Complete Genome Sequence of a Novel Potyvirus Infecting *Miscanthus Sinensis* (Silver Grass)

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Research Article

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Abstract

Here, we describe the full-length genome sequence of a novel potyvirus, tentatively named “miscanthus sinensis mosaic virus” (MsiMV), isolated from *Miscanthus sinensis* (silver grass) held in a post entry quarantine facility following its initial import into Western Australia, Australia. The MsiMV genome encompasses 9604 nucleotides (nt) encoding a 3071 amino acids (aa) polyprotein with conserved sequence motifs. The MsiMV genome is most closely related to sorghum mosaic virus (SrMV) with 74% nt and 78.5% aa sequence identity to the SrMV polyprotein region. Phylogenetic analysis based on the polyprotein grouped MsiMV with SrMV, sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV). This is the first report of a novel monopartite ssRNA virus in *Miscanthus sinensis* related to members of the genus *Potyvirus* in the family *Potyviridae*.

Main Text

Here, we describe the full-length genome sequence of a novel potyvirus, tentatively named “miscanthus sinensis mosaic virus” (MsiMV), isolated from *Miscanthus sinensis* (silver grass) held in a post entry quarantine facility following its initial import into Western Australia, Australia. The MsiMV genome encompasses 9604 nucleotides (nt) encoding a 3071 amino acids (aa) polyprotein with conserved sequence motifs. The MsiMV genome is most closely related to sorghum mosaic virus (SrMV) with 74% nt and 78.5% aa sequence identity to the SrMV polyprotein region. Phylogenetic analysis based on the polyprotein grouped MsiMV with SrMV, sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV). This is the first report of a novel monopartite ssRNA virus in *Miscanthus sinensis* related to members of the genus *Potyvirus* in the family *Potyviridae*.

Miscanthus sinensis, commonly known as silver grass, is a member of the family *Poaceae*, native to eastern Asia, where it is a keystone species in grasslands. In addition to ornamental applications, *M. sinensis* has been investigated for use as a biofuel crop due to its high biomass yield. Miscanthus has been found to be an alternate host for potyviruses infecting the *Poaceae* family, including switchgrass mosaic virus and barley yellow dwarf virus [3].

Potyviruses represent the largest and most economically damaging group of known plant RNA viruses [4]. Potyviruses are aphid-borne, infecting both monocotyledonous and eudicotyledonous angiosperms [7]. In response to viral infection, plants can disrupt viral transcripts via RNA interference using Dicer-like endonucleases [2]. Infections by potyviruses typically result in silencing responses predominantly using 21 and 22 nt small RNAs [8].

Most potyviruses have a monopartite genome encoding a long open reading frame (ORF) [5]. The polyprotein is proteolytically processed by three viral proteases into ten functional proteins: P1, HC-Pro, P3, 6K1, cylindrical inclusion (CI) protein, 6K2, viral genome-linked protein (VPg), NIa-Pro, nuclear inclusion b (NIb; also known as RNA dependent RNA polymerase), and capsid protein (CP) [12, 13].

A "Morning Light" cultivar of *Miscanthus sinensis* imported into Western Australia from the USA in 1985 was transferred to The Plant Quarantine Station, Rydalmere, New South Wales for virus screening, as per new import conditions for clonal grasses at the time [11]. Double-stranded RNA extracted from 38 g of mature leaf tissue run on a 0.75% agarose gel revealed bands suspected viral origin and subsequent mechanical inoculation of ground grass tissue onto plants of *Zea mays* cv. Supagold resulted in symptom expression [6]. The infected Morning Light cultivar was never released from Post Entry Quarantine and was maintained as a positive control for biological indexing. Sap from the infected plant showed the presence of filamentous viral particles approximately 600 nm in length (Fig. 1A) further supporting the presence of a viral pathogen.

Collection of RNA samples and *de novo* assembly of 21-22 nt small RNAs was performed as previously described [1] yielding a single 9604 nt scaffold encoding a single long ORF from 141 - 9353 nt and flanking non-coding sequences including a poly A tail in the 3'-end typical of potyviruses. Mapping of small RNA reads onto the MsiMV genome allowing no mismatches resulted in 11,370,140 aligned reads (~25K x coverage) of which 89.2%, 7.6% and 0.3% were 21 nt, 22 nt or 24 nt long, respectively. The high proportion of mapped 21 nt reads is consistent with them being derived from the antiviral silencing response [8].

Completeness of the genome assembly was further validated by mapping of both Illumina and long read Oxford Nanopore Technology (ONT reads > 500 bp) ribosomal depleted RNA-seq data (Fig. 1 D). Overall, 7154030 Illumina (~74.5K x coverage) and 21495 ONT (2140 x coverage) reads were mapped onto the MsiMV genome using bowtie [9] and minimap2 [10], respectively. All three technologies agreeded with the assembled MsiMV genome. Raw data available under BioProject PRJNA752836.

