PN-ADA-163

ANNUAL REPORT

Covering Period: October 2003- August 2004

Submitted to the Office Agriculture & Food Security
U.S. Agency for International Development

Molecular Marker-Assisted Breeding For Resistance to Whitefly-Transmitted Geminiviruses Infecting Tomato in Guatemala

CDR Grant Number: TA-MOU-01-C21-008

Project Duration: 2002 – 2005

Rehovot - August 17, 2004

ANNUAL REPORT

Covering Period: October 2003- August 2004

Submitted to the Office Agriculture & Food Security
U.S. Agency for International Development

Molecular Marker-Assisted Breeding For Resistance to Whitefly-Transmitted Geminiviruses Infecting Tomato in Guatemala

Principal Investigator: Dr. Henryk (Hanokh) Czosnek
Grantee Institution: The Hebrew University of Jerusalem, Israel

Collaborators:

Dr. Luis Mejia
University of San Carlos, Guatemala City, Guatemala

Dr. Douglas P. Maxwell
The University of Wisconsin, Madison, Wisconsin, USA.

Dr. Moshe Lapidot
Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel.

CDR Grant Number: TA-MOU-01-C21-008

Project Duration: 2002 – 2005

Rehovot - August 17, 2004

1. Scientific issues

Since the mid 1980s, tomato growing has been severely affected by geminiviruses transmitted by the whitefly *Bemisia tabaci*. One hundred percent disease incidence is common, leading to the complete loss of the crop. Insecticides generally provide insufficient control. Hence breeding for resistance is a major scientific and economical goal. The main goal of this project is to facilitate breeding by introducing molecular marker-assisted breeding technology into Guatemala using the development of locally adapted tomato cultivars resistant to whitefly-transmitted geminiviruses and other economically important pathogens as a model system.

The specific research objectives as stated in the project are to:

1. Introduce the principles of molecular marker-assisted breeding technology into a research program and into the M. Sc. Degree Program at Universidad de San Carlos, Guatemala City.
2. Determine whether the markers used to map genes for resistance to TYLCV and ToLCV (Eastern Hemisphere geminiviruses) identify identical loci in the tomato breeding lines selected for resistance to the Guatemalan geminiviruses.
3. Identify molecular markers linked to genes for resistance to whitefly-transmitted geminiviruses from Guatemala that are different than those in objective 2.
4. Determine if the field-selected resistant tomato germplasm is resistant to all three tomato-infecting whitefly-transmitted geminiviruses in Guatemala.
5. Combine the independent sources of resistance to whitefly-transmitted geminiviruses and evaluate this material in the field in Guatemala and Israel.
6. Use molecular markers to combine genes for resistance to whitefly-transmitted geminiviruses and other pathogens into improved resistant genotypes of tomatoes suited for the Guatemalan market.

2. Scientific achievements

2.1. Evaluation and selection of germplasm in Guatemala – December 2003 to April 2004

2.1.1. Germplasm utilized

Germplasm was collected from various breeding programs, which were focused on resistance to *Tomato yellow leaf curl virus* in the Mediterranean region or *Tomato leaf curl virus* in Asia. In all cases, begomovirus resistance had been introgressed from wild tomato species. In all cases, these lines or hybrids had moderate to high levels of viral resistance to a particular begomovirus species in the region where they were selected.

1. Population introgressed from *L. pimpinellifolium* and *L. peruvianum* (designed Pimpertylc J-13); obtained from Dr . H. Laterrot, INRA, France.
2. Population introgressed from *L. hirsutum*: hybrids FAVI 9, FAVI 12, and FAVI 13; obtained from Drs H. Czosnek and F. Vidavski, the Hebrew University of Jerusalem, Israel.
3. Line TY52 homozygous for the *Ty1* gene from *L. chilense*; obtained from Dr D. Zamir, the Hebrew University of Jerusalem, Israel.
4. Two lines, TY198 and TY197, with introgressions from *L. peruvianum* from Dr. M. Lapidot and colleagues, Volcani Center, Israel.

5. Line H24 with introgressions from *L. hirsutum* f. *glabratum*; obtained from Dr. P. Hanson. AVRDC, Taiwan.
6. Several lines with introgressions from *L. chilense*; obtained from Dr J. Scott, University of Florida, USA.

2.1.2. History of selection in the field in Guatemala

At the beginning of this project, several breeding lines were selected, with assistance from F. Vidavski, Hebrew University of Jerusalem, Israel (Tables 1 and 2). It was then decided that, since resistance to geminiviruses already seemed to have been fixed in these breeding lines, it would be desirable to introduce resistance to other pathogens and other traits of agronomic interest. Basic resistances to pathogens (such as to the fungi *Verticillium* and *Fusarium*) and other characters related to yield and fruit quality, such as fruit size and firmness, are essential before these genotypes can be released to the growers. The begomovirus resistant breeding lines were further evaluated and crossed to susceptible cultivars carrying other traits of interest (Table 3).

Table 1. Performance of parental lines (September, 2001 to February, 2002) and breeding lines which were selected.

Line and origin[1]	Av. DSI 30[2]	Av. DSI 60[2]	Av. weight/ fruit (gram)	Av. yield/ plant (gram)	Selected line
FAVI-9 (HUJI)					
F6-2522-1	1.45	1.68	36	235	G1h
F6-2522-2	1.08	1.48	39	499	G5h
F6-5211-1	1.67	2.17	24	130	
F6-5211-2	2.70	3.00	21	50	
F6-2312-1	1.22	1.97	93	293	
F6-2312-2	1.45	2.29	40	330	
F6-4111-1	1.49	1.98	61	868	G4h
F6-4111-2	2.85	1.80	35	272	
F6-5222-1	1.15	1.57	27	116	
F6-5222-2	1.24	1.82	33	119	
F6-2211-1	1.23	1.38	61	868	G3h, G13h
F6-2211-2	1.60	1.92	56	463	
FAVI-12 (HUJI)					
F1-2112-1	1.67	2.14	45	395	G2h
F1-2112-2	1.36	2.00	36	420	
FAVI-13 (HUJI)					
F2-211-1	1.80	2.63	62	291	
F2-211-1	1.89	2.50	0	0	
TY-198 (Volcani)					
L1-111-1	1.35	2.29	25	254	G11p
L1-111-2	1.39	2.39	28	264	
L1-112-1	1.61	2.18	35	690	
L1-112-2	1.51		32	410	
TY-197 (Volcani)					
L2-221-1	1.25	3.00	42	1,020	G12p, G19p
L2-221-2	2.09	2.25	32	418	
L2-211-1	1.91	2.00	35	390	
Control					
Elios (Peto)	2.66	2.94	11	44	

Line and origin[1]	Av. DSI 30[2]	Av. DSI 60[2]	Av. weight/ fruit (gram)	Av. yield/ plant (gram)	Selected line
8933 (Volcani)					
L3-132	1.36	2.71	19	309	
536 (Volcani)					
L5-1	2.17	2.80	28	210	G13p
HUJI					
VT-914	1.78	2.75	24	110	
HUJI					
Z1-1	2.63	2.90	15	282	
INRA					
P2-113 (1)	2.11	2.83	21	93	G10p
P2-113 (2)	2.03	2.61	20	277	
TY-200 (Volcani)					
L6	2.04	2.92	12	45	
IIHLD					
HC-7880	2.63	3.44	8	17	GC6
FLORIDA					
Fla 417-8	1.79	2.57	28	411	
Fla 496-11	1.49	1.70	72	995	G7c, G14c
Fla 476-19	-	-	-	-	
Fla 595-2	1.69	2.24	52	639	G9c
Fla 612-Y2	1.80	2.54	26	300	
Fla 619-2	1.65	2.07	56	498	
Fla 653-3	1.16	2.30	49	622	
Fla 658-2BK	1.14	1.74	49	1,010	G8c, G16c

[1]Hebrew University of Jerusalem, Israel (HUJI); The Volcani Center, Israel (Volcani); Institut Nationale de la Recherche Agronomique, France (INRA); Instituto de Investigaciones Horticolas "Liliana Dimitrova". Cuba (IIHLD); University of Florida (Florida). Elios is a commercial hybrid from Peto Seed Co.
[2]Disease severity index on a scale of 0-4, at 30 and 60 days alter transplant. A score lower that 2 is considered indicative of resistance.

Table 2. Performance of twelve resistant breeding lines compared to susceptible line GC6 (April to September, 2002).

Line	Origin	Av. DSI30	Av. DSI60	Av. weight /fruit (g)	Av. yield/ plant (g)
G1h	FAVI-9	1.11	1.48	81.4	1756
G4h	FAVI-9	1.08	1.50	88.6	1373
G13h	FAVI-9	1.22	1.50	96.2	2031
G15h	FAVI-9	1.19	1.12	89.7	1383
G2h	FAVI-12	1.08	1.58	58.6	1060
G17h	FAVI-13	1.92	2.44	66.0	588
G14c	Fla 496-11	1.40	1.54	89.0	1348
G9c	Fla 595-2	1.19	1.82	82.4	1742
G16c	Fla 658-2BK	0.95	1.21	68.4	2312
G11p	TY-198	1.68	2.46	38.1	1241
G19p	TY-197	1.17	1.70	55.7	1750
G10p	Pimper J-13	2.37	3.24	42.8	375
GC6	HC-7880	3.24	3.38	16.3	148

Table 3. Cultivars susceptible to begomoviruses but carrying resistance to other pathogens and other characters of interest.

Cultivar	*Origin and other characteristics*
Sun Coast	North Carolina, large fruit, VF_1F_2[1]
Rodade	South Africa, bacterial wilt, VF_1F_2[1], determinate growth
Very Firm	Israel, long shelf life, VF_1, determinate growth
M-82	Israel, processing, very firm, VF_1
HC-7880	Cuba, determinate growth, firm fruit, processing, VFSM

[1] Resistance to *Verticillium* (V), *Fusarium* race 1 (F_1) and race 2 (F_2), *Stemphyllium* (SM).

Table 4. Performance of R X S and R X R hybrids (Septeber/2001 to February, 2002).

No.	Hybrid	Av. DSI 30[3]	Av. DSI 60[3]	Av. weight/ fruit (gram)	Av. yield/ plant (gram)
H1	HC-7880 X F6-2211	1.46	2.09	44	1,077
H2	HC-7880 X F6-5221	1.52	2.21	37	912
H3	HC-7880 X P2-1132	2.00	2.87	24	574
H4	F6-2211 X P2-1132	1.32	1.89	54	878
H5	F6-5221 X P2-1132	1.38	1.75	46	598
H6	F6-5221 X Suscept.[1]	1.34	2.24	53	855
H7	F6-2211 X Suscept.[1]	1.39	1.78	49	1,423
H8	P2-1132 X Suscept.[1]	1.36	2.56	28	468
H9	HC-7880 X Suscept.[1]	1.87	2.96	19	155
H13	Marina (Sakata)[2]	2.29	3.12	18	303

[1]Susceptible line from the HUJI.
[2]Most popular commercial hybrid in the test area.
[3]Disease severity index on a 0-4 scale, at 30 andy 60 days after transplant. A score lower that 2 is considered indicative of resistance.

