Diseases Caused by Bacteria and Phytoplasmas

First Report of ‘*Candidatus Phytoplasma asteris*’ Associated with Witches’-Broom Disease of *Macadamia ternifolia* in China

Jianxin Chen,¹ Zejia Lv,¹ Yuqian Wei,¹ Aiting Zhou,¹ Ziqiang Wu,¹ Dezhou Shen,² and Jianrong Wu^{1,3,†}

¹ Yunnan Provincial Key Laboratory of Forest Disaster Warning and Control, Southwest Forestry University, Kunming 650224, China

² Forestry and Grassland Bureau of Dehong Prefecture, Dehong 678400, China

³ Key Laboratory of State Forestry Administration on Biodiversity Conservation in Southwest China, Southwest Forestry University, Kunming 650224, China

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Macadamia nut (*Macadamia ternifolia*) was first introduced into China from Australia in 1910, and the main cultivation areas were Yunnan and Guangxi. It can be used as both a fruit and a therapeutic drug, with high economic value. In March 2021, it was observed that the *M. ternifolia* was showing witches’-broom, leaf yellowing, and plexus bud in Dehong Prefecture, Yunnan Province, China. The terminal buds of infected plants were inhibited, and the lateral buds were stimulated to germinate into twigs in advance. It was named the *M. ternifolia* witches’-broom (MTWB) disease and was found in urban and rural areas of Mangshi, Lianghe, Yingjiang, Mangdong, Longchuan, and Longling cities and counties. More than 40% of the plants were infected on the seven areas surveyed. The lateral stems from symptomatic and asymptomatic plants were cut to small pieces. The tissues were treated by fixation, dehydration, and spraying gold. And the tissues were observed under a scanning electron microscope (Hitachi S-3000N) (Pathan et al. 2010). The nearly spherical bodies were found in the phloem sieve cells of symptomatic plants. Symptomatic and asymptomatic plants were collected from seven areas, ddH₂O was used as the negative control, and *Dodonaea viscosa* witches’-broom disease plants were used as the positive control. The total plants’ DNA extraction was conducted from 0.1 g of tissue using the CTAB method (Porebski et al. 1997), and DNA was stored at –20°C in a refrigerator in the Key Laboratory of Forest Disaster Warning and Control at the Southwest Forestry University. Nested PCR was employed to amplify the 16S rRNA gene with the primers P1/P7 and R16F2/R16R2 (Lee et al. 1993; Schneider et al. 1993). PCR amplicons of 1.8 kb and 1.2 kb were amplified (GenBank accessions MW892818, MW892819, MW892820, and MW892821). The direct PCR with primer pairs rp(I)F/rp(I)R (Lee et al. 2003) specific to the ribosomal protein (*rp*) gene yielded amplicons of approximately 1.2 kb (GenBank accessions

MZ442600, MZ442601, MZ442602, and MZ442603). The fragment from 21 samples was consistent with the positive control, confirming the association of phytoplasma with the disease. Interestingly, the phytoplasma 16S rRNA gene and *rp* gene were also amplified from the four samples of asymptomatic plant, and we speculated that the latent infection and hidden symptoms existed in macadamia nut (Moslemkhani and Sadeghi 2011). A BLAST analysis of the 16S rRNA sequences of MTWB phytoplasma showed that it has a 99% similarity with *Trema laevigata* witches’-broom phytoplasma (GenBank accession MG755412). The *rp* sequence shared 99% identity with *Salix tetradenia* witches’-broom phytoplasma (GenBank accession KC117314). An analysis with iPhyClassifier showed that the virtual restriction fragment length polymorphism pattern derived from the query 16S rDNA fragment of MTWB phytoplasma is most similar to the reference pattern of the 16Sr group I, subgroup B (OY-M, GenBank accession AP006628), with a pattern similarity coefficient of 0.99. The phytoplasma is identified as a ‘*Candidatus Phytoplasma asteris*’-related strain belonging to subgroup 16SrI-B. The phylogenetic tree was constructed based on 16S rRNA gene and *rp* gene sequences by using MEGA version 6.0 (Tamura et al. 2013) with the neighbor-joining method, and bootstrap support was estimated with 1,000 replicates. The result indicated that the MTWB phytoplasma formed a subclade in 16SrI-B and *rp*I-B, respectively. In 2013, macadamia nut showed leaf hardness phyllody and shoot proliferation caused by ‘*Ca. P. asteris*’ in Artemisa, Cuba. The concern is that the macadamia phytoplasma is closely related to the subgroup 16SrI-F, and it is significantly different from the Chinese strains (Pérez-López et al. 2013). In addition, the MTWB phytoplasma was graft-transmitted from infected to healthy plants in nursery conditions (Akhtar et al. 2009; Ikten et al. 2014). And the grafted plants were positive for the phytoplasma in the nested PCR assays. It is noteworthy that the plants were seriously damaged by aphids, and it was speculated that the insects of Homoptera caused the spread of the disease by sucking plant sap; thus, the aphids that transmit MTWB phytoplasma in China must be determined to control the MTWB disease. To the best of our knowledge, macadamia nut is a new host plant of ‘*Ca. P. asteris*’ in China. The newly emerged disease is a threat to macadamia nut.

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The author(s) declare no conflict of interest.

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[†]Indicates the corresponding author.
J. Wu; 1176279044@qq.com