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Research Letter

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In vitro and *in planta* antagonistic effects of plant growth-promoting rhizobacteria consortium against soilborne plant pathogens of *Solanum tuberosum* and *Solanum lycopersicum*

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One sentence summary: A bacterial consortium was selected for its in vitro and in planta biocontrol potential against two fungal pathogens of potatoes and tomatoes.

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ABSTRACT

Potatoes (*Solanum tuberosum* L.) and tomatoes (*Solanum lycopersicum* L.), among the main crops belonging to the Solanaceae family, are attacked by several pathogens. Among them *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Rhizoctonia solani* are very common and cause significant losses. Four plant growth-promoting rhizobacteria, *Azospirillum brasilense, Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae* and *Burkholderia ambifaria* were tested against these pathogens. *In vitro* antagonistic activities of single strains were assessed through dual culture plates. Strains showing antagonistic activity (*G. diazotrophicus, H. seropedicae* and *B. ambifaria*) were combined and, after an *in vitro* confirmation, the consortium was applied on *S. lycopersicum* and *S. tuberosum* in a greenhouse pot experiment. The bioprotection was assessed in pre-emergence (infection before germination) and post-emergence (infection after germination). The consortium was able to successfully counteract the infection of both *F. oxysporum* and *R. solani*, allowing a regular development of plants. The biocontrol of the fungal pathogens was highlighted both in pre-emergence and post-emergence conditions. This selected consortium could be a valid alternative to agrochemicals and could be exploited as biocontrol agent to counteract losses due to these pathogenic fungi.

Keywords: PGPR; biocontrol; *Rhizoctonia solani*; *Fusarium oxysporum* f. sp. *radicis-lycopersici*; *Solanum tuberosum*; *Solanum lycopersicum*

INTRODUCTION

Amid the most known plants cultivated to meet the nutritional needs of humans, the Solanaceae family is among the most important. This family is principally represented by potatoes (*Solanum tuberosum* L.) and tomatoes (*Solanum lycopersicum* L.) that are consumed worldwide for their high nutritional value while also containing large quantities of antioxidant molecules (Helmja *et al.* 2007). As in other crops, these species are attacked by several pathogens (i.e. fungi, bacteria, nematodes, oomycetes and viruses) that during the last decades developed resistance to

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commercially synthesized chemicals (Raaijmakers and Paulitz 2009).

Among the numerous fungal pathogens of potatoes and tomatoes, the present study addresses the biocontrol of the causal agent of root rot of tomato, *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) (Mcgovern 2015), and the causal agent of potato rhizoctoniosis – commonly known as black scurf – *Rhizoctonia solani* (Larkin 2020). Fungal pathogens represent not only a limiting factor in productive landscapes but also a severe threat for human health (Gull, Lone and Wani 2019). These issues, together with the high quality required by consumers and market limiting the utilization of chemicals, push farmers to pursue more environmentally friendly formulations (Sun *et al.* 2017). Rhizosphere microorganisms could represent a valid biocontrol agent (Tan *et al.* 2019). In particular, the use of plant growth-promoting rhizobacteria (PGPR) as biocontrol agents is gaining attention due to their sustainability and safety. Strains that belong to this group promote plant growth and development and protect the host plant from several pathogens.

Several studies already demonstrated biocontrol activity of some bacterial strains against fungal pathogens, belonging mainly to *Bacillus* and *Pseudomonas* genera (Siddiqui 2006). Among the less studied bacterial strains, those belonging to *Azospirillum brasilense, Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae* and *Burkholderia ambifaria* have demonstrated good biocontrol potential in *in vitro* preliminary results against *Lycopersicon esculentum* pathogens (Del Gallo *et al.* 2010). These strains showed good compatibility among each other inside host plant in gnotobiotic conditions, making them suitable for combination in consortia (Botta *et al.* 2013).

In the present work, we hypothesize that the consortium of these bacterial strains could positively counteract fungal infection of FORL and *R. solani* in *S. lycopersicum* and *S. tuberosum* plants. To verify this hypothesis, the antagonistic activity was first assessed *in vitro* so as to select effective strains, and also investigate the morphological changes of the mycelia by scanning electron microscopy (SEM). Once the consortium was obtained, the *in planta* bioprotection on tomatoes and potatoes was assessed in pre-emergence (infection before germination) and post-emergence (infection after germination) conditions. For the evaluation of the induced protection, the treated and infected experimental conditions (consortium + fungi) were compared to negative (no treatment or infection) and positive control (infection). The comparisons were carried out by assessing plant (i) survival; (ii) damages and (iii) development parameters (i.e. height, total chlorophyll content).

MATERIALS AND METHODS

Strains and growth conditions

Azospirillum brasilense ATCC 29710 (Eskew, Focht and Ting 1977), *G. diazotrophicus* ATCC 49037 (Gillis *et al.* 1989; Yamada, Hoshino and Ishikawa 1997), *B. ambifaria* PHP7 (Coenye *et al.* 2001) (originally provided by T. Heulin, C.P.B., CNRS, France) and *H. seropedicae* ATCC 35892 (Baldani *et al.* 1986) were utilized in this study. Strains were cultivated in T4 medium as described in Pagnani *et al.* (2020). The pathogens *R. solani* and FORL (originally provided by. G. Bonanomi, Department of Agriculture, University of Naples) were cultivated on potato dextrose medium (Sigma-Aldrich, St Louis, MO, USA). Working bacterial inocula and spore/mycelial solutions were prepared in different ways depending on the assay; details of preparations are described in each paragraph.

