

Some Biological and Molecular Characterization of Bean Common Mosaic Necrosis Virus Isolated from Soybean in Tehran Province, Iran

F. S. Abtahi, M. Koohi Hbib, and M. Khodaei Motlagh

Abstract—*Bean common mosaic necrosis virus* (BCMNV) is a potyvirus with a worldwide distribution. This virus causes serious economic losses in Iran in many leguminosae. During 2008, samples were collected from soybeans fields in Tehran Province. Four isolates (S1, S2 and S3) were inoculated on 15 species of Cucurbitaceae, Chenopodiaceae, Solanaceae and Leguminosae. *Chenopodium quinoa* and *C. amaranticolor*.

Did not developed any symptoms.all isolates caused mosaic symptoms on *Phaseolus vulgaris* cv. Red Kidney and *P. vulgaris* cv. Bountiful. The molecular weights of coat protein using SDS-PAGE and western blotting were estimated at 33 kDa. Reverse transcription polymerase chain reaction (RT-PCR) was performed using one primer pairs designed by L. XU *et al.* An approximately 920 bp fragment was amplified with a specific primer.

Keywords—ELISA, RT-PCR, SDS-PAGE, BCMNV.

I. INTRODUCTION

BEAN *common mosaic necrosis virus* (BCMNV), a potyvirus with a worldwide distribution, it was recently differentiated from *Bean common mosaic virus* (BCM) [5, 7] and new classification was approved[4]. The viruses were previously distinguished on the basis of reactions of differential cultivares [1, 6, 8]. BCMNV belongs to the genus potyviruses in the Potyviridae, a group of plant viruses characterized by a monoparticle, positive-sense, single stranded RNA genome encapsidated in flexuous rod shape particles. Viral RNA consists of about 10000 nucleotides [2]. Like other potyviruses, BCMNV is efficiently transmitted by aphids in a nonpersistent manner [2].

This virus includes mosaic, leaf distortion, stunting and letal necrosis in numerous bean (*Phaseolus vulgaris* L.) [1, 5, 6, 8].

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II. MATERIALS AND METHODS

A. Sample Collection

Samples were collected during the 2008 growing seasons from field-grown soybean (*Glycine max*) in Tehran Province. In this region, squashes are planted during early April and harvested from June to July. Virus infections become visible after the setting of the first fruits. Infected plants show symptoms such as: mosaic, leaf distortion, stunting leaf distortion and necrosis. Young leaves from some symptomatic plants were collected at random. All samples were kept in ice chests for transportation to the laboratory. Each plant sample was kept separately in a plastic bag at 4°C until analyzed.

B. Virus Identification

DAS-ELISA (double antibody sandwich ELISA) as described by Clark and Adams (1977) was used with a polyclonal antiserum against BCMNV (DSMZ-AS0230). A 200 µl aliquot of IgG was added to coat each well of plates. Each step of ELISA was followed by a 4-hr incubation at 37°C or a 12-hr incubation at 4°C. This was followed by three washes with a washing buffer. Ten milliliters of sample buffer, pH 7.4, was added to 1g tissue samples that had been ground in liquid nitrogen, and 200 µl of this extracted was added to each well. The reaction was read using a colorimeter at 405 nm after adding conjugate incubation with substrate for about one hour.

C. Host Range Studies

Four original isolates (S1, S2 and S3) recovered from infected soybean plants in the host range studied (from different locations) were maintained in *Phaseolus vulgaris* by sap inoculation. For plant assays, 15 species from 4 families were inoculated with the virus isolates. Sap prepared from leaves which were in 0.01 M Sodium phosphate buffer, pH 7, was rubbed onto leaves dusted with carborundum powder. The Leaves were then rinsed with water, and plants were maintained in an insect-proof screen house for observation. Symptoms on both inoculated and upper, uninoculated leaves

were recorded. Tests for latent infection were conducted by back-inoculation to *Phaseolus vulgaris* cv. Red kidney.