The hybrids resulting from crosses to the susceptible cultivars (Table 3) were evaluated during the later part of 2002 (Table 5).

Table 5. Performance of R X S hybrids (April to September, 2002).

Hybrid No.	Description of the hybrid and origin of the resistant breeding line	Av. DSI 30	Av. DSI 60	Av. weight/ fruit (g)	Av. yield/plant (g)
	FAVI-12				
H14	GF2 X Sun Coast	1.81	2.51	48.3	612
H15	GF2 X M82	1.67	2.54	35.3	1314
H16	GF2 X HC-7880	1.76	2.38	48.9	1340
	FAVI-13				
H17	GF17 X M82	2.31	2.97	31.3	704
H18	GF17 X Very Firm	1.65	2.65	46.3	770
H19	GF17 X Rodade	1.18	2.07	49.3	1251
H20	GF17 X HC-7880	1.86	3.04	31.9	990
	FAVI-9				
H21	GF3 X Sun Coast	1.87	2.18	70.3	1448
H22	GF3 X Rodade	1.38	1.81	64.8	1719

H23	GF1 X M82	1.77	2.48	34.5	1327
H24	GF1 X Very Firm	1.64	2.43	55.6	1446
H25	GF1 X HC-7880	1.33	1.93	35.6	1320
	HUJI				
H26	632	1.80	2.37	68.4	1245
	Control				
H13	Marina (Sakata)	3.07	3.36	15.2	136

Additional hybrids were made by crosses among some of the resistant lines (Table 6).

Table 6. Performance of R X R hybrids (April to September, 2002).

Hybrid	Description of the hybrid and origin of the resistant breeding line	Av. DSI 30	Av. DSI 60	Av. weight/ fruit (g)	Av. yield/plant (g)
	TY-198 X FAVI-12				
H27	GL11 X GF2	1.19	1.63	48.4	1590
	FAVI-12 X TY-197				
H28	GF2 X GL12	1.77	1.23	55.1	2372
	FAVI-13 X TY-198				
H29	GF17 X GL11	1.52	1.82	47.2	1582
	FAVI-13 X TY-197				
H30	GF17 X GL12	1.29	1.51	47.4	1478
	TY-198 X FAVI-9				
H31	GL11 X GF5	1.20	1.44	57.1	2088
	FAVI-9 X TY-197				
H32	GF1 X GL12	1.27	1.60	52.0	1877
	TY-198XPimperJ13				
H33	GL11 X GP10	2.06	2.87	38.7	1193
	PimperJ13XTY-197				
H34	GP10 X GL12	1.32	1.77	38.6	1781
	FAVI-13 X FAVI-12				
H35	GF17 X GF2	1.31	1.48	55.9	1622
	FAVI-9 X FAVI-12				
H36	GF1 X GF2	1.30	1.33	64.9	1940
	Control				
H37	Elios (Peto Seed Co.)	3.10	3.32	22.4	116

After the *International seminar on breeding of vegetable crops for resistance to whitefly transmitted geminiviruses*, that was held in Antigua Guatemala in January, 2003, we were approached the manager of a seedling producing company, Mr. Richard Rotter, called *Pilones de Antigua*. Mr. Rotter was interested in starting a vegetable seed company (tomatoes and peppers) and wanted to know if we would be willing to share our germplasm with him. Some begomovirus resistant lines were transferred to him in March, after the cycle of selection, these lines were G13h, G9c (Table 2) and T47-1 (a line selected from an early cross between a selection from Favi 9 and Lignon, a Cuban cultivar). Additionally, two other lines 31-4 (derived from a cross between G11p X G5h) and 36-4 (from a cross G1h X G2h). These lines were crossed to produce hybrids *LLanero 10*, *Llanero 11*, *Llanero 12* and *Maya Garden*. Additionally, based on previous results (Table 4), F. Vidavsky prepared seed of the H7 hybrid (G3h X susceptible line from his program), now called *Llanero 7*. The term *Llanero*, meaning *plainsman*, refers to the area in Sanarate where our trials are

located, which is known by the locals as *Los Llanos* or the plains. The hybrid called *Maya Garden* was produced crossing two lines with high resistance and good flavor, irrespective of shape or firmness, with the idea planting it in home or school gardens for domestic consumption. These hybrids were first planted by *Pilones de Antigua*, at different locations, in early 2004 and at least some are being considered for commercial release, even though the shape of their fruit is round and the level of firmness is not as high as required in most market situations. Tables 8 and 9 show the performance of some of the breeding lines and hybrids in Sanarate during the last trial that has been conducted (December/2003 to April/2004). In view of the promising results that were observed in the *Llanero* hybrids, the owners of *Pilones de Antigua* decided to go ahead with the initiative of the seed company, and this has just recently been formed under the name *Gentropic Seeds*.

Table 7. Symptom severity and partial yield of begomovirus resistant breeding lines (December/2003 to April/2004).

Line	Av. DSI30	Av. DSI45	Av. DSI60	Av. Fruit Wt.	Av. Yield (partial)
G13h	1,2	1.6	1.6	72	1,219
G9c	1.2	1.6	1.6	61	800
G16c	1.4	1.8	1.8	55	1,110
902	1.3	1.7	1.7	nd	nd
M82	2.1	3.0	3.1	nd	nd

Table 8. Symptom severity and partial yield of begomovirus resistant hybrids (December/2003 to April/2004).

Hybrid	Av. DSI30	Av. DSI45	Av. DSI60	Av. Fruit weight	Av. Yield (partial)
Llanero 7 (G3h X Favi susceptible)	1.2	1.7	1.7	59	1,997
Llanero 10 (G9c X T47-1)	1.1	1.5	1.6	71	1,277
Llanero 11 (G13h X T47-1)	1.0	1.5	1.5	95	1,262
Llanero 12 (G13h X G9c)	1.0	1.5	1.5	87	1,973
Maya Garden (31-4 X 36-4)	1.1	1.6	1.6	61	1,189
Elios	2.4	2.8	3.0	31	802
Marina	2.4	2.8	2.9	27	596
Silverado	2.3	2.8	2.9	39	955
Sheriff	2.3	3.0	3.1	20	446

From all of these hybrids, individual plants carrying desirable traits have been selected for several cycles. The most important criteria for selection have been resistance to begomoviruses along with fruit shape, fruit size, firmness and yield. The preferred fruit type for the Guatemalan market is a plum shape (the so called *Roma* type), with high firmness. There have been three cycles of selection up to now; March, 2003, September, 2003 and March, 2004. Along with the Guatemalan team, D. Maxwell has participated in all three evaluations, tomato breeders J Scott and F. Vidavski participated in the second and third evaluations respectively. As a result of these cycles of selection a wide array of resistant germplasm has been developed.

2.1.3. Selection in the field, September 2003 – August 2004

After the second cycle of selection (September, 2003), several of these lines were considered to be far enough along the selection process to participate in the formation of a new set of hybrids. These lines possessed the desired characters of fruit shape and firmness and could hopefully give origin to hybrids which could soon be released to the growers. The lines were given to *Gentropic Seeds* and approximately sixty hybrids (denominated XA hybrids) were produced and will be transplanted for evaluation in Sanarate and other locations in early July, 2004. After the third cycle of selection (March, 2004), several additional breeding lines with the desired qualities became available (Table 9). Several of these lines were again given to *Gentropic Seeds* for crossing and approximately 300 new XA hybrids are being produced at the present time. It must be stressed that *Gentropic Seeds* has agreed to pay the corresponding royalty fees for the use of these lines and that all the costs related to the crossing of the lines for the production of hybrids and the evaluation of these hybrids are being covered by the company.

From July 13 to 15, 2004, two hundred and twenty breeding lines and segregating populations were transplanted in Sanarate. This trial will be evaluated in October, 2004.

Some hybrids were produced by crosses between resistant and susceptible lines (Table 4; R X S. H1-H3 and H6-H9) and others by crosses among resistant lines (R X R; H4 y H5). One of the most popular cultivars in the area, the hybrid Marina (Sakata Seed Co.; H13) was used as a control.

Table 9. Lines selected in March 2004 with an overall rating above 80. Combinations of some of these lines are being used in the formation of around 300 hybrids to be evaluated at the end of 2004.

Line	Parent 1	Parent 2	Resistance	Generation	DSI	Stage	Shape	Firm.	Ln.	Color	WT	Yield	Vigor	Overall
21h-3-2-1	Sun Coast	G3h	h	F5	0	Red	60	25			160	45	40	90
122h-1-3	Favi	Favi	h	F4	0	Red	55	25		30	60	30	30	80
165h-1-a	Favi	Favi	h	F4	1.5	Gr	55				150	40	40	80
117h-1-a	Favi	Favi	h	F4	0	Gr	50		5		150	40	45	85
T47h-1-1-1	Favi9	Lignon	h	F7	0	Red	50	20			100	40	40	80
31hp-7-1-1	G11p	G5h	hp	F5	1.5	Red	50	20			110	40	40	80
25h-6-a	GC6	G5h	h	F4	1	Red	50	40	4	35	80	35	40	80
31hp-4-1-2	G11p	G5h	hp	F5	0	Red	48	20		30	60	40	40	80
43c-5-4-a	Marina	G9c	c	F5	1.5	Red	42	35	4	30	60	40	45	95
43-c-5-1-2	Marina	G9c	c	F5	0	Red	40	30	5	30	90	40	30	90
25h-4-1-a	GC6	G5h	h	F5	1.5	Red	40	35	4	35	90	45	45	90
31hp-4-1-1	G11p	G5h	hp	F5	1	Red	40	35	3	40	25	30	40	85
124h-1-3	Favi	Favi	h	F4	1.5	Red	40	25	4		70	50	45	85
43-c-	Marina	G9c	c	F5	1	Red	40	25	5		60	40	30	80

