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Comparative Susceptibility of Potato Cultivars to Verticillium Wilt Assessed via Wilt Severity and Subsequent Yield Reduction

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ABSTRACT

Experiments were conducted to investigate the relative susceptibility to Verticillium wilt (VW) of 10 local potato cultivars grown under greenhouse conditions and artificially inoculated with a mixture of *Verticillium dahliae* isolates in comparison with non-inoculated controls. VW effects were evaluated, 60 days post inoculation, via foliar symptoms intensity, plant growth and production parameters. In fact, for all cultivars pooled, the mean plant height was reduced by 20% whereas the weight of the aerial part and tuber were reduced by about 21 and 32%, respectively on inoculated compared with non-inoculated control plants. However, the response to *Verticillium* infection i.e. foliar symptoms, stunting effect and the resulting stem weight and yield reduction varied among cultivars. Foliar symptoms and reduction in yield were considered to be criteria in determining the degree of susceptibility to the pathogen. When comparisons are made based on foliar symptoms, all the tested cultivars exhibited varying degrees of susceptibility ranging from moderate to high. However, when based on yield reductions, 'Tango' and 'Elodie' were the only cultivars which had the lowest yield reductions and were classified as tolerant cultivars. The importance of the results, in relation to the scoring systems used for the assessment of cultivar's behavior against *V. dahliae*, is discussed.

Keywords: inoculation, leaf damage, plant growth, *Solanum tuberosum* L., scoring systems, tuber weight, Tunisia

INTRODUCTION

In potato production areas, premature plant death, declining tuber yields and diseased tubers are major problems caused by Verticillium field wilt (Powelson and Rowe 1993; Robinson *et al.* 2007). Verticillium wilt (VW) is one of the most important yield-limiting diseases in potato production. The main symptoms are plant stunting and unilateral foliar chlorosis and necrosis, wilting and desiccation, followed by early dying (Tsror (Lahkim) *et al.* 1990). Symptom development has been correlated with tuber yield decreases of up to 50% with some susceptible cultivars (Riedel and Rowe 1985) and even with the use of tolerant cultivars, 20-30% yield losses may occur if proper control measures are not utilized (Nachmias and Krikun 1985).

Verticillium spp. are widely distributed in most potato production soils because the pathogen is readily carried on seed potatoes and increased with regular cropping (Mace *et al*. 1974). The importance of infected seed potato stocks in disseminating *Verticillium* is well known. Furthermore, the potato vine is one of the best host-plant substrates for production of microsclerotia. The potato crop, thus, acts doubly in both the introduction and increase of this pathogen (Nachmias and Krikun 1985).

Integrated crop management systems are necessary to limit the losses caused by VW (Powelson and Rowe 1993). Rotation is one of the major components of VW management. However, many field crops and vegetables are susceptible to the pathogens causing this disease. In addition, *V. dahliae*'s resting structures (microsclerotia) can survive in the soil for more than 10 years (Keinath *et al*. 1991; Easton *et al*. 1992; Hawke and Lazarovits 1994; Tjamos and Fravel 1995; Heale and Karapapa 1999). Moreover, VW is difficult to control because of its broad host range, and the inefficacy of fungicides on pathogen once it reaches the xylem (Fradin and Thomma 2006).

Strategies to control VW mainly involve reduction of inocula in the soil and (or) the deployment of resistant cultivars (Larena *et al*. 2003). However, cultivars carrying resistance to VW are scarce (Rowe and Powlsen 2002; Larena *et al*. 2003), and the high pathogenic variability among *Verticillium* populations (Dobinson *et al*. 2000) render their resistance inefficient when the conditions are conducive for disease expression. Nevertheless, host resistance is still thought to be the most promising method of managing VW (Bae *et al*. 2008). Most widely grown potato cultivars are susceptible or moderately resistant to this disease (Mpofu and Hall 2002). In fact, cultivars 'Superior', 'Kennebec', 'Red Pontiac', 'Sebago', 'Irish Cobbler', and 'Norland' were most frequently infected (Slattery and Eide 1980; Alkher *et al*. 2009). However, a few commercial cultivars such as 'Ranger Russet,' 'Ranger Nugget,' 'Reddale' and 'Defender' are reported to have moderate levels of resistance to VW but these cultivars have not replaced the most widely grown varieties (Rowe and Powelson 2002; Frost *et al*. 2006; Alkher *et al*. 2009).

V. dahliae occurs in most agricultural soils, mainly because most soils have a potato cropping history (Ispahani *et al.* 2008). During a potato crop, large numbers of *V. dahliae* microsclerotia are formed on the senescing potato stems and petioles (Mol *et al*. 1996). In Tunisia, it has been hypothesized that potato has been important in the history of most Tunisian isolates of *V. dahliae*, including ones isolated from other crops, particularly race 2 isolates (Daami-Remadi *et al*. 2006). This hypothesis was supported by the cross-pathogenicity reported on artichoke, bell pepper, broccoli, cabbage, cauliflower, chili pepper, cotton, eggplant, mint, lettuce, potato, strawberry, tomato, and watermelon (Qin *et al*. 2006).

