Peruvian Journal of Agronomy
http://revistas.lamolina.edu.pe/index.php/jpagronomy/index

RESEARCH ARTICLE
https://doi.org/10.21704/pja.v7i1.1957

Received for publication: 03 January 2023
Accepted for publication: 24 April 2023
Published: 27 April 2023
ISSN: 2616-4477

Detection of *Furcraea Necrotic Streak Virus* (FNSV) in fique seed (*Furcraea macrophylla* Baker) to prevent the spread of the macana disease

Detección del virus de la raya necrótica del fique en semillas de fique (*Furcraea macrophylla* Baker) para prevenir la dispersión de la enfermedad de la macana

Maira Gamero[1], Daniel Ortiz[2], Gloria Barrera[1*]

*Corresponding author: mgamero@agrosavia.co
*https://orcid.org/0000-0003-2069-1868

Abstract

The fique crop has great potential for the development of the natural fiber market as a beneficial alternative for the protection of the environment. In Colombia, one of the main limitations of fiber production in fique plants is the viral disease Macana caused by *Furcraea Necrotic Streak Virus* (FNSV). This work aimed to validate the detection of FNSV in asexual planting material from one of the main producing areas and thus contribute to preventing the spread of the disease in the country. The analysis of plants from different geographic altitudes in Cauca, Colombia, showed a positive correlation with the prevalence of Macana disease (being more significant at higher altitudes) but not with the severity of the symptoms. The detection of FNSV on seeds by dot blot immunobinding assay (DBIA) using a polyclonal antibody IgY showed sensitivity (79 %) and specificity (80 %) when sprouts were analyzed, at the same time, for bulbils, the sensitivity was higher (100 %). Moreover, when sprouts were analyzed by the RT-PCR based on FNSV movement protein and polymerase-associated proteins, the sensitivity and specificity were 94 % and 50 %, respectively, in contrast, in the case of bulbils, the specificity was higher (100 %). Additionally, the results showed no uniformity in the distribution of the viral particles on vegetal tissue of infected plants, which is necessary to use the largest amount of tissue possible to perform the detection. As part of the optimization of the techniques, it was shown that plant tissue samples could be collected, transported, and stored on filter paper, allowing the detection of the virus 60 days after collection.

Keywords: *Macana, viral diagnosis, RT-PCR, DBIA, fique, FNSV, natural fiber, validation.*

Resumen

El cultivo de fique tiene un gran potencial para el desarrollo del mercado de la fibra natural, como alternativa beneficiosa para la protección del medio ambiente. Una de las principales limitaciones en la producción de fibra de fique en Colombia es la enfermedad viral Macana, causada por *Furcraea Necrotic Streak Virus*. El objetivo de este trabajo fue validar la detección del FNSV en material de siembra asexual procedente de una de las principales zonas productoras, con el fin de contribuir a la prevención de la enfermedad en el país. El análisis de las plantas provenientes de diferentes altitudes geográficas en Cauca, Colombia, mostró una relación positiva

How to cite this article:
Gamero, M., Ortiz, D., Barrera, G. (2023). Detection of *Furcraea Necrotic Streak Virus* (FNSV) in fique seed (*Furcraea macrophylla* Baker) to prevent the spread of the macana disease. *Peruvian Journal of Agronomy, 7*(1), 69-81 https://doi.org/10.21704/pja.v7i1.1957

[1] Centro de Investigación Tibaitatá, Mosquera, Colombia.
[2] Centro de Investigación Palmira, Popayán, Colombia.

con la prevalencia de la enfermedad de la Macana (a mayor altitud, mayor prevalencia), pero no hubo relación con la severidad de los síntomas. La detección del FNSV en semillas mediante inmunodetección por dot blot (DBIA) usando un anticuerpo polyclonal IgY, mostró una sensibilidad de 79% y una especificidad del 80%, en el caso de hijuelos, mientras que, para bulbillos la sensibilidad fue mayor (100%). Además, cuando los hijuelos fueron analizados por RT-PCR de dos regiones de genes específicos de proteína de movimiento y proteinas asociadas a la polimerasa del virus, la sensibilidad y especificidad fueron de 94 y 50%, respectivamente, mientras que, en bulbillos la especificidad fue más alta (100%). Adicionalmente, los resultados mostraron que no hay uniformidad en la distribución de las partículas virales en el tejido de plantas infectadas, siendo necesario el uso de gran cantidad de tejido para realizar la detección. Como parte de la optimización de las técnicas, se demostró que las muestras de tejido vegetal pueden ser recolectadas, transportadas y almacenadas en papel de filtro, lo que permite la detección del virus 60 días después de la recolección.

Palabras clave: *Macana, diagnóstico viral, RT-PCR, DBIA, fique, FNSV, fibra natural, validación.*

Introduction

The global demand for natural fibers (NF) has grown in recent years in response to environmental concerns and the depletion of petroleum resources (Safaripour et al., 2021). NF has a long history of serving humanity in a wide range of applications, and its importance was recognized by the United Nations and the Food and Agriculture Organization of the United Nations, declaring 2009 as the International Year of Natural Fibers. NF are sustainable materials characterized by biodegradability, low cost, and lightness. Their resources include lignocellulosic-like seed, bast, leaves, grass, bark, and proteins (Kozłowski et al., 2020). The fique (*Furcraea macrophylla* Baker), cultivated in the Andean region of Colombia, produces NF from leaves that are classified as hard fibers (Echeverri et al., 2015). The global market of natural fibers has shown significant growth with several commercial and industrial applications, including building materials, interiors of automobiles, medicine, and cosmetics, among others (Data Bridge Market Research, 2020). Currently, the fique fibers in Colombia are used for packaging agricultural products, agromantles, geotextiles, strings, ropes, and handicrafts (Ministerio de Agricultura y Desarrollo Rural [MADR], 2021). To properly exploit the potential of the Fique crop, it is necessary to overcome some limitations, including some associated with phytosanitary problems such as “macana” or necrotic streak disease of fique, which is caused by the positive RNA virus *Furcraea necrotic streak virus* - FNSV (Family: Tombusviridae, genus: *Macanavirus*) (Adams et al., 2013). The macana disease reduces fiber quality by progressive necrosis on tissue leaves, causing losses of up to 30 % of production (Flórez et al., 2013), and in advanced infections can lead to complete loss of the plant, increasing the shortage, which raises approximately 10 000 tons of fiber per year (MADR, 2021; Ortiz-González et al., 2021).

