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Relative Susceptibility of Nine Potato (*Solanum tuberosum* L.) Cultivars to Artificial and Natural Infection by *Rhizoctonia solani* as Measured by Stem Canker Severity, Black Scurf and Plant Growth

Mejda Daami-Remadi[1*] • Samia Zammouri[2] • Mohamed El Mahjoub[2]

[1] Centre Régional des Recherches en Horticulture et Agriculture Biologique, 4042, Chott-Mariem, Sousse, Tunisia
[2] Institut Supérieur Agronomique de Chott-Mariem, 4042, Chott-Mariem, Sousse, Tunisia

Corresponding author: * **daami_rm@yahoo.fr**

ABSTRACT

The relative susceptibility of nine potato cultivars against *Rhizoctonia solani* was tested via natural seed tuber infection and artificial culture substrate inoculation. The assessment was based on stem canker and black scurf severity and negative effect on plant growth and expected impact on yield. In both experiments, symptoms of Rhizoctonia stem canker were observed on all inoculated and naturally infested plants compared to non-infested controls. Furthermore, cultivars tested showed a variable reaction to *R. solani*, expressed by varying degrees of stem canker and black scurf severity and subsequent plant growth, but no totally immune cultivars were found. Under either artificial or natural infection by *R. solani*, cv. 'Spunta', and to a lesser degree 'Elodie', were shown to be the most susceptible to this pathogen whereas 'Alaska' and 'Daisy' were the most tolerant. However, although its higher susceptibility to Rhizoctonia disease and reduced growth compared to other cultivars tested, 'Spunta' showed the highest progeny tuber weight but with a pronounced incidence of black scurf.

Keywords: cultivar behaviour, disease incidence, inoculum source, seed tuber, yield

INTRODUCTION

Rhizoctonia solani Kühn [teleomorph *Thanatephorus cucumeris* (Frank) Donk] is a destructive soil-borne pathogen that causes diseases in many plant species world-wide (Carling *et al*. 2002). Rhizoctonia stem canker or black scurf is an economically important disease of potatoes around the world. It reduces the quality and the yield of potatoes and has become an important impediment for export of potatoes and self seed production in Tunisia (Daami-Remadi *et al*. 2008). Furthermore, when infected tubers are used as seeds, they will serve as inoculum sources for future potato crops because cold and wet soil conditions are favourable to sclerotia germination at seed tuber surfaces (**Fig. 1**). Thus, damage to potato plants by *R. solani* begins primarily beneath the soil surface where developing subterranean stems and stolons are particularly susceptible to attack (Banville 1978). Sources of infection include seed tubers and the soil itself, which may contain different types, as well as variable levels of inoculum (Jager *et al.* 1991). In fact, currently, *R. solani* isolates have been divided into 14 groups based on their anastomosis behaviour (Carling *et al*. 2002) and isolates belonging to AG-3, which usually attacks emerging shoots and survives as sclerotia on infected tubers (Roland 2006), are well adapted to proliferate over a range of soil water potentials well beyond the limits of their potato host and this must be one of the factors involved in its success as a soil-borne plant pathogen (Ritchie *et al*. 2006).

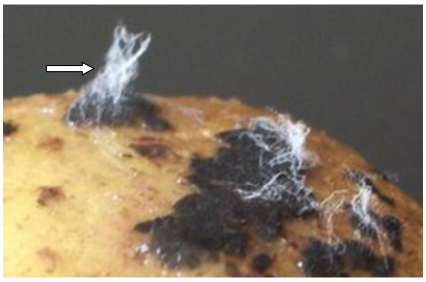

Fig. 1 Germinating *R. solani* sclerotia on potato seed tuber of 'Spunta'.

In Tunisia, Rhizoctonia disease is managed by cultural practices and methods that minimize prolonged contact of the plant or tubers with the pathogen (Daami-Remadi *et al*. 2008). Furthermore, as potato areas are limited, and rotation is often difficult to apply, the use of resistant cultivars would obviously improve the control of this disease.

Potato cultivars/breeding selections showed a range of susceptibility reactions to *R. solani*, precisely to black scurf and stem canker, but none of these was completely resistant to these diseases (Anderson 1982; Chand and Logan 1983; Jeger *et al*. 1996; Bains *et al*. 2002). In fact, according to a survey realized by the National Federation of potato growers in France, cvs. 'Ackersegen', 'Bea', 'Regale', 'Stella', 'Ultimus', 'Viola' were classified as less susceptible whereas 'Arrran', 'Banner', 'B.F.15', 'Bintje', 'Farfadette', 'Ke Pondy', 'Saskia' and 'Sirt' were susceptible (Betencourt 1971). In other recent surveys, 'Estima' and 'Kennebec' were shown to be moderately susceptible to Rhizoctonia disease (Simons and Gilligan 1997; Wolski *et al*. 2006) whereas 'Nicola', 'Rosvall' and 'Claustar' were reported to be susceptible (Tsror (Lahkim) and Peretz-Alon 2005). Furthermore, Rauf *et al*. (2005) found that, among 12 germplasm lines and cultivars of potato tested, the cultivars SH-5, SH-20 and the line CIP-9605 were found to be resistant to the disease causing fungus *R. solani* AG 3 (isolate SL-41) and that Faisalabad white was the most suscep-

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tible variety.

In Tunisia, as potato rhizoctonias were not deeply studied, cultivars' behaviour against this disease is still unknown although 'Nicola' and 'Spunta' seem to be susceptible to this pathogen (Daami-Remadi *et al*. 2008). 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. Furthermore, disease incidence and severity on daughter tubers is strongly influenced by the contaminated seed tuber and soil inoculum sources (Tsror (Lahkim) and Peretz-Alon 2005) and both inoculum sources have the ability to cause disease individually or in association (Platt *et al*. 1993; Wharton *et al*. 2007). Thus, the relative cultivar's susceptibility to Rhizoctonia disease was assessed, in the present study, via natural seed tuber infection and artificial culture substrate inoculation.

MATERIALS AND METHODS

Plant material: origin and preparation

Potato tubers of several cultivars (class A) used were kindly provided by the Technical Center of Potato, Essaïda, Tunisia. A fraction of tubers naturally infected by *R. solani* sclerotia (originating from an initial stock showing 5 to 10% of natural infection depending on cultivars) was used without any pathogen inoculation and their level of infection was determined according to the French scale used in Daami-Remadi *et al*. (2008). The second fraction of tubers was macroscopically free of black scurf symptoms and these were to be used for the inoculation trials.

Two weeks before planting, tubers were superficially disinfected in a commercial sodium hypochlorite solution (commercial bleach containing 12% of active chlorine) diluted to 5% for 5 min then rinsed with water and air dried. They were placed under environmental conditions favourable for pre-germination (15-20°C, 60-80% relative humidity and natural room light).

Germinating tubers (non-inoculated control, inoculated or naturally infected) were then individually planted in plastic pots (6 litres) containing a mixture of perlite and peat (25:75) previously sterilized for 60 min at 107°C. After emergence, plants were irrigated every 2 to 3 days.

