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Biological and Molecular Characterization of *Watermelon mosaic virus* Affecting Squash Plants Yield and Quality in Egypt

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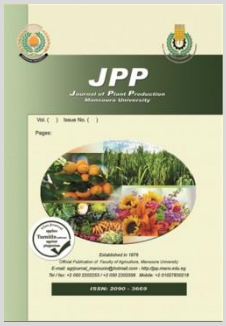

ABSTRACT

Survey of vegetable crops in different regions Ismailia Governorate in Egypt conducted detection of *Watermelon mosaic virus*. The virus detected using DAS-ELISA form naturally infected watermelon and squash plants, exhibiting severe yellow mosaic, vein clearing, blistering and distortion of leaves. Isolation and identification of WMV was carried out by studying host range, symptomatology, modes of transmission, serological reaction and electron microscopic examination. The molecular detection of WMV done using RT-PCR with specific primers designed for coat protein gene. RT-PCR products 825bp of coat protein gene of WMV cloned and then sequenced. The coat protein gene of the Egyptian isolate of WMV showed nucleotide sequences similarity ranged from 95%-99% with different isolates available in Gene Bank. The experiments carried out under greenhouse condition, (HRI) - (VRD) from2014 to 2017. Five squash inbred lines used making half diallel to produce 10 hybrids to determine performance some variables under WMV infection condition**.** A complete block design was used. The evaluation process performed through two experiments. First experience, cultivation the genotypes with a commercial hybrid after infection to evaluate the performance of genotypes for yield and some characteristics. Therefore the percentage of reduction in yield estimated. The second experiment without infection and evaluate the performance for the same variable. The study found that, there were significant differences between all the genotypes that were inoculated and noninoculated. The reducing in yield ranged 14.6: 30%. Therefore, the study recommended attention to the hybrid that achieved a decrease of 14.6% by continuing to work on it.

Keywords: Squash, Watermelon mosaic virus, DAS-ELISA, Transmission, Electron microscopy, RT-

INTRODUCTION

Immature squash fruit is a popular vegetable in Egypt. It grown in green houses, Squashes are generally divided into two groups (summer and winter squash). In this study we take light on the production of new squash hybrids through suitable breeding program by using half diallel. Squash get many viral diseases. The *potyviruses* watermelon mosaic virus (WMV) causes important yield losses in cucurbit crops, including *Cucurbitapepo* by reducing fruit production and quality (Purcifull*et al.*, 1984). Typical symptoms induced by WMV on susceptible melon plants include leaf mottling and mosaic, chlorosis, tip stunting or bunching, reduced fruit yield and quality. Virus is generally considered to rank first threat to plant production losses and causes economic disadvantages. Watermelon mosaic virus (WMV) is a type member of the genus Potyvirus and the family *Potyviridae* with approximately 750 nm long flexuous filamentous particles (Purcifull*et al.*, 1984). The genome of WMV is a single, positive-sense RNA of around 10035-nt long. It contains a unique large open reading frame coding for a polyprotein of 3217 amino acids; it contains nine putative cleavage sites, yielding ten putative functional proteins that have motifs conserved among homologous proteins of other potyviruses (Sharifi*et al.*, 2008). WMV can cause economically important diseases in several horticultural crops, mostly cucurbits and legumes, resulting in quality and yield losses. It can experimentally infect more than 170 plant species belonging to 27 families, including many weeds that can host the virus between crops (Shukla*et al*., 1994; Moradi, 2011). In order to identify the Egyptian isolate of WMV, biological, serological and molecular assays were performed. In addition, phylogenetic analyses were done using the nucleotide sequences of the partial coat protein gene of WMV isolate. So we work in this study for production of squash hybrids free of watermelon mosaic virus and good economic specifications and high yield.

MATERIALS AND METHODS

PART I.

Identification of the virus under study.

Source of virus isolates:

Several field visits were done in different watermelon and squash growing areas of Ismailia governorate in Egypt. The naturally infected watermelon and squash plants showing viral symptoms including severe yellow mosaic, vein clearing and blistering were collected. Infected leaf samples were kept in cool boxes after collecting from the field and stored at -80°C for later use for further study.

Propagation of virus isolates

Infected young leaves of squash plants were grinding in phosphate buffer solution (pH 7.2). The infectious sap was mechanically inoculated on *Chenopodium amaranticolor*as an indicator host. The single local lesion assay was used for biological purification of the isolate and propagated on healthy *Cucurbita pepo* plants.

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Virus identification:

Host range and indicators plants.

To study the response of the host plants to the studied isolate, nineteen plant species belonging to four families were mechanically inoculated with infectious crude sap expressed from squash plants. Seedling of each host species were inoculated and observed daily for symptoms development. An equal number of healthy seedlings of the same species and age were left without inoculation as a control. The mechanical inoculated plants were kept under observation in insect proof cages under greenhouse. Three weeks later, plants were examined visually for symptoms appearance. Symptomless plants were checked for virus infection by back inoculation onto *Chanopodium. amaranticolor* leaves and/or DAS ELISA technique.

Mechanical transmission.

