Impact of cucumber mosaic virus infection on the varietal traits of common bean cultivars in Iran

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Abstract Cucumber mosaic virus (CMV) appears to be one of the most widespread pathogens on beans. In the present research, 49 Iranian lines and cultivars of common bean (*Phaseolus vulgaris*) were screened for their reaction to CMV. Plants at primary leaf stage were inoculated with CMV by rub inoculation and then they were kept in an insect-proof growth chamber at 20 °C. Three weeks post-inoculation, inoculated plants were assayed based on their symptoms, growth rate, fresh and dry weights and virus titer. Results of the present study showed that a line, D81083, had moderate resistance, six lines and cultivars were found to be tolerant to the CMV and 42 lines were found to be susceptible, these plants exhibited severe symptoms and accumulated high levels of virus titer. However in the present research one moderately resistant line and six tolerant lines and cultivars were identified for use in breeding and cultivation and also for future on researches bean.

Keywords CMV · Iran · *Phaseolus vulgaris* · Resistance

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the staple crops in many parts of Africa, Asia and Latin America and serves as a source of primary nutrition to millions of people because of its high protein content [16]. In addition to human diet, leguminous crops are used for animal feed and the industrial production of citric acid [37]. Legumes like

other crops are attacked by several viral, fungal and bacterial pathogens [14]. Damaging consequences, caused by phytopathogenic virus attacks, are primarily manifested in the form of reduced plant yield, poor product quality, and extinction of infected plants [11, 26]. Virus disease surveys of French bean in Iran has shown the presence of eight virus species namely *Soybean mosaic virus* (SBMV), *Bean pod mottle virus* (BPMV), *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Bean yellow mosaic virus* (BYMV), *Bean leaf roll virus* (BLRV), *Cucumber mosaic virus* (CMV), and *Alfalfa mosaic virus* (AMV) [31] with the dominance of CMV in 10 % of the samples. Though all these viruses have potential to cause severe crop losses but their epidemics depends upon availability of vectors, type of variety and most important time of infection [32]. CMV, a type species of the genus *Cucumovirus* in the family *Bromoviridae*, being one of the most common plant virus is known to infects more than 1,300 species across the world [11].

CMV is a multicomponent virus consisting of three genomic single-stranded RNAs each encapsidated individually in a 28 nm diameter icosahedral particle. CMV is aphid transmitted in a stylet-borne non-persistent manner [12] and is seed borne in some hosts [38, 40]. CMV seed transmission in susceptible bean cultivars is up to 49 % [7] and is retained in the seed for about 27 months during storage [3]. CMV strains earlier described mainly based on host range, symptoms, vectors, serological properties and nucleic acid sequence [26] are currently divided into two major groups (subgroup 1 and subgroup 2) based on serological and sequence similarities [18].

CMV is the most prevalent virus detected in snap bean fields in some regions [21, 30]. In New York, up to 100 % of plants in some snap bean fields were infected with CMV [23]. The tepary bean (*Phaseolus acutifolius*) PI 309881

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Table 1 Lines and cultivars, place of collection and seed types of bean which were evaluated in this study

Line and cultivar name	Place of collection	Seed type	Line and cultivar name	Place of collection	Seed type
21240	K	Pinto bean	Sadri	K	Pinto bean
Ks51103	K	Pinto bean	Pak	K	White
Ks41197	K	White	Naz	K	Dark red
21201	K	Pinto bean	Oshtar	B	Red
21471	K	Pinto bean	TN-38-179	G	Cream
21213	K	Pinto bean	TN-38-14	G	Pinto bean
21182	K	Pinto bean	TN-38-145	G	Red
Ks41110	B	White	TN-38164	G	Cream
Ks21682	B	Pinto bean	TN-38-98	G	Cream
Ks21191	B	Pinto bean	TN-38-5	G	Pinto bean
Ks21676	B	Pinto bean	TN-38-130	G	Black
Ks21666	B	Pinto bean	TN-38-1033	G	Cream
D81083	B	Red	TN-38-138	G	Pink bean
Cos16	B	Pinto bean	TN-38-125	G	Cream
Aligodarz 1	B	Red	TN-38-1183	G	Red
Aligodarz 2	B	Red	TN-38-1374	G	Cream
Shazand	B	Red	TN-38-612	G	Pink bean
Dorsa	K	White	TN-38-1287	G	Pinto bean
Akhtar	K	Red	TN-38-49	G	Dark red
Goli	K/B	Red	TN-38-1487	G	Anasazi
Derakhshan	K	Red	TN-38-1252	G	Cream
Talash	B	Pinto bean	TN-38-732	G	Pinto bean
Daneshkadeh	K	White	TN-38-854	G	Cream
Shokoufa	K	White	TN-38-116	G	Black
Mehali Khomain	K/B	Pinto bean	–	–	–

