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Research Article

OCCURRENCE AND DISTRIBUTION OF *BEAN COMMON MOSAIC VIRUS* AND *BEAN YELLOW MOSAIC VIRUS* FROM COMMON BEAN FIELDS OF KERMAN PROVINCE, IRAN

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ABSTRACT

Common bean as an important and economic plant belongs to family Fabaceae. Among reported viruses from these crops, potyviruses are the most widespread viruses. In this survey for detection of *Bean yellow mosaic virus (*BYMV) and *Bean common mosaic virus* (BCMV), during 2010 growing season, a total of 520 common bean samples with mosaic and distortion were collected from several cities of Kerman province. The percentage of infection with BYMV and BCMV was 20.7 and 36.9, respectively. The highest percentage of virus infection was found in samples collected from Baft city. Among the collected weeds, only two samples of *Chenopodium quinoa* was infected with BYMV. Moreover, determining some characteristics of biological properties of two isolates, BYMV isolate (BY1) and BCMV isolate (BC2), revealed that host range and severity of symptoms for these isolates are different. The host range of BY1 was wider than BC2. Two fragments of 900 and 1450 nt length from the CP regions was amplified by RT-PCR using BYMV and BCMV specific primer pairs, respectively. BLAST analysis on the CP region of both isolates and comparison of their nucleotide sequence showed that BY1 and BC2 isolates are most similar to Japanese isolate of 90-2and Indian isolate of Sikkim, respectively.

Keywords: *BYMV, BCMV, DAS-ELISA and Coat Protein*

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.), is a highly variable species with a long history of cultivation. It is a major legume crop with significant nutritional importance that provides critical protein and calories worldwide and particularly in many underdeveloped countries (Jones, 1999). In many areas, common bean is the second most important source of calories after maize. The largest producers of dry beans are Brazil, Mexico, China, and the USA (FAO report). According to Iranian Ministry of Agriculture in 2009, different cultivars of common bean are grown in more than 790,000 ha annually in Iran.

Possible causes of low yield are the numerous pathogenic microorganisms, the most important of which are viruses that are a major yield reduction factor in bean production (Babovic, 2003). The list of viruses infecting beans is very long (Kumar *et al.*, 1994) but economically the most important ones are *Bean common mosaic virus* (BCMV) and *Bean yellow mosaic virus* (BYMV) which belong to *Potyvirus* genus and family *Potyviridae*. These two viruses can cause great damage to plant production, especially due to the presence of aphids vectors, which represent one of the important factors for the spread of the virus in the field (Spence and Walkey, 1995). The harmfulness of a given viral disease will depend on bean cultivar susceptibility, the stage of plant development at which the infection occurs, environmental factors and their effect on vector activity (Petrovic *et al.*, 2010).

Some of the viruses infecting common bean, including *Bean common mosaic virus* (BCMV), *Bean yellow mosaic virus* (BYMV) and *Cucumber mosaic virus* (CMV) were previously characterized based on biological and serological properties from the fields of south and central regions of Iran (Kaiser *et al.*, 1968). Considering the importance of bean crop in Kerman province, (as the widest province in Iran), and because of the lack of any information about the occurrence of damaging viruses in this province, the present study was conducted to assess the distribution of two potyviruses, BYMV and BCMV in the most important regions where common bean are grown in Kerman province.

MATERIALS AND METHODS

Survey

A virus survey was conducted in common bean grown under field conditions during the 2010 growing season at selected cities of Kerman provinces including Kerman, Baft, Jiroft, Bardsir and Sirjan (Figure 1). Weeds within the crops and at the margins of fields also were collected to assess the potential role as virus reservoirs. Leaf samples were obtained from symptomatic plants showing mosaic, mottling, yellowing, chlorotic and necrotic lesions and deformation. Furthermore, some samples with no obvious symptoms were randomly collected to exclude the possibility that plants were infected but tolerant. Each plant samples were labeled and brought to the laboratory by placing on ice and was kept at 4 ^{o}C until analyzed.

Figure 1: Map of Kerman province, showing the location of regions where field–grown bean crops were surveyed for virus diseases during the 2010 growing seasons

Virus Identification

Samples were tested by ACP-ELISA using specific commercial monoclonal antibody against potyviruses (DSMZ-AS-0573). The ELISA-positive samples from previous test were evaluated for viral infection using BYMV (DSMZ-AS-0471) and BCMV (DSMZ-AS-024) antisera in DAS-ELISA.

