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Syringa vulgaris is a new host for cucumber mosaic virus

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Summary. Virus-like symptoms consisting of light mosaic, chlorotic spots and oak chlorotic line patterns were observed on lilac plants (*Syringa vulgaris* L.) growing in a public garden in Imola (Emilia Romagna region, Italy). The causal agent was identified as cucumber mosaic virus (CMV) on the basis of biological, serological and nucleotide sequence properties of partial coat protein and movement protein genes, and the isolate was designated SYV. The CMV-SYV isolate caused mosaic symptoms on indicator plants of *Nicotiana tabacum* cv. Xanthi-nc, *N. rustica* and *Cucumis sativus*, while symptoms of local necrotic spots or pin points were observed on inoculated leaves of *Vigna unguiculata* and *Vicia faba*. To assess genetic differences between CMV-SYV and other known CMV isolates, phylogenetic analyses were carried out using 16 nucleotide sequences of coat protein and movement protein genes, including for SYV. The CMV-SYV isolate was most related to CMV subgroup IA isolates, and had 85.1-100% nucleotide sequence similarity to subgroup I isolates. This is the first report of CMV infecting *S. vulgaris*.

Keywords. Lilac, CMV, RT-PCR, subgroup identification.

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INTRODUCTION

Syringa vulgaris L. (lilac or common lilac; Oleaceae) is a popular spring-blooming ornamental shrub widely used in public and private gardens, for its large, showy and often fragrant inflorescences. Numerous lilac hybrids and cultivars have been developed for horticultural use. These selections exhibit variation in flower colour, period of blooming, and growth habit, and they are generally vegetatively propagated by rooting softwood cuttings to maintain genetic constancy (Hartmann *et al*., 1990). Risks of transmitting infectious diseases through vegetative propagation, particularly those caused by viruses, can be important. To combat virus diseases carried through vegetative propagules, adoption of preventive phytosanitary measures is essential, including correct pathogen diagnoses. Tomato mosaic virus (ToMV), arabis mosaic virus (ArMV), lilac leaf chlorosis virus (LLVC), toma-

to black ring virus (TBRV), tomato bushy stunt virus (TBSV), cherry leaf roll virus (CLRV), elm mottle virus (EMoV), strawberry latent ringspot virus (SLRSV), lilac ring mottle virus (LiRMoV) and tobacco rattle virus (TRV) have been previously described from lilac (Novák and Lanzová, 1975; Novák and Lanzová, 1977; van der Meer *et al*., 1976; van der Meer, 1976; Brunt, 1978; Canova and Betti, 1987; Castello *et al*., 1992; Cooper, 1993; Sharma-Poudyal *et al*., 2016).

Most of these viruses elicit similar symptoms in lilac, consisting mainly of chlorotic spots and line patterns on the leaves (van der Meer, 1976). This symptomatology is not indicative on the particular virus involved, and to get correct diagnoses, virus-specific detection methods are required. In this paper, we report the identification and characterization of a cucumber mosaic virus (CMV) isolate belonging to subgroup IA, from symptomatic lilac plants showing chlorotic ringspots and line patterns. This is the first documented serological and molecular evidence for the occurrence of CMV in lilac, causing similar symptoms to other viruses associated with that plant host.

MATERIALS AND METHODS

Since 2016, lilac plants showing virus-like leaf symptoms, including light mosaic, chlorotic ringspots and oak chlorotic line patterns (Figure 1), were observed in a public garden in Imola (Emilia Romagna region, Italy). Symptoms progressively and completely regressed after blooming, during the summer. In spring 2018, symptomatic leaves were collected from two plants, and were used in bioassay, serological and molecular tests.

In bioassays, three plants each of *Chenopodium quinoa*, *Nicotiana rustica*, *N. tabacum* cv. Xanthi and *Vigna unguiculata* cv. Black eye were mechanically inoculated with sap obtained by macerating symptomatic lilac leaves in 0.03 M phosphate buffer (pH 7). The same leaves were tested for the presence of CMV, alfalfa mosaic virus (AMV) and ToMV, using double-antibody sandwich (DAS)-ELISA commercial diagnostic kits (Bioreba AG; respectively, Art. Nr. 160675, 140575 and 152675 for the three viruses), including negative and positive controls. Samples from symptomatic and healthy leaves were also assessed for the presence of ilarviruses and nepovirus, using reverse transcription (RT)-PCR tests on total RNA extracted with the E.Z.N.A.® plant RNA kit (Omega Bio-tek, USA), and genus-specific primer pairs (Wei and Clover, 2008; Untiveros and Perez-Egusquiza, 2010). For the (RT)-PCR tests, the procedure of Parrella and Greco (2016) was used. Total RNA extracted from leaves of a healthy lilac plant was used as negative control, while total RNA extracted from tobacco plants infected with isolates of tomato ring spot virus (ToRSV) was used as positive control for nepovirus and parietaria mottle virus (PMoV) was used as positive control for ilarvirus (Parrella, 2002). Positive controls were derived from the collection of virus isolates at the Istituto per la Protezione Sostenibile delle Piante del CNR (IPSP-CNR), maintained as dehydrated leaves and stored over calcium chloride at 4°C.

