

High-throughput sequencing (HTS) for detection and discovery of pineapple mealybug wilt associated viruses (PMWaV): comparison of five approaches

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HTS technology are now routinely used for virus discovery, but have yet to replace more conventional approaches for virus detection. Here, we compared five different HTS methods for virus detection: 1) ribo-depleted total RNA, 2) Double-stranded RNA (dsRNA), 3) Virus-derived small interfering RNAs (siRNA), 4) Virion-associated nucleic acids (VANA), and 5) total RNA with Oxford Nanopore Technologies (ONT) and MinION platform. Pineapples from Reunion Island, displaying wilt symptoms, were tested in order to properly assess the performance and application range of each method.

From the combination of all generated sequences, we were able to assemble nearly full genomes of viruses known to be associated with the Mealybug wilt disease of pineapple: Four distinct *Pineapple mealybug wilt-associated virus* species (PMWaV1, -2, -3 and -5) and the Pineapple bacilliform comosus virus (PBCoV) were characterized. Moreover, the genomes of a *Sadwavirus* and three new viruses belonging to the *Ampelovirus* (n=2) and *Vitivirus* (n=1) genera were also fully assembled.

The comparison of the detection of each virus, using each tested method proves that all were suitable for the detection of all viruses, providing a sufficient coverage depth. The results showed that the ribo-depleted total RNA appears to be the best strategy to obtain nearly complete genomes, whether for RNA or DNA viruses, with or without poly-A tail. Conversely, total RNA with ONT failed for the complete reconstruction of most viral genomes.

Globally, the discovery of new viruses proves the importance of diagnostic HTS tests and their inclusivity in understanding disease etiology and further highlight the diversity of viruses associated with pineapple.