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Biological and molecular characterization of an isolate of *Sweet potato feathery mottle virus* from sweet potato

Maha Adel Kawanna[1] and Dalia Gamil Aseel [2]

[1]Department of Plant Pathology, Faculty of Agriculture, (El-Shatby), Alexandria University, 21545, **Egypt**.
[2]Plant Protection and Biomolecular Diagnosis Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, New Borg El-Arab City, 21934, **Egypt.**

***Correspondence:** mahakawanna@yahoo.com **Accepted: 0**4 April. 2019 **Published online:** 20 May. 2019

Sweet Potato feathery mottle virus designated SPFMV-N was isolated from naturally infected sweet potato plants showing vein clearing. SPFMV-N reacted positively with SPFMV antiserum but not with of *Sweet potato latent virus* (SPLV), *Cucumber mosaic virus* (CMV) and *Sweet potato mild mottle virus* (SPMMV) using indirect ELISA and dot blot immunoassay (DBIA). The experimental host range restricted to some species of family convolvulaceae. SPFMV-N could be transmitted mechanically or by grafting through *Ipomoea* species. *Myzus persicae* was more efficient followed by *A. craccivora*, *A. gossypii* then *A. nerii* in transmission of SPFMV-N, while, *Dactynotus sonchi* failed. SPFMV was assessed by real-time reverse transcriptase polymerase chain reaction (qRT-PCR) assay in infected leaves and tubers and confirmed by coat protein gene sequencing and phylogenetic tree. SPFMV-CP specific pair primers amplified one band of approximately 300bp long. The phylogenetic tree revealed that SPFMV-CP is closely related to SPFMV from Japan with nucleotide sequence similarity of 87%. Deduced amino acid sequence showed variations of SPFMV-Egypt when compared with other amino acid sequence of other SPFMV isolates. Protein analysis of SPFMV-N revealed a major protein band of Mw ~46kDa assumed to be the P3, and minor protein band of Mw ~38kDa for coat protein and 35kDa were also observed.

Keywords: Sweet potato; SPFMV; qRT- PCR; Protein profile; Sequencing; Phylogenetic tree.

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) is a dicotyledonous plant belongs to family *Convolvulaceae*. It is the third most important root crop in the world, ranking the seventh among all crops in global production (FAO 2000). Sweet potato plants are significantly affected by viral diseases. More than 14 virus diseases of sweet potato have been reported (Brunt et al. 1996). *Sweet potato feathery mottle virus* (SPFMV; genus: *Potyvirus*; family: *Potyviridae*) is the most common virus of sweet potato growing area and causes variable symptoms (Brunt et al., 1996). SPFMV has a very wide geographical distribution and now probably occurs wherever sweet potatoes are grown. It has been reported in Egypt (Abo El-Abbas et al., 1998 and Ashoub et al., 2008); China (Zhang and Ma 1998); Italy (Parrella et al. 2006); Kenya (Nyaboga et al. 2008); Korea (Ryun Kwak 2007 and 2015); Syria (Akel et al., 2010); Tanzania (Tairo et al., 2004); Uganda (Adikini et al., 2016); United states (Souto et al. 2003); Western Kenya (Opiyo et al., 2010a), Zimbabwe (Chavi et al., 1997). Some isolates of SPFMV led to economic losses, especially in the susceptible cultivars (Clark and Moyer, 1988).

This work was undertaken to identify and characterize the causal virus based on host range and symptomology, investigate the modes of transmission, quantify the virus using qRT-PCR, amplification of SPFMV-CP gene by RT-PCR, sequencing and phylogenetically by analysis of the coat protein sequence and detection the protein profile of SPFMV-N.

MATERIALS AND METHODS

Source of the virus

Sweet potato plants exhibiting vein clearing symptoms of leaves were observed in Nubaria, Egypt. Samples of the naturally infected sweet potato plants were collected in plastic bags. Each sample was divided into two parts; the first part was planted in pots containing sand and clay (1:1) and kept under greenhouse conditions for subsequent studies, while the second part was tested serologically for virus infection using indirect ELISA, DBIA.

Serological diagnosis

Source of antisera

The antisera of four viruses namely, SPFMV; *Sweet potato latent virus* (SPLV); Cucumber mosaic virus (CMV) and *Sweet potato mild mottle virus* (SPMMV) known to be widely distributed on sweet potato crop were used in this study. The antisera were obtained from the collection of Virology lab., Plant Pathology Dept., Faculty of Agriculture, Alexandria University, Egypt.

Indirect ELISA

Indirect enzyme – linked immunosorbent assay (indirect ELISA) as described by Koenig (1981) with some modifications was used for virus detection. The plant samples were ground in coating buffer (0.5 M sodium carbonate, pH 9.6) 1:10 (w/v). Wells were coated with antigens by adding 100 µl of each sample to the bottom of the well and incubated for 2 hours at 37°C or overnight at 4°C. Wells were blocked by 0.2% bovine serum albumin (BSA) and 1.0% gelatin in PBST and incubated for one hour. One hundred µl of virus-specific antiserum (2µg /ml) in serum buffer [PBS-Tween 20 containing 2% soluble ploy vinylpyrolidone (PVP) and 0.2% BSA] were added to each well and incubated for 2 hours at 37ºC.

Goat anti-rabbit gamma globulin conjugated to alkaline phosphatase (whole molecule, enzymatic activity 475 units /ml) obtained from Sigma Chem. Co. St Louis, Mo (Production # A 8025) was diluted 1:1000 in serum buffer and 100 µl were added to each well, followed by incubation for one hour at 37°C, Plates were washed three times with PBST between each step.

