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# Effect of *Fusarium* Species and Temperature of Storage on the Susceptibility Ranking of Potato Cultivars to Tuber Dry Rot

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## ABSTRACT

The relative susceptibility of 11 potato cultivars to *Fusarium solani*, *F. oxysporum* f. sp. *tuberosi*, *F. sambucinum*, and *F. graminearum* was assessed under different temperatures of storage. Obtained results revealed that these *Fusarium* species showed variable aggressiveness upon the 11 tested potato cultivars. In fact, *F. sambucinum* was the most aggressive on the majority of cultivars. *F. graminearum* showed comparable aggressiveness as *F. sambucinum* only on some cultivars. *F. oxysporum* f. sp. *tuberosi* and *F. solani* caused a relatively less severe dry rot on the cultivars tested. After 21 days of incubation at 15, 20, 25 and 30°C, none of the cultivars tested was completely resistant to all *Fusarium* species and only some of them showed lesser susceptibility to at the most one species. This is the case of 'Spunta', 'Mondial' and 'Nicola', the most cultivated in Tunisia, which tolerated at least one species of *Fusarium*: *F. oxysporum* f. sp. *tuberosi* for the two first cultivars and *F. solani* for the second. The rank order of susceptibility levels to Fusarium dry rot varied depending on *Fusarium* species and temperature of storage. When tubers were inoculated with *F. sambucinum* (the most aggressive species), cultivars placed in the less susceptible group at 30°C were classified as highly susceptible at 15°C. However, when tubers were inoculated with *F. oxysporum* f. sp. *tuberosi* (the least aggressive), cultivars placed on category of less susceptible were almost the same at all tested temperatures.

**Keywords:** cultivar, inoculation, *Solanum tuberosum* L., susceptibility, temperature range, Tunisia

## INTRODUCTION

Dry rot is an important post-harvest disease of potato (*Solanum tuberosum* L.) tubers that can be caused by several *Fusarium* species and it is of economic significance worldwide (Tivoli *et al*. 1985; Carnegie *et al*. 1998). It is particularly prominent in Tunisia under traditional and cold storage (Daami-Remadi and El Mahjoub 1996; Chérif *et al*. 2000). Thirteen *Fusarium* species were reported as causal agents of dry rot of potato worldwide (Secor *et al*. 2001; Cullen *et al*. 2005; Peters *et al*. 2008). *F. solani*, *F. oxysporum* f. sp. *tuberosi*, *F. sambucinum*, and *F. graminearum* were the predominant in Tunisia (Daami-Remadi *et al*. 2006a, 2006b). An effective control of Fusarium dry rot has been achieved with the fungicide fenpiclonil and the mixture of thiabendazole and imizalil (Carnegie *et al*. 1998). Nevertheless, resistance to the few chemicals registered for use on potato tubers for human consumption seems to be widespread among strains of *Fusarium* spp. (Kawchuk *et al*. 1994; Secor *et al*. 1994; Hanson *et al*. 1996; Gonzalez *et al*. 2002; Daami-Remadi and El Mahjoub 2006; Ocamb *et al*. 2007). Thus, it was be interesting to have more focus research on potato resistance and other alternative control methods.

Susceptibility assessment of potato cultivars to Fusarium dry rot was studied by several authors (Leach *et al*. 1981; Wastie *et al*. 1989; Hanson *et al*. 1996; Schisler *et al*. 1997; Esfahani 2005; Daami-Remadi *et al*. 2006a). However, all of the research conducted to date on the management of the Fusarium dry rot suggested that very few cultivars were highly resistant and none was immune (Scholte and Labruyère 1985). In fact, among other factors, the ranking of cultivars was affected by *Fusarium* species used for tuber inoculation (Hooker 1981). In Tunisia, the assessment of the susceptibility of local potato cultivars to the major causal agents revealed a cultivar by *Fusarium* species interaction and no cultivars were completely resistant to the whole *Fusarium* complex (Daami-Remadi and El Mahjoub 1996; Daami-Remadi *et al*. 2006a). However, the occurrence of mixed infections and the variation of their relative dominance in refrigerated and non-refrigerated stores, due to differences in ambient temperatures, are also possibly responsible of the variation of cultivars' behavior towards *Fusarium* species (Tivoli *et al.* 1985; Tivoli *at al*. 1988; Daami-Remadi *et al*. 2006b).

In fact, potato tuber dry rot incidence varied upon *Fusarium* spp. involved in disease development, soil, cultivars and environmental conditions such as temperature (Boyd *et al*. 1952; Seppanen 1983). The specific thermal requirements of Tunisian *Fusarium* species was determined at temperatures ranging from 5 to 40°C on tubers belonging to cv. 'Spunta' the most cultivated in Tunisia. An interaction between pathogens tested and temperature of storage was observed (Daami-Remadi *et al*. 2006b). Thus, due to complexity of resistance of potato tubers to all *Fusarium* species which also exhibited variable aggressiveness depending on temperatures of storage, this abiotic factor should be considered during the screening of resistance to the major *Fusarium* species. Consequently, the purpose of this study was to check if temperature may affect the different ranking orders of potato cultivar's susceptibility towards *Fusarium* spp.

