

Article - Agronomy/Soil Science

# Multiple Effect of Different Plant Growth Promoting Microorganisms on Beans (*Phaseolus vulgaris* L.) Crop

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Received: 2019.08.14; Accepted: 2020.03.30.

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## HIGHLIGHTS

- *Burkholderia* sp. 10N6 inoculation performed similar to *Rhizobium tropici* in greenhouse experiment.
- Treatments behaved differently in greenhouse and field conditions.
- Inoculated treatments showed similar shoot dry biomass compared to nitrogen fertilization.

**Abstract:** We evaluated the effect of combined *Rhizobium tropici*, *Trichoderma asperellum* and plant growth-promoting rhizobacteria (PGPR) in beans crop. The hypothesis that strains of *T. asperellum*, *R. tropici* and PGPR combined could improve growth, biomass accumulation and beans yield was tested under greenhouse and field conditions. The treatments consisted of control, mineral nitrogen application and inoculation, isolated and associated with the following microorganisms: *Rhizobium tropici*, *Bacillus subtilis*, *Trichoderma asperellum* and *Burkholderia* sp. 10N6. Results were evaluated by shoot dry weight (SDW) and root dry weight (RDW), number of nodules and yield components. In greenhouse environment all the microorganisms behaved similarly, and the treatments inoculated with *Burkholderia* sp. 10N6 (IBu) and *R. tropici* (IR) stood out regarding the production components. In field conditions the treatments IR and IRTBa presented the highest values of SDW and RDW. Our results suggest that inoculation with *R. tropici*, *T. asperellum* and PGPR may promote beans growth and bring benefits to shoot and root accumulation, increase the number of nodules as well as improve yield components, contributing to a sustainable agriculture.

**Keywords:** inoculation; bacteria; fungus; common bean.

## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a crop of great social and economic importance in Brazil. Besides being one of the main sources of protein for the population, it provides income for thousands of small farmers. Brazilian beans production in 2018 was about 2.97 million tons [1]. Among the different aspects related to cropping systems and beans production, N management stands out due to the high fertilizer costs [2]. Therefore, it is one of the main challenges, since insufficient fertilization rates may limit its potential and excessive rates are not only costly, but can promote negative environmental impact [3].

Biological nitrogen fixation (BNF) process can, at least partially, supply the requirements of this crop and ensure greater yield levels, up to 2500 kg ha<sup>-1</sup> [4,5]. Several species of *Rhizobium* have been described as capable of creating nodules and, in most cases, fixing N<sub>2</sub> with beans. In Brazil, *R. tropici* SEMIA 4077, 4080 and 4088 are the registered strains for use as inoculants in common beans (Normativa SDA13/2011).

Another group of beneficial soil microorganisms is represented by associative bacteria called plant growth-promoting rhizobacteria (PGPR) which are capable of promoting plant growth by means of several biological processes [6], including the production of several plant growth hormones as auxins [7], the capacity to induce plant resistance to diseases and stresses [8], to solubilize phosphate [9], and also biological nitrogen fixation [7,10].

Co-inoculation of *Rhizobium* and PGPR in legumes has received more attention recently [11,12,13]. The co-inoculation of *Pseudomonas* sp. with *Rhizobium leguminosarum* brings positive effects on the cultivation of lentils, since it provides plant growth and nutrient uptake [14], increases nodulation in some crops, such as in pigeon pea [(*Cajanus cajan* (L.) Mill sp.)], with the co-inoculation of *Pseudomonas fluorescens* and *Rhizobium* sp. [15]. Besides, this combination presents positive effects on grain yield, as reported for beans, inoculated with *Pseudomonas* and *Rhizobium* [16].

The fungi genus *Trichoderma* has been exploited as biocontrol agent against a range of plant pathogenic fungi because it antagonizes a number of plant pathogens [17]. In addition, it has been widely used as plant growth promoter [18,19]. The combination of biocontrol agents and PGPR increases disease suppression [20], improves crop yields and facilitates nutrient uptake by plants [21] over individual inoculations. In this way, this study was undertaken to test the hypothesis that the combination of *Trichoderma*, *Rhizobium* and PGPR strains could improve growth, biomass accumulation and yield in beans. The hypothesis was tested under greenhouse conditions as well as field to monitor the effects of individual and combined inoculation of these organisms.