D. Protein Analysis

The molecular weight of the viral coat protein was estimated by SDS PAGE (Sodium dodecyl sulfate poly acryl amid gel electrophoresis) as described by Laemmli (1970). A purified virion suspension and plant samples infected with the virus were mixed with the sample buffer, boiled at 100 °C for 5 minutes and subjected to electrophoresis on 12% poly acryl amid gel along with poly peptide size standard. The gel was stained with coomassie blue. The molecular weight of polypeptides from BCMNV was determined by comparison with the size standards. For western blot analysis, the purified virus and infected plant samples were electrophoresed as described previously. The separated proteins were transferred to nitrocellulose using the buffer styles of Sambrook *et al.* (1989). Probing of the western blots was done as described by Burgermeister and Koeing (1984) using the BCMNV antiserum to BCMNV (DSMZ- As 0230) at a dilution of 1:1000 (v/v).

E. Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA from BCMNV-infected plants was extracted using a phenol/chloroform protocol [3]. Three µl of RNA were submitted to reverse transcription in a final volume of 20 µl, using 2 µl PCR buffer 10x (0.5 M Tris-HCl, 0.7 M KCl, 0.1 M MgCl₂, pH 8), 1 µl DTT (100 mol/µl), 1 µl dNTPs (10 mmol/µl), 0.5 µl RNase inhibitors (10 mmol/µl) and 2 µl Reverse-DAG primer (100 pmol/µl) (5' - GCG TGG CAA TGA CAT - 3'; nucleotide position 8735-8749 on sequence L 31350) for one hour at 42°C with 0.5 µl MMLV reverse transcriptase (200 mmol/µl). 5 µl of the RT reactions were used for PCR using a 2.5 µl PCR buffer 10x, 0.8 µl MgCl₂, 0.9 µl dNTPs (10 mmol/µl), 0.3 µl Taq polymerase (5 unit/µl), 0.4 µl Reverse-NL3 (100 pmol) and 1 µl Forward-NL3 (100 pmol) (5' - CAG CTT GAA TTT GAT TCT GAT GAT GAC GTG-3'; 1057-1086 on sequence L 31350) oligonucleotides encompassing the N-terminal part of the coat protein coding region and the C-terminal part of the polymerase (N1b) (primers designed by L. Xu *et al.*, 1996). PCR reactions were performed by a first denaturation of the samples at 94°C for 3 minutes followed by 35 cycles at 94°C for 1 minute, 43 °C for 1 minute and 72°C for 1 minute and a final elongation step at 72°C for 10 minutes. PCR products were controlled by electrophoresis on 1% agarose gel (L. Xu *et al.*, 2002).

III. RESULTS

A. Host Range

The virus was isolated from infected soybean species from the families Cucurbitaceae, Amaranthaceae, Chenopodiaceae, leguminosae and Solanaceae that were tested. Mosaic, leaf distortion, black roots were observed on *Phaseolus vulgaris* cv. Red Kidney, *Phaseolus vulgaris* cv. Bountiful, *Glycin max* (Figs. 1 and 2) (Table I).

B. SDS-PAGE and Western Blot

Molecular weight of the capsid protein was determined as 33 kDa. Western blot analysis also revealed one band of approximately 33 kDa in the infected plant samples, while no band was found in the healthy plant extracts (Figs. 3 and 4).

C. RT-PCR

RT-PCR was carried out using the primers NL3/F and NL3/R (described previously) which resulted in a fragment of 920nts (Fig. 5).

IV. DISCUSSION

Soybean (*Glycin max*) belongs to the Leguminosae family and is one of the most important economic crops in the world. Leguminosae are threatened by viruses and there are many viruses that damage this family. BCMNV is a potyvirus with a worldwide distribution that causes serious economic losses in many leguminosae. The results of the host range studies, ELISA and RT-PCR indicate that Bcmnv isolates from Iran share the reported properties of the BCMNV. The isolates induced black root symptoms without local lesions. *Nicotiana benthamiana*, *N. tabacum*, *N. glutinosa* and *Pisum sativum* were immune to BCMNV isolates. Our SDS-PAGE pattern showed the presence of a protein of molecular mass 33 kDa, the expected size for the CP from BCMNV previously reported [6]. This 32 kDa protein was recognized by anti-BCMNV antibodies in Western blot assays, confirming the identity of the isolated viral particle as a potyvirus immunogenically related to BCMNV.

