Complete nucleotide sequence and genetic organization of Sunflower mild mosaic virus (SuMMoV)

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ABSTRACT

- Two potyviruses that infect sunflower (*Helianthus annuus* L.) crops have been reported in Argentina. Sunflower chlorotic mottle virus (SuCMoV) is the most widely distributed one, with two strains: common and ring spot, which have been completely characterized. Sunflower mild mosaic virus (SuMMoV) that produces a chlorotic mild mosaic on sunflower commercial crops was detected in the rural area of Paraná (Entre Ríos Province). This virus has been biologically and serologically characterized but not fully characterized at the molecular level. The aim of this work was to determine the complete sequence of SuMMoV and study its genomic organization.
- A total RNA extraction was made from a preparation enriched with this virus. The quality of preparation was confirmed through electron microscopy and RT-PCR with specific primers for capsid protein gene. Total RNA obtained was pyrosequenced and the sequences obtained were analyzed.
- Electron microscopy from rapid preparations of samples enriched with the virus showed high concentration of potyvirus-like particles. RT-PCR showed an aplicon of expected size. The pyrosequencing resulted in a large contig of 9686 nucleotides (nt) with a single open reading frame (ORF) of 3077 amino acids (aa). The ORF showed the highest levels of identity with *Tobacco etch virus* (TEV-L38714), 55.0% for nt and 54.8% for aa. The deduced genome organization is typical for a member of the *Potyviridae* family, and 10 common proteins mentioned for *Potyvirus* were identified: P1 (300 aa), HC-Pro (460 aa), P3 (349 aa), 6K1 (52 aa), CI (634 aa), 6K2 (53 aa), NIa-VPg (188 aa), NIa-Pro (241 aa), Nib (513 aa) and CP (287 aa). Nine putative protease cleavage sites were predicted computationally and by analogy with genome arrangements of other potyviruses.
- These results confirm that the SuMMoV is a member of *Potyvirus* genus, *Potyviridae* family that infects sunflower in Argentina and this is the first report of a full sequence of its genome.
- This study will contribute to understand its biology, diversity, evolution and begin molecular studies to obtain tolerance/resistance to disease.

Key words: mild mosaic – complete genome – pyrosequencing – potyvirus

INTRODUCTION

Plant virus diseases may cause economic losses by reducing yield and quality of plant products (Agrios 2005) and *Potyvirus* genus is by far the largest genus of plant viruses (Adams et al. 2005; Berger et al. 2005). In Argentina two potyviruses that infect sunflower (*Helianthus annuus* L.) crops have been reported (Lenardon 1994). One of them, Sunflower chlorotic mottle virus (SuCMoV), that causes chlorotic mottling symptoms, plant stunting and yield losses in sunflower (Dujovny et al. 1998; Dujovny et al. 2000; Lenardon et al. 2001) have been completely characterized. This one is the sunflower virus more prevalente in Argentina, since it has been detected in several provinces infecting cultivated and wild sunflowers (Lenardon et al. 2001) and weeds such as *Dipsacus fullonum* (Giolitti et al. 2009), *Helianthus petiolaris* (Dujovny et al. 1998) and *Ibicela lutea* (Bejerman et al. 2011). Furthermore, two biologically different strains of SuCMoV have been described in Argentina: the common (C) and the chlorotic ringspot (CRS) strains (Giolitti et al. 2010b) and a third strain was isolated in Brazil from *Zinnia elegans* (Maritan et al. 2004). The second sunflower potyvirus described in Argentina is the Sunflower mild mosaic virus (SuMMoV) that produces a chlorotic mild mosaic symptom (Fig. 1) on sunflower commercial crops and was detected in the rural area of Paraná (Entre Ríos Province) (Lenardon 1994). This virus is capable of infect a relatively wide host range and is transmitted in a non persistent manner for the aphid *Myzus persicae*, but is not transmited by seed or dodder (Giolitti et al. 2007). Its virion is a flexuous filament of 710 nm in length and has a single strand RNA of approximately 9,000 nt. The capsid protein sequence of SuMMoV has the highest percentages of identities with the *Tobacco etch virus* (TEV) with 63.9% for nucleotides and 69.2% for deduced amino acids (Giolitti et al. 2010a). The aim of this work was to determine the complete sequence of SuMMoV and study its genomic organization. Here, we present the first complete genome sequence of an isolate of SuMMoV, acquired using a novel non-directed next-generation sequencing approach and its relationship to other potyviruses.

