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An Evaluation of Bacterial Wilt (*Ralstonia solanacearum*) Resistance in a Set of Tomato Germplasm from the United States Department of Agriculture †

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Abstract: Bacterial wilt (BW), caused by *Ralstonia solanacearum*, is one of the devastating diseases in tomatoes (*Solanum lycopersicum* L.). The use of resistant cultivars and breeding for genetic resistance is the most effective, economical, and environmentally friendly management strategy for this disease. It is necessary to screen diverse germplasm and cultivated genotypes to identify resistant resources and to develop resistant cultivars in tomatoes to combat the changing pathogen isolates. This study evaluated 40 United States Department of Agriculture (USDA) tomato accessions for their BW resistance to the *R. solanacearum* isolate P822 under greenhouse conditions. The tomato plants were inoculated and visually assessed to observe their symptoms, and the disease severity was scored on a scale of 0 to 4 (0 = no leaf wilted, 1 = 25% of leaves wilted, 2 = 50% leaves wilted, 3 = 75% of leaves wilted, and 4 = 100% leaves wilted). Five accessions (PI 645370, PI 647306, PI 600993, PI 355110, and PI 270210) were observed as BW resistance, with PI 645370 showing the greatest resistance. The broad-sense heritability for BW resistance was estimated as 59.9% and 42.8% based on a 0–4 scale of disease incidence and the disease severity index, respectively. Two distinct clusters (sub-populations) were detected among 39 of the 40 accessions. The five identified BW-resistant accessions were distributed in both clusters, suggesting a likely difference in the genetic base among the five resistance accessions. The resistant accessions will contribute significantly to the tomato breeding program to develop new cultivars with BW resistance.

Keywords: tomato; *Solanum lycopersicum*; bacterial wilt; *Ralstonia solanacearum*; germplasm; disease resistance

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1. Introduction

Tomato (*Solanum lycopersicum* L.), a vital horticultural crop ranking second only to potato in economic value, belongs to the Solanaceae family and is extensively utilized fresh or in various processed forms [1]. With a rich nutritional profile, including vitamins, minerals, and bioactive compounds, tomatoes are associated with the prevention of chronic degenerative diseases [2], making tomatoes widely acknowledged as healthful foods, resulting in a substantial global increase in the cultivation acreage, production, and consumption of these nutritious fruits [3]. Globally, tomato production reached 182 million metric tons in 2018 [4], increasing to 186 million metric tons in 2020, as cited by Collins et al. (2022) [5]. Despite their economic importance, tomato cultivation faces challenges, such as cultivar selection, management practices, pests, diseases, and abiotic stresses [6–9]. Bacterial wilt (BW), caused by the soilborne bacterium *Ralstonia solanacearum* pathogen, stands out as a significant threat to

tomato crops, causing a significant loss of tomato fruit worldwide [10]. The selection and cultivation of tomatoes with inherent genetic resistance to BW are advocated to enhance the quality and longevity of tomato crop yields [3,11].

Ralstonia solanacearum ranks among the top 10 bacterial species in terms of scientific and economic importance in plant diseases [12]. It is a soilborne pathogen [13] causing vascular wilt disease by entering plants through wounds or root tips [14]. Classified into four phylogenic groups, this heterogeneous species complex includes strains with diverse geographical origins, host ranges, and pathogenic behaviors [15,16]. The primary source of infection is soil, where the pathogen can survive for several years in the absence of a host plant and establishes latent infections in native weeds, adding to the challenge of eliminating the pathogen [17,18]. Additionally, the pathogen can survive within a large temperature range of 10 °C to 41 °C and in varied environments [19]. Spread occurs through contaminated water, infested soil, and contaminated farming equipment [13]. *R. solanacearum* affects over 200 plant species, causing diseases like potato brown rot and bacterial wilt in tomatoes, tobacco, eggplants, and ornamentals, as well as Moko disease in bananas [14]. Symptoms include wilting during the day and recovery at night, progressing to plant death under severe conditions [14]. Bacterial wilt disease results in significant yield losses [20], impacting global crop productivity and causing annual losses exceeding USD 1 billion [12,21,22]. Tomato bacterial wilt, induced by the soil-dwelling bacterium *R. solanacearum*, is prevalent in the Southeastern United States. Under optimal conditions for disease development, it results in significant economic losses [23].

Several management strategies for BW have been suggested, including chemical fumigants, soil drainage, tillage practices, external nutrient supplements, and the use of resistant cultivars, transgenic plants, and microbes. However, each strategy has its limitations, and reliance on a single method often proves ineffective [24]. Chemical use, while temporarily effective, poses threats to beneficial microorganisms and human health [13,20]. Strategies like soil fumigation may not be economically feasible on a large scale, as quoted by Mcavoy et al. (2012) [25]. As a result, it is essential to formulate strategies that are both effective and environmentally friendly to decrease the incidence of this disease [26]. The development of resistant varieties emerges as the most effective, economical, and environmentally friendly approach to BW control [13,24,27].

Breeding for resistance involves identifying resistant sources and ongoing screening against the pathogen, providing a sustainable and long-term solution to mitigate the impact of BW [20,28]. The initial crucial step in any breeding program is germplasm collection, which is vital for crop improvement and diversity [29,30]. Genetic resources, mainly found in the wild due to natural selection, provide valuable materials for enhancing crops. In the case of BW resistance, wild species like *S. torvum*, *S. sisymbrifolium*, and *S. khasianum* contribute resistance resources for Solanum species like eggplants [29]. Additionally, some wild potato species exhibit BW tolerance [31]. Regarding tomatoes, potential sources of resistance to BW have been identified but are not extensively utilized, mainly due to their small fruit sizes and poor horticultural characteristics, which may not align with commercial tomato industry standards. These sources include L285 and selections from wild tomato accessions, namely PI 263722, PI 126408, PI 196298 (*Lycopersicon esculentum*), and PI 251323 (*Lycopersicon pimpinellifolium*) [32]. While specific BW-resistant genotypes have been identified and released, they often exhibit region-specific resistance, and their effectiveness may decline outside their regions of development due to environmental factors and variability among *R solanacearum* strains [33].