Pathogen

Isolates of *R. solani* were obtained during 2006 from Tunisian potato-growing areas (Essaïda, Gafsa, Testour, Kairouan) by isolating from tubers showing typical black scurf symptoms (two isolates) and plants with cankers at their stem bases (two isolates). Diseased tissues (stem canker or tuber periderm tissue with sclerotia) were rinsed thoroughly in tap water and cut into 0.5 cm^2 pieces. After surface-disinfesting in sodium hypochlorite (10%) for 3 min, the pieces were rinsed three times in sterile distilled water and dried on sterile filter paper. Pieces were plated on PDA (Potato Dextrose Agar) medium containing 300 mg/l of streptomycin sulphate (Pharmadrug Production Gmbh). Fungal cultures were incubated for 10 days at 25°C in the dark and were cleaned up by subculturing successively on PDA plates from the edge of actively growing colonies.

Isolates' pathogenicity was previously confirmed on potato plants 'Spunta' from which the pathogen was then re-isolated, purified and stored on PDA at 4°C until further use.

Mycelium and sclerotia of each isolate of *R. solani* were scraped from 10 days old cultures (Petri dishes, 9 cm in diameter) on PDA with a sterile flamed scalpel then mixed with other isolates with sterile distilled water (one plate per isolate and per litre). The pathogen mixture was homogenized by an electric mixer for 5 min before plant inoculation.

Assessment of the behavior of potato cultivars against *R. solani*

As sources of *R. solani* infection include seed tubers covered in sclerotia and mycelium, and the soil itself (Jager *et al.* 1991), the assessment of the behaviour of some potato cultivars against this pathogen was realized by using two sources of inoculum. In fact, in a first assay, seed tubers were naturally infected with *R. solani* sclerotia at level 2 according to the French scale described in Daami-Remadi *et al*. (2008).

For the second assay, apparently non-infested seed tubers of each cultivar were individually planted as described above and the inoculation was made by adding 100 ml of pathogen mixture to each pot at the collar level 10 days after plant emergence. Control plants were irrigated by a similar volume of sterile distilled water.

During growth period and until the end of both assays, plants were irrigated every 2 to 3 days.

Four and five weeks post-plantation of the different cultivars of the first and the second assay respectively, plants were uprooted and their roots were washed with tap water, air dried then examined for Rhizoctonia disease induction and severity. The collar necrosis index (CNI) and the index of progeny tubers (IPT) infection by *R. solani* sclerotia were determined for all treatments according to the scales used by Daami-Remadi *et al.* (2008) then averaged over the total number of developed stems or tubers. Other parameters such as tuber fresh weight and aerial and subterranean parts fresh and dry weights were also noted per plant to elucidate the effect of the pathogen x cultivar interaction on potato growth and yield.

The pathogen was isolated from diseased plants and from every treatment to confirm that *R. solani* was responsible for the observed symptoms.

Statistical analyses

The first assay was arranged in a completely randomized experimental design where the sole fixed factor corresponded to the different potato cultivars. Five replicates were used per treatment.

The second essay was arranged in a completely randomised factorial design where potato cultivars and treatments (inoculated or control) are both fixed factors. Ten replicates were used per treatment.

Data were analyzed using SPSS (Ver. 11) and subjected to analysis of variance and Fisher's least significant difference test, LSD at $p \leq 0.05$.

RESULTS

Relative susceptibility of potato cultivars to artificial inoculation with *R. solani*

Two weeks post-plantation, the first symptoms of Rhizoctonia stem canker were observed and were accompanied by delayed plant growth on inoculated plants as compared to controls. These symptoms progressed to necrosis and cankers at the stem bases of inoculated plants.

Stem canker developed following inoculation by *R. solani*

The collar necrosis index (CNI), noted five weeks post-plantation, varied depending on the potato cultivar used and the treatment realized; a significant interaction (at $p \leq 0.05$) was observed between both fixed factors. **Fig. 2** shows the variable reaction of cultivars tested to inoculation by *R. solani*. In fact, the highest CNI, of about 3.282, was recorded on 'Spunta' and to a lesser degree 'Elodie', 'Mondial' and 'Arinda' (2.676, 1.988 and 1.983, respectively) which seem to be the most susceptible to this disease where stem canker was even accompanied by black scurf on daughter tubers of 'Spunta' (**Fig. 3**). However, for 'Alaska' and 'Daisy', the CNI noted did not exceed 1 on a scale with a maximum of 5 used (Daami-Remadi *et al.* 2008). All control plants of the majority of cultivars tested did not develop stem canker symptoms, except for 'Spunta' and 'Tango',

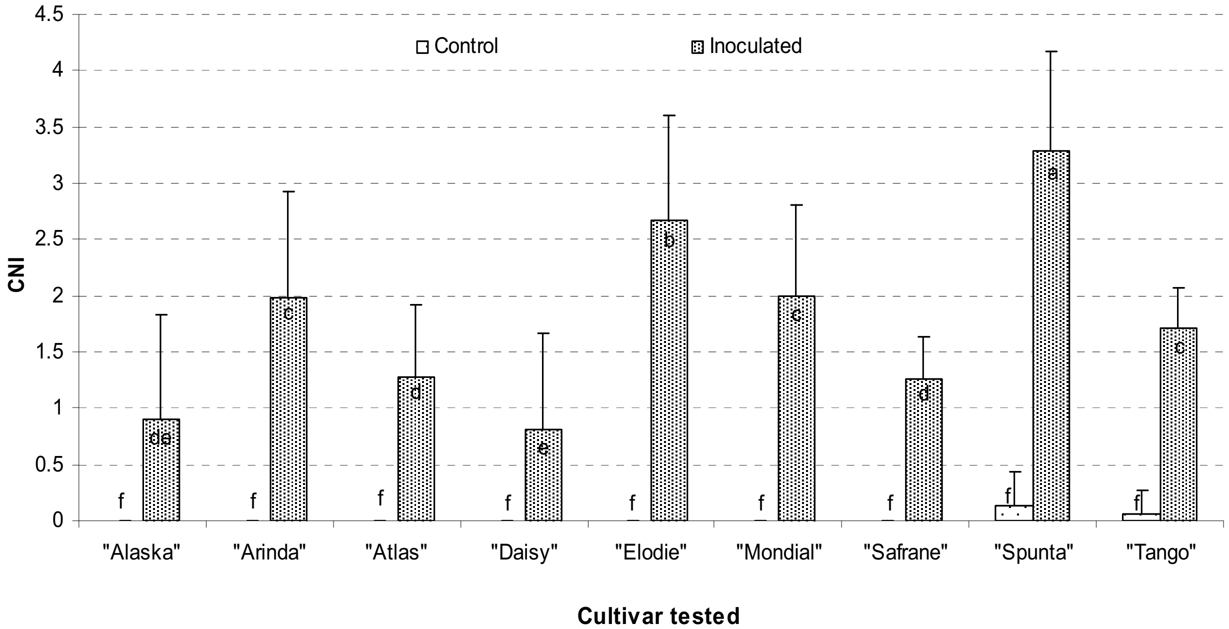

Fig. 2 Collar necrosis index (CNI), noted five weeks post-plantation, on inoculated potato plants of different cultivars compared to controls. A significant cultivar treatment interaction occurred and a two-way analysis was performed. For treatments (control or inoculated), bars (for cultivars) with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). The letters indicate analysis by cultivar tested for each treatment. Each bar represents the mean of 10 plants.

Fig. 3 Severity of stem canker (A) and black scurf (B), observed five weeks after plantation, on potato 'Spunta' plants inoculated by *R. solani.*

where a slight stem canker developed on some non-inoculated plants, and where the CNI was about 0.133 and 0.066, respectively.

