BIOCELL
2005, 29(2): 205-207

ISSN 0327 - 9545
PRINTED IN ARGENTINA

Brief Note

Natural infection of *Viola cornuta* (*Violaceae*) with *Cucumber mosaic virus*, subgroup I

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Keywords: *Viola cornuta*, *Cucumber mosaic virus*, electron microscopy, serology, AC-RT-PCR.

ABSTRACT: Plants of *Viola cornuta* displaying typical virus symptoms were observed during spring 2003 in a plant nursery in Córdoba, central Argentina. Electron microscopic examinations of symptomatic leaf samples revealed the presence of isometric virus-like particles about 30 nm in diameter. Subsequent serological analysis allowed the identification of the pathogen as a subgroup I strain of *Cucumber mosaic virus* (CMV). These results were confirmed by antigen capture - reverse transcription - polymerase chain reaction with specific CMV primers, and digestion with a restriction enzyme. This is the first report of CMV infecting *V. cornuta* in Argentina.

Introduction

Commercial floriculture has the potential to become an important industry in Argentina. Córdoba province, with an area of ca. 650,000 m^2 dedicated to this activity, occupies the seventh position in the national ranking (Informe INTA-JICA, 2003). Production can be affected by several factors, including plant pathogens such as fungi, viruses and, at a lesser extent, bacteria. There is, however, a lack of information concerning the occurrence and distribution of these infectious agents on the different ornamental crops grown in central Argentina.

During spring 2003, plants of *Viola cornuta* L. (*Violaceae*) showing flower color breaking, mild mosaic, leaf size reduction and general stunting were observed in a plant nursery located near La Granja (Córdoba province). The described symptomatology suggested the presence of a plant virus, and studies were conducted to elucidate the aetiology of the disease. This work reports on the identification of the disease causal agent, by means of electron microscope, serological and molecular techniques.

Materials and Methods

Samples were collected from *V. cornuta* plants showing the symptoms described above. Plant material was stored at –20°C, except for electron microscope observations (in this case, fresh leaf tissue was immediately processed).

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Received on March 29, 2004. Accepted on December 30, 2004.

Leaf dip preparations were made from portions of symptomatic leaves, cut several times with a razor blade in a drop of PBS pH 7 + 0.01% (w/v) sodium sulphite (Na_2SO_3). The leaf extracts were then transferred to carbon-coated Formvar grids for seven min. After washing with distillated water, these were negatively stained with 2% uranyl acetate and examined with a JEOL 1220 transmission electron microscope.

On the basis of the results obtained by microscopic analysis, symptomatic plants were tested for *Cucumber mosaic virus* (CMV) using DAS-ELISA (Clark and Adams, 1977). Leaf samples were ground in extraction buffer (PBS pH 7 + 0.05% Tween 20 + 2% polyvinyl pyrrolidone) at a 1:10 (w/v) dilution. Polyclonal and monoclonal antisera (anti CMV, and anti-CMV subgroups I and II) (Agdia Inc., Elkhart, USA) were used. Positive (supplied by Agdia) and negative (healthy *V. cornuta*) controls were placed on each microtitre plate. Absorbance at 405 nm was recorded after adding p-nitrophenyl phospate as substrate; values higher than three times the mean for healthy controls were considered positive.

Once the pathogen was serologically identified, an antigen-capture (AC) (Rowhani *et al*., 1995; López Lambertini *et al*., 1998) followed by reverse transcription-polymerase chain reaction (RT-PCR) was performed to confirm the presence of CMV in the analysed plants. For AC, PCR tubes were coated with 50 µl of a 1:10 (w/v) dilution of tissue extract in carbonated coating buffer, and incubated overnight at 4°C. RT-PCR was performed with two CMV-specific primers that flank the CMV capsid protein gene (Rizos *et al*., 1992). The following cycling parameters were used: 48°C for 45 min, 94°C for 4 min, (40 cycles of 94°C for 30 s, 40°C for 30 s, 68°C for 1 min), and a final cycle of 68°C for 7 min. The amplified fragment was then digested with restriction enzyme *Msp*I (Promega Corporation, Madison, USA) for subgroup identification (Rizos *et al*., 1992). Digestions were carried out at 37ºC for 4 h. Both, PCR and digestions products were run in agarose gels (1.4% and 3% respectively) and stained with ethidium bromide.

Results and Discussion

Electron microscopic observations revealed the presence of isometric particles, about 30 nm in diameter, in leaves of symptomatic *V. cornuta* plants (Fig. 1). The virus was identified as CMV by DAS-ELISA (data not shown). When testing with subgroup-specific antisera, the isolate under study was shown to belong to subgroup I. CMV infection was confirmed by AC-RT-PCR. As expected, a DNA fragment of approximately 870 bp was amplified from the analysed samples (Fig. 2). *Msp*I digestion of PCR products yielded two frag-

