

## IDENTIFICATION OF BACTERIAL WILT (*RALSTONIA SOLANACEARUM*) RESISTANCE IN CHERRY TOMATO

Taher, Dalia I. <sup>(1)\*</sup>, Chen, Jaw-Rong<sup>(2)</sup>, Fayad, A. M. <sup>(1)</sup>

<sup>(1)</sup> Self-Pollinated Vegetable Crops Research Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt

<sup>(2)</sup> World Vegetable Center, PO Box 42, Shanhua, Tainan 74199, Taiwan

Received: Feb. 27, 2022

Accepted: Mar. 17, 2022

**ABSTRACT:** Tomato (*Solanum lycopersicum*) is an important vegetable crop grown in tropical and subtropical areas of world. Bacterial wilt caused by *Ralstonia solanacearum*, is one of the most serious diseases causing substantial crop yield losses up to 100 percent under favorable climatic conditions. The identification of new sources is the first step toward the development of bacterial wilt resistant cultivars. The objective of this study was to evaluate 81 accessions of *S. lycopersicum* var. *cerasiforme* (cherry tomato) along with resistant and susceptible checks for resistance to the highly aggressive isolates of *R. solanacearum* Pss4 and Pss1632. Tomato accessions H7996 and L390 were used as resistant and susceptible checks, respectively. The resistant control (H7996) was resistant to Pss4, and moderately resistant to Pss1632. The accession VI005692 was highly resistant to Pss1632 strain. In addition, two accessions VI005936 and VI006074 were moderate resistant to Pss1632 strain. However, all cherry tomato accessions were susceptible to Pss4. These bacterial wilt-resistant tomato accessions may be of interest for the development of resistant rootstocks and/or cultivars that can be used to control bacterial wilt in tomato.

**Keywords:** Biotic stress, cultivated tomato, resistance, soil-borne diseases, wild relatives

### INTRODUCTION

Tomato (*Solanum lycopersicum*) is a member of the Solanaceae family that includes other major crop species such as potato, pepper, eggplant and tobacco and ornamental plants such as petunia (Willcox et al., 2003). Nutritionally, Tomato is one of the cheapest sources of vitamins and minerals. Fruits consist of a high percentage of carotenoids (80%), which are strongly associated with a reduced risk of cancer and cardiovascular diseases (Clinton, 1998; Giovannucci et al., 2002; Erba et al., 2013). It is consumed fresh or as processed products such as canned tomato, sauce, juice ketchup, stews and soup (Lenucci et al., 2006). Tomato is a globally important vegetable crop with an approximate global production of 182 million metric tons harvested from 4,848,384 hectares in 2017 (FAOSTAT, 2020). Egyptian cultivated area of tomato was 375, 276 ha with a productivity of 38.96 Mg ha<sup>-1</sup> (FAOSTAT, 2020). However, biotic stresses especially bacterial wilt induced by a soil-borne pathogen *Ralstonia*

*solanacearum* is one of the critical diseases affecting yield drastically even up to 100% (Manda et al., 2020; Barik et al., 2021). This pathogen penetrates the roots of the plant, then colonizes the xylem vessels and spreads through the vascular system of susceptible plants where its faster multiplication leads to wilting and ultimately to the host plant's rapid death (Genin and Denny, 2012; Huet, 2014; Mihovilovich et al. 2017). Based on geographical regions, *R. solanacearum* strains are divided into four phylotypes: 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 (Wicker et al., 2012). These phylotypes are able to infect Solanaceae crops, such as potato, tomato, and pepper (Lebeau et al. 2011). In addition, strains of *R. solanacearum* have conventionally been classified as five races based on host rang and six biovars on the basis of carbohydrate catabolism (Lebeau et al., 2011; Wang et al., 2012) Bacterial wilt of tomato is

\*Corresponding author: E-mail: [daliataher1981@gmail.com](mailto:daliataher1981@gmail.com)

caused by either race 1 or race 3 of *R. solanacearum* and, rarely by race 2 (Janse et al., 2004).

Bacterial wilt is highly widespread in most African countries, causing substantial crop yield losses (OEPP/PPO 2004; Mamphogoro et al., 2020). Numerous methods have been employed for controlling *R. solanacearum*, including chemical, physical and biological controls (Mbega et al. 2013; Kurabachew and Ayana, 2016). Various chemical methods have been used to control bacterial wilt over the years such as fumigants, algicide and sodium chloride bactericides. However, bacterial wilt has the capacity to quickly develop bactericides resistance (Nakaune et al., 2012; Mbega et al., 2013, Kurabachew and Wydra 2014, and Yuliar et al., 2015). Physical control such as soil solarization and hot water treatment were found to be ineffective and the disease still causes major profit loss (Huet, 2014). Biological control of bacterial wilt in tomato plants through natural enemies such as *Psaenibacillus macerans*, *Bacillus pumilus* and *Bacillus subtilis* has only been widely practiced in closed greenhouse (Liu et al., 2013; Wachowska et al., 2013). Due to the complex nature of the pathogen, no method is useful when applied alone, and economic considerations often influence the chemicals selected (Yuliar et al. 2015). Therefore, there is a need for new and more effective means of controlling bacterial wilt disease. Breeding resistant cultivars is still the most economical and environmentally promising strategy for managing bacterial wilt (Boshou, 2005; Huet, 2014). Identification of resistance sources is the first step toward the development of bacterial wilt resistant cultivars.

