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Biological and Molecular Studies on an Egyptian Potyvirus Isolate from *Hyocyamus muticus* L.

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A NEW isolate of potyvirus was isolated from naturally-infected henbane plants *(Hyocyamus muticus* L.) collected from the farm of Faculty of Pharmacy, Cairo University in January 2013. The naturally infected henbane plants exhibited severe mosaic, rugosity, blistering and malformation. Thermal inactivation point, dilution end point and longevity *in vitro* of virus isolate were found to be 65 ºC, 10^{-4} and 4 days, respectively. Electron micrograph of partial purified virus negatively stained with 2% phosphotungestic acid revealed the presence of filamentous viruses with size 1440 X14.3nm. Total RNA was extracted from infected henbane plant. Comparative nucleotide sequence analysis for virus showed a high degree of similarity (62 %) with four potyviruses accessions (EU482153: Potato virus Y isolate Foggia, KF850513: Potato virus Y isolate M3, AM184113: Henbane mosaic virus partial gene for polyprotein-PHYS/H isolate, AY166867: Potato virus Y strain N isolate). The virus isolate was published in GenBank with accession number KM497011.

Keywords: Potyvirus, Electron microscopy, Comparative Nucleotide sequence.

Potyvirus is the largest plant viruses genus causing significant losses in a wide range of crops (King *et al*., 2011; Revers and García, 2015) that is accounted for 40% of losses (Larsen *et al*., 2003; Yamamoto and Fuji, 2008). Horvath *et al*. (1988) showed that HMV infection has increased due to increasing the populations of *Datura stramonium* due to their application of nitrogen-containing fertilizers. Saha *et al*. (1997) reported that a mechanically transmissible filamentous virus causing severe mosaic with leaf blisters and malformation of *Datura metel* was identified as a strain of henbane mosaic potyvirus (HMV-Da) depending on its host range and electron-microscopy. This virus was restricted to species of *Solanaceae* and induced systemic symptoms in *Nicotiana* spp. and *Hyocyamus niger* but it was symptomless to *Lycopersicon esculentum, Capsicum annuum* and *Solanum* spp. In addition, HMV-Da particle was measured as 795 X 12 nm.

The genes of potyviruses that encoding a different proteins such as N terminal protein, helper component protease, protein P3, 6KD protein, cytoplasmic

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inclusion protein, 6KD protein 2, genome-linked protein, nuclear inclusion protein A, nuclear inclusion protein B and coat protein (Riechmann *et al*., 1992). Shukla *et al.* (1994) found that the genome of potyviruses is positive sense single stranded RNA of ~ 10000 nts with a VPg (virus protein genomic linked).

This investigation was carried out to identify a new Egyptian potyvirus isolated from naturally-infected *Hyocyamus muticus* L. plant showing mosaic, leaf blistering and malformation symptoms. Also, host range, particle size and morphology; *in vitro* properties as well as partial sequencing of the virus isolate were studied.

Materials and Methods

Source of the virus isolate

Fifteen infected henbane plants were collected in January 2013from the Experimental Farm of Faculty of Pharmacy, Cairo University. The plant samples were kept in sterile plastic bag then maintained at -20ºC for further study.

Detection of virus isolate by diagnostic plants

Three replica of each diagnostic plant for virus infecting henbane (*Chenopodium amaranticolor*, *Ch. quinoa, Cucumis sativus, Datura stramonium, Nicotiana glutinosa, N. rustica, N. tabacum* cvs. White Burley and Samsun, *Solanum demissum and S. tuberosum*) were selected according to Smith (1972). The diagnostic plants were grown under greenhouse conditions at Botany and Microbiology Department, Faculty of Science, Helwan University, Cairo, Egypt. One gram of naturally infected- henbane leaves was ground as a source of virus. The diagnostic plants were inoculated. Controls of corresponding plants were inoculated with the extraction buffer only. The inoculated plants were kept at moderate temperature (25-28ºC) in an insect proof greenhouse until external symptoms appeared.

Virus isolation and propagation

Infectious crude sap prepared from the infected henbane plants which gave positive results with diagnostic hosts and then mechanically inoculated on *S. demissum* for developing single local lesion. The local lesion produced was ground and used for the inoculation on healthy *H. muticus* seedlings leaves as a propagative host. The inoculated *H. muticus* plants were kept in an insect proof greenhouse until external symptom appeared.

