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Effect of rhizobacteria strains on the induction of resistance in barley genotypes against *Cochliobolus sativus*

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Summary Enhancement of the resistance level in plants by rhizobacteria has been proven in several pathosystems. This study investigated the ability of four rhizobacteria strains (*Pseudomonas putida* BTP1 and *Bacillus subtilis* Bs2500, Bs2504 and Bs2508) to promote the growth in three barley genotypes and protect them against *Cochliobolus sativus*. Our results demonstrated that all tested rhizobacteria strains had a protective effect on barley genotypes Arabi Abiad, Banteng and WI2291. However, *P. putida* BTP1 and *B. subtilis* Bs2508 strains were the most effective as they reduced disease incidence by 53 and 38% (mean effect), respectively. On the other hand, there were significant differences among the rhizobacteria-treated genotypes on plant growth parameters, such as wet weight, dry weight, plant height and number of leaves. *Pseudomonas putida* BTP1 strain was the most effective as it significantly increased plant growth by 15-32%. In addition, the susceptible genotypes Arabi Abiad and WI2291 were the most responsive to rhizobacteria. This means that these genotypes have a high potential for increase of their resistance against the pathogen and enhancement of plant growth after the application of rhizobacteria. Consequently, barley seed treatment with the tested rhizobacteria could be considered as an effective biocontrol method against *C. sativus*.

Additional keywords: Biocontrol, *Hordeum vulgare,* ISR, PGPR, spot blotch

Introduction

Plants have various mechanisms of resistance against pathogens. It has been proven that plant growth-promoting rhizobacteria (PGPR) capable of improving the growth and yield of crops by fixing atmospheric nitrogen, solubilizing insoluble phosphates and secreting hormones, such as IAA (Majeed *et al.*, 2015; Ahmed *et al.*, 2017), are also able to enhance plant resistance against pathogens by inducing the systemic resistance (ISR) (Pieterse *et al.*, 2002). This phenomenon can be systemic as PGPR are in soil on the plant roots while their positive effects appear on the above-ground plant parts. ISR is long-lasting and not conducive for developing resistance in the targeted pathogen. In addition, the activation of ISR-mediated defensive mechanism is overexpressed upon the subsequent pathogen challenge. Therefore, ISR phenomenon can be the basis of integrated pest management strategies in both field and greenhouse crops (Van Loon *et al.*, 1998; Ramamoorthy *et al.*, 2001; Zehnder *et al.*, 2001; Saravanakumar *et al.*, 2007).

This phenomenon has been proved in several plants against a broad range of bacterial, fungal and viral diseases, as well as against insects and nematodes (Van Loon *et al.*, 1998; Durrant and Dong, 2004; Bakker *et al.*, 2007; Choudhary and Johri, 2009; De Vleesschauwer and Höfte, 2009; Reglinski, 2009). Most PGPR-elicited ISR in plants belong to the genera *Pseudomonas* and *Bacillus* (Kloepper *et al.*, 2004; Choudhary and Johri, 2009). In the same context, a non-pathogenic *Pseudomonas putida* BTP1 strain has shown to enhance resistance in cucumber against *Pythium aphanidermatum*, and in bean and tomato against *Botrytis cinerea* (Ongena *et al.*, 1999; Ongena *et al.*, 2004; Adam *et al.*, 2008). In addition, the same effect has been shown on grapevine and potato plants against phylloxera (*Daktulosphaira vitifoliae*) and potato tuber moth (*Phthorimaea operculella* Zeller), respectively (Adam *et al.*, 2012; Adam *et al.*, 2013; Adam *et al.*, 2016). On the other hand, *Bacillus subtilis*

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Bs2500, Bs2504 and Bs2508 strains have also been demonstrated to induce systemic resistance in tomato and barley against *B. cinerea* and *Pyrenophora graminea*, respectively (Ongena *et al.*, 2008; Adam *et al.*, 2017).

