Phytopathologia Mediterranea (2013) 52, 3, 449–456

RESEARCH PAPERS

Efficacy of selected *Pseudomonas* strains for biocontrol of *Rhizoctonia solani* in potato

Moncef MRABET[1], Naceur DJEBALI[2], Salem ELKAHOUI[3], Youssra MILOUD[1], Sabrine SAÏDI[1], Belhassen TARHOUNI[4] and Ridha MHAMDI[1]

[1] Laboratory of Legumes, Center of Biotechnology of Borj Cédria (CBBC), BP 901, 2050 Hammam-Lif, Tunisia
[2] Laboratory of Molecular Physiology of Plants, Center of Biotechnology of Borj Cédria (CBBC), BP 901, 2050 Hammam-Lif, Tunisia
[3] Laboratory of Bioactive Substances, Center of Biotechnology of Borj Cédria (CBBC), BP 901, 2050 Hammam-Lif, Tunisia
[4] Technical Center of Potato and Artichoke, CTPTA, 2031 Essaïda, Tunisia

Summary. Thirty seven bacterial isolates from faba bean (*Vicia faba* L.) root-nodules were screened for their antagonistic activity against eight *Rhizoctonia solani* strains isolated from infected potato (*Solanum tuberosum* L.) tubers. Two bacterial strains (designated as Kl.Fb14 and S8.Fb11) gave 50% *in vitro* inhibition of *R. solani* mycelial growth. 16S rDNA sequence analysis indicated that strain Kl.Fb14 exhibited 99.5% identity with *Pseudomonas moraviensis*, and that S8.Fb11 exhibited 99.8% identity with *Pseudomonas reinekei*. Greenhouse trials in soil showed that strain S8.Fb11 reduced the percentage of sclerotia on potato tubers and amounts of tuber infection for the potato cultivars Spunta and Nicola. In a field trial conducted in South Tunisia, infection with *R. solani* reduced potato yield by approximately 40% for 'Spunta' and 17% for 'Nicola'; about 20% of the total tuber production was severely infected. However, when potato tubers were treated with strain S8.Fb11 prior to sowing, disease incidence was reduced to 6% of total production with low infection levels; potato yield was enhanced by about 6 kg per 10 m row in comparison to *R. solani* infected plants. The second selected *Pseudomonas* sp. (strain Kl.Fb14) did not affect either the levels of sclerotia on tubers or potato yield.

Key words: antibiosis, black scurf, biocontrol, potato, *Pseudomonas* sp.

Introduction

Rhizoctonia solani is a destructive and widespread soilborne plant pathogen causing diseases on potato (*Solanum tuberosum* L.) and other plant species (Ritchie *et al.*, 2006; Ahmadzadeh and Tehrani, 2009). The disease symptoms on potato include stem cankers and sclerotia on tubers (Eken *et al.*, 2000; Brewer and Larkin, 2005). The pathogen reduces the yield and quality of tubers, due to reduced size and number of tubers and the development of malformed tubers (Daami-Remadi *et al.*, 2008a), which can lead to quantitative yield losses of 50% (Keiser, 2008). While it is well-recognized that potato varieties differ in succesptibility to *R. solani*, no resistant cultivars are currently available (El Bakali and Martín, 2006; Djébali and Tarhouni, 2010). Many practices have been used to manage the diseases on potato, among which crop rotations, sowing in warm and dry conditions (Secor and Gudmestad, 1999; El Bakali and Martín, 2006) and use of fungicides (Parry, 1990) are the most common. However, biological control is considered a promising alternative to manage fungal diseases on potato (Recep *et al.*, 2009; Pérez-García *et al.*, 2011). The suppression of diseases by microbial agents takes place mainly in the rhizosphere and involves mechanisms that are yet to be reported (Van Loon, 2007; Badri *et al.*, 2009; Reinhold-Hurek and Hurek, 2011). Suppression of diseases with suitable crop rotations is generally associated with particular effects on soil biotic properties (Dick, 1992; Trabelsi *et al.*, 2012).

Corresponding author: M. Mrabet
Fax: +21 679325638
E-mail: moncef_mrabet@yahoo.fr

ISSN (print): 0031-9465
ISSN (online): 1593-2095

Legumes commonly cultivated in rotation with various crops -for their efficacy to improve soil mineral fertility- offer convenient microhabitats for some microbial species through the exudates that they release into the soil (Van Loon, 2007; Jensen *et al.*, 2010), and these plants have been suggested to play a role in plant disease suppression (Simon and Sivasithamparam, 1988; Stoddard *et al.*, 2010).

