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CHARACTERIZATION OF *TOBACCO MOSAIC TOBAMOVIRUS* (TMV-S) ISOLATED FROM SUNFLOWER (*HELIANTHUS ANNUUS* L.) IN EGYPT

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ABSTRACT

This is the first record of Tobacco mosaic *Tobamovirus* (TMV-S) on sunflower in Egypt. It was originally isolated from naturally infected sunflower plants growing in Giza Res. Station, showing systemic mosaic and spots. Purified TMV-S migrated as a single zone in density gradient column. Ultraviolet absorbance of TMV-S was typical of nucleoprotein with minimum and maximum at 247 and 260 nm respectively. The ratios of $A_{260/280}$ and $A_{max/min}$ were 1.2 and 1.1 respectively. Electron microscopy of purified virus showed the presence of rod shape particles with a size 300 nm. Titer of the prepared antisera as determined using ELISA was 1/2000. Electron microscopic examination of infected leaves of *N. clevelandii* founed various cytological abnormalities. Due to the non-availability of sources of resistance in Egypt to TMV-S in sunflower, a mutation breeding program was initiated. Seeds of two genotypes were subjected to four doses of gamma rays; 0, 100, 200 and 300 Gy from a ^{60}Co source. M1 and M2 generations were sown at the experimental farm of the Agricultural Res. Center to induce variability in the sunflower genotypes; Giza 102 and Sakha 53, which could be resistant to TMV-S. The statistical analysis indicated significant differences among irradiation doses on plant height; all used doses increased plant height comparing with the control. Inoculation with TMV-S caused decreases in plant height over all other factors. Number of leaves differed significantly according to the used cultivars, gamma ray doses and TMV-S. The highest number of leaves were found during M_2 generation, The differences in head diameter between cultivars were significant. The highest head diameter was observed in Giza 102 (21.7cm) in M_1 generation, followed by 20.2 and 20.0 cm for Giza 102 genotype developed through irradiation with 300 Gy in M_2 generation and Sakha 53 genotype developed through irradiation with 300 Gy in M_1 generation. Seed yield per plant differed significantly between all cultivars,

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irradiation doses and infection statuses. 100Gy of gamma ray irradiation doses decreased seed yield, while 200 and 300 Gy gamma ray dose increased seed yield per plant for both cultivars in both generations, The highest seed yield per plant were observed with Sakha 300 Gy in both generations, it was 104.1g (M_2) and 102.1g (M_1), while the highest seed index (100-seed weight) was noticed for Giza 102 genotype developed through irradiation with 200Gy in M_1 generation. It seems that 200 and 300 Gy treatments increased the most of studied characters in M_1 and M_2 generations in sunflower. The increments in mean of head diameter as a result of applying 300 Gy gamma ray dose increased seed yield/plant and seed index which finally improved seed yield. Both morphological characters and seed yield were reduced significantly as a result of virus infection. The effect of treatment with the virus was negative in all irradiated Sakha genotype developed through irradiation with100, 200 and 300 Gy, as well as and Giza 102 genotype developed through irradiation with100 Gy, because these genotypes tend to be resistant to TMV-S as ELISA test reported.

Keywords: *Tobacco mosaic virus* (TMV-S), *Tobamovirus,* sunfolwer, Purification, Antisera, Indirect ELISA, Electron microscopy.

INTRODUCTION

Sunflower *(Helianthus annuus)* is the most important source of edible oil in Zambia. It is widely grown and it is an important cash crop. Virus-like symptoms characterized by yellow blotching of leaves have been observed, especially among the newly introduced sunflower cultivars since 1988. Severe stunting of plants and almost 100% disease incidences has been recorded on some farms. Preliminary studies have indicated that *T. procumbens* is an alternate host to this viral disease and that aphids are capable of transmitting the disease from this host to sunflower (Muunga, pers. comm.). The disease is thought to be caused by *Sunflower yellow ring spot virus* (SYRSV) or *Sunflower yellow blotch virus* (SuYBV) genus ?*Umbravirus* reportedly infecting groundnut. A similar virus disease is reported to occur on sunfl ower in Kenya **(Theuri *et al.*, 1987).**

