

## GENETIC STUDIES AND EVALUATION OF SOME CUCUMBER GENOTYPES FOR CUCUMBER MOSAIC VIRUS (CMV) RESISTANCE AND SOME OTHER TRAITS

A.M. Abd Rabou<sup>1</sup>, Abeer M. Abo El-Wafa<sup>2</sup>  
and Nahla A. EL-Magawry<sup>1</sup>

1. Vegetables Breeding Dept. Hort. Res. Inst, ARC, Giza, Egypt.

2. Virus and Phytoplasma Res. Dept., Plant Pathology Res. Inst., ARC, Giza, Egypt.

### ABSTRACT

*Cucumber (Cucumis sativus L.) is one of the important vegetable crops in Egypt and the selection and planting of genotypes that are resistant to CMV is one of the most important components in an integrated disease control program. So, the objectives of this study were the identification of the resistance to CMV and estimating the genetic variance among ten cucumber inbred lines. This study was conducted during the period from 2017 to 2020. Selfing pollination to produce inbred lines and crossing to produce F<sub>1</sub>s were carried out in the greenhouse at Kaha Vegetable Research Farm (K.V.R.F), Kalubia at spring season in 2017, and horticultural evaluation of the inbred lines and their hybrids was carried out in the greenhouse at Kaha Vegetable Research Farm and Ismailia Experimental Station at spring season in 2020, while evaluation of the inbred lines was carried out at Ismailia Experimental Station during (2017/2018) and evaluation of the selection inbred lines and their hybrids to CMV was carried out under greenhouse conditions at Ismailia Experimental Station during (2018-2019). In order to determine the genetic polymorphism and discriminate among these genotypes, RAPD analysis was conducted on the isolated DNA samples from each genotype. Viral diseases are important problem for production of cucumber in Egypt. The most important of these viruses in Egypt is Cucumber mosaic virus (CMV). The CMV was tested serologically using indirect ELISA. CMV has a wide host range belonging to 4 families. Finger prints of the studied genotypes were conducted using 3 RAPD primers. The produced hybrids were infected by CMV. P1 and P7 are the best inbred lines due to their high yielding ability, good fruit and vegetative traits. But only P1 is considered the most promising inbred line, due to its resistance to CMV. These lines could be used in CMV resistance breeding programs to be released as a new cultivar, which possesses both high yielding and resistance. The general performances of the F<sub>1</sub> hybrids reflected the presence of three degrees of dominance effects complete, partial to over dominance and absence of dominance.*

Key words: *Cucumis sativus*, Inbred lines , Heterosis, Potence ratio, CMV, Fingerprint, RAPD.

### INTRODUCTION

Cucumber is a member of the *Cucurbitaceae* family, is native of Africa and Asia, where it has been consumed for 3,000 years. It is a popular fresh market product (Splittstoesser1984). Cucumber crop is one of the most important vegetable crops in Egypt. The total area cultivated in Egypt is 142000 acres producing 7-15 ton/fadden in addition, there are variable 5887 greenhouses on autumn season only produced 802644 and 25333 ton/Feddan respectively (Agriculture Ministry Statistics, 2018).

Several investigators recorded that diseases that are found on cucumber were considered the most destructive and cause considerable

losses in crop yield. Among the diseases' constraints, viral diseases that can affect plants and cause significant economic losses of 60 -70%, and in some instances the crop may still be harvested, but is of poorer quality and appearance. It was found that CMV in yam caused the average yield loss of around 30% by significantly reducing the mass of yam tubers. (Nagendran *et al* 2017).

CMV as a member of the genus *Cucumovirus* of the family *Bromoviridae*, is reported to infect 1287 plant species in 518 genera belonging to 100 families (Edwardson and Christie 1987). It is the most economically important plant viruses, causing damages in many agricultural crops (tomato, pepper, cucurbits, etc.) (Nagendran *et al* 2017). It is distributed worldwide, has the widest host range of all known plant viruses, and is transmitted in a non-persistent manner by almost sixty aphid species (Palukatis and Arenal 2003). Among cucumber viruses, CMV is considered as one of the most devastating viruses affecting cucumber (El-Beshehy and Sallam 2012, Megahed *et al* 2012, Farahat *et al* 2018 and Dermalah and Elsharkawy 2019), Lettuce (*Lactuca sativa*) (El-Borollosy and Waziri 2013), Cowpea (*Vigna unguiculata* L.) (Abd El -Aziz and Younes 2019), Banana (*Musa* spp.) (Nour El-Din *et al* 2013, El Dougdoug *et al* 2014 and Abdelsabour *et al* 2015), Geranium (*Pelargonium graveolens*) (Sofy and Soliman 2011) and Gladiolus (*Gladiolus grandifloras* Hort) (El Dougdoug *et al* 2014) plants in Egypt.

CMV can cause severe systemic mosaic symptoms such as leaf distortion and fruit lesions, which result in drastic reduction in marketable yield (Ben Chaim *et al* 2001). CMV infection causes fern leaf, stunting of vegetable crops and malformation of their fruits. The plants have light green foliage's, smaller than normal, yellow mottled, severe stunting and crinkled (Zitikaite *et al* 2011). Systemic mosaic, vein clearing, blistering, fruit malformation and stunted plant growth are the symptoms in cucumber plants (Rakib and Adhab 2012, El-Beshehy and Sallam 2012, Sallam *et al* 2012 and El Dougdoug *et al* 2014).

One of the most important components in an integrated disease control program is the selection and planting of cultivars that are resistant to pathogens. The term resistance usually describes the plant host's ability to

suppress or retard the activity and progress of a pathogenic agent, which results in the absence or reduction of symptoms (Steven *et al* 2000). In spite of the widespread distribution of CMV, the progress made in resistance breeding is rarely comparing to other diseases, therefore, the selection of cucumber resistant varieties for disease resistance appears to be the efficient means of controlling (Rahman *et al* 2016).

Improvement in cucumber can be achieved by assessing genetic variability and exploitation of heterosis. Cucumber is highly amenable for heterosis breeding because of the cross pollination and monoecious nature of the crop and it produces large numbers of seed and has a low seed rate per unit area, which provides utilization of heterosis breeding and has the possibility of improvement over its base population (Singh *et al* 2010). There is hybrid vigor in cucumber for fruit size and fruit number per plant (Hayes and Jones, 1916). Hybrid breeding offers opportunities for improvement in production, earliness, uniformity, quality, and resistance to pests and diseases. Considerable heterosis has been reported in cucumber (Ene *et al* 2019).

The objective of this study was identification of the resistance to CMV and estimating the genetic variance among ten cucumber inbred lines.

#### **MATERIALS AND METHODS**

This study was conducted during the period from 2017 to 2020. Selfing pollination to produce inbred lines and crossing to produce F1<sup>s</sup> were carried out in the greenhouse at Kaha Vegetable Research Farm (K.V.R.F), Kalubia at spring season in 2017, and horticultural evaluation of the inbred lines and their hybrids was carried out in the greenhouse at Kaha Vegetable Research Farm, Kalubia and Ismailia Experimental Station at spring season in 2020, while evaluation of the inbred lines was carried out at Ismailia Experimental Station during (2017/2018) and evaluation of the selection inbred lines and their hybrids to CMV was carried out under greenhouse conditions at Ismailia Experimental Station during (2018-2019).

##### **Plant material**

The genetic materials used in the present investigation included 12 different genotypes of cucumber presented in Table (1); three of them were collected from Main Vegetables and Hybrids Production Project

(M.V.H.P.P), four were collected from Gene Bank of Netherland in addition to three from Gene Bank of Sweden and two check hybrids (EL-SAFA and HADY) .Seven promising parents were selected out from this experiment as they showed resistance to CMV (Table 2).

**Table 1. The cucumber genotypes evaluated to CMV reaction in February 2018at Ismailia Experimental Station (First experiment).**

No.	Genotype	Source
1	22-5-8-9 CGN	Gene Bank of Netherland
2	17-10-24-4 CGN	Gene Bank of Netherland
3	67-90-34-3 CGN	Gene Bank of Netherland
4	2-99-25 NGB	Gene Bank of Sweden
5	224-6-5 NGB	Gene Bank of Sweden
6	19-11-14-2 CGN	Gene Bank of Netherland
7	15-17-9 NGB	Gene Bank of Sweden
8	1-18-7-22-18 DOKY	(M.V.H.P.P)
9	3-8-51 AYM	(M.V.H.P.P)
10	34-9-61 AYM	(M.V.H.P.P)
11	EL-SAFA	Horticulture Research Institute
12	HADY	Nun Hems co.

M.V.H.P.P = Main Vegetables and Hybrids Production Project

**Table 2. The cucumber genotypes used for half- diallel.**

No.	Genotype	Code
1	22-5-8-9 CGN	P1
2	17-10-24-4 CGN	P2
3	67-90-34-3 CGN	P3
4	224-6-5 NGB	P4
5	2-99-25 NGB	P5
6	3-8-51 AYM	P6
7	15-17-9 NGB	P7

### **Isolation and identification of CMV**

Samples of cucumber plants exhibiting mosaic, crinkle and deformation were collected from Agriculture College Farm of Ismailia Governorate. These samples were checked serologically against CMV antiserum provided by Serological Lab in Virus and phytoplasma Research Dept. A.R.C. Plant samples which gave positive reaction in the (indirect ELISA) as described by Clark and Adams (1977) test with CMV antiserum were used as a source of virus infection. Extracted sap of infected cucumber leaves was used to inoculate the following indicator hosts: *Nicotiana tabacum* cv. White Burley as systemic host, *Vigna unguiculata* cv. Local, was used as a local lesion host. To obtain virus isolate in a pure form, the single local lesion technique was followed according to Kuhn (1964) in biological purification of the virus isolate, these plants were inoculated with infected sap. Inoculated plants were kept in separate cages, as a source of virus infection. Necrotic local lesion induced by CMV on *Vigna unguiculata* cv. Local was back inoculated to *Nicotiana tabacum* cv. White Burley to obtain virus isolate in a pure form. The inoculum was prepared from CMV infected top cucumber leaves, ground in a mortar containing 0.1 M phosphate buffer, pH 7.0 (1: 2). The homogenate was filtrated through two layers of muslin, and the leaves of healthy plants were dusted with carborundum and rubbed gently with a cotton swab previously dipped into the suspension of virus inoculum.

### **Host range and symptomatology**

Plant species belonging to 4 different families (*Fabaceae*, *Chenopodiaceae*, *Solanaceae*, *Cucurbitaceae*) were mechanically inoculated with the virus isolate to study the host range. The cultivars tested are *Vigna unguiculata* cvs. Local; *Lupinus albus* cv. Giza 1 and cv. Giza 2; *Vicia faba* cv. Local; *Lycopersicon esculentum* cv. Local; *Capsicum annuum* cv. Local; *Datura metel*; *Nicotiana tabacum* cvs. White Burley; *Cucurbita pepo* cv. Local; *Cucumis sativus* cv. Local; *Cucumis melo* var. *flexuosus* Naud; *Cucumis melo* cv. Local; *Pisum sativum* cv. Eantasar 1 and Eantasar 2; *Chenopodium amaranticolor*; *Chenopodium album* and *Chenopodium quinoa*. An equal numbers of test plants were left without inoculation to serve as controls. Inoculated plants were observed

daily for 6 weeks. Development and severity of symptoms were recorded. Symptomless plants were back inoculated into indicator plants or checked serologically.

