

First report of Sugarcane mosaic virus in achira (*Canna edulis* Ker.) in Nariño, Colombia

Primer registro del Sugarcane mosaic virus en achira (*Canna edulis* Ker.) en Nariño, Colombia

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ABSTRACT

Achira (*Canna edulis* Ker.) is a cultivated species for handcrafted food products and starch production. In Colombia is estimated an achira cultivated area of 800 ha; in the department of Nariño there has been a disturbance of viral etiology, known by farmers as Streak Virus, due to its symptoms in the leaves, but without previous records in the area. The disease causes losses in performance, although they have not been established precisely. In order to clarify the nature of this pathology and the identity of the pathogen associated with

the problem, an investigation was carried out at the University of Nariño, by means of molecular tests of PCR and RT-PCR, sequencing, serology and electron microscopy, of foliar samples collected in the producing areas. The most outstanding symptoms in affected tissues were yellow mosaic, mottled, chlorotic streak and ribs discoloration, among others. There were no cytoplasmic inclusions similar to those produced by Potyvirus, nor viral particles were observed, nor serology positive results, but it was possible to achieve the amplification of a cDNA fragment, with specific primers for Potyvirus and 98% of homology of the sequences with

Sugarcane mosaic virus. This is the first SCMV report in achira in Nariño, Colombia.

Keywords: molecular detection; virus; symptoms; achira.

RESUMEN

La achira (*Canna edulis* Ker.) es una especie utilizada para la producción de almidón y alimentos artesanales. En Colombia, se estima un área cultivada de 800ha; en el departamento de Nariño, se viene presentando un disturbio de etiología viral, conocido por los agricultores como el rayado, por sus síntomas en las hojas, pero sin registros previos en esta zona. La enfermedad causa pérdidas en el rendimiento, aunque no se ha establecido con precisión. Con el objetivo de esclarecer la naturaleza de dicha patología y la identidad del patógeno asociado al problema, en la Universidad de Nariño, se realizó una investigación, mediante pruebas moleculares de PCR y RT-PCR, secuenciación, serología y microscopía electrónica, de muestras foliares colectadas en las zonas productoras. Los síntomas más sobresalientes en tejidos afectados fueron mosaico amarillo, moteado, rayado clorótico, aclaramiento de nervaduras entre otros. No se detectaron inclusiones citoplasmáticas similares a las producidas por Potyvirus, ni se observaron partículas virales, tampoco hubo resultados positivos con serología, pero sí se logró amplificación de un fragmento de cDNA, con cebadores específicos para Potyvirus y homología de 98% de las secuencias con el virus Sugarcane mosaic virus SCMV. Este es el primer reporte de SCMV en achira en Nariño, Colombia.

Palabras clave: detección molecular; virus; síntomas; achira.

INTRODUCTION

Achira (*Canna edulis* Ker.) belongs to the Cannaceae (Tanaka, 2004) family; it is of South American origin and is grown in Latin America, Caribbean, Australia and Asia (Corpoica, 2001; Rodríguez *et al.* 2003), mainly to extract starch from its rhizomes, which is used in food preparation such as bread, cakes and pasta. In Colombia, these activities are important for rural economy of several departments and farmers generally use local planting materials (Caicedo *et al.* 2003; Torres, 2004; Corpoica, 2001).

In Taiwan, Vietnam and southern China, the species is sown between 2,000 and 3,000m. a.s.l. (Kasemwong *et al.* 2003; Roca and Manrique, 2005) and in Colombia, the species is grown in areas with altitudes below 2,700m (Ariza León *et al.* 2013), it is estimated that the achira starch demand per year in the country is 1,681.65t. The main producers are the departments of Nariño with 502ha, Huila 81ha and Cauca 24ha (Agronet, 2016). The plantations are established with genotypes local of regional origin, selected by farmers (Caicedo *et al.* 2003).

Among the limiting factors for crop production are phytosanitary problems, and particularly diseases associated with viruses, since they significantly reduce yields reaching losses of 70% or more, and are hard to manage (Hull, 2014).