Viral phytopathogens are a significant threat to agricultural production systems worldwide and are difficult to manage due to the evolutionary capacity and adaptation of viruses to climate change, which has affected their geographic distribution and that of their vectors (Sarwar et al., 2020). Strategies to control viral diseases in plants are based on preventive measures for their dispersion, which include vector control and eradication of infected plants, among others (Rubio et al., 2020). The use of virus-free seed is recommended to control the spread of the disease; however, infected plants with FNSV can be asymptomatic, which is necessary to diagnose with laboratory methods (Toloza-Moreno et al., 2022).

For virus detection, the serological techniques are based on the specificity of the antigen-antibody interaction, where the most used methodologies are ELISA (enzyme-linked immunosorbent assay), DBIA (Dot Blot immunoassay), and TBIA (Tissue Blot Immunoassay) (Abd El-Aziz, 2019). These techniques have advantages that include sensitivity, simplicity, speed, and low cost, allowing their wide use in phytopathological diagnosis (Hsu, 2009; Boonham et al., 2014; Singh et al., 2021). With the advancement in the knowledge of macana disease and its causal agent, FNSV detection tests have recently been developed for the detection of FNSV in root

and leaf extracts of infected plants, including anti-FNSV IgY polyclonal antibodies and RT-PCR (Reverse Transcription Polymerase Chain Reaction) (Toloza-Moreno et al., 2022). In order to validate the usefulness of these diagnostic techniques and prevent the spread of the disease through infected seeds, it is necessary to determine the feasibility of the method on the planting material, optimize the protocols, as well as to establish the diagnostic parameters and their limitations (Jacobson, 1998). Among the most critical diagnostic validation parameters are specificity, sensitivity, positive and negative predictive values, and prevalence which guarantee the reproducibility of the techniques and the certainty of predicting the presence or absence of the pathogen to be diagnosed (Jacobson, 1998).

Therefore, this work aimed to study the prevalence and severity of the disease in one of the main fique-producing areas of Colombia and to validate the methods for the phytosanitary diagnosis of FNSV in asexual fique seed (bulbs and sprouts). It is expected to be able to contribute to the selection of seeds with phytosanitary quality to avoid the spread of the disease.

Materials and methods

Disease prevalence and severity evaluation

The disease prevalence and severity evaluation were carried out in Cauca which is the second higher fique producer department of Colombia. For prevalence analysis, 9 stablish fique crops were sampled in Totoró and Silvia municipalities (between 2 060 and 2 578 meters above sea level (m.a.s.l.), taking 95 adult fique plants (*F. macrophylla*) by using random and zigzag sampling. The height above sea level and the geographic coordinates were recorded at each sampling site. Disease prevalence was determined by the ratio of the number of plants with symptoms and the total number of plants evaluated. A scale was designed to determine the disease severity according to the leaf area affected (Table 1). Correlation analyses were performed using the linear model Y= mX+b with the STATGRAPHICS Centurion version XVI® program.

Seed collection

For seed collection, fique plants with asexual seeds (bulbils and sprouts) were selected from sampling mentioned above and were used for validation of the diagnostic methods. The samples included 26 plants with symptoms of infection on their leaves, and 19 without symptoms. The tools were disinfected with 5% hypochlorite and 10% alcohol before the collection procedure. The material was labeled and sent to the molecular biology laboratory of Tibaitatá Investigation Center for storage at -80ºC and subsequent processing.

Table 1. Scale for assessing the severity caused by the macana virus in fique leaves.

Grade	1	2	3	4	5	6
Severity	0 %	1 % – 10 %	11 % – 25 %	26 % – 50 %	51 % – 75 %	76 % – 100 %
Description	No visible disease symptoms such as chlorotic or necrotic lesions.	Chlorotic, necrotic spots or interveinal chlorosis cover up to 10 % of the leaf area.	Necrotic spots joined together to form necrotic streaks covering 10 % to 25 % of the leaf area.	Necrotic streaks cover 26 % to 50 % of the leaf area.	Necrotic streaks cover 51 % to 75 % of the leaf area.	Necrotic streaks range from 76 % to leaf death.
						

Asexual seed preparation for diagnostic

Each seed was weighed and divided into four segments of equivalent size to estimate the pathogen distribution. Each piece was macerated in a mortar by adding liquid nitrogen, and two aliquots were used: one for DBIA analysis and another for RNA extraction using the commercial RNAqueous® Phenol-free total RNA Isolation kit (Thermo Fisher Scientific), according to the manufacturer's instructions.

RT-PCR

We used two pairs of primers previously designed for Barrera & Belaich (2015): MP-F -5'-ATATCTACATGCGGCCTTGC -3' and MP-R-5'- GTTTGGGTTTCAGCGATGTT-3', which amplify a fragment of the gene coding for the movement protein (MP) of the FNSV, with an expected size of 492 base pairs (bp) and POL-F 5'- AGCCAGCTATACCACACAACC-3' and POL-R 5'-TACCACCAAGCGGTTAGCTT, which amplify a segment of polymerase-associated proteins (Pol) with an amplicon of 513 bp. According to the manufacturer's instructions, retro transcription was performed with the OneStep RT-PCR Kit (QIAGEN). The annealing temperature of the primers was 55° C. The products obtained were separated by electrophoresis on 1 % agarose gels in 1X TAE buffer and stained with SYBRSafe® (Invitrogen) for 60 minutes at 80 V. The size of the amplimers was estimated by comparison with a commercial molecular weight marker.

Dot blot Immunobinding Assay (DBIA)

The macerate of each seed was suspended in sterile water. Aliquots of 2µL of antigens was spotted onto a nitrocellulose membrane by triplicate and allowed to dry for 5 minutes at 37°C. Subsequently, the immunological test was performed according to the protocol by Toloza-Moreno et al., (2022) with some modifications: decreasing the concentration of the primary antibody to 5 µg/mL, using PBS 1X with 0.05 % Tween 20 as washing solution and using a commercial StartingBlock (PBS) Blocking Buffer (Thermo Scientific) with 0.05 % Tween 20. The membrane was washed with distilled water and allowed to dry at room temperature.

