



First report of citrus bent leaf viroid from Morocco

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Abstract

Citrus is one of the most economically important fruit crop in Morocco, with a total production of 2.2 million tonnes during 2016/17. Citrus bent leaf viroid (CBLVd) from the *Pospiviroidae* family and the *Apscaviroid* genus is one of the widely distributed citrus viroids, with no specific symptoms associated with its infection but responsible for leaf bending on Etrog citron. Najar et al. *Phytophathol Mediterr* 56: 409–420, (2017) reported that CBLVd affected, in mixed infection with other citrus viroids, the canopy volume and fruit quality of the Tunisian citrus cultivar Maltaise demi sanguine. In summer 2013, a field survey for the presence of CBLVd was conducted in commercial orchards of the Gharb region of Morocco. Twenty five samples without symptoms of viroid infection were randomly collected from one citrus orchard of Common Clementine (*Citrus clementina* hort. ex Tanaka). The rootstocks of these samples were not known. Total RNA were extracted from leaves around the tree canopy using RNeasy Plant Mini Kit (Qiagen, Germany) and assayed by reverse-transcription polymerase reaction chain (RT-PCR) with two sets of primers, CBLVd-F1/ CBLVd-R1 (Bernard and Duran-Vila 2006) and YI4F/YI4R (Khoo et al. *J PI Pathology* 99: 293, 2017); amplicons of the expected sizes (327 bp and 234 bp, respectively) were obtained from 15 samples. The resulting amplicons of 327 bp were sequenced in both directions and three sequences were deposited in GenBank under accession numbers MH200818, MH200819 and MH200820. Nucleotide blast analysis indicated 99% sequence identity with the Japanese isolate P2 (AB006735; Hataya et al. *Arch Virol* 143: 971–980, 1998), confirming the presence of CBLVd in the tested samples. The neighbour-joining phylogenetic analysis showed that the Moroccan CBLVd isolates were clustered in the same group as shown in Fig. 1. To the best of our knowledge, this study represents the first report of CBLVd in Morocco. Additional investigations on the occurrence and geographical distribution are being undertaken in Morocco's main citrus growing areas.

Keywords Citrus bent leaf viroid · Citrus · Morocco

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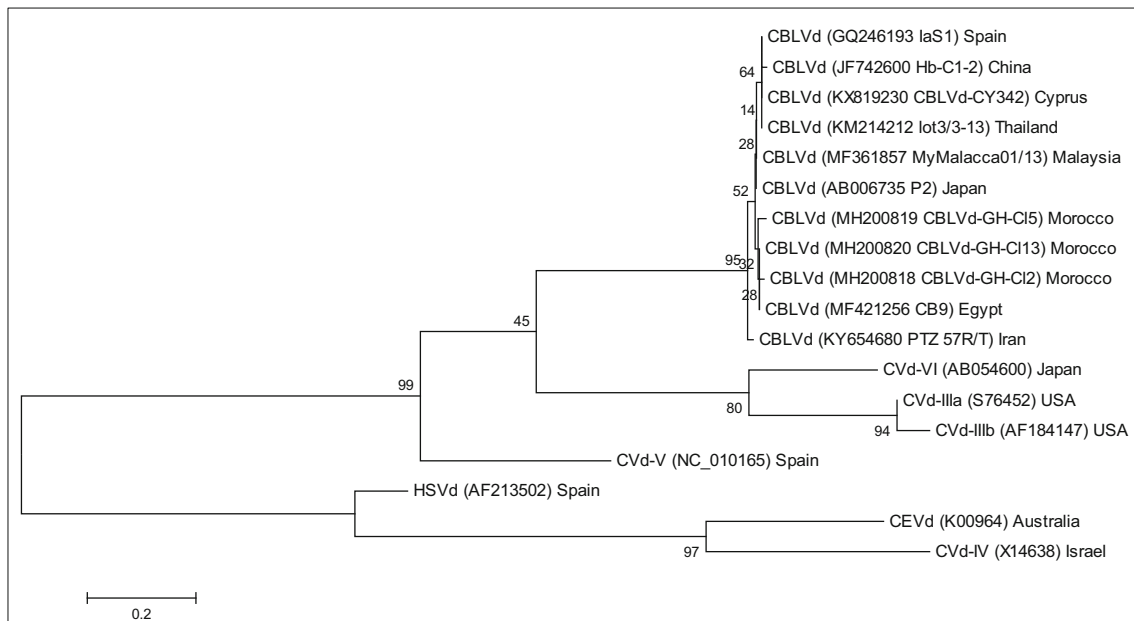


Fig. 1 Neighbor-joining phylogenetic tree of three Moroccan CBLVd isolates with various CBLVd isolates and other citrus viroids whose sequences are available in the NCBI database. The tree was generated and visualized using Mega 5.05 program (Tamura et al. 2011). Bootstrap replicates values (percentage) are indicated at the nodes. Scale bar

represents genetic distance. CVd-IV (Citrus viroid IV), CEVd (Citrus exocortis viroid), HSVd (Hop stunt viroid), CVd-V (Citrus viroid V), CVd-IIIa (Citrus viroid IIIa), CVd-IIIb (Citrus viroid IIIb), CVd-VI (Citrus viroid VI)

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