The protective effect of PGPR was often proved in dicots, such as cucumber, tobacco and *Arabidopsis*. However, the efficacy of PGPR in monocots against necrotrophic pathogens has been demonstrated only in a few cases (Van Loon, 2007; Van Wees *et al.*, 2008; Vlot *et al.*, 2008; Pinedra *et al.*, 2010). *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dast. [anamorph: *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.], the fungus causing spot blotch, is a common foliar pathogen of barley (*Hordeum vulgare*) and responsible for large economic losses in grain yield of cereals in North America (Mathre, 1990), Australia (Meldrum *et al.*, 2000) and Syria (van Leur *et al.*, 1997). Effective control of *C. sativus* can be achieved by the introduction of resistant cultivars as an important component of integrated disease management (Ghazvini and Tekauz, 2008).

Therefore, this work aimed mainly at studying the effect of four rhizobacteria strains (*P. putida* BTP1 and *B. subtilis* Bs2500, Bs2504, and Bs2508) against *C. sativus* on barley genotypes (Arabi Abiad, Banteng and WI2291) and on their ability to promote plant growth.

Materials and methods

Microbial strains and inoculum preparation

Rhizobacterial strains used in this study, *P. putida* BTP1 and *B. subtilis* Bs2500, Bs2504 and Bs2508, were provided by Prof. Philippe Thonart (Wallon Center for Industrial Biology, University of Liège, Belgium). *Pseudomonas putida* strain and *B. subtilis* strains were maintained for a short period at 4°C on King's B agar (King *et al.*, 1954) and 868 agar Petri dishes (20 g/l glucose, 10 g/l peptone, 10g/l yeast and 20 g/l agar) medium (Jacques *et al.*, 1999), respectively. For long-term maintenance, strains were stored at -80°C in cryotubes according to the manufacturer recommendations (Microban K; Prolab Diagnostic, Richmond Hill, Canada). For utilization, *P. putida* strain was grown on Casamino acids (CAA) broth medium (5 g/l CAA, 0.9 g/l K_2HPO_4 and 0.25 g/l $MgSO_4$) (Ongena *et al.*, 2002) for 24 h at 30±1°C, whereas *B. subtilis* strains were grown on 868 broth medium (20 g/l glucose, 10 g/l peptone and 10g/l yeast) for 48 h at 30±1°C. The cultures were then centrifuged at 10,000 rpm for 10 min. Supernatants were removed and bacterial cells were collected and resuspended in 10 mM $MgSO_4$ to a final concentration of 10^8 colony-forming units (CFU) per ml before use.

The fungal pathogen *Cochlibolus sativus* isolate Pt4 was provided by Dr. M.I.E. Arabi (Atomic Energy Commission of Syria). It was isolated from naturally infected barley leaves as described by Arabi and Jawhar (2003). The fungus was grown in 9 cm Petri dishes containing potato dextrose agar (PDA, Difco, Detroit, MI, USA) for 10 days at 22±1°C in the dark. The conidial suspension was prepared by harvesting conidia with 10 ml of sterile distilled water. After removing mycelial debris by filtration through several layers of cheesecloth, the conidial suspension was centrifuged for 5 min at 5,000 g and conidia were resuspended in 0.01% X-triton-100 to a final concentration of $2x10^4$ conidia/ml.

Barley genotypes

The three barley (*Hordeum vulgare* L.), genotypes, Arabi Abiad, Banteng and WI2291, used in the present study, were provided by Dr. M.I.E. Arabi (Atomic Energy Commission of Syria). They were chosen for their differential reaction to artificial inoculation with *C. sativus* (Arabi, 2005). WI2291 (susceptible) originated from the Waite Institute (Glen Osmond, Australia), Banteng (resistant) is a German genotype and Arabi Abiad (moderate resistant) is a local genotype (heterogeneous landrace).

