Diseases Caused by Bacteria and Phytoplasmas

Threeflower Tickclover (*Desmodium triflorum*) Is a New Host for Peanut Witches' Broom Phytoplasma, a 16SrII-V Subgroup Strain, in Taiwan

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Funding: This work was financially supported (in part) by grants-in-aid from the Ministry of Science and Technology (108-2628-B-005-003) and from the Ministry of Education (MOE) in Taiwan through the Advanced Plant Biotechnology Center from the Featured Areas Research Center Program within the framework of the Higher Education Sprout Project. Plant Dis. 105:209, 2021; published online as <https://doi.org/10.1094/PDIS-06-20-1303-PDN>. Accepted for publication 5 August 2020.

Threeflower tickclover (*Desmodium triflorum*) is a perennial herbaceous plant that belongs to the family Leguminosae. Threeflower tickclover widely grows at mid-low altitude regions in Taiwan and is commonly used as a traditional herbal medicine for the treatment of dysmenorrhea, muscle spasm, cough, pain, and poisoning. In March 2020, disease symptoms such as little leaf, phyllody, virescence, and witches' broom were observed on threeflower tickclover at the Sansheng Community Park in Mailiao, Yunlin County, Taiwan. Similar disease symptoms were observed on peanut infected with peanut witches' broom (PnWB) phytoplasma grown in the same area (Liu et al. 2015). Leaf samples collected from the healthy and symptomatic threeflower tickclover were used to extract total DNA and protein for polymerase chain reaction (PCR) and western blotting assays, respectively. Nested PCR was performed with the phytoplasma universal primer pair P1/

P7 followed by R16F2n/R16R2 for the amplification of 16S ribosomal RNA (rRNA) gene (Lee et al. 1993). A specific DNA fragment of expected size (1.2 kb) for 16S rRNA was only amplified from leaf samples of symptomatic threeflower tickclover. The nucleotide sequence of the amplified DNA fragment using primer pairs P1/P7 was deposited into GenBank (accession no. MT452308). BLAST analysis revealed that MT452308 shares 100% identity with GenBank accession NZ_AMWZ01000008 (complement [31109 to 32640]) of phytoplasma associated with PnWB disease (Chung et al. 2013). Based on the virtual restriction fragment length polymorphism pattern of MT452308 generated by iPhyClassifier, the phytoplasma detected in symptomatic threeflower tickclover could be classified into the 16SrII-V subgroup. For western blotting, the polyclonal antibody raised against Imp protein of purple coneflower witches' broom phytoplasma (Chien et al. 2020), which is identical to that of PnWB phytoplasma (accession no. ADD59806), was used. An expected signal of 19 kDa specific for Imp was only detected in threeflower tickclover exhibiting disease symptoms. Subsequent assays including PCR, DNA sequencing, and western blotting further confirmed that the gene encoding a SAP11-like protein (accession no. EMR14684) identified in PnWB phytoplasma was also found in samples of symptomatic threeflower tickclover and shares 100% identity with each other. Our results indicate that threeflower tickclover, a common weed in Taiwan, may act as an alternative natural host for PnWB phytoplasma and contributes to the spreading of phytoplasma disease.

References:

- Chien, Y.-Y., et al. 2020. Plant Dis. 104:1247.
Chung, W.-C., et al. 2013. PLoS One 8:e62770.
Lee, I. M., et al. 1993. Phytopathology 83:834.
Liu, C.-T., et al. 2015. Plant Signal. Behav. 10:e1107690.

The author(s) declare no conflict of interest.

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Keywords: threeflower tickclover, *Desmodium triflorum*, phytoplasma, peanut witches' broom disease, SAP11

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