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Short Paper

The first report of melon yellow spot virus *Orthotospovirus meloflavi* occurrence in Iran

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Abstract: During a survey in the spring of 2024, noticeable severe leaf yellow spots, yellowing, fruit necrosis, and chlorotic ring spots symptoms were observed in Cucumber plants cultivated near Isfahan City, central Iran, to assess Orthotospovirus infection. Total genomic RNA was individually extracted from the leaves of 5 symptomatic and one asymptomatic leaf sample. The extracted RNA samples were subsequently subjected to RT-PCR using an Orthotospovirus-universal primer pair. An amplicon of the expected size was obtained with all diseased samples, and a BLAST search of its nucleotide sequences exhibited a high level of nucleotide identity with several MYSP isolates. This is the first report of the melon yellow spot virus (MYSV) *Orthotospovirus meloflavi* occurrence in Iran.

Keywords: Iran, Isfahan, Phylogeny, *Cucurbitaceae*, MYSV

Introduction

Melon yellow spot virus (MYSV; *Orthotospovirus meloflavi*) was first identified in 1992 in Japan, causing spotted wilt symptoms and severe chlorosis on melon crops (Wu *et al*., 2024). It was also found that MYSV is persistently transmitted by *Thrips palmi* (McLeish *et al*., 2022). In addition to melons *Cucumis melo*, diseases caused by MYSV have been observed on cucumbers *Cucumis sativus* (Takeuchi *et al*., 2001), watermelon *Citrullus lanatus* (Iwaki *et al*., 1984), and balsam pear *Momordica charantia* (Takeuchi *et al*., 2009). MYSV has also been detected in several weed species commonly occurring around cucumber greenhouses (e.g. *Acalypha australis, Capsella bursa-pastoris, Conyza canadensis, Conyza sumatrensis, Gnaphalium purpureum, Lamium amplexicaule, Oxalis corniculata, Sonchus oleraceus, Stellaria media, Stellaria neglecta, Veronica persica*) (Quito-Avila *et al*., 2014). MYSV causes chlorotic spots, mosaic mottling, and yellowing on melon leaves, as well as mosaic patches on melon fruits and occasional mottling on cucumber fruits. These symptoms lead to decreased fruit quality and significant yield losses. Melon thrips *Thrips palmi* transmit MYSV persistently, primarily acquiring the virus during their first larval instar and transmitting it as adults. MYSV is not spread through soil contamination or seeds (Kato *et al*. 2000). At present, MYSV is considered to be one of the most serious threats to Cucumis production in the world. When infection occurs early in the season, yield losses of 30 to 60% have been observed. MYSV has been reported all over the world in the last decade on cucurbitaceous crops (Wu *et al*., 2024). *C. sativus* is a significant vegetable crop that is cultivated year-round in Iran.

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This research presents the first report on the m*elon yellow spot orthotospovirus* in Iran.

