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مقاله کوتاه پژوهشی

اولین گزارش از وقوع ویروس موزاییک طلایی فلفل و بتاستلایت همراه از فلفل دلمه‌ای در ایران

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چکیده

ویروس موزاییک طلایی فلفل (Pepper golden mosaic virus, PepGMV) یک ویروس بیماری‌زای گیاهی است که در سرتاسر جهان بر روی گونه‌های مختلف فلفل در مزارع باز و گلخانه‌ها وجود دارد. طی یک بررسی، در تابستان ۱۴۰۲ در گلخانه‌های فلفل در استان اصفهان، مرکز ایران، گیاهان فلفل دلمه‌ای با علائمی شبیه به بگوموویروس‌ها مانند پیچ‌خوردگی برگ، موزاییک شدید، بدشکلی و کاهش اندازه برگ مشاهده شدند. محصولات واکنش زنجیره‌ای پلی‌مراز به اندازه مورد انتظار (حدود ۵۰۰ جفت باز) مربوط به بخشی از ژن پروتئین پوششی از تمام نمونه‌های داری علایم به دست آمد. علاوه‌بر این، برای بررسی ارتباط همراهی یک بتاستلایت با جدایه‌های ایرانی ویروس موزاییک طلایی فلفل، یک قطعه ۱۲۵۰ جفت باز از ژنوم بتاستلایت همراه با موفقیت از یکی از جدایه‌های فلفل جمع‌آوری‌شده در استان اصفهان، با استفاده از جفت آغازگرهای عمومی مربوط به بتاستلایت های بگوموویروس‌ها (Beta01/Beta02) تکثیر شد. این مطالعه اولین گزارش از PepGMV و بتاستلایت مرتبط با آن از گیاهان فلفل در ایران می‌باشد.

کلمات کلیدی: اصفهان، بتاستلایت، بگوموویروس، آغازگرهای عمومی و اختصاصی.

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Short Research Article

First report of occurrence of pepper golden mosaic virus and the associated betasatellite from bell pepper in Iran

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Abstract

Pepper golden mosaic virus (PepGMV) is a plant virus that occurs worldwide on species of open fields and greenhouses that grown bell pepper. During a survey in Summer 2023 in pepper greenhouses of the Isfahan Province, central Iran, bell pepper plants were observed showing begomovirus-like symptoms, such as leaf curling, severe mosaic, bushy growth, and reduced leaf size. PCR products of the expected size (500 bp) corresponding to the region of the coat protein of begomoviruses were obtained from all of the symptomatic samples. Furthermore, to investigate the potential association of a betasatellite with an Iranian PepGMV isolate, a 1250 bp fragment of the betasatellite genome was successfully amplified from pepper sample collected in the Isfahan Provinces, using universal betasatellite primer pair Beta01/Beta02. This study highlights the initial report of PepGMV and the associated betasatellite infecting pepper plants in Iran.

Key words: Isfahan, Betasatellite, Begomovirus, Universal primers.

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Introduction

The bell pepper (*Capsicum annuum* L.) is a highly valuable vegetable crop due to its nutritional properties and commercial importance. It is a common crop in tropical and subtropical regions across the world. However, bell peppers are prone to various viral, fungal, and bacterial diseases, which results in substantial yield losses (Kenyon et al., 2014). Geminiviruses represent a diverse group of plant viruses that are characterized by their circular, single-stranded DNA genomes, which are packaged into isometric twinned particles. These viruses are known to infect a wide range of economically important crop species (Reddy et al., 2024). The genus *Begomovirus* is one of the important genera within the family *Geminiviridae*, which are responsible for major losses in a wide range of economically important crops such as tomatoes and pepper (Salehzadeh et al., 2022). Pepper golden mosaic virus (PepGMV) is a bipartite member of the genus which transmitted by whiteflies and naturally infects pepper, tomato, tobacco, and other solanaceous crops (Gautam et al., 2023). PepGMV is widely distributed globally and has been reported in mixed infection with other begomoviruses such as Pepper huasteco yellow vein virus (PHYVV), and more recently with Tomato yellow leaf curl virus (TYLCV). PepGMV-infected plants show a range of characteristic symptoms, such as yellow mosaic, leaf wrinkling, and plant stunting (Gautam et al., 2023), which can lead to significant crop losses. The host range of PepGMV was found to be narrow, limited to the family Solanaceae, and has been reported as a major viral pathogen in some economically important horticultural crops, such as tomatoes and peppers. Additionally, *Datura discolor*, *Nicandra physaloides*, *Cucurbita moschata*, *Cucurbita pepo*, *Sechium edule*, *Erythrina* spp., *and Nicotiana glauca* (an asymptomatic host) were found to be alternate hosts of the virus (Holguin-Peña et al., 2024).