Worldwide, several species of viruses affecting achira have been reported for years, Bean yellow mosaic virus, Potyvirus (BYMV) (Castillo *et al.* 1956), Tomato aspermy virus, Cucumovirus (TAV) (Hollings *et al.* 1971), Cucumber mosaic virus, Cucumovirus (CMV) (Lockhart, 1988), Canna yellow mottle virus, Badnavirus (CYMV) (Yamashita *et al.* 1985), Canna yellow streak virus, Potyvirus (CaYSV) (Monger *et al.* 2007), Tomato spotted wilt virus, Tospovirus (TSWV) (Moini *et al.* 2000) and Sugarcane mosaic virus, Potyvirus (SCMV) (Tang *et al.* 2016).

In Colombia, infections caused by CMV have been registered in the *Canna edulis* species (Castaño *et al.* 1994; Castaño *et al.* 1995; Reichel *et al.* 1995) and Banana streak virus, Badnavirus (BSV) (Reichel *et al.* 1996). Mixed infections of filamentous particles similar in their morphology to species of the genus Potexvirus (Reichel, 2007) and bacilliform particles similar to species of the genus Badnavirus have also been observed in achira plants with viral symptoms, from the department of Nariño (Reichel, data sin publish).

In the department of Nariño, achira is grown in the municipalities of La Cruz, San Pablo, El Tablón, Colón, Belén and Albán (Consolidado Agropecuario – Nariño, 2015). At present, there is no detailed knowledge of the virus species present in the crops, nor have been applied the diagnostic methods based on serological or molecular techniques that are available worldwide for these pathogens. In addition, their means of transmission and other biological characteristics of epidemiological interest are not known.

This situation motivated the development of the current research between the University of Nariño, Agrosavia and CIAT, aiming to establish solutions in the diagnosis and detection of viruses, through the standardization of serological and molecular protocols that serve as a starting point for such diseases management.

MATERIALS AND METHODS

Localization. This research was carried out at the University of Nariño (Pasto - Nariño) and at the CIAT International Center for Tropical Agriculture (Palmira - Valle del Cauca), under laboratory conditions.

Plant material. Plant samples were taken in La Cruz municipality, which is the main achira production area in Nariño and in an Achira germoplasm collection of AGROSAVIA (Obonuco-Pasto), with genotypes from the departments of Cundinamarca, Nariño and Huila.

Samples were taken from symptomatic and asymptomatic plants, based on symptoms reported for pathologies associated with viruses in achira (Castaño *et al.* 1994; Castaño *et al.* 1995; Reichel *et al.* 1995; Rajakaruna *et al.* 2014; Verchot & Rajakaruna, 2013). The sampling included the recollection of foliar tissues, which were labeled, wrapped in paper towels and placed in plastic bags for transport to the laboratories. The plant material was kept in a refrigerator at 4°C until processing.

Evaluation and visual description of viral symptoms. The monitoring and visual description of 10 achira plants was carried out for six weeks, with photographic record. The symptoms evolution was evaluated in each plant, from the first leaf outbreak, following recommendations of other authors (Armitage *et al.* 1990; Hunger & Sherwood, 1985; Lockhart, 1988; Monger *et al.* 2007; Pappu & Druffel, 2008; Zhang *et al.* 2011).

Virus detection by molecular tests

DNA extraction. Leaf samples from symptomatic achira plants were used, following the method of Prince *et al.* (1993), taking 0.4g of tissue in each case, which was frozen and macerated in liquid nitrogen using a sterile mortar and pestle. After the final wash in ethanol, the extracted nucleic acid was suspended in 30 to 50µL of Tris-EDTA buffer solution (10 mM Tris-HCl, pH 8.0, and 1mM EDTA, pH 8.0) and stored at 4°C until use.

