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Research Paper

Development of diagnostic molecular markers for marker-assisted breeding against bacterial wilt in tomato

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Bacterial wilt, caused by the *Ralstonia pseudosolanacearum* species complex, is an important vascular disease that limits tomato production in tropical and subtropical regions. Two major quantitative trait loci (QTL) of bacterial wilt resistance on chromosome 6 (*Bwr-6*) and 12 (*Bwr-12*) were previously identified in *Solanum lycopersicum* 'Hawaii 7996'; however, marker-assisted breeding for bacterial wilt resistance is not well established. To dissect the QTL, six cleaved amplified polymorphic sites (CAPS) and derived CAPS (dCAPS) markers within the *Bwr-6* region and one dCAPS marker near *Bwr-12* were developed, and resistance levels in 117 tomato cultivars were evaluated. Two markers, RsR6-5 on chromosome 6 and RsR12-1 on chromosome 12, were selected based on the genotypic and phenotypic analysis. The combination of RsR6-5 and RsR12-1 effectively distinguishes resistant and susceptible cultivars. Furthermore, the efficiency of the two markers was validated in the F_3 generation derived from the F_2 population between E6203 (susceptible) and Hawaii 7998 (resistant). Resistant alleles at both loci led to the resistance to bacterial wilt. These markers will facilitate marker-assisted breeding of tomato resistant to bacterial wilt.

Key Words: tomato, bacterial wilt, polygenic resistance, molecular marker, single nucleotide polymorphism, marker-assisted breeding.

Introduction

Bacterial wilt, caused by *Ralstonia solanacearum*, is one of the most destructive diseases that affects many plant species. The *R. solanacearum* species complex (*RSSC*) has been classified into races, biovars, and phylotypes based on host range, carbon source usage, 16S-23S rRNA gene sequence of the strains, respectively (Cho *et al.* 2018, Hayward 1991, Jeong *et al.* 2007). Genomic analysis and proteomic profiling of various strains of the pathogen collected from different countries classified *RSSC* into three species: *R. solanacearum* (Phylotype II), *R. pseudosolanacearum* (Phylotypes I and III), and *R. syzygii* (Phylotype IV). Further genomic analysis of the species classified *R.syzygii* into three subspecies named *syzygii*, *indonesiensis*, and *celebesensis* (Prior *et al.* 2016).

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The pathogen has a wide host range. Tomato and other *Solanaceae* plants are major hosts. The disease threatens the cultivation of these crops in tropical and subtropical regions and heated greenhouses in temperate regions because high temperatures are better suited for the pathogen and disease development. As a result, the pathogen causes substantial economic losses (Hayward 1991, Lopes and Rossato 2018). The pathogen moves into plant roots via natural openings, such as hydathodes, or damaged areas and proliferates in the xylem tissues. It then damages the xylem tissues and blocks the water flow, leading to the total collapse and death of susceptible plants (Bae *et al.* 2015, Lowe-Power *et al.* 2018). Xylem colonization and spread are necessary for bacterial wilt disease progress because mutations in xylem colonization rendered pathogen strains incapable of causing wilting in tomato plants (Schell 2000). Evaluation of core collections of the three fruit vegetables of *Solanaceae* crops (tomato, eggplant, and pepper) against different strains of the pathogen showed that resistance of tomato collections is low compared with eggplant and pepper (Lebeau *et al.* 2011).

Different control strategies, such as chemical, biological,

and cultural practices, can reduce bacterial wilt severity, but none of them are effective (Yuliar *et al.* 2015). Development and use of resistant cultivars is the most effective approach to control bacterial wilt (Abebe *et al.* 2016, Huet 2014, Scott *et al.* 2005, Wang *et al.* 2018). Breeding for bacterial wilt-resistant cultivars has been challenging due to the polygenic nature of resistance, broad host range, variability of the pathogen strains, and effect of environmental factors that directly influence the phenotypic expression of the disease (Danesh *et al.* 1994, Fegan and Prior 2005, Hayward 1991, Lee *et al.* 2011, Thoquet *et al.* 1996b, Tran and Kim 2010). Moreover, the resistance locus is linked to undesirable horticultural traits (Scott *et al.* 2005). *Solanum lycopersicum* 'Hawaii 7996' (hereafter Hawaii 7996) is a stable resistant resource against bacterial wilt across various geographic locations and different bacterial strains with the highest average survival rate of 97% (Wang *et al.* 1998). Analysis of bacterial wilt resistance using F_2, F_3, and recombinant inbred line (RIL) populations derived from a cross between Hawaii 7996 (resistant) and West Virginia 700 (susceptible) identified QTL on chromosomes 4, 6, and 11 (Thoquet *et al.* 1996a); 3, 4, 6, 8, and 10 (Thoquet *et al.* 1996b); 6 (Mangin *et al.* 1999); 6, 8, and 12 (Wang *et al.* 2000); 3, 4, and 6 (Carmeille *et al.* 2006); and 3, 6, and 12 (Wang *et al.* 2013). Among them, a QTL on chromosome 6 (*Bwr-6*) was stable when measured with different phenotyping criteria (area under disease progress curve and bacterial colonization), in different bacterial strains (race 3-phylotype II and race 1-phylotype I), and as measured in two different seasons (hot and cold) (Carmeille *et al.* 2006). *Bwr-12* was an active QTL specifically against Pss4 (race 1, biovar 4) (Wang *et al.* 2000). QTL on chromosome 6 (*Bwr-6*) and 12 (*Bwr-12*) are thought to be responsible for the stable resistance of Hawaii 7996. *Bwr-12* covers 2.8 cM between the SSR markers SLM12-12 and SLM12-2, controlling more than 50% of the phenotypic variation in some trials. *Bwr-6* was localized between SLM6-124 and SLM6-110 covering 15.5 cM and controlling up to 22.2% of the phenotypic variation (Wang *et al.* 2013). Although two QTL, *Bwr-6* and *Bwr-12*, were repeatedly confirmed as major contributors to bacterial wilt resistance in Hawaii 7996, the genetic nature of these critical QTL remained unidentified. We have previously conducted whole-genome resequencing of two susceptible cultivars (Heinz 1706 and BWS-3) and seven resistant cultivars (Hawaii 7996, Hawaii 7998, 10-BA-3-33, 10-BA-4-24, BWR-1, BWR-22, and BWR-23) of tomato and identified genome-wide single nucleotide polymorphisms (SNPs) in resistant and susceptible groups of cultivars (Kim *et al.* 2018). The highest number of polymorphic SNPs in coding regions were found on chromosome 12 (168 SNPs) followed by chromosome 6 (53 SNPs). These SNPs might be associated with resistance to bacterial wilt. Based on the SNP information generated by re-sequencing, an HRM marker (KHU-1) that is tightly linked to *Bwr-12* was developed; however, no tightly linked SNP-based molecular marker was developed to trace *Bwr-6* resistance due to the large interval (~12.7 Mbp) (Kim *et al.* 2018).

