International Research Journal of Plant Science Vol. 1(2) pp. 034-042, August 2010
Available online http://www.interesjournals.org/IRJPS

Full Length Research Paper

Development of single nucleotide polymorphism (SNP) markers for selection of Ve gene of tomato *Verticillium* wilt resistance

Ainong Shi[1,2], Richard Vierling[2], Richard Grazzini[3], Pengyin Chen[4], Homer Caton[1], and Yuejin Weng[2]

[1]Syngenta Seeds, 2369 330th Street, Slater, IA50244, USA;

[2]Indiana Crop Improvement Association and Department of Agronomy, Purdue University, West Lafayette, IN 47907, USA;

[3]GardenGenetics LLC, 131 Mendels Way, Bellefonte, PA 16823, USA;

[4]Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72701, USA.

Accepted 15 July 2010

Tomato Verticillium wilt, caused by *Verticillium dahliae* and *V. alboatrum* is a soil-born fungal disease. Use of host resistance is an effective and environment-friendly method to control this disease. Marker assisted selection (MAS) has become very important and useful in selection of Verticillium wilt resistance genes in tomato (*Solanuum lycopersicum* L. syn *Lycopersicon esculentum* Mill.). The objective of this research was to identify *Ve* gene-derived single nucleotide polymorphism (SNP)markers for MAS in tomato breeding. Six gene-derived SNP markers specific for *Ve* locus were identified and validated to be useful in selecting *Ve* resistance gene in different tomato germplasm.

Key words: Single nucleotide polymorphism, Tomato Verticillium wilt; Disease resistance

INTRODUCTION

Tomato Verticillium wilt, caused by *Verticillium dahliae* and *V. alboatrum* is a soil-born fungal disease. Use of host resistance is an effective and environment-friendly method to control this disease. The gene *Ve* was reported to confer this disease and mapped to chromosome 9 of tomato (*Solanuum lycopersicum* L. syn *Lycopersicon esculentum* Mill.) (Schaible et al., 1951; Diwan et al., 1999; Kawchuk et al., 2001). Kawchuk et al. (1994) identified a co-dominant random amplified polymorphic DNA (RAPD) marker associated to the *Ve* gene. Late, they developed co-dominant and allele-specific sequence characterized amplified region (SCAR) markers linked to *Ve* using the sequences from the RAPD marker (Kawchuk et al., 1998). Further, the two genes, *Ve1* and *Ve2* were cloned and their sequences were stored in GenBank, AF272366 and AF272367 for *Ve1*, AF365929 and AF365930 for *Ve2* (Kawchuk et al., 2001). Based on the sequences of the two genes, Acciarri et al. (2007) developed allele specific PCR-based markers for *Ve1* and *Ve2* and reported a cleaved amplified polymorphic sequences (CAPS) marker for *Ve2*. Recently, Kuklev et al. (2009) also reported a CAPS markers developed from the cloned homologies of Ve1 and Ve2 and showed polymorphism at the locus between resistant and susceptible plants. Fradin et al. (2009) showed that *Ve1*, but not *Ve2*, provides resistance in tomato against race 1 strains of *V. dahliae* and *V. albo-atrum* and not against race 2 and recognized that *Ve1* and *Ve2* were the two sections of *Ve* gene.

Marker assisted selection (MAS) has been widely and successfully used in selection for disease resistance by

*Corresponding author Email: ainong_shi@hotmail.com, ainong.shi@syngenta.com

applying genetic markers to identify and select specific genes or combine multiple resistance genes in tomato breeding (Foolad and Sharma, 2005; Foolad, 2007). Single nucleotide polymorphism (SNP) has been becoming to be the most useful as molecular marker in genome mapping, association studies, diversity analysis, and tagging of economic important genes in plant genomics because of their abundance and automated high-throughput genotyping, and the most cost effective genetic markers currently available (Rafalski, 2002; Giancola et al., 2006; Caicedo et al., 2007; Choi et al., 2007; Jones et al. 2009). SNPs have been discovered and verified in tomato (Labate and Baldo, 2005; Yang et al., 2004) and successfully used in selection resistance to bacterial speck and bacterial spot in tomato (Yang et al., 2005). It will be good to discover and validate all SNP markers between the resistance and susceptible alleles of *Ve1* and *Ve2* genes. However, it is much cheaper and easier to do partial sequences of *Ve1* and *Ve2* genes through PCR approach, which discovers and validates allele-specific SNP markers to identify and distinguish resistance and susceptible *Ve* gene in various tomato lines. The objective of this research was to identify *Ve* gene-derived SNP markers for MAS in tomato breeding.

