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Research Report

Development of a SCAR Marker Linked to Bacterial Wilt (*Ralstonia solanacearum*) Resistance in Tomato Line Hawaii 7996 Using Bulked-Segregant Analysis

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Abstract. We report the development of a codominant sequence characterized amplified region (SCAR) marker linked to bacterial wilt resistance in tomato line Hawaii 7996. Bulked segregant analysis was employed for rapid identification of RAPD markers linked to resistance genes. Genomic DNA from six resistant F_9 recombinant inbred lines (RILs) and six susceptible F_9 RILs, which derived from a cross between *S. lycopersicum* Hawaii 7996 (resistant parent) and *S. pimpinellifolium* WVa 700 (susceptible parent) were pooled in to an R-pool and an S-pool, respectively. A total of 800 RAPD primers were screened and only six primers (UBC#176, 205, 287, 317, 350, and 676) showed polymorphism between R- and S- pools. Of these, only two markers UBC#176 and 317 revealed a 100% linkage in the individual plants comprising the contrasting bulks. Of these, the marker UBC#176 was converted into a co-dominant SCAR marker and designated as SCU176-534. The marker SCU176-534 was confirmed by genotyping the individual of the R- and S- pools and gave the same result as UBC#176. When the marker SCU176-534 was further validated for association with resistance and its potential for maker-assisted selection (MAS) in 92 tomato lines and cultivars, the results showed that none of these carries the resistance gene. Thus, SCAR marker SCU176-534 can be used in early selection of resistant lines when Hawaii 7996 is used as a parent in a breeding program.

***Additional key words*:** BSA, Marker-assisted selection (MAS), *R. solanacearum*, *Solanum lycopersicum*, *Solanum pimpinellifolium*

Introduction

Bacterial wilt caused by *Ralstonia solanacearum* is a soil-borne disease which infects root and stem of the plant causing a sudden wilt. *R. solanacearum* is a genetically and hysiologically diverse pathogen. It has been divided into five races on the basis of differences in host range and six biovars on the basis of biochemical properties, in which race 1 (affects many solanaceous plants and other weeds)/biovars 1, 3, and 4 strains and race 3 (primarily affects potatoes)/ biovars 2 and N2 strains have affected potato cultivation so far (Denny and Hayward 2001; Hayward 1994). Fegan and Prior (2005) proposed a phylotype system. The pathogen was classified into four phylotypes. Strains belonging to phylotype III are only found in Africa, while strains belonging to phylotype IV are exclusively found originating from Indonesia. In contrast, the strains belonging to phylotype I and II are present on several continents. Nevertheless, the phylotype I was demonstrated to have the highest evolutionary potential as well as the highest virulence, with a worldwide prevalence and expanding (Lebeau et al, 2011; Wicker et al, 2012).

R. solanacearum targets primarily tomatoes but is also a problem for potatoes, tobacco, peppers, eggplant, bananas, ginger, cowpea, and peanut. Various strategies have been developed for controlling bacterial wilt, such as addition of

compost or solarization to change soil pH and reduce survival and activity of plant pathogens (Schonfeld et al., 2003), or soil fumigants (Hong et al., 2011; Ji et al., 2005; Pradhanang et al., 2005), which are hazardous to human health and environment. However, the broad host range of the pathogen as well as the existence in diverse strains with different virulence make efficient controlling of the disease very difficult. The most accepted and promising strategy is breeding resistant cultivars or grafting plants using resistant rootstocks. There are tomato varieties with some tolerance to bacterial wilt but variation in pathotype and strain within the pathogen make it difficult to utilize these varieties in some regions. Grafted plants have been found to be infected bacterial wilt due to materials used for rootstock became susceptible (Nakaho et al., 1996). Thus, breeding stable resistant varieties against diverse strains of the pathogen across regions are needed.

Traditional breeding for bacterial wilt resistance has been proven difficult for various reasons, including time-consuming, low efficiency, environmental effects on the development of disease, variation in pathogen populations, and association of resistance with small fruit size. In addition, so-called 'linkage drag', the inheritance of unwanted donor alleles in the same genomic region as the target locus, is difficult to overcome with conventional backcrossing, but can be addressed efficiently with the use of molecular markers. Marker-assisted selection (MAS) where selection is based on genotype greatly improves the efficiency of conventional selection and breeding. Selection based on genotype requires molecular markers that are tightly linked to trait of interest (Mohan et al., 1997), so that identification of breeder-friendly markers linked to genes and/or quantitative trait loci (QTL) controlling these traits is a high priority. The use of molecular marker to separate bacterial wilt resistance and undesirable horticultural traits, and to pyramid resistance genes from multiple sources, has been reported (Yang and Francis, 2005).

Tomato bacterial wilt resistance sources have been identified and cultivars with different levels of resistance have been developed by the Asian Vegetable Research and Development Center (AVRDC) and other groups (Scott et al., 2005). However, the resistance is not stable due to genetic diversity of the pathogen and environmental factors such as high temperature (Jaunet and Wang, 1999) and specific isolates (Hanson *et al*., 1996; Truong et al., 2008, Wang et al., 2013).