In this study, we further analyzed the *Bwr-6* region to dissect and develop a diagnostic molecular marker for this important QTL. Cleaved amplified polymorphic site (CAPS) and derived CAPS (dCAPS) markers were developed within *Bwr-6* and screened for their diagnostic potential using 117 tomato genotypes. Phenotypic and genotypic analysis using a wide range of germplasms enabled us to select RsR6-5 as a diagnostic marker for *Bwr-6* among the newly developed markers in the region. Consequently, this marker, in combination with RsR12-1, effectively distinguished bacterial wilt-resistant and wilt-susceptible tomato cultivars. The newly developed marker RsR6-5 together with RsR12-1 will promote marker-assisted breeding of tomato by targeting two major resistance QTL against bacterial wilt.

Materials and Methods

Plant materials

In total, 117 tomato cultivars were collected (either seed or genomic DNA sample) from different sources, including the Tomato Genetics Resource Center (TGRC), UC Davis; Kyung Hee University, Korea; National Agrobiodiversity Center (RDA-Genebank), Korea; and various commercial seed companies in Korea. The phenotype of 27 cultivars was confirmed by inoculation test in this study, 12 cultivars were inferred from previous reports and the phenotype of 78 cultivars was received from the respective company/supplier along with the genomic DNA sample (**Table 1**). Seeds were first sown in Petri dishes for germination, and germinated seeds were transferred to 128 cell seedling trays "(28 × 28 × 40, bottom 15 mm)" filled with bio mix (JM bio, Korea). The seedlings were grown in the Agricultural Experiment Station of Kyungpook National University in a glasshouse at an average temperature of 25–28°C and 16–8 h light-dark cycles. The seedlings were moved from the glasshouse to the growth chamber 3–4 days before inoculation for acclimatization to growth chamber conditions where they were kept post-inoculation. Four-week-old seedlings were used for inoculation.

Table 1. Summary of the phenotypic composition of tomato cultivars used in this study

Phenotyping	Number of cultivars	Bacterial wilt phenotype	
		Resistant	Susceptible
This study	27	11	16
Previous report	12	4	8
Company/supplier	78	2	76
Total	117	17	100

Disease evaluation of tomato cultivars for bacterial wilt resistance

R. pseudosolanacearum strain SL882, classified as race 1, biovar 4, and phylotype I (Lee *et al.* 2011), was cultured on casamino acid-peptone-glucose (CPG) medium (casamino acid, 1 g; peptone, 10 g; glucose, 5 g; and agar, 15 g per liter of distilled water) and incubated at 28°C for 48 h (Kelman 1954). The bacterial culture from the Petri dish (90 × 15 mm) was rinsed with distilled water and washed using a cotton swab to make the inoculum suspension and its concentration was adjusted to approximately 10^8 CFU/ml (OD_{600}~0.1) using a NanoDrop 2000/UV-Vis spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA).

Seedlings at one month after sowing were inoculated by dipping the roots in the bacterial suspension (Caldwell *et al.* 2017). Each seedling was grown in a 128-cell seedling tray (28 × 28 × 40, bottom 15 mm) was pulled out, and its roots were dipped in the bacterial suspension. The inoculated seedlings were transplanted into 50-cell seedling trays (45 × 45 × 50, bottom 32 mm) and kept in a growth chamber (temperature = 28°C, relative humidity = 70%, and 16–8 h light-dark cycles). The disease severity was evaluated based on a disease scale of 0 to 4, where 0 = no visible symptoms; 1 = 25% of leaves wilting; 2 = 50% of leaves wilting; 3 = 75% leaves wilting; 4 = all foliage is wilted, and the plant dies (Morel *et al.* 2018). The disease scale was determined based on visual observation of the degree of wilting. The average value of disease severity for ten plants was calculated per each line. Cultivars with mean disease severity scores of <2 were classified as resistant, while those with scores >2 were classified as susceptible to bacterial wilt.

Genomic DNA extraction

Genomic DNA of 27 cultivars which were phenotyped in this study was extracted from young leaf tissues with a modified cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson 1980). The concentration and quality of genomic DNA were measured using NanoDrop 2000/UV–Vis spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA).

Sequence analysis of Bwr-6 region and marker development

Non-synonymous SNPs between resistant and susceptible groups of tomato cultivars near *Bwr-6* within 15 candidate genes (18 SNPs) were previously identified by whole-genome resequencing (Kim *et al.* 2018). Each candidate gene contained one non-synonymous SNP except two genes, *Solyc06g051110.1* and *Solyc06g051140.2*, which have two and three SNPs, respectively. The nucleotide changes between bacterial wilt-resistant and wilt-susceptible groups of tomato varieties for these SNPs, along with the respective amino acid changes, are presented in **Table 2**. To dissect *Bwr*-6 and develop diagnostic markers, selected SNPs were converted to CAPS/dCAPS markers. Based on six non-synonymous SNPs located at 24669159, 34389374, 34399541, 35950028, 37049726, and 37186202, respectively) in *Bwr-6* (Kim *et al.* 2018), five CAPS (RsR6-1~RsR6-5) and one dCAPS (RsR6-6) markers were developed. An HRM molecular marker (KHU-1),

Table 2. List of candidate genes containing non-synonymous SNPs near *Bwr-6* and *Bwr-12*, adapted from Kim *et al.* (2018). SNPs indicated in bold were used to develop CAPS/dCAPS markers in this study

Candidate gene	SNP position (bp)	Nucleotide change		Amino acid change		Gene annotation (ITAG2.4)
		Susceptible	Resistant	Susceptible	Resistant	
Solyc06g035530.2	24,482,686	T	C	F	L	Gibberellin 20-oxidase-2
Solyc06g035620.2	24,667,815	A	G	Y	C	Scarecrow-like 1 transcription factor
Solyc06g035630.1	**24,669,159**	**T**	**C**	**L**	**P**	**GRAS family transcription factor**
Solyc06g036060.2	25,438,944	A	G	I	V	Zinc finger family protein
Solyc06g048580.1	31,287,788	T	C	L	S	Unknown protein
Solyc06g051110.1	34,213,688	G	T	L	F	Unknown protein
Solyc06g051110.1	34,214,085	C	T	A	V	Unknown protein
Solyc06g051140.2	34,285,452	A	C	E	A	Ubiquitin-conjugating enzyme 22
Solyc06g051140.2	34,285,469	G	A	M	I	Ubiquitin-conjugating enzyme 22
Solyc06g051140.2	34,285,734	C	A	A	D	Ubiquitin-conjugating enzyme 22
Solyc06g051150.1	34,291,655	C	A	L	I	Pentatricopeptide repeat-containing protein
Solyc06g051190.1	**34,389,374**	**A**	**G**	**T**	**A**	**RNA-dependent RNA polymerase family protein**
Solyc06g051210.2	**34,399,541**	**C**	**T**	**P**	**S**	**Bromodomain-containing protein**
Solyc06g053210.2	**35,950,028**	**T**	**C**	**X**	**Q**	**Ubiquitin | Homeobox leucine zipper protein**
Solyc06g054000.1	36,868,039	G	A	V	I	Unknown protein
Solyc06g054200.1	37,021,520	A	G	R	S	Calmodulin protein kinase
Solyc06g054230.2	**37,049,726**	**T**	**G**	**D**	**E**	**Calmodulin protein kinase**
Solyc06g054400.2	**37,186,202**	**T**	**C**	**I**	**T**	**Translation initiation factor**
Solyc12g009690.1	**2,941,301**	**A**	**G**	**H**	**R**	**LRR receptor-like serine/threonine-protein kinase**

