

# Pathogenicity and Aggressiveness of *Verticillium dahliae* Races from Non-Tomato Hosts towards Tomato Cultivars Possessing or not the *Ve* Gene

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## ABSTRACT

Race 2 strains are widely distributed, vary in aggressiveness and have rapidly supplanted race 1 strains in many tomato production areas in Tunisia and around the world. Based on the presence or not of Verticillium wilt symptoms on resistant (cv. 'Rio Grande') and susceptible (cv. 'Ventura') tomato cultivars, 8 *V. dahliae* isolates were typed as race 1, 7 isolates as race 2 and 1 isolate was non-pathogenic to tomato. Isolates from potato, artichoke, melon and olive belonged to *V. dahliae* race 2. Within the two *V. dahliae* races issued from non-tomato hosts, great variations in pathogenicity and aggressiveness were noted, as measured by leaf damage and plant height recorded 60 days post inoculation. All *V. dahliae* races isolates from tomato were aggressive to highly aggressive on eggplant cv. 'Bonica F1' but were non-pathogenic towards pepper cv. 'Chergui F1'.

**Keywords:** inoculation, leaf damage, *Lycopersicon esculentum* L., plant height, verticillium wilt

## INTRODUCTION

*Verticillium dahliae* Kleb. is a soil-borne fungus infecting more than 400 cultivated and weed plant species, worldwide, causing usually severe disease and substantial damage (Ligoxigakis 2009).

Physiologically and pathogenically distinct strains have been reported from a few hosts (Pegg and Brady 2002). However, most strains cannot be reliably differentiated morphologically (Dobinson *et al.* 1998). In fact, in a recent study, Jabnoun-Khiareddine *et al.* (2010a) have shown that, even when cultured on the same medium (PDA), huge morphological variability was noted within Tunisian *V. dahliae* isolates which have no relationship with their aggressiveness on tomato.

The differentiation of *V. dahliae* strains into race 1 and race 2 is based on their respective avirulence or virulence on tomato with *Ve*-mediated resistance (Bender and Shoemaker 1984).

In fact, wilt-resistant cultivars carry a single dominant gene coding for resistance (*Ve*), derived from a small-fruited, wild Peruvian cherry tomato (*Lycopersicon esculentum* var. *cerasiforme*: Gray Peru Wild) (Schaible *et al.* 1951), and that has been incorporated into most commercial cultivars. As early as 1957, however, *V. dahliae* isolates from California and Canada have been shown to induce typical disease symptoms (chlorosis, stunting and decreased yield) in cultivars carrying the *Ve* gene (Bender and Shoemaker 1984). Such isolates, named race 2 for their ability to overcome resistance conferred by the *Ve* gene, are now widely distributed, vary in aggressiveness (Pegg and Brady 2002), and have rapidly supplanted race 1 strains in many tomato production areas (Grogan *et al.* 1979; Ligoxigakis and Vakalounakis 1994; Dobinson *et al.* 2000; Pegg and Brady 2002).

In Tunisia, the appearance of *V. dahliae* race 2 in tomatoes has been described since 2006 (Daami-Remadi *et al.* 2006) and have been identified later in many tomato-growing regions. In fact, among a collection of 73 *V. dahliae* iso-

lates, recovered from susceptible and race 1-resistant cultivars, 44 were identified as race 2 and 29 as race 1, based on their pathogenicity towards differential tomato cultivars possessing or not the *Ve* resistance gene (Jabnoun-Khiareddine *et al.* 2007a). Furthermore, in a recent study, Jabnoun-Khiareddine *et al.* (2010b) have shown that under controlled conditions tomato cultivars, the most grown in Tunisia, exhibited varying degrees of susceptibility to Verticillium wilt (VW) ranging from moderate to high, as measured by leaf damage index and plant height. Moreover, when grown in a naturally *Verticillium*-infested soil (49 microsclerotia/g of soil), VW incidence, four months post-planting, was 100% for all the tested resistant cultivars. In fact, economic losses caused by this pathogen can reach 50% especially on tomato cultivars severely infected by *V. dahliae* (Jabnoun-Khiareddine *et al.* 2007a).

The origin(s) of race 2 have not been determined. The widespread cultivation of tomato cultivars with the *Ve* gene may have led to an increase in indigenous populations of race 2 isolates, or to the evolution of race 2 from either race 1 isolates or isolates not previously pathogenic to tomato (Grogan *et al.* 1979; Tjamos 1981; Dobinson *et al.* 1998; Pegg and Brady 2002).

In fact, *V. dahliae* is known for its widespread around the world; its longevity and survivability in the soil and its ability to infect a wide host range (Pegg and Brady 2002). This translates into plasticity and flexibility of the pathogen allowing it to infect, grow and develop in presence of its original or alternative hosts (Alkher *et al.* 2009). The pathogenicity of an isolate on one host does not predict which other host(s) may be susceptible to that isolate (Dobinson *et al.* 1998). In fact, although *V. dahliae* has a wide host range, it varies in its level of pathogenicity on different hosts (Bhat and Subbarao 1999). Isolates from one host are able to cause disease on other plant species but symptoms are often more severe on the host of origin. *V. dahliae* cross-pathogenicity was also reported from many hosts (Qin *et al.* 2006).