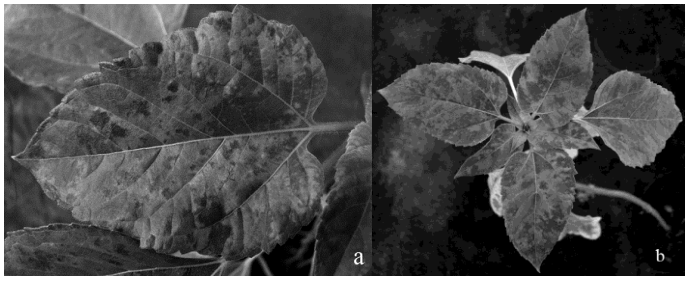

Figure 1: leaf (a) and plant (b) of sunflower showing mild mosaic symptoms.

MATERIALS AND METHODS

Plant sample: Original sunflower samples were collected from fields near Paraná city. Virus was maintained under greenhouse conditions by mechanical inoculation on sunflower and *Nicotiana occidentalis* plants. Infected leaves of *N. occidentalis* were collected 30 days after inoculation and stored at -70°C until its use.

Viral enrichment: To maximize the possibility of having high concentration of viral RNA proceeded to enrich a sample with virus particles. Twenty grams of infected plant tissue were ground 1/5 (w/v) in grinding buffer (0,1M $Na_3C_6H_5O_7$ pH 7) containing 0.1% thioglycolic acid and filtered through nylon gauze. Then was emulsified with 25% chloroform and clarified by centrifugation (11000xg for 15 min at 4ºC in a Beckman JA-14 rotor; Fullerton, CA, USA). The clarified was filtering through cotton and centrifuged (186000xg for 1 h at 4ºC in a Beckman 45Ti rotor) with 10 ml of 40% sucrose cushions. The precipitates obtained were suspended in 500 µl grinding buffer. The quality of the enrichment was checked through transmission electron microscopy (TEM) and RT-PCR. Rapid preparations (dips) for TEM were made floating formvar-coated grids on a drop of virus enriched preparation, washed with water and contrasted with 2% uranyl acetate solution (Kitajima 1997; Hari and Das 1998), then, the dips were inspected using a Jeol JEM EXII (Jeol, Tokio, Japan). The one step RT-PCR was performed using the "Access RT-PCR System" (Promega, Madison, WI, USA) according to the manufacturer's instructions, one pair of SuMMoV specific capsid protein (CP) primers (5' AGACTTGAAGCAATTTGTGC 3' and 5' AGGTCAAACCTCACTGC 3') and two microliters of virus enriched preparation, previously denatured for 5 min at 95ºC. One-step RT-PCR conditions were: 45 min to 48ºC; denaturation of 4 min at 94ºC; 40 cycles with denaturation of 30 seg at 94ºC, hybridization of 1.30 min at 53ºC and extension of 1 min at 68ºC; and a final extension of 7 min at 68ºC. RT-PCR products were analyzed by electrophoresis in 1.4% agarose gels in TAE buffer stained with ethidium bromide

