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RESEARCH PAPERS

Potato seed dressing with *Pseudomonas aeruginosa* strain RZ9 enhances yield and reduces black scurf

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Summary. A rhizospheric strain RZ9 of *Pseudomonas aeruginosa* was assessed for *in-vitro* growth inhibition of *Rhizoctonia solani* and effectiveness to control black scurf on potatoes (*Solanum tuberosum* L.) of the cultivars Spunta and Nicola, in greenhouse and field experiments. The strain RZ9 inhibited *R. solani* mycelial growth by more than 60% and completely inhibited the germination of sclerotia from infested potato tubers in *in-vitro* tests. In greenhouse assays, seed potato treatment with RZ9 cell suspension increased stem length, decreased the relative weight of infected potato tubers (by 67%), and increased the potato yield (by 16%) compared to pathogen-inoculated plants for both potato cultivars. In field trials conducted on sandy soils during 2012 and 2013, strain RZ9 reduced black scurf incidence and increased potato yield by an average of 5.3 t ha^{-1} for 'Spunta' and 5 t ha^{-1} for 'Nicola'. This study showed that the selected strain of *P. aeruginosa* is an efficient bacterium for enhancing yield and reducing black scurf of field-grown potatoes.

Key words: biocontrol, *Rhizoctonia solani*, *Solanum tuberosum*, yield.

Introduction

Black scurf of potato, caused by *Rhizoctonia solani* Kühn (teleomorph: *Thanathephorus cucumeris* Frank Donk), is one of the most devastating and destructive diseases of potato (El Bakali and Martín, 2006; Keiser, 2008), and this pathogen affects several other horticultural crops worldwide (De Curtis *et al.*, 2010). The pathogen causes cankers on host stems and the development of sclerotia on potato tubers (Bienkowski *et al.*, 2010). The infections start with germination of sclerotia on the surfaces of infected seed potatoes, and from soil residues, producing abundant hyphae that attack stolons and daughter tubers, causing severe reductions in tuber quality and yields (El Bakali and Martín, 2006).

In the last decade, the southern region of Tunisia has become an important area of potato production suitable for export to European countries during the period of tax exemption from January to March each year. However, severe black scurf on potato tubers has occurred in this region, reducing potato quality and yields, and this disease has become an impediment for potato exports (Djébali and Belhassen, 2010). Current management practices of the disease are mainly based on the use of synthetic fungicides such as pencycuron and azoxystrobin (Djébali and Belhassen, 2010). However, fungicide control has not been entirely effective, and black scurf remains a persistent problem (Brewer and Larkin, 2005; Huang *et al.*, 2012). In addition, using fungicides may have undesirable environmental and health consequences (Singh *et al.*, 2010). Therefore, there is increased demand for non-pesticide methods that reduce the impact of *R. solani* on potato crops. Hence, effective management of black scurf requires the implementation of an integrated approach, as reviewed in detail by Tsror (2010).

The biological control approach based on using natural antagonists such as rhizospheric bacteria constitutes a potential alternative that may help to re-

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duce chemical treatments (Singh *et al.*, 2012; Mrabet *et al.*, 2013). The bacterial genus *Pseudomonas*, and particularly the sub-group of fluorescent pseudomonads, are recognized for their active role in controlling plant fungal diseases, and are reported to extensively colonize potato rhizospheres (Diallo *et al.*, 2011). Recent reports showed that *Pseudomonas* root colonizing strains may hold promise for *Rhizoctonia* management (Ahmadzadeh and Tehrani, 2009; Singh *et al.*, 2010; Mrabet *et al.*, 2013). *Pseudomonas aeruginosa*, a fluorescent pseudomonad, was reported to produce diverse antifungal compounds playing key roles in the biocontrol of phytopathogenic fungi (Bajpai *et al.*, 2008; Höfte and Altier, 2010). This bacterium has received the status of the "Most eligible biological control agent" due to its unique characteristics of competitive root colonization, short generation time, ease of genetic manipulation and production of 'strong metabolic arsenals', such as siderophores, anti-phytopathogenic metabolites and plant growth regulators (Bano and Musarrat, 2003; Rane *et al.*, 2008). Laboratory and field experiments have shown the effectiveness of *P. aeruginosa* to control several fungal diseases such as damping-off in tomato caused by *Pythium* sp. (Buysens *et al.*, 1996), wheat blotch caused by *Septoria* sp. (Flaishman *et al.*, 1990), wilt of chickpea caused by *Fusarium* sp. (Anjaiah *et al.*, 1998), and southern blight caused by *Sclerotium* sp. (Brathwaite and Cunningham, 1980). To our knowledge, there has been no assessment of its effectiveness for control of diseases caused by *R. solani* in potato.

