Fine-mapping and Cloning of *Ty-1* and *Ty-3* and Mapping of a New Resistance Locus, "*Ty-6*"

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Begomovirus Resistance

- Major focus at University of Florida since 1990

- Resistance sources
  - *S. chilense* LA 2779
  - *S. chilense* LA 1932
  - *S. chilense* LA 1938
  - *S. lycopersicum* cv. 'Tyking'

- Inoculations
  - TYLCV presently
  - ToMoV – through 2006

Spring 2011 Survey

- 53 advanced lines
- CRBD
  - 2 reps
  - 4 plant plots
- Seedling TYLCV inoc.
- Genotyped at resistance loci
- Line evaluations:
  - Viral disease severity
  - Non viral foliar disease severity

Line	TY Gene		Line	TY Gene	
8259			8637		
8299			8638		
8300			8655		
8302			8656		
8347	3	4	8657	3	
8352	3		8680	3	
8353	3		8737		
8379	3		8738	3	
8383			8739	3	
8387			8741		
8467			8753		
8468			8779		
8469			8823	3	
8470			8824	3	
8472			8825	3	
8480	3		8826	3	
8501	3		8827		
8503			8840		
8600			8841		
8601	3		8843		
8602	3		8844	3	
8603			8845		
8604			Fla678	3	
8624			Fla685	3	
8625			Fla707	3	
8635			Fla726	3	4
8636	3			3	

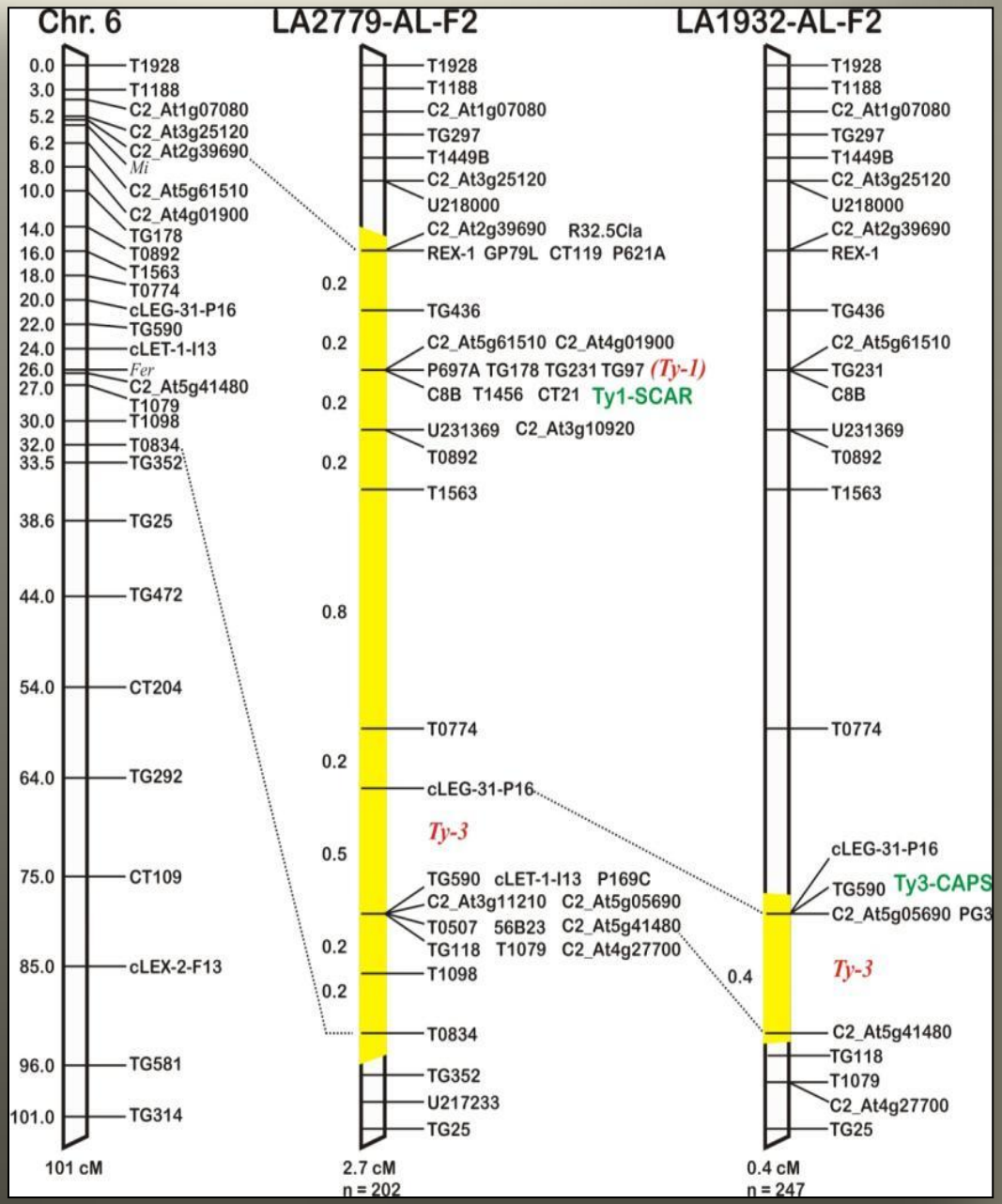
Non-*Ty-3* resistance
Ty-3 resistance

Mean non-viral foliar disease severity of advanced TYLCV-resistant breeding lines as affected by genotype at three resistance loci.

Genotype[1]	Mean Foliar DSI[2]		
	Ty-3	***Ty-4***	***ty-5***
+/+	6.1 a (*n* = 130)	4.6 b (*n* = 16)	5.4 a (*n* = 174)
+/-	5.7 b (*n* = 46)	6.0 a (*n* = 5)	4.4 b (*n* = 15)
-/-	5.3 c (*n* = 346)	5.6 a (*n* = 501)	5.7 a (*n* = 333)

[1] +/+ homozygous resistant; +/- heterozygous; -/- homozygous susceptible
[2] *DSI* disease severity index of non-viral infections (primarily bacterial spot and early blight) based on the Horsfall-Barratt scale, lower number means less disease; *n* number of plants in each group; means in column with same letter are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test

Ty-3 Fine Mapping



Ty-3 Fine Mapping

- Spring 2009: Screened ~10,500 F_2 plants for recombination

  ~300 total recombinants

  ~100 informative recombinants

Ty-3 Fine Mapping

- **Spring 2009:**

  Mapped within ~2.5 cM between M1 and M8

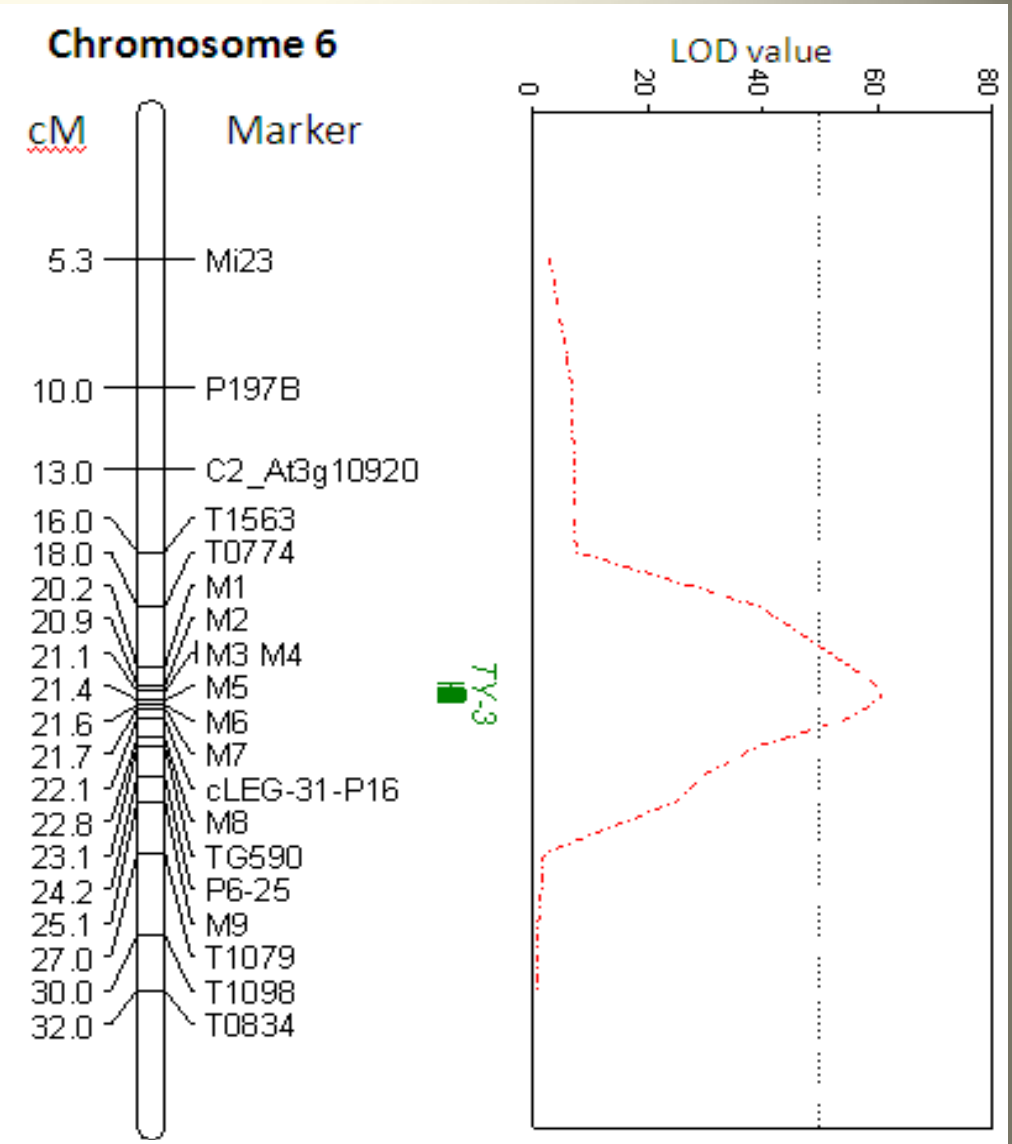


Figure 2. Mapping and interval analysis of *Ty-3* (map distance in cM) among the markers analyzed on chromosome 6; Interval analysis displaying LOD scores along Chromosome 6 for disease severity of approximately 300 recombinant plants. Map positions between 18 and 26 cM are based on recombination data from the present population.

