Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Short Notes

Sternbergia lutea, a new host of *Narcissus late season yellows virus*

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Summary. In autumn 2017, autumn daffodil plants with yellow-green stripes on the leaves were observed at a botanical garden in Budapest, Hungary. Indicator plants were inoculated, but symptoms did not develop. RT-PCR tests of the indicator plants were also negative for the viruses. *Potyvirus* specific ACP-ELISA and RT-PCR were carried out on the symptomatic *S. lutea* leaf samples. RT-PCR with universal potyvirus primers resulted in one, approx. 1700 base pair PCR product. Phylogenetic analysis of the nucleotide sequence of the coat protein demonstrated 98.78-99.51% identity with three Japanese isolates of *Narcissus late season yellows virus*. While unidentified potyvirus infection of autumn daffodil has been previously reported, sequence data have not been published. Therefore, this is the first report of *Sternbergia lutea* as a host of *Narcissus late season yellows virus*.

Keywords. *Potyvirus*, Hungary, daffodil.

Citation: J. Ágoston, A. Almási, K. Salánki, L. Palkovics (2021) *Sternbergia lutea*, a new host of *Narcissus late season yellows virus*. *Phytopathologia Mediterranea* 60(3): 403-407. doi: 10.36253/phyto-12709

Accepted: July 13, 2021

Published: November 15, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Assunta Bertaccini, Alma Mater Studiorum, University of Bologna, Italy.

INTRODUCTION

Sternbergia lutea (autumn daffodil, *Amaryllidaceae*) is an ornamental plant in the family (Bryan, 2002, 2005), which is grown for golden yellow flowers it produces in autumn, when other flowers are scarce. Leaves also appear in autumn (Bryan, 2002, 2005) and remain till the end of the following, unless damaged by hard frosts. Dry summers and full sun are necessary for flowering (Bryan, 2002, 2005).

In the autumn of 2017, bulbous plants in the botanical garden of the Hungarian University of Agriculture and Life Sciences, Buda Campus (Budapest, Hungary) were surveyed for possible virus infections. Several *Sternbergia lutea* plants showed heavy, 2–5 mm wide, yellow to yellow-green stripe

Figure 1. Symptoms of possible virus infection on leaves of *Sternbergia lutea.*

mosaic symptoms parallel to the veins on the entire length of leaves, that were indicative of possible virus infection (Figure 1).

Flowering of these plants has not been observed since the first year of planting in 2006, probably because the plants were planted below shadowing trees, but virus infections may have had also prevented flowering. There has been only one report of presumed potyvirus infection of autumn daffodil (Pleše, 1993) but the virus species was not identified, nor were sequence data published.

MATERIALS AND METHODS

Plant samples

A symptomatic clump of *S. lutea* plants were dug up in the autumn of 2017 from the Buda Campus botanical garden. Plants were potted and kept in an insect-free greenhouse in the first growing cycle, and were later planted in open-field conditions.

ELISA tests

In January of 2018 leaf samples were collected from the potted plants. ACP-ELISA tests were carried out by Agdia, based on the MAb PTY1 antibody (RRID:AB_2819158) (Jordan and Hammond, 1991). All tests were carried out in duplicate, according to the manufacturer's protocol. For each sample, 200 mg of leaf tissue was ground in 20 mL (1: 100 m:v) of indirect sample extraction buffer. Aliquots of 100 µL of the diluted sample were added to each test plate well, and then incubated at room temperature for 1 h. The wells were then emptied and washed seven times with phosphate buffered saline Tween (PBST). The monoclonal detection antibody, in mouse against potyvirus coat protein (Clone PTY1), was diluted 1: 100 (v:v) in conjugate buffer (ECI buffer), and 100 µL was added and incubated overnight at 4°C. Plates were then washed eight times with PBST. The enzyme conjugate polyclonal rabbit antibody against mouse IgG was diluted with ECI buffer to 1: 100 (v:v), and 100 µL was added to each plate well and incubated at room temperature. The plates were then washed eight times with PBST. Paranitrophenyl phosphate (pNPP) was added to PNP buffer at 1 mg mL^{-1}, and 100 µL was added to each plate well. Absorbance values were measured at 405 nm wavelength 30 min after addition of pNPP, using a Labsystem Multiskan MS ELISA reader. Positive controls were provided by the manufacturer, and negative controls were prepared from *Chenopodium amaranticolor* seedlings grown in an insect-free greenhouse. A sample was considered positive if the sample absorbance was at least three times greater than that of the negative control.

Herbaceous indexing

Chenopodium foetidum, *Nicotiana benthamiana* and *N. tabacum* 'Xanthi' plants were inoculated mechanically (0,02 M Sörensen's phosphate buffer, celite) with the sap of the same leaf used for ACP-ELISA testing. Plants were grown in an insect-free greenhouse, and symptom development was assessed during a 5-week post inoculation period.

