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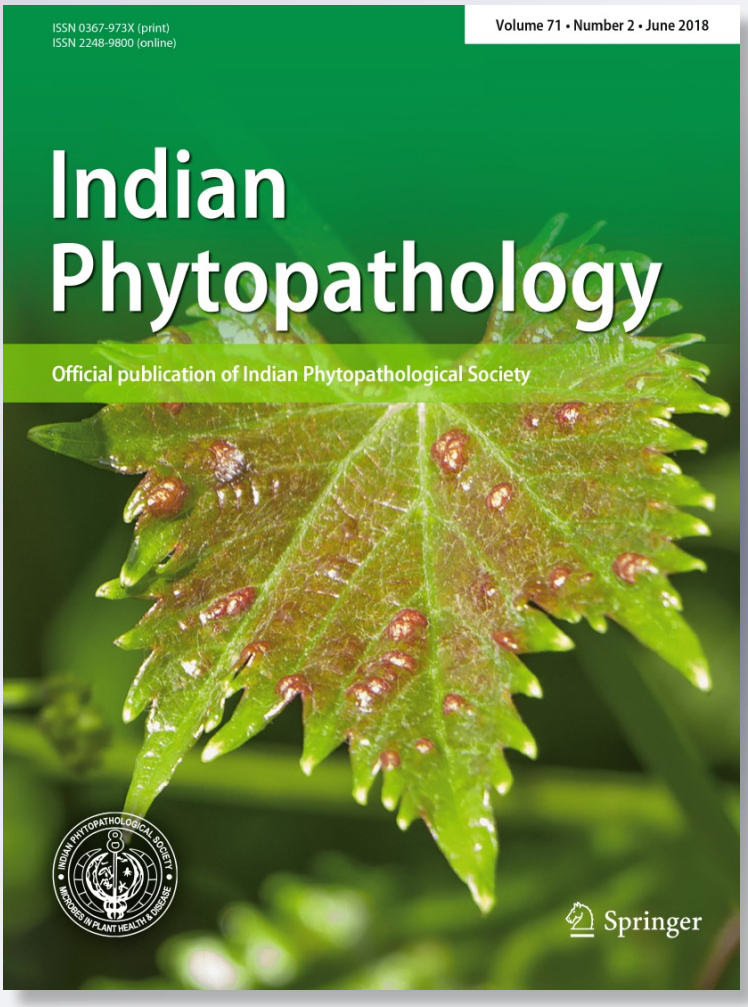

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RESEARCH ARTICLE

Increasing incidence of tomato big bud phytoplasma in Ranga Reddy District of Telangana State, India

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Abstract

Tomato is an important vegetable crop and India ranks second in area and production of tomato worldwide. During field surveys in Moinabad and Shamshabad Mandals in Ranga Reddy district of Telangana from 2014–2016, symptoms like big bud and phyllody were observed on tomato. The affected plants did not produce any fruit. The phytoplasma strains were detected and characterized using universal and nested primer pairs of phytoplasma with amplification of *16S rRNA* and *secA* genes. BLAST analysis of 1.25 kb 16S rDNA partial sequences of nested PCR products and 880 bp of *secA* gene products obtained from symptomatic TBB (tomato big bud) samples revealed 99% sequence identity with strains of '*Ca*. Phytoplasma australasia' (16Sr II group). Phylogenetic analysis and virtual RFLP analysis of 16SrDNA sequences of tomato big bud phytoplasma (TBBP) strain also suggested the closest relationship with '*Ca*. *P. australasia*'16Sr II-D subgroup related strain. Present study confirmed association of 16Sr II-D subgroup of phytoplasma associated with tomato big bud disease in Telangana State of India.

