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Characterization of tomato (*Solanum lycopersicum*) accessions for resistance to phylotype I and phylotype II strains of the *Ralstonia solanacearum* species complex under high temperatures

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Abstract

Bacterial wilt of tomato caused by *Ralstonia solanacearum* species complex (*RSSC*) causes substantial yield losses in the tropics and subtropics. Disease management options by chemicals are limited, and host resistance is the cheapest and easiest means of control. However, sources of bacterial wilt resistance in tomato are limited. The disease often coincides with higher temperatures in the tropics, and resistance sources that are more heat stable are particularly valuable for breeding of tropically adapted tomato cultivars. The objectives of this study were to identify tomato accessions that demonstrate relatively high bacterial wilt resistance under high temperatures and to identify accessions that may possess QTLs other than *Bwr-6* and *Bwr-12* (two major disease resistance QTLs against bacterial wilt), which could be exploited in future breeding. Sixty-seven tomato entries reported as bacterial wilt resistant were evaluated in a greenhouse against one strain each of phylotype I (Pss4) and phylotype IIB (Pss1632) of the *RSSC* (average temperature ≥29°C). Of those, five and 19 were homozygous for *Bwr-6* and *Bwr-12*, respectively, and six were homozygous for both QTLs. *Bwr-12* contributed to resistance against phylotype I strain but not against the phylotype II strain. *Bwr-6* contributed to resistance against both phylotype strains. Entries with both QTLs as a group performed relatively better against the phylotype I strain. Entry "94T765-24-79", which lacked *Bwr-6* and *Bwr-12*, demonstrated relatively high resistance against the phylotype II strain and may carry new QTL/s. As new bacterial wilt resistance QTLs are mapped and markers designed, pyramiding multiple bacterial wilt resistance QTLs into new varieties should be straightforward, thereby increasing the chances of obtaining stable resistance.

KEYWORDS

bacterial wilt, *Bwr-12*, *Bwr-6*, marker-assisted breeding, *Ralstonia solanacearum species complex*, *Solanum lycopersicum*

1 | INTRODUCTION

Tomato is a globally important vegetable crop with a total world production of ~182 million metric tons harvested from 4,848,384 hectares in 2017 (FAOSTAT Database, 2018). Widely grown in tropics and subtropics, tomato is an important source of vitamins A and C, antioxidants and carotenoids (García-Alonso et al., 2009; Perveen et al., 2015). Tomato, as a high value vegetable crop, encourages smallholders to switch from subsistence to commercial farming, thereby improving their income and livelihood (Hanson et al., 2016). Tomato production in the tropics and subtropics, however, is severely affected by bacterial wilt disease, caused by *Ralstonia solanacearum species complex* (*RSSC*; Safni et al., 2014). The *RSSC* are soil-borne bacteria which can attack >450 plant species in 50 families, including pepper, potato, tobacco, eggplant, ginger, banana and several other economically important crops (Hayward, 1994; Janse et al., 2004; Kunwar, Iriarte, et al., 2017; Kunwar, Paret, et al., 2017; Kunwar et al., 2015; Poussier et al., 2000). The pathogen exhibits wide phenotypic and genotypic variation and can survive for extended periods in irrigation water, soil and rhizosphere (Van Elsas et al., 2000; Van Elsas, Kastelein, Bries, & Overbeek, 2001). Consequently, bacterial wilt disease is often difficult to control. Strains within the *RSSC* were divided into five races and six biovars based on host range and utilization of disaccharides and hexose alcohols (Fegan & Prior, 2005). Later, strains were divided into four phylotypes based on phylogenetic analysis that also reflected geographical origin, with phylotype I, having its origin in Asia, phylotype II in America, phylotype III in Africa and phylotype IV in Indonesia (Fegan & Prior, 2005). Recently, Safni et al. (2014) using all the phenotypic, genotypic, multi locus sequence alignment (MLSA) and whole genome comparisons reclassified the species complex into three species, *R. solanacearum* (previously classified as phylotype II), *R. pseudosolanacearum* (previously classified as phylotypes I and III) and *R. syzygii* (previously classified as phylotype IV; Prior et al., 2016). Nevertheless, the phylotype system is still commonly used since it reveals both the evolutionary relationships and reflects the current species classification (Shutt, Shin, Waals, Goszczynska, & Coutinho, 2018). For ease of understanding, the pathogen strains used in this study will be referred to by their phylotypes.

Several sources of bacterial wilt resistance in tomato have been identified and studied extensively (Hanson et al., 1996; Scott, Wang, & Hanson, 2005; Wang, Hanson, & Barnes, 1998). Most resistance sources came from India, Philippines, Indonesia, Thailand, French West Indies and the United States (Boshou, 2005), and have been frequently exchanged among the major tomato breeding groups worldwide (Daunay, Laterrot, Scott, & Hanson, 2010; Ho, Chung, & Wang, 2013). It has been difficult to develop bacterial wilt resistant tomato cultivars due to the complex and polygenic inheritance of resistance, the association between resistance and poor fruit quality, highly variable pathogen strains and a complex interaction between bacterial wilt resistance and environmental factors such as soil pH, moisture and temperature (Daunay et al., 2010; Hai, Esch, & Wang, 2008; Hanson et al., 1996; Scott et al., 2005; Wang et al., 1998, 2013). Hanson et al. (1996) determined that resistance to bacterial wilt in tomato can be location-specific, and other studies have shown that the resistance can be strain-specific (Genin & Boucher, 2002; Ji et al., 2007; Lopes, Quezado-Soares, & De Melo, 1994; Prior, Steva, & Cadet, 1990; Wang et al., 1998). Wang et al. (1998) evaluated 35 tomato resistance sources in eleven countries and identified "Hawaii 7996" (H7996) as one of the most stable resistance sources. Genetic analysis based on a cross between H7996 and WVa700, a susceptible *S. pimpinellifolium* line, identified two major QTLs, one on chromosome 6 (*Bwr-6*; Carmeille et al., 2006; Thoquet et al., 1996; Wang et al., 2013) and the other on chromosome 12 (*Bwr-12*; Wang et al., 2013) which provided resistance to bacterial wilt. *Bwr-12* was mapped to a 2.8 cM region and controlled 18%–56% of the variation in resistance against phylotype I (race 1) strains but was not effective against phylotype II (race 3) strain (Wang et al., 2013). *Bwr-12* was associated with the suppression of bacterial growth in the stem. The *Bwr-6* genomic region spanned 15.5 cM and may include multiple QTLs. The location of *Bwr-6* differed slightly among phenotypic datasets and accounted for 12%–22% of the variation in resistance against few of the phylotype I (race 1) strains and one phylotype II (race 3) strain, tested in the study (Wang et al., 2013). Using markers designed for both *Bwr-6* (*SLM6-124*, *SLM6-118*, *SLM6-94*, *SLM6-17* and *SLM6-110*) and *Bwr-12* (*SLM12-2* and *SLM12-10*), Ho et al. (2013) found that these two QTLs are present in most bacterial wilt resistance sources. *Bwr-6* and *Bwr-12* provide useful resistance and have been incorporated in some commercial cultivars. It is prudent to identify new sources of resistance or to more effectively exploit known resistance sources, map new bacterial wilt resistance QTLs and pyramid them. Bacterial wilt disease often coincides with higher temperatures in the tropics and resistance sources that are more heat stable are particularly valuable for breeding of tropically adapted tomato cultivars. Over the past 40 years, the World Vegetable Center (WorldVeg) tomato breeding group has received germplasm with bacterial wilt resistance from different parts of the world, but these accessions have never been evaluated for resistance to bacterial wilt in a common trial. Therefore, the objectives of this study were to identify bacterial wilt resistant tomato entries that performed well under high temperature conditions and to identify those that may possess alternative resistance QTLs (other than *Bwr-6* and *Bwr-12*), which could be exploited in future breeding. To achieve these objectives, we assessed these resistance sources for disease reaction against one strain each of phylotype I (*R. pseudosolanacearum*) and phylotype II (*R. solanacearum*) by drench inoculation in the greenhouse, and we tested for the presence of *Bwr-6* and *Bwr-12* using linked molecular markers for each.

