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Testing the Susceptibility of Some Potato Cultivars to Black Scurf Disease Caused by *Rhizoctonia solani* Kühn.

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Abstract

Rhizoctonia stem canker and black scurf are among the most important diseases associated with potato (*Solanum tuberosum* L.) cultivation worldwide. Pathogenicity test of 19 isolates of *Rhizoctonia solani* Kühn obtained from four governorates in Syria revealed that isolates varied in their ability to cause disease with the isolate Rh15 being the most virulent in the development of the disease on potato plant under artificial infection. The relative susceptibility of seven commercial and local potato cultivars against *R. solani* was tested. The evaluation was based on black scurf severity and the negative impact of the disease on plant growth and expected effect on yield. The tested cultivars showed variable degrees of black scurf severity and consequent plant growth, but no completely immune cultivars were observed. Based on disease index (DI), potato varieties 'Agria', 'Ultra' and 'Labella' were highly susceptible to the disease; 'Spunta' was moderately resistant whereas 'Everest' was the most tolerant. Infection of the most susceptible cultivar "Afamia" resulted in the death of a large number of seedlings, large and deep canker on stems, with no formation of new tubers. Although 'Synergy' was moderately susceptible, and the black scurf incidence was higher than that of 'Everest' and 'Spunta', the loss of tubers weight was not significant compared to the previous two cultivars. The results suggested that use of tolerant and moderately resistant cultivars in Syria may help in reducing the development of black scurf on potatoes.

Keywords: Black scurf; cultivars; potato; *Rhizoctonia solani*; *Solanum tuberosum*; stem canker; susceptibility.

1. Introduction

Potato *Solanum tuberosum* L. is the third most important food crop in the world. In developing countries, potato production has greatly increased in the past two decades, and has now overtaken that in the developed world, indicating the increasing importance of potato as a main food crop to respond to the needs of increasing human populations (Birch *et al*., 2012). Potato cultivated area in the world reached more than 18 million hectares with a production of about 376.1 million tons (FAO, 2021). In Syria, potato production was estimated at 507,384 tons with a cultivated area of 22,369 hectares in 2016 (Annual Agricultural Statistical Group, 2016).

Stem canker and black scurf caused by *Rhizoctonia solani* Kühn. (telemorph *Thanatephorus cucumeris* (A.B. Frank) Donk is a serious disease of potato grown in cooler regions of the world (Yanar *et al*., 2005). *R. solani* causes appreciable yield losses each year, and losses caused by this pathogen have varied from 5% to 34% in different potato growing regions in the world (Carling and Leiner, 1990; Banville *et al*., 1996; Das *et al*., 2014). According to Keiser (2008), yield losses caused by black scurf disease reached 50%, resulting in important economic losses for potato growers. Abdo *et al*. (2012) confirmed the presence of the disease in most potato cultivation areas in Syria, where the infection rate of the disease was higher in the spring season than in autumn season with average incidence of 64.19% and 60.46% respectively.

Potato infection by Rhizoctonia diseases can occur at two different stages: infection of growing plants (Rhizoctonia stem canker) and infection of new tubers by sclerotia (black scurf). Either or both infection stages may be observed in potato crops (Banville *et al*., 1996; Ogoshi, 1987).

R. solani isolates can be classified into different anastomosis groups (AG), based on hyphal anastomosis in paired isolates grown in culture. Isolates belonging to the same AG are generally compatible and show a successful hyphal fusion, while isolates belonging to different AGs are usually incompatible and show unsuccessful anastomosis (Anderson, 1982; Carling, 1996; Carling *et al*., 2002; Kankam *et al*., 2021). Presently, 13 AGs are reported, several of which are divided into subgroups (Carling *et al*. 2002; Lees *et al*., 2002; Harikrishnan and Yang, 2004; Guleria *et al.*, 2007; Woodhall *et al*., 2007; Yang *et al*., 2015). Several studies confirm AG-3 as the main cause of both stem canker and black scurf of potato (Carling and Leiner, 1990; Moussa *et al.*, 2014). However, other AGs (AG-1, AG-2,1, AG-4, AG-5, AG-7, AG8, AG-9) have also been implicated in causing disease in potatoes (Okubara *et al*., 2008; Woodhall *et al*., 2007; Yanar *et al*., 2005; Campion *et al*., 2003; Lees *et al*., 2002; Sneh *et al*., 1996; Balali *et al*., 1995; Kankam *et al*., 2021). In Syria, two anastomosis groups (AGs) were identified: 47 isolates

