Biological assay and molecular characterization of apricot isolate of *Arabis mosaic virus*

(Received: 01.12. 2008; Accepted: 15.12.2008)

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ABSTRACT

Apricot is an economically important tree in Egypt and worldwide. A survey of stone fruits in different locations was carried out from 2005 to 2007 to identify viruses that infect apricot trees (Prunus armeniaca) in El-Fayium and Giza Governorates (middle Egypt). Samples from three apricot plantations were collected and tested for different viruses i.e. Apple mosaic virus (ApMV), Arabis mosaic virus (ArMV) , Prunus necrotic ring spot virus (PNRSV), Prune dwarf virus (PDV) and plum pox virus (PPV) were tested using double antibody sandwich enzyme-linked immunosorbent assay(DAS-ELISA). The infected trees showed reduced yield, lower fruit quality, chlorotic and yellowish symptoms on leaves similar to those of virus infection. Preliminary results revealed the presence of ArMV in the tested apricot trees. The isolated virus was biologically purified from single local lesions formed on Chenopodium amaranticolor. Identification of this virus was based on host range, transmissibility (mechanical, seed, graft and nematode). ArMV was able to infect only 25 plant species and varieties from 30 tested plants by mechanical or grafting; however nematode inoculation (Xiphinema americanum) was able to transmit ArMV from infected apricot and healthy apricot, peach, plum and grapes nursery (100 %). A high reliable sensitive RT-PCR assay was developed for the detection of ArMV. Primers were used to amplify the coat protein gene (419bp) RNA extracted from infected apricot leaves. The amplified fragment was cloned, sequenced and the nucleotide sequence was compared with other isolates available in GenBank. Sequence comparisons showed similarity ranging from 85 % to 89% of Austria isolates and Japan Kochi isolate, respectively.

Keywords: *Apricot, peach, DAS-ELISA-Nepoviruses, RNA isolation, RT-PCR, cloning and nucleotide sequencing.*

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INTRODUCTION

Arabis mosaic virus (ArMV) belongs to the genus *Nepovirus*, family *Comoviridae*. ArMV occurs in all temperate regions; the disease affects most stone fruits including sour cherry, almond, peach, apricot, plum, their wild and flowering counterparts and also some ornamental species like roses. ArMV is the most widespread virus disease of stone fruit trees. Stone fruits are important crops in most parts of the world (Gümüs *et al.*, 2007).

Symptoms vary according to species infected and strain of the virus. ArMV is transmitted by grafting; it is also mechanically transmitted to several herbaceous plants. Seed transmission rates vary between 5-7 % and

pollen transmission is reported to seeds and to the mother plants. No insect vectors are reported. ArMV is readily transmitted mechanically; it infects 93 species in 28 dicotyledonous families (Murant, 1970).

In nature, the virus is transmitted by nematodes belonging to the genera *Longidorus* and *Xiphinema*, through seed and pollen (Dijkstra and Khan, 2006). The virus occurs naturally in many species of wild and cultivated mono and dicotyledonous plants. ArMV has been reported from numerous vegetable crops infecting, sugar beet, strawberry, hostas (*Hosta* spp.), grapevine, rose, lily, olive, hop, cherry, orange blossom (*Choisya ternata*) and black currant. This virus causees important economic loss in yield, lower fruit quality and shortening the longevity of vines produced due to chlorotic and necrotic symptoms on inoculated and systemic leaves (Imura *et al.*, 2008 and Nölke *et al.*, 2009).

ArMV is a member of the genus *Nepovirus* in the *Comoviridae* family which has RNA genome and isometric particles about 30 nm in diameter (Brunt *et al.*, 1996). The genome consists of two single-stranded positive-sense RNAs, called RNA1 and RNA2 (Andret-Link *et al.*, 2004).

Due to widespread occurrence of latent viral infection, visual inspection is virtually useless. Field observations should be complemented by quick, sensitive and reliable laboratory testes. Reverse transcriptase–polymerase chain reaction (RT-PCR) using specific coat protein primers has been reported as one of the most sensitive methods for detection and identification of ArMV virus that infects hosts; olive and grapevine (Lockhart, 2006 and Nölke *et al.*, 2009).

One of the clones seems to be specific for an isolate of ArMV-H from a Belgian 'nettlehead'-diseased hop plant. Another clone detected all 11 ArMV-isolates from different plant species tested so far. The German ArMV-H or at least some of its isolates cannot be detected with the monoclonal antibody specific to ArMV-H; but strain differences may also be detected using the F(ab')2-based ELISA-procedure of (Adams *et al.*, 1987).