The assembled genome encodes a 3071 aa polyprotein with 74.0% nt and 78.5% aa identity to the SrMV polyprotein (KM025045). The polyprotein is predicted to cleave into 10 subdomains consistent with other potyviruses (Fig. 1 C).

A polyprotein phylogenetic analysis placed MsiMV within a SrMV and MDMV clade, and SCMV as a sister clade (Figure 2). The clade formation of these viruses may be reflective of the similarity of their plant hosts, which are all in the *Panicoidea* subfamily of *Poaceae*. The sequence similarity of these viruses could also explain overlaps in host susceptibility seen in SrMV, which can infect *Miscanthus*, corn, and sugarcane. The position of MsiMV as separate from the SrMV, SCMV, and MDMV clades further supports the classification of MsiMV as a novel species of potyvirus.

Declarations

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Ethics declarations

Conflicts of interest

The authors declare that they have no conflict of interest

Ethical approval

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

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Figures

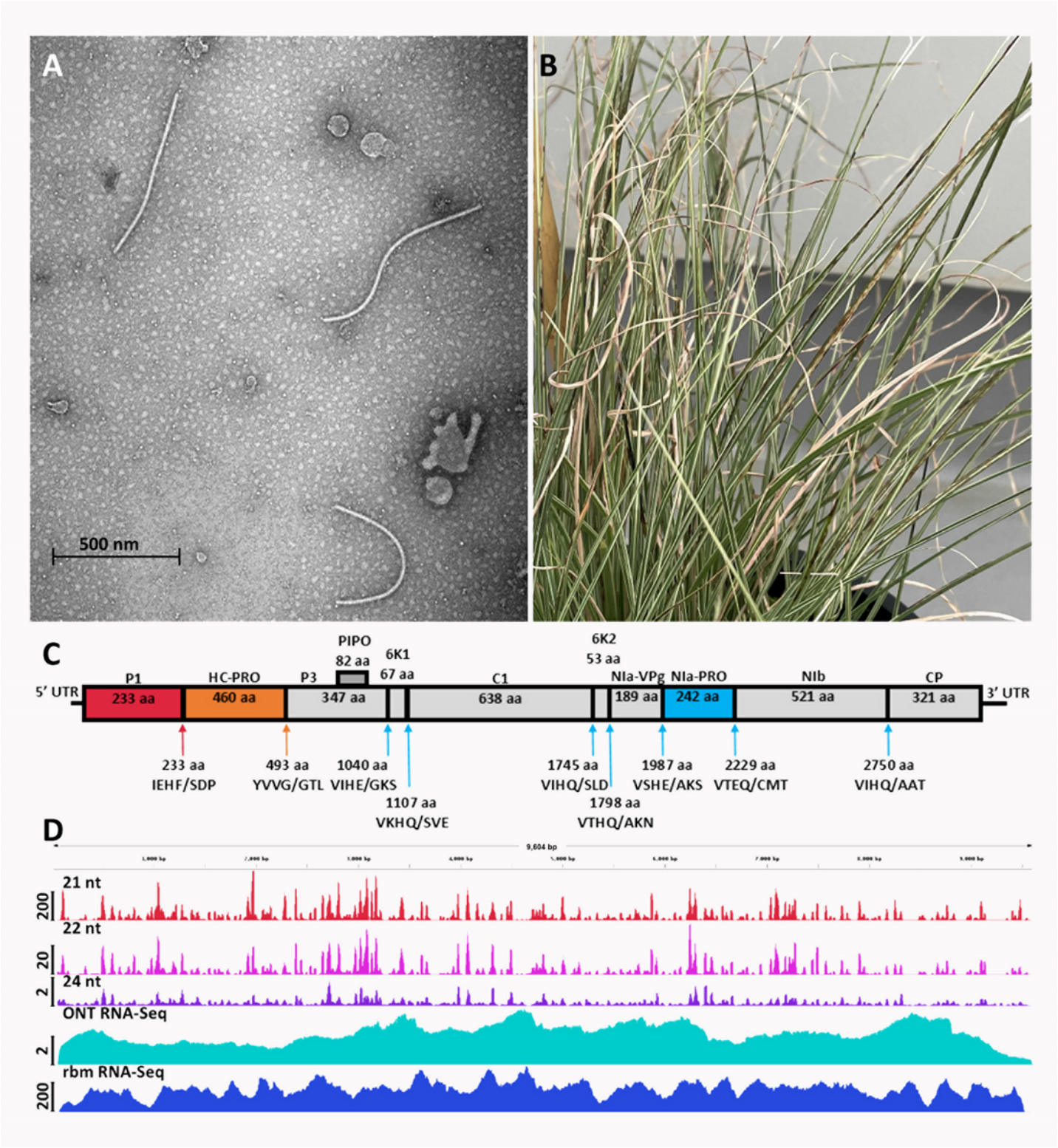

Figure 1

(A) Morphology of a MsiMV particle, viewed by transmission electron microscopy. (B) Miscanthus plant infected with MsiMV showing leaf mottling symptoms. (C) Schematic representation of the genome organization of MsiMV. The 5' (140 nt) and 3' (251 nt) untranslated regions are represented by solid horizontal bars while ORFs are depicted as open boxes. Putative polyprotein cleavage sites mediated by the P1, HC-Pro and NIa-Pro are indicated by red, orange and blue arrows, respectively. Protein domain

sizes in aa are indicated below the protein name. (D) Alignment of small RNAs, ONT and Illumina ribominus RNA-seq sequences onto the MsiMV genome. Scale bars on the Y-axis show total number of mapped reads (x1000).

