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Agronomic Efficiency and Phosphate Solubilization of *Pseudomonas fluorescens* and *Bradyrhizobium japonicum* in Leaf-Spray Inoculation and Seed Treatment in Soybean

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Abstract

The use of plant growth promoting bacteria (PGPB) that can solubilize phosphorus (P) has shown potential to improve nutrient availability in many crops such as soybean. This research aimed to evaluate agronomic efficiency and phosphorus solubilization through *Bradyrhizobium japonicum* and product to be registered *Pseudomonas fluorescens* (BR 14810) in soybean, at seed and leaf-spray inoculation. Four experiments with soybean (2020/21 crop) were installed in the following locations in the State of Goiás: Experimental Area of the Goiano Federal Institute, in Rio Verde, Bela Vista Farm, in Indiara, Bauzinho Farm, in Rio Verde, and Cachoeira Farm, in Doverlândia. The *B. japonicum* was inoculated in the seed of all treatments. It was tested three phosphate fertilization doses: 0, 50, and 100% recommended P dose, with and without *P. fluorescens*, at seed treatment and leaf-spray inoculation. The use of inoculation with *P. fluorescens* and *B. japonicum* increases nitrogen (N) content in grains and total N. The P content in dry mass, grains and total are increased using *P. fluorescens* and *B. japonicum*, confirming the ability to solubilize phosphates. Inoculation with *P. fluorescens* and *B. japonicum* is efficient for increasing shoot dry mass and productivity, can be used as a sustainable soybean management technology. Leaf-spray was more efficient than inoculation in seed treatment and can be used as an alternative mode of application. The results demonstrated that the product under test (*P. fluorescens*-BR 14810) can be used associated with *B. japonicum*, in ST or leaf-spray, resulting in increases of agronomic parameters and soybean yield.

Keywords: *Glycine max* L., P fertilization, solubilization, yield

1. Introduction

Humanity has always been concerned about food production to attend the increasing population and, for a long time, the solution was to expand agriculture to new areas. However, this scenario has changed in recent decades, first due to limitations of unexplored cultivable land, but also reinforced by the development of new technologies that allow higher yields, in addition to increasing environmental concerns, leading to agricultural practices aiming at achieving sustainable production. In this context, microbial inoculants with Plants Growth Promotion Bacterias (PGPB) have received increasing attention, gaining prominence and market scale in agriculture (Santos et al., 2019).

In the last decade, the use of inoculants containing microorganisms of "different type" has expanded. The idea is of combining strains or species acting in different microbial processes, so that the combined benefits of each one would result in higher benefits and yields. Examples of mixed inoculant are those combining microorganisms whose major processes are Biological Nitrogen Fixation-BNF (*Bradyrhizobium* spp., *Rhizobium* spp.) and phytohormone production (*Azospirillum* spp., *Pseudomonas* spp.), solubilization of phosphate (*Bacillus* spp.), or biological control (*Pseudomonas* spp., *Bacillus* spp.). If the microorganisms cannot be combined in a single product, they are manufactured separately and the bags containing each one is sold in the same package (Santos et al., 2019).

The success of microbial inoculation depends on the inoculation method, inoculum density, in addition to pH, temperature and soil moisture (Lopes et al., 2021). Although seed inoculation is the most used method, there are some limiting factors that can rapidly reduce the inoculum density or its ability to colonize the host plant, such as chemical treatments and allelochemical compounds produced in the germination of some species (Gautam, 2021; Lopes et al., 2021). Differences in compatibility between pesticides and inoculants depend on their active ingredient, formulation, time of application and period of contact with live microorganisms; however, in general, they have a high impact on cell survival and metabolism, affecting the microbial contribution to plant growth (Santos et al., 2019).

Besides phytohormones synthesis, beneficial properties associated with PGPB include BNF, phosphate and potassium solubilization, production of siderophores, detoxification of heavymetals, induction of plant systemic tolerance to abiotic and biotic stresses, production of hydrolytic enzymes, and production of exopolysaccharides (Vishwakarma et al., 2020). Such properties have been reported in several microorganisms, and the most cited carrying one or more of these properties are *Azospirillum* spp. (Cassán et al., 2021), *Pseudomonas* spp. (Zhang et al., 2018; Sandini et al., 2019), and *Bacillus* spp. (Ribeiro et al., 2018).