One hundred µl of the enzyme substrate, 0.5 mg/ml para-nitrophenylphosphate (Sigma#104) in 10% diethanolamine buffer, pH 9.8 were added to each well and incubated at room temperature (25°C) for about 30 minutes.

The ELISA values, measured by Multi Skan Ex ELISA reader, were expressed as absorbance at 405 nm. Absorbance values of at least double that of healthy control, were considered positive.

Dot blot immunoassay (DBIA)

DBIA of Powell (1987) optimized by Fegla et al., (2001) was used with the following steps and washes in between: (1) A grid consisting of 1cm squares was drawing on nitrocellulose membrane sheet (NSM, 0.45 nm, Bio- Rod Laboratories, Richmond, CA) with pencil. The sheet was cut to a size that accommodate the number of samples in an individual test, then dipped in 0.02 M carbonate buffer pH 9.6 and placed on filter paper to dry (2) Samples of leaves tested were triturated in carbonate buffer and centrifuged at 8000 rpm for 20 min (3) Two microliter of the sample was spotted in the center of each grid square then, the membranes were placed in blocking buffer {2% bovine serum albumin (BSA) and triton X-100 solution in carbonate buffer pH 9.6}, (4) soaking the nitrocellulose membrane with the virus antiserum for 2 h, (5) adding the goat anti-rabbit IgG conjugated to alkaline phosphatase gently agitated for one hour. Finally, the membranes incubated in freshly prepared 5- bromo- 4- chloro- 3- indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT) substrate solution for 15-30 min. After colour development, the reaction was stopped by washing the treated membrane in 0.01M Tris- HCl containing 0.05 M EDTA, pH 7.5. The positive reaction of DBIA was indicated by the development of purple colour on the blots. The negative reaction (virus-free samples) developed no or green colour.

Biological characterization of the isolated virus

Serological diagnosis indicated the involvement of SPFMV. One isolate of SPFMV designated as SPFMV-N was maintained on *Ipomoea nil* (Moyer et al., 1980) that served as a virus sources for subsequent studies. Except otherwise stated virus inocula were prepared by

grinding infected leaf tissues 1:5 (W/V) in 0.1M phosphate buffer, pH 7.0, containing 0.1% 2-mercaptoethanol. Leaves to be inoculated were first dusted with carborandum (600 mesh) before inoculation with a freshly prepared inoculum using forefinger.

Host range and symptomology

Thirty-three of plant species and cultivars belonging to 8 families were used to study the host range of the SPFMV-N. Ten seedlings of each tested plant species or cultivars were mechanically inoculated with inoculum and kept under greenhouse conditions. Indirect ELISA was used to confirm the infection with SPFMV.

Mode of transmission

Mechanical transmission

Plants of *I. nil* were used as a virus source. The infected leaves showing typical symptoms were ground 1:10 (w/v) using a mortar and pestle in 0.1M phosphate buffer, pH 7.0, containing 0.5% 2- mercaptoethanol. Leaves of healthy *Convolvulus arvensis*, *I. nil* and *I. setosa* plants, first lightly dusted with carborandum (600 mesh), then rubbed with forefinger previously soaked in the freshly prepared inoculum. Inoculated plants were observed for symptoms development and the infected plants were calculated.

Aphid transmission

Non viruliferous apterous forms of *Aphis craccivora* Koch*, A. gossypii* Glover, *A. nerii* Boyer, *Dactynotus sonchi* L. and *Myzus persicae* Sulz. were starved for 120 min and transferred to infected sweet potato leaves. Aphids were given an acquisition feeding period of 5 min before transferring them to healthy plants of *I. nil*, at the rate of 10 aphids/plant and left for 20 min feeding period. The same procedure was applied to the control host plant but by using virus-free apterous forms of each aphid species. The aphids finally were killed with an aphidicide, Lambada-Cyhalothrin (5%).

The plants were kept under insect proof cages and observed carefully for symptoms development. Percentage of successful transmission was recorded in each case. Indirect ELISA was used to confirm the transmission of SPFMV-N.

Grafting transmission

Longitudinal cuts were made near the top of infected and healthy stems of *I. nil* plants. Both stems were tied together with plastic tape, then kept in shady place under greenhouse conditions. The plants were observed for symptoms development. Percentage of successful transmission was recorded in each case. Indirect ELISA was used to confirm the transmission of SPFMV-N.

Molecular characterization of SPFMV-N

Total RNA extraction

Frozen leaves and tubers infected with SPFMV were ground to a fine powder in liquid nitrogen using a mortar and pestle. Total RNA was extracted using (QIAGEN RNeasy Plant Mini Kit, Germany), according to the manufacturers' directions. All total RNA samples were treated on column with DNase I using the RNase-Free DNase Set (QIAGEN, Germany). The DNase treatment was performed as an optional step during the RNA extraction based on the manufacturer's directions and stored at -80˚C.

cDNA synthesis

The total RNAs were reverse transcribed using SPFMV-CP reverse primer. In each reaction, 3 µL RNA (30 ng) were added to 17 µL of reaction mixture (2.5 µL of 5x RT reaction buffer; 2 µL of 10 mM/L dNTPs; 1 µL of SPFMV-CP reverse primer (10 pmol/µL); 0.2 µL of reverse transcriptase (200U) (Bio Labs, UK) and 11.3 µL of sterile H_2O. RT-PCR amplification was performed in a thermal cycler (Eppendorf, Germany). The program was performed at 42ºC for 2 hours; enzyme inactivation at 65 ºC for 20 min and final step at 4 ºC for 10 min.

