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

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Received: 28 Feb 2013. Published: 04 Aug 2013.

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 nucleotide identities that ranged from 52.2 to 93.5% (Table 1), except for BCuL30 and BCuL31 that were identical to each other. BCuL26 to BCuL29 sequences were deposited in GenBank (Accession Nos. JQ390618 to JQ390621) and were identical to each other. BCuL30 and BCuL31 sequences were deposited in GenBank (Accession Nos. EU377664). On the other hand, the sequence of the amplicons isolated from BCuL30 and BCuL31 (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 pineapple 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.

**Acknowledgements**

This research was supported in part by the grant C/5032-1 conceded to L. Hernandez-Rodriguez by the International Foundation for Science, Sweden.

**References**


**To cite this report:** Hernandez-Rodriguez L, Ramos-Gonzalez PL, Garcia-Garcia G, Javer Higginson E, Zamora-Rodriguez V, 2013. First report of *Pineapple bacilliform comosus virus* (PBCoV) and endogenous *Pineapple pararetrovirus-1* (ePPRV-1) in pineapple plants in Cuba. *New Disease Reports* 28, 2. [http://dx.doi.org/10.5197/j.2044-0588.2013.028.002] ©2013 The Authors This report was published on-line at www.ndrs.org.uk where high quality versions of the figures can be found.

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