קוד מחקר: 261-0291-99

נושא: יצירת אוכלוסית קוים איזוגנים של פלפל למיפוי יעיל של גנים המבקרים את הגדול בבתי-צמיחה

חוקר ראשי: דר' אילן פארן **מוסד:** מינהל המחקר החקלאי, ת.ד. 6 בית דגן 50250

חוקרים שותפים:

תקופת מחקר: 1997-1999

מאמרים:

תקציר

מטרת המחקר: יצירת אוכלוסייה של קוי אינטרוגרסיה של פלפל. קוים אלו מכילים מקטע אשר מאופיין בעזרת סמנים מולקולריים שמקורו ממין הבר של פלפל (CAPSICUM CHINENSE). אוכלוסיית הקווים מהווה סדרה שבה מקטעים שונים ממין הבר מוחדרים לקו בעל רקע תרבותי אחיד ובסה"כ כל גנום מין הבר מיוצג באוכלוסייה. אוכלוסייה זו מהווה משאב תמידי למיפוי (QTL) מולקולרי בפלפל וכמו כן מהווה כלי יעיל לזיהוי גנים המבקרים תכונות כמותיות הקשורות לאיכות ויבול הפרי.

מהלך העבודה: יצירת האוכלוסייה נעשתה ע"י סדרת הכלאות דחיקה מלאות BC3F2 לאחר דור, ובסלקציה לאזורים גנומים מסוימים, על סמך סמני AFLP ו- RFLP. 83% האוכלוסייה מונה 29 קוים המכילים כל אחד 1-3 אינטרגרסיות ממין הבר שבסה"כ מכסות מהגנום. מבין קוים אלו, 18 מקובעים לאינטרוגרסיה והשאר עדין הטרוזיגוטים לחלק או לכל האינטרוגרסיה. עבור הקווים הבלתי מקובעים ועבור האזורים הבלתי מכוסים בגנום יהיה צורך בהכלאות וסלקציות נוספות להשלמת האוכלוסייה שמופו QTL באוכלוסייה קודמת. אפיון ראשני של הקווים אפשר לזהות קוים המכילים QTL, דבר המעיד על יעילות האוכלוסייה למיפוי.

1999 final report:

Development of isogenic lines population of pepper for efficient mapping of genes controlling its production

Proposal number 261-0291-97

Ilan Paran

Department of Plant Genetics and Breeding

Agricultural Research Organization,

The Volcani Center,

P.O. Box 6, Bet Dagan, 50250,

Israel

FAX: 972-3-9669642

Email: vcparan@netvision.net.il

Abstract

The objective of this proposal was to construct introgression lines (IL) population in pepper. This population consists of lines each carries a defined chromosome region from the pepper wild species *Capsicum chinense* in a uniform genetic background of a bell-type *C. annuum* line. Such population will be used as a permanent mapping population in pepper and for efficient mapping of genes controlling quantitative traits (QTL) related to the crop production. The pepper IL population was constructed by backcrossing and marker assisted selection of chromosome segments from the donor parent. Currently, 29 lines were produced, each with 1-3 introgression that all together cover 83% of the donor genome. Out of the 29 lines, 18 are homozygous for the introgression, while for 11 IL the introgression is partly or completely heterozygous. For these lines and for the reminding 17% of the uncovered genome, further crossing and marker assisted selection will be used in order to develop a full set of introgression lines. Initial phenotypic evaluation of the IL confirmed the location of QTL for several fruit traits that were identified in other studies in pepper.

Background

Several genetic maps have been constructed in pepper in recent years (Lefebvre et al. 1995; Livingstone et al. 1999; Prince et al. 1993). However, the use of these maps to identify quantitative traits loci (QTL) controlling pepper production has been limited due to either low level of polymorphism or sterility of the progenies. Therefore, todate there is a very limited information on the molecular mapping of QTL in pepper. Recently, we mapped 55 QTL for 14 fruit yield and quality traits in an intra-specific F2 population from a cross of two *Capsicum annuum* lines (Ben Chaim et al. submitted). Although this population was efficient in identifying QTL, further verification, high resolution mapping and QTL introgression has been hindered because of the F2 population structure and the relative low level of polymorphism between the two parents.

The objective of this proposal was to construct a set of nearly isogenic lines (QTL-NILs) covering the entire genome of pepper, as a tool for precision QTL mapping. Such populations consists of a set of lines, each carrying a single defined chromosome segment originating from a donor exotic genotype in an otherwise uniform elite genetic background. This type of population is an efficient tool for QTL detection and mapping for the following reasons: 1) Wild species contain novel QTLs, with large effects. 2) QTL-NILs reduce the genetic variance that is not associated with the region containing the QTL. 3) QTL-NILs provide a permanent resource that can be tested in multiple conditions and therefore reduce the environmental variance. 4) QTL-NILs can be crossed to different inbreds thereby allowing to test the effect of the QTLs in different genetic backgrounds. 5) QTL-NILs allow the mapping of the effects to small intervals through recombination of a segment containing the QTL into smaller overlapping ones. The usefulness of such lines for the detection and high resolution mapping of QTL was demonstrated in tomato by Eshed and Zamir (1995) and Eshed et al. (1996).

Description of experiments and results

A summary of the steps of the construction of the introgression lines population is presented in Table 1. A BC1 population of 83 plants was constructed from an inter-specific cross between the *Capsicum chinense* accession PI 152225 and the *C. annuum* blocky type

inbred line PI 156 that was used as a recurrent parent. PI 152225 is characterized by a bushy growth habit and has small pungent fruits that weight 2 grams. PI 156 is a tall plant with very large fruits that weights 150-200 grams. Both fruits have mature red color. DNA was extracted from leaves of the BC1 plants and each plant was backcrossed to PI 156 to produce BC2 progeny. The BC1 map was constructed using AFLP markers developed by Keygene. Ten primer combinations were used to produce a total of 312 scorable bands. Mapping analysis was carried out using JoinMap 2.0 program at a LOD 4.0 for grouping the markers and resulted in 12 linkage groups and 6 unlinked markers.

