New Disease Reports

First report of *Pineapple bacilliform comosus virus* (PBCoV) and *endogenous Pineapple pararetrovirus*-1 (ePPRV-1) in pineapple plants in Cuba

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Mealybug wilt of pineapple (MWP) is considered one of the most destructive diseases of pineapple (*Ananas comosus*) crops worldwide. MWP have been related to five species of *Pineapple mealybug wilt-associated virus*-1 to 5 (PMWaV-1 to 5) (Closteroviridae: *Ampelovirus*), but an interaction with badnaviruses (Caulimoviridae: *Badnavirus*) has not been completely discarded (Sether *et al*., 2012). In Cuba, where MWP has been associated with 40% of crop losses, only PMWaV-2 and PMWaV-3 have been detected (Borroto *et al*., 2007; Hernandez *et al*., 2010a, b), and there is no evidence of badnavirus presence in the country. During a survey for badnaviruses infecting pineapple cv. Red Spanish in commercial fields in 2009-2011, 78 pineapple plants showing MWP symptoms (foliar reddening, leaves with tips curved down and dieback) were collected for further testing. Two pineapple plants cv. Red Spanish derived from *in vitro* apical meristem tissue culture were selected as negative controls for the PCR assays. Total nucleic acid was extracted from leaf samples according to Murray & Thompson (1980) and badnavirus sequences were amplified in PCR assays using the Badna-1A/Badna 4 degenerate primers as described (Gambley *et al*., 2008).

Fragments of the expected size (ca. 544 bp) were amplified from DNA extracts from forty symptom-bearing plants. However, PCR products of the same size were also obtained when DNA extracts from the two *in vitro* cultured plants (BCuL30 and BCuL31) were tested. Amplicons from the extracts of these two plants and from four randomly chosen MWP symptom-bearing plants (BCuL26 to 29) were selected for further characterisation. DNA fragments were gel-purified, cloned and four individual clones per infected plant sample were sequenced. Six sequences of 540 nt were recovered and showed nucleotides identities that ranged from 52.2 to 93.5% (Table 1), except for BCuLE30 and BCuLE31 that were identical to each other. BCuL26 to BCuL29 sequences were deposited in GenBank (Accession Nos. JQ390618 to JQ390621) and showed at least 80.1% nucleotide identity with *Pineapple bacillliform comosus virus* (PBCoV-Au) isolated in Australia (EU377664). On the other hand, the sequence of the amplicons isolated from BCuLE30 and BCuLE31 (JQ390622) showed nucleotide identities between 91.7 and 95.6% with isolates of *endogenous Pineapple pararetrovirus*-1 (ePPRV-1) from Australia and Hawaii (EU377674 and GQ395780). The obtained sequences confirmed the presence of PBCoV and ePPRV-1 in pineapples from Cuba. Phylogenetic analysis grouped BCuL26 to BCuL29 sequences and PBCoV within the same phylogenetic cluster, while BCuL30 grouped in an individual branch with ePPRV-1 supporting the previous results (Fig. 1). This is the first report of the PBCoV and ePPRV-1 presence in the Caribbean basin, results that highlight the need to implement certification procedures for the propagation material to reduce the risk of viral diseases affecting pineapple crop.

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References

Borroto-Fernández EG, Torres-Accosta JA, Laimer M, 2007. RT-PCR detection and protein-protein interaction of viral components of *Pineapple mealybug wilt-associated virus 2* in Cuba. *Journal of Plant Pathology* **89**, 435-439.

Gambley CF, Geering ADW, Steele V, Thomas JE, 2008. Identification of viral and non-viral reverse transcribing elements in pineapple (*Ananas comosus*), including two new badnavirus species. *Archive of Virology.* **153**, 1599-1604. [http://dx.doi.org/10.1007/s00705-008-0160-4]

Hernández L, Ramos PL, Rodríguez M, Peña I, Pérez JM, 2010a. First report of Pineapple mealybug wilt associated virus-3 infecting pineapple in Cuba. *New Disease Reports* **22**, 18. [http://dx.doi.org/10.5197/j.2044-0588.2010.022.018]

Hernández L, Ramos PL, Rodríguez M, Zamora V, Peña I, Pérez, JM, 2010b. Detección de ampelovirus asociados a la enfermedad de la marchitez de la piña (Ananas comosus (L.) Merr.) en la Isla de la Juventud, Cuba. [Detection of ampeloviruses associated to the mealybug wilt of pineapple (*Ananas comosus* (L.) Merr.) disease in the Island of Youth, Cuba.] *Revista CitriFrut* **27**, 39-46.

Murray MG, Thompson WF, 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* **8**, 4321-4325. [http://dx.doi.org/10.1093/nar/8.19.4321]

Sether DM, Melzer MJ, Borth WB, Hu JS, 2012. Pineapple bacilliform CO virus: Diversity, detection, distribution, and transmission. *Plant Disease* **96**, 1798-1804. [http://dx.doi.org/10.1094/PDIS-08-11-0718-RE]

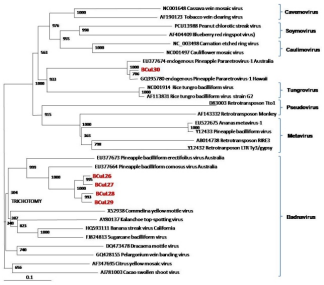

Figure 1

Table 1. Nucleotide identity percentages obtained by the comparison of badna1A/badna4 amplicon sequences recovered from DNA extracts of Cuban pineapples (BCuL26 to 30) and the homologue sequences of PBCoV-Au and *Pineapple bacilliform erectifolius virus* (PBErV) and ePPRV-1.

Virus isolate	BCuL26	BCuL27	BCuL28	BCuL29	BCuL30	PBCoV-Au	PBErV-Au	ePPRV-I-Au
BCuL27	93,5							
BCuL28	93,2	88,8						
BCuL29	91,2	90,5	91,8					
BCuL30	58,5	55,7	56,9	55,2				
PBCoV-Au	81,3	81,8	81,8	80,7	54,7			
PBErV-Au	73,2	72,1	72,5	72,3	52,7	71,5		
ePPRV-1-Au	56,8	56,2	54,0	54,8	95,6	55,0	53,5	
ePPRV-1-Hw	57,0	56,8	55,2	56,3	91,7	55,5	52,7	94,6

Figure 2

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