

Development of a 13-SNP Multiplex Assay for 5 Disease Resistance Loci in Tomato

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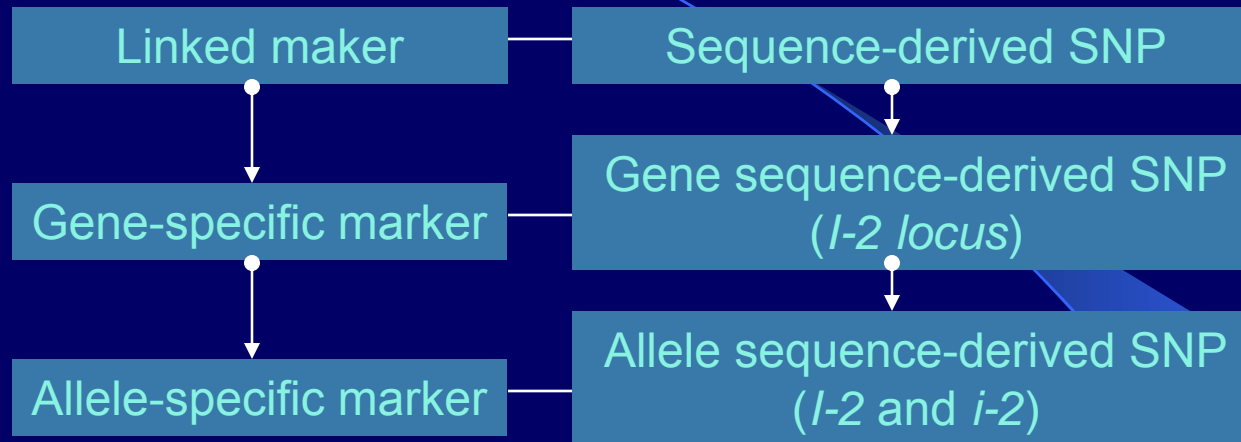


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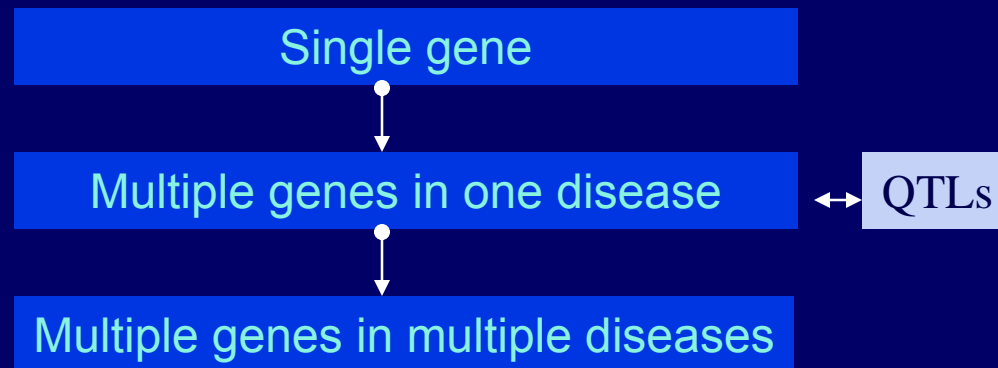


- **Genetic resistance is the primary method of controlling plant diseases**
- **Marker assisted selection (MAS) has become important and useful in the selection of disease resistance genes**
- **Single nucleotide polymorphism (SNP), because of its abundance and high-throughput scoring potential is becoming a powerful tool in genome mapping, association studies, diversity analysis, and tagging of important genes in plant genomics**

Molecular Marker Evolution



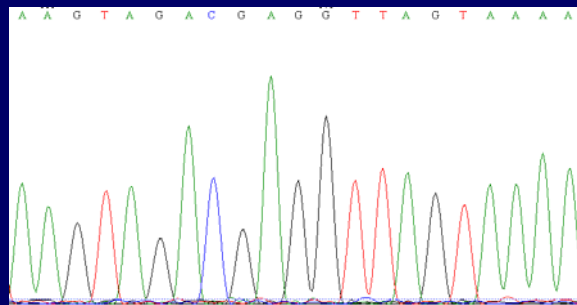
Molecular Assisted Selection



Single Nucleotide Polymorphism (SNP)

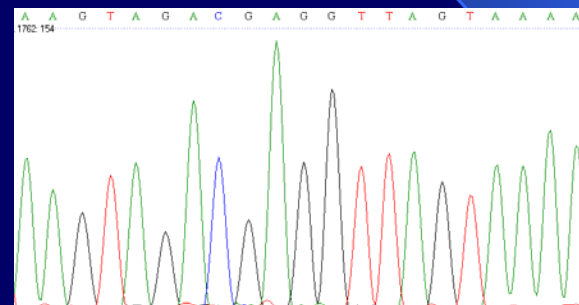
An example for Mi

Motella/LA2823	Mi	AAGTAGACGA G GTTAGTAAAAT
Mogeor/LA3471	Mi	AAGTAGACGA G GTTAGTAAAAT
NY07-464	mi	AAGTAGACGA C GTTAGTAAAAT
LA3130	mi	AAGTAGACGA C GTTAGTAAAAT



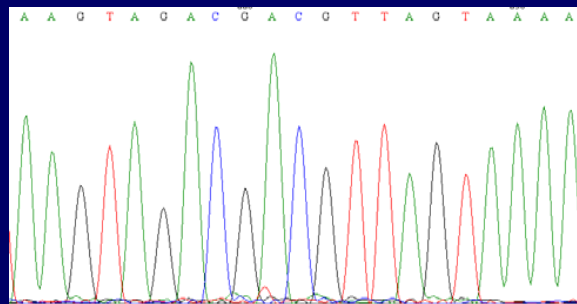
Motella

G



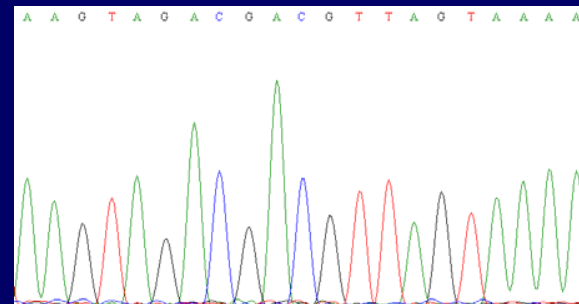
Mogeor

G



NY07-464

C



LA3130

C

Five Tomato Diseases



- A. Fusarium wilt (*Fusarium oxysporum* f. sp. *Lycopersici*) (image from apsnet)
- B. Verticillium wilt (*V. dahliae* and *V. albo-atrum*) (image from Cornell)
- C. Root-knot nematode (*Meloidogyne incognita*, *M. javanica*) (image from NCSU)
- D. Tomato mosaic virus (ToMV) (image from apsnet)
- E. Tomato spotted wilt virus (TSWV) (image from NCSU)