K Khomain bean research institute

B Brojerd bean research institute

G Iranian National Plant Gene-Bank

accession had previously been identified as CMV resistant, whereas no resistance was observed in 93 snap beans and 16 dry beans in American cultivars that were evaluated [15]. Recently, four Iranian cultivars Sadri, Shokoufa, Dorsa and Pak have been introduced suitable for cultivation in temperate-cold areas, tolerant to spotted spider mite and suitable for mechanized harvesting, respectively [2, 10, 35, 36].

RT4-4, a resistance gene, is identified in common bean cv. Othello that offered resistance to bean dwarf mosaic virus [39], and then it has been shown that this gene causes resistance to other viruses like CMV [28]. Some other resistance genes against CMV in other species were identified; *Ncm-1*, a single dominant resistance gene, had identified in lupin species [20], *RCY1*, a single dominant gene which is found in *Arabidopsis thaliana* ecotype C24, causes resistance to a yellow strain of CMV [34].

CMV is difficult to control because of its extremely broad natural host range, and the ability to be transmitted in a non-persistent manner by more than 60 species of aphids

[41]. Control of the aphid borne bean viruses through pesticides is impractical, as the aphid vectors complete their life cycle on other plants, such as, alfalfa, lettuce, celery [4, 24, 27]. The only practical solution to these problems is the incorporation of host-plant resistance into beans. Therefore, there is a need to evaluate resistant bean varieties to overcome this disease threat. Consequently, the purpose of the present study was to investigate the reaction of Iranian bean lines and cultivars to CMV.

Materials and methods

Plant materials and virus

Common bean (*P. vulgaris*) including 49 lines and cultivars were screened (Table 1). The CMV strain used in these tests was Fars isolate (provided by Plant Virology Research Center (PVRC), Shiraz, Iran). The virus was maintained on cucumber. Seeds of each cultivar or line

Fig. 1 Symptom scales of bean response to cucumber mosaic virus. **a** No visible symptoms, **b** mottling, **c** slight mosaic, **d** strong mosaic and malformation, **e** slight systemic necrosis

were planted in plastic pots containing potting mix (1:1:1, peat moss, perlite, and field soil).

Statistical analyses

The treatments consisted of the factorial combinations of 25 genotypes (cultivars and lines of bean) with and without virus (mock) inoculation. There was two pots per treatments in a completely randomized design. Two plants were evaluated for each pot. Main and interaction effects of experimental factors were determined from analysis of variance (ANOVA) using the GLM procedure in SAS [29]. *F* tests were conducted using the appropriate error term. Simple effects of virus treatments at each genotype were compared using the SLICE option for the least square means statement of SAS.

Virus inoculations

When the primary leaves were 50–75 % expanded (7 days after seeding), one primary leaf per plant was mechanically inoculated with a triturate of cucumber infected tissue in 0.1 M phosphate buffer (pH 8.5) and carborundum powder. A separate, sterile, cotton-tipped stick was used to inoculate each plant. After inoculation, plants were placed on a growth room (20 ± 2 °C, 16:8, light:dark).

Disease evaluations

All plants were evaluated 21 days postinoculation based on symptoms and vigor (leaf area and plant height), top dry and fresh weights, virus titer based on ELISA and the presence of viral RNA in plants. The symptoms score was performed from 0 to 5 (Fig. 1). Finally the reaction of lines and cultivars was clustered based on all morphological, serological and molecular data. The response of bean lines and cultivars against CMV were scaled as: (1) susceptible plants which contained viral RNA and developed symptoms of the disease, (2) tolerant plants which contained detectable amounts of viral RNA but showed a lack of, or delayed, symptoms, and (3) resistant plants which showed

neither the presence of virus RNA nor the symptoms of the disease [17].

Fresh and dry weights

Top fresh weights were determined by cutting the plants at soil level 21 days postinoculation. Plant tops were then dried in an oven at 70 °C for 3 days for top dry weight determination. The fresh and dry weights were measured and then were compared to those values from un-inoculated check plants to determine the percent reduction in dry weight.