In addition, and to confirm preliminary positive results obtained using CMV-specific ImmunoStrip® (Agdia Inc.) on symptomatic leaves from the two lilac plants, RT-PCR tests were also conducted using two pairs of CMV specific primers on total RNA isolated from infected and healthy lilac leaf tissues. Primer pair

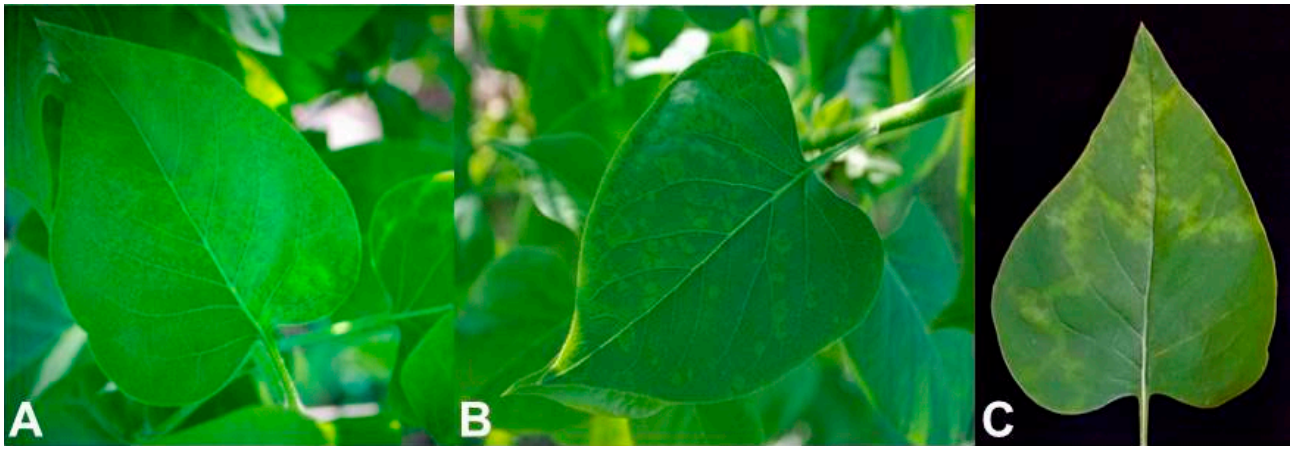

Figure 1. Natural symptoms on the leaves of *Syringa vulgaris* (lilac) plants infected by cucumber mosaic virus (CMV) isolate SYV, growing in a public garden (Northeast of Italy). Light mosaic (A), chlorotic spots (B) and oak chlorotic line patterns (C) were observed.

CMV1/CMV2 was used to amplify the partial coat protein gene (CP), and part of the 3'-noncoding region of CMV RNA3 (Singh *et al.* 1995; Parrella and Sorrentino 2009), and primer pair CMVMP1 (5'-ATGGCTTTC-CAAGGTACCA-3') and CMVMP2 (5'-CTAAAGAC-CGTTAACCACC-3') was used to amplify the entire Open Reading Frame (ORF) of the movement protein gene (MP). PCR conditions were as follows: initial denaturation cycle for 3 min at 92°C, followed by 35 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 60°C, extension for 1.5 min at 72°C. In the final cycle, the elongation time at 72°C was 7 min. PCR products of the expected length (approx. 500 bp for CMV1/CMV2 and 840 bp for CMVMP1/CMVMP2) were separated by electrophoresis in agarose gels and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega). DNA sequencing was carried out at Eurofins Genomics. Nucleotide sequence data were assembled using BioEdit Version 5.0.9 (Hall, 1999), and then compared with corresponding regions of the other CMV isolates retrieved from the GenBank database. Multiple nucleotide sequence alignments were performed by using the Clustal W program, while phylogenetic and molecular evolutionary analyses were inferred using the Maximum Likelihood method (Felsenstein, 1981), both packaged in the MEGA X software (Kumar *et al*., 2018). Peanut stunt virus (PSV; NC002040) was used as the outgroup to root the phylogenetic tree.