The identification of resistance varieties against BW is imperative [34], and it involves evaluating diverse genotypes from various regions for their responses to the pathogen. Screening methods include the sick plot method and the artificial inoculation of tomato plants with *R solanacearum* strains [29]. In the sick plot method, genotypes are planted in soil containing *R. solanacearum*, but an uneven pathogen distribution across the soil necessitates the use of artificial inoculation methods. These methods encompass soil drenching, where bacterial suspension is injected into root incisions; leaf clipping, involving clipping leaves

with bacterial suspension; and axil puncturing, where sterile needles are used to prick plant axils with the inoculum. Soil drenching has been found to significantly record the greatest BW incidences in screening tomatoes, brinjal, and chilies. Wilting symptoms are assessed using a BW score to measure resistance. In a study, thirteen genotypes from the USA, Taiwan, and Ghana were screened using the root dip technique, showing stable resistance in the H7996, LA0442, and LA0443 genotypes [35]. Another study evaluated fifty-five tomato genotypes and found seven greatly resistant genotypes, including RIL-118, Indam-1004, Arka Samrat, PKM-1, PED, EC-802390, and EC-816105, using the root-sectioned seedling dipping method [36]. This study aimed to identify tomato genotypes that are resistant to the bacterial wilt (BW) pathogen, providing valuable resources for future breeding initiatives. The goal is to incorporate these resistant genotypes into breeding programs, including genome-wide association studies and genomic selection, to facilitate the development of BW-resistant hybrids or cultivars. The specific objective was to evaluate the BW resistance of tomato germplasm sourced from the United States Department of Agriculture (USDA).

This paper presents insights drawn from a comprehensive evaluation presented in a Master's thesis by Makawa Phiri (2023) [37]. The research, conducted at the University of Arkansas, explored the complexities of understanding and enhancing bacterial wilt resistance in tomato crops. The results provide a foundation for the current study, which seeks to build upon the existing knowledge and contribute to the ongoing efforts to develop resilient tomato varieties.

2. Materials and Methods

2.1. Plant Materials and Bacterial Wilt Pathogen

Forty tomato accessions were obtained from the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) Germplasm Resources Information Network (GRIN). The majority (77.5%), specifically 31 of the 40 accessions, were originally from the United States; 2 were from Canada, and 1 each was from China, France, Guatemala, the Russian Federation, the Former Soviet Union, Spain, and the United Kingdom, respectively.

Ralstonia solanacearum strain P822 phylotype II sequevar 7 was used to test the tomato accessions for BW resistance. The *R. solanacearum* strain P822 was originally isolated from blueberry in Florida [38], and it was provided by Ana Maria Bocsanczy and David J. Norman at the University of Florida and stored at Alejandro Rojas's laboratory at the University of Arkansas, Fayetteville, AR for the bacterial growth, propagation, and preservation.

2.2. Greenhouse Experiment for Pathogen Tests

Screening of tomato accessions was conducted in a greenhouse at the Arkansas Agricultural Research and Extension Center, Fayetteville, AR between January 2023 and February 2023. Throughout the study, the greenhouse temperature and humidity were maintained at 21/18 °C in a day/night cycle and 73%, respectively.

Five tomato seeds of each accession were sown in pots (8.5 cm high, 8.5 cm top diameter, and 5.8 cm base diameter) placed in trays (52 cm long, 26 cm wide, and 6 cm high) containing a commercial potting mix (Berger, berger.ca, BM 6). The experiment was arranged in a completely randomized design (CRD) with three replicates. Immediately following seed sowing, the pots were irrigated with 150 mL of water every day for 35 days before exposure to the bacterium pathogen.

Additionally, 180 mL of liquid (0.5 teaspoons per gallon or 3.8 L) fertilizer (Miracle-Gro Water Soluble All Purpose Plant Food 24-8-16) containing ammoniacal nitrogen (N) (3.5%), urea nitrogen (N) (20.5%), available phosphate (P_2O_5) (8%), soluble potash (K_2O) (16%), boron (B) (0.02%), water-soluble copper (Cu) (0.07%), chelated iron (Fe) (0.15%), manganese (Mn) (0.05%), molybdenum (Mo) (0.0005), and water-soluble zinc (Zn) (0.06%) was applied in liquid form per pot 10 days after seed sowing and every 7 days in subsequent applications before the plants were exposed to bacterium pathogen.

During the experiment, thinning was carried out 20 days after planting to maintain 3 plants per pot and replicate. A total of 35 days after planting, seedlings were inoculated with *R. solanacearum* strain P822. The pathogen was tested for its virulence before being applied in this experiment. The pathogen was grown on Casamino acid–eptone–glucose (CPG) media maintained at 30 °C [39], where the CPG medium constituents were 1 g casamino acid (casein hydrolysate), 10 g peptone, 5 g glucose, and 17 agar for solid media (plates).

The substances were weighed and added to one cylinder jar, and 1 L of water was added. The mixture was autoclaved at 121 °C for 20 min (2 cycles). After autoclaving, the mixture was cooled down in a bath at 50 °C and then poured in Petri dishes. The colonies were streaked into new Petri dishes to multiply the pathogen, and they were incubated at 30 °C. On a solid medium, colonies of *R. solanacearum* are usually visible after 48–72 h of incubation at 28 °C.