MATERIALS AND METHODS

Plant materials

Forty-three tomato genotypes including released or commercial cultivars and accessions were used in this research (Table 1). Seeds of 23 tomato accessions (LA series) were obtained from the C.M. Rick Tomato Genetics Resource Center, Dept. of Plant Sciences, University of California, Davis, CA 95616 (http://tgrc.ucdavis.edu). The two lines NY07-461 and NY07-464 were obtained from Cornell University; refined selections from a cross between 'Brandywine' and 'Rose de Berne' (received as NY07-461 (Brandyrose #1) and NY07-464 (Brandyrose #2)) and they are very susceptible to Verticillium Wilt disease. The seeds of other 18 tomato genotypes were purchased from commercial sources. Thirteen tomato genotypes were reported to carry *Ve* gene, three susceptible, and other 27 unknown for Verticillium Wilt resistance.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was extracted from fresh leaves of greenhouse-grown plants using the CTAB (hexadecyltrimethyl ammonium bromide) method (Acciarri et al. 2007). PCR amplification was performed in an eppendorf thermal cycler following standard PCR procedures with minor modifications. Briefly, each 50 µl PCR reaction mixture consisted of 29.8 µl sterilized ddH_2O, 10 µl 5x Mango *Taq* reaction buffer (Bioline, London, UK), 3 µl $MgCl_2$ (25 *mM*), 1.5 µl dNTP (2.5 mM each), 1.5 µl each primer (5 µM), 0.2 µl Mango *Taq* DNA polymerase (5 U/µl) (Bioline, London, UK), and 2.5 µl template DNA (30 ng/µl). PCR procedure consisted of an initial denaturation step at 95 °C for 1 min and 20 second, 38 cycles of 30 second at 94 °C, 35 second at 54 °C, and 1 min and 20 second at 72 °C followed by an extension step at 72 °C for 5 min and a 4 °C soak. The PCR fragments were separated by gel electrophoresis with 1.5 % agarose gel in 0.5 X TAE buffer, stained with ethidium bromide, and visualized with UV light.

Three *Ve* gene-specific primer pairs, Ve1-f/r, Ve2-f1/r1 and Ve2-f2/r2 were designed from the sequences of the GenBank accessions: AF272366 and AF272367 for *Ve1*, and AF365929 and AF365930 for *Ve2* using the primer design tool – Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome) (Table S1). The three primer pairs were used to amplify DNA fragments by PCR as described above in eight tomato lines including Anahu, Bush Celebrity, NY07-461, NY07-464, Riesentraube, Mogeor, Peto 95-43, and Rehovot 13. The PCR fragments were sequenced in Purdue Genomics Core Facility, Purdue University, West Lafayette, IN 47907 (http://www.genomics.purdue.edu/). Sequences amplified from the primer pairs were submitted to GenBank using a DNA sequence submission and update tool – Sequin (http://www.ncbi.nlm.nih.gov/Sequin/).

SNP identification and genotyping

The sequences amplified from the primer pairs were aligned using the software BioEdit (http://www.mbio.ncsu.edu/bioedit/bioedit.html). SNP genotyping was done using Sequnom at the Division of Human Genetics in the Department of Genetics, Washington University, St. Louis, MO 63110 (http://hg.wustl.edu/info/Sequenom_description.html). Six SNPs were used for SNP genotyping in 43 tomato genotypes using Sequnom. The primers and the multiplex assay were list in Table S2.

RESULTS AND DISCUSSION

Three DNA fragments with the size of 996bp, 977bp, and 937bp were produced by the three primer pairs Ve1-f/r, Ve2-f1/r1 and Ve2-f2/r2 in all 43 tomato genotypes, respectively. A total of 18 sequences amplified from the three primer pair in tomato were stored in GenBank with the accessions, FJ686045, FJ686046, FJ809919-FJ809928, FJ985979-FJ985984.

From the multiple sequence alignment among the PCR fragments amplified from the primer pair Ve1-f/r with the corresponding DNA segments of the AF272366, AF272367, FJ464553 to FJ464557, three SNPs were observed (Figure. S1 Table S3). The two SNPs: Ve1-snp1 [A/T] and Ve1-snp3 [G/C] separated susceptible cultivars/lines from resistant ones but not the Ve1-snp2. All five susceptible cultivars/lines, NY07-461, NY07-464, Riesentraube, Moneymaker, and Craigella GCR26 had the base A at Ve1-snp1 and base G at Ve1-snp3, but seven resistant cultivars/lines, Mogeor, Peto 95-43, Bush Celebrity, Craigella GCR218, VFN-8, Motelle, and Craigella (AF272367) had the base T and C at the two SNPs, indicating the two SNP Ve1-snp1 and Ve1-snp3 were allele-specific SNPs for resistance *Ve1* allele and base A in Ve1-snp1 and the base G in Ve1-snp3 were specific for susceptible allele *ve1*. On contrast, the base T in Ve1-snp1 and the base C in Ve1-snp3 were

Table1. Polymorphism of the six SNP markers detected in 43 tomato cultivars/accessions for Verticullium wilt disease resistance genes *Ve* locus.

