Diseases Caused by Bacteria and Phytoplasmas

Waltheria indica Is a New Host of Phytoplasma Belonging to 16SrI-B Subgroup Associated with Virescence Symptoms in China

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Waltheria indica L. is a kind of medicinal plant belonging to the family Sterculiaceae distributed in China, whose extracts with many active compounds are used for treatment of rheumatism and sore pain (Hua et al. 2019). During September to November 2020, plants showing abnormal symptoms including floral virescence, leaf chlorosis, and leaflet were found in Dingan county of Hainan province, China, with about 70% incidence. The disease symptoms, suspected to be caused by the phytoplasma, a phloem-limited cell-wall-less prokaryotic pathogen that could not be cultured in vitro, severely impacted *W. indica* growth, resulting in financial loss and ecological damage in the location. For identification of the causal pathogen, the total DNA of symptomatic or symptomless *W. indica* samples was extracted using 0.10 g of fresh plant tissues using the CTAB method. PCR reactions were performed using primers R16mF2/R16mR1 (Lee et al. 1993) and AYgroEL/AYgroELR (Mitrovi et al. 2011) specific for phytoplasma 16S rRNA and *groEL* gene fragments. The target productions of the two gene fragments of phytoplasma were detected in the DNA from four symptomatic plant samples but not in the DNA from the symptomless plant samples. The PCR productions were sequenced, and the data were deposited in GenBank. The two gene fragments of the DNA extracted from the symptomatic plant samples were all identical,

with lengths of 1,340 bp for 16S rRNA (GenBank accession MW353909) and 1,312 bp for *groEL* (MW353709) gene sequence fragments, putatively encoding a sequence of 437 (*groEL*) amino acids. The phytoplasma strain was named as *W. indica* virescence (WiV) phytoplasma, WiV-hnda strain. A BLAST search based on the 16S rRNA gene fragment of WiV-hnda phytoplasma strain revealed the highest level of sequence identity (99.85%) with that of 16SrI aster yellows group members (16SrI-B subgroup), such as onion yellows phytoplasma strain OY-M (AP006628) from Japan (Oshima et al. 2004); periwinkle virescence phytoplasma strain PeV-hnhk (KP662136), chinaberry witches'-broom phytoplasma strain CWB-hnsy1 (KP662119), and CWB-hnsy2 (KP662120), all the strains from Hainan island of China (Yu et al. 2017). A BLAST search based on the *groEL* gene sequence fragment of WiV-hnda indicated 99.92% sequence identity with that of 16SrI aster yellows group members (16SrI-B subgroup) such as onion yellows phytoplasma strain OY-M (AP006628). Homology and phylogenetic analysis by DNAMAN 5.0 and MEGA 7.0 software indicated that the phytoplasma strains of WiV-hnda, OY-M, PeV-hnhk, CWB-hnsy1, and CWB-hnsy2 were clustered into one clade based on the 16S rRNA gene fragments. WiV-hnda, OY-M and Aster yellow witches'-broom (AYWB) (CP000061) phytoplasma strains were clustered into one clade based on the *groEL* gene fragments. To our knowledge, this was the first time that *W. indica* virescence disease induced by 16SrI-B subgroup phytoplasma strain was reported in China. Genetic analysis showed that WiV-hnda was closely related to the phytoplasma strains causing onion yellows in Japan, periwinkle virescence, and chinaberry witches'-broom disease in China.

References:

- Hua, Y. M., et al. 2019. J. Chin. Pharm. Sci. 28:468.
- Lee, I. M., et al. 1993. Phytopathol. 83:834.
- Mitrovi, J., et al. 2011. Ann. Appl. Biol. 159:41.
- Oshima, K., et al. 2004. Nat. Genet. 36:27.
- Yu, S. S., et al. 2017. Sci. Silva. Sin. 53:105.

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