A suspension of 10^6 CFU/mL was prepared using a virulent strain of isolate P822 of *R. solanacearum* following the method by Kim et al. (2016) [3] and Singh et al. (2015) [13]. The task was to prepare sterile deionized (DI) water for dilution and to test the virulence of *R. solanacearum* bacteria by reading the optical density (OD). To begin with, the laminar flow hood was sterilized using 70% alcohol. Then, a 50 mL Eppendorf tube; an inoculum loop, pipette, and tips; and a plate for OD reading were gathered.

Next, 1 mL of sterile DI water was poured into the Petri dish containing *R. solanacearum* colonies. The colonies were lightly loosened using the inoculum loop and then poured into the 50 mL Eppendorf tube. This process was repeated until almost all the colonies were scrapped. Sterile water was added to the Eppendorf tube until it reached the 50 mL mark. The bacterial suspension was prepared from the cultures where the inoculum dosage was adjusted to an OD of 0.3 at 600 nm (106 cfu/mL) using a spectrophotometer and read on GEN 5 software.

A soil-drenching inoculation method was followed, where a knife was used to injure plant roots by cutting through the soil 1–2 cm away from the stem base before inoculation (Figure 1). Before inoculation, the seedlings were kept without irrigation for a day, and 150 mL of the bacterial suspension was poured into the soil where the cut was made [40]. Following inoculation, the plants were watered with 150 mL of tap water daily.

Figure 1. Soil drenching inoculation method for *Ralstonia solanacearum* inoculum solution.

The degree of wilt in seedlings was evaluated on a scale of 0 to 4, with each seedling being assessed individually (Figure 2). A score of 0 indicated the absence of symptoms (no wilting), while a score of 1 indicated that 25% of the leaves were wilted. A score of 2 indicated that 50% of the leaves were wilted, and a score of 3 indicated that 75% of the leaves were wilted. A score of 4 indicated that all leaves were wilted or that the plant was dead [41,42].

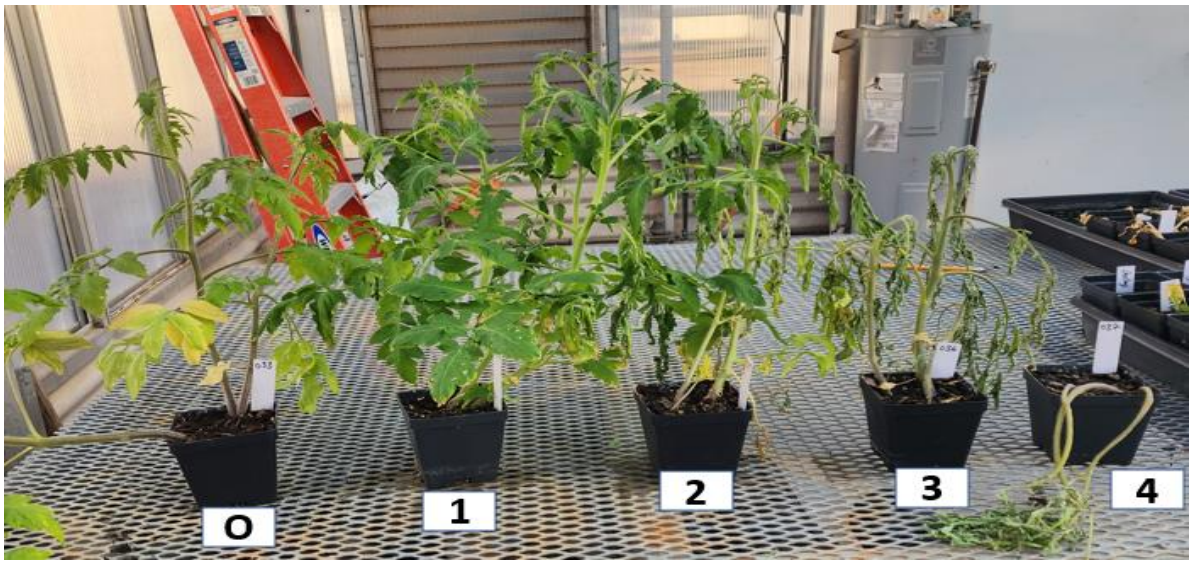


Figure 2. A 0–4 score scale for bacterial wilt incidence in tomato.

The disease severity index (DSI) was used to determine the BW reaction in each of the 40 tomato accessions and calculated as follows: DSI = 100 × ∑ (frequency × score of rating)/[(Total number of observations) × (maximal disease index)] [43].

2.3. Phenotypic Data Analysis

2.3.1. Statistical Model

The statistical model for the ANOVA analysis was as follows: Yij = μ + Gj +εij. I = 1, 2, 3 and j = 1. . .40, with μ representing the overall mean, Yij representing the response from the jth accession (Gj) (fixed effect), and eij representing the random error associated with the ijth observation.

2.3.2. ANOVA, Distribution, Descriptive Statistics, Pearson's Correlation, and Broad-Sense Heritability

The data were analyzed using JMP PRO 17. An analysis of variance (ANOVA) was conducted using the general linear model (GLM) procedure. The distribution of the data was visualized using 'Distribution'; descriptive statistics were estimated using 'Tabulate'; and the Pearson's correlation coefficients (r-value) and their *p*-values were calculated using the 'Multivariate Methods' option of JMP PRO 17, respectively.

The broad-sense heritability (H^2) was estimated as $H^2 = 100 \times \sigma^2_g/[\sigma^2_g + (\sigma^2_e/r)]$ [44], with σ^2_g being the total genetic variance, σ^2_e being the residual variance, and r being the number of replicates. The estimates for σ^2_g and σ^2_e were [EMS(G)-Var(Residual)]/r and Var(Residual), respectively. EMS(G) and Var(Residual) were obtained from the ANOVA table.

