Verify seedling screening method in seedling and greenhouse screening for tomato bacterial wilt

Abstract

Bacterial wilt in tomato plants is caused by *Ralstonia solanacearum* species complex. Tomato crops has economic significance, hence the loss of the disease is a huge one. Fortunately, it can be managed by resistant cultivars, which is promised to be an effective and environment friendly approach. For the screening of resistant cultivars, traditional screening processes can take a long time, but recently, published research found out a rapid screening method is applicable for tomato plants. Therefore, we aim to establish the cotyledon-screening method as a rapid and reliable alternative compare to traditional method. The method was undecided that how many days after inoculation is most applicable for measuring their reaction, hence we took wilting percentage from 5, 7 and 10 days after inoculation and select which set of data correlate reliable to the data from the traditional screening. The results showed that the 10th day after inoculation has the highest correlation coefficient. From carrying out the trials, we found out the advantages of the method as well as data that suggested when the method is appropriate and applicable**.** This experiment results provide us a preliminary cotyledon-screening method, more details will need further improvement through more modified experiment in the future.

Introduction

Bacteria wilt caused by *Ralstonia solanacearum* species complex (RSSC) is a prevalent disease that affects solanaceous plants in tropics, subtropics, and temperate regions. According to FAOSTAT, tomato (*Solanum lycopersicum* L.) is a major crop in Taiwan and its yield in 2020 was 97,603 tonnes. Meanwhile, the global yield of tomato was 186,821,216 tonnes, ranking 13th among 162 produced crops [1]. Given its dominating presence in the global market, the huge yield loss caused by bacterial wilt has serious economic implications worldwide. Based on a survey from 1991, the estimated losses of fresh market tomatoes caused by bacterial wilt from their surveyed fields was NT$ 496,000 per hectare in Taiwan [2].

Until recently, RSSC strains had been compartmentalized into 5 races based on host specificity [3] and 6 biovars based on carbohydrate utilization [4] but phylogenetic analysis had demonstrated that the heterogeneity of R. *solanacearum* can neither be confined to one species [5] nor taxonomically explained by the race/biovar system [6]. Different phylotypes had originated from different parts of the world and the resistant of a plant cultivar is highly associated with the dominant strains [7] as well as locality due to interaction with environmental factors [8][9]. The dominant population of RSSC in Taiwan is phylotype I, Race 1, biovar 3 or 4 [10]. The bacteria invade the plant through penetrating the root or through natural or artificial wounds and openings. Irreversible wilt and death occur soon after as they proliferate and colonize the xylem

tissue of the plant, blocking the path for water transportation. Besides its lethality, RSSC is a soilborne bacterium that has mechanisms of latent infection and a broad and expanding host range, including many weeds, which enable persistency and widespread distribution [11][12].

Among the diverse strategies of disease management such as chemicals, physical methods, biological control and certain practices, resistant cultivars are promised to be economically and environmentally sustainable solutions [13]. There are varied screening methods for resistant cultivars such as greenhouse screening and field evaluation [14][15]. However, these screening methods take a longer time to complete when compared to cotyledon-seedling screening method (CSM). According to a previous study, when applied on tomato plants, the cotyledon-seedling screening method uses a 6-7 days cotyledon-seedling for inoculation to make this method rapid and less resource dependent [16].

Hence, our objective is to establish a rapid screening method for tomato bacterial wilt resistance.

Materials and methods

Plant material:

A total of 151 tomato breeding lines (Table 1 and 2) were used to compare the screening methods in the greenhouse and the 8_{th} day stage of cotyledon seedlings. Susceptible variety L390 and resistant variety H7996 were involved in every trial. For cotyledon-seedling screening, each breeding line was laid out in a randomized complete block design (RCBD) with 3 replications. Each replication included 10 cotyledon seedlings. 5 cotyledon seedlings of each breeding line without inoculation were used as a negative control. For greenhouse screening, we apply 8 plants for each line. There is no block replication in greenhouse experiment due to insufficient seeds. The number of seedlings may vary depends on the germination rate.