Biological characters

Host range and symptomatology

Twenty seven seedling plants belonging to 6 families (*Asteraceae, Chenopodiaceae, Crucifrae, Cucurbitaceae, Fabaceae,* and *Solanaceae)* were maintained in an insect proof greenhouse. Plants were mechanically inoculated with virus-infected sap. The inoculated plants were kept in an insect proof greenhouse conditions until symptoms appeared.

In vitro properties

In vitro properties of the isolated virus [thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV)] were performed on leaves of *S. demissum* by using infectious crude sap obtained from virus infected *H. muticus* plants. The inoculated plants were kept in an insect proof greenhouse.

Partial purification of virus isolate: Virus purification was done according to modified method of Steere (1956). One hundred gram of fresh infected henbane leaves were homogenized in 0.1 M phosphate buffer pH 7.0 containing mercaptoethanol and clarified by adding cold n-butanol: chloroform (1:1). The upper aqueous phase was centrifuged at 6000 rpm for 30 min. The clarified supernatant was collected, concentrated by 4% NaCl and polyethylene glycol and then ultra-centrifuged at 40.000 rpm for 90 min. The pellet was suspended in phosphate buffer pH 7 and centrifuged at 6000 rpm for 20 min. Pellet was discarded and the resulted supernatant was the partially purified virus.

Calculation of virus concentration: Partially purified virus preparations were measured at range 200-300 nm wave length using ultraviolet spectrophotometer (Jasco, Model V-630, serial Noc285061148, Physics lab, Faculty of Science, Helwan University) in order to evaluate purity and estimate the concentration of the partially purified virus using equation as mentioned by Noordam (1973).

Virus concentration was estimated spectrophotometrically using an extinction coefficient of a potyvirus 2.5 (Saha *et al*., 1997).

Virus morphology: The partial purified virus particles were negatively stained by 2% phosphotungestic acid pH 6.8. The grid air dried then was examined using Jeol-Jem 1010 Transmission Electron Microscope (TEM), The Regional Center of Mycology and Biotechnology, Al-Azhar University.

Molecular characterization

Primer selection

Three oligonucleotide potyvirus primers were designed according to the coat protein nucleotide sequences of potyvirus published in National Center for Biotechnology Information (NCBI). The potyvirus primers were HMV [30F20 (+)5'-ACC ACT GAA GCA AAC CGA GA-3'& 788R20 (-)5'-CAT CTG GCG AAC ACC TAG CA-3'], Potato virus X (PVX) [87F22 (+)5'-CAG GGC TAT TCA CCA TAC CAG A-3' & 652R20 (-)5'-TTC CTG TGA TGC GGC CCC TA-3'] and Potato virus Y (PVY) [21F20 (+)5'-GCA GGA GGA AGC AAC AAG AA-3'& 734R22 (-)5'-GGT GGT GTG CCT CTC TGT GTT C-3'].

Total RNA extraction

The total RNA was extracted from 40 mg of fresh tissue of virus infected henban*e* leaves using Gene JET RNA Purification Kit (Thermo Scientific #K0731) with 300µl Lysis Buffer.

Synthesis of cDNA (RT-PCR)

RT-PCR was performed by RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific K1621) according to Malek *et al.* (2000).

Amplification of cDNA

To perform specific PCR amplification for the virus coat protein sequence RNAs, the primer set of HMV, PVX and PVY based on conserved and virus-specific sequences to amplify ~778 bp, 585 bp and 735 bp fragments respectively from coat protein length virus RNA. PCR was performed by Dream Taq DNA Polymerase kit (Thermo Scientific EP0702). The amplification was carried out using Veriti 96-Well Thermal Cycler. The initial denaturation cycle of the DNA was performed at 95°C for 5min followed by 35 cycles of denaturation at 95°C for 45 sec, annealing at 56°C for 45 sec for HMV reaction, 59.4 °C for PVX reaction and 56.3 °C for PVY reaction. The extension was done at 72°C for 60 sec while a single trailing cycle of long extension at 72°C for 10 min was carried out in order to ensure flush ends on the DNA molecules. Finally, the amplified DNA was electrophoresed on 1 % agarose gel and photographed using gel documentation system.

Sequencing

The purified PCR product was sequenced in two directions using high throughput Applied Biosystems 3730XL sequencers at Macrogen sequencing service, Korea.

Similarity and alignment analysis

The resulting nucleotide sequence of DNA was analyzed by using DAMAN-version 5.2.9. The phylogenic relationship and alignment analysis of viral coat protein gene sequences were compared to those of the GenBank and EMBL databases by advanced BLAST (Megablast) searches from the National Center for Biotechnology Information (NCBI).