BNF is the main source of nitrogen (N) for soybean crop. Bacteria of the genus *Bradyrhizobium* infect the roots of the plant via the root, forming the nodules. BNF can provide all the N that soybeans need. Soybean is the ideal crop in which to explore biological nitrogen fixation (BNF) as inoculation of the seed with efficient *Bradyrhizobium* strains provides rates of fixed atmospheric nitrogen (N_2) greater than 80% (Alves et al., 2006; Hungria et al., 2006) and high grain yield (Kaschuk et al., 2016; Moretti et al., 2020). In terms of nitrogen (N) fertilizer equivalents, the economic savings achieved using BNF in Brazil exceed 12 Tg of mineral N per year, worth over US$ 13 billion (Hungria & Mendes, 2015; Santos et al. 2019).

The genus *Pseudomonas* comprises a taxon capable of using a wide variety of simple or complex organic compounds. Consequently, they are distributed by soils and water, being important as plant pathogens, animals, and humans, with some strains related to the promotion of plant growth and biocontrol of phytopathogens (Zago et al., 2000).

It has been shown that plant growth-promoting bacteria like *Pseudomonas* can solubilize phosphorus (P) and make it more available to plants (Rathinasabapathi et al., 2018). Fernandez et al. (2012) evaluated *Pseudomonas* strains using an assay for phosphorus solubilization from tricalcium phosphate in buffered liquid media. Isolates that displayed a high degree of phosphorus solubilization also exhibited acid and alkaline phosphatase activities, extracellular protease, and hydrogen cyanide production, the last two traits being recognized for biocontrol of pathogenic microbes.

Acidification of the rhizosphere is a major mechanism by which phosphorus is solubilized from unavailable mineral sources, can also improve the activity of some of the phosphate-solubilizing enzymes (Rathinasabapathi et al., 2018). Microbes use the carbon sources from the plant roots and the soil environment. Glucose excreted from the roots can be taken up by the bacteria, which was converted to glucose 6-phosphate in the cytosol. However, this process requires ATP. Under phosphatelimiting conditions, the bacteria instead use the periplasmic pathway where glucose dehydrogenase and gluconate dehydrogenase in the membrane oxidize the glucose to acidic compounds, resulting in lower pH of the environment (Buch et al., 2008).

De Werra et al. (2009) made deletion mutants of *Pseudomonas fluorescens* CH0 lacking either glucose dehydrogenase (gcd) or gluconate dehydrogenase (gad) or both. It was proved that gluconic acid production was important for solubilizing phosphate from unavailable sources.

Phytate (*i.e.*, myo-inositol 1,2,3,4,5,6-hexakisphosphate) is made in plants as a storage form of phosphorus. This is also the most abundant organic form of phosphorus in the soil (Turner et al., 2002). There are multiple taxa of microbes that can mineralize phytic acid and make the phosphorus available including soil isolates of *Pseudomonas* spp. (Sun et al., 2017). Cho et al. (2003, 2005) has identified a phytase of histidine acid phosphatase family from P. syringae MOK1. Beta-propeller phytase in rhizosphere *Pseudomonas* sp. has been well-characterized also (Shen et al., 2016). Recent research has uncovered that P*seudomonas* and other soil bacteria also secrete glycerolphosphodiesterases that could degrade phospholipids, another organic source of phosphorus in the soil (Lidbury et al., 2017).

Information on the solubilization of iron and aluminum phosphates is scarce, although they are the predominant forms of phosphates (Raij, 1991). Furthermore, there are differences in the capacity and solubilization potential of microorganisms. The specific microorganism can solubilize only Ca-P, while others solubilize Al-P and Fe-P, and it should be considered that microorganisms can solubilize these phosphates in different intensities (Doyle et al., 1990; Silva Filho & Vidor, 2000).

Therefore, the use of bacterial inoculants is an alternative for the better utilization of phosphorus applied via fertilization and immobilized in the soil, besides promoting plant growth and development, resulting in higher yields.

Thus, the present research aimed to evaluate agronomic efficiency and phosphorus solubilization through *Bradyrhizobium japonicum* and *Pseudomonas fluorescens* (product to be registered) in soybean, in seed treatment and leaf-spray inoculation.

2. Materials and Methods

2.1 Location

Four experiments with soybean (crop 2020/21) were installed in the following locations in the State of Goiás: a) Experimental area of the Federal Institute of Goiano campus Rio Verde, located at latitude 17º47′53″ S, longitude 50º55′41″ W (Red Oxisol) and altitude 744 m, in Rio Verde; b) Bela Vista Farm, located at latitude 17º11′04″ S, longitude 49º59′04″ W (Yellow Oxisol) and altitude 586m, in Indiara; c) Bauzinho Farm, located at latitude 18º00′26″ S; longitude 50º31′02″ W (Red Oxisol) and altitude: 620 m, in Rio Verde, and d) Cachoeira Farm, located at latitude 16º42′21″ S; longitude 52º13′51″ W (Yellow Oxisol) and altitude: 623 m, in Doverlândia.

