Pak. J. Biotechnol. Vol. 3(1-2) 45-54 (2006) ISSN. 1812-1837

DETECTION AND IDENTIFICATION OF BEET MOSAIC POTY VIRUS (BTMV) INFECTING SUGAR BEET PLANTS IN EGYPT

R.A. Omar*, S.A. Sidaros*, S.A. El-Kewey*, Hayam S. Abd El-Kader**, A.A. Deif** and Jehan M. Abass**

* Dept of Agric. Botany, Fac. of Agric., Kafr El-Sheikh, Tanta Univ., Egypt, **Virus & Phytoplasma Res. Dept., Plant Pathology Res. Inst., ARC, Egypt
e-mail:hayamabdelkader68@yahoo.com

ABSTRACT:

The virus was isolated from naturally infected sugar beet plants and identified as BtMV according to symptomatology, host range, and modes of transmission, electron microscopy and immunological and molecular detection. Host range studies revealed that, BtMV infected 28 plant species belonging to 6 families. BtMV was not easily sap transmitted, not transmitted through seeds but was readily transmitted by *Myzus persicae* Sulz. and *Aphis fabae* Scorp. The electron microscope preparations revealed flexuous filamentous virus particles 715-775 nm in length. The purified virion protein of BtMV and the total protein extracted from infected beet tissues showed a strong cross reaction with Bean Yellow Mosaic virus (BYMV) polyclonal antibodies in dot blot immunoassay indicating a higher degree of relatedness. First strand cDNA synthesis of BtMV was synthesized by priming the 3 terminal poly (A) tail of the viral RNA with Oligo (dT). For cDNA amplification, Potyvirus specific primers corresponding to the non-coding region upstream of the coat protein gene was used. A PCR fragment of the expected size approximately 220 bp was amplified and its size was estimated by agarose gel electrophoresis.

INTRODUCTION:

Sugar beet crop has recently been introduced in Egypt, in order to meet with the increasing demand on sugar production. The cultivated area is increasing steadily and the ultimate goal is the gradual replacement of sugar cane by sugar beet crop as a main source of sugar (about 190, 000 to 200, 000 feddans in Egypt). Total sugar production was 0.5 million tons from sugar beet (Agricultural Economy yearbook, Ministry of Agriculture, 2003/2004). Sugar beet plant was found to be infected, world wide, with virus diseases, most important of which are aphid-borne. Beet mosaic virus, beet western yellows virus, beet yellows virus, beet curly top virus and cucumber mosaic virus are the most common viruses causing important diseases to sugar beet crop (Smith, 1972).

Beet mosaic virus (BtMV) is geographically wide spread and has been reported in USA (Howell and Mink, 1971; Lewellen, 1973), UK (Katis *et al.*, 1997), Germany (Briest and Kegler, 1987), Czechoslovakia (Polak, 1981), Slovakian (Mali, 2000; Glasa *et al.*, 2003), Netherlands (Dusi and Peters, 1999); Nigeria (Owolabi *et al.*, 1998), Iraq (Shawkat *et al.*, 1982). There isn't any recorded survey on BtMV in Egypt. Therefore this investigation was carried out to study a) isolation and identification of BtMV on the basis of host range, mode of transmission and electron microscopy and b) detection of BtMV using dot-blot immune binding assay and RT-PCR.

MATERIALS AND METHODS:

1. Isolation and identification:

Virus isolation: A field survey was conducted in the sugar beet growing areas in Kafr El-Sheikh. Sugar beet plants showing different symptoms ranged from mild to severe mosaic accompanied with distortion and malformation was observed associated with scattered spots within sugar beet cultivated

fields. Samples of naturally infected sugar beet plants were randomly collected. The virus was mechanically transmitted from infected sugar beet leaves to the tested plants. The single local lesion technique described by Kuhn, (1964) was used to obtain the virus isolate in pure form, and propagated on sugar beet plants as a virus source in subsequent experiments. Inoculated plants were kept under greenhouse conditions (25-30^{o}C) for 14-21 days and the symptoms were recorded afterwards. Sap extracted from diseased sugar beet leaves by phosphate buffer pH 7.6 was used in host range study.