In Tunisia, the behaviour of cultivars against Verti-

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cillium wilt is still unknown although cv. 'Spunta' usually yielded pathogen infection and exhibited wilt symptoms (Daami-Remadi *et al.* 2011). As cultivars used are quite specific to each country due to their aptitude to adaptation to local environmental conditions, conclusions on the behaviour of potato cultivars reported in others countries are not applicable in Tunisia. Consequently, information on comparative susceptibility of most cultured local potato cultivars will help growers make informed decisions regarding the management of this disease. Thus, due to the genetic diversity, lack of host specificity and pathogenic variability reported within *Verticillium* populations on several plant species (Strausbaugh 1993; Resende *et al.* 1994; Daayf *et al.* 1995; Bhat and Subbarao 1999; Dobinson *et al.* 2000; Tjamos *et al.* 2000; Mercado-Blanco *et al.* 2004; Daami-Remadi *et al.* 2011), the assessment of cultivar behaviour using a mixture of pathogen isolates (i.e. mimicking field conditions) is more appropriate to predict field resistance and to estimate the expected yield losses depending on cultivars.

MATERIALS AND METHODS

Plant material

Potato (*Solanum tuberosum* L.) seed tubers, belonging to 10 cultivars subscribed in the list A of the Tunisian varietal assortment, were tested and they were kindly provided by the Technical Center of Potato, Essaïda, Tunisia. Before use, tubers were superficially disinfected with a 10% sodium hypochlorite solution during 5 min, rinsed with tap water and air dried. They were kept under 15-20°C, 60-80% relative humidity and natural room light for pre-germination.

At the multi-germ stage, tubers were planted in plastic pots (25 cm diameter) containing a mixture of peat and perlite (2: 1), previously sterilized at 110°C for 1 h. After emergence, plants were watered every 2-3 days, depending on environmental conditions and plant's needs, until inoculation date.

Pathogen

Nine single-spore isolates of *V. dahliae* were used in the present study. They were isolated from diseased potato plants showing wilt symptoms and vascular discoloration removed from different geographical sites (**Table 1**). Their level of aggressiveness was assessed in a previous study (Daami-Remadi *et al.* 2011).

Table 1 Origins of *Verticillium dahliae* isolates tested and aggressiveness level on cv. 'Spunta'.

Isolate	Original host	Geographic origin	Aggressiveness level
Vd14	Potato	Nabeul	HA
Vd20	Potato	Sousse	HA
Vd23	Potato	Sousse	A
Vd28	Potato	Sousse	HA
Vd29	Potato	Sousse	HA
Vd30	Potato	Bizerte	MA
Vd31	Potato	Monastir	A
Vd57	Potato	Nabeul	MA
Vd89	Potato	Jendouba	A

* **HA:** Highly aggressive (LDI ≥3); **A:** Aggressive (LDI: 2-2.9); **MA:** Moderately aggressive (LDI: 1-1.9) according to Daami-Remadi *et al.* (2010).

They were cultured at 20°C on potato dextrose agar (PDA) medium amended with 300 mg/l of streptomycin sulphate (Pharmadrug Production Gmbh-Hambourg, Germany). Liquid cultures used for substrate inoculation were prepared on potato dextrose broth (PDB) and incubated at 20°C under continuous agitation at 150 rpm during 4 to 5 days. For their long term preservation, pathogen isolates were stored up to 12 months at -20°C in a 40% glycerol solution.

A mixture of isolates was used for plant inoculation and the mixed spore suspension obtained was adjusted to 10^7 spores/ml by a Malassez cytometer.

Potato inoculation and culture conditions

Ten days post-emergence of potato plants, inoculation was conducted by watering each potted plant with 100 ml of a conidial suspension (10^7 conidia/ml). Non-inoculated control (NIC) plants were watered with only 100 ml of sterile distilled water. Plants were placed under greenhouse conditions where temperatures ranged between 11 and 35°C (minima and maxima, respectively)

During all experimentation, plants were watered regularly, fertilized with a nutrient solution (20 N: 20 K_2O: 20 P_2O_5) (Manici and Cerato 1994) and kept clean from aphids and other pests that may interfere with VW symptom assessment.

VW severity was assessed, 60 days post-inoculation (DPI), based on both external and internal symptoms. In fact, the leaf damage index (LDI) was noted according to a 0-4 scale depending on symptom severity on leaves as previously described in Jabnoun-Khiareddine *et al.* (2006). This assessment was done for each stem individually and the mean for each plant was recorded.

Based on LDI records, cultivars were classed as follows: LDI: 0-0.9: Moderately resistant (MR), LDI: 1-1.9: Moderately susceptible (MS), LDI: 2-2.9: Susceptible (S), LDI ≥3: Highly susceptible (HS).

The effects of inoculations were also evaluated via some plant growth and production parameters. In fact, the length of all stems from the ground level was measured and the average per plant was used to calculate the mean height. However, for stems and tubers, the total weight per plant was recorded.

Based on yield reduction, compared to the non-inoculated controls, cultivars tested were also classified according to the method used by Susnoschi *et al.* (1976) as follows: Yield reduction ≤20%: Tolerant (T); Yield reduction: 20-40%: Susceptible (S); Yield reduction >40: Highly susceptible (HS).

Statistical analyses

Statistical analyses were performed, for all parameters measured, following a completely randomized factorial design where potato cultivars and treatments (inoculated or non-inoculated control) are both fixed factors. Five replicates were used per elementary treatment (i.e. per cultivar and per fungal treatment) and means were separated using Fisher's protected LSD test (at $P \leq 0.05$).