Results

Detection of the virus isolate by diagnostic hosts

Fifteen samples from naturally infected henbane plants (*Hyocyamus muticus* L.) exhibited viral symptoms on their leaves including severe mosaic, rugosity, blistering and malformation (Fig.1). These samples were mechanically inoculated on diagnostic hosts for viruses- infecting henbane and showed chlorotic local lesion with necrotic center appeared on inoculated leaf of *Chenopodium amaranticolor*, mosaic symptoms appeared on *Datura stramonium* and *Nicotiana tabacum* cv. White Burley. *N. tabaccum* cv. Sumsun showed necrotic lesions then systemic mosaic appeared on *N. glutinosa*. Chloresis then leaf malformation appeared on *N. rustica*, necrotic local lesions appeared on *S. demissum and Solanum tuberosum*. Moreover, no symptoms appeared on *Ch. quinoa and Cucumis sativus*.

Fig.1. ***Hyocyamus muticus*** **L., (A) healthy and (B) naturally-infected plant showing severe mosaic and blistering.**

Isolation and propagation of virus isolate

The virus isolate was inoculated into *Solanum demissum* plant. After 6 days post-inoculation, necrotic local lesions (pin point) appeared. Single local lesion was separated and used for inoculation of healthy *S. demissum* plant. The same type of local lesion appeared. These local lesions were used to inoculate *Hyocyamus muticus* L. as a propagative host. After 1 week post-inoculation, vein clearing and mild mosaic appeared then turned into severe mosaic after 2 weeks (Fig. 2).

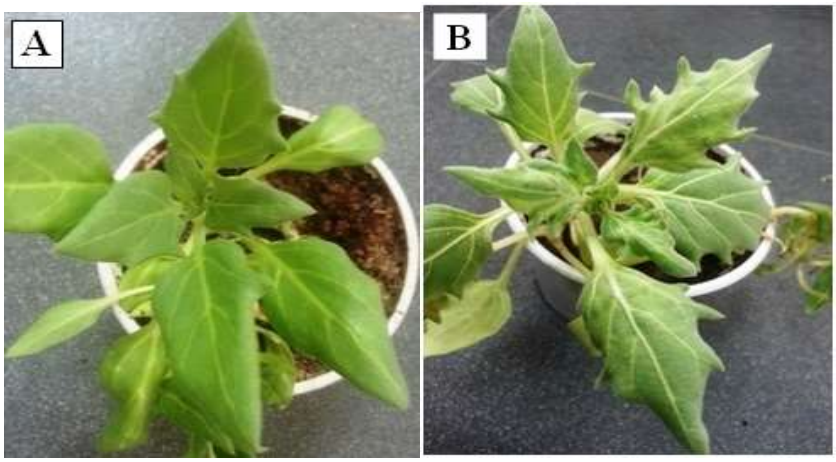


Fig 2. ***Hyocyomus muticus*** **L., (A) healthy and (B) infected- plant showing sever mosaic symptoms**

Host range and symptomatology

Virus isolate was tested on 27 plant species belonging to 6 families: *Asteraceae, Chenopodiaceae, Crucifrae, Cucurbitaceae, Fabaceae,* and *Solanaceae.*

From tested plants, 14 species reacted with different responses with virus isolate. These plants were *Capsicum annuum, Chenopodium amaranticolor, Datura metel, D. stramonium, H. muticus, Lycopersicon esculentum, Nicotiana glutinosa, N. rustica, N. tabaccum* cv. Kntaky, *N. tabaccum* cv. White Burely, *N. tabaccum* cv Samaun, *Petunia hybrida, Solanum demissum* and *S. tuberosum*as shown in Table 1. While the other 13 species exhibited no symptoms; these plants were: *Brassica alba, B. nigra, Ch. quinoa Cucumis sativus, Cucurbita pepo, Glycine max, N. tabaccum* cv. Kg23, *N. tabaccum* cv. Italy, *N. glauca, Phaseolus vulgaris, Vicia faba*, *Vign aunguiculata* and *Zinnia elegans.*

TABLE 1. Symptoms produced by virus isolate on different hosts.

