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Presence of Potyvirus in the Norte de Santander department and molecular characterization of NIb protein in Colombian SCMV isolates

Presencia de Potyvirus en el departamento de Norte de Santander y caracterización molecular de la proteína NIb en aislamientos colombianos de SCMV

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SCMV infected corn leaf.
Photo: G. Chaves-Bedoya

ABSTRACT

Potyviruses are the largest genus of plant viruses that cause significant losses over a wide range of crops. In this paper, the presence of potyvirus in different plant crops in the provinces of Ocaña and Pamplona located in the north and south of the Department of Norte de Santander (Colombia) was evaluated with RT-PCR analysis using universal oligonucleotides specific to the region that encodes the NIB protein. The results indicate the presence of several potyvirus in Pamplona in economically important crops such as corn (*Zea mays*), tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*) and zucchini (*Cucurbita pepo*). In Ocaña, potyvirus was found in bean (*Phaseolus vulgaris*), corn and pumpkin (*Cucurbita maxima)*. In corn, one of the most important crops, the presence of the *Sugarcane mosaic virus* (SCMV) was confirmed with nucleotide sequencing. This is the first report of this virus in the department. The presence of several potyviruses in different crops in Norte de Santander indicate an alarming phytosanitary condition that must be addressed with priority to establish detection and control systems that maximize production, ensure agricultural sustainability, and propose certification schemes and improvement programs to reduce economic losses.

Additional key words: Potyvirus; molecular diagnosis; phytopathology; plant virus.

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RESUMEN

Los potyvirus son el género más grande de virus de plantas que ocasionan pérdidas significativas en un amplio rango de cultivos. En este trabajo mediante análisis de RT-PCR con oligonucleótidos universales específicos para la región que codifica la proteína NIb, se evaluó la presencia de potyvirus en diferentes plantas de cultivo en las provincias de Ocaña y Pamplona ubicados al norte y sur del departamento de Norte de Santander respectivamente. Los resultados indican la presencia de potyvirus en la provincia de Pamplona en plantas de importancia económica como maíz (*Zea mays*), tomate (*Solanum lycopersicum*), papa (*Solanum tuberosum*) y calabacín (*Cucurbita pepo*); y en la provincia de Ocaña en plantas de frijol (*Phaseolus vulgaris*), maíz y ahuyama (*Cucurbita maxima*). En maíz se confirmó mediante secuenciación nucleotídica la presencia del virus del mosaico de la caña de azúcar (SCMV), por lo que este se constituye el primer reporte de este virus en el departamento. Los resultados positivos por RT-PCR de la presencia de potyvirus en diferentes cultivos en Norte de Santander indican la necesidad apremiante de establecer sistemas de detección y control que permitan maximizar la producción, asegurar la sustentabilidad agrícola, proponer esquemas de certificación y programas de mejoramiento para reducir las pérdidas económicas.

Palabras clave adicionales: Potyvirus; diagnóstico molecular; fitopatología; virus vegetal.

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INTRODUCTION

The potyviruses (genus Potyvirus family Potyviridae) is made up of 175 species (ICTV 2020). This viral genus, non-persistently transmitted by more than 200 species of aphids, has a great capacity for adaptation to new environments and hosts and can infect monocotyledonous and dicotyledonous plants, including crops and wild plants (Nigam *et al*., 2019). Among the virus genera that infect plants, potyviruses are the most studied from the point of view of the functional characterization of their proteins, interaction with the host, evolution, taxonomy and diagnosis (Revers and Garcia, 2015). They have a flexuous particle that is 700-750 nm long, and their genome consists of a single strand of RNA with approximately 10,000 nucleotides with positive polarity. This potyvirus encodes a large polyprotein that undergoes proteolytic processing, providing essential functions for replication and movement. In the 5'→3' direction, the proteins encoded by the potyviruses are P1, HC-Pro, P3N-PIPO, 6K1, CI, 6K2, VPg, NIa-Pro, NIb and CP. Among these proteins, NIb is an RNA dependent RNA polymerase or replicase whose function is viral genome replication (Hong and Hunt, 1996). The NIB genome sequence is well preserved and has been used for the design of universal primers for the detection of potyviruses (Ha *et al*., 2008; Zheng *et al*., 2010).

