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Screening Cultivated Eggplant and Wild Relatives for Resistance to Bacterial Wilt (*Ralstonia solanacearum*)

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Abstract: Bacterial wilt, caused by *Ralstonia solanacearum*, is highly diverse and the identification of new sources of resistance for the incorporation of multiple and complementary resistance genes in the same cultivar is the best strategy for durable and stable resistance. The objective of this study was to screen seven accessions of cultivated eggplant (*Solanum melongena* L.) and 40 accessions from 12 wild relatives for resistance to two virulent *R. solanacearum* strains (Pss97 and Pss2016; phylotype I, race 1, biovar 3). The resistant or moderately resistant accessions were further evaluated with Pss97 in a second trial under high temperatures (and also with Pss2016 for *S. anguivi* accession VI050346). The resistant control EG203 was resistant to Pss97, but only moderately resistant to Pss2016. One accession of *S. sisymbriifolium* (SIS1) and two accessions of *S. torvum* (TOR2 and TOR3) were resistant or moderately resistant to Pss97 in both trials. *Solanum anguivi* VI050346, *S. incanum* accession MM577, and *S. sisymbriifolium* (SIS1 and SIS2) were resistant to Pss2016 in the first trial. However, *S. anguivi* VI050346 was susceptible in the second trial. These results are important for breeding resistant rootstocks and cultivars that can be used to manage this endemic disease.

Keywords: *Solanum melongena*; wild relatives; bacterial wilt strains; phylotype I; disease resistance

1. Introduction

Bacterial wilt (BW), caused by *Ralstonia solanacearum,* is one of the most economically important soil-borne diseases of tomato (*Solanum lycopersicum* L.) and eggplant (*Solanum melongena* L.) in the tropics and subtropics [1]. This pathogen enters plant roots through wounds and multiplies rapidly in the vascular system, so that the xylem elements are filled with bacterial cells which block the xylematic flow, leading to yellowing of foliage, general wilting, and eventually plant death [2]. It was first described by Smith (1896) in potato (*Solanum tuberosum* L.), tomato, and eggplant. Bacterial wilt causes great losses because of its severe symptoms, wide geographic distribution, and unusually broad host range, which includes more than 200 plant species belonging to 53 different families [3,4]. In addition, *R. solanacearum* can survive in soil for many years [5].

Ralstonia solanacearum grows well at a temperature range of 28–32 °C, but it is also found in cold weather in Europe and North America [6,7]. Phenotypically, *R. solanacearum* strains are divided into four phylotypes based on geographical regions: phylotype I strains originate from Asia and Africa, phylotype II from the Americas, phylotype III from Africa and the surrounding islands, and phylotype IV from Indonesia [8,9]. These phylotypes are able to infect eggplant and other important *Solanaceae* crops, such as potato, tomato, and pepper (*Capsicum annuum* L.) [10].

Several methods have been used to control BW, including soil disinfection, soil amendment, biological and chemical controls, and resistant cultivars or rootstocks for grafting [11–14]. Chemical control is not economically practical, especially in the field, due to the localization of the pathogen inside the xylem and its ability to survive at high depths in the soil [15]. To date, there are no bactericides available to efficiently control bacterial wilt [16,17]. Antibiotics such as penicillin, ampicillin, tetracycline, and streptomycin have been reported as having little efficacy in repressing *R. solanacearum* growth [18], particularly in open fields. Previous studies have reported that biological control using different strains of *Pseudomonas fluorescens*, *Bacillus subtilis*, *B. amyloliquefaciens*, and rhizobacteria could suppress soil-borne diseases, including bacterial wilt, but validation on a larger scale is still needed [19–21]. Breeding for resistance to bacterial wilt is still the most appropriate, economical, and environmentally promising strategy for controlling this pathogen [2,22]. However, the development of resistant cultivars has been hampered by polygenic inheritance, and sometimes the association of resistance with horticulturally detrimental traits associated with the wild species (linkage drag) [22,23].