1.1. Symptomatology and host range: Plant species belonging to Amaranthaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Leguminosae, Malvaceae, Solanaceae and Graminaceae were inoculated with the extracted sap infected BtMV virus under greenhouse conditions (25-30^{o}C).

Virus symptoms were recorded regularly. The virus was purified biologically through two consecutive passages onto the local lesion host *Chenopoduim amaranticolor*. The resulting local lesions were singly back inoculated onto sugar beet plants. In all experiments, ten seedlings of each different host plant unless otherwise started were mechanically inoculated. An equal number of healthy seedlings of the same kind and age were inoculated with water and used as a control.

1.2. Modes of transmission:

1.2.1. Mechanical transmission: Infected sugar beet plants were homogenized in a sterilized mortar in the presence of few drops of phosphate buffer pH 7.6. The extracted sap was expressed through cheesecloth and used to inoculate twenty healthy seedlings of sugar beet, garden beet and spinach. Leaves of the inoculated seedlings were previously dusted with (600 mesh) carborundum.

1.2.2. Insect transmission: *Myzus persicae* Sulz. and *Aphis fabae* Scorp. were used to transmit the studied virus from infected sugar beet leaves to healthy plants. The above-mentioned aphids were starved for one hour. The virus free insects were allowed to feed on infected sugar beet leaves for acquisition feeding period of 5 min. The viruliferous (five aphids/plant) aphids were then transferred to healthy seedling of sugar beet and were allowed to feed for inoculation feeding period of 30 min, and then killed with Malathion (0.15%). The inoculated plants were then arranged in insect proof greenhouse. After 3 weeks, the number of plants, which showed virus symptoms, was recorded.

1.2.3. Seed transmission: Healthy seeds of *Abelmoschus esculentus* were sown under green house conditions. Growing seedlings were mechanically inoculated with the studied virus isolate. The infected plants showing typical symptoms of the disease were left to produce seeds, which were then collected and left to dry. The seeds obtained from infected plants were sown in clay pots under the green house conditions to test its ability to transmit the viral infection to the whole plant. The experiment was repeated three times.

1.3. Electron microscopy: The infected beet tissues were homogenized in 2 volumes of extraction buffer (0.5 M citrate buffer pH 6.5 containing 0.1% thioglycollic acid and 1 vol. of chloroform). The homogenate was filtered through three layers of muslin, and centrifuged at 10,000 g for 10 min using Beckman centrifuge Model J-21c with JA-20 rotor. The aqueous layer was taken and processed with two cycles of differential centrifugation (80,000 g for 2 h. and 105.000 g for 1 h) for high speed of 1st and 2nd cycles of centrifugation using Beckman L8-80M Ultracentrifuge with 90 Ti rotor. The pellets were resupspended in 0.05 M Na-citrate pH 7.2. Further purification was done by density gradient centrifugation in a 10 to 40% sucrose density gradient for 140 min. at 90,000 g using Beckman SW25.1 rotor. Virus zones were collected and concentrated at 120,000 g for 90 min. The purity of the virus preparation

was evaluated by measuring the optical densities of the diluted preparation at different wavelengths ranging from 220-320 min. on a spectrophotometer (Thomas *et al.,* 1997). BtMV particles have negatively stained with 2% phosphotungestic acid (PTA) as described by Sampson and Taylor (1968).

1.4. Dot-blot immunoassay: 0.1g BtMV infected beet leaves was homogenized in 500µl protein dissociation buffer (0.1 M Tris-HCl pH 7.2, 2% SDS, 2% β-mercaptoethanol, 10% sucrose, 0.005% bromophenol blue, 0.02 M PMSF, and 0.01 M EDTA) in microfuge tube. The tubes were centrifuged for 10 min at 5000 g. The tubes were boiled in a water bath for 10 min and then chilled on ice before blotting. Total proteins in the supernatant were serially diluted and 5 µl of each dilution was spotted onto the nitrocellulose membrane. The membrane was air dried and soaked in TBS (20 mM Tris-HCl pH 7.4, and 150 mM NaCl containing 3% BSA) for one hour at room temperature. The membrane was immuno developed using the protocol described by Cambra *et al.* (1994**)**. The total proteins were extracted in dissociation buffer as described under the Materials and Methods and 5µl of each were spotted on the nitrocellulose membrane. The membrane was probed with BYMV antisera (1:1000 dilution) as first antibody and anti- rabbit-Alkaline phosphatase conjugate as second antibody. The colour was developed after 5-15 minutes using BCIP-NBT substrate. Sample No.12: -ve: (Negative) the healthy plant shows no signal.

