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FIRST REPORT OF 16SRII-I SUBGROUP OF PHYTOPLASMA ASSOCIATED WITH PHYLLODY DISEASE OF *PARTHENIUM HYSTEROPHORUS* IN INDIA

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ABSTRACT

Parthenium hysterophorus is commonly known as "*Gajar Ghans*", is widely distributed weed worldwide. Parthenium is an alternative host for many phytoplasma diseases. In the present study, Parthenium with characteristic phytoplasma symptoms were observed while surveying the sugarcane fields at ICAR-Indian Institute of Sugarcane Research, Biological Control Centre, Pravaranagar, Maharashtra. Four phytoplasma infected Parthenium samples were collected and subjected to genomic DNA extraction followed by nested PCR assay. The 45% disease incidence was observed in five surveyed fields of sugarcane. Nested PCR assay results revealed the amplification of ~1.2 kb size amplicons in the four samples which were gel purified and sequenced. The sequence comparison and phylogenetic analyses of the first two Parthenium isolates (P1 and P2) having Acc. Nos. *viz.*, MT541822 and MT541823 revealed 98% and 97% sequence identity, respectively with the 'Opuntia ficus-indica' phytoplasma (Acc. No. JQ181545) belonging to 16SrII-I subgroup. To our knowledge this is first report of association of 16SrII-I subgroup of phytoplasma with Parthenium phyllody disease in India.

Key words: '*Candidatus* Phytoplasma aurantifolia', 16SrII-I subgroup, Parthenium phyllody.

Introduction

Parthenium hysterophorus belonging to family *Asteraceae,* is commonly known as "*Gajar Ghans*", is widely distributed in the Australia, Ethiopia, Indian subcontinent, South Asia, East Africa and Tropical America (Janke *et al.*, 2007). Parthenium is an important weed host for different bacterial, viral, phytoplasma diseases and several pests (Lakshmi and Srinivas, 2007).

In India, 16SrII-C and 16SrII-D subgroup of phytoplasma is known to infect Parthenium and cause "witches' Broom" disease (Yadav *et al.*, 2015). Moreover, phytoplasma 16Sr-I group '*Ca*. Phytoplasma asteris', is also known to infect Parthenium (Raj *et al.*, 2008). Parthenium plants present in the five sugarcane fields were observed with the characteristic phytoplasma symptoms while surveying for sugarcane pests and diseases at ICAR- Indian Institute of Sugarcane Research, Biological Control Centre, Pravaranagar, Maharashtra during July, 2019.

Materials and Methods

The phytoplasma affected Parthenium plants exhibited various phenotypic symptoms including phyllody, witches' broom, yellowing with smaller leaves, bunching and stunted growth of the plants (Fig. 1a-d). A total of four phytoplasma infected Parthenium plant samples were collected from the surveyed fields. Total genomic DNA was extracted from four isolates using the DNeasy plant mini kit (Qiagen, Germany). The nested PCR assay using 16S rRNA gene universal primers P1/P7 (P1: 52 - AAGAGTTTGATCCTGGCTCAGGATT-32 P7: 52 - CGTCCTTCATCGG CTCTT-32 Deng and Hiruki 1991; Schneider *et al.*, 1995) and R16F2n/R16R2 (R16F2n: 52