Assays for induction of resistance

Barley seeds of the three genotypes were surface sterilized by dipping in sodium hypochlorite (5%) for 5 min and washed

with sterilized distilled water three times for 3 minutes each time. Prior to sowing, sterilized seeds were soaked for 10 min in suspensions of 10^8 CFU/ml 0.01M $MgSO_4$ of the rhizobacteria strains. Untreated (control) seeds were soaked for 10 min in distilled water. Then, seeds were sown in 10 cm-plastic pots (four seeds of each genotype per pot) containing a sterilized potting substrate (Brill Substrate GmbH, KG, Germany). To ensure good colonization with the rhizobacteria, the soil substrate was mixed after its sterilization with the rhizobacteria suspensions (approximately 1l of 10^8 CFU/ml rhizobacterial suspension of each strain per 3 kg of substrate to obtain a final concentration of $3.3x10^7$ CFU/g soil substrate) or with an equal volume of sterilized distilled water per 3 kg of substrate for the untreated (control) seeds. Pots were arranged in a randomized complete block design with six pots per treatment and genotype as replicates (total of 24 plants). Plants were placed in a growth chamber at temperatures 22±1°C (day) and 17±1°C (night) with a 12-h photoperiod and 80-90 % relative humidity. All plants emerged from both treated and untreated seeds were inoculated with *C. sativus* at growth stage GS13 [three emerged leaves, based on the growth scale developed by Zadoks *et al.* (1974)] by depositing five drops (5µl each) of a conidial suspension ($2x10^4$ conidia/ml) on the second leaf of each plant. Experimental plants were initially incubated for 48h at 20°C, in darkness and under high humidity (> 90 %). Subsequently, they were placed in a growth chamber at 22 ± 1°C (day) and 17±1°C (night) with a 12-h photoperiod and 80-90% relative humidity. Fourteen days after inoculation, disease incidence was assessed and expressed as percentage of inoculation sites per leaf developing lesions (one lesion = 20%, 2 lesions = 40%, 3 lesions = 60%, 4 lesions = 80% and 5 lesions = 100%) (Fig. 1).

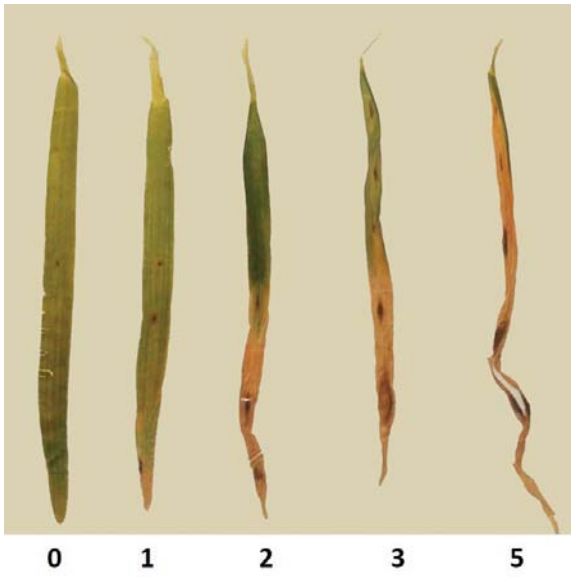

Figure 1. Development of symptoms over a period of 14 days on the second leaf of barley plants (Arabi Abiad genotype) inoculated with five drops (5µl each) of a conidial suspension ($2 x 10^4$ conidia/ml) of *Cochliobolus sativus*. Disease incidence was expressed as percentage of inoculation sites per leaf developing lesions (one lesion = 20%, 2 lesions = 40%, 3 lesions = 60%, 4 lesions = 80% and 5 lesions = 100%).

Estimation of rhizobacterial populations on barley roots

One gram of roots was collected from the rhizobacteria-treated barley genotypes 28 days after sowing and prior to their inoculation with *C. sativus*. Then, roots were washed with sterilized distilled water and crushed in sterilized pestle mortar with 2 ml sterilized distilled water to release the bacteria from tissues. One ml of the root solution was added to test tube containing 9 ml of 0.85% NaCl, and successive serial dilutions were prepared. Aliquots of 100 µl from dilution tubes were spread onto Luria-Bertani (LB) agar medium Petri dishes and incubated at 28°C for 1-2 days. Bacterial populations were estimated as CFU/g of roots.