In Tunisia, *V. dahliae* has been obtained from different hosts other than tomato, such as potato, eggplant, artichoke, melon, cucumber, squash, pepper and olive tree (Jabnoun-Khiareddine *et al.* 2006, 2007b, 2008). However, despite the economical importance of *V. dahliae* race 2 in Tunisia, it is not known whether *V. dahliae* isolates originated from non-tomato hosts, are able to cause disease on tomato cultivars carrying or not the *Ve* gene and to what extent. This information is important in locations where the above mentioned crops are widely grown and are considered for rotation with tomato. Furthermore, such information, about the race 2 population, would benefit breeding programs seeking to incorporate race 2 resistance or tolerance into tomato, as it would facilitate the selection of appropriate race 2 strains for screening purposes.

The objectives of the present study were to: (i) determine the race composition of some Tunisian *V. dahliae* isolates originated from non-tomato hosts; (ii) study the aggressiveness of the *V. dahliae* physiological races towards two differential tomato cultivars; (iii) test the pathogenicity of representatives of two races on two hosts, eggplant and pepper.

## MATERIALS AND METHODS

### Fungal material

Twenty four single-spore isolates of *V. dahliae* originating from different hosts and various Tunisian regions were used in the present study (Table 1). Two Tunisian reference isolates, race 1 (Vd18) and race 2 (Vd6), were also included in this study (Table 1).

Pathogen isolates were cultured at 20°C on potato dextrose agar (PDA) medium amended with 300 mg/l of streptomycin sulphate (Pharmadrug Production GmbH, Hamburg, Germany).

Liquid cultures used for plant inoculation were prepared on potato dextrose broth (PDB) and incubated at 20°C under continuous agitation at 150 rpm during 4 to 5 days. The spore suspension concentration used was adjusted to 10<sup>7</sup> spores/ml by a Malassez cytometer. For their long term preservation, pathogen isolates were stored up to 12 months at -20°C in a 25% glycerol solution (Robb 2000).

### Plant material

Two tomato (*Lycopersicon esculentum* L.) cultivars, possessing or not the *Ve* gene of resistance to *V. dahliae* race 1, 'Rio Grande' (*Ve*) and 'Ventura' (*ve*), respectively, were used for race typing and for a comparison of aggressiveness in local *V. dahliae* isolates.

Eggplant (*Solanum melongena* L., cv. 'Bonica F1') and pepper (*Capsicum annuum* L., cv. 'Chergui F1') were used to study the pathogenicity of some tomato *V. dahliae* race 1 and 2 isolates, previously characterized physiologically in Jabnoun-Khiareddine *et al.* (2007a).

Tomato (commercial sources: RACI Sementi S.R.L. and Peto-seed), eggplant (Clause) and pepper (Vilmorin) seeds were superficially disinfected by immersion in absolute ethanol for 2

min, followed by extensive rinsing in sterile distilled water. Seeds were sown in alveolus plates filled with previously sterilized peat. Seedlings were grown in a growth chamber at 24-26°C with 12-h photoperiod. They were watered daily and fertilized twice a week with a standard nutrient solution according to Pharand *et al.* (2002).

### Race typing and aggressiveness of *V. dahliae* races

For race typing analysis, 16 Tunisian *V. dahliae* isolates issued from non-tomato hosts, together with the two reference race 1 and race 2 isolates from tomato, were used for inoculation of tomato plants belonging to both differential cultivars i.e. 'Ventura' (*ve*) and 'Rio Grande' (*Ve*).

Twenty days-old tomato seedlings were uprooted from the substrate; their roots washed in tap water, trimmed, and dipped in the conidial suspension for 30 min. Non-inoculated control seedlings were dipped in sterile distilled water. Seedlings were then transplanted, immediately after inoculation, to pots (diameter = 17 cm) filled with a mixture of peat and perlite (2/3: 1/3), previously sterilized at 107°C for 1 h and maintained in glasshouse cell, for 60 days at 10-32°C (minimal and maximal temperatures, respectively) and a 12-h photoperiod. They were watered regularly and fertilized with a nutritious solution when needed (Pharand *et al.* 2002).

Thirty days after inoculation, isolates were typed as race 2 if they caused disease on both cultivars, as race 1 if they produced disease symptoms only on cv. 'Ventura', and as NP (non-pathogenic to tomato) if they did not produce wilt symptoms on either cultivar.

The evaluation of isolates aggressiveness was assessed 60 days post inoculation (DPI) based on the records of leaf damage index (LDI) which is calculated according to 0-4 scale (Jabnoun-Khiareddine *et al.* 2007a), and plant height. Furthermore, for the leaf damage index, these values which indicate the different levels of isolate aggressiveness are grouped in classes (Jabnoun-Khiareddine *et al.* 2010a) as follows: 0.0-0.9 (weakly aggressive isolates); 1.0-1.9 (moderately aggressive isolates); 2.0-2.9 (aggressive isolates); 3.0-4.0 (highly aggressive isolates).

### Pathogenicity of *V. dahliae* race 1 and race 2 towards eggplant and pepper

Pathogenicity of some Tunisian *V. dahliae* race 1 and 2 isolates, collected from tomato plants grown in different geographical regions and with varying degrees of aggressiveness towards 'Ventura' and 'Rio Grande' were carried out using the root dip method on 30 days-old eggplant ('Bonica F1') and pepper ('Chergui F1') seedlings.

Seedlings were treated (inoculated or not), cultured and maintained in the same conditions as previously described for tomato.

The evaluation of VW severity was assessed 60 DPI based on the records of plant height and the LDI.

