COVER SHEET

TITLE: Identifying and locating length and position of introgression on chromosome 6 influencing resistance to bacterial wilt and identifying markers for bacterial wilt resistance on chromosome 12

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ABSTRACT

Identifying and locating length and position of introgression on chromosome 6 influencing resistance to bacterial wilt and identifying markers for bacterial wilt resistance on chromosome 12

Bacterial wilt is a devastating disease effecting hundreds of species of plants and millions of people by destroying crops like the tomato. Conventional techniques to destroy the pathogen have been futile to this point and breeding for genetic resistance to the disease appears to be the most effective option. Using tomato germplasm field tested for resistance and susceptibility in Guatemala, PCR techniques and gel electrophoresis were used to obtain DNA fragments from specific chromosome regions believed to be important in resistance. These DNA fragments were then sequenced, and the sequences were compared from the susceptible and resistant germplasm to identify the location of introgressions associated with resistant germplasm. During this process, two separate introgressions were identified, where susceptible and resistant cultivars were different in sequences. Introgressions were located at 41.5 cM on chromosome 6 and 39 cM on chromosome 12. These introgressions were also associated with other resistant and susceptible germplasm, which had been evaluated in the field in Guatemala. The specific single nucleotide polymorphism associated with the resistant germplasm could be used in breeding programs employing marker-assisted selection methods.

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Introduction:

Bacterial wilt is a serious problem affecting plantations where tomatoes are grown throughout Guatemala and the rest of the world (Czosnek, 2004). Bacterial wilt is caused by *Ralstonia solanacearum*, a gram negative, rod-shaped bacterium (Champoiseau, 2008). It is a serious disease that affects tomatoes of southeastern Guatemala (Garcia et al., 2008); and it was recently discovered that the strain of *R. solanacearum* that affects tomatoes of Guatemala also is the most prevalent strain, phylotype I, sequevar 14 (race 1, biovar 3), found in Taiwan and the Philippines (Sanchez-Perez et al., 2008). The pathogen is soil-borne and can destroy entire plantations, which affectively destroys local people's income and way of life (Czosnek, 2004). Tomatoes are just one of 200 plants susceptible to this pathogen. Bacterial wilt is one of the most significant bacterial plant diseases due to its wide-spread occurrence in the tropics and subtropics combined with its ability to attack a wide variety of crops.

The first visible symptoms appear in the foliage (Champoiseau, 2008). The youngest leaves and ends of branches wilt. Soon after, the foliage will turn yellow and the plant will die (Fig. 1). In young stems dark brown streaks may become visible and highly susceptible plants may experience collapsing of stems. Cross sections of the stems may reveal brown discoloration. When a stem is freshly cut, a milky-white, sticky, dense mass is commonly observed, which indicates the presence of the bacterial wilt bacterium growing in the xylem of the stem.

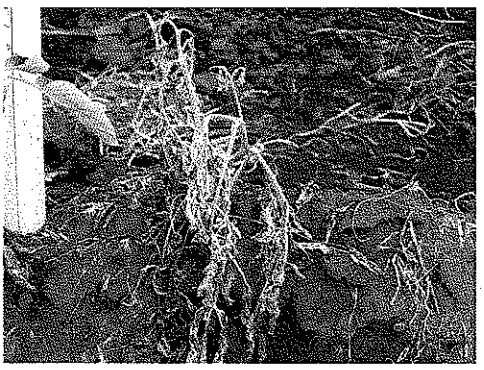

Figure 1. (left) Shows a tomato plant in Guatemala with bacterial wilt symptoms. The stems became discolored, and the foliage became a yellow coloration. (photograph, D. P. Maxwell)

The bacteria enter generally through damaged areas of roots, or points of emergence of lateral roots. It can also be distributed by insects through penetration of the stem (Champoiseau, 2008). Once inside the plant, the bacteria multiply rapidly inside the xylem in the vascular bundles. The bacterium is easily spread through water and soil (Czosnek, 2004). The pathogen can exist in water for several years (Champoiseau, 2008). This makes it very difficult to destroy. Soil fumigation is one way to destroy the pathogen but is environmentally damaging and

economically impractical (Czosnek, 2004). Since most methods will not effectively eliminate the disease, fields that become infected are often abandoned or converted to other crops, such as pasture for animals.

The most effective way to control the disease appears to be breeding tomato hybrids resistant to the disease (Czosnek, 2004). Levels of resistance have been found in many plants, including tomatoes. However, since multiple strains exist, hybrids resistance to one strain may be susceptible to another strain. Generally resistance from breeding is considered partial and often tomato breeders with private companies struggle to develop hybrids with good horticultural characteristics and high resistance. This becomes a goal and problem for developers of commercial tomato hybrids.

Tomatoes are a common food source throughout the world. In 2007, 124,400 acres were used to plant fresh market tomatoes. California and Florida each have ~40,000 acres specifically for tomatoes (Lucier, 2008). That isn't including tomatoes used only for processed food. Those acres produced $1.278 billion in tomatoes and 3.703 billion pounds of tomatoes. The tomato has many uses in food and the tomato is a world-wide marketed vegetable. The primary problem for tomatoes as a food source is disease, particularly in the tropical regions of developing countries. High quality tomatoes are often susceptible to diseases. Mapping the tomato genome is a world-wide effort involving many countries. With increased knowledge of the genome and genes encoded, the tomato can be better understood and bred for more efficient and resistant tomatoes. Tomato contains 12 chromosomes and each chromosome contains hundreds of markers and genes (Mueller et al., 2005). Different genes can be mapped and their function can be determined. One major project is to determine the function of the genes that code for resistance to bacterial wilt in tomatoes.

One resistant line of tomato is Hawaii 7996 (Czosnek, 2004) and this inbred is known to be resistant in Guatemala (Maxwell, pers. com.). This line has been highly successful as a dominant derived polygenic component of resistance. Several quantitative trait loci (QTL) are related to resistance to bacterial wilt in this inbred. Many strain specific QTLs were located for resistance for bacterial wilt associated with chromosomes 3, 4, 6, 7, 8, 10, and 12 (Thoquet et al., 1996; Wang et al., 2000). Hawaii 7996 lines likely have important QTLs near TG564 marker on chromosome 12 and TG73 marker on chromosome 6, as well as, a major QTL was located on chromosome 6 near TG118 marker. The QTL located on chromosome 6 is likely to be important world-wide according to recent research (Wang, pers. com.). Often QTLs are only important to specific strains but research indicates that the QTL on chromosome 6 may be relevant to all strains of the bacteria.

As indicated above, one of the primary techniques to manage the disease is through development of hybrids with resistance (Garcia et al., 2008). These bacterial wilt resistant hybrids should have higher yields and also produce high quality fruits. To produce these hybrids, it is necessary to first bred inbred tomato lines that are resistant to bacterial wilt. The genetic sequence and source of resistance of these inbreds will be very important in future breeding. After the plants resistance is determined, molecular markers can be used to search for introgressions that are associated with sequences that cause the resistance. Programs at the University of Florida as well as at Asian Vegetable Research and Development Center (AVRDC) in Taiwan have created germplasm that is resistant to the bacterial wilt pathogen (Garcia et al., 2008). Germplasm was obtained from these scientists at these institutions and planted in Guatemala in infested field and the level of resistance determined (Maxwell, pers. com.). These sources of bacterial wilt resistance are used in this project.

Objectives:

1. To determine the length of the QTL-introgression associated with bacterial wilt resistance in Hawaii7996 on chromosome 6 (Fig. 2a)
2. To determine if Hawaii7996 has an introgression on chromosome 12, specifically near the marker TG564 (Fig. 2b)
3. To determine if bacterial wilt resistant-inbred lines developed by the Guatemala Tomato Breeding Project have either of these introgressions

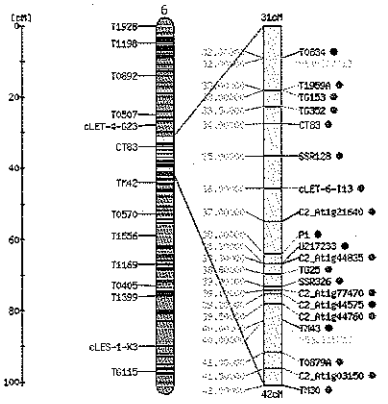

Figure 2a depicts chromosome 6 from SGN website, Tomato EXPEN 2000 diagram presenting the area on chromosome 6 where research was aimed. Multiple primers were designed for this region to isolate the introgression.

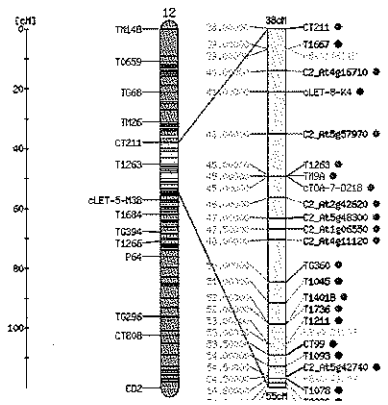

Figure 2b shows a diagram of Tomato EXPEN displaying chromosome 12, and the area of interest for the bacterial wilt introgression. Specifically 39 cM and 54.5 cM were targeted positions on the chromosome.

