New Disease Reports

First report of *Pineapple mealybug wilt associated virus-3* infecting pineapple in Cuba

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Pineapple (*Ananas comosus*) is a common crop in tropical and subtropical areas of the world. Crop yields are seriously affected by mealybug wilt of pineapple (MWP), a viral disease with mealybugs (*Dysmicoccus* spp.) as vectors (Sether *et al*., 2005). Viruses associated with MWP are members of the genus *Ampelovirus*, family *Closteroviridae*. In Hawaii, *Pineapple mealybug wilt-associated virus*- (PMWaV-1) infection has been correlated with 5 to 15% of ratoon crop yield reduction and losses of up to 30% of production due to premature or asynchronous fruit ripeness. However, in that region, the most widespread virus species is PMWaV-2, which causes up to 100% fruit loss (Sether & Hu, 2002). Conversely, PMWaV-2 is uncommon in Australia, a country where MWP also causes major reduction of pineapple fruit yield. In Australia, MWP symptoms are strongly correlated with infections by PMWaV-3 alone or by both PMWaV-1 and -3 (Gambley *et al*., 2008). Due to the high nucleotide identity and the conserved genome organization between PMWaV-1 and PMWaV-3, it is considered that these two viruses have similar deleterious effects on either growth rate or pineapple fruit yield in Hawaii (Sether *et al*., 2005, 2009). Currently, there are 5,310 ha of pineapple orchards in Cuba, with annual fruit production that reached 28,908 tonnes in 2009. MWP disease is an economic problem for pineapple production in the island, causing up to 40% crop losses (Anonymous, 1989). PMWaV-2 was first detected in a diseased pineapple plant from Ciego de Avila in 1998, with molecular characterisation further provided by Borroto-Fernández *et al*., (2007).

During a survey for PMWaVs in 2009, thirty pineapple plants showing typical symptoms of MWP (foliar reddening, leaves with tips curved down and dieback) were collected in the Island of Youth, western region of the country (Fig. 1). Total RNA was extracted using the *TRIzol LS Reagent* kit (Invitrogen, Scotland, UK). RT-PCR assays for PMWaV-1, PMWaV-2 and PMWaV-3 detection were performed using the 225/226, 223/224 and 263/264 primer pairs, respectively; these amplify fragments corresponding to the HSP70h protein ORF of each viral species (Sether *et al*., 2005). Fragments of expected size for PMWaV-2 (c. 610 bp) and PMWaV-3 (c. 490 bp) were simultaneously amplified from seventeen plants with appropriate symptoms. Amplicons corresponding to PMWaV-1 were not obtained. DNA bands were purified, ligated to pGEM®-T Easy vector (Promega, Madison, USA) and two individual clones derived from each virus per infected plant were sequenced. PMWaV-3 derived amplicons shared ≥ 98% sequence identity with each other, and a representative sequence was deposited in GenBank (PMWaV-3 Cu, Accession No. GU563497). Sequence comparisons showed the closest nucleotide identity (98%) to PMWaV-3 from Hawaii (DQ399259). On the other hand, a representative sequence of PMWaV-2 Cu amplicons (FN825676) showed the closest nucleotide identity (99%) to PMWaV-2 from Taiwan (EU769115.1) and Thailand (EU016675.1). Phylogenetic analysis based on the PMWaV-3 amplicon sequence of Cuban and reference closteroviruses, grouped PMWaV-3 Cu and PMWaV-3 isolates from Hawaii and Thailand within the same phylogenetic cluster (Fig. 2). The phylogenetic tree also supported the sequence divergence of the cluster composed by PMWaV-3 and PMWaV-1 from that of PMWaV-2Hw, GLRaV-1 and GLRaV-3 (type member of the genus *Ampelovirus*) as previously observed (Sether *et al*., 2009). Gathering all these data, this is the first report of the presence of the PMWaV-3 in Cuban pineapple fields and in the Caribbean basin.

Noteworthy is that pineapple plants affected by MWP were infected by both PMWaV-3 and PMWaV-2 which suggests that a complex of ampeloviruses may be widespread in Cuban pineapple fields. Results support the need to implement certification procedures for pineapple propagation materials to reduce the economic impact of MWP disease on pineapple crops in Cuba.

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Figure 1

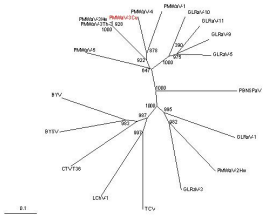

Figure 2

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