Sequencing: Total RNA was extracted from 200 µl of virus enriched preparation using a RNeasy Plant Mini kit (Qiagen, California, USA), according to manufacturer's recommendations. RNA was quantified in a NanoDrop Spectrophotometer ND-1000 (NanoDrop Technologies, Inc., Wilmington, DE, USA) and 200 µl (20 ng/µl) suspended in water were submitted to INDEAR (Rosario, Santa Fe, Argentina) for its pyrosequencing using 454 Genome Sequencer FLX Titanium System (Roche, Brandford, CT, USA). The step for the sample enrichment with RNA molecules with poly-A ends of the sequencing protocol was removal to avoid the loss of non-polyadenylated RNA viruses (Ahn et al. 2006). Contigs were assembled de novo from the dataset using Newbler v 2.6 software (Roche), edited manually and were subjected to both BLASTN and BLASTX analysis (NCBI 2011). Sequence and phylogenetic analysis were performed using the Lasergene 8.0.2 software package (DNASTAR, Inc., Madison, WI, USA). Complete genomic sequences of 28 related potyviruses, by protein BLAST (NCBI 2011) analysis of CP gene and the complete polyproteins sequences, including two isolates of SuCMoV (C and CRS), used for comparative analyses were retrieved from the GenBank. Cleavage sites of each protein were identified by sequence comparison to those of known potyviruses. Multiple sequences alignments produced by Clustal W for the full length nt sequence and the complete polyprotein aa sequence were used as input data for reconstructing phylogenetic trees by the Neighbor-Joining method. Statistical significance was estimated by performing 1000 replications of boopstrap resampling of the original alignment. Sequences used in comparisons with the SuMMoV were: *Bean common mosaic virus* (BCMV) AJ312437; *Beet mosaic virus* (BtMV) AY206394; *Bidens mottle virus* (BiMoV-SCSV) NC_014325; *Chilli veinal mottle virus* (ChiVMV) AM909717; Hardenbergia mosaic virus (HarMV) HQ161080; *Japanese yam mosaic virus* (JYMV) AB027007; *Johnsongrass mosaic virus* (JGMV) NC_003606; *Leek yellow stripe virus* (LYSV) NC_004011; *Lettuce mosaic virus* (LMV) NC_003605; *Lily mottle virus* (LiMV) AM048875; *Maize dwarf mosaic virus* (MDMV) NC_003377; *Narcissus yellow stripe virus* (NYSV) NC_011541; *Papaya ringspot virus* (PRSV) NC_001785; Pennisetum mosaic virus (PenMV) DQ977725; *Pepper severe mosaic virus* (PepSMV) AM181350; *Pepper veinal mottle virus* (PVMV) FJ617225; *Plum pox virus* (PPV) AY912055; *Potato virus A* (PVA) AJ131403; *Potato virus Y* (PVY) AY884984; *Sorghum mosaic virus* (SrMV) U57358; *Soybean mosaic virus* (SMV) NC_002634; *Sugarcane mosaic virus* (SCMV) JN021933; Sunflower chlorotic mottle virus (SuCMoV), isolate "C" (NC_014038) and "CRS" (GU181200); *Tobacco etch virus* (TEV) L38714; *Tobacco vein mottling virus* (TVMV) NC_001768; *Turnip mosaic virus* (TuMV) AB252127 and *Wisteria vein mosaic virus* (WVMV) NC_007216.

RESULTS

Viral enrichment: Dips of the viral enrichment checked in a TEM showed a high concentration of elongated and flexuous viral particles. RT-PCR of the RNA extracted from the viral enrichment showed a single band of the expected size for de CP gene of SuMMoV (data not shown).

