مجلـــة البحوث البيئية والطاقة جـامعة المنوفية قطاع خدمة المجتمع وتنمية البيئة

Isolation and identification of Potato aucuba mosaic virus naturally infecting potato plants in Egypt

عزل وتعريف فيروس موزيك أوكيوبا البطاطس من نباتات بطاطس مصابة طبيعيا في مصر

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العدد (۱۲)

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ABSTRACT

Potato aucuba mosaic virus (PAMV) was isolated from naturally infected potato (Solanum tuberosum, L. cv. Agria) plants, showing yellow spotting, leaf deformation, severe stunting and necrosis in potato tubers symptoms, suspected to be due to virus infection. Diseased leaf samples were collected from different localities in Menoufia Governorate, Egypt. The identification of isolated virus was based on symptomatology, host range, mode of transmission (mechanical- insect), DAS-ELISA, PCR, light microscopy and electron microscopy. Fifteen of potato cultivars were tested for their reaction to PAMV infection. Only thirteen potato cultivars showed virus symptoms and given positive reaction in ELISA test after mechanical inoculation with PAMV. While two cultivars showed symptoms and given negative reaction in ELISA test. PAMV infection affected some of photosynthetic pigments in infected potato leaves, i.e. chlorophyll and carotenoids and also reduced the concentration of total carbohydrates and starch in tubers.

Key words: *Potato aucuba mosaic virus* (PAMV), potato, serological tests, light microscopy, PCR, electron microscopy

INTRODUCTION

Potato (*Solanum tuberosum* L.) ranks the fourth place as the largest crops in the world. Also it is considered the first alternative for grain crops, and is used for human consumption, animal feed, and as a source of starch, carbohydrates, alcohol and protein (**Horton, 1992**). Potato is one of the most economic and important vegetable crops in Egypt. It grows in almost any season, except during very hot months in July and August and in most

of country (**Soliman, 2002**). **Ravinder** *et al.*, (**2014**) reported that, under field conditions, potato plants infect with several viruses during the growing season, resulting in decreased yield as well as deterioration in the quality of planting material. More than 20 different viruses have been recorded and all are considered pathogenic to potato (**Beemstar and Rozendal, 1972**). Leaf symptoms of this virus differ depending on virus strain and potato cultivar. Thus, the two types of symptoms are: The first type is bright yellow spots on the lower leaves, later coalescing to form large yellow or whitish spots, the second type of symptoms is necrotic spots, often leading to systemic or top necrosis (**Loebenstein, 2001**). **Khurana, (2004**) found that 20-40 % yield loss due to the incidence of PAMV on potato in India. In Egypt, **Gamal El din,** *et al.*, (**2005**) reported that PAMV caused a 33 % reduction in tuber yield.

PAMV have a single stranded RNA (ss RNA) with filamentous particles about 580 nm long and 11 nm wide and it belongs to genus *Potexvirus* family *Alphaflexiviridae* with cryptogram(R/* : */5 : S/ Ap (Potex virus group) (**Ravinder** *et al.*, **2014**).

DAS- ELISA test is a serological method for detection of diseases depending on specific antibodies and changing in color in the assay. This method use specific antibodies to coat the micro plate, which then trap antigens from bacteria , fungi and viruses. An enzyme- labeled specific antibody conjugate is then used for detection. The detection can be visualized or measured in a computer controlled plate ELISA-reader based on color changes resulting from the interaction between the substrate and the immobilized enzyme (Kassanis and Govier, 1972).

PCR and RT-PCR assays, which involve the enzymatic increase of DNA fragment defined by two oligonucleotide primers, have been used to diagnose a number of viral diseases (**Henson and French, 1993**). PCR based methods are more responsive than serological tests including ELISA. RT-PCR method was developed for rabid detection of PAMV with high degree of accuracy and sensitivity from leaves and tubers (**Raigond** *et al.*, **2013** and **Wu** *et al.*, **2018**).

One of the important deleterious effects of the virus infection on plant physiology is the reduction of chlorophyll content (**Subba** *et al.*, **1979**). The prevalence of this virus in the country encouraged us to conduct the present study which aims at the investigation and identification the virus by different methods as well as reaction of different potato cultivars grown

in Egypt to this virus and its effect on some physical and chemical analysis of infected potato plants.