## MATERIALS AND METHODS

### Pathogens

*Fusarium* spp. (*F. solani*, *F. graminearum*, *F. sambucinum*, and *F. oxysporum* f. sp. *tuberosi*) were isolated from potato tubers of different cultivars and showing typical symptoms of dry rot or from plants exhibiting partial or total wilting. They were cultured on potato dextrose agar (PDA) (Chemi-Pharma, Le Bardo, Tuni-

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sia) medium supplemented with 300 mg/l of streptomycin sulphate (Pharmadrug Production Gmbh, Hamburg, Germany). Their virulence was maintained by bimonthly inoculation of freshly wounded and healthy tubers and re-isolation on PDA plates. For their preservation, up to 12 months, monoconidial cultures were maintained at -20°C in a 20% glycerol (Chem-Lab NV Industriezone "De Arend", Zedelgem, Belgium) solution.

*Fusarium* species were identified based on several morphological and cultural criteria (Messiaen and Cassini 1968; Tivoli 1988; Leslie and Summerell 2006).

## Potato cultivars

Eleven potato cultivars were tested in the present study namely 'Arinda', 'Atlas', 'Bellini', 'Elodie', 'Fabula', 'Liseta', 'Mondial', 'Nicola', 'Oceania', 'Orla', and 'Spunta'. They were subscribed in the list A of the Tunisian varietal assortment and kindly provided by the Technical Center of Potato, Essaïda, Tunisia. They were stored for two months in the darkness at 6°C and bought to room temperature 3 h before use. Prior to inoculation, tubers were superficially disinfected with a 10% sodium hypochlorite (Aiglol Production, Zaouiet Sousse, Tunisia) solution during 5 min, rinsed with sterile distilled water and air dried.

## Inoculation and incubation

For each *Fusarium* species, the inoculum was composed of a mixture of four isolates chosen beforehand on the basis of their aggressiveness (Mejdoub-Trabelsi, unpublished data). Conidia were harvested by flooding each culture with sterile distilled water. The conidial suspension was recuperated, filtered through double layered cheese cloth and then the final concentration was adjusted to $10^7$ conidia/ml by using a Malassez cytometer (Fa. Laboroptik, Friedrichsdorf, Tiefe 0.2 mm, 0.0025 $mm^2$, HBG, Germany). Equal volumes of conidial suspensions of *F. solani*, *F. graminearum*, *F. sambucinum* or *F. oxysporum* f. sp. *tuberosi* were associated to obtain four types of mixtures ready for inoculation of potato tubers.

Before inoculation, disinfected potato tubers were wounded by removing a tuber plug (6 mm in diameter and depth) with a sterile cork-borer. These occasioned wounds were challenged with 100 µl of a conidial suspension mixture of each *Fusarium* species.

The treated tubers (two replicates of five tubers per elementary treatment) were placed in plastic bags to maintain a high humidity and then incubated in different incubators adjusted to 10, 15, 25 or 30°C for three weeks. After incubation period, tubers were cut along the longitudinal axis across the inoculation sites. The two perpendicular diameters of the lesion were recorded and the mean diameter was calculated for each site of inoculation.

Dry rot severity was also estimated by measuring the extent of the induced decay i.e. maximal width (w) and depth (d). The pathogen penetration into tubers was calculated based on the Lapwood *et al.* (1984) formula as follows:

$$\text{Penetration (mm)} = [w/2 + (d-6)]/2$$

Potato cultivars were then ranked for their susceptibility to *Fusarium* species based on the following below scale:

1. Less or moderately susceptible: penetration ≤ 4.5 mm;
2. Susceptible: 4.5 mm < penetration < 6 mm;
3. Highly susceptible: penetration ≥ 6 mm.

## Statistical analyses

Statistical analyses (ANOVA) for all parameters noted were performed following a completely randomized factorial design with three factors i.e. potato cultivars, fungal treatments (tubers inoculated with each *Fusarium* species and non inoculated tubers) and temperatures of storage. Means were separated using Fisher's protected least significant difference (LSD) or Student Newman-Keul's (SNK) tests (at $P < 0.05$).

## RESULTS

Mean diameter of dry rot lesions depended on fungal treatment, cultivars tested and temperature of incubation (**Table 1**). A significant (at $P \leq 0.05$) interaction was observed between the three fixed factors. In fact, all *Fusarium* mixtures of isolates were pathogenic to potato tubers. *F. sambucinum* was found to be the most aggressive at all temperatures tested (**Table 1; Fig. 1**). At 15°C, *F. sambucinum* isolates average a disease severity (lesion diameter) of 13.3 mm whilst *F. graminearum, F. solani* and *F. oxysporum* f. sp. *tuberosi* do not exceed 10.5 mm.

