



SHORT COMMUNICATION

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF AN ISOLATE OF *PELARGONIUM ZONATE SPOT VIRUS* INFECTING SUNFLOWER IN ARGENTINA**F. Giolitti¹, N. Bejerman¹, C. Nome¹, G. Visintin², S. de Breuil¹ and S. Lenardon^{1,3}**¹*Instituto de Patología Vegetal (IPAVE), Centro de Investigaciones Agropecuarias (CIAP), Instituto Nacional de Tecnología Agropecuaria (INTA), Camino 60 Cuadras Km 5,5, X5020ICA, Córdoba, Argentina*²*Facultad de Ciencias Agropecuarias, Universidad Nacional de Entre Ríos, Argentina*³*Facultad de Agronomía y Veterinaria, Universidad, Nacional de Río Cuarto, Argentina***SUMMARY**

Sunflower (*Helianthus annuus*) plants showing chlorotic concentric rings and line patterns on the leaves were observed in field crops near Paraná city (Entre Ríos, Argentina). Virus-enriched preparations examined with a transmission electron microscope contained quasi-spherical particles *ca.* 33 nm in diameter. Symptomatic sunflower samples were serologically negative for six known members of the family *Bromoviridae* when tested with commercial antisera, but in later tests gave a positive reaction with a *Pelargonium zonate spot virus* (PZSV) antiserum. The virus was mechanically transmitted to 16 plant species belonging to four families. Its complete genomic sequence, obtained by pyrosequencing, had an organization typical of members of the family *Bromoviridae*. Three contigs resulting from *de novo* assembly of deep sequencing reads showed 90.0%, 94.7% and 93.9% nucleotide identity with RNA-1 (GenBank accession No. AJ272327), RNA-2 (AJ272328) and RNA-3 (AJ272329), respectively, of *Pelargonium zonate spot virus* (PZSV), a member of the genus *Anulavirus* described in Italy. Therefore, the virus associated with chlorotic concentric rings and line pattern symptoms in sunflower was identified as an isolate of PZSV. To our knowledge, this is the first reference to PZSV infecting sunflower worldwide and the first report of its presence in South America.

Key words: PZSV, sunflower, pyrosequencing, *Bromoviridae*, chlorotic concentric rings, line pattern

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops in Argentina, with a planted area of 1,851,220 ha and a total yield of 3,340,520 tons reported in the 2011/2012 crop season (SIIA 2012). Several virus diseases of sunflower have been recorded worldwide and at least 36 viruses are known to affect this host naturally or following artificial inoculation (Brunt *et al.*, 1996). In most cases, sunflower-infecting viruses behave as minor pathogens, except for *Tobacco streak virus* (TSV) in India and Australia (Bhat *et al.*, 2002; Sharman *et al.*, 2008) and Sunflower yellow blotch virus (SuYBV) in Uganda (Aritua, 2006) both of which can cause major diseases. Two potyviruses have been detected on cultivated sunflower in Argentina, i.e. *Sunflower chlorotic mottle virus* (SuCMoV) and Sunflower mild mosaic virus (SuMMoV), both of which were isolated in the Entre Ríos province (Lenardon, 1994; Dujovny *et al.*, 1998; Giolitti *et al.*, 2012).

More recently, sunflower plants showing chlorotic concentric rings and line patterns, resembling symptoms produced by bromoviruses on other plant species, were observed in commercial crops in Entre Ríos during the 2003-2004 crop season (Fig. 1a). Similar symptoms had already been detected on sunflower in Mexico (Fucikovsky, 1976), but the associated pathogen had not been fully characterized.

Here, we describe the biological and molecular characterization of a virus pathogenic to sunflower, causing a disease that may become a problem for sunflower production in Argentina.