Direct PCR. Total DNA was used for direct PCR amplification using CaYMV-specific primers: CaYMV-3 and CaYMV-4, which produce a 565bp fragment (Momol *et al.* 2004). PCR was carried out using a denaturation cycle at 95°C for 5 min and 35 cycles of amplification (95°C for 60s, 60°C for 45s, 72°C for 60s), with a final extension at 72°C for 7 min. A mixture of 12.5µL of go taq master mix 2X Promega, 0.5µL of each primer, 9.5µL of water and 2µL of each DNA sample was prepared for a final volume of 25µL.

RNA extraction and RT-PCR. RNA extraction and cDNA synthesis were developed at CIAT (Palmira, Valle del Cauca). The RT-PCR technique was used according to the protocol described by Carvajal *et al.* (2014). Between 2 and 4µg of RNA and 200 to 400ng of random primers were denatured at 65°C for 5 min, followed by one minute on ice. The first cDNA chain was synthesized at 37°C for 1.5 hours after adding 5x first Strand buffer, 0.1 M DTT, 10mM dNTPs and 200 U of MMLV reverse transcriptase (invitrogen) to a final volume of 15µL. The quality of the cDNA was checked with a PCR of internal controls.

Once the cDNA was obtained, PCR tests were performed with specific primers for: BYMV (BYMV-F and BYMV-R) (Skelton *et al.* 2007), CaYSV (Canna-F1 and Canna-R2) (Monger *et al.* 2007), SCMV (S400-551 and S400-910) (Smith *et al.* 1994) and CMV and TAV (CPTALL-3 and CPTALL-5) (Choi *et al.* 1999), which produce fragments of 382, 695, 359 and 938 and 966bp, respectively. Primers and the positive control for SCMV were provided by Cenicaña.

In the PCR amplification tests, a mixture of 12.5µL of go taq master mix 2X Promega, 0.5µL of each primer, 9.5µL of water and 2µL of each cDNA sample was prepared for a final volume of 25µL. For the detection of BYMV, CaYSV and SCMV, 35 cycles were performed (95°C for 60s, 50°C for 45s, and 72°C for 60s), with a final extension of 72°C for 7 min. For Cucumovirus the same program was used, but using a temperature of the hybridization phase of 40°C for 60s.

Sequencing. The PCR amplification products were cleaned using the DNA precipitation method with polyethylene glycol and ethanol

(Ziv *et al.* 2007) and sequenced in MacroGen. The obtained sequences were compared by maximum mating and higher percentages of homology with those of reference in the GenBank, using the BLAST tool of the National Biotechnology Information Center (<http://www.ncbi.nlm.nih.gov/BLAST>) (Altschul *et al.* 1990).

Serological tests. Leaf tissue samples from asymptomatic and symptomatic achira plants were also subjected to serological tests of DAS-ELISA, with commercial antibodies (AGDIA) for the Potyvirus group, to verify the presence of viruses, following the methodology described by Clark & Adams (1977) and recommendations from other authors such as Reichel *et al.* (1997).

Electron Microscopy. It was carried out at the University of Antioquia. The resin immersion method was used to process foliar tissue from very young leaves, from symptomatic achira plants. Fixation was made in 2.5% glutaraldehyde and washed in saline buffer, postfixed in 1% osmium tetra-oxide, washed with buffer and propylene oxide. Then it was dehydrated in an ascending gradient of ethanol from 10% to 100%, to then imbibe it and include it in epoxy resin. Polymerization was carried out at 70°C, and ultrafine cuts at 60nm. Subsequently, the sections were contrasted with uranyl acetate and lead citrate and were observed under a Tecnai G2 F20 transmission electron microscope.

RESULTS AND DISCUSSION

Evaluation and visual description of viral symptoms. In the department of Nariño, the achira crops have a high incidence of virus, the evaluated plants showed a wide range of foliar symptoms, which start as a yellowing, mottled mosaics, and advance to the leaf distortion; especially on the edges, chlorotic streaking, vein clearing, leaf necrosis and sometimes dwarfism, as the most outstanding (Figure 1).

