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Evaluating The Resistance Of Tomato Cultivars To Algerian *Phytophthora Infestans* Genotypes Under Controlled Trial

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Abstract: Late blight is a destructive disease of solanaceous crops such as tomato (*Solanum lycopersicum* L.), caused by the Oomycete *Phytophthora infestans* (Mont.) de Bary. Late blight is generally controlled by fungicides applications which quickly become ineffective due to the appearance of new *P. infestans* genotypes which can overcome the resistance of improved tomato cultivars and cause total production losses. The aim of this study is to assess the resistance level of tomato cultivars under controlled conditions, inoculations were carried out on detached leaflets (cvs. Trakia, Saint Pierre and Marmande) using inoculums of the major *P. infestans* clonal lineages found in Algeria such as EU_13_A2 (n=1), EU_23_A1 (n=2) and EU_2_A1 (n=1) (three replicates of each isolate). This investigation showed that the choice of resistant cultivars can help control late blight and provide economic and environmental advantages by reducing the use of inputs.

Keywords: resistance; late blight; *Phytophthora infestans*; tomato

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is an important crop cultivated globally in tropical to temperate regions. It is the second-most-consumed vegetable after the potato [1].In Algeria, tomato is the fourth most important crop after potato, melon and onion. It is grown throughout the year, with winter cultivation taking place in greenhouses and summer cultivation in open fields. [2]. Often, diseases limit tomato production .But, late blight caused by the oomycete *Phytophthora infestans* (Mont.) de Bary is the most damaging disease in this crop [3]. This oomycete attacks all aerial parts of the plant. It causes leaf and stem necrosis, fruit loss and ultimately plant death [4].

In Algeria, three majors clonal lineages of *P.infestans* (EU_13_A2, EU_2_A1 and EU_23_A1) have been identified in commercial potato and tomato production regions [5]. However, most methods of controlling this pathogen are based on the application of expensive fungicides, which can be less effective when weather conditions are favorable for pathogen spread [6].

Or the emergence of new fungicide-resistant genotypes.

When, it is difficult to control *P. infestans* with fungicides; resistant cultivars provide an alternative means of disease control [7].

This study was conducted to evaluate the behaviour of three tomato cultivars against the major late blight clonal lineages using the detached leaflets test conducted under *in-vitro* conditions, the Aggressiveness components were measured including the incubation and latency period, lesion area and sporangia production. It is important to identify and characterise new sources of resistance and to develop new resistant cultivars

to make it easier to control late blight and offer economic and environmental benefits through reduced inputs.

2. Materials and Methods

2.1. Plant material

Three cultivars (Marmande, Saint Pierre and Trakia) were evaluated for their resistance to *P. infestans* under controlled conditions. The cultivars were grown in a glasshouse with a 25° - 20°C day/night cycle. Leaflets were harvested after nine weeks.

2.2. Phytophthora infestans isolates

Four isolates were selected for this investigation (Table 1). The isolates were obtained from samples of infected potato and tomato using isolation techniques which, consist of placing a small pieces of fresh samples of leaves, stems and fruits infected with *P. infestans* on potato slices and then putting them into closed Petri dishes. These were subsequently incubated at 15°C in the dark. After 4 to 5 days, the mycelium obtained was purified by repeated transfers into pea agar, which had been amended with antibiotics (30mg of Rifamycin and 200mg of Ampicillin). Pure cultures were kept at a temperature of 15°C. After isolation, genotyping was performed using 17 SSR loci [5]. Subsequently, the lineages were named according to the classic European nomenclature as defined by [8].

Table 1. Characteristics of isolates used in the detached-leaflets experiment.

Isolates	Area	Sampling years	Genotype
DZ-15-T25	Tipaza	2015	EU_23_A1
DZ-16-P01	Algiers	2016	EU_23_A1
DZ-15-P30	Algiers	2015	EU_13_A2
DZ-14-P18	Algiers	2014	EU_2_A1

2.3. Inoculation

Under controlled conditions, leaflets aged nine weeks were harvested and detached leaflets were inoculated on the abaxial side with a 20 µl drop of sporangial suspension (5 x 10^4 sporangia ml^{-1}). The inoculum was produced from *P. infestans* mycelium that had been grown on pea agar for 3 weeks. Two leaflets were placed on sterilised moist paper in Petri dishes and incubated at 18°C in a growth chamber for 16 hours in the light (three replicates for each isolate).

