



First report of the association of a '*Candidatus Phytoplasma asteris*' strain with *Crossandra infundibuliformis*

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Crossandra infundibuliformis (firecracker flower, Acanthaceae) is an evergreen, perennial, flowering plant which is native to South India and Sri Lanka, and valued medicinally (Vadivel & Panwal, 2016). Phytoplasmas are cell-wall-less unculturable prokaryotes transmitted by phloem-sucking leafhoppers and planthoppers that cause diseases in over a thousand plant species, including crop, ornamental and native plants worldwide (Rao *et al.*, 2017). So far, there are no records of phytoplasmas infecting *C. infundibuliformis*.

Crossandra infundibuliformis plants showing symptoms of leaf yellowing were observed in the Devanahalli area of Bengaluru, India during June 2016 (Figs. 1-2); 30-40% of the plants surveyed in an area of c. 0.2 hectares had symptoms. Leaf samples were collected from three diseased and three symptomless *C. infundibuliformis* plants and DNA was extracted from leaf midribs using the DNeasy plant mini kit (Qiagen, Germany). The extracted DNA was used as a template in nested PCR with primer pairs, P1/P7 (Schneider *et al.*, 1995) and R16F2n/R16R2 (Gundersen & Lee, 1996) that amplify the 16S rRNA gene, and primers SecAfor1/SecArev3 and SecAfor2/SecArev3 (Hodgetts *et al.*, 2008) which target the protein translocase subunit SecA (*secA*) gene. DNA extracted from the sesame phyllody phytoplasma (16SrI group; GenBank Accession No. KC920747) was used as a positive control. The ~1.2 kb and ~480 bp amplified products corresponding to the phytoplasma 16S rRNA and *secA* genes, respectively, were detected in all diseased *C. infundibuliformis* (CiLY) but not from the symptomless samples. Both 16S rDNA and *secA* amplicons were purified (WizardR SV Gel and PCR Cleanup System; Promega, USA), cloned (pGEM-T Easy Vector, Promega) and sequenced (ABA Biotech, India). The 16S rRNA (MT474158, MT474159) and *secA* (MT472835, MT472836) gene sequences from the symptomatic plants were deposited in GenBank. Sequence comparison and phylogenetic analysis (MEGA 7.0) of the 16S rRNA and *secA* gene sequences indicated that both shared 100% identity with members of the 16SrI phytoplasma group (formerly Aster yellows, '*Candidatus Phytoplasma asteris*'), and clustered with 16SrI phytoplasma group-related strains (Figs. 3-4). The iPhyClassifier analysis based on the 16S rDNA F2nR2 sequence of the CiLY phytoplasma (<https://plantpathology.ba.ars.usda.gov>) yielded similar RFLP patterns to those of 16SrI reference strains, subgroup B (M30790), with a similarity coefficient of 1.0. The virtual RFLP results suggested that the CiLY phytoplasma belongs to the 16SrI-B subgroup.

In India, '*Ca. P. asteris*' is the most widespread phytoplasma group, identified from 64 plant species (Rao *et al.*, 2017). In previous studies, *C.*

infundibuliformis plants exhibiting leaf yellowing symptoms were surveyed for phytoplasmas (Khasa *et al.*, 2016) but none were found. The present study is the first report of a phytoplasma associated with a leaf yellowing disease in *C. infundibuliformis*. The results have a significant phytosanitary impact for the epidemiology of the 16SrI phytoplasma group for the Acanthaceae family and related species, as well as, for 16SrI phytoplasma associated diseases in India and the region.

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Figure 1



Figure 2

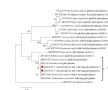


Figure 3

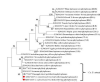


Figure 4

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