#### **CMV identification by indirect ELISA**

Samples were obtained from naturally infected cucumber plants grown in Ismailia governorate from (Agriculture College Farm). These samples tested by indirect ELISA test against CMV. This procedure was carried out following the standard methods of Hobbs *et al* (1987). Diseased plants were investigated depending on the visual external symptoms and serological detection.

Plant extracts were prepared by grinding tissues (0.5 g) in the presence of 4.5 ml antigen buffer to reach a dilution 1/10. The micro plates were loaded by 100 µl in each well. Plates were then incubated one hour at 37°C or light over at room temperature. Plates were unloaded and washed with PBS-Tween 1x, and 3 changes for 3 min each. Cross absorption was prepared with healthy cucumber sap and diluted 1/100 in serum buffer and filtrated with cheese-cloth. Primary antiserum was added in recommended dilution to filtrate stir, and incubated 45 min/37°C and then loaded with diluted primary antiserum (cross absorbed) 100 µl /well. The mixture was incubated for (1-1.5) h, at 37°C. The plates were unloaded and washed as before and loaded with secondary antiserum (conjugate) in recommended dilution in serum buffer. The plates were incubated for one hour at 37°C or overnight at room temperature. The plates were unloaded and washed as before and loaded with DiEAB substrate (0.25-0.3) mg/ml. which prepared immediately before use and incubated 30-60 min at room temperature then reading at absorbance of 405. The intensity of the resulting color from the reaction was measured using ELISA reader. The amount or rate of color change can then be used to measure the amount of antibody present (Hagag 2002).

#### **Calculation of CMV infection percentage and symptom intensity**

Inoculated plants by CMV were numbered and percentage of infection was determined as follows: Percentage of infection = (Number of diseased plants/Total number of plants) X 100. Symptom intensity values were used according to Joseph and Hesham (2002). Symptom intensity was

recorded according to the following scale [- =no symptom, += mild infection, ++= moderate infection and +++ = severe infection]

**Response of some cucumber genotypes and hybrids to artificial infection with CMV (at greenhouse)**

A greenhouse-pot experiment was conducted to determine the response of twelve genotypes of cucumber to mechanical inoculation with the tested isolated virus. It was carried out under greenhouse conditions at Ismailia Experimental Station.

This breeding program was started by using twelve genotypes of cucumber (22-5-8-9 CGN, 17-10-24-4 CGN, 67-90-34-3 CGN, 2-99-25 NGB, 224-6-5 NGB, 19-11-14-2 CGN, 15-17-9 NGB, 1-18-7-22-18 DOKY, 3-8-51 AYM,34-9-61 AYM, EL-SAFA and HADY).

Each genotype of the eighteen cucumber plants were sown in pots (25cm) (6 plants/pot,3 pots/genotype) served as replicates for virus inoculation. The same number of cucumber plants from each genotype was inoculated with distilled water served as control. Seeds of all test plants were grown in a mixed soil (clay: peat: sand 1:1:1 v/v/v), fertilized weekly and regularly irrigated. Four true leaves stage cucumber seedlings were mechanically inoculated with CMV isolate,10 days after inoculation of inoculated and non-inoculated cucumber plants, symptoms, percentage of infection and symptom intensity were recorded. The plants were observed and the systemically infected plants were counted until consistent numbers were reached (20-days post-inoculation).

Infection of the plants under the two experiments were carried out under greenhouse conditions at Ismailia Experimental Station. Seeds of 12 genotypes (First experiment) (Table 1) were sown first in foam trays under greenhouse in March 2018.In February 2019, seeds of 6 resistance genotypes in addition to one susceptible inbred line and their 21 half-diallel cucumber hybrids and 2 commercial hybrids (EL-Safa and Hady)as checks (Second experiment) (Table 3).

**Table 3. The cucumber genotypes were tested to CMV reaction in February 2019 at Ismailia Experimental Station(Second experiment)**

<b>No.</b>	<b>Parents</b>
<b>1</b>	<b>P1 (22-5-8-9 CGN)</b>
<b>2</b>	<b>P2 (17-10-24-4 CGN)</b>
<b>3</b>	<b>P3 (67-90-34-3 CGN)</b>
<b>4</b>	<b>P4 (224-6-5 NGB)</b>
<b>5</b>	<b>P5 (2-99-25 NGB)</b>
<b>6</b>	<b>P6 (3-8-51 AYM)</b>
<b>7</b>	<b>P7 (15-17-9 NGB)</b>
	<b>Hybrids</b>
<b>8</b>	<b>P1 X P2</b>
<b>9</b>	<b>P1 X P3</b>
<b>10</b>	<b>P1 X P4</b>
<b>11</b>	<b>P1 X P5</b>
<b>12</b>	<b>P1 X P6</b>
<b>13</b>	<b>P1 X P7</b>
<b>14</b>	<b>P2 X P3</b>
<b>15</b>	<b>P2 X P4</b>
<b>16</b>	<b>P2 X P5</b>
<b>17</b>	<b>P2 X P6</b>
<b>18</b>	<b>P2 X P7</b>
<b>19</b>	<b>P3 X P4</b>
<b>20</b>	<b>P3 X P5</b>
<b>21</b>	<b>P3 X P6</b>
<b>22</b>	<b>P3 X P7</b>
<b>23</b>	<b>P4 X P5</b>
<b>24</b>	<b>P4 X P6</b>
<b>25</b>	<b>P4 X P7</b>
<b>26</b>	<b>P5 X P6</b>
<b>27</b>	<b>P5 X P7</b>
<b>28</b>	<b>P6 X P7</b>
	<b>Check hybrids</b>
<b>29</b>	<b>EL-SAFA</b>
<b>30</b>	<b>HADY</b>



## DNA fingerprint of cucumber genotypes

### 1-DNA extraction

DNA extraction (20 µg) was carried out on the seven cucumber genotypes using the protocol described by High pure PCR-Template preparation kit (Roche Co. Germany). Integrity and quantity of the extracted DNA were estimated spectrophotometrically and visually verified on 1% agarose gel.

### 2-RAPD-PCR

The seven parents (Table 2) showed resistance, tolerance and susceptibility to CMV were selected to discover the molecular marker associated with CMV resistance by using RAPD assay. DNA amplification was carried out in a 25 µl reaction volume containing 1× Taq polymerase buffer, 200 µmoles of each nucleotide in dNTPs (i.e. ATP, TTP, GTP and CTP), 1.5 mM MgCl<sub>2</sub>, 2.5 unit Taq polymerase, 25 pmoles of decamer primer (Table 4) and 20ng genomic DNA in a programmable thermal cycler (Perkin Elmer Cetus, Gene Amp PCR System 2400). Amplification reactions were cycled 35 times for 1 min at 94 °C (denaturation), 1 min at 36 °C (annealing) and 2 min at 72 °C (extension) with a final extension step for 5 min. Amplification products were mixed with loading buffer (2 µl 40% glycerol and 0.025% bromophenol blue) and fractionated on 2% agarose-1×Tris-acetate-EDTA-ethidium bromide gel electrophoresis in 1×TAE buffer at 120V. RAPD bands were visualized and photographed on ChemiImager 5500, Alpha in notech gel documentation system (Williams *et al* 1990).

**Table 4. Sequences of the RAPD primers used in the present study.**

Primer code	Sequence (5`-3`)
CA-14	5`-CTCATGCTCT-3`
AH-18	5`-TCGCTCCGTT-3`
CA-12	5`-CTTTCGCCTC-3`

### Data analysis

Banding profiles generated by RAPD assay were separately compiled into a data matrix on the basis of presence (1) or absence (0) of bands. The binary matrices were used to estimate DNA polymorphisms and genetic relatedness of cucumber cultivars.

### **Horticultural evaluation**

Plants of thirty genotypes [seven parents, twenty-one hybrids, and two commercial hybrids (EL-Safa and Hady)] were grown in a greenhouse at Kaha Vegetable Research Farm, Kalubia and Ismailia Experimental Station at spring season in 2020, to be evaluated for their horticultural characters. Plants of all genotypes were raised using three replicates. Data were recorded for vegetative and fruit characteristics [average main stem length (cm), leaf area, number of female flowers per node, average fruit length (cm), fruit weight, early yield/plant (kg) and total yield per plant (Kg)].

### **Statistical analysis**

Data obtained were statistically analyzed according to the analysis of variance and L.S.D tests (Steel and Torrie 1960).

### **Genetic and statistical analysis**

#### **a. Potance ratio (P)**

$$P = \frac{\overline{F1} - \overline{M.P.}}{\frac{1}{2}(\overline{P2} - \overline{P1})} \quad (\text{Smith, 1952})$$

Where:-

- $\overline{F1}$  = First generation mean.

- $\overline{P1}$  = Mean of the smaller parent.

- $\overline{P2}$  = Mean of the larger parent.

-  $\overline{MP}$  = Mid-parent value =  $\frac{1}{2}(\overline{P1} + \overline{P2})$

The absence of dominance was assumed when the difference between the parents was significant and  $\overline{F1} - \overline{M.P.}$  was not significant. Complete dominance was assumed when potence ratio equaled to or did not differ from  $\pm 1.0$ . Meanwhile, partial dominance was considered when

potence ratio was between +1.0 and — 1.0, but was not equal to zero. Over dominance (Heterosis) was assumed when potence ratio exceeded  $\pm 1.0$ .

### b. Heterosis

Heterosis based on the mid and better parent value was estimated according to the following equation:

$$\text{Mid- parent heterosis} = \frac{\overline{F1} - \overline{M.P}}{\overline{M.P}} \times 100 \quad (\text{Sinha and Khanna, 1975})$$

Where:-

$\overline{M.P}$  = mean of the mid - parent.

$\overline{F1}$  = mean of the first hybrid generation

$$\text{Better parent heterosis} = \frac{\overline{F1} - \overline{H.P}}{\overline{H.P}} \times 100$$

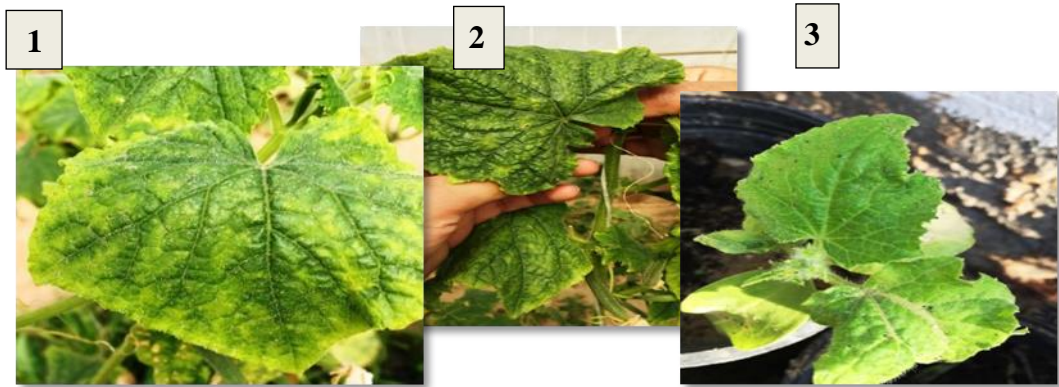
Where:  $\overline{H.P}$  Mean of the higher or better - parent.

