Scientia Agraria Paranaensis – Sci. Agrar. Parana.
ISSN: 1983-1471 – Online
DOI: https://doi.org/10.18188/sap.v20i4.28462

INOCULATION OF GROWTH-PROMOTING BACTERIA IN THE LETTUCE CROP

Fabiana Ribeiro Machado[1*], Fabio Ribeiro Machado[2], Vandeir Francisco Guimarães[1], Márcia de Moraes Echer[1]

SAP 28462 Received: 23/11/2021 Accepted: 01/03/2022
Sci. Agrar. Parana, Marechal Cândido Rondon, v. 20, n. 4, oct./dec., p. 395-404, 2021

ABSTRACT - Plant growth promoting bacteria (PGPB) are an alternative to increase the productivity of several crops, including lettuce. Several reports are found in the literature with the use of plant growth-promoting bacteria in lettuce cultivation, but most seek increments during the production of seedlings. Aiming to provide increments in the cultivation of lettuce in the field and to generate subsidies to technicians and producers, the present work aimed to study the influence of the application of commercial products based on *Azospirillum brasilense*, *Bacillus subtilis* + *B. megaterium* and *Pseudomonas fluorescens*, on agronomic characteristics and quality of crisp lettuce. The experiment was implemented in two locations, in a randomized block design, in a 4 x 3 factorial scheme, with four replications. The first factor refers to the application of different PGPB: control, without inoculation; inoculations with *A. brasilense*; *B. subtilis* + *B. megaterium* and *P. fluorescens*. The second factor was composed of three times of application of PGPB, the first being on the day of seedling transplantation (DAT), the second at 15 STR and the third on the day of seedling transplantation + 15 DAT. At 15 DAT, the relative chlorophyll content, plant height, head diameter, number of leaves per plant, leaf area, leaf dry mass, stem dry mass, root dry mass, total dry mass, root volume were evaluated. and root length. At harvest, the same variables analyzed at 15 DAT + leaf fresh mass, stem fresh mass, head fresh mass, root fresh mass, total fresh mass and leaf contents of nitrogen, phosphorus and potassium were analyzed. The inoculated plant growth-promoting bacteria did not promote a significant increase in the development of the lettuce cultivar Vera when compared to the control (without PGPB application).
Keywords: *Lactuca sativa* L., *Azospirillum brasilense*, *Bacillus subtilis*, *Pseudomonas fluorescens*.

INOCULAÇÃO DE BACTÉRIAS PROMOTORAS DE CRESCIMENTO NA CULTURA DA ALFACE

RESUMO - Na literatura são encontrados vários relatos com a utilização de bactérias promotoras de crescimento vegetal na cultura da alface, porém a maioria busca incrementos durante a produção de mudas. Visando proporcionar incrementos na cultura da alface a campo e gerar subsídios a técnicos e produtores, o presente trabalho teve como objetivo estudar a influência da aplicação de produtos comerciais a base de *Azospirillum brasilense*, *Bacillus subtilis* + *B. megaterium* e *Pseudomonas fluorescens*, sobre características agronômicas e qualidade da alface crespa. O experimento foi implantado em dois locais, em delineamento experimental de blocos ao acaso, em esquema fatorial 4 x 3, com quatro repetições. O primeiro fator, refere-se à aplicação de diferentes BPCV: controle, sem inoculação; inoculações com *A. brasilense*; *B. subtilis* + *B. megaterium* e *P. flurescens*. O segundo fator, foi composto por três épocas de aplicação das BPCV, sendo a primeira no dia do transplante das mudas (DAT), a segunda aos 15 DAT e a terceira no dia do transplante das mudas + 15 DAT. Aos 15 DAT foram avaliados o teor relativo de clorofila, altura de planta, diâmetro da cabeça, número de folhas por planta, área foliar, massa seca de folhas, massa seca de caule, massa seca de raiz, massa seca total, volume de raiz e comprimento de raiz. Na colheita foram analisadas as mesmas variáveis analisadas aos 15 DAT + massa fresca de folhas, massa fresca de caule, massa fresca da cabeça, massa fresca de raiz, massa fresca total e teores foliares de nitrogênio, fósforo e potássio. As bactérias promotoras de crescimento vegetal inoculadas não promoveram incremento significativo no desenvolvimento da cultura da alface cultivar Vera quando comparados ao controle (sem aplicação de BPCV).
Palavras-chave: *Lactuca sativa* L., *Azospirillum brasilense*, *Bacillus subtilis*, *Pseudomonas fluorescens*.

INTRODUCTION

The lettuce (*Lactuca sativa* L.) is considered the main leafy vegetable crop in Brazil, being cultivated in different ways, such as conventional, organic and hydroponic, and consumed mainly *in natura*. As it is a vegetable crop with a short cycle and a superficial root system, it has a high demand for nutrients, especially nitrogen, being supplied via chemical fertilization, through sources such as urea and ammonium sulfate (FILGUEIRA, 2013).

Several microorganisms can be found in the soil, and they can be divided according to the influence they can

[1]Western Paraná State University (Unioeste), Marechal Cândido Rondon *Campus*, Marechal Cândido Rondon, PR, Brazil. E-mail: fabrm2015@gmail.com. *Corresponding author.
[2]Cooperativa Agroindustrial de Cascavel (Coopavel), PR, Brazil.

cause on plants, being harmful, beneficial and neutral. Among the microorganisms characterized as beneficial, there are plant growth-promoting rhizobacteria (BRAGA JUNIOR, 2015).

The search for technologies that reduce the application of mineral fertilizers and, therefore, also reduce the economic-environmental costs generated by nitrogen fertilization, has generated more and more research with atmospheric nitrogen-fixing bacteria, such as, for example, their inoculation in seeds and plants in order to obtain greater crop increments (LOZADA et. al., 2018). Thus, inoculation with plant growth-promoting and nitrogen-fixing bacteria can be an alternative to reduce these costs (AGUIRRE et. al., 2018). In addition, biological nitrogen fixation enables the development of more sustainable agriculture, reducing damage to the environment and increasing product quality. The interactions between PGPB and plant roots promote mobilization and absorption of nutrients present in the soil solution, resulting in an increase in crop productivity (MANHÃES, 2014).

