Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(3): 3695-3697
Received: 01-03-2018
Accepted: 05-04-2018

Vijay Kumar Naik D
Department of Plant Pathology, SV. Agricultural College Tirupati, Andhra Pradesh, India

Bhaskara Reddy BV
Department of Plant Pathology, IFT, Regional Agricultural Research Station, Tirupati, Andhra Pradesh, India

Sailaja Rani J
Department of Plant Pathology, Agricultural College, Mahanandi, Andhra Pradesh, India

Sarada Jayalakshmi Devi R
Department of Plant Pathology, SV. Agricultural College Tirupati, Andhra Pradesh, India

Hari Prasad KV
Department of Entomology, S.V. Agricultural College, Tirupati, Andhra Pradesh, India

Correspondence
Bhaskara Reddy BV
Department of Plant Pathology, IFT, Regional Agricultural Research Station, Tirupati, Andhra Pradesh, India

"*Candidatus* Phytoplasma trifolii" associated with little leaf disease of *Solanum melongena* (Brinjal) in Andhra Pradesh, India

Vijay Kumar Naik D, Bhaskara Reddy BV, Sailaja Rani J, Sarada Jayalakshmi Devi R and Hari Prasad KV

Abstract
Solanum melongena is a major cultivated vegetable crop in India, phytoplasma disease symptoms like little leaf was observed on brinjal at farmer field, Tirupati area. The DNA was isolated from phytoplasma infected leaf samples and also from healthy leaf samples used as a control. The extracted DNA was amplified in PCR by using phytoplasma specific primers P_1/P_7 followed by R16F2n/R16R2. The PCR amplified 1250 bp product eluted from agarose gel were directly cloned in TA cloning vector pTZ57R/T. The phytoplasma clones of 1250bp were sequenced at automated DNA sequencing facility (Eurofin Genomics India Pvt. Ltd., Bangalore) and the sequence was submitted to GenBank (KP899062). BLAST analysis of the partial 16S rDNA sequence of the *Solanum melongena* phytoplasma revealed the highest sequence identity (95%) with phytoplasmas of the 16SrVI group "*Candidatus* Phytoplasma trifolii", including the Phytoplasma associated with brinjal from Nagpur (KX588712).

Keywords: nested PCR, 16S rDNA "*Candidatus* phytoplasma trifolii", little leaf disease, *Solanum melongena*, nested

Introduction
Phytoplasma are wall less prokaryote bounded by a unit membrane and have cytoplasm, ribosome and nucleic acid and filamentous or polymorphic in shape, bodies ranging from 0.15-1.0 µm in diameter and 0.5-1.8 µm in length. Phytoplasma transmitted from plant to plant by leafhopper and plant hopper (Florence and Cameron, 1978) [3]. Phytoplasmas are generally present in phloem sieve tubes and in the salivary glands of insect vectors. While phytoplasmas are multiply in the phloem, little is known about its mechanism.
A common symptom resulting from phytoplasma infection is phyllody, a condition in which a plant produces leaf like structures instead of flowers, Leaf yellowing, one of the common symptoms associated with the presence of these organisms, phytoplasma infected plants may also show virescence, the development of green flowers due to the loss of pigment in the petal cells and many phytoplasma infected plants acquire a bushy or witches' broom appearance due to changes in their normal growth patterns.
Solanum melongena known as eggplant or brinjal is a major cultivated vegetable crops in India. During the 2014 little leaf symptoms on brinjal were observed in farmer field at Tirupati area of Andhra Pradesh. The little leaf of brinjal was also reported from Bihar, India and Bangladesh. The infected plants are characterized by severe stunting and profuse branching, smalling of the leaves and phyllody of flowers. In early infection, no fruiting takes place. In late infection, fruits become malformed and seeds are shrivelled (Das and Mitra, 2004) [1].

Materials and Methods
In the year 2014 little leaf symptoms were observed in brinjal field at Tirupati. The infected samples were collected from brinjal field and also healthy sample used as a control. The total DNA from phytoplasma infected brinjal crop was extracted from leaves using the modified CTAB method (Murray and Thomson, 1980) [7].
The total DNA used as a template in first round PCR with P1/P7 primers (Deng and Hiruki. 1991; Smart *et al*. 1996) [2, 9] followed by nested PCR with phytoplasma specific primers R16F2n/R16R2 (Gundersen and Lee. 1996) [4]. The first round PCR and nested PCR were carried out sequentially in a final volume of 25 µl reactions containing 2.5 µl of (10X) PCR buffer, 2.0 µl (25 mM) $MgCl_2$, 0.5 µl (10 mM each) dNTPs, 1.0 µl (10 µM) each primers, 0.2 µl Taq DNA polymerase (5 u/ µl), and 2 µl template DNA (50 ng/ µl). The DNA was amplified by an initial denaturation of 94°C for 4 min followed by 35 cycles of

94°C for 30 seconds denaturation, 56°C for 1 min primer annealing (55°C for 1 min for nested PCR), 72°C for 2 min primer extension and final extension at 72°C for 10 min. The PCR products were analysed by electrophoresis in 1% (w/v) agarose gel. The DNA fragments in the gel were recorded using gel documentation system. The PCR amplified 1250bp DNA from gel slices was extracted using Gene JET Gel Extraction kit (Thermo scientific) as per the manufacturer's protocol.

