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Nucleotide Sequence of Capsid Protein Gene of *Bean yellow mosaic potyvirus* in Bean Plants from Al-Makhwah Governorate, Saudi Arabia

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ABSTRACT

The current study represents the first identification of *Bean Yellow Mosaic Virus* (BYMV) in bean plants (*Phaseolus vulgaris*) from Al-Makhwah Governorate, Saudi Arabia and nucleotide sequencing of capsid protein gene of BYMV. Thirty plant species and cultivars to twelve different families were mechanically inoculated by BYMV. Seventeen of them showed systemic symptoms mosaic, yellowing, vein clearing, stunting streaking mosaic, malformation and severe mosaic as a result of BYMV infection. *Chenopodium amaranticolor* and *Chinopodium quinoa* L. were found to be local lesion host after 4-6 days of inoculation. Two aphids, *Myzus persicae* Sluz. and *Aphis faba* were used to study the transmission of BYMV. *Aphis faba* was found to be the most effective vector with 60% of BYMV transmission. Immunological techniques namely Enzyme Linked Immuno Sorbent Assay (ELISA), Tissue Blot Immuno Binding Assay (TBIA) and Dot Blot Immune Binding Assay (DBIA) were amplified to study BYMV. Positive reaction was obtained and the prevalence of the virus in the flowers and seed parts was confirmed. The RT-PCR products were amplified from total RNA of bean plant tissues using specific primer BYMV1 and BYMV2 (designed on conserved sequences of BYMV Nib-CP). A cDNA fragment of 700 bp nuclear inclusion body and coat protein gene region primer (5'-Nib-CP 3') was amplified. Digoxigenin-labelled BYMV cDNA probe through Southern blot and Dot blot hybridization techniques were employed for the detection of BYMV infected bean plants. A strong positive reaction was observed with bean and faba bean infected with BYMV. A part of the 3' end of Nib-region and the 5' end of the CP region of BYMV Potyvirus Al-Makhwah KSA isolate (700 nt) was sequenced and analyzed (The DNA sequence submitted in GenBank acc.no. LC025531). Identity percentage of Al-Makhwah KSA isolate BYMV Potyvirus (Nib-CP) with a Japanese isolate was 99% and with USA gladiolus isolate and another Japanese isolate were 95% and 93%, respectively confirming that BYMV viral group is diversified mainly in some specific parts of genome, especially in the CP region, whereas Nib gene is very conservative.

Key words: Inclusion bodies, TBIA and DBIA, RT-PCR, sequencing and nucleic acid hybridization

INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is the considered as one of the most important legume crop in Saudi Arabia and other countries of the world. It is a significant source of nutrients because it consists of fiber, proteins, mineral salts and vitamins (Adams *et al.*, 1985). Bean is subjected to invasion by many diseases caused by several viruses. *Bean Yellow Mosaic Potyvirus* (BYMV) is a

very serious disease found in most beans growing worldwide. A virus disease is one of the most economically important diseases that damages the foliage and limits photosynthetic activity depending on the infection stage. Khalil and Erskine (2001) reported that loss due to BYMV disease can be reached up to 30% on susceptible cultivars of faba bean. This disease is characterized by symptomatic leaf samples showing stunting, vein clearing, mosaic, chlorosis, bright yellowing and leaf distortion (Park *et al.*, 1998; Desbiez and Lecoq, 2004). The genus *Potyvirus*, belonging to family Potyviridae is a large group of plant pathogenic viruses that encompasses 111 recognized and 86 tentative species infecting more than 30 plant families. In nature, most *Potyvirus*es are efficiently transmitted by aphids in a non persistent manner (Fauquet *et al.*, 2005). The BYMV contains a single molecule of linear, positive-sense, single-stranded ribonucleic acid ssRNA, about 9.6 kb in size which has a poly (A) tract at the 3'-end (Shukla *et al.*, 1994). The stage of development of a crop at the time of infection by a virus influences the severity of the disease, with the greatest reduction in plant growth and yield occurring when plants are infected during the earliest stage of their development (Hull, 2002). The BYMV is readily detected in leaf tissues of bean, faba bean and gladiolas by reverse transcription polymerase chain reaction RT-PCR (Vunsh *et al.*, 1990; Khattab, 2002; Mohammad and Angela, 2004; Al-Ani and Adhab, 2013). In this study, we reported the detection of BYMV in bean by serological method, RT-PCR technique and nucleic acid hybridization. Nucleotide sequence determined in this study was submitted to the accession no. LC 025531 and in our opinion this is the first report of BYMV affecting Bean in, Saudi Arabia, KSA.

