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Consortium of multifunctional microorganisms in soybean culture

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Abstract

The use of multifunctional microorganisms (MM) directly benefits the growth and development of plants due to the production of phytohormones and siderophores, supply of nutrients, and assimilation of atmospheric nitrogen (N_2), as well as, indirectly, by protecting plants against pathogens. In this way, the search for sustainable agricultural systems is of great importance in searching for cultivation technologies that provide productive increments and minimize production costs and negative environmental impacts. The study aimed to determine the effect of multifunctional microorganisms on gas exchange, grain yield, and production components in soybean plants. In the field experiment, 2019/2020 harvest, a randomized block design was used, with 32 replications. Soybean plants were treated with a consortium of microorganisms *Serratia marcensens* (BRM 32114) + *Bacillus* sp. (BRM63573), and, as a control, soybean plants were treated without intercropping. Soybean plants treated with microorganisms showed an increase in photosynthetic rate (16.65%), stomatal conductance (37.50%), the internal concentration of CO2 (10.36%), a mass of 100 grains (4.04%), and yield of grains (14.83%) about untreated plants. Therefore, using a consortium of multifunctional microorganisms, combining multiple functionalities from different microorganisms, shows the potential to increase the agronomic performance of soybean plants. Co-inoculation technology appears as a strategic component of achieving sustainable agriculture.

Keywords: glycine max; rhizobacteria; productivity; gas exchange.

Consórcio de microrganismos multifuncionais na cultura da soja

Resumo

A utilização de microrganismos multifuncionais (MM) beneficia diretamente o crescimento e desenvolvimento de plantas em decorrência da produção de fitormônios e sideróforos, suprimento de nutrientes e assimilação de nitrogênio atmosférico (N_2); bem como, indiretamente, por proteger as plantas contra patógenos. Dessa forma, a busca por tecnologias de cultivo que proporcionem incrementos produtivos e minimizem custos de produção e impactos negativos ao ambiente é de grande importância na busca por sistemas agrícolas sustentáveis. O objetivo do estudo foi determinar o efeito de microrganismos multifuncionais, sobre as trocas gasosas, produtividade de grãos e componentes de produção em plantas de soja. No experimento de campo, safra 2019/2020, utilizou-se o delineamento experimental de blocos casualizados, com 32 repetições. As plantas de soja foram tratadas com o consórcio de microrganismos *Serratia marcensens* (BRM 32114) + *Bacillus* sp. (BRM63573) e, como controle, plantas de soja tratadas sem o consórcio. Plantas de soja tratadas com microrganismos apresentaram aumento na taxa fotossintética (16,65%), condutância estomática (37,50%), concentração interna de CO_2 (10,36%), massa de 100 grãos (4,04%) e produtividade de grãos (14,83%) em relação às plantas não tratadas. Portanto, a utilização de consórcio de microrganismos multifuncionais, ou seja, combinação de múltiplas funcionalidades advindas de diferentes microrganismos, mostra potencial em aumentar a performance agronômica de plantas de soja. O uso da tecnologia de co-inoculação figura-se como componente estratégico para alcançar a agricultura sustentável.

Palavras-chaves: glycine max; rizobactérias; produtividade; trocas gasosas.

Introduction

Soybean (*Glycine max* (L.) Merr.) exhibits great socioeconomic value worldwide, and Brazil, in this scenario, was responsible, in the 2021/22 harvest, for the production of 144.7 million tons, in about 40 years. Million hectares, with average productivity of 3,026 kg ha-1 (CONAB, 2022). In general, this high productivity is associated with high doses of fertilizers and phytosanitary products, which, however, provide significant increases in production costs and are considered potential environmental pollutants (CHAGAS JUNIOR *et al.*, 2022).

In recent decades, Brazilian production has advanced in using sustainable technologies; however, significant challenges are encountered, especially those associated with climate change (SANTOS *et al.*, 2022). Thus, investment in research, development, and innovation is essential to reduce the intensive use of synthetic inputs and cultural management that negatively impact the environment (SILVA *et al.*, 2020b).

Within this context, using multifunctional microorganisms (MM) improves plant health and productivity due to the ability to act in nutrient cycling and supplementation. In addition, they act in the production of phytohormones such as indole acetic acid (IAA) and inhibit the development and action of pests and pathogens (CARDOSO; ANDREOTE, 2016). The microorganism–plant interaction can promote the growth of living organisms in the system and intensify the plants' tolerance to biotic and abiotic stresses (MORENO *et al.*, 2021).