Table 3. DNA marker information used in this study

Marker name	SNP position (bp)	Marker type	Primer sequence (5′→3′)	T_m (°C)	Restriction enzyme	Expected size (bp)	
						Susceptible	Resistant
RsR6-1	24,669,159	CAPS	F: GGAAATATTGGTTACAATCCAGTG	57.5	*Mnl*I	227	173, 54
			R: GAATACAACAAATCACTACCGGTC	59.3			
RsR6-2	34,389,374	CAPS	F: CTTCTTGATAGGACGACGTGATAT	59.3	*Rsa*I	87, 116	203
			R: CAATCAACGGATCACCCATTTTTC	59.3			
RsR6-3	34,399,541	CAPS	F: CTCTTTTTGCCAGATCTTGAATAG	57.5	*Mnl*I	214	116, 98
			R: CCATAGGTCAGCATCAAATTTCAA	57.5			
RsR6-4	35,950,028	CAPS	F: GTTTTCCTTGCAAATCATTTTGGC	57.5	*Mse*I	116, 97	213
			R: GTATATGTTGAGTTCACAATTCCC	57.5			
RsR6-5	37,049,726	CAPS	F: CTCAGAAACTGGATAAACTCGAAG	59.3	*Hinf*I	204	129, 75
			R: GGAGAAAGCAGCCAGCCATTTTT	60.6			
RsR6-6	37,186,202	dCAPS	F: CGGTGATGAGCAGGATTGATAAAA	59.3	*Hpy*CH4III	234	200, 34
			R: AGTCTTGGCCTTTGACGTGAAAGTGACACAAGAAG	60.6			
RsR12-1	2,941,301	dCAPS	F: GTTACACGAACAAGCTTAAATTTCTAGATTTATCCC	58.8	*Aci*I	203	168, 35
			R GTAATCAATTCGAAGGACCTGTC	64.9			

tightly linked to bacterial wilt resistance on chromosome 12, was developed based on an SNP (A/G) located at 2,941,301 bp (Kim *et al.* 2018). We converted this HRM marker, KHU-1, to a dCAPS marker (RsR12-1) to trace *Bwr-12* in this study (**Table 3**). The sequence of the target genes was retrieved from the Sol Genomics Network (https://solgenomics.net/). dCAPS finder 2.0 (http://helix.wustl.edu/dcaps/dcaps.html) NEBcutter (http://nc2.neb.com/NEBcutter2/) were used to find the appropriate restriction enzymes for the respective SNP site.

PCR amplification and gel electrophoresis

PCR reactions were carried out according to the manufacturer's instructions (SolGent Co., Ltd., Daejeon, Korea) in a total volume of 25 µl containing 1 µl genomic DNA, 2.5 µl 10X *e-Tag* reaction buffer, 0.5 µl of 10 mM dNTP mix, 1 µl of each forward and reverse primers, 0.125 µl Solg *e-Taq* DNA polymerase, and 18.875 µl of ddH_2O. PCR amplification was carried out using a Bio-Rad T100 thermocycler (Bio-Rad Laboratories, Inc.) with the following conditions: denaturing for 3 min at 95°C, followed by 34 cycles of 30 s at 95°C denaturation, 30 s at annealing temperature (which varied for different primer sets (**Table 3**)), 1 min at 72°C extension, and a final elongation step at 72°C for 5 min. PCR products were digested with the respective restriction enzymes. The reaction mixture consisted of 5 µl template PCR product, 1 µl reaction buffer, 0.1 µl of restriction enzyme, and 3.9 µl ddH_2O. The mixture was incubated at 37°C for 16 hrs and was carried out using Bio-Rad T100 thermal cycler (Bio-Rad Laboratories, Inc.). The digested product was mixed with 2 µl 6X DNA loading buffer and subjected to gel electrophoresis on a 3% agarose gel to visualize the polymorphic DNA bands. The details of CAPS/dCAPS marker information, including primer sequences, restriction enzymes, and the expected band sizes for resistant and susceptible tomato groups, are presented in **Table 3**.

Selection of F_3 and marker validation

To confirm the efficiency of the two QTLs, F_2 generation developed from a cross between E6203 (susceptible) and Hawaii 7998 (resistant) were screened with two markers RsR6-5 (*Bwr-6*) and RsR12-1 (*Bwr-12*). Ten F_2 plants homozygous for both markers (five resistant and five susceptible), four F_2 plants harboring only RsR6-5, and four F_2 plants harboring only RsR12-1 were selected for harvesting the F_3 seeds. The F_3 generation were inoculated and evaluated for disease resistance. Ten plants of each F_3 progeny were inoculated. The resistant and susceptible parents were included as controls during disease evaluation. The mean disease severity of ten plants was used to designate the resistance level of each progeny. Furthermore, the markers were evaluated for their diagnostic value using tomato cultivars (Yang *et al.* 2015).

Results

Phenotyping of tomato cultivars for bacterial wilt resistance

The resistance level of 27 cultivars against bacterial wilt disease was inoculated and confirmed in this study. Hawaii 7996, B-Blocking, Shincheonggang, BWR-20, Spider, High Power, 10-BA-3-33, 10-BA-4-24, IT 201664, Hawaii 7998, and Fighting were resistant with low mean disease severity scores (<2) while UC-134, LA1589, Purple Calabash, Florida8516, Heinz 1706, A-1, E6203, Moneymaker, Super Dotaerang, Anahu, Red Strong, Bluck Plum, Gold Nugget, VF-36, M82, and Dotaerang Red were susceptible with a mean disease severity score of >2.0 (**Fig. 1**). Previously reported susceptible cultivars, such as Moneymaker, Heinz 1706, and Super Dotaerang (Han *et al.* 2009, Kim *et al.* 2018), showed the highest mean disease severity indicating the presence of adequate disease pressure on inoculated plants. In addition, phenotypic information of 12 cultivars was inferred from previous reports and 78 cultivars were received from respective companies. In total, the