Table 1. Disease reaction of breeding lines for Pss4

Breeding lines	10 DAI Avg W%	Wilting percent						4 WAI Avg W%	Greenhouse scoring
		≤10%	≤20%	≤30%	≤40%	≤50%	≤60%		
220111	59.3	S	S	S	S	S	R	87.5	S
220112	100	S	S	S	S	S	S	75	S
220113	81.0	S	S	S	S	S	S	62.5	S
220114	100	S	S	S	S	S	S	62.5	S
220115	50	S	S	S	S	R	R	0	R
220116	90	S	S	S	S	S	S	50	S
220117	53.3	S	S	S	S	S	R	37.5	R
220118	58.9	S	S	S	S	S	R	0	R
220119	83.3	S	S	S	S	S	S	37.5	R

220120	100	S	S	S	S	S	S	50	S
220121	96.7	S	S	S	S	S	S	87.5	S
220122	96.3	S	S	S	S	S	S	50	S
220124	100	S	S	S	S	S	S	62.5	S
220126	55.6	S	S	S	S	S	R	37.5	R
220128	100	S	S	S	S	S	S	37.5	R
220129	23.7	S	S	R	R	R	R	37.5	R
220130	81.5	S	S	S	S	S	S	37.5	R
220132	93.3	S	S	S	S	S	S	87.5	S
220133	56.7	S	S	S	S	S	R	12.5	R
220134	41.7	S	S	S	S	R	R	62.5	S
220135	95.8	S	S	S	S	S	S	87.5	S
220136	71.1	S	S	S	S	S	S	75	S
220142	66.7	S	S	S	S	S	S	100	S
220144	46.7	S	S	S	S	R	R	100	S
220145	63.3	S	S	S	S	S	S	100	S
220146	93.3	S	S	S	S	S	S	75	S
220147	58.3	S	S	S	S	S	R	100	S
220148	71.5	S	S	S	S	S	S	100	S
220149	77.8	S	S	S	S	S	S	100	S
220150	50.0	S	S	S	S	R	R	75	S
220151	100	S	S	S	S	S	S	62.5	S
220152	83.3	S	S	S	S	S	S	75	S
220153	100	S	S	S	S	S	S	100	S
220154	100	S	S	S	S	S	S	100	S
220155	40.7	S	S	S	S	R	R	62.5	S
220156	25.9	S	S	R	R	R	R	62.5	S
220157	73.3	S	S	S	S	S	S	75	S
220158	66.7	S	S	S	S	S	S	87.5	S
220159	66.7	S	S	S	S	S	S	87.5	S
220160	100	S	S	S	S	S	S	100	S
220161	93.3	S	S	S	S	S	S	87.5	S
220162	100	S	S	S	S	S	S	100	S
220163	58.6	S	S	S	S	S	R	100	S
220164	73.3	S	S	S	S	S	S	100	S
220165	83.3	S	S	S	S	S	S	87.5	S
220331	83.3	S	S	S	S	S	S	100	S
220332	70	S	S	S	S	S	S	87.5	S
220333	100	S	S	S	S	S	S	12.5	R
220334	95.8	S	S	S	S	S	S	50	S
220335	81	S	S	S	S	S	S	62.5	S
220336	90	S	S	S	S	S	S	25	R
220337	88.9	S	S	S	S	S	S	37.5	R

220338	100	S	S	S	S	S	S	100	S
220339	89.7	S	S	S	S	S	S	87.5	S
220340	100	S	S	S	S	S	S	100	S
220341	100	S	S	S	S	S	S	87.5	S
220342	100	S	S	S	S	S	S	100	S
220343	100	S	S	S	S	S	S	100	S
220344	100	S	S	S	S	S	S	100	S
220345	96.3	S	S	S	S	S	S	100	S
220346	100	S	S	S	S	S	S	100	S
220347	91.7	S	S	S	S	S	S	87.5	S
220348	95.8	S	S	S	S	S	S	87.5	S
220349	100	S	S	S	S	S	S	100	S
220350	96.7	S	S	S	S	S	S	50	S
220352	91.7	S	S	S	S	S	S	100	S
220353	100	S	S	S	S	S	S	100	S
220354	96.7	S	S	S	S	S	S	100	S
220357	76.7	S	S	S	S	S	S	100	S
220358	100	S	S	S	S	S	S	87.5	S
220359	96.7	S	S	S	S	S	S	100	S
220360	100	S	S	S	S	S	S	100	S
220361	80	S	S	S	S	S	S	62.5	S
220362	100	S	S	S	S	S	S	87.5	S
22BW500	96.7	S	S	S	S	S	S	87.5	S
22BW501	70.8	S	S	S	S	S	S	87.5	S
HW7996	90	S	S	S	S	S	S	0	R
L390	100	S	S	S	S	S	S	100	S