Although several new viruses belonging to the genus *Tobamovirus* have been described **(Lewandowski, 2005).** *Tobamoviruses* are known as serious plant pathogens , in particular the type species, *Tobacco mosaic virus* (TMV-S), is considered one of the most

dangerous plant viruses. *Tobamoviruses* are easily transmitted mechanically, without the help of vectors. Geographical distribution of *Tobamoviruses* is world wide **(Lewandowski, 2005).** There is still an urgent need to fully characterize this disease in order to formulate appropriate control strategies against it. This paper was initiated to: (I) Dectection of virus (TMV-S) in sunflower plants by electron microscopy. (2) Production of antiserum specific to TMV-S (3) yield losses

MATERIALS & METHODS

Virus source and symptoms:

Samples of sunflower (L). G. Don. plants showing typical systemic mosaic and yellowing symptoms of TMV-S were collected from Agriculture Research Experimental Station (ARES).

Isolation and propagation

The virus was isolated by repeated single lesion isolation on leaves of *Chenopodium amaranticolor* L. and then propagated in *Nicotiana tabacum* L cv Samsun as described previously (**Da Silva *et al.*, 2008**).

1- Purification of TMV-S

The method used in this study is similar to that described by **Takahashi and Ahohara (1990)** with minor modification. Rate zonal centrifugations in the sucrose density-gradients were made instead of cesium chloride (CsCl) solutions. Beside that, the final pellet was resuspended in potassium phosphate buffer, pH 7.2 instead of citric acid buffer, pH 8.2.extraction buffer. Chloroform only was used instead of carbon tetrachloride and ethyl-ether. TMV-S infected sunflower leaves, propagated by sap-inoculation, were designated as a testing material. Purification was performed by putting 0.1 M sodium citrate buffer (pH 6.8, 3X w/v) in the infected leaves, grinding them for 30 min in a 4°C cold room, and then filtrating through cheese cloth. After stirring up 30% Chloroform for 10 min, several centrifugation steps were followed. The supernatant was centrifuged through a 10-40 % linear sucrose gradient in 50% potassium phosphate buffer, ph 7.5 at 32, 000 rpm for 2.5hr in a Bekman SW 60 rotor. Gradient columns were made and stored overnight at 4°C prior to use. The virus zones were collected with a bent tip hypodermal needle and syringe. The virus zone was diluted 1:1 with 0.05M potassium

phosphate buffer, pH 7.5, and the concentrated by high speed centrifugation (HSC). The resulting pellets were resuspended in 2ml of the buffer and stirring overnight at 4 °C. The purified virus preparation was estimated spectrophotometrically to evaluate the purity and concentration, using an extension coefficient $E^{0.1\%}$ 2.2 for TMV-S **(Converse and Martin, 1990)** with a spectronic 2000 spectrophotometer. The OD_{260} was converted to mg of virus/ml. Infectivity was tested on leaves of *C. quinoa.*

2- Electron microscope examination:

Purified virus preparation was negatively stained with 2% phosphotungestic acid (FTA), pH 7.0 and examined with an electron microscope **(Noordam, 1973; Kim and Lee, 1999)**). A drop of virus preparation was placed on carbon coated grid for 2min. After filtration, a drop of 2% phosphotungestic acid (FTA) was added. After 2min more the excess liquid was drained. The treated grid was examined using JEOL-JEM-1010), Faculty of Science, Al-Azhar University.

3. Antiserum and ELISA reagents production:

3.1. Production of antiserum specific to TMV-S:

3.1.1. Rabbit immunization:

New Zealand white rabbits, about 3 kg weight was used for antiserum raised against TMV-S. A total of 8.5 mg purified TMV-S were used for subcutaneous .