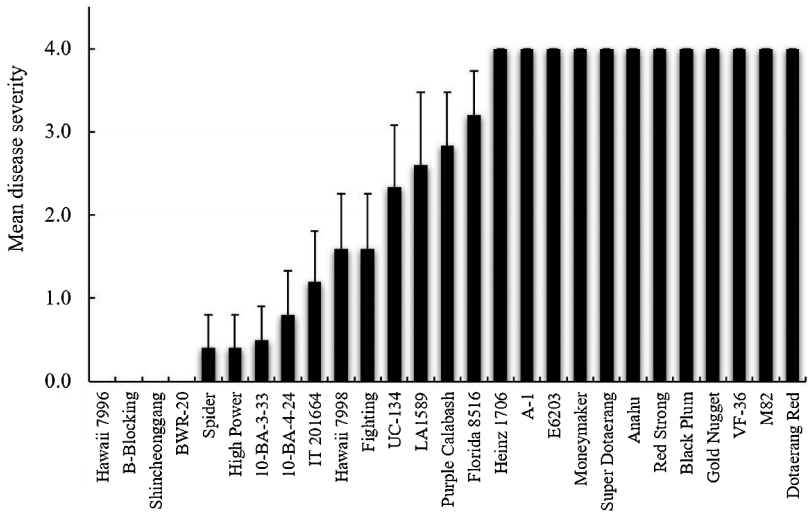

Fig. 1. Mean disease severity of tomato cultivars infected with *R. pseudosolanacearum* strain SL882, one month after inoculation. The disease severity was scored based on the disease scale of 0 to 4, where 0 = no visible symptoms; 1 = 25% of leaves wilting; 2 = 50% of leaves wilting; 3 = 75% of leaves wilting; and 4 = all foliage is wilted, and the plant dies.

collection includes 17 resistant and 100 susceptible cultivars (**Table 1**).

Screening of the SNP markers using phenotypic data of tomato cultivars

The primer sets were tested for polymorphism using six susceptible and five resistant cultivars. All primer sets resulted in a clear polymorphic band between susceptible and resistant tomato cultivars after enzyme digestion (**Fig. 2**). For selecting an accurate and reliable marker for *Bwr-6*, six markers (RsR6-1~6-6) were screened using 117 tomato cultivars (**Table 4**). The *Bwr-12* genotype of these cultivars was determined using RsR12-1. Hawaii 7996, Hawaii 7998, 10-BA-3-33, 10-BA-4-24, BWR-20, BWR-1, BWR-22, and BWR-23 had homozygous resistant genotypes with all six markers near *Bwr-6*. IT201664, High Power, Spider, SVTX6258, Super High Power, B-Blocking, Shincheonggang, Fighting, and Geumgang are either susceptible or heterozygous to RsR6-1, RsR6-2, and RsR6-3 while they are resistant to RsR6-4, RsR6-5, and RsR6-6 except for High Power, which is heterozygous to RsR6-4 and RsR6-5. Comparing the six markers based on the genotype of resistant cultivars, RsR6-1, RsR6-2, and RsR6-3 did not seem better candidate markers for *Bwr-6* because IT 201664, High Power, Spider, and Super High Power are susceptible to these markers.

Therefore, we considered RsR6-4, RsR6-5, and RsR6-6 for further analysis using the susceptible set of cultivars. Almost all bacterial wilt-susceptible cultivars had susceptible or heterozygous genotypes to RsR6-5 except Gold Sugar, Sinheukjinju, SV7160TC, and LA1589. Red Strong shows the resistant genotype to RsR6-4 and the susceptible genotype to RsR6-5. Cultivars resistant to RsR6-6 and heterozygous with RsR12-1, such as SV02444 TG, SV4224 TH, and SV0339TG, are expected to be resistant to bacterial wilt, but all were susceptible. In addition, Red Strong and SkyBall have homozygous resistant genotypes with RsR6-6 and RsR12-1, although both exhibit susceptible phenotype (**Table 4**).

The presence of resistant alleles in both *Bwr-6* and *Bwr-12* resulted in resistant phenotype while the absence of either of the two resulted in susceptible phenotype. This marker analysis indicated that cultivars homozygous resistant to RsR6-5 and either a homozygous resistant

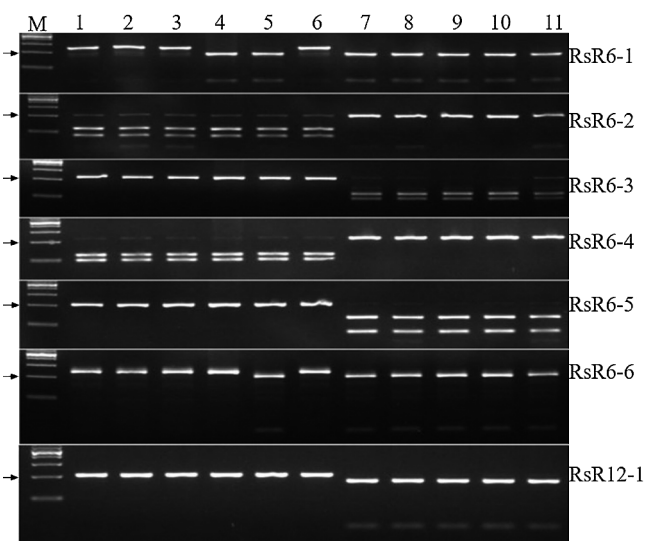

Fig. 2. Marker analysis of the newly developed CAPS/dCAPS in 11 tomato genotypes near *Bwr-6* (RsR6-1~RsR6-6) and *Bwr-12* (RsR12-1). Lanes 1 to 6 represent the bacterial wilt-susceptible group (M82, E6203, UC-134, VF-36, Gold Nugget, and Moneymaker) and lanes 7 to 11 represent the bacterial wilt-resistant group (Hawaii 7996, Hawaii 7998, 10-BA-3-33, 10-BA-4-24, and BWR-20). M, 1 kb DNA size marker. The arrows on the left indicate a 200 bp standard fragment.

Table 4. SNP marker genotype of resistant and susceptible tomato cultivars or lines used in this study

