

Article



# Performance of Aubergine Rootstocks against Verticillium dahliae Isolates in Southeastern Spain

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Abstract: Aubergine (Solanum melongena L.) (Solanaceae) is a widespread crop in the Mediterranean basin. Verticillium dahliae is one of the main soil-borne pathogens affecting the aubergine crop. Its control has traditionally been achieved by soil fumigation with chemical disinfectants. Restrictions on the use of chemical fumigants have led to the search for solutions in genetic resistance using rootstocks. In southeastern Spain, aubergines are grafted for the control of V. dahliae. Two Solanum torvum rootstocks (Hugo F1 and Torpedo) and a Solanum melongena hybrid (Javah F1) were tested against five isolates of V. dahliae obtained from grafted (A1 and A2) and ungrafted (Vd8, Vd17 and Vd66) aubergines compared with the susceptible cultivar Larne F1 under controlled conditions. Isolates from grafted plants infected all three rootstocks, with differences observed in the percentage of plants with symptoms and in the disease symptom severity. Three strains isolated from the ungrafted aubergines (Vd8, Vd17 and Vd66) infected Javah F1 rootstock. The Hugo F1 and Torpedo rootstocks showed a high level of resistance to V. dahliae, while Javah F1 was susceptible to the pathogen. The Hugo F1 and Torpedo rootstocks are suitable for mitigating the effects of Verticillium wilt in Mediterranean aubergine crops. Understanding the nature of the resistance from S. torvum could enhance the benefits of grafting or facilitate the introduction of resistance into commercial cultivars.

Keywords: Solanum melongena; Solanum torvum; graft; Verticillium wilt; soil-borne pathogen

# 1. Introduction

Aubergine (*Solanum melongena* L.) (Solanaceae) is a widespread crop originating in Asia. It is grown outdoors, in tropical and subtropical areas, and in greenhouses all over the world. The main producers are China and India, with a production of 38.2 and 12.7 M t, respectively. Spain is the tenth largest producer of aubergines in the world (276.320 t) and the second largest in Europe after Italy (307.430 t) [1].

*Verticillium dahliae* and *Fusarium oxysporum* f. sp. *melongenae* are the main soil pathogens affecting the aubergine crop in Spain [2–5]. *Verticillium dahliae* infects and damages spontaneous, woody and herbaceous crops [6–8]. It has a broad pathogenic capacity and a degree of parasitic specialization [8,9]. The symptoms are leaf yellowing, apical wilting, defoliation, reduced growth, the browning of the vascular system and partial or total desiccation of the plant [8,10–12]. In the absence of the cultivated host plant, this fungus can persist in the soil in form of microsclerotia for a long time (6 to 14 years) [11,13]. Mild climatic conditions favour the development of the disease and the appearance of symptoms [10,14]. The pathogenic variability of aubergine isolates is high, with different levels of aggressiveness depending on the cultivar [11] and with asymptomatic infectious isolates [4]. In Spain, symptoms of *V. dahliae* appear on aubergine crops in spring and



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**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). autumn when temperatures are mild for approximately 3 to 4 weeks. Climate change favours these conditions, so that damage caused by *V. dahliae* is increasing in aubergine crops in southeastern Spain (Andalucía, Valencia, Murcia and the Islas Baleares), where 94% of Spanish aubergines are produced. In Almería (Andalucía), the importance of Verticillium wilt in aubergines is greater due to the prolongation of the fungal activity period and the increase in the severity of symptoms [4,5].

In crops without sources of resistance, *V. dahliae* control has traditionally been achieved by reducing the inoculum in the soil prior to planting. Soil fumigation with chemical disinfectants (methyl bromide, chloropicrin or methyl isothiocyanate generators) has been used in horticultural and high-value crops, including aubergine [15].

