ISOLATION AND CHARACTERIZATION OF A GEMINIVIRUS ISOLATED FROM SUGAR BEET PLANTS IN EGYPT

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Sugar beet (*Beta vulgaris)* is considered as the second most important crop for sugar production because it supplies approximately 25% of the world raw sugar, (Megahed *et al*., 2015). It's cultivated in almost 41 countries with a total acreage usage close to ~20 million acres. Sugar beet has several advantages over sugar cane, such as less water requirements, shorter growth cycle, and better adaptation for sandy soil. Additionally, sugar beet foliage provides rich and thirst-quenching forage for animals during high temperature of the summer (Lukovic *et al*., 2009; Megahed *et al*., 2015). Beet is cultivated in 17 Egyptian governorates with an estimated total area of 550,000 acres, which provides approximately 8.9 MMT (million metric tons) used for sugar production (USDA, 2018). A major problem associated with sugar beet is the highest vulnerability to at least sixteen different viruses, seven out of those have seen already recorded in Egypt, (Mayo *et al*., 2005). Examples include, *Cucumber mosaic cucumovirus* (CMV), *Beet mosaic potyvirus* (BtMV), *Beet necrotic yellow vein benyvirus* (BNYVV), *Beet yellows closterovirus* (BYV)**,** *Beet western yellows luteovirus* (BWYV), *Beet necrotic ringspot virus* (BNRSV) and Beet curly top geminivirus (BCTV), which belongs to Geminivirus group, (Megahed *et al*., 2015).

Geminiviruses are characterized by their geminate structure and the unique Gemini shape of fused icosahedral viral particles, (Diana *et al*., 2013; Karyna *et al.*, 2016; El-Gaied *et al*., 2017). Geminiviruses are considered as a major threat for crop production, especially those transmitted through whiteflies. There are seven genera in Family *geminiviridae*, where genus *Begomovirus* is the largest genus among this family, (Stanley *et al*., 2005; Adams *et al*., 2013; Diana *et al*., 2013; Yaqin *et al*., 2014;

Wenxi *et al*., 2015; Karyna *et al*., 2016; Sayed *et al*., 2016). There are two main subgroups within the geminiviruses based on insect transmission. First group is transmitted by leafhoppers and 2nd group transmitted by whiteflies (WTG), (Goodman, 1977; Salem, 2018).

There are other many ways for geminiviruses transmission that include grafting and mechanical methods. No viral transmission through seeds was reported so far, (Alice *et al*., 2016). Geminiviruses symptoms vary depend on the developmental stage of the plant, plant cultivar, kind of virus, and infection time. Environmental conditions such as temperature, soil water contents, and physical and chemical properties of the soil are also important factors that affect viral infection. Symptoms like bright yellow mosaic, flower abscission, puckering of leaves, leaf rolling, leaf distortion, reduction in leaf size, chlorotic mottle and stunting of the infected plant were observed in infected plants, (Polston and Anderson, 1997; Yaqin *et al*., 2014).

Since geminiviruses considered as a major threat for crop production, we directed our effort towards detection and characterization of members of this group of viruses that affect sugar beet. We tried to answer some questions like: can other members of geminiviruses affect sugar beet? If yes, what is the mood of transmission? And how close would it be to TYLCV? To that end, biological, serological and molecular characterization of geminivirus that may infect sugar beet were also explored.

MATERIALS AND METHODS

Samples collection and virus detection

Samples from sugar beet plants exhibited geminivirus like symptoms (GVLS) and heavy infestation of whiteflies, were collected from several locations in different governorates (Fayoum, Giza and Kafr El-Shekh), during February and March 2015. Subsequently, collected samples were indexed after cultivation at the green house facility, and then sprayed with Bremor (1.5 ml/1l H_2O) to kill the immature and mature stages of the whiteflies (*Bemisia tabaci*). Infection with Geminivirus in collected plants was examined using ELISA followed by PCR. Indirect DAS-ELISA was performed as described by Clark and Adams (1977), using TYLCV antiserum specific for WTG and C4 antiserum specific for TYLCV Egyptian isolate. The PCR was performed using WTG specific primers (Abdallah *et al*., 1993) as described by Essam *et al*. (2004). Position and sequence of the used primers are listed in Table (1). Total DNA of collected plants was extracted using Qiagen DNA Extraction kit according to manufacture instruction. The PCR mixture (25 µL), contained 2 µM of each primer, 1X PCR buffer, 1.5 mM $MgCl_2$, 160 µM dNTPs, 5 µL of extracted DNA as a template and 0.5 unit Taq DNA Polymerase. PCR conditions were, 3 min at 94°C for one cycle, fol-

lowed by 35 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C, and finally one cycle of 3 min at 72°C.

