Evaluation of some cucumber inbred lines and their hybrids for *Cucumber mosaic virus* (CMV) resistance

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ABSTRACT

The major objective of our cucumber breeding program involves breeding for CMV disease resistance to reduce losses in quality and yield. Seven half diallel cross hybrids resistant to CMV were developed at the Vegetables Breeding Dept., Hort. Res. Inst., Egypt during March, 2006. Sixteen genetically different pure inbred lines of Cucumis sativus were used to develop the hybrids obtained from different sources and selected for their CMV disease-resistance trait. The inbred lines and one commercial (Beit alpha) cultivar as a susceptible control were examined to CMV resistance using biological, serological and molecular methods. The CMV isolate identified by Plant Path. Res. Inst. Virus and Phytoplasma Res. Dept. (ARC) was used in mechanical inoculation of all cucumber genotypes used during this study. The seeds of the genotypes were incubated and the seedlings were cultivated in foam trays with peat soil and kept under greenhouse conditions. At the cotyledon stage, i.e. before the development of the first true leaf, the seedlings were mechanically inoculated by rubbing with virus inoculum. Disease severity was assessed visually 7-10 days (on cotyledons) and 14-25 days (on true leaves) after inoculation with CMV. The results revealed that six out of sixteen cucumber inbred lines (Cus 260/1980, 6-5-23-2 Kaha, 1-180-309-18-105 Dokky, 5-57-22-17 Kaha, Cus 38/1991, and 25-2-1-90 Kaha) were found to be without systemic symptoms of CMV infection and proved to be resistant to CMV when tested by DAS-ELISA and RT-PCR. The promising accessions as sources of resistance have been intercrossed with leading commercial type (Beit-alpha) in half diallel system. In order to determine the genetic polymorphism and discriminate between cucumber inbred lines, RAPD-PCR analyses were conducted on the DNA isolated from each line. Dendrograms representing genetic distances were performed on the studied genotypes using the UPGMA (Unweighted Pair Group Method with Arithmetic Average). Twenty one cucumber hybrids obtained from the half diallel crossing between the six resistant genotypes and the local commercial cultivar (Beit-alpha) were subjected to CMV artificial inoculation in a separate greenhouse and symptoms were visually monitored for two months. Only seven cucumber hybrids showed high a level of resistance to CMV were screened in the greenhouse and evaluated for CMV resistance. The resistant hybrids obtained did not develop visual symptoms of CMV infection on cotyledons and true leaves. These resistant lines could serve as potential sources of resistance in breeding programs.

Key words: *Cucumber inbred lines, CMV resistance, RT-PCR, RAPD-PCR, DAS-ELISA, hybrids.*

INTRODUCTION

Cucumber is one of the most important vegetable crops in Egypt. Many viruses affect cucumber and cause mosaic diseases. *Cucumber mosaic virus* (CMV) is the most destructive and widespread disease of cucumber in Egypt and worldwide. Cucumber plants may become infected at any stage of growth, from emergence of the seedling to near maturity (Takanami, 1981). One of the most important components in an integrated disease control program is the selection of cultivars that are resistant to pathogens. Several races of cucumber make it difficult to use single-gene resistance. In some cases, resistance has been identified and incorporated into adapted lines.

Cucumber a member of the Cucurbitaceae family is native of Asia and Africa (Splittstoesser, 1984). In Egypt, the total cultivated area is 84261 and 5887 acres in greenhouses on autumn season with productivity of about 802644 and 25333 ton/acres, respectively Ministry of Agriculture statistics, 2006.

Cucumber mosaic virus (CMV), the type species of the genus Cucumovirus in the family of Bromoviridae is one of the most widespread plant viruses in the world (Chen *et al.*, 2001). CMV is probably the most widely distributed and important virus disease of cucumber (Zitter *et al.*, 1984). CMV is widespread in temperate regions and has an extremely wide host range, infecting plants in approximately 1000 species (Piazzolla *et al.*, 1998 and Francki *et al.*, 1991). CMV is transmitted by aphid vectors in non-persistent manner, a mode characterized by a rapid rate of virus acquisition and delivery, the absence of a latent period, and an increase in transmission efficiency following the pre-acquisition starvation of aphids (Ng and Perry, 2004).

One of the most important components in an integrated disease control program is the selection and planting cultivars 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, resulting in the absence or reduction of symptoms (Kolke *et al.*, 2000). The most effective means possible is to identify useful traits and transfer them through into breeding programs so, our emphasis on producing some resistances against CMV is based on the increasing environmental, economic and consumer concern with chemical control measures, and also reflects the observation that secondary diseases may cause major losses once varieties with limited resistance are released.