Keywords *16S rRNA* · Characterisation · *secA* gene · Tomato big bud phytoplasma (TBBP) · Virtual RFLP

Introduction

Phytoplasmas are cell wall-less bacteria-like microorganisms, which are generally transmitted by leafhoppers and can inhabit and propagate in both plant and insect vectors (Li et al. 2010). Phytoplasma diseases are increasingly important worldwide with a high economic impact on crop production and quality, costing losses of millions of dollars (Bertaccini et al. 2014). Plants infected by phytoplasmas exhibit a variety of symptoms that suggest profound disturbances to normal plant behaviour and physiology. The most important phytoplasma disease infecting vegetables are brinjal little leaf, potato purple top, big bud of tomato, chilli little leaf and witches' broom (Rao et al. 2017; Kumar et al. 2017). Tomato is grown worldwide for its edible fruits with hundreds of cultivars. During the year 2016–17, tomato production in India is estimated to be around 19.7 million tonnes which is 5.1% higher than the previous year. The major tomato growing States are Madhya Pradesh, Andhra Pradesh, Karnataka, Odisha and Gujarat with Andhra Pradesh on top. Major factors that limit tomato production besides its narrow genetic base are extreme susceptibility to biotic and abiotic stresses. Among biotic stresses, viruses are the major constraints causing severe yield and quality losses to tomato crop all over the world (Hanssen et al. 2010). Besides, phytoplasma diseases are also becoming a major constraint for the yield and quality of tomato crop production all over the world (Bertaccini et al. 2014). In an effort to investigate the changing pest dynamics in tomato under National Innovations in Climate Resilient Agriculture project, survey for phytoplasma diseases of tomato was carried out during five seasons (2014–16) in Ranga Reddy district of Telangana.

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Materials and methods

Surveys were made during August–October and November–January of *kharif* and *rabi*, respectively of 2014–16 across seven villages in Moinabad and Shamshabad Mandals of Ranga Reddy district of Telangana (Longitude: 79.2; Latitude: 7° 7′ 23.4624″N).

Phytoplasma suspected symptoms were recorded and incidence was calculated based on visual observations on 10 plants per spot of five randomly selected spots of 2 × 2 m and the average incidence was recorded. The samples were collected from plants showing phyllody and big bud symptoms during December 2016 for characterization (Fig. 1).

The total genomic DNA was extracted by CTAB method (Ahrens and Seemüller 1992) from five infected TBB samples. DNA isolated from periwinkle infected with BLL (Brinjal little leaf phytoplasma, 16SrII group, Acc No KX689253) maintained in the greenhouse was used as the positive control (Kumar et al. 2017). PCR amplification was performed with the phytoplasma universal primer pair P1/P7 (Deng and Hiruki 1991; Schneider et al. 1995) followed by nested primers R16F2n/R16R2 (Gundersen and Lee 1996). The amplification of *secA* gene was performed in semi-nested PCR assay using phytoplasma universal primers SecAfor1/SecArev3 (5′GARATGAAAACTGGR GAAGG3′/5′GTTTTRGCAGTTCCTGTC ATCC3′), followed by SecAfor2 (5′GATGAGGCTAGAACGCCT3′)/ SecArev3 as described by Hodgetts et al. (2008). The amplified *16S rRNA* and *secA* gene fragments of all five isolates were purified and sequenced. The sequences of PCR products were assembled using DNA Baser V.4 and aligned with phytoplasma group and/or subgroup representatives available in GenBank using Clustal W and the representative 16Sr DNA (Acc. No. MG251643) and secA gene sequences were submitted to GenBank.

The phylogenetic trees were constructed using neighbour joining method with MEGA 6 (Tamura et al. 2013) with 1000 bootstrap replications. The phytoplasma sequences corresponding to the R16F2n/R16R2 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 *Mollicutes* sp. phytoplasma 16Sr II-D subgroup (Acc. No. 410096) for assigning 16Sr sub-groups to ornamental phytoplasma strains analysed by the same restriction mapping utilizing AcaClone software generated RFLP sequences.