2.4. Genetic Diversity Analysis

2.4.1. DNA Extraction: Genotyping by Sequencing (GBS) and SNP Discovery

DNA (genome) was extracted from fresh leaves of tomato plants using the CTAB/SDS method. DNA sequencing was conducted using genotyping through the sequencing (GBS) approach [45] in pair-end sequencing, and libraries were sequenced using Illumina NovaSeq in the Biotechnology Center at the University of Wisconsin-Madison (https://biotech.wisc.edu/, (accessed on 22 December 2023)). The short-read sequences data were aligned to tomato genome reference *Solanum lycopersicum*, ITAG_4.0 (https://phytozome-next.jgi.doe.gov/info/Slycopersicum_ITAG4_0, (accessed on 22 December 2023)), and SNPs were postulated in a pipeline using TASSE–GBS [46] and Stacks 2 [47] (https://catchenlab.life.illinois.edu/stacks/, (accessed on 22 December 2023)). A total of 392,496 single nucleotide poly-

morphism (SNP) markers were discovered across 287 tomato genotypes distributed on 12 chromosomes of tomato and provided by UWBC.

2.4.2. Principal Component Analysis (PCA) and Genetic Diversity

The principal component analysis (PCA) and genetic diversity analysis were performed in 39 of the 40 tomato accessions (except PI 279565, listed in Table 1, because this accession did not have DNA sequencing data through GBS) in GAPIT 3 (Genomic Association and Prediction Integration Tool version 3) by setting PCA = 2 to 10 and NJ tree = 2 to 10 [48]. The phylogenetic trees were drawn using the neighbor-joining (NJ) method in GAPIT 3 and was also drawn using the Maximum Likelihood (ML) method in MEGA 7) [49] based on 4847 single nucleotide polymorphism (SNP) markers distributed on 12 chromosomes. The SNP set consisted of 4847 SNPs across the 39 accessions after filtering and keeping the SNPs with a minor allele frequency (MAF) of >3.5%, a missing allele value of <15%, and a heterogeneous rate of $\leq$30% in this study.

Table 1. List of 40 tomato (*Solanum lycopersicum* L.) accessions with their accession numbers (PI), names, origins, clusters (sub-population), bacterial wilt (BW) resistant scales, and disease severity index (DSI) values; significant difference at $p = 0.05$.

ACCESSION	NAME	ORIGIN	2_Cluster	BW_Score (Order by Lower Scale, Higher Resistant)	BW Score Significant at $p = 0.05$ Level	BW_DSI %	BW DSI Significant at $p = 0.05$ Level
PI645370	Venus	North Carolina, United States	Q2	0.0	I	0.0	G
PI600993	Liberator	United States	Q2	1.1	HI	27.8	FG
PI647305	Rosa o Monserrat	Spain	Q1	1.1	HI	27.8	FG
PI355110	Napoli	United States	Q1	1.2	GHI	30.6	EFG
PI270210	Sioux	United States	Q1	1.4	FGHI	36.1	DEFG
PI647184	Creole	United States	Q2	1.7	EFGH	41.7	CDEFG
PI636262	Favorite	Wyoming, United States	Q1	1.8	DEFGH	44.4	BCDEF
PI286255	Moneymaker	United Kingdom	Q1	1.9	CDEFGH	47.2	ABCDEF
PI339940	Chalks Early Jewel	United States	Q1	2.0	BCDEFGH	50.0	ABCDEF
PI601136	Baxters Early Bush Cherry	Texas, United States	Q1	2.0	BCDEFGH	50.0	ABCDEF
PI645390	CMVF 232	Nevada, United States	Q1	2.0	BCDEFGH	41.7	CDEFG
PI311109	Tomate Jocotillo	Guatemala	Q1	2.1	BCDEFGH	44.4	BCDEF
PI636205	T039	Oklahoma, United States	Q1	2.3	ABCDEFGH	58.3	ABCDEF
PI644794	Winsall	Illinois, United States	Q1	2.3	ABCDEFGH	58.3	ABCDEF
PI645389	H 2990	Nevada, United States	Q1	2.3	ABCDEFGH	58.3	ABCDEF
PI270234	Loran Blood	United States	Q1	2.4	ABCDEFGH	50.0	ABCDEF
PI270226	Early Santa Clara Canner	United States	Q1	2.6	ABCDEFGH	63.9	ABCDEF
PI639208	Black from Tula	Tula, Russian Federation	Q1	2.8	ABCDEFG	61.1	ABCDEF
PI547073	NC 8276	North Carolina, United States	Q2	2.9	ABCDEF	72.2	ABCDE
PI109836	Precoce des Halles	France	Q1	3.0	ABCDEF	66.7	ABCDEF
PI600930	Moran 3053	United States	Q2	3.0	ABCDEF	75.0	ABCD
PI205041	P.A. Young T162 FS-1	United States	Q1	3.1	ABCDE	69.4	ABCDEF
PI601449	Bealls Gourmet	United States	Q1	3.1	ABCDE	77.8	ABCD
PI647513	Red Pear	United States	Q1	3.1	ABCDE	77.8	ABCD
PI601118	VF 9209	California, United States	Q2	3.2	ABCDE	61.1	ABCDEF
PI279565	Caro Red	United States	no data	3.2	ABCDE	63.9	ABCDEF
PI339914	Coldset	Ontario, Canada	Q1	3.2	ABCDE	80.6	ABC
PI254655	Ker-1-M	United States	Q1	3.3	ABCD	63.9	ABCDEF
PI270232	Homestead	United States	Q1	3.3	ABCD	83.3	ABC
PI647566	Flora-dade	United States	Q2	3.3	ABCD	83.3	ABC
PI647196	Rutgers	United States	Q1	3.4	ABC	66.7	ABCDEF
PI647445	Zhongza No. 4	China	Q2	3.4	ABC	86.1	AB
PI601117	Peelmech	California, United States	Q2	3.5	AB	69.4	ABCDEF
PI279817	Scotia	Canada	Q1	3.6	AB	72.2	ABCDE
PI499370	Prevoskhodnyi 176	Former, Soviet Union	Q1	3.6	AB	77.8	ABCD
PI601098	Indiana 812	Indiana, United States	Q2	3.6	AB	88.9	A
PI645214	Floradel	Florida, United States	Q2	3.6	AB	88.9	A
PI645391	Florida MH-1	Michigan, United States	Q1	3.6	AB	88.9	A
PI645398	466 Jungs Improved	Wisconsin, United States	Q1	3.6	AB	80.6	ABC
PI647523	Wayahead VFNT Cherry	United States	Q1	3.8	A	72.2	ABCDE