MATERIALS AND METHODS

I. Isolation:

The virus was isolated from naturally infected potato plants growing under field condition at different location in Menoufia governorate, Egypt. Leaf samples showing, yellow flecking, severe stunting, leaf deformation (doubted to be due to virus infection) and positive ones were collected and examined serologically by ELISA (Clark and Adams, 1977). Samples were homogenized in a mortar and pestle, after addition phosphate buffer (, 0.1 M, 1:5 w/v, pH 7.2, Xu and Nie 2006), then the extracted liquid was approved through a double layer cheesecloth. Finally, the sap was back inoculated onto potato plants. Inoculated plants were reserved in the greenhouse, then used as a supplier of infection in the following experiments.

II. Identification:

A: Host range and symptomatology:

Seedling of twenty plants species and cultivars belong to six different botanical families, were mechanically inoculated with the isolated virus. The inoculated seedlings were grown under greenhouse conditions (25 \pm 5°C) and observed daily for 30 days for any virus symptoms. Inoculated plants showing visible or no visible symptoms were checked by a serological test (DAS-ELISA).

B-Mechanical transmission:

Crude sap was extracted from the infected tissues by using an electric homogenizer or by a mortar and pestle after adding phosphate buffer (1:5 w/v, 0.1 M, pH 7.2, **Xu and Nie, 2006**), then the extracted sap was filtrated through double layers of cheesecloth and obtained sap was used to inoculate the tested plants. The inoculated leaves were dusted with carborundum (600 mesh) and gently rubbed with a prepared inoculum, by using a cheese-cloth pad or by fore finger, then the inoculated leaves were washed with water for 15 min after inoculation (**Awad**, *et al.*, **1985**).

C- Aphid transmission:

In the course of studying insect transmission of the isolated virus, the green peach aphids (*Myzus persicae*), Black Cowpea aphids (*Aphis crassivora*) and Yellow Milkweeds (*Aphis nerii*) were used to study their ability to transmit the virus from infected on Cowpea plants (*Vigna unguiculata* L. cvs Black eye) to healthy ones, as described by (**Yiannis and Bryan, 1993**). Each plant to be inoculated received 15 viruliferous aphids. Control plants received the same number of virus-free insects.

D- DAS-ELISA:

The samples were tested in duplicate using DAS-ELISA as described by (**Clark and Adams, 1977**) according to the manufacture's instruction (Agdia Incorporated, USA). Positive reaction obtained with the specific antiserum.

E- Light microscopy:

Epidermal strips of both healthy and infected leaves of Burley tobacco plants were examined using light microscope. Strips were dipped for 5 min in 5 % triton X-100 and stained with mercuric bromophenol blue (Mazia *et al.*, 1953).

F- Electron microscopy:

The effect of PAMV infection on the cell or on the organelles as well as inclusion bodies detection were studied out according to **Mossop**, (1982). Ultra histopathological changes due to virus infection were studied using potato leaves, the work was done in TEM lab FARP. Faculty of Agriculture Research Park- Cairo University.

G- RT-PCR test:

1: Extraction of total RNA from plant tissues

The positive samples in ELISA test, were used to RT-PCR. Total RNA was isolated from the infected potato plants using RNA Purification Kit taken from BioFlux according to manufacturer's instructions.

2- primers:

The primers were designed by (**Ravinder** *et al.*, **2014**) for RT-PCR amplification of PAMV RNA based on the alignment of coat protein (CP) gene sequences of identified PAMV strains obtained from the NCBI website (GenBank).

The primer set of PAMV-F1 and PAMV-R were designed to amplify the entire CP gene of PAMV RNA 3 (Table 1).

Table 1: Two primers used in this study for detection of PAMV using RT-PCR.

