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Investigations on the Prevalence of Two Sweet Potato Viruses and their Potential Weed Reservoirs in Egypt

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GLOBALLY, sweet potato (*Ipomoea batatas* (L.) Lam., family *Convolvulaceae*), is an important vegetable crop, unluckily, the viral infection induces a significant yield loss. The current study aims to identify the viruses infecting sweet potato in Egypt and highlights the role of the interfacing weeds as viruses reservoir. The study covered both of transmission, host range, inclusion bodies, virus morphology, histopathology, serological diagnosis, genome identification using RT-PCR and multiplex RT-PCR. The results revealed that the potyviruses *sweet potato feathery mottle virus* (SPFMV) and *sweet potato virus G* (SPVG) are the most devastating viruses affecting sweet potato in Egypt. Coinfection with of both viruses was the common feature in both of sweet potato and its interfacing weeds. A wider host range in families Convolvulaceae, Chenopodiaceae and Euphorbiaceae, was detected. The infection symptoms on sweet potato were comparable to that detected in the interfaced weeds. The infection-incidence in perennial Convolvulaceae and Tiliaceae weed-species showed 100% virus incidence. The characteristic amorphous inclusion bodies for the detected viruses and the cytopathological alterations on the host cells were observed under Transmission Electron Microscopy (TEM). The nucleotide sequences of the partial coat protein gene of the detected viruses were compared to the isolate available in GenBank. This work recommends the importance of weed interfacing crops as virus reservoir, and in light, the importance of multidisciplinary research work to resolve the virus problems threats in potential crops. Identifying viruses and their incidence increase the efficiency of the management strategies to control the virus spread.

Keywords: Incidence, Interfacing weeds, RT-PCR, SPFMV, SPVG, Sweet potato, Virus.

Introduction

Globally, sweet potato (*Ipomoea batatas* (L.) Lam., family *Convolvulaceae*), is an important vegetable crop that grows in tropical and subtropical areas. It ranks the seventh most important food crop worldwide after wheat, rice, maize, potato, barley and cassava (Luan et al., 2007). It is an important source of carbohydrates and protein for human and animal consumption. In Egypt, sweet potato is one of the most economically important vegetable crops. The sweet potato cultivation in Egypt during 2018 occupied an area c. 28525.86 Fadden (Feddan 4200m^2), with average yield c. 11.767 Tons/ Fedden (FAOstat, 2020).

At present, 35 viral species have been reported to infect sweet potato crops worldwide (Souza et al., 2018) which comprises the major biotic constraint on sweet potato production worldwide. In 2005, 10-60% viral disease incidence was observed in sweet potato fields (Prasanth & Hegde, 2008) and the field monitoring indicated that 58% of plants were found to be virus-infected. According to Tibiri et al. (2019a), the

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severe infection symptoms were associated with the SPVD infecting sweet potato leading to yield reduction**.** Over 30 viruses infecting sweet potato assigned to nine families, including *Potyviridae*, *Bromoviridae*, *Caulimoviridae*, *Closteroviridae, Comoviridae, Flexiviridae, Geminiviridae, Luteoviridae, and Bunyaviridae,* have been dentified **(**Clark & Hoy, 2006; Adikini et al., 2016). In Africa, sweet potato was grown in many countries, but viruses infecting the crop have been studied mostly in sub-Saharan Africa where they cause yield reductions of 56–98% (Ngeve & Bouwkamp, 1991). The yield and quality of the storage roots were substantially decreased by virus diseases (Luan et al., 2007). Sweet potato virus disease (SPVD) is the name commonly used in Africa to describe a range of severe symptoms on sweet potato generally attributed to the virus infection stunting, shrinkage, crinkled, deformity, distorted leaves, a pale green mosaic, purpling on leaves, chlorotic yellowing, chlorosis, mosaic and vein-clearing (Gibson et al., 1998; Ma et al., 2019).

The most severe disease found in the cultivated sweet potato in Egypt which caused by two viruses from family *Potyviridae* namely *sweet potato feathery mottle virus* (SPFMV) and *sweet potato virus G* (SPVG) (Kwak et al., 2014; Ma et al., 2019). Since 2003, both of the two viruses (SPFMV and SPVG) were detected in naturally infected sweet potato plants grown in the Delta region in Egypt (IsHak et al., 2003).

Hondo et al. (2018), described the indirect enzyme-linked immune-sorbent assay (Indirect – ELISA), tissue blot and dot blot immune-binding assays both are **s**erological methods in which the reactions carried out using specific antibodies for SPFMV detection. While Tesfaye (2010), described the method used for SPVG detection.

Tugume et al. (2016), claimed that wild flora acts a virus reservoir causing significant losses in nearby crops, unfortunately, quite limited information about viruses in wild species is available. Viruses are better adapted to the wild plants, and infection in wild plants often symptomless; his study showed that 15.8% of the tested symptomless wild plants showed positive results for at least one virus. In spite of, these viruses having obvious symptoms in nearby cultivated sweet potatoes. Tugume et al. (2016), studied the SPCSV incidence in wild plants 5.4% of the tested wild species belonging to the family Convolvulaceae showed positive reaction using DAS-ELISA; out of the 27% showed virus-like symptoms. The role of weeds in crop diseases in Africa has not been well documented (Tibiri et al., 2019b).

Kwak et al. (2014) used the molecular assays by RT-PCR utilizing specific primers, multiplex RT-PCR and nucleotide sequencing, to examine the occurrence of SPFMV and SPVG. While IsHak et al. (2003), used the partial genomes sequences to study the viral phylogeny compared to the available data in GenBank. Also, Ma et al. (2019) tested sweet potato samples by one step RT-PCR using specific primers for each virus and the results confirmed the presence of SPFMV and SPVG and recorded as the main viruses in sweet potato in southern China.

Sweet potato feathery mottle virus (SPFMV; genus *Potyvirus*, family Potyviridae), the most common virus occurring virus in sweet potato. Virions are filamentous, not enveloped, usually flexuous, and with a modal length of 830–850nm. The genome consists of single-stranded linear RNA, with a poly(A) region (Loebenstein, 2015; Yan et al., 2020). The genomes range in size from 10,803 to 10,891 nucleotides (Wokorach et al., 2020). The virus can be transmitted mechanically to various hosts (Loebenstein, 2015).

Sweet potato virus G (SPVG, genus *Potyvirus*, family Potyviridae), SPVG was the second-most commonly occurring virus in sweet potato, it found mostly in mixed infections with SPFMV (Sivparsad & Gubba, 2013). SPVG is a single-stranded linear RNA virus. The complete genome sequence length was 10,781 nucleotides (Maina et al., 2016). SPVG was successfully transmitted mechanically to another hosts (Loebenstein & Lecoq, 2012).

The aim of the current study is to test and determine the incidence of SPFMV and SPVG in the cultivated sweet potato and their interfacing wild weeds showed virus-like symptoms. Virus characterization by its cytopathological examination by electron microscopy. In addition, the host range of the studied viruses (SPFMV and SPVG) and its genome identification using RT-PCR and multiplex RT-PCR, finally compared to that in database.

Materials and Methods

Source of virus isolates and field incidence on sweet potato

A total of 76 symptomless and virus-like symptoms sweet potato "cultivar A 195" samples were collected in October 2019 from El- Menufia governorate (Nile Delta agro-ecological zone) to detect the natural viral infection with viruses (*sweet potato feathery mottle virus* SPFMV and *sweet potato virus G* SPVG) and to study the virus incidence of natural infected sweet potato plants and associated weeds. The incidence of viral infection on the collected sweet potato samples was carried out by indirect enzyme-linked immune-sorbent assay (Indirect–ELISA) using specific polyclonal antibodies. The achieved results confirmed by molecular techniques (RT-PCR) utilizing specific primers. Samples maintained in pots in an insect-proof greenhouse at 20-25°C to be kept away from potential insect vectors.

Isolation and propagation of virus isolate

The naturally infected plants that showed positive SPFMV and SPVG by indirect-ELISA and RT-PCR used as a source of viral isolates. The virus' isolates were inoculated on leaves of *Chenopodium quinoa* and *Chenopodium amaranticolor* plants using one local lesion-technique (Price, 1945) and was kept under greenhouse condition and used as a virus source for the other identification tools. Single local lesions were then propagated on *Ch. amaranticolor* by mechanical inoculation on three times of purification was repeated according to Tang et al. (2013). Isolated viruses confirmed by studying the symptomatology, biological, serological and molecular studies.

Host range and symptomatology:

A total of 21 cultivated species belonging to six families (*Convolvulaceae, Euphorbiaceae, Leguminaseae, Cucurbitaceae***,** *Chenopodiaceae* and *Solanaceae***)**, as shown in Table 2. These species were used to study the host range of the SPFMV and SPVG. Ten seedlings of each tested plant species/cultivar were mechanically inoculated with viral inoculums obtained from positive naturally infected samples and kept under greenhouse conditions. Infected sweet potato plants were collected and tested for the viral infection by indirect-ELISA and RT-PCR.

Field sampling of weeds

The weeds associated with sweet potato field at El- Menufia governorate were excavated from the fields for virus detection. Fifty-four weed samples belonging to 18 species (3 samples/ species). These weeds were grouped into 10 plant families (dicotyledons & monocotyledons). Four perennials and 14 were of annual life duration. Each of the fifty-four samples was placed in a clean plastic bag, kept in an ice tank while transferred to the laboratory. Duplicate specimens were sent to Cairo University Herbarium (CAI), for taxonomic authentication. Similarly, a total of 76 sweet potato samples were also collected from the vicinity of the collected weeds samples for virus infection incidence.

Incidence of viral infection on the collected fifty-four weed samples were carried out by indirect enzyme-linked immune-sorbent assay (Indirect–ELISA) using specific polyclonal antibodies. RT-PCR utilizing specific primers were applied to the positive SPFMV and SPVG samples, to confirm the detected viruses.

Mechanical mode of transmission

Plants of naturally infected sweet potato (cultivar A 195) used as a virus source. The infected leaves showing typical symptoms as crinkled, distorted leaves, a pale green mosaic, purpling on leaves, chlorotic yellowing, chlorosis, mosaic, and vein-clearing were grinding 1:10 (w/v) using a mortar and pestle in 0.1M phosphate buffer, pH 7.0, containing 0.5% 2- mercapto-ethanol. The sap clarified through two-layer of cheesecloth; an equal number of healthy plants of the same age and cultivar were rubbed with buffer and kept serving as a control. Indicator plants and control were maintained in darkness for 16 to 24hrs before inoculation (Kawanna & Aseel, 2019). Leaves of healthy *Chenopodium amaranticolor*, *Ch. quinoa* and other hosts first lightly dusted with carborundum (600 mesh) as an abrasive prior to inoculation. Leaves (2-4), were inoculated per plant, and the inoculated leaves were briefly rinsed with tap water after inoculation. The inoculated plants were kept in greenhouse under daily observation up to 21 days for symptoms development. Plants showed no symptoms were checked by back inoculation to the indicator hosts; results were confirmed by RT-PCR.

Serological diagnosis (Indirect ELISA, DBIA & TBIA)

For the Indirect ELISA: the presence or absence of sweet potato viruses SPFMV and SPVG were determined at samples (cultivar A 195) obtained

from El- Menufia governorate by indirect-ELISA (Hampton et al., 1978) by using specific IgG against SPFMV and SPVG. The absorbance values using Vniskan ELISA reader were measured at 405nm. Absorbance values of the tested sample when exceeded more than twice of negative control were considered positive results.

