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RESEARCH PAPER

Suppression of crown and root rot of wheat by the rhizobacterium *Paenibacillus polymyxa*

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Summary. A seedling bioassay was developed for screening a wheat root-associated rhizobacterial strain of *Paenibacillus polymyxa* for ability to suppress crown and root rot pathogens of wheat. The primary aim was to evaluate the ability of *P. polymyxa* to suppress *Fusarium graminearum*, *F. culmorum*, *F. verticillioides* and *Microdochium nivale*, the fungal pathogens responsible for Fusarium crown and root rot and head blight of wheat in Algeria. Bioassays conducted under controlled conditions indicated that seed treatments with *P. polymyxa* strain SGK2 significantly reduced disease symptoms caused by all four fungal pathogens. Plant growth promotion (increased shoot and root dry weights), however, depended on the pathogen tested. Our results indicate that seed treatments with a biocontrol agent could be an additional strategy for management of wheat crown and root rot pathogens.

Key words: biocontrol, crown rot, *Paenibacillus polymyxa*, root rot, wheat.

Introduction

Wheat root-associated rhizobacteria have been tested by several researchers for ability to increase yields, improve the use of nitrogen fertilizer, reduce disease, and improve plant and seed quality. The modes of action were reported to be through production of plant hormones, polysaccharides, or antifungal compounds (Kloepper *et al.*, 1989; Lynch *et al.*, 1990; Raza *et al*., 2008; Beneduzi *et al*., 2012). The plant growth-promoting rhizobacterium (PGPR) *Paenibacillus polymyxa* was found associated with rhizospheres of several different plant species, and also as the dominant nitrogen-fixing bacterium associated with wheat roots (Ash *et al.*, 1993; Heulin *et al.*, 1994; Guemouri-Athmani *et al.*, 2000; Berge *et al.*, 2002; Dobbelaere *et al.*, 2003; Beneduzi *et al.*, 2012; Vacheron *et al.*, 2013). *P. polymyxa* has also been isolated from marine sediments (Lal and Tabacchioni, 2009), from various soils and rhizospheres of forest trees such as olive (*Olea europaea*; Blibech *et al*., 2012), rhizospheres of *Calendula officinalis* (Ait Kaki *et al*., 2013), and also from farm and processing plant environments, and raw and pasteurized milk (Postollec *et al.*, 2010).

The mode of action of plant growth stimulation by *P. polymyxa* has been attributed to production of auxin, indol-ethanol, and phenolic metabolites (Lebuhn *et al.*, 1997); chitinases, antifungal compounds (Mavingui and Heulin 1994; Selim *et al.*, 2005); exopolysaccharides involved in soil aggregation (Gouzou *et al.*, 1993; Bezzate *et al.*, 2000); and nitrogen fixation (Achouak *et al.*, 1999). Ability of *P. polymyxa* to suppress *Fusarium* species has been reported by Dijksterhuis *et al.* (1999) and He *et al.* (2009). He *et al.* (2009) tested the ability of the bacterium to suppress *Fusarium graminearum*. Examples of biocontrol

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agents that have been described for their antagonistic effect on *Fusarium* species include *Trichoderma harzianum* and *Pseudomonas* spp. (Hibar *et al.*, 2005; Alabouvette *et al.*, 2009).

Despite the expansion of wheat cultivation in Algeria, yields remain relatively low (1.8 t ha^{-1}) compared to yields in the USA, which can be as high as 7.3 t ha^{-1} (World Bank IBRD-IDA). Several factors impact crop yields in Algeria and these include fungal diseases such as common root rot (caused by *F. graminearum* and *F. culmorum*), loose smut (*Ustilago tritici*) and leaf rust (*Puccinia tritici-duri*) (Ezzahiri, 2001), poor cultural practices, soil nutrition, climatic conditions, and the use of traditional low-yielding local cultivars such as Hedba 3, Mohamed Ben Bachir (MBB), O.Zenati 368, and Bidi 17 (Hazmoune, 2000). In 2006, new approved cultivars and several other cultivars were authorized for wheat production, representing in total 32 cultivars of durum wheat (*Triticum durum*). The Vitron and Waha cultivars are the most requested on the market (Anonymous, 2006). Crown and root rot, and head blight, all caused by *Fusarium* species, such as *F. graminearum* and *F. culmorum*, often result in over 25% reductions in crop yields and impact on grain quality (FAO, 2003).

