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261-0240-97	איתור סמנים מולקולריים האחוזים לגנים המקנים עמידות:

תקציר הדו"ח:

אחד הגורמים העקריים המגבילים יצוד חמניות לפצוח בישראל הוא עלקת החמנית. צמח שפיל המסוגל לגרום לאובדן מוחלט של היבול. הוא העיקרי המגודל בארץ הינו ד"י 3 והוא רגיש לעלקת. בין זה נמצאו צמחים בודדים עמידים שמקורם בכל הנראה בהכלאות עם החניות עמו עמידות במהלך ההטבחה. במרבו ובלבני נעשית עבודת הטבחה ליצירת זני חמניות עמידים לפצוח אולם תורשת העמידות אינה ידועה.

מטרות המחקר היו: יצירת אובלוסיות מתפצלות לעמידות טיאפטרו בדיקת תורשת העמידות ומציאת סמנים מולקולרים האחוזים לגנים המקנים את העמידות. בדיקת ההתפצלות בדורות BC1, F2 הראתה שהעמידות לעלקת בחמנית לפצוח מבוקרת ע"י גן יחיד דומיננטי. נסרקו 300 סמני RAPD בין באלקים של DNA שנוצרו מצמחים עמידים ורגישים מהאובלוסיות המתפצלות. אולם עד כה לא נמצא שום פולימורפי. כמו כן, נסרקו סמני AFLP (15 פריימרים) בין שני קוי הורים קרובים הנבדלים בעמידותם.

עד כה נמצאו 3 בנדים פולימורפים בין שני הקוים. בנדים אלו ממופים בעת באובלוסיה המתפצלת לבדיקת התאמיות שלהם לגן לעמידות. בכוונתנו להמשיך ולסרוק סמנים נוספים של AFLP, RAPD למציאת שונות בין הבאלקים וקוי ההורים ולמפות סמנים פולימורפים ביחס לגן לעמידות.

Final report

Identification of molecular markers associated with genes conferring resistance to broomrape in sunflower (261-0240-95).

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Background

Confectionery sunflower has become an important crop in Israel due to its low water consumption and high value of the seeds. About 200,000 dunams of this crop are produced yearly in Israel. Sunflower broomrape (*Orobanche cumana*) is regarded as the most limiting factor to sunflower production in Israel as well as in many other countries in the Mediterranean region and in southeastern Europe. Production loss can reach 50% and even total loss of yield in extreme infestations.

Because of great difficulties in chemical eradication of broomrape, and costly effective treatments such as soil solarization, breeding for resistance remains the most promising approach to control the parasite. Resistance to broomrape was first identified in sunflower many years ago, but the appearance of new virulent races of broomrape required a continuos search for new resistance sources. All resistant sources reported todate were oil-type cultivars. Most

inheritance studies indicated that the resistance to broomrape in sunflower is controlled by a single dominant gene. For most resistant sources, allelism tests were not reported, therefore, it is not known how many different genes are present in sunflower. Recently, resistance to *Orobanche cumana* was identified in local populations of confectionery sunflower in Israel and it is being incorporated into elite cultivars of sunflower.

The aim of this proposal was to determine the inheritance of the resistance to broomrape in confectionery sunflower and to identify molecular markers linked to the resistance gene(s) and to use these markers for marker-assisted breeding.

Description of the results

A breeding confectionery resistant line 14-7 was crossed to a susceptible male sterile line A-62. The F1 was crossed with another susceptible line BD-4 to construct a test cross population and was sibmated to construct an F2 population. Both populations (a total of 140 plants) were planted in heavily infected soil of *Orobanche cumana* in Bet Dagan. Each plant was harvested for DNA extraction and was scored for resistance/susceptibility to broomrape. Resistance was determined if no emerged broomrape shoots were observed at maturity. Susceptibility was determined if plants were infected by broomrape. Susceptibility is also manifested by an inhibited growth of the plant. Ninety two percent (341/370 plants) of the controlled susceptible plants were infected. Therefore, the infection was considered highly efficient. Our data indicated that a single dominant gene controls the resistance in line 14-7 (97 resistant and 36

susceptible plants in the F2 population; Chi square for 3:1 segregation was 0.36, 0.5>P<0.75). Out of 178 F1 plants, six were infected by broomrape but this infestation appeared late in the growing season. An interesting observation was that F1 progeny from a cross between two susceptible plants exhibited a normal growth although they were infected by broomrape.

