BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF AN EGYPTIAN ISOLATE OF SOYBEAN MOSAIC VIRUS IN EGYPT.

Mohga M. El-Tahlawey, M. A. Amer and A. Mandour

Plant Virology Research Department, Plant Pathology Research Institute, A.R. C., Giza-Egypt.

ABSTRACT

Soybean mosaic Potyviridae (SbMV) was isolated from naturally infected soybean plants cvs. Giza 111 and Giza 35 grown at Giza Research Station Farm, Agricultural Research Centre, Giza. The inoculated indicator plants gave severe mosaic on soybean cv.Giza 111, mosaic and malformation on cv Giza 35, mild mosaic on cv. Giza 22, yellow mild mosaic on cv. Giza 83, vein clearing and mild mosaic on *Phaseolus vulgaris* cvs Contender. Chlorotic local lesion on *Chenopodium quinoa*. The virus was transmitted in non-persistent manner by aphids. Electron Microscopy showed aggregates of flexuous virus particles, degenerated mitochondria and inclusions appeared as pinwheel in cytoplasm of infected soybean leaves. Dot blot immunoassay (DBIA) and indirect enzyme linked immunosorbent assays (ELISA) were used as a serological method for detection and confirmation of SbMV isolate in infected tissues.A reverse transcription–polymerase chain reaction (RT-PCR) method was used to detect of local isolate of SbMV in infected plants using specific oligonucleotides primer. A major RT-PCR fragment (~340 bp) from the coat protein gene of the SbMV genome was detected.

Key words: SbMV, aphid transmission, DIBA, electron microscopy, RT-PCR

INTRODUCTION

Soybean mosaic virus (SbMV) belongs to the virus family Potyviridae **Shukla *et al*. (1994).** SbMV was first described in U.S.A. **(Clinton 1915).** The virus was isolated by many investigators in different countries .*i.e.* in U.S.**A (Pacumbaba, 1995),** India **(Nariani & Pingaley, 1960)**, Germany **(Quantz, 1961),** Bulgaria **(Vui-Yui, 1961),** Japan **(Iizuka & Yoshida, 1988),** Portugal (**De vasconcelos, 1964);** Africa (**Akhatova, 1969)**, Russian (**Reifman & Polivanova, 1969**), Soviet Far East **(Vaglav *et al*., 1970),** Taiwan **(Porto & Hagedorn, 1974 and Iizuka *et al*., 1994)**, Brazil **(Cho, *et al*., 1977)**, Korea (**Rossel & Thottapilly, 1993)** and Colombia **(Benscher *et al*., 1996)**. In Egypt, SbMV was isolated by many investigators **(Sabek *et al*., 1979; Kishtah *et al*., 1984 and Mandour 2002)**.

More than eight SbMV strains exist in Korea (**Kim *et al*., 2000)**. Detection and identification of SbMV strains is very important both for soybean cultivation and breeding SbMV-resistant cultivars. Although succeeded in discriminating SbMV strain G5 from non-G5 SbMV isolates using strain specific monoclonal antibodies, they had to use three antibodies because a single monoclonal antibody that could detect a single strain had not been identified. It is still difficult to differentiate SbMV strains by serological methods because most SbMV strains are serologically homogeneous **(Hill *et al*., 1994).** Also, it is not easy to raise strain specific antibodies. Therefore, the method based on the pathogenicity of SbMV isolates on differential soybean cultivars **(Kim *et al*., 2003)** has been used widely to identify SbMV strains. However, using differential cultivars is laborious and time consuming. SbMV is a flexuous, rode not enveloped shape d with a clear modal length of 700 nm; 15 nm wide, the containing 5.3% nucleic acid, 94.7% protein; and 0% lipid. The virion composed

of mono partite plus sense single stranded RNA (ssRNA) genome polyprotein and has base composition 24.3% guanosine (G), 29.9 % adenine (A), 14.9% cytosine (C), and 30.9% uracil (U) (Hill **& Benner, 1980).** The objectives of the present work aim to provide a biological and molecular characterization of an Egyption isolate of SbMV associated with diseases of soybean plants in Egypt.

MATERIALS AND METHODS

I-Source of virus isolate:

Naturally infected soybean plants (*Glycine max* L.) showing mosaic, yellowing, stunting, leaf curling and malformation were collected from two commercial soybean cultivars (Giza 111 and Giza 35) grown at Research Station Farm, Agricultural Research Centre, Giza. Samples were serologically checked against SbMV by indirect-ELISA according to **Hobbs *et al.*, 1987** using antisera kindly provided by Danish Government Institute of Seed Pathology, Denmark.