2 | MATERIALS AND METHODS

2.1 | Plant materials

A total of 67 tomato (*Solanum lycopersicum* L.) accessions were used in this study. They were found to have resistance to bacterial wilt by researchers in different organizations and kindly provided to

the World Vegetable Center (WorldVeg; Table 1). Accessions with the same or similar names but provided by different organizations/researchers were treated as separate entries. H7996, homozygous for both *Bwr-6* and *Bwr-12*, served as a resistant control, and "Pant Bahar" and "L390," both of which lack *Bwr-6* and *Bwr-12*, were included as susceptible controls.

2.2 | Bacterial strains and inoculum preparation

Phylotype I strain "Pss4" (race 1, biovar 3; Hai et al., 2008) and phylotype IIB strain, "Pss1632" (race 3, biovar 2), were used in the greenhouse experiments. Pss1632 was characterized as phylotype IIB (race 3, biovar 2) using three tests: the classical Hayward biovar sugar alcohol acidification test identified it as biovar 2 (Hayward, 1964), the phylotype-specific multiplex PCR of Fegan and Prior (2005) identified it as belonging to phylotype II, and finally, sequence analysis of the *egl* gene (Fegan et al., 1998) showed this strain belongs to sequevar 1. All members of sequevars 1 and 2 are race 3 biovar 2. Pss1632 will be referred to as phylotype II hereafter. Stored cultures of each strain were streaked on 2,3,5-triphenyltetrazolium chloride (TZC) medium (casamino acid [1 g], peptone [10 g], glucose [5 g] and 5 ml of 2, 3, 5-triphenyltetrazolium chloride [1%]; Kelman, 1954) and incubated for two days at 30°C. For inoculum preparation, several fluidal colonies were transferred to plates containing 523 media (Kado & Heskett, 1970) for multiplication at 30°C for 24 hr. The bacterial growth was suspended in sterile distilled water, and the optical density (OD) of the suspension was adjusted to 0.3 at 600 nm, corresponding to ~10^8 colony-forming units per millilitre (CFU/ml).

2.3 | Greenhouse experiment

Greenhouse experiments were conducted to screen the resistant tomato sources against phylotype I (Pss4) and phylotype II (Pss1632) strains, in the summers of 2017 and 2018, at WorldVeg, Taiwan. Entries were evaluated for reactions to each phylotype in separate trials each year. The greenhouse experiments were conducted following a randomized complete block design (RCBD) with two replications and twenty plants per replication per entry. Four-week-old seedlings, approximately at the four-leaf stage, were inoculated by pouring a bacterial suspension (20 ml of 10^8 CFU/ml) on the soil surface of each plant without wounding the roots. Three to four weeks after inoculation, when the disease incidence reached 100% in at least one of the susceptible controls, the number of healthy plants was counted for each entry, and the mean per cent survival was calculated. The plants were grown in the greenhouse under natural sunlight conditions throughout the experiments. The minimum and maximum temperature, in 2017, were 29 and 44°C, respectively, for phylotype I strain and 32 and 38°C, respectively, for phylotype II strain (average temperatures were 36.5 and 35°C for phylotype I and II trials, respectively) experiments. In 2018, minimum and maximum temperature for both phylotypes I and II trials were 19 and 39°C, respectively (the average temperature for both phylotype trials was 29°C).

2.4 | Characterization of bacterial wilt resistance sources in tomato for presence of *Bwr-6* and *Bwr-12* QTLs

Simple sequence repeat (SSR) markers reported to be associated with bacterial wilt resistance loci *Bwr-6* and *Bwr-12* (Wang et al., 2013) were used in this study. Primer sequences and detectable products of molecular markers used in the study are provided in Table 2. Genomic DNA was extracted from a section of a fresh young leaf (n = 20) using the protocol of Edwards, Johnstone, and Thompson (1991). For the extraction, 300 µl of extraction buffer (400 mM Tris-HCl, pH 7.5; 500 mM NaCl; and 50 mM EDTA, pH 8.0) was added to each leaf sample and vortexed on a high velocity bead beater (30 s). To this, 300 µl of SDS buffer (1%) was added, mixed gently and centrifuged (15 min at 6037 *g*). After centrifugation, 200 µl of supernatant was mixed with an equal volume of ice-cold isopropanol and centrifuged again (15 min at 6037 *g*). The resulting DNA pellet was rinsed with 400 µl of ice-cold ethanol (70%) and air-dried. The pellet was then resuspended in 300 µl of sterile deionized water and put in a water bath (65°C) for 30 min before storing at −20°C. For PCR amplifications using SSR markers, we used 2 µl of DNA, 0.1 µl of forward and reverse primers each (10 µM), 0.4 µl of deoxyribonucleotides, 6.4 µl of DNase-free water, 1 µl of buffer (10×) and 0.1 µl of *Taq* DNA polymerase. The PCR cycles consisted of denaturation at 94°C for 10 min, followed by 30 cycles of 94°C for 30 s, 55°C for 45 s and 72°C for 45 s and final extension at 72°C for 7 min. Amplified products were loaded onto 6% non-denaturing polyacrylamide gel (49.5 ml of 6% acrylamide solution; 35 µl TEMED and 1 ml of 10% APS) in TBE buffer. Gels were run for 50 min for *SLM6-17* and *SLM6-118* markers; 55 min for *SLM12-2* and *SLM12-2* markers; 60 min for *SLM6-94* and *SLM6-110* markers; and 70 min for *SLM6-124* marker. After electrophoresis, the gels were stained with ethidium bromide and visualized under UV light. The size of the bands obtained was determined using 50–700 bp DNA ladder used as a reference. Each co-dominant SSR marker was individually scored for each tomato line as a "2" if homozygous for the resistant allele (similar as H7996), "1" if heterozygous and "0" if the marker was the same as of the susceptible lines, Pant Bahar and L390.