Graphical genotyping of all the BC1 individuals allowed to calculate the percent recurrent parent genome content and to select plants that have the minimal amount of introgressions from the donor parent that cover its whole genome. The percent recurrent parent genome content in the BC1 population ranged from 87% to 60% and averaged 75% as expected in this generation. Nine BC1 plants each containing several introgressions were selected for further advancing the population.

A total of 180 BC2 plants (20 per each BC1) were planted and fingerprinted with the AFLP. In addition, DNA from 15 BC2 plants from each BC1 individual was pulled and used for RFLP analysis. tomato and pepper RFLP markers were chosen based on the map of Livingstone et al. (1999) to allow representation of all the pepper chromosomes. Ninety two RFLP markers were added to the map and allowed to align the map with that of Livingstone et al. (1999). The addition of the RFLP markers determined that most of the AFLP-based linkage groups in the BC1 map did not represent the whole pepper chromosomes (Figure 1). A sub set of 76 AFLP markers and all the RFLP markers were used to update the linkage map as presented in Figure 1. The map consists of 11 major linkage groups and 2 minor linkage groups that are assigned to chromosome 12 by Livingstone et al. (1999) and spans 2083 cM. Linkage groups 1 corresponds to chromosome 1 and 8 of pepper. This pseudo-linkage is a result of a reciprocal translocation that differentiates the two *Capsicum* species.

The percent recurrent parent genome content in the BC2 population ranged from 99% to 82% and averaged 92% compared to the expected 87.5% in the BC2 generation. Twenty four BC2 plants each containing 1-5 introgressions were selected for further advancing the population by backcrossing to the recurrent parent.

Three hundred and eighty BC3 (8-32 plants per each BC2) were planted and fingerprinted with the AFLP. The percent recurrent parent genome content in the BC3 population ranged from 100% to 93% and averaged 98% compared to the expected 94% in the BC3 generation. Fifty BC3 plants each containing 1-3 introgressions were selected for fixation of the introgressed regions by selfing.

Seven hundred and fifty BC3F2 plants were planted and fingerprinted with the AFLP and RFLP markers in the introgressed regions. A total of 29 introgression lines were obtained that all together cover 83% of the genome (Table 2). From these lines, only 18 were homozygous for the introgressed region, while the other 11 lines were partly or fully heterozygous.

Conclusions

Our inability to recover introgression lines that cover the entire donor genome resulted from two main reasons. First, BC1- BC3 selections were based on AFLP markers that, subsequently, have been determined to represent only partially the genome. Therefore, parts of the pepper chromosomes were not included in the initial selections. Second, selected plants were often sterile or produced non viable seeds. For several introgressed regions, BC3F2 plants homozygous for the introgression were sterile, and therefore, only heterozygous introgressions were obtained. In order to obtain introgression lines for the uncovered regions additional crossing and selection is required. For the remaining heterozygous regions additional selfing and selection will be performed. If sterility will be continued, these introgressions will be maintained as heterozygous regions.

Phenotypic evaluation of individual BC3F2 plants revealed some correspondence with fruit QTL identified in a different pepper mapping population (Ben Chaim et al. submitted). For example, plants with introgressions containing markers that correspond to fruit shape QTL in chromosome 3, 8 and 10 identified by Ben Chaim et al. had fruit shape that varied from that of the recurrent parent. This initial evaluation reveals the usefulness of the IL as a tool for QTL detection in pepper. Full scale phenotypic evaluation of the introgression lines will be carried put after more lines will be fixed and full genome coverage will be obtained. IL for selected QTL will be further used for high resolution mapping of the QTL and for introgressing the QTL to additional genetic backgrounds.

References

Ben Chaim A, Paran I, Grube R, Jahn MK, van Wijk R, Peleman J. QTL mapping of fruit related traits in pepper (*Capsicum annuum*). Submitted

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141: 1147-1162

Eshed Y, Gera G, Zamir D (1996) A genome wide search of a wild species alleles that increase horticutural yield of processing tomato. Theor. Apll. Genet. 93: 877-886

Lefebvre V, Palloix A, Caranta C, Pochard E (1995) Construction of an intra-specific integrated linkage map of pepper using molecular markers and doubled-haploid progenies. Genome 38: 112-121

Livingstone KD, Lackney VK, Blauth J, van Wijk R, Jahn MK (1999) Genome mapping in Capsicum and the evolution of genome structure in the Solanaceae. Genetics 152: 1183-1202

Prince JP, Pochard E, Tanksley SD (1993) Construction of molecular linkage map of pepper and a comparison of synteny with tomato. Genome 36: 404–417

Publications

Paran, B. Tanyolac, A. Palliox, M. Jahn, J. Rouppe-van-der-voort and J. Peleman. An integrated molecular map of pepper (*Capsicum* spp.). In preparation.

B. Tanyolac and I. Paran. Construction of introgression lines population in pepper. Plant Genomics Meeting. Maagan, Israel 1999. Poster.