For Dot Blot Immunoassay (DBIA) and Tissue Blot Immunoassay (TBIA): both reaction were carried out using specific antibodies for the detected SPFMV and SPVG. The tested samples (cultivar A 195), obtained from El- Menufia Governorate. Eight tested samples, one positive control sample and one negative control sample were printed directly on a nitrocellulose membrane in TBIA, and in the case of DBIA samples were grinding, purified and applied about 2µL on nitrocellulose tissue. The reactions were carried out as described by Lin et al. (1990).

RT-PCR and multiplex RT-PCR molecular detection of sweet potatoes viruses

Molecular characterization of the SPFMV and SPVG isolates was done by the RT-PCR analysis. For RT-PCR, total RNAs were extracted from samples (cultivar A195) obtained from El-Menofia governorate, about 100 mg of infected sweet potatoes leaves as a source of the virus using gene jet ™ RNA purification kit (Fermentas, USA). The extracted RNA was used as a template for one tube RT-PCR amplification reaction using Verso TM one-step RT- PCR kit (Thermo scientific) utilizing specific primers for the SPFMV SPFMV-1-F: 5`-TACACACTGCTAAAACTAGG-3` and SPFMV1-R: 5`-AGTTCATCATAACCCCATGA-3` and specific primers for SPVG SPG 3-F:5`-CAATGCCAAATGGAAGAATAG-3` and SPG3-R:5`- GCATGATCCAATAGAGGTTTTA -3`.

For each sample the RT-PCR was performed in 25µL total volume containing 4.75µL of nuclease-free water, 3ng/µL of total RNA, 12.5µL of one-step PCR master mix,3µL of 10µM of each primer, 1.25µL RT-Enhancer and 0.5µL Verso enzyme mix. Multiplex RT-PCR was done for SPFMV and SPVG in 25µL total volume containing 2µL of nuclease-free water, 3ng/µL of total RNA, 12.5µL of one-step PCR master mix, 3µL of 10µM of each primer of SPFMV and SPVG,0.5µL Verso enzyme mix and 1.25µL RT-Enhancer.

The first step in the RT reaction is incubation at 50˚C for 15min, followed by denaturation at 95˚C for 5min. The amplification reaction was performed through 35 cycles in T-Gradient thermal cycler (Biometra, Germany) starting with denaturation at 94˚C for 30sec, primer annealing at 55˚C for 30sec and extension at 72˚C for 60sec. The last step in this reaction is the final extension at the end of the 35th cycle was performed at 72˚C for 10min. The PCR products were stained with gel star (Lonza, USA) and analyzed by electrophoresis in 1.5% agarose gel and visualized by UV illumination using the Gel Documentation System (Gel Doc 2000, Bio-Rad, USA).

Nucleotide sequencing and bioinformatics study

SPFMV and SPVG specific bands of PCR fragment of the partial coat protein gene (products) were directly sequenced using Automated DNA sequencing. The forward and the reverse primers (SPFMV-F and SPFMV-R) and (SPVG-F and SPVG-R) those used for PCR were also used for DNA sequencing. The retrieved nucleotide sequences were analyzed Using DNAMAN Sequence Analysis Software (Lynnon Bio Soft. Quebec, Canada) and compared with the coat protein sequences of the SPFMV and SPVG isolates available in GenBank.

Histo-cytopathological studies by transmission electron microscopy (TEM)

Electron microscopy was used to observe the virus structure and to show the cytoplasmic inclusions of the viruses in the infected tissues. Some of these characteristics inclusions are often specific to viral families and are valuable for virus identification. The long flexuous particles of potyviruses can be observed by the transmission electron microscopy (TEM) of the leaf – dip preparation or in thin sections of infected sweet potato samples; confirming the identification of these viruses. These viruses can be identified by the production of characteristic pinwheel cytoplasmic inclusions induced by potyvirus infection (Tennant, 2015).

Healthy fresh sweet potato leaves (cultivar A 195) and infected leaves with SPFMV and SPVG were negatively stained in 2% phosphotungstic acid, according to Noordam (1973), Parrella et al. (2006) and mounted on carbon-coated copper grids (600 mesh). The grids were then examined by transmission electron microscope (TEM Joel - 1400) at the electron microscope unit faculty of Agriculture, Research Park, Cairo University (FARP). Images were captures by CCD camera (EMT) at different

magnifications ranging from (10000 x -80000 x).

For the ultrathin section of virus-infected tissues: the healthy and infected sweet potato leaves (cultivar 195) with SPFMV and SPVG were examined as sections including the midrib and leaf tissue from the two sides were cut with a razor blade, then fixed overnight in cold 2.5% glutaraldehyde prepared in 0.1M potassium phosphate buffer (PH 7.4) and postfixed in 1% osmium tetroxide (OSO4) in the same buffer for 3 hr. After staining overnight the samples were dehydrated according to Sivparsad & Gubba (2013), El-Banna et al. (2013) and Sun et al. (2019). The stained sections were examined by transmission electron microscope (TEM Joel- 1400) at the candidate magnification. At least 10 sections of each of the prepared samples were examined in The Electron Microscope Unit, Faculty of Agriculture, Research Park, Cairo University, (FARP). Electron micrographs were captured using CCD camera model AMT, Optronics camera with 1632x1632 pixel format as side-mount configuration.

Results

Virus incidence as single and mixed infection in sweet potato:

The incidence of SPFMV and SPVG potyviruses as tested in the randomly collected 76 sweet potato samples. The leaves showing SPVD sweet potato virus-like disease symptoms and symptomless plants as shown in Fig. 1. The serological test of these samples which verified by RT-PCR revealed that the infected samples with SPFMV showed the highest incidence percentage 12 infected out of the tested 76 samples (15.7%) and the infection with SPVG was 10.5% (8 out of the 76 samples). Four out of 76 tested sweet potatoes, samples showed a single infection of SPFMV with 5.2%. While, the mixed infection with both SPFMV and SPVG potyviruses was 10.4% (8 out of the 76 tested samples).

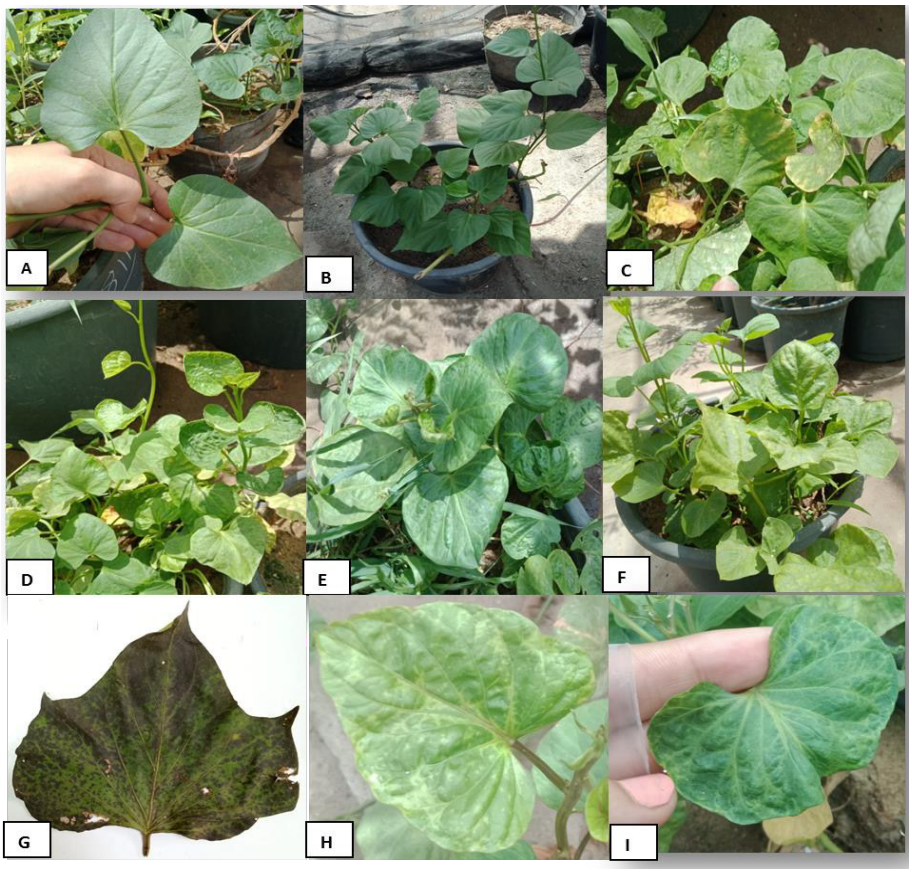

Fig. 1. Leaves showing SPVD sweet potato virus-like diseases and virus symptoms observed on sweet potato samples in the green hose experiment [A & B: Samples for healthy sweet potato plants, C& D: Yellow spotting, E: Chlorosis, yellow spotting, and leaf curling, F: Stunting, G: Purple spotted on leaves of infected plants, H: Vein chlorosis, yellow spotting, and warty leaf surface, and I: Chlorotic spotted and warty leaf surface]

Incidence of SPFMV and SPVG in weed samples

Incidence of viral infection in the fifty-four collected weed samples interfaced sweet potato tested by indirect–ELISA and the positive SPFMV and SPVG results, verified by RT-PCR, revealed presence of 100% virus infection in the weed species belonging to both of family *Convolvulaceae* and *Tiliaceae*, 75% in species of family *Euphorbiaceae* and 25% in family *Solanaceae*. Other species of families *Moraceae, Oxalidaceae, Compositae* showed 0.0% infection as outlined in Table 1.

The virus incidence in the weed samples interfaced with sweet potato, was 25% in perennial and 28.5% in annual (4 out of 14). The incidence of virus infection in individual/ species was 100% in *Convolvulus arvensis* var. *arvensis* and *Euphorbia heterophylla* and 66% in *Corchorus olitorius* (Table 1).

TABLE 1. Weed species interfacing the cultivated sweet potato

Plant name & family	Life duration	Virus incidence in Individual/species	Infected species/ family
Amaranthaceae			
Amaranthus hybridus L.	Annual	0.0%	0.0%
Compositae			
Biden pilosa L.	Annual	0.0%	0.0%
Xanthium strumarium L.	Annual	0.0%	
Convolvulaceae			
Convolvulus arvensis L. var. *arvensis*	Perennial	100%	100%
C. arvensis L. var. *linearifolius*	Perennial	66%	
C. arvensis L. *forma* 1 *	Perennial	33%	
Cyperaceae			
Cyperus rotundus L.	Perennial	0.0%	0.0%
Euphorbiaceae			
Euphorbia heterophylla L. (1)	Annual	100%	75%
E. heterophylla L. (2)	Annual	100%	
E. arguta Banks & Sol.	Annual	66%	
E. prosterata Ait.	Annual	0.0%	
Gramineae			
Cynodon dactylon (L.) Pres.	Perennial	0.0%	0.0%
Echinochloa colona (L.) Link	Annual	0.0%	
Solanaceae			
Abutilon theophrasti Medic	Annual	0.0%	25%
Hibiscus esculentus L.	Annual	0.0%	
Malva parviflora L.	Annual	0.0%	
Sida alba L.	Annual	33%	
Moraceae			
Morus alba L.	Perennial	0.0%	0.0%
Oxalidaceae			
Oxalis corniculatus L.	Annual	0.0%	0.0%
Solanaceae			
Solanum nigrum L.	Annual	0.0%	0.0%
Tiliaceae			
Corchorus olitorius L.	Annual	66%	100%

*= Amer & Hamed (2020).
Form the three replicate in each species 100% incidence= 3 plants infected; 66% incidence= 2 plants infected; 33% incidence= 1 plants infected and 0.0% incidence= no infected.