II-Isolation of SbMV:

Samples which reacted positively, were separated and used for mechanical inoculation to obtain the virus in relatively pure form. Single local lesion technique **(Kuhn, 1964)** was carried out to inoculate the local lesion host, *Chenpodium quinoa*. One lesion was separated, grinded in phosphate buffer and used to inoculate healthy soybean plant *cv.* Giza 111 which used as a source of virus.

III- Identification of the isolated virus:

The following was used to confirm virus identification and to characterize the SbMV isolate

1- Host range and diagnostic host studies:

Twenty six plant species and cvs belonging to four families were mechanically inoculated by the above mentioned virus infection sap obtained from infected soybean plants. Five seedlings of each test plant were used. The inoculated plants were kept under greenhouse conditions and observed for symptoms expression.

2- Aphid transmission

Non-viruliferous colonies of aphids, *Myzus persicae*,Sulz, *Aphis fabae* Scop and *Aphis craccivora* Koch. were maintained on Chinese cabbage seedlings in an insect proof cage. Aphids were starved for 30 min before they were transferred onto soybean plants infected with SbMV for 30 min. Viruleferious aphids were transferred onto healthy soybean seedlings and left to feed for 24 h, and then aphids were killed by sparing with 2%Malathion. Five aphids were used for each plant and five seedlings were used for each treatment which was repeated for three times. The plants were then observed for virus symptoms after four weeks. Plants were serologically checked by indirect-ELISA against SbMV presence.

3- Dot – Immunobinding assay (DIBA):

The procedure of **Lizarrage & Fernandez-Northcote (1989)** was followed. The leaf tissues (0.1 g) were ground in TBS (20 mM Tris bas, 500 mM NaCl, pH 7.5) in a 1: 10 (w/v) ratio and centrifuged at 12000 rpm at 4 °C in a microcentrifuge . The membrane was soaked in TBS containing 1% bovine serum albumin (BSA) overnight at room temperature and reacted with SbMV specific antirabbit antibody conjugate with alkaline phosphatase. The membrane was subjected to extensive washing in TBS buffer containing 0.1% Tween-20 and the reaction visualized by incubation in color solution (NBT/BCIB) as substrate.

4- Electron microscopy (Ultra-thin sections)

One-millimeter diameter disks were punched out from five young leaflets of soybean plants infected with SbMV. Disks were fixed and processed for electron microscopy according to **Spurr (1969).** Thin sections were cut from

selected pieces of the embedded tissue stained with uranyl acetate and lead citrate and viewed with a Philips EM300 electron microscope, Ain Shams Specialized Hospital.

5-Total RNA extraction from plant tissues and RT-PCR:

Viral RNA was isolated using the RNA isolation system (Promega, Corp., Madison, WI) according to the manufacture`s instructions. The complementary primer [MV1 5'-ATGCACACTCTTTTGGGCATGGGT-3'] and homologous primer [MV2 5'-GACAACAAATATTGCCGTACCTC-3'] specific for SbMV were used according to **Chen *et al.* (2004**).This pair of oligonucleotides amplifying a~340 bp fragments. RT-PCR was performed in 50 *ul* reaction mixtures containing 10 *ul* of AMV/ TFi 5 X reaction buffer, 1 *ul* of dNTP (10 mM each); 2 *ul* of MgSO4 (25 mM);1 *ul* of each primer (50 *pmol*); I *u*l of AMV reverse transcriptase (10 U/*ul*); 1 *ul* of TFi DNA polymerase (5 U*l* /*ul*) and 5 *ul* of the template RNA. Thermocycling was programmed as follows: cDNA synthesis at 48 °C for 45 min and RNA /cDNA primer denaturation at 94 °C for 2 min followed by 35 cycles for template denaturation at 94°C for 30 sec, primer annealing at 60°C for 1 min and extenstion at 72°C for 2 min and a final extenstion at 72°C for 7 min.

Analysis of RT-PCR amplified products:

Aliquots of 5 ul of RT-PCR amplified products were analysed on 1 % agarose gel in TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) at 100 V for 1 h. gel was stained with ethedium bromide. DNA Molecular Weight Marker IX (Roche Diagnostics GMBH) was used to determine the size of RT-PCR amplified products.

RESULTS AND DISCUSSION

Isolation and symptomatology

Naturally infected soybean plants showed mosaic, yellowing, stunting, leaf curling and malformation were collected from cvs Giza 111 and Giaza 35 growing at Research Station Farm, ARC, Giza during the growing season 2005. Samples which showed symptoms doubted to be SbMV, reacted strongly positive with indirect ELISA using antisera specific for SbMV. SbMV was successfully biologically purified from single local lesion induced on *Chenopodium quinoa* 6-8 days after inoculation **(Fig1)**. The purified virus isolate gave positive reaction when tested by indirect ELISA.