In order to determine which plants express CMV resistance, two tests can be used; an ELISA test (Enzyme-Linked ImmunoSorbent-Assay) is a commonly used procedure for the sero-diagnosis of plant viruses (Wei *et al.*, 2001). It can be quickly done in the field using a kit and a small sample of tissue and can give simple positive or negative results. The second test for the presence of the viral genome itself is PCR (Polymerase Chain Reaction). Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is a highly sensitive and specific method useful for the detection of rare transcripts or for the analysis of samples available in limiting amounts (Erlich, 1989 and Carding *et al.*, 1992). RT-PCR test is significant in the evaluation program because it can improve selection methods for resistance by eliminating escapes and asymptomatic genotypes.

Randomly Amplified Polymorphic DNAs (RAPDs), one of the most powerful and reliable methods (Williams *et al.*, 1990) were used to assess the genetic diversity present among cucumber genotypes. Furthermore,

RAPDs have several advantages over isozymes or RFLPs, such as speed, low cost and the use of small amounts of plant material (Curtis *et al.,* 2002). In the current study, (RAPD) technique has been applied to asses the genetic polymorphism and to develop fingerprint for each cucumber cultivar and successfully used in genetic diversity analysis of various cucurbits (Geng *et al.,* 2005). Although very advantageous, this technique is time consuming and expensive, and could not be performed on a large number of lines. All these detection methods are important tools to help the plant breeder to identify the plants for making crosses.

The objective of this study is using different cucumber inbred lines as sources of natural resistance to CMV for a breeding program and to select the top performing cucumber hybrids produced which are CMV resistant with an appropriate size and good fruit quality.

MATERIALS AND METHODS

This study was conducted during the period from 2003 – 2006. The breeding materials used included 16 different genotypes of cucumber (*Cucumis sativus*) (Table 1); 13 of them were collected from (I.M.V.H.P.P.), 3 were collected from Germany GeneBank in addition to Beit alpha variety (Asgrow co., U.S.A.). EL-SAFA and PASANDRA are two commercially available hybrids in the Egyptian market and also were included in the evaluation experiment. Self pollination was carried out between the cucumber inbred lines to produce F_1 hybrids under greenhouse conditions at Kaha, Vegetable Research Farm and Qalubia Egypt. The evaluation of resistance to CMV, using ELISA and RT-PCR methods was carried in the Biotechnology Lab, Improvement of the Main Vegetables and Hybrids Production Project (M.V.H.P.P), Veg. Crops Dept., Hort. Res. Inst., Dokki, Giza.

Virus isolation and identification

CMV isolate was identified on the basis of host range, symptomatology, and modes of transmission, serological tests and molecular methods according to Abdelkader *et al.,* (2006). CMV-Eg isolate was used in mechanical inoculation of the sixteen cucumber inbred lines as following: CMV infected tissues were ground in a sterilized mortar and pestle in 0.05 M Potassium phosphate buffer, pH 7.2 and then passed through a double layer of cheesecloth. Expressed sap was used in mechanical inoculation at the cotyledon stage, i.e. before the first true leaf developement. Inoculated seedlings and water sprayed control were kept under greenhouse conditions in isolated cages. Twenty five days post infection, all cucumber genotypes were inspected by DAS-ELISA and the negative samples were subjected to RT-PCR assay to confirm their negativity to CMV.

DAS-ELISA

ELISA kit supplied by LOEWE Biochemica, GmbH, Germany Cat. No. 07108PC was used for CMV detection in all cucumber genotypes used under current study. DAS-ELISA technique was carried out as described by Clark and Adams (1977) and the O.D. readings were recorded by ELISA reader (Sunrise, TECAN, Austria)

RT-PCR

Total RNA extraction was performed on six cucumber inbred lines which gave negative results by DAS-ELISA (Table 2) by using the protocol recommended by the Tri-reagent RNA kit (Sigma). 5 μg of T-RNAs extracted from cucumber plants were used as templates for cDNA synthesis. First strand cDNA synthesis was initiated with primer CMVCP-1 (5'-CCC CGG ATC CTG GTG GCC TT-3'), complementary to the conserved ultimate 3'-terminal 10 nucleotides of all CMV RNA 3 (coat protein gene). Second strand synthesis