In horticulture, the use of PGPB has the potential to increase production, with strains belonging to the genera *Azospirillum*, *Bacillus* and *Pseudomonas* gaining prominence. Within the genus *Azospirillum*, the species *Azospirillum brasilense* stands out, strains AbV5 and AbV6, which have the ability to increase nitrogen fixation and produce phytohormones that stimulate the development and growth of roots, thus increasing the area of absorption of water and nutrients. (NITROMIL, 2021). *Azospirillum brasilense* can confer greater tolerance of lettuce to the viruses of the *Tospovirus* group, the main agent causing plant death, and the use of *A. brasilense* associated with a rooting agent, attenuates the effects of the leaf miner at lower doses of N in topdressing (LIMA et al., 2017).

As for the genus *Bacillus*, the strains BRM 119 (*B. megaterium*) and BRM 2084 (*B. subtilis*) stand out, which colonize the rhizosphere of the plant, producing organic acids that act in the portion of the soil, which is in contact with the plant roots. Thus, the process of phosphorus solubilization begins, retained in the calcium, aluminum and iron present in the soil, leaving it readily available for absorption and assimilation by the plant. In this way, they act on the mineralization of phosphorus present in soil organic matter. In the genus Pseudomonas, one of the most used species is P. fluorescens, whose main function is to solubilize phosphorus retained in the soil, in addition to increasing nodulation and reducing pathogens (FERTIBIO, 2021).

The adoption of the practice of inoculation of PGPB in the lettuce crop would be a way to improve its development, agronomic characteristics, productivity gains, visual and chemical aspects and organoleptic characteristics of the product. In this context, this study aimed to verify the influence of *Azospirillum brasilense*, *Bacillus subtilis* + *Bacillus megaterium* and *Pseudomonas fluorescens*, on the agronomic characteristics and quality of curly lettuce Vera cultivar.

MATERIAL AND METHODS

The experiment was conducted in the field and divided into two trials, one being carried out at the Experimental Farm "Professor Doutor Antônio Carlos dos Santos Pessoa", geographical location 24°31'58"S 54°01'16"W, in the period from 30 November 2019 to January 12, 2020, belonging to the State University of West Paraná (Unioeste), Campus Marechal Cândido Rondon (PR). The other trial was conducted in an area provided by the São Maximiliano Maria Kolbe Seminary, geographical location 24°58'88"S 53°29'09"W from July 11 to August 23, 2020, located in the municipality of Cascavel (PR).

The soil of the microregion where the municipalities where the experiments were implemented is classified as Eutroferric RED LATOSOL, with a very clayey texture (SANTOS et al., 2018). The climate of the region is classified, according to Köppen, type Cfa, mesothermic (humid subtropical), with hot summers, tendency of rain concentration and average temperature above 22°C, winters with little possibility of frost and average temperature below 18°C.

The experimental design used was randomized blocks, in a 4 x 3 factorial scheme, with four replications, totaling 48 plots. The first factor refers to the application of different PGPB: control, without inoculation; inoculations with *A. brasilense*, *B. subtilis* + *B. megaterium* and *P. fluorescens*. The second factor was composed of three times of application of PGPB, the first being on the day of seedling transplantation (DAT), the second at 15 DAT and the third on the day of seedling transplantation + 15 DAT. Of the 48 plots, 16 plots consisted of four planting lines, with 2.1 m in length and 0.30 m spacing between plants, totaling 28 plants. In these plots, analyzes were performed fifteen days after seedling transplant + harvest. The other plots consisted of four planting lines, with 1.2 m in length and 0.3 m spacing between plants, totaling 16 plants. The spacing between each plot was 0.6 m and between the blocks, 1.0 m.

The treatments consisted of: T1 = control on the day of transplantation (control, without application of PGPB), T2 = strains of *Azospirillum brasilense* applied on the day of transplantation, T3 = strains of *A. brasilense* applied 20 days after transplantation, T4 = strains of *A. brasilense* applied on the day of transplantation + 15 days after transplantation, T5 = no application of PGPB on the day of transplantation + 15 days after transplantation, T6 = *Bacillus subtilis* + *B. megaterium* applied on the day of transplantation, T7 = *B .subtilis* + *B. megaterium* applied 15 days after transplantation, T8 = *B. subtilis* + *B. megaterium* applied on the day of transplantation + 15 days after transplantation, T9 = *Pseudomonas fluorescens* applied on the day of transplantation, T10 = *P. fluorescens* applied 15 days after transplantation, T11 = *P. fluorescens* applied on the day of transplantation + 15 days after transplantation and T12 = no application of PGPB on the day of transplantation + 15 days after transplantation.

The seedlings used were acquired from a reputable seedling producer in the city of Cascavel-PR and used Vera cultivar, which presents resistance to early flowering due to high temperatures and were transplanted when they had 4 to 6 definitive leaves.

The commercial inoculants used were *Azospirillum brasilense*, strains AbV5 and AbV6, *Bacillus subtilis* (CNPMS B2084 (BRM034840)) and *Bacillus megaterium* (CNPMS B119 (BRM033112)) and *Pseudomonas fluorescens*.

As base fertilization, 523.8 kg ha^{-1} of Super Simples, 116.6 kg ha^{-1} of potassium chloride, 88.8 kg ha^{-1} of urea and 8.7 kg ha^{-1} were applied. 1 of borax, for all treatments and in both assays. For the topdressing fertilization, 66.6 kg ha^{-1} of urea was applied 20 days after the seedlings were transplanted. Fertilization was carried out as recommended by the Fertilization and Liming Manual for the state of Paraná (SBCS/NEPAR, 2017).