RESULTS AND DISCUSSION

Mechanical inoculation from symptomatic lilac leaf samples induced local chlorotic lesions on *C. quinoa* and *V. unguiculata* observed 5 days post-inoculation, and vein clearing and light mosaic in *Nicotiana* spp. observed 14 days post-inoculation.

In double-antibody sandwich (DAS)-ELISA tests, only CMV was identified from both lilac plants. These results were confirmed by RT-PCR with CMV-specific primer pairs, and amplification products were obtained from the naturally infected lilac plants and mechanically inoculated plants, as well as from the PAE1 isolate of CMV (Parrella and Sorrentino, 2009) used as positive control. No amplification products were obtained from healthy plants (Figure 2). The sequences obtained from the two plants with the CMV1/CMV2 primers were identical, as well as those obtained with the CMVMP1/CMVMP2 primers, and these were deposited at GenBank (Acc. No. MH433673 for the partial CP gene and Acc. No. MH433674 for MP gene). The partial CP sequence of CMV-SYV was 96.5–98.6% similar

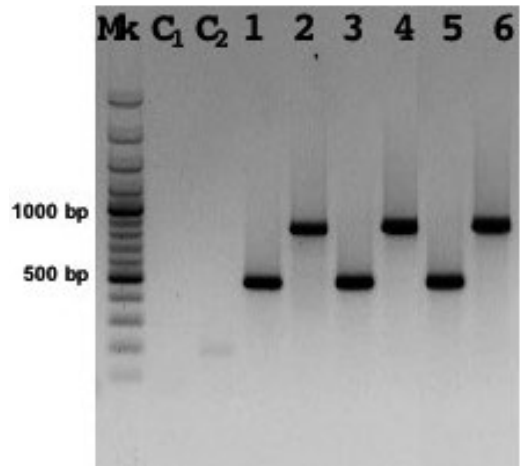

Figure 2. Results obtained with CMV1/CMV2 (lanes C1, 1, 3 and 5) and CMVMP1/CMVMP2 (lanes C2, 2, 4, and 6) primer pairs in reverse transcription (RT)-PCR tests, using total RNA extracted from healthy and symptomatic leaves of *Syringa vulgaris* (lilac): lane Mk, DNA ladder; lanes C1 and C2, negative controls (healthy lilac plant); lanes 1 and 2, amplicons obtained with RNA extracted from the lilac plant n. 1; lanes 3 and 4, amplicons obtained with RNA extracted from the lilac plant n. 2; lanes 5 and 6, positive controls (RNA of the CMV-PAE isolate; Parrella and Sorrentino, 2009).

to that of subgroup IA, 92.8–94.4% similar to subgroup IB and 77.0–76.5% similar to subgroup II, while the MP sequence was 96.4–99.0% similar to that of subgroup IA, 91.9–94.0% similar to subgroup IB and 79.2–79.6% similar to subgroup II. These results agree with those reported elsewhere concerning the nucleotide similarities within and among CMV isolate subgroups (Roossinck, 2002; Moury, 2004). Both of the CMV-SYV sequences obtained showed greatest sequence similarity at the nucleotide level with the CMV-Fny isolate (98.6% for the partial CP gene and 99.0% for the MP gene).

CMV1/CMV2 primers have been designed to produce amplicons of different sizes to distinguish CMV isolates belonging to subgroups I or II (Singh *et al.*, 1995; Parrella and Sorrentino, 2009). Based on the length of the amplicons obtained with these primers (487 bp), the CMV isolate from lilac (named SYV, from *Syringa vulgaris*) belonged to subgroup IA (Singh *et al*., 1995). This was further confirmed by the detection of one *Hpa*II restriction site in the 487 bp sequence, as previously proposed for the CMV isolates belonging to subgroup IA (Parrella and Sorrentino, 2009).