Identification of the isolated virus:

The isolated virus was identified as SbMV according to diagnostic host reaction, aphid transmission, electron microscopy, serological test and RT-PCR. Data presented in **Table (1)** reveal that out of 26 plants belonging to 14 species, 10 plants belonging to 4 species were susceptible to infection with SbMV. Among these plants, only one reacted by producing local lesions while the other 9 showed different types of systemic symptoms. Back inoculation from plants without symptoms to the indicator host revealed that they were virus-free. Indirect ELISA test ensured these results. It is clear from the obtained data that the hosts which react with the virus are belonging to Fabaceae and Chenopodiaceae. Concerning Fabaceae, *Glycine max* reacted with systemic symptoms ranging from vein clearing and mosaic (in case of Clark cv.), to mosaic (in case of Giza 111 and Giza 22 cvs.), to yellowing and mild mosaic symptoms (in case of Giza 83 cv.), mild mosaic (in case of Giza 35 cv.) In case of *Phaseolus vulgaris,* cultivar Giza 3 cv. gave mild mosaic symptoms and Pinto cv. gave vein yellowing and mild mosaic symptoms **(Fig 1)** and Sub blanc and Top crop cvs , gave no symptoms regard *Pisum sativum it* reacted with systemic symptoms from yellowing and mosaic in case of Lin clor and mild mosaic in case Tomas laxston cv.. Concerning *Chenopodium quinoa* it reacted

with chlorotic local lesions All the plants of *Cucurbitaceae* and *Solanaceae* tested and the other *Fabaceae* plants gave no symptoms.

This study deals with soybean mosaic virus, which was isolated from naturally grown soybean plants in Egypt. Host range studies indicated that the virus evoked vein clearing and mosaic on soybean Clark *cv.*, mosaic on Giza 111 and Giza 2 2 *cv*s., yellowing and mild mosaic on Giza 83 *cv*, mild mosaic on Giza 35 *cv* **(Fig 2)**, mild mosaic on *Phaseolus vulgaris* Giza 3 *cv*. and vein yellowing and mild mosaic Pinto *cv.*, yellowing and mosaic on *Pisum sativum* Lin clor *cv*., and mild mosaic on Tomas laxston *cv.*, chlorotic local lesions on *Chenopodium quinoa* **(Fig 1)**. These results are in agreement with those obtained by **Nariani & Pingaley, 1960; Akhatova, 1969; Cho, *et al*., 1977; Kishtah *et al*., 1984; Iizuka, *et al*., 1994; Benscher, *et al*., 1996 and Mandour 2002).**

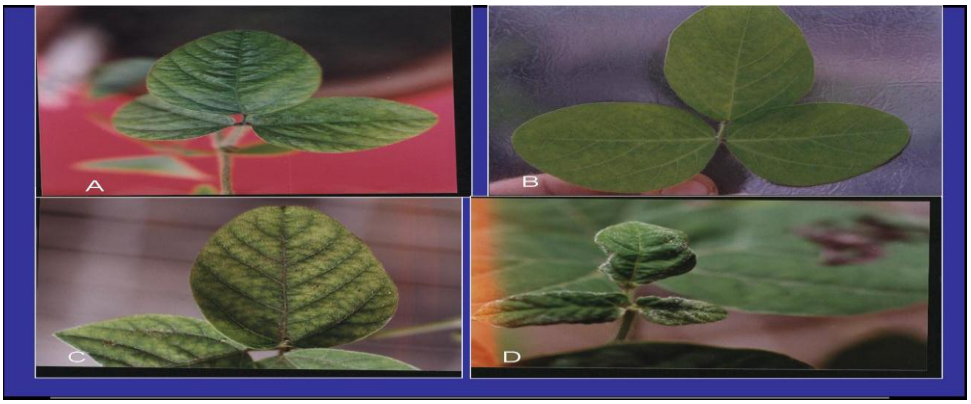

Fig (1) Symptoms of SbMV on soybean plants cvs. (A) Giza35 mild mosaic, (B) Giza111 mosaic, (C) Giza 83 yellowing and mosaic, (D) Clark. malformation and mosaic.