Screening molecular markers for linkage with the resistance gene was initiated by using the bulked segregant analysis approach. Two bulks of DNA were made, each from 15 resistant and susceptible plants. The two bulks were screened with 300 RAPD primers, however, we did not detect polymorphism between the bulks with any of these primers. On average, each primer amplified eight fragments, therefore, over 2000 loci were screened for polymorphism between the bulks. Our inability to detect polymorphism can result from several reasons. One is a lack of sufficient polymorphism between the parents involved in the cross used to make the segregating population. The original cross was made between a resistant line and a distantly related male sterile line A62. However, the F1 of this cross was sterile and therefore it could not be crossed again to A62. The F1 was therefore crossed with another susceptible line BD4 that is much closely related with the resistant line. This constrain decreased the level of variation in the cross and decreased the chance to identify polymorphism. A second factor that may have affected our ability to find polymorphism was that it is possible that some plants in the resistant bulk were in fact susceptible but they escaped the infection (as was observed for a low percentage of the controlled plants).

A second approach was taken to identify polymorphisms linked to the resistance gene. This was the screening of AFLP markers for polymorphism between the closely related breeding lines 14-7 (resistant) and BD4 (susceptible). So far 15 AFLP primers were used and 3 polymorphic bands present in 14-7 and absent in BD4 were identified. One example for such polymorphism is presented in Figure 1. Mapping of these polymorphisms in the segregating population is underway.

Conclusions

We will continue to screen RAPD and AFLP primers for polymorphism between the resistant and susceptible bulks as well as between the closely related parents. For AFLP analysis, we will use the 64 primer combinations in the Perkin Elmer and BRL AFLP mapping kits. For RAPD analysis, we will screen 500 additional random 10-mer primers (Operon). Polymorphic markers will be scored in individual plants and will be mapped relative to the resistance gene. We aim to identify markers within 1cM from the gene. Such markers will be cloned and converted to locus specific primers which will be used for marker-assisted breeding of confectionery sunflower in Israel.

Figure 1. AFLP between the closely related sunflower lines 14-1 (resistant) and BD4 (susceptible).