ACP-ELISA and DAS-ELISA were conducted according to Clark and Adams (1977) procedure. The antibodies were conjugated with alkaline phosphatase and P-nitrophenyl phosphate was used as a substrate. Absorbance was recorded at 405 nm using a multi scan ELISA reader (BioTek®, Elx 808) one hour after the addition of the substrate. Plants were considered infected when absorbance values reached two times the mean value of the healthy controls.

Host Range

For determination of some viral properties of detected viruses, two isolates, a BYMV isolate (BY1) and a BCMV isolate (BC2), were selected. One gram of infected leaves was ground in 5 ml of 0.05 M phosphate buffer (pH 7.0 containing 1 mM EDTA, 5 mM Na-DIECA and 5 mM thioglycolic acid) and the extracts were rubbed mechanically onto young *Chenopodium quinoa* leaves, previously dusted with Carborandom. Moreover, local lesions were induced post inoculation. After successive single local lesion passage to common bean plants, all plants that developed systemic mosaic symptoms within 4 weeks were tested by DAS-ELISA using specific antisera. Infected plants were kept at greenhouse as viral sources.

The host range and symptoms caused by each of these field isolates was tested by mechanical inoculation to a range of species belong to five families in greenhouse at 25-30^{o}C. The test plants were: *Chenopodium quinoa* Willd., *C.amaranticolor* Coste & Reyn., *Lactuca sativa* L., *Helianthus annus* L., *Cucurbita pepo* Duch., *Cucumis melo* L., *Pisum sativum* L., *Vicia faba* L., *Datura stramonium* L., *Lycopersicum esculentum* Mill., *Nicotiana benthamiana* Domin., *N. tabacum* L. *cv. Samsun and Phaseolus vulguris* L.

Each isolate was inoculated to five plants of each species and the symptoms were observed for 4 weeks thereafter. The occurrence and type of symptoms were recorded and inoculated leaves were assayed for the presence of viruses using DAS-ELISA.

RNA Extraction, RT-PCR and Sequencing

Total RNA was extracted from 100 mg of fresh leaf tissues ground in liquid Nitrogen using the RNeasy® Plant Kit according to the manufacture's protocol (Qiagen®, hatsworth, CA) and was reverse transcribed using MuMLV reverse transcriptase in RT-PCR reaction with specific primer pairs. These oligonucleotide primers were designed from the conserved sequences in the coat protein region of BYMV (BYMV-CPU: 5´-GTC GAT TTC AAT CCG AAC AAG-3´ and BYMV-CPD:5´-GGA GGT GAA ACC TCA CTA ATA C-3´) () and BCMV (Dbcmv: 5´ACC ACG CTG CAG CTA AAG AGA ACA 3´ and Ubcmv: 5´-AAT CTA GAT GAT ATC ATA CTC TCT A- 3´) (Jie and Xiao-ming, 2006 ; Xu and Hampton, 1996).

The cDNA was synthesized using 1 µl of total RNA, 1 µl *Taq* DNA polymerase, 0.5 µl MuMLV reverse transcriptase (200mmol/ µl), 4 µl *Taq* MuMLV buffer 10x, 0.5 µl RNA inhibitor (10 mmol/ µl), 1 µl dNTPs (10 mmol/ µl) (Vivantis company) and 2 µl reverse primer (100 pmol) in a total volume of 20 µl according to the manufacture's instruction. Afterwards, the cDNA was used in RT-PCR reaction with a total volume of 20 µl containing 5 µl PCR buffer 10X, 2µl MgC12 (50 mmol/ µl), 1µl dNTPs (10 mmol/ µl), 1µl of each Reverse and Forward primers (100 pmol), 0.5 µl Taq Polymeras (5 unit/ µl) and 5 µl cDNA.

Thirty reaction cycles were performed as follows: denaturation at 94 ^{0}C for 30s, annealing at 37 ^{0}C (for BYMV primer) or 50 ^{0}C (for BCMV primer) for 1min, and elongation at 72^{0}C for 2 min. A final 10 min elongation step at 72^{0}C was performed at the end of the 30 cycles.

These two mentioned primer sets are expected to amplify a 900 bp and 1450 bp PCR product within the viral coat protein region of BYMV and BCMV, respectively. PCR products were examined by electrophoresis in 1% agarose gel, stained by ethidium bromide and visualized by UV illumination.

