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Screening of Beneficial Microorganisms to Improve Soybean Growth and Yield

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HIGHLIGHTS

- We tested co inoculation in the development of soybean plants.
- Microorganisms provided better soybean development than in the control plants;
- Ab-V5 + *Trichoderma asperellum* pool provided 25% more soybean grain yield.

Abstract: The objective of this research was to identify the best microorganisms, alone or in mixture for total biomass gain (root + shoot), positive change in gas exchange, nutrient uptake (root, shoot and grain) and yield and yield components in the soybean crop. Trial under greenhouse conditions had the experimental design in a completely randomized scheme with 26 treatments and four replicates. The treatments consisted of the rhizobacteria BRM 32109, BRM 32110 and 1301 (Bacillus sp.), BRM 32111 and BRM 32112 (Pseudomonas sp.), BRM 32113 (Burkholderia sp.), BRM 32114 (Serratia sp.), Ab-V5 (Azospirillum brasilense) and 1381 (Azospirillum sp.), and the fungus Trichoderma asperellum (a mix of the isolates UFRA.T06, UFRA.T09, UFRA.T12 and UFRA.T52). Besides, the same isolates were combined in pairs, completing 16 combinations. Control treatments received no microorganism. Microorganisms applied isolated or in combination, provided biomass gain, positive gas exchange, increases in nutrients uptake at the shoot and grain, and improved grain yield and its components than control plants. Stood out the combination Ab-V5 + T. asperellum pool, which provided a 25% improvement in grain yield.

Keywords: g*lycine max*; bioagent; growth promotion; nutrient uptake; gas exchange; grain yield.

INTRODUCTION

The inoculation of plant growth promoting rhizobacteria (PGPR) represents a strategic alternative for sustainable agricultural systems [1]. This group of microorganisms can benefit the plant via multiple mechanisms, which have been divided into direct and indirect stimulation. A co-inoculation consists of adding

more than one recognized beneficial microorganism to the plants in order to maximize their contribution to plant development.

The plant growth promotion by Trichoderma sp is due to the production of specific metabolites, such as growth stimulants (phytohormones), hydrolytic enzymes, siderophores, antibiotics, and carbon and nitrogen permeases. These characteristics allow it to act as a growth bio-promoter, ensuring that the plant has access to nutrients solubilized by the fungus (especially phosphates), which are not available in the soil [2]. The ability of the fungus to colonize roots is a critical factor for its interference with plant growth and productivity [3].

Studies of Embrapa Rice and Bean and the Federal Rural University of Amazonia allowed the identification of beneficial rhizobacteria (BRM 32109, BRM 32110, BRM 32111, BRM 32112, BRM 32113, BRM 32114, 1301, AbV5 and 1381), collected from upland rice fields [4] and four isolates of the fungus Trichoderma asperellum (UFRA.T06, UFRA.T09, UFRA.T12, UFRA.T52), collected from reforestation rhizosphere soil and Amazon native forest [5],[6] . After selection and characterization, research carried out in the greenhouse proved the potential of these microorganisms, since significant increases were observed in the gas exchange and biomass production of irrigated [7] and upland rice [8]. The same species of beneficial microorganisms can promote the growth of several plant species [9]. Thus, the objective of this research was to identify the best microorganisms, alone or in the mixture for total biomass gain (root + shoot), positive change in gas exchange, nutrient uptake (root, shoot and grain), yield components and grain yield of the soybean crop.

MATERIAL AND METHODS

The study was conducted in a greenhouse condition at the headquarters of Embrapa Rice and Bean, in the municipality of Santo Antônio de Goiás, GO, Brazil. Arable layer soil (0-0.20 m) of a dark red Latosol (Árico) [10] of a pasture of Urochloa brizantha for more than 20 years was used. The soil chemical characteristics were determined according to the methods described by [11] (Table 1).

Table 1. Soil characteristics used to fill the posts where soybean (*Glycine max* (L.) Merril) plants were planted in the experiment conductin 2018 at Embrapa Rice and Beans

pH	Ca^{2+}	Mg^{2+}	Al^{3}	$H^{+} + Al^{3}$	P	K	Cu^{2+}	Zn^{2+}	Fe^{3+}	Mn^{2+}	SOM	Clay	Silt	Sand
(H_2O)														
----------mmolc dm^{-3}----------							----------mg dm^{-3}----------				----------g kg^{-1}----------			
5.0	7.3	2.1	1	26	0.6	56	2.2	1.1	37.5	16.1	19.91	444	127	429

*SOM – Soil Organic Matter

Three weeks before soybean sowing, the 10 kg pots were completely filled with soil and fertilized with 10 grams of N-P_2O_5-K_2O (5-30-15). For nitrogen supply, the liquid inoculant (*Bradyrhizobium japonicum*) was pulverized in the seeding groove, including the control treatment. For this, 5 mL of the commercial product "Grap Nod" was diluted in 900 mL of water, and 10 mL of the solution was applied per pot.

The experimental design was a completely randomized scheme with 26 treatments (Table 2) and four replicates, under greenhouse conditions. Treatments consisted of the microbiolization of soybean seeds with microorganisms, isolated, and in mixing pairs. Besides, the application of microbial suspension on the 7^{th} and 21^{st} at the soil and soybean seedlings, respectively.

Table 2. Treatments (isolated microorganisms and/or a mixture of microorganisms) of the beneficial microorganisms with soybean (*Glycine max* (L.) Merril) experiment, conducted in 2018 at Embrapa Rice and Beans.

