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SHORT COMMUNICATION

Bean Yellow Mosaic Virus in Soybean from Argentina

Ramón E. Campos[1,*], Nicolás Bejerman[1,*], Claudia Nome[1], Irma G. Laguna[1,2] and Patricia Rodríguez Pardina[1]

1 Instituto de Patología Vegetal (IPAVE), Centro de Investigaciones Agropecuarias (CIAP), Instituto Nacional de Tecnología Agropecuaria (INTA), Camino 60 Cuadras km 5 ½ X5020ICA, Córdoba, Argentina

2 Consejo de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917 (C1033AAJ), Ciudad Autónoma de Buenos Aires, Argentina

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Correspondence
P. Rodríguez Pardina, Instituto de Patología Vegetal (IPAVE), Centro de Investigaciones Agropecuarias (CIAP), Instituto Nacional de Tecnología Agropecuaria (INTA), Camino 60 Cuadras km 5 ½ X5020ICA, Córdoba, Argentina.
E-mail: rodriguez.patricia@inta.gob.ar

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*These two authors contributed equally to this work.

Abstract

An association of *Bean yellow mosaic virus* (BYMV), (genus *Potyvirus*), with yellow mosaic leaf symptoms on soybean in Argentina was determined by enzyme-linked immunosorbent assay, a result confirmed by transmission electron microscopy and reverse transcription-polymerase chain reaction using a degenerate primer targeting the C-terminal region of BYMV's NIb. The sequence analysis showed that the isolate had 80.6–91.6% identities with other BYMV isolates from different strain groups, indicating that it is a strain of BYMV.

Introduction

Soybean (*Glycine max* [L.] Merrill) is one of the most valuable crops in the world, not only as an oil seed crop and feed for livestock, but also as a good source of protein for the human diet and biofuel feedstock (Masuda and Goldsmith 2009). Argentina is one of the top soybean exporting countries in the world. Soybean production in Argentina has steadily expanded over the last three decades increasing land area and production from 344 000 ha and 496 000 t in 1974, to 18 764 900 ha and 48 878 800 t in 2011 (FOASTAT 2011). The number of diseases affecting soybean crops and their disease severity has also increased, particularly during the years 1990s. Eleven viruses have been identified infecting soybean crops in Argentina since the late 1970s: *Soybean mosaic virus* (SMV) *Alfalfa mosaic virus* (AMV), *Tobacco streak virus* (TSV) and *Groundnut ringspot virus* (GRSV) were detected in all the soybean producing areas of Argentina, (Laguna et al. 2008), *Peanut mottle virus* (PMV) was only found in Córdoba Province (where 90% of peanut is cultivated) (Laguna et al. 2008), *Soybean stunt virus* (SSV) in Salta Province, (Laguna et al. 2008), while *Cowpea mild mottle virus* (CpMMV) and four begomoviruses (*Soybean blistering mosaic virus*, *Tomato yellow spot virus*, *Bean golden mosaic virus* and *Euphorbia mosaic virus*-Peru) were detected in the north-west region of the country (Laguna et al. 2008; Rodríguez Pardina et al. 2010). During the 2010–2011 growing season, a survey was carried out in different soybean producing areas of Argentina. The samples were collected and brought to laboratory and tested by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) or by plate-trapped antibody-enzyme-linked immunosorbent assay (PTA) (Clark and Adams 1977; Mowan and Dawson 1987) using SMV, AMV, TSV, CpMMV, GRSV antisera and by nucleic acid hybridization assay using probes for begomoviruses (Rodríguez Pardina et al. 2010). Some of the tested samples were negative for all viruses.

Material and Methods

Leaf samples of soybean plants showing mosaic and blistering symptoms in the field conditions were collected from Casbas, province of Buenos Aires. The symptomatic leaves were ground 1 : 5 (w/v) using phosphate buffer (pH 7, 0.01 M) supplemented with silicon carbide (600 mesh) as abrasive. The resulting slurry was rubbed onto soybean healthy plants. After inoculation, the plants were transferred into a insect-proof greenhouse and kept for observation of symptom development.

Leaf-dip preparations were made from portions of symptomatic leaves cut into several pieces with a razor blade in a drop of PBS pH 7 + 0.01% (w/v) sodium sulphite (Na_2SO_3). The leaf extracts were then transferred to carbon-coated Formvar grids for seven min. After washing with distilled water, the grids were negatively stained with 2% uranyl acetate and examined under a Jeol JEM EXII transmission electronic microscope (TEM) (Jeol, Tokyo, Japan). Based on the symptoms and the evidence observed by TEM, the symptomatic plants were therefore tested by indirect ELISA (Mowan and Dawson 1987), using an *anti-potyvirus* group monoclonal antibody (AGDIA Inc) and by DAS-ELISA using two different -*Bean yellow mosaic virus* (BYMV) commercial antisera (AGDIA Inc and BIOREBA AG). Samples were considered positive when OD410 values were higher than the mean of the healthy controls plus three times the standard deviations.