MATERIALS AND METHODS

Source of samples: Eight samples from naturally infected bean fields showing, mosaic, chlorosis bright yellowing, stunting, vein clearing and leaf malformation were collected from open field from AL-Makhwah Governorate in Southern region of Saudi Arabia during season 2011-2012.

Host range and symptomatology: Thirty plant species and cultivars belonging to twelve different families (Table 1) were mechanically inoculated by BYMV to study the host range. Five plants of each species and cultivars were inoculated with infected sap. In addition, five plants were inoculations with the sap prepared from healthy plants to serve as controls. Back inoculated were made on *Chenopodium amaranticolor* L. and *C. quinoa* in order to check virus in symptomless plants. On the other hand, all tested plants were serologically tested using indirect ELISA method.

Mode of transmission

Mechanical transmission: The mechanical transmissibility of the virus was assayed by sap inoculation using 0.1 M phosphate buffer, pH7.0, 1% sodium EDTA and carborandum. Immediately after inoculation the test plant leaves were washed with distilled water using an atomizer. Inoculated plants were kept in insect-proof greenhouse and visually inspected for typical viral symptoms.

Insect transmission: *Myzus persicae* Sulz and *Aphis fabae* Scop were tested to find out whether or not they transmit the virus. Colonies of the aphids were collected from the field and reared on cabbage seedlings under insect-proof cages for a long period of experimental time. Filial generations were used in sub sequent. Insects were fasted for 1 h before transferring it to infected bean plants. The aphids were left to feed for 10 min and then transferred to 10 healthy bean seedlings at rate of 10 insects per plant for inoculation feeding period of 24 h. The same procedure was applied for the control, except that virus-free aphids were used. Insects were killed by 0.2% acephate. The inoculated seedlings were kept in an insect-proof cage till symptoms appearance and

Table 1: Host range and reaction of the different host inoculated with the isolated virus

Families	Host plant	Symptoms	ELISA reading
Chenopodiaceae	<i>Chenopodium quinoa</i>	CLL	0.872+
	<i>Chenopodium amaranticolor</i> cost and Ryn	CLL	0.724+
Cucurbitaceae	<i>Cucumis sativus</i> L.	0	0.122-
	<i>Cucurbita pepo</i> L.	0	0.112-
Fabaceae	<i>Phaseolus vulgaris</i> L.	M-Y-Mal-VC-D-Stu	0.992+
	<i>Phaseolus coccineus</i> L.	M-Y-Mal-VC-D-Stu	0.989+
	<i>Phaseolus iunatus</i> L.	0	0.100-
	<i>Vigna unguiculata</i> L.	M-Y-Vc-D-Stu	0.975+
	<i>Vicia faba</i> L.	Y-Vc	0.979+
	<i>Lupinus albus</i>	S.M	0.892+
	<i>Trifolium hybridum</i>	Y-M	0.792+
	<i>Trifolium</i>	Y-M	0.752+
Solanaceae	<i>Nicotina glutinosa</i> L.	0	0.113+
	<i>Petunia hybrid</i> vilm	M	0.689+
	<i>Solanum tuberosum</i> L.	0	0.101-
	<i>Solanum lycopersium</i> L.	0	0.111-
Lamiaceae	<i>Ocimum basilicum</i>	M-Y-Vc-S	0.899+
	<i>Mentha longifolia</i>	M	0.692+
	<i>Mentha arvensis</i> L.	M	0.859+
	<i>Ruta graveolens</i>	0	0.100-
	<i>Coleus forskohlii</i>	0	0.111-
Alliaceae	<i>Allium cepa</i>	0	0.114-
Myrtaceae	<i>Dianthus cayophyllata</i>	0	0.102-
	<i>Dianthus</i>	0	0.102-
Amaryllidoideae	<i>Narcissus tazetta</i>	M-Y	0.952+
Geraniaceae	<i>Centaurea cineraria</i>	0	0.118-
Asteraceae	<i>Chrysanthemum morifolium</i>	0	0.109-
Theaceae	<i>Camellia sinensis</i>	M	0.940+

CLL: Chlorotic local lesion, Y: Yellowing, SM: Server mosaic, Mal: Malformation, 0: No symptoms, D: Pod deformation, Stu: Stunting, Vc: Vein clearing, M: Mosaic, -: Negative reaction, +: Positive reaction, *Control of indirect ELISA absorbance at 405 nm = 0.244

after two weeks the plants were checked for presence virus isolate using ELISA. Percentage of insect transmission was calculated for all treatments.