Grafting onto resistant rootstocks could also provide an alternative solution to manage soil-borne pathogens, including bacterial wilt, in *Solanaceous* crops [24,25]. However, bacterial wilt resistance may vary with location, temperature, and strain differences of the pathogen [26]. Further identification of resistance sources to bacterial wilt for breeding, and introgression of resistance genes into eggplant rootstock/cultivar is the best strategy to improve the chances of durable resistance for managing bacterial wilt [2]. Eggplant is related to a large number of wild relatives, which are largely an unexplored source of resistance against bacterial wilt [25]. Therefore, we hypothesize that exploring the broad diversity of eggplant wild relatives for tolerance or resistance to bacteria may lead to the discovery of new sources of resistance that can be exploited for breeding new tolerant eggplant varieties or rootstocks [22]. In order to test this hypothesis, we evaluated seven accessions of cultivated eggplants and 40 accessions from 12 wild relatives for resistance to *R. solanacearum* strains, Pss97 and Pss2016, which form part the predominant virulence group in Taiwan, in order to identify sources of resistance which could be of interest for developing new rootstocks and for breeding in this crop.

2. Material and Methods

The experimental investigation reported here was carried out at the greenhouses of the World Vegetable Center (WorldVeg), Taiwan.

2.1. Seeds and Plant Growth Conditions

Seeds of seven accessions of cultivated eggplant and 40 accessions of 12 wild relatives of eggplant were obtained from the genebanks of Universitat Politècnica de València (Spain) and the World Vegetable Center (WorldVeg) (Table 1). Eggplant accessions EG203 and EG048 were used as resistant and susceptible checks, respectively. Before sowing, seeds were soaked in water for one day, and then another day in 500 ppm GA_3 (Gibberellic acid) to improve seed germination [27]. After these treatments, seeds were washed with water and directly sown in 3 inch diameter plastic pots containing a steam sterilized soil mixture (3:1:1:1 ratio of soil, rice hulls, sand, and compost) and moved to WorldVeg's greenhouse (16/8 h day/night). Temperature ranged from 23 °C to 36 °C and relative humidity from 81.5% to 84.1% during the evaluation trials. Seedlings were watered daily and fertilized weekly with an NPK (Nitrogen, Phosphorus, Potassium) 15-15-15 fertilizer. Four-week-old plants (four to six fully expanded true leaves) were tested for *R. solanacearum* resistance, as described below.

Table 1. Cultivated eggplant and wild relatives used for evaluation for resistance to bacterial wilt.

Taxa and Accession Code [a]	Country of Origin	Genepool	Accession Code in Germplasm Collection
S. anguivi			
ANG1	Ivory coast	Secondary	BBS119
ANG2	Ivory coast	Secondary	BBS125/B
VI048764	Thailand	Secondary	VI048764
VI050346	Unknown	Secondary	VI050346
VI050392	Unknown	Secondary	VI050392
S. campylacanthum			
CAM5	Tanzania	Secondary	MM680
CAM6	Tanzania	Secondary	MM700
CAM8	Kenya	Secondary	MM1426
S. dasyphyllum			
DAS1	Uganda	Secondary	MM1153
S. elaeagnifolium			
ELE1	Senegal	Tertiary	MM1627
ELE2	Greece	Tertiary	ELE2
S. incanum			
MM577	Israel	Primary	MM577
INC1	Israel	Primary	MM664
S. insanum			
INS1	Sri lanka	Primary	SLKINS-1
INS2	Sri lanka	Primary	SLKINS-2
INS3	Japan	primary	MM498
VI034853	Malaysia	Primary	VI034853
VI037989	Thailand	Primary	VI037989
VI040123	Thailand	Primary	VI040123
VI040350	Thailand	Primary	VI040350
VI041106	Thailand	Primary	VI041106
VI041189	Thailand	Primary	VI041189
VI054957	Lao People's Democratic Republic	Primary	VI054957
VI054962	Lao People's Democratic Republic	Primary	VI054962
VI054964	Lao People's Democratic Republic	Primary	VI054964
VI054967	Lao People's Democratic Republic	Primary	VI054967
VI046583	Vietnam	Primary	VI046583
S. lichtensteinii			
LIC1	South Africa	Secondary	MM674
LIC2	Iran	Secondary	MM677
S. linnaeanum			
LIN1	Spain	Secondary	JPT0028
LIN3	Tunisia	Secondary	MM195
VI042691	Italy	Secondary	VI042691
VI042692	Italy	Secondary	VI042692
VI042740	Colombia	Secondary	VI042740
S. melongena			
MEL1	Ivory coast	Cultivated	BBS-118/B
MEL2	Ivory coast	Cultivated	BBS-146
MEL3	Ivory coast	Cultivated	BBS-175
MEL4	Sri Lanka	Cultivated	7145
MEL5	Sri Lanka	Cultivated	8104
MEL6	Sri Lanka	Cultivated	Ampara
ANS26	Spain	Cultivated	ANS26
S. pyracanthos			
PYR1	Unknown	Secondary	SOLN-66
S. sisymbriifolium			
SIS1	Unknown	Tertiary	SOLN-78
SIS2	Unknown	Tertiary	1180
S. tomentosum			
TOM1	South Africa	Secondary	MM992
S. torvum			
TOR2	Sri Lanka	Tertiary	SLKTOR-2
TOR3	Unknown	Tertiary	55953