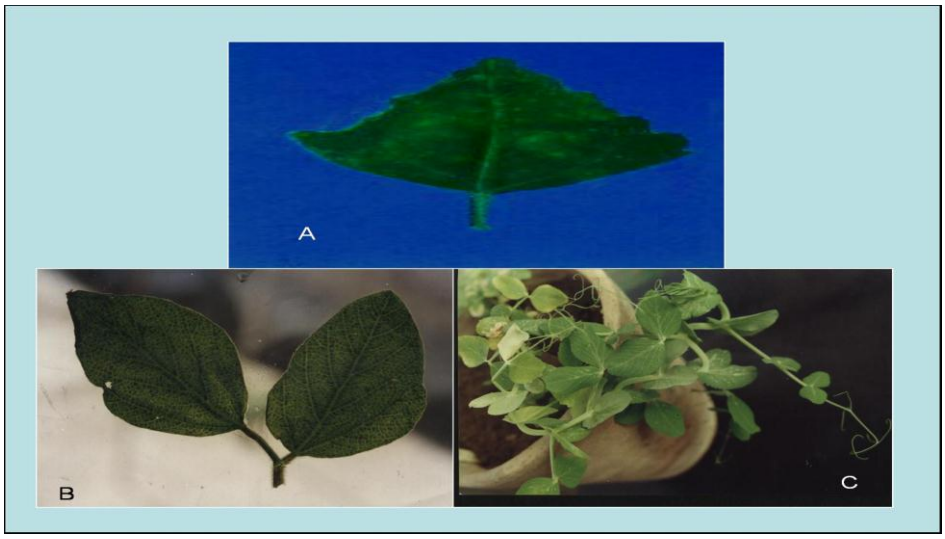

Fig (2). (A) Symptom of SbMV on (*Ch. quinoa*) chlorotic local lesions, (B) Symptoms of SbMV on (*Phaseolus vulgaris*) cv. Giza 3 mild mosaic, (C) Symptom of SbMV on (*Pisum sativum*) cv. Lin clor yellowing and mosaic.

Regarding to insect transmission, five viurileferous of Myzus persica can transmit SbMV, in non- persistent manner from the tested plants after 30 min acquisition and 1 h inoculation feeding periods. Results shown in Table (2) indicated that SbMV was transmitted also by Aphis craccivora and A. *fabae*. In this study, for SbMV aphid transmission, *M. persica* was found to be more efficient than *A. fabae* and *A. cracceivora* insects when they allowed to feed on soybean infected plants.The rewspective averages of infeted plants were 77.5, 32.5 and 25%, respectively. This result was confirmed by the findings of **Sabek, *et al.* (1979); Morales, *et al*. (1990); Kosaka & Fukunishi (1993); Benscher *et al*. (1996) and Mandour (2002).**

Table (1). Reaction of different plants to SbMV inoculation.

Inoculated plants	Symptoms	Infection	Indirect ELISA
Family:- Fabacea			
Glycine max L.*cvs*.			
Clark	VC+Mo	+	+
Giza 111	Mo	+	+
Giza 22	Mo	+	+
Giza 83	Y+MMo	+	+
Giza 35	MMo	+	+
Phaseolus vulgaris cvs			
Giza 3	MMo	+	+
Sub blanc	0	-	-
Top crop	0	-	-
Pinto	VY+MMo	+	+
*Vicia faba cvs.*Giza 716	0	-	-
Giza 3	0	-	-
Giza 204	0	-	-
Vigna unguiculata cvs	0	-	-
Creame 97			
Creame use	0	-	-
Crème 7	0	-	-
Pisum sativum cvs			
Lin clor	Y+Mo	+	+
Tomas laxston	MMo	+	+
Little marvel	0	-	-
Trifolium p*retense*	0	-	-
Family:- Cucurbitaceae			
Cucurbita pepo	0	-	-
Family:- Chenopodiaceae			
C .amarnticolor	0	-	-
C.quinoa	Ch.LL	+	+
Family:- Solanaceae			
Nicotiana tabacum	0	-	-
N. glutinosa	*0*	-	-
N. clevelandii	*0*	-	-
N .a rusica	0	-	-

* MMo= Mild mosaic, Mo= Mosaic, Y= Yellowing, 0= No symptoms, VC= Vein clearing, YV= Vein yellowing, Ch. LL = Chlorotic local lesion.** As determined by ELISA test + = ELISA positive reaction, - = ELISA negative reaction.

Table (2). Precentage of transmission of SbMV from infected soybean to healthy soybean plants by viruleferous Aphids:

Aphid	No. of inoculated plant	No. of infected plant	% infection
Myzus persicae	40	31	77.5
Aphis craccivora	40	10	25
Aphis fabae	40	13	32.5

Dot – Immunobinding assay (DIBA):

Dot-blot-immunoassay of total protein extracted from SbMV infected tissues. 12 samples collected from open field are positively reacted with SbMV antisera diluted 1-1000. The total proteins were extracted in phosphate buffer and 10 microliters of each were spotted on the nitrocellulose membrane. The membrane was immune-developed using 1-1000 dilution of SbMV antiserum as first antibody and 1-7500 dilution of anti- rabbit-alkaline phosphatase conjugated as second antibody. The colour was developed after 5-15 minutes using BCIP-NBT substrate. Row No 4: (Negative) the healthy plant shows no signal**. (Fig 2).**