Table 2. Disease reaction of breeding lines for Pss1632

Breeding lines	10 DAI Avg W%	Wilting percent						4 WAI Avg W%	Greenhouse scoring
		≤10%	≤20%	≤30%	≤40%	≤50%	≤60%		
220411	82.3	S	S	S	S	S	S	100	S
220412	100	S	S	S	S	S	S	100	S
220413	96.7	S	S	S	S	S	S	100	S
220414	100	S	S	S	S	S	S	100	S
220415	90	S	S	S	S	S	S	100	S
220416	86.7	S	S	S	S	S	S	100	S
220417	88	S	S	S	S	S	S	100	S
220418	90.5	S	S	S	S	S	S	75	S
220419	61.1	S	S	S	S	S	S	75	S
220420	100	S	S	S	S	S	S	75	S
220421	93.3	S	S	S	S	S	S	75	S
220422	95.2	S	S	S	S	S	S	75	S

220424	90	S	S	S	S	S	S	100	S
220425	100	S	S	S	S	S	S	100	S
220426	96.7	S	S	S	S	S	S	75	S
220427	90.5	S	S	S	S	S	S	100	S
220428	53.3	S	S	S	S	S	R	75	S
220429	95.8	S	S	S	S	S	S	62.5	S
220431	33.3	S	S	S	R	R	R	87.5	S
220432	100	S	S	S	S	S	S	100	S
220433	100	S	S	S	S	S	S	100	S
220434	53.3	S	S	S	S	S	R	100	S
220435	66.7	S	S	S	S	S	S	100	S
220436	66.7	S	S	S	S	S	S	100	S
220437	83.3	S	S	S	S	S	S	100	S
220438	100	S	S	S	S	S	S	100	S
220440	100	S	S	S	S	S	S	100	S
220442	100	S	S	S	S	S	S	100	S
220443	100	S	S	S	S	S	S	87.5	S
220444	100	S	S	S	S	S	S	100	S
220445	96.3	S	S	S	S	S	S	100	S
220446	100	S	S	S	S	S	S	87.5	S
220447	91.7	S	S	S	S	S	S	75	S
220448	100	S	S	S	S	S	S	100	S
220449	100	S	S	S	S	S	S	100	S
220450	86.7	S	S	S	S	S	S	75	S
220451	100	S	S	S	S	S	S	75	S
220452	95	S	S	S	S	S	S	100	S
220453	100	S	S	S	S	S	S	100	S
220454	60	S	S	S	S	S	S	100	S
220455	100	S	S	S	S	S	S	100	S
220456	53.3	S	S	S	S	S	R	87.5	S
220457	85	S	S	S	S	S	S	100	S
220458	86.7	S	S	S	S	S	S	75	S
220459	100	S	S	S	S	S	S	62.5	S
220460	77.8	S	S	S	S	S	S	100	S
220461	100	S	S	S	S	S	S	87.5	S
220462	100	S	S	S	S	S	S	100	S
220463	95.8	S	S	S	S	S	S	100	S
220464	100	S	S	S	S	S	S	87.5	S
220465	100	S	S	S	S	S	S	87.5	S
220466	96.7	S	S	S	S	S	S	100	S
220467	81.5	S	S	S	S	S	S	25	R
220468	94.4	S	S	S	S	S	S	87.5	S
220469	94.4	S	S	S	S	S	S	100	S