Restrictions on the use of chemical fumigants have led to the search for solutions in genetic resistance, using rootstocks resistant to the main soil pathogens such as *V. dahliae*. Some authors have reported high levels of resistance to *V. dahliae*, *F. oxysporum* f. sp. *melongena* and *Meloidogyne* spp. in *Solanum torvum*, *S. sisymbrifolium* and *S. incatum* and in hybrids of *S. lycopersicum* with *S. hirsutum* or with *S. habrochaites* (Solanaceae) used as tomato rootstocks. However, some *S. torvum* rootstocks present problems in nursery management, low and irregular germination due to seed dormancy [16] and incompatibility with the aubergine cultivars used. Selections of these species or interspecific hybrids have been evaluated as eggplant rootstocks with different results, sometimes inconsistent, against different pathogens (including *V. dahliae*) [15,17–25].

*Solanum torvum* seems to be the rootstock that best protects aubergine against soil pathogens, especially *Meloidogyne* spp. and *V. dahliae* [17,25,26]. Plants with symptoms of *V. dahliae* infection were observed in aubergine plants grafted on *S. torvum* in Mallorca (Spain).

The aim of this work was to evaluate the performance of several commercial rootstocks of aubergine against *V. dahlae* isolates obtained from grafted and ungrafted aubergine plants.

# 2. Materials and Methods

## 2.1. Isolates and Host Plants

Five isolates of V. dahliae were used (Table 1). Two isolates, A1 and A2, were obtained from aubergine plants grafted on the Javah F1 rootstock showing advanced symptoms of Verticillium wilt (reduced growth, leaf yellowing and defoliation). For isolations, stem segments of 2 mm in thickness were cut with a sterilized scalpel from affected plants, and their surface was disinfected by immersion in a solution of sodium hypochlorite at 1% for one minute, followed by two rinses in sterile distilled water (SDW). The washed pieces were dried on sterile filter paper and plated on potato dextrose agar (PDA) medium supplemented with 100 ppm streptomycin sulphate (Sigma Aldrich Company, St. Louis, MO, USA). The plates were incubated at  $22 \pm 1$  °C in the dark for five days. *Verticillium* spp. were observed to grow out of the vascular bundles. Selected colonies were subcultured on PDA without antibiotics [27]. Vd8, Vd17 and Vd66 isolates were obtained from the mycotheca of the Plant Protection Department of Instituto Murciano de Investigación y Desarrollo Agrario y Medioambiental (IMIDA) and were isolated from aubergine plants grown in greenhouses in Murcia (Spain) showing symptoms of Verticillium wilt. The isolates were identified as V. dahliae on PDA without antibiotics by the absence of yellow or orange pigmentation on the back of the colony, the production of unicellular conidia and microsclerotia [28].

Table 1. Verticillium dahliae isolates used in the experiments.

Isolate	Origin	Date	Host
Vd 8	Cartagena (Murcia)	February 1984	S. melongena cv. Bonica
Vd 17	El Palmar (Murcia)	May 1988	S. melongena
Vd 66	Cieza (Murcia)	December 1992	S. melongena
A1	Mallorca (Islas Baleares)	June 2012	S. melongena grafted on Javah F1 rootstock
A2	Mallorca (Islas Baleares)	June 2012	S. melongena grafted on Javah F1 rootstock

#### 2.2. Production of Inoculum

The inoculum was obtained by blending 18-day-old cultures on PDA with sterile distilled water. The concentration of conidia was determined by a hemacytometer (Neubauer chamber Marienfeld) and adjusted with SDW to  $1 \times 10^5$  conidia mL<sup>-1</sup>.

#### 2.3. Susceptibility Assay

Three commercial rootstocks for aubergine with different degrees of resistance to *V. dahliae*, Hugo F1 (moderate/intermediate resistance), Javah F1 (moderate/intermediate resistance) and Torpedo (high resistance), and one susceptible cultivar (Larne F1) were used in the assay as a control (Table 2). The rootstocks were selected for their favourable nursery behaviour (high germination rates and uniform development), as well as their compatibility with the cultivars commonly grown in the Mediterranean region. The Larne F1 cultivar (aubergine), which is typically cultivated in Spain, was selected as a reference due to its sensitivity to *V. dahliae*.