### Statistical analyses

Statistical analyses were performed, for all parameters measured, following either a completely randomized design where treatments (isolates or non-inoculated control) were the only fixed factor or following a completely randomized factorial design where tomato cultivars and fungal treatments (pathogen isolates and non-inoculated control) were the fixed factors. Five replicates (i.e. 5 plants) were used per elementary treatment.

Data were statistically analyzed by SPSS Software version 11 and subjected to analysis of variance and Fisher's least significant difference test, LSD at  $P \leq 0.05$ .

## RESULTS

### Race typing

The majority of *V. dahliae* isolates tested in the present study and which are issued from different hosts have induced typical VW symptoms 15 to 30 DPI, depending on

**Table 1** *Verticillium dahliae* isolates tested and their origins.

Isolates	Original plant hosts	Origins
Vd6, Vd13, Vd17, Vd18, Vd45, Vd95	Tomato	Sousse
Vd66	Tomato	Monastir
Vd72, Vd97	Tomato	Nabeul
Vd14, Vd81	Potato	Nabeul
Vd9, Vd23, Vd26	Potato	Sousse
Vd31	Potato	Monastir
Vd30	Potato	Bizerte
Vd64, Vd80	Melon	Sousse
Vd8	Eggplant	Monastir
Vd12	Eggplant	Sousse
Vd37, Vd98	Olive	Sousse
Vd67, Vd119, Vd120	Artichoke	Sousse

**Table 2** Origin and number of *V. dahliae* isolates and their pathogenicity towards two differential tomato cultivars.

Origin and number of isolates in each group	Pathogenicity <sup>a</sup>		Race typing
	'Ventura'	'Rio Grande'	
Potato (1)	-	-	Non-pathogenic
Potato (4); Eggplant (2)	+	-	Race 1
Artichoke (1); Olive (1)			
Potato (2); Melon (2); Artichoke (2); Olive (1)	+	+	Race 2

<sup>a</sup> Based on symptom development during a 4-week observation period. Cv. 'Ventura' was used as the susceptible (*ve*) and cv. 'Rio Grande' as the resistant (*Ve*) tomato cultivars.

isolates and tomato cultivars used. In fact, VW starts as yellow, V-shaped blotches that narrow at the leaf margins. This is accompanied, sometimes, by inner vein yellowing, which begins unilaterally. These yellow areas grow over time, turn brown, and then the leaf dies. Defoliation and reduction of the foliage size can also be observed.

Based on the presence or not of these symptoms on the two differential tomato cultivars, i.e. 'Ventura' (*ve*) and 'Rio Grande' (*Ve*), *V. dahliae* isolates were classified into three groups (Table 2).

Vd14, Vd23, Vd26 and Vd30, isolates from potato, Vd8 and Vd12, isolates from eggplant, Vd120 isolate from artichoke and Vd98 isolate from olive, all caused important disease symptoms on 'Ventura' but were non-pathogenic or slightly pathogenic on 'Rio Grande', and were defined as tomato race 1. However, the other isolates from potato (Vd9 and Vd81), artichoke (Vd67 and Vd119), melon (Vd64 and Vd80) and olive (Vd37) were pathogenic to both tomato cultivars and were typed as race 2. Only one isolate, Vd31, issued from potato, was non-pathogenic to tomato. Thus, out of the 16 tested isolates, issued from non-tomato hosts, 50% were typed as race 1 and 43.75% as race 2.

It is interesting to note that *V. dahliae* isolates, issued either from similar or different hosts, differ in their abilities to cause VW disease towards tomato cultivars. These pathogenic differences can be observed in the case of potato, artichoke and olive isolates which have behaved differently towards tomato cultivars. In fact, some isolates from potato

belonged to race 1, and others to race 2, while the other was non-pathogenic to tomato.

## Aggressiveness of *V. dahliae* race 1 and 2

### 1. Leaf damage index

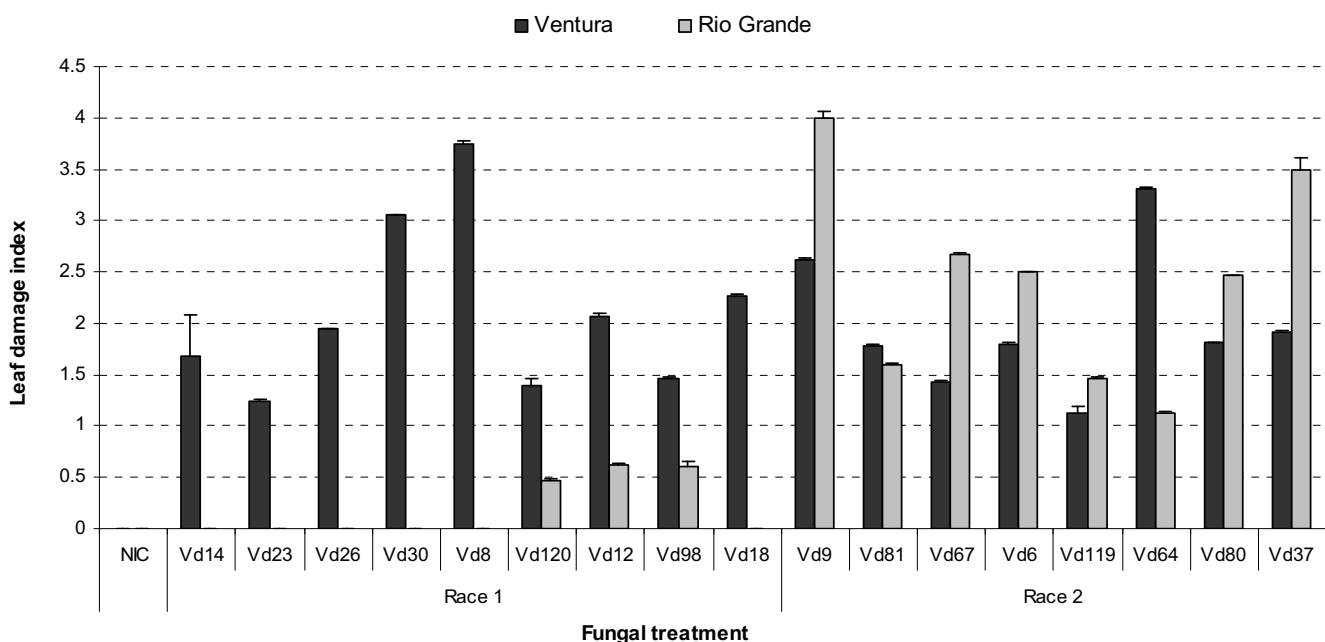
All tomato plants inoculated with *V. dahliae* race 1 and race 2 isolates showed typical VW symptoms. However, at the end of the assay i.e. 60 DPI, all tomato plants showed leaf symptoms of variable intensity depending on the tested tomato cultivars and the fungal treatments realized (Fig. 1); a significant ( $P \leq 0.01$ ) interaction was noted between both fixed factors. All inoculated tomato plants showed significantly greater LDI than the non-inoculated control plants which were symptomless.