Objective

- To identify SNP markers for *I-2* of Fusarium wilt resistance, *Mi* of root-knot nematode resistance, *Sw-5* of TSWV resistance, *Tm-2* of ToMV resistance, and *Ve* of Verticillium wilt resistance
- To develop a multiplex SNP assay for marker assisted selection (MAS) of the five genes

Outline

- I. **Develop allele-specific SNP marker for *Sw-5***
- II. **Select SNP markers for *I-2*, *Mi*, *Tm-2*, and *Ve***
- III. **Develop a 13-SNP assay for the 5 genes**
- IV. **SNP genotyping for the 5 genes in tomato germplasm**

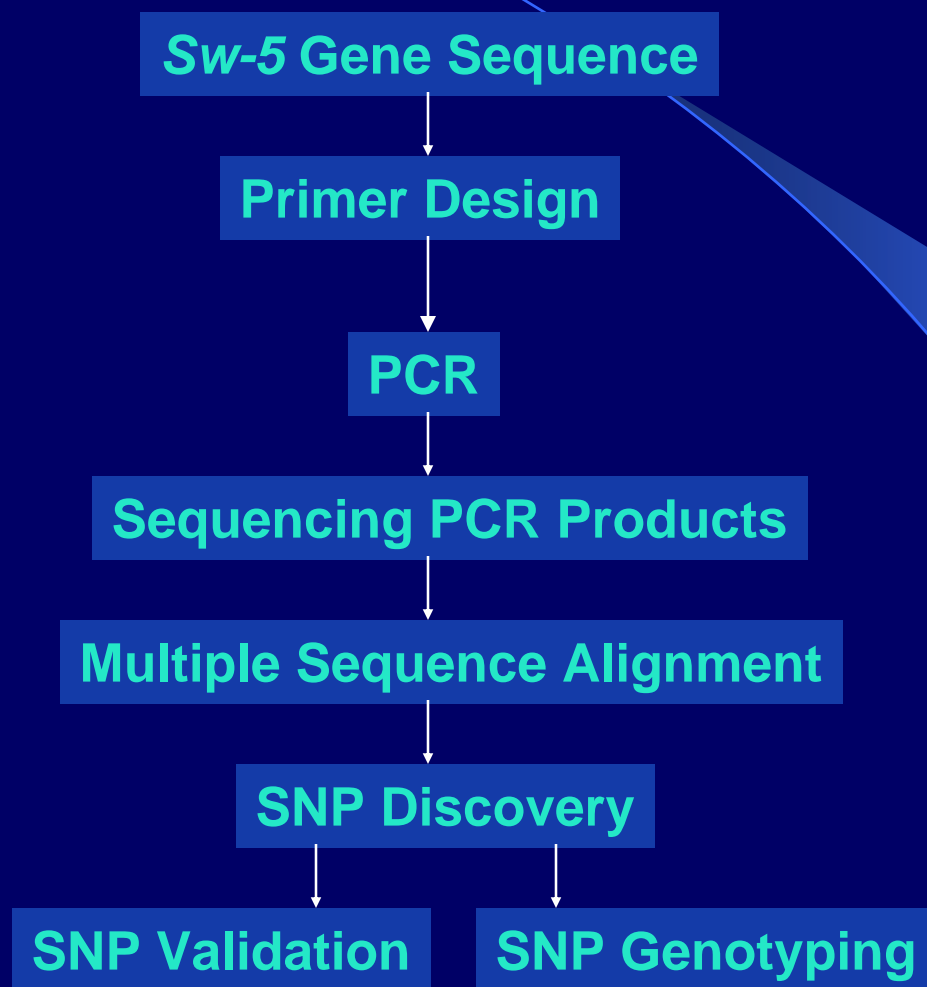
I. Develop Allele-specific SNP Marker for Sw-5

- Sw-5 is the most broadly deployed TSWV resistance gene utilized in tomato breeding because of its durability and stable resistance (Boiteux and Giordano, 1992; Stevens et al., 1992, 1994, 1995).
- Sw-5 gene has been cloned and its sequence was deposited in GenBank with the accession AY007366.