Plant Species	Rootstock	Company	Resistance	
Solanum torvum	Hugo F1	Akira Seeds	<sup>1</sup> IR Verticillium sp.	
<i>Solanum</i> sp.	Javah F1	Takii Europe B.V.	<sup>1</sup> IR Verticillium sp.	
Solanum torvum	Torpedo	Ramiro Arnedo S.A.	<sup>2</sup> HR Verticillium sp.	
Solanum melongena	Larne F1	Semillas Fitó S.A.	-	
1	2 **** 1 * 1			

Table 2. Rootstocks used in the experiments.

<sup>1</sup> IR: intermediate resistance. <sup>2</sup> HR: high resistance.

The seeds were disinfected with a 1.5% sodium hypochlorite solution for 4 min and then washed several times in SDW before sowing. Seedlings were grown in autoclaved peat (120 °C for one hour) in trays with 150 cells of 25 cm<sup>3</sup> each. They were maintained in a growth chamber at 24  $\pm$  1 °C, under a 14:10 (L:D) photoperiod and 60  $\pm$  10% relative humidity.

The plants were transplanted into pots (0.2 L) when they had the first two true leaves. They were grown in an autoclaved peat (Klasmann TS3; Klasmann-Deilmann GmbH, Saterland, Germany) and perlite (Projar, S.A. Co., Valencia, Spain) mixture (2:1) under identical climatic conditions to those described above.

Plants were inoculated seven days after transplanting (3–4 true leaves). Each plant was inoculated with 10 mL of conidial suspension ( $1 \times 10^5$  conidia mL<sup>-1</sup>), resulting in  $1 \times 10^6$  conidia per plant [5,11]. A hole (1 cm deep) was made on each side of the plant, and the inoculum was introduced into it. An uninoculated control (10 mL of SDW) was used for each cultivar. For each cultivar and isolate, 15 plants were evaluated. Groups of five plants were replicated three times for each cultivar and isolate. The assay was completed after seven weeks. Two weeks were allowed between each replicate to condition the culture chamber and prepare for the next replicate.

The evaluation was conducted seven weeks after inoculation and involved the measurement of the following parameters.

# (1) Verticillium wilt incidence

At the end of the assay, each plant was tested for the presence or absence of fungal infection by using the methodology by Tello et al. [29]. Four stem samples (1 mm thick sections) were taken from each plant. These were disinfected by immersion in a 1% sodium hypoclorite solution for one minute, rinsed in SDW, dried on sterile filter paper and plated on PDA culture medium. The samples were incubated for a period of five days at a temperature of  $22 \pm 1$  °C, after which they were examined under a microscope for the presence of morphological features and cultural characteristics of *V. dahliae*. These included the presence of verticillate conidiophores, hyaline conidia and microsclerotia.

The incidence of Verticillium wilt was calculated as the ratio of infected plants to the total number of plants.

(2) Disease symptom severity

The Verticillium wilt severity of each plant was evaluated according to the following scale: 0 = no symptoms; 1 = chlorosis of the lower leaves; 2 = chlorosis of 30-50% of the leaves; 3 = chlorosis of more than 50% of the leaves; 4 = generalised chlorosis and leaf necrosis and/or wilting of the plant; 5 = death of the plant (Figure 1).



**Figure 1.** Symptom scale to assess infection of aubergine plants by *Verticillium dahliae*. The numbers represent the level of disease incidence, where 0 = no symptoms; 1 = chlorosis of the lower leaves; 2 = chlorosis of 30–50% of the leaves; 3 = chlorosis of more than 50% of the leaves.