No.	Tomato cultivar/line	Type	Company/Supplier[a]	SNP marker genotype[b]							Reference[c]
				RsR6-1	RsR6-2	RsR6-3	RsR6-4	RsR6-5	RsR6-6	RsR12-1	
Bacterial wilt resistant											
1	Hawaii 7996	Inbred line	KHU	R	R	R	R	R	R	R	This study
2	Hawaii 7998	Inbred line	KHU	R	R	R	R	R	R	R	This study
3	10-BA-3-33	Inbred line	KHU	R	R	R	R	R	R	R	This study
4	10-BA-4-24	Inbred line	KHU	R	R	R	R	R	R	R	This study
5	BWR-20	Inbred line	KHU	R	R	R	R	R	R	R	This study
6	BWR-1	Inbred line	KHU	R	R	R	R	R	R	R	Kim *et al.* (2018)
7	BWR-22	Inbred line	KHU	R	R	R	R	R	R	R	Kim *et al.* (2018)
8	BWR-23	Inbred line	KHU	R	R	R	R	R	R	R	Kim *et al.* (2018)
9	IT 201664	Inbred line	RDA	H	S	S	R	R	R	R	This study
10	High Power	F_1 hybrid	Dae Yeon seed co.	H	S	S	H	H	R	R	This study
11	Super High Power	F_1 hybrid	Dae Yeon seed co.	S	S	S	R	R	R	R	Kim *et al.* (2018)
12	Spider	F_1 hybrid	Takii Korea	S	S	S	R	R	R	R	This study
13	B-Blocking	F_1 hybrid	Takii Korea	H	H	H	R	R	R	H	This study
14	Shincheonggang	F_1 hybrid	Farm Hannong	H	H	H	R	R	R	H	This study
15	Fighting	F_1 hybrid	Takii Korea	H	H	H	R	R	R	H	This study
16	SVTX6258	F_1 hybrid	Monsanto Korea	H	H	H	R	R	R	R	Supplier
17	Geumgang	F_1 hybrid	Monsanto Korea	H	H	H	R	R	R	H	Supplier
Bacterial wilt susceptible											
18	M82	Inbred line	TGRC	S	S	S	S	S	S	S	This study
19	E6203	Inbred line	TGRC	S	S	S	S	S	S	S	This study
20	VF36	Inbred line	TGRC	R	S	S	S	S	S	S	This study
21	Moneymaker	Inbred line	TGRC	R	S	S	S	S	S	S	This study
22	A-1	Inbred line	TGRC	S	S	S	S	S	S	S	This study
23	Anahu	Inbred line	TGRC	S	S	S	S	S	R	S	This study
24	Black Plum	Inbred line	TGRC	S	S	S	R	S	R	S	This study
25	Florida 8516	Inbred line	TGRC	S	S	S	S	S	S	S	This study
26	Gold Nugget	Inbred line	TGRC	R	S	S	S	S	R	S	This study
27	Purple Calabash	Inbred line	TGRC	H	H	S	H	S	R	S	This study
28	UC-134	Inbred line	TGRC	S	S	S	S	S	S	S	This study
29	Heinz 1706	Inbred line	TGRC	S	S	S	S	S	S	S	This study
30	New Yorker	Inbred line	TGRC	S	S	S	S	S	R	S	Jung *et al.* (2014)
31	Miniheuksu	F_1 hybrid	Asia Seed Co., Ltd.	R	S	S	S	S	R	S	Kim *et al.* (2018)
32	TY Unique	F_1 hybrid	Asia Seed Co., Ltd.	R	S	S	S	H	R	H	Supplier
33	Shinsugar Yellow	F_1 hybrid	Asia Seed Co., Ltd.	H	H	H	H	H	R	H	Supplier
34	Red Strong	F_1 hybrid	Bunong Seed	R	S	R	R	S	R	R	This study
35	Sun Star	F_1 hybrid	Bunong Seed	H	S	S	S	S	H	S	Supplier
36	Black Eagle	F_1 hybrid	Bunong Seed	R	S	H	H	S	S	H	Supplier
37	Tamla	F_1 hybrid	Bunong Seed	H	S	H	H	S	H	S	Supplier
38	Bntoskna	F_1 hybrid	Bunong Seed	H	S	S	H	S	H	H	Supplier
39	TY Izzang	F_1 hybrid	Bunong Seed	H	S	H	H	S	R	S	Supplier
40	Candy Plus	F_1 hybrid	Bunong Seed	H	S	H	H	S	H	S	Supplier
41	Super Star	F_1 hybrid	Bunong Seed	H	S	S	S	S	H	H	Supplier
42	Red Zenith	F_1 hybrid	Bunong Seed	H	S	H	H	S	R	H	Supplier
43	TY Hunter	F_1 hybrid	Bunong Seed	R	S	H	H	S	H	H	Supplier
44	TY One Top	F_1 hybrid	Bunong Seed	S	S	S	H	S	H	H	Supplier
45	Yureka	F_1 hybrid	Bunong Seed	S	S	H	H	S	H	H	Supplier
46	Black Ace	F_1 hybrid	Bunong Seed	R	S	S	S	S	S	H	Supplier
47	Oasis	F_1 hybrid	Bunong Seed	S	S	H	S	S	H	S	Supplier
48	TY Megaton	F_1 hybrid	Bunong Seed	S	S	S	S	S	R	S	Supplier
49	Gold Sugar	F_1 hybrid	Bunong Seed	R	S	H	H	R	R	S	Supplier
50	Dotaerang Red	F_1 hybrid	Dong seo seed	H	H	S	S	S	R	S	This study
51	Black Ball	F_1 hybrid	Dongoh Seed	R	S	H	H	S	H	H	Supplier
52	Kolmi	F_1 hybrid	Dongoh Seed	S	S	H	R	H	R	S	Supplier
53	Sky Ball	F_1 hybrid	Dongoh Seed	R	S	H	H	S	R	R	Supplier
54	Starbuck	F_1 hybrid	Farm Hannong	R	S	S	S	S	S	S	Supplier
55	Olleh TY	F_1 hybrid	Farm Hannong	S	S	H	H	S	R	S	Supplier
56	Rafito	F_1 hybrid	Farm Hannong	H	S	S	S	S	H	R	Supplier
57	Big Wonderful	F_1 hybrid	Gonong Seed	R	S	H	H	S	H	S	Supplier
58	TY Carnival	F_1 hybrid	Gyeongwon	R	S	H	H	S	H	H	Supplier
59	Legend Summer	F_1 hybrid	Haesung Seed Plus	S	S	S	S	S	R	S	Supplier
60	Daewang	F_1 hybrid	Jeil Seed Bio	H	S	S	S	S	S	S	Supplier

Table 4. (continued)