Inoculum was prepared by homogenizing infected squash leaves with few drops of phosphate buffer (pH 7.2) in a sterilized mortar. Leaves of host plants previously dusted with carborandum (600 mech) were rubbed with the forefinger or with a cheesecloth pad previously soaked in the inoculum. The plants were rinsed with tap water and kept in the insect proof greenhouse.

Insect transmission.

Two aphid species namely, *Myzuspersicae*sulz and *Aphis faba*scop, were checked for their ability to transmit the isolated virus. *Myzuspersicae*sulz and *Aphis faba*scop were maintained on virus- free cabbage plants for *Myzuspersicae*sulz and healthy squash for *Aphis faba*scop and kept under insect-proof cages in the greenhouse. The aphids were starved for one hour and then transferred to feed for 30 minutes acquisition feeding period on diseased plants. At the end of the feeding period, aphids were transferred to healthy plants at a rate of 10 aphids / plant. After 24 hours feeding period the insects were killed by spraying all tested plants with an effective insecticide (malathion 0.2%).

ELISA test.

ELISA test was carried out to detect WMV in the collected samples using the method described by ELISA kits (Bioreba AG, USA) and according to Clark and Adams (1977).

Electron microscopy (Ultra-thin sections).

One-millimeter diameter disks were punched out from five young leaflets of squash plants infected with watermelon mosaic virus (WMV). Disks were fixed and processed for electron microscopy according to Spurr, (1969). Thin sections were cut from selected pieces of the embedded tissue stained with uranyl acetate and lead citrate and viewed with a Philips EM300 electron microscope.

Molecular characterization:

To continuing the complete the identification and diagnoses of the virus molecular characterization study was carried out during 2019.

RNA extraction.

RNA extraction from leaf samples was carried out using RNeasy Plant Mini Kit(QIAGEN) according to the instructions of manufacturer.

Primers for the partial coat protein gene of WMV.

The sense primer WMV-F (5`-GAA TCA GTG TCT CTG CAA TCA GG-3`) and the antisense primer WMV-R (5`-ATT CAC GTC CCT TGC AGT GTG-3`) were designed to amplify 825 bp of the coat protein gene of WMV (Sharifi *et al*., 2008).

One-step RT-PCR.

One-step RT-PCR reactions were carried out using the " iScript One Step qRT-PCR Kit " (BIOMATIK) in 25 µL reaction volume. Each reaction contained 1 µL of the RNA extract (40 ng of total RNA), 12.5 µLi Green Mastermix, 1.5 µL of 10 µM of each primer, 0.5 µL of qRT-PCR Enzyme Mix and 25 µL of nuclease-free water. Synthesis of cDNAwas done at 42°C for 30 min and denaturation at 95°C for 10 min followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and a final cycle of 72°C for 10 min. 5 µL of PCR products were loaded in 1% agarose gels with 100 bp DNA ladder (BIOMATIK) and pictures were taken under UV light with digital imaging system gel doc (Syngene Bio Imagins, IN Genius).

Molecular cloning.

DNA cloning was carried out to insert RT-PCR products (coat protein gene of WMV) into pGEM-T Easy vector (Promega) and according to manufacturer's instructions. The PCR products were ligated into the vector using ligase enzyme (provided with the kit). The recombinant plasmids were transformed into *Escherichia coli* DH5α strain. Recombinant plasmids were isolated using Wizard Plus SV Minipreps DNA Purification System (Promega) from some colonies then digested with restriction enzyme *Eco*RI and fractionated on 1% agarose gel in 0.5X TBE buffer.

Nucleotide sequencing of the RT-PCR products.

The nucleotide sequencing was carried out at Macrogen Company. The nucleotide sequences of the coat protein gene of WMV isolates were analyzed and compared using MEGA X (Kumar *et al*., 2018) and DNAMAN 8 Sequence Analysis Software (Lynnon, BioSoft) with different isolates from Gene Bank belonging to WMV.

PART II.

The effect of virus infection on yield and quality of *Cucurbita pepo* genotypes.

This study was conducted for developing inbred lines characterized by good horticultural traits and produces promising hybrids of squash, this work were carried out under greenhouse condition at Kaha Research Station and Dokki, Vegetable Research Department, Horticulture Research Institute, Agriculture Research Center.

Selfing program for insure the purity of inbreed lines:

The five parental inbred lines Table (1) were at a high degree of homozygosity since they were selfed for five successive generations in the early and late summer seasons of both 2014 and 2016 years.

Table 1. List of the squash genotypes used in the hybridization program.

Genotypes	Source	Sex expression
P_1(138)	Cornell University	Monoecious
P_2 (108-1R)	Cornell University	Monoecious
P_3 (215-3L)	Cornell University	Monoecious
P_4 (217-6R)	Cornell University	Monoecious
P_5 (144-5)	Cornell University	Monoecious

Hybridization

Seeds from five inbred lines were sown in seedling trays under greenhouse conditions in 25th February 2014 for summer season. At the three leaves stage, seedlings were transplanted in Dokki greenhouse. At the flowering stage, crossing took place using all possible combination of a half diallel mating design giving a total of 10 crosses. Crossing technique was done using special clips as recommended by (Andeweg 1956). Fruits reached maturing stage within 40 –

50 days after pollination. Seeds were taken away from fruit, cleaned and spread for drying.