3. Results

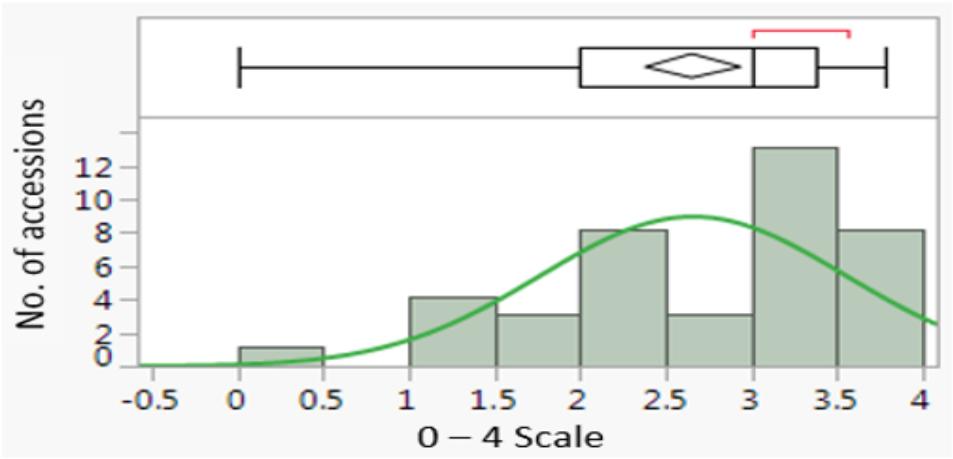
3.1. Parameters and Distributions of Bacterial Wilt Resistance

The investigation focused on evaluating BW symptoms in tomato plants, employing a 0-4 severity scale. An analysis of the data from 40 tomato accessions on day 11 post-symptom onset revealed a rightward skew in the distribution, indicating an increased susceptibility to BW (Figure 3a). Among the 40 accessions assessed, the PI 645370 accession demonstrated the greatest resistance with a score of 0, while the other four accessions, PI 647305, PI 600993, PI 355110, and PI 270210, exhibited scores below 1.5 (Table 1), suggesting resistance to the BW pathogen *R. solanacearum* strains P822. These five accessions emerge as promising candidates for BW resistance, providing valuable insights for the prospective development of more robust tomato crops.

On the other hand, the seven accessions, namely PI 279817, PI 499370, PI 645391, PI 645398, PI 645214, PI 601098, and PI 647523, showed scores of 3.6 or higher on a scale of 4 (Table 1), indicating that they are more susceptible to BW and may be used as susceptible controls to screen BW resistance in tomato germplasm and as susceptible parents in genetic studies.

The recorded scores for BW resistance in the 40 tomato accessions had an average value of 2.7, a standard deviation (Std Dev) of 0.9, a standard error (Std Err) of 0.14, and a coefficient variation (CV) of 33.5% (Figure 3a). These statistics suggest that the population had a large range (3.8) and variation (0.9 Std Dev and 33.5% CV) in BW resistance. Among the 40 genotypes, the top five genotypes with a score of 0 or an average mean of <1.5 demonstrated great levels of BW resistance, showing their potential to be used in breeding programs targeting the development of new cultivars or lines with BW resistance.

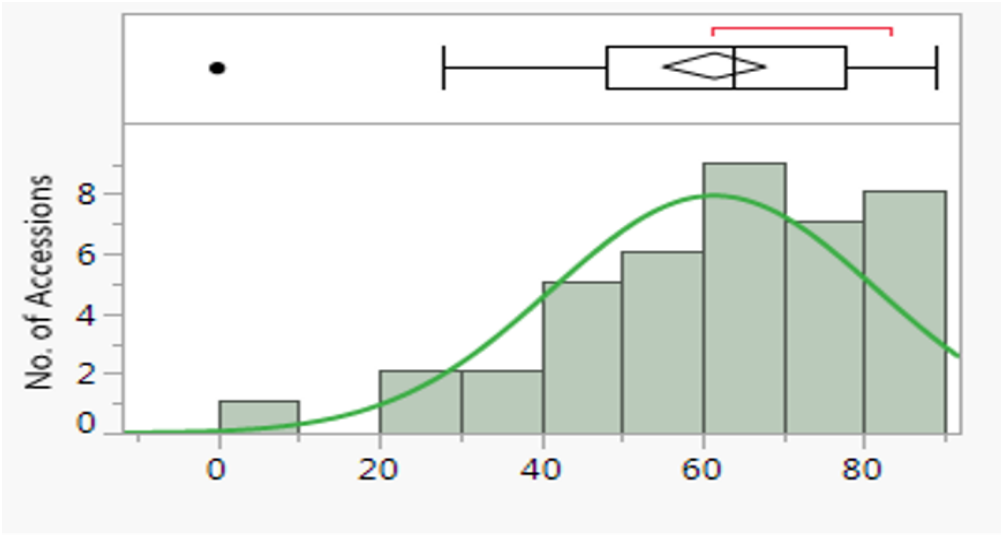
For the DSI, an average value of 61.5, a standard deviation (Std Dev) of 20.06, a standard error (Std Err) of 3.17, and a coefficient variation (CV) of 32.6% (Figure 3b) were detected. These statistics suggest that the population had a large range (88.8) and variation (20.06 Std Dev and 32.6% CV) in BW DSI. Among the 40 genotypes, PI 645370 had a 0 DSI and demonstrated the greatest level of BW resistance, and both PI 600993 and PI 647305 had 27.8% DSI, showing mediate resistance (Table 1). The three accessions also had the top greatest resistance, with 0 or 1.1 scores based on the 0–4 score scale (Table 1), thus indicating the potential to be used in breeding programs to develop new cultivars or lines with BW resistance.