The bootstrap maximum-likelihood trees for nucleotide sequences of the CMV-SYV isolate and the two corresponding genomic regions of CMV isolates retrieved from the GenBank database and identified by the CMV1/CMV2 (partial CP gene) and CMVMP1/CMVMP2 (MP ORF) primer pairs, are presented

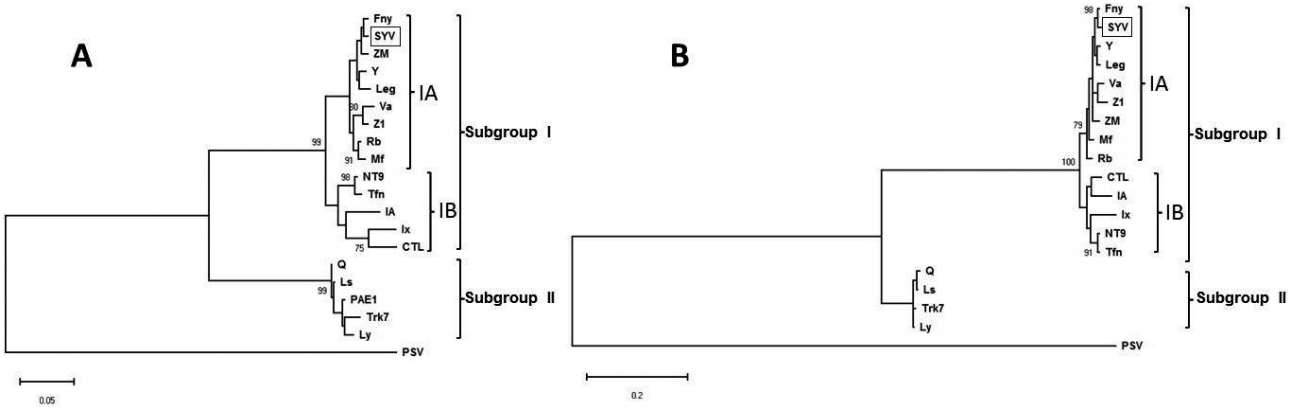

Figure 3. Phylogenetic trees derived from nucleotide sequences of the CMV RNA fragments obtained with the primer pairs CMV1/CMV2 and CMVMP1/CMVMP2 of SYV with the corresponding nucleotide regions of previously reported CMV isolates retrieved from GenBank (accession numbers and, when available, natural host plants and geographic origins are reported in parentheses for each CMV isolate): ZM (JN180311; *Zea mais*; South Korea); Rb (GU327365; *Rudbeckia hirta* var. pulcherrima; South Korea); Z1 (GU327368; *Cucurbita pepo*, South Korea); Fny (D10538; *Solanum lycopersicum*; United States of America); Y (D12499; Japan); Leg (D16405; *Vigna unguiculata*, Japan); Va (JX014248; *Vigna angularis* var. nipponensis, South Korea); Mf (AJ276481; South Korea); NT9 (D28780; *Solanum lycopersicum*; Taiwan); Tfn (Y19626; *Solanum lycopersicum*; Italy); Ix (U20219; *Ixora* sp.; Philippines); CTL (EF213025; *Brassica chinensis*; China); IA (AB042294; Indonesia); Q (M21464; Australia); Trk7 (L15336; *Trifolium repens*; Hungary); Ly (AF198103; *Lupinus angustifolius*; Australia); Ls (AF127976; USA); PAE1 (FN257306; *Passiflora edulis*; Italy); PSV (NC002040). The position of the SYV isolate is shown in the box. Peanut stunt virus (PSV) was used as an outgroup. Phylogenetic analyses were performed employing the maximum likelihood method packaged in the MEGA X software. Bootstrap values ≥ 70% are indicated at each node.

in Figures 1A and 1B. Analyses for both fragments showed that CMV isolates could be divided into two subgroups, as subgroup I and II. As expected, subgroup I was further divided into subgroups IA and IB. For CMV-SYV, this analysis showed that it belongs to subgroup IA and is closely related to the CMV-Fny, in both the genetic regions studied. Therefore, these results further confirmed that CMV-SYV belongs to subgroup IA of CMV.

Several viruses have been described in lilac (Brunt, 1978; Canova and Betti, 1987; Cooper 1993; Sharma-Poudyal *et al*., 2016). However, until now the presence of CMV infections in this host have not been reported. Results of the present study indicate that the CMV-SYV isolate systemically infected lilac plants causing symptoms including light mosaic, chlorotic ringspots and oak chlorotic line patterns on the host leaves (Figure 1). CMV is efficiently transmitted by more than 75 species of aphids (Hemiptera: *Aphididae*), in a non-persistent, stylet-borne manner, and these insects are the main vectors disseminating CMV in nature (Jacquemond, 2012). Nevertheless, since lilac is generally vegetatively propagated by basal shoots or root sprouts, it is important that mother-plants are carefully checked for virus infections to avoid spreading virus-infected material. To our knowledge, this is the first report of natural CMV infections in lilac.

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