Serological detection

Indirect ELISA: The microtitre plates were coated with 200 μL well⁻¹ infected bean samples extracted in coating buffer, pH 9.6, 1 g tissue 5 mL buffer. The plates were covered and incubated at 37°C for 2 h. This method was described by Koenig (1981).

Dot blot immunoassay (DBIA): Healthy and infected beans samples were ground in phosphate buffer pH 9.5 (1:5 w/v). The DBIA was obtained as described by Lin *et al.* (1990).

Tissue blot immunoassay: Tissue blot immunoassay (TBIA) was used for detection of BYMV as described by Lin *et al.* (1990). Membrane was washed three times and incubated in substrate buffer (PBS containing 730 $\mu\text{g mL}^{-1}$ of Nitrobluetetrazolium (NBT) and 500 $\mu\text{g mL}^{-1}$ of 5-bromo-4-chloro-3-indolyl phosphate (BCIP), pH = 9.5) for 5 min at RT with shaking. Violet to purple color indicated. After the color has appeared, rinse membrane quickly with H₂O then air-dry.

RNA extraction and Reverse Transcription-Polymerase Chain Reaction (RT-PCR): Viral RNA was extracted from 50 mg of infected and healthy bean leaves using RNase Plant Mini Kit (Qiagen) according to manufacturer's instructions. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was done using Qiagen oneStep RT-PCR enzyme Mix (OneStep RT-PCR kit, Qiagen). Primer BYMV1/ BYMV 2 pair for *Bean yellow mosaic virus* (BYMV) was used this resulted in an amplification product 700 bp nuclear inclusion body and coat protein gene region primer (5' N1b-CP 3') by Uga (2005). The RT was performed in a thermocycler (Biometra Co.).

Eight micro litter of the RNA extraction and PCR product NIB-CP for BYMV and 2 μ L of loading dye (methylene blue) were analyzed on 1% agarose gel dissolved in 1XTAE buffer (Sambrook *et al.*, 1989), in the presence of 0.5 mg mL⁻¹ ethidium bromide and electrophoresis at 100 v. The DNA was visualized using an UV transilluminator (wavelength 245 nm) and 100 bp plus DNA ladder (Vivantes) was used as a size standard.

Southern blot and dot blot hybridization assays: Digoxigenin-11-dUTP-labeled cDNA probe, corresponding to BYMV was prepared by using dig labeling nucleotide mix (Roche, Boehringer Mannheim, Indianapolis). The specificity of the probes was determined southern blot hybridization by Southern (1975). This reaction was used to confirm that PCR amplified fragments by PCR was BYMV 5' NIB-CP gene using the non- radioactive dig labeled probe. The leaf tissues both plants bean and faba bean infected BYMV dot blots were carried out according to Loebenstein *et al.* (1997). Five micro litter of the supernatant were spotted onto nitrocellulose membrane. The DNA was fixed on the membranes by Ultraviolet (UV) cross-linked for 30 sec. Prehybridization, hybridization and immunological detection were carried out using the GeniusII DNA Labelling and Detection Kit (Boehringer Mannheim, IN System).

Partial nucleotide sequence BYMV: Amplified RT-PCR product of BYMV isolate was purified with GFXTM PCR purification kit (Amersham Pharmacia Biotech Inc. USA) and sent to Colors for research, Egypt for determined the sequence. Sequences of BYMV AL. Makhwah isolates 700 bp was checked for homologous sequences in GeneBank using the BLAST program available online (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic analysis of the BYMV isolate using DNAMAN software program to determine the phylogenetic relationship with published sequence in GenBank. Acc. Nos. of BYMV isolates was done using: AB439729 Japan Nakazono-Nagaoka *et al.* (2009), AM884180 Taiwan Chen *et al.* (2001), AY192568 USA Hammond and Hammond (2003), D83749 Japan Nakamura *et al.* (1996) and D28819 Japan Nakamura *et al.* (1994).

