Identification and molecular characterization of *Bean yellow mosaic virus* infecting French bean in Himachal Pradesh

P. N. Sharma¹ · Vivek Sharma¹ · Anuradha Sharma¹ ·
Kajal Rajput¹ · S. K. Sharma¹

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Abstract French bean (*Phaseolus vulgaris* L.), is one of the most widely grown vegetable crop. Disease samples showing yellow mosaic symptoms on leaves and pods were collected from Himachal Pradesh and inoculated on common bean cv. Jawala through sap inoculation. The virus successfully transmitted by mechanical inoculation produced yellow mosaic, leaf distortion, curling, wrinkling of leaves followed by stunting of plants. The identity of the virus as *Bean yellow mosaic virus* (BYMV) was established through Double antibody sandwich-enzyme linked immunosorbent assay, multiple sequence alignment and phylogenetic analysis of the coat protein gene sequence amplified by reverse transcription-polymerase chain reaction. The cp gene contained 819 nucleotides potentially coding for 273 amino acids. The sequence showed 83–99 % nucleotide and 89–99 % amino acid sequence identities with other BYMV isolates/strains and shared maximum identity with BYMV strain reported from *Gладиолус* sp. in Japan. This study constitutes the first report of BYMV occurrence on *P. vulgaris* in Himachal Pradesh.

Keywords BYMV · French bean · RT-PCR · DAS-ELISA · Coat protein

French bean (*Phaseolus vulgaris* L.), is one of most commonly cultivated green vegetable throughout the world including India. The crop is mainly cultivated by the small and marginal hill farmers of Himachal Pradesh, Jammu &

Kashmir and Uttarakhand over an area of about 26.75 thousand hectares [1]. The crop is attacked by a variety of plant pathogens and among these, *Bean yellow mosaic virus* (BYMV) a member of family *Potyviridae* and species of the genus *Potyvirus* [7], is widely distributed throughout the world where legumes are grown. BYMV causes economic damage in susceptible bean cultivars that react with apical bud necrosis, leading to plant death. The virus was first reported by Pierce [18], from the Red Valentine variety while working on the relative resistance of bean varieties to common bean mosaic (Bean virus 1). In India, BYMV is reported to infect many legumes (family *Fabaceae*) including chickpea (*Cicer arietinum*) [4], green beans (*P. vulgaris*) [5], soybeans (*Glycine max*) [17], faba beans (*Vicia faba*) [11] and non leguminous plants, Cape gooseberry (*Physalis peruviana*) [12], *Fressia* sp, [13], vanilla (*Vanilla planifolia*) [<http://www.ncbi.nlm.nih.gov/nuccore/AY845012>]. Its specificity was first suspected not only from the fact that it produced symptoms distinct from that of the *Bean common mosaic virus* (BCMV), but also because it affected the varieties like Corbet Refugee, Great ‘Northern U1 No.1 and Robust resistant to common mosaic. The virus was designated as Bean virus 2 (BYMV). The virus is not seed-borne in french bean, but transmitted to a small percentage in *Lupinus albus*, *L. luteus*, *Melilotus alba*, *Pisum sativum* and *V. faba* [3, 16].

The survey of bean growing area of Himachal Pradesh was conducted to record the incidence of virus diseases. During the survey, plants exhibiting yellow mosaic symptoms on leaves and pods of french beans variety Falguni were collected from farmer’s field in Rajol area of Kangra district (Fig. 1a). Infected plants were stunted and possessed few pods which were deformed and contained no or few seeds. To study sap transmissibility of virus, the samples were inoculated on healthy plant of common bean

✉ P. N. Sharma
pns1960@gmail.com

¹ Molecular Plant Pathology Laboratory, Department of Plant Pathology, College of Agriculture, CSK HP Agril University, Palampur, HP 176062, India

Fig. 1 Symptoms of *Bean yellow mosaic virus* on naturally infected french bean plants (a) and on sap inoculated plants of susceptible cv. Jawala (b) and Maximum Parsimony relationship phylogram of the

coat protein gene of test isolate of BYMV with related isolates of BYMV at amino acid level using MEGA6 software (c)

cv. Jawala through sap inoculation method as described by Sharma et al. [19]. BYMV is known to produce variable type of symptoms depending upon host and virus strain which include yellow mosaic or mosaic spots on infected leaves including stunting, leaf distortion, deformation, cupping wrinkling and malformation. Some of the isolates of BYMV also cause bud necrosis which finally lead to plant death [3, 6]. The test isolate was transmitted successfully by sap inoculation and produced disease syndrome similar to that of reported by various workers on different host plants including the common bean [3, 6, 9, 20]. On sap inoculated plants of susceptible common bean cv. 'Jawala', initially yellowish mosaic symptoms (Fig. 1b) appeared on first trifoliate leaves after 7 days of inoculation followed by drooping of the leaflets, leaf curling, wrinkling, distortion and malformation. The plants were stunted and possessed few pods. The pods of infected plants also showed yellow mosaic symptoms and such pods were stunted and deformed.

The disease samples both from natural and sap inoculated plants were subjected to Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) test to confirm the involvement of associated virus. The polyclonal antibodies of BYMV, *Bean common mosaic virus* (BCMV) and *Cucumber mosaic virus* (CMV) known to infect common bean were used as per specifications and protocol of the kit (Bioreba, Switzerland). The present isolate reacted positively with BYMV specific antiserum in

DAS-ELISA test and did not react with BCMV and CMV antiserum.

