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BRIEF REPORT

Biological and molecular characterization of a highly divergent johnsongrass mosaic virus isolate from *Pennisetum purpureum*

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Abstract The complete genome sequence (9,865 nucleotides) of a highly divergent johnsongrass mosaic virus isolate (JGMV-CNPGL) was determined using Illumina sequencing. This isolate infected 10 genotypes of gramineous plants including maize. A comparative analysis of the complete genome showed 80 % nucleotide (nt) sequence identity (86 % amino acid (aa) sequence identity) to a johnsongrass mosaic virus isolate from Australia. The coat protein (CP) identity values, however, were lower than those for the whole genome (78 % and 80 % for nt and aa, respectively) and were close to the species demarcation values (77 % nt and 80 % aa). Unexpectedly, the amino-terminal portion of CP of JGMV-CNPGL showed only 38 % sequence identity to other JGMV isolates. The biological implications of this sequence divergence remain to be elucidated.

Elephant grass (*Pennisetum purpureum*) is a perennial grass species belonging to the family *Poaceae*, which includes maize, sorghum, wheat, rice, oat, barley, millet, brachiaria and panicum. Elephant grass, also known as napier grass, was first described in 1827 by Heinrich Schumacher in Africa, where this species originated [1]. The introduction of elephant grass into Brazil occurred in 1920, and this plant adapted to different soil types, except for poorly drained soils [2]. In the last 15 years, it has been used for feeding cattle due to its nutritional quality and yield [3]. The potential of elephant grass as a biomass source for bioethanol production has also been recognized [4].

Elephant grass is propagated by vegetative means using mature stems [3], an agricultural practice that favors the accumulation of viruses in crop plants (?references?). Until now, viruses of three genera (*Potyvirus*, *Mastrevirus,* and *Sobemovirus*) and an unclassified virus of the family *Luteoviridae* have been reported to infect elephant grass worldwide. In Brazil, johnsongrass mosaic virus has been reported recently by our research group [5].

Johnsongrass mosaic virus (JGMV) belongs to the genus *Potyvirus* (family *Potyviridae*), causing mosaic symptoms and severe yield losses in several monocot species. This virus is transmitted in a non-persistent manner by some aphid species: *Aphis craccivora, Aphis gossypii, Myzus persicae* and *Rhopalosiphum maidis* [6, 7]. JGMV was first described in Australia as maize dwarf mosaic virus [8] and later as a johnsongrass strain of sugarcane mosaic virus based on its biological and serological properties [7, 9]. However, Shukla and Ward [9] demonstrated, based on nucleotide and amino acid sequences of the coat protein (CP), that this virus was indeed a member of a new potyvirus species, which was given the name *Johnsongrass mosaic virus.* Previous reports have demonstrated the occurrence of this virus in Australia [8, 10, 11], South America [5, 12, 13], Nigeria [14] and the USA [6, 15]. In

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the USA, JGMV was first isolated in Texas and described as an MDMV isolate capable of infecting oat, johnsongrass and maize [6]. Now, this strain is designated as JGMV-MDO (maize dwarf oat strain) [15]. JGMV has also been reported in Venezuela infecting several varieties of maize [12, 16] and in Colombia infecting *Brachiaria* spp. (JGMV-Brac) [13]. A distinct JGMV isolate in Brazil infecting *Pennisetum purpureum* was reported previously by our group, and its CP gene was sequenced [5].

Here, we have investigated the biological characteristics of this Brazilian JGMV isolate, hereafter designated as JGMV-CNPGL (Centro Nacional de Pesquisa de Gado de Leite strain), and determined its complete genome sequence. Potyvirus-like flexuous and filamentous viral particles were observed in leaves of *P. purpureum*. Leaf tissues were fixed with 2 % phosphotungstic acid and then examined by transmission electron microscopy (Fig. 1A). The host range and symptomatology of this virus were determined by mechanical inoculation of infected leaf extracts (Fig. 1C) into 14 putative host plants. These plants were kept in greenhouse conditions and monitored for 30 days for the appearance of symptoms. The first symptoms were observed 13 days post-inoculation (dpi), and the infection was confirmed by RT-PCR using specific primers JG8352-F (CAAAGCCCCATACTTGTCGG) and JG9413-R (TTAGCCCCACGGTATGAATG). Ten inoculated plants were susceptible to JGMV-CNPGL, showing two main types of symptoms: chlorotic veins observed in *Zea mays* 2B587, *Zea mays* 3646H1(Fig. 1B) and *Pennisetum glaucum* (Millet ADR500) and mosaic symptoms in

