Pathological and Molecular Characterisation of Lettuce Big-Vein Virus and Associated Virus from Egypt

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ABSTRACT

Lettuce (*Lactuca sativa*) is known to be attacked by many viruses causing disease epidemics among which Lettuce big-vein virus (s) is (are) shown here to cause considerable quality and yield losses. In winter 2014, samples suspected of being infected with lettuce big-vein disease viruses (*Mirafiori lettuce* big-vein *virus*, MLBVV and *Lettuce* big-vein *associated virus*, LBVaV) were collected from naturally infected plants grown at different locations, from two governorates in Egypt (El-Qalyubia and El-Fayoum). The characteristic symptom of the disease was seen as the development of a clear region along the major veins of leaf due to the disappearance of chlorophyll making the veins appear as if they were bigger than normal. Along with this symptom, leaves were crinkled and head size was reduced and assumed elongated shape. Three isolates (two of MLBVV, 1 and 2 and one of LBVaV) were detected in lettuce specimens giving negative reaction to *cucumber mosaic virus* (CMV) and *lettuce mosaic virus*(LMV)to specific polyclonal antisera using DAS-ELISA. Detection was based on the RT-PCR technique with a pair of specific primers for coat protein gene of both MLBVV and LBVaV as it resulted in the generation of a unique PCR amplification product of approximately 233 bp for MLBVV and approximately 360bp for LBVaV. It was also proven that MLBVV and LBVaV isolated here are transmissible by the fungal vector *Olpidium virulentus*. The fungus was isolated from plant roots and seeds andits identity was confirmed using the PCR technique. The length of the product amplified was approximately 632 bp using a specific pair of primers for the fungal ITS region. Purification of MLBVV and LBVaV was achieved and the two viruses were detectable and distinguishable by polymerase chain reaction (PCR) and electron microscopy. Samples with symptoms that were positive by RT-PCR to MLBVV and LBVaV were gel-purified and sequenced using specific forward and reverse primers. Sequences related to the three virus isolates (MLBVV1, MLBVV2 and LBVaV1) were submitted to Genbank database under the accession numbers, LT721898, LT721899 and LT721900. The highest similarity percentage was observed between MLBVV1 and MLBVV2 and a Spain isolate was 70.3% and 69.7%, respectively. While, the highest similarity percentage between LBVaV1 and Brazil isolate was 100%.To the best of our knowledge this is the first report of lettuce big-vein disease in Egypt.

Key words: Mirafiori lettuce big-vein virus, Lettuce big-vein associated virus,ELISA, RT-PCR.

INTRODUCTION

Lettuce (*Lactuca sativa*) is an important dietary vegetable in most parts of the world. Consumption of lettuce has some health benefits attributed to the presence of vitamin C, phenolic compounds and high fibre content (Mulabagal*et al.*, 2010). Approximately 10978.9feddan of lettuce are grown in Egypt with a total production of 101392 tons for calendar year 2016(FAO Statistics Database, 2018).

The plant is commonly attacked by several viruses of which cucumber mosaic virus and lettuce mosaic virus have been detected in Egypt (Allam and Ismail, 1972; Fegla.,1990; Abdel-Aziz, 2001; El-Shamy, 2011; El-Borollosy and Waziri, 2013). In contrast, lettuce big-vein disease (LBVD) caused by *lettuce big-vein virus* (LBVV) is a serious malady that has been reported in many different areas of the world, but never in Egypt.

Lettuce big-vein associated virus (LBVaV, genus: Varicosavirus,Kuwata*et al*., 1983), was thought to be the causal agent. However, a discovery by Roggreo*et al.* in 2000 and later confirmed by Lot *et al.* in 2002, showed the presence of a second virus, *Mirafiori lettuce big-vein virus* (MLBVV, genus: Ophiovirus), in infected lettuce plants demonstrating typical symptoms.

Both viruses are believed to be naturally transmitted by the fungus *Olpidiumvirulentus* (*O. brassicae*,koganezawa*et al*., 2005). The resting spores of the fungus can persist for over 20 years in soil and can retain the ability to transmit the disease for over 15 years (Campbell, 1985 and Campbell, 1996).

The present investigation has therefore been conducted to isolate the two new viruses suspected of being involved in lettuce big-vein disease in Egypt and to characterise the obtained isolates on both pathological and molecular basis.