In vitro antagonistic activity

In vitro antifungal activity of the single strains, and subsequently of the consortium, were evaluated by dual culture method on potato dextrose agar (PDA; Sigma-Aldrich, St Louis, MO, USA) Bacterial single strains were taken from active T4 broth cultures during the *log* phase line, spread on the edges of Petri dish (Ø90 mm) at 2.5 cm away from the center and incubated at 28°C. Since *A. brasilense* showed poor fungal inhibition, a bacterial consortium was prepared combining broth cultures of *B. ambifaria*, *G. diazotrophicus* and *H. seropedicae*; 10 mL of each strain grown on T4 (at *log* phase) was collected and transferred in a sterile 50-mL tube. Bacterial consortium was carefully vortexed, spread on the edges of the Petri dish and incubated as previously described. After 24 h (48 h for *G. diazotrophicus* and consortium), a 5-mm-diameter disk of actively growing fungal mycelium was positioned at the center of the plates. Control plates were prepared by placing fungal disks at the center of PDA Petri dishes. Plates were incubated at 28°C, till the fungal mycelia completely covered the control plate. Antagonistic activity was estimated by comparing the fungal growth of the dual culture plates with that of control plates and calculating the inhibition percentages as follows:

$$\mathrm{I}\,\% = \frac{(\text{mm growth control} - \text{mm growth dual culture})}{\text{mm growth control}} \times 100.$$

PGPR–pathogen interactions study by SEM

The dual culturing interaction zones were examined by SEM. The growing mycelia within the interaction zone between PGPR consortium and fungal strain were sampled, transferred in sterile Petri dishes and fixed overnight at 4°C in glutaraldehyde (2.5% in 0.05 M phosphate buffer, pH 7.3). Fixed samples were washed three times in distilled water and dehydrated through subsequent 15-min treatments of 30, 50, 70% ethanol (v/v). Before SEM analysis, samples were treated with hexamethyldisilazane (HMDS; Sigma-Aldrich, St Louis, MO, USA), left to dry for few seconds under hood flow, mounted on to stubs by means of carbon tape and covered with chromium. The observations were performed by a Gemini SEM 500 SEM (Zeiss, Oberkochen, Germany); all the micrographs were acquired using an accelerating voltage of 5 kV and in SE2 signal (type II secondary electrons).

In planta antagonistic activity

The *in planta* antagonistic activities of the consortium against *R. solani* and FORL were carried out on *S. tuberosum* (var. Agata) and *S. lycopersicum* (var. San Marzano), respectively. The pre-emergence was assessed on seeds (for *S. tuberosum* the seed tuber presented visible sprouts <1 mm), while post-emergence was assessed on plantlets with first leaf of main stem unfolded.

For the pre-emergence experiments, the inoculation of the seeds was performed by dipping the seeds in an Erlenmeyer flask (1 L) containing the consortium solution (10^{10} CFU mL^{-1}). All the bacteria were present at the same amount.

Seeds were left immersed in the inoculum at constant shaking (150 rpm) for 20 min at room temperature. Seeds were then recovered through a funnel, set up with a perforated aluminum sheet and left to dry overnight. All the procedures were performed in aseptic conditions. The final density of the bacteria on seeds (10^6 CFU g^{-1}) was estimated by plating serial dilutions

on T4 agar plates and plate counting after 48 h of incubation at 28°C.

For the post-emergence experiments, the plantlets were inoculated after transplanting with 10 mL of a consortium solution of 10^6 CFU mL^{-1} (adjusted spectrophotometrically at 600 nm) directly at the seedling base.

Infections were induced with *R. solani* mycelial solution (3 g L^{-1}) and FORL spore suspensions (10^6 mL^{-1}) following the procedures described by Khan, Bora and Borah (2017) and Roberti *et al.* (2015), respectively.

Each experiment consisted of four experimental units arranged as follows: (i) seed/plant with consortium inoculation and no fungal infection (PGPR); (ii) seed/plant with consortium inoculation and fungal infection (PGPR+F); (iii) seed/plant with fungal infection only (F); and (iv) seed/plant without consortium inoculation and fungal infection (C). For *S. tuberosum*, each experimental unit consisted of 25 pots with two sprouted seeds/plants per pot (6.5 L filled with commercial common soil), while for *S. lycopersicum* each experimental unit consisted of 8 pots with five seeds/plants per pot (3 L filled with commercial common soil). Experiments were carried out in greenhouse under natural photoperiod. The experiments were followed until infected plants showed evident disease symptoms (i.e. stem canker for potato plants and root rot/wilt for tomato plants).