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(85.45%) belonged to AG3, only one isolate (1.81%) belonged to AG1, and 7 isolates (12.72%) remained unidentified (Abdo *et al*., 2012). A molecular study, using specific primers, showed that 78.95% of *R. solani* isolates belonged to the sub-group AG3- PT (Abo Akel *et al*., 2022).

Diseases caused by *R. solani* are traditionally controlled by the use of fungicides (Grosch *et al.,* 2005; Lahlali and Hijri, 2010). However, they have minor impact and may cause environmental pollution (Jiang *et al.,* 2005; Kurzawińska and Mazur, 2008). Consequently, a combination of crop rotation and resistant varieties offers the most practical and effective measure to control the disease (Scholten *et al*., 2001). Accordingly, the selection and cultivation of resistant potato cultivars has become one of the most economical and effective way to control tuber black scurf (Naz *et al.,* 2008).

The purpose of this study was to evaluate the susceptibility of some potato cultivars grown in Syria to *R. solani* under artificial infection.

2. Materials and Methods

2.1. Sample collection and pathogen isolation

Potato tubers showing typical symptoms of black scurf were collected from four different governorates (Homs, Aleppo, Daraa, Damascus countryside) in Syria. Samples were washed carefully under running tap water to remove the adjacent soil particles, surface sterilized with 1% sodium hypochlorite for 2 min, rinsed three times with sterile water and then were dried between two sterilized filter papers. Infected parts were cut using sterilized scalpel into small pieces (3-5 mm), transferred to plates of PDA supplemented with streptomycin sulfate (120 mg l^{-1}) to suppress bacterial growth, then incubated at 25 ± 1ºC for 7 days. Plates were daily observed for mycelial growth. Hyphal tips of mycelium emerging from the infected pieces were transferred to fresh plates of PDA (Sinclair and Dhingra, 2019; Naffaa *et al*., 2021). Pure cultures of *R. solani* isolates were identified microscopically on the basis described by Ogoshi (1996). The identified isolates were subcultured on PDA slants and kept at 4ºC for further studies.

2.2. Preliminary pathogenicity test

Nineteen isolates of *R. solani*, identified in another study as AG3 (Abo Akel *et al*., 2022), were used for pathogenicity tests. Potato tubers (cv. 'Spunta'), relatively similar in size with 6-7 buds, were surface sterilized by soaking in sodium hypochlorite (1%) for 3 min, then in ethanol 70% for one minute. After that, they were washed several times with sterilized water. Experimental layout Soil (clay, sand and peat 1: 1: 2 v) was sterilized twice in autoclave at 121ºC for 30 min. One tuber was planted at a depth of approximately 5 cm in a 50-cm plastic pot containing 6 kg of sterilized soil mixture. Each tuber was inoculated by adding 700 g of sterilized sand with 5 mycelial discs (5 mm) which were taken at the periphery of 7-day-old fungal colonies, then 500 ml of distilled water were added, and covered with the soil mixture (Simons and Gilligan, 1997). The same amount of autoclaved sand without mycelial discs was added to control. Three pots were used as replications for each isolate as well as for the control. The plants were grown in winter season at ambient temperature. The plants were harvested 5 weeks after planting. The average plant length was calculated. Since most plants did not form tubers, and some seedlings were killed (damping-off), a preliminary assessment of the pathogenicity was based on the percent of plant showing stem canker symptoms according to the following formula:

Disease incidence % = [(plant number in control – plant number in treatment) / plant number in control] × 100.