MATERIALS AND METHODS

Plant Sampling and Virus Identification

Samples suspected of being infected with lettuce big-vein disease viruses (*Mirafiori lettuce big-vein virus*, MLBVVand *Lettuce* big-vein *associated virus*, LBVaV, were collected separately in plastic bags from naturally infected plants grown at different locations, from two governorates in Egypt (El-Qalyubia and El-Fayoum) during the winter season of 2014. Lettuce cv. Balady seedlings were used for biological detection of big-vein

viruses (MLBVV and LBVaV) in collected samples. Identification was performed depending on DAS-ELISA and RT-PCR.

Seed samples were also collected and used to confirm the existence of big-vein viruses and *Olpidiumvirulentus*usingthe same molecular techniques as a proof of that the disease is transmitted by seeds.

Detection by DAS-ELISA

Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) technique (Clark and Adams, 1977) was used for virus-detection using a standard procedure. Specific antiserum against *cucumber mosaic virus* (CMV) and *lettuce mosaic virus*(LMV) (ELISA kits) were supplied by LOEWE biochemical (Germany).

Detection by reverse transcription-polymerase chain reaction (RT-PCR)

RNA extraction from leaves and seeds

Samples giving positive reaction with biological detectionand those giving negative reaction with CMV and LMV antisera by DAS-ELISA were used to confirm the identity of MLBVV and LBVaV. Total RNA was extracted from infected leaf and seed tissues using RNA Mini preps super Kit EaZy (E-Z-N-A) according to manufacturer instructions.

RT-PCR analysis

The Oligonucleotide primer sequence used were: MLBVV sense (5'CAG CAC TTT TTG GAT TTT GTC C'3), MLBVV antisense (5'AGA GAA GCC TGT TCC TGC AA'3), LBVaV sense (5'TCA TCC CCC AGT TCA CAA A'3) and LBVaV antisense (5'ATG TTC TTCGCC ACC TGT CT'3), designed based on sequence of coat protein (CP) gene (Araya *et al*., 2011). RT and PCR were carried out sequentially in the same tube using QIAGEN One Step RT-PCR Kit in a volume of 25 µl each containing 3 µl of template RNA, 3 µl antisense primer (10uM), 3 µl sense primer (10uM), 1 µldNTPs mix and 5 µl QIAGEN One Step RT-PCR buffer 5x. Amplifications were performed with a thermal cycler. The RT-PCR programme consisted of two cycles of cDNA synthesis; first 50°C for 15 minutes and the second 95 °C for 1 minute followed by 30 cycles, each with 30s at 94°C for denaturation, 30s at 60°C for annealing and 30s at 72°C for elongation. Reaction mixtures were then incubated at 72°C for 10 min for final extension. PCR products were separated on a 2% agarose gel in TBE buffer, stained with ethidium bromide and photographed under UV light.

Isolation and identification of *Olpidiumvirulentus*

Isolation of single sporangium

Baiting the *Olpidium* from the infected soil collected from the field (two governorates, El-Qalyubia and El-Fayoum)by young seedlingswas conducted according to Weber and Webster(2000). A single sporangial isolate was obtained from culture after one week from transplanting the seedlings. As described by Lin *et al.* (1970), lettuce plants were dug out and roots washed by running tap water to remove the soil and homogenised by mortar and pestle in 20 ml of chilled (2°C) 0.5 M glycine-NaOH buffer (PH 7.6). The filtrate was collected in a beaker and kept in an ice bath, and drops of filtrate were streaked over the surface of chilled, sterile 2% water agar in Petri plate. The agar surface was examined by light microscopy and single mature sporangium was isolated then transferred to a small drop of chilled sterile tap water on slide by removing a plug of agar on which the sporangium was located. After that, theslide was examined to ensure the presence of a single sporangium and then the slide was washed into the one-litreglass beaker containing 15-day old lettuce cv. Balady seedlings in sterile sand soil (1/2 kg) and maintained until symptom appearance (about 4 weeks).

Roots of lettuce plants taken from positively reacting lettuce cv. Balady(expressing lettuce big-vein disease symptoms) were dug out, washed by running tap water to remove the soil and labeled and divided into twoportions, the first portion was kept to air dry, while theother portion was later used to confirm the identity of *Olpidiumvirulentus* by polymerase chain reaction (PCR).

Identification of *Olpidiumvirulentus*bypolymerase chain reaction (PCR)

DNA extraction from leaves and seeds

DNA was isolated from lettuce roots and seeds according to the protocol of Keb-Llanes*et al.* (2002).