The level of tuber decay estimated based on the mean pathogen penetration (**Table 2**) also depended on cultivars, fungal treatments used for inoculation and temperatures of incubation as a significant interaction was recorded between the three fixed factors. In fact, *F. sambucinum* caused of the severest dry rot regardless of cultivar tested when tubers were stored at 15, 20 and 25°C. However, at 30°C, *F. graminearum* caused the most severe dry rot as compared to the other fungal treatments. Significant differences in disease severity (penetration) (**Fig. 2**) were observed among *Fusarium* species used for tuber inoculation.

When inoculated by *F. sambucinum*, tubers belonging to 7 ('Nicola', 'Atlas', 'Fabula', 'Bellini', 'Liseta', 'Arinda' and 'Elodie') out 11 cultivars tested were found to be highly susceptible showing a mean penetration reaching or exceeding 6 mm (**Table 3**). Since the rest of cultivars were as well

**Table 1** Fusarium dry rot severity (diameter. in mm) noted on potato tubers depending on different mixture of *Fusarium* isolates used for tuber inoculation at different temperatures of incubation.

| Temperature | 15°C | | | | | 20°C | | | | | 25°C | | | | | 30°C | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivar/ treatment | Co | F. sol | F. oxy | F. sam | F. gra | Co | F. sol | F. oxy | F. sam | F. gra | Co | F. sol | F. oxy | F. sam | F. gra | Co | F. sol | F. oxy | F. sam | F. gra | a* |
| 'Spunta' | 9 | 9.8 | 10.6 | 12.5 | 10.8 | 8.5 | 11.2 | 10.7 | 13.2 | 10.9 | 8.2 | 9.6 | 9.7 | 11.6 | 10.4 | 8.3 | 19.8 | 10.3 | 10 | 12.5 | 10.9 c |
| 'Mondial' | 8.2 | 10.4 | 12.1 | 11.3 | 10.5 | 9.1 | 11.1 | 10.3 | 11.7 | 12.1 | 8.8 | 12.1 | 10.8 | 12.1 | 11.7 | 8.7 | 9.7 | 9.8 | 10.6 | 11.3 | 10.6 cd |
| 'Nicola' | 9.7 | 8.5 | 8.8 | 12.2 | 9.5 | 8.7 | 11.1 | 11.2 | 14.5 | 15.1 | 8.4 | 9 | 11.7 | 15.2 | 12.9 | 8.6 | 8.8 | 9.1 | 12.5 | 10.5 | 10.8 cd |
| 'Atlas' | 8.6 | 9.9 | 9.9 | 12.9 | 10.7 | 8.5 | 11.7 | 11.4 | 12.5 | 12.6 | 8.9 | 10.1 | 9.8 | 11.3 | 11.3 | 9 | 11.1 | 10.4 | 10.2 | 10.4 | 10.6 cd |
| 'Oceania' | 8.8 | 9.3 | 10.2 | 10.5 | 9.4 | 7.9 | 8.4 | 9.6 | 12.2 | 8.7 | 9.1 | 12.2 | 10.4 | 11.5 | 10.4 | 8.5 | 13.9 | 8.8 | 11 | 9.5 | 10.0 e |
| 'Fabula' | 9.6 | 10.4 | 10.1 | 18.7 | 10.1 | 9.3 | 9.5 | 10.5 | 12.4 | 10.4 | 10.1 | 11.8 | 11.6 | 13 | 11.1 | 9.5 | 9.7 | 9.5 | 12.4 | 16.9 | 11.3 b |
| 'Orla' | 8.1 | 10.4 | 10.2 | 14.1 | 10.6 | 9.8 | 10.2 | 10.4 | 11.1 | 11.2 | 8.9 | 9.7 | 10.2 | 9.9 | 9.8 | 9.7 | 20.1 | 9.5 | 10.3 | 11.7 | 10.8 cd |
| 'Bellini' | 7.6 | 10 | 10.7 | 14.8 | 9.5 | 8.6 | 11.8 | 11.8 | 13.5 | 12 | 7.9 | 9.1 | 10.3 | 11.9 | 8.4 | 8.3 | 10 | 10 | 11.1 | 11 | 10.4 d |
| 'Liseta' | 9.3 | 11.3 | 10.1 | 14.7 | 13.1 | 8.8 | 13 | 13.6 | 14.4 | 15.6 | 7.8 | 13 | 11.9 | 14.6 | 12.3 | 8.5 | 10.2 | 11.4 | 12.8 | 12.2 | 11.9 a |
| 'Arinda' | 8.7 | 10.9 | 9.6 | 13 | 10.9 | 10 | 12.7 | 11.4 | 13.4 | 12.3 | 8.6 | 9.4 | 9.3 | 11.1 | 11.3 | 7.9 | 12.1 | 11 | 16.5 | 16.9 | 11.4 b |
| 'Elodie' | 9.3 | 10.4 | 11 | 11.6 | 10.5 | 9.1 | 12 | 11.1 | 12.9 | 10.6 | 9.2 | 11.2 | 10.4 | 13.7 | 11.4 | 9.2 | 21.1 | 9.6 | 10 | 20.1 | 11.7 ab |
| Mean b | 8.8 | 10.1 | 10.3 | 13.3 | 10.5 | 8.9 | 11.2 | 11.1 | 12.9 | 12.0 | 8.7 | 10.7 | 10.6 | 12.4 | 11.0 | 8.7 | 13.3 | 9.9 | 11.6 | 13.0 | |
| Mean c* | 10.6 b | | | | | 11.2 b | | | | | 10.7 a | | | | | 11.3 a | | | | | |

