SUMMARY ARTICLE
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Molecular Detection of Citrus Greening Causal Bacterium Affecting on Mandarin in Bangladesh

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Citrus greening, also known as huanglongbing, is a serious disease that infects most of the citrus cultivars and causes enormous loss to the citrus industry worldwide. A fastidious, unculturable, gram-negative bacterium is responsible for this biotic stress that impedes nutrient and sugar transport from roots to leaves by blocking phloem. As a result, plant experiences a critical scarcity of its essentials and leaves become yellow with blotchy mottling symptom (Figure 1) that is not uniform across the leaf, leaves drop, shoots become stunted, and the branches gradually start to dieback. Three species were identified previously - *Candidatus Liberibacter asiaticus* (Las), widespread in Asia and North America is the most catastrophic of them to citrus cultivation. It has an unusual mode of transmission and spread; by infecting citrus seedling through vegetative propagation (grafting). The sap-sucking insect vector Asian citrus psyllid, adds to this abiotic stress thereby making it difficult to control them by all available traditional measures. Detection of the disease is not easy by applying conventional techniques like electron microscopy, bioassay on indicator plants, or ELISA, serological techniques, and dot-blot DNA hybridization. In the case of visual inspection, it can be easily confused with a nutrient deficiency symptoms. Polymerase chain reaction (PCR) based detection is the most reliable and accepted for citrus greening confirmation.

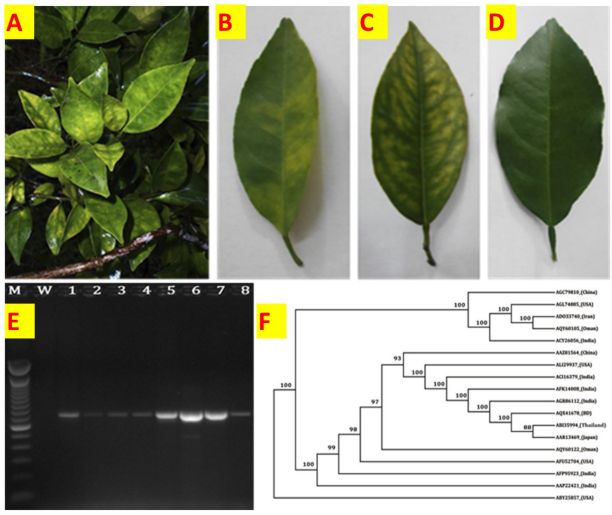

Figure 1. A) Field symptoms of citrus greening (Huanglongbing) of mandarin caused by *Candidatus Liberibacter asiaticus*, B) Citrus greening showing yellowing and mottling of the leaf, C) Yellowing of leaf due to nutrient deficiency, D) Healthy leaf, E) Electrophoresis on 1.2% agarose gel of DNA amplified with primers A2/J5 from water (lane W), mandarin orange infected with *Candidatus Liberibacter asiaticus* (lane 1, 2, 3, 4, 5, 6, 7, 8), Lane M: 100 bp ladder (Invitrogen, Carlsbad, CA, USA), F) Phylogenetic tree based on translated amino acid sequences of ribosomal protein genes of β- operon showing the positions of Bangladeshi isolate and representatives of *Candidatus*

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liberibacter asiaticus strains available in the GenBank.

To identify and confirm the citrus greening infection, symptomatic leaf samples were collected from three citrus growing regions of Bangladesh. Genomic DNA was extracted from leaf midribs following the standard protocol of extracting bacterial DNA from plant tissue. In this study, a PCR protocol was described with minor modification of previously published literature. Species-specific primer pairs (A2/J5) were used to identify *Candidatus Liberibacter asiaticus* targeting the ribosomal protein genes of the *rpl*KAJL-*rpo*BC operon (β-operon) with expected amplicon size of Las-specific DNA. A partial sequencing was done through outsourcing and sequence data were deposited to NCBI (National Center for Biotechnology Information). The sequence data were compared with other Las sequences available in the GenBank database and phylogenetic relationship was determined.

An asymmetric pattern of yellowing in leaves is a typical preliminary identifying character for the detection of citrus greening (Figure 1). Further detection by PCR with Las-specific primers followed by gel electrophoresis produced an amplicon size of about 703 bp which confirmed the *Candidatus Liberibacter asiaticus* infection in studied areas (Figure 1). When BLAST (Basic Local Alignment Search Tool) homology was compared, partial sequence data (GenBank accession # KX822011) of ribosomal protein gene showed 99% similarity with other available sequences in the GenBank database. Constructed phylogenetic tree from translated protein indicated a close relationship of studied isolate with Indian, Thai, and Japanese isolates (Figure 1). It can be suggested that the causal bacteria might be introduced by infected materials from where Bangladesh imported new germplasm for varietal development. These findings confirmed the existence of *Candidatus Liberibacter asiaticus* as a causal pathogen of citrus greening on mandarin and revealed that the root cause of the citrus decline was not due to micronutrient deficiencies or disorder in Bangladesh. The current study is just a beginning and has exposed the way of in-depth study on this damaging bacterium, to investigate the genetic diversity of pathogen and host range. The results may facilitate the policy level to initiate an immediate management strategy to control the causal pathogen or insect vector for sustainable citrus cultivation in Bangladesh.

Reference: Tipu, M.M.H., Rahman, M.M., Islam, M.M., Elahi, F-E, Jahan, R., and Islam, M.R. (2020) Citrus Greening Disease (HLB) on *Citrus reticulata* (Mandarin) Caused by *Candidatus Liberibacter asiaticus* in Bangladesh. Physiological and Molecular Plant Pathology, 112:101558.

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