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Original Article

First Report of the Occurrence of Tomato Spotted Wilt Virus in Epipremnum Aureum in Iran

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Abstract

Background and Aims: Epipremnum aureum (pothos), a perennial ornamental plant belonging to the family Araceae, is widely cultivated as a decorative houseplant and greenhouse ornamental species. Viral diseases are among the most important constraints affecting the quality, marketability, and propagation of ornamental plants. During a survey conducted in January 2023 in ornamental plant greenhouses in Maragheh, Yazd, Shiraz, Tehran, and Bushehr, Iran, pothos plants showing chlorotic and necrotic spots, yellowing, concentric ringspots, mosaic patterns, and leaf deformation were observed. Because these symptoms were similar to those caused by orthotospoviruses, the present study aimed to investigate the possible natural infection of E. aureum by Tomato spotted wilt virus (TSWV), currently classified as Orthotospovirus tomatomaculae.

Material and methods: Twenty-five symptomatic pothos leaf samples and one asymptomatic sample used as a negative control were collected and subjected to total RNA extraction. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using the degenerate orthotospovirus primer pair Tospo_GENs/Tospo_GENas, which amplifies a conserved region of the nucleocapsid gene. The amplified fragment was purified and sequenced by Sanger sequencing. The obtained sequence was compared with representative TSWV sequences available in GenBank using BLASTn and phylogenetic analysis.

Results: RT-PCR generated an amplicon of the expected size, approximately 420 bp, from all symptomatic samples, whereas no amplification was observed from the asymptomatic control plant. Sequence analysis confirmed that the amplicon corresponded to TSWV. The Iranian pothos isolate showed high nucleotide identity with previously reported TSWV isolates from other geographical regions, supporting its classification as TSWV.

Conclusions: To our knowledge, this is the first confirmed report of TSWV naturally infecting E. aureum in Iran. The detection of TSWV in pothos highlights the potential role of ornamental foliage plants as reservoirs of orthotospoviruses in greenhouse production systems. Further surveys, vector monitoring, full-genome sequencing, and biological assays are recommended to determine the epidemiological significance of TSWV infection in pothos and its potential risk to nearby ornamental and vegetable crops.

Keywords: *Epipremnum aureum,* golden pothos, ornamental plants, Tomato spotted wilt virus, tomato spotted wilt orthotospovirus, Orthotospovirus.

Introduction

Plant viruses infect a wide range of cultivated, ornamental, and wild plant species and are responsible for substantial economic losses worldwide (16). Among them, Tomato spotted wilt virus (TSWV), currently classified as *Orthotospovirus tomatomaculae*, is one of the most economically important plant viruses because of its broad host range, efficient thrips-mediated transmission, and ability to cause severe disease in both green-house and field crops (2, 15, 30, 39). TSWV has been reported from numerous botanical families and infects a

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wide range of vegetable, field, ornamental, and weed hosts (24, 30). TSWV is transmitted in a persistent-propagative manner by several thrips species, particularly *Frankliniella occidentalis*, *Thrips tabaci*, and related vectors (6, 19). The virus is acquired by thrips during the larval stage and subsequently transmitted by adults, making vector management a major component of disease control (3, 19). In greenhouse systems, the combination of susceptible ornamental hosts, continuous plant production, and persistent thrips populations can facilitate virus survival and spread (11, 13, 20). Ornamental plants may therefore serve not only as economically affected crops but also as epidemiologically important reservoirs for TSWV and other orthotospoviruses (13, 20, 21, 30).

The symptoms induced by TSWV vary depending on host species, cultivar, plant age, virus isolate, environmental conditions, and vector pressure (5, 17, 34, 38). Typical symptoms include chlorotic or necrotic spots, concentric ring spots, line patterns, leaf deformation, mosaic, bronzing, venial necrosis, stem necrosis, stunting, wilting, and flower malformation (13, 20, 2, 30). In ornamental crops, these symptoms are particularly important because even mild foliar distortion or discoloration can significantly reduce marketability (20–22, 28). In addition, some infected ornamental plants may remain asymptomatic or express symptoms only after a latent period, complicating visual diagnosis and increasing the risk of silent virus dissemination through vegetative propagation and plant trade (20, 21, 28).

TSWV has been reported from many ornamental hosts, including chrysanthemum, impatiens, petunia, cyclamen, gloxinia, gerbera, begonia, zinnia, pelargonium, lisianthus, and several foliage ornamentals (8, 11, 13, 20–22, 25, 28, 30). Previous studies have emphasized that ornamental plants can act as important reservoirs of TSWV in protected cultivation systems, especially where susceptible hosts and thrips vectors coexist (11, 13, 20, 21). However, the host status of many ornamental foliage plants remains insufficiently documented in several regions, including Iran.

