Diseases Caused by Bacteria and Phytoplasmas

First Report of a '*Candidatus Phytoplasma aurantifolia*'-Related Strain (16SrII-V) Associated with Phyllody, Virescence, and Shoot Proliferation of Sweet William (*Dianthus barbatus*) in Taiwan

S. Lee,¹ Y.-J. Lee,¹ C.-Y. Chang,¹ and C.-C. Chu^{1,2,†}

¹Department of Plant Pathology, National Chung Hsing University, Taichung 402, Taiwan

²Advanced Plant Biotechnology Center, National Chung Hsing University, Taichung 402, Taiwan

S. Lee, Y.-J. Lee, and C.-Y. Chang contributed equally.

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Sweet William (*Dianthus barbatus*) is an ornamental belonging to the Caryophyllaceae family; the species produces clusters of flowers that come in various colors and are grown commonly as garden plants (Lim 2014). In February 2021, sweet William plants showing symptoms typical of phytoplasma diseases were found in a garden located in Wufeng District, Taichung, Taiwan (24°04'37.6"N 120°43'20.4"E). Infected plants exhibited virescence and phyllody symptoms and produced an abnormal number of new shoots from the base of the flowers/flower-like structures as well as the base of the plants. Among the 15 plants grown in the area, two exhibited such symptoms. The two symptomatic plants, along with five symptomless plants, were sampled. Two flower-like structures were collected from each of the symptomatic plants, and two flower samples were collected for each symptomless plant. Total DNA were extracted from each sample using the Synergy 2.0 Plant DNA Extraction Kit (OPS Diagnostics) and subjected to diagnostic PCR using primers P1/P7 (Schneider et al. 1995). All four symptomatic samples produced a 1.8-kb amplicon and the 10 symptomless samples did not. The amplification products were diluted 50-fold and used in a second round of PCR using primers R16F2n/R16R2 (Gundersen and Lee 1996). Again, only the symptomatic samples produced an expected 1.25-kb amplicon. A sample was selected for each plant and the PCR products from the first round of

PCR were cloned using the pGEM-T Easy Vector System (Promega Inc., Madison, WI) and sequenced (three clones per sample). Fragments of the 16S rRNA gene (1,248 bp; GenBank accession MW788688) were analyzed using iPhyClassifier (<https://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi>). Sequences obtained from the two infected plants were identical and were classified to the 16SrII-V subgroup with similarity coefficients of 1.0; they also shared 98.6% similarity with the sequence of a '*Candidatus Phytoplasma aurantifolia*' reference strain (accession U15442). BLASTn results indicated that the 16S rRNA gene sequences detected were identical to those of 16SrII-V phytoplasmas affecting mungbean (accession MW319764), lilac tasselflower (accession MT420682), peanut (accession JX403944), and green manure soybean (accession MW393690) found in Taiwan. To corroborate the above results, 16SrII group-specific primers were used to conduct nested and seminested PCR targeting the pathogen's 16S rRNA gene (outer primers: rpF1C/rp(I)R1A; inner primers: rp(II)F1/rp(II)R1; Martini et al. 2007) and immunodominant membrane protein gene (*imp*; outer primers: IMP-II-F1/IMP-II-R1; inner primers: IMP-II-F2/IMP-II-R1; Al-Subhi et al. 2017). In both assays, the symptomatic samples produced the expected amplicons and the symptomless samples did not. The coding sequence of the *imp* gene (519 bp; accession MW755353) was the same among all symptomatic samples, and shared 100% identity with that of the peanut witches'-broom phytoplasma (16SrII; accession GU214176). To our knowledge, this is the first report of a 16SrII-V phytoplasma infecting sweet William in Taiwan. Since 16SrII-V phytoplasmas have also been found infecting mungbean and peanut in Taiwan (Liu et al. 2015), the findings here suggest that by serving as a natural host in the field, sweet William may potentially contribute to the spread of 16SrII-V phytoplasmas to food crops.

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[†]Indicates the corresponding author.
C.-C. Chu; cchu9@nchu.edu.tw