2.5 | Statistical analysis

The per cent survival data were arcsine square root transformed for statistical analysis. One-way ANOVA was conducted to test the significant difference among entries for bacterial wilt resistance using R (R Core Team, 2016). Mean separation was conducted using LSD at p = .05. In 2018, seeds of "CRA66" did not germinate in the phylotype I trial, and this entry was therefore not included in statistical analysis for that year. The tomato entries were divided into five groups based on the genotypes of *Bwr-12* and *Bwr-6* SSR markers. A linear mixed model was applied to test for differences in groups and genotypes nested in groups using R software. Replication was considered a random effect. A Tukey multiple comparison procedure was used to test for differences in groups, and a sliced F test was used to test for difference in genotypes for each level of group. Since

TABLE 1 Distribution of bacterial wilt resistance QTLs (*Bwr-12* and *Bwr-6*) in a set of resistance tomato sources and their reactions to phylotype I and II strains of the *Ralstonia solanacearum species complex* (RSCC)

				Bwr-12		*Bwr-6*					Survival (%)[†] (*SEM*)			
											Phylotype I (Pss4)		Phylotype II (Pss1632)	
	Entry	Origin	Source	*SLM 12-2*	*SLM 12-10*	*SLM 6-124*	*SLM 6-118*	*SLM 6-94*	*SLM 6-17*	*SLM 6-110*	2017	2018	2017	2018
Group-1[‡]	F7-80-pink	Philippines	UPLB	+	+	+	H	+	+	+	77 a[§] (14)	75 ab (3)	59 ab (19)	59 a–d (9)
	F7-80-465-10-pink	Philippines	W.Veg	+	+	+	H	+	H	+	64 a–e (4)	78 ab (0)	52 a–c (9)	59 a–d (9)
	MT-11	Malaysia	MARDI	+	+	–	–	H	H	H	57 a–h (15)	50 c–i (2)	28 c–m (6)	33 g–p (7)
	CRA 84-26-3 129	Guadeloupe	INRA	+	+	–	–	–	H	–	57 a–h (34)	45 c–j (0)	18 h–n (5)	30 h–q (4)
	CRA 84-23-1 115	Guadeloupe	INRA	+	+	–	–	–	–	–	50 a–i (23)	41 e–l (8)	7 mn (7)	7 st (7)
	CRA 84-58-1 170	Guadeloupe	INRA	+	+	–	–	–	–	–	43 a–j (30)	41 e–l (2)	28 c–m (6)	33 g–p (10)
	CRA 84-69 119	Guadeloupe	INRA	+	+	–	–	–	–	–	39 a–j (39)	32 h–o (14)	13 j–n (0)	23 l–s (0)
	CRA 84-25-1 128	Guadeloupe	INRA	+	+	–	–	–	–	–	39 a–j (26)	33 h–o (7)	18 h–n (5)	27 k–r (0)
	CRA 84-41 111	Guadeloupe	INRA	+	+	–	–	–	–	–	34 a–j (34)	23 k–q (0)	13 j–n (0)	19 o–t (19)
	L180 (VI005591)	Taiwan	GRSU	+	+	–	–	–	–	+/OB	30 a–j (30)	39 f–m (9)	12 k–n (12)	34 g–p (4)
	Caraibe 128	Guadeloupe	INRA	+	+	–	–	–	–	–	34 a–j (21)	38 g–m (2)	10 l–n (10)	30 i–q (7)
	King Kong 3-11	–	NIVOT	+	+	–	–	–	–	–	33 a–j (10)	34 h–n (4)	16 i–n (3)	10 r–t (10)
	T-89 (Biang)	–	Sri Lanka	+	+	–	–	–	–	–	29 a–j (17)	7 q–r (7)	16 i–n (3)	0 t (0)
	Redlander	Australia	Australia	+	+	–	–	–	–	–	29 b–j (2)	37 h–n (4)	20 g–n (7)	25 k–s (6)
	B9-1	India	India	+	+	–	–	–	–	–	24 d–j (10)	44 c–k (9)	0 n (0)	10 r–t (10)
	TBL-3	–	NIVOT	+	+	–	–	–	–	–	19 d–j (19)	22 l–r (22)	16 i–n (3)	16 p–t (3)
	TBL-2	–	NIVOT	+	+	–	–	–	–	–	9 h–j (10)	32 h–o (5)	0 mn (0)	14 q–t (14)
	TBL-1	–	NIVOT	+	+	–	–	–	–	–	11 g–j (3)	29 h–n (2)	7 k–n (7)	7 st (7)
	TBL-4	–	NIVOT	+	+	–	–	–	–	–	15 e–j (12)	35 i–p (2)	12 mn (12)	10 r–t (10)
Group-2	LS 89	–	Japan	+	–	+	+	+	+	+	66 a–d (24)	60 b–f (4)	22 f–n (22)	36 f–o (13)
	H7998S	USA	UF	+	–	+	+	+	+	+	62 a–f (17)	74 ab (17)	38 a–i (8)	53 b–f (8)
	H7998M	USA	NIVOT	+	–	+	+	+	+	+	53 a–i (20)	74 ab (17)	33 c–l (15)	41 e–l (2)
	S/T2	Philippines	UPLB	–	–	+	+	+	+	+	24 d–j (11)	10 p–r (10)	44 a–g (11)	43 d–k (0)
	GA 1565 (PI 263722)	USA	UF	–	–	+	+	+	+	+	12 g–j (12)	0 r (0)	38 a–i (8)	21 n–s (3)
Group-3	**H7996**	**USA**	**UF**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**72 a–c (6)**	**59 b–g (5)**	**50 a–d (7)**	**48 c–h (3)**
	TmL114-42	Philippines	W.Veg	+	+	+	+	+	+	+	65 a–d (8)	84 a (7)	40 a–i (3)	50 b–g (2)
	TML114	Philippines	Philippines	+	+	+	+	+	+	+	59 a–g (14)	64 a–d (0)	38 a–i (8)	56 a–e (8)
	H7997	USA	UF	+	+	+	+	+	+	+	60 a–f (18)	61 b–f (7)	45 a–f (19)	48 c–h (6)
	R3034-3-10-N-UG	Philippines	W.Veg	+	+	+	+	+	+	+	53 a–i (2)	66 a–c (2)	37 b–j (4)	65 a–c (8)
	MT-1	MARDI	Malaysia	+	+	+	+	+	+	+	39 a–j (9)	50 c–i (2)	10 l–n (10)	14 q–t (14)

(Continues)

TABLE 1 (Continues)