Treatment	Microorganism(s)
1	BRM 32109
2	BRM 32110
3	BRM 32111
4	BRM 32112
5	BRM 32113
6	BRM 32114
7	1301
8	1381
9	*T. asperellum* pool
10	Ab-V5
11	BRM 32114 + BRM 32110
12	BRM 32114 + Ab-V5
13	BRM 32114 + *T. asperellum* pool
14	BRM 32110 + Ab-V5
15	BRM 32110 + *T. asperellum* pool
16	Ab-V5 + *T. asperellum* pool
17	1381 + 1301
18	1381 + Ab-V5
19	1301 + Ab-V5
20	1381 + BRM 32114
21	1301 + BRM 32114
22	1381 + BRM 32110
23	1301 + BRM 32110
24	1381 + *T. asperellum* pool
25	1301 + *T. asperellum* pool
26	Control (no microorganism)

The main characteristics of the isolates of rhizobacteria: *Bacillus sp.* (BRM 32109, BRM 32110 and 1301), *Pseudomonas sp.* (BRM 32111 and BRM 32112), *Burkholderia sp.* (BRM 32113), *Serratia sp.* (BRM 32114), *Azospirillum brasilense* (Ab-V5) and *Azospirillum* sp. (1381) are described in Table 3. The rhizobacteria are stored and preserved in the collection of Multifunctional Microorganisms of Embrapa Rice and Beans. The *T. asperellum* pool is stored and preserved in the Collection of Fungal Cultures of the Plant Protection Laboratory of the Federal Rural University of Amazonia.

Table 3. Collection code, geographical origin, biochemical characteristics and taxonomic classification of the eight rhizobacteria isolates used to treat soybean seeds

Code[A]	Origin[B]	Color[C]	Biochemical[D]					Taxonomic[E]
			AIA[F]	Celul.[G]	phos[ph]or	Sider.[I]	Biofilm[J]	
BRM 32109	GO/Brazil	White		+	+		+	*Bacillus* sp.
BRM 32110	PA/Brazil	White		+	+	+	+	*Bacillus sp.*
BRM 32111	PA/Brazil	Yellow		+	+	+	+	*Pseudomonas sp.*
BRM 32112	GO/Brazil	Yellow		+	+	+	+	*Pseudomonas* sp.
BRM 32113	PA/Brazil	Pink	+	+		+	+	*Burkholderia sp.*
BRM 32114	PA/Brazil	Pink	+	+	+	+	+	*Serratia* sp.
Ab-V5	PR/Brazil	Yellow	**+**	+	+	+	+	*Azospirillum sp.*
1381		White	**+**	+	+	+	+	*Azospirillum sp.*
1301		White	**+**	+	+	+	+	*Bacillus sp.*

Number code of rhizobacterial isolates in the Microorganisms and fungi Multifunction Embrapa Rice and Beans collection geographical origin of each isolate; C, D, colony color, Biochemical characterization and Taxonomic classification of each isolate, described by [11]; F acid idol acetic producer; Gcelulase producer; Phosphatase producer; Isiderophers producer; biofilm producer. The methodology is described in [11].

Bacterial suspensions for seed microbiolization and applications were prepared with water from cultures grown for 24 hours in solid medium 523 [13] at 28 ºC, and the suspension concentration of each of the bacteria was fixed in spectrophotometer for A540 = 0.5 (108 CFU mL^{-1}, Colony-Forming Unit). Soybean seeds were immersed in a suspension of microorganisms, according to treatment, and the control seeds were immersed in water for a period of 4 hours under constant agitation at 25 °C. For *T. asperellum*, each

isolate was grown in Petri dishes containing PDA (dextrose and potato agar) for 5 days and bioformed [5]. Seed treatment was carried out at concentrations of 10 g of *T. asperellum* powder per 1 kg of seeds [4], [5]. The suspension concentration of each *T. asperellum* pool isolate was fixed at 108 ml^{-1} conidia, and then the isolates were mixed in equal volumes.

Soybean seed microbiolization was based on the methodology used for rice seeds microbiolization, proposed by [4], with an adaptation. Before proceeding soybean microbiolization, we performed a test to determine the optimal period of time for seeds and bacterial suspension contact, The seeds were submersed in a suspension containing water and the isolate BRM 32110 (*Bacillus* spp.), during different times period (2 hours, 4 hours, 6 hours and 16 hours), and only water (control treatment), totaling 5 treatments and 4 repetitions. Seeds were taken out from suspension dried at room temperature, and sown in 500 mL cups. The best time period was determined by identifying the highest seedling sown, 15 days after sowing. The treatment that provided the best results was the microbiolization for 4 hours. Therefore, this methodology was used in this experiment.

Ten soybean seeds, cultivar BRS 6970IPRO, were sown per pot in August 2018. Four days after occurred germination, and the thinning of plants was done, maintaining three plants per pot. The cultural practices were carried out according to the recommendations and needs of the crop aiming at keeping the pots free of weeds, diseases and pest insects.

Microbial suspension similar to that used for soybean seed microbiolization was applied to the soil at 7th days after sowing (DAS) and sprayed onto soybean plants at 21th DAS. These applications were made as follows:

- Application to the soil: microbial suspension (100 mL, 10^8 CFU mL^{-1} for each rhizobacterium and 10^8 conidia mL^{-1} for *T. asperellum* isolates) or water (control) was applied to each plot (vase);
- Application to the seedlings: microbial suspension (30 mL, 10^8 CFU mL^{-1} for each rhizobacterium and 10^8 conidia mL^{-1} for *T. asperellum* isolates) or water (control) was applied to each plot. Hand sprayer with a constant pressure supplied by a CO_2 pressure source and a type of conical nozzle (TX-VS2) on 21th DAS was used.

The following evaluations were carried out:

Measurements were performed using an infrared gas analyzer (LCpro +, ADC BioScientific, Hoddesdon, England) (Table 4). A soybean plant per pot was used to perform measurements of gas exchange, totaling four plants for each treatment. Gas exchange measurements were performed in the central leaflets of the upper third of soybean plants (completely expanded and exposed to sunlight).

Table 4. Gas exchange parameters obtained from IRGA (infrared gas analyzer).