Full genome and partial coat protein (coat protein) gene sequences have significantly clarified the taxonomic status of several viruses, therefore, the identity of the virus was further confirmed by amplifying the CP gene of the test virus. Total RNA was isolated from 100 mg of infected plant leaves using Trizol (Life Technology). The integrity of total RNA was analyzed on 1 % denaturing gel using leaves from healthy plants as negative control. cDNA was synthesized using MMLV reverse transcriptase (USB) in a reaction volume of 20 µl, containing 1 µg of total RNA, 1 µl of oligodT₍₁₀₋₁₈₎ as per the manufacturer's instructions. For reverse transcription-polymerase chain reaction (RT-PCR), BYMV specific primer pair BYMV-pnsF (5'-TCA GATCAAGAGCAACTCAATGCA-3') and BYMV-pnsR (5'-GACGGATACTCTAAATACGAACA-3') designed from the BYMV sequences available in the NCBI, Genbank (www.ncbi.nlm.nih.gov) using primer3 software program available online (www.frado.wi.mit). RT-PCR amplification was performed in 50 µl reaction volume using 5 µl of 10 × Taq buffer, 3 µl of 25 mM MgCl₂, 4 µl of 2 mM dNTPs mix, 2 µl of 10 mM forward and reverse primer, 2.0 µl of cDNA, 0.2 µl of 5U/µl Taq polymerase (Merck Genei) and final volume was adjusted with nuclease free water. Amplification was performed in GeneAmp PCR system 9700 (Applied Biosystems) with initial denaturation of 94 °C for 5 min followed by 35 cycles at 94 °C for 15 s,

annealing at 48 °C for 40 s and extension at 72 °C for 1 min with a final extension of 5 min at 72 °C. The PCR products were electrophoresed on 1.2 % agarose gel in 0.5 × TAE buffer stained with ethidium bromide (0.5 µg/ml) and visualized under Gel Doc system. The RT-PCR amplification of the genomic RNA extracted from yellow mosaic disease samples generated an 832 bp amplicon. Desired RT-PCR product was eluted from gel using DNA Gel Extraction Kit (Auprep GELX Life tech. Pvt Ltd, India) as per the manufacturer's instructions.

Eluted PCR product was ligated into a pGEM-T easy plasmid vector (Promega Corp., Madison, WI) according to the manufacturer's instructions and transformed into *E. coli* strain DH5 α competent cells. Plasmid containing the CP gene insert was isolated by alkali lysis method, lyophilized and both the strands of recombinant plasmid were custom sequenced using T7 and SP6 primers. The sequences obtained were BLAST analyzed (www.ncbi.nlm.nih.gov/blast) and the complete nucleotide sequence obtained after alignment was submitted to NCBI GenBank vide accession number KC011006. The nucleotide sequences of test virus and other BYMV strains/isolates obtained from NCBI Genbank were used for phylogenetic analysis. The nucleotide sequences were translated using ExPASy Translate Tool (www.expasy.org/tools/dna.html). The multiple alignment of the sequence was done using ClustalW (www.ebi.ac.uk/Tools/msa/clustalw2/) and phylogenetic tree was constructed by maximum parsimony approach using MEGA6 software [22].

BLASTN nucleotide sequence analysis of the cloned product revealed that the present isolate of BYMV consisted of complete CP (819) and partial 3'UTR region. Based on CP gene amino acid sequence homology analysis with other isolates of BYMV, the putative proteolytic cleavage site (Q/S) between the NIb protein and the CP was identified. The predicted cleavage site is identical to other BYMV isolates. The tripeptide motif asparagine-alanine-glycine (NAG) was also present in the BYMV isolate at a/a position 9 of CP. The nucleotide and the amino acid sequence analysis of the test isolate showed 83–99 % nucleotide and 89–99 % amino acid homology with other BYMV isolates (Data not shown). The present isolate showed maximum identity with BYMV strain from *Gladiolus* sp. (AB029436, AB029439, AB439729) reported in Japan (99 %). The NAG associated with aphid transmission in potyviruses was reported in the present isolate [2]. The similar NAG motif has also been reported at same position in various BYMV isolates (GDD, MB4 and S) by Guyatt et al. [8], Hammond and Hammond [10] and Nakamura [14, 15].

The results of phylogenetic analysis further confirmed the identity of the test virus as BYMV of genus *Potyvirus* and family *Potyviridae*. Phylogenetic tree constructed at amino acid sequence level to study phylogenetic relationship of the test isolate with other BYMV isolates/strains

infecting different hosts clustered test isolate with other BYMV isolates/strains (Fig. 1c). The isolate showed closest relationship with BYMV strains reported from *Gladiolus* sp. (AB029436, AB029439, AB439729) in Japan and close relationship with BYMV isolates reported from *Vicia faba* (AY192568, D83749) in USA and Japan, *P. vulgaris* (EU082123) in Australia and *Freesia* sp. (AM748702) in India. Similarly, high sequence similarity was also observed between the strains of single potyvirus species by Shukla and Ward [21] and Ward et al. [23]. On the basis of high sequence similarity of the test virus with other isolates of BYMV, the sequence of test virus was identified as the CP gene of BYMV which is cleaved from a larger protein by NIa protease.

In conclusion, DAS-ELISA test and CP gene analysis of the test virus established the association of BYMV with yellow mosaic of french bean and constitutes the first authentic record of BYMV on french bean (*P. vulgaris*) from Himachal Pradesh named as BYMV-HP isolate.

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