



First report of the association of a 'Candidatus Phytoplasma asteris'- related strain with *Plumbago auriculata* leaf yellowing in India

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Plumbago auriculata (Plumbaginaceae) is a perennial ornamental shrub with many therapeutic values and native to South Africa (Saji & Antony, 2015). Several groups of phytoplasma have been reported to infect ornamental and medicinal plants all over the world (Rao *et al.*, 2018). However, no phytoplasma has been found so far in *P. auriculata*.

Leaf yellowing symptoms on *P. auriculata* plants (Fig. 1) were observed in Baramati, Pune, India in March 2016 with a disease incidence of 8-10%. To verify the presence of phytoplasma, three symptom-bearing and three asymptomatic *P. auriculata* samples were collected and DNA was extracted using the CTAB protocol. PCR was performed in a Mastercycler (Eppendorf, Germany) (Rao *et al.*, 2014) and DNA from the sesame phyllody phytoplasma (16SrI group) (GenBank Accession No. KC920747) was used as a positive control. The 16S rRNA gene was amplified from all the symptomatic *P. auriculata* leaf yellowing (PaLY) samples but not from the asymptomatic samples using P1/P7 (Schneider *et al.*, 1995) followed by R16F2n/R2 primer pairs (Gundersen & Lee, 1996) in nested PCR. Amplicons of the expected size (c. 1.25 kb) were purified and directly sequenced (MN239503 and MN239504). BLAST analysis showed that these 16S rRNA gene sequences shared 100% identity with phytoplasmas in the 16SrI group (MG252367, MK440284, MK440282, KX15181 & KT957205). A phylogenetic tree was constructed using the neighbour-joining method with MEGA 7.0 (Fig.2).

The R16F2n/R2 sequence of the PaLY phytoplasma was subjected to *in silico* RFLP using the *iPhyClassifier* online tool (<https://plantpathology.ba.ars.usda.gov>). The *iPhyClassifier* analysis indicated that the virtual RFLP patterns derived from the 16S rDNA F2n/R2 fragment of the PaLY phytoplasma strain with restriction enzymes were similar to those of a phytoplasma strain from group 16SrI, 'Candidatus Phytoplasma asteris' (M30790), formerly Aster Yellow Group, subgroup B, with a similarity coefficient of 1.0. Based on sequence and RFLP results, the PaLY phytoplasma is classified as a member of the phytoplasma subgroup 16SrI-B.

Phytoplasmas have been recorded from plant species in the Plumbaginaceae such as *Limonium sinuatum* in Canada, Europe and Israel (Rao *et al.*, 2018). In India, the 'Ca. P. asteris' group is the most widespread

group and has been found in 64 plant species (Rao *et al.*, 2017). Phytoplasmas in subgroup 16SrI-B have been associated particularly with diseases in pineapple, sesame, sugarcane, squash, rose and fennel. The present study reports *P. auriculata* as a new host for the 16SrI-B phytoplasma subgroup worldwide.

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Figure 1

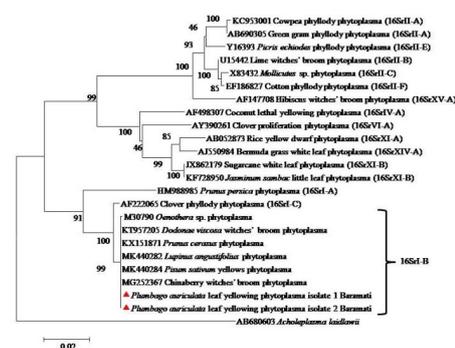


Figure 2

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