Calculations of diagnostic parameters (Statistical analysis)

To calculate the diagnostic validation parameters, a contingency table was used for the variables: infected plants (symptomatic/asymptomatic) and uninfected plants and results of the diagnostic technique (Jacobson, 1998) (Table 2).

Since it has been shown that RT-PCR and DBIA techniques can detect asymptomatic infections (Barrera & Belaich, 2015, Barrera et al., 2015), positive plants with both techniques in the absence of symptoms were taken as true positives.

The confidence interval (95 % CI) for each parameter was estimated according to the exact or Clopper -Pearson method (Brown et al., 2001).

The results were evaluated using the kappa index to determine the concordance between the two techniques, using the formula:

$$K = \frac{P_0 - P_e}{1 - P_0}$$

P_0 is the proportion of observed concordance, and P_e is the proportion of expected concordance due to chance (Gómez et al., 2007). The index was categorized according to the parameters of Landis & Koch (1977) where the concordance is very weak if it presents values lower than 0.20, weak if it is between 0.21 and 0.40; moderate between 0.41 and 0.60; good between 0.61 and 0.80 and very good if it is higher than 0.80.

Printing foliar samples on filter paper cards.

The usefulness of filter paper cards was evaluated to optimize protocols and validate an effective transportation costs. Prints were made from various parts of a diseased fique leaf on a paper card and allowed to dry at room temperature. Then, they were cut and suspended in sterile distilled water. This suspension was used for DBIA analysis at days 0, 2, 8, 15, 22 and 40

days after application and viral RNA extraction and RT-PCR at days 0, 2, 8, 16 and 60 days after application.

Results and discussion

Disease prevalence and severity evaluation

From total analyzed plants, 51 showed typical symptoms of the macana virus, characterized by necrotic lines on the upper and lower sides of the leaf because of cell death in the vascular bundles (Morales et al., 1992). The prevalence of the disease ranged from 5 % to 41 %, and according to regression analysis, showed a positive linear correlation with altitude, R^2=0.8164 (Figure 1a), indicating that sites at higher altitudes favor the presence of the disease. This could be related with the low temperatures presented at higher altitudes, which could generate a greater interaction between the vector and the virus promoting the spread of the pathogen. Additionally, the plant´s defense mechanisms could be affected by environmental conditions being more susceptible to the infection. These results are consistent with those reported by Aguilar et al. (2015), who found that the prevalence of potato virus X is reduced in conditions associated with high temperatures.

Considering that the temperatures in sampling sites ranged between 8 °C – 18 °C this could suggest that the FNSV is expressed in a wide temperature range. In the context of climate change, the temperature increase may likely affect the prevalence, persistence, and distribution of plant viral infections (Rodriguez, 2012; Hull, 2014) including the Macana disease. On the other hand, disease severity showed a low correlation with altitude (R^2=0.0652), and values ranged from 9 % to 55 % from 2000 to 2600 m.a.s.l. (Figure 1b). These results differ from that of Kubota & Ng (2016), who found higher severity of a viral disease at a higher temperature.

Detection of FNSV in asexual seed

The infection was detected in sprouts and bulbils. The Macana is a systemic disease which distribution depend on viral capacity to move long-distance through the phloem (Kappagantu et al., 2020), suggesting more successful infection in closer tissue to the pathogen entrance in the roots. In this case, the bulbils are born in the inflorescence of the plant, found in the upper part of it, while the sprouts are born at the base of the plant very close to the ground, being more possible found virus presence on it. Viral detection in plants with Macana disease using both techniques DBIA and RT-PCR and using fragments of whole seed (4/4) showed that the virus is not homogeneously distributed since only 28 % of the seeds were positive in the four segments analyzed by RT-PCR (Figure 2a) and 55 % were positive in the four fragments by DBIA (Figure 2b). In any case, a sample was considered positive if it had at least one of the 4 positive segments with both techniques. Due to the differences in the distribution of the pathogen in the seeds, it is necessary to process the whole plant material to increase the probability of virus detection.

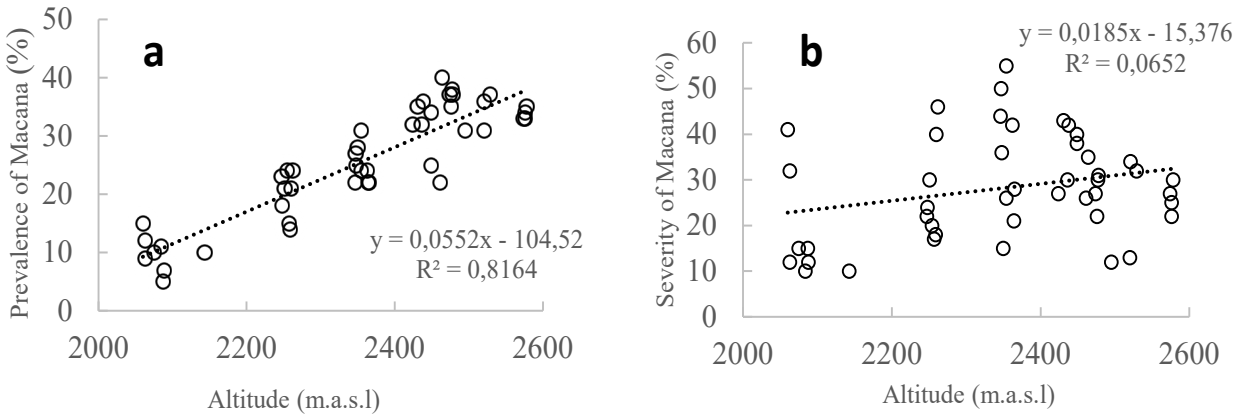


Figure 1. Correlation between altitude and Macana disease. (a) Prevalence (b) Severity

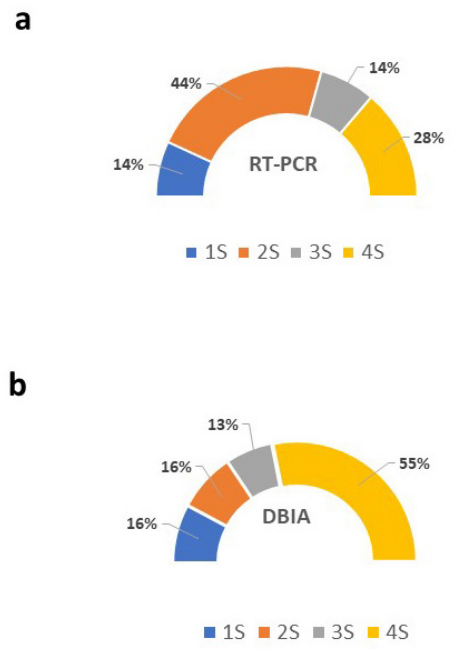


Figure 2. Positive segments (S) for viral detection by RT-PCR (a) and DBIA (b). The numbers indicate the segments of asexual seed analyzed, since it was divided into 4 parts.