The tomato line Hawaii 7996 (H 7996) was found as the most stable and durable resistance source to *R. solanacearum* in worldwide multi-locations evaluations (Wang et al., 1998). However, some studies carried out last decade have shown that the expression and the level of this resistance varied depending on the strain used in testing trials. Thus, within the phylotype I, Hawaii 7996 was shown to be highly susceptible to the Taiwanese strains Pss190 and Pss366, whereas it has been found moderately resistant to the Taiwanese strains Pss4 and Pss358, and highly resistant to strains GMI1000 from French Guyana as well as CMR134 from Cameroon (Lebeau et al., 2011). When testing this resistance against all phylotypes, Lebeau et al. (2011) demonstrated that it was phylotype- as well as strain-specific. Therefore, we can assume that this resistance is controlled by genes/QTLs involved in specific relationships or not with the different strains of the bacterium. During the last two decades, several mapping studies using different populations derived from the interspecific cross between *S. lycopersicum* H7996 and *S. pimpinellifolium* WVa 700 demonstrated the quantitative and oligogenic character of the resistance in the H 7996. Two major QTL have been located on chromosomes 6 and 12, *Bwr-6* with non-specific effects against strains of phylotypes I and II, and *Bwr-12* with specific effects against strains of phylotype I (Thoquet et al., 1996; Wang et al., 2000; Carmeille et al., 2006). Recently, *Bwr-6* was located along a 15.5-cM region on chromosome 6 whereas *Bwr-12* was located more precisely in 2.8-cM interval on chromosome 12 (Wang et al., 2013). SSR markers were detected tightly linked to *Bwr-12* and can be used for MAS for Phylotype I strains. Dissection and fine-mapping of *Bwr-6* region is ongoing in the AVRDC (Jaw-Fen Wang, personal communication).

Miao et al. (2009) have been developed two dominant SCAR markers, $TSCAR_{AAT/CGA}$ and $TSCAR_{AAG/CAT}$ associated with bacterial wilt resistance using different materials. These markers were reported to be located 4.6 cM and 8.4 cM, respectively, from a resistance gene, *TRSR*-1, and have been suggested useful for breeding for bacterial wilt resistance via MAS. Another SNP markers and other PCR-based markers associated with bacterial wilt resistance genes on chromosome 6 (C2_ At1g44835 and C2_At4g10030) and 12 (SSR20) have been reported (Mejía et al., 2009). However, further efforts are needed to develop reliable PCR-based markers for screening for bacterial wilt resistance in tomato. Success of identification of markers linked to resistance genes or objective traits using BSA and RAPD methods have been reported (Du et al., 2011; Iglesias-Andreu et al., 2010; Khampila et al., 2008; Makandar and Prabhu, 2009; Parihar et al., 2010; Shobha and Thimmappaiah, 2011; Singh et al., 2011; Zhang et al., 2008; Truong et al., 2013a; b). In this study, we identified RAPD markers associated with the resistance gene to Korean *R. solanacearum* isolate in tomato line H-7996 using bulked-segregant analysis (BSA) and converted into SCAR markers.

Materials and Methods

Plant Materials and DNA Extraction

Resistant genotype of *Solanum lycopersicum* Hawaii 7996 (H 7996), susceptible genotype of *S. pimpinellifolium* West

Table 1. Genotype of RILs comprising R- and S-pools and parents using polymorphic RAPD markers

Line	Marker						Percentage of surviving plants (%)[z]	
	UBC#176 (900bp fragment)	UBC#205	UBC#287	UBC#317	UBC#350	UBC#676	Guatemala	Taiwan
Hawaii7996 (Resistant check)	H	H	H	H	H	H	84	88
WVa700 (Susceptible check)	W	W	W	W	W	W	22	31
RIL-26	H	H	H	H	W	H	91	59
RIL-32	H	H	H	H	H	H	95	55
RIL-41	H	H	H	H	H	H	94	71
RIL-74	H	H	H	H	H	H	94	75
RIL-162	H	W	W	H	H	W	95	89
RIL-200	H	H	H	H	H	H	94	89
RIL-30	W	W	W	W	W	W	21	35
RIL-79	W	W	W	W	W	W	18	39
RIL-158	W	W	W	W	W	W	1	35
RIL-170	W	W	W	W	W	W	0	25
RIL-182	W	W	W	W	W	W	28	25
RIL-183	W	W	W	W	W	W	11	40

[z]Mejía et al. (2009); H: sequence for H7996; W: sequence for WVa700

Verginia 700 (WVa 700), and 92 tomato genotypes, which belong to *S. lycopersicum*, were provided by Vegetable research Division, National Institute of Horticultural & Herbal Science (NIHHS), Korea. The seeds were sown in the 72-well tray using potting substrate (Seoul Bio Co., Ltd., Korea). Genomic DNA of H7996, WVa700 and 92 tomato genotypes were extracted from leaves of young seedlings (3 to 4 true leaves) using DNeasy Plant Kit (96-well format) from QIAGEN (Qiagen GmbH, Hilden, Germany). The DNA concentration was measured on a Nanovue spectrophotometer (GE Healthcare, Little Chalfont, Buckinghamshire, U.K). The quality of the DNA was inspected using agarose gel electrophoresis and spectral absorbance (the A_{260}/A_{280} ratio). Genomic DNA of 12 F_9 recombinant inbred lines (RILs) were provided by Bacteriology Unit, AVRDC-The World Vegetable Center, Taiwan. The F_9 RIL was derived from a cross between H-7996 (*S. lycopersicum*, resistant) and WVa 700 (*S. pimpinellifolium*, susceptible) (Thoquet et al., 1996). This cross was made in France and advanced up to F_3 using single seed descent (SSD) method (Tigchelaar and Casali, 1976). Seeds of F_3 lines were then sent to the Institute of Plant Breeding of the University of the Philippines Los Banos for generation advance to produce the F_5 recombinant inbred lines. Generation advance of H7996 × WVa700 mapping population from F_6 to F_9 generation was made at AVRDC. The six resistant F_9 recombinant inbred lines (RILs) (RIL#26, 32, 41, 74, 162, and 200) and six susceptible F_9 RILs (RIL#30, 79, 158, 170, 182, and 183) were selected based on percentage of wilted plant from disease evaluations conducted in Guatemala (Mejía et al., 2009) and Taiwan (Truong, 2007), which is shown in Table 1.