The first experiment:

The parents and crosses seeds taken from the previous step plus control seeds (jasmine), it was divided into two parts. Every part was sown in seedling trays under greenhouse conditions on1st march 2017. At the three true leaves stage, the plants were moved to the greenhouse condition. Artificial inoculations were done by rubbing the cotyledons and/or first and second true leaves with a mixture of inoculum. One part of them got inoculated by watermelon mosaic virus inoculum. The insecticides were not sprayed in the trial so as to enhance the population of whitefly. Tob increase inoculum pressure. Symptoms were recorded one to two weeks after inoculation.

Symptom score and yield assessment:

Squash genotypes susceptibility to infection with WMV.

The following data were recorded:

The disease symptoms and degree of infection were recorded according to the following scale.

(a) (+1) = Low infection: The symptoms appeared after two week of inoculation as vein clearing followed by mild mosaic.

(b) (+2) = Moderate infection: In this case the symptoms appeared as yellow veins and leaf distortion.

(c) (+3) = High infection: The symptoms in this case appeared as severe yellow mosaic, the developing trifoliate leaves become deformed basis as described by Walkey and Pink (1984).

The second experiment:

Evaluation for parents and their hybrids.

Seeds of the five parental lines and their ten hybrids (15 genotypes) as well as check variety (Jasmin),at the same inoculums stage, seedlings were transplanted to the greenhouse 540 (9×60) m 2 in a randomized complete blocks design with 3 replications. Seedlings were transplanted into 1 rows in the bed, (row 4 m length and 1.0 m width). The space between plants was 1.00 m. The common agriculture practices, i.e., irrigation, fertilization and pest control procedure for commercial squash production were followed. The horticultural characters (yield and fruits characters) were recorded as follows:-

1- Yield characters: total yield (Kg/plant) and early yield (the first three harvests) (Kg/plant).

2- Fruits characters: Fruit length (cm), fruit diameter (cm), fruit weight (gm) and number of fruits per plant.

3- Percentage total yield losses.

Statistical analysis:

Data were statistically analyzed, using analyses of variance (ANOVA) with the Stat soft statistical package (MSTATC) software program (Michigan State University, East Lansing, MI, USA). Probabilities of significance among genotypes compared with the least significant difference L.S.D. (P≤0.05) were used to according to Gomez and Gomez (1984).

Table 2. Sources of variation, degree of freedom and expected mean squares.

Sources	Degree of freedom	Expected mean square
Blocks	(b-1)	$\sigma^2 e + g\sigma^2 b$
Genotypes	(g-1)	$\sigma^2 e + b\sigma^2 g$
Error	(g-1) (b-1)	$\sigma^2 e$

Where: b= Blocks g= Genotype (parents + hybrids) $\sigma^{2=}$ Variance e= Error

RESULTS AND DISCUSSION

Sample collection for viruses.

Field inspection of different vegetable growing areas of Ismailia governorate in Egypt was done. The naturally infected watermelon and squash plants showed viral symptoms including severe yellow mosaic, vein clearing, blistering and distortion of leaves were collected (Fig. 1).

Fig. 1. (a) Symptoms of WMV on squash very mosaic and vein clearing (b) leaf distortion and severe yellow mosaic.

ELISA test.

The collected leaf samples were initially tested using ELISA kits (BIOREBA) and many samples gave positive reaction indicating the presence of the WMV in the collected samples. The ELISA tests were done on 86 samples; 46 samples of watermelon plants and 40 samples of squash (Table 3) using specific antibodies for WMV provided with kit (Bioreba, USA).

Table 3. The samples tested by ELISA for WMV and percentage of infection.

Crop	No. of samples	No. of positive (+) samples	No. of negative (-) samples	Percentage of infection (%)
Squash	40	14	26	35%
Watermelon	46	10	36	21.7%

The table (3) showed that, 14 samples of squash were WMV positive with 35% of infection and 10 samples of watermelon were WMV positive with 21.7% of infection.

Virus identification:

Host range and indicators plants.

Host range and symptomatology.

Variable symptoms were observed on the infected squash plants. The virus infection showing yellow, severe mosaic, green blisters, vein clearing, malformation. The virus was propagated on squash plants which developed the same symptoms as those in naturally infected plants. The isolate of WMV show different patterns of symptoms on cucurbits hosts as vein clearing, mild mosaic, severe mosaic, vein bending, green blisters and malformation (Table 4 and Fig. 2).

Incidence of WMV was confirmed by back inoculation to *Ch. amaranticolor*. The tested plants could be divided accorded their reactions into two groups:

1- Susceptible hosts to WMV.

a- Plants reacted with systemic symptoms.

Systemic symptoms were observed on the tested cucurbitaceous plant species, *Phaseolus vulgaris* and *Lens culiaris*. Symptoms of vein clearing were appeared within 8 days after inoculation and systemic symptoms, generally, appeared 9-11 days after inoculation.

b- Plants reacted with local lesions.

WMV isolate produced necrotic local lesions on the inoculated leaves of *Chanopodium. amaranticolor* Fig. (2), *Ch. quinoa*6-8 days after inoculation.