Virus isolation, transmission and host range

A group of 150-200 virus free whiteflies, were given an acquisition access period on collected beet plants for about two days. Groups of whitefly each consists of 20-30 insect, were transferred to insect proof wooden cage to inoculate different healthy plant species, including beet, cotton (*Gosypium barbadense* L. Giza 83), family *Malvaceae*, Faba bean (*Vicia faba* L. Giza 402) family *Leguminosae*, tomato (*Solanum lycopersicum* L.) and tobacco (*Nicotiana tabacum*), family *Solanaceae*. Different modes of virus transmission (insect, grafting, and mechanical), were used. Insect transmission was conducted as described above. For grafting, healthy tomato plants were inoculated by wedges-grafting as described by Mansour and Al-Muss (1992), using virus-infected beet plants. For mechanical transmission**,** young leaves from infected beet plants were grinded in 0.1 M phosphate buffer, pH 7 and the crude sap rubbed the healthy plant leaves were dusted by carborundum. Phosphate buffer was used as a negative control. All inoculated plants were caged at 23°C in greenhouse. Virus presence in all inoculated plants was detected after 15- 20 days of inoculation by PCR as above described using HD3 and HD4 and TYLCV-CP specific primers, and symptoms were weekly-recorded.

Electron microscope examination

Small pieces of both infected and healthy beet leaves were taken and fixed in 5% cold glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2 for 4 h at 4°C, followed by washing with 0.01 sodium phosphate buffer (SPB), pH 7.2 for 10 min. The specimens were post fixed in 1% Osmiun tetra oxide in SPB for 2 h at 4°C. The specimens were treated with ascending gradient of cold ethanol started with 30% till 100% at 4°C (1 h for each concentration). For post dehydration the propylene oxide (PO) was added for 30 min at 4°C. The propylene oxide was then replaced with mixture of PO and resin medium 1:1 (v/v) for 2 h, 1:2 (v/v) for 4 h, 1:3 (v/v) overnight and finally with pure resin for 4 h at 4°C. The specimens were incubated at 37°C for 72 h. The ultrathin sections were prepared using the ultramicrotone and silver sections were picked into the cupper nylon coated grids. The selected sections were stained with a mixture of 2% uranyl acetate and acetone (1:1, v/v) for 20 min at room temperature followed by staining with Reynold lead citrate for 20 min. The grad was washed with water and dried on a filter paper, then was examined under the electron microscopy (Phillips), as described by Paliwal (1978).

Molecular characterization

PCR fragment for a part of viral genome located in the core region of the geminivirus *C1* gene was amplified by PCR using HD3 and HD4 primers and

subjected to nucleotide sequence determination.

The geminivirus infecting beet was further characterized by Rolling circle amplification (RCA) combined with restriction fragment amplification (RFLP). Circular DNA was amplified using TempliPhi^{TM} kit (GE Healthcare) according to the manufacture described protocol. Two µL containing 10 ng of total DNA extracted from geminivirus infected plants was dissolved in 1x sample buffer, and denatured at 95°C for 3 min and then ice cooled down for 1 min. Components were incubated for 20 h at 30°C after adding the reaction buffer. Reaction was stopped by heating at 65°C for 10 min. Aliquots of ~300 ng of PCR products were digested by restriction enzymes *Hpa*II (Biolabs) for 1 h. Restriction products were run on 2% agarose gel which was subsequently stained using ethidium bromide.

RESULT AND DISCUSSION

Detection of geminiviruses in beet plants

Plant samples with (GVLS) were collected from three different governorates in Egypt during February and March of 2015. Main symptoms observed were leaf curling, leaf rolling and vein banding of leaves and stunting growth (Fig. 1**)**. Collected plants were tested with ELISA and PCR for the presence of viral antigen(s) and viral DNA, respectively.

Begomoviruses cause great loss to the agriculture sector worldwide, (Zhou, 2013; Leke *et al*., 2015). They infect large number of dicotyledonous plants, which include thirty-seven genera classified within seventeen families. Host plants for geminiviruses include, but not limited to, fiber crops, vegetables, ornamentals and uncultivated vegetation, (Zhou, 2013; Abouseadaa *et al*., 2015; Karyna *et al*., 2016).

Globally, sugar beet (*Beta vulgaris*) is the second-best crop, after sugarcane, used for sugar production. Sugar beet is more advantageous over sugar cane because it needs less time to grow, less water requirements, and its fast adaptation to non-rich soil. Furthermore, sugar beet foliage provides rich and green forage for animals during summer, (Lukovic *et al*., 2009; Megahed *et al*., 2015). The above-mentioned factors encouraged farmers to grow more sugar beets, which has led to a significant increase in beet cultivation and production in Egypt that estimated 9.5 million metric ton in 2018.

DAS-ELISA

Indirect DAS-ELISA was conducted using two different antisera; the first was TYLCV polyclonal antiserum, which reacts with WTGs and the second was C4 antiserum, specific for TYLCV Egyptian isolate. Both antisera were used in dilution of 1:1000. Healthy beet with no apparent symptoms was used as a negative control. Results showed that 22 out of 27 tested beet plants reacted positively with TYLCV. No reactivity was observed when C4 antiserum was used (Fig. 2).

