

# Virus prevalence and mixed infections in yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) crops in Valle del Cauca, Colombia

## Prevalencia e infección mixta de virus en cultivos de maracuyá amarillo (*Passiflora edulis* f. *flavicarpa*) en el Valle del Cauca, Colombia

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### ABSTRACT

Yellow passion fruit crop is affected by multiple phytosanitary problems. Among the main ones are viruses of the genera *Potyvirus* (soybean mosaic virus, SMV), *Cucumovirus* (cucumber mosaic virus, CMV), *Tymovirus* (tymovirus passiflorae, PYMV) and *Begomovirus* (passionfruit leaf distortion virus, PLDV). Information about the prevalence and interaction between these viruses is scarce. The objective of this study was to verify the prevalence and identify the types of mixed viral infections of potyvirus, cucumovirus, tymovirus, and begomovirus in passion fruit crops in Valle del Cauca, Colombia. Passion fruit leaf samples with symptoms were collected and their nucleic acids were purified. Virus identification was performed by PCR using universal primers by viral genus, and specific primers for the SMV, CMV, PFYMV and PLDV viruses. The incidence of each virus by municipality was analyzed and the severity of the symptoms was compared with the diagnosis made. In total, 66 samples were collected in the municipalities of Toro, Dagua, Roldanillo, Bolívar, La Unión and El Cerrito. It was established that the most prevalent viruses are PLDV followed by SMV and CMV. The presence of PYMV was not detected in the samples analyzed. The presence of double and triple infections among potyvirus, cucumovirus and begomovirus were identified for the first time, finding greater severity of symptoms with a greater number of viruses identified per sample. This research provides key results for the design of control strategies for viral diseases in passion fruit.

Keywords: *Begomovirus*; *Cucumovirus*; Plant viruses; *Potyvirus*; Virus identification.

### RESUMEN

El cultivo de maracuyá amarillo es afectado por múltiples problemas fitosanitarios. Entre los principales, están los virus de los géneros *Potyvirus* (soybean mosaic virus, SMV), *Cucumovirus* (cucumber mosaic virus, CMV), *Tymovirus* (passion fruit yellow mosaic virus, PFYMV) y *Begomovirus* (passionfruit leaf distortion virus, PLDV). Información acerca de la prevalencia e interacción entre estos virus es escasa. El objetivo del presente estudio fue verificar la prevalencia e identificar los tipos de infecciones virales mixtas de potyvirus, cucumovirus, tymovirus y begomovirus en cultivos de maracuyá en Valle del Cauca, Colombia. Muestras foliares de maracuyá con síntomas virales fueron recolectadas y se purificaron sus ácidos nucleicos. La identificación de los virus se realizó por PCR empleando cebadores universales por género viral, y cebadores específicos para los virus SMV, CMV, PFYMV y PLDV. Se analizó la incidencia de cada virus por municipio y se comparó la severidad de los síntomas con el diagnóstico realizado. En total se colectaron 66 muestras en los municipios de Toro, Dagua, Roldanillo, Bolívar, La Unión y El Cerrito. Se estableció que los virus con mayor prevalencia fueron PLDV, seguido por SMV y CMV. No se detectó la presencia de PFYMV en las muestras analizadas. Se identificó por primera vez la presencia de infecciones dobles y triples entre potyvirus, cucumovirus y begomovirus, encontrándose mayor severidad de síntomas a mayor número de virus identificado por muestra. La presente investigación provee resultados clave para el diseño de estrategias de control de enfermedades virales en maracuyá.

Palabras clave: *Begomovirus*, *Cucumovirus*, Identificación de virus, *Potyvirus*, Virus vegetales.

## INTRODUCTION

Currently, passion fruit crops in Colombia are established from coastal areas up to 1500 m a.s.l., with about 10 000 ha and an approximate production of 165 000 tons per year<sup>-1</sup> (Agronet, 2022). The Meta, Antioquia, Huila and Valle del Cauca departments concentrate 63% of the cultivated area and the highest yields (MADR, 2021). Despite this potential, yellow passion fruit crops are being affected by different insect pests and pathogens that decrease yields and reduce fruit quality, making the use of agrochemicals necessary (Ocampo *et al.* 2021).

Pathogens that reduce passion fruit quality and crop yields include plant-parasitic nematodes, fungi, bacteria, and viruses (Ortiz-Paz *et al.* 2012; Ocampo Pérez *et al.* 2022). Viruses can reduce the shelf life of passion fruit and affect plant growth, decreasing production by more than 80% (Vaca-Vaca *et al.* 2016; Asande *et al.* 2023; Kiptui *et al.* 2020; Ramos-González *et al.* 2020).

Currently, five virus genera have been reported affecting the yellow passion fruit crop in Colombia: *Potyvirus*, *Cucumovirus*, *Tymovirus*, *Begomovirus* y *Cilevirus* (Benschler *et al.* 1996; Chavez *et al.* 1999; Morales *et al.* 2001; 2002; Vaca-Vaca *et al.* 2017; Roy *et al.* 2023).

Soybean mosaic virus (SMV) belongs to the genus *Potyvirus*, family *Potyviridae* and species *Soybean mosaic virus*. It has a positive-sense, single-stranded RNA (+ssRNA) genome of 9,583 nt, a viral protein genome-linked (VPg) covalently linked to the 5' end and a poly-A tail at the 3' end (Zhang *et al.* 2009; Jaramillo *et al.* 2018). Several aphid species, including *Aphis gossypii* and *Myzus persicae* were recognized as non-persistent transmitters of this virus, which also spreads through seeds (Domier *et al.* 2007; Jossey *et al.* 2013; Hajimorad *et al.* 2018). In *Passiflora*, SMV causes chlorosis, leaf mosaic and epinasty in early stages of infection, followed by leaf hardening, defoliation and even premature death of infected plants (Benschler *et al.* 1996).

Cucumber mosaic virus (CMV) belongs to the genus *Cucumovirus*, family *Bromoviridae* and species *Cucumber mosaic virus*. It contains a positive-sense, single-stranded RNA (+ssRNA) genome divided into three segments (RNAs 1, 2, and 3), and is transmitted by seeds and by more than 80 species of aphids (*Aphis gossypii* among the most effective) in a non-persistent manner (Palukaitis *et al.* 1992; Chandankar *et al.* 2013; Cardona *et al.* 2022a). The characteristic symptoms of this virus consist of a bright yellow mottling on the leaves, which is more intense on leaves close to the site of infection (Gioria *et al.* 2002). Likewise, roughness and chlorosis of leaves and bleaching of fruits have been associated with the initial infection of this virus in *Passiflora* (Lan *et al.* 2020).

Passion fruit leaf distortion virus (PLDV) belongs to the genus *Begomovirus*, family *Geminiviridae* and species *Passion fruit leaf distortion virus*. It contains a single-stranded circular DNA (ssDNA) genome consisting of two components: DNA-A of 2,600 nt and a DNA-B of 2,572 nt (Vaca-Vaca *et al.* 2017). It is transmitted through the biological vector whitefly (*Bemisia tabaci* complex)

and, in *Passiflora*, causes symptoms such as yellow mosaic and deformation of leaves and fruit (Vaca-Vaca *et al.* 2016; 2017).

