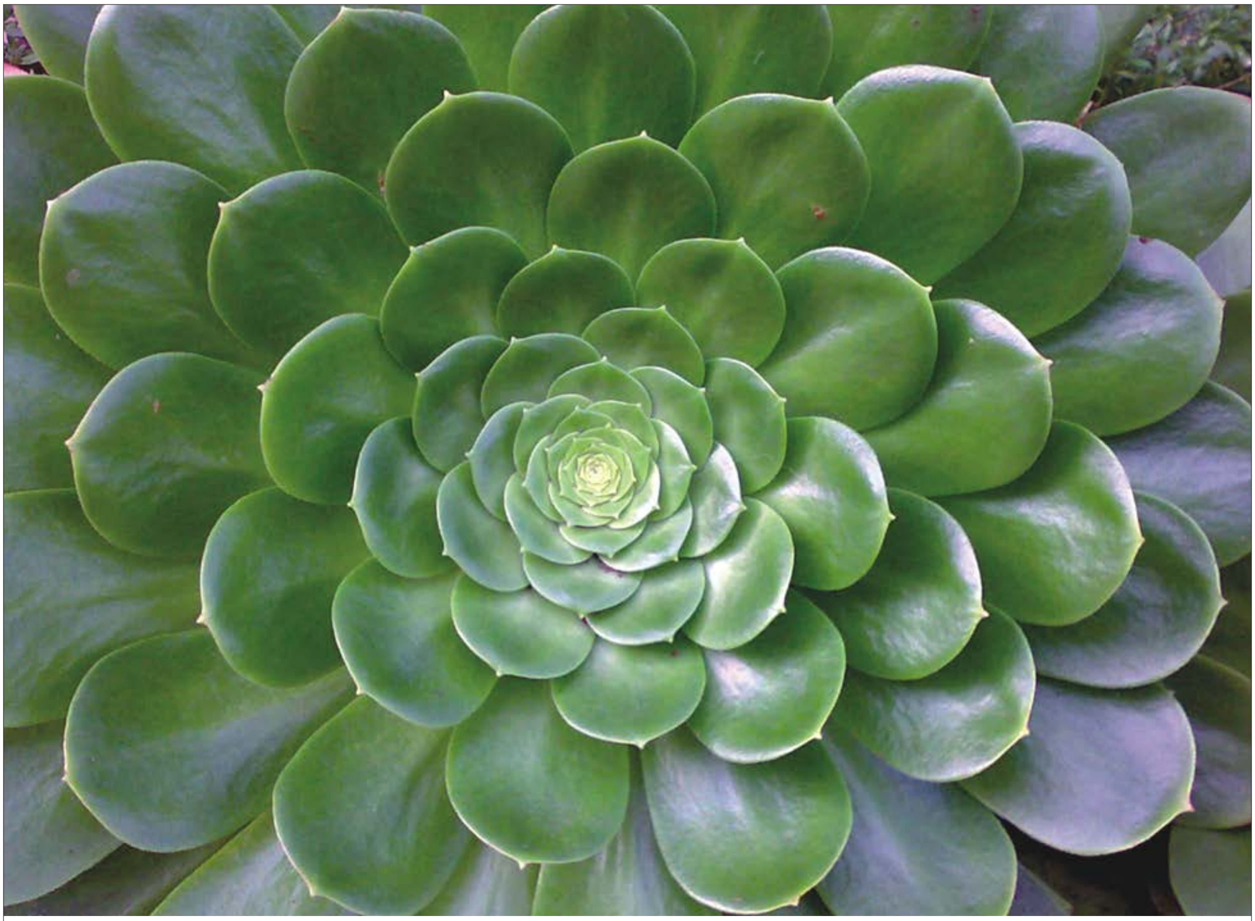

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Comparative Susceptibility of Some Local Potato Cultivars to Four *Fusarium* Species Causing Tuber Dry Rot in Tunisia

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Abstract: Comparative susceptibility of local potato cultivars to *Fusarium graminearum*, *F. sambucinum*, *F. solani* and *F. oxysporum* f.sp. *tuberosi*, causing tuber dry rot is investigated in the current study for the first time in Tunisia. Obtained results revealed that these *Fusarium* species showed variable aggressivity upon the 11 tested potato cultivars. In fact, *F. graminearum* was the most aggressive on the majority of cultivars; *F. sambucinum*, *F. solani* and *F. oxysporum* f.sp. *tuberosi*, showed a comparable aggressivity on some used cultivars. After 21 days of incubation at 25-27°C, all inoculated tubers of all cultivars showed dry rot symptoms with different degree revealing that cultivar's resistance was distinct against *F. oxysporum* f.sp. *tuberosi*, *F sambucinum*, *F. graminearum* and *F. solani*. Furthermore, no cultivars were completely resistant to the whole *Fusarium* complex and only some cultivars showed a lesser susceptibility to at least two species this is the case for Mondial and Spunta against *F. sambucinum* and *F. oxysporum* f.sp. *tuberosi* and the case of Liseta against *F. graminearum* and *F. oxysporum*.

