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Short Communication

The first report of the occurrence of alfalfa mosaic virus on *Tropaeolum majus* and *Rudbeckia hirta* in Iran

Mehrdad Salehzadeh[a], Alireza Afsharifar[a]*, Arezoo Pakdel[b], Tayebeh Kiani[c]

[a] Plant Virology Research Center, School of Agriculture, Shiraz University, Shiraz, I. R. Iran
[b] Plant Protection Department, Faculty of Agriculture, Bu-ali Sina University, Hamedan, I. R. Iran
[c] Plant Pathology, Shahid Chamran University, Ahvaz, I. R. Iran

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ABSTRACT- *Tropaeolum majus* (garden nasturtium) and *Rudbeckia hirta* are common ornamental plants that can be susceptible to plant virus infections, leading to symptoms that adversely affect their ornamental appeal and commercial production. In this research, severe leaf chlorosis was observed on *T. majus* and *R. hirta* plants in green spaces in Shiraz and Bushehr, Iran (2023). Total RNA was extracted from five symptomatic leaf samples of each species, as well as one asymptomatic sample as a negative control. RT-PCR with alfalfa mosaic virus (AMV)-specific primers successfully amplified the expected 780 bp fragment in all symptomatic samples, but not in the asymptomatic control. Nucleotide sequencing confirmed the 780 bp size of the amplicon, and analysis revealed that the AMV isolates infecting *T. majus* (IR-GN01-Shiraz and IR-GN02-Bushehr) were most similar to an Iranian AMV isolate (Acc. No. MW_014931.1), while those infecting *R. hirta* (IR-AN04-Shiraz and IR-AN03-Bushehr) were the most similar to Chinese AMV isolates. This study is the first report of AMV infecting *T. majus* and *R. hirta* in Iran, a noteworthy finding, considering the extensive cultivation of these ornamental plants and the potential impact of AMV on their visual and economic value.

INTRODUCTION

Tropaeolum majus, commonly known as garden nasturtium, is a ground-covering, flowering annual herb that is indigenous to the Andes Mountains in South America. This ornamental species is known for its edible leaves and flowers, which contain a peppery flavor (Jens & Birger, 1993), and the flowers exhibit a range of yellow, red, or orange colors, with orange being the most prevalent (Bloem et al., 2014). *Rudbeckia hirta*, commonly referred to as Black-Eyed Susan, is a North American flowering plant of the *Asteraceae* family, native to the eastern and central regions of the continent. *R. hirta* is generally used in parks, gardens, borders, and also as a cut flower (Vardeman et al., 2022). Viral diseases pose a significant threat to these ornamental plants, inducing a wide range of symptoms that can diminish their decorative worth and result in substantial financial loss. The vegetative propagation and global trade of infected plant materials can worsen the detrimental impact of viral diseases on the economic value of ornamental plants (Kumari et al., 2021).

Alfalfa mosaic virus (AMV) primarily infects herbaceous plants, but its host range naturally includes more than 600 species within 70 families. This virus is commonly transmitted by a large number of aphid species (more than 15 species) in a non-persistent manner (Bol, 2003). In recent years, AMV has reportedly occurred on various ornamental plants, including *Euonymus japonica* var. *microphylla* (Bellardi et al., 1994), *Viburnum opulus* L. (Parrella et al., 2011), *Hibiscus rosa-sinensis* (Parrella et al., 2012), *Lavandula* × *intermedia* (Vrandečić et al., 2013), several petunia cultivars (Pilić et al., 2019), *Araujia sericifera* (Parrella et al., 2013), and numerous *Lavandula* spp., *Origanum* spp., *Aubrieta* spp., *Dahlia* spp., and *Zantedeschia* spp. (Fletcher, 1987).

Over the past few years, *T. majus* and *R. hirta* have gained popularity as ornamental plants. However, their cultivation has been partially marginalized by a rise in virus-related diseases (Parrella et al., 2012; Salehzadeh et al., 2024). This is largely attributed to the vegetative propagation of these plants, primarily through seeds and cuttings, as well as the international trade of unchecked plant materials, which has exacerbated the widespread dissemination of viral pathogens among cultivated garden nasturtiums globally. Therefore, it is crucial to identify the scientific identification of viral agents affecting *T. majus* and *R. hirta* (Gera & Zeidan, 2004). Ornamental plants serve several essential purposes in landscape design. They provide key benefits, such as adding aesthetic appeal to the landscape by introducing color, texture, and variety, creating visually stunning settings that enhance the overall beauty of an area. The objective of this research was to identify the type of virus affecting *Tropaeolum majus* and *Rudbeckia hirta* plants showing viral symptoms in the regions of Bushehr and Shiraz.