Number of stems formed per plant following inoculation by *R. solani*

The mean number of stems per plant, noted five weeks post-plantation, varied depending on the potato cultivar used and the treatment realized; a significant interaction (at $p \leq 0.05$) was observed between both fixed factors. **Fig. 4** shows that substrate inoculation by *R. solani*, 10 days post-emergence, did not negatively affect the number of stems developed per plant except for 'Spunta' where this parameter was reduced by about 38% compared to the non-inoculated control. In the case of 'Atlas' and 'Alaska', the number of stems formed was 17% higher in inoculated plants than in non-inoculated controls.

Stem dry weight following inoculation by *R. solani*

The stem dry weight, noted five weeks post-plantation, varied depending on the potato cultivar used and the treatment realized; the interaction (at $p \leq 0.05$) between both fixed factors was non-significant but each factor individually affected this parameter significantly. In fact, stem dry weight was reduced by 10% on inoculated compared to non-inoculated control plants (**Fig. 5**). Moreover, the lowest value of variable stem dry weight was noted on 'Spunta' and 'Elodie' (**Fig. 6**), which seem to be most affected by Rhizoctonia disease and which showed to be the most susceptible; this depended on the CNI parameter (**Fig. 2**). However, the highest stem dry weight was noted on 'Mondial' and at a lesser degree 'Atlas' and 'Safrane'. The other cultivars tested showed intermediate stem dry weights (**Fig. 6**).

Root dry weight following inoculation by *R. solani*

The root dry weight, noted five weeks post-plantation, varied depending on the potato cultivar used and the treatment realized; the interaction (at $p \leq 0.05$) between both fixed factors was non-significant but each factor individually affected this parameter significantly. In fact, root dry weight was reduced by 19% on inoculated plants compared to non-inoculated controls (**Fig. 7**).

Fig. 8 shows the variability in root dry weight noted on tested cultivars. In fact, the lowest root biomass was recorded on 'Spunta' and to a lesser degree 'Alaska' and 'Daisy'. However, the highest root dry weight was noted on 'Safrane' and 'Atlas' (**Fig. 8**) as was also the case for the stem dry weight (**Fig. 6**). The other cultivars tested showed intermediate root dry weights.

Progeny tuber weight following inoculation by *R. solani*

The progeny tuber fresh weight, noted five weeks post-plantation, varied only depending on the treatment realized while the potato cultivar used had no significant effect on this parameter.

Fig. 9 shows that the tuber fresh weight was significantly reduced by about 17% on inoculated plants compared to non-inoculated controls. This illustrates the nega-

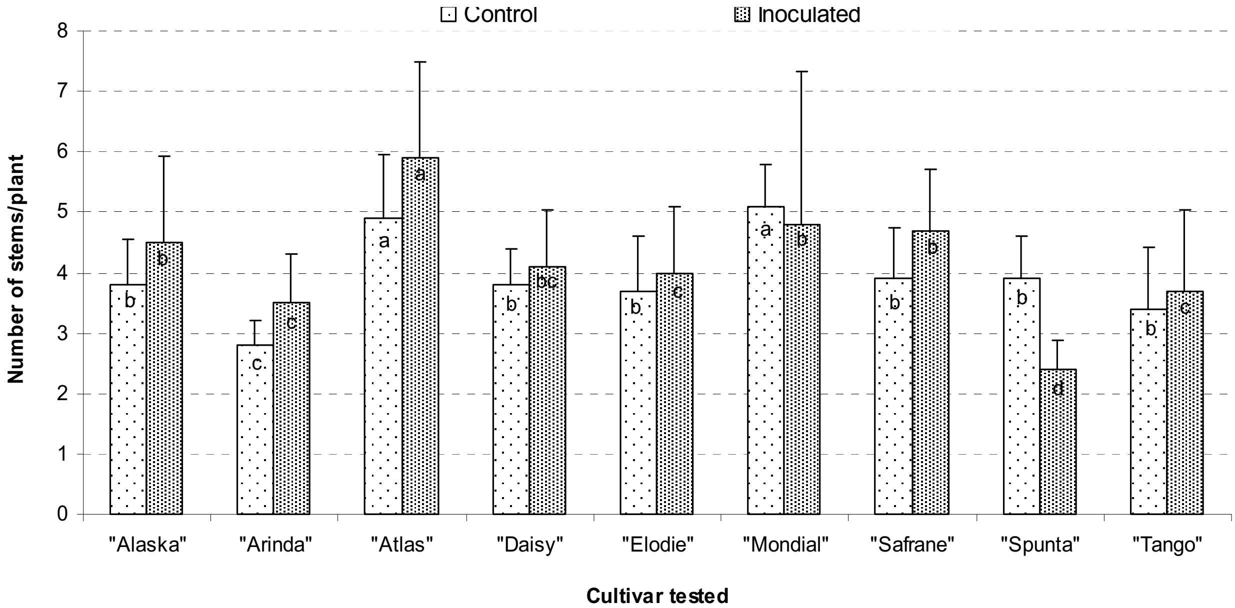

Fig. 4 Number of stems per plant noted, five weeks post-plantation, on inoculated potato plants of different cultivars compared to controls. A significant cultivar treatment interaction occurred and a two-way analysis was performed. For treatments (control or inoculated), bars (for cultivars) with the same letter are not significantly different according to Fisher's protected least significant difference LSD test (p≤0.05). The letters indicate analysis by cultivar tested for each treatment. Each bar represents the mean of 10 plants.

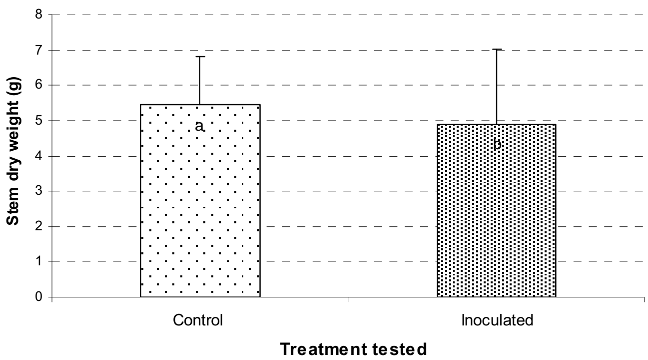

Fig. 5 Stem dry weight noted, five weeks post-plantation, on potato plants inoculated by *R. solani* compared to non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test (p≤0.05). Each bar represents the mean of 90 plants.

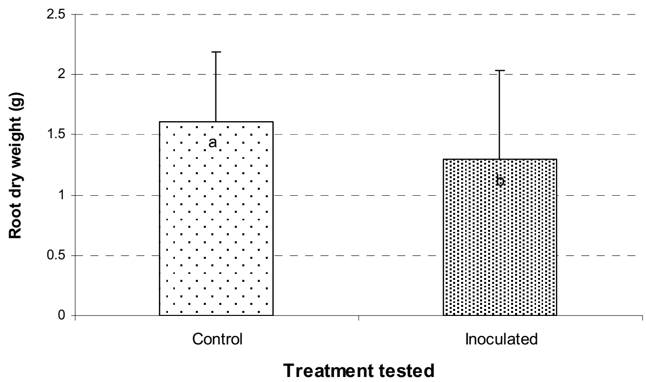

Fig. 7 Roots dry weight noted, five weeks post-plantation, on potato plants inoculated by *R. solani* compared to non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test (p≤0.05). Each bar represents the mean of 90 plants.