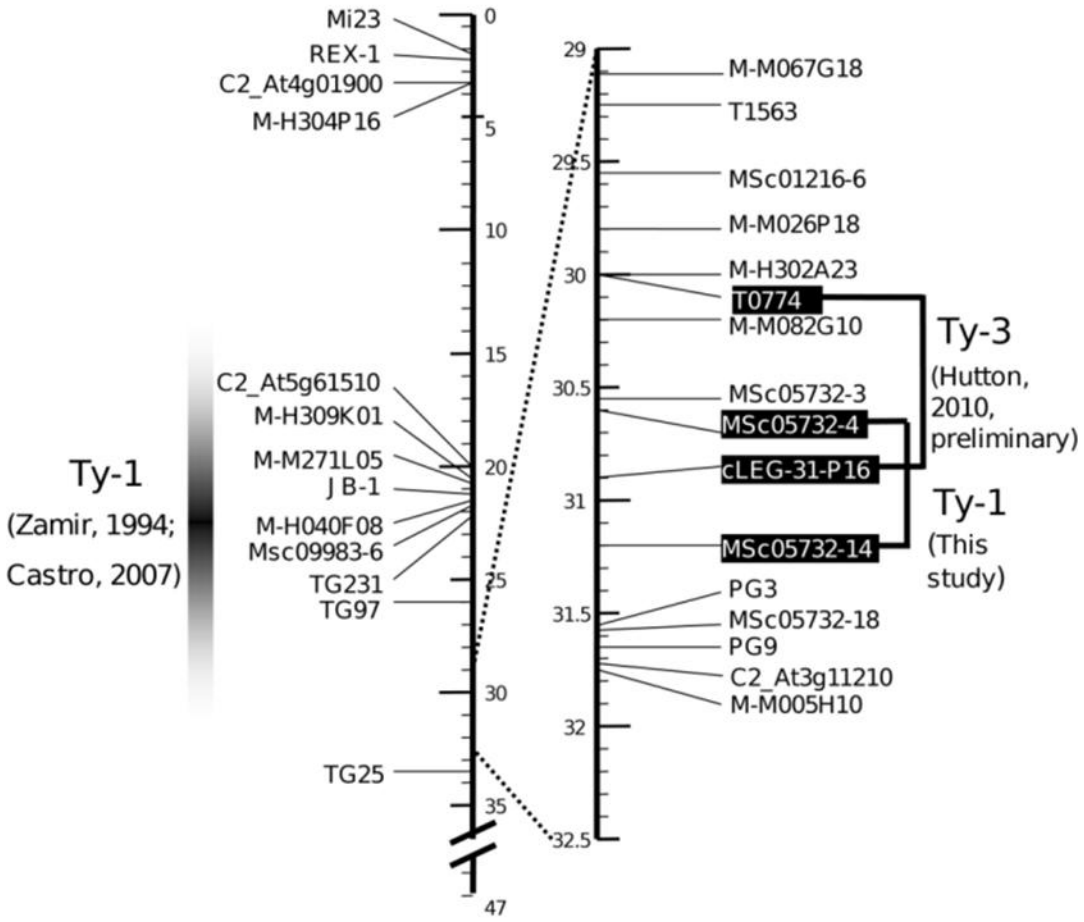
Ty-3 Fine Mapping

B

Tomato chromosome 6

Physical Position (Mb)	Molecular Marker	Controls: Tygress	Fla. 7776	Fla 8680	(Fla. 7776 x RIL 554) F_1	(Fla. 7776 x RIL 157) F_1	Recombinant Class: A (4)	B (1)	C (2)	D (1)	E (3)	F (1)	G (1)	H (6)	I (1)	J (1)	K (1)	L (2)	M (2)
30.027	T0774_UF	/	-	+	/	/	+	+	+	+	+	+	+	-	-	-	-	-	-
30.280	SL_2.40ch06_30.280	/	-	+	/	/	+	+	+	+	+	+	+	-	-	-	-	-	-
30.696	SL_2.40ch06_30.696	/	-	+	/	/	+	+	+	+	+	+	+	-	-	-	-	-	-
30.797	EcoRI0039C03	nd	-	+	/	/	-	+	+	+	+	+	+	+	-	-	-	-	-
30.815	HBa0161K22	/	-	+	/	/	-	-	+	+	+	+	+	+	-	-	-	-	-
30.816	**UF_TY3-P1**	/	-	+	/	/	-	-	+	+	+	+	+	+	-	-	-	-	-
30.821	UF_TY3-P3	/	-	+	/	/	-	-	+	+	+	+	+	+	+	-	-	-	-
30.849	UF_TY3-P11	nd	-	+	/	/	-	-	+	+	+	+	+	+	+	-	-	-	-
30.852	FOS00169A13	/	-	+	/	/	-	-	+	+	+	+	+	+	+	-	-	-	-
30.853	UF_TY3-P12	/	-	+	/	/	-	-	+	+	+	+	+	+	+	-	-	-	-
30.862	UF_TY3-P15	/	-	+	/	/	-	-	+	+	+	+	+	+	+	-	-	-	-
30.873	EcoRI0066C10	/	-	+	/	/	-	-	+	+	+	+	+	+	+	-	-	-	-
30.873	UF_TY3-P18	/	-	+	/	/	-	-	+	+	+	+	+	+	+	-	-	-	-
30.876	UF_TY3-P19	/	-	+	/	/	-	-	+	+	+	+	+	+	+	-	-	-	-
30.887	**UF_TY3-P23**	/	-	+	/	/	-	-	-	+	+	+	+	+	+	-	-	-	-
30.890	UF_TY3-P24	/	-	+	/	/	-	-	-	+	+	+	+	+	+	-	-	-	-
30.891	SL_2.40ch06_30.891	/	-	+	/	/	-	-	-	-	+	+	+	+	+	+	-	-	-
30.962	HBa0045I03	/	-	+	/	/	-	-	-	-	-	+	+	+	+	+	-	-	-
30.970	HBa0135O04	nd	-	+	/	/	-	-	-	-	-	-	+	+	+	+	+	-	-
30.978	EcoRI0020O03	nd	-	+	/	/	-	-	-	-	-	-	-	+	+	+	+	+	-
30.991	cLEG-31-P16	nd	-	+	/	/	-	-	-	-	-	-	-	+	+	+	+	+	+
31.499	P6-25	/	-	+	/	/	-	-	-	-	-	-	-	+	+	+	+	+	+
	Mean TYLCV DSI	0.7^{G}	4.0^{A}	0.0^{H}	2.4^{B}	2.0^{C}	4.0^{A}	4.0^{A}	1.3^{EF}	1.5^{EF}	1.6^{DE}	1.9^{CD}	1.5^{EF}	1.5^{EF}	1.2^{F}	4.0^{A}	4.0^{A}	4.0^{A}	4.0^{A}

