Identification of 16SrII group phytoplasma in fecting commonbean(Phaseolusvulgaris L) in Karnataka

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***Abstract*:** Common bean (Phaseolus vulgaris L) belongs to Fabaceae family and important herbaceous plant grown worldwide as fodder and human food crop. Recently symptoms of yellowing and size reduction of leave and witches broom symptoms have been observed in farmer's field near Hessaraghatta. For conformation of phytoplasma infection symptomatic and asymptomatic common bean samples were collected from field and by using a modified CTAB methodtotal DNA was extracted. Detection of phytoplasma was done by using specific primers P1/P7 and secYF2/R2 for 16Sr DNA and secY genes. The productsof expected size 1.7 kb and 1.5 kb were obtained by PCR, cloned sequenced and compare with the 16S rRNA and sec gene phytoplasma sequences available in the NCBI database.16Sr RNA and secY gene sequences of common bean phytoplasma sharedhighest nucleotide identity with '*Candidatus* Phytoplasma australasiae' (Ca.P. australasiae) 16Sr II group isolates from different parts of the world. Phylogenetic analysis also revealed the current study of phytoplasma closely clustered with the *Ca*. P. australasiae16Sr II group. The virtual RFLP pattern of common bean 16S rDF2nR2 fragment is showing similar reference patterns of all previously established 16Sr IIgroups. This is the identification of 16SrIIPeanut WB group phytoplasmainfecting commonbean in Karnataka.

***Key words*:** Common bean, 16SrRNA and sec gene,16Sr II Peanut WBgroup.

Introduction

Common bean (Phaseolus vulgaris L) is a diploid self-pollinating species,and commonly known as dwarf bean and kidneybean belongs to the family Fabaceae, grown as a human food crop and fodder worldwide.It originatesfrom Mesoamerica (Bitocchi et al 2012) .In the developing world it plays a key role in reducing malnutrition as well as generating income for otherwise low-income households. Across southern Asia Grain legume crops suffer huge losses due to disease caused by Begomoviruses (Qazi et al., 2007). The genus Phaseolus has over 50 species, and common bean (Phaseolus vulgaris L.) or rajma is one of them, accounting for 90% of cultivated species throughout the world. Common bean is the leading producer in Brazil. In India, both bushy and trailing types of common bean are grown in different part of the country, seeds as an importance source of rich protein (23%), which is a key component of the cropping system. Seeds are also rich in iron, phosphorus and calcium. The green leaves and fresh pods are used as vegetable in the diet, predominantly in eastern parts of India and vegetarian population of Uttar Pradesh state. The major limitation for cultivation of common bean (Phaseolus vulgaris L.) is Golden mosaic disease caused by whitefly-transmitted geminiviruses (Qazi et al., 2007). Now days common bean crop is infected by number of diseases among them occurrences of phytoplasma diseases are common. Plants showing yellowing and size reduction of leave and witches broom symptoms have been observed in farmer's field near Hessaraghatta.

Materials and methods

Leaf samples were collected from the common bean plants exhibiting yellowing and size reduction of leave and witches broom from the major common bean growing areas from Hessaraghatta, Karnataka, India.

Symptomatic and symptomless plants were collected and total DNA was extracted from modified CTAB method (swarnalatha et al 2013).A PCR was done by using universal primer pairs P1/P7 (Deng and Hiruki 1991) followed by nested PCR with R16F2n/R2 primers (Gundersen and Lee 1996) further SecY gene of common bean phytoplasm was amplified by SecYF2 (II) and SecYR1 (II) (Lee et al., 2010). DNA amplification was performed and 16S rRNA and SecY gene amplified products were purified, cloned and selected clones were sequenced from Eurofins Genomics India Pvt. Ltd (Karnataka, India) with automated sequencing ABI PRISM 3730(Applied Biosystems). Sequences of Common bean phytoplasma isolates, full length 16Sr RNA and SecY gene sequences derived were queried using iPhyClassifier online tool (Zhao et al., 2009).The sequence results of phytoplasma were analyzed using NCBI (www.ncbi.nlm.nih.gov) blast search, followed by Bioedit sequence alignment editor (version 5.0.9) (Hall, 1999), to determine percentage sequence similarities with other species and multiple sequence alignments using ClustalX (Thompson et al., 1997). Phylogenetic tree was generated using the neighbor joining method with 1000 bootstrapped replications, to estimate evolutionary distances between all pairs of sequences simultaneously with MEGA 5.0 software (Tamura et al., 2011).

