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Research Article

Inoculation and co-inoculation with multifunctional rhizobacteria for the initial development of soybean[1]

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ABSTRACT

Soybean inoculation and co-inoculation with multifunctional rhizobacteria is a sustainable alternative that may contribute to plant growth and increased agricultural production, making the product more competitive, as well as reducing costs for the producer. This study aimed to evaluate the effect of inoculation and co-inoculation with multifunctional *Serratia* sp. and *Bacillus* sp. rhizobacteria on the early development of soybean. A completely randomized experimental design was used, with four treatments and ten replicates, totaling 40 experimental plots. The treatments consisted of the microbiolization of soybean seeds and a control treatment: BRM 32114 (*Serratia* sp.) isolate; BRM 63573 (*Bacillus* sp.) isolate (formerly named 1301); co-inoculation with BRM 32114 + BRM 63573; and control (without microbiolization). BRM 32114 and BRM 63753, both isolated and combined, were efficient to improve the initial development of soybean seedlings, providing significant effects for most of the analyzed variables (length, total surface, root volume and root, shoot and total biomass), when compared to the control treatment.

KEYWORDS: *Glycine max*, *Bacillus* sp., *Serratia* sp.

RESUMO

Inoculação e coinoculação com rizobactérias multifuncionais para o desenvolvimento inicial da soja

A inoculação e coinoculação da soja por rizobactérias multifuncionais é uma alternativa sustentável que pode contribuir com o crescimento das plantas e com o aumento da produção agrícola, tornando o produto mais competitivo e, ainda, diminuir os custos para o produtor. Objetivou-se avaliar o efeito da inoculação e coinoculação com rizobactérias multifuncionais *Serratia* sp. e *Bacillus* sp. no desenvolvimento inicial da soja. O delineamento experimental foi o inteiramente casualizado, com 4 tratamentos e 10 repetições, totalizando 40 parcelas experimentais. Os tratamentos consistiram na microbiolização das sementes de soja e tratamento controle: isolado BRM 32114 (*Serratia* sp.); isolado BRM 63573 (*Bacillus* sp.) (antes nomeado 1301); coinoculação com BRM 32114 + BRM 63573; e controle (sem microbiolização). BRM 32114 e BRM 63753 isolados e combinados foram eficientes para melhorar o desenvolvimento inicial de plântulas da soja, proporcionando efeitos significativos para a maioria das variáveis analisadas (comprimento, superfície total, volume de raiz e biomassa de raiz, parte aérea e total), quando comparados ao tratamento controle.

PALAVRAS-CHAVE: *Glycine max*, *Bacillus* sp., *Serratia* sp.

INTRODUCTION

Soybean (*Glycine max*) is considered one of the most important products of the Brazilian agribusiness, with an estimated production of 125.47 million tons in the 2021/2022 crop season (Conab 2022). However, high soybean yields are usually associated with the use of high dosages of fertilizers and pesticides, among other factors, which are highly costly and a potential source of environmental pollutants (Chagas Junior et al. 2022). In this context, the use of multifunctional rhizobacteria may be an alternative to reduce the environmental risks caused by the inadequate and sometimes excessive use of agricultural inputs and pesticides, contributing to plant growth and increased agricultural production, making the product more competitive and also reducing production costs (Braga Junior et al. 2018, Diaz et al. 2019).

Multifunctional rhizobacteria (also known as plant growth-promoting rhizobacteria - PGPR) are microorganisms that can live freely in the rhizosphere, form symbiotic associations with plants and colonize the internal tissues of plants

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(endophytic bacteria) (Glick 2012, Wang et al. 2012). These bacteria are known for being able to enhance plant growth, especially in the root system (Kumar et al. 2019, Basu et al. 2021), providing a greater access to nutrients and water (Reddy 2013, Ahemad & Kibret 2014). This growth is stimulated by different mechanisms of action of multifunctional rhizobacteria, such as biological nitrogen fixation - BNF (N), phosphate solubilization (P) (Mehta et al. 2014), production of siderophores (Lakshmanan et al. 2015) and of growth-promoting compounds, such as some phytohormones (auxins, cytokinins and indoleacetic acid - IAA) (Glick 1995), among others.