2.3. Susceptibility of potato cultivars to R. solani (Rh15)

Susceptibility of 7 commercial and local potato cultivars ['Spunta', 'Afamia', 'Benella', 'Everest', 'Ultra', 'Synergy', 'Agria', and 'Labella'] against the most virulent isolate *R. solani* (Rh15) was tested under greenhouse conditions. The isolate Rh15 was chosen based on the results of the preliminary pathogenicity test. Planting and inoculation methods were the same as described above for the pathogenicity test. Nine plots were used as replications for each cultivar as well as for the control. The plants were fertilized with a balanced NPK (2-3g/ liter of water), and watered when needed.

Average of plant lengths at the beginning of flowering stage, stem canker incidence (%), number and weight of tubers and the ratio of infected progeny tubers were noted 120 days after planting (Woodhall *et al*., 2008). The *tuber surface area covered with sclerotia* was used as a general method to evaluate potato black scurf severity based on the following rating scale: **0**: no sclerotia present, **1**: (< 1%), **2**: (2–10%), **3**: (11–20%), **4**: (21–50%), **5**: (≥ 51%) of tuber area covered. Disease index (DI) and relative resistance index (RRI) were calculated by the following formulas (Zhang *et al*., 2014).

$$DI = \frac{(n0 \times 0) + (n1 \times 1) + (n2 \times 2) + (n3 \times 3) + (n4 \times 4) + (n5 \times 5)}{y \times 5}$$

Where *nx* = number of tubers in severity class *x*,*y* = total number of tubers

$$PRI = 1 - \frac{DI_x}{DI_{\max}}$$

DI_x = disease index of the observed tuber, DI_{max} = the maximum disease index of all cultivars.

Black scurf resistance was measured with the relative resistance index (RRI) as follows: 0.00–0.39 = highly susceptible (HS), 0.40–0.59 = moderately susceptible (MS), 0.60–0.79 = moderately resistant (MR), 0.80–0.99 = highly resistant (HR), 1 = immune (I) (Zhang *et al*., 2014).

2.4. Statistical analysis:

One – way analysis of variance was carried out using SPSS15 statistical program at $P \leq 0.05$ (Gomez and Gomez, 1984).

3. Results and Discussion

3.1. Pathogen isolates

Nineteen fungal isolates were obtained from sclerotia of *R. solani* on potato tubers and identified based on their morphological characteristics (Table 1).

Table 1: *Rhizoctonia solani* isolates and their isolation sources

R. solani isolates	Isolates sources	
	Season	Area
Rh1, Rh2, Rh3, Rh4, Rh6, Rh7, Rh9, Rh11, Rh12, Rh13, Rh15, Rh17, Rh18	Spring (February, March, April)	Homs
Rh5, Rh19	Spring (February, March, April)	Aleppo
Rh10, Rh16	Summer (August, September)	Damascus countryside (Saasa)
Rh8, Rh14	Summer (August, September)	Daraa

3.2. Preliminary pathogenicity test

Nineteen *R. solani* isolates were tested for their pathogenicity to potato cultivar 'Spunta' in pots under artificial infection during winter season at ambient temperature. Isolates' pathogenicity was evaluated based on the percent of plants showing stem canker symptoms. It was not possible to assess the disease severity based on tuber surface area covered by sclerotia because a large number of plants did not form any new tubers, and this may be due to the prevailing environmental conditions during the experiment period, and also to the experiment duration. So, a preliminary evaluation of the isolates pathogenicity was based on their ability to cause stem cankers. Isolates showed significant differences of potato stem canker incidence (% plants showing stem canker). Isolates Rh4, Rh8, and Rh17 seemed to be non-pathogenic, whereas infection percentage varied between 2.26% for the isolates Rh14 and 38.64% for the isolates Rh2, Rh9 and Rh15 (Table 2).