No.	Tomato cultivar/line	Type	Company/Supplier[a]	SNP marker genotype[b]							Reference[c]
				RsR6-1	RsR6-2	RsR6-3	RsR6-4	RsR6-5	RsR6-6	RsR12-1	
61	Hongboseok	F_1 hybrid	Jeil Seed Bio	S	S	S	S	S	S	S	Supplier
62	Jeilheukjinju	F_1 hybrid	Jeil Seed Bio	S	S	S	R	S	R	S	Supplier
63	Dotaerang Myeongpum	F_1 hybrid	Jeil Seed Bio	S	S	S	S	S	S	S	Supplier
64	Heukryong	F_1 hybrid	Jeil Seed Bio	S	S	S	S	S	S	H	Supplier
65	Minijaok	F_1 hybrid	Jeil Seed Bio	S	S	S	R	S	R	S	Supplier
66	Sinheukjinju	F_1 hybrid	Jeil Seed Bio	H	R	S	R	R	R	S	Supplier
67	Super Dotaerang	F_1 hybrid	Koregon seed	H	H	S	S	S	R	S	This study
68	Lezaforta F_1	F_1 hybrid	Mifko seed	S	S	S	S	S	H	S	Supplier
69	Unicorn	F_1 hybrid	Monsanto Korea	H	S	S	S	S	R	S	Supplier
70	SV 7160 TC	F_1 hybrid	Monsanto Korea	R	S	H	R	R	R	S	Supplier
71	SV02444 TG	F_1 hybrid	Monsanto Korea	S	S	H	S	S	R	H	Supplier
72	Bacchus	F_1 hybrid	Monsanto Korea	R	S	H	H	S	R	S	Supplier
73	SV4224 TH	F_1 hybrid	Monsanto Korea	S	S	H	S	S	R	H	Supplier
74	SV0339TG	F_1 hybrid	Monsanto Korea	S	S	H	S	S	R	H	Supplier
75	Tiara	F_1 hybrid	Nongwoo Bio Co., Ltd.	H	S	S	S	S	R	S	Kim *et al.* (2018)
76	TY SenseQ	F_1 hybrid	Nongwoo Bio Co., Ltd.	S	S	H	H	S	H	S	Supplier
77	Redpang	F_1 hybrid	Nongwoo Bio Co., Ltd.	S	S	S	S	S	R	S	Supplier
78	Titichal	F_1 hybrid	Nongwoo Bio Co., Ltd.	S	S	H	H	S	R	S	Supplier
79	TY Altorang	F_1 hybrid	Nongwoo Bio Co., Ltd.	S	S	H	H	S	R	S	Supplier
80	Beta Tiny	F_1 hybrid	Nongwoo Bio Co., Ltd.	S	S	S	H	H	H	S	Supplier
81	TY Tiny	F_1 hybrid	Nongwoo Bio Co., Ltd.	S	S	H	H	S	H	S	Supplier
82	Cupirang	F_1 hybrid	Nongwoo Bio Co., Ltd.	S	S	S	S	S	R	S	Supplier
83	Minichal	F_1 hybrid	Nongwoo Bio Co., Ltd.	S	S	S	H	H	H	S	Supplier
84	TY Sispen	F_1 hybrid	Nongwoo Bio Co., Ltd.	S	S	H	H	S	R	S	Supplier
85	Black Change	F_1 hybrid	Nongwoo Bio Co., Ltd.	H	S	H	H	S	H	H	Supplier
86	Mulya	F_1 hybrid	RDA	S	S	S	S	S	R	S	Kim *et al.* (2018)
87	Sigyo 1 ho	F_1 hybrid	RDA	S	S	S	S	S	R	S	Kim *et al.* (2018)
88	Broadley	F_1 hybrid	RDA	R	S	S	S	S	R	S	Kim *et al.* (2018)
89	Yulwon	F_1 hybrid	RDA	S	H	H	R	H	R	S	Kim *et al.* (2018)
90	Hoyong	F_1 hybrid	Sakata Korea	H	S	S	S	S	R	H	Kim *et al.* (2018)
91	Tosama	F_1 hybrid	Sakata Korea	S	S	S	H	S	H	S	Supplier
92	Super Sun Road	F_1 hybrid	Sakata Korea	H	S	S	H	S	H	H	Supplier
93	Super Top	F_1 hybrid	Sakata Korea	S	S	S	S	S	H	H	Supplier
94	Lokousan Maru	F_1 hybrid	Sakata Korea	S	S	S	S	S	H	S	Supplier
95	Taiyau	F_1 hybrid	Sakata Korea	S	S	S	S	S	H	H	Supplier
96	Taihu	F_1 hybrid	Sakata Korea	R	S	H	S	S	H	H	Supplier
97	Super Top	F_1 hybrid	Sakata Korea	S	S	S	S	S	H	H	Supplier
98	Tiger	F_1 hybrid	Samsung Seeds	H	S	S	S	S	R	S	Supplier
99	Chalstone TY	F_1 hybrid	Sky seed	S	S	H	H	S	R	H	Supplier
100	TY Marathon	F_1 hybrid	Sky seed	S	S	S	S	S	R	H	Supplier
101	Rapsody	F_1 hybrid	Syngenta Korea	H	S	S	H	S	S	S	Supplier
102	Madison	F_1 hybrid	Syngenta Korea	H	S	S	S	S	S	S	Supplier
103	Ricophin-9	F_1 hybrid	Syngenta Korea	H	S	S	H	H	R	S	Supplier
104	Duine	F_1 hybrid	Syngenta Korea	H	S	S	S	S	H	S	Supplier
105	Dafnis	F_1 hybrid	Syngenta Korea	S	S	H	H	S	H	H	Supplier
106	Komodo	F_1 hybrid	Syngenta Korea	S	S	H	H	S	H	H	Supplier
107	Tory	F_1 hybrid	Syngenta Korea	S	S	H	H	S	H	H	Supplier
108	Mamirio	F_1 hybrid	Syngenta Korea	R	S	H	H	S	H	H	Supplier
109	European Rapsodie	F_1 hybrid	Syngenta Korea	H	S	S	H	S	S	S	Supplier
110	Trio Plus	F_1 hybrid	Taeyang seed	R	S	H	H	S	R	H	Supplier
111	Kang Jeok	F_1 hybrid	Taeyang seed	R	S	H	H	S	H	S	Supplier
112	Dotaerang TY Winner	F_1 hybrid	Takii Korea	S	S	H	S	S	R	S	Supplier
113	Doterang Plus	F_1 hybrid	Takii Korea	S	S	S	S	S	R	S	Supplier
114	Dotaerang Solar	F_1 hybrid	Takii Korea	S	S	S	S	S	R	H	Supplier
115	Cuty	F_1 hybrid	Takii Korea	S	H	H	H	H	R	S	Supplier
116	Dotaerang Diamond	F_1 hybrid	Takii Korea	S	S	S	S	S	R	S	Supplier
117	LA1589	Wild species	TGRC	R	S	R	R	R	R	S	This study

[a] Company/supplier of seed or DNA sample: KHU = Kyung Hee University, RDA = National Agrobiodiversity Center (RDA-Genebank), TGRC = Tomato Genetics Resource Center. All tomato genotypes belong to *Solanum lycopersicum* species except LA1589 (*Solanum pimpinellifolium*).

[b] SNP marker genotype: R = resistant, S = susceptible, H = heterozygous.

[c] Reference for the phenotypic information of the tomato cultivars used in the study. Supplier's phenotypic information was obtained via personal communication from the company.