Line	Parent 1	Parent 2	Resistance	Generation	DSI	Stage	Shape	Firm.	Ln.	Color	WT	Yield	Vigor	Overall
5-1-3														
43c-5-4-b	Marina	G9c	c	F5	1.5	Red	40	35	4	30	70	45	45	80
15h-1-1-a	G2h	M82	h	F5	1.5	Red	40	30	4	30	90	40	40	80
136h-1-a	GC6XG3h	SCXG2h	h	F4	1.5	Gr	40		4		90	40	40	80
105h-1-a	F6-2211	Favi	h	F6	1.5	Gr	38		6		120	40	40	90
43c-2-1-a	Marina	G9c	c	F5	1.5	Gr	38		5		100	35	40	80
44hp-1-2-a	Marina	GP10XG1h	hp	F5	1.5	Red	38	35	4	35	80	35	40	80
173c-1-a	Scott	Scott	c	F6	1	Red	38	40	4	35	80	35	35	80
43c-4-1-a	Marina	G9c	c	F5	1.5	Brk	37	40	6		110	40	40	90
43c-2-1-1	Marina	G9c	c	F5	1	Red	37	25	5		90	40	40	80
44hp-1-2-1	Marina	G10pXG1h	hp	F5	1	Red	37	25	4	35	50	40	40	80
105h-1-3	F6-2211	Favi	h	F6	1.5	Gr	37		5		60	30	30	80
137h-1-4	Marina	G2h	h	F4	1	Gr	37		5		50	45	40	80
25h-2-1-2	GC6	G5h	h	F5	2	Red	35	25	5		90	45	40	95
105h-1-4	F6-2211	Favi	h	F6	1	Red	35	25	5		80	30	30	85
143-1-1	Marina	GC6XM82		F4	1	Red	35	25	4	35	70	40	40	85
172c-2-a	Scott	Scott	c	F6	0	Red	35	30	3	30	60	40	45	85
43c-4-3-a	Marina	G9c	c	F5	2	Pk	35	35	6		110	45	40	85
143-1-2	Marina	GC6XM82		F4	1	Pk	35	30	5		40	35	35	80
124h-1-a	Favi	Favi	h	F4	1.5	Gr	35		6		140	40	40	80
43c-4-1-3	Marina	G9c	c	F5	1	Red	34	30	5		100	40	45	80
T44h-2-1-a	Favi9	Elios	h	F7	2	Gr	34		7		120	35	40	80
GA-1	?	?	?	?	1	Red	33	20	5.5	40	40	40	40	90
171c-2-1	Scott	Scott	c	F6	1	Pk	33	35	5		50	40	40	90
171c-2-a	Scott	Scott	c	F6	1	Red	33	35	3	30	60	40	35	90
LR171c-1-a	Scott	Scott	c	F6	1	Gr	33		4		60	45	40	90
173c-2-a	Scott	Scott	c	F6	1	Pk	33	35	5		80	40	40	90
124h-1-2	Favi	Favi	h	F4	1.5	Red	33	35	5	30	50	45	45	85

Line	Parent 1	Parent 2	Resistance	Generation	DSI	Stage	Shape	Firm.	Ln.	Color	WT	Yield	Vigor	Overall
173c-2-1	Scott	Scott	c	F6	1.5	Red	33	30	5	40	70	30	30	85
173c-2-2	Scott	Scott	c	F6	0	Red	33	30	4	45	80	35	35	85
171c-1-SB	Scott	Scott	c	F6	1	Pk	33	40	5	35	40	40	30	85
43c-4-1-2	Marina	G9c	c	F5	1	Red	33	35	5	35	40	40	45	85
173c-1-1	Scott	Scott	c	F6	1.5	Red	33	30	5	35	80	35	40	80
T44h-2-1-b	Favi9	Elios	h	F7	1.5	Gr	32		6		100	40	40	85
136h-4-1	GC6X G3h	SCXG2 h	h	F4	2	Red	28	25	6		100	35	40	85
43c-4-3-1	Marina	G9c	c	F5	1	Red	22	40	7		100	45	45	90
43c-4-1-1	Marina	G9c	c	F5	1	Gr	22		7		50	35	40	80

2.2. Identification of loci linked to resistance against Guatemalan geminiviruses in the selected tomato breeding lines utilizing the markers used to map genes for resistance to TYLCV and ToLCV (Eastern Hemisphere geminiviruses)

2.2.1. Background

Several research programs have introduced genes for resistance to begomoviruses from wild tomato species into *L. esculentum*. As indicated above the main breeding lines being used in Guatemala, which have high levels of begomovirus resistance, are those derived from the program of Drs F. Vidavski and H. Czosnek (*S. habrochaites* (*L. hirsutum*) as resistance source) and those from Dr. J. Scott in Florida (*L. chilense* as resistance source). Thus, our resistance gene tagging efforts have concentrated on tagging genes from these sources, and mainly the lines with *L. hirsutum* resistance genes. The molecular tagging approach involved the development of PCR primers from RFLP probes that are located in the region of known introgressions for *Ty1* gene from *L. chilense* in chromosome 6 (Theor. Appl. Genet. 88:141-146) and for *Ty2* gene from *L. hirsutum* in chromosome 11 (J. Amer. Soc. Hort. Sci. 125:15-20).

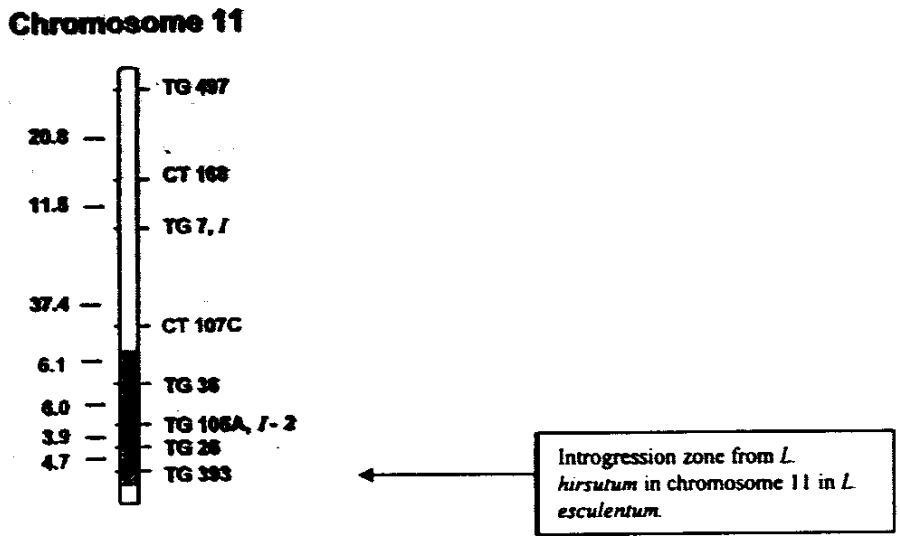
2.2.2. Application of PCR-based tagging approach

The general approach was to sequence RFLP probes located in the region of the *Ty1* or *Ty2* gene introgression. These probes were identified from the published papers and the chromosome maps at the Solanum Genome Network site (www.sgn.cornell.edu). These sequences were used to search for matching gene sequences in GenBank and the *Arabidopsis thaliana* genome data base (mips.gsf.de/projects/plants). PCR primers were designed to match the conserved sequences in the exons between *L. esculentum* and *A. thaliana*, so that at least one intron and more, if possible, would be amplified by the PCR primer pair. The PCR fragments were cloned and sequenced or sequenced directly. These sequences were compared with DNA software such as GCG or DNAMAN.

2.2.3. Chromosome 11 introgression for Ty2 gene:

The *Ty2* gene from *L. hirsutum* var. *glabratum accession B6013* is located in chromosome 11 (Fig. 1) (Hanson et al. J. Amer. Soc. Hort. Sci. 125:15-20). The probes TG105, TG26 (pUC8, PstI, 2,400 bp), TG30 (PUC8, EcoRI, 2,000 bp), TG36 (pUC8, PstI, 3,200 bp) and TG393 (pGEM4z, PstI, 1,200 bp), which map to this region, were obtained and sequenced. The resistant line, H24, homozygous for *Ty2* gene was supplied by Peter Hanson from Asian Vegetable Research Development Center. DNA of *Solanum habrochaites* (*L. hirsutum*) accessions 1928, and 1353 and *S. habrochaites* f. glabratum LA 1223 (Amer. J. Botany 88:1888-1902) was supplied by D. Spooner, USDA and Department of Horticulture, University of Wisconsin-Madison. Susceptible lines *L. esculentum* line was *L. esculentum* var. *cerasiforme* and M82.

Figure 1. Chromosome 11. Arrow indicates location of introgression from *L. hirsutum* reported by Hanson et al. (J. Amer. Soc. Hort. Sci. 2000, 125:15-20).



Design of PCR primers for amplification of fragments from the region of the Ty2-gene introgression involved development of primers for RFLP markers using the sequence of the ends of the markers TG36, TG30, TG105, TG26, CT120, and TG393. From these effort, the primer pair for the sequence of TG105(M13F) had a high nt identity with (95%) with *Solanum demissium* BAC clone, AC091627, at 50,320..50,486 was most informative and only this data is provided. Primer P105s (5'-ctt cag aat tcc tgt ttt agt cag ttg aac c-3') and P105c (5'-atg tca cat ttg ttg ctt gga cca tcc-3') gave a single 450-bp fragment with various genotypes of tomato. These PCR fragments were directly sequenced (Fig. 2).

Conclusions: In a region of about 160 nt, the sequence of H24 with the *Ty-2* gene introgression from *S. habrochaites* for ToLCV resistance shares 10 SNPs and one large indel with the three accessions of *S. habrochaites*. These are unique for the H24 and the three wild species accessions and clearing demonstrate that an introgression for *S. habrochaites* has occurred in this region of chromosome 11. Since these SNPs and the indel are not present in 902h, G1h, and G2h, which all have begomovirus resistance derived from *S. habrochaites*, it is concluded that this TG105 locus does not contain the resistance gene for these lines. TY52, TY50, G1h, *cerasiforme*, and HC7880 have identical

sequences in this region and G1h is resistant to begomoviruses in Guatemala and the other three are susceptible in Guatemala and TY52 has the *Ty-1* gene introgression in chromosome 6.
The results with the locus TG105 show that this PCR-based tagging method can be used to tag regions of introgressions for wild species.