1.5. Amplification of BtMV non-coding region by PCR: 10µl of total RNA was added to a primer annealing reaction mixture contains 6µl of 5X first strand buffer (250mM Tris-HCl. pH 8.3, 375mM KCl, and 15mM $MgCl_2$), 3µl of 0.1M dithiothreitol (DTT), 1µg of complementary primer (MBtMV) and sterile H_2O to a final volume of 30µl. The annealing reaction mixture was denatured by heating at 70 °C for 5 min and primers annealed at room temperature for 30-45 min. The annealed reaction was added to 20µl of a cDNA reaction mixture containing: 4µl of 5X first strand buffer, 2ul of 0.1 M DTT, 1µl of RNasin (40 units, Promega Corp., Madison, US), 5µl of 0.3 M B-mercaptoethanol, 2.5µl of 10mM dNTPs (2.5 mM each dGTP, dATP, dTTP, and dCTP), and 1µl of Moloney murine leukemia virus (200U/µl) reverse transcriptase (Promega, Corp.). Reactions were mixed briefly, and incubated for 1-1.5 hour at 42ºC. Amplification were performed in thin-walled PCR tubes and contained the following reaction mixture: 5ul of 10X PCR buffer (1X is 10mM Tris-HCl, pH 8.3, 50 mM KCl, and 0.001% gelatin), 3µl of 25mM $MgCl_2$ (1.5 mM final concentration), 1µl of 10mM dNTPs, 1µl each of 50 pm of forward primer (PBtMV) 5'-GTAGTGGTCTCGGTATCTA TA-3' and reverse primer (MBtMV) 5'-GTC TCTTGCACAAGAACTATAACC-3' DNA primer 2.5 units of Taq DNA polymerase (Promega) and sterile H_2O to a volume of 45µl. The cDNA mixture (5µl) was added to the PCR reaction and amplified in a DNA thermocycler (Biometra) with the following cycling parameters: denaturation at 94ºC for 30 sec, primer annealing at 55ºC for 30 sec, and extension at 72ºC for 45 sec for 35 cycles with a final extension at 72ºC for 7 min.

Southern hybridization: After agarose gel electrophoresis, the gel was soaked into a DNA denaturing buffer (1.5 M NaCl, 0.5 N NaOH) with constant agitation to denature the DNA. The gel was neutralized by soaking for 30 min in neutralization solution (1 M Tris pH 7.4, 1.5 M NaCl) at room temperature with gentle agitation. The nitrocellulose membrane was wetted with deionized water and then immersed in the transfer buffer 20xSSC (3M NaCl 0.3 M odium Citrate-$2H_2O$). The wetted nitrocellulose membrane was placed on top of the gel, 2 pieces of 3 MM paper (cut exactly the same size as the gel) was placed on top of the wet nitrocellulose membrane. A stack of paper towels was placed on the 3 MM papers

and 500 gm weight was also placed above this setup. The DNA transfer was allowed to proceed for 8-24 hours as described by (Southern, 1975). The membrane was then washed with 6X SSC and placed between two sheets of 3 MM paper till dry. The DNA was cross-linked under UV light for 2 min between 2,500 and 10,000 μJoules/cm^2. The membrane was hybridized according to Boehringer Mannheim guide manual. DIG-labeled DNA probe amplified from the cloned coat protein sequence of BtMV (Abass, 2004) was used in Southern hybridization assays to assess specificity.

