

Chromosome 12, 39 cM

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Bacterial Wilt (BW) of tomatoes has become a serious constraint to tomato production in the south eastern Guatemala. This disease is caused by *Ralstonia solanacearum* and the strain (phylotype I, sequevar 14 (race 1, biovar 3) in Guatemala is the same phylotype as in Taiwan (Sanchez-Perez et al. 2008. Plant Pathology 57:320-331). The objective of this research was to determine if there was an introgression on chromosome 12 in germplasm with resistance to Bacterial Wilt. Wang et al. (2000, MPMI 13:6-13) reported that a “strong” marker (QTL) for resistance was located between CT120 and CT156 (tomato-EXPEN 1992, 30 to 72.7 cM) on chromosome 12. [On the SGN website, the CT120 marker is located on the Tomato-EXPEN 1992 map on chr. 11 at 86.2 cM and chr. 12 at 30 cM and on the Tomato-EXPEN 2000 map on chr. 10 at 0 cM and chr. 11 99 cM. For the CT156, the locations are 72.7 cM on chr. 12 for the Tomato-EXPEN 1992 map, and 0 cM for chr. 8 and 97 cM for chr. 12 for Tomato-EXPEN 2000 map.] These authors concluded that this QTL on chr. 12 is more important than the QTL on chromosome 6 at 43.3 cM (TG73, EXPEN 2000) for strain Pss4.

PCR primers were designed from the T1667 marker at 39 cM chr. 12 (EXPEN 2000 map) by E. Ewert and tested on various germplasm sources. There were SNP and indel differences between the H7996 (resistant line) and the WVa700 (susceptible line) used as parents in production of a RIL population (Thoquet et al., 1996. MPMI 9:826-836). The H7996 sequence was detected in one other highly resistant inbred, and in the resistant RILs, 26, 41 and 74. The WVa700 sequence was associated with L390 (susceptible line from AVRDC) and three BW-susceptible inbred lines that had resistance to begomoviruses and the susceptible RILs, 170 and 158. Other germplasm is currently being evaluated.

The RILs were supplied by Dr. J.-F. Wang and evaluated in Guatemala as part of the CDR-USAID grant to Drs. Luis Mejia and Hanokh Czosnek. The phenotype of the RIL evaluated in Guatemala corresponded to the phenotype of the RILs in Taiwan (Taiwan data produced by J.-F. Wang, pers. com.). The RIL population was originally developed by Thoquet et al. (1996. MPMI 9:826-836) from F2 populations of the cross of H7996 x WVa700 (*S. pimpinillifolium*).

Primers (designed by E. Ewert)

P12-39 F1: GAT TCA ACT TAT GCA GAG AGG G
P12-39 R1: CCT CTC TCG GAA TTT TGT AAC

Annealing Temperature: 53°C, a single PCR fragment was produced, which was directly sequenced.

Sequence of p12-39 F1/R1 RIL26 (resistant to BW), 462 bp

```
1      GATTCAACTT ATGCAGAGAG GGAATACCTT GTGTCCATCA AAAAGTCAGA ATATGAAACG
61     ATAAAAAAC AATGGAAGGT TTGTCATATT CTCAGCTTGG GCAGGGTTGG TTGAAAAGTA
121    AAATGTTTCT GATTCTTCAT TTAATCAATC TTTCTAGGTG TTTTCCTTCA ATTAGCAAAA
181    TGTTCCTTCT TGTAACAGCG ATAAATTAAT TCTAGATCTG TTAAGGTGCT ATATAAGATC
241    CTTTTCTTTC CTACTTCTAT TTTGATGCTC TTGTAATATA ATGTTTCATT TGGGAAGTCT
301    ATCCCTATGT TTTCCTTTTG TCTAGAAGGC TCCCATGACA AGAAGATTGT CTCTGAGATA
361    ATTGTGTTGA AAGTGTTAAT TTAATTCTAA TGTTTATCGA TGTCTCTGTA ACAGAGCATC
421    TCTAAGGAGC AGGCAAAAAG AGTTACAAAA TTCCGAGAGA GG
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Sequence of p12-39 F1/R1 Gh13 (Susceptible to BW), 462 bp

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1      GATTCAACCT TATGCAGAGA GGAATACCT TGTGTCCGTC AAAAAGTCAG AATATGAANC
61     GATAAAAAAC CAATGGAAGG TTTGTCATAT TCTCAGCTTG GACAGGGTTG GTTGAAAAGT
121    AAAATGTTTC TGATTCTTCA TTTACTCAAT CTTTNTAGGT GTTTTCCTTC AATTAGCAAA
181    ATGTTTTTCA TTTGTAACAGC GATAATTTAA TTCTAGATCT GTTAAGGTGC TATAAGATCC
241    TTTTCTTTCC TACTTCGATT TTGATGCTCT TGTAATTTTA ATGTTTCATT TGGGAAGTCT
301    ATCCCTATGT TTTCCTTTTG TCTAGAAGGC TCCCATGACA AGAAGATTGT CTTTGAGATA
361    ATTGTGTTAA AAGTGTTAAT TTAATTCTAA TGTTTATCGA TGTCTCTGTA ACACAGCATC
421    TCTAAGGAGC AGGCAAAAAG AGTTACAAAA TTCCGAGAGA GG
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Comparison of RIL26 (R) and Gh13 (S) with p12-39 F1/R1

Upper line: Gh13
bottom line: RIL26