The effects of these bacteria may be maximized by a technique known as co-inoculation, which consists of inoculating the soybean seed with more than one microorganism, being a new biotechnological tool to improve the soybean development and yield by reducing the use of synthetic inputs, thus contributing to the current sustainable farming practices (Hungria et al. 2017). Silva et al. (2020a) observed a higher production of root and total biomass in soybean plants co-inoculated with the multifunctional rhizobacterial isolates BRM 32114 (*Serratia* sp.) and BRM 63573 (*Bacillus* sp.) (formerly named 1301). However, despite many years of research, there is still a great variability of responses for the use of co-inoculation with multifunctional microorganisms, that is, some results show advantages in relation to inoculation and others do not. In addition, there are questions to be answered regarding the effect of rhizobacteria on the initial development of soybean seedlings.

Therefore, this study aimed to evaluate the effect of inoculation and co-inoculation with the multifunctional rhizobacteria *Serratia* sp. and *Bacillus* sp. in the early development of soybean.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse under controlled conditions, at the Embrapa Arroz e Feijão, in Santo Antônio de Goiás, Goiás state, Brazil (16º28’00”S, 49º17’00”W and altitude of 823 m), in January 2022. The climate of the region is AW (tropical Savanna), according to the Köppen classification, with an average temperature of 23.3 ºC and average rainfall of 1,428 mm.

A completely randomized experimental design was used, with four treatments and ten replicates, totaling 40 experimental plots.

The treatments consisted of the microbiolization of soybean seeds with isolates and a control treatment: BRM 32114 (*Serratia* sp.) isolate; BRM 63573 (*Bacillus* sp.) isolate (formerly named 1301); co-inoculation of BRM 32114 + BRM 63573; and control (without microbiolization). These microorganisms were identified, stored and preserved in the collection of multifunctional organisms of the Embrapa Arroz e Feijão.

Bacterial suspensions for seed microbiolization were prepared with water obtained from cultures grown in solid medium (nutrient agar), being subsequently grown in liquid medium (nutrient broth) (Kado & Heskett 1970) in an incubator under constant stirring, for 24 hours, at 28 ºC. The concentration was fixed at 10^8 CFU (colony forming units) mL^{-1}, determined in a spectrophotometer at A540 = 0.5. Microbiolization was carried out by soaking the soybean seeds of the NS 6906 IPRO cultivar in each respective microorganism suspension, while control seeds were immersed in water. All the treatments were maintained for four hours under constant stirring at 25 ºC (Filippi et al. 2011, Silva et al. 2020a). Twenty seeds of each treatment were used for 100 mL of suspension. In the treatment with co-inoculation, 50 mL of the suspension of each microorganism were used, which were mixed until a homogeneous solution was obtained before adding the soybean seeds.

For each experimental unit, 500 mL plastic cups filled with medium-textured soil from the surface layer (0.00-0.20 m) were used (the soil was not autoclaved and chemical analysis was not performed), without fertilization, where two seeds were sown per cup. Thinning was carried out after seedling emergence, leaving one seedling per cup. At 15 days after sowing, the soil of each cup was washed to remove the root system of soybean seedlings. The removed seedlings were then photographed with a digital camera and the obtained images analyzed using the WinRHIZO 2012 software, Regent Instruments Inc., Quebec City, QC, Canada (Arsenault et al. 1995). The following analyzes were performed using the aforementioned software: total root length (cm), root diameter (mm), total root surface (cm^2) and root volume (cm^3).

Then, the roots and shoots of the soybean seedlings were stored separately in kraft paper bags properly identified, which were transferred to a forced circulation oven at 65 ºC, until reaching constant mass. The root, shoot and total dry matter were obtained using a precision scale (accuracy of 0.0001 g). The data were submitted to analysis of variance and significant means compared by the Fisher's LSD test at 5 % of probability, using the R software, version 3.5.0 (R Core Team 2016). The principal component analysis (PCA) was performed to describe correlations between response variables and treatments. The principal components were used as response variables, when the result of the correlation test produced r ≥ 0.50. Before running the PCA analyses, the data were standardized, so that each variable was kept with zero mean and unit variance (Hair et al 2005). The two-dimensional graphical correlation graphic correlated the isolated bacteria, the co-inoculated bacteria, the control and the response variables through the R statistical software.

