



*Phytopathological Report*

## First report of *Orthospovirus impatiensnecromaculae* infecting lettuce (*Lactuca sativa*) in Guanajuato, Mexico

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### ABSTRACT

**Background/Objective.** Determine the presence of *Orthospovirus impatiensnecromaculae* (Impatiens necrotic spot virus, INSV) in lettuce crops in Guanajuato, Mexico.

**Materials and Methods.** Symptomatic lettuce samples were collected from a commercial field in Guanajuato, Mexico, in May 2024. The detection and identification in 12 individual samples was performed with INSV ImmunoStrips and reverse transcription-PCR (RT-PCR) and corroborated by Sanger sequencing.

**Results.** Ten of the 12 lettuce samples tested positive for *O. impatiensnecromaculae* by ImmunoStrips. The identity of the virus was confirmed through RT-PCR and Sanger sequencing (GenBank: PQ151798).

**Conclusion.** The presence of INSV in lettuce crops from Guanajuato, Mexico highlights the need for continuous monitoring and implementation of integrated disease management strategies to mitigate the spread and impact in this region.

**Keywords:** Impatiens necrotic spot virus, ImmunoStrips, RT-PCR, Trips

## INTRODUCTION

Lettuce (*Lactuca sativa*), a cold-season, annual and autogamous species, is one of the most cultivated plants in the world. In May of 2024, Iceberg lettuces exhibiting tan to dark brown necrotic spots, distorted leaves, and stunted growth (Figure 1), were observed in a commercial field located in Guanajuato, Mexico (DD: 21.242194, -100.881768). Additionally, a high infestation of western flower thrips (*Frankliniella occidentalis*) was determined in the same area, the hosts being corn plants in an adjacent field and some lettuce plants. This thrips species is known to vector multiple plant viruses. Based on this information and the scientific literature, the objective was to identify the presence of *Orthotospovirus impatiensnecromaculae* (Impatiens necrotic spot virus, INSV) in the lettuce crop in Guanajuato, Mexico.

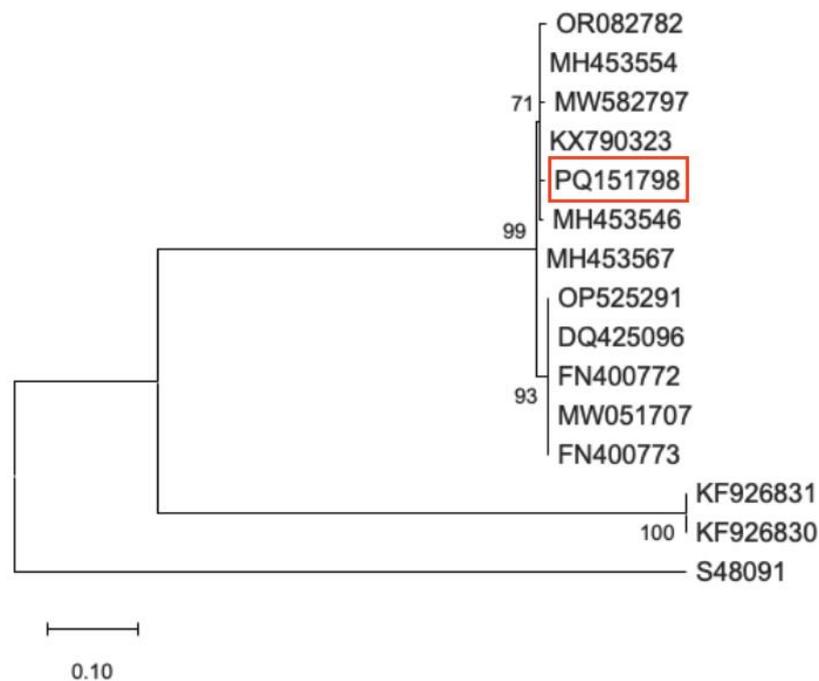


**Figure 1.** Symptomatic lettuce plants infected by *Orthotospovirus impatiensnecromaculae* observed during the field survey.

