New Disease Reports

First report of a '*Candidatus* Phytoplasma asteris' (16SrI group) associated with little leaf disease of *Solanum melongena* (brinjal) in India

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Received: *07 Feb 2012.* ***Published:*** *16 Nov 2012.* ***Keywords:*** *BLL phytoplasma, 16S ribosomal DNA, RFLP*

During November 2007, brinjal little leaf (BLL) symptoms (Fig. 1) were observed in approximately 20% of the brinjal (*Solanum melongena*) plants growing in the fields of Bihar, India, leading to the suspicion of a phytoplasma infection. To test for the presence of phytoplasma, genomic DNA was isolated from the leaf midribs of ten plants with and four plants without symptoms, and the phytoplasma DNA amplified by nested PCR with the universal primers P1/P7 (Deng & Hiruki, 1991) followed by R16mF2/R16mR1 (Gundersen & Lee, 1996), as previously described (Khan *et al*., 2004). The nested PCR amplicons of 1.4 kb corresponding to the phytoplasma 16S ribosomal DNA were cloned into pDRIVE vector (Qiagen GmbH, Germany). No PCR amplicons were observed for the symptomless plants. Twelve positive clones containing 16S ribosomal DNA of phytoplasma were sequenced and found sharing a 99.93% of sequence identity. Sequences of two clones were deposited in GenBank (Accession Nos. JQ518317 and JQ518318). *In silico* RFLP patterns were generated from the phytoplasma 16S ribosomal sequences using the gel plotting program pDRAW32 (http://www.acaclone.com/) and a phylogenetic tree was constructed using the neighbour-joining method of MEGA 4 (Tamura *et al*., 2007).

BLAST analysis revealed that the Bihar phytoplasma detected in brinjal showed 98% 16S rDNA sequence identity with those of phytoplasmas from group 16SrI ('*Candidatus* Phytoplasma asteris'). The Bihar phytoplasma also showed only 84%, 74% and 72% 16S rDNA sequence identity respectively with those of the previously reported BLL phytoplasmas in India (EF186820, EU375486) and Bangladesh (AF228052) belonging to the 16SrVI group (‘*Ca.* Phytoplasma trifolii’). Phylogenetic analysis (Fig. 2) evidenced that the phytoplasma associated with little leaf in brinjal in Bihar separated as a new phylogenetic branch within the 16SrI group cluster. *In silico* restriction fragment length polymorphism (RFLP) patterns were generated (Wei *et al*., 2007) for the Bihar BLL phytoplasma and the 16SrVI BLL phytoplasma reported earlier in India (EF186820) as well as the 16SrI Indian phytoplasmas identified in sandal spike (EF198362) and withania (DQ151998) (Fig. 3) with 13 restriction enzymes (*Alu*I, *Bam*HI, *Bfa*I, *Dra*I, *Eco*RI, *Hae*III, *Hha*I, *Hin*fI, *Hpa*I, *Hpa*II, *Kpn*I, I, and *Taq*I). All the RFLP profiles of the Bihar BLL phytoplasma were similar to those of the 16SrI phytoplasmas, except for the *Alu*I and *Kpn*I RFLP patterns that differed from those exhibited by the 16SrVI BLL phytoplasma (EF186820). RFLP and the sequence results confirmed that the Bihar BLL phytoplasma is closely related to the phytoplasma group 16SrI and may represent a new subgroup within this group. This is the first report of a 16SrI phytoplasma affecting brinjal in India. The fact that two different phytoplasma groups (16SrVI and 16SrI) have been associated with little leaf diseases in brinjal may have further significant impact on disease epidemiology and control in India.

Acknowledgements

The authors are grateful to the Department of Biotechnology, Government of India for funding.

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Figure 1

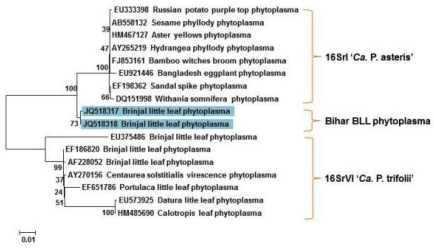

Figure 2

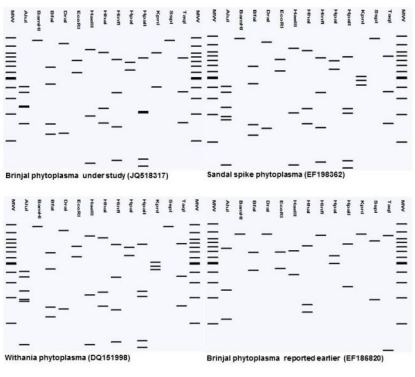

Figure 3

To cite this report: Kumar J, Gunapati S, Singh SP, Lalit A, Sharma NC, Tuli R, 2012. First report of a '*Candidatus* Phytoplasma asteris' (16SrI group) associated with little leaf disease of *Solanum melongena* (brinjal) in India. *New Disease Reports* **26**, 21. [http://dx.doi.org/10.5197/j.2044-0588.2012.026.021]

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