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AAGCTTGGTGCAATGACATTATACAATCAACAAAGAATAGAAAGGAGATAAAAAGCACTATCCAAATCCC
-----
-----
TCCATTTTCCTTTTTTTGATTATTCCAGCTACCAAGACAACCACTAGGGGCAGTCCTTTGCATTTTTCTG
CTACTGCTTGACTCACATCTTGTAGTTCAAGCGGGCAATCTTCCTTTGAAACACTTTTTTCTGCAACAA
TTGGCAACTCTCTTCTGTTGTGAGGAATGGAAGAGAATAAGGATCAGTATGGTACTTGACTTGCTTACCC
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CATCCCATACCATAACAATCCCACATATCATCCAATACAATGAGATATCTCTTCCCATTATTTTCTCCT
CAACATGTCGGCAAGAACATCAACCGTAGCTCCATTGTCGTTGAAACCTGTAACCTTGACTGAAAATATCT
TGTAATAACTCTATCCGGTTATACGTTTGAGAAATGATGCACCATGCTCGAACATCAAAGCGAGAAACAA
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ACCTCCTCATCAGTTACAAGATTGCTATGTCGATCCGGCAGATGTTTTAATGGAGCAGCCACATAGTGAG
-----
TTGGCTTAAATAAGTTTTTTTTAACTTAAAATATGTTATTTGAGTATTGACAAACAATAAATAAGTC
AAAATCAGCTTTTAAGTCAGTTTGACCACGCTCTCTTAT
```

Sw-5 – AY007366

Procedure for development of Sw-5 specific SNP markers



AAGCTTGGTGCAATGACATTATACAATCAACAAAGAATAGAAAGGAGATAAAAAGCACTATC

TCCATTTTCCTTTTTTTGATTATTCCAGCTACCAAGAC**AACCACTAGGGGCAGTCCTT**TGCA
CTACTGCTTGACTCACATCTTGTAGTTCAAGCGGGCAATCTTCTTTTGAACACTTTTTTC
TTGGCAACTCTCTTCTGTTGTGAGGAATGGAAGAGAATAAGGATCAGTATGGTACTTGACT
ACTTCTTCAAGTCGAGTTGTTACGACTATTCTGCTTCTAATTCCAACATCTGAAAAGAAAG
CATCCCATACCATACAATCCCACATATCATCCAATACAATGAGATATCTCTTCCCATTAAAT
CAACATGTCGGCAAGAACATCAACCGTAGCTCCATTGTCGTTGAAACCTGTAACCTTGACTG
TGTAATAACTCTATCCGGTTATACGTTTGAGAAATGATGCACCATGCTCGAACATCAAAGC
TAATGTCATTATTGTACAACCTTTCTAGCAATTGTCGTTTTCCCTTGTCTCCCATGCCTACA
AACGTCTAGCTCATTTGTACCTCTAGTCAGATAATCAATTAGTTCTTCTGCTTGTTTCTCAA
ACCTCCTCATCAGTTACAAGATTGCTATGTCGATCCGGCAGATGTTTTAAT**TGGAGCAGCCA**

TTGGCTTAAATAAGTTTTTTTTAACTTAAATATGTTATTTTGGAGTATTGACAAACAATAA
AAAATCAGCTTTTAAGTCAGTTTGACCACGCTCTCTTAT

Sw-5: AY007366

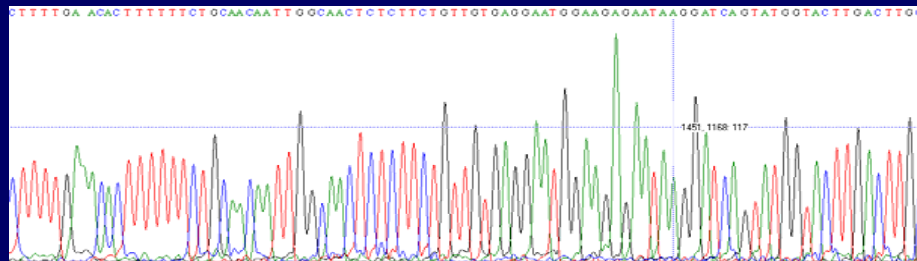
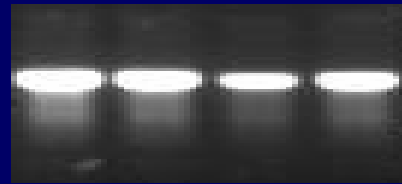
Primer design

PCR

PCR Product Sequencing

Tool: Primer-BLAST

Sw5-f1 **AACCACTAGGGGCAGTCCTT**
Sw5-r1 **CTCACTATGTGGCTGCTCCA**
 (TGGAGCAGCCACATAGTGAG)



Multiple Sequence Alignment

- Tool - BioEdit

AY007366-sw5b	AACCACTAGGGGCAGTCCTTTGCATTTTTCTGCTACTGCTTGACTCACATCTTGTAGTTTCAGGCGGGCAATCTTCCTTTTG	
LA3667	-----	100
NY07-464	-----	100
LA2823	-----	100
AY007366-Sw5a	AACCACTAGGGGCAGTCCTTTGCATTTTTCTGCTACTGCTTGACTCACATCTTGTAGTTCAAGCGGGCAATCTTCCTTTTG	
AY007366-sw5b	AATTGGCAACTCTCTTCTGTTGTGAGGAATGGAAGAGAATAAGGATCAGTATGGTACTTGACTTGCTTACCCACTTCTTCA	
LA3667	-----	200
NY07-464	-----	200
LA2823	-----	200
AY007366-sw5b	TTCTGCTTCTAATTCCATCATCTGGAAAAGAAAGCCTTAAGTCATCCCATAACATAACAATCCACATATCATCCAATACAAT	
LA3667	-----W-----	300
NY07-464	-----A-----	300
LA2823	-----A-----	300
AY007366-sw5b	TAATTTTCTCCTCAACATGTCGGCAAGAACAATCAACCGTAGCTCCATTGTCGTCGGAACCTGTAAGTACTTGACTGAAAATATC	
LA3667	-----A-----Y-R-----G-----M-C-R	400
NY07-464	-----G-----T-A-----A-----T--	400
LA2823	-----G-----T-A-----A-----T--	400
AY007366-sw5b	TTATACGTTTGAGAAATGATGCACCATGCTCGAACATCAAAGCGAGAAACAATAATGTCATTATTGTACAACCTTCTAGCA	
LA3667	-----	500
NY07-464	-----	500
LA2823	-----	500
AY007366-sw5b	CTCCCATGCCTACAATTGGGACAACGTCTAGCTCATTGTACCTCTAATCAGATAATCAATTAGTTCTTCTGCTTTATTCTC	
LA3667	-----R-----KW-----	600
NY07-464	-----	600
LA2823	-----	600
AY007366-sw5b	ATCAGTTACAAGATTGCTATGTCGATCCGGCAGATGTTTTAATGGAGCAGCCACATAGTGAG	662
LA3667	-----	662
NY07-464	-----	662
LA2823	-----	662

SNP Discovery

Accession	Gene	GenBank accession	Sw5-snp1	sw5-snpS	snp3	snp4	snp5	snp6
Stevens	Sw-5b	AY007366	31279 T	31392 A	31415 C	31417 G	31434 G	31459 C
LA3667	Sw-5	FJ686041	218 W	331 A	354 Y	356 R	373 G	398 C
LA3433	sw-5	FJ686040	218 A	331 G	354 T	356 A	373 A	398 T
Riesentraube	sw-5	FJ686039	218 A	331 G	354 T	356 A	373 A	398 T
LA2823	sw-5	FJ686042	218 A	331 G	354 T	356 A	373 A	398 T
Stevens	Sw-5a	AY007366	22586 A	22699 A	22722 T	22724 A	22741 G	22766 C

* Sw-5 consisted of Sw-a and Sw-b. Sw5-a is located at 20824-24561 base and Sw5-b at 29513-33253 base of the GenBank accession AY0073366 that was cloned from the tomato cultivar "Stevens" (Folkertsma et al 1999). Sw-b is the functional allele for conferring resistance to TSWV (Spasova et al., 2001).