Fig. 2. symptomology of WMV (a) mosaic and malformation on *Cucurbita pepo* cv. Cavili, (b) severe mosaic and yellow on *Cucurbita pepo* cv. Eskandarni, (c) severe mosaic and Green blisters on *Cucumis sativus* cv. Balady (d) necrotic local lesions on *Chanopodium amaranticolor.*

1-Unsusceptible plants.

These plant species were not susceptible to WMV infection. These plants are: *Viciafaba, Lupinus termis, Datura stromonium, D. metal, Nicotiana glutiosa, Ni. tabacum, Ni. Clevelandii* and *Ni. arusica*. Host range studies for diagnosis will usually be most useful for those infecting a relatively narrow range of plants (Matthewes 1991).

The general outlook at the results in table (4) indicates that the studies isolate of WMV had a wide host range between members of the family *Cucurbitaceae*. On the other hand, the virus infects few species of *Chenopodiaceae* and *Fabaceae*. Data in table (4) show the virus induced severe mosaic symptoms on *Cucumis sativus cv.*Balady, *Cucumis melo, Luffa aegyptiaca* and *Citrullus lanatus cv.*Giza 2.

Table 4. The reaction of different hosts to Watermelon mosaic virus.

Family	Host plant	Symptoms
Cucurbitaceae	*Cucurbitapepo* cv. Cavili	M+MAL
	Cucurbitapepo cv. Eskandarni	SM+Y
	Cucurbita maxima cv. Wintersquash	SM
	Cucumismelo	SM+VC
	Cucumissativus cv. Balady	SM+GB
	Citrulluslanatus cv. Giza 2	M+MAL
	Luffaaegyptica	SM+MAL
Chenopodiaceae	*Ch. amaranticolor*	NLL
	Ch. quinoa	NLL
Fabaceae	*Phaseolus vulgaris* cv. Giza 4	M
	Lens culiaris cv. Giza 9	mM
	Viciafaba cv. Giza 3	NS
	Lupinustermis cv. Lupine	NS
Solanaceae	*Nicotianatabacum*	NS
		NS
		NS
		NS
		NS
		NS

NLL = Necrotic local lesions, SM = severe mosaic, M= mosaic, mM = mild mosaic, Y= yellowing, MAL = malformation, VC = Vein clearing, GB = Green blisters, NS = No symptoms.

Also, the virus induced severe mosaic, green blisters and malformation symptoms on Eskandarani squash plants. The obtained results also showed that the virus induced necrotic local lesions on *Chanopodium. amaranticolor,Ch. quinoa,* while the virus isolate induced mild mosaic on *Lens culiaris* and induced mosaic on *Phaseolus vulgaris*. These results are in agreement with those obtained by Van Regenmortel (1971) who found a systemic vein-banding, mosaic and leaf-distortion on *Luffa aegyptica and Cucurbita pepo cv*. Cavili. The virus WMV induced chlorosis, mosaic and green blisters. Fischer and Lockhart (1974) reported that the Moroccan isolate of WMV induced mosaic and leaf blisters on *Cucumis sativus, Citrullus lanatus, Cucumis melo, Cucurbita pepo* and *Cucurbita maxima*. The obtained data in table (4) confirmed the results of Purcifull and Hiebert (1979), Al-Musa and Mansour (1982), Abd El-Salam *et al.* (1991), Kosaka and Fukunishi (1997), Licoq *et al*. (2001) and Dukic *et al*. (2006).

Mechanical transmission:

Obtained results revealed that WMV was easily transmitted mechanically to indicator hosts as *Chenopodium amaranticolor* which showed local lesions.

Insect transmission:

Two aphid species namely *Myzus persicae*sulz and *Aphis fabae*scop were used to transmit WMV. Results in table (5) revealed that WMV was transmitted by both genera in a non-persistent manner. *Myzus persicae*sulz was found to be more efficient than *Aphis fabae*scop. Percentage of transmission for *Myzus persicae*sulz was 80%, while it was 40% for *Aphis fabae*scop. Similar results were obtained by Van Regenmortel (1971), Al-Musa and Mansour (1982), Purcifull *et al*. (1984), Nameth *et al*. (1986), Licoq *et al*. (2001) and Mahmoud, Ashgan (2005).

Table 5.Transmission of Watermelon mosaic virus by aphids.

Aphid	No. of inoculated plants	No.of infected plants	% Infection
Myzuspersicae	10	8	80
Aphis fabae	10	4	40

Electron microscopic examination:

The ultra-thin section of WMN-infected leaves showed virus particles aggregates, scroll degenerated mitochondria in cytoplasm of infected squash leaves (Fig. 3, 4 and 5), representing Potyvirus group. This result is an agreement with those obtained by Van Regenmortel (1971), Purcifull et al. (1984), Nameth et al. (1986), Sidek and Sako (1996), El-Kewey et al. (1995) and Mahmoud, Ashgan (2005).