Tomato wild relatives have been frequently used in the genetic improvement of cultivated tomato, as sources for resistance to biotic and abiotic stresses and also for quality traits (Hanson et al., 1996; Scott et al., 2005), as well as for the development of rootstocks. The objective of this study was to evaluate 81 accessions of *S. lycopersicum* var. *cerasiforme* (cherry tomato) along with resistant and susceptible checks for resistance to the highly

aggressive isolates of *R. solanacearum* Pss4 and Pss1632 for selection of novel sources of resistance for development of resistant cultivars that can be used globally to manage bacterial wilt disease sustainably.

## MATERIALS AND METHODS

### 1. Plant materials and growth conditions

The disease screening trial was carried out at greenhouses of World Vegetable Center (WorldVeg), Taiwan. Seeds of 81 accessions of *S. lycopersicum* var. *cerasiforme* were obtained from the genebank of WorldVeg (Table 1). Due to low germination, four and six tomato accessions were not evaluated for resistance to bacterial wilt strains Pss4 and Pss1632, respectively. Tomato accessions H7996 and L390 were used as resistant and susceptible checks, respectively. Seeds were sown in 9-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 the greenhouse for evaluation with a photoperiod of 16/8 h day/night, average temperature ranged from 24.2 to 29.1°C and average humidity ranged from 87.6 to 97.9 %. Seedlings were watered daily and fertilized weekly with an NPK 15-15-15 fertilizer. Plants were arranged randomly according a randomized complete block design (RCBD) with three replications and 8 plants per entry in each replication (i.e., 24 plants per accession and resistant and susceptible checks). Four-week-old plants (4-6 fully expanded true leaves) were tested for *R. solanacearum* resistance.

### 2. Disease assessment

Bacterial wilt strains Pss4 and Pss1632 were collected from Tainan and Yunlin Counties respectively in Taiwan. These strains belong to the predominant virulence group. Pss4 strains collected from infected tomato plants and identified as genotype race 1, biovar 3, phylotype 1. Pss1632 strains collected from infected potato plants and identified as genotype race 1, biovar 2 and phylotype 2. This identification was conducted through host range (Buddenhagen et

Identification of bacterial wilt (*Ralstonia solanacearum*) resistance in cherry tomato

al., 1962), biovar test (He et al., 1983; Cook et al., 1989) and molecular markers (Fegan and Prior 2005) at Bacteriology unit of WorldVeg. Bacterial strains stored at -80°C and were cultured on 2, 3, 5-triphenyl tetrazolium chloride-amended medium TTC, (Kelman 1954) and incubated at 30°C for 2 days. Then several typical fluid white colonies with pink center

were transferred from TTC to 523 medium (Kado and Heskett 1970), and incubated at 30°C overnight for multiplication. Bacterial mass from overnight cultures was transferred and suspend in water, adjusted the concentration until the optical density (O.D) value reach 0.3 at the wavelength of 600 nm (about 10<sup>8</sup> cfu/ml).

**Table 1. Means of wilting percentage and disease index, and resistance category in 77 cherry tomato accessions and controls (H7996 and L390) evaluated against *Ralstonia solanacearum* strains Pss4 and Pss1632 at four weeks after inoculation**

Accession code	Strain Pss4			Accession No.	Strain Pss1632		
	Wilting (%)	Disease index	RC		Wilting (%)	Disease index	RC
VI005556	75.00	68.33	S	VI005512	91.67	91.67	S
VI005512	100.00	100.00	S	VI005543	83.33	80.00	S
VI005543	100.00	100.00	S	VI005544	83.33	78.33	S
VI005544	91.67	90.00	S	VI005555	83.33	80.00	S
VI005555	100.00	98.33	S	VI005556	83.33	83.33	S
VI005557	100.00	100.00	S	VI005557	75.00	71.67	S
VI005558	100.00	100.00	S	VI005558	91.67	90.00	S
VI005560	100.00	100.00	S	VI005560	91.67	90.00	S
VI005562	100.00	100.00	S	VI005562	75.00	71.67	S
VI005569	100.00	100.00	S	VI005569	66.67	60.00	S
VI005571	100.00	96.67	S	VI005571	75.00	58.33	S
VI005578	100.00	96.67	S	VI005578	75.00	68.33	S
VI005579	91.67	90.00	S	VI005579	83.33	81.67	S
VI005580	100.00	100.00	S	VI005580	91.67	86.67	S
VI005581	100.00	100.00	S	VI005581	83.33	80.00	S
VI005584	100.00	98.33	S	VI005584	100.00	96.67	S
VI005585	100.00	100.00	S	VI005585	83.33	78.33	S
VI005599	100.00	98.33	S	VI005599	100.00	93.33	S
VI005692	75.00	70.00	S	VI005692	0.00	0.00	R
VI005862	100.00	100.00	S	VI005862	83.33	75.00	S
VI005891-A	100.00	95.00	S	VI005891-A	100.00	93.33	S
VI005891-B	100.00	96.67	S	VI005891-B	83.33	83.33	S
VI005892	91.67	86.67	S	VI005892	83.33	80.00	S
VI005896	100.00	98.33	S	VI005896	100.00	95.00	S
VI005927	100.00	96.67	S	VI005927	91.67	90.00	S
VI005936	91.67	91.67	S	VI005936	37.50	27.50	R
VI006074	100.00	100.00	S	VI006074	33.33	23.33	R
VI006090	100.00	100.00	S	VI006090	91.67	88.33	S
VI006399	100.00	98.33	S	VI006399	66.67	65.00	S
VI006557	100.00	100.00	S	VI006557	100.00	95.00	S
VI006587	100.00	98.33	S	VI006587	100.00	98.33	S
VI006630	100.00	100.00	S	VI006630	91.67	78.33	S
VI006789	100.00	95.00	S	VI006789	100.00	93.33	S
VI006842	100.00	100.00	S	VI006842	100.00	100.00	S
VI006906	100.00	98.33	S	VI006906	100.00	96.67	S
VI006917	100.00	100.00	S	VI006917	91.67	90.00	S