In preliminary work (unpublished), a novel rhizospheric strain of *P. aeruginosa* (strain RZ9), isolated from healthy faba bean plants grown in the region of Hajeb Laayoun, Central Tunisia, was shown to have *in-vitro* antagonistic activity against a wide range of phytopathogenic fungi (including *Fusarium* spp., *Alternaria* spp., and *Phoma* spp.). However, effects of this strain towards *R. solani* were not known. The objectives of the present study were to examine the effect of the strain RZ9 of *P. aeruginosa* on *R. solani* growth, and to determine its effectiveness for control of black scurf on potato in a greenhouse and in field trials in Tunisia.

Materials and methods

Microbial strains and culture conditions

Strain RZ9 of *Pseudomonas aeruginosa* (Accession number KF152932), previously isolated from the faba bean rhizosphere and preselected for its antagonistic activity against a wide range of fungal species was used in this work. The bacterium was maintained on solid Luria-Bertani (LB) medium at 28°C and conserved on liquid LB medium supplemented with 25% glycerol at –80°C. Four *R. solani* strains; RS1.2, RS2.2, RS3.2, and RS5.2 were isolated from potato tuber-borne sclerotia collected from different cropping areas in Tunisia (Mrabet *et al.*, 2013) and used for *in-vitro* antibiosis tests. The *R. solani* strains were cultivated on potato dextrose agar (PDA) at 25°C in darkness and conserved at 4°C. Strain RS5.2 (Accession number: KF152934) was used to prepare *R. solani* inocula for tests on plants.

In vitro antibiosis tests on fungal mycelia

Antibiosis of strain RZ9 of *P. aeruginosa* against *R. solani* strains RS1.2, RS2.2, RS3.2, and RS5.2 was tested with a dual culture method (Mrabet *et al.*, 2011). Briefly, an agar disk (9 mm in diam.) of fresh growing fungal mycelium (4 d) of each *R. solani* strain was placed in the centre of each 90 mm diam. Petri dishe containing PDA medium. Strain RZ9 of *P. aeruginosa* was applied in two opposite streaks at approximately 20 mm from the fungal plug (Figure 1) and the Petri plates were incubated at 25°C. The ability of the bacterium to inhibit the *R. solani* strains was assessed by measuring the diameter of mycelial growth after 3 d incubation. Six replicates were used for each antibiosis interaction, and growth inhibition was recorded according to the following formula:

$$\text{Growth inhibition } (\%) = ((A\text{-}B)/A) \times 100,$$

where A was the diameter of mycelial growth in the control (not inoculated with strain RZ9), and B was the diameter of mycelia growth in the presence of the bacterial strain.

Preparation of microbial inocula

Cell suspensions of strain RZ9 of *P. aeruginosa* were prepared by scraping the cells from a single colony on a Petri plate and transferring them to liquid LB medium and shaking at 150 rpm at 28°C for 24h. Several dilutions of the RZ9 cell suspension were prepared by addition of sterile LB broth medium. The optical density at a wave length of 600nm (OD600) was measured with a spectrophotometer

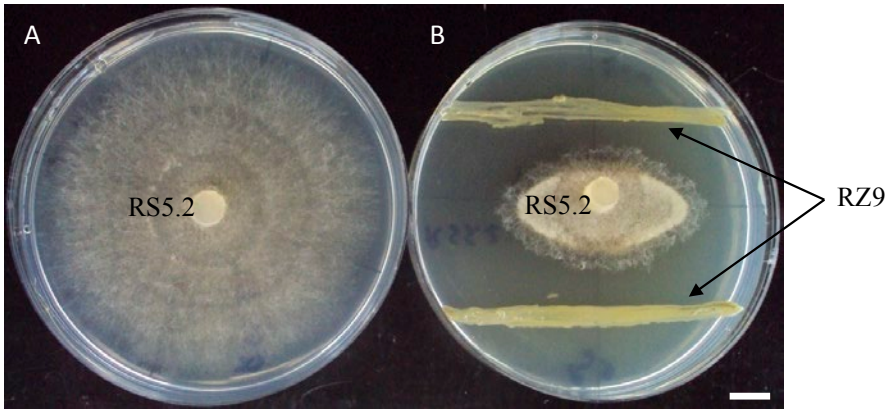

Figure 1. *In vitro* antibiosis activity of strain RZ9 of *Pseudomonas aeruginosa* against the strain RS5.2 of *Rhizoctonia solani* on PDA after 4 d incubation at 25°C. A, control; B, antagonistic activity of strain RZ9. Bar = 1 cm.