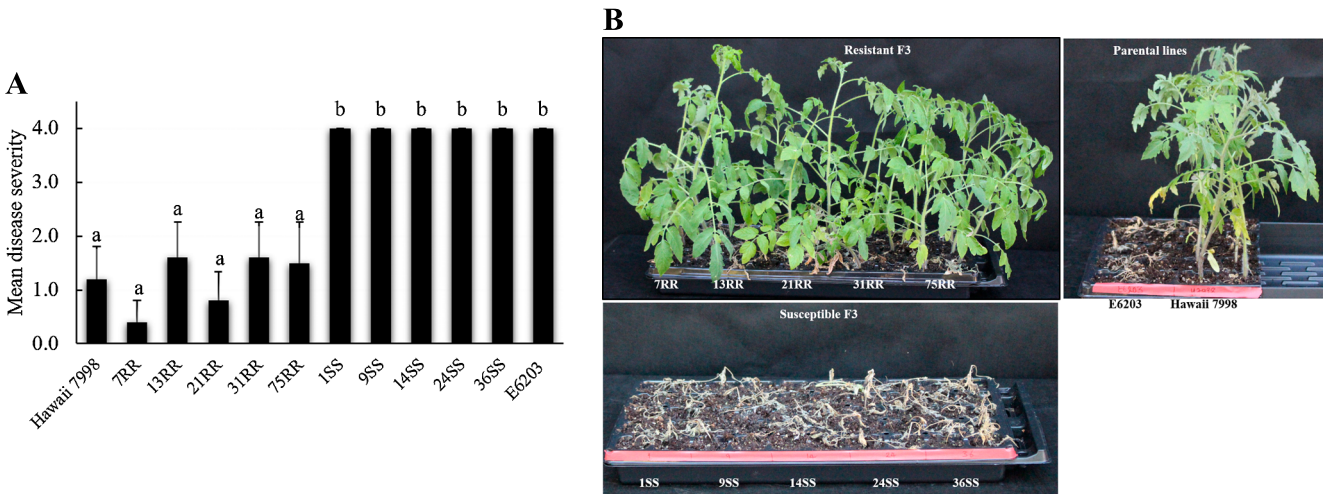

Fig. 3. Evaluation of F_3 generation selected by RsR6-5 and RsR12-1 for resistance to *R. pseudosolanacearum*. (A) Mean disease severity of F_3 generation developed from a crossing between E6203 and Hawaii 7998 one month after inoculation with *R. pseudosolanacearum* strain SL882. The genotypes of the F_3 lines are either homozygous resistant or susceptible at RsR6-5 and RsR12-1. The F_3 lines are followed by RR (carrying resistant alleles of both *Bwr6* and *Bwr-12*) or SS (carrying susceptible alleles at both loci). Different letters on the bars indicate a significant difference ($P < 0.05$) of mean disease severity. (B) Photographs of homozygous F_3 generation and parental lines one month after inoculation with SL882.

or heterozygous genotype to RsR12-1 exhibited a resistant phenotype. However, homozygous susceptible or heterozygous genotypes with RsR6-5 exhibited a susceptible phenotype regardless of the RsR12-1 genotype. One exception to this was High Power, which exhibits a resistant phenotype although it has a heterozygous genotype with RsR6-5. The genotype of RsR6-5 is highly correlated with the bacterial wilt phenotype of tomato cultivars and selected for tracing *Bwr-6*. The diagnostic accuracy of the markers was evaluated and RsR6-5 and RsR12-1 combination was resulted in 94.1% true positive rate and 100% true negative rate (**Supplemental Table 1**). Taken together, RsR6-5 and RsR12-1 should be used for effective marker-assisted selection of bacterial wilt resistance in tomatoes

Validation of the RsR6-5 and RsR12-1 markers using F_3 populations

To validate the efficiency of the two markers for selecting resistant lines in the segregating population, 10 F_3 generations homozygous resistant and susceptible (each five lines), and eight F_3 generation carrying only Bwr-6 or Bwr-12 (each four lines) were developed from E6203 (susceptible) and Hawaii 7998 (resistant) were selected based on RsR6-5 and RsR12-1 genotype. Hawaii 7998 is one of the entries in international set of bacterial wilt resistant lines evaluated in twelve fields and showed an average of 90% survival rate (Wang *et al.* 1998). The resistance of Hawaii 7998 and Hawaii 7996 was derived from the same origin, PI 127805A (*S. pimpinellifolium*) (Daunay *et al.* 2010, Scott *et al.* 2005). Hence, genetic resistance to bacterial wilt in these two lines might be governed by same gene.

Ten plants of each F_3 generation, along with the two parental lines, were inoculated with *R. pseudosolanacearum* strain SL882 for disease evaluation. The two parental lines exhibited distinct differences in bacterial wilt resistance as expected, with mean disease scores of 4.0 ± 0.0 and 1.2 ± 0.61, respectively. The mean disease severity between homozygous resistant and susceptible genotypes in the F_3 generation was significantly different (**Fig. 3A**). Lines with homozygous resistant genotypes exhibited highly resistant phenotypes with mean disease severity scores ranging from 0.4 ± 0.40 to 1.6 ± 0.65, which were not significantly different from that of the resistant parent. On the other hand, homozygous susceptible F_3 exhibited highly susceptible phenotypes with mean disease severity scores of 4.0 ± 0.0, which were similar to the susceptible parent (**Fig. 3A**, **3B**). F_3 progenies carrying resistant allele only in RsR6-5 or RsR12-1 were susceptible (**Supplemental Fig. 1**). These results suggest that the combination of RsR6-5 and RsR12-1, which are associated with *Bwr-6* and *Bwr-12*, respectively, are predictive for bacterial wilt resistance.

Discussion

Genetic resistance is the most effective control strategy for bacterial wilt of tomato, and multiple breeding programs have been engaged in developing resistant lines by incorporating resistance from different resistant sources (Daunay *et al.* 2010, Wang *et al.* 1998). Genomic regions linked to bacterial wilt resistance in the well-known resistant cultivar, Hawaii 7996, were identified on different chromosomes, and some regions were detected against specific strains (Thoquet *et al.* 1996a, 1996b, Wang *et al.* 2000). *Bwr-3* and *Bwr-4* were associated with resistance against phylotype II strains, while *Bwr-12* was specific to

Phylotype-I (Carmeille *et al.* 2006). In contrast, *Bwr-6* was associated with various strains from different phylotypes (I and II) (Thoquet *et al.* 1996a) and consistently detected under various conditions (Carmeille *et al.* 2006, Geethanjali *et al.* 2010, Mangin *et al.* 1999, Wang *et al.* 2013). Genetic analysis of bacterial wilt resistance in *S. lycopersicum var. cerasiforme* 'L285' identified three QTL on chromosomes 6, 7, and 10 (Danesh *et al.* 1994). The genomic regions associated with bacterial wilt resistance on chromosome 6 in both Hawaii 7996 and L285 were co-localized. Furthermore, the most stable QTL for bacterial wilt resistance in eggplant was also identified in chromosome 6 and syntenic with *Bwr-6* of tomato (Salgon *et al.* 2018). All these suggest the significance of *Bwr-6* in bacterial wilt resistance in tomato and likely in other Solanaceae crops. The development of functional markers for such broad-spectrum QTL is essential to facilitate marker-assisted breeding.

Genetic analysis using only segregating populations is time-consuming, cost-inefficient, and the developed markers may be specific to certain resistant lines (Pascual *et al.* 2016). In this regard, we used a complementary approach to dissect the *Bwr-6* region, using germplasm collections and F_3 generation to validate our result. SNP-based CAPS/dCAPS markers near *Bwr-6* were developed and validated. A total of 117 tomato germplasms were screened with newly developed markers for *Bwr-6* genotypes, and the corresponding phenotypic information was used to explore the efficiency of each marker.

Among 17 resistant cultivars used to screen the markers in the *Bwr-6* region, four cultivars (IT 201664, High Power, Super High Power, and Spider) had susceptible genotypes with RsR6-1, RsR6-2, and RsR6-3. On the other hand, all resistant cultivars had homozygous resistant genotypes with RsR6-4, RsR6-5, and RsR6-6 except High Power, which has a heterozygous genotype with RsR6-4 and RsR6-5. Similarly, all cultivars exhibiting resistant phenotypes have homozygous resistant genotypes with RsR12-1, except B-Blocking, Shincheonggang, Fighting, and Geumgang which are heterozygous to this marker. The true positive rate of RsR6-1, RsR6-2, and RsR6-3 combined with RsR12-1 was 47.1% whereas that of RsR6-4 and RsR6-5 was 94.1% and that of RsR6-6 was 100%. Based on these observations, we hypothesized that the three markers (RsR6-4, RsR6-5, and RsR6-6) are better predictors of resistance conferred by *Bwr-6* than RsR6-1, RsR6-2, and RsR6-3.

