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Resistance to Taiwanese race 1 strains of *Ralstonia solanacearum* in wild tomato germplasm

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Abstract A total of 252 wild *Solanum* accessions and one population of 49 introgression lines of LA716 were screened for resistance to a race 1/biovar 4/ phylotype I strain Pss186 of *Ralstonia solanacearum*. Most wild tomato accessions were highly susceptible. However, five accessions of *S. pennellii*, i.e. LA1943, LA716, LA1656, LA1732 and TL01845 were resistant to strain Pss186. These accessions were then challenged against two other race 1/phylotpye I strains Pss4 and Pss190, which were more aggressive. All the five *S. pennellii* accessions were susceptible to Pss4, but displayed high to moderate resistance to Pss190 with a percentage of wilted plants ranging from 0% to 60%. Pss190 is an aggressive strain that made a resistant tomato line Hawaii 7996 susceptible. Thus, the results found in this study provide evidence of the presence of strain-specific resistance. LA3501, which has an introgression segment on chromosome 6, was found to be resistant to Pss186 among the screened introgression lines. This confirms the importance of resistance trait loci on chromosome 6 that have been identified by other studies. This is the first report of *S. pennellii* being resistant to bacterial wilt. These new resistant sources will provide breeders with more resources to breed for stable resistance to bacterial wilt of tomato.

Keywords Bacterial wilt · Germplasm evaluation · Strain-specific

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Introduction

Bacterial wilt caused by race 1 strains of *Ralstonia solanacearum* is one of the most important diseases that limit tomato production in the tropics and subtropics. As a soil-borne pathogen, the bacteria enter tomato plants through the roots, colonize vascular tissue and cause wilting symptoms (Denny 2006). Race 1 strains are difficult to control because of their soil-borne nature and their extremely wide host range including many weeds which favour its widespread distribution and persistence in the environment (Hayward 2000). Chemical control for soil-borne diseases is usually unsuccessful and uneconomical and the only commercial pesticides available for controlling *R. solanacearum* are chemical fumigants. Thus, host plant resistance has been a major strategy for managing bacterial wilt in tomato.

Resistance sources to bacterial wilt in tomato have been identified and cultivars with different levels of resistance have been developed (Scott et al. 2005).

However, breeding for stable resistance is challenging due to the fact that resistance in tomato to bacterial wilt can be location-specific (Hanson et al. 1996). *Ralstonia solanacearum* is highly heterogeneous. It has been divided into five races based on their host range and into six biovars based on their ability to utilize specific sugar alcohols and disaccharides (Denny 2006). Recently, four phylotypes were distinguished based on sequence analysis of the internal transcribed spacer region, the endoglucanase (*egl*) gene, and the *hrpB* gene (Fegan and Prior 2005). In this study, we focus on strains that have been characterized as race 1, biovar 3 or 4, and phylotype I isolated from tomato in Taiwan. These strains displayed different level of aggressiveness according to Jaunet and Wang (1999).

Tomato accessions have been evaluated with different strains and biovars of race 1 of *R. solanacearum* and with different screening methods, and only few accessions have been found to be resistant (Gonzalez and Summers 1996; Jaworski et al. 1987). There are only a few *Solanum pimpinellifolium* or *S. lycopersicum* accessions being used as sources of resistance in breeding programmes globally (Scott et al. 2005). Thus, there is a need to identify more diverse resistance sources to help overcome the highly variable strains of the pathogen.

Resistance to plant pathogens has been identified from several sources of wild tomato germplasm (Egashira et al. 2000; Pico et al. 2000; Pim et al. 1993; Rosello et al. 1999). In addition, several potential sources for resistance to tomato bacterial disease that can be easily crossed with *S. lycopersicum* cultivars have been used successfully for genetic studies (Astua-Monge et al. 2000; Francis et al. 2000). However, systemic evaluations of wild tomato germplasm for resistance to bacterial wilt have not been conducted. For example, Jaworski et al. (1987) evaluated 2,064 tomato accessions in the field with natural and artificial inoculation of indigenous strains of race 1 biovar 1, including 72 *S. pimpinellifolium*, 60 *S. peruvianum*, 4 *S. habrochaites*, and 6 *S. habrochaites* f. *glabratum* (previous known as *L. hirsutum* f. *glabratum*). In this study GA 1405-1-2 BWT, a selection from PI 251323 (*S. pimpinellifolium*) was the only wild accession among the four selected resistant materials.