The application of the commercial inoculants tested was performed manually, being applied to the seedlings still in the polyethylene trays of 200 cells, with the aid of a precision pipette in each of the seedlings. For application at 15 days after transplanting the seedlings, a backpack sprayer propelled with CO_2 was used. The inoculant dose applied on the day of seedling transplantation and 15 days after transplantation was 0.1 L ha^{-1} of each formulation of commercial inoculants used, as recommended by the manufacturer for registered cultures.

Irrigation was performed daily, in the late afternoon (around 5:00 pm) from transplant to harvest, by micro-sprinkler in the municipality of Marechal Cândido Rondon and by drip in Cascavel, available at the place where the tests were carried out. Weed management was performed manually. There was no need to carry out pest and disease control, due to their non-appearance.

Chlorophyll content (CC) was evaluated using a Minolta® chlorophyll meter, model Spad-502 Plus, every 7 days, from the day of seedling transplant until the day of harvest. Measurements were performed in the afternoon, before irrigation. For these determinations, measurements were carried out at three points per leaf, in three leaves of the middle third per plant, being carried out in the four central plants of each plot.

Plant height (HEI, cm), plant head diameter (HDIA, cm), number of leaves per plant (NLP), plant leaf area (ARF, cm^2), root length per plant (RL, cm), root volume (RV), fresh plant leaf biomass (FLB, g), fresh plant stem biomass (FPSB, g), fresh plant head biomass (FPHB, g), fresh plant root biomass (FRB, g) and total fresh plant biomass (TFB, g), plant head dry biomass (PHDB, g), plant leaf dry biomass (LDB, g), plant stem dry biomass (SDB, g), plant root dry biomass (RDB, g), total dry biomass per plant (TDBP, g) and foliar analysis for nitrogen content (N, g Kg^{-1} of dry mass), phosphorus (P, g Kg^{-1} of dry mass) and potassium (K, g Kg^{-1} of dry mass) absorbed.

On the 15th day after transplanting the seedlings, CC, HDIA, NLP, ARF, RL, RV, FLB, FPSB, FPHB, FRB and TFB were analyzed. The harvest was carried out 42 days after transplanting the seedlings and the following evaluations were performed: CC, HDIA, NLP, ARF, RL, RV, FLB, FPSB, FPHB, FRB and TFB absorbed leaf N, P, K contents.

Plant height was measured from the ground to the apex of the plants individually, in 4 random plants per plot. The head diameter was measured in two directions and the circumference area in cm² was then calculated. The number of leaves was determined by manually detaching them from the plant with subsequent counting. To determine the leaf area, a measuring device (model Li 3100C) was used. The root length was determined from one end of the root to the other and the root volume was determined with the displacement of water in a graduated cylinder (50 mL).

To determine fresh biomass, each of the materials analyzed were weighed on a Quanta digital scale, with three places after the comma. As for the dry biomass, the materials were deposited in kraft paper bags and dried in a drying oven with air circulation/renewal, at a temperature of 65°C for 72 h, until constant weight. After this period, the materials were weighed on a Marte® precision digital scale, with three places after the decimal point.

Leaf analyzes to determine the nutritional levels of N, P and K present in the leaves were performed at the Primorlab laboratory, located in Assis Chateaubriand (PR). The data obtained were tabulated and statistically analyzed using the Genes program, and the averages were compared using the Tukey test, at a 5% error probability.

RESULTS AND DISCUSSION

Table 1 shows the values relative to the averages for chlorophyll levels at 21, 28, 35 days after transplanting the seedlings (DAT) and in the harvest of the plants (CLT) at 42 DAT, with the inoculation of plant growth-promoting bacteria (PGPB) and application time for the municipalities of Marechal Cândido Rondon and Cascavel, both in the state of Paraná. It can be observed that there was no difference regarding the chlorophyll content and in the harvest of the plants for any of the PGPB application time. Almeida (2016) in a study with inoculation of *Azospirillum* in iceberg lettuce seedlings, observed that, for total chlorophyll, the treatment that received *Azospirillum* sp. (NC6+NC8) did not differ statistically from the treatment that received *Azospirillum* sp. (NC6+NC8) x *A. brasilense* (AbV-5+AbV-6), however, the interaction showed to reduce the efficiency of the chlorophyll content.

It can be observed in Table 2 that there was no significant response for chlorophyll content, plant height, head diameter, number of leaves and leaf area at 15 DAT of lettuce seedlings, in the two studied municipalities. In the experiment carried out in the municipality of Marechal Cândido Rondon, it can be observed that, even with no significant response to the analyzed variables, there was a reduction in the values of CC, NLP and ARF, when compared to the other treatments, with the inoculation of *Azospirillum brasilense*.

TABLE 1 - Chlorophyll content with the application of plant growth-promoting bacteria (PGPB), at 21, 28, 35 days after transplanting the lettuce seedlings (DAT) and at harvesting the plants (CLT) at 42 DAT, in the municipalities of Marechal Cândido Rondon and Cascavel (PR).