S. melongena (Checks)			
EG048	Denmark	Cultivated	VI046095
EG203	India	Cultivated	VI045276

[a] Accessions with VI codes are from the World Vegetable Center genebank, while the others are from Universitat Politècnica de València.

2.2. Pathogen and Resistance Assays in First Trial

Inoculations were conducted with two virulent *R. solanacearum* strains (Pss97 and Pss2016). The Pss97 strain was isolated from infected eggplants from Pingtung County of southern Taiwan in 1991, and belongs to the predominant virulence group in Taiwan. Pss2016 was isolated from infected tomatoes grafted on eggplant rootstocks from Yilan County of northern Taiwan in 2015. Both strains were identified as phylotype I, race 1, biovar 3, based on identification conducted through host range [28], biovar test [29–31], and molecular markers [8] at the Bacteriology unit of WorldVeg. Bacterial strains stored at −80 °C were cultured on a 2,3,5-triphenyl tetrazolium chloride-amended (TTC) medium [32] and incubated at 30 °C for two days. Then, several typical fluid white colonies with pink centers from TTC medium were transferred to 523 medium [33] and incubated at 30 °C overnight for multiplication. The bacterial mass from overnight cultures was transferred and suspended in water, and the concentration was adjusted to an optical density of 0.3 at 600 nm wavelength (about 10^8 cfu/mL).

Roots of accessions and checks were injured with a knife by cutting through the soil 1–2 cm away from the stem base before inoculation. The inoculum volume was determined as the ratio between bacterial suspension and potting mixture, to a proportion 1:10 (*v*/*v*). Hence, 30 mL of bacterial suspension (10^8 cfu/mL) was poured into each pot (3 inch) and the inoculated plants were kept in a plastic greenhouse [34]. Plants were watered in excess two times a day after inoculation to maintain the soil moisture high. Two plants per accession and check without inoculation were used as negative controls. Plants were arranged according to a randomized complete block design (RCBD), with three replications and eight plants per accession in each replication (24 plants per accession and resistant and susceptible checks). All accessions and check plants were kept in a greenhouse after inoculation (28.4 ± 2.0 °C, 16/8 h day/night, and humidity 81.5 ± 2.0 %) and the bacterial wilt severity was evaluated once a week for four weeks using wilting percentage (W%) and disease index (DI), based on a disease rating scale (0–5) (Figure 1), where 0 = no symptoms, 1 = one leaf partially wilted, 2 = two or three leaves wilted, 3 = all leaves wilted except the top two or three leaves, 4 = all leaves wilted, 5 = plant dead [35].

Wilting percentage (W%) was calculated following the formula: W% = (Nw/Nt) × 100, where Nw = number of wilted plants, and Nt = total number of plants. The disease index (DI; %) was calculated using the following formula: DI = ((N0 × 0 + N1 × 1 + N2 × 2 + N3 × 3 + N4 × 4 + N5 × 5)/(Nt/5)) × 100, where N0 to N5 = number of plants with disease rating scale values from 0 to 5, and Nt = total number of plants. The resistance reaction of accession was based on the W% and DI at the fourth week after inoculation (WAI), and categorized by DI at the fourth WAI. Accessions with DI from 0% to 30% were considered resistant (R), above 30% to 40% were moderately resistant (MR), above 40% to 50% were moderately susceptible (MS), and over 50% as susceptible (S) [36].