Passion fruit yellow mosaic virus (PFYMV) belongs to the genus *Tymovirus*, family *Tymoviridae* and species *Tymovirus passiflorae*. It contains a positive-sense, single-stranded RNA (+ssRNA) genome of 6,088 nt and its natural transmission mechanism has not yet been identified. However, its experimental infection has been achieved through mechanical transmission and by the chrysomelid beetle *Diabrotica speciosa*, exclusively affecting plants of the genus *Passiflora*, causing symptoms such as yellow nets, bright yellow mosaic and leaf roughness (Crestani *et al.* 1986; Jaramillo *et al.* 2019).

Plants are commonly affected by several viruses simultaneously, and understanding the interactions between these causal agents is critical to comprehending viral pathogenesis, which in turn is essential to developing efficient management strategies (Syller, 2012). Plant viruses co-infecting the same host can interact synergistically, causing more severe symptoms than when a single infection occurs (Lamichhane & Venturi, 2015), or antagonistically, when a low virulence virus strain prevents or reduces subsequent infection by a homologous virus (Syller, 2012; Chávez-Calvillo *et al.* 2016). It has been observed that, in some cases, the outcome depends on the order in which infection occurs (Chávez-Calvillo *et al.* 2016; Riska & Hisashi, 2020).

A previous report on virus in yellow passion fruit crops in Colombia revealed the presence of potyvirus in samples from the central west of the country between the departments of Valle del Cauca and Risaralda, which was identified as SMV by serological tests (Benschler *et al.* 1996). In other study, Chavez *et al.* (1999), using serological tests, identified the presence of potyvirus (SMV strain) and tymovirus in yellow passion fruit leaf samples collected in nine departments of Colombia. Subsequently, these viruses were identified by molecular techniques as SMV and PFYMV (Morales *et al.* 2001; 2002). Vaca-Vaca *et al.* (2016) reported for the first time the incidence of begomovirus in yellow passion fruit crops in Valle del Cauca. This begomovirus was characterized at the molecular level, being different from other begomoviruses reported worldwide, and was named PLDV because of the symptoms it causes in passion fruit (Vaca-Vaca *et al.* 2017). Recently, Roy *et al.* (2023) identified SMV in a passion fruit leaf sample from the department of Meta and detected Passion fruit green spot virus (PFGSV), a *Cilevirus* transmitted by mites of the genus *Brevipalpus* in a leaf sample from the department of Casanare.

The objective of this paper was to analyze the prevalence and identify the types of mixed infections among viruses belonging to the genera *Potyvirus*, *Tymovirus*, *Cucumovirus* and *Begomovirus* in the main producing areas of yellow passion fruit in Valle del Cauca. The results obtained provide an updated perspective on the viral diversity limiting passion fruit production in Valle del Cauca.

## MATERIALS AND METHODS

**Collection of plant material.** During 2017, a collection of yellow passion fruit leaves with viral symptoms was conducted in six municipalities of Valle del Cauca in Colombia. Among five and ten plants with viral symptoms were randomly collected in each crop. The sampling sites were geo-referenced, and the samples were transported in a styrofoam cooler with cooling gel bags at  $\approx 10^{\circ}\text{C}$  to the Laboratorio de Sanidad y Microbiología Agrícola de la Universidad Nacional Sede Palmira for further processing and molecular analysis. Once in the laboratory, each sample composed of 3 leaves of normal size ( $\geq 10$  cm in length) or 4 leaves of smaller size, was stored at room temperature ( $25^{\circ}\text{C}$ ) with silica gel, in plastic bags with hermetic seal, thus achieving dehydration and stabilization of the RNA for at least 6 months (Munguti *et al.* 2016; Ruiz-Vargas *et al.* 2024).

### Purification of nucleic acids.

**Extraction and Visualization of Genomic DNA.** From each sample, 20 to 30 g of dry leaf tissue was taken and macerated with liquid nitrogen in a mortar until a fine powder was obtained. The powder obtained was transferred to a 1.5 ml microtube and genomic DNA extraction was performed using the cetyl trimethyl ammonium bromide (CTAB) methodology reported by Doyle *et al.* (1990). Quantification and verification of the quality of the extracted DNA was performed by 0.8% (w/v) agarose gel electrophoresis, which was run in TAE 1X at 70 volts for 50 minutes. Ethidium bromide staining ( $10\text{ ng}/\mu\text{l}$ ) was used, and the Thermo Scientific<sup>TM</sup> GeneRuler 1Kb DNA Ladder Molecular Weight Marker was used as a reference standard. Development was performed on BioRad Gel Doc XR+ imaging system, consisting of a transilluminator and Quantity One v.4.6.5 software.

**RNA Extraction and cDNA Synthesis.** Total RNA extraction was performed from each sample using TRIzol $\rightarrow$  reagent (Invitrogen<sup>TM</sup>) following the protocol established by the manufacturer. The determination of RNA purity and concentration was performed on 0.8% agarose gels. From the total RNA extracted from each sample, complementary DNA (cDNA) was synthesized via reverse transcription (RT) using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific<sup>TM</sup>) following the manufacturer instructions.

### Virus diagnosis.

**DNA Virus Diagnosis.** Begomovirus and PLDV were detected from the purified total DNA by Polymerase Chain Reaction (PCR). Universal primers MP16 and MP82 were used, which amplify a fragment between 400-500 bp of the N-terminal region of the capsid protein (Cp) (Umaharan *et al.* 1998). In the samples that tested positive, specific detection of PLDV was performed using the species-specific primers PLDV-F23 and PLDV-R405 that amplify a 450 bp fragment of Cp (Vaca-Vaca *et al.* 2020). The amplified products were visualized on 0.8% agarose gels.

**RNA Virus Diagnosis.** From the cDNA synthesized from each sample collected, cucumovirus, CMV, potyvirus, SMV and PFYMV were detected by PCR using universal primers by viral genus and specific viruses. To detect viruses of the genus *Cucumovirus*, the universal primers CMV-F 5'- and CMV-R 5' that amplify a 586-bp fragment of the Cp gene were used (Herrera-Vásquez *et al.* 2009). To detect potyvirus, the universal primers Nib2F and Nib3R that amplify a 350 bp fragment of the Nib (nuclear inclusion protein b) gene were used (Zheng *et al.* 2010). Cucumovirus-positive samples were evaluated for CMV with the species-specific primers CMV cp-F and CMV cp-R that amplify a 229-bp fragment of the Cp gene, designed by Rivera-Toro *et al.* (2020) for the detection of CMV in chili peppers (*Capsicum* sp.). Potyvirus-positive samples were evaluated with SMV species-specific primers designed in this research work: SMV F269 (5'-TGG-GTG-TGG-TTA-TGA-ATG-G-3') and SMV R562: (5'-CTG-TTT-GGT-GTT-GTT-TTG-GAA-GT-3'), which amplify a 313 bp fragment of the Cp gene. Finally, to detect PFYMV, the following species-specific primers were designed and used: PFYMV-R536 (5'-AAC-CCA-AAC-GAG-AGA-GAC-3') and PFYMV-F294 (5'-AAA-CTC-CCA-TCA-ACA-CCA-A-3'), which amplify a 260 bp fragment of the Cp gene. The amplified products of each virus were visualized on 0.8% agarose gels.

**Species-specific Primer Design.** The nucleotide sequences of SMV (GenBank KY249373.1) and PFYMV (GenBank AF467107.1) reported by Jaramillo *et al.* were used to design the species-specific primers for SMV and PFYMV. (2018) y Morales *et al.* (2002), respectively. The design was performed with the CLC Main Workbench 7.9 software. (Qiagen<sup>®</sup>) and its specificity was validated using the Primer Blast program (Ye *et al.* 2012).