In fact, legumes could stimulate the growth of several soil microorganisms including those antagonistic to pathogenic fungi, which could result in the reduction of some plant diseases (Mrabet *et al.*, 2011). Faba bean (*Vicia faba*) is considered a suitable grain legume to be used in crop rotations for its positive effects on soil mineral and health properties and on the yield of subsequent crops (Peoples *et al.*, 2009; Jensen *et al.*, 2010; Pypers *et al.*, 2011). Faba bean is widely used in crop rotations with potato in Tunisia. This plant is known to stimulate rhizospheric competency of some microorganisms (McEwen *et al.*, 1989; Lupwayi and Kennedy, 2007). The present research work aimed to assess bacteria associated with faba bean root nodules for the biocontrol activity against *R. solani*, assessing their impacts on potato yields under greenhouse and field conditions.

Materials and methods

Bacterial and fungal strains

Thirty-seven bacterial strains were isolated from faba bean root nodules after surface disinfection with mercuric chloride (0.1%) according to the protocol of Mhamdi *et al.* (2002). The bacterial strains were maintained on Luria-Bertani (LB) medium (Miller, 1972) supplemented with 25% glycerol, and the isolates were stored at -80°C. Eight *R. solani* strains (RS1.1, RS1.2, RS1.3, RS2.2, RS2.3, RS3.2, RS5.1, RS5.2), from a local collection of the Laboratory of Legumes, Centre of Biotechnology of Borj-Cédria, Tunisia which were previously isolated from infected potato tubers grown in different regions of Tunisia, were used. They were maintained on Potato Dextrose Agar (PDA) medium (Pronadisa) at 25°C in darkness.

Molecular characterization of selected bacterial strains

The 16S rDNA from the bacterial isolates was amplified by PCR using primers fD1 and rD1 (Weisburg *et al.*, 1991) as previously described by Mhamdi *et al.* (2002). PCR products were purified from agarose gels using the Wizard SV Gel and PCR-clean up system from Promega. The amplified fragments were sequenced using the same primers and assembled using the CAP program available on the NCBI website (http://www.ncbi.nlm.njh.gov/blast). Partial 16S rDNA sequences were deposited in Genbank under accession numbers JQ023167 for strain S8Fb11 (1319 nt) and JQ023168 for strain Kl.Fb14 (883 nt).

In vitro antibiosis tests

The antibiosis tests of bacteria against fungi were performed using the methods of Mrabet *et al.* (2011). For each *R. solani* strain, fungal plugs (9 mm diam.) from a 5-day-old culture on PDA were placed in the centre of 90 mm diam. Petri dishes containing PDA. Each bacterial strain was applied in two opposite streaks on the test plate agar medium, *c.* 20 mm from the fungal plug. The ability of the bacterial strains to inhibit the fungal growth was assessed by measuring the diameter of mycelial colony growth (mm) after 3 days of incubation at 24°C. Six replicate plates were used for each *R. solani*/bacterial strain combination (treatment).

Preparation of inocula

Bacterial inocula were grown in Erlenmeyer flasks (2 L capacity) containing 500 mL LB broth by inoculating the broth with a single colony of either strain S8.Fb11 or Kl.Fb14, and shaking at 150 rpm, 28°C for 48 h. Fungal inocula were each prepared separately in 1 L of potato dextrose broth (PDB) by inoculating ten fresh plugs (each 9 mm diam.) from a culture of each *R. solani* strain. and incubating at 24°C for 9 days in darkness. The growing fungal mycelia were then homogenized with an electric mixer for 5 min to prepare inoculum. The inoculum concentration was determined by dilution plating on PDA.

Potato varieties and growth conditions

The potato 'Spunta' and 'Nicola' which are widely cultivated in Tunisia (Djébali and Tarhouni, 2010), were used as host plants in this study. Certified seed tubers were obtained from the Technical Center of Potato and Artichoke (CTPTA), Essaïda, Tunisia. Tubers were ascertained as visibly free from black

scurf. Before germination, similarly sized tubers (3–5 cm diam.) were surface disinfected in 5% sodium hypochlorite solution for 5 min, and then rinsed with sterile distilled water. Tubers were placed under favourable conditions for pre-germination (*c.* 20°C, 70% of relative humidity) for 2 weeks before planting. Each germinated tuber was then planted in a 30 cm diam. pot (15 L capacity) filled with a mixture of field soil (from the Cap Bon region, in northern Tunisia) and peat at a ratio of 2:1, Physical and chemical characteristics of the field soil used are given in Table 1.