## 2.4. Statistical Analysis

Statistical analysis of affected plants and disease symptoms was performed by using R, version 4.2.2 [30], and RStudio, version 2022.12.0 [31].

The uninoculated (control) plants showed no symptoms and were excluded from statistical analysis.

(1) Verticillium wilt incidence

To know the importance of the factors of the affected plants, i.e., Solanum rootstock and *V. dahliae* isolate, and their interactions, a generalized linear mixed model with binomial distribution and logit link function was fitted, with replication as a random factor. Calculations were performed using the glmer functions of the lme4 software package, version 1.1-34 [32]. The significance of the factors was tested with likelihood ratio tests comparing hierarchically nested models, using the anova function of the stat package [30].

For significant factors, pairwise comparisons of the proportions of affected plants were made by using Fisher's exact test, with the fisher.multcomp function of the RVAideMemoire package [33].

#### (2) Disease symptom severity

Means and medians of the symptoms of the Solanum rootstock and *V. dahliae* isolate combinations were calculated. Percentile confidence intervals for the medians were calculated by using bootstrap, with the groupwiseMedian function of the rcompanion software package [34].

Symptoms were also analysed by using rank-based non-parametric methods for the analysis of ordinal data in factorial designs [35]. From the midranks of the observations, the non-parametric relative effect of treatment was estimated, and its confidence intervals and the significance of the factors Solanum rootstock and *V. dahliae* isolate and their interaction were tested with the ANOVA-type statistic by using the rankFD function of the rankFD software package [36,37].

When the main factors Solanum rootstock and *V. dahliae* isolate and their interactions were significant, pairwise comparisons were made by using the mctp·function of the nparcomp software package [38], with its default settings (Tukey-type contrast, global pseudo-rank estimation method, Fisher asymptotic approximation method and 95% CIs), and a p < 0.05 was adopted as a threshold of statistical significance.

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# 3. Results

## 3.1. Verticillium Wilt Incidence

There was a highly significant effect of the factors rootstock (LRT(3) = 115.2, p < 0.001) and isolate (LRT(4) = 118.3, p < 0.001). The interaction showed a trend towards significance (LRT(12) = 20.708, p < 0.055) (Table 3).

**Table 3.** Verticillium Wilt Incidence. Generalized linear mixed model fits and analysis of deviance for testing the significance of the factors Solanum rootstock (St) and *Verticillium dahlae* isolate (Vd) and their interaction.

	Goodness-of-Fit Test				Analysis of Deviance						
$N^{1}$	Model	Npar <sup>5</sup>	AIC <sup>6</sup>	Df <sup>7</sup>	logLik <sup>8</sup>	Deviance 9	Test	Factor tested	LRT <sup>10</sup>	$\Delta df$	Pr(>Chisq) <sup>11</sup>
1	St <sup>2</sup> + Vd <sup>3</sup> + St:Vd <sup>3</sup> + (1   R <sup>4</sup> )	21	111.63	39	-34.813	69.625					
2	$St^2 + Vd^3 + (1   R^4)$	9	108.33	51	-45.166	90.333	1 vs. 2	St:Vd	20.708	12	0.055
3	$St^{2} + (1   R^{4})$	5	218.71	55	-104.353	208.707	2 vs. 3	Vd	118.374	4	$<2.2 \times 10^{-16}$ ***
4	$Vd + (1   R^4)$	6	217.56	54	-102.78	205.559	2 vs. 4	Sc	115.226	3	$<2.2 \times 10^{-16}$ ***

<sup>1</sup> N = number of model. <sup>2</sup> St = Solanum rootstock. <sup>3</sup> Vd = *Verticillium dahlae* isolate. <sup>4</sup> R = replicate. <sup>5</sup> npar = number of model parameters. <sup>6</sup> AIC = Akaike Information Criterion. <sup>7</sup> df = degrees of freedom. <sup>8</sup> logLik = the log-likelihood for the model. <sup>9</sup> deviance = deviance for the model. <sup>10</sup> LRT = likelihood ratio test statistic. <sup>11</sup> Pr(>Chisq) = *p*-value.