The relationships between LDI and plant height, stem weight and tuber weight were compared using Pearson's correlation analysis.

RESULTS

Verticillium wilt severity

Typical symptoms of VW were observed, 15 to 20 DPI, on all inoculated potato plants independent of the cultivar. However, at the end of the assay i.e. 60 DPI, the intensity of leaf symptoms was variable depending on cultivars and treatments tested (**Fig. 1**); a significant interaction was recorded between both fixed factors (at $P \leq 0.05$).

In fact, none of the cultivars tested showed LDI values less than 1 and consequently, no resistant cultivar was found among the plant material tested and according to our experiment conditions. 'Alaska' (**Fig. 2**), 'Timate', 'Atlas' and 'Tango' showed the highest LDI records of more than 3 and they were, thus, categorized as highly susceptible on the basis of leaf damage intensity occasioned by pathogen inoculation. However, 'Mondial' (**Fig. 2**) exhibited the least wilt severity (LDI of about 1.2) and was classed as moderately susceptible whereas the remaining cultivars were found to be susceptible to VW disease (LDI ranged between 2 and 3).

Nevertheless, on 'Tango', 'Arinda', 'Spunta', 'Daisy', 'Elodie' and 'Safrane' plants, weak wilt symptoms (LDI < 1) were also noted on non-inoculated control plants and were probably due to a latent infection of seed tubers but the LDIs recorded on inoculated plants were greater.

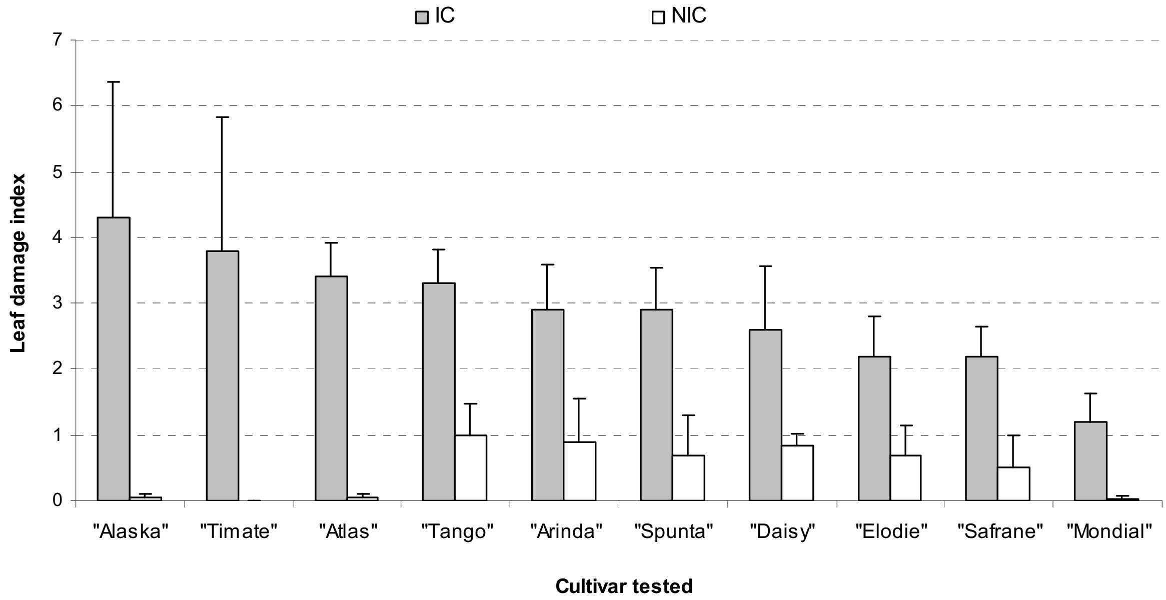

Fig. 1 Leaf damage index noted, 60 days post-inoculation, on potato plants of different cultivars inoculated with *V. dahliae* compared to the non-inoculated controls. (IC) inoculated with a mixture of nine *V. dahliae* isolates; (NIC) non-inoculated control; 11 < T < 35°C.