Parameters	A Photosynthetic rate	E Transpiratory rate	gs Stomatal conductance	Ci Internal CO_2 concentration	EiC Instantaneous Carboxylation Efficiency
Unit	(μmol CO_2 m^{-2} s^{-1})	(mmol H_2O m^{-2} s^{-1})	(mol H_2O m^{-2} s^{-1})	(μmol mol^{-1})	(μmol m^{-2} s^{-1}) ($\mu mol\ mol^{-1})^{-1}$)

*The instantaneous efficiency of carboxylation (EiC) was calculated by the ratio between A and Ci [13].

The readings were made in the period between 08:00, and 10:00 am in the full bloom of soybean plants. The equipment was regulated to use concentrations of 370-400 mol mol^{-1} CO_2 in the reference air used in the IRGA photosynthesis chamber. The flux of photosynthetically active photons (DFFFA) used was 1200 μmol [quanta] m^{-2} s^{-1}. The minimum equilibrium time established for the reading was 2 minutes.

When the soybean plants reached full bloom stage, the shoot of one plant per pot was randomized removed; washed in water; packed in paper bags, previously identified; dried in an oven with forced-air circulation at 65 ºC for 72 h and, then, weighed. Roots were measured in the two left plants after harvesting grain yield and yield components using the same methodology. Dry biomass of shoot, root and total (shoot + root) were determined.

When soybean plants reached physiological maturity at 116 days after sowing, the harvest occurred. The number of pods per plant, number of grains per pot, mass of 100 grains and grain yield were determined. Grain yield was determined by weighing total grains harvested from each pot, and the moisture content of the grains was corrected to 13% and converted to g^{-1} plant. Number of pod per plant was determinate by counting pods number in each pot and dividing by two. Number of grains per pod was determinate by counting the number of grains of a sample of 10 pods randomly collected and divided by 10. Mass of 100 grains, randomly collected, were weighed and the moisture content of the grains corrected to 13%.

Shoot, root and grains after drying were ground (Willey mill) for analysis and determination of nutrients content (N, P, K and Fe). Nitrogen was extracted with H_2SO_4. From the extracted solution, the N concentration was determined using the Kjeldahl distillation method [14]. Phosphorus and exchangeable K were extracted with a Mehlich1 extracting solution (0.05 M HCl in 0.0125 M H_2SO_4). The extracts were calorimetrically analyzed for P, and flame photometry was used to analyze K. Iron was determined in a Mehlich1 extract by atomic absorption [11].

The data were submitted to analysis of variance, and when significant, the means were compared by the LSD test ($\alpha \leq 0.05$). The SAS Statistical Software, SAS Institute, Cary, NC, USA (SAS, 1999) was used.

RESULTS AND DISCUSSION

The analysis of variance revealed that there were differences in the values of A and EiC in soybean, cultivar BRS 6970IPRO, inoculated and treated with the different microorganisms, isolated and in combination (Table 5). Thus, soybean treated with combinations 1301 + BRM 32110 and 1301 + Ab-V5 had the highest A values when compared to treatments 1381 + *T. Asperellum* pool, 1301 + 32114, 1381 + 32114, 1381 + 32110, 1381 + Ab -V5, Ab-V5 + *T. Asperellum* pool, BRM 32109, BRM 32111 and 1301, but did not differ statistically from the other treatments or the control. Soybean plants co-inoculated with 1301 + Ab-V5 also provided highest EiC, compared to the control treatment. In our research we could see that microorganisms provided differences in only one variable (EiC). Therefore, we can see that microorganisms used in this trial had little effect on gas exchange. However, these microorganisms can have effect in other variables that affects plant development with effect on grain yield. [7] reported that although microorganisms did not have high effect on gas exchange in relation to the control treatment, they provided significant improvements on irrigated rice yield.

Table 5. Photosynthesis (A), transpiration (E), stomatal conductance (gs), CO_2 internal concentration (Ci) and instantaneous carboxylation efficiency (EiC) of soybean plants, BRS 6970IPRO, co-inoculated with beneficial microorganisms, isolated and in combination.

Microorganisms	A	E	Gs	Ci	EiC
	($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	($mmol\ H_2O\ m^{-2}\ s^{-1}$)	($mol\ H_2O\ m^{-2}\ s^{-1}$)	($\mu mol\ mol^{-1}$)	($\mu mol\ m^{-2}\ s^{-1}$) ($\mu mol\ mol^{-1}$)$^{-1}$
32109	8.05 c	3.96	0.79	341	0.024 c
32110	12.67 abc	3.37	0.58	299	0.043 abc
32111	9.99 bc	3.75	0.68	296	0.035abc
32112	12.18 abc	2.86	0.44	280	0.044 abc
32113	11.02 abc	2.92	0.54	308	0.038 abc
32114	20.96 abc	2.79	0.51	265	0.076 ab
1301	10.01 bc	2.89	0.51	256	0.040 abc
1381	11.60 abc	3.56	0.68	297	0.042 abc
Trichoderma pool	18.58 abc	3.59	0.63	318	0.060 abc
Ab-V5	19.51 abc	3.75	0.79	318	0.060 abc
32114 + 32110	12.32 abc	3.70	0.51	289	0.044 abc
32114 +Ab-V5	15.18 abc	3.66	0.60	278	0.053 abc
32114 + *T.* pool	24.88 ab	2.42	0.40	292	0.079 ab
32110 + Ab-V5	12.97 abc	3.85	0.91	312	0.043 abc
32110 + *T.* pool	17.45 abc	3.39	0.52	292	0.059 abc
Ab-V5+ *T.* pool	9.29 c	4.36	1.03	341	0.028 c
1381 + 1301	12.55 abc	3.47	0.64	283	0.045 abc
1381 + Ab-V5	8.95 c	3.55	0.60	322	0.027 c
1301 + Ab-V5	25.24 a	4.03	0.79	312	0.080 a
1381 + 32114	9.21 c	3.51	0.58	255	0.049 abc
1301 + 32114	7.55 c	2.93	0.48	320	0.023 c
1381 + 32110	9.64 c	2.27	0.38	273	0.037 abc
1301 + 32110	25.25 a	3.59	0.58	361	0.066 abc
1381 + *T.* pool	6.87 c	3.29	0.51	303	0.026 c
1301 + *T.* pool	12.36 abc	2.88	0.49	278	0.044 abc
No microorganism	10.25 abc	3.88	0.68	324	0.032 bc