RESULTS AND DISCUSSION

The studied virus was identified as beet mosaic virus (BtMV) on the basis of symptomatology, host range, mechanical and insect transmission, electron microscopy and ensured by immunological and molecular tests.

1. Symptomatology and Host range: Studied BtMV was mechanically inoculated in fifty-one plant species belongs to 9 families. The host range and the reaction of different plants are recorded in Table (1). The tested plants were divided into two types according to their reactions on the host plant; susceptible and non-susceptible hosts.

1.1 Susceptible hosts to BtMV: Results demonstrated in Table (1) show that the BtMV isolate was able to infect 28 plant species belonging to 6 families i.e. Amaranthaceae, Chenopodiaceae Compositae, Curcubitaceae, Leguminosae and Malvaceae. Susceptible hosts were divided into 2 groups: systemic and local lesion hosts.

a. Host plants with systemic symptoms: The following plant species have found to be systemically infected with the studied BtMV isolate. *Beta vulgaris* cv. top (Fig. 1), cv. Pleno, cv. Asospoly, cv. Gazela, cv. Oscarpoly, cv. Raspoly (Fig. 2), cv. Panther, cv. Negma, cv. Bamela, cv. Montbianko, cv. Kawemira, cv. Lola, cv. Gloria, cv. Delmon, *Beta patellaris*, *Beta vulgaris* var. rapa, *Spinacea oleracea*, *Melilotus indicus* L., *Pisum sativum* L., *Vicia faba*, *Glycin max*. *Abelmoschus esculentus*, (Fig. 3), *Cucurbita pepo* L. and *Sonchus oleraceus* L.

b. Host plants with local lesion symptoms: *Chenopodium amaranthicolor* (Fig. 4), *Ch. album, Amaranthus caudatus* L. and *Beta vulgaris*.

Table -1: The reaction of different hosts to BtMV isolated from sugar beet

Tested plants		% Inf.	Inc.	Symp.
Common Name	Scientfic name		(days)	
Fam. Amaranthaceae				
Globe maranth	*Gomphrena globosa* L.	0.0	-	No
	Amaranthus caudatus L.	10.0	6-7	N LL
Fam. Chenopodiaceae				
Sugar beet	*Beta vulgaris* cv. Top	100.0	7-10	VC-CL L-Mal-M
	cv. Pleno	96.0		Vb-mM-CLL-Pls
	cv. Asospoly	93.5		Vb-LR-M
	cv. Gazela	86.0		NC-Mot
	cv. Oscarpoly	89.2		VC-Mot
	cv. Raspoly	100.0		LR-CLL-M
	cv. Panther	100.0		LR-CLL-Mal-Pls
	cv. Negma	95.3		Vb-mM-CLL
	cv. Bamela	93.0		Vb-M-CLL
	cv. Montbianko	89.0		VC-Mot
	cv. Kawemira	95.5		Vb-Pls-M
	cv. Lola	93.2		VC-M-Mal
	cv. Gloria	100.0		Vb-CLL-M
	cv. Delmon	97.3		Vb-CLL-M
Red beet	*Beta patellaris*	100.0		Vb-M
Fodder beet	*Beta vulgaris* var. rapa	100.0		Vb-M
White beet	*Beta vulgaris*	97	10	VC-M
Spinach	*Spinaceae oleracea*	100.0		VC-M-Mal-Stu
Goose food	*Chenopodium amaranticolor*	100.0	5-7	NLL
Pig weed	*Ch. Album*	100.0	7	NLL
Fam. Compositae				
Lettuce	*Lactuca sativa L.*	0.0	-	No
Zinnia	*Zinnia elegans* Jacq	0.0	-	No
Milk Thistle	*Sonchus oleraceus* L.	72.0	10	Vc-mM
Pot Marigold	*Calendula officinalis* L.	0.0	-	No
Fam. Cruciferae				
Turnip	*Braassica rapa* L.	0.0	-	No
Cabbage	*B. oleracea* L. var cabitata	0.0	-	No
Cauliflower	*B. oleraceae* L. var. botrities	0.0	-	No
Radish	*Raphanus sativus* L.	0.0	-	No
Rocket salad	*Eruca sativa* Mill	0.0	-	No
	Cheiranthus cheiri L.	0.0	-	No
Stock	*Maththiola incana* R.Br.	0.0	-	No
Fam. Curcubitaceae				
Squash	*Cucurbita pepo* L.	50.0	6	M
Cucumber	*Cucumis sativus* L.	0.0	-	No

.