Symptomatic sunflower leaf samples collected near Paraná city were ground 1:5 (w/v) in 0.01 M phosphate buffer, pH 7, containing 0.1% Na₂SO₃, and silicon carbide (600 mesh) as abrasive. The resulting slurry was rubbed on the leaves of healthy sunflower and *Nicotiana glutinosa* plants, which were successfully infected and used as inoculum for mechanical transmission to 200 healthy sunflower seedlings (cv. Contiflor 17 DRM) at the V2 growth stage (Schneider and Miller, 1981). The experiment was divided into four replicates of 50 plants each. To assess seed transmission, four plots were sown, each with 250 seeds from

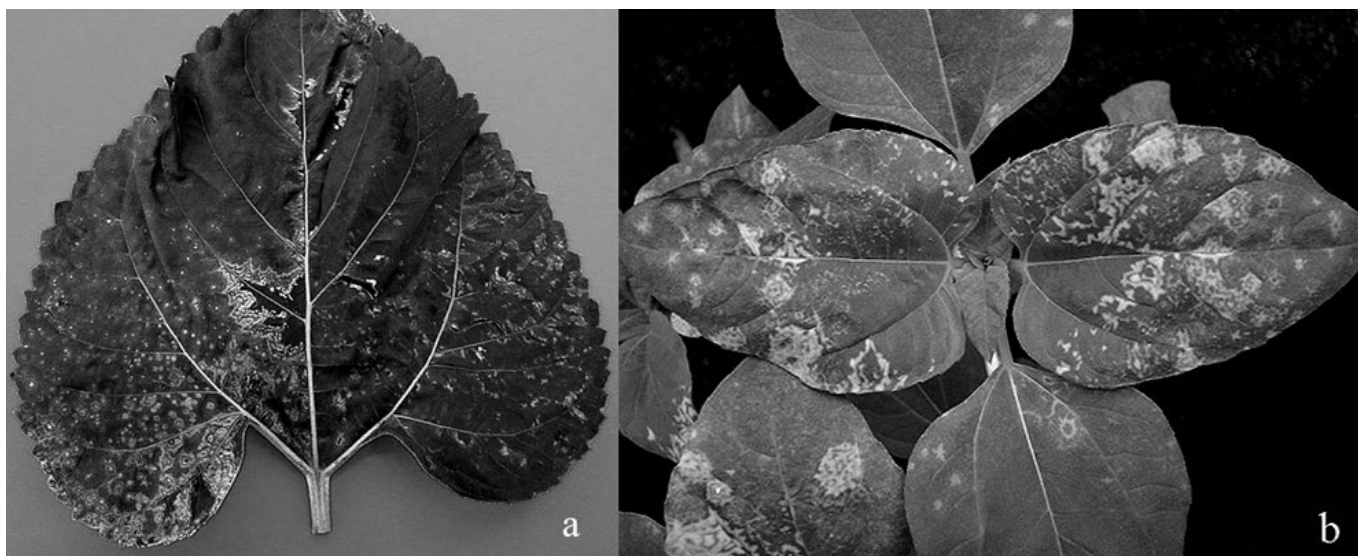


Fig. 1. Chlorotic concentric rings and line pattern symptoms observed on a leaf of naturally infected sunflower (a), and leaves of inoculated sunflower plants (b).

manually inoculated and systemically infected sunflower plants of cv. Contiflor 17 DRM.

An experimental host range was mechanically inoculated, which comprised no less than 25 plants each of 26 different plant species belonging to seven families, grown under greenhouse conditions ($22 \pm 3^\circ\text{C}$).

Plants were observed for symptom expression and four weeks post inoculation leaf samples were collected and used to back-inoculate sunflower or *N. glutinosa* plants. Concentrated, partially purified virus preparations were prepared from leaves of systemically infected *N. glutinosa* plants ground 1/5 (w/v) in extraction buffer (Jones *et al.*, 2006) treated with 25% chloroform and subjected to one cycle of differential centrifugation. To break the emulsion the extract was centrifuged in an Avanti J-25 centrifuge with a JA-20 rotor (Beckman Coulter, USA) for 15 min at 8,500 rpm and 4°C . The aqueous phase was collected and centrifuged in a XL-90 ultracentrifuge with a 90 Ti rotor (Beckman, USA) for 1 h at 40,000 rpm and 4°C . The pellet was resuspended in 0.005 M boric acid buffer, pH 7.

The quality of the preparation was checked by transmission electron microscopy (TEM). Formvar-coated grids were floated on a small drop of concentrated virus suspension, negatively stained with 2% uranyl acetate solution (Kitajima, 1997) and examined with a Jeol JEM EXII (Jeol, Japan).

DAS-ELISA was performed on diseased sunflower samples with commercial kits against six members of the family *Bromoviridae* occurring in Argentina: *Apple mosaic virus* (ApMV) and *Prunus necrotic ringspot virus* (PNRSV) (Bioreba, Switzerland); *American plum line pattern virus* (APLPV), *Cucumber mosaic virus* (CMV), *Prune dwarf virus* (PDV), and *Tobacco streak virus* (TSV) (Agdia, USA) (Nome Huespe *et al.*, 2012).