RESULTS AND DISCUSSION

In all the treatments with multifunctional rhizobacteria (BRM 32114, BRM 63573 and BRM 32114 + BRM 63573), the plants showed a significantly greater length, total surface and root volume than the control (Table 1). The root length of the seedlings treated with BRM 63573 was 74 % larger than the control, being 62 % larger than the control with the BRM 32114 isolate and co-inoculation. BRM 32114 provided a total root surface 74 % greater and a total volume 83 % larger than the control treatment, followed by the BRM 63573 isolate, with an increase of 70 and 71 %, respectively, and co-inoculation, with 57 and 62 %.

Corroborating the present results, Sousa et al. (2019) studied irrigated rice seedlings treated with the BRM 32114 isolate and found a root length up to 30.4 % higher than in the untreated seedlings (control). The results found in the present study may be partially explained by the ability of these bacteria to produce indoleacetic acid (IAA), which is a phytohormone responsible for promoting root growth and cell elongation (Martins 2015, Taiz et al. 2017). According to Hungria (2011), the ability of multifunctional rhizobacteria to promote a greater root development may lead to several other beneficial effects in plants, such as a greater absorption of water and minerals, resulting in more vigorous and productive plants.

The treatments with inoculation of the BRM 32114 and BRM 63573 rhizobacteria and the treatment with co-inoculation (Table 1) showed the same results in this study, highlighting that co-inoculation was not more efficient than inoculation for the root development of soybean seedlings. However, co-inoculation was significantly superior to the control and may provide other benefits in relation to inoculation, such as greater biological nitrogen fixation and yields, which were not evaluated in this study. It is expected that this technique would potentiate the beneficial effects of these microorganisms in plants, that is, that the productive results obtained with co-inoculation exceed those obtained with the use of bacteria in isolation (Ferlini 2006, Bárbaro et al. 2008).

Regarding biomass production, all treatments were superior to the control for root, shoot and total biomass (Table 2). For root biomass, co-inoculation showed the greatest increase when compared to the control (45 %), followed by BRM 63573 (36 %) and BRM 32114 (35 %). The treatment using BRM 32114 was the one that provided the highest shoot biomass production, being significantly superior to the control (66 %), followed by BRM 63573 (44 %) and co-inoculation (25 %). For total biomass, the treatment with BRM 32114 was also the one with

Table 1. Total root length, root diameter, total root area and root volume of soybean seedlings (NS 6906 IPRO cultivar) as a function of treatments with rhizobacteria, at 15 days after sowing.

Treatments	Root length	Root diameter	Total root area	Root volume
	cm	mm	cm^2	cm^3
BRM 32114 (*Serratia* sp.)	41.61 a*	1.14 a	155.65 a	0.44 a
BRM 63573 (*Bacillus* sp.)	44.88 a	1.07 a	151.64 a	0.41 a
BRM 32114 + BRM 63573	41.61 a	1.06 a	139.96 a	0.39 a
Control	25.73 b	1.10 a	89.34 b	0.24 b

* Means followed by the same letter in the column do not differ by the Fisher's LSD test at 5 % of significance, using the R statistical software.

Table 2. Root, shoot and total dry matter of soybean seedlings (NS 6906 IPRO cultivar) as a function of treatments with rhizobacteria, at 15 days after the sowing.

Treatments	Root dry matter	Shoot dry matter	Total dry matter
		g	
BRM 32114 (*Serratia* sp.)	0.0473 a*	0.2008 a	0.2481 a
BRM 63573 (*Bacillus* sp.)	0.0477 a	0.1734 ab	0. 2211 a
BRM 32114 + BRM 63573	0.0510 a	0.1535 b	0.2046 a
Control	0.0351 b	0.1208 c	0.1559 b

* Means followed by the same letter in the column do not differ by the Fisher's LSD test at 5 % of significance, using the R statistical software.

the highest increase, when compared to the control (59 %), followed by BRM 63573 (42 %) and co-inoculation (31 %). However, treatments with the isolated and co-inoculated microorganisms were similar for root and total biomass, and co-inoculation showed significantly lower results than BRM 32114 for shoot biomass.

