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First Report of a 16SrII-A Subgroup '*Candidatus* Phytoplasma Aurantifolia'-related Strain Infecting *Pachyrhizus erosus* in Taiwan

Y.-C. Chiu[1,2] | C.-H. Huang[3] | J.-Y. Yang[1,2,4,5]

[1]Institute of Biochemistry, National Chung Hsing University, Taichung, Taiwan | [2]Doctoral Program in Microbial Genomics, National Chung Hsing University and Academia Sinica, Taichung, Taiwan | [3]Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan | [4]Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan | [5]Advanced Plant and Food Crop Biotechnology Center, National Chung Hsing University, Taichung, Taiwan

Correspondence: J.-Y. Yang (jyang@nchu.edu.tw)

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Keywords: 16SrII phytoplasma | jicama | *Pachyrhizus erosus*

Pachyrhizus erosus, commonly known as jicama or yam bean, is a leguminous crop valued for its crisp, edible tubers which are rich in vitamin C, essential micronutrients and bioactive compounds (Jaiswal et al. 2022). In July 2025, *P. erosus* plants displaying severe witches'-broom-like symptoms were observed in Mailiao, Yunlin, Taiwan (23°45′04.6″N 120°16′57.6″E). Diseased plants exhibited excessive shoot proliferation and shortened internodes, resulting in a dense canopy (Figure 1A,B). Floral abnormalities, including phyllody, virescence and floral sterility were also observed (Figure 1C). To identify the agent, genomic DNA was extracted from diseased tissues using a Plant Genomic DNA Purification Kit (GeneMark, Taiwan), followed by nested PCR targeting the conserved 16S rRNA gene using phytoplasma universal primer pairs P1/P7 and R16F2n/R16R2, as described by Lee et al. (1993).

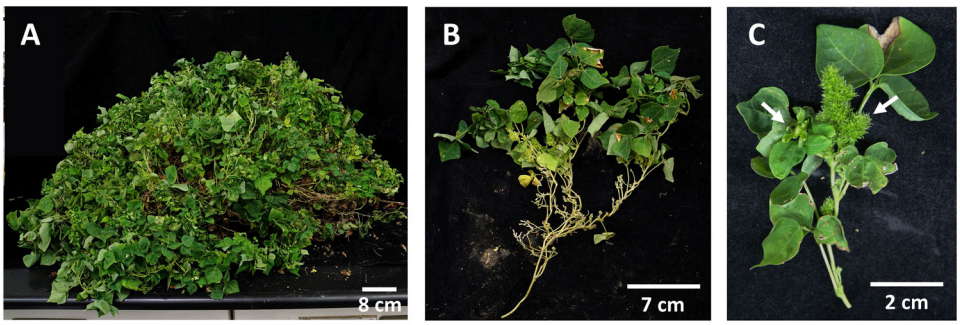


FIGURE 1 | Symptoms of witches'-broom disease in *Pachyrhizus erosus* observed in Mailiao, Yunlin, Taiwan: diseased plants of *P. erosus* exhibiting (A) witches'-broom symptoms, (B) dense, bushy canopy caused by excessive shoot proliferation and shortened internodes and (C) floral abnormalities, including virescence, phyllody and floral sterility (arrows).

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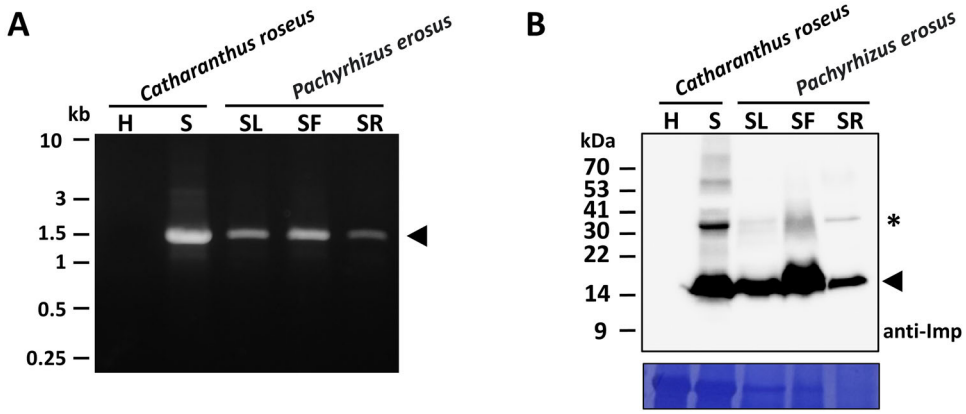
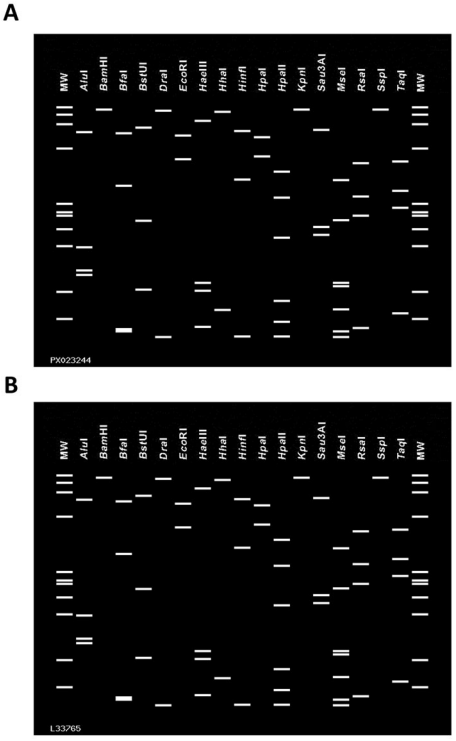


