



Detection of *Begomovirus capsicummusive* (PepGMV) and associated damage in habanero pepper (*Capsicum chinense*) in Tabasco

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ABSTRACT

Background/Objective. This study aimed to detect viral agents in four *Capsicum chinense* cultivars in Tabasco, Mexico; determine disease incidence across five developmental stages; and quantify habanero pepper yield.

Experimental development. A field bioassay was established using the cultivars Palenque F1, Jaguar, Orange, and PX-11459057 in a completely randomized design. ImmunoStrips® (Agdia Inc.) were used to detect *Orthospovirus tomatomaculae* (tomato spotted wilt virus, TSWV), *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV), and *Orthospovirus iridimaculaflavi* (iris yellow spot virus, IYSV). PCR was employed for *Begomovirus* detection. Viral incidence was evaluated at five time points during the crop cycle, and fruit yield (kg plant⁻¹ y t ha⁻¹) and quality were assessed. ANOVA with Tukey's test ($p \leq 0.05$) was applied.

Results. ImmunoStrips® tests were negative for all four analyzed viruses. *Begomovirus capsicummusive* (pepper golden mosaic virus, PepGMV) tested positive by PCR in cultivars Palenque F1, Jaguar, and PX-11459057, with genetic similarity exceeding 98%. Viral symptoms included interveinal chlorosis, mosaic, leaf deformation, and dwarfing. Incidence surpassed 95% at 113 days after transplanting (dat). Fruit production was negatively affected in both quantity and quality.

Conclusion. The presence of *Begomovirus capsicummusive* (PepGMV) was confirmed in habanero pepper in Tabasco, associated with interveinal chlorosis, leaf deformation, and dwarfing, with incidence exceeding 95 % at 113 ddt. Fruit production ranged from 0.1 to 1.41 kg ha⁻¹.

Keywords: PepGMV, *Begomovirus capsicummusive*, Habanero pepper, Yield



INTRODUCTION

Habanero pepper (*Capsicum chinense*) is a crop with high adaptability to different climates and altitudes, and it is also a nutritional source of compounds used in food, cosmetics, and pharmaceuticals (Aguirre-Hernández and Muñoz-Ocoter, 2015). In Mexico, its production exceeds 31 000 tons, with Tabasco ranking as the third-largest producer (SIAP, 2023).

The crop is grown mainly in open fields (85%), where it is exposed to attacks by multiple pathogens, particularly viruses transmitted by insect vectors (thrips, whiteflies, aphids, others), by seed, or by mechanical contact (Parisi *et al.*, 2020). Among the most relevant viruses reported in Mexico are *Orthospovirus tomatomaculae* (tomato spotted wilt virus–TSWV), *Cucumber mosaic virus* (cucumber mosaic virus–CMV), *Alfalfa mosaic virus* (alfalfa mosaic virus–AMV), *Orthospovirus iridimaculaflavi* (iris yellow spot virus–IYSV), and *Begomovirus capsicummusive* (pepper golden mosaic virus–PepGMV), the latter spread by *Bemisia tabaci*. *Tobamovirus tabaci* (tobacco mosaic virus–TMV) alone causes estimated annual losses of 21 billion dollars in the United States (Miller, 2024). The International Committee on Taxonomy of Viruses (ICTV) recognizes four genera within the family *Geminiviridae*: *Mastrevirus*, *Topocuvirus*, *Curtovirus*, and *Begomovirus* (Fauquet *et al.*, 2008).

Begomovirus species stand out for their diversity, transmission by whitefly species, and ability to infect dicotyledonous plants. Their genome may be monopartite or bipartite (Argüello *et al.*, 1994; Gutiérrez *et al.*, 2004; Gafni and Epel, 2002). More than 200 *Begomovirus* species have been described (King *et al.*, 2012). These viruses induce mosaic symptoms, chlorosis, deformation, and dwarfing, and they are considered among the most important groups of plant pathogens worldwide.

