Diseases Caused by Bacteria and Phytoplasmas

First Report of Phytoplasma Belonging to 16SrXXXII Group Associated with Witches'-Broom Symptoms in *Trema tomentosa* in China

Shao-shuai Yu,¹ Qing-hua Tang,¹ Yuan Wu,² Ming-xing Lin,¹ Rui-ling Zhao,¹ Wei-wei Song,¹ and Wei-quan Qin^{1,†}

¹ Coconut Research Institute of Chinese Academy of Tropical Agricultural Sciences, Hainan Innovation Center of Academician Team, Wenchang 571339, China

² Hainan Duoyan Arecanut Industry Development Company Limited, Qionghai 571400, China

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Trema tomentosa (Roxb.) Hara, belonging to Ulmaceae, is a significant economic and ecological plant in China (Ma et al. 2014). The plants displayed abnormal symptoms including witches'-broom, internode shortening, and leaf chlorosis, as shown in the supplementary figure, that seriously affected their growth, causing financial loss and ecological damage in Hainan province of China. During August through September 2020, these plants with symptoms were first found and collected in Dingan and Qinghai counties of Hainan province, China, with about 60% incidence separately. For molecular detection of the causal pathogen, the total DNA of *T. tomentosa* samples was extracted using 0.10 g of fresh plant leaves based on the CTAB method. Polymerase chain reaction (PCR) was carried out using the primers R16mF2/R16mR1 (Lee et al. 1993) and secAfor1/sArev3 (Hodgetts et al. 2008) specific for phytoplasma 16S rRNA and *secA* gene fragments, which resulted in production from the four diseased plant samples' DNA but not the four symptomless ones. These PCR products were sequenced and the data deposited in GenBank. The two gene fragments of the DNA extracted from the diseased samples were identical, with length of 1,303-bp 16S rRNA (GenBank accession MW138004) and 587-bp *secA* (MW139904) gene fragments, putatively encoding a 195 (*secA*) amino acids sequence separately. The phytoplasma strain was named as *Trema tomentosa* witches'-broom (TtWB) phytoplasma, TtWB-hn strain. A BLAST search based on the 16S rRNA gene fragment of TtWB

phytoplasma revealed the highest level of sequence identities (more than 99%) with that of 16SrXXXII Malaysian periwinkle virescence (MaPV) group members (Nejat et al. 2013), such as the phytoplasma strains of MaPV (EU371934), *Camptotheca acuminata* witches'-broom (CAWB) (MH141804), oil palm disease (EU498728), Malaysian yellow dwarf coconut disease (MaYDCD) (EU498727), and *Elaeocarpus zollingeri* yellows (EZY) (LC257960). Homology and phylogenetic analysis using DNAMAN 5.0 and MEGA 7.0 software indicated that the phytoplasma strains of TtWB, MaPV, CAWB, oil palm disease, MaYDCD, and EZY were clustered into one clade with 99% bootstrap value based on the 16S rRNA gene fragments. Based on the *secA* gene sequence analysis, the phytoplasma strains of TtWB, MaPV (FJ755005), and EZY (LC257967) were clustered into one clade with 100% bootstrap value. In addition, phylogenetic and computer-simulated restriction fragment length polymorphism analyses based on the nearly full-length 16S rRNA gene sequence of the TtWB phytoplasma strain (MW138004) indicated that the TtWB phytoplasma strain is more closely related to the 16SrXXXII-A subgroup than to the other subgroups within 16SrXXXII group (Nejat et al. 2013; Wei et al. 2007). It may represent a new subgroup within the 16SrXXXII group, designated as 16SrXXXII-D subgroup, which is distinct from the other phytoplasma subgroups within the 16SrXXXII group. To our knowledge, this is the first report indicating the occurrence of the phytoplasma strain belonging to 16SrXXXII-D subgroup associated with witches'-broom disease in *T. tomentosa* in Hainan province, a tropical island of China. The analysis of genetic variation based on the 16S rRNA and *secA* gene fragments indicated that the TtWB-hn strain was more closely related to the phytoplasma strains infecting periwinkle, oil palm, and coconut palm in Malaysia, *C. acuminata* in Yunnan province of China, and *E. zollingeri* in Japan.

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[†]Indicates the corresponding author.
E-mail: W. Q. Qin; qwq268@163.com