RESULTS

Host range and symptomatology: Reaction of thirty plant species and cultivars belonging to twelve different families shown in Fig. 1 and Table 1, were studied by mechanical inoculation with the virus isolate. The symptoms appeared on the host plants were confirmed by using indirect ELISA method. The host plants could be divided into three groups as follows: A-plant species reacted only with local symptoms: The virus produced chlorotic local lesions on the inoculated leaves of *Chenopodium amaranticolor* and *C. quinoa* within 4-6 days after inoculation B-plants reacted with systemic infection: The isolated virus caused yellow mosaic, stunting and malformation on *Phaseolus vulgaris* L. cvs. Elixir, Alhama, Banga Reverjard and *Vicia faba* L. It also showed mosaic and malformation on *Pelargonium zonta*, yellow and vein clearing on *Lupinus albus*, pod deformations and reduced seed size on *Phaseolus vulgaris* L. and *Vicia faba* L. Sever mosaic caused by the virus appeared on *Trifolium hybridum* and breaking color on flowers of *Pelargonium zonta* within 14-21 days of inoculation. C-Plants were not infected with the virus isolated. Result in Table 2 showed that thirteen plant species were not susceptible to the isolated virus such as *Cucumis sativus* L., *Cucurbita pepo* L., *Vigna unguiculata* L., *Coleus forskohli*, *Ruta graveolens*, *Solanum tuberosum* L., *Solanum lycopersicum*, *Nicotina glutinosa*, *Allium cepa*, *Dianthus caryophyllata*, *Dianthus barbatus*, *Centaurea cineraria* and *Chrysanthemum morifolium* L. Symptomless infection was confirmed by back inoculation on *C. amaranticolor* and assay host plant.

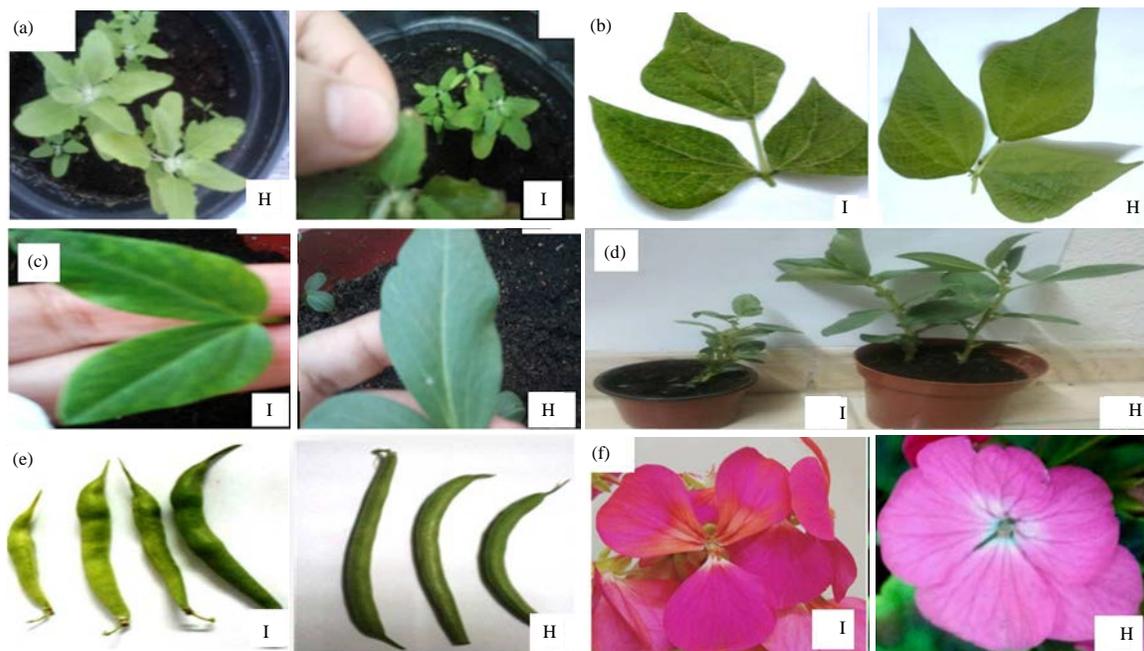


Fig. 1(a-f): (a) Symptoms caused by BYMV on H: Healthy leaves of *Chenopodium amaranticolor* and I: Infected leaf chlorotic local lesion, (b) Symptoms caused by BYMV on leaves of *Phaseolus Vulgaris*. cv. Elixir. I: infected leaves yellow mosaic and malformation, H: Healthy leaves, (c) Symptoms caused by BYMV on *Vicia faba* L. I: infected leaf yellow mosaic, H: Healthy leaf, (d) Symptoms caused by BYMV on *Vicia faba* L. I: infected plant showing stunting and H: Healthy plants, (e) Symptoms caused by BYMV on Pods of *Phaseolus vulgaris* cv. Elixir. I: infected pods showing deformations, mosaic and reduced seed size, H: Healthy pods and (f) Symptoms caused by BYMV on flowers of *Pellarjonium zonate* I: infected flowers showing color breaking, H: Healthy flower