Total RNA was extracted from virus-enriched preparation using the RNeasy Plant Mini kit (Qiagen, USA) and

submitted to INDEAR (Rosario, Santa Fe, Argentina) for pyrosequencing with a 454 Genome Sequencer FLX Titanium System (Roche, USA). Reads were assembled *de novo* from the dataset using Newbler v 2.6 software (Roche, USA) and subjected to both BLASTN and BLASTX analyses (NCBI, 2011). Sequence and phylogenetic analyses were performed using the Lasergene 10 software package (DNA-STAR, USA). Multiple sequence alignments produced by ClustalW for the full-length nucleotide (nt) sequences of RNA-1, RNA-2 and RNA-3, and deduced protein sequences were used as input data for constructing phylogenetic trees using the Neighbor-Joining method. The 5' and 3' termini of the three RNAs were determined by comparisons with those of the Italian isolated of PZSV (It).

GenBank sequences used for comparison belonged to members of the following genera:

- (i) *Alfavirus*: *Alfalfa mosaic virus* (AMV) (HQ316635, HQ316636, HQ316637).
- (ii) *Anulavirus*: *Amazon lily mild mottle virus* (ALiMMV) (AB724113, AB724113, AB724113); *Pelargonium zonate spot virus* (PZSV) (AJ272327, AJ272328, AJ272329).
- (iii) *Bromovirus*: *Broad bean mottle virus* (BBMV) (NC_004008, NC_004007, NC_004006); *Brome mosaic virus* (BMV) (GU584131, GU584130, GU584129); *Cassia yellow blotch virus* (CYBV) (AB194806, AB194807, AB194808); *Cowpea chlorotic mottle virus* (CCMV) (AF325739, AF325740, AF325741); *Melandrium yellow fleck virus* (MYFV) (NC_013266, NC_013267, NC_013268); *Spring beauty latent virus* (SBLV) (NC_004120, NC_004121, NC_004122).
- (iv) *Cucumovirus*: *Cucumber mosaic virus* (CMV) (AB188234, AB188235, AB188236), *Gayfeather mild mottle virus* (GfMMV) (NC_012134, NC_012135, NC_012136); *Peanut stunt virus* (PSV) (AB360968, AB360969, AB360970); *Tomato aspermy virus* (TAV) (NC_003837, D10663, NC_003836).

Table 1. Reaction of 26 species to mechanical inoculation with PZSV isolate Arg, DAS-ELISA reaction and symptoms expressed.

Family	Plant species	Infection (%)	DAS-ELISA reaction	Symptoms
<i>Amaranthaceae</i>	<i>Amaranthus retroflexus</i>	0	0	-
	<i>Gomphrena globosa</i>	0	0	-
<i>Asteraceae</i>	<i>Bidens pilosa</i>	0	0	-
	<i>Bidens subalternans</i>	65	+	ch
	<i>Chrysanthemum coronarium</i>	66.1	+	ch
	<i>Cichorium intybus</i>	24.79	+	
	cv. Radicheta	100	+	ch
	cv. Achicoria	0	0	-
	<i>Dipsacus fullonum</i>	19.23	+	ch
	<i>Helianthus petiolaris</i>	44.44	+	ch
	<i>Helianthus tuberosus</i>	0	0	-
	<i>Matricaria recutita</i>	62.16	+	ch
<i>Lactuca sativa</i>	0	0	-	
<i>Cucurbitaceae</i>	<i>Cucumis metuliferus</i>	0	0	-
<i>Chenopodiaceae</i>	<i>Chenopodium amaranticolor</i>	100	+	ll
	<i>Chenopodium quinoa</i>	100	+	ll
<i>Fabaceae</i>	<i>Arachis hypogea</i>	0	0	-
	<i>Desmodium tortuosum</i>	0	0	-
	<i>Glycine max</i>	8.33	+	ch
	<i>Pisum sativum</i>	100	+	ch
<i>Scrophulariaceae</i>	<i>Antirrhinum majus</i>	0	0	-
<i>Solanaceae</i>	<i>Capsicum annuum</i>	20	+	ch
	<i>Nicotiana glutinosa</i>	100	+	ch
	<i>Nicotiana occidentalis</i>	100	+	ch
	<i>Nicotiana rustica</i>	75	+	ch
	<i>Petunia hybrida</i>	7.14	+	cry
	<i>Solanum lycopersicum</i>	1.46		
	cv. San Marzano	0	0	-
	cv. De la Plata	0	0	-
	cv. Roma VF	0	0	-
	cv. Río Grande	5.56	+	ch
<i>Solanum tuberosum</i>	0	0	-	

ch= chlorotic concentric rings and linear patterns, cry: cryptic, ll= local lesions and (-) no symptoms. With (+) and without (0) DAS-ELISA positive plants.