The fragments of CP genes were sequenced by BIONEER Company (South Korea) and analyzed using Basic Local Alignment Search Tool (BLAST) available online and deposited in NCBI.

RESULTS

Distribution of BYMV and BCMV

Leaf samples were collected from 520 plants at five cities shown in table 1. Both two viruses (BYMV and BCMV) were detected in samples collected from all five cities. Out of 310 samples (59.6%), which were found to be infected with potyviruses, 192 and 108 samples were infected with BYMV and BCMV respectively. Of the samples tested, the percentage of single infection with BYMV and BCMV and double infection was 36.9%, 20.7% and 12.9%. The incidence of BCMV was higher in each city compared with the other virus tested. The infection rate of BCMV in collected common bean plants was 49% in Baft, 37.5% in Kerman, 30.7% in Sirjan, 25.8% in Jiroft and 29.3% in Bardsir, while the infection rate of BYMV was 28.7% in Baft, 15.4% in Kerman, 17.8% in Sirjan, 19% in Jiroft and 19.5% in Bardsir.

To determine potential reservoir hosts for BYMV and BCMV, some weed samples were collected, most of which were symptomless. Out of 32 samples, two *Chenopodium quinoa* samples from Baft fields were positive for BYMV.

Host Range

Two isolates (BY1 and BC2) was tested for determination of host range by sap inoculation to 13 plant species and plants subsequently were tested for the presence of BYMV and BCMV by DAS-ELISA (Table 2).

The biologically purified BY1 and BC2 isolates after successive single local lesion transfers, were inoculated to a range of plant species. The host range and severity symptoms of BY1 and BC2 isolate on the test plants were different as presented in Table 2. The severest symptoms displayed in plants infected with BY1 isolate which induced wilting and mosaic of the tested plant species (Figure 2).

Table 1: Incidence of BYMV and BCMV viruses in common bean plants from samples collected in five cities of Kerman province during 2010

Location	Samples collected	Number of samples positive by DAS-ELISA	
		BCMV	BYMV
Baft	153	75	44
Kerman	136	51	21
Sirjan	101	31	18
Jiroft	89	23	17
Bardsir	41	12	8
Total	520	192	108

Table 2: Symptoms induced on test plants mechanically infeted by BY1 and BC2 isolates

Family	Species	Symptoms*		DAS-ELISA result	
		BY1	BC2	BY1	BC2
Chenopodiaceae	*Chenopodium quinoa* Willd.	CLL/M	CLL/-	+	-
	C.amaranticolor Coste & Reyn.	CLL/M	-/-	+	-
Compositae	*Lactuca sativa* L.	CLL/-	-/-	-	-
	Helianthus annus L.	CLL/M	-/-	+	-
Cucurbitaceae	*Cucurbita pepo* Duch.	-/-	-/-	-	-
	Cucumis melo L.	-/-	-/-	-	-
Leguminosae	*Phaseolus vulgaris* L.	NLL/M,W	CLL/M, W	+	+
	Pisum sativum L.	NLL/M	NLL/-	+	-
	Vicia faba L.	CLL/M, W	-/L	+	+
Solanaceae	*D. stramonium* L.	CLL/L	-/-	+	-
	Lycopersicum esculentum Mill.	NLL/M	-/-	+	-
	N. benthamiana Domin.	NLL/L	-/-	+	-
	N. tabacum L. cv.Samsun	NLL/L	-/-	+	-