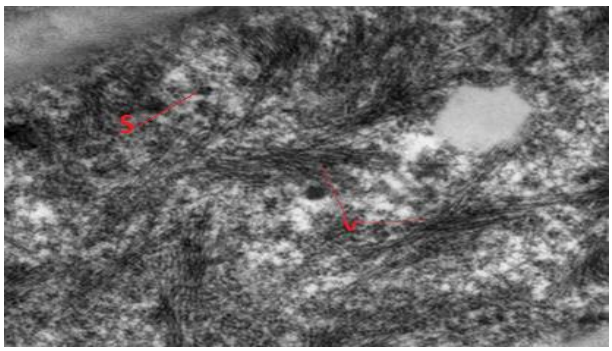

Fig. 3. Virus particles aggregates (V) and Scroll (S) in cytoplasm in Squash leaf cells infected with WMV. Mag: 40000X.

Fig. 4. Degenerated mitochondria (M) and extensively virus particles (V) in cytoplasm in Squash leaf cells infected with WMV. Mag: 15000X.

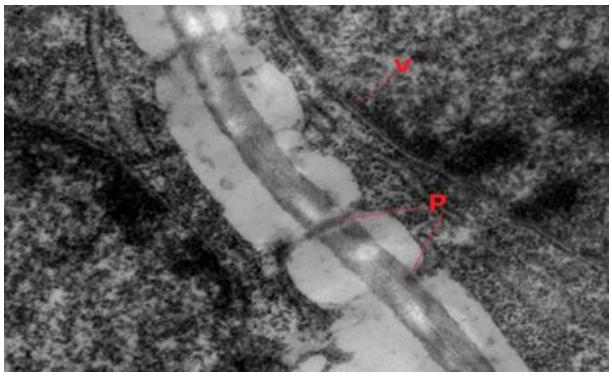

Fig. 5. Virus particle (V) and Plasmodesmata connecting the cells (P) in cytoplasm in Squash leaf cells infected with WMV. Mag: 30000X.

Molecular characterization:

One step RT-PCR and cloning.

Vegetable leaf samples collected from growing areas were subjected the RT-PCR assay. RT-PCR was carried out to amplify coat protein gene of watermelon mosaic virus using set of primers WMV-F and WMV-R, as described above. Those set of primers succeeded to amplify the expected size bands for coat protein gene of WMV at 825 bp (Fig. 6). The samples were selected from previous positive ELISA tests.

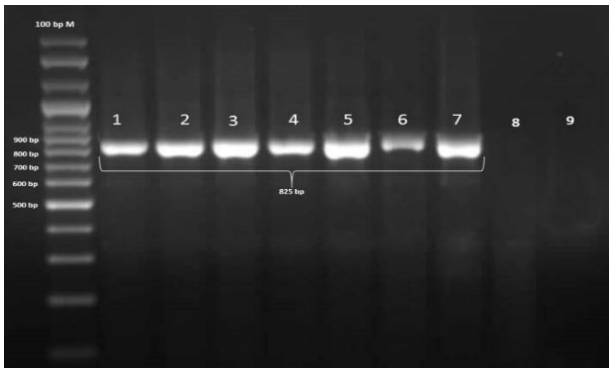

Fig. 6. Agarose gel electrophoresis of RT-PCR amplified products. M: 100 bp DNA ladder (Promega); from 1 to 3: three infected samples of watermelon; from 4 to 7: four infected samples of squash; 8,9: two negative samples.

RT-PCR products were succefully inserted into pGEM-T Easy vector and the recombinant plasmids were transformed into *Escherichia coli* DH5α strain. Recombinant plasmids were isolated successfully from different colonies using Wizard Plus SV Minipreps DNA Purification System. Digestion with restriction enzyme *Eco*RI and fractionation on 1% agarose gel in 0.5X TBE buffer was done ending with positive results.

Nucleotide sequencing.

Coat protein gene for the isolate of WMV was sequenced in Macrogen Company (South Korea). The nucleotide sequence was submitted to the Gene Bank under the accession numbers MN966673. The sequenced coat protein gene was used in phylogenetic analysis using MEGA X (Kumar *et al*., 2018) and the Optimal Alignment Method of DNAMAN 8 software (Lynnon BioSoft) to study the relationship between the WMV isolates used in this study and those isolates available in Gene Bank belonging to WMV (Fig. 7). Thecoat protein gene of the Egyptian isolate of WMV showed nucleotide sequences similarity ranged from 95%-99% with different WMV isolates available in Gene Bank. This result is similar with results of several investigators (Sharifi *et al*., 2008 and Moradi 2011).

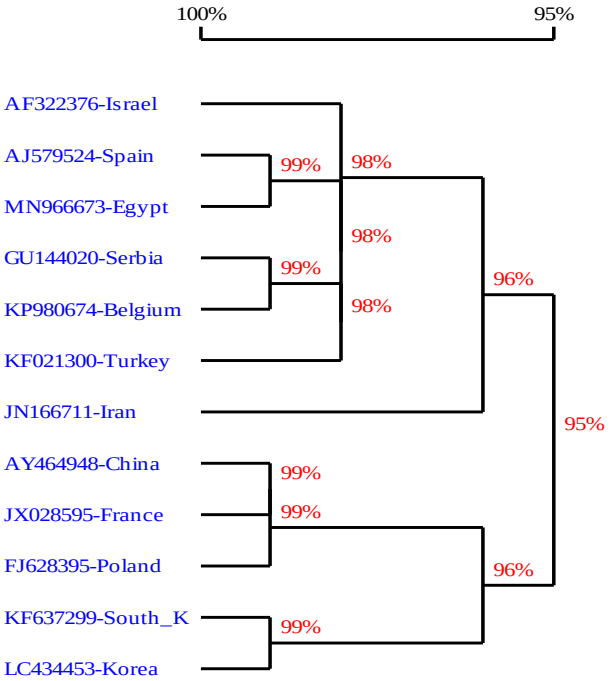

Fig. 7. Phylogenetic tree showing relationships between the Egyptian isolate of WMV and several isolates of WMVbased on the nucleotide sequences of their CP genes.