Figure 1. Genetic map and introgression lines derived from a cross of *Capsicum annuum* x *C. chinense*. Marker types are tomato clones for RFLP (TG, CT and CD), pepper RFLP clones

(PG). AFLP markers are presented as the primer combination followed by the size of the mapped fragment. EF is a pepper RFLP obtained from Dr. U. Bonas (Martin Luther University, Germany). ZDS, EPOXY, PDS, PSY, GGPPS are genes from the carotenoids biosynthesis pathway. Q6 and O12 are RAPD markers. pFK2 is potato fructokinase cDNA obtained from Dr. D. Granot, The Volcani Institute, Israel. Distances in cM are to the left of each linkage group. Linkage groups are numbered according to the map of Livingstone et al. (1999). Introgressions are presented as thin bars (homozygous) or dashed bar (heterozygous) to the right of the linkage group. Thick bars represent AFLP-based linkage groups.

Table 1. Steps in the development of pepper introgression lines

83 BC1 plants
- 10 AFLP primer combinations
- 312 AFLP markers in 12 linkage groups
- 6 markers unlinked
- Average % recurrent parent genome: 75%
- 9 BC1 plants selected

180 BC2 (20 per each BC1 plant)
- Addition of 92 RFLP to the map
- Total map length: 2083 cM
- Average % recurrent parent genome: 92%
- 24 BC2 selected with 1-5 introgressions

380 BC3 (8-32 per each BC2)
- Average % recurrent parent genome: 98%
- 50 BC3 selected with 1-3 introgressions

750 BC3F2 (10-30 per each BC3)
- 29 introgression lines that cover 83% of the genome
- 18 lines are fixed for the introgressed region
- 11 lines are heterozygous for the introgressed region

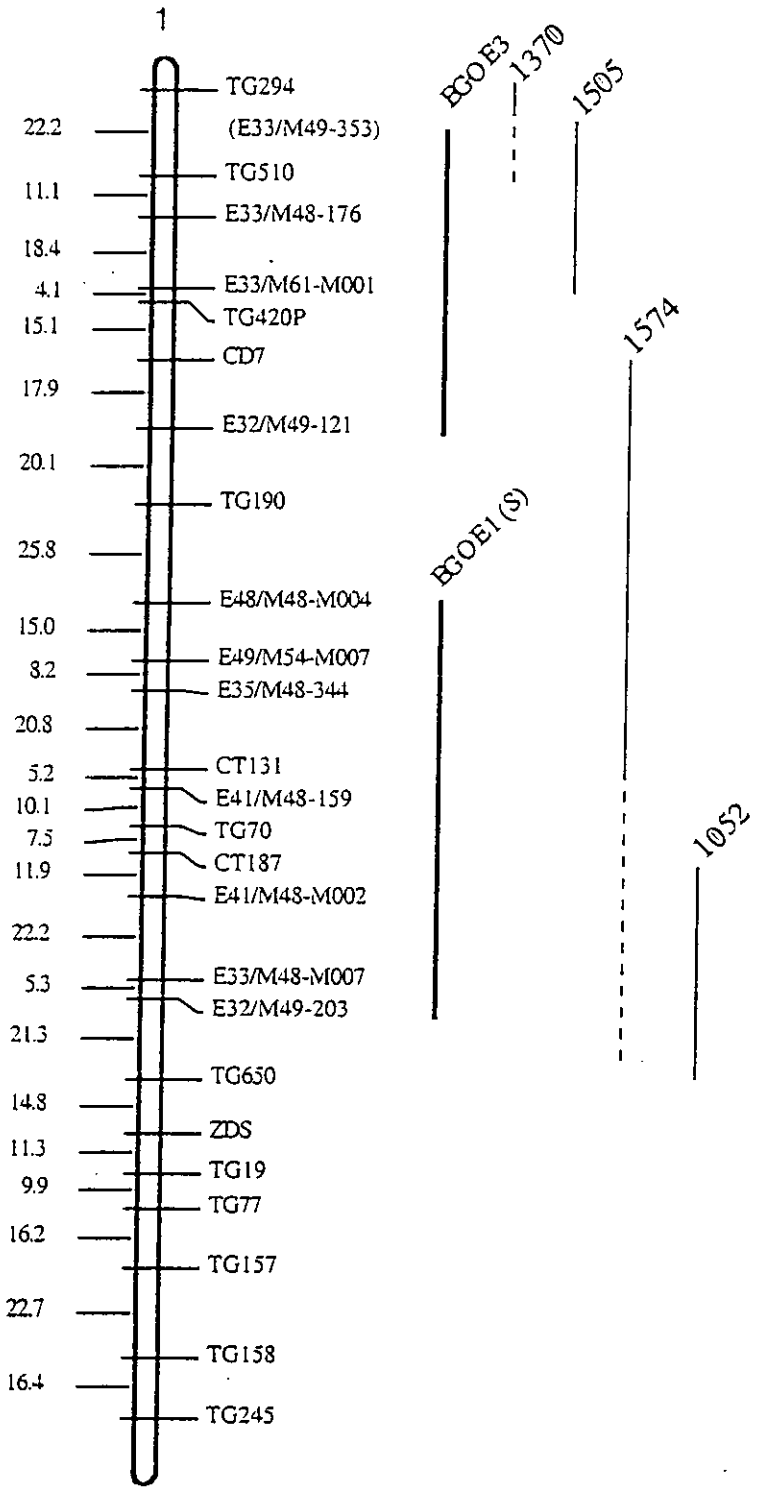
Table 2. Summary of pepper introgression lines