Host range reactions and symptomatology.

The two isolates SPFMV and SPVG were tested on 21 species belonging to six families. Inoculation of each isolate of SPFMV and SPVG induced symptoms on 12 out of the 21 species tested namely: (*Euphorbia heterophylla, E. millii, E. pulcherrima, E. peplus, Chenopodium amranticolor, Ch. quinoa, Ch. wild, Convolvulus arvensis, Ipomea batatas, I. cairica, I. carnea and Ricinus communis*) belonging to three families (*Convolvulaceae, Euphorbiaceae* and *Chenopodiaceae*). Inoculated plants showed various symptoms such as yellowing, mosaic and chlorosis. Naturally, infected sweet potato plants showed symptoms of SPFMV and SPVG as vein clearing, yellowing, mosaic, chlorosis and stunting, although no symptoms appeared on infected *Euphorbia millii* and *E. pulcherrima* (Table 2 and Figs. 1, 2).

TABLE 2. Reaction of different host range inoculated mechanically with SPFMV and SPVG

Family	SPFMV	SPVG
	Symptom	
Chenopodiaceae *Ch. quinoa* Willd.	Local spots turned to systemic chlorosis	Local spots to systemic chlorosis
Ch. album L. subsp. *amranticolor*	Local spots to systemic chlorosis	Local spots to systemic chlorosis
Ch. wild	Mosaic, chlorotic, yellowing	Mosaic, chlorotic, yellowing
Convolvulaceae: *Ipomea batatas* L.	Chlorotic, vein clearing, yellowing	Chlorotic, yellowing chlorotic
I. cairica (L.) Sweet	Chlorotic, vein clearing, yellowing	Chlorotic, vein clearing, yellowing
I. carnea Jacq.	Mosaic, chlorotic,yellowing	Mosaic, chlorotic, yellowing
Convolvulus arvensis L.	Yellowing, chlorotic	Yellowing, chlorotic
Cucurbitaceae *Cucumis sativas* L.	No symptoms	No symptoms
Euphorbiaceae *Euphorbia heterophylla* L.	Yellow spotting, chlorotic spotted and warts on the surface of the leaves	Yellow spotting, chlorotic spotted
E. pulcherrima Willd. ex Klotzsch	No symptoms	No symptoms
E. millii Des Moul.	No symptoms	No symptoms
E. peplus L.	Chlorotic, yellowing	Chlorotic, yellowing
Ricinus communis L.	Crinkle, chlorotic	Chlorotic, yellowing
Leguminaseae *Vigna unguiculata* (L.) Walp.	No symptoms	No symptoms
Solanaceae *Lycopersicon esculentum* Mill.	No symptoms	No symptoms
Nicotiana glutinosa L.	No symptoms	No symptoms
N. tabacum L. *var. White burly*	No symptoms	No symptoms
N. benthemiana Domin	No symptoms	No symptoms
Petunia hybrid Vilm.	No symptoms	No symptoms
Datura metel L.	No symptoms	No symptoms
D. stramonium L.	No symptoms	No symptoms

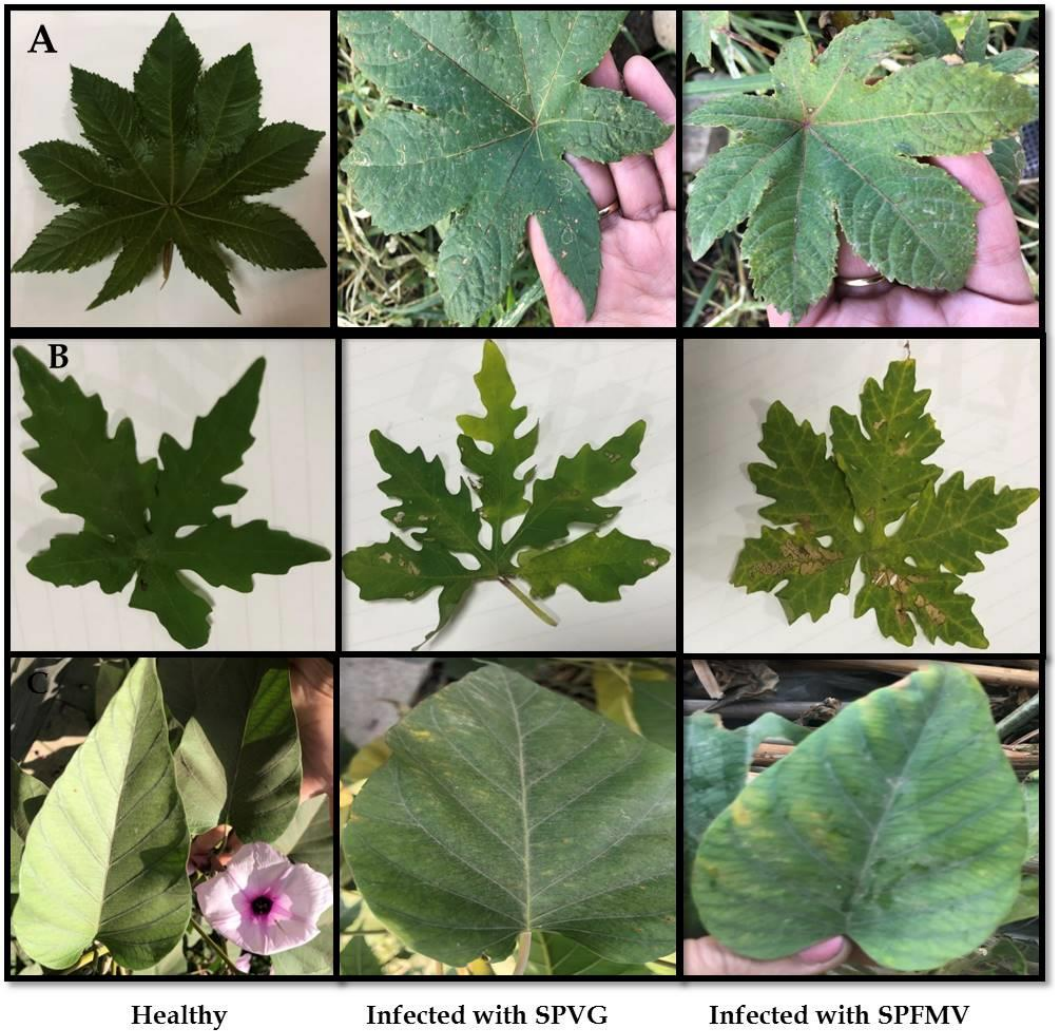

Fig. 2. Symptoms on different host range [A: Healthy *Ricinus communis* and infected sample with SPVG showing crinkle and chlorotic also infected sample with SPFMV showing chlorotic and yellowing; B: Healthy *Ipomaea cairica* and infected sample with SPVG showing chlorotic also infected sample with SPFMV showing chlorotic and vein clearing; C: Healthy *Ipomaea carnea* and infected sample with SPVG showing yellowing also infected sample with SPFMV showing chlorotic, mosaic and yellowing]

The mixed infection with both SPFMV and SPVG potyviruses was detected in four species namely: *Convolvulus arvensis, Euphorbia arguta, Sida alba* and *Corchorus olitorius*. While *Euphorbia heterophylla* showed mixed infection in some individuals and others with a single SPVG virus infection (Table 3 and Fig. 3). The virus infected weeds revealed the presence of both SPFMV and SPVG potyviruses in 31.4%; out of them 5.5% showed single SPVG infection while others had mixed infection of SPFMV and SPVG potyviruses (Table 3 and Fig. 3).

Isolation and propagation of virus isolate:

The SPFMV and SPVG were isolated from the infected sweet potato leaves, biologically purified by mechanical inoculation on *Ch. amaranticolor* and *Ch. quinoa* using one local lesion-technique and was kept under greenhouse condition and used as a virus source for the other identification tools.

Weeds infection with SPFMV and SPVG.

SPFMV and SPVG were successfully identified in different wild species expressing various symptoms similar to that detected on sweet potato as shown in Fig. 4 . These symptoms such as yellow spotting, chlorotic spotted, warts on the surface of the leaves, yellow spotting and purple spotted on *Euphorbia heterophylla* and *E. arguta* leaves. Also, yellow spotting and purple spotted on leaves on *Sida alba* and yellow

spotting, chlorotic spotted and warts on the surface of the leaves on *Corchorus olitorius* (Table 3 and Fig. 3). Seventeen samples of the tested weeds belonging to five different species were infected with SPFMV and SPVG as mixed infection and 3 samples of *E. heterophylla* were infected with a single infection of SPVG as shown in Fig. 5.

Mechanical transmission

Mechanical transmission results showed that SPFMV and SPVG were successfully transmitted mechanically from infected to the healthy sweet potato plants, *Chenopodium album* sub sp. *amaranticolor*, *Ch. quinoa* and other host range. Inoculated plants were positive for both viruses (Table 2) and results were confirmed using PCR.

Serological diagnosis using Indirect-ELISA, DBIA and TBIA:

Indirect -ELISA detected SPFMV and SPVG in leaves of infected sweet potato plants, the values of tested materials for infectivity with SPFMV and SPVG showed the range values between 0.80 to 1.00 for the infected plants and the control value was 0.200.

The DBIA and TBIA techniques were found to be sensitive to detect SPFMV and SPVG in the studied sweet potato leaves (cultivar A 195). Infected samples turned to purple color as shown in positive control, while healthy ones were pale or remained green as shown in negative control. In the DBIA technique within SPFMV detection for eight tested samples almost five samples S1, S2, S4, S5 and S7 were positive, while for SPVG detection three samples S2, S6 and S7 were positive (Fig. 6). Also, the same eight samples were tested by TBIA the same results observed as shown in Fig. 7.

Molecular Detection using RT-PCR:

The total RNA isolated from each of the infected sweet potato samples with SPFMV and SPVG was used as a template for the one-step RT-PCR amplification. Electrophoresis analysis of RT-PCR product showed a single amplified fragment at 356bp for sweet potato feathery mottle virus (SPFMV) and 286 bp for sweet potato virus G (SPVG), as shown in Fig. 8.

TABLE 3. Wild species interfacing the infected sweet potato with diverse virus symptoms

Plants	Detected viruses	Viral symptom	
Family & Species		**Mixed infection with SPFMV and SPVG**	**SPVG**
		Convolvulaceae	
Convolvulus arvensis L. var. *arvensis*	SPFMV & SPVG	yellow spotting, Chlorotic spotted	-
C. arvensis L. var. *linearifolius*	SPFMV & SPVG	yellow spotting and Chlorotic spotted	-
C. arvensis L. *forma* 1 *	SPFMV & SPVG	yellow spotting and Chlorotic spots	-
		Euphorbiaceae	
Euphorbia heterophylla L. (1)	SPFMV & SPVG	yellow and chlorotic spotting and warts on leaf surface	-
E. heterophylla L. (2)	SPVG	-	yellow spotting and Chlorotic spots
E. arguta Banks & Sol.	SPFMV & SPVG	yellow spotting and Purple spotted on leaves	-
E. prosterata Ait.	-ve	-ve	-ve
		Solanaceae	
Sida alba L.	SPFMV & SPVG	yellow spotting and Purple spotted on leaves	-
		Tiliaceae	
Corchorus olitorius L.	SPFMV & SPVG	yellow spotting, Chlorotic spotted and warts on the leaf surface	-

*= Amer & Hamed (2020).