PGPB	Marechal Cândido Rondon (PR)			
	21 DAT	28 DAT	35 DAT	CLT
No inoculation	17.85 ns	20.30 a	15.42 ns	12.07 ns
Azospirillum brasilense	18.22 ns	18.38 a	16.27 ns	12.63 ns
Bacillu subtilis + B. megaterium	18.42 ns	18.64 a	15.69 ns	12.46 ns
Pseudomonas fluorescens	18.67 ns	17.40 a	15.12 ns	12.29 ns
Inoculation times				
DT	18.33 ns	18.79 ns	12.24 ns	15.52 ns
15 DAT	18.03 ns	18.72 ns	12.47 ns	15.76 ns
DT + 15 DAT	18.50 ns	18.54 ns	12.39 ns	15.60 ns
Averages	18.29	18.68	12.36	15.63
CV(%)	8.64	11.35	5.73	9.06
	Cascavel (PR)			
No inoculation	16.62 ns	18.95 ns	18.77 ns	17.67 ns
Azospirillum brasilense	17.42 ns	18.40 ns	19.35 ns	18.19 ns
Bacillu subtilis + B. megaterium	17.26 ns	18.82 ns	18.72 ns	17.87 ns
Pseudomonas fluorescens	16.98 ns	18.81 ns	18.75 ns	17.85 ns
Inoculation times				
DT	17.18 ns	18.66 ns	18.91 ns	17.80 ns
15 DAT	17.18 ns	18.99 ns	19.01 ns	18.04 ns
DT + 15 DAT	16.85 ns	18.59 ns	18.77 ns	17.84 ns
Averages	18.74	18.90	17.64	17.07
CV(%)	6.32	8.04	7.13	4.63

*Equal lowercase letters do not differ from each other in the column, by Tukey's Test, at 5% error probability. DT = day of transplanting lettuce seedlings, ns = not significant.

TABLE 2 - Chlorophyll content (CC), plant height (HEI, cm), plant head diameter (HDIA, cm), number of leaves per plant (NLP) and plant leaf area (ARF, cm²) of lettuce, with the application of plant growth-promoting bacteria (PGPB), 15 days after transplanting (DAT), in the municipalities of Marechal Cândido Rondon and Cascavel (PR).

PGPB	Marechal Cândido Rondon (PR)				
	CC	HEI	HDIA	NLP	ARF
No inoculation	16.32 ns	7.72 ns	12.17 ns	7.27 ns	114.90 ns
Azospirillum brasilense	14.77 ns	7.47 ns	11.65 ns	4.22 ns	100.37 ns
Bacillu subtilis + B. megaterium	16.12 ns	7.15 ns	12.12 ns	7.42 ns	131.14 ns
Pseudomonas fluorescens	16.40 ns	7.15 ns	11.67 ns	7.60 ns	118.81 ns
Averages	15.91	7.37	11.90	7.38	116.31
CV(%)	10.41	6.47	9.15	9.61	21.06
	Cascavel (PR)				
No inoculation	17.97 ns	6.66 ns	11.31 ns	7.75 ns	92.95 ns
Azospirillum brasilense	18.57 ns	6.34 ns	10.50 ns	7.31 ns	75.70 ns
Bacillu subtilis + B. megaterium	17.80 ns	6.47 ns	10.34 ns	7.50 ns	73.45 ns
Pseudomonas fluorescens	17.82 ns	6.44 ns	11.00 ns	7.69 ns	80.43 ns
Averages	18.04	6.48	10.79	7.56	80.63
CV(%)	6.80	7.59	5.82	7.35	20.28

*ns = not significant.

Ferreira et al. (2011), studying the lettuce culture, observed an increase in the length of the aerial part of the seedlings, when *Bacillus subtilis* was applied to the substrate, at a concentration of 1.0%. In their study on growth-promoting microorganisms in lettuce, Domingues et al. (2021), also did not observe significant difference for number of leaves, leaf area, fresh biomass of the aerial part and dry biomass of the total aerial part, with application of *Trichoderma* spp., B. *subtilis* and *Azospirillum brasilense*.

Table 3 shows the average values of leaf dry biomass, stem dry biomass, root dry biomass, total dry biomass, root volume and root length, in inoculation with PGPB at 15 DAT of lettuce seedlings, in the two studied municipalities. It can be seen in Table 3 that lettuce plants that were not inoculated with any bacteria had a higher average stem dry biomass, when compared to those that received inoculation. Statistically, this treatment was superior only in the treatment that received inoculation with *Pseudomonas fluorescens*, not differing from the other treatments that received *Bacillus subtilis* + B. *megaterium* and *Azospirillum brasilense*. Although the plants that received inoculation with *Pseudomonas fluorescens* were inferior to those that did not receive the application of PGPB, there was no significant difference for this variable.

TABLE 3 - Plant leaf dry biomass (LDB, g), plant stem dry biomass (SDB, g), plant head dry biomass (PHDB, g), plant root dry biomass (RDB, g), total plant dry biomass (TDB, g), root volume (RV) and root length (RL) of lettuce plant, with the application of plant growth-promoting bacteria (PGPB), at 15 DAT, in Marechal Cândido Rondon and Cascavel (PR).

PGPB	Marechal Cândido Rondon (PR)					
	LDB	SDB	RDB	TDB	RV	RL
No inoculation	0.575 ns	0.039 a*	0.038 ab	0.653 ns	0.620 ns	8.722 ns
Azospirillum brasilense	0.504 ns	0.034 ab	0.034 b	0.572 ns	0.565 ns	8.915 ns
Bacillu subtilis + B. megaterium	0.499 ns	0.033 ab	0.038 ab	0.569 ns	0.610 ns	8.295 ns
Pseudomonas fluorescens	0.446 ns	0.028 b	0.043 a	0.517 ns	0.675 ns	9.885 ns
Averages	0.506	0.033	0.038	0.578	0.617	8.954
CV(%)	16.82	9.97	8.67	15.53	27.58	8.86
	Cascavel (PR)					
No inoculation	0.34 ns	0.02 ns	0.05 ns	0.40 ns	0.89 ns	10.19 ns
Azospirillum brasilense	0.29 ns	0.01 ns	0.05 ns	0.35 ns	0.83 ns	9.89 ns
Bacillu subtilis + B. megaterium	0.27 ns	0.01 ns	0.04 ns	0.33 ns	0.84 ns	9.71 ns
Pseudomonas fluorescens	0.29 ns	0.01 ns	0.04 ns	0.34 ns	0.70 ns	9.98 ns
Averages	0.30	0.01	0.04	0.36	0.82	9.94
CV(%)	18.91	38.01	10.74	17.67	14.59	7.32

*Equal lowercase letters do not differ from each other in the column, by Tukey's Test, at 5% error probability. ns = not significant.