				Bwr-12		*Bwr-6*					Survival (%)[†] (*SEM*)			
											Phylotype I (Pss4)		Phylotype II (Pss1632)	
	Entry	Origin	Source	*SLM 12-2*	*SLM 12-10*	*SLM 6-124*	*SLM 6-118*	*SLM 6-94*	*SLM 6-17*	*SLM 6-110*	2017	2018	2017	2018
Group-4	94T765-24-79	USA	UMN	–	–	–	–	–	–	–	14 f–j (14)	31 h–p (18)	37 b–j (0)	41 e–l (5)
	CRA 84-15-3 133	Guadeloupe	INRA	–	–	–	–	–	–	–	14 f–j (14)	7 qr (7)	16 i–n (3)	7 st (7)
	LA3501	–	TGRC	–	–	–	–	–/OB	–	OB	0 j (0)	0 r (0)	17 h–n (17)	28 j–r (9)
	L390 (VI005795)	**Taiwan**	**GRSU**	–	–	–	–	–	–	–	**10** h–j **(10)**	**0 r (0)**	**0** n **(0)**	**0 t (0)**
	Pant Bahar	**India**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**–**	**0 j (0)**	**0 r (0)**	**0 n (0)**	**0 t (0)**
Group-5	LE 415 Anagha	India	India	H	H	–	–	–	–	–	75 ab (3)	50 c–i (11)	30 c–m (7)	38 e–n (2)
	CRA 84-51-1 153	Guadeloupe	INRA	H	H	H	H	H	H	H	56 a–h (22)	43 d–l (3)	10 l–n (10)	27 k–r (4)
	CRA 84-6-6 95	Guadeloupe	INRA	H	H	–	–	–	–	H	47 a–j (14)	43 d–l (3)	20 g–n (7)	25 k–s (2)
	CRA 84-38-1 116	Guadeloupe	INRA	H	H	–	–	–	–	–	45 a–j (23)	34 h–n (0)	20 g–n (7)	34 g–p (0)
	CRA 84-57-1 140	Guadeloupe	INRA	H	H	–	–	–	–	–	44 a–j (21)	44 d–k (5)	7 mn (7)	0 t (0)
	CRA 84-47-1 152	Guadeloupe	INRA	H	H	–	–	–	–	–	41 a–j (14)	23 k–q (0)	12 k–n (12)	30 s–q (4)
	CRA 84-39-1 132	Guadeloupe	INRA	H	H	–	–	–	–	–	32 a–j (32)	32 h–o (5)	29 c–m(11)	34 g–p (4)
	CRA 84-59-1 117	Guadeloupe	INRA	H	H	–	–	–	–	–	37 a–j (24)	19 m–r (0)	13 j–n (0)	17 p–t (17)
	CRA 66	Guadeloupe	UF	H	H	H/OB	–	H	–	H	33 a–j (3)	NG	62 a (17)	56 a–e (5)
	Arthaloka F1	Indonesia	EW seeds	H	H	–	–	–	–	–	30 a–j (30)	16 n–r (3)	23 e–n (5)	34 g–p (3)
	S2/T96	Philippines	UPLB	H	H	–	–	–	–	–	24 c–j (24)	34 h–n (4)	23 e–n (0)	10 r–t (10)
	S2/T44	Philippines	UPLB	H	H	–	–	–	–	–	27 c–j (14)	25 j–q (12)	13 k–n (13)	0 t (0)
	TW-3	Thailand	KKU	H	H	–	–	–	–	–	30 a–j (4)	29 i–p (2)	19 h–n (0)	28 j–q (6)
	T-245	Sri Lanka	HORDI	H	–	H	H	H	H	H	27 c–j (14)	25 j–q (2)	7 mn (7)	7 st (7)
	L285 (VI005795)	Taiwan	GRSU	H	H	H	H	H	H	H	32 a–j (19)	37 h–n (4)	51 a–c (3)	73 a (5)
	Idola F1	Indonesia	EW seeds	+	H	H	H	+	+	+	44 a–j (21)	63 a–e (6)	30 c–m (0)	45 d–j (3)
	GA219 (PI 126408)	USA	–	–	–	+	–	+	–	H	41 a–j (8)	33 h–o (19)	29 c–m (2)	40 e–m (6)
	GA219	USA	UF	–	–	–	–	+	–	H	30 a–j (7)	32 h–o (5)	27 d–m (4)	40 e–m (0)
	LS1811-2-8	Japan	NIVOT	+	H	–/OB	–	–/OB	–	H/OB	28 b–j (6)	35 h–n (8)	17 h–n (17)	7 st (7)
	LS1811-2-14	Japan	NIVOT	+	–	–/OB	–	–/OB	–	–/OB	23 d–j (23)	52 c–h (4)	22 f–n (9)	17 p–t (3)
	94T765-24-74	USA	UMN	–	–	–	–	H	–	H	20 d–j (20)	22 l–r (22)	40 a–h (7)	67 ab (11)
	94T766-05, 76	USA	UMN	–	–	–	–	–/OB	–	H	20 d–j (20)	0 r (0)	34 c–k (12)	47 c–i (2)
	GA 1405 (PI 251323)	USA	UF	–	–	H	–	H	+	+	15 f–j (15)	28 j–q (9)	47 a–e (5)	56 a–e (2)
	Fla 973600-1	USA	UF	–	–	–	–	–	–	OB	14 f–j (14)	10 p–r (10)	28 c–m (9)	18 o–t (5)
	Fla 973243-1	USA	UF	–	–	–	–	–	–	+	7 ij (7)	0 r (0)	25 e–m (12)	22 m–s (9)

(Continues)

TABLE 1 (Continues)

Entry	Origin	Source	*Bwr-12*		*Bwr-6*					Survival (%)† (*SEM*)			
										Phylotype I (Pss4)		Phylotype II (Pss1632)	
			SLM 12-2	*SLM 12-10*	*SLM 6-124*	*SLM 6-118*	*SLM 6-94*	*SLM 6-17*	*SLM 6-110*	2017	2018	2017	2018
Fla 973579-1	USA	UF	H	–	–	–	–	–	+	7 ij (7)	12 o–r (12)	25 e–m (2)	21 n–s (3)
Fla 973237-1	USA	UF	H	–	–	–	–	–	+	0 j (0)	7 qr (7)	39 a–i (9)	27 k–r (4)
Fla 973587-3	USA	UF	–	–	–	–	–	–	OB	0 j (0)	10 p–r (10)	25 e–m (2)	16 p–t (3)
Fla 7421	USA	UF	–	–	–	–	–	–	+	7 ij (7)	0 r (0)	16 h–n (4)	16 p–t (3)
Ratan	–	Bangladesh	–	–	–	–	–	–	+	12 (12)	18 m–r (5)	14 j–n (14)	21 n–s (3)
BRS-1	Australia	QDPI	–	+	–	–	–	–	–	15 f–j (15)	23 k–q (5)	10 l–n (10)	0 t (0)
Rodade	S. Africa	QDPI	–	+	–	–	–	–	–	14 f–j (14)	24 k–q (11)	7 mn (7)	7 st (7)