Recent studies show the beneficial effects of multifunctional microorganisms in increasing shoot dry biomass and productivity of soybean (SILVA *et al.*, 2020b; CHAGAS JUNIOR *et al.*, 2022) and common bean (*Phaseoulus vulgaris*) (REZENDE *et al.*, 2021a); growth promotion of rice (*Oryza sativa* L.) (FERNANDES *et al.*, 2021; PARRALES *et al.*, 2022) and accumulation of N in maize plants (*Zea mays*) (MORENO *et al.*, 2021). In addition, the use of different beneficial microorganisms at the same time, defined as co-inoculation or consortium of microorganisms, has been gaining market share, since it adds different mechanisms of action, producing potential synergistic effects, compared to the use of isolated microorganisms (FLAUZINO *et al.*, 2018).

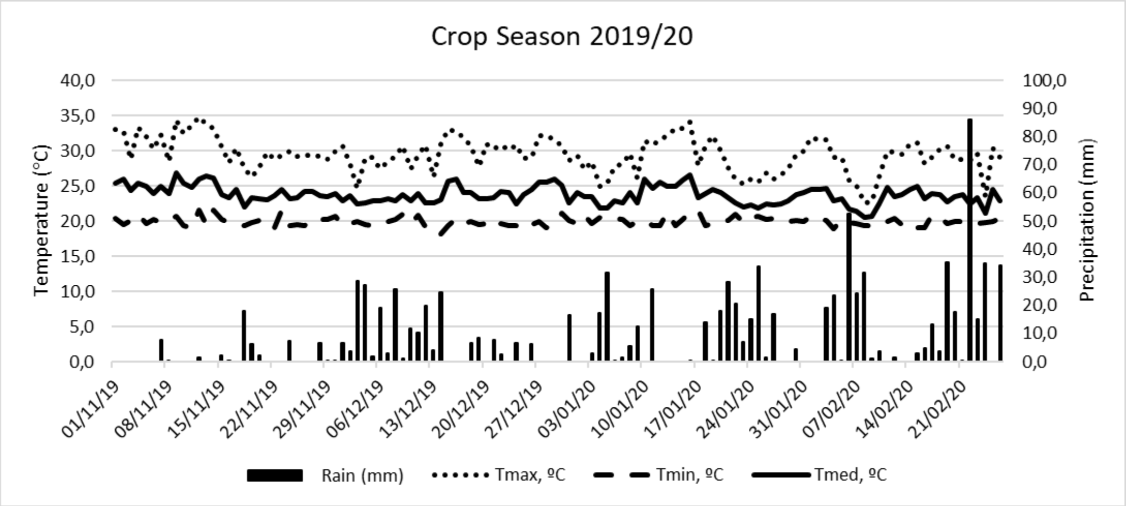
Thus, this study aimed to verify the effect of co-inoculation *Serratia marcescens* (BRM 32114) *+ Bacillus* sp. (BRM63573) on agronomic performance (gas exchange, grain yield, and yield components) of soybean plants.

Material and Methods

The experiment was carried out in the 2019/2020 harvest at the Experimental Station of Embrapa Arroz e Feijão, located in Santo Antônio de Goiás, GO, Brazil, 16º28'00" S and 49º17'00" W coordinates, and at 823 m altitude. The climate is tropical savanna Aw (tropical with wet summers and dry winters) according to the Köppen classification (KÖPPEN, 1918). The average annual precipitation is between 1500 and 1700 mm, and the average annual temperature is 22.7 °C, varying annually from 14.2 °C to 34.8 °C. Additionally, temperature and rainfall data were monitored during the 2019/2020 harvest (Figure 1).

Before implementing the experiment, soil fertility (medium texture latosol) was determined in the 0-0.20 m layers according to the methodology proposed by Teixeira *et al.* (2017), with the following results: pH (H_2O) = 6.3; Ca^{2+} = 18.8 mmolc dm^{-3}; Mg^{2+} = 7.2 mmolc dm^{-3}; H + Al^{3+} = 11 mmolc dm^{-3}; Al^{3+} = 0 mmolc dm^{-3}, P = 3.1 mg dm^{-3}; K^{+} = 142 mg dm^{-3}; Cu^{2+} = 0.6 mg dm^{-3}; Zn^{2+} = 1.5 mg dm^{-3}; Fe^{3+} = 6.7 mg dm^{-3}; Mn^{2+} = 13.8 mg dm^{-3} and organic matter = 31.2 g kg^{-1}.

Figure 1. Temperature and rainfall during the soybean cycle in the 2019/2020 harvest. Santo Antônio de Goiás, Goiás, Brazil.



