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Occurrence of viruses in Calla and Peruvian lily in Tuscan nurseries and evidence of new viral records in Italy

A. Luvisi [1,2 (*)], D. Rizzo [3], L. Stefani [3], A. Panattoni [2], A. Materazzi [2]

[1] *Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Centro Ecotekne Università del Salento, Via Provinciale Lecce Monteroni, 73100 Lecce, Italy.*

[2] *Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, Università di Pisa, Via del Borghetto, 80, 56124 Pisa, Italy.*

[3] *Servizio Fitosanitario Regionale, Regione Toscana, Via Ciliegiole, 99, 51100 Pistoia, Italy.*

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Abstract: In order to evaluate the health status of Calla and Peruvian lily in Tuscan nurseries, 18 viruses belonging to six families and one unassigned virus were assayed. Tests were carried out on 90 *Zantedeschia aethiopica* plants and 48 *Alstroemeria* spp. plants collected from 12 Tuscan nurseries in two years, via RT-PCR tests. *Z. aethiopica* was mainly affected by viruses belonging to the *Potyviridae* family, with the main infection caused by Dasheen mosaic virus (DsMV) and Zantedeschia mild mosaic virus (ZaMMV). Even if *Alstroemeria* spp. plants were affected by *Potyviridae* family viruses too, higher infection rates were recorded for *Betaflexiviridae*, where Lily symptomless virus infected more than half of plants. This is the first known report of Lily mottle virus (LMoV) in *Alstroemeria* spp. and *Z. aethiopica* or ZaMMV in *Alstroemeria* spp. in Italy.

1. Introduction

Calla and Peruvian lily are commonly cultivated in Tuscany (Italy) and they represent one of the main cut flower productions. Calla lily [*Zantedeschia aethiopica* (L.) Spreng] is known to be susceptible to at least 13 virus species, mainly belonging to *Potyviridae*, *Bunyaviridae,* and *Tombusviridae* (Huang *et al*., 2007). Peruvian lily (*Alstroemeria* spp.) has become one of the most popular cut flowers worldwide. It has been reported as the natural host of various plant viruses, including members of *Potyviridae*, *Betaflexiviridae* or *Bunyaviridae* (Park *et al*., 2010).

In Tuscany, virus surveys were carried out on various woody plants such as grapevine (Rizzo *et al*., 2012; 2015 a) but to our knowledge no reports are available for Calla and Peruvian lily in Italy. In order to evaluate the health status of these plants in Tuscan nurseries, various viruses were assayed, belonging to the following families: *Betaflexiviridae* [Alstroemeria carla virus (AlCV), Lily symptomless virus (LSV)], *Bromoviridae* [Cucumber mosaic virus (CMV)], *Bunyaviridae* [Impatiens necrotic spot virus (INSV), Iris yellow spot virus (IYSV), Tomato spotted wilt virus (TSWV)], *Comoviridae* [Arabis mosaic virus (ArMV), Broad bean wilt virus 1 (BBWV-1), Broad bean wilt virus 2 (BBWV-2)], *Potyviridae* [Alstroemeria mosaic virus (AlMV), Bean yellow mosaic virus (BYMV), Dasheen mosaic virus (DsMV), Konjac mosaic virus (KoMV), Lily mottle virus (LMoV), Turnip mosaic virus (TuMV), Zantedeschia mosaic virus (ZaMV), Zantedeschia mild mosaic virus (ZaMMV)], *Tombusviridae* [Carnation mottle virus (CarnMV)] and unassigned [Tobacco rattle virus (TRV)]. An additional aim of this survey was to evidence new viral records in Italy for these widespread cultivations, due to the intense international exchanges that characterize the commercialization of lily.

2. Materials and Methods

Tests were carried out on 90 *Z. aethiopica* plants and 48 *Alstroemeria* spp. plants collected from 12 Tuscan nurseries in two years. Plants showed symp-

(*) Corresponding author: andrea.luvisi@unisalento.it
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toms such as foliar chlorosis, yellow spot and stripes. Total RNA was extracted from foliar tissue (2 g) using RNeasy Plant Mini Kit (Qiagen, Netherlands) protocol, modified according to MacKenzie *et al*. (1997). Tissues (2 g) were ground using a Tissue lyser (Qiagen) adding 5 ml of grinding buffer (4.0 M guanidine isothiocyanate, 0.2 M sodium acetate pH 5.0, 25 mM EDTA, 2.5% PVP-40 and 2.0% sodium bisulfate) just before use. The homogenate (1 ml) was transferred to a 1.5 ml tube and 100 µl of 20% sarkosyl were added. After 2 min centrifugation, 600 µl were transferred to a QIAshredder spin column (Qiagen) placed in a 2 ml collection tube. The subsequent steps of RNA extraction were according to the manufacturer's protocol. The extracted RNA was then retro-transcribed into cDNA using the iScript cDNA Synthesis kit (Biorad, USA). For each sample, 2 µl of cDNA were amplified in a total volume of 20 µl containing 1X HotMaster Buffer, 0.5 µg/µl BSA and 1 U of HotMaster Taq DNA Polymerase (Eppendorf, Germany). Primers and RT-PCR parameters were chosen following the protocols reported in Table 1. 18S rRNA was used as internal control (Osman and Rowhani, 2006). Finally, 10 µl of the amplification mix was electrophoresed in a 1.5% agarose gel in TAE buffer [40 mM Tris base, 20 mM sodium acetate, 1 mM EDTA pH (8.0)]. The amplified cDNA fragments were visualized on a UV transilluminator.

