

## Diseases Caused by Viruses

### First Report of Three Pineapple Mealybug Wilt-Associated Viruses in Queen Victoria Pineapples in Reunion Island

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Mealybug wilt of pineapple is one of the most destructive diseases of pineapple (*Ananas comosus*) in the world (Sether et al. 2005). Viruses in three distinct species of the genus *Ampelovirus* (family *Closteroviridae*), *Pineapple mealybug wilt-associated virus-1*, *virus-2*, and *virus-3*, have been identified in several pineapple-growing regions worldwide such as Hawaii (Hu et al. 1993), Australia (Wakman et al. 1995), Central and South America (Boroto et al. 1998), Ecuador (Alvarez et al. 2015), and recently West Africa (Nyarko and Asare-Bediako 2019). The 'Queen Victoria' cultivar is the most widely cultivated pineapple in Reunion Island and is the main fruit crop exported. From October 2016 to February 2018, leaves from four pineapples (cv. Queen Victoria) from four different plots showing symptoms of wilt disease were collected in Reunion Island. Three sets of primers were used in reverse transcription polymerase chain reaction (RT-PCR) for the specific detection of members of each virus species: PMW1dF/R for pineapple mealybug wilt-associated virus-1 (PMWaV-1) (Gambley et al. 2009), PMWaV2-223/224 for PMWaV-2 (Sether et al. 2005), and Wilt3dF/R for PMWaV-3 (Gambley et al. 2009). Primer sets were designed from the RdRp genes for PMWaV-1 and -3, and from the HsP70 gene for PMWaV-2. Expected DNA fragments of 303, 610, and 424 nucleotides (nt) were obtained from the four samples for PMWaV-1 and -2,

and from one sample for PMWaV-3, respectively. The fragments were directly sequenced in both directions, assembled, and analyzed (Geneious version R11.1.2). The four consensus sequences (accession nos. MT447832 to 835), obtained from the 303-nt DNA fragments, shared 91.6 to 95.8% nt and 95.5 to 97% amino acid (aa) identities with Hawaiian (AF414119, MH704740) and Australian (EF467924, EF467925, and EF463006) isolates of PMWaV-1. The four consensus sequences (MT469951 to 954), obtained from the 610-nt DNA fragments, shared 98.3 to 99.8% nt and 97.7 to 100% aa identities with Hawaiian isolates of PMWaV-2 (AF283103, MH704741). The single consensus sequence (MT469955), obtained from the 424-nt DNA fragments, shared 96 to 97.3% nt and 100% aa identities with Hawaiian (DQ399259, MH704742) and Australian (EF467918) isolates of PMWaV-3. To further confirm the presence of members of PMWaV-1, -2, and -3 in the four pineapple samples, three specific primer sets designed from the coat protein (CP) genes were used (FJ08-1/2 [Shen et al. 2009] CP229/CP230, and CP231/CP232 [Hernandez-Rodriguez et al. 2014], respectively, for each virus). Amplicons of the expected sizes were obtained from the four samples for PMWaV-2, and from two samples for PMWaV-1 and -3. The direct sequencing of the amplicons confirmed the previous results. The consensus sequences of the CP of PMWaV-1 (MT990947 to 948), PMWaV-2 (MT990949 to 952), and PMWaV-3 (MT990953 to 954) shared 98 to 100% nt and 99 to 100% aa identities with several isolates of the viruses from Hawaii (MH704740 to 742, MN539274), Taiwan (LC507819), and Cuba (DQ225114). To our knowledge, this is the first report of PMWaV-1, -2, and -3 on wilt diseased pineapples in Reunion Island. The spread of this ampelovirus complex in the pineapple fields of Reunion represents potentially a major threat for this agricultural export sector.

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