was primed with degenerated primer CMVCP-2 (5'-CCC CGG ATC CAC ATC AYA GTT TTR AGR TTC AAT TC-3'), corresponding to nucleotide 1102 to 1126 in the coat protein gene. The oligonucleotide primers were bought from Metabion GmbH (Lena-Christ-Strasse 44, D-82125 Martinsried/ Germany). Primer annealing reaction mixtures were carried out in 6 μl of 5X first strand buffer (250 mM Tris-HCl pH 8.3, 375 mM KC1, and 15 mM $MgCl_2$), 3 μl of 0.1 M dithiothreitol (DTT), 1 μg of complementary primer (CMVCP-1) and sterile H_2O to a final volume of 30 μl. The reaction mixtures were denatured by heating at 100°C for 5 min and placed on ice to prevent renaturation of the RNA and primer. 20 μl of a cDNA reaction mixtures containing: 4 μl of 5X first strand buffer, 2 μl of 0.1 M DTT, 1 μl of RNAs in (40 units, Promega Corp., Madison, USA), 5 μl of 0.3 M β-mercaptoethanol, 2.5 μl of 10 mM dNTPs (2.5 mM each dGTP, dATP, dTTP, and dCTP), and 1 μl of Moloney murine leukemia virus (200 U/μl) reverse transcriptase (Promega, Corp) were added to each tube. Reactions were mixed briefly, and incubated for 1-1.5 hr at 42°C. Amplifications were performed in thin-walled PCR tubes and contained the following reaction mixture: 2.5 μl of 10X PCR buffer (IX is 10 mM Tris-HCl, pH 8.3, 50 mM KC1, and 0.001% gelatin), 1.5 μl of 25 mM $MgCl_2$ (1.5 mM final concentration), 0.5 μl of 10 mM dNTPs, 0.5 μl each of 50 pm/λ primer (CMVCP-1 & CMVCP-2), 2.5 units of Taq DNA polymerase (ABgene, U.K.) and sterile H_2O was added to a final total volume of 25 μl. 2.5 μl of the cDNA mixtures were added to the PCR reactions and amplified in a DNA thermocycler (Perkin Elmer Cetus, Gene Amp. PCR System 2400) with the following cycling parameters: denaturation at 94°C for 30 sec, primer annealing at 55°C for 30 sec, and extension at 72°C for 45 sec for 35 cycles with a final extension at 72°C for 7 min.

Plant material and DNA extraction

DNA extraction (20 μg) was carried out on the seventeen 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.

Table (1): Sources of cucumber accessions used in the current study.

No.	Genotype	Source
1	5-57-22-17KAHA	(I.M.V.H.P.P)
2	1-180-309-18-105 DOKY	(I.M.V.H.P.P)
3	Cus 260/1980	Gene Bank of Germany (India)
4	1-19-299-2 KAHA	(I.M.V.H.P.P)
5	6-5-23-2 KAHA	(I.M.V.H.P.P)
6	6-7-22-19-52 DOKY	(I.M.V.H.P.P)
7	30-7-22-103 KAHA	(I.M.V.H.P.P)
8	1-18-7-22-18 DOKY	(I.M.V.H.P.P)
9	1-26-27-19 KAHA	(I.M.V.H.P.P)
10	Cus 38/1991	Gene Bank of Germany (Georgia)
11	25-2-1-90 KAHA	(I.M.V.H.P.P)
12	Cus 461/1985	Gene Bank of Germany (China)
13	4-30-22-1 KAHA	(I.M.V.H.P.P)
14	25-2-22-15 KAHA	(I.M.V.H.P.P)
15	30-6 KAHA	(I.M.V.H.P.P)
16	64-10-205-2-87 KAHA	(I.M.V.H.P.P)
17	Beit alpha	Asgrow co. - USA

RAPD-PCR

Six promising parents (Table 2) showed resistances to CMV were selected to discover the molecular marker associated with CMV resistance by using RAPD assay. DNAs amplification were 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 $MgCl_2$, 2.5 unit Taq polymerase (Bio Allaiance), 25 pmoles of decamer primer (Operon Technologies, Alameda, USA) (Table 3) and 20 ng 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-1xTris-acetate-EDTA-ethidium bromide gel electrophoresis in 1×TAE buffer at 120 V. RAPD bands were visualized and photographed on ChemiImager 5500, Alpha Innotech gel documentation system.

Table (2): The cucumber accessions used for fingerprinting.

No	Genotype	code
1	5-57-22-17KAHA	P1
2	1-180-309-18-105 DOKY	P2
3	Cus 260/1980	P3
4	6-5-23-2 KAHA	P4
5	Cus 461/1985	P5
6	25-2-1-90 KAHA	P6
7	Beit alpha	P7

Table (3): Sequences of the RAPD primers used in the present study.

Primer code	Sequence (5`-3`)
AL-12	CCC AGG CTA C
AL-14	TCG CTC CGT T

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. Data analyses were performed using the software UPGMA (Unweighted Pair Group Method with Arithmetic Average). Both monomorphic and polymorphic bands were used to calculate pair-wise genetic similarity among cucumber cultivars using Jaccard's coefficient (Jaccard, 1908) according to the equation:

$$\text{Jaccard's coefficient} = \frac{N_{AB}}{N_{AB} + N_A + N_B}$$

as where N_{AB} is the number of bands shared by samples, N_A the amplified fragments in sample A, and N_B represents fragments in sample B. Coefficients values were then used to create similarity matrices.