Note: The experiment was conducted in greenhouse at WorldVeg. "+" and "–" indicate homozygous for resistant (H7996) and susceptible (L390 and Pant Bahar) allele, respectively. The resistant and susceptible controls are indicated in bold. The letters "M" and "S" after H7998 represent the source from which the seeds were obtained i.e., Scott. J for H7998S and Monma. S for H7998M. Abbreviations: EW seeds = East-West Seeds, Indonesia; GRSU = Genetic Resources and Seed Unit at the WorldVeg, Taiwan; H = heterogeneous; HORDI = Horticultural Crops Research and Development Institute, Sri Lanka; INRA = Institut National de la Recherche Agronomique, Guadeloupe; KKU = Khon Kaen University, Thailand; MARDI = Malaysian Agricultural Research and Development Institute; NG = no germination; OB = other bands; QDPI = Queensland Department of Primary Industries, Australia; *SEM* = Standard error of mean; TGRC = Tomato Genetic Resource Center, University of California, Davis; UF = University of Florida; UNM = University of Minnesota; UPLB = Institute of Plant Breeding, University of the Philippines at Los Baños; W.Veg = World Vegetable Center, Taiwan; "/" = mixture of susceptible, resistant or other bands.

†Each phylotype was evaluated separately in the greenhouse. The seedlings were inoculated through soil drench where 20 ml of 10^8 CFU/ml of bacterial suspension was poured on the base of each plant with no root wounding. The data for percentage survival were transformed using arcsine square root and represent means of two replications with 20 plants per replication. The greenhouse experiments were conducted in the summer of 2017 and 2018 at The World Vegetable Center, Taiwan.

‡Tomato entries were divided into five groups based on their genotypes of *Bwr-12* and *Bwr-6*. Group 1, 2, 3, 4 and 5 consisted 19, 5, 6, 5 and 32 tomato entries, respectively. Entries in "Group 1" and "Group 2" consisted *Bwr-12* and *Bwr-6* QTLs, respectively. Entries in "Group 3" consisted both *Bwr-12* and *Bwr-6* QTLs. Entries in "Group 4" lacked both QTLs and entries in "Group 5" produced heterogenous bands (mixture of genotypes) at *Bwr-12* and/or *Bwr-6*.

§The per cent survival data were arcsine square root transformed and one-way ANOVA was conducted to test the significant difference among entries using R (R Core Team, 2016). Columns followed by the same letter do not differ significantly based on LSD at $p = .05$.

TABLE 2 The SSR markers used for screening bacterial wilt resistance sources of tomato, WorldVeg, Taiwan (modified from Ho et al., 2013)

Resistant loci	Marker name	Repeat motif	Forward primer sequence 5–3	Reverse primer sequence 3–5	Resistant band (bp)[a]	Susceptible band (bp)[a]
Bwr-12	*SLM12-2*	$(TA)_{11}$	ATCTCATTCAACGCACACCA	AACGGTGGAAACTATTGAAAGG	~200 (2 bands)	~250 (2 bands)
	SLM12-10	$(AT)_{21}$	ACCGCCCTAGCCATAAAGAC	TGCGTCGAAAATAGTTGCAT	300	250
Bwr-6	*SLM6-124*	$(TAT)_{10}$	CATGGGTTAGCAGATGATTCAA	GCTAGGTTATTGGGCCAGAA	280	300
	SLM6-118	$(AAT)_{18}$	TCCCAAAGTGCAATAGGACA	CACATAACATGGAGTTCGACAGA	210/240	180/205
	SLM6-17	$(TA)_{12}$	TCCTTCAAATCTCCCATCAA	ACGAGCAATTGCAAGGAAAA	190/200	200/210
	SLM6-94	$(TA)_{33}$	CTAAATTTAAATGGACAAGTAATAGCC	CACGATAGGTTGGTATTTTCTGG	270/290/330/360	290/310/340/370
	SLM6-110	$(ATT)_{22}$	AGAATGCGGAGGTCTGAGAA	ATCCCACTGTCTTTCCACCA	270/290	290/310

[a]Some of the markers resulted in more than one band separated by "/".

Group 5 was heterogeneous at *Bwr-6* and/or *Bwr-12* loci, this group was not included in group comparisons.

3 | RESULTS

3.1 | Resistance to bacterial wilt differs among resistance tomato sources

Conditions favoured bacterial wilt development in all the greenhouse trials as evidenced by low survival of plants in L390 (0%–10%) and Pant Bahar (0%), and high percentages of wilted plants in H7996 (28%–52%; Table 1). The mean survival of all the entries inoculated with phylotype I and phylotype II strains was consistent between the two years (40% and 42% in 2017 and 2018, respectively, for phylotype I strain; and 27% and 33% in 2017 and 2018, respectively, for phylotype II strain; Table 1). Survival means for each trial were below 50%, which indicates high bacterial wilt pressure. Overall, the mean survival of all the entries was higher in response to phylotype I strain than to phylotype II strain by 13% in 2017 and by 9% in 2018 which indicates that the phylotype II strain was more virulent than the phylotype I strain. For some heterogeneous entries (i.e., entries in which the resistance alleles are not fixed), we observed discrepancies between 2017 and 2018 in response to a given phylotype, which is expected for such genotypes.

One-way ANOVA detected significant differences among entries in response to both phylotype I and phylotype II strains (Table 1). There was no significant difference between the L390 and Pant Bahar, the two susceptible controls, in terms of per cent survival, regardless of whether they were inoculated with phylotype I or phylotype II strain. H7996 performed significantly better than L390 and Pant Bahar susceptible controls across all the trials (Table 1). Overall, the group of Hawaii entries (H7996, H7998S, H7998M and H7997) and the entries from the Philippines (F7-80-465-10-pink, F7-80-pink, TML114, R3034-3-10-N-UG) stood out as the most consistently resistant entries against both phylotypes I and II strains (Table 1). There were no significant differences between the three Hawaii entries, H7996, H7997 and H7998, and these were among the most resistant entries, in response to both phylotypes. None of the entries tested in this study performed significantly better than the Hawaii entries in response to both strains. However, a few entries were statistically similar to the Hawaii entries and were consistently resistant to both phylotypes across both years, such as F7-80-465-10-pink, F7-80-pink, MT-11, TML114, R3034-3-10-N-UG and LE 415 Anagha.