Therefore, the aim of this study is to evaluate two core collections of wild tomato germplasm for resistance to race 1, phylotype I strains of *R. solanacearum*. First, a strain with lower aggressiveness was used. Then selected resistant accessions were evaluated with two other strains with higher aggressiveness. Because the selected resistant accessions were mostly *S. pennellii*, a population of introgression lines of LA716 (*S. pennellii*) was evaluated in order to identify possible chromosomal locations of resistant QTL.

Materials and methods

Plant materials

Two core collections of wild tomato were evaluated in this study in order to cover the majority of tomato gene pools available. They included 109 accessions from the wild tomato core collection of the AVRDC—The World Vegetable Center (AVRDC) and 143 accessions from the core collection of the C. M. Rick Tomato Genetic Resource Center (TGRC) located at the University of California, Davis. Overall, there were 252 accessions evaluated including 14 accessions of *S. cheesmaniae*, 17 of *S. chilense*, 10 of *S. chmielewskii*, 52 of *S. lycopersicum*, 37 of *S. habrochaites*, 10 of *S. neorickii*, 19 of *S. pennellii*, 42 of *S. peruvianum*, and 51 of *S. pimpinellifolium*. All seeds of tested accessions were provided by the Genetic Resource and Seed Unit (GRSU), AVRDC. The TGRC core collection includes accessions representing the genetic diversity of wild tomato and was established by the late Dr. Charles M. Rick. The collection was acquired and multiplied by GRSU at AVRDC. A population of 50 introgression lines that derived from a cross between *S. lycopersicum* cv M82 and *S. pennelli* LA716 (Eshed and Zamir 1994), and the two parents were evaluated to explore the possible chromosomal locations of QTL associated with resistance to *R. solanacearum*. However, line LA3487 (IL3-2), which contains a chromosome 3 segment of *S. pennellii* failed to set and was not tested due to lack of seed.

In each evaluation, Hawaii 7996 (H7996) and L390 were used as resistant and susceptible controls, respectively. Before sowing, seeds were treated by soaking in 0.25× Clorox (6% sodium hypochlorite) for 5 min and then rinsed under running water for

15 min and sown immediately in 5 cm diam pots. Seeds of H7996 and L390 were sown 2 days later than the wild accessions and introgression lines. The potting mixture used consisted of sand, soil, rice husks and compost in the ratio of 1:3:1:1 which had been steam-sterilized. Three week-old seedlings with four fully expanded true leaves were used for the evaluation.

Bacterial strains and plant inoculation

Strains of *R. solanacearum* Pss4 (race 1, biovar 3), Pss186 (race 1, biovar 4), and Pss190 (race 1, biovar 4) isolated from tomato were provided by the Bacteriology Unit, AVRDC. These strains have been characterized as phylotype I using the phylotype-specific multiplex PCR developed by Fegan and Prior (2005; Jaw-Fen Wang, unpublished data). These strains were used because of their difference in aggressiveness. Overall, Pss190 was the most aggressive followed by Pss4 and then Pss186 (Jaunet and Wang 1999). When screening wild tomatoes in the preliminary evaluation and LA716 introgression lines, strain Pss186 was used. The preliminary evaluation was conducted over seven batches from February 8, 2006 to July 21, 2006. Pss4 and Pss190 were used later to evaluate the stability of the resistance of the selected accessions.

Stored cultures of Pss4, Pss186 and Pss190 kept at −80°C were streaked on tetrazolium medium (TTC; Kelman 1954) and incubated at 30°C for 2 days. Several typical fluidal single colonies from TTC plates were transferred to 523 medium (Kado and Heskett 1970) for multiplication at 30°C for 24 h. Then a dense bacterial suspension of each strain was prepared. A total of 0.1 ml of the suspension was spread on one fresh 523 plate and kept at 30°C for 24 h. Bacterial masses were harvested and suspended with distilled water until an O.D. value reached 0.3 at the wavelength of 600 nm (about 10^8 CFU ml^{-1}). Seedlings with four fully expanded true leaves (about 3 weeks old) were inoculated by pouring 20 ml of the above suspension on the soil surface of each pot. When evaluating the introgression lines in the field, the suspension was further diluted five times with distilled water (approximately 2×10^7 CFU ml^{-1}).