Figure 2. Sequence from PCR fragments amplified with primer pair P105s/P105c from tomato breeding lines and three wild species accessions of hir = *S. habrochaites* (*L. hirsutum*)(Amer. J. Botany 88:1888-1902). 902h, breeding line with resistance to TYLCV (Phytopathology 88:910-914) with resistance derived from *S. habrochaites*; G9c line selected in Guatemala from Fla 595-2 with resistance to TYLCV and ToMoV from *S. chilense* (J. Scott); G2h, line selected from hybrid FAVI 12 (begomovirus resisted derived from 902h); HC7880, open pollinated cultivar from Cuba susceptible to begomoviruses in Guatemala; Cera, *S. lycopersicum* var. *cerasiforme* collected in Sanarate, Guatemala, susceptible to begomoviruses; G1h, line selected from hybrid FAVI 9 (begomovirus resistance derived from 902h); TY52, isolines with *Ty-1* gene from *S. chilense* (Zamir et al. TAG 88:141-146); TY50, isoline to TY52 without *Ty-1* gene for TYLCV resistance; H24, line with *Ty-2* gene introgressed from a *S. habrochaites* into chromosome 11 in the region of the TG105 marker (Hanson et al. J. Amer. Soc. Hort. Sci. 125:15-20).

```
902h        TTTGCAAACCCCTAACAAATAGACTAAGCCCTTTAACTTGTTT.AAGAGATGTCAATTTA     59
G9c         TTTGCAAACCCCTAACAAATAGACTAAGCCCTTTAACTTGTTT.AAGAGATGTCAATTTA     59
G2h         TTTGCAAACCCCTAACAAATAGACTAAGCCCTTTAACTTGTTT.AAGAGATGTCAATTTA     59
HC7880      TTTGCAAACCCCTAACAAATAGACTAAGCCCTTTAACTTGTTTtAAGAGATGTCAATTTA     60
Cera        TTTGCAAACCCCTAACAAATAGACTAAGCCCTTTAACTTGTTTtAAGAGATGTCAATTTA     60
G1h         TTTGCAAACCCCTAACAAATAGACTAAGCCCTTTAACTTGTTTtAAGAGATGTCAATTTA     60
TY52        TTTGCAAACCCCTAACAAATAGACTAAGCCCTTTAACTTGTTTtAAGAGATGTCAATTTA     60
TY50        TTTGCAAACCCCTAACAAATAGACTAAGCCCTTTAACTTGTTTtAAGAGATGTCAATTTA     60
H24         TTTGtAAACCCCTAACAAATAGACaAAaCCCTTcAACTTGTTTtAAGAcATGTaAATTTA     60
hir 1353    TTTGtAAACCCCTAACAAATAGACaAAaCCCTTcAACTTGTTTtgAGAcATGTaAATTTA     60
hir 1928    TTTGtAAACCCCTAACAAATAGACaAAatCCTTcAACTTGTTTtAAGAcATGTaAATTTA     60
hir 1223    TTTGtAAACCCCTAACAAATAGACaAAaCCCTTcAACTTGTTTtAAGAcATGTaAATTTA     60
Consensus   tttg*aaaccccctaacaaatagac*aa* cctt*aacttgttt  aga*atgt*aattta

902h        TGTATTTATATTTACAAAACTAAAATTTAATCACTATATACAACGTAATTTTCCGACAAA     119
G9c         TGTATTTATATTTACAAAACTAAAATTTAATCACTATATACAACGTAATTTTCCGACAAA     119
G2h         TGTATTTATATTTACAAAACTAAAATTTAATCACTATATACAACGTAATTTTCCGACAAA     119
HC7880      TGTATTTATATTTAtAAAACTAAAATTTAATCACTATATACAACGTAATTTTCCGACAAA     120
Cera        TGTATTTATATTTAtAAAACTAAAATTTAATCACTATATACAACGTAATTTTCCGACAAA     120
G1h         TGTATTTATATTTAtAAAACTAAAATTTAATCACTATATACAACGTAATTTTCCGACAAA     120
TY52        TGTATTTATATTTAtAAAACTAAAATTTAATCACTATATACAACGTAATTTTCCGACAAA     120
TY50        TGTATTTATATTTAtAAAACTAAAATTTAATCACTATATACAACGTAATTTTCCGACAAA     120
H24         TGTATTTATATTTAtAAAACTAAAATTTAAcCACTATATACAACGTAATTTTCCGACgAA     120
hir 1353    TGTATTTATATTTAtAAAACTAAAATTTAAcCACTATATACAACGTAATTTTCCGACgAA     120
hir 1928    TGTATTTATATTTAtAAAACTAAAATTTAAcCACTATATACAACGTAATTTTCCGACgAA     120
hir 1223    TGTATTTATATTTAtAAAACTAAAATTTAAcCACTATATACAACGTAATTTTCCaACgAA     120
Consensus   tgtatttatattta aaaactaaaatttaa*cactatatacaacgtaattttcc ac*aa

902h        GGAGTGTCG.............AGCCAAGGTAGGTCCTG                          145
G9c         GGAGTGTCG.............AGCCAAGGTAGGTCCTG                          145
G2h         GGAGTGTCG.............AGCCAAGGTAGGTCCTG                          145
HC7880      GGAGTGTCG.............AGCCAAGGTAGGTCCTG                          146
Cera        GGAGTGTCG.............AGCCAAGGTAGGTCCTG                          146
G1h         GGAGTGTCG.............AGCCAAGGTAGGTCCTG                          146
TY52        GGAGTGTCG.............AGCCAAGGTAGGTCCTG                          146
TY50        GGAGTGTCG.............AGCCAAGGTAGGTCCTG                          146
H24         GGAGTGTCGctcgacacccctt ggaCCAAGGTAGGTCCTc                        160
hir 1353    GGgGTGTCGctcgacacccctt ggaCCAAGGTAGGTCCTc                        160
hir 1928    GGgGTGTCGctcgacacccctt ggaCCAAGGTAGGTCCTc                        159
hir 1223    GGAGTGTCGctcgacacccctt ggaCCAAGGTAGGTCCTc                        159
Consensus   gg*gtgtcg**********************ccaaggtaggtcct*
```

For the TG105 locus, the PSNP105s/PSNP105c-PCR fragment was sequenced for 11 tomato lines and the three S. habrochaites accessions. For the 11 tomato lines, there were three SNP-indel patterns, the esculentum type, the habrochaites type, and the chilense type. Lines G1h, HC7880, M82, G3h had the *esculentum* type, lines G2h, 902h, G9c, and GL11 had the *chilense* type, and lines H24 and CLN2498-68 had the *habrochaites* type. Thus, the lines derived from 902 in Guatemala, existed in only the *esculentum*- and *chilense*-type patterns and not the *habrochaites* type.

2.2.4. Testing of other primer pair for introgressions of the resistance gene in 902 in chromosome 11 in the region of Ty-2 gene.

2.2.4.1. Aldehyde Oxidase primers (PALO, Chromosome 11):
The TG105 sequence had a high identity to the *S. demissum* BAC (AC091627) and there were several gene sequences associated with this BAC clone. One gene, aldehyde oxidase (exon nt numbers 76,163 – 76,310 to 77,520 – 77,821) has a 97% nt identity with the aldehyde oxidase of tomato (mRNA sequence, AF258814). Two PCR primers were designed (PALO2s/PALOs) to give an amplification of an intron, which is present in the *S. demissum*. PCR with this primer pair produced multiple bands, two bands of around 1.1 and 1.2 kb and one band of the expected size of 666 bp. The band of the expected size from G1h and *S. habrochaites* 1928 was cloned, despite repeated trials the band corresponding to HC7880 could not be cloned. This multiple band situation was not improved when annealing temperature was raised from 50 to 57°C and these primers were not used for further analyses.

2.2.4.2. TG30 Locus:
PCR primer pair (P30s/P30c) amplified a 500-bp fragment and the sequence was identical for HC7880, M82, G1h, G2h, G3h, G9c, 902, H24 (Ty2 gene), GL11, CLN2443A (Ty2 gene), CLN2498-68. Thus, there is no evidence for an introgression from any wild species in this region of these lines.

2.2.4.3. TG26, T1660 and cLET24J2 region:
The primers were designed as follows. The M13F TG26 sequence (complete sequence, App. I) had a 94% and 96% nt identity for 189 nt with 46,465..46,654 and for 172 nt with 46,783..46,952, respectively, of *S. demissum* BAC, AC136471 (123,428 nt). Two other RFLP probe markers (cLET24J2 and T1660) also had high nt identity with this BAC clone sequence. The sequence 10..450 of cLET24J2 had a 97% nt identity with 74,065..74,505 of *S. demissum* and a 78% nt identity with the calmodulin mRNA (NM_101103). These sequences also gave positive matches with *A. thaliana* (AC007190, gene At1g12310). Two putative exons were identified in the *S. demissum* sequence; one corresponded to 430..565 and 1..476 of cLET24J2 with 71,548..71,614 and 74,515..74,066, respectively, for *S. demissum*. Two primers, PCAL1s and PACL1c, were designed to amplify the putative intron with a fragment sequence of about 2,500 bp. For the T1660 marker sequence, it had a 82% nt identity with *A. thaliana* (AF069299, 31,303..31,427), which indicated the presence of an exon. Additionally, there was a high nt identity of T1660 regions, 4..63, 66..141, and 143..279 with *S. demissum* AC136471 for regions 70,226..70,286, 70070..70145, and 69030..69166, respectively. The *A. thaliana* region corresponds to a putative ribosomal protein and the exons for this gene were used to match regions of *S. demissum* (68,683..70,248) and then design primers. This is the region of *S. demissum* BAC that corresponds to T1660 sequence. Primers PRib3s and PRib3c were designed from the *S. demissum* sequence (regions 69,110..69,132, and 69,130..70,110) that was conserved with *A. thaliana*. Because the *S. demissum* BAC AC136471 was not annotated, the GlimmerM (ver. 3) program at TIGR web site was used to detect putative ORFs.

The ORFs that appeared to be mostly likely genes as determined by length of exons over 1,000 bp were used to detect regions of high identity with *A. thaliana*. For reference, the tomato RFLP marker probes sequences for TG26, T1660, and cLET24J2 correspond to nt number 46,600, 69,800, and 73,500, respectively. Putative ORF #53 (77,727..79,701) had high identity with *A. thaliana* (AB018109, eg. 49,447..49,778) and the exons from *A. thaliana* were compared with the sequence of *S. demissum* and two primers (P53s and P53c), which would give a PCR fragment of 790 bp for *S. demissum*. Putative ORF #69 (117,424..119,162) had a high identity (96%) with AF049900 of *L. esculentum* mRNA for gibberellin 20-oxidase and also for *A. thaliana* (85%, AL161563). The mRNA sequence for *L. esculentum* was compared with *S. demissum* and two sets of primers (P69s2/P69c2 and P69s1/P69c1) designed from putative exons to amplify different introns.

2.2.4.4. Ribosomal Protein primers (Chromosome 11):
Fragments corresponding to HC7880, G1h and *S. habrochaites* 1928 were cloned and sequenced. The fragment corresponding to *L. hirsutum* 1928 was larger (around 900 bp) than the one from HC7880 and G1h (around 650 bp). These fragment sequences were aligned and it was found that 1928, which is "*L. hirsutum*" DNA, differs substantially from the *L. esculentum* sequence. There is for example a large intron which is missing in the *L. esculentum* sequence. There is one SNP between the G1h and HC7880, an A/G substitution. The HC7880 allele was designated G and the G1h allele g (Table 10).

Table 10. Genotypes of parental lines and segregating progeny for the ribosomal protein sequence.

Line	Phenotype	Genotype
G1h	R	g/g
HC7880	S	G/G
F3-11	R	g/g
F3-15	R	g/g
F3-18	S	g/g
F3-31A	S	g/g

Since two susceptible plants had the G1h allele, this region was not thought to be involved in resistance for the G1h phenotype.

2.2.4.5. Calmodulin protein primers (PCal1, Chromosome 11):
A PCR fragment of around 2.3 kb was amplified from the parental lines, HC7880, G1h, G2h, G3h and 1928. An attempt was made to clone this fragment, the digestion of plasmid DNA with EcoRI and BstZ1, however, produced unexplainable results and this effort was abandoned.
2.2.4.6. TG26 locus:
PCR primers (P26s/P26c) gave fragments which were sequenced. Three resistant lines -G1h, G2h, G3h, and one susceptible line M82 had identical sequences: there were 16 SNPs between the sequence for these lines and *S. habrochaites* 1928, which does not support an introgression in this region from *S. habrochaites* in these lines. It would be interesting to test H24 with these primers.

