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ORIGINAL PAPER

Incidence, Distribution and Molecular Characterization of Pea Seed-borne Mosaic Virus (PSbMV) in Egypt

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ABSTRACT

Pea seed-borne mosaic virus is an important disease of pea crop and has a wide host range. The field survey was conducted in 2020 and 2021 in different locations of pea-growing fields of four governorates in Egypt to determine the incidence of the virus. Symptoms of virus infection were observed in 25.2 and 28.3 % of total surveyed fields at nine of ten regions in 2020 and 2021, respectively with an incidence ranged between 2 to 28.7%. Naturally infected pea leaves showed symptoms of severe mosaic, downward leaf rolling, vein banding, yellowing, and interveinal chlorosis. The isolated virus was identified based on the symptoms developed on diagnostic hosts, seed transmission, and molecular techniques. The seed transmission tests indicated that seed germination was affected by the virus, as it decreased the germination rate by 56%. The virus was transmitted through seeds where reverse transcription-polymerase chain reaction was used to detect the virus in seedlings produced from infected seeds and the percentage of seed transmission was 18%. 335 bp of the potyvirus coat protein gene and 800 bp of nuclear inclusion protein (*NIb*) gene of the virus were amplified using a set of degenerate and specific primers, respectively. The two sets of primers succeeded to amplify the partially coat protein gene of a potyvirus and a portion of the *NIb* gene of the virus. The amplified product was successfully cloned and sequenced. Results of sequence analysis showed similarity ranging between 95-100% compared with twelve reported isolates of the virus. The Egyptian isolate of Pea seed-borne mosaic virus was submitted in the GenBank under the accession number ON075784.

Keywords: Pea Seed-borne Mosaic Virus, PSbMV, Pea, *Pisum sativum*, Distribution, Molecular Characterization

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INTRODUCTION

Pea (*Pisum sativum* L.) is a food legume crop, in Egypt pea is grown in the winter season for local consumption and exportation. It is a source of protein for humans and livestock where the seeds contain a great amount of protein and carbohydrates (Abou El-Salehein *et al*. 2019). The total growing area is 4091.9 feddans with a production of 153233 tons (FAO, 2020). Pea plants are susceptible to many viruses which include 35 viruses (Hampton 1984). Pea seed-borne mosaic virus (PSbMV; genus *Potyvirus*, family *Potyviridae*) which infects pea crops worldwide, is economically important due to its high rate of seed transmission and causes serious losses in peas yield and quality (Coutts *et al*. 2009). Many commercial cultivars of peas are susceptible to PSbMV, this susceptibility confirmed the fact that this virus is transmitted vertically through generations by seeds besides its transmission by aphid vectors in a non-persistent manner. PSbMV was initially reported under different names and discovered for the first time in Czechoslovakia by Musil (1966) then it was reported in 35 countries **(**Maury and Khetarpal 1992 and Makkouk *et al*., 1993). In Egypt, PSbMV has been detected in pea (Abdelmaksoud *et al*., 2000 and El-Banna *et al*., 2008) and in cowpea forage (*Vigna unguiculata* L. Walp.) (Kararah *et al*., 2014). PSbMV has filamentous particles and measures approximately 770 nm in length and 12 nm in width. It can induce the production of inclusion bodies that are pinwheel in shape and aggregate in the mesophyll cells (Hampton *et al*., 1981; Wang *et al*., 1991 and Makkouk *et al*., 1993). PSbMV can infect host range limited to the *Fabaceae* family such as pea (*Pisum sativum*), faba bean (*Vicia faba*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), and pasture legumes such as alfalfa (*Medicago sativa*) and vetch (*Vicia* spp.) (Makkouk *et al*. 2012; Gheshlaghi *et al*. 2019; Almási *et al*. 2020). The virus causes a wide variety of symptoms depending

on the virus isolate and host, such as downward leaf rolling, mild mosaic, vein clearing, vein banding, and mild stunting. Seed coat cracking, seed discoloration, shrunken seeds, and the reduced size of the seeds are also observed due to PSbMV infection (Šafářová *et al*. 2008; Congdon *et al*. 2017; Gheshlaghi *et al*. 2019; Almási *et al*. 2020). The presence of symptoms on the seed coat of field peas is not an indicator of PSbMV infection within the embryo of the seed (Astier *et al*. 2007; Khetarpal and Maury 1987; Latham and Jones 2001). The viral genome is monopartite consisting of positive-sense single-stranded RNA of 9924 nucleotides, while the viral transcriptome consists of 9618 nucleotides coding for a polyprotein of 364 KDa that is further divided into nine functional proteins (Johansen *et al*. 1991; Makkouk *et al*. 2012). The coat protein (CP) of PSbMV is responsible for the encapsulation of the viral genome (Andersen and Johansen 1998). Enzyme-linked immunosorbent assays are the most common method for PSbMV detection and RT-PCR assays are also available to reliably detect PSbMV (van der Vlugt *et al*. 1999; Safarova *et al*. 2014). Several pathotypes of PSbMV have been distinguished based on molecular genetic analysis of the viral genome **(**Johansen 1996; Hjulsager *et al*. 2002; Giakountis *et al*. 2015). Especially the genes encoding the CP (Wylie *et al*. 2011). Four pathotypes of PSbMV were distinguished by their ability to overcome *sbm* resistance genes present in differential host pea lines. (Johansen *et al*. 1991 and Cerna *et al*. 2017). This study aimed to determine the incidence of Pea seed-borne mosaic virus associated with pea plants, evaluate the rate of seed transmission, identification, and molecular characterization of the isolated virus.