UV-VIS Dual Beam, (UVS-2700, LABOMED), and cells for each dilution were counted by dilution plating. An OD600 value of 0.52 which corresponded to approximately 10^7 cfu mL^{-1} was then used as a standard calibration for the preparation of other RZ9 inocula.

The *R. solani* inoculum was prepared by subculturing 25 fungal agar discs (9 mm diam.) from 4 d-old cultures in 1 L potato dextrose broth medium for 10 d at 25°C in darkness and without shaking. The mycelium obtained was crushed with an electric stick blender (Black and Decker, SB2000) at 10,000 rpm (three times for 30 s) and directly used for plant inoculation. The concentration of viable mycelial fragments in the suspension was assessed by dilution plating.

Antibiosis test on detached sclerotia and on infested potato tubers

The effect of strain RZ9 of *P. aeruginosa* on *R. solani* mycelium development from sclerotia was determined on both detached sclerotia and infected potato tubers of the cultivar Spunta. In the first test, sclerotia were detached from infected potato tubers collected in the field, surface disinfested with sodium hypochlorite (5%) for 1min, and washed five times with sterile distilled water to remove remaining sodium hypochlorite. The sclerotia were then dried on sterile filter paper and placed on PDA amended with 1% (v/v) of the RZ9 cell suspension (containing ca 10^7 cfu mL^{-1}). The bacterial suspension was mixed into the molten PDA just prior to pouring the medium in the plates. Two hours later, the sclerotia were deposited equidistantly on the surface of the medium (three sclerotia per Petri plate; Figure 2). Plates of PDA without bacterial amendment were inoculated similarly as experimental controls. All inoculated plates were incubated at 25°C, and six replicate plates were used for each modality. Mycelia development from the sclerotia was monitored for up to 2 weeks.

In the second test, heavily infected potato tubers were surface disinfested with 5% sodium hypochlorite for 5min, washed five times with sterile distilled water, and the dried on sterile filter paper. The tubers were then sprayed with either a cell suspension (containing *c.* 10^7 cfu mL^{-1}) of strain RZ9 of *P. aeruginosa*, previously grown on LB medium, or with sterile LB medium as an experimental control. The tubers were then placed inside plastic bags containing wet filter paper to maintain adequate humidity. Each plastic bag contained three potato tubers and a total of six replicate bags were used for each treatment. The germination of sclerotia was checked after 3 weeks of incubation at 25°C.

Greenhouse biocontrol assays

Assays were conducted in the greenhouse, using 15 L capacity plastic pots containing a 2:1 mixture of a sandy soil and commercial peat. During the experiment, average conditions in the greenhouse were *c.* 40% RH, 14 h photoperiod, 28°C. Fungicide-free cer-