Fam. Leguminosae				
Sweet clover	*Melilotus indicus* L.	93.0	10	SM-mM
Pea	*Pisum sativum* L.	40.0	8	Vb-SM-mM
Broad bean	*Vicia faba* L.	60.0	10	mM
Soya bean	*Glycin max*	60.5	8	mM
Bean	*Phaseolus vulgaris* L.	0.0	-	No
Cowpea	*Vigna sinensis*	0.0	-	No
Fam. Malvaceae				
Cotton	***Gossypium barbadence***	0.0	-	No
Okra	*Abelmoschus esculentus*	70.0	10	Vb-Vc-mM-Mal-Mot-ygr.
Fam. Solanaceae				
Stramony	*Datura stramonium* L.	0.0	-	No
Tomato	*Lycopersicon esulontum*	0.0	-	No
Egg plant	Mill	0.0	-	No
Tobacco	*Solanum melongena* L.	0.0	-	No
Tobacco	*Nicotiana glutinosa*	0.0	-	No
Wild tobacco	*N. tabacum* cv. Sammsun	0.0	-	No
	N. rustica L.			
Fam. Graminaceae				
Maize	*Zea mays* L.	0.0	-	No.
wheat	*Triticum sativum*	0.0	-	No.

The symbols indicate the following symptoms:

VC: Vein Clearing Vb: Vein banding Cll: Chlorotic local lesions Nll: Necrotic local lesion

Mal: Malformation Pls: Plisters M: Mosaic mM: Mild mosaic

SM: Severe mosaic LR: Leaf roll Mot: Mottling Stu: Stunting

Ygr: Yellowish green In = Infection, Inc= Incubation period, Symp. = Symptoms.

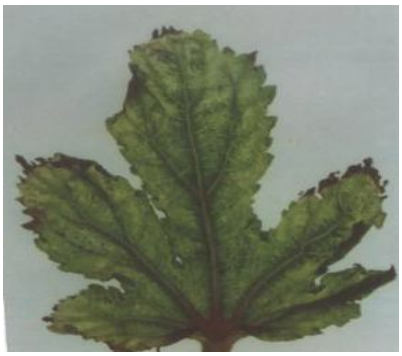

Fig. -1: Severe mosaic, malformation and plasters induced by BtMV on leaves of *Beta vulgaris* cv. top

Fig. - 2: Chlorotic spots and leaf roll induced by BtMV on *Beta vulgaris* cv. Raspoly

Fig. (3): Vein clearing, vein banding, malformation and mosaic induced in *Abelmoschus esculentus* as a result of infection with BtMV.

Fig. (4): Necrotic local lesions induced on *Ch. amaranticolor* infected with BtMV.

1.2. Unsusceptible hosts: About 23 plant species were found to be unsusceptible to the studied BtMV strain as shown in Table (1). The obtained results were in agreement with results obtained by Bennett (1965) and Lewellen (1973. The following tested hosts developed systemic symptoms as a result of infection with BtMV: *Beta vulgaris, Beta patellaris; Spinacea oleracea*; *Melilotus indicus* L.*; Pisum sativum* L.; *Vicia faba; Glycin max; Abelmoschus esculentus; Cucurbita pepo L.* and *Sonchus aleraceus* L. The results have in agreement with that obtained by Bennett (1965), Howell and Mink (1971), Lewellen (1973), Halliwell and Johnson (1988), Katis *et al*. (1997), Dusi and Peters (1999).

2. Modes of transmission:

2.1. Mechanical transmission: Data presented in Tale (2) elucidate the following:

1. The BtMV isolated from sugar beet could not be transmitted by infectious crude sap to *Beta vulgaris* cv. top (sugar beet), *Beta vulgaris* (White beet) and *Spinacea oleracea* (Spinach)
2. The percentage of infection was increased from 15 to 30% and from 75 to 95% when the infected crude sap was diluted by distilled water (1: 2 v/v), and by phosphate buffer pH 7.6 (1: 1 v/v) respectively.

