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Comparison of sequence-dependent and sequence-independent approaches for detecting sugarcane viruses in a plant quarantine context

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Implementation of sensitive and accurate methods is essential for the detection of viruses in asymptomatic plants. In this study, three diagnostic approaches were compared for detecting sugarcane viruses at the CIRAD sugarcane quarantine of Montpellier (Visacane): 1/ Sequence-dependent polymerase chain reaction (PCR) and reverse transcription (RT)-PCR, 2/ Sequence-independent virion associated nucleic acid (VANA)-based metagenomics with Illumina sequencing (VANA-Illumina), and 3/ Sequence-independent VANA-based metagenomics with Oxford Nanopore MinION sequencing (VANA-MinION). Ninety-six sugarcane samples were individually tested by PCR or RT-PCR for three known sugarcane-infecting viruses: sugarcane yellow leaf virus (SCYLV), sugarcane white streak virus (SWSV), and sugarcane mild mosaic virus (SCMMV). These 96 samples were subsequently distributed into 19 pooled samples of 4-6 individuals from a same geographical location, and processed using the two VANA approaches. SCYLV was detected by VANA-Illumina and VANA-MinION in six of seven pooled samples that contained at least one RT-PCR positive sample. SWSV tested positive by the two VANA approaches in six pooled samples with 1-5 PCR positive samples. Four of six pooled samples containing SCMMV RT-PCR positive samples tested also positive by VANA-Illumina and VANA-MinION. One of these six samples was positive by VANA-Illumina only and the remaining was negative using both VANA approaches. SCMMV was also detected by VANA-Illumina in one pooled sample that was considered free of SCMMV by RT-PCR. Fifty-three (93%) of the 57 detection results (19 pooled samples x 3 viruses) were identical regardless of the diagnostic approach. VANA-Illumina and VANA-MinION approaches also resulted in the detection of additional viruses not tested by PCR or RT-PCR, including sugarcane bacilliform virus and two unknown geminiviruses that were found by VANA-Illumina and VANA-MinION in five and two pooled samples, respectively. Metagenomics-based diagnosis of sugarcane viruses appears very promising although the sensitivity of this approach remains to be improved.

Mots clés: Sugarcane - Diagnostic - PCR - HTS.