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Growth-promoting bacteria change the development of aerial part and root system of canola

Bactérias promotoras de crescimento alteram o desenvolvimento da parte aérea e sistema radicular da canola

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Abstract

Plant growth promoting rhizobacteria (PGPR) are soil bacteria that have the ability to colonize the rhizosphere and favor the growth of plants through several mechanisms, such as phytohormone production, biological fixation in nitrogen (BNF), increased efficiency in uptake of nutrients in the soil, and greater tolerance to water stress or disease attack. The objective of this work was to evaluate the effects of inoculation of different growth promoting bacteria on the development of canola plants. The experiment was conducted in a greenhouse with a completely randomized design. Four treatments were evaluated: control - without inoculation, *Azospirillum brasilense* of strain Ab-V5, *Rhizobium* sp. strain 8121, and *Bacillus* sp. strain CM. The height parameter of plants was evaluated weekly, starting at 25 days after sowing (DAS). At the end of the evaluations, at 53 DAS, the length of the main root, the dry mass of the aerial part and the root system were measured. The canola showed a linear increase in height from 25 to 53 DAS. *Bacillus* sp. negatively influenced the growth of the plants, reducing their height in periods 25, 32 and 39 DAS in relation to the control group. *A. brazilense.* yielded 2.64 times more dry root mass (0.7 g plant^{-1}) than the control plants (0.3 g plant^{-1}). Even though *Rhizobium* sp. produced an increase in the length of the main root, this was not reflected in an increment of dry mass in the root system. The greatest development of the root system was provided by *A. brazilense*.
Key words: Sustainable agriculture. Agricultural biostimulant. PGPR. Rhizobacteria. Winter culture. Biodiesel.

Resumo

As rizobactérias promotoras de crescimento de plantas (PGPR) são bactérias do solo que possuem a capacidade de colonizar a rizosfera e favorecem o crescimento das plantas através de diversos mecanismos, tais como produção de fitohormônios, a fixação biológica no nitrogênio (FBN), aumento da eficiência na captação de nutrientes do solo, maior tolerância ao stress hídrico ou ao ataque de doenças. Objetivou-se nesse trabalho avaliar os efeitos da inoculação de diferentes bactérias promotoras de crescimento sobre o desenvolvimento da parte aérea e sistema radículas de plantas canola. O experimento foi realizado em condições de casa de vegetação com delineamento inteiramente casualizado. Foram avaliados quatro tratamentos: controle - sem inoculação, *Azospirillum brasilense* da estirpe Ab-V5, *Rhizobium* sp. estirpe 8121, *Bacillus* sp. estirpe CM. O parâmetro altura de plantas foi avaliado semanalmente, iniciando-se aos 25 dias após a semeadura (DAS). No término das avaliações,

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aos 53 DAS, foram mensurados o comprimento da raiz principal, a massa seca da parte aérea e do sistema radicular. A canola apresentou aumento em altura dos 25 aos 53 DAS. *Bacillus* sp. influenciou negativamente o crescimento das plantas, reduzindo a altura das mesmas nos períodos 25, 32 e 39 DAS em relação ao controle. *A. brazilense* proporcionou 2.64 vezes mais massa seca de raízes (0,7 g planta^{-1}) em comparação ao controle (0,3 g planta^{-1}). O *Rhizobium* sp. apesar de proporcionar aumento no comprimento da raiz principal, este não refletiu em incremento de massa seca no sistema radicular. O maior desenvolvimento do sistema radicular foi proporcionado por *A. brazilense*.
Palavras-chave: Agricultura sustentável. Bioestimulante agrícola. PGPR. Rizobactérias. Cultura de inverno. Biodiesel.

Introduction

In ten years, worldwide consumption of nitrogen (N), phosphorus (P) and potassium (K) increased approximately 18%, reaching a volume of around 184 million tons. This increase in the demand for chemical fertilizers has been even more significant in developing countries, where, in the period mentioned - 2005 to 2014 - consumption grew by 22% (IFA, 2016).

In Brazil, the fertilizer market moved slightly more than 30 million tons in 2015, an amount that provided the segment with revenues of US $ 12.5 billion (ANDA, 2015; ABIQUIM, 2015). However, in this segment there is great external dependence. Among the primary macronutrients, 82% of N, 58% of P and 94% of K are from other countries (IBRAM, 2015). Although the sector is highly profitable, problems related to pollution, contamination and imbalances perpetrated by chemical fertilizers continue (BHARDWAJ et al., 2014). The use of chemical fertilizers without real necessity induces salinization of the soil, accumulation of heavy metals, eutrophication of the water, accumulation of nitrate and liberation in the air of nitrogen and sulfur (SAVCI, 2012).

This worrying social and economic context has encouraged research around agricultural substances called biostimulants, whose market is in rapid expansion at the global level (CALVO et al., 2014). Agricultural biostimulants are products composed of substances or microorganisms with the potential to provide several benefits to plants, including increased efficiency in soil nutrient uptake and greater tolerance to water stress or disease attack (DU JARDIN, 2015). Within this universe of microorganisms that are beneficial to plants, of particular interest are rhizobacteria (MINZ et al., 2013) which, as the denomination suggests, inhabit the rhizosphere, a portion of the soil under direct influence of the roots of the plants (SOUZA et al., 2015). In this environment develops the calls plant growth-promoting rhizobacteria (PGPR).