Bacterial Strain and Plant Inoculation

Isolate of *R.solanacearum* was isolated from tomato plant with symptom of bacterial wilt from plastic-house of NIHHS, Suwon, Korea. Strain was purified and was grown on tetrazolium chloride (TZC) medium (Schaad, 1988) at 28 ± 2°C for 48 h. Bacterial masses were harvested from 48-hour-culture TZC plates and suspended with distilled water. Concentration of inoculum was 10^7 cfu/mL (OD = 0.14). Seedlings with four fully expanded true leaves (about three-week old) were inoculated by wounding the roots and dipped in bacterial suspension. Inoculated seedlings were maintained in greenhouse at around 25-35°C. Disease evaluations were done at 10, 20, and 30 days after inoculation. Severity of wilting symptoms of individual inoculated plants was rated on a scale of 1 to 5, where: 1 = no visible symptoms; 2 = one to less than half of the foliage wilting; 3 = about half of the foliage wilting; 4 = nearly all of the foliage wilting; 5 = the whole plant wilting and dead. Disease index (DI): DI was calculated following the formula (Winstead and Kelman, 1952): $DI = [(N_1 \times 1 + N_2 \times 2 + N_3 \times 3 + N_4 \times 4 + N_5 \times 5)/(N_T \times 5)] \times 100$; where N_1 to N_5 are the number of plants at each scale, and N_T is number of total plants.

Bulk segregation analysis

An equal amount of DNA from six resistant F_9 recombinant inbred lines (RILs) (RIL#26, 32, 41, 74, 162, and 200)

and six susceptible F_9 RILs (RIL#30, 79, 158, 170, 182, and 183), which selected by Mejía et al. (2009) were pooled in to an R-pool and an S-pool, respectively (Michelmore et al., 1991). These pools were used to screen RAPD primers, which showed polymorphism between the parents. Once DNA bands were found corresponding to the resistant parent and R-pool, or to the susceptible parent and S-pool, as well as revealed a 100% linkage in the individual plants comprising the contrasting bulks, the bands were cloned and sequenced.

RAPD analysis

A total of 800 UBC (University of British Columbia) RAPD primers (synthesized by Bioneer, Daejeon, Korea) were pre-screened on the parents and reference resistant and susceptible inbred lines. The PCR reactions were performed in Eppendorf Mastercycler Gradient (Minnesota, USA). The 15 μL reaction volume included 2.5 mM $MgCl_2$ (Roche, Seoul, Korea), 200 μM deoxyribonucleotide triphosphate mix (Roche), 10 X PCR buffer, 25 mM $MgCl_2$, 1 U of *Taq* DNA polymerase (Genet Bio, Chungnam, Korea), and 0.25 μM of random primer and 5-10 ng of genomic DNA. The amplification reactions were carried out using the following thermal profile: 94°C for 3 min (1 cycle); 94°C for 1 min, 37°C for 1 min, 72°C for 2 min (40 cycles); 72°C for 7 min (1 cycle). Amplified products were incubated with a 1:10,000 dilution of the SYBR Green I nucleic acid gel stain (Invitrogen, Massachusetts, USA) for 20 minutes and separated on a 1% agarose gels using 0.5 X TBE buffer for three and half hours at 120 V and photographed under UV light. A 100 bp molecular ladder (Promega, Tokyo, Japan) was used as a molecular weight marker.

Cloning and sequencing RAPD fragments

The RAPD fragments obtained from H7996 and WVa700 were excised from 1% agarose gels and purified with a QIAquick gel extraction kit (Qiagen, Hilden, Germany). The fragment was cloned using TOPO TA Cloning kit following the manufacturer's instructions (Invitrogen, Massachusetts, USA). Twenty white colonies of each transformant were selected to analyse transformants by PCR. Plasmid DNA was extracted using Core-one plasmid miniprep kit (Seoul, Korea) and sent to the sequencing company CoreBio (Seoul, Korea) for sequencing. The sequence was analyzed using the program BioEdit 7.0 (Hall, 1999)

Primer design

Primers were designed according to the sequence obtained using the program Primer3 4.0 (Rozen and Skaletsky, 2000). Oligonucleotide primers were synthesized by Bioneer Corp. (Daejeon, Korea).

SCAR analysis

Each PCR reaction was carried out in a total reaction volume of 25 μL containing 15-20 ng of genomic DNA, 200 μM deoxyribonucleotide triphosphate mix (Roche), 10 X PCR buffer, 25 mM $MgCl_2$, 1 U of *Taq* DNA polymerase (Genet Bio, Chungnam, Korea), and 0.25 μM of each primer. PCR was performed on an Eppendorf Mastercycler Gradient. The amplification profile consisted of an initial denaturation for 5 min at 94°C followed by 35 cycles of PCR amplification under the following parameters: 20 sec at 94°C, 1 min at the annealing temperature of 55°C, and 1 min of primer elongation at 72°C. A final incubation at 72°C for 10 min was programmed to allow completion of primer extension. Amplified products were visualized on an agarose gel as described previously. A 100 bp ladder (Promega, Tokyo, Japan) was used as a molecular weight marker.