Verlaan, et al. 2011

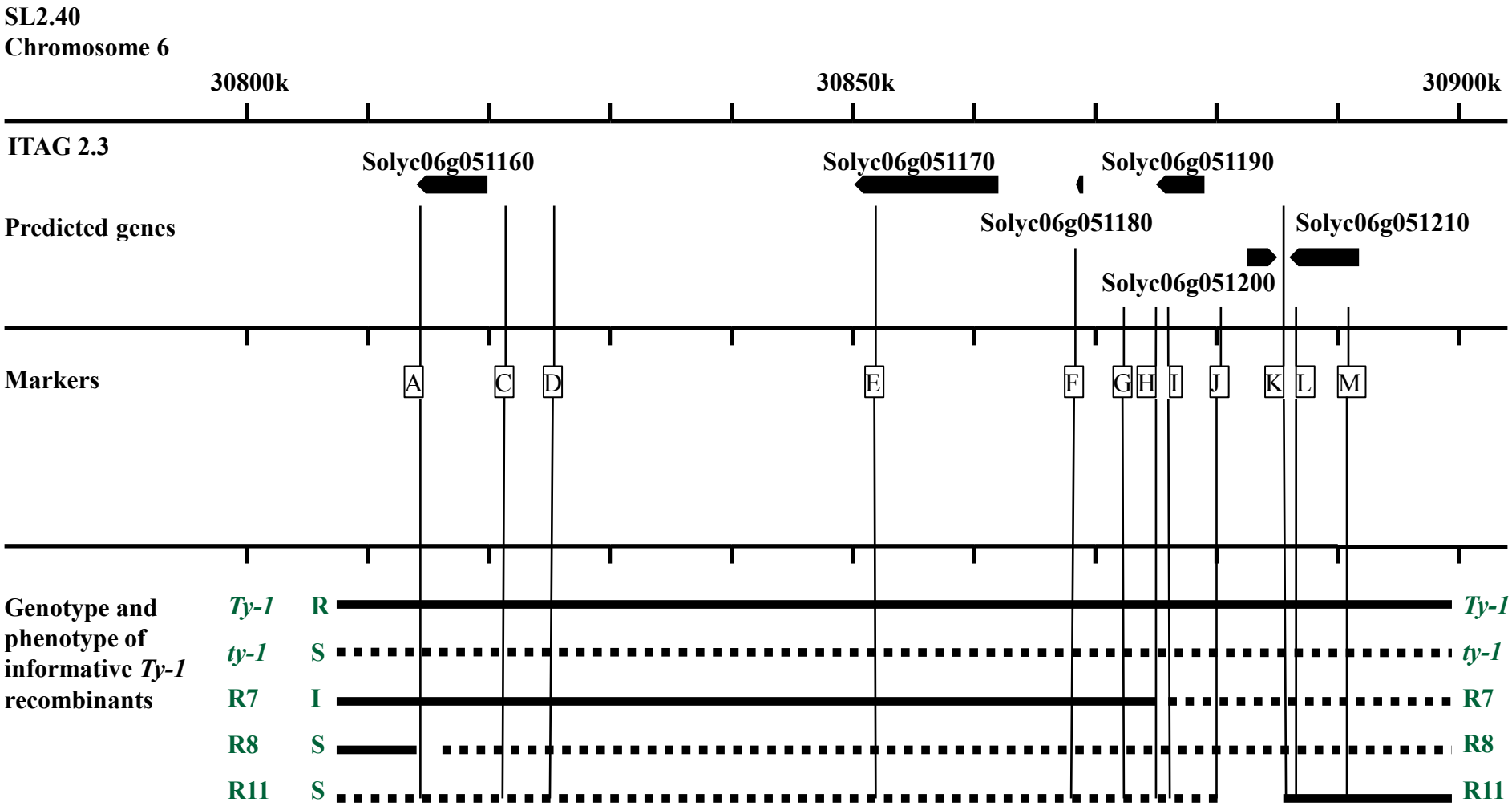


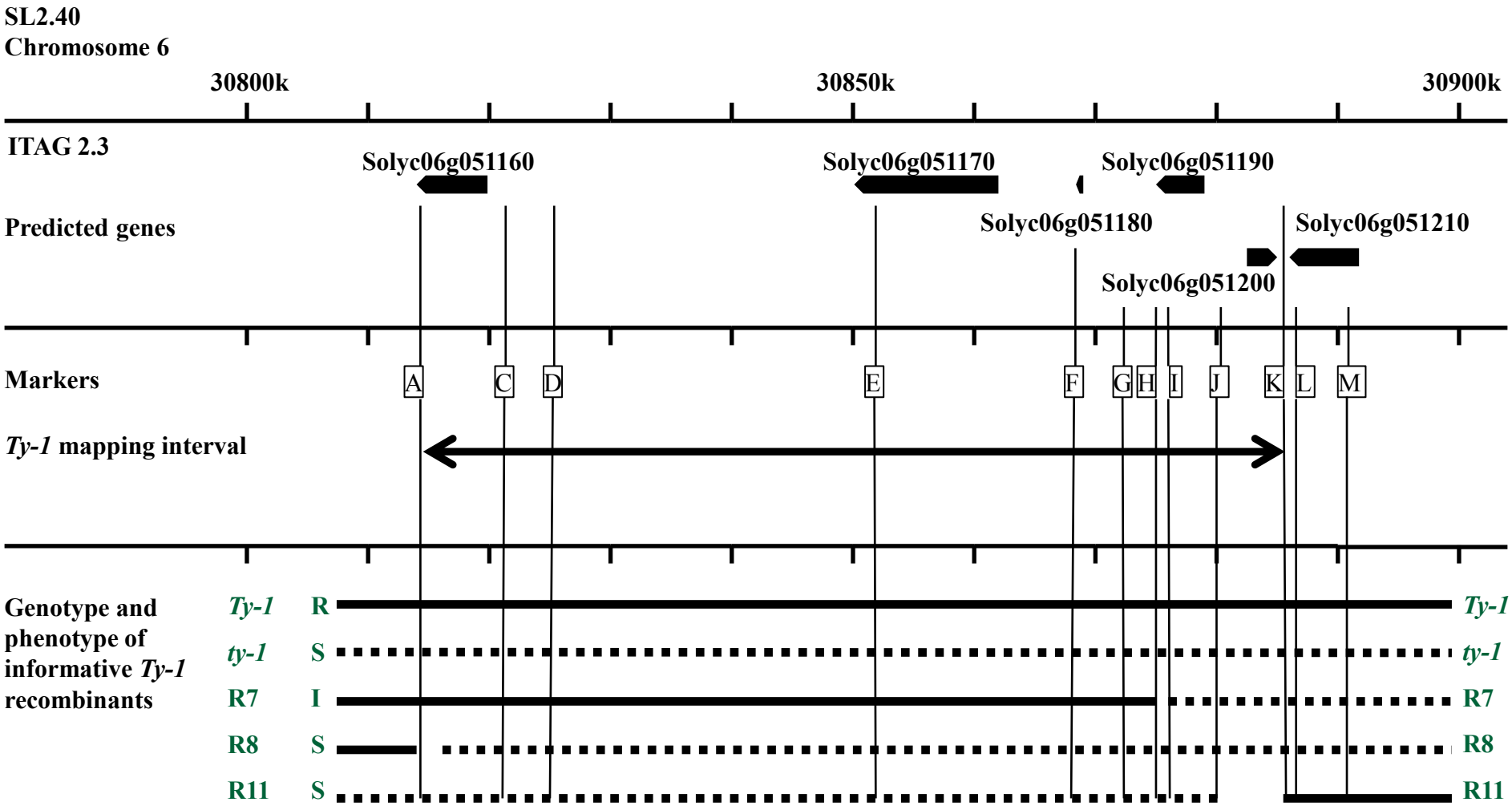
Ty-1 Fine Mapping

A

Tomato chromosome 6

Physical Position (Mb)	Molecular Marker	Controls: Tygress	Controls: Fla. 7776	Controls: Ty-1 RIL	Recombinant Inbred Lines: R6/R9/R13	R2/R3/R10	R8	R7	R1	R4/R12	R5	R11
2.322	Mi23	/	-	+	-	-	-	-	-	-	-	-
3.571	M-H309K01	/	-	+	-	-	-	-	-	-	-	-
26.206	TG97	/	-	+	-	-	-	-	-	-	-	-
29.260	T1563	nd	-	+	-	-	-	-	-	-	-	-
30.027	T0774_UF	/	-	+	+	+	+	+	-	-	-	-
30.523	MSc05732-3	/	-	+	-	+	+	+	-	-	-	-
30.300	MSc05732-4	/	-	+	-	+	+	+	+	-	-	-
30.696	SL_2.40ch06_30.696	/	-	+	-	-	+	+	+	+	-	-
30.815	**HBa0161K22**	/	-	+	-	-	+	+	+	+	+	-
30.816	UF_TY3-P1	/	-	+	-	-	-	+	+	+	+	-
30.821	UF_TY3-P3	/	-	+	-	-	-	+	+	+	+	-
30.852	FOS00169A13	/	-	+	-	-	-	+	+	+	+	-
30.873	EcoRI0066C10	/	-	+	-	-	-	+	+	+	+	-
30.873	UF_TY3-P18	/	-	+	-	-	-	+	+	+	+	-
30.875	WU_M27	/	-	+	nd	nd	-	+	nd	nd	nd	-
30.876	UF_TY3-P19	nd	-	+	-	-	-	-	+	+	+	-
30.880	WU_M29	nd	-	+	nd	nd	-	-	nd	nd	nd	-
30.885	**WU_M31**	nd	-	+	nd	nd	-	-	nd	nd	nd	+
30.887	UF_TY3-P23	nd	-	+	-	-	-	-	+	+	+	+
30.890	UF_TY3-P24	nd	-	+	-	-	-	-	+	+	+	+
30.891	SL_2.40ch06_30.891	nd	-	+	-	-	-	-	+	+	+	+
30.970	HBa0045I03	nd	-	+	-	-	-	-	+	+	+	+
31.040	SL_2.40ch06_31.040	/	-	-	-	-	-	-	-	-	-	-
31.499	P6-25	/	-	-	-	-	-	-	-	-	-	-
	Mean TYLCV DSI	0.8^{D}	4.0^{A}	1.5^{C}	4.0^{A}	4.0^{A}	4.0^{A}	2.8^{B}	0.0^{E}	0.0^{E}	0.0^{E}	4.0^{A}