Detection and quantification of SPFMV-CP gene by quantitative real time polymerase chain reaction (qRT-PCR)

The qRT-PCR reaction consists of, 10µl of 2x Quantitech SYBR® Green RT Mix (BioLine, Germany), 1µL of each 10pmol/µL SPFMV-CP forward primer (5`-GCCGATTTTAACACCAGATGGA-3`) and SPFMV-CP reverse primer (5`-GGTTGCCCACTGTTATTTCCTT-3`), (primers were designed using coat protein gene sequence; accession no. AF439637) according to Kokkinos and Clark (2006), 1 µL cDNA (50 ng) and up to 7 µL of RNase free water 20 µL. Samples were spun before loading in the Rotor's wells. The real time PCR program was performed as follows: initial denaturation at 95 °C for 10 min.; 40 cycles of 95 °C for 15 sec.; annealing at 60 °C for 30 sec and extension at 72 °C for 30 sec. This reaction

was performed using a Rotor-Gene-6000-system (QIAGEN, USA). A 18S rRNA-F (TACCTGGTTGATCCTGCCAGTAG) and 18SrRNA-R (CCAATCCCTAGTCTGCATCGT) were used for reference gene. A 5 µL Real –Time PCR products were separated by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under UV light. Scoring for the presence or absence of DNA fragments was aided using a 1.5 kb DNA ladder (Promega, USA).

Data analysis:

Delta Delta Threshold cycle ($\Delta\Delta C_T$) expression values were calculated for RNA samples of SPFMV to determine gene expressions using 18S rRNA (reference gene) and the other SPFMV-CP gene. **$\Delta\Delta\ C_T$ expression = 2 $^{(-\Delta\Delta\ CT)}$**, the equations show the mathematical model of the relative expression ratio for the real time PCR. The ratio of the target gene is expressed in sample versus control in comparison to reference gene (Schmittgen and Livak 2008).

Amplification of SPFMV-CP gene using RT-PCR, sequencing and phylogenetic analysis

A total volume of 50 µL, containing 10 µL (10X PCR buffer), 1 µL of each 10pmol/µL SPFMV-CP forward (5'-CTTCAGTGACGTTGCTGAAGC-3') and reverse (5'-AAGAGGTTATGTATATTTCTAGTAA-3') primers according to (Ashoub et al., 2008), 0.5 µL of 1U Taq DNA polymerase (BioLine, Germany) and 1 µL of cDNA template and up to 36.5 µL mile Q water, was used. To amplify DNA fragments of 300 bp from the cDNA of SPFMV, PCR conditions for SPFMV-CP gene was as follows: one cycle of 2 min at 95°C and 30 cycles (1 min at 95°C, 1 min at 52°C, 1 at 72°C, followed by 10 min final extension at 72°C in a thermocycler. A PCR mix without template was used as negative control. PCR products were separated by electrophoresis as described by (Shaikhaldein et al. 2018).

PCR products were purified by using PCR clean up column kit (Maxim biotech INC, USA). DNA sequence for CP gene was performed by Sigma company. The sequence was analyzed using NCBI-BLAST (http://bast.ncbi.nlm.nih.gov/Blast.cgi) to confirm identify the SPFMV-CP. DNA sequence was alignment and compared with other SPFMV isolates available in the GenBank database. The sequences were used for comparison using MEGA 4 (Tamura et al., 2007), and phylogeny was tested with bootstrap method. The phylogenetic tree was analyzed and generated based on UPGMA statistic method. In addition, the DNA sequences were translated to deduce amino acids and aligned using the ClustalW2 Multiple Sequence program (Larkin et al. 2007; Rice et al. 2000).

Detection of protein pattern for SPFMV-N in sweet potato leaves and tuber by SDS-PAGE

Proteins of SPFMV-N isolated from leaves and tuber of sweet potato as well as healthy plants were separated by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) as described by Nelson et al. (1984). Gel electrophoresis was performed using the Mini-PROTEIN II vertical gel electrophoresis system (Bio**–**Rad); 5% staking gel and 12% resolving gel as following: Protein samples were diluted 3:1 (V/V) with sample loading buffer and denatured by heating at 90ºC for 5 min followed by cooling on ice. The samples were loaded into the gel and electrophoresis was carried out at about 80V in IX Tris /glycine-SDS running buffer. After electrophoresis, the gel was stained by shaking for 1 h in Coomassie brilliant blue R-250 stain and de-stained with de-staining solution overnight until the bands were clearly defined and photographed using white light.

RESULTS

Serological diagnosis

Serological diagnosis using indirect ELISA and DBIA (Fig. 1) confirmed the involvement of SPFMV. While, negative reaction was obtained with SPLV, CMV or SPMMV antisera.

Biological characterization of SPFMV-N

Host range and symptomology

The host range and the reactions of different plant species mechanically inoculated with SPFMV-N are summarized in Table (1). Results showed that, the host range of SPFMV-N is narrow. It restricted only in plant species belonging to family convolvulaceae out of the 8 tested plant families.

SPFMV-N induced chlorotic spots (Fig. 2A), green vein banding and yellowing (Fig. 2B, C) and mottle on the subsequent leaves (Fig. 2 D, E) of sweet potato cv. Abis.

