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Differential susceptibility of citrus cultivars toward blast and black pit in Tunisia caused by *Pseudomonas syringae* pv. *Syringae*

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Abstract
Pseudomonas syringae pv. *syringae* responsible of citrus blast and black pit, occur in several citrus-growing region around the world. Evaluated strains of *P. syringae* pv. *syringae* were virulent to citrus plants. Moreover, interactions between cultivars and strains were proved. The susceptibility of thirty-three citrus cultivars in detached leaves and eight cultivars in detached fruits to four virulent strains of *Pseudomonas syringae* pv. *syringae* were evaluated. In addition, eight citrus cultivars were evaluated by steam inoculation to six virulent strains of *Pseudomonas syringae* pv. *syringae*.
The detached young leaf test appeared to be more convenient and gave a more accurate prediction. This test has the additional advantages that symptom development requires less time than in fruits test. Moreover, leaves are available for longer period of time than fruits. This method provided a rapid and reproducible screening system of cultivar susceptibility to bacterial blast of citrus.
Our results of detached leaf test revealed that citrus cultivars 'Thompson Navel' and 'New Hall' were the most susceptible and, 'Eureka' cultivarseemed to be less susceptible to citrus blast disease. In fact, the severity value obtained on 'Thompson Navel' leaves was 87.5%. Steam inoculation showed also that 'Thompson Navel' cultivar was the most susceptible to the blast of citrus and 'Eureka' cultivar was found the less susceptible to the disease.
However 'Eureka' and 'Swett Lime' cultivars seemed to the most susceptible to black pit disease. The highest severity values (93.75%) were obtained on 'Eureka' fruits.
Eureka cultivar displayed the lowest susceptibility to blast of citrus but revealed to be very susceptible to black pit of citrus. A lack of correlation between cultivars susceptibility against black and blast of citrus is proved.

Keywords: *Pseudomonas syringae* pv. *syringae*, citrus cultivars, susceptibility, blast and black pit

1. Introduction
Pseudomonas syringae is a polyphagous phytopathogenic bacterium associated with more than 180 species of both annual and perennial crops, including vegetables, fruits and ornamental plants [2] (. This bacterium was found as an epiphyte on the phyllosphere in many geographic areas [2]. Characteristic disease symptoms of blast appear on leaves and twigs. Blast lesions usually develop firstly on the leaf petiole, or wing of susceptible hosts as small water–soaked or dark spots. These symptoms expand rapidly in both directions, upward toward the leaf mid-vein and downward into the axil and twig. The petiole and twig tissues become severely damaged by girdling, then collapse. The leaves wither, curls, dry become brown, and eventually drop. Necrosis in the twig is generally limited and usually progress slowly. The lesions of infected twigs tissues, beginning at the margins become reddish brown to chestnut colored and may resemble scab or calluses [3].
The disease is associated with cool, damps weather and physical injuries to host caused by wind or hail [4].
In Tunisia, *Pseudomonas syringae* pv. *syringae* causing citrus blast of twigs and leaves and black pit of fruit was reported by Boubaker [5] on sour orange (*citrus aurantium*). In fact, the use of cultivars with low susceptibility may be one of the most appropriate methods of disease control.However, all over the world there is a little information about susceptibility of this disease toward *Pseudomonas syringae* pv. *syringae*. The most susceptible cultivars to blast of citrus are orange (C. *sinesis*) and Grapefruit (C. *paradisi*) while lemon (*Citrus limon*) was the most susceptible to black pit of citrus [3].
The aims of this study were to (i) assess, under controlled environment conditions, the susceptibility of citrus cultivars to blast and black pit in Tunisia (ii) study strains virulence of *Pseudomonas syringae* pv. *syringae* using, *ex vivo* method based on pathogen inoculation on fruits and detached young leaves and also *in vivo* method based on pathogen inoculation on stems of citrus cultivars.

2. Materials and methods
Plant material
Three types of plant material were used in this experiment:
1. Two years-old citrus plants from citrus nursery of Tunisia were used. Plants were grown in plastic bag (dimension 1L). After inoculation, plants were kept in the greenhouse in individual plastic bag filled with a substrate composed of peat and sand and watered each three days. Cultivars used were 'Thompson Navel', 'Meski Ansli', 'Cassar', 'MA3', 'Malti Petit Pierre', 'Star Ruby, 'Sweet Lime' and 'Eureka'.
2. Young leaves were collected from a Tunisian citrus nursery. Thirteen citrus cultivars were evaluated which are 'Thompson Navel', 'New Hall', 'Double Fine',

'Bearss Lime', 'Ortanique', 'Hernandina', 'Valencia Late', 'Malti Petit Pierre', 'MA3', ' Star Ruby', 'Eureka', 'Sweet Lime', 'Lemon Baldi'. They were collected at the same phonological stage. Young leaves were picked up the same day that the inoculation was performed and were stored at 4°C under high humidity conditions until inoculation.

3. Fruits used in this study were maintained at 4°C until inoculation. The Cultivars used for this experiment were 'Thompson Navel', 'Meski Ansli', 'Cassar', 'MA3', 'Malti Petit Pierre', 'Star Ruby', 'Sweet Lime' and 'Eureka'.