Testing leaves for the presence of rhizobacteria

Small leaf samples were excised from rhizobacteria-treated plants. The samples were surface sterilized with 5% sodium hypochlorite for 3 min and washed three times (for 3 min each time) with sterilized distilled water. Samples were left to dry on sterile filter paper. Then they were transferred under aseptic conditions onto Petri dishes containing LB agar medium. The dishes were incubated for 72 h at 28±1°C in the dark.

Assessment of growth of rhizobacteria-treated barley plants

Six weeks after sowing, the experimental barley plants were harvested and plant growth parameters, such as plant height (measured from the soil level to the top of the main plant stem), number of leaves, wet weight (measured on the above soil level plant parts) and dry weight (above soil level plant parts were dried at 65°C for 48h and weighted), were recorded.

Statistical analysis

Statistical analyses were performed using STAT-ITCF programme at 0.05 significance level (P=0.05) (Anonymous, 1988). Data were subjected to analysis of variance (ANOVA) for the determination of differences in the means between treatments. Differences between means were tested for significance using the Student-Newman-Keuls Test.

Results

Resistance induced by rhizobacterial strains in barley plants

The results showed that all tested rhizobacterial strains (*P. putida* BTP1 and *B. subtilis* Bs2500, Bs2504, and Bs2508) used in this study exhibited a protective effect on barley genotypes Arabi Abiad, Banteng and WI2291 against *C. sativus*. There was a significant difference among these strains concerning their ability to increase the resistance level in barley. However, *P. putida* BTP1 and *B. subtilis* Bs2508 strains were the most effective, as they significantly reduced the mean disease incidence on the three genotypes by 53 and 38%, respectively, compared to the control plants (Table 1). In addition, a significant difference was observed between barley genotypes used in this study. Banteng was the least susceptible genotype, while WI2291 and Arabi Abiad were very susceptible (Table 1). Moreover, of all the three genotypes tested, Arabi Abiad and Banteng were the most and the least responsive genotypes to rhizobacterial strains, respectively (Table 1). More specifically, the disease incidence in Arabi Abiad plants treated with BTP1 Bs2500, Bs2504, and Bs2508 strains decreased by 58, 41, 27 and 50%, respectively, as compared to control plants, whereas in Banteng plants, the disease incidence decreased by 41, 10, 13 and 15%, respectively, as compared to control plants (Table 1).

The density of the rhizobacterial population on the roots of the experimental barley plants ranged between 2.2 and 11.1x10^6 CFU/g of roots (Table 2), which shows that the rhizobacterial strains were readily established and maintained on barley roots. Furthermore, the present study showed that the rhizobacteria did not migrate from the roots to the leaf tissues, as no rhizobacteria were isolated from the plant leaves. Therefore, the inducing agent and the phytopathogen remained localized on different

Table 1. Effect of rhizobacterial strains of *Pseudomonas putida* (BTP1) and *Bacillus subtilis* (Bs2500, Bs2504 and Bs2508) on the disease incidence (%) on barley genotypes Arabi Abiad, Banteng and WI2291 inoculated with *Cochliobolus sativus*.

Treatment	Disease incidence (%)			Mean
	Arabi Abiad	Banteng	WI2291	
Control*	70.4** ± 3.8 a***	31.2 ± 3.8 a	74.4 ± 5.6 a	58.7 ± 3.4 a
BTP1	29.6 ± 3 d	18.4 ± 3.2 b	35.2 ± 3.7 d	27.7 ± 2.1 d
Bs2500	41.6 ± 4.3 bc	28 ± 3.5 ab	60 ± 4.2 bc	43.2 ± 2.7 b
Bs2504	51.2 ± 3.7 b	27.2 ± 3.8 ab	57.6 ± 4.4 bc	45.3 ± 2.7 b
Bs2508	35.2 ± 3.3 cd	26.4 ± 3.6 ab	48 ± 3.8 c	36.5 ± 2.3 c

* Plants inoculated with *C. sativus* and not treated with rhizobacteria
** Mean of 6 replicates
*** Means followed by the same letters do not differ significantly at P<0.01 according to Newman-Keul's test

Table 2. Population density (CFU/g of roots) of rhizobacterial strains *Pseudomonas putida* (BTP1) and *Bacillus subtilis* (Bs2500, Bs2504 and Bs2508) on the roots of barley genotypes Arabi Abiad, Banteng and WI2291, as estimated 28 days after sowing and prior to their inoculation with *Cochliobolus sativus*.