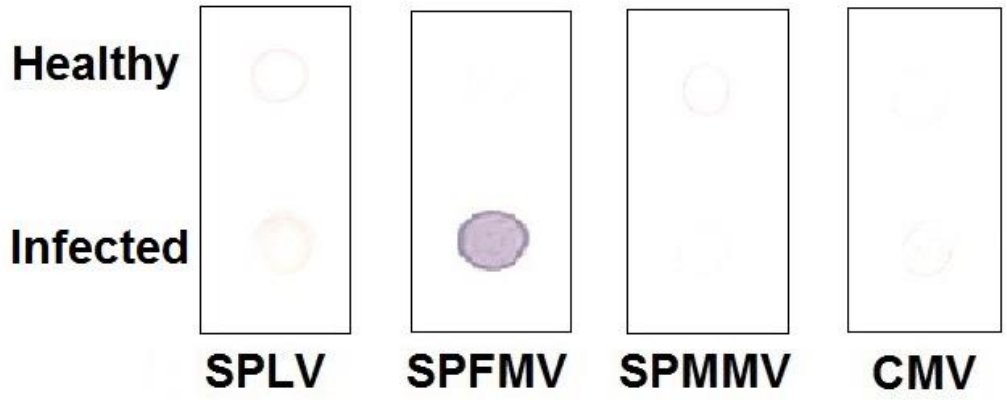

Figure. 1: Serological diagnosis by DBIA using four antisera SPFMV, SPLV, CMV and SPMMV.

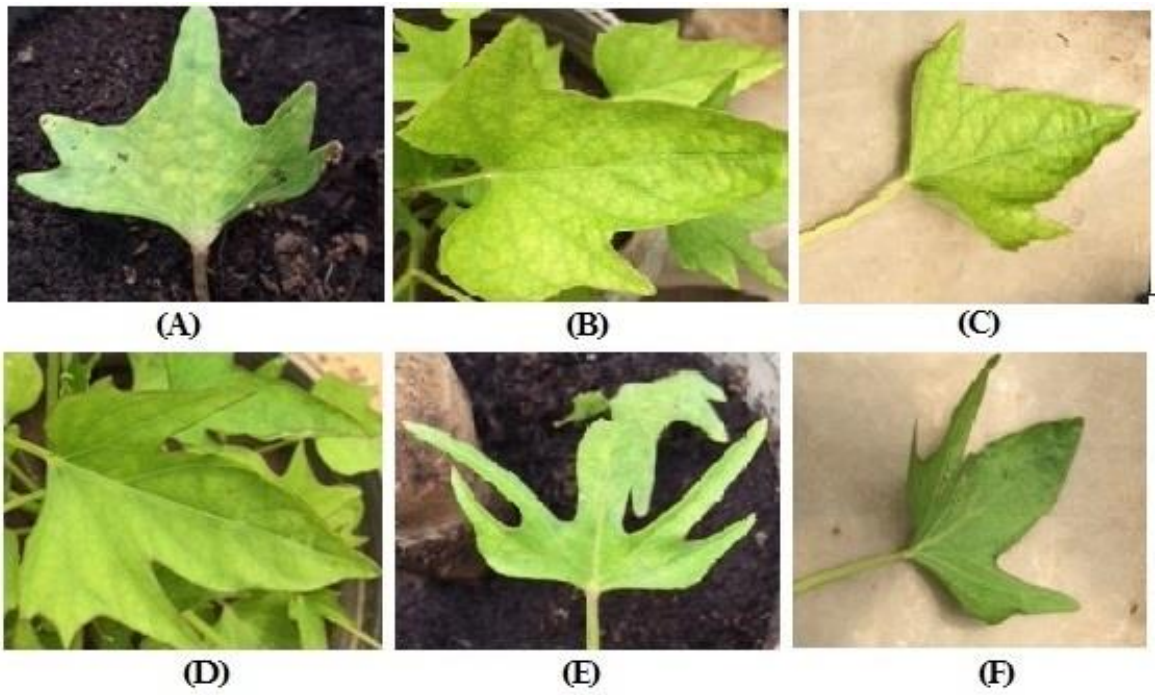

Figure. 2: (A) chlorotic spots on a sweet potato leaf, (B), (C) green vein banding and yellowing, (D), (E) mottle symptoms on sweet potato leaf, (F) healthy leaf.

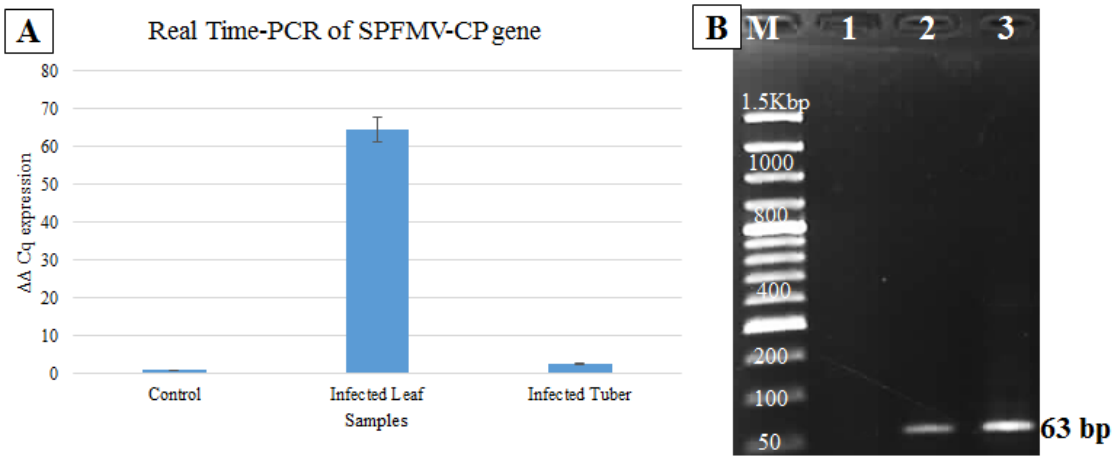

Figure. 3 A) Histogram of quantitative estimation of SPFMV-CP gene expression for infected sweet potato leaves and tuber. B) Real Time-PCR products of SPFMV-CP gene by agarose gel electrophoresis; M, 1.5 Kbp DNA marker; Lane1, Healthy control; Lane 2, SPFMV-N infected leaves; Lane 3, SPFMV-N infected tuber.