Multiple comparisons were made with Fisher's exact test for the different levels of the factors Solanum rootstock and *V. dahliae* isolate. Since the interaction was close to significance, comparisons were also made for each rootstock–isolate combination (Table 4).

Table 4. Proportion of plants affected and pairwise comparisons using Fisher's exact test.

St $^{1}$ Vd $^{2}$	A1	A2	Vd17	Vd66	Vd8	Mean
Larne F1	0.600 ab	0.867 a	0.067 c	0.067 c	0.333 bc	0.387 B
Hugo F1	0.267 bc	0.200 bc	0.000 c	0.000 c	0.000 c	0.093 C
Javah F1	1.000 a	1.000 a	0.133 bc	0.067 c	1.000 a	0.640 A
Torpedo	0.133 bc	0.267 bc	0.067 c	0.000 c	0.000 c	0.093 C
Mean	0.500 AB	0.583 A	0.067 C	0.033 C	0.333 B	0.303

<sup>1</sup> St = Solanum rootstock and <sup>2</sup> Vd = *Verticillium dahlae* isolate. Different capital letters indicate significant differences within the mean values of the rootstocks or within the mean values of the isolates with a *p*-value  $\leq 0.05$  according to Fisher's exact test. Different lowercase letters indicate significant differences between rootstock and isolate combinations with a *p*-value  $\leq 0.05$  according to Fisher's exact test.

Only plants on the Javah F1 rootstock and Larne F1 cultivar were infected when inoculated with the five isolates. The Javah F1 rootstock exhibited a higher mean proportion of affected plants (0.640) than the Larne F1 cultivar (0.387). However, it only became more susceptible when inoculated with the Vd8 isolate with 100% of plants affected compared with 33.3% of the susceptible cultivar (Table 4).

The mean values of the affected plants show that the S. torvum rootstocks (Hugo F1 and Torpedo) were equally resistant to each other (9.3%) and significantly more resistant than the Larne F1 cultivar and the interspecific rootstock Javah F1, which was the most susceptible (64%).

Differences in the aggressiveness of the isolates were evident when inoculating the Javah F1 rootstock and the Larne F1 cultivar, but not when inoculating the Hugo F1 and Torpedo rootstocks.

Isolates from grafted plants (A1 and A2) were more aggressive (50% and 58.3% of plants affected) than isolates from ungrafted plants, except for isolate Vd8, which was similar in aggressiveness (33.3%) to isolate A1.

# 3.2. Disease Symptom Severity

The percentage of plants with symptoms was closely related to the percentage of plants from which the pathogen was isolated.

For each combination of plant material and *Verticillium* isolates, the mean severity index and median are shown in Figure 2.



**Figure 2.** Severity of disease symptoms for each combination of *V. dahliae* isolates and Solanum rootstocks and Larne F1 cultivar. Medians and their 95% confidence intervals (in circles and blue lines) and means (black asterisks) are depicted. To facilitate comparisons, the data are organized into facets according to isolates (**A**) or rootstocks (**B**). In (**A**), the term "all" refers to the entire set of isolates for each rootstock, while in (**B**), it refers to the complete set of rootstocks for each isolate.

All cultivars exhibited symptoms on the leaves, except for the Hugo F1 rootstock inoculated with the Vd66 isolate. No plant showed symptoms on 50% or more of its leaves (value 3 on the symptom scale). Yellowing symptoms were observed on the lower leaves of the S. torvum Hugo F1 and Torpedo rootstocks inoculated with isolates Vd66 and Vd8. However, *V. dahliae* was not isolated from these rootstocks.

The ANOVA-type statistic obtained from the non-parametric analysis of the relative effects shows a significant effect of the factors rootstock and isolate and their interaction on the symptoms (Table 5).