* Means followed by the same letter do not differ from each other by the LSD test. BRM 32109, BRM 32110 and 1381 - *Bacillus sp*., BRM 32111 *Burkholderia sp*., BRM 32112 and BRM 32113 - *Pseudomonas sp,* Ab-V5 - *Azospirillum sp.,* 1301 - *Azospirillum brasilense, T. asperellum* pool (UFRA-06, UFRA- 09, UFRA-12 and UFRA-52).

The 1381 (*Azospirillum sp*) + BRM 32114 (*Serratia sp*.) treatment differed statistically from the other treatments for greater shoot biomass production, including the control treatment (Table 6). In addition to

soybean inoculation with *Bradyrhizobium*, the use of growth-promoting bacteria of the genus *Azospirillum* has been promising, as they may increase the root system and the volume of soil explored, thus influencing soybean nodulation and efficient nutrient absorption, which consequently provides higher biomass production and crop yield [15]. In a similar study, there was also an increase in shoot biomass and roots in soybean plants inoculated with *Azospirillum sp* [16]. Bacteria of the genus *Serratia sp* also promote plant growth and increase biomass production through phytohormones production, P solubilization and increased root development, allowing for greater nutrient absorption [7].

Soybean plants treated with the isolate 1301 (*Bacillus sp*.) and mixture 1301 + BRM 32114 (*Serratia* sp.) were significantly superior to control plants for root dry biomass (Table 6). The 1301 + BRM 32114 treatment also provided higher total biomass of soybean plants, being significantly superior to the control treatment. These results showed that the co-inoculation of the soybean with beneficial microorganisms, isolated or in combination, can provide significant increases in shoot and root biomass. Likewise, [17] showed that soybean inoculation with *Bacillus subtilis* increased shoot and root dry biomass in Gurupi and Araguaçu regions.

Table 6. Dry matter of shoot, root and total (shoot + root) of soybean, BRS 6970IPRO, co-inoculated with several beneficial microorganisms, isolated and in combination.

Microorganism	Shoot	Root	Total
	----------grams plant^{-1}----------		
32109	5.07 cde	22.14 cd	27.22 cd
32110	4.93 cde	26.07 bcd	31.00 bcd
32111	4.63 de	24.85 bcd	29.48 bcd
32112	4.53 e	21.87 cd	26.40 cd
32113	5.62 bcde	26.27 bcd	31.89 bcd
32114	5.44 bcde	20.45 d	25.90 d
1301	5.20 bcde	37.95 ab	43.15 ab
1381	6.43 bcde	26.87 bcd	25.90 d
T. asperellum pool	6.78 ab	28.95 bcd	35.73 abcd
Ab-V5	5.29 bcde	24.21 bcd	29.50 bcd
32114 + 32110	5.10 cde	24.58 bcd	29.69 bcd
32114 +Ab-V5	6.13 bcd	26.91 bcd	33.04 bcd
32114 + *T. asperellum* pool	5.68 bcde	15.47 d	21.16 d
32110 + Ab-V5	6.42 abc	20.21 d	26.64 cd
32110 + *T. asperellum* pool	5.48 bcde	35.72 abc	41.21 abc
Ab-V5 + *T. asperellum* pool	6.09 bcde	24.69 bcd	30.79 bcd
1381 + 1301	5.58 bcde	27.08 bcd	32.66 bcd
1381 + Ab-V5	6.20 bcd	21.83 cd	28.03 cd
1301 + Ab-V5	5.80 bcde	24.95 bcd	30.76 bcd
1381 + 32114	7.84 a	27.16 bcd	35.00 bcd
1301 + 32114	6.45 abc	43.45 a	49.90 a
1381 + 32110	5.79 bcde	25.59 bcd	31.38 bcd
1301 + 32110	5.76 bcde	29.21 bcd	34.97 bcd
1381 + *T. asperellum* pool	6.11 bcd	25.16 bcd	31.27 bcd
1301 + *T. asperellum* pool	5.93 bcde	18.32 d	24.26 d
No microorganism	5.51 bcde	22.89 cd	28.40 bcd

*Means followed by the same letter do not differ from each other by the LSD test. BRM 32109, BRM 32110 and 1381 - *Bacillus sp*., BRM 32111 *Burkholderia* sp., BRM 32112 and BRM 32113 - *Pseudomonas sp*, Ab-V5 - *Azospirillum sp*., 1301 - *Azospirillum brasilense, T.asperellum* pool (UFRA-06, UFRA- 09, UFRA-12 and UFRA-52).

Some genera of PGPR favor plant growth directly by synthesizing growth regulators (such as auxins) as well as solubilizing phosphates and zinc and/or producing siderophores [18]. Other microorganisms act indirectly by preserving plant growth through biological control, phytoalexin production, or induce plant resistance in the presence of pathogens [19]. In our study, the beneficial microorganisms evaluated, as shown in Table 3, are auxin, cellulase, siderophores and exopolysaccharides producers and phosphate solubilizers. Therefore, the higher development of soybean plants due to microorganisms treatment is likely because of the production of these composts.

Regarding nitrogen (N) uptake and accumulation in the shoot, none of the treatments were significantly superior to the control. The treatments BRM 32110, BRM 32110 + Ab-V5, BRM 32114 + *T. asperellum* pool, BRM 32109, BRM 32111, 1301, BRM 32112, *T. asperellum* pool, 1381 + *T. asperellum* pool were significantly higher than the others treatments (Table 7).

