

Viral treasure hunt in European outermost territories: how metagenomics boosts the discovery of novel viral species in tropical and sub-tropical crops germplasm

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A comparative assay of 8 viral metagenomic protocols based on next generation sequencing (NGS) was performed on 6 tropical and subtropical crops (banana, garlic, sugarcane, sweet potato, vanilla and yam) originating from the Azores, Guadeloupe, Madeira and Réunion. Roche 454-based sequencing of purified dsRNA and virion-associated nucleic acids (VANA) proved the best strategies for recovering plant virus sequences following a first screening performed on a set of 96 plant samples. These two metagenomics approaches were further used on a total of 740 plant samples of the 6 targeted crops. Following demultiplexing, reads were either mapped against viral references or assembled into contigs and annotated using Blast analyses, leading to the identification and molecular characterization of 5 new viral species in garlic, 10 in yams, 7 in sugarcane, and 3 in vanilla. Sequences hinting at the possible presence of additional novel agents were also identified.

In addition, a systematic search was performed on publicly available EST resources for the 6 targeted crops in order to identify yet uncharacterized viral agents. For all newly identified viruses, corresponding EST sequences were downloaded, assembled in contigs and taxonomic relationships were established.

Sequences assembled from NGS reads and EST datamining were used to design specific primers for the detection of the novel viral species, allowing studies of their prevalence and diversity in germplasm collections conserved in European outermost regions and providing insights into transmission modes of some of these novel viruses.

The potential of metagenomics for plant virus discovery and diagnostics will be discussed, together with the risk of complicating the exchange of plant material following the discovery of new virus species in conserved plant germplasm.

Mots-clés : Virus, tropical germplasm, metagenomics, diversity, diagnostics.