Epipremnum aureum is a widely cultivated ornamental foliage plant valued for its tolerance to indoor conditions, rapid vegetative growth, and use in interior landscaping. Because it is commonly propagated vegetatively and maintained in greenhouse and nursery environments, infected plants may contribute to the long-distance movement and local persistence of plant viruses. TSWV has previously been reported from *E. aureum* in Korea (25), but there has been no confirmed report of natural infection of pothos by TSWV in Iran.

During surveys conducted in summer 2024 in ornamental greenhouses in Maragheh, Tehran, Shiraz, Bushehr, and Yazd, Iran, pothos plants showing chlorotic and yellow ring spots, leaf deformation, and distortion were observed. The present study was therefore conducted to detect and identify the causal virus associated with these symptoms using RT-PCR, Sanger sequencing, and phylogenetic analysis. The results provide molecular evidence for the natural occurrence of TSWV in *E. aureum* in Iran and expand current knowledge of the host range and epidemiological importance of this virus in ornamental production systems (21, 25, 30, 39).

Methods and Materials

Sample Collection

During a survey conducted in January 2023, *Epipremnum aureum* plants showing virus-like symptoms were observed in ornamental greenhouses in Maragheh, Yazd, Shiraz, Tehran, and Bushehr, Iran. The symptoms included white to yellow chlorotic spots, concentric ring spots, mosaic patterns, and leaf malformation (Figure 1). Similar symptoms have been reported in TSWV-infected ornamental hosts and other susceptible plants (11, 13, 20, 21, 25, 30). Five symptomatic leaf samples were collected from each city, giving a total of 25 symptomatic samples. In addition, one asymptomatic *E. aureum* plant was sampled and used as a negative control.

Leaf samples were placed in sterile plastic bags, transported to the laboratory under chilled conditions, and stored at −80°C until RNA extraction. Information on sampling location, symptom type, and sample code was recorded for each sample.

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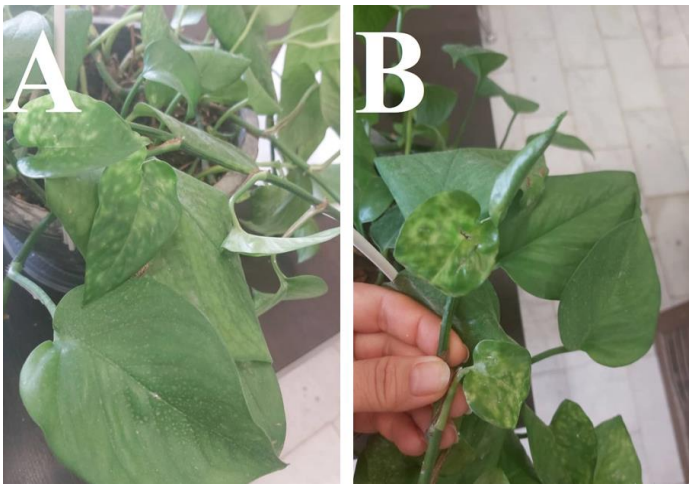

Fig. 1. Symptoms observed on *Epipremnum aureum* plants infected with TSWV in Bushehr province. A: severe chlorotic spots and leaf deformation on *E aureum* B: yellow ringspots and leaf deformation on *E aureum*.

RNA Extraction and RT-PCR Detection of TSWV

Total RNA was extracted from symptomatic and asymptomatic leaf tissues using TRIzol reagent according to the manufacturer's instructions. RNA concentration and purity were assessed spectrophotometrically, and the extracted RNA was used for complementary DNA synthesis.

Detection of Tomato spotted wilt virus was performed by RT-PCR using the degenerate primer pair NSm-F/NSm-R, which amplifies an approximately 420-bp fragment of the NSm gene located on the M RNA segment. Molecular detection using RT-PCR is a widely used approach for identifying plant viruses, includeing orthotospoviruses, in symptomatic plant tissues (21, 30, 40). The primer sequences were as follows: NSm-F, 5′-GCTTAAATAAGTGACATTAAAAATACTATATTTCATC-3′; and NSm-R, 5′-AGAGCAATCAGTGCATCAGAAATATACCTATTATAC-3′.

PCR amplification was performed in a final volume of 25 µL containing 12 µL of 2× PCR master mix, 9 µL of nuclease-free water, 1 µL of cDNA template, and 2 µL of each primer at 10 µM. The thermal cycling conditions were as follows: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 60 s, annealing at 58°C for 60 s, and extension at 72°C for 60 s; followed by a final extension at 72°C for 10 min.