As a group, race 1 isolates were almost as aggressive on the susceptible cultivar 'Ventura' (mean LDI  $\approx 2.1$ ) as race 2 isolates (mean LDI  $\approx 2$ ). On the average, race 2 isolates were more aggressive on the resistant cultivar 'Rio Grande' (mean LDI  $\approx 2.4$ ), than on the susceptible 'Ventura' (mean LDI  $\approx 2$ ). On 'Rio Grande', race 1 isolates caused no or slight symptoms.

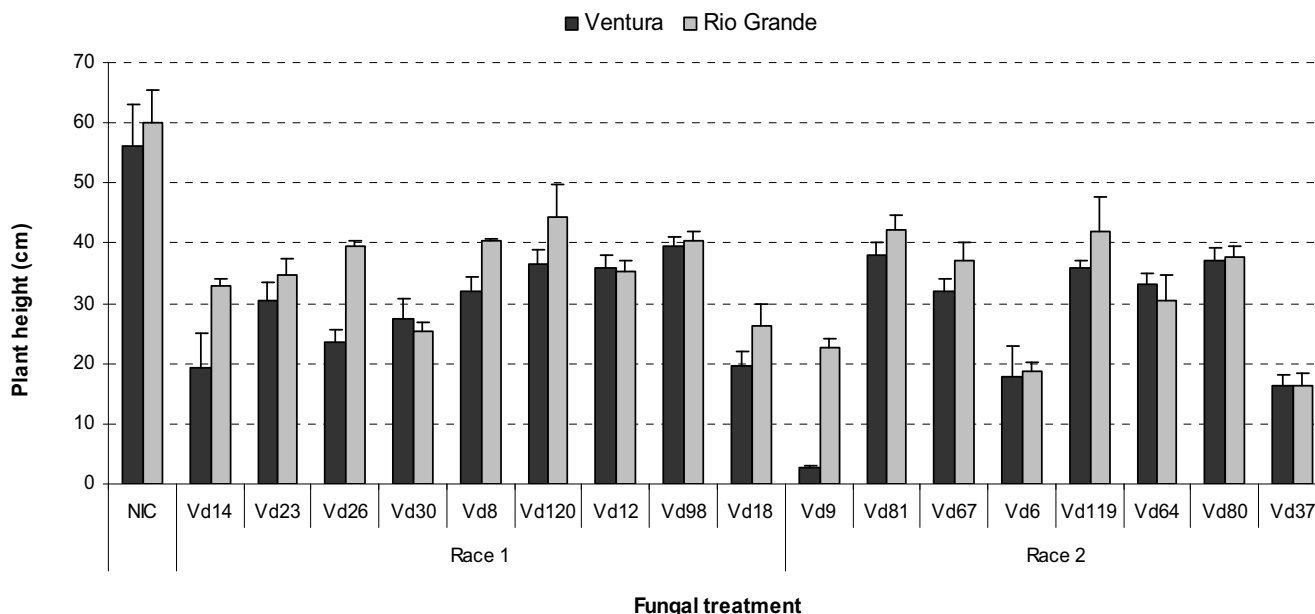
However, according to our experiment conditions, isolates inside both races differ in their aggressiveness from weak to moderate and high (Fig. 1). In fact, for race 1 isolates, LDI records varied from 0.47 noted on 'Rio Grande' plants inoculated with Vd120 to 3.75 noted on 'Ventura' plants inoculated with Vd8 issued from eggplant. For race 2 isolates, LDI records varied from 1.12 noted on 'Ventura' plants inoculated with Vd119, to 4, noted on 'Rio Grande' plants inoculated with Vd9, isolated from potato. These results reflect the presence of great variation in aggressiveness within *V. dahliae* species and even within its physiological races.

This aggressiveness variation within *V. dahliae*, as measured by LDI records on inoculated plants, seems not to be related to isolates hosts. This could be noted in the case of the two race 1 isolates issued from eggplant, Vd8 and Vd12, which were respectively highly and moderately aggressive towards 'Ventura'. In other cases, some race 1 isolates were moderately aggressive on 'Ventura', although they are issued from different hosts as Vd26, Vd120 and Vd98 issued respectively from potato, artichoke and olive.