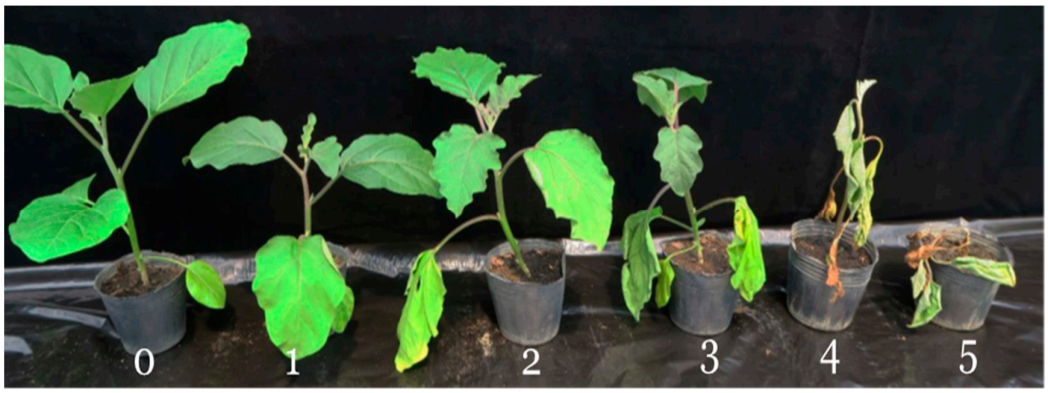

Figure 1. Disease rating scale 0–5 according to [35], where 0 = no symptoms, 1 = only one leaf partially wilted, 2 = two or three leaves wilted, 3 = all leaves except two or three wilted, 4 = all leaves wilted, and 5 = plant dead.

2.3. Evaluation of Resistant and Moderately Resistant Accessions in Second Trial

Accessions classified as R or MR, based on DI ≤ 40%, as described above, along with checks were re-evaluated using the same resistance screening protocol in the same season to confirm the resistance in accessions identified at WorldVeg's greenhouse under high temperatures (34.8 ± 2.0 °C, 16/8 h day/night, humidity 84.1 ± 2.0%). Due to limited seed numbers and low germination rates in accessions of *S. incanum* (MM577), *S. sisymbriifolium* (SIS1 and SIS2), and *S. torvum* (TOR2 and TOR3), these accessions were not re-evaluated for resistance to Pss97 in the second trial.

3. Results

The resistance reaction and category of seven accessions of cultivated eggplant and 40 accessions of wild relatives against *R. solanacearum* strains Pss97 and Pss2016 at four weeks of inoculation is presented in Table 2. The susceptible check (EG048) displayed the expected reactions to strains Pss97 and Pss2016. In this way, all EG048 plants wilted and died rapidly two and three weeks after inoculation by Pss97 and Pss2016, respectively. The resistant check (EG203) was resistant to Pss97, with less than 10% of W% and DI, and moderately resistant and moderately susceptible to Pss2016, with 50% and 62.5% of W% and 31.1% and 48.8% of DI in the two trials.

Table 2. Evaluation of resistance of 47 cultivated and wild relatives of eggplant accessions against *Ralstonia solanacearum* strains Pss97 and Pss2016 at four weeks after inoculation.

