



## First report of a 16SrII-D phytoplasma 'Candidatus Phytoplasma australasia' associated with a tomato disease in India

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Tomato (*Solanum lycopersicum*) is an important cash crop in India. Recently, symptoms of leaf yellowing and curling, little leaf, severe stunting and phyllody (Fig. 1) were observed in tomato fields in the Meerut district of western Uttar Pradesh with incidence of 5-8%. To investigate whether or not a phytoplasma is associated with the disease, eight tomato plants with indicative symptoms and eight without were collected from two fields belonging to Samaspur-Surani village, Meerut.

Total DNA was extracted from midribs (DNeasy Plant Mini Kit, Qiagen, Germany) and used as template in a nested PCR assay to amplify the phytoplasma 16S rRNA gene using universal phytoplasma primers P1/P7 (Deng & Hiruki, 1991) and R16F2/R16R2 (Gundersen & Lee, 1996). Bands of expected size (1.8 and 1.26 kb, respectively) were produced in all samples from symptom-bearing plants, but not for the symptomless ones (Fig. 2). Three PCR products amplified with P1/P7 samples were selected and purified (QIAquick Gel Extraction kit, Qiagen, Germany) and directly sequenced. The resultant 16S rDNA sequences showed 100% of sequence identity with each other, and the consensus sequence was deposited in GenBank (Accession No. JX104335). BLAST analysis showed the highest sequence identity (100%) with that of members of group 16SrII 'Candidatus Phytoplasma aurantifolia' including the Papaya yellow crinkle phytoplasma strain Y10097 designated as 'Ca. Phytoplasma australasia' that belongs to subgroup 16SrII-D. Phylogenetic analysis supported BLAST comparisons since the Meerut tomato phytoplasma (Tomato-TMT) clustered in a single distinct branch that embraces 'Ca. Phytoplasma australasia' (Fig. 3). iPhyClassifier (Zhao *et al.*, 2009) was used to perform sequence similarity and generate virtual restriction

fragment length polymorphism (RFLP) profiles. The 16S rDNA sequence of Tomato-TMT isolates exhibited RFLP profiles identical to those of the reference strain Y10097, 'Ca. P. australasia', subgroup 16SrII-D (Fig. 4). To our knowledge, this is the first report of a 16SrII-D phytoplasma in India affecting the tomato crop. The results have significant phytosanitary impact for the epidemiology of 16SrII phytoplasma diseases in the tomato industry of Uttar Pradesh, India.

### Acknowledgements

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Figure 1

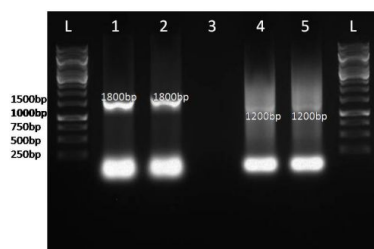


Figure 2

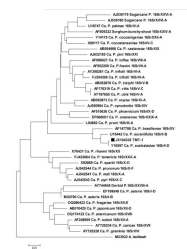


Figure 3

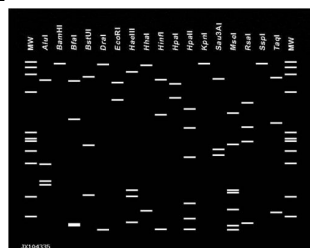


Figure 4

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