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Isolation and Partial Characterization of Apple Mosaic *Ilavirus* (ApMV) in Egypt

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A newly described *ilarvirus* (Family: *Bomoviridae*, Genus *ilarvirus*, Subgroup III), which infects apple trees, is identified as apple mosaic *ilarvirus* (ApMV) in the present study for the first time in Egypt. The virus induced chlorotic patches and/or mosaic on the leaves of infected apple trees. Additional symptoms included vein-banding and/or bright patterns of the leaves were noticed. Host range studies showed that ApMV could infect plants belonging to families: *Amaranthaceae, Chenopodiaceae, Compositae (Asteraceae), Cucurbitaceae, Euphorbiaceae, Fabaceae* and *Solanaceae*. Electron microscopy (EM) of purified virus revealed isometric and quasi-isometric particles approximately 25nm and 28x30-32 nm in diameter, respectively.

ApMV was detected in tissues of infected trees by ELISA, immunosorbent electron microscopy (ISEM) and tissue blotting immuno- binding assay (TBIA). Other techniques, *i.e.* agar gel double diffusion and reverse transcription polymerase chain reaction (RT-PCR), have been used to compare between the two isolates of ApMV obtained from apple (cv. Anna or M.M. 106 rootstock) with other *ilarvirus*, prunus necrotic ring spot virus (PNRSV).

Serologically, agar gel double diffusion test revealed that the two isolates of ApMV are closely related (close proximity), but distantly related to PNRSV. The same conclusion occurred when RT-PCR was used.

Key words: Apple mosaic virus (ApMV), ACLSV, EM, ISEM, PNRSV and reverse transcription polymerase chain reaction (RT-PCR).

Apple (*Malus demostica*) is one of the most widely grown fruit crops worldwide. Apple mosaic *ilarvirus* (ApMV) is common in most areas of the world where apple and stone fruits are cultivated. It exists as a number of strains that cause a diversity of symptoms on many rosaceous species *i.e.*, rose (Thomas,1984); almond (Digiaro *et al.*, 1992); *Prunus* sp. (Digiaro and Savino, 1993) and apple (Fidan, 1994). Hop (*Humulus lupulus*); filberts (*Corylus avellana*) and horse chestnut (*Aesculus hippocastanum*) are nonrosaceous hosts in which ApMV has been reported to occur in nature (Sweet and Barbara, 1979; Sano *et al.*; 1985; Savino *et al.*, 1985; Postman and Cameron, 1987 and Klein and Husfloen, 1995).

ApMV is the type member of the *ilarvirus* genus. ApMV has a worldwide distribution and induces a large variety of symptoms in 65 species belonging to

19 families. Virus is transmitted by mechanical inoculation; by grafting (of roots); possibly not transmitted by seed; but probably transmitted by pollen to the pollinated plants (Brunt *et al.*, 1997). Recently, serological and polymerase chain reaction (PCR) procedures have been developed for the detection and serotyping of ApMV (Candresse *et al.*, 1995; Turk, 1996 and Menzel *et al.*, 2002). According to serological reactions, ApMV and its membership prunus necrotic ring spot *ilarvirus* are identical and can be used as synonyms (Smith and Skotland, 1986). On the other hand, many investigators showed that ApMV was distantly related to PNRSV (Sano *et at.*, 1985; Rowhani *et al.*, 1994 and Candresse *et al.*, 1998).

Therefore, the purpose of this study was to identify an *ilarvirus* infecting apple through mechanical transmission, virus purification, morphology of virus particles, serological studies (*i.e.* DAS-ELISA, immunosorbent electron microscopy and tissue blotting immuno-binding assay), as well as its relationship with other *ilarvirus* such as prunus necrotic ring spot virus (PNRSV), was taken in consideration.