Validation of diagnostic techniques of the virus in asexual seeds

Suitable material for validation purposes of diagnostic techniques for plant pathogens should include one of the following matrices: purified virus, plant material naturally or artificially infected with the microorganism, or plant material enriched with the microorganism (van der Vlught et al. 2007), so for this work, vegetative seeds from plants with visible symptoms of necrotic streak disease and purified viruses were used as positive controls. Also, since there is no gold standard for diagnosing macana and both techniques have previously been shown to be capable of detecting asymptomatic infections (Toloza-Moreno et al., 2022), positive plants with both techniques were taken as true positives (TP) as infected plants, and negative plants with both methodologies were taken as true negatives (TN) as uninfected plants. The detection of viral presence in bulbils showed that DBIA technique was positive in 16 bulbils which 10 were TP, and 5 samples were TN, corresponding to uninfected plants (Table 3, Appendix S1). On the other hand, with RT-PCR, 10 bulbils analyzed were true positive amplifying a fragment of MP, all corresponding to infected plants. The negative samples (11) presented only 5 true negatives (Table 3, Appendix S2).

In the case of sprouts, 16 were positive with DBIA, 15 infected and one uninfected, while the test was negative in 8 plants, 4 infected and 4 uninfected (Table 3). The RT-PCR technique detected the virus in 16 plants, 15 of which were infected, while the test was negative in 5 plants, 1 infected and 4 non-infected (Table 3).

Both tests detected the virus in 9 asymptomatic plants, confirming that the phytopathological diagnosis of the macana virus should not be based solely on the presence of symptoms since these are not observed with the naked eye in the early stages of the disease. They can also be confused with symptoms associated with other diseases or abiotic stress (Jeong et al., 2014). Similarly, other factors influence the appearance of symptoms or not, such as genotypic variability and intrinsic components of the virus, environmental conditions such as light intensity, temperature, and altitude, and nutritional conditions of the plant (van Der Want & Dijkstra, 2006).

On the other hand, 11.5 % of the plants with symptomatology were negative with both methodologies, which can be attributed to an error in symptom identification, problems that could be related to the tissue type analyzed, or the technique used. There are several critical factors inherent to the plant tissue that can alter the specificity of an assay; these can be enzyme inhibitors, degrading agents, factors that cause nonspecific binding, and substances previously applied to plants, such as pesticides and growth regulators, among others (Cardwell et al., 2018).

In the case of RT-PCR, this can be affected by the presence of RNases, polysaccharides, tannins, or plant polyphenols released during cell lysis which decreases the quality and yield of nucleic acid extraction. Also, some reagents used for RNA extraction can inhibit enzymatic reactions carried out in retro transcription (Lacroix et al., 2016). While with DBIA, cross-reactions with proteins from other pathogens may occur (Seida, 2017), generating false positives, so it is necessary to test with opportunistic microorganisms or environmental contaminants

Table 2. Diagnostic test parameters for a detection technique and contingency table for their assessment.

Test result	Infected plants		Healthy plants	Total
	Symptomatics	**Asymptomatics**		
Positive	TP	TP	FP	TP+TP+FP
Negative	FN	FN	TN	FN+FN+TN
Total	TP+FN		FP+TN	N

Notes: Sensitivity (Se): TP/ (TP + FN), Specificity (Sp): TN/ (TN + FP), Positive Predictive Value (PPV): TP/ (TP + FP), False Predictive Value (FPV): TN/ (TN + FN), Prevalence (P): (TP+FN) /N. TP: True positives; FP: False positives; FN: False negatives; TN: True negatives; N: sample size.

closely related to the pathogen to be detected (Cardwell, et al., 2018). All these factors may explain the discrepancy obtained in the results (25.5 %), which is also confirmed by the kappa index that shows a moderate agreement between both methodologies: 0.44 in the case of bulbils and 0.48 in sprouts.

The estimated diagnostic indicators for sensitivity, specificity, positive predictive value, negative predictive value, and prevalence are shown in Table 2. The most sensitive technique for bulbils was DBIA, with a sensitivity of 100 % compared to RT-PCR, which obtained 63 %. In contrast, the most specific technique was RT-PCR, with 100 %, whereas DBIA obtained 45 %. For DBIA, the PPV and NPV values were 63 % and 100 %, respectively, while for RT-PCR, the results were 100 % and 45 %, respectively.

In the case of sprouts, RT-PCR showed the highest diagnostic sensitivity with 94 % compared to DBIA (79 %). The most specific test was DBIA (80 %), while RT-PCR showed 50 %. For DBIA, the PPV and NPV values were 94 % and 50 %, while, with RT-PCR, these parameters obtained similar results, 79 % and 80 %, respectively.

Table 3. Contingency tables for bulbils (a and b) and sprouts (c and d) with each diagnostic technique performed. Below are the diagnostic parameters obtained from each table.

BULBILS

(a)	**INFECTED PLANTS**	**UNINFECTED PLANTS**	
DBIA	+	-	**Total**
+	10	6	16
-	0	5	5
Total	10	11	21

(b)	**INFECTED PLANTS**	**UNINFECTED PLANTS**	
RT-PCR	+	-	**Total**
+	10	0	10
-	6	5	11
Total	16	5	21

SPROUTS

(c)	**INFECTED PLANTS**	**UNINFECTED PLANTS**	
DBIA	+	-	**Total**
+	15	1	16
-	4	4	8
Total	19	5	24