Materials and Methods

During a greenhouse survey conducted in the spring of 2024, Cucumis (*C. sativus*), plants exhibiting suspected Orthotospovirus infection symptoms, such as severe leaf yellow spots, yellowing, fruit necrosis, and chlorotic ring spots (Fig. 1), were collected from Shahinshahr city (Isfahan province, Iran), in spring 2024 and kept at −80 °C until further processing. To confirm Orthotospovirus infections, total RNA was extracted from five symptomatic and one asymptomatic sample of *C. sativus* plants using TRIzol reagent (Sinaclone.Iran), followed by reverse transcription-polymerase chain reaction (RT-PCR) assays employing a set of degenerate Orthotospovirus primers (Tospo_GENs, Tospo_GENas) targeting a 420-nucleotide segment of the M (NS_M protein) gene (Bald-Blume *et al*., 2017). PCR was carried out in a 25 µl reaction mixture containing 12 µl PCR mastermix (Denazist, Iran), 9 µl H_2O, 2 µl of each primer (10 µM), and 2 µl cDNA. The mixture was initially denatured at 94 °C for 5 minutes, followed by amplification through 35 cycles, each cycle consisting of denaturation at 94 °C for 60 seconds, annealing at 57 °C for 60 seconds, and extension at 72 °C for 60 seconds, with a final cycle at 72 °C for 10 minutes. Subsequently, the amplification products were resolved on 1% (w/v) Tris-acetate agarose gels and stained with ethidium bromide. The RT-PCR analysis yielded a DNA fragment of the expected size (~420 bp) in all of the five examined symptomatic samples, while no RT-PCR product was obtained from the asymptomatic leaf sample. Subsequently, the RT-PCR products from two samples were purified and directly sequenced (one-direction sequenced) by Sinohe Inc., Iran. The obtained DNA sequences were analyzed using the nucleotide Blast Program (NCBI) and then aligned and compared with some other corresponding sequences available in the GenBank using MEGA version V 8.0. A phylogenetic tree reconstructed based on the alignment of a 420 base pair fragment of RNA-M (NS_M protein gene) of the genome of two Melon yellow spot virus (MYSV) isolates from *Cucumis sativus* in comparison with the same region of the homologous gene of ten exotic MYSV isolates retrieved from GenBank. The Maximum Likelihood (ML) Method in MEGA-8.0 Software was Utilized for phylogenetic tree reconstruction (Tamura *et al*., 2007). An isolate of Tomato spotted wilt virus from Spain (AC: HQ537114.1) was used as an outgroup species. Iranian isolates clustered with Asian isolates (China and Indian isolates). Branches with less than 86% support were excluded from consideration. Nodes that support less than 86% of bootstrap values are not reported. The tree depicts isolated numbers and geographical origins. Further PCR assays targeting other viral groups, including a degenerate primer pair of potyviruses Nib1/Nib3R (Gibbs and Mackenzie, 1997); a degenerate primer pair of begomoviruses $primer^{BC}$ (Deng *et al*., 1994) / $primer^{181V}$ (Rojas *et al.,* 1993) and tobamoviruses degenerate primer pair TobamodF/TobamodR (Li *et al*., 2018), was conducted to explore the possibility of MYSV mixed infection with other significant viruses infecting *C. sativus*.

Results and Discussion

PCR products of the expected size (approximately 420 bp) were obtained from all of the symptomatic samples (five samples each of *C. sativus* plants), while no amplified products were generated from any of the healthy leaf samples of *C. sativus* plants. Amplified fragments from two samples were gel purified using a PCR purification kit (Qiagen Co.) and sequenced directly using a paired-end sequencing strategy (Sinohe Co. Iran). Multiple alignments of the obtained of Iranian nucleotide sequences (corresponding to a part of NSM gene) showed that Iranian isolates are most similar to Chinese and Indian isolates of the MYSV. Based on this, the PQ429064 isolate is most similar (98.1%) to an MYSV isolate from China (AC: OQ784918.1; isolate: HNTG; host: *Cucumis melo*) And PQ429065 isolate.... it is most similar (98.9%) to Indian (AC: OQ559640.1; isolate: IN-Kar; host:

C. sativus) isolates of the MYSV. In phylogenetic analysis, Iranian isolates with grouped Asian strains, particularly Chinese and Indian isolates (Fig. 2), suggest a possible introduction through agricultural product trade. Further PCR assays targeting various viral groups, including potyviruses, tobamoviruses and begomoviruses, yielded no positive results.

Figure 1 Symptoms observed on *C. sativus* plants infected with MYSV in Shahinshahr; Isfahan province. A: severe ring spot and tissue chlorosis on *C. sativus* fruit B: yellow spots on *C. sativus* leaves.