2.2.4.7. T1660 locus:
PCR primers (P69s2/P69c2) gave an 820 bp-fragment which was sequenced. The sequence for the susceptible M82 and HC7880 lines was identical to the begomovirus resistant line G3h. Thus, there is no evidence for an introgression in this region. The abi sequence indicated that they are homozygous for this region. When the sequence was used in a BLAST search, a >97% nt identity was obtained for 8..174 and 745..820 with 784..950 and 949..1023 for the mRNA for gibberellin 20

oxidase-3 (AF04990). The intron region for tomato should be compared with the intron for *S. demissum*, which was used to design the primers.

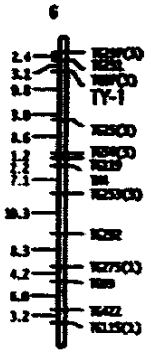
2.2.4.8. P120 primers (Chromosome 11)
Three sets of primers corresponding to this region were designed, 120S-120ASC, 120S-120C and 120S-120AC. Only primer pair 120S-120ASC produced a single band (around 450 bp); this band, corresponding to parental genotypes HC7880, G1h, G2h and G3h, was sent for direct sequencing. Increasing the temperature of annealing to 57^{0}C with primer pair 120S-120AC resulted in the loss of the three bands that had been observed (0.4, 1.1 and 2 kb).

2.2.4.9. Probe 393 (Chromosome 11)
The PCR products corresponding to this probe (around 900 bp, primers P393s/P393c) were sequenced directly . No differences were observed between the parental lines HC7880, M82, G1h, G2h and G3h except G2h may have one indel at about 90 nt (an A).

2.2.5. Chromosome 6 introgression for Ty1 gene

For the *Ty1* gene, clones of probes TG297 and TG436 in chromosome 6 that are located in the introgressed region from *L. chilense* in the resistant isoline TY52 (Theor. Appl. Genet. 88:141-146) (Fig. 3) were sequenced. TY52 and TY50, isoline to TY52 without the introgression, were supplied to Dani Zamir, Hebrew University of Jerusalem (HUJ). The RFLP map of the introgression was provided by H. Czosnek, which showed that introgression was TG97 and TG232.

Fig. 3. Chromosome 6. Introgression from *L. chilense* (From Zamir et al. 1993. Theor. Appl. Genet. 88:141-146).



TG436 is located in region between *Ty1* and TG25.

For primer design, the RFLP probes CD67 (no sequence), TG97 (PstI, 1.4 kb, no good sequence), CT216c (EcoRI, 1.7 kb), TG232 (PstI, 1,415 bp), TG297 (PstI, 1,807 bp), TG436 (PstI, 1377 bp, HUJ; PstI, 1036 bp, Cornell University) were sequenced. Sequences for each probe were compared with data bases for *Arabiopsis* and GenBank. CT216c had one exon and one intron match with AT5g59550 (no primers designed). TG232 had 70% identity with for 28 – 496 nt with AC009991 (PTG232s1/PTG232c1). TG297 had an excellent match with AT5g22770 and 8 exons were identified (P297s3, P297s6, P297c6, P297c8). The sequence for the Cornell University probe TG436 matched exon 5 and 6 of At2g47680 (ATP-dependent RNA helicase A) (PCT436s5b/PCT436c6).

The most useful information was with PCR primer pairs for TG436 locus: PCT436s5b (5' cct tcc aac ata cta tgc act tga gc 3'), PCT436c6 (5' ccc aga aaa ctt gta aag aac ggc 3'). This primer pair gave a PCR fragment of about 900 bp, which was cloned and sequenced for *S. habrochaites*

LA1223, G1h, TY52, TY50 and HC7880. Two indels were detected (Fig. 4) among these five genotypes and TY52 and G1h shared the same indel pattern. As these were the two genotypes that had resistance to begomoviruses, two PCR primers were designed to amplify a 300-bp fragment (PSNP436s, 5' tct gca agt cgc atc gga agg tat gc 3'; PSNP436c, 5' gta tgg gcc acc tgg cat gca cct cg 3') that would amplify the region with the two indels.

This primer pair was used to amplify PCR fragment, which was sequenced, from 10 additional genotypes. The genotypes were divided into two groups based on the two indels (Fig. 4). Those that have *esculentum*-type sequences and those that had the TY52 or *chilense*-type sequences. Those in the *esculentum*-type were TY50, HC7880, M82, G3h, GL11, CLN2443A (Ty 2 gene), CLN2498-68, and H24 (Ty 2 gene). The *chilense*-type sequence group included TY52 (Ty 1 gene), G1h, G2h, 902h, and G9c. G9c has resistance to TYLCV and ToMoV derived from *S. chilense*, so it may have the Ty 1 gene. Lines with resistance in Guatemala to begomoviruses were in both groups, which indicated that this region is not the location of the introgression from *S. habrochaites*, ie for G3h.

Because of the presence of the chilense-type sequence in two resistant lines from Guatemala, G1h and G2h, the presence or absence of these two indels was determined in F3 populations from four crosses. Lines with resistance to begomoviruses G1h And G2h were crossed to susceptible parental lines HC-7880 and M82. The resulting F1 hybrids were planted at the beginning of 2002 (Table 1). The F2 populations were planted in early 2003 and resistant and susceptible plants (the most extreme phenotypes) were selected in March, 2003 (see web site for images: http://www.plantpath.wisc.edu/GeminivirusResistantTomatoes/CDR/Mar03/H16RS.htm). DNA was extracted from pooled tissue of the progeny of these plants (around 15 seedlings). This DNA, along with DNA from the parental lines, was used during this period (July, 2003) to screen for the indel marker in region 436 of Chromosome 6

Table 11. Hybrids evaluated in Guatemala from September to April 2002.

Hybrid	Description	DSI 30	DSI 60	Average Weight/fruit (g)	Average yield/plant (g)
H15	GF2 X M82	1.67	2.54	35.3	1,314
H16	GF2 X HC-7880	1.76	2.38	48.9	1,340
H23	GF1 X M82	1.77	2.48	34.5	1,327
H25	GF1 X HC-7880	1.33	1.93	35.6	1,420

Two approaches were used to analyze the F3 populations. In one case, fluorescent-labeled primers were designed to be used with primers PSNP436s or PSNP436c for amplification of the sequence containing one of the two indels in that region. The PR fragments were analyzed with a Gene Scan single lane system in order to determine their length; those with a deletion were expected to be shorter by two nucleotides. This is what was detected. The other approach involved the sequencing of PCR fragments from the primer pair PSNP436s/PSNP436c and scoring each sequence for *esculentum*-type, *chilense*-type or heterozygous. It was very easy to detect the heterozygous genotypes as the sequence would go out of frame exactly at the position of one indel and then into frame at the exact position of the second indel.

Results obtained with fluorescent (FAM) labeled primers and sequence data are shown in Table 3. HC7880, M82 and GF3 have genotype E/E (homozygous for *esculentum* type, AA deletion followed by AC insertion). G1h and G2h have genotype e/e (homozygous, *chilense* type, AA insertion followed by AC deletion). For the resistant phenotype, there are 2, 13, and 5 plants in class E/E, E/e, and e/e, respectively. For the susceptible plants, there are 7, 5, and 4 plants in class E/E, E/e, and e/e, respectively. When the data are combined across both susceptible and resistant

phenotypes (9, 18, 9 plants for the E/e, E/e, e/e, respectively), the segregation fits a 1:2:1 ratio, which would be expected for a random selection of plants.

Conclusion: Thus, the combined data are not supportive of a linkage between the resistant phenotype in the G1h and G2h lines and the molecular marker TG436. It is possible that these indels can be used as a marker for the *Ty-1* gene as they are present in TY52 and G9c.

Figure 4. Sequence of PCR fragment with primer pair (PSNP436s/ PSNP436c) for *S. habrochaites* (*L. hirsutum*) LA1223, and HC7880, open pollinated cultivar from Cuba susceptible to begomoviruses in Guatemala; G1h, line selected from hybrid FAVI 9 (begomovirus resistance derived from 902h); TY52, isolines with *Ty-1* gene from *S. chilense* (Zamir et al. TAG 88:141-146); TY50, isoline to TY52 without *Ty-1* gene for TYLCV resistance.

```
hir-1223    AAAAGTTTGTGGAGATAGTCCTTAAAGCAGGCAGAATACTGCACCCAAGGGAGTGACATA    60
TY50        AAAAGTTTGTGGAGATAGTCCTTAAAGCAGGCAGAATACTGCACCCAAGGGAGTGACATA    60
HC7880      AAAAGTTTGTGGAGATAGTCCTTAAAGCAGGCAGAATACTGCACCCAAGGGAGTGACATA    60
TY52        AAAAGTTTGTGGAGATAGTCCTTAAAGCAGGCAGAATACTGCACCCAAGGGAGTGACATA    60
G1h         AAAAGTTTGTGGAGATAGTCCTTAAAGCAGGCAGAATACTGCACCCAAGGGAGTGACATA    60
Consensus   aaaagtttgtggagatagtccttaaagcaggcagaatactgcacccaagggagtgacata

hir 1223    GTTGATCAATAAAGTAGGGAAAA..CCATGAGGTCTCGGGTTATTAAAATCTGAGTAGAG    118
TY50        GTTGATCAATAAAGTAGGGAAAA..CCATGAGGTCTCGGGTTATTAAAATCTGAGTAGAG    118
HC7880      GTTGATCAATAAAGTAGGGAAAA..CCATGAGGTCTCGGGTTATTAAAATCTGAGTAGAG    118
TY52        GTTGATCAATAAAGTAGGGAAAAaaCCATGAGGTCTCGGGTTATTAAAATCTGAGTAGAG    120
G1h         GTTGATCAATAAAGTAGGGAAAAaaCCATGAGGTCTCGGGTTATTAAAATCTGAGTAGAG    120
Consensus   gttgatcaataaagtagggaaaa**ccatgaggtctcgggttattaaaatctgagtagag

hir 1223    TCAAAACCACTAGGTGATTCTCATTTGCACAAGACCCCAAACCTTGGTGGACAGATTTAC    178
TY50        aCAAAACCACTAGGTGATTCTCATTTGCACAAGACCCCAAACCTTGGTGGACAGATTTAC    178
HC7880      aCAAAACCACTAGGTGATTCTCATTTGCACAAGACCCCAAACCTTGGTGGACAGATTTAC    178
TY52        aCAAAACC..TAGGTGATTCTCATTTGCACAAGACCCCAAACCTTGGTGGACAGATTTAC    178
G1h         aCAAAACC..TAGGTGATTCTCATTTGCACAAGACCCCAAACCTTGGTGGACAGATTTAC    178
Consensus   *caaaacc**taggtgattctcatttgcacaagaccccaaaccttggtggacagatttac

hir 1223    CCGGTACCTGTGTTAGTGGGACTTGGGAGGTAGCATGTACTCGATAGAATTAGTCGAGGT    238
TY50        CCGGTACCTGTGTTAGTGGGACTTGGGAGGTAGCATGTACTCGATAGAATTAGTCGAGGT    238
HC7880      CCGGTACCTGTGTTAGTGGGACTTGGGAGGTAGCATGTACTCGATAGAATTAGTCGAGGT    238
TY52        CCGGTACCTGTGTTAGTGGGACTTGGGAGGTAGCATGTACTCGATAGAATTAGTCGAGGT    238
G1h         CCGGTACCTGTGTTAGTGGGACTTGGGAGGTAGCATGTACTCGATAGAATTAGTCGAGGT    238
Consensus   ccggtacctgtgttagtgggacttgggaggtagcatgtactcgatagaattagtcgaggt
```