In the experiment carried out in Marechal Cândido Rondon, the root dry biomass of lettuce plants presented a significant result and a significant difference between those that received the inoculation of *Pseudomonas fluorescens* and *Azospirillum brasilense*. For the other variables, such as leaf dry biomass, total dry biomass, root volume and root length, no significant difference was observed. The same occurred in the lettuce experiment in Cascavel (PR). Cipriano et al. (2013), in a study with hydroponic lettuce, found greater root length, shoot dry biomass and root dry biomass, with the inoculation of *Pseudomonas* spp. of the fluorescent group. The same authors attributed these results to the production of metabolites by these microorganisms, which possibly benefited the growth of lettuce plants.

Table 4 shows the averages for chlorophyll content, plant height, head diameter, number of leaves per plant and leaf area per lettuce plant, inoculated with PGPB, at the times of application for the municipalities studied. Despite having presented a statistical difference for the number of leaves per lettuce plant, in the Cascavel test, the averages obtained in Tukey’s test, at 5% error probability, did not differ from each other, that is, there was no difference statistics between treatments that received inoculation with PGPB.

Almeida (2016) in their research with nitrogen application and inoculation of *Azospirillum brasilense* in iceberg lettuce did not show a significant response regarding the leaf area of the plants. Evaluating the effect of Pseudomonas inoculation in corn. As mentioned, Ferreira et al. (2011) observed an increase in the length of the aerial part of lettuce seedlings when *Bacillus subtilis* was applied to the substrate at a concentration of 1.0%. Domingues et al. (2021), in a study on growth-promoting microorganisms in lettuce, found no significant difference for number of leaves, leaf area, fresh shoot biomass and total shoot dry biomass, with the application of *Trichoderma* spp., *Bacillus subtilis* and *Azospirillum brasilense.*

TABLE 4 - Chlorophyll content (CC), plant height (HEI, cm), plant head diameter (HDIA, cm), number of leaves per plant (NLP) and plant leaf area (ARF, cm2) of lettuce, with the application of plant growth promoting bacteria (PGPB), at different times, in the municipalities of Marechal Cândido Rondon and Cascavel (PR).

PGPB	Marechal Cândido Rondon (PR)				
	CC	HEI	HDIA	NLP	ARF
No inoculation	12.07 ns	19.38 ns	31.44 ns	40.37 ns	3886.41 ns
Azospirillum brasilense	12.63 ns	19.48 ns	31.81 ns	40.77 ns	3784.94 ns
Bacillu subtilis + B. megaterium	12.46 ns	19.41 ns	31.81 ns	40.52 ns	3867.81 ns
Pseudomonas fluorescens	12.29 ns	19.27 ns	31.89 ns	40.02 ns	3713.51 ns
Inoculation times					
DT	12.24 ns	19.47 ns	31.36 ns	39.55 ns	3700.25 ns
15 DAT	12.47 ns	19.45 ns	31.87 ns	41.50 ns	3925.34 ns
DT + 15 DAT	12.39 ns	19.23 ns	31.98 ns	40.22 ns	3813.92 ns
Averages	12.36	19.38	31.74	40.42	3813.17
CV(%)	5.73	5.21	3.51	9.51	10.35
	Cascavel (PR)				
No inoculation	17.67 ns	23.44 ns	31.12 ns	43.31 a*	4646.82 ns
Azospirillum brasilense	18.19 ns	23.12 ns	30.98 ns	41.02 a	4310.01 ns
Bacillu subtilis + B. megaterium	17.87 ns	22.23 ns	30.65 ns	38.83 a	4019.19 ns
Pseudomonas fluorescens	17.85 ns	22.73 ns	30.54 ns	39.69 a	4350.48 ns
Inoculation times					
DT	17.80 ns	23.18 ns	30.91 ns	41.89 ns	4494.80 ns
15 DAT	18.04 ns	22.92 ns	30.73 ns	40.30 ns	4430.01 ns
DT + 15 DAT	17.84 ns	22.53 ns	30.83 ns	39.95 ns	4076.81 ns
Averages	17.89	22.88	31.74	40.71	4333.88
CV(%)	7.13	9.47	3.51	7.29	13.06

*Equal lowercase letters do not differ from each other in the column, by Tukey's Test, at 5% error probability. DAT = days after transplanting lettuce seedlings. DT = day of transplanting lettuce seedlings. ns = not significant

In Table 5, it can be seen that there was no statistical difference between treatments, for the averages of fresh leaf biomass, fresh stem biomass, fresh head biomass, fresh root biomass and total fresh biomass at lettuce harvest, in the test in the municipality of Marechal Candido Rondon. Although there was no statistical difference, lettuce plants inoculated with *Bacillus subtilis* + B. *megaterium* showed lower means for these variables.

Menezes (2019), in the test on lettuce growth and production, in response to rhizobacteria and nitrogen, observed that *Azospirillum brasilense* increased the fresh biomass of the shoot and the number of leaves. However, Souza et al. (2018), also working with lettuce plants, observed that the isolated application of *A. brasilense* did not provide this increase.

Almeida (2016) in their research with nitrogen doses and inoculation with *Azospirillum* spp., in the iceberg lettuce cultivate Lucy Brown, did not find a significant difference for head diameter, number of leaves, leaf area, fresh and dry plant biomass, corroborating this study, since there was also no significant difference between the inoculants used. Other authors, however, such as Fasciglione et al. (2012), in studies with lettuce seeds cv. Elisa, observed that, when the seeds were inoculated with *A. brasilense* and without saline stress, they presented higher fresh biomass.