In the present study, seedling bioassays were used to assess the effects of *P. polymyxa* SGK2, isolated in Algeria from a durum wheat rhizosphere and identified using a polyphasic approach (Guemouri-Athmani *et al.*, 2000). The bacterium was tested against three *Fusarium* spp. and *M. nivale*, which are major pathogens of durum wheat in Algeria.

Materials and methods

Soil and plant cultivars

Seeds of wheat (*T. durum* cv. Hoggar/Vitron) were obtained from the Technical Institute for Crops (ITGC, Algeria). This cultivar was selected by the International Maize and Wheat Improvement Center (CIMMYT, Mexico) and introduced in Algeria in 1986. *T. durum* cv. Hoggar/Vitron is a semi-early cultivar. Soil was sampled in Oued Smar (ITGC Experimental Station), 12 km east of Algiers. Ten samples, each collected at depth of 10 cm, were pooled for soil analyses. Soil analyses were performed according international validated methods at the National Institute of Agronomy (INA, Algiers), and the Olsen method was used to determine available phosphorus. Based on physical and chemical characteristics, this soil was a calcareous silty clay loam (Table 1).

Fungal pathogens and bacterial strain

The fungal cultures used include *F. graminearum*, *F. culmorum*, *F. verticillioides* and *M. nivale* (Table 2). Fungal species were identified using several morphological characteristics (size and form of macroconidia, presence or absence of microconidia, colour of the mycelium, presence or absence of chlamydospores) when grown on Potato Dextrose Agar (PDA) (Burgess *et al.*, 1994). Virulence of the isolates was tested on wheat seedlings before bioassays were conducted. Symptoms observed in wheat seedling bioassays indicated that virulence was not lost during routine maintenance of the fungi in culture. The bacterial strain, *P. polymyxa* SGK2, was isolated from

Table 1. Characteristics of soil at ITGC (Oued Smar).

Physical analysis[a]	**Coarse sand**	**Fine sand**	**Coarse silt**	**Fine silt**	**Clay**	**Permeability**
	11%	8%	47%	4%	30%	10.2 cm h^{-1}
Total minerals	**pH (H_20)**	**P_2O_5**[b]	**K_2O**	**$CaCO_3$**	**Salinity**	**CEC**[c]
	8.5	0.003%	0.02%	14.5%	0.1 dS m^{-1}	22.6 cmol kg^{-1}
Carbon and Nitrogen	**Organic matter**	**Total C**	**Total N**	**C/N**		
	2.06%	1.19%	0.12%	9.9		

[a] Coarse sand (> 200 μm), fine sand (50 to 200 μm), coarse silt (20 to 50 μm), fine silt (2 to 20 μm), clay (< 2 μm).
[b] According to Olsen method.
[c] Cationic Exchange Capacity.

Table 2. List and origin of fungal phytopathogens used in this study.

Fungal species	Code	Origin	Isolation year
Fusarium culmorum	FC01/08	*Triticum durum* cv. Vitron, ITGC (crown region)	2008
Fusarium graminearum	FG04/08	*T. sativum* cv. Hiddab 1220, ITGC (crown region)	2008
Fusarium verticillioides	FV01/07	*T. sativum* cv. Latino, INA (spike)	2007
Microdochium nivale	MN01/08	*T. durum* cv. Vitron, ITGC (crown region)	2008

the rhizosphere of durum wheat grown in Tiaret soil using the immunotrapping technique (Mavingui *et al.*, 1992) and identified using a polyphasic approach (phenotyping with the API50CHB microtube system and genotyping with ARDRA and REP-PCR techniques (Guemouri-Athmani *et al.*, 2000). This strain was selected for its strong xyloglucan activity (Athmani-Guemouri *et al.*, 2014) among 111 strains isolated from durum wheat rhizospheres, of plants grown on soils with different crop rotation histories (including wheat from 1 to more than 100 years).