Materials and Methods

Orchards survey and virus sources:

Visual inspections followed by ELISA test was carried out in apple orchards (cv. Anna and M.M.106 rootstock) grown in Agriculture Research Centre, Ministry of Agic., Qunater region and newly reclaimed land during 2000-2001. Two ApMV isolates were obtained from cv. Anna and M.M.106 rootstock. The cv. Anna isolate was used in all experiments of the present study. Meanwhile, the second isolate of M.M.106 rootstock was only used in relationship studies (*i.e.* agar gel double diffusion test and RT-PCR detection) with other isolate from cv. Anna, as well as the other related virus (PNRSV) that membership in *ilarvirus* group.

Concerning PNRSV, it was previously isolated and purified by Ghanem (2000b) from plum trees (cv. Hollywood) grown at El-Saff location, Giza governorate.

Mechanical transmission:

Fresh ApMV-naturally infected leaves and flower petals of apple trees (cv. Anna) were ground in 0.05M sodium phosphate buffer ph 8.0 containing 0.2% (w/v) ascorbic acid, 0.2% (w/v) nicotine and 0.83% (w/v) polyvinylpyrrolidone. The extracts were rubbed on the following test plants: *Amaranthus retroflexus* L.; *Beta vulgaris* L.; *Chenopodium quinoa* Willd; *Chenopodium murale* L.; *Cucumis sativus* L. cv. Beit Alpha; *Cucurbita pepo* L.; *Datura stramonium* L.; *Gomphrena globosa* L.; *Helianthus annuus* L.; *Lactuca sativa* L.; *Nicotiana benthamiana* L.; *N. clevelandii* Gray; *N. glutinosa* L.; *Ricinus communis* L. and *Vigna unguiculata* L. Walp. cv. Balady. Fifteen plants of each species were tested, control plants were buffer treated only. Plants were kept in the greenhouse for symptom appearance. Virus infection was confirmed by ELISA test.

Virus purification:

ApMV was purified from cucumber cv. Beit Alpha cotyledons according to the method of Sano *et al.* (1985). All stages of the purification were performed under

cold (6°C) condition. Infectivity was assayed on cucumber cv. Beit Alpha by counting local lesions.

Electron microscopy (EM) of purified virus:

The carbon-coated grid was floated on drop of purified virus for 5 min, stained with 2% potassium phosphotangistic acid, then air dried. Grid was then examined with Joel electron microscope at the Electron Microscopy Unit, Fac. of Sci., Cairo Univ.

Serological tests:

Different serological techniques were used to detect ApMV, *i.e.* ELISA test (DAS-ELISA); immunosorbent electron microscopy (ISEM); tissue blotting immuno-binding assay (TBIA) and agar gel double diffusion test.

1- DAS-ELISA:

DAS-ELISA was essentially performed, as previously described by Dominguez *et al.* (1998) and Moury *et al.* (2001) using commercially available kits (ApMV-Pab. and PNRSV-Pab which kindly provided from Dr. Boscia, Bari Univ., Bari, Italy) and apple chlorotic leaf spot virus (ACLSV) was obtained from Sanofi Phytodiagnosis, Paris, France. Periodical DAS-ELISA tests (approximately one per month) were conducted up to 4 months started from spring to summer season 2000. Samples included leaves, petioles and flower petals of apple trees (cv. Anna and M.M.106), as well as infected test plants.

2- Immunosorbent electron microscopy (ISEM):

ISEM technique was used as described by Ghanem (2000a). The antibodies performed in this study included ApMV polyclonal antibody (ApMV-Pab) to detect ApMV antigens in the infected tissues of apple trees.

3- Tissue blotting immuno-binding assay (TBIA):

The procedure of TBIA was conducted according to Knapp *et al.* (1995). The TBIA was used to detect ApMV in different tissues of apple trees (leaves, stems and leaf petioles). Nitroblue tetrazolium (NBT) / 5 bromo-4-chloro-3 indolyl phosphate (BCIP) complex give purple color in positive reactions (Abdel-Salam, 1998).

4- Agar gel double diffusion test:

This experiment was conducted to determine the serologic relationship between ApMV and PNRSV as a membership in *ilarvirus* group. Agar gel double diffusion test was done in Petri dishes using 0.7% agar containing 0.85% sodium chloride and 0.1% sodium azide. Two isolates of ApMV isolated from apple cv. Anna and M.M.106 rootstock and one isolate of PNRSV isolated from plum were used in this experiment.