Bacterial strains identification and inoculums preparation

Identification of *Pseudomonas syringae*

Plates with individual colonies were examined under binocular microscope. Fluorescents bacterial colonies similar in appearance to *Pseudomonas syringae* were selected and purified on NA amended with 5% of sucrose (SNA). For further analysis, strains were stored in 15% glycerol solution at −20°C.

All strains were identified according to the biochemical and physiological test, according to the procedures described by Lelliott *et al* [6], and by Braun-Kiewnick and Sands [7]: fluorescence on King's B medium (KB), levan production, oxidase activity, pectolytic activity, arginine dihydrolase activity and tobacco hypersensitivity (LOPAT tests). Gram reaction of the strains was determined using 3% KOH [8].

Five Tunisian strains of *Pseudomonas syringae* pv. *syringae* BAT 13, FT4, BC5, BT6, and BCL1 isolated from citrus necrosis and a reference strain DAPP-PG 115obtained from prof. Roberto Buonaurio (Bacterial Collection of the Plant Protection Unit, Department of Agricultural, Nutritional and Environmental Sciences, University of Perugia, Italy) were used. For the inoculums preparation, bacteria were grown on NA for 24 h at 25°C, suspended in sterile distilled water and spectrophotometrically adjusted to 10^8 colony forming units (CFU) per ml.

Leaves, fruits and plants inoculation

To evaluate cultivar susceptibility, leaves, fruits and plants of citrus cultivars were inoculated by *Pseudomonas syringae* pv. *syringae* strains.

Firstable, leaves were dipped in a solutionof sodium hypochlorite (1% active hypochlorite) for 5 min, rinsed three times in sterile distilled water and excess water removed with a filter paper. Detached leaves were inoculated with a 2 µl drop of the bacterial suspension deposited on a fresh wound made with a scalpel [9]. The inoculation was performed in the petiole of the leaf. Inoculated leaves were placed on a sterile filter paper over water agar (10 g agar l−1) in sterile squared Petri dishes. Noninoculated leaves treated with sterile distilled water were used as control. The Petri dishes were sealed with a piece of parafilm and incubated at 24 ◦C, 16 h light and 18 ◦C,8 h darkness, for 5 days in a controlled plant tissue culture chamber(JSCC-250CP). Experiment consisted of three replicates of two leaves for each cultivar (one wound per leaf).

Fruits inoculation was carried out on mature fruits, purchased in a local market, according to the procedure described by Young [10] (Fruit was inoculated with 10µL of bacterial suspension 10^8 CFU/ml. Fruits treated with sterile distilled water were used as control. All fruits were covered with plastic bags and placed in a humid chamber. The diameter of the lesions was recovered 7 days after the inoculation. The experiment consisted of three replicates of one fruit per cultivar (four inoculations per fruit).

Citrus plants were wounded at six sites on the stem. Each wound site was inoculated with10 µL of bacterial suspension 10^8 CFU/ml. Three replicates of two plants for each cultivar were used for each bacterium (six wounds per plant and 36 wounds sites per strain). Noninoculated plants were used as control plants. The length of inoculated wound sites developing necrosis was recorded 10 weeks after inoculation.

Experimental design

In vivo, strains virulence and cultivars susceptibility experiment was a complete randomized design. Seven treatments (BAT 13, FT4, BC5, BT6, BCL1, DAPP-PG 115 and sterile distilled water) were used.

Ex vivo, strain virulence and cultivar susceptibility experiment for leaves and fruits was a complete randomized design. Five treatments (BAT 13, BC5, BT6, DAPP-PG 115 and sterile distilled water) were used.

Assessment of infection severity and statistical analysis

Five severity index levels (*I*) were established in order to quantify the intensity of infections on fruits and leaves. The severity index ranged from 0 to 4 depending on the absence or presence of infection and its intensity according to the following scale: 0, no infection; 1, necrosis limited to the inoculation point; 2, necrosis affecting the inoculation point and the leaf midvein or presence of a necrotic area of less than 5-mm diameter in fruits; 3, necrosis expanding through the midvein and additional veins in leaves or necrotic area of 5–10-mm

diameter on fruits; and 4, necrosis of more than 50% leaf surface or necrotic area higher than 10-mm diameter on fruits). Severity (*S*) was calculated for each treatment according to the following formula:

$$S = \frac{\sum_{n=1}^{N} In}{N \times Imax} \times 100$$

Where *In* is the corresponding severity index, *N* is the number of inoculated leaves or fruits per replicate and *Imax* is the maximum severity index (corresponding to 4).

Statistical analysis was performed by IBM SPSS Statistics 20. A one way analysis of variance (ANOVA) was performed and means were compared using Duncan's multiple rang test by the least significant difference test (p<0.05).

3. Results

Bacterial identification

Bacterial strains were characterized on the basis of LOPAT and GATTa tests. The five strains used were Gram negative by KOH test, oxidase negative, levane positif on NSA medium and able to induce HR on tobacco leaves. The five Tunisian strains were arginine dihydrolase negative and able to macerate potato slices. Strains were positive for gelatin liquefaction and aesculin hydrolysis while being negative for tyrosinase activity and tartrate utilization (G+A+T-Ta-) [11]. According to biochemical tests (LOPAT and GATTa), the five Tunisian strains were identified as *Pseudomonas syringae* pv. *syringae.*

Strain virulence

Differences in virulence among *P. syringae pv. syringae* strains were investigated in order to study the cultivar susceptibility.

