



Tomatoes showing yellow leaf curl symptoms in the island of Grenada exhibit an infection with *Tomato yellow leaf curl virus* either alone or in combination with *Potato yellow mosaic virus*

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In March 2007, severe symptoms of leaf curling and yellowing resembling tomato yellow leaf curl disease were observed on tomato (*Solanum lycopersicum*) plants with a very high incidence in six sites on Grenada Island (Fig. 1; Table 1). Eleven leaf samples from tomato presenting the strongest symptoms were collected. Samples were tested for the presence of begomoviruses using polymerase chain reaction (PCR) assay with sets of degenerate primers designed to amplify parts of the DNA-A and DNA-B components (Table 1; Delatte *et al.*, 2005; Rojas *et al.*, 1993). PCR products of the expected sizes, obtained with all DNA-A and DNA-B sets of primers for nine and three tomato samples, respectively, suggested the presence of Old World monopartite and New World bipartite begomoviruses. The nine partial DNA-A PCR products obtained with primers FD382-RD1038 were cloned and sequenced (EMBL-GenBank-DDBJ Accession Nos. FM163453 to FM163459, FM163462, FM163463). The highest nucleotide identity of 99% (BLASTn, NCBI) was obtained with the Old World monopartite *Tomato yellow leaf curl virus*-Israel (TYLCV-IL) isolates from Caribbean Islands (EF490995, AF024715). Similarly, the three partial DNA-B sequences obtained with primers PBL1V2040-PCRC1 (FM163460, FM163461 and FM163464) shared the highest nucleotide identity of 96% with the New World bipartite *Potato yellow mosaic virus*-Trinidad [Trinidad & Tobago] (PYMV-TT[TT], AF039032) DNA-B.

To confirm the molecular characterisation of the begomoviruses, full-length viral genomes were amplified from two PCR-positive samples (Table 1) by rolling-circle amplification, cloned using a set of restriction enzymes and sequenced (Inoue-Nagata *et al.*, 2004). The complete DNA-A genome sequences obtained with *NcoI* (FR851297, FR851298), with 100% nucleotide identity, showed the highest sequence identity of 99% with isolates of TYLCV-IL ([Texas], AF039032; [Puerto Rico], AF039032). The complete DNA-A and DNA-B genome sequences obtained with *Sall*, *BamHI* and *EcoRI* (FR851299 to FR851302) showed the highest sequence identity of 96% with the Trinidad & Tobago strain of PYMV DNA-A and DNA-B (AF039031 and AF039032, respectively; Umaharan *et al.*, 1998). The phylogenetic reconstruction with publicly available complete genome sequences confirmed the relationship of Grenada isolates of TYLCV-IL with the isolates from the United States, the Caribbean Islands and Central America, and of PYMV with the unique isolate of TT strain described in Trinidad & Tobago (Fig. 2).

To our knowledge, this is the first report of the Old World TYLCV and the New World PYMV implicated in yellow leaf curl disease on tomato in

Grenada. This description confirms the invasion of the Lesser Antilles in the Caribbean from north to south by the Israel strain, also called the "severe" strain of TYLCV. The proximity between the island of Grenada and South America, where the "severe" strain of TYLCV has never been described, to our knowledge represents a new occurrence of first importance for the regional management of emerging crop diseases and regulatory institutions.

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Figure 1

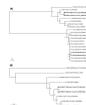


Figure 2

Accession	Host	Year	Location	Genome Type	TYLCV-IL (%)	PYMV (%)
EF490995	Tomato	2005	Caribbean Islands	Monopartite	99	0
AF024715	Tomato	2005	Caribbean Islands	Monopartite	99	0
FM163453	Tomato	2007	Grenada	Monopartite	99	0
FM163459	Tomato	2007	Grenada	Monopartite	99	0
FM163462	Tomato	2007	Grenada	Monopartite	99	0
FM163463	Tomato	2007	Grenada	Monopartite	99	0
AF039031	Tomato	1998	Trinidad & Tobago	Bipartite	0	96
AF039032	Tomato	1998	Trinidad & Tobago	Bipartite	0	96

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