PCR products were separated by electrophoresis on a 1% agarose gel prepared in Tris-acetate-EDTA buffer, stained with ethidium bromide, and visualized under UV illumination. Amplicon size was estimated by comparison with a DNA molecular size marker.

Sequencing and Sequence Analysis

Representative RT-PCR products were selected from each geographical region based on the clarity and intensity of the electrophoretic bands. Two representative amplicons from each region were submitted for bidirectional Sanger sequencing, except for Maragheh, from which one representative amplicon was sequenced. Sequencing was performed by Sinuhe Company, Shiraz, Iran.

Raw forward and reverse chromatograms were inspected, trimmed, and assembled to obtain consensus sequences. The obtained sequences were compared with sequences available in GenBank using the BLASTn program of the National Center for Biotechnology Information. Multiple sequence alignment was performed using ClustalW implemented in MEGA version 8.0. Phylogenetic relationships were inferred using the Maximum Likelihood method in MEGA 8.0. Bootstrap analysis was performed with 100 replicates, and only bootstrap values above the selected threshold were shown on the tree. An isolate of *Impatiens necrotic spot orthotospovirus* was included as an outgroup.

Detection of Possible Mixed Infections

To determine whether the symptomatic *E. aureum* samples were co-infected with other common plant viruses, additional PCR or RT-PCR assays were performed using primer pairs specific or degenerate for selected virus groups. These included primers for Cucumber mosaic virus, begomoviruses, and tobamoviruses, following previously described molecular detection approaches (35–37, 40). PCR products were analyzed by agarose gel electrophoresis as described above.

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Results

Disease Symptoms and RT-PCR Detection

Virus-like symptoms were observed on *E. aureum* plants collected from ornamental greenhouses in Maragheh, Yazd, Shiraz, Tehran, and Bushehr. The most common symptoms were chlorotic and yellow spots, concentric ring spots, mosaic patterns, and leaf deformation (Fig. 1). These symptoms were consistent with those reported for TSWV and related orthotospoviruses in ornamental and horticultural hosts (11, 13, 20, 21, 25, 30).

RT-PCR using the NSm-F/NSm-R primer pair amplified a DNA fragment of the expected size, approximately 420 bp, from all 25 symptomatic samples. No amplification was observed from the asymptomatic *E. aureum* sample used as a negative control. These results indicated that the symptomatic plants were infected with an orthotospovirus closely related to TSWV.

Sequence Identity and BLAST Analysis

Representative amplicons from the surveyed regions were sequenced and deposited in GenBank under accession numbers PQ306600–PQ306607. BLASTn analysis confirmed that all sequences corresponded to TSWV. The Tehran isolate PQ306602 showed the highest nucleotide identity, 98.21%, with a TSWV isolate from South Korea, MT842841.1, obtained from *Petasites japonicus*. The Maragheh isolate PQ306605 showed 97.39% identity with a TSWV isolate from the USA, KU179602.1, obtained from *Hoya* sp.

The Shiraz isolates PQ306603 and PQ306604 showed 98.52% and 98.69% identity, respectively, with the Spanish TSWV isolate FM163373.1, obtained from *Solanum lycopersicum*. The Yazd isolates PQ306606 and PQ306607 showed 98.62% and 98.29% identity, respectively, with the Spanish isolate FM 163371.1, also from *S. lycopersicum*. The Bushehr isolates PQ306600 and PQ306601 showed 97.31% and 97.18% identity, respectively, with the same Spanish isolate FM 163371.1. These levels of sequence identity support the identification of the Iranian pothos isolates as TSWV and are consistent with the broad host range and genetic diversity reported for this virus (17, 24, 30, 32, 34, 39).

Phylogenetic Analysis

A Maximum Likelihood phylogenetic tree was constructed using the partial NSm gene sequences obtained in this study and representative TSWV sequences retrieved from GenBank. The Iranian *E. aureum* isolates grouped within the TSWV clade, confirming their identity as TSWV. The Tehran isolate clustered close to Asian isolates, whereas the Maragheh isolate was placed near the American TSWV isolate obtained from *Hoya* sp. The Shiraz and Yazd isolates grouped with Spanish isolates, while the Bushehr isolates formed a distinct sister cluster separated from the other Iranian and reference isolates (Figure 2).