Following to stem inoculation of plants cultivar Thompson Navel, four groups of virulence were registrated: group 1 represented by BCL1which appeared to be the less virulent causing a length of stem necrosis of 0.494 cm; group 2 by DAPP-PG 115,with a length of steam necrosis of 0.627 cm. BAT 13 formed the third group causing a length of stem necrosis of 0.666cm and group 4 by BT6, FT4 and BC5seemed to be the most virulent with necrosis value of0.847, 0.847 and 0.844cm, respectively (Table1).

Table 1: Virulence level of BAT 13, FT4, BC5, BCL1, BT6 and DAPP-PG 115, seven weeks after the inoculation of Thompson Navel plants.

Cultivar	DAPP-PG 115	BAT 13	BT6	FT4	BC5	BCL1
Thompson Navel	0.627±0.0048 b	0.666±0.0083c	0.847±0.0048d	0.847± 0.0048 d	0.844±0.0048 d	0.494± 0.0096 a

Small letters are for comparison of means in the same row.
Least significant difference: means followed by the same letter do not differ significantly ($P< 0.05$).

The one-way analysis of variance showed a significant difference between means $p \leq 0.05$.

In fact, two strains, a Tunisian strain BAT 13 and an Italian strain DAPP-PG115, were less virulent than FT4, BC5 and BT6. Thus, BCL1 was weakly virulent pathogen. Strains BT6, FT4, and BC5 were highly virulent, producing necrosis more developed.

Cultivar susceptibility

A dark brown to black color lesions was developed in the tissue of leaves. Thus, the symptoms on inoculated detached leaves started to be observed 48 h after inoculation and the measurements were after 5 days of inoculation. In fruits, the symptoms were observed from 5 days after inoculation. Control inoculations with sterile water did not produce any symptoms. Moreover, the symptoms on inoculated plants started to be observed from 15 days after inoculation.

Detached leaves test

Leaf inoculations caused small lesions, moderate lesion in leaves on two of 13 citrus cultivars, and caused large to very large lesions on nine of 13 cultivars, tested (Table2).

Means lesion rating of leaves for all tested cultivars 5 days after inoculation by DAPPG 115, BAT13, BT6 or BC5 applied at 10^8 CFU/ml were 1.26±0.39; 1.92±0.55; 2.55±0.64 and 2.46±0.62, respectively. On every cultivar, BT6 and BC5 were significantly more virulent than BAT13 and DAPP-PG115; but BAT13 was significantly more virulent than DAPP- PG115 on the 13 used cultivars.

Table 2: Mean lesion ratings of leaves of 13 citrus cultivars inoculated with *Pseudomonas syringae* pv. *syringae* incubated during 5 days at controlled culture chamber.

cultivars	Inoculums	Leaf lesion rating after inoculation with 10^8 [x] CFU/ml
New Hall	Water (control)	0 a
	DAPPG-115	2.16±0.52 b
	BAT13	2.83±0.68 c
	BT6	3.35±0.76d
	BC5	3.5± 0.76 d
T. Navel	Water (control)	0 a
	DAPPG-115	2.16± 0.52 b
	BAT13	2.83± 0.68 c
	BT6	3.66± 0.78 d
	BC5	3.5± 0.76 d
Ortanique	Water (control)	0 a
	DAPPG-115	1.66±0.47 b
	BAT13	2.5±0.64 c
	BT6	3.16±0.72 d
	BC5	3±0.7 d
Star Ruby	Water (control)	0 a
	DAPPG-115	1.5±0.44 b
	BAT13	2.5±0.64c
	BT6	3±0.7d
	BC5	3±0.68d
Malti petit pierre	Water (control)	0 a
	DAPPG-115	1.66±0.44 b
	BAT13	2.5±0.64c
	BT6	3.16±0.72 d
	BC5	3.16±0.7 d
Double Fine	Water (control)	0 a
	DAPPG-115	1.66±0.47b
	BAT13	2.5±0.64 c
	BT6	3.16±0.72d
	BC5	3 ±0.7d
Lemon Baldi	Water (control)	0 a
	DAPPG-115	1± 0.37 b
	BAT13	1.66±0.52c
	BT6	2.66±0.66d
	BC5	2.5 ±0.64d
Valencia Late	Water (control)	0 a
	DAPPG-115	1.16±0.4 b
	BAT13	1.83±0.55 c
	BT6	2.66±0.66d
	BC5	2.5±0.64d
Sweet Lime	Water (control)	0 a
	DAPPG-115	1.16±0.37 b
	BAT13	2±0.57c
	BT6	2.83±0.68 d
	BC5	2.66±0.66 d
Hernandina	Water (control)	0 a
	DAPPG-115	0.66±0.28b
	BAT13	1.16±0.44bc
	BT6	1.66±0.52c
	BC5	1.5±0.5c
MA3	Water (control)	0 a
	DAPPG-115	0.66±0.28b
	BAT13	1.16±0.44bc
	BT6	1.5±0.5c
	BC5	1.5±0.5c
Eureka	Water (control)	0 a
	DAPPG-115	0.5±0.28 b
	BAT13	0.66±0.33bc
	BT6	1±0.4c
	BC5	1±0.4c
Bearss Lime	Water (control)	0 a
	DAPPG-115	0.5±0.23 b
	BAT13	0.83±0.37bc
	BT6	1.16±0.44c
	BC5	1.16±0.44c

