



First detection of bell pepper endornavirus infecting bell pepper in Guanajuato, Mexico

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ABSTRACT

Background/Objective. Bell pepper (*Capsicum annuum*) is one of the most important agricultural crops of Mexico. Globally, over 60 virus species have been reported infecting *Capsicum* spp., some of them are associated with significant losses in yield and quality. The objective of this report is to determine the presence of bell pepper endornavirus (BPEV, *Alphaendornavirus capsici*) in bell pepper crops in Guanajuato, Mexico.

Experimental development. Samples of bell pepper plants were collected from a commercial greenhouse in Guanajuato, Mexico, in May 2024. A symptomatic plant (mosaic, leaf yellowing, and dwarfing symptoms) was analyzed by high throughput sequencing (HTS). Detection and identification were performed on individual samples using reverse transcription PCR (RT-PCR) and corroborated by Sanger sequencing. A phylogenetic analysis was conducted to investigate genetic diversity of the virus.

Results. A partial sequence of BPEV was generated by HTS and *de novo* assembly (GenBank: PZ103604), this virus was part of a mixed infection in the symptomatic plant, including *Begomovirus capsicumhuastecoense* (pepper huasteco yellow vein virus) and Cucumber mosaic virus. In addition, five bell pepper plants, including symptomatic and asymptomatic, tested positive for BPEV using RT-PCR, the identity of the virus was confirmed through Sanger sequencing and phylogenetic analysis.

Conclusion. The detection of BPEV in commercial bell pepper crops in Guanajuato highlights the importance of continuous monitoring of emerging viruses, including possible mixed infections with other viruses, and further studying their impact on crop health and productivity. Although BPEV was detected in symptomatic plants, the observed symptoms cannot be attributed to it as it was detected as part of a co-infection. To our knowledge, this is the first report of BPEV infecting bell pepper in Mexico.

Keywords: *Alphaendornavirus*, Bell pepper endornavirus, Detection, HTS, Bell pepper, Mexico



INTRODUCTION

Bell pepper (*Capsicum annuum*) is one of the most important agricultural crops of Mexico. According to public data from the Mexican government, national production was around 580,000 tons in 2025, with Sinaloa, Sonora, Jalisco and Guanajuato the main producing states (https://nube.agricultura.gob.mx/cierre_agricola/). More than 60 virus species have been reported infecting *Capsicum* spp., many of which are responsible for significant yield and quality losses worldwide (Petrov *et al.*, 2024).

Endornaviruses can infect a wide range of hosts, such as plants, fungi and oomycetes. Several plants of economic and nutritional importance can be infected by endornaviruses, plants such as beans, cereals, cucurbits and peppers have reported infections. These viruses cause no visual symptoms and results in minor losses in fruit quantities and weight loss (Paudel *et al.*, 2024).

Bell pepper endornavirus (BPEV, *Alphaendornavirus capsici*) is an RNA virus that infects bell pepper plants, transmitted vertically through infected seeds. BPEV does not cause apparent symptoms; however, its interactions with other viruses have become a topic of increasing interest, as coinfections involving BPEV may influence disease development and symptom expression (Paudel *et al.*, 2024 and Escalante, 2018). To further aid the future investigations surrounding these interactions, the objective of this report is to determine the presence of BPEV in bell pepper crops in Guanajuato, Mexico.

EXPERIMENTAL DEVELOPMENT

In 2024, a sample from a bell pepper plant cultivar ‘Magno’ displaying mosaic, leaf yellowing, and dwarfing symptoms was collected from a commercial greenhouse in the state of Guanajuato. Due to confidentiality considerations, further details regarding the exact location of the site where the symptomatic plant was collected cannot be disclosed. To investigate the etiology of the symptoms and the possible presence of a viral infection, a high throughput sequencing (HTS) analysis was performed at Tecnológico de Monterrey. Briefly, through the use of the MagMAX Viral Kit in combination with the KingFisher Apex System from Thermo Fisher Scientific (USA), total RNA was extracted from the plant’s leaves. The extracted RNA was subjected to library preparation using TruSeq Stranded Total RNA Library Prep Gold (Illumina, USA) and sequenced on the Illumina NovaSeq 6000 platform yielding over 30 million reads which were subsequently assembled using SPAdes (Bankevich *et al.*, 2012). From that assembly, 25 *de novo* contigs (ranging from 220 to 5,465 nts) were identified using BLASTn with high similarity (>97% nt identity) to several viral accessions available in GenBank. These included viruses such as *Begomovirus capsicumhuastecoense* (pepper huasteco yellow vein virus), cucumber mosaic virus, and BPEV.

Specifically, 10 contigs showed homology to BPEV (GenBank: JN019858), including the longest generated contig (5,465 nts), which was later deposited at GenBank under accession number PZ103604. BLASTx of the longest contig showed a similarity of 99% to the polyprotein of a BPEV isolate from the USA (GenBank: WPT38552). Likewise, the same contig clustered with members of the genus *Alphaendornavirus* (Figure 1); for the phylogenetic analysis, the MEGA12 software was used to construct a maximum likelihood tree using 1,000 bootstrap replicates and considering the best-fitting nucleotide substitution model, identified as GTR+G+I.

