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Disease Notes



# First Report of a 16Sr I-B Phytoplasma Associated with Phyllody and Stem Fasciation of Flax (*Linum usitatissimum*) in India

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Flax or linseed is grown as a fiber or oilseed crop in tropical and temperate regions. It is commercially cultivated in many countries of the world including Canada, China, India, the United States, Ethiopia, Pakistan, Russia, Poland, and Argentina (1). In December 2013, symptoms suggestive of phytoplasma infection were noticed on flax in different experimental fields of Central Research Institute for Jute and Allied Fibres (CRIJAF) research farm, Barrackpore, India, and the incidence was less than 2%. Because incidence of phytoplasma diseases are increasing worldwide, occurrence of a phytoplasma in a new geographical area poses an imminent threat. The infected plants showed floral virescence, phyllody, and stem fasciation (flattened stem). Floral malformation was very conspicuous with abnormal structures replacing normal flowers. All the floral parts, including petals, turned into green leaves. Total DNA was extracted from leaf mid veins of three symptomatic and three asymptomatic plants using a DNeasy Plant Mini Kit (Qiagen). PCR was carried out with the phytoplasma-specific

universal P1/P7 primer set followed by nested primer pair R16F2n/R16R2 (2), resulting in DNA amplicons that were 1.8 kb and 1.2 kb, respectively, in all symptomatic samples tested. No amplification was observed with DNA from symptomless samples. This suggested association of a phytoplasma with the disease. The five purified nested PCR products were cloned in a pGEM-T Easy vector (Promega) and sequenced. One of the sequences that proved to be identical to the others was deposited in GenBank (Accession No. KJ417660). The consensus sequence was analyzed by NCBI BLAST and found to share 99% similarity with the 16Sr DNA sequence of the '*Candidatus* Phytoplasma asteris' reference strain (GenBank HQ828108), which belongs to 16SrI group. The phylogenetic tree based on 16SrDNA sequence of phytoplasmas belonging to group 16SrI and other distinct phytoplasma groups also showed that the phytoplasma clustered with members of group 16SrI (3). The nested PCR product of R16F2n/R16R2 was digested using restriction enzymes *AluI*, *BfaI*, *BstU*, *HhaI*, *HpaI*, *KpnI*, *MseI*, and *RsaI*. The RFLP patterns were compared with those of known phytoplasma strains (2) and they matched the patterns for aster yellows subgroup B (16Sr I-B). Subsequently, the *iPhyClassifier* 16Sr group/subgroup classification based on similarity (4) analyses showed that the studied strain had 16SrDNA sequences in the 16SrI-B group with a similarity coefficient of 1.00. To the best of our knowledge, this is the first report of 16SrI-B phytoplasma associated with flax in India.

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