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First occurrence of bean common mosaic virus in soybean [Glycine max] from India

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Abstract The incidence of Bean Common Mosaic Virus (BCMV) in soybean cultivated in western region of Tamil Nadu state, India, was confirmed by symptomatology and reverse transcription-polymerase chain reaction (RT-PCR). Sequence analysis showed nucleotide and amino acid identities of 100% for the isolate BCMV TN1 with BCMV isolate from China (GenBank Accession No. KJ807806) and the isolates soymosaic2 and soymosaic3 showed highest identity of 98.4% with the BCMV isolate (GenBank Accession No. KJ807807) in soybean from China. This is the first report on the emergence of BCMV in soybean in India.

Keywords Potyvirus · Bean common mosaic virus · RT-PCR

Soybean [*Glycine max*] otherwise known as 'golden bean' or 'miracle bean', is one of the premier agricultural crops in India. Soybean, with over 40% proteins and 20% oil has now been recognised all over the world as a potential supplementary source of edible oil and nutritional food. Soybean cultivation has grown rapidly and India is the fifth largest producer in the world accounting for 4% of total global production. Soybean is known to be infected by several major pathogens and yield loss due to disease, insects and weed species ranges from 20 to 100% (IPM package for soybean bulletin, 2014). Major viral diseases of national and regional importance are yellow mosaic viruse (genus *Begomovirus*; family Geminiviridae) and bud blight caused by the species Tobacco streak virus (genus *Ilarvirus*; family Bromoviridae) (IPM package for soybean bulletin, 2014).

Bean common mosaic virus (BCMV) is a member of the genus *Potyvirus* belonging to the family Potyviridae. The virus members have monopartite flexuous filamentous particles of about 750 nm in length encapsidating a positive sense single stranded RNA genome of about 10 kb size (Bhadramurthy and Bhat 2009). The BCMV is commonly transmitted by inoculation of sap by several aphid species in a non-persistent manner. A high proportion of 3 to 95% seed and pollen transmission also has been recorded (Zaumeyer and Thomas 1957). In Frenchbean, BCMV produces mosaic and yellow mottling symptoms that appear as a light green-yellow and dark green mosaic pattern on trifoliate leaves. Leaf discoloration is usually accompanied by puckering, blistering, distortion and a downward curling and rolling. The presence of BCMV in India was reported in *Vigna unguiculata* and *Phaseolus vulgaris* (Sachchidananda *et al*. 1973), in *Vanilla planifolia* (Bhadramurthy and Bhat 2009) and in *Lablab purpureus* (Udayashankar *et al*. 2011). Around the world, this virus is known to infect crops such as soybean, mungbean, yambean and many leguminous weed plants.

During the summer season 2014-15, a total of 392 soybean genotypes were screened for incidence of viral diseases at experimental farms of Department of Pulses of Tamil Nadu

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Fig. 1 Symptomatic leaves showing mosaic, leaf distortion and puckering

Agricultural University, Coimbatore, Tamil Nadu, India. Of these, three entries viz., CSB0808, CSB0904 and UGM70 showed mosaic, leaf distortion and puckering symptoms (Fig. 1). The disease incidence in these entries ranged between 23.8 to 37.4%. The symptomatic leaf samples along with non-symptomatic healthy leaves of those three entries were collected from the field at the flowering stage and the total RNA was extracted using RNeasy Plant Mini Kit (Qiagen, Valencia, CA) and complementary DNA (cDNA) was synthesised (RevertAid™, Fermentas, India). The polymerase chain reaction was performed using different primer sets of RNA viruses viz., Tospovirus (Li *et al*. 2011), Tobacco streak virus (Rajamanickam and Karthikeyan 2014), Potyvirus (Hsu *et al*. 2005) and Tobacco mosaic virus (Letschert *et al*. 2002). The RNA extracted from the symptomatic leaves of entries CSB0808, CSB0904 and UGM70 yielded an amplicon of the size of 1.1 kb product with the genus *Potyvirus* group specific degenerate primer pair PNIbF1/PCPR1 (Hsu *et al*. 2005) corresponding to the 3' end of NIb gene and 5' end of the coat protein gene (Fig. 2). Out of 10 samples tested for each entry, 3 samples were positive by PCR analysis. The RT-PCR amplicons were gel purified (GeneJET, Fermentas, India) and each fragment was sequenced bi-directionally (Excelris, Ahmedabad).

