

Detection of *Bean yellow mosaic virus* and *Cucumber mosaic virus* infecting gladiolus in Argentina

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SUMMARY

Among the factors affecting floral production worldwide, viral diseases can account for significant losses. However, relatively little is known about the presence of viral agents infecting ornamental plants in Argentina, and this information is essential for disease management strategies. This paper reports on the first detection of *Bean yellow mosaic virus* and *Cucumber mosaic virus*, subgroup I, infecting gladiolus (*Gladiolus x hortulanus*) in the country. Both viruses were identified by electron microscopy, serology and reverse transcription - polymerase chain reaction.

Key words: floriculture, plant disease, mixed infection, *Potyvirus*, *Cucumovirus*.

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RESUMEN

Entre los factores que afectan la producción de flores a nivel mundial, las enfermedades virales pueden ocasionar significativas pérdidas. Sin embargo, relativamente poco se sabe sobre la presencia de virus en especies ornamentales cultivadas en Argentina, y dicha información es esencial para el diseño de estrategias de manejo de enfermedades. En este artículo se informa por primera vez sobre la presencia de *Bean yellow mosaic virus* y *Cucumber mosaic virus*, subgrupo I, en gladiolo en el país. Ambos virus fueron identificados mediante microscopía electrónica, serología y transcripción reversa - reacción en cadena de la polimerasa.

Palabras clave: floricultura, enfermedades de plantas, infección mixta, *Potyvirus*, *Cucumovirus*.

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Viral diseases cause significant economic losses to floriculture, due to the pathogen's ability to induce alterations in the morphology and colour of flowers and vegetative organs. Since commercial floriculture is an expanding activity in Argentina, the early diagnosis of diseases of ornamental plants, along with the accurate identification of their causal agents, is essential for establishing management measures.

In November 2004, virus-like symptoms, which included leaf chlorotic mosaic and flower colour breaking, were observed in gladiolus (*Gladiolus x hortulanus*, cv. Rose Supreme) plants grown in a plant nursery located in the province of Corrientes (Argentina). In order to elucidate the aetiology of the disease, leaf samples from 25 plants were collected and stored at -70 °C (except for electron microscopy studies, in which fresh leaf tissue was used). 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 (Na₂SO₃). The leaf extracts were then transferred to carbon-coated Formvar grids for seven minutes. After washing with distilled water, the grids were negatively stained with 2% uranyl acetate and examined with a JEOL 1220 transmission electron microscope. Leaf dip observations revealed the presence of isometric particles about 30 nm in diameter and flexuous filamentous particles about 750 nm long (Fig. 1). All the samples were positive by DAS-ELISA (Clark & Adams, 1977) using anti-*Bean yellow mosaic virus* (BYMV, genus *Potyvirus*, family *Potyviridae*) antibodies (Bio-Rad Laboratories, Inc.), except for the healthy controls. DAS-ELISA with anti-*Cucumber mosaic virus* (CMV, genus *Cucumovirus*, family *Bromoviridae*) antibodies (Agdia, Inc.) showed that 20 of these 25 plants were co-infected with this virus. Further serological tests with CMV subgroup-specific antibodies placed the studied isolates into subgroup I. Identity of viruses was confirmed by reverse transcription-polymerase chain reaction (RT-PCR), with primers specific to BYMV and CMV (Vunsh *et al.*, 1990; Rizos *et al.*, 1992). Prior to RT-PCR analysis, 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. As expected, DNA fragments of ca. 750 bp and 870 bp respectively were amplified from the analysed samples. Digestion of CMV PCR products with the restriction endonuclease *MspI* (Promega Corporation, Madison, USA) yielded two fragments of ca. 340 and 540 bp. This pattern allowed the classification of the isolate within subgroup I (Rizos *et al.*, 1992), in accordance with the serological results.

BYMV and CMV are very ubiquitous plant viruses, and have been frequently found infecting gladiolus in several regions of the world (Katoch *et al.*, 2003). In Argentina, CMV is known to affect various horticultural and ornamental crops, such as snapdragon (*Antirrhinum majus* L.) and viola (*Viola cornuta* L.) (Arneodo *et al.*, 2005a,b). The present findings constitute, however, the first record of this virus on gladiolus plants. On the other hand, there is only one pre-

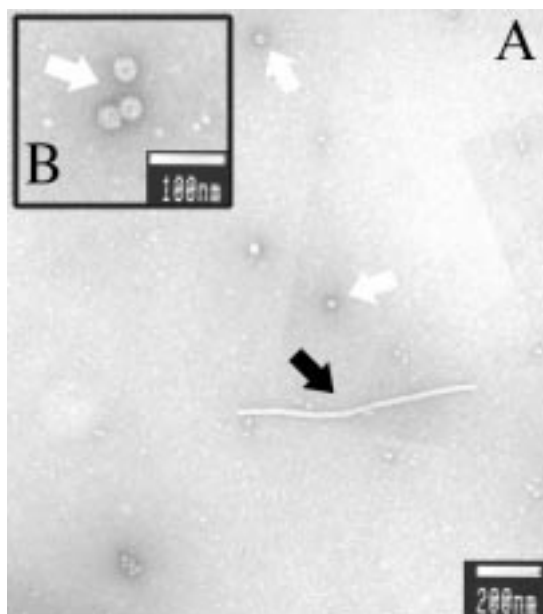


Figure 1: A. Particles of BYMV (black arrow) and CMV (white arrows) in sap of infected gladiolus leaf. B. Detail of CMV particles.

vious report indicating the possible presence of BYMV in the country, on faba bean (*Vicia faba* L.), pea (*Pisum sativum* L.), and bean (*Phaseolus vulgaris* L.) crops (Von der Pahlen, 1962). By the time he carried out the study, this author based his conclusions on the mechanical inoculation of the virus to a range of diagnostic hosts, as well as on its physical properties. The present work represents the first identification of BYMV in Argentina by means of electron microscopy, serological and molecular techniques.

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