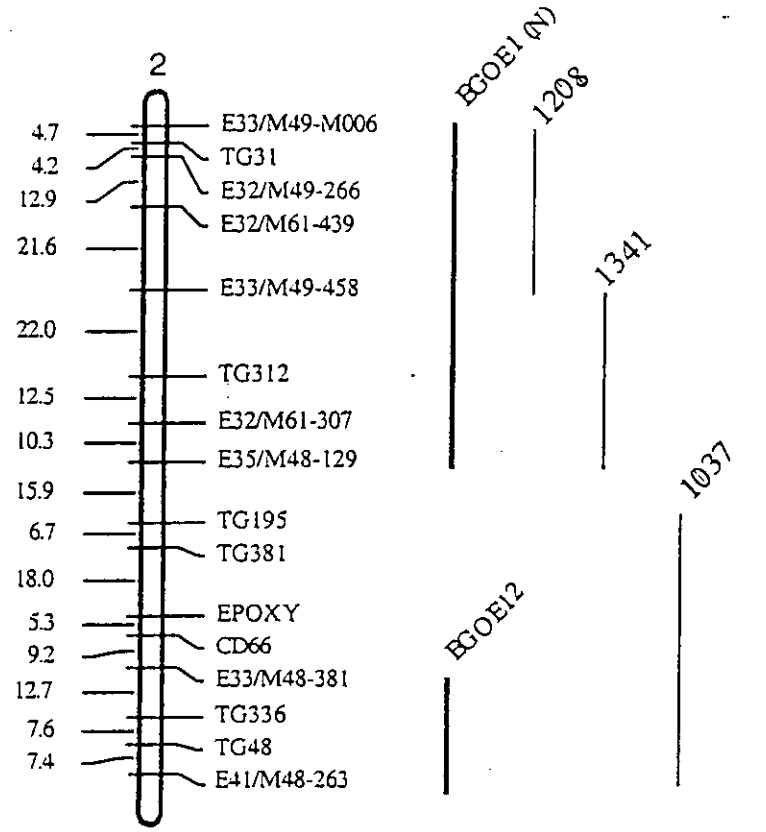
Chrom.	Line Number	Targeted introgression		Non targeted introgression		Remarks
		Fixed	Unfixed	Fixed	Unfixed	
1	1370	TG294	TG510	(7) CD54: E33/M49-M015	(11) TG105	
	1505	E33/M49-253: E33/M61-M001			(12) TG381-CD66	
	1574	CD7: CT131	TG650	(4) TG587, (9) CD32		
	1052	CT187: TG650				
2	1208	E33/M49-M006: E33/M49-458			(6) PG159	Check TG19, TG157
	1341	E33/M49-458: E35/M48-129	TG105: TG36		(13) Tg53, (3) E35/M48-M006: E33/M49-179	
	1037	TG195: E41/M48-263				
3	1585-126	TG558: TG74			(4) E33/M49-M014, (2) E41/M48-263	
	1608-243	TG102: TG74	TG74: CD4		(6) CT184: CD25	
	1608-232	TG417: CD4			(4) E33/M49-M014 (6)) CT184: CD25	
4	1024	TG135: E33/M61-M005	TG62: CT50		(5) TG419, (6) CT184, (7) CT114	
	1135	E32/M61-M003: TG62				
	1315	E33/M61-335: TG587	E33/M49-M014		(5) CD74, (12) TG516, (UL) TG53	
5	1174	TG123: E35/M48-M007			(6) CD25	
	1026-11	E32/M61-M008: TG419	TG419: CD74			Check TG123

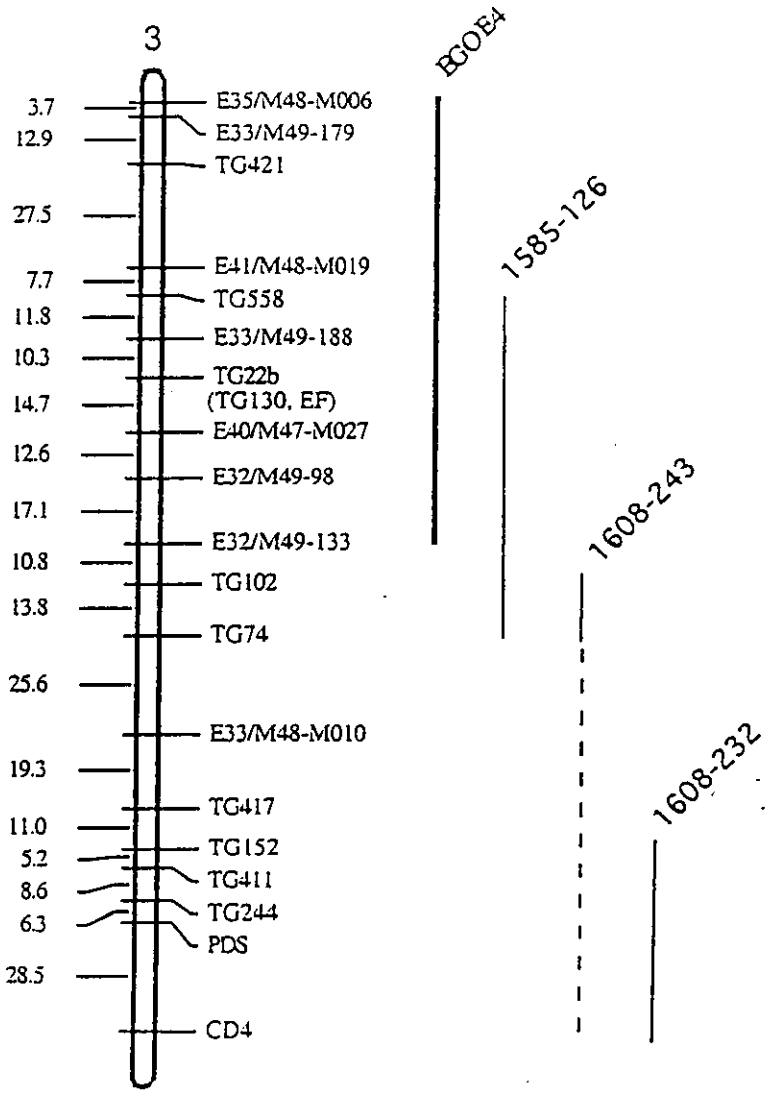
Table 2 (continued). Summary of pepper introgression lines