Plant family	Plant species	Symptoms
Chenopodiaceae	*Ch. amaranticolor*	Circular chlorotic local lesions with necrotic center appeared after 4 days post-inoculation.
Solanaceae	***C.*** *annuum*	Vein necrosis appeared after one week post-inoculation then crinkle and malformation after 2 weeks developed.
	D. metel	Necrotic local lesions after 5 days post-inoculation appeared then crinkle and systemic mild mosaic appeared on new leaves after two weeks post-inoculation. Finally after 3 weeks, it was turned into severe mosaic, blistering and malformation.
	D. stramonium	Systemic mosaic symptoms developed after 10 days post-inoculation.
	H. muticus	Vein clearing, mild mosaic appeared after 1 week post-inoculation then turned into sever mosaic after 2 weeks.
	L. esculentum	Chlorotic local lesions appeared after 5 days post-inoculation then mosaic and crinkle developed after 3 weeks.
	N. glutinosa	Necrotic local lesion appeared after 1 week post-inoculation. Then systemic mosaic symptoms, leaf malformation and stunting developed after 2 weeks.
	N. rustica	Chlorosis appeared after 12 days post-inoculation then developed to crinkle and malformation after 3 weeks.
	N.tabaccum cv. Kntaky	Mild mosaic appeared after 3 weeks post-inoculation.
	N. tabaccum cv. White Burely	Systemic mosaic symptoms and crinkle appeared after 2 weeks post-inoculation.
	N. tabaccum cv. Samaun	Systemic mosaic symptoms appeared after 20 days post-inoculation.
	P. hybrida	Mild mosaic appeared after 3 weeks post-inoculation.
	S. demissum	Necrotic local lesions appeared after 6 days post-inoculation.
	S. tuberosum	Necrotic local lesions appeared after 5-6 days post-inoculation.

In vitro properties

Infectious crude sap that extracted from infected *H. muticus* L. leaves was used to determine the properties of the isolated virus. The thermal inactivation point (TIP), dilution end point(DEP) and longevity *in vitro* (LIV) at room temperature (25-28°C) of the isolated virus were found to be 65°C, 10^{-4} and 4 days respectively.

Ultraviolet extinction spectra of partial purified virus

The concentration of virus preparation was estimated, it was 0.77mg / 100g fresh leaves using spectrophotometric measurements at 260 nm. The absorption ratio A260 / A280, A280 / A260 and Amax /Amin were 1.093, 0.914 and 1.072 respectively as represented in Table 2.

TABLE 2. Absorption spectrum of partial purified virus isolate .

Amax (nm) at	Amin (nm) at	A260 / A280	A280 / A260	Amax/Amin	Yield mg / 100g
267	249	1.093	0.914	1.072	0.77

Morphological characters

Electron microscopic examination of partial purified preparation of virus isolate demonstrated the presence of flexuous filamentous virions with 1440 nm long and 14.3 nm wide as shown in Fig. 3.

Fig 3. TEM micrograph of partial purified virus showing long filamentous virion negatively stained with 2% phosphotungestic acid. Bar represents 100 nm, 80.000 X.

Molecular characterization of virus isolate

PCR amplification of the coat protein gene of the virus isolate

The total RNA prepared from infected henbane leaf was reverse transcribed by RevertAid First Strand cDNA Synthesis Kit and minus sense of each three different primers for coat protein gene of henbane mosaic virus (HMV), Potato virus X (PVX) and Potato virus Y (PVY). The resulting complementary DNA

(cDNA) was amplified by adding each forward (+) specific primer. Only HMV primers produce amplified PCR product as shown in Fig 4. The amplified cDNA was in the expected size calculated (~778 bp). The authenticity of the resulting PCR product (~778 bp) was verified by DNA sequencing after purification of DNA fragment from agarose gel using rapid and efficient kit.

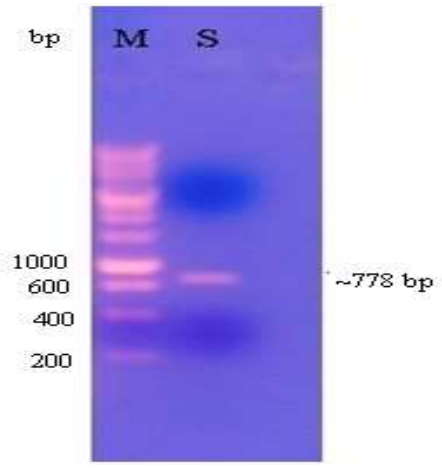


Fig. 4.1% agarose gel electrophoresis showing the PCR product of isolated virus coat protein gene using henbane mosaic virus specific primers forward (30F20) & reverse (788R20). Total RNA was extracted from henbane leaves infected with virus isolate. The right arrow pointed to the amplified PCR product (~778 bp). Lane 1, Molecular weight DNA (~200 bp ladder) and Lane 2, amplified PCR product(S).