SL2.40
Chromosome 6
30800k
30850k
30900k
ITAG 2.3
Solyc06g051160
Solyc06g051170
Solyc06g051190
Predicted genes
Solyc06g051180
Solyc06g051210
Solyc06g051200
Markers
A
C
D
E
F
G
H
I
J
K
L
M
Genotype and phenotype of informative Ty-1 recombinants
Ty-1 R
ty-1 S
R7 I
R8 S
R11 S
Ty-1
ty-1
R7
R8
R11


SL2.40
Chromosome 6
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Solyc06g051160
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Solyc06g051190
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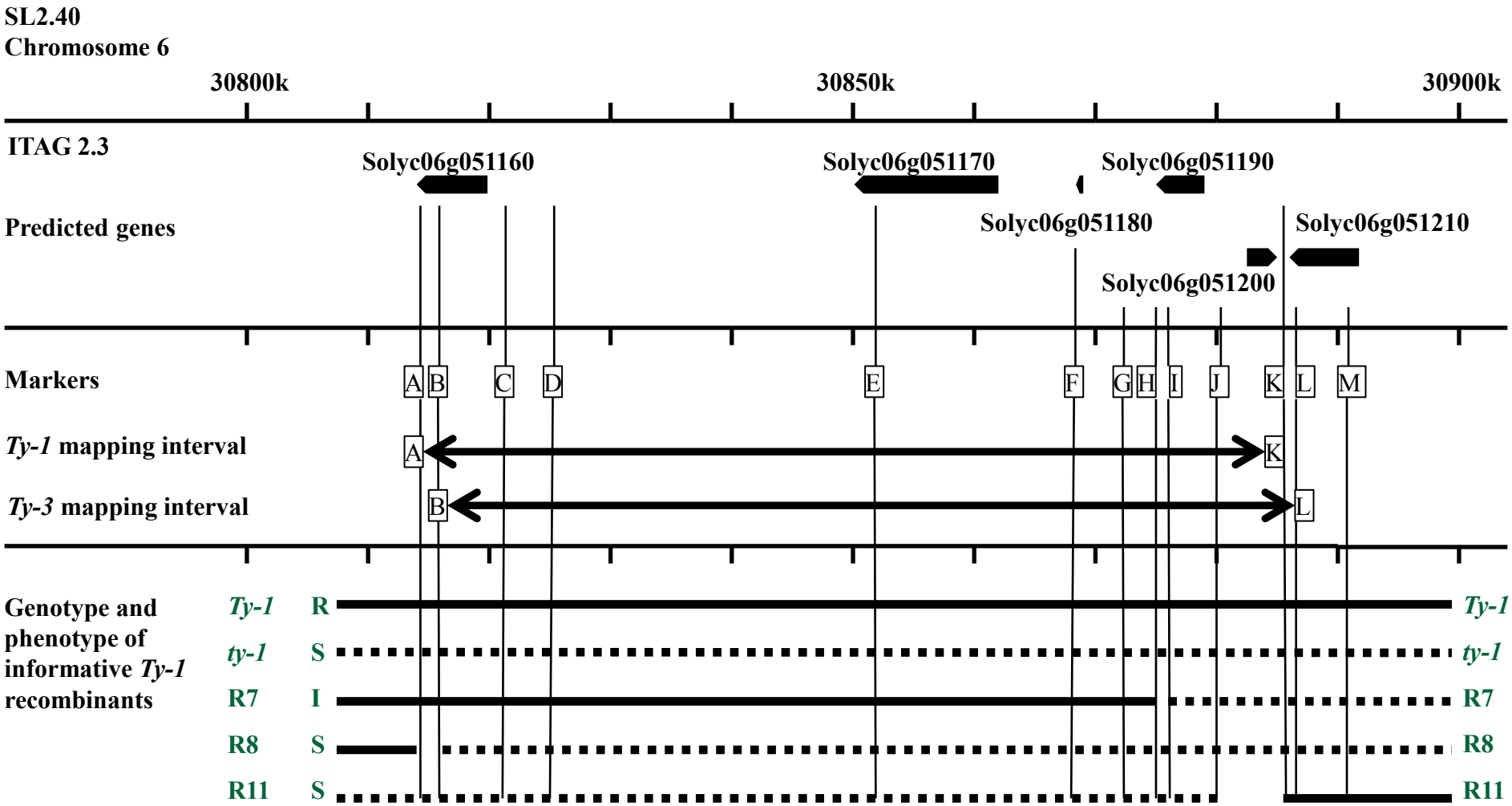
Solyc06g051180
Solyc06g051210
Solyc06g051200
Markers
A
C
D
E
F
G
H
I
J
K
L
M
Ty-1 mapping interval
Genotype and phenotype of informative Ty-1 recombinants
Ty-1 R
ty-1 S
R7 I
R8 S
R11 S
Ty-1
ty-1
R7
R8
R11


SL2.40
Chromosome 6
30800k
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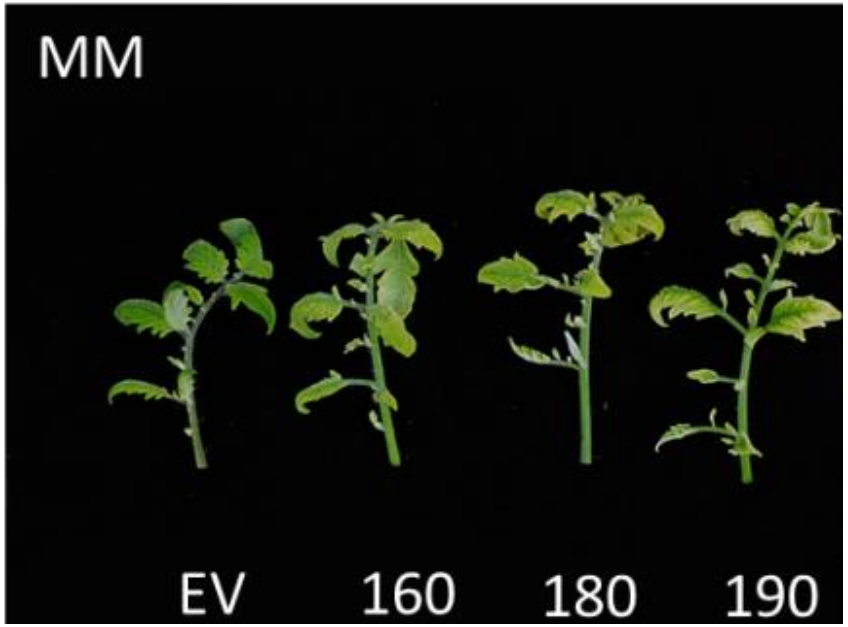
ITAG 2.3
Solyc06g051160
Solyc06g051170
Solyc06g051190
Predicted genes
Solyc06g051180
Solyc06g051210
Solyc06g051200
Markers
A B C D E F G H I J K L M
Ty-1 mapping interval
Ty-3 mapping interval
Genotype and phenotype of informative Ty-1 recombinants
Ty-1 R
ty-1 S
R7 I
R8 S
R11 S