These results were in accordance with that reported by Bennett, (1965); Howell and Mink, (1971); Lewellen, (1973); Polak, (1981); Katis *et al.*, (1997); Dusi and Peters, (1999) and Mali, (2000)

Table-2: Mechanical transmission of BtMV to certain

Host plant	Crude Sap		Sap diluted with distilled water (1: 2)		Sap diluted with phosphate buffer pH 7.6 (1: 1)	
	Inf./ Inoc.	%	Inf./I noc.	%	Inf./In oc.	%
Beta vulgaris cv. Top *Beta vulgaris*	0/20	0	3/20	15	19/20	95
	0/20	0	4/20	20	15/20	75
Spinacia oleracea	0/20	0	6/20	30	18/20	90

3.2. Insect transmission: *Myzus persicae* Sulz and *Aphis fabae* were used to transmit BtMV from infected sugar beet to healthy seedlings of sugar beet cv. Top. Starved aphids were fed on diseased leaves for 5 min. then they were transferred to healthy seedling for inoculation feeding period of 30 min. Results demonstrated in Table (3) showed that *Myzus persicae* transmitted the virus to 70% only of the inoculated plants. However, *Aphis fabae* transmitted the virus to 30% only of the inoculated plants. BtMV is transmitted by *Myzus persicae* Sulz in non-persistent manner (Mali, 2000). BtMV was also transmitted by *Aphis fabae* Scop (Polak, 1981; Dusi and Peters, 1999).

Table -3: Insect transmission of BtMV from infected sugar beet leaves to sugar beet seedling (each treatment repeated twice).

Insects	Inf./Inoc.	% Infection
Myzus persicae	7/10	70
Aphis fabae	3/10	30

3.3. Seed transmission: Seeds from *Abelmoschus esculentus* artificially infected with BtMV isolate were sown in clay pots under green house conditions. The resultant seedlings were checked for the presence of BtMV according to external symptoms. The obtained results demonstrated that seeds could not transmit BtMV isolate. The germination ability of resultant seeds reduced from 92 % to 70 % as a result of infection with BtMV.

3.4. Shape and size of virus particles: Electron microscopic examination of purified virus obtained from sugar beet infected with BtMV (Fig. 5) reveled that BtMV had flexuous filamentous particles about 715-775 nm in length. Our result is similar in this respect to those reported by Owolabi *et al.*, (1998)

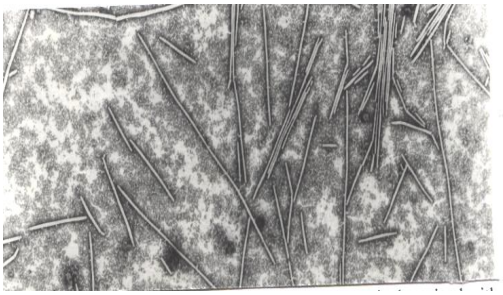

Fig.-5: Electron micrograph of BtMV purified particles negatively stained with 2% PTA (X 22,500)

4. Immunological detection:

4.1. Detection of BtMV by dot blot immunoassay: Dot- blot- immunoassay of virion protein and total protein extracted from BtMV infected tissues were performed according to Mansfield *et al.* (1994). Figure (6) shows that proteins extracted from eleven infected beet tissues and spotted on the nitrocellulose membrane were positively reacted with BYMV antisera diluted 1-1000. The membrane was developed using 1-1000 dilution of BYMV antisera as first antibody and 1- 7500 dilution of anti-rabbit-alkaline phosphatase conjugate as second antibody. The colour was developed after 5-15 minutes using the BCIP-NBT chromogenic substrate.