Both pre-emergence and post-emergence samples were analyzed for plant germination/survival (%), damage grade, plant height (cm), root length (cm), true leaves (n°), branches (n°) and total chlorophyll content (mg/g) assessed spectrophotometrically according to Pace *et al.* (2020). For *S. lycopersicum*, the damage grade was estimated as follow: 0 = no damage; 1 = damage extension up to 3 mm; 2 = damage extension of 3–6 mm; 3 = damage extension of 6–9 mm; 4 = damage extension of 9–12 mm; and 5 = damage extension > 12 mm or plant death. For *S. tuberosum*: 0 = no damage; 1 = damage extension up to 5 mm; 2 = damage extension of 5–10 mm; 3 = damage extension of 10–15 mm; 4 = damage extension of 15–20 mm; and 5 = damages extension > 20 mm or plant death.

Statistical analysis

The differences in mean values between the experimental conditions were analyzed by one-way analysis of variance. Fisher's LSD post-hoc test was applied for results comparisons and separation of the means was performed at three levels of significance: 0.1, 1 and 5% ($P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively). Statistical analysis was performed using Microsoft XLSTAT 2016 statistical software (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

In vitro antagonistic activity

The inhibition percentages of the single strains against *R. solani* and FORL are shown in Table 1. All strains showed more antagonistic activity toward *R. solani* than FORL (+36%). The PGPR consortium formed by *B. ambifaria*, *G. diazotrophicus* and *H. seropedicae* showed same antagonistic activity as single strains. Regarding *R. solani*, no statistically significant differences ($P > 0.05$) were observed among the inhibition percentages obtained by the single strains and the bacterial consortium (68% on average), except for *A. brasilense*, which showed the lowest inhibition percentage ($P < 0.05$). Different results were observed for the inhibition against FORL: the most effective strain ($P < 0.05$) was *H. seropedicae* while the lowest inhibition percentage ($P < 0.05$) was observed in *A. brasilense*. In any case, for both pathogens the combination of the three bacterial strains resulted in an efficient inhibition, statistically comparable ($P > 0.05$) with those obtained for *H. seropedicae*.

Table 1. Results of *in vitro* antagonistic activity of single bacterial strains and bacterial consortium (PGPR) against *R. solani* and FORL.

Strains	Inhibition %	
	R. solani	FORL
Azospirillum brasilense	18.1 b	12.2 c
Burkholderia ambifaria	68.9 a	48.4 b
Gluconacetobacter diazotrophicus	66.6 a	49.9 ab
Herbaspirillum seropedicae	69.6 a	52.8 a
PGPR	68.0 a	52.3 a
LSD	4.5	3.5

Inhibition percentages were calculated from results of three independent experiments repeated twice. Results followed by different case letters are not significantly different according to Fisher's LSD post-hoc test ($P < 0.05$).

Although previous studies reported antagonistic activities by *A. brasilense* toward several soilborne plant pathogens (Bashan and de-Bashan 2010 and references therein), particularly against fungal diseases caused by *Alternaria*, *Bipolaris* and *Fusarium* (López-Reyes *et al.* 2017), our results suggested low *in vitro* biocontrol ability against both *R. solani* and FORL. Since *in vitro* dual culture method is a high-throughput screening for antifungal activity and has high correlation with results that can be obtained with *in planta* experiments (Shehata *et al.* 2016), *A. brasilense* strain was not further investigated.

Instead, the results obtained with *B. ambifaria*, *G. diazotrophicus* and *H. seropedicae* are in accordance with literature data. For these strains, the biocontrol activity was already described for the inhibition against several fungal pathogens. *Burkholderia ambifaria* is a very versatile biocontrol species that presents suppressing ability against many soilborne plant pathogens of several crops, including FORL and *R. solani* (Li *et al.* 2002), by the production of several metabolites (Simonetti *et al.* 2018). *Gluconacetobacter diazotrophicus*, by producing secondary metabolites and antifungals, counteracts *F. oxysporum* in *Ipomoea batatas* (Logeshwarn, Thangaraju and Rajasundari 2011). Finally, *H. seropedicae* already showed interesting biocontrol activity against *Fusarium oxysporum* f. sp. *cubense* (Weber, Muniz and Vitor 2007). For these two latter strains, as far as we know, our study is the first report describing the antagonistic activity against *R. solani*.

PGPR–pathogen interactions study by SEM

Interaction zones of dual culture of the PGPR consortium and fungal pathogens were investigated by SEM. Figure 1 shows the micrographs obtained for *R. solani* mycelium cultured alone (CONTROL) and cultured with the consortium (PGPR). At 500× magnification, the control (Fig. 1A) showed continuous and overlapping hyphae. On the contrary, in the presence of the consortium (Fig. 1B) mycelium appearance changed, showing irregular and discontinuous development. At 1000× magnification, the differences were much more appreciable: the control micrograph (Fig. 1C) showed normal and undamaged structures, while a marked degradation was evident in the PGPR one (Fig. 1D), especially at the level of the hyphal apex.