Polymerase chain reaction (PCR)

PCR amplification was carried out in a total volume of 25 µl and a pair of specific primers to the *O. virulentus* internal transcribed spacer (ITS region) capable of generating an amplification fragment with expected length of 632bp was used: a forward primer (ITS1) 5′- TCC GTA GGT GAA CCT GCG G-3′and a reverse primer (ITS4) 5′- TCC TCC GCT TAT TGA TAT GC-3′(Sasaya and Koganezawa, 2006). The PCR amplification was carried out in a total volume of 25µl containing 3µl of template DNA, 12.5µl Dream Taq Green PCR master mix and 0.5µl 10uM of each primer. PCR amplification was performed as one cycle at 95°C for 5 min followed by 35 cycles each with 45s at 95°C for denaturation, 1 min at 60°C for annealing and 2 min at 72°C for elongation. Reactions were then incubated at 72°C for 10 min for final extension. The PCR products were analysed as above.

Purification procedure

MLBVV and LBVaVwere purified according to the method described by Roggero *etal.* (2000).

The two viruses were purified from infected lettuce plants with slight modifications.

One hundred gram frozen infected leaves, harvested four weeks post inoculation were mixed with 500 ml (1:5 v/v) cold extraction buffer [10mM phosphate buffer, pH 7.0, containing 20mM sodium sulfite, 10mM disodium diethyl dithiocarbamate (Na-DIECA) and 5mM disodium ethylenediaminetetraacetic acid (Na-EDTA)] and homogenised in Waring blender.

The first step of sedimentation of virions by low speed centrifugation, then the viruses precipitated from the supernatant with 6% polyethyleneglycol (PEG 6000) and 30mM NaCL, followed by 2 cycles of differential centrifugation and finally pellets were suspended in 1ml of 1mM phosphate buffer. The virus yield was determined spectrophotometrically. Preparations were negatively stained and examined with a transmission electron microscope.

Partial sequencing of big-vein virus coat protein (CP) gene and Alignment

The amplified products of CP gene (233 bp for MLBVV and 360 bp for LBVaV) were purified using Centri-Sep spin columns. The products were sequenced by the use of a Big Dye terminator cycle sequencing kit and resolved on an ABI PRISM model 310 automated DNA sequencer at Sigma Company. Pair-wise and multiple DNA sequence alignment were carried out using ClustalW software programme version 1.82 http://www.ebi.ac.uk./clustalw/, (Thompsonet*etal*., 1994). A phylogenetic tree demonstrating the nucleotide sequence distance among the compared elements was established using DNAMAN version 5.1 (LynnonCorp., Canada).

RESULTS

Isolation and initial characterisation

Field observation revealed that the most common symptoms observed on naturally infected plants in two governorates (El-Fayoum and El-Qalyubia) in Egypt were severe clearing around leaf veins, stunting, thickening of the main veins, crinkling leaves, reduction of head size and deformed lettuce heads (Fig. 1). These symptoms were suspected of being caused by lettuce big-vein pathogen [*Mirafiori lettuce big-vein virus* (MLBVV) and *Lettuce big-vein associated virus* (LBVaV)]. Applying the DAS- ELISA technique with the use of two polyclonal antisera specific to two viruses, namely, *Cucumber mosaic virus* (CMV) and *lettuce mosaicvirus* (LMV) has proven that these symptoms were not belonging to CMV or LMV.

Identification of viruses throughRT-PCR

Identification of two virus isolates from lettuce leaves and seeds was confirmed using the RT-PCR technique with a pair of specific primers for coat protein gene of each virus and the generation of a unique PCR amplification product of approximately 233 bp for MLBVVand approximately 360 bpfor LBVaV (Fig. 2).

Isolation of single sporangium

The method used for obtaining single sporangium isolate of *Olpidiumvirulentus*as described by Lin *et.al* (1970) permitted the routine testing of homogenous isolate (Fig.3).

Symptoms of lettuce big-vein disease (LBVD) appeared on lettuce plants inoculated with single sporangium isolated from soil collected from El-Qalyubia governorate after about 7 weeks from sowing. However, no symptoms were observed on plants similarly inoculated with single sporangium isolated from El-Fayoum governorate.