Table 2: Percentage of transmission of the virus isolate by insects

Insects species	No. of inf. Pl./No of inoc. Pl	Infection (%)
<i>Myzus persicae</i> sulz	5/10	50
<i>Aphis fabae</i>	6/10	60

Modes of transmission

Mechanical transmission: The isolated virus was readily transmitted by mechanical inoculation of sap extracted from infected bean leaves to *Phaseolus vulgaris* L. cv. Elixir and *Chenopodium amaranticolor*.

Insects transmission: Both green peach aphid (*Myzus persicae* Sulz.) and *Aphis fabae* instead of faba were found to be vectors for the virus under investigation.

The virus inoculation study on *C. amaranticolor* showed 60% successful transmission by *A. faba* whereas only 50% by *M. persicae* Sulz.

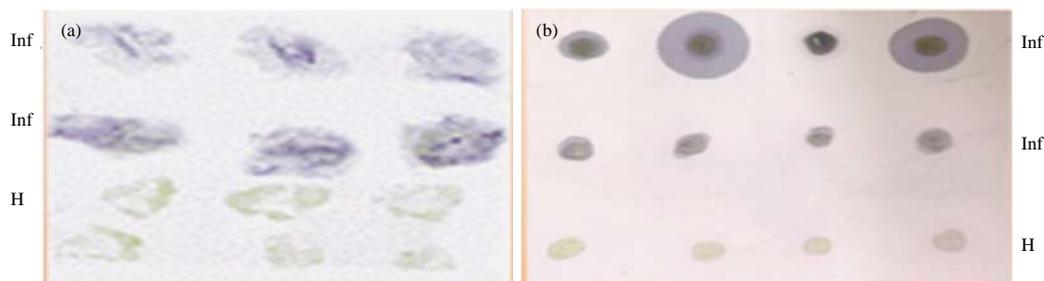


Fig. 2(a-b): Detection of BYMV by (a) Tissue blot immunoassay and (b) Dot blot immunoassay. Inf: Infected samples and H: Healthy samples

Serological diagnosis of the isolated virus using

Indirect ELISA: The virus isolate was detected by in direct ELISA against antiserum of BYMV, *Broad bean stain virus* (BBSV), *Pea seed born mosaic potyvirus* (PSbMV) and *Potato virus Y* (PVY). Positive reaction was obtained only with the virus isolate and its corresponding antiserum but it did not react with sap from healthy plant. This result indicated that, the virus isolate under study was related serologically to BYMV.

Dot blotting immunobinding assay (DBIA) and tissue blotting immunobinding assay (TBIA): The BYMV was readily detected immunologically using DBIA and TBIA (Fig. 2). Positive reaction was indicated by development of purplish-blue color, whereas in negative reaction tissues from healthy plants remain green.

Extraction of total nucleic acid: The RNA was extracted using RNase Plant Mini Kit (Qiagen). Figure 3 showed the viral genomic RNA integrity of the BYMV, lane 1 and RNA extracted from healthy bean plant, lane 2.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR): The RT-PCR products were amplified from the total RNA of bean plant tissues with different symptoms by the specific primer BYMV1 and BYMV2. A cDNA fragment of 700 bp was amplified from the total RNA extracted from the bean plants infected with BYMV. The cDNA fragments reflected a part of the 3' end of NIB region and the 5' end of the CP region of a BYMV. Agarose gel electrophoresis analysis of the amplified PCR products is demonstrated in Fig. 4. However, no product was amplified from healthy bean plants using the same procedure.