* Corresponding author: Professor of Plant Virology Research Center, School of Agriculture, Shiraz University, Shiraz, I. R. Iran
E-mail address: afshari@shirazu.ac.ir
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MATERIALS AND METHODS

In the green spaces of Shiraz and Bushehr cities, southern Iran (January 2023), severe yellow mosaic symptoms were observed on the leaves of *T. majus* and *R. hirta* plants (Fig. 1). To determine the presence of AMV infection in the plant material, five symptomatic leaf samples from each plant species and each city were selected for further analysis by total RNA extraction from 10 symptomatic samples and an asymptomatic one, using TRIzol reagent (Denazist, Iran). The extracted RNA samples underwent RT-PCR analysis, employing a set of degenerate primers (AMV-F and AMV-R) to amplify a 780-nucleotide fragment corresponding to the coat protein (CP) gene of AMV (Masoumi et al., 2012). The PCR reaction mixture and cycling conditions were 25 µL total volume, containing 12 µL PCR master mix (Denazist, Iran), 9 µL H_2O, 2 µL of each 10 µM primer, and 1 µL cDNA. This was followed by an initial denaturation at 94 °C for 5 minutes, and then 35 cycles of 94 °C for 60 seconds (denaturation), 58 °C for 60 seconds (annealing), and 72 °C for 60 seconds (extension), with a final extension at 72 °C for 10 minutes. The PCR amplicons were visualized on a 1.5% agarose gel stained with ethidium bromide.

RESULTS AND DISCUSSION

RT-PCR products of the expected size (~780 bp) were obtained for all 20 symptomatic samples. No amplification was observed in the asymptomatic *T. majus* and *R. hirta* leaf samples (Fig. 2). The amplified products of the samples per plant species and city were eluted from the gel and subjected to the direct sequencing (Sinohe Inc., Shiraz, Iran). Sequences of the Iranian AMV-isolates were aligned and compared to other AMV nucleotide sequences (AMV-CP) that had been already deposited in the NCBI database. Comparisons were made via MEGA software (version 8.0).

Sequence analysis revealed that the Iranian AMV isolates infecting *T. majus* plants (IR-GN01-Shiraz and IR-GN02-Bushehr) were closely related to other AMV isolates in GenBank. They showed a maximum similarity identity of 98.7% and 99.4%, respectively, to another Iranian AMV isolate (Acc. No. MW_014931.1), and the lowest similarity identity of 95.9% and 96.7%, respectively, to a USA AMV isolate (Acc. No. MT_596817) (Fig. 3). Similarly, the Iranian AMV isolates infecting *R. hirta* plants (IR-AN04-Shiraz and IR-AN03-Bushehr) showed a maximum similarity identity of 98.9% and 99.6%, respectively, to a Chinese AMV isolate (Acc. No. OL_706266.1), and the lowest similarity identity of 96.9% and 97.2%, respectively, to an Italian AMV isolate (Acc. No. MT_09322211.1) (Fig. 3).

Phylogenetic analysis using the maximum likelihood (ML) method in MEGA version 8.0 software (Tamura et al., 2007), with 100 bootstrap replicates, showed that the Iranian AMV isolates infecting *T. majus* and *R. hirta* were resolved into distinct cluster groupings with other Iranian and Asian AMV isolates, respectively (Fig. 3).

To assess potential co-infections with other viruses (Salehzadeh et al., 2023; Salehzadeh., 2022), PCR reactions were performed using cucumber mosaic virus-specific primer pairs and two degenerate primer pairs corresponding to begomoviruses and tobamoviruses. However, no additional viral infections were detected.

To our current knowledge, this study represents the first report of alfalfa mosaic virus infecting *T. majus* and *R. hirta* plants in Iran. Due to the economic importance of *T. majus* and *R. hirta* cultivation, as well as the detrimental effects of AMV on the growth, yield, and quality of various plant species, it is crucial to comprehend the genetic diversity of AMV viruses affecting these plants. This knowledge allows for the development of specific treatments, the identification of resistant plant varieties, and the formulation of efficient management practices.

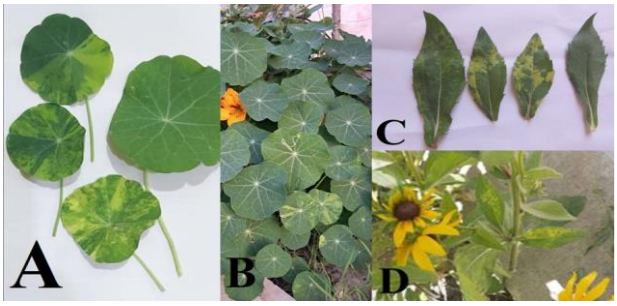

Fig. 1. The presence of mosaic and chlorosis symptoms on leaves of *T. majus* (A and B) and *R. hirta* (C and D) plants infected with alfalfa mosaic virus (AMV) in the surveyed regions of southern Iran (Shiraz and Bushehr cities).