LSD (Cultivars × Fungal treatments × Temperatures) = 1.49 mm at $P \leq 0.05$

a Mean diameters per cultivar independently of temperatures of storage and fungal treatments

b Mean diameters per fungal treatment and per temperature independently of cultivars tested

c Mean diameters per temperature independently of fungal treatments and cultivars tested

* Means affected with the same letter are not significantly different at $P \leq 0.05$

Co: untreated control; F. sol, F. oxy, F. sam, F. gra: Mixture of *Fusarium* isolates of *F. solani*, *F. oxysporum* f. sp. *tuberosi*, *F. sambucinum*, *F. graminearum*, respectively.

**Table 2** Fusarium dry rot severity (penetration. in mm) noted on potato tubers depending on different mixture of *Fusarium* isolates used for tuber inoculation at different temperatures of incubation

| Temperature | 15°C | | | | | 20°C | | | | | 25°C | | | | | 30°C | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivar/ treatment | Co | F. sol | F. oxy | F. sam | F. gra | Co | F. sol | F. oxy | F. sam | F. gra | Co | F. sol | F. oxy | F. sam | F. gra | Co | F. sol | F. oxy | F. sam | F. gra | a* |
| 'Spunta' | 3.4 | 4.1 | 4.5 | 7.3 | 5.1 | 3.6 | 4.8 | 4.2 | 6.9 | 5.6 | 2.8 | 4.2 | 4.5 | 5.3 | 4.8 | 2.5 | 9.6 | 4.6 | 3.6 | 5.3 | 4.8 cde |
| 'Mondial' | 3.4 | 4.7 | 4.5 | 5.2 | 5.1 | 3.2 | 4.4 | 4.3 | 5.2 | 5.2 | 3.2 | 5.5 | 4.8 | 5.5 | 4.8 | 2.7 | 4.5 | 3.6 | 4.4 | 4.5 | 4.4 f |
| 'Nicola' | 4.6 | 3.8 | 3.6 | 8 | 4.7 | 3.1 | 5 | 4.6 | 9.7 | 8.8 | 2.7 | 4.2 | 6.5 | 7.9 | 5.2 | 3 | 3.6 | 3.8 | 7 | 5.3 | 5.3 b |
| 'Atlas' | 3.6 | 4.5 | 4.2 | 7.6 | 5.1 | 2.9 | 5 | 5.5 | 6.8 | 6.2 | 3.6 | 4.6 | 4.4 | 5.7 | 5.7 | 3.1 | 4.1 | 3.5 | 5 | 4.1 | 4.8 def |
| 'Oceania' | 3.2 | 4.3 | 4.3 | 4.8 | 3.8 | 2.9 | 3 | 3.2 | 6 | 2.8 | 3.2 | 7.8 | 4.7 | 7 | 5.6 | 3.3 | 6.6 | 3.5 | 5.6 | 4.4 | 4.5 ef |
| 'Fabula' | 3.2 | 5.1 | 4.6 | 11.3 | 4.8 | 4.1 | 3.8 | 4.1 | 6 | 4.3 | 3.9 | 5.1 | 4.5 | 6 | 5.1 | 3.1 | 4.2 | 3.7 | 6.5 | 9.7 | 5.2 bc |
| 'Orla' | 3.3 | 5 | 4.7 | 8.6 | 6.7 | 3.1 | 4 | 4 | 4.7 | 5.3 | 3.3 | 4.3 | 3.9 | 4.6 | 4.2 | 4.3 | 10.8 | 3.6 | 4.4 | 6.2 | 5.0 bcd |
| 'Bellini' | 3 | 4.1 | 4.6 | 7.3 | 3.8 | 3 | 5.4 | 5.6 | 6.8 | 5.7 | 3.5 | 4.4 | 4.9 | 5.6 | 3.3 | 4.4 | 5.1 | 4 | 5.8 | 5.4 | 4.8 def |
| 'Liseta' | 3.1 | 5.7 | 4.6 | 7.9 | 5.9 | 3.5 | 6.4 | 6.6 | 7.2 | 7.7 | 2.2 | 7 | 6.4 | 7.2 | 6 | 3.8 | 5.2 | 5.2 | 7.1 | 7.4 | 5.8 a |
| 'Arinda' | 3.3 | 4.6 | 4 | 6.5 | 5 | 4.1 | 6.4 | 5 | 6.9 | 6.7 | 3.4 | 4.3 | 4.1 | 5.1 | 7.6 | 2.8 | 7.3 | 5.3 | 9 | 11.7 | 5.7 a |
| 'Elodie' | 3.1 | 5 | 4.1 | 6.6 | 5 | 4 | 5 | 4.8 | 6.8 | 6.1 | 3.8 | 4.5 | 4.8 | 11 | 5.4 | 4.4 | 8.9 | 5.6 | 4.4 | 8.1 | 5.6 a |
| Mean b | 3.4 | 4.6 | 4.3 | 7.4 | 5.0 | 3.4 | 4.8 | 4.7 | 6.6 | 5.9 | 3.2 | 5.1 | 4.9 | 6.4 | 5.2 | 3.4 | 6.4 | 4.2 | 5.7 | 6.6 | |
| Mean c* | 4.9 b | | | | | 5.1 ab | | | | | 5.0 b | | | | | 5.2 a | | | | | |