In this study, the occurrence of ArMV on Apricot trees was detected, based on data obtained by nematode inoculation and seed transmission. In addition, different diagnostic methods using RT-PCR for the detection of the *Arabis mosaic virus* infecting Apricot trees was applied. Also, partial nucleotide sequence of the coat protein gene of an Egyptian isolate of ArMV is reported.

MATERIALS AND METHODS

Source of virus isolates

A survey of stone fruits in different locations was carried out from 2005 to 2007 to identify viruses infecting apricot trees (*Prunus armeniaca*) growing in El-Fayium and Giza Governorates. Samples from three apricot plantations were collected.

For biological purification, single local lesion technique was carried out (Kahn, 1964) using *Chenopodium amaranticolor* Cost & Reyn as a local lesion host. Whereas *N. tabacum* L. var. White Burley and *Cucumis sativas* L. were used as propagative hosts for the present experiments.

Modes of transmission

Mechanical transmission (Host rang and symptomatology)

In preliminary transmission tests; mechanical transmission of PNRSV from infected rose to herbaceous hosts was found to be unsatisfactory. Therefore, the following buffers were used for mechanical transmission: 0.01 M phosphate buffer, pH 7.2, containing 2.5% nicotine, 0.01 M phosphate buffer, pH 7.2, containing 0.001 M

Na-DIECA, 0.01 M phosphate buffer, pH 7.8, containing 0.001 M Na-DIECA and 2.5% nicotine mixed with activated charcoal (100 mg ml-1, w/v), and 0.05 M phosphate buffer, pH 7.2, containing 2% sodium sulfite.

Graft transmission

Ten seedlings of each apricot (*Prunus armeniaca* L. cvs. Amar, Canino and Hammawy), peach (*Prunus persica* cvs. Florida prince, GF305, Nemagarde and Soltany) and plum (*Prunus domistica* cvs. Clymax and Mariana) were graft-inoculated from the ArMV source obtained during May and June 2006. The inoculum consisted of 2: 3 buds per each seedling.

Seed transmission

Seeds were separately harvested from healthy and ArMV-infected apricot trees. Approximately one hundred seeds were sown in sterilized soil under insect-proof cages after germination of apricot (cv. El-Amar). Seeds were treated by elimination of the shell in of case urgent necessity of material (1-3 weeks). The resultant seedlings were observed for symptoms appearance. Symptomless plants were checked for virus infection by back inoculation onto *Ch. amaranticolor* and ELISA test to detect the percentage of seed transmitted.

Nematode transmission

The procedures used were those described by Gibbs and Gower (1960) and Brown and Trudgill (1983). Experiments were done with 25 cm 3 plastic pots maintained in temperature controlled cabinets with three-week-old seedlings of *Chenopodium quinoa* used as mechanically infected sources of ArMV. Virus-free nematode groups, mainly adults and fourth-stage juveniles, were given access to these virus-source plants for four weeks. They were then extracted, counted and in groups of two or five placed in clean 25 cm^3 pots in which *Chenopodium quinoa* virus free bait plants were planted three. The excised portion of root was tested by ELISA for the presence of virus and those bait plants which had virus detected in the excised portion of their roots were used as virus-source plants in the subsequent test.

Serological test

Apricot and peach leaf samples were tested for *Apple mosaic virus* (ApMV), *Arabis mosaic virus* (ArMV) , *Prunus* necrotic *ring sopt virus* (PNRSV), *Prune dwarf virus* (PDV) and *plum pox virus* (PPV), All viruses tested in triplicate using conventional double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) according to the manufacturer`s instructions (Sanofi - Santi animal, France). Optical density was measured at λ= 405 nm in an ELISA micro well reader (Dynatech Immunoassay MR 7000). Samples with an absorbency of at least twice the healthy controls were considered as a positive for the presence of the virus. Also, the methods were used to confirm identification of ArMV on host range and mode of transmission.