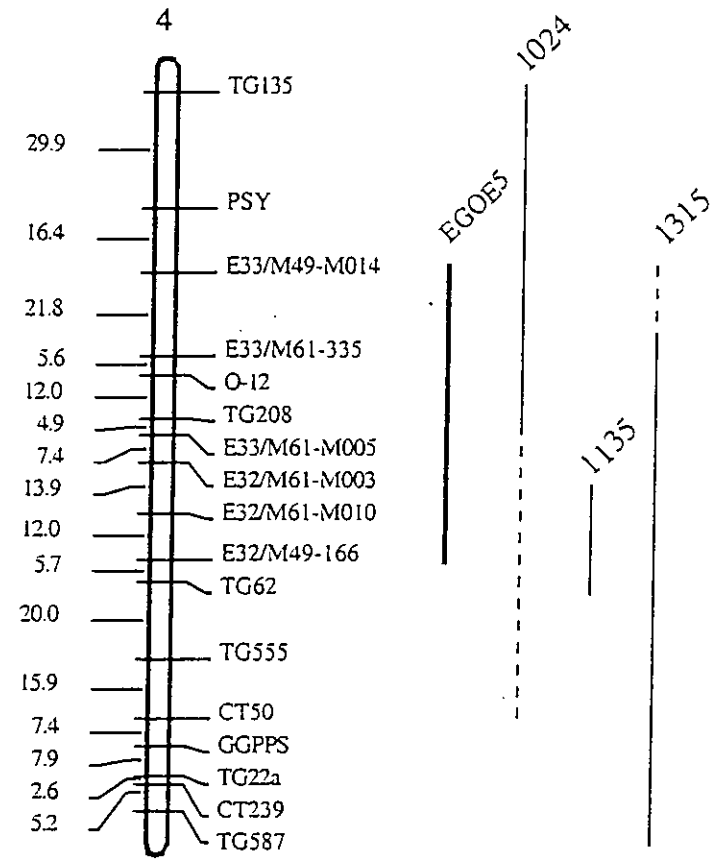
Chrom.	Line Number	Targeted introgression		Non targeted introgression		Remarks
		Fixed	Unfixed	Fixed	Unfixed	
6	1608-		E33/M49-M013:		(3) TG555: CD4, (4) E33/M49-M014	

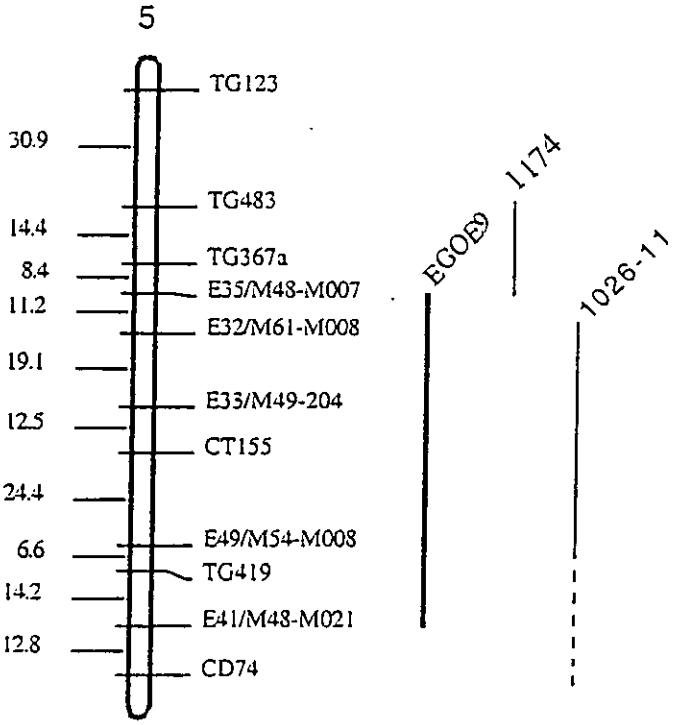
	237		CD25			
7	1115 1193 1243-76	E41/M48-M022: E33/M49-M015 E33/M49-M015: E33/M49-M012 E33/M49-M004: CT114		(10) E35/M48-M005: TG395	(4) TG62-TG587	Check TG128
9	1562 1413	E35/M48-M010: CD32 E33/M48-310: CT79	E32/M61-90: E41/M48-79	(1) TG294		
10	1007 1579 1243	TG408: TG422 E35/M48-M005: E40/M47-M023 E35/M48-M005: TG395	TG408		(13) TG53, (2) TG294 (4) TG62: TG587	
11	1104 1341	E32/M49-481: TG105	TG105: TG36	(2) E33/M49-458: E35/M48-129	(6) PG159 (13) TG53, (3) E35/M48-M006: E33/M49-179	Check TG421, TG558
12	1070 1625-248 1535	TG194: CT168 TG557: TG516 E33/M48-261: E48/M48-308	CT168: TG557	(7) E33/M49-M004	(4) CT239: TG587 (4) TG62	Check TG216, CT114
13	1071	TG176			(4) CT239-TG587	