Results

A total of 800 RAPD primers, which were successfully used in previous studies (Truong et al. 2013a;b), were screened between the two parents H7996 (H) and WVa700 (W). Of these, 23 polymorphic primers were used to screen R- and S- pools, but only six primers showed polymorphism between R- and S-pools (Fig. 1). Three of these (UBC#176, 317, and 676) were associated in a coupling phase linkage with the bacterial wilt resistance, amplifying the polymorphic fragments only in the resistant parent. The other three RAPD

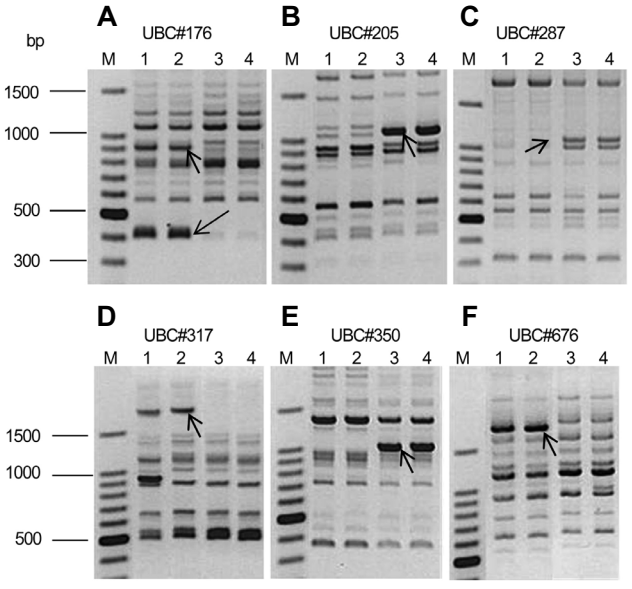


Fig. 1. Agarose gel electrophoresis of RAPD primers (A: UBC#176, B: UBC#205, C: UBC#287, D: UBC#317, E: UBC#350, F: UBC#676) showed polymorphism between R- and S-pools. Lanes M, 100 bp molecular ladder, 1, resistant parent H7996; 2, R pool; 3, susceptible parent WVa700; 4, S pool. Polymorphic markers are indicated by arrows.

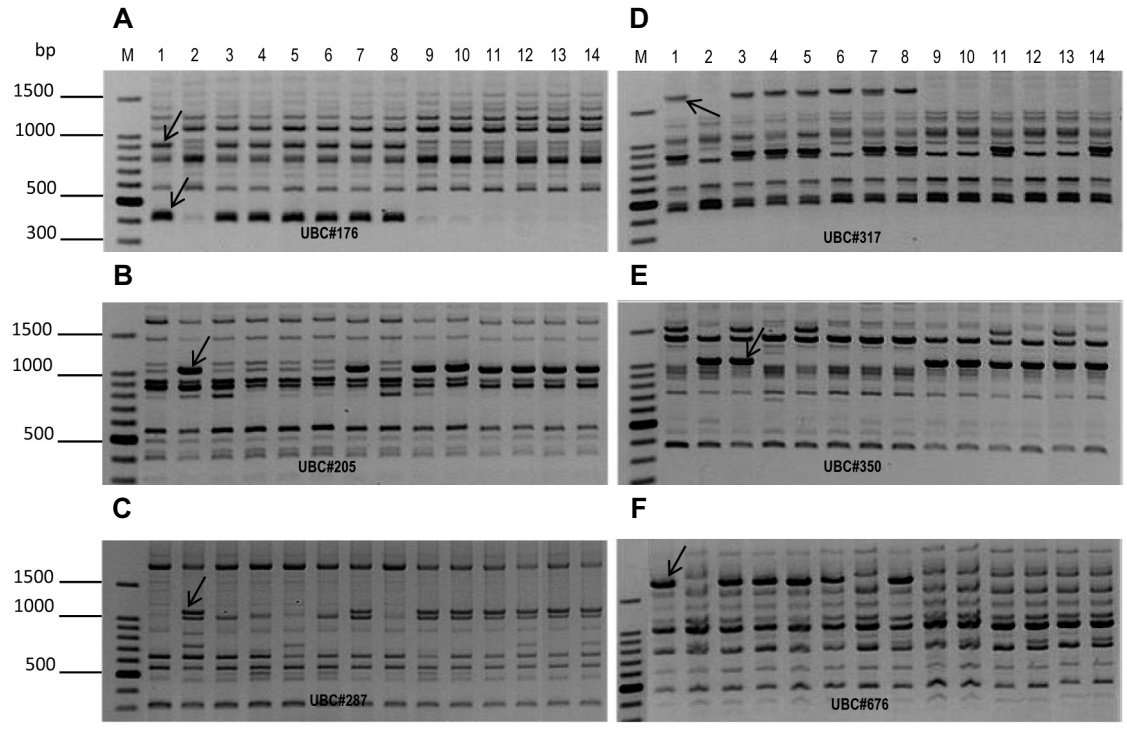


Fig. 2. RAPD primers (A: UBC#176, B: UBC#205, C: UBC#287, D: UBC#317, E: UBC#350, F: UBC#676) showed polymorphism between R- and S-pools screened on individuals comprising the bulks. Lanes M, 100 bp molecular ladder, 1, resistant parent H7996; 2, susceptible parent WVa700; 3-8, resistant RILs: RIL#26, 32, 41, 74, 162, and 200, respectively; 9-14, susceptible RILs: RIL#30, 79, 158, 170, 182, and 183, respectively. Polymorphic markers are indicated by arrows.