**Table 1. Cont.**

VI006921	91.67	90.00	S	VI006921	91.67	86.67	S
VI007556	91.67	86.67	S	VI007556	83.33	75.00	S
VI007560	100.00	100.00	S	VI007560	100.00	93.33	S
VI007564	100.00	100.00	S	VI007564	100.00	93.33	S
VI007568	100.00	96.67	S	VI007568	91.67	83.33	S
VI007571	100.00	100.00	S	VI007571	83.33	71.67	S
VI009092	100.00	95.00	S	VI009092	91.67	85.00	S
VI009093	100.00	96.67	S	VI009093	83.33	78.33	S
VI009100	100.00	100.00	S	VI009100	91.67	85.00	S
VI009101	100.00	100.00	S	VI009101	58.33	41.67	MS
VI009102	91.67	90.00	S	VI009102	83.33	80.00	S
VI009403	ND	ND	ND	VI009403	ND	ND	ND
VI009450	91.67	86.67	S	VI009450	100.00	86.67	S
VI009649	100.00	95.00	S	VI009649	58.33	55.00	S
VI009650	83.33	76.67	S	VI009650	91.67	88.33	S
VI009739	100.00	100.00	S	VI009739	91.67	91.67	S
VI009936	100.00	100.00	S	VI009936	100.00	100.00	S
VI010095	100.00	100.00	S	VI010095	100.00	100.00	S
VI029818	ND	ND	ND	VI029818	ND	ND	ND
VI030133	100.00	100.00	S	VI030133	75.00	73.33	S
VI030143	100.00	100.00	S	VI030143	100.00	96.67	S
VI030144	100.00	98.33	S	VI030144	100.00	98.33	S
VI030154	100.00	98.33	S	VI030154	100.00	91.67	S
VI030155	100.00	100.00	S	VI030155	91.67	83.33	S
VI030361	100.00	100.00	S	VI030361	58.33	50.00	MS
VI030676	ND	ND	ND	VI030676	ND	ND	ND
VI030679	ND	ND	ND	VI030679	ND	ND	ND
VI030690	100.00	100.00	S	VI030690	ND	ND	ND
VI037951	75.00	75.00	S	VI037951	41.67	31.67	MR
VI037955	100.00	100.00	S	VI037955	100.00	100.00	S
VI040002	100.00	100.00	S	VI040002	83.33	78.33	S
VI040033	100.00	98.33	S	VI040033	91.67	85.00	S
VI040174	100.00	98.33	S	VI040174	91.67	81.67	S
VI040273	100.00	96.67	S	VI040273	91.67	85.00	S
VI040288	100.00	100.00	S	VI040288	100.00	98.33	S
VI041105	91.67	91.67	S	VI041105	66.67	61.67	S
VI041154	100.00	100.00	S	VI041154	83.33	83.33	S
VI044914	100.00	96.67	S	VI044914	ND	ND	ND
VI045785	83.33	80.00	S	VI045785	100.00	100.00	S
VI057404	100.00	100.00	S	VI057404	100.00	100.00	S
VI057409	100.00	98.33	S	VI057409	91.67	83.33	S
VI057430	100.00	100.00	S	VI057430	100.00	96.67	S
VI057431	100.00	100.00	S	VI057431	91.67	88.33	S
VI059336	100.00	100.00	S	VI059336	100.00	76.67	S
VI063893	100.00	100.00	S	VI063893	100.00	100.00	S
L390	100.00	100.00	S	L390	100.00	96.67	S
H7996	8.33	8.33	R	H7996	58.33	55.00	S

Resistance category (RC) was performed according to the disease index at the fourth week after inoculation; R = resistant (0–30%), MR = moderately resistant (>30–40%), MS = moderately susceptible (>40–50%), S = susceptible (>51%).