Nogueira (2019), in their study with the inoculation of lettuce seeds with *A. brasilense* strain sp. 245, found superior results for fresh shoot biomass, dry biomass and head diameter after 47 DAT. Lima et al. (2017) found that the application of this bacterium, associated with nitrogen fertilization, promoted an increase in fresh shoot biomass. Sehata et al. (2016), evaluating the effect of *Bacillus subtilis*, associated with nitrogen application in lettuce, observed a significant increase in the number of leaves, fresh biomass, dry biomass and total yield.

TABLE 5 - Fresh plant leaf biomass (FLB, g), fresh plant stem biomass (FPSB, g), fresh plant head biomass (FPHB, g), fresh plant root biomass (FRB, g) and total fresh plant biomass (TFB, g) of lettuce, with the application of plant growth-promoting bacteria (PGPB), at different times, in the municipalities of Marechal Cândido Rondon and Cascavel (PR).

PGPB	Marechal Cândido Rondon (PR)				
	FLB	FPSB	FPHB	TFB	BFT
No inoculation	304.31 ns	46.00 ns	350.31 ns	7.33 ns	357.64 ns
Azospirillum brasilense	295.12 ns	44.46 ns	339.58 ns	7.30 ns	346.88 ns
Bacillu subtilis + B. megaterium	299.42 ns	44.85 ns	344.27 ns	7.11 ns	351.38 ns
Pseudomonas fluorescens	293.87 ns	44.14 ns	338.02 ns	7.09 ns	345.11 ns
Inoculation times					
DT	294.34 ns	43.55 ns	337.89 ns	7.14 ns	345.03 ns
15 DAT	308.50 ns	46.81 ns	355.31 ns	7.16 ns	362.47 ns
DT + 15 DAT	291.70 ns	44.23 ns	335.94 ns	7.32 ns	343.25 ns
Averages	298.18	44.86	343.05	7.21	350.25
CV(%)	12.00	12.64	11.62	10.02	11.49
	Cascavel (PR)				
No inoculation	341.91 a	37.78 ns	379.69 a	12.90 ns	392.59 a
Azospirillum brasilense	321.38 a	35.18 ns	356.56 a	12.97 ns	369.54 a
Bacillu subtilis + B. megaterium	279.01 a	30.15 ns	309.17 a	11.93 ns	321.10 a
Pseudomonas fluorescens	313.08 a	32.85 ns	345.94 a	13.23 ns	359.16 a
Inoculation times					
DT	333.13 ns	35.78 ns	368.91 ns	13.76 ns	382.67 ns
15 DAT	314.79 ns	34.35 ns	349.14 ns	12.53 ns	361.62 ns
DT + 15 DAT	293.62 ns	31.85 ns	325.47 ns	11.97 ns	337.44 ns
Averages	313.85	33.99	347.84	12.75	360.59
CV(%)	16.03	19.56	16.31	16.33	13.06

*Equal lowercase letters do not differ from each other in the column, by Tukey's Test, at 5% error probability. DAT = days after transplanting lettuce seedlings. DT = day of transplanting lettuce seedlings. ns = not significant.

In Table 6, it can be seen that plants inoculated with the bacteria showed no significant difference between the application times for total dry biomass. However, plants inoculated with *Azospirillum brasilense* showed higher values when applied simultaneously at DT + 15 DAT, with an average of 18.79 g. With the inoculation of this bacterium only in the DT, an average of 4.83 g was obtained, with a difference between these two values of 21%.

TABLE 6 - Total dry biomass (g) of lettuce plants, after harvest, with the application of plant growth-promoting bacteria (PGPB), at different times, in the municipality of Marechal Cândido Rondon (PR).

PGPB	Inoculation time		
	DT	15 DAT	DT + 15 DAT
No inoculation	16.24 Aa	16.24 Aa	16.24 Aab
A. brasilense	14.83 Ba	16.63 ABa	18.79 Aa
B. subtilis + B. megaterium	17.86 Aa	16.67 Aa	15.33 Aab
P. fluorescens	16.62 Aa	17.63 Aa	14.08 Ab
Averages	16.43	16.43	16.43
CV(%)	12.70	12.70	12.70

*The same lowercase letters in the column and uppercase in the row, do not differ from each other in the column, by Tukey's Test, at 5% error probability. DAT = days after transplanting lettuce seedlings. DT = day of transplanting lettuce seedlings.

Table 7 shows the averages of leaf dry biomass, stem dry biomass, head dry biomass, root dry biomass, root volume and root length at harvest of lettuce plants, cultivated in the two test municipalities, with no significant difference between the treatments. Menezes (2019) found that the application of *Bacillus subtilis*, when applied to the soil, reduced the total and commercial shoot dry biomass, total plant and root dry biomass and the nitrogen accumulated in the shoot of lettuce plants cultivar Vera. This reduction was probably due to the competition for nutrients contained in the substrate, between the plants and the microorganism.

Pishchik et al. (2016) found that the use of *Bacillus subtilis* in the lettuce crop promoted increases both in dry biomass and in the total levels of nitrogen accumulated in the shoot and chlorophyll content, probably due to the production of auxin and higher concentration of organic solutes in vegetable vacuoles. Madhaiyan et al. (2010), in studies with tomato and red pepper seeds *in vitro*, verified a greater length of the roots, with the inoculation of *Azospirillum brasilense*, due to the production of indoleacetic acid (IAA). The authors also observed that the bacterium promoted an increase in the aerial part, confirming its growth-promotion capacity.

TABLE 7 - Plant leaf dry biomass (LDB, g), plant stem dry biomass (SDB, g), plant head dry biomass (PHDB g), plant root dry biomass (RDB, g), volume root length (RV, m³) and root length (RL, cm), after the harvest of lettuce plants, with the application of plant growth-promoting bacteria (PGPB), at different times, in the municipalities of Marechal Cândido Rondon and Cascavel (PR).