Genotype	Population density of rhizobacteria strains (CFU/g roots)			
	BTP1	Bs2500	Bs2504	Bs2508
Arabi Abiad	9.4	11.1	4.2	8.6
Banteng	4.7	10.9	2.2	3.9
WI2291	7.2	4.7	3	8.8

plant organs showing that the disease suppression was due to induction of resistance in the host plant.

Effect of rhizobacteria on plant growth parameters

Effect on wet weight

The results showed that all rhizobacterial strains (BTP1, Bs2500, Bs2504, Bs2508) had a positive effect on the wet weight of the three barley genotypes (Arabi Abiad, Banteng and WI2291). Bacterial strains differed in their effect on the wet weight, but BTP1 and Bs2508 strains were the most effective, as they significantly increased the mean wet weight by 32, 28.8 and 36.2% and by 22.7, 11.1 and 27.3% in Arabi Abiad, Banteng and WI2291 genotypes, respectively, compared to the control plants (Table 3). On the other hand, Arabi Abiad genotype was more responsive to rhizobacterial treatments than the two other genotypes (Banteng and WI2291), as the general effect of all rhizobacterial strains on wet weight increased by 23.7% in Arabi Abiad genotype (Table 3).

Effect on dry weight

The results showed that all rhizobacterial strains (BTP1, Bs2500, Bs2504, Bs2508) had a positive effect on the dry weight of the three barley genotypes (Arabi Abiad, Banteng and WI2291). However, BTP1 and Bs2508 strains were the most effective, as they significantly increased the mean dry weight by 27.9, 17.6 and 24% and by 23.3, 8.8 and 18% in Arabi Abiad, Banteng and WI2291 genotypes, respectively, compared to the control plants (Table 3).

Effect on plant height

There were significant differences in the ability of the rhizobacterial strains tested (BTP1, Bs2500, Bs2504, Bs2508) to increase the mean plant height of Arabi Abiad, Banteng, and WI2291 genotypes. However, BTP1 and Bs2508 strains were the most effective as they significantly increased the mean plant height by 15.2, 8 and 17.6% and by 11.7, 7.3 and 13.3% in Arabi Abiad, Banteng and WI2291 genotypes, respectively, compared to the control plants (Table 3).

Effect on number of leaves

The results showed that all rhizobacterial strains (BTP1, Bs2500, Bs2504, Bs2508) increased significantly the mean number of leaves of the three barley genotypes (Arabi Abiad, Banteng and WI2291). However, BTP1 and Bs2508 strains were the most effective, as they increased significantly the mean number of leaves by 26, 18.8 and 27.2% and by 13.7, 6.3 and 17.3% in Arabi Abiad, Banteng and WI2291 genotypes, respectively, compared to the control plants (Table 3).

Discussion

Several studies reported that some rhizobacterial strains could be used as biocontrol agents against pests (Zehnder *et al.*, 1997; Zehnder *et al.*, 2001; Haas and Défago, 2005; Reglinski, 2009). In the present work, the protective effect of rhizobacteria

Table 3. Effect of rhizobacterial strains of *Pseudomonas putida* (BTP1) and *Bacillus subtilis* (Bs2500, Bs2504 and Bs2508) on plant growth parameters of barley genotypes Arabi Abiad, Banteng and WI2291 inoculated with *Cochliobolus sativus*.