In Mexico, losses caused by viral diseases in crops range from 20 to 100% (Anaya-López *et al.*, 2003). Even without visible symptoms, viral infections significantly reduce plant productivity, affecting both fruit size and number (Hull, 2014). Given the impact of viral diseases on habanero pepper production in Mexico (Argüello *et al.*, 2003), it is essential to accurately identify the causal agents and determine their incidence in different cultivars. In Tabasco, there are no previous reports on the specific detection of viruses or the recognition of associated symptoms. Therefore, the aim of this study was to detect viral agents causing diseases with characteristic viral symptoms using serological and molecular techniques in four habanero pepper cultivars from Tabasco, and to determine their incidence and yield under field conditions.

EXPERIMENTAL DEVELOPMENT

Study area. The study was conducted in Arroyo Hondo Abejónal, Cárdenas, Tabasco, which has a warm–humid tropical climate (Am(g)w), average temperatures of 26 °C, abundant summer rainfall, and a prolonged dry period beginning in March. The area lies on an alluvial plain composed of recent Quaternary sediments (Palma-López *et al.*, 2007).

Plant material. Four habanero pepper cultivars were evaluated: Jaguar, Orange, Palenque F1, and PX-11459057. For field establishment, 45-day-old seedlings measuring 15 cm in height were used, obtained from the Barbosa nursery (Muna, Yucatán).

Experimental design. The bioassay was established on 13 February 2024 to assess the phytosanitary and productive performance of four habanero pepper cultivars. The

experiment was conducted in a completely randomized design with four treatments (cultivars) and four replications. Each experimental plot consisted of four 15-m rows, with 1.5 m between rows. Plants were spaced 0.5 m apart, and the useful plot consisted of the two central 13-m rows (52 plants each).

Agronomic management. Plastic mulch and drip fertigation were used with an NPK formula of 218-196-180 kg ha⁻¹ (López-López *et al.*, 2018). No insecticides were applied during the cycle, in order to allow whitefly infestation for natural virus inoculation. Only one initial application was made to control defoliating insects. Fungicides and bactericides were applied preventively to avoid the occurrence of other types of diseases.

Virus detection by serological testing with ImmunoStrips®. A total of 64 rapid tests were performed on symptomatic plants to detect TSWV, CMV, AMV, and IYSV following the manufacturer's manual. Leaf tissue from five symptomatic leaves taken from the upper part of different plants (0.15 g per sample) was macerated in 3 mL of the kit's buffer solution (Agdia Inc) until a homogeneous extract was obtained. The ImmunoStrip® was then inserted into the extract and left at room temperature (30 °C) for 10 to 20 minutes.

Detection of Begomovirus by PCR. Composite leaf samples consisting of 10 symptomatic apical leaves from each cultivar were collected. The samples were processed at the BioCiencia S.A. de C.V. laboratory (Monterrey, N.L.). DNA extraction was carried out using the Plant DNAzol® method. For *Begomovirus* detection, the primers prV-324/prC-889 targeting the *cp* coat protein gene were used (Wyatt and Brown, 1996). PCR amplifications were performed in a 25 µL reaction volume with primers prV-324/prC-889 (509–565 bp fragments), and the products were visualized by electrophoresis on a 1% agarose gel. Sequencing of the amplified fragments, in both directions, was conducted at the Psomagen laboratory (Maryland, USA). The resulting sequences were compared with accessions deposited in the NCBI GenBank database using the BLAST system.

Description of viral symptoms and incidence of symptomatic plants. The incidence percentage was recorded at five time points: 28, 49, 71, 94, and 113 days after transplanting (dat). To do this, the number of plants showing viral symptoms was quantified relative to the total number of plants in the useful plot. Symptoms observed on foliage, flowers, fruit, and the general appearance of the plants were also documented.

Fruit size, color, and yield. The diameter and length of 40 fruits per cultivar were measured using a digital caliper (ENVR brand). Variations in the typical fruit color of each cultivar were documented before physiological maturity. The scale established in the technical guide for the varietal description of habanero pepper (SAGARPA and SNICS, 2014) was used to determine fruit size and color. The average weight of harvested fruits was obtained from the four useful plots of each cultivar. Production was expressed as kg of fruit per plant, and yield was estimated.