Virtual gel plotting and In-silico restriction enzyme digestion of F2n/R2 fragment was done using online iPhyClassifier tool (Bertaccini andDuduk 2009).For the classification of phytoplasma 16Sr RNA gene into different groups and subgroups on the basis of RFLP analysis were employed by prescribed restriction enzymes (Wei et al., 2007). A virtual 3.0% agarose gel electrophoresis image was generated after in silico restriction digestion, and these virtual PCR–RFLP patterns were used for finer differentiation of commonbean phytoplsama isolates from the existing members of the peanut witches-broom group (16SrII).

Results and discussion

During field survey, the common bean plants showing the distinctive phytoplasma-like yellowing and size reduction of leave and witches broom was observed. In earlier studies as well similar symptoms caused by phytoplasma on fruits, vegetables, cereals and trees crops were reported (Bertaccini and Duduk 2009). The phytoplasma classification is based on the highly conserved 16S rRNA gene does not always give the molecular distinction. The most useful and reliable taxonomic information is provided by the sequencing of both SecY gene with 16S ribosomal gene for the finer differentiation of phytoplasma group. Using universal primer pairs P1/P7 specific to 16S rRNA region of phytoplasma 16SrRNA gene of phytoplasma was amplified from beaninfected samples collected from fields (Gundersen and Lee 1996). In the nested PCR using primers R16F2n/R16R2 the PCR products obtained were re-amplified, which yielded a strong PCR amplicon of approximately 1.2 kb DNA fragment (Fig. 1).

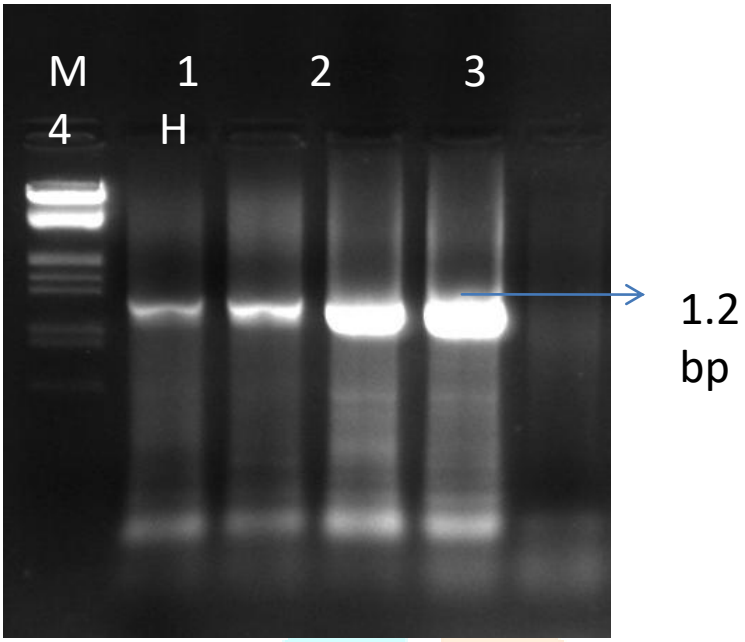

Fig.1.Gel picture showing PCR amplification of phytoplasma samples (1.2 kb)
M: Molecular weight marker
Lanes 1-4 Amplification of Phytoplasma
Lanes 5: Healthy sample

Similarly, by using the primer pair SecYF1/SecYR1 (lee et al, 2010) the SecY gene of common bean phytoplasma isolates was amplified. The resulted PCR product 1.6 kb size was corresponding to the SecY region of phytoplasma (Fig. 2).

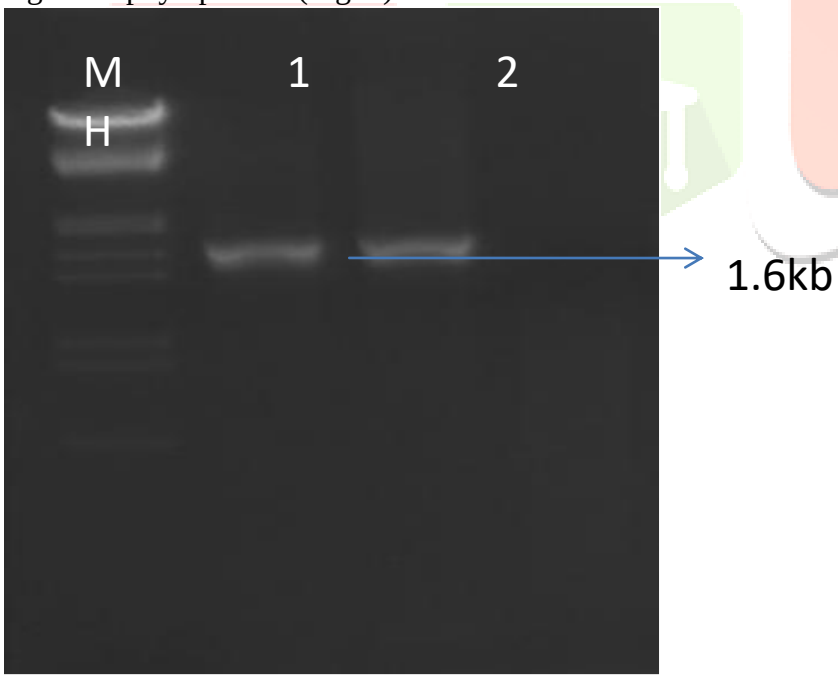

Fig.2.Gel picture showing PCR amplification of sec phytoplasma samples (1.6 kb)
M: Molecular weight marker
Lanes 1-2 Amplification of sec Phytoplasma
Lanes 3: Healthy sample