Pathogenicity test

Seven-day-old plantlets of 'Spunta' grown in a greenhouse (40% RH, 12h light/12h dark diurnal cycle) were each inoculated with 100 mL of a mycelial suspension (10^6 cfu mL^{-1}) for each *R. solani* strain, with the inoculum suspension applied at the stem base of each plantlet. The ability to induce sclerotia on progeny tubers was checked after 3 months. The severity of disease was evaluated based on sclerotia formation (Brewer and Larkin, 2005) on a six point scale, where 0 = no visible sclerotia, 1 = 1% of tuber surface covered by sclerotia, 2 = 2–5%, 3 = 5–10%, 4 = 10–15%, and 5 ≥15%. A negative control consisting of non-inoculated plantlets was included.

Table 1. Main physical and chemical characteristics of two soils used in this study.

Characteristic	Cap Bon	Sned-Gafsa
Clay (%)	8	12
Silt (%)	7	5
Sand (%)	85	83
C.E (µs/cm)	512	1940
pH	8.0	8.4
N (g/kg)	1.37	0.51
Na (meq/100 g)	1.62	0.63
K (meq/100 g)	1.85	0.33
Ca (meq/100 g)	5.67	0.48
Mg (meq/100 g)	1.37	1.73
P_2O_5 (ass ppm)	109	13

Greenhouse assay

Ten similar germinated tubers (3–5 cm diam.) of each cultivar were immersed in either of the two bacterial suspensions (S8.Fb11 or Kl.Fb14 at 10^8 cfu mL^{-1}) or in a commercial fungicide pencycuron (Monceren®, Bayer Crop Science India Ltd.) for 20 min and planted in a two:one mixture of soil (Table 1) and peat. Fungal inocula consisted on 100 mL suspensions of the mixed inoculum of the eight *R. solani* strains (10^6 cfu mL^{-1}), previously prepared separately. The fungal suspension was inoculated at the stem bases of potato plantlets at the four-leaf growth stage. For each potato cultivar, the following treatments were applied: (i) without bacteria and *R. solani* (control treatment), (ii) inoculated with *R. solani*, (iii) *R. solani* strains and treated with pencycuron at a concentration of 10 µg mL^{-1}, (iv) treated with bacterial strain S8.Fb11 or Kl.Fb14, or (v) tubers treated with strain S8.Fb11 or Kl.Fb14 and plantlets inoculated with *R. solani*. For each treatment five replicate plantlets were treated. Each plantlet was fertilized with commercial mineral fertilizers containing 10 mg of di-ammonium phosphate, 10 mg of ammonium nitrate, and 10 mg of potassium sulphate at 30 and 60 days after plantating. The plantlets were irrigated as required.

Plants were harvested 90 days post planting and the following parameters were recorded: total weight of progeny tubers, yield of infected tubers and the severity of tuber infection with sclerotia.

Field trial

The field biocontrol trial was conducted in the south of Tunisia (Sned-Gafsa) using the potato 'Spunta' and 'Nicola'. The main soil characteristics of the Sned-Gafsa site are given in Table 1. Experimental plots were 10 m long and three rows wide with a total of 100 seed potato tubers per treatment. The experiment was arranged in three blocks. Fungal inocula consisted of *R. solani* strain RS5.2 which was previously isolated from infected potato tuber from this region. Before planting, tubers inoculated with *R. solani* were immersed in a suspension of the *R. solani* strain RS5.2 (10^6 cfu mL^{-1}), then dried in darkness before treating with bacterial suspension of either strain S8.Fb11 or Kl.Fb14 (10^7cfu mL^{-1}), or with the fungicide fludioxonil (200 ppm), by immersion for 5 min. A negative control of non-treated potato tubers was included. A drip irrigation system

was applied and fertilisation added during the plant development stage. The total quantities of fertilizers added were equivalent to 107 kg of nitrogen 50 kg of phosphorus pentoxide and 180 kg of potassium oxide. Weeds were manually removed from the plots.

Plants were harvested 5 months post planting and the following parameters were recorded: the total weight of tubers, the weight of infected tubers, and severity of sclerotia infection.

Statistical analyses

Data were subjected to analysis of variance (ANOVA) using the STATISTICA software version 5.1 (www.statsoft.com), and means were compared with Duncan's multiple range test ($P<0.05$).

Results

In vitro antibiosis assays

The ability of the 37 bacterial strains to inhibit mycelial growth of the eight *R. solani* strains was assessed on PDA medium. Eleven strains did not show any antagonistic effects on any of the *R. solani* strains. Twentty four bacterial strains gave variable amounts of inhibition of the eight *R. solani* strains ranging from 0 to 20%. Two bacterial strains (S8.Fb11 and Kl.Fb14) showed antagonistic activities against all the *R. solani* strains. The *in vitro* inhibition of mycelial growth ranged from 21 to 46% for strain S8.Fb11, and from 30 to 44% for *strain* Kl.Fb14 (Table 2).