[x] Leaves ratings: 0, no infection; 1, necrosis limited to the inoculation point; 2, necrosis affecting the inoculation point and the leaf midvein; 3, necrosis expanding through the midvein and

additional veins in leaves; and 4, necrosis of more than 50% leaf surface.
[y] unlike letter indicate significant differences among inoculum within each variety.
Least significant difference: means followed by the same letter do not differ significantly (P< 0.05).
Small letters are for comparison of means in the same row.
Each value is expressed as mean ± SD.

The ANOVA of leaves lesion rating of the13 citrus cultivars to the four *P. syringae* pv. *syringae* strains using the detached leaves assay showed a significant effect of cultivar ($P< 0.05$), strains ($P<0.05$)and interaction between cultivars x strains ($P< 0.05$).
Detached leaves from the 13 cultivars showed different levels of infection (Figure 1) and a wide range of susceptibility (severity values ranging from 12.5 % to 87.5%) among citrus cultivars inoculated with DAPPG-115, BAT13, BT6 and BC5 strains (Figure 2).

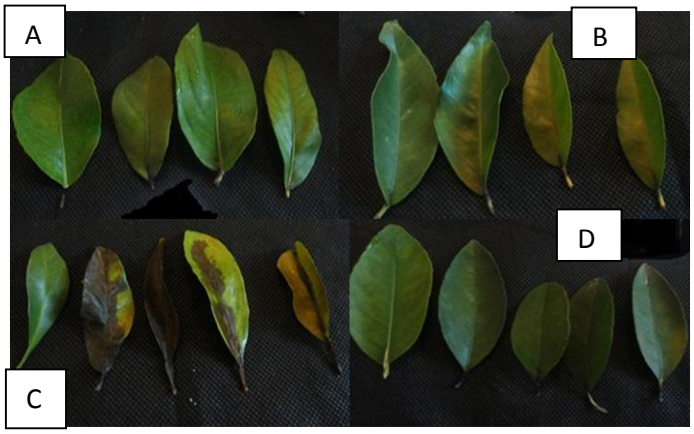

Fig 1: Lesion developed in detached leaf of cvs. Eureka (A), MA3 (B), Double Fine(C) and Bearss Lime (D) inoculated with the four tested strains.

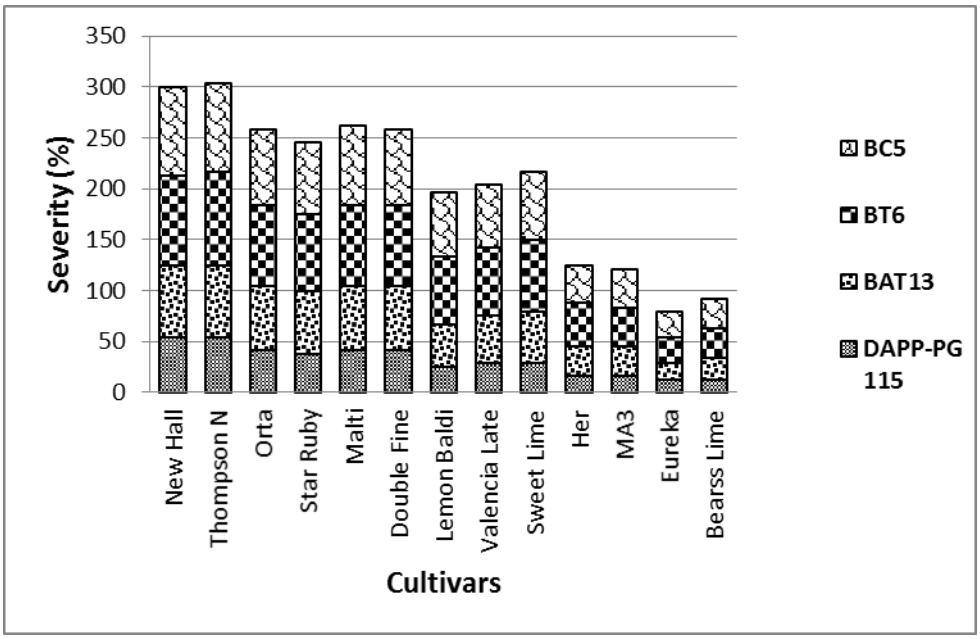

Fig 2: Severity of infections (%) caused by *P. syringae* pv. *syringae* DAPP-PG 115, BAT13, BT6 and BC5 on detached leaves from 13 citrus cultivars.