Mating design and greenhouse experiment

The experiments were carried out for the F1 progeny of twenty one crosses between the six promising cucumber inbred lines resistant to CMV (Parents). The parents were crossed in half diallel cross design using Griffing's second method (Griffing, 1956; Garretsen and Keuls, 1978; Mądry and Ubysz Borucka,

1982). Fifteen of the crosses were between the six parents themselves, and six crosses occurred between the six parents and the commercial sensitive cultivar (Beit alpha). The crossing scheme is presented in Table 4.

The parents were cultivated in the greenhouse in the summer of 2004. For the main part of the experiment, Plants were grown in rows with an area as 2.5 m long and 1 m wide ridges, seedlings were sown at a distance of 50 cm apart. The plants were raised on their main stem and the other lateral branches were pruned at the bud stage.

All agricultural practices were applied following the recommendation of Ministry of Agriculture and Land Reclamation.

Table (4): Layout of the half diallel cross design based on the Griffing's second method for six cucumber inbred lines.

Cucumber inbred lines	P1	P2	P3	P4	P5	P6
P1						
P2	P1xP2					
P3	P1xP3	P2xP3				
P4	P1xP4	P2xP4	P3xP4			
P5	P1xP5	P2xP5	P3xP5	P4xP5		
P6	P1xP6	P2xP6	P3XP6	P4xP6	P5xP6	
P7 (Beit alpha)	P1xP7	P2xP7	P3xP7	P4xP7	P5xP7	P6xp7

RESULTS AND DISCUSSION

1. Virus isolation and identification

Upon mechanical inoculation of the seventeen cucumber inbred lines with CMV isolate, eleven cucumber genotypes showed mosaic symptoms at the cotyledon stage. A symptom developed later on, included severe systemic mosaic and yellowing on the young leaves, while crinkling and blisters on the older ones (Fig. 1 B). Six out of sixteen cucumber accessions were symptomless and appeared healthy (Fig. 1 A).

Fig. (1) :(A) Symptoms expression on cucumber plants upon mechanical inoculation with cucumber plants showing no symptoms. (B): cucumber plants showing systemic mosaic on the young leaves (arrows), crinkling and blistering on the old leaves.

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P2	P1xP2					
P3	P1xP3	P2xP3				
P4	P1xP4	P2xP4	P3xP4			
P5	P1xP5	P2xP5	P3xP5	P4xP5		
P6	P1xP6	P2xP6	P3XP6	P4xP6	P5xP6	
P7 (Beit alpha)	P1xP7	P2xP7	P3xP7	P4xP7	P5xP7	P6xp7

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Evaluation of resistant cucumber inbred lines by

1- DAS-ELISA

Cucumber plants obtained upon mechanical inoculation during the growing season 2005 were evaluated for CMV disease severity by using DAS-ELISA (Table 5) and Fig. (2). Among 17 cultivated cucumber genotypes only six genotypes, 5-57-22-17 KAHA, 1-180-309-18-105 DOKKY, CUS 260/1980, 6-5-23-2 KAHA, CUS 461/1985 and 25-2-1-90 KAHA were found to be negative to CMV and considered phenotypically resistant. These genotypes were completely symptomless during mechanical inoculation with CMV and confirmed negative when tested by RT-PCR (Fig. 3).

Table (5): Detection of CMV infection in cucumber parental genotypes using DAS-ELISA during March, 2004 .O.D. readings after one hr incubation with pNpp substrate. Readings greater than twice the A_{405} of healthy control considered positive (+ve) and reading below it considered negative (-ve).

No.	Genotypes	O.D. readings	Results
1	5-57-22-17KAHA	0.120	-ve
2	1-180-309-18-105 DOKKY	0.139	-ve
3	Cus 260/1980	0.131	-ve
4	1-19-299-2 KAHA	0.507	+ve
5	6-5-23-2 KAHA	0.140	-ve
6	6-7-22-19-52 DOKKY	0.513	+ve
7	30-7-22-103 KAHA	0.675	+ve
8	1-18-7-22-18 DOKKY	0.546	+ve
9	Cus 38/1991	0.516	+ve
10	1-26-27-19 KAHA	0.489	+ve
11	Cus 461/1985	0.138	-ve
12	25-2-1-90 KAHA	0.140	-ve
13	4-30-22-1 KAHA	0.552	+ve
14	25-2-22-15 KAHA	0.906	+ve
15	30-6 KAHA	0.606	+ve
16	64-10-205-2-87 KAHA	0.459	+ve
17	Beit alpha	0.606	+ve