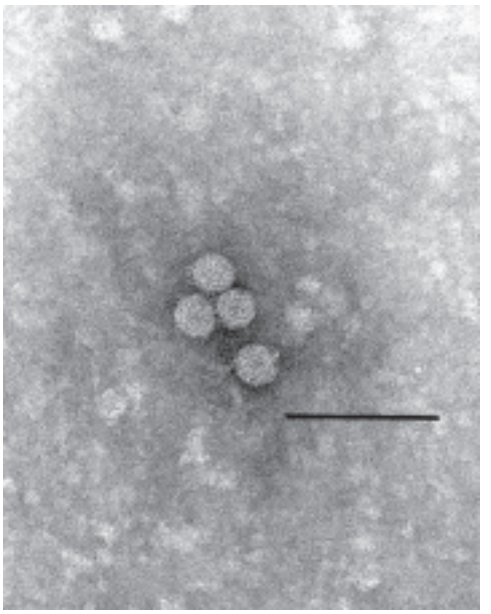

FIGURE 1. Electron micrograph of a leaf dip preparation stained in 2% uranyl acetate, showing CMV particles. Bar: 100 nm.

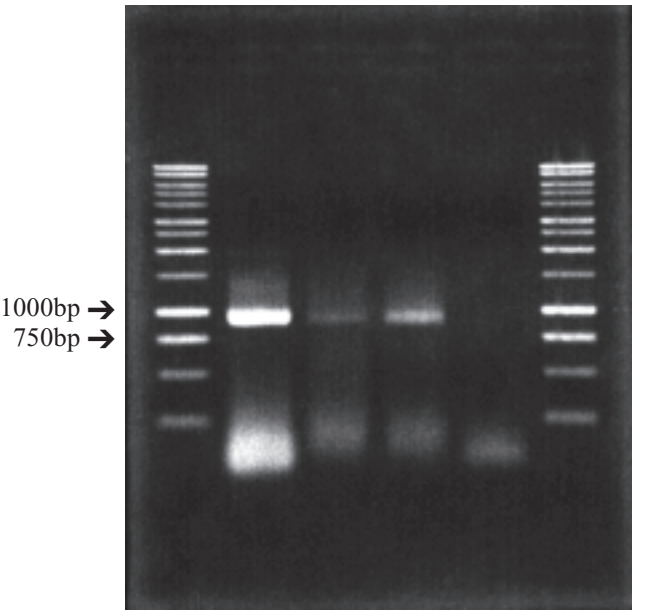

FIGURE 2. RT-PCR amplification of CMV RNA. M: 1 kb DNA ladder (Promega). Lane 1: positive control (CMV-infected *Trifolium repens*). Lanes 2 and 3: different *V. cornuta* symptomatic plants. Lane 4: negative control (healthy *V. cornuta*).

ments of approximately 340 and 540 bp (Fig. 3). This pattern allowed the classification of the isolate within subgroup I, in accordance with the serological results.

CMV, an ubiquitous plant virus, infects a wide range of plant species around the world, among which many ornamentals, and new hosts for this virus are described

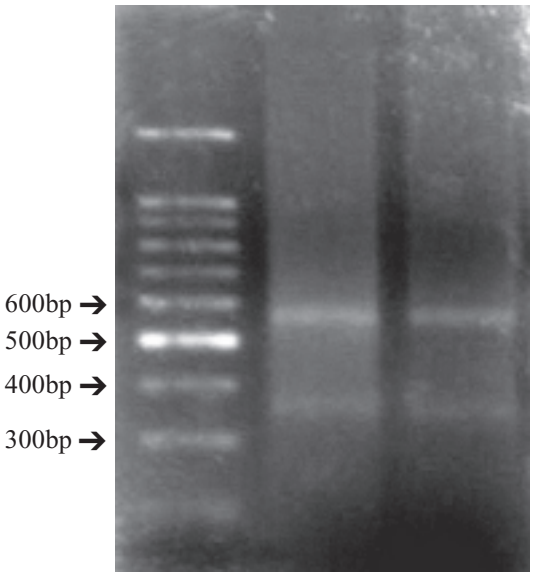

FIGURE 3. Subgrouping of the CMV isolate by restriction enzyme analysis. M: 100 bp DNA ladder (Promega). Lanes 1 and 2: *Msp*I digestions of RT-PCR products from different *V. cornuta* symptomatic plants.

every year. Subgroup I strains seem to be more frequent than those of subgroup II (Crescenzi *et al*., 1993) and, in general, induce a more severe disease (Boari *et al*., 2000; Hord *et al*., 2001). In Argentina, the virus has already been detected in various crops, including *Apium* sp., *Lactuca sativa*, *Calendula officinalis* and several *Cucurbitaceae* (compiled by Fernández Valiela, 1995). More recently, it has been recorded infecting *Capsicum annuum* (Atencio *et al*., 1997), *Glycine max* (Laguna *et al*., 2002), *Phaseolus vulgaris* (Rodríguez Pardina *et al*., 2002) and *Arachis hypogaea* (de Breuil *et al*., 2002). This paper reports, for the first time, the occurrence of CMV on *V. cornuta*.

CMV has a very broad range of natural reservoirs. It can be easily transmitted by more than 80 aphid species during brief probes, as well as by mechanical transmission and by seed (Gallitelli, 2000). Therefore, the disease can spread rapidly in plant nurseries or under greenhouse conditions. The accurate identification of the pathogenic agent constitutes the first step towards its management.

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