PART II.

The plants of the various entries were screened for symptom development and severity during the growth period. The test was carried out during the summer season.

Regarding to Table (6) plants of each parent and hybrid were planted in paired inoculated and noninoculated. The plants of the various entries were covered for symptom development and severity during the growth period. The test was carried out during the summer season. The average summer temperature at the experiment site was about 35°C. Under our inoculation conditions there were essential differences in the degree of symptom development among the different genotypes. So there were significant differences between the entire genotype in all characteristics.

Performance of total yield:

Regarding to Table (6) when comparing parents and hybrids under inoculation and un inoculation conditions. The results showed that, there were significant differences between all the genotypes under study whether were inoculation and uninoculation.

1-Uninoculation genotype.

The hybrids (P_2xP_5) 108-1R x 144-5 was obtained the total yield (1187.76) gm/plant follows (P_4xP_5), (P_1xP_4), (P_2xP_4), (P_1xP_2), (P_1xP_5), (P_3xP_5) compared with control was obtained 993.4 gm/plant and the seven squash hybrids outperformed their parental lines.

Comparing inoculated with uninoculated genotype. The data showed decrease total yield in this way, (P_2xP_5) 1025.06 gm, (P_4xP_5) 954.81 gm, (P_2xP_4) 854.10 gm, (P_1xP_2) 840.75gm, (P_3xP_4) 820.54 gm, (P_3xP_5) 818.03gm, (P_2xP_3) 802.32gm respectively. Juan *et al.* (1995) Proved that, WMV inoculated squash hybrid XPH-1719 produced a lower virus-infected fruit yield than 'Pavo'. The results attributed to lines P_1, P_3 and P_5 got sever symptoms but line P_2 and P_4 got moderate symptoms. And due to the early fruit yield reduction.

Table 6. Effect of watermelon mosaic virus on characteristics of squash plants grown in the greenhouse under inoculation and uninoculation conditions.

		Characteristics					
Genotypes	**Treatment**	**Total yield gm /plant**	**Early yield gm/plant**	**Average fruit weight/gm**	**Average fruit length/cm**	**Average fruit width / cm**	**Fruit number /plant**
P_1 (138)	inoculated	517.90	271.63	94.90	14.06	3.01	5.45
	Uninoculated	681.67	387.24	101.06	14.70	2.93	6.76
P_2(108-1R)	inoculated	701.07	431.03	100.26	13.90	3.40	7.13
	UnInoculated	954.79	575.04	105.56	14.20	3.03	8.51
P_3(215-3L)	inoculated	415.33	207.23	103.03	12.40	3.08	3.60
	Uninoculated	583.10	292.07	99.20	13.40	3.14	5.87
P_4(217-6R)	inoculated	660.65	276.06	99.06	12.90	3.10	6.70
	Uninoculated	862.31	350.60	110.30	14.10	3.00	7.80
P_5(144-5)	inoculated	579.06	319.13	101.16	12.20	3.00	5.70
	Uninoculated	824.87	405.20	105.20	13.70	3.03	7.80
P_1 X P_2	inoculated	840.75	413.16	102.10	13.10	3.30	8.20
	uninoculated	1006.04	498.25	103.87	14.03	3.01	9.60
P_1 X P_3	inoculated	730.13	321.05	100.16	11.90	3.10	7.31
	uninoculated	910.43	418.26	97.03	13.80	3.15	9.31
P_1 X P_4	inoculated	845.29	470.00	100.16	13.20	3.04	8.50
	uninoculated	1055.90	593.20	104.03	14.80	3.00	10.23
P_1 X P_5	inoculated	805.53	417.15	105.06	12.80	3.10	7.60
	uninoculated	1005.97	510.17	111.30	13.90	3.04	9.05
P_2 X P_3	inoculated	802.32	352.03	99.26	13.10	3.20	8.10
	uninoculated	987.35	438..03	103.13	13.50	2.99	9.60
P_2 X P_4	inoculated	854.10	491.20	109.16	12.60	3.11	7.70
	uninoculated	1018.17	575.27	111.06	13.80	3.00	7.80
P_2 X P_5	inoculated	1025.06	642.26	103.06	12.10	3.00	9.86
	uninoculated	1187.76	750.03	110.10	14.50	2.98	10.70
P_3 X P_4	inoculated	820.54	381.08	105.10	11.60	3.00	7.50
	uninoculated	987.35	475.03	109.13	13.60	3.02	9.40
P_3 X P_5	inoculated	818.03	409.04	108.10	11.90	3.10	7.40
	uninoculated	997.14	501.11	110.20	13.80	3.00	9.20
P_4 X P_5	inoculated	954.81	483.10	102.03	13.10	2.91	9.41
	uninoculated	1125.13	592.10	109.03	14.20	3.00	10.32
Control	inoculated	788.14	318.00	107.15	12.20	3.00	7.20
	uninoculated	993.40	402.10	110.03	14.30	2.97	9.19
LSD5 %5		0.813	0.581	0.376	0.831	0.063	0.65

WMV effect on total yield gm/plant.