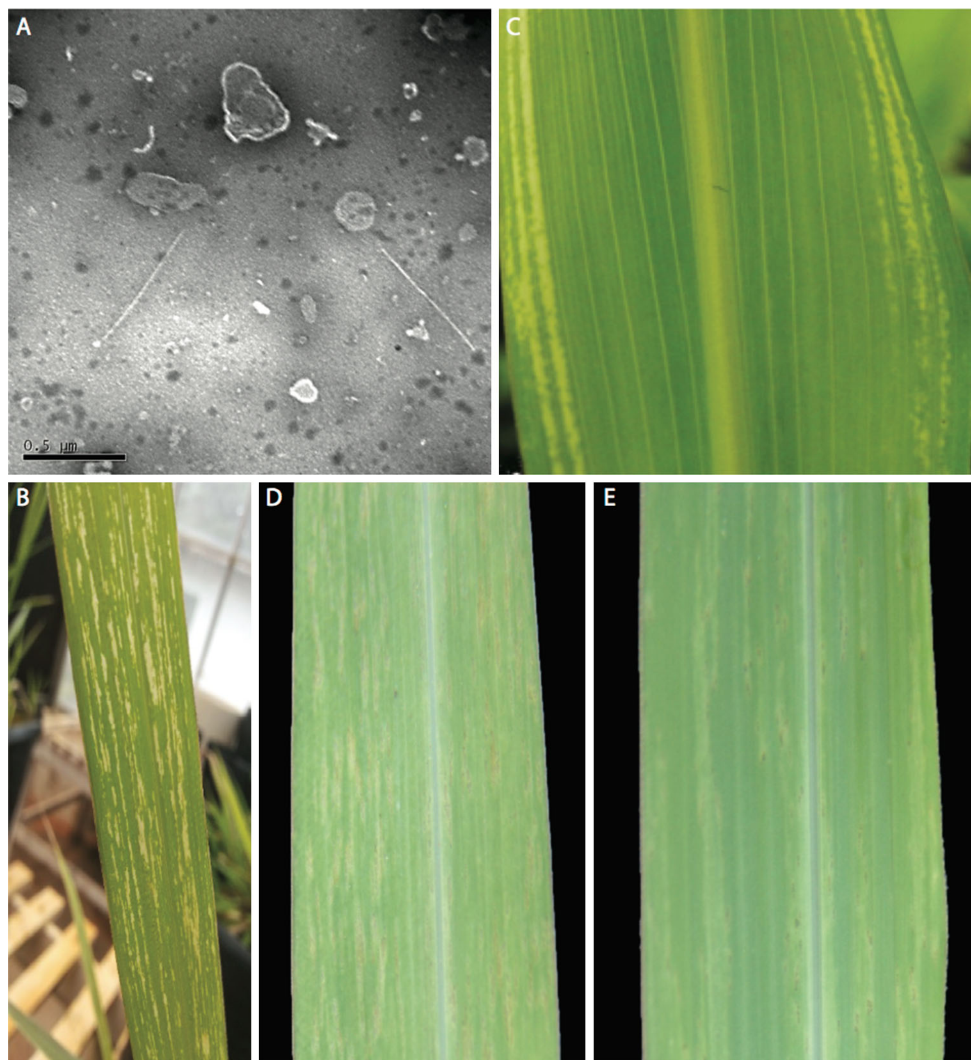

Fig. 1 (A) Virus particles of johnsongrass mosaic virus isolate JGMV-CNPGL (Centro Nacional de Pesquisa de Gado de Leite strain) infecting *Pennisetum purpureum*. (B) Symptoms of isolate JGMV-CNPGL in *Zea mays* 3646H1. (C) Symptoms induced by the JGMV-CNPGL isolate in *Pennisetum purpureum*. (D) Symptoms of isolate JGMV-CNPGL in *Panicum maximum* cv. Mombaça; (E) Symptoms of isolate JGMV-CNPGL in *Sorghum bicolor* BRS332