Total RNA extraction and RT-PCR

For RNA extraction, the EZ-10 Spin Column Total TNA Minipreps Super Kit (BIO BASIC INC) was used. The total RNA from infected and uninfected apricot tissues according to the kit procedure, 10 µl of total extracted RNA were mixed with 25 pmol of the specific reverse primer (AP2: 5`-CATTAACTTAAGATCAAGGATTC-3`) designed by Pantaleo *et al.*, (2001), then 3. 5 µl free nuclease water was added to final volume of 15 µl. The mixture was heated to 70°C for 5 min and then cooled on ice for 2 min. This mixture was used for reverse transcription at 37°C for one hr with 200 units of M-MLV reverse transcription (Promega, USA) in 1X RT buffer, 25 units of rRNasin

ribonuclease inhibitor (Promega, USA) and 10 mmol of each of dATP, dTTP, dCTP and dGTP. PCR was used to amplify 419 nucleotides of ArMV using the specific primers forward (AP1: 5`-AATACCCCGGGTGTTACATCG-3`) and reverse AP2 primer designed to amplify partial coat protein found in RNA 2 (Pantaleo *et al.*, 2001). PCR was performed in 50 µl total volume mixture containing 5 µl of RT mixture (cDNA), 20 pmol of each AP1 and AP2 primers, 10 mmol of each of four dNTPs, 25 µM of $MgCl_2$, 2.5 units *Taq* polymerase (Promega, USA), and 5 µl of 10X PCR buffer. PCR was carried out in the Biometra T-Gradient thermocycler with one initial denaturation cycle at 94°C for 2 min followed by 35 cycles of amplification with temperature profiles of 30 s at 94°C, 30 s at 50°C and 1 min at 72°C. Thirty five cycles were applied followed by one final extension cycle at 72°C for 7 min and the PCR amplified products were analyzed in 1.5% agarose gel, stained with ethidium bromide, then scanned with UV illuminator.

DNA cloning and sequencing

The amplified CP fragment was ligated directly into PCR® II - TOPO® vector (Invitrogen, Carlsbad, CA, USA). The recombinant plasmids were introduced into *E. coli* strain DH5α as described by manufacturer's instructions. DNA was prepared from selected white colonies, digested with *Eco*RI and fractionated on agarose gel using 1Kb DNA ladder (Stratagene, Germany). Clones having 419 bp insert were selected for dideoxy sequencing using ABI PRISMTM Dye Terminal Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS (University of Wisconsin-Madison, Biotechnology Dept., USA) and performed on 377 XL automated DNA Sequencer (Applied Biosystem Co.). The nucleotide sequence was compared and analyzed using DNAMAN Sequence Analysis Software (Lynnon BioSoft, Quebec, Canada) with those of ArMV isolates available in GenBank.

RESULTS AND DISCUSSION

Biological characterization

Several samples of naturally infected apricot fruits and leaves exhibiting symptoms that suspected to be due to virus infection (Fig.1, A, B, C and D) were collected from El-Fayium and Giza Governorates. Out of theses samples, the isolated virus gave positive reaction with ArMV using DAS-ELISA whereas these samples gave negative reactions with other *Prunus* viruses, i.e., ApMV, PNRSV, PDV and PPV. The virus was biologically isolated using single local lesion technique from local lesion produced on *Chenopodium Album*, *Ch. amaranticolor* and *Ch quinoa* (Fig.1 E, F and G). *N. tabacum* cv. White Burley reacted with systemic symptoms (Fig.1 H) was used as a maintain host for further studies. The virus isolate was identified as ArMV according to the symptomology, host range, stability in sap, and modes of transmission, serological reaction and molecular biology. The most common symptoms induced by ArMV are malformation and brownish spots on fruit and leaves associated with several forms of deformation including stunting. The symptoms vary depending on the host plant and cultivar, season and year. Many infections with ArMV are latent and the plants do not show symptoms. ELISA test showed that ArMV virus was detected in grapevine and stone fruits (apricot and almond) in Lebanon, Albania, and Turkey respectively (Gümüs *et al.*, 2007)

In earlier work on plant viruses, host range was used as an important criterion in diagnosis. Such information may be still important, or even crucial in certain

circumstances (Farzadfar *et al.*, 2004 and Navalinskienė *et al.*, 2006). Serological methods (enzyme-linked immunosorbent assay) are routinely employed for these purposes because they allow sensitive, specific, and simultaneous analysis of many samples in a single microplate or membrane (Garnsey and Cambra, 1991).