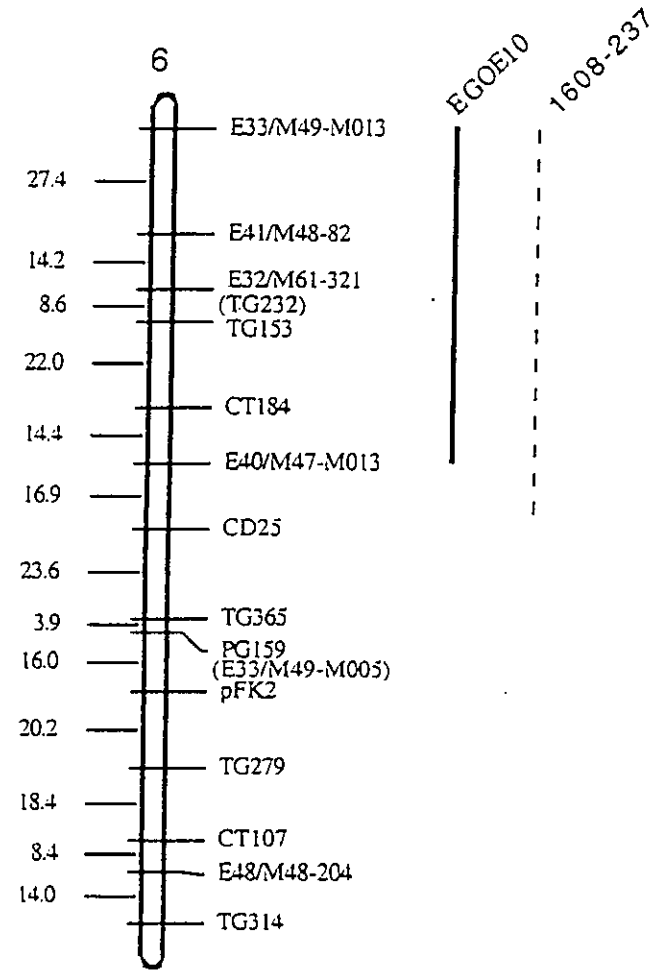

1
TG294
22.2
(E33/M49-353)
TG510
11.1
E33/M48-176
18.4
E33/M61-M001
4.1
TG420P
15.1
CD7
17.9
E32/M49-121
20.1
TG190
25.8
E48/M48-M004
15.0
E49/M54-M007
8.2
E35/M48-344
20.8
CT131
5.2
E41/M48-159
10.1
TG70
7.5
CT187
11.9
E41/M48-M002
22.2
E33/M48-M007
5.3
E32/M49-203
21.3
TG650
14.8
ZDS
11.3
TG19
9.9
TG77
16.2
TG157
22.7
TG158
16.4
TG245
BGOE3
1370
1505
1574
BGOE1(S)
1052

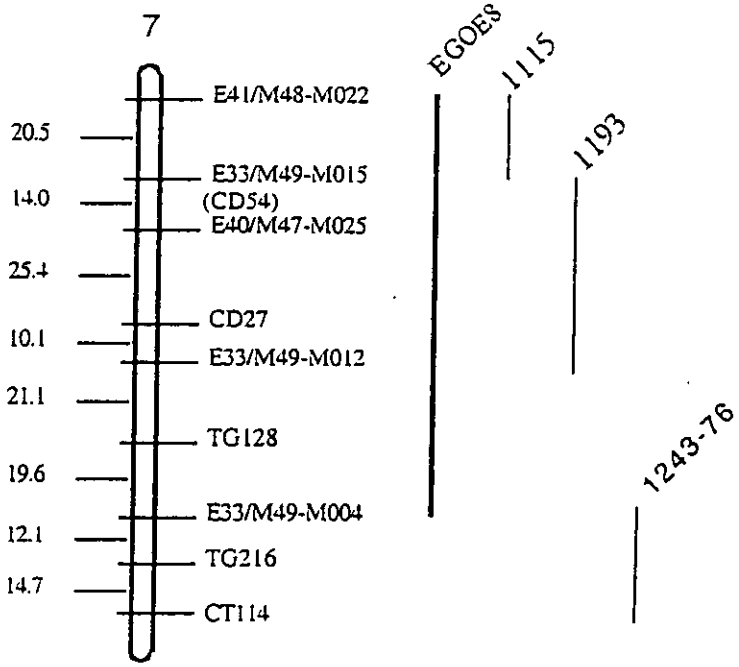

2
4.7
4.2
12.9
21.6
22.0
12.5
10.3
15.9
6.7
18.0
5.3
9.2
12.7
7.6
7.4
E33/M49-M006
TG31
E32/M49-266
E32/M61-439
E33/M49-458
TG312
E32/M61-307
E35/M48-129
TG195
TG381
EPOXY
CD66
E33/M48-381
TG336
TG48
E41/M48-263
BGOE1 (N)
1208
1341
1037
BGOE12

