Diseases Caused by Viruses

First Report of Apricot Vein Clearing-Associated Virus Infecting *Prunus domestica* Revealed by High-Throughput Sequencing in Morocco

R. Tahzima,^{1,2,†} R. Qessaoui,³ Y. Foucart,² S. Massart,¹ and K. De Jonghe^{2,†}

¹ University of Liège (ULg) Gembloux Agro-Bio Tech, Department of Plant Pathology, Gembloux, Belgium

² Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Plant Sciences Unit, Merelbeke, Belgium

³ Ibn Zohr University, National School of Applied Sciences, Agadir, Morocco

Funding: Funding was provided by FOD Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu, project EURAVELCH (RI 16/A-198). Plant Dis. 105:1231, 2021; published online as <https://doi.org/10.1094/PDIS-09-20-1966-PDN>. Accepted for publication 23 October 2020.

Plum (*Prunus domestica* L., Rosaceae) trees, like many stone fruit trees, are known to be infected by numerous plant viruses, predominantly as consequence of their clonal mode of propagation and perennial cultivation (Jelkmann and Eastwell 2011). Apricot vein clearing-associated virus (AVCaV) is a member of the genus *Prunivirus* in the family *Betaflexiviridae*. AVCaV was first reported in Italy infecting apricot (*P. armeniaca* L.) associated with foliar vein clearing symptoms (Elbeaino et al. 2014). It has also been detected in various *Prunus* species, such as plum, Japanese plum (*P. salicina* L.), sour cherry (*P. cerasus* L.), Japanese apricot (*P. mume* L.), apricot, and peach (*P. persica* L.) sourced from Asian and European countries (Marais et al. 2015), as well as in the ornamental myrobalan (*P. cerasifera* L.) in Australia (Kinoti et al. 2017). In 2018, during the vegetative season, a survey was carried out in two different apricot and plum orchards in the southern region of Agdez (Agadir, Morocco), where stone fruit trees are grown. Five branches with leaves were sampled from three apricot and three plum trees of unknown cultivars, all asymptomatic. Total RNA was extracted from 100 mg of plant tissue (leaves and cambial scraping) using an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany), and separate samples (one per species) were used for library preparation (NEBNext Ultra RNA library kit; New England

BioLabs, U.S.A.) and sequencing (Illumina NextSeq v2, totRNA sequencing) at Admera Health (U.S.A.). All generated reads (6,756,881) from the plum sample were quality filtered and submitted to the VirusDetect pipeline (Zheng et al. 2017). The plum cDNA library, a total of 20 viral contigs (68 to 1,928 bp), mapped to several AVCaV accessions in GenBank. A reference mapping (CLC Genomics Workbench 12, Qiagen) was conducted against all four available AVCaV full genomes (KM507062, KM507063, KY132099, and HG008921), revealing 100% coverage of the full sequence (8,358 nt) with 97 to 98% nucleotide (nt) identities (BLASTn). Analysis of the derived sequences allowed identifying the location of the four predicted open reading frames (ORFs) (i.e., ORF1, 6,066 nt/2,021 aa; ORF2, 1,383 nt/460 aa; ORF3, 666 nt/221 aa; and ORF4, 420 nt/139 aa), previously described for the AVCaV genome (Elbeaino et al. 2014). The amino acid sequences of the encoded proteins of AVCaV isolate from Morocco also shared 97 to 98% identities with the corresponding sequences of complete genome AVCaV isolates in GenBank. To confirm the detection of AVCaV in the three plum samples, specific RT-PCR primers (VC37657s, 5'-CCATAGCCACCCCTTTT-CAA-3'; VC28239a, 5'-GTCGTCAAGGGTCCAGTGAT-3') (Elbeaino et al. 2014) were used, and the expected 330-bp fragment from the replicase gene was amplified in all three samples and subsequently sequenced (MT980794 to 96). Sanger sequences were 100% identical to the corresponding HTS-derived sequence. This is the first report of AVCaV infecting plum in Africa. The incidence of AVCaV in Moroccan *Prunus* species is unknown. Plum trees from the surveyed orchards were also confirmed to be coinfecting with little cherry virus 1 using HTS. Further investigation is required to determine the impact of AVCaV on these asymptomatic plum trees and other stone fruit species.

References:

- Elbeaino, T., et al. 2014. Virus Res. 181:1.
- Jelkmann, W., and Eastwell, K. C. 2011. Page 153 in: Virus and Virus-like Diseases of Pome and Stone Fruits. APS Press, St. Paul, MN.
- Kinoti, W. M., et al. 2017. Plant Dis. 101:1337.
- Marais, A., et al. 2015. PLoS One 10:e0129469.
- Zheng, Y., et al. 2017. Virology 500:130.

The author(s) declare no conflict of interest.

Keywords: *Prunus*, *Betaflexiviridae*, *Prunivirus*, HTS, next generation sequencing (NGS)

[†]Indicates the corresponding author.

E-mail: K. De Jonghe; kris.dejonghe@ilvo.vlaanderen.be