As observed in this study, Silva et al. (2020b) found higher root and total biomass production in soybean plants inoculated with BRM 63573 and co-inoculated with BRM 32114 and BRM 63573. However, unlike in our results, these authors did not find significant increases in shoot biomass, when compared to the control (without microorganisms). Braga Júnior et al. (2018) observed that the soybean inoculation with *Bacillus subtilis* increased the shoot and root dry biomass of soybean plants.

Similarly to the present study, authors such as Gitti et al. (2012), Zuffo et al. (2015) and Zuffo et al. (2016) also did not observe a significant response to the use of co-inoculation, when compared to inoculation, regarding plant development. However, this technique may bring other benefits, such as greater biological nitrogen fixation and phosphorus solubilization, among others, which may reflect in higher soybean yields (Hungria et al. 2013).

Regarding the principal component analysis, it was verified that the variability of the treatments with the isolated (BRM 32114 and BRM 63573) and combined (BRM 32114 + BRM 63573) rhizobacteria in the analyzed variables (length, total surface, diameter, root volume and shoot, root and total biomass) were best described by two principal components (PC) representing 99 % of the data variation, that is, PC1 (79.5 %) and PC2 (19.5 %) (Figure 1).

The factor map (biplot) shows groups of variables (arrows) indicating positive and negative

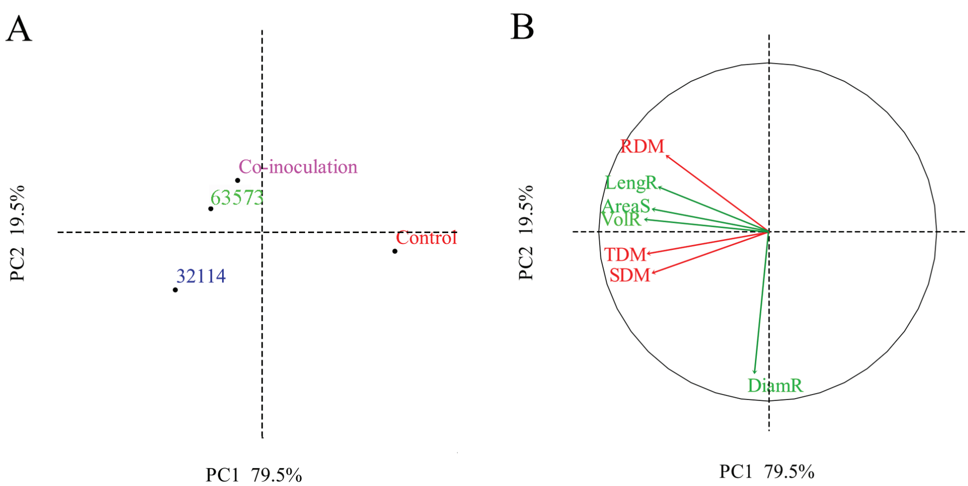

Figure 1. Principal component analysis (PCA) of PC1 x PC2 explaining the correlations between evaluated variables and four treatments. A) BRM 63573 (*Bacillus* sp.), BRM 32114 (*Serratia* sp.), co-inoculation (BRM 63573 + BRM 32114) and control treatment; B) variables or relationship circle. RDM: root dry matter (g); TDM: total dry matter (g); SDM: shoot dry matter (g); LengR: total root length (cm); AreaS: total root surface (cm^2); VolR: root volume (cm^3); DiamR: root diameter (mm).

correlations with each principal component (PC), where the arrow length indicates the magnitude of each response for each PC (Figure 1). For example, PC1 was not positively correlated with any of the analyzed variables. On the other hand, PC2 was positively correlated with root length, total surface, total volume and root dry matter, while being negatively correlated with the other variables.

According to the representational quality of the treatments with isolated and combined microorganisms for the analyzed variables, BRM 63573 and co-inoculation (BRM 32114 + BRM 63753) presented the highest positive correlation for length, total surface, root volume and root dry matter (Figure 1). BRM 32114 was positively correlated with root diameter, shoot dry matter and total dry matter. The control treatment (without rhizobacteria) was not positively correlated with any of the analyzed variables.