Methods and Materials:

To begin with, a Pure Gene extraction kit was used to extract the DNA samples for tomato germplasm (Table 1) using the specific factory directions of extraction for the kit.

Table 1. Displays the Code numbering for germplasm, their source, and resistance of germplasm to *Ralstonia solanacearum*.

UW#	Germplasm code	R. sol. Reaction[a]	Source of germplasm
G1	H7996	0.5	AVRDC, Dr. J.-F. Wang[b]
G3	WVa700[c]	4	AVRDC, Dr. J.-F. Wang
G19	RIL170[d]	3.5	AVRDC, Dr. J.-F. Wang
G23	RIL200[d]	1	AVRDC, Dr. J.-F. Wang
G51	GTc546	4	Semillas Tropicales, Dr. L. Mejía
W144	Not available	1.5	Private Seed Co., Dr. D. P. Maxwell
W157	Not available	2.5	Private Seed Co., Dr. D. P. Maxwell
W300	Not available	1	Private Seed Co., Dr. D. P. Maxwell

[a] Resistance of germplasm to *Ralstonia solanacearum* from a 0 = highly resistant to 4 = plants dead. Reaction of lines provided by scientists who provided the germplasm.
[b] AVRDC, Asian Vegetable Research Development Center, Taiwan.
[c] *Solanum pimpinellifolium* cv. West Virginia, susceptible
[d] RIL, recombinant inbred line number from cross of H7996 by WVa700 (Wang, pers. com.)

Primer design strategies:

Primers were designed from COSII markers found in the Sol Genomics Network database(Table 2). Each marker chosen only appears in one location in the entire genome otherwise multiple and different genes would have been detected by the primers. The markers chosen were approximately 3 cM or more apart, when choosing other positions on the chromosome to test. This was in order to increase the chance of locating an introgression on that chromosome. These primers spanned at least 10 cM from the initial position of an introgression on both sides.

These marker sequences identified on the SGN web site were analyzed by using the nucleotide blast search on the National Center for Biotechnology Information web site to find appropriate sequence matches with other plant sequences to create primers. The sequence matches represented only exons, or sequences that will be coded into proteins. The PCR primer sites were designed from the exon sequences as this would increase the chance that the PCR primers would anneal to the unknown sequences of an introgression. It was preferable to have an intron between two exons since sequence differences are most likely to occur in introns (non-coding regions).

When designing the primers, we used the marker DNA rather than the match DNA. Primers were designed to amplify approximately 800-1000 base pair fragments of DNA. Primers designed were between 18 and 30 base pairs in length and the primers had at least half cytosine or guanine. Also, the primers had a guanine or cytosine at the 3' end. It is important that the primers are not self-complementary or they should be under 1 kcal melting energy. Also, the melting temperature was between 50 and 60 degrees Celsius.

In order to create the reverse primer, the sequence selected must be reversed and complemented. These reverse primers will have similar characteristics in regards to melting temperature and will exhibit the same percent of cytosine and guanine. The sequence's complement will be written and then the complemented sequence will be written 5' to 3', ie, reversed. Forward and reverse primers are required to perform the PCR. These were then ordered through Biotechnology Center of UW-Madison. Lists of the primers used are in Table 2.

Table 2. displays primer coding, with position and marker it was derived from. The table indicates the organism the primers were designed from and whether the primers produced evidence of an introgression.

Primer Name	Sequence(5'->3')	Chromosome	Target Positoin(cM)	Marker identification	Organism	Introgression
P6-25F2	GGT AGT GGA AAT GAT GCT GCT C	6	25	C2_At1g77470	Vitis vinifera	No
P6-25R5	GCT CTG CCT ATT GTC CCA TAT ATA ACC	6	25	C2_At1g77470	Vitis vinifera	No
P6-32F1	CAT TGT TGT TGC TCC TCA G	6	32	TO834	Vitis vinifera	No
P6-32R1	CCT TTC TCC ATT CCA TCC AC	6	32	TO834	Vitis vinifera	No
P6-32R2	CTG CTC CTT CCA CTA AAT ATA ACT G	6	32	TO834	Vitis vinifera	No
P6-37F1	GAG AAG ATG ACT GTT CTT GTT G	6	37	C2_At1g21640	Vitis vinifera	No
P6-37R1	CCA GCA GCT GTT GAG TAA G	6	37	C2_At1g21640	Vitis vinifera	No
P6-39.1F1	GCC TCT GAT GAT CGT GGG	6	39.1	C2_At1g77470	Vitis vinifera	No
P6-39.2R1	CTT AAC CGC TGA CTT TGC	6	39.1	C2_At1g77470	Vitis vinifera	No
P6-41F1	CAT TAT TTC CAT GTT GCA AAA GCT CC	6	41.5	C2_At1g03150	Solanum lycopersicum	Ineffective
P6-41F2	GTA GGA CTA AAG GGT GGT AGA TAG GAG C	6	41.5	C2_At1g03150	Solanum lycopersicum	Yes
P6-41F3	TGG GTG ATA TAG CTG ACG ACT GAT G	6	41.5	C2_At1g03150	Solanum lycopersicum	Yes
P6-41F4	CAA ATA TAA GCT TGA AGG TAG GAC	6	41.5	C2_At1g03150	Solanum lycopersicum	Yes
p6-41F5	GAA ATA ATA TGC CTA AAG CTC TCC	6	41.5	C2_At1g03150	Solanum lycopersicum	Yes
P6-41R1	CAT TCA CCT TGC CCT TCA ACT TTT CC	6	41.5	C2_At1g03150	Solanum lycopersicum	Ineffective
P6-41R2	CTT GGA CCT AGG ATC TGT TGC TGG	6	41.5	C2_At1g03150	Solanum lycopersicum	Yes
P6-41R3	TAG TGA AAT CCG CCT ACA CCA GCC	6	41.5	C2_At1g03150	Solanum lycopersicum	Yes
p6-41R4	CAC GGA AGG GAG TAT AAG AGA ATG	6	41.5	C2_At1g03150	Solanum lycopersicum	Yes
P6-41R5	CAT GAA GAG GCC AGA ATA CAC C	6	41.5	C2_At1g03150	Solanum lycopersicum	Yes
P12-39F1	GAT TCA ACT TAT GCA GAG AGG G	12	39	T1667	Solanum lycopersicum	Yes
P12-39F2	GGA ATA CCT TGT GTC CGT C	12	39	T1668	Solanum lycopersicum	Ineffective
P12-39R1	CCT CTC TCG GAA TTT TGT AAC	12	39	T1669	Solanum lycopersicum	Yes
P12-39R2	CCC AAA TCA AAG TTG TAG AAT GAG TAT G	12	39	T1670	Solanum lycopersicum	Ineffective
P12-54.5F1	GCT TTA ATT AGC GAT ACT CAG TGA TGG	12	54.5	C2_At5g42740	Solanum lycopersicum	Ineffective
P12-54.5F2	CAG CAC AGA AAA CAG ACC CG	12	54.5	C2_At5g42740	Solanum lycopersicum	Yes
P12-54.5R1	CTT CTC CTT AAC ATG TGC TGC C	12	54.5	C2_At5g42740	Solanum lycopersicum	Ineffective
P12-54.5R2	GGC TAC ATC AAT TGG ATC AAC ATT CG	12	54.5	C2_At5g42740	Solanum lycopersicum	Yes

PCR methods:

Primers were initially diluted to a 10 uM solution. The diluted primers, Taq polymerase, deionized water, deoxyribonucleotide triphosphate mixture (dNTP's), $MgCl_2$, and PCR buffer from Promega were necessary for PCR reactions. Five micro liters of DNA (about 50 ng) were used for each plant DNA sample. The following conditions were used for each PCR reaction mixture, plus the appropriate controls.

- 5 micro liters of deionized water
- 5 micro liters of PCR buffer
- 2.5 micro liters of $MgCl_2$
- 2.5 micro liters of dNTP's
- 0.1 micro liters of Taq polymerase
- 2.5 micro liters of forward primer
- 2.5 micro liters of reverse primer

The mixture was placed in the thermal cycler with the TGen53 program. This program corresponds to an annealing temperature of 53 degrees C. The volume of each tube was 25 micro liters and the lid was heated. The program details are listed below:

1. 94° C for 3 minutes
2. 94° C for 30 seconds
3. 53° C for 1 minute
4. 72° C for 1 minute
5. Repeat step 2 to 5 35 times before going to step 6
6. 72° C for 10 minutes
7. 4° C for ever

When the PCR program was completed, the samples were stored at -20 C until analyzed. An electrophoresis gel was the first step. Gel electrophoresis was performed with 1.2% agarose and 0.5x TBE. The mixture was heated and stirred until the agarose was dissolved into a homogeneous mixture. The gel was poured into a form and when the agarose was solid, the lanes were loaded with 7 micro liters of PCR reaction sample and 3 micro liters of loading dye. A Promega DNA marker ladder was used in one lane. When all samples were loaded into the gel, the current was turned on to approximately 130 volts.

When the DNA had been separated, the gel was removed and placed in a solution of ethidium bromide. Since ethidium bromide is toxic, gloves were used. The gel was exposed to UV light, which activates the ethidium bromide, and a photograph of the gel was taken and saved for further use. Analysis of the gel was done, which included estimating the size of the DNA bands (fragments), differences between different samples, and ensuring that the water remains uncontaminated (no PCR DNA fragments). From here, there were many options based on the results.