The experimental design was in randomized blocks, with two treatments and 32 replications. The treatments consisted of the use or not of the consortium of microorganisms in soybean plants. The MM consortium used was *S. marcescens* (BRM 32114) + *Bacillus* sp. *(*BRM63573), and the control treatment was untreated soybean plants. The plots had dimensions of 5 m x 10 m, and the helpful area consisted of the three central lines, disregarding 0.50 m on each side. Co-inoculation of microorganisms (growth promoters) and the inoculant Bradyrhizobium japonicum were applied in the sowing furrow with the aid of the Micron® equipment. *B. japonicum* belongs to the Grap Nodl® formulation and the microorganisms *S. marcescens* (BRM 32114) and *Bacillus* sp. (BRM63573) were collected in upland rice yield. The information in parentheses is the access code to the collection of pathogenic and multifunctional microorganisms of the Embrapa Rice and Beans, and the passport data can be consulted at the link: (https://am.cenargen.embrapa.br/amconsulta/colecao?id =6allelomicro).

Bacterial suspensions of *S. marcescens* (BRM 32114) and *Bacillus* sp. (BRM63573) were prepared separately with nutrient broth, grown for 24 h in solid medium 523 (KADO; HESKETT, 1970), at 28 °C, and the concentration fixed in a spectrophotometer A540 = 0.5 (108 CFU). The broth was kept in a cold chamber, and the microorganisms were mixed in the Micron® chamber on the day of planting, using 300 ml ha^{-1} of each rhizobacteria suspension. For the inoculant *B. japonicum*, the dosage used was 600 ml ha^{-1}, following the manufacturer's recommendation.

Soybean sowing took place on November 2, 2019, using 25 seeds per m², and seedling emergence occurred four days after sowing. The transgenic soybean cultivar, BRS 6970IPRO, early cycle and characterized with tolerance to the herbicide glyphosate and with the intact RR2 PROTM technology was used (EMBRAPA, 2015). The base fertilization, applied in the sowing furrows, was calculated according to the chemical characteristics of the soil according to the recommendations of Sousa; Lobato (2003). Cultural practices to keep the area free from weeds, diseases and insects followed the technical recommendations for soybean cultivation (SEIXAS *et al.*, 2020).

Gas exchange

Measurements of gas exchange were performed at 57 days after the emergence (DAE) of soybean plants, the period corresponding to the full flowering of the crop. Photosynthetic rates (A, µmol CO_2 m^{-2} s^{-1}) and transpiratory (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}) and internal CO_2 concentration (Ci, µmol mol^{-1}) were carried out on the middle third of the upper leaves (completely expanded and with good sun exposure). The evaluations were performed with a portable infrared gas analyzer (LCpro +, ADC BioScientific), from 8:00 am to 10:00 am. The equipment was configured to use concentrations of 370-400 mol-1 CO_2 in the air, and the photosynthetically active photon flux density (PPFD) was equal to 1200 µmol [quanta] m^{-2} s^{-1}. The minimum settling time for reading performance was 2 minutes.

Productivity and production components

The grain yield of the crop was determined by harvesting the soybean mechanically after grain maturation (102 days after sowing), in the useful area of each plot. Ten plants were manually collected within the useful area to determine the number of pods per plant (NVP), the number of grains per pod (NGV), and the weight of 100 grains (M100, g). The yield was determined by harvesting each plot's useful area, with the grains drying (moisture correction to 13%) and expressed in kg ha^{-1}.

Statistical analysis

Data were submitted for analysis of variance, and when the F test detected significance, the means were compared by the LSD test (p ≤0,05). Blocks and all block interactions were considered random effects. The statistical package SISVAR 5.6 was used.

Results and Discussion

The use of co-inoculation of *S. marcescens* (BRM 32114) + *Bacillus* sp. (BRM63573) promoted a significant increase in gas exchange in soybean plants, except for the transpiration rate (Table 1). The increase was 16.65% for photosynthetic rate, 37.50% for stomatal conductance, and 10.36% for an internal concentration of CO_2, compared to control plants. Hormonal substances originating from the metabolism of microorganisms, such as IAA (indole-acetic acid), also plant hormones. As a result, more outstanding production of AIA in the system can directly influence the architecture of the plant's root system and stimulate the formation of lateral roots, which are responsible for the increase in the absorption of nutrients and water (RONDINA *et al.*, 2020). Although this component (IAA content) was not evaluated in our study, the greater robustness of the root system, probably due to the higher production of IAA, may have promoted increases in the nutrient content, photosynthetic efficiency, and productive potential of soybean plants.

Similar results were obtained by Silva *et al.* (2020a), who showed a 75% increase in the photosynthetic rate of soybean plants treated with *Bacillus sp.* and *Pseudomonas sp.*, and Lima *et al.* (2022) who showed a 66.7% increase in the photochemical efficiency of soybean plants inoculated with *Rhizobium* and, thus, demonstrated the nutritional and biochemical benefits arising from the microorganisms.