It is also interesting to remark that three race 1 isolates



**Fig. 1** Leaf damage index noted 60 days post-inoculation on cvs. 'Ventura' and 'Rio Grande' plants inoculated with isolates of *V. dahliae* race 1 and race 2 compared to the non-inoculated control. (NIC) non-inoculated control;  $10^{\circ}\text{C} < T < 30^{\circ}\text{C}$ ; (Vd9, Vd14, Vd23, Vd26, Vd30, Vd31 and Vd81) *V. dahliae* isolates from potato; (Vd67, Vd119 and Vd120) isolates from artichoke; (Vd8 and Vd12) isolates from eggplant; (Vd64 and Vd80) isolates from melon; (Vd37 and Vd98) isolates from olive. LSD (Cultivars  $\times$  Fungal treatments interaction) = 0.1186 at  $P \leq 0.05$ .



**Fig. 2** Plant height noted 60 days post-inoculation on cvs. 'Ventura' and 'Rio Grande' plants inoculated with isolates of *V. dahliae* race 1 and race 2 compared to the non-inoculated control. (NIC) non-inoculated control; 10°C < T < 30°C; (Vd9, Vd14, Vd23, Vd26, Vd30, Vd31 and Vd81) *V. dahliae* isolates from potato; (Vd67, Vd119 and Vd120) isolates from artichoke; (Vd8 and Vd12) isolates from eggplant; (Vd64 and Vd80) isolates from melon; (Vd37 and Vd98) isolates from olive. LSD (Cultivars × Fungal treatments interaction) = 4.93 cm at  $P \leq 0.05$ .

from non-tomato hosts were as aggressive as, and even more aggressive than the reference race 1 isolate from tomato (Vd18), as for Vd12 and Vd8 issued from eggplant and Vd30, issued from potato.

Similar variation in aggressiveness was also noted within race 2 isolates, regardless of their origin. As an example, Vd81 and Vd119, issued respectively from potato and artichoke were moderately aggressive towards both tomato cultivars. Moreover, isolates from the same host, as Vd81 and Vd9, from potato, were respectively moderately to highly aggressive on 'Rio Grande'. Similarly, isolates from melon, Vd64 and Vd80, were moderately to aggressive on 'Rio Grande', and highly to moderately aggressive on 'Ventura', respectively.

As shown in **Fig. 1**, some race 2 isolates from non-tomato hosts, such as Vd9 and Vd37 isolates obtained from respectively potato and olive, were more aggressive than the reference race 2 isolate, Vd6, on both differential cultivars.

## 2. Plant height

Plant height, noted at 60 DPI, varied depending on tomato cultivars and fungal treatments realized; a significant ( $P \leq 0.01$ ) interaction was noted between both fixed factors. All inoculated tomato plants inoculated with *V. dahliae* race 1 and 2 isolates, showed significantly lesser height than the non-inoculated control plants, according to our plant inoculation and growth conditions (**Fig. 2**). In fact, a reduction as high as 95.37% has been noted on tomato 'Rio Grande' plants inoculated with Vd9 isolated from potato. The majority of *V. dahliae* isolates tested had reduced this growth parameter by more than 30% on both cultivars plants comparatively to non-inoculated control plants.

For race 1 isolates, reduction in plant height varied from 29.54 to 65.84% on 'Ventura' and from 26 to 58% on 'Rio Grande', while for race 2 isolates, this reduction in plant height ranged between 32.56 and 95.37% and between 29.67 and 72.67% on 'Ventura' and 'Rio Grande', respectively.

As shown in **Fig. 2**, variation was noted within *V. dahliae* races as for height reduction noted on inoculated plants, regardless of isolate's origin. In fact, as an example, Vd9 and Vd81 isolates from potato have occasioned height reduction of 95.37 and 32.56% on 'Ventura' and of 62.33% and 29.67% on 'Rio Grande' plants, respectively.

Moreover, *V. dahliae* isolates which have occasioned greater foliar symptoms, as Vd30, Vd8, Vd9, Vd37, have also caused important height reduction of both cultivars. This could translate the aggressiveness factors used by these pathogens to affect the expression of both foliar damage and aerial part elongation of tomato plants.

We also notice, that some *V. dahliae* isolates that were slightly pathogenic or non-pathogenic via LDI records, were able to affect the height development of inoculated plants (**Fig. 2**). In fact, this is the case of Vd14 and Vd23 which were non-pathogenic on 'Rio Grande' but have, in the contrary, reduced plant growth of the same cultivar by 45 and 42%, respectively.

## Pathogenicity of *V. dahliae* race 1 and race 2 isolates towards eggplant

### 1. Leaf damage index

All *V. dahliae* race 1 and race 2 isolates have induced on eggplant 'Bonica F1' plants VW symptoms which severity, as measured via LDI records, was significantly ( $P \leq 0.01$ ) higher than that of the non-inoculated control plants which have remained symptomless (**Fig. 3**).

All race 1 and race 2 isolates were aggressive to highly aggressive on eggplant. In fact, LDI records noted on plants inoculated with race 1 isolates were not significantly different from those caused by race 2 isolates. However, on average, race 2 isolates were more aggressive (LDI = 3.53) than race 1 isolates (LDI = 3.18) on 'Bonica F1' plants. Race 2 isolate Vd66 from tomato was the most aggressive (LDI = 4).