In vitro effects of *Paenibacillus polymyxa* SGK2 on fungal pathogens

An *in vitro* assay was carried out to test the ability of *P. polymyxa* SGK2 to suppress fungal pathogens on PDA (Difco Laboratories) and King's B media (KB, bioMérieux,). The bacterial strain was grown overnight in tenfold diluted Tryptic Soy Broth (TSB/10, Becton Dickinson). A 50 µL droplet of this overnight bacterial culture washed by centrifugation and resuspended in sterile 0.85 % KCl was spotted on culture solid media (PDA or King's B) near the edge of each Petri dish, and then incubated at 30°C. After 2 d, a disc (7 mm diam.) of a fungal culture grown on PDA for 7 d was placed at the centre of the Petri dish. The Petri dishes were then incubated in the dark at 28°C for 7 d before assessment. The experiment was repeated three times. The iron deficient KB medium was used to test whether competition for iron is the mode of action of *P. polymyxa* SGK2, and PDA was used to test for the production of antifungal compounds by the bacterium.

In planta assays for fungal antagonism

Preparation of fungal inoculum

The fungal inoculum was prepared in 250 mL capacity Erlenmeyer flasks containing 54 wheat seeds in 22 mL water and sterilized (120°C for 20 min) (modified from Chabot *et al.*, 1993). Flasks were then separately inoculated by the four fungal pathogens. Each flask contained five discs (6 mm in diameter) of the test fungus, a 7 d PDA culture. The flasks were incubated under light at 25°C for 7 d, with agitation of the flasks every 3 d.

Seed treatment with Paenibacillus polymyxa SGK2

Wheat seeds of *T. durum* cv. Hoggar/Vitron were surface-sterilized with saturated calcium hypochlorite (2% vol/vol) for 2 h and 10% H_2O_2 (30 min) under partial vacuum, and then rinsed profusely in sterile water (Amellal *et al.*, 1998). *P. polymyxa* SGK2 bacterial cells were applied to surface-sterilized wheat seeds by dipping them in a bacterial suspension grown for 2 d in TSB/10 medium, washed by centrifugation and resuspended in sterile 0.85 % KCl (final concentration: 10^6 colony forming units mL^{-1}) at 30°C for 4 h (100 seeds in 100 mL bacterial inoculum; approx. 10^6 bacterial cells $seed^{-1}$). Inoculated seeds were dipped in a sterile 1% methylcellulose solution (mass/volume) in order to optimize bacterial adhesion on the seed surfaces. Coated seeds were dried overnight under sterile laminar air flow. A 3 mL bacterial suspension (10^6 colony-forming units mL^{-1}) grown in TSB/10 medium was also added on the soil surface after sowing. Wheat seeds of the control treatment were surface sterilized, rinsed in sterile water and sown directly into the soil.

Seedling bioassay

The bioassay system consisted of pots (5 cm diam × 10 cm height) filled with 100 g of field soil (from Oued Smar), which was maintained at 60% water holding capacity. Durum wheat seeds were sown at a depth of 2 cm, with five seeds per pot. The fungal inoculum was prepared in Erlenmeyer flasks containing sterile wheat seeds and water (as above), which were respectively inoculated with spore sus-

Table 3. *In vitro* effects of *Paenibacillus polymyxa* SGK2 on the four fungal pathogens on two growth media. Petri dishes were incubated at 28°C for 7 d, and presence and sizes of inhibition zones were assessed.

Medium	*Fusarium culmorum*	*Fusarium graminearum*	*Fusarium verticillioides*	*Microdochium nivale*
PDA	-	-	+	-
King B	++	+	++	++

-, absence of inhibition zone (identical to the control).
+, diameter of inhibition zone 0.5 to 1 cm.
++, diameter of inhibition zone > 1 cm.

pension containing (10^6 spores/mL) of each *Fusarium* sp. Flasks were incubated under light at 25°C for 15 d, with agitation of the flasks every 3 d to ensure optimal development of the fungi.