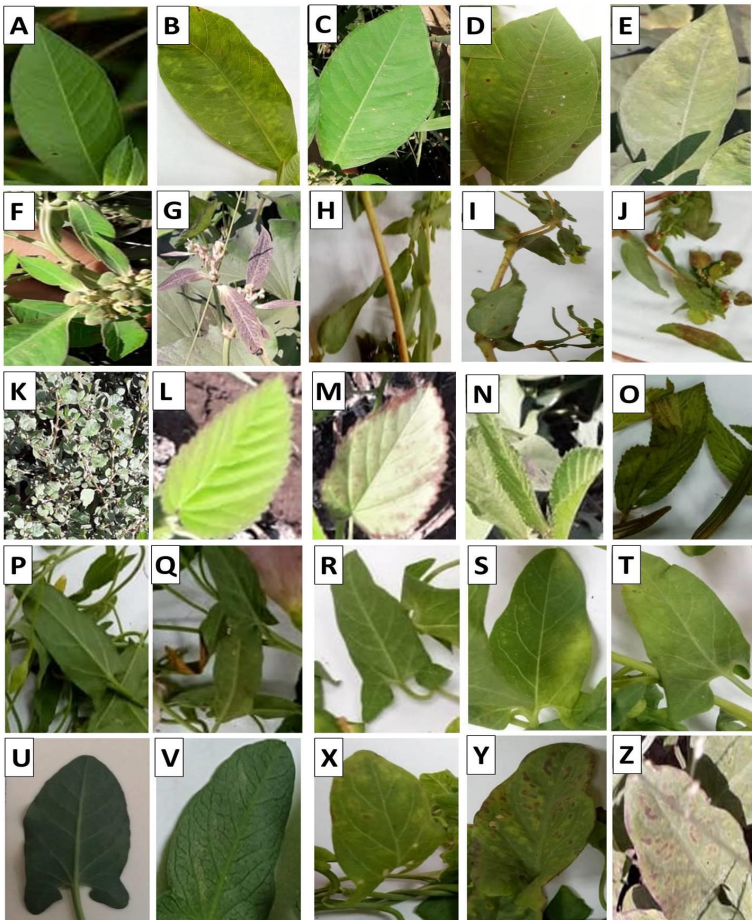

Fig. 3. Symptoms on wild species interfacing the infected sweet potato [A-G: *Euphorbia heterophylla;* A: Healthy, B-E: Infected *E. heterophylla* (1) & F: Healthy, G: Infected *E. heterophylla* (2); H- J: *Euphorbia argute*, H: Healthy & I-J: infected; K: Healthy *E. prosterata*; L-M: *Sida alba*, L: Healthy & M: Infected; N-O: *Corchorus olitorius*, N: Healthy & O: Infected; P-Q: *Convolvulus arvensis* var. *linearifolius*, P: Healthy & Q: Infected; R-T: *C. arvensis* forma 1, R: Healthy, S & T: Infected; U-Z: *C. arvensis* var. *arvensis*, U: Healthy & V-Z: Infected.

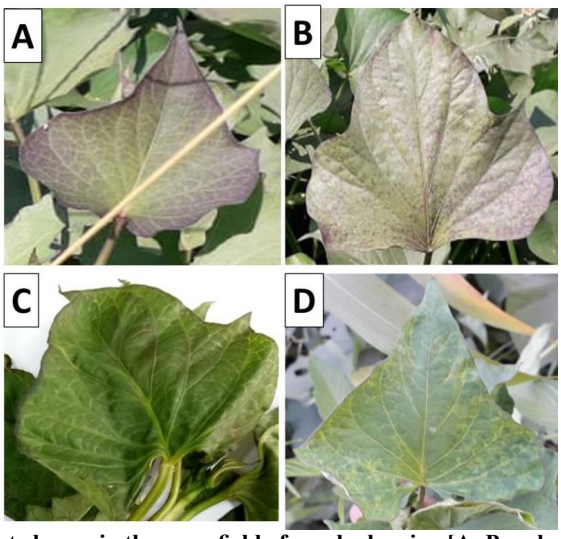

Fig. 4. Symptoms on Sweet potato leaves in the same field of weeds showing [A: Purple spotted on leaves, B: Purple spotted and chlorosis, yellow spotting, C: Leaf curling, Chlorotic spotted and warts and D: Chlorosis, yellow spotting, leaf curling and vein clearing]

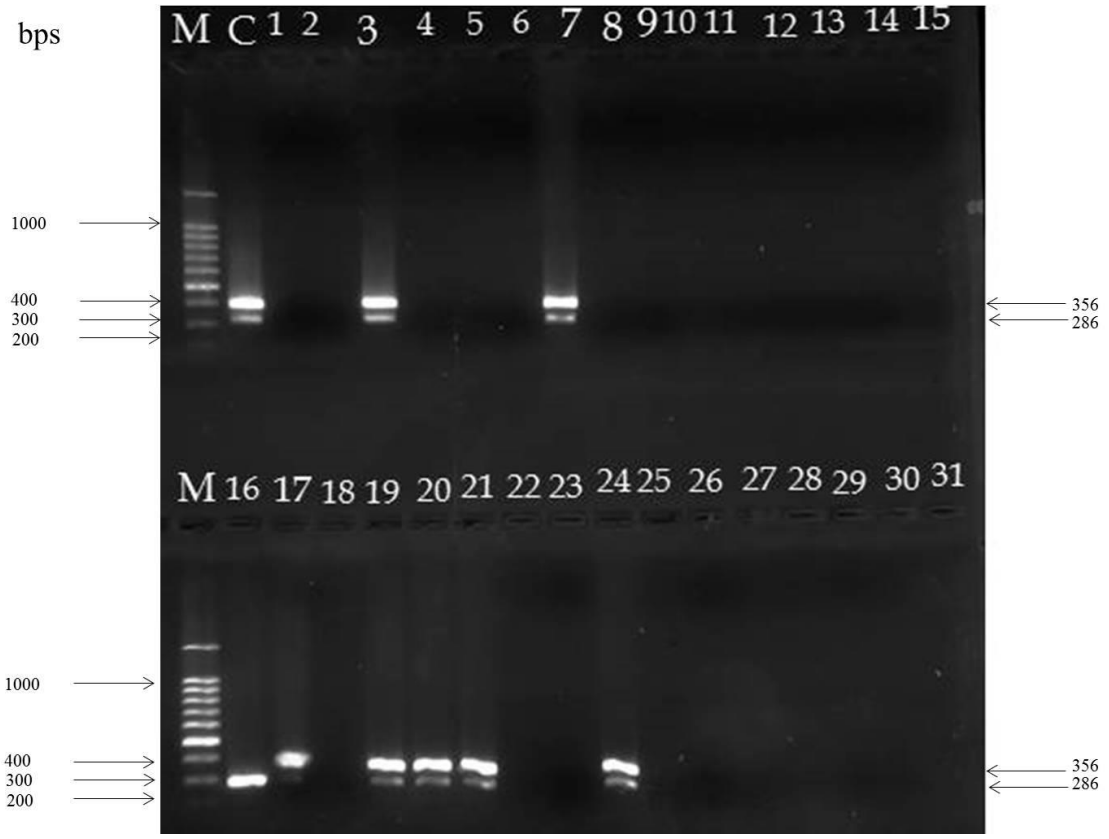

Fig. 5. Agarose gels showing the RT-PCR amplicons from total RNA extracted from weeds [C: Control, sample 3, 7, 17, 19, 20, 21 and 24 confirming the presence of S1 sweet potato feathery mottle virus (SPFM, V) with a band at 356bp and, S2: Sweet potato virus G (SPVG) with a band at 286bp by multiplex PCR and one single infection with sweet potato virus G (SPVG) individually at sample 16, M shows the 100bp molecular weight marker]

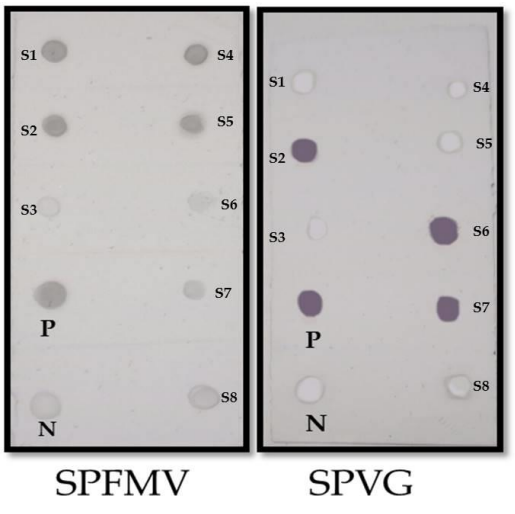

Fig. 6. DBIA on nitrocellulose membrane (NCM) [S1-S8: Tested sweet potato samples, P: Infected sample showing purple color as detection of SPFMV and SPVG in infected sweet potato t leaves and N: Healthy negative control]

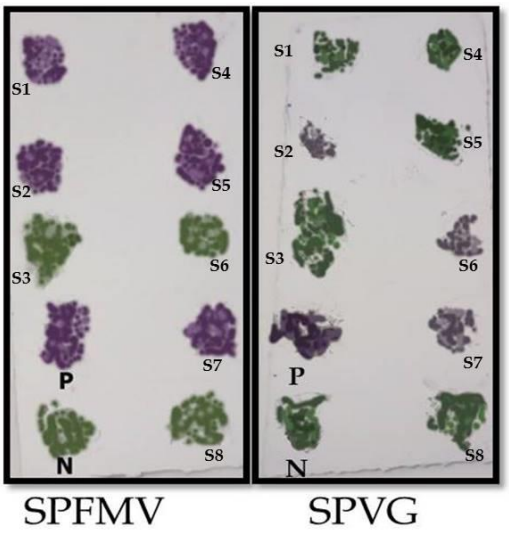

Fig.7. TBIA on nitrocellulose membrane (NCM) [S1-S8: Tested sweet potato samples, P: Infected sample showing purple color as detection of SPFMV and SPVG in infected sweet potato leaves, and N: Healthy (-ve) control]