Fig. 2 Phylogenetic analysis using nucleotide sequences of the polyprotein region of potyviruses. The blue color indicates viruses that infect dicotyledonous plants, and the green color indicates viruses infecting monocots. The complete nucleotide sequence of JGMV-CNPGL ORF1 was aligned with other potyvirus sequences (Supplementary Table 1) using the translation alignment tool implemented in Geneious 9.1. The phylogenetic tree was inferred using the FastTree algorithm implemented in Geneious 9.1 (color figure online)

Brachiaria brizantha cv. Arapoty, *Brachiaria brizantha* cv. Xaraés, *Panicum maximum* cv. Mombaça (Guineagrass) (Fig. 1D), *Panicum maximum* cv. Massai, *Panicum maximum* C12, BRS Capileto, and *Sorghum bicolor* BRS332 (Fig. 1E). JGMV-CNPGL did not infect *Triticum aestivium* BRS264 (wheat), *Hordeum vulgare* L. VCU-CPAC (barley), *Crotalaria juncea* or *Glycine max* (soybean) under greenhouse conditions. The JGMV-CNPGL host range was similar to that reported for JGMV-N [14] and JGMV-MDO [15]. More importantly, JGMV-CNPGL infected maize, suggesting that this virus represent a potential threat to this crop in Brazil, one of the largest maize producers.

For genome sequencing of JGMV-CNPGL, viral particles were partially purified as follows. Infected leaves were

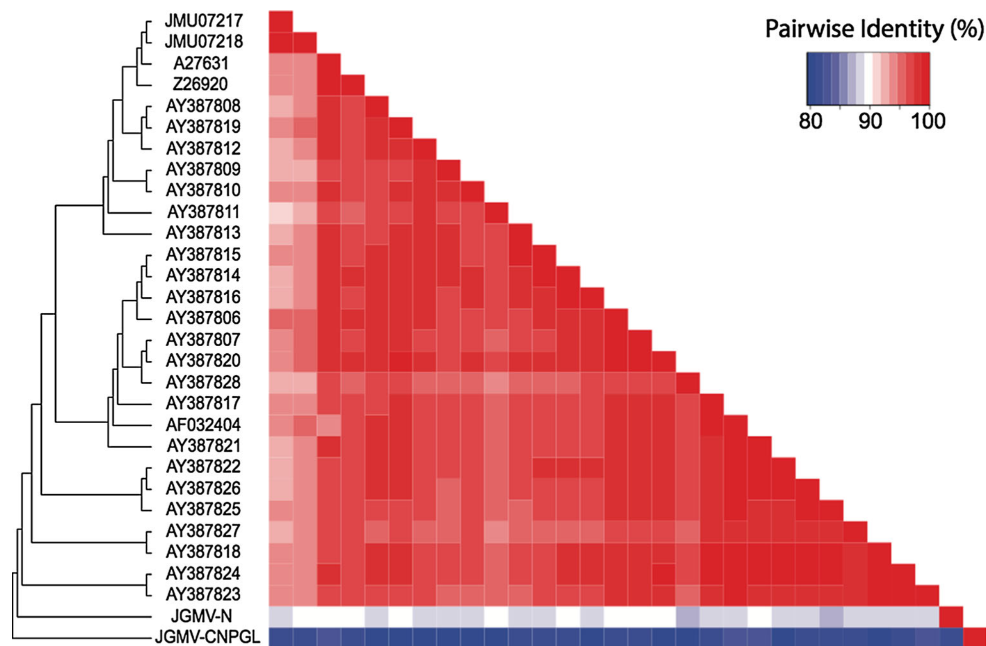

Fig. 3 Pairwise comparison matrix of CP protein sequences from several JGMV isolates. The identity values are presented in the lower triangular matrix, ordered according to the phylogenetic tree. The color represents the identity values (blue to red). The Brazilian isolate (JGMV-CNPGL) was compared to other JGMV sequences from the GenBank database, originating from Australia (A27631, Z26920, AF032404, AY387806-AY387828), the USA (JMU07217 and JMU07218J) and Nigeria (P83574J). The phylogenetic tree was inferred using the same dataset, and the branches were proportionally transformed using FigTree v. 1.4.2 (available at http://tree.bio.ed.ac.uk/software/figtree/) (color figure online)