Same situation was underlined by the analysis of the interaction zone between FORL and the consortium (Fig. 2). Both at 500×

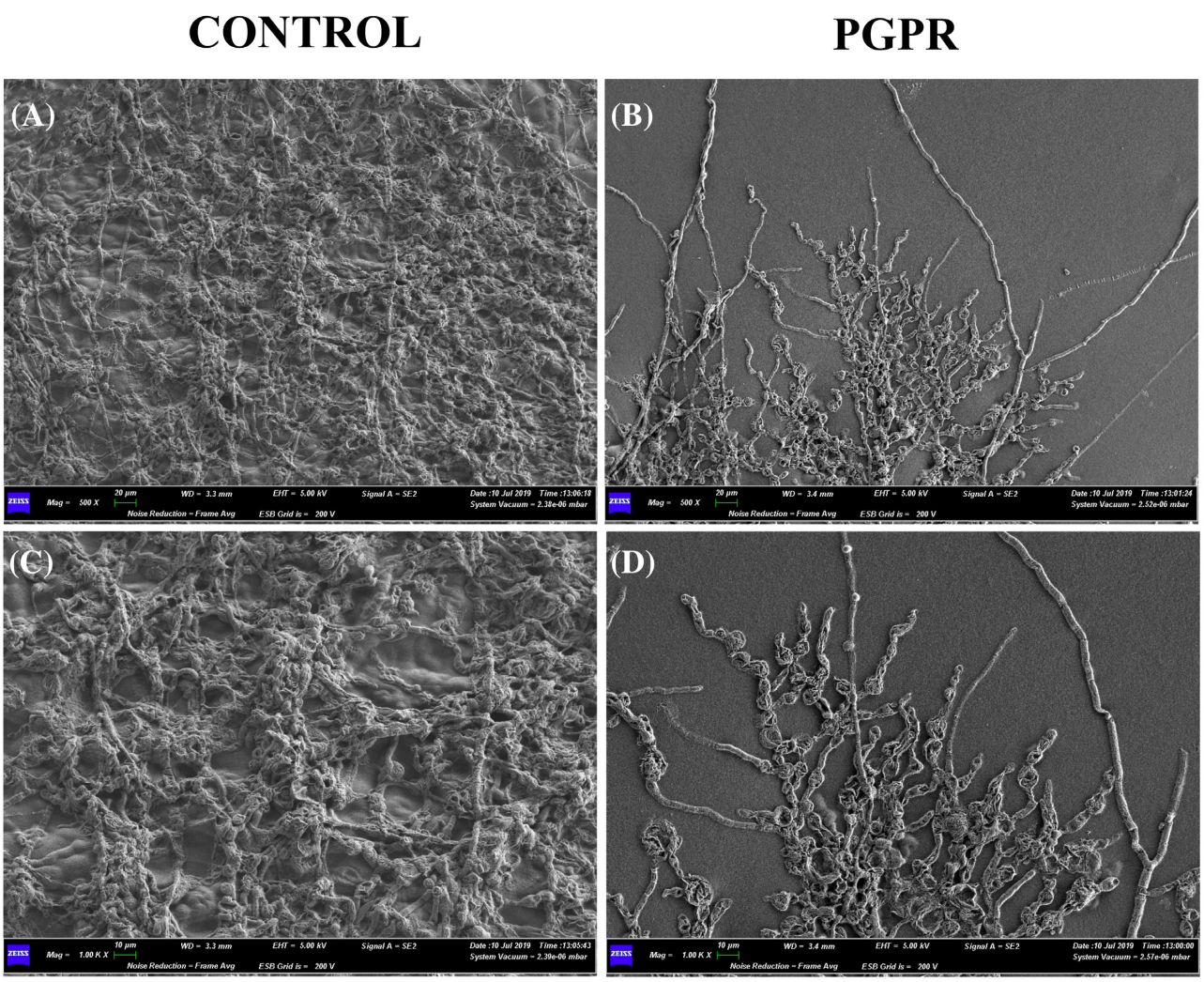

Figure 1. Interactions of bacterial consortium and *R. solani* studied by SEM. The micrographs show the differences in hyphal morphology between fungal strain cultivated alone – CONTROL, at ×500 **(A)** and ×1000 **(C)** magnification, and in dual culture with bacterial consortium – PGPR, at ×500 **(B)** and ×1000 **(D)** magnification.

and 1000× magnification, control micrograph showed continuous, undamaged structures while when the fungal strain is co-cultivated with the consortium, we observed discontinuity and degradation.

These changes in mycelial growth and hyphal structures are in accordance with the literature. Actually, several authors quoted morphological and cellular changes, while investigating the interaction zones between fungal and bacterial growth by different microscopic techniques (Deora, Hashidoko and Islam 2005; Xing, Guo and Lee 2005; Begum *et al.* 2008; de los Santos-Villalobos, Barrera-Galicia and Miranda-Salcedo 2012; Kamruzzaman *et al.* 2015; Sharma *et al.* 2019).

In planta antagonistic activity

Figure 3A shows the comparison of the four experimental conditions of pre-emergence *S. tuberosum* plants. In the PGPR condition, the presence of the consortium promoted greatest plant growth and development with respect to the control (C). The experimental condition with the presence of the single fungal infection (F) presented no plant germination and the tubers were completely rotted. Therefore, the fungal infection caused a very serious damage in the pre-emergence experiment. This negative impact was abundantly hindered by the antagonistic activity of the bacterial strains (PGPR+F), which allowed a normal development of plants like those observed for the control (C). These visual results were confirmed by plant growth and by development parameters analyzed and reported in Table 2. Both aerial part and root systems were positively affected by the PGPR treatment and presented best lengths ($P < 0.05$) and branches development ($P < 0.05$). In association with fungal infection (PGPR+F), plant growth and development promotion seemed to decrease in favor of plant protection from the disease. In particular, in PGPR+F condition, the direct and indirect mechanisms of action of PGPR were slightly less effective due to the presence of the fungal pathogen compared to the condition without this biotic stress (PGPR). For PGPR+F experimental condition, no germination losses and lesions were registered and same sizes of control plants were recorded ($P > 0.05$). Anyway, the bacterial promotion in PGPR+F has been kept in terms of true leaves development and total chlorophyll content; for these parameters, lower results than PGPR condition ($P < 0.05$), but still higher than controls ($P < 0.05$), were recorded.