Primer pairs	Primer sequence	Location in genome*	Product size (bp)
PAMV-F1	5'-ACCCAAGCGTTCCTATATACTC -3'	5176-5197	
PAMV-R2	5'-GGTCAAATCATTGCCAGCAATC -3'	5514-5535	360 bp

^{*}nt number on accession #NC003632

3- cDNA synthesis

Two µg of RNA, and 1.5 µl of 10 µM of the complementary primers (PAMV-R2) were mixed in a sterile RNase-free microcentrifuge tube with nuclease-free water to a final volume of 15 µl. The tubes were heated to 70°C for 5 min, then cooled immediately on ice and spun briefly to collect the solution at the bottom of the tube. The following components were added to the annealed primer/template: 5 µl of 5X M-MLV reaction buffer [250 mM of Tris-HCl (pH 8.3), 375 mM of KCl, 15 mM of MgCl₂, and 50 mM of DTT (dithiothretol)], 2 µl of 10 mM deoxynucleoside triphosphates (dNTPs, 25 units of RNasin® ribonuclease inhibitor, and 200 units of M-MLV RT enzyme (Promega) and nuclease-free water to final volume of 25 µl. the tubes were mixed gently by flicking the tubes and incubated for 60 min at 37°C.

4- Polymerase chain reaction (PCR):

PCR amplification was performed in a final volume of 25 μ l as the following: 2.5 μ l of cDNA, 2.5 μ l of 2.5 mM of dNTPs, 2.5 μ l of 10X buffer, 2.5 μ l of 25 mM MgCl₂, 1 μ l of each forward and reverse sense primer at 10 μ M, 0.2 μ l *Taq* DNA polymerase, and water.

The temperature report of the PCR cycle was pre-incubation at 95°C for 5 min leading to 35 cycles of melting at 94 °C for 30 sec, annealing at different temperature for 30 sec and synthesis at 72 °C for 45 sec followed by an extension of 72°C for 7 min. The amplified products were analyzed by electrophoresis on 1 % agarose gel, stained with ethidium bromide solution alongside DNA Ladder. And visualized with UV illumination using Gel Documentation System (Gel Doc 2000 BIO RAD). The expected size of the PCR product was 360 bp for PAMV .

III-Susceptibility of some local and imported potato varieties to virus infection:

This experiment was carried out to determine the reaction of fifteen potato cultivars to virus infection. An isolate of PAMV which previously identified by ELISA from Menoufia Governorate, was used for inoculums preparation. The inoculums were prepared by grinding potato leaves collected from PAMV- infected plants, in 0.01 M Potassium phosphate buffer, pH 7.1 in a ratio 1:4. When the potato seedlings reached the 6-8 leaf stage, 300 mesh carborundum was dusted on five seedlings of each of the tested cultivars. The filtered inoculums were rubbed on the dusted leaves. Five other seedlings were inoculated with sap extracted from healthy potato leaves in the same buffer. Seedlings were kept in a greenhouse (25-30 °C) and observations were recorded throughout the four weeks of the experiment and tested by ELISA.

IV- Effect on chemical analysis of potato plants:

A- Photosynthetic pigments:

For measuring of photosynthetic pigments, i.e. chlorophyll a, b and carotenoids, a vegetative sample from the fifth leaf was taken and the pigments were extracted by 85% aqueous acetone according to (Fadeel' method, (1962). The absorbance was determined using Carl- Zeis Spectrocolourimeter at the wave lengths of 440, 644, 662 nm. The data of obtained results was statically analyzed according to **Snedecor and Cochran (1980).**

B-Total carbohydrate contents (%) were determined in tubers according to the method described by Dogras and Psomakelis, (1991).

C- Starch contents (%) was determined using the method of **Dogras and Psomakelis**, (1991).

RESULTS

I-Isolation:

Potato aucuba mosaic virus was isolated from naturally infected potato plants (Solanum tuberosum L. cv. Agria) grown in Menoufia governorate, Egypt. The diseased plants showed, yellow spots, necrosis, severe stunting and leaf deformation, Fig (1). Samples collected, were used to inoculate healthy potato plants and other indicator plants by mechanical inoculation under greenhouse conditions as described before and symptoms were developed after

different inoculation period. These symptoms were similar to those previously observed on naturally infected potato plants.

