


First Report of Moroccan Watermelon Mosaic Virus in Pumpkin Plants in Brazil

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Cucurbita moschata is widely cultivated in Brazil, and zucchini lethal chlorosis virus, squash mosaic virus, papaya ringspot virus, and watermelon mosaic virus have been reported as viral pathogens in this crop in Brazil. Leaf samples of *C. moschata* showing mosaic, blistering, and yellowing symptoms were collected from a commercial field in Petrolina, Pernambuco State, and a commercial field in Juazeiro, Bahia State, in February 2023. To identify viruses that infected cucurbit plants in Brazil, three pooled samples showing virus-like symptoms (plants from the genera *Cucurbita* and *Cucumis* and other cucurbit plants, including watermelon and chayote) were analyzed by high-throughput sequencing (HTS). The total RNA was extracted from the semipurified virus using the protocol described by Blawid et al. (2017). The cDNA library was constructed from one RNA sample, which was composed of three pooled RNA samples (the genera *Cucurbita* and *Cucumis* and other cucurbit plants), using the TruSeq Stranded Total RNA with Ribo-Zero Plant kit (Illumina, San Diego, CA, U.S.A.), and sequenced by HTS using the NovaSeq 10G scale (150-bp paired ends). De novo assembly of total reads was performed using Megahit (Li et al. 2015), and the resulting contigs were analyzed using BLASTx with RefSeq viral proteins 2023 (NCBI) in Geneious Prime (Biomatters, Auckland, New

Zealand). A total of 88,028,898 reads were obtained, and 407,500 contigs (mean length: 514 nt) were assembled. Two contigs showed higher amino acid sequence identities (95.4% of 3,124 amino acids in polyprotein and 87.2% of 203 amino acids in P1 protein) with *Moroccan watermelon mosaic virus* in the genus *Potyvirus* of the family *Potyviridae* (McKern et al. 1993), a virus that had not been previously reported in Brazil. The complete genome was assembled by the read mapping to the contigs as references. The assembled complete genome of Moroccan watermelon mosaic virus (MWMV; LC775353) was 9,713 nt, not counting the poly(A) tail, and 217,278 reads were aligned to the genome with a mean coverage of 3,369.6 and a pairwise identity of 99.0%. The assembled genome encoded a polyprotein with a higher amino acid sequence identity of 97.82% with the Moroccan isolate (OQ847413). To confirm the presence of this virus in individual samples, RT-PCR was performed with specific primers (MWMV-F: 5'-ATTGTCTGATGAAAGAGCACA-3' and MWMV-R: 5'-CAGCTTCAGTCGCAACAAG-3'), targeting the cylindrical inclusion gene (the expected size of 598 bp). Eleven field samples of pumpkin plants (six from a field in the Juazeiro region and five from the Petrolina region) were analyzed using RT-PCR, and one sample from Juazeiro and five samples from Petrolina were positive for MWMV. One replicon of each region was sequenced (Juazeiro, OR338305; Petrolina, OR338306), and they showed higher nucleotide identities of 97.0% with each other and 96.4 and 97.7%, respectively, with the isolate from Morocco (OQ847413). Samples positive for MWMV were tested for the presence of other viruses previously reported in Brazil. All five samples from Petrolina were positive in the RT-PCR assay as a mixed infection with zucchini yellow mosaic virus (ZYMV) and cucurbit whitefly-borne yellows virus. Also, four out of five samples were positive for papaya ringspot virus (PRSV). On the other hand, in one sample positive for MWMV from Bahia state, no mixed infection with ZYMV and PRSV was observed. This is the first report of the occurrence of MWMV in Brazil and South America, associated with mosaic, blistering, and yellowing disease symptoms in pumpkin plants.

References:

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