Similar situation was obtained in the post-emergence experiment. Figure 3B shows the comparison of the four experimental conditions of post-emergence of *S. tuberosum* plants. Plants

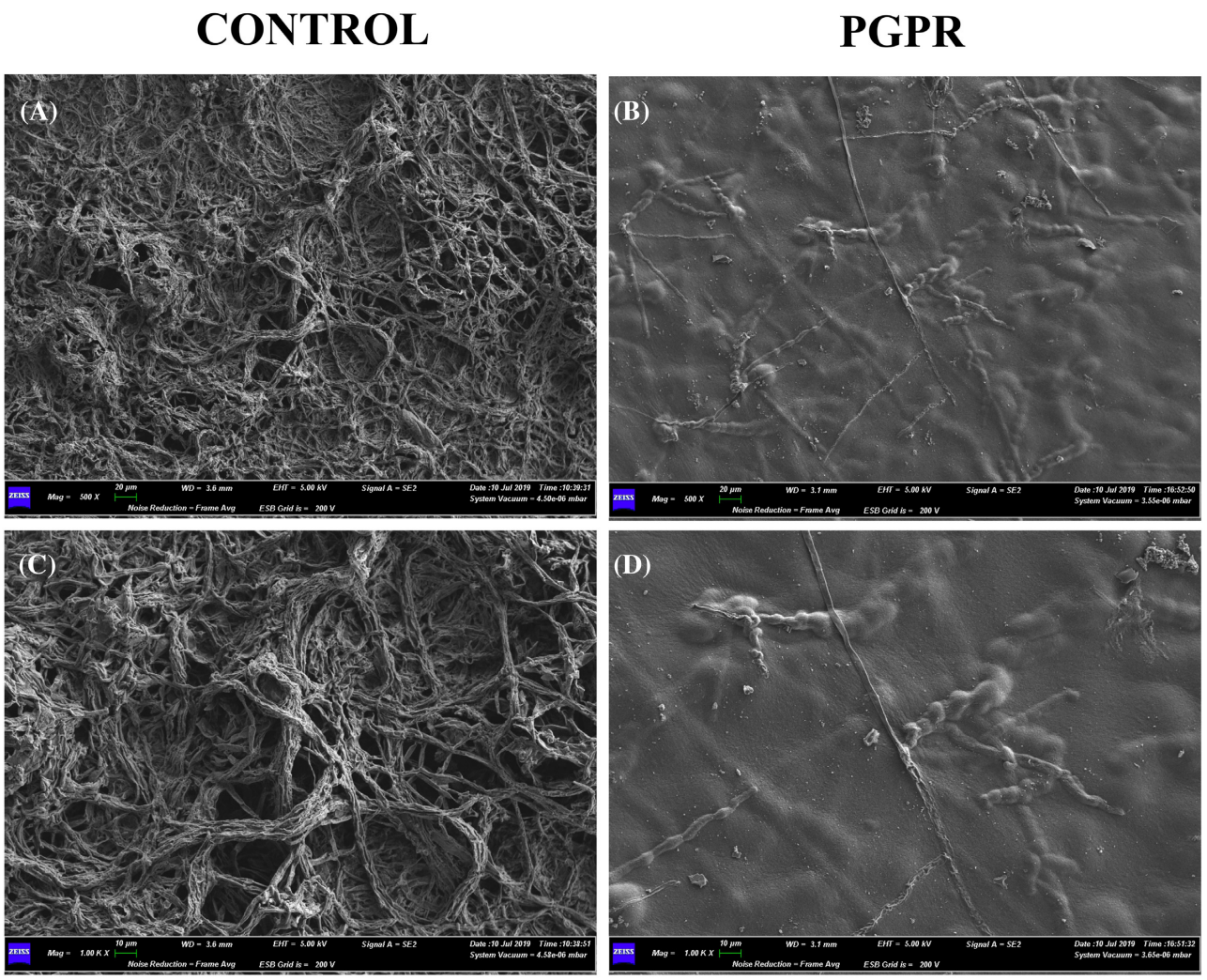

Figure 2. Interactions of bacterial consortium and FORL studied by SEM. The micrographs show the differences in hyphal morphology between fungal strain cultivated alone – CONTROL, at ×500 **(A)** and ×1000 **(C)** magnification, and in dual culture with bacterial consortium – PGPR, at ×500 **(B)** and ×1000 **(D)** magnification.

