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NEW DISEASE REPORT

First report of *Moroccan watermelon mosaic virus* and other viruses infecting watermelon (*Citrullus lanatus*) in Uganda

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Watermelon (*Citrullus lanatus*) ranks among the top ten fruits cultivated globally, being prized for its fresh, nutritious fruit. In Uganda, watermelon is a significant fruit and cash crop, however, watermelon production is constrained by both abiotic and biotic factors, including viruses (Masika *et al.*, 2022a). Fifteen leaf samples (four diseased and one non-symptomatic) were collected in 2020 from three commercial watermelon farms in Kween, Masaka and Mukono districts, Uganda. Two sets of leaf tissue were collected, each containing 12 diseased and three non-symptomatic leaf samples. One set was analysed using high-throughput sequencing (HTS) and the other was kept at -80°C to confirm the HTS results by RT-PCR and Sanger sequencing. Total RNA was extracted according to Lodhi *et al.* (1994); the HTS libraries were constructed from one RNA sample containing a pool of the 15 leaves using the TruSeq Stranded Total RNA with a Ribo-Zero Plant kit (Illumina Inc., USA), and sequenced by HTS using the HiSeq 2500 platform (Illumina Inc., USA) (150 bp paired ends) in South Africa. *De novo* assembly of HTS data was performed using the Genome Detective virus tool (Vilsker *et al.*, 2019), generating a report containing the viral consensus sequences. A total of 8,682,658 raw reads were obtained (http://www.ncbi.nlm.nih.gov/bioproject/1191351). After cleaning and removal of non-viral reads, 7,038,818 and 865,648 reads were obtained. From the clean reads and assembled contigs, six viruses: *Citrullus lanatus cryptic virus* (CiLCV), *Cucumis melo cryptic virus* (CmCV), *Moroccan watermelon mosaic virus* (MWMV), pepo aphid-borne yellows virus (PABYV), *Watermelon crinkle leaf-associated virus* (WCLaV), and one novel virus, *Escherichia coli virus*, known to infect prokaryotes were identified. Such novel viruses were also reported by Togoobat *et al.* (2023). The complete genome of MWMV (GenBank Accession No. OM718862) was 9,672 nt without the poly(A) tail, and the PABYV genome (OM802496) was 5,798 nt.

Since sequencing was conducted on pooled samples, we recognise the potential risk of chimera formation during the generation of consensus sequences. As a result, we confirmed the presence of viruses in the second set of samples which had been frozen. RT-PCR was performed with specific primers (MWMV-F: 5'-CATTCTCAACTTGGTGGGACGC-3' and R: 5'-CTGATGCTCTCCTCTGTGACAAG-3') for the cylindrical inclusion gene of MWMV (582 bp), and PABYV primers according to Masika

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FIGURE 1 Viral symptoms observed on watermelon plants during the 2020 survey in Uganda included leaf mosaic patterns and yellowing.

et al. (2022b). Of the15 individual samples, 13 and 11 tested positive for MWMV and PABYV, respectively. One MWMV-positive amplicon was Sanger sequenced (Masaka, PQ397541) and showed 97% sequence identity with the HTS genome isolates. Nine of the 15 samples were infected with both viruses. One non-symptomatic sample from Kween tested positive for both viruses, while the two others were singly infected by MWMV. In addition, two diseased samples were singly infected with PABYV and MWMV, respectively. The singly-infected samples generally exhibited mild leaf symptoms, while mixed infections involving both viruses resulted in more severe symptoms, including mosaic patterns, distortion and pronounced growth retardation. The other identified viruses in our HTS analysis are likely latent or asymptomatic in watermelon under the conditions we surveyed. While detailed quantitative data on disease incidence was not undertaken, observational estimates indicated that approximately 30–40% of plants in the affected fields exhibited virus-like symptoms, including mosaic patterns, leaf mottling, vein clearing, and stunting (Fig. 1). This is the first report of MWMV in watermelon plants in Uganda; the reports of CiLCV, CmCV and WCLaV require further confirmation.

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