LSD (Cultivars × Fungal treatments × Temperatures) = 1.23 mm at $P \leq 0.05$
a Mean penetration per cultivar independently of temperatures of storage and fungal treatments
b Mean penetration per fungal treatment and per temperature independently of cultivars tested
c Mean penetration per temperature independently of fungal treatments and cultivars tested
* Means affected with the same letter are not significantly different at $P \leq 0.05$
Co: untreated control; F. sol, F. oxy, F. sam, F. gra: Mixture of *Fusarium* isolates of *F. solani*, *F. oxysporum* f. sp. *tuberosi*, *F. sambucinum*, *F. graminearum*, respectively.

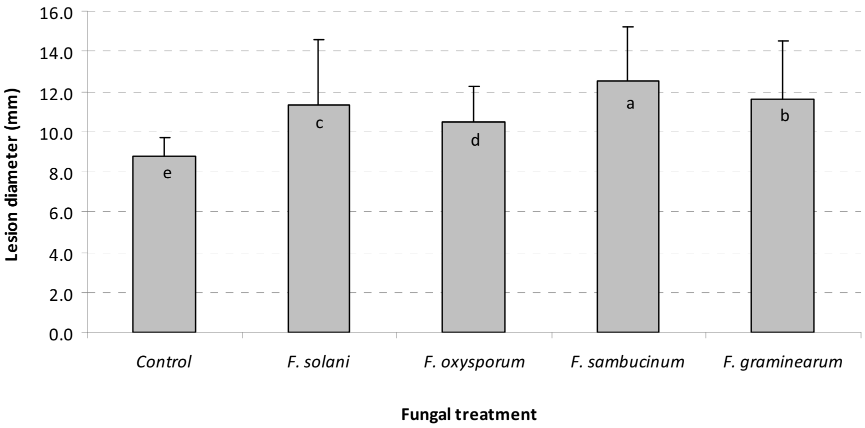


**Fig. 1 Effect of inoculation with four *Fusarium* species (mixture of isolates) on potato tubers lesion diameters noted after 21 days of incubation.** Bars with the same letter are not significantly different according to Student Newman-Keul's (SNK) test ($P \leq 0.05$).

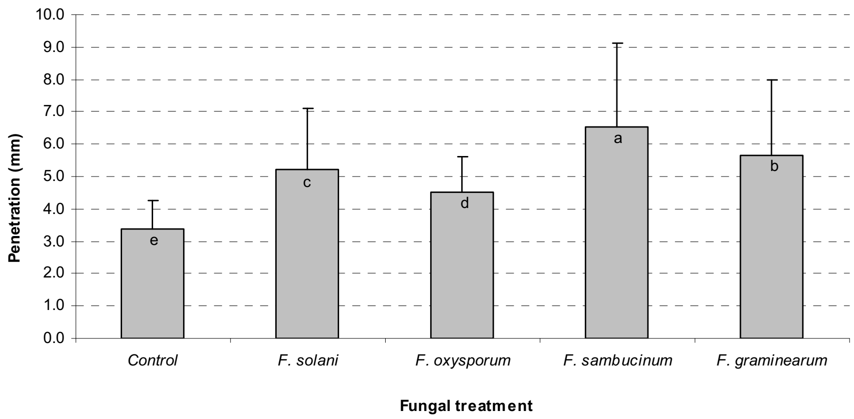


**Fig. 2 Effect of inoculation with four *Fusarium* species (mixture of isolates) on potato tubers dry rot development noted after 21 days of incubation.** Bars with the same letter are not significantly different according to Student Newman-Keul's (SNK) test ($P \leq 0.05$).

susceptible to this pathogen, none of the cultivars tested was tolerant to *F. sambucinum*. However, when tubers were inoculated by *F. oxysporum* f. sp. *tuberosi*, 7 ('Spunta', 'Mondial', 'Atlas', 'Oceania', 'Fabula', 'Orla', 'Arinda') out of 11 cultivars exhibited tolerance whereas none showed any high susceptibility to the mixture of this pathogen.