The 16S rDNA sequence comparison showed that the closest relative to strain Kl.Fb14 was the type strain of *Pseudomonas moraviensis*, with 99.8% sequence identity. The closest relative to strain S8.Fb11 was the type strain of *Pseudomonas reinekei*, with 99.5% sequence identity.

Greenhouse assay

Rhizoctonia solani strains were tested for their ability to induce sclerotia on potato tubers and all were found to be highly infectious and produced many sclerotia. For more simplicity and to better assess the biocontrol efficacy of both selected bacteria, an inoculum composed of a mixture of the eight *R. solani* strains was prepared. When the mixture of *R. solani* strains was inoculated alone, 100% of the progeny tubers were infected with sclerotia, with a severity score of 3, making these tubers below market quality (Table 3). *Pseudomonas* sp. strain S8.Fb11 reduced the proportion of infected tubers to 40% for cv. 'Spunta' and to 74% for cv. 'Nicola'. The severity score did not exceed 1, i.e. 1% of the tuber surface covered with sclerotia, in both cases (Table 3). However, the weight of infected tubers was reduced for cv. 'Spunta' only. *Pseudomonas* sp. strain Kl.Fb14 strain did not significantly affect sclerotia development or severity score on progeny tubers for both cultivars.

Field trial

The field experiment was conducted in Sned-Gafsa using the RS5.2 strain of *R. solani* which originated from this region. The analysis of variance of the total weight of potato production showed that this depended on the potato variety (contributing 81%), and on the biocontrol treatment (contributing 19%) ($P£0.001$). As there was no block effect (P ³ 0.05), results are presented as the average of data collected from the three blocks. The non-infected potato tubers had low levels of infection: 5% of 'Spunta' tubers and 2% 'Nicola' tubers were infected. When compared to experimental controls, infection by *R. solani* strain RS5.2 significantly reduced potato yield for both cultivars, mainly for 'Spunta' (Figure 1). Potato infec-

Table 2. Mean growth inhibition (%) of eight *Rhizoctonia solani* isolates in Petri-dishes antibiosis tests, by two bacterial strains from faba bean root-nodules.

Strain	*R. solani* isolate[a]							
	RS1.1	RS1.2	RS1.3	RS2.2	RS2.3	RS3.2	RS5.1	RS5.2
Strain S8.Fb11	21.2^{c}	25.3^{c}	24.2^{c}	33.7^{b}	37^{b}	35.5^{b}	45.6^{a}	33^{b}
Strain Kl.Fb14	33.3^{bc}	33.3^{bc}	35.3^{b}	40.9^{a}	35.2^{b}	43.9^{a}	30.8^{bc}	29.9^{c}

[a] In the same row, values with different letters are statistically different (Duncan test, $P<0.05$). Each value is an average of six replicates.

Table 3. Mean tuber weights, weights of infected tubers and disease severity scores for potato plants ('Spunta' and 'Nicola') grown after different *Rhizoctonia solani*, bacterial strain or fungicide combination treatments were applied in a greenhouse assay.

Treatment	'Spunta'			'Nicola'		
	Total tuber weight (g/ plant)	Infected tubers weight (g/ plant)	SI [a]	Total tuber weight (g/ plant)	Infected tubers weight (g/ plant)	SI [a]
Control	436 a	0 c	0	449 ab	0 b	0
Inoculated with *R. solani*[b]	514 a	514 a	3-5	556 ab	556 a	2–3
Inoculated with *R. solani* and treated with pencycuron	621 a	0 c	0	614 a	0 b	0
Treated with strain S8.Fb11	432 a	0 c	0	558 ab	0 b	0
Inoculated with *R. solani* and treated with strain S8.Fb11	553 a	166 b	1	593 ab	421 a	1
Treated with strain Kl.Fb14	379 a	0 c	0	399 b	0b	0
Inoculated with *R. solani* and treated with strain Kl.Fb14	588 a	581 a	3–5	405 b	405 a	2–3

[a] The score of infection (SI) is deduced from the sclerotia development scale published by Brewer and Larkin (2005).
[b] A mixture of eight *R. solani* isolates grown separately was used as inoculum.
Each value is average of five replicates. Values with different letters in the same column are significantly different (Duncan's multiple range test at $P<0.05$).

tion from the *R. solani* treatment averaged 21% for 'Spunta' and 14% for 'Nicola'. The greatest infection scores were most often found with 'Spunta' (Figure 2). Treatment with *Pseudomonas* sp. strain S8.Fb11 or the fungicide fludioxonil increased potato yield for both cultivars inoculated with *R. solani*. Yield increases of 6 kg per 10 m row for 'Spunta' and 1 kg for 'Nicola' were recorded. The icidence of tuber infection was reduced to 7% for both cultivars, which was a greater reduction than with the fludioxonil application. Similar to our results under greenhouse conditions, in this field trial no significant biocontrol effect was found from *Pseudomonas* sp. strain Kl.Fb14.