The pots treated with strain SGK2 or untreated were replicated: four replicates per treatment × nine treatments (control/no fungus and no bacterium, *F. culmorum* with and without SGK2, *F. graminearum* with and without SGK2, *F. verticillioides* with and without SGK2, *M. nivale* with and without SGK2) and were completely randomised. A treatment without fungal pathogen but with 2.5 g of sterile macerated wheat seeds was also included as a further control treatment.

After one-month incubation, the severity of the symptoms on crowns of resulting wheat plants was scored using a scale from 0 to 3 (Vakalounakis and Fragkiadakis, 1999) as follows:

0 = no symptoms; 1 = darkening or brown colouring of crowns, less than 25%; 2 = darkening or brown colouring of crowns, ranging from 25 to 50%; and 3 = darkening or brown colouring of crowns, greater than 50%.

A disease index (DI) was calculated using the following formula:

$$DI = [(0 \times F0) + (1 \times F1) + (2 \times F2) + (3 \times F3)]/N$$

where F = the number of plants for each severity symptom scale, and N = the total number of plants.

Plant growth was measured at the end of the experiment as the shoot and root fresh and dry weights. Shoots and roots were weighed after drying at 80°C for 3 d.

Statistical analyses

Statistical analyses (ANOVA) were performed using the Statgraphics Centrion software (version XV) to analyse plant weight data. Fisher's least significant difference (LSD) test was used for multiple-range analysis. The Friedman test was used to compare the disease indices before and after treatment with SGK2 strain with a significance level of 5% applied.

Results

In vitro effects of *Paenibacillus polymyxa* SGK2 on fungal pathogens

The *in vitro* effects of *P. polymyxa* SGK2 were tested on two culture media (PDA and King's B). The antagonistic effects of strain SGK2 were observed for all the four fungal species tested on the iron-deficient King's B medium (Table 3). In contrast, only growth of *F. verticillioides* was slightly inhibited by the bacterial strain on iron-rich PDA medium. This suggests that competition for iron through the production of siderophores could be one of the main mechanisms involved in fungal growth inhibition of *Fusarium* species by *P. polymyxa* SGK2. The inhibition of *F. verticillioides* by *P. polymyxa* SGK2 on PDA medium, an iron rich medium, indicates that diffusible antifungal compounds may also play roles in the ability of strain SKG2 to inhibit some fungal pathogens. Therefore, competition for iron through the production of siderophores may not be the only mechanism involved in the suppression of fungal pathogens by strain SKG2 (Figure 1).

In planta assays for fungal antagonism

Analysis of variance (ANOVA) estimates indicated no statistically significant effect of the treatments, fungal pathogen or the strain SGK2, on root (Table 4) or shoot (Table 5) fresh weights ($P>0.60$). In contrast, effects of fungal pathogen inoculation on root

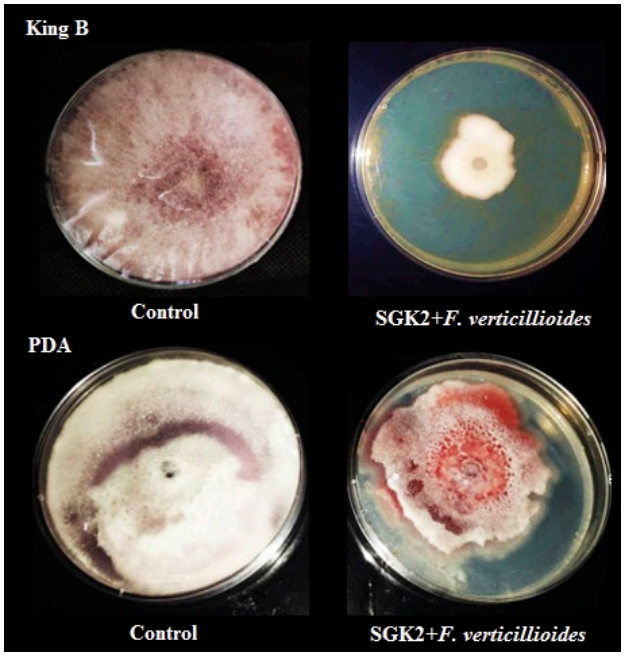

Figure 1. Direct confrontation test between *Paenibacillus polymyxa* SGK2 and *Fusarium verticillioides* on the King's B and PDA media after 7 d incubation at 28°C in the dark.