## MATERIAL AND METHODS

### Inoculants and experimental design

Common beans (*P. vulgaris* L.) were inoculated in furrow with commercial products containing *Rhizobium tropici* SEMIA 4088, *Bacillus subtilis* QST 713 and *Trichoderma asperellum* SF 04 in the following concentration: 10<sup>9</sup> CFU/mL, 10<sup>9</sup> CFU/mL, 10<sup>9</sup> CFU/g, respectively. Besides, *Burkholderia* sp. 10N6, from PGPR bank of Microbial Molecular Biology Laboratory (State University of Ponta Grossa) was tested due to its capacity to fix nitrogen, produce AIA, protease, siderophore, and solubilize phosphate in vitro trials. *R. tropici*, *B. subtilis* and *T. asperellum* were applied at the following doses: 2.5 ml, 2 ml and 1 g per kg of seeds, respectively. *Burkholderia* sp. 10N6 was applied at the dose of 2.5mL per kg of seed after growing in liquid Digs medium [22] until the concentration of 10<sup>8</sup> CFU/mL. The bean seeds were chemically treated with Standak® Top (Piraclostrobin + Methyl Thiophanate + Fipronil) at a dose of 2 mL per kg of seeds.

Both experiments included two controls, (1) non-inoculated control (NI), and (2) non-inoculated control receiving N-fertilizer (NI+N). In field experiment, plants received 150 kg N ha<sup>-1</sup> of urea in the R5 phenological stage (pre-flowering) and in the greenhouse experiment, received 0.30 g pot<sup>-1</sup> of urea at V4 (third open trifoliolate). Beside the controls, the following treatments, were included: (3) Inoculated with *R. tropici* (IR), (4) Inoculated with *R. tropici* + *B. subtilis* (IRBa), (5) Inoculated with *R. tropici* + *T. asperellum* (IRT), (6) Inoculated with *R. tropici* + *Burkholderia* sp. (IRBu), (7) Inoculated with *B. subtilis* (IBa), (8) Inoculated with *T. asperellum* (IT), (9) Inoculated with *Burkholderia* sp. (IBu) and (10) Inoculated with *R. tropici* + *T. asperellum* + *B. subtilis* (IRTBa). The experiment was conducted under a complete randomized design in greenhouse and randomized block design in field conditions, totaling ten treatments. In greenhouse, twelve pots were cultivated per treatment and in field, four replicates of 18 m<sup>2</sup> each.

## Sites description and field management

The greenhouse experiment was conducted from November of 2017 to January, 2018 in Ponta Grossa State University. Beans were grown in 8 L pots, using a mixture of sand and vermiculite (1:1) as substrate. All vessels received addition of macro and micronutrients as described by [23] and were irrigated before sowing. Bacteria inoculation was performed in furrow close to the seeds using micropipettes.

The field experiment was conducted from January to April 2018 at the Estação Experimental Agrícola Campos Gerais (EEACG), located in Palmeira - PR (25°25'43, 50 "S, 50°3'14.46" O), with elevation of 831 m. Chemical and physical analysis of the 0-20 cm soil layer are showed in Table 1. The soil is classified as medium texture Cambisol. There was no base fertilization at the time of beans sowing, since the area received 15 Mg ha<sup>-1</sup> of sewage sludge in December 2017. The climate is classified as temperate (Cfb), according to Köppen classification and during the experiment 662 mm accumulated as precipitation, with maximum temperature of 26.2 °C in March and minimum of 15.6 °C in April.

**Table 1.** Chemical and physical properties of the soils (0–20 cm) before sowing.

CHEMICAL											PHYSICAL		
pH	(H+Al)	K	Ca	Mg	Al	CEC	T <sub>CEC</sub>	BS	MO	P	Clay	Silt	Sand
CaCl <sub>2</sub>	mmolc dm <sup>-3</sup>							%	g dm <sup>-3</sup>	mg dm <sup>-3</sup>	g kg <sup>-1</sup>		
4.4	126	2.5	20	14	26.8	162.5	36.5	22.46	24	18	303	254	443

CEC - Cation Exchange Capacity (H+Al+Ca+Mg+K); T<sub>CEC</sub> - Ca+Mg+K; BS - Base Saturation (T<sub>cec</sub>/CEC) × 100

The experimental plots were 2.25 m (width) x 8 m (length) and the seed lines were spaced 0.45 m, with fifteen plants per meter. Inoculants were applied to furrows by direct spraying. The area was desiccated before sowing and before harvesting to standardize beans senescence. Herbicides and insecticides were used in all treatments, according to technical recommendations.