Virus titer

Indirect-ELISA was performed for detection and quantifying ration of virus titer in the CMV-inoculated lines and cultivars [6]. The second trifoliolate leaves of each plant 21 days postinoculation were taken and tested using a rabbit anti CMV polyclonal antiserum with a 1:350 dilution produced by Plant Virology Research Center (PVRC), Shiraz, Iran. Goat anti-rabbit-IgG alkaline phosphatase conjugate (Promega, USA) was used as secondary antibody with a 1:7,500 dilution. Six replicates were applied for each samples.

For this assay the sap from top leaves was extracted in extraction buffer (1:10 ratio) [0.1 M K_2HPO_4 , 0.15 % 2- β -mercaptoethanol, pH 8.5]. Absorbance was measured using an ELISA reader anthos 2,020 at 405 nm. If the OD of plants was equal or more than R ($R = X + 3 SD$, X is the mean of negative control, SD is the standard deviation of negative controls) described as infected plants.

RNA extraction

RNA extraction was performed using the RNX-Plus solution (CinnaGen, Tehran, Iran) according to the manufacturer's instructions. Around 0.1 gr of leaf was ground and then one ml RNX-Plus solution was added and after shaking 500 μ l of chloroform was added and shaken gently for five min, centrifuged for 10 min at 13,000 rpm, 4 °C

(Allegra 64R), supernatant was transferred into another RNase free tube. Added one volume of isopropanol, then incubated at -20°C for 20 min, centrifuged at 4°C , 10 min, 13,000 rpm, the supernatant was discarded and the plate was washed by cold ethanol (70 %). The plate was dried and then resuspended in 30 μl of DEPC water and stored at -20°C .

Viral cDNA synthesis and PCR

The reverse transcription was performed using the hyperScript RT premix kit as described by the manufacturer (GeneAll, South Korea). The PCR reaction was performed in a total volume of 25 μl containing: 2 μl of cDNA, 2 unit *Taq* DNA polymerase (CinnaGen, Tehran, Iran), 0.5 μl of 10 mM dNTP mix, 2.5 μl of $10\times$ PCR Buffer, 0.8 μl of 50 mM MgCl_2 , 1 μl of 10 μM of each forward and reverse primers, CMV-F: GTAGACATCTGTGACGCGA, CMV-R: GCGCGAAACAAGCTTCTTATC [8], rRNA-F: AACGGCTACCACATCC AAGG and rRNA-R: TCATTACTCCGATCCCGAAG [22] and 14.4 μl of sterile water. The PCR reaction was carried out in a SensQuest thermocycler (Germany) thermal cycler as follows: initial denaturing at 94°C for 2 min, 35 cycle of 94°C for 40 s, annealing temperature at 60°C for 30 s, extension at 72°C for 90 s, then followed with one cycle at 72°C for five min as final extension.

Results

Reaction of lines and cultivars to CMV

Forty-nine lines and cultivars of different types of beans were screened. Resulted data showed that the mechanical inoculation method was efficient enough for inoculation of bean lines. Cultivated bean lines showed different symptoms ranging from no symptoms to severe malformation and necrosis (Fig. 1). The effects of CMV on length, fresh and dry weights of common bean lines and cultivars are summarised in Tables 2 and 3, and the reaction of lines and cultivars to the virus 21 days postinoculation is shown in Table 3. Statistical analysis showed significant differences in disease severity rate among varieties (Table 2). Morphological and statistical analyses showed that the lines and cultivars were significantly different at 0.05 (Table 2). Consequently each infected line was compared with its healthy check plants by paired sample T test analysis. Leaf area of the two top leaves of plants were measured by leaf area meter DELTA-T and showed that this parameter was not different at 0.01 and is not a good parameter for screening at post inoculation time. Other parameters used in these assays showed significant difference between

Table 2 Mean squares and significance levels from ANOVA for effects of cucumber mosaic virus on length (L) and fresh (FW) and dry (DW) weights among the bean genotypes (lines and cultivars)

Source	DF	L	DW	FW
Genotype	24	11140.6**	0.71**	36.11**
Virus	1	3814.1**	1.63**	92.63**
Genotype $\times$ virus	24	694.8 ^{ns}	0.65**	38.87**
Genotype $\times$ virus $\times$ pot (Error a)	50	437.5	0.17	9.60
Sampling error (Error b)	100	456.4	0.16	7.69
CV (%)	–	18.16	23.63	17.93