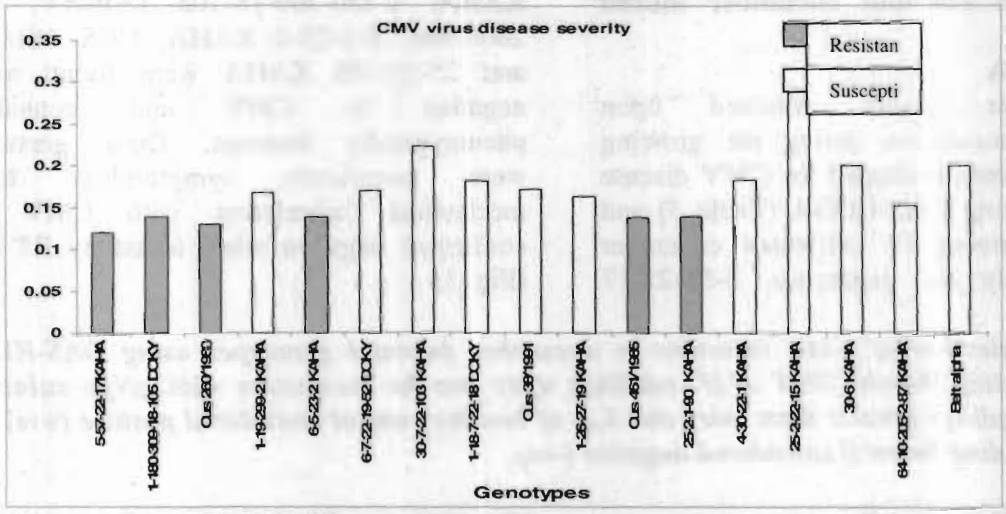

Fig. (2):O.D. readings of DAS-ELISA of seventeen cucumber genotypes up on mechanical inoculation with CMV. Six genotypes ids: (5-57-22-17KAHA, 5-57-22-17KAHA ,Cus 260/198, 6-5-23-2 KAHA ,Cus 461/1985. and 25-2-1-90 KAHA) were found to be negative.

2-RT-PCR

The six cucumber genotypes which gave negative results by DAS-ELISA were confirmed to be negative also by RT-PCR in comparison with the commercial variety Beit alpha. Fig. (3) showed that no amplified products appeared in the six tested cucumber lines while a band of molecular weight 650 bp appeared with the commercial variety Beit alpha indicating that this cultivar is susceptible to CMV infection and the other six inbred lines were resistant to CMV. These resistant lines may lead to the development of cucumber cultivars with improved CMV resistance.

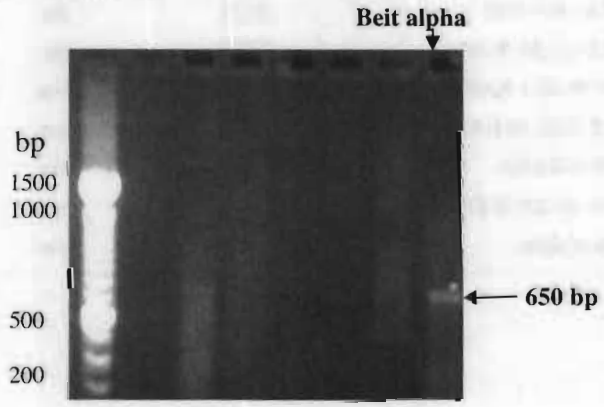

Fig. (3): Agarose gel electrophoresis showing the RT-PCR product amplified from total RNAs extracted from six cucumber inbred lines (P1 to P6) and Beit alpha cultivar (P7). The arrow points to the amplified PCR product of expected size 650 bp of the CMV/CP gene using the two specific primers CMVCP1 and CMVCP2. M: Molecular weight DNA marker (100 bp ladder).

RAPD-PCR

RAPD-PCR was used to evaluate the genetic diversity between the seven cucumber genotypes (6 resistant inbred lines and one commercial cultivar; Beit alpha) using two RAPD primers AL-14 and AL-12. Out of five RAPD primers used only two successfully generated both polymorphic and monomorphic banding patterns. A total of twenty six fragments detected between the seven cucumber genotypes; three of them were monomorphic and twenty two were polymorphic with an average of 88.4 % polymorphism (Table 7). Level of polymorphism varied from one primer to another (Geng *et al.*, 2005). The two RAPD primers (AL-12 and AL-14) showed high level of polymorphism (93 % and 83% respectively). The resulted amplified fragments are shown in Fig. (4 A &B) and Tables (6&7). Although a high degree of polymorphism was revealed by using RAPD primers, it failed in generating molecular markers related to either resistance or susceptibility of CMV disease in cucumber. Cluster analysis classified the seven genotypes into two main sublusters (Fig. 4B). The first main subcluster consisted of (P6 and P7) which is divided into one subcluster (P3), while the second main subcluster is consisted of (P2 and P4) which is devided into two sub-clusters (P1 & P5). The lowest genetic similarity (0.20) were observed between P7 and each of the parents while the highest genetic similarity index was scored between the two parents P2&P4 (0.90) followed by P3 &P6 (0.77). Primer AL-14 revealed fragments of 1200, 1100 and 500 bp which were present only in the DNA fragments of 5 resistant genotypes (P1, P2, P3, P4 and P5) and also revealed fragment of 700 bp which was present only in the DNA of the susceptible genotype (P7). A band of 800 bp was generated in all cucumber genotypes. When the cucumber genotypes were tested against primer AL-12, a band of 600 bp was generated, this fragment could be considered a negative marker for CMV resistance in all cucumber genotypes except the P7, the susceptible variety.