Where: -  $\overline{B.P}$  Mean of the better – parent.

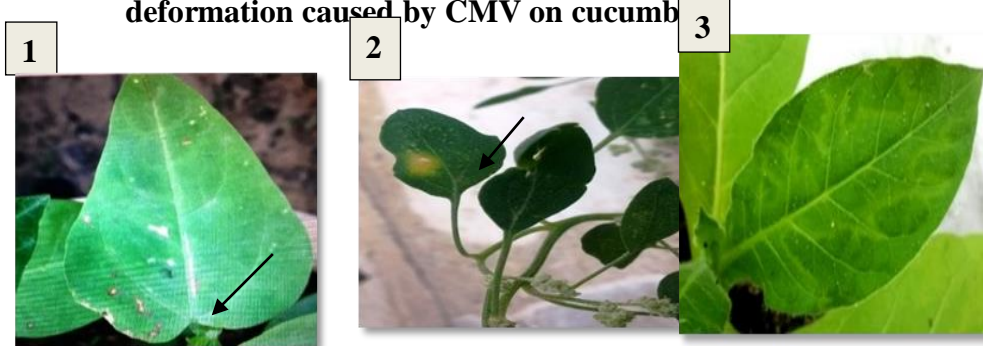
## RESULTS AND DISCUSSION

### Isolation and identification of CMV

Samples of cucumber plants exhibiting mosaic, crinkle and deformation were collected from Agriculture College Farm of Ismailia Governorate, Ismailia experimental Station, Agric. Res. Center and Agriculture College Farm (Fig. 1). These samples were checked serologically against CMV antiserum using Indirect ELISA. Samples which reacted positively with CMV were collected separately and used for virus inoculation. The virus isolate was biologically purified as mentioned before (Materials and Methods) and re-inoculated onto *Nicotianatabaccum* cv. White Burley plant were then used as a propagative host for the virus isolate. Upon inoculation with CMV on *Vigna unguiculata* cv. Local showed necrotic local lesions (brown lesion) (Fig. 2). These results are in agreement with those reported by Eid *et al* (1984) and Rakib and Adhab (2012), *Nicotianatabaccum* cv. White Burley showed mosaic (Fig. 2). This result is in agreement with those reported by Zitikaite *et al* (2011) and El Dougdoug *et al* (2014).



**Fig.1, 2. Naturally infected cucumber plants showing viral symptoms mosaic and crinkle, 3- showing vein clearing, mosaic and deformation caused by CMV on cucumb**



**Fig. 2 Showing indicator hosts produced by CMV .1- necrotic local lesions (brown lesion) on *Vigna unguiculata* cv. Local; 2- necrotic local lesion on *Chenopodium quinoa* and 3- mosaic on *Nicotiana tabacum* cv. White Burley.**

#### **Host range and symptomatology**

The tested plants reacted with different response and reaction of susceptibility with CMV. Seventeen hosts belonging to 4 families were inoculated mechanically with the virus isolate.

**Symptoms appeared on the plants might be grouped into three categories**

**A-Plants reacted with systemic symptoms**

Symptoms started to appear 12-15 days after inoculation on cucumber cv. with by CMV which showed mosaic and deformation (Fig1). These results are in agreement with Megahed *et al* (2012) and Sultana *et al* (2014). In addition to the previously mentioned hosts, the following plants showed systemic symptoms: *Capsicum annuum* cv. Local showed green vein banding and mosaic; *Nicotiana tabacum* cv. White burley showed vein clearing and mosaic; *Lycopersicon esculentum* cv. Local showed mottle and *Cucumis sativus* cv. Local showed vein clearing, mosaic, deformation and stunting (Fig. 2 and Table 5). These results are in agreement with those reported by Iqbal *et al* (2011), Rakib and Adhab (2012) and El- Dougdoug *et al* (2014). *Pisum sativum* cv. (Eantasar1 and Eantasar2) showed vein clearing and mottle; *Vicia faba* cv. Local showed mottle; *Cucurpita pepo* cv. Local showed vein clearing and mottle; *Cucumis melo* var. *flexuous* Naud showed mottle and *Cucumis melo* cv. Local showed vein clearing (Fig 3 and Table 5). These results are in agreement with those reported by Zitikaite (2002), Zitikaite *et al* (2011) and Sultana *et al* (2014).



**Fig. 3. Showing hosts produced by CMV.1- vein clearing on *Pisum sativum* cv. Eantasar1; 2-mosaic on *Capsicum annuum* cv. Local3-green vein banding on *Capsicumannuum* cv. Local and 4-vein clearing and mosaic on *Cucumis sativus* cv. Local**

**Table 5. Reaction of different hosts to infection with the CMV.**

Family	Species	External Symptom
<i>Cucurbitaceae</i>	<i>Cucumissativus cv. Local</i>	M-D-VC-S
	<i>Cucurpitapeppo cv. Local</i>	MO-VC
	<i>Cucumis melo var. flexuous Naud</i>	MO
	<i>Cucumismelo cv. Local</i>	VC
<i>Solanaceae</i>	<i>Lycopersiconesculentum cv. Local</i>	MO
	<i>Capsicumannuum cv. Local</i>	M-GVB
	<i>Datura metal</i>	NS
	<i>Nicotianatabaccum cv. White burley</i>	VC-M
<i>Fabaceae</i>	<i>Vigna unguiculata cv. Local</i>	VC-NLL-MO
	<i>Pisum sativum cv. Eantasar 1</i>	VC-MO
	<i>Pisum sativum cv. Eantasar 2</i>	VC-MO
	<i>Lupin albus cv. Giza 1</i>	NS
	<i>Lupin albus cv. Giza 2</i>	NS
	<i>Vicia faba cv. Local</i>	MO
<i>Chenopodiace</i>	<i>Chenopodium amaranticolor</i>	NS
	<i>Chenopodium album</i>	NS
	<i>Chenopodium quinoa</i>	CLL

(NS) No symptoms. (MO) Mottle. (M) Mosaic. (VC) Vein clearing (CLL) Chlorotic Local Lesion. (NLL) Necrotic local lesion. (GVB) Green Vein Banding. (S) Stunting. (D) Deformation.

#### **B- Plants reacted with local symptoms**

Up on inoculation with CMV on *Chenopodium quinoa* and *Vigna unguiculata cv. Local* reacted with necrotic lesions (Fig 2 and Table 5). This result was in agreement with Paradies *et al* (2000), Zitikaite (2002) and Iqbal *et al* (2011).

#### **C- Plants with no symptoms**

No symptoms were observed on the following inoculated plants: *Lupin albus cv. (Giza 1 and Giza 2)*; *Chenopodium amaranticolor*; *Chenopodium album* and *Datura metal*.

**Response of some cucumber genotypes to artificial infection with CMV (at greenhouse)**

**1- Evaluation of cucumber genotypes for CMV resistance**

Data obtained on the reaction of cucumber genotypes evaluated for CMV resistance under artificial infection conditions in the 2018 season are presented in Table (6).

**Table 6. Percentage of infection and symptom intensity of cucumber genotypes with CMV by artificial infection.**

Genotypes	Symptoms	S/T* replicates			Percentage of infection	Symptom intensity	Interaction category**
		R1	R2	R3			
22-5-8-9 CGN(P1)	NS	0/6	0/6	0/6	-	-	R
17-10-24-4 CGN (P2)	NS	0/6	0/6	0/6	-	-	R
67-90-34-3 CGN (P3)	Mosaic	1/6	2/6	1/6	22.2%	+	T
224-6-5 NGB (P4)	Mosaic	1/6	3/6	3/6	38.9%	+	T
2-99-25 NGB (P5)	Mosaic	2/6	2/6	4/6	44.4%	+	T
3-8-51 AYM (P6)	Mosaic	2/6	2/6	2/6	33.3%	+	T
15-17-9 NGB (P7)	Severe mosaic	4/6	4/6	5/6	72.2%	+++	S
19-11-14-2 CGN	Mosaic	3/6	4/6	5/6	66.7%	++	MT
34-9-61 AYM	Severe mosaic	5/6	3/6	5/6	72.2%	+++	S
1-18-7-22-18 Doky	Mosaic	3/6	4/6	5/6	66.7%	++	MT
EL- Safa (check)	Mosaic	2/6	4/6	3/6	50%	++	MT
Hady (check)	Mosaic	2/6	4/6	3/6	50%	++	MT

\*=No. of symptomatic plant(S)/ Total number of tested plants(T). NS= No symptoms. - = Resistance (R), + = Tolerance (T), ++ = Moderately Tolerance (MT), +++ =Susceptible(S). \*\* [(0-10) Resistance (R), (10.10-45) = Tolerance (T), (45.10-70) = Moderately Tolerance (MT), (70.10-10) =Susceptible(S).] Reference (El-Bramawy and El-Beshehy 2011).

Upon mechanical inoculation of the cucumber inbred lines with CMV isolate, eight cucumber genotypes(67-90-34-3 CGN, 19-11-14-2 CGN, 224-6-5 NGB, 2-99-25 NGB, 3-8-51 AYM, 1-18-7-22-18 DOKY,EL-SAFA and HADY) showed mosaic and two cucumber genotypes(15-17-9 NGB and 34-9-61 AYM) showed severe mosaic symptoms at the cotyledon stage. (22-5-8-9 CGN and 17-10-24-4 CGN) cucumber genotypes were symptomless and appeared healthy. A symptom developed later on included systemic mosaic and severe mosaic on the young leaves while crinkling and deformation on the older ones (Fig. 1 and 2).



This virus was isolated in previous studies from cucumber, by other investigators in different countries (El-Beshehy and Sallam 2012, Megahed *et al* 2012, Farahat *et al* 2018 and Derbalah *et al* 2019).

Data concerning the percentage of infected plants and the symptom intensity are presented in (Table 6). The results showed that genotypes 15-17-9 NGB and 34-9-61 AYM revealed highest percentage of artificial infection (72.2%) and (72.2%), respectively.

On the contrary, the lowest percentage of artificial infection was obtained by the genotypes 67-90-34-3 CGN and 3-8-51 AYM (22.2%) and (33.3%) respectively. While the genotypes 22-5-8-9 CGN and 17-10-24-4 CGN were resistant to CMV.

On the other hand, the symptom intensity of artificial infection is presented in (Table 6). Two genotypes (15-17-9 NGB and 34-9-61 AYM) were susceptible to CMV. While four genotypes (19-11-14-2 CGN, EL-SAFA, HADY and 1-18-7-22-18 DOKY) were moderately tolerant to CMV. Four genotypes (67-90-34-3 CGN, 224-6-5 NGB, 2-99-25 NGB and 3-8-51 AYM) were tolerant to CMV, and two genotypes (22-5-8-9 CGN and 17-10-24-4 CGN) were resistant to CMV.

This result is in agreement with Munshi *et al* (2008) who found in his study on cucumber lines that 8 genotypes were categorized as resistant, 13 as moderately resistant, 9 as moderately susceptible and one as susceptible to CMV. Sharma *et al* (2013) found that 26 collections of pumpkin were highly resistant to CMV.