## Plant sampling and harvesting

In the greenhouse experiment, three evaluations were performed, totaling four replicates for each evaluation. At V4, shoot dry weight (SDW) and root dry weight (RDW) were evaluated. At R6, SDW, RDW, number of nodules (NN) and their respective weight (NDW) were evaluated. At R9, the harvest was carried out, thus determining productivity per pot and yield components: number of pods per plant (PPL), number of grains per pod (GP) and weight of one thousand grains (WTG).

In field experiment, SDW and RDW at V4 and R6 were evaluated. The evaluations were performed on ten plants per plot. Harvesting was performed on 5.4 m<sup>2</sup> per plot, and yield components PPL, GP and WTG were determined in two remaining meters per plot, by collecting ten plants.

## Statistical analyses

Treatments differences in both experiments were tested through analysis of variance (ANOVA) after normality, variance homoscedasticity and outlier's absence requirements check. When significance at p<0.05 was found, means were compared using Tukey test at p<0.05. To summarize the data variance, we performed a principal component analysis for each of the experiments. All analysis was performed using R language v 3.5.1 [24].

## RESULTS

### Effect of PGPM inoculation on beans grown under greenhouse conditions

Shoot dry weight (SDW) at V4 showed no statistical difference between treatments and at R6 the uninoculated and fertilized with mineral N (NI+N) treatment was statistically higher than the control without inoculation (NI) and co-inoculated treatments with *R. tropici* + *B. subtilis* (IRBa), *R. tropici* + *T. asperellum* (IRT) and *R. tropici* + *T. asperellum* + *B. subtilis* (IRTBa) (Table 2). However, the treatments inoculated with *R. tropici* (IR), *R. tropici* + *Burkholderia* sp. 10N6 (IRBu), *B. subtilis* (IBa), *T. asperellum* (IT) and *Burkholderia* sp. 10N6 (IBu) were statistically equal to the NI+N. For root dry weight (RDW), treatments IBa, IT and IBu presented the highest values at V4 (Table 2). In R6, only the NI+N treatment stood out statistically from the

others, presenting 2.42 g more than average treatments that received inoculation. For nodulation, there was no statistical difference between treatments (Table 2).

**Table 2.** Effect of inoculation with plant growth promoting microorganisms (PGPM) on shoot dry weight (SDW) and root dry weight (RDW) at stage V4 and SDW, RDW, nodule number (NN) and nodule dry weight (NDW) at stage R6 of common bean cultivated in greenhouse conditions.

Treatment <sup>a</sup>	V4		R6			
	SDW	RDW	SDW	RDW	NN	NDW
	g		g		n° pl <sup>-1</sup>	mg pl <sup>-1</sup>
NI	1.58 a	0.675 ab	6.74 b	4.55 b	3.00 a	0.00 a
NI+N	1.51 a	0.659 ab	12.71 a	6.88 a	2.00 a	0.00 a
IR	1.58 a	0.721 ab	10.01 ab	4.45 b	33.75 a	61.0 a
IRBa	1.83 a	0.781 ab	8.74 b	4.35 b	40.25 a	75.0 a
IRT	1.55 a	0.629 b	8.60 b	4.23 b	54.50 a	164.0 a
IRBu	1.79 a	0.804 ab	10.63 ab	4.53 b	45.25 a	120.0 a
IBa	1.65 a	0.860 a	10.33 ab	4.44 b	42.75 a	86.0 a
IT	1.73 a	0.850 a	10.00 ab	4.71 b	47.25 a	58.0 a
IBu	1.71 a	0.862 a	9.97 ab	4.73 b	24.50 a	52.0 a
IRTBa	1.71 a	0.726 ab	8.38 b	4.21 b	46.00 a	97.0 a

<sup>a</sup> NI non-inoculated and without N-fertilizer; NI+N non-inoculated with 0.30 g pot<sup>-1</sup>, IR inoculation with *R. tropici* SEMIA 4088, IRBa *R. tropici* + *B. subtilis*, IRT *R. tropici* + *T. asperellum*, IRBu *R. tropici* + *Burkholderia* sp. 10N6, IBa *B. subtilis*, IT *T. asperellum*, IBu *Burkholderia* sp. 10N6, IRTBa *R. tropici* + *T. asperellum* + *B. subtilis*. Data represent the means of three (V4 stage) and four (R6 stage) replicates and when followed by the same letter, within each column are not statistically different (Tukey,  $p \leq 0.05$ ).