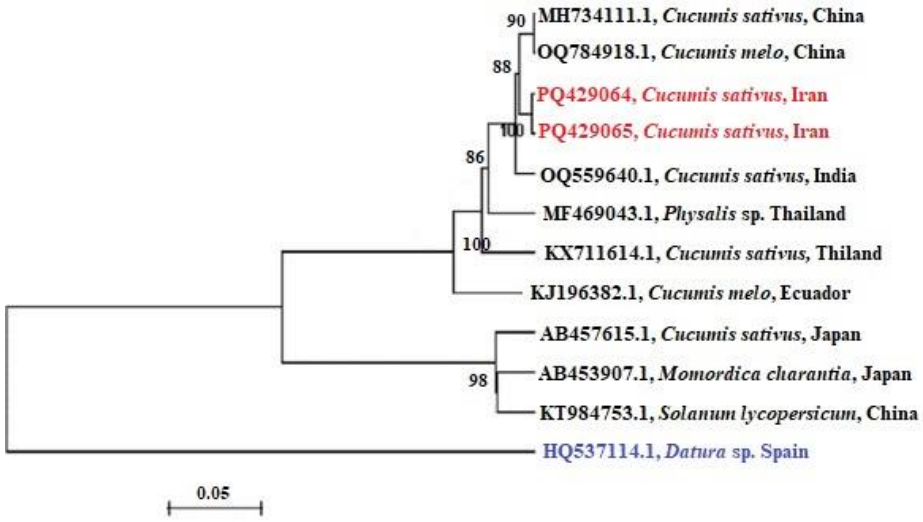

Figure 2 A phylogenetic tree was reconstructed based on the alignment of a 420 base pair fragment of the RNA-M (NSM protein gene) from two Iranian melon yellow spot virus (MYSV) isolates obtained from *C. sativus*, compared to the same region of the homologous gene from ten exotic MYSV isolates retrieved from GenBank. The Maximum Likelihood (ML) method in MEGA 8.0 software was utilized for the tree reconstruction. An isolate of Tomato spotted wilt virus from Spain (AC: HQ537114.1) served as the outgroup species. Branches with less than 86% support were excluded from consideration, and nodes with bootstrap values below this threshold are not reported. The tree indicates the isolated numbers and their geographical origins.

This suggests that there is no mixed infection of MYSV with other viruses. The detection of MYSV in *C. sativus* plants in Iran represents the first report of this virus in Iran. Given the economic importance of cucurbitaceous crops and related industries in southern Iran and the potential of emerging viruses to rapidly spread and impact crop production, ongoing research aims to elucidate the genetic variations, pathogenicity, host range, and distribution of MYSV in *Cucurbitaceae* plants under greenhouse and open-field conditions in Iran.

Statements and Declerations

Competing Interests

Authors declare they have no financial interests.

Author Contributions

All authors contributed to the study conception and design, Material preparation, data collection and nucleotide sequence analysis were performed by Alireza Afsharifar and Mehrdad Salehzadeh. The first draft of the manuscript was written by Alireza Afsharifar, and all authors commented on previous versions of the manuscript. All authors read and approved the final file of the manuscript.

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References

Bald-Blume, N., Bergervoet, J. H. and Maiss, E. 2017. Development of a molecular assay for the general detection of tospoviruses and the distinction between tospoviral species. Archives of virology, 162: 1519-1528.

Deng, D., McGrath, P. F., Robinson, D. J. and Harrison, B. D. 1994. Detection and differentiation of whitefly-transmitted geminiviruses in plants and vector insects by the polymerase chain reaction with degenerate primers. Annals of applied Biology, 125(2): 327-336.

Gibbs, A. and Mackenzie, A. 1997. A primer pair for amplifying part of the genome of all potyvirids by RT-PCR. Journal of Virological Methods, 63(1-2): 9-16.

Iwaki, M., Honda, Y., Hanada, K., Tochihara, H., Yonaha, T., Hokama, K. and Yokoyama, T. 1984. Silver mottle disease of watermelon caused by tomato spotted wilt virus. Plant Disease, 68(11): 1006-1008.

Kato, K., Handa, K. and Kameya-Iwaki, M. 2000. Melon yellow spot virus: a distinct species of the genus Tospovirus isolated from melon. Phytopathology, 90(4): 422-426.