DNA sequence analysis:

If further analysis was needed, the fragments were sequenced. These DNA samples were mixed with exo-nuclease I (Epicentre) and shrimp alkaline phosphatase (Promega Corp.). Each DNA sample required two separate reactions. One reaction was with the forward primer and the other was with the reverse primer. Each DNA sample needed two different strip tubes, with the same treatment listed below for each strip tube:

- 1 micro liter of exo-nuclease
- 1 micro liter of shrimp alkaline phosphatase
- 5 micro liters of sample DNA

The strip tubes were placed into the PCR machine where they underwent the Cut&Kill program to degrade all single stranded DNA (the primers). The details regarding the program are as follows:

1. 37° C for 15 minutes
2. 80° C for 15 minutes

When the program was completed, for each PCR reaction mixture (two tubes) 1 micro liter of forward primer was added to one tube and the other tube of the same sample received 1 micro liter of the reverse primer. Next, the strip tubes had the following UW-Biotechnology Center solutions added for each strip tube being prepared:

- 2 micro liters of Big Dye (Biotechnology Center, UW-Madison)
- 3 micro liters of Big Dye Buffer
- 7 micro liters of deionized water

The samples were then placed in the thermal cycler to undergo the program BTSEQ. The details of the program are as follows:

1. 96° C for 10 seconds
2. 55° C for 15 seconds
3. 60° C for 3 minutes
4. Repeat steps 1 to 3 35 times before moving to step 5
5. 72° C for 7 minutes
6. 4 forever

For cleaning the samples a special magnetic plate is required. The strip tubes were placed into the magnetic plate. Next, 10 micro liters of magnetic beads were pipetted into each tube along with 80 micro liters of 80% ethanol. The strip tubes remained in the magnetic plate for the duration of the cleanup. After three minutes, all of the liquid was removed from the tubes with a

pipette. Then, 150 micro liters of 80% ethanol were added to each strip tube. After three minutes, the liquid was removed. Lastly, 50 micro liters of deionized water were added to each strip tube and the tubes were closed and sent to the Biotechnology Center run for sequence analysis. This involves separation of each ssDNA length and detection of the chromophore attached to each ssDNA. The sequence file from the Biotechnology Center was visualized with Chomas software and sequence assembled and compared using DNAMan software.

Results and discussion:

The quality of the extracted DNA from 12 tomato lines (Table 1) was evaluated by using the PCR primer pair, P6-25F2/R5 (Table 2), which is know to give PCR fragments, if the DNA quality is good. This primer pair amplifies a tomato genomic DNA fragment at 25 cM on chromosome 6 for the Ty3 introgression, which is associated with resistance to begomoviruses. All 12 samples (Fig. 3) produced a~320-bp fragment. There were no PCR fragments in the water control. This is what was expected because all of these tomato lines are susceptible to the begomoviruses and these lines vary in resistance and susceptibility to the *R. solanacearum*. These results indicate that the quality of the DNA for these tomato DNA samples is good and could be used in further experiments to determine the introgressions associated with resistance to *R. solanacearum*.

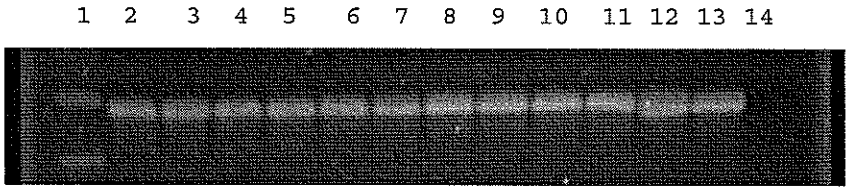

Fig. 3. Agarose gel of the PCR reaction using the primers, P6-25F2/R5, on the 12 tomato samples listed in Table 1. Lane 1, DNA marker, lane 2-13, tomato samples, lane 14, water control. PCR fragment size is about 320 bp.

Evaluation of an introgression on chromosome 6:

The results from Wang et al. (2000) indicated that an introgression for resistance to *R. solanacearum* may occur between 32 cM and 45 cM on chromosome 6. PCR primers were designed for molecular markers in this region and PCR fragments from bacterial wilt resistant and susceptible inbred lines were sequenced to determine if an introgression was present and its length. The primer pair P6-32F1/R1 was used to produce fragments of DNA corresponding to 32 cM on chromosome 6. Initially the P6-32F1/R1 primer pair did not produce any fragments. So a new reverse primer was designed, P6-32R2, which was run with the P6-32F1. This pair produced ~400-bp fragments for 8 samples. The concentrated, single PCR fragments were sequenced. Sequence analysis showed that all samples were identical and no differences were seen between

the 5 resistant and 3 susceptible lines. The partial sequence for the resistant tomato line G1 is listed below:

```
TCTGTTTTTTTTTCGCTTTATTGTGCTCTGTTGTTGCTCCTCAGGTTTCTTCCTCTCCAT   60
CAAGAAATTGATAGTTTTGTACACATTTTTAATTTACCTGCCATCCAAACATCTCACCGG  120
GTACAAAATCTCAATTTAGTTAACGTGTGTGTCTGTAGGTTGCCACGATAAATAAAAAAT  180
TAACAGGGTACTTGTGCGTTATTCCTATGAACGTTATTAAATATTGATCCTAGTCTAATA  240
TCTATAACTATCATTAACAGCTTTTGGATTGTGAAATTAGTTGGTTCTAACTACTGCTAG  300
TCTGGGTACGGGTTTGCTAATGAACAATTTTCATTTCAGTTATATTTAGTGGAAGGAGCA  360
G                                                              361
```

Since the sequences for both the resistant and susceptible tomato lines were identical, it was concluded that there was no introgression at 32 cM on chromosome 6.

A set of P6-37 primers was run looking for further evidence of an introgression on chromosome 6 at 37 cM. Initially, using the TGen53 program (PCR annealing temperature of 53 C) two fragment sizes were observed. The fragments were ~900 bp and 500 bp but the larger fragment length was a significantly strong band. In an attempt to eliminate the smaller band the DNA samples were run using TGen57 program with an annealing temperature of 57 C, which would increase the specificity of the primer annealing to the tomato DNA. This produced single, concentrated fragments for the listed samples (Table 1), but G3 and G19, two susceptible inbreds, did not give fragments. So the initial fragments from the TGen53 PCR were sequenced as well as the two samples from the TGen57 PCR, including the two resistant tomato lines, G1 and G23. No differences in genomic sequence between resistant and susceptible inbred lines were found for 856 nucleotides.

```
GAGAAGATGACTGTTCTTGTTGAACCTGAGGTGCATGATATATTTGCAAGAATCCCAGGC   60
TTTGGGTTTGTTCAGACCTTCTATAGTCAAGATACCAGGTGGGATGATTCATAACTGTAC  120
GTGCAAAAGCATTGTTCTTTTCGTTTATTTTTCCCTCTGTGTGCTCATTACTTCACAGAG  180
AGGTGTGACCTTGCCTGATTTACCAAATGCAGCGATCTTCATGAGAGGGTTGACTTTGTT  240
GCGTGTTTGGGAGGAGATGGTGTGATACTCCATGCATCAAATATATTTCGAGGTGCTGTT  300
CCACCCGTCATCTCATTTAACCTAGGATCCCTTGGATTTCTCACTTCCCATCCAGTATGC  360
CTCTCAACTTAAATTTAAGCTTTTATTTATTATCTATAAATACTAATGTTTTGCTTCTTG  420
TCATCAATTCATACAGTTTGAAGATTACAAGAAGGATCTTAGAAAAGTCATCCATGGAAA  480
CAACACGCTAGATGGTGTGTATATAACTCTAAGGATGCGTCTCCGATGTGAGATATTCCG  540
AAGTGGGAAAGCAATGCCTGGAAAGGTGTTTGATGTCCTAAATGAAGTTGTAGTTGATCG  600
TGGTTCTAATCCATACCTGTCAAAAATAGAGTGTTATGAACATGACCACCTCATAACCAA  660
GGTGCGGTTTATATTCTTAAACATCAATTTGCACTGGCAAAGGATTTAGAGAGCGATCTG  720
TTTTAATATTTTGGATCACGTTTCTTCTAGAATTTAAAAGAAGTGGATCCAGCAAGTGCA  780
CTTCTGAATTAATGAGCAAGAGATGAAAGAATAATCTTGTCGTACTGTATTTTATTTGGC  840
AGGTGCAAGGTGATGG                                               856
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Since all breeding lines had identical DNA sequences at 37 cM on chromosome 6, it was concluded that this is not a point of introgression for bacterial wilt resistance. This confirms results found by Wang et al. (2000).

A P6-39.1 primer pair was designed to search for the introgression presumed to be on chromosome 6. The P6-39.1 primer pair was used to obtain PCR fragments to sequence at 39.1

cM on chromosome 6. The samples produced a concentrated, single PCR fragment of ~470 bp for 12 tomato lines (Fig. 4).

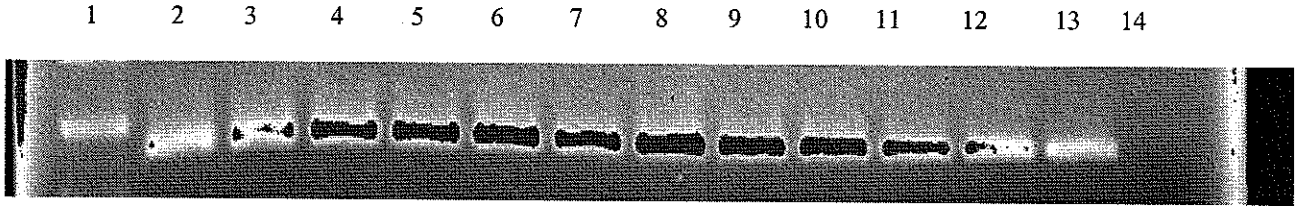


Fig. 4. Displays an agarose gel for the PCR reaction products with P6-39.1 primer pair. Lane 1 contains a DNA marker, and lanes 2-13 contain 12 DNA tomato inbred lines (Table 1). Lane 14 is the water control, indicating no contamination. All PCR fragments are approximately 470 bp.

No differences in the sequences for the resistant and susceptible lines were found. Since there were no differences between resistant lines and susceptible lines, there is no introgression at 39.1 cM on chromosome 6. The partial sequence from the resistant line G1 for chromosome 6 at 39.1 cM is listed below:

```
GATTGATGTGGTGCGCCAGCAGATTCAGGACTTTGCTAGTACTCAGAGCATCTCATTTGG    60
GTAATTTTTTCCTTTAATTTCTTATATTCTATGAGCTTGAATGATGAATAGTGGTGCATA   120
TTCAGGAATTTTCTATATAAAGTATATATAAGTGAACAATGTAACATTCACCTCAACTCT   180
ATAGGCTATATACATGTACAGGCACTCTACTTTACAAAATTGTCTTCTTCTTAGACTTGT   240
AATGTTATCATATTGTTCCAAATTCTGTCTCTGACTTTTCGCTAGACTAAAAAATGGCAA   300
AGGGTAGTATATAATGGATGAGTTAAGGTGCCATCTTTTCTACTAGGAAG             350
```

For the marker at 41.5 cM, multiple primer pairs were used with the 12 samples. The primer pairs were P6-41F1/R1, P6-41F2/R2, P6-41F3/R3, P6-41F2/R3, and P6-41F3/R2. The PCR reactions for each primer pair were analyzed by gel electrophoresis. PCR fragments of different sizes were obtained from the different primer pairs. The P6-41F1/R1 primer pair produced weak PCR ~1000-bp fragments. The P6-41F2/R2 and P6-41F3/R3 primer pairs produced concentrated PCR ~500-bp fragments. The P6-41F2/R3 primer pair produced weaker ~400-bp fragments and the P6-41F3/R2 primer pair produced weak ~550-bp fragments. All samples were sequenced, and only the sequence from the P6-41F2/R2 and P6-41F3R3 primer pairs were capable of being analyzed. The other sequences were not clear enough to analyze with any confidence. Two positions in the sequence showed differences among the lines and these were consistent between resistant and susceptible lines.

There was a single nucleotide polymorphism (SNP) near nt 203 and two nt insertion-deletions (INDEL) near nt 317. The resistant lines (G1 and G23) contained a G at the SNP position and contained 10 consecutive T's near the INDEL. Susceptible lines (G3 and G19) contained a C at the SNP position and 12 consecutive T's at the INDEL. Complete sequence for G1 and G3 are below for P6-41F4/R4 primers.

Resistant tomato line G1:

```
CAAATATAAGCTTGAAGGTAGGACTAAATGGTGGTAGATAGGAGCCAATTAAGTAGAAAG    60
AGTCTAGAGTCCTTTAAGAATACCGAAAATCTGAAGATTAAGCCAAAATTGCCAGAATTG   120
AAAATAATATGCCTAAAGCTCTCCCCTAACTCTTACCTTCTTTTTCAAGAGCAAATTGTT   180
TTCTAAAGGTGGATGGAGGAAATGGGATTTTCTCGAGGAATATTCTTCAGCAGCACTGTT   240
TGATGCAGGGTATACATTAACTTTGACTTTATAGCTTCTTTTTTTTTTGTCATATAGTTG   300
GCAAAGAATTGATTGAATTTAGATCAAACAATAGGTTGTAGCAAGGAATGGCAAGGATAA   360
GCATTCTGAATTTCATTTCTCTTTCAAGAATTGGGGATGGAAAGTAGATTTCATGGGCTG   420
GGGTAGGCGGATTTCTCTAATTCTATTCCCCTAGATGCTAACACTATGTTTCTGAAAATT   480
TCCAGCAACAGATCCTAGGTCCAAGGCAGATGCAAGTAATCATTCTCTTATACTCCCTTC   540
CGTG                                                           544
```

Susceptible tomato line G3:

```
CAAATATAAGCTTGAAGGTAGGACTAAATGGTGGTAGATAGGAGCCCATTAAGTAGAAAG    60
AGTCTAGAGTCCTTTAAGAATACCGAAAATCTGAAGATTAAGCCAAAATTGCCAGAATTG   120
AAAATAATATGCCTAAAGCTCTCCCCTAACTCTTACCTTCTTTTTCAAGAGCAAATTCTT   180
TTCTAAAGGTGGATGGAGGAAATGGGATTTTCTCGAGGAATATTCTTCAGCAGCACTGTT   240
TGATGCAGGGTATACATTAACTTTGACTTTATAGCTTCTTTTTTTTTTTGTCATATAGT    300
TGGCAAAAAATTGATTGAATTTAGATCAAACAATAGGTTGTAGAAAGGAATGGCAAGGAT   360
AAGCATTCTGAATTTCATTTCTCTTTCAAGAATTGGGGATGGAAAGTAGATTTCATGGGC   420
TGGTGTAGGCGGATTTCCCTAATTCTATTCCCCTAGATGCTAACACTATGTTTCTGAAAA   480
TTTCCAGCAACAGATCCTAGGTCCAAGGCAGATGCAAGAAATCATTCTCTTATACTCCCT   540
TCCGTG                                                         546
```

The sequence for the PCR fragment from P6-41BF/R and P6-41CF/R primer pairs showed no differences between lines. The entire sequence for the resistant tomato line G1 for the 41.5 cM for primer pairs B and C are below:

Resistant line G1 using primer pair P6-41BFR:

```
GTACCTGAAGAGATACTTGTTGAGAGAGTGGTTGGCCGTAGACTAGATCCTGTAACTGGG    60
AGAATATACCATTTGAAGTATTCTCCGCCAGAGACCGATGAAATCGCTGCAAGGCTTACC   120
CAGCGCTTTGATGATACAGAAGAAAAGGCATGTTAATGCATGAATACGTACATTATTGTT   180
GCTACAACTGCTGTAGGGCTTTTCCTTTTGTATGAAGCTTTTAAATCACGTAAACTTTTT   240
TAATTCTTATTTGGTGGAAGCCTACCGTTTGTCCCATTGGTCATTTCTTGACCTTCTAAT   300
CAAACCATTTTAATCTATTCTCCTCCTGGAAATTTTGTTGCAGGTCTCGAAATTTCCATT   360
TTCAACTAAATCTTTCTTTTAGCTATTTCCTTTATCATTCTTGCTGTCCTTTTCCCTTGC   420
TTACTCAAGTCGCTTTGTAGATAACTGACTTAAGTATAGGTTTCTATAACATTATAAATA   480
ATGTTGTGTGATCAGCTTCATTTTTGTCTAATTTCTTTTTCAATTTACCGTTTACAGGTG   540
AAGCTGCGTCTGCACACTCACCGTCAAAATGTGAATCAGTTCTCTCAATGTACAAAC      597
```

Resistant line G1 using primer pair P6-41CFR:

```
GTCTGCACACTCACCGTCAAAATGTGGAATCAGTTCTCTCAATGTACAAAGATACTATAT    60
TCCAGGTATTCTTTTTGCCACAGATGTGGTGCAGTTGCTTCACTCGGGAATGCAGATTGA   120
AACACTCATCAGTCACCATCAAAGTGTGGAATCATTCCTTATATACTTGATTCATAGTTT   180
ATTGGGTTGTTTGATGGTTTACAAGGTCTTGGTTAACAGGAATATAGTCCATAAAAGACA   240
ATGTGGAAGGGACTCGGTGGAAATGGTTCGATGAGAAAGTAGTTTGGTTTTCTATTTGGC   300
AAAAGAACAATGATATGGTTATAAAATTTCGTTGTTTGACATGTAGGTTACAAAATGATT   360
ACTTTAAATTATCTTCAGTTCATCCAGGTCATCCTTCCTCATAACAGTTGTAAAACAAAG   420
GAAAGCCAAAGCAAAAGGGCTATACATCCTCTTTTATCTTAAAATTTACTCAATAAGCTA   480
AAGGAAGAGTAAATCCTTTTGAGTATTACATTCATACTAATCAATCTGGGGGGAAATATA   540
CTTAATCAAGGACTCCCCTTCCCTCTTCAATGAACAAGGATACTGTATGTTCTTTCTTTC   600
CATACGCGTGCATGATCGTGTTGTTATCATTTCCTGGTGTTAAAACTTCCATCTTCTCTG   660
AGCTATTTTAATTTCAGGTGGACGGGAGCGTTTCCAAAAAGGAAGTATTTGCTCAAATTG   720
ATGGTGCATTAACTCCCCTAAAAGGGGCAAAGGAGTGAAGATTTGGAACCGGGGTGTCCA   780
GAGATCAGGAGGATCATCGAAGACAAAAGAG                                 811
```

The introgression that exists at 41.5 cM on chromosome 6 was confirmed using the primers P6-41F4/R4, and P6-41F5/R5. The P6-41F4/R4 primers produced the best quality sequence to be analyzed. There were significant differences in this portion of the allele between resistant and susceptible lines (Fig. 5). The P6-41A, P6-41B, and P6-41C primers amplified different portions of the genome than the P6-41F4/R4 primers and no differences were identified at the A, B, or C positions. Comparison of G1 and G3 sequence amplified by P6-41F4/R4 is below indicating 98.7% nucleotide identity with differences emphasized.

Upper line: Susceptible G3 DNA sequence derived from P6-41F4/R4, 546 bp

Lower line: Resistant G1 DNA sequence derived from P6-41F4/R4 , 544 bp

```
1     CAAATATAAGCTTGAAGGTAGGACTAAATGGTGGTAGATAGGAGCCCATTAAGTAGAAAG
                                                      |
1     CAAATATAAGCTTGAAGGTAGGACTAAATGGTGGTAGATAGGAGCCAATTAAGTAGAAAG

61    AGTCTAGAGTCCTTTAAGAATACCGAAAATCTGAAGATTAAGCCAAAATTGCCAGAATTG

61    AGTCTAGAGTCCTTTAAGAATACCGAAAATCTGAAGATTAAGCCAAAATTGCCAGAATTG

121   AAAATAATATGCCTAAAGCTCTCCCCTAACTCTTACCTTCTTTTTCAAGAGCAAATTCTT
                                                                  |
121   AAAATAATATGCCTAAAGCTCTCCCCTAACTCTTACCTTCTTTTTCAAGAGCAAATTGTT

181   TTCTAAAGGTGGATGGAGGAAATGGGATTTTCTCGAGGAATATTCTTCAGCAGCACTGTT

181   TTCTAAAGGTGGATGGAGGAAATGGGATTTTCTCGAGGAATATTCTTCAGCAGCACTGTT

241   TGATGCAGGGTATACATTAACTTTGACTTTATAGCTTCTTTTTTTTTTTTGTCATATAGT
                                              ||
241   TGATGCAGGGTATACATTAACTTTGACTTTATAGCTTC..TTTTTTTTTTGTCATATAGT

301   TGGCAAAAAATTGATTGAATTTAGATCAAACAATAGGTTGTAGAAAGGAATGGCAAGGAT
            |                                     |
299   TGGCAAAGAATTGATTGAATTTAGATCAAACAATAGGTTGTAGCAAGGAATGGCAAGGAT

361   AAGCATTCTGAATTTCATTTCTCTTTCAAGAATTGGGGATGGAAAGTAGATTTCATGGGC

359   AAGCATTCTGAATTTCATTTCTCTTTCAAGAATTGGGGATGGAAAGTAGATTTCATGGGC
```

```
421   TGGTGTAGGCGGATTTCCCTAATTCTATTCCCCTAGATGCTAACACTATGTTTCTGAAAA
          |               |
419   TGGGGTAGGCGGATTTCTCTAATTCTATTCCCCTAGATGCTAACACTATGTTTCTGAAAA

481   TTTCCAGCAACAGATCCTAGGTCCAAGGCAGATGCAAGAAATCATTCTCTTATACTCCCT
                                              |
479   TTTCCAGCAACAGATCCTAGGTCCAAGGCAGATGCAAGTAATCATTCTCTTATACTCCCT

541    TCCGTG

539    TCCGTG
```

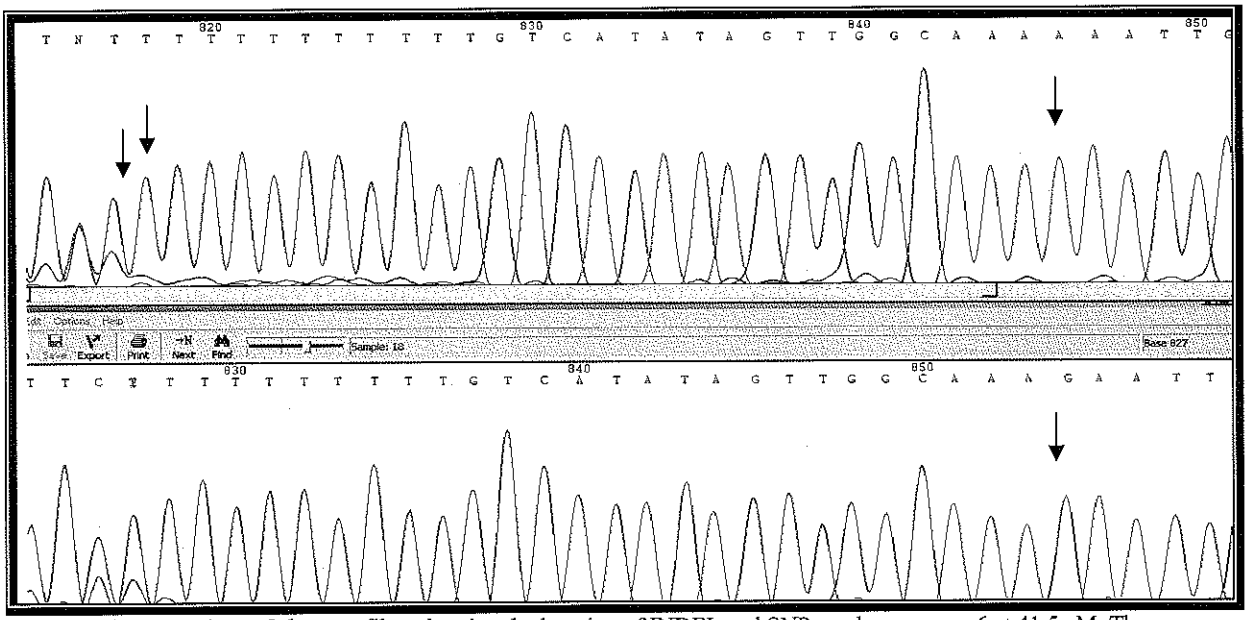


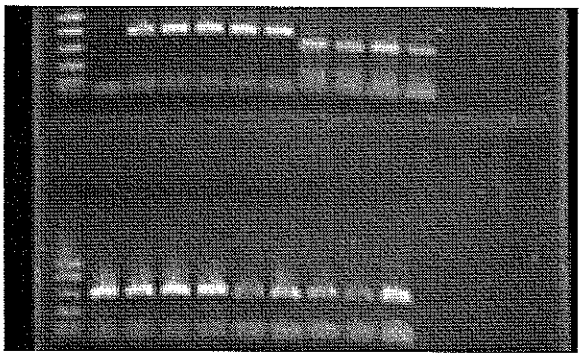
Figure 5. Shows portions of chromas files, showing the location of INDEL and SNP on chromosome 6 at 41.5 cM. The top sequence is the susceptible G51 and the bottom sequence is the resistant G1 as it was amplified by the P6-41F4/R4 primers.

In regard to chromosome 6, it was concluded that an introgression for bacterial wilt was present in the expected region as the QTL identified by Wang et al. (2000). We found that the introgression did exist at 41.5 cM on chromosome 6. No evidence to support the existence of an introgression at any other points was discovered including: 25 cM, 37 cM, or 39.1 cM. Thus, without further experimentation the exact length of the introgression cannot be determined, but it can be concluded that the introgression must not extend lower than 39.1 cM on chromosome 6 and likely exceeds past 41cM on chromosome 6.

Evaluation of an introgression on chromosome 12:

Through research on bacterial wilt, it was found that there was significant evidence that an introgression was located on chromosome 12 (Wang et al., 2000). This location was believed to be important for strong bacterial wilt resistance so inquiry into the length and exact position of the introgression began. Wang et al. (2000) predicted an introgression to be between 45 cM and 55 cM. So, P12-54.5 primers were designed to discover if there was an introgression on chromosome 12 at 54.5 cM. The P12-54.5F1/R1 and P12-54.5F2R2 primer pairs were run simultaneously. When fragments were analyzed using gel electrophoresis, the P12-54.5F1/R1 primer pair produced two PCR fragments of ~500-bp and 700-bp (Fig. 6). The smaller fragment was more concentrated. The water was not contaminated. The P6-54.5F2/R2 primer pair produced strong single fragment, which was ~ 700-bp. The P6-54.4F2/R2 primer pair samples were sequenced. We found that W300(R), W157(R), G51(S), G1(R), G23(R), and W144 (R) matched 100 percent identity at this position. We found these samples differed from G3(S) and G19(S) by a SNP near 625 bp. G3 and G19 were matched at 100% identity. The G3 and G19 had an A at this position and the other samples contained a C at this point. Complete sequence of G1 and G3 are below, shown in that order.

1 2 3 4 5 6 7 8 9 10 11



12 13 14 15 16 17 18 19 20 21

Figure 6 An electrophoresis gel, showing five samples amplified with P12-54.5 primers (lane 3-7), as well as four samples P6-39 primers (8-11). In lane two it is clear that there is no contamination of water control. Two DNA ladders run along side the samples in lanes 1 and 12. Four samples are run for P6-41F3/R3 (lanes 13-16) and P6-41F2/R2(lanes 17-21).

Resistant line G1:

```
CTGCGTGCTCCAAGGGATGCAGTCATCAGCACAGAAAACAGACCCGTTCTTCATGTTGCA    60
AAATAGTGACGGGAAGAATGTTGTTCCTGAGGTTTGGCATGTACTTGATAAGATCCGAGA   120
CTTCTCTGAGAGGGTTCGCAGTGGTTCCTGGGTAATATCGATCATCAAAGAGATTAATTT   180
AAGCAATACTGCTAAGTTTCTAATCAAGAGGCAATTCTTGATAGGTTGGAGCCACAGGAA   240
AAGCATTGAAAGATGTTGTAGCTGTTGGTATAGGTGGCAGTTTCTTGGGCCCTTTGTTCG   300
TACACACAGCACTTCAAACAGGTATGTCTATGACCCTCTCATCCAAACAAAAGCTATTCA   360
TTTGTTTTGCTTTCTTTGTTGTCTAGTTTTTAACTTGCCAAATGTTAAACTTTTAAACCT   420
GGCATATTACAGAAGCGGAGGCTAGTGAGTGTGCAAGAGGGCGCCAGTTGCGATTGTAAG   480
GAATTATTTAAACCAATTTTTGTTGCCGCCCTGCATCTATTGCCATACATGTTTGTGAAG   540
GTGTCCCTCTTTAAATAGTCCAATATTTCTAGTTTAGAGCAAAGAAAACGCTCTCTTTCT   600
GAATTGTGAGAATAGTTATATTTAAATTTCTTTGTGGTTGCAGTCTCGCGAATGTTGATC   660
CAATTGATGTAGCC                                                 674
```

Susceptible line G3:

```
CTGCGTGCTCCAAGGGATGCAGTCATCAGCACAGAAAACAGACCCGTTCTTCATGTTGCA   60
AAATAGTGACGGGAAGAATGTTGTTCCTGAGGTTTGGCATGTACTTGATAAGATCCGAGA  120
CTTCTCTGAGAGGGTTCGCAGTGGTTCCTGGGTAATATCGATCATCAAAGAGATTAATTT  180
AAGCAATACTGCTAAGTTTCTAATCAAGAGGCAATTCTTGATAGGTTGGAGCCACAGGAA  240
AAGCATTGAAAGATGTTGTAGCTGTTGGTATAGGTGGCAGTTTCTTGGGCCCTTTGTTCG  300
TACACACAGCACTTCAAACAGGTATGTCTATGACCCTCTCATCCAAACAAAAGCTATTCA  360
TTTGTTTTGCTTTCTTTGTTGTCTAGTTTTTAACTTGCCAAATGTTAAACTTTTAAACCT  420
GGCATATTACAGAAGCGGAGGCTAGTGAGTGTGCAAGAGGGCGCCAGTTGCGATTGTAAG  480
GAATTATTTAAACCAATTTTTGTTGCCGCCCTGCATCTATTGCCATACATGTTTGTGAAG  540
GTGTCCCTCTTTAAATAGTCCAATATTTCTAGTTTAGAGCAAAGAAAACGCTCTCTTTCT  600
GAATTGTGAGAATAGTTATATTTACATTTCTTTGTGGTTGCAGTCTCGCGAATGTTGATC  660
CAATTGATGTAGCC                                                 674
```

Differences were found between lines associated with 54.5 cM on chromosome 12. The most resistant line, G1, was identical to the most susceptible line, G51, and most lines matched these two sequences. G3 matched G19, which did not match G1. We compared the complete sequence of G3 to G1 and revealed a 99.85% match, with a single nucleotide difference.

Upper line: Susceptible line G3 derived from P12-54.5F2R2, 674 bp
Lower line: Resistant line G1 derived fromP12-54.5F2R2, 674 bp

```
1    CAGCACAGAAAACAGACCCGTTCTTCATGTTGCACTGCGTGCTCCAAGGGATGCAGTCAT

1    CAGCACAGAAAACAGACCCGTTCTTCATGTTGCACTGCGTGCTCCAAGGGATGCAGTCAT

61   AAATAGTGACGGGAAGAATGTTGTTCCTGAGGTTTGGCATGTACTTGATAAGATCCGAGA

61   AAATAGTGACGGGAAGAATGTTGTTCCTGAGGTTTGGCATGTACTTGATAAGATCCGAGA

121  CTTCTCTGAGAGGGTTCGCAGTGGTTCCTGGGTAATATCGATCATCAAAGAGATTAATTT

121  CTTCTCTGAGAGGGTTCGCAGTGGTTCCTGGGTAATATCGATCATCAAAGAGATTAATTT

181  AAGCAATACTGCTAAGTTTCTAATCAAGAGGCAATTCTTGATAGGTTGGAGCCACAGGAA

181  AAGCAATACTGCTAAGTTTCTAATCAAGAGGCAATTCTTGATAGGTTGGAGCCACAGGAA

241  AAGCATTGAAAGATGTTGTAGCTGTTGGTATAGGTGGCAGTTTCTTGGGCCCTTTGTTCG

241  AAGCATTGAAAGATGTTGTAGCTGTTGGTATAGGTGGCAGTTTCTTGGGCCCTTTGTTCG

301  TACACACAGCACTTCAAACAGGTATGTCTATGACCCTCTCATCCAAACAAAAGCTATTCA

301  TACACACAGCACTTCAAACAGGTATGTCTATGACCCTCTCATCCAAACAAAAGCTATTCA
```

```
361   TTTGTTTTGCTTTCTTTGTTGTCTAGTTTTTAACTTGCCAAATGTTAAACTTTTAAACCT

361   TTTGTTTTGCTTTCTTTGTTGTCTAGTTTTTAACTTGCCAAATGTTAAACTTTTAAACCT

421   GGCATATTACAGAAGCGGAGGCTAGTGAGTGTGCAAGAGGGCGCCAGTTGCGATTGTAAG

421   GGCATATTACAGAAGCGGAGGCTAGTGAGTGTGCAAGAGGGCGCCAGTTGCGATTGTAAG

481   TGTGAAGGAATTATTTAAACCAATTTTTGTTGCCGCCCTGCATCTATTGCCATACATGTT

481   TGTGAAGGAATTATTTAAACCAATTTTTGTTGCCGCCCTGCATCTATTGCCATACATGTT

541   GTGTCCCTCTTTAAATAGTCCAATATTTCTAGTTTAGAGCAAAGAAAACGCTCTCTTTCT

541   GTGTCCCTCTTTAAATAGTCCAATATTTCTAGTTTAGAGCAAAGAAAACGCTCTCTTTCT

601   GAATTGTGAGAATAGTTATATTTAAATTTCTTTGTGGTTGCAGTCTCGCGAATGTTGATC
                                  |
601   GAATTGTGAGAATAGTTATATTTACATTTCTTTGTGGTTGCAGTCTCGCGAATGTTGATC

661   CAATTGATGTAGCC

661   CAATTGATGTAGCC
```

This comparison shows that G3 differs from G1 by a single nucleotide, near 625 bp. It was determined that this position cannot be an introgression for bacterial wilt resistance, since the resistant lines were equivalent to susceptible lines. The different alleles must be a result of another mutation. The G3 sample is a different species of tomato, *S. pimpinellifolium* (Sharma, 2008). The other samples are primarily *S. lycopersicum* or lines bred between wild species and *S. lycopersicum*. This indicates that most of the lines should be similar but the origin of the difference found at 54.5 cM may be due to an allele from *S. pimpinellifolium*. Since *S. pimpinellifolium* is closely related to *S. lycopersicum*, these two species should have similar sequences but not entirely the same. This could account for the difference between G3 and all other lines. Since G19 is a recombinant line bred from G1 x G3, it is reasonable that G19 has the allele from G3 at this position. It was concluded that the allele seen in G3 and G19 is due to an *S. pimpinellifolium* allele and that at 54.5 cM on chromosome 12 there is no introgression for bacterial resistance.

To further search for the introgression on chromosome 12, P12-39 primers were designed. DNA samples were amplified isolating sequence at 39 cM on chromosome 12 using the primer pairs P12-39F1/R1 and P12-39F2/R2. The P12-39F1/R1 primer pair produced concentrated single PCR ~500-bp fragments using electrophoresis techniques but the P12-39F2/R2 primer pair did not produce fragments. No contamination was seen in the water control. We sequenced the P12-39F1/R1 samples and found very interesting data. We found multiple differences between the most resistant line W300(R) and G51(S). The resistant line G1 matched W300(R) and G51(S) matched two susceptible lines G3 and G19 with 100% identity. We also found that G51(S) matched 100% identity with two lines with low resistance, W157 and W144. Complete sequence for G1(R) and G3(S) were obtained and partial sequence of W300(R) was recovered.

Resistant line G1:

```
GATTCAACTTATGCAGAGAGGGAATACCTTGTGTCCATCAAAAAGTCAGAATATGAAACG   60
ATAAAAAACCAATGGAAGGTTTGTCATATTCTCAGCTTGGGCAGGGTTGGTTGAAAAGTA  120
AAATGTTTCTGATTCTTCATTTACTCAATCTTTCTAGGTGTTTTCCTTCAATTAGCAAAA  180
TGTTTTCACTTGTAACAGCGATAAATTAATTCTAGATCTGTTAAGGTGCTATATAAGATC  240
CTTTTCTTTCCTACTTCTATTTTGATGCTCTTGTAATATAATGTTTCATTTGGGAAGTCT  300
ATCCCTATGTTTTCCTTTTGTCTAGAAGGCTCCCATGACAAGAAGATTGTCTCTGAGATA  360
ATTGTGTTGAAAGTGTTAATTTAATTCTAATGTTTATCGATGTCTCTGTAACAGAGCATC  420
TCTAAGGAGCAGGCAAAAAGAGTTACAAAATTCCGAGAGAGG                    462
```

Susceptible line G3:

```
GATTCAACTTATGCAGAGAGGGAATACCTTGTGTCCGTCAAAAAGTCAGAATATGAAACG   60
ATAAAAAACCAATGGAAGGTTTGTCATATTCTCAGCTTGGACAGGGTTGGTTGAAAAGTA  120
AAATGTTTCTGATTCTTCATTTACTCAATCTTTCTAGGTGTTTTCCTTCAATTAGCAAAA  180
TGTTTTCACTTGTAACAGCGATAATTTAATTCTAGATCTGTTAAGGTGCTATAAGATCCT  240
TTTCTTTCCTACTTCTATTTTGATGCTCTTGTAATTTTAATGTTTCATTTGGGAAGTCTA  300
TCCCTATGTTTTCCTTTTGTCTAGAAGGCTCCCATGACAAGAAGATTGTCTTTGAGATAA  360
TTGTGTTAAAAGTGTTAATTTAATTCTAATGTTTATCGATGTCTCTGTAACAGAGCATCT  420
CTAAGGAGCAGGCAAAAAGAGTTACAAAATTCCGAGAGAGG                     461
```

Resistant line W300:

```
TCCTCTCTCCGGAATTTTGTAACTCTTTTTGCCTGCTCCTTAGAGATGCTCTGTTACAGA   60
CGATAAACATTAGAATTAAATTAACACTTTCAACACAATTATCTCAGAGACAATCGACAT  120
TTCTTGTCATGGGAGCCTTCTAGACAAAAGGAAAACATAGGGATAGACTTCCCAAATGAA  180
ACATTATATTACAAGAGCATCAAAATAGAAGTAGGAAAGAAAAGGATCTTATATAGCACC  240
CGCTGTTACAAGTGAAAACATTTTGCTAATTGAAGGTTAACAGATCTAGAATTAATTTAT  300
AAAACACCTAGAAAGATTGAGTAAATGAAGAATCAGAAACATTTTACTTTTCAACCAACC  360
CTGCCCAAGCTGAGAATATGACAAACCTTCCATTGGTTTTTTATCGTTTCATATTCTGAC  420
CATTTTTGATGGACACAAGGTATTCCCTCTCTGCATAAGTTGAAT                 465
```

During analysis, it was found that on chromosome 12 at 39 cM there were significant differences in the sequences of DNA. The samples G23(R), W300(R), and G1(R) matched with 100% identity; and susceptible lines or those inbreds with low resistance had 100% identity, G3, G19, G51, W157, and W144. G1(R) matched G3(S) with 98.7% identity and showed multiple points of mutation in the allele. Complete

sequence comparison of G1(R) and G3(S) is below and Fig. 7 and 8 show comparisons of Chromas files, with points of differences between lines.

Upper line: Resistant line G1 derived from P12-39F1R2,462 bp
Lower line: Susceptible line G3 derived from P12-39F1R1,461 bp

```
1      GATTCAACTTATGCAGAGAGGGAATACCTTGTGTCCATCAAAAAGTCAGAATATGAAACG
                                          |
1      GATTCAACTTATGCAGAGAGGGAATACCTTGTGTCCGTCAAAAAGTCAGAATATGAAACG

61     ATAAAAAACCAATGGAAGGTTTGTCATATTCTCAGCTTGGGCAGGGTTGGTTGAAAAGTA
                                            |
61     ATAAAAAACCAATGGAAGGTTTGTCATATTCTCAGCTTGGACAGGGTTGGTTGAAAAGTA

121    AAATGTTTCTGATTCTTCATTTACTCAATCTTTCTAGGTGTTTTCCTTCAATTAGCAAAA

121    AAATGTTTCTGATTCTTCATTTACTCAATCTTTCTAGGTGTTTTCCTTCAATTAGCAAAA

181    TGTTTTCACTTGTAACAGCGATAAATTAATTCTAGATCTGTTAAGGTGCTATATAAGATC
                                 |                          ||
181    TGTTTTCACTTGTAACAGCGATAATTTAATTCTAGATCTGTTAAGGTGC..TATAAGATC

241    CTTTTCTTTCCTACTTCTATTTTGATGCTCTTGTAATA.TAATGTTTCATTTGGGAAGTC
                                            ||
239    CTTTTCTTTCCTACTTCTATTTTGATGCTCTTGTAATTTTAATGTTTCATTTGGGAAGTC

300    TATCCCTATGTTTTCCTTTTGTCTAGAAGGCTCCCATGACAAGAAGATTGTCTCTGAGAT
                                                                  |
299    TATCCCTATGTTTTCCTTTTGTCTAGAAGGCTCCCATGACAAGAAGATTGTCTTTGAGAT

360    AATTGTGTTGAAAGTGTTAATTTAATTCTAATGTTTATCGATGTCTCTGTAACAGAGCAT
             |
359    AATTGTGTTAAAAGTGTTAATTTAATTCTAATGTTTATCGATGTCTCTGTAACAGAGCAT