This result agrees with the results obtained in ArMV-Lv from privet (*Ligustrum vulgare*, Lv) and ArMV from Italy propagated on *Chenopodium quinoa*, and *Chenopodium amaranticolor* respectively (Gerola *et al.*, 2008). The virus was easily sap transmissible to several plant species belonging to 9 families. The results are reported in Table (1) and Fig (1) showing symptoms of different hosts. The following hosts did not become infected upon inoculation: *Datura metal* L., *Datura stramonium*, *Vigna unguiculata*, *Prunus armeniaca* L.cv. Canino and *Zinnia elegans* L. These results were confirmed using back assay. The obtained results of inoculated plants are in agreement with those reported for ArMV before Brown and Trudgill (1983) and Samuitienė *et al.* (2008). Also the results agree with ArMV-Lv from privet (*Ligustrum vulgare*, Lv) and ArMV from Italy was propagated on *Chenopodium quinoa*, and *Chenopodium amaranticolor* respectively (Gerola *et al.*, 2008). *Xiphinema americanum* was able to transmit ArMV from infected apricot and *Ch. amaranticolor* to apricot, peach, plum and grapes nursery (100%). These results also agree with those of Derek and Brown (1986) and Lockhart (2006). For seed transmission, data indicated that the virus could be transmitted through seeds. Seedlings resulted from seeds previously collected from infected plants showed external symptoms and positive ELISA results in rates between 5% to 6% in apricot seeds.

Seed transmission is a common feature and was found in at least 15 species out of 12 plant families with up to nearly 100% of the progeny being infected (Murant, 1970). But this type of spread is of little importance in crops that are propagated vegetatively like hops, grapes, etc. Spread of ArMV by plant contact in the field seems to be rare if it occurs at all. Some evidence exists for pollen transmission of ArMV in hops (Eppler, 1983), but no proof has yet been furnished that healthy mother plants can be infected by virus-carrying pollen. In non-cultivated vegetation, spread occurs initially by seed and, secondarily, over shorter distances by nematode transmission (McNamara, 1980). Symptomatic host plants and inoculated test-plants were tested for ArMV infection by DAS-ELISA. The reaction was considered positive when absorbance at 405 nm was more than twice the mean of healthy (negative) controls by (Samuitienė *et al.* 2008).

The above results confirm that ArMV is widespread on perennial, apricot , causes harmful diseases, retards plant growth, damages some or all parts of a plant, distorts its standard properties, reduces the aesthetic quality and marketability. Virus-infected plants are more susceptible to fungal and bacterial pathogens which lead to premature death.

Table (1): Reaction of host plants inoculated mechanically, grafting and nematode transmitted by ArMV.

Infected host	Mode of transmission	Symptoms	ELISA test
Family: *Amranthaceae*			
Gompherena globosa L.	Mech.	S.M	+
Family: *Apiacaeae*			
Apium graveolens var. *dulce*	Mech. & Nema.	C. L.L.	+
Family: *Caryophyllaceae*			
Dianthus caryophyllus L.	Mech. & Nema.	C. L.L.	+
Family: *Chenopodiaceae*			
Chenopodium Album	Mech. & Nema.	L.L	+
Chenopodium amaranticolor Coste & Reyn.	Mech. & Nema.	L.L	+
Chenopodium quinoa Wild	Mech. & Nema.	C. L.L	+
Family: *Compositeae*			
Zinnia elegans L.	Mech.	0	-
Family: *Cucurbitaceae*			
Cucumis sativas L. cv.Beta Alfa	Mech.	M	+
Family: *Fabaceae*			
Pisum sativum L. cv. Linkolin	Mech.	M	+
Phaseolus vulgaris	Mech.	M	+
Vigna unguiculata	Mech.	0	-
Family: *Rosacae*			
Prunus armeniaca L. cvs. Amar	Graf., Mec &	M	+
Canino	Nema.	0	+
Hammawy	Graf., Mec &	mM	+
Prunus domestica L. cvs. Clymax	Nema.	M	+
Mariana	Graf., Mec &	M	+
Prunus persica L. cvs: Florida prince	Nema.	M	+
GF305	Graf., Mec &	M	+
Nemagarde	Nema.	M	+
Soltany	Graf., Mec &	mM	+
Family: *Vitidaceae*			
Vitis vinifera L.cvs: Superior	Nema.	M	+
Tomson seedless	Graf., Mec &	M	+
Flam seedless	Nema.	0	-
Family: *Solannceae*			
Datura metal L.	Graf., Mec &	0	-
Datura stramonium L.	Nema.	V.C.	+
Nicotiana glutinosa L.	Graf., Mec &	S.M	+
Nicotiana rustica	Nema.	M	+
*Nicotiana tabacum*L. *var. White Burly*	Graf. & Nema.	V.C.	+
Petunia hybrida Vilm cv. Rosa of Heaven	Graf. & Nema.	S.M	+
Physalis floridana L.	Graf. & Nema.	S.M	+

Mech. = Mechanical transmission. Nema. = Transmitted by nematode, Graf. = Transmitted by grafting, L.L. = Local lesion, C.L.L. = Chlorotic Local Lesion, M= Mosaic, mM = mild Mosaic, V.C. = Systemic vein clearing, S.M = Sever mosaic, 0 = no symptoms and + = Positive, - + Negative (in ELISA test)