(v) *Illarvirus: American plum line pattern virus* (APLPV) (NC_003451, NC_003452, NC_003453); *Apple mosaic virus* (ApMV) (NC_003464, NC_003465, NC_003480); *Asparagus virus 2* (AV-2) (NC_011808, NC_011809, NC_011807); *Citrus leaf rugose virus* (CiLRV) (NC_003548, NC_003547, NC_003546); *Elm mottle virus* (EMoV) (NC_003569, NC_003568, NC_003570); *Fragaria chiloensis latent virus* (FCILV) (NC_006566, NC_006567, NC_006568); *Humulus japonicus latent virus* (HJLV) (NC_006064, NC_006065, NC_006066); *Parietaria mottle virus* (PMoV) (NC_005848, NC_005849, NC_005849); *Prunus necrotic ringspot virus* (PNRSV) (JN416771, JN416772, JN416773); *Spinach latent virus* (SPLV) (NC_003808, NC_003809, NC_003810); *Tobacco streak virus* (TSV) (FJ403375, FJ403376, FJ403377); *Tulare apple mosaic virus* (TAMV) (NC_003833, NC_003834, NC_003835).

Oleavirus: Olive latent virus 2 (OLV-2) (C_003673, C_003674, C_003671) (Bujarski *et al.*, 2012).

The biological and molecular characterization of the agent associated with symptomatic sunflowers in Argentina showed that it is a new isolate of *Pelargonium zonate*

spot virus (PZSV-Arg). These findings were confirmed by positive DAS-ELISA reactions using a virus-specific antiserum (DSMZ, Braunschweig, Germany).

PZSV is readily mechanically transmitted from infected plants (Gallitelli, 1982), and was sap-transmitted with a high efficiency to sunflower (98.5%), its natural host in Argentina (Fig. 1b). This virus was isolated originally from tomato (*Solanum lycopersicum*) in Italy as a strain of *Tobacco streak virus* (Martelli and Cirulli, 1969) and was later reported to affect plants of *Pelargonium zonale* showing concentric chlorotic rings in the leaves, from which it took its name (Gallitelli, 1982). Further records came from tomato in Spain (Luis-Arteaga and Cambra, 2000), France (Gebre-Selassie *et al.*, 2002) and, recently, in the USA (Gulati-Sakhuja *et al.*, 2009). PZSV is seed-transmitted in *N. glutinosa* and *Diplotaxis eruroides*, both with an efficiency of ca. 5% (Vovlas *et al.*, 1989) and in tomato with an efficiency of ca. 29% (Lapidot *et al.*, 2010). However, transmission through sunflower seeds has not been observed in this study, either because of the different host species or to the fact that different viral isolates may vary in their ability to be seed-transmitted (Lapidot *et al.*, 2010).

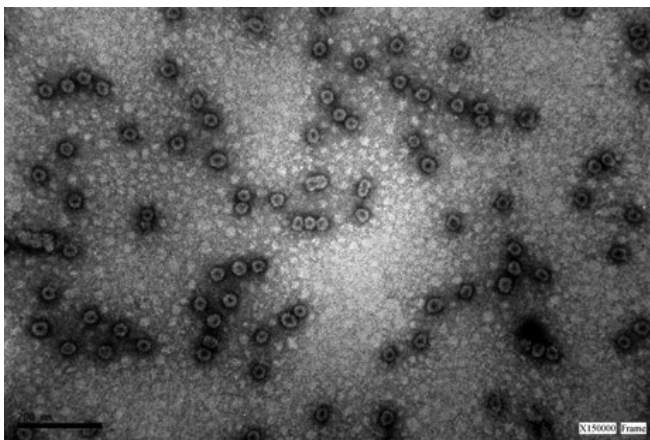


Fig. 2. Negatively stained particles of PZSV-Arg from a concentrated preparation from symptomatic tissues of naturally infected sunflower leaves. Bar = 200 nm.