(d)	**INFECTED PLANTS**	**UNINFECTED PLANTS**	
RT-PCR	+	-	**Total**
+	15	4	19
-	1	4	5
Total	16	8	24

DIAGNOSTICS PARAMETERS	**(a)** (95 % CI)	**(b)** (95 % CI)	**(c)** (95 % CI)	**(d)** (95 % CI)
Sensitivity	100 % (65.5-99.1)	62.5 % (35.8-83.7)	79 % (53,9-93,0)	94% (67.7-99.6)
Specificity	45 % (18.1-75.4)	100 % (46.2-98.1)	80 % (29.8-98.9)	50% (17.4-82.5)
Positive predictive value	63 % (35.9-83.7)	100 % (65.5-99.1)	93.75 % (67.7-99.6)	79% (53.9-93.0)
Negative predictive value	100 % (46.2-98.1)	45.4 % (18.4-75.4)	50 % (17.4-82.5)	80% (29.8-98.9)
Prevalence	48 % (26.4-69.7)	76.2 % (52.4-91)	79.2 % (57.2-92)	67% (44.6-83.5)

Note. 95% CI: 95% Confidence Interval

The primary method of multiplication of fique is through asexual seeds, with bulbils being the most abundant planting material since one plant can produce up to 3000 seeds, which are used for planting in new areas. In contrast, sprouts are mainly used for replanting established crops, and one plant can produce between 30 and 35 seeds, which is insufficient for planting large areas of crops (Cadena de Producción del Fique [CADEFIQUE], 2006). Bearing in mind that bulbils are the most used seeds for sowing new crops, the RT-PCR technique is recommended for viral detection in this type of propagule since it has a sensitivity of 62.5 % and a specificity of 100 %, which guarantees that a healthy plant is correctly identified, since it will not show false positives, thus allowing the selection of seeds with high phytosanitary quality for sowing.

Likewise, this technique has a PPV of 100 % and a high associated prevalence (76.2 %), which ensures the conditional probability that plants with a positive test have the disease.

In this sense, specificity and sensitivity allow measuring the validity of a diagnostic test and do not vary with prevalence, i.e., they are inherent characteristics of the test, whereas predictive values indicate the probability that the test provides a correct diagnosis and depend on the prevalence of the disease in the population where the test is applied (Pallás & Villa, 2019). Therefore, if the prevalence is high, a positive result tends to confirm the presence of the disease, whereas, if the prevalence is low, a positive result will not allow affirming its existence.

In the case of sprouts, although the RT-PCR technique has a high sensitivity (94 %), its specificity is low (50 %); therefore, it has a high probability of detecting a high number of false positives, which would lead to discard many healthy seed and increase the number of plants to be tested, resulting in high costs and effort for the producer. For this reason, the DBIA technique is more appropriate because it presents high percentages of both sensitivity and specificity (79 % and 80 %, respectively), which translates into a high probability of detecting diseased and healthy plants, in addition to being a more economical technique compared to the molecular technique. Similarly, the observed prevalence was 79.2 %, increasing the positive test's predictive capacity to 94 %.

Printing of foliar samples on filter paper cards

Tests were conducted to determine the usefulness of printing diseased leaves on chemically treated filter paper to facilitate the shipment of samples from the field to the laboratory, avoid the use of expensive refrigeration equipment, and ensure the viability of the virus on the matrix.

Viral detection in diseased foliar prints on filter paper cards was maintained until day 60 post application at room temperature using RT-PCR (Appendix S3).

In the case of DBIA, the results were positive up to 40 days after the prints on the card of a diseased leaf sample from Cauca (Appendix S4).

The cards are composed of chaotropic agents and other substances that lyse the cells and immobilize the genetic material inhibiting its degradation and preventing the proliferation of contaminating bacteria, which allows the collection and storage of different types of biological matrices for long periods at room temperature and constitute an effective way of sending samples to the laboratory for diagnosis (Burgoyne, 1996; Cardona-Ospina et al., 2019). The cards have been employed on a wide range of viruses transported at room temperature and characterized by molecular methods in the laboratory, ranging from several days to several weeks (Thorne & McElhinney, 2017), which is confirmed in this work.

Conclusions

The prevalence of the macana disease in fique plants in the department of Cauca is related to the altitude, suggesting that environmental conditions associated with higher altitude favor the disease. In contrast, the severity of the disease was not dependent on altitude, which indicates that the virus has a wide adaptation to the climate and can severely affect crops from 2000 m.a.s.l to 2600 m.a.s.l.

Because some infected plants may not show symptoms, and the virus distribution is not homogeneous in whole tissue plant, it is

necessary to send asexual seeds from healthy and diseased plants to the laboratory and process them thoroughly to ensure the detection of the pathogen. The diagnostic techniques analyzed in this work are beneficial as a preventive measure since there is currently no effective diagnostic method to prevent viral spread from planting material. The RT-PCR technique is recommended for bulbils and DBIA for sprouts, and the suspected plant material can be printed on filter paper, which maintains the viability of the virus for long periods until its detection in the laboratory.

Aknowledgements

We are grateful to the Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA) for providing physical and human resources. This work was supported by grants from the Colombian Ministry of Agriculture and Rural Development (MADR), through the administrative code number TV18.

Author contributions

MG: Laboratory sample processing, execution experimental design, review of statistical analysis of results, revision of the manuscript, discussion of results.

DO: Field work carried out, execution experimental design, review of statistical analysis of results, discussion of results, manuscript review.

GB: Conceptualization of the work, experimental design, statistical analysis of results, discussion of results, support, and supervision of the study.

Conflicts of interest

The signing authors of this research work declare that they have no potential conflict of personal or economic interest with other people or organizations that could unduly influence this manuscript.

ID ORCID and e-mails

Gamero, M. mgamero@agrosavia.co
https://orcid.org/0000-0003-2069-1868

Ortiz, D. dfortiz@agrosavia.co
https://orcid.org/0000-0001-6743-9374

Barrera, G. gbarrera@agrosavia.co
https://orcid.org/0000-0001-82454388

References

Abd El-Aziz, M. (2019). Three modern serological methods to detect plant viruses. *Journal of Plant Science and Phytopatholog*, *3*, 101–106. https://doi.org/10.29328/journal.jpsp.1001039

Adams, M. J., King, A. M. Q., & Carstens, E. B. (2013). Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses. *Archives of Virology, 158*(9), 2023–2030. https://doi.org/10.1007/S00705-013-1688-5

Aguilar, E., Allende, L., Del Toro, F. J., Chung, B. N., Canto, T., & Tenllado, F. (2015). Effects of elevated CO2 and temperature on pathogenicity determinants and virulence of potato virus X/Potyvirus-Associated synergism. *Molecular Plant-Microbe Interactions, 28*(12), 1364-1373. https://doi.org/10.1094/MPMI-08-15-0178-R

Barrera, G., & Belaich, M. (2015). *Descripción morfológica y molecular de un aislamiento colombiano del virus de la raya necrótica del fique*. XI Congreso Argentino de Virología.