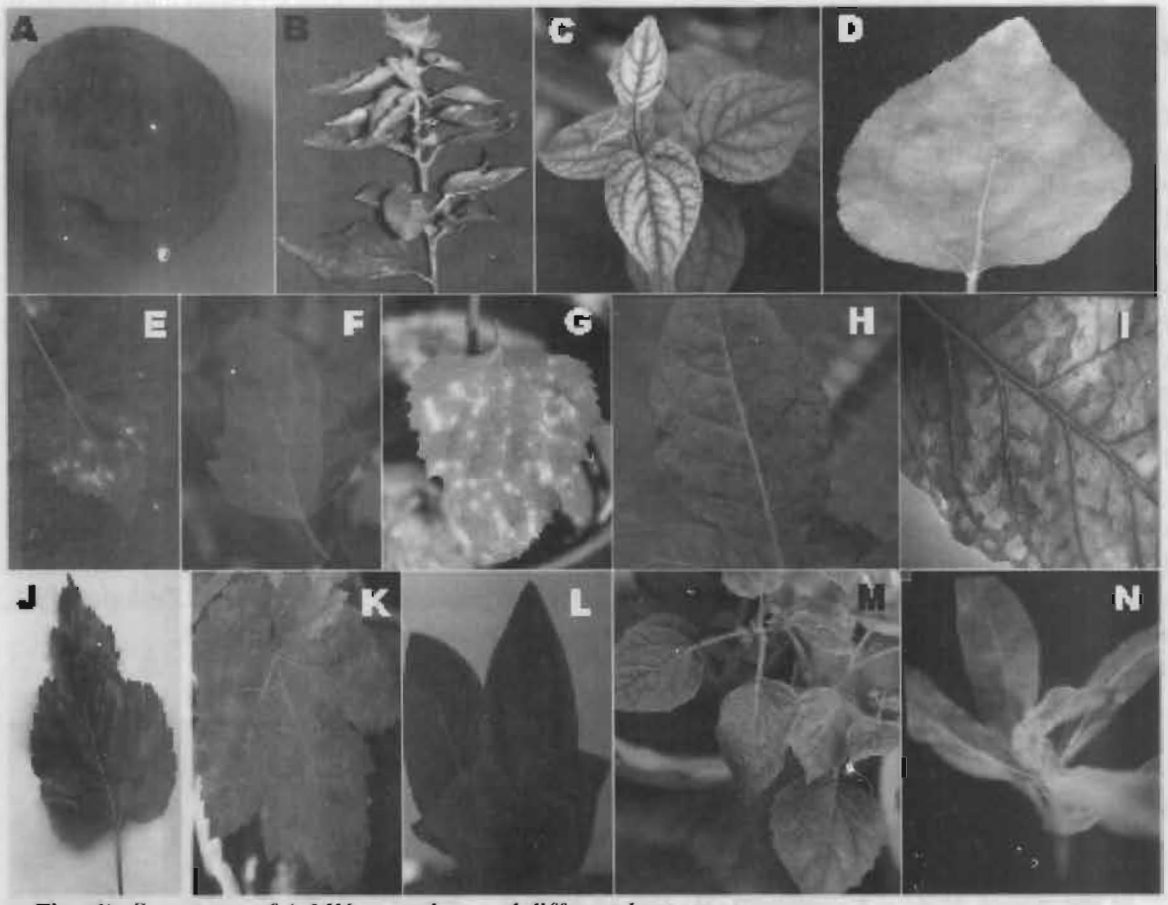

Fig. (1): Symptoms of ArMV on apricot and different host range.

A. Natural infestation of ArMV on apricot fruit (Prunus armeniaca cv. El-Amar) showed malformation and brownish spots.

B. Artificial infection by grafting transmission on (Prunus armeniaca cv. El-Amar) showed typical symptoms (yellows, malformation and rosetting).

C. Artificial infection by nematode transmitted on (Prunus armeniaca cv. Local) showed systemic mosaic and chlorotic veins.