### Effect of PGPM inoculation on beans grown under field conditions

In V4 the treatments inoculated with IR, *R. tropici* + *B. subtilis* (IRBa), IRT, IRBu, IBa and IRTBa presented the highest values of SDW and were statistically equal to each other. In R6, the treatments inoculated with IR, IRTBa, IRT and IRBu were statistically higher than the non-inoculated control (NI), presenting an increase of 67.3%, 63.7%, 62.5% and 61.8% respectively (Table 3). All treatments that received inoculation or co-inoculation were statistically equal to NI+N treatment. However, NI + N, IRBa, IBa, IT and IBu treatments did not differ statistically from NI.

For RDW, in V4, IR and IRTBa treatments were statistically higher than NI treatment and NI+N treatment. IRTBa co-inoculation provided an increase of 57.1% in relation to the NI and 49.1% in relation to NI+N, whereas only *R. tropici* inoculation presented 52.7% of root increase in relation to the NI and 44.9% compared to NI+N. In R6, only IRTBa co-inoculation showed statistical difference from NI, presenting root growth of 47.8%. The other inoculated treatments were statistically equal to IRTBa and NI (Table 3).

**Table 3.** Effect of inoculation with plant growth promoting microorganisms (PGPM) on shoot dry weight (SDW) and root dry weight (RDW) at stage V4 and SDW, RDW at stage R6 of common bean cultivated in field conditions.

Treatment <sup>a</sup>	V4		R6	
	SDW	RDW	SDW	RDW
	g		g	
NI	14.18 bc	3.34 cd	71.02 b	15.51 b
NI+N	12.31 c	3.52 bcd	107.00 ab	20.04 ab
IR	26.31 a	5.10 a	118.84 a	20.88 ab
IRBa	23.59 ab	4.51 abc	101.53 ab	18.07 ab
IRT	27.25 a	4.67 ab	115.41 a	17.98 ab
IRBu	18.84 abc	3.88 bcd	114.93 a	19.64 ab
IBa	19.59 abc	4.32 abcd	106.75 ab	18.33 ab
IT	12.66 c	3.29 d	105.08 ab	19.96 ab
IBu	15.3 bc	3.55 bcd	100.31 ab	19.85 ab
IRTBa	19.91 abc	5.25 a	116.26 a	22.93 a

<sup>a</sup> NI non-inoculated and without N-fertilizer; NI+N non-inoculated with 150 kg of N ha<sup>-1</sup>, IR inoculation with *R. tropici* SEMIA 4088, IRBa *R. tropici* + *B. subtilis*, IRT *R. tropici* + *T. asperellum*, IRBu *R. tropici* + *Burkholderia* sp. 10N6, IBa *B. subtilis*, IT *T. asperellum*, IBu *Burkholderia* sp. 10N6, IRTBa *R. tropici* + *T. asperellum* + *B. subtilis*. Data represent the means of four replicates and when followed by the same letter, within each column are not statistically different (Tukey,  $p \leq 0.05$ ).

### Effect of PGPM inoculation on bean yield components

The NI+N treatment was statistically higher and accounted for the highest yield (8.86 g) of beans under greenhouse conditions, which can be explained by the higher number of pods per plant (PPL) (11.60), found in this same treatment (Table 4). The IR, IBa and IBu treatments were statistically higher than NI, showing 2.84 g, 2.46 g and 2.38 g increment, respectively. Although the variables weight of thousand grains (WTG) and GP did not show statistical difference between the treatments, IBu was highlighted as presented the highest WTG value (227.97 g), which was 37.6% higher than NI+N and 5.28% higher than standard IR treatment (Table 4).