For seedling evaluations, inoculated plants were rated at 1, 2, 3 and 4 weeks after inoculation using a 0–5 scale; where 0=no symptoms, 1=one leaf partially wilted, 2=2–3 leaves wilted, 3=4 or more leaves wilted, 4=all leaves wilted, 5=plant dead (Winstead and Kelman 1952). The percentages of wilted plants (PWP) at 4 weeks after inoculation (wai) were calculated following the formula $PWP=(N_W/N_T)\times100$, where N_T is number of total tested plants and N_W is number of wilted plants. Plants in the field experiment were rated once a week after transplanting using a 0–5 scale; where 0=no symptoms, 1=<20% leaves wilted, 2=20% to <60% leaves wilted, 3=60% to <100% leaves wilted, 4=all leaves wilted, 5=plant collapsed or dead. The PWP at 6 weeks after transplanting was calculated.

The relative area under the disease progress curve (RAUDPC; Fry 1978) was calculated in the following manner: First, PWP was used to calculate AUDPC, which expresses the dynamics of disease development according to Shaner and Finney (1977), following the formula $AUDPC = \sum_{i=1\,\mathrm{to}\,n-1}[(Y_{i+1}+Y_i)/2]\times[X_{i+1}-X_i]$, where Y_i is PWP at the ith observation (i=1 being the first observation point), and X_i is time at the ith observation, and n is total number of observations. Second, AUDPC was then divided by the number of days from inoculation to the end of observation period.

To determine the presence of the pathogen in plants showing no symptoms at the time of final rating, a printing method was used. Plants showing no symptoms were harvested, and the roots and all leaves were removed. The remaining stem was washed with tap water, rinsed in distilled water, sprayed with 70% alcohol, and blotted dry on paper towels. Each plant was sectioned at the stem midpoint and 2 cm from the stem tip with a sterilized razor blade. The cut surface of the top section and the lower cut surface of the middle section were pressed tightly on the plate surface for 5 s per print and five prints were made continuously for each cut surface. The medium used was a semi-selective medium of *R. solanacarum* called SM1 (Tsai et al. 1985). The SM1 plates were incubated at 30°C for 3 days. When a fluidal bacterial mass was observed on at least one print, the sample was scored as positive for pathogen colonization; then the percentage of colonized plants (PCP) was calculated for each section following the formula $PCP=((N_C+N_W)/N_T)\times100$, where N_T is number of total plants, N_W is number of wilted plants, and N_C is number of plants showing positive colonization. If there were doubts about the identity of the cultured

bacteria, a bacterial mass was streaked on SM-1 for observing typical colonies of the pathogen.

Experimental design and data analysis

The accessions of wild tomatoes were evaluated over seven batches with 1 week between batches due to limited space in the greenhouse. Each evaluation was laid out as a randomized complete block design (RCBD). Accessions with a final PWP equal or <60% were selected for confirmation. Four confirmation experiments were conducted over time at different seasons and greenhouse locations and against different pathogen strains (Table 2). When more than one strain was used in one experiment, a split-plot design was followed with 'strain' as the main-plot and 'plant materials' as the subplot. Screening of introgression lines of LA716 at the seedling stage was conducted in the same way. All experiments consisted of two replications and ten plants per replication. When evaluating LA716 introgression lines in the field, RCBD was followed with three replications and 12 plants per replication at a spacing of 60 cm between lines and 40 cm between rows in a plot size of 2.4×1 m. Basal fertilizer (15% N, 15% P_2O_5, 15% K_2O, and 4% MgO) was broadcast at a rate of 120 kg ha^{-1} over the field before bulking up the beds.

All percentage data were transformed by arcsine square-root for the analyses of variance. In the combined analysis of variance across experiments, and in experiments involving more than one strain where the split-plot design was used, the data were analyzed using the Proc Mixed procedure of SAS (SAS Institute, Inc., Cary, NC, USA). The entry mean comparison was performed under each experiment or strain when the entry × experiment or entry × strain was significant. The significant differences were determined at $P<0.05$ by LSD.