D. Severe mosaic symptoms appeared on apricot leaves Prunus armeniaca cv. Canino produced by ArMV grafting transmitted.

E. Local lesion on Chenopodium quinoa infected with ArMV.

F. Local lesion on Chenopodium Album infected with ArMV.

G. Local lesion and then systemic chlorotic mottle appeared on Chenopodium amranticolor infected with ArMV.

H. Nicotiana tabacum White Burley produced systemic mosaic and blisters.

I. Sever mosaic appeared in Nicotiana rustica mechanical infected with ArMV.

J. Nematode transmission on (Vitis vinifera cv: Superior) showed mosaic and malformation on leaves.

K. Chlorotic blotches on (Vitis vinifera.cv: Tomson seedless) grafting transmitted with ArMV.

L. Petunia hybrida Vilm cv. Rosa of Heaven showed systemic vein clearing.

M. Sever mosaic and systemic chlorosis showed on Physalis floridana.

N. Gompherena globosa showed severe mosaic and leaf twisting by mechanical transmitting with ArMV.

PCR amplification

RNA extracted from apricot plants infected with ArMV were reverse transcribed using AP2 specific primer followed by polymerase chain reaction (PCR) to produce ArMV cDNA. No PCR product was obtained when cDNA generated from uninfected plant using same procedures (Fig. 2, L2). Specific bands in agarose gel of the analyzed products were obtained after electrophoresis corresponding to the expected size of amplification product of 419 bp, confirming ArMV identity (Fig. 2).

On this subject, the pathogen was identified by using specific primers for amplifying the cDNA segment by RT-PCR. Amplifications and detection of coat protein region using these primers (AP1/ AP2) resulted in a cDNA size of approximately 419 bp. This result agrees with those obtained by (Pantaleo *et al.*, 2001 and Samuitienė *et al.*, 2008) as they reported that specific bands in polyacrylamide gel of the analyzed products after electrophoresis at a position corresponding to the expected size of amplification product of 420 bp and 421 were obtained, confirming ArMV identity of ornamental plants and olive in crude extracts from Italy and Lithuania. DAS-ELISA and RT-PCR, together with classical biological methods, increased the reliability of virus identification in complex infections. Recently, the ArMV virus has been described as an agent of viral disease in *Hyacinthus* and *Lycopersicon esculentum* in Lithuania (Navalinskienė, *et al.*, 2006 and Zitikaite *et al.*, 2006) by using RT-PCR analysis.

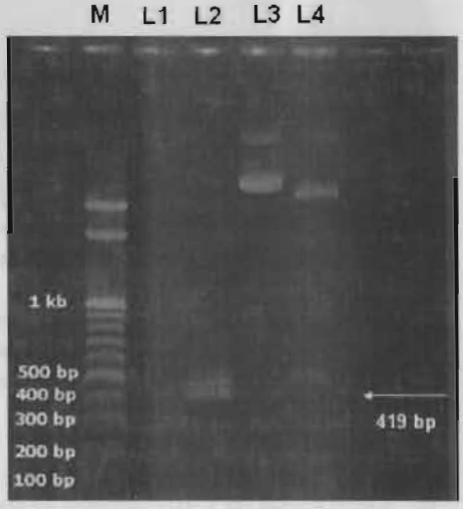

Fig. (2): 1% Agarose gel in TBE buffer stained with ethidium bromide showed RT-PCR amplification of the ArMV-CP using AP1 and AP2 primers and enzyme digestion of the cloned ArMV-CP fragment. M: 100 bp marker, L1: RT-PCR for RNA extracted from Healthy plants, L2: RT-PCR for RNA extracted from infected plants, L3: undigested clone of ArMV-cp fragment and L4: digested clone of ArMV-CP fragment using EcoRI restriction Enzyme.

Partial sequence of ArMV-*CP*

In order to partially sequence the ArMV-CP gene, the PCR product was cloned into PCR® II - TOPO® vector (Invitrogen, Carlsbad, CA.). The recombinant plasmid was then introduced into *E. coli* strain DH5α and the white colonies were subcultured in LB media with ampicillin. The plasmid was isolated and purified using Wizard plus SV Purification System (Promega). The recombinant plasmid was purified and digested with *Eco*RI restriction enzyme and two bands were obtained as shown in (Fig. 2, L4), one for the insert (with extra about 30 nucleotides as a part from the poly linker site of the vector) which is about 450 bp and the other for the plasmid which about 4 kb.

Nucleotide sequencing of the RT-PCR amplified fragment in the recombinant plasmid for the partial coat protein of ArMV (Fig. 3) was done to determine if this PCR fragment was from Nepovirus group or not and to compare the sequence from this isolate with those of other ArMV isolates available in the GenBank. The sequenced fragment of ArMV-Eg was submitted to the GenBank under accession No EU289226.