Statistical analysis. The assumptions of normality and homogeneity of variance were verified (Lidman, 1974). Incidence (%) data were transformed using the arcsine $\sqrt{X}$ function prior to ANOVA. ANOVA was performed on the experimental data from the completely randomized design, and means were compared using Tukey's test ($p \leq 0.05$), with the Statgraphics Plus 5.1 software for Windows (Polhemus, 1980).

Virus detection with ImmunoStrips® in seedlings and field plants. The ImmunoStrips® tests were negative for the four analyzed viruses (TSWV, CMV, AMV, and IYSV) in all four cultivars (Table 1). These viruses were likely not present at the time of analysis, and the foliar symptoms cannot be attributed to them. These serological tests do not necessarily call into question their recognized sensitivity and effectiveness in virus detection (Vásquez-Gutiérrez *et al.*, 2024), suggesting instead the presence of other viruses not detectable by the ImmunoStrips used (Agdia EMEA, 2024). Chew-Medinaveitia *et al.* (1995) reported similar findings in *C. annuum*, with negative serological results in plants showing clear viral symptoms, as observed in this study.

Virus detection by PCR. The cultivars Palenque F1, PX-11459057, and Jaguar showed amplicons of 545, 509, and 565 bp, respectively. BLAST sequence analysis revealed an identity range of 98.35–99.02%, corresponding to *Begomovirus capsicummusive* (pepper golden mosaic virus–PepGMV) (Table 1). Therefore, the symptoms observed in habanero pepper in Cárdenas, Tabasco, are considered to be associated with the presence of *B. capsicummusive*, a *Begomovirus* of economic importance. The virus was detected in three of the four habanero pepper cultivars (Palenque F1, Jaguar, and PX-11459057), representing the first report for Tabasco. This detection agrees with the findings of Gracia-Medrano *et al.* (2022) in Yucatán, where PepGMV was identified in more than 90% of *Capsicum chinense* samples. The symptoms observed—mosaic, interveinal chlorosis, leaf curling, and leaf deformation—were consistent with PepGMV infections (Brown *et al.*, 2005; Lozano-Durán *et al.*, 2011).

Table 1. Percentage similarity of PCR-obtained sequences of viruses associated with habanero pepper plants.

Treatment	Cultivar	<i>Begomovirus</i> (PCR)	Accession	Percent similarity (%)	Viral species
1	Palenque Fi	Positive	HE967533	98.35	<i>Begomovirus capsicummusive</i>
2	Jaguar	Positive	HE967533	99.02	<i>Begomovirus capsicummusive</i>
3	Orange	Negative	-	-	-
4	PX-11459057	Positive	LN848769	98.53	<i>Begomovirus capsicummusive</i>

Note. N/D = Not detected

The cultivar Orange tested negative by PCR for *Begomovirus*. This may be due to a low viral load in the samples (González-Garza, 2017; Morales *et al.*, 2017) or to symptoms associated with other viruses. It is suggested that conventional diagnosis be complemented with pre-amplification techniques such as rolling circle amplification (RCA) to improve sensitivity (Morales *et al.*, 2017; Sánchez *et al.*, 2021), as well as molecular analysis for other virus genera associated with the observed symptoms.

Viral symptoms. Before transplanting, plants of the four cultivars showed no viral symptoms. At 31 dat, foliar symptoms of interveinal chlorosis were observed on newly emerged leaves in the apical zone, along with foliar mosaic, leaf rolling, leaf curling, and reduced size of new leaves (Figures 1 A, B, C, D, and E). At 69 dat, the number of diseased plants increased, and reductions in leaf blade size and plant dwarfing were detected; flower

bud drop was also observed (Figures 1 F and I). At 125 dat, in addition to the previous damage, fruit deformation and reduced fruit size were recorded (Figures 1 G and H). In the cultivar Palenque F1, fruits showed loss of green color, turning pale (Figure 1 G).



Figure 1. Habanero pepper plants showing different viral symptoms. **A)** Interveinal chlorosis; **B)** mosaic; **C)** leaf rolling; **D)** curling of apical leaves; **E)** reduced size of new leaves; **F)** flower abortion; **G)** fruit deformation and discoloration; **H)** reduced fruit size; **I)** viral symptoms expressed at different plant ages.