**Incidence Analysis.** The incidence percentage (positive samples/total samples\*100) of the different viruses (Agris, 2005) was calculated by locality and municipality. The distribution of single, double and triple infections in the different municipalities was determined. The correlation between the altitudinal gradient 924 - 1692 m a.s.l., and the presence of viruses was evaluated by Pearson correlation (cor.test function, Stats R package) (R Core Team, 2013). The correlation between this altitudinal gradient and the presence of single, double and triple infections was also estimated.

## RESULTS AND DISCUSSION

**Detection of begomovirus and PLDV in yellow passion fruit crops.** A total of 66 passion fruit leaf samples at different phenological stages were collected. The discrimination of the samples by municipality is shown in Table 1. Of the total number of samples analyzed, 45.5% were positive for begomovirus. In general, begomoviruses were distributed in five of the six municipalities evaluated, and in four of them they presented an incidence higher than 50% (Table 1).

The localities with the highest incidence of begomovirus belong to the municipalities of El Cerrito and Bolívar. In contrast, the samples from the municipality of La Unión were the only ones found to be free, not only of begomovirus, but also of the other three RNA viruses evaluated in this research: cucumovirus, potyvirus and tymovirus (Table 1).

Of the begomovirus-positive samples, 50% were found to correspond to PLDV, a virus previously detected in yellow passion fruit crops in the municipalities of Palmira and La Unión, in the department of Valle del Cauca (Vaca-Vaca *et al.* 2016; 2017). Fifteen of the 30 samples in which begomovirus was detected gave negative results when evaluated by PCR for PLDV. This suggests that there is another virus or viruses of this genus infecting yellow passion fruit crops in the department.

### Detection of potyvirus, SMV, cucumovirus, CMV and PFYMV in yellow passion fruit crops.

(a) Potyvirus and SMV. Of 66 samples analyzed, 21.2% were found to be infected with potyvirus. Table 1 shows the diagnosis by municipality. Of these samples, 85.7% were identified as SMV using specific primers. In addition, 2 of the 14 samples in which the presence of virus of the *Potyvirus* genus was detected gave negative results when evaluated by RT-PCR for SMV, which suggests the presence of another virus of this genus in passion fruit crops in Valle del Cauca. The highest incidence of potyvirus was observed in yellow passion fruit crops located in the municipality of Toro, followed by the municipalities of Dagua and Roldanillo. Finally, samples from crops located in the municipalities of El Cerrito and La Unión were free of infestation by pathogens of this viral genus (Table 1).

Table 1. Incidence of viral infections in yellow passion fruit crops in Valle del Cauca, Colombia.

| Municipality | Collected samples | PCR results | <i>Begomovirus</i> | PLDV        | <i>Potyvirus</i> | SMV         | <i>Cucumovirus</i> | CMV         | PFYMV |
|--------------|-------------------|-------------|--------------------|-------------|------------------|-------------|--------------------|-------------|-------|
| Bolívar      | 17                | Positive    | 16                 | 8           | 1                | 1           | 0                  | 0           | 0     |
|              |                   | Incidence % | <b>94.1</b>        | <b>47.1</b> | 5.9              | 5.9         | 0.0                | 0.0         | 0.0   |
| Dagua        | 18                | Positive    | 1                  | 1           | 4                | 3           | 1                  | 0           | 0     |
|              |                   | Incidence % | 5.6                | 5.6         | <b>22.2</b>      | 16.7        | 5.6                | ND          | 0.0   |
| El Cerrito   | 2                 | Positive    | 2                  | 1           | 0                | 0           | 1                  | 1           | 0     |
|              |                   | Incidence % | <b>100.0</b>       | <b>50.0</b> | 0.0              | 0.0         | <b>50.0</b>        | <b>50.0</b> | 0.0   |
| La Unión     | 11                | Positive    | 0                  | 0           | 0                | 0           | 0                  | 0           | 0     |
|              |                   | Incidence % | 0.0                | 0.0         | 0.0              | 0.0         | 0.0                | 0.0         | 0.0   |
| Roldanillo   | 10                | Positive    | 6                  | 3           | 2                | 2           | 3                  | 3           | 0     |
|              |                   | Incidence % | <b>60.0</b>        | <b>30.0</b> | <b>20.0</b>      | <b>20.0</b> | <b>30.0</b>        | <b>30.0</b> | 0.0   |
| Toro         | 8                 | Positive    | 5                  | 2           | 7                | 6           | 8                  | 7           | 0     |
|              |                   | Incidence % | <b>62.5</b>        | <b>25.0</b> | <b>87.5</b>      | <b>75.0</b> | <b>100.0</b>       | <b>87.5</b> | 0.0   |
| Total        | 66                | Positive    | 30                 | 15          | 14               | 12          | 13                 | 11          | 0     |
|              |                   | Incidence % | <b>45.5</b>        | <b>22.7</b> | <b>21.2</b>      | 18.2        | 19.7               | 16.7        | 0.0   |

Incidences greater than 20% in each municipality are indicated in bold. ND, not determined. PLDV: passionfruit leaf distortion virus, SMV: soybean mosaic virus, CMV: cucumber mosaic virus, PFYMV: passion fruit yellow mosaic virus.

In general, potyviruses were identified in four of the six municipalities included in this study, with an incidence higher than 20% in three and an incidence of 87.5% in one. In a study reported by Benschler *et al.* (1996) it was indicated that SMV was detected in three species of the *Passifloraceae* family in the departments of Valle del Cauca and Risaralda: passion fruit (*P. edulis f. flavicarpa* Degener), granadilla (*P. ligularis* Juss.) and badea (*P. quadrangularis* L.). Subsequently, in other reports and in this study, the prevalence of this pathogen has been recorded in yellow passion fruit crops in the department of Valle del Cauca for at least 26 years (Benschler *et al.* 1996; Morales *et al.* 2001). Likewise, SMV was detected in passion fruit plants in the department of Meta (Roy *et al.* 2023). The persistence of this virus in yellow passion fruit and other passion fruit crops in this department and, in general, in the country, is explained by the ability of this virus to be transmitted to the following generations by seed (Domier *et al.* 2007; Cardona *et al.* 2022a;b) and by the use of seeds of unknown phytosanitary quality

by farmers, due to a shortage of certified nurseries for obtaining virus-free seeds (Rodríguez *et al.* 2016).

(b) Cucumovirus and CMV. PCR analysis detected the presence of cucumovirus in 19.7% of the total samples analyzed and the distribution in the different municipalities are shown in Table 1. Of these samples, 84.6% amplified the CMV capsid protein gene fragment. Additionally, 2 of the 13 samples in which the presence of virus of the genus *Cucumovirus* was detected gave negative results when evaluated by PCR for CMV. This suggests that there is another virus or viruses of this genus infecting passion fruit crops in the department.

The highest incidence of cucumovirus was observed in yellow passion fruit crops located in the municipalities of Toro and Roldanillo. In contrast, samples from the localities tested in the municipalities of Bolívar and La Unión were free of this viral

pathogen (Table 1). Possibly, the crops sampled in these localities were initiated with cucumovirus-free plant material, and adequate management of aphids and the natural vector of this virus was done (Chandankar *et al.* 2013; Cardona *et al.* 2022a).