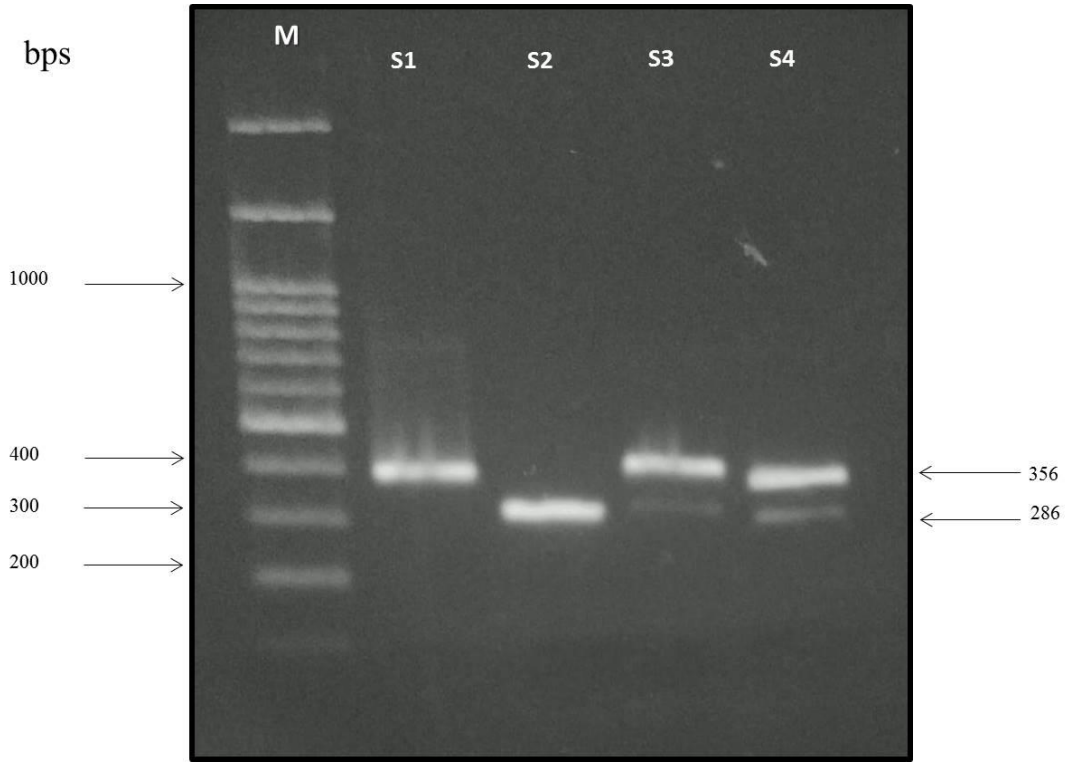

Fig. 8. RT-PCR analysis of total RNA extracted from symptomatic *Ipomoea potato* on agarose gel showing amplicons confirming the presence of (S1) sweet potato feathery mottle virus (SPFMV) individually at 356bp; (S2) sweet potato virus G (SPVG) individually at 286bp, (S3 and S4) multiplex PCR for SPFMV and SPVG; respectively [Lane 1 shows the 100bp molecular weight marker]

Nucleotide sequencing:

The purified RT-PCR fragment representing a partial coat protein gene of SPFMV and SPVG was directly sequenced and compared with corresponding sequences of other isolates of SPFMV and SPVG available in GenBank. The genetic codes were translated into the equivalent amino acid using the standard universal code. The obtained data were assembled using DNAMAN software. Multiple sequences alignment of the nucleotide sequences of the partial coat protein gene (Egyptian Isolate) were done with the corresponding sequences of the following SPFMV isolate available in GenBank: Egy-_ SPFMV, Argentina_-KF386, Australia-_MF572, China-_KY296451, S._Korea- _KP1156, Japan-_ NC_001841, USA-_MH782227, Brazil-_ MF185715, Peru- _FJL55666, S._ Africa-_MH023, Uganda-_KP729265, Spain-_ KU511268, E._Timor-_MF5720 and Kenya-_ MH264535.

Also sequence alignment of the nucleotide sequences of the partial coat protein gene (Egyptian Isolate) was done with the corresponding sequences of the following SPVG isolate available in GenBank: Egy-_SPVG, Argentina-_NC_01, China-_MK392509, Brazil-_MF185716, S-_ Korea-_JN6138, USA-_JN613805, E-_Timor-_ KX2798, S-_Africa-_MH023 and Taiwan-_ KF790759 (Fig. 9 A).

The nucleotide sequences of the SPFMV showed a range of 87% and 94 % similarity with the different SPFMV isolates in the GenBank as outlined in the phylogenetic trees (Fig. 9 A). While, the phylogenetic tree of the Egyptian isolate SPVG based on the nucleotide sequence showed a range of 88% and 100% similarity with the different SPVG isolates in the GenBank (Fig. 9 B).

Morphology of viruses

The morphology of SPFMV and SPVG particles was studied by electron microscopy (TEM) using leaf dip preparation negatively stained with 2% phosphotungstic acid. Cylindrical filamentous particles of viruses were observed, and which are typical of potyviruses as shown in Fig. 10.

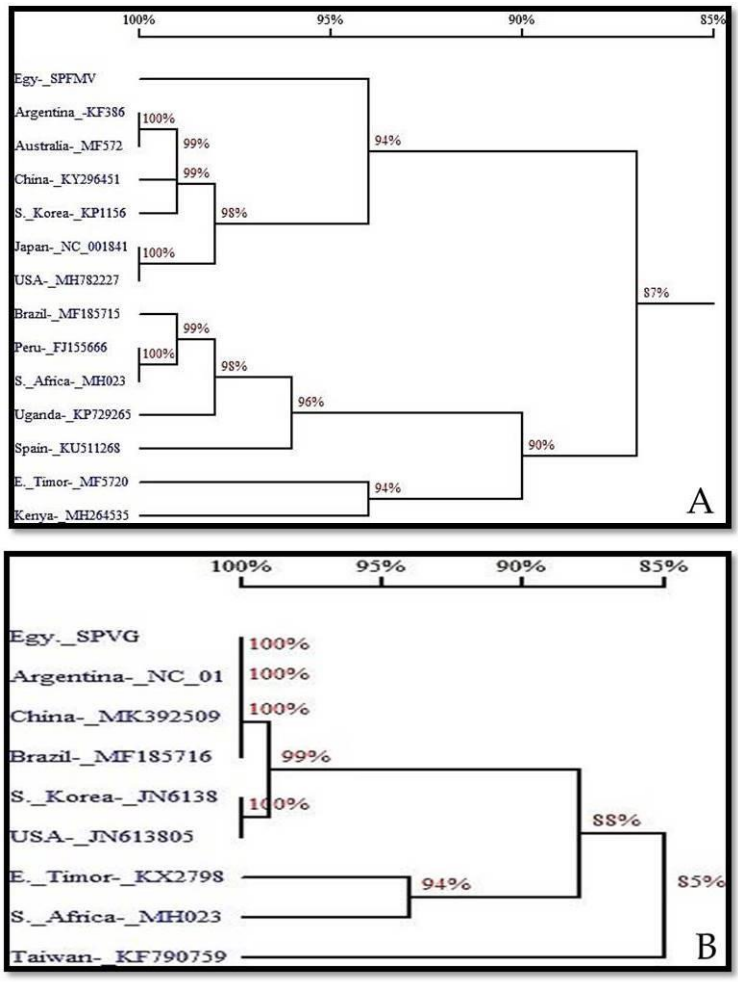

Fig.9. Phylogenetic tree showing the molecular relationships between the obtained virus isolates in this study based on the nucleotide sequences of the partial CP gene and the GenBank isolates [A: Isolate of SPFMV and B: Isolate of SPVG]

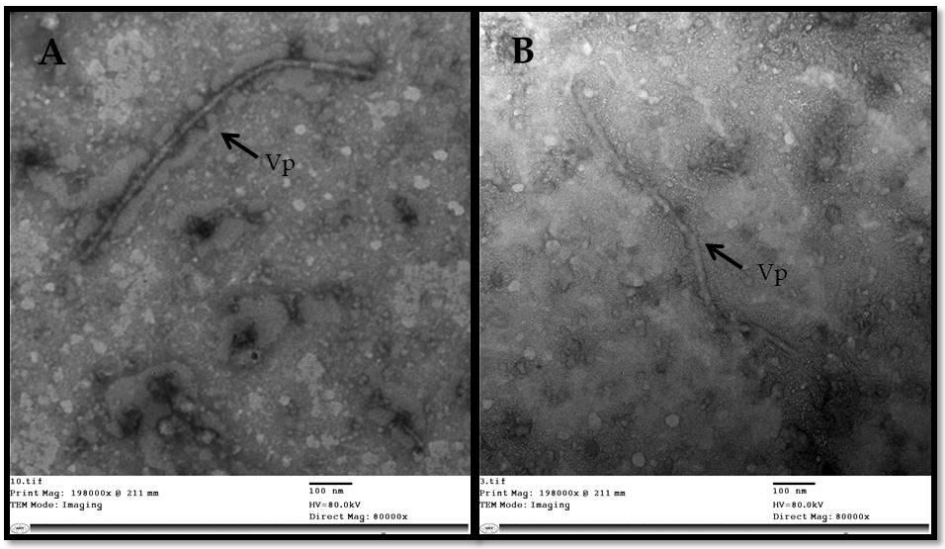

Fig. 10. Transmission electron micrographs of negatively stained (A-B) crude leaf sap from symptomatic sweet potato plants showing cylindrical filamentous of virus particles (Vp) [Magnification x 80000]

Cytopathology of virus-infected sweet potato tissues

Leaf tissues from sweet potato plant (cultivar A 195) infected with SPFMV and SPVG revealed many ultrastructural changes due to the viral infection. The changes were observed in the whole-cell and the cell content as cell wall, nucleus, chloroplast, and mitochondria.

The ultrastructural changes in the sweet potato cells showing abnormalities in the cell and extended the cell wall, also in many cells lost its thickness shape, size, and integrity (Fig. 11). The chloroplast of infected plants with SPFMV and SPVG were totally affected and appeared very small in size compared with the chloroplast of healthy cells. The chloroplasts were generally disorganized with irregular shape and the outer membrane of the chloroplast showed severe degradation; also shrank collapsed chloroplasts were shown. The grana were observed to be separated or totally destroyed and chloroplasts contained much more starch grains compared to those of healthy cells (Fig. 12).

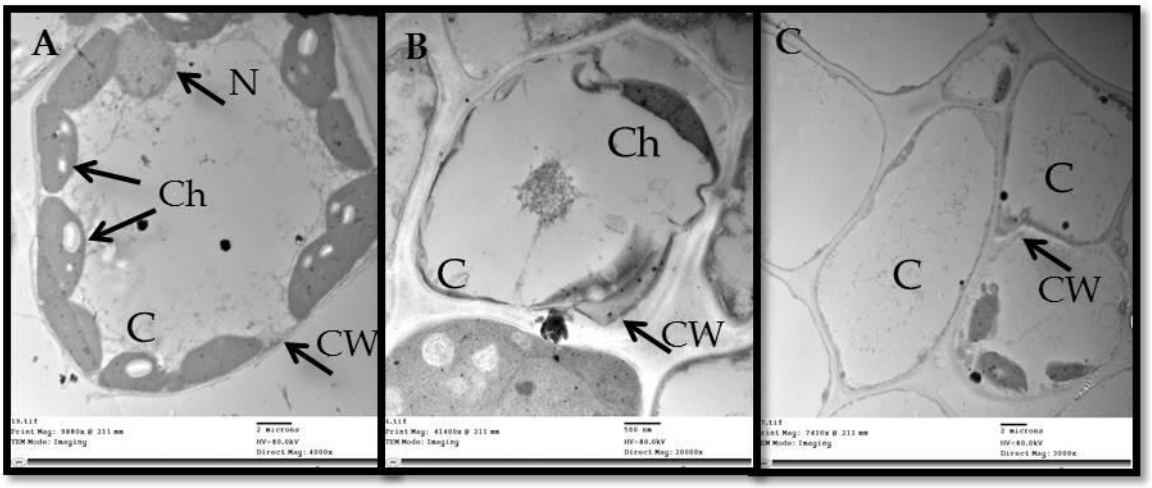

Fig. 11. Ultrathin sections of sweet potato leaves infected with SPFMV and SPVG, showing cell abnormalities [A: Healthy sweet potato plant cell (C) with normal cell wall (Cw), nucleus (N) and chloroplast (Ch); B: Infected leaf with SPVG showing abnormalities extended into the cell wall (Cw) with reduced thickness, size and integrity; C: Infected plant with SPFMV showing fully destroyed cells (C) with no integrity]