Purified virus preparation was emulsified with an equal volume of Freud's complete adjuvant for the first and incomplete adjuvant for six subsequent injections at weekly intervals. Subcutaneous injections were made into the neck area of the rabbit by pulling up the loose skin and inserting a 22-gauge needle between the skin and muscle tissue **(Hampton *et al.*, 1990 and Da Silva *et al.*, 2008).**

3.1.2. Rabbit bleeding and blood collection:

Rabbits were bled one week after the last injection along five weeks, from the right ear. The blood was collected, left to clot at 37°C in an incubator for 1-2 hrs, and then kept at 4°C overnight. Antiserum was separated through centrifugation at 5,000 rpm for 3 min and stored at -20°C dispended as small aliquots in coated tubes until used for titer determination and other serological tests.

3.1.3. Determination of antiserum titer:

Checkerboard titration was used to determine optimal conditions for indirect micro plate ELISA for TMV-S antisera.

Clarified sap of virus infected and control squash leaves were diluted at 1/5, using phosphate buffer, pH 7.5, containing 0.85% NaCl. TMV-S antiserum preparations were diluted with the serum buffer, 1/500. 1/1000, 1/500, 1/1000, 1/1500, 1/2000, 1/2500, 1/3000, 1/3500, and 1/4000, respectively. The reaction was done between infected clarified extract and its induced antiserum by indirect ELISA test **(Converse and Martin, 1990)**

3.2. ELISA reagents production:

3.2.1. Purification of the immunogamaglobulin G (IgG):

Gamaglubulins were purified from the antisera using the caprylic acid method recorded by **Steinbuch and Audran (1969).**

1. One ml of virus antiserum was added to 0.06M sodium acetate buffer, pH 4.8 (1:2).
2. IgG was dialyzed against this buffer (o,o6 M sodium acetate) for about 24 hours (three times) at 4°C.
3. While stirring vigorously drop-wise 0.082 ml caprylic acid were added with continuous stirring for 30 min. at room temperature, then centrifuged at 8,000 rpm for 10min.
4. The supernatant was collected and dialyzed twice against 0.05M phosphate buffer, pH 7.2, for 4 hrs at 4°C.
5. The resulting IgG was diluted with distilled water to make 4 ml and an equal volume of saturated ammonium sulphate solution were added at room temperature while stirring and the stirring was continued for 30 min.
6. After centrifugation at 8,000 rpm for 10 min., the pellet was collected and suspended in 1ml distilled water followed by dialysis three times against 0.05M phosphate buffer, pH 7.2, for 24-48 hrs at 4°C. If necessary the IgG was centrifuged clear 10 min at 8,000 rpm. The IgG was then adjusted to 1mg/ml (A_{280} nm = 1.4) and stored at -20 °C in coated tubes until use.

3.2.2. Conjugation of IgG with alkaline phosphatase:

1. The bottle of alkaline phosphatase (0.7mg AP Sigma P-5521, 2,000 units) was washed with 3.2M ammonium sulfate, pH 7.0.

2. The precipitate was collected after centrifugation 20 min at 6.000 rpm and dissolved in 0.350 ml IgG (1mg/ml) at the rate of 2mg AP/1ml IgG.
3. The conjugate (IgG-AP) was dialyzed 5 times in PBS buffer, pH 7.5. The last dialysis, PBS buffer without NaN3 was used.
4. Gluteraldehyde (1%) was added to a concentration of 0.06% (V IgG-AP × 0.06 = V_g) and the mixture was incubated 4 hrs at room temperature with gentle stirring. A light yellow-born color was developed.
5. Gluteraldehyde was then removed by dialysis 5 times in PBS buffer with NaN3 (0.02%), the volume of the conjugate was measured and BSA (bovine serum albumin, Sigma A-4503) at the rate of 5mg BSA to 1ml solution was added.
6. The conjugate either dispended as small aliquots or diluted with glycerol to 50% (v/v), and stored at -20°C in coated tubes, where it should remain stable for many months. Because of volume changes and possible gama-globulin losses during the conjugation procedure all references to the use of conjugates are in terms of dilutions of the conjugate rather than at absolute concentrations.