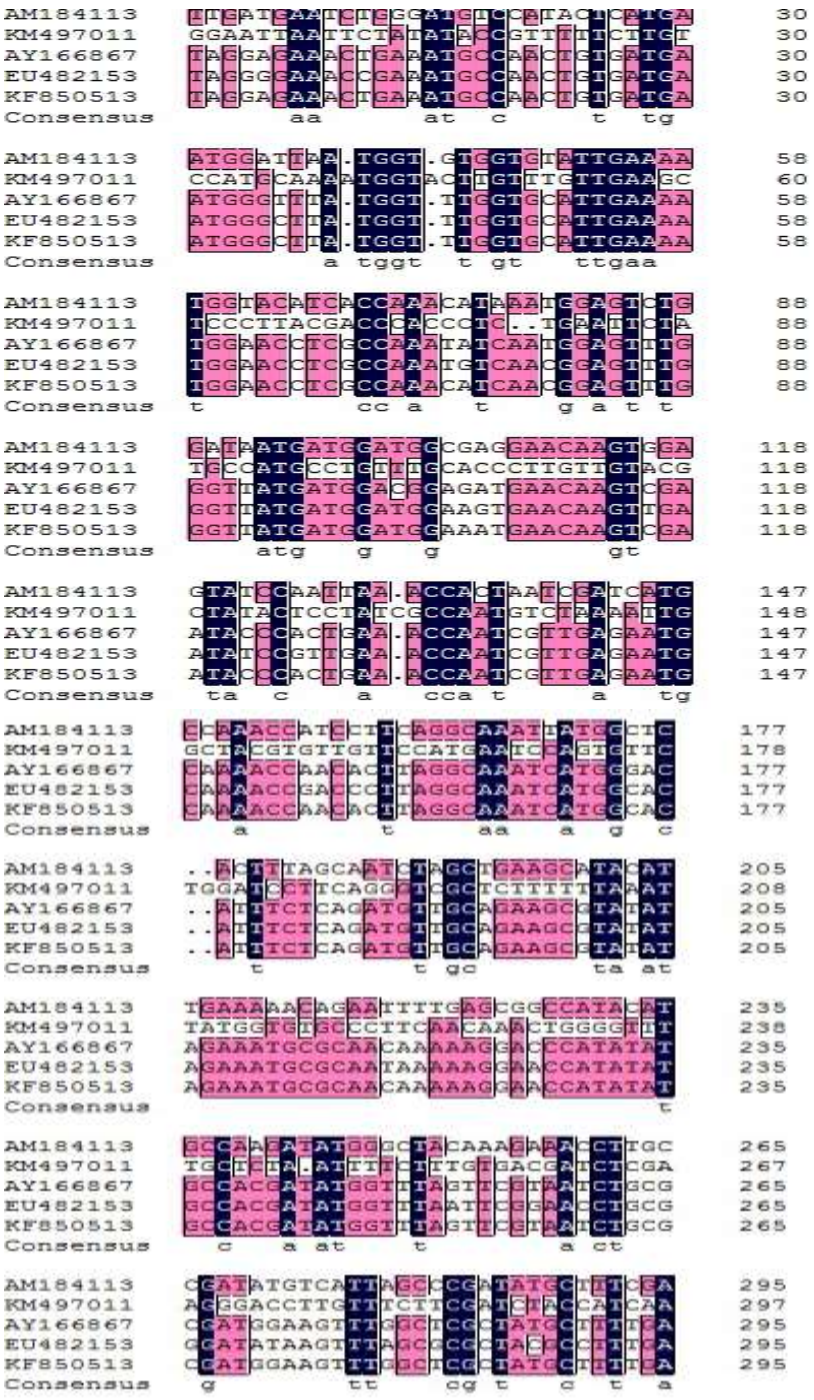
Automated DNA sequencing.

Partial nucleotide sequence of the virus coat protein gene of the current study revealed a size of 778 bp. The 778 bp was aligned with four published sequences of potyviruses:

1. AM184113 (Hungary-isolate) Henbane mosaic virus partial gene for polyprotein-PHYS/H isolate was reported by Salamon *et al*. (2008).
2. AY166867 (American-strain) Potato virus Y strain N isolate was reported by Nie & Singh (2003).
3. EU482153 (Foggia-isolate) Potato virus Y isolate Foggia was reported by Mascia *et al*. (2010).
4. KF850513 (M3-isolate) Potato virus Y isolate M3 was reported by Quintero-Ferrer *et al*. (2014) as shown in Fig 5A.

In the present study, the Egyptian potyvirus isolate under study was published in GenBank under accession number KM497011.