Discussion

The main focus of previous studies on the potential benefits of using faba bean in cropping systems were mainly linked to the ability of this crop plant to enhance soil nitrogen content (Hauggard-Nielsen *et al.*, 2008; Peoples *et al.*, 2009; Jensen *et al.*, 2010). The effect of faba bean-associated bacteria on disease reduction in subsequent crops has received little attention. The present study represents a first step towards increasing knowledge of the positive effects that could arise from faba bean root-associated bacteria on the susceptibility of potato to the fungal pathogen *R. solani*.

Two faba bean root-nodule isolates belonging to the genus *Pseudomonas* were selected for their ability to inhibit *in vitro* mycelial growth of *R. solani*. *Pseudomonas* has been described among root nodule endophytic bacteria in different legume species (Ibáñez *et al.*, 2009) and as an efficient root-colonizer of different crops (Walsh *et al.*, 2001; Meyer *et al.*, 2010). Moreover, this genus was reported as the most active and dominant group of bacteria in the rhizosphere (Shoda, 2000). In the present study, the two selected *Pseudomonas* strains caused an average mycelial growth inhibition of over 30% across the eight *R. solani* strains tested. Previous research has described *Pseudomonas* species that showed high levels of inhibition of the *in vitro* growth of *R. solani* mycelia (Brewer and Larkin, 2005; Adesina *et al.*, 2007).

Both candidate strains in the present study were assessed in biocontrol assays under greenhouse conditions and in the field, applied to two cultivars of potato. Results showed that *Pseudomonas* sp. strain S8.Fb11 significantly reduced disease incidence on potato tubers for both cultivars. The percentage of in-

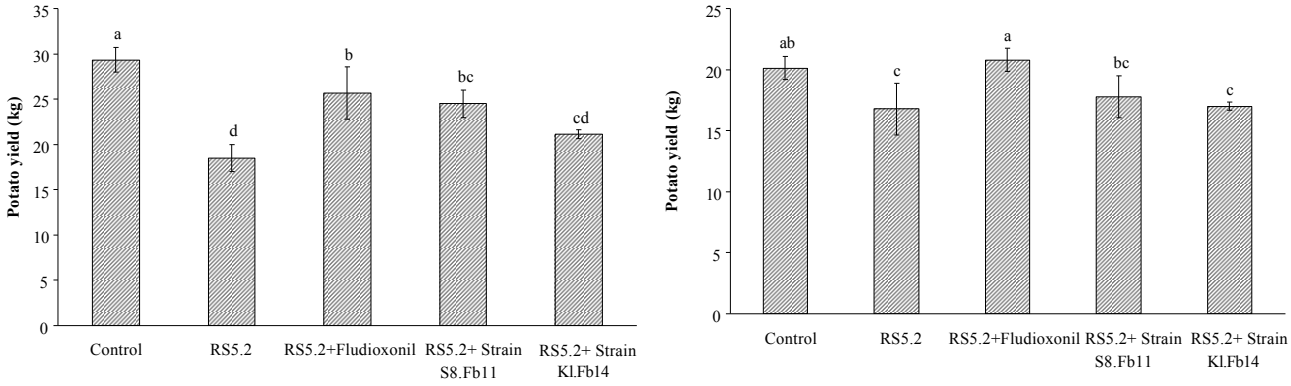

Figure 1. Mean tuber yields (kg/10 m row) from the potato cultivars 'Spunta' (A) or 'Nicola' (B) inoculated with *Rhizoctonia solani* isolate RS5.2 and treated with a fungicide (fludioxonil) or two different bacterial strains (S8.Fb11 or K1.Fb14), applied in a field trial. Means accompanied by different letters are significantly different according to Duncan's multiple range test at $P<0.05$.