Percentage decrease in yield gm/plant.

In Table (7) the percentage decrease in yield for every inoculated parent and hybrid P_2xP_5= 14.6%, P_4xP_5= 15.2%, P_2xP_4=16%, P_3xP_4=17.1%, P_1xP_2=17.5%, P_3xP_5=18%, P_2xP_3=19%, P_1xP_5= P_1xP_3 = 20%, P_1xP_4= 20.1%, control=20.7%, P_4=23%, P_2=23.8%, P_1=26%, P_3=29%, P_5= 30% respectively. Ferdinand *et al.* (2011) showed that, yield losses of summer squash to aphid-transmitted viruses are often ranging from 20 to 80%. The results agreement with Marc *et al.* (1997) on cantaloupe studied that, control plants were severely stunted (44% reduction in shoot length) and had poor fruit yield (62% loss) compared to transgenic plants, and most of their fruits (60%) were unmarketable. the fruit yield reduction.

Uninoculated genotype performance of early yield gm/plant.

In Table (6) showed that, highly significant differences for genotypes, parents and crosses for early yield, P_2xP_5 obtained high early yield (750.03) gm, P_1 x P_4 (593.20)gm, P_4xP_5 (592.10)gm, P_2 x P_4 (575.27) gm …..etc and control (402.10) gm respectively Table (8).

Inoculated genotype performance of early yield gm/plant.

Data presented in Table (6) showed that, highly significant values were observed for the studied genotypes, parents and hybrids. P_2xP_5 recorded the highest value (642.26) gm, P_2xP_4 (491.20) gm, P_4xP_5 (483.10) gm, P_1xP_4 (470.00) gm ….. etc and control (402.10)gm. The results

attributed to the infected degree of symptoms whereas lines P_3, P_5 got sever symptoms and P_2, P_4 got moderate symptoms.

Uninoculated genotype performance of average fruit weight / gm.

Regarding to average fruit weight, there were highly significant differences among the evaluated entries. $P_1xP_5$and P_2xP_4 obtained highly average fruit weight 111.30 gm, 111.06 gm respectively without significant differences between them. P4, P_2xP_5, P_3xP_5 and control (110.3 – 110.1 – 110.2 – 110.03 gm) respectively, without significant differences between them. This result reflected agreement with Ferdin *et al*. (2011) said that, WMV inoculated squash fruit marketable size at maturity with an average weight of 0.14 kg.

Inoculated genotype performance of average fruit weight/gm.

The estimates of mean performance for Inoculated hybrids showed that, the crosses recorded highly significant differences, P_2xP_4 (109.16), P_3xP_5 (108.10) and control (107.15). Kamooh, (1987) showed significant differences between WMV infected and non- infected watermelon plants. The average fruit weight of the infected cultivars was (1.59-2.63 and 2.59 kg) respectively, while average fruit weight of the same cultivars was (4.35- 5.77 and 5.68 kg) under the non- infected condition. These results showed that all genotypes were affected by the WMV infection for average fruit weight which resulted in the reduction of their early yield and total yield.

Uninoculated genotype performance of average fruit length/cm.

In Table (6) showed that, significant differences for genotypes, parents and crosses for average fruit length, P_1xP_4 (14.8) cm, p1(14.7) cm, P_2xP_5 (14.5) cm and control (14.3) cm.

Inoculated genotype performance of average fruit length/cm.

Concerning average fruit length, data presented in Table (6) showed that, P_1(14.06) cm, P_2(13.9)cm, P_1xP_4 (13.2) cm, P_4xP_5 (13.1) cm. The effect of Tomato Yellow Leaf Curl Virus on tomato plants, Moshe et al. (1997) proved that, Inoculated Tomato Yellow Leaf Curl Virus tomato plants produced small size fruits. This results due to the small size of the fruit as a result of being affected by the infection.

Uninoculated genotype performance of average fruit width/cm.

Regarding fruit width / cm in Table (7) the data calculated that, significant differences for genotypes parents and crosses for average fruit width. P_1xP_3 (3.15) cm, P_3 (3.14)cm, P_1xP_5 (3.04) cm, P_5,P_2(3.03)cm, P_3xP_4 (3.02)cm…etc and control (2.97)cm.

Inoculated genotype performance of average fruit width/cm.

The results of the comparison among the mean performances inoculation and un inoculation conditions.

The data inoculated plants showed that, P_2(3.40)cm, P_1xP_2 (3.30)cm, P_2xP_3 (3.20)cm, P_2xP_4 (3.11)cm….. etc and control (3.00)cm. Nameth *et al*. (1985a) indicated that, when WMV1 was present in combined with WMV2 melon fruits were small and poorly formed. This is due to the small size of the fruit as a result of being affected by the infection.

Uninoculated genotype performance of fruit number/plant.