Therefore, the highest severity values were obtained on 'Thompson Navel' and 'New Hall' leaves inoculated with BT6 (91.66%) and BC5 (87.5%). Those cultivars developed necrosis that expanded through the veins and for some strains, through the leaf surface. Moreover, detached leaves from 'Oratanique', 'Star Ruby', 'Malti Petit Pierre', 'Double Fine'cultivars inoculated with all tested strains showed different levels of infection and a wide range of susceptibility according to the strains virulence and also the susceptibility of the citrus cultivars. Thus, leaves inoculated by BT6 showed severity values of 79.16%, 75%, 79.16 % and 79.16%, respectively.
Detached leaves of 'Henandina' and 'MA3'cvs. Exhibited a degree of severity with values between 16.66% and 41.66%. For the leaves of 'Eureka 'and 'Bearss Lime'cvs. Inoculated by BT6, the results showed severity values of 25% and 29.16 %, respectively. In case of,'Lemon Baldi', 'Valencia Late' and 'Sweet Lime' leaves inoculated with BT6 generated severity values varying from 66.66 % and 70.83%, respectively.

Detached fruits test

The results from the fruit inoculation assay showed also a significant effect of cultivar ($P < 0.05$), strain ($P <0.05$) and strains x cultivars interaction ($P < 0.05$). Higher necrotic spot was registered by both of BT6 and BC5 strains. The smaller necrotic spots were exhibited by BAT13 and DAPP- PG115. Mean lesions diameter for all tested cultivars after application of 10^8 CFU/ml of BAT13 and DAPP-PG115, BT6 and BC5 were 0.518±0.079, 0.542±0.085, and 0.69±0.069 and 0.684±0.064cm, respectively (figure 3).

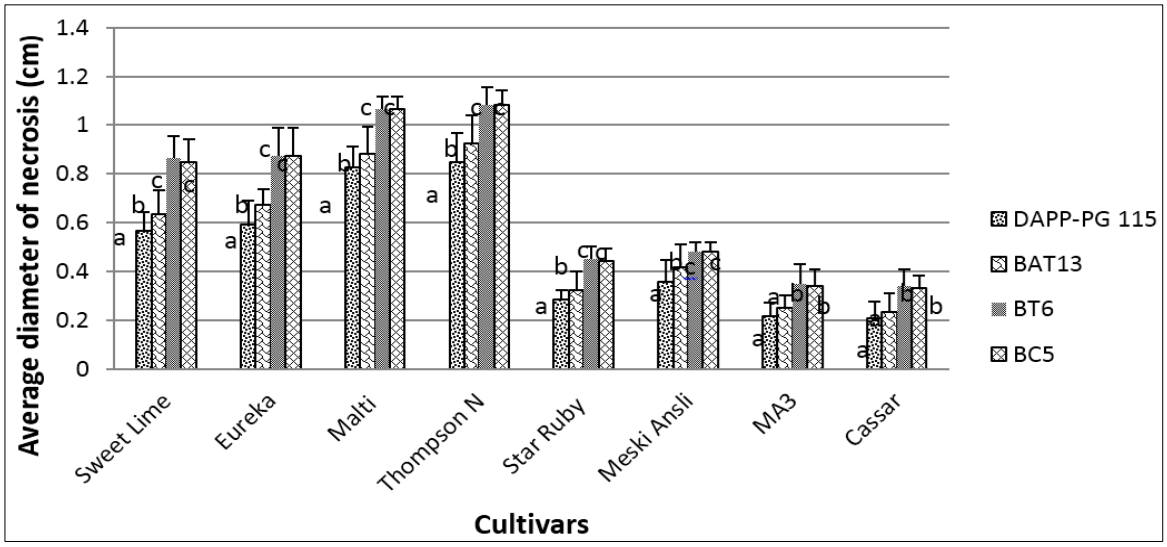

Fig 3: Average diameter (cm) of necrosis caused by *P. syringae*pv. s*yringae* DAPP-PG 115, BAT13, BT6 and BC5 on detached fruits from 8 citrus cultivars. Bars indicates the standard erreurs. *Least significant difference: means followed by the same letter do not differ significantly (P< 0.05).*

In fact, fruits from Eureka, Sweet Lime cvs. inoculated with the T6 strain developed necrotic areas higher than 10-mm diameter and the severity values were 93.75 % and 91.66%%, respectively. On fruits of 'Malti Petit Pierre', 'Thompson Navel'cvs. necrotic spots were of 5–10-mm diameter and the severity values for these cultivars were 77.083% and 79.16%, respectively. Citrus paradisi 'Star Ruby' fruits developed progressive necrotic spots smaller than 5-mm diameter in more than 68% of inoculations, and severity values were 62.5%. Fruits from 'Meski Ansli' cv. developed necrotic areas of 5–10-mm diameter in more than 62% inoculations, and severity values were 70.83 % (figures 4 and 5). Cultivars 'MA3', 'Cassar' fruits developed progressive necrotic spots smaller than 5-mm diameter in more than 66%of inoculations and severity values for these cultivars were 54.16% and 52.08 %, respectively.

Fig 4: Necrotic spots developed in cvs. Star Ruby (A) Sweet Lime (B) Eureka (C)and Meski Ansli (D) inoculated with BAT 13.