Paramete	N	Mean	Min	Max	Range	Std Dev	Std Err	CV
BW_score	40	2.7	0.0	3.8	3.8	0.9	0.14	33.5

(**a**)

Figure 3. *Cont.*



(**b**)

Figure 3. (**a**) Distribution of scores (0–4) for bacterial wilt incidence in 40 tomato accessions. X-axile represents the 0–4 scale of bacterial wilt incidence; Y-axile represents the number of accessions; the bracket represents the peak of the distribution; and the green line represents the theoretical normal distribution. (**b**) Distribution of bacterial wilt disease severity index (DSI) % in 40 tomato accessions. X-axile represents the 0–4 scale of bacterial wilt incidence; Y-axile represents the number of accessions; the bracket represents the peak of the distribution; and the green line represents the theoretical normal distribution.

The ANOVA showed a significant genotype (accession) effect (p = 0.0003 < 0.001) for the disease scale and p = 0.0177 < 0.05 (Table 2), indicating that there were significant differences for the BW resistance level (0–4 scale). Among the 40 accessions, the PI 645370, PI 647305, PI 600993, PI 355110, and PI 270210 accessions had the lowest scores, ranging from 0 to 1.5, showing that the five accessions were BW-resistant.

Table 2. ANOVA table for bacterial wilt incidence and disease severity index (DSI) among the 40 tomato accessions.

BW	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F	EMS	H^2%
BW Score	Genotype	39	92.584	2.374	2.493	0.0003	$\sigma^2_e + r\sigma^2_G$	59.9
	Error	80	76.185	0.952			σ^2_e	
	C. Total	119	168.769					
BW_DSI	Genotype	39	47,138.31	1208.67	1.7507	0.0177	$\sigma^2_e + r\sigma^2_G$	42.8
	Error	80	55,231.48	690.39			σ^2_e	
	C. Total	119	102,369.79					

The estimated broad-sense heritability (H^2) values were 59.9% and 42.8% for the BW resistance scale and DSI, respectively, indicating that genetic factors accounted for a significant portion of the variation in BW resistance among the tomato genotypes (accessions) tested, and demonstrating that BW resistance is inheritable (Table 2).

3.2. Principal Component Analysis (PCA) and Genetic Diversity Analysis

A PCA was conducted for 39 of the 40 tomato accessions (except PI 279565 listed in Table 1). The 39 accessions were divided into two distinct clusters or sub-populations,

represented by red (Q1) and blue (Q2) colors in both the PCA plot (Figure 4A) and phylogenetic tree (Figure 4B) based on the neighbor-joining (NJ) algorithm in GAPIT 3. The Q2 sub-population was the majority with 28 accessions, accounting for 71.8% of the total population, and Q1 had 11 accessions (28.2%) (Table 1, Figure 4).