Molecular markers can help in selecting the tolerant/susceptible genotypes. For this purpose RAPD-PCR was conducted to descriminate between the studied genotypes RAPD-PCR was successfully used in fingerprinting the resistant cucumber genotypes and the Beit-alpha genotype and revealed high degree of polymorphism It is preferable to isolate and characterize more DNA markers in cucumber for more powrfull genomic studies such as gene mapping and marker – assisted selection.

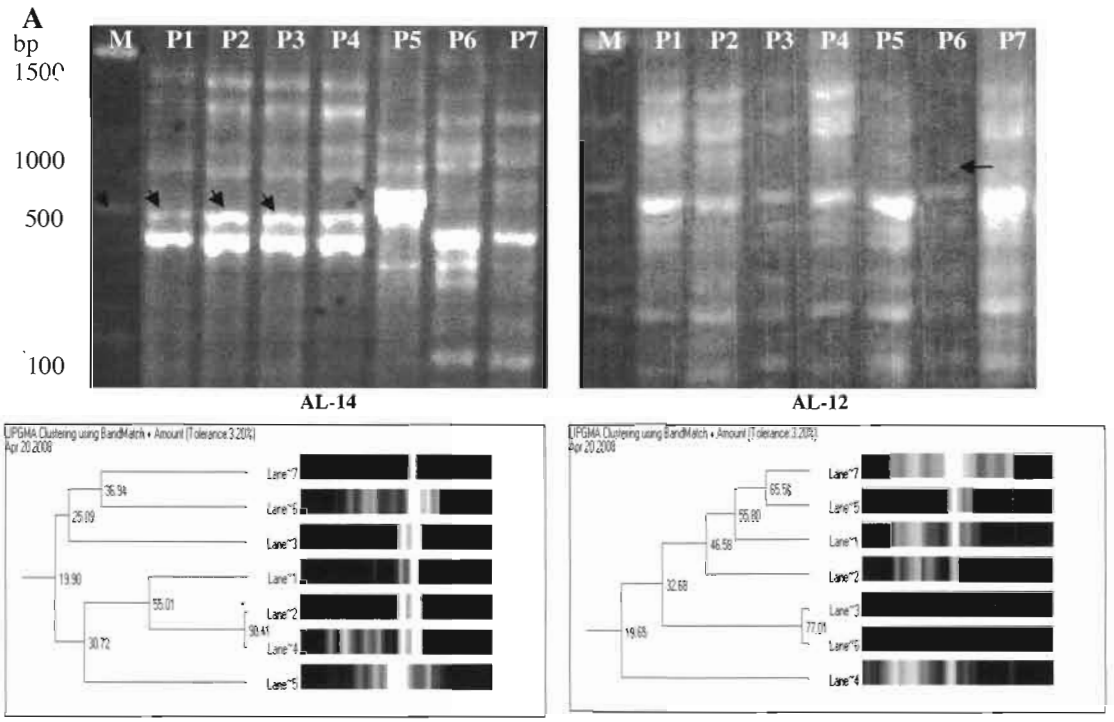

Fig. (4). (A): Photographs presenting RAPD-PCR products obtained from 6 cucumber inbred lines (L1 to L6) and the commercial variety Beit alpha (L7) using two RAPD primers (AL-14 & AL-12). (B): Dendrograms of the genetic distances among the seven cucumber parent genotypes based on RAPD-PCR analysis.

3.3.1 Evaluation of F1 hybrids for CMV resistance under greenhouse conditions

The results showed that seven cucumber hybrids (P1XP2, P1XP4, P1XP5, P1XP6, P2XP5, P2XP6, and P4XP6) which came from the parents (P1, P2 and P4) were screened in the greenhouse and evaluated for CMV resistance. These hybrids exhibited improved CMV resistance in greenhouse and by DAS-ELISA (Table 8 and Fig. 5). On the other hand, the commercial hybrids (EL-SAFA and PASANDRA) were found to be highly susceptible to CMV infection as all hybrids which contain the line P7.These results were in agreement with (Havey, 1996) who evaluated three cucumber lines TMG1, SMR18, and Marcetmar 76 to CMV resistance and found that TMG1 line had the highest resistance than the two other lines (SMR18 and Marcetmar 76), and after crossing the TMG1 with ST8-5 line (susceptible) he found that the (ST8-5xTMG1) hybrid was resistant to CMV, but the other hybrids were susceptible. Our results were also in accordance with the data obtained by Ghai *et al.*, (1998) who evaluated 14 cucurbit lines to CMV resistance and found that P2 and P3 was the best male parents to CMV resistance and P11 was the best female parent to CMV resistance. After crossing them, he found that the best hybrids resistant to CMV were P6xP4 then P11xP3.