In general, cucumoviruses were identified in four of the six municipalities included in this study. Three of these had an incidence higher than 30% and one had an incidence of 100%. In previous studies, CMV was found in gulupa (*P. edulis f. edulis* Sims) crops in Antioquia (Cardona *et al.* 2022a;b).

(c) Tymovirus PFYMV. Of the 66 samples analyzed, none amplified by PCR the expected 262 bp fragment for the detection of tymovirus PFYMV (Table 1). Crestani *et al.* (1986) reported by serological assays (ELISA) the presence of a virus of the *Tymovirus* genus, PFYMV, which was detected in Brazil affecting yellow passion fruit crops. In Colombia, Morales *et al.* (2001) reported the detection and partial genome sequence of a virus like the PFYMV (pathogenically and antigenically) in yellow passion fruit crops located in the departments of Antioquia, Caldas, Santander and Valle del Cauca. Recently, Jaramillo *et al.* (2019) published the complete genome of the PFYMV virus affecting passion fruit in Colombia, warning that the partial sequence of PFYMV (AF467107.1) reported in the GenBank database by Morales *et al.* (2001), showed some errors at the 3' end, specifically in the ORF coding for the capsid protein. This could explain the negative results obtained in this study, since the primers designed in this research work were created on the region having errors, i.e. the PFYMV capsid protein gene reported by Morales *et al.* (2001).

It is suggested to design new primers on the new sequence reported by Jaramillo *et al.* (2019) or use those already elaborated by these authors to conduct future PFYMV detection studies in passion fruit crops. In the department of Antioquia, Cardona *et al.* (2022 a;b) documented the presence of this virus in both symptomatic and asymptomatic samples of gulupa, with an incidence ranging from 30 to 90%. This finding was achieved by amplification of the aforementioned primers Tymo\_F\_CP and qTymo\_R\_CP designed by Jaramillo *et al.* (2019).

### **Mixed infections of three viral genera in yellow passion fruit crops.**

Plant diseases are commonly the result of interactions between complexes of microorganisms, and hardly of isolated pathogens (Lamichhane & Venturi, 2015). In this investigation, null infections (no infections detected), single (a single virus genus identified), and mixed infections by two (double infections) and three (triple infections) virus genera were detected in the same sample.

Table 2 shows the incidence of mixed infections in 66 samples collected in passion fruit crops, where begomoviruses (PLDV) have the highest incidence in single infections (28.8%), followed by cucumoviruses and potyviruses. At the level of double infections, an incidence of 6.1% can be observed for the combinations *Potyvirus* + *Cucumovirus* and *Potyvirus* + *Begomovirus*, while the

combination *Cucumovirus* + *Begomovirus* had an incidence of 4.5%. Finally, at the level of triple infections, the simultaneous presence of the three viral genera, *Potyvirus*, *Cucumovirus* and *Begomovirus*, was determined in four of the samples evaluated, confirming the joint presence of SMV, CMV and PLDV. This highlights the high variability of interactions that occur naturally in agroecosystems, useful information for the management of viral diseases in plants (Syller, 2012).

Multiple infections were observed in five of the six municipalities evaluated (Table 2). In municipalities such as Bolivar, Dagua, El Cerrito, and Roldanillo, both single and double infections were observed. In the municipality of Toro, only double and triple infections were found, and it was the only municipality where triple infections were detected (Table 2). Such unpredictable pathogen-induced biological and epidemiological effects are the result of constant battles between the genes of the different viruses and the host plant (Liang *et al.* 2016). Likewise, the municipality of La Unión stood out because none of the viral genera included in this study were detected in the samples from the two localities of this municipality included in this study (Table 2). Therefore, it is suggested that the visualized symptoms ranging from leaf lamina chlorosis, interveinal chlorosis, epinasty, yellow mosaic, to leaf roughness and deformation, in the crops sampled in these two localities, may be due to nutritional deficiencies or other stress factors in the plant (Datnoff *et al.* 2007; Hull, 2009).

The main symptoms observed in passion fruit samples positive for virus infections were leaf roughness and deformation (LRD), epinasty (EPI), chlorosis (C) and yellow mosaic (YM) (Figure 1). The samples in which a greater number of symptoms were observed corresponded to simple viral infections by begomovirus (Figure 2c).

Additionally, greater symptom severity was observed in samples in which mixed infections, both double and triple, were detected (Figure 2), suggesting the existence of synergistic interactions (Lamichhane & Venturi, 2015). These results are consistent with the study conducted by Riska & Hisashi (2020) in which mixed artificial inoculation of East Asian passiflora virus and East Asian passiflora distortion virus belonging to the genus *Potyvirus* was performed on *Passiflora foetida* plants, and it was observed that the severity of symptoms after double inoculation was greater than that observed after single infection with either of the two potyviruses. On the other hand, Syller (2012) reported CMV in synergistic co-infections with a potyvirus, potato virus Y (PVY) and suggested that the outcome of interactions between potyviruses and other viruses depends on the co-evolution between these and the host plant.

On the other hand, no significant correlation ( $p > 0.05$ ) was found in this study between the viral infections (genera and species) detected and the altitudinal range where the infected samples came from (data not shown). There was also no significant correlation between the altitudinal range of the samples and the type of infection detected in the samples: single, double and triple infections (data not shown).

Table 2. Types of mixed viral infections identified in yellow passion fruit crops in Valle del Cauca, Colombia.

| Type of mixed infection |                   |             | Simple    |         |             | Double           |                   |                   | Triple                     | Nule        |
|-------------------------|-------------------|-------------|-----------|---------|-------------|------------------|-------------------|-------------------|----------------------------|-------------|
| Municipality            | Collected samples | PCR results | C (CMV)   | P (SMV) | B (PLDV)    | P (SMV)+ C (CMV) | C (CMV)+ B (PLDV) | P (SMV)+ B (PLDV) | P (SMV)+ C (CMV)+ B (PLDV) | No virus    |
| Bolívar                 | 17                | Positive    | 0         | 0       | 15 (8)      | 0                | 0                 | 1 (1+0)           | 0                          | 1           |
|                         |                   | Incidence % | 0         | 0       | <b>88.2</b> | 0                | 0                 | 5.9               | 0                          | 5.9         |
| Dagua                   | 18                | Positive    | 0         | 2 (1)   | 0           | 1 (1+0)          | 0                 | 1 (1+1)           | 0                          | 14          |
|                         |                   | Incidence % | 0         | 11.1    | 0           | 5.6              | 0                 | 5.6               | 0                          | 0           |
| El Cerrito              | 2                 | Positive    | 0         | 0       | 1 (1)       | 0                | 1 (1+0)           | 0                 | 0                          | 0           |
|                         |                   | Incidence % | 0         | 0       | <b>50</b>   | 0                | <b>50</b>         | 0                 | 0                          | 0           |
| La Unión                | 11                | Positive    | 0         | 0       | 0           | 0                | 0                 | 0                 | 0                          | 11          |
|                         |                   | Incidence % | 0         | 0       | 0           | 0                | 0                 | 0                 | 0                          | <b>100</b>  |
| Roldanillo              | 10                | Positive    | 2 (2)     | 0       | 3 (1)       | 0                | 1 (1+1)           | 2 (2+1)           | 0                          | 2           |
|                         |                   | Incidence % | <b>20</b> | 0       | <b>30</b>   | 0                | 10                | <b>20</b>         | 0                          | <b>20</b>   |
| Toro                    | 8                 | Positive    | 0         | 0       | 0           | 3 (2+2)          | 1 (1+0)           | 0                 | 4 (4+4+2)                  | 0           |
|                         |                   | Incidence % | 0         | 0       | 0           | <b>37.5</b>      | 12.5              | 0                 | <b>50</b>                  | 0           |
| Total                   | 66                | Positive    | 2         | 2       | 19          | 4                | 3                 | 4                 | 4                          | 28          |
|                         |                   | Incidence % | 3.3       | 3.3     | <b>28.8</b> | 6.1              | 4.5               | 6.1               | 6.1                        | <b>42.4</b> |

Incidence greater than 20% is indicated in bold. Nomenclature for viral genera: Cucumovirus (C), Potyvirus (P) and Begomovirus (B). The number of samples that tested positive for the specific viruses is indicated in parentheses: C (CMV), P (SMV) y B (PLDV).