^{ns} not significant

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

healthy and infected plants (Table 2). The lines with the most potential for resistance in the first replicate were tested further in the growth chamber tests. It was concluded therefore that line D81083 was moderately resistant. Six lines including 21,213, Ks21666, Daneshkadeh, Sadri, TN.38.1033 and TN.38.1183 were evaluated as tolerant with slight absorbance at 405 nm and symptom ratings less than 1.5 and there was no difference between healthy and infected plants at 0.05 (Tables 2 and 3). The other 42 lines and cultivars which were screened, evaluated as susceptible to CMV (Table 3). Molecular analysis of resistant and tolerant lines showed virus band in all of them and a 520 bp band was amplified in each case (Fig. 2).

Discussion

Screening for sources of resistance to diseases is the first step in a genetic improvement breeding program and resistant bean varieties could potentially form the basis of sustainable diseases management strategies. Common bean plants inoculated with virus were considered as resistant if no systemic virus movement or symptoms occurred on any leaves other than the inoculated primary leaf [33]. Resistant plants were normal in size and appearance except for the inoculated leaf, which at times showed symptoms or even died and fell off. Common bean plants susceptible to CMV exhibited symptoms on top trifoliate leaflets and reduction in plant fresh and dry weights, high absorbance at 405 nm, indicating systemic virus movement. The range of symptoms in susceptible plants can vary among plants and also plants might be infected by other seed borne viruses or be influenced environmentally. In the present study, the reaction of 49 Iranian common bean lines and cultivars were assessed to CMV. Because of the detection of CMV in all plants by serology or molecular techniques in repeated experiments, based on these criteria, it was concluded that all the lines and cultivars evaluated in this study were

Table 3 Reaction of lines and cultivars to cucumber mosaic virus 21 days after inoculation. Results for ELISA represent the mean of six samples

Line/cultivars	Virus	Mean			Mean scale	Absorbance at 405 nm	PCR	CMV response
		L	DW	FW				
21240	With	71.00	1.073	14.77	0.5	1.1/+	0	S
	Without	83.00	2.05	5.60	–	–	–	–
	<i>p</i> value	0.42	0.28	0.71	–	–	–	–
Ks51103	With	75.50	1.56	12.85	1.66	0.92/+	0	S
	Without	79.00	1.71	14.47	–	–	–	–
	<i>p</i> value	0.97	0.62	0.46	–	–	–	–
21201	With	61.03	1.01	12.30	0.375	0.866/+	0	S
	Without	69.25	1.39	13.17	–	–	–	–
	<i>p</i> value	0.58	0.33	0.69	–	–	–	–
21471	With	117.00	1.84	15.7	0.6	0.95/+	0	S
	Without	129.5	1.98	22.05	–	–	–	–
	<i>p</i> value	0.40	0.63	0.005	–	–	–	–
21213	With	115.25	1.61	14.50	1.25	0.55/–	+	T
	Without	124.75	1.67	16.75	–	–	–	–
	<i>p</i> value	0.52	0.84	0.311	–	–	–	–
21182	With	133.13	2.04	13.27	1.5	0.78/+	0	S
	Without	135.75	2.25	16.1	–	–	–	–
	<i>p</i> value	0.86	0.466	0.20	–	–	–	–
Ks21191	With	100.16	1.68	14.75	0.125	1.18/+	0	S
	Without	128.00	2.81	20.35	–	–	–	–
	<i>p</i> value	0.06	0.0003	0.01	–	–	–	–
Ks21676	With	72.50	1.07	12.66	1.14	0.8/+	0	S
	Without	92.75	1.66	17.27	–	–	–	–
	<i>p</i> value	0.177	0.044	0.04	–	–	–	–
Ks21666	With	106.25	1.327	13.00	1.42	0.6/–	+	T
	Without	118.00	1.67	13.82	–	–	–	–
	<i>p</i> value	0.43	0.24	0.70	–	–	–	–
D81083	With	51.25	1.52	16.85	0	0.657/–	+	MR
	Without	80.25	1.65	19.25	–	–	–	–
	<i>p</i> value	0.055	0.66	0.28	–	–	–	–
Aligodarz 1	With	133.50	1.55	17.49	0.875	0.91/+	0	S
	Without	138.00	1.83	18.05	–	–	–	–
	<i>p</i> value	0.76	0.34	0.8	–	–	–	–
Shahzand	With	169.75	1.55	16.55	1.71	0.81/+	+	S
	Without	173.25	2.09	17.00	–	–	–	–
	<i>p</i> value	0.81	0.067	0.84	–	–	–	–
Akhtar	With	83.87	1.83	16.40	1.66	1.03/+	+	S
	Without	88.75	1.89	19.35	–	–	–	–
	<i>p</i> value	0.743	0.81	0.18	–	–	–	–
Goli	With	164.75	1.44	13.60	1.375	0.97/+	0	S
	Without	165.75	1.57	15.00	–	–	–	–
	<i>p</i> value	0.95	0.65	0.53	–	–	–	–
Derakhshan	With	66.87	1.41	12.18	1.5	0.7/–	+	S
	Without	90.00	2.34	17.72	–	–	–	–
	<i>p</i> value	0.124	0.002	0.01	–	–	–	–