New species of potyvirus are identified every year. By 2019, 167 species had been reported (Nigam *et al*., 2019). By mid-2020, 175 species had already been identified (ICTV, 2020). The data confirmed that this genus is one of the more important groups of viruses that represent an economic risk and a threat to crops around the world.

Most diseases caused by potyviruses have symptoms such as mosaics, speckles, chlorotic rings or discoloration and deformation in foliage, flowers, fruits and stems, and necrosis of various tissues. Most cause severe delays in young plants and drastically reduce yields (Agrios, 2005). The severity of losses is proportional to the time the plant has been infected, i.e. how young the plant was when infected (Revers and Garcia, 2015).

Diagnosis of viral diseases can be addressed from different approaches, ranging from symptomatology in indicator plants, electron microscopy techniques, enzyme assays such as ELISA tests, and molecular methods including Polymerase Chain Reaction (PCR). The latter is the main route of identification and diagnosis in potyviruses (Díaz *et al*., 2010).

Molecular detection techniques can overcome many deficiencies of conventional assays, especially if they depend on PCR. PCR-based techniques are generally more specific and faster than conventional techniques (Mirmajlessi *et al*., 2015). One variant of PCR is RT-PCR, which is the molecular method for detecting RNA plant viruses. The design of primers for virus amplification is critical to the success in amplifying RNA targets through RT-PCR. Primers with a wide range of specificity should be designed from highly conserved genomic sequences. Different universal primers have been described for the detection of potyvirus (Langeveld *et al*., 1991; Pappu *et al*., 1993; Gibbs and Mackenzie, 1997; Chen *et al*., 2001; Ha *et al*., 2008; Zheng *et al*., 2010).

In Colombia, this potyvirus has been reported in crops such as the tree tomato (Ayala *et al.,* 2010), papaya (Chaves-Bedoya and Ortiz-Rojas, 2015; Ortiz-Rojas and Chaves-Bedoya, 2017), potato (Riascos *et al.,* 2017) and recently pepper (Rivera-Toro *et al.,* 2021). Breeding programs need to know which viral agents, such as potyviruses, are present in the field. For the Department of Norte de Santander – Colombia, this information is very limited. This is the first study that looked for the presence of potyvirus infecting crop plants such as potatoes, corn, beans, peas and pumpkins in the provinces of Pamplona and Ocaña in Norte de Santander. The results suggest that, in both provinces, there was a broad presence of potyviruses infecting economically important crops; strategies for vector control must be improves to decrease viral incidence and economic losses in the region.

MATERIALS AND METHODS

Field samples. Plant samples from different crops were collected in the provinces of Pamplona and Ocaña in the months of November and December, 2018. The sampling methodology consisted of visually identifying symptoms in crops and collecting the necessary foliar tissue. The sampling included major crops of economic importance, such as corn (*Zea mays*), potatoes (*Solanum tuberosum*), beans (*Phaseolus vulgaris*), zucchini (*Cucurbita pepo*), tree tomatoes (*Solanum betaceum*), tomatoes (*Solanum lycopersicum*), onions (*Allium fistolosum*), peas (*Pisum sativum*), carrots (*Daucus carota*), peppers (*Capsicum annuum*), tobacco (*Nicotiana tabacum*), cassava (*Manihot esculenta*) and arracacha (*Arracacia xanthorrhiza*). The leaves of collected plants were placed in polystyrene containers with ice to keep them fresh and were taken to the research laboratory FITOBIOMOL at the Universidad Francisco de Paula Santander in the city of Cucuta. The leaves were stored in a Thermo Scientific Forma 8800 Series freezer at -70ºC until processing.

RNA extraction. Symptomatic leaves of different crop plants were used for the RNA extraction using the reagent TRIzol® (Invitrogen), starting with 100 mg of tissue according to the manufacturer's instructions. The RNA pellet was diluted in 75 μL of DEPC water. The RNA was quantified in a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific) and stored in 15 μL of aliquots to prevent freezing, thawing, possible degradation of the entire sample. The RNA was used for reverse transcription and PCR reactions. A sample of papaya (*Carica papaya*) infected with the potyvirus PRSV was used as a positive control.