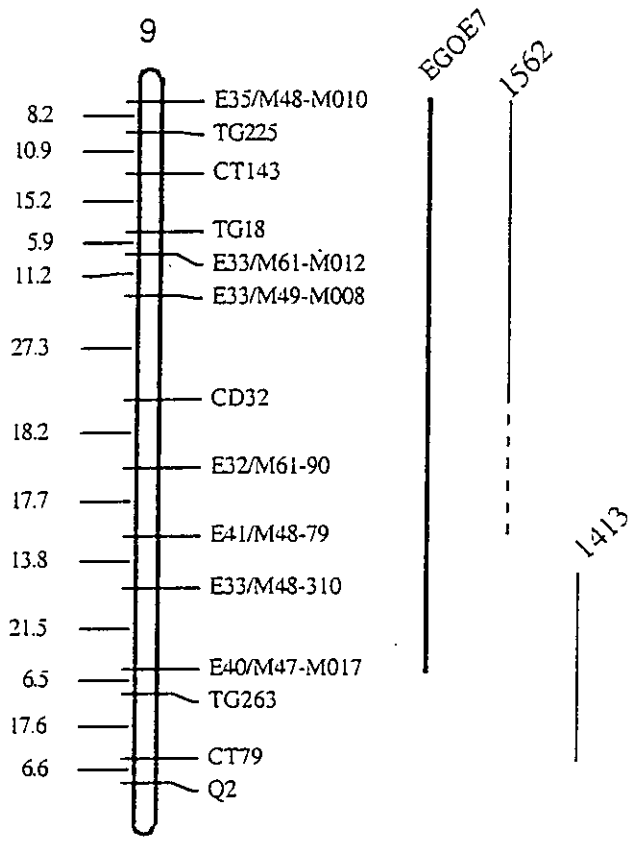

3
3.7
12.9
27.5
7.7
11.8
10.3
14.7
12.6
17.1
10.8
13.8
25.6
19.3
11.0
5.2
8.6
6.3
28.5
E35/M48-M006
E33/M49-179
TG421
E41/M48-M019
TG558
E33/M49-188
TG22b
(TG130, EF)
E40/M47-M027
E32/M49-98
E32/M49-133
TG102
TG74
E33/M48-M010
TG417
TG152
TG411
TG244
PDS
CD4
BGOE4
1585-126
1608-243
1608-232

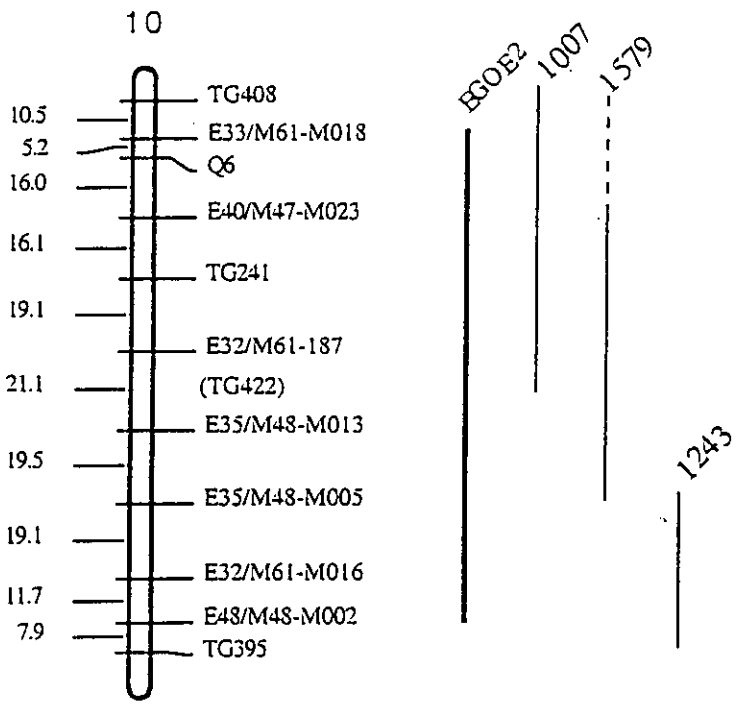

4
TG135
29.9
PSY
16.4
E33/M49-M014
21.8
E33/M61-335
5.6
O-12
12.0
TG208
4.9
E33/M61-M005
7.4
E32/M61-M003
13.9
E32/M61-M010
12.0
E32/M49-166
5.7
TG62
20.0
TG555
15.9
CT50
7.4
GGPPS
7.9
TG22a
2.6
CT239
5.2
TG587
EGOE5
1024
1135
1315