It was possible to see higher phosphorus (P) uptake and accumulation in plants treated with microorganisms (Table 7). The highlight was soybean plants treated with BRM 32110 (*Bacillus* sp.) + *T. asperellum* pool compared to the control. Our results corroborate those of [20], found that in a soybean experiment, several *Bacillus* isolates with natural phosphate fertilization provided higher phosphorus content in the shoots of soybean plants and soil than in the control treatment (without *Bacillus* inoculation). Furthermore, *Trichoderma* fungi are also indicated as efficient for solubilizing phosphates and increasing soil fertility and plant growth [21]. Therefore, the association of the *Bradyrizhobium*, *Bacillus* sp. and *Trichoderma* pool may have favored the higher phosphorus accumulation in soybean shoots in response to the higher root system development and the ability of these microorganisms to perform phosphorus solubilization.

The treatment BRM 32113 (*Burkholderia sp.*) was superior to control treatment for potassium accumulation in soybean plants (Table 7). Potassium is fundamental in regulating the osmotic potential of cells and increasing the specific surface of the root system [22]. In this experiment, the isolate BRM 32113 provided higher K accumulation in the shoots, although it had no effect on root biomass. This suggests greater availability of K in the soil solution and possible improvement in the absorption mechanism of this nutrient but not in the biomass accumulation.

BRM 32111 (*Pseudomonas* sp.), 1381 (*Azospirillum* sp.) + BRM 32110 (*Bacillus* sp.) and 1381 (*Azospirillum* sp.) + *T. asperellum* pool differed statistically from control treatment, providing a greater accumulation of iron in soybean plants (Table 7). Both rhizobacteria and *Trichoderma asperellum* pool produce siderophores, which can be defined as small peptide molecules containing side chains and functional groups that can provide a high-affinity set of ligands to coordinate ferric ions [23]. Siderophores can chelate ferric ion with high affinity, allowing its solubilization and extraction from most mineral or organic complexes [24]. In soil, siderophore production activity plays a central role in determining the ability of different microorganisms to improve plant development. Microbial siderophores enhance iron uptake by plants that are able to recognize the bacterial ferric-siderophore complex [25] and are also important in the iron uptake by plants in the presence of other metals such as nickel and cadmium [25]. Soybean plants benefit from siderophores production by facilitating the acquisition of Fe, which participates in various enzymatic functions, including protein synthesis [26]. Evaluating the effect of beneficial rhizobacteria on upland rice plants, [7] also observed higher values of Fe and other plant nutrients.

Table 7. Nitrogen (N), phosphorus (P), potassium (K) and iron (Fe) nutrient contents of the shoot of BRS 6970IPRO soybean plants, treated with beneficial microorganisms, isolated and in combination.

Microorganism	N	P	K	Fe
	----------g kg^{-1}----------			
32109	46.42 abc	3.76 bcd	18.96 abc	97 bcd
32110	48.76 a	3.87 abcd	17.38 abcdef	96 cd
32111	46.65 ab	3.85 bcd	18.72 abcd	120 a
32112	45.46 abc	3.70 bcd	18.75 abcd	97 bcd
32113	43.02 bcde	3.44 d	20 a	94 cd
32114	44.43 abcd	3.62 cd	17.63 abcde	94 cd
1301	46.32 abc	4.03 abc	19.04 ab	101 abcd
1381	41.97 cde	3.57 cd	16.74 bcdefg	107 abcd
T. asperellum pool	46 abc	3.71 bcd	16.93 abcdef	113 abcd
Ab-V5	44.71 abcd	3.61 cd	17.47 abcde	95 cd
32114 + 32110	45.12 abcd	3.79 bcd	16.92 abcdefg	111 abcd
32114 +Ab-V5	44.36 abcd	3.69 bcd	14.34 fgh	100 abcd
32114 + *T. asperellum* pool	46.69 ab	3.96 abcd	14.89 efgh	101 abcd
32110 + Ab-V5	46.70 ab	4.07 abc	14.72 efgh	99 abcd
32110 + *T. asperellum* pool	45.02 abcd	4.34 a	14.73 efgh	103 abcd
Ab-V5 + *T. asperellum* pool	42.60 bcde	4.15 ab	14.91 efgh	112 abcd
1381 + 1301	41.96 cde	4.16 ab	14.77 efgh	103 abcd
1381 + Ab-V5	40.77 de	3.66 bcd	16.06 bcdefgh	96 bcd
1301 + Ab-V5	44.09 bcd	3.62 cd	14.11 gh	100 abcd
1381 + 32114	39.31 e	3.75 bcd	14.32 fgh	114 abcd
1301 + 32114	44.43 abcd	3.70 bcd	15.52 efgh	92 d
1381 + 32110	43.73 bcde	3.60 cd	14.56 efgh	119 ab
1301 + 32110	43.78 bcde	3.58 cd	15.88 cdefgh	93 d
1381 + *T. asperellum* pool	45.42 abc	3.90 abcd	13.33 h	116 abc
1301 + *T. asperellum* pool	43.45 bcde	3.62 cd	15.78 defgh	109 abcd
No microorganism	44.36 abcd	3.64 bcd	16.17 bcdefgh	94 d

* Means followed by the same letter do not differ from each other by the LSD test. BRM 32109, BRM 32110 and 1381 - *Bacillus sp*., BRM 32111 *Burkholderia sp*., BRM 32112 and BRM 32113 - *Pseudomonas sp*, Ab-V5 - *Azospirillum sp*., 1301 - *Azospirillum brasilense*, *T.asperellum* pool (UFRA-06, UFRA- 09, UFRA-12 and UFRA-52).