The virus was successfully transmitted to 16 of 26 plant species in four families (Table 1). Back-inoculated sunflower and *N. glutinosa* plants developed typical symptoms when experimentally infected symptomatic plants were used as inoculum source, but did not show symptoms when the inoculum came from symptomless plants, except with *Petunia hybrida* that was latently infected. PZSV-Arg induced symptoms similar to those described for other isolates of this virus in *Chenopodium quinoa*, *C. amaranticolor*, *Capsicum annuum*, *N. glutinosa* and *N. rustica* (Luis-Arteaga and Cambra, 2000; Gebre-Selassie *et al.*, 2002; Liu and Sears, 2007; Lapidot *et al.*, 2010). The Spanish isolate induced systemic symptoms in *Gomphrena globosa* (Luis-Arteaga and Cambra, 2000) and the Israeli isolate local symptoms in *Lactuca sativa* (Lapidot *et al.*, 2010), neither of which reacted with PZSV-Arg. By contrast, *Pisum sativum* reacted to PZSV-Arg infection but not to the French isolate of the same virus (Gebre-Selassie *et al.*, 2002). As mentioned, *P. hybrida* showed no symptoms when inoculated with PZSV-Arg, but displayed systemic symptoms when inoculated with the Spanish and French isolates (Luis-Arteaga and Cambra, 2000; Gebre-Selassie *et al.*, 2002). The major difference among experimental host ranges is that PZSV-Arg infects tomato at a low percentage, whereas this plant species is the main host of other isolates (Vovlas *et al.*, 1989; Luis-Arteaga and Cambra, 2000; Gebre-Selassie *et al.*, 2002; Liu and Sears, 2007; Gulati-Sakhuja *et al.*, 2009; Lapidot *et al.*, 2010). The reaction of tomato to PZSV-Arg may be cultivar-dependent, as observed for cucumber (Lapidot *et al.*, 2010). Tissue samples from inoculated host range plant, that had been lyophilized and stored at -20°C reacted positively when tested by DAS-ELISA using the afore mentioned PZSV-specific antiserum.

TEM observations of concentrated virus preparation revealed plenty of quasi-spherical particles *ca.* 33 nm in diameter (Fig. 2), resembling very much those of members of the family *Bromoviridae*. This prompted DAS-ELISA

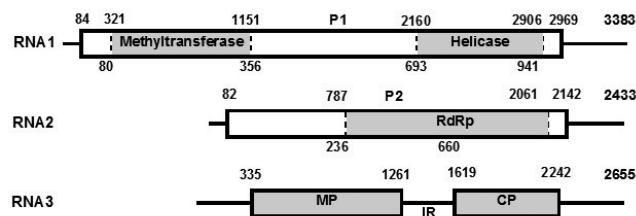


Fig. 3. Schematic representation of the genomic organization of the three RNAs of PZSV-Arg. ORFs are displayed as boxes and grey areas represent the encoded proteins (methyltransferase, helicase, RdRp, MP and CP). Numbers above the boxes indicate the nucleotides in which each fragment begins and ends, and numbers below the boxes, the amino acid at which each protein starts and ends.

checking of diseased sunflower samples with antibodies to six members of the family *Bromoviridae* reported from Argentina: APLPV, ApMV, CMV, PDV, PNRSV and TSV, which induce symptoms on different plant species similar to those produced by PZSV on sunflower (Nome Huespe *et al.*, 2012). All samples were serologically negative for all these viruses.

A dataset of 54,506 reads was generated by pyrosequencing totaling 21,516,806 nt, which represents an average read length of 394.76 bp. *De novo* assembly yielded 655 isotigs, grouping 90.92% of the reads. BLAST analysis indicated that 6, 11 and 176 of these isotigs were highly related to RNA-1, RNA-2 and RNA-3 (AJ272327, AJ272328 and AJ272329) of PZSV-It, with coverages of 85 \times , 121 \times , 290 \times , respectively. The greater abundance of RNA-3 observed is probably because this is the most abundant RNA species encapsidated by PZSV particles (Finetti-Sialer and Gallitelli, 2003). The complete sequence of the three RNAs was submitted to GenBank under the accession Nos. JQ350736, JQ350739 and JQ350740. The genomic structure of these RNAs is highly similar to that reported for PZSV-It (Finetti-Sialer and Gallitelli, 2003).