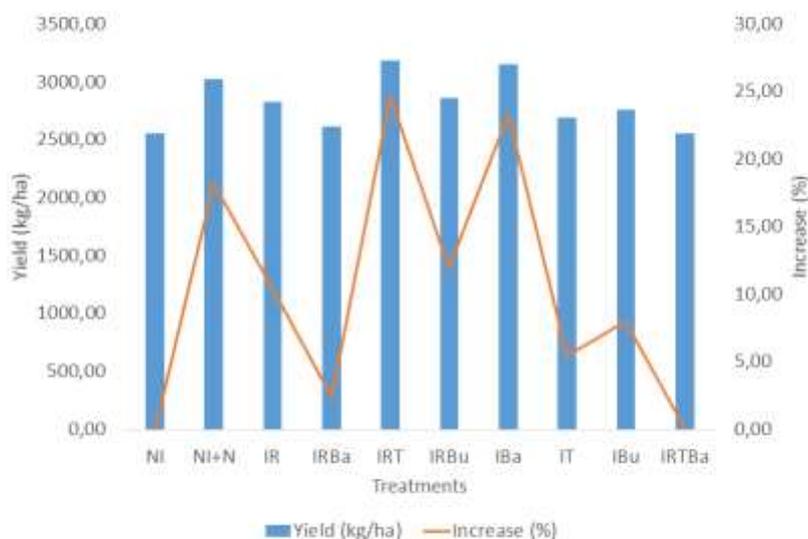
In field, grain yield did not show statistical difference among treatments, however, the highest yields were found in IRT (3185.50 kg ha<sup>-1</sup>) and IBa (3152.64 kg ha<sup>-1</sup>) treatments. The IBa treatment stood out regarding WTG and GP, however, it was not statistically different from the other treatments. PPL values of the IRT treatment were statistically higher than all other treatments, IRT treatment presented approximately 13 more pods per plant than uninoculated treatment (NI) (Table 4).

**Table 4.** Effect of inoculation with plant growth promoting microorganisms (PGPM) on yield components in common bean cultivated in greenhouse and field conditions.

Treatment <sup>a</sup>	Greenhouse				Field			
	PPL	GP	YIELD	WTG	PPL	GP	YIELD	WTG
	n <sup>o</sup> pl <sup>-1</sup>	n <sup>o</sup> pod <sup>-1</sup>	g	g	n <sup>o</sup> pl <sup>-1</sup>	n <sup>o</sup> pod <sup>-1</sup>	kg ha <sup>-1</sup>	g
NI	5.6 bc	2.97 a	2.95 d	165.65 a	12.95 c	4.59 a	2556.05 a	238.00 a
NI+N	11.6 a	3.84 a	8.86 a	214.58 a	21.35 ab	4.63 a	3021.24 a	259.58 a
IR	8.20 bc	3.58 a	5.79 b	216.52 a	20.35 ab	4.51 a	2824.39 a	242.10 a
IRBa	6.60 bc	3.26 a	4.67 bcd	221.29 a	23.15 ab	4.39 a	2616.73 a	233.00 a
IRT	7.00 bc	3.40 a	4.58 bcd	195.30 a	25.08 a	5.36 a	3185.50 a	241.50 a
IRBu	6.00 bc	3.20 a	3.63 cd	191.00 a	21.68 ab	4.97 a	2860.57 a	243.30 a
IBa	8.60 ab	3.18 a	5.41 bc	197.12 a	19.95 ab	5.23 a	3152.64 a	267.38 a
IT	5.00 c	3.25 a	3.39 cd	214.03 a	17.4 bc	4.58 a	2694.41 a	254.30 a
IBu	7.40 bc	3.22 a	5.33 bc	227.97 a	20.43 ab	4.60 a	2760.42 a	252.63 a
IRTBa	5.40 bc	3.10 a	2.81 d	172.07 a	16.75 bc	4.18 a	2556.54 a	232.03 a