Virus incidence. The incidence of habanero pepper plants with viral symptoms was directly proportional to crop age (Figure 2). At 28 dat, the cultivars Jaguar (3%) and Orange (1%) showed initial viral symptoms. At 49 dat, during the flowering stage, incidence ranged from 3 to 14%. In the early fruiting stage (71 dat), incidence increased to 24% in Jaguar and Orange. At 94 dat, during the productive stage, incidence was highest in Jaguar (61%), Orange (54%), and Palenque F1 (48%), while the cultivar PX-11459057 showed only 25%.

Finally, at 113 dat, all cultivars had incidence levels above 95%, with noticeable effects on fruit size.

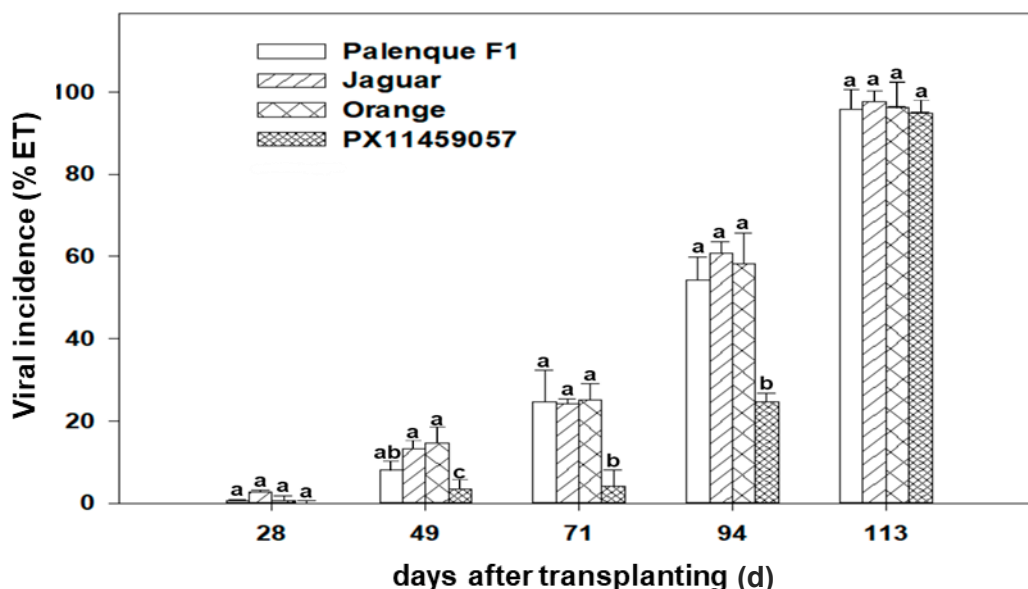


Figure 2. Incidence of viral symptoms in habanero pepper plants at different crop development stages. Note. ET = standard error, d = days.

The results show an increasing pattern in incidence percentage as the phenological cycle progressed for all four cultivars. This pattern agrees with the findings of Mejía-Teniente *et al.* (2015), who reported sustained increases in diseased plants showing viral symptoms of *Begomovirus capsicummusivi*–PepGMV and *Begomovirus capsicumhuastecoense*–PHYVV in habanero pepper under field conditions in Yucatán. In this study, whiteflies were not managed, which may have favored high viral spread (Brown *et al.*, 2005; Villa-Castorena *et al.*, 2014).

Fruit yield. Figure 3 shows the mean yield per harvest (kg of fruit per plant) for the cultivars Palenque F1, Jaguar, Orange, and PX-11459057. The analysis of variance of the average accumulated yield from six harvests per plant showed significant differences ($p < 0.05$) in fruit production (Table 2). The cultivars Jaguar and Orange showed viral presence from a very early stage, resulting in damage to plant structure and reduced yield. Palenque F1 and PX-11459057 developed symptoms later and with less impact on production (Table 2). Average fruit yield was 0.23 and 0.10 kg per plant for the cultivars Jaguar and Orange, respectively. Palenque F1 yielded 0.93 kg per plant, and the highest value was recorded for PX-11459057 at 1.41 kg per plant. PX-11459057 showed lower incidence of symptomatic plants at early stages, which resulted in higher accumulated yield (18.92 t ha⁻¹), far surpassing the yields of the cultivars Jaguar and Orange.