420    CTCTAAGGAGCAGGCAAAAAGAGTTACAAAATTCCGAGAGAGG

419    CTCTAAGGAGCAGGCAAAAAGAGTTACAAAATTCCGAGAGAGG
```

Since differences between resistant and susceptible lines were consistent with this position, it was concluded that this is an introgression associated with bacterial wilt

resistance. Resistance is associated with the allele that G1 contains and susceptibility is associated with the allele found in G3.

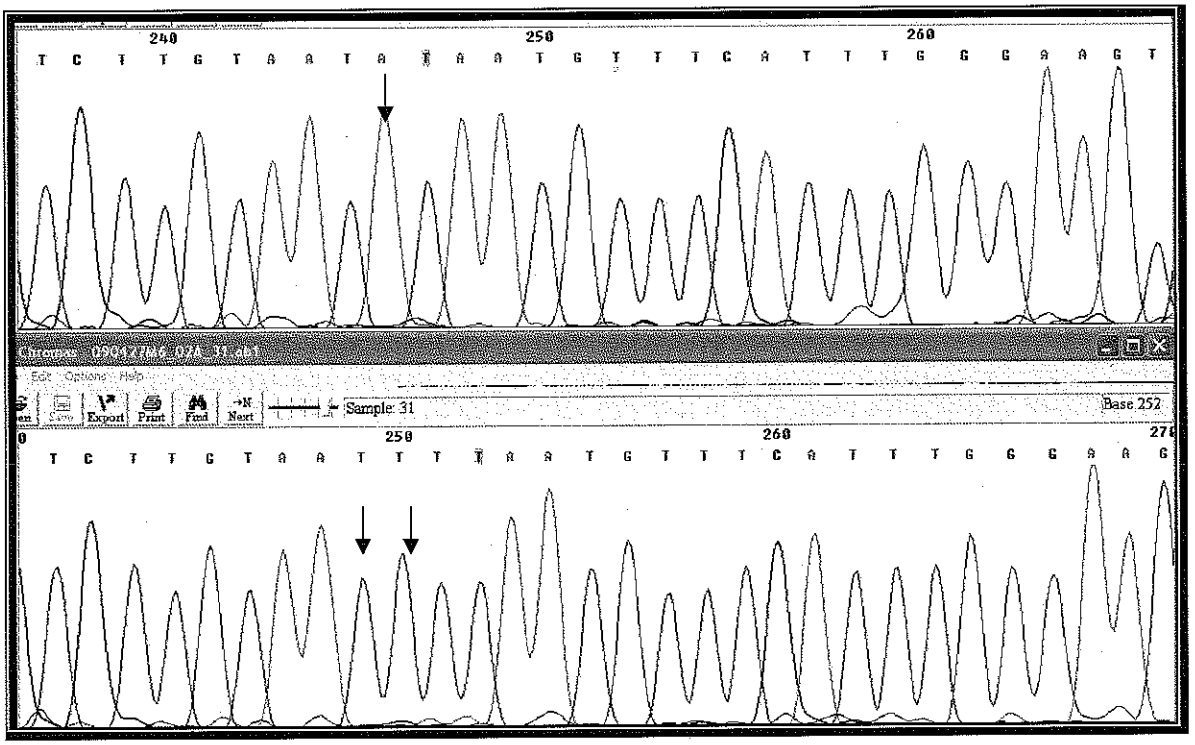


Figure 7. Shows Chromas files showing points of mutation on chromosome 12 at 39 cM as amplified by P6-39F1/R1 primers. The resistant line G1 is shown as the upper diagram and the lower line is the susceptible G3.

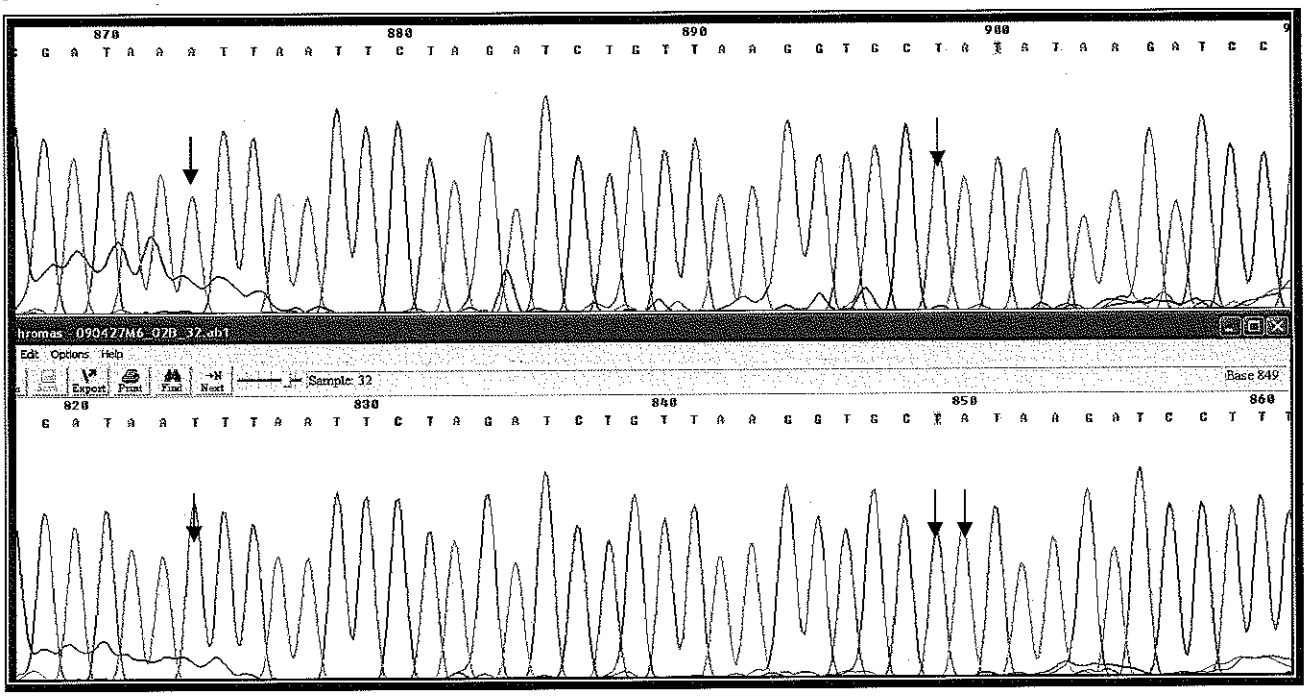


Figure 8. displays chromas files for P6-39F1/R1 primers amplifying portions of sequence showing mutations between the resistant line G1(lower) and susceptible line G3(upper).

In conclusion, in regard to chromosome 12 it was confirmed an introgression does exist. Specifically at 39 cM on chromosome 12 there is an introgression for bacterial wilt resistance. There is no introgression for bacterial wilt at 54.5 cM on chromosome 12. The length of the introgression is unclear. These DNA sequence results support the conclusions from the report by Wang et al. (2000) on the location of a QTL on chromosome 12.

Tomato breeding line	Chromosome 6, 41.5 cM	Chromosome 12, 39 cM
G1	Resistant	Resistant
G3	Susceptible	Susceptible
G19	Resistant	Susceptible
G23	Resistant	Resistant
G51	Susceptible	Susceptible
W144	Susceptible	Susceptible
W157	Resistant	Susceptible
W300	Resistant	Resistant

Table 3. Displays breeding lines and their resistant or susceptible alleles relating to bacterial wilt resistance.

Additional Research:

Additional research will be needed to complete our understanding of the pathogen/host relationship as well as further understand the nature of the gene that codes for resistance. For further research, the primers associated with introgressions should be used to amplify the DNA of other samples to support the fact that these are introgressions located on chromosomes 12 and 6. Further samples will confirm and support conclusions made throughout this article. Also, the function of genes associated with bacterial wilt resistance should be determined in order to understand the disease resistance further. Other markers should be developed to determine the other introgression positions on other chromosomes and the length of these introgressions should be identified. In addition the length of the introgression on chromosome 12 and 6 should be studied in more detail. These data from this effort should be used to develop molecular markers for plant breeders to use.

Acknowledgements:

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Works Cited

Allen, Aurther. "A Passion for Tomatoes." Smithsonian Magazine Aug. 2008. Smithsonian Institute. Feb. 2009 <http://www.smithsonianmag.com/science-nature/passion-for-tomatoes.html>.

Buring, J. E., S. Liu, J. M. Gaziano, and H. D. Sesso. "Dietary lycopene, tomato-based food products and cardiovascular disease in women." The Journal of Nutrition 133 (2003): 2336-341. PubMed. July 2003. Harvard University. 30 Jan. 2009 <www.ncbi.nlm.nih.gov/pubmed/12840203>.

Champoiseau, Patrice G. "Bacterial Wilt of Tomato." Ralstonia solanacearum. 12 Sept. 2008. The United States Department of Agriculture - National Research Initiative Program. 3 Feb. 2009 <http://plantpath.ifas.ufl.edu/rsol/Trainingmodules/BWTomato_Module.html>.

Czosnek, H. Develop tomato breeding lines with resistance to Ralstonia solanacearum and begomoviruses for Guatemala and Central America. Guatemala and Central America. Aug. 2004. The Hebrew University of Jerusalem.Oct. 2008.

Garcia, Brenda E., Luis Mejia, and Douglas P. Maxwell. Evaluation of molecular markers associated with bacterial wilt resistance in the RILs from Hawaii7996 x WVa700. Aqua Blanca, Jutiapa, Guatemala. 4 Aug. 2008. University of Wisconsin-Madison and San Carlos University, Guatemala City. Oct. 2008.

Kent, George C. "Wilt Diseases." *Encyclopedia Americana*. 2009. Grolier Online. 15 Jan. 2009 <http://ea.grolier.com/cgi-bin/article?assetid=0420390-00>.

Lucier, Gary. "Background statistics: Fresh-market Tomatoes." 10 June 2008. The Economics of Food, Farming, Natural Resources, and Rural America. Economic Research Service. United States Department of Agriculture. 2 Feb. 2009 <http://www.ers.usda.gov/News/tomatocoverage.htm>.

Lucier, Gary. "Vegetables and Melons: Tomatoes." 30 Oct. 2008. The Economics of Food, Farming, Natural Resources, and Rural America. Economic Research Service. United States Department of Agriculture. 2 Feb. 2009 <http://www.ers.usda.gov/Briefing/Vegetables/tomatoes.htm>.

Lukas, Mueller, and Lukas, A. "The SOL Genomics Network. A Comparative Resource for Solanaceae Biology and Beyond." Sol Genomics Network. 3 May 2005. Cornell University. 6 Jan. 2009 <http://www.sgn.cornell.edu/>.

Mangin, B., P. Thoquet, J. Olivier, and N. H. Grimsley. "Temporal and Multiple Quantitative Trait Loci Analyses of Resistance to Bacterial Wilt in Tomato Permit the Resolution of Linked Loci." Genetics 151 (1999): 1165-172. Genetics. Mar. 1999. Genetics Society of America. 31 Jan. 2009 <http://www.genetics.org/cgi/content/full/151/3/1165>.

Sharma, Arun, Liping Zhang, Davud Nuni-Liu, Hamid Ashrafi, and Majid R. Foolad. "A Solanum lycopersicum crossed with Solanum pimpinellifolium Linkage Map of Tomato Displaying Genomic Locations of R-Genes, RGAs, and Candidate Resistance/Defense-Response ESTs." International Journal of Plant Genetics 2008 (2008):1-18.

Thoquet, P. , J. Olivier, C. Sperisen, P. Rogowsky, P. Prior, G. Anais, B. Mangin, B. Bazin, R. Nazer, and N. Grimsley. Polygenic resistance of tomato plants to bacterial wilt in the French West Indies. Mol. Plant Microbe Interact. (1996) 9:826-836.

Wang,J.-F., J. Olivier, P. Thoquet, F. Mangin, L. Sauviac, and N.H. Grimsley. Resistance to tomato line Hawaii7996 to Ralsonia solanacearum Pss4 in Taiwan is controlled mainly by a major strain-specific locus. Mol. Plant Microbe Interact. (2000) 13:6-13.

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