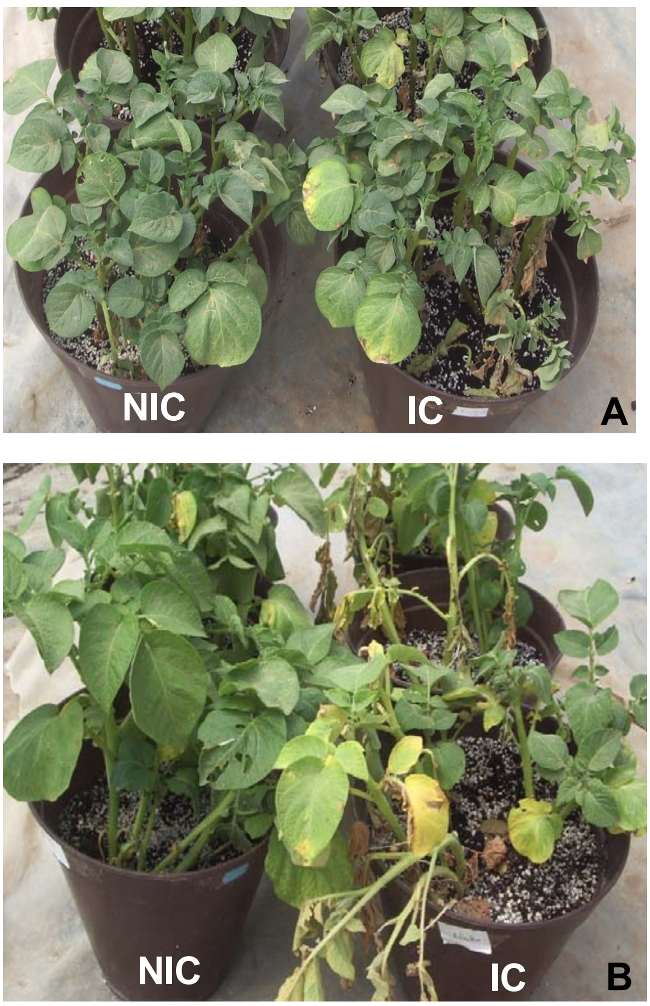

Fig. 2 Early dying symptoms observed, 60 days post-inoculation, on potato plants of 'Mondial' (A) and 'Alaska' (B) inoculated with *V. dahliae* compared with the non-inoculated control plants. (IC) inoculated with a mixture of nine *V. dahliae* isolates; (NIC) non-inoculated control; 11 < T < 35°C.

Plant height

The mean plant height, noted at 60 DPI, was significantly (at $P \leq 0.05$) related to cultivars and fungal treatment, independently as no significant interaction was noted between both fixed factors.

Fig. 3 indicated that the highest plant height (of about 25 cm) was recorded on 'Elodie', 'Daisy' and 'Safrane' whereas the lowest (15 cm) was noted on 'Arinda'. The other cultivars had intermediate records (height ranging between 15 and 20 cm).

It is important to note that cultivars showing greater plant height were shown to be susceptible via LDI records (comprised between 2 and 2.9). Moreover, plants of significantly comparable LDI records were found to have significantly different heights. For example, 'Alaska' and 'Mondial', classed as highly and moderately susceptible, respectively via LDI records, had significantly similar plant heights.

Although plant height, for all cultivars and fungal treatments combined, was found to be negatively correlated to LDI ($R = -0.422$, $P \leq 0.01$), the intensity of response to pathogen inoculation was variable depending on cultivars used.

Data shown in **Fig. 4** indicated that, for all cultivars pooled, the mean plant height was reduced by 20% on inoculated compared with non-inoculated plants showing the adverse effects of *V. dahliae* on this growth parameter.

Aerial part weight

The aerial part weight, noted at 60 DPI, depended significantly (at $P \leq 0.05$) on cultivars and fungal treatments tested, independently; the interaction between both fixed factors was insignificant.

Fig. 5 shows that the highest aerial part weight, for all fungal treatments combined, was noted on 'Safrane' plants (~75 g) and to a lesser degree o 'Atlas', 'Elodie' and 'Mondial' (50-60 g) whereas the lowest weight was recorded on 'Timate' plants.

It is important to note that cultivars showing the greater weight of the aerial part were previously shown to be susceptible or highly susceptible based on LDI records. Furthermore, plants of significantly comparable LDI records were found to have significantly different stem weights as is the case of 'Atlas' and 'Timate'. Moreover, 'Alaska' and 'Mondial', classed as highly and moderately susceptible, respectively via LDI records, showed significantly similar

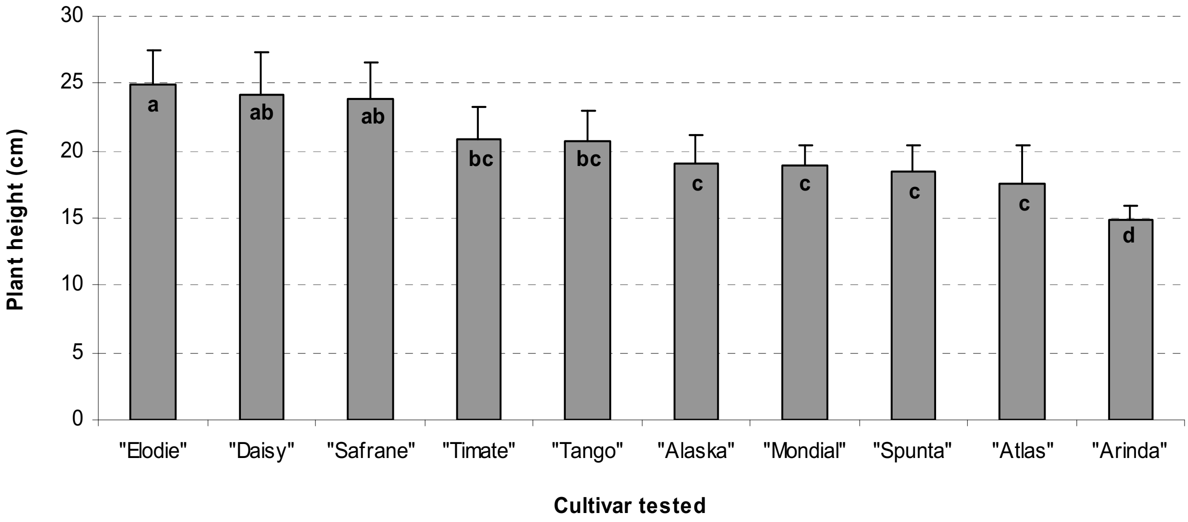

Fig. 3 Plant height recorded, 60 days post-inoculation, on the 10 potato cultivars tested. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($P \leq 0.05$). For each potato cultivar, data presented is the mean height for inoculated and non-inoculated plants; 11 < T < 35°C.