Key words: *Solanum tuberosum*, *Fusarium* complex, inoculation, assessment, susceptibility, aggressivity

Introduction

Several studies on potato *Fusarium* dry rot showed that causal agents had an important specificity and a variable aggressivity between countries. In the world, dry rot incidence depended on *Fusarium* spp., soil, local climatic conditions and cultivars. *F. roseum* var. *sambucinum*, *F. solani* var. *coeruleum* and *F. oxysporum* are the most common causal agents of tuber dry rot (Adams and Lapwood, 1983; Tivoli *et al*., 1986a, 1988; Theron and Holz, 1989, 1990; Desjardins and Gardner, 1991; Desjardins *et al*., 1992; Manici and Cerato, 1994; Choiseul, 1996; Carnegie *et al*., 2001; Daami-Remadi *et al*., 2006). Moreover, tuber susceptibility was found to be distinct to *F. solani*, *F. roseum* and to the other *Fusarium* species (Jellis, 1975; Hooker, 1981; Jellis and Starling, 1983; Corsini and Pavek, 1986; Tivoli *et al*., 1986a; Wastie *et al*., 1989). Specific cultivar reactions were observed against *F. solani*, *F. sulphureum* (Wastie and Bradshaw, 1993) and *F. oxysporum* (Huaman *et al*., 1989); and the ranking of tested cultivars was affected by *Fusarium* species used for tuber inoculation (Hooker, 1981).

Incidence of potato dry rot and occurrence of fungicide resistance (Tivoli *et al*., 1986b; Hide *et al*., 1992; Desjardins *et al*., 1993; Nolte, 1993; Hanson *et al*., 1996; Holley and Kawchuk, 1996; Choiseul, 1996; Platt, 1997; Thomson and Waterer, 1999; Peters *et al*., 2001) led to more focus research on tuber resistance and other control alternatives. Wild *Solanum* species are tested by several

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authors and Wiersema (1977) found that some resistant cultivars to nematodes, obtained via several crosses between *Solanum andigenum* and *S. vernei*, showed a very high degree of resistance to *Fusarium* sp. As minor differences in resistance within *S. tuberosum* are known, *S. spegazzinii*, *S. acaule* and *S. kurtzianum* showed resistance to root infection by *F. eumartii* in infested soil (Hooker, 1981). Furthermore, the wild species *Solanum ehrenbergii*, *S. spegazzinii*, *S. chacoense* and *S. vernei* were reported to be highly resistant to *F. solani* var. *coeruleum* and *F. sulphureum* (Huaman *et al*., 1989). A multitude of crossings were realized between clones and cultivars having various degree of resistance to *F. sulphureum* and *F. coeruleum* (Wastie and Bradshaw, 1993). However, several authors have reported difficulty in combining high resistance to *F. sambucinum* and to *F. coeruleum* in cultivars and progenies of *S. tuberosum* ssp. *tuberosum* (Huaman *et al*., 1989).

Some authors studied behaviour of one or two *Fusarium* species and comparison of different observations and their transposition to another species is hazardous due to variations in biological particularities of each pathogen.

Currently, it is clear that resistance to *Fusarium* species was genetically distinct and also inherited (Corsini and Pavek, 1986; Wastie *et al*., 1989). Therefore, these authors specified that cultivar resistance must be assessed for each *Fusarium* specie separately.

In Tunisia, screening of local potato cultivars for resistance to *Fusarium* is indispensable due to the involvement of a *Fusarium* species complex is disease development and especially after appearance of benzimidazole-resistant isolates of *F. sambucinm* (Triki *et al*., 2001; Chérif *et al*., 2001; Daami-Remadi and El Mahjoub, 2004, 2006). Furthermore, these *Fusarium* species are present as mixed infections on potato tubers in refrigerated and unrefrigerated stores (Daami-Remadi *et al*., 2006) and some of them are also wilt pathogens (Ayed *et al*., 2006a, b). Thus the major objective of this study is to assess the local potato cultivar's susceptibility to the major *Fusarium* species.