'W' = 'A/T', 'Y' = 'C/T', and 'R' = 'A/G'

SNP Validation and Genotyping by Sequenom

Cultivar/Accession	Gene at <i>Sw-5</i> locus	Sw5-f1/r1	Sw5-f2/r2	Sw5-snp1	Sw5-snp2
Talladega	<i>Sw5-b^z</i> & <i>Sw5-a</i>	+	+	A T	A A
Sophya	<i>Sw5-b</i> & <i>Sw5-a</i>	+	+	A T	A A
BHN-444	<i>Sw5-b</i> & <i>Sw5-a</i>	+	+	A T	A A
LA3667	<i>Sw5-b</i> & <i>Sw5-a</i>	+	+	A T	A A
Anahu (LA0655)	<i>sw5-b</i>	+	-	A A	G G
LA0656	<i>sw5-b</i>	+	-	A A	G G
VFNT Cherry (LA1221)	<i>sw5-b</i>	+	-	A A	G G
LA1802	<i>sw5-b</i>	+	-	A A	G G
Royal Red (LA2088)	<i>sw5-b</i>	+	-	A A	G G
Mobox (LA2821)	<i>sw5-b</i>	+	-	A A	G G
Motella (LA2823)	<i>sw5-b</i>	+	-	A A	G G
UC-204C (LA3130)	<i>sw5-b</i>	+	-	A A	G G
LA3432	<i>sw5-b</i>	+	-	A A	G G
LA3433	<i>sw5-b</i>	+	-	A A	G G
Mogeor (LA3471)	<i>sw5-b</i>	+	-	A A	G G
LA3473	<i>sw5-b</i>	+	-	A A	G G
Peto 95-43 (LA3528)	<i>sw5-b</i>	+	-	A A	G G
Riesentraube	<i>sw5-b</i>	+	-	A A	G G
Bush Celebrity	<i>sw5-b</i>	+	-	A A	G G
NY07-461	<i>sw5-b</i>	+	-	A A	G G
NY07-464	<i>sw5-b</i>	+	-	A A	G G

II. Selection of SNPs for *I-2*, *Mi*, *Tm-2* and *Ve*

- *I2* is resistant to Fusarium wilt and the gene has been cloned (GenBank Accession: AF118127) (Simons et al., 1998).
- *Mi* is resistant to root-knot nematode. *Mi-1.1* and *Mi-1.2* have been cloned (AF039682 and AF039682) (Milligan et al., 1998).
- *Tm-2* is resistant to tomato mosaic virus. *Tm-2*, *Tm-2²*, and *tm-2* at *Tm-2* locus have been cloned (AF536199, AF536200, and AF536201) (Lanfermeijer et al., 2003).
- *Ve* is resistant to Verticillium wilt. *Ve1* and *Ve2* were cloned [(AF272366 and AF272367 for *Ve1*, and AF365929 and AF365930 for *Ve2* (Kawchuk et al., 2001); FJ464554 - FJ464557 for *Ve1* and FJ464558 - FJ464561 for *Ve2* (Fradin et al., 2009)]. The *Ve1*, but not *Ve2*, provided resistance in tomato against race 1 strains of *V. dahliae* and *V. albo-atrum* (Fradin et al., 2009).

One SNP selection for *I-2* of Fusarium wilt resistance

AF118127

AGAGACTCACAGAGTTATGGATC**G**ATCATGATGGGAGT

GACGAA

03F-7

AGAGACTCACAGAGTTATGGATC**G**ATCATGATGGGAGT

GACGAA

MoneyMaker

AGAGACTCACAGAGTTATGGATC**C**ATCATGATGGGAGT

GACGAA

	AF118127	<i>I-2</i>	3274	G
	03F-7	<i>I-2</i>	143	G
	MoneyMaker	<i>i-2</i>	143	C

Yu and Zou. 2008. A co-dominant molecular marker of Fusarium wilt resistance gene *I-2* derived from gene sequence in tomato. *Hereditas* (Beijing) 30(7): 926—932

One SNP selection for *Mi* of root-knot nematode resistance

Name	Allele at <i>Mi</i>	Sequence around <i>Mi23-snp1</i> SNP location
EU033927. M82-1-8	mi	GGAACTATTAAGTAGACGACCGTTAGTAAAATAACAAGCA
EU033930. LA2184	mi	GGAACTATTAAGTAGACGACCGTTAGTAAAATAACAAGCA
EU033932. LA3858	Mi3	GGAACTATTAAGTAGACGACGGTTAGTAAAATAACAAGCA
EU033926. Gh2	Mi	GGAACTATTAAGTAGACGACGGTTAGTAAAATAACAAGCA
Motella /LA2823	Mi	GGAACTATTAAGTAGACGACGGTTAGTAAAATAACAAGCA
Mogeor / LA3471	Mi	GGAACTATTAAGTAGACGACGGTTAGTAAAATAACAAGCA
LA0655	Mi	GGAACTATTAAGTAGACGACGGTTAGTAAAATAACAAGCA
NY07-461	mi	GGAACTATTAAGTAGACGACCGTTAGTAAAATAACAAGCA
NY07-464	mi	GGAACTATTAAGTAGACGACCGTTAGTAAAATAACAAGCA
LA3130	mi	GGAACTATTAAGTAGACGACCGTTAGTAAAATAACAAGCA
LA2396	mi	GGAACTATTAAGTAGACGACCGTTAGTAAAATAACAAGCA

Stuart,S., Williamson,V.M., Garcia,B.E. and Maxwell,D.P. 2007. A co-dominant SCAR marker for detection of the *Mi-1* locus for resistance to root-knot nematode in tomato germplasm.

<http://www.plantpath.wisc.edu/pp-old/GeminivirusResistantTomatoes/Markers/MAS-Protocols/Mi23-SCAR.pdf>

Part of SNPs for *Tm-2* of Tomato mosaic virus resistance

Name	Gene	2059	2162	2288	2300	2308	2311	2313	2323	2333	2334	2352	2356	2363	2372	2381	2390	2397
AF536201	<i>Tm-2</i>	C	T	T	G	T	T	A	T	G	G	T	C	C	A	G	G	C
AF536200	<i>Tm-2</i>	C	T	T	G	T	T	A	T	G	G	T	C	C	A	G	G	C
AF536199	<i>tm-2</i>	T	C	A	A	C	G	C	C	A	A	G	T	T	G	C	C	A
LA0655	<i>tm-2</i>	T	C	A	A	C	G	C	C	A	A	G	T	T	G	C	C	A
LA0656	<i>tm-2</i>	T	C	A	A	C	G	C	C	A	A	G	T	T	G	C	C	A
LA3528	<i>tm-2</i>	T	C	A	A	C	G	C	C	A	A	G	T	T	G	C	C	A
LA1221	<i>Tm-2</i>	C	T	T	G	T	T	A	T	G	G	T	C	C	A	G	G	C
