Molecular characterization of phytoplasma of 16SrI-B group association with *Acalypha indica* in India

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Abstract The typical phytoplasma symptoms of little leaf, yellowing, chlorosis, witches' broom, and stunting growth were observed on *Acalypha indica* plants during the field survey conducted at Lucknow and surrounding districts in year 2015–2016. To confirm the association and possibility of phytoplasma etiology, PCR assays were performed using universal primer pairs (P1/P6) and nested primer pairs (R16F2n/R2) in a total of five diseased samples along with control. A ~1.2 Kb amplicon was observed in nested PCR assay in diseased sample; however, no band was observed in control sample. The positive amplicons were sequenced for 16S rDNA and used for the virtual RFLP analysis and phylogenetic studies. BLASTn search showed 99–100% sequence identities with the '*Candidatus* phytoplasma asteris' members (16SrI group) and phylogenetic analysis showed closest relationship with member of 16SrI group. The virtual RFLP assigned it as a member of 16SrI-B subgroup. This is the first record of phytoplasma association of '*Ca. P. asteris*' subgroup B with *A. indica* in the world.

Keywords *Acalypha indica* · Mollicutes · *Candidatus* phytoplasma asteris · Little leaf · Witches broom

Introduction

Phytoplasma (earlier—mycoplasma-like organism (MLO)) are cell wall-less Mollicutes that colonize plant phloem sieve tube elements. They are emerging and cause devastating losses in crops and natural ecosystems worldwide. Phytoplasma are transmitted from one plant to others by phloem-feeding insects, primarily leafhoppers, plant hoppers, and psyllids (Bertaccini et al. 2014). They have small genomes and cause diseases in many economically important plants. Generally Phytoplasma causes different symptoms of yellowing, stunting of plants, proliferation of shoots, phyllody, verscence, and reduced size in plants which affects their economic value (Bertaccini 2015). The '*Ca. P. asteris*' 16SrI group is the major group infecting different plant species worldwide (Madhupriya 2016).

Acalypha indica occurs widely throughout the tropics of the old world. It belongs to the family Euphorbiaceae and a common weed in many parts of Asia including India, Pakistan, Yemen, Sri Lanka, and throughout Tropical Africa and South America (Ramachandran 2008). It has been introduced to the areas of the new world with favorable climates. *A. indica* L. is the most frequently and abundantly occurring weed plant in north India during the rainy season. The plants have medicinal properties and are useful in bronchitis, asthma, pneumonia, and rheumatism. Its roots and leaves have laxative properties. This plant is held in high esteem in traditional Tamil Siddha medicine as it is believed to rejuvenate the body (Ramachandran 2008). In Africa, the leaves are cooked and eaten as a

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vegetable and also browsed by cattle (Schmelzer and Gurib-Fakim 2008).

In the last few years, many reports focused on the emergences related to new phytoplasma diseases or new outbreaks of already known ones. Diagnosis of phytoplasma through nested PCR assays using universal primers allow the detection of a wide range of unknown phytoplasmas associated with plants (Gundersen-Rindal and Lee 1996). In the current investigation, we are reporting an association of ‘*Ca. P. asteris*’ subgroup B with *A. indica* in the world for the first time by nested PCR, sequence analysis, and RFLP.

Materials and methods

During survey of Lucknow district in 2015–2016, little leaf and witches’ broom symptoms were recorded in *A. indica*, which were collected for the characterization of associated phytoplasma. Five symptomatic and non-symptomatic leaves samples were collected. Total genomic DNA were isolated according to Ahrens and Seemuller (1992) from symptomatic and non-symptomatic leaf samples and isolated DNA were subjected to PCR with universal primer pairs (P1/P6) (Deng and Hiruki 1991). PCR product was used as template DNA (1:20) and nested PCR was done with R16F2n/R2 primer (Gundersen-Rindal and Lee 1996). 1% gel electrophoresis was done to check the presence of phytoplasma. Amplified products were eluted (Nucleo spin, Germany) and directly sequenced. The sequenced products were assembled using BioEdit and used for BLASTn analysis. Phylogenetic analysis was done by MEGA 5.1 (Tamura et al. 2011) with identical sequences available in GenaBank and representatives of different groups of phytoplasma.

The *A. indica* phytoplasma strain sequence corresponding to the F2nR2 region was subjected to in silico RFLP analysis using pDRAW32 program developed by AcaClone Software (<http://www.acaclone.com>) and compared with representative sequences of the ‘*Ca. P. asteris*’ subgroups for assigning 16Sr subgroups classification of to *A. indica* phytoplasma strains analyzed by the same restricting mapping utilizing AcaClone software generated RFLP sequences.

Results and discussions

During September 2015–2016, little leaf symptoms, leaf yellowing, and witches’ broom with a 2.3% disease incidence were observed on *A. indica* plant at Lucknow

Fig. 1 *A. indica* plants showing typical witches’ broom, little leaf, and leaf yellowing symptoms at Lucknow place of UP, India. (a) Healthy and b diseased plant)

place of Uttar Pradesh, India (Fig. 1). In nested PCR analysis, all five symptom-bearing plant samples showed ~1.2 kb amplicon, which was purified and processed for sequencing. BLASTn analysis of the 16S rRNA gene sequence from *A. indica*, assembled nucleotide sequence (~1.2 kb) showed a maximum 99–100% sequence identity with those of the ‘*Candidatus* phytoplasma asteris’ members (16SrI group). During phylogenetic analysis, *A. indica* isolate (KX139546) clustered with members of ‘*Ca. P. asteris*’ and subgroup B (Fig. 2). Virtual RFLP using pDRAW32 program developed by AcaClone Software, the isolate under study showed similar banding patterns with reference strains of (GenBank Acc. No. M30790), a member of 16SrI-B subgroup, hence our isolate may be assigned as a member of the same subgroup strain (Fig. 3). In the present study, we report the association of ‘*Ca. P. asteris*’ (16SrI-B group) with *A. indica* plant in Uttar Pradesh, India which might be having an economic impact on other commercial crops and needs further investigations. In India, Singh and Gupta (2011) reported witches’ broom disease on *A. indica* only based on the symptomatological studies and could not identify the causal pathogen at its taxonomical level. To our knowledge, this is the first report of 16SrI-B subgroup phytoplasma on *A. indica* plant in India. Weeds like *Ageratum conyzoides*, *Arundo donax*, and *Codiaeum variegatum* (Tiwari et al. 2012, 2014, 2015) has already been reported from Uttar Pradesh, India in recent years as a host of 16SrI group and present study reporting another host of 16Sr I group phytoplasma from the same state.

It is well studied that weed plants often work as a reservoir/green bridge of pathogens as well as insect vectors during the off-season of economic crops and are transmitted to commercially important crops during their

Fig. 2 Phylogenetic tree based on 16SrDNA constructed by neighbor-joining method showing the relationships among *Aclypha* phytoplasma isolate and reference phytoplasma strains. Accession numbers are specified in the tree. *A. laidlawii* was used as an out group

Reference sequence of 16Sr IB

Aclypha phytoplasma

Fig. 3 Restriction site map of F2nR2 region of reference phytoplasma subgroup with 17 restriction enzymes (*AluI*, *BamHI*, *BfaI*, *BstUI*, *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *MboI*

(*Sau3AI*), *MseI*, *RsaI*, *SspI*, and *TaqI*) generated using pDRAW software. Phytoplasma isolate (Acc. No. KX139546) produced virtual RFLP profile identical to reference phytoplasma (16Sr-IB, M30790)

growing seasons by the transmitting insect vectors and thus play important role in the survival and transmission of the disease/pathogen round the year.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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