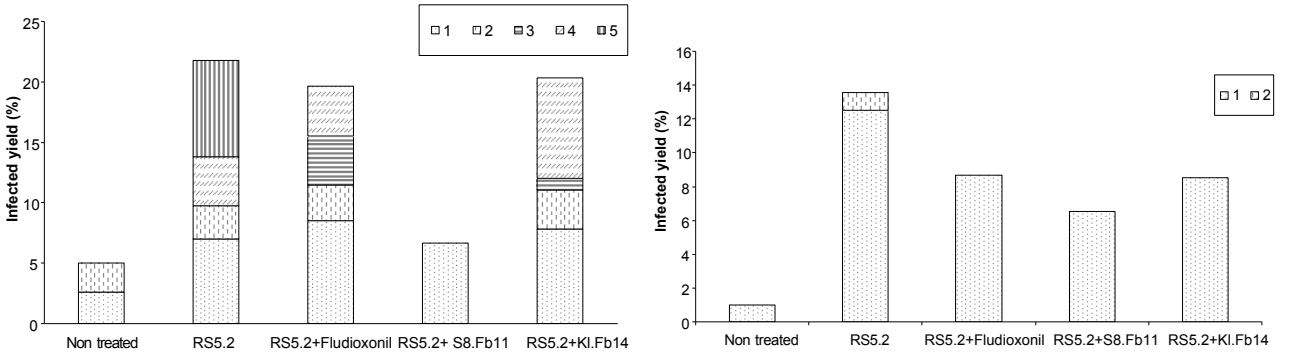

Figure 2. Mean proportions of potato tubers of the cultivars 'Spunta' (A) or 'Nicola' (B) in severiy scores 1 (low disease) to 5 (severe disease) (Brewer and Larkin, 2005), from plants grown in a field trial, which were inoculated with *Rhizoctonia solani* isolate RS5.2 and treated with a fungicide (fludioxonil) or two different bacterial strains (S8.Fb11 or K1.Fb14).

fected tubers was reduced to 40% for 'Spunta', better than values reported by Brewer and Larkin (2005). Moreover, the infected tubers from the strain S8.Fb11 treatment had reduced disease severity (sclerotia infection score <1 using the scale published by Brewer and Larkin, 2005), indicating that these potatoes were still marketable. The *Pseudomonas* sp. strain Kl.Fb14 had no effect on disease incidence; thus, the *in vitro* mycelia growth inhibition did not automatically translate into biocontrol under soil conditions for this strain. The biocontrol mechanisms involving host plants and the diverse microbial composition of the rhizosphere are more complex than simple antagonism as demonstrated in Petri plates (Ahmadzadeh and Tehrani, 2009; Mrabet *et al.*, 2011). This could be due to low levels of root colonization and/or to variable gene expression by the inoculated biocontrol agents under environmental conditions (Duffy and Defago, 1999; Walsh *et al.*, 2001). The biocontrol efficacy of selected bacterial strains must, therefore, be assessed in a range of conditions.

In the field trial, only the local *R. solani* strain RS5.2 was used in order to prevent the introduction of other pathogenic *R. solani* strains to the region. The field experiment showed that *R. solani* reduced potato yield, which confirms previous findings (Brewer and Larkin, 2005; Keiser, 2008). The proportional yield reduction was greater for the cultivar 'Spunta'

(40%) than for 'Nicola' (17%), which emphasizes the greater susceptibility of 'Spunta' to *R. solani*, as reported by Daami-Remadi *et al.* (2008b). *Pseudomonas* sp. strain S8.Fb11 significantly increased yield of potatoes from plants inoculated with the fungal pathogen. Efficacy in reducing the *R. solani* disease incidence in field trials for both cultivars was even greater than the reduction resulting from treatment with a standard fungicide, fludioxonil. This result could, in part, be explained by the fact that fungicides cannot move systemically from seed tubers to roots (Paulitz *et al.*, 2002). In contrast, *Pseudomonas* sp. strain Kl.Fb14 did not improve potato yield.

Brewer and Larkin (2005) reported that current cultural and chemical controls of *R. solani* are not completely effective and *Rhizoctonia* diseases of potatoes remain as persistent problems. Thus, the selection of candidate antagonistic biocontrol agents is a promising strategy to reduce disease incidence in potato crops, or at least to reduce the use of fungicides. *Pseudomonas* sp. strain S8.Fb11 showed an ability to survive at contrations of 7500 ppm of the fungicides pencycuron, fludioxonil, or azoxystrobin (data not shown). This is of considerable importance since soils cultivated with potato are mainly treated with at least one of the three fungicides or their analogues, and thus, strain S8.Fb11 could be used even in soils contaminated with these pesticides. Previous investigations reported that *Pseudomonas* sp. improved growth and performance of legumes (Tilak *et al.*, 2006; Ahmadzadeh and Tehrani, 2009; Egamberdieva *et al.*, 2010); the present study demonstrates an additional value to strains of this genus, namely their potential to protect potatoes which are commonly grown in rotation systems with legumes.

Further investigations on the ability of the faba bean-associated root bacteria to control various pathogenic fungi interacting with intercrops are needed to enhance understanding of how these cropping systems can be used to manage important plant diseases caused by fungi.