The aim of this study was to detect and identify a geminivirus that is locally spread in sugar beet fields in Egypt using DAS- ELISA and PCR. To that end, sugar beet fields from three different locations were selected and beet plants with GVLS were collected. The most remarked symptoms were leaf curling, leaf rolling and vein banding of leaves and plants stunting. We also noticed that theses GVLS plants were accomplished by heavy infestation of whiteflies. GVLS plants were tested with DAS-ELISA using C4-antisera specific to TYLCV Egyptian isolate and TYLCV polyclonal antiserum specific to WTG. C4 antisera gave negative results with all the examined plants, whereas the TYLCV polyclonal antiserum reacted positively. These results were expected where C4 antiserum is specific for the movement protein of TYLCV-Eg and TYLCV polyclonal antiserum has common epitops for WTGs. This finding indicated that the virus detected in sugar beet belongs to WTG but not TYLCV Egyptian isolate.

Serological techniques are extremely useful for the identification, quantitative assay and routine detection of plant viruses, (Clark and Adams, 1977; El-Gaied *et al.,* 2014; Lonoce *et al*., 2016). Our results agreed with (Akad *et al*., 2004; El-Gaied *et al*., 2014), they were able to trap plant viruses using ELISA with specific antibodies. It also agreed with (Megahed *et al*., 2015), who used DAS-ELISA to detect Beet necrotic yellow vein, Beet curly top geminivirus, Beet mosaic potyvirus, Cucumber mosaic cucumovirus, and Beet yellow clostero virus in Beet plants.

PCR

Eight samples plants were examined for ELISA, PCR analysis using WTGs specific primers (HD3 and HD4). Five out of eight examined plants gave the expected molecular weight (659 bp) as well as the positive control, while negative result was obtained by using healthy plant samples (Fig. 3).

Isolation of WTG from infected beet plants

Beet plants were exposed to viruliferous whiteflies for 20 days and then examined for the presence of geminiviruses by PCR using WTGs- specific primers (HD3 and HD4) (Fig. 4A) and TYLCV coat protein- specific primers (CP-F and CP-R) (Fig. 4B). PCR amplicons were observed when HD3/ HD4 primers (lanes 1-4). No PCR amplicons were obtained when CP-F/ CP-R primers (Fig. 4B, lanes 1-6).

Furthermore, symptoms appeared on beet plants after 40-45 days of inoculation with viruliferous whiteflies including deformation of small leaves, vein banding, curling and upward cup shape of leaves, infected plants appeared to be stunted compared with the control (non-inoculated plants) which exhibited no viral-infection (Fig. 5).

PCR using WTG- specific primers gave positive results when tested against

beet- extracted DNA, 20 days post exposure to viruliferous whiteflies, indicating that the virus belongs to WTG. A 659 bp-amplicon was obtained from infected beet plants, whereas, no amplification was detected using TYLCV-Cp specific primers. The 659 bp- amplicon was sequenced and blasted using GenBank database.

Virus modes of transmission and host range

In host range experiment, different plants species such as; cotton, faba bean, tomato and tobacco, were subjected to viral infection by using viruliferous whiteflies. Inoculated plants were tested for viral infection 15-20 days post inoculation by PCR using WTG specific primers. Specific PCR fragments were amplified from all tested plants except faba bean plants (Fig. 6 A, B, C, D). To test the different modes of virus transmission, infection was confirmed using PCR 15-20 days post grafting and mechanical transmission in tomato and beet plants, respectively, (Fig. 6 E, F). Virus symptoms in host range experiment were noticed 30-45 days post inoculation on inoculated plants (cotton, tomato and tobacco), while no symptoms were observed on faba bean (Fig. 7 panels 1, 2 and 3). Grafted plants (tomato) showed downward cup shape and curling of the leaves, while the mechanically transmitted plants (beet) showed identical symptoms including upward cup shape and curling of the leaves and stunting of plants 30-45 days post transmission (Fig. 7 panel 4).

Host range experiment using *viruleferous* whiteflies was carried out and followed by PCR verification using WTG specific primers. Infected plants showed symptoms comparable with those observed in infected beet plants. These results were in accordance with those seen in TYLCV and pepper leaf curl virus, (Essam *et al*., 2004; El-Gaied *et al*., 2014), respectively. PCR results indicated that the virus obtained from beet was detected in tomato, nicotiana, and cotton plants but not in faba beans. Also results are in accordance with Shirazi *et al*. (2014) who successfully used cucumber, pepper, and tomato plants as host range to transmit TYLCV.