dry weight (P<0.001; Table 6) and shoot dry weight (P=0.01; Table 7) were significant. There were also significant effects of bacterial inoculation on root dry weight (P<0.03; Table 6) and shoot dry weight (P<0.02; Table 7). The interaction between the fungal pathogen and *P. polymyxa* SGK2 was not significant for shoot and root dry weights.

Effects of fungal pathogens and *Paenibacillus polymyxa* strain SGK2 on disease index

The effects of *P. polymyxa* strain SGK2 on wheat seedlings in the presence of the three *Fusarium* species and *M. nivale* were evaluated based on the severity of the symptoms on the crown tissue, using a disease index scale from 0 to 3. The mean disease index in control pots inoculated with the pathogen alone ranged from 2.2 to 3.0 (Figure 2), demonstrating that the fungal inoculation preparation was effective and caused disease symptoms on wheat seedlings. Using the disease index rating, the ability of seed treated *P. polymyxa* strain SGK2 to suppress disease symptoms was also observed. Irrespective of the pathogen species tested, in the presence of the biocontrol agent, decreases in disease indices ranged from 71 to 87%. In pots treated with biocontrol agent *P. polymyxa* the decrease in disease index was 75% in the presence of *F. graminearum*, 78% in the presence of *F. culmorum*, 72% in the presence of *F. verticillioides* and 87% in the presence of *M. nivale*. Friedman tests evidenced a global significant difference (P<0.046) between treatments with or without inoculation with *P. polymyxa* SGK2.

Effects of fungal pathogens and *Paenibacillus polymyxa* strain SGK2 on root dry weights

When compared to the control pots without fungal inoculum, only pathogen control pots inoculated with *F. culmorum* or *F. graminearum* showed significant decreases in mean root dry weights (P<0.001)

Table 4. ANOVA for "Root fresh weight" in mg - type III Sum of squares

Source	Sum of squares	Ddl	Mean square	F	Probability
Main effects					
A: *Paenibacillus*	264.5	1	264.5	0.16	0.69
B: fungi	2706.6	3	902.2	0.54	0.66
Interaction					
AB	57.3	3	19.0833333	0.01	0.99
Residue	40127.5	24	1671.97916		
Total (corrected)	43155.9	31			

All F values are based on the mean square residual error.

Table 5. ANOVA for "Shoot fresh weight" in mg - type III Sum of squares

Source	Sum of squares	Ddl	Mean square	F	Probability
Main effects					
A: *Paenibacillus*	712.5	1	712.5	0.16	0.69
B: fungi	8825.1	3	2941.7	0.66	0.59
Interaction					
AB	1.1	3	0.4	0.00	1.00
Residue	107689.7	24	4487.1		
Total (corrected)	117228.4	31			

All F values are based on the mean square residual error.

Table 6. ANOVA for "Root dry weight" in mg - type III Sum of squares

Source	Sum of squares	Ddl	Mean square	F	Probability
Main effects					
A: *Paenibacillus*	39.6	1	39.6	5.79	0.024
B: fungi	186.4	3	62.1	9.08	0.0003
Interaction					
AB	14.7	3	4.9	0.72	0.55
Residue	164.2	24	6.8		
Total (corrected)	404.9	31			

All F values are based on the mean square residual error.

Table 7. ANOVA for "Shoot dry weight" in mg - type III Sum of squares

Source	Sum of squares	Ddl	Mean square	F	Probability
Main effects					
A: *Paenibacillus*	118.2	1	118.2	7.23	0.01
B: fungi	229.6	3	76.5	4.68	0.01
Interaction					
AB	137.2	3	45.7	2.80	0.06
Residue	392.5	24	16.4		
Total (corrected)	877.4	31			

All F values are based on the mean square residual error.