There was no amplification in the healthy bean samples which served as negative controls. The amplified PCR products of 16S rRNA and sec genes from infected plants of common bean were subsequently cloned and sequenced.

F2nR2 primed fragment of 16Sr RNA gene sequences of common bean phytoplasma isolate under study were compared with different groups of phytoplasma retrieved from the database. The common bean phytoplasma isolate showed maximum nt identity of 99% with the Peanut WB group (16Sr II). These results were well supported by a phylogenetic analysis showing the common bean phytoplasma isolates forming separate group with the above mentioned phytoplasma within the 16SrII Peanut WB group (Fig.3).

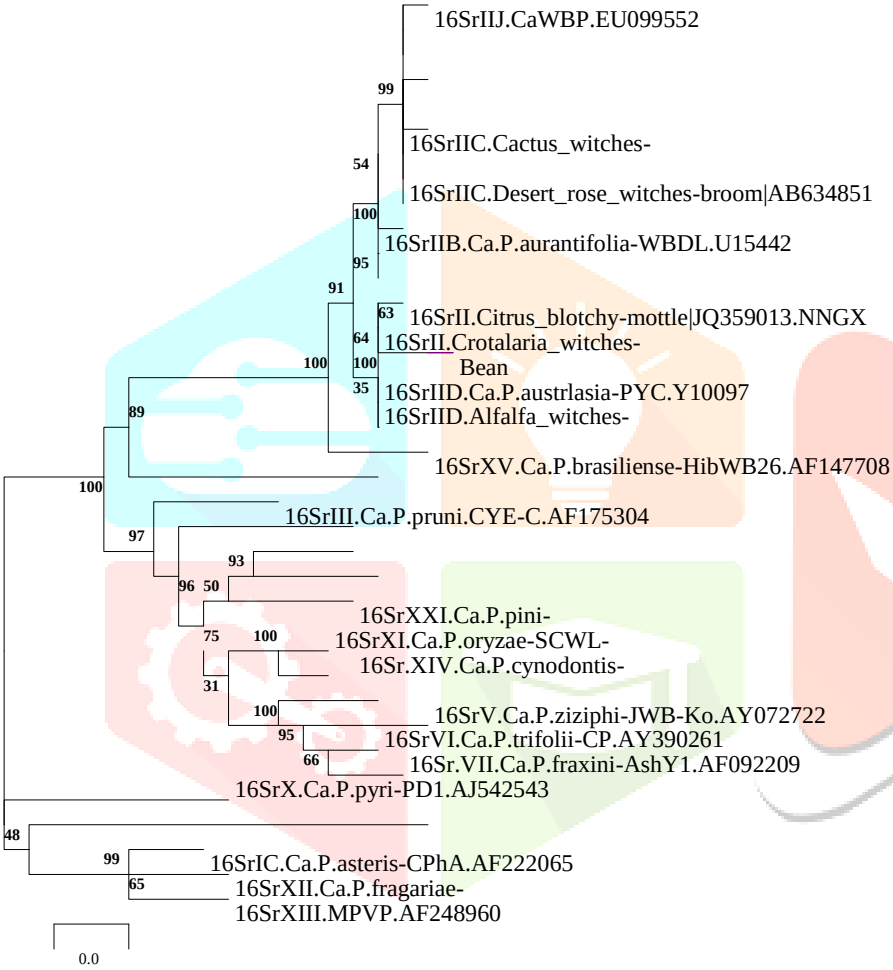

Fig 3.Phylogenetic trees constructed from aligned complete nucleotide sequences of bean phytoplasma 16SrDNA with other sequences retrieved from the database using Neighbor joining algorithm.

Similarly, SecY gene sequences of common bean phytoplasma isolate were compared with the different groups of phytoplasma. The analysis showed that common bean phytoplasma in the current study showed highest nucleotide identity of 99% with 16SrII pea nut witches broom. In contrast, common bean phytoplasmaisolate showed less than 89% identity with the other members of different groups. These results were well supported by phylogenetic analysis, showing the SecY gene of common bean phytoplasma isolate closely clustered with phytoplasma belonging to 16SrII Peanut WB group (Fig. 4).