Regarding the root nutrient accumulation, no treatment differed from the control for N, P and Fe (Table 8). For N, treatments BRM 32114, BRM 32112, 1381 + Ab-V5, 1381 + 32114 and 1301 + *T. asperellum* pool were significantly higher than treatments 32114 + Ab-V5 and 1301 + 32110. For P, only treatment BRM 32113 was significantly superior to treatments 1301 + 32110, 32110 + *T. asperellum* pool, 32114 + Ab-V5, Ab-V5 and *T. asperellum* pool treatments. For iron (Fe), the treatments Ab-V5, 1301, 32114 + Ab-V5, 1301 + 32110, *T. asperellum* pool, 1381 + 32110 and 1301 + 32114 were significantly higher than treatments 1301 + *T. asperellum* pool and 32114.

Differently, for K, the treatments BRM 32111, BRM 32112, BRM 32114, BRM 32113, BRM 32110 and 1381 + 32114 provided higher accumulation in the roots when compared to the control (Table 8). As already mentioned, the K accumulation in the soybean roots can be a result of your increased soil solution availability. In evaluating carrot plants that were inoculated with rhizobacteria isolated from the *Crotalaria spectabilis* rhizosphere, [27] found higher K content in plant roots.

Table 8. Nitrogen (N), phosphorus (P), potassium (K) and iron (Fe) nutrient contents of root of BRS 6970IPRO soybean plants, treated with beneficial microorganisms, isolated and in combination.

Microorganism	N	P	K	Fe
	---------- g kg^{-1}----------			-------mg Kg^{-1}------
32109	14.16 abc	1.25 bc	3.38 defg	47.05 abcde
32110	13.37 abc	1.33 abc	5.20 abcd	46.51 abcde
32111	13.50 abc	1.45 abc	5.77 a	37.94 abcdef
32112	16.05 a	1.90 a	5.67 ab	36.83 bcdef
32113	13.93 abc	1.53 abc	5.49 abc	41.93 abcdef
32114	16.06 a	1.55 abc	5.66 ab	31.88 ef
1301	10.57 bc	1.27 abc	3.34 efg	53.31 ab
1381	13.87 abc	1.47 abc	4.77 abcdefg	41.54 abcdef
T. asperellum pool	10.82 abc	1.23 bc	4.02 abcdefg	50.56 abc
Ab-V5	10.78 abc	1.23 bc	3.78 cdefg	53.54 a
32114 + 32110	13.75 abc	1.35 abc	3.08 g	38.90 abcdef
32114 +Ab-V5	9.39 c	1.19 bc	3.66 cdefg	52.68 ab
32114 + *T. asperellum* pool	14.12 abc	1.82 ab	3.74 cdefg	35.36 cdef
32110 + Ab-V5	14.42 abc	1.60 abc	3.96 abcdefg	39.93 abcdef
32110 + *T. asperellum* pool	10.64 abc	1.24 bc	3.67 cdefg	50.50 abc
Ab-V5 + *T. asperellum* pool	13.90 abc	1.49 abc	3.89 bcdefg	33.88 def
1381 + 1301	11.26 abc	1.55 abc	4.26 abcdefg	48.21 abcde
1381 + Ab-V5	14.87 ab	1.61 abc	3.83 bcdefg	39.24 abcdef
1301 + Ab-V5	12.41 abc	1.47 abc	3.38 defg	46.48 abcde
1381 + 32114	14.87 ab	1.74 abc	5.04 abcde	39.27 abcdef
1301 + 32114	13.65 abc	1.57 abc	3.91 bcdefg	48.79 abcd
1381 + 32110	10.65 abc	1.34 abc	4.25 abcdefg	48.97 abcd
1301 + 32110	8.98 c	1.18 c	3.32 efg	50.99 abc
1381 + *T. asperellum* pool	12.10 abc	1.43 abc	2.99 g	43.09 abcdef
1301 + *T. asperellum* pool	15.76 ab	1.75 abc	4.94 abcdef	29.34 f
No microorganism	11.52 abc	1.59 abc	3.13 fg	49.76 abcd

* Means followed by the same letter do not differ from each other by the LSD test. BRM 32109, BRM 32110 and 1381 - *Bacillus sp*., BRM 32111 *Burkholderia sp*., BRM 32112 and BRM 32113 - *Pseudomonas sp*, Ab-V5 - *Azospirillum sp*., 1301 - *Azospirillum brasilense*, *T.asperellum* pool (UFRA-06, UFRA- 09, UFRA-12 and UFRA-52).

No treatments differed from the control treatment in the accumulation of macronutrients N, P and K in the grains (Table 9). Treatments 1301 + *T. asperellum* pool, BRM 32110, BRM 32112 and 1381 accumulated more N than treatment 32111. In relation to P, treatments 1301 + 32110, 32114 + *T. asperellum* pool and 32110 + *T. asperellum* pool were significantly higher than treatments BRM 32109, BRM 32111, Ab-V5, *T. asperellum* pool, 1381, BRM 32114, BRM 32112, BRM 32113 and 1381 + 32114. The treatments BRM 32114, *T. asperellum* pool, 1301, 32114 + Ab-V5, 32114 + *T. asperellum* pool and 32110 + Ab-V5 accumulated higher amount of K than treatments Ab-V5, 1381 + 32110, 1301 + 32110 and 32110 + *T. asperellum* pool.

The BRM 32113 microorganism differed significantly from control treatment for grain Fe accumulation (Table 9). Nutrient concentration in the seed is expressed as a function of the values found in the constituent reserve part. These values may vary among species, cultivars, and also the environmental conditions to which they are subjected. Nutrients stored in the seeds are essential because they are responsible for maintaining the seedling in the first days of emergence and also allow more nutritious grain for food [28]. Emphasis should be given to the isolate BRM 32113 in relation to the nutrient accumulation of soybean plants, as it provided higher K content in shoots and roots and Fe in grains. The greater absorption of nutrients by plants treated with beneficial microorganisms is due, in part, to the greater development of the roots and the increase of the root hairs [29]. Higher root growth can be stimulated by the production of phytohormones allowing greater access to nutrients and, therefore, greater absorption of these.