5- Detection of ApMV and PNRSV using RT-PCR:

RT-PCR technique was also performed for the same purpose as previously explained in agar gel double diffusion test.

Reverse transcriptase-PCR (RT-PCR) technique was run according to Hammond *et al.* (1998) and Rosner *et al.* (1998). One to two µg of total nucleic acids was used

to prepare first strand cDNA using 10 pmol of JC-10 as a primer (5'-TTCATCGACAACGAAGAC-3'). Polymerase chain reactions using either the universal primer, JC-12 (5'-GCAATCATACCCACGCTG-3') or JC-16 (5'-CCTCCCTCCGATTTAGAC-3'), in combination with primer JC-10 were performed to amplify PCR products of 641 bp, (primer JC-10/JC12) and 291 bp (primer JC-16/JC-10), respectively, in reaction conditions as described by Hammond *et al.* (1998).

Results and Discussion

Source of the virus and virus isolation:

The virus under investigation was isolated from naturally infected apple trees (cv. Anna) grown in newly reclaimed land. Identification of the virus depended on symptomatology, mechanical transmission, host range, diagnostic hosts reaction, virus purification, morphology of virus particles and serological tests, *i.e.* DAS-ELISA, ISEM, TBIA, agar double diffusion test and RT-PCR.

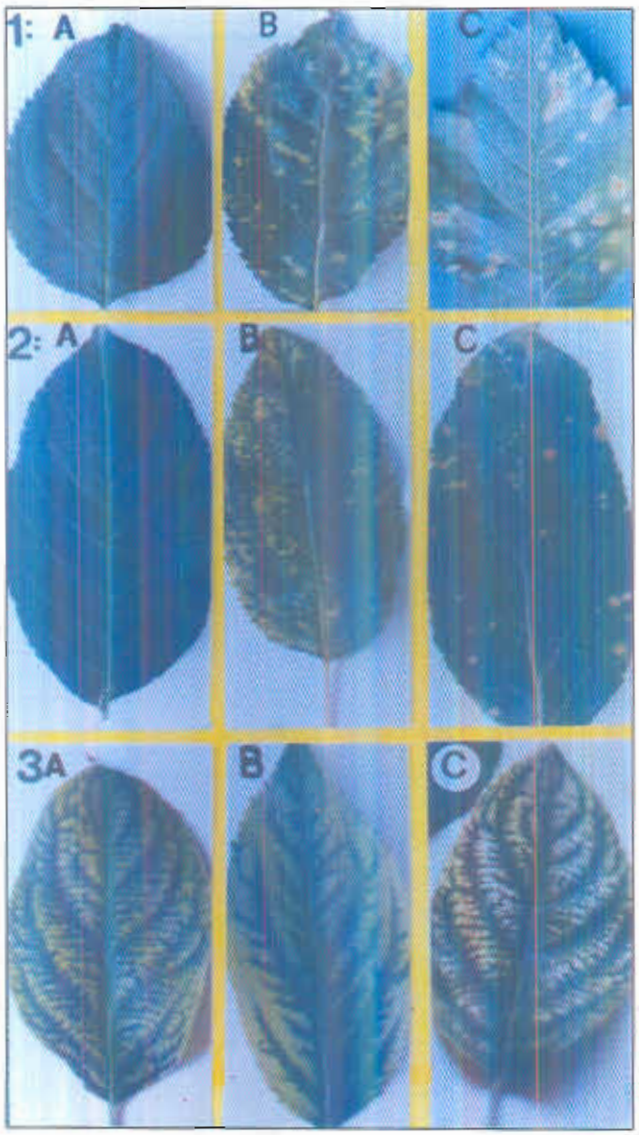
Survey and symptoms of ApMV:

