Diseases Caused by Bacteria and Phytoplasmas

Occurrence of Phytoplasma Belonging to 16SrII Group Associated with Witches'-Broom Symptoms in *Emilia sonchifolia* in Hainan Island of China

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Phytoplasmas are cell wall-less and phloem-limited plant pathogenic prokaryotes that are unable to cultured in vitro, causing complex syndromes with specific symptoms in their plant hosts, such as witches'-broom, virescence, yellows, and phyllody (IRPCM 2004). *Emilia sonchifolia* is a medicinal plant belonging to family Asteraceae, mainly used as a traditional Chinese medicine with the function of anti-inflammatory, analgesic, antibacterial, antiviral, antitumor, hypoglycemic, and immune regulation (Zhao et al. 2019). In October to November 2020, plants affected by abnormal symptoms including witches'-broom, internode shortening, leaf chlorosis, and leaflet were found in Hainan Province. These disease symptoms, suspected to be induced by phytoplasma, had about 50% infection rates in the field, causing economic loss and ecological damage. Total DNA of symptomatic or asymptomatic *E. sonchifolia* samples were extracted using a rapid extraction kit for plant genomic DNA (Aidlab Biotechnologies Co., Ltd, Beijing, China). PCR was performed using primers R16mF2/R16mR1 (Lee et al. 1993) and secAfor1/sArev3 (Hodgetts et al. 2008) specific for phytoplasma 16S rRNA and *secA* gene fragments. PCR products of phytoplasma 16S rRNA and *secA* gene were obtained from the DNA of three symptomatic plant samples but not from 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 two gene fragments of the DNA extracted from the diseased samples were identical, with lengths of 1,324 bp 16S rRNA (GenBank MW353971) and 760 bp *secA* (MW353710), putatively encoding 253 (*secA*) amino acid sequence. The phytoplasma strain was named as *Emilia sonchifolia* witches'-broom (EsWB) phytoplasma, EsWB-hnda strain. A BLAST search based on the 16S rRNA gene fragment of EsWB-hnda showed the highest level of sequence identities

(99.92 to 100%) with that of 16SrII peanut witches'-broom group members, such as *Celosia argentea* witches'-broom phytoplasma strain (KX426374), *Desmodium ovalifolium* witches'-broom phytoplasma strain (GU113152), *Crotalaria* witches'-broom phytoplasma strain (EU650181), Peanut witches'-broom phytoplasma strain (JN681274), and *Cleome* witches'-broom phytoplasma strain (EU513212), all from Hainan; and Cassava witches'-broom phytoplasma strain (KM280679) from Vietnam. A BLAST search based on the *secA* gene sequence fragment of EsWB-hnda showed 99.47 to 99.61% sequence identity with that of 16SrII peanut witches'-broom group members such as *Echinacea purpurea* witches'-broom phytoplasma strain (CP040925), *Cicer arietinum* phyllody phytoplasma strain (KX358585), *Daucus carota* phyllody phytoplasma strain (KX358580), and *Spinacia oleracea* phyllody phytoplasma strain (KX358579). Analysis using iPhyClassifier (Wei et al. 2007) based on the 16S rRNA gene sequence of EsWB-hnda indicated that it was a member of the 16SrII-V subgroup. Phylogenetic analysis was performed by neighbor-joining method using MEGA version 7.0 with 1,000 bootstrap value (Kumar et al. 2016). The results showed that the phytoplasma strains associated with witches'-broom symptoms in *E. sonchifolia*, *Celosia argentea*, *D. ovalifolium*, *crotalaria*, peanut, cleome, and cassava were clustered into one clade with 98% bootstrap value based on the 16S rRNA gene sequence fragments, and the phytoplasma strains of *Echinacea purpurea* witches'-broom, *Cicer arietinum* phyllody, *Daucus carota* phyllody, and *S. oleracea* phyllody were clustered into one clade with 100% bootstrap value based on the *secA* gene sequence fragments. To our knowledge, this is the first report of a phytoplasma belonging to the 16SrII-V subgroup associated with *Emilia sonchifolia* witches'-broom disease in Hainan, with comparatively close relationship to 16SrII peanut witches'-broom group phytoplasma strains infecting the plants like peanut, *D. ovalifolium*, and cleome from the same island of China and cassava from Vietnam.

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