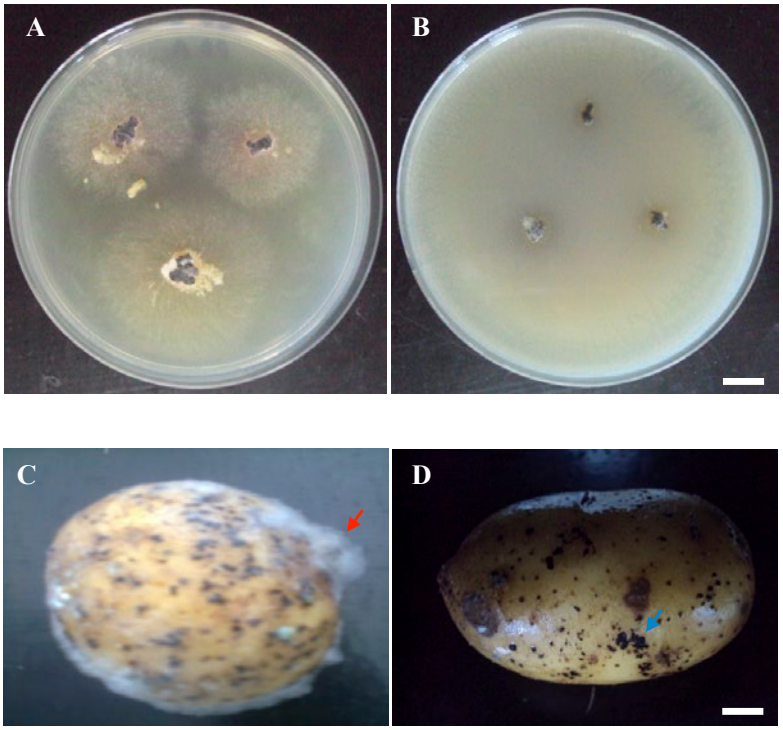

Figure 2. Effects of the strain RZ9 of *Pseudomonas aeruginosa* on *Rhizoctonia solani* mycelium emergence from detached sclerotia (A, B) and on mycelia development from infected potato tubers (C, D) incubated at 25°C. A, PDA medium showing mycelia growing from sclerotia; B, PDA medium mixed with RZ9 cell suspension (*c.* 10^7 cfu ml^{-1}) at 1% (v/v) showing growth inhibition of mycelium emergence from detached sclerotia; C, infected potato tuber sprayed with the LB medium showing the emergence of *R. solani* mycelium from sclerotia (Red arrow); D, infected potato tuber sprayed with *P. aeruginosa* strain RZ9 cell suspension grown in LB medium and showing the inhibition of *R. solani* mycelium development (Blue arrow). Bars = 1 cm.

tified seed potatoes of 'Spunta' and 'Nicola' (3–5 cm diam.) obtained from the Technical Centre of Potato and Artichoke (CTPTA, Tunisia) were used. The seed potatoes were treated by immersion for 5 min either in a cell suspension (containing ca 10^7 cfu mL^{-1}) of strain RZ9 of *P. aeruginosa* or in a preparation of fludioxonil (250 mg L^{-1}; Maxim® 100 FS, Syngenta). The seed potatoes were planted immediately after treatment, one per pot, and the plants were grown for 2 weeks, until they reached a stage of four leaves. The plants were then inoculated with *R. solani* by drenching the base of the stems of each plant with a 50 mL aliquot of a fungal suspension (containing *c.* 10^6 cfu mL^{-1}).

For each potato variety the following modalities were distinguished: (1) non-treated and non-inoculated plants (control), (2) *P. aeruginosa*-treated plants but not inoculated with *R. solani* (RZ9), (3) untreated but *R. solani*-inoculated plants (RS5.2), (4) fungicide-treated and *R. solani*-inoculated plants (RS5.2 + Fludioxonil), and (5) *P. aeruginosa*-treated and *R. solani*-inoculated plants (RZ9 + RS5.2). Five replicate pots were used for each modality.

Each growing plant was fertilized with commercial fertilizers (receiving a total of 20 g of diammonium phosphate, 20 g of ammonium nitrate, and 20 g of potassium sulphate) applied at 30 and 60 d after planting with a half of the total quantity for each application. During the vegetative stage, plant growth was assessed by measuring stem length at 30 d post planting. Harvest was performed 3 months after planting and the total fresh weight of tubers produced by each plant was recorded. The relative weight of infected potato tubers per plant was assessed as the weight of infected tubers relative to that of total tubers. Disease scoring was done by visual evaluation of the level of infection of potato

daughter tubers, using a severity scale which included six levels, where 0 = no visible sclerotia, 1 = *c.* 1% of tuber surface covered by sclerotia, 2 = 2–5%, 3 = 5–10%, 4 =10–15%, and 5 greater than 15% tuber surface covered (Anonymous, 2009).

Field biocontrol trials

Field trials were conducted in September of 2012 and 2013 in the region of Sned-Gafsa (South Tunisia), which is known for high prevalence of black scurf on potato (Djébali and Belhassen, 2010; Mrabet *et al.*, 2013). The soil type was sandy with physical and chemical characteristics previously reported by Mrabet *et al.* (2013). Both trials consisted of three 25 m long experimental blocks, each consisting of 12 rows of potato plants. Each block was divided into four 3-row plots, where one of the following modalities was applied: (1) non treated and non-inoculated plants (control), (2) untreated but *R. solani*-inoculated plants (RS5.2), (3) fungicide-treated and *R. solani*-inoculated plants (RS5.2 + Fludioxonil), and (4) *P. aeruginosa*-treated and *R. solani*-inoculated plants (RZ9 + RS5.2). The experiment was arranged in a randomized complete block design with three replicate plots for each modality. Each plot contained 210 plants (70 per row). The distances separating two consecutive potato seed tubers were 30–35 cm in the row and 75–80 cm between two adjacent rows. Fungicide-free certified seed potatoes of cultivars Spunta and Nicola obtained from the Technical Centre of Potato and Artichocke (CTPTA, Tunisia) were used. The three randomized blocks for 'Spunta' and those for 'Nicola' were symmetrically juxtaposed in the same field plot. Just before planting, germinated seed potatoes (sprouts <1 cm long) were treated with a cell suspension of strain RZ9 of *P. aeruginosa* (containing *c.* 10^7 cfu mL^{-1}) or with the fungicide fludioxonil (250 mg a.i. L^{-1}) by immersion for 5 min. The treated tubers were planted 10–15 cm deep. At 21 d post-planting (four leaf stage), the plants were drench-inoculated in modalities involving *R. solani*. For this, 50 mL aliquots of RS5.2 mycelial suspension (containing *c.* 10^6 cfu mL^{-1}) were inoculated at the stem base of each plants. The plots were drip-irrigated and chemical fertilization was adapted to the plant development stage. The fertilizers were applied equally at 0, 45, and 90 d post planting. The total quantities of fertilizers added per ha were 107 kg of nitrogen (N), 50 kg of phosphorus (P_2O_5) and 180 kg of potassium (K_2O). Weeds were mechanically removed. At 5 months post-planting, a 10 m median section of each plot was used to harvest approximately 30 plants from each row and each modality. The total fresh weights of daughter tubers were measured for each of the three rows of the elementary plots and the production was calculated (t ha^{-1}). The weights of infected potato tubers were also recorded.