RNA extraction, RT-PCR, and cloning

Total RNA was extracted from the same symptomatic leaf of *Sternbergia lutea* collected for ELISA and

inoculation tests, and from leaves of the inoculated herbaceous indexing plants, 5 weeks post inoculation. The protocol of White and Kaper (1989) was used. RT-PCR was carried out with universal potyvirus primers poty7941 and poly T_2 (Salamon and Palkovics, 2005). The primers amplified the C terminal part of the RNA-dependent RNA polymerase (RdRp, NIb) including the highly conserved GNNSGQP motif, the complete coat protein (CP) sequence, and the complete 3' untranslated region (UTR) to the first few bases of the polyA tail. PCR products were separated in 1% (w/v) agarose gel electrophoresis in 1× Tris-Borate-EDTA (TBE) buffer. The gel was stained with ethidium bromide and the products were visualized and photographed under UV light. Amplicons of the expected size (approx. 1700 nt) were purified using the High Pure PCR Product Purification Kit (Roche). The PCR product was cloned into pGEM®-T Easy vector (Promega), following manufacturer's instructions. *Pst*I digestion of the purified PCR product was carried out to verify its ability to be digested for further cloning.

Additionally, RT-PCR was carried out for *Cucumber mosaic virus* (CMV), using the protocol described by Nemes and Salánki (2020). CMV is known to have a wide host range and also infects monocotyledonous bulbous ornamental plants (van Rijn *et al.*, 1995; de Best *et al.*, 2000). The sample was also tested for *Narcissus latent virus* (NLV) and *Narcissus mosaic virus* (NMV), using multiplex RT-PCR (He *et al.*, 2019), as these viruses have been reported to infect daffodils in Hungary (Ágoston *et al.*, 2020).

Koch's postulates

To fulfill Koch's postulates, an asymptomatic *S. lutea* plant was mechanically inoculated with sap from the symptomatic *S. lutea* plant in April 2019, as described above. Molecular identification for virus from the inoculated plant was performed as described above.

Sequencing, and sequence analyses

The cloned fragment was sequenced in both directions using M13 forward and reverse primers in an ABI Prism automatic sequencer (BaseClear B.V.). Nucleotide sequence identities were determined by BLAST analyses. Further phylogenetic analyses were carried out with the MEGA-X program (Kumar *et al.*, 2018). ClustalW (Larkin *et al.*, 2007) multiple sequence alignment was carried out (gap opening penalty 15.00, gap extension penalty 6.66 for pairwise and multiple alignment, transition weight 0.5, ClustalW weight matrix, no negative matrix was used) on the complete coat protein (CP) sequences.

A Maximum Likelihood phylogenetic tree (Felsenstein, 1981) was constructed with the Hasegawa-Kishino-Yano model (Hasegawa *et al.*, 1985) with gamma distribution and invariant sites. This substitution model had the lowest Bayesian Information Criterion (5682.807). To test the phylogeny the tree was bootstrapped 1,000 times (Felsenstein, 1985).

RESULTS AND DISCUSSION

Mechanical inoculation of herbaceous test plants *C. foetidum*, *N. benthamiana* and *N. tabacum* 'Xanthi' with plant sap of symptomatic *Sternbergia* leaf resulted in plants remaining symptomless throughout the 5 week observation period. RT-PCR tests were also negative. Most potyviruses with monocotyledonous hosts do not infect dicotyledonous plants (Mowat *et al.*, 1988). In the ACP-ELISA tests, the absorbances were 0.947 and 0.953, while respective negative controls were 0.034 and 0.047, indicating the presence of *Potyvirus* infections. Universal potyvirus group specific RT-PCR resulted in an approx. 1700 nt product. RT-PCR tests for CMV, NLV and NMV were negative.

The mechanically inoculated *S. lutea* plant showed the same symptoms as the original infected plant (Figure 1), but the symptoms appeared only in the next growing season, in October of 2019, approx. 7 months after inoculation. Flowering of the inoculated *S. lutea* was not observed, possibly because the bulb was not of flowering size. RT-PCR of the inoculated plant also resulted in the expected PCR products size of approx. 1700 nt.

The nucleotide sequence of the cloned PCR product from the original symptomatic *S. lutea* plant, coding the complete coat protein region, included a unique *Pst*I recognition site (GenBank accession No. MK783944). *Pst*I digestion of the original PCR product and from the inoculated plant resulted the two predicted fragments. This indicated identification of a single *Potyvirus* in the infected leaf tissues and in the inoculated plant, confirming the virus as the agent causing the observed disease (fulfilment of Koch's postulates). BLAST analysis of the CP nucleotide sequence revealed greatest identity with Hungarian (MK132194 – 98.64%, MK132193 – 98.34%), Australian (MH886515 – 96.47%), and Japanese (LC158450 – 99.51%, LC158451 – 99.39%, LC158467 – 98.78%) isolates of *Narcissus late season yellows virus* (NLSYV) deposited in GenBank.