In conclusion, from data presented in Table (6) the genotypes 22-5-8-9 CGN and 17-10-24-4 CGN were resistant to infection with CMV. While the genotypes 15-17-9 NGB and 34-9-61 AYM were susceptible to infection with CMV. The genotypes 67-90-34-3 CGN, 224-6-5 NGB, 2-99-25 NGB, 3-8-51 AYM, 19-11-14-2 CGN, EL-Safa, Hady and 1-18-7-22-18 DOKY were tolerant to infection with CMV. These inbred lines could be used for CMV resistance breeding programs to be released as new cultivars, which possess high yielding under CMV infection.

## **2. Evaluation of cucumber hybrids for CMV resistance**

Data obtained 28 entries of cucumber (7 parents and 21 F1 hybrids) and 2 check hybrids and their reaction to artificial infection with CMV in the 2019 season. The symptom intensity of artificial infection was presented in (Table 7).

**Table 7. Symptom intensity CMV of Cucumber genotypes and hybrids.**

NO.	Genotype	symptoms	Symptom Intensity	Interaction category
1	P1	-	-	R
2	P2	-	-	R
3	P3	Mild mosaic	+	T
4	P4	Mild mosaic	+	T
5	P5	Mosaic	+	T
6	P6	Mild mosaic	+	T
7	P7	Severe mosaic	++	S
8	(P1) X (P2)	-	-	R
9	(P1) X (P3)	Mosaic	+	T
10	(P1) X(P4)	-	-	R
11	(P1) X (P5)	-	-	R
12	(P1) X(P6)	-	-	R
13	(P1) X (P7)	Severe mosaic	++	S
14	(P2) X (P3)	Mosaic	+	T
15	(P2) X(P4)	Mosaic	+	T
16	(P2) X (P5)	-	-	R
17	(P2) X(P6)	-	-	R
18	(P2) X (P7)	Severe mosaic	++	S
19	(P3) X(P4)	Severe mosaic	++	S
20	(P3) X (P5)	Mosaic	+	T
21	(P3) X(P6)	Mosaic	+	T
22	(P3) X (P7)	Severe mosaic	++	S
23	(P4) X (P5)	Severe mosaic	++	S
24	(P4) X(P6)	-	-	R
25	(P4) X (P7)	Severe mosaic	++	S
26	(P5) X(P6)	Mosaic	+	T
27	(P5) X (P7)	Severe mosaic	++	S
28	(P6) X (P7)	Severe mosaic	++	S
29	EL-SAFA	Severe mosaic	++	S
30	HADY	Severe mosaic	++	S

- = Resistance (R) + = Tolerance (T), ++ =Susceptible(S).

The line (P7) was susceptible to CMV, while the lines (P3), (P4), (P5) and (P6) were tolerant and other parents (P1), (P2), were resistant to CMV. The line (P2) was common to 3 resistant hybrids [(P1) X (P2), (P2) X(P5) and (P2) X (P6)], in addition the line (P6) was common to 3 resistant hybrids [(P1) X(P6), (P2) X(P6) and (P4) X(P6)]. On the other hand, the results showed that the two commercial hybrids used as check were susceptible as all hybrids which contain the line P7.

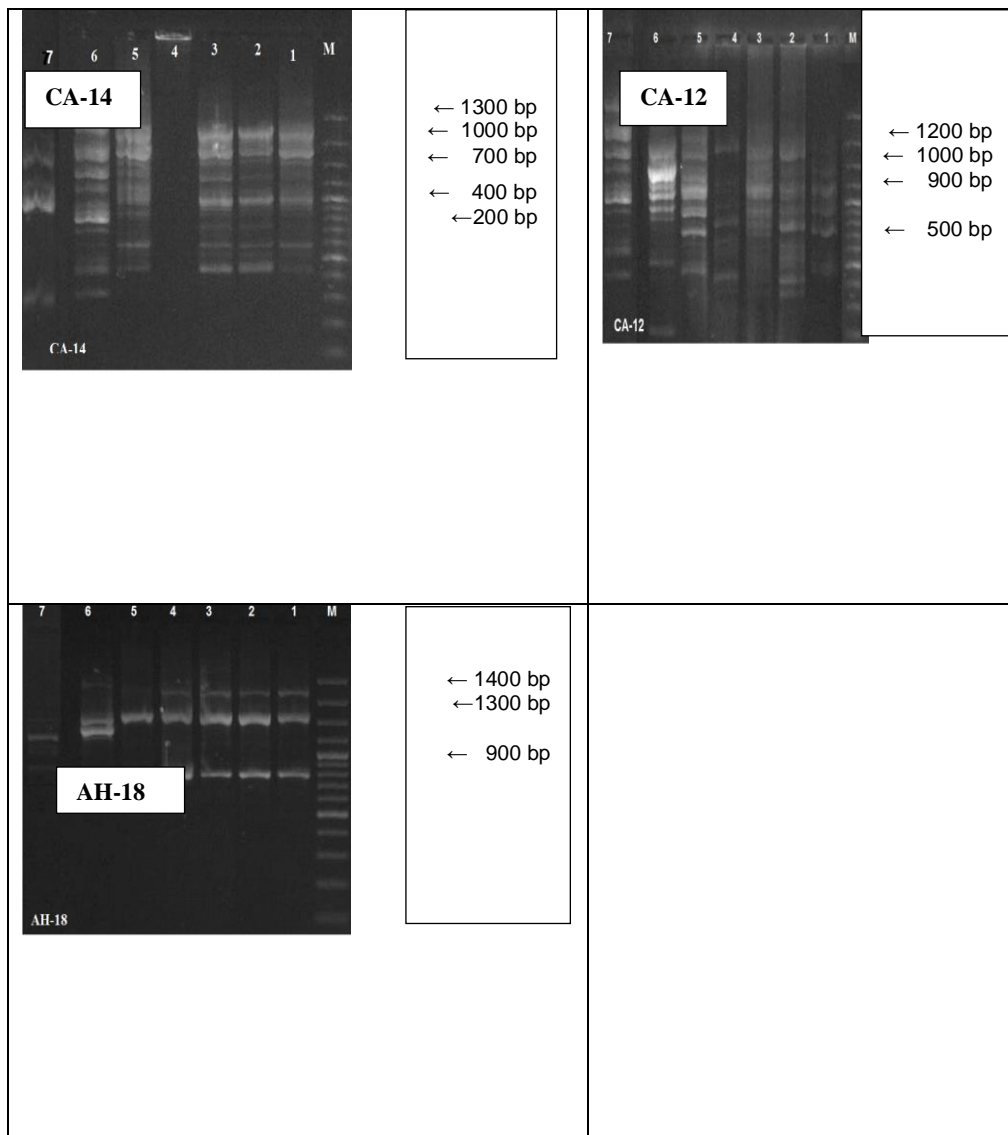
These results revealed that, the resistance to CMV in cucumber is completed and this is in agreement with (Havey 1996) who evaluated 3 genotypes cucumber for resistance to CMV and TMG1 line had the higher resistance compared to the other lines (SMR18 , Marcetmar 76) , and after crossing them with ST8-5 line (susceptible) he found that (ST8-5 X TMG1) hybrid was resistant , but the other hybrids were susceptible and (Ghai *et al* 1998) who evaluated 14 Cucurbita lines for resistance to CMV and found that P2 and P3 were the best male parents for resistance to CMV and P11 was the best female parent for resistance to CMV. After crossing them, the best hybrids resistant to CMV were P6 X P4 and then P11 X P3.

#### **DNA fingerprint of cucumber genotypes**

RAPD-PCR was used to evaluate the genetic diversity of seven cucumber inbred lines (6 resistant and one susceptible) using 3 RAPD primers. The three primers successfully amplified DNA fragments for the cucumber genotypes

In the seven cucumber parents, the three RAPD primers generated polymorphic banding patterns; all of them generated only both polymorphic and monomorphic banding patterns.

A total of thirty-two fragments were obtained 7 for AH-18, 13 for CA-12 and 12 for CA-14 visualized across the seven cucumber parents. Level of polymorphism varied from one primer to another. The three RAPD primers (CA-12, CA-14 and AH-18) showed high level of polymorphism (83.3%, 92.3% and 85.7% respectively) The resulted amplified fragments are shown in Fig. (4).



**Fig. 4. Photographs presenting 3RAPD products of the 7 different genotypes of cucumber using 3 primers.**

**Table 8. Amplification patterns among the seven cucumber genotypes using three RAPD primers.**

Primer	M. Wt.	Presence or absence of bands							No. polymorphic bands
		1	2	3	4	5	6	7	
CA-14	1450	0	0	0	0	1	1	0	10
	1350	0	0	0	0	0	0	0	
	1300	1	1	1	0	1	1	0	
	1100	1	1	1	0	1	1	0	
	1000	1	1	1	0	1	1	1	
	800	1	1	1	0	1	1	1	
	700	1	1	1	0	1	1	1	
	600	1	1	1	0	1	1	0	
	400	1	1	1	0	1	1	0	
	300	1	1	1	0	1	1	0	
	200	1	1	1	0	1	1	1	
	150	0	0	0	0	0	0	0	
	Total	9	9	9	0	10	10	4	
	CA-12	1500	0	0	0	0	0	1	
1400		0	0	1	1	1	1	1	
1300		0	1	1	0	1	1	1	
1200		1	1	1	0	1	1	1	
1100		1	1	0	1	1	1	1	
1000		1	1	1	1	1	1	1	
900		1	1	1	1	1	1	1	
800		1	1	0	1	1	1	1	
700		0	1	0	1	1	1	1	
600		0	1	1	0	1	1	1	
500		0	0	1	1	1	1	1	
400		0	0	0	1	1	0	1	
300		0	0	0	0	0	1	0	
Total		5	8	7	8	11	12	12	
AH-18	1500	0	0	0	0	0	1	0	6
	1400	1	1	1	1	1	0	0	
	1300	1	1	1	1	1	1	1	
	1200	0	0	0	0	0	1	1	
	1100	0	0	0	0	0	0	1	
	1000	0	0	0	0	0	0	1	
	900	1	1	1	1	1	0	1	
Total	3	3	3	3	3	3	5		

The summary of RAPD polymorphism detected between the seven cucumber parents were generated by the three RAPD primers is presented in Table (8). The three primers amplified a total of 32 amplicons, four of them were monomorphic and twenty eight fragments were polymorphic with an average of 87.5% polymorphism (Table 9).