MATERIALS AND METHODS

Incidence of PSbMV in pea fields:

To determine the distribution of PSbMV, a field survey was conducted in different locations of four governorates in Egypt (Fayoum, Beni Suef, Qalyubia, and Menoufia) in 2020 and 2021 as presented in Table (1). One hundred and ninety-nine of pea fields from ten locations of the major pea growing areas in four governorates were surveyed and samples were collected through the flowering and fruiting stages based on visual symptoms related to the PSbMV virus. All collected samples were placed in polythene bags, stored at 4°C, and tested for the virus infection through RT-PCR. The incidence of symptoms expression (percentage of plants with PSbMV symptoms) was estimated for each field by visual inspection of 100 plants following a W pattern (crossing the rows) as described by Fletcher (1993). Disease incidence was also estimated based on RT-PCR as the percentage of virus-positive samples relative to the total number of tested samples.

Plant material, sap inoculation, and identification:

Samples of pea leaves showing virus-related symptoms (Fig. 1) were collected from different locations in four governorates (mentioned before) during the 2020 and 2021 growing seasons. The collected leaf samples were checked by RT-PCR assay. Samples of pea leaves that gave positive results with RT-PCR were used for virus isolation and propagation on pea seedlings by mechanical inoculation. The single local lesions technique on *Chenopodium amaranticolor* leaves described by Kuhn (1964) was used for the biological purification of the virus. The resulting single local lesion on *Ch. amaranticolor* was used to propagate the virus on pea plants as a source of the virus. All host species were mechanically inoculated using the sap of PSbMV infected pea leaves after grinding leaf tissues with sterilized pestles and mortars in 0.1 M sodium phosphate buffer pH 7.2, at a ratio of 1:2 (tissue weight: buffer volume). The inoculated plants were observed for symptoms development after virus inoculation. Healthy plants were inoculated with the buffer used as negative controls. The results were confirmed by RT-PCR using specific PSbMV primers.

Host range and diagnostic host reactions:

A total of nineteen plant species were selected as host range and diagnostic hosts belonging to five families, *Chenopodiaceae, Fabaceae, Cucurbitaceae, Solanaceae*, and *Brassicaceae* were mechanically inoculated and kept in an insect prof greenhouse. These hosts included *Ch. amaranticolor, Ch. quinoa, Ch. album, Lens culinaris, Pisum sativum, Cicer arietinum, Lathyrus annuus, Phaseolus vulgaris, Vigna unguiculata, Vicia faba, Cucumis sativus, Cucurbita pepo, Cucumis melo, Gomphrena globosa, Nicotiana tabacum, Nicotiana glutinosa, Lycopersicon esculentum, Brassica oleracea. L. var. botrytis* and *Brassica oleracea var. capitata*. The inoculated plants were examined daily up to 35 days for symptoms development under greenhouse conditions.

Transmission through pea seeds:

Around 100 seeds of pea (*Pisum sativum* cv Balady) were sown in a plastic house where the

temperature ranged from 20-25°C. After two weeks from sowing, pea seedlings were mechanically inoculated with PSbMV and 30 plants were left uninoculated and served as healthy control. The inoculated plants were kept in the greenhouse. After two weeks from inoculation, the presence of the virus in pea plants was checked by RT-PCR. Plants that gave positive reactions were labeled and kept till seed maturity and checked for virus presence. After maturity, healthy and infected plants were harvested, and their seeds were collected for replanting in pots (25 cm diameter) containing sterilizing soil. Seedlings were kept in the greenhouse. The germination rate was estimated for each healthy and infected seedling. The percentage of seed transmission was calculated according to Kararah *et al*. (2014) as several seedlings showing symptoms and gave positive reaction with PSbMV divided on the number of immerged seedlings X 100.

Extraction of total RNA:

RNA extraction from pea samples was carried out using Simply P Total RNA Extraction Kit (Bio Flux, Cat # BSC52S1) according to the manufacturer instructions.

Reverse transcriptase-polymerase chain reaction (RT-PCR):

In this investigation, two sets of primers were used: First, the sense primer U335: (5` GAATTCATGRTNTGGTGYATHGANAAYG G 3`) and the antisense primer D335: (5` GAGCTCGCNGYYTTCATYTGNRHDWKN GC 3`) were used to amplify 335 bp of the potyvirus coat protein (Langeveld *et al*. 1991). Using the Verso TM one-step RT-PCR kit, the extracted RNA was employed as a template for a one-tube RT-PCR amplification experiment (Thermo Scientific). RT-PCR was carried out in a 25 µL total volume including 4.75 µL of nuclease-free water, 3 ng/L total RNA, 12.5 µL of one-step PCR master mix, 3 µL of 10 mM primers, 0.5 µL of Verso enzyme mix, and 1.25 µL of RT-Enhancer. The RT reaction began with 15 min of incubation at 50°C, followed by 2 min of denaturation at 95°C. In an applied biosystems DNA Thermal Cycler (Proflex PCR system; Applied Biosystems, Waltham, MA, USA), the amplification reaction was run through 35 cycles, starting with denaturation at 95°C for 1 min, primer annealing at 55°C for 1 min, and extension at 72°C for 1 min. The final extension was conducted at 72°C for 7 min at the end of the 35th cycle. The PCR products were electrophoresed for 1 hour at 100 V in a 1.5% agarose gel in 0.5 X Tris-borate-EDTA (TBE) buffer, stained with EZview nucleic acid dye (Biomatik, Kitchener, ON, Canada), and viewed under UV light. Second, according to Naimuddin and Akram (2021) primer combination (PSbMV-F: 5` GGGGGCCATGGACCGTAGGA 3` and PSbMV-R: 5` CGAAGCGCTGTCTCCGCGAT 3`) to detect PSbMV by targeting a part of the *NIb* gene (~800 bp) and the RT reaction began with 15 min of incubation at 50°C, followed by 2 min of denaturation at 95°C, followed by PCR with initial denaturation at 94°C for 1 min and 35 cycles of denaturation at 94°C for 30 s, annealing at 54°C, extension at 72°C for 5 min, and one cycle of final extension at 72°C.