Nucleic acid hybridization

Southern blot: The non-radioactive digoxigenin- labelled BYMV cDNA probe was used to confirm the authenticity of the PCR products. The amplified amplicons of the 3' end of NIB and the 5' end of the CP region, were used for detection of the BYMV using Southern blot hybridization technique. Southern hybridization analysis using the cDNA probe revealed intensens signals as shown in Fig. 5. The probes hybridized with DNA products, 700 bp, that were observed after amplification of the PCR from BYMV infected bean plants. No hybridization signals could be detected in the PCR amplification product of healthy bean plant.

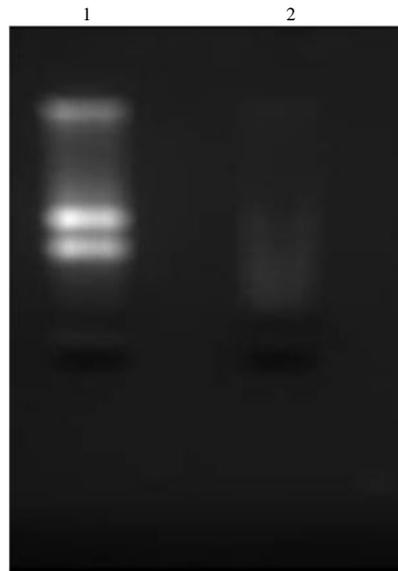


Fig. 3: 1% Agarose gel electrophoresis for RNA extracted from bean plant, Lane 1: RNA extraction from *Bean yellow mosaic virus* and Lane 2: RNA extraction from healthy bean plant

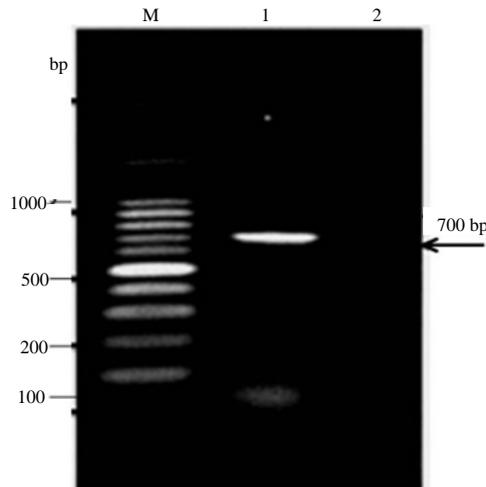


Fig. 4: Agarose gel electrophoresis of the amplified 3' end of N1b region and the 5' end of the CP region fragment using primers BYMV1 and BYMV2. Lane 1: PCR products of bean plant, the correct size is 700 bp and Lane 2: A healthy bean sample. M: 100 bp plus DNA ladder (Vivantes)

Dot blot hybridization: The reaction between dig-labeled cDNA probe specific for BYMV and bean and faba bean plants infected BYMV using dot blot technique was demonstrated in Fig. 6. Strong positive reaction of dot blot hybridization was observed with bean and faba bean infected with BYMV lanes 1 and 2, respectively. Also strong blue signal reaction was observed with PCR product positive control (lane: P). Healthy leaves of bean (negative controls) gave no reaction in dot blot hybridization technique.

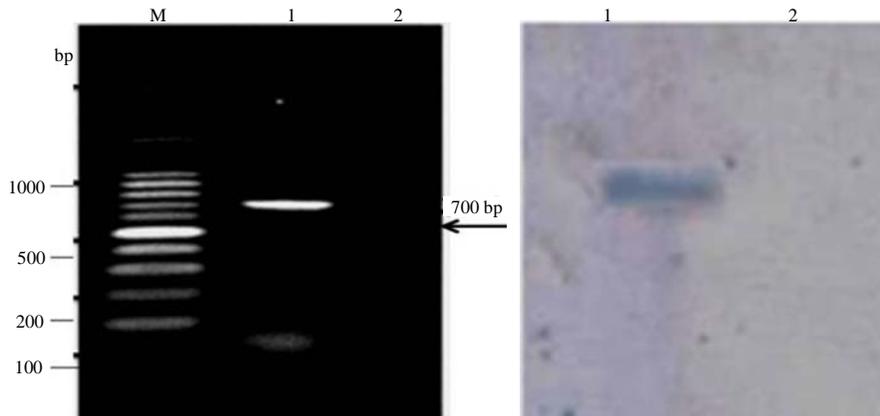


Fig. 5: Southern blot hybridization analysis using BYMV1/BYMV2 for Nib and the 5' end of the CP regions. Positive reaction was shown in lanes 1

