Identification of *Lily symptomless virus* Infecting *Lilium martagon* ‘Pink Taurade’ in Ohio

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*Lily symptomless virus* (LSV) is a member of the *Carlavirus* genus of the *Betaflexiviridae* family. The viral genome is a single molecule of linear single-stranded RNA approximately 8.4 Kb in length encoding six open reading frames (ORF) that include the viral replicase ORF at the 5’ terminus and the capsid protein ORF at the 3’ terminus (2,5). The virus has a host range restricted to the *Liliaceae* family and is transmitted by aphids non-persistently (1,5).

In the spring of 2012, a *Lilium martagon* ‘Pink Taurade’ plant showing stunting and an interveinal chlorosis symptom resembling a nutrient deficiency (Fig. 1) was submitted to the Ohio Plant Diagnostic Network as part of a Farm Bill funded survey of viruses infecting ornamental hosts. The plant was collected from a block of approximately fifty container-grown lilies and was one of only a few showing the symptoms. The sample tested positive for LSV and negative for the *Potyvirus* group, *Alfalfa mosaic virus*, *Apple mosaic virus*, *Arabis mosaic virus*, *Broad bean wilt virus*, *Carnation latent virus*, *Carnation mottle virus*, *Carnation necrotic fleck virus*, *Carnation ringspot virus*, *Coupea mosaic virus*, *Cucumber mosaic virus* (CMV), *Impatiens necrotic spot virus*, *Peanut stunt virus*, *Pelargonium flower break virus*, *Prunus necrotic ringspot virus*, *Tobacco etch virus*, *Tobacco mosaic virus*, *Tobacco ringspot virus*, *Tomato aspermy virus*, *Tomato bushy stunt virus*, *Tomato mosaic virus*, *Tomato ringspot virus*, *Tomato spotted wilt virus*, and *Watermelon mosaic virus* by enzyme-linked immunosorbent assays (ELISA) using commercially available antibodies (Agdia Inc., Elkhart, IN).

For immunocapture reverse transcription (IC-RT), magnetic beads conjugated with sheep anti-rabbit IgG were incubated with polyclonal rabbit anti-LSV IgG (Agdia Inc., Elkhart, IN) as previously described (3). Leaf tissue samples were extracted, incubated with LSV-IgG conjugated beads, washed, and cDNAs synthesized from bound virions (3). Three full length LSV genome sequences (accession numbers NC_005138.1, HM222522.1, AM422452.2) were used to design two sets of primers to amplify the 5’ region of the RNA dependent RNA polymerase (RdRp) gene (LSVRdRpFWd34, 5’-CGACAAGTTCATAACAGTTA-
CTAG-3'; LSVRdprev1082, 5'-GCAGACTGCTTATCAGGTTTCTTC-3') and the capsid protein (CP) gene (LSVCPrfwd7112, 5'-CGATTAGACTCTCAGTTATG-3'; LSVCPRev8037, 5'-GGTAGTTCGTCGCTTCAATG-3'; Integrated DNA Technologies Inc., Coralville, IA) of the LSV genome. Five µl of cDNA or sterile water was used as a template for PCR reactions. Amplification was performed in 25 µl reactions [1.5 mM MgCl$_2$, 0.2 mM dNTP mix, 0.2 µM primer pair, 0.625 units GoTaq Flexi polymerase (Promega Inc., Madison, WI)] with the cycling parameters: 94°C (2 min); 30 cycles of 94°C (45 sec); 52°C (30 sec), 48°C (30 sec); 72°C (60 sec); and a final extension of 72°C (10 min). Both primer pairs amplified distinct products of expected size: approximately 1050 bp for the RdRp markers and 925 bp for the CP markers (Fig. 2). The amplicons were excised, purified from the agarose, and ligated into pGEM-T vector as previously described (3). Colonies were screened for inserts which were subsequently sequenced. Sequences were assembled, subjected to pairwise and multiple sequence alignments (3), and the ORFs were translated (Genedoc v. 2.6.001, 2000).

![Fig. 2. PCR detection of LSV from cDNAs synthesized from immunocaptured virions with RdRp-specific (Lane 1) and CP-specific (Lane 2) primers. Water controls with RdRp (Lane 3), CP (Lane 4), and M13 sequencing (Lane 5) primers. Tobacco streak virus Hosta isolate capsid protein clone (Lane 6) with M13 primers used as a positive PCR control. M = 1 Kb DNA ladder (250, 500, 750, 1000, and 1500 bp markers indicated). Electrophoresis was performed in 0.8% agarose at 100 volts for 60 min in 1X TAE buffer. Gel was stained with ethidium bromide. RdRp and CP amplicons are 1049 bp and 926 bp, respectively.](image)

Five RdRp and five CP clones were sequenced (The Ohio State University Plant Microbe Genomics Facility) and the ORF sequences deposited in GenBank (accession numbers KC884544-KC884553). The CP amplicon clones were all 926 nucleotides (nt) and encompassed the 876 nt CP ORF. The clones were 99.3-100% identical to one another with a 99.7% mean, and when translated shared 98.2-100% predicted amino acid (aa) identity with a 99.1% mean. The predicted CP was 291 amino acids. The RdRp amplicon clones were all 1049 nt long and encompassed the 5' 1025 nt of the LSV replicase ORF. Four of the five clones were 99.4-99.8% identical to one another with a 99.6% mean. The fifth clone differed at 111 nt sites and was only 88.8-89.2% identical to the other four. When translated, that clone had 99% predicted aa identity to the other four which in turn had 99.1-100% aa identity to each other. Of the 341 predicted amino acids of the 5' terminus of the replicase ORF, only three positions consistently differed: aa 119 was a glutamine or histidine; aa 280 was an alanine or valine; aa 315 was a phenylalanine or leucine. Thus the differences in the
111 nt sites between the two populations of clones did not translate into differences in the predicted amino acid sequence of the replicase region studied here, which supports work by others reporting LSV genome sequence heterogeneity in ORF 1 (2).

BLASTn searches of the NCBI database using default settings (100% query coverage) with the LSV-Lilium CP ORF sequences resulted in several 99% matches with LSV isolates from South Korea (accession numbers JX962776.1, AF103784.1, JN830615.1) and China (accession number AJ564638.1). BLASTn searches using the two LSV-Lilium RdRp ORF sequences resulted in 89% and 99% matches with LSV isolates from China (accession numbers HM222522.1, AJ564638.1) and India (accession number AM422452.2), respectively. The results presented here represent the first confirmed report of LSV infecting Lilium spp. in Ohio. The symptoms observed on Lilium martagon ’Pink Taurade’ are inconsistent with the curl-stripe/basal stripe disease caused by LSV on lilies, as well as the necrotic fleck disease when LSV is associated with CMV, and the streak-mottle of leaves when associated with Tulip breaking virus on L. speciosum varieties (1), and are more characteristic of a nutritional deficiency. The test plant has been on a weekly fertility regime for the past six months using a 2-1-1 slow release liquid fertilizer, and the growing media pH (7.2) is within the 6.0-7.5 range recommended for Lilium spp. (4) suggesting the interveinal chlorosis and stunting may not be due to a lack of available nutrients. Whether or not these symptoms can be attributed solely to LSV remains to be determined. Ohio perennial growers should benefit from these findings by gaining awareness of the potential threat aphid-transmissible LSV poses to their bulb production operations.

**Literature Cited**