Table 9. Nitrogen (N), phosphorus (P), potassium (K) and iron (Fe) nutrient contents of grains of BRS 6970IPRO soybean plants, treated with beneficial microorganisms, isolated and in combination.

Microorganism	N	P	K	Fe
	---------- g kg^{-1}----------			-------mg Kg^{-1}------
32109	56.50 abcd	4.81 f	13.57 abcd	72.48 ab
32110	57.44 ab	5.36 abcde	12.90 abcdef	54.52 bc
32111	55.12 d	4.90 ef	13.36 abcde	59.34 bc
32112	57.35 ab	5.07 cdef	13.57 abcd	55.12 bc
32113	56.05 abcd	5.07 cdef	13.15 abcdef	89.81 a
32114	55.96 abcd	5.05 cdef	13.99 a	60.97 bc
1301	56.21 abcd	5.25 abcdef	13.84 abc	62.12 bc
1381	57.23 abc	5.04 cdef	13.37 abcde	57.00 bc
T. asperellum pool	55.45 bcd	5.02 cdef	13.92 ab	59.38 bc
Ab-V5	56.55 abcd	4.95 def	12.06 f	65.16 bc
32114 + 32110	55.81 abcd	5.34 abcde	13.44 abcde	66.78 bc
32114 +Ab-V5	56.06 abcd	5.45 abc	13.84 abc	73.36 ab
32114 + *T. asperellum* pool	55.86 abcd	5.63 ab	13.84 abc	65.81 bc
32110 + Ab-V5	56.97 abcd	5.35 abcde	13.83 abc	64.05 bc
32110 + *T. asperellum* pool	55.75 abcd	5.61 ab	12.56 def	68.18 bc
Ab-V5 + *T. asperellum* pool	55.25 cd	5.08 cdef	13.52 abcde	53.61 bc
1381 + 1301	57.15 abcd	5.24 abcdef	12.74 bcdef	61.25 bc
1381 + Ab-V5	56.94 abcd	5.36 abcde	12.73 bcdef	60.97 bc
1301 + Ab-V5	57.04 abcd	5.42 abcd	13.50 abcde	61.57 bc
1381 + 32114	56.26 abcd	5.11 cdef	12.87 abcdef	47.94 c
1301 + 32114	55.71 bcd	5.18 bcdef	12.81 abcdef	69.05 b
1381 + 32110	56.26 abcd	5.47 abc	12.37 ef	68.91 b
1301 + 32110	56.70 abcd	5.69 a	12.40 def	71.68 ab
1381 + *T. asperellum* pool	57.77 a	5.49 abc	13.39 abcde	55.22 bc
1301 + *T. asperellum* pool	56.17 abcd	5.43 abcd	13.22 abcdef	62.73 bc
No microorganism	56.88 abcd	5.33 abcde	13.05 abcdef	60.25 bc

*Means followed by the same letter do not differ from each other by the LSD test. BRM 32109, BRM 32110 and 1381 - *Bacillus sp*., BRM 32111 *Burkholderia sp*., BRM 32112 and BRM 32113 - *Pseudomonas sp*, Ab-V5 - *Azospirillum sp*., 1301 - *Azospirillum brasilense*, *T.asperellum* pool (UFRA-06, UFRA- 09, UFRA-12 and UFRA-52).

The Ab-V5 (*Azospirillum brasilense*) + *T. asperellum* pool co-inoculation was the treatment that provided number of pods per plant, 100 grain weight and soybean yield significantly higher than the control treatment (Table 10). For the number of grains per pod, the treatments BRM 32113; BRM 32111; 1301; *T. asperellum* pool; 1301 + Ab-V5; 1381 + BRM 32114 and BRM 32114 + Ab-V5 were superior compared to control treatment. (Table 10). In a study conducted by [30], soybean co-inoculation with *Bradyrhizobium spp*. and *A. brasilense* provided a 16% increase in grain yield compared to the control treatment (without inoculation). While [31] found that the application of Trichoderma sp. Soybean seeds and sprouts yielded 12% higher grain yield than control plants (without inoculation). These results allow inferring that soybean plants treated with rhizobium and co-inoculated with beneficial microorganisms increased their growth and grain yield significantly. Since the '90s, [32] had already described that *A. brasilense*, associated with *Bradhyrizobium*, can bring benefits to soybean crop, such as higher development, higher nodulation, BNF efficiency and higher grain yield. While [33] reported that *T. asperellum* could promote higher growth and yield of soybean plants through the production of phytohormones, higher nutrient absorption and use efficiency, in addition to greater control of harmful microorganisms.

Table 10. Number of pods per plant (NPP), the number of grains per pod (NGP), mass of 100 grains (M100) and grain yield (GY) of soybean, BRS 6970IPRO, co-inoculated with beneficial microorganisms, isolated and in combination.