Taxa and Accession Code [a]	Pss97			Pss2016		
	W% ± SE [b]	DI ± SE [c]	Resistance Category [d]	W% ± SE	DI ± SE	Resistance Category
Solanum anguivi						
VI050346	100 ± 0	100 ± 0	S	41.7 ± 11.0	23.3 ± 9.8	R
ANG1	100 ± 0	96.7 ± 3.3	S	95.8 ± 4.2	45.8 ± 14.0	MS
ANG2	100 ± 0	100 ± 0	S	100 ± 0	59.2 ± 6.0	S
VI048764	100 ± 0	92.5 ± 3.8	S	100 ± 0	91.7 ± 4.2	S
VI050392	100 ± 0	100 ± 0	S	95.8 ± 4.2	66.7 ± 9.8	S
S. campylacanthum						
CAM5	95.8 ± 4.2	95.8 ± 4.2	S	95.8 ± 4.2	51.7 ± 3.6	S
CAM6	100 ± 0	97.5 ± 2.5	S	ND	ND	
CAM8	90.5 ± 9.5	83.6 ± 9.9	S	100 ± 0	70 ± 1.4	S
S. dasyphyllum						
DAS1	100 ± 0	100 ± 0	S	100 ± 0	100 ± 0	S
S. elaeagnifolium						
ELE1	100 ± 0	98.7 ± 1.3	S	83.3 ± 8.3	52.5 ± 3.8	S
ELE2	100 ± 0	100 ± 0	S	66.7 ± 6.7	61.3 ± 9.6	S
S. incanum						

MM577	100 ± 0	95 ± 2.5	S	66.7 ± 8.3	23.3 ± 7.4	R
INC1	100 ± 0	100 ± 0	S	100 ± 0	76.7 ± 5.7	S
S. insanum						
INS1	100 ± 0	98.3 ± 1.7	S	100 ± 0	96.7 ± 3.3	S
INS2	95.8 ± 4.2	82.5 ± 6.6	S	100 ± 0	74.2 ± 2.2	S
INS3	100 ± 0	100 ± 0	S	ND	ND	
VI034853	100 ± 0	93.3 ± 4.4	S	95.8 ± 4.2	89.2 ± 1.7	S
VI037989	100 ± 0	100 ± 0	S	100 ± 0	89.2 ± 1.7	S
VI040123	100 ± 0	97.5 ± 2.5	S	95.8 ± 4.2	82.5 ± 10.1	S
VI040350	100 ± 0	100 ± 0	S	95.8 ± 4.2	86.7 ± 3	S
VI041106	100 ± 0	93.3 ± 4.4	S	100 ± 0	90 ± 3.8	S
VI041189	100 ± 0	91.7 ± 8.3	S	100 ± 0	75.8 ± 5.5	S
VI054957	100 ± 0	100 ± 0	S	100 ± 0	92.5 ± 5.2	S
VI054962	100 ± 0	95 ± 3.8	S	100 ± 0	93.3 ± 3	S
VI054964	95.8 ± 4.2	90.8 ± 6.8	S	100 ± 0	82.5 ± 6.6	S
VI054967	100 ± 0	97.5 ± 1.4	S	62.5 ± 31.5	89.2 ± 6.5	S
VI046583	100 ± 0	97.5 ± 2.5	S	91.7 ± 8.3	50.3 ± 2.6	S
S. lichtensteinii						
LIC1	100 ± 0	100 ± 0	S	95.8 ± 4.2	90 ± 6.6	S
LIC2	100 ± 0	100 ± 0	S	100 ± 0	55 ± 7.6	S
S. linnaeanum						
LIN1	95.8 ± 4.2	95.8 ± 4.2	S	100 ± 0	86.7 ± 4.4	S
LIN3	100 ± 0	100 ± 0	S	100 ± 0	91.7 ± 5.1	S
VI042691	100 ± 0	100 ± 0	S	100 ± 0	76.7 ± 7.1	S
VI042692	100 ± 0	100 ± 0	S	100 ± 0	77.5 ± 2.9	S
VI042740	100 ± 0	100 ± 0	S	95.8 ± 4.2	79.2 ± 3.6	S
S. melongena						
MEL1	100 ± 0	97.5 ± 2.5	S	100 ± 0	100 ± 0	S
MEL2	75 ± 12.5	100 ± 0	S	100 ± 0	81.1 ± 3.9	S
MEL3	100 ± 0	100 ± 0	S	100 ± 0	89.2 ± 4.2	S
MEL4	100 ± 0	98.3 ± 1.7	S	100 ± 0	100 ± 0	S
MEL5	91.7 ± 8.3	75.8 ± 8.7	S	100 ± 0	89.2 ± 0.8	S
MEL6	100 ± 0	96.7 ± 3.3	S	100 ± 0	100 ± 0	S
ANS26	100 ± 0	100 ± 0	S	100 ± 0	100 ± 0	S
S. pyracanthos						
PYR1	100 ± 0	100 ± 0	S	100 ± 0	84.2 ± 5.1	S
S. sisymbriifolium						
SIS1	33.3 ± 22	33.3 ± 22	MR	41.7 ± 4.2	12.5 ± 0	R
SIS2	37.5 ± 0	37.5 ± 0	MR	33.3 ± 4.2	9.2 ± 2.2	R
S. tomentosum						
TOM1	75 ± 0	71.7 ± 0.8	S	100 ± 0	95.8 ± 4.2	S
S. torvum						
TOR2	16.7 ± 11	5.8 ± 3.0	R	91.7 ± 8.3	64.2 ± 5.5	S
TOR3	12.5 ± 0	10.8 ± 1.7	R	38.9 ± 14.7	32.2 ± 16.4	MR
S. melongena(Checks)						
EG048	100 ± 0	98.3 ± 1.7	S	100 ± 0	88.3 ± 3.0	S
EG203	0 ± 0	0 ± 0	R	50 ± 9.6	31.1 ± 8.7	MR

