New Disease Reports

First report of *Pepper veinal mottle virus*, Pepper yellows virus and a novel enamovirus in chilli pepper (*Capsicum* sp.) in Rwanda

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In January 2016, 35 leaf dried samples of chilli pepper (*Capsicum* sp.) were submitted to Fera Science Ltd, from six districts in Rwanda: Ruhango, Nyanza, Rulindo, Kayonza, Nyagatare and Kirehe. The samples were sent in following the appearance of a suspected virus in the chilli pepper crops. Symptoms before drying included distorted leaves, stunting and mosaic (Fig. 1). Chilli pepper is an important horticultural crop in Rwanda; in 2017, 27 tones chilli pepper were exported, mainly to the UK, The Netherlands and Belgium, worth approximately US $54,000 (National Agricultural Export Development Board, Rwanda).

Following a previous finding of an unknown potyvirus in a sample of *Capsicum* with the same symptoms from Rwanda in June 2015, the samples were tested by ELISA with generic potyvirus antisera from the Leibniz-Institut DSMZ (Braunschweig, Germany). A potyvirus was detected by ELISA in all the samples. To try to identify the potyvirus by sequencing, the samples were tested by PCR using the P9502 and CPUP potyvirus primers (van der Vlugt *et al*., 1999). However, the presence of a potyvirus could not be confirmed in this way. The samples were also tested by ELISA for known potyviruses found in *Capsicum*, including *Chilli veinal mottle virus* (DSMZ) and *Potato virus Y* (Bioreba, Reinach, Switzerland). These ELISA tests were negative. Therefore, one of the samples from the Ruhango region was screened using an Illumina MiSeq as described by Adams *et al*. (2014). Sequences for the following viruses were derived from the MiSeq run and added to GenBank: *Pepper veinal mottle virus* (PVMV, genus *Potyvirus*; GenBank Accession No. MG470801, Pepper yellows virus (PeYV, genus *Polerovirus*; MG470802) which is a newly described virus detected in pepper (Lotos *et al*., 2017), *Cucumber mosaic virus* (CMV, genus *Cucumovirus*; MG470798, MG470799 and MG470800) and a novel virus which is tentatively a member of the genus *Enamovirus* (MG470803). Real time PCR assays were designed (Table 1) to PVMV and PeYV and all the samples were tested using these assays. Both PVMV and PeYV were detected in samples from Ruhango, Nyanza and Nyagatare. However, PeYV but not PVMV, was detected in samples from Kayonza, Rulindo and Kirehe.

Following the sequencing results the samples were also tested by ELISA to confirm CMV infection (Agdia, Elkhart, Indiana, USA). CMV was detected in the sample tested by next generation sequencing (Ruhango region) and in samples from Nyanza and Rulindo, but not in the samples from Kayonza, Nyagatare and Kirehe.

This is the first confirmed report of PVMV and PeYV in Rwanda and the first detection of a novel enamovirus. Work is ongoing to try to characterise the enamovirus.

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Figure 1

Table 1. Primer and probe sequences of real time PCR (TaqMan) assays designed to detect *Pepper veinal mottle virus* (PVMV) and Pepper yellows virus (PeYV).

Assay	Sequence 5'-3'
PVMV-Forward	CCATATCTTGGCATGTAA
PVMV-Reverse	CATGGCGCACTTCAGTAA
PVMV-Probe	[FAM]ATTGCGCTTTTCAATGTACGCCTCAGC[BHQ1]
PeYV-Forward	GCTCTTTATCCGGTGGGT
PeYV-Reverse	CGCTGTGGTCATCATTTC
PeYV-Probe	[FAM]AAACCAAAGCGCACTATAGAGCAGGGAATTG[BHQ1]

Figure 2

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