The artificial infection with some *R. solani* isolates resulted in a significant reduction in plant height. The average plant *height*s ranged between 24.43 cm for the isolate Rh15 and 35.53 cm for the isolate Rh14 compared to the control (34 cm). In general, no correlation was observed between stem canker incidence and plant height. The disease did not negatively affect the plant growth for some isolates, whereas other isolates significantly reduced the plant growth. However, Rh15 isolated from Homs province, which led to the highest percentage of plants showing stem canker (38.64%) with significant reduction in plant growth (28.15%) compared to the control, was chosen for susceptibility test of some potato cultivars to *R. solani* infection.

Table 2: Pathogenicity of *Rhizoctonia solani* isolates on 'Spunta' potato cultivar under artificial infection

Isolates	Infection percentage %(1)	Average of plant heights (cm)	Isolates	Infection percentage %(1)	Average of plant heights (cm)
Rh1	31.82 ef	34.7 jk	Rh11	22.73 cd	27.5 bcd
Rh2	38.64 g	29.11cde	Rh12	13.64 b	25.92 ab
Rh3	22.73 cd	34.47 jk	Rh13	34.09 fg	30.67 efg
Rh4	0 a	27.51 bcd	Rh14	2.26 a	35.53 k
Rh5	34.09 fg	31.25 efghi	Rh15	38.64 g	24.43 a
Rh6	36.37 fg	26.33 abc	Rh16	27.28 de	32.67 ghijk
Rh7	18.18 bc	25.83 ab	Rh17	0 a	32.09 fghij
Rh8	0 a	34.1 ijk	Rh18	27.28 de	31.13 efgh
Rh9	38.64 g	31.44 efghi	Rh19	27.28 de	29.63 def
Rh10	20.46 c	26.33 abc	Control	-	34 hijk
LSD5%	6.72	2.9	LSD%	6.72	2.9

Values followed by the same letter do not differ significantly according to (LSD) least significant difference ($P \leq 0.05$).

These results are in according with other previous studies (Balkan and Wenham, 1973; Abdo *et al*., 2012). Pathogenicity test of 12 isolates showed significant differences between isolates where RS7 was the most virulent isolate in the development of the stem canker and black scurf disease on potato cv. 'Spunta' in Egypt (Abdel-Sattar *et al*., 2017). *R. solani* isolates, even under similar conditions, showed significant differences in infection severity and induced symptoms, suggesting the involvement of genetic factors in virulence differences (Rubio *et al*., 1996). Variance of isolates in pathogenicity was also attributed to difference between (AGs) where *R. solani* AGs, other than AG3, usually have low virulence against potato (Balkana and Wenham, 1973; Yanar *et al*., 2005; Khandaker *et al*., 2011; Abdel-Sattar *et al*., 2017). Jaradat *et al*. (2023) showed that *R. solani* AG-3PT was the primary pathogen associated with potato stem canker and black scurf diseases in Jordan. Carling and Leiner (1990) showed that virulence of *R. solani* isolates on potato may be affected by the source of isolates, where isolates recovered from lesions were more virulent than those obtained from sclerotia. Truter and Wehner (2004) found that isolates obtained from stem lesions or from sclerotia on tubers were more virulent than isolates obtained from asymptomatic tubers and soil.

3.3. Susceptibility of potato cultivars to Rhizoctonia solani infection

Based on the preliminary pathogenicity test results, the most virulent isolate (Rh15) was used for cultivar susceptibility test. Seven commercial and local potato cultivars were tested under artificial infection in pots. Tested cultivars showed variable degrees of black scurf

severity and subsequent plant growth and yield, but no completely immune cultivars were found.