The region's climate, according to the Köppen-Geiger classification, is classified as Aw (tropical climate with dry season). The test sites were sampled 60 days in advance for the evaluation of physical and chemical characteristics. Table 1 shows the chemical and granulometric characterization of each area. The population of native rhizobia in the soil in each area was estimated by counting in Petri dishes. The methodologies followed are described in Normative Instruction nº 30 of MAPA (Ministério da Agricultura, pecuária e abastecimento) (MAPA, 2010).

Table 1. The soil's chemical and granulometric characteristics in four locations in the state of Goiás, Brazil (crop 2020/21)

Local	pH_{H_2O}	P	K	Fe	Mn	Zn	Ca	Mg	Al	Organic matter	Saturation by bases	Areia	Silte	Argila	Native rhizobia*
		------------ mg dm^{-3} ------------					-- $cmol_c\ dm^{-3}$ --			g dm^{-3}	------------------ % ------------------				UFC g $soil^{-1}$
IF Goiano campus Rio Verde	6.1	15.2	255	9.0	60.2	6.0	4.4	1.3	0.03	34.9	55.6	36.2	17.4	46.4	4.8×10^3
Bela Vista Farm. Indiara	6.3	29.6	138	22.5	38.2	3.0	3.8	1.1	0.00	22.4	65	61	15	24	2.6×10^4
Bauzinho Farm. Rio Verde	6.1	22.3	302	13.8	22.6	4.0	3.5	0.92	0.02	28.6	58	30	14	56	3.2×10^3
Cachoeira Farm. Doverlândia	6.2	18.5	280	21.3	18.6	3.5	2.8	0.88	0.03	21.8	61	46	18	36	4.1×10^3

Note. Extractors: Mehlich 1 (P, K, Fe, Zn e Mn); KCl 1 N (Ca, Mg e Al); * Analysis performed 48h before planting.

The planting date was between the 2nd and 5th of November 2020. The rainfall during the period was 833 mm in the area of the IF Goiano (Rio Verde); 768 mm at Fazenda Bela Vista (Indiara); 852 mm at Fazenda Bauzinho (Rio Verde) and 791 mm at Fazenda Cachoeira (Doverlândia).

2.2 Experimental Design and Treatments

The cultivar used was Bônus® (8579 RSF IPro), planting density of 280,000 seeds ha^{-1}. In all locations, the predecessor crop was corn and the product Standak Top®: insecticide Fipronil from the pyrazole group, the fungicides Pyrclostrobin from the strugirulin group, and Methyl Thiophanate from the benzimidazole group (2 mL kg $seeds^{-1}$) was used in the seed treatment. After treatment of the seeds, they were dried in the shade for 4 hours and then received the inoculation treatments.

The research was installed in a randomized complete block design with 10 treatments and six replications. The plots were 6 m long × 4 m wide, totaling 24 m^2, separated by 1 m wide corridors. The results were subjected to analysis of variance, and average were separated by the Tukey test (5%) using Sisvar® software (Ferreira, 2019).

In all treatments, the inoculant Atmo® was used in seed treatment (ST), with strains of *Bradyrhizobium japonicum* (SEMIA 5079 and 5080; 5.0×10^9 CFU mL^{-1}), 100 mL 50 kg of $seeds^{-1}$. As standard inoculant of

Pseudomonas fluorescens, the product Fertibio Phospro® (BR 14810; 2.0 × 10^8 CFU mL^{-1}) was used, 50 ml 50 kg of $seeds^{-1}$. The inoculant evaluated was *Pseudomonas fluorescens* (BR 14810; 2.0 × 10^8 CFU mL^{-1}), 100 ml 50 kg of $seeds^{-1}$, in seed treatment (ST) and 200 mL ha^{-1} in leaf-spray inoculation (V3-V4).

All plots were fertilized with KCl (150 kg ha^{-1}; 00:00:52). The treatments consisted of different doses of phosphate fertilization, three levels being tested: 0, 50 and 100% of the recommended phosphorus dose, with and without the inoculant with *P. fluorescens* via seed treatment and leaf-spray inoculation. The recommended dose of phosphorus for the four areas was 80 kg ha^{-1} of P_2O_5 or the equivalent of 400 kg ha^{-1} of super simple fertilizer (Table 2).

2.3 Analyzed Parameters

At 35 days after emergence (DAE), the following parameters were evaluated: aerial dry mass and N and P contents and number and dry mass of nodules. Five plants were collected from the central area of the second row of each plot. At harvest, aerial dry mass, grain yield (13% moisture), N and P contents, and total N and P in grains were evaluated, and the 8 central lines of each plot were harvested.