LA2088	<i>Tm-2</i>	C	T	T	G	T	T	A	T	G	G	T	C	C	A	G	G	C
LA3432	<i>Tm-2</i>	C	T	T	G	T	T	A	T	G	G	T	C	C	A	G	G	C
LA3433	<i>Tm-2</i>	C	T	T	G	T	T	A	T	G	G	T	C	C	A	G	G	C
LA3471	<i>Tm-2</i>	C	T	T	G	T	T	A	T	G	G	T	C	C	A	G	G	C

		7270	7280	7290	7300	7310
AF536201	<i>Tm-2 a</i>	G	T	G	A	A
AF536200	<i>Tm-2</i>	T	A	T	T	G
AY742887	<i>Tm-2nv</i>	T	A	T	T	G
FJ817598	LA3433 <i>Tm-2</i>	T	A	T	T	G
FJ817597	LA1221 <i>Tm-2</i>	T	A	T	T	G
FJ817599	LA3528 <i>tm-2</i>	T	A	T	T	G
FJ817596	LA0656 <i>tm-2</i>	T	A	T	T	G
FJ817595	LA0655 <i>tm-2</i>	T	A	T	T	G
AF536199	<i>tm-2</i>	T	A	T	T	G

		8100	8110	8120	8130	8140
AF536201	<i>Tm-2 a</i>	T	C	G	A	C
FJ817606	LA3471 <i>Tm-2 a</i>	T	C	G	A	C
FJ817603	LA2088 <i>Tm-2 a</i>	T	C	G	A	C
FJ817602	LA1221 <i>Tm-2 a</i>	T	C	G	A	C
AF536200	<i>Tm-2</i>	T	C	G	A	C
AY742887	<i>Tm-2nv</i>	T	C	G	A	C
FJ817605	LA3433 <i>Tm-2</i>	T	C	G	A	C
FJ817604	LA3432 <i>Tm-2</i>	T	C	G	A	C
FJ817601	LA0656 <i>tm-2</i>	T	C	G	A	C
FJ817600	LA0655 <i>tm-2</i>	T	C	G	A	C
FJ817607	LA3528 <i>tm-2</i>	T	C	G	A	C
AF536199	<i>tm-2</i>	T	C	G	A	C

		8490	8500	8510	8520	8530	8540	8550	8560	8570	8580
AF536201	<i>Tm-2 a</i>	G	T	T	A	C	A	A	C	A	A
FJ817606	LA3471 <i>Tm-2 a</i>	G	T	T	A	C	A	A	C	A	A
FJ817603	LA2088 <i>Tm-2 a</i>	G	T	T	A	C	A	A	C	A	A
FJ817602	LA1221 <i>Tm-2 a</i>	G	T	T	A	C	A	A	C	A	A
AF536200	<i>Tm-2</i>	G	T	T	A	C	A	A	C	A	A
AY742887	<i>Tm-2nv</i>	G	T	T	A	C	A	A	C	A	A
FJ817605	LA3433 <i>Tm-2</i>	G	T	T	A	C	A	A	C	A	A
FJ817604	LA3432 <i>Tm-2</i>	G	T	T	A	C	A	A	C	A	A
FJ817601	LA0656 <i>tm-2</i>	G	T	T	A	C	A	A	C	A	A
FJ817600	LA0655 <i>tm-2</i>	G	T	T	A	C	A	A	C	A	A
FJ817607	LA3528 <i>tm-2</i>	G	T	T	A	C	A	A	C	A	A
AF536199	<i>tm-2</i>	G	T	T	A	C	A	A	C	A	A

Identification of SNPs for *Ve1* of Verticillium wilt Resistance

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AF272367      TTTGAGCTTGCCTGATTGTCAAATTCAGGCCCTTTGGATGAATCACTTTCTAAGCTTCACCTTTCTCTCTTTTGCCAACCTGACCAGAACAAATCTCTCT
              AF272366      .....A.....
              NY07-461      .....A.....
              NY07-464      .....A.....
              Riesentraube .....A.....
              Mogeor        .....T.....
              Peto 95-43     .....T.....
              Bush Celebrity .....T.....

AF272367      CAGCGGGAGCCTTCTGCATATATATTGAGCTCCCCTCGTTGCAGCAGCTTTTTCTTTACAGAAATCAATTTGTTGGCCAAGTCGACGAATTCGCAAT
              AF272366      ..G.....
              NY07-461      ..A.....
              NY07-464      ..A.....
              Riesentraube .....A.....
              Mogeor        ..G.....
              Peto 95-43     ..G.....
              Bush Celebrity ..G.....

AF272367      CACTTTCTTCCAACCTCTTTAGAGGGACAGTGCCCTTGACCTCATTGGGAGGCTGAGCAACCTTCAAGACTGGAGCTTTCTTACAATAAAGTACTGACTGT
              AF272366      .....G.....
              NY07-461      .....G.....
              NY07-464      .....G.....
              Riesentraube .....G.....
              Mogeor        .....C.....
              Peto 95-43     .....C.....
              Bush Celebrity .....C.....
    
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Variety	Gene at Ve locus	GenBank accession	Ve1-snp1		Ve1-snp2		Ve1-snp3	
			Location	Base	Location	Base	Location	Base
Craigella	Ve1	AF272367	2199	T	2752	G	3041	C
Mogeor	Ve1	FJ809927	50	T	603	G	892	C
Peto 95-43	Ve1	FJ809928	50	T	603	G	892	C
Bush Celebrity	Ve1	FJ686046	50	T	603	G	892	C
NY07-461	ve1	FJ809926	50	A	603	A	892	G
NY07-464	ve1	FJ809925	50	A	603	A	892	G
Riesentraube	ve1	FJ686045	50	A	603	A	892	G

Identification of SNPs for Ve2

Tomato variety	Gene at Ve locus	GenBank accession	Ve2-snp2		Ve2-snp3		Ve2-snp4	
VFN-8	Ve	AF365929	2827	G	2949	T	2990	C
VFN-8	Ve	AF365930	2771	G	2893	T	2934	C
VFN-8	Ve	FJ464562	2771	C	2893	C	2934	T
Motelle	Ve	FJ464561	2771	C	2893	C	2934	T
Craigella GCR218	Ve	FJ464559	2771	C	2893	C	2934	T
Craigella GCR26	ve	FJ464558	2771	G	2893	T	2934	C
Moneymaker	ve	FJ464560	2771	G	2893	T	2934	C
NY07-464	ve	FJ985984	457	G	579	T	620	C
NY07-461	ve	FJ985983	457	G	579	T	620	C
Rehovot 13	ve	FJ985982	457	G	579	T	620	C
Peto 95-43	Ve	FJ985981	457	C	579	C	620	T
Bush Celebrity	Ve	FJ985980	457	C	579	C	620	T