Through symptoms and DAS-ELISA confirmation, ApMV and sometimes ACLSV were the two viruses detected in the collected samples of surveyed apple. In early summer, apple trees (cv. Anna and M.M.106 rootstock) infected by ApMV showed yellow patches or spots restricted between veins and the leaf edges (Figs. 1&2 A-C). Sometimes, in June and July, apple leaves tested of both cultivars (cv. Anna and M.M.106 rootstock) showed veinclearing and veinbanding (Fig. 3 A&B) which extended to bright patterns on older leaves (Fig. 3C). Using ApMV-Pab antiserum, virus was most reliable in early spring but viral concentrations often fell to undetectable levels later in the summer season. These preliminary results confirm that the symptoms of Egyptian isolate similar to ApMV isolates infecting apple (Brunt *et al.*, 1997). Also, it is similar to a number of *ilarviruses* infecting horse chestnut (Sweet and Barbara, 1979); hop (Klein and Husfloen, 1995) and apricot trees (Dominguez *et al.*, 1998).

Furthermore, in some apple orchards, ACLSV was detected either alone or in dual infection by ApMV. Such results were confirmed using ACLSV Pab antiserum. Data in Table (1) show that the levels of infection were 16.5 and 17.5% for ApMV and 32.1 and 34.4% for ACLSV in apple trees cv. Anna and M.M.106 rootstock, respectively. Dual infection of ApMV and ACLSV was observed in 6.8% and 7.7% apple trees cv. Anna and M.M.106 rootstock, respectively. These results indicate a significant influence on viral incidence, in case of rootstock. Such results are in accordance with those of Boyé and Gentit (1998); Khoury (1998), Pallas *et al.* (1998) and Ghanem *et al.* (2002) who demonstrated that these viruses are characterized as widespread, systemic infection, graft transmitted, cause trees decline on sensitive rootstock and spread through the vegetation production.

Symptoms in herbaceous test plants:

ApMV was transmitted from naturally infected leaves and petals of apple trees to different test plants as shown in Table (2). Symptoms appeared 5-10 days after inoculation. No symptoms appeared on control plants.



Figs. 1&2. ApMV-infected apple in orchards showing yellow patches or spots restricted between veins in both of rootstock M.M.106 (Fig. 1: A, B & C) and cv. Anna (Fig. 2: A, B &C).

Fig. 3. In summer season, old leaves showing veinclearing (A), veinbanding (B) followed by bright patterns (C).

Table 1. Infection percentages of ApMV and ACLSV viruses detected in apple trees by ELISA-DAS in Egypt during spring season, 2000

Source of plant material	Virus(es) detected	No. of trees: infected/tested	Infection (%) *
cv. Anna	ApMV	19/115	16.5
	ACLSV	62/193	32.1
	ApMV+ACLSV	6/87	6.8
M.M.106 rootstock	ApMV	31/177	17.5
	ACLSV	41/119	34.4
	ApMV+ACLSV	7/91	7.7

* Data are based on DAS-ELISA of collected samples.

Table 2. Host range of apple mosaic virus (ApMV)

Test plant	Symptoms of virus in:		Serologic detection in:	
	inoc. leaves	uninoc. leaves	inoc. leaves	uninoc. leaves
***Amaranthaceae*:**				
Amaranthus retroflexus L.	NCL *	L	+	+ **
Gomphrena globosa L.	L	0	+	-
***Chenopodiaceae*:**				
Chenopodium murale L.	NLL	0	+	-
Ch. quinoa Willd	ChLL	0	+	-
Beta vulgaris L.	0	0	-	-
***Compositae (Asteraceae)*:**				
Helianthus annuus L.	0	MM	-	+
Lactuca sativa L.	0	0	-	-
***Cucurbitaceae*:**				
Cucumis sativus L. (cv. Beit Alpha)	ChLL	0	+	-
Cucurbita pepo L.	0	0	-	-
***Euphorbiaceae*:**				
Ricinus communis	0	MM	-	+
***Leguminosae (Fabaceae)*:**				
Vigna unguiculata (L.) Walp.	0	MM	-	+
***Solanaceae*:**				
Datura stramonium L.	NLL	0	+	-
Nicotiana benthamiana L.	ChLL	0	-	+
N. clevelandii Gray	0	0	-	-
N. glutinosa L.	0	MM	-	+

* ChLL= chlorotic local lesion, L= latent infection, MM= mild mottle or mosaic, NLL= necrotic local lesion, NCL= necrotic creamy lesion and 0= not infected.