For the detection of INSV in field samples, 12 symptomatic plants were randomly collected. The samples were analyzed with ImmunoStrips specific for this virus (Agdia, USA). This technique is based on lateral flow immunochromatography, using specific antibodies that produce visible lines (indicators) when immersed in the sample extract (sap). According to this analysis, 10 of the 12 samples analyzed were positive.

The plant material was shipped and stored and the Bioengineering Center at Tecnológico de Monterrey, campus Queretaro, where the molecular analysis was performed. To confirm the presence of INSV, total RNA was extracted from the 12 lettuce samples using TRIzol reagent as per manufacturer instructions (Meng and Feldman, 2010). For the reverse transcription-PCR (RT-PCR), specific primers (INSVF: 5'CCCAACTGCCTCTTTAGTGC3' and INSVR: 5'GGACAATGGATCTGCTCTGA3') were used, following the protocol described by González-Pacheco and Silva-Rosales (2013), which amplify a genomic fragment of the S segment. Reverse Transcriptase (Revert Aid) and DNA Polymerase (Dream Taq) were from ThermoFisher Scientific (USA). As a result,

the RT-PCR reaction amplified the expected product (250-bp fragment) in the same 10 lettuce samples previously detected by ImmunoStrips. Amplified products were sent for Sanger sequencing at Macrogen Inc. (Korea), and sequences identical to each other (GenBank: PQ151798) showed 99% identity with an INSV isolate reported in the USA (GenBank: KX790323). A phylogenetic analysis was performed based on the sequenced region and the sequences of selected isolates of *O. impatiensnecromaculæ* and *O. tomatomaculæ* (tomato spotted wilt virus, TSWV) using the MEGA7 software (Figure 2).



**Figure 2.** Maximum-likelihood phylogenetic tree of *Orthotospovirus impatiensnecromaculæ* (Impatiens necrotic spot virus, INSV) isolates available in GenBank. Sequence generated during this study is indicated with red color. GenBank: S48091 corresponds to *Orthotospovirus tomatomaculæ* (tomato spotted wilt virus, TSWV) and is included in the phylogenetic analysis as outgroup.

In Mexico, *O. impatiensnecromaculæ* is identified in tomatillo (*Physalis ixocarpa*) and bell pepper (*Capsicum* spp.) in Guanajuato and Querétaro (González-Pacheco and Silva-Rosales, 2013). During the preparation of this manuscript, INSV was reported in Iceberg lettuce from samples collected in the State of Mexico and Puebla, and showed symptoms of yellowing, brown necrotic spots and ring spots on the leaves and midribs (Quevedo *et al.*, 2024). Recently, there were severe outbreaks of *O. impatiensnecromaculæ*, with incidences up to 27%, in commercial fields of lettuce in California, USA, and linked to high populations of *F. occidentalis*, the natural vector of this virus (Koike *et al.*, 2008; Kuo *et al.*, 2014). Since this insect plays a crucial role in the spread of the virus, it is possible that, in Guanajuato, the proximity of various crops facilitates the movement of thrips vectors between fields, causing the infestation of more susceptible crops, such as lettuce.

This is the first report of INSV infecting lettuce plants in the Bajío region, Mexico. The Bajío is one of the most important agricultural areas in Mexico and includes the states of Guanajuato, Aguascalientes, Querétaro, Jalisco, and Michoacán. Therefore, effective management practices should be implemented, emphasizing the importance of studying putative vectors to prevent the spread of *O. impatiensnecromaculae* to other commercial fields and avoid economic losses. In that sense, the development of INSV-resistant cultivars and integrated pest management approaches will be crucial in addressing these challenges and ensuring the long-term health and productivity of crops in the region, including lettuce.

#### **Conflicts interest**

No conflict of interest exists concerning the research.

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#### **Author contributions**

All authors participated in writing the paper and internal review, later, all authors have read and approved the final manuscript.

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