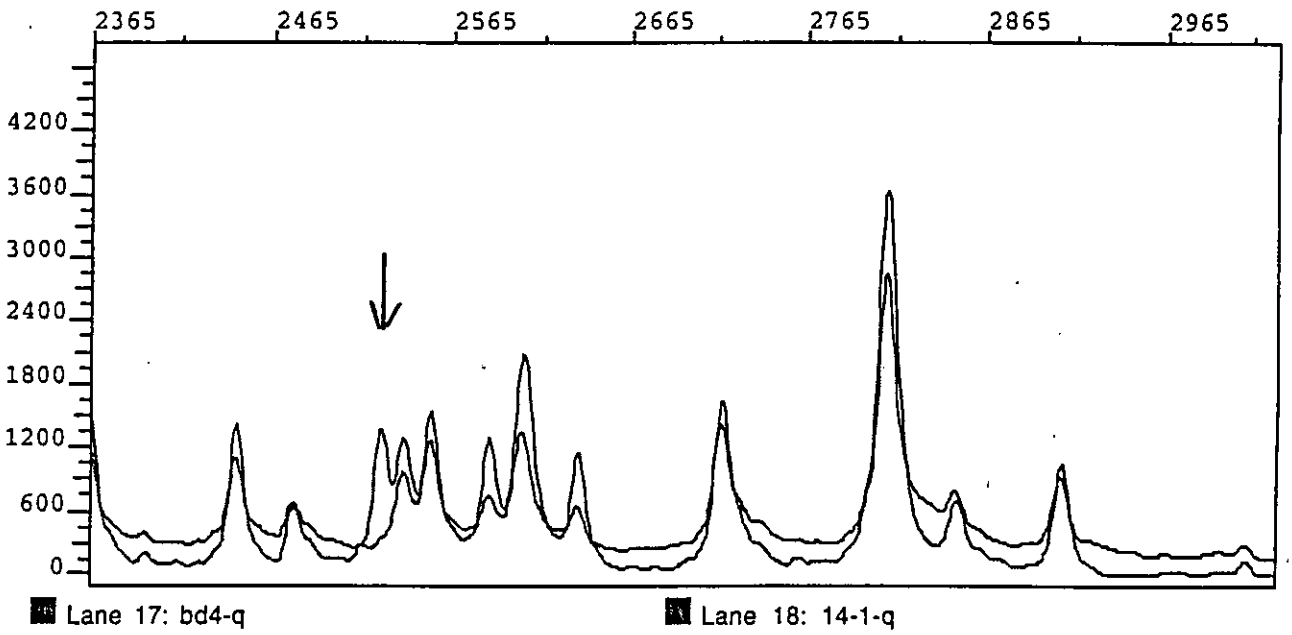
The AFLP reactions were performed with the AFLP Plant Mapping Kit of Perkin Elmer, according to the manufacturer's protocol. In short, the DNA (0.5 microgram) was digested with *Eco*RI and *Mse*I, ligated to adaptors and amplified in two successive steps. In the first amplification (preselective amplification), the primers used included the respective restriction enzyme adaptor sequence plus recognition site plus one base extension (A for *Eco*RI primer and C for *Mse*I primers). In the second step (selective amplification), the complexity of the amplification products was further reduced by using primers with extensions of an additional two bases. The *Eco*RI primers were labeled with fluorescent dyes which facilitated the visualization of the amplification products by a DNA sequencer. The amplification products were resolved in 6% denaturing polyacrylamide gels by means of an ABI sequencer 373 in the DNA sequencing unit at the Weizmann Institute of Science, Rehovot. In each lane a size standard (GeneScan-500) was included which allowed the sizing of the amplified products. The results were analyzed using the GeneScan Analysis program for displaying and sizing the peaks (each peak represents an amplification product). The top bar indicates the size of the peaks in scan number. The numbers to the left of the electropherogram indicate the amplitude of the peaks. An arrow indicates a polymorphic peak.

Applied Biosystems
Model 672
GeneScan 1.2.2-1

Results -- 17B, 18B, (Stacked)
Run Date: 3/7/1997
Gel File: RUN-168 Gel

Points 2365 To 3031
faran
seq-a

Date: 10/20/1997



Not For Use In Diagnostic Procedures

סכום

1. מטרות המחקר לתקופת הדוח.

בדיקת התורשה של העמידות לעלקת בחמנית לפצוח ואיתור סמנים מולקולרים האחוזים לגנים המבקרים את העמידות.

2. עיקרי הניסויים והתוצאות.

בוצעו הכלאות בין הורה רגיש ועמיד וגודלו אוכלוסיות BC1,F2, F1. התפצלות העמידות באוכלוסיות אלו מראה על כך שהעמידות מבוקרת ע"י גן יחיד דומיננטי. שתי שיטות יושמו למציאת שונות של סמנים מולקולרים. א. סריקה של באלקים מצמחים עמידים ורגישים מאוכלוסית F2 ע"י סמני RAPD וסריקת סמני AFLP על DNA מקוי הורים. עד כה לא נמצאו סמנים בתאחיזה לגן לעמידות.

3. המסקנות המדעיות וההשלכות לגבי יישום המחקר והמשכו. תורשת העמידות של גן יחיד מאפשרת שמוש בסמנים אחוזים לגן לעמידות לצרכי סלקציה. עקב השונות הנמוכה בין קוי ההורים שהתקבלה עקב אילוצי הכלאה (עקרות זכרית) לא הצלחנו עד כה לזהות סמנים פולימורפים.

4. הבעיות שנותרו לפיתרון ו/או השינויים במהלך העבודה.

יש צורך להמשיך בסריקת מספר רב יותר של סמנים בכל אחת משתי השיטות ומיפוי הסמנים באוכלוסיות המתפצלות.

5. האם הוחל כבר בהפצת הידע שנוצר בתקופת הדו"ח.

לא.