Acknowledgements

The authors wish to thank the Technical Center of Potato and Artichoke, Essaïda, Tunisia (CTPTA) for the facilities provided to conduct this research. They also thank Ms Sarra Bachkwel for her technical assistance in DNA sequencing. Dr Ezzeddine Aouani from the CBBC, Tunisia and Dr Maren J. Friesen from the University of Southern California, USA assisted with review of the manuscript of this paper.

Literature cited

Adesina M.F., A. Lembke, R. Costa, A. Speksnijder and K. Smalla, 2007. Screening of bacterial isolates from various European soils for *in vitro* antagonistic activity towards *Rhizoctonia solani* and *Fusarium oxysporum*: Site-dependent composition and diversity revealed. *Soil Biology and Biochemistry* 39, 2818–2828.

Ahmadzadeh M. and A.S. Tehrani, 2009. Evaluation of fluorescent pseudomonas for plant growth promotion, antifungal activity against *Rhizoctonia solani* on common bean, and biocontrol potential. *Biological Control* 48, 101–107.

Badri D.V., T.L. Weir, D. Van Der Lelie and J.M. Vivanco, 2009. Rhizosphere chemical dialogues: plant-microbes interactions. *Current Opinion in Biotechnology* 20, 642–650.

Brewer M.T. and R.P. Larkin, 2005. Efficacy of several potential biocontrol organisms against *Rhizoctonia solani* on potato. *Crop Protection* 24, 939–950.

Daami-Remadi M., S. Zammouri and M. El Mahjoub, 2008a. Effect of the level of seed tuber infection by *Rhizoctonia solani* at planting on potato growth and disease severity. *The African Journal of Plant Science and Biotechnology* 2, 34–38.

Daami-Remadi M., S. Zammouri and M. El Mahjoub, 2008b. 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. *The African Journal of Plant Science and Biotechnology* 2, 57–66.

Dick W.A. 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. *Agriculture, Ecosystems and Environment* 40, 25-36.

Djébali N. and T. Belhassen, 2010. Field study of the relative susceptibility of eleven potato (*Solanum tuberosum* L.) varieties and the efficacy of two fungicides against *Rhizoctonia solani* attack. *Crop Protection* 29, 998–1002.

Duffy B.K. and G. Defago, 1999. Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol isolates. *Applied and Environmental Microbiology* 65, 2429–2438.

Egamberdieva D., G. Berg, K. Lindström and L.A. Räsänen, 2010. Co-inoculation of *Pseudomonas* spp. with *Rhizobium* improves growth and symbiotic performance of fodder galega (*Galega orientalis* Lam.). *European Journal of Soil Biology* 46, 269–272.

Eken C., E. Demirci and F. Sahin, 2000. Pathogenicity of the fungi determined on tubers from potato storages in Erzurum, Turkey. *Journal of Turkish Phytopathology* 29, 61–69.

El Bakali A.M. and M.P. Martín, 2006. Black scurf of potato. *Mycologist* 20, 130–132.

Hauggaard-Nielsen H., B. Jørnsgaard, J. Kinane and E.S. Jensen, 2008. Grain legume-cereal intercropping: the practical application of diversity, competition and facilitation in arable and organic cropping systems. *Renewable Agriculture and Food Systems* 23, 3-12.

Ibáñez F., J. Angelini, T. Taurian, M.L. Tonelli and A. Fabra, 2009. Endophytic occupation of peanut root nodules by opportunistic *Gammaproteobacteria*. *Systematic and Applied Microbiology* 32, 49–55.

Jensen E.S., M.B. Peoples and H. Hauggaard-Nielsen, 2010. Faba bean in cropping systems. *Field Crops Research* 115, 203–216,

Keiser A., 2008. *Rhizoctonia solani*- a fungal disease with multiple symptoms: means of preventive and curative control. In: *Potato Research for a Production of Quality*. Information Day, February 2008, Changins (In French).

Lupwayi N.Z. and A.C. Kennedy, 2007. Grain legumes in Northern Great Plains: impacts on selected biological soil processes. *Agronomy Journal* 99, 1700–1709.

McEwen J., R.J. Darby, M.V. Hewitt and D.P. Yeoman, 1989. Effects of field beans, fallow, lupins, oats, oilseed rape, peas, raygrass, sunflowers and wheat on nitrogen residues in the soil and on the growth of a subsequent wheat crop. *Journal of Agricultural Science Cambridge* 115, 209–219.