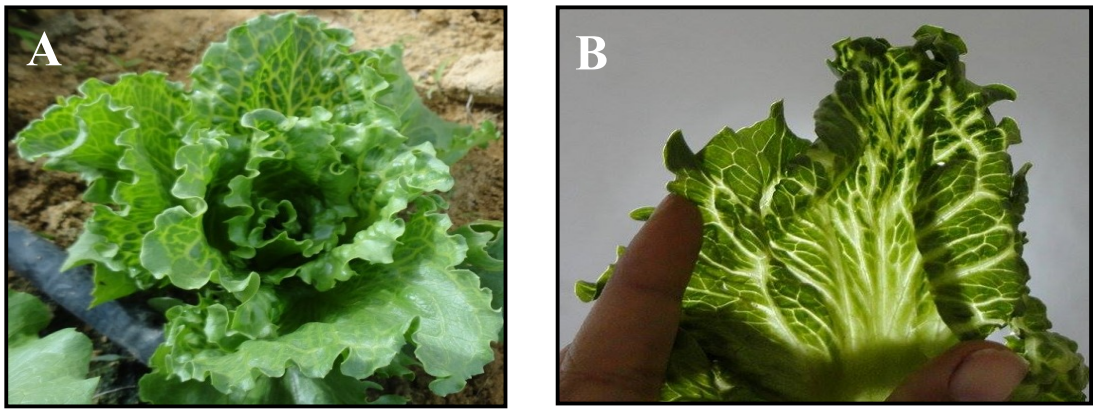

Fig. 1: Naturally infected lettuce plants, showing reduction in head size (A) and vein clearing and thickening of the main veins of leaves (B) suspected of being naturally infected with lettuce big-vein virus.

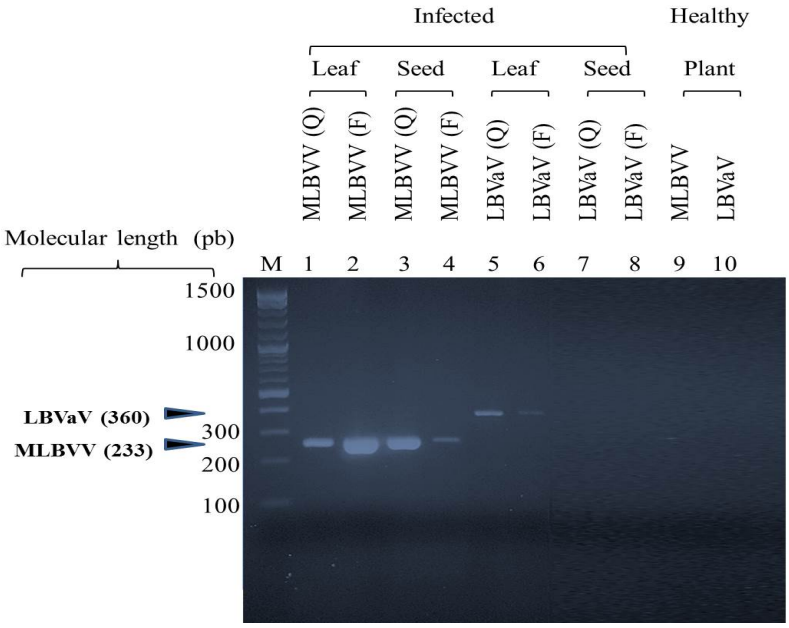

Fig. 2: Agarose gel electrophoresis showing the PCR amplification products of coat protein gene of MLBVV and LBVaV from lettuce. M,100bp Marker; lane1, MLBVV1 of lettuce leaf from El- Qalyuba; lane2, MLBVV2 of lettuce leaf from El-Fayoum; lane3, MLBVV from lettuce seed from El-Qalyuba; lane4, MLBVV of lettuce seed from El- Fayoum; lane5, LBVaV1 of lettuce leaf from EL-Qalyubia isolate; lane6, LBVaV2 of lettuce leaf inoculated with El-Fayoum isolate; lane7, LBVaV of lettuce seed from EL-Qalyubia; lane8, LBVaV of lettuce seed from EL-Fayoum; lane9, MLBVV from healthy plant leaf; lane10, LBVaV from healthy plant leaf.

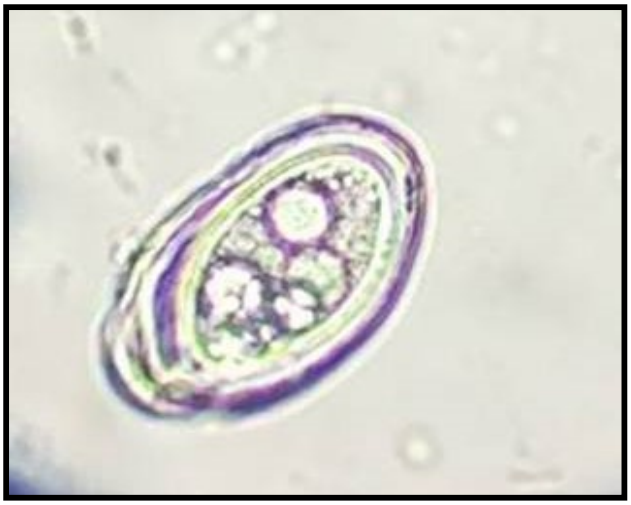

Fig. 3: Single sporangium of *Olpidium virulentus* extracted from lettuce root as described by Lin *et.al* (1970) and streaked on agar surface (Magnification X400).

