First report of johnsongrass mosaic virus in Argentina

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Abstract

Potyriviruses are responsible for important economic losses in maize crop. This work reports the detection of johnsongrass mosaic virus, a potyvirus affecting maize in Argentina. Electron microscopy studies, mechanical transmission tests and molecular assays based on the CP gene were performed. To our knowledge, this is the first detection of JGMV in Argentina.

Keywords *Zea mays* L. · Potyvirus · Johnsongrass mosaic virus · Symptomatology · Mechanical transmission · CP sequence · Phylogenetic analysis

Maize (*Zea mays*) is one of the most important cereal crops in Argentina. The country ranks fifth in maize production and third in exports in the world (USDA 2018). The cultivated area in Argentina exceeds 6 million hectares, producing over 40 million tons ('Bolsa de Cereales' 2019). In Córdoba province, the cultivated area was over 2.2 million hectares in 2017/18, with a production of above 11 million tons (BCCBA 2019). This crop can be affected by multiple viruses of several taxonomic groups worldwide, including members of the family *Potyviridae*. Specific potyviruses, such as maize dwarf mosaic virus (MDMV), sugarcane mosaic virus (SCMV), wheat streak mosaic virus (WSMV), and johnsongrass mosaic virus (JGMV), were shown to cause the devastating maize lethal necrosis disease (MLN) in synergistic co-infection with maize chlorotic mottle virus (MCMV), a virus belonging to the genus *Machlomovirus* of family

Tombusviridae (Redinbaugh and Stewart 2018; Stewart et al. 2017). In Argentina, earlier studies revealed the presence of MDMV, SCMV, WSMV and MCMV, as well as their synergistic interaction, causing MLN to maize crop (Giménez Pecci and Laguna 2012; Giménez Pecci et al. 2017). The present work aimed to determine the presence of JGMV in maize samples collected in Córdoba province, Argentina.

JGMV was first reported in Australia as maize dwarf mosaic virus (MDMV) (Taylor and Pares 1968), and subsequently detected in several countries, including Brazil, Colombia, Nigeria, USA and Venezuela (Garrido and Trujillo 1993; McDaniel and Gordon 1989; Morales et al. 1996; Seifers et al. 2005; Silva et al. 2013). JGMV infection causes mosaic and chlorotic vein symptoms in several monocotyledonous species. The virus is spread by some aphid species in a non-persistent manner; and can also be mechanically transmitted, but not through seed (Shukla and Teakle 1989).

Maize plants showing symptoms associated with viral infection were collected in the northern area of Córdoba province, in the localities of Jesús María, Pozo del Tigre and Villa del Totoral in 2004. Symptomatic leaf samples were lyophilized and stored for future analysis. The affected plants showed leaf symptoms of mosaic with fine chlorotic streaks and ringspots (Fig. 1a). Healthy maize plants were mechanically inoculated using sap from symptomatic leaves extracted with phosphate buffer (0.01 M Na₂HPO₄/NaH₂PO₄, pH 7 + 0.1% Na₂SO₃) and 600-mesh silicon carbide as abrasive; the inoculated plants produced similar symptoms 15 days after inoculation (Fig. 1b). Electron microscopy observations of leaf-dip preparations revealed the presence of typical flexuous-filamentous potyvirus particles ~750 nm long and ~12 nm wide (Fig. 1c).

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Fig. 1 Symptoms of JGMV-Arg isolate in maize (*Zea mays*) collected from the field in Córdoba province (a) and induced by mechanical inoculation in healthy maize plants (b). Leaf-dip preparation from symptomatic maize samples showing flexuous-filamentous particles typical of potyviruses (c)