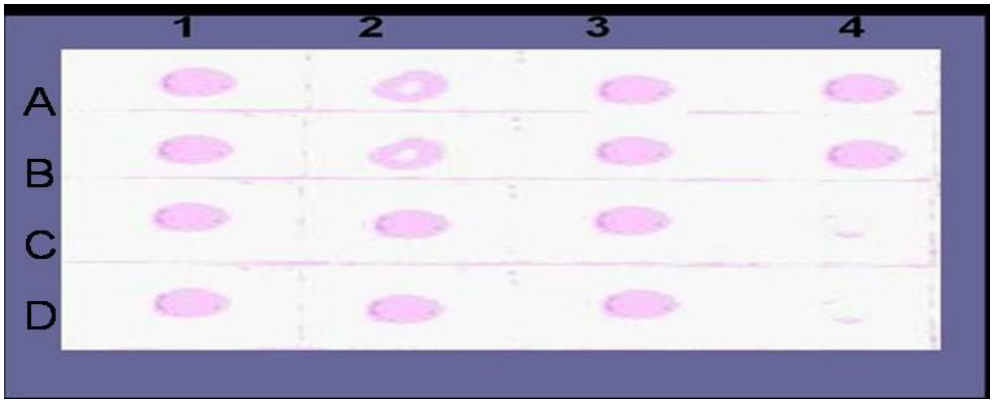

Fig (3): Dot blot immunoassay for detection of SbMV in infected plant tissue (Row 1, Row 2 and Row 3 A,B,C and D). No reaction was observed between the anteserum and uninfected plant tissue (Row 4 C &D).

Electron microscopic examination

The ultra-thin section of SbMV-infected leaves showed aggregates filamentous virus particles, degenerated mitochondria and pinwheel (characteristic for SbMV), in the cytoplasm, representing Potyviridae group **(Fig 4).** Electron Microscopy studies reveal that SbMV has been observed the formation of inclusion bodies which associated with SbMV infection.

Similar results were reported by **Tu, (1973 and 1976); Morales *et al*. (1990) and Mandour (2002)** who reported that SbMV induces the formation of inclusion bodies and virus particles aggregates in the cytoplasm.

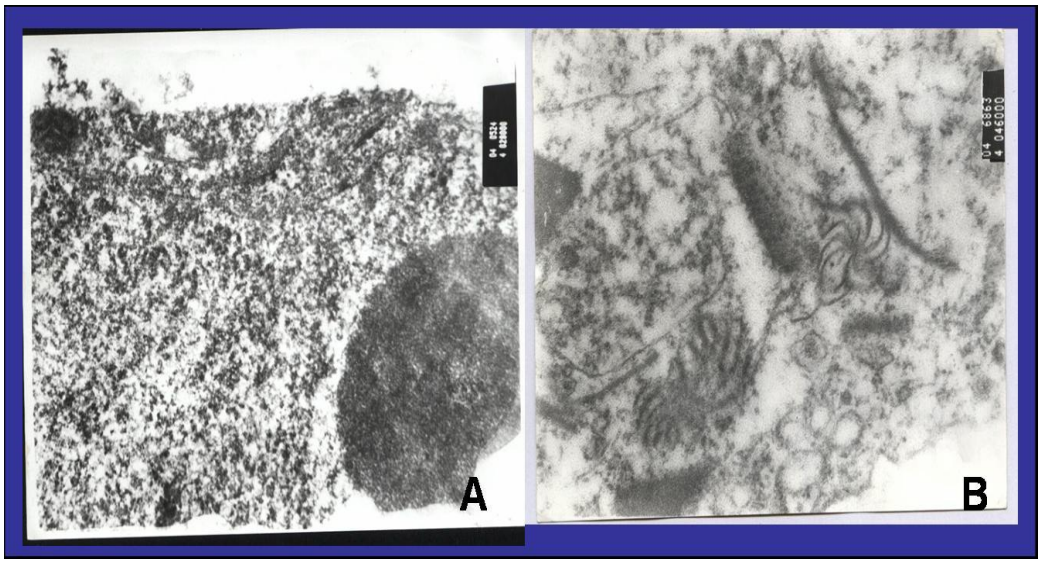

Fig (4) .(A) The ultra-thin section of SbMV-infected leaves showed aggregates filamentous virus particles, (B) Inclusion pinwheel, degenerated mitochondria and virus aggregates in cytoplasm of soybean leaf cells infected` with SbMV.