Materials And Methodes

During a survey in the summer of 2024 in pepper greenhouses in Isfahan Province, central Iran, bell pepper plants were observed showing begomovirus-like symptoms such as leaf curling, severe mosaic, bushy growth, and reduced leaf size (Fig. 1). To verify the begomovirus infection, five symptomatic leaf samples and one asymptomatic pepper sample were collected and total DNA was extracted from the samples using a CTAB-based method (Gawel & Jarret, 1991). PCR reactions were performed using a begomovirus degenerate primer pair B^C (Deng *et al*., 1994) and 181^V (Rojas *et al*., 1993). PCR products of the expected size (c. 500 bp) corresponding to the intergenic and coat protein regions of begomovirus genome were obtained from all of the five symptomatic samples while not from asymptomatic leaf tissue of bell pepper, implying the association of a begomovirus with the tested samples. To identify potential co-infections of other viruses alongside PepGMV in the infected samples, PCR reactions were conducted using four different primer pairs. These included a specific primer pair (CMV CP-F, CMV CP-R) designed to amplify a fragment of CMV genome (Rizos et al., 1992), a degenerate primer pair for Potyviruses (Nib2F, Nib3R, Gibbs & Mackenzie, 1997).

Results and Discussion

The amplified fragments from one sample were gel-purified using a PCR purification kit (Qiagen Co.) and sequenced directly using a paired-end sequencing strategy (Sinohe Co. Iran). Nucleotide sequencing of the 500 bp fragment and an initial comparison of its sequence to the corresponding regions of begomovirus genomes using the NCBI BLAST database indicated that the DNA fragment is most similar to the corresponding region of the pepper golden mosaic virus (PepGMV) genome. The nucleotide sequence alignment of the PCR product showed that the Iranian isolate IR-PepGMV-02 shared 98% identity with the corresponding region of the Pepper golden mosaic virus (PepGMV) genome, and therefore the causal virus was considered as the Iranian isolate of PepGMV. Multiple nucleotide sequence alignment of the obtained sequences with the corresponding sequences in the GenBank database revealed that the Iranian isolate IR-PepGMV-02 shares the highest identity (98.27%) with an Indian PepGMV isolate (accession number KC846888, host: *Cucumis sativus*, Isolate: 50A, Host country: India) and the lowest identity (94.65%) with an isolate of

PepGMV from Nicaragua (acc no. AJ_842138.1; Host: *Lycopersicum esculentum*, isolate NID3C, country Nicaragua).