220470	96.7	S	S	S	S	S	S	100	S
220471	85	S	S	S	S	S	S	87.5	S
220472	88.9	S	S	S	S	S	S	87.5	S
220473	100	S	S	S	S	S	S	100	S
220474	100	S	S	S	S	S	S	100	S
220475	93.3	S	S	S	S	S	S	87.5	S
220476	100	S	S	S	S	S	S	100	S
220477	100	S	S	S	S	S	S	87.5	S
220478	63	S	S	S	S	S	S	75	S
220479	80	S	S	S	S	S	S	100	S
220480	86.7	S	S	S	S	S	S	100	S
220481	93.3	S	S	S	S	S	S	75	S
220482	76.7	S	S	S	S	S	S	87.5	S
220483	88.9	S	S	S	S	S	S	50	S
220485	80	S	S	S	S	S	S	75	S
220486	89.6	S	S	S	S	S	S	37.5	R
220487	92.6	S	S	S	S	S	S	50	S
220488	100	S	S	S	S	S	S	87.5	S
22BW50	76.7	S	S	S	S	S	S	75	S
22BW50	57.9	S	S	S	S	S	R	50	S
HW7996	81.65	S	S	S	S	S	S	12.5	R
L390	88.15	S	S	S	S	S	S	100	S

Cotyledon-seedling screening:

Seed treatment and sowing

Seeds used was initially treated with 35% hydrochloric acid and 10% tri-sodium phosphate. However, they were treated for again with surface sterilization before sowing. Seeds were treated with 1% sodium hypochlorite (NaClO) (bleach) for 5 min and three washes of sterilized water for 5 minutes. The sterilized seeds were placed on sterilized wet cotton and tissue paper beds in a tissue culture container, then incubated in a growth chamber at 25°C in the dark for 3 days. After germination, the seedlings are exposed to a set 12h photoperiod (light intensity 4891 Lux; 90 µ-mole/s.m2; the distance between lamp and seedling is 30 cm). 7-day-old cotyledon-seedlings will be used for inoculation.

Pathogen preparation

Pss4 (Phylotype I, Biovar 3, Race 1) was isolated from tomato collected from Shanhua, Tainan District, Taiwan in 1988 for resistance screening. This strain was used to inoculate 80 breeding lines (Table 1).

Pss1632 (Phylotype II, Biovar 2, Race 3) was isolated from potato collected from Dounan, Yunlin District, Taiwan in 2009 for resistance screening. This strain was used to inoculate 77 breeding lines (Table 2).

The selected strain from storage culture was streaked on Triphenyltetrazolium chloride (TTC) plate and incubated at 30 °C for 2 to 3 days. Before the day of inoculation, the culture was multiplied on 523 medium and stored at 30 °C overnight. Bacterial mass from overnight cultures is transferred and suspended in water. Concentration of inoculum was adjusted until the O.D. value reach 0.3 at the wavelength of 600 nm (about 10^8 CFU/ml). For cotyledon-seedling, the inoculum was further distilled to a concentration of 10^6 CFU/ml as it was founded to be the optimum concentration in previous trials.

Inoculation

The cotyledon-seedling was inoculated by root-dipping for 1 minute in a 10^6 CFU/ml bacterial suspension. The inoculated seedling was transferred to an empty petri dish to enable 5 minutes of air exposure for better infection results. Seedlings were transferred to 2 ml of microfuge tube with 2.0 ml of sterilized water and kept in a growth chamber at 28°C with 12h photoperiod (light intensity 4891 Lux; 90 µ-mole/s.m2; Distance between lamp and seedling is 30 cm).

Evaluation

The evaluation was carried out at 5, 7, and 10 days after inoculation (DAI) and the most appropriate day for evaluation will be determined from the results that correlate best with the results from greenhouse screening. During the evaluation, we gave either one of two assessments for the plant which were negative (uninfected) and positive (infected). Infected cotyledon-seedlings are with symptoms of wilting stem and cotyledon, constriction at the stem and obvious blackening at the growth point (Fig.2).