Table 3 continued

Line/cultivars	Virus	Mean			Mean scale	Absorbance at 405 nm	PCR	CMV response
		L	DW	FW				
Talash	With	131.13	1.32	10.39	2.125	0.82/+	0	S
	Without	143.25	1.59	14.87	—	—	—	—
	<i>p</i> value	0.42	0.36	0.05	—	—	—	—
Daneshkadeh	With	130.37	1.24	13.90	0.166	0.625/—	+	T
	Without	155.75	1.87	17.35	—	—	—	—
	<i>p</i> value	0.092	0.036	0.12	—	—	—	—
Mehali khomain	With	154.5	1.78	14.59	1.87	0.9/+	0	S
	Without	174.25	2.51	18.47	—	—	—	—
	<i>p</i> value	0.187	0.015	0.08	—	—	—	—
Sadri	With	139.75	1.59	13.13	0.75	0.755/+	0	T
	Without	162.25	2.04	16.10	—	—	—	—
	<i>p</i> value	0.134	0.127	0.18	—	—	—	—
Pak	With	72.25	0.84	13.35	1	0.92/+	0	S
	Without	91.75	1.37	10.91	—	—	—	—
	<i>p</i> value	0.193	0.07	0.27	—	—	—	—
TN.38.145	With	82.62	1.16	11.57	3	0.933/+	0	S
	Without	93.50	1.34	12.75	—	—	—	—
	<i>p</i> value	0.465	0.53	0.60	—	—	—	—
TN.38.164	With	138.75	1.44	13.54	2	0.66	0	S
	Without	178.75	2.04	16.05	—	—	—	—
	<i>p</i> value	0.009	0.043	0.26	—	—	—	—
TN.38.1033	With	129.50	1.42	12.52	1	0.63	0	T
	Without	171.87	1.52	17.67	—	—	—	—
	<i>p</i> value	0.006	0.74	0.02	—	—	—	—
TN.38.1183	With	55.00	1.15	12.07	1	0.675	0	T
	Without	92.75	3.00	28.13	—	—	—	—
	<i>p</i> value	77.63	0.0001	0.0001	—	—	—	—
Oshtar	With	179.25	1.75	16.13	0.5	1.1/+	+	S
	Without	183.13	1.97	17.10	—	—	—	—
	<i>p</i> value	0.794	0.46	0.66	—	—	—	—
Negative control	—	—	—	—	—	0.52	—	—

The *p* value is based on an *F* test for simple main effects of virus for each genotype using the slice option in SAS

S susceptible, T tolerant, MR moderate resistance, + positive ELISA, PCR 0 PCR was not done, Negative control not treatment plants. $R(X + 3SD) = 0.709$

L plant length, DW dry weight, FW fresh weight, C control plants (mouck inoculated)

infected by CMV Fars isolate in different degrees. The ELISA absorbance values were considerably different between various cultivars or lines that were assayed. These variable reactions indicated different levels of susceptibility to the virus. Although using ELISA to measure virus titer has been common approach in assessing plant resistance, ELISA results have not always been directly correlated with symptoms and therefore comprehensive quantitative rating system represents a more reliable way for direct measurement of the effect of virus on the plant [33].

