



# First report of '*Candidatus Phytoplasma asteris*' associated with flat stem disease of spinach (*Spinacia oleracea* L.) in India

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During 2019, spinach (*Spinacia oleracea* L.) belonging to the family Chenopodiaceae (var. All Green) expressing symptoms of flat stem, indicative of phytoplasma infection were observed at the seed production block of the Indian Institute of Vegetable Research (IIVR) farm (Uttar Pradesh, India). The disease incidence ranged from 10–20%. To confirm the phytoplasma presence, total DNA was extracted from the stem tissue of 7 symptomatic samples along with 4 asymptomatic samples. The amplification of ribosomal DNA was done by nested PCR assay with universal primer pairs P1/P7 followed by nested primer pair 3Fwd/3Rev (Manimekhalai et al. 2010). Semi-nested PCR assay was performed to amplify *secA* gene using SecAfor1/SecArev3 and SecAfor2/SecArev3 primer pairs (Hodgetts et al. 2008). Amplicons of ~1.3 kb corresponding to 16S rDNA region and ~480 bp to *secA* region were yielded only from symptomatic samples. Amplicons were sequenced directly from 3 randomly selected samples. Sequences showed 100% identity among themselves and were submitted directly to NCBI database (GenBank Accession No. MW137921 and MW139906). Pairwise analysis of 16S rDNA and *secA* gene indicated maximum identity with two Polish aster yellows phytoplasma strains infecting sugar beet (KU720554, 100% sequence identity) in different parts of the globe and rapeseed phyllody phytoplasma (CP055264, 99.8% sequence identity), both belonging to the 16SrI-B phytoplasma sub-group ('*Candidatus Phytoplasma asteris*'). Further in *iPhyClassifier* analysis, the virtual RFLP pattern using 17 restriction

enzymes of 16S rDNA gene sequence confirmed the close association of spinach flat stem with 16SrI-B subgroup phytoplasma strain (AP006628) with a similarity coefficient 1.00. Previously, association of 16SrI subgroup phytoplasma with spinach samples showing yellowing was reported from Iran (Tazehkand et al. 2010). To our knowledge, this is the first report of the 16SrI-B subgroup phytoplasma associated with spinach in India.

## Declarations

**Ethical approval** The authors declare that they have not used humans and/or animals on their studies.

**Conflict of interest** Authors declare that they have no conflict of interest.

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