New Disease Reports

A '*Candidatus* Phytoplasma asteris' isolate associated with bud proliferation disease of cowpea in India

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Vigna unguiculata subsp. *unguiculata*, commonly known as the cowpea or black eyed pea, is one of the most important legumes across the semi-arid tropics valued for its pods and dried seeds. Cowpea plants severely affected with bud proliferation disease were observed during July to September 2009 in New Delhi, India. The infected plants were devoid of normal leaf patterning, flowers and fruits. The leaves present were thicker and dark green in colour compared to the healthy plants and showed extensive bud proliferation on the main shoot (Fig. 1). Most of the plants were stunted. Such features are usually associated with phloem-inhabiting wall-less bacteria, the phytoplasmas (class *Mollicutes*).

To investigate the association of phytoplasmas with the disease, ten plants with symptoms and two symptomless plants were collected from the botanical garden at the University of Delhi. Stem tissues were pulverised in liquid nitrogen and processed for genomic DNA extraction by the CTAB method (Doyle & Doyle, 1990). Purified DNA was used as template (10ng/µl) for the 16S ribosomal DNA amplification in a nested-polymerase chain reaction with phytoplasma specific primer pairs P1/P7 (Deng & Hiruki, 1991) and R16F2n/R2 (F2n/R2) (Gundersen & Lee, 1996). Amplification products resolved on a 1.2% agarose gel revealed amplicons of expected size (~1.25 kb) for the symptom-bearing plants only. Three PCR products were gel-extracted and purified using QIAquick Gel Extraction Kit (QIAGEN, USA), sequenced bi-directionally and aligned. All three sequences obtained shared 100% identity with each other and the consensus sequence of the Vigna bud proliferation phytoplasma was submitted to GenBank (Accession No. HM449952). BLAST searches revealed the 16S rDNA sequence of the cowpea phytoplasma to share 99% identity with those of phytoplasmas of 16SrI group, '*Candidatus* Phytoplasma asteris' members, including *Brassica napus* phyllody (JN193482.1), periwinkle little leaf (AB646266.1) and maize bushy stunt (HQ530152.1) phytoplasmas.

Three F2n/R2 amplicons were subjected to restriction fragment length polymorphism analysis (RFLP) using six restriction endonucleases (REs), as per manufacturer's instructions (Fermentas, Lithuania). The REs were randomly selected on the basis of their ability to assign a 16SrI member(*Alu*I, *Mse*I, *Sau*3AI, *Taq*I) and which could differentiate within 16SrI subgroups (*Bfa*I, *Hha*I). The digestion products were run on a 6% polyacrylamide gel and visualised under a gel-documentation system (Bio-Rad, USA). The RFLP patterns obtained matched the profiles of phytoplasmas belonging to 16SrI-B subgroup reported in previous studies (Lee *et al*., 1998). A dendrogram constructed by the maximum parsimony method of MEGA v4.01 using reference phytoplasma 16S rDNA sequences reported earlier, confirmed that the Vigna bud proliferation phytoplasma is related to the 16SrI group (Fig. 2). A 16SrXII-B strain of '*Ca.* Phytoplasma australiense' has been associated with witches' broom and small leaves of *V. unguiculata* var. *sesquipedalis* in Australia (Saqib *et al*., 2006). A Vigna little leaf phytoplasma belonging to group 16SrV has also been reported (De La Rue *et al*., 2001). However, this is the first report of '*Ca*. P. asteris' affecting *Vigna* in India and worldwide. The identification of symptoms of bud proliferation disease and the association with a 16SrI-B phytoplasma for cowpea is a significant tool to identify and locate infectious plants in the field and prevent spread of the phytoplasma to the whole crop.

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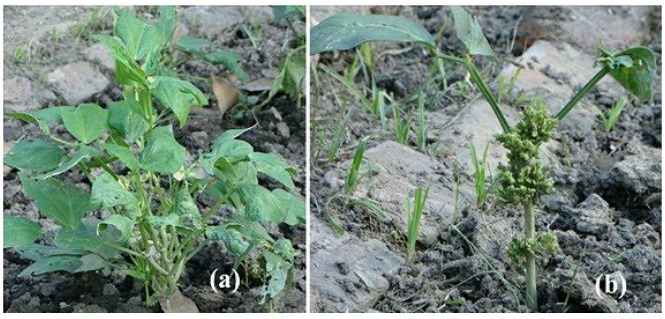

Figure 1

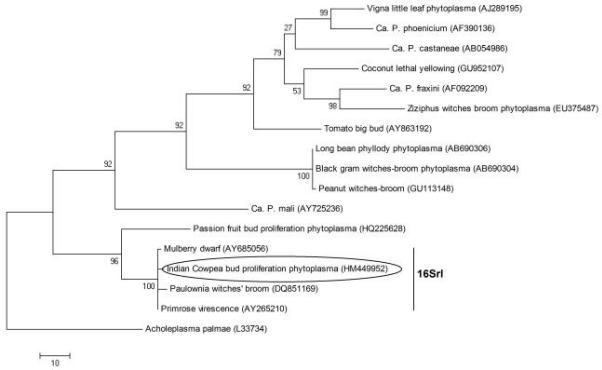

Figure 2

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