Comparative Reaction of Potato Cultivars to Sclerotium rolfsii Assessed by Stem Rot and Tuber Decay Severity

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ABSTRACT

Experiments were conducted to evaluate the relative susceptibility of 11 local potato cultivars to Sclerotium rolfsii by using several stem and tuber disease parameters. Based on stem rot severity, noted three weeks post-inoculation, pathogen penetration, and percentage of rotten tissue, recorded after 8 days of incubation at 30°C, none of the cultivars tested was resistant to S. rolfsii. However, there was a variable degree of susceptibility in which cultivar ‘Tango’ was found to be the most susceptible whilst ‘Daisy’ was the most tolerant to the disease. The most severe stem rot attributed to pathogen inoculation was observed, three weeks post-inoculation, on ‘Tango’ and ‘Spunta’ plants. For all cultivars combined, the percentage of rotten tuber tissue was related to the lesion diameter formed on the tuber surface and to the pathogen penetration.

INTRODUCTION

Potato (Solanum tuberosum L.) is an economically important crop worldwide. It is classified as the third most important food crop after wheat and rice (Wang et al. 2008; Schieber and Aranda Saidaha 2009; Visser et al. 2009). In Tunisia, it is a strategic crop (Azzouz 1996) with an average yield of about 14 t/ha (Djebali and Tarhouni 2010). As potato is frequently grown in monoculture and rotated with other vegetable crops (Daami-Remadi et al. 2011), it is threatened by several soil-borne fungi. Amongst these fungal pathogens, agents causing wilt and root and tuber rots are responsible of yield losses (Priou and El Mahjoub 1999).

Sclerotium rolfsii is a soil-borne plant pathogen of worldwide occurrence that infects more than 500 plant species (Aycoc 1966; Mordue 1974; Punja 1985; Wokocha 2001; Ozgonen et al. 2010). Sclerotium wilt, incited by S. rolfsii is an important field disease of potato particularly in tropical, subtropical and warm temperate areas (Aycoc 1966; de Icochea 1981).

The large number of sclerotia produced by S. rolfsii, their survival ability for several years, and the abundant growth rate of the fungus are key factors making this pathogen of major importance (Punja 1988). The first confirmed report of losses due to the pathogen in the USA was made by Rolfs in 1892 on tomato (Lycopersicon esculentum Miller) in Florida (Aycoc 1966). The disease was particularly severe, in Alessandria located in Northern Italy, on potato cv. ‘Monalisa’ causing 5 to 15% yield losses because of premature plant death and rotting of tubers (Garibaldi et al. 2006).

In Tunisia, the disease has been observed, since 2006, on potato plants and rotting tubers (Daami-Remadi et al. 2007) and was found to cause serious soft rot disease under the same thermal conditions of traditional improved storage or non-stored potatoes, where temperature ranges between 25 and 35°C (Daami-Remadi et al. 2010). Moreover, the reaction of potato cultivars to stem rot disease has not been well established, although cv. ‘Spunta’ usually showed pathogen infection and exhibited typical symptoms (Daami-Remadi et al. 2007, 2010). To the best of our knowledge, there are limited published data concerning the relative susceptibility of commercial potato cultivars to S. rolfsii, in Tunisia and the world at large, with the exception of the work of Holm et al. (1987) and Garibaldi et al. (2006). Thus, as potato production areas are limited under Tunisian conditions and rotation is often difficult to implement, assessing the local cultivars for their reaction to S. rolfsii is needed. This would consequently identify the use of resistant or less susceptible cultivars which would obviously reduce economic loss and damage attributed to the disease.

Due to the occurrence of S. rolfsii in soil and plants and their effects on plants and tubers, the present work conducted to assess the susceptibility of local cultivars to stem and tuber infection and rots.

MATERIALS AND METHODS

Plant material

Relatively healthy and undamaged potato (Solanum tuberosum L.) tubers belonging to 11 cultivars mentioned in A class (cultivars distributed to farmers for growing potatoes for consumption and/or common seed production for late season crop) of the Tunisian varietal assortment, were tested. They were kindly provided by the Technical Potato Center, Essaïda, Tunisia. Just before use, tubers were washed to remove excess soil, superficially disinfected with a 10% sodium hypochlorite solution for 5 min, rinsed with sterile distilled water and air dried.