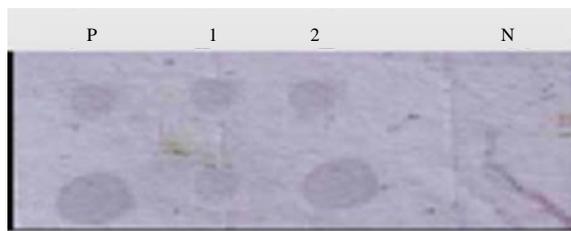


Fig. 6: Dot blot hybridization of Dig labeled cDNA probe BYMV and target BYMV PCR amplification nucleic acid. Lane P: PCR blot for BYMV Nib-CP gene as a positive control. Lanes: (1, 2) dot blot from infected leaves of bean and faba bean, respectively. Lane: N healthy leaves of bean as a negative control

Partial nucleotide sequence BYMV: The RT-PCR of bean infected BYMV was purified with GFX™ PCR kit. The RT-PCR of 700 bp was sequenced and the analysis of amplified 700bp revealed that this DNA fragment BYMV-Al-Makhwah KSA isolate was amplified within the specific 3' end of Nib region and the 5' end of the CP region (Nib-CP gene). A multiple alignment was done along with sequences previously obtained which have already been used as reference sequences in other studies (Nakamura *et al.*, 1994, 1996; Hammond and Hammond, 2003; Chen *et al.*, 2001; Nakazono-Nagaoka *et al.*, 2009) and from the Genbank sequence data. The following sequences were used in the comparisons: Japan BYMV from Gladiolus hybrid cultivar (AB439729), BYMV from *Eustoma russellianum* (AM884180) Taiwan, BYMV from gladiolus (AY192568) USA and BYMV from broad bean and bean (D28819 and D83749) Japan, respectively. The phylogenetic homology tree based on multiple sequence alignments showed that the BYMV-Al-Makhwah KSA isolate about 99% similarity with Japan BYMV isolate (AB439729). In addition it showed 93-95% homology with USA gladiolus BYMV isolate and Japan BYMV (AY192568 and D83749), respectively. The phylogenetic homology tree based on multiple sequence alignments is presented in Fig. 7. BYMV is a member of *Potyvirus*. The DNA sequence was submitted in GenBank (acc. no.LC025531) and the author believes this is the first report of the Bean yellow mosaic virus affecting Bean in Al-Makhwah Governorate, KSA.

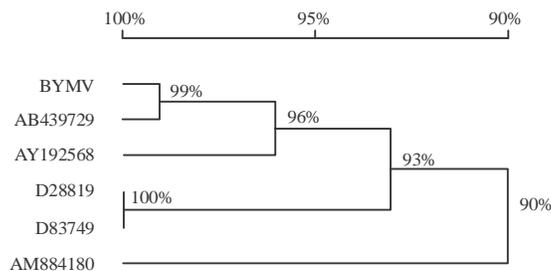


Fig. 7: Phylogenetic tree based on multiple sequence alignments of the 3' end of N1b region and the 5' end of the CP region (N1b-CP gene) of the BYMV with reference to international isolates