Results and discussion

During the survey two major symptoms, viz. phyllody and big bud (Fig. 1a–c) were recorded during August–October and November–January of *kharif* and *rabi*, respectively of 2014–16 across seven villages in Moinabad and Shamshabad Mandals of Ranga Reddy district of Telangana. Incidence of phyllody and big bud symptoms was recorded and found to be 1, 2.5 and 5% during the crop seasons of 2014, 2015 and 2016, respectively.

A 1.8 kbp amplicons of 16S rDNA in direct and ~1.2 kb in the nested PCR assay were obtained from five isolates, respectively, similar to phytoplasma positive sample of brinjal little leaf (BLL) (data not shown). When phytoplasma universal primer pairs *SecAfor1/SecArev2* and *SecAfor1/ SecArev3* were used, products of ~880 and ~480 bp were amplified from DNA of TBB samples. No amplification was observed in direct and nested/semi-nested PCRs with same set of primers specific for 16S rRNA and *secA* genes when DNA from asymptomatic tomato plants was used.

In the pairwise sequence comparison of the *16S rRNA* gene partial sequence (1.25 kb) of five TBB phytoplasma strains showed their maximum identity of 98–99% with '*Candidatus* Phytoplasma australasia' related strains

Fig. 1 Symptoms of tomato big bud disease; **a** big bud symptoms; **b** shoot proliferation with big bud and phyllody symptoms; **c** sterile inflorescence

(16SrII-D). The phylogenetic analysis of 16S rDNA (Acc. No. MG251643) and *secA* gene (Acc. No. MG251644), sequences of TBB phytoplasma strains clustered together with the corresponding phytoplasma strains of subgroups II-D (Figs. 2, 3). Virtual RFLP analysis of *16SrRNA* gene of TBBP strain using 17 restriction enzymes revealed that the strain produced RFLP profiles identical to those of phytoplasma strains similar to the restriction profiles of the reference strain of Mollicutes sp.(16Sr II-D) phytoplasma (Y10096) classified in 16SrII-D subgroup (Fig. 4).

Phytoplasma diseases such as big bud, tomato dwarf, tomato stunt, stolbur and tomato yellows have been reported on tomato in different parts of the world (Gibb et al. 1996; Granett and Provvidenti 1974; Gungoosingh-Bunwaree et al. 2007; Serrone et al. 2001; Shaw and Kirkpatrick 1993; Vellos and Lioliopoulou 2007; Vibio et al. 1996; Zimmerman-Gries and Klein 1978). Among these, the tomato big bud phytoplasma is now becoming common in different countries and has been found to be caused by different phytoplasma species. In India, Varma (1979) detected big bud like symptoms on tomato plants from North India. But the molecular characterization of the phytoplasma strain associated with tomato big bud in India was recently confirmed as

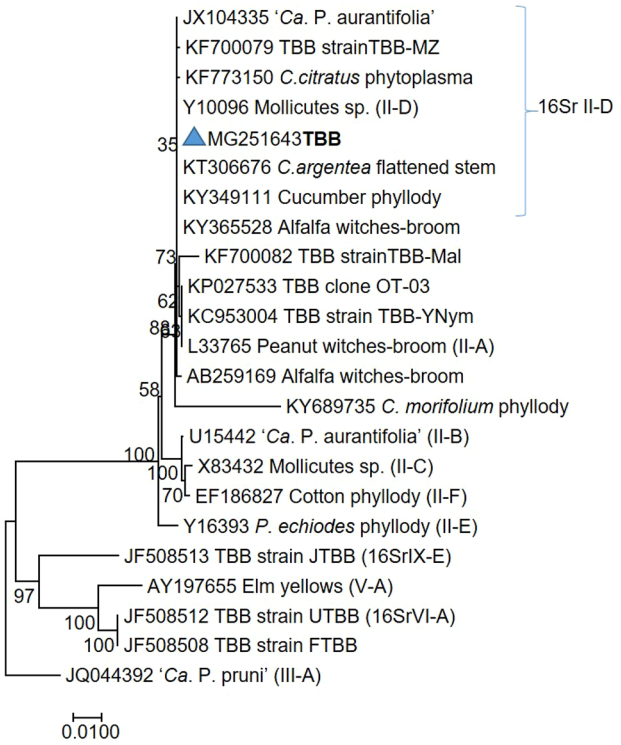

Fig. 2 Phylogenetic tree based on 16S rDNA constructed by neighbour joining method showing the relationships among tomato big bud (TBB) phytoplasma and selected phytoplasma strains. GenBank accession numbers are specified in the tree. Bootstrap values are expressed as percentage of replications