### 2. Plant height

Apart from leaf damage, height of eggplant plants inoculated with *V. dahliae* isolates was significantly reduced ( $P \leq 0.01$ ), compared with the non-inoculated control plants (**Fig. 4**). This height reduction has even exceeded 90% on plants inoculated with Vd66 isolate.

As shown in **Fig. 4**, race 2 isolates were, on average, more aggressive than race 1 isolates on 'Bonica F1' plants, causing a mean height reduction of 72.75%, against 58.25% caused by race 1 isolates. This height reduction caused by race 1 varied from 43.07 to 70.40% and from 58.25 to 90.51%, for race 2 isolates. This reflects the presence of

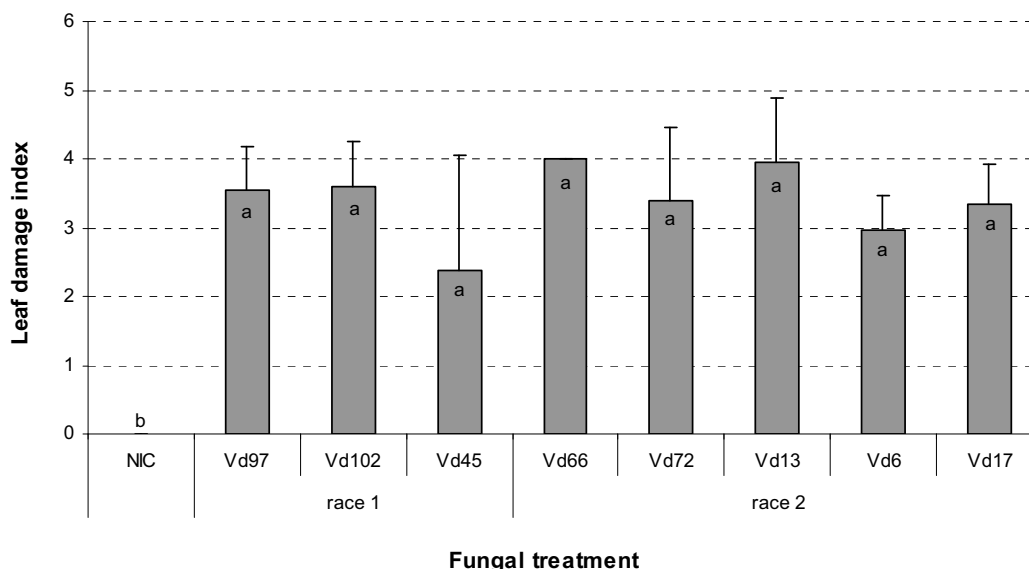


Fig. 3 Leaf damage index noted 60 days post-inoculation on cv. 'Bonica F1' plants inoculated with isolates of *V. dahliae* race 1 and race 2 compared to the non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ( $P \leq 0.05$ ); (NIC) non-inoculated control;  $10^{\circ}\text{C} < T < 30^{\circ}\text{C}$ ; (Vd97-Vd17) *V. dahliae* isolates from tomato.

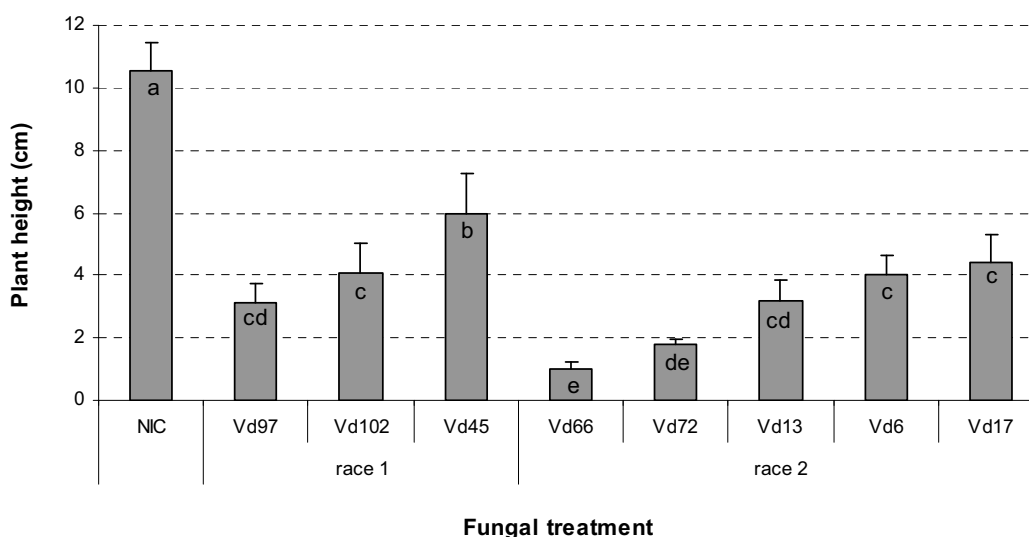


Fig. 4 Plant height noted 60 days post-inoculation on cv. 'Bonica F1' plants inoculated with isolates of *V. dahliae* race 1 and race 2 compared to the non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ( $P \leq 0.05$ ); (NIC) non-inoculated control;  $10^{\circ}\text{C} < T < 30^{\circ}\text{C}$ ; (Vd97-Vd17) *V. dahliae* isolates from tomato.

variations within *V. dahliae* races, despite their common host of origin, which is tomato. In fact, significant differences were noted concerning the height of plants inoculated with some isolates within each race, as race 2 isolates Vd6 and Vd66 and race 1 isolates, Vd45 and Vd102.