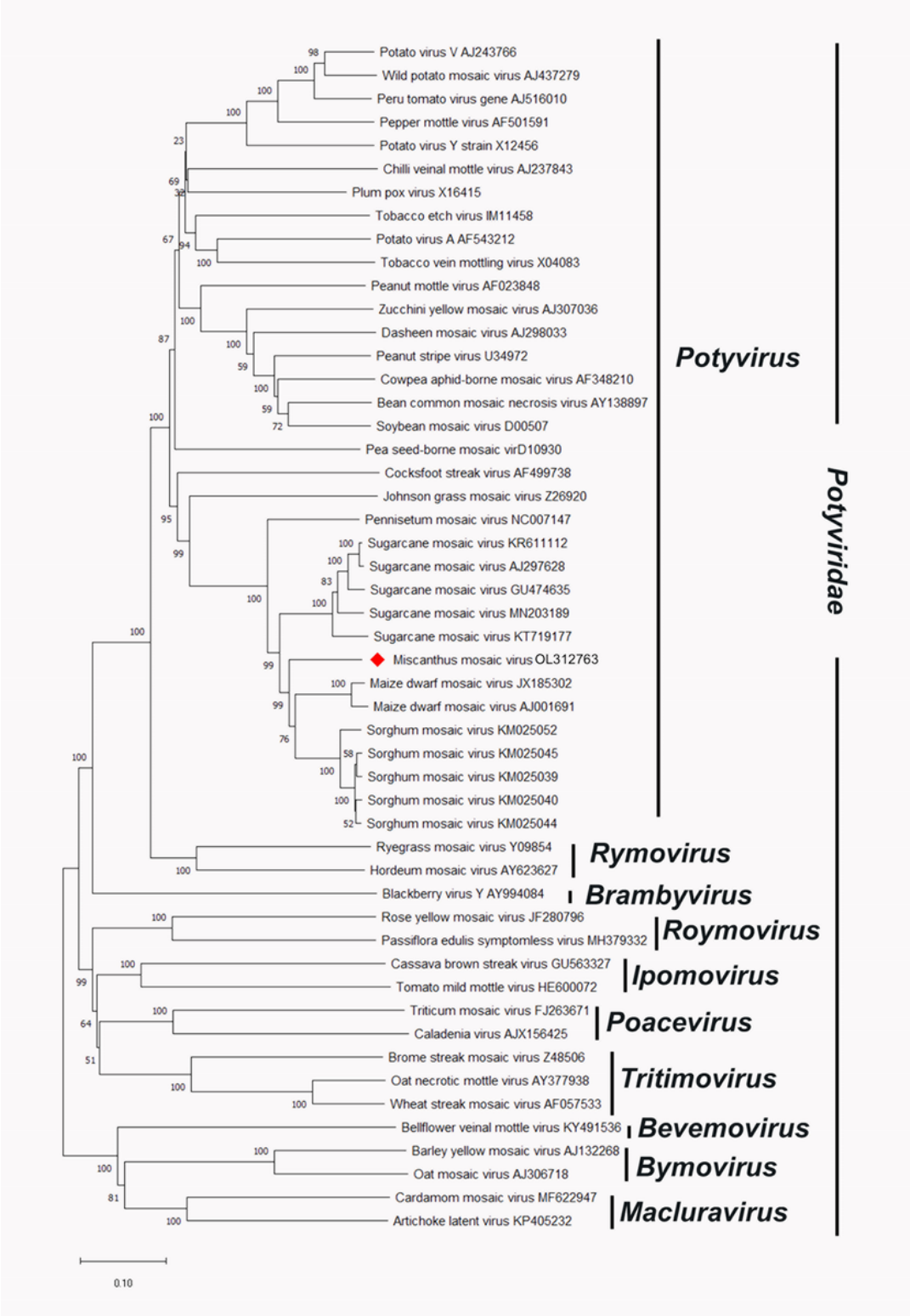

Figure 2

Neighbour joining tree of polyprotein aa sequences of MsiMV and selected members of the Potyviridae family. Bootstrap analysis was applied using 1000 bootstrap replicates. Percent bootstrap values are

indicated at each node.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- MsiMVgenome.fasta