Identification of *Olpidium virulentus* by polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) was performed on total DNA extracted from lettuce root and seed samples infested with *O. virulentus* with the use of a pair of primers specific to a sequence in the internal transcribed spacer (ITS) region. Analysis of the PCR product was carried out by agarose gel electrophoresis. The length of the product amplified was approximately 632 bp (Fig. 6). A positive reaction of PCR was obtained with root inoculated with a single sporangium isolated from El-Qalyubia governorate and seeds collected. While, a negative reaction was obtained with healthy tissue and infected plants (roots and seeds) form El-Fayoum.

Virus purification

The ultraviolet absorbance of the purified virus preparation was typical for nucleoproteins with a minimum at 240 nm and a maximum at 260nm. The ratio of A260 / A280 was 1.94. The yield of purified virus was 1.2mg/100g infected lettuce leaves.

Electron microscopy

Electron microscopy of a purified preparation of MLBVV and LBVaV complex showed MLBVVashighly kinked filaments about 3 nm in width forming masses of undetermined length, while LBVaV was observed as a few straight rigid rod-shaped particles of a modal length of about 300 nm and a width of 18 nm (Fig. 5).

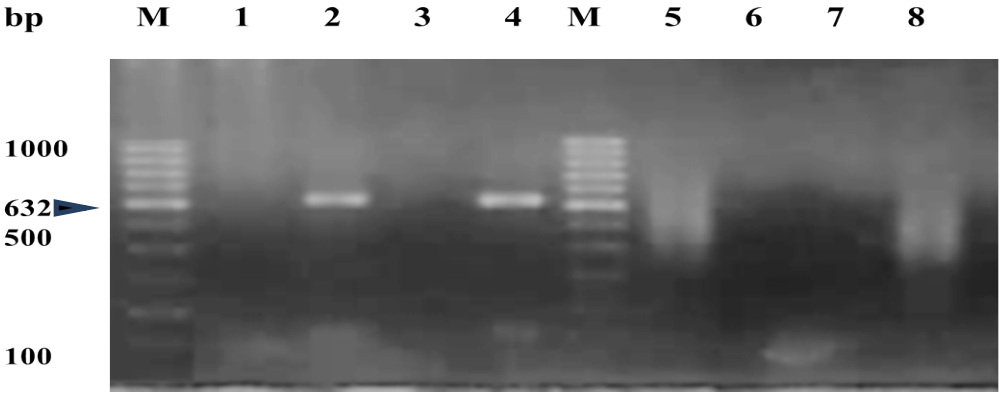

Fig. 4: Agarose gel electrophoresis showing the PCR amplification product (arrowed at 632bp) of ITS region of *Olpidium virulentus*.M, Marker; lane1, healthy roots; lane2,infested roots from Qalyubia;lane3, healthy seeds; lane4, infested seeds from Qalyubia; lane5, infested roots from Fayoum; lane6, healthy roots; lane7, healthy seeds; lane8,infested seeds from Fayoum.