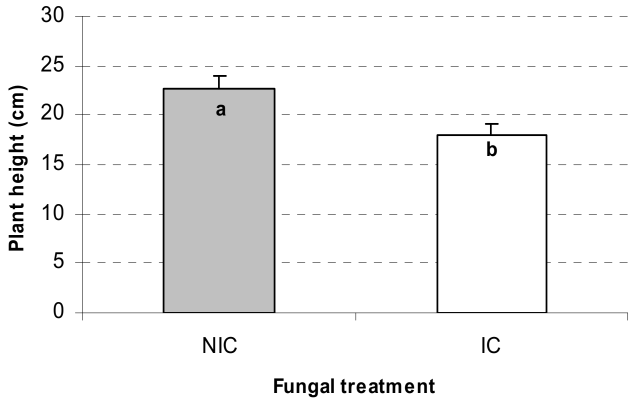

Fig. 4 Plant height recorded, 60 days post-inoculation, on potato plants (all cultivars pooled) inoculated with *V. dahliae* compared with the non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($P \leq 0.05$); (IC) inoculated with a mixture of nine *V. dahliae* isolates; (NIC) non-inoculated control; 11 < T < 35°C.

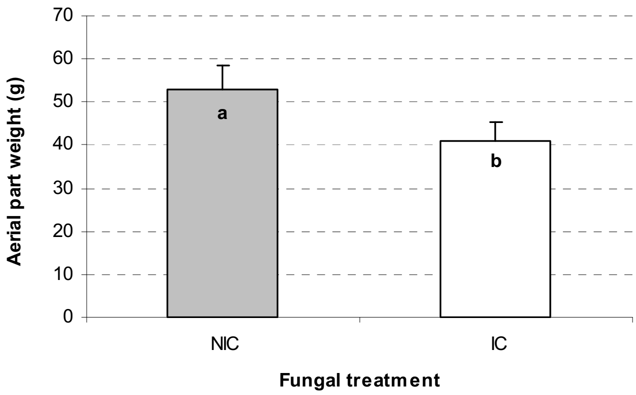

Fig. 6 Aerial part weight recorded, 60 days post-inoculation, on potato plants (all cultivars pooled) inoculated with *V. dahliae* compared with the non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($P \leq 0.05$); (IC) inoculated with a mixture of nine *V. dahliae* isolates; (NIC) non-inoculated control; 11 < T < 35°C.

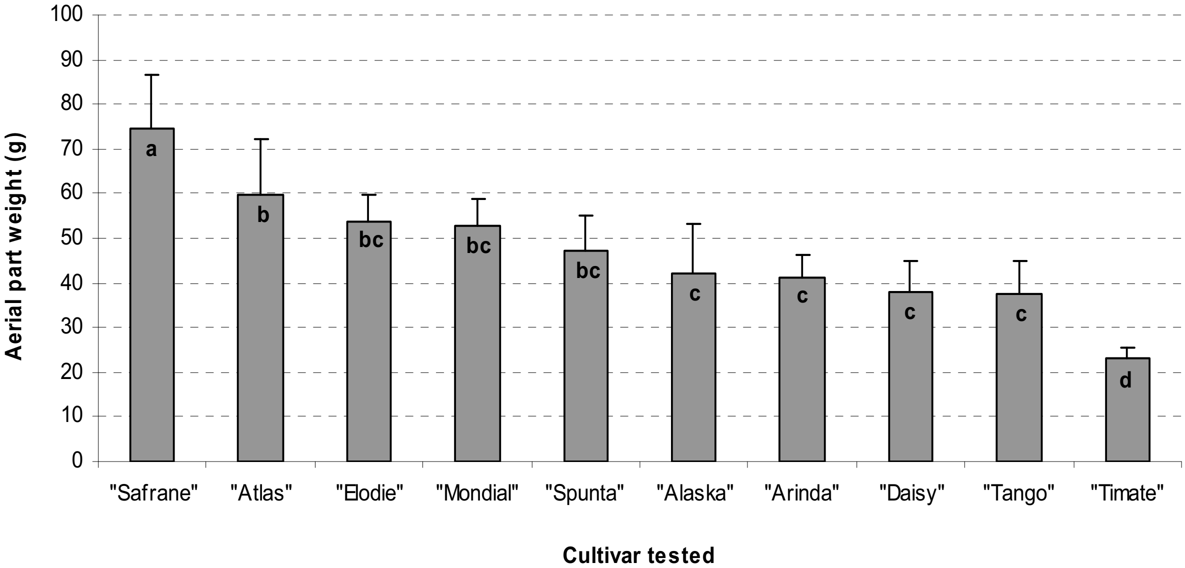

Fig. 5 Aerial part weight recorded, 60 days post-inoculation, on the 10 potato cultivars tested. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($P \leq 0.05$). For each potato cultivar, data presented is the mean weight for inoculated and non-inoculated plants; 11 < T < 35°C.