2.3. RFLP – tagging of resistance genes to begomoviruses in a Guatemalan breeding line

Since no region of introgression had been detected for the resistance genes in 902 derived lines, an RFLP approach was also tried. DNA from two susceptible lines (HC7880 and M82) and one line (G1h) resistant to the begomoviruses in Guatemala and derived from FAVI 9 (resistance genes from 902) were subjected to standard RFLP analysis with ^{33}P-labelled probes for each of the 12 chromosomes. DNAs were digested with five restriction enzymes: *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, or *Xba*I. Chr1, top TG301, middle TG19; Chr2, top TG227; Chr3, middle TG129; Chr4, top TG146, middle TG62, bottom TG450 ; Chr5, bottom TG23, bottom CD74; Chr6, top TG231, TG119, middle TG54, bottom TG422; Chr7, top TG438, bottom CD54, Chr8, top TG41, middle TG282; bottom TG402; Chr9, top TG10, bottom TG8; Chr11, top TG194, bottom TG30, TG26 (TY2 gene introgression region); Chr12, middle CT79, bottom CD2.

Conclusions: No polymorphisms were detected for these 125 combinations of probes (25) and restriction enzymes (5).

2.4. Introduction of molecular based tagging of genes for use in a breeding program at San Carlos University.

Several options for molecular gene tagging were considered originally. The Volcani Institute group had limited success with the PCR markers for *Verticillium* wilt resistance and Hebrew University was using the REX markers for the nematode resistance gene (Mi). It was decided that this marker would be use to introduce these methods into the biotechnology curriculum at San Carlos University. The technique involved PCR and restriction enzyme digestions, which could be performed in Dr. Mejia's laboratory at San Carlos University. Also, it would be very useful to develop breeding lines for Guatemala that had both resistance to begomoviruses and root knot nematode. This has not been possible with the Ty-1 gene, since this gene for TYLCV resistance is very closely linked to the *Mi* gene.

2.4.3. Primers REXF1/REXR2 (Chromosome 6) – REX locus

The REX primers amplify a molecular marker linked to *Mi* gene (root knot nematode resistance) in Chromosome 6 (Williamson et al. 1994. TAG 87:757-763). This primer pair amplifies a single band of around 750 bp from tomato breeding lines and hybrids. The REX fragment is then digested with *Taq*I restriction enzyme, and if a TaqI site (TCGA) is present, the REX fragment is linked to the *Mi* gene (ie an introgression from *S. peruvianum*). This gives a digestion of two fragments, 570 and 160 bp. The *esculentum* sequence does not have the sequence associated with the *Taq*I restriction site. A heterozygous plant would give three bands after digestion.

An M. Sc. student, Carolina Zea, at San Carlos University did her thesis research on the application of this marker for evaluation of germplasm in the breeding lines being developed in this CDR project. A cross was made between Marina, a commercial cultivar with the N gene (*Mi* gene) and the hybrid H5 (G1h x G10p) giving population H44. This H44 population was evaluated in the field in March 03 and plant 29 was resistant to begomoviruses. This plant was identified by the REX marker in Guatemala as being heterozygous for the *Mi* gene. One potential problem with digestions is the possibility that incomplete digestions could produce erratic results. In order to verify digestion results, several seedling progenies (F3 plants) were produced in Guatemala and the DNA from these plants was brought to Wisconsin and amplified with the REX primers. The amplified fragments were sent for direct sequencing, using the REXR2 primer. Sequence analysis of the REX PCR fragment clearly distinguished among the three classes of plants, homozygous for the *Taq*I site, heterozygous, and homozygous for no *Taq*I site. The presence of the *Taq*I site corresponds to the resistance gene marker and is associated with a SNP for A/C, T for *Mi* gene and G for *Mi*+ gene or *L. esculentum* phenotype (susceptible). A commercial hybrid, Better Boy, reported to have the *Mi* gene (N) was heterozygous (A/C), G1h and G2h were homozygous for the *Mi* gene marker (A/A), and G3h, HC7880, M82 and *L. esculentum* var. *cerasiforme* were homozygous for the *Mi*+ gene (susceptible allele) marker (C/C).

At the Tomato Round Table Meeting in Guatemala in 2002, Dr. Judith Milo from the Hebrew University of Jerusalem reported that the REX locus could be used to tag the *Ty1* gene (*chilense*) for resistance to TYLCV. So we wanted to compare the REX locus sequence for a line with *Ty1* gene (TY52), a line with *S. habrochaites* (*L. hirsutum*) (902), a control cultivar that is homozygous for the resistance Mi gene (*Mi/Mi*, Motelle) and the susceptible lines (*Mi+/Mi−*, Moneymaker and M82). Dr. Valerie Williamson supplied seeds of Motelle and Moneymaker, Dr. Favi Vidavski seeds of the 902 breeding line and M82, and Dr. H. Czosnek seeds for the TY52 and TY50 from D.

Zamir. The TaqI site (CAPS marker) was evident in Motelle (TaqI site present) and restriction site was lacking in the REX fragment for the susceptible line (M82) (Fig. 5). The susceptible line had a SNP at this position and the sequence was C instead of A, as was present in Motelle. This result is consistent with the expected results for the TaqI digestions (Williamson et al. TAG 87:757). The three presumably susceptible lines, M82, TY50 and *L. esculentum* var. *cerasiforme* had the identical sequences for this 600 nt region. Four lines, Motelle, TY52, G2h, and 902, had the A nt for this TaqI site SNP. This was unexpected as TY52 and G2h and 902 were thought to be susceptible to root knot nematode. These three lines were subjected to a standard bioassay for root knot resistance. TY52 and 902 were found to be susceptible in the bioassay conducted at Hebrew University of Jerusalem (F. Vidavski, pers. com.) and G2h was resistant to root knot nematode at UC-Davis (V. Williamson, pers. com.). **Thus, in two cases the presence of the *TaqI* SNP (A) gave false positive results.** In this 600-nt region of the REX fragment, there are nine SNPs between the three susceptible lines (TY50, M82 and cerasiforme) and the three lines Motelle, 902, and G2h. However, only seven of these SNPs were shared by TY52 and Motelle. There were four SNPs that could distinguish TY52 from Motelle. It was concluded that three types of sequences could be recognized by the unique SNPs for the REX locus, ie, *esculentum* type (M82), *peruvianum* type (Motelle), and *chilense* type (TY52). Dr. Valerie Williamson noted that the *chilense*-type sequence had two *TaqI* sites, and thus would give three fragments when digested with *TaqI* restriction enzyme. SNPs associated with the *peruvianum*-type sequence were easily detected in commercial hybrids (heterozygous for the REX locus) such as Better Boy and Marina.

Because 902 is the main source of resistance to begomoviruses being used in Guatemala and also the Tomato MERC countries (Palestinian Authority, Lebanon, Jordan, Egypt, Morocco, and Tunisia), it was decided that a new PCR-based method for detection of the *Mi* gene should be developed. Dr. S. Tanksley, Cornell University, had developed a PCR-based method (CAPS) for detection of the *Mi* gene and it is protected by intellectual property by the Cornell University Foundation. Dr. Maxwell negotiated with the Cornell University Foundation to evaluate the Tanksley PCR-based method (Cornell method). He was given permission to evaluate the marker for research only and that they could not be used in a breeding program. The Cornell method was evaluated with the same germplasm as had been tested for the REX primers. As with the REX locus CAPS marker, the Cornell method gave false positives for TY52 and 902. The expected results for linkage to the *Mi* gene were obtained with Motelle and G2h. The PCR fragments from the Cornell primer pair were sequenced and as before the TY52 could be distinguished from Motelle and M82 by several SNPs, thus the Cornell PCR fragment could distinguish between *chilense*-type, *peruvianum*-type and *esculentum*-type sequences. **The surprise was that 902 had the *chilense*-type sequence and not the *peruvianum* type sequence.** Unfortunately, this information can not be used to develop a specific PCR-based system, which would distinguish the three types of sequences, because of the intellectual property agreement with Cornell University Foundation.

Dr. Valerie Williamson's group cloned the Mi gene region and sequenced a BAC clone (U81378) and the sequence for two genomic regions for the Mi1.2 and Mi1.1 gene (Plant Cell 10:1,307-1,319). Several primers specific to the promoter sequence of the functional gene, Mi.12, were designed and tested. From this effort, primer Mi12F1 (5' gca att cta gat cta gct att tgt tgt tc 3') and Mi12R2 (5' cct gct cgt tta cca tta ctt ttc caa cc 3') gave the most encouraging results. This primer pair gave a 720 bp and 620 bp fragment with Motelle (resistant) and Moneymarker (susceptible), respectively. For the heterozygous cultivars, Better Boy and Marina, two fragments (620 and 720 bp) were obtained. These data are consistent with the results from the REX locus and the Cornell locus. Six other lines that are thought to be susceptible to room knot nematode all gave fragments of 620 bp. G2h, which is resistant, gave a 720 bp fragment. Line Ty52, which gives false positives with the REX locus and Cornell locus gave a 1,000-bp fragment. Also, G9c, which

as the *chilense*-type sequence for REX locus and Cornell locus, had a 1000-bp fragment. These PCR primers should be useful to follow the *TY1* gene as it gives a distinct fragment size from the *esculentum*- and *peruvianum*-type fragments. The fragment from *S. habrochaites* (*L. hirsutum*) was 800 bp. Line 902 gave the *peruvianum*-type fragment, 720 bp, which would be a false positive. These fragments were sequenced and compared to the published sequence for the BAC clone in GenBank. Surprisingly, the sequences from the 620 bp or the 720 bp fragments did not match sequences of this BAC clone, which means that a different region of the tomato genome had been amplified. These two sequences were sent to Dr. Valerie Williamson (UC-Davis) and her group matched the sequence obtained from Motelle (*peruvianum* type) with *Mi1.4* in the 2p group of *Mi* genes in this same region and the sequence from Moneymaker (*esculentum* type) with the *Mi1F* in the 1e region. Thus, the sequences did match those in the region of the Mi gene clusters of Chromosome 6, but they did not match the original region (promoter of Mi1.2) used to design the primers. In June 2004, Seah et al. (2004. TAG 108:1635-1642) reported that the nematode-resistant region has the *Mi-1* gene and 6 other homologs, which are grouped into two clusters (four in one and three in the other) and are separated by 300 kb. The REX locus is located in this 300-kb region. The organization of this Mi gene region in susceptible tomato was also report by Seah et al. (2004), and they suggested that there has an inversion of the 300-kb region between the two clusters. Also, they found two additional Mi-1 homologs that mapped to chromosome 5.