first ground in liquid nitrogen, then homogenized in PBS-EDTA and β-mercaptoethanol, and filtered with cheese-cloth. The filtrate was then centrifuged through a sucrose cushion at 33,000 × *g* for 2 h, and the pellet was resuspended in PBS. From this enriched virus fraction, total RNA was extracted using an RNeasy Plant Mini Kit (QIAGEN, Valencia, CA), following the manufacturer's manual. RNA samples were sequenced at Catholic University of Brasilia (UCB) using a Nextera DNA Library Preparation Kit and an Illumina MiSeq platform. The paired-ends reads (7,402,180) were quality-filtered, the adapter sequences were removed, and contigs were assembled *de novo* using CLC Genomics Workbench version 6.0.3. The assembled contigs were compared using blastx against the Viral RefSeq database, and contigs related to JGMV were selected and mapped to the reference genome of JGMV (NC-003606). The complete genome sequence was 9,865 nucleotides (nt) long with two open reading frames (ORFs), and 28,967 reads aligned back to it. ORF1, which encompasses 9,414 nt, encodes a large polyprotein consisting of 3,059 amino acids (aa). After proteolytic cleavage, the proteins P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-pro, NIb and CP are predicted. ORF2 (2,793-3,068 nt), embedded in the P3 cistron, encodes the P3N+PIPO protein (91aa), which was identified by the presence of the conserved motif G_1A_6. Several motifs previously reported to be conserved among potyviruses have been found, including HXDGXSG and FIIRGR in P1 [17], GDD in NIb, Y/FK/RNK in HC-Pro [18] and DAG in the CP. However, the KITC motif of HC-Pro, which is involved in the interaction of the viral particle and the stylus [19], is absent in the JGMV-CNPGL isolate. The lack of this conserved motif has been reported for other potyviruses such as soybean mosaic virus [20] and wisteria vein mosaic virus [21]. The 5' and 3' untranslated regions (UTR) were 237 nt and 451 nt long, respectively, and both were confirmed by RACE. The JGMV-CNPGL genome was deposited under GenBank accession number KT833782.

The complete nucleotide sequence of JGMV-CNPGL ORF1 was aligned with other potyvirus sequences (Supplementary Table 1) using the translation alignment tool implemented in Geneious 7.1 [22]. As shown in Fig. 2, JGMV isolates clustered with canna yellow streak virus. All the viruses grouped in this clade infect only monocots, suggesting an ancestral adaptation towards these hosts. A comparison of the JGMV-CNPGL genome sequence with those of other potyviruses revealed highest nucleotide sequence identity (80 % nt and 86 % aa) to a JGMV isolate from Australia (NC-003606). The nucleotide sequence identity between these viruses was 70 % for P1, 76 % for HC-Pro, 85 % for P3, 86 % for 6K1, 83 % for CI, 83 % for

6K2, 81 % for VPg, 84 % for NIa-pro, 85 % for NIb, 78 % for CP, 41 % for 5'UTR and 85 % for 3'UTR. Figure 3 presents the full comparison matrix sequence and phylogenetic analysis based on CP aa sequences of JGMV-CNPGL and other JGMV isolates from three different countries (Australia, United States and Nigeria).

To establish a new potyvirus species, its members must share CP identity values lower than 76-77 % nt and 80 % aa. The identity between the CP of JGMV-CNPGL and JGMV Australian isolate was 78 % nt and 82 % aa, respectively, slightly above the threshold for new species demarcation [23]. Although these two isolates belong to the same species, the N-terminal region of CP from JGMV-CNPGL is highly divergent in the region from aa residues 9 to 85, showing only 38 % identity in this region. Interestingly, another JGMV isolate infecting *Panicum maximum* was recently reported in Brazil (GenBank accession number 289893). Although this isolate shares 80 % sequence identity with JGMV Australia, it cannot infect *P. purpureum* and maize, unlike JGMV-CNPGL. These observations indicate that these differences among JGMV isolates lead to biological variations regarding host range, for instance. The implications of this amino-terminal variation in JGMV-CNPGL remain to be investigated. Considering that *P. purpureum* and *P. maximum* originated in Africa, it is reasonable to assume that JGMV also emerged in the same area. More-intense sampling efforts may help to understand its evolutionary history and adaptations to distinct hosts.

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Compliance with ethical standards

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