Statistical analyses

Statistical analyses were performed using Statistica software version 5.1 (www.statsoft.com) and a statistics package (Microsoft Excel 2007). In all experiments, one-way ANOVA, followed by Fisher's least significant difference (LSD) test (P=0.05), were used to determine statistically significant differences between mean values.

Results

In vitro inhibition of *Rhizoctonia solani* mycelial growth by *Pseudomonas aeruginosa* strain RZ9

On control plates, strains RS1.2, RS2.2, RS3.2, and RS5.2 of *R. solani* completely colonized the 90mm-diameter Petri plates in 4 days (Figure 1) and presented similar radial growth (P>0.05 – data not shown). In the presence of strain RZ9 of *P. aeruginosa*, the radial growth of the four strains of *R. solani* was inhibited by 65 to 70% (Figure 1, Table 1).

Table 1. Antibiosis of the strain RZ9 of *Pseudomonas aeruginosa* against four *Rhizoctonia solani* strains isolated from field infected potato tubers collected from the north, the centre, and the south of Tunisia. Growth inhibition (%) = (A-B)/A) x 100; where: A; diameter of mycelia growth in control growth, B; diameter of mycelia growth in antibiosis test.

R. solani strain	*In vitro* growth inhibition (%)[a] by the strain RZ9 of *P. aeruginosa*
RS1.2	65(±0.6) b
RS2.2	68(±3.2) b
RS3.2	70(±0.2) a
RS5.2	65(±1.7) b

[a] Values with different letters are statistically differents according to Fishers LSD test at P = 0.05. Values in parentheses are standard errors of the means.

Inhibition of sclerotium germination

While colonies of *R. solani* developed from all sclerotia incubated on non-amended PDA, the presence of strain RZ9 of *P. aeruginosa* in the medium completely inhibited the emergence of mycelium from sclerotia (Figure 2). Similarly, when infected potato tubers were sprayed with cell suspensions of the bacterium and incubated at 25°C, no mycelium emergenced from sclerotia, while abundant mycelia developed on untreated tubers (Figure 2).

Biocontrol of *Rhizoctonia solani* under greenhouse conditions

Plant emergence and growth

Plant emergence was more rapid in the treatments involving strain RZ9 of *P. aeruginosa* for both potato cultivars. There was also a positive trend in later plant growth from the bacterium treated seed tubers. At 30 d post planting, mean of stem length in modalities involving strain RZ9 was 48% greater than in non-treated and non-inoculated plants (control), 164% greater than untreated but *R. solani*-inoculated plants (RS5.2), and 93% greater than fungicide-treated and *R. solani*-inoculated plants (RS5.2 + Fludioxonil) (Figure 3).

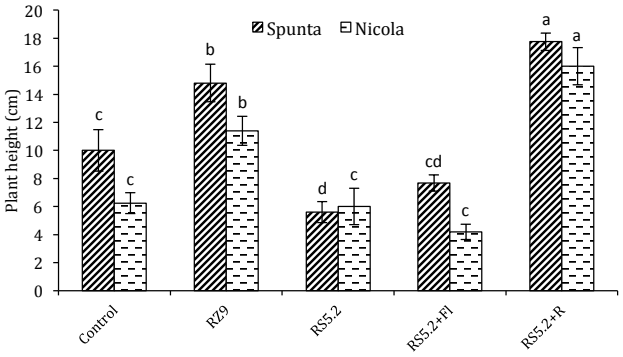

Figure 3. Mean heights of plants of the potato cvs 'Spunta' and 'Nicola' at 30 d post-plantating in the biocontrol assay of *Rhizoctonia solani* under greenhouse conditions. For each cultivar, values with different letters are statistically different (Fishers LSD test; $P < 0.05$). Bars represented standard errors. RZ9: *P. aeruginosa*; RS5.2: *R. solani*; fludioxonil: fungicide chemical.