FIGURE 2 | Molecular detection of phytoplasma in *Pachyrhizus erosus* exhibiting witches'-broom symptoms: (A) nested PCR targeting the 16S rRNA gene using genomic DNA from diseased leaves (SL), flowers (SF) and roots (SR) of *P. erosus*. H and S correspond to the negative and positive controls, respectively. Arrowhead indicates the 1.2-kb phytoplasma-specific amplicon. (B) Western blot analysis of total protein extracts probed with anti-immunodominant membrane protein (Imp) antibody raised against the peanut witches'-broom phytoplasma. Arrowhead indicates the specific signal of Imp (19 kDa) (upper panel). As a loading control, the large subunit of Rubisco was visualized with Coomassie brilliant blue staining (lower panel). Non-specific signals were indicated by asterisks.



C

x: L33765
y: PX023244

Enzyme	N_x	N_y	N_{xy}
AluI(AGCT)	4	4	4
BamHI(GGATCC)	1	1	1
BfaI(CTAG)	4	4	4
BstUI(CGCG)	3	3	3
DraI(TTTAAA)	2	2	2
EcoRI(GAATTC)	2	2	2
HaeIII(GGCC)	4	4	4
HhaI(GCGC)	2	2	2
HinfI(GANTC)	3	3	3
HpaI(GTTAAC)	2	2	2
HpaII(CCGG)	6	6	6
KpnI(GGTACC)	1	1	1
MseI(TTAA)	7	7	7
RsaI(GTAC)	4	4	4
Sau3AI(GATC)	3	3	3
SspI(AATATT)	1	1	1
TaqI(TCGA)	4	4	4

FIGURE 3 | Virtual RFLP patterns generated by *i*PhyClassifier. Virtual RFLP profiles were obtained from in silico digestion of the 1.2-kb 16S rRNA gene fragment of (A) *Pachyrhizus erosus* witches'-broom phytoplasma (PX023244) and (B) peanut witches'-broom phytoplasma (L33675), and (C) comparative analysis of the virtual RFLP patterns of PX023244 (x) and L33675 (y). N_x and N_y represent the total number of DNA fragments generated by enzyme digestion in patterns x and y, respectively, and N_{xy} indicates the number of DNA fragments shared between the two patterns.

A 1.2-kb amplicon was amplified exclusively from diseased leaves, flowers and roots, suggesting the presence of a phytoplasma (Figure 2A). Genomic DNAs extracted from uninfected and *Echinacea purpurea* witches'-broom phytoplasma-infected *Catharanthus roseus* plants were used as negative and positive controls, respectively.

To verify phytoplasma infection, western blotting was performed using a polyclonal antibody targeting the immunodominant membrane protein (Imp) of the peanut witches'-broom phytoplasma, a 16SrII group '*Candidatus* Phytoplasma aurantifolia' strain prevalent in Taiwan. A specific signal was detected only in the leaves, flowers and roots of diseased plants, providing further evidence of phytoplasma infection (Figure 2B). To further identify the phytoplasma strain, the DNA fragment amplified from diseased *P. erosus* using the P1/P7 primer pair was sequenced directly and a partial 16S rRNA gene sequence of the *P. erosus* witches'-broom phytoplasma was submitted to GenBank

(Accession No. PX023244). BLAST analysis revealed that this sequence was identical to those of members of the 16SrII phytoplasma group, 'Ca. P. aurantifolia' subgroup 16SrII-A. The virtual RFLP analysis using *i*PhyClassifier (Zhao et al. 2013) further confirmed the BLAST comparisons by showing a similarity coefficient of 1.00 between the *P. erosus* phytoplasma and the reference strain of 16SrII-A subgroup (L33765) (Figure 3).

To our knowledge, this is the first report of witches'-broom disease in *P. erosus* caused by a '*Ca*. P. aurantifolia'-related strain belonging to the 16SrII-A subgroup, both in Taiwan and worldwide, highlighting its potential threat to the health and sustainable production of leguminous crops in Taiwan.

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