Before inoculation, roots of accessions and checks were injured with a knife by cutting through the soil 1 to 2 cm away from the stem base. A mount of 40 ml of bacterial suspension ( $10^8$ cfu/ml) was poured into each pot and kept the inoculated plants in a plastic greenhouse (Hanson et al. 1996). Plants were evaluated once a week for four weeks using the wilting percentage (W%) and disease index (DI) based on a disease rating scale (0 - 5), 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 (Winstead and Kelman 1952). Wilting percentage (W%) was calculated following the formula  $W\% = (Nw / Nt) \times 100$ , where Nw = number of wilted plants; and, Nt = total number of plants. The disease index (DI) was calculated using the following formula  $DI = [(N0 \times 0 + N1 \times 1 + N2 \times 2 + N3 \times 3 + N4 \times 4 + N5 \times 5) / (Nt / 5)] \times 100$ , where N0 to N5 = number of plants having disease rating scale values from 0 to 5; and, Nt = total number of plants. Accessions with DI from 0% to 30% were considered as resistant (R), above 30% to 40% as moderately resistant (MR), above 40% to 50% as moderately susceptible (MS), and over 50% as susceptible (S) according to Aslam et al. (2017).

### 3. Morphological and horticultural traits in bacterial wilt-resistant accessions

Vegetative growth parameters including growth habit (dwarf, determinate, semi-determinate and indeterminate), leaf attitude (horizontal, erect, semi-erect) and anthocyanin coloration of leaf veins were recorded at 55 days after transplanting. At flowering stage, number of flower per inflorescence, petal length (cm), sepal length (cm), and style type stamen length (cm) were recorded. After fruit harvesting, fruit shape, presence of jointless pedicel, firmness, cracking, fruit fasciation, fruit weight (gm), fruit length (cm), fruit width (cm), pedicel length (mm), number of locules, skin color of ripen fruit, interior flesh color (pericarp), and soluble solids (TSS%) were measured on 10 harvested fruits per replication. Fruit firmness (g/cm<sup>2</sup>) was measured using a hand penetrometer (2 mm) on opposite cheeks at the center of each fruit. The

probe was inserted to the bioyield point. The TSS in juice of tomato fruits was estimated by a hand refractometer according to the Association of Official Agricultural Chemists (AOAC, 1965).

### Results

The resistance reaction and category of 81 accessions of *S. lycopersicum* var. *cerasiforme* against *R. solanacearum* strains Pss4 and Pss1632 at four weeks of inoculation is presented in Table 2. The susceptible check (L390) displayed the expected reactions of high susceptibility to strains Pss4 and Pss1632 with the values 100% and 96.67%, respectively. All L390 plants wilted and died rapidly two and three weeks after inoculation by Pss4 and Pss1632, respectively. Bacterial wilt symptoms were appeared one week after inoculation in susceptible check as well as *S. lycopersicum* var. *cerasiforme* accessions. The resistant check (H7996) was resistant to Pss4, with value 8.33 for W% and DI, and it was moderately resistant to Pss1632, with values 58.33% and 55 for W% and DI, respectively. It is worth mentioning that all of the 81 accessions of wild tomato were susceptible to Pss4, with a range 75 –100% for W% and 68.33 –100% for DI. Out of 81 wild accessions screened for resistance to Pss1632, VI005692 accession was immune or highly resistant to Pss1632 strain. In addition, two accessions VI005936 and VI006074 were moderate resistant, with 33.3% and 37.5% of W% and 33.3% and 37.5% of DI, respectively. However, VI037951 accession was moderate susceptible with 41.76% of W% and 31.76% of DI%.

Morphological and horticultural traits including vegetative growth, flowering and fruit parameters in bacterial wilt-resistant tomato accessions are presented in Table 2. Three types of growth habit were observed among resistant accessions. VI005692 accession had dwarf type. In addition, determinate and semi determinate types were observed in VI006074 and VI037951, respectively. Anthocyanin coloration of leaf veins was absent among the genotypes. Number of flowers per inflorescence exhibited either high or low. Highest number of flowers per inflorescence was recorded in VI006074, but the lowest number of flowers per inflorescence was

recorded in dwarf tomato accession VI005692. The tomato accession varied in respect to style type. It was slightly exerted, inserted, and at the same level as stamen in VI005692, VI006074 and VI037951, respectively. This could be used to differentiate tomato accessions at flowering stage as wider variation has seen in the study. Most of vegetative and flowering parameters were not observed in VI005936. Fruit data was not varied among tomato accessions in terms of fruit shape, cracking and skin color of ripen fruit. Interestingly, fruit cracking was not observed in all resistant tomato accessions. Jointless pedicel was present in resistant tomato accessions except VI037951. Fruits were soft in VI006074 and VI037951, but fruit firmness was medium in

VI005692 and VI005936. Wide variations were observed in fruit weight, fruit length, fruit width, pedicel length, and number of locules among tomato accessions. The fruit weight ranges from 15.9 to 3.5 gm, fruit length ranges from 2.7 to 1.4 cm and fruit width ranging from 3 to 1.3 cm. The highest pedicel length was present in VI005692 accession, but the lowest pedicel length was in VI006074 accession. The skin color of all accessions was yellow. The interior flesh fruit of all resistant tomato accessions was red except VI006074 was pink. Finally, the soluble solids content (SSC) was measured, and highest SSC values were found in VI006074 accession.