### Relationship between the observed symptomatology and the type of virus infection detected in yellow passion fruit crops.

The 93.3% of the samples showed typical symptoms of virus infection (Figure 1) such as bulging (B), chlorosis (C), interveinal chlorosis (iC), defoliation (D), deformations (DEF), dwarfing (Dw), epinasty (EPI), yellow mosaic (YM), leaf roughness and deformation (LRD), and fruit roughness and deformation (FRD). A total of 6.7% of the samples showed no symptoms.

In the samples with single *Begomovirus* infections (Figure 2), symptoms such as: epinasty, leaf roughness and deformation were observed in the 19 positive samples, yellow mosaic in 14 samples, chlorosis in 11 samples, interveinal chlorosis in three samples, and defoliation in two samples. Of the two samples in which single *Potyvirus* infection was detected, epinasty was observed in two, and chlorosis, yellow mosaic, roughness and fruit deformation were also observed in one of them. In the two samples with simple infection by *Cucumovirus*, yellow mosaic, roughness and deformation of leaves were observed, and in one of them chlorosis was also observed. In the four samples with double *Potyvirus* + *Cucumovirus* infection, chlorosis, yellow mosaic, roughness and leaf deformation were observed, and defoliation was also observed in one of them. In the three samples in which double *Cucumovirus* + *Begomovirus* infection was identified, roughness and deformation of leaves and yellow mosaic were observed, in one there was also chlorosis and in another there were also epinasty and bulging. Of the four samples in which double *Potyvirus* + *Begomovirus* infection was detected, one was asymptomatic, three showed epinasty, two showed chlorosis, two showed leaf roughness and deformation, one showed interveinal chlorosis, and one showed defoliation. In the four samples in which triple *Potyvirus* + *Cucumovirus* + *Begomovirus*

infection was detected, roughness and leaf deformation were observed. Chlorosis was also observed in three of them, dwarfing was observed in one sample, and yellow mosaic and deformations were also observed in another. Fruit deformation was observed in one sample.

Other authors have also associated different symptoms with the viral infections detected, such as Benschel *et al.* (1996) who observed yellow mosaic, epinasty and defoliation in passion fruit samples inoculated with SMV *potyvirus*. Likewise, Chavez *et al.* (1999), when inoculating potyvirus and tymovirus in different plants, observed that: (a) in *Passifloras*, interveinal chlorosis, yellow mosaic, chlorosis, deformations, (b) in beans (*Phaseolus vulgaris*), necrosis of veins, roughness and deformation of leaves, and yellow mosaic, and (c) in *Physalis floridiana* interveinal chlorosis. Morales *et al.* (2001) reported the identification of *Potyvirus* SMV causing deformations, yellow mosaic and ringed spots, and tymovirus PFYMV causing yellow mosaic with a tendency to form bands on the leaves. Vaca-Vaca *et al.* (2017) found yellow mosaic and leaf and fruit roughness and deformation in samples diagnosed with *Begomovirus* PLDV.

In conclusion, the prevalence of viruses affecting yellow passion fruit crops in the main producing municipalities of the department of Valle del Cauca was determined. The presence of single, double and triple infections was established among viruses of the *Potyvirus*, *Cucumovirus* and *Begomovirus* genera, associated with symptoms such as roughness and deformation of leaves, yellow mosaic, chlorosis, epinasty, roughness and deformation of fruits. In symptomatic samples with double and triple infections, a greater severity of symptoms was apparently observed, suggesting synergistic interactions between the viruses involved.