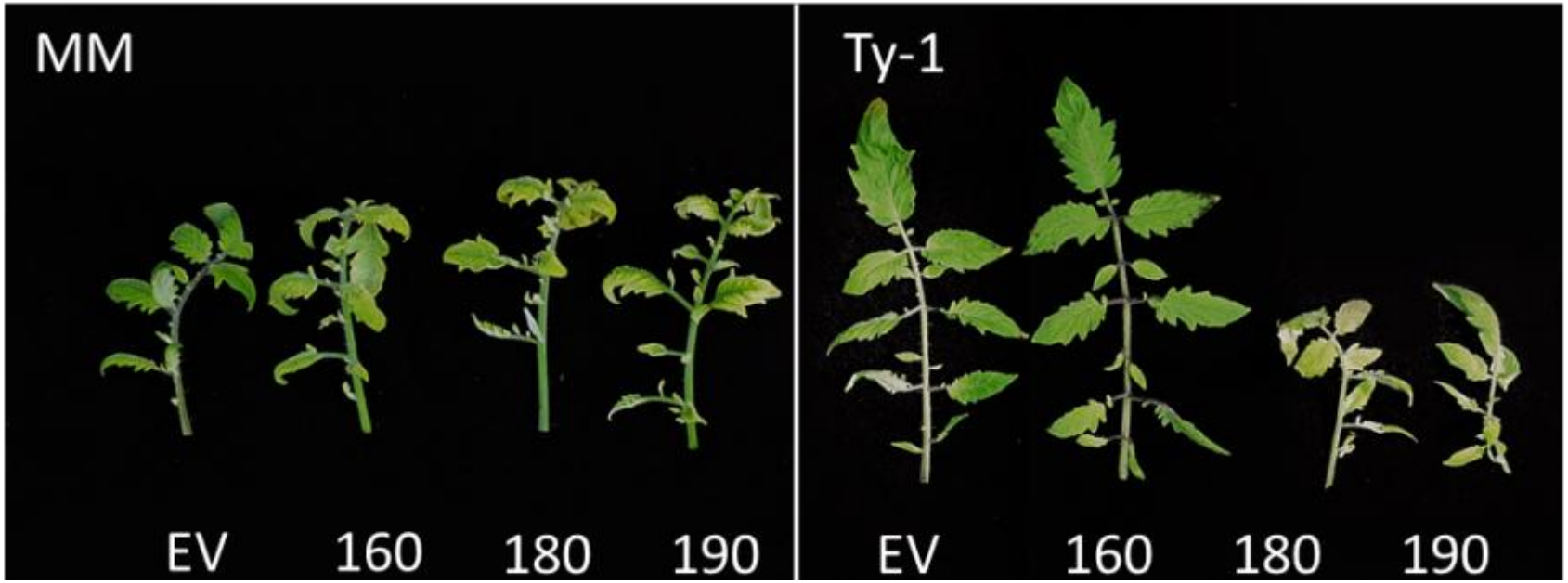
Candidate genes, VIGS constructs

- 160: Unknown function
- 170: RNA-dependent RNA polymerase (failed!)
- 180: RNA-dependent RNA polymerase
- 190: RNA-dependent RNA polymerase
- 200: Ribosomal protein (failed)

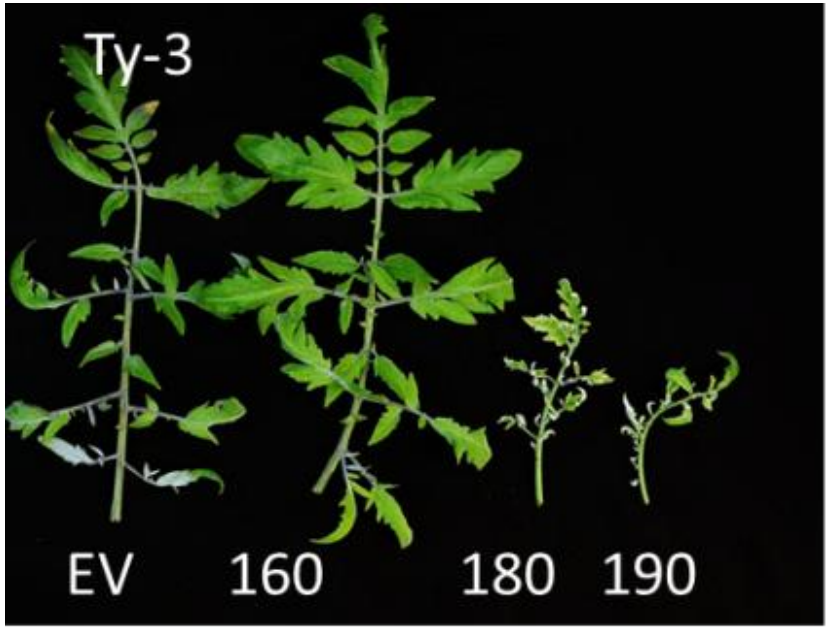
Silencing of candidate genes



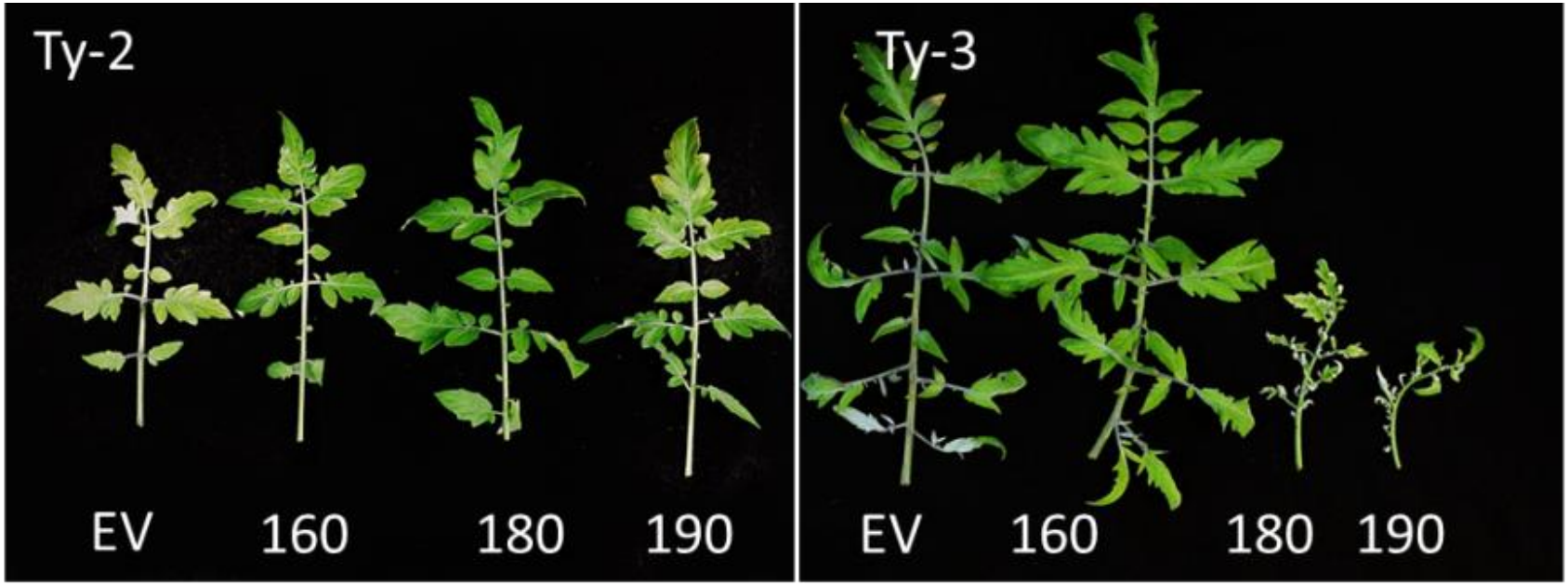
Silencing of candidate genes



Silencing of candidate genes

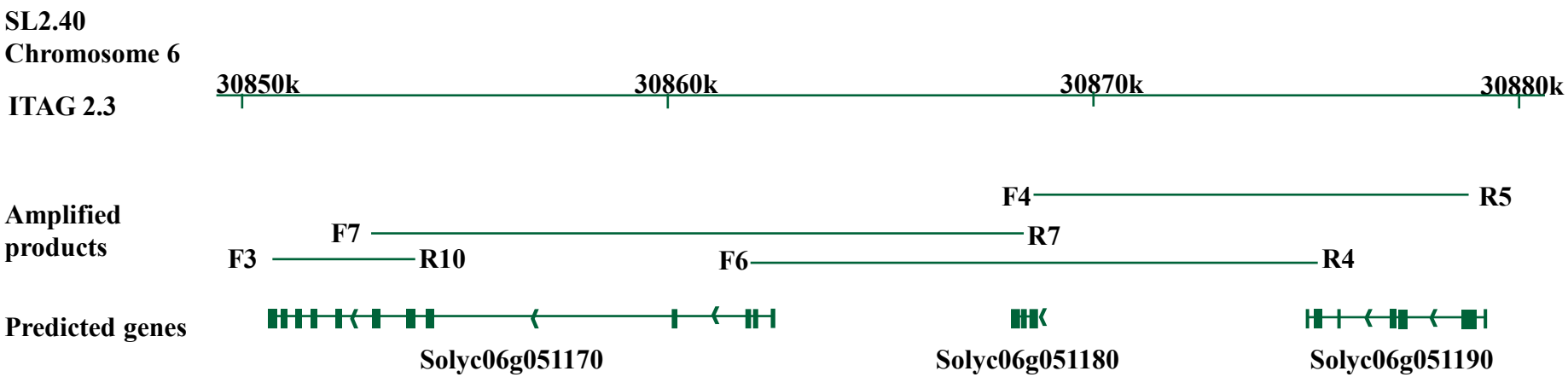


Silencing of candidate genes



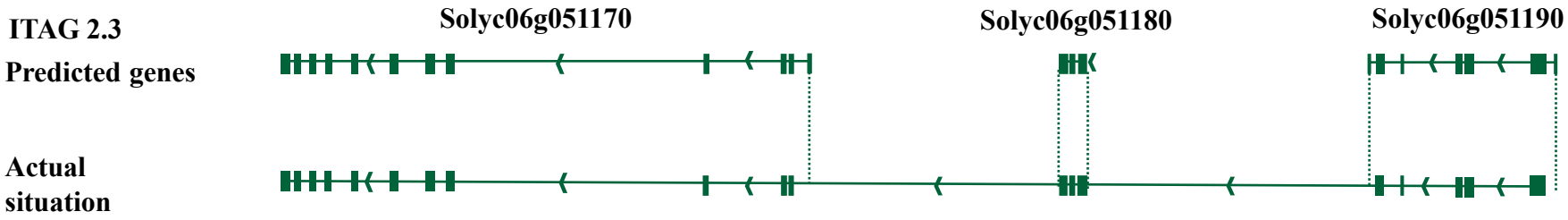
180 and 190, susceptibility!?