The markers were further compared using the susceptible panel of germplasms. Some cultivars exhibiting susceptible phenotypes, such as Red Strong, are resistant to RsR6-4, RsR6-6, and RsR12-1. However, all cultivars having homozygous resistant genotypes with RsR6-5 and RsR12-1 exhibited resistant phenotypes. In summary, 99% and 87% true negative rate was obtained for RsR6-4 and RsR6-6 while RsR6-5 resulted in 100% true negative rate genotypes in combination with RsR12-1 (**Supplemental Table 1**). These results suggest that RsR6-5 is the best diagnostic marker to trace *Bwr-6* associated with bacterial wilt resistance. Diagnostic markers developed based only on a segregating population may not fully correlate with the trait when tested in diverse germplasms, hindering their utilization for marker-assisted selection in a broad set of breeding germplasms (Niewohner *et al.* 1995). Therefore, utilization of a wide range of germplasms, including inbred lines, commercial F_1 hybrids, and wild species, for validating developed markers is essential before deployment to end-users, including breeders and farmers.

The diagnostic potential of RsR6-5 coupled with RsR12-1 for bacterial wilt resistance in tomato was tested using a broader set of germplasms (Bartkiewicz *et al.* 2018, Yang *et al.* 2015) and can be used for marker-assisted selection in commercial breeding programs. Without determining the genotyping results for another major resistance QTL, *Bwr-12*, determination of *Bwr-6* genotype with the RsR6-5 marker alone is able to predict resistant and susceptible phenotypes in the tomato cultivars used in this study with 94.1% and 96% accuracy, respectively. High Power, SV7160TC, Gold Sugar, Sinheukjinju, and LA1589 showed non-matching genotypes. On the other hand, *Bwr-12* genotyping determined by RsR12-1 alone was able to predict resistant and susceptible phenotypes with 100% and 66% accuracy, respectively. Resistance conferred by the *Bwr-12* genotype shows a dominant inheritance pattern (Kim *et al.* 2018). RsR6-5 and RsR12-1 combination was resulted in 94.1% of true positive rate and 100% true negative rate showing the highest diagnostic accuracy compared to other marker combinations.

Heterozygous to RsR6-5 and either heterozygous or homozygous resistant to RsR12-1 yielded susceptible phenotypes except for High Power, which suggests that the *Bwr-6* resistance allele might be recessive; however, this should be further validated using a segregating population. Recessive gene resistance to bacterial disease has been reported in Arabidopsis and rice. *R. solanacearum* resistance in *Arabidopsis thaliana* is governed by a recessively inherited gene (*RRS1-R*) (Deslandes *et al.* 2002). Similarly, among more than 43 resistance genes identified so far for bacterial blight in rice caused by *Xanthomonas oryzae* pv. *oryzae*, 16 are inherited as recessive traits (Kim 2018, Vikal and Bhatia 2017).

Extensive QTL mapping studies have been conducted to identify genomic regions associated with bacterial wilt resistance (Carmeille *et al.* 2006, Geethanjali *et al.* 2010, Mangin *et al.* 1999, Thoquet *et al.* 1996a, 1996b, Wang *et al.* 2000, 2013). The release of the tomato reference genome (Tomato Genome Consortium 2012) and availability of whole-genome resequencing data for various tomato cultivars (Lin *et al.* 2014, The 100 Tomato Genome Sequencing Consortium 2014) facilitated comparisons among tomato genotypes using genome-wide SNP markers for different traits. The adequate number of SNPs among tomato genotypes can be used to saturate markers nearby previously identified QTL regions. Accordingly, whole-genome

resequencing of bacterial wilt resistant and susceptible tomato cultivars revealed genome-wide SNPs that were candidates for distinguishing the two groups of tomato, with the highest number of non-synonymous SNPs identified on chromosomes 12 and 6 (Kim *et al.* 2018). Analysis of SNPs near *Bwr-12* in the same study discovered molecular marker (KHU-1) tightly linked to bacterial wilt resistance; this marker was used to discriminate resistant and susceptible tomato cultivars. The analysis of SNPs near *Bwr-6* and the development of diagnostic markers in this study will pave the way toward identifying candidate genes and facilitating resistance gene pyramiding.

RsR6-5 is located in the coding region of *Solyc06g054230.2*, which encodes a putative calmodulin protein kinase, suggesting that this gene could be a possible candidate gene for bacterial wilt resistance in tomato. The nucleotide substitution of guanine to thymine at 128 bp changes the amino acid aspartate to glutamate. The majority of plant disease resistance genes encode nucleotide-binding site-leucine-rich repeat (NBS-LRR) proteins (McHale *et al.* 2006). However, the role of calmodulin proteins in the response of plants to biotic and abiotic stresses has also been reported (Cheval *et al.* 2013, Zeng *et al.* 2015). Calmodulin is a calcium-binding protein and regulates downstream calcium signal-related responses. The expression of tomato calmodulin genes is significantly altered upon pathogen infection. Functional analysis revealed that the silencing of *SlCaM2* (*Solyc10g081170.1.1*) and *SlCaM6* (*Solyc03g098050.2.1*) in tomato reduced its resistance to tomato rattle virus and *Pythium aphanidermatum* and decreased the expression of downstream signaling and defense-related genes (Zhao *et al.* 2013). Transcriptome analysis of bacterial wilt-resistant (LS-89) and susceptible (Ponderosa) cultivars indicated an approximately 30-fold increase of a putative calmodulin-binding family protein in response to *R. solanacearum* infection in resistant cultivars, while the analogous response in susceptible cultivars was very limited (Ishihara *et al.* 2012). These findings suggest that *Solyc06g054230.2* may play an important role in bacterial wilt resistance in tomato; although, further analysis is required to elucidate the gene function.

In conclusion, SNPs near *Bwr-6* were analyzed to search for markers tightly linked to this QTL. A total of 117 tomato germplasms were used to validate newly developed markers near this QTL. Among the analyzed markers, RsR6-5 is tightly linked to bacterial wilt resistance derived from *Bwr-6*. Consequently, this marker, in combination with RsR12-1, effectively predicted bacterial wilt-resistant and susceptible cultivars. The significance of these markers was further validated using an F_3 generation developed from a crossing between resistant and susceptible parents. F_3 lines that had resistant genotypes with RsR6-5 and RsR12-1 exhibited resistant phenotypes, while susceptible to the same markers exhibited susceptible phenotypes. The SNP-based diagnostic marker to *Bwr-6* was not identified in previous studies, and the newly developed marker in this study (RsR6-5) will help to trace this locus in marker-assisted breeding of tomato cultivars that are resistant to the devastating effects of bacterial wilt.

Author Contribution Statement

JML conceived and designed the experiments. AMA, JC and YK performed the experiments. CSO, IY, and ISN provided experimental materials. AMA and JML wrote and revised the manuscript, and all co-authors contributed and approved the final draft of the manuscript.

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