The RNA 2 of the *Arabis mosaic nepovirus* (ArMV) encodes a polyprotein (coat protein region) from which protein 2A is released by proteolytic cleavage at the N-terminus in Grapevine plant. Nepoviruses have been divided into three subgroups(A-C) based on the length of RNA 2, serological properties of the virions and cleavage site specificity of the viral proteinase (Mayo and Robinson, 1996) .The complete genome of the grapevine isolate NW of ArMV (subgroup A) has been determined by Wetzel *et al.* (2004).

A multiple alignment was done along with previously obtained sequences by Imura *et al.* (2008) and from the GenBank sequence data. The following sequences were used in the comparisons of five different ArMV isolates available in GenBank with the obtained Egyptian isolate. Germany grapevine cultivar Pinot gris, (NC_006056); Japan: Niigata naturally infected narcissus plant (AB279740); Japan: Kochi "naturally infected lily cv. Casablanca" (AB279741); Austria (X55460); and Japan: Hiroshima naturally infected butterbur plant (AB279739) were analyzed using DNAMAN software.

The phylogenetic tree of partial nucleotide sequence alignment (Fig. 4) comparisons showed that the percentage of similarity is ranging from 85-89% of the five reported isolates of ArMV with the Egyptian isolate (ArMV-Eg). The results indicated that the highest sequence similarity was found between the Egyptian isolate of ArMV and isolates from Japan which is 89%, while the lowest was found with ArMV isolates from Austria isolate which is 85%.The similarity of the nucleotide sequences suggested that the ArMV-Eg isolate may be a different strain due to the low similarity (85-89 %) with other isolates in the Gene Bane.

The phylogenetic homology tree based on multiple sequence alignments (Fig. 4) of the ArMV- Eg isolate, revealed that the (*CP*) of the ArMV -NW isolate is closely related to the naturally infected grapevine cultivar Pinot gris of Germany and naturally infected lily cv. Casablanca (Imura *et al.*, 2008) revealed that the ArMV isolate appeared lowest ArMV Austria isolate (Bertioli *et al.*, 1991) Therefore, the ArMV-Eg is probably considered a new strain of ArMV.

ArMV, which causes a significant disease in apricot trees *(Prunus armeniaca)* species, the most commonly used methods of diagnosis still rely on biological indexing using either woody or herbaceous indicator plants. While serological reagents are available for these viruses, the use of ELISA for routine screening is limited to their detection in herbaceous indicator plants, or to specific times of the year from young leaf tissue or flower blossoms obtained from their woody hosts. It is anticipated that the methods described in this research will facilitate the development of rapid and sensitive diagnostic techniques that can be used the year-round for the specific detection of these and other important viral pathogens directly in their woody hosts.

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1          AATACCCCGGGTGTTACATCGATGAGGATGGGAATTTTGAAATTGAAATACGTCCCCCAT
1            I  P  R  V  L  H  R  *  G  W  E  F  *  N  *  N  T  S  P  I

61         ATATCGAACTCCACTGCGTTTACTTAGTGCACAGGCGGCGAGCTCATTTACGAGTACGCT
21           Y  R  T  P  L  R  L  L  S  A  Q  A  A  S  S  F  T  S  T  L

121        GAATTTCTATGCAATTTCAGGGCCAATTGCCCCAAGTGGAGAAACTGCTAGGATGCCAGT
41           N  F  Y  A  I  S  G  P  I  A  P  S  G  E  T  A  R  M  P  V

181        GGTAGTAATCAATGAAATTGCACTTCCGGACCTTTCTGTTCCATCTTTTCCTGATGATTA
61           V  V  I  N  E  I  A  L  P  D  L  S  V  P  S  F  P  D  D  Y

241        TTTCCTTTGGGTGGACTTTTCAGCATTTACTGTTGATACTGAAGAGTATGTAATAGGGTC
81           F  L  W  V  D  F  S  A  F  T  V  D  T  E  E  Y  V  I  G  S

301        TAGGTTTTTTGACATTACCTCGACCACTAGTACTGTCCACCTTGGTGATAATCCTTTTGC
101          R  F  F  D  I  T  S  T  T  S  T  V  H  L  G  D  N  P  F  A

361        CCATATGATAGCTTGCCATGGACTTCACCATGGAATCCTTGATCTTAAGTTAATG
121          H  M  I  A  C  H  G  L  H  H  G  I  L  D  L  K  L
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Fig. (3): Nucleotide sequences and its deduced amino acids of 419 nt fragment amplified from ArMV-CP gene.