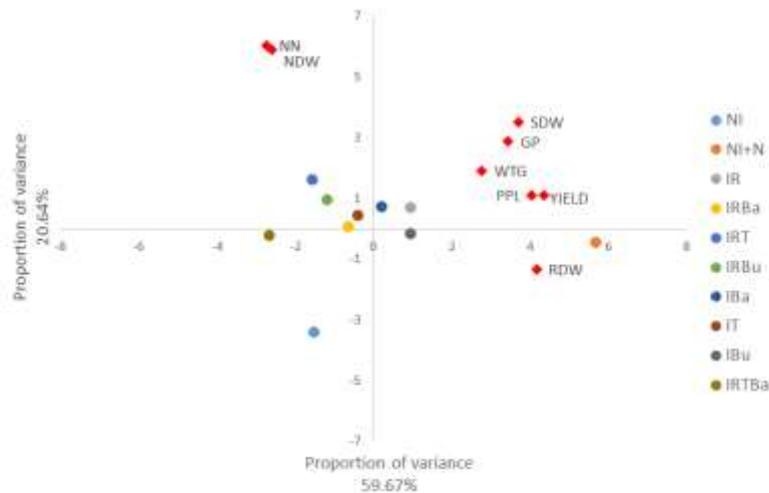
<sup>a</sup> NI non-inoculated and without N-fertilizer; NI+N non-inoculated with 150 kg of N ha<sup>-1</sup> in field and 0.30 g pot<sup>-1</sup> in greenhouse, IR inoculation with *R. tropici* SEMIA 4088, IRBa *R. tropici* + *B. subtilis*, IRT *R. tropici* + *T. asperellum*, IRBu *R. tropici* + *Burkholderia* sp. 10N6, IBa *B. subtilis*, IT *T. asperellum*, IBu *Burkholderia* sp. 10N6, IRTBa *R. tropici* + *T. asperellum* + *B. subtilis*. Data represent the means of three (V4 stadium) and four (R6 stadium) replicates and when followed by the same letter, within each column are not statistically different (Tukey,  $p \leq 0.05$ ).

IRT and IBa treatments showed a yield increase of 24.63% and 23.34%, respectively compared to control (NI). It is superior than the increase registered for NI+N treatment, which was equivalent to 18.2% (Figure 1). The yield levels achieved in the experiment were higher than the average yield found for Paraná state, which was 1340 kg ha<sup>-1</sup> in the 2017/18 season [25].

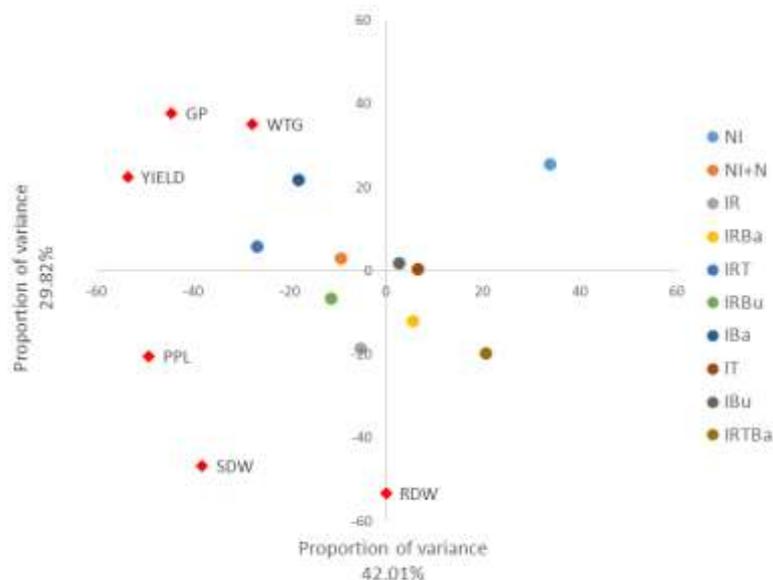


**Figure 1.** Effect of inoculation and co-inoculation in planting furrow on bean crop yield in the field. NI noninoculated and without N-fertilizer; NI+N non-inoculated with 150 kg of N ha<sup>-1</sup>; IR inoculation with *R. tropici* SEMIA 4088; IRBa *R. tropici* + *B. subtilis*; IRT *R. tropici* + *T. asperellum*; IRBu *R. tropici* + *Burkholderia* sp. 10N6; IBa *B. subtilis*; IT *T. asperellum*; IBu *Burkholderia* sp. 10N6; IRTBa *R. tropici* + *T. asperellum* + *B. subtilis*.

The principal component analysis summarized 80.31% of total variance for the greenhouse experiment and 71.83% for the field experiment (Figures 2 and 3). The greenhouse analysis showed all the microorganisms treatments behaving similarly, and the controls (NI+N and NI) differing from the group. The variables were positively correlated and the IBu and IR based treatments presented higher values for yield components. In the field experiments, the variables were also positively related, but the correlations were weaker compared with greenhouse experiment, what may indicate higher random effects over data. The treatments NI and IRTBa differed from the others that composed a group.



