New Disease Reports

First report of *Arabis mosaic virus* in potato (*Solanum tuberosum*), identified by nanopore sequencing

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Tubers of the potato breeding line 06-6065-2 were received at SASA in 2014 from a UK potato grower for the initiation of pathogen-tested nuclear stocks. The line was tested by DAS-ELISA and found negative for the potato viruses: *Beet ringspot virus, Potato latent virus*, *Potato leaf roll virus*, *Potato mop top virus*, *Potato virus A*, *Potato virus M*, *Potato virus S*, *Potato virus V*, *Potato virus X* and *Potato virus Y*. The breeding line also tested negative for pospiviroids using a DIG-labelled RNA probe (Monger & Jeffries, 2015). However, inoculation to a range of indicator plants produced symptoms of chlorosis and mottling on some: *Capsicum annuum, Chenopodium murale, C. quinoa, Nicotiana benthamiana, N. bigelovii, N. debneyi* and *N. occidentalis* P1. The *C. quinoa* plants produced the most severe symptoms (Fig. 1). Plants that did not show symptoms were *C. amaranticolor, Datura metel, N. clevelandii* and *N. tabacum* (cv. White Burley). The breeding line was maintained as microplants which showed no symptoms; however, chlorotic symptoms developed in glasshouse-grown plants (Fig. 2).

Subsequently, potato-derived RNA was subjected to deep sequencing using the MinION nanopore sequencing platform from Oxford Nanopore Technologies (ONT, UK). At present only RNA with a polyA tail can be sequenced directly using this method. mRNA was extracted from microplant tissue using Dynabeads (Invitrogen, USA). Sequencing was performed on the MinION flow cell, F10-MIN 106 R9 using the direct RNA sequencing kit, SQK-RNA002 (ONT), with approximately 140 ng of mRNA. The MinKnow software (ONT) generated a file of passed fastq reads (1,470,019). These reads were analysed using the Kaiju web server (http://Kaiju.binf.ku.dk/server). The Kaiju analysis classified 339,384 of the reads and identified 5,252 reads as *Arabis mosaic virus* (ArMV). The ArMV finding in 06-6065-2 was confirmed using an immunostrip (Agdia, USA) and a real-time assay (Monger & Mumford, 2010).

The ArMV RNA1 and 2 sequences were extracted from the passed nanopore reads by mapping them to reference sequences (GenBank Accession Nos. AY017339 and AY303786) with Minimap2 (https://github.com/lh3/minimap2). The output alignment files, ArMV_RNA1.sam and ArMV_RNA2.sam, were visualised with the reference sequences using Tablet (https://ics.hutton.ac.uk/tablet/download-tablet/). 3,814 reads mapped to RNA1 and 5,857 reads mapped to RNA2. The contigs from both alignments were missing the first 11 bases at the 5′ end and had missing and undetermined bases along their lengths. The accurate sequence of the coat protein was obtained by RT-PCR with primers designed from the MinION sequence (MN227140). The sequence identity with other ArMV isolates on the NCBI database was determined using the Megalign program (Lasergene 15, DNASTAR, USA). At the amino acid level, the highest identity (97%) was two UK isolates, from bee-associated pollen (MH614321, plant species unknown) and lilac (D10086). The phylogenetic relationship with other ArMV isolates from different countries and host species was examined (Fig 3).

ArMV is transmitted naturally to plants via nematodes in the soil and this is probably the method by which the tubers had acquired the virus. Potato is an intensively tested and managed crop worldwide, and given that the virus has not been reported previously, implies that this virus is a rare event in potato.

References

1. Monger WA, Jeffries C, 2015. Detection of *Potato spindle tuber viroid* and other related viroids by a DIG labelled RNA probe. In: Lacomme C, ed. *Methods in Molecular Biology: Plant Pathology Techniques and Protocols*. New York, USA: Humana Press, 259-272. http://dx.doi.org/10.1007/978-1-4939-2620-6_19
2. Monger WA, Mumford R, 2010. The improvement of diagnostic capabilities for the soft-fruit industry: the development of rapid and sustainable alternatives for strawberry virus testing. https://horticulture.ahdb.org.uk/sites/default/files/research_papers/SF 84. Accessed 13 April 2020.
3. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731-2739. http://dx.doi.org/10.1093/molbev/msr121

Figure 1

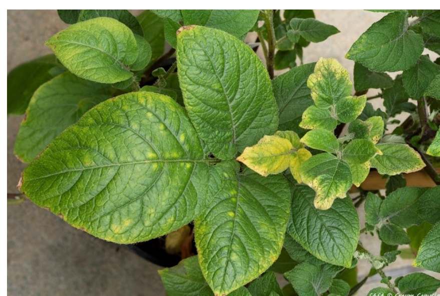

Figure 2

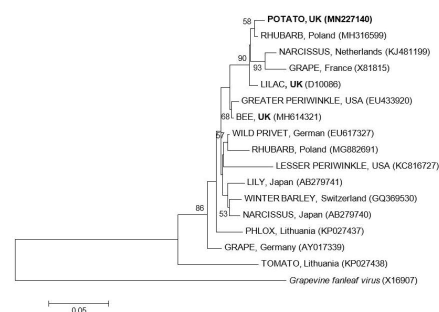

Figure 3

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