Sequencing: A dataset of 54,506 sequences were obtained. De novo assembly yielded 655 contigs including the 90.92% of readings, BLAST analysis indicated that 12 of these contigs were highly related to *Potyvirus* and the longest was approximately 9,800 pb in length. The analysis of this fragment revealed a sequence of 9,718 nucleotides (nt) in length with a open reading frame 1 (ORF1) consisting of 9,411 nt, with an ATG start codon and a TAA stop codon, located at positions 161-163 and 9,572-9,574, respectively. The 5´ untranslated region (UTR) was of 160 nt and the 3´ UTR 147 nt, followed by a poly-A tail. The base composition of the viral genomic RNA was adenine 32.95%, cytosine 19.91%, guanine 22.77%, and uracil 24.37%. ORF1 encoded a polyprotein of 3,137 amino acids (aa) and its comparison with that of other known potyviruses revealed 9 putative proteinase cleavage sites that gives rise to 11 proteins: P1 (360 aa), HC-Pro (460 aa), P3 (349 aa), 6K1 (52 aa), CI (634 aa), 6K2 (53 aa), VPg (188 aa), NIa (241 aa), NIb (513 aa), CP (287 aa) and PIPO protein (ORF2) (Chung et al. 2008) embedded within the P3 cistron encoded a protein of 75 aa) (Fig. 2). Expected potyvirus motifs of FRNK in the HC-Pro, GDD in the NIb, and NAG in the CP (instead of DAG) are conserved.

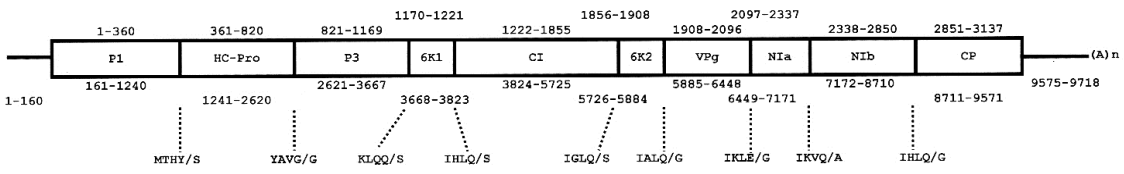

Figure 2: Scheme of genome structure and predicted cleavage sites of a polyprotein of SuMMoV

Sequence identities between SuMMoV and the analyzed potyviruses were between 49.9-59.0% at the nt level for whole genome and 42.0-54.8% at the aa level for ORF1. Sequence identity at the nt level for 5´ and 3´ NCRs between those viruses and SuMMoV were found to be 27.5-51.2% and 21.4-54.8%, respectively. Furthermore, at the nt and aa level for coding regions ranged from 31.2-39.3% and 10.6-22.6% in P1, 48.7-58.2% and 40.0-56.2% in HC-Pro, 37.3-44.7% and 22.1-30.1% in P3, 44.7-63.8% and 28.8-59.6% in 6K1, 53.6-62.1% and 48.5-64.1% in CI, 40.5-57.7% and 24.5-44.2% in 6K2, 50.5-61.4% and 43.2-60.6% in VPg, 36.1-55.5% and 11.6-55.6% in NIa, 59.0-65.9% and 58.0-68.9% in NIb and 57.8-63.6% and 54.3-67.3% in CP, respectively (Table 1). For PIPO protein the identities were 40.4 and 45.2% for nt and 15.3 and 20.3% for aa when SuMMoV was compared with HarMV and SMV, respectively.

Table 1: Comparison of percentage of similarities of nucleotides sequences of whole genome (ssRNA) and noncoding regions, and amino acid sequences of ORF1 and protein coding regions between SuMMoV and 28 other potyviruses.