Meyer B.J., M.P. Lutz, M. Frapolli, M. Péchy-Tarr, L. Rochat, C. Keel, G. Défago and M. Maurhofer, 2010. Interplay between wheat cultivars, biocontrol Pseudomonads, and soil. *Applied and Environmental Microbiology* 76, 6196–6204.

Mhamdi R., G. Laguerre , M.E. Aouani , M. Mars and N. Amarger, 2002. Different species and symbiotic genotypes of field rhizobia can nodulate *Phaseolus vulgaris* in Tunisian soils. *FEMS Microbiology Ecology* 41, 77–84.

Miller J.H., 1972. *Experiment in Molecular Genetics*. Cold spring Harbor Laboratory, New York, NY, USA.

Mrabet M., E. Abdellatif , K. Zribi , R. Mhamdi and N. Djébali, 2011. *Sinorhizobium meliloti* can protect *Medicago truncatula* from infection by *Phoma medicaginis*. *Phytopathologia Mediterranea* 50, 183–191.

Parry D.W., 1990. Diseases of potato. In: *Plant Pathology in Agriculture*. Cambridge University Press, Cambridge, UK, pp. 276–280.

Paulitz T.C., R.W. Smiley and R.J. Cook, 2002. Insights into the prevalence and management of soil-borne cereal pathogens under direct seeding in the Pacific Northwest USA. *Canadian Journal of Plant Pathology* 24, 416-428.

Peoples M.B., H. Hauggaard-Nielsen and E.S. Jensen, 2009. Chapter 13. The potential environmental benefits and risks derived from legumes on rotation. In: *Agronomy Monograph 52* (D.W. Emerich, H.B. Krishnan, ed.), Nitrogen fixation in Crop Production Am. Soc. Agron., Crop Sci. Soc. Am., and Soil Sci. Soc Am. Madison, Wisconsin, USA, 349-385.

Pérez-García A., D. Romero, A. de Vicente, 2011. Plant protection and growth stimulation by microorganisms: biotechnological applications of Bacilli in agriculture. *Current Opinion in Biotechnology* 22, 187–193.

Pypers P., J-M. Sanginga, B. Kasereka, M. Walangululu and B. Vanlauwe, 2011. Increased productivity through integrated soil fertility management in cassava-legume intercropping systems in the highlands of Sud-Kivu, DR Congo. *Fields Crops Research* 120, 76–85.

Recep K., S. Fikrettin, D. Erkol and E. Cafer, 2009. Biological control of the potato dry rot caused by *Fusarium* species using PGPR strains. *Biological Control* 50, 194–198.

Reinhold-Hurek B. and T. Hurek, 2011. Living inside plants: bacterial endophytes. *Current Opinion in Plant Biology* 14, 435–443.

Ritchie F., M.P. McQuilken and R.A. Bain, 2006. Effect of water potential on mycelial growth, sclerotial production, and germination of *Rhizoctonia solani* from potato. *Mycological Research* 110, 725–733.

Secor G.A. and N.C. Gudmestad, 1999. Managing fungal diseases of potato. *Canadian Journal of Plant Pathology* 21, 213–221.

Shoda M., 2000. Bacterial control of plant diseases. *Journal of Bioscience and Bioengineering* 89, 515–521.

Simon A. and K. Sivasithamparam, 1988. Crop rotation and biological suppression of *Gaeumannomyces graminis* var. *tritici* in soil. *Transactions of the British Mycological Society* 91, 279–286.

Stoddard F.L., A. Nicholas, D. Rubiales, J. Thomas and A.M. Villegas, 2010. Integrated pest management in faba bean. *Field Crops Research* 115, 308–318.

Tilak K.V.B., Ranganayaki N. and C. Manoharachari, 2006. Synergistic effects of plant-growth promoting rhizobacteria and *Rhizobium* on nodulation and nitrogen fixation by pigeonpea (*Cajanus cajan*). *European Journal of Soil Science* 57, 67–71.

Trabelsi D., H. Ben Ammar, H. Mengoni and R. Mhamdi, 2012. Appraisal of the crop-rotation effect of rhizobial inoculation on potato cropping systems in relation to soil bacterial communities. *Soil Biology and Biochemistry* 54, 1–6.

Van Loon L.C., 2007. Plant responses to plant growth-promoting rhizobacteria. *European Journal of Plant Pathology* 119, 701–711.

Walsh U.F., J.P. Morrissey and F. O'Gara, 2001. *Pseudomonas* for biocontrol of phytopathogens: from functional genomics to commercial exploitation. *Current Opinion in Biotechnology* 12, 289–295.

Weisburg W., Barns S.M., Pelletier D.A. and D.J. Lane, 1991. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* 173, 697–703.

Accepted for publication: February 13, 2013