** CLL: Chlorotic local lesions, M: Mosaic, NLL: Necrotic local lesion, W: Wilting, L: Latent symptom*

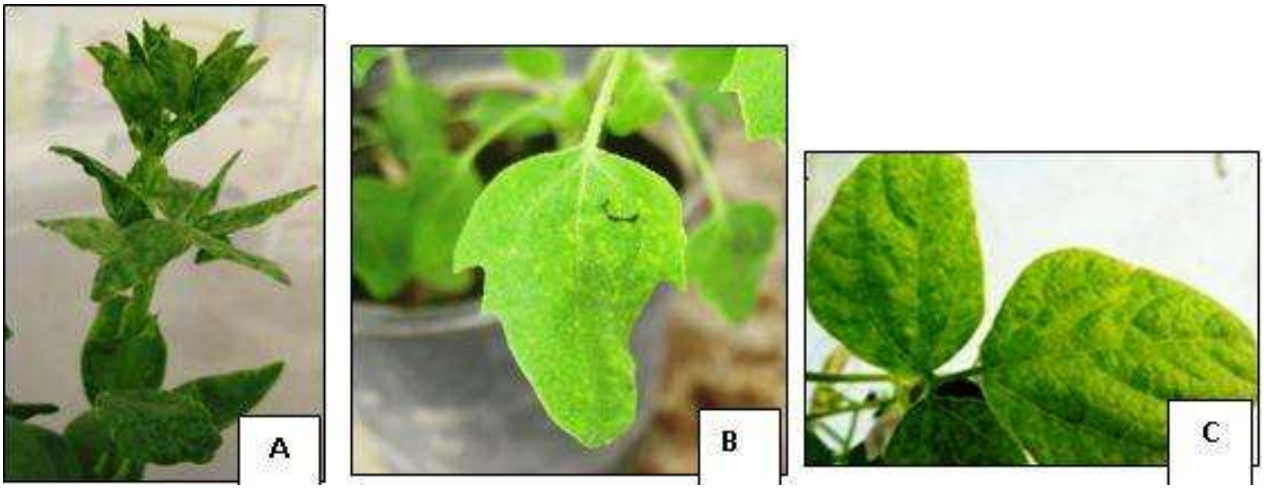

Figure 2: Symptoms induced by the BY1 isolate on A) *Vicia faba*, B) *Chenopodium quinoa* and by the BC2 isolate on C) *Phaseolus vulgaris*

RT-PCR

In RT-PCR test, DNA fragments of the expected size (907) were amplified from CP region of RNA genome of BY1isolate (Figure 3B). Predicted size PCR products (approx1450 bp) were also amplified from BC2 isolate with primer pair Dbcmv/Ubcmv (Figure 3A). None of the primer pairs generated specific PCR products from nucleic acid extracts of non-infected plant tissues.

Nucleotide (nt) and amino acid (aa) sequences were analyzed using Vector NTI advance 11 software (Invitrogen Corp., Carlsbad, CA) and compared with the NCBI databases using the Basic Local Alignment Search Tool (BLAST) programs. BLAST alignment with the database confirmed the similarity between the CP sequences of BY1 and BC2 isolate and the CP sequences of other BYMV and BCMV isolates in databases, respectively.