Acronym	ssRNA	ORF1	5´NCR	ssRNA	P1	HC-Pro	P3	6K1	CI	6K2	VPg	NIa	NIb	CP	3´NCR
BCMV	50.8	45.3	35.2	50.8	12.6	46.0	23.5	33.3	54.8	30.2	50.8	46.5	59.7	58.6	24.6
BiMoV-SSV	51.8	47.0	31.7	51.8	21.2	46.2	29.5	51.0	55.1	40.4	49.5	41.1	60.3	59.2	35.9
BtMV	50.9	46.0	43.2	50.9	13.6	47.5	25.9	33.3	53.6	44.2	53.2	44.4	61.3	57.1	45.1
ChiVMV	51.9	46.6	35.8	51.9	16.3	52.5	23.1	37.3	51.3	37.3	50.3	43.3	60.3	64.6	36.3
HarMV	51.4	44.0	35.5	51.4	15.0	46.0	22.8	33.3	53.6	26.4	49.2	43.6	58.0	57.5	31.7
JGMV	49.9	42.0	37.6	49.9	12.7	40.0	22.3	39.2	48.5	30.2	46.0	40.6	58.0	54.3	35.7
JYMV	53.0	47.5	39.0	53.0	17.9	50.9	23.8	47.1	55.0	32.1	51.1	46.5	63.5	61.1	36.0
LiMV	51.8	48.7	45.8	51.8	20.8	50.9	30.0	39.2	56.3	35.8	44.1	48.5	64.6	61.5	32.6
LMV	53.8	47.8	35.6	53.8	19.7	52.4	29.6	41.2	51.0	39.6	54.3	47.3	65.5	56.7	38.4
LYSV	51.2	44.9	27.8	51.2	12.5	46.3	23.9	53.8	52.7	35.8	44.7	42.1	63.3	59.7	27.9
MDMV	51.2	44.6	32.3	51.2	18.5	41.4	26.3	34.6	52.0	34.0	47.1	42.5	59.5	57.4	31.2
NYSV	53.4	48.9	42.1	53.4	17.6	52.2	26.4	35.3	56.6	34.0	55.1	48.1	64.0	66.3	39.7
PenMV	51.2	44.8	30.7	51.2	17.9	41.6	25.1	34.6	52.3	34.0	46.5	41.7	60.5	59.1	36.6
PepSMV	52.5	46.4	33.3	52.5	14.2	47.4	28.1	42.3	53.9	40.4	44.1	41.9	63.4	61.2	39.7
PPV	53.1	50.2	27.5	53.1	15.1	53.1	28.3	45.1	59.6	39.6	56.9	49.0	65.4	59.2	35.9
PRSV	50.7	44.3	51.2	50.7	13.0	47.0	24.9	45.1	53.5	24.5	44.7	42.1	58.2	58.5	40.3
PVA	54.6	49.6	34.5	54.6	22.6	52.8	29.3	35.3	57.3	41.5	51.6	11.6	66.0	56.9	21.4
PVMV	52.3	46.4	34.2	52.3	14.9	50.5	24.0	39.2	53.5	35.8	51.3	42.5	60.5	65.1	35.4
PVY	51.7	47.4	40.6	51.7	21.1	48.6	27.5	51.0	55.6	36.5	50.5	43.6	59.5	61.7	35.7
SCMV	50.4	44.3	31.9	50.4	18.5	41.4	25.4	30.8	50.1	30.2	44.4	40.8	63.0	57.4	33.6
SMV	51.1	44.8	36.6	51.1	12.6	45.7	24.1	33.3	54.3	35.8	48.1	41.9	60.5	59.7	29.4
SrMV	50.6	44.3	33.3	50.6	18.9	41.6	23.1	28.8	51.0	37.7	46.5	42.9	60.1	56.6	31.9
SuCMoV-C	52.0	46.4	45.2	52.0	19.8	48.0	24.0	49.0	55.6	38.5	45.7	41.5	60.3	62.7	39.9
SuCMoV-CR	51.7	46.6	42.1	51.7	20.1	47.8	24.6	49.0	56.1	38.5	46.2	41.1	60.3	61.9	39.9
TEV	59.0	54.8	32.8	59.0	18.1	56.2	30.1	59.6	64.1	35.8	60.6	55.6	68.9	67.3	44.5
TuMV	52.9	48.6	34.9	52.9	15.9	52.0	22.6	41.2	58.2	32.1	53.7	48.1	66.3	61.0	54.8
TVMV	54.6	49.3	33.3	54.6	20.1	54.9	27.8	47.1	57.7	39.6	43.2	46.4	62.9	58.0	32.9
WVMV	51.6	44.9	35.3	51.6	10.6	48.1	22.1	35.3	54.8	34.0	48.7	43.2	60.0	56.8	30.3

Phylogenetic comparison, at the level of aa sequencee of ORF1 (Fig. 3) and nt sequence of whole genome (data not shown) of SuMMoV with other potyviruses revealed that it shared the greatest evolutionary relationships with TEV, TVMV and PVA, in all cases the statistical significance exceeds 94%.