Cloning of phytoplasma 16S rDNA

The PCR products eluted from agarose gels were directly cloned in TA cloning vector pTZ57R/T (Instaclone PCR cloning kit, Fermentas, USA) as per the manufacturer's protocol. The phytoplasma clones of 1250bp were sequenced at automated DNA sequencing facility. (Eurofin Genomics India Pvt. Ltd., Bangalore). Sequence phylograms were constructed from aligned sequences using neighbor-joining method and boot strap option using Mega 7.0 software (Tamura *et al*., 2007) [10].

Results and Discussion

Phytoplasma infected brinjal plants shows the production of little leaf symptoms (Fig.1). DNA was isolated from phytoplasma infected and healthy brinjal leafs by CTAB method. The amount of DNA and purity of DNA (260/280 ratio) was measured in Nanodrop spectrophotometer. This DNA used as template for running of the nested PCR with universal primers P1/P7 and R16F2n/R16R2.

16S rDNA from brinjal plant collected from Tirupati was amplified in PCR using 16S rDNA specific primers R16F2n/R16R2 and obtained 1250 bp product in all isolates (Fig. 2). The 1250bp product was eluted from agarose gel was cloned into a pTZ57R/T vector and sequenced and the sequence was submitted to GenBank (KP899062).

The sequence obtained in this study was compared with those of known phytoplasmas in the database (NCBI) by constructing phylograms (Fig. 3) using neighbor-joining method and boot strap option using Mega 7.0 software and found to be 95 % similar to the members of the 16SrVI: Clover proliferation, that contains Phytoplasma associated with citrus from Nagpur (KX588712).

Kumar *et al*. (2012) [6] reported little leaf disease of brinjal caused by '*Candidatus* Phytoplasma asteris' at brinjal field of Bihar, India and Kelly *et al*. (2009) [5] reported little leaf disease of brinjal caused by '*Candidatus* Phytoplasma asteris' in Bangladesh. In our study we reported little leaf disease of brinjal in Andhra Pradesh, causal agent is '*Candidatus* Phytoplasma trifolii'. To our knowledge, this is the first report of the association of a '*Candidatus* Phytoplasma trifolii' isolate with little leaf of brinjal in Andhra Pradesh.

Fig 1: Phytoplasma infected brinjal plant shows little leaf symptom

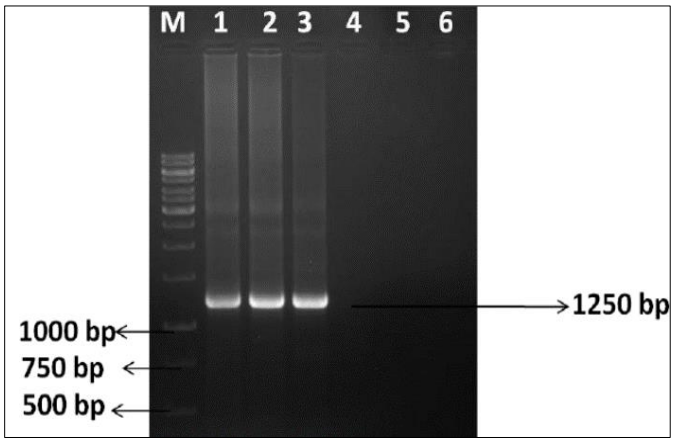

Fig 2: Amplification of phytoplasma 16S rDNA with R16F2n/R16R2 primers with DNA of phytoplasma infected *Solanum melongena* plants

Lanes: M-1 Kb DNA ladder, Lanes: 1, 2, 3-phytoplasma infected *Solanum melongena* plants, Lanes: 4, 5, 6-healthy *Solanum melongena* plants.

