New Disease Reports

Identification of *Nectarine stem pitting-associated virus* infecting *Prunus persica* in Hungary

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Nectarine stem pitting-associated virus (NSPaV; genus *Luteovirus*) was first described in the USA in imported nectarine (*Prunus persica*) trees held in post-entry quarantine (Bag *et al*., 2015). It was later identified in peach and nectarine plants in California (Villamor *et al*., 2016) and in China (Lu *et al*., 2017), and in *Prunus mume* in Japan (Candresse *et al*., 2017).

A peach sample showing severe yellow leaf symptoms (Fig. 1) was collected from a *Prunus persica* 'Baby Gold' tree from a 13-year-old organic orchard in Szob (Pest County) in Hungary in May 2011. A small RNA library was prepared from the sample and submitted for next-generation sequencing on an Ion Torrent Personal Genome Machine. Eighteen contigs were assembled from 227,878 reads using the VirusDetect pipeline (Zheng *et al*., 2017) showing 95 to 100% homology to NSPaV sequences available in GenBank (Accession Nos. NC_027211, KT273409 and KT273410). These covered about 25% of the NSPaV genome. A further 75 contigs revealed 95 to 100% similarity to *Plum pox virus* (PPV) sequences available in GenBank covering 65% of the PPV genome. The presence of NSPaV in the sample was confirmed by specific primers based on the NSPaV contig sequences (F: 5′-TTGGTTACCACCAATGCGAC-3′, R: 5′-AGAGGCGAAGACATCACTTTACTAG-3′; 415 bp product) and also by the NSPaVF-NSPaVR primers described by Bag *et al*. (2015) yielding a 459 bp band by RT-PCR. The 415 bp NSPaV PCR product (isolate 9/4) was sequenced and deposited in GenBank (KY829024). The sequence shared 96 to 98% nucleotide identity with the NSPaV isolates available in GenBank (NC_027211, KT273409 and KT273410).

Thirteen additional peach samples from the same orchard, all showing the same leaf yellowing symptoms, were tested for NSPaV and PPV. NSPaV was detected by RT-PCR in all the samples using both primer pairs mentioned above but not in healthy controls. PPV was also identified in all 13 samples using the P1-P2 universal primers (Wetzel *et al*., 1991) by RT-PCR and using DAS ELISA (Loewe). The 415 bp NSPaV PCR product obtained from one of the samples (isolate 2/6) was also sequenced and deposited in GenBank (KY626337). The sequence showed 96 to 97% nucleotide identity with NSPaV sequences in GenBank (NC_027211, KT273409 and KT273410) and shared 99% nucleotide identity with NSPaV isolate 9/4.

Screening of a larger number of symptomatic and asymptomatic samples in the affected field is in progress in order to establish the relationship between NSPaV and PPV in the aetiology of the disease. To our knowledge, this is the first report of NSPaV in Hungary and in Europe.

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Figure 1

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