2.4. Aggressiveness components

The incubation period (IP), was evaluated by daily observations of the first symptoms, while the latency period (LP), was expressed through daily observations of the initial sporangia production. Subsequently, lesion area (LA) was measured five days after inoculation, according to the formula provided by [9]: the lesion area was calculated as LA =1/4 x π x length x width of necrosis. Sporangia production (SP) was assessed seven days after inoculation .The infected leaflets were washed in 10 ml of sterilised distilled water and the number of sporangia were quantified with a Malassez cell. The sporangia were expressed as the number of sporangia per ml.

2.5. Data analysis

All statistical analyses, including analysis of variance and Tukey HSD test, were performed using the software R v.3.3.2. (The R Foundation for Statistical Computing, 2016).

3. Results

Isolates produced a shorter incubation period on cv. Marmande (Table.2), but longer on cv. Saint Pierre, ranged from 3 days (EU_13_A2, cv. Marmande) to 5 days (EU_23_A1, cv. Saint Pierre). The same results were noticed with a latency period that ranged from 3.5 days for (EU_13_A2, cv. Marmande) to 6 days for (EU_2_A1, cv. Saint Pierre). The EU_13_A2 and EU_2_A1 clonal lineages showed significant results with all cultivars ($p \leq 0.001$). However, there was no significant difference in incubation and latency period between EU_23_A1 clonal lineages and cultivars ($p \geq 0{,}001$). A short incubation and latency period means that the pathogen is able to attack a cultivar more quickly, indicating its susceptibility, as in the case of cv. Marmande.

The lesion area was larger on the cv. Marmande with all isolates compared to the Saint Pierre and Trakia cultivars, where sizes ranged from 68.56 mm² (EU_23_A1, cv. Trakia) to 377.07 mm² (EU_23_A1, cv. Marmande). Furthermore, sporangia production was more significant in the cv. Marmande with isolates of EU_13_A2 and EU_23_A1 lineages, compared to the Saint Pierre and Trakia cultivars. While, the sporulation rate varied from 3.1 x 10^4 sporangia ml^{-1} (isolate EU_23_A1, cv. Trakia) to 45.73 x 10^4 sporangia ml^{-1} (isolate 23_A2, cv. Marmande) (Fig. 1).

The EU_13_A2 and EU_23_A1 clonal lineages showed significant results on lesion area and sporulation with all cultivars. Whereas, the EU_2_A1 lineage had no significant effect on all cultivars tested ($p=0.49$ for lesion area and $p=0.83$ for sporulation). This result suggests, that the isolates have different levels of virulence(Figure 1).