Table 1. Reactions of different plant species to inoculation with SPFMV-N.

Host plant		Reaction to Inoculation	Reaction of indirect ELISA*
Family & Scientific name	English name		
Fam. Amaranthaceae			
Amaranthus caudatus L.	Foxtail amaranth	-	-
Gomphrena globose	Globe amaranth	-	-
Fam. Asteraceae			-
Lactuca sativa	Lettuce	-	
Senecio vulgaris	Common groundsel	-	-
Sonchus oleraceus L.	Common sowthistle	-	-
Fam. Chenopodiaceae			
Beta vulgaris var. cicla	Swiss chard	-	-
Chenopodium album	Goosefoot	-	-
C. amaranticolor Coste and Reyen		-	-
C. quinoa Wild		-	-
Spinacia oleracea	Spinach	-	-
Fam. Convolvulaceae			
Convolvulus arvensis	Bindweed	M	+
Ipomoea batatus cv. Mabroka	Sweet potato	Cs, M, LC	+
Ipomoea b. cv. Abis	Sweet potato	Cs, VC, Mot	+
I. nil	Morning glory	Cs, VC, M	+
I. setosa	Morning glory	Cs, VC, VB, M	+
Fam. Cruciferae			
Brassica oleraceae L.	Cabbage	-	-
Brassica rapa L.	Turnip	-	-
Eruca sativa	Salad Rocket	-	-
Raphanus sativus	Raddish	-	-
Fam. Cucurbitaceae			
Cucumis melo L.	Cantaloup	-	-
Cucurbita pepo L.	Squash	-	-
Cucumis sativus L.	Cucumber	-	-
Fam. Fabaceae			-
Glycine max L.	Soybean	-	-

Lathyrus odoratus L.		-	-
Phaseolus vulgaris L.	Common bean	-	-
Pisum sativum L.	Pea	-	-
Vicia faba L.	Faba bean	-	-
Vigna sinensis L.	Cow pea	-	-
Fam. *Solanaceae*			
Capsicum annum L.	Pepper	-	-
Datura stramonium L.	Stramony	-	-
Lycopersicon esculentum L.	Tomato	-	-
Nicotiana glutinosa L.	Tobacco	-	-
Solanum melongena L.	Egg plant	-	-
Solanum nigrum L.	Black nightshade	-	-

Abbreviations: CS=chlorotic spots, LC=leaf curling, M=mosaic, Mot=mottle, VB= vein banding, VC=vein clearing, Y=yellowing and - =not infected.

*Indirect ELISA was used 15 and 30 days after inoculation. (+)= positive, (-)= negative reaction.

No symptoms developed on and no virus was recovered from *Amaranthus caudatus*, *Gomphrena globosa*, *Lactuca sativa*, *Senecio vulgaris*, *Sonchus oleraceus, Chenopodium album, C. amaranticolor, C. quinoa, Spinacia oleracea, Brassica oleraceae, Brassica rapa*, *Eruca sativa*, *Raphanus sativus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita pepo, Glycine max*, *Lathyrus odoratus*, *Phaseolus vulgaris*, *Pisum sativum, Vicia faba*, *Vigna sinensis*, *Capsicum annum*, *Datura stramonium, Lycopersicon esculentum, Nicotiana glutinosa, Solanum melongena, S. nigrum* (Table 1).

Mode of Transmission

Mechanical transmission

Results showed that SPFMV-N were mechanically transmitted from *I. nil* to *C. arvensis*, *I. nil* and *I. setosa* having transmission rate of 40, 50%, and 40%, respectively. Inoculated plants were confirmed to be virus free using indirect ELISA.

Aphid transmission

Results presented in Table (2) revealed that SPFMV-N was transmitted in a nonpersistent manner by four of six aphid species tested. *M. persicae* Sulz transmitted the virus with the highest rate 55% followed by *A. craccivora* Koch, *A. gossypii* Glover then *A. nerii* Boyer with transmission rate of 35, 20 and 10%, respectively. *Dactynotus sonchi* L. could not able to transmit the isolated virus.

Grafting transmission

SPFMV-N can be transmitted easily by grafting from *I. nil* plants to other *I. nil* plants. The transmission rate was 50%.

Molecular characterization of SPFMV-N

Quantitative Real-Time PCR of SPFMV-CP gene expression

The relative amounts of viral RNA for target SPFMV-CP of both leaves and tuber was compared with the amount of viral RNA of healthy sweet potato and the results normalized to 18S rRNA gene. As shown in Figure (3A), the quantitative estimation of SPFMV-CP gene expression indicated that, the highest expression level was observed in sweet potato leaves infected by SPFMV (64.51) but the low level of gene expression of SPFMV-CP in case sweet potato tuber infected by SPFMV (2.46).

Figure (3B) showed the Real-Time PCR of the size expected bands 63 bp for SPFMV-N in sweet potato leaves and tuber.

Amplification of SPFMV-CP gene using RT-PCR, sequencing and phylogenetic analysis

cDNAs of SPFMV isolate were subjected to PCR amplification using the sense and antisense specific primers to detect and amplify the CP gene. SPFMV-CP specific primers amplified one band with approximately 300 bp (Figure 4A).