Genotype	Treatment	Wet weight (g)	Dry weight (g)	Plant height (cm)	No of leaves
Arabi Abiad	Control*	2.69** ± 0.1 c***	0.43 ± 0.01 c	37.6 ± 0.5 c	7.3 ± 0.2 c
	BTP1	3.55 ± 0.1 a	0.55 ± 0.02 a	43.3 ± 0.7 a	9.2 ± 0.3 a
	Bs2500	3.24 ± 0.1 b	0.52 ± 0.01 ab	40.3 ± 0.7 b	7.8 ± 0.3 bc
	Bs2504	3.22 ± 0.1 b	0.48 ± 0.02 bc	38.4 ± 0.8 bc	7.6 ± 0.3 bc
	Bs2508	3.3 ± 0.1 b	0.53 ± 0.01 a	42 ± 0.5 a	8.3 ± 0.1 b
	General effect****	23.7	20.93	9	12.67
Banteng	Control*	2.22 ± 0.1 d	0.34 ± 0.01 b	31.4 ± 0.4 b	9.6 ± 0.2 b
	BTP1	2.86 ± 0.1 a	0.4 ± 0.02 a	33.9 ± 0.4 a	11.4 ± 0.3 a
	Bs2500	2.3 ± 0.1 c	0.36 ± 0.02 a	32.2 ± 0.5 b	10 ± 0.2 b
	Bs2504	2.33 ± 0.1 c	0.35 ± 0.02 b	32.5 ± 0.6 b	9.7 ± 0.2 b
	Bs2508	2.57 ± 0.1 b	0.37 ± 0.01 a	33.7 ± 0.5 a	10.2 ± 0.3 b
	General effect****	13.29	8.82	5.33	7.55
WI2291	Control*	2.82 ± 0.1 d	0.5 ± 0.01 b	39.8 ± 0.5 c	8.1 ± 0.2 c
	BTP1	3.84 ± 0.1 a	0.62 ± 0.02 a	46.8 ± 0.6 a	10.3 ± 0.3 a
	Bs2500	3 ± 0.1 cd	0.53 ± 0.03 b	45 ± 0.7 ab	8.8 ± 0.2 bc
	Bs2504	3.1 ± 0.1 c	0.51 ± 0.03 b	41.4 ± 0.8 c	8.4 ± 0.3 c
	Bs2508	3.59 ± 0.1 b	0.59 ± 0.02 a	45.1 ± 0.6 ab	9.5 ± 0.2 b
	General effect****	19.92	12.5	12	14.19

* Plants infected with *C. sativus* but not treated with rhizobacteria
** Means of 6 replicates
*** Means followed by the same letters do not differ significantly at *P<0.01* according to Newman-Keul's test.
**** Increase of mean growth of plants treated with different rhizobacterial strains compared to the control plants.

strains *P. putida* BTP1 and *B. subtilis* Bs2500, Bs2504 and Bs2508 on three barley genotypes against *C. sativus* was demonstrated. Results showed that treatment of barley seeds with any of the rhizobacterial strains tested led to a significant reduction in disease incidence in Arabi Abiad and WI2291 genotypes. However, in the case of Banteng genotype, a significant reduction in disease incidence was observed only on plants treated with BTP1 strain. These results are in agreement with precedent studies carried out with the same rhizobacterial strains on tomato and bean against *Botrytis cinerea,* and on barley against *Pyrenophora graminea* (Ongena *et al*., 2004; Adam *et al*., 2008; Ongena *et al*., 2008; Adam *et al*., 2017). *In vitro* studies showed that *P. putida* BTP1 strain could not inhibit *C. sativus* mycelial growth, which implies that there was no direct antagonism between these two organisms (rhizobacterium and fungal pathogen) (unpublished data). This is further supported by precedent work demonstrating that *P. putida* BTP1 did not excrete any fungitoxic compounds (Ongena *et al*., 1999). Thus, the resistance induced by BTP1 strain is unlikely to be related to the production of any antibiotic molecule with plant defense-stimulating activity. In the present study, all rhizobacterial strains used colonized very well the barley roots (between 2.2 and 11.1 x 10^6 CFU/g of roots). These results are in agreement with our precedent studies on tomato, which showed that *P. putida* BTP1 cell density was 3.0 (± 2.1) x 10^6 CFU/g on the roots at the time of inoculation of plants with *B. cinerea* (Adam *et al*., 2008). Raaijmakers *et al*. (1995) showed that the threshold population density of *P. putida* strain WCS358 and *P. fluorescens* strain WCS374 for a significant suppression of Fusarium wilt of radish was