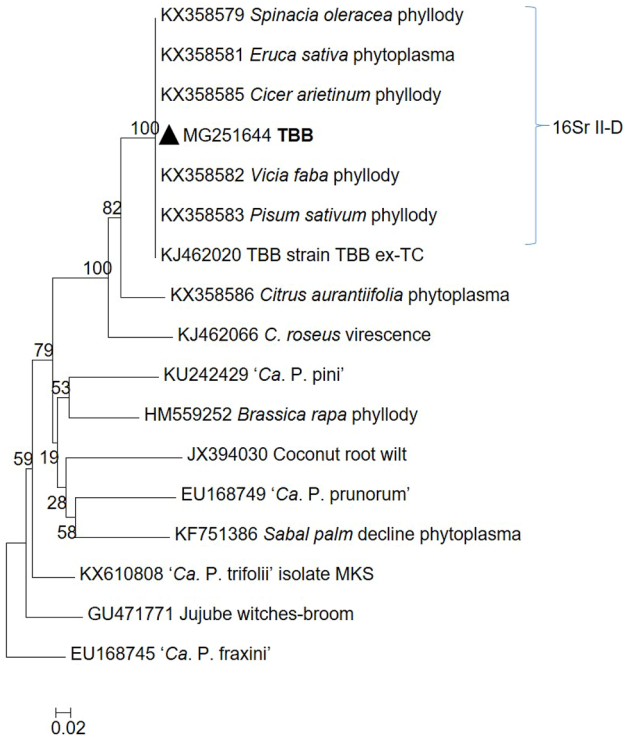

Fig. 3 Phylogenetic tree constructed by neighbor-joining method showing the relationships between phytoplasmas' strains of tomato big bud (TBB) phytoplasma (marked by the triangles) and reference phytoplasma strains of *secA* gene fragment. '*Ca*. P. fraxini' phytoplasma (EU168745) was used as an outgroup. Accession numbers are specified in the tree. Bootstrap values are expressed as percentage of replications

'*Ca*. P. australasia' subgroup D from Uttar Pradesh and Karnataka (Singh et al. 2012; Swarnalatha and Reddy 2014). In the present study we have also reported occurrence of 16Sr II-D subgroup associated with TBB disease of tomato from Telangana state which indicate that TBB disease is spreading to new areas in India. Our study also confirms utilization of *secA* gene in characterization of TBB disease. The increasing incidence of disease in areas of Ranga Reddy district of Telangana during last 3 years alarm us to study the spread sources and other epidemiological factors for the increasing incidence of the disease. Phytoplasma belonging to 16SrII-D subgroup was found as the most widely distributed phytoplasma strain on other vegetables and horticultural crops in India (Rao et al. 2017). It needs immediate attention towards further studies on factors involved in epidemiological significance of the disease.

