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www.publish.csiro.au/journals/apdn *Australasian Plant Disease Notes*, 2010, **5**, 117–119

First report of '*Candidatus* Phytoplasma asteris' (16SrI) associated with little leaf of cotton and luffa in India

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Abstract. Cotton and luffa plants with little leaf symptoms were observed during February 2010 at New Delhi, India. Nested polymerase chain reaction with phytoplasma 16S rDNA-specific primers efficiently detected the pathogen in the diseased plants. Sequence comparisons showed homology with the members of '*Candidatus* Phytoplasma asteris', group 16SrI. Phylogenetic analysis also assigned the cotton and luffa little leaf phytoplasma to the 16SrI cluster.

The Malvaceae member *Gossypium hirsutum*, commonly known as upland cotton is a world-wide valuable fibre-yielding crop. *Luffa cylindrica* belongs to the family Cucurbitaceae and is widely cultivated as a young edible vegetable crop and a fibre crop from mature fruits. During February 2010, these two plant species were observed to have little leaf symptoms at New Delhi, India. Leaves showed reduction in size to nearly one-third in cotton while to nearly one-fifth in luffa (Fig. 1). Such symptoms have previously been linked to phytoplasma diseases. To identify the presence of the pathogen, petioles from four symptomatic and two symptomless plants were collected for DNA isolation using cetyltrymethyl ammonium bromide as previously described (Saghai-Maroof *et al*. 1984). These were used as templates in a nested polymerase chain reaction (PCR) using phytoplasma-specific 16S rDNA primers P1/P7 (Deng and Hiruki 1991). The PCR products from the first round were diluted (1 : 30) and used in the subsequent round with R16F2/R2 (Gundersen and Lee 1996). Amplicons of expected size (~1.25 Kb) were observed in all the samples from symptomatic plants, while they were absent in reactions with the symptomless samples (Fig. 2). The PCR product from each sample was gel extracted, purified (QIAquick gel extraction kit, Qiagen, Germantown, MD,