ANOVA-Type Statistic	Statistic	df1	df2	<i>p</i> -Value
Rootstock	60.7205	2.6059	205.2054	0
Isolate	10.9794	3.8751	205.2054	0
Rootstock:Isolate	1.9955	9.8844	205.2054	0.036

**Table 5.** Non-parametric analysis of disease symptoms. ANOVA-type statistic from the relative effect of Solanum rootstocks and *V. dahlae* isolate and their interaction.

Considering each simple factor independently, significant differences can be observed among all cultivars. The interspecific hybrid rootstock Javah F1 exhibited the highest mean symptom severity (1.827), followed by the Larne F1 cultivar (1.360), the Torpedo rootstock (0.653) and the Hugo F1 rootstock (0.160).

Sixteen days after inoculation, yellowing and wilting symptoms were observed on the basal leaves of the Javah F1 rootstock, with greater prevalence and severity being observed in plants inoculated with isolates A1 and A2. Seven days later, symptoms increased in severity in plants inoculated with isolates A1 and A2, while those inoculated with isolate Vd 8 exhibited only mild symptoms. At this time, plants inoculated with isolates Vd 17 and Vd 66 showed no symptoms.

Grafted plant isolates A1 and A2 induced more severe symptoms (1.45 and 1.433, respectively) compared with ungrafted plant isolates Vd66 and Vd17 (0.55 and 0.6, respectively), although not as severe as Vd8 (0.967).

The interaction effect is shown in Figure 3, where the relative effects for each isolate and cultivar are plotted. All isolates induced similar relative symptom rates in the Solanum rootstocks Hugo F1 and Torpedo. This is not applicable to the susceptible cultivar Larne F1. Isolates from grafted plants resulted in higher symptom levels compared with two isolates from ungrafted aubergines (Vd17 and Vd66). For the interspecific hybrid rootstock Javah F1, only one of the grafted isolates (A1) differed from ungrafted isolates Vd66 and Vd17.



**Figure 3.** Relative effects of disease symptoms and their 95% confidence intervals of each combination of Solanum rootstocks and *V. dahliae* isolates. To facilitate comparisons, the data are organized into facets according to isolates (**A**) or rootstocks (**B**). In (**A**), the term "all" refers to the entire set of isolates for each rootstock, while in (**B**), it refers to the complete set of rootstocks for each isolate. Within each facet, the letters correspond to multiple comparisons made with all Rootstock:Isolate combinations, except for "all" facets which are made with each single factor. The letters have been simplified to make comparisons within each facet.

#### 4. Discussion

Five isolates of *V. dahliae* were inoculated to test the performance of three commercial aubergine rootstocks against a cultivar of aubergine.

Inoculation experiments were conducted to assess the effectiveness of various aubergine rootstocks in controlling Verticillium wilt. The assay results indicate that *S. torvum* cultivars, Hugo F1 and Torpedo, were effective rootstocks. Only a small percentage of plants (9.3%) were affected by the pathogen and exhibited mild symptoms of chlorosis on the lower leaves at the end of the assay. These rootstocks exhibited resistance when inoculated with isolates from the IMIDA fungus collection and showed low susceptibility when inoculated with isolates from grafted plants. This behaviour is consistent with that obtained by other authors. Zhou et al. [20] reported that S. torvum had a disease rate of 15% and a disease index of 12.5% at the end of the trial. They also found that the first symptoms appeared 5 days later than in susceptible or moderately resistant cultivars, indicating resistance. Cürük et al. [23] reported a 51% reduction in the symptom index (0.983 vs. 2.0) and a 43% reduction in the disease index (1.9 vs. 3.333) in plants grafted on S. torvum compared with ungrafted plants grown on substrates inoculated with  $1 \times 10^6$  conidia mL<sup>-1</sup>. Garibaldi et al. [25] found that the onset of symptoms was delayed by five days in S. torvum plants compared with the susceptible variety Black Bell when inoculated with  $1 \times 10^7$  CFU mL<sup>-1</sup> isolates from the rootstock and the variety. At the end of the trial, which was 72 days after inoculation, symptoms were observed in 20–27% of S. torvum plants and 87–100% of Black Bell plants.