Table 2. Inoculation and fertilization treatments were used in soybean experiments in four locations in Goiás, Brazil (crop 2020/21)

Nº	Tratamento	P fertilization
1	*B. japonicum* (Control)	0%
2	*B. japonicum* (Control)	50%
3	*B. japonicum* (Control)	100%
4	*B. japonicum* + *P. fluorescens* ST	0%
5	*B. japonicum* + *P. fluorescens* ST	50%
6	*B. japonicum* + *P. fluorescens* ST	100%
7	*B. japonicum* + *P. fluorescens* leaf-spray	0%
8	*B. japonicum* + *P. fluorescens* leaf-spray	50%
9	*B. japonicum* + *P. fluorescens* leaf-spray	100%
10	*B. japonicum* + Fertibio Phospro® ST	50%

Note. *** According to the soil analysis, the doses of P should correspond to 0, 50, and 100% of the recommendation for soybean cultivation.

3. Results

Regarding the variables: number and dry mass of nodules per plant, there were no differences between treatments, except for Bela Vista Farm where the *B. Japonicum*+ *P. fluorescens* ST treatment without P resulted in greater nodule dry mass than the controls (Table 3).

Table 3. Number of nodules, shoot dry mass of nodules, dry mass of aerial part per plant evaluation, in soybean inoculated with *Bradyrhizobium japonicum* and *Pseudomonas fluorescens* and phosphate fertilization (crop 2020/21)

Treatments	P Fertilization	IF Goiano			Bauzinho Farm			Bela Vista Farm			Cachoeira Farm		
		Nº nodules $plant^{-1}$	Dry mass nodules (mg $plant^{-1}$)	Shoot dry mass (g $plant^{-1}$)	Nº nodules $plant^{-1}$	Dry mass nodules (mg $plant^{-1}$)	Shoot dry mass (g $plant^{-1}$)	Nº nodules $plant^{-1}$	Dry mass nodules (mg $plant^{-1}$)	Shoot dry mass (g $plant^{-1}$)	Nº nodules $plant^{-1}$	Dry mass nodules (mg $plant^{-1}$)	Shoot dry mass (g $plant^{-1}$)
B. japonicum (Control)	0%	19.7 bc	116.36 a	82.67 b	20.65 a	289.23 a	101.71 c	21.83 a	115.28 c	90.26 e	16.3 b	115.97 a	89.25 d
B. japonicum (Control)	50%	19.9 bc	117.79 a	99.78 ab	21.55 a	122.32 a	116.30 b	22.88 a	116.57 bc	100.90 d	17.51 ab	119.73 a	101.58 c
B. japonicum (Control)	100%	24.1 a	118.58 a	99.41 ab	20.61 a	124.34 a	125.67 ab	22.93 a	116.38 bc	109.70 c	19.63 a	117.38 a	110.36 bc
B. japonicum + *P. fluorescens* ST	0%	18.5 c	117.82 a	101.95 a	20.93 a	123.74 a	125.36 ab	22.68 a	118.48 a	118.05 b	17.5 ab	119.51 a	105.01 c
B. japonicum + *P. fluorescens* ST	50%	20.3 abc	120.13 a	107.95 a	22.18 a	123.66 a	129.11 a	23.16 a	117.27 ab	120.30 b	17.61 ab	117.50 a	118.86 ab
B. japonicum + *P. fluorescens* ST	100%	20.5 abc	118.38 a	110.24 a	19.51 a	124.92 a	128.49 ab	23.01 a	117.34 ab	120.98 b	18.28 ab	117.33 a	120.28 ab
B. japonicum + *P. fluorescens* leaf-spray	0%	21.7 abc	117.95 a	108.15 a	21.83 a	125.30 a	134.35 a	22.06 a	118.33 ab	124.18 a	18.36 ab	117.31 a	120.12 ab
B. japonicum + *P. Fluorescen* leaf-spray	50%	23.3 ab	119.47 a	110.07 a	22.40 a	124.78 a	131.97 a	22.96 a	117.41 ab	129.77 a	16.45 b	118.82 a	118.86 ab
B. japonicum + *P. Fluorescens* leaf-spray	100%	19.2 c	118.64 a	110.16 a	22.81 a	124.49 a	128.93 a	23.08 a	118.20 ab	130.92 a	17.65 ab	118.28 a	127.66 ab
B. japonicum + Fertibio Phospro® ST	50%	20.8 abc	119.48 a	115.61 a	21.30 a	124.38 a	131.18 a	23.01 a	117.64 ab	131.54 a	17.51 ab	117.50 a	106.22 c
CV (%)		6.09	0.88	3.28	8.88	1.66	2.36	9.64	2.47	2.69	8.56	2.27	2.54

Note. * Means followed by the same letter in the column are not different according to the comparison of Tukey's test (5%).

