Received: 15 December 2024 | Accepted: 12 February 2025

DOI: 10.1002/ndr2.70022

New Disease Reports Open Access WILEY

NEW DISEASE REPORT

First detection of saffron dwarf virus, wheat dwarf virus, wheat dwarf virus-associated alphasatellite and a new putative potyvirus species in saffron in Iran

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Funding information
University of Tehran, Grant/Award Number: 73148924/6/19; fond national de la recherche scientifique; Iran National Science Foundation, Grant/Award Number: 4000914

KEYWORDS
Crocus sativus, high-throughput sequencing

Saffron (*Crocus sativus*, Iridaceae), the most valuable spice in the world, is propagated vegetatively through corms and cultivated widely in Iran (Moradi et al., 2021). Several viruses have been reported in saffron in Iran: saffron latent virus, saffron yellow mosaic virus and beet western yellows virus (Parizad et al., 2018; Tavoosi et al., 2024; Haseli et al., 2024).

During 2022, a total of 100 leaf tissue samples were collected from diseased (leaf curling, mosaics and mottling) (Figure 1) and asymptomatic saffron plants growing in cultivated fields in Fars and Tehran provinces in Iran (an area of 8,385 and 1,650 ha, respectively). The diseased and asymptomatic samples were pooled together into two groups of 50. Viral particles were enriched from the pooled samples using the virion-associated nucleic acid extraction protocol (Maclot et al., 2021). Libraries were prepared with the Illumina TruSeq PCR-Free preparation kit, and sequencing was performed on the NovaSeq 6000 platform with paired-end reads (2 × 150 bp). A total of 2,305,263 raw reads were obtained by high-throughput sequencing (HTS) and trimmed to 1,609,204 high-quality reads (with a quality score above

FIGURE 1 Leaf curling, mosaic and mottling symptoms in saffron leaf samples collected from Fars and Tehran provinces, Iran

TABLE 1 Species-specific primers designed in this study for PCR-based verification of high-throughput sequencing results (saffron dwarf virus, SaDV; saffron Iran virus. SaIRV; wheat dwarf virus, WDV; wheat dwarf virus-associated alphasatellite. WDVaA). Primers were used with the following PCR mix and cycling conditions: Mango *Taq* polymerase (Bioline, Belgium) with dNTPs (0.20 mM), $MgCl_2$ (2.00 mM), and PCR primers at a final concentration of 0.20 μM, the cycling profile included an initial step at 94°C for 60 seconds, followed by 40 cycles of 94°C for 20 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 180 seconds

Primer	Sequence (5′-3′)	Product size (bp)
SaDV-F	TCAGTCGGAAATTGCCTGGA	496
SaDV-R	GACGTACTGTCCTTCACCCA	
SaIRV-F	TGATAGCTCGTTGTCGCCAT	569
SaIRV-R	ATGCCTCAATCATTGCTGCG	
WDV-F	AGAAGGTCGGATCCAAGTCG	576
WDV-R	AACCCAGACGAAGATTGGCT	
WDVaA-F	GGAAGAATCGCCATCAACGG	376
WDVaA-R	CTTCTCAAGTACGCGTTGCC	

32) using BBDuK (version 38.84) with default parameters within Geneious Prime 2020.2. After *de novo* contig assembly using the SPAdes assembler (version 3.13.0), 6,608 contigs were generated. The tblastx comparison was conducted with a local database created using the viral refseq database (downloaded from NCBI, November 2021). The tentative viral contigs were extended by iterative mapping using Geneious assembler (custom sensitivity mode, with mismatch set according to the demarcation criteria of each virus). This resulted in three sequences with high nucleotide identity with known species: a sequence of 2,538 nt with 98% identity to wheat dwarf virus (WDV; GenBank Accession No. KU877917); a sequence of 2,726 nt with 96% identity to saffron dwarf virus (SaDV, BK067261); and a sequence of 1,486 nt with 89% identity to wheat dwarf virus-associated alphasatellite (WDVaA, PP445014). In addition, a contig of 9,548 nt (PQ740911) had limited homology (<71%) with various potyviruses, and the putative polyprotein (3,113 aa) shared 62% identity with Narcissus yellow stripe virus (BBE01234), suggesting that this sequence might correspond to a new potyvirus based on the demarcation threshold for members of the genus (Inoue-Nagata et al., 2022). To confirm the presence of the viruses detected in the pooled samples, PCR/RT-PCR assays were developed using novel specific primer pairs (Table 1). Amplified cDNA fragments of the expected size were sequenced bi-directionally, and showed 93–100% identity with the corresponding genome sequences obtained by HTS.

These detections correspond to the first report of the presence of WDV (PQ740908), WDVaA (PQ740910) and SaDV (PQ740909) in saffron plants in Iran. The discovery of a putative new potyvirus (PQ740911), tentatively named *Potyvirus crociranense* (saffron Iran virus, SaIRV) will be further investigated. HTS data is available under Bioproject PRJNA1187297. Additional research is necessary to determine the prevalence and effects of these viruses on saffron crop.

ACKNOWLEDGEMENTS

A. Dizadji, A. Golnaraghi and S. Massart contributed equally to this report. The authors would like to acknowledge the financial supports of Iran National Science Foundation (INSF, Project No. 4000914), Fond National de la Recherche Scientifique (FNRS, Belgium; Grant J.0149.20) and University of Tehran (73148924/6/19).

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How to cite this article: Valouzi, H., Dizadji, A., Golnaraghi, A., Salami, S.A., Selmi, I., Fontdevila Pareta, N. et al. (2025) First detection of saffron dwarf virus, wheat dwarf virus, wheat dwarf virus-associated alphasatellite and a new putative potyvirus species in saffron in Iran. *New Disease Reports*, vol.51, e70022. https://doi.org/10.1002/ndr2.70022