Primers. The positive-sense primer NIb2F and complementary NIb3R were used for PCR amplification. These primers were designed to amplify conserved sequences corresponding to the positions 7619-7641 and 7945-7968 of the *Potato virus Y* (PVY), accession No. AF522296. This pair of primers has been reported as the most effective in detecting potyvirus (Gibbs and Mackenzie, 1997; Thompson *et al*., 2003).

RT-PCR. The total RNA samples of each plant sample were used as a template to amplify the conserved NIB region of the potyviral genome. Reverse transcription was performed using the enzyme M-MuLV Reverse Transcriptase (New England) in a reaction volume of 25 μL, with 1 μL of the total RNA extract. Reverse transcription was performed at 48ºC for 45 min and then at 94ºC for 2 min to stop the reaction. The PCR amplification was initially performed as described Zheng *et al*. (2010). The PCR products were separated with electrophoresis at 100 V in 1.0% agarose gel and TAE buffer. The bands were visualized using GelRed®. The samples that were amplified to the expected size, as compared to the positive control, were assumed to be a successful detection of potyvirus.

Cloning and sequencing PCR products. Because of its economic importance and widespread presence in Norte de Santander, corn samples were used for further analysis. The PCR products of the expected size were purified using a QIAquick PCR Purification Kit (Quiagen) and cloned into the Invitrogen TOPO™ TA Cloning Kit vector following the manufacturer's instructions. Cloning was used to transform competent cells using a BIO-RAD MicroPulser™ electroporation

device. Plasmid DNA was purified using a PureLink® Quick Plasmid purification kit (Invitrogen). Cloning was confirmed with restriction analysis and sequencing in triplicate. The consensus sequence in each case was used to identify the most related sequences in the GenBank (Altschul *et al*., 1997).

Sequence analysis. The alignment of nucleotides and deduced amino acid sequences was done with MegAlign. The manual editing of sequences was done using EditSeq (Lasergene, DNASTAR, Madison, WI). The phylogenetic relationships of the sequences were inferred by comparing the nucleotide sequence of Colombian SCVM isolates with sequences reported in the GenBank based on their nucleotide similarity and host. It has been reported that SCMV clusters in accordance with the host (Chaves-Bedoya and Ortiz-Rojas, 2012). The analyses were performed using the Maximum Likelihood test with 1000 iterations to estimate the confidence of the grouping. The GTR (General Time Reversible) model was used as the nucleotide replacement model. In cases where the resampling values were below 50%, the nodes were collapsed using TreeGraph 2 (Stover and Muller, 2010).

Results and Discussion

Crop plant sample origin and preliminary assays

Rumex crispus, a weed present in some crop plots, was included. Weeds are considered alternative hosts that can act as a source and reservoir of viruses that could then infect nearby crop plants, contributing to the prevalence and temporary distribution of a virus in crops (Juarez *et al*., 2019). In total, 18 samples from the province of Pamplona and 22 samples from the province of Ocaña were collected (Tab. 1).

RNA extraction and RT-PCR

The oligonucleotides used in this study reproducibly amplified a band of 350 base pairs of a papaya sample infected with PRSV VR5 isolate (Chaves-Bedoya and Ortiz-Rojas 2015), a potyvirus from Villa del Rosario (Norte de Santander-Colombia), indicating the efficiency of the oligos. RNA was extracted from the plant samples indicated in table 1 as described in the methodology. Reverse transcription was performed with each of the RNAs using the NIB3R

Table 1. Potyviral NIb amplification with RT-PCR in crop samples from two provinces of Norte de Santander – Colombia.

Crop	Province	Samples	RT-PCR (+)	RT-PCR (-)
Allium fistolosum	Pamplona	1	0	1
Arracacia xanthorrhiza	Pamplona	1	0	1
Capsicum annuum	Ocaña	3	0	3
Cucurbita pepo	Pamplona	3	3	0
Cucurbita maxima	Ocaña	2	2	0
Daucus carota	Pamplona	1	0	1
Manihot esculenta	Ocaña	1	0	1
Nicotiana tabacum	Ocaña	1	0	1
Pisum sativum	Pamplona	1	1	0
Rumex crispus	Pamplona	1	1	0
Phaseolus vulgaris	Pamplona	1	1	0
	Ocaña	5	2	3
Solanum betaceum	Pamplona	1	0	1
Solanum licopersicum	Pamplona	4	1	3
	Ocaña	4	0	4
Solanum tuberosum	Pamplona	2	2	0
Zea mays	Pamplona	2	1	1
	Ocaña	6	3	3
Total		40	17	23

oligonucleotide. cDNAs were used to perform PCRs under these conditions. As shown in table 1, both in Ocaña and Pamplona, to the north and south of the department of Norte de Santander respectively, the presence of potyvirus was detected in crops such as *Cucurbita pepo, Solanum lycopersicum, Zea corn, Cucurbita maxima, Phaseolus vulgaris, Pisum sativum, Solanum tuberosum* and in the arvense *Rumex crispus.*