Analysis of the genomic sequence showed that the total length of RNA-1 was 3,383 nt. This RNA contains one long open reading frame (ORF) starting at AUG codon (nt position 84-86) and extending up to a UGA stop codon (nt position 2970-2972), which encodes a polypeptide of 962 aa residues (protein 1, P1) with a predicted molecular mass of 108.73 kDa. The 5' and 3' non-coding regions (NCRs) have a length of 83 nt and 414 nt, respectively. The 5' NCR of RNA-1 is 5 nt longer than the 5' NCR of PZSV-It. A motif scan of the deduced P1 aa sequence of PZSV-Arg showed that this protein contains a methyltransferase (Met) domain in its N-terminal half (aa 80 to 356) and a helicase (He) domain in its C-terminal region (aa 693 to 941), which is consistent with the molecular features of members of the family *Bromoviridae* (Fig. 3).

RNA-2 is 2,433 nt in length and codes for a polypeptide of 687 aa residues (protein 2, P2) with a predicted molecular mass of 79.16 kDa. The AUG start and the UAA stop codons are located at nt positions 82-84 and 2143-2145,

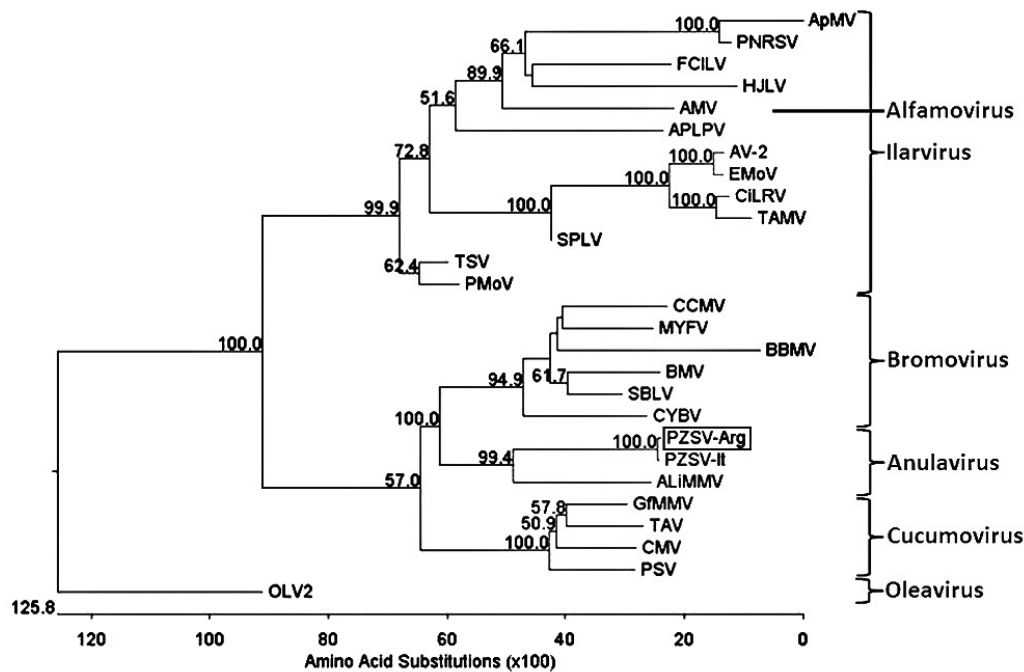


Fig. 4. Neighbour joining phylogenetic tree constructed with the complete deduced amino acid sequence of RdRp of PZSV-Arg, compared with known sequences of 26 other bromoviruses belonging to six different genera. Numbers at the nodes are bootstrap values over 50%. Statistical significance was estimated by performing 1000 replications of bootstrap resampling of the original alignment.

respectively. P2 contains an RNA-dependent RNA polymerase (RdRp) domain (aa 236 to 660) (Fig. 3). The 5' and 3' NCRs have a length of 81 nt and 291 nt, respectively and its 3' NCR is two nt shorter than the 3' NCR of PZSV-It.