Cultivar/accession	Reported *Ve* gene[a]	Ve1-snp1	Ve1-snp2	Ve1-snp3	Ve2-snp1	Ve2-snp2	Ve2-snp4	Proposed *Ve1* and *Ve2* alleles	Proposed *Ve* Allele
Dad's Sunset	*?*	A A[a]	A G	C G	C C	G G	C C	*ve1ve2*	*ve*
Brandywine Red Landis	*?*	A A	A G	C G	C C	G G	C C	*ve1ve2*	*ve*
Brandywine Sudduth	*?*	A A	A G	C G	C C	G G	C C	*ve1ve2*	*ve*
Brandywine Yellow	*?*	A A	A G	C G	C C	G G	C C	*ve1ve2*	*ve*
Great White	*?*	A A	A G	C G	C C	G G	C C	*ve1ve2*	*ve*
LA0159	*?*	A A	A G	C G	C C	G G	C C	*ve1ve2*	*ve*
LA0316	*?*	A A	A G	C G	C C	G G	C C	*ve1ve2*	*ve*
Anahu (LA0655)	*?*	A A	A G	C G	C C	G G	C C	*ve1ve2*	*ve*
LA0656	*?*	A A	A G	C G	C C	G G	C C	*ve1ve2*	*ve*
LA1802	*?*	A A	A G	C G	C C	G G	C C	*ve1ve2*	*ve*
NY07-461	*ve*	A A	A G	C G	C C	G G	C C	*ve1ve2*	*ve*
NY07-464	*ve*	A A	A G	C G	C C	G G	C C	*ve1ve2*	*ve*
Riesentraube	*ve*	A A	A G	C G	C G	G G	C C	*ve1ve2*	*ve*
Tomatoberry	*?*	A A	A G	C G	C G	G G	C C	*ve1ve2*	*ve*
Aunt Rubys	*?*	A A	A G	C G	C G	G G	C C	*ve1ve2*	*ve*
LA1792	*?*	A A	A G	C G	C G	G G	C C	*ve1ve2*	*ve*
LA2503	*?*	A A	A G	C G	C G	G G	C C	*ve1ve2*	*ve*
Rehovot 13 (LA3129)	*?*	A A	A G	C G	C G	G G	C C	*ve1ve2*	*ve*
LA3386	*?*	A A	A G	C G	C G	G G	C C	*ve1ve2*	*ve*
LA3433	*?*	A A	A G	C G	C G	G G	C C	*ve1ve2*	*ve*
Sugary	*?*	A T[b]	G G	C C	C C	C G	C T	*(Ve1ve1)(Ve2?)*	*Ve*
Talledaga	*Ve*	A T	G G	C C	C C	C G	C T	*(Ve1ve1)(Ve2?)*	*Ve*
LA2934	*?*	T T[b]	G G	C C	C G	C G	C C	*Ve1(Ve2?)*	*Ve*
LA1269	*?*	T T	G G	C C	C G	G G	T T	*Ve1(Ve2?)*	*Ve*
Swt Chelsea	*Ve*	T T	G G	C C	C G	C G	C T	*Ve1(Ve2?)*	*Ve*
VFNT Cherr (LA1221)	*Ve*	T T	G G	C C	C G	C G	T T	*Ve1(Ve2?)*	*Ve*
BHN-444	*Ve*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
Bush Celebrity	*Ve*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
Golden Girl	*Ve*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
Royal Red (LA2088)	*Ve*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
Mobox (LA2821)	*Ve*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
Motelle (LA2823)	*Ve*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
UC-204C (LA3130)	*Ve*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
Mogeor (LA3471)	*Ve*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
Peto 95-43 (LA3528)	*Ve*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
Sophya	*Ve*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
Carbon	*?*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
Cherokee Purple	*?*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
Ontario 7710 (LA2396)	*?*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
LA3473	*?*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
LA3667	*?*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
CLN2264F (LA4285)	*?*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*
Swt Cluster	*?*	T T	G G	C C	C G	C C	T T	*Ve1Ve2*	*Ve*

[a] '*Ve*' is the resistance allele and '*ve*' is the susceptible allele of Verticillium wilt disease resistance reported in previous research.
[b] The SNP type such as A A and T T signify a homogenous SNP type, and A T signifies a heterogonous SNP type.

specific for the resistance allele *Ve1*. The Ve1-snp1 [A/T] was also reported by Acciarri et al. (2007) and the base A existed in the susceptible line L98A and the base T in the resistant cultivars Mogeor and ISPORT L13/3.3 in their

Table S1. Six primers and their characteristics

Primer name [a]	Primer Sequence (5' --> 3')	Tm (℃)	Location in AF272367 [b]	Location in AF272366	Location in AF365930	Location in AF365929
Ve1-f	TTTGAGCTTGCGTGATTGTC	63.90	2150-2169	657-676	-	-
Ve1-r	TTGAGATCGGGGAACTTTTG	63.80	3145-3126	1652-1633	1646-1627	1702-1683
Ve2-f1	CGAATTTCAGGCCCTATTGA	60.03	-	-	670- 689	726-745
Ve2-r1	TTGAGATCGGGGAACTTTTG	60.04	3145-3126	1652-1633	1646-1627	1702-1683
Ve2-f2	TCCTAGTCTTGCGCTCCAAT	59.98	3814-3833	2321-2340	2315-2334	2371-2390
Ve2-r2	TCTTTTCCACCCTCATCGTC	60.05	-	-	3251-3232	3307-3288

[a] The forward primer Ve1-f and the reversal primer Ve1-r are designed from the GenBank accession AY272236 and AY272367 of the *Ve1* gene, and the two forward primers Ve2-f1 and Ve2-f2 and the two reversal primers Ve2-r1 and Ve2-r2 from AF365930 and AF365929 of the *Ve2* gene for tomato Verticillium wilt disease resistance using the primer design tool Primer-BLAST. The Ve1-r is the same primer of the Ve2-r1.
[b] The primer location presenting the corresponding site in the GenBank accessions. For example, the primer Ve1-f is located at 2150-2169 sites of the GenBank accession AF272367 and at 657-676 sites in AF272366.