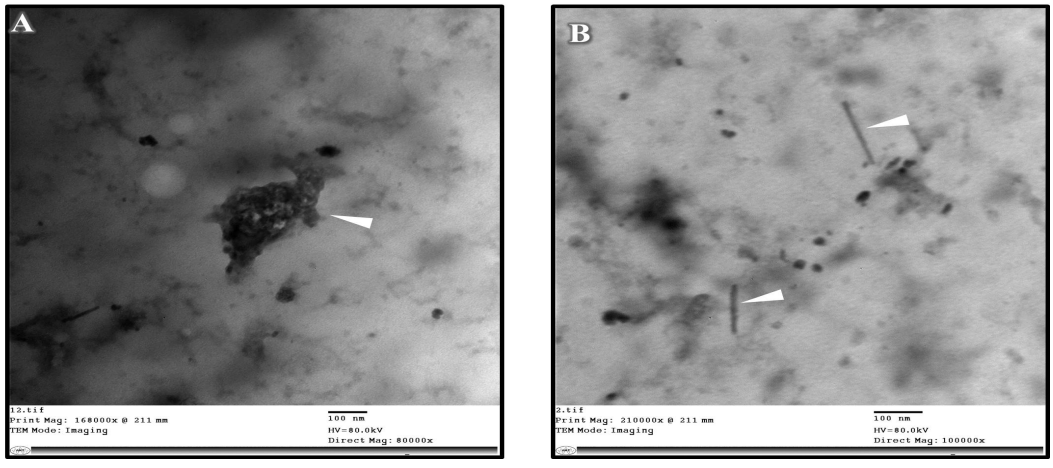

Fig. 5: Electron micrograph of partially purified lettuce big-vein viruses (MLBVV and LBVaV), stained with 2% uranyl acetate, showing (arrow heads) MLBVV supercoiled particle of undetermined length (A) and LBVaV rod-shaped particles with 300nm in length and 18 nm in width (B).

Sequencing and sequence analysis

The amplicons obtained for the two viruses (MLBVV and LBVaV) were purified and sequenced using ABIPRISM model 310 automated DNA sequencer.Sequences related to the three virus isolates were submitted to Genbank database under the accession numbers given in Table 1.

Table 1: Accession numbers of partial coat protein gene sequences of two isolates of *Mirafiori lettuce big-vein virus* (MLBVV1 and MLBVV2) and an isolate of *Lettuce big-vein associated virus* (LBVaV) involved in this study and deposited in GenBank.

Virus isolate*	Accession #
MLBVV1	LT721898
MLBVV2	LT721900
LBVaV1	LT721899

*, MLBVV1 and LBVaV1 isolated from Qalyubia and MLBVV2 isolated from Fayoum

Multiple alignment for cDNA sequences of the two isolates of MLBVV with those of 5 MLBVV isolates and LBVaV with 6 isolates retrieved from the GenBank made available through the NCBI was conducted using ClustalW software programme version 1.82 http://www.ebi.ac.uk./clustalw/, (Thompson*et al.,* 1994), a phylogenetic tree demonstrating the nucleotide sequence distance among the compared elements was established (Fig. 6 and 7).

The Egyptian isolates used in this study proved identity amounting to 70.3%, 69.7 and 100%, respectively (Table 2 and 3).

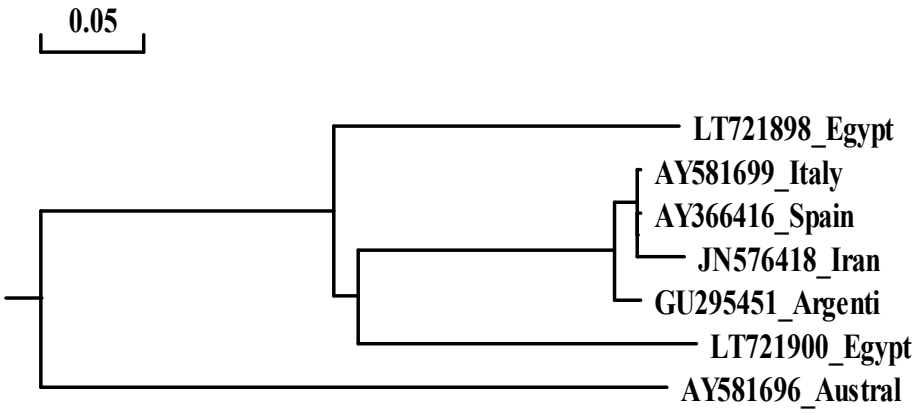

Fig. 6: A phylogenetic tree demonstrating the evolutionary relationship among the two isolates of *Mirafiorilettucebig-veinvirus*(MLBVV1, Accession # LT721898 and MLBVV2, Accession # LT721900) and different isolates from the Genbank based on the partial nucleotide sequence of coat protein gene using DNMAN 5 software.

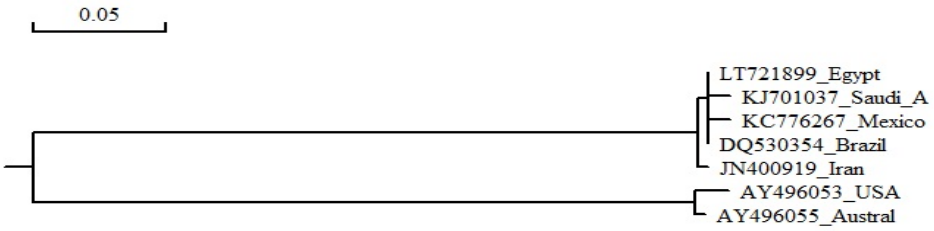

Fig. 7: A phylogenetic tree demonstrating the evolutionary relationship among *lettuce big-vein associated virus* (LBVaV) and different isolates from the Genbank based on the partial nucleotide sequence of coat protein gene using DNMAN 5 software.