PGPB	Marechal Cândido Rondon (PR)					
	LDB	SDB	PHDB	RDB	RV	RL
No inoculation	14.01 ns	1.51 ns	15.52 ns	0.72 a*	7.22 ns	21.03 ns
Azospirillum brasilense	14.56 ns	1.54 ns	16.10 ns	0.65 a	7.07 ns	22.03 ns
Bacillu subtilis + B. megaterium	14.41 ns	1.59 ns	16.00 ns	0.62 a	6.98 ns	20.35 ns
Pseudomonas fluorescens	13.92 ns	1.58 ns	15.50 ns	0.61 a	7.04 ns	21.19 ns
Inoculation times						
DT	14.13 ns	1.61 ns	15.74 ns	0.65 ns	6.99 ns	21.72 ns
15 DAT	14.58 ns	1.57 ns	16.15 ns	0.65 ns	7.20 ns	20.42 ns
DT + 15 DAT	13.97 ns	1.49 ns	15.46 ns	0.65 ns	7.04 ns	21.32 ns
Averages	14.23	1.55	15.78	0.65	7.08	21.15
CV(%)	14.41	17.26	12.68	13.29	11.07	9.09
	Cascavel (PR)					
No inoculation	18.31 ns	2.13 ns	20.44 ns	1.06 ns	21.50 ns	12.56 ns
Azospirillum brasilense	15.62 ns	1.80 ns	17.42 ns	0.98 ns	18.41 ns	12.09 ns
Bacillu subtilis + B. megaterium	17.51 ns	2.02 ns	19.53 ns	1.07 ns	20.59 ns	12.87 ns
Pseudomonas fluorescens	16.54 ns	1.91 ns	18.45 ns	1.12 ns	19.56 ns	12.39 ns
Inoculation times						
DT	18.08 ns	2.04 ns	20.13 ns	1.13 ns	21.26 ns	13.33 ns
15 DAT	16.60 ns	2.02 ns	18.62 ns	1.04 ns	19.67 ns	11.97 ns
DT + 15 DAT	16.29 ns	1.84 ns	18.13 ns	0.99 ns	19.12 ns	12.12 ns
Averages	16.99	1.96	18.96	1.06	20.01	12.47
CV(%)	14.35	16.40	14.03	16.99	13.67	17.66

*Equal lowercase letters do not differ from each other in the column, by Tukey's Test, at 5% error probability. DAT = days after transplanting lettuce seedlings. DT = day of transplanting lettuce seedlings. ns = not significant.

In Table 8 presents the results related to the comparison of the averages of the foliar contents of nitrogen (N), phosphorus (P) and potassium (K) in the lettuce crop after harvest for the test in the two municipalities, where, it can be observed, that there was no significant difference between treatments. As observed in the present work, the application of *Bacillus subtilis* + B. *megaterium, Azospirillum brasilense* and *Pseudomonas fluorescens* did not present statistical difference for chlorophyll content, plant height, head diameter, number of leaves, leaf area, fresh leaf biomass, fresh stem biomass, fresh head biomass, root mill biomass, total fresh biomass, dry leaf biomass, dry stem biomass, dry head biomass, dry root biomass, root volume, root length and leaf N contents , P and K, in lettuce cultivar Vera.

Regarding TDB, a statistical difference can be observed in the interaction between treatments, in the trial carried out in the municipality of Marechal Cândido Rondon, where lettuce plants, when inoculated with *Azospirillum brasilense*, showed higher values, in the DT + 15 DAT, 21% to more than that treatment that occurred in the DT. Regarding the time of application in DT + 15 DAT, it was found that plants inoculated with *A. brasilense* were 25% higher than those inoculated with *Pseudomonas fluorescens*, however, compared to the control (without inoculation), there was no difference statistics for any of the treatments. What may have occurred was a competition between the plants and the inoculated microorganisms for nutrients available in the soil solution.

The inoculated microorganisms may not have survived in the soil, due to the adverse conditions of the medium to which they were inserted, or even the applied amount of inoculants was not sufficient to lead to an increase in the culture. Even, under conventional cultivation conditions, the open field may have influenced the colonization, survival, multiplication and development of the plant growth-promoting bacteria used.

TABLE 8 - Values of nitrogen (N, g Kg^{-1}), phosphorus (P, g Kg^{-1}) and potassium (K, g Kg^{-1}), found in the leaf tissue of lettuce plants harvested, with the application of bacteria plant growth promoters (PGPB), at different times, in Marechal Cândido Rondon and Cascavel (PR).

PGPB	Marechal Cândido Rondon (PR)		
	N	P	K
No inoculation	31.72 ns	5.02 ns	50.80 a*
Azospirillum brasilense	34.00 ns	5.45 ns	53.32 a
Bacillu subtilis + B. megaterium	36.07 ns	5.77 ns	57.31 a
Pseudomonas fluorescens	34.17 ns	5.52 ns	57.78 a
Inoculation times			
DT	33.98 ns	5.33 ns	54.78 ns
15 DAT	34.53 ns	5.37 ns	54.78 ns
DT + 15 DAT	33.85 ns	5.62 ns	54.85 ns
Averages	34.12	5.44	54.80
CV(%)	13.31	12.28	11.82
	Cascavel (PR)		
No inoculation	31.11 ns	6.51 ns	68.23 ns
Azospirillum brasilense	27.71 ns	6.28 ns	65.17 ns
Bacillu subtilis + B. megaterium	32.67 ns	6.55 ns	65.80 ns
Pseudomonas fluorescens	31.76 ns	6.36 ns	66.64 ns
Inoculation times			
DT	30.50 ns	6.17 ns	66.92 ns
15 DAT	32.68 ns	6.49 ns	66.63 ns
DT + 15 DAT	29.25 ns	6.62 ns	65.82 ns
Averages	30.81	6.42	66.45
CV(%)	16.58	16.50	13.81

*Equal lowercase letters do not differ from each other in the column, by Tukey's Test, at 5% error probability. DAT = days after transplanting lettuce seedlings. DT = day of transplanting lettuce seedlings. ns = not significant.