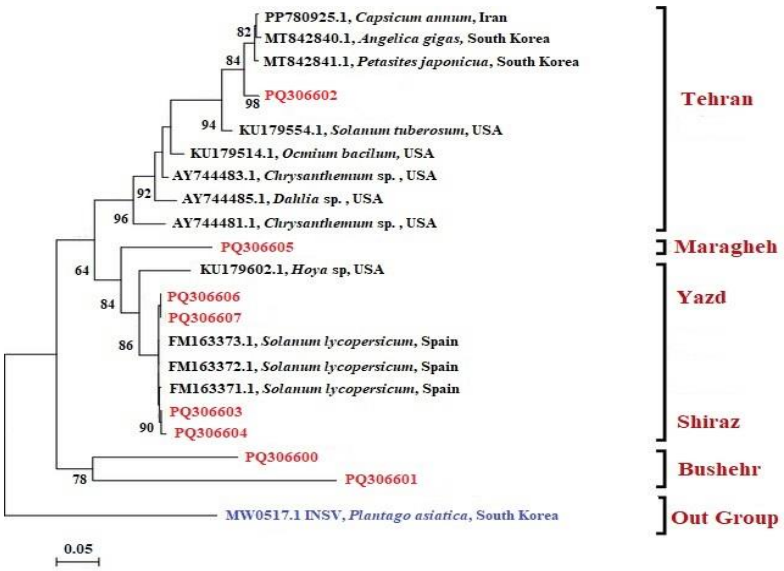

Fig. 2. Phylogenetic analysis of Iranian Tomato spotted wilt virus (TSWV) isolates obtained from *Epipremnum aureum*. The phylogenetic tree was constructed based on the alignment of a 420-bp fragment of the M RNA segment encoding the NSm movement protein. Iranian TSWV isolates from *E. aureum* were compared with 12 representative TSWV isolates retrieved from GenBank. The tree was generated using the Maximum Likelihood method implemented in MEGA version 8.0. An isolate of *Impatiens necrotic spot orthotospovirus* (INSV) from South Korea (GenBank accession no. MW0517.1) was used as an outgroup. Iranian isolates clustered with different TSWV isolates from other regions of the world. Bootstrap values lower than 84% were not considered significant and are not shown at the nodes.

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However, because the phylogenetic analysis was based on a short 420-bp fragment of the NSm gene, the inferred relationships should be interpreted cautiously. The data are sufficient for molecular confirmation of TSWV infection but are not sufficient to determine the precise geographical origin or introduction pathway of the Iranian isolates. Full-genome sequencing and broader sampling would be required for more robust inference of virus diversity, population structure, and possible introduction routes (17, 31, 34, 39).

Detection of Mixed Infections

No PCR products were obtained using primer pairs targeting Cucumber mosaic virus, begomoviruses, or tobamoviruses. These results suggest that the tested samples were not infected with the screened viruses. However, because only selected virus groups were tested, the absence of other viral co-infections cannot be ruled out completely. Broader detection approaches, such as high-throughput sequencing or additional diagnostic assays, would be required to exclude the presence of other viruses.

Discussion

This study provides molecular evidence for the natural infection of *Epipremnum aureum* by Tomato spotted wilt virus in Iran. TSWV was detected by RT-PCR in all symptomatic pothos samples collected from five Iranian cities, and sequence analysis of representative isolates confirmed their identity as TSWV. To our knowledge, this is the first report of TSWV infecting *E. aureum* in Iran.

The detection of TSWV in pothos is epidemiologically important because *E. aureum* is widely propagated vegetatively and commonly maintained in ornamental greenhouses, where thrips vectors may facilitate virus spread (19, 20, 21, 30). Infected pothos plants may therefore serve as a source of primary inoculum for other ornamental or vegetable hosts grown nearby (13, 20, 2, 30). Further studies should include broader surveys, vector monitoring, full-genome sequencing, biological indexing, and evaluation of the role of pothos in TSWV epidemiology under greenhouse conditions.

Mixing plant viruses has become a major challenge. It is in controlling these disease agents (38). We used PCR to find other viruses in the infected samples. We looked for co-infections of other viruses alongside TSWV using four different primer pairs. However, no PCR products were obtained using these primer pairs. This shows that other viruses did not infect the tested plants along with TSWV have found many different viruses in canna in many countries (39).

TSWV was first reported from *E. aureum* by lee et al., (2022) (40). The TSWV symptoms reported by these researchers matched the symptoms sampled from *E. aureum* in this study. The samples came from different cities of Iran. This is the first report of Tomato spotted wilt virus on *E. aureum* in Iran.

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Disclosure

None

Conflict of Interest

No conflict of interest is declared.

Funding

None

Ethics Approval and Consent to Participate

Not applicable

Data Availability Statement

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All data generated or analyzed during this study are included in this article.

Author Contributions

All authors contributed to the study design. Alireza Afsharifar and Mehrdad Salehzadeh prepared materials, collected data, and analyzed nucleotide sequences. Alireza Afsharifar wrote the first draft. All the authors commented on earlier versions. All authors read and approved the final file of the manuscript.

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