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First report of *Ethiopian tobacco bushy top virus* and its associated satellite RNA infecting common bean (*Phaseolus vulgaris* L.) in Zambia

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During surveys for common bean viruses in Central Province of Zambia in April 2018, symptoms of bushy top, deep green curled branches and patchy leaf chlorosis were observed on five plants in a 2-ha farmer's field. Total RNA was isolated from symptomatic leaf samples using the CTAB method (Chang et al. 1993). The RNA from one sample (CP414-1) was used to construct a cDNA library with the Illumina TruSeq RNA Library Prep Kit (Illumina, San Diego, CA), followed by high-throughput sequencing (HTS) on the Illumina MiSeq platform that generated ~3.1M single-end raw reads of ~300 nucleotides (nt) each. A total of 355,885 reads showed hits to Ethiopian tobacco bushy top virus (ETBTV; *Umbravirus*), ETBTV satellite RNA (satRNA) and peanut mottle virus (PeMoV, *Potyvirus*) based on BLASTn analysis. The full-length genomes of ETBTV (4239-nt; MT225089), its satRNA (521-nt; MT225092) and PeMoV (9,643-nt) were assembled from the HTS reads using Geneious R11.1.2 (Biomatters, Auckland, New Zealand). The obtained complete genome sequences of ETBTV (MT225089) and ETBTV satRNA (MT225092) shared 88% and 95% nt identities, respectively with the corresponding viral (KJ918748) and satRNA (KJ918747) sequences of isolate 18-2 (Abraham et al. 2014). The near complete PeMoV genome was 89% identical to isolate Liaoning (MH270528). The HTS results were validated by two-step RT-PCR analyses of the five field-collected samples using newly designed primer pairs (data not shown). All five samples gave the expected 988-bp ETBTV-specific and 521-bp satRNA-specific DNA bands while three samples produced the expected 2100-bp PeMoV-specific fragment. The virus specificities of the agent specific PCR fragments were ascertained by Sanger sequencing (ETBTV: MT225090-91; ETBTV satRNA: MT225093-94; PeMoV: MT900843-44) and they shared 98-100% identities with their corresponding HTS-derived sequences. To further probe for the presence of an ETBTV helper virus, the samples were screened by RT-PCR with the

degenerate primer pair Lu1-mod-F/C2R3 that was modified from Robertson et al. (1991). The expected 245-bp DNA bands was obtained from all five samples, indicating the presence of a possible luteovirus or polerovirus target in these samples. The BLASTn analyses of the two Sanger sequenced gel-eluted products (MT900845-46) showed that they shared 100% identity with each other and 96% nt identity with cowpea polerovirus 1 (CPPV1, KX599163). Leaf tissue extracts from a common bean plant that was confirmed by RT-PCR to be positive for all four agents were rub-inoculated onto *Nicotiana occidentalis* and common bean (Sutter Pink) plants (n=5 each) at the three fully expanded leaf stage, with a buffer inoculation as control. Systemic foliar symptoms consisting of leaf deformation, stunting and leaf bushy top were observed on all ten plants, 10 days post-inoculation whereas the control plants remained symptomless. All the test plants were screened by RT-PCR as described above. The results showed that all five *N. occidentalis* plants were positive for ETBTV+ETBTVsatRNA, the five common bean plants tested positive for ETBTV+satRNA+PeMoV, and all 10 plants of both species were negative for CPPV1. To the best of our knowledge, this is the first report of ETBTV, ETBTV satRNA and CPPV1 infecting common bean in Zambia, and the first molecular based confirmation of PeMoV occurrence in the country. Ongoing studies are focused on determining the extent of the disease spread and assessment of its economic impact.

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