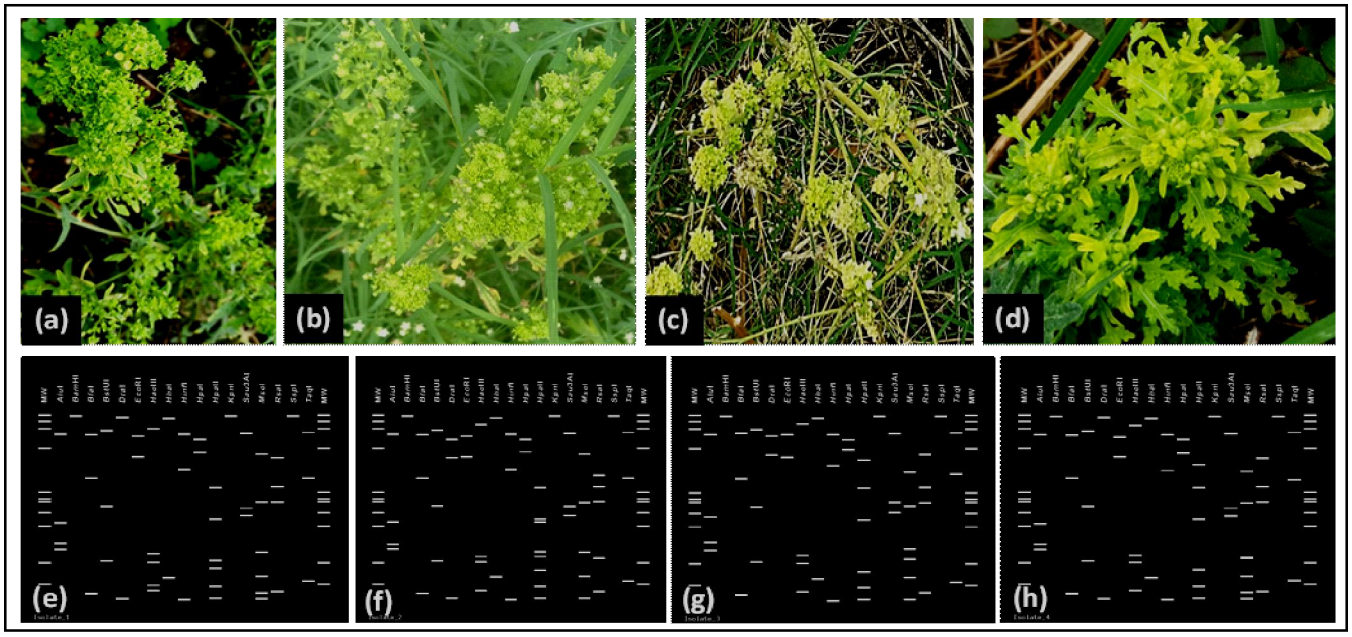

Fig. 1: Natural occurrence of phyllody disease in Parthenium characterized by different symptoms including witches broom like appearance (a), phyllody (b), yellowing with smaller leaves and bunching (c) and stunted growth (d). The virtual restriction fragment length polymorphism (RFLP) profile of the four phytoplasma isolates (e, f, g and h).