Amplicons of approximately 350 bp, as shown in figure 1, that matched the control were presumed to correspond to some potyvirus. A total of 17 out of 40 samples were positive for the RT-PCR for potyvirus (Tab. 1).

The PCR technique with potyvirus-specific primers is reliable and sometimes is the only laboratory procedure that leads to positive results. Recently, the successful case of detection of potyvirus by RT-PCR using specific oligonucleotides in *Canna edulis* Ker., was reported, while microscopy and serological techniques were negative in the same case (Betancourt *et al*., 2020).

In Colombia, studies have been carried out to characterize or identify potyvirus associated with different crops such as tree tomatoes and potatoes in the Department of Antioquia, which found the PYV (Ayala *et al*., 2010; Gutiérrez and Marín, 2018). On the other hand, SCMV (*Sugarcane mosaic virus*) has been reported as infecting unconventional hosts such as african oil palm (*Elaeis guineensis*) and achira (*Canna edulis* Ker.), both of which are reported in the Department of Nariño, in southwest Colombia (Morales *et al*., 2002; Betancourt *et al*., 2020). This is the first report of SCMV infecting corn in Norte de Santander although no information was found for the rest of Colombia.

In this study, the presence of potyvirus in a weed plant commonly known as "cow tongue" (*Rumex crispus*) was detected. The importance of these weeds and alternative hosts during the occurrence and spread of viral plant diseases is due to the fact that it is an integral part of viral epidemiology and can function as reservoirs of viruses and vectors throughout the year (Srinivasan *et al*., 2013). Alternative hosts serve as reservoirs of viral inoculum in live plant material that is present between the harvest of one crop and the planting of the next (Ranabhat *et al*., 2018).

The greatest effect from potyviruses was in Pamplona. Cucurbitaceae and corn were infected in both provinces, while tomato crops were affected in Ocaña. These results indicate a high presence of potyviruses infecting different crops. Under field conditions, plants can be infected with more than one virus, and the broad distribution of these plant pathogens in Norte de Santander indicates that efficient control measures are not being implemented to mitigate or prevent diseases. There is no availability of antiviral compounds to cure diseased plants, so early and accurate detection of plant-infecting viruses is essential. Plant diseases caused by viruses can be effectively controlled when management measures are applied at the beginning of the disease development or by planting virus-free crops (Joo-Jin *et al*., 2014).

Phylogenetic analysis of SCMV's NIb

Among the positive results obtained with RT-PCR, corn samples were selected for confirmation with SCMV. The results of the BLAST (Basic Local Alignment Search Tool) analysis (Camacho *et al*., 2009) indicated that the sequences corresponded to the region approximately between position 7479-7774 of the complete genomic sequences of the *Sugarcane mosaic virus* (SCMV). Partial nucleotide sequences recovered from the NIb region of SCMV in Norte de Santander were reported in the GenBank with the access numbers MN607183 and MN607184. Table 2 shows the sequences used to build phylogenetic relationships of the new SCMV sequences with those already reported around the world.

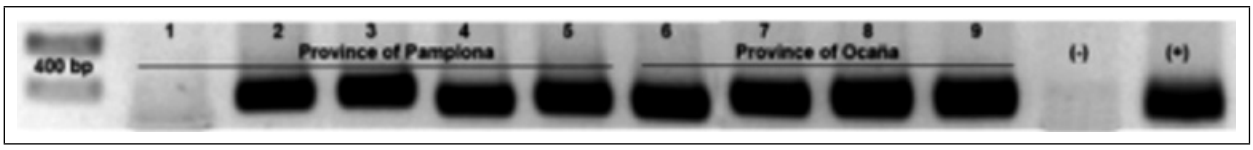

Figure 1. 1.0% agarose electrophoresis gel showing RT-PCR amplification of potyviruses NIb regions from crop plants in two provinces of Norte de Santander. 1, Tree tomato (*Solanum betaceum)*; 2, zucchini (*Cucurbita pepo*); 3, corn (*Zea mays*); 4, tomato (*Solanum lycopersicum*); 5, potato (*Solanum tuberosum*); 6, pea (*Pisum sativum*); 7, bean (*Phaseolus vulgaris*); 8, corn (*Zea mays*); 9, pumpkin (*Cucurbita maxima*); (-) negative control; (+) positive control.