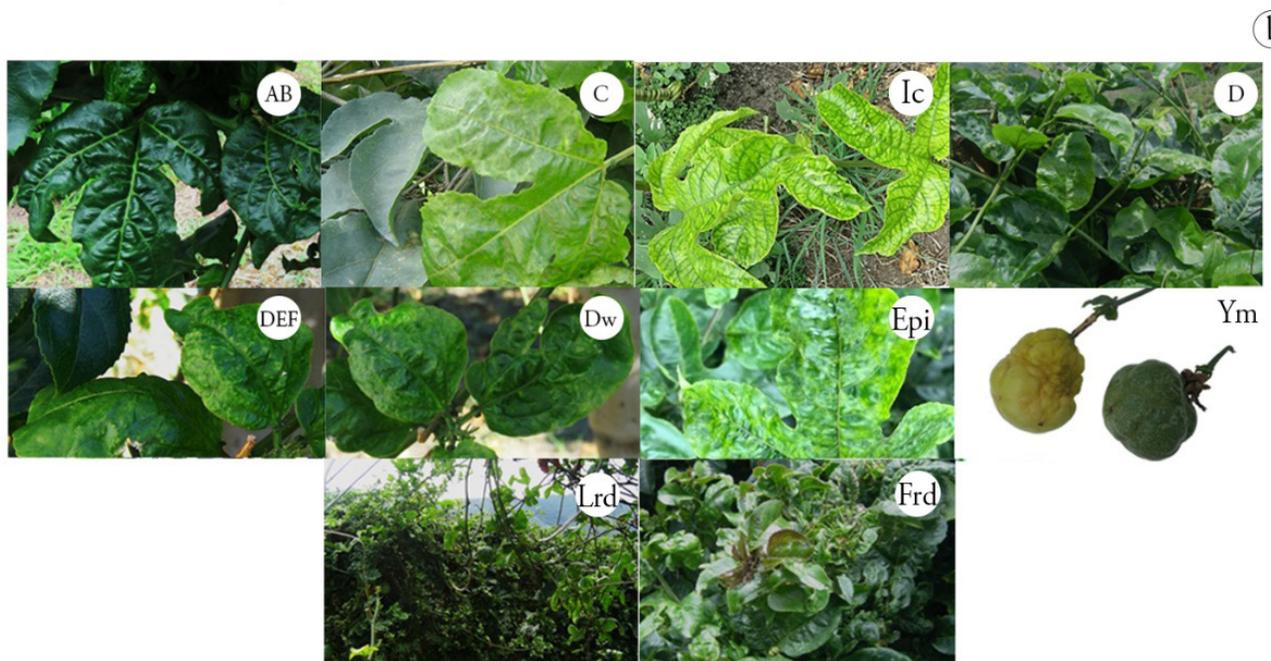
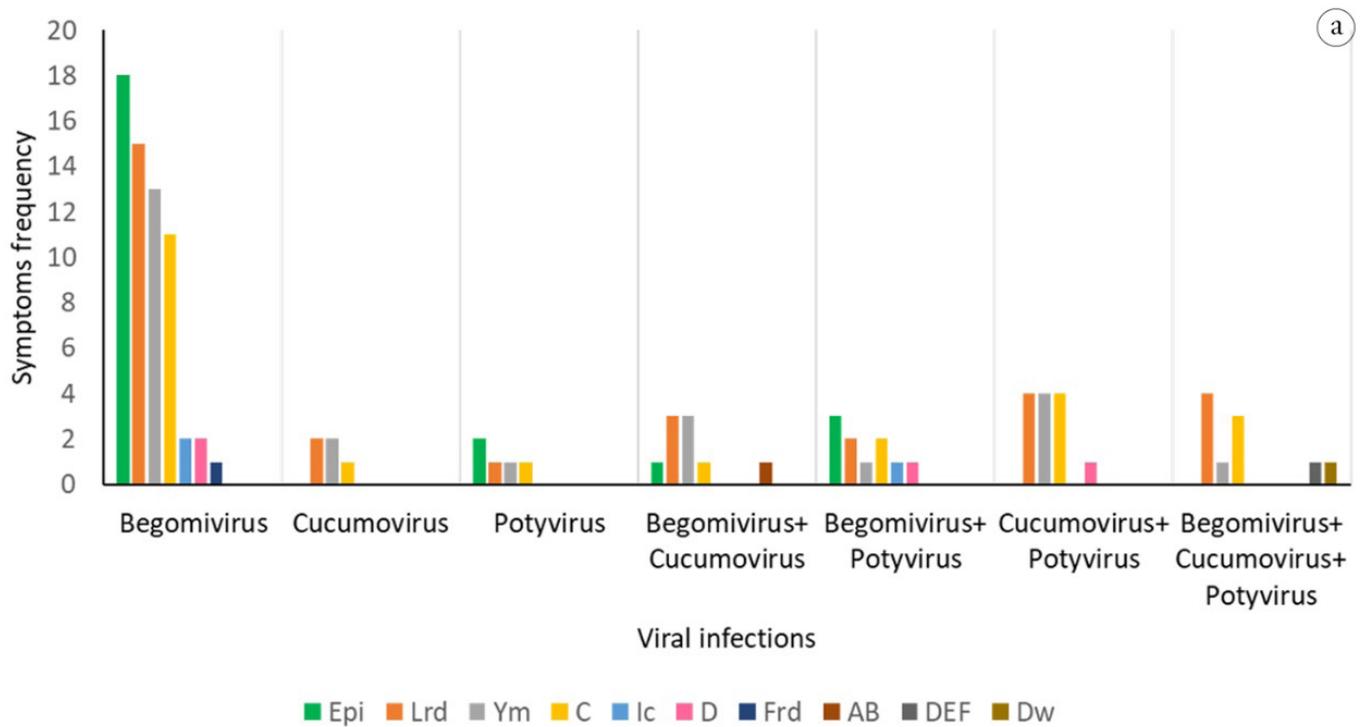


Figure 1. a) Symptoms frequency associated with single, double and triple viral infections b) photography of symptoms: AB (Bulging), C (Chlorosis), Ic (Interveinal chlorosis), D (Defoliation), DEF (Deformities), Dw (Dwarfism), Epi (Epinasty), Ym (Yellow mosaic), Lrd (Leaf Roughness and deformation), Frd (Fruit Roughness and deformation).



Figure 2. Symptomatology observed in yellow passion fruit plants with different viral infections. Simple infections: a) *Cucumovirus* (CMV); b) *Potyvirus* (SMV); c) *Begomovirus* (PLDV). Double infections: d) [*Potyvirus* (SMV) + *Cucumovirus* (CMV)]; e) [*Cucumovirus* (CMV) + *Begomovirus*]; f) [*Potyvirus* (SMV) + *Begomovirus*]. Triple infections: g and h) [*Potyvirus* (SMV) + *Cucumovirus* (CMV) + *Begomovirus*] and i) [*Potyvirus* (SMV) + *Cucumovirus* (CMV) + *Begomovirus* (PLDV)].

The results obtained in this study indicate that begomovirus PLDV prevails over SMV and CMV as a viral agent that is limiting passion fruit production in Valle del Cauca. This information is quite useful for the producer, since it allows him not only to orient his cultural activities towards the control of these phytopathogens but also to undertake future breeding programs aimed at obtaining materials tolerant to viruses that really affect and limit the production of this *Passiflora* in Valle del Cauca.

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Francy Jhoana Ceballos-Burgos: Data screening, formal analysis, research, visualization, writing the original draft, proofreading and editing. Jorge Iván Pérez-López: research, proofreading and editing. Andres Felipe Nieto-Cárdenas: research, proofreading and editing. Diana Marcela Rivera-Toro: research, proofreading and editing. John Albeiro Ocampo: research, proofreading and editing. Karina López-López: Conceptualization, Data screening, Formal analysis, Methodology, writing the original draft, proofreading and editing. Juan Carlos Vaca-Vaca: Conceptualization, Methodology, Acquisition of funding, Project management, Supervision, writing original draft, proofreading and editing.

## REFERENCES

- AGRIOS, G.N. 2005. Plant Pathology. Fifth edition. Elsevier Academic Press. USA. 167p.
- AGRONET. 2022. Red de información y comunicación del sector agropecuario de Colombia, Agronet MinAgricultura. Available from Internet in: <https://www.agronet.gov.co/estadistica/Paginas/home.aspx?cod=1>