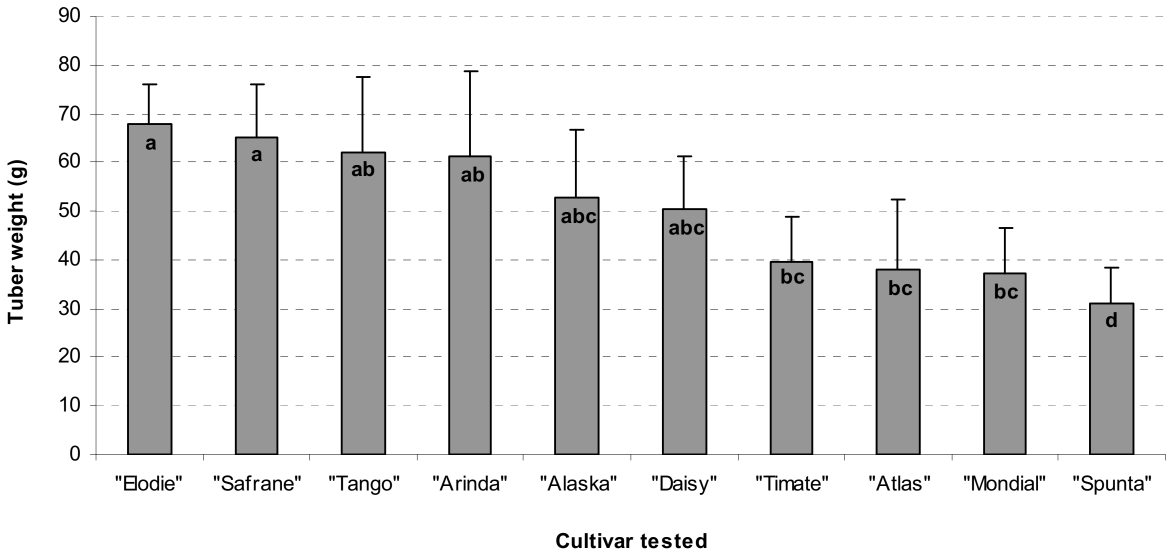

Fig. 7 Tuber weight recorded, 60 days post-inoculation, on the 10 potato cultivars tested. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($P \leq 0.05$). For each potato cultivar, data presented is the mean weight for inoculated and non-inoculated plants; 11 < T < 35°C.

weights of aerial parts.

Although stem weight, for all cultivars and fungal treatments combined, was shown to be negatively correlated to LDI ($R = -0.297$, $P \leq 0.01$), the response to pathogen inoculation was variable depending on cultivars used.

Data shown in **Fig. 6** indicates that, for all cultivars pooled, the aerial part weight was reduced by about 21% on inoculated compared with non-inoculated control plants.

Tuber weight

The tuber weight, noted at 60 DPI, varied significantly (at $P \leq 0.05$) with cultivars and fungal treatments tested, independently; the interaction between both fixed factors was insignificant.

Fig. 7 shows that the highest tuber weight, for all fungal treatments, was recorded on 'Elodie' and 'Safrane' plants (~65 g) and to a lesser degree on 'Tango' and 'Arinda' (~60 g) whereas the significantly lowest weight was noted on 'Spunta' plants.

It is interesting to note that cultivars showing the greater tuber weights were previously shown to be susceptible ('Elodie' and 'Safrane') or highly susceptible ('Tango') based on LDI records. Furthermore, plants of significantly comparable LDI records were found to have significantly different tuber weights as is the case of 'Arinda' and 'Spunta' (61.4 and 31.2 g, respectively). Moreover, 'Alaska' and 'Safrane', classed as highly and moderately susceptible respectively via LDI records, showed significantly comparable tuber weights.

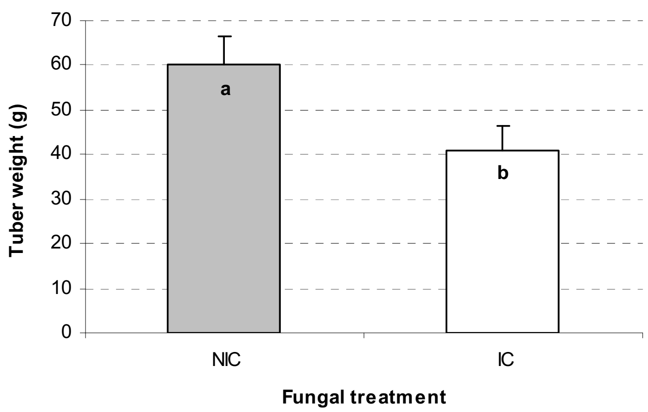

Fig. 8 Tuber weight recorded, 60 days post-inoculation, on potato plants (all cultivars pooled) inoculated with *V. dahliae* compared with the non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($P \leq 0.05$); (IC) inoculated with a mixture of nine *V. dahliae* isolates; (NIC) non-inoculated control; 11 < T < 35°C.

Although tuber weight, for all cultivars and fungal treatments combined, was found to be negatively correlated to LDI ($R = -0.377$, $P \leq 0.01$), the response to inoculation varied depending on the cultivar used.

Fig. 8 shows that, for all cultivars pooled, tuber weight was reduced by about 32% on inoculated compared with non-inoculated plants.

Relation between cultivar susceptibility degree and subsequent growth and yield reduction

T Data presented in **Table 2** indicates that for the highly susceptible cultivars based on LDI records ('Alaska', 'Atlas', 'Tango' and 'Timate'), reduction in plant height compared to the non-inoculated control varied from 14.7 to 27.5%. However, for susceptible cultivars ('Arinda', 'Daisy', 'Elodie', 'Safrane' and 'Spunta'), this decrease ranged between 12.8 and 26.8% compared to 14.7% recorded on the moderately susceptible cultivar 'Mondial'.