Electron microscopy:

Sections of leaves exhibiting acute symptoms was prepared for electron microscopic examination. Leaf specimens were cut 1-2 cm long then fixed in 2.5% glutaraldehyde in potassium phosphate buffer, pH 7.4. Specimens were washed in cold buffer and postfixed in 1% osmium tetroxide in the same buffer for 3 h. Sections of leaves from healthy control plants were similarly prepared for electron microscopy. After staining overnight in 1% uranyl acetate (Gardner, 1967), the leaf specimens were dehydrated in ethanol-acetone series and embedded in Spurr's medium (Spurr, 1969). Ultra-thin sections were cut with glass knife on LKB ultramicrotome, mounted on copper grids and stained for 10 min with a mixture of an equal volume of saturated uranyl acetate and acetone followed by lead citrate **(Kim& Lee, 1999 and Da Silva *et al.*, 2008).**

Irradiation and field experiments:

Dry seeds (10% moisture content) of two sunflower cultivars: Giza 102 and Sakha 53 (obtained

from the Oil Crops Research Department, Field Crops Research Institute, ARC) were exposed to 0, 100, 200 and 300 Gy of gamma rays at a dose rate of 0.8 Gy min^{-1} at the National Center for Radiation Research and Technology, Atomic Energy Authority. Irradiated seed lots and non-irradiated controls were grown (immediately after irradiation) on 15th of June, 2009 at Giza Research Station, ARC to give M_1 generation. At harvest, data were recorded on morphological and yield components characters. Seeds of each genotype were bulked separately.

In M_2 generation, seeds from each irradiated M_1 treatment as well as controls were grown on 21st of June, 2010 to obtain M_2 plants. At harvest, data were recorded on

In M_1 and M_2 generations, a split-split plot design with four replications was used for each generation. The two infection statuses were devoted to main plots, two cultivars to subplots and four irradiated doses to sub-sub plots. The irradiated and non-irradiated seeds were sown in plots; each plot consisted of five rows, 4 meters long, 70 cm apart and 20 cm between hills. Random samples of 20 individual plants for both un-infected (healthy) and infected with TMV-S per treatment were used to measure studied characters: plant height (cm), number of leaves, head diameter (cm), seed yield/plant (g.) and 100-seed weight (seed index) (g.)

All data were statistically analyzed by the software **CoStat (2005)** in consultation with the analysis of variance **(Gomez and Gomez, 1984)**. The means were compared using Least significant Difference (LSD) at p=0.05 as outlined by **Duncan (1995).**

RESULTS & DISCUSSION

In Egypt, This first reported the occurrence of a new *Tobamovirus* infect cultivars sunflower. The virus was serologically with TMV-S even though it shared the same antigenic sites. Then *the Tobacco mosaic virus* sunflower strain (TMV-S- S) has been used for the new virus *Tobamovirus.* Do not learn to know precisely an isolate TMV-S which effect sunflower approach to any strains for TMV-S, after an analysis Nucleotide sequence special for the virus and this will be later.

1- Isolation and propagation:

The virus isolate, TMV-S was isolated from infected sunflower. After biological purification through single lesion transfers on *C. amaranticolor*, the resulting viruse was propagated on either *N. rustica* on *Helianthus annuus*.

Transmission studies:

Mechanical transmission:

TMV-S was easily transmitted mechanically to sunflower inoculated plants with showed chlorotic local lesions followed by systemic chlorosis **Figure (1).** It causes systemic chlorosis on seed heads **(Figure 2).**

3- Virus purification and ELISA kit production:

3-1- Purification of the virus isolate:

Purified TMV-S migrated as a single zone, 3 to 4 cm respectively below the minsicus of the density gradient column. This zone was found infections when tested in the local lesion host plant and gave typical ultraviolet absorption spectrum of nucleoprotein with a maximum and a minimum at 260 and 247, respectively **(Figure 3).**

$A_{260/280}$ and $A_{max/min}$ ratios were 1.2 and 1.1 for TMV-S. These results are inagreement with other investigators (**Brunt *et al.*, 1996**).