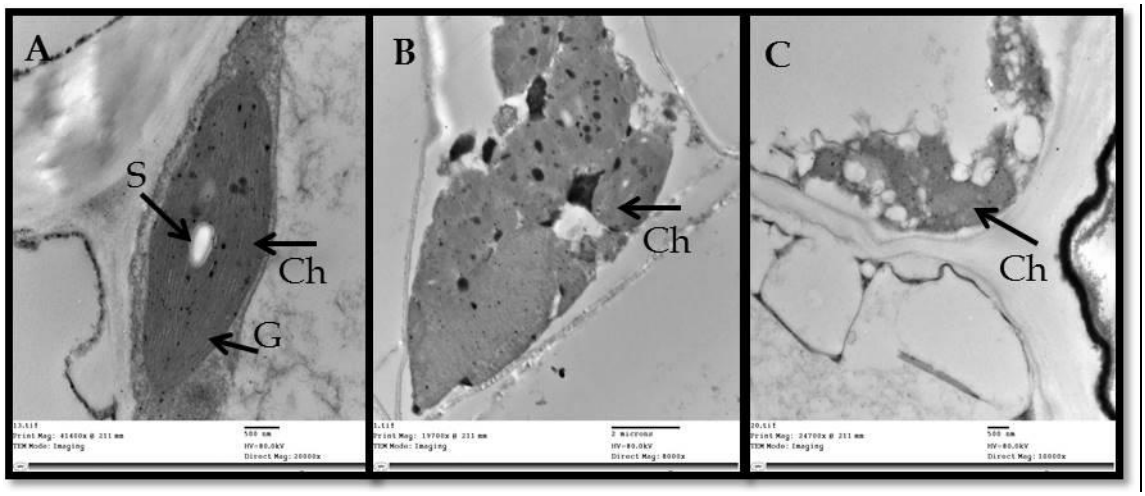

Fig. 12. Ultrastructure of sweet potato leaves infected with SPFMV and SPVG [A: Healthy sweet potato plant chloroplast (Ch) with a large granule of starch (S) at the center and normal regular grana (G); B: Infected plant leaves with SPVG showing decayed chloroplast (Ch) with irregular shape and destroyed grana; and C: Infected plant leaves with SPFMV showing fully destroyed chloroplast (Ch) with no integrity]

In addition, the nucleus was absent sometimes or the viruses were also able to induce some abnormalities in size enlarged or elongated or in shape as nucleus misshape and the nuclear membrane of the infected cells were indentation, protrusion, decomposed and destroyed. The chromatin becomes denser (Fig. 13). Numerous vesicles were observed in the cytoplasm of the infected tissues (Fig. 14).

The cytoplasm of mesophyll cells was highly granulated and contained differently shaped (scrolled shaped) of inclusion bodies either in the cytoplasm (pinwheels, laminated pinwheels, paramural bodies, membranous bodies, and scrolls) or in the nucleus (crystalline) or adjacent to and inside the chloroplast which seemed abnormal in structure as a reaction of infected tissues with SPFMV and SPVG. Plasmodesmata were increased in infected tissues (Fig. 14). Also, potyvirus pinwheel inclusion bodies and laminated inclusion bodies were observed in cells**.**

Discussion

The current study was carried out on sweet potato grown in El Menufia governorate (Nile Delta Agro-ecological zone), in Egypt. Out of the six viruses previously reported on sweet potato (SPFMV, SPVG, *sweet potato chlorotic stunt virus* SPCSV, *sweet potato mild mottle virus* SPMMV, *sweet potato latent virus* SPLV and *sweet potato leaf curl virus* SPLCV); SPFMV and SPVG were the only detected viruses infecting sweet potato "cultivar A195" in the studied field. This detection was in consistent to that of IsHak et al. (2003), who reported that SPFMV and SPVG caused severe symptoms on sweet potato in Egypt, these symptoms including stunting, yellowing, and leaf curling.

The incidence of SPFMV and SPVG were 15.7% and 10.5%; respectively and the results confirmed that SPFMV was the most dominant in the studied samples of sweet potato "cultivar A195". The high incidence percentage of SPFMV and SPVG in El- Menufia, is related to the large scale cultivation of sweet potato (about 40%) in this governorate along the Egyptian land. These results agreed with Bondok (2013), who reported that viral diseases considered to be one of the most important problems affecting sweet potato and the incidence was up to 34.5% in Egypt. Also, Kim et al. (2017), who reported that the incidence of SPFMV on sweet potato showed the highest infection rate at 87.0% in 2012; dropped to 20.7% in 2013, and then increased to 35.3% in 2014. The observed SPFMV incidence in Egypt was agreed with Kwak et al. (2007), who also claimed that SPFMV considered one of the most prevalent and damaging viruses that widely infecting sweet potato crop in Korea. While SPFMV infection in Kenya was reported to infect up to 90% of the cultivated within the three crop cycle (Karyeija et al., 1998).

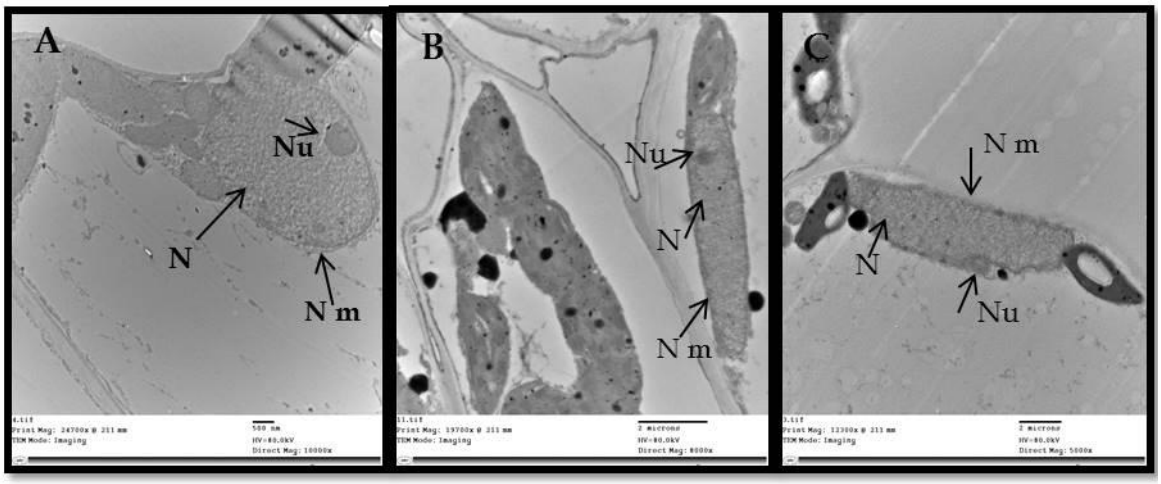

Fig. 13. A: Healthy sweet potato plant regular nucleus (N) with its normal nucleolus (Nu) and normal regular nuclear membrane (Nm) B: Ultra-thin leaf sections from an infected plant with SPVG showing enlarged elongated nucleus (N) and degradation of the nucleolus (Nu) which became very small, C: Ultra-thin leaf sections from an infected plant with SPFMV showing misshaped nucleus (N) and destroyed the nuclear membrane (Nm) and nucleolus (Nu)

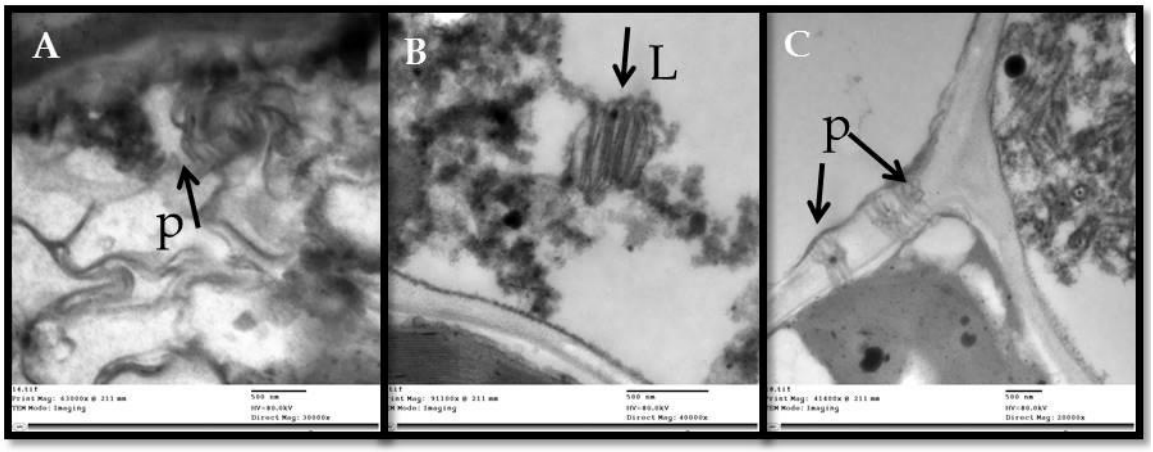

Fig.14. A: Ultra-thin sections of leaf from an infected sweet potato plant showing pinwheel inclusion bodies (P). B: Ultra-thin leaf sections from an infected plant showing laminated inclusion bodies (L) and C: Infected plant showing a large number of plasmodesmata of infected tissues and pinwheel inclusion bodies (P) inside it

The obtained results showed the presence of 5.2% single infection on sweet potato by SPFMV, on the other hand, SPVG not traced in single infection in the tested sweet potato samples of "cultivar A 195". The two viruses SPFMV and SPVG were detected mostly as mixed infection with 10.4% in the tested samples. This result reported earlier in Egypt by IsHak et. al. (2003). While higher rates of SPVG infecting sweet potato in Korea were detected by Kim et. al. (2017) reached to 57.8%, 13.5% and 18.6% in 2012, 2013 and 2014; respectively. The SPFMV infecting sweet potato in Uganda reached c. 70% (Tugume et al., 2016).