Table 2. SCMV isolates used to build the phylogenetic tree. SCMV´s NIb sequences from Colombia are in bold.

No	Accession	Host	Origin	Year	No	Accession	Host	Origin	Year
1	MH093730	Corn	Kenya	2018	28	JX047389	Corn	China	2017
2	MH093728	Corn	Kenya	2018	29	JX047386	Corn	China	2017
3	MF467404	Corn	Tanzania	2020	30	JX047383	Corn	China	2017
4	MH093734	Corn	Kenya	2018	31	JX047382	Corn	China	2017
5	MF467398	Corn	Tanzania	2020	32	JN021933	Corn	China	2011
6	MF467397	Corn	Tanzania	2020	33	JX047381	Corn	China	2017
7	MF467394	Corn	Tanzania	2020	34	JX047402	Corn	China	2017
8	MH093727	Corn	Kenya	2018	35	DQ647651	Corn	Thailand	2016
9	MF467396	Corn	Kenya	2020	36	KY006657	Corn	Ecuador	2016
11	MF467401	Corn	Tanzania	2018	37	KU886553	Corn	China	2018
12	MF467400	Corn	Tanzania	2020	38	JX047431	Corn	China	2017
13	MF467399	Corn	Tanzania	2020	39	JX047427	Corn	China	2017
14	MF467395	Corn	Tanzania	2020	40	JX047419	Corn	China	2017
15	MF467393	Corn	Tanzania	2020	41	JX047409	Corn	China	2017
16	MK481076	Corn	Kenya	2020	42	JX047408	Corn	China	2017
17	MK481075	Corn	Kenya	2019	43	KF744392	Corn	Rwanda	2014
18	JX047415	Corn	China	2019	44	MG932078	Corn	Kenya	2018
19	JX047424	Corn	China	2017	45	MH093720	Corn	Kenya	2018
20	JX047412	Corn	China	2017	46	MH205604	Corn	Kenya	2018
21	JX047392	Corn	China	2017	47	MG932077	Corn	Kenya	2018
22	JX047416	Corn	China	2017	48	MH795798	Sugar cane	Nigeria	2018
23	JX047426	Corn	China	2017	49	MH093723	Corn	Kenya	2018
24	JX047401	Corn	China	2017	50	MN607183	Corn	Colombia	2019
25	JX047405	Corn	China	2017	51	MN607184	Corn	Colombia	2019
26	JX047387	Corn	China	2017	52	EU091075	Sugar Cane	México	2011
27	KT275937	Papaya	Colombia	2017	--	-	-	-	-

The phylogenetic tree (Fig. 2) was built including 50 partial sequences of the NIB region of SCMV recovered at GenBank (Clark *et al*., 2016). Most sequences were reported in China and Africa. In the phylogenetic tree, two groups are formed; the first group included isolates from Asia, Africa, Ecuador (KY006657) and Colombia (generated in this study). The second group had SCMV sequences reported in Africa.

The nucleotide distance matrix indicated that the percentages of similarity of the Colombian SCMV isolates with the African SCMV isolates that make up group II was 88.7%-90.1%, and the similarity was greater than 97% with the other isolates, including those from China and Africa. Among African SCMV sequences, the similarity was greater than 98%. The Colombian sequences had a similarity of 100%.

The potyvirus NIb protein is a RNA-dependent RNA polymerase (RdRP) that is absolutely required for potyviral genome replication and is a multifunctional protein. RdRPs are the only proteins encoded by all RNA viruses, making them ideal for evolutionary RNA virus analysis (Shen *et al*., 2020). The high similarity between the NIB sequences was due to the high degree of sequence conservation that resulted from the replicase function of this protein.