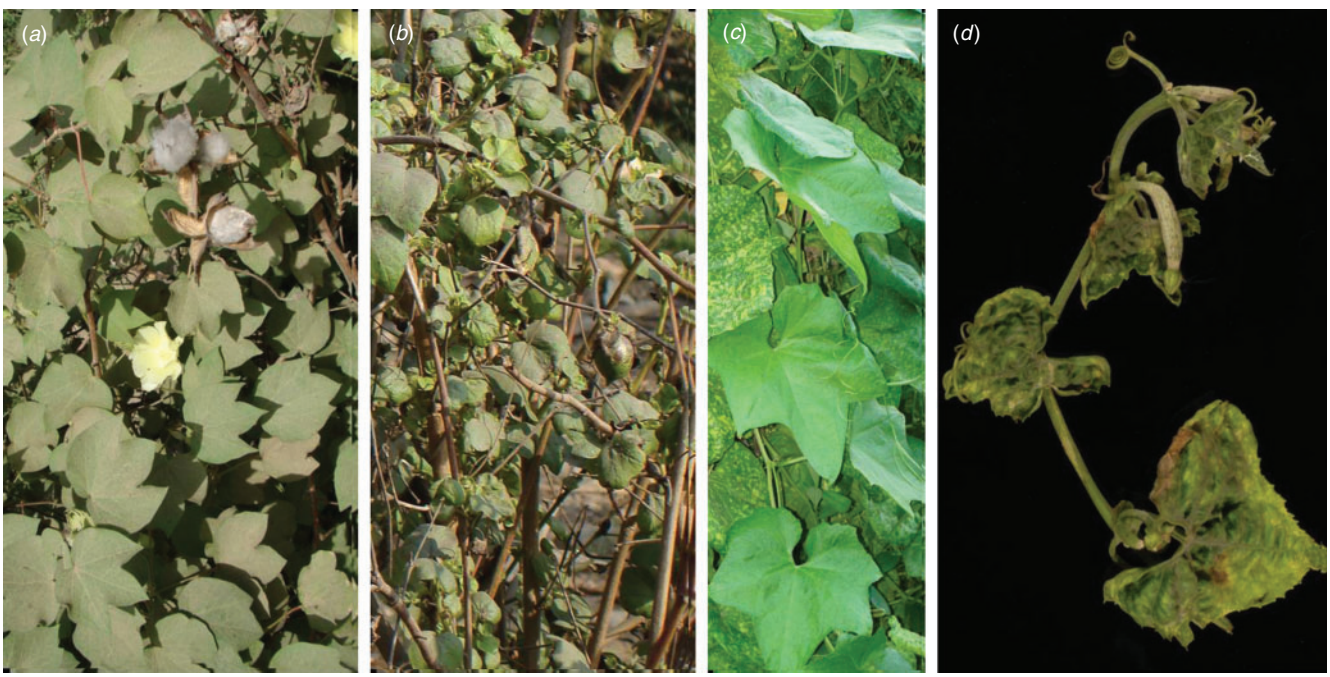

Fig. 1. (*a*) Healthy cotton plant. (*b*) Infected cotton plant showing little leaf symptoms. (*c*) Healthy luffa plant. (*d*) Infected luffa twig showing small yellowing leaves.

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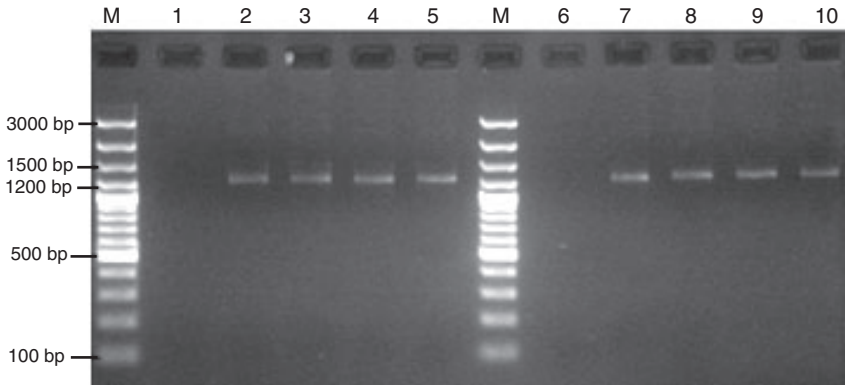

Fig. 2. Agarose gel (1.2%) showing ≈1.25 Kb band obtained by nested polymerase chain reaction with phytoplasma-specific 16S rDNA primers P1/P7 and R16F2/R2. Lanes 1, 6: symptomless samples. Lanes 2–5: samples of symptomatic cotton. Lanes 7–10: samples of symptomatic luffa. M-100 bp DNA marker (MBI Fermentas, Vilnius, Lithuania).

USA) and directly sequenced. Analysis of both the sequences using the BLAST tool from http://www.ncbi.nlm.nih.gov/ showed 99% sequence similarity to several 16SrI phytoplasma members, namely sesame phyllody (AB558132.1), Japanese spurge yellows (AB551736.1), *Eryngium foetidum* witches' broom (GU113155.1), periwinkle little leaf (GU113147.1), mulberry yellow dwarf (GQ249410.1) phytoplasmas. The partial 16S rDNA sequences were submitted to GenBank with Accession Nos. HM467914 and HM467913 for cotton and luffa little leaf phytoplasma, respectively. A dendrogram was