```
1      GATTCAACTTATGCAGAGAGGGAATACCTTGTGTCCGTCAAAAAGTCAGAATATGAAA
      |
1      GATTCAACTTATGCAGAGAGGGAATACCTTGTGTCCAATCAAAAAGTCAGAATATGAAA
      |
61     CGATAAAAAACCAATGGAAGGTTTGTGCATATTCTCAGCTTGGACAGGGTTGGTTGAAAAG
      |
60     CGATAAAAAACCAATGGAAGGTTTGTGCATATTCTCAGCTTGGGCAGGGTTGGTTGAAAAG
      |
121    TAAAATGTTTCTGATTCTTCATTTACTCAATCTTTCTAGGTGTTTCCTTCAATTAGCAA
      |
120    TAAAATGTTTCTGATTCTTCATTTACTCAATCTTTCTAGGTGTTTCCTTCAATTAGCAA
      |
181    AATGTTTTCACTTGTAACAGCGATAATTTAATTCTAGATCTGTTAAGGTGC..TATAAGA
      |
180    AATGTTTTCACTTGTAACAGCGATAAATTAATTCTAGATCTGTTAAGGTGCTATATAAGA
      |
239    TCCTTTTCTTCTACTTCTATTTTGTGCTCTTGTAATTTAATGTTTCATTTGGGAAG
      |
240    TCCTTTTCTTCTACTTCTATTTTGTGCTCTTGTAATA.TAATGTTTCATTTGGGAAG
      |
299    TCTATCCCTATGTTTTCTTTTGTCTAGAAGGCTCCCATGACAAGAAGATTGTCTTTGAG
      |
299    TCTATCCCTATGTTTTCTTTTGTCTAGAAGGCTCCCATGACAAGAAGATTGTCTCTGAG
      |
359    ATAATTGTGTTAAAAGTGTTAATTTAATTCTAATGTTTATCGATGTCTCTGTAACAGAGC
      |
359    ATAATTGTGTTGAAAAGTGTTAATTTAATTCTAATGTTTATCGATGTCTCTGTAACAGAGC
      |
419    ATCTCTAAGGAGCAGGCAAAAAGAGTTACAAAATTCCGAGAGAGGA
      |
419    ATCTCTAAGGAGCAGGCAAAAAGAGTTACAAAATTCCGAGAGAGGA
```

Blast Search: (search sequence = RIL26, resistant to BW)

Matched the following with greater than 90% accuracy to the following on NCBI database:

>  [gb|AC209588.1](#)  Solanum lycopersicum DNA sequence from clone LE_HBa-146I19 on chromosome 12, complete sequence
Length=122513

Identities = 455/465 (97%), Gaps = 3/465 (0%)

```
Query 1      GATTCAACTTATGCAGAGAGGGAATACCTTGTGTCCATCAAAAAGTCAGAATATGAAAC 60
          |||
Sbjct 34613   GATTCAACTTATGCAGAGAGGGAATACCTTGTGTCCGTCAAAAAGTCAGAATATGAAAC 34671

Query 61     GATAAAAAACCAATGGAAGGTTTGTTCATATCTCAGCTTGGGCAGGGTTGGTTGAAAAGT 120
          |||
Sbjct 34672   GATAAAAAACCAATGGAAGGTTTGTTCATATCTCAGCTTGGACAGGGTTGGTTGAAAAGT 34731