To confirm the presence of BPEV, the original plant was evaluated by reverse transcription PCR (RT-PCR) using RevertAid Reverse Transcriptase, DreamTaq GoTaq Polymerase, and newly designed primers. The primers employed were BPEV_1591F (5'-ACAAGCTGGCCATGTTGAAT-3') and BPEV_2396R (5'-GAAGTCACCAGTCTGCAACA-3') designed based on multiple BPEV sequences available in GenBank, which amplify a product of 807 bp in the BPEV polyprotein. The one-step RT-PCR amplification was performed under the following conditions: 30 min at 52 °C, 40 cycles of 30 s at 94 °C, 45 s at 58 °C, and 1 min at 72 °C, and a final step of 7 min at 72 °C. Because this study represents the first detection of BPEV in Mexico, no previously confirmed positive sample was available to serve as a positive control. A no-template control (nuclease-free water) was included in all RT-PCR reactions. Finally, virus identity was supported by Sanger sequencing of RT-PCR products.

Additionally, five bell peppers were randomly collected, including symptomatic and asymptomatic, from the same commercial greenhouse and tested using the above-described assay. All the investigated plants tested positive via RT-PCR and Sanger sequencing confirmed the identity of the virus.

BPEV has previously been reported in Poland (Minicka *et al.*, 2023), Slovakia (Tomašechová *et al.*, 2019) and the USA (Dayal *et al.*, 2025), mainly in bell pepper plants. To our knowledge, this is the first report of BPEV infecting bell pepper plants in Mexico. Although the symptoms of a BPEV infection are not visible, given their cryptic nature, its presence may have effects on abiotic stress or interact with other viruses infecting the plant and should be further investigated.

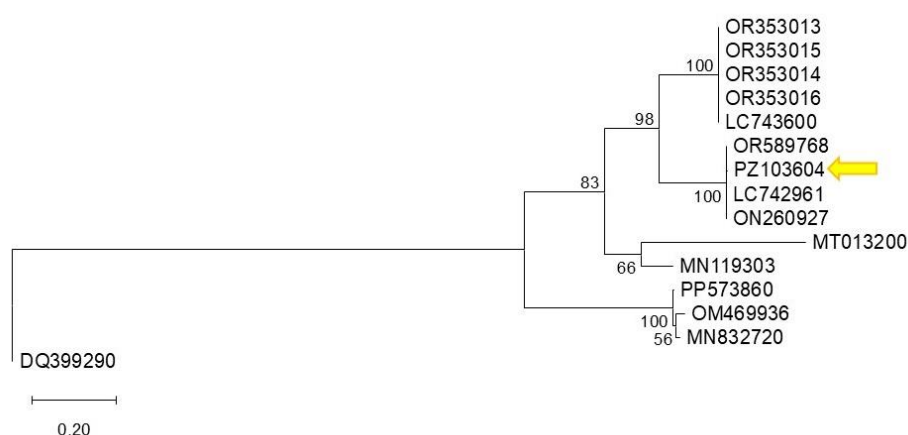


Figure 1. Phylogenetic tree constructed using the Maximum Likelihood method based on the bell pepper endornavirus (BPEV, *Alphaendornavirus capsici*) polyprotein sequence and other *Alphaendornavirus* sequences available in GenBank. Reference sequences were selected from GenBank based on their annotation as BPEV isolates and sequence availability. The analysis was performed using the corresponding nucleotide substitution model. Bootstrap support values (1,000 replicates) are indicated at the nodes, showing only those greater than 50%. Sequence PZ103604 (indicated by an arrow) clusters with closely related sequences with high bootstrap support, suggesting a close phylogenetic relationship. Sequence DQ399290 (*Betaendornavirus*) was used as the outgroup. The scale bar represents 0.20 substitutions per site.

CONCLUSIONS

The detection of BPEV in commercial bell pepper crops in Guanajuato highlights the importance of continuous monitoring of emerging viruses, including possible mixed

infections with other viruses, and further studying their impact on crop health and productivity. Although BPEV was detected in symptomatic plants, the observed symptoms cannot be attributed to BPEV because the virus is generally considered symptomless and was detected as part of a co-infection.

Further studies into viruses such as BPEV could aid in protecting us against future problems or prove useful in limiting the effect of other viruses. To our knowledge, this is the first report of BPEV infecting bell pepper in Mexico.

Limitations. Sampling was done in a sole greenhouse with a limited number of plants.

Conflicts of Interest. The authors declare that they have no conflicts of interest regarding the research, authorship, and/or publication of this report.

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Authors' Contributions. All authors contributed to this work and revised the final version of the manuscript.

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