**Figure 2.** Principal component analysis (PCA) generated from shoot dry weight (SDW), root dry weight (RDW), weight of thousand grains (WTG), yield, number of pods per plant (PPL), number of grains per pod (GP), number of nodules (NN) and nodule dry weight (NDW) under the condition of greenhouse in bean crop.



**Figure 3.** Principal component analysis (PCA) generated from shoot dry weight (SDW), root dry weight (RDW), weight of thousand grains (WTG), yield, number of pods per plant (PPL) and number of grains per pod (GP) under the condition of field in bean crop.

## DISCUSSION

The associations between microorganisms and plants have been studied for a long time, but its application in agriculture, partially or totally replacing chemical fertilizers, is still moderate. Common beans (*Phaseolus vulgaris* L.) are a cheap source of protein for growing world population, however, there are some concerns about the ability of biological nitrogen fixation to support plant growth and yield [26].

In greenhouse, the NI+N treatment (nitrogen in cover) was highlighted in terms of grain yield in pots. This result may be associated with a higher accumulation of SDW and RDW in the greenhouse at R6 (Figure 2, Table 2). The yield is positively correlated with SDW, RDW, WTG, PPL and GP data, as shown in principal component analysis (Figure 2). In a recent study [27], the authors found higher yields, shoot dry weight, number of pods per plants and number of grains per plant when inoculation with *R. tropici* plus nitrogen fertilization at sowing (20 kg ha<sup>-1</sup> of N) was used. However, the treatment that received inoculation only allowed a production of shoot dry weight equivalent to fertilized treatment, demonstrating that inoculation with rhizobium is equivalent to nitrogen fertilizer, however, with lower production cost. Other study [28] found results in SDW parameter: the control fertilized with mineral N was statistically higher than the treatment inoculated with *R. tropici* CIAT 899.

In the field experiment, at R6, all treatments that received PGPR inoculation were statistically equal to each other for SDW, however, IR, IRT, IRBu and IRTBa treatments were statistically higher compared to uninoculated treatment (NI), showing that inoculation with PGPR improves beans' biomass accumulation. The authors [28] found similar responses with common bean in non-sterile soil: shoot biomass increased with co-inoculation with *R. tropici* CIAT 899 + *B. elkanii* 29w when compared to plants inoculated only with *R. tropici* CIAT 899. However, co-inoculated plants did not differ statistically from the control, inoculated with *R. tropici* alone, which also happened in our study.

The use of PGPR with *Rhizobium* is justified by the ability to stimulate root growth, increasing the number of infection sites available for inoculated *Rhizobium* strains [29]. In addition, they are used as attempts to improve plant nodulation, BNF and nutritional status [30, 31, 32]. In our experiment, IRBu treatment presented the highest SDW at R6, being statistically equal to NI+N treatment and the IRT treatment the highest NN and NDW in a greenhouse, demonstrating that the interaction between these microorganisms is favorable to beans development. Regarding the nodulation parameters, it is clear that the number of nodules (NN) and the weight of nodules (NDW) are correlated (Figure 2).

Nodules found in the NI, NI+N, IBa, IT and IBu treatments might be occupied by nodulating bacteria from bean seeds or substrate, since used sand and vermiculite was not previously sterilized. A recent study [33] reported that *Azospirillum* was the fourth most abundant genus recovered from bacterial rRNA from bean seeds, concluding that *Azospirillum* indeed comprises a significant component of *P. vulgaris* seed microbiome. In addition, these authors found an abundance of *A. brasilense* within intercellular spaces of common beans fibrous stem pith cells using confocal microscopy. In addition, [28] found *Bradyrhizobium elkanii* colonization in intracellular spaces of bean nodules, while colonization by *R. tropici* CIAT 899 was intracellular.

Interactions between rhizobia-legume-PGPR may contribute to rhizobia-legume symbiosis, as the use of PGPR induces legume nodules development, improves plant growth and BNF [34] and the use of *Trichoderma* controls phytopathogenic fungi and bacterial strains and produces plant growth metabolites [35]. Growth stimulation is perceived through increased biomass, productivity, stress resistance and increased nutrient absorption [36]. In greenhouse the IRT treatment was responsible for the largest NN, presenting approximately 20 more nodules than the IR treatment. In the field experiment, the same treatment presented the highest grain yield (3185.50 kg ha<sup>-1</sup>), being superior to NI+N treatment and resulting in 24.63% increase (Figure 1) compared to NI treatment. The co-inoculation of *R. tropici* and *T. asperellum* also influenced yield components, resulting in higher values of pod number and grain number per pod.