A few entries showed phylotype-specific resistance. Eight entries demonstrated relatively high resistance to the phylotype I strain (F7-80-pink, F7-80-465-10-pink; LS 89, H7998S, H7997, TmL114-42-N-H.T.P early, TML114 and LE 415 Anagha; Table 1). These entries had statistically similar or better survival percentage than H7996, and all were significantly better than both the susceptible controls, L390 and Pant Bahar (Table 1). Six additional entries (MT-11, CRA 84-26-3 129, CRA 84-23-1 115, H7998M, R3034-3-10-N-UG and CRA 84-51-1 153), were statistically similar

TABLE 3 The presence of two major QTLs (*Bwr-6* and *Bwr-12*) associated with bacterial wilt resistance in a set of resistance tomato sources and their disease reaction to phylotype I and phylotype II strains of *Ralstonia solanacearum species complex* (RSCC) in the greenhouse

				Phylotype I‡ (Pss1632)			Phylotype II‡ (Pss1632)		
	Group†	*Bwr-12*	*Bwr-6*	Mean survival§ (%)	p^x (within group)	p^y (among groups)	Mean survival§ (%)	p^x (within group)	p^y (among groups)
2017	1 (*n* = 19)	+	–	36 b¶	.0016	<.0001	18 b	0.0023	<.0001
	2 (*n* = 5)	–	+	43 ab	.0012		34 a	0.4381	
	3 (*n* = 6)	+	+	58 a	.3127		36 a	0.0355	
	4 (*n* = 5)	–	–	7 c	.7726		14 b	0.0293	
2018	1 (*n* = 19)	+	–	38 b	<.0001	<.0001	23 b	<0.0001	<.0001
	2 (*n* = 5)	–	+	43 b	<.0001		38 a	0.0615	
	3 (*n* = 6)	+	+	63 a	.0443		46 a	0.0008	
	4 (*n* = 5)	–	–	7 c	.0171		15 b	0.0008	

Note: The experiments were conducted in the summer seasons of 2017 and 2018 at The World Vegetable Center, Taiwan. "+" and "–" indicate homozygous for resistant (H7996) and susceptible (L390 and Pant Bahar) alleles, respectively. p^x and p^y represent significant difference within group and between groups, respectively (at $p = .01$). A linear mixed model was used to test for differences in groups and genotypes nested in groups. A Tukey multiple comparison procedure was used to test for differences in groups, and sliced *F* test was used to test for difference in genotypes for each level of group.

†Tomato entries were divided into four groups based on their genotypes of *Bwr-12* and *Bwr-6*. Entries in "Group 1" and "Group 2" consisted *Bwr-12* and *Bwr-6* QTLs, respectively. Entries in "Group 3" consisted both *Bwr-12* and *Bwr-6* QTLs. Entries in "Group 4" lacked both QTLs, and entries in "Group 5" produced heterogenous bands (mixture of genotypes) at *Bwr-12* and/or *Bwr-6*.

‡Each phylotype was evaluated separately in the greenhouse. The seedlings were inoculated through soil drench where 20 ml of 10^8 CFU/ml of bacterial suspension was poured on the base of each plant with no root wounding.

§Each value represents group means across each group. The survival percentage for each genotype in a group was transformed using arcsine square root before calculating group means.

¶Column means followed by the same letter do not differ significantly across groups based on Tukey's multiple comparison.

to H7996 and significantly better than Pant Bahar in both years (significantly better than L390 only in 2018 but not in 2017; Table 1). Fourteen entries showed a susceptible reaction to the phylotype I strain, including all six Florida (Fla) entries, and were statistically similar to susceptible controls, L390 and Pant Bahar, and had significantly less survival than H7996 across both phylotype I trials (Table 1). LA3501 and Pant Bahar were the most susceptible entries with 0% survival across both phylotype I trials (Table 1).

Twenty-one entries were relatively resistant across the two phylotype II trials and were statistically similar to H7996 and had significantly better survival than susceptible controls L390 and Pant Bahar (i.e., F7-80-pink, F7-80-465-10-pink, MT-11, CRA 84-58-1 170, H7998 [S and M], S/T2, TmL114-42-N-H.T.P early, TML114, H7997, R3034-3-10-N-UG, 94T765-24-79, LE 415 Anagha, CRA 84-39-1 132, CRA 66, L285, Idola F1, GA219 [PI 126408], GA219, 94T765-24-74, 94T766-05-76, GA 1405 [PI 251323]; Table 1). Additionally, CRA66 (*Solanum lycopersicum* var. *cerasiforme*), the source of resistance of Caraibe 128 and CRA-84 prefixed lines (Daunay et al., 2010; Henderson & Jenkins, 1972; Prior, Grimault, & Schmit, 1994) was one of the most resistant entries across both phylotype II trials. Twenty-one entries were not significantly different from L390 and Pant Bahar for reaction to the phylotype II strain.

Three entries, CRA 84-15-3 133, 94T765-24-79 and LA3501, along with two susceptible controls, L390 and Pant Bahar, were negative for all the *Bwr-6* and *Bwr-12* markers and were consistently susceptible across phylotype I and phylotype II strains in all trials, except 94T765-24-79. Mean survival in 94T765-24-79 was statistically similar to H7996 and significantly higher than Pant Bahar and L390 across both phylotype II trials.

3.2 | *Bwr-6* and/or *Bwr-12* were common in bacterial wilt resistance sources of tomatoes collected from different origins

Of the 67 entries genotyped for *Bwr-12* and *Bwr-6*, only five, including susceptible checks L390 and Pant Bahar, completely lacked both QTLs. The remaining 62 entries were homozygous or heterogeneous (mixtures of resistant and susceptible genotypes) for all or parts of *Bwr-12* and/or *Bwr-6*. The tomato entries could be broadly grouped into five categories based on the genotypes of *Bwr-6* and *Bwr-12*: Group 1, (19 entries), was homozygous for *Bwr-12* (14 of which lacked *Bwr-6* and five tested positive for only some *Bwr-6* markers); Group 2, (five entries), was homozygous for *Bwr-6* (two of which lacked *Bwr-12* and three tested positive for only one *Bwr-12* marker); Group 3, (six entries), was homozygous for both QTLs; Group 4, (five entries) completely lacked both QTLs; and Group 5, (32 entries), was heterogeneous at *Bwr-6* and/or *Bwr-12* loci (Table 1).

All the Hawaii entries were homozygous for resistance alleles at both *Bwr-6* and *Bwr-12* QTLs, except for H7998, which produced a resistant band with only one of the two *Bwr-12* markers. However, it is important to note that *SLM12-2* and *SLM12-10* flank *Bwr-12* and H7998 may also have *Bwr-12*, but that there may have been a crossover between the gene and *SLM 12-10*. Both seed sources

of H7998 (i.e., H7998M from Dr. Monma at the National Research Institute of Vegetable, Ornamental Plants and Tea [NIVOT], Japan and H7998S from Dr. Jay Scott at the University of Florida) showed the same marker pattern. All the Philippine entries were homozygous for *Bwr12* and *Bwr-6* except for F7-80-pink and F7-80-465-10-pink, which produced heterogeneous bands with one and two SSR markers of *Bwr-6*, respectively. Fifteen entries bred by INRA (Institut National de la Recherche Agronomique, Guadeloupe) were tested in this study, out of which seven were homozygous for *Bwr-12* and seven were heterogeneous (and one lacked both QTLs). Most INRA-developed entries (i.e., 12 out of the 16) lacked *Bwr-6*. CRA 66, which was one of the most resistant entries across both phylotype II trials, produced heterogeneous bands at *Bwr-12* (Table 1). L285, a wild tomato accession from the WorldVeg gene bank, and an important source of resistance (Danesh, Aarons, McGill, & Young, 1994), also produced heterogeneous bands with all the *Bwr-6* and *Bwr-12* markers (Table 1). The six Florida (Fla.) entries lacked *Bwr-12* and seemed to contain only the region of *Bwr-6* detected by *SLM6-10* (Table 1).