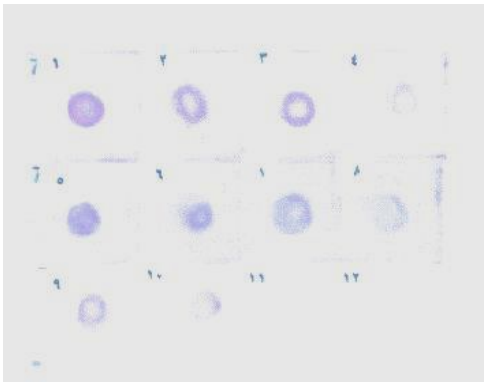

Fig (6). Dot-blot-immunoassay of total protein extracted from BtMV mechanically infected tissues. Sample (1): BtMV virion protein. Samples from 1 to 11 are positively reacted with BYMV antisera diluted 1-1000. Sample (12): Healthy plant showing no signal.

Detection of BtMV by RT-PCR and Southern hybridisation:Reverse transcription–polymerase chain reaction (RT-PCR) was performed on total RNA extracted from 0.02mg leaf tissues by using High pure RNA tissue extraction kit (Roche, Diagnostics). The quality of RNA isolated depends on the source of the tissue being used as starting material. The yields of total RNA per gram of infected tissue were obtained 120µg/0.02 mg of tissues. The purity of the RNA sample, as indicated by A260/A280 ratios, was 1.2. In addition to absorbance ratios, RNA quality was evaluated by agarose gel electrophoresis. RNA isolated using this method showed the characteristic 2:1 ethidium bromide staining ratio of 28S to 18S RNA, indicating no significant degradation.

The RNA was reverse transcribed by the MMLV reverse transcriptase. The reverse transcription reaction was primed with either the Oligo-dT as minus-sense primers. The resulting complementary DNA (cDNAs) was amplified by PCR after adding MBtMV and PBtMV primers. Figure (7) shows the amplification products obtained from infected beet by using primers MBtMV and PBtMV. The expected product size of the PCR product is 220 bp. Figure (7A) shows the amplification products obtained from infected sugar beet. Southern blot hybridization technique was used to confirm the authenticity of the PCR products of BtMV/non-coding region obtained. The PCR products were resolved on 1% agarose gel and transferred onto a nitrocellulose membrane as described under the Materials and Methods. Hybridization was performed using the BtMV specific digoxigenin labeled DNA probe. The results showed that the DNA probe was successfully hybridized with all PCR products of the non-coding region of BtMV amplified with the primers MBtMV and PBtMV (220 bp) as shown in Fig. (7 B).

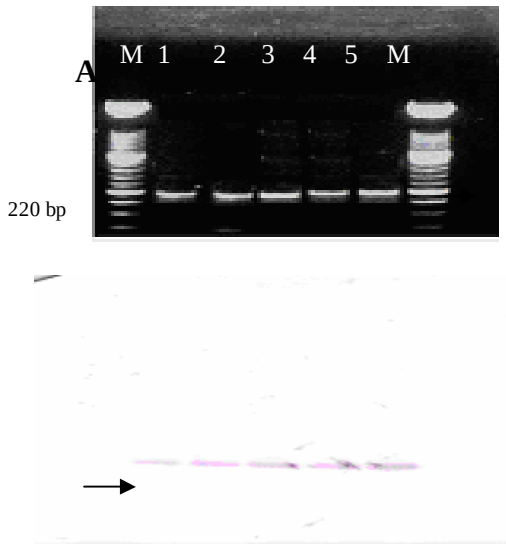

Figure (7): (A): 1% agarose gel electrophoresis showing the PCR amplification products of BtMV non-coding region (UTR). Lanes 1,2,3,4, and 5 the PCR products amplified from different infected tissue samples using a specific primer set known as MBtMV and PBtMV designed in the Non-coding region sequence of the BtMV genome.

(B): Nitrocellulose membrane showing Southern hybridization signals of the amplified products presented above in Fig (A). BtMV/DNA probe labeled with dig-11-dUTP was used in the hybridization technique. The correct size of the PCR products shown in both figures was 220bp as indicated by arrow. M: Molecular weight DNA Marker.