Mogeor	Ve	FJ985979	457	C	579	C	620	T

III. A 13-SNP Multiplex Assay for 5 Genes by Sequenom

SNP_ID	2nd-PCR	1st-PCR	UEP_MASS	UEP_SEQ
I2-SNP03	ACGTTGGATGCCGAAAGGAGTGGCATTAC	ACGTTGGATGTCAATATCTTCGTCCTCCC	8003.2	TACAGAGACTCACAGAGTTATGGATC
M23-snp1	ACGTTGGATGGCCCCGACGGAACTATTAAG	ACGTTGGATGTGGGAAAAAAGTGATCTCTC	6174	CGGAACTATTAAGTAGACGA
Tm2RS-snp1	ACGTTGGATGTAGTTGGGCCAATGCTTAC	ACGTTGGATGTATGGCTAGCGGTATACCTC	7494.9	TGGTGAATATTGGTAGAAATATAG
Tm2RS-snp2	ACGTTGGATGTTCATAGCCCGAATGGGTTG	ACGTTGGATGGGAATCCTGTAAGTAACAGC	7103.6	CCGAATGGGTTGTCTGAAGTAAC
Tm2R-snp5	ACGTTGGATGATCTTCTCAATCTCCATAGC	ACGTTGGATGCTCTTAGATGACTTCCTTCC	6936.5	gCAATCTCCATAGCAAACCTCATC
Ve1-snp1	ACGTTGGATGTCAGGAACTGTGCTAGAGAG	ACGTTGGATGAATTCAGGCCCTTTGGATG	5949.9	GAGAGAAAGTGAAGCTTAG
Ve1-snp2	ACGTTGGATGAAAAAGCTGCTGCAACGAGG	ACGTTGGATGTGAAGGACTCTCAGAGCTTG	7097.6	agATATATGCAGGAAGGCTCCCG
Ve1-snp3	ACGTTGGATGAGAGGTTGAATTGCTGCTAC	ACGTTGGATGAGGCTGAGCAACCTTTCAAG	5451.6	ACTTGCATCAACAGTCAA
Ve2-snp1	ACGTTGGATGGGAATCGATCCATTCAGGTG	ACGTTGGATGTGGCCAAGTCGACGAATTC	6732.4	ccagCCATTCAGGTGGTTGTTT
Ve2-snp2	ACGTTGGATGTGGGAAGCTACAAATGCTTG	ACGTTGGATGAGACTTGAAAGCTCTGAGGG	5780.8	GAATCACTAGACCTGTCAA
Ve2-snp4	ACGTTGGATGTGGTCTGGTTTCAACTCTG	ACGTTGGATGCCTTTGAAGGAAACAGAGGC	6863.5	ggTTGCAAATGACGTTAAGAGG
sw5-SNP1	ACGTTGGATGGGTATGGGATGACTTAAGGC	ACGTTGGATGACCCACTTCTTCAAGTCGAG	7308.8	ACTTAAGGCTTTCTTTTCCAGATG
sw5-SNP2	ACGTTGGATGTTTCTCCTCAACATGTCGGC	ACGTTGGATGGGTGCATCATTTCTCAAACG	5212.4	AACATGTCGGCAAGAAC

IV. SNP Genotyping by Sequenom in Tomato

(1) I2-SNP03 SNP marker for I-2 allele

Line	Name	Gene reported	I2-SNP03		M23-snp1	Mi	Tm2 RS-snp1	Tm2 R-snp2	Tm-2	Ve1-snp1	Ve1-snp2	Ve1-snp3	Ve	sw5-SNP1	sw5-SNP2	Sw-5
			I-2	I-2												
97	NY07-461	susceptible to all	CC		CC		CC	TT		AA	AG	CG		AA	GG	
98	NY07-464	susceptible to all	CC		CC		CC	TT		AA	AG	CG		AA	GG	
84	Riesentraube	susceptible to all	CC		CC		CC	TT		AA	AG	CG		AA	GG	
LA2088	Royal Red	<i>Asc, I, Tm-2, Ve</i>	CC		CC		TT	CC	Tm-2	TT	GG	CC	Ve	AA	GG	
LA1792		<i>Hero</i>	CC		CC		CC	TT		AA	AG	CG		AA	GG	
LA0655	Anahu	<i>Mi</i>	CC		GG	Mi	CC	TT		AA	AG	CG		AA	GG	
LA0656		<i>Mi</i>	CC		GG	Mi	CC	TT		AA	AG	CG		AA	GG	
LA1221	VFNT Cherry	<i>Mi, Tm-2a, Ve</i>	CC		GG	Mi	TT	CC	Tm-2	TT	GG	CC	Ve	AA	GG	
LA2396	Ontario 7710	<i>Pto</i>	CC		CC		CC	TT		TT	GG	CC	Ve?	AA	GG	
LA3129	Rehovot 13	<i>Pto^2</i>	CC		CC		CC	TT		AA	AG	CG		AA	GG	
LA1802		<i>Sm</i>	CC		CC		CC	TT		AA	AG	CG		AA	GG	
LA3433		<i>Tm-2</i>	CC		CC		TT	CC	Tm-2	AA	AG	CG		AA	GG	
LA3473		<i>Ty-1</i>	CC		CG	Mi?	CC	TT		TT	GG	CC	Ve?	AA	GG	
LA3386			CC		CC		CC	TT		AA	AG	CG		AA	GG	
LA3528	Peto 95-43	<i>Asc, I I-2, Sm, Ve</i>	CG	I-2	CC		CC	TT		TT	GG	CC	Ve	AA	GG	
LA3471	Mogeor	<i>Frl, I, I-2, Mi, Pyl, Tm-2a, Ve</i>	CG	I-2	GG	Mi	TT	CC	Tm-2	TT	GG	CC	Ve	AA	GG	
LA2823	Motelle	<i>I-2, I, Mi, Sm, Ve</i>	CG	I-2	GG	Mi	CC	TT		TT	GG	CC	Ve	AA	GG	
148	Sophya	<i>I-2, Mi, Sw-5, Tm-2, Ve</i>	CG	I-2	CG	Mi	TT	CC	Tm-2	TT	GG	CC	Ve	AT	AA	Sw-5
LA4285	CLN2264F	<i>I-2, Ph-3, Sm, Tm-2</i>	CG	I-2	CC		TT	CC	Tm-2	TT	GG	CC	Ve	AA	GG	
LA2821	Mobox	<i>I-2, I, Sm, Ve</i>	CG	I-2	CC		CC	TT		TT	GG	CC	Ve	AA	GG	
150	BHN-444	<i>I-2, Sw-5, Ve</i>	CG	I-2	CC		CC	TT		TT	GG	CC	Ve	AT	AA	Sw-5
147	Talledaga	<i>I-2, Sw-5, Ve</i>	CG	I-2	CC		CC	TT		AT	GG	CC	Ve	AT	AA	Sw-5
LA3130	UC-204C	<i>I-2, I, Ve</i>	CG	I-2	CC		CC	TT		TT	GG	CC	Ve	AA	GG	
LA3667		<i>Sw-5</i>	CG	I-2?	CC		CC	TT		TT	GG	CC	Ve?	AT	AA	Sw-5

IV. SNP Genotyping by Sequenom in Tomato

(2) M23_snp1 SNP marker for *Mi* allele

Line	Name	Gene reported	I2-SNP 03	I-2	M23 - snp1	Mi	Tm2R S-snp1	Tm2 R-snp2	Tm-2	Ve1 - snp 1	Ve1-snp2	Ve1-snp 3	Ve	sw5 - SN P1	sw5-SNP 2	Sw-5
148	Sophya	<i>I-2, Mi, Sw-5, Tm-2, Ve</i>	C G	I-2	C G	Mi	T T	C C	Tm-2	T T	G G	C C	Ve	A T	A A	Sw-5
LA0655	Anahu	<i>Mi</i>	C C		G G	Mi	C C	T T		A A	A G	C G		A A	G G	
LA0656		<i>Mi</i>	C C		G G	Mi	C C	T T		A A	A G	C G		A A	G G	
LA1221	VFNT Cherry	<i>Mi, Tm-2a, Ve</i>	C C		G G	Mi	T T	C C	Tm-2	T T	G G	C C	Ve	A A	G G	
LA3471	Mogeor	<i>Frl, I, I-2, Mi, Pyl, Tm-2a, Ve</i>	C G	I-2	G G	Mi	T T	C C	Tm-2	T T	G G	C C	Ve	A A	G G	