II-Identification:

A-Host range and symptomatology:

Twenty plant species belong to six botanical families were inoculated mechanically with virus isolate. The reaction of different plants to PAMV was classified to different categories according to their reaction (Table1 and Fig 1).

B- Mechanical inoculation:

Results showed that PAMV was readily mechanically inoculated by sap extracted from infected potato leaves, using fore finger with (carborundum 600 mesh and Phosphate buffer pH7.2) as abrasive as described before.

C- Aphid transmission:

Myzus persicae, Aphis crassivora and Aphis nerii were checked for ability to transmit the virus isolate. The results showed that three mentioned aphid species were found to be able to transmit PAMV, from infected seedling (Vigna unguiculata.L cvs Black eye) to healthy ones in a non-persistent manner by three species after 20-30 minutes acquisition feeding period. The percentages of transmission were 80, 73.3 and Tr.33%, respectively (Table 2).

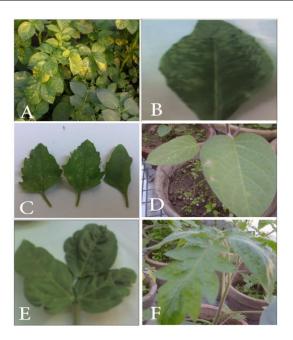


Fig (1):

Reaction of some plants to mechanical inoculation with the isolated virus: A: Yellow spotting, leaf deformation and severe stunting *Solanum tuberosum* L. cv. Agria. B. Mosaic on *Nicotiana tabacum* L C. Local lesions on *Chenopodium quinoa* L. D. Necrotic local lesions on *Datura stramonium* L. E. Local lesions and Leaf deformation on *Vigna unguiculata*.L cvs Black eye. F. Mosaic and blisters on leaves of *Lycopersicum esculentum* M.cv. Super strene B.

Table (1): Susceptibility and reaction of different plants to mechanical inoculation with the isolated PAMV, under greenhouse conditions

Host plant tested					ELISA	
Family	Scientific name	English name	Variety	Symptoms induced	test	
1-Amaranthaceae	Gomphrena globosa L.	Globeamaranth	_	NLL	+	
2.Chenopodiaceae	Ch album Ch.amaranticolor. Ch. quinoa. Wild	Ouares Lamb'S Gooses Foot	_ _ _	CLL CLL CLL	+ + + +	
3- Curcurbitaceae	Cucumis sativus L.	Cucumber	Balady	R	-	
4- Labiatae (Laminaceae)	Ocimum basilicum. L	Sweet basil	_	YM	+	
5- Leguminosae	Glycine max L. Medicago sativa L. Phaseolus vulgaris Pisum sativum L. Trifolium alexadrium Vicia fabae L. Vigna unguiculata	Soya bean alfalfa Common bean Garden pea Egyptian clover Broad bean Cowpea	Lee — Giza 6 Mister B — Balady Black eye	R Y &M LC & M M&VC Y &M BLL & SN CLL &LD	- + + + + +	
6- Solanaceae	Datura stramonium L	Jimson-weed	_	M&MO	+	

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Capsicum annum L.	Pepper	_	M&MO	+
Lycopersicum esculentum M.	Tomato	Calif.wander	SM& LD	+
Nicotiana tabacum L.	Tobacco		LD & M	+
Nicotiana glutinosa L.	Wirginia plant	White-Burly	M	+
Nicotiana debneyi L.	_	_	LL&M	+
Solanum nigrum L.	Solanum	_	RS&M	+

Abbreviation of symptoms:

BLL = black local lesions LC = leaf curling RS = ring spots

CLL = chlorotic local lesion M = mosaic SM = severe mosaic

LD = leaf deformation NLL=necrotic local lesions SN = stem necrosis

MO = mottling N = necrosis VC = vein clearing

Y = yellowing symptoms YB = yellow blotches YM = yellow mosaic

R = Resistance + = Positive reaction - = Negative reaction

Table (2): Insect transmission ability of PAMV by three aphid species.

