

NEW DISEASE REPORT

First report of *Potato spindle tuber viroid* infecting tree tomato in Kenya in mixed infection with *Potato virus Y*

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Potato spindle tuber viroid (PSTVd) is composed of a single stranded circular RNA and was first described causing disease in potato in 1922. PSTVd is also known to cause disease in pepper and tomato but it can be symptomless in other species (CABI, 2021). In Kenya it has been detected in *Solanum coagulans* (Skelton et al., 2019).

Leaf samples of tree tomato (*Solanum betaceum*) showing virus-associated symptoms, including leaf malformation and mottling, and veinal necrosis (Fig. 1), were collected from 14 farms during a survey in four counties in Kenya: Embu, Machakos, Meru and Tharaka-Nithi. Sequencing was done on a pooled sample, formed by selecting one sample from those collected from each of the farms. A single representative leaf sample from each farm was selected, and pooled into the single sample for high throughput sequencing (HTS) analysis. RNA was extracted using a CTAB method (Gambino et al., 2008) then taken for cDNA synthesis. Sequencing was done on the Ion Torrent S5 XL (Thermo Fisher Scientific, USA) platform resulting in nine million reads with an average read length of 146 bp. The CLC Genomics workbench (QIAGEN, Germany) was used to trim 15 nucleotides from the 3' end of all raw sequence reads and those less than 20 bp in length were discarded. A query of a local download GenBank virus database, revealed 26 reads aligning to PSTVd, and 1440 aligning to members of the *Potato virus Y* (PVY) complex. Reference-guided genome assembly for PSTVd was conducted using the exemplar strain (GenBank Accession No. NC002030) and resulted in a 359 bp consensus genome sequence, which was added to GenBank (MZ054164). BLAST results showed a 97.78% nucleotide alignment to a PSTVd isolate from the USA (JX280944).

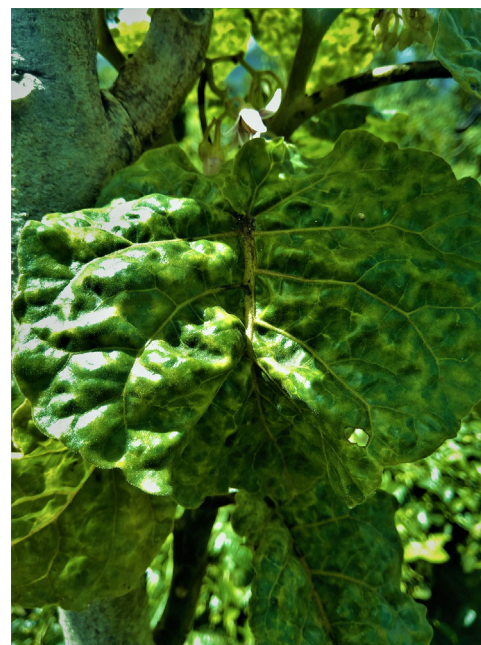


FIGURE 1 Leaf infected with *Potato spindle tuber viroid* and *Potato virus Y* showing mottling and malformation

Primers were designed and used to validate the HTS PSTVd results (PSTVd F 5'-CGGAACAACTCGTGGTTCCTG-3'; PSTVd R 5'-AGGAACCAACTGCGGTTCCTG-3'), while the F3/B3 primers described by Przewodowska et al. (2015) were used to test for PVY.

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The assays were also used to test individual samples from each farm. Both PSTVd and PVY were detected in four of seven samples from a farm in Embu, and six of 11 samples from a farm in Meru. Only one sample, from Embu, tested positive for single infection by PVY. One PSTVd RT-PCR product from each farm, was Sanger sequenced (MZ054165 and MZ054166) and had 98.06% and 98.31% nucleotide identity to MZ054164. The sample which tested positive only for PVY did not show any observable difference in symptoms compared with those which tested positive for both PSTVd and PVY (i.e. leaf malformation and mottling and veinal necrosis).

PSTVd has a broad experimental host range, including tree tomato in which it was reported to be symptomless (CABI, 2021; Singh, 1973). Further investigation is required to determine if PSTVd affects yield in tree tomato and to identify other reservoir hosts of the viroid so that mitigation measures can be applied.

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