** + or - =positive or negative reaction with ELISA.

Data in Table (2) show that among 15 plant species tested, eleven were susceptible to virus infection by mechanical inoculation. The virus was not transmitted into *Beta vulgaris* L., *Cucurbita pepo* L., *Lactuca sativa* L., *Nicotiana clevelandii* Gray. Chlorotic spots developed on inoculated cucumber (cv. Beit Alpha) cotyledons (Fig. 4 A & B), *Amaranthus retroflexus* L. (Fig. 5), *Chenopodium quinoa* Willd (Fig. 6 A & B), *Chenopodium murale* L., *Datura stramonium* L. and *Nicotiana benthamiana* L., 5-10 days after inoculation. *Helianthus annuus* L., *N. glutinosa* L. and *Vigna unguiculata* (L.) Walp reacted with systemic mild mosaic. Infectivity was lost when the extracts of young leaves were stored for more than one day at –20° C. The results were confirmed by ELISA test. Results of the present investigation are in agreement with those recorded by many investigators (Thomas, 1984 and Fidan, 1994) who demonstrated that symptoms of ApMV appeared within 1-2 weeks after inoculation.

Purification of ApMV:

Yield of purified virus from ApMV – infected cotyledons of cucumber was 3 mg/kg fresh weight. Purified virions (Fig. 7) were infective to cucumber, *Chenopodium quinoa* plants. It infected cucumber producing chlorotic spots on the inoculated cotyledons. The virus did not infect cucumber plants systemically. Also, ApMV could be transferred from cucumber to cucumber after 4-5 successive passages. Such results are similar to those of ApMV (Sweet and Barbara, 1979; Edward *et al.*, 1984 and Brunt *et al.*, 1997). While, these results were in contrast with those of Sano *et al.* (1985) who cleared that ApMV isolated from hop could not be transferred from cucumber to cucumber. ApMV isolated from hop is intermediate between ApMV and PNRSV. They also indicated that hop spherical virus (ApMV) differs biologically from that of apple or peach, although the two viruses are closely related serologically.

Detection of ApMV by electron microscopy:

Examination of ApMV-purified preparations revealed the presence of isometric particles 25nm in diameter and some quasi-isometric (slightly irregular shape) particles 28nm x 30-32nm (Fig. 7 A & B) similar to those reported for ApMV (Brunt *et al.*, 1997 and Kurihara *et al.*, 1998).

Serological studies :

Three different serological tests were conducted to detect ApMV in apple trees (leaves, stems and petioles).

(A) Detection of ApMV by immunosorbent electron microscopy (ISEM):

The use of ISEM resulted in an increased number of virus particles trapped on the grid as seen by comparing uncoated and ApMV-antibody-coated grids, Also, the attachment of antibody to virus particles, the decoration step, increases the apparent (Fig. 8) width of the particles about three times making the observation easier and the diagnosis quicker. The decoration confirms the identity of the virus. This result indicates that, similar to ELISA, ISEM is probably very useful for the detection of ApMV where smaller numbers of samples are to be examined and where identification of a particular virus by serology and morphology is required.

Such results confirm those of Savino *et al.* (1995); James and Howell (1998); Ghanem (2000b) and Ghanem *et al.* (2002) who suggested that ISEM appears to be as alternative to ELISA and provides a useful diagnostic tool for virus detection. Also, ISEM proved to be sensitive, rapid and reliable test.