[a] Accessions with VI codes are from the World Vegetable Center genebank, while the others are from Universitat Politècnica de València. [b] Indicates the means of three replications of wilt percentage (W%) at the fourth week after inoculation. Means followed by ± standard error (± SE). [c] Indicates the means of three replications of disease index (DI) at fourth week after inoculation. Means followed by ± standard error (± SE). [d] Resistance category according to the DI at the fourth week after inoculation. R = resistant (0–30%), MR = moderately resistant (>30–40%), MS = moderately susceptible (>40–50%), S = susceptible (>51%). ND indicates no data due to the limited number of seeds or low germination.

None of the evaluated eggplant genotypes were immune or highly resistant to both strains. All accessions of cultivated eggplant were susceptible to Pss97 and Pss2016, with a range 75.8–100% of W% and 81.1–100% of DI, respectively. Of the 40 wild accessions screened for resistance to Pss97 in the first trial, two accessions—TOR2 and TOR3, of *S. torvum*—were resistant, with 16.7% and 12.5 % of W% and 5.8% and 10.8% of DI, respectively. Two accessions—SIS1 and SIS2, of *S. sisymbriifolium*—were moderately resistant, with 33.3% and 37.5% of W% and 33.3% and 37.5% of DI, respectively. In

the first trial, *S. anguivi* VI050346, *S. incanum* MM577, and two accessions—SIS1 and SIS2, of *S. sisymbriifolium*—were classified as resistant to Pss2016, with ranges of 9.2–23.3% for DI and 33.3–66.7% for W%. *Solanum torvum* TOR3 was classified as moderately resistant to Pss2016, with 32.2% of DI and 38.9% of W%, and *S. anguivi* ANG1 was moderately susceptible, with 95.8% of W% and 45.8% of DI. It is worth mentioning that all cultivated eggplant accessions and more than 85% of the wild relative accessions tested in the first trial were susceptible to both bacterial wilt strains (Figure 2), and wilt symptoms were appeared one or two weeks after inoculation.