RNA-3 is 2,655 nt in size, i.e. 4 nt longer than PZSV-It, and comprises two non-overlapping ORFs, encoding a movement protein (MP) of 309 aa residues and the capsid protein (CP) of 208 aa; both proteins are one aa shorter than the same proteins of PZSV-It. These two ORFs are separated by an intergenic region (IR) of 357, which is 3 nt longer than that of PZSV-It. The 5' NCR consists of 334 nt and precedes the first ORF that begins with an AUG start codon located at nt position 335-337 and ends with a UGA stop codon located at nt position 1262-1264. The second ORF begins with an AUG start codon at nt position 1619-1621 and ends with a UAA stop codon at nt position 2243-2245. The 3' NCR consists of 413 nt (Fig. 3). The 5' and 3' termini sequences of the three RNAs are identical to the homologous sequences of PZSV-It. PZSV-Arg has the same nt context regions for start codons of the four ORFs, the GDD conserved motif of RdRp and the 11 nt sequence of internal control regions of 5' NCRs of RNA-1, RNA-2 and in the IR of RNA-3 as in PZSV-It (Finetti-Sialer and Gallitelli, 2003; Gallitelli *et al.*, 2005).

The comparison of PZSV-Arg sequences with those of other 26 members of the family *Bromoviridae* showed the highest degrees of identity with homologous sequences of PZSV-It. In particular, RNA-1, -2 and -3 nt sequences of PZSV-Arg with the comparable sequences of PZSV-It, were 90%, 94.7% and 93.9% identical, respectively.

Identities between the predicted aa sequences of P1, P2, MP and CP of PZSV-Arg and those of PZSV-It were 92.6%, 92.6%, 98.4% and 95.2%, respectively (data not shown).

Phylogenetic trees based on a comparison of the nt sequences of RNA-1, -2 and -3, and aa sequences of P1, methyltransferase and helicase domains, P2, movement protein, capsid protein (data not shown) and RdRp domain (Fig. 4) showed that PZSV-Arg was allocated with a high level of confidence in a cluster with PZSV-It and ALIMMV, a recently described putative novel member of the genus *Anulavirus* (Fuji *et al.*, 2013), which is clearly separated from clades comprising members of the genera *Alfamovirus*, *Bromovirus*, *Cucumovirus*, *Iilarvirus* and *Oleavirus*.

This study provides information of a new sunflower disease in Argentina. This finding urges us to pursue studies aimed at avoiding or restraining the potential damages caused by this disease to sunflower or other economically important crops of our country. To our knowledge, this is the first record of PZSV infecting sunflower worldwide and the first report of its presence in South America.

REFERENCES

- Aritua V., 2006. Outbreak of virus-like disease on sunflower in Uganda. Internet resource: <http://www.africancrops.net/news/may06/sunflower.htm>.
- Bhat A.I., Jain R.K., Kumar A.M.R., Varma A., 2002. Serological and coat protein sequence studies suggest that necrosis