Barrera, G., Cuartas, P., & Villamizar, L. (2015). Desarrollo de anticuerpos policlonales para la detección del virus de La Macana del fique. *Biomédica ,35*(1), 50–78

Boonham, N., Kreuze, J., Winter, S., van der Vlugt, R., Bergervoet, J., Tomlinson, J., & Mumford, R. (2014). Methods in virus diagnostics: From ELISA to next generation sequencing. *Virus Research, 186*, 20–31. https://doi.org/10.1016/J.

VIRUSRES.2013.12.007

Brown, L. D., Cai, T. T., & DasGupta K. (2001) Interval estimation for a binomial proportion. *Statist Sci, 16*(2), 101–133. https://doi.org/10.1214/ss/1009213286

Burgoyne, L. A. (1993). *Solid medium and method for DNA storage* (U. S. Patent No. US5496562A).

Cadena de Producción del Fique (2006). *Guía ambiental del subsector fiquero*. Ministerio de Agricultura y Desarrollo Rural.

https://repository.agrosavia.co/bitstream/andle/20.500.12324/12855/43452_54923.pdf?sequence=1&isAllowed=y

Cardona-Ospina, J. A., Villalba-Miranda, M. F., Palechor-Ocampo, L. A., Mancilla, L. I., & Sepúlveda-Arias, J. C. (2019). A systematic review of FTA cards® as a tool for viral RNA preservation in fieldwork: Are they safe and effective?. *Preventive Veterinary Medicine*, *172*, 104772. https://doi.org/10.1016/j.prevetmed.2019.104772

Cardwell, K., Dennis, G., Flannery, A. R., Fletcher, J., Luster, D., Nakhla, M., Rice, A., Shiel, P., Stack, J., Walsh, C., & Levy, L. (2018). Principles of diagnostic assay validation for plant pathogens: A basic review of concepts. *Plant Health Progress*, *19*(4), 272–278. https://apsjournals.apsnet.org/doi/full/10.1094/PHP-06-18-0036-RV

Data Bridge Market Research (2020). *Global Natural Fibers Market – Industry Trends and Forecast to 2029*. https://www.databridgemarketresearch.com/reports/global-natural-fibers-market

Echeverri, R. D., Franco, L. M., & González, M. R. (2015). *Fique en Colombia*. Fondo Editorial ITM. https://repositorio.itm.edu.co/bitstream/handle/20.500.12622/2013/Fique-en%20Colombia.pdf?sequence=1&isAllowed=y

Flórez, R., Beltrán Acosta, C., & Toro, S. I. (2013). Análisis económico e impacto del virus de la macana en el cultivo de fique. In A.Smith, C. Beltrán-Acosta, & A. Cotes (eds,). *Avances en el estudio del virus de la macana en el cultivo de fique (Furcraea spp.): compilación de artículos técnicos* (pp. 21–29). Corpoica. https://repository.agrosavia.co/handle/20.500.12324/19556

Gómez González, C., Pérez Castán, J. F., & Martin, J. L. R. (2007). Capítulo 8: Pruebas diagnósticas. Concordancia. *SEMERGEN - Medicina de Familia*, *33*(10), 509–519. https://doi.org/10.1016/S1138-3593(07)73955-2

Hsu, Ht. (2009). Development of Enzyme Linked, Tissue Blot and Dot Blot Immunoassays for Plant Virus Detection. In Burns, R. (eds) Plant Pathology. Methods in Molecular Biology,508, pp. 15–25). https://doi.org/10.1007/978-1-59745-062-1_2

Hull, R. (2014). *Plant Virology*. Academic Press, London.

Jacobson, R. (1998). Validation of serological assays for diagnosis of infectious diseases. *Revue Scientifique et Technique*, *17*(2), 469–526. https://doi.org/10.20506/rst.17.2.1119

Jeong, J. J., Ju, H.J., & Noh, J. (2014). A Review of Detection Methods for the Plant Viruses. *Research in Plant Disease, 20*(3), 173–181. https://doi.org/10.5423/RPD.2014.20.3.173

Kappagantu, M., Collum, T. D., Dardick, C. & Culver, J. N. (2020). Viral Hacks of the Plant Vasculature: The Role of Phloem Alterations in Systemic Virus Infection. *Annual Review of Virology, 7*(1), 351-370. https://doi.org/10.1146/annurev-virology-010320-072410

Kozłowski, R. M., Mackiewicz-Talarczyk, M., & Barriga-Bedoya, J. (2020). 20-New emerging natural fibres and relevant sources of information. In R. M. Kozłowski & M. Mackiewicz-Talarczyk (eds.), *Handbook of Natural Fibres* (pp.747–787). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-818398-4.00022-0

Kubota, K., & Ng, J. C. K. (2016). Lettuce chlorosis virus P23 Suppresses RNA Silencing and Induces Local Necrosis with Increased Severity at Raised Temperatures. *Phytopathology*, *106*(6), 653–662. https://doi.org/10.1094/phyto-09-15-0219-r

Lacroix, C., Renner, K., Cole, E., Seabloom, E. W., Borer, E. T., & Malmstrom, C. M. (2016). Methodological Guidelines for Accurate Detection of Viruses in Wild Plant Species. *Applied and Environmental Microbiology*, *106*(6), 653–662. https://doi.org/10.1128/AEM.03538-15

Landis, J. R. & Koch, G. G. (1977). The measurement of observer agreement for categorical data. *Biometrics, 33*(1), 159–174.

Ministerio de Agricultura y Desarrollo Rural (2021). *Cadena Agroindustrial del Fique* [PowerPoint slides]. https://sioc.minagricultura.gov.co/Fique/Documentos/2021-03-31%20Cifras%20Sectoriales.pdf

Morales, F., Castaño, M., Calvert, L., & Arroyave, J. A. (1992). Furcraea Necrotic Streak Virus: An Apparent New Member of the Dianthovirus Group. *Journal of Phytopathology, 134*(3), 247–254. https://doi.org/10.1111/j.1439-0434.1992.tb01233.x

Ortiz-González, D., Paredes-Martínez, O. E., & García-Parra, M. A. (2021). Rehabilitación del cultivo de fique (*Furcraea macrophylla*) mediante poda "descope" en Cauca, Colombia. *Centro Agrícola*, *48*(1), 5–13. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0253-57852021000100005&lng=es&tlng=

Pallás, J. A. M., & Villa, J. J. (2019). Sensibilidad y Especificidad. In J. M. Argimon Pallàs & J. J. Villa (eds.), *Métodos de investigación clínica y epidemiológica* (pp. 339–344). Elsevier.