Non- insect methods of viral transmission, such as grafting and mechanical transmission, were carried out, and indicated the presence of virus in infected plants. These results were in accordance with (El-Gaied *et al*., 2008) who reported that strawberry leaf curl geminivirus was successfully transmitted by grafting and by mechanical method. In addition, (Henry *et al*., 2013) showed transmission of Cassava brown streak virus using grafting method under controlled conditions. This also was observed with (El-Gaied *et al*., 2014) who was able to transmit pepper leaf curl virus to pepper plants using both grafting and mechanical transmission.

Electron microscope examination

Ultrathin-sections of the infected beet plants were prepared from samples inoculated with virulefrous whiteflies.

Sections of infected leaves were examined compared to the healthy one using electron microscopy. Figure (8) represents the ultrathin section of healthy beet plants compared with infected beet plants. The results showing the circular inclusion bodies aggregated in infected cells. In addition**,** some cytopathological effects are observed as severe damage in chloroplasts and abnormal build-up of many enlarged plastoglobuli, vesicles, deformation of nucleus, degenerated mitochondria, disorganized and malformation of chloroplasts. Virus particles appeared in chloroplast, also wide intra-spacing were also observed between cells**.**

Ultrathin sections were prepared from infected beet plants and examined under electron microscopy. Results showed that circular inclusion bodies accumulated in the cytoplasm of infected cells. In addition, some cytopathological effects and anatomical deviations on the cell components indicated severe deformation in chloroplasts and abnormal build-up of many enlarged plastoglobuli vesicles and uneven thickening of cell wall of infected beet. These results were agreed with, (Magdy *et al.,* 2012), who examined ultra-thin section of infected tomato under electron microscope and the examination showed internal anatomical changes with great damage, disorganized, malformation in chloroplasts accompanied with wide intra-spacing were also observed between cells. Abnormal build-up of many enlarged plastoglobuli, vesicles, deformation of nucleus, degenerated mitochondria were also observed.

Geminivirus DNA analysis

The 659 bp- amplicon that was obtained from infected beet plants using WTG- specific primers (Fig. 4A) was sequenced and blasted against GenBank database. Sequence analysis revealed high homology to the core region of C1 gene of geminiviruses (Fig. 9-a). A phylogenetic tree was constructed based on nucleotide sequence alignments and confirmed that the beet virus belongs to the Begomoviruses (Fig. 9-b)**.** Sequence analysis revealed high homology to the core region of *C1* gene of geminiviruses. A phylogenetic tree was constructed based on nucleotide sequence alignments and confirmed that the beet virus belongs to the geminiviruses which consider as a new isolate of TYLCV**.** These results agreed with (Sayed *et al.,* 2016), who used phylogenetic relationships to determine the relationship of TYLCV isolate with selected begomoviruses in Kingdom Saudi Arabia.

Detection and conformation of rolling circle amplicons (RCA)

The presence of the geminivirus in four infected samples from different plants (beet, tomato, tobacco, and cotton) was confirmed by the rolling circle amplification (RCA) (Fig. 10, lanes 2-5, respectively). A typical RCA products profile was produced from all samples. The amplicon sizes ranged from 250-1400 bp, expected sizes for DNA-A and DNA-B. No PCR amplicons were detected in healthy beet (Fig. 10, lane 6).

RCA combined with RFLP were enriched the detection and diagnosis of geminiviruses, since they show more suitability and sensitivity with the small DNA sample for geminiviral screening (Haible *et al*., 2006; Jeske *et al*., 2010). RCA combined with RFLP was used in our study to confirm the presence of geminiviruses infection in tested samples which visualized a typical RCA/RFLP profile from all infected samples

SUMMARY

Sugar beet is one of the main sources for sugar manufacturing in Egypt, however its cultivation and growing can be severely hampered due to viral infection. In this study beet plants infected with geminivirus-like symptoms (VLS), such as leaf curling, leaf rolling, and stunting plants growth, were collected from different several locations and examined for the presence of geminivirus through indirect DAS ELISA using two antiserums, namely C4 antiserum specific for tomato yellow leaf curl virus (TYLCV), which gave negative results, and TYLCV polyclonal antiserum which is common for any whitefly transmitting geminiviruses (WTGs) which gave positive results. Plants were tested by PCR using WTGs specific primers, positive results were obtained. Virus transmission from infected beet plants to different host plants was also been assessed. Our data revealed the ability of the virus to infect tobacco, tomato and cotton while faba bean gave negative result. Electron microscope examination showed virus-like particles scattered in the cells of positive plant. Additionally, significant cytopathological effects due to viral infection were observed in the nuclei and many other organelles. Furthermore, the typical geminivirus amplicons from rolling circle amplification (RCA) combined with restriction fragment digestion amplification, which were detected in those infected host plants. Our results demonstrated that the isolated virus from infected beet plants belongs to the whitefly-transmitted geminiviruses (WTGs). Based on transmission patterns, virus host range, and sequence analysis of C1 region of viral genome, we suggest that the virus presented in this study can be considered as a new isolate of Tomato yellow leaf curl virus (TYLCV).