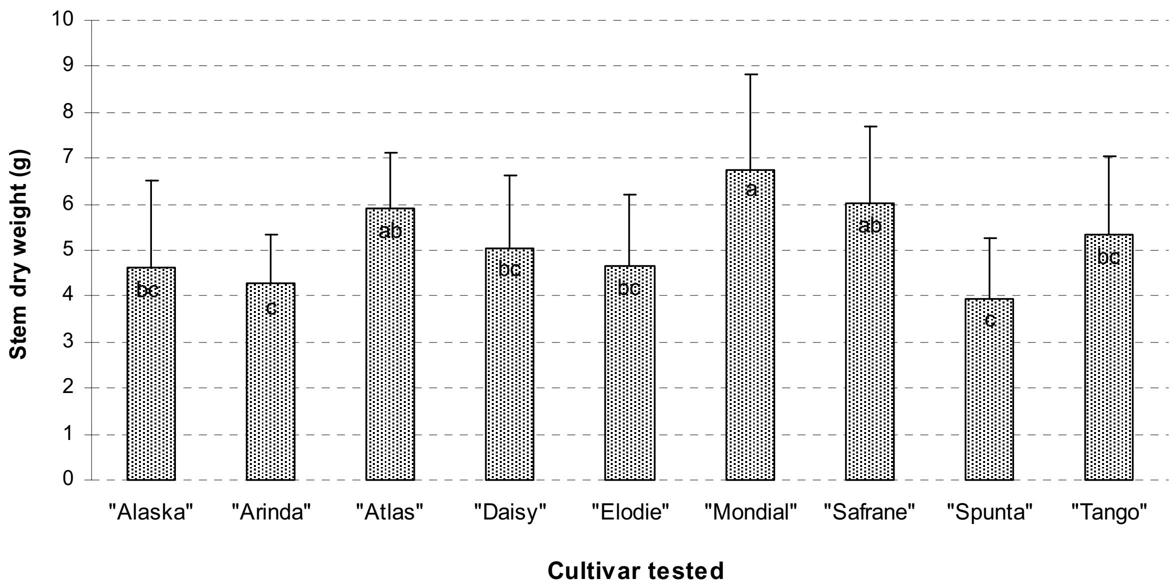

Fig. 6 Stem dry weight noted, five weeks post-plantation, on potato plants of different cultivars. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test (p≤0.05). Each bar represents the mean of 20 plants.

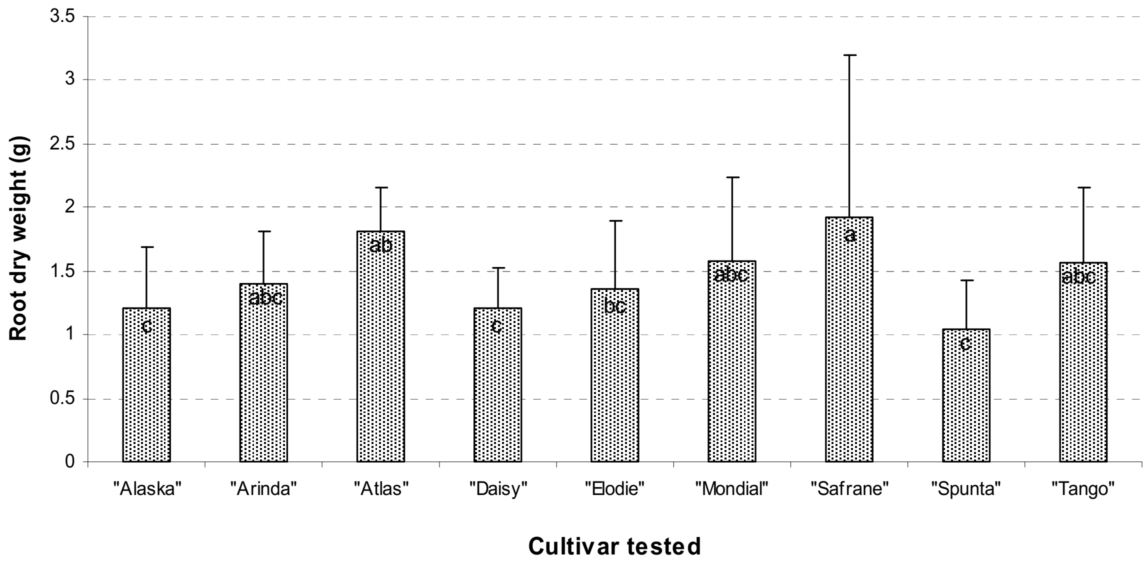

Fig. 8 Roots dry weight noted, five weeks post-plantation, on potato plants of different cultivars. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test (p≤0.05). Each bar represents the mean of 20 plants.

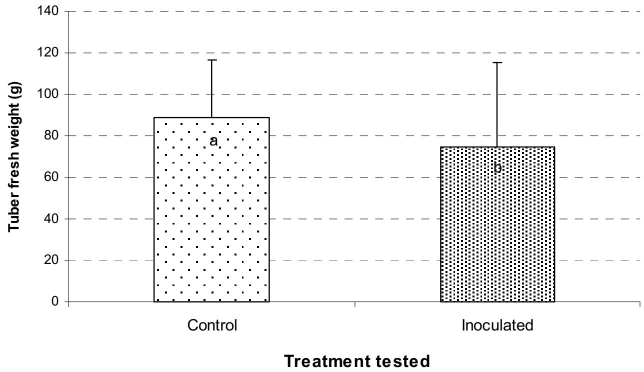

Fig. 9 Progeny tubers fresh weight noted, five weeks post-plantation, on potato plants inoculated by *R. solani* compared to non-inoculated control. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test (p≤0.05). Each bar represents the mean of 90 plants.

tive effect of *R. solani* on the expected yield. All potato cultivars tested had significantly similar tuber fresh weights.

Relative susceptibility of potato cultivars under natural infection by *R. solani*

1. Stem canker developed under natural infection by *R. solani*

The CNI, noted four weeks post-plantation, varied significantly depending on the potato cultivar used. In fact, the severity of variable stem canker expressed by cultivars in the presence of natural infection by *R. solani* had the highest CNI of about 3.03 in 'Spunta' and to a lesser degree in 'Atlas', 'Elodie' and 'Safrane' (**Figs. 10, 11**) with a CNI of about 2.012, 1.48 and 1.462, respectively; these cultivars seem to be the most susceptible to this disease. However, the lowest CNI of about 0.295 was noted on 'Alaska'; the CNI of 'Mondial', 'Tango' and 'Daisy' did not exceed 1; they seem to be less susceptible or tolerant to Rhizoctonia disease.

'Tango', showing a CNI of 0.637 and classed as less susceptible by CNI, induced on the developed stem canker an atypical formation of sclerotia-like structures at the stem bases and roots (**Fig. 12**). A similar phenomenon occurred normally on tubers, a condition commonly known as black scurf. Fungal isolations made on 'Tango' plants showing these symptoms revealed that *R. solani* is involved in this phenomenon.