Because of their importance as pathogens, potyviruses have been studied more than other viruses (Revers and Garcia, 2015). However, in Colombia and the Department of Norte de Santander studies are scarce; much more remains to be explored.

Effective control and sustainable management of viral diseases in plants should be based on ecological and

epidemiologically robust strategies that minimize the risk of epidemics and loss of associated crops (Ranabhat *et al*., 2018). Agricultural losses from pathogens can transform local economies and significantly reduce a food base resource for a community, affecting its well-being (Scholthof, 2007).

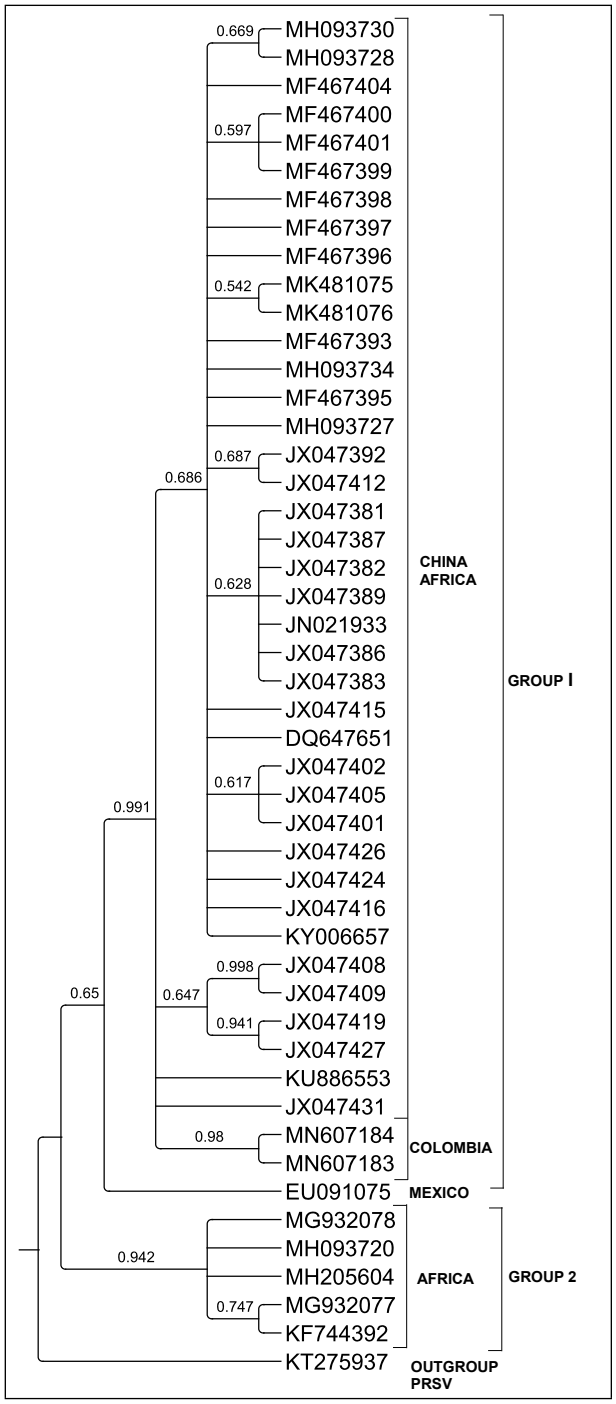

Figure 2. Phylogenetic tree with partial SCMV NIb sequences from China, Africa and Colombia.

CONCLUSIONS

In Norte de Santander, potyviruses were detected in crops such as *Cucurbita pepo*, *Solanum lycopersicum*, *Zea corn*, *Cucurbita maxima*, *Phaseolus vulgaris*, *Pisum sativum*, *Solanum tuberosum* and in the arvense *Rumex crispus*.

The analysis of nucleotide sequences obtained from the corn samples confirmed the presence of the *Sugarcane mosaic virus* (SCMV); this is the first report of its presence in Norte de Santander.

Crops in Norte de Santander have precarious phytosanitary conditions because they are mostly infected with potyviruses. More studies are needed to identify other potyviral species present in the field in Norte de Santander, which would help develop appropriate and effective techniques that can treat diseases and achieve sustainable agriculture.

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Declaration of Interest Statement. The authors declare no conflict of interest.

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