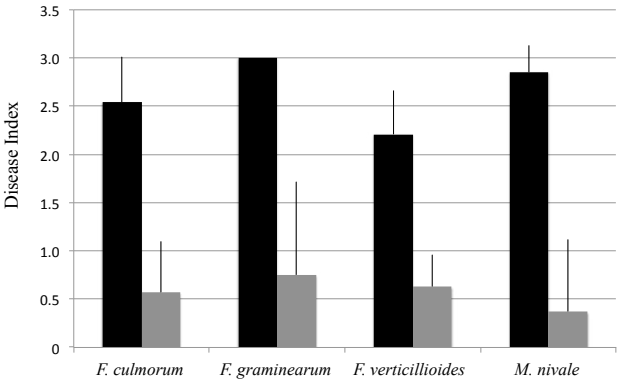

Figure 2. Mean crown rot disease indices after *Paenibacillus polymyxa* SGK2 inoculation of wheat seedlings. Durum wheat seedlings were either infected by the fungal pathogen (black bars), or inoculated by the fungal pathogen and the *P. polymyxa* SGK2 strain (grey bars). The disease indices ranged from 0 (no disease) to 3 (maximum disease with crown infection surface ≥ 50 %). Standard deviations of the means are indicated. Friedman test showed a global difference (P<0.046) between treatments with or without inoculation with *P. polymyxa* SGK2 strain.

(Figure 3). *F. culmorum* reduced root dry weight by 29% and *F. graminearum* reduced root dry weight by 59%. When compared to the control pots without the fungal inoculum, however, there were no significant reductions in root dry weight in pots inoculated with *F. verticillioides* or *M. nivale.*

Significant effects of strain SGK2 inoculation was observed on root dry weight (P<0.03) of durum wheat plants in the presence of *F. culmorum* or *F. graminearum* (Figure 3). Seed treatments with *P. polymyxa* SGK2 restored the root dry weight significantly. When compared to the control treatment, root dry weight was restored by 90% in pots inoculated with *F. culmorum*, and by 70% in pots inoculated with *F. graminearum*.

Effects of fungal pathogens and *Paenibacillus polymyxa* strain SGK2 on shoot dry weights

When compared to the control pots without the fungal inoculum, pathogen control pots inoculated with *F. culmorum, F. verticillioides* or *M. nivale* showed significant decreases in mean shoot dry weights (P=0.01) (Figure 4). No significant decrease in shoot dry weight was observed in the presence of *F. graminearum.* The reduction in shoot dry weight

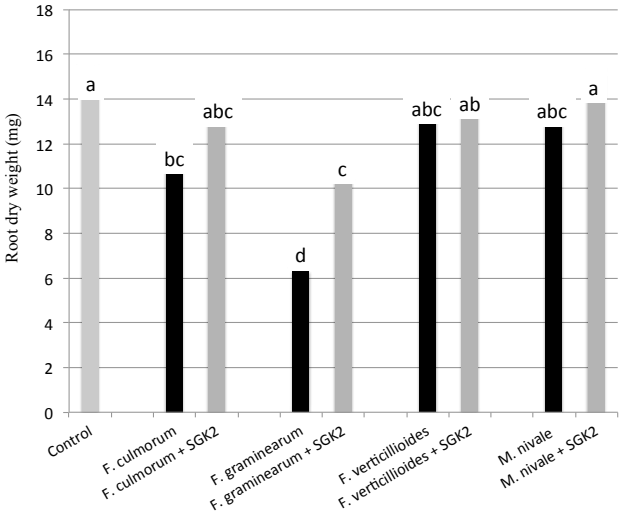

Figure 3. Effects of inoculation with *Paenibacillus polymyxa* SGK2 on root growth of durum wheat inoculated with four fungal species, as measured by mean root dry weight. Durum wheat was either infected by the fungal pathogens (black bars), or inoculated by the fungal pathogen and the *P. polymyxa* SGK2 strain (grey bars). Values accompanied by different letters are significantly different according to LSD tests (P<0.05).