Table 2: Estimates of evolutionary percent identity between the sequences of the two Egyptian *Mirafiori lettuce big-vein virus* (MLBVV1, Accession # LT721898 and MLBVV2, Accession # LT721900) isolates reported here and some other isolate sequences deposited in the Gen

	AY366416 Spain	AY581696 Australia	AY581699 Italy	GU295451 Argentina	JN576418 Iran	LT721898 Egypt	LT721900 Egypt
AY366416-Spain	100%						
AY581696-Australia	39.8%	100%					
AY581699-Italy	99.4%	40.4%	100%				
GU295451-Argenti	97.1%	40.4%	97.1%	100%			
JN576418-Iran	97.7%	39.8%	97.7%	95.3%	100%		
LT721898-Egypt	70.3%	39.2%	70.3%	70.3%	69.2%	100%	
LT721900-Egypt	69.7%	38.8%	69.7%	68.5%	68.5%	66.5%	100%

Table 3: Estimates of evolutionary percent identity between the Egyptian isolate of *Lettuce big-vein associated virus* (LBVaV, Accession # LT721899) reported here and other isolate sequences deposited in the GenBank database based on nucleotide sequence alignment of coat protein genes.

	LT721899 Egypt	AY496053 USA	AY496055 Australia	DQ530354 Brazil	JN400919 Iran	KC776267 Mexico	KJ701037 Saudi A.
LT721899-Egypt	100%						
AY496053-USA	48.3%	100%					
AY496055-Australia	49.1%	98.3%	100%				
DQ530354-Brazil	100.0%	48.3%	49.1%	100%			
JN400919-Iran	99.1%	48.3%	49.1%	99.1%	100%		
KC776267-Mexico	99.1%	47.4%	48.3%	99.1%	98.3%	100%	
KJ701037-Saudi A.	99.1%	47.4%	48.3%	99.1%	98.3%	98.3%	100%

DISCUSSION

The naturally infected plants that were growing in different locations in Egypt and from which the specimens used as a source of thebig-vein virus isolates were collected suggested that lettuce big-vein disease might be existing in Egypt. Characterising the obtained isolates was based on serological reactivity and molecular detectability.

The negative reaction observed with the lettuce specimens used as a source ofthe three isolates (two of MLBVV, 1 and 2 and one of LBVaV)when tested by DAS-ELISAand specific polyclonal antisera for *cucumber mosaic virus* (CMV) and *lettuce mosaic virus* (LMV)proved that the three isolates suspected of causing lettuce big-vein diseaseused in this study were not contaminated by either of the two most commonly encountered viruses in lettuce, CMV and LMV. Detection of the three isolates suspected of being implicated in the disease was based on the RT-PCR technique with a pair of specific primers for coat protein gene of both MLBVV and LBVaV.

The generation of a single PCR amplification product of 233 bp for MLBVVand approximately 360 bpfor LBVaV when RT-PCR technique was applied with a pair of specific primers to the MLBVV and LBVaV investigated here validates the identity of the two viruses. This finding is supported by a similar result previously obtained by Araya *et al.* (2011).

The identity of the fungus,*Olpidium virulentus* proven to be the vector of the isolates obtained in this study was confirmed using the PCR technique. The length of the product amplified was approximately 632 bp using a specific pair of primers for the ITS region. The length of the amplified PCR fragment was in agreement with that obtained by Sasaya and Koganezawa(2006).

Electron microscopy of a purified preparation of MLBVV and LBVaV complex showed the presence of MLBVVparticles as highly kinked filaments, about 3 nm in diameter masses of undetermined length and this finding agrees with those of Roggero*et al*., (2000) and Lot *et al*. (2002).

A few straight rigid rod-shaped particles of LBVaV, the modal dimensions of the particles were found to be 300 nm in length and 18 nm in diameter. Those results correspond with those reported by other investigators (Kuwata*et. al*., 1983, Vette*net al*., 1987and Lot *et al*., 2002).

When sequence alignment along with phylogenetic analysis of CP gene nucleotide sequence of MLBVV1 and MLBVV2 (accession numbers, LT721898 and LT721900, respectively) was conducted with 5 different isolate sequences and LBVaV1 (accession number, LT721899)with those of 6 various LBVaVisolates retrieved from the GeneBank, the highest similarity percentage was observed between MLBVV1 and MLBVV2 and Spain isolatewith 70.3% and 69.7% similarity, respectively. Likewise, the highest similarity percentage was between LBVaV1 and Brazil isolate with similarity percentage of 100%.

