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الدكتوراة

الدرجة:

عنوان الرسالة: دراسات بيوتكنولوجية علي نباتات الخيار  
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قسم: الخضر فرع: تاريخ منح الدرجة: ٢١ / ٨ / ٢٠٠٨

### المستخلص العربي

الهدف الرئيسي لهذا البحث دراسة مقاومة بعض سلالات الخيار للفيروس (CMV) وانتاج بعض الهجن المناسبة تحت ظروف الصوب في مصر ودراسة بعض صفاتها بستانيا ووراثيا ولهذا الغرض أجريت التجارب في محطة بحوث الخضر بقها- محافظة القليوبية في الفترة من ٢٠٠٣ وحتى ٢٠٠٦.

وفى هذه الدراسة تم الحصول على ١٦ 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 وقد تمت العدوي الفيروسيه ميكانيكيا للنباتات في مرحلة الأوراق الفلجية وقد تم تحديد شدة الإصابة المرضية عن طريق ملاحظة الاعراض مظهرها بعد ٧-١٠ ايام على الأوراق الفلجية و بعد ١٤ - ١٨ يوم على الأوراق الحقيقية من بداية العدوي بالفيروس. اظهرت النتائج ان هناك ٦ 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 هي:

٦- ٢٦٠/١٩٨٠، Cus DOKKY، ١٠٥-١٨-٣٠٩-١٨٠-١-١٧KAHA، ٥٧-٢٢-٥-٥٧ (٥-٢٣-٢ KAHA، Cus ٤٦١/١٩٨٥. and ٢٥-٢-١-٩٠ KAHA)

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 DNA الذى تم عزله من كل سلالة على حدة وقد تم ايضا تحديد المسافات الوراثية بين السلالات تحت الاختبار واستنتاج شجرة التقارب الوراثي Dendrograms باستخدام البرنامج المتخصص (UPGMA).

تم الحصول على احدى وعشرون 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 ٧ هجن فقط اظهرت مستوي عالي من المقاومة الفيروسيه تحت ظروف الصوبة بعد تقييمها للتأكد من مقاومتها لفيروس موزايك الخيار حيث لم يتم ظهور أي اعراض للإصابة المرضية على الاوراق الفلجية او الحقيقية. وتم كذلك تقييم لبعض الصفات الاقتصادية البستانية في السلالات والهجن وكذلك اجراء دراسة وراثية لبعض هذه الصفات. وقد تم التوصل الي الهجين P١XP٢ الذي يعتبر مبشرا للزراعة تحت الصوب البلاستيكية بعد مزيد من الاختبارات الموسعة.

الكلمات الدالة: الخيار - الهجن - فيروس CMV - درجة التوريث - طول الساق - طول الثمرة - عدد الأزهار الموثثة علي العقدة - محصول النبات الواحد.

## SUMMARY

This study was conducted during the period from 2003 to 2006. The breeding materials used included 17 different genotypes of cucumber (*Cucumis sativus*, L.). 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 to obtain cucumber inbred lines which were used to produce F<sub>1</sub> hybrids. The work was carried out under greenhouse conditions at Kaha, Vegetable Research Farm, Qalubia governorate Egypt. The evaluation of resistance to CMV, using ELISA and RT-PCR methods was carried out at biotechnology Lab, Improvement of the Main Vegetables and Hybrids Production Project (I.M.V.H.P.P), Veg. Res., Dept., Hort. Res. Inst., Dokki, Giza.

### 1. Genotypes evaluation

#### b. Evaluation for CMV

The sixteen inbred lines of cucumber in addition to one commercial (Beit-Alpha) cultivar as a susceptible genotype were examined to CMV resistance by using biological, serological and molecular methods. The CMV isolate identified by Virus and Phytoplasma Res. Dept., Plant Path. Res. Inst., (ARC), was used for mechanical inoculation to all cucumber genotypes included in this study. The seeds of genotypes were incubated and the seedlings were cultivated in transplanting trays with mixture of peat moss, vermiculite, macro and micro elements which were kept under greenhouse conditions. At the cotyledon stage, the seedlings were mechanically inoculated by rubbing with virus inoculums. Disease severity was assessed visually at 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 were used as sources of resistance and crossed with the commercial type (Beit-Alpha) in a half di-allele mating. In order to determine the genetic polymorphism and discriminate among cucumber inbred lines, RAPD-PCR analysis was conducted on the DNAs isolated from each line. Dendrograms representing genetic distances were performed on the studied genotypes using the UPGMA (Unweighted Pair Group Method with Arithmetic Average).

### **b. Horticultural evaluation**

Seventeen cucumber genotypes were evaluated in a greenhouse during the two seasons of 2003 and 2004 to study their performance.

1-Three genotypes, i.e. 3, 7 and 9, gave the longest stem in both seasons with significant differences compared with the other genotypes of this study.

2-The result showed that line 3 gave significantly the longest fruit compared with the other genotypes in both years.

3-Fruit diameter was significantly the greatest in lines 3 and 12 compared with the other genotypes in both years.

4- Line (3) gave significantly the heaviest fruit weight in both seasons of study.

5-The lines 1, 4 and 15 had significantly the highest number of female flowers per node without significant differences among them in the two seasons. On other hand, the results indicated that lines 3 and 11 had the lowest number of female flowers per node in both seasons.

6-Fruits of lines 9 and 11 had bitter taste in the two seasons.

7-Lines 3 and 11 gave white spines but line 9 gave black spines on the fruits in the two seasons.