With respect to fruit number/plant. The data exhibited that, the parental lines values ranged from 5.45 (P_1) to 8.51 (P_2) / plant, and their F_1 hybrids ranged from 7.8 (P_2x P_4) to 10.7 (P_2 x P_5), while check hybrid (Jasmine) recorded 9.19.

Table 7. The percentage total yield loses for infected watermelon mosaic virus squash plants genotype and Jasmin (control).

Genotype	Percentage total yield loses
P_1(138)	26%
P_2 (108-1R)	23.8%
P_3 (215-3L)	29%
P_4 (217-6R)	23%
P_5 (144-5)	30%
P_1XP_2	17.5%
$P_1X\ P_3$	20%
P_1XP_4	20.1%
P_1XP_5	20%
P_2XP_3	19%
P_2XP_4	16%
P_2XP_5	14.6%
P_3XP_4	17.1%
P_3XP_5	18%
P_4XP_5	15.2%
Control (Jasmin)	20.7%

Inoculated genotype performance of fruit number /plant.

In Table (8), the highest parent mean value was reflected by parental line (108-1R) (P2) while, the parental line (215-3L) (P3) showed the lowest mean value for the same trait. And their F1 hybrids ranged from 7.31 (P1 x P3) to 9.86 (P2 x P5), while check hybrid (Jasmine) recorded 7.20. The results agreement with Ferdinand et al. (2011) said that, the majority squash genotype WMV resistant produced 3 to 9 fruits per plant. This was the result of stunting of plants and deformation of leaves because the leaves are the basis of the plant's food.

Table 8. Infection rate for infected watermelon mosaic virus squash plants genotype and Jasmin (control).

Genotype	Infection rate		
	Severe	Moderate	Low
P_1	+3		
P_2		+2	
P_3	+3		
P_4		+2	
P_5	+3		
P_1XP_2			+1
$P_1X\ P_3$		+2	
P_1XP_4		+2	
P_1XP_5		+2	
P_2XP_3			+1
P_2XP_4			+1
P_2XP_5			+1
P_3XP_4			+1
P_3XP_5			+1
P_4XP_5			+1
Control (Jasmin)		+2	

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التوصيف البيولوجي والجزيئي لفيرس موزايك البطيخ علي محصول وجودة نبات الكوسة في مصر

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تم عزل فيرس موزايك البطيخ من الحقول المزروعة بالكوسة في مناطق مختلفة بمحافظة الاسماعيلية. تم تعريف الفيرس عن طريق دراسة المدي العوائلي وطرق الانتقال والاسيرولوجي باستخدام DAS-ELISA والفحص بالميكروسكوب الالكتروني لقطاعات في الانسجة المصابة . وكذلك بدراسة جزئية الاستخلاص coat- protein بواسطة انزيم البلمرة المتسلسل النسخ العكسي للحلمض النووي للفيرس RT- PCR مع بادئات متخصصة مصممة لجين غلاف البيروتين ثم دمج ناتج PCR(825PB) في داخل ناقل متخصص ثم الادخال في البكتريا لاكثار الجين المطلوب ثم بعد ذلك اجراء تحليل للتتابع النيوكلوتيدي للمقارنة مع العزلات الاخري في بنك الجينات Gene Bank وجد ان العزلة WMV المصرية تتشابه بنسبة ٩٥% - ٩٩% مع العزلات المختلفة المتاحة في Gene Bank . اجريت هذه التجارب تحت ظروف البيوت المحمية بافسام بحوث الخضر بالدقي التابع لمعهد بحوث البساتين – مركز البحوث الزراعية – مصر خلال ٥ مواسم متتالية من ٢٠١٤ حتي ٢٠١٧ باستخدام خمس سلالات مرباة داخليا من الكوسة *Cucurbita pepo* L واستخدم برنامج التهجين بين السلالات الخمس بنظام التهجين النصف دائري (٥×٥) لانتاج ١٠ هجن. وتم اجراء العدوي الصناعية بالحقن بفيرس موزايك البطيخ . ثم تم اجراء عملية التقييم باستخدم القطاعات كاملة العشوائية في ٣ مكررات من خلال تجربتين كالاتي :- التجربة الاولي : زراعة الاباء والهجن ومعهم هجين تجاري (جاسمين) وذلك بعد العدوي الصناعية ل تقييم اداء السلالات والهجين من حيث المحصول المبكر والكلي للنبات – قطر وطول الثمرة – متوسط وزن الثمرة – عدد الثمار علي النبات .التجربة الثانية : زراعة الاباء والهجن ومعهم هجين تجاري (جاسمين) بدون عدوي (كنترول) وتم تقييم اداء السلالات والهجين من حيث المحصول المبكر والكلي للنبات – قطر وطول الثمرة – متوسط وزن الثمرة – عدد الثمار علي النبات . ولذلك قدرت النسبة المئوية للنقص في المحصول الكلي - وتبين من الدراسة وجود فروق معنوية بين كل الطرز الوراثية الذي تم حقنه والكنترول – كما تراوح النقص في المحصول بين ١٤.٦ % الي ٣٠% - ولذا توصي الدراسة بادخال الهجين الذي حقق ١٤.٦ % باستمرار العمل عليه لادخاله في الزراعة المصرية .