It is also to note that within each race, isolates causing greater leaf damage have also caused greater height reduction, according to our inoculation and plant growth conditions.

All *V. dahliae* isolates were successfully re-isolated on PDA, from all inoculated plants.

#### Pathogenicity of *V. dahliae* race 1 and race 2 isolates towards pepper

No foliar symptom were observed on inoculated pepper 'Chergui F1' plants, neither on non-inoculated control plants, 60 DPI, according to our inoculation and plant growth conditions. Race 1 and 2 isolates were non-pathogenic towards pepper and were not also able to cause any significant reduction in inoculated plant height comparatively to the control plants.

## DISCUSSION

The present study emphasis, for the first time in Tunisia, the pathogenicity as well as the aggressiveness of *V. dahliae* isolates from non-tomato hosts towards resistant ('Rio Grande') and susceptible ('Ventura') tomato cultivars, as measured by leaf damage and plant height.

Based on the presence or not of VW symptoms on the two differential tomato cultivars, 8 *V. dahliae* isolates were typed as race 1, 7 isolates as race 2 and 1 isolate was non-pathogenic towards tomato. Within each race, isolates were originated from different hosts. In fact, despite the relatively small population tested, pathogenic variation has been noted. Among 7 isolates issued from potato, 4 were identified as race 1, 2 as race 2 and 1 was non-pathogenic to tomato. This pathogenic variation has been demonstrated within host adapted subpopulations of *V. dahliae*, including those pathogenic on cotton, potato and tomato (Puhalla and Bell 1981; Powelson and Rowe 1993; Daayf *et al.* 1995). Indeed, Uppal *et al.* (2007) have suggested three generators of variability in order to explain the high level of variability in pathogenicity observed among 43 *Verticillium* spp. isolates originating from infected potato tubers. In fact, it may

be the result of (i) the ability of isolates to produce pathogenicity factors, such as toxins which are known for their local effect in inducing necrosis that can lead to wilting, as well as for their abilities to trigger host defense mechanisms (Zhen and Li 2004); (ii) the ability to exchange genetic material between isolates from the same vegetative compatibility group (VCG), which can lead to increasing the genetic diversity within and among populations (Bhat *et al.* 2003; Bellahcene *et al.* 2005); (iii) the multi-host characteristic of the species (Bhat and Subbarao 1999; Pegg and Brady 2002).

In the present study, isolates collected from potato, artichoke, melon and olive belonged to *V. dahliae* race 2. In this sense, Ligoxigakis and Vakalounakis (1994) found that among 92 *V. dahliae* isolates originated from 12 host species belonging to different botanical families, one isolate, issued from cucumber, was identified as race 2. Similarly, Dobinson *et al.* (1996) mentioned that *V. dahliae* isolates from potato were also identified as race 2.

In a recent study, Ligoxigakis (2009) mentioned that out of 105 isolates of *V. dahliae* tested, 67 were identified as race 2. Among these isolates, 50 were obtained from 21 cultivated species: bean, broccoli, cabbage, cauliflower, chickpea, chicory, cucumber, eggplant, endive, melon, ochrus pea, olive tree, pepper, potato, Romaine lettuce, radish, summer squash, marigold, tomato, vetch, and watermelon, as well as 17 isolates obtained from 9 weed species proved to belong to race 2. Among these plant species, Ligoxigakis (2009) has identified 20 worldwide new hosts of *V. dahliae* race 2.

In the present study, potato, melon and olive tree are identified for the first time in Tunisia, as new hosts of *V. dahliae* race 2, whereas artichoke may join the new hosts of race 2 in Tunisia as well as in the world. The ability of these isolates from non-tomato hosts to infect both susceptible and resistant tomato cultivars is not surprising as *V. dahliae* is known for its high level of diversity and pathogenic variability (Pegg and Brady 2002; Alkher *et al.* 2009). The recent studies of Alkher *et al.* (2009) revealed that *V. dahliae* can quickly adapt to host defenses and increase its pathogenicity on either the original or alternative hosts. In fact, Shittu *et al.* (2009) mentioned that historically, *Verticillium* spp. have proven very adept at expanding host range by moving onto new plant species.

Giving that in Tunisia, tomato is frequently grown in short rotations with potato, melon, artichoke and other crops, and that this crop is sometimes intercropped with olive trees, tomato weakly aggressive isolates will be able to gain pathogenicity on tomato. In fact, changes in pathogenicity among fungi can be gradual in their appearance, relatively stable and genetic in nature or sudden and cyclic (Alkher *et al.* 2009).

In a recent study, Daami-Remadi *et al.* (2011) have shown that Vd18 which is a race 1 isolate issued from tomato, as well as isolates from melon, artichoke and olive (Vd80, Vd69 and Vd37, respectively) were aggressive to highly aggressive towards potato 'Spunta' plants. Moreover, isolates from potato, Vd14, Vd23 and Vd30 which are aggressive to highly aggressive to potato 'Spunta' plants (Daami-Remadi *et al.* 2011), have been shown, in the present study to be moderately to highly aggressive to susceptible tomato 'Ventura' plants. In fact, this *V. dahliae* cross-pathogenicity was already documented on several hosts including cotton, mint, lettuce, broccoli, cabbage, cauliflower, strawberry, artichoke, bell and chili pepper, eggplant, potato, tomato, and watermelon (Bhat and Subbarao 1999; Pegg and Brady 2002; Qin *et al.* 2006; Alkher *et al.* 2009).

