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Molecular characterization of phytoplasma associated with phyllody of *Pedalium murex* - a common weed in coconut plantations

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Abstract Phytoplasma belonging to subgroup 16SrII-A and identified as "*Candidatus* Phytoplasma *australasiae*"- was found constantly associated with phyllody of *Pedalium murex*, a common medicinal cum succulent weed in coconut ecosystem prevalent in South India. Infected *P. murex* showed stunted growth with reduced leaf size, shortened internodes and the characteristic transformation of floral parts into leafy structures typical to that of phytoplasma disease. This weed could possibly serve as a reservoir of sesamum phyllody in this region but no link with root (wilt) disease in coconut could be established based on molecular characterization studies. This is the first report of the association of 16SrII-A group phytoplasma with pedalium phyllody from the world.

Keywords Phytopathogenic mollicutes · 16SrII-A phytoplasma · Sesamum phyllody · Coconut root (wilt) disease

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Introduction

Pedalium murex Linn. (Family: Pedaliaceae) is a medicinal cum succulent weed observed in the coconut plantations in coastal tract of southern India. This herb shows the presence of alkaloids, flavanoids, tannins and phenolic compounds which are responsible for its medicinal properties (Rajashekar *et al.* 2012). The weed grows very prolific after the summer rains in the Onattukara region. Weeds, in general, are plants "out of place" that compete for light, space, nutrients and water with the main crop. In addition, weeds are suspected reservoirs of pests and pathogens including phytoplasma and serve as source of residual inoculum for the cultivated main crops. Plant diseases particularly phytopathogenic mollicutes infected weeds are of paramount importance in serving as alternate / collateral hosts in the process of disease spread. Onattukara region which includes taluks of Mavelikkara, Karthikapalli, Chengannur and Karunagapally with geographical position ranging from 9°3′16″N Latitude to 76°36′50.46″E Longitude is well known for the cultivation of two important crops *viz.,* coconut [*Cocos nucifera* Linn.(Family: Arecaceae)] and sesamum [*Sesamum indicum* Linn. (Family: Pedaliaceae)]. Both these crops suffer from phytoplasmal diseases such as root (wilt) disease (RWD) in coconut and phyllody in sesamum significantly affecting the yield and livelihood security of the farming community. Former one infecting coconut is a non-lethal, debilitating malady with constant association of 16Sr XI group phytoplasma (Manimekalai *et al.* 2010) and the latter one infecting

sesamum completely transforms the floral parts into vegetative structures belongs to 16Sr II group phytoplasma (Akhtar *et al.* 2008). Yield loss as high as 968 million nuts (Solomon and Geetha 2004) and seed yield loss up to 33.9 percent (Abraham *et al.* 1977) were reported from coconut and sesamum respectively, due to the phytoplasmal diseases. Weeds in the region were thus carefully examined for the presence of phytoplasma-induced symptoms so as to determine the occurrence of any linking factor of sesamum phyllody and coconut RWD by thorough characterization of phytoplasma in them. In this investigation during October 2013, some pedalium plants in coconut plantations from this region showed phyllody symptoms typical to that of phytoplasma infection. Infected plants showed stunted growth with reduction in leaf size, shortening of internodes and transformation of floral parts into leafy structures. Though phytoplasma has been reported to be associated with this disease by light and electron microscopy (Misra and Joshi 1983) molecular characterization has not been undertaken yet.

Materials and methods

Total DNA was extracted from the leaf midrib and flowers of five symptomatic and non-symptomatic *P. murex* samples using cetyltrimethylammonium bromide (CTAB) method (Lodhi *et al.* 1994). The DNA extracted from grassy shoot disease affected sugarcane served as the positive control. The 16S rDNA gene was amplified by Polymerase Chain Reaction (PCR) using primer pairs P1/P7 (Deng and Hiruki 1991; Schneider *et al.* 1995) nested with R16 F2n / R16 R2 (Gundersen and Lee 1996). The thermal conditions of the primary PCR were as follows: initial denaturation was maintained at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 55°C for 1 min and extension at 72°C for 2 min. The final extension was done at 72°C for 10 min. The primary PCR product was diluted to 1:10 with sterile distilled water and two micro-litre of the diluted product was used for nested PCR with primer pair R16F2n/R16R2. An annealing temperature of 56°C was followed in the nested PCR. All the PCR amplifications were carried out in Techne Flexigene thermal cycler. The amplicons were resolved in 1.2% agarose gels and documented using Biorad Molecular Imager Gel doc XR system, USA. PCR products amplified with P1/P7 alone and nested with R16F2n/R16R2 were eluted (QIAquick Gel Extraction kit, Qiagen, Germany) and directly sequenced. A database search of sequences homologous to the16S rDNA sequences under study was performed by BLAST analysis at National Centre for Biotechnology Information (NCBI) (Altschul *et al.* 1990).