Detection of SbMV using RT-PCR:

RT-PCR was performed on total RNA extracted from 30 mg infected and uninfected plant materials using SV- Total RNA Isolation System. The RNA was reverse transcripted by AMV reverse transcriptase. The reverse transcription reaction was primed with the complementary primer specific for SMV. The resulting complementary DNA (cDNA) was amplified by PCR after adding the complemantary and homologous primers.The expected size is~ 340 bp. Obtained data in **Fig (5)** illustrate the agarose gel electrophoresis of RT-PCR amplified SbMV-CP cDNA from infected soybean Giza 35 *cv.* (lane 1 *cv.Phaseolus vulgaris* Giza 3 *cv*. (lane 2); soybean leaves Giza 111 *cv.* (lane 3). No amplified fragments of cDNA were obtained from uninfected soybean leaves Giza 35 *cv*. (lane 4).

PCR is an extremely sensitive and specific technique for the amplification of genomes , and became widely used as adiagnostic technique for infection by phytoplasma **(Schaff *et al*., 1992)**, bacteria **(Minsavage *et al*., 1994)**, viroids **(Rezaian *et al*.,1992)** and plant viruses belonging to several different groups (e.g.,the geminiviruses, luteoviruses and potyvirus groups **(Henson and French, 1993; Langeveld *et al*., 1991; Robertson *et al*., 1991 and Rojas *et al*., 1993)** .RT-PCR has been used to detect plant viruses **(Singh *et al*., 1995)**. The assay has been applied to enhance detection sensitivety of potyviruses such as plum pox virus **(Wetzel *et al*., 1991)** sugarcane mosaic virus **(Smith and Van de Velde, 1994)**, zucchini yellow mosaic virus **(Thomson *et al*., 1995)** , two sweet potato viruses **(Colinet *et al*., 1994)** and SbMV **(Omunyin *et al*., 1996).**

Fig. (5): 1.2% agarose gel electrophoresis showing PCR amplification of SbMV using specific SbMV1 and SbMV2 primers (~340 bp) from infected soybean Giaza 35 cv. ,infected *Phaseolus vulgaris* Giza 3 cv. and soybean leaves Giza 111 cv. (lane1 2 and 3). Healthy soybean plants showing no amplified product (lane 4). M: DNA Molecular Weight Marker IX (Roche Diagnostics GMBH).

REFERENCES

Akhatova, F.K. (1969). Soybean mosaic (in Russian), Vest. Soil. Khoz. Nauk Alma-Aea. 12 (5): 79–82 (English summary in an annotated bibliography of soybean diseases, 1882–1974. J.N. Sinclair & O.D. Dhingra, 1975); P. 72.

Benscher. D, Pappu, S.S.; Niblett, C.L.; Varon. De. Agudelo, F.; Morales, F.; Hodson, E.;Alvarez, E.; Acosta, O.; Lee, R.F.and De. Agudelo, F.V. (1996). A strain of soybean mosaic potyvirus infecting Passiflora spp. in Colombia. Plant Dis., 80 (3): 258 – 262.

Chen, J.; Zheng, H.Y.; Lin, L.; Adams, M.J.; Antoniw, J.F.; Zhao, M. F.; Shang,Y.F. and Chen, J.P. (2004). A virus related to soybean mosaic virus from *Pinellia ternate* in China and its comparison with local SbMV isolates. Arch. Virol., 149 (2) : 349-363.

Cho, E.K.; Chung, B.J. and Lee, S.H. (1977). Studies on identification and classification of soybean virus diseases in Korea. II. Etiology of a necrotic disease of *Glycine max*. Plant. Dis. Rept., 61: 313 – 317.

Clinton, G.P. (1915). Notes on plant diseases of Connecticut. Connecticut State Agr. Expr. Sta. Ann. Rept. 1915: 446 – 447.

Colinet, D.; J. Kummert; R.; Lepoivre and Semal, J. (1994). Identification of distinct potyviruses in mixedly- infected sweetpotato by the polymerase chain reaction with degenerate primers. Phytopathology, 84 : 65-69.

De vasconcelos, F.A.T. (1964). Contribuicao para o estudo virus do mosaico da soja. Anais Do Instituto Superior De Agronom ,26: 181 – 221.

Henson, J.M. and French, R. (1993). The polymerase chain reaction and plant disease diagnosis. Ann. Rev. Phytopathology ,31 : 81-89.

Hill, J.H. and Benner, H.I. (1980). Properties of soybean mosaic virus and its isolated protein. Phytopathol. Z., 97: 272 – 281.

Hill, J.H.; Benner, H.I. and Van Deusen, R.A. (1994). Rapid differentiation of soybean mosaic virus isolates by antigenic signature analysis. J. Phytopathol., 142:152-162.

Hobbs, H. A.; Reddy, D. V. R.; Rasjeshwari, R. and Reddy, A. S. (1987). Use of direct antigen coating and protein a coating ELISA procedures for detection of three peanut viruses. Plant Dis., 71: 747-749.