Table 1. Photosynthetic and transpiration rates, stomatal conductance and internal CO_2 concentration of soybean plants treated with *S. marcescens* (BRM 32114) + *Bacillus* sp. (BRM 63573), 2019/2020 harvest, Santo Antônio de Goiás, Goiás.

Treatments	**A**	**E**	**gs**	**Ci**
Microbial	**µmol CO_2 m $^{-2}$ s $^{-1}$**	**mmol H_2O m^{-2} s $^{-1}$**	**mol H_2O m^{-2} s^{-1}**	**µmol mol^{-1}**
Serratia marcescens (BRM 32114) + *Bacillus* sp. (BRM 63573)	13,31a*	3,04	0,22a	245a
Control (no microorganisms)	11,41b	3,20	0,16b	220b
Factor		ANOVA factors (p value)		
Microorganisms (M)	0,000	0,974	0,000	0,012
CV (%)	15,00	12,75	19,35	16,44

*Means followed by the same letter do not differ by the LSD test (P<0.05).

Soybean plants treated with the microbial consortium *S. marcescens* (BRM 32114) + *Bacillus* sp. (BRM63573) showed a significant increase in grain yield (14.83%) and weight of 100 grains (4.04%) (Table 2). On the other hand, the number of pods per plant (NVP) and the number of grains per pod (NGV) were similar to the control treatment.

The association of bacteria of the genera *S. marcescens* and *Bacillus* sp. proved efficient in promoting benefits to soybean plants. The first belongs to the Enterobacteriaceae family, is facultative gram-negative, and can be isolated from water, soil, plants, and insects. It is important to mention that *S. marcenses*, isolated BRM 32114, is capable of solubilizing non-labile phosphorus and producing siderophores, in addition to synthesizing lytic enzymes such as chitinases and β-1,3-glucanases and, therefore, has a high potential to act as a biological control (FRASCA *et al.*, 2021). The second, *Bacillus* sp. (BRM 63573), belongs to the Bacilliaceae family, currently includes more than 60 species, are gram-positive, endospore formers, and are commonly isolated from soils and plants in anaerobic or aerobic systems. In addition, they promote an increase in the availability of P in the soil, which directly influences the biochemistry and physiology of plants (CHAGAS *et al.*, 2017; SCHWAB; AGUIAR, 2019), with consequent beneficial effects on increased seed germination rate and seedling emergence.

Braga Junior *et al.* (2021) reported the beneficial effect of the application of *B. subtilis* on soybean plants, resulting in productivity increases of 26.6% and 15.4% in the 2015/16 and 2016/17 harvests; in addition to stimulating plant growth through the synthesis of indole acetic acid (IAA) and the solubilization of non-labile phosphorus. Gagné-Bourque *et al.* (2015) observed an acceleration in seed germination and seedling growth rate and an increase in the productivity of soybean plants co-inoculated with *B. distachyon* + *B. subtilis*.

Table 2. The number of pods per plant (NVP), number of grains per pod (NGV), weight of 100 grains (M100), and grain yield of soybean plants treated with *S. marcescens* (BRM 32114) + *Bacillus* sp. (BRM 63573), 2019/2020 harvest, Santo Antônio de Goiás, Goiás.

Treatments	**NVP**	**NGV**	**M100**	**Produtividade**
Microbial	**unit**	**unit**	**g**	**kg ha^{-1}**
Serratia marcescens (BRM 32114) + *Bacillus* sp. (BRM 63573)	84	2	16,73 a	4336 a
Control (no microorganisms)	81	2	16,08 b	3776 b
Factor		ANOVA factors (p value)		
Microorganisms (M)	0,220	0,598	0,002	0,001
CV (%)	11,26	11,06	5,01	13,00

Means followed by the same letter do not differ by the LSD test (p<0.05).

Currently, it is essential to ensure sustainable food production systems and, consequently, the implementation of resilient agricultural practices that increase production and productivity while helping to protect, restore and conserve ecosystem services is necessary. In this sense, the co-inoculation of multifunctional microorganisms, as shown in the present study, is a sustainable technology that acts intrinsically and extrinsically on plants and provides a production increase and stability of the ecological balance (REZENDE *et al.*, 2021b).

Conclusion

The use of the consortium of multifunctional microorganisms (co-inoculation), *S. marcescenss* (BRM 32114) + *Bacillus* sp. (BRM63573), promoted an improvement in the agronomic performance of soybean plants, with an increase of 14.83% in productivity.

The use of this technique is promising due to the increases in plant productivity through a sustainable technology to the production system and the reduction of the use of inputs, minimizing production costs.

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