Table 3 showed that 'Everest' cultivar had significantly lower black scurf incidence (31.15%), and lower percent of stem canker (11.1%) than the other six cultivars. Number and weight of tubers and plant length were also less affected by the infection. 'Spunta' showed black scurf incidence of 38.26%. Although the significant decrease in the number of tubers compared to the previous cultivar, no significant differences were observed between them in tuber weight loss and plant length reduction. This can be explained by the formation of few and relatively large tubers. A previous study reported that 'Spunta' was the most susceptible cultivar to this pathogen. However, 'Spunta' produced the highest progeny tuber weight but with a noticeable incidence of black scurf (Daami-Remadi *et al*., 2008). In contrast, according to Djébali and Belhassen (2010) investigation, 'Spunta' showed the least percentage of infection of progeny tubers by *R. solani* sclerotia at harvest.

Although the infected tubers incidence in 'Synergy' cultivar was relatively high (55.4%), this did not significantly affect the number and weight of the tubers, as the reduction in both was not significant compared to the less affected 'Everest' cultivar, and the stem canker incidence did not exceed 22.2%. Cultivars 'Labella', 'Agria' and 'Ultra' had a high rate of infected tubers and stem canker (76.67 and 67.8%, respectively in 'Agria'). The infection resulted in a significant yield loss.

Infection of the local variety 'Afamia' resulted in the death of a large number of seedlings, stem canker of all remaining plants, a relatively weak vegetative growth, and no formation of new tubers. Therefore, it was not possible to assess the effect of infection on plant growth and yield. This cultivar was ranked as highly susceptible to infection by *R. solani*.

Figure 1: Different degrees of covered area from the surface of potato tubers by *Rhizoctonia solani* sclerotia under artificial infection.

Table 3: Susceptibility of seven potato cultivars to the black scurf disease caused by *Rhizoctonia solani* Kühn (Rh15) under artificial infection

Cultivar		Percentage of progeny tubers infection (%)	Percentage of stem canker %	Number of tubers	Relative reduction in tubers Number %	Average weight of tubers (g)	Relative reduction in tubers weight %	Average length of plants (cm)	Relative reduction in plant length %
'Synergy'	*Infected*	55.4 c	22.2 b	9.56	22.47 a	425.36	23.34 a	46.9	13.47 ab
	Control	-	-	12.33	-	567.92	-	54.2	-
'Labella'	Infected	61.6 c	56.7 d	5.67	52.75 c	212	48.14 b	27.67	17.25 bc
	Control	-	-	12	-	408.83	-	33.44	-
'Spunta'	Infected	38.26 b	33.3 c	9.83	37.27 b	358.55	25.36 a	28.99	16.36 abc
	Control	-	-	15.67	-	480.35	-	34.66	-
'Agria'	Infected	76.67 d	67.8 e	3.67	63.3 d	179.3	56.93 c	38.39	17.64 c
	Control	-	-	10	-	416.33	-	46.61	-
'Ultra'	Infected	60 c	56.7 d	5.2	52.73 c	274.83	44.12 b	36.16	12.99 a
	Control	-	-	11	-	491.78	-	41.56	-
'Everest'	*Infected*	31.15 a	11.1 a	13	27.78 a	412.7	19.8 a	30.31	12.42 a
	Control	-	-	18	-	514.56	-	34.61	-
'Afamia'	*Infected*	-	100 f	-	-	-	-	22.9	40.22 d
	Control	-	-	16.67	-	245.11	-	38.31	-
LSD at 5%		6.73	10.12		7.99		7.07		4.1

Values followed by the same letter in the same column do not differ significantly according to LSD test (at $P \leq 0.05$).

Table 4 shows the evaluation of tuber black scurf resistance of the 7 tested potato cultivars. All cultivars were obviously infected, and black scurf severity induced by Rh15 varied significantly among cultivars tested. These results showed also that there were no immune cultivars, but that most were susceptible. 'Everest' was highly resistant (HR) with fewer and smaller sclerotia on tubers, and small superficial lesions scattered on stems. Only 'Spunta' showed moderate resistance. However, 'Agria', 'Ultra' and 'Labella' were highly susceptible to the disease, with disease index more than 34. The most susceptible cultivar 'Afamia' exhibited post-emergence stem death, large and deep canker on stems, without formation of new tubers. Although 'Synergy' was moderately susceptible (MS), and the black scurf incidence was higher than that of 'Everest' and 'Spunta', the loss of tubers weight was not significant compared to the previous two cultivars.