Moreover, the homology tree of partially sequenced coat protein gene (KM497011) revealed relatively high degree of similarity (62 %) with the previous four potyviruses. Multiple sequencing alignments were generated using DAMAN-version 5.2.9 (Fig. 5B).


AM184113
KM497011
AY166867
EU482153
KF850513
Consensus


AM184113 324
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AM184113 354
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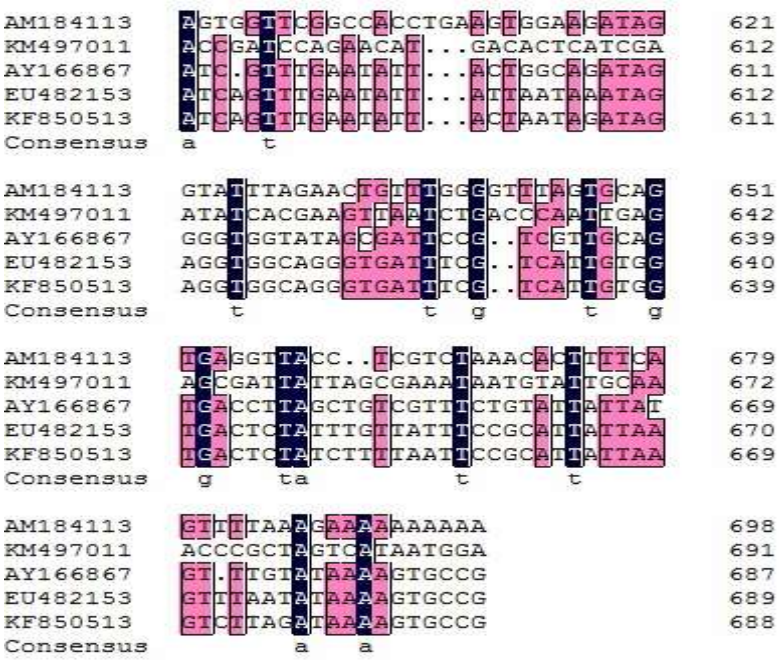

Fig. 5A. Multiple sequence alignment of partial CP gene sequence of the current studied virus (KM497011) with four different isolates of potyviruses. Accessions numbers indicated above were as following: AM184113: Henbane mosaic virus partial gene for polyprotein-PHYS/H isolate, AY166867: Potato virus Y strain N isolate,EU482153: Potato virus Y isolate Foggia, KF850513: Potato virus Y isolate M3.

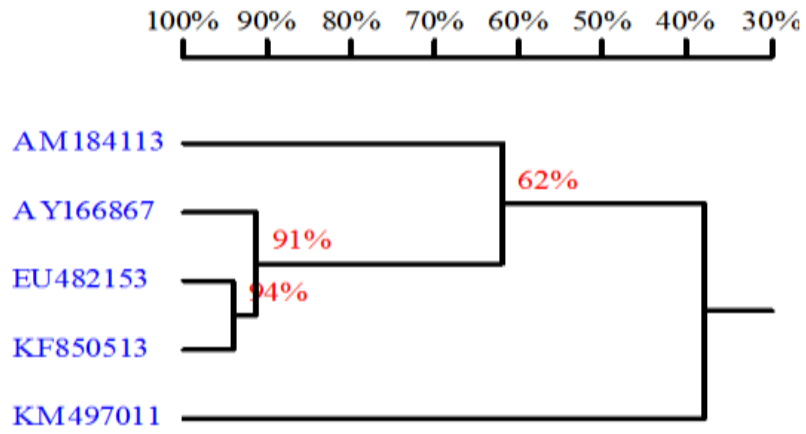

Fig 5 B. Homology tree of partial CP gene sequence of the virus (KM497011) with a previous four different isolates of potyviruses.

The homology tree of KM497011 revealed some degree of similarity (~62%) with other four potyviruses isolates.

Discussion

The viruses of the *Potyviridae* infect wide range of economical crops (Edwardson, 1974). Severe mosaic, rugosity, blistering and malformation exhibited on the leaves of naturally infected henbane plants (*Hyocyamus muticus* L.).Fifteen diseased samples were collected in January 2013 from the Experimental Farm of Faculty of Pharmacy, Cairo University