**Table 9. Total number of amplicons, monomorphic and polymorphic amplicons as revealed by RAPD primers among seven cucumber genotypes.**

Primer	Total number of amplicons	Polymorphic amplicons	Monomorphic amplicons	% of polymorphism
CA-14	12	10	2	83.3%
CA-12	13	12	1	92.3%
AH-18	7	6	1	85.7%
<b>Total</b>	<b>32</b>	<b>28</b>	<b>4</b>	<b>87.5%</b>

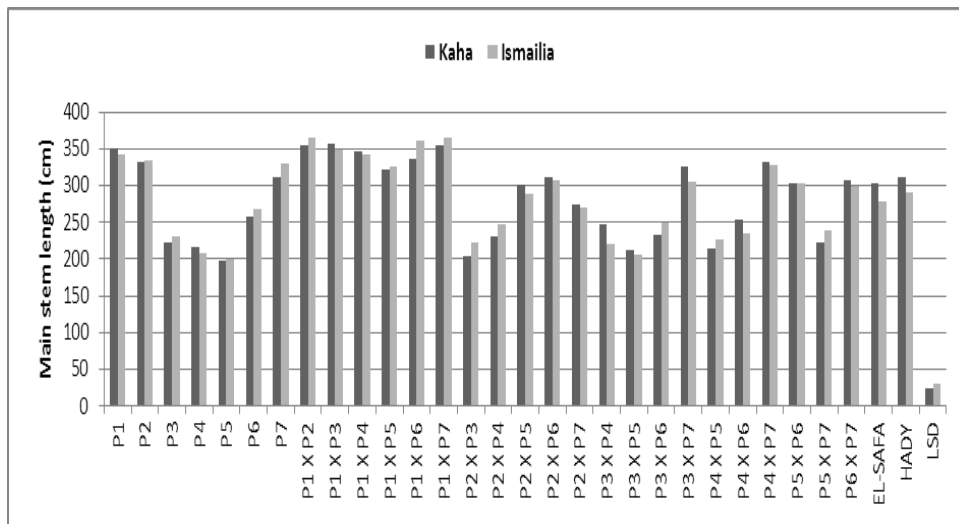
### **Evaluation of cucumber genotypes for horticultural characteristics**

This study was conducted in a greenhouse during 2020 in two locations (Kaha and Ismailia). Thirty cucumber genotypes (7 parents, 21F<sub>1</sub> hybrids and the 2 commercial hybrids (E-Safa and Hady) were used.

#### **1. Main stem length (cm)**

Data obtained on the main stem length of 30 cucumber genotypes in two locations are illustrated in Fig. (5). The results indicated that parents 1, 2 and 7 were the highest in main stem length in two locations respectively, with significant differences from the other parents. The results revealed also that most of hybrids which contain P1 gave higher significantly main stem length compared with the other hybrids studied in both locations. The results showed that, this line is tolerant to CMV (Table 7). So, it might be recommended to be evaluated on a large scale and tested for other

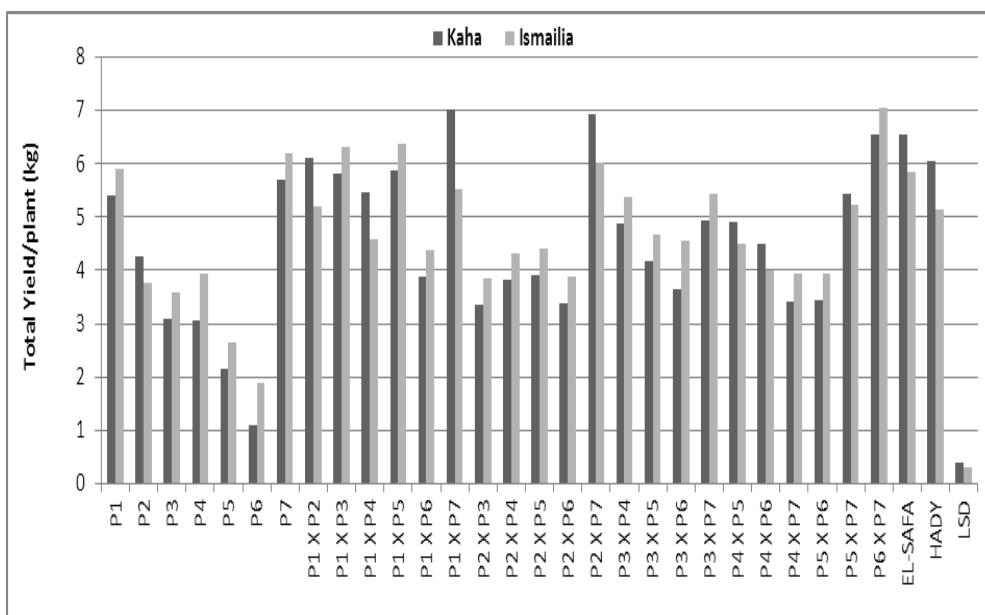
horticultural characteristics. These results are in agreement with Melisa and Wehner (2006) who stated that synecious cucumbers usually have earlier and more concentrated harvests than monoecious cucumbers e.g. 'Dasher II' (tall, synecious, slicing type) which was the best performing cultivar in the character of main stem length.



**Fig. 5. Main stem length (cm) of cucumber genotypes in Kaha and Ismailia.**

## 2. Total yield/plant (kg)

Data obtained on total yield/plant of 7 parents, their 21 hybrids and 2 commercial hybrids in two locations are presented in Fig. (6). The results indicated that parents 7 and 1 had the highest of total yield/plant in two locations respectively, with significant differences from the other parents. On the other hand, the results revealed also that the most of hybrids which contain P7 and P1 (respectively) gave significantly higher total yield/plant compared with the other hybrids studied in both locations. The hybrids of the parent P7 were susceptible to CMV, but the hybrids of the parent P1 were resistant or tolerant to CMV Table (7). So, it might be recommended that P1 is recommended for new breeding program.

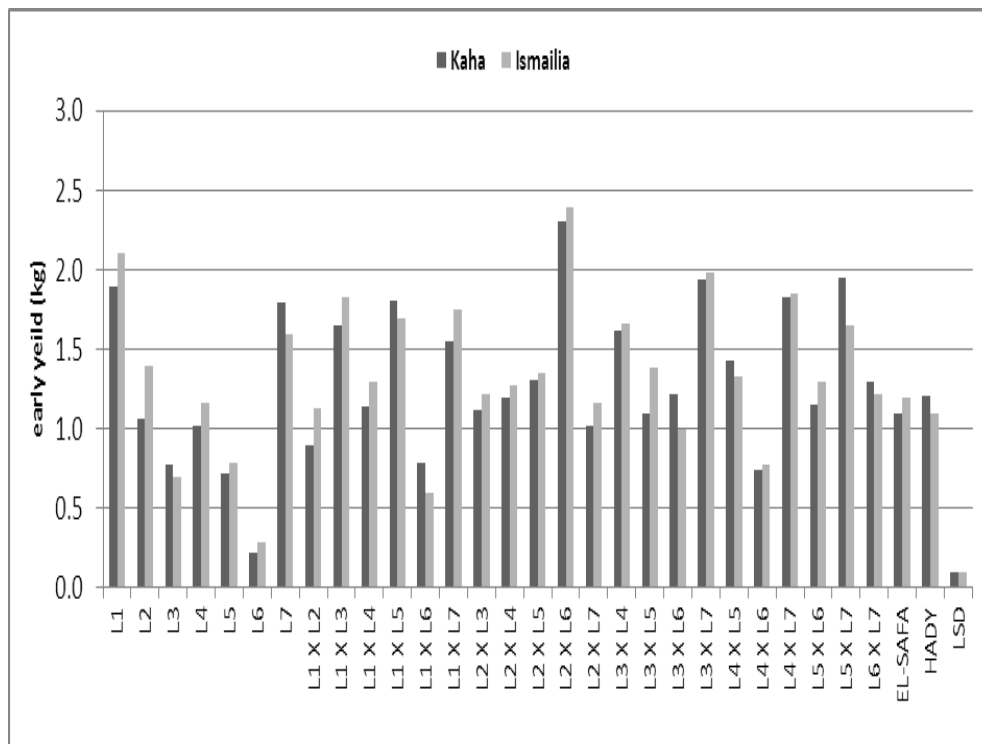


**Fig. 6. Total Yield/plant (kg) of cucumber genotypes in Kaha and Ismailia.**

### **3. Early yield/plant (kg)**

Data obtained on early yield/plant of 30 cucumber genotypes during 2020 in two locations (Kaha, Ismailia) are presented in Fig. (7). The results obtained showed that P1 and P7 produced significantly earlier yield/plant compared with the other used parents in the two locations. The results indicated that the hybrid P2XP6 gave the highest early yield/plant significant differences as compared with the other hybrids in two locations. On the other hand, P6 gave the least early yield/plant in the two locations with significant difference from other parents.

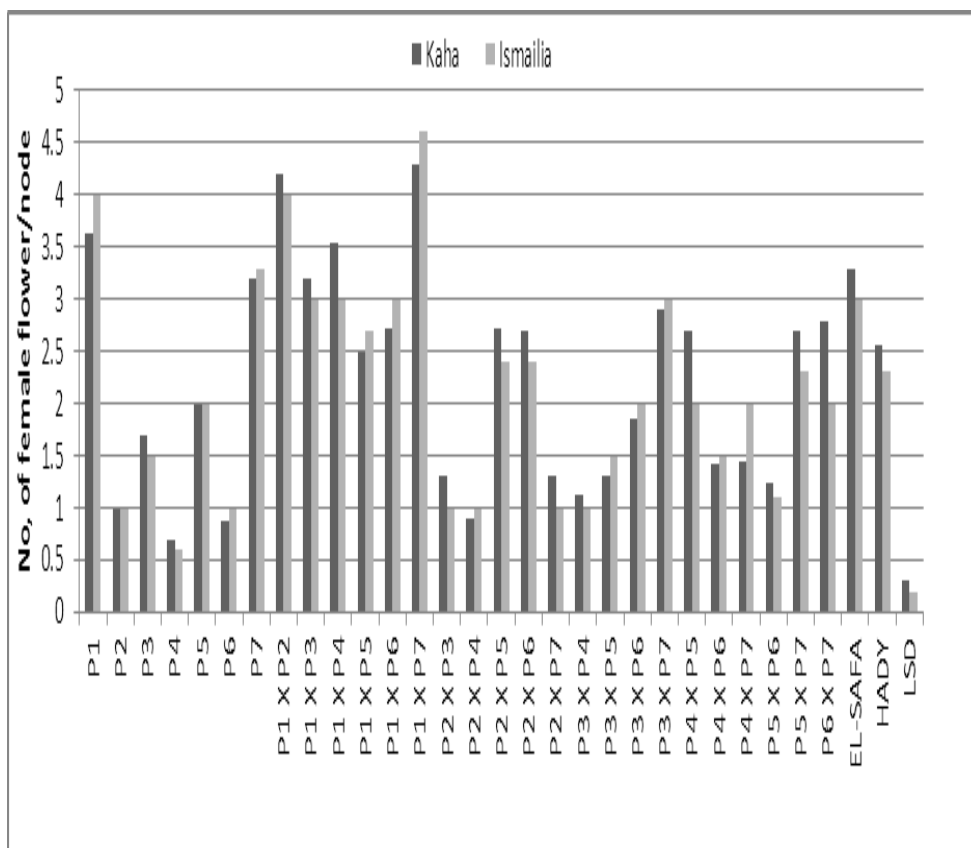




**Fig. 7. Early Yield/plant (kg) of cucumber genotypes in Kaha and Ismailia.**

#### **4. No. of female flower/node**

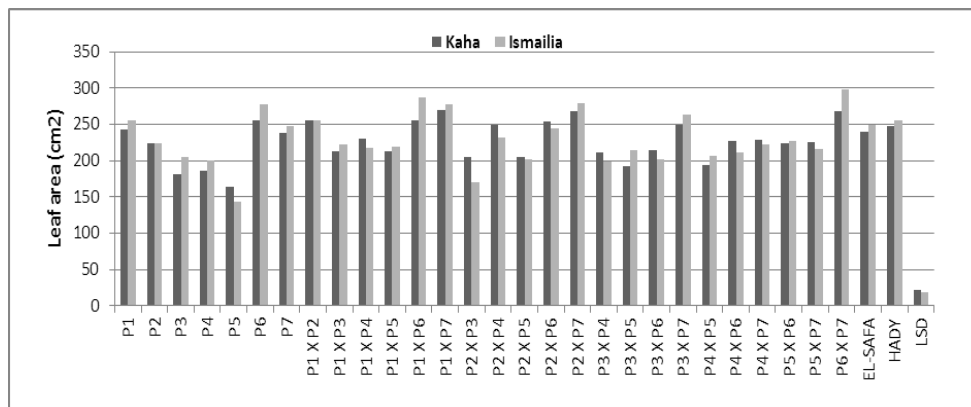
Data obtained on No. of female flower/node of 7 parents, their 21 hybrids and 2 commercial hybrids in two locations are illustrated in Fig. (8). The results indicated that parents 7 and 1 had the highest of No. of female flower/node in two locations, without significant differences among them in Ismailia. By contrast, there were significant differences between them in Kaha. On the other hand, the results revealed that P1XP7 and P1XP2 (respectively) gave significantly high No. of female flowers/ node compared with the other hybrids studied in both locations. P1XP7 was the best in this trait but susceptible to CMV. On the other hand, P1XP2 had lower No. of female flowers/node than P1XP7 but was resistance to CMV (Table 7).