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Table (1): Primers used for PCR detection of Geminivirus.

Primer	Sequence 5→3	Additional coding site	location in viral genome	Expected amplicon
CP-F	CG**GAATTC**ACATGTCGAAGCGACCAGG	*BamH*I	467-1253	787 bp
CP-R	CG**GGATCC**TTAATTTGATAATGAATC	*EcoR*I		
HD-3	CG**GGATCC**AAACAGGTCAGCACATTTCC	*BamH*I	391-1049	659 bp
HD-4	GG**GGTACC**TATATGAGGAGGTAGGTCC	*Kpn*I		

*Restriction recognition sites (bold and underlined) that added to the 5 end of each primer.

Fig. (1): Sugar beet plants collected from open field associated with heavy infestation of whiteflies and showing virus like symptoms such as, curling, rolling and vein banding of leaves.

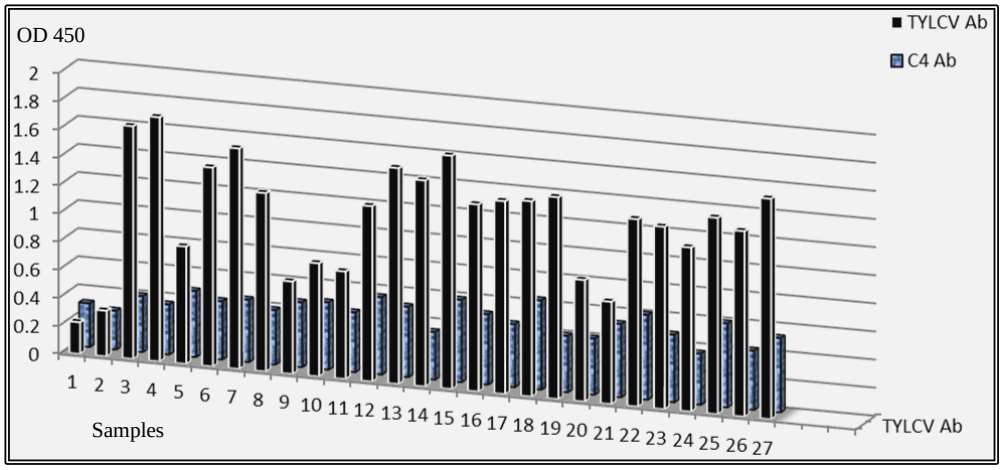

Fig. (2): A histogram shows the detection of WTG in collected beet plants by DAS ELISA using TYLCV and C4 antisera. Column No.1: represents the negative control.

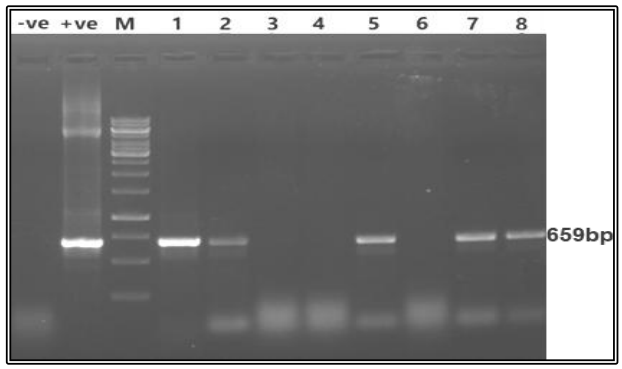

Fig. (3): Amplified PCR fragments of 659 bp (lanes 1, 2, 5, 7 and 8) produced using WTG primers (HD3 and HD4) for eight VLS beet plants collected from the open fields. While lanes (3, 4 and 6), showed negative results. The cloned TYLCV genome (lane +ve) was used as positive control. Lane -ve represents healthy beet plant

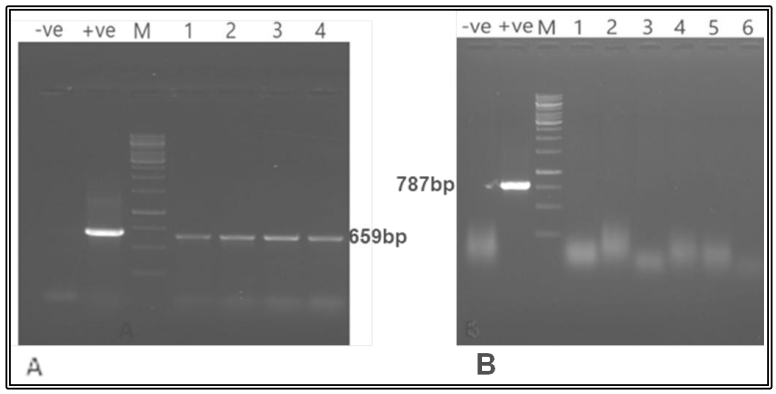

Fig. (4): PCR analysis using HD3 and HD4 for five beet plants (A), lane (-ve) represents negative control from healthy plant. Lane (+ve) represents positive control produced from cloned TYLCV genome. Lane M represents the 1Kb ladder. Lane (1-4) represent infected beet plants which gave positive results of 659 bp as well as the positive control. PCR analysis using CP-F and CP-R primers for seven beet plants (B), lanes: (1-6) represent infected plants. Lane (-ve) represents healthy beet plant. Lane (+ve) represents positive control produced from cloned TYLCV genome of 787 bp. Lane M represents the 1Kb ladder.