Table S2 Primer properties in the SNP assay for Sequenom SNP genotyping

SNP_ID [a]	SNP_ type	2nd-PCRP	1st-PCRP	UEP_ MASS	UEP_SEQ
Ve1-snp1	A/T	ACGTTGGATGTCAGGAACTGTGCTAGAGAG [b]	ACGTTGGATGAATTTCAGGCCCTTTGGATG	5949.9	GAGAGAAA
Ve1-snp2	A/G	ACGTTGGATGAAAAAGCTGCTGCAACGAGG	ACGTTGGATGTGAAGGACTCTCAGAGCTTG	7097.6	agATATATG
Ve1-snp3	C/G	ACGTTGGATGAGAGGTTGAATTGCTGCTAC	ACGTTGGATGAGGCTGAGCAACCTTTCAAG	5451.6	ACTTGCATC
Ve2-snp1	C/G	ACGTTGGATGGGAATCGATCCATTCAGGTG	ACGTTGGATGTGGCCAAGTCGACGAATTTC	6732.4	ccagCCATTC
Ve2-snp2	C/G	ACGTTGGATGTGGGAAGCTACAAATGCTTG	ACGTTGGATGAGACTTGAAAGCTCTGAGGG	5780.8	GAATCACTA
Ve2-snp4	C/T	ACGTTGGATGTGGTGCTGGTTTCAACTCTG	ACGTTGGATGCCTTTGAAGGAAACAGAGGC	6863.5	ggTTGCAAA

[a] SNP_ID such as Ve1-snp1 is a SNP specific for *Ve1* locus and the name is the same as the one list in Table 4, 5 and 6.
[b] SNP primers were designed using Assay Design 3.1 software from Sequenom.

research, which was the same as ours. The exception was AF272366 that was reported to have the *Ve1* gene (Kawchuk et al., 2001) but it had the A base in Ve1-snp1 and base G in Ve1-snp3 showed the bases as the susceptible allele did and not same as the AF272367. Both of AF272366 and AF272367 were cloned from Craigella and contained *Ve1* gene (Kawchuk et al., 2001) but five SNPs between them were observed. Acciarri et al. (2007) also reported seven SNPs and one Del marker between the sequences amplified from the two resistance cultivars, Mogeor and ISPORT L13/3.3 and one susceptible line L98A. Among the eight markers, SNPs [G/C] and [A/T] located at 1739 base and 2199 base of the AF272367 sequence also showed difference between AF272366 and AF272367. The base G in the SNP [G/C] and T in the SNP [A/T] existed in the corresponding sites of AF272367 were also observed at the resistant lines Mogeor and ISPORT L13/3.3 but not in the susceptible line L98A. In other way, the base C and base A in the AF272366 were found in the susceptible line L98A but not in the resistant ones from their research. From the six sequences of PCR fragments in our data, the base T in Ve1-snp1 and base C in Ve1-snp3 from the resistant cultivars was also observed at the corresponding sites of AF272367 and not in AF272366, but the base A and G in the two SNPs were found in the AF272366. From the *Ve1* gene cloned sequences, Fradin et al. (2009) reported the base T in Ve1-snp1 and base C in Ve1-snp3 existed in the resistant cultivars VFN-8, Motella, and Craigella GCR218 and the base A and G in the two SNPs in the

Table S3. Three SNPs detected from the six sequences amplified from the primer pair Ve1-f/r plus eight corresponding sequences from the *Ve1* gene clones

Tomato variety [a]	Gene at Ve1 locus	GenBank acession	Ve1-snp1 [b]		Ve1-snp2		Ve1-snp3	
Craigella	Ve1	AF272367	2199	T	2752	G	3041	C
Craigella	Ve1 ?	AF272366	706	A	1259	G	1548	G
VFN-8	Ve1	FJ464557	706	T	1259	G	1548	C
Motelle	Ve1	FJ464556	706	T	1259	G	1548	C
Craigella GCR218	Ve1	FJ464553	706	T	1259	G	1548	C
Craigella GCR26	ve1	FJ464554	706	A	1258	G	1547	G
Moneymaker	ve1	FJ464555	706	A	1258	G	1547	G
Riesentraube	ve1	FJ686045	50	A	603	A	892	G
NY07-461	ve1	FJ809926	50	A	603	A	892	G
NY07-464	ve1	FJ809925	50	A	603	A	892	G
Peto 95-43	Ve1	FJ809928	50	T	603	G	892	C
Mogeor	Ve1	FJ809927	50	T	603	G	892	C
Bush Celebrity	Ve1	FJ686046	50	T	603	G	892	C

[a] Craigella is the cultivar used for clone of the *Ve1* gene of AF272367 and AF272366 (Kawchuk et al.. 2001, GenBank accessions AF272366 and AF272367). VFN-8, Motelle, and Craigella GCR218 are the resistance cultivars used for clones of the Ve1 gene (Fradin et al. 2009, GenBank accessions FJ464557, FJ464556, and FJ464553). Craigella GCR26 and Moneymaker are susceptible cultivars used for clones of the ve1 allele (Fradin et al. 2009, GenBank accessions FJ464554 and FJ464555). NY07-461, NY07-464, and Riesentraube are Verticillium wilt disease susceptible cultivars/lines and Mogeor, Peto 95-43, and Bush Celebrity contain the *Ve1* gene for Verticillium wilt disease resistance.