Diagnostic susceptible hosts for viruses-infecting henbane were *Chenopodium amaranticolor* (chlorotic local lesion with necrotic center), *Datura stramonium*, *Nicotiana tabacum* cv. White Burley and *N. tabaccum* cv. Sumsun (mosaic symptoms), *N. glutinosa* (necrotic lesions followed by systemic mosaic), *N. rustica* (chloresis followed by leaf malformation), on *Solanum demissum* and *S. tuberosum* (necrotic local lesions). No symptoms appeared on *Ch. quinoa and Cucumis sativus.* These results are in agreement with those obtained by Lovisolo and Bartels (1970) and Salamon (1989) who found that the diagnostic susceptible host species for HMV were *D. stramonium* (severe mosaic), *N. glutinosa, N. tabacum* cvs. White Burley and Samsun (necrotic local lesions and then mosaic) while diagnostic insusceptible host species were *Amaranthus* ssp., *C. sativus* and *Ch. quinoa*. Also, Delgado-Sanchez and Grogan (1970) found that potato virus y may be confused with tobacco etch and henbane mosaic viruses, which produce somewhat similar symptoms and have similar host ranges. Tobacco etch virus infects *D. stramonium* and produces necrotic wilting of Tabasco pepper and etching of the tobacco leaves. Henbane mosaic virus occurs less frequently in the common hosts of tobacco etch and potato Y viruses; it can infect *D. stramonium* but not Tabasco pepper. While, Purcifull and Edwardson (1981) reported that the potato virus X infection for *D. stramonium* in the form of small chlorotic rings followed by mottling, veinal chlorosis or veinal necrosis but Salamon (1989) found that henbane mosaic virus infect *D. stramonium* in the form of severe mosaic. As far as the authors are aware, the obtained results are the first record for Egyptian potyvirus isolate from *H. muticus*. Hamilton (1932) and Horvath *et al.* (1988) isolated HMV from *D. stramonium* showing wilt disease. Govier and Plumb (1972) isolated HMV from *Atropa belladonna* L., *D. inermis* L., *D. stramonium* L., *Hyoscyamus niger* L., *N. tabacum* L., *Physalis alkekengi* L. and *S. dulcamara* L. plants.

Walkey (1991) reported that the importance of host symptom to applied plant virologist. The field symptoms give the first indication to identity of virus and in the laboratory the symptoms produced in a host plant range may be of considerable value. The nature and the severity of disease symptoms will determine the economic importance of a virus, in the terms of reduced quality and yield loss.

Twenty seven species belong to six different families were used to determine the host range of the isolated virus using mechanical inoculation. The obtained results showed that 14 species reacted with different responses with virus isolate belonging to two families (*Chenopodiaceae* and *Solanaceae*). These results are in agreement with those obtained by Saha *et al.* (1997), contrary to our results Horvath *et al.* (1988) and Saha *et al.* (1997) could not detect HMV on *Capsicum annuum* and Saha *et al.* (1997) not detected HMV on *Lycopersicon esculentum* and *Solanum* spp. While, in the present study, virus isolate produce no symptoms on 13 plants belonging to six families *(Asteraceae, Crucifrae, Chenopodiaceae, Cucurbitaceae, Fabacea and Solanaceae)*.These plants were *Brassica alba, B. nigra, Ch. quinoa, C. sativus, Cucurbita pepo, Glycine max, N. tabaccum* cv. Kg23, *N. tabaccum* cv. Italy, *N. glauca, Phaseolus vulgaris, Vicia faba, Vigna unguiculata* and *Zinnia elegans.* These results were disagreed with Horvath *et al.* (1988) who detected HMV on *Ch. quinoa, C. sativus and Phaseolus vulgaris.*

Matthews (1991) reported that stability of the virus TIP, DEP and LIV as measured by infectivity (often in crude extracts) was an important criterion in attempting to establish group of viruses, control virus distribution and elimination of viruses both *in vitro* and open fields. In the present study, TIP, DEP and LIV of virus isolate were 65°C, 10^{-4} and 4 days respectively. These results were relatively in agreement with those reported by Sheikh *et al.* (2012) recorded that a TIP of a potyvirus has been isolated from *Wedelia trilobata* plants in Aligarh district of state Uttar Pradesh (India) was between 55°C -60°C, DEP was within 10^{-4} and LIV at 20°C was within 24 hrs. Delgado-Sanchez and Grogan (1970) showed that the TIP of PVY strains ranged between 52-62°C, DEP was 10^{-2}- 10^{-3} and inactivated within 48-72 hrs at room temperature.