Fig.2 Comparison between negative (uninfected) and positive (infected) cotyledon-seedlings. The right seedling shows signs of damping off, constriction at the stem and obvious blackening at the collar region. Its stem has also lost its pigment due to colonization of bacteria.

Wilting percentage (W%) was derived from dividing wilted cotyledon-seedlings with the total number cotyledon-seedlings of the same breeding line.

$$Wilting\ \% = \frac{Wilted\ plants}{Total\ plant\ number} x10$$

Greenhouse screening:

Environment of greenhouse

Seedlings was sown in 2-inch-diameter plastic pot in the disease screening house. Data of temperature and humidity were collected by a multiple function data logger at 1 hour intervals.

Seed treatment and sowing

Seeds for greenhouse screening (treated with 35% hydrochloric acid and 10% tri-sodium phosphate) were directly sown in 2-inch-diameter plastic pots with potting mixture (3:1:1:1 ratio of soil, rice hulls, sand, compost) in the greenhouse. The seedlings are kept in a well-ventilated greenhouse and plants are irrigated daily, except a day before inoculation.

Pathogen preparation

For inoculation of greenhouse plants, pathogen preparation was similar differing only in the concentration of inoculum, which was 10^8 CFU/ml.

Inoculation

Seedlings with 4 to 6 true leaves (about 3 to 4 weeks depends on season) were used for inoculation. Soil drenching was used where 20 ml bacterial suspension (O.D.600 = 0.3, about 10^8 CFU/ml) was poured.

Evaluation

Evaluation was carried out every week up to 4 weeks and the resistance reaction of breeding lines will be based on the W% at the fourth week after inoculation (4 WAI). During evaluation, we gave either one of two assessments for the plant which is uninfected and infected. Infected plants can show one or more of these symptoms: obvious wilting of leaves starting from the root upwards, black spots and discolouration of stem and a collapsed stem (Fig.3 and 4). Evaluation was carried out

in the morning to reduce the possibility of signs of wilting due to high temperature and susceptible plants that showed symptoms were severed at the stem during inspection.

Fig.3 (up) and 4 (down) Comparison between negative (uninfected) and positive (infected) plant. The plant in Fig.4 had obvious wilting of leaves starting from the root upwards and discolouration on its stem but had yet to completely collapse.

The W% was calculated as follows:

$$Wilting\ \% = \frac{Wilted\ plants}{Total\ plant\ number} x100$$

Data analysis:

Data analysis was done by R studio with correlation coefficient for W% at 4 WAI (Greenhouse scoring) of tomato plant and lab scoring of cotyledon-seedlings.

Results

Germination rate

Germination rate of cotyledon-seedlings differed for different breeding lines. Table 3 indicated germination rate of cotyledon-seedlings out of 50 seeds. Germination rate of susceptible and resistant checks were not included.

Table 3. Germination rate of cotyledon-seedling

Breeding lines for Pss4	Germination rate	Breeding lines for Pss1632	Germination rate
220111	30	220411	45
220112	15	220412	47
220113	33	220413	36
220114	34	220414	40
220115	32	220415	42
220116	31	220416	43
220117	43	220417	28
220118	21	220418	24
220119	39	220419	16
220120	40	220420	34
220121	34	220421	48
220122	31	220422	30
220124	35	220424	38
220126	26	220425	32
220128	31	220426	45
220129	42	220427	23
220130	40	220428	39
220132	43	220429	43
220133	48	220431	22
220134	26	220432	26
220135	27	220433	14
220136	29	220434	44
220142	16	220435	30
220144	44	220436	11
220145	32	220437	13
220146	38	220438	18
220147	16	220440	33
220148	34	220442	8
220149	29	220443	35
220150	18	220444	36
220151	18	220445	40
220152	26	220446	27