Depending on the experiment being conducted, tubers were either kept at 15-20°C, 60-80% relative humidity under natural room light for pre-sprouting (for pot experiment) or used directly for inoculation (tuber experiment). After sprout induction (occurrence), tubers were planted (one tuber per pot) in plastic pots (25 cm diameter) containing a mixture of peat and perlite (3:4: 1/4), previously sterilized at 110°C for 1 h. After emergence, potato plants were watered every 2-3 days, depending on environmental conditions and plant’s needs, until inoculation date.
Pathogen

*S. rolfsii* SS1 isolate, originally obtained from rotted potato tubers, was used for tuber and plant inoculation. Its pathogenicity as well as its aggressiveness was previously characterized on potato cv. ‘Spunta’ (Daami-Remadi et al. 2007, 2010). The pathogen was cultured for 6 to 10 days at 25°C on potato dextrose agar (PDA) medium amended with 300 mg/l of streptomycin sulphate (Pharmadrug Production GmbH-Hamburg, Germany) before use. For plant inoculations, a mixture of mycelium and sclerotia, prepared by adding 10 plates of the pathogen grown on media to one liter of sterile distilled water, was used.

Tuber inoculation and rot severity assessment

Tubers were wounded by a 6-mm diameter disinfected cork borer but not inoculated were used per cultivar tested. Tubers on PDA. Inoculated tubers were placed in moistened trays and completely randomized within a growth chamber.

Because of disease evaluation to confirm the results and ascertain that the symptoms observed were indeed induced by *S. rolfsii*. Simi- larly, stem segments of diseased tissues were surface disinfected in 0.5% sodium hypochlorite solution for 5 min and rinsed three times with sterile distilled water. Dried segments were then plated (3 pieces per plate) on PDA supplemented with streptomycin sulphate (300 mg/l), and incubated for 7 days at 25°C to isolate the pathogen. Some disinfected stem sections were also placed in a humid chamber at 25°C for visual observation of the fan-like mycelium development, which is characteristic of morphological growth of *S. rolfsii* pathogen.

Statistical analyses

As all non-inoculated plants and tubers were symptomless; therefore, only the data of inoculated plants and tubers were considered in the statistical analyses. The disease severity parameters were analyzed using Analysis of variance (ANOVA) to assess treatment effects (cultivars) on lesion diameter and stem rot in the various experiments. The effect of treatment on lesion diameter (tuber rot) and stem rot were also compared by computing treatment means using Statistical Analysis System (SPSS). Means were separated using Fisher’s protected LSD test (at *P* ≤ 0.05).

The relationships between the surface lesion diameter on tubers, penetration and the percentage of rotten tissue were compared using Pearson’s correlation analysis (SPSS Ver. 11) where *P* = 0.05 was considered statistically significant.

RESULTS

Analysis of variance of cultivar’s data indicated that all the variables were affected significantly (*P* ≤ 0.01) by the plant material tested (Table 1).

Effect of potato cultivars on tuber surface lesion diameter incited by *S. rolfsii*

Inoculated tubers of all cultivars, showed fan-like mycelial growth, forming symmetrical circles around the site of inoculation, typical of *S. rolfsii* infection. However, all non-inoculated tubers were symptomless. Significant (*P* ≤ 0.05) differences were found in the mycelial growth, as measured by mean colony diameter noted after 72 h of incubation at 30°C. Data shown in Fig. 1 indicates that tuber surface lesions in excess of 4 cm in diameter were recorded on tubers of all cultivars. The cv. ‘Bellini’, showed the highest external lesion growth on tubers (6 cm) while the cvs. ‘Eloide’ and ‘Daisy’ did not girdle the inoculated tubers within 6-8 days.

<table>
<thead>
<tr>
<th>Disease ratings/Source</th>
<th>df</th>
<th>Mean square</th>
<th>F value *</th>
</tr>
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<tbody>
<tr>
<td><strong>Lesion diameter</strong></td>
<td></td>
<td></td>
<td>5.899**</td>
</tr>
<tr>
<td>Cultivars</td>
<td>10</td>
<td>3.920</td>
<td></td>
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<tr>
<td>Error</td>
<td>99</td>
<td>0.665</td>
<td></td>
</tr>
<tr>
<td><strong>Pathogen penetration</strong></td>
<td></td>
<td></td>
<td>3.565**</td>
</tr>
<tr>
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<td>22.205</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>99</td>
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<tr>
<td><strong>Rotten tissue</strong></td>
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<td></td>
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</tr>
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<tr>
<td><strong>Stem rot</strong></td>
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<td>11.601**</td>
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<tr>
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<tr>
<td>Error</td>
<td>55</td>
<td>0.874</td>
<td></td>
</tr>
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</table>

*Values followed by ** are statistically significant at *P* ≤ 0.01. df: degree of freedom.