**Table 2. Morphological and horticultural traits in bacterial wilt-resistant cherry tomato accessions identified in the present study**

Traits	WorldVeg genebank code			
	VI005692	VI005936	VI006074	VI037951
<b>Vegetative growth</b>				
Growth habit	Dwarf	ND	Determinate	Semi-determinate
Leaf attitude	Horizontal	ND	Horizontal	Semi-erect
Anthocyanin coloration of leaf veins	Absent	Absent	Absent	Absent
<b>Flowering data</b>				
Number of flower per inflorescence	6	ND	13.4	8
Petal length (cm)	1.4	ND	1.3	1.4
Sepal length (cm)	1.2	ND	0.8	0.7
Style type	Slightly exerted	ND	Inserted	Same level as stamen
Stamen length (cm)	1	ND	1	0.9
<b>Fruit data</b>				
Fruit shape	Round	Round	Round	Mixture (Round, High-round)
Presence of jointless pedicel	Present	Present	Present	Absent
Firmness	Medium	Medium	Soft	Soft
Cracking	None	ND	None	None
Fruit fasciation	Smooth	Smooth	Smooth	Slight
Fruit weight (gm) (N=10)	15.9	9	3.5	9.6
Fruit length (cm) (N=10)	2.7	1.4	1.8	2.3
Fruit width (cm) (N=10)	3	1.3	1.8	2.4
Pedicel length (mm)	9.3	6	5.4	7.2
Number of locules (N=10)	2.4	2	2	2.1
Skin color of ripen fruit	Yellow	Yellow	Yellow	Yellow
Interior flesh color (pericarp)	Red	Red	Pink	Red
Soluble solids (%)	5.4	6	7.8	5.5

## Discussion

Bacterial wilt is one of the major diseases of tomato and other solanaceous plant, the damage of BW is spreading beyond tropical and subtropical regions worldwide (Mansfield et al., 2012). Identification of sources of tolerance or resistance to disease is a first step for conventional breeding of these traits. In the last decades, some examples of genetic transfer of interest from wild species to the cultivated species have been established (Prohens et al., 2017). Numerous sources for resistance to bacterial wilt have been found in *S. pimpinellifolium* and cultivated tomato such as, Hawaii 7996, Hawaii 7997, and Hawaii 7998 (Scott et al., 2005; Carmeille et al., 2006; Alsam et al., 2017). Resistance in current resistant commercial cultivars is mostly derived from two major sources *S. pimpinellifolium* and *S. lycopersicum* var. *cerasiforme* (Hanson et al., 1998). Breeding bacterial wilt-resistant tomato varieties is difficult because resistance is often dependent on pathogen strain, which is highly affected by environmental conditions such as soil type, temperature, pH and moisture (Wang et al., 1998; Prior et al., 2016; Kunwar et al., 2019).

Our study focused on identification of bacterial wilt resistance in *S. lycopersicum* var. *cerasiforme* (cherry tomato) because it is very close to the cultivated tomato. In addition, previous genetic studies have shown that introgression of disease resistance from *S. lycopersicum* var. *cerasiforme* may be easier and faster (Ranc et al., 2008). Desirable traits were found in cherry tomatoes including disease resistance, fruit abscission, soluble solids content, fruit size, flavor, texture, and post-harvest quality (Kwon et al., 2009). Cherry tomatoes were developed to enrich the tomato market with new competing commercial choices (Mukherjee et al. 2020; Rodriguez et al. 2011). These reasons encouraged us to explore more stable sources of resistance to bacterial wilt in *S. lycopersicum* var. *cerasiforme*. In our study, we evaluated 81 accessions of *S. lycopersicum* var. *cerasiforme* along with resistant and susceptible checks for resistance to the highly aggressive

isolates of *R. solanacearum* Pss4 and Pss1632. The accession VI005692 showed a high level of resistance to Pss1632 strain and could be an appropriate source for breeding resistant tomato cultivars. Moreover, it may also be useful as a resistant standard tomato line to bacterial wilt in future pathological studies. In addition, the results indicate that accessions VI005936 and VI006074 were moderately resistant to Pss1632 strain. Many factors affect bacterial wilt resistance such as, plant age, inoculum concentration, temperature, inoculation method (Singh et al., 2014). Previous research found that the mechanisms of resistance could be due to a higher concentration of secondary metabolism, such as polyphenols and steroidal glycoalkaloids, which prevent bacterial movement into the vicinity of the plant system (Vasse et al., 2005; Namesy et al., 2019; Rakha et al., 2020). Chemical analysis in resistant and susceptible tomato accessions might enable us to identify mechanisms of bacterial wilt resistance in *S. lycopersicum* var. *cerasiforme*.