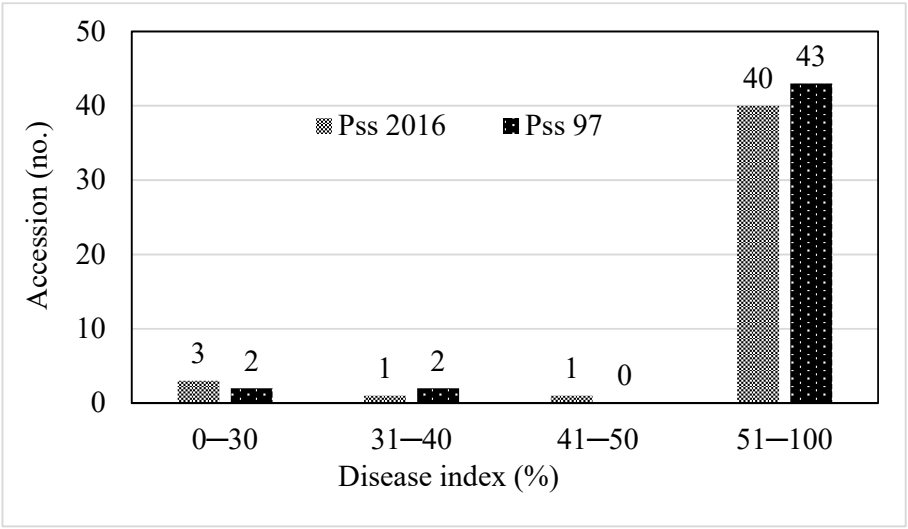

Figure 2. Frequency distribution of bacterial wilt resistance based on disease index (DI) for cultivated and wild relative eggplant accessions evaluated resistance to *Ralstonia solanacearum* strains Pss97 and Pss2016 at four weeks after inoculation.

Second Trial

Six out of 40 accessions of wild relatives of eggplant (VI050346, MM577, SIS1, SIS2, TOR2, and TOR3) that were identified as resistant or moderately resistant in the first trial (i.e., those that scored ≤40% for DI), along with the checks, were screened again in a second trial at high temperatures using the same *R. solanacearum* strains of Pss97. *Solanum anguivi* VI050346 was susceptible to both Pss97 and Pss2016. *S. sisymbriifolium* SIS1 was resistant to Pss97, with 17.5% of DI. Two accessions—TOR2 and TOR3, of *S. torvum*—were moderately resistant to Pss97 (Table 3).

Table 3. Re-evaluation of the resistance of selected resistant and tolerant accessions against *Ralstonia solanacearum* strains Pss97 and Pss2016 at four weeks after inoculation in the second trial.

Taxa and Accession Code [a]	Pss97			Pss2016		
	W% [b]	DI [c]	Resistance Category [d]	W%	DI	Resistance Category
Solanum anguivi						
VI050346	100.0	99.2	S	95.8	81.7	S
S. incanum						
MM577	100	100	S	ND	ND	ND
S. sisymbriifolium						
SIS1	20.8	17.5	R	ND	ND	ND
SIS2	62.5	51.7	S	ND	ND	ND
S. torvum						
TOR2	54.2	36.7	MR	ND	ND	ND
TOR3	44.4	33.3	MR	ND	ND	ND
S. melongena (checks)						
EG048	100.0	100.0	S	100.0	100.0	S
EG203	8.3	2.5	R	62.5	48.8	MS

[a] Accessions with VI codes are from the World Vegetable Center genebank, while the others are from Universitat Politècnica de València. [b] indicates the means of three replications of wilt percentage (W%) at fourth week after inoculation. Means followed by ± standard error (± SE). [c] indicates the means of three replications of disease index (DI) at fourth week after inoculation. Means followed by ± standard error (± SE). [d] Resistance category according to the DI at fourth week after inoculation. R = resistant (0–30%), MR = moderately resistant (>30–40%), MS = moderately susceptible (>40–50%), S = susceptible (>51%). ND indicates no data due to the limited number of seeds or low germination.

4. Discussion

Bacterial wilt, caused by *R. solanacearum*, has been ranked second in the list of the most scientifically and economically important bacterial pathogens [2,15]. Resistance to *R. solanacearum* has been reported in some tomato cultivars, such as Hawaii 7996, Hawaii 7997, and Hawaii 7998 [37], but these cultivars have not been widely accepted due to poor horticultural traits, such as small fruits, linked with bacterial wilt resistance [38]. Grafting susceptible tomato cultivars onto bacterial wilt-resistant eggplant rootstocks provides good control, especially during the hot–wet season, and can minimize problems caused by flooding [14,25]. This technology has been adopted by WorldVeg on a large scale in the Philippines, Vietnam, and Taiwan to control bacterial wilt in tomatoes. A number of bacterial wilt resistant eggplant rootstocks, such as EG203, EG195, EG190, and TS03, have been successfully developed and released by WorldVeg to manage bacterial wilt in tomato, but resistance levels are not stable under different environmental conditions. These reasons encouraged us to explore the germplasm for more stable sources of resistance to bacterial wilt in the cultivated eggplant genepool and in a representation of the highly genetically diverse eggplant wild relatives [39].