Aphid specie	No. of inoculated plants	No. of infected plants	Transmission (%)
Myzus persicae (Sulz)	15.0	12.0	80.0
Aphis crassivora (Koch)	15.0	11.0	73.3
Aphis nerri (Boyer)	1°.0	°.0	rr.rr

D- DAS ELISA:

The virus was detected by DAS-ELISA test, positive reaction obtained with the specific antiserum.

E-Inclusion bodies

Epidermal strips of both healthy and infected tobacco (*N. tabacum* L.cv.White Burley) plants with PAMV, were stained with mercuric bromophenol blue and examined using light microscope. Examinations revealed the presence of cytoplasmic amorphous inclusion in infected cell (Fig. 2).

F- Electron microscopy:

In ultrathin sections of potato plants (*Solanum tuberosum* L.cv. Agria), leaf cells infected with showed major cell organelles like chloroplast was erratic. The chloroplast of potato leaf cells infected with PAMV were suffer severe destructure of the lamellar system with starch granules (arrow), deformations and wrinkles in cell wall and Swollen stroml thylakoids (arrow) (Fig. 2).

G- Amplification of the RT-PCR:

RT-PCR amplification of viral RNA was carried out on the total RNA isolated from infected Potato plants. Electrophoresis investigation of RT-PCR product showed a single amplified fragment of ~360 bp and no fragments were amplified from the RNA extracted from symptomless or healthy plants. Electrophoresis analysis of RT-PCR product showed that amplified fragment of ~ 360 bp was obtained from coat protein gene of PAMV (Fig. 2).

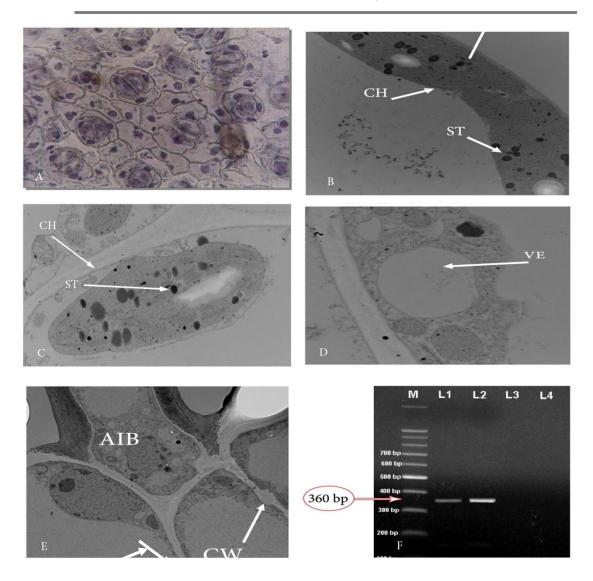


Fig (2): **A**: Light microscopy of epidermal cells from infected White Burley tobacco leaf with PAMV stained with mercuric bromophenol blue, showed amorphous cytoplasmic inclusion bodies (AIB).**B**. Transmission of electron micrograph of mesophyll cell of potato leaves (cv. Agria) infected with PAMV, showed disorganized (ch) with starch granules . **C& D**: Transmission of electron micrograph of mesophyll cell of potato leaves (cv. Agria) infected with PAMV, showed the cytoplasm is highly vesiculated

with amorphous inclusions.**E:**.Transmission of Electron micrograph of mesophyll cell of potato leaves (cv. Agria) infected with PAMV, showed deformations and wrinkles in cell wall with amorphous inclusions .**E:** Agarose gel electrophoresis analysis of RT-PCR amplified products. M: 100 bp DNA ladder; L1, L2: two Potato plants infected with PAMV; L3, L4: healthy plant sample as –be control.

III- Susceptibility of some local and imported potato varieties to virus infection :

Seedling of all potato cultivars inoculated with the sap prepared from PAMV-infected plants showed virus symptoms within two to three weeks post inoculation. These symptoms varied from irregular bright yellow blotch on leaves followed by black necrosis which spread internally into the leaflet veins and petioles, to stem necrosis and sometimes the plants die. Only thirteen potato cultivars infected showed virus symptoms and given positive reaction in ELISA test after mechanical inoculation with PAMV. While two cultivars showed symptoms and given negative reaction in ELISA test (Table 3).

