



First report of cassava torrado-like virus, cassava polero-like virus and cassava new alphaflexivirus associated with cassava frogskin disease in Brazil

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Cassava (*Manihot esculenta* Crantz) plants with typical symptoms of frogskin disease (CFSD) have been observed since 2012 in Cruz das Almas, Bahia, Brazil. The above ground parts of the plants were asymptomatic, however, the roots showed typical symptoms of woody-like appearance, thickened cork-like peel, opaque aspect, and coalescent lip-like slits in a honeycomb pattern. Plants did not test positive for phytoplasma based on a nested-PCR reaction for the 16S region (Alvarez et al. 2009). Therefore, we hypothesized a viral infection. To verify our hypothesis, total RNA was extracted from 0.5 g of plant tissue using CTAB followed by reverse transcription and polymerase chain reaction with specific primers for: (i) cassava frogskin-associated virus – CsFSaV; (ii) ‘cassava torrado-like virus’ – CsTLV; (iii) ‘cassava polero-like virus’ – CsPLV and (iv) ‘cassava new alphaflexivirus’ – CsNAV, as described by Carvajal-Yepes et al. (2014). Amplicons were obtained for CsTLV (RNA1: 834 bp, RNA2: 719 bp), CsNAV (1227 bp) and CsPLV (1001 bp). No amplicons were obtained for CsFSaV. PCR products were sequenced in both directions, manually edited and deposited in the NCBI database: CsTLV (MN194209, MN194210 and MN194211) and RNA 2 (MN194212, MN194213 and MN194214), CsPLV (MN172358, MN207078 and MN207079) CsNAV (MN207080, MN207081 and MN207082). A BLASTn analysis of the viral sequences

showed 96–100% identity with CsTLV (RNA1 polyprotein: KC505250), CsTLV (RNA2 polyprotein: KC505251); CsPLV (RNA-dependent RNA polymerase and coat protein: KF885738) and CsNAV (replicase, TGB1, TGB2, and coat protein: KC505252 and KY288516), respectively (E-value <0.001). The three putative virus species (CsTLV, CsNAV and CsPLV) were described for the first time by Carvajal-Yepes et al. (2014) on association with CFSD together with CsFSaV in Colombia. To our knowledge this is the first report of these three viruses associated with CFSD in Brazil.

References

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