In our study, all accessions of *S. lycopersicum* var. *cerasiforme* were susceptible to Pss4, indicating that Pss4 has higher virulence than Pss1632. The early wilt symptoms appeared one week after inoculation, and most of the plants were completely wilting after two weeks. Similarly, Hoque et al., (1981) found that a high incidence of bacterial wilt in tomato was observed 15 days after inoculation at the early stage of growth. In the present study, accessions VI005692, VI005936 and VI006074 were susceptible to Pss4, but were previously reported to be resistant to another strain Pss1632. Also, Truong et al., (2008) found five accessions of *S. pennellii* were found to have significant tolerance to Pss186 and Pss190, but not Pss4. This may indicate potential strain-specific nature of resistance in these accessions. These bacterial wilt-resistant accessions may be of interest for the development of resistant rootstocks and/or cultivars that can be used to manage bacterial wilt in tomato. Therefore, further studies are needed to evaluate resistant sources against a broader array of bacterial wilt strains with a wider diversity to determine their potential use in future breeding.

## REFERENCES

- AOAC (1965). Association of Official Agricultural Chemists, Official Methods of Analysis. Washington, D.C. 10th ed.
- Aslam, M. N.; Hussain, M. A. and Raheel, M. (2017). Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm. J. Plant Dis. Prot., 124: 585-590.
- Barik, S.; Ponnampalani, N.; Acharya, G. C.; Sandeep, V.; Singh, T. H.; Kumari, M.; Srinivas, P. and Shankar, S. (2021). Genetic analysis of bacterial wilt resistance in eggplant (*Solanum melongena* L.). Eur. J. Plant Pathol., 160: 349-364.
- Boshou, L. A. (2005). broad review and perspective on breeding for resistance to bacterial wilt. In Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex. Allen, C.; P. Prior and A. C. Hayward, (Eds.); American Phytopathological Society: St. Paul, MN, USA; pp. 225-238.
- Buddenhagen, I. W. (1962). Designations of races in *Pseudomonas solanacearum*. Phytopathology, 52: 726.
- Carneille, A.; Caranta, C.; Dintinger, J.; Prior, P.; Luisetti, J. and Besse, P. (2006). Identification of QTLs for *Ralstonia solanacearum* race 3 in tomato. Theor. Appl. Genet., 113: 110-121.
- Clinton, S. K. (1998). Lycopene: Chemistry, biology, and implications for human health and disease. Nutr. Rev., 56: 35-51.
- Cook, D. (1989). Genetic diversity of *Pseudomonas solanacearum*: Detection of restriction fragment length polymorphisms with DNA probes that specify virulence and the hypersensitive response. Mol. Plant Microbe Interact., 2: 113.
- Erba, D.; Casiraghi, M. C.; Ribas-Agustí, A.; Cáceres, R.; Marfà, O. and Castellari, M. (2013). Nutritional value of tomatoes (*Solanum lycopersicum* L.) grown in greenhouse by different agronomic techniques. J. Food Compost Anal., 31(2): 245- 251.
- FAOSTAT (2020). Tomato production in 2019, Crops/Regions/World list/Production Quantity (pick lists). UN Food and Agriculture Organization, Corporate Statistical Database. Retrieved 3 March 2021.
- Fegan, M. and Prior, P. (2005). How complex is the '*Ralstonia solanacearum* species complex? In Bacterial wilt Disease and the *Ralstonia solanacearum* Species Complex. Allen, C.; P. Prior and A. C. Hayward (Eds.) Saint Paul, MN: APS Press; pp 449-461.
- Genin, S. and Denny, T. P. (2012). Pathogenomics of the *Ralstonia solanacearum* Species Complex. Annu. Rev. Phytopathol., 50: 67-89.
- Giovannucci, E.; Rimm, E. B.; Liu, Y.; Stampfer, M. J. and Willett, W. C. (2002). A prospective study of tomato products, lycopene, and prostate cancer risk. J. Natl. Cancer Inst., 94: 391-398.
- Hanson, P. M.; Licardo, O.; Wang, J. F. and Chen, J. T. (1998). Diallel analysis of bacterial wilt resistance in tomato derived from different sources. Plant. Dis., 82: 74-78.
- Hanson, P. M.; J. F. Wang; O. Licardo; G. L. Hartman; Y. C. Lin and J. Chen (1996). Variable reaction of tomato lines to bacterial wilt resistance at several locations in southeast Asia. HortScience, 31: 143.
- He, L.Y. (1983). Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Dis., 67: 1357-1361.
- Hoque, M.O.; HO, M. L. and Chowdhury, B. C. (1981). Screening tomato varieties for resistance to bacterial wilt. Bangladesh. J. Agril. Res. 6: 55.
- Huet, G. (2014). Breeding for resistances to *Ralstonia solanacearum*. Front. Plant Sci., 5: 715.
- Janse, J. D.; Van Den Beld, H. E.; Elphinstone, J.; Simpkins, S.; Tjou-Tam-Sin, N. N. and J. Van Vaerenbergh (2004). Introduction to Europe of *Ralstonia solanacearum* biovar 2, race 3 in *Pelargonium zonale* cuttings. J. Plant Pathol., 86: 147-155.