Further phylogenetic analyses were carried out with the following NLSYV GenBank sequences:

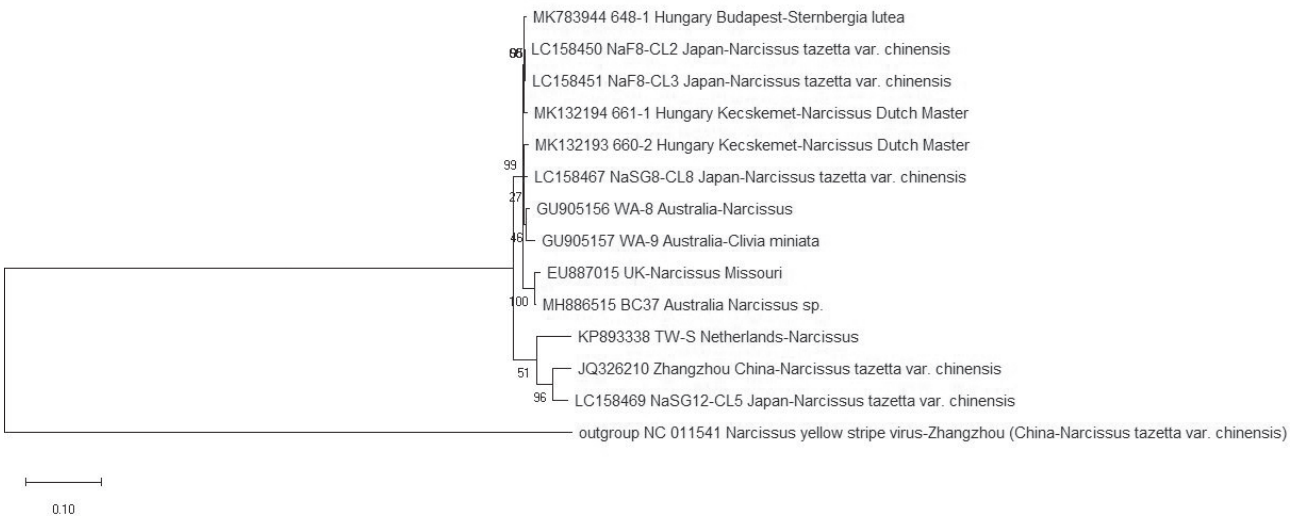

Figure 2. Phylogenetic tree of *Narcissus late season yellows virus* nucleotide sequences of the complete coat protein regions.

EU887015, GU905156, GU905157, JQ326210, KP893338, LC158450, LC158451, LC158467, LC158469, MH886515, MK132193, MK132194, MK783944, and for the outgroup sequence of *Narcissus yellow stripe virus* (GenBak accession number NC_011541) was used. After production of the first ClustalW alignment (Larkin *et al.*, 2007) on the complete CP sequence tested, DNA evolutionary models were applied, and a Maximum Likelihood phylogenetic tree (Felsenstein, 1981) was constructed (Figure 2).

The phylogenetic tree divided the virus isolates in two clades. One clade consisted of three NLSYV strains from *Narcissus* sp. from the Netherlands, China and Japan, while the other was composed of ten strains, eight collected from *Narcissus* and two isolated from different plant species, i.e., *Clivia miniata* from Australia and the Hungarian strain from *S. lutea*. With the exception of the GenBank accession GU905157 from *C. miniata* from Australia, the other NLSYV strains present in the analysis derived from *Narcissus* varieties. This is because daffodil is the main host of NLSYV, although not all the cultivars were reported to be susceptible to this virus (Mowat *et al.*, 1988).

Only one presumable *Potyvirus* infection has been previously reported from *S. lutea*, where electron microscopy of the leaf tissue showed cylindrical inclusions and filamentous particles in the cytoplasm (Pleše, 1993), but the virus was not identified to species level. The present report confirms the presence of a potyvirus in *S. lutea*, which has been identified as *Narcissus late season yellows virus*.

ACKNOWLEDGEMENTS

This research was supported by the project EFOP-3.6.1-16-2016-00006 "The development and enhancement of the research potential at John von Neumann University". The study was funded by the Hungarian Government and co-financed by the European Social Fund, and by the Ministry for Innovation and Technology within the framework of the Thematic Excellence Programme 2020- Institutional Excellence Subprogram (TKP2020-IKA-12) for research on plant breeding and plant protection.

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