Molecular cloning and nucleotide sequence analysis:

pGEM®-T Easy vector (Promega) was used to ligate the PCR product that obtained using the degenerate primers (U335/D335) and the transformed recombinant plasmids into *E. coli* strain DH5α were produced. White colonies were selected for DNA isolation, then the DNA was digested with *Eco*RI, and fractionated on 1% agarose gels. The nucleotide sequence of clones having expected inserts were selected for dideoxy sequencing with ABI 377XL automated DNA sequencing instrument, using 36 cm well to read plates and a 5% Long Ranger (FMC) acrylamide gel. Data were analyzed using ABI™ version 3.0 of Sequencing Analysis. All sequencing procedures were completed in Macrogen Company (South Korea). The nucleotide sequence of PSbMV was compared and evaluated with those of PSbMV isolates in GenBank using DNAMAN 8 Sequence Analysis Software (Lynnon BioSoft, Canada).

RESULTS

Disease incidence:

To determine the occurrence and distribution of PSbMV, surveys were conducted in 2020 and 2021 in different locations of some pea-growing fields in Egypt (mentioned before). In 2020, symptoms of PSbMV infection were observed in 25.2% of total pea surveyed fields at nine of ten regions as presented in table (1). The percentage of incidence varied greatly not only between regions but also between fields in the same region where the incidence ranged between 2 to 27.5% but this percentage reached 35% in two farms in Tamiya region of Fayoum governorate. Fields in which pea plants exhibited symptoms in 2021 growing season represented 28.32 % of total visited fields with an incidence of 2 to 28.7 % as indicated in Table (1).

Table (1): Occurrence of Pea Seed-borne Mosaic Virus (PSbMV) in pea fields in four governorates during 2020 and 2021.

Governorates	Regions	Fields with symptomatic plants/total		Symptomatic samples/ total		Incidence percentage (%) based on visual symptoms		PCR detection positive samples/ total	Observed symptoms
		2020	2021	2020	2021	2020	2021		
Fayoum	Fayoum	3/12	4/10	10/300	18/400	3.3	4.5	11/30	M, S
	Tamiya	6/15	5/12	150/600	137/500	25	27.4		SM, LR,
	Itsa	2/10	3/10	45/200	47/300	22.5	15.6		Y, S
	Senours	1/10	3/12	2/100	17/300	2	5.7		Y
Beni Suef	Sedmant	4/10	3/8	110/400	86/300	27.5	28.7	15/22	SM, LR, S, VB
	Beba	2/15	1/10	8/200	2/100	2.5	2		Y, S
Qalyubia	Kanater	3/8	2/8	16/300	18/200	5.3	9	7/20	M, LR
	Kaha	0/7	1/10	0/700	2/100	0.0	2		NS
Menoufia	Menoufia	4/10	3/9	75/400	62/300	18.7	20.7	9/18	SM, VC
	Quesna	1/6	2/7	65/600	25/200	10.8	12.5		MM, S

M: mosaic; S: stunting; SM: severe mosaic; LR: leaf rolling; Y: yellowing; VB: vein banding; NS: no symptoms; VC: vein clearing; MM: mild mosaic.

Visual symptoms, isolation, and identification of PSbMV:

Naturally infected pea leaves with PSbMV showed symptoms of severe mosaic, downward leaf rolling (Fig. 1A) vein banding, interveinal chlorosis (Fig. 1B), and yellowing (Fig. 1C) compared to healthy ones (Fig. 1D). Also, seeds showed discoloration, deformation, and reduced size (Fig. 1E) and crack of the seed coat (Fig. 1F) when compared with healthy seeds (Fig. 1G). The virus was successfully isolated from naturally PSbMV-infected pea plants which were collected from four governorates and propagated on *Pisum sativum* by mechanical inoculation. Forty-two out of ninety naturally infected samples with PSbMV gave a positive reaction with RT-PCR at the expected size.

Fig. (1): Symptoms of Pea Seed-borne Mosaic Virus (PSbMV) on naturally infected pea plants. (A): severe mosaic and downward leaf rolling; (B): vein banding and interveinal chlorosis; (C): yellowing; (D): healthy pea plants; (E): seeds showing discoloration, deformation, and reduced size; (F): seed coat cracking, and (G): healthy seeds.

