



First report of a '*Candidatus Phytoplasma trifolii*'-related strain associated with soybean bud proliferation and seed pod abortion in Iran

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During a survey in 2016, soybean (*Glycine max*) plants in commercial fields in Mazandaran and Golestan provinces, Iran exhibited symptoms including bud proliferation and aborted seed pods, but showed no stunting or growth reduction (Fig. 1). Approximately 25% of plants were diseased. Leaf and pod samples were collected from five diseased plants from Golestan province and ten from Mazandarn province, and two asymptomatic plants were collected from a field without symptoms in Golestan province.

Total genomic DNA was extracted using a CTAB-based method (Doyle & Doyle, 1987). The DNA was used as template in direct PCR using the phytoplasma-universal primers P1/P7 (Ahrens & Seemüller, 1992) designed to amplify the 16S ribosomal RNA gene, followed by nested PCR using the R16MF2/R16MR1 primers (Gundersen & Lee, 1996). Reactions containing template DNA from all of the diseased plants yielded PCR products of the expected size (1,400 bp). All of the asymptomatic plants tested negative for phytoplasmas. Two isolates, one each from Golestan (SG7) and Mazandaran province (SG13), were sequenced directly using primers R16MF2/R16MR1 on both strands by Macrogen (Korea). The sequences of the two isolates were identical. Consensus nucleotide sequences of 1,250 bp were deposited in GenBank (Accession Nos. MG992479 and MG992480). A BLAST search revealed that the sequences shared 98% identity to rDNA of '*Candidatus Phytoplasma trifolii*' (MF564268) associated with tomato disease in Turkey and '*Ca. P. trifolii*' associated with a new disease in chilli pepper in Mexico. The new isolates (SG7 and SG13) associated with soybean bud proliferation and seed pod abortion (SBPSPA) disease have 98.24% sequence identity to the '*Ca. P. trifolii*' reference strain (AY390261) and therefore represent a '*Ca. P. trifolii*'-related strain. In a phylogenetic tree constructed using the

neighbour joining algorithm, the SBPSPA phytoplasma obtained from soybean in Iran clustered with 'clover proliferation' group (16SrVI) strains.

There are numerous reports of '*Ca. P. trifolii*' in Iran, associated with cucumber and zucchini phyllody, salix proliferation and date yellows. There are reports of phytoplasmas in soybean including '*Ca. P. asteris*' in the USA (Mollov *et al.*, 2014), and peanut witches' broom phytoplasma in Malawi and Mozambique (Lava *et al.*, 2011). To our knowledge, this is the first report of '*Ca. P. trifolii*' in soybean worldwide.

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Figure 1

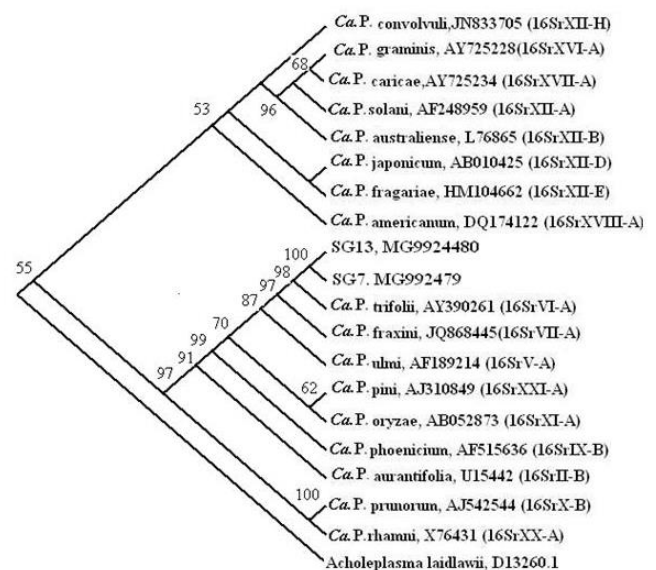


Figure 2

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