- Gene prediction wrong?
- 170, 180 and 190 similar function
- Design primers that overlap predicted genes



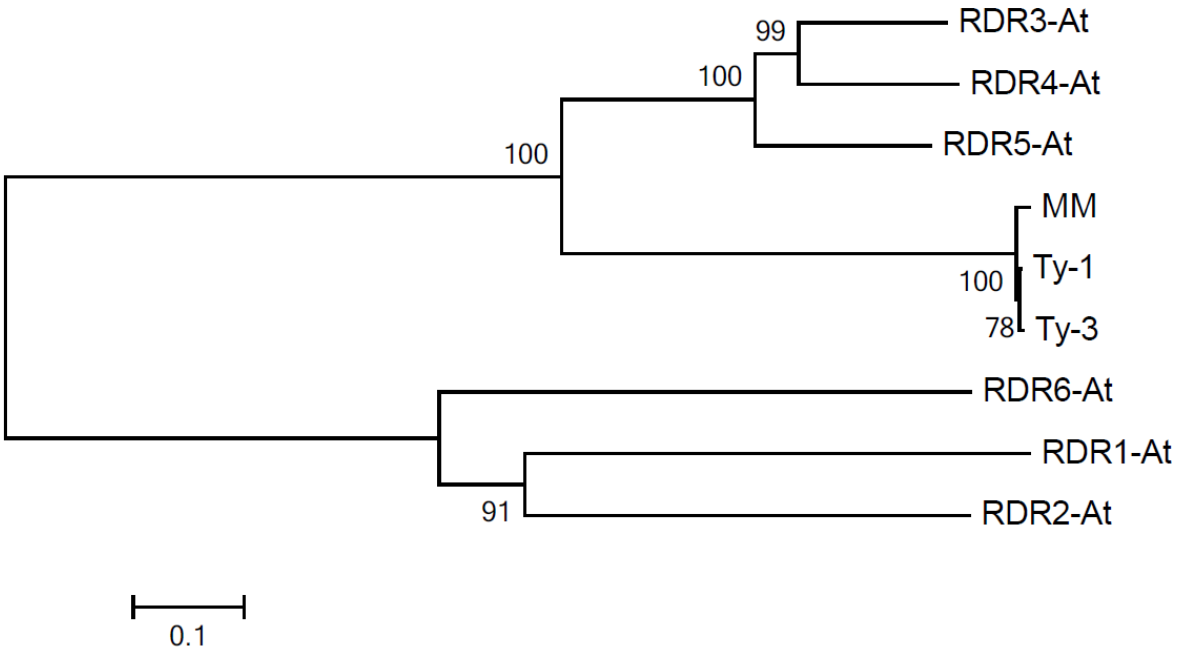
Full length cloning

- GeneRacer (Invitrogen) used to amplify and obtain sequence of 5 prime and 3 prime end
- New primers designed based on obtained sequence
- 3.1 kb fragment amplified and dTOPO cloned>sequenced



RNA-dependent RNA polymerases

- *Ty-1*/*Ty-3* are RDR3, 4, 5 homologs



RNA-dependent RNA polymerases

Catalytic domain

RDR1-At	N	E	C	S	G	S	D	L	D	G	D	I	Y	F	V	C	W
RDR2-At	N	E	C	S	G	G	D	L	D	G	D	Q	F	F	V	S	W
RDR6-At	N	E	A	S	G	S	D	L	D	G	D	L	Y	F	V	A	W
RDR3-At	D	E	I	A	G	G	D	F	D	G	D	M	Y	F	I	S	R
RDR4-At	D	E	I	A	G	G	D	F	D	G	D	M	Y	F	I	S	R
RDR5-At	D	E	I	A	G	G	D	F	D	G	D	M	Y	F	I	S	R
Ty-1	T	E	I	A	N	G	D	F	D	G	D	M	Y	W	V	S	I
Ty-3	T	E	I	A	N	G	D	F	D	G	D	M	Y	W	V	S	I
MM	T	E	I	A	N	G	D	F	D	G	D	M	Y	W	V	S	I

RNA-dependent RNA polymerases

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
RDR3-At	V	S	P	R	-	V	C	D	S	K	T	D	K	V	G	C	E	L	D	K	T	Q	E	L
RDR4-At	G	F	S	W	-	V	C	D	S	R	T	D	M	V	-	C	E	L	H	K	T	Q	S	S
RDR5-At	A	S	S	R	-	A	S	D	S	T	T	D	K	V	-	C	D	L	N	K	I	Q	T	S
Ty-1	P	S	C	I	S	V	Q	D	C	T	A	D	K	V	A	G	E	L	V	K	M	Q	L	S
Ty-3	P	S	C	I	S	L	L	D	C	T	A	D	I	V	A	G	E	L	V	K	M	Q	L	P
MM	-	-	-	-	C	R	Q	N	Y	S	L	N	K	T	V	R	K	M	A	N	T	R	F	S

4 aa deletion

Take home message

- *Ty-1* and *Ty-3* are allelic
- The annotation of the tomato genome in the *Ty-1*/*Ty-3* region was not correct
- *Ty-1* and *Ty-3* code for an *RDR* with an atypcial DFDGD catalytical domain for which no clear function has been described yet
- Reduced introgression sizes:
  - *Ty-1* (~175 Kb)
  - *Ty-3* (~70 Kb)
  - Planned for release in 2013

Spring 2011 Survey

- 53 advanced lines
- CRBD
  - 2 reps
  - 4 plant plots
- Seedling TYLCV inoc.
- Genotyped at resistance loci
- Line evaluations:
  - Viral disease severity
  - Non viral foliar disease severity

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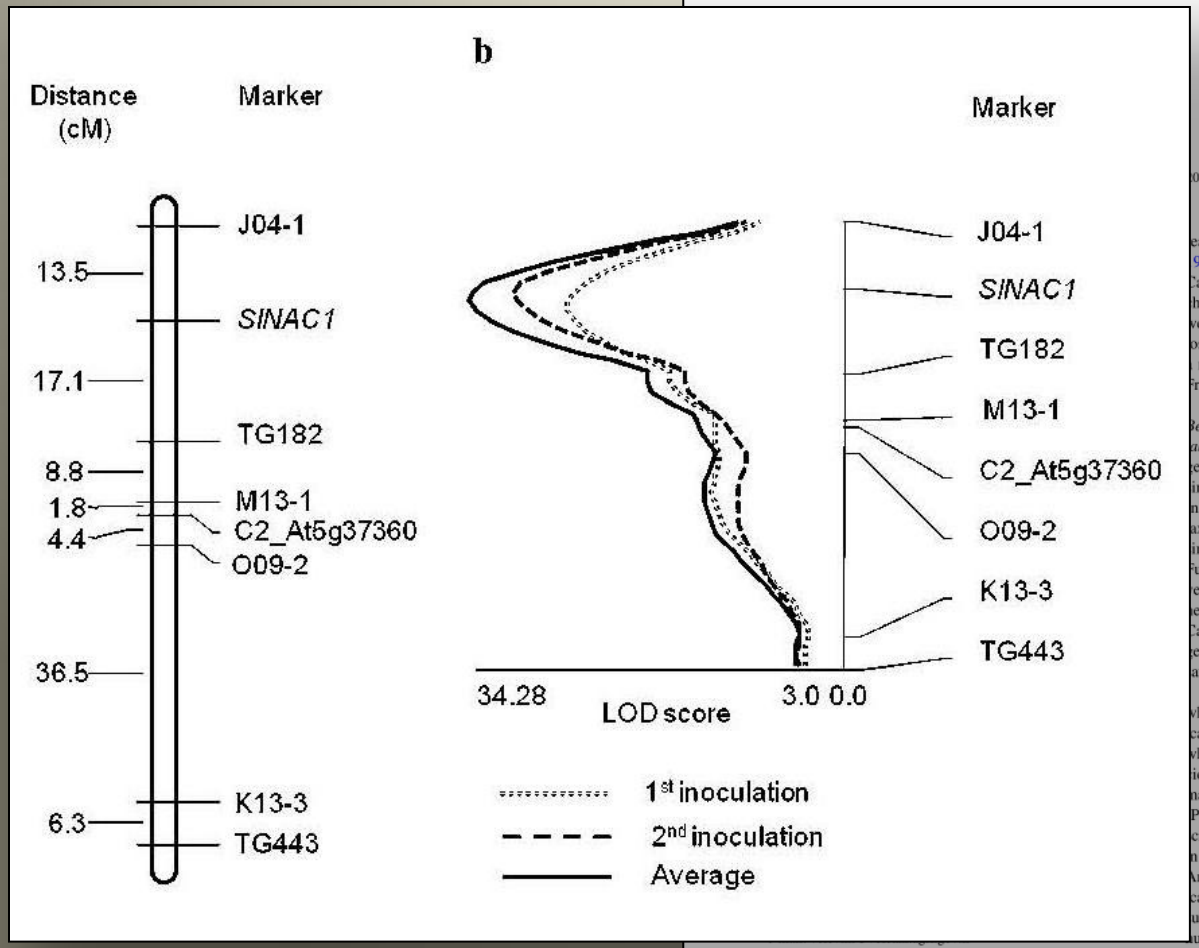
8503			8840		
8600			8841		
8601	3		8843		
8602	3		8844	3	
8603			8845		
8604			Fla678	3	
8624			Fla685	3	
8625			Fla707	3	
8635			Fla726	3	4
8636	3			3	

ty-5



Theor Appl Genet (2009) 119:519–530
DOI 10.1007/s00122-009-1060-z

ORIGINAL PAPER

Molecular dissection of *Tomato leaf curl virus* resistance in tomato line TY172 derived from *Solanum peruvianum*

0 May 2009

eached worldwide distribution (Czosnek and Laterrot 997; Polston and Anderson 1997; Moriones and Navas-Castillo 2000). TYLCV induces a severe tomato disease haracterized by yellowing and cupping of apex leaves as well as stunted plant growth coupled with significant yield osses. In many tomato-growing areas, TYLCV has become major limiting factor in tomato production (Lapidot and Friedmann 2002).