- ASANDE, L.K.; OMBORI, O.; ODUOR, R.O.; NCHORE, S.B.; NYABOGA, E.N. 2023. Occurrence of passion fruit woodiness disease in the coastal lowlands of Kenya and screening of passion fruit genotypes for resistance to passion fruit woodiness disease. *BMC Plant BiolOgy*. 23:544. <https://doi.org/10.1186/s12870-023-04546-8>
- BENSCHER, D.; PAPPU, S. S.; NIBLETT, C. L.; VARON DE AGUDELO, F.; MORALES, FRANCISCO JOSÉ; HODSON DE JARAMILLO, E.; ÁLVAREZ, E.; ACOSTA, O. 1996. A strain of soybean mosaic virus infecting *Passiflora* spp. in Colombia. *Plant Disease*. 80(3):258-262. <https://doi.org/10.1094/pd-80-0258>
- CARDONA, D.; GALLO GARCÍA, Y.; HIGUITA, M.; HOYOS SÁNCHEZ, R.; GUTIÉRREZ SÁNCHEZ, P.A.; MARÍN MONTOYA, M. 2022a. Detección molecular de virus en cultivos, plántulas y semillas de gulupa (*Passiflora edulis* f. *edulis*) en el Oriente de Antioquia. *BioAgro*. 34(2):125-138. <http://www.doi.org/10.51372/bioagro342.3>
- CARDONA, D.; HIGUITA, M.; POSADA, J.; GALLO, Y.; MARÍN, M.; GUTIÉRREZ, P. 2022b. Viruses infecting purple passion fruit (*Passiflora edulis* f. *edulis*) in southwestern Antioquia, Colombia. *Archives of Phytopathology and Plant Protection*. 55(12):1394-1409. <https://doi.org/10.1080/03235408.2022.2098588>
- CHANDANKAR, V.D.; MONDHE, M.K.; BHOYAR, P.R.; NINAWA, B.N.; JADESHA, G. 2013. Biophysical characterization, host range and transmission studies of Cucumber mosaic virus. *The Bioscan*. 8(2):437-441.
- CHAVEZ, B.; VARON DE AGUDELO, F.; MORALES, FRANCISCO JOSÉ; CASTAÑO, M.; ARROYAVE, JOSÉ A.; GÁLVEZ E.; GUILLERMO E. 1999. Reconocimiento, transmisión y hospederas de patógenos virales del maracuyá (*Passiflora edulis* Sims) en Colombia. *Fitopatología Colombiana*. 3(1): 24-31.
- CHÁVEZ-CALVILLO, G.; CONTRERAS-PAREDES, C.A.; MORA-MACIAS, J.; NOA-CARRAZANA, J.C.; SERRANO-RUBIO, A.A.; DINKOVA, T.D.; CARRILLO-TRIPP, M.; SILVA-ROSALES, L. 2016. Antagonismo or synergism between papaya ringspot virus and papaya mosaic virus in *Carica papaya* is determined by their order of infection. *Virology*. 489:179-191. <https://doi.org/10.1016/j.virol.2015.11.026>
- CRESTANI, O.A.; KITAJIMA, E.W.; LIN, M.T.; MARINHO, V.L.A. 1986. Passion fruit yellow Mosaic virus, a new tymovirus found in Brazil. *Phytopathology*. 76(9):951-955.
- DATNOFF, L.E.; ELMER, W.H.; HUBER, D.M. 2007. Mineral nutrition and plant disease. *American Physiological Society*. St. Paul, Minnesota. USA. 278p.
- DOMIER, L.L.; STEINLAGE, T.A.; HOBBS, H.A.; WANG, Y.; HERRERA-RODRIGUEZ, G., HAUDENSHIELD, J.S., MCCOPPIN, N.K.; HARTMAN, G.L. 2007. Similarities in Seed and Aphid Transmission Among *Soybean mosaic virus* Isolates. *Plant Disease*. 91(5):546-550. <https://doi.org/10.1094/PDIS-91-5-0546>
- DOYLE, J.J.; DOYLE, J.L.; BAILEY HORTORIUM, L.H. 1990. Isolation of plant DNA from fresh tissue. *Focus*. 12(1):13-15.
- GIORIA, R.; ESPINHA, L.M.; REZENDE, J.A.M.; GASPAR, J.O.; KITAJIMA, E.W. 2002. Limited movement of *Cucumber mosaic virus* (CMV) in yellow passion flower in Brazil. *Plant Pathology*. 51(2):127-133. <https://doi.org/10.1046/j.1365-3059.2002.00678.x>
- HAJIMORAD, M.R.; DOMIER, L.L.; TOLIN, S.A.; WHITHAM, S.A.; SAGHAI MAROOF, A. 2018. Soybean mosaic virus: a successful potyvirus with a wide distribution but restricted natural host range. *Molecular Plant Pathology*. 19(7):1563-1579. <https://doi.org/10.1111/mp.12644>
- HERRERA-VÁSQUEZ, J.A.; ALFARO-FERNÁNDEZ, A.; CÓRDOBA-SELLÉS, M.C.; CEBRIÁN, M.C.; FONT, M.I.; JORDÁ, C. 2009. First Report of *Tomato torrado virus* Infecting Tomato in Single and Mixed Infections with Cucumber mosaic virus in Panama. *Plant disease (USA)*. 93(2):198. <https://doi.org/10.1094/PDIS-93-2-0198A>
- HULL, R. 2009. *Comparative Plant Virology*. Elsevier Academic Press. San Diego. 400p.
- JARAMILLO MESA, H.; MARÍN MONTOYA, M.; GUTIÉRREZ SÁNCHEZ, P.A. 2018. Molecular characterization of Soybean mosaic virus (SMV) infecting Purple passion fruit (*Passiflora edulis* f. *edulis*) in Antioquia, Colombia. *Archives of Phytopathology and Plant Protection*. 51(11-12):617-636. <https://doi.org/10.1080/03235408.2018.1505411>
- JARAMILLO MESA, H.; MARÍN MONTOYA, M.; GUTIÉRREZ SÁNCHEZ, P.A. 2019. Complete genome sequence of a Passion fruit yellow mosaic virus (PFYMV) isolate infecting purple passion fruit (*Passiflora edulis* f. *edulis*). *Revista Facultad Nacional de Agronomía Medellín (Colombia)*. 72(1):8643-8654. <https://doi.org/10.15446/rfnam.v72n1.69438>
- JOSSEY, S.; HOBBS, H.A.; DOMIER, L.L. 2013. Role of soybean mosaic virus-encoded proteins in seed and aphid transmission in soybean. *Phytopathology*. 103(9):941-8. <https://doi.org/10.1094/PHYTO-09-12-0248-R>
- KIPTUI, L.J.; TOROITICH, F.J.; KILALO, D.C.; OBONYO, M. 2020. Interaction between Cowpea Aphid-Borne Mosaic Virus Isolates and Its Effect on *Passion Fruit* Woodiness Disease on *Passiflora edulis* Sims and *Passiflora ligularis* Juss. *Advances in Agriculture*. 2020 2020:8876498. <https://doi.org/10.1155/2020/8876498>