Diagnostic host reactions:

The reaction of diagnostic hosts to virus infection is shown in figure (2) and summarized in table (2). Mechanically inoculated plants induced various symptoms expression including mild mosaic, severe mosaic, vein banding, downward leaf rolling, and necrotic or chlorotic local lesions. Some hosts showed no symptoms but gave a positive reaction when detected by RT-PCR (symptomless). Responses of diagnostic hosts can be divided into four categories: (1) Hosts gave only local symptoms (chlorotic or necrotic lesions) on *Ch. amaranticolor, Ch. quinoa.* (2) Hosts gave only systemic symptoms: *Pisum sativum, Vicia faba, Cicer arietinum, Vigna unguiculata, Lens culinaris, Lathyrus annuus,* and *Phaseolus vulgaris* (3) Hosts didn't show symptoms (symptomless hosts), but the presence of PSbMV in these plants was detected by RT-PCR: *Nicotiana tabacum,* and *Gomphrena globosa.* (4) Hosts didn't show any symptoms of PSbMV and gave negative reaction with RT-PCR: *Beta vulgaris, Cucumis melo, Cucurbita pepo, Cucurbita moschata, Nicotiana glutinosa, Brassica oleracea L. var. botrytis* and *Brassica oleracea L. var. capitata.*

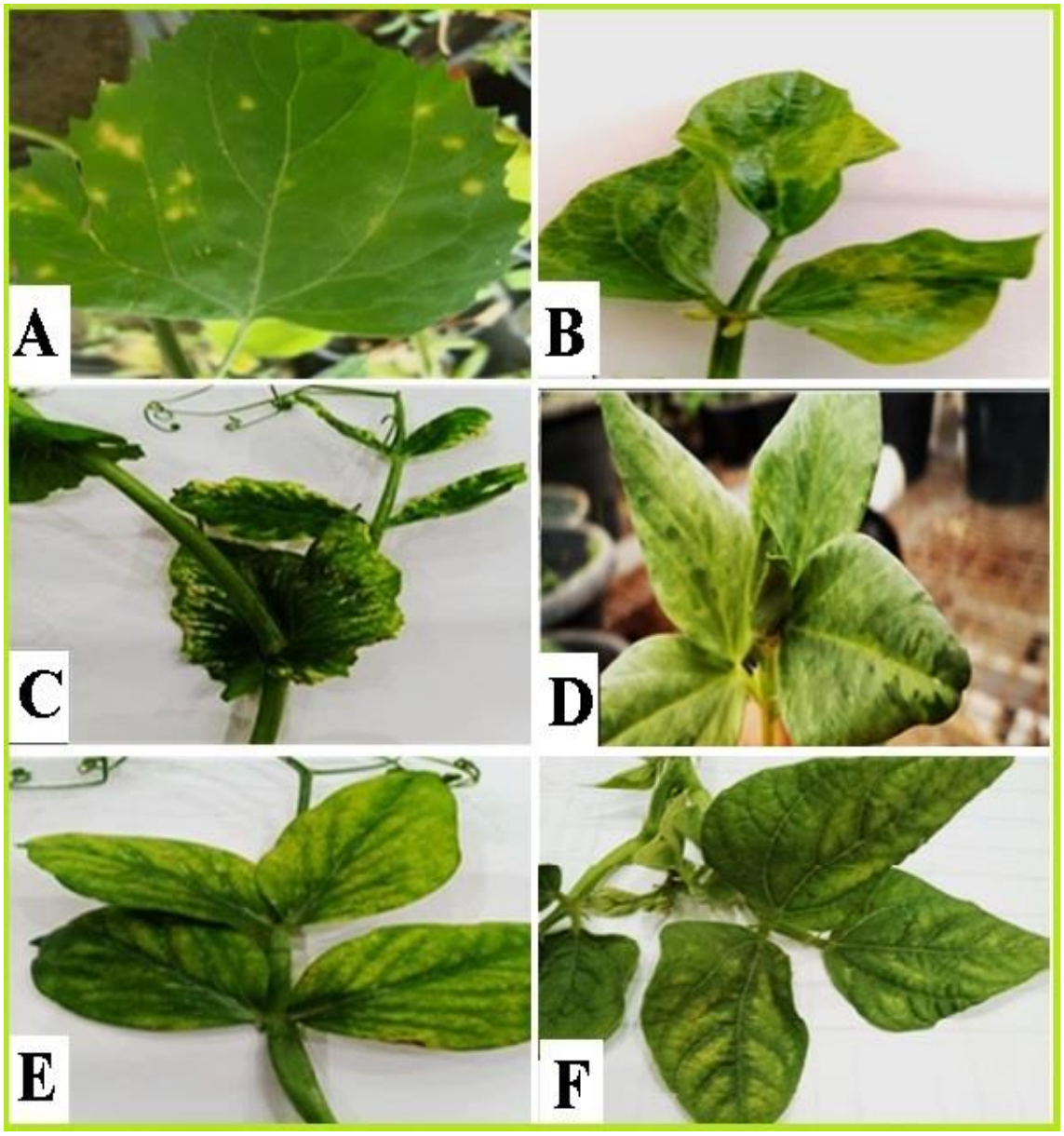

Fig. (2): Symptoms of Pea Seed-borne Mosaic Virus (PSbMV) as a result of responses of some diagnostic host plants for the mechanical inoculation. (A): chlorotic local lesions in the inoculated *Chenopodium amaranticolor* leaf; (B): severe mosaic on *Vigna unguiculata*; (C and E): severe mosaic, downward leaf rolling and vein banding on *Pisum sativum*; (D and F): systemic mild mosaic on *Vicia faba* and *Phaseolus vulgaris* respectively.

Table (2): External symptoms of PSbMV on diagnostic hosts after mechanical inoculation.