**Fig. 8. No. of female flower/node of cucumber genotypes in Kaha and Ismailia.**

### 5. Leaf area (cm<sup>2</sup>)

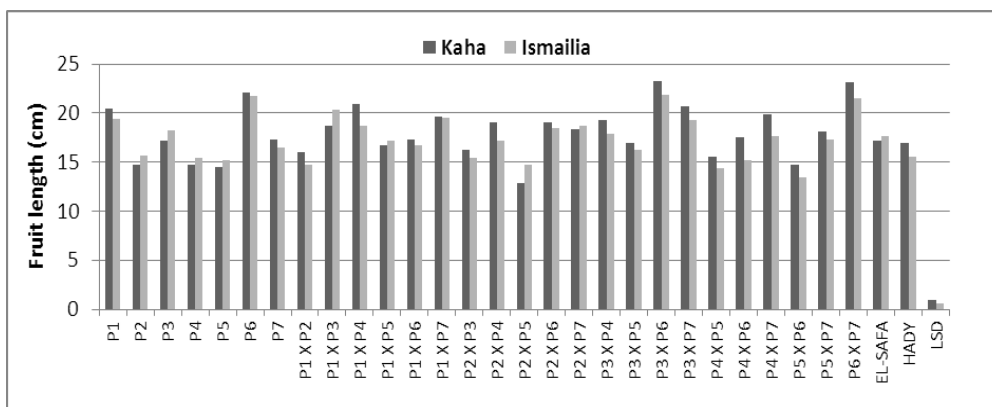
Data obtained on Leaf area are presented in Fig. (9). The results showed that P6 produced significantly greater Leaf area compared with the other studied parents in Ismailia and without significant differences from P1 and P7 in Kaha. The results indicated that the hybrid P6XP7 gave the highest leaf area and showed significant differences as compared with the other hybrids in Ismailia and without significant differences with another 10 hybrids in Kaha location.



**Fig. 9. Leaf area (cm<sup>2</sup>) of cucumber genotypes in Kaha and Ismailia.**

### 6. Fruit length (cm)

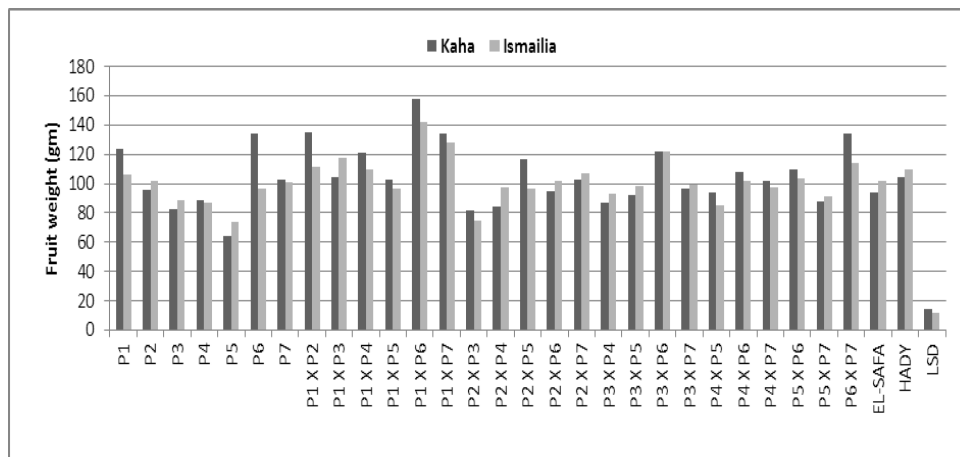
Data obtained on fruit length of 7 parents, their 21 hybrids and 2 commercial hybrids in two locations are illustrated in Fig. (10). The best value of Egyptian marketable fruit length on spring season from 16-17 cm, so, P3 had the best fruit length between the others parents in two locations. The two commercial hybrids (EL-safa and Hady) had the best fruit length as well as 7 hybrids in two locations.



**Fig. 10. Fruit length (cm) of cucumber genotypes in Kaha and Ismailia.**

## 7. Fruit weight (gm)

Data obtained on fruit weight of 7 parents, their 21 hybrids and 2 commercial hybrids in two locations are illustrated in Fig. (11). The best value of Egyptian marketable fruit weight on spring season from 90-100 gm, so, P2, P3, P4 and P7 revealed the best fruit weight among the others parents in two locations and along with P1 in Ismailia. The two commercial hybrids (EL-safa and Hady) had the best fruit weight as well as 9 hybrids in two locations.



**Fig. 11. Fruit weight (gm) of cucumber genotypes in Kaha and Ismailia.**

These results are in agreement with Delaney and lower (1987) who evaluated individual plants of the cross made between two determinate cucumber varieties (99 and 67 cm) and one indeterminate type of cucumber (220 cm), the results showed significant difference in means of stem length between the two hybrids (315 and 278 cm) respectively. Besides, Awad (1996) also worked on seven cultivars and inbred lines of cucumber and their F<sub>1</sub> hybrids which were obtained by diallel crosses. The results showed highly significant differences among genotypes for all the studied variables and the hybrids were found superior than the means of parents for main stem length. Besides Ghaderi and Lower (1979) evaluated parents, F<sub>1</sub>, F<sub>2</sub> and backcross generations of two cucumber hybrids and found that there

were differences in stem length between different generations, but the better parent in two hybrids had the highest values.

**Genetic components: -**

**Mid parent and better parent heterosis**

Mid parent heterosis for stem length (Table 10 and 11) varied from -22% to 30% when all the two types of heterosis are considered. Desirable positive MP heterosis was observed in 12 F<sub>1</sub> crosses, which value of 3 F<sub>1</sub> crosses which 0 and 6 F<sub>1</sub> crosses were negative. On the same time 0 results observed for better parent heterosis for this trait which varied from -34% to 13%. Desirable positive MP heterosis was observed in 6 F<sub>1</sub> crosses, where value of 2 F<sub>1</sub> crosses were 0 and 13 F<sub>1</sub> crosses were negative.

**Table 10. Number of superior crosses showing significant heterosis.**

	MP			BP		
	-	0	+	-	0	+
<b>Stem length</b>	<b>6</b>	<b>3</b>	<b>12</b>	<b>13</b>	<b>2</b>	<b>6</b>
<b>Fruit length</b>	<b>10</b>	<b>-</b>	<b>11</b>	<b>13</b>	<b>-</b>	<b>8</b>
<b>Fruit weight</b>	<b>1</b>	<b>-</b>	<b>20</b>	<b>8</b>	<b>1</b>	<b>12</b>
<b>No. female flowers/plant</b>	<b>8</b>	<b>-</b>	<b>13</b>	<b>14</b>	<b>3</b>	<b>4</b>
<b>Total yield/plant</b>	<b>3</b>	<b>-</b>	<b>18</b>	<b>8</b>	<b>-</b>	<b>13</b>
<b>Early yield/plant</b>	<b>6</b>	<b>-</b>	<b>15</b>	<b>12</b>	<b>-</b>	<b>9</b>
<b>Leaf area</b>	<b>8</b>	<b>-</b>	<b>13</b>	<b>12</b>	<b>1</b>	<b>8</b>

For fruit length, the extent of variation was from -38% to 16% for all types of heterosis with 11 and 8 crosses showing significantly positive MP and BP heterosis, respectively. However, the important direction of heterosis for this trait either in positive or negative is depending on the breeder's point of view in respect to produce short or long fruit types.

Significant heterosis up to 40% over MP and 34% over BP were recorded for fruit weight. 20 crosses out yielded MP and 12 crosses significantly out yielded BP.

**Table 11. Values of mid parent heterosis (MP) and better parent heterosis % for studied traits.**

Genotypes	Stem length		Fruit length		Fruit weight		No. female flowers/ plant		Total yield/plant		Early yield/plant		Leaf area	
	MP%	BP%	MP%	BP%	MP%	BP%	MP%	BP%	MP%	BP%	MP%	BP%	MP%	BP%
P1 x P2	-19	-19	16	14	6	7	-53	-70	8	-12	-22	-27	-22	-27
P1 x P3	9	-7	11	5	5	-1	25	-9	33	7	72	24	72	24
P1 x P4	22	0	11	7	4	-3	3	-39	-7	-22	34	16	34	16
P1 x P5	-10	-28	9	5	5	-9	-13	-30	49	8	38	3	38	3
P1 x P6	0	-9	13	-1	15	13	-7	-39	13	-26	29	-24	29	-24
P1 x P7	9	7	9	1	24	20	26	15	-9	-11	-5	-16	-5	-16
P2 x P3	-22	-34	-9	-16	-21	-26	-20	-33	5	3	16	-13	16	-13
P2 x P4	-9	-26	11	10	4	-4	25	0	12	9	-1	-9	-1	-9
P2 x P5	8	-14	-5	-6	10	-5	60	20	38	18	23	-4	23	-4
P2 x P6	2	-8	-1	-15	2	0	140	140	37	3	183	71	183	71
P2 x P7	8	7	-16	-25	7	5	60	0	21	-3	-36	-46	-36	-46
P3 x P4	0	-5	6	-2	6	6	-5	-33	42	36	79	43	79	43
P3 x P5	-4	-11	-3	-11	21	11	-14	-25	49	30	87	76	87	76
P3 x P6	0	-7	10	1	31	26	60	33	66	27	102	43	102	43
P3 x P7	22	2	8	4	21	10	9	-25	11	-12	31	-13	31	-13
P4 x P5	11	13	-6	-33	5	-2	54	0	36	14	36	14	36	14
P4 x P6	-1	-12	-18	-30	11	5	88	-25	36	1	6	-34	6	-34
P4 x P7	24	0	7	-4	14	3	30	-25	-23	-37	-20	-38	-20	-38
P5 x P6	30	13	-27	-38	22	7	-27	-45	73	48	141	65	141	65
P5 x P7	20	-4	-1	-11	7	-9	-10	-33	18	-16	18	-19	18	-19
P6 x P7	18	5	-19	-23	40	34	20	-25	74	270	-50	-71	-50	-71

Heterosis of No. female flowers/ plant ranged from -53% to 140% for MP and -70% to 140% for BP. Desirable positive MP heterosis was observed in 13 F<sub>1</sub> crosses, which 8 F<sub>1</sub> crosses exhibited desirable and 4 crosses showed desirable positive BP heterosis which 14 F<sub>1</sub> crosses exhibited desirable for this trait. Also, Heterosis of total yield/plant ranged from -23% to 74% for MP and -37% to 270% for BP.