Tjamos (1981) suggested that this broadening of host range could take place in the field after introduction of tomato cultivation. Moreover, isolates of monoculture are generally less virulent whereas those of multiculture are very virulent; it was possible to obtain isolates with mixture of genotypes or different pathotypes of *V. dahliae* from areas with diversified cropping systems (Tjamos 1981). In fact, many parameters which define every region (crops, rotations, monoculture, etc.) may intervene in genetic diver-

sity of *V. dahliae* population (Korolev *et al.* 2000).

Furthermore, *V. dahliae* isolates from olive were able to cause disease on susceptible as well resistant tomato cultivars, according to our inoculation and plant growth conditions. In the same sense, Tjamos (1981) and Cherrab *et al.* (2002) showed that the isolates from olive were pathogenic on a susceptible variety of tomato. This finding should be taken into consideration in breeding programs against VW of olive. In fact, VW of olive has been reported in Tunisia since 2006 (Triki *et al.* 2006). Thus, in order to restrict its distribution, the intercropped crops in olive orchards could play an important role in reducing level of inoculum in the soil and cut down the occurrence of new and may be higher virulent strains of *V. dahliae* in olive orchards.

Within the tomato race 2 groups, some variation in disease severity on tomato and eggplant has been noted. It is important to note that this variability observed among *V. dahliae* isolates was based on disease incidence evaluated as wilting symptoms together with its effect on plant height. In fact, *V. dahliae* isolates are known to vary in pathogenicity as well as in virulence (Tjamos 1981; Davis and Everson 1986; Strausbaugh 1993; Davis *et al.* 1994; Resende *et al.* 1994; Daayf *et al.* 1995; Tsrer *et al.* 2001; Vallad *et al.* 2006; Uppal *et al.* 2007). Similar observations have been noted within Tunisian and Canadian *V. dahliae* race 2 isolates from tomato (Dobinson *et al.* 1998; Jabnoun-Khiaredine *et al.* 2010b) and for isolates of *V. dahliae* pathogenic on potato, cotton and lettuce (Asworth 1983; Joaquim and Rowe 1991; Vallad *et al.* 2006). In fact, regardless of the race, a continuum of virulence exists among isolates of *V. dahliae* pathogenic on tomato, suggesting that the genetic control of virulence may differ from that controlling race specificity (Grogan *et al.* 1979; Tjamos 1981; Pegg and Brady 2002). Indeed, based on these observations, it has been suggested that despite their common ability to cause disease on tomato cultivars carrying the *Ve* resistance gene, race 2 isolates from geographically separate regions and even within one geographical site, may be genetically distinct (Tjamos 1981; O'Garro and Clarkson 1988; Baergen *et al.* 1993). Thus, results of our study may reveal genetic variation within the Tunisian race 2 population, which may demonstrate that this race type has multiple origins. In fact, correlations between pathogenicity (pathotypes) and Vegetative Compatibility Groups (VCGs) on certain hosts have been reported for *V. dahliae* (Joaquim and Rowe 1991; Daayf *et al.* 1995). Isolated VCGs act, in fact, as genetically isolated lineages that can evolve divergent pathogenicity and vegetative viability traits (Leslie 1993). In a recent study, Korolev *et al.* (2009) showed that *V. dahliae* race 1 isolates were assigned to vegetative compatibility groups VCG2A and VCG2B while race 2 isolates were assigned to VCG4B and these results are similar to those of Dobinson *et al.* (1998). However, Nagao *et al.* (1997) found that Japanese isolates of *V. dahliae* race 2 were vegetatively closely related with those of *V. dahliae* race 1 suggesting that these race 2 isolates derived from those of *V. dahliae* race 1. Thus, Vegetative or heterokaryon compatibility is a powerful tool to assign the natural populations of fungi into subgroups based on their genetic diversity (Sanei *et al.* 2008).

In the present study, *V. dahliae* race 2 isolates, as well as race 1 isolates, were aggressive to eggplant 'Bonica F1' but were non-pathogenic towards pepper 'Chergui F1'. In fact, recently, Korolev *et al.* (2009) mentioned that race 2 isolates were all aggressive to eggplant. Similarly, Nagao *et al.* (1997) found that all race-2 isolates severely infected eggplant, but disease severity was significantly different between isolates and that, on the contrary, none of the race-2 isolates affected foliar symptoms on sweet pepper. In fact, according to Vigouroux (1971), eggplant and pepper, respectively, can constitute a preferential and an occasional host for *V. dahliae*. Isolates from different hosts were also shown to be highly virulent on eggplant (Ciccarase *et al.* 1987).

Even if each group of isolates would be more aggressive on its original host, it caused various levels of infection

on the alternate host. Indeed, several studies have reported variability in the ability of *V. dahliae* isolates, collected from one host species, to infect other host species (Tjamos *et al.* 2000; Mercado-Blanco *et al.* 2004). This suggests that potato, melon, artichoke, eggplant, olive or other hosts are potential reservoirs for both *V. dahliae* races and in any case should not be considered in a rotation sequence, especially where tomato follows these crops. In this case, other biological control methods including the use of indigenous antagonists (Jabnoun-Khiareddine *et al.* 2009a, 2009b), in combination with other control measures such as solarization, may be more efficient in controlling this disease.

In conclusion, our study showed that *V. dahliae* is characterized by a high pathogenic flexibility; however, little is known about the genetics behind local *V. dahliae* populations. Additional studies are needed to better characterize the level of genetic variation within the local *V. dahliae* populations.

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