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TABLE I
REACTION OF SELECTED INDICATOR PLANT SPECIES TO BMNV

Families	Test plants	Symptoms in leaves
Cucurbitaceae	<i>C. pepo</i> cv. <i>Khoy</i>	-
	<i>Chenopodium quinoa</i>	nll
Chenopodiaceae	<i>C. amaranticolor</i>	-
Leguminosae	<i>P. vulgaris</i> cv. <i>Red Kidney</i>	nll
	<i>P. vulgaris</i> cv. <i>Bountiful</i>	nll
	<i>Vigna unguiculata</i>	-
	<i>Vicia faba</i>	-
	<i>Glycin max</i>	br, nll
Solanaceae	<i>Datura stramonium</i>	-
	<i>Nicotiana tabacum</i> var <i>samsun</i>	-
	<i>N. glutinosa</i>	-
	<i>N. rustica</i>	-
	<i>N. cocker</i>	-
	<i>Physalis alkekengi</i>	-
Papilionaceae	<i>Cicer arietinum</i>	-

Br= Black roots

cll= Chlorotic local lesion

nll= Necrotic local lesion.



Fig 1. Symptoms of chlorotic local lesions and distortion. on *Phaseolus vulgaris* cv. Red Kidney.



Fig 2 . Symptoms of black root on *Glycin max*.

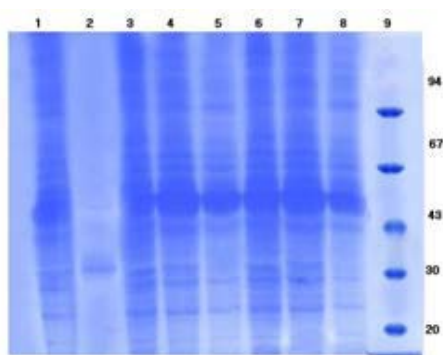


Fig 3. Denaturing polyacrylamide gel electrophoresis of infected plants (line 1, 2, 4, 6), healthy plant (8, 5, 3, 7) and molecular weight marker proteins (line 9).

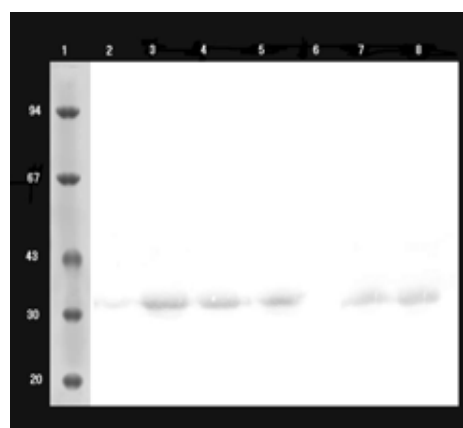


Fig 4. Electro blot immunoassay (Western blotting) of capsid protein of BCMNV with antiserum to BCMNV. Figures on left indicate the position and the molecular weights of the markers protein, lane 1: marker proteins, 2, 3, 4, 5, 7, 8 infected plants and 6 healthy plant (Marker related previous step).

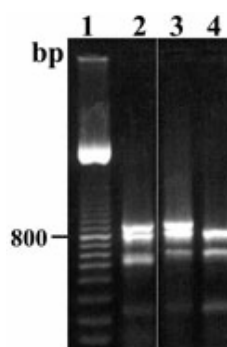


Fig 5. 1% agarose gel electrophoresis analysis of RT-PCR products amplified with NL3F/NL3R primer pair .Lanes 2-4 lane 1 ladder.
(1= 100 PB DNA Ladder, 2= S1, 3= S2 and 4= S3)