Further, the partial sequences of the CP gene for SPFMV was aligned and compared with other SPFMV-CP isolates of different geographical overseas available in the GenBank database. The phylogenetic relationships were generated using MEGA4. The phylogenetic tree revealed that SPFMV-CP is closely related to SPFMV from Japan with nucleotide sequence similarity of 87% (Figure 4B).

The results of deduced amino acids sequence showed that variations with SPFMV, i.e. D → S, T→ A, H→Q, V→A, L→F and E→K substitutions in SPFMV-N isolate from Egypt compared with amino acids of SPFMV isolates from USA, Japan

and China (Figure 4C).

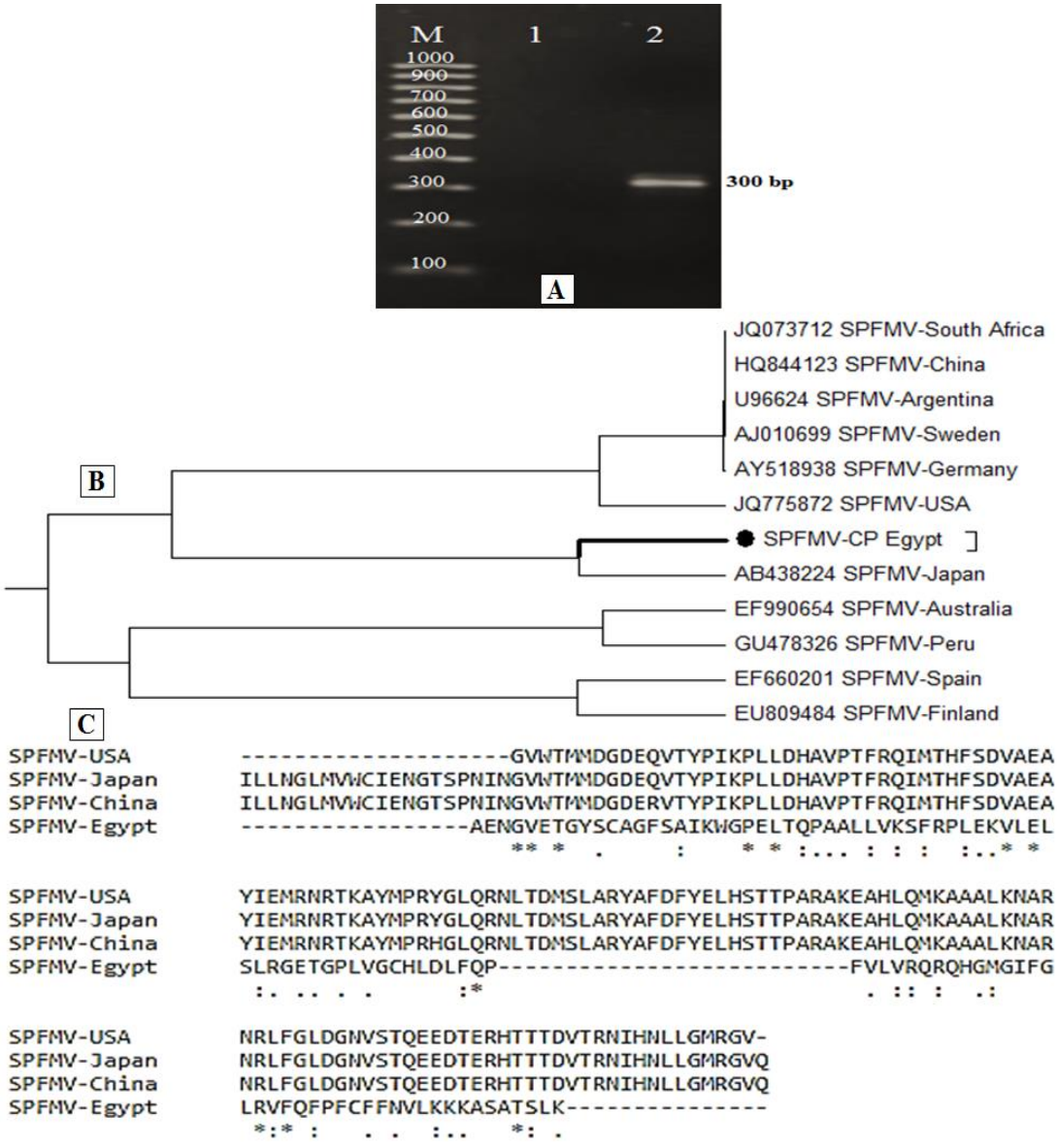

Figure. 4 A) PCR products of SPFMV-CP gene by 2% agarose gel electrophoresis; M, 1 Kbp DNA marker; Lane1, Negative control (Healthy sweet potato plant); Lane 2, sweet potato leaf infected with SPFMV-N. B)

Phylogenetic tree of the coat protein gene of SPFMV-N isolate, the phylogenetic tree was established from the other nucleotide sequences of the SPFMV- coat protein genes available in the GenBank. C) Multiple alignment of deduced amino acids of SPFMV-CP with different deduced amino acids of SPFMV isolates available in Genbank. Completely conserved residues across all the aligned sequences marked with an asterisk (*) below. Absent amino acids are indicated by dashes (-), degeneracy by (:) and substitution by (.).