Iizuka, N.; Charchar, M.J.D. and Charchar, M.J.d. A. (1994). Classification of strains of soybean mosaic virus and seed transmissibility. Relatorio. Tecnico. do. Proje to. Nopo. Brasileiro. de. Cooperacao. em. Pesquisa. Agricola. Nos. cerrados. 1987-1992. ref 226 – 236.

Kim, Y. H.; Kim, O. S.; Lee, B. C.; Im, D. J. and Choi, J. K. (2000). Distribution and diversity of soybean mosaic virus strains in Korea. Korean J. Plant Pathol., 16:179.

Kim, Y. H.; Kim, O. S.; Lee, B. C.; Moon, J. K.; Lee, S. C. and Lee, J. Y. (2003). G7H, a New soybean mosaic virus strain: Its virulence and nucleotide sequence of CI gene. Plant Dis., 87:1372-1375.

Kosaka, Y. and Fukunishi, T. (1993). Attenuated isolates of soybean mosaic virus derived at a low temperature. Plant Dis., 77: 882 – 886.

Kishtah, A. A.; Tolba, M. A.; El-Sherbeeny, M. H.; Hassan, M. Z. and Safia Abdalla, T. (1984). Studies on some soybean culitvars and lines resistant to soybean mosaic virus in Egypt. Agricultural Research Review, 62. (2): 347 – 352.

Kuhn, C. W. (1964). Separation cowpea virus mixtures. Phytopathology ,54: 739-740.

Langeveld, S. A. ;Dore, I. M.; Memelink, I.; Derks, A.R.L.M.; C.I.M. Van der Vlugt, C.I. M.; Asjes, C.J. and Bol, J.E. (1991). Identification of potyviruses using polymerase chain reaction with degenerate primers. J. gen. Virology, 72 : 1531-1541.

Lizarrage, C. and Fernandez-Northcote, E.N. (1989). Detection of potato virus X and Y in sap extracts by amodified indirect enzyme – linked immunosorbent assay on nitrocellulose membranes (NCM-ELISA). Plant Dis. 73 : 11-14.

Mandour, A. M. (2002). Studies on effect of environmental factors on some soybean viruses. M.Sc. Thesis. Institute of Environmental Studies and Research. Ain Shams University.

Minsavage, G. V.; Thomson, C. M.; Hopkins, D. L. ;Leite, R. M. V. C. and Stall, R. E. (1994). Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. Phytopathology, 84 : 461-456.

Morales, F. J; Messen, A. I; Castano, M. and Calvert, L. (1990). Detection of a strain of soybean mosaic virus affecting tropical forage species of Centrosema. Plant Dis., 74: 648 – 651.

Nariani, T. K. and Pingalely, K. V. (1960). A mosaic diseases of soybean (*Glycine max* (L.) Merr.) Ind. Phytopath., 13: 130 – 136.

Omunyin, M.E.; Hill, J.H. and Miller, W.A. (1996). Use of unique RNA sequence specific oligonucleotide primers for RT-PCR to detect and differentiate soybean mosaic virus strain. Plant Dis., 80 : 1170-1174.

Pacumbaba, R.P. (1995). Seed transmission of soybean mosaic virus in mottled and non-mottled soybean seeds. Plant Dis., 79 (2): 193 – 195.

Porto, M.D. and Hagedorn, D.J. (1974). Susceptibility of *Phaseolus lathyroides* to soybean mosaic virus. Plant Dis. Reptr., 58: 322 – 326.

Quantz, L. (1961). Investigations on the common bean mosaic and the soybean virus. Phytopathol. Z., 43: 79 – 101.

Reifman, V.G. and polivanova, T.A. (1969). [Virus diseases of soybean in Soviet Far East.] Trudy Biol-pochy. Inst. Dal-Nevost fil Sib. otd. ANSSR : 83 – 104.

Rezaian M.A.; Krake, L.R. and Golino, D.A. (1992). Common identity of grapevine viriods from USA and Australia revealed by PCR analysis . Intervirology, 34 : 38-43.

Robertson, N.L; French, R. and Gray, S.M. (1991). Use of group specific primers and polymerase chain reaction for the detection and identification of luteoviruses. J. gen. Virology, 72 : 1473-1477.

Rojas, M.R.; Gilbertson, R.L.; Russell, D.R. and Maxwell, D.R. (1993). Use of degenerate primers in the polymerase chain reaction to detect whitefly transmitted geminiviruses. Plant Dis., 77 : 340-347.

Rossel, H.W. and Thottappilly, G. (1993). Seed transmission of viruses in soybean (*Glycine max*) in relation to sanitation and international transfer of improved germplasm. Seed Sci. and Technol., 21 (1): 25 – 30.

Sabek, A. M.; Tolba, M. A. and Kishtah, A. A. (1979). Two strains of soybean mosaic virus isolated from naturally infected soybean 3^{ed} Egypt Phytopathol. Congress 117 – 129.