- disease on sunflower in India is caused by a strain of *Tobacco streak ilarvirus*. *Archives of Virology* **147**: 651-658.
- Brunt A.A., Crabtree K., Dallwitz M.J., Gibbs A.J., Watson L., Zurcher E.J., 1996. Known susceptibilities of Compositae, Plant Viruses Online: Descriptions and Lists from the VIDE Database. Internet resource: <http://biology.anu.edu.au/Groups/MES/vide/>.
- Bujarski J., Figlerowicz M., Gallitelli D., Roossinck M.J., Scott S.W., 2012. Family *Bromoviridae*. In: King A.M.Q., Adams M.J., Carstens E.B., Lefkowitz E.J. (eds) *Virus Taxonomy*. Ninth Report of International Committee on Taxonomy of Virus, pp. 965-976. Elsevier-Academic Press, London, UK.
- Dujovny G., Usugi T., Shohara K., Lenardon S.L., 1998. Characterization of a *Potyvirus* infecting sunflower in Argentina. *Plant Disease* **82**: 470-474.
- Finetti-Sialer M., Gallitelli D., 2003. Complete nucleotide sequence of *Pelargonium zonate spot virus* and its relationship with the family *Bromoviridae*. *Journal of General Virology* **84**: 3143-3151.
- Fucikovsky Z.L., 1976. Enfermedades y plagas del girasol en México. In: Alonso L.C. (ed.) *Enfermedades y Daños de Herbicidas en el Cultivo del Girasol*, pp18-19. Koipe, Madrid, Spain.
- Fuji S., Kikuchi M., Ueda S., Toda T., Furuya H., Fukumoto F., Hanada K., 2013. Characterization of a new anulavirus isolated from Amazon lily plants. *Archives of Virology* **158**: 201-206.
- Gallitelli D., 1982. Properties of the tomato isolate of *Pelargonium zonate spot virus*. *Annals of Applied Biology* **100**: 457-466.
- Gallitelli D., Finetti-Sialer M., Martelli G.P., 2005. *Anulavirus*, a proposed new genus of plant viruses in the family *Bromoviridae*. *Archives of Virology* **150**: 407-411.
- Gebre-Selassie K., Delecolle B., Gognalons P., 2002. First report of an isolate of *Pelargonium zonate spot virus* in commercial glasshouse tomato crops in Southeastern France. *Plant Disease* **86**: 1052.
- Giolitti F., de Breuil S., Bejerman N., Lenardon S., 2012. Complete nucleotide sequence and genetic organization of Sunflower mild mosaic virus (SuMMoV). In: Hall A. (ed.) *Proceedings 18th International Sunflower Conference, Mar del Plata & Balcarce, Argentina*: 997-1002.
- Gulati-Sakhuja A., Sears J.L., Nuñez A., Liu H.-Y., 2009. Production of polyclonal antibodies against *Pelargonium zonate spot virus* coat protein expressed in *Escherichia coli* and application for immunodiagnosis. *Journal of Virological Methods* **160**: 29-37.
- Jones A.T., McGavin W.J., Gepp V., Zimmerman M.T., Scott S.W., 2006. Purification and properties of blackberry chlorotic ringspot, a new virus species in subgroup 1 of the genus *Ilarvirus* found naturally infecting blackberry in the UK. *Annals of Applied Biology* **149**: 125-135.
- Kitajima E.W., 1997. *Microscopia Electrónica de Virus de Plantas*, Universidad de Sao Paulo, Brazil.
- Lapidot M., Guenoune-Gelbart D., Leibman D., Holdengreber V., Davidovitz M., Machbash Z., Klieman-Shoval S., Cohen S., Gal-On A., 2010. *Pelargonium zonate spot virus* is transmitted vertically via seed and pollen in tomato. *Phytopathology* **100**: 798-804.
- Lenardon S.L., 1994. Síntomas de etiología viral en cultivos de girasol. In: Pereyra V.R., Escande A.R. (eds) *Enfermedades del Girasol en la Argentina*. Manual de Reconocimiento, pp 99-103. INTA, Buenos Aires, Argentina.
- Liu H.-Y., Sears J.L., 2007. First report of *Pelargonium zonate spot virus* from tomato in the United States. *Plant Disease* **91**: 633.
- Luis-Arteaga M., Cambra M.A., 2000. First report of natural infection of greenhouse-grown tomato and weed species by *Pelargonium zonate spot virus* in Spain. *Plant Disease* **84**: 807.
- Martelli G.P., Cirulli M., 1969. Le virosi delle piante ortensi in Puglia. III. Una maculatura gialla del pomodoro causata dal virus della necrosi perinervale del tabacco (*Tobacco streak virus*). *Phytopathologia Mediterranea* **8**: 154-156.
- NCBI, 2011. National Center for Biotechnology Information. BLAST: Basic Local Alignment Search Tool. Internet resource: <http://blast.ncbi.nlm.nih.gov/>.
- Nome Huespe S.F., Docampo D.M., Conci L.R., 2012. Atlas Fitopatológico Argentino. Internet resource: <http://www.fitopatoatlas.org.ar>.
- Schneider A.A., Miller J.F., 1981. Description of sunflower growth stages. *Crop Science* **21**: 901-903.
- Sharman M., Thomas J.E., Persley D.M., 2008. First report of *Tobacco streak virus* in sunflower (*Helianthus annuus*), cotton (*Gossypium hirsutum*), chickpea (*Cicer arietinum*) and mung bean (*Vigna radiata*) in Australia. *Australasian Plant Disease Notes* **3**: 27-29.
- SIIA, 2012. Sistema Integrado de Información Agropecuaria, Ministerio de Agricultura Ganadería y Pesca. Internet resource: <http://www.sii.gov.ar/index.php/series-por-tema/agricultura>.
- Vovlas C., Gallitelli D., Conti M., 1989. Preliminary evidence for an unusual mode of transmission in the ecology of *Pelargonium zonate spot virus* (PZSV). *Proceedings 4th Plant Virus Epidemiology Workshop, Montpellier, France*: 302-305.