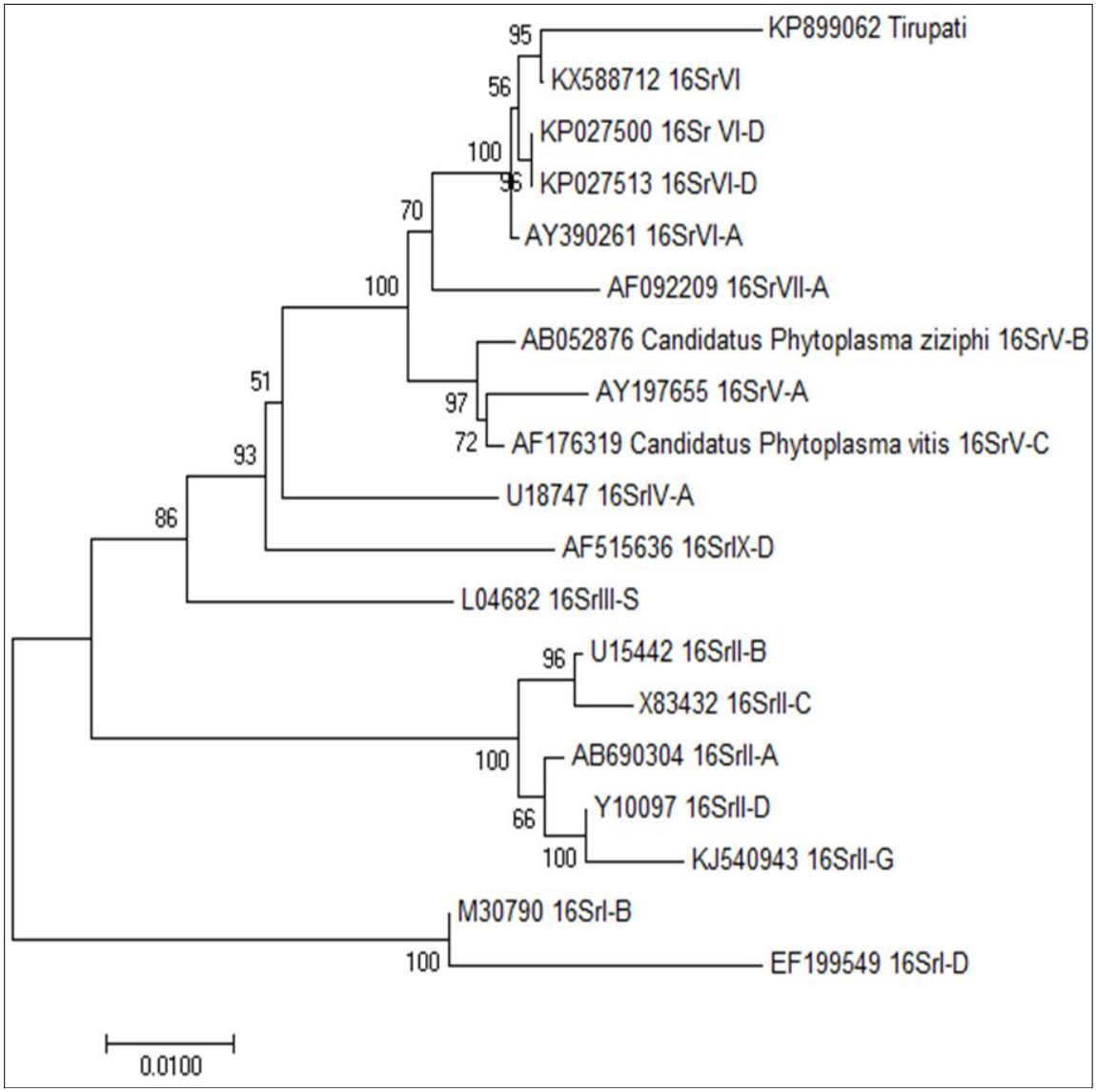

Fig 3: Phylogenetic tree showing the genetic relationship of AP little leaf of brinjal phytoplasma to other phytoplasmas based on 16S rDNA sequences

Acknowledgments
We would like to thanks to ADR, Regional Agricultural Research station for providing necessary facilities for conducting this study and also thanks to Dr. B.V. Bhaskara Reddy, Principal Scientist, Institute of Frontier Technology for his guidance during this study.

References

1. Das AK, Mitra DK. Detection of brinjal little leaf phytoplasma in situ by light and fluorescence microscopy. Indian Phytopathology. 2004; 57(2):242-244.
2. Deng S, Hiruki C. Amplification of 16S rRNA genes from culturable and non-culturable Mollicutes. Journal of Microbiological Methods. 1991; 14:53-61.
3. Florence ER, Cameron HR. Three dimensional structure and morphology of mycoplasrna like bodies associated with albino disease of *Prunus avium.* Phytopathology. 1978; 68:75-80.
4. Gundersen DE, Lee IM. Ultrasensitive detection of phytoplasmas by nested PCR assays using two universal primer pairs. Phytopathologia Mediterranea. 1996; 35:144-151.
5. Kelly PL, Arocha Y, Dider SZ. First report of a 16SrI, '*Candidatus* Phytoplasma asteris' isolate affecting eggplant and *Mikania* sp. in Bangladesh. Plant Pathology. 2009; 58:789.
6. Kumar J, Gunapati S, Singh SP, Lalit A, Sharma NC, Tuli R. First report of a 'Candidatus Phytoplasma asteris' (16SrI group) associated with little leaf disease of Solanum melongena (brinjal) in India. New Disease Reports. 2012; 26:21.
7. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research. 1980; 8:4321-4326.
8. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual, CSHL Press. New York, 2001; 2.
9. Smart CD, Schneider B, Blomquist CL, Guerra LJ, Harrison NA, Ahrens U, *et al.* Phytoplasma Specific PCR primers based on sequences of the 16S-23S rRNA spacer region. Applied and Environmental Microbiology. 1996; 62(8):2988-2993.
10. Tamura K, Dudley J, Nei M, Kumar S. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution. 2007; 24:1596-1599.