Results

Resistance to bacterial wilt in wild tomatoes

Over the seven screenings of wild tomato germplasm, the maximum temperature ranged from 31°C to 32.1°C and minimum temperature from 25.7°C to 27.3°C. These conditions were favourable for the development of bacterial wilt, which was indicated by the complete wilting of L390 plants in each test (Table 1). The similar disease pressure over batches resulted in a similar severity on the resistant control H7996, which had PWP at 4 wai ranging from 0% to 5%. The final PWP ranged from 10% to 100% among the 252 genotypes screened. Most accessions were highly susceptible with the exception of a few of S. *pennelli* and *S. chmielewskii* (Table 1). Accessions having PWP equal to or <60% were *S. chmielewskii* accession LA1317, and seven accessions of *S. pennellii*: LA1926, LA1943, LA716, LA1272, LA1656, LA1732, and TL01845.

Stability of selected resistant accessions

In order to evaluate the stability of resistance in selected wild accessions, the materials were evaluated under different environments and against different *R. solacearum* strains. Information about each experiment is summarized in Table 2. Experiments 1 and 2 were conducted in the summer. Experiment 1 was conducted in a screen-house without a temperature control facility. Experiment 2 was conducted in a

Table 1 Summary of preliminary screening of wild tomatoes over seven batches [temperature ranges over batches were 31–32.1°C (max.)/25.7–27.3°C (min.); RH ranged from 75.5–86.2% (max.) to 57.5–74.5% (min) over batches] for resistance to a *R. solanacearum* strain Pss186 (race 1/biovar 4/phylotype I)

Species[a]	Percentage of wilted plants	
	Range[b]	Mean[b]
S.cheesmanii (14)	90.0–100.0	98.7
S. chilense (17)	100.0	100.0
S. chmielewskii (10)	50.0–100.0	87.8
S. esculentum (52)	85.0–100.0	98.5
S. hirsutum (37)	88.9–100.0	99.4
S. parviflorum (10)	90.0–100.0	98.5
S. pennellii (19)	10.0–100.0	69.8
S. peruvianum (42)	90.0–100.0	99.2
S. pimpinellifolium (51)	80.0–100.0	97.5
Hawaii 7996 (resistant control)	0.0–5.0	3.0
L390 (susceptible control)	100.0	100.0

[a] The number of accessions screened of each species is indicated in parentheses.

[b] Values are ranges and means of percentages of wilted plants of accessions of each species and the controls over batches.

Table 2 Details of confirmation experiments

Experiment	Inoculation date (day/month/year)	Strain used	Max. T [a]	Min. T [a]	Max. RH[a]	Min. RH[a]
1	23/06/2006	Pss186	31.9±1.6	27.3±0.9	85.1±2.1	74.4±4.9
2	09/08/2006	Pss4, Pss186	28.9±1.6	26.4±0.8	90.9±3.8	55.8±5.0
3	13/10/2006	Pss4, Pss186, Pss190	30.5±1.8	25.0±2.5	87.4±6.3	68.4±4.0
4	26/12/2006	Pss190	29.0±2.2	25.7±1.4	62.2±8.2	55.9±6.5

[a] Values are means of each record from inoculation day until the last recording day; *max.* and *min. T* maximum and minimum temperature, *max*. and *min. RH* maximum and minimum relative humidity

glasshouse with a temperature control facility, and this made the temperature range narrower and the relative humidity (RH) range larger than in experiment 1. Experiment 3 was conducted in the autumn, while experiment 4 was in the early winter. Both experiments were conducted in a glasshouse with a heating facility. The reactions of selected accessions to Pss186 were repeated three times (Table 3). Combined analysis showed a significant interaction between experiment and entry. The respective controls, H7996 and L390, did not vary significantly over the experiments. In general, all tested accessions had similar reactions over the experiments, with the exception of LA716, LA1317, and LA1656. These accessions displayed a higher disease incidence in experiment 1. This could be due to the higher temperature and prolonged RH in this experiment. LA1317 had significantly higher disease incidence in experiment 1. LA1943 and LA1732 were more stable than the others against Pss186, as they had similar reactions as H7996 in two of the three confirmation experiments.