However, the decrease in stem weight varied between 15.2 and 42.5% for the highly susceptible cultivars compared with 12.7 to 28.3% and 31.4% for the susceptible and the moderately susceptible cultivars, respectively. Nevertheless, the range of decrease in tuber weight was 17.9 to 46%, 19.3 to 41.3%, and 37.8% for the highly susceptible, susceptible and moderately susceptible cultivars (based on LDI records), respectively. Thus, **Table 2** reveals the variable response of cultivars tested to *Verticillium* infection i.e. stunting effect and the resulting stem weight and yield reduction were found to vary among cultivars with different degrees of susceptibility to wilt agent.

Nevertheless, based on yield reduction records according to Susnoschi *et al.*'s (1976) method (**Table 2**), some cultivar classification has changed for five cultivars out of 10 tested. In fact, 'Timate' and 'Tango', classed as highly susceptible based on LDI, have become susceptible and tolerant (yield reduction less than 20%), respectively, on the basis of yield decrease scoring system. In the same way, 'Elodie' moved from susceptible to tolerant according to both classification methods used, respectively. However, 'Arinda' and 'Mondial' showed an increased degree of susceptibility (from susceptible and moderately susceptible to highly susceptible and susceptible, respectively) when the yield decrease scoring system was used. The remaining cultivars tested showed similar degrees of susceptibility accor-

Table 2 Growth and yield reduction of ten potato cultivars tested and their classification based on LDI and yield reduction records.

Cultivars	LDI	LDI based classification	Height reduction (%)*	Stem weight reduction (%)*	Yield reduction (%)*	Classification based on yield reduction *
'Alaska'	4.3	HS	18.6	42.5	44.0	HS
'Timate'	3.8	HS	24.4	22.1	36.4	S
'Atlas'	3.4	HS	14.7	15.2	46.0	HS
'Tango'	3.3	HS	27.5	21.5	17.9	T
'Arinda'	2.9	S	17.3	12.7	41.3	HS
'Spunta'	2.9	S	20.4	14.6	39.2	S
'Daisy'	2.6	S	24.4	16.8	20.4	S
'Elodie'	2.2	S	12.8	15.8	19.9	T
'Safrane'	2.2	S	26.8	28.3	27.5	S
'Mondial'	1.2	MS	14.7	31.4	37.8	S

HS: Highly susceptible (LDI ≥3); **S:** Susceptible (LDI: 2-2.9); **MS:** Moderately susceptible (LDI: 1-1.9) according to Daami-Remadi *et al.* (2010) method; **T:** Tolerant (Yield reduction ≤20%); **S:** Susceptible (Yield reduction: 20-40%); **HS:** Highly susceptible (Yield reduction >40) according to Susnoschi *et al.* (1976) method.
* Reduction compared to the non-inoculated controls

ding to both scoring systems adopted.

Data presented in **Table 2** also indicated that for some cultivars as is the case of 'Alaska' and 'Atlas', greater yield reductions (44 and 46%, respectively) were associated with higher degrees of wilting (higher LDI records) due to Verticillium infection. However, for 'Mondial', showing the lowest LDI value, an important yield reduction of about 37.8% was recorded.

DISCUSSION

The present study reports, for the first time in Tunisia, the evaluation of local potato cultivars for resistance to Verticillium wilt caused by *V. dahliae*. Cultivar behaviour was assessed by using a mixture of pathogen isolates obtained from different regions in Tunisia and exhibiting varying degrees of aggressiveness on 'Spunta' (Daami-Remadi *et al.* 2011). Plant resistance depends, in fact, on the aggressiveness of the tested isolates (Beye and Lafay 1988).

Moreover, VW effects were evaluated via foliar symptoms, plant growth and production parameters, as a precise detection and quantification of resistance to *V. dahliae* in potato. Furthermore, as evaluation of resistance is often done by observing symptom development (Davis *et al.* 1983; Platt and Sanderson 1987) and comparing yields with plants grown in noninfested soil (Susnoschi *et al.* 1975, 1976), two scoring systems were used in this study to compare the susceptibility/resistance of potato cultivars to *V. dahliae*, based on both LDI records and reductions in yield.

The results of the present study conclusively demonstrate the adverse effects of VW on yield depending on the cultivar tested. In fact, for all cultivars pooled, the mean plant height was reduced by 20% whereas the aerial part weight and tuber weight were reduced by about 21 and 32%, respectively on inoculated compared with non-inoculated plants. However, the response to *Verticillium* infection i.e. foliar symptoms, stunting effect and the resulting stem weight and yield reduction, varied among cultivars and no clear relationships could be established between these parameters even in the presence of significant correlations between foliar symptoms and the subsequent reductions in plant height, stem and tuber weights. Moreover, assessment of plant height, contrary to foliar symptoms, is independent of cultivar maturity characteristics and cultivar resistance (Zambino and Anderson 1981); plant height was used as a criterion only to estimate disease development. However, when comparisons are made based on foliar symptoms, the tested cultivars are classed as moderately susceptible (LDI: 1-1.9), susceptible (LDI: 2-2.9) or highly susceptible (LDI ≥3). These important foliar symptoms, which are responsible for early senescence of the inoculated plants, are related to significant reductions in yield of most of the tested cultivars. In fact, when comparisons are made based on reductions in yield, the tested cultivars are classed as highly susceptible (yield reduction >40), susceptible (yield reduction: 20-40%) or tolerant (yield reduction ≤20%).