Furthermore, to investigate the potential association of a betasatellite with the IR-PepGMV-03 isolate, a 1250 base pair (bp) fragment of the associated betasatellite genome was successfully amplified from pepper isolates collected in the Isfahan Province (IR-PepGMV-03), using the universal betasatellite primer pair Beta01/Beta02 (Briddon et al., 2001). Following direct sequencing, the BLAST search revealed 99.21% identity with a betasatellite isolate reported from Jordan (acc no. MT_316190.1 betasatellite name Cotton leaf curl Gezira betasatellite; isolate: Okra/ Jordan -J10-47; host *Abelmoschus esculentus;* country: Lebanon: associated virus: Okra leaf curl virus). A maximum likelihood phylogenetic tree was constructed using a 500-nucleotide fragment of the begomoviruses-CP gene of the Iranian PepGMV isolates, characterized in the current study, along with nine exotic PepGMV sequences. The analysis conducted using MEGA8.0 software with 100 bootstrap replicates, revealed the existence of two major clusters (Groups 1 and 2, Figure 2). Cluster 1 includes the PepGMV isolate from *C. annum* (in this study), along with four PepGMV isolates, primarily originating from *C. annum* in the Mexico, and one isolate from *Cucumis sativus* in India. The second cluster (Group 2) consists of four Nicaraguan PepGMV isolates reported from *Solanum lycopersicum* (tomato) and *C. baccatum*. In the phylogenetic analysis of betasatellites associated with pepper golden mosaic virus (PepGMV) isolates depicted in Figure 2, a 1250 base pair fragment of betasatellite DNA was used to construct a tree. The tree includes one PepGMVB isolate from *Capsicum annuum* from this study and 13 exotic betasatellite isolates from GenBank database. Analysis was conducted using the Maximum Likelihood (ML) method in MEGA-8 software, with an isolate of *Tobacco leaf curl Pusa virus* as the outgroup

The Iranian PepGMVB isolate clustered with Asian isolates from Jordan and Israel, indicating a genetic relationship among these isolates. Branches with less than 62% support were excluded from consideration, and nodes with less than 62% bootstrap values are not reported. The tree provides insights into the genetic similarities and differences among the betasatellites associated with various PepGMV isolates, highlighting the clustering patterns and evolutionary relationships within this group of viruses.

To identify potential co-infections of other viruses alongside PepGMV in the infected samples, PCR reactions were conducted using four different primer pairs. These included a specific primer pair (CMV CP-F, CMV CP-R) designed to amplify a fragment of CMV genome (Rizos et al., 1992), a degenerate primer pair for Potyviruses (Nib2F, Nib3R, Gibbs & Mackenzie, 1997), and a degenerate primer pair for the *Tobamovirus* genus (TobamodF/TobamodR, Li et al., 2018). However, no PCR products were obtained using these primer pairs, indicating the absence of other virus co-infections with PepGMV in the tested plants. This study highlights the initial report of PepGMV and its associated betasatellite impacting pepper plants in Iran.

Figure 1. Severe mosaic and distortion symptoms on pepper leaves, accompanied by plant stunting, caused by PepGMV infection in a greenhouse in Isfahan.

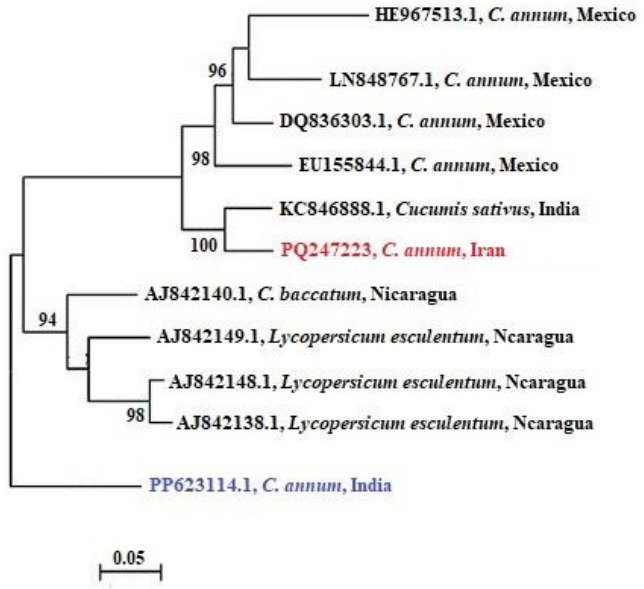

Figure 2. A phylogenetic tree constructed based on the alignment of a 500 base pair fragment of Av1-DNA from a pepper golden mosaic virus (PepGMV) isolate from *Capsicum. annum* and 10 exotic PepGMV isolates retrieved from GenBank. The tree was constructed using the Maximum Likelihood (ML) method in MEGA-8 software. An isolate of *Chili leaf curl virus* (acc no. PP623114) was used as an outgroup. The Iranian isolate clustered with Asian isolate from India. Branches with less than 94 % support and nodes with less than 94% bootstrap values were excluded from consideration. The tree provides information on the isolate numbers and their geographical origins.