(B) Detection of ApMV using TBIA test:

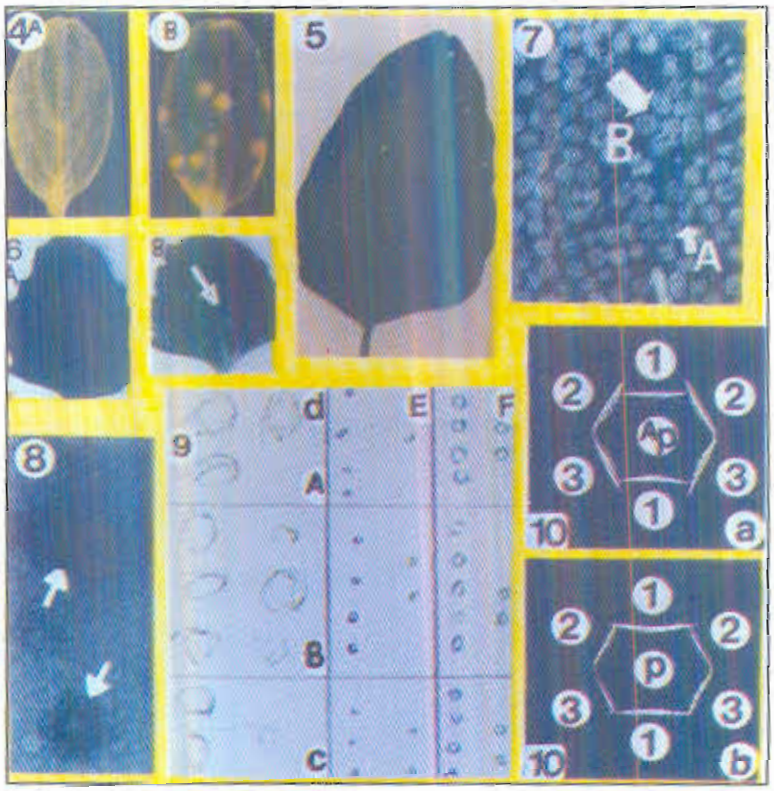
Applying TBIA test, ApMV-AS detected ApMV antigen in all samples of apple (leaves, petioles and stems) collected from cv. Anna, rootstock M.M.106 and grafted trees (Fig. 9 A, B & C). The virus could not be detected in some leaves especially in summer season. This is probably due to effect of temperature degrees, uneven distribution and low concentration or presence of the virus in a form not detectable by this method (*e.g.* perhaps present as naked RNA). Similarly, uneven distribution has been observed by tissue blotting with other viruses, such as papaya ring spot virus in papaya petioles (Lin *et al.*, 1980); plum pox virus (sharka) in apricot (Dicenta and Audergon, 1995); viruses of *Malus* sp. and *Prunus* sp. (Knapp *et al.*, 1995); prune dwarf virus (Ghanem, 2000a); tomato mosaic *tobamovirus* & cucumber mosaic *cucumovirus* (Fegla *et al.*, 2001) and apple chlorotic leaf spot *trichovirus* (Ghanem *et al.*, 2002).

(C) Serologic relationship between ApMV and PNRSV:

Serological relationships among the two virus isolates from apple (cv. Anna and M.M.106 rootstock) and PNRSV isolated from plum were investigated using the antisera to ApMV and a PNRSV serotype in agar-gel diffusion test. As shown in Fig. (10 A & B) confluent precipitation lines without spurs were formed by ApMV-infected cv. Anna and ApMV-infected M.M.106 rootstock tissues when tested in adjacent wells against each of the two antisera (ApMV-AS or PNRSV-AS). Meanwhile, by contrast reactions of partial identity (with spurs) occurred when ApMV and PNRSV antigens were detected in adjacent wells against each of ApMV-AS and PNRSC-AS antisera. Such results confirm the identity of ApMV as an *ilarvirus* since ApMV-As is known to react with other *ilarviruses* (Sano *et al.*, 1985 and Sanchez-Navarro and Pallas, 1997). Furthermore, such result confirm that both isolates of ApMV (from cv. Anna and M.M.106 rootstock) were indistinguishable serologically (close proximity) from ApMV but distantly related to PNRSV. Results of the present investigation confirm those reported by other researchers (Postman and Cameron, 1987 and Brunt *et al.*, 1997) who reported that ApMV is distantly related to PNRSV. Also, this result had already been observed by Sano *et al.* (1985) who mentioned that hop spherical ApMV isolated from hop was closely related to apple or peach-ApMV, but only distantly related to PNRSV.