The results of the trial indicated that the Javah F1 rootstock was more susceptible than the Larne F1 cultivar. Miles et al. [19] found that the aubergine cultivar Millionaire, when grafted onto the Javah F1, Red Scorpio and Meet rootstocks (*S. melongena* hybrids), was infected at the same level as the ungrafted cultivar (97–100% of plants).

Isolates obtained from plants grafted onto Javah F1 (A1 and A2) affected more plants of the Larne F1 cultivar and the Javah F1rootstock than those obtained from ungrafted aubergines. They produced more symptoms in sensitive materials than in resistant ones. Xu et al. [11] discovered variations in the pathogenicity and aggressiveness of a group of *V. dahliae* isolates from aubergine crops. Of the isolates, 25% were classified as strongly virulent (with a disease severity index (DSI) above 45.1 and defoliation), 45% as moderately virulent (with a DSI between 25.1 and 45 and no defoliation) and 30% as weakly virulent (with a DSI below 25 and no defoliation). As in the work by Gómez et al. [5], none of the isolates caused defoliation or plant death. However, 3 of the 12 isolates studied by Xu et al. [11] were defoliants.

The precise origin of *S. torvum* resistance against *V. dahliae* remains unclear. Wang et al. [39] proposed that a complex multigene process is involved in the resistance of *S. torvum* against *V. dahliae*. This could explain the difference in the behaviour of Hugo F1 and Torpedo, which exhibited a similar percentage of affected plants but displayed differences in the severity of symptoms.

Partial infection of resistant rootstocks and differences in susceptibility between interspecific hybrids and commercial cultivars could be influenced by inoculum density or temperature, as pointed out by Miles et al. [19] when evaluating *S. melongena* hybrid rootstocks and tomato rootstocks.

The inoculum density used in the experiments  $(1 \times 10^6 \text{ conidia per plant})$  is similar to that used by other authors:  $1 \times 10^7 \text{ CFU mL}^{-1}$  [25]; 0.9 to  $2.3 \times 10^6 \text{ conidia mL}^{-1}$  [4];  $1 \times 10^6 \text{ conidia mL}^{-1}$  [11,15,20,23].

However, under natural conditions, performance against the pathogen is more dependent on variations in soil temperature than under controlled conditions. In growing seasons with temperatures unfavourable to the pathogen, rootstock performance is better, disease incidence is generally lower, and grafting provides better quality yield [19,24]. In addition, Zhou et al. [40] observed that the use of *S. torvum* as a rootstock improved soil health by producing higher levels of oxidoreductases and increased populations of microorganisms (actinomycetes and bacteria) in the rhizosphere of plants.

Grafting is an expensive practice with difficult nursery management, mainly due to heterogeneous seed germination of some *S. torvum* rootstocks [18]. Seeds of *S. torvum* germinate slowly, and it may take about 30 days to reach germination, with percentages

varying between 15% and 50% [17]. To remedy these shortcomings, some authors consider the breeding of interspecific hybrids of Solanum species [17,21].

## 5. Conclusions

The selection of lines with better nursery performance must be performed without losing the level of resistance to pathogens. Further research is needed to better understand the interactions between *Verticillium dahliae* and *Solanum torvum*, the pathogenic variability of isolates of this fungus and the nature of *S. torvum* resistance to improve grafting performance or to introduce general or specific resistance into plant material. The *S. torvum* rootstocks tested could be suitable for mitigating the effects of Verticillium wilt in Mediterranean aubergine crops. Although the results are promising under controlled conditions, fields validation is necessary.

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