Data were analyzed using Sigma-Plot software (version 11; Systat Software, San Jose, CA). The software was used to perform analysis of variance (ANOVA). Data expressed in percent were converted to arcsin values. $P < 0.05$ was considered to be significant.

Table 1 - List of references for primers and RT-PCR conditions

Target	RT-PCR assay
Comoviridae	
ArMV	Faggioli *et al*., 2005
BBWV-1	Ferrer *et al*., 2008
BBWV-2	Ferrer *et al*., 2008
Betaflexiviridae	
AlCV	Spence *et al*., 2000
LSV	Lim *et al*., 2009
Bromoviridae	
CMV	Faggioli *et al*., 2005
Bunyaviridae	
IYSV	Kritzman *et al*., 2000
INSV	Liu *et al*., 2009
TSWV	Mumford *et al*., 1994
Potyviridae	
AlMV	Spence *et al*., 2000
BYMV	Ganesh Selvaraj *et al*., 2009
DsMV	Wen-Chi *et al*., 2010
KoMV	Wen-Chi *et al*., 2010
LMoV	Lim *et al*., 2009
TuMV	Wen-Chi *et al*., 2010
ZaMV	Kwon *et al*., 2003
ZaMMV	Wen-Chi *et al*., 2010
Tombusviridae	
CarnMV	Cevik *et al*., 2010
Unassigned	
TRV	Wei *et al*., 2009

3. Results

The health status of Calla and Peruvian lily as determined by the present survey is reported in Table 2. *Z. aethiopica* was mainly affected by viruses belonging to the *Potyviridae* family. More than two plants out of three were infected by DsMV and 50%

Table 2 - Health status of Calla lily (*Zantedeschia aethiopica*) and Peruvian lily (*Alstroemeria* spp.) expressed as percentage of infected plants

Target	*Zantedeschia aethiopica*	*Alstroemeria* spp.
Comoviridae		
ArMV	-	-
BBWV-1	-	-
BBWV-2	-	-
Betaflexiviridae		
AlCV	-	-
LSV	-	56.3 a
Bromoviridae		
CMV	-	12.5 b
Bunyaviridae		
IYSV	-	-
INSV	3.3 c *	-
TSWV	3.3 c	-
Potyviridae		
AlMV	-	-
DsMV	66.7 a	13.5 b
KoMV	-	-
LMoV	16.7 b	12.5 b
TuMV	-	-
ZaMMV	50.0 a	6.3 c
Tombusviridae		
CarnMV	-	-
Unassigned		
TRV	-	-

* Values in the same column followed by the same letter do not differ significantly according to Duncan's multiple range test (P=0.05).

Table 3 - Sequence of isolates of Zantedeschia mild mosaic virus and Lily mottle virus isolate detected in Italy