obtained from condition F showed evident damages and less development with respect to the control. These negative indicators of the infection are absent in the presence of the bacterial consortium (PGPR+F): in this condition also, plants reached better growth than the control, comparable to the ones inoculated by PGPR. In Table 2, plant growth and development parameters of post-emergence experiment are reported. Also in this case, the presence of the bacterial consortium alone (PGPR) promoted the growth of the plant. In particular, this effect was more visible in the aerial part (best results of height, true leaves and total chlorophyll with respect to the control, $P < 0.05$) than in the root (similar results of control, $P > 0.05$). The post-emergence infection was more attenuated with respect to the pre-emergence one, with a percentage of survival of 65%. Nevertheless, survived plants presented scarce development of aerial part (length, true leaves, branches, total chlorophyll) with respect to the control ($P < 0.05$). Root length of F condition presented no statistically significant differences than the control ($P > 0.05$); however, plants showed root rot and damages with an extension > 20 mm. In PGPR+F condition, the bacteria counteracted effectively the fungal infection (100% of survival and damages with an extension of 5–10 mm in PGPR+F vs 65% of survival and damages with an extension >20 mm in F), promoting a normal development of the plants. Number of branches and total chlorophyll content and root length of PGPR+F were statistically comparable both to the control and the PGPR conditions ($P > 0.05$). Plants of PGPR+F also presented same height of the control ($P > 0.05$) but a higher number of true leaves ($P < 0.05$). For all the investigated parameters, the PGPR+F condition was statistically separated from the F one ($P < 0.05$).

Figure 4 shows the comparison of the four experimental conditions of pre- (Fig. 4A) and post-emergence (Fig. 4B) in *S. lycopersicum* plants. In both experiments, FORL infection caused failures in F condition, with damping off (Fig. 4A) and wilt (Fig. 4B). On the contrary, the concurrent presence of the bacterial consortium in PGPR+F condition counteracted the pathogenic action of the fungus, promoting regular plant development.

In Table 2, plant growth and development parameters recorded for pre- and post-emergence experiments of *S. lycopersicum* plants are shown.

In the pre-emergence experiment, a very low rate of complete germination, 13%, due to high damping-off occurrence was recorded. Survived plants presented damages with an extension > 12 mm. The effects of fungal infection in F condition were evident in terms of plant height and total chlorophyll. Positive results were recorded when there was the concurrent presence

Table 2. Results of *in planta* pre-emergence (PRE) and post-emergence (POST) antagonistic activity of bacterial consortium (PGPR) against *R. solani* in *S. tuberosum* and FORL in *S. lycopersicum*.

	S. tuberosum					*S. lycopersicum*				
					PRE-EMERGENCE					
Parameter	**CNT**	**PGPR**	**PGPR+F**	**F**	**LSD**	**CNT**	**PGPR**	**PGPR+F**	**F**	**LSD**
Germination (%)	100	100	100	0	–	100	100	100	13	–
Damages (grade)	0	0	1	5	–	0	0	0	5	–
Plant height (cm)	14.0 b	26.0 a	13.0 b	–	4.1	4.4 a	4.5 a	4.2 a	1.7 b	0.5
Root length (cm)	10.2 b	13.1 a	10.0 b	–	2.4	1.7 c	6.9 a	3.9 b	1.5 c	1.5
True leaves (n°)	7.5 c	18.9 a	12.5 b	–	2.6	2.3 bc	3.6 a	3.0 ab	1.7 c	0.9
Branches n°	5.5 a	6.1 a	3.8 b	–	1.0	–	–	–	–	–
Chl (a + b)	55.7 c	86.6 a	67.9 b	–	4.9	37.6 c	74.4 a	37.6 b	16.5 d	2.9
					POST-EMERGENCE					
Survival (%)	100	100	100	65	–	100	100	100	25	–
Damages (grade)	0	0	2	5	–	0	0	0	5	–
Plant height (cm)	26.8 b	50.0 a	31.4 b	18.5 c	5.3	5.4 a	5.6 a	5.8 a	3.9 b	0.6
Root length (cm)	14.0 ab	16.2 a	16.5 a	11.2 b	3.0	3.2 c	4.7 a	4.4 b	1.8 d	0.6
True leaves n°	30 c	51.3 a	40.4 b	14.3 d	6.7	9.0 c	15.0 a	11.0 b	2.9 d	1.8
Branches n°	9.6 a	10.3 a	9.4 a	5.5 b	1.9	–	–	–	–	–
Chl (a + b)	13.5 b	17.5 a	15.3 ab	8.2 c	2.9	49.5 c	58.1 a	49.5 b	18.9 d	3.79

Results followed by different case letters are not significantly different according to Fisher's LSD post-hoc test ($P < 0.05$). In the table: CNT, no bacterial inoculation, no fungal infection (control); PGPR, bacterial inoculation with consortium without fungal infection; PGPR+F, bacterial inoculation with consortium and fungal infection; F, no bacterial inoculation + fungal infection; LSD, Least Significant Difference.

of bacterial consortium: in PGPR+F condition a complete germination of seeds, and plant with no damages were recorded.

Also, in the post-emergence experiment huge losses were recorded, with a survival rate of 25% and survived plants with damages with an extension > 12 mm. The negative impact of infection in the presence of the fungus alone (F condition) was evident on all plant growth and development parameters (F condition statistically grouped apart from the control). Instead, positive results were recorded when there was the concurrent presence of bacterial consortium (PGPR+F), with 100% survival rate and plants without any evident symptoms.