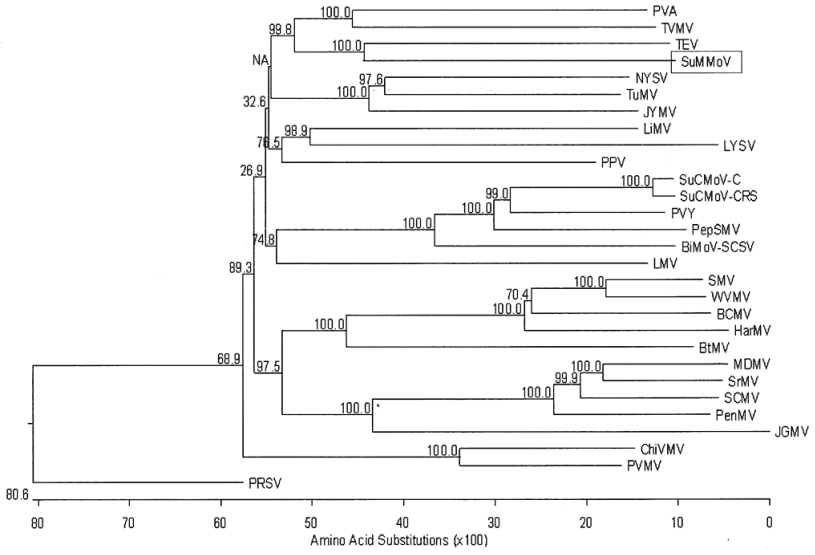

Figure 3: Phylogenetic trees of amino acid sequences of complete polyproteins of SuMMoV and 28 other potyviruses analyzed.

DISCUSSION

The pyrosequencing method used here to obtain the full genome sequence of the SuMMoV was simple, rapid, highly accurate, and relatively inexpensive compared to traditional sequencing strategies. Here, a enriched sample with viral RNA was used and satisfactory results were obtained, similar results were obtained when enriched samples with dsRNA were used (Roossinck et al. 2010).

The genomic map obtained from SuMMoV presents two NCRs and 11 protein coding regions, typical regions of the genome of a member of the *Potyvirus* genus (Shukla et al. 1994; Adams et al. 2005; Berger et al. 2005; Chung et al. 2008) confirming previous studies which mention the SuMMoV as a member of this genus (Giolitti et al. 2007; Giolitti et al. 2010a). Additionally, the star and stop codons, cleavage sites for proteins, percentage of base composition, and the conserved regions are common between potyviruses and were cited for other such as ChiVMV, HarMV, Butterfly flower mosaic virus (BFMV) and Passion fruit woodiness virus (PWV) (Chen et al. 2008; Tsai et al. 2008; Adams and Research 2011; Wylie and Jones 2011a; Wylie and Jones 2011b). According to current criteria to discriminate between different potyviruses on the basis of the nt sequence of whole genome would be expected to exceed the limit of 80% (Berger et al. 2005) to be considered a distinct species in the genus *Potyvirus*, when SuMMoV was compared with other 28 potyviruses never exceeded this threshold. When the aa sequence of ORF1 was compared we found a series of identities of 42.0-54.8%, which is included in the range of 24.4-80.9% (Adams et al. 2005), which allows to consider to SuMMoV a separate species. Furthermore, when were compared all protein coding regions at nt and aa level, the ranges of identities obtained never reached the necessary percentages (Adams et al. 2005) for consider to SuMMoV one of the species with which it was compared, including CP gene. This gene showed a range of identities for the aa sequence of 54.3-67.3%, not reaching 80% identity (Adams et al. 2005; Berger et al. 2005) necessary to be considered one of the viruses analyzed. Phylogenetic comparisons of the genome of SuMMoV revealed that it shared greatest evolutionary relationship with TEV, PVA and TVMV, over 75 % by what it is considered significant (Adams et al. 2005), these viruses infect solanaceous host and is clearly separated from the group of viruses that infect grasses and legumes hosts. This should be studied as SuMMoV was isolated from sunflower, a *Asteraceae.* Complete sequences of viruses are essential for understanding its biology, diversity and evolutionary history, for devising new control strategies and evaluating its risk (Achon et al. 2007). For understanding the molecular mechanisms of pathogenesis and replication (Baric 2006) and allows to development of full-length biologically active cDNA clones, for positive-strand RNA plant viruses that are indispensable for investigating the functions of viral genes and control elements as well as generating virus-derived gene expression and silencing vectors (Peremyslov and Dolja 2007). This is the first report of complete genomic sequence of SuMMoV and its genomic organization. This work clearly supports the idea that is a new member of the *Potyvirus* genus detected naturally infecting sunflower in Argentina.