[b] The SNP location and type such as Ve1-snp1 is located at 2199 base of the sequence of AF272367 with T base in the location for the SNP

Table S4. One SNP detected among six DNA sequences amplified from the primer pair Ve2-f1/r1 plus two GenBank Accessions of *Ve2* locus

Variety [a]	Gene	GenBank accession	Ve2-snp1	
			Location	Base
VFN8	Ve2	AF365929	1441	G
VFN8	Ve2	AF365930	1385	G
Peto 95-43	Ve	FJ809919	716	G
Bush Celebrity	Ve	FJ809920	716	G
Mogeor	Ve	FJ809921	716	G
Anahu	ve	FJ809922	716	C
NY07-461	ve	FJ809923	716	C
NY07-464	ve	FJ809924	716	C

[a] VFN8 is the cultivar that was used for clone the Ve gene (Kawchuk et al.. 2001). AF365929 is the accession in GenBank which is the *Lycopersicon esculentum* verticillium wilt disease resistance protein *Ve2* (*Ve2*) gene, complete cds. AF365930 is the *Lycopersicon esculentum* verticillium wilt disease resistance protein *Ve2* (*Ve2*) mRNA, complete cds. NY07-461, NY07-464, and Anahu are Verticillium wilt disease susceptible cultivars/lines and Mogeor, Peto 95-43, and Bush Celebrity are Verticillium wilt disease resistant cultivars.

susceptible cultivars Craigella GCR26 and Moneymaker, and showed the same results for the two SNPs as in this research.

From the multiple sequence alignment among the PCR fragments amplified from the primer pair Ve2-f1/r1 with the corresponding DNA segments of AF365929 and AF365930. One SNP Ve2-snp1 [C/G] was observed and the base G in Ve2-snp1 was observed in the three resistant cultivars/lines and the base C in the three susceptible ones (Table 4S). The SNP Ve2-snp1 [C/G] was also reported by Acciarri et al. (2007) at the corresponding location of *Ve2* sequences. The base G in the SNP was found in the resistant lines Mogeor and ISPORT L13/3.3 and base C in the susceptible line L98A in their research.

From the multiple sequence alignment among the PCR fragments amplified from the primer pair Ve2-f2/r2 with the corresponding DNA segments of AF365929,

Table S5. Three SNPs detected among six DNA sequences amplified from the primer pair Ve2-f2/r2 plus seven GenBank Accessions of *Ve2* locus

Tomato variety [a]	Gene at Ve locus	GenBank acession	Ve2-snp2 [b]		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

[a] VFN-8, Motelle, and Craigella GCR218 are the resistance cultivars used for clones of the Ve2 gene (Kawchuk et al.. 2001; Fradin et al. 2009; GenBank accessions AF365929, AF365930, FJ464559, FJ464561, and FJ464562). Craigella GCR26 and Moneymaker are susceptible cultivars used for clones of the ve2 allele (Fradin et al. 2009, GenBank accessions FJ464558 and FJ464560). NY07-461, NY07-464, and Rehovot 13 are Verticillium wilt disease susceptible cultivars/lines and Mogeor, Peto 95-43, and Bush Celebrity are Verticillium wilt disease resistant cultivars.

[b] The SNP location and type such as Ve2-snp2 is located at 2827 base of the sequence of AF365929 with G base in the location for the SNP.

AF365930, and FJ464558 to FJ464562, three SNPs were observed (Fig. 2S and Table 5S). The three susceptible cultivars/lines, NY07-461, NY07-464, and Rehovot had the base G at Ve2-snp2, base T at Ve2-snp3, and base C at Ve2-snp4. The three resistant cultivars/lines, Mogeor, Peto 95-43, Bush Celebrity had the base C, C, and T at the three SNPs, respectively. The three SNPs were also identified by Acciarri et al. (2007) and the same results were reported that the base G at Ve2-snp2, base T at Ve2-snp3, and base C at Ve2-snp4 were observed at the corresponding location of sequences from the susceptible cultivars and the base C, C, and T at the three SNPs in the resistant line. However, the base G, T, and C at the three SNPs for the susceptible cultivars/lines in our research and from the data reported by Acciarri et al. (2007) were also found in the corresponding locations of AF365929 and AF365930 sequences which were proposed to be *Ve2* resistance gene sequences (Kawchuk et al.. 2001). The reason needs to be further verified. Acciarri et al. (2007) have not published their sequences in GenBank yet and did not compare their sequences to AF365929 and AF365930 in their article. The *Ve1* and *Ve2* genes were cloned and isolated from the tomato germplasm VFN8 and verified their function as encoding a class of cell-surface glycoproteins with receptormediated endocytosis-like signals and leucine zipper or PEST sequences in potato (*Solanum tuberosum* ssp. *tuberosum*) cultivar De´sire´e, which is highly susceptible to verticillium wilt (Kawchuk et al. 2001). The tomato germplasm VFN8 was reported to be cultivar Craigella was reported in the GenBank accessions AF272366 and AF272367 for the tomato used for gene clone in the article. However, the tomato verticillium wilt disease resistance *Ve1*, and no cultivar information was reported in the GenBank accessions AF365929 and AF365930 for the tomato verticillium wilt disease resistance *Ve2*. However, SNPs do be observed in the corresponding locations of the sequences of *Ve1* and *Ve2* genes. From more recent *Ve2* cloned sequences, Fradin et al. (2009) reported tomato VFN-8 had the same results as ours for resistance allele. The base C, C, and T at the three SNPs were also reported in the resistant lines, Motelle (GenBank acc. FJ464561) and Graigella GCR218 (FJ464559) and the base G, T, and C in the three SNPs were reported in the susceptible lines, Craigella GCR26 (FJ464558) and Moneymaker (FJ464560) (Table S5)