REFERENCES

Abass, Biological and serological studies on beet mosaic virus (BtMV) in Egypt. Ph.D.

thesis, Faculty of Agriculture, Tanta University Pp.120 (2004)

Bennett, C.W., Partial or complete restriction of two mosaic viruses to parenchyma tissue. Plant reporter.**49:** 375-.377(1965)

Briest,E. and H.Kegler, A contribution on the differentiation of viruses affecting beet. Archiv-fur-Phytopathologie-und-Pflanzen schutz **23:** 3-11(1987)

Cambra, M., M. Asensio, M.T. Gorris, E. Perez, E. Camarasa, J.A. Garcia, J.J. Moya, D. Lopez Abell, C.Vela and A. Sanz, Detection of plum pox poty virus using monoclonal antibodies to structural and non-structural proteins. Eppo Bulletin. **24:** 569-577 (1994).

Dusi, A.N. and D. Peters, *Beet mosaic* virus: its vector and host relation-ships. Journal of Phytopathology, **147:** 293-298 (1999).

Glasa, M. O. Kudela and Z. Subr, Molecular analysis of the 3 terminal region of the genome of beet mosaic virus and its relation with other poty viruses. Arch. Virol. **148:** 1863-1871 (2003)

Halliwell, R.S and J. Johnson, Virus diseases of spinach in the Texas winter garden. Miscell aneous-Publication-Texas-Agricu-tural-Experiment-Station, MP-1656, Pp. 4 (1988)

Howell, W.E. and G.I. Mink, The relationship between volunteer sugar beets and occurrence of *Beet mosaic* and beet western yellows viruses in Washington beet fields. Plant disease Report **55**: 767-678 (1971).

Katis, N., G. Kokinis, I. Eleftherohorinos and P. Jones, Arable weeds identified as new sources of *Beet mosaic* poty virus in Greece. Annals of Applied Biology **130**: 255-260 (1977)

Kuhn, C.W., Separation of cowpea virus mixtures. Phytopathology **54**: 739-740(1987)

Lewellen, R.T. Inheritance of *Beet mosaic* virus resistance in sugar beet. Phytopathology **63**: 877-881. (1973)

Mali, V.R. Natural infection of beet by *Beet mosaic* poty virus (BtMV) in west Slovakian. Zeitschrift fuer Pflanzenkran kheiten und. Pflanzenschutz. (Print) **107:** 539-547. (2000)

Mansfield, H.R., M.D. Stern and D.E. Oherby. Effect of beet pulp and animal by-product on milk yield and *in vitro* fermentation by rumen microorganisms. J. Dairy Sci. **77:** 205-216. (1994)

Owolabi, T.A., M.A. Taiwo, G.A. Thottappilly, S.A. Shoyinka, E. Proll and F. Rabensteein. Properties of a virus causing mosaic and leaf curl disease of *Celosia argentea* L. in Nigeria Acta Virologica. **42:** 133-139 (1988)

Polak, I., Studies of virus diseases of sugar beet and its resistance to these diseases in Czechoslovakia. Principles of plant resistance to diseases and pests. 9-12. Bucharest, Romania. (1981)

Sampson,P.T.and R.H. Taylor. Acomparison of the electron microscope microprecipiton tests and indicator plants for the detection of potato viruses S, X and V. Phytopathology **58**: 489-493. (1968)

Shawkat, A.L.B. G.I. Fegla and N.A. Kasem. Effect of beet mosaic virus infection on sugar beet and Swiss chard, and inhibition of virus transmission by mineral oil. Alex. Sci. Exch. Vol. 3, No. 1. (1982)

Smith, K.M. A Text Book of plant virus disease. 3rd Ed. Longman Group Ltd. London, PP. 684 (1972)

Southern, E.M. Detection of specific sequences among DNA fragments separated by gel-eectrophoresis. Journal of Molecular Biology **98**: 403-517 (1975)

Thomas, J.E.; Geering, A.D.W.; Gambley, C.F.; Kessling, A.F. and White, M. (1997). Purification, properties and diagnosis of banana bract mosaic poty virus and its distinction form abaca mosaic poty virus. Phytopathology **87**: 698-705.