- Kado, C. and Heskett, M. (1970). Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. *Phytopathology*, 60: 969-976.
- Kelman, A. (1954). The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology*, 44: 693-695.
- Kunwar, S.; Che Hsu, Y.; Fen Lu, S.; Fen Wang, J.; Jones, J.; Hutton, S.; Paret, M. and Hanson, P. (2019). Characterization of tomato (*Solanum lycopersicum*) accessions for resistance to phylotype I and phylotype II strains of the *Ralstonia solanacearum* species complex under high temperatures. *Plant Breed.*, 139: 389-401.
- Kurabachew, H. and Ayana, G. (2016). Bacterial wilt caused by *Ralstonia solanacearum* in Ethiopia: status and management approaches: a review. *Int. J. Phytopathol.*, 5: 107- 119.
- Kurabachew, H. and Wydra, K. (2014). Induction of systemic resistance and defense-related enzymes after elicitation of resistance by rhizobacteria and silicon application against *Ralstonia solanacearum* in tomato (*Solanum lycopersicum*). *Crop Prot.*, 57: 1- 7.
- Kwon, Y. S.; Park, S. G. and Yi, S.I. (2009). Assessment of genetic variation among commercial tomato (*Solanum lycopersicum* L) varieties using SSR markers and morphological characteristics. *Genes and Genomics*, 31(1): 1-10.
- Lebeau, A.; Daunay, M. C.; Frary, A.; Palloix, A.; Wang, J. F.; Dintinger, J.; Chiroleu, F.; Wicker, E.; et al. (2011). Bacterial wilt resistance in tomato, pepper, and eggplant: genetic resources respond to diverse strains in the *Ralstonia solanacearum* species complex. *Phytopathology*, 101: 154-165.
- Lenucci, M. S.; Cadinu, D.; Taurino, G. P. and Alessandro, D. G. (2006). Antioxidant composition in cherry and high-pigment tomato cultivars. *J. Agric. Food. Chem.*, 54: 2606.
- Liu, J.; Sui, Y.; Wisniewski, M.; Droby, S. and Liu, Y. (2013). Review: utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. *Int. J. Food Microbiol.*, 167: 153- 160.
- Mamphogoro, T. P.; Babalola, O.O. and Aiyegoro, O.A. (2020). Sustainable management strategies for bacterial wilt of sweet peppers (*Capsicum annum*) and other Solanaceous crops. *J. Appl. Microbiol.*, 129: 496-508.
- Manda, R.; Addanki, V. and Srivastava, S. (2020). Bacterial wilt of solanaceous crops. *Int. J. Chem. Stud.*, 8(6): 1048-1057.
- Mansfield, J.; Genin, S.; Magori, S.; Citovsky, V.; Sriariyanum, M.; Ronald, P.; Dow, M.; Verdier, V.; et al. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol. Plant Pathol.*, 13: 614- 629.
- Mbega, E. R.; Adriko, J.; Mortensen, C. N.; Wulff, E. G.; Lund, O. S. and Mabagala, R. B. (2013). Improved sample preparation for PCR-based assays in the detection of *Xanthomonads* causing bacterial leaf spot of tomato. *Br. Biotechnol. J.*, 3: 556-574.
- Mihovilovich, E.; Lopes, C.; Gutarra, L.; Lindqvist-Kreuzer, H.; Aley, P.; Priou, S. and Bonierbale, M. (2017). Protocol for Assessing Bacterial Wilt Resistance in Greenhouse and Field Conditions. *International Cooperators' Guide*. pp. 35. Lima: International Potato Center.
- Mukherjee, D.; Maurya, P.; Bhattacharjee, T.; Banerjee, S.; Chatterjee, S.; Mal, S. et al. (2020). Assessment of Breeding Potential of Cherry Tomato [*Solanum lycopersicum* var. *Cerasiforme* (Dunal) A. Gray] Grown under Open Field to Identify Desirable Alleles. *Inter. J. of Vegetable Sci.*, 9: 2152-2171.
- Nakaune, M.; Tsukazawa, K.; Uga, H.; Asamizu, E.; Imanishi, S.; Matsukura, C. and Ezura, H. (2012). Low sodium chloride priming increases seedling vigor and stress tolerance to *Ralstonia solanacearum* in tomato. *Plant Biotechnol.*, 29: 9-18.