The seven SNPs except Ve2-snp3 were used to identify and validate *Ve* gene by SNP genotyping using Sequenom among 43 tomato cultivars/lines. Ve1-snp1 showed three types [A A], [A T], and [T T], Ve1-snp2 two types [A G] and [G G], and Ve1-snp3 two types [C G] and [C C], Ve2-snp1 two types [C C] and [C G], Ve2-snp2 three types [G G], [C G], and [C C], and Ve2-snp4 three types [C C], [C T] and [T T] (Table 1).. The haplotypes of Ve1-snp1, Ve1-snp2 and Ve1-snp3 consisted of two combinations [A A][A G][C G] and [T T][G G][C C] among the 43 soybean lines except Sugary and Talledaga with combination [A T][G G][C C] (Table 1). 20 cultivars/lines showed [A A][A G][C G] for the three *Ve1* SNPs, indicating they contained the susceptible gene *ve1.* The very susceptible lines NY07-461 and NY07-464 belonged to this group and no one out of the 20 lines has been reported to carry the Verticillium wilt *Ve* resistance genes. 21 cultivars/lines showed [T T][G G][C C] of the three *Ve1* SNPs, indicating they contained the resistance gene *Ve1*.

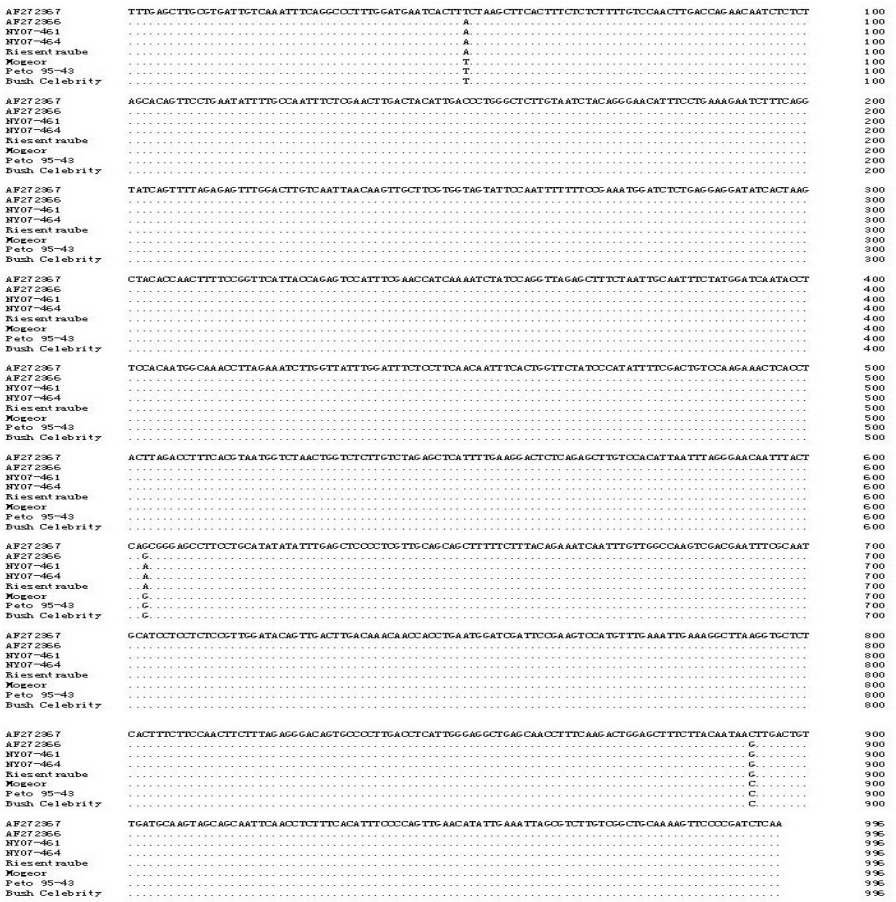

Figure S1. Multiple sequence alignment among PCR fragments amplified from the primer pair Ve1-f/r with the corresponding DNA segments of the AF272366 and AF272367. AF272366 is the accession in GenBank that contains the sequence of the *Lycopersicon esculentum* verticillium wilt disease resistance protein (*Ve1*) mRNA, complete cds, and AF272367 is the accession of *Lycopersicon esculentum* Verticillium wilt disease resistance protein (*Ve1*) gene, complete cds (Kawchuk et al., 2001, GenBank Accession AF272366 and AF272367). NY07-461, NY07-464, and Riesentraube are Verticillium wilt disease susceptible cultivars/lines and Mogeor, Peto 95-43, and Bush Celebrity contain the *Ve1* gene for Verticillium wilt disease resistance.