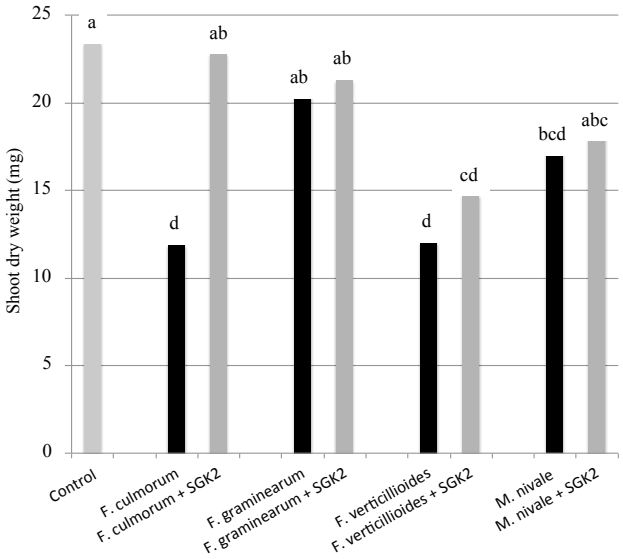

Figure 4. Effects of inoculation with *Paenibacillus polymyxa* SGK2 on shoot growth of durum wheat inoculated with four fungal species, as measured by mean shoot dry weight. Durum wheat was inoculated with either the fungal pathogens (black bars), or inoculated with the fungal pathogens and the *P. polymyxa* SGK2 strain (grey bars). Values accompanied by different letters are significantly different according to LSD tests (P<0.05).

was 49% with *F. culmorum*, 49% with *F. verticillioides* and 25% with *M. nivale*.

Significant effect of strain SGK2 inoculation was observed on mean shoot dry weight ($P<0.03$) only in pots with *F. culmorum* (Figure 4). Seed reatments with *P. polymyxa* SGK2 restored the shoot dry matter significantly (by 95%) when compared to the uninoculated controls.

Colonization of wheat seedlings by *Paenibacillus polymyxa*

Roots from pots treated with the pathogens and *P. polymyxa* SGK2 were observed after 30 d of incubation using a light microscope (×40 magnification). Lysis of the pathogen mycelia was observed from all root samples treated with strain SGK2.

Discussion

The use of conventional methods to manage plant diseases with agrichemicals has limitations. There is increasing public concern regarding the effects of agrichemicals on the environment and human health. Therefore, a key research goal for sustainable agricultural production is the exploitation of naturally occurring environmentally-friendly mechanisms for disease control. In this study we evaluated the effects of *P. polymyxa* SGK2 (Guemouri-Athmani *et al.*, 2000) on Fusarium crown rot, root rot and head blight wheat pathogens, using a seedling bioassay technique.

Compared to the earlier studies of He *et al.* (2009) on biocontrol of *F. graminearum* by *P. polymyxa*, the present study compared four different fungal species responsible for wheat diseases in Algeria. He *et al.* (2009) shown that *P. polymyxa* strains W1-14-3 and C1-8-b were able to control *F. graminearum* in greenhouse conditions. Compared to a control treatment, these strains respectively reduced disease severity by 57 and 55%. As well, in a study dealing with biocontrol of *Fusarium* and plant-growth promotion by *Paenibacillus* strains isolated from soil, Xu and Kim (2014) demonstrated the *in vitro* and *in vivo* antagonistic effects of *P. polymyxa* strain SC09-21 against *F. oxysporum* f.sp. *radicis-lycopersici*.

We have demonstrated that seedling bioassays can be used for screening biocontrol agents of wheat pathogens using disease index, and also to evaluate the effects of biocontrol agents on plant growth.

The present study also demonstrated that wheat pathogens impact shoot and root growth of wheat seedlings quite differently, as measured by fresh and dry weight estimates. While *F. graminearum* significantly reduced only root growth, *F. verticillioides* reduced only shoot growth. Inoculation with *P. polymyxa* SGK2 improved significantly the root growth in the presence of *F. graminearum*. Inoculation with this strain, however, did not improve shoot growth of seedlings infected by *F. verticillioides* and *M. nivale*. Strain SGK2 did not improve growth, but protected plants from the pathogen, which also causes reduced plant growth. The mechanisms by which *Fusarium* species impact seedling growth and their ability to colonize plant roots and stems may differ.