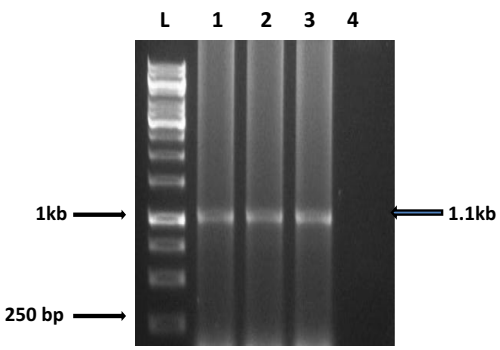

Fig. 2 RT-PCR detection of *Potyvirus* using degenerate primers, PNIbF1/PCPR1 [L = 1kb ladder, (Genei, Bangalore); Lane1, 2 & 3 = template from symptomatic soybean plants; Lane 4 = template from non-symptomatic soybean plants]

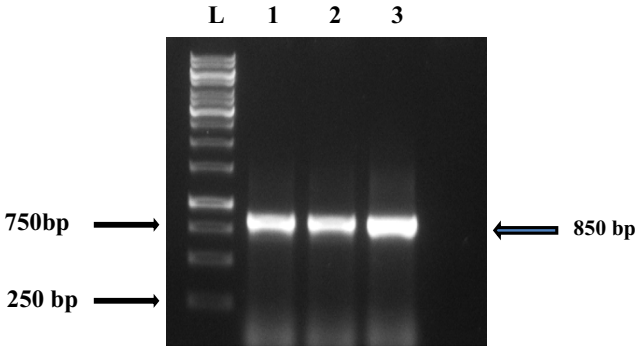

Fig. 3 RT-PCR detection of BCMV using specific primers, AIB90/91 [L = 1kb ladder, (Genei, Bangalore); Lane 1, 2 & 3 = template from symptomatic soybean plant; Lane 4 = template from non-symptomatic soybean plants]

The partial sequences were subjected to NCBI BLAST analysis in which 100% similarity was observed with the species *Bean common mosaic virus*; the isolate with which maximum identity observed was from China (GenBank Accession Nos. KJ807806). The cDNA synthesised from the RNA of symptomatic leaves were subjected to RT-PCR using BCMV specific primer AIB90/AIB91 corresponding to 3' end of NIb gene and 3' end of CP gene (Bhadramurthy and Bhat 2009) which yielded a 850 bp product (Fig. 3). All three sequences were submitted in National Centre for Biotechnology Information (NCBI) GenBank (KU213642, KU739386 and KX380786) for the isolates CSB0808, CSB0904 and UGM70 respectively.

The nucleotide sequence of the amplicon BCMV TN1 representing the region from 8323 nt co-ordinate (NIb region) to 9300 nt co-ordinate in the BCMV genome were compared with those of other BCMV isolates in multiple alignment in CLUSTAL-W programme in the Bio-edit software. Within this region compared, BCMV- TN1 isolate from Tamil Nadu exhibited 100% identity with BCMV isolates from China

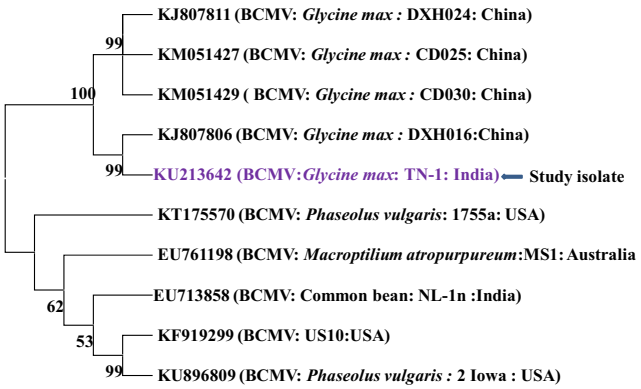

Fig. 4 Phylogenetic dendrogram based on the alignment of partial nucleotide sequence of NIb and coat protein gene of BCMV- TN-1 with that of selected BCMV isolates . Values at nodes represent the percentage bootstrap cores (1000 replicates) only values more than 50 are shown