## **2. Evaluation of cucumber hybrids**

### **a. Evaluation of F<sub>1</sub> hybrids for CMV resistance**

Twenty one cucumber hybrids obtained from the half di-allele crossing between six resistant genotypes and 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 out of the twenty cucumber hybrids showed high level of resistance to CMV after screening in the greenhouse and evaluation for CMV resistance. The resistant hybrids obtained did not develop visual symptoms of CMV infection on cotyledons or true leaves. These resistant lines could serve as potential sources of resistance in breeding programs.

#### **b. Evaluation of cucumber hybrids for horticulture characters**

This study was conducted in a greenhouse during the two seasons of 2005 and 2006. Thirty cucumber genotypes i.e., 7 parents, 21 hybrids and 2 commercial hybrids (EL-Safa and Pasandra) were evaluated as follows.

1. Parent ( $P_3$ ) gave the longest stem in both seasons. The hybrids which included  $P_3$  gave significantly long stems compared with the other hybrids in the two seasons without significant differences with commercial hybrid (Pasandra) and another 3 hybrids in the first season. On the contrary,  $P_7$  had the shortest main stem in the two seasons with significant differences compared with other parents.

2. The  $P_3$  exhibited the greatest fruit length in the two seasons. The results indicated that hybrid ( $P_3$ ) X ( $P_6$ ) gave the highest fruit length with significant differences compared with other hybrids in the two seasons of study.

3. Parents  $P_6$  had significantly the highest fruit diameter in both seasons. The results showed also that the hybrids ( $P_1$ ) X ( $P_6$ ) and ( $P_3$ ) X ( $P_6$ ) gave the highest fruit diameter with significant differences with other hybrids in the two seasons.

4. Parent  $P_3$  gave the highest the fruit weight in the two seasons. The results pointed out that the hybrid ( $P_1$ ) X ( $P_3$ ), ( $P_3$ ) X ( $P_6$ ) and ( $P_5$ ) X ( $P_6$ ) had significantly the highest fruit weight compared with the other hybrids in the two seasons.

5. Parent  $P_1$  had significantly the highest number of female flowers per node in the two seasons. The results showed that hybrid ( $P_1$ ) X ( $P_2$ ) had

significantly the highest number of female flowers per node compared with the other hybrids in the two seasons.

6. Parent P<sub>1</sub> had significantly the highest early yield / plant in the two seasons. The results indicated also that the hybrid (P<sub>1</sub>) X (P<sub>2</sub>) gave the highest early yield / plant with significant differences with the other hybrids in the two seasons.

7. Parent P<sub>4</sub> had significantly the highest total yield / plant in the two seasons. The results showed also that the hybrid (P<sub>1</sub>) X (P<sub>2</sub>) gave the highest total yield / plant with significant differences compared with the other hybrids in both seasons.

8. Fruit of P<sub>5</sub> had bitter taste in the two seasons. Also, the fruits of six hybrids which included P<sub>5</sub> had bitter taste.

9. Parents P<sub>3</sub> and P<sub>5</sub> had white spines on the fruits in the two seasons. On the contrary, the fruits of the other lines were smooth. All hybrids which contained P<sub>3</sub> or P<sub>5</sub> had white spines on the fruits in both seasons.

### **3. Genetical studies**

1. Cucumber main stem length was studied genetically using the cross (P<sub>3</sub> × P<sub>7</sub>). Mid-parent heterosis and high-parent heterosis had values ranged between 30.6% and 8.1%, respectively. Over dominance of main stem length character towards the high parent was obtained. This character was controlled by one gene. Broad and narrow sense heritability were 80.4% and 70.4%, respectively.

2. Fruit length was studied using the cross (P<sub>1</sub> × P<sub>4</sub>). Mid-parent heterosis and high-parent heterosis had negative values (-5% and -15.9%, respectively). The results indicated that partial dominance of fruit length character towards the short parent and controlled by one gene. Broad and narrow sense heritability were 80.5% and 75.04% respectively.

3. Fruit diameter was studied in the cross (P<sub>1</sub> × P<sub>4</sub>). Mid-parent heterosis had positive value (0.5%) but high-parent heterosis had negative value (-8.9%). The results indicated partial dominance of fruit diameter character towards the high parent was recorded. This character was found to be

4. Fruit weight was studied using the cross ( $P_1 \times P_4$ ). Mid-parent heterosis had positive value (11.9%) but high-parent heterosis had negative value (-5.63%). The positive potence ratio (P) with value (0.65) indicated partial dominance of fruit weight character towards the heavy fruit parent and this character was controlled by three genes. Broad sense heritability estimates were moderate (68%) whereas narrow those of sense heritability were low (32.8%).

5. Number of female flowers per node was studied in the cross ( $P_1 \times P_5$ ). Mid-parent heterosis had positive value (0.0%) but high-parent heterosis had negative value (-45.6%). The potence ratio (P) with value (0.0) indicated no dominance was observed and nine genes were found to be effective in the performance of this character. Broad and narrow sense heritability were 92.63% and 91.7%, respectively.

6. Total yield character was studied in the cross ( $P_1 \times P_5$ ). Mid-parent and better parent heterosis values were 97.8% and 19%, respectively. Potence ratio (P) gave the value 1.5 indicating over dominance of total yield per plant towards the high parent and this character was controlled by poly gene (25 genes). Broad sense heritability estimates were moderately high (78.5%) for fruit weight whereas narrow those of sense heritability were moderate (55.9.8%).

7. Bitter fruit taste character was studied using the cross ( $P_1 \times P_5$ ) and found that this character was dominant and controlled by one pair of genes.

8. The spines produced on the fruit character was studied using the hybrid ( $P_1 \times P_5$ ) and found that smooth fruit was dominant and this character is singly inherited.