Rodriguez, N. (2012). Enfermedades en especies de importancia económica en Colombia transmitidas por hongos vectores de partículas virales. [Bachelor Thesis, Pontificia Universidad Javeriana]. Repositorio Institucional de la Pontificia Universidad Javeriana https://repository.javeriana.edu.co/bitstream/handle/10554/11769/RodriguezPaipillaNathalie2012.pdf?sequence=1

Rubio, L., Galipienso, L., & Ferriol, I. (2020). Detection of Plant Viruses and Disease Management: Relevance of Genetic Diversity and Evolution. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.01092

Safaripour, M., Hossain, K. G., Ulven, C. A., & Pourhashem, G. (2021). Environmental impact tradeoff considerations for wheat bran-based biocomposite. *Science of The Total Environment*, *781*, 146588. https://doi.org/10.1016/j.scitotenv.2021.146588

Sarwar, M., Shad, N. A., & Batool, R. (2020). Integrated management of vectored viral diseases of plants. In L. P. Awasthi (eds.), *Applied Plant Virology*, (pp. 707–724). Academic Press. https://doi.org/10.1016/B978-0-12-818654-1.00050-5

Seida, A. A. (2017). Monoclonal antibodies and Polyclonal antibodies: A Brief Comparison. *Adv Tech Clin Microbiol*, *1*(2).

Singh, K. P., Jahagirdar, S. & Sarma, B. K. (2021). *Emerging Trends in Plant Pathology*. Springer.

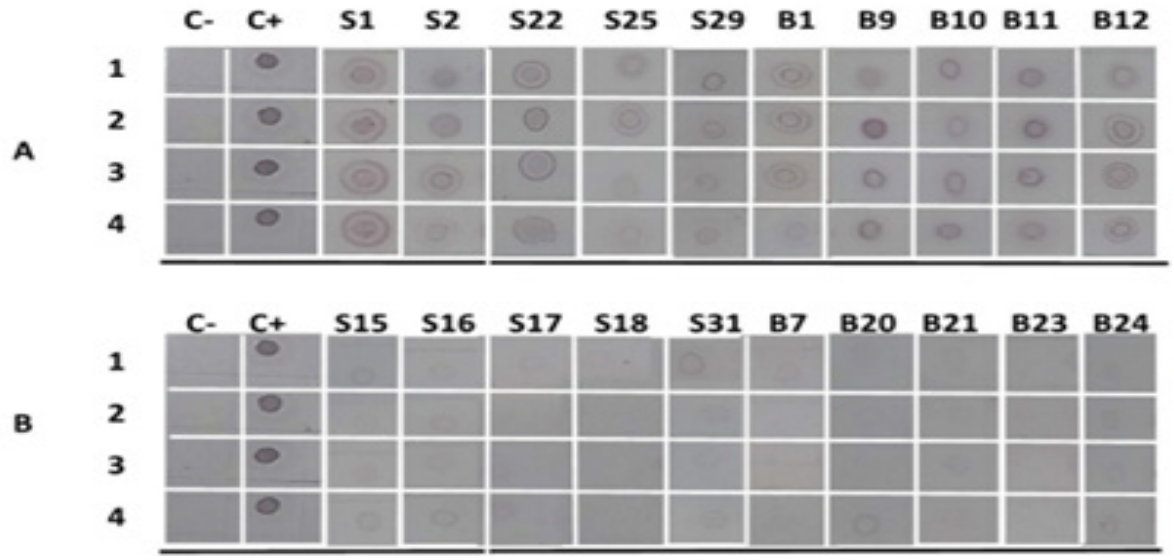
Thorne, L., & McElhinney, L. (2017). *Protocol for virus FTA card production and FTA cards assays protocols*. file:///C:/Users/OscarDGI/Downloads/Attachment_0.pdf

Toloza-Moreno, D., Villamizar-Rivero, L., Cuartas-Otalora, P., & Barrera-Cubillos, G. (2022). Immunodetection of Furcraea Necrotic Streak Virus-FNSV in fique plants (*Furcraea macrophylla* Baker) using a polyclonal antibody IgY produced in chicken egg yolk. *Journal of Immunological Methods*, *503*, 113232. https://doi.org/10.1016/j.jim.2022.113232

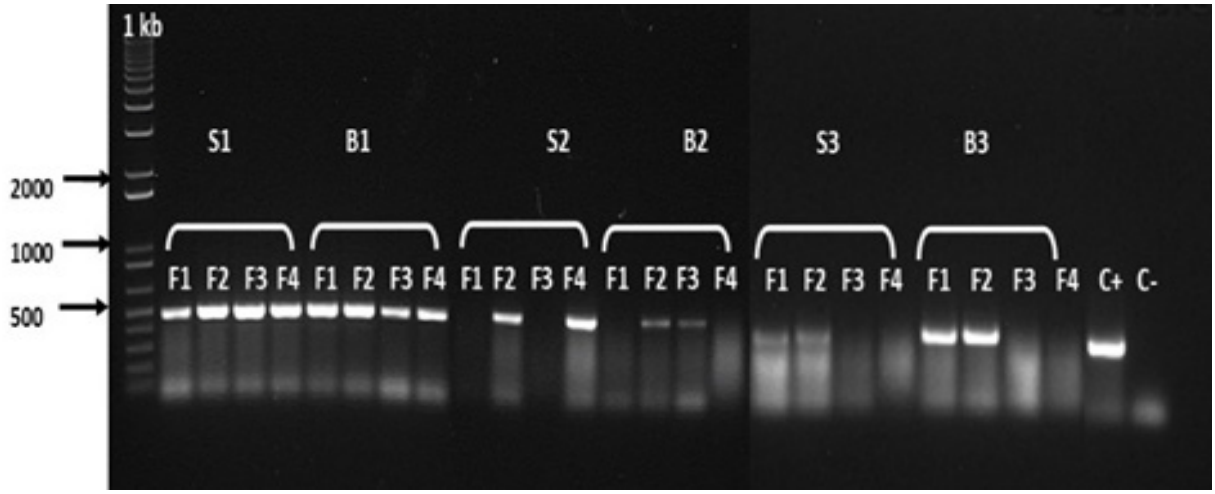
van der Want, J. P. H., & Dijkstra, J. (2006). A history of plant virology. *Archives of Virology 151*(8), 1467–1498. https://doi.org/10.1007/s00705-006-0782-3

van der Vlught, R. A. A., Verbeek, M., and Bonants, P. J. M. (2007). Explanatory document for the validation of detection methods for plant pests. *Plant Research International* (B.V. Report 135EN). https://library.wur.nl/WebQuery/wurpubs/fulltext/301443