The five *S. pennellii* accessions, which showed a lower incidence to Pss186, were selected and inoculated with two other strains. The aggressiveness displayed by Pss4, Pss186, and Pss190 on L390 and H7996 was as expected (Table 4). Pss4 and Pss186 caused a similar percentage of wilting of H7996 plants (5% to 15%), and Pss190 caused a complete wilting of H7996. The entry × strain interaction was significant, indicating individual entries had different reactions against different strains. All entries were highly susceptible to Pss4 and showed similar reactions to Pss186 and Pss190 based on visual

Table 3 PWP of selected accessions 28 dai with Pss186 in three confirmation experiments

Entry	Experiment 1			Experiment 2			Experiment 3		
S. chmielewskii									
LA1317	80.0	b[a]	A[b]	25.0	cd	B	n.t.		
S. pennelli									
LA716	40.0	cd	A	35.0	cd	A	0.0	d	B
LA1272	64.3	c	A	55.0	bc	A	n.t.		
LA1656	40.0	cd	A	0.0	e	B	10.0	cd	AB
LA1732	20.0	de	A	55.0	bc	A	40.0	b	A
LA1926	55.0	cd	A	75.0	ab	A	n.t.		
LA1943	25.0	cde	A	35.0	cd	A	10.0	cd	A
TL01845	n.t.			20.0	de	A	25.0	bc	A
Hawaii 7996	5.0	e	A	0.0	e	A	15.0	bcd	A
L390	100.0	a	A	90.0	a	A	100.0	a	A

n.t. = not tested

[a] Means followed by the same lower case letters are not significantly different within the same column based on $LSD_{0.05}$ (within-experiment comparisons).

[b] Means followed by the same upper case letters are not significantly different within the same row based on $LSD_{0.05}$ (between-experiment comparisons).

Table 4 PWP, PCP-m and PCP-t of selected accessions 28 dai with Pss186, Pss190 and Pss4 in experiment 3

Entry	PWP									PCP-m						PCP-t					
	Pss4			Pss186			Pss190			Pss186			Pss190			Pss186			Pss190		
LA716	100.0	a[a]	A[b]	0.0	c	B	0.0	c	B	25.0	b	A	0.0	c	A	5.0	bc	A	0.0	c	A
LA1656	100.0	a	A	10.0	bc	B	15.0	bc	B	10.0	b	A	35.0	bc	A	5.0	bc	A	10.0	c	A
LA1732	100.0	a	A	40.0	b	B	50.0	b	B	45.0	b	A	55.0	b	A	40.0	b	A	40.0	b	A
LA1943	100.0	a	A	10.0	bc	B	20.0	bc	B	10.0	b	A	35.0	bc	A	0.0	c	A	10.0	c	A
T L01845	100.0	a	A	25.0	b	B	10.0	bc	B	45.0	b	A	15.0	bc	A	30.0	b	A	5.0	c	B
Hawaii 7996	5.0	b	B	15.0	bc	B	100.0	a	A	15.0	b	B	100.0	a	A	10.0	bc	B	100.0	a	A
L390	100.0	a	A	100.0	a	A	100.0	a	A	100.0	a	A	100.0	a	A	100.0	a	A	100.0	a	A

[a] Means in the same column followed by the same lower case letters are not significantly different based on $LSD_{0.05}$ (within-strain comparisons).

[a] Means in the same row followed by the same upper case letters are not significantly different based on $LSD_{0.05}$ (between-strain comparisons).

wilting symptoms. LA716 was the most resistant, and had no wilted plants against Pss186 and Pss190. The surviving plants were assayed to determine whether they were colonized by the pathogen. The PCP was always lower in the top-section than in the middle-section of the stems in plants of all entries inoculated with both strains. No resistant accessions were immune to *R. solanacearum*. For Pss186, five *S. pennellii* accessions had tolerances similar to H7996 resulting in a similar PCP. H7996 was highly susceptible to Pss190, while the *S. pennellii* accessions except LA1732 displayed good tolerance to Pss190 (0% to 10% colonized plants).