- Namisy, A.; Chen, J.; Prohen, J.; Metwally, E.; Elmahrouk, M. and Rakha, M. (2019). Screening of cultivated eggplant and wild relatives for resistance to bacterial wilt (*Ralstonia solanacearum*). *Agriculture*, 9(7): 157.
- OEPP/EPPO (2004). *Ralstonia solanacearum*. *EPPO Bull* 34: 173- 178.
- Prior, P.; Ailloud, F.; Dalsing, B. L.; Remenant, B.; Sanchez, B. and Allen, C. (2016). Genomic and proteomic evidence supporting the division of the plant pathogen *Ralstonia solanacearum* into three species. *BMC Genomics*, 17: 90.
- Prohens, J.; Gramazio, P.; Plazas, M.; Dempewolf, H.; Kilian, B.; et al. (2017). Introgressomics: a new approach for using crop wild relatives in breeding for adaptation to climate change. *Euphytica*, 213: 158.
- Rakha, M.; Namisy, A.; Chen, J. R.; El-Mahrouk, M. E.; Metwally, E.; et al. (2020). Development of interspecific hybrids between a cultivated eggplant resistant to bacterial wilt (*Ralstonia solanacearum*) and eggplant wild relatives for the development of rootstocks. *Plants*, 9(10):1405.
- Ranc, N.; Muñoz, S.; Santoni, S. and Causse, M. (2008). A clarified position for *Solanum lycopersicum* var. *cerasiforme* in the evolutionary history of tomatoes (Solanaceae). *BMC Plant Biol.*, 8: 130.
- Rodriguez, G. R.; Munos, S.; Anderson, C.; Sim, S. C.; Michel, A.; Causse, M.; et al. (2011). Distribution of SUN, OVATE, LC, and FAS in the tomato germplasm and the relationship to fruit shape diversity. *Plant Physiol.*, 156: 275-285.
- Scott, J.W.; Wang, J. F. and Hanson, P. M. (2005). Breeding tomatoes for resistance to bacterial wilt, a global view. *Acta Hort.*, 695: 161-172.
- Singh, A. K.; Pan, R. S. and Bhavana, P. (2014). Genetic studies for quantitative traits and resistance to bacterial wilt in brinjal (*Solanum melongena*). *Int. J. Innov. Hortic.* 3(2): 154-157.
- Truong, H. T.; Esch, E. and Wang, J. F. (2008). Resistance to Taiwanese race 1 strains of *Ralstonia solanacearum* in wild tomato germplasm. *Eur. J. Plant Pathol.* 122:471-479.
- Vasse, J.; Danoun, S. and Trigalet, A. (2005). Microscopic studies of root infection in resistant tomato cultivar Hawaii. In *Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex*. Allen, C.; P. Prior and A. C. Hayward, (Eds.); APS Press: St. Paul, MN, USA; pp. 285-291.
- Wachowska, U.; Kucharska, K.; Jedryczka, M. and Łobik, N. (2013). Microorganisms as biological control agents against fusarium pathogens in winter wheat. *Pol. J. Environ. Stud.*, 22: 591-597.
- Wang, J. F.; Hanson, P. and Barnes, J. A. (1998). Worldwide evaluation of an international set of resistance sources to bacterial wilt in tomato. In *Bacterial Wilt Disease: Molecular and Ecological Aspects*; Prior, P.; C. Allen and J. Elphinstone (Eds.); Springer: Berlin, Germany; pp. 269-275.
- Wicker, E.; Lefeuvre, P.; De Cambiaire, J. C.; Lemaire, C.; Poussier, S. and Prior, P. (2012). Contrasting recombination patterns and demographic histories of the plant pathogen *Ralstonia solanacearum* inferred from MLSA. *ISME. J.*, 6: 961-974.
- Willcox, J. K.; Catignani, G. L. and Lazarus, S. (2003). Tomatoes and Cardiovascular Health. *Crit. Rev. Food. Sci. Nut.*, 43: 1-18.
- Winstead, N.N. and Kelman, A. (1952). Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. *Phytopathology*, 42: 623-634.
- Yuliar, Y.; Nion, Y. A. and Toyota, K. (2015). Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. *Microbes Environ* 30: 1- 11.

## التعرف على مقاومة الذبول البكتيري (*Ralstonia solanacearum*) في الطماطم الكرزية (*Solanum lycopersicum* var. *cerasiforme*)

داليا إبراهيم طاهر<sup>(1)</sup> - جاورونج تشين<sup>(2)</sup> - عاطف فياض<sup>(1)</sup>

<sup>(1)</sup> قسم محاصيل الخضار ذاتية التلقيح - معهد بحوث البساتين- مركز البحوث الزراعيه - الجيزه - مصر

<sup>(2)</sup> المركز العالمي للخضار بتايوان

### الملخص العربي

الطماطم (*Solanum lycopersicum*) هي محصول نباتي مهم يزرع في المناطق الاستوائية وشبه الاستوائية. الذبول البكتيري الناجم عن *Ralstonia solanacearum*، هو أحد أخطر الأمراض التي تسبب خسائر كبيرة في المحصول تصل إلى 100% في ظل الظروف المناخية المواتية للمرض. تحديد مصادر المقاومة الجديدة هو الخطوة الأولى نحو تطوير أصناف مقاومة للذبول البكتيري. الهدف من هذه الدراسة هو تقييم عدد 81 سلالة من *S. lycopersicum* var. *cerasiforme* (الطماطم الكرزية) لمقاومة العزلات شديدة الإصابة من الذبول البكتيري Pss4 و Pss1632. تم استخدام صنف الطماطم H7996 و L390 كأصناف مقاومة وحساسة، على التوالي. كان الصنف الكنترول المقاوم (H7996) مقاومًا لـ Pss4 ومتوسط المقاومة لـ Pss1632. سلالة الطماطم VI005692 كانت شديدة المقاومة لسلالة Pss1632. بينما كانت سلالاتان الطماطم VI005936 و VI006074 متوسطة المقاومة لسلالة Pss1632. ومع ذلك، كانت جميع سلالات الطماطم الكرزية عرضة للإصابة لـ Pss4. قد تكون هذه السلالات المقاومة ذات أهمية لتطوير أصول للتطعيم عليها أو أصناف مقاومة يمكن استخدامها للسيطرة على الذبول البكتيري في الطماطم.