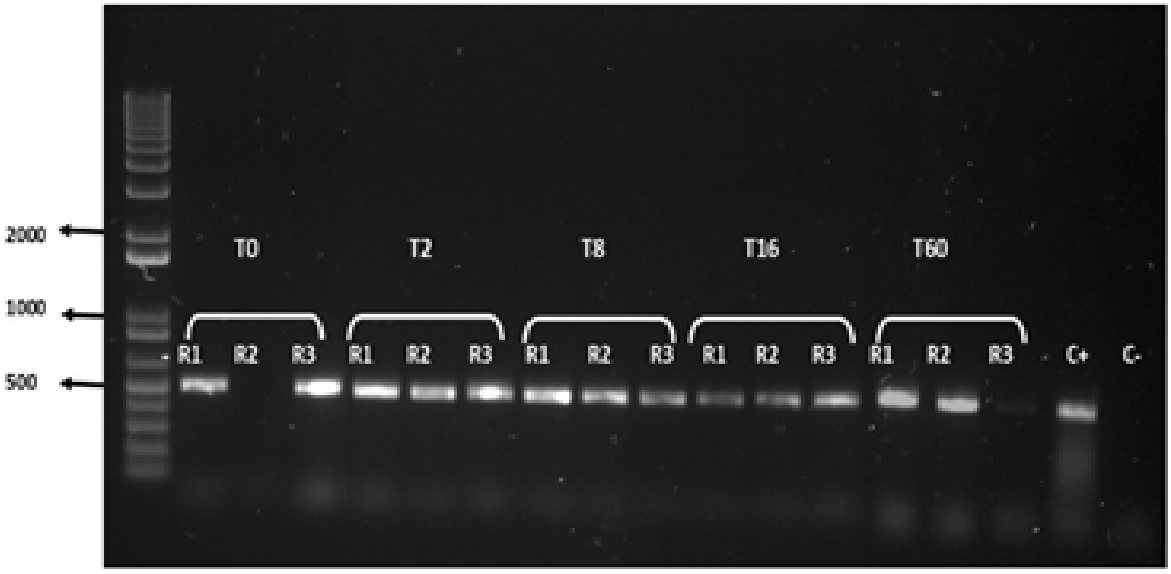
Appendix



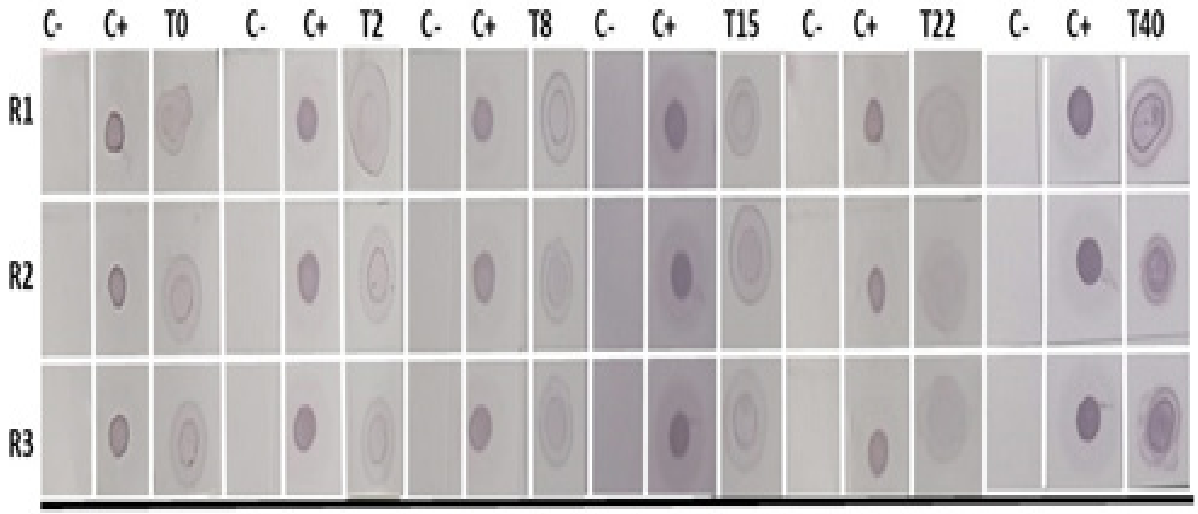
Appendix S1. DBIA using anti-FNSV IgY against Macana virus. Positive (A) and negative (B) results of the DBIA test on 5 sprouts (S) and 5 bulbils (B). Each row corresponds to a fragment analyzed from the same sample. C+: positive control (purified "Macana" virus); C-: negative control (water).



Appendix S2. Detection of FNSV by RT-PCR of a region of the Movement Protein (MP) gene in symptomatic plants. S: sprout; B: bulbil; F: fraction; C+: positive control; C-: negative control. Molecular weight marker: 1 Kb (Invitrogen™).



Appendix S3. Detection of FNSV in diseased foliar prints on filter paper cards by amplifying of a region of the polymerase-associated proteins gene (Pol). Times (T) 0, 2, 4, 8, 16, and 60 days. R1, R2, and R3: Replicates of the same sample. C+: positive control (purified viral); .C-: negative control (ultrapure water). Molecular weight marker: 1 Kb (Invitrogen™).



Appendix S4. DBIA for the detecting of FNSV in diseased leaves prints on filter paper cards at times (T) 0, 2, 8,15,22, and 40 days post-application. Rows correspond to replicates. C+: positive control (purified viral) C-: negative control (water).