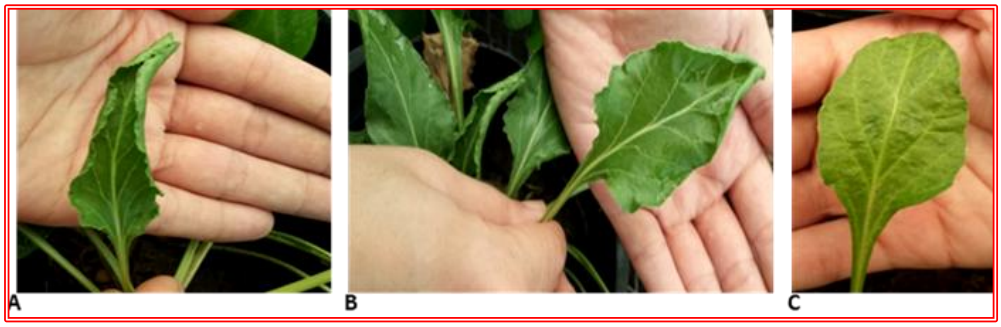

Fig. (5): Beet plants showing upward cup shape, curling and deformation of new leaves, 45 days post inoculation with viruliferous whiteflies (A, B), compared with, healthy plants (C).

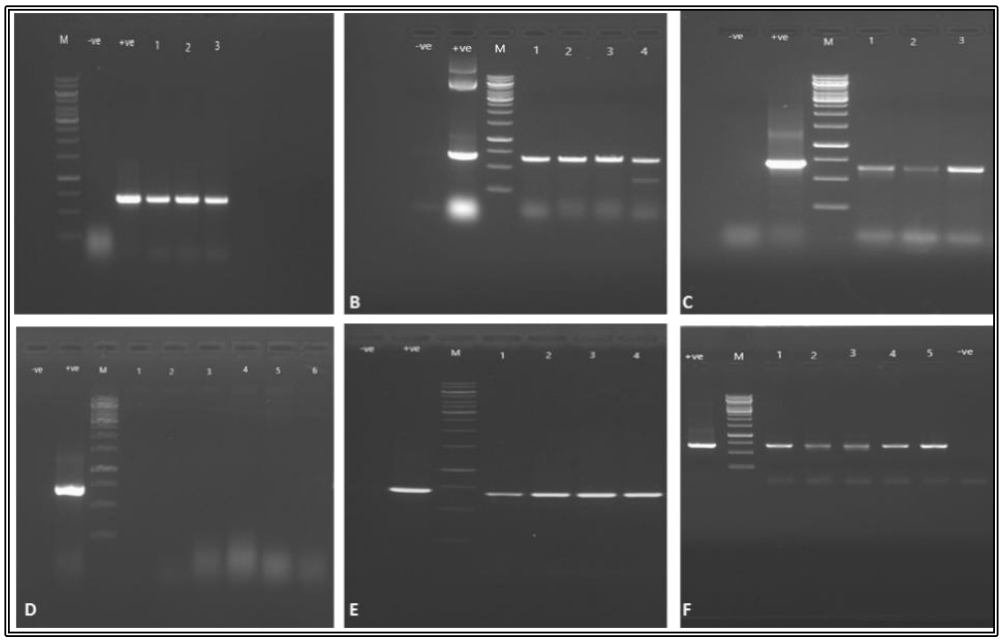

Fig. (6): Represents the detection of geminivirus by PCR using HD3 & HD4 primers in different host range, grafted and mechanically transmitted plants. Lane M: 1 Kb ladder, lane +ve: represents PCR produced from cloned TYLCV genome as a positive control of 659 bp, while lane -ve represents negative control produced from healthy plants. A: *Nicotiana*, B: tomato, C: cotton, D: bean, E: grafted tomato plants and F: mechanically inoculated Beet plants.