3-1-2- Electron microscopy:

Extracted virus particles from infected sunflower leaves were observed by an electron microscope using a dip method. Typical rod shape virus particles, 300 nm long were found **(Figure 4).** Such results agree with the diameter values reported for virions of TMV-S by **(Kim and Lee, 1999).**

4- Serologic studies

4-2- Production of antiserum specific to TMV-S:

Polyclonol antibodies raised against TMV-S was prepared. The antisera produced against TMV-S had titers of 1/2000 as determined by indirect ELISA test (Table 1). In the present work, the polyclonal antibody raised against TMV-S had a virus-specific titer of 1:2000, which was successfully used in ELISA technique. The concentration of IgG after purification and the IgG conjugated with alkaline phosphatase was 1:1000.

One of the major goals in the present work is to produce ELISA

reagents which can be used as a rapid method for TMV-S detection. **(Converse and Martin, 1990).**

5- Cytological studies:

5-1- Electron microscopy:

Electron microscopic examination of infected leaves of *N. clevelandii* revealed various cytological abnormalities which have been absent in healthy tissues. These abnormalities have been absent in healthy tissues. These abnormalities are.

1- ultra thin section of TMV-S infected *N. clevelandii* leaf showing nucleus which is misshapen and the chromatines are degenerated **(Figures 5, 6).** Cytological studies revealed abnormalities in nucleus described for many other *Tobamoviruses* **(Francki *et al.*,1985). Sabanadzovic *et al.*, (2008)** showed that virus particles were plentiful, forming large layered aggregates in the cytoplasm of infected petunias by TMV.

They often associated with plasmalemma and other membranes. The grana and intergrana appeared abnormal (Fig7) Nuclei were notably abnormal, usually appear as more or less ellipsoidal bodies, they contained intranuclear inclusion of various sorts that may affect the nucleolus or the size and shape of the nucleus. Sometimes the nuclear membrane have became obscure and broken and also showed virus like particles in the cytoplasm (Fig 8).

Previous identification of intracellular TMV by electron microscopy has been mentioned by **Thomas (1964)** extension of distorted encircling a mitochondria and

containing a large and numerous TMV particles.

They are typical for many Tobamoviruses. **Ladipo1 *et al.*(2003)** observed the same cytopathological changes in infected cells of *N. Benthamiana*, massive cytoplasmic inclusions consisting of stacked plate-like layers of virus particles. Each layer was a lateral aggregate of the rod-like particles with the particle ends in register. Presumably, due to fixation artifacts, the plate-like layers were also found in disordered

arrangements. Also **Dae *et al.*(2005)** mentioned that cells infected with TMV-S in the pepper cultivars of Cheongyang and Wangshilgun had the typical ultrastructures of tobamovirus as the stacked-band structure and multiple spiral aggregate (SA). The

cells infected with TMV-S had large numbers of *Tobamovirus* particles and accumulated in the cytoplasm and vacuole. TMV-S had the typical ultrastructures of *Tobamovirus* as the stacked and structure in cytoplasm.

Electron microscopic examination will contribute towards a rapid and clear identification of virus diseases of plants and will be useful for diagnostic purposes in agriculture and in plant phytopathology **(Bernd and Günther, 2009).**

Effect of Gamma ray irradiation treatments on sunflower characters in M_2 generation

The mean values of studied characters for two sunflower cultivars in M_1 and M_2 generation after irradiation with gamma ray doses are presented in Table (2).

Significant differences were found between the two sunflower cultivars, gamma ray doses and treatment with TMV-S for plant height, number of leaves, head diameter, seed yield y). In this respect, A land seed index in both M_1 and M_2 generations. The highest genotype was Sakha 53 (Table 2). The highest plant height was observed in Sakha 53 treated with 200 Gy in M_2 generation (187.0 cm), followed by Giza 102 treated with 300 Gy (183.0 cm). The statistical analysis indicated significant differences among irradiation doses on plant height; all used doses increased plant height comparing with the control. Inoculation with TMV-S caused decreases in plant height over all other factors.