The incidence of viral SPFMV and SPVG infection in the weed-interfaced sweet potato "cultivar A 195" crop, were verified in the fifty-four weed samples by RT-PCR, revealed the presence of 100% virus infection in weed species belonging to both family *Convolvulaceae* and *Tiliaceae* decreased to 75% in species of the family *Euphorbiaceae* and was 25% in family *Solanaceae* (Table 1). This mixed infection of SPFMV and SPVG, was in matching with the data obtained by Tugume et al. (2016), in East Africa, who reported that many weeds contained double or triple infection of SPFMV, SPMMV and SPCSV. The current work revealed that both of the cultivated sweet potato "cultivar A 195" and their interface weed species had the same mixed SPFMV and SPVG infection. This result is contradicted with that of Tugume et al. (2016), who reported that the co-infection of SPCFV and the other three viruses infected weed species was in contrast to that of the cultivated sweet potato. At the Convolvulaceae family level, the high incidence of the virus infection in individual/ species it was 100% in *Convolvulus arvensis* (Table 1), is inconsistent with that of Tugume et al. (2016). The detection of weed species in both *Tiliaceae, Euphorbiaceae* and *Solanaceae* as SPFMV-reservoir in this work provides a new addition to the field of virus-host range. This range was restricted to the wild and native *Ipomoea* spp. and related genera occurred throughout tropics (Karyeija et al., 1998). *Euphorbia heterophylla* is among the weed species interfaced with the cultivated sweet potato, the plant harbor both of mixed infection (SPFMV and SPVG) that dominating the single SPVG infection. Karyeija et al. (1998) reported that in Uganda, the introduced and naturalized *Euphorbia heterophylla* is SPFMV host. Despite, dominance and high incidence/individual of mixed infection (SPFMV and SPVG) in perennial (*Convolvulus arvensis*), the incidence was 25% in perennial and 28.5% in annual (Table 1). Perennials may be forming the most probable source of virus for the further sweet potato cultivations. However, the current study revealed no virus incidence in wild perennial grass species (*Cynodon dactlyon*; Table 1) interfaced with sweet potato. Some grass species resistant to viruses showed the high potential of acting as a reservoir of generalist plant viruses and infecting the perennial grasses in the same habitat (Hily et al., 2014). The weed species interface sweet potato showed virus (SPFMV and SPVG) symptom as yellow spotting, chlorotic spotted, warts on the surface of the leaves, yellow

spotting and Purple spotted (Table 3 and Fig. 3), these symptoms of generally comparable to that of the infected sweet potato with the same viruses in the same habitat (Table 2 and Fig. 2). This work on weed interfacing crops in lights the importance of these weeds as virus reservoir, Wosula et. al. (2012) and Tibiri et al. (2019a & b) reported that weeds are good sources and reservoirs for important crop pathogens as SPFMV and SPVG. Also, Okonya et. al. (2019) reported global potato yield losses due to pathogens, insects, and weeds.

Naturally infected sweet potato plants grown in Egyptian conditions showing symptoms and reacted positively with serological and molecular testes were used as a source of viral isolates. The SPFMV and SPVG Egyptian isolates inoculated on leaves of healthy *Chenopodium quinoa* and *Ch. amaranticolor* plants and gave chlorotic local lesions were then propagated on *Ch. amaranticolor* and the identity of two viruses were confirmed by symptomatology, serological, biological and molecular studies. these results agreed with that described by Untiveros et al. (2008) who isolated SPFMV and SPVG by three consecutive single-lesion transfers on *Ch. amaranticolor*. Also, agreed with Souto et al. (2003) and Ateka (2004) who isolated SPFMV successfully on *Chenopodium quinoa* and *Ch. amaranticolor*.

The host range study provided a novel data of new natural hosts of the studied SPFMV and SPVG viruses in Egypt. The achieved results confirmed that these viruses were transmitted mechanically to 12 species (out of the tested 21 species, Table 2), namely: (*Euphorbia heterophylla, E. millii, Euphorbia pulcherrima, E. peplus, Chenopodium. amranticolor, Ch. quinoa, Ch. wild, Convolvulus arvensis, Ipomea batatas, I. cairica, I. carnea* and *Ricinus communis*). The detected hosts belonging to *Convolvulaceous, Chenopodiaceae* and *Euphorbiaceae* in current work was supported by the finds of Clark et al. (2012) which confirmed that SPFMV infect *Chenopodiaceae* and *Convolvulaceae,* and Loebenstein et al. (2009) which confirmed that SPVG could infect both families. In addition, Karyeija et al. (1998) stated that SPFMV and SPVG infected *Euphorbiaceae.* On the contrary, Bondok (2013) and Tugume et al. (2016), were restricted the host range to the species of family *Convolvulaceae*. The two viruses induce similar symptoms on the biological indicator plants as described earlier by Li et al. (2012).

The mechanical transmission showed that SPFMV was successfully transmitted mechanically and these results agreed with that given by Kawanna & Aseel (2019) in Egyptian conditions**.** Also, SPVG were transmitted mechanically and this achieved results agreed with Loebenstein & Lecoq (2012) who reported this mode of transmission for both of SPVG and SPFMV. In contrast, Souto et al. (2003) and Bondok (2013) reported that, SPFMV was difficult to be inoculated mechanically.

SPFMV and SPVG were successfully detected in sweet potato and weeds serological testing using the indirect ELISA and these resulted agreed with Rännäli et al. (2008, 2009), who used this technique for detection of both viruses infecting sweet potato. Also, used nitrocellulose membrane (NCM)-ELISA to confirm the presence of SPFMV and the results were positive and these results agreed with Loebenstein & Lecoq (2012), and using specific antisera in (NCM-ELISA) to test the presence of SPVG the results were positive as mentioned by Trenado et al. (2007).

Reverse-transcription PCR as single and/ or multiplex RT-PCR assays using SPFMV and SPVG specific primers used to amplify partial coat protein gene fragments at 356 bp and 286 bp; respectively (Kwak et al., 2014). Further confirmation with direct DNA sequencing and phylogenetic analysis for the coat protein gene of SPFMV and SPVG was performed and recorded as an Egyptian isolate. Sequence analysis and the phylogenetic tree among the DNA nucleotide sequences showed an identity that ranged between 87% to 94 % for SPFMV and 88% to 100% for SPVG isolates; respectively (Figs. 9A and 9B) when compared with the different SPFMV and SPVG isolates in the GenBank.

Cytopathological studies as further confirmation for the presence the viral infection with SPFMV and SPVG by TEM examination of negatively stained partially purified preparations of the virus to clear the presence of filamentous flexuous particles and showed the cytopathological effects described by Parrella et al. (2006). Several cytopathological alterations were also observed through investigating the ultra-thin sections using EM, chloroplast and mitochondria were totally affected (Fig. 12). Different abnormalities into

the cell wall as thickness shape, size and integrity, the nucleus was absent sometimes or misshaped, enlarged, elongated and the chloroplast of infected plants with SPFMV and SPVG were totally destroyed, shrunken and irregular infected chloroplasts contained much more starch grains compared to those of healthy cells as described by IsHak & El-Deeb (2004). Also, potyvirus pinwheel inclusion bodies and laminated inclusion bodies were observed in cells as described by Sivparsad & Gubba (2013) and described by Loebenstein & Lecoq (2012) which confirmed that SPVG caused these features which observed in the cytoplasm of epidermal, mesophyll, and vascular cells of infected *Ipomoea nil and I. setosa.* The results revealed that SPFMV and SPVG have destroyed the cell content as described by IsHak & El-Deeb (2004).

This work recommends the importance of weed interfacing crops as a virus reservoir, and in light of the importance of multidisciplinary research work namely, symptoms, serology, ultrastructure, molecular, etc. to resolve the virus identification, especially that infect the potential crops.

Conclusion

The incidence of the potyviruses, including sweet potato feathery mottle virus (SPFMV) and sweet potato virus G (SPVG) in the cultivated sweet potato can be minimized through the virus control in its interfacing wild weeds. This study also recommends the use of multidiscipline approaches to verify the virus identity in the infected crop and its interfacing weeds.

Conflict of interest: The authors reported no potential conflict of interest.

Authors contribution: Amira A. Mazyad carried out the practical part under the supervision of the four coauthors. The manuscript construction, revision and data analysis were carried out in participation of all the authors, each in its speciality.

Ethical approval: Not applicable.

References

Amer, W.M., Hamed, A.B (2020) Biosystematic study of *Convolvulus arvensis* morphotypes in Egypt. *Tackholmia* (in press).

Adikini, S., Mukasa, S.B., Mwanga, R.O., Gibson, R.W. (2016) Effects of Sweet potato feathery mottle virus and Sweet potato chlorotic stunt virus on the yield of sweetpotato in Uganda. *Journal of Phytopathology*, **164**(4), 242-254.

Ateka, E.M. (2004) Biological and molecular characterization of potyviruses infecting sweet potato. *Unpublished PhD Thesis*, University of Nairobi, Kenya.

Bondok, A. (2013) "*Studies on Some Viral Diseases of Sweet Potato in Egypt*". Lambert Academic Publishing, pp.7-32.

Clark, C.A., Hoy, M.W. (2006) Effects of common viruses on yield and quality of Beauregard sweet potato in Louisiana. *Plant Disease*, **90**(1), 83-88.

Clark, C.A., Davis, J.A., Abad, J.A., Cuellar, W.J., Fuentes, S., Kreuze, J.F., Valkonen, J.P. (2012) Sweetpotato viruses: 15 years of progress on understanding and managing complex diseases. *Plant Disease*, **96**(2), 168-185.

El-Banna, O.M., Mikhail M.S., El-Attar, A.K., Aljamali, A.A.R. (2013**)** Molecular and Electron Microscope Evidence for an Phytoplasma with Sesame Phyllody in Egypt. *Egyptian Journal of Phytopathology*, **41**(2), 1-14.

FAOSTAT (2020) http://www.fao.org/faostat/fr/#data/QC/ visualize. Accessed, 8 Feb 2020.

Gibson, R.W., Mpembe, I., Alicai, T., Carey, E.E., Mwanga, R.O.M., Seal, S.E., Vetten, H. J. (1998) Symptoms, aetiology and serological analysis of sweet potato virus disease in Uganda. *Plant Pathology*, **47**(1), 95-102.

Hampton, R.O., Beczer, L., Hagedorn, B., Bos, L., Inonye, T., Barnett, O., Musil, M., Meiners, L. (1978) Host reactions of mechanically transmissible Legume viruses of North temperate zone. *Phytopathology,* **68**, 989-997.

Hily, J.M., Garcia, A., Moreno, A., Plaza, M., Wilkinson, M.D., Fereres, A., Garcia-Arenal, F. (2014) The relationship between host lifespan and pathogen reservoir potential: an analysis in the system Arabidopsis thaliana-Cucumber mosaic virus. *PLoS Pathogens*, **10**(11). https://doi.org/ 10.1371/journal.ppat.1004492.

Hondo, A., Damaiyanti, K.C., Hafizh, M.F., Amanatillah, N.E., Damayanti, T.A. (2018) Penyakit Bercak Kuning Ubi Jalar di Bogor, Jawa Barat. *Jurnal Fitopatologi Indonesia*, **14**(2), 69.

IsHak, J., El-Deeb, S.H. (2004) Investigating the effects of Sweetpotato chlorotic stunt virus (SPCSV) infection to sweetpotato plants using light and electron microscopy/Licht-und elektronenmikroskopische Untersuchungen von Süßkartoffelpflanzen nach Infektion mit dem Sweetpotato chlorotic stunt virus. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/*Journal of Plant Diseases and Protection*, 362-370. https://www.jstor.org/stable/43215588.

IsHak, J.A., Kreuze, J.F., Johansson, A., Mukasa, S.B., Tairo, F., El-Abbas, F.A., Valkonen, J.P.T. (2003) Some molecular characteristics of three viruses from SPVD-affected sweet potato plants in Egypt. *Archives of Virology*, **148**(12), 2449-2460.

Karyeija, R.F., Gibson, R.W., Valkonen, J.P.T. (1998) The significance of sweet potato feathery mottle virus in subsistence sweet potato production in Africa. *Plant Disease,* **82**(1), 4-15.

Kawanna, M.A., Aseel, D.G. (2019) Biological and molecular characterization of an isolate of Sweet potato feathery mottle virus from sweet potato. *Bioscience Research.* **16**(2), 1515-1527.

Kim, J., Wook Yang, J., Kwak, H.R., Kim, M.K., Seo, J.K., Chung, M.N., Lee, G.S. (2017) Virus incidence of sweet potato in Korea from 2011 to 2014. *The Plant Pathology Journal*, **33**(5), 467.