3.3 | The presence of *Bwr-6* contributes to resistance against both phylotypes I and II whereas *Bwr-12* contributes to resistance against phylotype I only

A Tukey multiple comparison test revealed significant differences among groups possessing different genotypes of *Bwr-6* and *Bwr-12* for reactions to particular phylotype (Table 3). Also, there were significant differences among entries within groups (Table 3). Group 1 entries, possessing *Bwr-12*, had significantly higher mean survival in response to phylotype I strain than Group 4 (lacking both QTLs; Table 3). However, there was no significant difference in mean survival between Group 1 and Group 4 in response to the phylotype II strain (Table 3). Group 2, possessing *Bwr-6*, had significantly higher mean survival than Group 4 in response to both phylotypes I and II (Table 3). Group 3, possessing both *Bwr-12* and *Bwr-6*, in response to phylotype I strain, showed higher mean survival compared with all other groups, being statistically significant in one of the 2 years (Table 3). All the entries within Group 3 (except MT-1) likewise stood out as the most resistant entries against the phylotype I strain, with mean survival statistically similar to H7996 and significantly higher than L390 and/or Pant Bahar (Table 3).

4 | DISCUSSION

The bacterial wilt screening in this study was conducted under high temperatures in the greenhouse (min/max = 29/44°C; average = 36.5°C in 2017, and min/max = 19/39°C; average = 29°C in 2018). High bacterial wilt pressure was observed across all the greenhouse experiments as evidenced by high wilting (28%–52%) on resistant check H7996. Wang et al. (2013) also evaluated H7996 against Pss4 (phylotype I) strain in two WorldVeg trials, one in a temperature-controlled greenhouse (28–31°C) and a second in a screenhouse trial (21–31°C), and found mean per cent wilting of 24% and 19.8%, respectively. Overall, HW7996 performed better in response to Pss4 (with survival rates 76%–80.2%) in the Wang trials relative to this study, probably due to the cooler temperatures.

Mew and Ho (1977) found that increased soil temperatures could impair the level of resistance to bacterial wilt in certain tomato varieties. In Taiwan and other parts of SE Asia, it has been noted that varieties with *Bwr-12*-based resistance tend to show more wilting under high temperatures. However, high temperatures, especially in the rainy season, are often confounded with high soil moisture (personal communication with Peter Hanson). Nevertheless, many farmers in the tropics grow tomato in the open field under high and fluctuating temperatures and crops often sustain high losses from bacterial wilt. As average temperatures are increasing in many parts of the tropics due to the effects of climate change, bacterial wilt incidence and severity can be expected to rise, increasing disease pressure on available bacterial wilt resistant varieties. It is important to better understand how expression of bacterial wilt resistance is affected by temperature and other environmental factors and to identify resistance sources and QTLs that sustain or enhance resistance under such high temperatures.

In our screening, we did not uncover a new bacterial wilt resistance source superior to H7996, but the study did reveal several interesting entries that merit further investigation for the presence of additional bacterial wilt resistance QTL. Entry 94T765-24-79 lacked *Bwr-12* and *Bwr-6* but showed moderate resistance to Pss1632 (37% and 41% survival in 2017 and 2018, respectively); this entry may carry new QTL/s that could be combined with *Bwr-12* and *Bwr-6*. Its sister line, 94T765-24-74, also showed relatively high resistance to Pss1632, and marker analysis for *Bwr-6* revealed heterogeneous pattern at *SLM6-110* and presence of a non-H7996 band at *SLM6-94*, indicating the possibility of new allele at that locus. The H-prefixed (Hawaii) entries and four entries from the Philippines found to be highly resistant to Pss4 and Pss1632 in this study were previously identified by Wang et al. (1998) as among the most resistant in a worldwide screening trial; these entries were homozygous for *Bwr-6*, *Bwr12* or both (Ho et al., 2013). They probably possess other resistance QTL/s in addition to *Bwr-6* and *Bwr-12*. Carmeille et al. (2006) identified three additional QTLs in H7996 located on chromosomes 3, 4 and 8 and associated with resistance to race 3 phylotype II strains and that explained 3.2%–29.8% of the phenotypic variation, depending on the season. The phenotyping trials of the H7996 x WV700 RIL population that led to mapping of *Bwr-12* and *Bwr-6* were conducted in Asia and Reunion Island, and it would be useful to assess this valuable RIL population for reaction to the bacterial wilt pathogen in African and Latin American hotspots to uncover additional QTLs in H7996. LE 415 Anagha from India, heterogeneous for *Bwr-12* but lacking *Bwr-6*, demonstrated relatively high resistance to both Pss4 and Pss1632. A study conducted in Bangalore, India reported LE 415 to be highly resistant to bacterial wilt in the field (Prasanna, 2012; Singh & Malhotra, 2013). The presence of *Bwr-12* in LE 415 Anagha afforded good resistance to Pss4 in this