In general, production of antifungal compounds by biocontrol agents affects their abilities to prevent plant diseases. The ability of a *P. polymyxa* strain isolated from the rhizosphere of watermelon to produce anti-fungal compounds was reported in an earlier study (Raza *et al.*, 2010). In the present study, when *in vitro* effects of *P. polymyxa* SGK2 were tested on agar media, all four fungal species were inhibited on the iron-deficient King's B medium. In contrast, only *F. verticillioides* growth was slightly inhibited by the strain SGK2 on PDA medium. This suggests that competition for iron through the production of siderophores could be one of the main mechanisms involved in fungal growth inhibition of *Fusarium* species by *P. polymyxa* SGK2. Studies conducted by Yu *et al.* (2011) has shown that siderophores play important roles in mediating inhibition by *Bacillus subtilis* of *F. oxysporum*, which causes wilt in peppers. Further studies are needed to understand the mode of action of *P. polymyxa* SGK2. However, iron chelation may not be the only mechanism involved in the antibiosis, and diffusible antifungal compounds may be produced by strain SKG2, as inhibition zones were also observed on an iron-rich PDA medium. Mavingui and Heulin (1994) have shown that *P. polymyxa* isolated from wheat rhizospheres produces chitinase enzymes that suppressed *Gaeumannomyces graminis* var. *tritici (Ggt)*, the causal agent of take-all in wheat.

In the present study, when seedling roots were observed with light microscopy after 30 d of incubation, lysis of pathogen mycelium was observed from all root samples treated with *P. polymyxa* SGK2. Formation of biofilms by *P. polymyxa* SGK2 was also observed (data not shown). This may partially ex-

plain the effect of strain SGK2 to reduce disease, and improve root and shoot dry weights, in some of the pathogen-biocontrol combinations tested.

The results from this study are encouraging, as *F. culmorum* is a major causative agent of Fusarium crown rot, root rot and head blight in cereals in Algeria. Natural epidemics have resulted in severe yield losses, reduced quality, and grain contamination by mycotoxins. *F. culmorum* is more aggressive on wheat in warm areas (Eslahi, 2012), where water stress and drought conditions increase the susceptibility of plants rather than the virulence of the fungi (Scherm *et al*., 2013; Motallebi *et al*., 2015). Management of these soil-borne phytopathogens by seed treatments with *P. polymyxa* may offer an alternative or additional strategies to conventional disease management methods.

Successful colonization of host rhizospheres and plant tissues by introduced microbes is key for effective control of soil-borne pathogens and promotion of plant growth (Hebbar *et al*., 1992a; Hebbar *et al*., 1992b; Ploetz, 2005). It is postulated that production of antifungal substances, rapid growth rates, the capacity to utilize a wide range of carbon sources exuded by roots, and to lesser extent the production of extracellular enzymes, may facilitate biocontrol agent colonization of host roots (Hebbar *et al*., 1992c). Thangavelu and Gopi (2015) demonstrated the importance of root colonization and survival of biocontrol agents for effective suppression of *F. oxysporum* causing wilt in bananas. When compared to bacterial strains isolated from rhizospheres of banana plants, those isolated from within the roots (endophytic) were not only better root isolates, but survived longer on banana roots and suppressed the wilt pathogen. Our results also demonstrated that *P. polymyxa* SGK2, with its ability to form biofilms on the surfaces of wheat roots, an indication of close association with the roots, is an efficient suppressor of the wheat crown and root pathogens tested.

The present study has clearly shown that *P. polymyxa* strain SGK2 is able to suppress *Fusarium* spp. and *M. nivale*. However, effects of the biocontrol agent on disease severity and plant growth largely depend on the *Fusarium* species and *M. nivale* involved in pathogenesis. Our study has also shown that several mechanisms may be involved in pathogen suppression. To better explain the pathways involved in plant pathogen-host-biocontrol agent interactions, further research will be needed.

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