Families	Test plants	Symptoms	RT-PCR
Chenopodiaceae	*Chenopodium quinoa*	CLL	+
	Chenopodium amaranticolor	CLL	+
	Beta vulgaris	NS	-
Fabaceae	*Cicer arietinum*	M	+
	Lens culinaris	M, VC	+
	Pisum sativum	VB, MM, SM, LR	+
	Vicia faba	MM	+
	Lathyrus annuus	M, Y	+
	Phaseolus vulgaris	MM	+
	Vigna unguiculata	SM	+
Cucurbitaceae	*Cucurbita pepo*	NS	-
	Cucumis melo	NS	-
	Cucurbita pepo	NS	-
	Cucurbita moschata	NS	-
Solanaceae	*Nicotiana tabacum*	NS	+
	Gomphrena globosa	NS	+
	Nicotiana glutinosa	NS	-
	Lycopersicon esculentum	NS	-
Brassicaceae	*Brassica oleracea L. var. capitata*	NS	-
	Brassica oleracea L. var. botrytis	NS	-

CLL: chlorotic local lesions; NS: no symptoms; M: mosaic; VB: vein banding; MM: mild mosaic; SM: severe mosaic; LR: leaf rolling; Y: yellowing.

Seed transmission:

The seed transmission results indicated that, seed germination was significantly affected by PSbMV, as seed germination was decreased by 56 % compared with healthy once. The virus was transmitted through pea seeds where the seedlings raised from the infected seeds were detected by R-TPCR and the percentage of seed transmission was 18%.

One-step RT-PCR and cloning:

Pea samples collected from growing areas (mentioned before) were subjected to RT-PCR assays. RT-PCR was carried out to amplify ~335 bp of the coat protein gene of potyvirus using a set of degenerate primers (U335/D335) and ~800 bp of NIb gene of PSbMV using a set of specific primers (PSbMV-F/PSbMV-R) as described above (Fig. 3). Those two sets of primers succeeded to amplify the expected size bands for the partially coat protein gene of potyvirus and partially *NIb* gene of PSbMV. RT-PCR product that obtained using the degenerate primers (U335/D335) was successfully inserted into pGEM-T Easy vector and the recombinant plasmids were transformed into *Escherichia coli* DH5α strain. Recombinant plasmids were isolated successfully from different colonies using Wizard Plus SV Minipreps DNA Purification System. Digestion with restriction enzyme *Eco*RI and fractionation on 1.5% agarose gel in 0.5X TBE buffer was done ending with positive results.

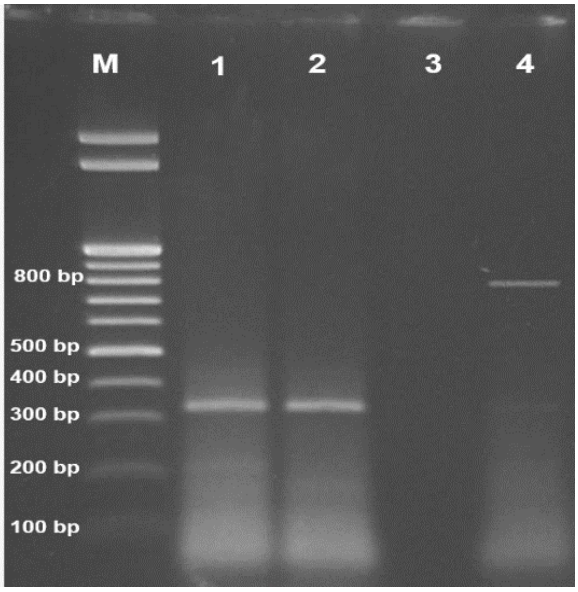

Fig. (3): Agarose gel electrophoresis of RT-PCR products of PSbMV. M: 100bp DNA ladder; 1 and 2: samples detected using degenerate primers for potyviruses; 3: healthy plant control; 4: sample infected with PSbMV detected using specific primers of PSbMV.

Nucleotide sequencing:

The partial coat protein gene of PSbMV that obtained using the degenerate primers (U335/D335) was sequenced in Macrogen Company (South Korea). The sequenced partially coat protein gene was used in phylogenetic analysis using the Optimal

Alignment Method of DNAMAN 8 software (Lynnon BioSoft) to study the relationship between the PSbMV isolate used in this study and those isolates available in GenBank. Sequence comparisons showed similarity ranging between 95-100% of the twelve reported isolates of PSbMV with the Egyptian isolate. The results indicated that the highest sequence similarity was found between the Egyptian isolate (ON075784) and PSbMV isolates from Australia, Czech Republic, Greece, Germany, UK, Denmark, New Zealand, and USA at 100%, while the lowest sequence similarity was found with two isolates from Denmark and one from UK at 95% (Fig. 4).

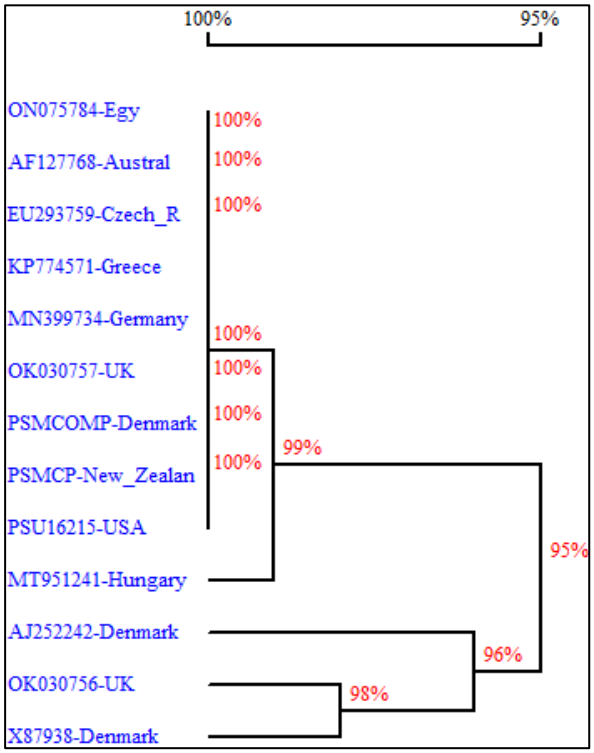

Fig. (4): phylogenetic tree showing similarity percentage between PSbMV Egyptian isolate and reported isolates based on nucleotide sequences.