In both pre-emergence and post-emergence experiments, plant height was not significantly affected by PGPR treatment, both in the presence and in the absence of fungal infection (PGPR, PGPR+F and control grouped in the same statistical group, $P > 0.05$). On the other hand, root length, true leaves and chlorophyll contents were positively affected by the presence of PGPR, which promoted the development of these plant components, with respect to the control, either in the presence or in the absence of fungal infection (PGPR and PGPR+F lengths higher than control, $P < 0.05$). This stimulating activity was in all the cases slightly decreased by the fungal infection, because all plant and development parameters of PGPR+F condition were lower than PGPR alone ($P < 0.05$). In any case, the PGPR+F condition showed no results lower than the control condition. Therefore, as shown for *S. tuberosum,* it seems that plant stimulation is diminished in order to counteract the infection of the pathogen.

The bacterial antagonistic activity against *R. solani* has been widely studied and reviewed over the years on several plant species. Recent scientific literature comprises works mostly related to the use of *Trichoderma* spp. (Woo *et al.* 2014; Chao and Wen-ying 2019), a fungus that provides many bioformulations for *R. solani.* A promising bacterial genus widely investigated is *Bacillus*, characterized by the production of a wide range of antagonistic molecules effective against this pathogen (Wu and Liu 2019; Hussain and Khan 2020). Moreover, a good biocontrol activity against *R. solani* was also displayed by a commercial *B. ambifaria* strain, with results comparable to strains belonging to *Bacillus* spp. and *Trichoderma* spp. (Larkin 2016). Finally, another widely studied genus is *Streptomyces* spp. (Gowdar, Deepa and Amaresh 2018; Liotti, da Silva Figueiredo and Soares 2019). All these bioformulations showed effective *R. solani* containment, contributing to the development of healthy plants, thanks to their PGP properties.

Several evidences have also shown the efficacy of bacterial strains against FORL pathogen (Mcgovern 2015). Among them, *Bacillus megaterium* and *Burkholderia cepacia* can reduce FORL infection up to 75 and 88%, respectively (Omar, O'Neill and Rossall 2006). In Kamou *et al.* (2015), among 384 isolates, good FORL biocontrol potential was showed by seven strains isolated from rhizospheres of wheat (*Triticum aestivum*), tomato (*S. lycopersicum*) and pepper (*Capsicum annum*) cultivated in Central Macedonia, Northern Greece and on Zakynthos island. These strains, belonging to *Pseudomonas chlororaphis*, *Bacillus cereus*, *Serratia marcescens* and *Serratia rubidaea* species, reduced infection impact under controlled conditions (i.e. gnotobiotic system and pots). Two strains of *P. chlororaphis* and *S. marcescens*, significantly decreased severity of FORL disease, reaching results like healthy control plants in pot experiments under natural conditions. Moreover, recent studies on the rhizosphere of Algerian native plants allowed the isolation of several rhizobacteria with good biocontrol activity against FORL. In Zamoum *et al.* (2015), a *Streptomyces* species isolated from *Solanum caeruleatus* showed the highest biocontrol of FORL root rot and the greatest plant growth-promoting effects. The work of Goudjal *et al.* (2016) revealed that a *Streptomyces asterosporus* isolated from *Solanum nigrum* presents a good biocontrol effect against FORL, together with a good plant growth promotion on tomato seedlings.

Mixed inoculations of rhizobacteria are an effective strategy for the management of plant pathogens (El Komy *et al.* 2020 and references therein). Mechanisms involved in this biocontrol activity include ecological niche competition with pathogens, promotion of the defense system of the plant and inhibitory molecules production (e.g. antibiotics, siderophores)