For molecular characterization, total RNA was extracted from systematically soybean infected tissues using RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. cDNA was synthesized using M-MLV reverse transcriptase (Promega, Madison, USA) and oligo-dT/*Not*I primer (Tsuneyoshi et al. 1998). PCR was performed using a potyvirus-specific degenerate primer targeting the C-terminal region of NIb gene and an oligo-dT primer, as described before (Gibbs and Mackensie 1997). The PCR product was cloned into pGEM T-Easy Vector (Promega). Plasmids mini preps were prepared from three clones using QIAprep Spin Miniprep kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The clones were sequenced in both directions by the Unidad de Genómica, Instituto de Biotecnología-INTA (Argentina).

The nucleotide and deduced amino acid sequences were compared with those of other potyvirus available in GenBank (www.ncbi.nlm.nih.gov), and database searches were carried out using the Blast*n* algorithm (Altschul et al. 1990). Multiple sequence alignments of nucleotide and deduced amino acid sequences were performed with Clustal W (Thompson et al. 1994). Maximum-likelihood tree was generated with MEGA 5 (Tamura et al. 2011), using the GTR + G model. A recombination analysis based on the complete CP gene was performed using the software RDP3 (Martin et al. 2010).

Results and Discussion

All inoculated plants developed symptoms similar to those observed in the field (Fig. 1). Flexuous filamentous particles typical of potyviruses were observed by electron microscopy of dip preparations (Fig. 2). The samples were positive in Indirect ELISA using anti-potyvirus group monoclonal antibody; furthermore, a

Fig. 1 Inoculated soybean plants showing mosaic and blistering symptoms

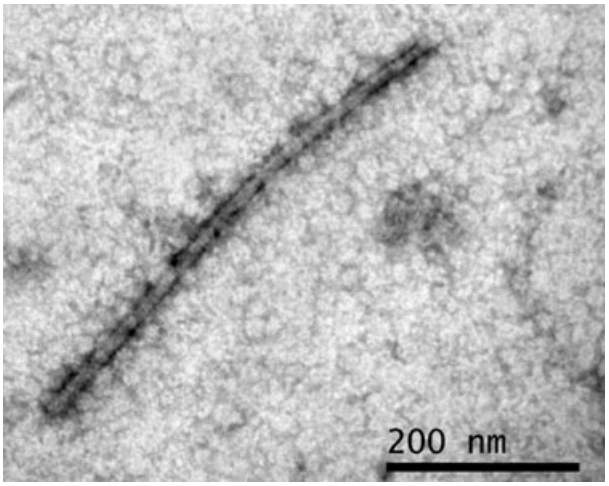

Fig. 2 Flexuous filamentous particles, typical of potyviruses observed under electronic microscope on dip preparations.

Table 1 *Bean yellow mosaic virus* isolates, belonging to different strain groups, used in comparative sequence analyses and percent nucleotide (nt) sequence identities with the Argentinean isolate of this virus

Strain group	Isolate	GenBank accession number	nt identity (%)
Broad bean	FBMj	EU082112	86.4
	90-2	D89545	86.8
Canna	Cgz	EF592168	84.8
	Chz	DQ060521	84.4
General	GDD	AY192568	83.9
	Mi	X81124	82.9
Lupin	LP-1	EU082119	90.8
	RLut1	EU082124	91.2
Monocot	35-1	AB097090	91.6
	VM-23	AY845012	91.5
Pea	I	S71232	80.6
	CS	D00604	82.0
W	W	DQ641248	86.2

very weak reaction was observed in DAS-ELISA when two different commercial BYMV antisera were used, which should indicate cross-reaction against the CP of this virus.

A 1629 nucleotides (nt) sequenced fragment was obtained and its analysis (Lasergene v. 8.0.2) showed that the potyvirus detected in soybean is a BYMV isolate. The fragment amplified consisted of an uninterrupted reading frame that contained a part of the NIb-coding region (nt 1–636), the complete CP-coding region (nt 637–1455) and the 3′-NCR (nt 1456–1629). The sequence was deposited in the GenBank DNA database (Accession No KC731531). When the nt sequence of the complete coat protein (CP) of BYMV–Arg isolate was compared with other BYMV isolates (Wylie et al. 2008), a 80.6 to 91.6 % identity was obtained (Table 1). Phylogenetic analysis based on the complete coat protein nt sequences showed that the BYMV Argentinean isolate formed a monophyletic cluster, which was placed between monocot and lupin polytypic groups of BYMV (Fig. 3). Furthermore, no evidence of recombination was found within the CP of BYMV-Arg. According to the observed in the phylogenetic tree, it should be interesting to evaluate the BYMV-Arg host range not only to determine if it overlaps with that of other strain groups, but also the host specialization of the BYMV isolate infecting soybean, which has not been studied yet.