The leaf samples were serologically tested by DAS-ELISA against JGMV, MDMV, sorghum mosaic virus (SrMV) and SCMV using commercial antisera (Loewe Biochemica GmbH, Germany), and retested with our antisera against Argentine isolates of MDMV and SCMV (IPAVE-CIAP-INTA). The samples reacted positively only with JGMV antisera, although with weak colour development (absorbance values about 0.18, with a cut-off value of 0.08 and a positive control of 1.11). Total RNA was extracted using Trizol reagent (Invitrogen, CA, USA). Reverse transcriptions followed by PCRs were carried out using MMLV reverse transcriptase (Promega, Madison, WI, USA), KAPA HiFi HotStart DNA polymerase (KAPA Biosystems, USA), and the primers JGMV-F (5'-AGAGATGTACAAGAACGCCATCGC-3') and JGMV-R (5'-CATCCTTTAACGTCAGATAGACGG-3'), designed to amplify a ~ 1000-bp fragment of the coat protein (CP) gene of JGMV. The RT-PCR products, obtained

in triplicate from symptomatic plants, were purified using the Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced in both directions (Macrogen, South Korea). The obtained sequences were assembled into the final consensus sequence (906 nucleotides) using Staden Package program v 2.0.0b10 (available at <http://staden.sourceforge.net/>) and ORF finder tool (<https://www.ncbi.nlm.nih.gov/orffinder/>). BLAST analysis of the obtained sequence confirmed the identification of JGMV. The complete nucleotide (nt) sequence of the CP gene of the Argentine JGMV isolate (JGMV-Arg) was deposited in GenBank under accession number MK411416.

Pairwise identity analyses between the JGMV-Arg sequence and those available in GenBank from Australia, Brazil, Ethiopia, Kenya, Nigeria and USA were carried out using the MUSCLE-based pairwise alignment option implemented in SDT v1.2 (Muhire et al. 2014). The percentages of nucleotide sequence identities ranged from 76.0% to 98.2%,

Fig. 2 Phylogenetic relationship (neighbour-joining method) based on the CP amino acid sequence of the Argentine JGMV isolate (indicated with an asterisk) with other 32 JGMV isolates from Australia, Brazil, Ethiopia, Kenya, Nigeria and USA. The scale bar indicates pairwise nucleotide distance. Bootstrap values are shown as percentages of 1000 replications (values below 50% are not shown). Origin and access number are indicated for each CP amino acid sequence

showing the lowest and highest values with the isolate from Brazil (KC333416.1) and USA (U07218.1), respectively. Comparison of CP deduced amino acid (aa) sequence revealed the lowest identity value of 77.6% with the isolate from Ethiopia (AUB13349.1) and the highest value of 99.0% with the isolate from USA (AAA16873.1), as shown in the color-coded matrix of pairwise identity scores (Online Resource 1). These results are consistent with the *Potyvirus* species demarcation convention based on CP identity (Adams et al. 2005).

The neighbour-joining (NJ) and maximum-likelihood (ML) phylogenetic trees were constructed based on the full CP aa sequences using MEGA 5 (Tamura et al. 2011), with the best-fitted substitution model (JTT + G) and 1000 bootstrap replicates. The topology of the NJ (Fig. 2) and ML (data not shown) trees indicated that JGMV-Arg shared the closest evolutionary relationships with those from USA (AAA16872.1 and AAA16873.1). The phylogenetic relationships between the JGMV isolates support the results obtained by pairwise comparison (Online Resource 1).

To our knowledge, this is the first report of JGMV in Argentina. The recent detection of this virus in samples collected some years ago may be largely attributable to the high similarity of the symptoms caused by potyviruses on maize, so JGMV infection could be easily mistaken with those of the other potyviruses present in the same area where the samples were collected, such as MDMV, SCMV and WSMV (Giménez Pecci et al. 2017). Also, it might be expected that JGMV would be more widespread in the country since the aphid vectors, such as *Aphis craccivora*, *Aphis gossypii*, *Myzus persicae* and *Rhopalosiphum maidis*, are present in

the country, as well as the host plants belonging to the family *Poaceae*, like sorghum, johnsongrass, panicum and brachiaria (Ortego et al. 2004; Shukla and Teakle 1989). Further studies are needed to understand the current situation in terms of geographical distribution, incidence/prevalence and potential contribution to MLN of this viral disease in Argentina.

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Compliance with ethical standards

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

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

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