**Disease Note**

# FIRST REPORT OF CITRUS BENT LEAF VIROID IN MALAYSIA

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Citrus is among eight major fruit crops grown in Malaysia, with a total production of 36,450 tons in 2013. Citrus bent leaf viroid (CBLVd, genus *Apscaviroid*, family *Pospiviroidae*), is widely distributed in citrus with no specific symptoms associated with its infection but responsible for leaf bending on Etrog citron. Leaf samples of citrus species such as calamondin [*Citrofortunella microcarpa* (Bunge) Wijnands], kaffir lime (*Citrus hystrix* DC.), key lime [*C. aurantifolia* (Cristm.) Swingle], mandarin orange (*C. reticulata* Blanco), pomelo (*C. maxima* Merr.), sweet orange [*C. sinensis* (L.) Osbeck] with stunting, leaf yellowing and epinasty were collected from different states in Malaysia. The rootstocks of these samples were not known. Total nucleic acids were extracted from leaves around the tree canopy and tested by RT-PCR with two sets of primers, CBLV-CM/CBLV-CP (Ashulin *et al*., 1991) and the newly designed YI4F/YI4R (5'-CGGAGACTTCTTGTGGTTCC-3' and 5'-CTTGGAAGTCCGCTCGACTA-3', respectively). Altogether 21 of 133 citrus samples were positive for CBLVd. The resulting amplicons of 328 bp and 234 bp in size were cloned. Sequence analysis revealed 95-99% identity with CBLVd isolate Jp (accession No. AB006734), confirming the presence of CBLVd (KU194472, KX823338-KX823343) in the tested samples. No particular symptoms were observed in the test samples correlated with the presence of CBLVd, thus the observed symptoms may not be induced by this viroid. To the best of our knowledge, this is the first report of CBLVd in Malaysia. Additional investigations on geographical distribution, epidemiology and economic impact are essential to formulate CBLVd management strategies in Malaysia.

Ashulin L., Lachman O., Hadas R., Bar-Joseph M., 1991. Nucleotide sequence of a new viroid species, citrus bent leaf viroid (CBLVd) isolated from grapefruit in Israel. *Nucleic Acids Research* **19**: 4767.

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# *CANDIDATUS* LIBERIBACTER ASIATICUS CAUSING CITRUS HUANGLONGBING ON *CITRUS SINENSIS* IN BANGLADESH

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Citrus huanglongbing (greening) is one of the important diseases of citrus in Asia (Bové, 2006). The symptoms include blotchy chlorosis and/or mottling of the leaves, stunted growth, poor root growth, small, green, and malformed fruits and decline of the trees. The disease is caused by the phloem-limited unculturable fastidious bacteria *Candidatus* Liberibacter asiaticus (CLas), *Ca.* L. africanus (CLaf), and *Ca.* L. americanus (CLam) (Bové, 2006; Jagoueix *et al*., 1994). Among them CLas is the most widespread (e.g., Asia, Brazil and North America). In recent years the presence of huanglongbing was suspected in different regions of Bangladesh. To establish whether this is the case, in July, 2016 symptomatic plant samples of *Citrus sinensis* were collected from four citrus-growing areas of the country: Jaintapur (Sylhet), Akbarpur (Moulvibazar), Dighinala (Khagrachari) and Hathazari (Chittagong). The samples were analyzed by polymerase PCR using the 'CLas' specific primers A2 (5'-TATAAAGGTTGACCTTTCGAGTTT-3') and J5 (5'-ACAAAAGCAGAAATAGCACGAACAA-3') which amplify a partial sequence of the β-operon (*rpl*KAJL-*rpo*BC operon) of ribosomal protein genes (Hocquellet *et al*., 1999). A product 703 bp in size specific to *Ca.* L. asiaticus was amplified, sequenced and deposited in GenBank (accession No. KX826950). The nucleotide sequence of the recovered CLas shared 100% sequence identity with other *Ca.* L. asiaticus strains from NCBI database (e.g. KC477384, KT164844, KC133065). To our knowledge this is the first molecular-based detection of *Candidatus* Liberibacter asiaticus infecting *Citrus sinensis* in Bangladesh.

Bové J.M., 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. *Journal of Plant Pathology* **88**: 7-37.

Hocquellet A., Toorawa P., Bové J.M., Garnier M., 1999. Detection and identification of the two *Candidatus* Liberobacter species associated citrus huanglongbing by PCR amplification of ribosomal protein genes of the operon. *Molecular and Cellular Probes* **13**: 373-379.

Jagoueix S., Bové J.M., Garnier M., 1994. The phloem-limited bacterium of greening disease of citrus is a member of the subdivision of the proteobacteria. *International Journal of Systematic Bacteriology* **44**: 379-386.

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