In shoot dry mass, all treatments with *B. japonicum* + *P. fluorescens* were greater than the control without phosphate fertilization. The treatment with *B. japonicum* + *P. fluorescens* without P had an average increase of 23% when applied in seed treatment and 33.25% when leaf-spray inoculation, in relation to the control without P (Table 3). At Cachoeira Farm, the treatment *B. japonicum* + *P. fluorescens* without P in leaf-spray resulted in higher shoot dry mass than in seed treatment and also compared to the standard treatment Fertbio Phospro® TS + 50% P (Table 3).

The N content in dry mass, in IF Goiano, in the *treatment B. japonicum* + *P. fluorescens* in ST without the use of P, resulted in higher values than the control without phosphate fertilization; in the other areas there was no difference (Table 4).

Table 4. Nitrogen evaluation in soybean inoculated with *Bradyrhizobium japonicum* and *Pseudomonas fluorescens* and phosphate fertilization (crop 2020/21)

Treatments	P Fertilization	IF Goiano			Bauzinho Farm			Bela Vista Farm			Cachoeira Farm		
		N contents dry mass (g kg^{-1})	N contents grains (%)	Total N (kg ha^{-1})	N contents dry mass (g kg^{-1})	N contents grains (%)	Total N (kg ha^{-1})	N contents dry mass (g kg^{-1})	N contents grains (%)	Total N (kg ha^{-1})	N contents dry mass (g kg^{-1})	N contents grains (%)	Total N (kg ha^{-1})
B. japonicum (Control)	0%	61.73 b	5.3 c	143.2 e	76.97 a	5.63 b	151.55 d	67.82 a	6.05 c	161.48 d	65.87 b	5.88 c	177.33 f
B. japonicum (Control)	50%	64.34 ab	6.0 b	182.9 d	75.97 a	6.23 a	188.95 d	68.22 a	6.25 ab	180.55 cd	67.41 ab	6.2 b	192.99 ef
B. japonicum (Control)	100%	64.61 ab	6.3 ab	246.8 ab	76.00 a	6.26 a	253.80 a	67.24 a	6.26 ab	261.22 a	66.59 ab	6.26 b	242.21 b
B. japonicum + *P. fluorescens* ST	0%	65.75 a	6.4 a	183.0 d	76.62 a	6.30 a	186.08 c	68.00 a	6.33 ab	181.11 cd	68.03 ab	6.35 ab	199.16 de
B. japonicum + *P. fluorescens* ST	50%	66.66 a	6.3 ab	200.4 c	77.13 a	6.38 a	201.86 bc	66.94 a	6.36 a	189.58 bc	69.15 ab	6.61 a	212.67 cd
B. japonicum + *P. fluorescens* ST	100%	64.68 ab	6.4 a	258.4 ab	77.25 a	6.26 a	262.91 a	67.17 a	6.41 a	271.67 a	67.88 ab	6.56 a	266.28 a
B. japonicum + *P. fluorescens* leaf-spray	0%	65.97 a	6.4 a	201.8 c	77.67 a	6.36 a	199.51 bc	68.05 a	6.51 a	207.73 b	68.11 ab	6.40 ab	213.31 cd
B. japonicum + *P. fluorescens* leaf-spray	50%	66.44 a	6.4 ab	210.8 c	76.40 a	6.33 a	209.57 b	67.60 a	6.33 ab	206.63 b	69.53 a	6.48 ab	219.04 c
B. japonicum + *P. fluorescens* leaf-spray	100%	64.88 ab	6.4 ab	265.4 a	76.62 a	6.26 a	271.09 a	67.88 a	6.45 a	271.67 a	68.45 ab	6.56 a	280.73 a
B. japonicum + Fertibio Phospro® ST	50%	65.33 ab	6.3 ab	197.0 cd	76.15 a	6.33 a	199.99 bc	68.33 a	6.45 a	202.41 b	68.77 ab	6.38 ab	209.10 cd
CV (%)		2.26	2.51	4.93	1.98	2.13	4.55	2.96	3.30	3.93	2.76	2.42	3.78

* Means followed by the same letter in the column are not different according to the comparison of Tukey's test (5%).

In all areas, the N content in the grains and total N were higher in treatments with *B. japonicum* + *P. fluorescens* and Fertbio Phospro® TS+50%P, compared to the control without phosphate fertilization. The treatment *B. japonicum* + *P. fluorescens*, without P, in leaf-spray and ST, obtained respectively 47.5 kg and 32 kg more total N than the control without P (Table 4). The treatments with inoculation of *P. fluorescens* under evaluation had the same performance as the standard treatment Fertbio Phospro® TS+50%P (Table 4).