DISCUSSION

Pea seed-borne mosaic virus (PSbMV) is one of the most economically important seed-transmitted viruses infecting pea plants. The exchange of infected germplasm material causes the worldwide spread of the virus (Maury and Khetarpal 1992; Makkouk *et al*. 1993; Jones 2004; Kararah *et al*. 2014; Gheshlaghi *et al*. 2019; Almási *et al*. 2020). In the present study, surveys were conducted in two successive seasons, during 2020 and 2021 in different locations of pea-growing fields in Egypt to determine the incidence of PSbMV and understand its epidemiology in our country. Results of the incidence indicated that PSbMV infection was widespread in pea-growing fields where, in 2020, symptoms of PSbMV infection were observed in 25.2% of total pea surveyed fields at nine of ten regions with a percentage of an incidence ranging between 2 to 27.5% while this percentage increased in 2021. Incidence of PSbMV infection in two farms of Fayoum governorate was greater than 30% because these farms used pea seeds produced in the previous year in cultivation and the spread of aphids, which increase the percentage of virus infection. PSbMV survival between seasons depends on seeds as the main reservoir (Ali and Randles 1997). The incidence of PSbMV was varied greatly between regions and this is in agreement with Ali and Randles (1997). The incidence and field spread of PSbMV in sampled crops depend on seed source, variety, locality, and active aphid vectors (Jones 2004). The seed transmission of PSbMV plays an important role in the early spread of the virus before aphid arrival (Congdon *et al*. 2016). Many pathotypes of the virus have been reported based on the symptom's reaction of hosts to PSbMV (Makkouk *et al*. 1993; Ali and Randles 1998; Chatzivassiliou *et al*. 2016; Congdon *et al*. 2017; Gheshlaghi *et al*. 2019). Mechanically inoculated plants induced various symptoms expression including systemic symptoms such as mild mosaic, severe mosaic, vein banding, downward leaf rolling, and necrotic or chlorotic local lesions. Some hosts showed no symptoms but gave a positive reaction when detected by RT-PCR (symptomless). This might be due to the low titer of the virus in these plant species. Host reactions obtained in this study were similar to those reported by (Makkouk *et al*. 1993; Congdon *et al*. 2017; Gheshlaghi *et al*. 2019). On other hand, our finding revealed that some of the hosts differed in their reactions from those previously reported. In this study, *Phaseolus vulgaris* L. reacted systemically with PSbMV and gave mild mosaic. These findings are in line with those of Almási *et al*. (2020) who reported that *Phaseolus vulgaris* cv. Maxidors gave strong systemic symptoms with mosaic when mechanically inoculated with PSbMV. The seed transmission results indicated that seed germination was significantly affected by PSbMV and the percentage of seed transmission was 18% (Khetarpal and Maury 1987) found that PSbMV was transmitted in the lentils with a percentage of 32-44% and through faba bean seeds with low percentage of seed transmission. Whereas Shukla *et al*. (1994)

reported that, seed transmission of the virus in lintel at a range of 0.2-44% and in pea at a range of 0.3-80%. Abraham and Makkouk (2002) found that the transmission of PSbMV through seeds reached 17 % in 31% of the lots when examining 270 lentil seed lots. Two sets of primers used in this study succeeded to amplify the expected size bands for the partially coat protein gene of a potyvirus and a portion of the *NIb* gene of PSbMV. The comparison between the PSbMV Egyptian isolate and those isolates available in GenBank is based on the Sequence and phylogenetic analysis of the partial coat protein gene of PSbMV. The results showed the percentage of similarity ranged from 95 to 100% of the twelve reported isolates of PSbMV with the Egyptian isolate (Accession Number: ON075784). Based on resistance trials, the most common strains of PSbMV were divided into four pathotypes: (P-1, P-2 or L-1, P-3, and P-4) and they are linked to known resistance genes (*sbm1*, *sbm2*, *sbm3*, and *sbm4*) of pea differential lines (Makkouk *et al*. 2014; Giakountis *et al*. 2015). Phylogenetic analysis for the nucleotide sequence of partial coat protein confirms the identity of PSbMV. BLAST involved the Egyptian isolate in a distinct cluster from Denmark and UK isolates and in the same cluster with Australia, Czech Republic, Greece, Germany, UK, Denmark, New Zealand, and USA isolates. According to our phylogenetic analysis, the PSbMV Egyptian isolate can be classified as the pathotype P-1 (Giakountis *et al*. 2015; Gheshlaghi *et al*. 2017; Almási *et al*. 2020).

Our study findings provide important information about the incidence of PSbMV and determine its epidemiology in different locations of our country. This study also clarified the molecular characteristics of the PSbMV Egyptian isolate. We recommend for future survey which could help in studying the genetic diveristy of PSbMV.