Results of the present study suggest that RT-PCR may provide a more definite criterion than morphological inspection and/or ELISA in the selection of resistant lines. The leaf area data were variable and, consequently, not as useful in grading lines for resistance or susceptibility. When partial resistance was present, the variable reactions were not unexpected [33]. In this research plants evaluated after 21 days based on some researches [33] despite showing difference in morphological parameters including top fresh/dry weights and plant height between control and treated plants, yet for most lines there was non statistical

Fig. 2 PCR detection of cucumber mosaic virus specific 520 bp and plant 18S rDNA specific 459 bp DNA fragment in resistant lines and cultivars of common bean plants, electrophoresis on a 1 % agarose gel and staining by ethidium bromide. 1 size marker (GeneRuler™

1 kb DNA ladder, Fermentas), 2 healthy common bean, 3 CMV infected common bean as a positive control, 4 Ks21666, 5 D81083, 6 Daneshkadeh, 7 Derakhshan, 8 21213, 9 Akhtar

significant difference (Table 3). It is concluded that plants should be checked after longer growth period as suggested by other researchers [5, 12]. This investigation showed that line D81083 had moderate resistance to CMV and the virus was not detected in it by ELISA, while the virus can replicate in this line with low titer. The line D81083 is therefore recommended for breeding program of common bean (Table 3). Our results showed that 21213, Ks21666, Daneshkadeh, Sadri, TN.38.1033, and TN.38.1183 lines were tolerant to CMV (Table 3) and could be used as potential genetic source in bean breeding programs as well and should be checked by insect virus infection in field condition. Morphological and serological experiments suggest that some lines and cultivars may be resistance to CMV but in molecular analysis the CMV-RNA were detected (Fig. 2). The validation of the reaction of bean lines and cultivars to CMV in the field condition remain to be addressed.

Previous study in our laboratory has shown that 21,213 and 21,182 lines are resistant to tomato yellow leaf curl virus [22], while the result of present study showed that the line 21,213 was tolerant to CMV and the reaction of 21,182 line was susceptible. Moreover, Talash and Derakhshan cultivars have been evaluated as resistant to BCMV [19], but we showed that Derakhshan cultivar was susceptible to CMV. In addition, it has been shown that line ks51103 is resistance to bacterial common blight (*Xanthomonas axonopodis* pv. *phaseoli*) [25], whereas our result showed that this line was susceptible to CMV. The greenhouse screening is only a quick indication of which lines should be tested further, and the potential resistant lines have to be tested in the field [13].

Identification markers linked to resistant gene for characterization of resistance genes to CMV and transfer of these genes to tolerant bean plants could be carried out but inoculations with other virus strains may be useful in determining which resistance gene or genes is present.

The identification of the genetic systems responsible for these reduced viral accumulations could enable pyramiding them with other systems controlling different responses and thus enhancing the durability of the resistance [5]. It would be of interest to ascertain the stability of resistance found in D81083 and other resistance lines when cultured at

different environmental conditions like variable temperature. In this research, plants were inoculated by mechanical method, some lines may have resistance to insect vectors as was found in other researches for different viruses and different plants [1, 9]. It would be interesting theory to check the interactions between viruses, lines and viral vectors.

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References

1. Azizi A, Mozafari J, Shams-bakhsh M. Phenotypic and molecular screening of tomato germplasm for resistance to tomato yellow leaf curl virus. *Iran J Biotechnol.* 2008;6:1999–2206.
2. Beyzaei E, Dorri HR, Ghanbari AA, Gaffari Khaligh H, Rahmani Ghobadi A, Taheri Mazandarani M, Shahraeen N, Yousefi M Sadri. A new large seed chiti bean cultivar suitable for cultivation in temperate-cold areas of Iran. *Seed Plant Improv J.* 2012; 28(2):335–7.
3. Bos L, Matt DZ. A strain of cucumber mosaic virus, seed-transmitted in beans. *Neth J Plant Pathol.* 1974;80:113–23.
4. Bruckart WL, Lorbeer JW. Cucumber mosaic virus in weed hosts near commercial fields of lettuce and celery. *Phytopathology.* 1976;66:253–9.
5. Cebolla-Cornejo J, Soler S, Gomar B, Soria MD, Nuez F. Screening capsicum germplasm for resistance to tomato spotted wilt virus (TSWV). *Ann Appl Biol.* 2003;143:143–52.
6. Clark ME, Adams AN. Characteristics of microplate methods of enzyme linked immunosorbent assay for detection of plant viruses. *J Gen Virol.* 1977;34:475–83.
7. Davis RF, Hampton RO. Cucumber mosaic virus isolates seed-borne in *Phaseolus vulgaris*: serology, host pathogen relationships and seed transmission. *Phytopathology.* 1986;76:999–1004.
8. De Blas C, Borja MI, Saiz M, Romero J. Broad spectrum detection of cucumber mosaic virus (CMV) using the polymerase chain reaction. *J Phytopathol.* 1994;141:323–9.
9. Dintinger J, Boissot N, Chiroleu F, Hamon S, Reynaud B. Evaluation of maize inbreds for maize strip virus and maize mosaic virus resistance: disease progress in relation to time and accumulative number of plant hoppers. *Phytopathology.* 2005;95: 600–7.
10. Dorri HR, Beizaie E, Ghanbari AA, Assadi B, Gaffari Khaligh H, Shahraeen N, Yousefi M, Dadivar M, Lak MR, Hassani Mehraban A Dorsa. A new white bean cultivar, tolerant to tow spotted