Based on the molecular criteria suggested for species discrimination within the *Potyviridae* family (Adams et al. 2005), the virus isolate associated with blistering and mosaic symptoms on soybean in Argentina was identified as a strain of BYMV, which we named BYMV-Arg. Since the first report of BYMV on French beans from the USA and the Netherlands in 1925, it spread worldwide, with a broad host range including beans, faba bean, lupins and clover (Wylie et al. 2008) and both monocotyledonous and dicotyledonous ornamental crops such as bulbous iris, lisianthus, gentian, freesias and some orchids (Kumar et al. 2009). BYMV has also been detected, only by serological techniques, infecting soybean in USA and Iran (Malvick 1992; Golnaraghi et al. 2002,);. however, both the antisera used to detect it, and the OD

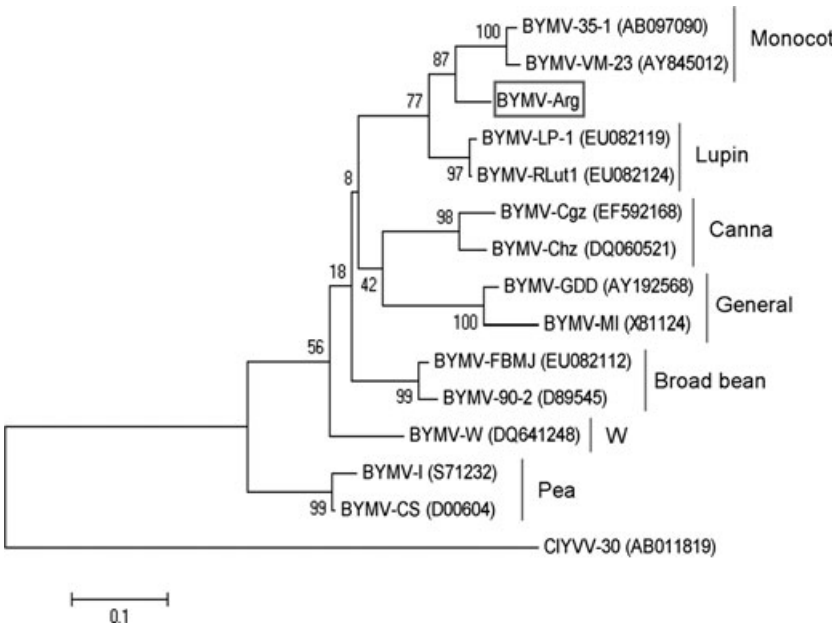

Fig. 3 Maximum-likelihood tree showing the phylogenetic relationship between *Bean yellow mosaic virus* (BYMV) isolated in Argentina with 13 other BYMV isolates from different subgroups. A *Clover yellow vein virus* (ClYVV) isolate was used as the outgroup sequence. The alignment was produced using the complete coat protein nucleotide sequences. Names of the seven distinct polytypic groups, which are labelled on the right side of the tree, are based on natural hosts. The phylogram is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer it. Phylograms were generated with MEGA 5 (Tamura et al. 2011) using the GTR + G model. Numbers at branches indicate the percentage of 1000 bootstrap replications where values were above 50%. The evolutionary distance scale is in the units of the number of nucleotide substitutions per site.

values of the positive reactions were not reported; therefore, we cannot shed light on our DAS-ELISA results, which showed very weak reaction when BYMV antisera was used.

Many strains of BYMV have been reported, which are distinguished by pathogenicity, serological properties or molecular diversity. Based on the phylogenetic relationship, the BYMV isolates were recently divided into 6 polytypic (general group, which included isolates from 8 different species, Monocot, Lupin, Broad Bean, Canna and Pea) and 1 monotypic group (W), named according to their original natural isolate host (Wylie et al. 2008). Based on its phylogenetic relationships, the Argentinean soybean isolate should constitute a new monotypic strain group within BYMV, which could be designated Soy. The very weak reaction shown by this isolate when tested against the BYMV commercial antisera could point out the variability found in this monotypic strain group, which should be considered not only in future studies, but also to develop specific anti-BYMV-Arg antibodies to use in epidemiological studies of this virus. The very weak reaction shown by BYMV-Arg when tested against the BYMV commercial antisera could be related with the BYMV isolates used to prepare the antisera, which possibly have been obtained from isolates that are not related to that of Argentina. To our knowledge, not only this is the first report of *Bean yellow mosaic virus* infecting soybean in Argentina, but also is the first molecular characterization of a BYMV isolate from soybean and the first BYMV sequence from South America.

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