# Disease Note

## Diseases Caused by Viruses

### First Report of Saffron-Associated Mastrevirus 1 from Saffron in Iran

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In spring 2022, 40 leaf samples of saffron plants harboring a wide variety of symptoms, including curling, yellowing, mosaic, dwarfing, and leaf malformation, were collected from three Khorasan provinces in Iran. These samples were processed using the virion-associated nucleic acid-based metagenomics approach (Moubset et al. 2022). Notably, 147 contigs with a size of >500 bp distributed among 35 samples with multiple symptomatic patterns (curling, yellowing, mosaic, dwarfing, and leaf malformation) shared >90% nucleotide identity with saffron-associated mastrevirus 1 (SaM1) (Martínez-Fajardo et al. 2024). This virus has recently been detected from transcriptomic datasets from saffron (*Crocus sativus* L.) collected in India. SaM1 was proposed to belong to a new species of the *Mastrevirus* genus (*Geminiviridae* family) and was tentatively named *Saffron-associated mastrevirus 1* (Martínez-Fajardo et al. 2024). In addition, contigs assigned to saffron potyviruses, including saffron latent virus, turnip mosaic virus, and bean yellow mosaic virus, were assembled from the 40 saffron samples, pinpointing that no conclusion could be made on the causal virus of observed symptoms. Total DNAs of the 40 saffron samples were further extracted using the DNeasy Plant Mini Kit (Qiagen) and were tested for the presence of SaM1 using a primer pair amplifying a 327-bp-long fragment located in the coat protein of SaM1 (CSAV_2F 5′-TTTAAGTCAGGGTCTGGAGATG-3′ and CSAV_3R 5′-GGCATACTGTAACCTCGTCTTC-3′). Amplification conditions were as follows: an initial denaturation at 95°C for 10 min, 30 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and a final extension step at 72°C for 10 min. This PCR test confirmed that 28/40 samples tested positive for SaM1. Total DNA from one sample (#69-32) testing positive for SaM1 was further amplified using Phi29 DNA polymerase (TempliPhi, GE Healthcare) by rolling circle amplification (RCA) as previously described (Shepherd et al. 2008). RCA products were used as a template for PCR amplification of the complete genome of SaM1 using abutting primers (CSAV_PST1F 5′-CTGCAGTTGCGGTAAGTCTATGTTGGCTG-3′ and CSAV_PST1R 5′-CTGCAGAGACAACGATTCCCAAAATTACTTTGTTCC-3′). Amplification conditions were as follows: an initial denaturation at 95°C for 10 min, 30 cycles at 95°C for 30 s, 55°C for 1 min, and 72°C for 2 min and 30 s, and a final extension step at 72°C for 10 min. Amplification products of approximately 2.7 Kbp were gel purified, ligated to pGEM-T Easy (Promega), and sequenced by standard Sanger sequencing using a primer walking approach (Azenta, Germany). The arrangement of open reading frames within the 2,724-nt circular DNA of the CSAV_A9PstI clone (accession no. PQ392009) is similar to those reported for mastreviruses, including the coat protein, the movement protein, the replication-associated protein (Rep), and the RepA protein. In addition, the CSAV_A9PstI sequence shares 95.3% genome-wide identity with the SaM1 consensus sequence assembled from transcriptomics data (Martínez-Fajardo et al. 2024). Overall, these results indicate that SaM1 is widely present in saffron in different regions of Northeast Iran. In addition, while all tested plants were infected by several viruses, the etiology of SaM1 remains to be determined precisely. To our knowledge, this is the first report of SaM1 from saffron in Iran.

*References*:

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The author(s) declare no conflict of interest.

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