220153	13	220447	19
220154	22	220448	44
220155	33	220449	39
220156	30	220450	37
220157	35	220451	37
220158	50	220452	26
220159	48	220453	40
220160	46	220454	38
220161	43	220455	15
220162	32	220456	20
220163	24	220457	23
220164	40	220458	22
220165	34	220459	42
220331	16	220460	32
220332	22	220461	44
220333	45	220462	36
220334	35	220463	26
220335	23	220464	26
220336	46	220465	27
220337	37	220466	39
220338	48	220467	35
220339	30	220468	34
220340	24	220469	23
220341	18	220470	41
220342	27	220471	25
220343	42	220472	15
220344	38	220473	41
220345	30	220474	37
220346	29	220475	35
220347	15	220476	45
220348	36	220477	43
220349	30	220478	35
220350	35	220479	45
220352	14	220480	35
220353	33	220481	38
220354	35	220482	37
220357	17	220483	22
220358	47	220485	36
220359	48	220486	47
220360	47	220487	unknown
220361	40	220488	21
220362	18	22BW500	40
22BW500	45	22BW501	21
22BW501	32		

Environment of greenhouse

The average daily maximum temperature from 6th of July 2022 to 10th Aug 2022 was 34.68 °C and the average daily minimum temperature was 26.68°C (Fig. 1). The average relative humidity was RH79.16%.

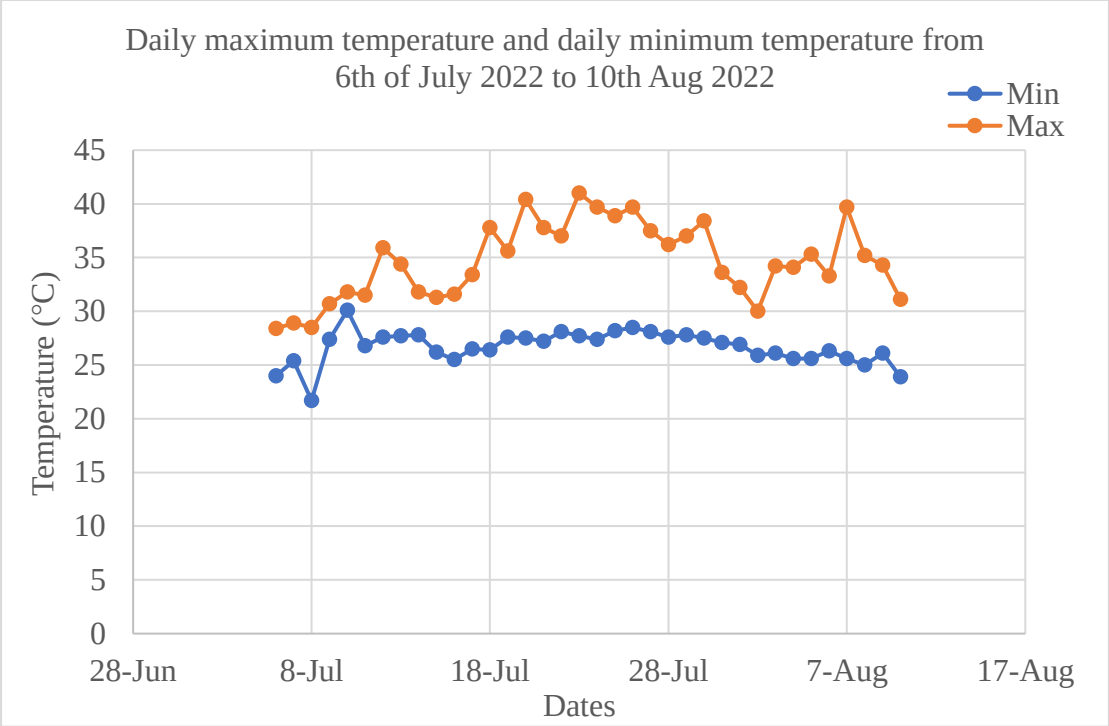

Fig. 1 Daily maximum temperature and daily minimum temperature from 6th of July 2022 to 10th Aug 2022

Correlation coefficient of greenhouse scoring and lab scoring

For Pss4, the value of Pearson's correlation coefficient between greenhouse method and cotyledon-seedling method was 0.08, 0.1 and 0.24 respectively at 5 days DAI, 7 DAI and 10 DAI (Table 3).

For Pss1632, the value of Pearson's correlation coefficient between greenhouse scoring and cotyledon-seedling method was 0.1, 0.14 and 0.19 respectively at 5 DAI, 6 DAI and 10 DAI (Table 4).