Resistance to *R. solanacearum* has been identified in previous studies in a number of accessions of cultivated eggplant (*S. melongena*) and in distant wild relatives, such as *S. capsicoides, S. sisymbriifolium S. sessiliflorum, S. stramonifolium, S. virginianum, S. grandiflorum, S. hispidum, S. torvum, S. nigrum, S. americanum,* and *S. scabrum* [39]. In our study, no immunity was found in the materials tested, however, two accessions of *S. torvum* (TOR2 and TOR3) and two accessions of *S. sisymbriifolium* (SIS1 and SIS2) were observed to be resistant or moderately resistant to one or both of the bacterial wilt strains tested. These results confirm earlier findings that found high levels of bacterial wilt resistance in these two wild species [25,40]. Both accessions belong to the tertiary genepool of eggplant and are therefore promising for introgression breeding in eggplant [39]. Rootstocks of *S. torvum* accessions have been used in several studies and were highly resistant to bacterial wilt, and resulted in a good fruit yield in the scion [41,42]. *Solanum sisymbriifolium* rootstocks showed resistance against bacterial wilt disease under sick plots in field conditions [40]. In addition, *S. incanum MM577* was observed to be resistant to Pss2016, although these results should be confirmed. This species belongs to the secondary genepool of eggplant and can be crossed with cultivated eggplant [43]. In fact, lines of *S. melongena* with introgressions from *S. incanum* have been obtained recently [44]. This is the first report of bacterial wilt resistance in *S. incanum*, which has also been reported as resistant to *Fusarium oxysporum* f. sp. *melongenae* [42], another harmful soil-borne disease for Solanaceae crops.

Differences in bacterial wilt resistance levels between the first and second trials were evident in accessions of *S. anguivi, S. sisymbriifolium* (SIS2), and *S. torvum*. The susceptibility or reduction of resistance in the second trial second could be due to the higher temperatures observed in the second trial, which reached 36 °C. Similar results were found in tomato and tobacco (*Nicotiana tabacum*), where resistant cultivars become susceptible when exposed to temperatures above 28 °C [26,45]. In addition, soil moisture and soil temperature may have influenced the resistance reaction of the genotypes [46]. Although we did not study the mechanisms of resistance, the resistance present in identified genotypes could be due to a higher concentration of secondary metabolism, such as polyphenols and steroidal glycoalkaloids, that prevent bacterial movement into the vicinity of the plant system [16,47]

All cultivated eggplant accessions and more than 85% of the wild relative accessions screened in our study were susceptible to bacterial wilt. The early wilt symptoms appeared one week after

inoculation in most of susceptible accessions, and were completely wilted after two weeks. Similarly, a high incidence of bacterial wilt in tomato was observed 15 days after inoculation at the early stage of crop growth [48]. Also, other authors [46] found that most of the susceptible genotypes displayed a susceptible reaction in their early stages of growth (10 to 20 days after inoculation).

5. Conclusions

Among the eggplant genotypes tested in our experiments, high levels of resistance were detected in *S. sisymbriifolium* and *S. torvum,* for both strains. In addition, *S. incanum* (MM577) and *S. anguivi* (VI050346) displayed resistance to Pss2016. However, resistance in *S. sisymbriifolium* (SIS2) and *S. anguivi* (VI050346) might be decreased or broken down when temperature increases, as occurred in the second trial. Hence, an evaluation of bacterial wilt resistance under different environmental conditions would provide a better understanding of the resistance mechanisms of these sources and their potential interest for offering broad and stable resistance, which is required for the development of cultivars with durable resistance. Our results made it possible to identify some new sources of resistance to bacterial wilt in wild relatives of eggplant from very different origins. These materials may be of interest for the development of resistant rootstocks and/or cultivars that can be used to manage bacterial wilt in eggplant and also, when used as rootstocks, in tomato.

Author Contributions:

A.N., J.-R.C., M.R., designed experiments, collected data, conducted statistical analysis; and writing manuscript; J.P. provided plant materials and reviewed the manuscript; E.M and M.E. supervised the student, written and reviewed the manuscript.

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