First report of alstroemeria mosaic virus in South Africa

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Received: 18 September 2020 / Accepted: 2 November 2020 / Published online: 5 November 2020
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Abstract

This is the first report of the presence of alstroemeria mosaic virus (AIMV) in South Africa, and describes the second complete genome to date. AIMV was identified from a sample of *Alstroemeria pulchella* via RNAtag sequencing and RT-PCR. The diversity and distribution of AIMV, as well as the potential threat to the cutflower industry, still needs to be determined.

Keywords *Potyvirus* · *Alstroemeria* · RNAtag-seq · South Africa

Alstroemeria species are native to South America but both species and cultivars have become important in the international ornamental and cut flower trades. In South Africa, *Alstroemeria* is cultivated for the cut flower industry, with localized production in the Western Cape, North-West and Gauteng provinces. While viral diseases are known to affect *Alstroemeria* in South Africa, no putative causal agents have been previously identified.

Commercial propagation is typically performed vegetatively (Spence et al. 2000), leading to the associated accumulation of viruses in cut flower crops. A diverse group of at least 17 viruses from ten different genera are known to infect *Alstroemeria* (Hassani-Mehraban et al. 2019), which includes alstroemeria mosaic virus (AIMV). AIMV is a member of the family *Potyviridae* (genus: *Potyvirus*), which has flexuous filamentous particles (680–900 nm) that encapsidate a non-segmented polyadenylated single-stranded RNA genome (Adams et al. 2005). Infection of plants with AIMV results in

the reduction of both the number and the quality of flower stems (van Zaayen et al. 1992), as well as chlorotic streaks parallel to the leaf midribs; and is thought to be the virus most commonly infecting *Alstroemeria* (van Zaayen et al. 1994). AIMV was discovered almost 40 years ago in the United Kingdom (Brunt and Phillips 1981). Despite it being the most important virus of *Alstroemeria*, a complete genome of a strain from Ecuador only became available in 2019 (Mosquera-Yuqui et al. 2020).

In August 2019 a sample of *A. pulchella* was collected in a private garden (GPS coordinates: 25.781 S; 28.292 E). The plant showed symptoms of chlorosis and interveinal mosaic (Fig. 1) on the leaves. Total RNA was isolated using a GeneJET plant RNA purification kit (Thermo Scientific, Vilnius Lithuania). The extracted RNA was used as the partial input for an RNAtag-seq library, which was prepared according to Shishkin et al. (2015). The resulting library was sequenced using an Illumina HiSeq 2500 instrument (Illumina, San Diego, CA, United States) (paired-end 2 × 125 nt) at the Agricultural Research Council - Biotechnology Platform (ARC-BTP), Pretoria, South Africa.

The dataset was demultiplexed and separated from thirty-one unrelated datasets, using the Je suite (Girardot et al. 2016). This resulted in a total of 1,701,788 paired-end reads, which were trimmed for adapter and barcode content, as well as read length, using CLC Genomics Workbench 9 (Qiagen Bioinformatics, Aarhus, Denmark). Trimming parameters: min read length = 20 bp; quality limit = 0.05; trim adapter sequences (Illumina universal adapter: 5' AGATCGGAAGAG 3'; RNAtag-seq adapter: 5' TACACGACGCTCTTCCGATC

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13314-020-00409-5>.

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Fig. 1 Viral symptoms associated with a plant of *Alstroemeria pulchella*, maintained as an ornamental

TNNNNNNNT 3'). Trimming resulted in 1,499,784 trimmed reads, with an average length of 113.5. Raw trimmed reads were submitted as an NCBI sequence read archive with BioProject number PRJNA657267. The trimmed read dataset was assembled using metaSPAdes 3.14.0 (Nurk et al. 2017) and viral contigs identified using blastn with the viral refseq database as reference sequences (Altschul et al. 1990).

The AIMV genome from this study (AIMV-RSA) had an average coverage of 273x and is 9778 nt in length, excluding the poly-A-tract (GenBank accession: MT892648). The polyprotein gene of AIMV-RSA is located between 180 and 9365 nt. The genome shares a 98.5% average nucleotide identity with AIMV Als1 from Ecuador (GenBank accession: MK440140) and 98.9% average amino acid identity with the cognate polyprotein amino acid sequence, which were determined using the ani and aai calculators from the enveomics collection (Rodriguez-R and Konstantinidis 2016). According to species demarcation limits, these values indicate that the viruses from Ecuador and South Africa are both strains of AIMV (Adams et al. 2005).

The polyprotein sequence was aligned against 24 cognate sequences of the most closely related members of the genus *Potyvirus*, using ClustalW within BioEdit 7.2.5 (Hall 1999). The determination of the best-fit maximum-likelihood model and generation of the subsequent phylogeny (LG + G + I; 1000 bootstrap replicates) was performed with MEGA X (Stecher et al. 2020). The resulting maximum-likelihood phylogeny (Fig. 2) confirms the close relationship between the South African and Ecuadorian strains of AIMV, as well as

illustrating that AIMV is phylogenetically isolated from other extant potyviruses.

The presence of AIMV was confirmed using RT-PCR. AIMV-specific primers were designed, which amplified a portion of the coat protein domain of the polyprotein, with the following sequences and primer binding sites: AIMV-CP-F (5'-CACCTGCAAGCAAAGAGATTCTGA-3'; 8629-8651 nt); AIMV-CP-R (5'-TCGCATCTCGTAAGTTCCTGATC-3'; 9119-9141 nt). RT-PCR was carried out as a two-step reaction, using Promega GoScript™ Reverse Transcriptase and GoTaq® *Taq* polymerase (Promega, Madison, WI, United States), according to the manufacturer's instructions. Gel electrophoresis confirmed the presence of an amplicon of the expected size (513 bp). The identity of the amplicon was confirmed with bidirectional Sanger sequencing (Inqaba Biotec, Pretoria, South Africa).

This is the first report of AIMV in South Africa. The apparent lack of additional plant viruses suggests that the observed symptoms are associated with AIMV, however Koch's postulates were not confirmed. Conserved motifs associated with aphid transmission have been associated with AIMV (Mosquera-Yuqui et al. 2020), with *Myzus persicae* and *Neotoxoptera formosana* being known vectors (Yasuda et al. 1998). *M. persicae* has a widespread distribution in South Africa (Daiber and Schöll 1959) suggesting the existence of an efficient vector population. Potential control mechanisms could include reducing *M. persicae* vector populations and the production of virus-free planting material, which can be generated through meristem-tip culture (Hakkaart and Versluijs

Fig. 2 Maximum likelihood phylogeny based on the amino acid sequences of the polyprotein of the RSA isolate of alstroemeria mosaic virus (indicated by a solid circle marker) and selected members of the genus *Potyvirus*. The phylogeny represents the tree with the highest log likelihood and was generated in MEGA X using the Le Gascuel model,

gamma distributed with invariant sites. Bootstrapping was applied (1000 replicates) and the percentage of trees in which the associated taxa clustered together is shown next to the branches. Bootstrap percentages lower than 50 are not shown

1988). Further research should include determining how prevalent AIMV is within the cut flower and horticultural

industries in South Africa, as well as other *Alstroemeria* infecting viruses.

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