



First report of *Tomato leaf curl New Delhi virus* and the whitefly *Bemisia tabaci* Asia1 species on tomato in the Seychelles

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In July 2017, severe symptoms of leaf curling resembling those of tomato leaf curl disease and outbreaks of whiteflies (more than 50 individuals per plant) were observed on tomato (*Solanum lycopersicum*) plants in open fields and in greenhouses on the island of Praslin in the Seychelles (Fig. 1). Symptomatic tomato leaf samples and whiteflies were collected (Table 1).

Three tomato leaf samples were tested for the presence of begomoviruses using PCR assays with a set of degenerate primers designed to amplify the coat protein gene of the Old World begomovirus DNA-A component (Clust4CP-F342/Clust4CP-R1032; Séka *et al.*, 2016). PCR products of the expected size were obtained from one sample, suggesting the presence of an Old World begomovirus. To characterise the complete genome, DNA-A and -B molecules were amplified by rolling-circle amplification, cloned using *EcoRV* and *PstI* restriction enzymes and sequenced. BLASTn analysis showed that the DNA-A sequence (2,740 nucleotides; GenBank Accession No. MH511991) shared the highest nucleotide identity (99%) with potato isolates of *Tomato leaf curl New Delhi virus* (ToLCNDV; KC874503 and AY286316) from India. The DNA-B sequence (2,695 nucleotides; MH511990) shared the highest nucleotide sequence identity (99%) with a potato isolate of *Tomato leaf curl New Delhi virus* (ToLCNDV; FN356024) also from India. Maximum-likelihood phylogenetic trees constructed with publicly available begomovirus sequences (MEGA6; Tamura *et al.*, 2013), confirmed the genetic relationship of the Seychelles isolate of ToLCNDV with isolates from India (Fig. 2). PCR diagnosis performed with specific primers designed to amplify the coat protein ORF of ToLCNDV DNA-A and direct sequencing of amplicons confirmed the presence and the identity of ToLCNDV in two tomato samples from two different plots (Table 1). In parallel, total DNA was extracted from 20 female whiteflies (Table 1), to identify their species. The core region of the mitochondrial cytochrome oxidase I gene was amplified and sequenced with the general primer set C1-J-2195 and L2-N-3014 (Simon *et al.*, 1994). Sequences 755 bp in length were obtained. A single haplotype was retrieved and showed 100% pairwise sequence identity (BLASTn) with Thai isolates of Asia1 *B. tabaci* species (KR110108).

To our knowledge, this is the first report of the bipartite Old World ToLCNDV associated with leaf curl disease on tomato in the Seychelles and more widely in sub-Saharan Africa. Despite a viral description based on a small number of symptomatic tomato samples from the same locality and the lack of information regarding the outbreak and natural occurrence of ToLCNDV on tomato crops in the Seychelles, this description confirms

the spread of ToLCNDV outside the Indian sub-continent. The concomitant description of ToLCNDV and *B. tabaci* Asia1 species in the Seychelles is consistent with the fact that both are considered native to Asia (Moriones *et al.*, 2017, and Rekha *et al.*, 2005; respectively). This report is of prime importance for regional management of emerging diseases on vegetable crops since ToLCNDV causes diseases in a large number of plant species belonging to different families including the Cucurbitaceae, Fabaceae, Malvaceae and Solanaceae (Moriones *et al.*, 2017).

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Figure 1

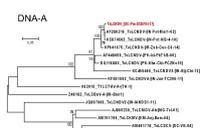


Figure 2

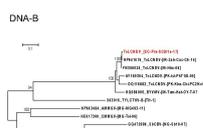


Table 1. Origin of tomato leaf samples and *Bemisia tabaci* whiteflies sampled on tomato in Praslin Island (Seychelles) and results of PCR testing. Each leaf sample was collected on a different plant and plot; whiteflies were collected from different plants from the same plot.

Sample ID	Locality	Date of sampling (day/month/year)	GPS coordinates	PCR diagnosis
Tomato - SC100	Baie Sainte-Anne	26/07/2017	4°18'46.83S 55°41'09.99E	ToLCNDV-DNA-A ¹
Tomato - SC10	Baie Sainte-Anne	26/07/2017	4°18'46.83S 55°41'10.00E	ToLCNDV-DNA-A ¹
Tomato - SC16	Baie Sainte-Anne	26/07/2017	4°19'46.84S 55°41'10.97E	-
Bemisia - BE01	Baie Sainte-Anne	23/07/2017	4°18'47.55S 55°41'13.72E	ASIA1 ²

¹Sample from which a complete genome of *Tomato leaf curl New Delhi virus* has been cloned and sequenced.
²Degenerate primers designed to detect Old World begomoviruses: Clust4CP-F342 / Clust4CP-R1032 (Séka *et al.*, 2016).
³Specific primers designed to detect ToLCNDV DNA-A: F314-ToLCNDV-A (5'-TCTAGGCGCAGATGCTCTCTC-3') R1170-ToLCNDV-A (5'-CAGGATACCTAAGGACCTCT-3').
⁴DNA barcoding based on partial sequencing of mitochondrial cytochrome c oxidase subunit I (COI) gene after PCR amplification with the primer set C1-J-2195 and L2-N-3014 (Simon *et al.*, 1994).

Figure 3

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