The findings from our study are in accordance with those of Susnoschi *et al.* (1976) showing that the main effects of the disease consisted in early senescence and a reduction in tuber size which were considered as criteria in determining the degree of susceptibility to the pathogen (Susnoschi *et al.* 1976; Plasencia and Banttari 1997).

Based on two scoring systems, LDI and yield reductions, cultivars 'Alaska', 'Timate' 'Atlas', 'Arinda', 'Spunta', 'Daisy', 'Safrane' and 'Mondial' are either susceptible or highly susceptible. These results are similar to those of many other researchers such as Ayers (1952), McLean (1955), Guthrie (1960), Busch (1966), Hunter *et al.* (1968), and Susnoschi *et al.* (1976) showing that all the commonly grown varieties are susceptible to VW and exhibit varying degrees of susceptibility, ranging from moderate to high. However, recently, most widely grown potato cultivars are reported to be susceptible or moderately resistant to this disease (Mpofu and Hall 2002; Rowe and Powelson 2002; Frost *et al.* 2006) and potato cultivars carrying resistance to VW are still scarce (Uppal *et al.* 2007).

However, 'Tango' and 'Elodie' produced satisfactory yield (lowest yield reduction compared to non-inoculated control) despite important leaf damage (LDI = 3.3 and 2.2, respectively) and were then found to be tolerant. In fact, tolerance is defined as no significant reduction in yield, despite a high degree of infection in the plant (Nachmias and Krikun 1985). Susnoschi *et al.* (1976) reported a similar response of the tolerant cultivars 'Désirée', 'Alpha' and 'Baraka' who showed only limited yield losses, despite high levels of stem and tuber infection. Similarly, Corsini and Pavek (1996) reported that the species hybrids that produced enough seed for meaningful yield trials showed apical stem colonization by *V. dahliae*, although wilt symptoms remained very mild. In fact, many researchers have mentioned that the correlation between stem colonization and symptom expression was low (Susnoschi *et al.* 1976; Jansky and Rouse 2000; Bae *et al.* 2008). Stem colonization is typically considered to be a better method of resistance evaluation than symptom expression because it measures actual pathogen levels in plant tissues (Bae *et al.* 2008).

The tolerance of potato varieties have been related to the strong haulm development and lateness of maturity (Bae *et al.* 2008). In fact, late season vigor appears to be one of the key factors in the potato plant's ability to survive these diseases long enough to produce a good yield of high quality potatoes, but this vigor must be combined with normal tuber bulking (Corsini and Pavek 1996). However, in the case of our study, 'Tango' and 'Elodie' seem not to be late maturing cultivars as the total foliage is almost damaged.

These tolerant varieties are undesirable because they add inoculum to successive potato crops even when rotation practices are used (Bae *et al.* 2008). Jansky and Rouse (2000) mentioned that it is important to identify clones that allow high levels of *Verticillium* to develop in their stems, and to eliminate them from the breeding population.

The reaction of 'Mondial' was different from that described for all the other tested cultivars. Foliar symptoms noted on this cultivar were lower than that of the others, and reduction in yield was the same as for the susceptible cultivars. It is possible that 'Mondial' is a late maturing cultivar showing lesser symptoms, as found also by Susnoschi *et al.* (1976) when testing the resistance of potato cultivars to VW. Similarly, Bae *et al.* (2008) mentioned that late maturing clones did not show foliar symptom expression even though *V. dahliae* was detected in stems and often expressed immature plant resistance, which is usually accompanied by low yield. In fact, the lack of success in finding adequate levels of resistance may reflect previous confounding differences in maturity with differences in resistance. To address this complication, clones could be grouped based on maturity and then assessed for symptoms within maturity groups (Bae *et al*. 2008).

The present data highlights the difficulty in using symptom expression to identify cultivars with true VW resistance and emphasis the need of evaluation of stem colonization (levels of the pathogen in stems). In fact, Bae *et al.* (2008) mentioned that, instead of counting CFU (colony-forming units) in stems, resistance could be determined by counting the number of stems that contain any fungal propagules, which is easy to do.

Conclusions on the behaviour of local potato cultivars towards VW are not decisive since testing for resistance/ tolerance under greenhouse conditions is extremely difficult with regard to obtaining good symptom expression and yield data. Consequently, field trials where plot sizes are large and replications are important are needed to evaluate, under Tunisian conditions, the effect of VW on yield reductions of the most grown potato cultivars. In fact, different environmental factors and possibly races of the pathogen nullify the tolerance obtained under other conditions (Susnoschi *et al.* 1975). Depending on severity, time of occurrence and growing season, potato yields and tuber size may be substantially reduced (Davis 1985). Moreover, the interactions between *V. dahliae* and many other pathogens must be considered in planning trials to screen potato clones or cultivars for resistance or tolerance to VW, failing to do so may result in *Verticillium* tolerant genotypes being scored as 'susceptible' (Tsror (Lahkim) *et al.* 1990).

Findings from our results are still important as VW continues to threat potato production in Tunisia and highlights the urgent need of breeding potato clones with useful levels of resistance especially under Tunisian conditions.

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