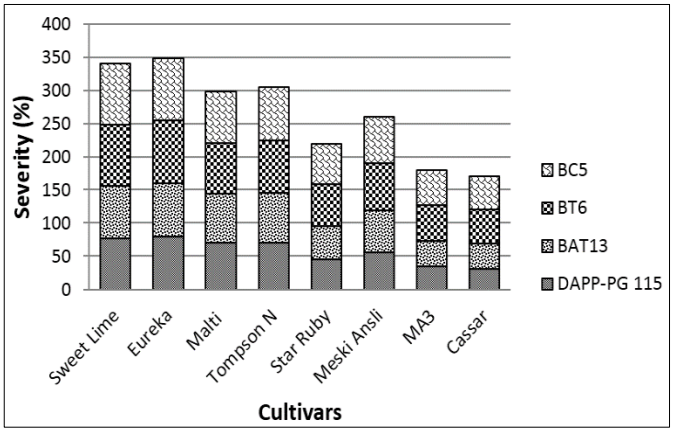

Fig 5: Severity of infections (%) caused by *P. syringae* pv. *syringae* DAPP-PG 115,
BAT13, BT6 and BC5 on detached fruits from 8 citrus cultivars.

Steam inoculation test

The steam inoculation of different cultivars revealed that BT6, FT4 and BC5 strains seemed to be similarly virulent in each cultivar according to the mean length of the necrosis (Table 2). For FT4 strain, the values, ranged between 0.63 cm (Eureka) and 1.108 cm (Malti Petit Pierre). In fact, our results showed large differences in disease response among cultivars using the six pathogen strains. The obtained results revealed that some genotype display a low susceptibility against *Pseudomonas syringae* pv. s*yrinage* infection as cultivar Eureka. However, Thompson Navel was very susceptible to blast of citrus (Figure 6).

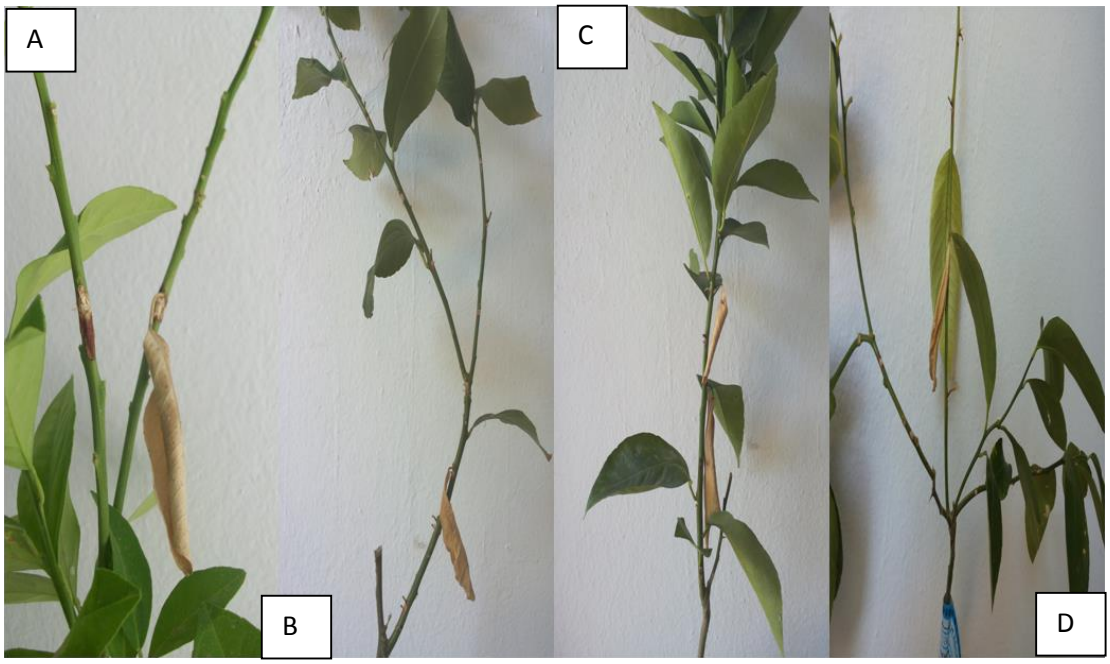

Fig 6: Steams necrosis developed for Thompson Navel (A), Eureka (B), MA3 (C) and Swett Lime (D) cvs. Ten weeks after inoculation with BAT13.

Cultivars of 'Malti Petit Pierre' and 'Star Ruby' appeared susceptible, 'Sweet Lime' and 'Meski Ansli' cvs. exhibited an intermediate infection level. 'MA3' and 'Cassar' cvs. appeared less susceptible than 'Sweet Lime' and 'Meski Ansli' cvs.

The Clementine cultivars 'MA3' and 'Cassar' inoculated with FT4 developed a necrosis with the length mean of 0.74 and 0.73cm, respectively (Table 3).