There was no increase of root dry weight parameter due to co-inoculation. Values of RDW of IRTBa co-inoculation (22.93 g) was statistically equal to IR (20.88 g) at R6, in field conditions. However, IRTBa was statistically higher than NI, presenting an addition of 7.42 g of RDW. This result agrees with [37] study, which did not observe statistical difference in RDW between *Rhizobium* and PGPR co-inoculation and only *Rhizobium*. Similarly, [31] found no statistical difference between shoot and root dry weight of common bean inoculated with *R. tropici* CIAT 899 strain alone or with *Paenibacillus polymyxa* DSM 36 strain. The IR and IRTBa treatments presented the highest values of RDW and SDW demonstrating the existence of a positive correlation between these variables (Figure 3).

The treatments NI+N, IRT and IBa were responsible for the best results regarding bean yield, which is also positively correlated with WTG and GP (Figure 3). GP and WTG variables showed no significant differences among treatments under greenhouse and field conditions. For the PPL variable, in greenhouse,

NI+N treatment was statistically higher than the others, presenting 11.6 pods per plant. On the other hand, in the field, IRT treatment was statistically higher compared to others, with 25.08 pods per plant. [35] found some strains of *Trichoderma* that could stimulate early stages of growth in beans, potentially leading to the use of these strains as novel bioinoculants in agriculture with potential for increased crop yields. Greenhouse productivity was higher with NI+N treatment. In field conditions, despite there was no statistical difference between treatments, comparing the treatments that received inoculation, IRT and IBa presented the highest yields, both numerically higher than NI+N treatment. Other study [38] found maximum soybean yield with the co-inoculation of *B. japonicum* and *B. subtilis* over an uninoculated control. Also, [37] found extra abundant nodulation of the common bean with co-inoculation *Bacillus megaterium* + CIAT 899. Additionally, [39] reported an increase of shoot dry weight as a result of co-inoculation of common bean with *Bacillus megaterium* (M-3) strain and *Rhizobium* strain. In studies with other legumes, synergism between *Bacillus* and *Bradyrhizobium* in the rhizosphere has been shown to increase nodulation and plant biomass [34].

The greenhouse experiment behavior was not seen under field conditions (Figures 2 and 3). This may indicate that soil fauna in field conditions influenced the treatments. Similar results were reports by [28] who found less prominent response in non-sterile soil as a result of possible interactions with other soil microorganisms. The same was observed for the yield components, that presented higher variation in field conditions reflecting the greater environmental variation. Field experiments with plant growth promoting rhizobacteria (PGPR) usually show high variation [40]. Many factors like nitrogen fertilization, soil type, genetics, climatic conditions, can influence the development of crops and therefore, inoculation response [41].

In field conditions, the treatments did not show a clear pattern, however in greenhouse conditions IBu treatment was positively highlighted. In other study, [42] found that soil inoculation with *Burkholderia* sp. LD-11 promoted biomass accumulation and improved instantaneous water use efficiency in greenhouse maize crop. Also, [43] reported that inoculation with *B. fungorum* UFLA 04-155 in beans promoted shoot dry matter and nutrient content compared to uninoculated plants not fertilized with nitrogen.

Many studies have considered combining rhizobia and PGPR strains to find sustainable biofertilizers as an alternative to chemical fertilizers [44]. To increase the inoculation efficiency, there is the need to better understand the interaction among the inoculated microorganisms and the host plant. Besides, it is essential to find genotypes more responsive to the plant-microorganism interaction to improve development and yield of various agricultural cultures.

## CONCLUSION

Our results suggest that combined inoculation between *R. tropici*, *T. asperellum* and PGPR may promote beans growth and bring benefits to shoot and root accumulation, increasing the number of nodules as well as yield components. Although the greenhouse and field results did not converged, inoculation with *Burkholderia* sp. 10N6 in greenhouse seemed to promote beans growth. Further studies should be performed under different soil and climate conditions to confirm these results.

**Acknowledgments:** To CNPq, CAPES and Araucaria Foundation for their financial support. To Microbial Molecular Biology Laboratory and EEACG for their scientific support. Comercial AG for the availability of agricultural inputs.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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