Potato production and disease development

In the *R. solani* modality, the potato yield was decreased by 8% compared to the control. This was associated with 100% of tubers presenting sclerotia on their surfaces for both potato cultivars (Table 2), with infection scores of 3 to 5. The application of *P. aeruginosa* to RS5.2-inoculated plantlets resulted in re-

Table 2. Mean of total tuber weights and weights of infected tubers of potato cultivars Spunta and Nicola in biocontrol assays against *Rhizoctonia solani* with the strain RZ9 of *Pseudomonas aeruginosa* under greenhouse conditions. The assays involved the application of *R. solani* strain RS5.2, the strain RZ9 of *P. aeruginosa*, and the fungicide fludioxonil, separately or in combinations.

Treatment	'Spunta'			'Nicola'		
	Total tuber weight (g/ plant)	Infected yield (%)[a]	Score[b]	Total tuber weight (g/ plant)	Infected yield (%)[a]	Score[b]
Control	419 (±5.4) ab	0 (±0) c	0	413 (±13.3) a	0 (±0) c	0
P. aeruginosa (RZ9)	432 (±5.7) ab	0 (±0) c	0	421 (±14.1) a	0 (±0) c	0
R. solani (RS5.2)	393 (9.3) b	100 (±0) a	3-5	381 (±2.3) a	100 (±0) a	3-4
RS5.2 + fludioxonil	434 (±17.3) ab	27 (±5.7) b	1-2	397 (±12.0) a	23 (±7.2) b	1
RS5.2 + RZ9	462 (±3.1) a	33 (±11.2) b	1-2	438 (±15.1) a	19 (±4.6) b	1

Each value is an average of five replicates. The values in the same column accompanied by different letters are significantly different (Fishers LSD test at $P = 0.05$). Values in parentheses represent sdandard deviations.

[a] The percentage of infected yield represents the proportion of infected yield to the total yield.

[b] Black scurf severity score.

duced black scurf severity score (1–2) and increasing potato yield by 17% for 'Spunta' and 15% for 'Nicola', and decreased the weight of infected potato daughter tubers by 67% for 'Spunta' and 81% for 'Nicola'. Moreover, the bacterial inoculation resulted in low infection black scurf severity scores of 1-2. These results were comparable to those obtained with fludioxonil application to *R. solani*-inoculated plantlets (Table 2).

Field biocontrol of *Rhizoctonia solani*

Analysis of variance showed that there were no row and block effects ($P \geq 0.05$) for potato yields as well as for relative weight of infected potato daughter tubers for each modality. In the first field trial (2012 growing season), inoculation with strain RS5.2 of *R. solani* resulted in significantly decrease of potato yield, assessed by the total tuber weight, when compared to the control (Table 3). Potato yield was reduced by 27% (6.5 t ha^{-1}) for 'Spunta'and 19.7% (3 t ha^{-1}) for 'Nicola'. The relative weight of infected daughter tubers of *R. solani*-inoculated plants were 74% for 'Spunta' and 68% for 'Nicola' (Figure 4), mainly presenting high black scurf severity scores (4-5). However, in the modality of plants treated with *P. aeruginosa* and inoculated with *R. solani*, potato yields increased by 35% (6.25 t ha^{-1}) for 'Spunta' and 31% (3.7 t ha^{-1}) for 'Nicola', compared to plants inoculated with *R. solani* RS5.2 alone. Similar results were obtained in the control and in the fludioxonil modality (Table 3). Moreover, *P. aeruginosa* significantly reduced tuber infection by RS5.2 to about 35% for both potato varieties (Figure 4), with low severity scores ranging from 1 to 2.

In the second field trial (2013 growing season), inoculation with *R. solani* reduced the potato yield compared to the control, by 23% (5.5 t ha^{-1}) for 'Spunta' and 19% (3 t ha^{-1}) for 'Nicola' (Table 3). However, the yield in the biocontrol modality, involving strain RZ9 of *P. aeruginosa* and strain RS5.2 of *R. solani*, was enhanced by 22% (4.3 t ha^{-1}) for 'Spunta' and 52% (6.3 t ha^{-1}) for 'Nicola' compared to plants inoculated with *R. solani* alone (Table 3). The improvements were similar to the results obtained in the fludioxonil treated and *R. solani* inoculated plants, and were also greater than for the control, particularly for 'Nicola'. The incidence of infection with *R. solani* was only about 27% for tubers of cultivar Spunta and 26% for 'Nicola' in the inoculation with *R. solani* modality with black scurf severity score of 2-3. In the *P. aeruginosa*-treated and *R. solani*-inoculated (RZ9 + RS5.2) modality, only 4% and 1% of the tubers of the two cultivars had black scurf, with very low severity scores, giving reductions in disease incidence of 88% for 'Spunta' and 95% for 'Nicola' (Figure 4).