Query 121    AAAATGTTTCTGATTCTTCATTTACTCAATCTTTCTAGGTGTTTTCTTCAATTAGCAAA 180
          |||
Sbjct 34732   AAAATGTTTCTGATTCTTCATTTACTCAATCTTTCTAGGTGTTTTCTTCAATTAGCAAA 34791

Query 181    ATGTTTTCACTTGTAACAGCGATAAATTAATTCTAGATCTGTTAAGGTGCTATATAAGAT 240
          |||
Sbjct 34792   ATGTTTTCACTTGTAACAGCGATAAATTAATTCTAGATCTGTTAAGGTGC--TATAAGAT 34849

Query 241    CCTTTTCTTTCTACTTCTATTTTGATGCTCTTGTA--TATAATGTTTCATTTGGGAAGT 299
          |||
Sbjct 34850   CCTTTTCTTTCTACTTCTATTTTGATGCTCTTGTAATTTAATGTTTCATTTGGGAAGT 34909

Query 300    CTATCCCTATGTTTTCTTTTGTCTAGAAAGGCTCCCATGACAAGAAGATTGTCTCTGAGA 359
          |||
Sbjct 34910   CTATCCCTATGTTTTCTTTTGTCTAGAAAGGCTCCCATGACAAGAAGATTGTCTTTGAGA 34969

Query 360    TAATTGTGTTGAAAAGTGTTAATTTAATTCTAATGTTTATCGATGTCTCTGTAACAGAGCA 419
          |||
Sbjct 34970   TAATTGTGTTAAAAGTGTTAATTTAATTCTAATGTTTATCGATGTCTCTGTAACAGAGCA 35029

Query 420    TCTCTAAGGAGCAGGCCAAAAAGAGTTACAAAATTCCGAGAGAGG 462
          |||
Sbjct 35030   TCTCTAAGGAGCAGGCCAAAAAGATTTACAAAATTCCGAGAGAGG 35073
```

Matched the following with greater than 90% accuracy to the following on SOL genomics network:

>[C12HBa0146I19.1](#) AC209588.1 htgs_phase:3 submitted_to_sgn_as:C12HBa0146I19
upload_account_name:italy

Length = 122,513

Score = 829 bits (418), Expect = 0.0
Identities = 455/465 (97%), Gaps = 3/465 (0%), Frame = +1 / +1

```
Query: 1      gattcaacttattgcagagaggggaataccttgtgtccatcaaaaagtcagaatatgaaac 60
          |||
Sbjct: 34613   gattcaacttattgcagagaggggaataccttgtgtccgtcaaaaagtcagaatatgaaac 34671

Query: 61     gataaaaaaccaatggaaggtttgtcatattctcagcttgggcaggggttggtgaaaagt 120
          |||
Sbjct: 34672   gataaaaaaccaatggaaggtttgtcatattctcagcttggacaggggttggtgaaaagt 34731
```

Query: 121 aaaatgtttctgattcttcatttactcaatctttctaggtgttttccttcaattagcaaa 180
 |||||
Sbjct: 34732 aaaatgtttctgattcttcatttactcaatctttctaggtgttttccttcaattagcaaa 34791

Query: 181 atgttttcacttgtaacagcgataaattaattctagatctgtaagggtgctatataagat 240
 |||||
Sbjct: 34792 atgttttcacttgtaacagcgataaatttaattctagatctgtaagggtgc--tataagat 34849

Query: 241 ccttttctttcctacttctattttgatgctcttgtaa-tataatgtttcatttggaagt 299
 |||||
Sbjct: 34850 ccttttctttcctacttctattttgatgctcttgtaatttaatgtttcatttggaagt 34909

Query: 300 ctatccctatgttttcctttgtctagaaggctcccatgacaagaagattgtctctgaga 359
 |||||
Sbjct: 34910 ctatccctatgttttcctttgtctagaaggctcccatgacaagaagattgtctctgaga 34969

Query: 360 taattgtggtgaaagtgttaatttaattcctaagtttatcgatgtctctgtaacagagca 419
 |||||
Sbjct: 34970 taattgtggtgaaagtgttaatttaattcctaagtttatcgatgtctctgtaacagagca 35029

Query: 420 tctctaaggagcaggcaaaaagagttacaaaattccgagagagg 462
 |||||
Sbjct: 35030 tctctaaggagcaggcaaaaagatttacaaaattccgagagagg 35073