TYLCV belongs to the family *Geminiviridae*, genus *Begomovirus*, and transmitted by the whitefly *Bemisia abaci* in a circulative and persistent manner. The virus enome is composed of a single (monopartite) circular ingle-stranded DNA molecule of about 2,800 nucleotides. n the past, there has been some confusion regarding the axonomy of TYLCV. Several begomoviruses, inducing imilar symptoms in tomato, were all named TYLCV. Further analyses of these viruses showed that the tomato ellow leaf curl disease (TYLCD) is induced by a heteroge-eous complex of begomoviruses (Moriones and Navas-Castillo 2000). Most of the isolates have a monopartite enome, and recently a TYLCV isolate containing a DNA-β atellite was identified (Khan et al. 2008).

The management of TYLCV is difficult because its hitefly vector populations can reach high numbers. Chem-cal control methods have been only partially effective, hile raising concerns that the vector may develop pesti-ide resistance and that intense application of pesticides ay have deleterious environmental consequences Palumbo et al. 2001). Physical barriers such as fine-mesh creens and UV-absorbing plastic sheets or screens are used n the Mediterranean region to protect crops (Cohen and Antignus 1994; Antignus et al. 2001). However, such phys-cal barriers add to production costs and may also result in uboptimal light conditions, overheating, and increased umidity, which can hamper appropriate plant growth and

Selective genotyping at the *SlNAC1* locus of resistant and susceptible F_2 tomato plants grown in Spring 2009 and test for transmission disequilibrium

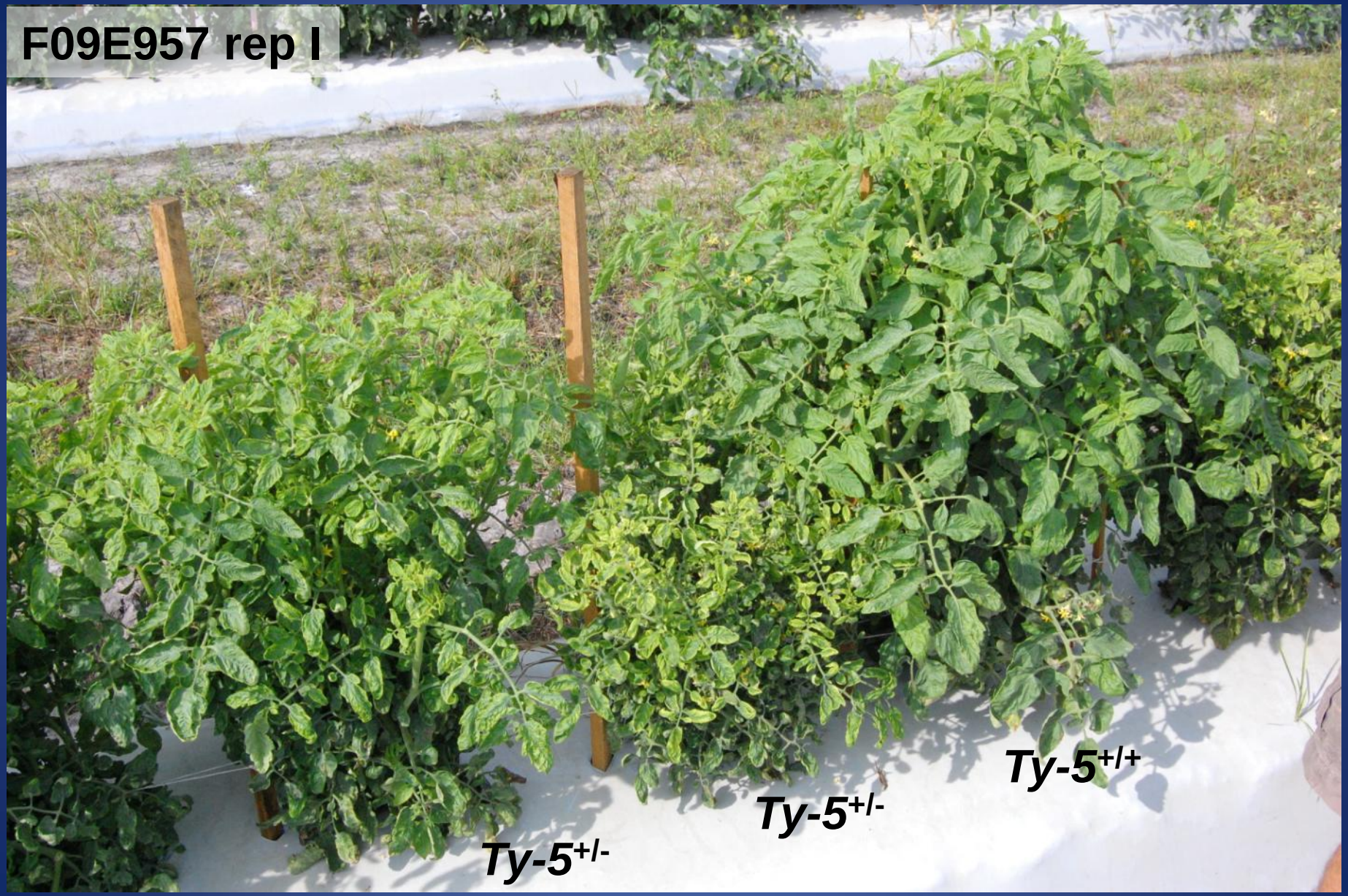
Family	Phenotype	DSI	Total no.	Plant nos. at the *SlNAC1* locus[z]			Transmission Disequilibrium *(P)*
				+/+	+/-	-/-	
(7655B x 8753) F_2	Resistant	0.5 2.0	21	14	7	0	
(8248 x 8753) F_2		1.0 – 2.0	12	11	0	1	
Total			33	25	7	1	
							<.0001
(7655B x 8753) F_2	Susceptible	3.5 4.0	21	0	11	10	
(8248 x 8753) F_2		3.5	9	0	6	3	
Total			30	0	17	13	

[z] +/+ homozygous resistant, +/- heterozygous, -/- homozygous susceptible; *DSI* disease severity index.

Disease severity and *SINAC1* genotype for parents and F_2 generation, Fall 2009.

Line	Plant No.	Genotype at the *SINAC1* locus	Mean DSI
Fla. 7547 (7547)	18	-/- (S parent)	4.0 a[z] ± 0.0
Fla. 344	18	+/+ (R parent)	1.1 d ± 0.1
	34[y]	+/+	1.8 c ± 0.1
(7547 x Fla. 344) F_2	39	+/-	3.4 b ± 0.1
	28	-/-	3.3 b ± 0.1

[z] Mean separation in columns by DMRT at $P \leq 0.05$.
[y] F_2 segregation fits 1:2:1 and 3:1 (+/-, -/-:+/+) ratios at P = .9 - .5 with χ^2 values of 0.669 (2dF) and 0.223 (1dF), respectively.