2. Number of stems formed per plant under natural infection by *R. solani*

When seed tubers naturally infected by *R. solani* were used, the number of stems noted per plant, four weeks post-plantation, depended significantly (at p≤0.05) on the potato cultivar tested. In fact, the lowest stem number developed per plant, between 2 and 3, was recorded for 'Atlas' and at a lesser degree for 'Spunta' (**Fig. 13**); the latter cultivar also showed the highest stem canker severity (**Fig. 10**). However, 'Arinda', 'Daisy' and 'Tango' showed 7 to 8 stems per plant even when their initial seed tubers were infected with *R. solani* sclerotia at planting.

3. Stem dry weight obtained under natural infection by *R. solani*

The stem dry weight noted, four weeks post-plantation, varied depending on the potato cultivar used. All cultivars derived from seed tubers naturally infected by *R. solani* at planting had significantly comparable stem dry weights of about 6 to 9 g except for 'Spunta', which showed the lowest dry weight of about 3 g (**Fig. 14**); incidentally, it also had the lowest stem number per plant (**Fig. 13**).

4. Root dry weight obtained under natural infection by *R. solani*

In the presence of natural seed tuber infection by *R. solani* at planting, all potato cultivars tested showed significantly (at p≤0.05) variable root dry weights four weeks post-plantation. In fact, two groups of cultivars – based on root dry weight – could be distinguished (**Fig. 15**): the first one contained primarily 'Elodie', 'Safrane', 'Mondial' and 'Daisy', with more than 1.5 g, and the second one, including 'Spunta', had the lower root dry weight, followed by 'Atlas', 'Arinda' and 'Tango', which were intermediate to both groups.

5. Progeny tuber weight obtained under natural infection by *R. solani*

The progeny tuber fresh weight noted, four weeks post-plantation, varied depending on the potato cultivar used. In fact, **Fig. 16** shows that 'Spunta' plants had the highest tuber fresh weight (about 90 g), and to a lesser degree 'Elo-

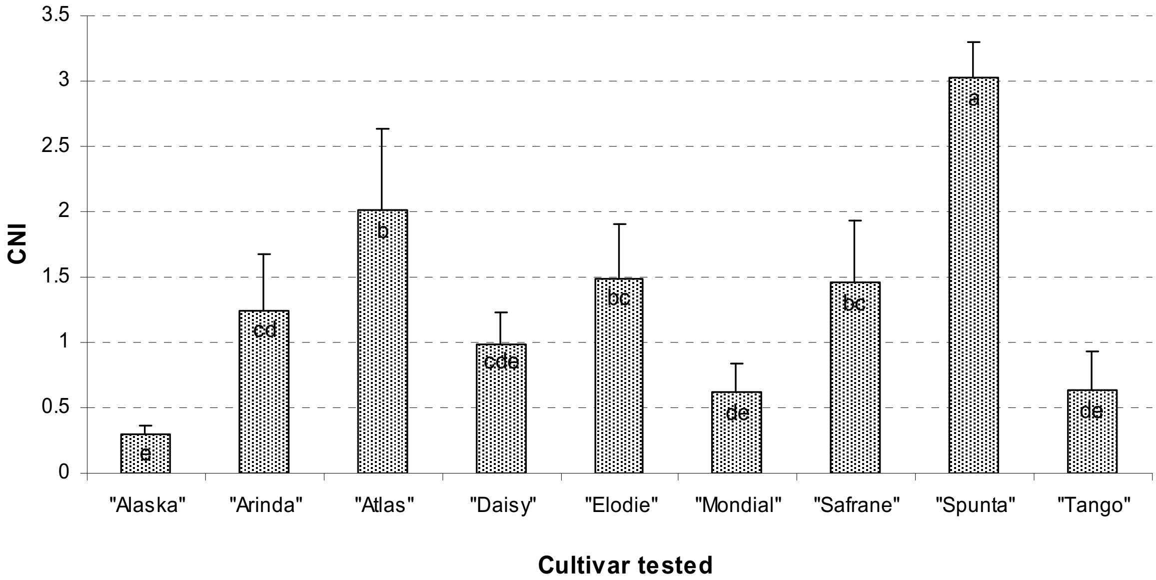

Fig. 10 Collar necrosis index (CNI), noted four weeks post-plantation, on potato plants of different cultivars naturally infected by *R. solani*. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). Each bar represents the mean of 5 plants.

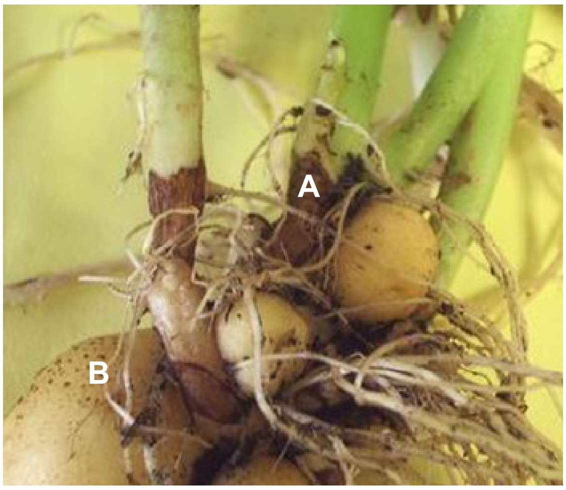

Fig. 11 Severity of stem canker (A) and black scurf (B), observed four weeks after plantation, on potato cv. 'Safrane' derived from seed tuber naturally infected by *R. solani* at planting.

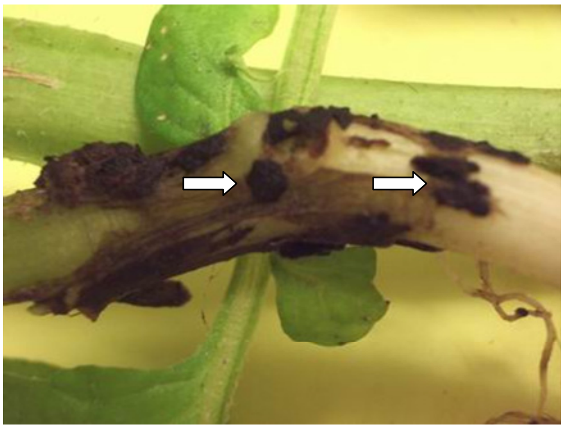

Fig. 12 Sclerotia of *R. solani* formed on stem canker, formed four weeks after plantation, on potato cv. 'Tango' derived from seed tuber naturally infected at planting.