REFERENCES

Achon, M.A., L. Serrano, N. Alonso-Dueñas and C. Porta. 2007. Complete genome sequences of *Maize dwarf mosaic* and *Sugarcane mosaic virus* isolates coinfecting maize in Spain. Arch Virol 152: 2073-2078.

Adams, M. and R. Research. 2011. Cleavage sites in the family *Potyviridae*. Descriptions of Plant Viruses. http://www.dpvweb.net/potycleavage/index.html#intro.

Adams, M.J., J.F. Antoniw and C.M. Fauquet. 2005. Molecular criteria for genus and species discrimination within the family *Potyviridae*. Arch Virol 150: 459-479.

Agrios, G.N. 2005. Plant Pathology, Elsevier Academic Press.

Ahn, H.I., J.Y. Yoon, J.S. Hong, H.I. Yoon, M.J. Kim, J.H. Ha, M.J. Rhie, J.K. Choi, W.M. Park and K.H. Ryu. 2006. The complete genome sequence of *Pepper severe mosaic virus* and comparison with other potyviruses. Arch Virol 151: 2037-2045.

Baric, R.S. 2006. Synthetic Viral Genomics. In Working Papers for Synthetic Genomics: Risks and Benefits for Science and Society. 2007. M.S. Garfinkel, D. Endy, G.L. Epstein and R.M. Friedman**:** 35-81.

Bejerman, N.E., F. Giolitti, S. De Breuil and S. Lenardon. 2011. *Ibicella lutea* un nuevo hospedante alternativo del Sunflower Chlorotic mottle virus (SuCMoV). 2º Congreso Argentino de Fitopatología, Mar del Plata, 1-3 de junio de 2011.: 244.

Berger, P.H., M.J. Adams, O.W. Barnett, A.A. Brunt, J. Hammond, J.H. Hill, R.L. Jordan, S. Kashiwazaki, E. Rybicki, N. Spence, D.C. Stenger, S.T. Ohki, I. Uyeda, A. van Zaayen, J. Valkonen

and H.J. Vetten. 2005. Family *Potyviridae*. In Virus Taxonomy, Classification and Nomenclature of Viruses, Eighth Report of the International Committee on Taxonomy of Viruses. C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger and L.A. Ball. San Diego, CA, Elsevier Academic Press**:** 819-841.

Chen, J., Y.-H. Shi, M.-Y. Li, M.J. Adams and J.-P. Chen. 2008. A new potyvirus from butterfly flower (*Iris japonica* Thunb.) in Zhejiang, China. Arch Virol 153: 567-569.

Chung, B.Y.-W., W.A. Miller, J.F. Atkins and A.E. Firth. 2008. An overlapping essential gene in the *Potyviridae*. PNAS 10515: 5897-5902

Dujovny, G., T. Masaya, H. Koganesawa, T. Usugi, K. Shohara and S.L. Lenardon. 2000. Molecular characterization of a new *Potyvirus* infecting sunflower. Arch Virol 145: 2249-2258.