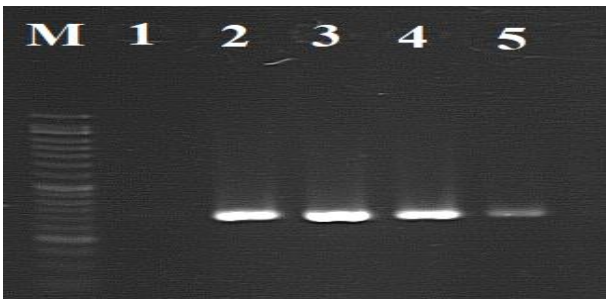

Fig. 2. Agarose gel electrophoresis of RT-PCR amplicon (approximately 780 bp fragment), using the AMV-specific primer pair (AMV-F and AMV-R) from naturally infected *T. majus* and *R. hirta* leaves. Lane M contains the 100 bp DNA size ladder (Denazist, Iran). Lanes 2 and 3 correspond to symptomatic *T. majus* plants (IR-GN01-Shiraz and IR-GN02-Bushehr, respectively), while lanes 4 and 5 correspond to *R. hirta* plants (IR-AN04-Shiraz and IR-AN03-Bushehr, respectively) that were positive for AMV detection. Lane 1 represents a healthy, asymptomatic control plant.

Phylogenetic analysis was performed and constructed based on alignments of 780 nucleotide sequences of an Iranian isolate of alfalfa mosaic virus (AMV) compared to sequences of other AMV isolates from GenBank. The tree was generated using the maximum likelihood (ML) method packaged in MEGA-8.0. While providing accession numbers of the virus isolates, we used cucumber mosaic virus (CMV, AB448694.1, *Nicotiana tabacum*, Syria) from the genus *Cucumovirus* (Salehzadeh, 2018) as an outgroup. Branches with lower than 97% support were excluded from consideration. The phylogenetic tree contains sequences from various plant species, such as *Sechium edule* from Italy, *Nicotiana tabacum* from Germany, *T. majus* from Shiraz and Bushehr, *Wisteria sinensis* from Iran, *Vicna minor* from Iran, *Plantago* sp. from Iran, *Nicotiana glutinosa* from China, *Medicago*

sativa from China, *R. hirta* from Shiraz and Bushehr, *Glycine max* from the USA, and *Nicotiana tabacum* from Syria (Fig. 3).

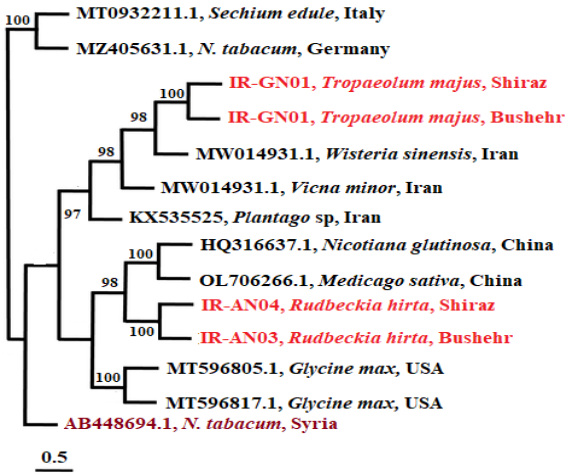

Fig. 3. A phylogenetic tree of an Iranian isolate of alfalfa mosaic virus (AMV) based on the nucleotide sequences of a coat protein gene portion. Multiple sequence alignment was performed using the maximum likelihood algorithm implemented in the MEGA 6.0 software package. The partial AMV-CP genes analyzed in this study are highlighted in red.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Mehrdad salehzadeh and Alireza Afsharifar; Methodology: Mehrdad salehzadeh and Alireza Afsharifar; Software: Mehrdad salehzadeh and Arezoo Pakdel; Validation: Mehrdad salehzadeh, Alireza Afsharifar, Arezoo Pakdel, and Tayebeh Kiani; Investigation: Mehrdad salehzadeh and Alireza Afsharifar; Resources: Arezoo Pakdel and Tayebeh Kiani; Data curation: Arezoo Pakdel and Tayebeh Kiani; Writing—original draft preparation: Mehrdad salehzadeh and Alireza Afsharifar; Writing—review and editing: Alireza Afsharifar; Visualization: Mehrdad salehzadeh and Arezoo Pakdel; Supervision: Alireza Afsharifar; Project administration: Alireza Afsharifar; Funding acquisition: Alireza Afsharifar.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

This work is not related to experimental animals or specific human diseases that requires publication and approval of publication ethics.

DATA AVAILABILITY

The authors declare that the datasets are available from the corresponding author on request.

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