Susceptible lines gave either the 620-bp fragment (cerasiforme, M82, G3h, G16c, TY50) or the 1000 bp fragment (TY52 and G9c). The 620-bp fragments were sequenced except for M82: they all were identical to the *esculentum*-type sequence for Moneymaker. The 720-bp fragment, characteristic of the *peruvianum*-type sequence from Motelle, was for G1h gave the *peruvianum*-like sequence.

In conclusions, both the CAPS markers for the REX locus and the Cornell locus give false positives with 902 and TY52. When the fragments are sequenced, the *peruvianum*- and *chilense*-type sequences can be identified by unique SNP patterns. The Mi12F1/Mi12R2 primers give four different size fragments for each type, *esculentum*- (620 bp), *peruvianum*- (720 bp), *hirsutum*- (800 bp), and *chilense*-type (1,000 bp). Line 902 appears to be chimeric in this region with sequences both from *chilense* (Cornell locus) and *peruvianum* (REX and Mi12F1/Mi12R2 loci) and this may explain the difficulty in developing a PCR-based method to distinguish 902 from Motelle. This is important since most of the resistance to begomoviruses is being derived from 902.

One last point, G2h, which was selected from FAVI 12, has both resistance to root knot nematode and begomoviruses and has the excepted sequence and PCR fragments for the *Mi-1* gene. This is an excellent line to use to incorporate resistance for both begomoviruses and root knot nematode into the various programs in the tomato MERC project.

Fig. 5. Alignment of sequences for the REX locus for TY52 (*Ty1* gene *Ty1/Ty1*, L. *chilense* resistance to TYLCV), 902 (*L. hirsutum* resistance to begomoviruses), Motelle (*Mi/Mi*, homozygous for resistance to root knot nematode), TY50 (isolines to TY52, *Ty1+/TY1+*), M82 (*Mi+/Mi+*, susceptible to begomoviruses and root knot nematode), and cera (*L. esculentum* var. *cerasiforme*, susceptible to begomoviruses)

```
TY52-R2       TGCAAGCAAATTGACTAGCTTGACGTAAGGGATCTGCACTTACATCGGTATCCTGTTGAG     60
902-R2        TGCAAGCAAATTGACTAGCTTGACGTAAGGGATCTGCACTTgCATCGGTATCCTGTTGAG     60
Motelle-REX   TGCAAGCAAATTGACTAGCTTGACGTAAGGGATCTGCACTTgCATCGGTATCCTGTTGAG     60
G2hREX        TGCAAGCAAATTGACTAGCTTGACGTAAGGGATCTGCACTTgCATCGGTATCCTGTTGAG     60
TY50-R2       TGCAAGCAAAgTGACTAGCTTGACGTAAGGGATCTGCACTTACATCGGTATCCTGTTGAG     60
M82REX        TGCAAGCAAAgTGACTAGCTTGACGTAAGGGATCTGCACTTACATCGGTATCCTGTTGAG     60
CERREX        TGCAAGCAAAgTGACTAGCTTGACGTAAGGGATCTGCACTTACATCGGTATCCTGTTGAG     60
Consensus     tgcaagcaaa tgactagcttgacgtaagggatctgcactt catcggtatcctgttgag
```

```
TY52-R2       TTGCATAACCAGAAACCGTGGACTTTGCTTTGACTTTTTTACCTGATTCACGATGGACAT   120
902-R2        TTGCATAACCAGAAACCGTGGACTTTGCTTTGACTTTTTTACCTGATTCACGATGGACAT   120
Motelle-REX   TTGCATAACCAGAAACCGTGGACTTTGCTTTGACTTTTTTACCTGATTCACGATGGACAT   120
G2hREX        TTGCATAACCAGAAACCGTGGACTTTGCTTTGACTTTTTTACCTGATTCACGATGGACAT   120
TY50-R2       TTGCATAACCAGAAACCaTGGACTTTGCTTTGACTTTTTTACCTGATTCACGATGaACAT   120
M82REX        TTGCATAACCAGAAACCaTGGACTTTGCTTTGACTTTTTTACCTGATTCACGATGaACAT   120
CERREX        TTGCATAACCAGAAACCaTGGACTTTGCTTTGACTTTTTTACCTGATTCACGATGaACAT   120
Consensus     ttgcataaccagaaaccc tggactttgctttgacttttttacctgattcacgatg acat

TY52-R2       CTCTCTCCTCTAATTCAGCTTCAGATAATAGATCATAACTCTTGCCATTGCAGGCATTAT   180
902-R2        CTtTCTCCTCTAATTCAGCTTCAGATAATAGATCATAACTCTTGCCATTGCAGGCATTAT   180
otelle-REX    CTtTCTCCTCTAATTCAGCTTCAGATAATAGATCATAACTCTTGCCATTGCAGGCATTAT   180
G2hREX        CTtTCTCCTCTAATTCAGCTTCAGATAATAGATCATAACTCTTGCCATTGCAGGCATTAT   180
TY50-R2       CTtTCTCCTCTAATTCAGCTTCAGATAATAGATCATAACTCTTGCCATTGCAGGCATTAT   180
MM82REX       CTtTCTCCTCTAATTCAGCTTCAGATAATAGATCATAACTCTTGCCATTGCAGGCATTAT   180
CERREX        CTtTCTCCTCTAATTCAGCTTCAGATAATAGATCATAACTCTTGCCATTGCAGGCATTAT   180
Consensus     ct tctcctctaattcagcttcagataatagatcataactcttgccattgcaggcattat

TY52-R2       CCTTCTTAACCATACTGGATTTATTGGAGAACCCATCATTTTCACCATCAGAAGACCTCT   240
902-R2        CCTTCTTAACCATACTGGATTTATTGGAGAACCCATCATTTTCACCATCAGAAGACCTCT   240
Motelle-REX   CCTTCTTAACCATACTGGATTTATTGGAGAACCCATCATTTTCACCATCAGAAGACCTCT   240
G2hREX        CCTTCTTAACCATACTGGATTTATTGGAGAACCCATCATTTTCACCATCAGAAGACCTCT   240
TY50-R2       CCTTCTTAACCATACTGGATTTATTGGAGAACaCATCATTTTCACCATCAGAAGACCTCT   240
M82REX        CCTTCTTAACCATACTGGATTTATTGGAGAACaCATCATTTTCACCATCAGAAGACCTCT   240
CERREX        CCTTCTTAACCATACTGGATTTATTGGAGAACaCATCATTTTCACCATCAGAAGACCTCT   240
Consensus     ccttcttaaccatactggatttattggagaac catcattttcaccatcagaagacctct

TY52-R2       TGGGACTAGAAGTGGGTAAGGCTGAAGAGGGAGCAACAGAAGGTCGCGAATTGCATCGAT   300
902-R2        TGGGACTAGAAGTGGGaAAGGCTGAAGAGGGAGCAACAGAAGGTCGCGAATTGCATaGAT   300
Motelle-REX   TGGGACTAGAAGTGGGaAAGGCTGAAGAGGGAGCAACAGAAGGTCGCGAATTGCATaGAT   300
G2hREX        TGGGACTAGAAGTGGGaAAGGCTGAAGAGGGAGCAACAGAAGGTCGCGAATTGCATaGAT   300
TY50-R2       TGGGACTAGAAGTGGGTAAGGCTGAAGAGGGAGCAACAGAAGGTCGCGAATTGCATaGAT   300
M82REX        TGGGACTAGAAGTGGGTAAGGCTGAAGAGGGAGCAACAGAAGGTCGCGAATTGCATaGAT   300
CERREX        TGGGACTAGAAGTGGGTAAGGCTGAAGAGGGAGCAACAGAAGGTCGCGAATTGCATaGAT   300
Consensus     tgggactagaagtggg aaggctgaagagggagcaacagaaggtcgcgaattgcat gat

TY52-R2       CCTTTTGTGAAGAATCTGCAGCTTTAACACTCAACAAAGATAGAGTACTATCCAGATCTT   360
902-R2        CCTTTTGTGAAGAATCTGCAGCTTTAACACTCAACAAAGATAGAGTACTATCCAGATCTT   360
Motelle-REX   CCTTTTGTGAAGAATCTGCAGCTTTAACACTCAACAAAGATAGAGTACTATCCAGATCTT   360
G2hREX        CCTTTTGTGAAGAATCTGCAGCTTTAACACTCAACAAAGATAGAGTACTATCCAGATCTT   360
TY50-R2       CCTTTTGTGAAGAATCTGCAGCTTTAACACTCAACAAAGATAGAGTACTATCCAGATCTT   360
M82REX        CCTTTTGTGAAGAATCTGCAGCTTTAACACTCAACAAAGATAGAGTACTATCCAGATCTT   360
CERREX        CCTTTTGTGAAGAATCTGCAGCTTTAACACTCAACAAAGATAGAGTACTATCCAGATCTT   360
Consensus     cctttgtgaagaatctgcagctttaacactcaacaaagatagagtactatccagatctt

TY52-R2       GCCCAGCCTGCTGTTCCTTTTTAACTTGACCTGTTCCAGCACTACCTTTGCTTGCACTAG   420
902-R2        GCCCAGCCTGCTGTTCCTTTTTAACTTGACCTGTTCCAGCACTACCTTTGCTTGCACTAG   420
Motelle-REX   GCCCAGCCTGCTGTTCCTTTTTAACTTGACCTGTTCCAGCACTACCTTTGCTTGCACTAG   420
G2hREX        GCCCAGCCTGCTGTTCCTTTTTAACTTGACCTGTTCCAGCACTACCTTTGCTTGCACTAG   420
TY50-R2       GCCCAGCCTGCTGTTCCTTTTTAACTTGACCTGTTCCAGCACTACCTTTGCTTGCACTAG   420
M82REX        GCCCAGCCTGCTGTTCCTTTTTAACTTGACCTGTTCCAGCACTACCTTTGCTTGCACTAG   420
CERREX        GCCCAGCCTGCTGTTCCTTTTTAACTTGACCTGTTCCAGCACTACCTTTGCTTGCACTAG   420
Consensus     gcccagcctgctgttccttttttaacttgacctgttccagcactacctttgcttgcactag

TY52-R2       TGTCCTTCCGGTCAGACAAGGAGACCCTTGCTACCTTTTCCTTCCTGGAGATGTCATCAC   480
902-R2        TGTCCTTCCGGTCAGACAAGGAGACCCTTGCTACCTTTTCCTTCCTGGAGATGTCATCAC   480
Motelle-REX   TGTCCTTCCGGTCAGACAAGGAGACCCTTGCTACCTTTTCCTTCCTGGAGATGTCATCAC   480
G2hREX        TGTCCTTCCGGTCAGACAAGGAGACCCTTGCTACCTTTTCCTTCCTGGAGATGTCATCAC   480
TY50-R2       TGTCCTTCCGGTCAGACAAGGAGACCCTTGCTACCTTTTCCTTCCTaGAGATGTCATCAC   480
M82REX        TGTCCTTCCGGTCAGACAAGGAGACCCTTGCTACCTTTTCCTTCCTaGAGATGTCATCAC   480
CERREX        TGTCCTTCCGGTCAGACAAGGAGACCCTTGCTACCTTTTCCTTCCTaGAGATGTCATCAC   480
Consensus     tgtccttccggtcagacaaggagacccttgctacctttteettcct gagatgtcatcac

                                                    TCGA
TY52-R2       ATATTTTTTCCATAGAATCCTGGGGATTACATGTCAAGGAATCTCGAAGTTCTCTCGCTT   540
902-R2        ATATTTTTTCCATAGAATCtTGGGGATTACATGTCAAGGAATCTCGAAGTTCTCTCcCTT   540
Motelle-REX   ATATTTTTTCCATAGAATCtTGGGGATTACATGTCAAGGAATCTCGAAGTTCTCTCcCTT   540
G2hREX        ATATTTTTTCCATAGAATCtTGGGGATTACATGTCAAGGAATCTCGAAGTTCTCTCcCTT   540
TY50-R2       ATATTTTTTCCATAGAATCCTGGGGATTACATGTCAAGGAATCTCGcAGTTCTCTCcCTT   540
M82REX        ATATTTTTTCCATAGAATCCTGGGGATTACATGTCAAGGAATCTCGcAGTTCTCTCcCTT   540
CERREX        ATATTTTTTCCATAGAATCCTGGGGATTACATGTCAAGGAATCTCGcAGTTCTCTCcCTT   540
Consensus     atatttttttccatagaatc tggggattacatgtcaaggaatctcg agttctctc ctt
```

```
G2hREX      ATATTTTTTCCATAGAATCtTGGGGATTACATGTCAAGGAATCTCGAAGTTCTCTCcCTT    540
TY50-R2     ATATTTTTTCCATAGAATCCTGGGGATTACATGTCAAGGAATCTCGcAGTTCTCTCcCTT    540M82REX
ATATTTTTTCCATAGAATCCTGGGGATTACATGTCAAGGAATCTCGcAGTTCTCTCcCTT    540
CERREX      ATATTTTTTCCATAGAATCCTGGGGATTACATGTCAAGGAATCTCGcAGTTCTCTCcCTT    540Consensus
atatttttccatagaatc tggggattacatgtcaaggaatctcg agttctctc ctt

TY52-R2     TTCTCTTAATCGGAGAATCATTATTGTCACACTTCCCCTTATGCGTTGACACATCGGAAA    600
902-R2      TTCTCTTAATCGGAGAATCATTATTGTCACACTTCCCCTTATGCGTTGACACATCGGAAA    600
Motelle-REX TTCTCTTAATCGGAGAATCATTATTGTCACACTTCCCCTTATGCGTTGACACATCGGAAA    600
G2hREX      TTCTCTTAATCGGAGAATCATTATTGTCACACTTCCCCTTATGCGTTGACACATCGGAAA    600
TY50-R2     TTCTCTTAATCGGAGAATCATTATTGTCACACTTCCCCTTATGCGTTGACACATCGGAAA    600
M82REX      TTCTCTTAATCGGAGAATCATTATTGTCACACTTCCCCTTATGCGTTGACACATCGGAAA    600
CERREX      TTCTCTTAATCGGAGAATCATTATTGTCACACTTCCCCTTATGCGTTGACACATCGGAAA    600
Consensus   ttctcttaatcggagaatcattattgtcacacttccccttatgcgttgacacatcggaaa
```