approximately 10^5 CFU/g of roots (Raaijmakers *et al*., 1995). Previous studies on tomato and bean (Ongena *et al*., 2002; Adam *et al*., 2008; Ongena *et al*., 2008) support our results that rhizobacterial strains are not able to migrate from the roots to the leaf tissues through the plant. Thus, both the resistance-inducing agent and the plant pathogen seem to remain localized on different plant organs, indicating that disease suppression could be due to induction of a systemic resistance phenomenon in the plant. Rhizobacterial strains *P. putida* BTP1 and *B. subtilis* Bs2500, Bs2504 and Bs2508 differed in their protective effect on barley genotypes Arabi Abiad, Banteng, and WI2291 against *C. sativus*. However, *P. putida* BTP1 and *B. subtilis* Bs2508 were the most effective strains. In addition, landrace genotype Arabi Abiad was the most responsive to rhizobacterial treatments with respect to the increase in resistance compared to Banteng and WI2291 genotypes. Therefore, there is a potential to increase the level of resistance of genotype Arabi Abiad to infection by *C. sativus* by using rhizobacteria. Banteng genotype was less susceptible to *C. sativus* compared to the other two genotypes. Generally, rhizobacteria strains could induce some resistance mechanism in Banteng genotype. Our results are in agreement with precedent studies, which showed that host genotypes differ in their expression of induced resistance, and that the highly susceptible genotypes were more responsive to induced resistance than the resistant genotypes (Dann *et al*., 1998; Resende *et al*., 2002; Tucci *et al*., 2011; Walters *et al*., 2011a; Córdova-Campos *et al*., 2012; Adam *et al*., 2017).

On the other hand, our results showed that rhizobacterial strains stimulated some of the plant growth parameters (i.e. wet weight, dry weight, plant height and number of leaves) under pathogen pressure in all barley genotypes tested. These results are in agreement with those of Orhan *et al*. (2006) studies on raspberry, which showed that colonization of plant roots and rhizosphere with rhizobacterial strains increased significantly the plant growth in terms of yield, cane length, number of clusters per cane and number of berries per cane. Several studies reported that applying *P. fluorescens* Pf5 on sugar beet, barley, corn, blueberry and tomato led to findings similar to those of our studies (De Silva *et al*., 2000; Cakmakci *et al*., 2001; Ataoglu *et al*., 2004; Turan *et al*., 2004). Furthermore, several PGPR may affect plant growth through the production and release of gibberellins as phytohormones; the growth of red pepper plants and alder plants was enhanced by treatment with some PGPR strains producing gibberellins (Gutiérrez-Mañero *et al*., 2001; Joo *et al*., 2005). Çavuşoğlu and Kabar (2008) demonstrated that some plant growth regulators (PGRs), such as gibberellic acid (GA_3), kinetin (KIN), benzyladenine (BA) and ethylene (E), overcome the negative effect of salt stress on percentage of seed germination, radicle elongation and fresh weight. Previous studies showed that *P. putida* BTP1 strain secretes N-alkylated benzylamine derivative (NABD), an elicitor who plays an important role in the elicitation of the ISR phenomenon in bean and tomato plants against *B. cinerea* (Ongena *et al*., 2008). Thus, we could suggest that the benzylamine derivative produced by BTP1 and which is similar to benzyladenine, might play an important role in stimulation of the plant growth.