Table (3): Viral susceptibility using local and imported varieties of potatoes.

Potato Cultivar	ELISA	Symptoms	Potato cultivars	ELISA	Symptoms
1-Alpha	+	M & D	10- Hermes	-	M
2- Anova	-	SID	11-Lady rosetta	+	N+D
3- Berne	+	YS	12- Nicola	+	YS& D
4-Diamant	+	N+C	13- Mondial	+	SID &M
5- Cara	+	SM	14- Nicola	+	M
6- Christal	+	M	15- Spunta	+	LD
7- Falour	+	M+D			
8- Gala	+	YS			

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Abbreviation of symptoms and ELISA:

+ : Positiv reaction M: Mosaic

-: Negative reaction N : Necrosis

LD: Leaf distortion SID: Slight distortion

C : Crinkle SM: Severe mosaic

YS: Yellow spot

IV. Viral Effect on Physical and chemical analysis of potato plants: A- Photosynthetic pigments:

Obtained data in table (3) revealed that the virus damaged the photosynthetic pigments, i.e. chlorophyll a, chlorophyll b and carotenoids, that determined in fresh leaf samples of potato, compared with healthy potato leaves.

B-<u>Total carbohydrate contents</u>:

Results in table (3) showed that viral infection reduced the concentration of total carbohydrate by about (41.33%) compared with healthy potato plants .

C- Starch contents:

Data presented in table (3) showed that viral infection increased the concentration of starch by about (12.810%) compared with healthy potato plants (14.471 %).

Table(3): Effect of PAMV infection on some photosynthetic pigments and some chemical compounds in potato under field conditions.

Characters	Chlorophyll A (mg/g d.w)	Chlorophyll B (mg/g d.w)	Carotenoids (mg/g d.w)	Total Carbohydrates (%)	Total Starch
Healthy	2.110	1.713	0.905	50.480	12.810
Diseased	0.772	0.646	0.403	30.620	14.471

DISCUSSION

Potato aucuba mosaic virus (PAMV) was isolated from naturally infected potato plants (Solanum tuberosum L.) grow in different locations in Menoufiagovernorate in Egypt. This virus was isolated before from obviously infected potato plants by (Awad, et al.,1985 and Gamal Eldin et al.,2005).

PAMV has a great wide host range, it can naturally infect many herbaceous and some woody plant hosts (150 species in 22 families) and is transmissible to over 430 species of 51 dicotyledonous family (**Kollmer and Larson, 1960**).

Host range and symptomatology studies showed that several hosts were reacted with local symptoms on the new leaves. These hosts includes: *Chenopodium amaranticolor*, *Ch. quinoa*, and *Ch. album*. These results were also reported by (Kollmer and Larson, 1960; Box, 1975; Awad, et al.,1985 and Gamal El-din et al.,2005).

Hosts reacted with systemic symptoms, these systemic symptoms varied from mosaic, mild to severe mottle, chlorosis, chlorotic ring spots, leaf deformation and stunting, these hosts are, *Ocimum bacilicum, Phasiolus vulgaris, Pisum sativum, Trifolium alexadrium* L., *Glycine max L., Vicia fabae* L, *Vigna unguiculata* L., *Datura stramonium* L., *Capsicum annum* L., *Lycopersicum esculentum., Nicotiana tabacum* L., *Nicotiana glutinosa* L., *Nicotiana debneyi* L. and *Solanum nigrum* L. (Awad, *et al.*,1985 and Gamal El- din *et al.*,2005).

Hosts were appeared no reaction, one of these hosts was *Cucumis* sativus. Similar results were also reported by other authors, (**Awad**, et al.,1985).

The isolated virus easily transmitted by mechanical means. These results agreed completely with those reported for PAMV by (Mossop 1982; Awad, et al.,1985; Gamal El- din et al.,2005 and Fox, et al., 2016).

Regarding to data of insect transmission, indicated that *Myzus* persicae was able to transmit PAMV efficiently in the non-persistent manner with the highest rate (80%) .While *Aphis crassivora* (73.3%) and *Aphis nerri* (20%).This result is similar to that obtained by (**Yiannis and Bryan**, (1993). While *Aphis nerii* could not transmit the virus, this result is similar to that obtained by (**David** *et al.*, (1993).

