



First report of *Cymbidium mosaic virus* on orchids in Paraguay

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Ornamental flower growers in Paraguay have been encouraged to produce orchids by an increased demand over the last decade and the improvement in cultivation techniques. During 2014 and 2015, leaf samples showing virus-like symptoms (Figs. 1-4) including flower colour breaking, leaf necrosis and necrotic spotting, were observed in plants of five orchid genera (*Cattleya*, *Dendrobium*, *Miltonia*, *Oncidium* and *Phalaenopsis*) grown in commercial greenhouses in the Paraguayan municipalities of Asuncion and Caacupé.

Ultra-thin sections of foliar samples from all genera were examined by transmission electron microscopy and revealed the presence of aggregates of flexuous particles in the cytoplasm of cells in at least one sample from every orchid genus (Fig. 5). Leaf extracts from symptomatic plants of all genera reacted positively with polyclonal antiserum against *Cymbidium mosaic virus* (CymMV), genus *Potexvirus*, in a plate trapped antigen-ELISA. Total RNA was extracted from twenty symptomatic leaf samples (*Cattleya*, n=4; *Dendrobium*, n=5; *Miltonia*, n=3; *Oncidium*, n=5; and *Phalaenopsis*, n=3), and analysed by one-step RT-PCR amplification using specific primers for CymMV (forward: 5'-GGTGCAGGCAGCATAGAG'-3, reverse: 5'-TAATCATGGGAGAGCCCACT'-3), *Orchid fleck virus* (OFV) (forward: 5'-TGTCATAGCCGACATAAACACC'-3, reverse: 5'-TGTAGAGCTTGCGAGATACAGG'-3) and *Odontoglossum ringspot virus* (ORSV) (forward: 5'-CCAAACACAACAAGCTCGAA'-3, reverse: 5'-CCGTAGTTGTGCGATTCTGC'-3). Amplicons of the expected size for CymMV (681 bp) were obtained with total RNA extracted from all samples. In addition, at least one plant of each genus was found to also be

infected with either OFV or ORSV; two viruses previously reported in Paraguay (González-Segnana, 1989; Ramos-González *et al.*, 2016). Three RT-PCR amplicons for CymMV, obtained from different plants of each genus (fifteen in total), were purified with PCR Clean-Up System (Promega, USA) and directly sequenced (Macrogen Inc., Korea). Sequences of two amplicons were deposited in GenBank (Accession Nos. MG774929 and MG774930). Comparisons of the nucleotide sequences revealed 98 to 99% identity with the corresponding nucleotide sequences of the coat protein gene of CymMV deposited in GenBank (AY571289 and AB541544).

This is the first report of CymMV in Paraguay. Further surveys are necessary to determine the occurrence of this virus in commercial greenhouses throughout Paraguay. Recommendations for suitable crop management should be shared between growers to avoid the spread of these viruses.

References

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Figure 1



Figure 2



Figure 3



Figure 4

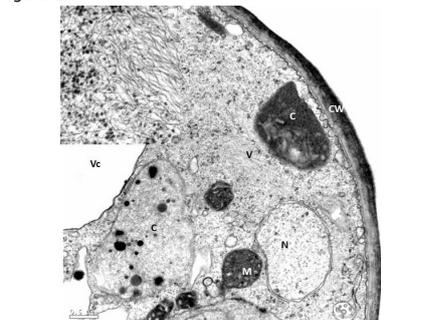


Figure 5

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