Desirable positive MP heterosis was observed in 18F<sub>1</sub> crosses, which 3 F<sub>1</sub> crosses exhibited desirable and 13 crosses showed desirable positive BP heterosis which 8 F<sub>1</sub> crosses exhibited desirable for this trait. Same results observed in early yield/plant, Desirable positive MP heterosis was observed in 15 F<sub>1</sub> crosses, which 6 F<sub>1</sub> crosses exhibited desirable and 9

crosses showed desirable positive BP heterosis which 13 F<sub>1</sub> crosses exhibited desirable for this trait.

Mid parent heterosis for Leaf area varied from -16% to 19% when all the two types of heterosis are considered. Desirable positive MP heterosis was observed in 13 F<sub>1</sub> crosses, which value of 8 F<sub>1</sub> crosses were negative. On the same results observed for better parent heterosis for this trait which varied from -27% to 13%. Desirable positive BP heterosis was observed in 8 F<sub>1</sub> crosses, which value of 1 F<sub>1</sub> crosses were 0 and 12 F<sub>1</sub> crosses were negative. Al-Araby (2004), Bairagi *et al* (2002), and Mule *et al* (2012) in cucumber, as well as, Al-Araby *et al* (2019) on cucumber, showed various values of heterosis results for different characters.

### **Potence ratio**

Potence ratio which measure the average degree of dominance over all loci (Table 12 and 13), was found to be less than unity for stem length (11 crosses), fruit length (6 crosses), fruit weight (8 crosses), No. female flowers/ plant (10 crosses), total yield/plant (6 crosses), leaf area (9 crosses) and early yield (10 crosses). This revealing that such traits were controlled by partial dominance. However, no dominance was found in the inheritance of No. female flowers/ plant (one cross). On the other hand, the remainder crosses in all traits exhibited the potence ratio greater than unity, indicating the role of over- dominance in the inheritance of stem length (10 crosses), fruit length (15 crosses), fruit weight (13 crosses), No. female flowers/plant (7 crosses), total yield/plant (15 crosses), leaf area (12 crosses) and early yield (11 crosses). All studied traits were controlled by both complete and partial dominance. These mean that: No. female flowers/plant was controlled by all 3-types of dominance. However, the others studied traits were controlled by only one type or two types namely by partial and over-dominance.

The general performances of the F<sub>1</sub> hybrids reflected the presence of two degrees of dominance effects; that is, complete and partial to over dominance, only no. female flowers/plant had absence of dominance, involved in inheritance of characters. However, partial to over dominance response in inheritance of most traits contributes to the genetic basis of heterosis which agreed well with the observations.

**Table 12. Potence ratio of 21 cucumber hybrids.**

Genotypes	Stem length	Fruit length	Fruit weight	No. female flowers/ plant	Total yield/plant	Early yield/plant	Leaf area
P1 x P2	26.66-	6.79	10.74	1.00-	0.35	3.33-	3.75
P1 x P3	0.52-	2.12	0.79	0.67	1.36	1.85	1.79
P1 x P4	0.98	3.46	0.59	0.04	0.36-	2.15	0.06-
P1 x P5	0.40-	2.37	0.33	0.54-	1.28	1.13	0.38
P1 x P6	0.01-	0.91	8.07	0.13-	0.25	0.42	2.40
P1 x P7	5.15	1.07	8.65	2.71	3.53-	0.38-	6.51
P2 x P3	1.18-	1.23-	3.05-	1.00-	2.53	0.48	4.52-
P2 x P4	0.39-	14.52	0.45	1.00	4.75	0.07-	1.68
P2 x P5	0.30	3.18-	0.62	1.80	2.22	0.84	0.43
P2 x P6	0.20	0.08-	0.92	0	1.14	2.78	0.24-
P2 x P7	7.84	1.53-	3.44	1.00	0.84	1.79-	1.04
P3 x P4	0.06	0.75	7.13	0.11-	9.09	3.14	1.33-
P3 x P5	0.63-	0.33-	2.29	1.00-	3.28	14.33	1.31
P3 x P6	0.06-	1.13	6.90	3.00	2.12	2.46	1.06-
P3 x P7	1.13	2.40	2.25	0.20	0.42	0.62	0.27-
P4 x P5	5.47-	8.58-	0.68	1.00	1.85	1.88	1.23
P4 x P6	0.10-	1.07-	1.96	3.50	1.03	0.11	0.71-
P4 x P7	1.00	0.62	1.37	0.41	1.02-	0.71-	0.37-
P5 x P6	2.03	1.55-	1.62	0.80-	4.38	3.04	0.25
P5 x P7	0.78	0.05-	0.40	0.30-	0.45	0.39	0.34
P6 x P7	1.51	3.48-	8.68	0.33	1.39-	0.66-	1.96

**Table 13. Number of superior crosses showing significant potence.**

	-	0	+
Stem length	10	-	11
Fruit length	10	-	11
Fruit weight	1	-	20
No. female flowers/ plant	7	1	12
Total yield/plant	3	-	18
Early yield/plant	6	-	15
Leaf area	8	-	13



These results in agreement with Abd Rabou (2008) which found the positive potence ratio was (0.65) which indicated partial dominance of fruit weight character towards the heavy fruit parent. Also, Ragab (1984) and Li-Jian *et al* (1995) reported in their studies that the partial dominance case was noticed for the heavy fruit over light. Mid-parent heterosis had positive value (11.9%) but high-parent heterosis had negative value (-5.63%) The results agree with those of Moushumi and Sirohi (2006) for fruit length, fruit diameter, and fruit weight in cucumber despite using dissimilar hybrids evaluated in other environments which means that the results can be considered a general response for the crop. Also, Das *et al* (2019) found that for vine length, they were more than  $\pm 1$ , the ' $\pm$ ' sign indicates the direction of dominance of either parent, for 11 crosses and within  $\pm 1$  in 4 crosses, indicating overdominance and partial dominance, respectively, for inheritance of this trait. And potence ratio of fruit length expressed overdominance in 14 crosses and partial dominance in a single hybrid. In case of fruit diameter, 12 hybrids exhibited overdominance, 3 hybrids showed partial dominance. Overdominance occurred in 11 crosses and partial dominance in 4 hybrids for inheritance of number of fruits per plant.

#### **CONCLUSION**

It could be concluded that P1 and P7 are promising inbred lines due to its high yielding ability, good fruit and vegetative traits. But only P1 is considered the most promising inbred line, due to its resistance to CMV. It would be recommended for further evaluation on a large scale.

## REFERENCES

- Abd El-Aziz, M. H. and H.A. Younes (2019).** Detection of Cucumber mosaic virus in infected cowpea plants (*Vigna unguiculata* L.) from northern Egypt. *Novel Research in Microbiology Journal*.3(2): 326-340.
- Abdelsabour, G. A. K.; W. Ahmed and Y. M. M. Sabry (2015).** Coat protein gene of new isolate of cucumber mosaic virus infecting Banana in Egypt. *Journal of Microbiology, Biotechnology Food Sciences*; (2) 177-181.
- Abd Rabou, A. M. (2008).** Biotechnological Studies on Cucumber Plants. Ph.D. Thesis, Fac. Agric. Cairo University, 141p.
- Al-Araby, A. A. (2004).** Breeding studies on cucumber crop (*Cucumis sativus* L.). M.Sc. Thesis, Fac. Agric., Tanta, Univ., Egypt.
- Al-Araby, A.A. M.E. Ahmed, S.A. Omran and A.M. Abosh (2019).** Heterosis and combining ability in cucumber (*Cucumis sativus* L.) using line × tester analysis. *Egypt. J. Plant Breed.* 23(6):1169– 1194.
- Awad, M. M. W. (1996).** Genetic studies on some economic characters in diallel crosses under high temperature conditions in cucumber (*Cucumis sativus* L.). Ph.D. Thesis, Fac. Agric. Cairo University.
- Bairagi, S. K.; D. K. Singh and H. H. Ram (2002).** Studies on heterosis for yield attributes in cucumber (*Cucumis sativus* L.). *Veg. Sci.* 29:75–77.
- Ben -Chaim A., R.Grube, Lapidot, M. M., Jahn, and I. Paran (2001).** Identification of quantitative trait loci associated with resistance to cucumber mosaic virus in *Capsicum annuum*. *Theor. Appl. Genet.* 102: 1213-1220.
- Clark, M.F. and A.N. Adams (1977).** Characteristics of the microplate method of enzyme linked immune sorbent assay for the detection of plant viruses. *J. of Gen. Virology*, 34: 475-483.
- Das, S. P., Amit Ranjan Mandal, P. K. Maurya, T. Bhattacharjee, S. Banerjee, A. K. Mandal and A. Chattopadhyay (2019).** Genetic control of economic traits and evidence of economic heterosis in crosses involving monoecious cucumber genotypes. *International Journal of Vegetable Science*, 10: 1-22.
- Delaney, D. E. and R. L. Lower (1987).** Generation means analysis of plant characters in cross between two determinate cucumber lines and *Cucumis sativus* var. *hardwickii*. *J. Amer. Soc. Hort. Sci.* 112:707-711.
- Derbalah, A. S. H. and M. M. Elsharkawy (2019).** A new strategy to control Cucumber mosaic virus using fabricated Ni Nanostructures. *Journal of Biotechnology* 306, 134–141.
- Edwardson, J. R. and R. G. Christie (1987).** Viruses infecting forage legumes - Gainesville., No. 1, p. 143–1214.
- Eid, S.A., A.A. Kishtah and A.A. Abu-Zeid (1984).** *Nicotiana glauca* L., A natural host for cucumber mosaic virus. *Agricultural Research Review* Vol.62, No.2 :367-378.