LA2823	Motelle	<i>I, I-2, Mi, Sm, Ve</i>	C G	I-2	G G	Mi	C C	T T		T T	G G	C C	Ve	A A	G G	
LA3473		<i>Ty-1</i>	C C		C G	Mi?	C C	T T		T T	G G	C C	Ve?	A A	G G	
LA2088	Royal Red	<i>Asc, I, Tm-2, Ve</i>	C C		C C		T T	C C	Tm-2	T T	G G	C C	Ve	A A	G G	
LA1792		<i>Hero</i>	C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA2396	Ontario 7710	<i>Pto</i>	C C		C C		C C	T T		T T	G G	C C	Ve?	A A	G G	
LA3129	Rehovot 13	<i>Pto^2</i>	C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA1802		<i>Sm</i>	C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA3433		<i>Tm-2</i>	C C		C C		T T	C C	Tm-2	A A	A G	C G		A A	G G	
LA3386			C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA3528	Peto 95-43	<i>Asc, I, I-2, Sm, Ve</i>	C G	I-2	C C		C C	T T		T T	G G	C C	Ve	A A	G G	
LA4285	CLN2264F	<i>I-2, Ph-3, Sm, Tm-2</i>	C G	I-2	C C		T T	C C	Tm-2	T T	G G	C C	Ve	A A	G G	
LA2821	Mobox	<i>I, I-2, Sm, Ve</i>	C G	I-2	C C		C C	T T		T T	G G	C C	Ve	A A	G G	
LA3130	UC-204C	<i>I, I-2, Ve</i>	C G	I-2	C C		C C	T T		T T	G G	C C	Ve	A A	G G	
LA3667		<i>Sw-5</i>	C G	I-2?	C C		C C	T T		T T	G G	C C	Ve?	A T	A A	Sw-5
150	BHN-444	<i>I-2, Sw-5, Ve</i>	C G	I-2	C C		C C	T T		T T	G G	C C	Ve	A T	A A	Sw-5
147	Talledaga	<i>I-2, Sw-5, Ve</i>	C G	I-2	C C		C C	T T		A T	G G	C C	Ve	A T	A A	Sw-5
97	NY07-461	susceptible to all	C C		C C		C C	T T		A A	A G	C G		A A	G G	
98	NY07-464	susceptible to all	C C		C C		C C	T T		A A	A G	C G		A A	G G	
84	Riesentraube	susceptible to all	C C		C C		C C	T T		A A	A G	C G		A A	G G	

IV. SNP Genotyping by Sequenom in Tomato

(3) SNP markers for *Tm-2* locus

Line	Name	Gene reported	I2-SNP 03	I-2	M23-snp1	Mi	Tm2R S-snp1	Tm2 R-snp2	Tm-2	Ve1 -snp 1	Ve1-snp2	Ve1-snp3	Ve	sw5 - SNP 1	sw5-SNP 2	Sw-5
LA2088	Royal Red	<i>Asc, I, Tm-2, Ve</i>	CC		CC		TT	CC	<i>Tm-2</i>	TT	GG	CC	Ve	AA	GG	
LA1221	VFNT Cherry	<i>Mi, Tm-2a, Ve</i>	CC		GG	Mi	TT	CC	<i>Tm-2</i>	TT	GG	CC	Ve	AA	GG	
LA3433		<i>Tm-2</i>	CC		CC		TT	CC	<i>Tm-2</i>	AA	AG	CG		AA	GG	
LA3471	Mogeor	<i>Frl, I, I-2, Mi, Pyl, Tm-2a, Ve</i>	CG	I-2	GG	Mi	TT	CC	<i>Tm-2</i>	TT	GG	CC	Ve	AA	GG	
148	Sophya	<i>I-2, Mi, Sw-5, Tm-2, Ve</i>	CG	I-2	CG	Mi	TT	CC	<i>Tm-2</i>	TT	GG	CC	Ve	AT	AA	Sw-5
LA4285	CLN2264F	<i>I-2, Ph-3, Sm, Tm-2</i>	CG	I-2	CC		TT	CC	<i>Tm-2</i>	TT	GG	CC	Ve	AA	GG	
97	NY07-461	susceptible to all	CC		CC		CC	TT		AA	AG	CG		AA	GG	
98	NY07-464	susceptible to all	CC		CC		CC	TT		AA	AG	CG		AA	GG	
84	Riesentraube	susceptible to all	CC		CC		CC	TT		AA	AG	CG		AA	GG	
LA1792		<i>Hero</i>	CC		CC		CC	TT		AA	AG	CG		AA	GG	
LA0655	Anahu	<i>Mi</i>	CC		GG	Mi	CC	TT		AA	AG	CG		AA	GG	
LA0656		<i>Mi</i>	CC		GG	Mi	CC	TT		AA	AG	CG		AA	GG	
LA2396	Ontario 7710	<i>Pto</i>	CC		CC		CC	TT		TT	GG	CC	Ve?	AA	GG	
LA3129	Rehovot 13	<i>Pto</i> ^{Δ2}	CC		CC		CC	TT		AA	AG	CG		AA	GG	
LA1802		<i>Sm</i>	CC		CC		CC	TT		AA	AG	CG		AA	GG	
LA3473		<i>Ty-1</i>	CC		CG	Mi?	CC	TT		TT	GG	CC	Ve?	AA	GG	
LA3386			CC		CC		CC	TT		AA	AG	CG		AA	GG	
LA3528	Peto 95-43	<i>Asc, I, I-2, Sm, Ve</i>	CG	I-2	CC		CC	TT		TT	GG	CC	Ve	AA	GG	
LA2823	Motelle	<i>I, I-2, Mi, Sm, Ve</i>	CG	I-2	GG	Mi	CC	TT		TT	GG	CC	Ve	AA	GG	
LA2821	Mobox	<i>I, I-2, Sm, Ve</i>	CG	I-2	CC		CC	TT		TT	GG	CC	Ve	AA	GG	
150	BHN-444	<i>I-2, Sw-5, Ve</i>	CG	I-2	CC		CC	TT		TT	GG	CC	Ve	AT	AA	Sw-5
147	Talledaga	<i>I-2, Sw-5, Ve</i>	CG	I-2	CC		CC	TT		AT	GG	CC	Ve	AT	AA	Sw-5
LA3130	UC-204C	<i>I, I-2, Ve</i>	CG	I-2	CC		CC	TT		TT	GG	CC	Ve	AA	GG	
LA3667		<i>Sw-5</i>	CG	I-2?	CC		CC	TT		TT	GG	CC	Ve?	AT	AA	Sw-5

IV. SNP Genotyping by Sequenom in Tomato

(4) SNP markers for Ve allele

Line	Name	Gene reported	I2-SNP 03	I-2	M23-snp1	Mi	Tm2 RS-snp1	Tm2 R-snp2	Tm-2	Ve1-snp 1	Ve1-snp2	Ve1-snp3	Ve	sw5-SNP 1	sw5-SNP 2	Sw-5
147	Talledaga	<i>I-2, Sw-5, Ve</i>	C G	I-2	C C		C C	T T		A T	G G	C C	Ve	A T	A A	Sw-5
LA2088	Royal Red	<i>Asc, I, I, Tm-2, Ve</i>	C C		C C		T T	C C	Tm-2	T T	G G	C C	Ve	A A	G G	
LA1221	VFNT Cherry	<i>Mi, Tm-2a, Ve</i>	C C		G G	Mi	T T	C C	Tm-2	T T	G G	C C	Ve	A A	G G	
LA3528	Peto 95-43	<i>Asc, I, I-2, Sm, Ve</i>	C G	I-2	C C		C C	T T		T T	G G	C C	Ve	A A	G G	
LA3471	Mogeor	<i>Frl, I, I-2, Mi, Pyl, Tm-2a, Ve</i>	C G	I-2	G G	Mi	T T	C C	Tm-2	T T	G G	C C	Ve	A A	G G	