Number of leaves differed significantly according to the used cultivars, gamma ray doses and TMV-S. The highest number of leaves were found during M_2 generation, it was 36.7 (for Sakha 53 genotype developed through irradiation with 200 Gy) followed by 35.7 (for Giza 102 genotype developed through irradiation with 300 Garge spectrum of variability for morphological characters was isolated and characterized by **Jambhulkar and Joshua (1999),** among them 3 were for chlorophyll, 9 for leaf, 3 for stem, and 8 for capitulum. All of them bred true in subsequent generations. Distinct mutants were yellow leaf vein, fasciation and zigzag stem. Three characters were mutated in the wrinkled leaf mutant: the lamina was dark green and highly wrinkled, the petioles were thick and shortened and the ray florets were dissected. These novel mutations have not been

reported so far, among the large number of mutations for morphological characters that were isolated and characterized for their inheritance pattern earlier (**Luczkiewicz, 1975**). Single recessive gene and two genes with complementary effect controlled most of them. Similar gene action for various morphological mutants has also been reported (**Miller, 1992**). Genetic analysis of the fascinated mutant in our studies showed that it is governed by a single recessive gene.

The differences in head diameter between cultivars were significant. The highest head diameter was observed in Giza 102 (21.7cm) in M_1 generation, followed by 20.2 and 20.0 cm for Giza 102 genotype developed through irradiation with 300 Gy in M_2 generation and Sakha 53 genotype developed through irradiation with 300 Gy in M_1 generation.

Seed yield per plant differed significantly between all cultivars, irradiation doses and infection statuses. 100Gy of gamma ray irradiation doses decreased seed yield, while 200 and 300 Gy gamma ray dose increased seed yield per plant for both cultivars in both generations, The highest seed yield per plant were observed with Sakha 300 Gy in both generations, it was 104.1g (M_2) and 102.1g (M_1), while the highest seed index (100-seed weight) was noticed for Giza 102 genotype developed through irradiation with 200Gy in M_1 generation.

It seems that 200 and 300 Gy treatments increased the most of studied characters in M_1 and M_2 generations in sunflower. The increments in mean of head diameter as a result of applying 300 Gy gamma ray dose increased seed yield/plant and seed index which finally improved seed yield.

Both morphological characters and seed yield were reduced significantly as a result of virus infection. The effect of treatment with the virus was negative in all irradiated Sakha genotype developed through irradiation with100, 200 and 300 Gy, as well as and Giza 102 genotype developed through irradiation with100 Gy, because these genotypes tend to be resistant to TMV-S as ELISA test reported.

Figure 1. chlorotic local lesions followed by systemic chlorosis on sunflower inoculated with the virus isolate.

Figure 2. Symptoms shown include systemic chlorosis and reduction in size of seed heads.

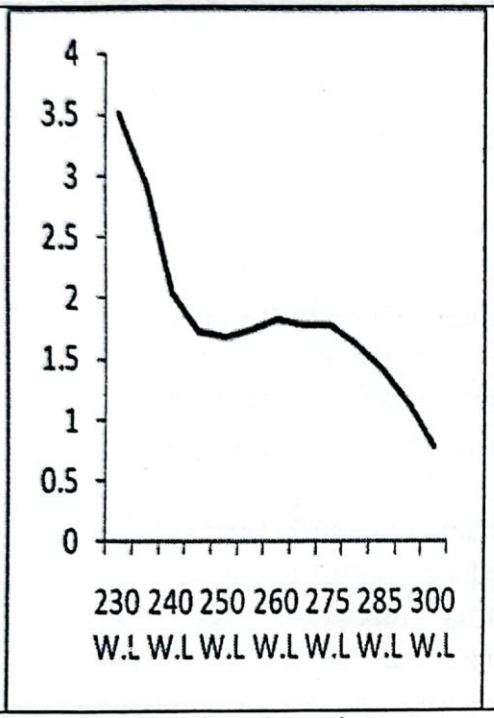

Figure 3. Ulraviolet absorption spectra of purified TMV-S

Figure 4. Particles of purified TMV-S preparation obtained from infected sunflower. The scale bar represents 0.5 μm.

Table 1. Absorbance values of TMV-S antiserum.