- El-Beshehy, E.K.F. and A.A.A. Sallam (2012).** Partial characterization of an isolate of cucumber mosaic virus from Ismailia Governorate. *International Journal of virology* 8(1):90-97.
- El-Borollosy, A. M. and H. M. A Waziri. (2013).** Molecular characterization of a cucumber mosaic cucumovirus isolated from lettuce in Egypt. *Annals of Agricultural Science* 58(1), 105–109.
- El-Bramawy, M. A.S. A. and E. K. F. El-Beshehy (2011).** The resistance of Bean Yellow Mosaic Virus (BYMV) in faba bean (*Vicia faba* L.) with diallel analysis. *Journal of Biology and Life Science*, 2(1), 1-15.
- El-DougDoug, K.A., A.R. Sofy, G.A. Hameed and R.A. Dawood (2014).** Intraspecific Diversity of Cucumber mosaic Cucumoviridae in Egypt. *International Journal of Virology* 10 (2):.94-102.
- Ene, C.O., P.E. Ogbonna, C.U. Agbo, and U.P. Chukwudi (2019).** Heterosis and combining ability in cucumber (*Cucumis sativus* L.). *Inform. Proc. Agric* 6:150–157.
- Farahat, A.S., A.A. El-Morsi, H.E. Soweha, A.R. Sofy and E.E. Refaey (2018).** Metabolic changes of cucumber plants due to two Cucumber mosaic virus Egyptian isolates. *Arab Univ. Journal of Agricultural, Special Issue*, 26(2C).
- Ghaderi, A. and R.L. Lower (1979).** Gene effects of some vegetative characters of cucumber. *J. Amr. Soc. Hort. Sci.* 104:(141-144).
- Ghai, T.R., S. Jaswinder, S. K. Arora and J. Singh (1998).** Genetic studies for resistance to powdery mildew and Cucumber mosaic virus in summer squash (*Cucurbita pepo* L.). *Punjab-Vegetable-Grower*. 30-34; 5 ref.
- Hagag, S. Z. (2002).** Quantitative determination methods and classification of Cucumber mosaic virus by enzyme linked immunosorbent assay. M.Sc. Thesis, School of Agric. And Biolog. Scie, Osaka Prefecture Univ., Japan. 77pp.
- Hanan, N. El-Din, R. Salem and K. S. Abdalla (2013).** Cloning and characterization of *cucumber mosaic virus* coat protein gene from infected banana plants in Egypt. *Egypt. J. Genet. Cytol.*, 42:151-161.
- Hayes, H.K., and D.F. Jones (1916).** First generation crosses in cucumbers. *Conn. Agr. Exp. Sta. Ann. Rep* 40 (1917):319–322. New Haven, CT.
- Havey, M. J.(1996).** CMV resistance in three sources of cucumber. Report Cucurbit-Genetics Cooperative. No. 19, 32-33.
- Hobbs, H.A., D.V.R. Reddy, R. Rasjeshwari and A.S. Reddy (1987).** Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanutviruses. *plant-disease* 71: 747-749.
- Iqbal, S., M. Ashfaq and H. Shah (2011).** Biological characterization of Pakistani isolates of Cucumber mosaic cucumovirus (CMV). *Pak. J. Bot.*, 43(6): 3041-3047.
- Joseph, O. K. and F. N. Hesham (2002).** Evaluation of broad bean cultivars for resistance to Broad bean mottle virus using a simplified enzyme-linked immunosorbent assay. *Subtropical Plant Science*, 54: 29-33.2002.
- Kuhn, C.W.(1964).** Separation of cowpea virus mixtures. *Pytopathology* 54:739-740.

- Li-Jian, W., J. W. Li and Z. D. Wei (1995).** Genetic Analysis for major Agronomic Characters in Cucumber (*Cucumis sativus* L.). *Acta-Horticulturae*. 402:388-391.
- Megahed, A.A, H. A. El dougdougK, B.A. Othman, L S. M. ashin, Ibrahim, M.A. and A. R. Sofy (2012).** A new Egyptiansatellite strain of Cucumber Mosaic Cucumovirus. *International Journal of Virology* 8(3):240-257.
- Melisa, F. C. and T. C. Wehner (2006).** Cucumber crop information choosing a patio cucumber. <http://cuke.hort.ncsu.edu/cucurbit/wehner/601/hsposter1.ppt>
- Mule, P. N. V., V. A. Khandelwal, D. A. Lodam, P. P. Shinde and A. B. Patil (2012).** Heterosis and combining ability in cucumber (*Cucumis sativus* L.) *Madras Agric. J.* 99 (7-9): 420-423.
- Munshi, A. D., P. Bishwajit and M. Bikash (2008).** Genetics of resistance to Cucumber mosaic virus in *Cucumis sativus* var. hardwickii R. *Alef.Euphytica*. 164:501–507
- Moushumi, S., and P.S. Sirohi (2006).** Gene action of quantitative characters including yield in cucumber (*Cucumis sativus* L.). *Indian J. Hort* 63(3):341–342.
- Nagendran, K., K.K. Panddey, A.B. Rai and B. Singh (2017).** Viruses of vegetable crops: symptomatology, diagnostics and management. *IIVR Technical Bulletin No.75, IIVR, Varanasi*, pp.48.
- Palukatis, P. and G. F. Arenal (2003).** Cucumoviruses. *Adv Virus Res* 62:241–323.
- Paradies, F., M. FinettiSialer, D. Gallitelli, M.A. Castellano, A. Di Franco, M. Digiario, G.P. Martelli, and M.A. Yilmaz (2000).** Partial characterization of cucumber mosaic virus isolates from citrus and grapevine. *Journal of Plant Pathology*, **82** (2), 133-145.
- Ragab, M.A. E. (1984).** Studies on the inheritance of some economic characters in Cucumber (*Cucumis sativus* L.). *Fac. Agric. Mansoura University*. Ph. D. Thesis 141p.
- Rahman, S., A. Akanda, I. H. Mian, K. A. Bhuiyan, and M. Hossain (2016).** New sources of resistance to Cucumber mosaic virus in *Capsicum annuum*. *Journal of Crop Science and Biotechnology*. 19 (3): 249 ~ 258.
- Rakib, A. A. and M. A. Adhab (2012).** Protection of melon plants against Cucumber mosaic virus infection using *Pseudomonas fluorescens* biofertilizer. *African Journal of Biotechnology* Vol. 11(100), pp. 16579-16585.
- Sallam, A.A.A; A. Youssef Sahar, E.K.F. El-Beshehy and M.H.A. Abu-Hatem (2012).** Anatomical and ultrastructural changes in cucumber (*Cucumis sativus* L.)leaves infected with cucumber mosaic virus(CMV). *Agricultural researchJournal, Suez Canal university* vol. 12(2):119-125.
- Sharma, M., P. D. Thakur, D. Gupta and A. K. Thakur (2013).** Identification of viruses and screening of summer squash (*Cucurbita pepo*) germplasm against viral disease under controlled conditions. *Indian Journal of Agricultural Sciences* 83(4): 426–30.
- Singh, H.K., S. Pandey, A. Tiwari, and M.C. Singh (2010).** Heterosis and combining ability for yield and contributing traits in cucumber (*Cucumis sativus* L.). *Veg. Sci* 37(1):64–66.

- Sinha, S. K. and R. Khanna (1975).** Physiological, biochemical and genetic basis of heterosis. *Adv.Agron.* 27: 123-174.
- Smith, H.H. (1952).** Fixing transgressive vigor in *Nicotiana rustica*. Heterosis, Iowa State Coll., Ames, Iowa, EE. UU.161-74.
- Sofy, A. R. and A. M. Soliman (2011).** Molecular identification of a cucumber mosaic virus subgroup I Egyptian isolate from Geranium based on bioinformatics analysis of CP gene sequence. *Egyptian journal virology*,8:178-194.
- Splittstoesser, W.E. (1984).** Vegetable growing handbook Second Edition. Westport, Connecticut: AVI Publishing. p. 209.
- Steel, R. G. D. and J. H. Torrie (1960).** Principles and procedures of statistics with special reference to the Biological Sciences. Mc Graw Hill Book Company. Inc. New York.
- Steven, T., M. G.Kolke, F. Calven, S. Richard and J. Michell (2000).** Plant Disease Management for Organic Crops. Publication Number: 7252 :6 pp.
- Sultana, R., A. M. Akanda, M. A. S. Haque, A. Majumdar and M. A. Z. Al-Munsur (2014).** An investigation to virus like diseases of marigold. *Journal of Bioscience and Agriculture Research.* 18.10.2014, Vol. 02(01): 23-35.
- Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey (1990).** DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, 18, 6531– 6535.
- Zitikaite, I. (2002).** Viruses of cucumber plants and identification of their agents. *Biologija.* No.2,1392-0146.
- Zitikaite, I., J. Staniulis, L. Urbanaviciene and M. Zizyte (2011).** Cucumber mosaic virus identification in pumpkin plants. *Agriculture*, vol. 98, No. 4, p. 421–426.

## دراسات وراثية وتقييم لبعض التراكيب الوراثية من الخيار لمقاومة فيروس موزايك الخيار (CMV) وبعض الصفات الاخرى

أيمن محمد عبد ربه<sup>1</sup>, عبير محمد ابو الوفا<sup>2</sup> و نهلة احمد المغاوري<sup>1</sup>

1. قسم تربية الخضر - معهد بحوث البساتين- مركز البحوث الزراعية- جمهورية مصر العربية

2. قسم الفيروس والفيثوبلازما - معهد بحوث امراض النباتات - مركز البحوث الزراعية- جمهورية مصر العربية

تعد الأمراض الفيروسية مشكلة هامة لإنتاج الخيار في مصر. وتعتبر التربية لمقاومة الأمراض الفيروسية وبخاصة فيروس موزايك الخيار (CMV) احد الأهداف الاساسية التطبيقية لبرامج التربية في الخيار وذلك بهدف تقليل الخسائر الكمية والنوعية للمحصول. و قد تم عزل فيروس موزايك الخيار من نباتات مصابه طبيعيا في مزرعه كليه الزراعة بمحافظة الإسماعيلية وتم تعريف الفيروس بواسطة اختبار الاليزا الغير مباشر باستخدام المصل المضاد لفيروس موزايك الخيار. وتم تنقيه الفيروس علي العوائل المشخصة ودراسة المدى العائلي له. في هذه الدراسة تم الحصول على 10 سلالات مربية تربية داخلية من مصادر مختلفة لاستخدامها في انتاج هجن لمقاومة CMV وتم اختيار 7 سلالات منها بدرجات مقاومة مختلفة للاصابة الفيروسية من خلال تقدير نسبة الاصابة وشده الاصابة لانتاج 21 هجين عن طريق التهجين النصف دائري وتم تقييم السلالات السبعة و21 هجين مع هجينين تجاريين (الصفاء وهادي) للاصابة بفيروس CMV داخل الصوبة الزراعية بمحطة البحوث بالإسماعيلية خلال موسمي (2017/2018 , 2018/2019). تم اجراء تحليل البصمة الوراثية لسلالات الخيار السبعة وذلك لتحديد الاختلافات الوراثية بينها باستخدام تقنية RAPD-PCR وذلك على DNA الذي تم عزله من كل سلالة على حدة وقد تم ايضا تحديد المسافات الوراثية بين السلالات تحت الاختبار. وتم دراسة قوة الهجين و القدرة علي التوريث للصفات البستانية علي الهجن المنتج واطهرت النتائج النهائية للتقييم المرضي و البستاني للسلالات ان P1 و P7 من السلالات الواعدة بسبب القدره المحصوليه العاليه، المواصفات الخضرية والصفات البستانية الجيدة. لكن P1 أظهر مقاومة عالية للفيروس، لذا يمكن استخدامه في برنامج متقدم لإنتاج الهجن ذات الصفات التجارية المقاومة للفيروس .

المجلة المصرية لتربية النبات 24(4): 743-779 (2020)