Our studies showed that the effect of *P. putida* BTP1 and *B. subtilis* Bs2508 strains on stimulation of the plant growth and resistance to *C. sativus* in barley plants was greater than that of the other strains (Bs2500 and Bs2504). This is in agreement with recent studies which reported that PGPR could be used to replace chemical fertilizers/pesticides and to stimulate the growth of tomato plants directly or indirectly via availability of many essential plant nutrients, phytohormones, or through suppression of plant diseases (Ahmed *et al*., 2017). Several studies showed that the application of rhizobacterial strains in rice, potato and cotton crops reduced the incidence of charcoal root rot (*Macrophomina phaseolina*), late blight (*Phytophthora infestans*) and bacterial leaf blight (*Xanthomonas citri* pv. *malvacearum*), respectively. Furthermore, they increased the yield

compared to untreated plants (Yasmin *et al*., 2016; Rizvi *et al*., 2017; Adrees *et al*., 2019).

Consequently, the present study investigated for the first time the effect of rhizobacteria on induction of resistance and promotion of plant growth on three barley genotypes. Induced resistance by seed treatment with PGPR is considered one of the most important biocontrol methods against diseases, especially for crops that are grown over large areas. Finally, more research is needed to determine the effects of PGPR strains on barley plants under field conditions and on the defense mechanisms responsible for resistance to *C. sativus*.

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Επίδραση στελεχών ριζοβακτηρίων στην επαγωγή ανθεκτικότητας γονοτύπων κριθαριού εναντίον του μύκητα *Cochliobolus sativus*

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Περίληψη Η ενίσχυση του επιπέδου αντοχής των φυτών από τα ριζοβακτήρια έχει αποδειχθεί σε πολλά παθοσυστήματα. Στην παρούσα μελέτη διερευνήθηκε η ικανότητα τεσσάρων στελεχών ριζοβακτηρίων (*Pseudomonas putida* BTP1 και *Bacillus subtilis* Bs2500, Bs2504 και Bs2508) να προάγουν την ανάπτυξη σε τρεις γονοτύπους κριθαριού και να τα προστατεύουν έναντι του φυτοπαθογόνου μύκητα *Cochliobolus sativus*. Τα αποτελέσματά μας έδειξαν ότι όλα τα στελέχη ριζοβακτηρίων που δοκιμάστηκαν είχαν προστατευτική επίδραση στους γονοτύπους κριθαριού Arabi Abiad, Banteng και WI2291. Ωστόσο, τα στελέχη *P. putida* BTP1 και *B. subtilis* Bs2508 ήταν τα πιο αποτελεσματικά καθόσον μείωσαν τη συχνότητα της ασθένειας κατά 53 και 38% (μέση επίδραση), αντίστοιχα. Από την άλλη πλευρά, υπήρξαν σημαντικές διαφορές μεταξύ των γονοτύπων κιθαριού που δέχτηκαν την επέμβαση με ριζοβακτήρια ως προς διάφορες παραμέτρους ανάπτυξης των φυτών, όπως το νωπό βάρος, το ξηρό βάρος, το ύψος των φυτών και ο αριθμός των φύλλων. Το στέλεχος *P. putida* BTP1 ήταν το πιο αποτελεσματικό καθόσον αύξησε σημαντικά την ανάπτυξη των φυτών κατά 15-32%. Επιπλέον, οι ευπαθείς στο παθογόνο γονότυποι κριθαριού Arabi Abiad και WI2291 εμφάνισαν την καλύτερη ανταπόκριση στα ριζοβακτήρια. Αυτό σημαίνει ότι οι συγκεκριμένοι γονότυποι έχουν υψηλό δυναμικό για αύξηση της αντοχής τους στο παθογόνο και ενίσχυση της ανάπτυξής τους μετά από εφαρμογή ριζοβακτηρίων. Ως εκ τούτου, η επέμβαση σε σπόρους κριθαριού με τα παραπάνω ριζοβακτήρια μπορεί να θεωρηθεί ως μια αποτελεσματική μέθοδος βιολογικής αντιμετώπισης του φυτοπαθογόνου μύκητα *C. sativus*.