Antiserum dilutions	**ELISA reading for antisera**	
	A 405 nm	
	H	Inf.
1/500	0.212	0.537
1/000	0.199	0.403
1/500	0.175	0.373
1/2000	**0.145**	**0.350**
1/2500	0.180	0.139
1/3000	0.065	0.120
1/3500	0.042	0.076
1/4000	0.039	0.058

Figure 5. Electron micrograph of ultra thin section of TMV-S infected *N. clevelandii* leaf showing nucleus which is misshapen and the chromatines are degenerated (×12,000).

Figure 6. Magnified part of fig 4 the misshapen nucleuos, and the mitochondrion which begon in degeneration ×20,000).

Figure 7. An electron micrograph of swollen chloroplast (CL) . Note that the grana and intergrana lamella were degenerated (×20,000).

Figure 8. Electron micrograph of mesophyl cell of TMV-S infected N. clevelandii showing virus like particles in the cytoplasm
(×20,000).

Table (2). **Effect of TMV-S infection on mean values of morphological, yield and yield component characters for two irradiated sunflower cultivars in M_1 and M_2 generations in two successive seasons 2009 and 2010.**

Genotype	Gamma ray Doses	Infection status	Plant height (cm)		No. of leaves		Head diameter (cm)		Seed yield plant $^{-1}$(g)		100-seed weight (g).		ELISA
			2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	
Sakha 53	Control	Control	141.3	156.7	29.0	32.0	17.7	16.3	86.7	82.4	8.1	8.2	
		Infected	128.7	142.7	26.7	29.0	12.7	11.4	66.0	67.5	6.0	6.1	+
	100 Gy	Control	152.0	166.7	31.7	34.7	13.3	12.0	74.0	75.5	7.6	7.7	
		Infected	151.3	168.0	28.3	31.3	12.3	11.1	73.0	74.5	6.2	6.2	-
	200 Gy	Control	169.0	187.0	33.7	36.7	15.0	14.0	94.3	96.2	7.4	7.6	
		Infected	159.3	176.7	31.0	34.0	13.0	11.7	78.5	80.1	9.0	9.2	-
	300 Gy	Control	150.0	170.0	25.0	27.0	20.0	18.6	102.1	104.1	9.7	9.8	
		Infected	149.0	161.0	30.3	33.3	19.3	17.9	96.4	98.3	8.4	8.5	-
Giza 102	Control	Control	140.0	155.0	21.3	23.3	15.0	13.7	69.0	70.5	7.0	7.1	
		Infected	132.0	146.0	22.7	24.7	13.3	12.1	59.6	60.8	4.9	5.0	+
	100 Gy	Control	144.7	160.3	23.3	25.3	18.3	15.0	69.1	70.6	6.8	6.9	
		Infected	143.7	159.3	28.0	30.7	16.3	16.9	66.6	67.9	5.6	5.7	+
	200 Gy	Control	166.7	166.0	25.0	27.3	18.0	16.6	86.1	84.7	16.5	8.9	
		Infected	143.0	158.3	30.0	33.0	16.3	15.0	67.7	68.8	8.8	6.7	-
	300 Gy	Control	153.3	183.0	32.7	35.7	21.7	20.2	99.8	101.8	8.8	8.9	
		Infected	145.0	165.0	25.0	27.0	15.0	13.7	93.4	95.2	8.7	8.7	-
LSD 5%			10.85	2.64	2.97	3.13	2.64	2.52	6.95	9.16	7.45	1.26	

+ = Positive - = Negative

REFERENCES

Bernd , Z. and Günther, Z (2009). Rapid diagnosis of plant virus diseases by transmission electron microscopy. Journal of Virological Methods **162:** 163-169.

Brunt, A.A., Crabtree, A., Dallwitz, M.J., Gibbs, A.J. and Watson, L. (1996). Viruses of plants. Description and lists from the VIDE Database. CAB International Walling for U.K., 1484 pp.

CoStat Pro. (2005). CoHort software. Version 6.311, 798 Lighthouse Ave. PMB 320, Monterey, CA, 93940, USA

Converse, R.H., and Martin, R.R. (1990). ELISA methods for plant viruses. In: Serological methods for detection and identification of viral and bacterial plant pathogens. 179-196pp. Hompton, R.O.; Ball, E.M.; and DeBoer, S.H. (eds.). The American Phytopathological Society, St. Paul, USA.