Microorganism	NPP	NGP	M100	GY
	Unit	Unit	g	g plant^{-1}
32109	46.50 bcdef	1.96 cdef	18.61 bc	34.00 cde
32110	51.75 abcde	2.07 abcde	18.16 bc	28.60 abcde
32111	40.62 ef	2.15 ab	19.37 ab	33.84 de
32112	49 bcdef	1.96 def	18.42 bc	35.08 bcde
32113	39.37 f	2.17 a	19.37 ab	33.04 e
32114	44.75 cdef	2.06 abcde	18.92 abc	34.87 cde
1301	51.62 abcde	2.14 ab	17.63 c	38.82 abcde
1381	57.50 abc	1.98 bcde	18.56 bc	41.97 abcd
Trichoderma pool	44.12 cdef	2.14 ab	18.59 bc	34.45 cde
Ab-V5	41.62 def	2.07 abcde	19.28 abc	33.10 e
32114 + 32110	52.12 abcde	2.04 abcde	18.96 abc	40.28 abcde
32114 +Ab-V5	44.37 cdef	2.11 abcd	19.41 ab	36.35 abcde
32114 + *T.* pool	43.50 cdef	2.05 abcde	18.43 bc	32.88 e
32110 + Ab-V5	50.50 abcdef	2.05 abcde	18.39 bc	38.34 abcde
32110 + *T.* pool	53.00 abcd	2 abcde	18.24 bc	39.08 abcde
Ab-V5 + *T.* pool	61.87 a	1.80 f	20.51 a	44.42 a
1381 + 1301	54.62 abcd	1.99 abcde	19.21 abc	42.01 abc
1381 + Ab-V5	51.75 abcde	2.07 abcde	18.83 abc	40.04 abcde
1301 + Ab-V5	47.87 abcdef	2.13 abcd	18.95 abc	38.15 abcde
1381 + 32114	47.12 abcdef	2.13 abc	18.63 bc	37.38 abcde
1301 + 32114	49.87 abcdef	2.04 abcde	18.25 bc	37.24 abcde
1381 + 32110	50.50 abcef	2.07 abcde	18.63 bc	38.94 abcde
1301 + 32110	42.75 cdef	2.10 abcde	19.01 abc	34.06 cde
1381 + *T.* pool	54.37 abc	2.08 abcde	19.31 abc	43.07 ab
1301 + *T.* pool	53.12 abcd	1.93 ef	18.75 bc	38.60 abcde
No microorganism	49.62 bcdef	1.93 ef	18.48 bc	35.49 bcde

* Means followed by the same letter do not differ from each other by the LSD test. BRM 32109, BRM 32110 and 1381 - *Bacillus sp*., BRM 32111 *Burkholderia sp*., BRM 32112 and BRM 32113, *Pseudomonas sp*, Ab-V5 - *Azospirillum sp*., 1301 - *Azospirillum brasilense*, *T.asperellum* pool (UFRA-06, UFRA- 09, UFRA-12 and UFRA-52).

In summary, we highlight the co-inoculation of the Ab-V5 + *T. asperellum* pool, as it provided, on average, 25% increase in soybean yield (Table 10). These results can be explained by the higher number of pods per plant and a higher mass of 100 grains. Isolate Ab-V5 (*Azospirillum brasilense*) and *T. asperellum* are the only microorganisms in this study that are already commercially used as growth promoters in various crops and have their proven benefits, especially in higher soybean yield.

The association of PGPR and fungi, such as *Trichoderma* sp., with plants, expands the zone of root absorption, increasing the contact surface with the soil, favoring greater absorption of nutrients such as P, zinc, copper and K [34] and N [35], resulting in increased plant tolerance to abiotic and biotic stresses [36]. Similarly, PGPR provide an increase in plant yield by the ability to increase the efficiency of nutrient uptake, especially low mobility such as Fe and P. Besides the function of fixing the N from the air in the roots, some selected bacteria have the capacity to make soluble the phosphorus present in the soil and make it available to the vegetable [37]. They can also produce auxins such as indole-3-acetic acid, increasing the root length of the plants, thus leading to greater uptake of nutrients from the soil [38]. All these characteristics together can provide higher soybean grain yield in comparison to the control treatments, as observed in our trial.

Our results are promising and highlight the potential of a combination of beneficial microorganisms co-inoculated with *Bradyrhizobium* on soybean growth/ development. Positive physio-agronomic responses such as the increase of photosynthetic rate and carboxylation efficient, nutrients content, dry matter production and grain yield were obtained. Emphasis should be given to the technology used, beneficial microorganisms, where it is the low cost of investment, easy application and use, not polluting, and it is part of a desired sustainable context in modern agriculture. In addition, the results are even more relevant because they are microorganisms that were selected from the rice root system [4] and provided growth improvement in the soybean plant. Industries are looking for beneficial microorganisms that provide improvements in several crops for economic purposes. The same species of beneficial microorganisms can lead to greater growth and development of plants in several species [39,9]. The results are commercially important as these microorganisms have yielded good results in irrigated rice [7], upland rice [8] and soybean crops (present trial). Our focus is to evaluate the performance of beneficial microorganisms in production

systems (field conditions) since they recommend the rotation of crops as one of the main pillars for the sustainable intensification of agriculture. Therefore, additional studies should be performed under field conditions to confirm the good results obtained under controlled conditions.

The effect of beneficial microorganisms on physiological activities, biomass production, nutrient accumulation and crop yields varies in laboratory, greenhouse and field trials. Because soil is an unpredictable environment and sometimes makes it difficult to achieve an intentional result. Climate variations also have a major impact on the effectiveness of microorganisms, as well as the presence of other natural soil microorganisms, but sometimes unfavorable growth conditions in the field are expected as normal agricultural functioning [40]. There are several studies performed with the genera of microorganisms tested in this study and several others that report their ability to stimulate plants through substances that promote plant growth through different mechanisms of action, which are still being investigated [41]. Thus, research conducted in this sector demands to understand the development of these microorganisms in the plant, seeking the effectiveness of this technique, the development of new management methods that ultimately enable high production and low use of fertilizers. Various other researches on different crops, microorganisms, and environmental conditions must be undertaken to find the most efficient species of microorganisms in different conditions and also for each crop and region.

CONCLUSION

The tested microorganisms provided increases in gas exchange, biomass production, yield components, grain yield and nutrient accumulation in soybean biomass.

The mixture of the microorganisms Ab-V5 + *T. asperellum* pool provided the highest grain yield of soybean, being 25% higher than the control treatment. These results can be explained by the greater number of pods and a mass of 100 grains provided the soybean plants by this mixture of microorganisms.

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