These results agree with other research. In fact, Bains *et al*. (2002) reported that potato cultivars showed variation in susceptibility to *R. solani*, but no cultivars were totally resistant to the black scurf disease. Yanar *et al*. (2005) showed that some of tested potato cultivars were highly susceptible to black scurf disease, but some cultivars had higher levels of resistance than the local susceptible cv. 'Batum' in Turkey. Potato cultivars showed different degrees of resistance to *R. solani*, but no completely resistant cultivars have been observed (Daami-Remadi *et al*., 2008; Djébali and Belhassen, 2010; Khandaker *et al*., 2011; Thangavel *et al*., 2014). In contrast, Singh *et al*. (2021) showed that out of eighteen potato varieties, three expressed immune response to stem canker and black scurf in India.

Otrysko and Banville (1992) suggested that range of susceptibilities may not indicate varying levels of resistance to *R. solani*, but may be due to the different levels of maturity of the cultivars. Bains *et al*. (2002) reported that cultivars with late maturity showed comparatively low levels of the disease, whereas, early and mid-maturing cultivars showed comparatively high levels of the disease, with some exceptions. The differences in cultivar susceptibility may be due to both inheritance and maturity levels of the cultivars. But these conclusions do not seem to be fully applicable to the cultivars tested in this study. The early-season cultivar 'Everest' showed a high level of resistance, while the early-season local cultivar 'Afamia' was in contrast highly susceptible. The other cultivars showed a range of susceptibility reactions to *R. solani*, and almost all of them are semi-early, except the early season cultivar 'Synergy'. This may indicate that the genotype of the variety is the most important factor in the resistance process.

Zhang *et al*. (2014) reported that the susceptibility was relatively stable across years, but some moderately resistant and susceptible cultivars may be changed from moderate resistance to moderate susceptibility or from moderate susceptibility to moderate resistance.

Some researchers have confirmed that the difference in susceptibility to infection is due to the nature of resistance in potato varieties (Zhang *et al*., 2016). Moreover, other factors, such as environment conditions, plant vigor, cuticular stricture, tuber maturity, genetic factors, and pathogenicity that affect the expression of potato resistance may also be the causes that affect potato resistance evaluation (Bains *et al*., 2002; Djébali and Belhassen, 2010; Leach and Webb, 1993; Otrysko *et al*., 1992).

Table 4: Assessment of susceptibility of seven potato cultivars to *Rhizoctonia solani* (Rh15) under artificial infection.

Cultivars	Disease Index (DI)	Relative Resistance Index (RRI)	Resistance evaluation
'*Synergy*'	26.81	0.41	MS
'Labella'	45.43	0	HS
'Spunta'	16.36	0.64	MR
'Agria'	35.85	0.21	HS
'Ultra'	34.02	0.25	HS
'*Everest*'	9.19	0.8	HR
'*Afamia*'	-	-	HS

4. Conclusion

The relative susceptibility test of seven commercial and local potato cultivars grown in Syria showed a range of susceptibility to the black scurf disease caused by *R. solani*, and no totally immune cultivars were found. Most of the tested cultivars were highly susceptible to the disease. 'Everest' was the most resistant cultivar whereas 'Spunta' was moderately resistant, and the local cultivar 'Afamia' was the most susceptible.

To our knowledge, this is the first study demonstrating the relative susceptibility of some potato cultivars grown in Syria to *R. solani*. In fact, it is very important to estimate the susceptibility degree to potato cultivars based on the density of the sclerotia formed on the tubers, which (in addition to the soil-borne inoculum) are considered important sources for the initiation of Rhizoctonia disease in potato plant. Further studies are needed to evaluate the susceptibility of other potato cultivars cultivated in Syria to stem canker and black scurf disease.

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