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Incidence of a *Candidatus* Phytoplasma Associated with Phyllody Disease of *Solanum trilobatum*

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ABSTRACT

Changes in climatic conditions has resulted in the expression of newer diseases due to vector multiplication in case of virus and phytoplasma. In Solanum trilobatum, a typical phyllody symptoms with a pale green colouration in veinal and interveinal region with pinkish patches on leaf lamina , leaf curling with reduced size of upper young leaves developing in to phyllody symptoms were observed . PCR amplicons of expected size (1.8 kb) were obtained in direct PCR amplifications with most of the symptomatic plant samples, followed by nested-PCR amplifications yielded phytoplasma-specific PCR amplicons (1.2 kb) from all plants with phyllody symptoms. Probably this is the first report of occurrence of Candidatus phytoplasma of S. trilobatum in India

Key words: *Solanum trilobatum, Phytoplasma, PCR, Phyllody*

INTRODUCTION

Solanum trilobatum (L.) (Family: *Solanaceae*) the night shade (pea eggplant) is one of the important medicinal shrub grows as a thorny creeper with bluish white flower and become a climber, more commonly found in Southern India. *S. trilobatum* is a pervasively used Indian medicine to cure various human diseases. It was distributed extensively in the southern parts of India and has been used in herbal medicine to treat various diseases like respiratory problems, bronchial asthma, cough and tuberculosis. Its commercial cultivation and promotion in Tamil Nadu has led to its increased popularity and use as a medicinal crop. Most *Solanaceae* family members are prone to fungal, bacterial and viral diseases. These can be easily spread *via* weed species of the family or/and by its vectors. Among them, the phytoplasmas have established as a unique group of plant pathogens[3] and can cause significant yield losses in many economically important crops around the world[7]. It is a wall-less, intracellular, non-helical prokaryotes that colonize plant phloem sieve cells and insects, which were formerly known as mycoplasma-like organisms[1]. Phyllody symptoms were observed in plants of *S. trilobatum* at several places in and around TNAU of Coimbatore District, Tamil Nadu during August 2017 to November 2017.

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The symptoms in the field initially included a pale green colouration in veinalandinterveinal region with pinkish patches on leaf lamina (Fig. 1), curled and reduced size of upper young leaves that progressively developed to crowding of leaves and individual leaves were thicker than normal and leathery in texture (Fig. 2), with cessation of internode elongation. It caused significant damage to the plants by severe stunting, cessation of floral parts with no fruits in fields. The entire apical shoots were converted into twisted reduced leaves closely arranged on the top of the stem, with very short internodes. Infections that occurred later in the season caused characteristic symptoms, such as virescence, phyllody, and witches' broom (Fig. 3).

Fig. 1: Pale green in veinal and interveinal region with pinkish patches on leaf lamina

Fig. 2: Phyllody symptom

Infected plant **Healthy plant**

Young leaf samples were collected and total genomic DNA was extracted from the midribs of leaves from plants with typical symptoms and two symptomless plants using a CTAB method[2]. The qualitative and quantitative assessments of the DNA samples were checked by agarose gel electrophoresis with a nucleic acid standard. The DNA samples were suspended in sterile DNase free milli-Q water and then stored at −20°C. Direct and nested PCR amplifications of 16S rRNA gene with the phytoplasma-specific universal primers P1/P7 and R16F2n/R16R2, respectively were used for confirmation of the phytoplasma associated with *S. trilobatum*phyllody. Total DNA extracts from all plant and insect samples were amplified in a direct PCR assay using primer pairs P1/P7 in the first round of amplification. Reactions were executed in a 50 µl volume. Each reaction had 200 µM of dNTPs, 1.25 U Taq polymerase (Promega), 2 mM $MgCl_2$ and 1 µL of template DNA (10–100 ng/ µL). Thermocycler conditions were as follows: initial denaturation at 95°C for 60 s; denaturation at 95°C 30 s, annealing at 58°C for 30 s and extension at 72°C for 1 min for 35 cycles; and a final extension step of 10 min at 72°C; for the nested step, the thermocycler conditions were the same, except 30 cycles were used. The amplified products of the direct PCR assay was diluted to 1:40, and 1 µL was used as a template and re-amplified with internal primers R16F2n/R16R2 in nested PCR assays[4,5,6]. Healthy *S. trilobatum*plant samples and sterile DNase free milli-Q water were used as negative controls. The amplifications were carried out in a programmable thermocycler (Eppendorf PCR master cycler™). Although the PCR amplicons of expected size (1.8 kb) were obtained in direct PCR amplifications with most of the symptomatic plant samples, followed by nested-PCR amplifications yielded phytoplasma-specific PCR amplicons (1.2 kb) from all plants with phyllody symptoms (Fig. 4&5).

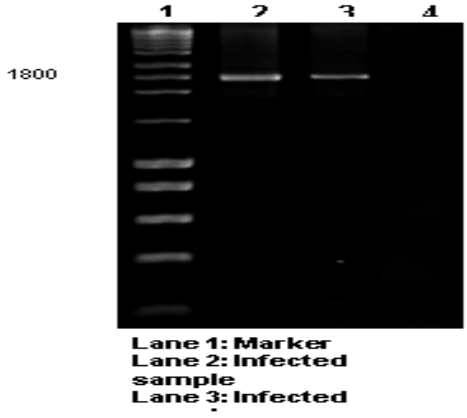
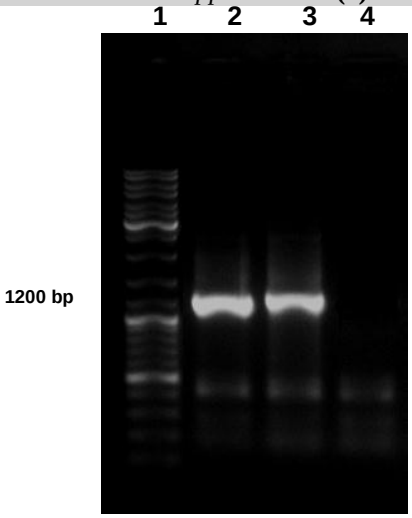


Fig. 4: PCR product obtained with primer pair P1 and P7



Lane 1: Marker
Lane 2: Infected sample
Lane 3: Infected sample
Lane 4: Healthy sample

Fig. 5: PCR product obtained with primer pair R16F2n/R16R2

Symptomless plants and water controls yielded no expected amplicons in nested PCR assays. In this case, the phytoplasma insect vectors, leaf hopper (Orosiusorientalis (Matsumura) [albicinctus (Distant)]) were observed in the infected fields and it may occasionally feed on the cultivated plant, causing monocyclic epidemics. Here in we describe the visual symptoms, provide further details on the phytoplasma associated with the phyllody disease, and provide evidence for its means of first occurrence in India

In the past years, phytoplasmaphyllody had a substantial negative effect on the production of *S. trilobatum* in Tamil Nadu. Effective and reliable vector and disease management methods are required to combat this emerging disease. An extensive research on its management has currently been underway by our group. Therefore, the outcomes of the study described here will provide basic and invaluable knowledge for future research on the disease management and host-vector-pathogen interactions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors GT and KR designed the study, supervised and facilitated the research and wrote the first draft of the manuscript. Authors GT, SP and PR performed the experiments and analyzed the results obtained in the study. All authors read and approved the final manuscript.

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