Farmers call the disease as “yellow streak” because of its predominant symptom, which they correlate to a decrease in the yield of starch. Canna economically speaking can be separated in two main groups, those used as source of food, i.e. *C. edulis* and those used for ornamental purposes, i.e. *C. indica* and related hybrids. Because ornamental and edible cannas can have different leaf color and can also have variegations in their leaves, the expression of viral symptoms on them are somewhat different depending on the variety and could lead to confusion when observing samples in the field (Rajakaruna *et al.* 2014). In this case, the plantations studied were planted with two different varieties (green or black), none of those are known to have variegations in their leaves, suggesting that the observed symptoms are due to physiological responses possibly caused by the presence of virus. Similar symptoms were reported in China in achira plantations affected by SCMV (Tang *et al.* 2016). Also, similar symptoms description due to Potyvirus infections in canna have been reported by several authors (Alexandre *et al.* 2017; Zakubanskiy *et al.* 2017; Kumari *et al.* 2016; Monger *et al.* 2007). Other virus species like a Tomato aspermy virus, Cucumovirus (TAV) (Hollings *et al.* 1971), Cucumber mosaic virus, Cucumovirus

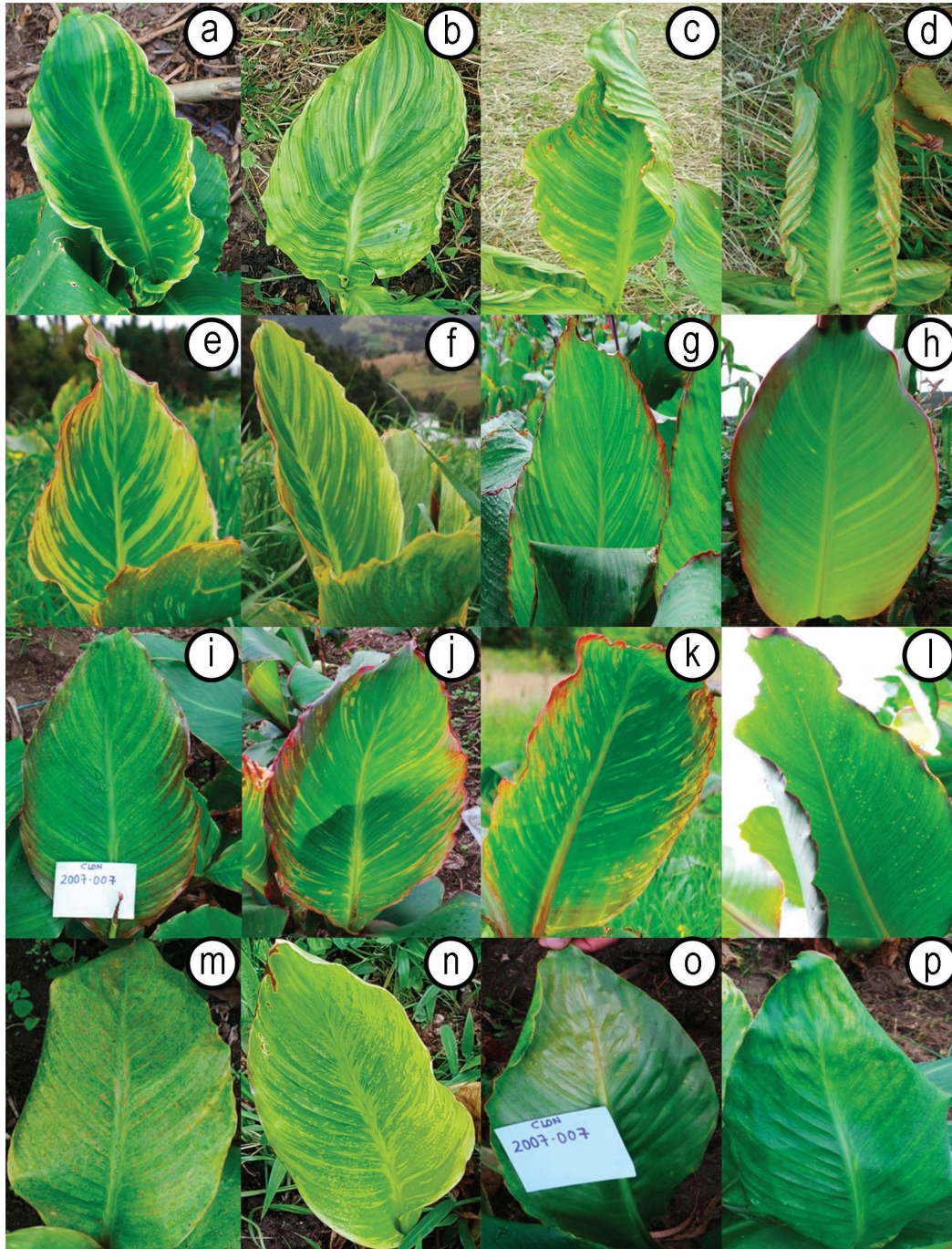


Figure1. a.-b. symptoms with veins deformation and chlorosis; c.-d. chlorosis and vein necrosis together with leaf rolling; e.-f. chlorotic bands on leaves; g.-h. symptoms of chlorotic veins and streaks; i.-k. symptoms of mosaics with different tonalities (yellow, white, green, orange and purple), set to deformation of the edge of the leaves; l. - n. symptoms of streaks and chlorotic spots; o.-p. symptoms of chlorotic speckling on the final third of the leaf.

(CMV) (Lockhart, 1988), Canna yellow mottle virus, Badnavirus (CYMV) (Yamashita *et al.* 1985), because similar symptoms, but none were detected in this study.