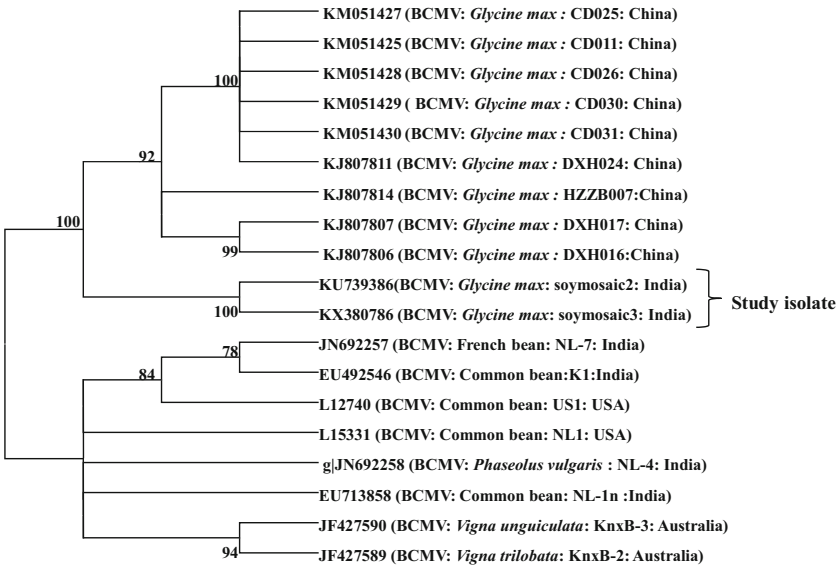

Fig. 5 Phylogenetic dendrogram based on the alignment of partial nucleotide sequence of coat protein gene of soymosaic2 and soymosaic3 with that of selected BCMV isolates. Values at nodes represent the percentage bootstrap scores (1000 replicates) only values more than 50 are shown

(Accession no. KJ807806). The nucleotide sequence of amplicon from soymosaic2 and soymosaic3 isolates representing the region from 8961 nt co-ordinate to 9698 nt co-ordinate were compared with other BCMV isolates. In this region, soymosaic2 and soymosaic3 isolates exhibited highest percent identity of 97% with BCMV isolate from China (Accession no. KJ807807). However, identity with BCMV isolates from Frenchbean and common bean from India was considerably low (90%).

In the phylogenetic tree comparing the amplicons from 8329 to 9300 nt co-ordinate all the BCMV isolates from soybean clustered together; the TN1 and China isolate KJ807806 occupied a separate branch (Fig. 4). In the phylogenetic tree comparing amplicons from 8961 to 9698, the soymosaic2 and soymosaic3 isolates grouped with all the soybean BCMV isolates form China, well separated from BCMV isolates from Frenchbean and common bean from India (Fig. 5).

BCMV is considered as a pathogen of Frenchbean and cowpea in many countries (Verma and Gupta 2010; Sachchidananda *et al*. 1973). It has been reported for its presence in crops viz., *Vanilla planifolia* (Bhadramurthy and Bhat 2009) and lablab (Udayashankar et al. 2011) in India. Zhou *et al* (2014) and Lee *et al* (2015) reported the presence of BCMV in soybean from the countries China and South Korea respectively. Until now, BCMV infecting soybean has not been characterised from India. BCMV was associated with the soybean plants showing mosaic mottling symptoms and diagnosis at the right time and destroying the infected plants will eliminate the spread of the disease. This is the first evidence for the BCMV infection in soybean in India.

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