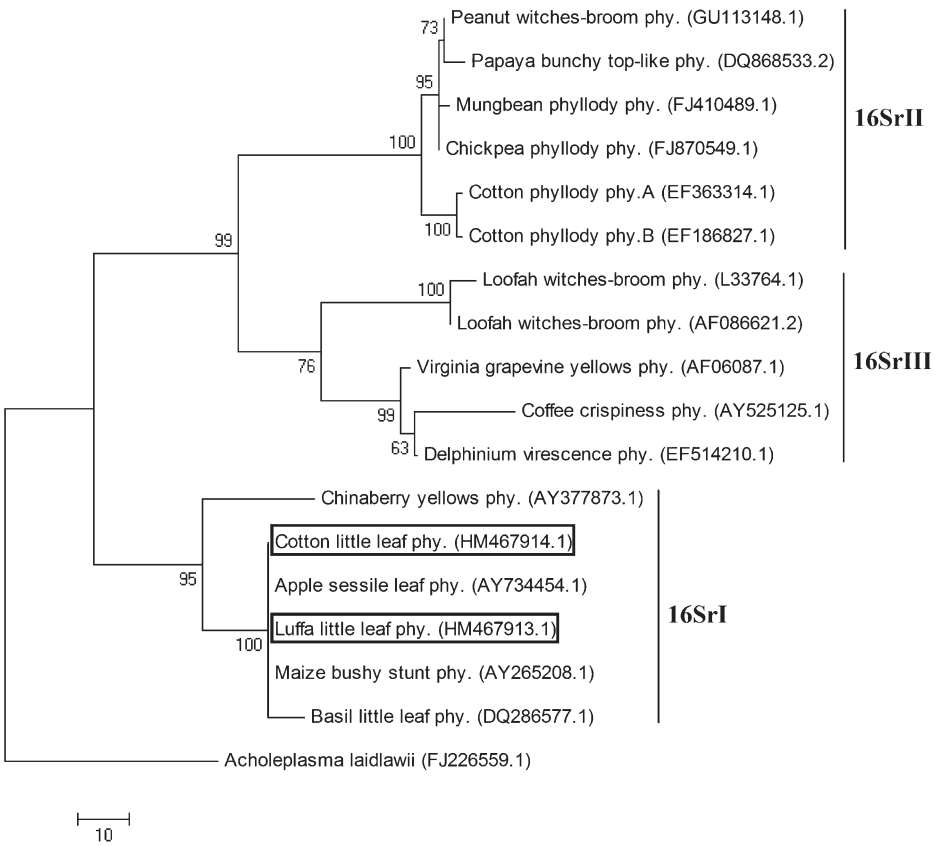

Fig. 3. Phylogenetic tree based on the partial 16S rDNA sequences of phytoplasmas from the NCBI database. Cotton and luffa little leaf phytoplasma New Delhi isolates are boxed. The tree was constructed using maximum parsimony method of MEGA v.4.1 with 100 replicates (bootstrap values are shown at each node). *Acholeplasma laidlawii* was used as an outgroup for rooting the tree.

constructed using maximum parsimony method of MEGA software v.4.1 (Tamura *et al*. 2007) with phytoplasma 16S rDNA sequences as raw data. The evolutionary tree depicted the cotton and luffa little leaf phytoplasma to cluster within the 16SrI clade (Fig. 3), inferring their close relationship with the aster yellow members. The earlier reported cotton (EF186827.1, EF363314.1) and luffa (AF086621.2, L33764.1) phytoplasmas took distinct positions in the 16SrII and 16SrIII cluster. Thus, the sequence and phylogenetic analysis assign the phytoplasma associated with little leaf of cotton and luffa to '*Ca*. Phytoplasma asteris'.

Molecular investigations to date have shown phytoplasmas to be associated with cotton phyllody in Italy, Burkina Faso, Mali (Khan *et al*. 2002; Martini *et al*. 2007; Marzachi *et al*. 2009) and luffa witches' broom in Brazil (Montano *et al*. 2007). This is the first record of a phytoplasma associated with both these economically important crops in the Indian subcontinent. Additionally, cotton and luffa are hereby reported as the two new hosts of 16SrI group phytoplasmas.

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Manuscript received 28 June 2010, accepted 6 October 2010