Virus detection by molecular tests

Direct PCR. None of the collected samples amplified by direct PCR. Thus, there was no presence of Canna yellow mottle virus (CaYMV) Badnavirus (Caulimoviridae family) using specific primers.

In exploration studies in Brazil, Alexandre *et al.* (2017) reported that Badnavirus amplifications were not obtained in a *Canna* species, with symptoms of chlorotic mosaic, but subsequent studies using RT-PCR showed the presence of a Potyvirus in the same samples.

RT-PCR. Negative results were obtained for the TAV, CMV, BYMV and CaYSV viruses, but positive results were obtained for the Sugarcane mosaic virus (SCMV) Potyvirus, in (20/30) symptomatic samples examined, corresponding to 66%. One of the gels is shown in figure 2.

Molecular diagnostic tests revealed that a high proportion of plants were infected with SCMV, pointing to an association with the “yellow stripped” disease in the department of Nariño. In China,

Tang *et al.* (2016) similarly detected SCMV infecting *Canna* spp. While the similarity of symptoms of the “yellow stripped” disease might be confused with those of caused by CaYSV, (Rajakaruna *et al.* 2014), there is a phylogenetic relationship between these two viruses as shown by Chauhan *et al.* (2015), whom showed that CaYSV (Potyvirus), a virus that affects achira, belongs to the subgroup of SCMV.

Sequencing. Sanger sequencing of the sugarcane mosaic virus (SCMV) RT-PCR amplification products were performed and the sequences compared for maximum identity and coverage against the available sequences deposited at the Gene Bank of the National Center for Biotechnology Information (NCBI), using the BLAST tool of the National Biotechnology Information Center. The

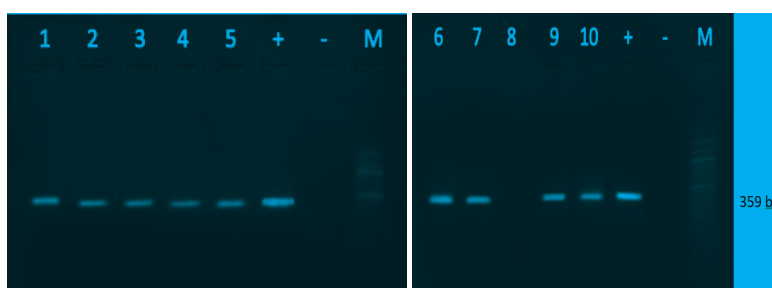


Figure 2. 1% of agarose gel, Lanes 1 to 5 and 6 to 10 cDNA samples from achira plant tissue, M: molecular weight marker of 100 bp (Thermoscientific), +: positive control, SCMV cDNA donated by CENICAÑA, -: Negative control, reaction mix without cDNA.