The reactions of selected accessions to Pss190 were confirmed in another experiment, and the combined analysis did not show significant interaction between experiment and entry (Table 5). The controls, H7996 and L390, were fully susceptible to Pss190 in the two experiments. LA1317 and LA1926 showed a susceptible reaction similar to the controls. Among the entries, LA716 and TL01845 were the most resistant over two experiments. Resistance to Pss190 in these wild tomatoes should be confirmed in summer under higher mean temperature and RH conditions to ensure its stability.

Reactions of LA716 introgression lines to Pss186

Under the greenhouse conditions (mean maximum temperature, 31.8°C; mean minimum temperature, 29.4°C), introgression lines (ILs) of LA716 were highly susceptible when inoculated with an inoculum density of 10^8 CFU ml^{-1}. The PWP of ILs ranged from 80% to 100%. All tested ILs showed similar severity and disease progress compared to M82 and the susceptible check L390 (data not shown). The presence of a high temperature range during this experiment may have contributed to this high severity, which was observed among the experiment with Pss186 (Table 3). Seedlings of the ILs were inoculated with a lower inoculum dose and transplanted to the field. Here, the mean temperature ranged from 26.6°C to 17.9°C and the total rainfall was 47 mm during the experiment. Judging from the control lines, the disease pressure in the field experiment was lower

Table 5 PWP of selected accessions inoculated with Pss190 in two confirmation experiments

Entry	Experiment 3		Experiment 4	
S. chmielewskii				
LA1317	n.t.		100.0	a
S. pennelli				
LA716	0.0	c[a]	15.0	bc
LA1272	n.t.		55.0	b
LA1656	15.0	Bc	25.0	b
LA1732	50.0	B	60.0	b
LA1926	n.t.		75.0	ab
LA1943	20.0	Bc	40.0	b
TL01845	10.0	Bc	0.0	c
Hawaii 7996	100.0	a	95.0	a
L390	100.0	a	100.0	A

n.t. = not tested

[a] Means followed by the same lower case letters are not significantly different in column-wide comparisons based on $LSD_{0.05}$ (within-experiment comparisons).

than that in the greenhouse experiment due to the lower temperatures and/or lower initial inoculum dose. Plants of *S. pennellii* LA716 were not adapted to the high soil moisture conditions found in the field. The experimental field was irrigated immediately after transplanting and it rained immediately afterwards (9 mm in the first 4 days), which prolonged the high soil moisture conditions. Only four plants per replication survived 1 week after transplanting; however, all plants of M82 and the ILs grew well in the experiment. The PWP of the introgression lines ranged from 27.8% to 100%; while those of LA716 and M82 were 25% and 86.1%, respectively. When evaluating the final PWP, only LA3501 had a significantly lower incidence than M82 and was not significantly different from LA716. Analyzing data of the RAUPDC, four ILs, LA3476, LA3501, LA3510, and LA3517, displayed significantly lower RAUPDC than M82. These four ILs also showed significantly higher disease progress than LA716 (Table 6). The results indicated possible QTL located in the introgressed segments present in these four lines presumably contributing to partial resistance.

Discussion

Planting resistant materials has been the main strategy to control tomato bacterial wilt caused by race 1 strains of *R. solanacearum*. However, limited resistance sources have been used in breeding programmes (Scott et al. 2005). This study evaluated resistance in 252 accessions of tomato germplasm belonging to nine species against three of race 1 phylotype I strains of *R. solanacarum*, and five accessions of *S. pennellii* were found to have significant tolerance to Pss186 and Pss190, but not Pss4.

The frequency of resistance sources found from tomato germplasm has been low as shown by previous studies. Jaworski et al. (1987) evaluated 2,064 tomato accessions against race 1/biovar 1 strains and identified only four selections to be highly tolerant. Among them, three selections were from *S. lycopersicum* and one from *S. pimpinellifolium*. Similarly, Gonzalez and Summers (1996) found five accessions to have some degree of resistance against two strains of race 1 biovar 1, and eight accessions were resistant to two strains of race 1 biovar 3 among 233 accessions screened. More recently, partial resistance to a strain of race 3 biovar2 phylotype II was detected in one accession belonging to *S. peruvianum*, and one *S. lycopersicon* var. *cerasiforme* accession among the 82 accessions screened (Carmeille et al. 2006b). Summarizing the results from previous studies, it may be worthwhile to more intensively evaluate the accessions of *S. lycopersicon* var. *cerasiforme*, *S. pimpinellifolium*, and *S. pennellii* for resistance to the pathogen.