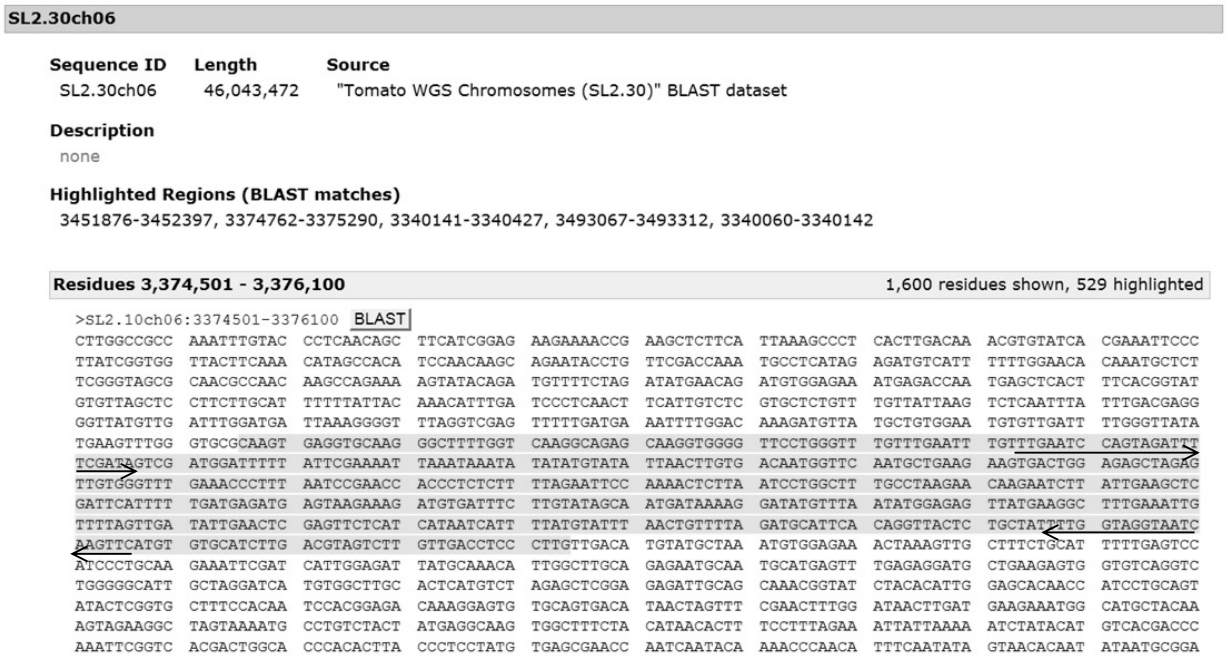
fragments (UBC#205, 287, and 350) amplified polymorphic fragments only in the susceptible parent and thus were associated in repulsion phase linkage with bacterial wilt resistance. Of these, primer UBC#176 produced two polymorphic fragments (400 and 900 bp) (Fig. 1). These primes were then used to analyze the twelve individuals constituting the bulks to determine whether there was significant linkage to the resistance trait. However, only markers UBC#176, which generated 900-bp fragment and 400-bp fragment, and UBC#317, which generated about 2,5 kb fragment revealed a 100% linkage in the individual plants comprising the contrasting bulks, whereas the other markers revealed 91.7% (Table 1, Fig. 2).

The 400 and 900 bp-fragments generated from marker UBC#176 were successfully cloned and sequenced. Sequencing results showed that the terminal 10 bases of 5' to- 3' matched the sequence of primer. These sequences were blasted against the Sol Genomics Network (SGN) database using blast. Sequence of 534 bp sub-clone matched five regions of sequence SL2.30ch06 (3451876-3452397, 3374762-3375290, 3340141-3340427, 3493067-3493312, and 3340060-3340142), and highest matched region was 529 nucleotides (Fig. 3). Sequence of 1190-bp sub-clone matched eight regions of sequence SL2.30ch06 (35280581-35281765, 42456472-42456552, 20792258-20792341, 34953293-34953371, 3069470-3069550, 1833469-1833533, 13442265-13442315, 27940857-27940929), and highest matched region was 1,185 nucleotides (Fig. 4). Two SCAR primer pairs (SCU176-1190-F1R1 and SCU176-1190-F2R2) were designed covering the sequence of 1190-bp sub-clone and one primer pair covering 534-bp sub-clone (SCU176-534) (Table 2). These primers were used to screen the two parents and the pools. There was no polymorphism produced from primers SCU176-1190-F1R1 and SCU176-1190-F2R2 regardless of different PCR conditions tested. Primer SCU176-534 showed polymorphism between the parents and the pools. The marker SCU176-534 was confirmed by genotyping the individual RILs comprising the R- and S-pools and the two parents and resulted the same as UBC#176. About 30 bp presented in the resistant parent but absented in the susceptible parent (Fig. 5).

Ninety two tomato lines were evaluated for bacterial wilt resistance. Of these, only one line (TRxVC11-2)-9-1F4) had no symptom of wilting and could be considered as moderate resistance. The tomato lines were genotyped using the polymorphic SCAR and RAPD markers; however, none of them carries the resistance gene (Table 3).