DISCUSSION

Bean (*Phaseolus vulgaris*) is an important food legumes in Saudi Arabia and other countries of the world. *Bean yellow mosaic virus* is a common disease of bean because of the multiplicity of mode of transmission (Elbadry *et al.*, 2006; Radwan *et al.*, 2008; Alkhalaf *et al.*, 2009; Dorriv *et al.*, 2013). Seventeen plants species out of thirty plants species and varieties, showed systemic symptoms mosaic, yellowing, vein clearing, stunting streaking mosaic, malformation and severe mosaic as a result of infection plant by BYMV. *Chenopodium amaranticolor* and *Chenopodium quinoa* L., indicator plants were found to be local lesion host. These results are in agreement with those by Bos (1970), Mckirdy *et al.* (2000), Khattab (2002), Hemida (2005), Al-Khalaf *et al.* (2010), Al-Ani (2011) and Al-Ani and Adhab (2013). The BYMV was easily transmitted mechanically by infection sap. This explains the widespread of this virus and affects the epidemiology of mechanically transmitted virus. This result is in full agreement with those reported by Parrella and Castellano (2002), Shahwan (2007), Al-Khalaf *et al.* (2010) and Bashir *et al.* (2013). Two aphids, *Myzus persicae* Sluz. and *Aphis fabae* were used to study the transmission of BYMV. *Myzus persicae* Sluz. was able to transmit BYMV in non-persistent manner. The percentage of transmission was ranged from (50-60%), *Aphis faba* was the most efficient vector (60%). These vectors were reported to play an important role in the spread of BYMV in the field. These results are in a agreement with those obtained by Al-Ani (2011) and Al-Ani and Adhab (2013). In this study, immunological techniques namely Enzyme Linked Immuno Sorbent Assay (ELISA), Tissue blot immuno binding assay (TBIA) and Dot blot immune binding assay (DBIA) were applied for the current study of BYMV. These techniques are rapid, require low concentration of the virus and little amount of antiserum and highly sensitive in recognizing closely related viruses. These results are in agreement with those recorded by Khattab (2002), Al-Khalaf *et al.* (2010), El-Bramawy and El-Beshehy (2012), Soleimani *et al.* (2012) and Dorriv *et al.* (2013). The RT-PCR based assay using specific primer BYMV1/2 designed based on conserved sequences of BYMV N1b-CP allowed the detection of a wide array of unknown BYMV associated with plants (Katoch *et al.*, 2002b; Ganesh *et al.*, 2009a, b). The majority of primers used in PCR is derived from (N1b-CP) sequences which contain highly conserved and variable regions that have been used to develop pathogen-specific PCR primer for BYMV potyvirus detection. Detection of BYMV potyvirus group in infected bean samples using RT-PCR with specific primers showed a fragment of 700 bp. Similar result was mentioned by Vunsh *et al.* (1990), Katoch *et al.* (2002a), Uga (2005) and Kaur *et al.* (2014).

Hybridization method that employ nonradioactive labels for detection of plant viruses has increased In recent years, with Digoxigenin (dig)-labeled probes (Harper and Creamer, 1995). In the present study, a simple, specific and rapid method for the detection of BYMV infected bean plant was used. Southern blotting from samples of infected and healthy plants were applied onto nitrocellulose membrane was applied, followed by hybridization with infected plant using cDNA probe of NIB-CP genes (700 bp) for BYMV. The same results were reported by Barnett *et al.* (1987) and Vunsh *et al.* (1990). Nucleic acid dot blot hybridization with BYMV with a strong positive reaction was observed with bean and faba bean infected with BYMV (Gilbertson *et al.*, 1991; Brown and Bird, 1992; Harper and Creamer, 1995). Dot blot hybridization is very sensitive than squash blot and would be is used for detection of a small quantity of viral DNA in plant tissues but it gives a non-specific colored background so it can be used in routine (Loebenstein *et al.*, 1997). Hybridization method that employs nonradioactive labels for detection of plant viruses has increased in the recent years, with Digoxigenin (dig)-labeled probes (Farag *et al.*, 2005). The dig-labeled probes combined with colorimetric visualization were capable of detecting different types of viruses with a high degree of specificity.

A part of the 3' end of NIB region and the 5' end of the CP region of BYMV *Potyvirus* Al-Makhwah KSA isolate (700 nt) was sequenced and analyzed. Data showed that the identity percentage of Al-Makhwah KSA isolate BYMV potyvirus (NIB-CP) with a Japanese isolate was 99% and with USA gladiolus isolate and another Japanese isolate were 95 and 93%, respectively. Based on these results, we confirmed that BYMV viral group was diversified mainly in some specific parts of the genome, especially in the CP region that is the most variable part of the viral genome (Shukla *et al.*, 1988; Ward *et al.*, 1992), whereas the NIB gene is very conservative and very useful in detecting a wide spectrum of BYMV isolates. The sequencing BYMV was started from the primers which were covering mainly CP-NIB gene (Oertel *et al.*, 1997; Lecoq *et al.*, 2000). Sequencing of CP and NIB genes is often used for phylogenetic analyses of many species of the genus *Potyvirus* (Handley *et al.*, 1998; Alegria *et al.*, 2003; Gadiou *et al.*, 2008; Wylie and Jones, 2009; Campos *et al.*, 2014). Several techniques for detecting BYMV are now available ELISA, dot-blot hybridization, sequencing and PCR seemed to be the most promising technique for detection of low amounts infected plants of BYMV (Scott *et al.*, 1989; Efaisha, 2005; Ganesh *et al.*, 2009a; Choi *et al.*, 2013). The present work represents the first identification of BYMV in bean plants in Al-Makhwah, KSA.

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