In silico restriction analysis and virtual gel plotting with restriction endonucleases *Alu*I, *Bam*HI, *Bfa*I, *Bst*UI, *Dra*I, *Eco*RI, *Hae*III, *Hha*I, *Hin*fI, *Hpa*I, *Hpa*II, *Kpn*I, *Sau*3AI, *Mse*I, *Rsa*I, *Ssp*I and *Taq*I were obtained

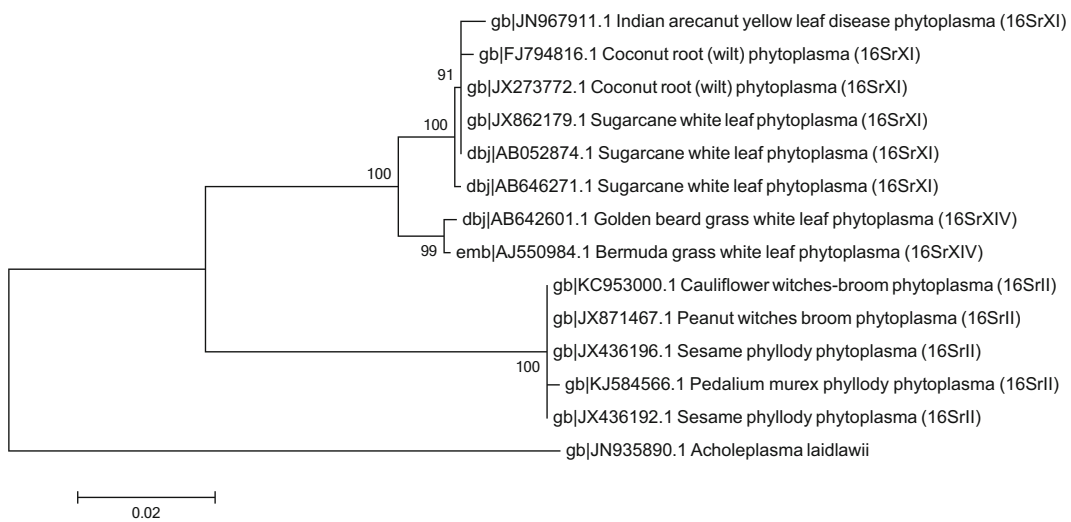

Fig. 1 Phylogenetic tree comparing the 16S rDNA sequences of *Pedalium murex* phyllody phytoplasma with 16Sr XI, 16Sr XIV and 16Sr II groups of phytoplasma employing *A. laidlawii* as outgroup

using the *i*Phy-Classifier online tool (http: //www.plantpathology.ba.ars.usda.gov/cgibin/resource/iphyclassifier.cgi) as described by (Zhao *et al*. 2009). The restriction pattern obtained was compared with the virtual RFLP gel of different phytoplasmal groups. To analyze the relatedness of the pedalium phyllody phytoplasma with other phytoplasmal groups, phylogenetic tree was constructed with partial 16Sr DNA sequences using Mega 6.0 software by neighbour joining method with 1000 replications for bootstrap analysis.

Results and discussion

Amplicons of approximately 1.2 kb was obtained from all the symptomatic samples by using universal primers P1/P7-R16F2n/R16R2. Asymptomatic samples were devoid of any amplicon at desired base pair level. The sequences obtained from primary PCR with a length of 1685 bp (Accession No.KJ873878) and nested PCR with 1172 bp (Accession No.KJ584566) were deposited in NCBI GenBank. Blast analysis of the 16S rDNA gene sequence of pedalium phyllody phytoplasma showed 99% similarity to the nucleotide sequences of phytoplasmas associated with cauliflower witches'-broom (KC953000), peanut witches'-broom (JX871467), tomato big bud (JQ923436) and sesamum phyllody (JX436196).

Analysis with *i*PhyClassifier software revealed that the 16S rDNA sequence of pedalium phyllody phytoplasma shared 99.8% similarity with that of the '*Candidatus* Phytoplasma *australasia*' reference strain (GenBank accession: Y10097). The virtual RFLP pattern derived from the 16S rDNA fragment was identical (similarity coefficient 1.00) to the reference pattern of 16Sr group II, subgroup A (GenBank accession: L33765). Combining the sequence analysis and virtual RFLP pattern generated by *i*PhyClassifier, the phytoplasma causing pedalium phyllody was identified as '*Candidatus* Phytoplasma *australasia*'-related strain belonging to subgroup 16SrII-A.

Phylogenetic analysis of 16S rDNA sequences revealed that *P. murex* phyllody phytoplasma clustered with sesamum phyllody phytoplasma but its association with coconut RWD belonging to 16Sr XI group could not be established on account of distant phylogenetic link (Fig. 1). Sweep net surveys in the locality also indicated the presence of the vector of sesame phyllody, *Orosius albicinctus*. Molecular detection of phytoplasma in vector is in progress. Present studies indicate that pedalium phyllody could possibly serve as a reservoir of sesamum phyllody in the region but not for coconut RWD. To our knowledge, this is the first report in the world on the association of 16SrII-A group phytoplasma with pedalium phyllody.

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