The concentration of virus preparation 0.77mg / 100g fresh leaves using spectrophotometric measurements at 260 nm. The UV spectral analysis of a nucleoprotein showed maximum and minimum absorptions were 267 nm and 249 nm, respectively. The absorption ratio A260/A280, A280/A260 and Amax/Amin were 1.093, 0.914 and 1.072, respectively. These results were in agreement with obtained by Sheikh *et al.* (2012) who recorded that the purified potyvirus had been isolated from *Wedelia trilobata* plants in Aligarh showed the typical UV spectrum of a nucleoprotein with a maximum absorption at 260 nm and minimum absorption at 246 nm. The A260 / A280 ratio was 1.21 ± 0.04.

The electron microscopy examination of partially purified preparation of the virus isolate demonstrated the presence of flexuous filamentous viruses with length of 1440 nm and width of 14.3 nm. These results showed great difference with that reported by Horvath *et al.* (1988) and Saha *et al.* (1997).Our results strongly indicate that the isolated virus is a new Egyptian long flexuous potyvirus isolate.

The molecular characters of the virus under study were investigated. Total RNA from virus infected *Hyoscyamus muticus* was extracted, and converted to cDNA and amplified via PCR reactions using specific primer: henbane mosaic virus (HMV), potato virus X (PVX) and potato virus Y (PVY).

Successful PCR product was obtained by henbane mosaic virus "HMV" primer [30F20 (+) and788R20 (-)] using Taq-DNA polymerase. The annealing step was optimized by adjusting annealing temperature to 56°C for 45 sec. The size of the PCR products of specific amplified gene was ~778 bp. The virus isolate under current study was published in Genbank with accession number KM497011.

However, the presumptive virus isolate under study showed some degree of similarity (62 %) with all the other potyviruses accessions (EU482153: Potato virus Y isolate Foggia, KF850513: Potato virus Y isolate M3, AM184113: Henbane mosaic virus partial gene for polyprotein-PHYS/H isolate, AY166867: Potato virus Y strain N isolate.

These results indicated that the virus under study might be either a new isolate of henbane mosaic virus (HMV) as showed by biological and morphological studies or might be a new isolate of potatao virus Y (PVY) since the nucleotide sequence similarity is relatively high (88%) with PVY Foggia isolate.

Shukla and Ward (1989a & 1989b) found that the N terminus was the only large region in the entire coat protein that is unique to potyviruses and contains virus - specific epitopes. Gambino *et al*. (2008) reported the importance of validating RNA extraction procedure for different sample matrixes and the ability of the extraction method to provide a suitable nucleic acid free of PCR inhibitors from each sample matrix because plants are known to contain a lot of possible PCR inhibitors such as polysaccharides.

In conclusion, the virus under study might be a new isolate of PVY or new isolate of HMV infecting *Hyoscyamus muticus* L. In order to confirm the identity of the virus isolate, further molecular investigations should be done emphasizing on the full viral genome sequences.

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دراسات بيولوجية و جزيئية علي عزلة فيروس البوتي المصرية من نبات السكران

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تم عزل فيروس بوتي جديد من نباتات السكران مصابة إصابة طبيعية وقد تم تجميعها في شهر يناير عام ٢٠١٣ من مزرعة كلية الصيدلة، جامعة القاهرة. أظهرت النباتات المصابة إصابة طبيعية أعراضا ظاهرية ألا وهي تبرقش شديد وإنخفاضات علي سطح الورقة و تغضن و تشوه الأوراق. و بدراسة درجة ثبات الفيروس في العصير الخام ، وجد أن له درجه حرارة مثبطة لنشاطه و كانت ٦٥ درجة مئوية بينما نقطة التخفيف التي تفقده القدرة علي إحداث العدوي كانت $١٠^{-٤}$ و كذلك يستطيع البقاء حيا في درجه حرارة الغرفة لمدة لاتزيد عن ٤ أيام. وأظهر الفحص بالميكرسكوب الإلكترونى للفيروس المنقى جزئيا عن وجود جزيئات خيطية ومرنة بحجم ١٤٤٠ X ١٤.٣ نانوميتر عند صباغتها بصبغة حامض الفسفوتنجستيك السالبة. تم إستخلاص الحامض النووى الكامل RNA لنبات السكران المصاب بالفيروس. أوضح التتابع النيوكلتيدي المقارن للفيروس نسبة تشابه عالية (٦٢٪) مع اربع فيروسات بوتي بأرقام مسجلة كالآتي: EU482153: عزلة فيروس البطاطس Foggia Y ، KF850513: عزلة فيروس البطاطس M3 Y ، AM184113: الجين الجزئي لعديد البروتينات لعزلة فيروس تبرقش السكران-PHYS/H , AY166867: عزلة فيروس البطاطس N Y. وتم تسجيل العينة فى بنك الجينات برقم مسجل KM497011.