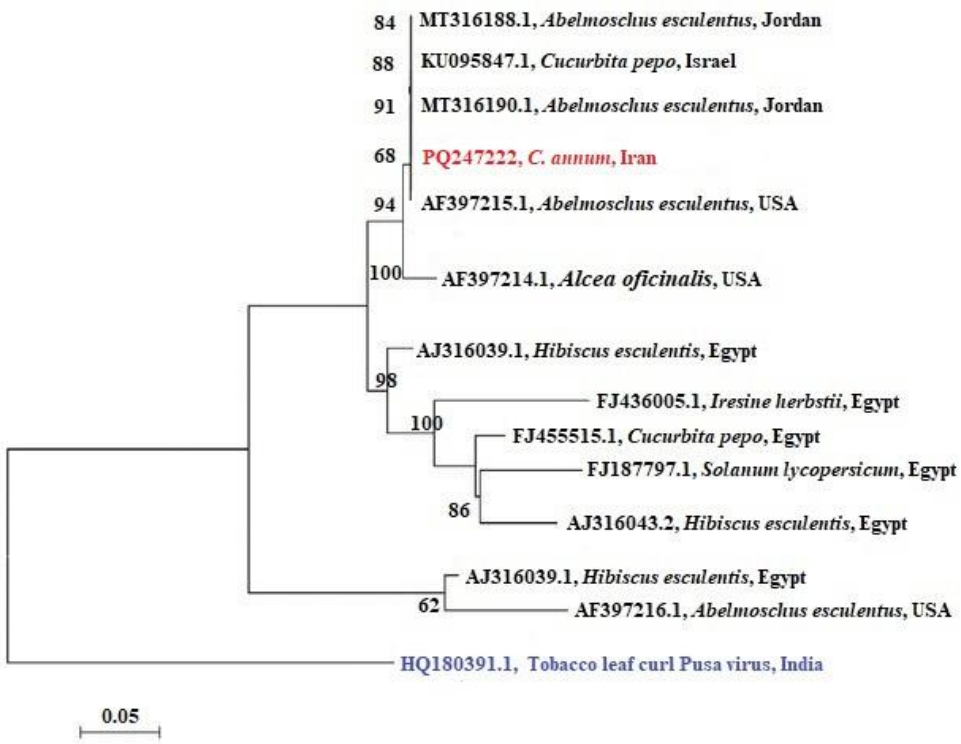

Figure 3. A phylogenetic tree constructed based on the alignment of a 1250 bp fragment of the associated betasatellite with *Capsicum annum* sample and 13 exotic betasatellite isolates retrieved from GenBank. The tree was constructed using the Maximum Likelihood (ML) method in MEGA-8 software. An isolate of *Tobacco leaf curl pusa virus* (AC: HQ180391.1) was used as an outgroup. The Iranian isolate clustered with Asian isolates from Jordan and Israel. Branches with less than 62 % support and nodes with less than 62% bootstrap values were excluded from consideration. The tree provides information on the isolate numbers and their geographical origins.

With the expansion of greenhouse cultivation of vegetables products, particularly peppers, in Iran, the prevalence of significant pathogenic viruses, such as Pepper mild mottle virus, (PMMoV) (Salehzadeh et al., 2021) , Tomato brown rugose fruite virus (ToBRFV) (Salehzadeh et al., 2021) , Chilli leaf curl virus (ChiLCV) (Salehzadeh et al., 2022), and Alfalfa mosaic virus (AMV) (Salehzadeh et al., 2024), has increased. Therefore, it is crucial to identify and assess the distribution of these harmful viruses in order to minimize damage and prevent their spread across the country.

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