Although there are studies that prove the existence of significant responses regarding the association of lettuce with these microorganisms, other studies also do not demonstrate a significant response for promoting the growth and development of inoculated cultures.

The lack of significant responses regarding the use and association of *Bacillus subtilis* + *B. megaterium*, *Azospirillum brasilense* and *Pseudomonas fluorescens* and curly lettuce plant, may be associated with a series of factors, such as temperature, soil moisture, soil texture, pH, bacteria-host plant ratio (specific exudates, phytotoxins and bacteriocins), competitive capacity with native bacteria, inoculation methods and growth conditions.

New studies are needed around these microorganisms in lettuce culture, seeking positive responses regarding their use, in order to seek better increments to this culture and improve the quality of the final product. Not just for the lettuce crop, but for vegetable crops in general.

CONCLUSION

The inoculated plant growth-promoting bacteria did not promote a significant increase in the development of the lettuce cultivating Vera, when compared to the control (without BPCV application).

REFERENCES

ALMEIDA, W.M. **Nitrogen doses and inoculation with Azospirillum sp. in iceberg lettuce cv. Lucy Brown.** 2016. 79 p. Dissertation (Master's in Plant Production) - Federal University of Mato Grosso, Sinop, 2016.

AGUIRRE, P.F.; OLIVO, C.J.; RODRIGUES, P.F.; FALK, D.R.; ADAMS, C.B.; SCHIAFINO H.P. Forage yield of coastcross-1 pastures inoculated with *Azospirillum brasilense*. **Acta Scientiarum.** Animal Science, v.40, n.1, p.159-165, 2018.

BRAGA JUNIOR, G. M. **Efficiency of Bacillus subtilis in the biocontrol of phytopathogens and plant growth promoter.** 2015. 87p. Dissertation (Master's in Plant Production) - Federal University of Tocantins, Gurupi, 2015.

CIPRIANO, M.A.P.; PATRICIO, F.R.A.; FREITAS, S.S. Potential of rhizobacteria to promote root rot growth and control in hydroponically cultivated lettuce. **Summa Phytopathologica**, v.39, n.1, p.51-57, 2013.

DOMINGUES, S.C.O.; CARVALHO, M.A.C.; RABELO, H.O.; MOREIRA, E.S.; SCATOLA, L.F.; DAVID, G.Q. Growth-promoting microorganisms in lettuce. **Nativa**, v.9, n.2, p.100-105, 2021.

FASCIGLIONE, G.; CASANOVAS, E.M.; QUILLEHAUQUY, V. BARASSI, C.A. *Azospirillum* improves lettuce growth and transplantation in saline conditions. **Journal of the Science of Food and Agriculture**, v.92, n.12, p.2518-2523, 2012.

FERREIRA, J.T.P.; SANTOS, T.M.C.; ALBUQUERQUE, L.S.; SANTOS, J.V.; CARDOSO FILHO, J.A.; RAMALHO NETO, C.E. Isolation and selection of growth promoting bacteria of the genus *Bacillus* and its effect on two varieties of lettuce (*Lactuca sativa* L.). **International Research Journal of Microbiology**, v.2, n.2, p.70-78, 2011.

FERTIBIO. **Biological Phosphorus Solubilizer**. Fertibio PHOSPRO - *Pseudomonas fluorescens*. Ponta Grossa, PR, 2021. Available at: <https://www.fertibio.com.br/produtos/fertibio-phospro/>. Accessed on: 15 Nov. 2021.

FILGUEIRA, F.A.R. **New horticulture manual:** modern agrotechnology in the production and commercialization of vegetables. 3rd ed. to see. and amp. Viçosa, MG: Ed. UFV, 421p., 2013.

LIMA, A.A.; VENTUROSO, L.R.; SILVA, B.A.A.; GOMES, A.F.; SCHIMIDT, O. Efficiency of inoculation of *Azospirillum brasilense* associated with rootstock in lettuce growth and production. **Green Journal of Agroecology and Sustainable Development**, v.12, n.2, p.233-240, 2017.

LOZADA, J.A.R.; SILVEIRA, K.C.; SILVA, L.J.; BALDOTTO, M.A.; BALDOTTO, L.E.B. Selection of diazotrophic bacteria isolated from wastewater treatment plant sludge at a poultry slaughterhouse for their effect on maize plants. **Ceres Magazine**, v.65, n.1, p.85-92, 2018.

MADHAIYAN, M.; POONGUZHALI, S.; KWON, S.W.; AS, T.M. *Bacillus methyltrophicus* sp. nov., a methanol-utilizing, plant growth-promoting bacterium isolated from rice rhizosphere soil. **International Journal of Systematic and Evolutionary Microbiology**, v.60, n.10, p.2490-2495, 2010.

MORNINGS, N.E. **Plant growth-promoting bacteria and substrates in Bromeliaceae and Cactaceae.** 182p. Dissertation (Master's in Plant Production) - State University of Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, 2014.

MENEZES, A.P.M. **Lettuce growth and yield in response to rhizobacteria and nitrogen.** 71p. Thesis (Doctorate in Plant Production) - UFA, Rio Branco - AC, 2019.

NITROMIL. **Biological Inoculants**. Technology at the root. Nitro1000 Grasses - *Azospirillum brasilense* - AbV5 and AbV6. Cascavel, PR, 2021. Available at: <https://www.nitro1000.com.br/informativoTrigoMilho.php>. Accessed on: 15 Nov. 2021.

NOGUEIRA, E.D. **Evaluation of growth parameters of lettuce (*Lactuca sativa*) inoculated with *Azospirillum brasilense* strain sp. 245**. 2019. 75p. Dissertation (Master's in Organic Agriculture) - Federal Rural University of Rio de Janeiro, Seropédica, 2019.