

RESUMEN

Las enfermedades virales constituyen uno de los principales factores limitantes del cultivo de *Lilium spp.* Los virus más comunmente encontrados en *Lilium spp.* en diferentes países son *Lily symptomless virus* (LSV), *Lily mottle virus* (LMoV) y *Cucumber mosaic virus* (CMV). El objetivo de esta tesis fue evaluar la presencia de LSV, LMoV y CMV en *Lilium* en Argentina, y en el caso de que estos virus estuvieran presentes se planteó estimar su incidencia en diferentes híbridos cultivados y localidades de producción, así como evaluar diferentes alternativas para obtener plantas de sanidad controlada. Se trabajó con híbridos de *Lilium longiflorum* (Ll), Asiáticos (As), Orientales (Or), *Longiflorum* × Asiáticos (LA), *Longiflorum* × Orientales (LO) y Orientales × Trompeta (OT). Se llevó a cabo el reconocimiento de la sintomatología asociada a la infección viral y se identificaron áfidos posibles vectores de virus en parcelas de producción de *Lilium*. Se evaluaron plantas sintomáticas y asintomáticas mediante DAS-ELISA y RT-PCR. Para esto se utilizaron anticuerpos policlonales específicos contra las proteínas de la cápside de LSV, LMoV y CMV, así como *primers* degenerados y específicos, respectivamente. Se pudo detectar la presencia de los tres virus. Las secuencias del gen de la proteína de la cápside (CP) y de aminoácidos de la CP de LSV, LMoV y CMV, aislados desde plantas cultivadas en Argentina, presentaron una elevada similitud con las de otras cepas de los mismos virus registradas en el GenBank. Asimismo, se detectaron infecciones virales en bulbos provenientes de Holanda, lo cual indica una posible vía de entrada de los virus. La incidencia de virosis fue evaluada por medio de DAS-ELISA en plantas de bulbos provenientes de siete localidades de Argentina entre las latitudes 26° 56' S y 43° 03' S, y longitudes 65° 21' O y 71° 29' O. Los virus detectados en orden decreciente fueron LSV (42,1%), LMoV (35,6%) y CMV (20,0%) y se encontraron en infecciones simples o mixtas. La incidencia de virus varió entre los híbridos desde 36% (O 'Montecristo') a 94,7% (Ll 'Avita') en 2006, y desde 38,9% (OT 'Yelloween') a 82,1% (LO 'Triumphator') en 2007, con una incidencia total de 64,1% y 70,7% en 2006 y 2007, respectivamente. También se encontró una variación en la incidencia entre localidades. La mayor incidencia de virus (89,6 y 87,6% en 2006 y 2007, respectivamente) se observó en Bahía Blanca, y la menor incidencia fue detectada en Trevelli (47,4%) en 2006 y en Malargüe (48,6%) en 2007. La alta incidencia de virus en el cultivo de *Lilium* puede estar asociada a la utilización de bulbos infectados para la propagación y a la falta de medidas de control de vectores. Para ajustar un sistema de obtención de bulbos de sanidad controlada se utilizó material infectado con virus de híbridos cultivados en Argentina. Los bulbillos fueron producidos por *scaling ex vitro* o por multiplicación *in vitro* con o sin tratamiento de termoterapia y/o quimioterapia. Posteriormente, se extrajeron y cultivaron los ápices meristemáticos. La obtención

de plantas sanas fue dependiente de los híbridos, de los virus presentes y de los tratamientos aplicados. La aplicación de termoterapia *ex vitro* en escamas de bulbos durante la diferenciación de bulbillos (*scaling*) y la obtención de meristemas desde dichos bulbillos se propone como una innovación para un protocolo exitoso de obtención de bulbos de *Lilium spp.* de sanidad controlada.

ABSTRACT

Viral diseases are one of the main limiting factors in lily production. The viruses most commonly found in lilies worldwide are *Lily symptomless virus* (LSV), *Lily mottle virus* (LMOV) and *Cucumber mosaic virus* (CMV). The aim of this thesis was to evaluate the presence of LSV, LMOV and CMV in *Lilium* in Argentina, and when present, to estimate their incidence in different hybrids and localities, and also to evaluate different alternatives to produce virus-free plants. Hybrids evaluated belong to six groups: *Lilium longiflorum* (LI), Asiatics (As), Orientals (Or), *Longiflorum* × Asiatics (LA), *Longiflorum* × Orientals (LO) and Orientals × Trumpet (OT). Different symptoms associated to viral infection were recognized and aphid species with potential capacity to transmit viruses were identified in *Lilium* production plots. Symptomatic and asymptomatic plants were evaluated by DAS-ELISA and RT-PCR using polyclonal antiserum against the capsid protein of LSV, LMOV and CMV, and with degenerate and specific primers, respectively. The gene nucleotide and amino acid sequences of the coat protein of LSV, LMOV and CMV were highly similar to those from other isolates of the same viruses registered in the GenBank. Infections were detected in bulbs imported from the Netherlands, which indicates a possible way of entry of viruses into our country. Virus incidence was evaluated by DAS-ELISA in bulbs coming from seven Argentinean localities. The areas surveyed were between latitude 26° 56' S and 43° 03' S, and longitude 65° 21' W and 71° 29' W. Virus infection in decreasing order were LSV (42.1%), LMOV (35.6%), and CMV (20.0%) and single or mixed infections were detected. Virus infection varied among hybrids from 36.0% (Oriental 'Montecristo') to 94.7% (*Lilium longiflorum* 'Avita') in 2006, and from 38.9% (OT 'Yelloween') to 82.1% (LO 'Triumphator') in 2007, with an overall infection incidence of 64.1 and 70.7% in 2006 and 2007, respectively. A variation in virus infection incidence among localities was also detected. The highest virus infection incidence (89.6 and 87.6% in 2006 and 2007, respectively) was observed in Bahía Blanca (38° 44' S, 62° 16' W). The lowest virus infection incidences, were 47.4% in Trevellin (43° 03' S, 71° 29' W) in 2006, and 48.6% in Malargüe (35° 28' S, 69° 35' W) in 2007. The high occurrence of viruses infecting lily crops in Argentina could be due to the use of infected bulbs during propagation and also to the absence of preventive virus vector control measures. The starting material to set a system for obtaining virus-free plants were infected hybrids grown in Argentina. For bulblet production, scales or scale sections were cultured *ex vitro* or *in vitro*, with or without thermotherapy and /or chemotherapy. Then, meristem-tips were cultured *in vitro*. Different percentages of virus-free plants were obtained depending on the hybrid, the treatment and the viruses present in infected tissues. The application of thermotherapy to bulb scales during the differentiation of bulblets (*scaling*) and the extraction of

meristem-tips from these bulblets is an innovative proposal for a successful protocol for obtaining virus- free *Lilium spp* plants.

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