Data shown in **Table 3** indicate that *F. solani* was more aggressive than *F. oxysporum* f. sp. *tuberosi* on 10 of cultivars tested and developed a less severe dry rot on the majority of cultivars as compared to *F. graminearum*. Consequently, local cultivar's tolerance or susceptibility was found to vary depending on *Fusarium* species used for tuber inoculation.

Independently of temperatures tested (pooled data of all temperatures), *F. sambucinum* exhibited the highest aggressiveness on the majority of cultivars. *F. graminearum* showed a comparable aggressiveness, as *F. sambucinum*, on 5 cultivars. Furthermore, even when the screening of tuber resistance to dry rot was assessed depending on temperature effect, these two pathogens developed the most severe symptoms at 15, 20 and 25°C whilst at 30°C, *F. solani* caused the most severe dry rot.

**Table 3** Variation of the level of susceptibility of potato cultivars to dry rot caused by *Fusarium sambucinum* depending on temperatures of storage.

| Temperature/ level of susceptibility | Less susceptible | Susceptible | Highly susceptible |
|---|---|---|---|
| 15°C | - | 'Mondial'. 'Oceania' | 'Spunta'. 'Nicola'. 'Atlas'. 'Fabula'. 'Orla'. 'Bellini'. 'Liseta'. 'Arinda'. 'Elodie' |
| 20°C | - | 'Mondial'. 'Orla' | 'Spunta'. 'Nicola'. 'Atlas'. 'Oceania'. 'Fabula'. 'Bellini'. 'Liseta'. 'Arinda'. 'Elodie' |
| 25°C | - | 'Spunta'. 'Mondial'. 'Atlas'. 'Orla'. 'Bellini'. 'Arinda' | 'Nicola'. 'Oceania'. 'Fabula'. 'Liseta'. 'Elodie' |
| 30°C | 'Spunta'. 'Mondial'. 'Orla'. 'Elodie'. 'Oceania' | 'Atlas'. 'Oceania'. 'Bellini' | 'Nicola'. 'Liseta'. 'Arinda' |

**Table 4** Variation of the level of susceptibility of potato cultivars to dry rot caused by *Fusarium oxysporum* f. sp. *tuberosi* depending on storage temperature.

| Temperature/ level of susceptibility | Less susceptible | Susceptible | Highly susceptible |
|---|---|---|---|
| 15°C | 'Spunta'. 'Mondial'. 'Nicola'. 'Atlas'. 'Oceania'. 'Arinda'. 'Elodie' | 'Fabula'. 'Orla'. 'Bellini'. 'Liseta' | |
| 20°C | 'Spunta'. 'Mondial'. 'Oceania'. 'Fabula'. 'Orla' | 'Nicola'. 'Atlas'. 'Bellini'. 'Arinda'. 'Elodie' | 'Liseta' |
| 25°C | 'Spunta'. 'Atlas'. 'Fabula'. 'Orla'. 'Arinda' | 'Mondial'. 'Oceania'. 'Bellini'. 'Elodie' | 'Nicola'. 'Liseta' |
| 30°C | 'Mondial'. 'Nicola'. 'Atlas'. 'Oceania'. 'Fabula'. 'Orla'. 'Bellini' | 'Spunta'. 'Liseta'. 'Arinda'. 'Elodie' | |

The present assessment also revealed that none of the cultivars used behaved as resistant to all the tested *Fusarium* species but in majority, they tolerated at the most one species as is the case for 'Spunta', 'Mondial', 'Nicola', 'Atlas', 'Orla', 'Bellini' and 'Arinda'. 'Oceania' and 'Fabula', however, exhibited tolerance to two species (respectively *F. oxysporum* f. sp. *tuberosi*/*F. graminearum* and *F. solani*/*F. oxysporum* f. sp. *tuberosi*), according to our experimental conditions of inoculation and incubation, whereas 'Liseta' and 'Elodie' were found to be susceptible to all tested *Fusarium* spp.

Data shown in **Tables 3** and **4** indicate that the rank order of susceptibility levels to Fusarium dry rot varied depending on *Fusarium* species and temperatures of storage suggesting instability of the behaviour of potato cultivars under different conditions leading to unexpected tuber loss. This phenomenon is more evident for the most and the least aggressive *Fusarium* species such as *F. sambucinum* and *F. oxysporum* f. sp. *tuberosi*.

Moreover, all of the cultivars ranked as highly susceptible to *F. sambucinum* (**Table 3**) were classified in the susceptible or less susceptible group when tested against *F. oxysporum* f. sp. *tuberosi* at the majority of incubation temperatures tested (**Table 4**).

Furthermore, *Fusarium* species were present as mixed infections on potato tubers at all temperature of stores. Consequently, when evaluating cultivars for disease resistance or for effectiveness or disease control measures, assessment should be performed toward most *Fusaria* species. Our results demonstrated that among the 11 tested cultivars, 'Mondial' was found to be less susceptible followed by 'Oceania', 'Atlas' and 'Bellini' with equal level of susceptibility. Cultivars 'Liseta', 'Arinda', 'Elodie' were classified as the most susceptible to *Fusarium* species (**Tables 3, 4**). These three cultivars showed similar susceptibility when comparisons were made base on mean penetration as dry rot severity parameter (**Table 2**).