Discussion

Strain RZ9 of *P. aeruginosa* exhibited strong inhibition of the *in vitro* growth of four *R. solani* strains with different geographic origins. This suggests that the bacterium has a wide range of inhibitory activity against *R. solani* and corroborates previous reports on this species (De Curtis *et al.*, 2010; Verma *et al.*,

Table 3. Mean potato yields (t/ha) in the field biocontrol trials of *Rhizoctonia solani* (RS5.2) with the strain RZ9 of *Pseudomonas aeruginosa*, conducted in the region Sned-Gafsa during 2012 and 2013 growing seasons, using the potato cultivars Spunta and Nicola.

Year	Cultivar	Potato yield (t/ha)[a]			
		Control	RS5.2	RS5.2 + Fludioxonil	RS5.2 + RZ9
2012	Spunta	24.0 (±0.9)a	17.5 (±0.8)b	25.0 (±1.4)a	23.7 (±0.6)a
	Nicola	14.8 (±0.5)a	11.8 (±0.7)b	16.0 (±0.4) a	15.5 (±1.1)a
2013	Spunta	24.9 (±0.7)a	19.2 (±0.6)b	25.8 (±1.1)a	23.6 (±0.9)a
	Nicola	14.8 (±0.4)b	12.0 (±0.3)c	17.5 (±0.5)a	18.3 (±0.7)a

[a] Values in each row with different letters are significantly different according to Fishers LSD test at $P = 0.05$. Values in parentheses are standard deviations.

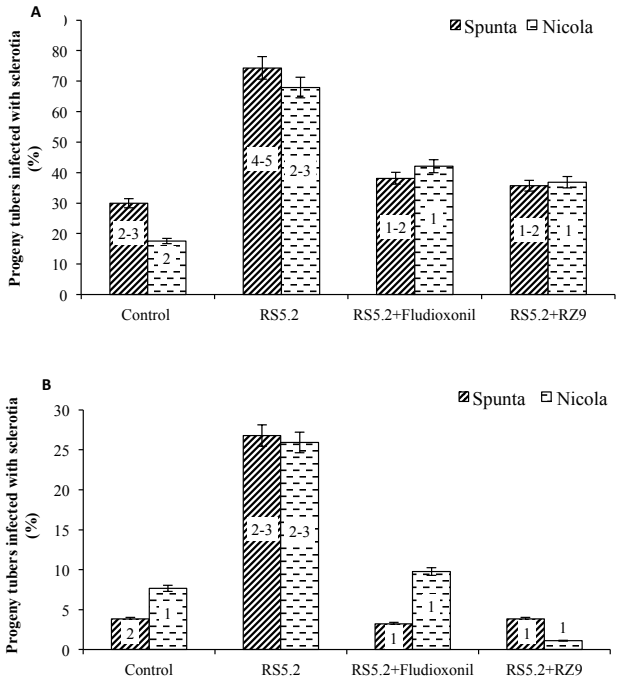

Figure 4. Relative weight of infected potato daughter tubers (%) in field trials conducted in the Sned-Gafsa region for potato cvs 'Spunta' and 'Nicola' during two growing seasons in 2012 (**A**) and 2013 (**B**). Bars represented standard errors. The percentage of infected daughter tubers represents the proportion of infected daughter tuber weight compared with the total weight of daughter tubers. RZ9: *Pseudomonas aeruginosa*; RS5.2: *Rhizoctonia solani*; fludioxonil: chemical fungicide. Values inside the histograms represent the black scurf severity scores.

2014). With the aim to assess the efficacy of potato seed dressing with strain RZ9 of *P. aeruginosa* for the biocontrol of *R. solani*, greenhouse and field trials were conducted. Two potato cultivars Spunta and Nicola, largely cultivated in Tunisia (Djébali and Belhassen, 2010), were used in this study.