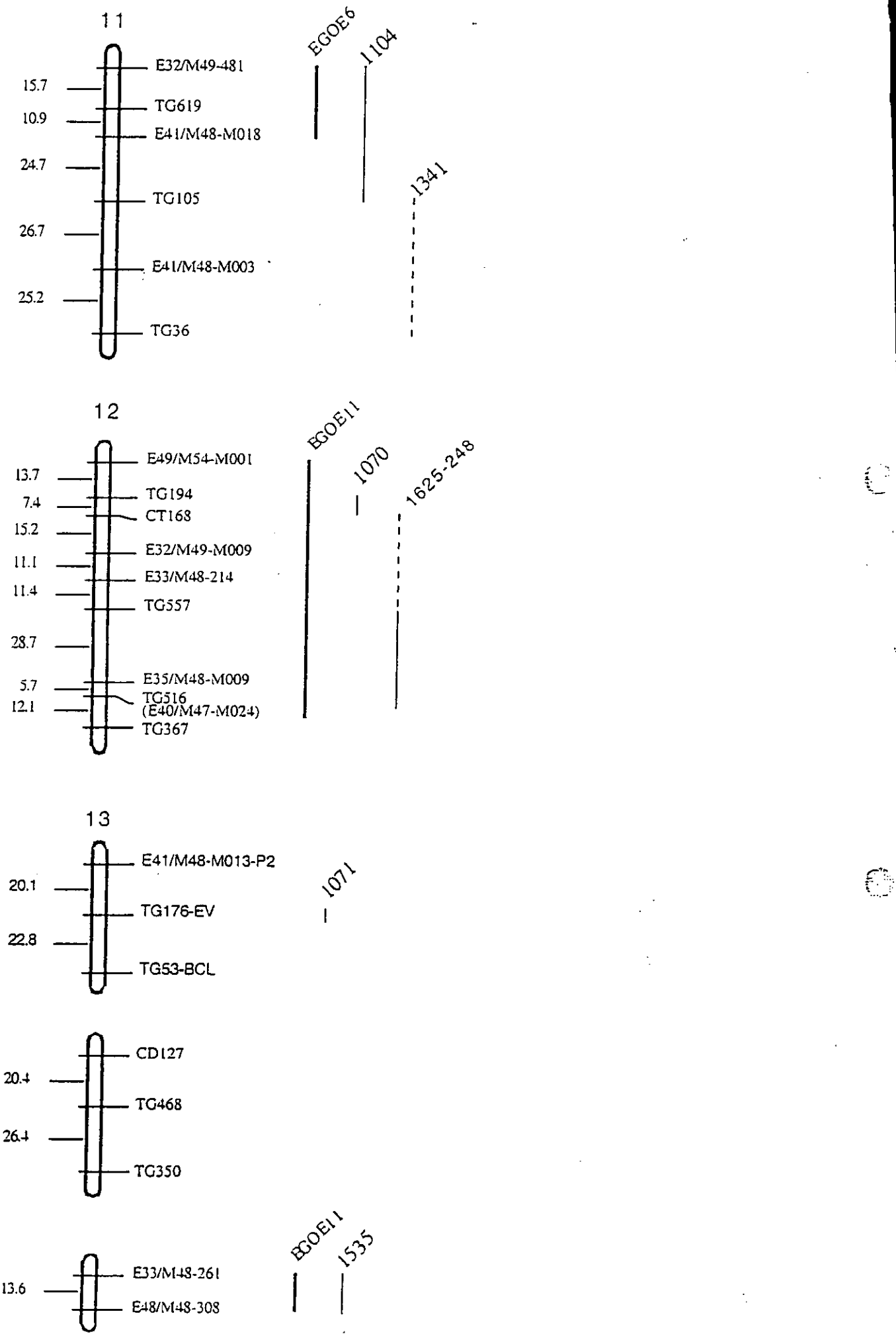

5
TG123
30.9
TG483
14.4
TG367a
8.4
E35/M48-M007
11.2
E32/M61-M008
19.1
E33/M49-204
12.5
CT155
24.4
E49/M54-M008
6.6
TG419
14.2
E41/M48-M021
12.8
CD74
EGOE9
1174
1026-11


6
E33/M49-M013
27.4
E41/M48-82
14.2
E32/M61-321
8.6
(TG232)
TG153
22.0
CT184
14.4
E40/M47-M013
16.9
CD25
23.6
TG365
3.9
PG159
16.0
(E33/M49-M005)
pFK2
20.2
TG279
18.4
CT107
8.4
E48/M48-204
14.0
TG314
EGOE10
1608-237


7
E41/M48-M022
20.5
E33/M49-M015
14.0
(CD54)
E40/M47-M025
25.4
CD27
10.1
E33/M49-M012
21.1
TG128
19.6
E33/M49-M004
12.1
TG216
14.7
CT114
EGOE8
1115
1193
1243-76


9
8.2
10.9
15.2
5.9
11.2
27.3
18.2
17.7
13.8
21.5
6.5
17.6
6.6
E35/M48-M010
TG225
CT143
TG18
E33/M61-M012
E33/M49-M008
CD32
E32/M61-90
E41/M48-79
E33/M48-310
E40/M47-M017
TG263
CT79
Q2
EGOE7
1562
1413


10
10.5
5.2
16.0
16.1
19.1
21.1
19.5
19.1
11.7
7.9
TG408
E33/M61-M018
Q6
E40/M47-M023
TG241
E32/M61-187
(TG422)
E35/M48-M013
E35/M48-M005
E32/M61-M016
E48/M48-M002
TG395
EGOE2
1007
1579
1243


11
E32/M49-481
15.7
TG619
10.9
E41/M48-M018
24.7
TG105
26.7
E41/M48-M003
25.2
TG36
EGOE6
1104
1341
12
E49/M54-M001
13.7
TG194
7.4
CT168
15.2
E32/M49-M009
11.1
E33/M48-214
11.4
TG557
28.7
E35/M48-M009
5.7
TG516
12.1
(E40/M47-M024)
TG367
EGOE11
1070
1625-248
13
E41/M48-M013-P2
20.1
TG176-EV
22.8
TG53-BCL
1071
CD127
20.4
TG468
26.4
TG350
E33/M48-261
13.6
E48/M48-308
EGOE11
1535

סיכום עם שאלות מנחות

1. מטרות המחקר:

יצירת אוכלוסיית קוי אינטרוגרסיה של פלפל המכילים מקטעים מוגדרים ע"י סמנים מולקולריים ממין בר של פלפל C. chinense ברקע אחיד של פלפל תרבותי.

2. עיקרי הניסויים והתוצאות:

לאחר סדרת הכלאות דחיקה, יצירת מפה מולקולרית של פלפל וסלקציות המבוססות על סמנים מולקולריים לאינטרוגרסיות ממין הבר. נוצרו 29 קוים המכסים 83% מגנום מין הבר.

3. המסקנות המדעיות וההשלכות:

בעיות פוריות בצאצאים הומוזיגוטים לאלל הבר וסלקציה המבוססות על סמני AFLP המכסים את הגנום באופן חלקי לא אפשרו קבלת סט מלא של קוים הומוזיגוטים המכסים את כל הגנום. הערכה פנוטיפית וגנטית של הקוים איפשרה זיהוי קוים המכילים QTL לתכונות פרי.

4. הבעיות שנותרו לפתרון:

מאחר וחלק מהקוים עדיין הטרוזיגוטים וכמו כן 17% של גנום מין הבר אינו מיוצג באוכלוסיה, יש צורך בהכלאות נוספות וסלקציות להשלים את יצירת קוי האינטרוגרסיה.

5. הפצת הידע:

עד כה נמצא מאמר אחד בהכנה וכמו כן תוצאות העבודה הוצגו בכנוס מדעי - ראה פרוט בדו"ח המצורף.