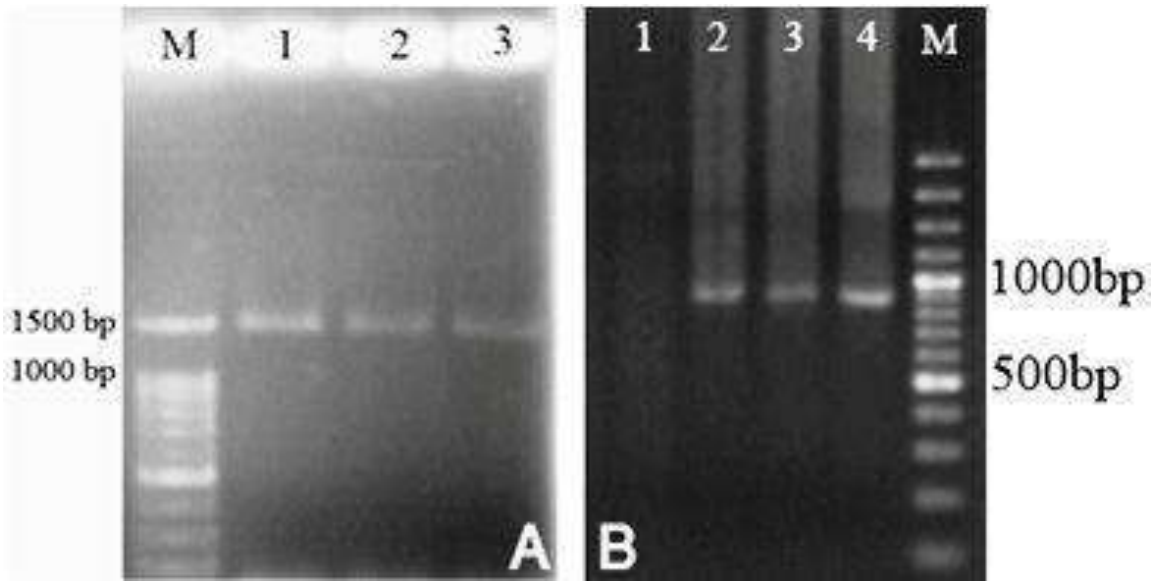

Figure 3: Agarose gel electrophoresis of RT-PCR amplifications of the coat protein from BC2 (A) and BY1 (B) isolates using Dbcmv/Ubcmv and BYMV-CPU/BYMV-CPD primer pairs, respectively. A. lane 1-3: BC2-infected Bean, M: 100bp molecular weight marker. B. lane 1 uninfected control of *C. quinoa*, lane 2: BY1-infected *C. quinoa*, lane3 and 4: BY1-infected Bean, M: 100bp molecular weight marker

To investigate the relationship of the BY1 and BC2 isolates to other isolates, 18 nucleotide sequences for BYMV and 11 nucleotide sequences for BCMV were retrieved from Genbank entries. Homological relationships were inferred using the DNAMAN version 4.02 (Lynnon BioSoft) software based neighbor-joining method with a 1,000 replicate bootstrap value. Grouping of isolates was similar, no matter whether the neighbor-joining or maximum parsimony method was used. BYMV isolates were grouped in five clusters (Pea, Canna, General, Dicot and Monocot), of which the BY1 isolate together with Australian isolate of FBD1 and Japanese isolate of 90-2 grouped in Dicot cluster (Figure 4). The homology tree also showed that the BY1 isolate was very close to 90-2, with a high level of homology (96.8%) between the two isolates.

The CP region sequence of BC2 isolate was also compared with other BCMV isolates to determine phylography. The phylogenetic tree of all 12 sequences (including BC2) grouped into four distinct clades numbered I to IV (Figure 5). The alignment showed that BC2 isolate together with Aus, MS1, KnxB-3, NL-1n, B-29K and Zacatecas grouped in cluster II. The BC2 isolate showed closest identity with Sikkim isolate from India (98.1% identity).