2.5. Web site

Details and photographs of field plots can be found on the web site prepared by Dr. D.P. Maxwell: http://www.plantpath.wisc.edu/GeminivirusResistantTomatoes/CDR/

3. Plans for next year

3.1. Seedling production and hybrid seed production

In March 2004 several begomovirus resistant tomato lines and hybrids were transferred to a Guatemalan seed company called *Pilones de Antigua* (see pages 6 and 7). These lines were crossed to produce hybrids *Llanero 7, LLanero 10, Llanero 11, Llanero 12* and *Maya Garden*. These hybrids were first planted by *Pilones de Antigua*, at different locations, in early 2004 and at least some are being considered for commercial release. In view of the promising results that were observed in the *Llanero* hybrids, the owners of *Pilones de Antigua* decided to go ahead with the initiative of the seed company, and this has just recently been formed under the name *Gentropic Seeds*. This company will be devoted to the production of seeds for Guatemala and Central America.

3.2. Field evaluation of additional populations

From July 13 to 15, 2004, two hundred and twenty breeding lines and segregating populations were transplanted in Sanarate (see table 9, page 8). This trial will be evaluated in October, 2004. selections will be made that will be evaluated in March 2005.

3.3. DNA markers linked to resistance to begomoviruses

SNPs and indels have been used to show that the resistance in GF lines is not associated with the same introgression of *Ty1* and *Ty2* genes in Chromosome 6 and 11, respectively. Hence we will continue to use the RFLP approach which has been started during the last year of research. Several markers for each of the 12 chromosomes will be used to evaluate chromosomal regions for polymorphisms.

4. Managerial issues

No major issues.

5. Special concerns

During the last field assays, it appears that problems caused by Bacterial wilt were responsible for damages unrelated to the presence of whitefly-transmitted geminiviruses. To deal with this growing problem, we have asked for the support of US AID to tackle this problem. In August 2004, we have submitted a proposal entitled "Develop tomato breeding lines with resistance to *Ralstonia solanacearum* and begomovirus in Guatemala and Central America" (project C25-037).

6. Strengthening

6.1. Training in the use of DNA markers

The Guatemalan PI and three faculty members from San Carlos University spent 6 weeks at the University of Wisconsin (May 15 to June 30, 2004). This training dealt mainly with marker assisted selection, identification of markers linked to geminivirus resistance in samples selected in Guatemala. These researchers were: Julieta Ortiz (Department of Chemistry), Sergio Melgar (Department of Biology) and Daisuke Furusawa (a Japanese volunteer working with us in the lab, College of Agronomy).

6.2. Workshop "Molecular methods for diagnosis of pathogens and breeding for resistance in vegetables"

The workshop was held in Antigua from February 25 to February 27, 2004. The first two days dealt with molecular methods for diagnosis of pathogens and breeding for resistance in vegetables. The last day was devoted to a symposium on Bacterial Wilt. Information about the meeting can be found at: http://www.concyt.gob.gt/noticias.html
Attending the meeting was:
Drs D. Maxwell, M. Nakhla and M. Havey, from the University of Wisconsin, USA.
Dr F. Vidavsky, from the Hebrew University of Jerusalem, Israel.
Dr Jaw-Fen Wang from the Asian Vegetable Research and Development Center, Taiwan.

There were approximately 30 participants in the workshop (faculty, students and technicians from San Carlos University, other private universities, the public sector and the private productive sector). There were around 60 participants in the symposium on bacterial wilt, including tomato producers.

Dr Mathew Metz from USAID participated in the workshop.

6.3. Visit to Dr J. Scott at the University of Florida

During the 1st International Symposium on Tomato Diseases and the 19th Annual Tomato Disease Workshop which were held at Orlando, Florida, 20-24 June, 2004, Drs L. Mejia, F. Vidavski, M. Lapidot, M. Nakhla and D.P. Maxwell have visited Dr J. Scott at the Gulf Coast Research and

Education Center in Bradenton, Florida. The researchers have seen the tomato breeding fields for resistance to ToMoV and TYLCV and had a discussion with Dr J. Scott and his lab members about the use of molecular markers to tag resistance to begomoviruses.

7. Collaborative activities

7.1. Visits and field evaluations

Following the workshop, the tomato fields in Sanarate have been visited by the team of the University of San Carlos headed by Dr L Mejia and RE Teni. Drs F Vidavski, M Havey, DP Maxwell, M Nakhla, J-FWang and M Metz have participated in the evaluation and selections.

7.2. Production of advanced breeding lines

About 20 g of seeds of (GF1 x commercial breeding line) have been produced by a commercial company in Israel at 1.5 cents/seed. The seeds have been sent mid-October 2003 to Dr Mejia, Guatemala. Five hybrids, Llanero 7, Llanero 10, Llanero 11, Llanero 12 and Maya Garden have been produced at Pilones de Antigua. These hybrids were produced from crosses between geminivirus resistant lines that have been selected in Guatemala. All five hybrids are tested at different locations in Guatemala in 2004.

7.3. Workshop

See 6.2.

7.4. Linkage with Middle East Regional Cooperation (MERC) Program

Dr D.P. Maxwell has presented the results of the breeding program in Guatemala during the annual meeting of the MERC program on classical and marker assisted breeding for TYLCV resistance, held in Chania, Crete, Greece, July 12 to 21, 2004. Dr Maxwell has also presented this program during the visit to the Biotechnology Center in Heraklion, July 22, 2004.

8. List of papers and abstracts

Mejía, L., R. E. Teni, A. Sánchez, A. Pallides, F. Vidavski, H. Czosnek, L. Cohen, M. Lapidot, J. W. Scott, M. J. Havey, M. K. Nakhla and D. P. Maxwell. 2004. Evaluation of tomato germplasm and selection of breeding lines for resistance to begomoviruses in Guatemala. 4th International Geminivirus Symposium, Capetown, South Africa, February 16-24, 2004.

Mejía, L., R.E. Teni, A. Sánchez, A. Pallides, F. Vidavski, H. Czosnek, M. Lapidot, J. Scott, M.J. Havey, M.K. Nakhla and D.P. Maxwell. 2004. Evaluation of tomato germplasm and selection of breeding lines for resistance to Begomoviruses in Guatemala. Abstract. First International Symposium on Tomato Diseases and 19th Annual Tomato Disease Workshop (Orlando, Florida, 20-24 June, 2004).

Zea, C., A. Sánchez, L. Mejía, M.A. Arevalo, F. Vidavski, M. Salus, C. Martin and D.P. Maxwell. 2004. Problems associated with the use of molecular markers for tagging the Mi gene in tomato

breeding lines. Abstract. First International Symposium on Tomato Diseases and 19th Annual Tomato Disease Workshop (Orlando, Florida, 20-24 June, 2004).

Mejía, L., Teni, R. E, Vidavski, F., Czosnek, H., Lapidot, M., Nakhla, M.K., and Maxwell, D.P. (2004), Evaluation of tomato germplasm and selection of breeding lines for resistance to begomoviruses in Guatemala. Submitted to Acta Horticulturae.