Discussion

Inheritance of resistance to bacterial wilt in tomato can be



SL2.30ch06

Sequence ID	Length	Source
SL2.30ch06	46,043,472	"Tomato WGS Chromosomes (SL2.30)" BLAST dataset

Description
none

Highlighted Regions (BLAST matches)
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Residues 3,374,501 - 3,376,100 — 1,600 residues shown, 529 highlighted

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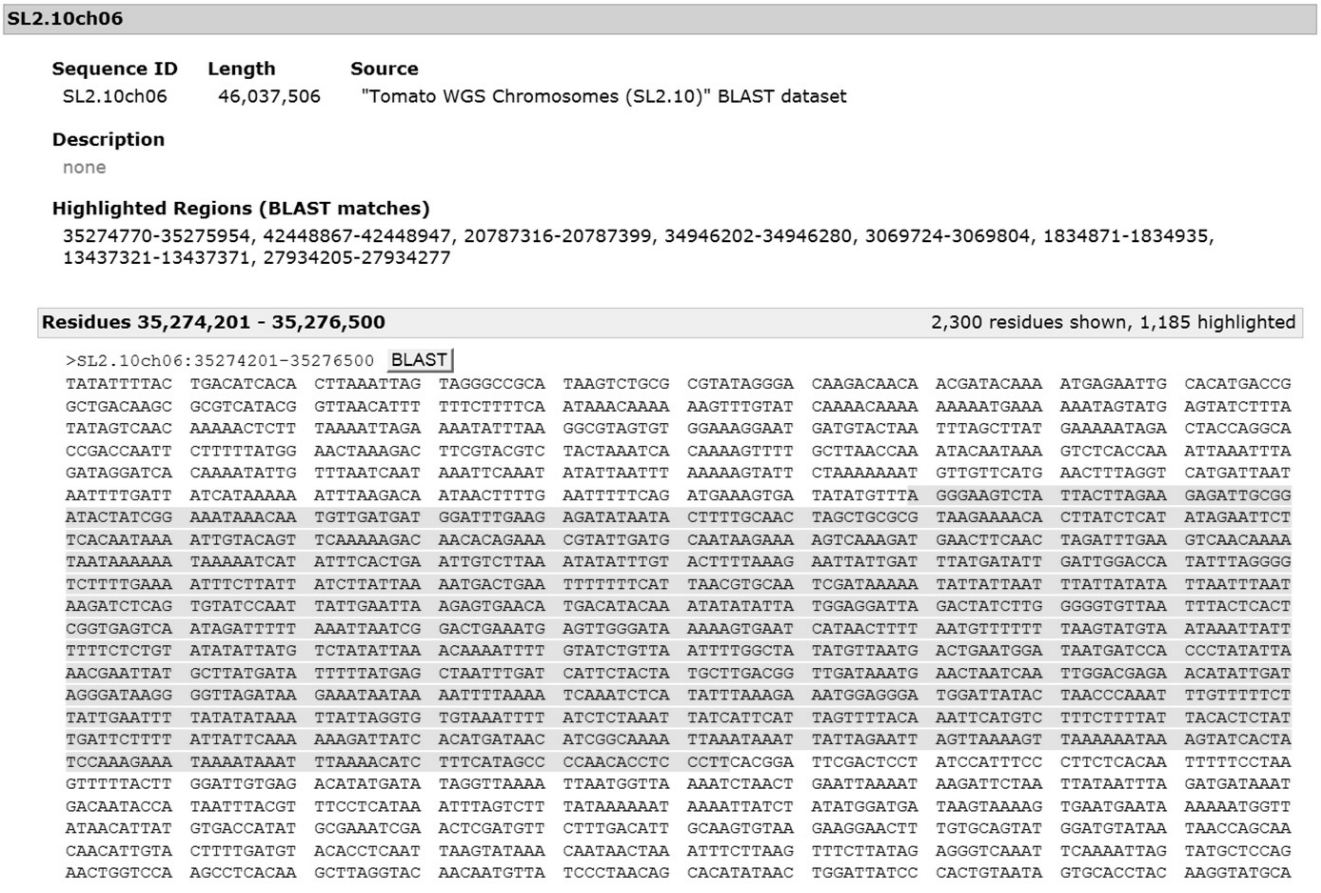
Fig. 3. Blast result of 534 -base-pair sub-clone sequence with tomato genome from Solanum Genomics Network (SGN). The arrows indicate primer positions of new SCAR markers.



SL2.10ch06

Sequence ID	Length	Source
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Description
none

Highlighted Regions (BLAST matches)
35274770-35275954, 42448867-42448947, 20787316-20787399, 34946202-34946280, 3069724-3069804, 1834871-1834935, 13437321-13437371, 27934205-27934277

Residues 35,274,201 - 35,276,500 — 2,300 residues shown, 1,185 highlighted

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AACGAATTAT GCTTATGATA TTTTTATGAG CTAATTTGAT CATTCTACTA TGCTTGACGG TTGATAAATG AACTAATCAA TTGGACGAGA ACATATTGAT
AGGGATAAGG GGTTAGATAA GAAATAATAA AATTTTAAAA TCAAATCTCA TATTTAAAGA AATGGAGGGA TGGATTATAC TAACCCAAAT TTGTTTTTCT
TATTGAATTT TATATATAAA TTATTAGGTG TGTAAATTTT ATCTCTAAAT TATCATTCAT TAGTTTTACA AATTCATGTC TTTCTTTTAT TACACTCTAT
TGATTCTTTT ATTATTCAAA AAAGATTATC ACATGATAAC ATCGGCAAAA TTAAATAAAT TATTAGAATT AGTTAAAAGT TAAAAAATAA AGTATCACTA
TCCAAAGAAA TAAAATAAAT TTAAAACATC TTTCATAGCC CCAACACCTC CCTTCACGGA TTCGACTCCT ATCCATTTCC CTTCTCACAA TTTTTCCTAA
GTTTTTACTT GGATTGTGAG ACATATGATA TAGGTTAAAA TTAATGGTTA AAATCTAACT GAATTAAAAT AAGATTCTAA TTATAATTTA GATGATAAAT
GACAATACCA TAATTTACGT TTCCTCATAA ATTTAGTCTT TATAAAAAAT AAAATTATCT ATATGGATGA TAAGTAAAAG TGAATGAATA AAAAATGGTT
ATAACATTAT GTGACCATAT GCGAAATCGA ACTCGATGTT CTTTGACATT GCAAGTGTAA GAAGGAACTT TGTGCAGTAT GGATGTATAA TAACCAGCAA
CAACATTGTA CTTTTGATGT ACACCTCAAT TAAGTATAAA CAATAACTAA ATTTCTTAAG TTTCTTATAG AGGGTCAAAT TCAAAATTAG TATGCTCCAG
AACTGGTCCA AGCCTCACAA GCTTAGGTAC AACAATGTTA TCCCTAACAG CACATATAAC TGGATTATCC CACTGTAATA GTGCACCTAC AAGGTATGCA
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Fig. 4. Blast result of 1190 -base-pair sub-clone sequence with tomato genome from Solanum Genomics Network (SGN).