study and may hint at additional resistance QTLs. CRA66, a small-fruited tomato which was used as the main source of resistance in INRA, Guadeloupe, presented heterogeneous bands at *Bwr-6* markers *SLM6-94* and *SLM6-110* and a unique band at *SLM6-94*, and also showed relatively high resistance to Pss1632 (62% and 56% survival in 2017 and 2018, respectively; Table 1). The origin of CRA66 is controversial, and while some suggest it is a local landrace from Guadeloupe, others speculate that it is derived from OTB2, a large-fruited line developed in Japan and based on North Carolina line NC1953-4N. OTB2 is segregating for bacterial wilt resistance and fixed for fusarium wilt resistance (Daunay et al., 2010). In a previous study conducted at WorldVeg, Taiwan, Ho et al. (2013) found that CRA 66 possessed resistance alleles at *Bwr-6* but not *Bwr-12*. In this study, we used CRA 66-S seeds provided by Jay Scott from the University of Florida but Ho et al. (2013) used seed from Philippe Prior of INRA (coded CRA66P). If CRA66 is a landrace from the French West Indies, it is possible that there was some genetic diversity between the two seed sources which may explain the difference in results between the two studies. GA-1405, a selection from a cross between *S. lycopersicum* and *S. pimpinellifolium*, jointly released by the ARS/USDA and the University of Georgia, was homozygous for part of *Bwr-6* and showed high resistance against phylotype II. This entry was reported by (Jaworski et al. 1987) to be highly tolerant to an endemic strain belonging to race 1. It is likely that such entries showing susceptible reactions to Pss4 and Pss1632 are in fact resistant to specific pathogen strains where they were bred. All the Florida (Fla) entries were susceptible to the phylotype I strain, and only three out of six (Fla 973237-1, Fla 973243-1 and Fla 973579-1) were more resistant than Pant Bahar and L390 across both Pss1632 trials (Table 1). Although Phylotype II (*R. solanacearum*) is endemic in Florida, genetic diversity within the phylotype is probably large and screening and selection for resistance in Florida may have led to varieties resistant to different local strains. The introgression line LA3501, which carries a *S. pennellii* introgression segment on chromosome 6 near the *Bwr-6* region, was susceptible to Pss4 but was previously reported to be resistant to another phylotype I strain, Pss186 (race 1 biovar 4; Hai et al., 2008). This may indicate potential strain-specific nature of resistance in LA3501. Such entries with potential strain-specific resistance may be utilized to supplement or enhance broad-based resistance of more stable sources like H7996. Additional collaborative research is needed to test these resistance sources against a broader array of diverse *Ralstonia* strains to identify any strain-specific resistances so as to utilize them in future breeding to enhance spectrum of resistance against *RSSC*.

It is interesting that *Bwr-6* and *Bwr-12* were common in homozygous or heterogeneous states in many entries, supporting the likelihood of frequent exchange among bacterial wilt breeding programs over the years (Daunay et al., 2010). Yet, only a few entries were homozygous for both QTLs, and this suggests the difficulty of combining and fixing multiple bacterial wilt resistant QTLs through conventional disease screening (Foolad & Panthee, 2012). Availability of effective co-dominant markers linked to *Bwr-12* and *Bwr-6* enables effective early generation selection and has equipped WorldVeg and other programs to incorporate these important resistance QTLs in new breeding lines.

Although *Bwr-6*- and *Bwr-12*-associated QTLs were present in the majority of the bacterial wilt resistant tomato entries evaluated in this study and a few other recent studies (Hanson et al., 2016; Ho et al., 2013; Wang et al., 2013), the exact source of these QTLs has not been determined. The reasons for this include a lack of exact pedigree information for the majority of the resistant entries, including those for Hawaii; frequent exchange of resistant sources between major bacterial wilt breeding programs in the world; and limited availability of resistant tomato sources when breeding for resistance to bacterial wilt began (Daunay et al., 2010). Finding strong bacterial wilt resistance in sources without *Bwr-12* and *Bwr-6* may be challenging, and it will be prudent for breeders to exhaustively exploit all unique sources of bacterial wilt resistance that are available to them. Out of 15 CRA 84- prefixed entries, eight were heterogeneous for *Bwr-12* or all or parts of *Bwr-6* which seems surprising since they are all inbred lines. However, it is important to consider that most of these entries were bred before *Bwr-12* and *Bwr-6* were mapped or markers were designed. Progeny selection would depend on disease screening assays. Bacterial wilt resistance is often incomplete, and wilted plants could occur even for highly resistant entries such as H7996, which is homozygous at both *Bwr-12* and *Bwr-6*; thus, attaining homozygosity of bacterial wilt resistance alleles was difficult. Development of lines using a single plant heterozygous for bacterial wilt resistance QTLs followed by repeated self-pollination without further selection would eventually have reduced the frequency of heterozygotes and resulted in lines composed of mixtures of genotypes homozygous for susceptible or resistance alleles (heterogeneous).

Wang et al. (2013) determined that *Bwr-12* contributed to stable resistance against phylotype I but not phylotype II strains. In this study, most entries homozygous for *Bwr-12* also performed relatively well against the phylotype I strain (Table 3). However, some entries homozygous for *Bwr-12* such as TBL-1 to TBL-4 performed poorly and the reasons for this are unclear. It is possible that they may have been some recombination in close proximity to the locus or they may contain different alleles at the *Bwr-12* locus, a point that needs further investigation. In our trials, *Bwr-12* did not provide significant resistance to the phylotype II strain which is in agreement with Wang et al. (2013). Ho et al. (2013) tested 16 bacterial wilt resistant tomato entries for reactions to three phylotype I strains differing in virulence and demonstrated that the presence of both *Bwr-6* and *Bwr-12* QTLs provided stable resistance compared to entries with *Bwr-12* alone. However, in contrast to the indication of Wang et al. (2013) that *Bwr-12* has a larger effect on resistance against phylotype I strains than *Bwr-6*, we did not observe differences between *Bwr-6* and *Bwr-12* against the phylotype I strain in this study. It is possible that higher temperatures in this study may have weakened the expression of *Bwr-12*, thus making it less effective against the phylotype I strain. This study also found that entries containing both *Bwr-6* and

Bwr-12 (Group 3) performed better overall against the phylotype I strain and were better than entries with either *Bwr-6* or *Bwr-12*, alone, which may indicate an additive or complementary effect between the two QTLs. In fact, all the Group 3 entries were among the most resistant entries against phylotype I (Table 3). Wang et al. (2013) had demonstrated *Bwr-6* to be effective against a few phylotype I strains and one phylotype II (race 3) strain. In this study, the effectiveness of *Bwr-6* against phylotype I and phylotype II was observed in both years which was consistent with that observed by Wang et al. (2013).

Breeding tomato for resistance to bacterial wilt is difficult because resistance is often dependent on pathogen strain and is highly affected by environmental factors (Hanson et al., 1996; Lopes et al., 1994; Prior et al., 1990). Thus, identification of new sources of stable resistance that are useful on a global scale against this genetically diverse pathogen is a high priority. We evaluated sixty-seven tomato entries indicated by donors to be sources of bacterial wilt resistance that were collected or received and maintained at the WorldVeg, Taiwan; these entries were tested for disease reaction, at high temperatures in the greenhouse, against phylotype I and phylotype II strains of *Ralstonia*, using drench inoculation assay. We further characterized these resistance sources for the presence of two major bacterial wilt resistant QTLs, *Bwr-12* and *Bwr-6*. Combining the results, we are able to identify novel resistance sources that performed well under high temperature which do not have the known QTLs. As new bacterial wilt resistance QTLs are mapped and markers designed, pyramiding multiple bacterial wilt resistance QTLs into new varieties should be more straightforward, thereby increasing the chances of obtaining stable resistance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

PH, YH and SL designed the experiments. SK and SL coordinated the greenhouse experiments. SK and YH coordinated the genotyping experiments. SK, PH and JW analysed the data. SK and PH wrote the manuscript. JW, JJ, SH and MP edited the manuscript.

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