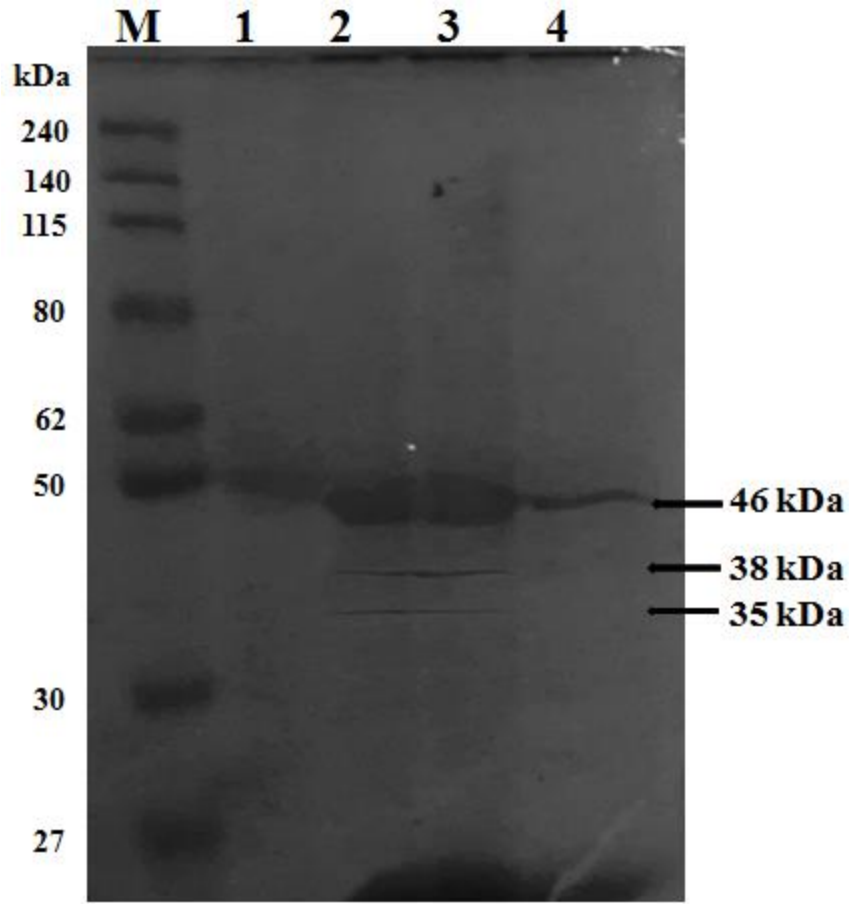

Figure. 5 SDS-PAGE analysis showing the protein profile of sweet potato infected by SPFMV-N. where M, protein marker; Lane 1, Healthy plant (sweet potato control), Lane 2-3, Infected sweet potato leaves, Lane 4, Infected sweet potato tuber.

Table 2. Transmission of SPFMV-N by some aphid species

Aphid species*	Transmission	
	Rate**	%
***Aphis craccivora* Koch**	7/20	35
***A. gossypii* Glover**	4/20	20
***A. nerii* Boyer**	2/20	10
***Dactynotus sonchi* L.**	0/20	0.0
***Myzus persicae* Sulz.**	11/20	55

* Ten aphids per plant were used.
** No. of infected plants / No. of tested plants.

Detection of proteins pattern for SPFMV-N in sweet potato leaves and tuber by SDS-PAGE

Protein analysis of healthy leaves (sweet potato) and SPFMV-N extracted from leaves and tuber tissues of sweet potato preparations using SDS-PAGE revealed a major protein band of Mw ~46kDa, and this was assumed to be the P3. Minor protein bands of Mw ~38kDa for coat protein and 35kDa were also observed. Moreover, we observed that the proteins of sweet potato plant (healthy) and viral proteins of SPFMV-N were degraded upon storage at -20°C over time (Figure 5). The molecular weight of coat protein subunit of the isolated virus was approximately 38 kDa.

Discussion

Sweet potato is one of the most important tuber crops worldwide. Yield of sweet potato is

affected by a number of factors including infection by several pathogens. Viruses are a major biotic constraint on sweet potato. One isolate of *Sweet potato feathery mottle virus* (SPFMV) designated as SPFMV-N isolated from Nubaria location, Alexandria governorate, Egypt. Partial characterization of the obtained isolate was based on biological and molecular studies.

The host range of SPFMV-N is narrow, constrained only in plant species belonging to family convolvulaceae out of the 8 tested plant families. Such results are agreed with Moyer and Salazar (1989) and Wolters et al., (1990). On the contrary, Moyer et al., (1980) reported that SPFMV isolate of North Carolina can infect *Nicotiana benthamina* and *Chenopodium amaranticolor*.

The range of symptoms associated with infection by SPFMV are as much influenced by host genotype and environment as by virus strain or isolate (Clark and Moyer 1988). SPFMV-N induced chlorotic spots, green vein banding and yellowing mottle on the subsequent leaves of sweet potato cv. Abis. Sweet potato plants infected with SPFMV developed faint chlorotic spots and vein clearing (Tairo et al., 2004). Symptoms varied according to the host plant, plant cultivar and environmental factors.

Symptoms produced on the differential hosts by the isolated virus resemble to some extent, those induced by other isolates of SPFMV recorded by Moyer and Kennedy (1978); Brunt et al., (1996); Chavi et al., (1997). SPFMV induced chlorotic local lesions on *C. amaranticolor* and *C. quinoa* and *vein clearing like symptoms in Cucumis sativus* (Chavi et al., 1997). Our isolate failed to infect these hosts. The main natural host of SPFMV is sweet potato, although the virus also, occurs in wild Ipomoea species which are considered as reservoirs of SPFMV (Clark et al., 1986).

Virus transmission is one of the most important factors in understanding a virus outbreak and its epidemiology (Kim et al., 2015). Thus, several ways of virus transmission were investigated in the current work. SPFMV-N were mechanically transmitted from *I. nil* to *C. arvensis*, *I. nil* and *I. setosa* with transmission rate of 40, 50%, and 40%, respectively. Moyer and Kennedy (1978) revealed that, SPFMV can easily transmitted to *I. nil* plants.