## DISCUSSION

The evaluation of local potato cultivars for resistance to Fusarium dry rot was previously investigated in Tunisia. However, the present study reports for the first time a susceptibility ranking of local potato cultivars to *Fusarium* species at four different temperatures. Since temperature is the major factor affecting population dynamic and community structure of *Fusarium* species (Saremi and Burgess 2000), its incorporation into susceptibility ranking of Tunisian cultivars seems compulsory.

The results of the present study conclusively demonstrate that the rank order of cultivar's susceptibility to the four *Fusarium* species, actually involved in potato dry rot development in Tunisia, varied widely among cultivars indicating independent behavior against each causal agent. This supports the previous findings indicating that resistance to these pathogens is genetically distinct (Corsini and Pavek 1986; Wastie *et al.* 1989). Similarly, many other authors (Seppanen 1983; Wastie and Bradshaw 1993; Lui and Kushalappa 2002; Esfahani 2005) have also reported that cultivars react differently to *Fusarium* species.

In order to mimic the field conditions, comparative susceptibility of potato cultivars to Fusarium dry rot is assessed by artificially inoculation with a mixture of four isolates per *Fusarium* species, and not only one isolate per species, hence the originality of this work.

The present study revealed that the majority of cultivars highly susceptible to *F. sambucinum* were rated tolerant to *F. oxysporum* f. sp. *tuberosi* (**Table 3**). *F. sambucinum* was shown to be the most aggressive on the majority of potato cultivars tested. *F. graminearum* showed a comparable aggressiveness as *F. sambucinum* on 7 cultivars. These results, which indicate the higher aggressiveness of *F. sambucinum*, as compared to the other *Fusarium* species, are in agreement with several reports from different countries: France (Tivoli and Jouan 1981), South Africa (Theron and Holz 1989), Scotland (Wastie *et al.* 1989), USA (Secor and Salas 2001), Tunisia (Daami-Remadi and El Mahjoub 2004; Ayed *et al.* 2006), and the UK (Peters *et al.* 2008). However, *F. graminearum* used in the present study was not more aggressive than *F. sambucinum* as previously reported in Daami-Remadi *et al.* (2006a); the differences in plant material, pathogen aggressiveness and inoculation method may give additional information on potential sources of these variations.

Estrada *et al.* (2010) signaled, based on a 2004-2005 survey of potatoes stores in the North-Central potato-producing region of the USA, that the predominant causes of dry rot were *F. graminearum* and *F. sambucinum*. However, in Tunisia, *F. graminearum* and *F. sambucinum* were classified within the most aggressive group. Differences in disease severity and relative importance of pathogens involved in dry rot development may be attributed to several biotic (cultivars, physiological age, wound dimension, pathogen aggressiveness, etc) and abiotic factors (mainly temperature and humidity) (Lozoya Saldaña and Hernández Vilchis 2001; Lui and Kushalappa 2002; Daami-Remadi *et al.* 2006a; Choiseul *et al.* 2007).

*F. oxysporum* f. sp. *tuberosi* behaved, in the present assessment, as the least aggressive species even though its frequent involvement in disease fusarial dry rot and wilt complex. In fact, in a previous study, Daami-Remadi *et al.* (2006a) recorded a severe dry rot caused by *F. oxysporum* f. sp. *tuberosi*, as compared to *F. sambucinum* and *F. gramine-*

*arum*, on 4 cultivars amongst 11 tested. The investigations of Theron and Holz (1989) indicated that among fourteen *Fusarium* spp. isolates, involved in dry rot and stem-end rot lesions on potato tubers collected in South Africa, *F. oxysporum* f. sp. *tuberosi* and *F. solani* were the predominant agents and *F. oxysporum* was the most pathogenic. Thanassoulopoulos and Kitsos (1985) indicated a variability of the infection frequency of this pathogen due to cultivar effect. Similarly, Manici and Cerato (1994) suggested that *F. oxysporum* f. sp. *tuberosi* isolates may differ in pathogenicity as dry rot or wilt agents. As, in the present study, virulence of *F. oxysporum* f. sp. *tuberosi* isolates was already verified on potato tubers before testing cultivar's behavior and temperature effect, it is possible that their ability to cause wilt was more prominent than dry rot. Thereby, *F. oxysporum* f. sp. *tuberosi* isolates had little effect on the cultivars tested.

The obtained results gave additional informations on the effect of mixed inoculation. In fact, all previous Tunisian studies showed the aggressiveness of individual inoculation of one isolate per *Fusarium* species. In this paper, the objective is to clarify the behaviour of Tunisian cultivars by mimicking, as much as possible, natural conditions. Therefore, a mixture of local isolates of each species appears as an appropriate inoculum to screen for *Fusarium* resistance or susceptibility. The results showed that tuber inoculation with mixtures of isolates belonging to the same species exhibited variation in *Fusarium* aggressiveness as already described. Novelty of our findings lies in the fact that mixed inoculations revealed similar patterns of reaction when trials were conducted with a single isolate per *Fusarium* species. In fact, *F. sambucinum* and *F. graminearum* were always the most aggressive when compared to *F. solani* and *F. oxysporum* f. sp. *tuberosi*.