CONCLUSION

In the current study, the authors concluded that symptoms of PSbMV infection were observed in 25.2% and 28.32% of total pea surveyed fields in 2020 and 2021 respectively. The seed germination was significantly affected by PSbMV, as it decreased the germination rate by 56%. The virus was transmitted through seeds where the seedlings raised from the infected seeds were checked by R-TPCR and the percentage of seed transmission was 18%. The two sets of primers used in this study succeeded to amplify the expected size bands for the partially coat protein gene of a potyvirus and a portion of the *NIb* gene of PSbMV. The partial coat protein gene of PSbMV was submitted to GenBank under the accession number ON075784. The sequence comparisons showed the percentage of similarity ranged from 95-100% of twelve reported isolates of PSbMV with the Egyptian isolate of PSbMV.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

REFERENCES

Abdelmaksoud, H.M.; Gamal Eldin, A.S.; Ibrahim, S.M. and Sallam, A.A. 2000. Nucleotide sequence of the coat protein gene of pea seed borne mosaic *Potyviridae* (Egyptian strain). Proc. 7th Arab Congress of Plant Protection, Amman-Jordan 7:349.

Abou El-Salehein, E.H.; El-Gammal, M.I.; Salem, I.M. and Omar, E.R. 2019. Utilization of friendly fertilizers as an organic and NPK fertilizers on peas (*Pisum sativum* L.). Int. J. Environ., 8(2): 85-94.

Abraham, A. and Makkouk, K.M. 2002. The incidence and distribution of seed-transmitted viruses in pea and lentil seed lots in Ethiopia. Seed Sci. Technol., 30: 567-574.

Ali, A. and Randles, J.W. 1997. Early season survey of pea viruses in Pakistan and the detection of two new pathotypes of pea seedborne mosaic potyvirus. Plant Dis., 81(4): 343-347.

Almási, A.; Boros, R.; Salánki, K. and Barna, B. 2020. First report on pea seed-borne mosaic virus in Hungary. Acta Phytopathol. Entomol. Hung., 55(2): 161-166.

Andersen, K. and Johansen, I.E. 1998. A single conserved amino acid in the coat protein gene of pea seed-borne mosaic potyvirus modulates the ability of the virus to move systemically in *Chenopodium quinoa*. Virology, 241: 304-311.

Astier, S.; Albouy, J.; Maury, Y.; Robaglia, C. and Lecog, H. 2007. Principles of Plant Virology; Genome, Pathogenicity, Virus Ecology. Enfield, NH, USA: Science Publishers 494 pp.

Cerna, H.; Černý, M.; Habánová, H.; Šafářová, D.; Abushamsiya, K.; Navrátil, M. and Brzobohatý, B. 2017. Proteomics offers insight to the mechanism behind *Pisum sativum* L. response to pea seed-borne

mosaic virus (PSbMV). J. Proteomics, 153: 78-88.

Chatzivassiliou, E.K.; Giakountis, A. Kumari, S.G. and Makkouk, K.M. 2016. Viruses affecting lentil (*Lens culinaris Medik*.) in Greece; incidence and genetic variability of bean leafroll virus and pea enation mosaic virus. Phytopathol. Medi., 55(2): 239-252.

Congdon, B.S.; Coutts, B.A.; Renton, M.; Banovic, M. and Jones, R.A.C. 2016. Pea seed-borne mosaic virus in field pea: widespread infection, genetic diversity and resistance gene effectiveness. Plant Dis., 100(12): 2475-2482.

Congdon, B.S.; Coutts, B.A.; Renton, M.; van Leur, J.A.G. and Jones, R.A.C. 2017. Seed fractionation as a phytosanitary control measure for Pea seed-borne mosaic virus infection of field pea seed-stocks. Eur. J. Plant Pathol., 148: 733-737.

Coutts, B.A.; Prince, R.T. and Jones, R.A.C. 2009. Quantifying effects of seedborne inoculum on virus spread, yield losses, and seed infection in the pea seed-borne mosaic virus-field pea pathosystem. Phytopathology, 99: 1156-1167.

El-Banna, O.M.; Ibrahem, A.H.; Khattab, E.A.H. and El-Kammar, H.F. 2008. Isolation, identification, purification and production of ELISA reagent for pea seed-borne mosaic potyvirus affecting *Pisum sativum* L. plants in Egypt. Egypt. J. Virol., 5(2): 157-172.

FAO. 2020. FAOSTAT. FAO Statistics Division. Food and Agriculture Organization of the United Nations, Rome (Available online at) http://www.fao.org/faostat/en/#data

Fletcher, J.D. 1993. Surveys of virus diseases in pea, lentil, dwarf and broad bean crops in South Island, New Zealand. N. Z. J. Crop Hortic. Sci., 21(1): 45-52.

Gheshlaghi, T.M.; Shahraeen, N. and Rakhshandehroo, F. 2017. Report on host range and serological detection of pea seed borne mosaic virus from lentil (*Lens culinaris Medik*). The second national congress of monitoring and forecasting in plant protection, 9 March 2017. Gorgan: Gonbadcavoos University.

Gheshlaghi, T.M.; Shahraeen, N. and Rakhshandehroo, F. 2019. Serological and molecular characteristics of pea seed borne mosaic virus (PSbMV) from lentil (*Lens culinaris Medik* L.) in Iran. Arch. Phytopathol. Pflanzenschutz., 52(9-10): 873-883.

Giakountis, A.; Skoufa, A.; Paplomatas, E.I.; Tokatlidis, I.S. and Chatzivassiliou, E.K. 2015. Molecular characterization and phylogenetic analysis of a Greek lentil isolate of pea seed-borne mosaic virus. Phytoparasitica, 43(5): 615-628.

Hampton, R.O. 1984. Compendium of Pea Diseases, Pea seed-borne mosaic (p 34-35). American Phytopathological Society. 57pp.