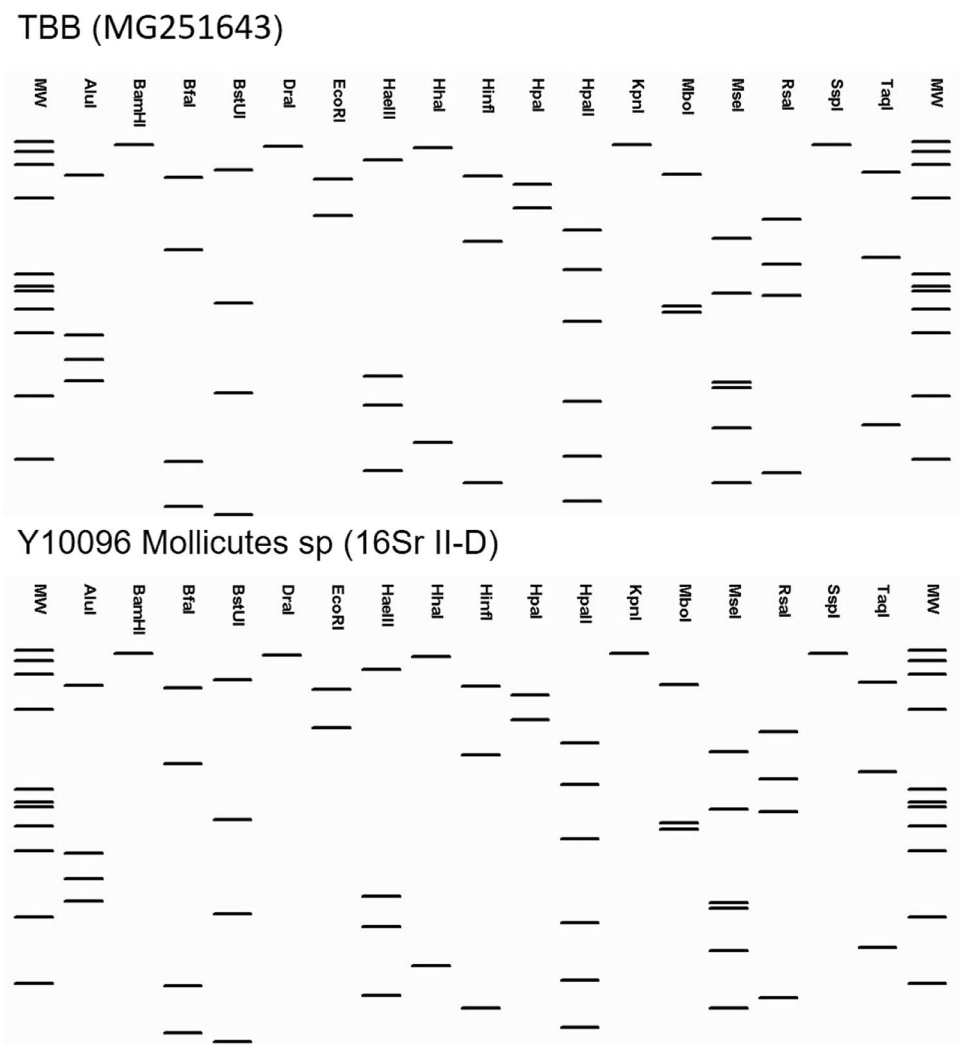

Fig. 4 Virtual RFLP patterns from in silico digestion of R16F2n/R2 fragments of the phytoplasma infecting tomato in India (MG251643) and reference strain (Y10096) with 17 restriction enzymes (*Alu*I, *BamH*I, *Bfa*I, *BstU*I, *Dra*I, *Eco*RI, *Hae*III, *Hha*I, *Hinf* I, *Hpa*I, *Hpa*II, *Kpn*I, *Mbo*I (*Sau3A*I), *Mse*I, *Rsa*I, *Ssp*I, and *Taq*I) usingpDRAW software. MW, DNA from phiX174 digested with *Hae* III (from top to bottom, of 1353; 1078; 872; 603; 310; 281; 271; 234; 194; 118 and 72 bp)

References

Ahrens U, Seemüller E (1992) Detection of DNA of plant pathogenic mycoplasma-like organisms by a polymerase chain reaction that amplifies a sequence of the *16S rRNA* gene. Phytopathology 82:828–832

Bertaccini A, Duduk B, Paltrinieri S, Contaldo N (2014) Phytoplasmas and phytoplasma diseases: a severe threat to agriculture. Am J Plant Sci 5:46–62