Dae H. K, Jeom D. C., Jae H. K, Jenog S. K and Eui K. C. **(2005).** Ultrastructural characteristics of necrosis and stunt disease in red pepper by the mixed infections of Tobacco mosaic virus-U1 or Pepper mild mottle virus and Pepper mottle virus. Plant Pathol. J. 21(3) : 252-257.

Da Silva,R.M., De Souto, R.E., Pedroso, J.C., Arakava,R., Almeida, A.M.R., Barboza, A.L and Vida, J.B. (2008). Detection and Identification of TMV infecting tomato under protected cultivation in Paraná State. Brazilian Archives of Biology and Technology. 51(5): 903-909.

Francki, R.I.B, Milne, R.G and Hatta, I (1985) Atlas of Plant iruses,Vols I and II, CRC, Boca Raton, 222 and 284 pp.

Gardner, W.S. (1967). Electron microscopy of barley stripe mosaic: Comparative cytology of tissues infected during different stages of maturity. Phytopathology 57: 1315 - 1326.

Gomez, K. A. and Gomez, A.A (1984). Statistical Procedures for Agricultural Research, 2nd End, John Wiley and Sons, New York, pp:229-308

Hampton, R., Ball, E. and Beboer, S. (1990). Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens. The American Phytopathological Society St. Paul, Minsota, USA, 389pp.

Jambhulkar, S. J. and Joshua, D.C. (1999). Induction of plant injury,
chimera, chlorophyll and morphological mutations in sunflower using gamma rays. Helia 22: 63-74.

Kim, H.M and Lee, K.J. (1999). Characteristics of Tobacco Mosaic Virus Isolated from Wasabi (*Eutrema wasabi*) in Korea. Plant Pathol. J. 15(4) : 247-250.

Ladipo1, J.L., Koenig, 1, R. and Lesemann, D.E. (2003). *Nigerian tobacco latent virus*: a new *Tobamovirus* from tobacco in Nigeria. *European Journal of Plant Pathology* **109:** 373–379.

Lewandowski D.J (2005) Genus *Tobamovirus*. In: Virus taxonomy. Eighth report of the International Committee on the Taxonomy of Viruses. Eds. C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, L.A. Ball. Elsevier, San Diego: 1009–1014.

Luczkiewicz, T. (1975). Inheritance of some characters and properties in sunflower (*Helianthus annuus* L.). Genetica Polonica 10: 167-184.

Miller, J. F. (1992). Update on inheritance of sunflower characteristics. Proc. 13th Intern. Sunflower Conf., Pisa (Italy) 2: 905-945.

Dunca, D. B., 1995. Multiple range and multiple F-test Biometrics, 11:1-42

Noordam, D. (1973). Identification of Plant Viruses. Methods and Experiments. Center for Agric. Publishing and Documentation, Wageninen, Netherlands, 207 pp.

Sabanadzovic, S., Abou Ghanem-Sabanadzovic, N., Henn, A., and Lawrence ,A. (2008). Characterization of a petunia strain of turnip vein – clearing virus. Journal of Plant Pathology. **90** (3): 505-509.

Steinbuch, M. and Audran, R. (1969). The isolation of IgG from

mammalian sera with the aid of caprylic acid. *Archives of Biochemistry and Biophysics* 134:279-284.

Spurr, A.R. (1969). Low viscosity epoxy resin embedding medium for electron microsopy. Ultrastruc. Res., 26:31– 43.

Takahashi, Y. and Ahohara, K. (1990). Practical detection of wasabi strain of tobacco mosaic virus by using ''cocktail'' monoclonal antibodies. *Ann. Phytopathol. Soc. Jpn.* 56:621-627.

Theuri, J.M., K.R. Bock, and R.D. Woods. (1987) Distribution, host range, and some properties of a virus disease of sunfl ower in Kenya. Tropical Pest Management 33(1): 202–207.

Thomas, A.S. (1964). Assembly and aggregation of *Tobacco Mosaic virus* in tomato. The Journal of cell Biology. 21: 253-264.