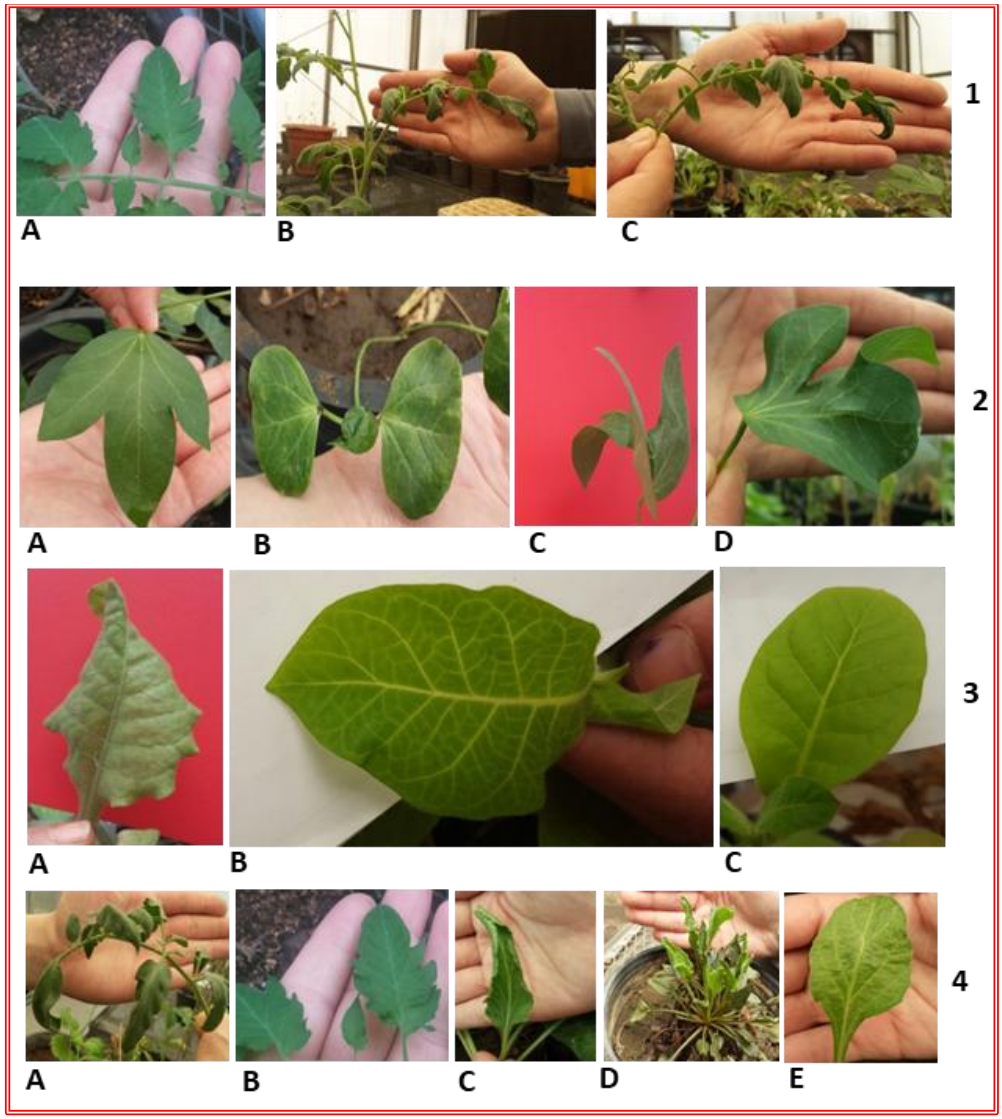

Fig. (7): Photos represent the viral-infection symptoms on tomato, cotton and *Nicotiana* plants after 30-45 days post inoculation with viruliferous whiteflies (upper three panels), and symptoms seen on tomato and beet plants after 30-45-day post grafting and mechanical transmission, respectively (panel 4). A in the panel (1), is a healthy tomato plant, where B and C showing symptoms appeared on tomato. A in the panel (2) represents a healthy cotton plant, while B, C, and D represent symptoms seen on cotton plants including deformation of leaves, curling and stunting. Symptoms appeared on panel (3) in A and B in *Nicotiana* plants, including curling, interveinal yellowing and stunting of new leaves and a healthy symptomless *Nicotiana* plant in C. In the panel (4), A, represent symptoms on tomato plants after grafting. Figure C, and D, including curling and downward cup shape on mechanically inoculated beet plants, while B and E are healthy tomato and beet plants, respectively.