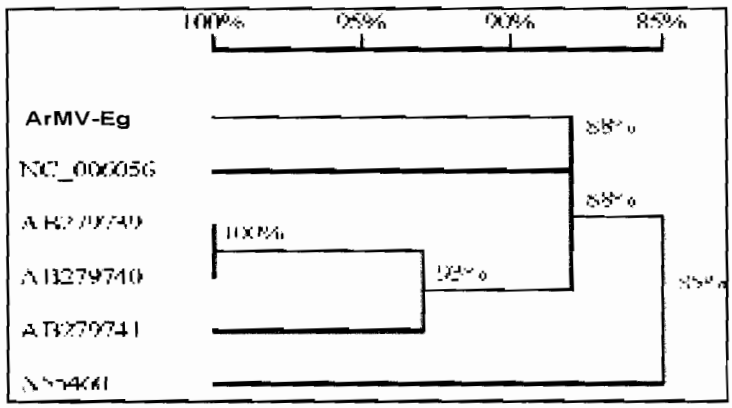

Fig. (4): The phylogenetic homology tree based on multiple sequence alignments of the ArMV Egyptian isolate compared to previously sequenced isolates.

Mosaic, systemic vein clearing and severe mosaic symptoms were noticed in Egypt two years ago on apricot trees in El-Fayium and Giza Governorates. The disease is still under recognition and investigation but needs more attention as a serious problem that could become uncontrollable and may be fast spreading *via* nematode vector or by seed transmission.

On the basis of DNA sequences, it can be concluded that the ArMV was an organism as ssRNA viruses; *Picornavirales; Comoviridae: Nepovirus*; Subgroup A. This is the first report of an ArMV in Apricot trees in Egypt. Also it may be a new strain of ArMV due to

the low similarity (85-89 %) with other isolates available in the Gene Bane.

ACKNOWLEDGEMENTS

We are grateful to Dr. Wafaa M. A. El – Nagdi , Nematology Laboratory, Plant Pathology Department, National Research Centre, Dokki, Giza- Egypt for her assistance in detecting nematode in the cultivated soil under natural infected apricot, also for technical assistance and supplied cultures of (*X. spp.)* the source of nematode used in transmitting the virus.

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الملخص العربي

التعريف البيولوجى و الجزيئى لعزلة المشمش من فيروس تبرقش الارابس

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تعتبر اشجار المشمش من الاشجار الاقتصادية فى مصر والعالم .و قد لوحظت الاعراض لاول مرة فى محافظتى الفيوم والجيزة.و تظهر الأعراض الجهازية على أشجار المشمش كنقص فى حجم و جودة الثمار و موت انسجة النبات ولقد تمت التنقية البيولوجية و التعريف بواسطة البقعة الموضعية على العوائل المشخصة لهذا الفيروس من تطعيم و نقل بواسطة البذور والنيماتودا . و قد استخدم اختبار السيرولوجى للكشف عن الفيرس باستخدام عدد من الامصال المضادة المتخصصة لعدد من الفيروسات التى تصيب الاشجار الخشبية . و كانت النتيجة ان الاشجار المصابة بالفيرس اظهرت تفاعلا موجبا مع النباتات المصابة . اختبر فيرس موزيك المشمش فى ٢٥ نوع نباتى و ٣٠ جنس وتم الاختبار بواسطة النقل الميكانيكى والتطعيم و النقل بالنيماتودا و النقل بالبذرة . وظهر ان النيماتودا تنقل الفيرس بنسبة ١٠٠% من المشمش المصاب الى العوائل المشخصة الاخرى ولقد تم الفحص البيولوجى على واحد من أهم العوائل المشخصة لهذا الفيروس . و قد استخدمت الطرق الحديثة التى تعتمد على استخدام الحمض النووى فى الكشف عن العزلة المصرية تحت الدراسة. ولذلك تم اللجوء الى استخدام الطرق الأكثر تطورا . بأستخدام تفاعل البلمرة التسلسل و وذلك باستخدام بادئ متخصص فى منطقة الغلاف البروتينى فى الكشف عن الفيروس وقد وجد أن هناك حزمة من الحمض النووى المتخصصة لهذا الفيروس حجمها حوالى ٤١٩ قاعدة نيوكلوتيدية باستخدام البادئ المتخصص للأشجار المشمش .تم دراسة التتابع النوكلتيدى لمقطع من جين الغلاف البروتينى (٤١٩) نيوكلوتيدة وتم مقارنة التاتج بعزلات أخرى تم الحصول عليها من بنك الجينات ووجد أن نسبة التشابة بين العزلة المصرية تحت الدراسة وبين العزلات الاخرى المتاحه ببنك الجينات تتراوح بين ٨٥-٨٩ % و هذا يقودنا لاحتمال اننا امام غزلة جديدة لفيروس موزيك الارابس .