Table (6): Amplification patterns among the seven cucumber genotypes using two RAPD primers

Primer	Amplicon size (bp)	Presence or absence of bands P1	P2	P3	P4	P5	P6	P7	NO. polymorphic bands
	1300	1	1	1	1	1	0	0	
	1200	0	0	0	0	1	0	0	
	1100	1	1	1	1	0	1	1	
	1000	0	0	0	0	0	1	1	
	900	0	1	0	1	1	1	1	
	800	1	1	1	1	1	1	1	
AL-14	700	0	0	0	0	0	1	1	13
	600	1	1	0	1	1	1	1	
	500	1	1	1	1	1	0	0	
	400	1	1	1	1	0	1	1	
	300	0	0	1	0	1	1	1	
	200	0	0	0	0	0	1	1	
	100	0	0	0	0	1	1	1	
	1500	1	0	0	0	0	0	0	
	1300	1	0	0	0	0	0	0	
	1200	0	0	1	1	1	0	0	
	1100	1	1	0	0	0	0	1	
	1000	0	0	1	1	1	0	0	
	800	1	1	0	0	1	0	0	
AL-12	700	0	1	0	1	1	0	0	10
	600	0	0	0	0	0	0	1	
	500	0	0	0	0	0	1	0	
	400	1	1	1	1	1	1	1	
	300	0	1	1	1	0	1	1	
	200	1	1	0	1	1	1	1	
	100	1	1	1	1	1	1	1	

Table (7): Total number, monomorphic and polymorphic amplicons as revealed by RAPD primers among seven cucumber genotypes.

Primer	Total number of amplicons	Polymorphic amplicons	Monomorphic mplicons	% of polymorphism
AL-14	**14**	**13**	**1**	**93 %**
AL-12	**12**	**10**	**2**	**83 %**
Total	**26**	**23**	**3**	**88.4 %**

Table (8): Evaluation of CMV resistant cucumber hybrids by DAS-ELISA during September (2005).

	Cucumber genotypes	O.D readings	Results
1	P1	0.139	-ve
2	P2	0.138	-ve
3	P3	0.141	-ve
4	P4	0.139	-ve
5	P5	0.143	-ve
6	P6	0.137	-ve
7	P7	0.867	+ve
8	P1XP2	0.129	-ve
9	P1XP3	0.603	+ve
10	P1XP4	0.142	-ve
11	P1XP5	0.139	-ve
12	P1XP6	0.120	-ve
13	P1XP7	0.528	+ve
14	P2XP3	0.519	+ve
15	P2XP4	0.576	+ve
16	P2XP5	0.138	-ve
17	P2XP6	0.124	-ve
18	P2XP7	0.519	+ve
19	P3XP4	0.576	+ve
20	P3XP5	0.453	+ve
21	P3XP6	0.591	+ve
22	P3XP7	0.594	+ve
23	P4XP5	0.447	+ve
24	P4XP6	0.137	--ve
25	P4XP7	0.603	+ve
26	P5XP6	0.450	-+ve
27	P5XP7	0.516	+ve
28	P6XP7	0.552	+ve
29	EL-SAFA	0.630	+ve
30	PASANDRA	0.609	+ve

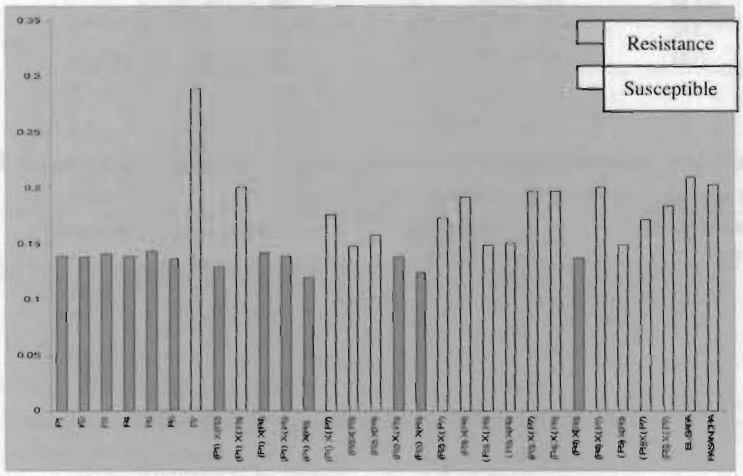

Fig. (5): O.D. readings of DAS-ELISA of twenty one cucumber genotypes in field spread tests for CMV resistance. Eight cucumber hybrids: (P1X2, P1X4, P1X5, P1X6, P2X5, P2X6, and P4X6) were identified with resistance to cucumber mosaic virus (CMV). The commercial hybrids (EL-SAFA and PASANDRA) were highly susceptible to CMV.