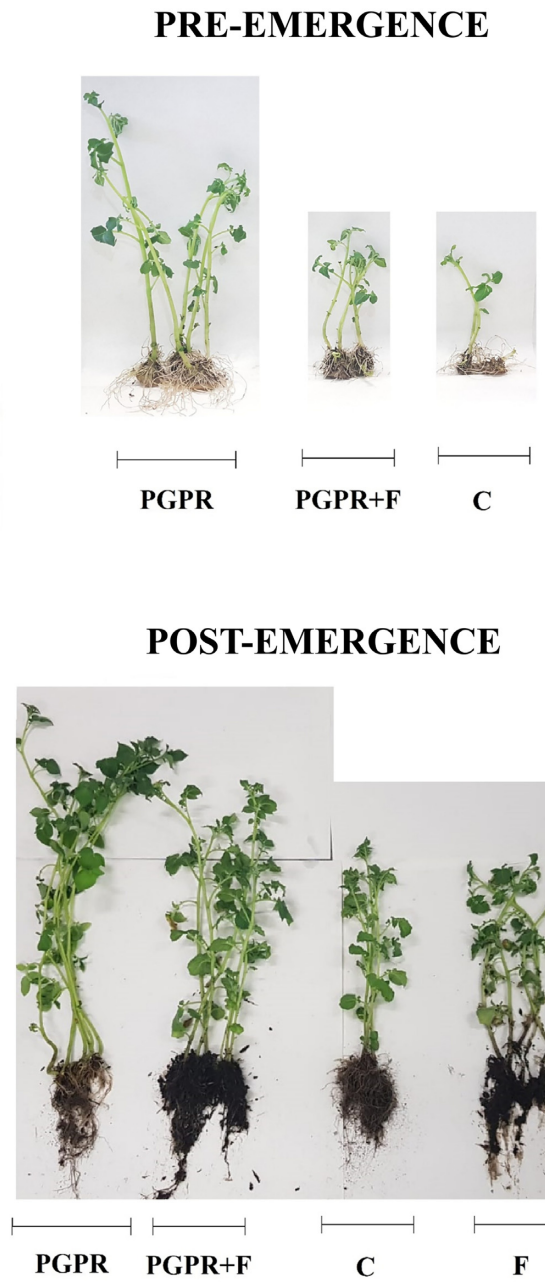

Figure 3. Results of *in planta* pre-emergence **(A)** and post-emergence **(B)** antagonistic activity of the bacterial consortium against *R. solani* in *S. tuberosum*. In the figure: PGPR, seed/plant with consortium inoculation and no fungal infection; PGPR+F, seed/plant with consortium inoculation and fungal infection; C, seed/plant without consortium inoculation and fungal infection; F, seed/plant with fungal infection only.

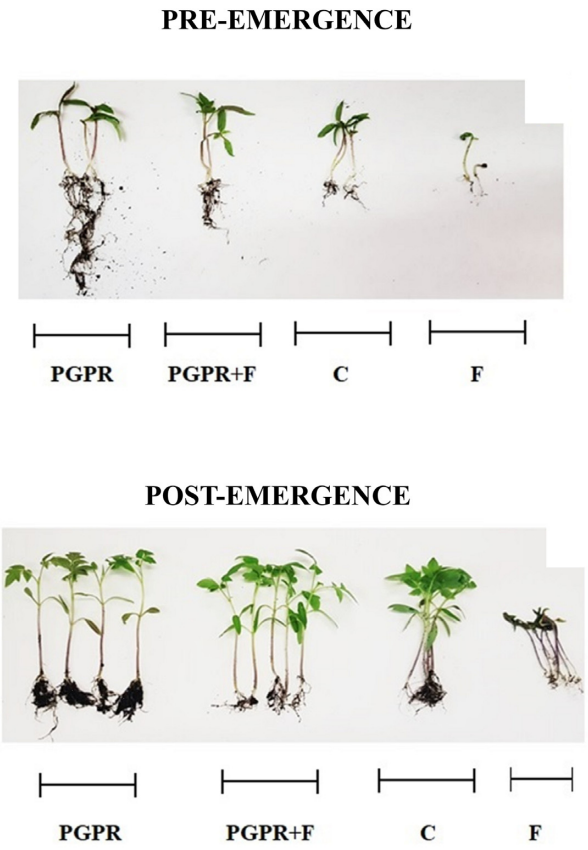

Figure 4. Results of *in planta* pre-emergence **(A)** and post-emergence **(B)** antagonistic activity of the bacterial consortium (PGPR) against FORL in *S. lycopersicum*. In the figure: PGPR, seed/plant with consortium inoculation and no fungal infection; PGPR+F, seed/plant with consortium inoculation and fungal infection; C, seed/plant without consortium inoculation and fungal infection; F, seed/plant with fungal infection only.

(Bouizgarne 2013). However, the antagonistic capabilities demonstrated during the *in planta* experiment could differ in large-scale seed germinators and in open fields. This is due to the presence of several variables that may affect the rhizobacteria viability on the seeds, in presence of environmental abiotic and biotic factors (Siddiqui 2006). However, previous studies on consortia containing *G. diazotrophicus*, *H. seropedicae* and *B. ambifaria* strains showed that these bacteria can effectively colonize host plants, helping them to thrive (Botta *et al.* 2013; Pagnani et al. 2018, 2020).

CONCLUSIONS

Nowadays, one way of plant fungal disease control is the use of resistant cultivars. However, the genetic manipulation still presents to many unsolved issues (e.g. fungal pathovars' resistant to products of resistance genes, that directly or indirectly detect pathogenic effectors and trigger effective defense responses, and field trial scale up of new technologies) and the use of agrochemicals remains among the most utilized techniques, with serious consequences on the health of ecosystems and of human beings. Consumer pressure placed by and laws introduced to limit the use of agrochemicals are pushing agriculture toward effective and sustainable techniques to manage fungal infections of crops. Biocontrol agents could respond to this need. However, many studies consider only *in vitro* antagonistic effects. In this perspective, it is important to produce increasing scientific evidence to support the use of biocontrol agents. They include not only microorganisms that can protect the plant against pathogens but also microorganisms that can stimulate plant growth, limiting or removing the use of agrochemicals. In this study, a PGPR consortium turned out to be a promising tool for sustainable agriculture showing good biocontrol activity against *R. solani* and *F. oxysporum* f. sp. *radicis-lycopersici*. Further studies are needed to test their effectiveness in nurseries and in open field, or greenhouse experiments and pilot trials. However,

our results suggest a promising applicability of this consortium as biocontrol agent against these fungal pathogens.

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