Deng S, Hiruki C (1991) Amplification of *16S rRNA* genes from culturable and nonculturable Mollicutes. J Microbiol Methods 14:53–61

Gibb KS, Davis R, Constable F (1996) Tomato big bud phytoplasma. A widespread and successful phytoplasma in Australia. Int Organ Mycoplasmol Lett 4:280

Granett AL, Provvidenti R (1974) Tomato big bud in New York State. Plant Dis Rep 58(211):214

Gundersen DE, Lee I-M (1996) Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathologica Mediterrenea 35:144–151

Gungoosingh-Bunwaree A, Bertaccini A, Benimadhu SP (2007) Presence of phytoplasma infections in tomato plants in Mauritius. Bull Insectol 60:151–152

Hanssen IM, Gutiérrez-Aguirre I, Paeleman A, Goen K, Wittemans L, Lievens B, Vanachter ACRC, Ravnikar M, Thomma BPHJ (2010) Cross-protection or enhanced symptom display in greenhouse tomato co-infected with different *Pepino mosaic virus* isolates. Plant Pathol 59:13–21

Hodgetts BN, Mumford R, Harrison N, Dickinson M (2008) Phytoplasma phylogenetics based on analysis of *secA* and 23SrRNA gene sequences for improved resolution on candidate species of *Candidatus Phytoplasma*. Int J Syst Evol Microbiol 58:1826–1837

Kumar M, Priya M, Rao GP (2017) Molecular characterization, vector identification and sources of phytoplasmas associated with brinjal little leaf disease in India. 3 Biotech 7:1–11

Li ZN, Song JG, Zhang CP, Yu XQ, Wu KK, Wu WJ, Wu YF, Xiang Y (2010) Berberis phyllody is a phytoplasma-associated disease. Phytoparasitica 38:99–102

Rao GP, Madhupriya Thorat V, Manimekalai R, Tiwari AK, Yadav A (2017) A century progress of research on phytoplasma diseases in India. Phytopathogenic Mollicutes 7:1–38

Schneider B, Seemüller B, Smart C, Kirkpatrick BC (1995) Phylogenetic classification of plant pathogenic mycoplasmalike organisms or phytoplasmas. In: Razin S, Tully JG (eds) Molecular and diagnostic procedures in mycoplasmology. Academic Press, San Diego, pp 369–380

Serrone PD, Merzachi C, Bragaloni M, Galeffi P (2001) Phytoplasma infection of tomato in central Italy. Phytopathol Mediterranea 40:137–142

Shaw ME, Kirkpatrick BC (1993) The beet leafhopper transmitted virescence agent causes tomato big bud disease in California. Plant Dis 77:290–295

Singh J, Rani A, Kumar P, Baranwal VK, Saroj PL, Sirohi A (2012) First report of a 16SrII-D phytoplasma *Ca.* Phytoplasma australasia associated with a tomato disease in India. N Dis Rep 26:14

Swarnalatha P, Reddy MK (2014) Duplex PCR for simultaneous detection of Begomovirus and Phytoplasma from naturally infected tomato. Pest manag in Hortic Ecosyst 20:59–68

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis (MEGA) software version 6.0. Mol Biol Evol 30:2725

Varma JP (1979) Occurrence of tomato big bud like disease in Haryana. Sci Cult 45:205–207

Vellos E, Lioliopoulou F (2007) Detection and characterization of phytoplasmas infecting tomato plants in Greece. Bull Insectol 60:157–158

Vibio M, Meglioraldi S, Lee IM, Davis RE, Bertaccini A (1996) Genomic variability in 16S rI-G phytoplasma infecting tomatoes in Italy and Bulgaria. Int Organization for Mycoplasmol Lett 4(260):261

Zimmerman-Gries S, Klein M (1978) A tomato big budlike disease of tomato in Israel and its association with mycoplasma like organisms. Plant Dis Rep 62(590):594