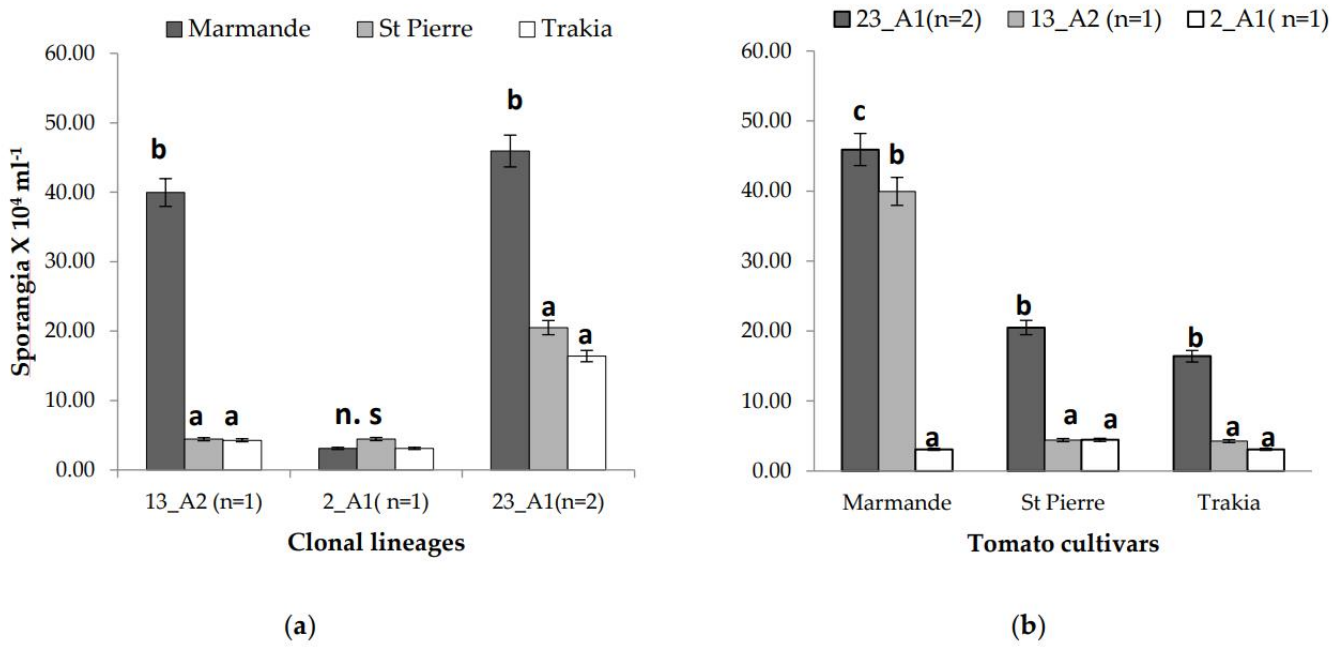

Figure 1. The rate of sporulation production expressed as the number of sporangia x 10^4 ml^{-1} (**a**) Comparison between tomato cultivars; (**b**) Comparison of *P.infestans* clonal lineages.

Table 2. Mean values of aggressiveness components.

	Cultivars	13_A2 (n=1)	2_A1(n=1)	23_A1(n=2)	mean
IP (days)	Marmande	3.00 a	4.00 a	3.89 a	3.61
	St Pierre	4.00 b	4.75 b	5.00 b	4.62
	Trakia	4.33 c	4.00 a	4.88 b	4.63
	Pr(>F)	1.65e-08 ***	0.00363 **	0.929	4.78e-14 ***
LP (days)	Marmande	3.50 a	5.00 a	4.89 a	4.44
	St Pierre	4.20 b	6.00 b	5.69 b	5.23
	Trakia	4.44 b	5.83 b	5.71 b	5.34
	Pr(>F)	0.00866 **	<2e-16 ***	0.703	0.000158 ***
LA (mm^2)	Marmande	248.57 c	189.71 a	377.07 b	282.54
	St Pierre	154.88 b	156.61 a	145.87 a	150.30
	Trakia	68.56 a	75.62 a	99.70 a	87.54
	Pr(>F)	1.56e-05 ***	0.491	3.02e-05 ***	0.000355 ***
SP (10^4 ml^{-1})	Marmande	$39.95x10^4$ b	$3.1x10^4$ a	$45.93x10^4$ b	$36.8x10^4$
	St Pierre	$4.42x10^4$ a	$4.45x10^4$ a	$20.50x10^4$ a	$13x10^4$
	Trakia	$4.27x10^4$ a	$3.1x10^4$ a	$16.40x10^4$ a	$11.3x10^4$
	Pr(>F)	0.00288 **	0.837	0.0153 *	5.38e-05 ***

IP: Incubation period; LP: Latency period; LA: Lesion area. SP: Sporangia production. The mean aggressiveness components are expressed using Tukey's HSD test with α=0.05.The statistical significance is expressed using asterisk (*p < 0.05; **p < 0.01; ***p < 0.001).

4. Discussion

The data of aggressiveness components obtained from the detached leaflet assay can be a reliable predictor of cultivars and isolates behaviour under field conditions [10]. In our case, the cv. Marmande was very susceptible, compared with cvs. Saint Pierre and Trakia which showed a good level of resistance to all *P.infestans* clonal lineages.

The sensitivity of Marmande can be explained by the fact that this cultivar is extensively cultivated, and its partial resistance has been overcome by local *P.infestans* populations in Algeria. In contrast, the resistance of other two cultivars (cvs. Saint Pierre and Trakia) was higher due to their limited cultivation. Our results are in agreement with those of [11], who observed the adaptation of the *P. infestans* population to the locally dominant cultivars, which had overcome their partial resistance.

We also noticed that the isolates behaved differently according to their genotype, with some of them proving to be very aggressive, such as EU_23_A1. This can be explained by the adaptation of this genotype to tomato cultivars [2]. Compared with EU_13_A2 genotype, which was less aggressive on tomato cultivars except for the Marmande. The reason is that this genotype has only been found on potato under field conditions in Algeria, so it is well adapted to potato [5]. Similarly, certain lineages such as EU_13_A2 and EU_6_A1 in Europe are identified as potato specialists [12]. Therefore, they do not adapt to substrates other than potato, and their virulence decreases on tomato [13].

5. Conclusion

Our experiments were conducted under controlled conditions. However, in the field, many factors such as cultivars-pathogens interactions and climate can affect a cultivar's resistance to a pathogen, causing cultivars to move from resistant to susceptible. It is essential to assess cultivars' behaviour, particularly those that have exhibited a notable level of pathogen resistance in the field. Searching for cultivars with resistance to late blight would be a positive step towards enhancing tomato production. This approach would also reduce the need to use fungicides, which can adversely affect both the environment and human health.

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Conflicts of Interest: "The authors declare that they have no conflict of interest".

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