Diseases Caused by Bacteria and Phytoplasmas

Tephrosia purpurea Represents a New Host of 16SrII-V Subgroup Phytoplasma Associated with Witches'-Broom Disease in China

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Tephrosia purpurea is a medical plant with excellent insecticidal activity belonging to the family Leguminosae distributed throughout southern of China (Pei et al. 2013). During January to February 2021, plants showing abnormal symptoms including witches'-broom, internode shortening, leaf chlorosis and leaflet formation were found in Ledong County of Hainan Province, a tropical island in China, with about 60% incidence. The *T. purpurea* disease symptoms were suspected to be induced by phytoplasma, a phloem-limited prokaryotic pathogen that cannot be cultured in vitro and that causes severe financial loss and ecological damage to the island. Total DNA from the symptomatic and asymptomatic samples of *T. purpurea* were extracted using 0.10 g of fresh plant leaves and branches by the CTAB method (Doyle and Doyle 1990). 16S rRNA and *secA* gene sequence fragments of phytoplasma were detected through PCR amplification using primers R16mF2/R16mR1 (Gundersen and Lee 1996) and *secA*for1/sArev3 (Hodgetts et al. 2008). The two gene sequence fragments of phytoplasma were obtained from the DNA of six symptomatic plant samples but not from the DNA of six asymptomatic plant samples. These amplified products were sequenced, and the data were deposited in GenBank. The two gene sequence fragments of the DNA obtained from the diseased plant samples were all identical, with a length of 1,335 bp for the 16S rRNA (GenBank accession MW616560) and 729 bp for the *secA* gene (MW603929). The *secA* gene fragment putatively encodes for 242 amino acids. The phytoplasma strain was named as

Tephrosia purpurea witches'-broom (TpWB) phytoplasma, TpWB-hnld strain. The 16S rRNA gene sequence fragment of TpWB-hnld was analyzed by the online tool iPhyClassifier (Wei et al. 2007), indicating that the pathogen strain was a member of subgroup 16SrII-V and a '*Candidatus* Phytoplasma aurantifolia'-related strain. BLAST analysis based on the 16S rRNA gene sequence fragment of TpWB-hnld showed 100% sequence identity with that of peanut witches'-broom group members (16SrII group), such as cassava witches'-broom phytoplasma (KM280679) and *Cleome* sp. phytoplasma (KM280677); BLAST analysis based on the *secA* gene sequence fragment of TpWB-hnld showed 100% sequence identity with that of peanut witches'-broom group members (16SrII group), such as sesame phyllody phytoplasma (JN977044). Homology and phylogeny were analyzed using the software DNAMAN 5.0 and MEGA 7.0, indicating that TpWB-hnld and other subgroup 16SrII-V phytoplasma strains, including cassava witches'-broom phytoplasma, *Cleome* sp. phytoplasma, *Crotalaria* witches'-broom phytoplasma (EU650181) and *Desmodium ovalifolium* witches'-broom phytoplasma (GU113152), were clustered into one clade with 98% bootstrap value based on the 16S rRNA gene sequence fragments; TpWB-hnld and sesame phyllody phytoplasma were clustered into one clade based on the *secA* gene sequence fragments. Multiple alignment based on the 16S rRNA gene sequence fragment showed that the TpWB-hnld phytoplasma strain showed 98% sequence identity with TpWB phytoplasma strain (HG792252) belonging to 16SrII-M subgroup reported in India (Yadav et al. 2014). To our knowledge, this was the first time that 16SrII-V subgroup phytoplasma associated with *Tephrosia purpurea* witches'-broom disease was identified in China. Molecular analysis based on the 16S rRNA and *secA* gene sequence fragments indicated that TpWB-hnld phytoplasma was a member of subgroup 16SrII-V and a '*Ca. P. aurantifolia*'-related strain.

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