Table 3: Mean length of steams necrosis (cm) of eight citrus cultivars inoculated with *Pseudomonas syringae* pv. *syringae* (DAPP-PG 115, BT6, BCL1, FT4, BC5 and BAT13), ten weeks after inoculation.

cultivars	DAPP- PG115	BT6	BCL1	FT4	BC5	BAT13
Thompson N	0.977±0.009 bD	1.2dE	0.655±0.012aE	1.2dE	1.197±0.004dE	1.061±0.004cD
Malti	0.93±0.004 bC	1.108 ±0.004dD	0.608 ±0.008aD	1.108 8±0.008 dD	1.102 ±0.004dD	0.983cC
Star Ruby	0.927 ±0.004bC	1.102±0.004dD	0.577±0.004aD	1.102±0.012 dD	1.097±0.004dD	0.977±0.004 cC
MA3	0.627±0.004bB	0.738±0.004dB	0.472±0.06aB	0.741dB	0.736±0.004dB	0.680±0.004cB
Cassar	0.625bB	0.733dB	0.447±0.004aB	0.738±0.004dB	0.736±0.004dB	0.66cB
Eureka	0.522±0.096bA	0.625±0.008dA	0.330±0.012aA	0.633dA	0.619±0.004dA	0.552±0.004cA
Sweet Lime	0.641bB	0.855±0.01dC	0.527±0.004aC	0.861±0.004dC	0.852±0.004dC	0.658±0.008cB
Meski Ansli	0.636±0.004bB	0.852±0.009C	0.522±0.004aC	0.855±0.004dC	0.847±0.004dC	0.655±0.012cB

Least significant difference: means followed by the same letter do not differ significantly (P< 0.05).
Capital letters are for comparison of means in the same column.
Small letters are for comparison of means in the same row.
Each value is expressed as mean ± standard errors.

4. Discussion

Symptoms on detached leaves were characteristic of citrus blast. Area shading from brown to black, which usually extends rapidly in both directions to the base of the leaf blade and expanding through the leaf surface. Lesions developed in the tissue of leaves and the severity of the infection depending on the cultivars susceptibility and strains virulence.

Fruits, developed typical black pit spots in correspondence of the inoculation sites. However, differences in size of lesions depending on the cultivars susceptibility and strains virulence were noted. The severity of the infection depended also of strains virulence and the susceptibility of the citrus cultivars. In plants, symptoms were characteristic of citrus blast. Necrotic areas on twigs were developed and enlarged. Later the infected tissue becomes reddish-brown to chestnut-colored dry scab. Difference in virulence among *P. syringae* pv. *syringae* strains investigated during this study was demonstrated. In fact, Tunisian BT6, FT4 and BC5 strains behaved similarly virulent in each cultivar and were the most virulent strains. On the contrary, CL1 was weakly virulent.

A wider range of severity was obtained on detached leaves test (12.5–91.66%) and on fruit test (31.25–93.75%). Detached young leaves appear to be more convenient for inoculations of *P. syringae* pv. *syringae* than fruits because leaves are available through the growing season and also could be obtained from seedlings or micropropagated plants grown in the greenhouse. The detached leaves test has the additional advantage that symptom development is quicker (48 h) than in fruits test (5 days). The Citrus cultivars tested varied widely in susceptibility of plant, leaf and fruit to the virulent strains of *P. syringae* pv. *Syringae*. The susceptibility of citrus cultivars was not the same towards black pit and citrus blast.

Based on the research that was conducted in this study, we found a different level of susceptibility among citrus cultivars

and against *Pseudomonas syringae*pv. S*yringae* strains. In particular, a low susceptibility was observed in citrus plants for 'Eureka'cv. Which turn out to be the less susceptible toward citrus blast disease in Tunisia. On the contrary, 'Thompson Navel'cv. Seemed to be the highest susceptible and 'Malti Petit Pierre' and 'Star Ruby'cvs. Were susceptible. While 'Sweet Lime' and 'Meski Ansli' cvs. Register an intermediate infection level. Moreover, 'MA3' and 'Cassar'cvs. Were less susceptible than Meski Ansli and 'Sweet Lime' cultivars to *Pseudomonas syringae* pv. *syringae.* In the detached leaves test, 'Thompson Navel' and 'New Hall' cvs. showed the highest susceptibility against blast of citrus caused by *Pseudomonas syringae* pv. *syrinage*, but 'Ortanique,'Star Ruby','Malti Petit Pierre' and 'Double Fine' cvs. were susceptible. 'Eureka' and 'Bearss Lime'cultivars displayed the lowest susceptibility to blast of citrus. 'Lemon Baldi', 'Valencia Late' and 'Sweet Lime' cvs. were considered moderate susceptible to blast of citrus and 'Hernandina' and 'MA3'cvs. were found less susceptible than sweet orange. Cultivars such as, 'Eureka' and 'Sweet Lime' were found highly susceptible to *P. syringae* pv. *syringae* in the detached fruit test. On the contrary, cultivars could be considered less susceptible 'MA3' and 'Cassar' cvs., developed the lowest levels of infection against black pit of citrus. Moreover, 'Thompson Navel 'and 'Malti Petit Pierre' cvs. Were found susceptible to *P. syringae* pv. s*yringae* in the detached fruit test. In addition, 'Meski Ansli' cv. was found moderately susceptible and 'Star Ruby' turn out to be less susceptible than cultivar Meski Ansli. Thus, 'Eureka' cultivar displayed the lowest susceptibility to blast of citrus are those showing the highest susceptibility to black pit of citrus. A lack of correlation between cultivars susceptibility against black and blast of citrus is proved. In fact, blast of citrus leaves and steams, and black pit of citrus fruit, are caused by some strains of *Pseudomonas syringae* [12]. Blast results in expanding lesions on citrus leaves and stems, which leads to defoliation of trees in severe cases. For *Citrus sinensis*, 'Yafa', 'Valencia' and 'Washington Navel' cvs and for *C. reticulata* 'Clementine', 'Fremont', 'Satsuma' and 'Mineola Tangelo' cvs. and also *C. paradisi* 'Star Ruby' cv. were found the most susceptible cultivars in citrus plants toward citrus blast disease [13]. In addition, some studies have reported that the hypo dermic injections of the leaves of sweet orange, sour orange and tangerine showed the same results. However, lemon leaves produced less effect than on the other three species, indicating a greater degree of resistance. This is also in agreement with observations in the orchards where lemon lesions when formed on the leaves and twigs are fewer and smaller than on the sweet orange [14]. Moreover, sweet-orange leaves and twigs are more susceptible than lemon leaves and twigs The most susceptible cultivars to blast of citrus are orange (*C.* sinensis) and Grapefruit (*C.* paradisi) while lemon (Citrus limon) was the most susceptible to black pit of citrus [13].