Table 2. Average habanero pepper fruit production per plant and potential yield per hectare.

Cultivar	Average production (kg per plant)	Estimated yield (t ha ⁻¹)
Palenque F1	0.93 b	12.51 b
Jaguar	0.23 a	3.09 a
Orange	0.10 a	1.38 a
PX-11459057	1.41 c	18.92 c

Fruit quality. Significant differences were observed in fruit diameter among cultivars ($p < 0.05$), but not in fruit length ($p > 0.05$). According to the varietal description scale for habanero pepper by SAGARPA and SNICS (2014), all fruits were classified as small (Table 3). In all cultivars, fruit size was smaller than the reported genetic potential, which may be attributed to the association with viral symptoms caused by *Begomovirus capsicummusive*, affecting fruit size and coloration. Viral damage may also be responsible for the reduction in the typical intense green color observed in some cultivars, such as Palenque F1.

Table 3. Average diameter and length of habanero pepper fruits harvested from plants associated with *Begomovirus capsicummusive*.

Cultivar	Diameter (cm)	Fruit diameter scale (SAGARPA, SNICS, 2014)	Length (cm)	Fruit length scale (SAGARPA, SNICS, 2014)
Palenque F1	2.87 b	Small	4.1 a	Short
Jaguar	2.81 b	Small	3.6 a	Short
Orange	2.45 a	Small	3.8 a	Short
PX-11459057	2.98 b	Small	4.1 a	Short

All cultivars showed fruit dimensions and weight below the potential values reported for Palenque F1, Jaguar, Orange, and PX-11459057 (Ramírez-Meraz *et al.*, 2018; López-López *et al.*, 2018; Agromora, 2021; Bayer, 2024). This type of morphological damage has been reported for *Geminivirus* in *Capsicum* spp., where viral proteins have been shown to interfere with auxin and gibberellin hormone signaling, affecting plant architecture and fruit development (Ascencio-Ibáñez *et al.*, 2008). From an agronomic perspective, the reduction in yield and fruit size may be attributable to the association with *Begomovirus capsicummusive*, which compromises crop profitability compared with the maximum yields reported by Ramírez-Meraz *et al.* (2018) under intensive management—more than 30 t ha⁻¹ for Jaguar and up to 32.5 t ha⁻¹ in other hybrids, according to Ramírez and Méndez-Aguilar (2020).

These results reinforce the need for accurate diagnosis of the viruses present in Tabasco to support integrated management strategies that include cultivars with genetic tolerance, protection of seedlings at early stages, and effective vector management (Lapidot and Friedmann, 2002; Velásquez-Valle *et al.*, 2014).

CONCLUSIONS

The association of *Begomovirus capsicummusive* (PepGMV) was confirmed in three of the four evaluated cultivars (Palenque F1, Jaguar, and PX11459057) through PCR testing. The high incidence of plants with viral symptoms (greater than 95%) may be associated with the low yield (0.1 to 1.4 kg ha⁻¹) and small fruit size of habanero pepper in Tabasco. The cultivar PX-11459057 showed late incidence at 94 dat (25%), which contributed to its higher yield (1.41 kg ha⁻¹).

Limitations

This study has several limitations that should be considered when interpreting the results. First, no insecticides were applied during the crop cycle in order to avoid interfering with the activity of insect vectors such as *Bemisia tabaci*, thereby allowing viral infection to occur naturally. In addition, the internal physiological effects of viral infection on

biochemical or gene expression parameters were not analyzed, which represents an important opportunity for future research. Likewise, because no viruses were detected with the immunological tests after two sampling rounds, PCR testing for *Begomovirus* was conducted.

Conflict of interest

The authors declare that there is no conflict of interest.

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