Diseases Caused by Viruses

First Report of Apricot Vein Clearing-Associated Virus Infecting Flowering Apricot (*Prunus mume*) in the United States

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Apricot vein clearing-associated virus is the type species of genus *Prunivirus*, family *Betaflexiviridae*. Apricot vein clearing-associated virus (AVCaV) was first discovered from an Italian apricot tree (*Prunus armeniaca*) showing leaf vein clearing and mottling symptoms (Elbeaino et al. 2014). Since then, AVCaV has been reported in symptomatic and asymptomatic plants from other countries (Kinoti et al. 2017; Kubaa et al. 2014; Marais et al. 2015). In 2018, a domestic selection of a flowering apricot (*P. mume* 'Peggy Clarke') (PC01) with no discernible foliar virus-like symptoms was received from a private *Prunus* collection located in California for inclusion in Foundation Plant Services (University of California, Davis) collection. Total RNA was isolated from PC01 leaves using a MagMax Plant RNA Isolation Kit. The RNA was analyzed for a panel of 15 *Prunus*-infecting viruses (Diaz-Lara et al. 2020) by reverse-transcription quantitative PCR (RT-qPCR). Also, to screen sap-transmissible viruses, homogenate of young leaves of PC01 was rubbed onto leaves of herbaceous plants, *Chenopodium amaranticolor*, *C. quinoa*, *Cucumis sativus*, and *Nicotiana clevelandii* (Rowhani et al. 2005). The source PC01 tested negative for 15 screened viruses. Interestingly, vein-clearing symptoms were observed on leaves of *C. quinoa* and *C. amaranticolor* plants. These results suggested the presence of a mechanically transmissible virus in PC01. To determine the identity of the mechanically transmissible agent, symptomatic *C. quinoa* and PC01 plant were advanced for high-throughput sequencing analysis. Aliquots of RNA from PC01 and *C. quinoa*

were rRNA depleted and used for cDNA library preparation with an Illumina TruSeq Stranded Total RNA kit. Short raw reads (75 bp) were trimmed, de novo assembled, and subsequently annotated using the tBLASTx algorithm as described (Al Rwahnih et al. 2018). A total of 47,261,138 and 8,812,296 single-end reads were obtained from PC01 and inoculated *C. quinoa*, respectively. The only viral agent detected from PC01 and inoculated *C. quinoa* was AVCaV with 33,950 and 14,295 mapped contigs, respectively. The de novo assembly generated near-complete contigs resembling AVCaV genome from both PC01 and *C. quinoa*, which shared 99.8% identity at nucleotide level. The longest contig (8,342 nt, 73.5× coverage depth) obtained from PC01 was further completed using a SMARTer RACE kit. The complete genome sequence of AVCaV-PC01 was 8,364 nt long (GenBank MK170158). The best hit in alignment of full-length virus genome to GenBank corresponded to KY132099.1 with 98% identity. Additionally, AVCaV infection was confirmed in both PC01 selection and the symptomatic *C. quinoa* by RT-PCR as described (Marais et al. 2015). Lastly, symptomatic leaves of *C. quinoa* were used in a leaf-dip method to visualize virus particles by transmission electron microscope. As a result, flexuous rod-shaped virions were observed from leaf dips of *C. quinoa* plants. Additionally, RT-PCR screening of 47 *Prunus* samples that originated from the same private collection resulted in detection of AVCaV in a plum sample (*P. domestica* 'Persian Green'). To our knowledge, this is the first report of AVCaV in California, U.S.A. Furthermore, mechanical transmission of an AVCaV isolate infecting flowering apricot to herbaceous hosts was confirmed. Field surveys and biological studies are underway to determine the prevalence of AVCaV and assess its effect on tree performance.

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