The stability of selected resistance sources is a concern, as both temperature and strain can affect the final severity of bacterial wilt on tomato. It is known that several tomato varieties display a higher severity of bacterial wilt under higher temperatures in a controlled environment (Krausz and Thurston 1975)

Table 6 PWP and RAUPDC of selected introgression lines after inoculation with Pss186 in the field in comparisons with LA716 and M82

Entry	PWP[a]	LA716	M82	RAUPDC[b]	LA716	M82
LA3476 (IL1-1)	72.2	**	ns	54.6	**	*
LA3501 (IL6-2)	27.8	ns	**	28.5	*	**
LA3510 (IL8-1)	63.9	**	ns	47.6	**	**
LA3517 (IL10-3)	66.7	**	ns	53.2	**	*
Hawaii 7996	0.0	*	**	0.0	**	ns
L390	91.7	**	ns	73.4	**	ns
LA716	25.0			18.8		
M82	86.1			67.7		

Mean comparisons between LA716 and M82 with introgression lines by LSD (significant at $^{**}P<0.01$ and $^{*}P<0.05$)

ns not significant

[a] Means of final PWP (6 weeks after transplanting)

[b] Means of RAUPDC

and in the field (Prior et al. 1996). The resistance to Pss186 in *S. pennelli* LA1943, LA1926, LA1272, LA1732 and TL01845 was consistent under different seasons and environmental conditions. Another two *S. pennelli* LA716 and LA1656 as well as *S. chmielewskii* LA1317 could be sensitive to high temperature, as they expressed higher disease incidence, but not complete breakdown, in the first experiment, when the temperature was higher.

Strain-specific resistance was found in *S. pennellii* accessions. These accessions were resistant to Pss186 and Pss190, but not to Pss4. In tomato, the strain-specific nature of resistance to *R. solanacearum* has been reported (Krausz and Thurston 1975). Strain-specific QTL have already been identified in Hawaii 7996, a resistant tomato variety (Danesh and Young 1994; Wang et al. 2000). Therefore using well-characterized strains in screening is important for future resistance deployment. Hawaii 7996 was fully susceptible to Pss190 but resistant to Pss186 and Pss4. In previous studies, Hawaii 7996 was found to be highly resistant when evaluated in fields located in 11 countries and territories (Wang et al. 1997). However, it displayed partial resistance against a race 3/biovar 2/phylotype II strain isolated from potato in Reunion (Carmeille et al. 2006b) and three strains of a new pathogenic variant of *R. solanacearum* named phylotype II/4NPB, described recently (Wicker et al. 2007). Therefore, it is necessary to evaluate the new *S. pennellii* resistance sources reported here against strains with a wider diversity to determine their potential use.

Among the LA716 introgression lines, only LA3501 (IL6-2) showed a higher level of resistance than M82 and a similar level of resistance as LA716 against Pss186 in the field evaluation. The IL6-2 carried an introgression segment on chromosome 6, where the resistance gene *Bwr-6* is located (Carmeille et al. 2006a). Anchor RFLP markers in the segments, like TG25 (Danesh and Young 1994), TG153 (Carmeille et al. 2006a; Danesh and Young 1994; Mangin et al. 1999; Thoquet et al. 1996; Wang et al. 2000), TG162 and TG240 (Mangin et al. 1999; Wang et al. 2000) have been detected and are associated with resistance to bacterial wilt in Hawaii 7996 when it has been challenged with different strains of race 1 and race 3. Therefore, the resistance gene in this region could be essential for stable resistance to bacterial wilt in tomato. Due to missing LA3487 (IL3-2) in our study, we could not rule out the possible association of this chromosomal region with the resistance in LA716, although no QTL has been detected in this region.

Breeding a super tomato variety that could be resistant against all known tomato-pathogenic *R. solanacearum* strains seems unrealistic, particularly when more diverse strains continue to be reported. As our knowledge on the diversity of the strains increases, it is critical to know how these different strains are distributed globally and how they interact with known sources of resistance in tomato. This information could then be used to select the proper resistance sources and to set the geographical coverage of a breeding programme.

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