



First report of a '*Candidatus Phytoplasma asteris*'-related strain (16SrI-B subgroup) associated with witches' broom disease in *Cucurbita pepo* in India

G.P.Rao*, Gopala, S. Goel and A. Rao

Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012, India

*E-mail: gprao_gor@rediffmail.com

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Summer squash (*Cucurbita pepo*, Cucurbitaceae) is an important vegetable crop cultivated all over the world for its tender fruits which are used as a cooked vegetable. In India, pumpkins, squash and gourds occupy 0.36 million hectares which is the highest area of cucurbit cultivation in the world (FAO, 2010). Different groups of phytoplasmas have been reported to be associated with cucurbits, including '*Candidatus Phytoplasma asteris*' in *Momordica charantia* in Myanmar, '*Ca. P. australasia*' in *Cucurbita pepo* and *Cucumis sativus* in Iran, and '*Ca. P. pruni*' in *Cucurbita moschata*, *Lagenaria siceraria*, *Luffa cylindrica* and *Sicana odorifera* in Brazil (Salehi *et al.*, 2015).

During May 2016, witches' broom symptoms were observed on 7% of summer squash plants (*C. pepo* cv. Pusa Pasand) in an experimental field of the Indian Agricultural Research Institute, New Delhi, India (Fig. 1). Representative plants were tested for the presence of phytoplasmas. DNA was extracted from three symptom-bearing and two asymptomatic *C. pepo* plants with a CTAB method and were used as template in a nested PCR assay primed by primer pairs P1/P7 (first round) and R16F2n/R16R2 (nested round) for the 16S rRNA gene (Gundersen & Lee, 1996). DNA fragments of 1.2 kb were amplified in the nested round from DNA extracted from all three symptom-bearing samples but not from any of the asymptomatic plants. A representative nested round PCR product of *C. pepo* phytoplasma strain was purified and sequenced directly. The *C. pepo* phytoplasma strain (GenBank Accession No. KY471168) shared 100% nucleotide sequence identity with the 16S rDNA sequence of a 16SrI-B phytoplasma strain reported in North American grapevine (KX236148) and 99.68% identity to the reference strain of '*Ca. P. asteris*' (M30790), and is therefore identified as a '*Ca. P. asteris*'-related strain. Phylogenetic analysis with selected reference strains indicated that the phytoplasma clustered together with member strains of 16SrI-B subgroup (Fig. 2). Phytoplasma strains associated with *C. pepo* phyllody disease have been identified in Egypt and Iran, and were assigned to '*Ca. P. australasia*' (Omar *et al.*, 2012;

Salehi *et al.*, 2015).

'*Candidatus Phytoplasma asteris*' is reported to infect several plant species in India (Rao *et al.*, 2010) but to the best of our knowledge this is the first report of a '*Ca. P. asteris*'-related strain causing witches' broom disease of *C. pepo* in India or globally. The impact of witches' broom disease on cucurbit yield, the distribution of disease and its epidemiology in India are currently under investigation.

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Figure 1

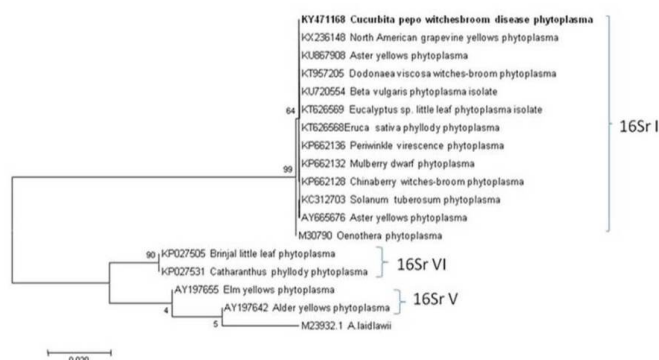


Figure 2

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