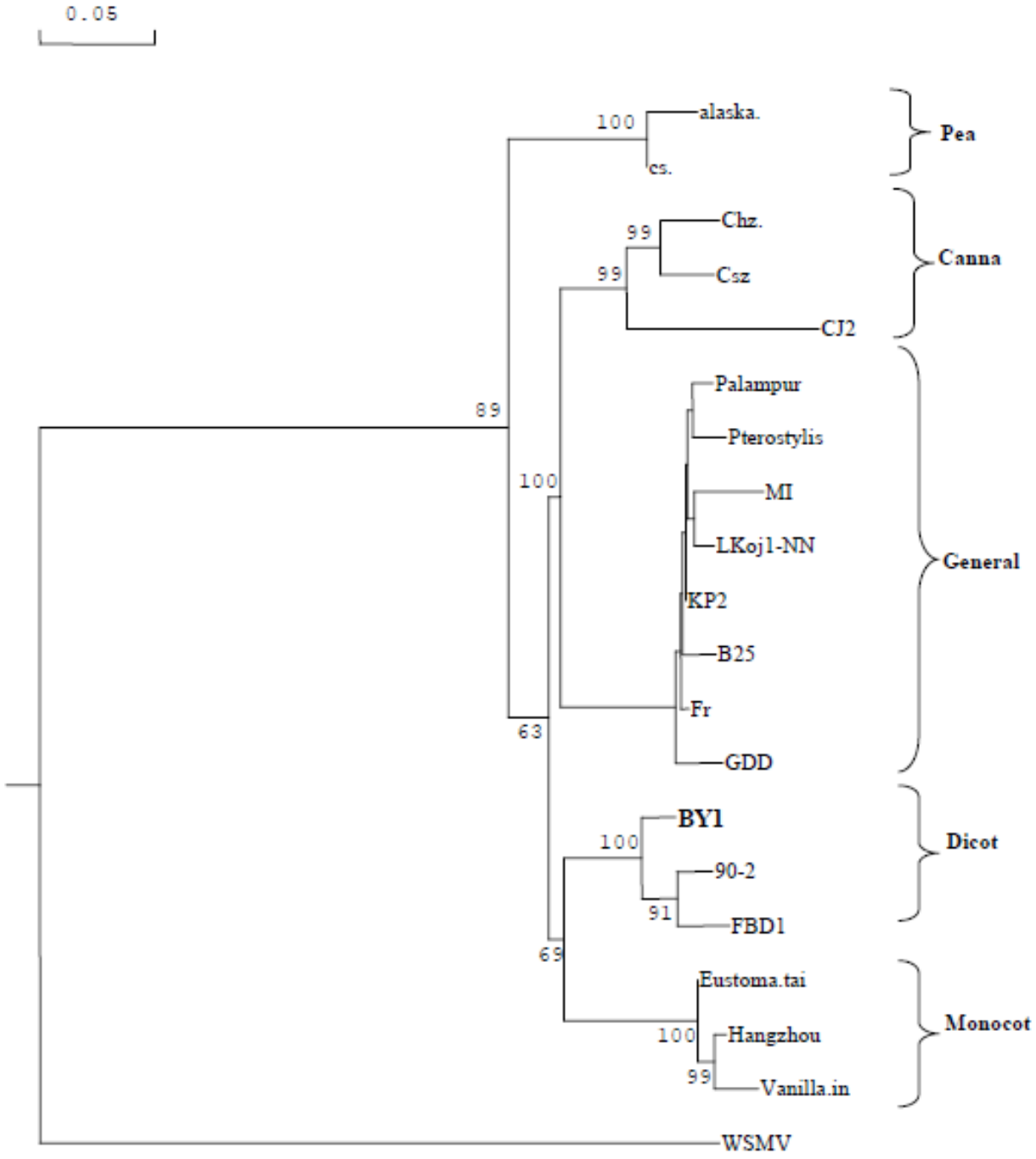

Figure 4: Phylogenetic tree showing the relationship between different BYMV isolates including the BY1 isolate based on coat protein sequences. Isolate names and GenBank accession numbers are as follows: Alaska (GU126690), Eustoma.Tai (AM884180), M-I (X81124), Hangzhou (AJ311371), CJ-2 (JQ012792), CS (AB373203), 90-2 (AB439731), Chz (DQ060521), Kp2 (JX173278), Fr (FJ492961), Csz (EF592169) Pterostylis (AF185960), Vanilla.In (FJ752701), Palampur (AM398198), GDD (AY192568), LKoj1-NN (AF192782), B25 (AM884181), FBD1 (EU082117). The tree was constructed using DNAMAN software with 1000 bootstrap replicates. Values at nodes indicate significance in bootstrap analysis.

