 14], and ^{1245}GSGKSX$_3$P^{1253}, ^{1331}DECH^{1334}, ^{1360}SATPP^{1364}, ^{1460}VATNIIENGVTL^{1471} and ^{1508}QRL GRVGR^{1515} were also found in the CI protein. Other motifs were detected in the NIb and CP proteins: ^{2626}GDD^{2628} in NIb, and ^{2800}DAG^{2802} and ^{2912}WCIEN^{2916} in CP [15]. A putative protein PIPO (81 aa) with the conserved consensus nucleotide sequence GAAAAAAA [5] was also found within the SuRBV P3 gene between nucleotides 2847 and 3089.

A BlastP search of the complete, deduced sequence of the polyprotein encoded by SuRBV revealed that it shared the highest amino acid identities with polyproteins encoded by members of the PVY subgroup of the genus *Potyvirus*. Comparison of the complete genome sequence of SuRBV with other select PVY subgroup members showed a nucleotide identity ranging from 62.3% to 70.6% (Supplementary Table 1), which is below the threshold value used to discriminate between species and strains (76%) within the genus *Potyvirus* [3]. However, comparison of the SuRBV CP coding region with those of bidens mosaic virus (BiMV), SuCMoV, PVY and pepper severe mosaic virus (PepSMV) showed that the deduced amino acid sequence identities were above the species demarcation threshold of 80%. The amino acid sequences of the remaining coding regions of SuRBV were unambiguously below the threshold for species demarcation estimated by Adams et al. [2], with the exception of the VPg coding region (Supplementary Table 1). These results suggest that the complete genome sequences, rather than the CP coding region, should be used as a criterion for species demarcation of closely related potyviruses, as was suggested by Adams et al. [2] and Romay et al. [12]. Furthermore, analysis of the polyprotein cleavage sites showed differences between SuRBV and closely related viruses (Fig. 1), which is another criteria established by the ICTV [3] to demarcate species between distinct potyviruses.

The phylogenetic relationship between SuRBV and the other potyviruses is illustrated in a phylogenetic tree based on multiple alignments of the deduced amino acid sequence of the entire polyprotein (Fig. 2), which shows that SuRBV clusters together with BiMV and SuCMoV within the PVY subgroup. These results provide clear evidence of species demarcation, consistent with the pairwise sequence comparisons shown in Supplementary Table 1, and suggest that SuRBV, BiMV and SuCMoV could have a common ancestral origin that also includes PVY and PepSMV. No evidence of recombination was detected between SuRBV and other members of the PVY subgroup.

The data obtained in this study suggest that this recently discovered sunflower-infecting virus represents a distinct species in the genus *Potyvirus*, which we have tentatively named "*Sunflower ring blotch virus*". Furthermore this new potyvirus is closely related to the PVY subgroup

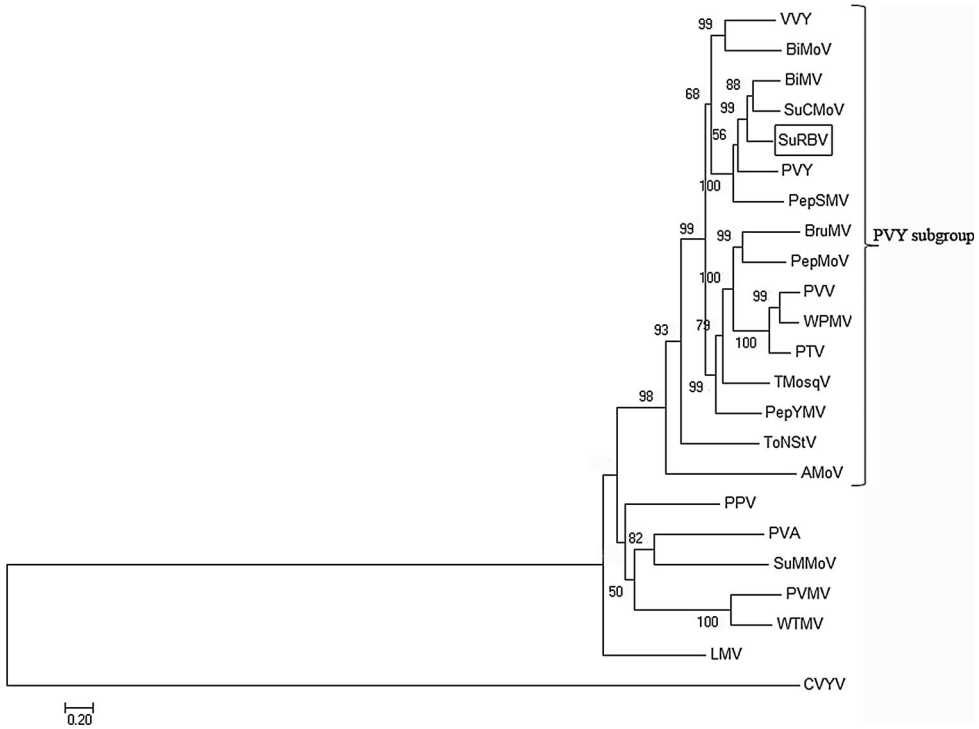

Fig. 2 Phylogenetic tree based on the alignment of the deduced amino acid sequences of the polyprotein of SuRBV and representative members of the genus *Potyvirus*. A maximum-likelihood phylogenetic tree was obtained using the PhyML software (LG+I+G+F model). The values on the branches show percentage support (out of 1000 bootstrap replications); only bootstrap values (%) above 50 are shown. The scale bar indicates the number of substitutions per site. The viruses used to construct the tree and their accession numbers are: arracacha mottle virus (AMoV; DQ925486), bidens mosaic virus (BiMV; KF649336), bidens mottle virus (BiMoV; AF538686), brugmansia mosaic virus (BruMV; JX874139), lettuce mosaic virus (LMV; AJ306288), pepper mottle virus (PepMoV; EU586128), pepper severe mosaic virus (PepSMV; AM181350), pepper veinal mottle virus (PVMV; KR002568), pepper yellow mosaic virus (PepYMV; AB541985), plum pox virus (PPV; AM157175), peru tomato virus (PTV; AJ516010), potato virus A (PVA; KF977085), potato virus V (PVV; KT985459), potato virus Y (PVY, KR528584), sunflower chlorotic mottle virus (SuCMoV; GU181199), sunflower mild mosaic virus (SuMMoV; JQ350738), tobacco mosqueado virus (TMosqV; KT834407), tomato necrotic stunt virus (ToNStV; JX846918), vervena virus Y (VVY; EU564817), wild potato mosaic virus (WPMV; AJ437279), wild tomato mosaic virus (WTMV; KM401435). The polyprotein encoded by cucumber vein yellowing virus (CVYV; AY578085) was used as outgroup

members SuCMoV and BiMV, which infect *Asteraceae* and were isolated in South America [4, 13].

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Compliance with ethical standards

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

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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