SPFMV-N was transmitted in a nonpersistent manner by *M. persicae* Sulz with the highest rate 55% followed by *A. craccivora* Koch, *A. gossypii* Glover then *A. nerii* Boyer with transmission rate of 35, 20 and 10%, respectively. Such results agreed with those of Moyer and Kennedy (1978) and Brunt et al. (1996). Aphids are known to be an important tool for naturally distribution of SPFMV. *Dactynotus sonchi* L. could not able to transmit the isolated virus. The efficiency of transmission could be varied according to virus isolate and aphid species.

SPFMV-N can be transmitted easily by grafting from *I. nil* plants to other *I. nil* plants with transmission rate of 50%. These results are agreed with Prasanth and Hegde (2008) and Rukarwa et al. (2010) who showed that grafting can transmit SPFMV to *I. setosa*. Loebenstein et al., (2015) used grafting to test the freedom of viruses of sweet potato.

The quantitative estimation of SPFMV-CP gene expression indicated that, the highest expression level was observed in sweet potato leaves infected by SPFMV (64.51) while the low level (2.46) in case sweet potato tuber infected by SPFMV. Our data generally are agreed with those of Kokkinos and Clark (2006), who found that, the titers of SPFMV isolate were very low and this titer differed according to the plant cultivar tested and the period after inoculation.

The expected size of the bands for SPFMV-N in sweet potato leaves and tuber of Real-Time PCR was 63 bp. Similar results have been reported by Kokkinos and Clark (2006) who reported that the primer/probe SPFMV set amplifies a 63 bp fragment from the coat protein gene.

Using a pair of SPFMV-CP specific primers amplified single band of approximately 300 bp. Our data agree with those of Ashoub et al. (2008). Moreover, Opiyo et al. (2010b) found that the PCR products of the size expected for SPFMV (703 bp) were amplified from double-infected samples by both sPCR and mPCR but no amplicons were obtained from healthy plants. RT-PCR was very efficient in distinguishing healthy and SPFMV-infected plants.

The partial sequence of CP gene for SPFMV-N was aligned and compared with SPFMV-CP isolates sequences available in the GenBank database. The highest similarity obtained between SPFMV-N and Japan isolate with 87%. Nearly our results are in agreement with those of Ryun Kwak et al. (2007) who found that the group O was contained isolates K2 (Korea), O (Japan), TZ4 (Tanzania) and Arua10a (Uganda) to five samples (96-4, 152, 342, 583-1, and 607-2) were used. The CP sequences with similarity 97.4-99.6% and 97.1-100% of these isolates and aa levels,

respectively. Moreover, group RC including (70.6%) of isolates K1 (Korea), XN3 (China), and S (Japan). The CP sequences were identical 96.9-99.2% with group RC and 95.9-99.4% identical at aa levels. While, no SPFMV strains have been found in Korea belonging to group EA and C. Also, the SPFMV isolates CW137 and IS90 from Korea had similarity 98- 99% with the RC strain, and the SPFMV-GJ122 isolate had identity 96-98% with the O strain (Ryun Kwak et al., 2015**)**.

The results of deduced amino acids sequence showed variations with SPFMV, i.e. D → S, T→ A, H→Q, V→A, L→F and E→K substitutions in SPFMV-N isolate from Egypt. Our results are agreed with those of Ryun Kwak et al. (2007)**.**

Protein analysis of sweet potato leaves or tuber tissue infected with SPFMV-N and healthy leaves using SDS-PAGE revealed a major protein band of Mw ~46kDa, which be expected to be the P3. Minor protein bands of Mw ~38kDa for coat protein and 35kDa were also observed. Our notice that, the proteins of sweet potato plant (healthy) and viral proteins of SPFMV-N were degraded during storage at -20°C over time. Abad et al., (1992) obtained a major protein band of purified virus suspensions with molecular weight about 40 kDa and some additional bands of 46, 35, 31, and 27 kDa. The protein band 46 kDa was not detectable after exposing of the purified preparation to an additional cesium chloride density gradient centrifugation run; it considered as host protein. The other proteins may referred to partial degradation of the viral capsid protein and they differed according to the purification procedure followed. Furthermore, the molecular weight of coat protein subunit of the isolated virus was approximately 38 kDa. Such results were in harmony with those of Moyer and Cali (1985) and Brunt et al., (1996).

CONCLUSION

We can conclude that serological diagnosis with antisera specific for four common viruses; SPFMV, SPLV, CMV and SPMMV affecting sweet potato using indirect ELISA and DBIA revealed the presence of SPFMV with samples collected from naturally infected sweet potato showing vein clearing symptoms. The virus isolate is designated as SPFMV-N. Partial characterization of SPFMV-N was achieved based on host range, mode of transmission, qRT-PCR, amplification of SPFMV-CP gene using RT-PCR, sequencing and sequence analysis with phylogenetic tree and proteins pattern.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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To achieve this goal, this study was carried out at Plant Pathology Department, Faculty of Agriculture, Alexandria University and Plant Protection and Biomolecular Diagnosis Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications.

AUTHOR CONTRIBUTIONS

MAK performed the serological and biological studies of SPFMV-N. Also, she wrote and revised the manuscript. DGA designed the experiment of the molecular characterization such as; qRT-PCR and data analysis, specific PCR, sequencing, bioinformatics analysis, protein profile, participated in the writing and the revision of the manuscript. All authors read and approved the final version.

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