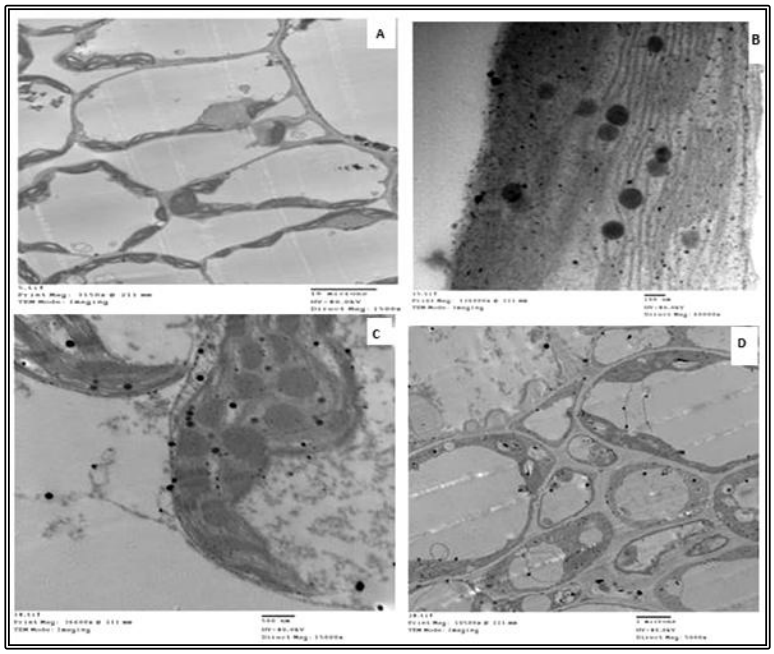

Fig. (8): Electron microscopy examination showed appearance of internal symptoms in the form of anatomical deviations of intensive damage in chloroplasts and change in the build-up of many enlarged plastoglobuli, vesicles. A: Healthy plant. B: virus particles aggregated in chloroplast. C: virus particles and malformation of chloroplast. D: wide intra-spacing between cells of infected plants

CTGTATCCGAGCTCAGCGCCTGTCTGGGTCGCTGACCCGCCAGTCAAGACCTACGTGGAGA
AAGACGGAGACTTCATTGATTTTGGAGTTTTCCAAATCGATGGCAGATCAGCTAGAGGAGG
TCAGCAATCTGCCAACGACGCATATGCCGAAGCACTCAATTCAGGCAGTAAATCCGAGGCC
CTCAATATATTAAAAGAGAAGGCCCCAAAGGACTATATTTTACAATTTCATAATTTAAGTT
CAAATTTAGATAGGATTTTTAGTCCTCCTTTAGAAGTTTATGTTTCTCCATTTCTTTCTTC
TTCTTTTAATCAAGTTCCAGATGAACTTGAAGAGTGGGTCGCCGAGAACGTCGTGTCTTCC
GCTGCGCGGCCATGGAGACCTAATAGTATTATCATCGAGGGTGATAGCAGAACAGGCAAAA
CAATGTGGGCCAGGTCCCTAGGCCCACATAACTATTTATGTGGACATCTAGACCTAAGCCC
AAAGGTGTACAGTAATGATGCGTGGTACAACGTCATTGATGACGTAGACCCGCATTATTTA
AAGCACTTCAAGGAATTCATGGGGGCCCAGAGGGACTGGCAAAGCAACACAAAATACGGGA
AGCCCATTCAAATTAAAGGGGGAATTCCCACTATCTTCCTCTGCAATCCAGGACCTACCTC
CTCATATAGGGTACCCCCTG

Fig. (9-a): Nucleotide sequence of the amplified PCR fragment using HD3 and HD4 primers which located in core region of C1 gene.

Fig. (9-b): Phylogenetic tree produced by the alignment of the amplified nucleotide sequence with the GenBank databases.

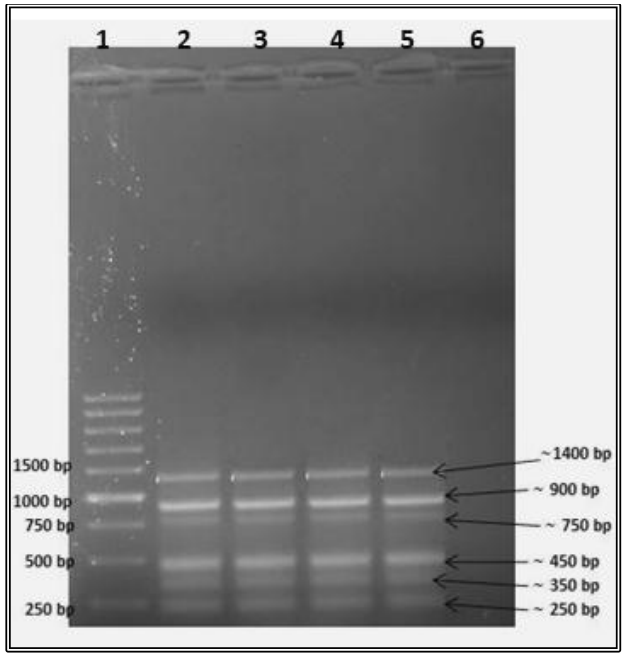

Fig. (10): Detection of geminivirus rolling circle amplicons in four infected plant samples. Lane 1: DNA marker; lanes 2, 3, 4, 5 represent Beet, Tomato, Tobacco, and cotton plants, respectively, while lane 6 represents healthy beet plant.