(D) Detection of ApMV and PNRSV using RT-PCR:

RT-PCR assay was performed with samples infected by ApMV or PNRSV. The data of amplifications products using either the primer pair designed to amplify different serotypes are shown in Fig. (11). Using restriction enzyme (*Mse* I) in RFLP analysis, a specific product about 700 base-pairs (bp), clearly visualized in the ethidium bromide stained agarose gel, was obtained with all the ApMV-infected samples and purified ApMV. The primer pair JC-10/JC-12 amplified only the 300bp fragment from the PNRSV-infected samples. This result indicated that the primers



Figs. 4, 5 & 6: ApMV-developed symptoms after mechanical inoculation of indicator plants:

Fig. 4: *Cucumis sativus* L. cv. Beit Alpha showing chlorotic spots on cotyledons (A: healthy & B: infected).

Fig. 5: *Amaranthus retroflexus* L. showing creamy local lesions.

Fig. 6: *Chenopodium quinoa* Willd exhibited chlorotic local lesions (A: healthy & B: infected).

Fig. 7: Electron micrographs showing isometric particles, 25 nm in diameter (A) and quasi-isometric (slightly irregular shape) 28x30-32nm (B).

Fig. 8: Immuno-electron microscopy of ApMV-antigens treated with ApMV-antiserum showing decorated isometric particles.

Fig. 9: TBIA test illustrating the detection of ApMV in blotted-cut leaves (D), petioles(E) and stems(F) of different orchards: (A) cv. Anna, (B) M.M.106 rootstock and (C) Six months grafted trees.
Healthy cuts= green color & Infected cuts= purple color

Fig. 10: Agar gel double diffusion test of ApMV and PNRSV using antiserum against (A) ApMV-AS and (B) PNRSV-AS.
1= PNRSV-infected-samples, 2= ApMV-infected apple cv. Anna and 3= ApMV-infected apple M.M.106 rootstock, Ap= ApMV-antiserum, P= PNRSV-antiserum.

pair allowed the simultaneous amplification of ApMV and PNRSV, it would sometimes be interesting to process the amplification products so as to be able to identify the viruses which were present in the indexed samples. These results are in accordance with those of Rowhani *et al.* (1994) and Hammond *et al.* (1998) who cleared that the restriction digestion of universal PCR products (that used in the present study) and the other primer could discriminate among PNRSV isolates. Moreover, Sano *et al.* (1985); Postman and Cameron (1987); Brunt *et al.* (1997); Candresse *et al.* (1998) and Spiegel *et al.* (1999) in their studies on PNRSV and ApMV revealed that ApMV was serologically distant related to PNRSV.