Thus, based on the results of the present study, it can be concluded that the inoculation and co-inoculation of the soybean crop with the multifunctional rhizobacteria isolates BRM 32114 and BRM 63753 are efficient to improve the root development of seedlings, providing a greater root length, total surface and volume, as well as a higher biomass production (Figure 1). It can also be observed that the evaluated isolates did not differ from each other, as well as that inoculation and co-inoculation were significantly similar for all the analyzed variables.

The results found in this paper are very relevant, since a greater root development (Figure 2) allows plants to explore a greater soil volume, thus providing a greater access to water and nutrients from the soil, what may directly reflect on higher yields. In addition, this study is also important from a commercial point of view, since agricultural companies are looking for multifunctional microorganisms with potential to improve plant development and possibly agricultural production, especially for a crop of great economic interest such as soybean. Moreover, the use of multifunctional rhizobacteria is a sustainable technology that does not degrade the environment and reduces the use of synthetic inputs, besides having a low cost (Braga Junior et al. 2018, Diaz et al. 2019). Therefore, studies on this subject are very important to contribute to a more sustainable and productive agriculture.

Photos: Mariana Aguiar Silva

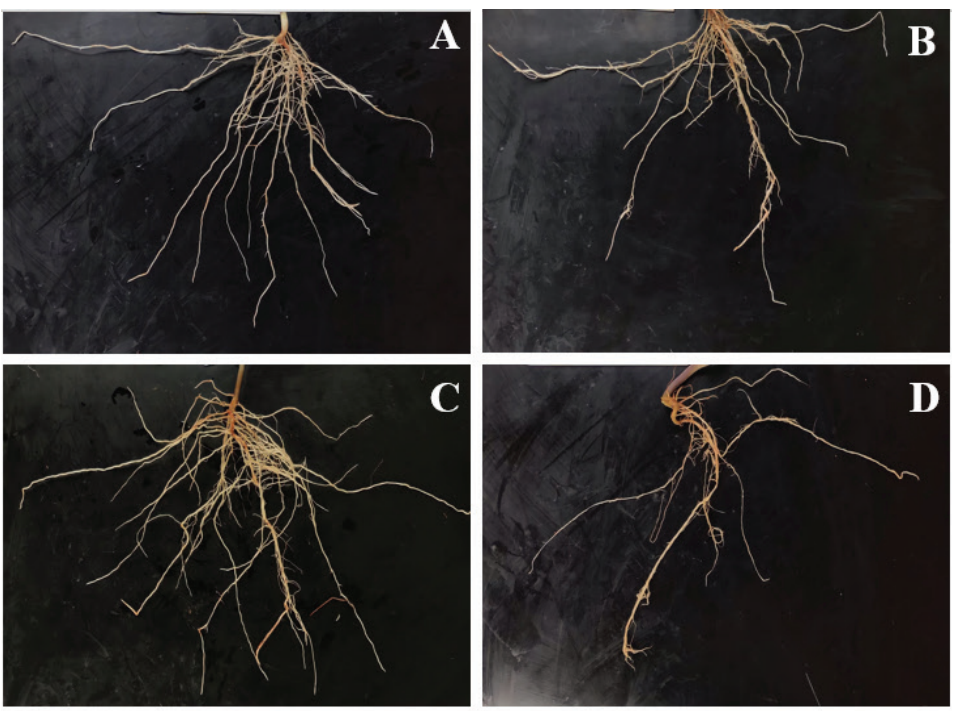

Figure 2. Root system of soybean seedlings submitted to microbiolization with multifunctional rhizobacteria. A) BRM 32114 (*Serratia* sp.); B) BRM 63753 (*Bacillus* sp.); C) co-inoculation (BRM 32114 + BRM 63753); D) control (without microbiolization). The soybean seedlings were photographed at 15 days after sowing with a digital camera and the images analyzed using the WhinRHIZO 2012 software.

CONCLUSION

The multifunctional rhizobacteria BRM 32114 (*Serratia* sp.) and BRM 63753 (*Bacillus* sp.) isolates, both isolated and combined, are efficient to improve the initial development of soybean seedlings, providing significant effects for length, total surface, root volume and root, shoot and total biomass.

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