Further, Fawcett et *al*.[15]]reported that black pit results, in dark – colored, sunken blemishes on fruits, particularly lime and lemon, of up to 35 mm in diameter [16]. On lemon fruit, black pit symptoms developed within 5 days. In fact, fruit lemon cultivar appeared to be the most susceptible to black pit and the mandarin group the most resistant, although slightly susceptible. Sweet-orange fruit is less susceptible than lemon fruits [14].

In a previous study, the mandarin group, except 'Dancy tangerine', showed no effect in three days and only a maximum of 1 to 2 mm in nine days. The 'Lisbon, 'Villa Franca' and 'Eureka'response was similar, but the 'Rough lemon' (*Citrus jambhiri*) was less susceptible. For the mandarin group, the 'King', 'Clementine mandarin'and 'Dancy Tangerine' were moderately resistant and the 'Satsuma orange'seemed to be resistant. The sweet-orange group was fairly susceptible. 'Navel', 'Valencia' and the varieties known as 'Parson's Brown' and 'Pineapple' orange and'Lue Gim' Gong were exhibited a compared reaction. The pummelo group, represented by 'Marsh seedless','McCarty', 'Duncan' and 'Triumph', were only a little less susceptible than the sweet oranges. The lime fruits represented by the 'Mexican', 'Tahiti' and 'Rangpur limes' appeared to be intermediate, but the 'Kusaie lime'was about equal to the grape fruit varieties [14].

In the citrons, the spots were slow in development, but finally became as large as those in the grapefruit. The Sampson tangelo (tangerine x pummelo) appeared to be intermediate in susceptibility [14].

5. Conclusion

From the present work, we could conclude that detached leaves test assay in petri dishes is more convenient and most reliable for determining the pathogenicity of *Pseudomonas syringae* pv. *syringae* on citrus and also for the screening of cultivars susceptibility against blast of citrus *in vitro*. In addition, detached fruits test could be used for *in vitro* screening of cultivars susceptibility towards black pit of citrus. Our results showed that Eureka cv. was less susceptible to blast of citrus while, MA3 and Cassar cvs seemed to be less susceptible to black pit and therefore could be used to replant citrus orchards mainly in the humid and hail regions where the environmental conditions are favorable to blast and black pit of citrus.

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7. References

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Abstract Citrus blast and black pit that became increasingly important bacterial diseases are caused by Pseudomonas syringae pv. syringae. This study aimed to evaluate the antibacterial potential of Bacillus species strains and garlic extracts against two P. syringae isolates (BAT13 and DAPP-PG115). The Bacillus species strains were isolated from symptomless citrus leaves. Under in vitro conditions, 21 Bacillus species strains and garlic extract displayed antibacterial activity against the pathogen. Under greenhouse conditions, antagonistic bacteria, garlic Epub 2013 May 31. Pseudomonas syringae pv. tomato DC3000: a model pathogen for probing disease susceptibility and hormone signaling in plants. Xin XF1, He SY. Author information.Â Since the early 1980s, various strains of the gram-negative bacterial pathogen Pseudomonas syringae have been used as models for understanding plant-bacterial interactions. In 1991, a P. syringae pathovar tomato (Pst) strain, DC3000, was reported to infect not only its natural host tomato but also Arabidopsis in the laboratory, a finding that spurred intensive efforts in the subsequent two decades to characterize the molecular mechanisms by which this strain causes disease in plants. Diseases Caused by Pseudomonas syringae. Crown Gall Disease of Nursery Crops. Potential Impact of Cyanobacteria on Crop Plants.Â Altering soil pH affected the susceptibility of peach to Pseudomonas syringae (Weaver, 1975). The nature of the soil at the planting site can affect tree susceptibility in an indirect way. Clay soils typically were free of nematodes in South Carolina, and losses from peach tree short-life in these soils were minimal.Â Overwintering and survival of Pseudomonas syringae pv. syringae and symptom development in peach trees. Plant Disease 68:468-470. Hattingh, M.J., Roos, I.M.M., and Mansvelt, E.L. 1989. Infection and systemic invasion of deciduous fruit trees by Pseudomonas syringae in South Africa. Plant Disease 73:784-789. Hawkins, J.E. 1976.