Hampton, R.; Mink, G.; Bos, L.; Inouye, T.; Musil, M. and Hagedorn, D. 1981. Host differentiation and serological homology of pea seed-borne mosaic virus isolates. Neth. J. Plant Pathol., 87(1): 1-10.

Hjulsager, C.K.; Lund, O.S.; and Johansen, I.E. 2002. A new pathotype of pea seed-borne mosaic virus explained by properties of the P3-6k1- and viral genome-linked protein (VPg)-coding regions. Mol. Plant Microbe Interact., 15(2): 169-171.

Johansen, E.; Rasmussen, O.F.; Heide, M. and Borkhardt, B. 1991. The complete nucleotide sequence of pea seed-borne mosaic virus RNA. J. Gen. Virol., 72: 2625-2632.

Johansen, I.E. 1996. Intron insertion facilitates amplification of cloned virus cDNA in *Escherichia coli* while biological activity is reestablished after transcription *in vivo*. PNAS USA, 93: 12400-12405.

Jones, R.A.C. 2004. Using epidemiological information to develop effective integrated virus disease management strategies. Virus Res., 100(1): 5-30.

Kararah, M.A.; El-Banna, O.M.; Zein, S.N. and Abd-Elrehiem, A.F. 2014. Biological and serological studies on pea seed-borne mosaic virus (PSbMV) on cowpea forage crop *(Vigna unguiculata* L. Walp.). Egypt. J. Virol., 11(2): 288-310.

Khetarpal, R.K. and Maury, Y. 1987. Pea seed-borne mosaic virus: a review. Agronomie, EDP Sciences, 7(4): 215-224.

Kuhn, C.W. 1964. Separation of cowpea virus mixture. Phytopathology, 54: 739-740.

Langeveld, S.A.; Dore, I.M.; Memelink, J.; Derks, H.J. M.; van der Vlugt, C.I.M.; Asjes, C.J. and Bol, J.F. 1991. Identification of potyviruses using polymerase chain reaction with degenerate primers. J. Gen. Virol., 72: 1531-1541.

Latham, L.J. and Jones, R.A.C. 2001 Alfalfa mosaic and pea seed-borne mosaic viruses in cool season crop, annual pasture, and forage legumes: susceptibility, sensitivity, and seed transmission. Aust. J. Agric. Res., 52: 771-790.

Makkouk, K.M.; Kumari, S.G.; van Leur, J.A.G. and Jones, R.A.C. 2014. Control of plant virus diseases in cool-season grain legume crops. Adv. Virus Res., 90: 207-254.

Makkouk, K.M.; Pappu, H. and Kumari, S.G. 2012. Virus diseases of peas, beans, and faba bean in the Mediterranean region. Adv. Virus Res., 84: 367-402.

Makkouk, K.M.; Kumari, S.G. and Bos, L. 1993. Pea seed-borne mosaic virus: occurrence in faba bean (*Vicia faba*) and lentil (*Lens culinaris*) in West Asia and North Africa, and further information on host range, transmission characteristics, and purification. Neth. J. Plant Pathol., 99(3): 115-124.

Maury, Y. and Khetarpal, R.K. 1992. Pea seed-borne mosaic virus. Pages 74-92 in: Plant disease of international importance: Diseases of vegetables and oil seed crops. Vol II. H.S. Chaube, U.S. Singh, A.N. Mukhopadhyay, and J. Kumar, eds. Prentice Hall, Inc., Englewood Cliffs, NJ.

Musil, M. 1966. Über das Vorkommen des Blattrollens der Erbse in der Slowakei (Vorrlaufige Mitteilung). Biology, 21: 133-138.

Naimuddin, K. and Akram, M. 2021. Molecular characterization of pea seed borne mosaic potyvirus associated with apical rosette in field pea (*Pisum sativum* L.) in India. Arch. Phytopathol. Pflanzenschutz., 54(19-20): 1753-1763.

Safárová, D.; Brázda, P. and Navrátil, M. 2014. Effect of artificial dsRNA on infection of pea plants by pea seed-borne mosaic virus. Czech J. Genet. Plant Breed., 50: 105-108.

Safárová, D.; Navrátil, M.; Petrusová, J.; Pokorný, R. and Piáková, Z. 2008. Genetic and biological diversity of the pea seed-borne mosaic virus isolates occurring in Czech Republic. Acta Virol., 52(1): 53-57.

Shukla, D.D.; Ward, C.W. and Brunt, A.A. 1994. The potyviridae. CAB international Walling ford, U.K. 516 pp.

van der Vlugt, R.A.; Steffens, P.; Cuperus, C.; Barg, E.; Lesemann, D.E.; Bos, L. and Vetten, H.J. 1999. Further evidence that shallot yellow stripe virus (SYSV) is a distinct potyvirus and reidentification of welsh onion yellow stripe virus as a SYSV strain. Phytopathology, 89(2): 148-155.

Wang, D.; Woods, R.D.; Swaby, A.G. and Cockbain, A.J. 1991. Detection of pea seed-borne mosaic virus virion and pinwheel inclusion body proteins in leaf and seed tissues of pea (*Pisum sativum* L) by immunogold labelling. Agronomie, 11: 387-394.

Wylie, S.J.; Coutts, B.A.; and Jones, R.A.C. 2011. Genetic variability of the coat protein sequence of pea seed-borne mosaic virus isolates and the current relationship between phylogenetic placement and resistance groups. Arch. Virol., 156: 1287-1290.