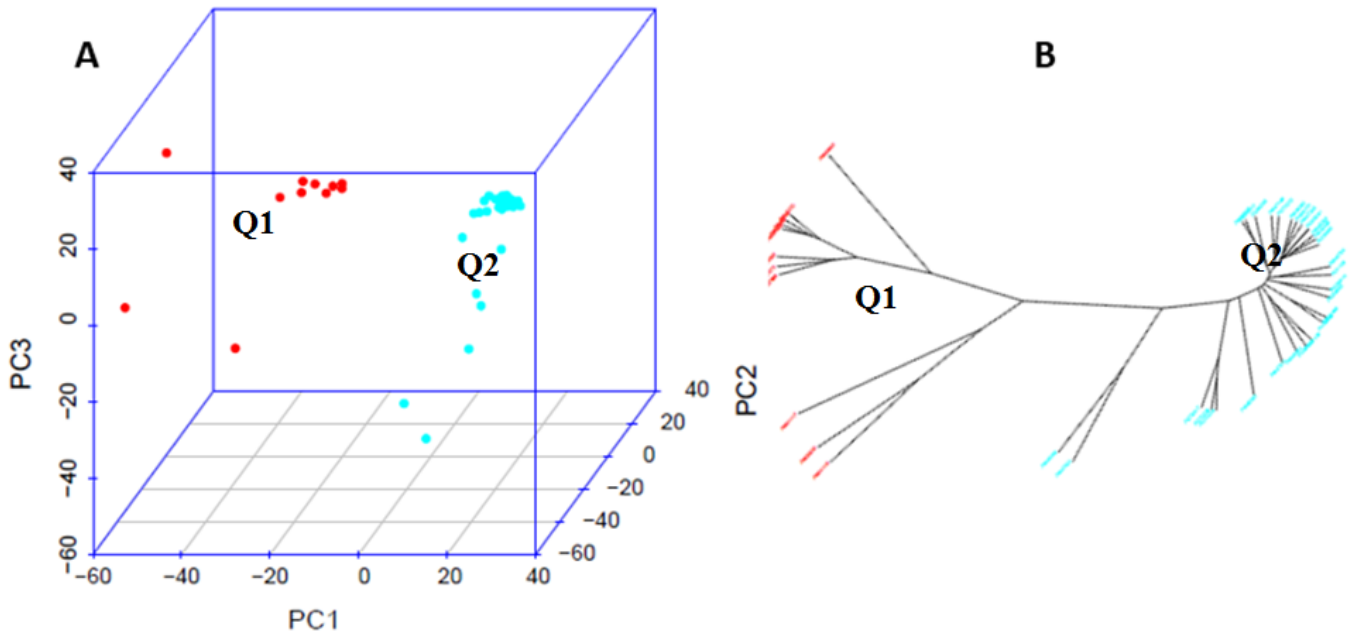


Figure 4. Population genetic diversity analysis in 39 of the 40 USDA tomato germplasm accessions (except PI 279565 listed in Table 1 because it did have DNA sequencing data through GBS). (**A**) Three-dimensional graphical plot of the principal component analysis (PCA) and (**B**) two sub-populations of phylogenetic tree drawn using GAPIT 3.

Two distinct clusters were also observed among the 39 tomato accessions in the phylogenetic tree postulated using the Maximum Likelihood (ML) method in MEGA 7 (Figure 5). The top five greatly BW-resistant accessions, namely PI 645370, PI 647305, PI 600993, PI 355110, and PI 270210, were arranged into the two clusters (Q1 and Q2) marked red and square-shaped in Figure 5 and Table 1 with the accession information, indicating a different genetic base (background). Interestingly, despite most of the accessions in the study originating from the USA, the genotype diversity analysis revealed diverse genetic differences among the USA accessions, among different countries, and among USA states (Figure 5). Four of the five resistant accessions were originally collected from the United States of America, while one was collected from Spain (Table 1 and Figure 5). This highlights the availability of genotypes that are resistant to the *R. solanacearum* isolate P822, which is common in the USA. The sup-population Q1 contains three BW-resistant accessions, namely two from the USA and one from Spain, and the Q2 has two BW-resistant accessions from the USA. This information can be used to identify and utilize BW-resistant genotypes in breeding programs to develop more resilient tomato crops.

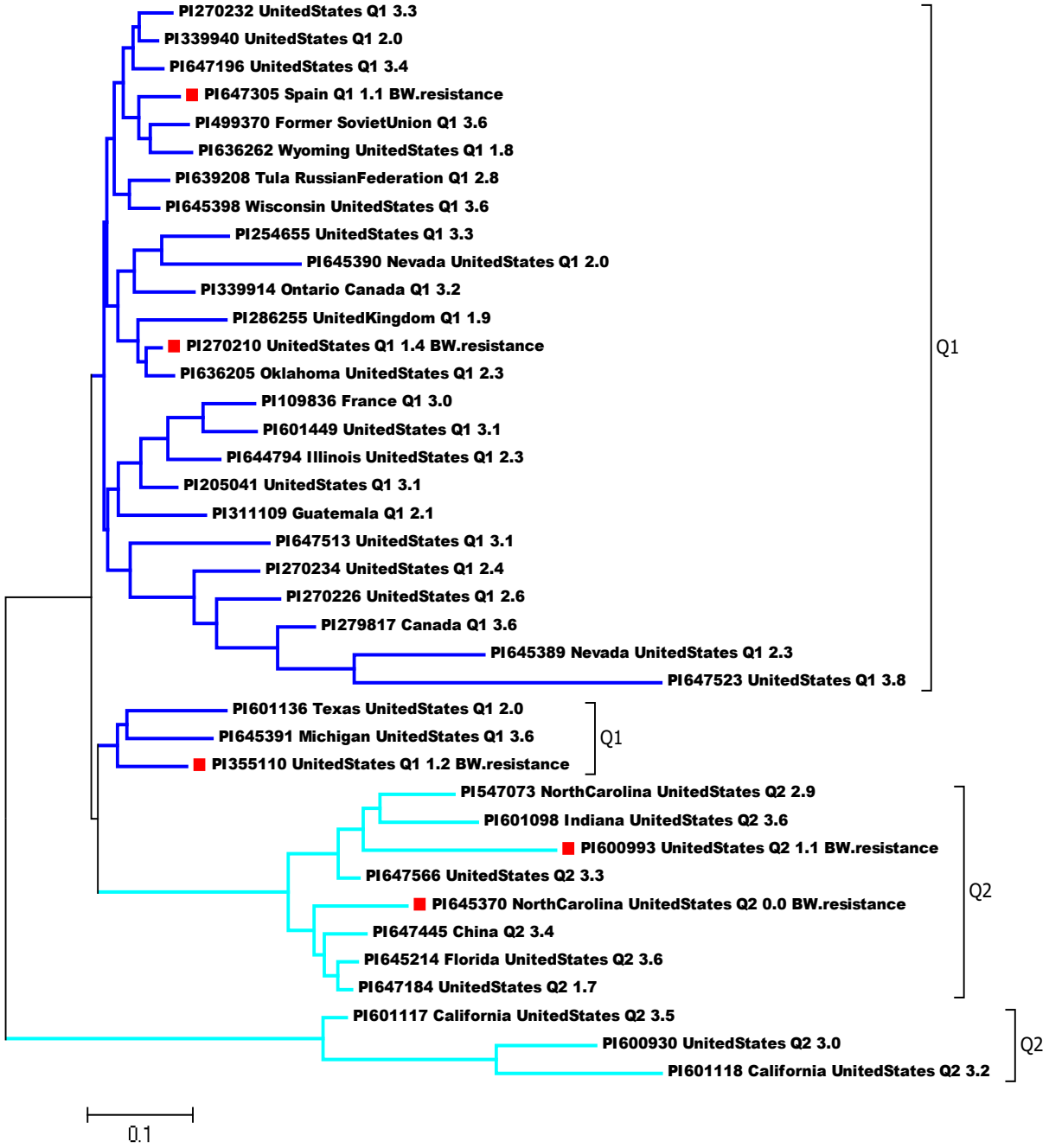


Figure 5. Phylogenetic tree among 39 tomato accessions drawn using MEGA 7 through the Maximum Likelihood (ML) method, where the accession number (PI), origin, cluster (Q1 and Q2), and bacterial wilt (BW) scale are merged as each taxon name in the tree; the red square shapes are the 5 greatest BW-resistant accessions with disease rates of less than 1.5; and two clusters (sub-populations) are observed among the 39 accessions.