Overall, resistance to the major *Fusarium* species is controlled by independent genetic mechanisms which were known to complicate breeding efforts (Huaman *et al*. 1989). In fact, several authors have reported difficulty in combining high resistance to *F. sambucinum* and *F. coeruleum* in cultivars and progenies of *Solanum tuberosum* ssp. *tuberosum*. Our results suggest that there were evidence that the screening of local potato cultivars against four *Fusarium* species implicated in dry rot development at more than one temperature is original but also difficult. This difficulty is attributed to the complexity of *Fusarium* species. They were among the most globally important plant pathogens because a plethora of hosts are affected by one or more species of this fungal genus, as mentioned by Estrada *et al.* (2010). Incidentally, Leach and Webb (1981) have reported difficulty in combining high resistance to *F. coeruleum* and *F. sambucinum* in one clone.

In the same way, on the purpose of finding cultivars with resistance to more than one species, others authors have used more than one isolate to search for Fusarium dry rot resistance in potato tubers. Ayers (1956) and Leach and Webb (1981) both reported that there are few cultivars highly resistant to both *F. sambucinum* and *F. solani*. This is likely, because resistance to *F. sambucinum* and *F. solani* were under separate genetic control in the cultivars screened (Corsini and Pavek 1986). Later, Burkhart *et al.* (2007) found non-additive genetic variance when screening with a mixture of two isolates of *F. sambucinum* and one isolate of *F. solani*.

The temperature factor during storage aggravates the situation. Regardless, in trying to assess local cultivar's susceptibility to these Fusaria, it appears that 'Spunta', 'Mondial', and 'Nicola', the most cultivated in Tunisia, tolerated at least one *Fusarium* species: *F oxysporum* f. sp. *tuberosi* for the two first cultivars and *F. solani* for the second. However, 'Oceania' and 'Fabula' tolerated two species i.e. *F. oxysporum* f. sp. *tuberosi*/*F. graminearum* and *F. oxysporum* f. sp. *tuberosi*/*F. solani*, respectively. In confirmation with what has been said, suggesting its aggressiveness, there is no cultivar tolerating *F. sambucinum*. In this paper, we have reported that the rank order of susceptibility levels to dry rot varied depending on *Fusarium* species and on temperature of storage. When tubers were inoculated with *F. sambucinum*, cultivars placed in the less susceptible group at 30°C were classified as highly susceptible at 15°C. However, when tubers were inoculated with *F. oxysporum* f. sp. *tuberosi*, cultivars placed on category of less susceptible were almost the same at all tested temperatures. This variability of behavior of cultivars to *F. sambucinum* may be due to a large thermal spectrum of this pathogen suggesting an important ability of adaptation. This rich activity makes the ranking of cultivar's susceptibility more difficult and unstable. Our data are in agreement with previous data demonstrating the implication of temperature as a significant background noise in the varietal susceptibility to Fusarium dry rot. Indeed, Theron and Holz (1990) signaled that the different cultivars did not react uniformly to *Fusarium* spp. at different temperatures and they suggested that when evaluating cultivars for disease resistance or for effectiveness or disease control measures, test should be performed at standardized temperature. In the same way, Saremi and Burgess (2000) reported the strong effect of temperature on the community structure of *Fusarium*. They found that communities of *Fusarium* species were significantly different at different temperatures. In the light of the findings of this investigation, the importance of temperature on assessment of the local potato cultivar's susceptibility to *Fusarium* species is confirmed. Results shown in this work concerning *F. solani* inoculated as combined isolates joins findings of Daami-Remadi *et al.* (2006b) who indicated that *F. solani* (one isolate used for inoculation), seems to have its thermal optimum of *in vivo* development situated between 30 and 35°C. However, *F. sambucinum* and *F. graminearum* showed their highest aggressiveness at temperatures less than 25°C for all cultivars pooled like the investigations previously taken in Tunisia with each inoculum type.

The data exposed in the current study imply that a strategy of optimally combining cultivar resistance could potentially be integrated into a programme to manage Fusarium dry rot and Verticillium wilt. In fact, the susceptibility of many commonly grown potato cultivars to Verticillium wilt was investigated in Tunisia (Daami-Remadi *et al.* 2010) and the results revealed that the majority of tested cultivars showed different resistance to both diseases, exceting "Arinda", cultivar with same susceptibility to both *Fusarium* and *Verticillium* species.

Due to the shown variability of the level of susceptibility to each *Fusarium* species depending temperature of storage, the presence of these *Fusaria* as mixed infections on potato tubers, further studies are also needed for checking potato cultivar's behavior toward dry rot agents based on bi-, tri-, and tetra- inoculations mimicking natural infections.

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