The haplotypes of Ve2-snp1, Ve2-snp2 and Ve2-snp4 mainly consisted of two combinations {[CC]/[CG]}[G G][C C] and [GG][C C][T T] among the 43 soybean lines. 20 lines showed {[CC]/[CG]}[G G][C C] for the three *Ve2* SNPs, indicating they contained the susceptible gene *ve2*, and 17 lines showed [CG][C C][T T] containing the resistance gene *Ve2*, but six lines showed exception (Table 1). Among the 43 soybean lines, Mogeor was identified to have two sequences of the *Ve1* and *Ve2* genes (Acciarri et al. 2007). Twelve were reported to carry the Verticillium wilt *Ve* resistance gene (Table 1, http://tgrc.ucdavis.edu). Recently, Fradin et al. (2009) identified that *Ve1*, but not *Ve2*, provides resistance in tomato against race 1 strains of *V. dahliae* and *V. albo-atrum* and not against race 2 strain. Therefore, all Ve2-derived SNPs were regarded as linked markers for Verticillium wilt *Ve* resistance. After all, the two *Ve1*-derived SNP markers, Ve1-snp1 and Ve1-snp3 were regards as allele-specific SNP markers for the *Ve* gene, which can distinguish the resistance allele *Ve* and the susceptiobe allele *ve* in tomato lines.

In this research, an effective procedure was used to develop gene-derived SNP markers for *Ve1* and *Ve2* of tomato Verticillium wilt disease. One *Ve1* gene-specific primer pair Ve1-f1/r1 was designed from AF272366 and AF272367 and two *Ve2* gene-specific primer pairs Ve2-f1/r1 and Ve2-f2/r2 from AF365929 and AF365930 using the primer design tool Primer-BLAST. After run PCR reactions in different tomato cultivars/lines with and without the *Ve* resistance gene, six PCR products amplified from each primer pair were sequenced. SNPs were discovered from these sequences by multiple sequence alignment. SNP markers were verified and used to identify *Ve* alleles among 43 tomato