- LAMICHHANE, J.R.; VENTURI, V. 2015. Synergisms between microbial pathogens in plant disease complexes: a growing trend. *Frontiers in Plant Science* (Suiza). 6:385. <https://doi.org/10.3389/fpls.2015.00385>
- LAN, H.; BAOCHUN, L.; PENG, Z.; XU, D.; WANTING, W.; YANJIE, Y.; ZUJIAN, W.U. 2020. Cucumber mosaic virus infection modulated the phytochemical contents of *Passiflora edulis*. *Microbial Pathogenesis*. 138:103828. <https://doi.org/10.1016/j.micpath.2019.103828>
- LIANG, Z.; DICKISON, V.; SINGH, M.; XIONG, X.; NIE, X. 2016. Studies of tomato plants in response to infection with PVX and difference PVY isolates reveal a remarkable PVX-PVY<sup>NTN</sup> synergism and diverse expression profiles of genes involved in different pathways. *European Journal of Plant Pathology*. 144(1):55-71. <https://doi.org/10.1007/s10658-015-0750-4>
- MINISTERIO DE AGRICULTURA Y DESARROLLO RURAL, MADR. 2021. Cadena de Pasifloras. Indicadores e instrumentos. Available from Internet in: <https://sioc.minagricultura.gov.co/Pasifloras/Documentos/2020-06-30%20Cifras%20Sectoriales.pdf>
- MORALES, F.J.; LOZANO POTES, I.; CASTAÑO, M.; ARROYAVE, J.A.; VELASCO, A.C.; VARON DE AGUDELO, F. 2002. Partial characterization of a tymovirus infecting passion fruit in Colombia, South America. *Journal of Phytopathology (USA)*. 150(4-5): 292-296.
- MORALES, F.J.; LOZANO POTES, I.; MUÑOZ, C.; CASTAÑO ZAPATA, M.; ARROYAVE, J.A.; VARÓN DE AGUDELO, F.; CHAVEZ, B.; CASTILLO, G.P. 2001. Caracterización molecular de los virus que afectan al maracuyá (*Passiflora edulis* Sims) y otras passifloras en Colombia. *Fitopatología Colombiana*. 25(2):99-102.
- MUNGUTI, F.M.; KILALO, D.C.; MACHARIA, M.; MAGIRI, E.N.; KINYUA, J.K.; HOLTON, T.A. 2016. Evaluation of *Passiflora edulis* leaf sample storage methods on RNA quality and suitability for use in RT-PCR assays. *Annual Research & Review in Biology*. 10(1):1-8. <https://doi.org/10.9734/ARRB/2016/24497>
- OCAMPO PÉREZ, J.; MORILLO CORONADO, Y.; ESPINAL AGUILAR, F.J.; MORENO CABRERA, I. 2022. Tecnología para el cultivo del maracuyá (*Passiflora edulis* f. *flavicarpa* Degener) en Colombia. Universidad Nacional de Colombia, y Corporación Colombiana de Investigación Agropecuaria – Agrosavia Palmira. Palmira, Colombia. 100p.
- OCAMPO, J.; MARÍN, V.; URREA, R. 2021. Agromorphological characterization of yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener) reveals elite genotypes for a breeding program in Colombia. *Agronomía Colombiana*. 39(2):156-176. <https://doi.org/10.15446/agron.colomb.v39n2.91622>
- ORTIZ-PAZ, R.A.; GUZMÁN-PIEDRAHITA, O.A.; OCAMPO, J.A. Identification of plant parasitic nematodes in the germplasm bank of yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener) in Colombia. 2012. *Acta agronómica (Colombia)*. 61(4):267-276.
- PALUKAITIS, P.; ROOSSINCK, M.J.; DIETZGEN, R.G.; FRANCKI, R.I.B. 1992. Cucumber Mosaic Virus. *Advances in Virus Research*. 41:281-348. [https://doi.org/10.1016/S0065-3527\(08\)60039-1](https://doi.org/10.1016/S0065-3527(08)60039-1)
- R CORE TEAM. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Available from Internet in: <http://www.r-project.org/>
- RAMOS-GONZÁLEZ, P.L.; SANTOS, G.F.; CHABI-JESUS, C.; HARAKAVA, R.; KITAJIMA, E.W.; FREITAS-ASTÚA, J. 2020. Passion Fruit Green Spot Virus Genome Harbors a New Orphan ORF and Highlights the Flexibility of the 5'-End of the RNA2 Segment Across Cileviruses. *Frontiers in Microbiology*. 11:206. <https://doi.org/10.3389/fmicb.2020.00206>
- RISKA, M.N.; HISASHI, I. 2020. Effects of Coinfection with East Asian *Passiflora* Virus and East Asian *Passiflora* Distortion Virus on *Passiflora Foetida*. *Journal of General Plant Pathology (Alemania)*. 86(3):211-18. <https://doi.org/10.1007/s10327-020-00913-7>
- RIVERA-TORO, D.M.; VACA-VACA, J.C.; LÓPEZ-LÓPEZ, K. 2020. Detection and molecular characterization of the cucumber mosaic virus in chili pepper (*Capsicum* spp. L.) crops. *Agronomía Colombiana*. 38(2):279-286. <https://doi.org/10.15446/agron.colomb.v38n2.82975>
- RODRÍGUEZ, M.H.; NIÑO, E.N.; CUTLER, J.; LANGER, J.; CASIERRA-POSADA, F.; MIRANDA, D.; BANDTE, M.; BÜTTNER, C. 2016. Certificación de material vegetal sano en Colombia: un análisis crítico de oportunidades y retos para controlar enfermedades ocasionadas por virus. *Revista Colombiana de Ciencias Hortícolas*. 10(1):164-175. <https://doi.org/10.17584/rcch.2016v10i1.4921>
- ROY, A.; GUILLERMO, L.M.; NUNZIATA, S.; PADMANABHAN, C.; RIVERA, Y.; BRLANSKY, R. H.; HARTUNG, J. 2023. First report of Passion fruit green spot virus in yellow Passion fruit (*Passiflora edulis* f. *flavicarpa*) in Casanare, Colombia. *Plant disease*. 107(7):2270 <https://doi.org/10.1094/PDIS-09-22-2267-PDN>
- RUIZ-VARGAS, N.; RAMANAUSKAS, K.; TYSZKA, A.S.; BRETZ, E.C.; YEO, M.T.S.; MASON-GAMER, R.J.; WALKER, J.F. 2024. Transcriptome data from silica-preserved leaf tissue reveal gene flow patterns in a Caribbean bromeliad. *Annals of Botany*. 133(3): 459-472. <https://doi.org/10.1093/aob/mcae002>

- SYLLER, J. 2012. Facilitative and antagonistic interactions between plant viruses in mixed infections. *Molecular Plant Pathology*. 13(2):204-216. <https://doi.org/10.1111/J.1364-3703.2011.00734.X>
- UMAHARAN, P.; PADIDAM, M.; PHELPS, R. H.; BEACHY, R. N.; FAUQUET, C. M. 1998. Distribution and diversity of geminiviruses in Trinidad and Tobago. *Phytopathology*. 88(12):1262-1268. <https://doi.org/10.1094/PHYTO.1998.88.12.1262>
- VACA-VACA, J.C.; CARRASCO-LOZANO, E.C.; LÓPEZ-LÓPEZ, K. 2017. Molecular Identification of a new begomovirus infecting yellow passion fruit (*Passiflora edulis*) in Colombia. *Archives of Virology (Austria)*. 162(2):573-576. <https://doi.org/10.1007/s00705-016-3098-y>
- VACA-VACA, J.C.; CARRASCO-LOZANO, E.C.; RODRÍGUEZ-RODRÍGUEZ, M.; BETANCUR-PÉREZ, J.F.; LÓPEZ-LÓPEZ, K. 2016. Primer reporte de un begomovirus presente en maracuyá amarillo [*Passiflora edulis* f. *flavicarpa* (Degener)] en Valle del Cauca, Colombia. *Revista Colombiana de Biotecnología*. 18(2):56-65. <https://doi.org/10.15446/rev.colomb.biotec.v18n2.52904>
- VACA-VACA, J.C.; JARA-TEJADA, F.; LÓPEZ-LÓPEZ, K. 2020. Partial molecular characterization of begomoviruses isolated from weeds collected in tomato crops in the southeast of Valle del Cauca, Colombia. *Revista Colombiana de Ciencias Hortícolas*. 14(1):115-124. <https://doi.org/10.17584/rcch.2020v14i1.10434>
- YE, J.; COULOURIS, G.; ZARETSKAYA, I.; CUTCUTACHE, I.; ROZEN, S.; MADDEN, T.L. 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC bioinformatics*. 13(134):1-11. <https://doi.org/10.1186/1471-2105-13-134>
- ZHANG, C.; HAJIMORAD, M.R.; EGGENBERGER, A.L.; TSANG, S.; WHITHAM, S.A.; HILL, J.H. 2009. Cytoplasmic inclusion cistron of Soybean mosaic virus serves as a virulence determinant on Rsv3-genotype soybean and a symptom determinant. *Virology*. 391(2):240-248. <https://doi.org/10.1016/j.virol.2009.06.020>
- ZHENG, L.; RODONI, B.C.; GIBBS, M.J.; GIBBS, A.J. 2010. A novel pair of universal primers for the detection of potyviruses. *Plant Pathology*. 59(2):211-220. <https://doi.org/10.1111/j.1365-3059.2009.02201.x>