Dujovny, G., T. Usugi, K. Shohara and S.L. Lenardon. 1998. Characterization of a *Potyvirus* infecting sunflower in Argentine. Plant Dis 825: 470-474.

Giolitti, F., N. Bejerman, S. de Breuil and S. Lenardon. 2010a. Caracterización molecular del mosaico suave del girasol. 5to Congreso Argentino de Girasol, Buenos Aires.: 319-320.

Giolitti, F., N. Bejerman and S. Lenardon. 2009. *Dipsacus fullonum*: an Alternative Host of Sunflower chlorotic mottle virus in Argentina. J Phytopathol 157: 325-328.

Giolitti, F., S. de Breuil and S. Lenardon. 2007. Caracterización parcial del virus causal del "Mosaico Suave del Girasol". 4to Congreso Argentino de Girasol: 379-380.

Giolitti, F.J., N.E. Bejerman, S. de Breuil and S.L. Lenardon. 2010b. Identification and Characterization of a New Strain of Sunflower chlorotic mottle virus, a Potyvirus Infecting Asteraceae in Argentina. J Phytopathol 158: 536-541.

Hari, V. and P. Das. 1998. Ultra Microscopic Detection of Plant Viruses and Their Gene Products. In Plant Virus Disease Control. A. Hadidi, R.K. Khetarpal and H. Koganesawa. St. Paul, Minnesota**:** 417-427.

Kitajima, E.W. 1997. Microscopia electrónica de virus de plantas. Centro de Microscopía Electrónica de apoyo a la investigacion agropecuaria. Escuela Superior de Agricultura "Luiz de Queiroz" Sao Paulo, Brasil, Universidad de Sao Paulo, Brasil.

Lenardon, S.L. 1994. Síntomas de etiología viral en cultivos de girasol. In Enfermedades del Girasol en la Argentina. Manual de reconocimiento. V.R. Pereyra and A.R. Escande**:** 99-103.

Lenardon, S.L., F. Giolitti, A. León, M.E. Bazzalo and M. Grondona. 2001. Effects of Sunflower chlorotic mottle virus infections on sunflower yield parameters. Helia 2435: 55-66.

Maritan, A.C., J.O. Gaspar and L.E.A. Camargo. 2004. Identificação e caracterização de um Potyvirus isolado de *Zinnia elegans*. Fitopatol bras 291: 28-33.

NCBI. 2011. National Center for Biotechnology Information. BLAST: Basic Local Alignment Search Tool. http://blast.ncbi.nlm.nih.gov/.

Peremyslov, V.V. and V.V. Dolja. 2007. Cloning of Large Positive-Strand RNA Viruses. Current Protocols in Microbiology. 7: 16F.1.1-16F.1.26.

Roossinck, M.J., P. Saha, G.B. Wiley, J. Quan, J.D. White, H. Lai, F. Chavarría, G. Shen and B.A. Roe. 2010. Ecogenomics: using massively parallel pyrosequencing to understand virus ecology. Mol Ecol 191: 81-88.

Shukla, D.D., C.W. Ward and A. Brunt. 1994. The *Potyviridae*. Wallingford, U.K., CAB International.

Tsai, W.S., Y.C. Huang, D.Y. Zhang, K. Reddy, S.H. Hidayat, W. Srithongchai, S.K. Green and F.-J. Jan. 2008. Molecular characterization of the CP gene and 3'UTR of *Chilli veinal mottle virus* from South and Southeast Asia. Plant Pathol 573: 408-416.

Wylie, S.J. and M.G.K. Jones. 2011a. The complete genome sequence of a Passion fruit woodiness virus isolate from Australia determined using deep sequencing, and its relationship to other potyviruses. Arch Virol 156: 479-482.

Wylie, S.J. and M.G.K. Jones. 2011b. Characterisation and quantitation of mutant and wild-type genomes of Hardenbergia mosaic virus isolates co-infecting a wild plant of *Hardenbergia comptoniana*. Arch Virol 156: 1251-1255.