Kwak, H.R., Kim, M.K., Jung, M.N., Lee, S.H., Park, J.W., Kim, K.H., Ko, S.J., Choi, H.S. (2007) Genetic diversity of sweet potato feathery mottle virus from sweet potatoes in Korea. *The Plant Pathology Journal,* **23**, 13-21.

Kwak, H.R., Kim, M.K., Shin, J.C., Lee, Y.J., Seo, J.K., Lee, H.U., Choi, H.S. (2014) The current incidence of viral disease in Korean sweet potatoes and development of multiplex RT-PCR assays for simultaneous detection of eight sweet potato viruses. *The Plant Pathology Journal*, **30**(4), 416.

Li, F., Xu, D., Abad, J., Li, R. (2012) Phylogenetic relationships of closely related potyviruses infecting sweet potato determined by genomic characterization of Sweet potato virus G and Sweet potato virus 2. *Virus Genes,* **45**(1), 118-125.

Lin, N.S., Hsu, Y.H., Hsu, H.T. (1990) Immunological detection of plant viruses and a mycoplasmalike organism by direct tissue blotting on nitrocellulose membranes. *Phytopathology,* **80**, 824-828.

Loebenstein, G. (2015) Control of sweet potato virus diseases. In: "*Advances in Virus Research*" (Vol. 91, pp. 33-45). Academic Press. https://doi.org/10.1016/bs.aivir.2014.10.005**.**

Loebenstein, G., Lecoq, H. (2012) Viruses and virus diseases of vegetables in the Mediterranean basin. *Academic Press*. https://www.bookdepository.com/Viruses-Virus-Diseases-Vegetables-Mediterranean-Basin-84-Gad-Loebenstein/9780123943149.

Loebenstein, G., Thottappilly, G., Fuentes, S., Cohen, J. (2009) **"***Virus and Phytoplasma Diseases. The Sweetpotato*". (pp. 105-134). Springer Sciences Business Media BV, Dordrecht, The Netherlands.

Luan, Y.S., Zhang, J., Liu, D.M., Li, W.L. (2007) Molecular characterization of sweet potato leaf curl virus isolate from China (SPLCV-CN) and its phylogenetic relationship with other members of the Geminiviridae. *Virus Genes*, **35**(2), 379-385.

Ma, S., Zheng, Q., Ye, J., Feng, W., Zhou, G., Zhang, T. (2019) Identification of viruses infecting sweet potato in southern China by small RNA deep sequencing and PCR detection. *Journal of General Plant Pathology*, **85**(2), 122-127.

Maina, S., Edwards, O.R., Barbetti, M.J., de Almeida, L., Ximenes, A., Jones, R.A. (2016) Deep sequencing reveals the complete genome sequence of Sweet potato virus G from East Timor. *Genome Announcements*, **4**(5). https://doi.org/10.1128/genomeA.00957-16.

Ngeve, J.M., Bouwkamp, J.C. (1991) Effects of sweet potato virus disease (SPVD) on the yield of sweet potato genotypes in Cameroon. *Experimental Agriculture,* **27**(2), 221-225. https://doi.org/10.1017/S0014479700018858.

Noordam, D.D. (1973) "*Identification of Plant Viruses: Methods and Experiments*". Center for Agriculture Publishing and Documentation, Netherlands, ISBN-13: 9789022004647, 207p.

Okonya, J.S., Ocimati, W., Nduwayezu, A., Kantungeko, D., Niko, N., Blomme, G., Kroschel, J. (2019) Farmer reported pest and disease impacts on root, tuber, and banana crops and livelihoods in Rwanda and Burundi. *Sustainability*, **11**(6), 1592. https://doi.org/10.3390/su11061592.

Parrella, G., Stradis, A.D., Giorgini, M. (2006) Sweet potato feathery mottle virus is the casual agent of sweet potato virus disease in Italy. *Plant Pathology*, **55**(6). https://doi.org/10.1111/j.1365-3059.2006.01476.x.

Prasanth, G.,Hegde, V. (2008) Occurrence of Sweet potato feathery mottle virus and Sweet potato leaf curl Georgia virus on sweet potato in India. *Plant Disease,* **92**(2), 311-311.

Price, W.C. (1945) Accuracy of the local lesion for measuring virus activity. IV. Southern Bean Mosaic Virus. *American journal of Botany*, **32**(10), 613-619.

Rännäli, M., Czekaj, V., Jones, R.A.C., Fletcher, J.D., Davis, R.I., Mu, L., Valkonen, J.P.T. (2008) Molecular genetic characterization of Sweet potato virus G (SPVG) isolates from areas of the Pacific Ocean and southern Africa. *Plant Disease*, **92**(9), 1313-1320.

Rännäli, M., Czekaj, V., Jones, R.A.C., Fletcher, J.D., Davis, R.I., Mu, L., Valkonen, J.P. (2009) Molecular characterization of Sweet potato feathery mottle virus (SPFMV) isolates from Easter Island, French Polynesia, New Zealand, and southern Africa. *Plant Disease,* **93**(9), 933-939.

Sivparsad, B.J., Gubba, A. (2013) Identification and distribution of viruses infecting sweet potato (Ipomoea batatas L.) in KwaZulu-Natal province, South Africa. *South African Journal of Plant and Soil,* **30**(3), 179-190.

Souto, E.R., Sim, J., Chen, J., Valverde, R.A., Clark, C.A. (2003) Properties of strains of Sweet potato feathery mottle virus and two newly recognized potyviruses infecting sweet potato in the United States. *Plant Disease*, **87**(10), 1226-1232.

Souza, C.A., Rossato, M., Melo, F.L., Pereira-Carvalho, R.C. (2018) Complete Genome Sequences of Sweet potato feathery mottle virus and Sweet potato virus G from Brazil. *Genome Announc*, **6**(5), e01567-17.

Sun, Z.Y., Zhang, Y.H., Xu, Q.Q., Chen, Z.X., Xie, L., Mao, B.Z. (2019) First report of sweet potato Feathery Mottle Virus infecting *Dendrobium candidum* in China. *Plant Disease*, **103**(5), 1047-1047.

Tang, J., Ward, L.I., Clover, G.R.G. (2013) The diversity of Strawberry latent ring spot virus in New Zealand. *Plant Disease*, **97**, 662-667.

Tennant, P. (Ed.). (2015) "*Virus Diseases of Tropical and Subtropical Crops*" (Vol. 3). CABI.

Tesfaye, T. (2010) Survey and serological detection of sweet potato (*Ipomoea batatas* (L.) Lam.) infecting viruses in ethiopia. *Doctoral Dissertation*, Addis Ababa University.

Tibiri, E.B., Tiendrébéogo, F., Pita, J.S., Somé, K., Bangratz, M., Néya, J.B., Barro, N. (2019a) Molecular and biological features of sweet potato leaf curl virus in Burkina Faso. *Acta Scientific Microbiology*, **2**, 170-177.

Tibiri, E.B., Somé, K., Pita, J.S., Tiendrébéogo, F., Bangratz, M., Néya, J.B., Barro, N. (2019b) Effects of sweet potato feathery mottle virus, sweet potato chlorotic stunt virus and their co-infection on sweet potato yield in Western Burkina Faso. *Open Agriculture*, **4**(1), 758-766.

Trenado, H.P., Lozano, G., Valverde, R.A., Navas-Castillo, J. (2007) First report of Sweet potato virus G and Sweet potato virus 2 infecting sweet potato in Spain. *Plant Disease,* **91**(12), 1687-1687.

Tugume, A.K., Mukasa, S.B., Valkonen, J.P. (2016) Mixed infections of four viruses, the incidence and phylogenetic relationships of Sweet potato chlorotic fleck virus (Betaflexiviridae) isolates in wild species and sweetpotatoes in Uganda and evidence of distinct isolates in East Africa. PlOS ONE, **11**(12). https://doi.org/10.1371/journal.pone.0167769.

Untiveros, M., Fuentes, S., Kreuze, J. (2008) Molecular variability of sweet potato feathery mottle virus and other potyviruses infecting sweet potato in Peru. *Archives of Virology,* **153**(3), 473-483.

Wokorach, G., Otim, G., Njuguna, J., Edema, H., Njung'e, V., Machuka, E.M., Echodu, R. (2020) Genomic analysis of Sweet potato feathery mottle virus from East Africa. *Physiological and Molecular Plant Pathology,* **110**, 101473. https://doi.org/10.1016/j.pmpp.2020.101473.

Wosula, E.N., Clark, C.A., Davis, J.A. (2012) Effect of host plant, aphid species, and virus infection status on transmission of Sweetpotato feathery mottle virus. *Plant Disease*, **96**(9), 1331-1336.

Yan, K.R., Zhang, Y.H., Yang, C.B., Ma, C.N., He, B.W., Mao, B.Z. (2020) First report of sweet potato Feathery Mottle Virus infecting *Chrysanthemum morifolium* in China. *Plant Disease*, (ja). https://doi.org/10.1094/PDIS-10-19-2156-PDN.

دراسة انتشار اثنين من فيروسات البطاطا الحلوة و الأعشاب البرية المحتملة كمستودع لها في مصر

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تعد البطاطا الحلوة (العائلة العليقية) علي المستوي العالمي محصول نباتي هام ، ولسوء الحظ ، تسبب العدوى الفيروسية خسارة كبيرة فيه. تهدف الدراسة الحالية إلى التعرف على الفيروسات التي تصيب البطاطا الحلوة في مصر وتسلط الضوء على دور الحشائش المصاحبة كمستودع للفيروسات. غطت الدراسة النقاط الآتية: الانتقال، مدى العائل ، الأجسام المميزة للفيروس ، مورفولوجيا الفيروس ، تشريح الخلايا المصابة ، التشخيص السيرولوجي ، تحديد الجينوم باستخدام RT-PCR المتعدد .

أوضحت النتائج أن فيروس البطاطا الحلوة (SPFMV) وفيروس البطاطا الحلوة (SPVG) هما من أكثر الفيروسات التي تصيب البطاطا الحلوة في مصر. كانت الإصابة المزدوجة بكلا الفيروسين هي السمة الشائعة في كل من البطاطا الحلوة والأعشاب المصاحبة لها. تم الكشف عن مدي أوسع في العائلات هي العليقية والرمرامية واللبينية. وقد رصدت أعراض الإصابة على البطاطا الحلوة مماثلة لتلك التي ظهرت علي الأعشاب المصاحبة لها. أكما أظهرت النتائج أن معدل حدوث العدوى في أنواع الأعشاب المعمرة من العائلة العليقية والعائلة التيلية هي 100%. لوحظت الأجسام غير المتبلورة المميزة للفيروسات المكتشفة والتغيرات المرضية داخل خلايا العائل تحت المجهر الإلكتروني النافذ. تمت مقارنة تسلسلات النيوكليوتيدات لجينات بروتين الغلاف الجزئي للفيروسات المكتشفة بالعزلة المتوفرة في بنوك الجينات. يوصي هذا العمل بأهمية التعامل مع الأعشاب البرية لأنها مستودع للفيروسات و أهمية العمل البحثي متعدد التخصصات لحل تهديدات مشاكل الفيروسات في المحاصيل الاقتصادية. يؤدي التعرف على الفيروسات ومدى إصابتها إلى وضع إستراتيجيات ذات كفاءة للسيطرة على انتشار الفيروس.