Table 2. Primer sequences of SCAR markers

Primer name	Forward primer	Reverse primer
SCU176-534:	TTGAACCAAGAATCTATTCG	GAACTTGAATGCCTACCAAA
SCU176-1190F1R1	TGCGGATACTATCGGAAATA	CAACTCATTTCAGTCCGATT
SCU176-1190F2R2	TCACTCGGTGAGTCAAT AGAT	TTTGCCGATGTTATCATGT

Table 3. Genotype of tomato germplasm using polymorphic SCAR and RAPD markers

Accession name[z]	IT number[y]	Disease Index	Genotype							
			UBC#176 (900-bp fragment)	UBC#176 (400-bp fragment)	SCU176-534	UBC#205	UBC#287	UBC#317	UBC#350	UBC#676
C16	32897	60	S	S	S	S	S	S	S	S
C24(F5)	32900	8	S	S	S	S	S	S	S	S
Carter's Fruit	32909	64	S	S	S	S	S	S	S	S
CL9-0-0-2-4-0	32920	50	S	S	S	S	S	S	S	S
CL9d-03-6-0-0-0	32922	34	S	S	S	S	S	S	S	S
CL123-2-4-0-0-1	32946	8	S	S	S	S	S	S	S	S
Cornel	32974	48	S	S	S	S	S	S	S	S
DD17	32981	86	S	S	S	S	S	S	S	S
PI356914	33129	66	S	S	S	S	S	S	S	S
PI365917	33131	72	S	S	S	S	S	S	S	S
VC8-1-2-1	33195	76	S	S	S	S	S	S	S	S
VF 1402	33208	88	S	S	S	S	S	S	S	S
VC11-1 VG	33223	84	S	S	S	S	S	S	S	S
(TRxVC48-1)-17-2F4	116926	2	S	S	S	S	S	S	S	S
(TRxVC11-2)-9-1F4	116930	0	S	S	S	S	S	S	S	S
VC13-2-2P	116962	2	S	S	S	S	S	S	S	S
Known You#1	116982	62	S	S	S	S	S	S	S	S
84-134-IT-2-1	142192	70	S	S	S	S	S	S	S	S
101T6×Oaejeong	160383	72	S	S	S	S	S	S	S	S
Fireball	180788	88	S	S	S	S	S	S	S	S
Ace55 VF	180789	46	S	S	S	S	S	S	S	S
ACC39	201640	66	S	S	S	S	S	S	S	S
Local check	201652	70	S	S	S	S	S	S	S	S
RAVID	207028	18	S	S	S	S	S	S	S	S
U14889	207029	56	S	S	S	S	S	S	S	S
Livingston beauty	207221	68	S	S	S	S	S	S	S	S
Chernoploana (Black fruit)	207225	56	S	S	S	S	S	S	S	S
Peto 94C	207231	88	S	S	S	S	S	S	S	S
Ledy finger	207234	78	S	S	S	S	S	S	S	S
CLN2413J	219661	10	S	S	S	S	S	S	S	S
L06082	219666	88	S	S	S	S	S	S	S	S
Sogoang (Suceptabile check)		92	S	S	S	S	S	S	S	S
H7996 (Resistant check)		0	R	R	R	R	R	R	R	R

[z]selected tomato accessions from RDA germplasm. The remaining tomato genotypes (accession numbers are in parentheses), which were negative for the R allele, were Veletenskiy (229553), Zevat (207241), ESI (IT: 207245), Kitaiskoi (207248), Gigant 72 (203284), Voin (203288), Privoljskii (203295), Early Scarlet (203411), Mortgage lifter (203471), Principe Borghese (203475), Yellow Delicious (203478), Beefsteak mixed (203480), Progress (203250), Avrora (203251), Rogiziansky (203263), Rannii Uzbekistana (203266), Vilina (203270), Harkovskii 179 (203273), Fukuju (116983), Summer Pink#2 (116985), Apollo FNR (116987), Pusa Ruhy (136576), Birganj Local (136592), First in field (32993), Heinz 1548 (33018), June pink (33033), Ponderosa (33034), Pope (33137), Row pac (33144), Rutgers FM (33148), Rutgers PS (33149), Sekeo (33153), Sioux (33162), Supermarket (33167), Tamu saladette (33168), Anahu R-2 (32885), Best of All (32891), Burpee's Marglobe (32896), Burpee's Big Boy Hybrid (181221), Mepuguan (199443), Bunhong 18 (199576), White skin (33212), Y13-152A-10-64 (33213), Oregon cherry (33216), KL2 (33038), LA1441-70L-17-2 (33060), NFR-3 (33091), Heungjin 4 (33101), Pieraline (33219) , BP11981 (203274) , VF145 FS (33206) , VF145 GUS (33207), 85-Ha-1 (142196), ACC50 (201641), VF Gardener (33209), Ventura 'pat' (33218), and CL607-1-0 TT 151 (33220).