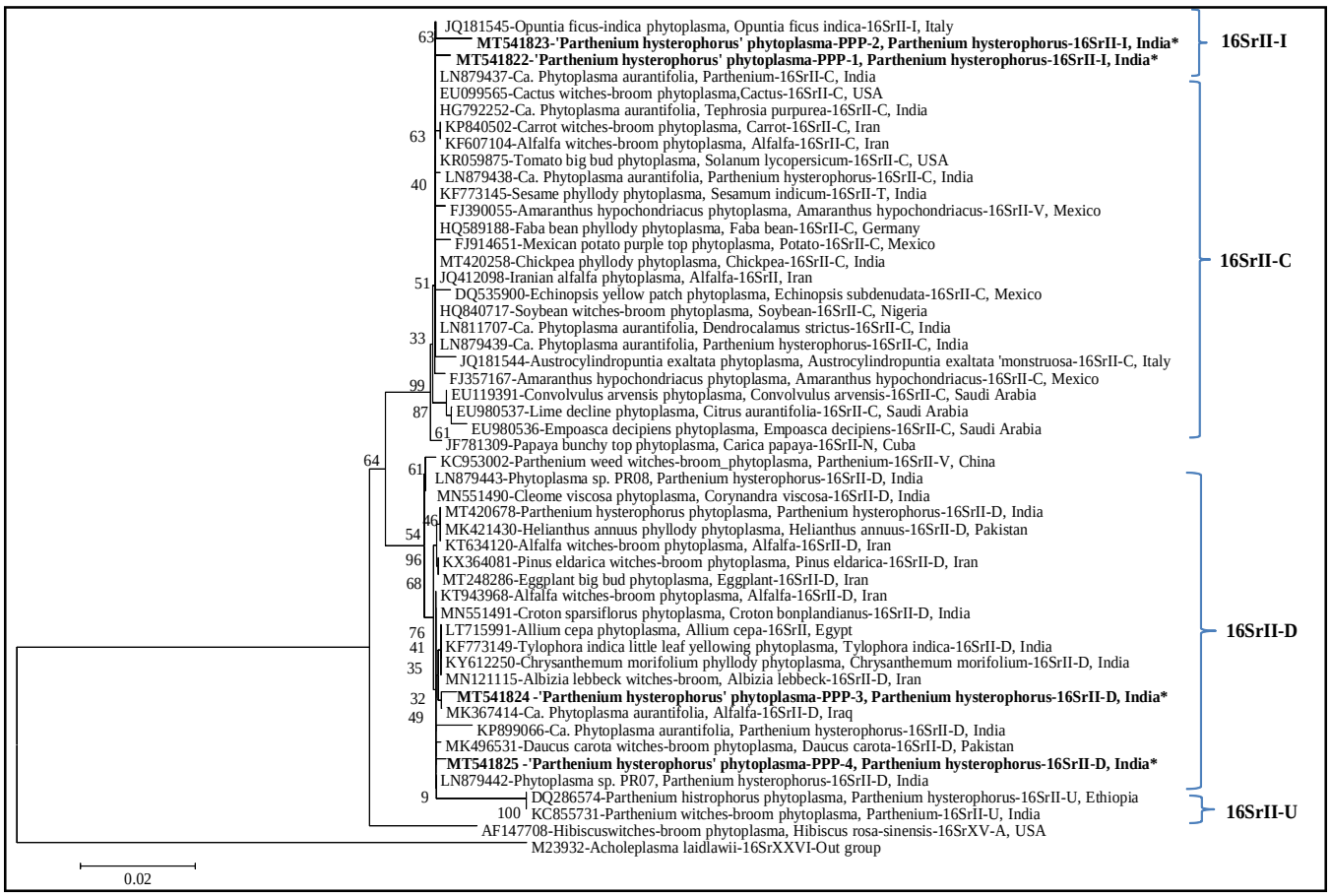

Fig. 2: Phylogenetic tree based on evolutionary history of 16S rRNA gene sequences of four Parthenium phytoplasma isolates was inferred using the Neighbor-Joining method with the available phytoplasma strains in the NCBI GenBank. The optimal tree with the sum of branch length = 0.25423830 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test at 1000 replicates is shown next to the branches. Evolutionary analyses were conducted in MEGA6. *Parthenium phytoplasma isolates from the present study are indicated in bold font.

-GAA ACGACTGCTAAGACTGG-32; R16R2: 52 -TGACGGGCGGTGTGTACAAACCCCG-3') (Gunderson and Lee, 1996) revealed the amplification of ~1.8 kb and ~1.2 kb size amplicons in the first and second rounds, respectively from four Parthenium isolates and in a positive control (data not shown). The amplicons were gel eluted following manufacturer's protocol of PCR purification kit (Sigma-Aldrich, USA) and outsourced for double pass Sanger sequencing and quality of the nucleotides ensured based on the q value.

Results and Discussion

The nucleotide sequence comparison was made by the CLUSTAL W multiple alignments of P1 and P2 isolates having Acc. Nos. MT541822 and MT541823 shared 98% and 97% sequence identity with the 'Opuntia ficus-indica' phytoplasma from Italy (Acc. No. JQ181545) belonging to 16SrII-I subgroup (Fig. 2). The phylogenetic dendrogram of the four isolates was constructed by Neighbour-Joining Method using MEGA 6.0 software program (Tamura *et al.*, 2013) which revealed the close clustering of P1 and P2 isolates with the known sequences of 16SrII-I subgroup of phytoplasma available in the NCBI GenBank. Moreover, virtual restriction fragment length polymorphism (RFLP) profile of 16Sr RNA genes P1 and P2 isolates through computer-simulated *i*Phyclassifier program (https://plantpathology.ba.ars.usda.gov/; Zhao *et al.*, 2009) revealed 0.61 and 0.58 coefficient pattern similarities, respectively, with new subgroup of phytoplasma (16SrII-I) (Fig. 1e-f). The sequence comparison of P3 and P4 isolates having Acc. Nos. MT541824 and MT541825 revealed 96% and 98% sequence identity with '*Ca*. Phytoplasma aurantifolia' (16SrII-D) (Fig. 2), with the similarity coefficient of 0.60 and 0.67 (Fig. 1g-h), respectively, which is known to occur on Parthenium in India (Yadav *et al.*, 2015). In phylogenetic analysis, P3 and P4 isolates clustered closely with the isolates belonging to the 16SrII-D subgroup (Fig. 2). To our knowledge this is the first report of association of 16SrII-I subgroup of phytoplasma (Opuntia ficus-indica phytoplasma) with "phyllody disease" in Parthenium in India.

Conflict of Interest: All the authors declare that they do not have any conflict of interest

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Data Availability: Sequence information of four isolates is available under accession numbers P1: MT541822, P2: MT541823, P3: MT541824, P4: MT541825.

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