First Report of Amazon Lily Mild Mottle Virus in Peony in the United States

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Lemoine's disease of peony (LDP) symptoms include root galling and stunted growth, making it a major concern for the peony industry (Garfinkel and Chastagner 2016). In an effort to elucidate the causal agent of the disease, three viruses (citrus leaf blotch, cycas necrotic stunt, and lychnis mottle) have previously been detected in peony (*Paonia lactiflora* Pall.) (Gress et al. 2017; Shaffer et al. 2019). Even though the viruses have been found in symptomatic plants, they also infect a significant number of asymptomatic materials. This indicates that they are not the sole causal agents of LDP, and for this reason total RNA was extracted as previously described (Poudel et al. 2013) from six plants never examined for viruses before, with two having the characteristic LDP galls. A high-throughput sequencing library was prepared using the Illumina TruSeq Stranded Total RNA with Ribo-Zero Plant kit, and pooled material was sequenced using the Illumina NextSeq500 platform. Bioinformatics analysis using VirFind (Ho and Tzanetakis 2014) identified the three aforementioned viruses as well as a fourth, unpublished one. One asymptomatic sample was infected by Amazon lily mild mottle virus (ALiMMV), a member of the genus *Anulavirus*. The three viral RNAs were represented by 146,820, 107,323, and 319,262 75-bp single-end reads, out of a total of over 70 million, covering 99, 78, and 90% (94% for the entire genome) of the respective molecules. The consensus sequence was taken from the read mappings with at least 10× coverage of the sequence submitted to GenBank (accession nos. MT362772 to MT362774). There are no nucleotide (nt) or amino acid (aa) cutoffs for species demarcation in the genus *Anulavirus* (Bujarski et al.

2019; Elbeaino et al. 2020). However, the sequences showed 77 to 81% nt identity to the reference virus sequences (NC 018402 to NC 018404) with the aa identities of the four virus proteins ranging from 86 to 91%, indicating that this is a distinct ALiMMV isolate infecting peony. We developed a reverse transcription polymerase chain reaction (RT-PCR) detection method and assayed the presence of the virus in 284 peony samples (both symptomatic and asymptomatic) from a private collection in Arkansas (140) and the University of Michigan Nichols Arboretum (144). The RT was performed following the Poudel et al. (2013) protocol. Primers 1368F (5'-AAGGGGTTGGTTCTCAAGAGG-3')/1958R (5'-ATTAA GAGCATCAAAATCCGCCG-3') (Integrated DNA Technologies) that amplify a 590-nt fragment of RNA3 were utilized in PCR amplification. The reactions initiated at 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a final extension of 72°C for 10 min. Four samples (the sequenced isolate from Arkansas and three from Michigan) tested positive, and amplicon sequencing revealed a 98 to 100% nt identity to the original isolate. One of the Michigan plants was reported as stunted, but the other samples were asymptomatic. ALiMMV is efficiently transmitted by seed and mechanical means (Fuji et al. 2013). Given the biological properties of ALiMMV and the fact that peony is propagated through root cuttings in commercial settings, it is important for materials to be tested before propagation. Given that none of the four viruses identified recently is associated with LDP, our efforts have shifted to addressing the possibility that LDP is caused by a combination of viruses.

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