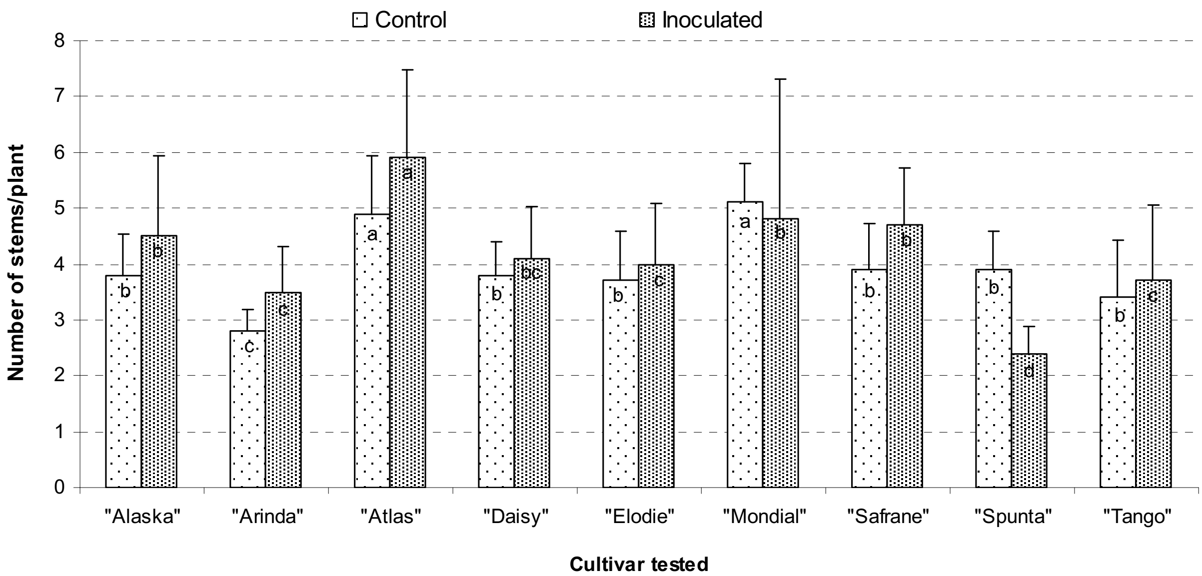

Fig. 13 Number of stems per plant noted, four weeks post-plantation, on potato plants of different cultivars derived from seed tuber naturally infected by *R. solani* at planting. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). Each bar represents the mean of 5 plants.

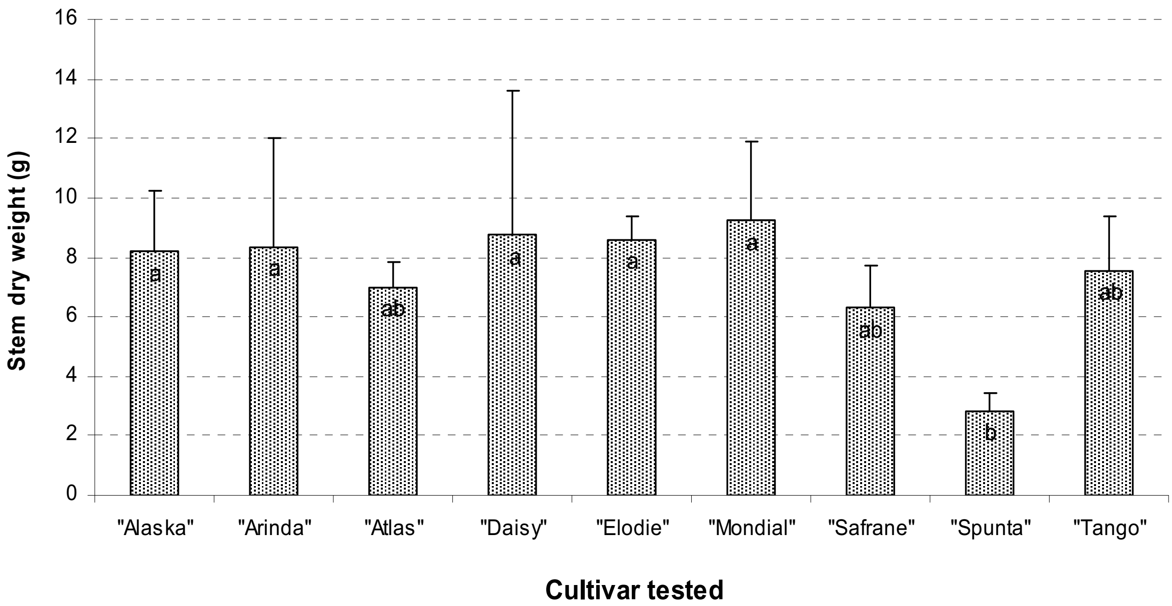

Fig. 14 Stem dry weight noted, four weeks post-plantation, on potato plants of different cultivars derived from seed tuber naturally infected by *R. solani* at planting. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). Each bar represents the mean of 5 plants.

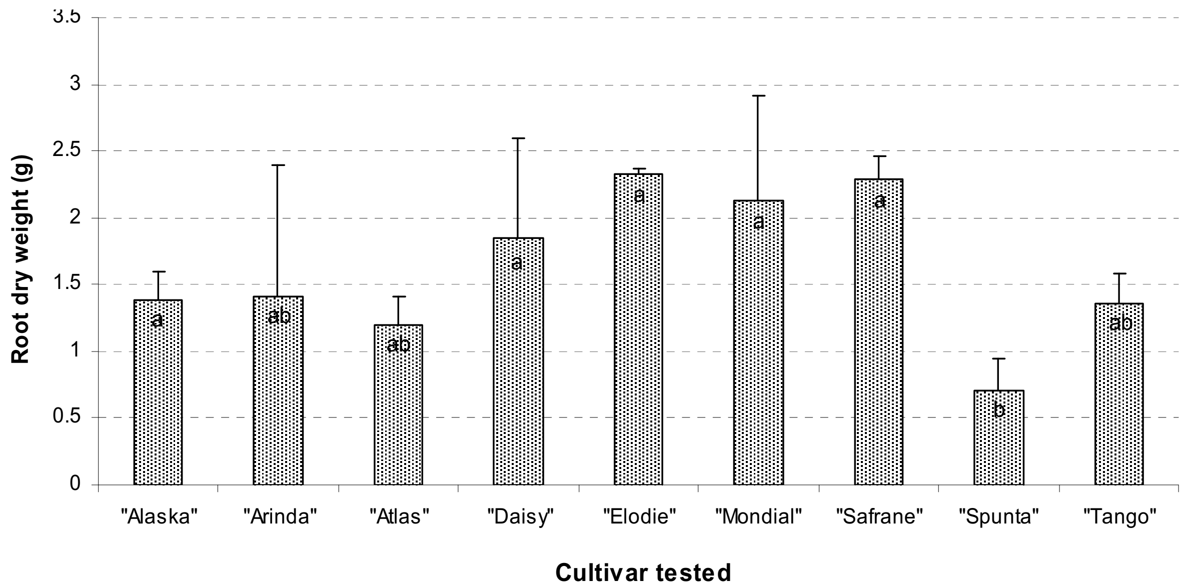

Fig. 15 Roots dry weight noted, four weeks post-plantation, on potato plants of different cultivars derived from seed tuber naturally infected by *R. solani* at planting. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). Each bar represents the mean of 5 plants.

die', 'Atlas' and 'Arinda'. However, 'Mondial' and 'Safrane' produced the lowest quantity, 35 to 45 g, of progeny tubers under experimental conditions.

6. Black scurf severity on progeny tubers under natural infection by *R. solani*

The indexes of progeny tuber (IPT) infection by *R. solani* sclerotia (black scurf), four weeks post-plantation, on potato plants derived from seed tubers naturally infected at planting varied significantly (at $p \leq 0.05$) depending on the cultivar used. Severe black scurf occurred on progeny tubers (**Fig. 17**) of 'Spunta', 'Daisy', 'Tango' (**Fig. 18**) and 'Alaska' where the IPT value lay between 1.13 and 1.66. However, the least incidence of black scurf occurred on 'Atlas' and 'Elodie' where the IPT was about 0.08; it less than 0.6 for the other cultivars tested.