Schaff, D.; Lee, I. and Davis, R.E. (1992). Sensetive detection and identification of mycoplasma- like organism in plants by polymerase chain reaction . Biochem. Biophys. Res. Commun., 186 : 1503-1509.

Shukla, D. D.;Ward, C. W. and Brunt, A. A. (1994). The Potyviridae. CAB International, Wallingford, UK.

Singh, R. P.; Kurz, J. and Boiteau, G. (1995). Detection of stylet-biorne and circulative potato viruses in aphids by duplex reverse transcription polymerase chain reaction. J. Viro. Methods, 55 : 133-143.

Smith, G.R. and Van de Velde, R. (1994). Detection of sugarcane mosaic virus and fiji disease virus in diseased sugarcane using the polymerase chain reaction. Plant Dis., 78 : 557-561.

Spurr, A. R. (1969). A low viscosity epoxy resin-embedding medium for electron microscopy. Ultrastruc. Res. 26 – 31.

Thomson, K.G.; Dietzgen, R.G.; Gibbs, A.J.; Tang, Y.C.; Liesack, W.; Teakle, D.S. and Stackebrandt, E. (1995). Identification of zucchini yellow mosaic potyvirus by RT-PCR and analysis of sequence variability. J. Virol. Methods, 55: 83-96.

Tu, J.C. (1973). Electron microscopy of soybean root nodules infected with soybean mosaic virus. Phytopathology, 63: 1011 – 1017.

Tu, J.C. (1976). Localization of infections of soybean mosaic virus, it mottled soybean seeds. Microbiol., 14: 150 – 151.

Vaglav, V.; Radman, L.; Batinica, J.; Ristanovic, M.; Dimic, N.; Numic, R. and Bes, A. (1970). Contributions to the knowledge of diseases and pests of soybean in the productive regions of Bosnia. Zashtita Bilja, 21: 229 – 236.

Vui-Yui, D. (1961). The mosaic disease of the soybean varieties in Bulgaria [in Russian, English summary]. Rast. Zasht. (Bulgaria), 9: 20 – 26.

Wetzel, T.; Candresse, T.; Ravelonardo, M. and Dunez, J. (1991). A polymerase chain reaction assay adapted for plum pox potyvirus detection. J. Virol. Methods, 33 : 355-365.

الوصف البيولوجى والجزيئى للعزلة المصرية لفيروس التبرقش فى فول الصويا فى مصر

مهجــة عبد الرحمن الطحلاوى ، محمــود أحمد عــامر ، أيمن محمد مندور
قسم بحوث الفيروس، معهد بحوث أمراض النباتات، مركز البحوث الزراعية، الجيزة- مصر

تم عزل فيروس التبرقش فى فول الصويا من نباتات فول الصويا أصناف كلارك وجيزة١١١، جيزة ٢٢، جيزة ٨٣، وجيزة٣٥. أظهرت النباتات المشخصة المصابة بالفيروس أعراضا عبارة عن تبرقش و شفافية العروق علي نباتات فول الصويا صنف كلارك وتبرقش علي صنف جيزة١١١و جيزة ٢٢؛ تبرقش خفيف مع اصفرار علي صنف جيزة ٨٣ وتبرقش خفيف علي صنف جيزة ٣٥ ؛ وكذلك تصاب بعض أصناف الفاصوليا والبسلة بهذه العزلة كما أعطت هذه العزلة أعراض عبارة عن بقع ميته علي نباتات الزربيح. أظهرت تجارب النقل الحشري أن الفيروس ينتقل بطريقة غير باقيه بواسطة حشرات المن. كما أظهرت دراسات الميكروسكوب الإلكتروني أن الفيروس يسبب تشوة للميتوكوندريا ويعطى أجسام محتواة عبارة عن pinwheel وهذا الشكل هو ما يميز مجموعة فيروسات Potyvirus وخاصة فيروس SbMV. تم استخدام الطرق السيرولوجية مثل طريقة الأليزا وطريقة البصمة الأمينوجينية فى الكشف عن هذا الفيروس وذلك باستخدام ألنتيسيرم المتخصص لهذا الفيروس. كما تم استخدام تفاعل البلمرة المتسلسل العكسى النسخ كطريقة أكثر حساسية ودقة فى الكشف عن العزلة المصرية لفيروس التبرقش فى نباتات فول الصويا وقد أظهرت النتائج وجود حزمة من الحامض النووى الديوكسى الناتج من هذا التفاعل وزنها الجزيئى حوالى ٣٤٠ نيوكليتدة وذلك باستخدام بادئ متخصص فى منطقة الغلاف البروتينى لهذا الفيروس.