LA2823	Motelle	<i>I, I-2, Mi, Sm, Ve</i>	C G	I-2	G G	Mi	C C	T T		T T	G G	C C	Ve	A A	G G	
148	Sophya	<i>I-2, Mi, Sw-5, Tm-2, Ve</i>	C G	I-2	C G	Mi	T T	C C	Tm-2	T T	G G	C C	Ve	A T	A A	Sw-5
LA2821	Mobox	<i>I, I-2, Sm, Ve</i>	C G	I-2	C C		C C	T T		T T	G G	C C	Ve	A A	G G	
150	BHN-444	<i>I-2, Sw-5, Ve</i>	C G	I-2	C C		C C	T T		T T	G G	C C	Ve	A T	A A	Sw-5
LA3130	UC-204C	<i>I, I-2, Ve</i>	C G	I-2	C C		C C	T T		T T	G G	C C	Ve	A A	G G	
LA2396	Ontario 7710	<i>Pto</i>	C C		C C		C C	T T		T T	G G	C C	Ve?	A A	G G	
LA4285	CLN2264F	<i>I-2, Ph-3, Sm, Tm-2</i>	C G	I-2	C C		T T	C C	Tm-2	T T	G G	C C	Ve?	A A	G G	
LA3473		<i>Ty-1</i>	C C		C G	Mi?	C C	T T		T T	G G	C C	Ve?	A A	G G	
LA3667		<i>Sw-5</i>	C G	I-2?	C C		C C	T T		T T	G G	C C	Ve?	A T	A A	Sw-5
97	NY07-461	susceptible to all	C C		C C		C C	T T		A A	A G	C G		A A	G G	
98	NY07-464	susceptible to all	C C		C C		C C	T T		A A	A G	C G		A A	G G	
84	Riesentraube	susceptible to all	C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA1792		<i>Hero</i>	C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA0655	Anahu	<i>Mi</i>	C C		G G	Mi	C C	T T		A A	A G	C G		A A	G G	
LA0656		<i>Mi</i>	C C		G G	Mi	C C	T T		A A	A G	C G		A A	G G	
LA3129	Rehovot 13	<i>Pto^2</i>	C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA1802		<i>Sm</i>	C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA3433		<i>Tm-2</i>	C C		C C		T T	C C	Tm-2	A A	A G	C G		A A	G G	
LA3386			C C		C C		C C	T T		A A	A G	C G		A A	G G	

IV. SNP Genotyping by Sequenom in Tomato

(5) SNP markers for Sw-5 allele

Line	Name	Gene reported	I2-SNP 03	I-2	M23 - snp 1	Mi	Tm2R S-snp1	Tm2 R-snp 2	Tm-2	Ve1 - snp 1	Ve1 - snp 2	Ve1 - snp 3	Ve	sw5 - SN P1	sw5-SNP 2	Sw-5
148	Sophya	<i>I-2, Mi, Sw-5, Tm-2, Ve</i>	C G	I-2	C G	Mi	T T	C C	Tm-2	T T	G G	C C	Ve	A T	A A	Sw-5
150	BHN-444	<i>I-2, Sw-5, Ve</i>	C G	I-2	C C		C C	T T		T T	G G	C C	Ve	A T	A A	Sw-5
147	Talledaga	<i>I-2, Sw-5, Ve</i>	C G	I-2	C C		C C	T T		A T	G G	C C	Ve	A T	A A	Sw-5
LA3667		<i>Sw-5</i>	C G	I-2?	C C		C C	T T		T T	G G	C C	Ve?	A T	A A	Sw-5
97	NY07-461	susceptible to all	C C		C C		C C	T T		A A	A G	C G		A A	G G	
98	NY07-464	susceptible to all	C C		C C		C C	T T		A A	A G	C G		A A	G G	
84	Riesentraube	susceptible to all	C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA2088	Royal Red	<i>Asc, I, Tm-2, Ve</i>	C C		C C		T T	C C	Tm-2	T T	G G	C C	Ve	A A	G G	
LA1792		<i>Hero</i>	C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA0655	Anahu	<i>Mi</i>	C C		G G	Mi	C C	T T		A A	A G	C G		A A	G G	
LA0656		<i>Mi</i>	C C		G G	Mi	C C	T T		A A	A G	C G		A A	G G	
LA1221	VFNT Cherry	<i>Mi, Tm-2a, Ve</i>	C C		G G	Mi	T T	C C	Tm-2	T T	G G	C C	Ve	A A	G G	
LA2396	Ontario 7710	<i>Pto</i>	C C		C C		C C	T T		T T	G G	C C	Ve?	A A	G G	
LA3129	Rehovot 13	<i>Pto²</i>	C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA1802		<i>Sm</i>	C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA3433		<i>Tm-2</i>	C C		C C		T T	C C	Tm-2	A A	A G	C G		A A	G G	
LA3473		<i>Ty-1</i>	C C		C G	Mi?	C C	T T		T T	G G	C C	Ve?	A A	G G	
LA3386			C C		C C		C C	T T		A A	A G	C G		A A	G G	
LA3528	Peto 95-43	<i>Asc, I-2, Sm, Ve</i>	C G	I-2	C C		C C	T T		T T	G G	C C	Ve	A A	G G	
LA3471	Mogeor	<i>Frl, I-2, Mi, Pyl, Tm-2a, Ve</i>	C G	I-2	G G	Mi	T T	C C	Tm-2	T T	G G	C C	Ve	A A	G G	
LA2823	Motelle	<i>I-2, Mi, Sm, Ve</i>	C G	I-2	G G	Mi	C C	T T		T T	G G	C C	Ve	A A	G G	
LA4285	CLN2264F	<i>I-2, Ph-3, Sm, Tm-2</i>	C G	I-2	C C		T T	C C	Tm-2	T T	G G	C C	Ve	A A	G G	
LA2821	Mobox	<i>I-2, Sm, Ve</i>	C G	I-2	C C		C C	T T		T T	G G	C C	Ve	A A	G G	
LA3130	UC-204C	<i>I-2, Ve</i>	C G	I-2	C C		C C	T T		T T	G G	C C	Ve	A A	G G	

Summary

One 13-SNP multiplex assay was developed
one for *I-2*,
one for *Mi*,
two for *Sw-5*,
three for *Tm-2* locus, and
six for *Ve* locus

Further research

More SNPs, more genes, and more diseases

Linked SNP	Single gene
Gene sequence-derived SNP	Multiple genes in one disease
Allele sequence-derived SNP	Multiple genes in multiple diseases

QTLs

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Thank you!

