Diseases Caused by Bacteria and Phytoplasmas

Occurrence of a 16SrII-V Subgroup Phytoplasma Associated with Witches'-Broom Disease in *Melochia corchorifolia* in China

Shao-shuai Yu,^{1,†} Yuan Wu,² and Wei-wei Song¹

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

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

Funding: This work was supported by the Hainan Provincial Natural Science Foundation of China (320RC743) and Fundamental Research Funds for the Central Non-profit Research Institute of Chinese Academy of Tropical Agricultural Sciences (1630152021005). Plant Dis. 106:754, 2022; published online as <https://doi.org/10.1094/PDIS-07-21-1375-PDN>. Accepted for publication 17 August 2021.

Melochia corchorifolia L. is a plant belonging to the family Sterculiaceae; extracts from this plant have been reported to inhibit melanogenesis (Yuan et al. 2020). During September to November 2020, plants showing abnormal symptoms including witches'-broom, leaf chlorosis, and leaflet and internode shortening were found in Dingan county of Hainan province, China, with about 50% infection rates in the field. The disease symptoms were suspected to be caused by the phytoplasma, a plant-pathogenic prokaryote that could not be cultured in vitro. Aiming to confirm the pathogen causing the symptoms, total DNA of symptomatic or asymptomatic *M. corchorifolia* samples was extracted by the CTAB method (Doyle and Doyle 1990) using 0.10 g of fresh plant leaves using a rapid extraction kit for plant genomic DNA (CTAB Plant Genome DNA Rapid Extraction Kit, Aidlab Biotechnologies, Beijing, China). PCR reactions were performed using primers R16mF2/R16mR1 (Gundersen and Lee 1996) specific for phytoplasma 16S rRNA gene fragments. PCR products of phytoplasma 16S rRNA gene sequences were obtained from the 10 symptomatic plant samples but not from the DNA of the asymptomatic plant samples. The PCR products were cloned and sequenced by Biotechnology (Shanghai) Co., Ltd. (Shanghai, China), and the data were deposited in GenBank. The sequences of 16S rRNA gene fragments amplified from the DNA extracted from the diseased plant samples were all identical, with a length of 1,336 bp for the 16S rRNA (GenBank accession MZ353520). Nucleotide BLAST search based on the 16S rRNA gene

fragment of the phytoplasma strain showed 100% sequence identities with that of 16SrII peanut witches'-broom group members, such as cassava witches'-broom phytoplasma (KM280679), *Cleome* sp. phytoplasma (KM280677), *Tephrosia purpurea* witches'-broom phytoplasma (MW616560), *Desmodium triflorum* little leaf phytoplasma (MT452308), and peanut witches'-broom phytoplasma (JX403944). Analysis of the 16S rRNA gene sequence of McWB-hnda strain by interactive online phytoplasma classification tool iPhyClassifier (Zhao et al. 2009) indicated that the phytoplasma strain is a member of the 16SrII-V subgroup. The phytoplasma strain was named as *Melochia corchorifolia* witches'-broom (McWB) phytoplasma, McWB-hnda strain. Phylogenetic analysis performed by MEGA 7.0 employing the neighbor-joining method with 1,000 bootstrap values (Kumar et al. 2016) indicated that the McWB-hnda phytoplasma strain was clustered into one clade with the phytoplasma strains of *T. purpurea* witches'-broom, *Cleome* sp., peanut witches'-broom, cassava witches'-broom, and *D. triflorum* little leaf with 97% bootstrap value; McWB-hnda phytoplasma strain identified in the study and *Melochia corchorifolia* phyllody phytoplasma strain (KX150461) belonging to 16SrI-B subgroup previously identified in the Hainan Island of China by Chen et al. (2017) are in two independent clades. To our knowledge, this is the first report of a 16SrII-V subgroup phytoplasma associated with McWB disease in Hainan Province, a tropical island of China. The phytoplasma strain identified in the study was relatively close to 16SrII peanut witches'-broom group phytoplasma strains associated with witches'-broom or little leaf diseases in the plants like peanut, *T. purpurea*, cassava, and *D. triflorum*. Our finding in the study indicated that *M. corchorifolia* may act as an alternative natural host not only for 16SrI-B subgroup phytoplasma but also for 16SrII-V subgroup phytoplasma, which would contribute to the spreading of the related phytoplasma diseases.

References:

- Chen, W., et al. 2017. J. Plant Pathol. 99:291.
- Doyle, J. J., and Doyle, J. L. 1990. Focus 12:13.
- Gundersen, D. E., and Lee, I. M. 1996. Phytopathol. Mediterr. 35:144.
- Kumar, S., et al. 2016. Mol. Biol. Evol. 33:1870.
- Yuan, X. H., et al. 2020. J. Cosmet. Dermatol. 19:2421.
- Zhao, Y., et al. 2009. Int. J. Syst. Evol. Microbiol. 59:2582.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: phytoplasma, *Melochia corchorifolia* witches'-broom, 16SrII-V subgroup, molecular detection

[†]Indicates the corresponding author.
S.-s. Yu; hzyuys@163.com