DISCUSSION

The relative susceptibility of nine potato cultivars to Rhizoctonia disease was assessed, in the present study, via natural seed tuber infection and artificial culture substrate inoculation. Both methods were used in screening studies of the behaviour of potato cultivars against *R. solani* (Hide and Bell 1978; Hide *et al.* 1985, 1989, 1992; Csinos and Stephenson 1999; Tsror (Lahkim) and Peretz-Alon 2005). This assessment of host resistance or susceptibility was based on the quantification of symptoms (stem canker and black scurf), plant growth parameters and expected impact on yield.

In both experiments, symptoms of Rhizoctonia stem canker were observed, accompanied by heterogeneous plant growth depending on the cultivar used. Whether plants were artificially inoculated or naturally infected compared to non-infested controls especially affected plant growth. The infection of potato plants by *R. solani* was initiated, as reported by Platt *et al.* (1993), by soil-borne and tuber-borne

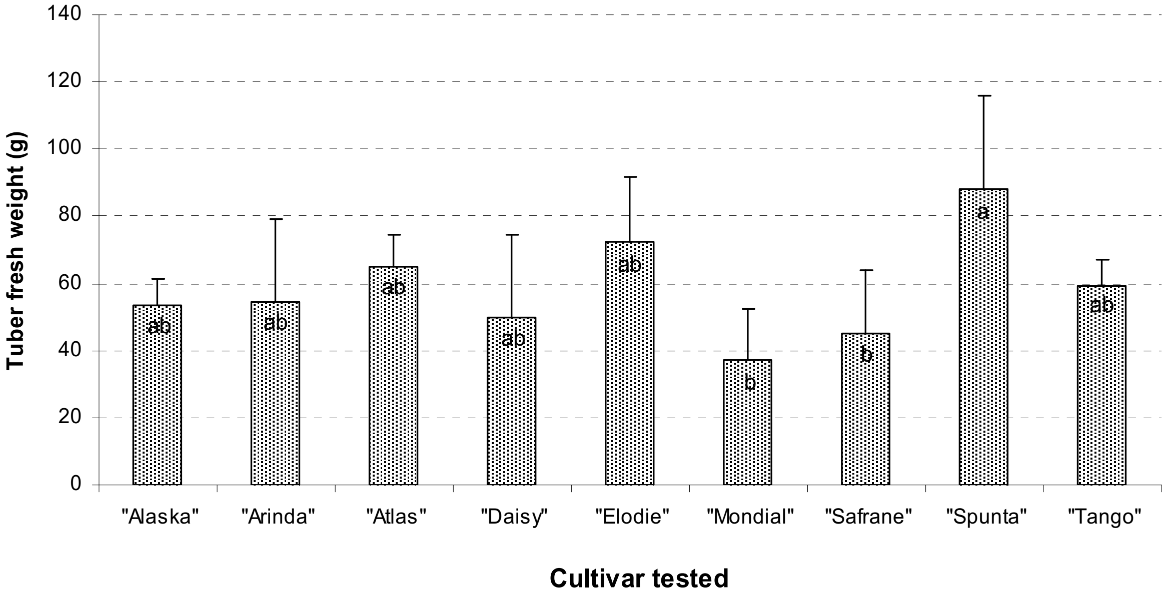

Fig. 16 Progeny tubers fresh weight noted, four weeks post-plantation, on potato plants of different cultivars derived from seed tubers naturally infected by *R. solani* at planting. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). Each bar represents the mean of 5 plants.

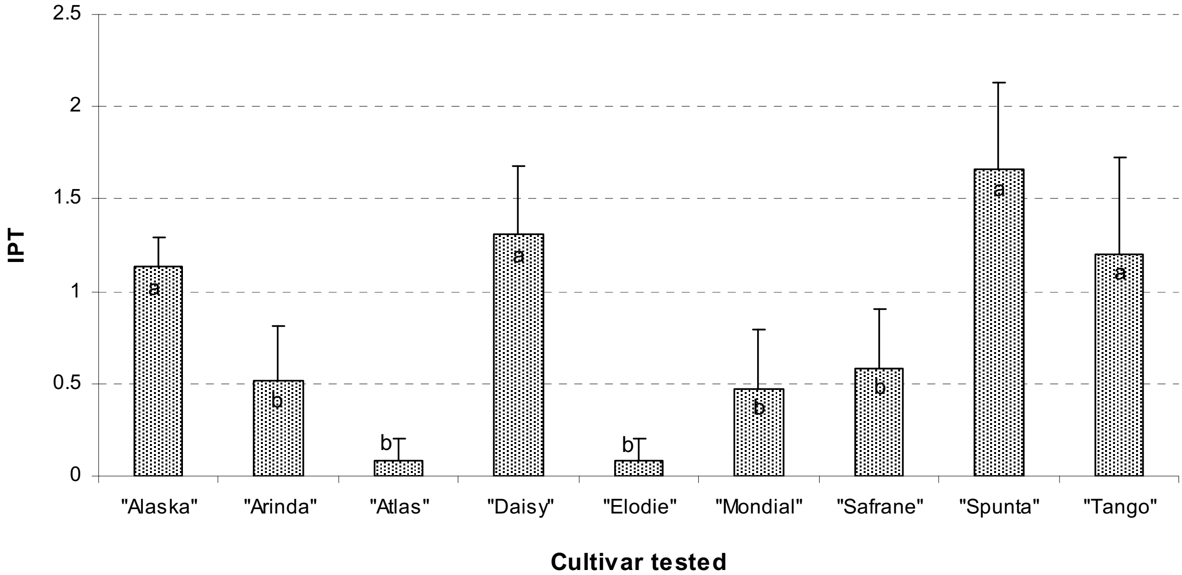

Fig. 17 Indexes of progeny tuber (IPT) infection by *R. solani* sclerotia noted four weeks post-plantation, on potato plants of different cultivars derived from seed tubers naturally infected at planting. Bars with the same letter are not significantly different according to Fisher's protected least significant difference LSD test ($p \leq 0.05$). Each bar represents the mean of 5 plants.

Fig. 18 Black scurf (A) and stem canker (B) observed, four weeks post-plantation, on potato 'Tango' derived from seed tubers naturally infected at planting.

inoculum. The induced stem canker was shown to delay shoot emergence (Hide *et al.* 1992), to decrease the number and length of stems and even to cause increased variation in stem length; Hide *et al.* (1989b) also reported that these effects were cultivar-dependent.

Both experiments converged on the variable reaction of the cultivars tested to *R. solani*, via substrate inoculation or natural seed tuber infection, on the varying degrees of stem canker severity and on the absence of totally immune cultivars. These results are in agreement with those of El Bakali and Martin (2006), who reported that potato varieties differ in their susceptibility to *Rhizoctonia* and on the degree of resistance to sclerotia formation on tubers; these authors also claimed that to date no variety has been found with immunity to stem lesions. Furthermore, all shoots of cultivars we tested showed stem canker symptoms, as also found by Hide *et al.* (1989a).

Plants derived from seed tubers naturally infected by *R. solani* sclerotia at planting showed, four weeks later, a stem canker score variable between 0.295 and 3.03. In contrast, when seed tubers were artificially inoculated 10 days post-emergence, this parameter lay between 0.816 and 3.282 five weeks post-plantation. These results showed that soil-borne inoculum is potentially as damaging as seed-borne inoculum which is particularly effective in causing disease because of its close proximity to developing sprouts and stolons, as mentioned by Wharton *et al*. (2007). However, our findings are not in agreement with those of El Bakali and Martin (2006) who found that tuber inoculum is more important than soil inoculum claiming that it represented, in their study, the primary cause of disease.