Table 1 Analysis of variance of Sclerotium disease ratings on potato cultivars inoculated with Sclerotium rolfsii.
Effect of potato cultivar on tuber rot severity induced by *S. rolfsii*

In the inoculated tubers, disintegration of tuber tissue and subsequent rotting were observed, whereas no rot developed on the non-inoculated tubers. Tuber rot severity varied significantly ($P \leq 0.05$) among the cultivars tested. The lesion depth recorded after 8 days of incubation at 30°C ranged from between 10 to 16 mm (Fig. 2). The majority of cultivars tested showed significantly similar rot severities.
Correlations analysis, for all cultivars pooled, showed a statistically significant positive correlation between the percentage of rotten tubers and the lesion diameter ($r = 0.224, P = 0.0181; n = 110$) when data was combined for cultivars. Nevertheless, analysis made on each cultivar showed that these parameters were significantly correlated only in the case of cv. ‘Atlas’ ($r = 0.689, P = 0.028; n = 10$).

Correlations analysis, for all cultivars pooled, showed a significant positive correlation between lesion depth (pathogen penetration) and the percentage of rotten tissue ($r = 0.383, P = 0.00004; n = 110$). However, when correlation analysis was done for each cultivar separately, the parameters were found to be significantly correlated only in the case of cvs. ‘Spunta’ and ‘Timate’ ($r = 0.755, P = 0.012; n = 10$ and $r = 0.765, P = 0.010; n = 10$, respectively).

### Effect of potato cultivar on stem rot severity

Inoculated plants showed typical symptoms of stem rot with variable severity depending on the cultivar when assessed after three weeks of inoculation. Initially, infected stem tissues were soft, depressed, and brownish. The Southern blight fungus caused sudden wilting as the first symptom, on the severely affected plants, followed by the appearance of a fan-like, white fungal mycelia at the collar region and even the subsequent formation of sclerotia which first appeared as white nodules, but later turned brown.

Data presented in Fig. 5 showed that the most severe stem rot (of about 3–4) was observed on ‘Tango’ and ‘Spunta’ plants whereas ‘Arinda’ and ‘Bellini’ resulted in a disease score of 2 (DSI). However, all the other cultivars showed significantly similar stem rot severity and infection score did not exceed 1.

The isolation on PDA of the pathogen from diseased plants confirmed the involvement of *S. rolfsii* in the symptoms observed and assessed.

### DISCUSSION

The present study reports, for the first time in Tunisia, the evaluation of the relative susceptibility of local potato cultivars to stem and tuber rots caused by *S. rolfsii*. In general potato cultivar susceptibility or resistance to this pathogen has not been quantified in many parts of the world, compared to the abundant literature on *S. rolfsii* involvement in other plants such as peanut (Branch and Csinos 1987; Smith et al. 1989; Brenneman et al. 1990; Besler et al. 1997). In a previous research, Holm et al. (1987) described the resistance of an oblong russet ‘Ute Russet’ potato cultivar to leaf-roll necrosis and *S. rolfsii*. The cultivar ‘Patronis’ was also reported to be highly susceptible to potato wilt caused by *S. rolfsii* (Bakr and Khan 1981) in another study. Similarly, Garibaldi et al. (2006) mentioned the susceptibility of cultivars ‘Hermes’ and ‘Monalisa’ based on premature plant death and rotted tubers. Potato cultivars have also been reported to vary in their degree of susceptibility and reaction to *S. rolfsii*, but the researchers noted that many current cultivars have not been well characterized with respect to their reaction (Browne et al. 2002; Davis et al. 2007). Thus, more comparative studies for both stem and tuber rot intensity would yield much needed data and information in this pathosystem on potato.

Cultivar resistance or lower susceptibility to the disease could constitute a promising alternative method of controlling stem and tuber rots caused by *S. rolfsii*. This could be
integrated with the other cultural practices such as management of crop residue and crop rotation (Backman et al. 1984; Franke et al. 1998). Previous research has shown that significant yield losses due to the disease can occur when potatoes are grown continuously or in short rotations with other crops susceptible to the disease, such as tomato (Aycock 1966; de Icochea 1981). Furthermore, Tunisian climate conditions prevailing in late spring and autumn provide conditions such as bioavailability of the pathogen and higher temperature conducive to stem decay development. Soft rot or Sclerotium blight continues to be a threat to potato production in Tunisia by affecting the most important crops (during spring and autumn). Our research has identified cultivar resistance/susceptibility to S. rolfsii under potted and storage incubation conditions. Various stem rot and tuber rot parameters were utilized to assess cultivar resistance or susceptibility to this disease. This research also indicates the urgent need of testing other disease management measures such as biocontrol with indigenous antagonists, other than host-resistance alone, since this is a serious pathogen on potatoes and other crops.

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