Li, Y., Tan, G., Lan, P., Zhang, A., Liu, Y., Li, R. and Li, F. 2018. Detection of tobamoviruses by RT-PCR using a novel pair of degenerate primers. Journal of Virological Methods, 259: 122-128.

McLeish, M. J., Zamfir, A. D., Babalola, B. M., Peláez, A., Fraile, A. and García-Arenal, F. 2022. Metagenomics show high spatiotemporal virus diversity and ecological compartmentalisation: Virus infections of melon, *Cucumis melo*, crops, and adjacent wild communities. Virus Evolution, 8(2): veac095.

Quito-Avila, D. F., Peralta, E. L., Martin, R. R., Ibarra, M. A., Alvarez, R. A., Mendoza, A. and Ochoa, J. 2014. Detection and occurrence of melon yellow spot virus in Ecuador: an emerging threat to cucurbit production in the region. European Journal of Plant Pathology, 140: 193-197.

Rojas, M. R., Gilbertson, R. L., Russell, D. R. and Maxwell, D. P. 1993. Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Disease, 77(4): 340-347.

Takeuchi, S., Okuda, M., Hanada, K., Kawada, Y. and Kameya, M. 2001. Spotted wilt disease of cucumber *(Cucumis sativus*) caused by Melon yellow spot virus. Japanese Journal of Phytopathology, 67(1): 46-51.

Takeuchi, S., Shimomoto, Y. and Ishikawa, K. 2009. First report of Melon yellow spot virus infecting balsam pear (*Momordica charantia* L.) in Japan. Journal of General Plant Pathology, 75(2): 154-156.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. MEGA 4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24(8): 1596-1599.

Wu, H., Liu, M., Li, W., Wang, M., Xiu, J., Peng, B. and Gu, Q. 2024. Development and Application of Droplet Digital PCR Assay for the Detection of Watermelon Silver Mottle Virus and Melon Yellow Spot Virus. Horticulturae, 10(3): 199.

اولین گزارش از وقوع ویروس لکه‌زرد خربزه در ایران

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چکیده: در طی یک بررسی در بهار ۱۴۰۳، علایم لکه‌های زرد شدید روی برگ، زردی، علایم لکه حلقوی سبزرد در میوه، چروکیدگی‌هایی با بافت فرورفته در محل لکه‌های حلقوی در گیاهان خیار کشت شده در نزدیکی شهر اصفهان، مرکز ایران مشاهده شد. و برای ارزیابی احتمال آلودگی به ارتوتوسپویروس‌های بیمارگر گیاهی مورد بررسی قرار گرفت. RNA ژنومی کل به‌صورت جداگانه از برگ‌های ۵ جدایه دارای علایم و یک جدایه به‌ظاهر سالم به‌عنوان کنترل منفی استخراج شد. نمونه‌های RNA استخراج شده متعاقباً با استفاده از یک جفت آغازگر دژنره ارتوتوسپوویروس‌ها با واکنش زنجیره‌ای پلیمراز معکوس مورد بررسی قرار گرفتند. در نمونه‌های آزمایش شده دارای علایم در واکنش RT-PCR، یک قطعه با اندازه مورد انتظار ۴۲۰ جفت‌بازی در تمام نمونه‌های دارای علایم سنتز شد درحالی‌که، در نمونه کنترل منفی هیچ قطعه‌ای تکثیر نشد. زیر هم‌چینی‌توالی‌های نوکلئوتیدی جدایه‌های ایرانی هویت قطعات تکثیر شده را Melon yellow spot virus (*Orthotospovirus meloflavi*, MYSV) را نشان دادند. در ادامه واکاوی درخت تبارنما براساس جدایه‌های ایرانی و جدایه‌های گزارش شده از مناطق مختلف جهان، مشخص گردید جدایه‌های ایرانی بیشترین تشابه را جدایه‌های آسیایی MYSV دارند. براساس بررسی‌های ما این اولین گزارش از وقوع MYSV در ایران است.

واژگان کلیدی: ارتوتوسپوویروس، ایران، اصفهان، خیار