Table 4. Correlation between wilting percentage of greenhouse at 4 WAI and cotyledon- seedling screening at 5 DAI, 7 DAI and 10 DAI for bacteria strain Pss4 and Pss1632.

	Day of evaluation		
Bacterial strain	5 DAI	7 DAI	10 DAI
Pss4	0.08	0.10	0.24

Pss1632	0.11	0.14	0.19

The correlation at 10 DAI was the highest for both bacterial strains at 0.24 for Pss4 and 0.19 for Pss1632. Hence, we used data of 10 DAI to further determine the appropriate W% to be used as criteria to differentiate susceptible and resistant breeding lines for seedlings.

Consistency

For Pss4, the consistency of W% were 82.5, 82.5, 82.5, 82.5 78.8 and 80 respectively for W% of cotyledon-seedling ≤10%, ≤20%, ≤30%, ≤40%, ≤50% and ≤60% (Table 5).

Table 5. Consistency of wilting percentage (W%) for bacterial strain Pss4

Number of breeding line	W% of cotyledon-seedling						GH Scoring
	≤10%	≤20%	≤30%	≤40%	≤50%	≤60%	
Susceptible	87	87	85	85	80	73	72
Resistant	0	0	2	2	7	14	15
Consistency	82.5	82.5	82.5	82.5	78.75	80	

For Pss1632, the consistency of W% were 94.9, 94.9, 94.9 93.7, 93.7 and 88.6 respectively for W% of cotyledon-seedling ≤10%, ≤20%, ≤30%, ≤40%, ≤50% and ≤60% (Table 6).

Table 6. Consistency of wilting percentage (W%) for bacterial strain Pss1632

Number of breeding line	W% of cotyledon-seedling						GH Scoring
	≤10%	≤20%	≤30%	≤40%	≤50%	≤60%	
Susceptible	79	79	79	78	78	74	75
Resistant	0	0	0	1	1	5	4
Consistency	94.9	94.9	94.9	93.7	93.7	88.6	

Discussion

From the environmental data, it is an optimum environment for bacterial wilt disease under the greenhouse condition, where high temperatures and humidity is favourable [17].

When comparing both methods, the results shown two major advantages of CSM which were time saving and the option to apply the screening regardless of the growth season and throughout the whole year. CSM took 32 days to complete all trials but greenhouse screening took 60 days. Furthermore, CSM is not confined to the growth season of the plants, guaranteeing it a higher throughput. For the operation

techniques, CSM required more technical training but fewer human resources. When considering space capacity, under same amount of test lines, CSM required only a growth chamber, compared to greenhouse method which an entire greenhouse was needed. Branching off on the topic of space, another reason why CSM can have a higher throughput is also because the small space requirement allows it to carry out more test lines at once.

Cotyledon-seedling screening provides a controlled environment for plant to interact directly with disease without interference from other factors. Hence, its selection is more rigid and specific. Greenhouse screening allows plant to interact with disease and environment in a less controlled environment.

Knowing these differences, we can infer where the cotyledon-seedling screening method is applicable and where they are not. For example, seedling screening may be used for pathology purposes as selected cultivars will have high specificity of resistance. Its high throughput and shorter time guarantee it to be a good preliminary screening method too when there are many germplasm lines to be screened. This completed our objective where we needed to establish a rapid screening method to shorten the time of screening.

Greenhouse screening can be used for breeding purposes. The selected cultivars can be either high or moderately resistant based on different breeding purpose.

Another interesting point from this experiment was that the resistant cultivar Hawaii 7996 did not show resistance in the seedling stage. For the bacterial strain Pss4, the wilting percentage was high in CSM (90%) but low in greenhouse screening (0%). For the bacterial strain Pss1632, the result is similar (81.65% for CSM and 12.5% for greenhouse screening). In the future, we might need to find a resistant check to as a CSM resistant check.

Acknowledgments

This research was supported by World Vegetable Centre. A special thanks to the staff of the Bacteriology and Tomato Breeding unit for providing their assistance.