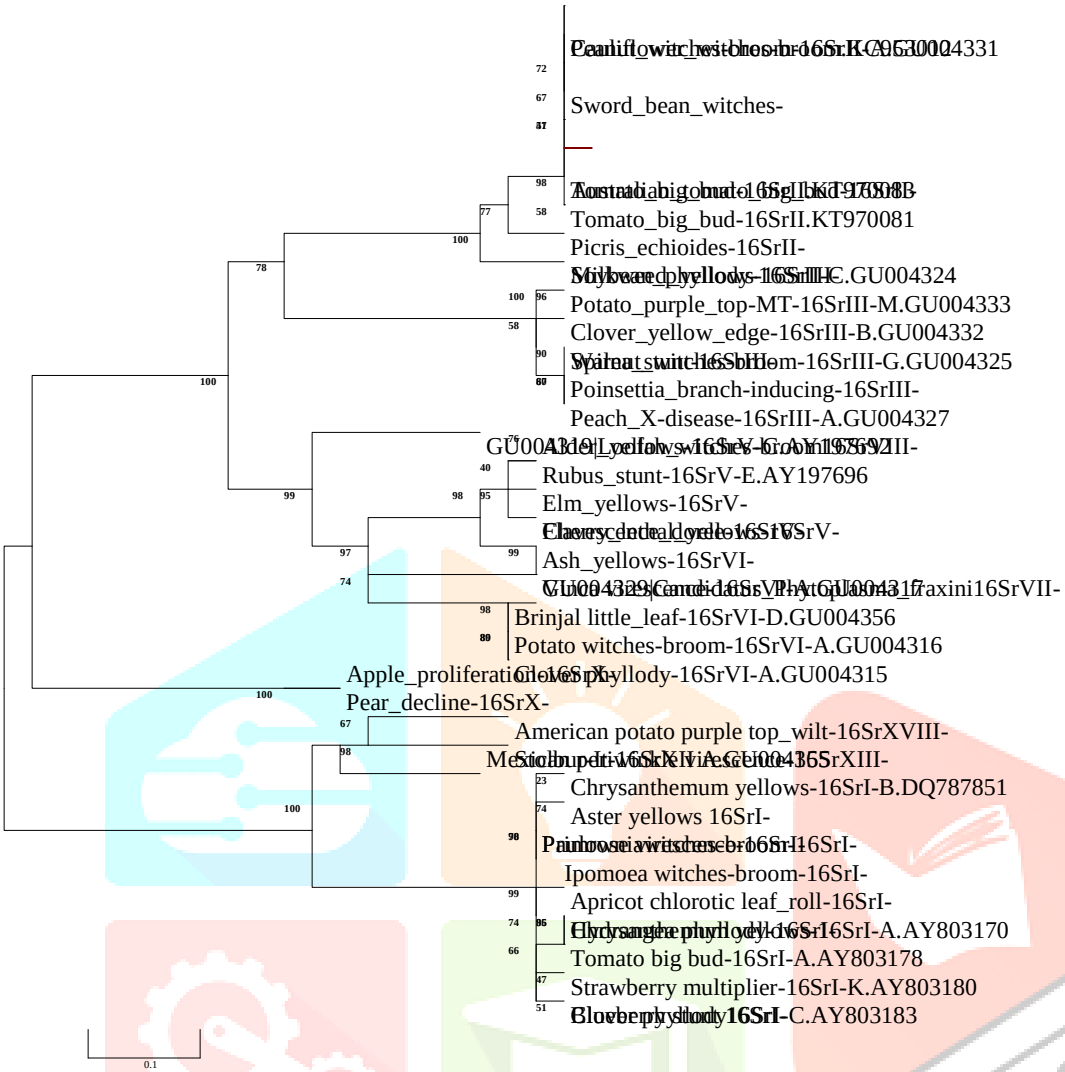

Fig 4.Phylogenetic trees constructed from aligned complete nucleotide sequences of bean phytoplasma sec with other sequences retrieved from the database using Neighbor joining algorithm.

Based on the analysis of 16S rRNA and SecY gene, the identified common bean phytoplasma isolate in the present study belonged to the Peanut WB groupSimilarly, diverse group of phytoplasma associated with common bean disease have been identified and they belonged to 16SrI (Zamora et al., 2012), group.The RFLP and in Silico restriction analysis indicated that common bean phytoplasma was identical to 16SrII Peanut WB group. Most of the phytoplasma associated with bean belongs to the 16SR II group. Distinct phytoplasma members of diverse groups are associated with have also been associated with common bean around the world. This is the identification of 16SrII Peanut WB group phytoplasma infecting common bean in Karnataka.

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