Zantedeschia mild mosaic virus isolate 2079 polyprotein gene, partial cds (GenBank: KF156666)
TCATTGAGTACCAACCCCAACAGTCCGATCTGTTTAATACTCGCGCGTCACAAACCCAATTCAATAATTGGTATGATGCGATCAAAAATGAG- TATGGGGTTGATGATAGTCAGATGCAGAGAATCATGAATGGCTTCATGGTGTGGTGTCTCGAGAATGGGACATCACCAAACATAAATGGCGTGTGGGT- TATGATGGATGGGGATGAACAAGTAGAATTTCCACTAAAACCAATGGTGGAGAATGCCAAGCCTACGCTGCGTCAAATAATGCACCACTTTTCAGACG- CAGCCGAGGCTTACATTGAACTTAGGAATGCCGCTGCCCCATATATGCCTAGATATGGGTTGCTGCGGAACTTAAGAGACAGAGGTCTAGCACGCTTCG- CATTCGACTTCTATGAAGTCACTTCAAAGACACCAGATCGTGCTAGAGAAGCTGTAGCGCAGATGAAGGCAGCAGCGCTAAACAATGTTTCCACAAG- GATGTTTGGATTGGATGGAAATATTGCAACTGCCACGGAGAACACTGAAAGGCACACTGCTAAGGATGTAAGTCCGAGCATGCACTCGCTACTCGG- GATCTCAGCCTTGCAGTAAAGGAGCTGGAAACAGCCCACAGTTATTGTCTTGCGATAGGGTTTAAATAGCCGTACTATTGTCGTTGCTAGATGTTG- CAGTGTGGGCCTCCCACCTTAAGGTTTATCAGTGTGGCTTTCCACCTAGTTCCTTACATTGCGCATAGTATGTGT
Lily mottle virus isolate 2409 coat protein gene, partial cds (GenBank: KF156662.1)
TGCTGGGGCCTCTAGCTCCACACAAACGAGTCGCCCAACACGTCCAGAGATTGCCGCGGTCGATGTAGCACCACAACAGAGCTCTGAGGCTA- GAGTGCGTGATCGTGATGTTGATGCTGGCACCGTGGGAACATACCAAATCCCTCGACTGAAAGCACTGGCAACAAAGATTAACGTACCCAAGGT- CAAGGGGCGAACAATAGTGAACACTGGGCACCTTGTGAACTACAACCCAGACCAAACAGATATTTCAAATACAAGGTCAACCCAGAAGCAATTTGA- GACCTGGCATAACGCTGTGAAAGATGAGTATGGTCTCAACGACGAGAGTATGGCTCTCGCAATGAATGGTCTGATGGTTTGGTGCATAGAGAATGG- CACCTCACCAAACATAAATGGCGTGTGGCTCATGATGGACGGAGATCAGCAAGTTGAATTTCCTTTACGTCCTATACTTGAACACGCAAAACC- GACGCTGCGCCAAATTATGGCGCATTTCTCAAACCTCGCTGAAGCTTATATTGAGAAGCAAAATTTGGAGAAACCGTACATGCCTAGGTACGGCCTT- CAGCGAAATCTCACCGATTTCAATCTAGCACGATTTGCTTTTGATTTCTATGA

of tested plants were infected by ZaMMV. Lower infection rates were reported for viruses belonging to *Bunyaviridae.* Even if *Alstroemeria* spp. plants were affected by *Potyviridae* viruses too, higher infection rates were recorded for *Betaflexiviridae*, where LSV infected more than half of plants. Further infections were caused by *Bromoviridae* viruses. *Comoviridae*, *Tombusviridae* or TRV infections were not found in both plants.

In *Z. aethiopica*, mixed infections were set at 40% for DsMV/ZaMMV, 6.7% for DsMV/LMoV/ZaMMV and 3.3% for DsMV/LMoV(data not shown). In *Alstroemeria* spp., mixed infections were set at 6.3% for LSV/ZaMMV, LSV/LMoV, DsMV/ZaMMV, CMV/LSV or CMV/DsMV/LSV.

With regard to *Alstroemeria* spp., the sequence obtained from a ZaMMV amplicon (GenBank accession no. KF156666 (Table 3) had 99% nucleotide identity with the corresponding fragment of a reference ZaMMV isolate (GenBank accession no. AY626825). Further isolates of LMoV were detected (GenBank accessions no. KF156667, KF156668, KF156669). Each further isolate had 99% nucleotide identity with the corresponding fragment of a reference ZaMMV isolate. All four isolates are different but had 99% nucleotide identity.

The sequence obtained from LMoV amplicon (GenBank accession no. KF156662) (Table 3) had 94% nucleotide identity with the corresponding fragment of a reference LMoV isolate (GenBank accession no. JN703466). The same LMoV amplicon was obtained from a *Z. aethiopica* sample as well. Further isolates of LMoV were detected in both species with 100% nucleotide identity with KF156662 (GenBank accessions no. KF156663, KF156664, KF156665).

4. Conclusions

Various viral infections, mainly due to viruses belonging to two families, *Betaflexiviridae* and *Potyviridae*, seem to affect Calla and Peruvian lily in Tuscan nurseries. Most detected viruses are frequently reported for both plants and mixed infection of virus belonging to *Potyviridae* are quite common in *Z. aethiopica* (Huang *et al*., 2007). However, some evidence is rarer.

To our knowledge this is the first report of LMoV in *Alstroemeria* spp. and *Z. aethiopica* or ZaMMV in *Alstroemeria* spp. in Italy, while ZaMMV was recently identified in Taiwan (Huang and Chang, 2005) and in Italy (Rizzo *et al*., 2015 b).

This report puts in evidence how widespread the virus is within these common plants and the need for constant monitoring of the health status for flower production. Even if some of viruses that affect Calla and Peruvian lily may be eradicated by thermotherapy (Panattoni *et al*., 2013) and heat treatment may help in *Bromoviridae* control (Luvisi *et al*., 2015), prevention represents the preferred method of virus control.

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