results indicated the resemblance of the amplified sequences to the id. AF006734.1, the partial coding sequence of the sugar cane mosaic virus coat protein, isolate Brisbane, of Australian origin. The matching fragment had a length of 286bp with no gaps and a homology of 98% to the consensus sequence of the experimental samples of (Nariño) Colombia.

Serological tests. Serological tests of DAS-ELISA were performed with commercial serological kits (AGDIA) for the genus Potyvirus in which no positive results were obtained. Negative and positive control for the assay were satisfactory (data not shown). The results suggest that perhaps a low titer of viral particles precluded the assay from recognizing the pathogen and reinforce the notion that molecular assays have higher sensitivity than serological tests. Nonetheless, in the available literature about the Agdia Poty test, there is no information about the sensitivity of the assay to different strains of SCMV. The same reported (Alexandre *et al.* 2017), in serological studies with polyclonal antibodies to Potyvirus in *Canna*.

Electron Microscopy. The tissues examination under observation in transmission electron microscope did not confirm the presence of viral particles and inclusion bodies (Figure 3). This situation is

not indicative of the absence of virus because its detection requires a correct selection of samples. On the other hand, the concentration of virus in plant tissues can be low, which makes it difficult to detect it for electron microscopy.

Different viruses have been reported that are infecting the *Canna* spp., in countries such as the United Kingdom, Belgium, Holland, France, Italy, Austria, Israel, Kenya, Iran, India, China, Thailand, Japan, the United States and Brazil (Zakubanskiy *et al.* 2017).

In conclusion, this is the first report of the Sugarcane Mosaic Virus, Potyvirus (SCMV) infecting achira crops (*Canna edulis* ker.) in Nariño (Colombia), through the use of molecular detection techniques. The epidemiological implications on the crop are serious, because its propagation is vegetative. It is necessary to initiate studies related to vectors and cleaning of planting material.

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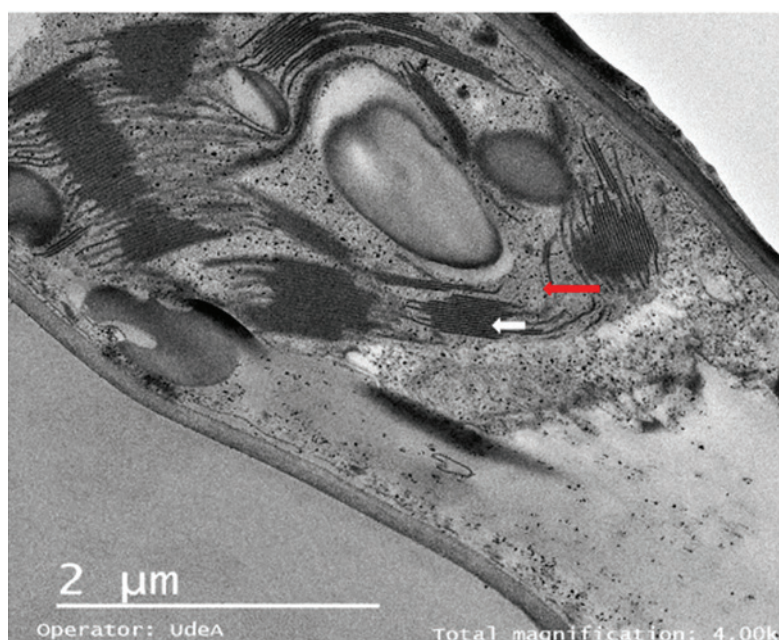


Figure 3. Electron micrographs of achira leaf tissue of the green variety. White arrow grana thylakoid and red arrow Chloroplast stroma.

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