- spider mite, for cultivation in temperate -cold areas of Iran. *Seed Plant Improv J.* 2012;28(1):155–7.
11. García-Arenal F, Palukaitis P. Cucumber mosaic virus. In: García-Arenal F, Palukaitis P, Mahy BWJ, Van Regenmortel MHV, editors. *Encyclopedia of Virology*. 3rd ed. Oxford: Academic Press; 2008. p. 614–9.
 12. Gildow FE, Shah DA, Sackett WM, Butzler T, Nault BA, Fleischer SJ. Transmission efficiency of cucumber mosaic virus by aphids associated with virus epidemics in snap bean. *Phytopathology*. 2008;98:1233–41.
 13. Gillaspie AGJ. New method for screening cowpea germ plasm for resistance to cucumber mosaic virus. *Plant Dis.* 2006;90:611–4.
 14. Graham PH, Vance CP. Legumes: importance and constraints to greater use. *Plant Physiol.* 2003;131:872–7.
 15. Griffiths P. Breeding snap beans for cucumber mosaic virus (CMV) resistance. *101st annual international conference of the American society for horticultural science*. Austin, Texas 2004: 869.
 16. Hall R. *Compendium of bean diseases*. Minnesota: APS Press, The American Phytopathological Society; 1991.
 17. Hernández-Verdugo S, Guevara-González RG, Rivera-Bustamante RF, Oyama K. Screening wild plants of *Capsicum annuum* for resistance to pepper huasteco virus (PHV): presence of viral DNA and differentiation among populations. *Euphytica*. 2001;122:31–6.
 18. Hong JS, Ohnishi S, Masuta C, Choi JK, Ryu KH. Infection of soybean by cucumber mosaic virus as determined by viral movement protein. *Arch Virol.* 2007;152:321–8.
 19. Hormozi-Nejad MH, Mozafari J, Rakhshandehroo F. Responses of Iranian bean cultivars to bean common mosaic virus (BCMV). 18th Iranian plant protection congress. Hamadan: University of Bu-Ali Sina; 2008. p. 24–7.
 20. Jones RAC, Latham LJ. Natural resistance to cucumber mosaic virus in lupin species. *Ann Appl Biol.* 1996;129:523–42.
 21. Larsen RC, Miklas PN, Eastwell KC, Grau CR, Mondjana A. A virus disease complex devastating late season snap bean production in the Midwest. *Annals reports of bean improvement cooperative, Michigan State University, Michigan*; 2002. (45): 36–37.
 22. Montazeri Hedesh R, Shams-Bakhsh M, Mozafari J. Evaluation of common bean lines for their reaction to tomato yellow leaf curl virus-Ir2. *Crop Prot.* 2011;30:163–7.
 23. Nault BA, Shah DA, Taylor AG. Viruses and aphids everywhere in New York snap bean fields in 2005. In *Proceedings of the 2006 Empire State Fruit and Vegetable EXPO*. Cornell Coop. Exten., Syracuse, NY; 2006. pp. 74–76.
 24. Nault BA, Shah DA, Dillard HR, McFaul AC. Seasonal and spatial dynamics of alate aphid dispersal in snap bean fields in proximity to alfalfa and implications for virus management. *Environ Entomol.* 2004;33:1593–601.
 25. Osdaghi E, Alizadeh A, Shams-bakhsh M, Lak MR. Evaluation of common bean lines for their reaction to the common bacterial blight pathogen. *Phytopathol Mediterr.* 2009;48:461–8.
 26. Palukaitis P, Roossinck MJ, Dietzgen RG, Francki RIB. Cucumber mosaic virus. *Adv Virus Res.* 1992;41:281–348.
 27. Rist DL, Lorbeer JW. Relationships of weed reservoirs of cucumber mosaic virus (CMV) and broad bean wilt virus (BBWV) to CMV and BBWV in commercial lettuce fields in New York. *Phytopathology*. 1991;81:367–71.
