First Report of Citrus Greening Disease and Associated Bacterium

"Candidatus Liberibacter asiaticus" from Bhutan. Y. S. Ahlawat and V. K. Baranwal, Plant Virology Unit, Division of Plant Pathology, Indian Agricultural Research Institute, Pusa, New Delhi-110012 India; Thinlay and Doe Doe, National Plant Protection Centre, Royal Government of Bhutan, Semtokha, Bhutan; and S. Majumder, Plant Virology Unit, Division of Plant Pathology, Indian Agricultural Research Institute, Pusa, New Delhi-110012 India. Plant Dis. 87:448, 2003; published on-line as D-2003-0218-01N, 2003. Accepted for publication 28 January 2003.

During July 2002, surveys of mandarin orchards were conducted in Punakha Valley and Wangdue districts of Bhutan. Symptoms of the greening disease were observed in most of the orchard. The incidence of disease was recorded up to 30% in 24 private orchards with more than 5,000 trees total. Affected trees were generally stunted with leaves showing symptoms of mottling. Sometimes, symptoms were seen only on one part of the canopy. The greening disease is caused by a fastidious phloem restricted bacterium, "Candidatus Liberibacter asiaticus" in Asian countries and "Candidatus Liberibacter africanus" in African countries. To confirm the presence of this bacterium causing greening disease in Bhutan, 33 leaf samples were collected from seven locations in Bhutan and stored at –80°C. Petioles and midribs were used for extraction of DNA using DNeasy Plant Mini Kit (Qiagen Gmbh, Hilden, Germany). Polymerase chain reaction (PCR) was initially performed with a sample from Rimchu, Bhutan using primer pair 5′TATAAAAGGTGGACCTTT CGAGTTT/5′ACAAAAGCAGAAATAGCACGAACAA previously designed for amplification of ribosomal protein genes of beta-operon of two liberibacter species (1). An amplicon of approximately 700 bp was obtained. The size of the PCR product is similar to that amplified from "Candidatus Liberibacter asiaticus". The amplicon was cloned in pGEM-T easy vector and sequenced. The clone was 703 nt long and showed 100% sequence homology with the corresponding sequence of "Candidatus Liberibacter asiaticus" confirming that "Candidatus Liberibacter asiaticus" is the cause of greening disease in Bhutan. Later, one sample from each location was analyzed and found to be positive to greening. To our knowledge, this is the first report of this bacterium and greening disease in Bhutan, and citrus greening appears to be the main cause of declining citrus in the Punakha Region of Bhutan.