Materials and Methods

Pathogens

Fusarium spp. were isolated from tubers showing typical symptoms of dry rot or potato plants partially or totally wilted. It is a fungi complex composed of *F. solani*, *F. graminearum* (isolated on 2003), *F. sambucinum* and *F. oxysporum* f. sp. *tuberosi* (isolated on 2004) from tubers cv. Spunta showing typical symptoms of dry rot. These *Fusarium* species are grown at 25°C on PDA for one week. They are stored at -20°C in 20% glycerol solution for long term preservation.

Potato cultivars

Potato cultivars tested in the present study are listed in Table 1. They are provided by the Technical Potato Center of Tunisia and they are stored for two months in the darkness at 6°C and bought to room temperature 3 h before use.

Table 1: Characteristics of potato cultivars tested for their tolerance to four *Fusarium* species causing potato dry rot in Tunisia

Cultivars*	Tuber shape	Skin color	Flesh	Dry matter (%)
Alaska	oblong	yellow	white	19.2
Asterix	oblong, prolonged	red	clear yellow	21.3
Atlas	oblong	yellow	yellow	19.1
Fabula	oval	pale yellow	pale yellow	-
Latona	oblong	yellow	yellow	19.0
Liseta	oblong	yellow	yellow	19.0
Mondial	oblong, prolonged	yellow	yellow	19.0
Platina	oblong	yellow	yellow	18.5
Safrane	oblong	yellow	yellow	19.2
Spunta	oblong, prolonged	yellow	yellow	18.5
Timate	oblong	yellow	pale yellow	19.0

*Potato cultivars registered in the list A by the Tunisian Technical Potato Center (CTPT)

Tests of potato cultivar's susceptibility to Fusarium species

Tubers of different tested cultivars are superficially disinfected with a solution of 10% sodium hypochlorite, for 5 min and then rinsed abundantly with sterile distilled water. Container and alveolus plaques used for inoculated tubers incubation, are washed before use, dipped for 24 h in sodium hypochlorite solution then rinsed with sterile distilled water.

Dimension of inoculation sites is of 6 mm diameter and depth. Inoculation technique consists of deposing an agar disc (6 mm diameter) colonized by pathogen at occasioned wounds. Tuber incubation is realized at 25-27°C for 21 days, in a growth chamber, at high relative humidity. Every elementary treatment is repeated twenty times (ten tubers x two wounds).

After incubation period, tubers were cut longitudinally via sites of inoculation. Parameters of dry rot induced (maximal width (w) and depth (d)) are noted. The pathogen penetration within tubers is calculated following formula of Lapwood *et al*. (1984) where:

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

Cutltivar's susceptibility to *Fusarium* species was estimated following the below scale:

- Less or moderately susceptible: mean penetration $\leq$ 10 mm
- Susceptible: 10 mm <mean penetration < 15 mm
- Highly susceptible: mean penetration $\geq$ 15 mm

Statistical analyses (ANOVA) are performed following a completely randomized factorial design where cultivars and *Fusarium* spp. are both fixed factors. Means are separated using Fisher's protected LSD test ($p \leq 0.05$).

Results

Mean pathogen penetration noted after 21 days of incubation at 27°C varied upon tested cultivars and *Fusarium* spp. used for tuber inoculation (Table 2). A significant interaction ($p \leq 0.05$) was observed between both fixed factors. This interaction was traduced by variable degrees of dry rot development depending on tested cultivars and *Fusarium* spp. under similar conditions of inoculation and incubation.

Among the 11 tested cultivars, nine were found to be highly susceptible to *F. graminearum* showing a mean penetration reaching or exceeding 15 mm. However, this pathogen induced a dry rot of lesser importance on cv. Liseta. However, cvs. Atlas, Platina (Fig. 4), Spunta, Fabula, Alaska (Fig. 1 and 2), Astérix, Mondial, Safrane, Timate and, at a lesser degree, Latona were moderately susceptible.