To the best of our knowledge and according to the available literature, this is the first report in Egypt where both,MLBVV and LBVaV, isolates and *O. virulentus*have been isolated and identified.

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الملخص العربي

التوصيف المرضى والجزيئى لفيروس العرق الكبير فى الخس والفيروسات المرتبطه فى مصر

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أظهرت الملاحظات الحقلية التى أجريت أن أكثر الاعراض شيوعا على نباتات الخس المصابة طبيعيا والتى تم جمعها من محافظتين مختلفتين في جمهورية مصر العربية كانت تشبه اعراض مرض العرق الكبير في الخس. أظهر الكشف بطريقة الإيلايسا المباشرة المزدوجة بإستخدام أمصال مضادة لاثنين من الفيروسات وهما فيروس موزاييك الخيار(CMV) وفيروس موزايك الخس (LMV) عن عدم تواجد اي منهما في العينات.

تم تأكيد تعريف عزلات الفيروسات المعزولة بإستخدام الـــ RT-PCR حيث ظهر ناتج مضاعفة واحد للتفاعل بطول ٢٣٣ زوج من القواعد لفيرسالعرق الكبير في الخس (MLBVV) وآخر بطول ٣٦٠ زوج من القواعد للفيرس المصاحب للعرق الكبير في الخس (LBVaV) وذلك بإستخدام زوج متخصص من البادئات لجين الغلاف البروتينى لكل منهما. كما تم إثبات أن عزلات فيرس العرق الكبير في الخس والفيرس المصاحب (MLBVV وLBVaV) تنتقل بالفطر *Olpidiumvirulentus* وذلك بعزل الفطر من جذور النباتات المصابة وكذلك من البذور المصابة طبيعيا. وتم تعريف الفطر بواسطة تفاعل البوليمريز المتسلسل (PCR) حيث ظهر ناتج مضاعفة للتفاعل بطول ٦٣٢ زوج من القواعد بإستخدام زوج متخصص من البادئات لمنطقة الـــ ITS. وأمكن بإستعمال التقنيات الجزيئية (النسخ العكسي وتفاعل البوليمريز المتسلسل) إثبات وجود كل من الـــMLBVV والـــ LBVaV والفطر الناقل *Olpidiumvirulentus* في البذور حيث كانت النتيجة ايجابيةفي كل الحالات.

تم عمل تنقية لفيروسي مرض العرق الكبير في الخس من أوراق خس مصابة، واتضح ان منحنى امتصاص الاشعة فوق البنفسجية للتحضيرات النقية جزئيا مطابقة لتلك المعروفة عن البروتينات النووية، وان متوسط نسبة A280/A260 كانت ١,٩٤. وقد أمكن الحصول على كميات من الفيروس النقى بلغت ١,٢ مجم/ ١٠٠ جم من الوزن الرطب لأوراق الخس المصابة. وأظهرت الدراسات بواسطة المجهر الالكترونى للتحضير المنقي جزئيا وجود دقائق جزيئات فيروس MLBVV الخيطية بقطر ٣ نانوميتر ولكن لم نتمكن من تحديد الطول بسبب إلتفاف الدقائق. كما ثبت وجود دقائق فيروس الـــ LBVaV العصويه بطول ٣٠٠ نانوميتر وعرض ١٨ نانوميتر.

تم تنقية ناتج مضاعفة تفاعل إنزيم البوليميريز المتسلسل (PCR) ومعرفة التتابع النيوكلوتيدي لعزلات هذه الدراسة وتم تسجيلها في بنك الجينات تحت رقم LT721898، LT721899 وLT721900. ثم تمت مقارنة التتابعات وتحليل شجرة النسب الوراثية، وقد ثبت وجود درجة تشابة بين عزلتى فيروس العرق الكبير في الخس (MLBVV1 وMLBVV2) والعزلة المسجلة من أسبانيا وكانت نسبة التشابه٧٠,٣% و٦٩,٧% على التوالي. كما ثبت وجود درجة تشابه عالية وصلت ١٠٠% بين عزلة فيروسLBVaV1 والعزلة الميكسيكية.

وطبقاً لما توفر لدينا من معلومات من خلال التراث البحثي المسجل والمتاح نستطيع القول بأن هذا البحث هو أول تسجيل لهذان الفيروسان(MLBVV وLBVaV) في مصر.