In our experiments, plant inoculation occurred following the irrigation of inoculum suspension at the collar level and this, according to Wharton *et al*. (2007), favours the infection of plant organs developed in proximity to the inoculum such as stem bases. This phenomenon was shown to occur mostly in the early part of the plant growth cycle, which is also the case in this screening. Hide *et al*. (1985) showed that by inoculating seed tubers at the time of planting with *R. solani* to stimulate seed tuber infection, much stem canker is caused although shoots could eventually grow from diseased plants. However, Hartill (1989) reported less susceptibility of stems after plant emergence, and later infection which occurred mainly on tubers and tuber-bearing stolons. Although Hide *et al.* (1985) had reported the resistance of shoots to infection by *R. solani* after aerial leaves development, a severe stem canker was developed in both experiments and especially on plants inoculated 10 days post-emergence. This disease severity may be attributed to the virulence of the inoculum used and to the conducive post-planting conditions such as regular irrigation every 2 days. In fact, Hide and Firmager (1989) reported that moisture can greatly affect the transmission of inoculum from seed tubers to the crop and the subsequent development of the disease. Moreover, the incidence and severity of symptom expression are influenced, according to Csinos and Stephenson (1999), by several factors or components such as infection efficiency and time from inoculation to symptom expression or incubation period. Furthermore, a positive correlation between the density of infection structures and disease severity was also involved in pathogen penetration and symptom progression on stems and consequently, disease severity (Marshall and Rush 1980; Demirci and Döken 1998). Other factors such as soil-borne and tuber-borne inoculum density, temperature and soil moisture were also shown to affect the development and severity of Rhizoctonia stem canker on potato (Bolkan *et al.* 1974; Simons and Gilligan 1997; Kyritsis and Wale 2002).

The present results confirmed, via both screening methods used, that 'Spunta' and to a lesser degree 'Elodie' were the most susceptible to *R. solani* whereas 'Alaska' and 'Daisy' were the most tolerant in both cases, i.e. artificial or natural infection by this pathogen. These findings will have practical consequences in disease management and may also explain the greater and higher incidence of potato rhizoctonias in Tunisia because in some local potato areas, 'Spunta' is cultured in monoculture for more than one crop within a year (early, season and late crops). In the same way, Gilligan *et al*. (1996) reported that replenishment of inoculum (initially originating from seed tuber, debris or soil) to soil was rapid following the growth of a susceptible crop, evidently of a susceptible cultivar, with comparatively high levels of infection and disease, and this phenomenon occurred even in long rotations.

Substrate inoculation by *R. solani*, 10 days post-emergence, did not negatively affect the number of stems developed per plant except for 'Spunta', which was shown to be the most susceptible to stem canker severity. Moreover, this number was reduced by about 38% in inoculated plants compared to the non-inoculated controls. In addition, even in the presence of natural seed tuber infection by *R. solani*, 'Spunta' developed the fewest stems per plant associated with the highest stem canker severity. This result confirmed the findings of Hide *et al*. (1985, 1989b) who showed that severe stem canker can be associated with a decrease in the number and length of stems on the infected plants. In contrast, Zimmer (1988) reported that there was no definite correlation between the Rhizoctonia index and the number of stolons or stems produced.

Variable stem and root dry weights were noted. Following natural and artificial infection by *R. solani*, 'Spunta' plants were most negatively affected by the pathogen, as assessed by these parameters. Moreover, the stem and root dry weights reductions, on inoculated compared to non-inoculated control plants (**Figs. 5, 7**), varied between 10 and 19%, respectively for the majority of cultivars tested. These results are in agreement with those of Hide *et al.* (1985, 1989b) who found that inoculating decreased the mean weight of foliage per plant and delayed tuber initiation; delayed foliage and tuber growth were the direct result of severe stem canker.

On some cultivars tested, stem canker severity was not always associated with reduced plant growth as is the case of the artificially inoculated 'Mondial' plants which had a relatively severe stem canker (CNI of about 2) but showed the highest stem dry weight. Similarly, Hide *et al.* (1985) recorded an increase in the foliar dry matter in infected plants due to below-ground lesions interfering with the downward transport of assimilates.

Substrate inoculation by *R. solani*, 10 days post-emergence, significantly reduced the tuber fresh weight by about 17% more in inoculated than in non-inoculated control plants: this illustrates the negative effect of *R. solani* on the expected yield. However, in 'Spunta', which showed the most severe stem canker produced under natural infection by *R. solani*, the highest fresh tuber weight was associated with most severe black scurf on progeny tubers.

The reduction of tuber weight induced by substrate inoculation by *R. solani* was also reported by Banville (1978) who showed that severe infection can reduce yield. In the same way, Hartill (1989) reported that infection by *R. solani* increased the number of tubers initiated but reduced their total weight. Hide *et al*. (1992) found that inoculating delayed plant growth and decreased tuber numbers and yield.

The reduced yield due to the presence of *R. solani* depended on the inoculum source. In fact, Hide *et al.* (1985), in inoculation experiments, found that soil-borne, but not seed-borne, inoculum was associated with a loss in yield. However, Platt *et al.* (1993) reported that infected potato seed tubers used or planted in contaminated soil can cause a reduction in the marketable tuber yield.

Severe stem canker observed on 'Spunta' and 'Elodie' was accompanied by severe black scurf on progeny tubers. These findings are in accordance with most reports indicating that the severity of stem canker in the growing crop is positively correlated with the subsequent formation of black scurf on progeny tubers (Banville 1978; Chand and Logan 1982; Read *et al.* 1989); other authors reported difficulties in relating stem canker and black scurf (Hide *et al.* 1989a). However, Frank and Leach (1980) asserted that soil-borne inoculum is more ideally situated to infect tubers. Downgraded tuber quality is not only attributed to tuber-borne sclerotia or black scurf symptoms (Jager *et al.* 1991), but also to the possible development of malformed (misshapen) tubers, alteration of their target size and number in addition to a reduction in their marketable yield (Hide *et al.* 1973; Frank 1978, 1981; Anderson 1982; Carling *et al.* 1989; Read *et al*. 1989; Jeger *et al*. 1996).

Finally, although the pot experiments are not conclusive in identifying, with certainty, resistance among cultivars and although they do not fully reflect the reality under field conditions, the present results are important because they elucidated the behaviour of potato cultivars, via several assessment parameters, against *R. solani* in the presence of two sources of inoculum (natural seed tuber infection or culture substrate inoculation) which act independently in disease development. Furthermore, in both our experiments convergent results were obtained regarding the most susceptible or the most tolerant cultivars, the absence of resistance and the variable reaction of cultivars tested. These conclusions will be useful for a future disease control strategy as the cultivar factor had a significant effect not only on disease severity but also on fungicide efficacy as reported by Zimmer (1988a, 1988b). These results should be confirmed under field conditions, in different crops within a year, where disease-free seed tubers could be planted in infested fields and naturally infected seed tubers in non-infested soils to elucidate more the effect of cultivar when considering both possible sources of inoculum that contribute to Rhizoctonia disease development in Tunisia.

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