F09E957 rep I
Ty-5+/-
Ty-5+/-
Ty-5+/+

F09E972 rep I
Ty-5-/-
-/-
-/-

Spring 2011 Survey

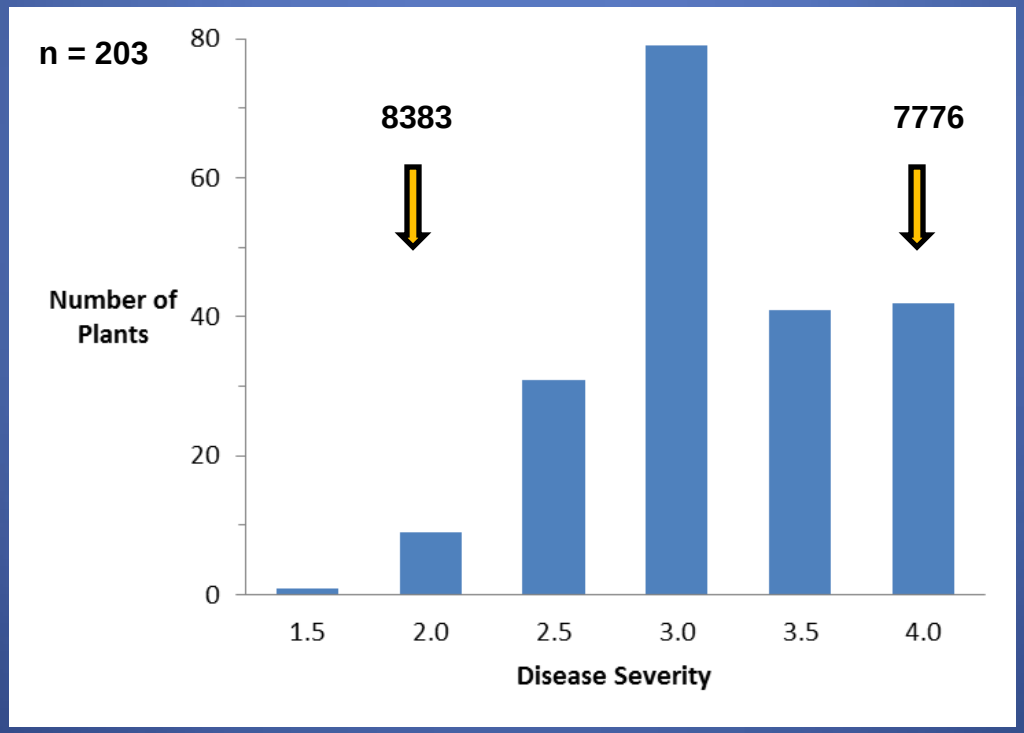
- 53 advanced lines
- CRBD
  - 2 reps
  - 4 plant plots
- Seedling TYLCV inoc.
- Genotyped at resistance loci
- Line evaluations:
  - Viral disease severity
  - Non viral foliar disease severity

Line	TY Gene			Line	TY Gene		
8259				8637			
8299			5	8638			5
8300			5	8655			5
8302				8656			5
8347	3	4		8657	3		
8352	3			8680	3		
8353	3			8737			5
8379	3			8738	3		
8383				8739	3		
8387			5	8741			
8467			5	8753			5
8468				8779			5
8469			5	8823	3		
8470			5	8824	3		
8472			5	8825	3		
8480	3			8826	3		
8501	3			8827			
8503				8840			5
8600				8841			
8601	3		5	8843			
8602	3			8844	3		
8603				8845			5
8604			5	Fla678	3		
8624				Fla685	3		
8625			5	Fla707	3		
8635				Fla726	3	4	
8636	3				3		

Ty-6 Mapping

Ty-6 Mapping

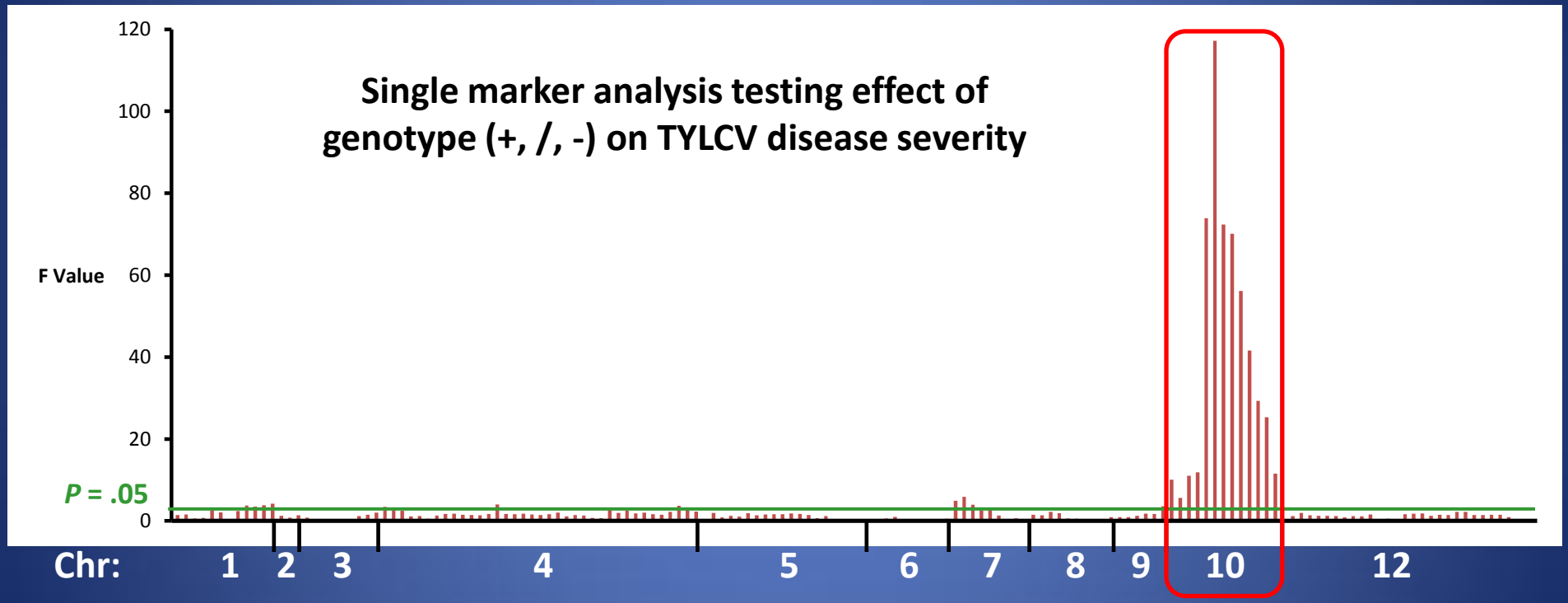
- Population: (7776 x 8383) F2
- Resistance Source: *S. chilense* LA2779



Ty-6 Mapping

- Genotyping accomplished through University of Florida SolCAP Community Project
- Subset of 384 SNPs optimized for processing and fresh market
- 158 polymorphic SNPs for (7776 x 8383) population

Ty-6 Mapping
(7776 x 8383) F_2



13/13 markers
P < .0001

Ty-6 Mapping
(7776 x 8383) F_2

Genotype	Mean DSI[z]
-	3.9 A
I	3.2 B
+	2.5 C

[z] Mean separation in columns by Duncan's Multiple Range Test at $P \leq 0.05$.

Chr 10 Marker	SL2.40 position (Mb)	F Value
1	3.024	10.0
2	3.991	5.7
3	4.260	11.1
4	4.721	11.9
5	60.348	73.9
6	62.488	117.2
7	62.755	72.3
8	62.811	70.1
9	63.003	56.1
10	63.160	41.6
11	63.665	29.3
12	63.928	25.3
13	64.754	11.5

Ty-6 Confirmation

TYLCV Disease Severity of Five Inbred Lines, Fall 2012

Line	Genotype	Source	Mean TYLCV DSI
Fla. 7804	susc.		4.0 A
Fla. 8383	*Ty-6*	LA2779	1.6 B
Fla. 8638B	*ty-5*, (*Ty-6*?)	Tyking/LA1938	0.5 C
Fla. 8753	*ty-5*, (*Ty-6*?)	Tyking/LA1938	0.5 C
Fla. 726	*Ty-3*, *Ty-4*	LA1932	0.0 D

[z] Mean separation based on Duncan's Multiple Range Test at $P \leq 0.05$.

Ty-6 Confirmation

(8638B x 7804) F2 Population

Source	DF	F Value	*P*
ty-5	2	88.15	<.0001

R-Square = 0.47

ty-5 Genotype	*N*	Mean DSI[z]
-	71	3.0 A
I	74	3.0 B
+	73	1.5 C

[z] Mean separation in columns by Duncan's Multiple Range Test at $P \leq 0.05$.

Ty-6 Confirmation

(8638B x 7804) F2 Population

Source	DF	F Value	*P*
Ty-6	2	50.55	<.0001

R-Square = 0.31

Ty-6 Genotype	*N*	Mean DSI[z]
-	80	3.3 A
I	91	2.4 B
+	56	1.7 C

[z] Mean separation in columns by Duncan's Multiple Range Test at $P \leq 0.05$.

Ty-6 Confirmation

(8638B x 7804) F2 Population

Source	DF	F Value	*P*
ty-5	2	152.04	<.0001
Ty-6	2	96.40	<.0001
ty-5 *x* Ty-6	4	0.98	0.42

R-Square = 0.73

ty-5	*Ty-6*	*N*	Mean DSI[z]
-	-	21	3.7 A
-	/	33	2.9 B
-	+	17	2.1 C
/	-	28	3.7 A
/	/	27	2.8 B
/	+	19	2.3 C
+	-	22	2.1 C
+	/	30	1.4 D
+	+	20	0.9 E

[z] Mean separation by DMRT at $P \leq 0.05$.

Conclusions

- *Ty-6*
  - "novel" begomovirus resistance locus identified
  - Derived from *S. chilense* (??)
  - Likely corresponds to *qTy10.1* identified by Hanson *et al.* from Fla. 456
  - Additive effect
  - Complements *ty-5* and (presumably) *Ty-3* well
- Next steps
  - Improved marker development for MAS
  - Testing in combination with other loci
  - Fine mapping…gene identification

Thanks!