The P content in shoot dry mass, in grains and total P in all areas, were higher in treatments with inoculation of *B. japonicum* + *P. fluorescens*, regardless of the P_2O_5 dose, when compared to the controls (Table 5). Except for the treatment *B. japonicum* + *P. fluorescens* ST, without P, which did not differ from controls with 50% and 100% of the recommended dose of phosphate fertilization (Table 5).

Table 5. Phosphorus evaluation in soybean inoculated with *Bradyrhizobium japonicum* and *Pseudomonas fluorescens* and phosphate fertilization (crop 2020/21)

Treatments	P Fertilization	IF Goiano			Bauzinho Farm			Bela Vista Farm			Cachoeira Farm		
		P contents dry mass (g kg^{-1})	P contents grains (%)	Total P (kg ha^{-1})	P contents dry mass (g kg^{-1})	P contents grains (%)	Total P (kg ha^{-1})	P contents dry mass (g kg^{-1})	P contents grains (%)	Total P (kg ha^{-1})	P contents dry mass (g kg^{-1})	P contents grains (%)	Total P (kg ha^{-1})
B. japonicum (Control)	0%	3.99 c	0.50 d	13.4 f	4.22 c	0.53 c	14.25 e	3.80 e	0.76 cd	20.20 fg	3.72 c	0.82 cd	25.07 ef
B. japonicum (Control)	50%	4.32 b	0.66 d	20.2 ef	4.81 b	0.66 c	20.20 e	4.16 d	0.66 d	19.13 g	4.12 b	0.66 d	20.64 f
B. japonicum (Control)	100%	4.80 a	1.02 c	40.1 d	5.32 a	1.09 b	44.40 bcd	4.80 bc	1.15 c	48.01 cd	4.78 a	1.17 bc	45.41 bcd
B. japonicum + *P. fluorescens* ST	0%	4.38 b	0.98 c	28.2 e	5.28 a	1.14 b	33.79 d	4.62 c	1.08 bc	31.03 ef	4.32 b	1.17 bc	36.81 de
B. japonicum + *P. fluorescens* ST	50%	4.83 a	1.31 b	41.8 cd	5.32 a	1.31 ab	41.67 cd	4.96 ab	1.31 ab	39.29 de	4.69 a	1.31 ab	42.37 cd
B. japonicum + *P. fluorescens* ST	100%	4.93 a	1.41 ab	56.9 ab	5.24 a	1.28 ab	53.97 ab	5.09 ab	1.41 ab	60.67 ab	4.72 a	1.41 ab	57.60 ab
B. japonicum + *P. fluorescens* leaf-spray	0%	4.92 a	1.61 a	50.8 bc	5.42 a	1.31 ab	41.24 cd	5.06 ab	1.66 a	53.16 bc	4.74 a	1.61 a	53.96 bc
B. japonicum + *P. fluorescens* leaf-spray	50%	4.98 a	1.51 ab	50.3 bc	5.24 a	1.53 a	50.80 bc	5.09 ab	1.60 a	52.21 bc	4.84 a	1.51 ab	51.18 bc
B. japonicum + *P. fluorescens* leaf-spray	100%	5.00 a	1.53 ab	64.1 a	5.34 a	1.35 ab	58.44 a	5.12 a	1.55 a	65.29 a	4.79 a	1.53 ab	65.47 a
B. japonicum + Fertibio Phospro® ST	50%	4.97 a	1.53 ab	48.0 bc	5.22 a	1.31 ab	41.66 cd	5.09 ab	1.60 a	50.14 bc	4.79 a	1.53 ab	50.21 bc
CV (%)		3.28	14.55	13.56	2.36	13.10	14.19	2.69	12.53	12.34	2.54	16.22	16.12

Note. * Means followed by the same letter in the column are not different according to the comparison of Tukey's test (5%).

The treatment *B. japonicum + P. fluorescens*, without P, presented an average increase of 16% of P in dry mass in ST and 28% in leaf-spray, compared to the control without P. This same treatment showed an average increase of 17.5 kg ha^{-1} of total P in ST and 31.6 kg ha^{-1} in leaf-spray, compared to the control without P. Evidencing that foliar spray had better performance than inoculation in seed treatment (Table 5). The treatments with inoculation of *P. fluorescens* under evaluation had the same performance as the standard treatment Fertbio Phospro® TS+50%P (Table 5)

Regarding productivity, the treatment *B. japonicum + P. fluorescens*, ST + 50%P presented higher yields than the control without P. The *treatment japonicum + P. fluorescens*, ST + 100% P, presented higher yields than the control 0 and 50%P (Table 6).

