Diseases Caused by Bacteria and Phytoplasmas

Identification of 16S rXXX-A Phytoplasma Associated with Salt Cedar (*Tamarix chinensis*) Witches'-Broom in Southern Xinjiang of China

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Salt cedar is an ornamental shrub/moderate tree species native to Asia and East Europe, and grows in salt-alkali soil, desert, and other dry areas, which plays an important role in wind prevention and sand fixation as well as maintaining ecological balance. Salt cedar witches'-broom (SCWB), which was extremely pernicious to salt cedar, was first observed and reported in Xi'an, China, in 2005 (Zhao et al. 2005). Witches'-broom symptoms were observed on 20 out of 150 (13.3%) salt cedar plants surveyed from the Alar region and 10 out of 86 (11.6%) plants from the Akesu region in southern of Xinjiang in May 2020. The damaged plants were compared with asymptomatic plants; the major symptoms included branches clustered and intersegment shortened and coarsened, giving rise to the formation of clusters. Total plant DNA was extracted from phloem tissues with asymptomatic symptoms and phloem tissues with witches'-broom symptoms by a CTAB-based DNA extraction method (Green et al. 1999). The 16S rRNA gene and the phytoplasma universal primers P1/P7 and rpF1/rpR1 of the ribosomal protein (rp) gene were used for polymerase chain reaction (PCR) amplification by using the extracted plant total DNA as the template. The PCR product was used as the template, and the R16F2n/R16R2 primer was used for nested PCR amplification of the 16S rRNA gene after the amplification was completed. The results

show that no product was obtained in asymptomatic plants. When DNA samples from witches'-broom symptomatic plants were used as templates, fragments with lengths 1,219 and 1,174 bp, corresponding to the 16S rRNA gene and rp gene, were obtained. 16S rRNA gene was sequenced and deposited in GenBank under accession number MW447513. BLAST analysis revealed that the partial 16S rRNA sequence of the phytoplasma associated with *Periplocaaphylla* witches'-broom showed the highest sequence identity (99.67%) to salt cedar witches'-broom phytoplasma, '*Candidatus* Phytoplasma tamaricis' (accession no. FJ432664). Phylogenetic and molecular evolutionary analyses were conducted using MEGA-X (Kumar et al. 2018). Results showed that the SCWB and 16S rXXX group's '*Ca. P. tamaricis*' (GenBank accession FJ432664) had the highest affinity. A virtual restriction fragment length polymorphism was done to determine the subgroup (Zhao et al. 2009). The 16S rDNA sequence from the *Tamarix chinensis* Lour. plant showed 99.3% similarity with that of the '*Ca. P. tamaricis*' reference strain (GenBank accession FJ432664), suggesting that the phytoplasma in this study belongs to a '*Ca. P. tamaricis*'-related strain. Therefore, it can be stated that SCWB belongs to the 16S rXXX group. The partial rp sequences only shared 84.74% sequence similarity with that of '*Ca. P. prunorum*' (MG383523) of the apple proliferation group, a known subgroup 16S rX. BLAST analysis based on the partial rp sequences showed that it shares less than 90% similarity with that of any known phytoplasma; we suspect that this is due to a lack of sequenced rp gene sequences for the 16S rXXX group. To our knowledge, this is the first report of salt cedar witches'-broom phytoplasma in Xinjiang province, China. As a consequence, we conclude the SCWB phytoplasma rp gene belongs to 16S rXXX-rp group, which is also the first report about the 16SrXXX-rp group. SCWB1 is the only strain in the 16S rXXX group, and it is the representative strain of the 16S rXXX-A subgroup (Zhao et al. 2009). So, the SCWB disease we found in southern Xinjiang belongs to the 16S rXXX-A subgroup.

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