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الملخص العربي

التقييم المرضى لبعض سلالات وهجن الخيار ومدى مقاومتها لفيروس موزايك الخيار باستخدام التقنيات الجزيئية

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**** قسم بحوث الفيروس و الفيتوبلازما- معهد بحوث أمراض النباتات- مركز البحوث الزراعية- جمهورية مصر العربية

تعتبر التربية لمقاومة الأمراض الفيروسية وبخاصة فيروس موزايك الخيار احد الأهداف الاساسية التطبيقية لبرامج التربية في الخيار وذلك بهدف تقليل الخسائر الكمية و النوعية للمحصول. وقد تم انتاج 7 هجن خيار مقاومة لفيروس موزايك الخيار خلال موسم مارس 2006 بقسم بحوث تربية الخضر – معهد بحوث البساتين عن طريق ال half diallel cross. وفى هذه الدراسة تم الحصول على 16 سلالة مرباة تربية داخلية من مصادر مختلفة لاستخدامها فى انتاج هجن لمقاومة للفيروس. وقد تم اختبار مدى مقاومة هذه السلالات لفيروس موزايك الخيار ومقارنتها بالصنف التجارى الحساس بيتا الفا باستخدام الطرق البيولوجية و السيرولوجية و الجزيئية. ولقد تم الحصول على عزلة فيروس موزايك الخيار معرفة من قسم بحوث الفيروس و الفيتوبلازما - معهد بحوث امراض النباتات لاستخدامها فى عمل العدوى الميكانيكية لأصناف الخيار المستخدمة فى هذه الدراسة.
تم زراعة بذور اصناف الخيار داخل صناديق من الفوم محتوية على بيئة زراعية صناعية (بيتموس) ووضعت تحت ظروف الصوبة. وقد تمت العدوي الفيروسية ميكانيكيا للنباتات في مرحلة الأوراق الفلقية (قبل ظهور الأوراق الحقيقية) وقد تم تحديد شدة الاصابة المرضية عن طريق ملاحظة الاعراض مظهريا بعد 7-10 ايام علي الأوراق الفلقية و بعد 14 – 18 يوم علي الأوراق الحقيقية من بداية العدوي بالفيروس. اظهرت النتائج ان هناك 6 سلالات من الستة عشر سلالة المختبرة لم تظهر عليها اى اعراض اصابة جهازية بعد العدوى بفيروس موزايك الخيار وقد تم التأكد من انها مقاومة للفيروس عند اختبارها باختبارات الاليزا و تفاعل البلمرة المتسلسل وهذه السلالات هى:
25-2-1-90 KAHA. Cus 461/1985 , 6-5-23-2 KAHA, Cus 260/1980, 5-57-22-17KAHA , 5-57-22-17KAHA
وقد تم عمل تهجين بين سلالات الخيار الستة المقاومة للفيروس فيما بينها وبين الصنف التجارى الحساس بيتا الفا باستخدام التزاوج (half diallel mating) للحصول على الهجن.وقد تم اجراء تحليل البصمة الوراثية لسلالات الخيار الستة المقاومة للفيروس بالإضافة الي صنف بيتا الفا الحساس وذلك لتحديد الإختلافات الوراثية بينها باستخدام تقنية RAPD-PCR وذلك على ال DNA الذى تم عزله من كل سلالة على حده وقد تم ايضا تحديد المسافات الوراثية بين السلالات تحت الاختبار واستنتاج شجرة التقارب الوراثى Dendrograms باستخدام البرنامج المتخصص (UPGMA) .تم الحصول على احدي وعشرين هجينا من خلال التهجينات التى تمت فيما بين السلالات الستة المقاومة للفيروس وبين الصنف التجارى بيتا الفا ,ثم تم عمل عدوي صناعية لهذه الهجن بفيروس موزايك الخيار فى صوبة منفصلة وتم متابعة الاعراض المظهرية يوميا و لمدة شهرين. تم الحصول على 7 هجن فقط اظهرت مستوي عاليا من المقاومة الفيروسية تحت ظروف الصوبة بعد تقييمها للتأكد من مقاومتها لفيروس موزايك الخيار حيث لم يتم ظهور أي اعراض للإصابة المرضية على الاوراق الفلقية او الحقيقية مما يجعل هذه السلالات من المصادر الواعدة المقاومة للفيروس والتى يمكن استخدامها في برامج التربية.