[y]genotypes with IT numbers are present in RDA genebank. R: genotypes were the same with resistance test results; S: genotypes were the same with susceptibility test results.

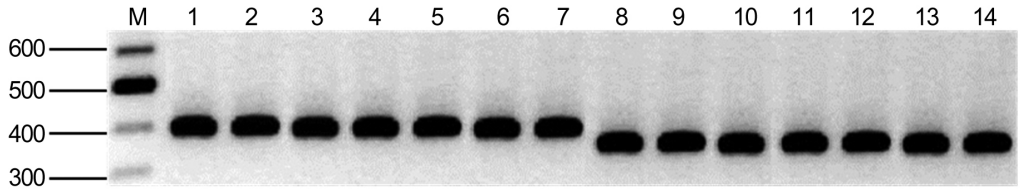

Fig. 5. PCR products of SCAR marker SCU176-534. Lanes M, 100 bp molecular ladder, 1, resistant parent H7996; 2-7, resistant RILs: RIL#26, 32, 41, 74, 162, and 200, respectively; 8, susceptible parent WVa700; 9-14, susceptible RILs: RIL#30, 79, 158, 170, 182, and 183, respectively.

complicated by interaction between the plant genotype and pathogen strains as well as the effects of the environment on resistance expression (Hayward, 1991). Resistance to bacterial wilt in tomato cultivar H7996 has been studied and reported to be a stable resistance source (Wang et al., 1998). The mode of inheritance of resistance in H7996 could vary depending on strains and inoculation methods used according to previous studies (Carmeille et al., 2006; Grimault et al., 1995; Mangin et al., 1999; Thoquet et al., 1996; Truong, 2007; Truong et al., 2008; Wang *et al.*, 2000). Progenies derived from a cross between the resistant cultivar H7996 and the susceptible WVa700 have been used for studying the genetic control of the resistance in H7996, and common QTLs associated with the resistance were detected on chromosome 6 in all studies used the same cross (Carmeille et al., 2006; Geethanjali et al., 2010; Mangin et al., 1999; Thoquet et al., 1996; Truong, 2007; Wang et al., 2013; Wang et al., 2000). Furthermore, one QTL on chromosome 12 was suggested to be specific to strain Pss4 (Wang et al., 2000). Recently, another QTL associated with the stable resistance in H7996 was detected on chromosome 12 (Wang et al., 2012).

Mejia et al. (2009) reported six RILs presented sequences of two markers on chromosome 6 and one marker on chromosome 12, which were associated with bacterial wilt resistance. In this study we used these resistant RILs to create the resistant DNA bulk to screen RAPD markers associated with bacterial wilt resistance. Thus, at these loci, resistant lines such RIL-162 and RIL-26 could have inherited the susceptible parent's WVa700 allele. Resistant inbred line presenting the susceptible parent (WVa700) sequence associated with bacterial wilt resistance on chromosome 12 have been reported (Mejía et al., 2009).

Two fragments generated from markers UBC#176 were selected to convert into SCAR marker. Fragment generated from markers UBC#317 was not chosen for conversion into SCAR marker because it was greater than 2.5 kb and is not easy to detect deletion/insertion. The UBC#176 fragments were successfully cloned and sequenced. 534 and 1190-bp sub-clones of UBC#176 fragments were identified and matched 99% of sequence of SL2.30ch06. Thus, this marker belongs to chromosome 6 of tomato. Interestingly, five regions in SL2.30ch06 matched parts of 534-base-pair sub-clone sequence. Of these, only regions 3374762-3375290, 3340141-3340427 covered 99% and 98%, respectively. Whereas, eight regions in SL2.30ch06 matched the 1190-base-pair sub-clone of UBC#176, but only one region 35280001-35282300 covered 99.6%, and the remained only 6-7%. Thus, 534-base-pair sub-clone sequence could be belonging to a family of genes located on chromosome 6. In previous studies, QTLs linked to bacterial wilt resistance already were identified along large segment on chromosome 6 (Thoquet et al., 1996; Wang et al., 2000; Carmeille et al., 2006, Truong, 2007, Wang et al., 2013). Further studies using near-isogenic lines would be necessary to fine-map the QTLs in this region. Therefore, much more new polymorphic new markers linked to the trait are needed in this region on chromosome 6. The 534-bp fragment was successfully converted into a codominant SCAR marker, but not 1190-bp fragment. The new SCAR marker SCU176-534 generated about 400 bp from resistant parent and about 370 bp from susceptible parent. The failure of the SCAR marker derived from 1190-bp sub-clones of RAPD marker UBC#176 to produce polymorphism could be caused by mismatches in nucleotide in the priming sites as reported in previous studies (Gupta et al., 2006; Horejsi et al., 1999; Paran and Michelmore, 1993; Truong et al., 2013a).

In previous studies, several PCR-based markers associated with bacterial wilt have been identified (Mejía et al., 2009; Miao et al., 2009). We have screened our materials using two SCAR markers $TSCAR_{AAT/CGA}$ and $TSCAR_{AAG/CAT}$ (Miao et al., 2009). However, there was no polymorphism (data not shown). The six RAPD markers and the new SCAR marker were used to genotype 92 tomato accessions from RDA genebank; however, none of them carries the resistant allele of the marker. Thus, tomato line (TRxVC11-2)-9-1F4 exhibiting no wilting was just escaped in the screening trial, what is in agreement with the fact that none accession carried resistance allele. Thus, the markers developed in this study will be useful for early selection of tomato germplasm for resistance to *R. solanacearum* in breeding programs.

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