Using light microscope, inclusion bodies were observed in epidermal strips of tobacco White Burley leaves infected with the isolated virus. Infected samples reacted positively to mercuric bromophenol blue. This result indicated that these amorphous inclusions are protein in nature. This result is in harmony with those recorded by (Gamal El-din et al., (2005).

The electron micrograph of PAMV in this study, revealed the significal changes of the structure of chloroplasts and formation of virus – induced inclusions in them are observed. They occurred large zones of cytoplasm, that is a typical sign of PAMV infection. This result was in agreement with that of (Mikrobiol, (2007).

The present study evidenced the successful use of RT-PCR to detect PAMV in total nucleic acid extracts from infected potato plants, only one day is required for positive identification of the virus from infected tissue, amplified products for PAMV was established as follows: The size of the major product from PAMV-infected tissue was identical to that of the 360 bp from the CP gene of PAMV; the specific primers did not amplify viral cDNA from extracts of uninfected potato plants. Two PAMV CP gene specific primers sets (PAMV-1F and PAMV-R1) in this study, were designed and were highly specific, sensitive and useful in RT-PCR for detection and amplifying PAMV CP gene sequence from total RNAs extracted from potato leaves. Both primers were designed by (Ravinder et al., (2014) with specificity to PAMV RNAs and did not produce amplicons from potato leaves infected with several other viruses or a viroid.

Susceptibility of fifteen local and imported potato varieties to virus infection were studied for susceptibility to infection with PAMV. Only thirteen potato cultivars infected showed virus symptoms and given positive

reaction in ELISA test after mechanical inoculation with PAMV. While two cultivars showed symptoms and given negative reaction in ELISA test. Similar results were obtained and described by (Awad, et al., (1985).

Present study indicated that PAMV infection reduced some of photosynthetic pigments in potato leaves, i.e. chlorophyll A, B, carotenoids and total chlorophyll compared with healthy potato plants. The obtained results are similar to that obtained by (Subba et al., (1979) and Gamal Eldin et al., (2005). Present results indicated that PAMV decreased the total carbohydrates and starch content in potato tubers, compared with healthy potato plant. This decrease was due to viral infection caused destruction of chlorophyll pigments in infected leaves and consequently in the process of photosynthesis and the result was the lack of the content of total carbohydrates in infected plants. The obtained results are similar to those obtained by (Subba et al., (1979).

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عزل وتعريف فيروس موزيك أوكيوبا البطاطس من نباتات بطاطس مصابة طبيعيا في مصر

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الملخص العربى:

تم عزل فيروس موزيك أوكيوبا البطاطس من نباتات البطاطس (صنف أجريا) المصابه طبيعيا في الحقل من أماكن مختلفة من محافظة المنوفيه ولوحظت أعراض متفاوتة من بقع صفراء وتجعد أوراق وتقزم وبقع ميتة علي الدرنات. ثم عرفت هذه العزلة باستخدام الطرق البيولوجيه (الأعراض -المدى العوائلي والعوائل المشخصه) وطرق النقل (ميكانيكي وحشري) وأيضا تم التعريف بإستخدام الطرق السيرولوجيه مثل (طريقة الإليزا المباشرة) وكذلك تم التعريف بإستخدام الميكروسكوب الضوئي والإلكتروني . تم إختبار حساسية ١٥ صنف من نباتات البطاطس تجاه الإصابة بهذا الفيروس. حيث وجد ١٣ صنف ظهرت عليهم أعراض و أعطوا نتيجة موجبة مع إختبار الإليزا. بينما صنفان ظهرت عليهم الأعراض وأعطوا نتيجة سالبة مع الإليزا. أدت الإصابة بفيروس أوكيوبا البطاطس الي تقليل بعض الصبغات في الأوراق مثل (كلوروفيل أ و ب وكذلك الكاروتينات) وأدت الإصابة أيضا الي خفض تركيز الكربوهيدرات الكلية وزيادة تركيز النشا داخل درنات البطاطس المصابة.