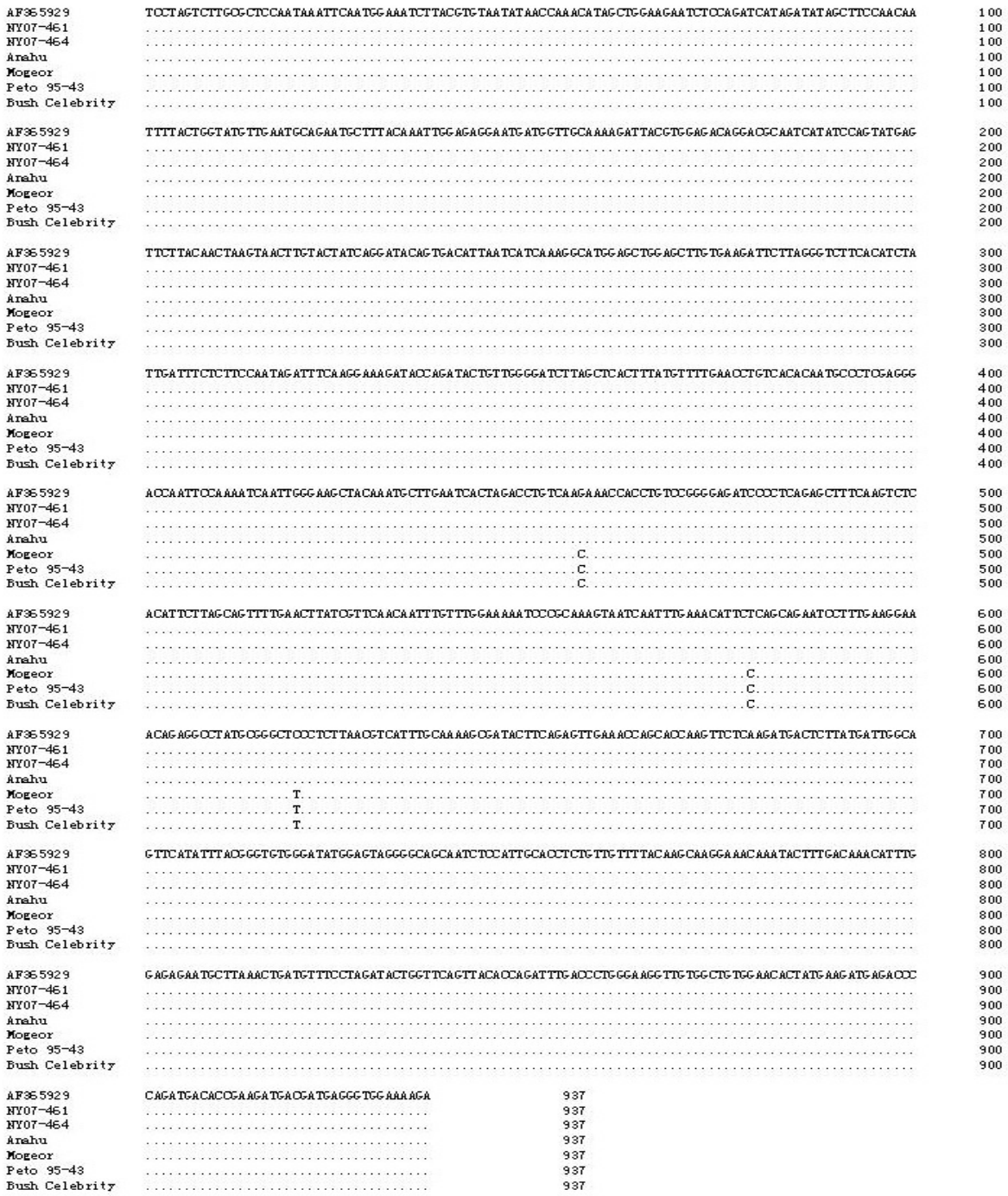

Figure S2. Multiple sequence alignment among PCR fragments amplified from the primer pair Ve2-f2/r2 with the corresponding DNA segments of the AF365329. AF365929 is the accession in GenBank that contains the sequence of the *Lycopersicon esculentum* verticillium wilt disease resistance protein *Ve2* (*Ve2*) gene, complete cds (Kawchuk et al., 2001, GenBank Accession AF365929). NY07-461, NY07-464, and Anahu are Verticillium wilt disease susceptible cultivars/lines and Mogeor, Peto 95-43, and Bush Celebrity are Verticillium wilt disease resistant cultivars.

cultivars/lines by SNP genotyping using Sequnom technology. Finally, three *Ve1* and three *Ve2* gene-derived SNP markers were identified and the *Ve* alleles were postulated for these cultivars/lines from this

research (Table 1). Therefore, this research will provide breeders a tool in MAS for *Ve* genes of tomato Verticillium wilt resistance and a method to identify gene-derived SNP markers in tomato breeding.

REFERENCES

Acciarri N, Rotino GL, Tamietti G, Valentino D, Voltattorni S, Sabatini E (2007). Molecular markers for *Ve1* and *Ve2 Verticillium* resistance genes from Italian tomato germplasm. Plant Breed. 126: 617-621.

Caicedo AL, Williamson SH, Hernandez RD (2007). Genome-wide patterns of nucleotide polymorphism in domesticated rice. PLoS Genet. 3: 1745-56.

Choi IY, Hyten DL,Matukumalli LK (2007). A soybean transcript map: gene distribution, haplotype and single nucleotide polymorphism analysis. Genetics 176: 685-696.

Diwan N, Fluhr R, Eshed Y, Zamir D,Tanksley SD (1999). Mapping of *Ve* in tomato: a gene conferring resistance to the broadspectrum pathogen, *Verticillium dahliae* race 1. Theor. Appl. Genet..98: 315-319.

Fradin EF, Zhang Z, Ayala JCJ, Castroverde CDM, Nazar RN, Robb J, Liu C, Thomma BPHJ (2009). Genetic dissection of Verticillium wilt resistance mediated by tomato *Ve1*. Plant Physiology 150: 320-332.

Foolad MR, Sharma A (2005). Molecular markers as selection tools in tomato breeding. Acta Hort 695: 225-240.

Foolad MR (2007). Genome mapping and molecular breeding of tomato. Internat. J. Plant Genomics. Article ID 64358, 52 pages (doi:10.1155/2007/64358)

Giancola S, McKhann HI, Berard A, Camilleri C. Durand S, Libeau P, Roux F, Rebound X, Gut Brunel D (2006). Utilization of three high-throughput SNP genotyping methods, the GOOD assay, Amplifluor and Taqman, in diploid and polyploidy plants. Theor. Appl. Genet..112: 1115–1124.

Jones E, Chu W, Ayele M, Ho J, Bruggeman E, Yourstone K, Rafalski A, Smith OS, McMullen MD, Bezawada C, Warren J, Babayew J, Basu S, Smith S (2009). Development of Single Nucleotide Polymorphism (SNP) Markers for Use in Commercial Maize (*Zea Mays* L.) Germplasm. Mol Breed. 24:165-176.

Kawchuk LM, Lynch DR, Hachey J, Bains PS, Kulcsar F (1994). Identification of a codominant amplified polymorphic DNA marker linked to the Verticillium wilt resistance gene in tomato. Theor Appl Genet..89: 661-664.

Kawchuk LM, Hachey J, Lynch DR (1998). Development of sequence characterized DNA markers linked to a dominant Verticillium wilt resistance gene in tomato. Genome 41: 91—95.

Kawchuk LM, Hachey J, Lynch DR, Kulcsar F, van Rooijen G, Waterer DR, Robertson A, Kokko E, Byers R, Howard RJ, Fischer R, Prufer D (2001). Tomato *Ve* disease resistance genes encode cell surface-like receptors. PNAS 98: 6511—6515.

Kuklev MY, Fesenko IA, Karlov GI (2009). Development of a CAPS marker for the Verticillium wilt resistance in tomatoes. Russian J. Genet. 45:575–579.

Labate JA, Baldo AM (2005). Tomato SNP discovery by EST mining and resequencing. Mol. Breed. 16: 343-349.

Rafalski JA (2002). Application of single nucleotide polymorphisms in crop genetics. Curr. Opin. Plant Biol. 5: 94–100.

Schaible L, Cannon OS, Waddoups B (1951). Inheritance of resistance to Verticillium wilt in a tomato cross. Phytopathol. 41: 986—990.

Yang W, Bai X, Kabelka E, Eaton C, Kamoun S, van-der-Knaap E, Francis D (2004). Discovery of single nucleotide polymorphisms in *Lycopersicon esculentum* by computer aided analysis of expressed sequence tags. Mol Breed. 14: 21-34.

Yang W, Miller SA, Scott JW, Jones JB, Francis DM (2005). Ming tomato genome sequence databases for molecular markers: application to bacterial resistance and marker assisted selection. Acta Hort (ISHS) 695: 241-250.