4. Discussion

Bacterial diseases triggered by *R. solanacearum*, a devastating pathogen causing a threat to many plants worldwide [50], are among the major phytopathological problems of solanaceous crops. Information on the sources of resistance and inheritance is necessary to devise efficient and successful breeding strategies for developing resistant cultivars. Resistant tomato accessions have been found in other studies that are specific to different isolates of the pathogen. In this study, the use of a 0–4 scale and the DSI to assess disease severity are a widely accepted methods for evaluating tomato genotypes' resistance to

BW [51]. Out of the forty genotypes (accessions) tested in this study, five genotypes were found to be resistant to the *R. solanacearum* isolate P822, with many genotypes being susceptible to the bacteria isolate. The discovery of many genotypes that are susceptible to BW is consistent with previous studies that have reported a high degree of variability in the resistance to the disease among tomato genotypes. In a previous study conducted by Hai et al. (2008) [50], 252 wild tomato accessions were evaluated with Taiwanese race 1 strains, and five accessions were identified to be resistant to the Pss186 strain. In another study that screened 285 tomato accessions screened, 4 showed high resistance against the pathogen [3]. Consistent with prior research, including the current study, several tomato genotypes (accessions) with resistance to BW were identified. Our result aligns with that in Hai et al.'s (2008) [50] report, which highlighted the limited frequency of discovering resistance resources in tomato germplasm. The identification of PI 645370 as an extremely resistant genotype and promising candidate genotypes PI 647306, PI 600993, PI 355110, and PI 270210 for BW resistance is particularly important, as it suggests that these genotypes may possess genetic traits that make them more resilient to the disease. Previous studies have documented the existence of BW-resistant genes in tomatoes, including the RRS1-R gene [52]. As such, similar genes could probably be present in the current genotypes under investigation. If the presence of resistance genes is verified in these genotypes through further investigations, they would represent a significant addition to the collection of identified resistant tomato genotypes, such as Hawaii 7996 [53]. This would expand the selection of tomato cultivars with desirable traits, potentially enabling breeders to develop more effective and sustainable strategies for controlling disease outbreaks and enhancing crop productivity. The confirmation of resistance genes in this genotype would also contribute to a deeper understanding of the molecular mechanisms underlying tomato–pathogen interactions, which could inform the development of novel approaches for enhancing crop resilience and ensuring food security. Future studies should strive to confirm the presence of resistance genes in this genotype and to elucidate their functional significance. This is particularly important due to the increasing prevalence of BW in many tomato-growing regions worldwide.

Broad-sense heritability is a critical parameter in quantitative genetics, reflecting the proportion of phenotypic variance attributable to genetic differences among individuals [54]. In the context of this study on BW resistance in tomatoes, broad-sense heritability estimates were calculated to assess the genetic basis of resistance. The study found that BW resistance exhibited a broad-sense heritability of 59.9% based on a 0–4 scale and 42.8% based on the DSI. These estimates suggest that BW resistance is a moderately heritable trait in tomatoes, indicating that genetic factors play a significant role in determining resistance levels. This aligns with previous findings by Boakye-Mensah (2020) [55] that have reported high broad-sense heritability for BW incidence in tomatoes. It's worth noting that heritability estimates can be influenced by various factors, including the genetic complexity of the trait, environmental conditions, and the methods used for estimation. Therefore, while the estimated heritability provides valuable insights into the genetic control of BW resistance, it's essential to consider these factors when interpreting the results [56]. Overall, the findings underscore the importance of genetic factors in determining BW resistance in tomatoes and suggest that selecting for resistant crops could be an effective strategy in breeding programs aimed at developing cultivars with enhanced resistance to this destructive disease.

The clustering of genotypes in the principal component analysis (PCA) and in the phylogenetic tree can have important implications for plant breeding and conservation [57]. For example, by identifying genetically diverse genotypes, breeders can develop tomato cultivars with improved resistance to diseases and environmental stressors. Similarly, conservation efforts can focus on preserving the genetic diversity represented by both sub-populations to ensure the long-term viability of tomato production systems [57]. In this study, a PCA and phylogenetic analysis identified two distinct sub-populations of tomato genotypes (accessions) with different levels of genetic diversity, and the five BW-resistant accessions distributed in the two sub-populations provide different genetic bases

for breeders to choose from. These findings can inform breeding and conservation efforts to develop more resilient and sustainable tomato production systems.

5. Conclusions

This study evaluated bacterial wilt (BW) resistance in 40 USDA tomato germplasm accessions. Five accessions, namely PI 645370, PI 647306, PI 600993, PI 355110, and PI 270210, were observed as being BW-resistant, with PI 645370 having the greatest resistance. The broad-sense heritability was estimated as 59.9% based on a 0–4 scale of disease incidence and 42.8% based on the DSI of disease severity for BW resistance. Two distinct clusters (sub-populations) were shown among 39 of the 40 accessions, consisting of 3 and 2 BW-resistant accessions in each cluster, respectively, suggesting the presence of different genetic bases in the five resistant accessions. The identification of resistant tomato genotypes for BW resistance provides valuable information for plant breeding programs to develop BW-resistant tomato cultivars.

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