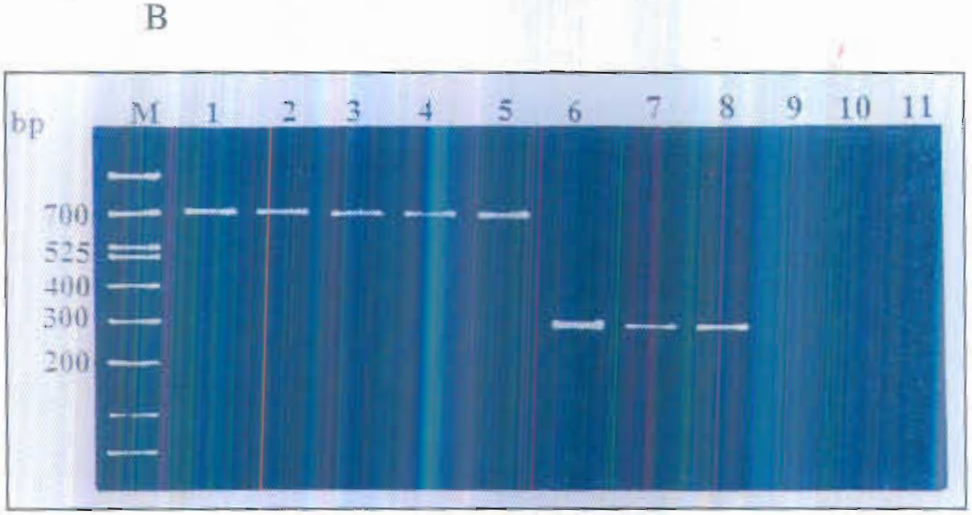


Fig. 11: Agrose gel electrophoresis of RT-PCR from ApMV or PNRSV–infected samples as well as purified viruses: Lane M, DNA marker, lane 1, ApMV-infected cv. Anna, lane 2 ApMV-infected M.M.106 rootstock, lane 3 ApMV-infected grafted apple, lane 4 ApMV-infected cucumber cotyledons, lane 5, purified ApMV, lane 6, PNRSV-infected plum (cv. Hollywood), lane 7, PNRSV-infected cucumber cotyledon, lane 8 purified PNRSV, lane 9, buffer and lanes 10, &11, stock extracts of healthy cucumber cotyledons.

In conclusion, the results of the present study demonstrate the potential of serologic tests of agar gel double diffusion test and RT-PCR-based assay to serve as useful tools for differentiation among ApMV and PNRSV or their serotypes. Also, validation of the reliability and specificity of RT-PCR assay can be employed in monitoring of spatial and temporal spread of strains in field studies.

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عزل وتعريف فيروس موزيك التفاح في مصر

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تم فى هذه الدراسة عزل وتعريف فيروس موزيك التفاح لأول مرة فى مصر. ويعطى الفيروس أعراض موزايك وانخفاض بين العروق ثم ابيضاض بين العروق في موسم الصيف على أوراق أشجار التفاح سواء الأصل M.M.106 rootstock أو على الصنف المنزرع cv. Anna.

وقد بينت دراسات الحصر أن كل من الأصل والصنف المنزرع قابلين للإصابة والتأثر بالفيروس . وقد أوضحت الدراسة أن الفيروس ينتقل ميكانيكيا إلى العديد من العوائل المشخصة التابعة لعائلات نباتية مختلفة هي النرجسية والرمرامية والمركبة والقرعية السوسبية والبقولية بالإضافة إلي الباذنجانية. وأظهرت دراسات الميكروسكوب الالكتروني على تحضيرات الفيروس المنقى وجود نوعين من الجزيئات: الأولى كروية أقطارها ٢٥ نانوميتر والأخرى شبة كروية يتراوح أقطارها ما بين ٢٨ x ٣٠-٣٢ نانوميتر. وأكدت الدراسة كفاءة كل من اختبار الاليزا والارتباط المناعى باستخدام المجهر الإليكتروني (ISEM) وإختبار البصمة النسيجية DBIA فى الكشف عن الفيروس في أنسجة الأشجار المصابة.

علاوة على ذلك استخدم اختبار الترسيب المزدوج واختبار تفاعل البلمرة المتسلسل (PCR) في الكشف عن عزلتين من فيروس موزايك التفاح (ApMV) ومقارنته بإحدى الفيروسات التابعة لنفس الجنس *ilarvirus* وهو فيروس التنكرز الحلقي في البرقوق (PNRSV) . فقد أظهرت نتائج اختبار الترسيب المزدوج في الاجار تطابق العزلتين المتحصل عليهما من أشجار التفاح cv. Anna والأصل M.M.106 rootstock حيث تقابلا بدون تكوين مهماز طرفى بينما تقابل كل من العزلتين مع الفيروس PNRSV بتكوين مهماز طرفي وهذا يدل على وجود علاقة قرابة سيرولوجية بين عزلات التفاح المتطابقتين معا وعزلة البرقوق PNRSV لكنهم غير متطابقتين . وقد أكد اختبار PCR أيضا عدم تطابق الفيروسين ApMV و PNRSV.