 28. Seo YS, Rojas MR, Lee JY, Jeon JS, Ronald P, Lucas WJ, Gilbertson RL. A viral resistance gene from common bean functions across plant families and is up-regulated in an non-virus-specific manner. *Proc Natl Acad Sci USA.* 2006;103:11856–61.
 29. SAS Institute Inc., The SAS System for Windows. Release 9.0. Statistical Analysis Systems Institute, Cary, NC, USA; 2002.
 30. Shah DA, Dillard HR, Mazumdar-Leighton S, Gonsalves D, Nault BA. Incidence, spatial patterns and associations among viruses in snap bean and alfalfa in New York. *Plant Dis.* 2006;90:203–10.
 31. Shahraeen N, Ghotbi T, Dezaje EA, Sahandi A. A survey of viruses affecting French bean (*Phaseolus vulgaris*) in Iran includes a first report of Southern bean mosaic virus and bean pod mottle virus. *Plant Dis.* 2005;89:1012.
 32. Spence NJ, Walkey DGA. Variation for pathogenicity among isolates of bean common mosaic virus in Africa and a reinterpretation of the genetic relationship between cultivars of *Phaseolus vulgaris* and pathotypes of BCMV. *Plant Pathol.* 1995;44:527–46.
 33. Strausbaugh CA, Myers JR, Forster RL, McClean PE. A quantitative method to screen common bean plants for resistance to bean common mosaic necrosis virus. *Phytopathology*. 2003;93:1430–6.
 34. Takahashi H, Miller J, Nozaki Y, Sukanto TM, Shah J, Hase S, Ikegami M, Ehara Y, Dinesh-Kumar SP. RCY1, an *Arabidopsis thaliana* RPP8/HRT family resistance gene, conferring resistance to cucumber mosaic virus requires salicylic acid, ethylene and a novel signal transduction mechanism. *Plant J.* 2002;32:655–67.
 35. Taheri Mazandarani M, Beyzaei E, Dorri HR, Ghanbari AA, Salehi P, Taheriyoon G, Mohammadkhani R, Shahraeen N, Hassani Mehraban A Shokoufa. Shokoufa a new high yielding white bean cultivar for cultivation in temperate and cold areas of Iran. *Seed Plant Improv J.* 2010;26(1):141–4.
 36. Taheri Mazandarani M, Beyzaei E, Dorri HR, Ghanbari AA, Salehi P, Taheriyoon G, Mohammadkhani R, Shahraeen N, Hassani Mehraban A Pak. Pak, a new white bean cultivar suitable for mechanized harvesting. *Seed Plant Improv J.* 2010;26(1):144–6.
 37. Todorović J, Vasić M, Todorović V. Dry and spring bean. Grafomark, Institute of Field and Vegetable Crops, Novi Sad, Faculty of Agriculture, Banja Luka, Serbia and BiH; 2008.
 38. Tomlinson JA, Karter AL. Studies on the seed transmission of cucumber mosaic virus in chickweed (*Stellaria media*) in relation to the ecology of the virus. *Ann Appl Biol.* 1970;66:381–6.
 39. Wang HL, Sudarshana MR, Gilbertson RL, Lucas WJ. Analysis of cell-to-cell and long-distance movement of a bean dwarf mosaic geminivirus-green fluorescent protein reporter in host and nonhost species: identification of sites of resistance. *Mol Plant Microbe Interact.* 1999;12:345–55.
 40. Yang Y, Kim KS, Anderson EJ. Seed transmission of cucumber mosaic virus in spinach. *Phytopathology*. 1997;87:924–31.
 41. Zehnder GW, Murphy JF, Sikora EJ, Kloepper JW. Application of rhizobacteria for induced resistance. *Eur J Plant Pathol.* 2001;107:39–50.