Table 6. Yield (kg ha^{-1}) evaluation in soybean inoculated with *Bradyrhizobium japonicum* and *Pseudomonas fluorescens* and phosphate fertilization (crop 2020/21)

Treatments	P Fertilization	IF Goiano	Bauzinho Farm	Bela Vista Farm	Cachoeira Farm	Average
B. japonicum (Control)	0%	2685 e	2694 d	2680 e	3015 e	2.766
B. japonicum (Control)	50%	3050 d	3030 bc	2889 de	3111 de	3.020
B. japonicum (Control)	100%	3928 b	4052 a	4168 a	3864 b	4.004
B. japonicum + *P. fluorescens* ST	0%	2853 e	2954 cd	2859 de	3137 de	3.200
B. japonicum + *P. fluorescens* ST	50%	3182 cd	3162 bc	2981 bcd	3214 cd	3.318
B. japonicum + *P. fluorescens* ST	100%	4018 ab	4195 a	4283 a	4055 b	4.248
B. japonicum + *P. fluorescens* leaf-spray	0%	3145 cd	3134 bc	3188 bc	3333 c	3.175
B. japonicum + *P. fluorescens* leaf-spray	50%	3322 c	3309 b	3262 b	3378 c	3.267
B. japonicum + *P. fluorescens* leaf-spray	100%	4180 a	4326 a	4212 a	4274 a	4.290
B. japonicum + Fertibio Phospro® ST	50%	3128 d	3158 bc	3139 bc	3275 cd	2.939
CV (%)		4.76	4.65	2.99	2.91	

Note. * Means followed by the same letter in the column are notdifferent according to the comparison of Tukey's test (5%).

In relation to *japonicum* + *P. fluorescens* treatments, leaf-spray, without P, produced 409 kg ha^{-1} more than the control without P and there was no difference from the control with 50%P. When the dose was 50%P, it produced 501 kg ha^{-1} more than the control without P and 247 kg ha^{-1} than the control with 50%P, except for Bauzinho Farm, where the productivity of this treatment compared to the 50%P witness had no difference (Table 6).

The treatment *japonicum* + *P. fluorescens*, leaf-spray, with 100% P, obtained higher yields than all the controls, producing on average 1524 kg ha^{-1} more than the control without P and 286 kg ha^{-1} than the control 100 % P. At Bauzinho Farm and Bela Vista Farm, the treatment *japonicum* + *P. fluorescens*, leaf-spray, with 100% P, did not differ from the 100%P control, but produced an average of 159 kg ha^{-1} more (Table 6).

Higher yield averages were observed in the treatment with *Pseudomonas fluorescens*, leaf-spray inoculation and 100% P, compared to the other treatments, in the experiments of Cachoeira Farm (4.274 kg ha^{-1}) and Experimental area of the Federal Institute of Goiano campus Rio Verde (4.180 kg ha^{-1}).

The treatments with inoculation of P. *fluorescens* under evaluation had the same performance as the standard treatment Fertbio Phospro® TS+50%P, except for the area in IF Goiano where its productivity was lower than the treatment *P. fluorescens* + *B. japonicum*, leaf-spray and ST, 100% P (Table 6).

4. Discussion

The present experiment showed that treatments with application of *B. japonicum* + *P. fluorescens* increased shoot dry mass, nitrogen and phosphorus concentration in the plant, grains and, consequently, yield.

The use of PGPB positively influences the absorption of nutrients that is linked to total dry matter production and nutrient concentration in the plant. The amounts of nutrients exported are directly proportional to the productivity and concentration of nutrients in the grains (Oliveira Júnior et al., 2020). Guimarães et al. (2021) reported that the inoculation of *P. fluorescens* in soybean increased morphometric parameters reflecting dry mass gain, and higher nutrient content (NPK) of plant tissues.

In the present study, the n content in grains and total N were higher in treatments with *B. japonicum* + *P. fluorescens* (Table 4). P. fluorescens contributes to BNF indirectly, improving root architecture and nodule formation by *Bradyrhizobium* sp. The proposed mechanisms by which PGPB can improve the nodular activity of *Rhizobium* are: production of binding proteins in the cell membrane (Burns et al., 1981), production of antimicrobial agents (Li & Alexander, 1988), stimulation of root colonization by mycorrhizal fungi, which result in changes in root morphology (Meyer & Linderman, 1986).