Considering the greenhouse and field trials, results showed that the inoculation of *R. solani* to potato plants caused high levels of tuber infection of up to 100% in the greenhouse and 74% under field conditions. These levels of disease were associated with yield reductions up to 6.5 t ha^{-1} in 'Spunta' and 3 t ha^{-1} in 'Nicola'. Such disease impacts corroborate previous reports of yield losses of up to 30% and increases of relative weights of infected potato daughter tubers (Keiser, 2008; Wilson *et al.*, 2008; Tsror, 2010). The difference in *Rhizoctonia* disease prevalence in the trials conducted in 2012 and 2013 indicates changes in the persistence of the inoculated *R. solani* which have been previously attributed to soil conditions (Kühn *et al.*, 2009; Djébali and Belhassen, 2010). The survival of inoculum is determinant of *R. solani* pathogenicity since black scurf on tubers occurs mainly at the end of potato culture life-cycle (Djébali and Belhassen, 2010).

The greenhouse experiments conducted in this study showed that seed treatment with strain RZ9 of *P. aeruginosa* to *R. solani*-inoculated plantlets was associated with an average increase in tuber weight of 16% for 'Spunta' and 21% for 'Nicola' with corresponding 67 and 81% decreases, respectively, in the relative weight of infected potato daughter tubers, compared to *R. solani* inoculated plants. In an analogous study, Brewer and Larkin (2005) reported that *P. fluorescens* strains led to about 40% reduction of black scurf development. Likewise, some *P. aeruginosa* strains were found to effectively control *R. solani* on bean plants and to enhance yields (Siddiqui and Shaukat, 2003; Verma *et al.*, 2014). Results of the present study also showed that strain RZ9 of *P. aeruginosa* enhanced potato plant emergence and stem length, suggesting a plant growth promoting effect of this strain. Various strains of *P. aeruginosa* have been reported to produce phytohormones (indolacetic acid), and to provide host plants with nutrients through phosphate solubilisation and iron sequestration activities (Höfte and Altier, 2010; Verma *et al.*, 2014), thus enhancing plant growth. Strain RZ9 of *P. aeruginosa* did not produce any disease symptoms (necrotic lesions, chlorosis, rotting) when inoculated onto potato plantlets or potato tuber slices (data not shown).

In both of the field trials reported here, treatment of seed potatoes with strain RZ9 of *P. aeruginosa* caused yield increases in the presence of *R. solani* inoculum. These yield improvements (up to 35% for 'Spunta' and 52% for 'Nicola') were greater than those reported in studies with other fluorescent pseudomonads (about 10%; Diallo *et al.*, 2011). Seed tuber treatment with strain RZ9 was also associated with decreases in the relative weight of black scurf-affected daughter tubers by 53 and 88% for 'Spunta', and by 46 and 95% for 'Nicola' in 2012 and 2013, respectively, in comparison to the *R. solani* inoculated plants. The fact that in 2013 the reduction of black scurf development was greater than occurred in 2012 could be linked to the large amounts *R. solani* infec-

tion in 2012. The decrease of black scurf obtained in this study was greater than results obtained using *Trichoderma harzianum* for the biocontrol of *Rhizoctonia* diseases, which were reported to be reduced by 11-31% (Wilson *et al.*, 2008).

Our results revealed that the strain RZ9 of *P. aeruginosa* completely inhibited the emergence of *R. solani* mycelium from detached-sclerotia and from infected potato tubers. This is of very important since black scurf in potato is, in part, due to infected seed potatoes. This finding unveils the antagonistic efficacy of strain RZ9 of *P. aeruginosa* and could explain how antagonistic bacteria act on seed tuber-borne *R. solani* and hence prevent the infection of potato daughter tubers. The main effects of *P. aeruginosa* against phytopathogenic fungi involve antibiosis, nutrient depletion, hyperparasitism, and stimulation of plant defence pathways (Bakker *et al.*, 2007; Berry *et al.*, 2010; De Curtis *et al.*, 2010; Yin *et al.*, 2013). However, the mechanisms of biocontrol activity of the strain RZ9 of *P. aeruginosa* to against *R. solani* on potato still need to be explored.

The present research has highlighted the effectiveness of seed potato dressing with a novel strain of *P. aeruginosa* for control of *Rhizoctonia* disease in potato 'Spunta' and 'Nicola'. Results obtained using the selected strain RZ9 were similar to those achieved with applications of the commercial fungicide; fludioxonil. This indicates that the selected bacterium could be a source of an antifungal compound with high activity against *R. solani*. Beside the progress in the potential use of the bacterium to control the black scurf in potato, further studies are under way to identify potentially effective metabolites from strain RZ9 of *P. aeruginosa* that may be formulated as bio-fungicide. Particular attention will be given to the toxicity tests and the applicable doses to ensure the safe use of the strain in biocontrol strategies. This will provide more information for a effective delivery of a new formulation of RZ9 biocontrol inocula or its secondary bio-products for the eco-friendly treatments of potato against black scurf.

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