When inoculated by *F. sambucinum*, tubers belonging to four within the 11 tested cultivars (Fabula, Atlas, Astérix and Timate) were highly susceptible showing mean a penetration superior or

Table 2: Aggressivity of four *Fusarium* species noted, after 21 days of incubation at 25-27°C, on potato tubers belonging to 11 different local cultivars as measured by mean pathogen penetration (mm) into inoculated tubers

	Alaska	Asterix	Atlas	Fabula	Latona	Liseta	Mondial	Platina	Safrane	Spunta	Timate
F. gram	17.9	17.7	20.0	18.3	12.7	3.6	14.9	19.2	15.2	18.3	14.7
F. samb.	14.1	15.1	16.7	19.1	12.5	12.8	5.4	11.6	12.6	5.8	15.5
F.oxysp.	11.2	14.5	12.9	17.6	11.0	4.8	5.5	10.9	13.0	6.5	14.9
F. solani	11.7	18.3	10.9	16.9	12.9	16.7	9.1	14.9	14.0	11.4	14.4

LSD (cultivars x *Fusarium* spp.) = 2.4 mm ($p \leq 0.05$). *F. gram*: F. *graminearum*, *F. samb.*: *F. sambucinum*, *F. oxysp.*: *F. oxysporum* f. sp. *tuberosi*

Fig. 1: Dry rot occasioned by *F. sambucinum* (left) and *F. graminearum* (right) on tubers of the cultivar Alaska noted after 21 days of incubation at 25-27 C

Fig. 2: Dry rot occasioned by *F. oxysporum* f.sp. *tuberosi* (left) and *F. solani* (right) on tubers of the cultivar Alaska noted after 21 days of incubation at 25-27 C

Fig. 3: Dry rot occasioned by *F. oxysporum* f.sp. *tuberosi* on tubers of the cultivar Astérix noted after 21 days of incubation at 25-27 C

Fig. 4: Dry rot occasioned by *F. sambucinum* (left) and *F. graminearum* (right) on tubers of the cultivar Platina noted after 21 days of incubation at 25-27°C

equal to 15 mm. However, in the case of *F. oxysporum*, three within the 11 used cultivars, Fabula, Astérix (Fig. 3) and Timate, were highly susceptible whereas Alaska, Atlas, Latona, Platina and Safrane were found to be susceptible to this pathogen.

When *F. solani* was used for tuber inoculation, five within the 11 tested cultivars showed a higher susceptibility; this is the case of Asterix, Fabula, Liseta, Platina and Timate cultivars. However, Safrane, Latona, Spunta, Atlas and Alaska (Fig. 2) were susceptible to this pathogen whereas a lesser susceptibility to *F. solani* was observed on Mondial.

This study showed that Mondial, Liseta and Spunta tolerated at least two *Fusarium* species according to our inoculation and incubation conditions whereas the other tested cultivars were found to be susceptible to all tested *Fusarium* species.

Concerning the pathogen aggressivity, *F. graminearum* was the most aggressive on the majority of tested cultivars but, on certain cultivars, *F. sambucinum*, *F. solani* and *F. oxysporum* also showed a similar aggresivity to that of *F. graminearum*.

Finally, local cultivar's reaction to the tested *Fusarium* species varied upon pathogen used for tuber inoculation. Therefore, their tolerance or susceptibility is distinct depending on *F. graminearum*, *F sambucinum*, *F. solani* and *F. oxysporum*.

Discussion

In the present study, the screening of local potato cultivars against four *Fusarium* species actually implicated in dry rot development is original because it has never been investigated in Tunisia. Furthermore, this originality is also attributed to the site-specific of this kind of research due to the multiple variations concerning pathogen aggressivity and plant material between different countries.

Obtained results showing the interaction between dry rot agents and cultivars reflect the incidence of this post-harvest disease. This observation is supported by the fact that every *Fusarium* species can be as aggressive as the most aggressive ones on certain tested cultivars. *F. graminearum* was shown to be the most aggressive on the majority of tested potato cultivars. *F. sambucinum* showed a comparable aggressivity as *F. graminearum* on three cultivars.

This result concerning higher aggressivity of *F. graminearum* comparatively to other tested species is original because all comparative studies realized all over the world have shown that *F. sambucinum*, *F. solani* and *F. oxysporum* are the most common aggressive *Fusarium* species on potato (Tivoli and Jouan, 1981; Tivoli *et al*., 1986a, 1988; Theron and Holz, 1989, 1990;

Wastie *et al*., 1989; Desjardins *et al*., 1992; Choiseul, 1996; Carnegie *et al*., 2001; Vitale *et al*., 2004; Daami-Remadi and El Mahjoub, 2004; Ayed *et al*., 2006a). Present result concerning aggressivity of *F. graminearum*, according to our inoculation and incubation conditions, do not converge to that obtained by Tivoli and Jouan (1981) who classified this pathogen within the lesser aggressive *Fusarium* group and *F. sambucinum* within the most aggressive one.