Reference

[1] Food and Agriculture Organization of the United Nations. (2020). FAOSTAT statistical database. https://www.fao.org/faostat/en

[2] 康慧鳳、王添成、Hartman,G. L. (1991).〈Survey of Bacterial Wilt on Fresh Market Hybrid Tomatoes in Taiwan〉，《植物保護學會會刊》，33:2 卷，頁 197-203。

[3] Buddenhagen, I., Sequeira, L., and Kelman, A. (1962). Designation of races in Pseudomonas solanacearum. Phytopathology 52:726.

[4] Hayward, A. C. (1964). Characteristics of Pseudomonas solanacearum. J. Appl. Bacteriol. 27, 265–277. doi: 10.1111/j.1365-2672.1964.tb04912.x

[5] Hayward, A. C. (1964). Characteristics of Pseudomonas solanacearum. J. Appl. Bacteriol. 27:265-277.

[6] A. Lebeau, M.-C. Daunay, A. Frary, A. Palloix, J.-F. Wang, J. Dintinger, F. Chiroleu, E. Wicker, P. Prior. (2010) Bacterial Wilt Resistance in Tomato, Pepper, and Eggplant: Genetic Resources Respond to Diverse Strains in the Ralstonia solanacearum Species Complex. Bacteriology Vol. 101, No. 1,

[7] Lopes, C. A., Quezado-Soares, A. M., and de Melo, P. E. (1994). Differential resistance of tomato cultigens to biovars I and III of Pseudomonas solanacearum. Plant Dis. 78:1091- 1094.

[8] Hanson, P. M., Wang, J.-F., Licardo, O., Hanudin, Mah, S. Y., Hartman, G. L., Lin, Y.-C., and Chen, J.-T. (1996). Variable reaction of tomato lines to bacterial wilt evaluated at several locations in Southeast Asia. HortScience 31:143-146.

[9] Lin, C. H., Hsu, S. T., Tzeng, K. C., & Wang, J. F. (2008). Application of a Preliminary Screen to Select Locally Adapted Resistant Rootstock and Soil Amendment for Integrated Management of Tomato Bacterial Wilt in Taiwan. Plant disease, 92(6), 909–916.

[10] Hsu, S.-T. (1991). Ecology and control of Pseudomonas solanacearum in Taiwan. Plant Prot. Bull. (Taiwan) 33:72-79.

[11] Hartman, G. L., and Elphinstone, J. G. (1994). Advances in the control of Pseudomonas solanacearum race 1 in major food crops. Pages 157-177 in: Bacterial Wilt: The Disease and its Causative Agent, Pseudomonas solanacearum. A. C. Hayward and G. L. Hartman, eds. CAB International, Wallingford, UK.

[12] Denny, T.P. (2006) Plant pathogenic Ralstonia species. In Plant-Associated Bacteria ed. Gnanamanickam, S.S. pp. 573–644

[13] Yuliar, Yanetri Asi Nion, Koki Toyota. (2015). Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. Microbes Environ. 30(1): 1–11.

[14] Tarek R. E., Samuel Jacquiod, Eman H. N., Søren J. Sørensen, Kornelia Smalla. (2020). Biocontrol of bacterial wilt disease through complex interaction between tomato plant antagonists, the indigenous rhizosphere microbiota and Ralstonia solanacearum. Front. Microbiol., Sec. Plant Pathogen Interactions.

[15] Techawongstien, S., Thummabenjapone, P., Bolkan, H. (2009). Screening tomato varieties for their bacterial wilt resistance over seasons in northeast Thailand. Acta Horticulturae, (808), 263–268.

[16] Singh N., Kumar R., Ray SK. (2018). An Innovative Approach to Study Ralstonia solanacearum Pathogenicity in 6 to 7 Days Old Tomato Seedlings by Root Dip Inoculation. Bio Protoc.

[17] Toyota, Koki. (2004). Effect of moisture conditions and preincubation at low temperature on bacterial wilt of tomato caused by Ralstonia solanacearum. microbes and environments - Microbes environments. 19. 244-247. 10.1264/jsme2.19.244.