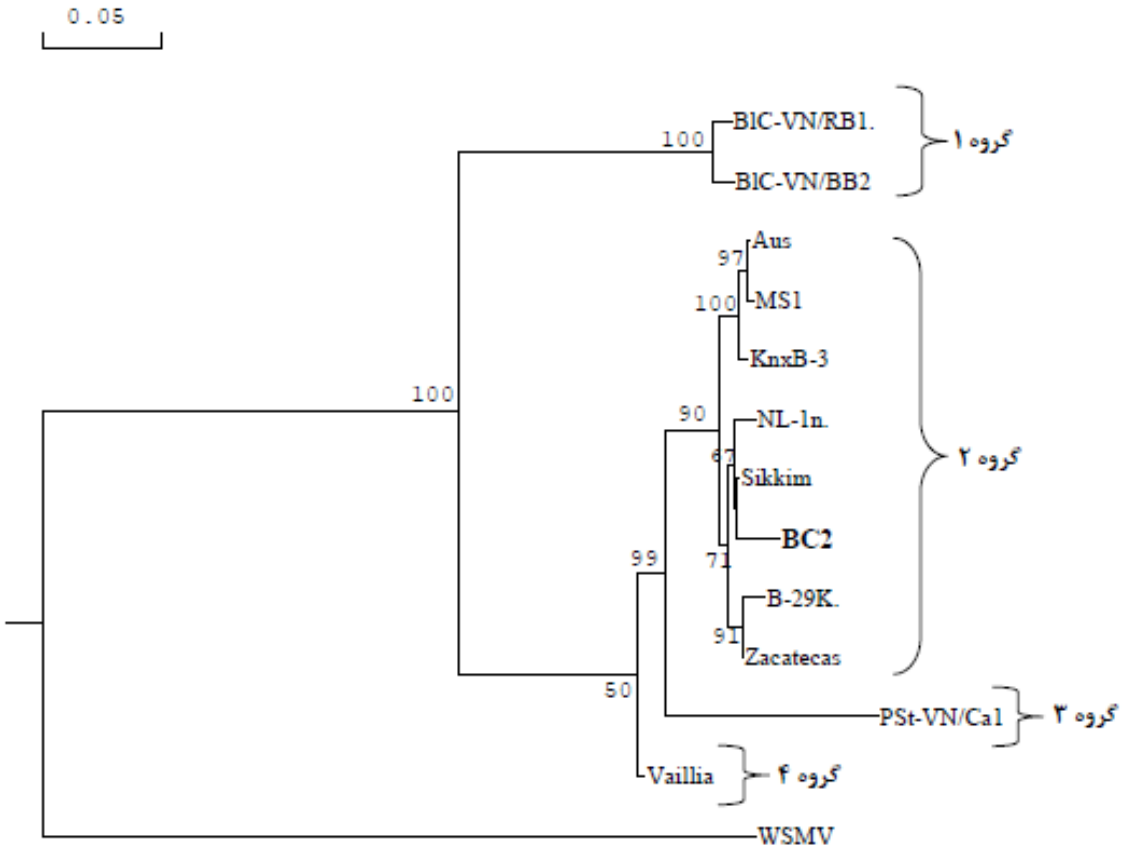

Figure 5: Phylogenetic tree generated from the alignment of the CP nt sequences of BC2 isolate and some selected isolates of BCMV. Isolate names and GenBank accession numbers are as follows: Aus (DQ054366), Sikkim (JQ753313), B1C-VN/RB1 (DQ925420), MS1 (EU761198), NL-1n (EU713858), Vanilla (AJ429522), KnXB-3 (JF427590), B-29K (FJ157245), B1C-VN/BB2-6 (DQ925423), Zacatecas (AF328753), PSt-VN/Ca1 (DQ925419). The tree was constructed using DNAMAN software with 1000 bootstrap replicates. Values at nodes indicate significance in bootstrap analysis.

DISCUSSION

Bean common mosaic virus and *Bean yellow mosaic virus* are two bean infecting potyviruses that are widely distributed in bean crops throughout the world (Galvez and Morales, 1989; Gilbertson *et al.*, 2002).

In this survey, leaf samples were collected from 520 plants at five cities of Kerman provinces. The occurrence of BCMV and BYMV in the fields surveyed and analysis of DAS-ELISA data indicates that these viruses are widely distributed in the selected cities of Kerman province, which were tested. Any location was not found to be virus-free. In this study, the infection rate of both viruses in Baft city was higher than other cities. Our finding is in agreement with those of some other authors who mentioned BCMV is more important than BYMV in common bean fields of Iran and other parts of the world (Shahraeen *et al.*, 2005; Gilbertson *et al.*, 2002). A significant proportion of the plants surveyed were symptomatic but tested negative by ELISA for BCMV or BYMV. It was discovered subsequently that, for many of these samples, the mosaic and deformation symptoms observed in the field probably are linked to infection by some other viruses such as *Cucumber mosaic virus* and *Alfalfa mosaic virus* (Gilbertson *et al.*, 2002) or may be result of physiological damages.

The biological characterization of the selected isolates of each virus was performed based on expression and type of symptoms on a number of species belonging to five families. From the reactions produced in

these hosts, we noticed high level of biological variability between BY1 and BC2 isolates. Isolates differed greatly in the number of plant species that become infected and in resultant symptoms. Whereas BY1 isolate infected many hosts systematically, BC2 isolate caused systemic infection in only a few of the species tested. The symptoms produced by BY1 were similar to those described by Al-Khalaf *et al.,* (2008) for SV205-85 isolate. It is difficult to identify these isolates by the reaction to indicator plants. Because of the wide host range of the virus, it seems that BYMV could become an important pathogen on other crop in addition to bean.

The presence of BYMV and BCMV viruses were confirmed using specific RT-PCR. The specificity of these primers was confirmed through nucleotide sequence analysis. These primers will be useful for rapidly screening bean germplasm where seed contamination by isolates of the two viral species occurs.

The present work represents the first identification of BCMV and BYMV in Kerman province by means of serological and molecular techniques. The distribution of BCMV and BYMV in this area hereby reported will be useful for breeders to incorporate virus resistance into bean cultivars where any or both of the two viral species occur.

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