Obtained results also revealed, according to incubation conditions, that *F. solani* was more aggressive than *F. graminearum* in five tested cultivars. Similarly, *F. oxysporum* developed the most important dry rot on four cultivars comparatively to *F. sambucinum* and *F. graminearum*. This finding illustrates the interaction *Fusarium* x potato cultivars and joins other reports concerning the distinct degree of resistance of potato cultivars to *Fusarium* species even after their inoculation and incubation in the same conditions (Seppanen, 1981; Jellis and Starling, 1983; Corsini and Pavek, 1986; Wastie *et al*., 1989; Platt, 1992; Choiseul, 1996; Mortazavi-Bak and Nasr-Esfarani, 1998, Ayed *et al*., 2006b). This result confirms a previous comparative susceptibility assessment of 16 potato cultivars to *F. sambucinum*, *F. graminearum*, *F. culmorum* (european isolates) and local *F. solani* (Daami-Remadi and El Mahjoub, 1996) where, moreover, intra-specific variations were observed within european *F. sambucinum* and local *F. solani* isolates. Therefore, local cultivar's resistance was concluded to be distinct to *F. sambucinum*, *F. graminearum*, *F culmorum* and *F. solani*.

The screening of local cultivar's resistance is enhanced by the increasingly incidence of the disease, the qualitative evolution of the dry rot causal agents and also by the appearance of benzimidazole-resistant strains of *F. sambucinum* in Tunisia (Daami-Remadi and El Mahjoub, 2004, 2006). An intra-specific difference of *F. sambucinum* was observed for thiabendazole-resistant and thiabendazole-sensitive strains (Tivoli *et al*., 1986b; Desjardins *et al*., 1993; Choiseul, 1996). Moreover, according to Choiseul (1996), it seems that potato cultivars could affect even efficacy of fungicides used for *Fusarium* dry rot control and a significant interaction between fungicides, *Fusarium* species and cultivars was observed.

The most common cultivars planted in Tunisia such a Mondial, Spunta and Timate were susceptible to *Pythium aphanidermatum* causing potato tuber leak (Priou *et al*., 1997). This study revealed their susceptibility to *F. graminearum* and at a lesser degree to *F. solani*. Therefore, the screening for cultivar resistance to all *Fusarium* spp. is more important than to one *Fusarium* species but highly susceptible to others as suggested by Tivoli *et al*., (1986a). The present assessment demonstrated that no cultivars appeared resistant to all tested *Fusarium* species but some of them tolerated at least two species. This finding is supported by the fact that Leach and Webb (1981) have reported difficulty in combining high resistance to *F. coeruleum* and *F. sambucinum* in one clone.

Concerning the host inoculation stage used in this screening for resistance to *Fusarium* dry rot, tubers were stored at 6°C during two months and placed at room temperature prior the test. A similar method was followed by Theron and Holz (1987) and this for breaking tuber natural resistance observed at harvest. The tested tubers physiological age is optimal because they remained turgescent even after 21 days of incubation. We detailed this physiological age criterion because Tivoli *et al*. (1986a) reported that this parameter is an important factor for assessing potato cultivars and that disease level is lower in younger tubers.

Our method of inoculation was used in many studies dealing with dry rot and other tuber rot agents (Tivoli *et al*., 1986a; Huaman *et al*., 1989; Priou *et al*., 1997). Furthermore, Wastie *et al*. (1989) found no year x cultivar interactions for 15 cultivars tested over 8 years under Scottish conditions. However, other studies signaled variation in the ranking of cultivars infected with *F. coeruleum* between years (Lees *et al*., 1998) but several authors suggested that one year's assessment of cultivars resistance to dry rot is sufficient (Leach and Webb, 1981; Huaman *et al*., 1989; Wastie *et al*., 1989) as followed in the present study.

As potato tuber resistance to all *Fusarium* species is complex, other studies are required to include tuber physiology in dry rot susceptibility because this criterion is influenced by plantation date, fertilization, irrigation, soil quality, conservation duration and temperature. Furthermore, as an interaction of the *Fusarium* complex with temperatures of incubation engendered a variable aggressivity (Daami-Remadi *et al*., 2006), the effect of temperature will be included in our future studies on differential susceptibility of some potato clones to these *Fusarium* species.

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