Diseases Caused by Viruses

First Report of Gentian Kobu-sho-Associated Virus Infecting Peony in the United States and the Netherlands

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Lemoine's disease of peonies (LDP) is associated with root galls that could lead to stunted growth and reduced flowering. In the quest to identify the causal agent(s) of LDP, two symptomatic plants (cultivars Alice Crousse [AC] and Alice Harding [AH]) were sampled in Arkansas in 2019 and sequenced as described (Shaffer et al. 2019). Gentian Kobu-sho-associated virus (GKaV) was present in both plants. The contigs from AH were mapped to the reference sequence of GKaV (AB698918; Kobayashi et al. 2013), yielding 87% of the ~23-kb genome, which was completed by Sanger sequencing (GenBank accession no. MW646307) as per Thekke-Veetil et al. (2013). Sample AC was coinfecting with cycas necrotic stunt virus (CNSV) and AH with CNSV, citrus leaf blotch virus, and lychnis mottle virus. *Gentiana triflora* Pall. and *G. scabra* Bunge plants with Kobu-sho disease symptoms that include galls/tumors on all plant parts were also positive for GKaV (Iwade et al. 2006; Kodama et al. 2004). The striking similarity between symptoms of the two diseases led to the development of a GKaV screening protocol to determine its presence in LDP-affected material. Primers GKaVF (5'-TTAGTGATGAGTGCCTTTTCC-3') and GKaVR (5'-CTGCCAGTCTTCTGTGAACC-3'), which amplify a 574-nt region of the virus, were used to screen 144 peony leaf samples from the University of Michigan's Nichols Arboretum collection. Thirty-two plants were stunted and 112 displayed normal growth. Nineteen (59%) of the stunted plants tested positive for GKaV compared with eight (6.5%) of the symptomless plants.

Partial GKaV genome sequences of three isolates from stunted Michigan plants were deposited in GenBank (MW646310–12) along with three GKaV isolates from Arkansas collected at the same location and time as AC and AH (MW646308, 9, and 13); two had LDP symptoms and the status of the third was unknown. In 2020 four peony root samples from the Netherlands were sequenced as described in Hammond et al. (2021) to identify the causal agent of root galls in three samples. GKaV was present in two, cultivar Paul M. Wild and No. 40391499, and the nearly complete genome sequences were deposited in GenBank (MW916234–5). Paul M. Wild was coinfecting with cucumber mosaic virus and tobacco rattle virus and No. 40391499 with a novel amalgavirid. The third, symptomatic cultivar Many Happy Returns, was infected with CNSV while the fourth, symptomless cultivar Itoh, was infected with CNSV and Amazon lily mild mottle virus (Shaffer et al. 2021). Percent pairwise identities between sequences were calculated using SDT version 1.2 (Muhire et al. 2014). The six partial GKaV sequences from Michigan and Arkansas shared 92 to 100% nt (98 to 100% aa) identity. Analysis of the three near full length GKaV genomes presented in this communication and the type isolate (NC020252) showed 87 to 91% nt (93 to 97% aa) identity. This report provides evidence that GKaV infects peony and is present in Europe and North America. The association of GKaV with LDP is not established, but the virus has been detected in 59% of the plants showing disease symptoms and in <7% of asymptomatic plants. We hypothesize that, as in the case of gentian, GKaV has an extended incubation period in peony (Kobayashi et al. 2013) and its titer may fluctuate between seasons, as has been well established for other crops (Villamor et al. 2022). The industry does not perform virus cleanup routinely; propagation material should be tested for GKaV to minimize its spread because the virus may be associated with LDP in at least some cultivars.

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