Sandini et al. (2019), demonstrated that, inoculation P. *fluorescens* in corn, promoted plant growth and yield at both levels, increasing plant biomass accumulation by 24 and 20%, relative to the non-inoculated control, at standard or high levels, respectively. The grain yield increased by 29 and 31%, relative to the non-inoculated control, under standard and high levels of technology, respectively. Under both situation, plant growth and grain yield improved by *P. fluorescens* was equivalent to the application of 100% of the recommended N fertilizer, even when the amount of N fertilizer applied to the crop was reduced by 25%, without compromising yield.

In the present study, the p content in shoot dry mass, in grains and total P in all areas, were higher in treatments with *B. japonicum* + *P. fluorescens* regardless of the P_2O_5 dose when compared with the controls (Table 5). This suggests that the inoculation of PGPB improves phosphorus availability and, consequently, reduces the need for phosphate fertilization.

Bashan and Bashan (2004) said that increased biomass and P contents in plants are indicative of the effect of phosphate solubilization that can result in plant growth and development. It has been shown that plant growth-promoting bacteria like *Pseudomonas* can solubilize P and make it more available to plants (Chien et al., 2011).

P. fluorescens is highly positive in the synthesis of siderophores, phosphate solubilization (CaHPO4 2H2O), and of 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Hungria et al., 2021). Glick et al. (1998) proposed that PGPB strains possessing ACC-deaminase can hydrolyze ACC, the precursor of ethylene in higher plants, producing α-ketobutyrate and ammonia which, in turn, can promote plant growth. Recent research has uncovered that *Pseudomonas* and other soil bacteria also secrete glycerolphosphodiesterases that could degrade phospholipids, another organic source of phosphorus in the soil (Lidbury et al., 2017).

Guimarães et al. (2021) inoculated *P. fluorescens* in ST soybean, without P and with 50% and 100% of the recommended dose and found that this bacterium promotes growth and productivity gains, related to its phosphate solubilization potential, with half of the recommended dose of phosphate fertilizer.

It comes to the form of inoculation, in most of the parameters evaluated, the leaf-spray inoculation treatments obtained superior results in relation to Inoculation ST. The treatment *B. japonicum* + *P. fluorescens* with 100% of phosphate fertilization, presented yield averages higher than the average of Brazilian productivity (3,523 kg/ha) and of the state of Goiás (3,714 kg/ha) (Table 6) (CONAB, 2021).

The leaf inoculation method is an alternative for seeds with chemical treatment that end up reducing bacterial survival (Puente et al., 2018). In this Standak ® Top, composed of two fungicides and an insecticide, was the product used for seed treatment. Rodrigues et al. (2020) report this product affects the survival of *B. japonicum* and, mainly, of *B. elkanii* cells, with a drastic decrease verified after 7 days of contact (Rodrigues et al., 2020). Pre-inoculated soybean seeds are with Standak® Top for up to 90 days, often showing zero recovery of rhizobia cells from the seeds (Hungria & Nogueira, 2019).

The leaf spray inoculation was beneficial when using *Azospirillum brasilense* in sorrh (Nakao et al., 2014), soybean (Puente et al., 2018) and corn (Fukami et al., 2017; Galindo et al., 2020; Fukami et al., 2016; Barbosa et al., 2021). Machado et al. (2020) report that when applying *Bacillus subitils* leaf route in corn, higher height parameters and significant productivity increase. Positive results were also obtained with leaf inoculation of *Azospirillum brasilense* and *Pseudomonas fluorescens* in brachiaria pastures (Hungria et al., 2021).

The results of the present study demonstrated that the product under test (*P. fluorescens*-BR 14810) can be used associated with *B. japonicum*, in ST or leaf-spray, resulting in increases of agronomic parameters and soybean yield. Results compatible with the product containing the same strain of *P. fluorescens*, already registered and marketed: Fertibio Phospro®, were obtained, conferring recommendation for use and registration of the technology in soybean crop.

5. Conclusions

The use of inoculation with *Pseudomonas fluorescens* and *Bradyrhizobium japonicum* increases n content in grains and total N, indicating that *P. fluorescens* collaborates with *B. japonicum* facilitating BNF.

The p content in dry mass, grains and total are increased using *P. fluorescens* and *B. japonicum*, confirming the ability to solubilize phosphates, facilitating their absorption by plants and making possible reduction of the use of phosphate fertilization in soybean crop.

Inoculation with *P. fluorescens* and *B. japonicum* is efficient for increasing shoot dry mass and productivity, confirming its ability to promote plant growth, and can be used as a sustainable soybean management technology

Leaf-spray was more efficient than inoculation in seed treatment and can be used as an alternative mode of application.

The results of the present study demonstrated that the product under test (*P. fluorescens*-BR 14810) can be used associated with *B. japonicum*, in ST or leaf-spray, resulting in increases of agronomic parameters and soybean yield.

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