PGPRs occur naturally in the soil and are able to provide benefits to plants through various mechanisms which can be grouped into four modes of action: i) synthesis of substances that can be assimilated directly by plants, ii) nutrient mobilization, iii) induction of the plant to stress tolerance and iv) disease tolerance (GARCIA-FRAILE et al., 2015). Thus, the use of PGPR presents great potential in the construction of strategies related to bioprotection and for increasing the productivity of plants cultivated at the global level (FARINA et al., 2012).

Canola (*Brassica napus* L.) is an oleaginous plant belonging to the cruciferous family that was developed through the genetic improvement of rapeseed (MILCIADES-MELGAREJO et al., 2014). At the global level, rapeseed / canola cultivation currently covers an area of 33.7 million hectares, with the world's largest producers being the European Union, Canada and China, respectively. Grain production is close to 69 million tons and oil production reaches about 28 million tons, which makes rape / canola the third most important species in the world production of vegetable oil, behind soybeans only and the palm (USDA, 2017).

In Brazil, good adaptability to the South region has made it possible for the crop to be an interesting alternative for winter cultivation, in succession to soybeans. According to data from Companhia Nacional de Abastecimento, the area planted with canola in Brazil was slightly more than 46 thousand hectares in 2017. Rio Grande do Sul is the largest producing State, with a cultivated area of 43.3 thousand hectares (CONAB, 2017). The oil content in canola grains (on average, 38% in Brazil) makes the crop an interesting alternative for the production of biodiesel, which is already widely used on the European continent. In this respect, it is important to emphasize the qualities of canola oil for the production of biofuels and lubricants. The higher erucic acid content, present in some varieties of rapeseed / canola, enables the production of industrial oils with high stability and high resistance at elevated temperatures (TOMM et al., 2009).

Besides potential for growth of the cultivated area at both the national and global levels and the economic importance of canola oil, adds to this the fact of great responsiveness of the crop to fertilization, especially by nitrogen (KAEFER et al., 2014). These facts and characteristics make canola assume special relevance in studies that contribute to reduction in the use of chemical fertilizers and to the sustainable production of biodiesel at low cost.

In this context, the present research aimed to evaluate the effects of inoculation of different growth promoting bacteria on the development of canola plants.

Material and Methods

The experiment was carried out in a greenhouse belonging to the Department of Agronomy of the State University of Londrina (UEL), State of Paraná, Brazil (23° 23 Latitude S and 51° 11’ Longitude W altitude 566 m). The soil used as substrate to compose the experimental units was a Red Latosol eutroferric, with the following chemical attributes: pH ($CaCl_2$) 5.6; Ca, Mg, K e Al, respectively, 8.04; 1.46; 1.54 and 0.00 in $cmol_c\ dm^{-3}$, P ($mg\ dm^{-3}$) 102; organic matter ($g\ kg^{-1}$) 26.2. Canola seeds used were of the Iapar PCI-0801 variety that has an early cycle (120 days), high oil content (40%) and good quality cake, 35% ether extract, and thousand grain weight of 3.19 g (ARAUJO et al., 2017).

Azospirillum brasilense strain Ab-V5, *Rhizobium* sp. strain 8121, and *Bacillus* sp. strain CM of the collection of bacterial isolates from the biochemistry and biotechnology department of the State University of Londrina were used. Isolated colonies of each strain used, obtained from growth on solid medium Dygs (2 g of glucose; 1.5 g peptone; 2 g yeast extract; 0.5 g K_2HPO_4; 0.5 $MgSO_4$; distilled water q.s.p. 1 L, at pH 6.0) were multiplied in 5 mL of medium, cultured for 48 hours under shaking in an orbital shaker incubator, under agitation of 160 rpm and temperature of 28 ° C.

After this growth period, the cultivation was stopped and the concentration determined after a reading of 640 nm spectrometry, set at an optical density of 0.5 x 10 9 cells mL^{-1}. Sterilization of the seed coat was carried out with sodium hypochlorite 2.0% (3 min) under immersion and shaking, washed five times with distilled water. The inoculation was performed at a concentration of 20 mL kg-1 of seeds, in plastic bags for each batch of seed, under agitation for 2 minutes.

The experimental design was completely randomized and composed of four treatments, control - without inoculation, *Azospirillum brasilense* strain Ab-V5, *Rhizobium* sp. strain 8121, and *Bacillus* sp. strain CM. The experimental units were composed of three-liter polyethylene vases. For filling, a 1: 1 mixture of soil and sand was used, both sterilized previously in an autoclave. At sowing, three seeds per experimental unit were used, remaining, after thinning at 11 days after sowing (DAS), one plant per vase.

The plant height variable was evaluated weekly, starting at 25 DAS. At the end of the evaluations, at 53 DAS, the length of the main root, the dry mass of the aerial part and the root system were measured. The height of the aerial part was measured with a graduated ruler from the ground level to the apical meristem. At the end of the experiment, the aerial part was separated and taken to the oven at 60 ° C until constant weight, and then weighed in an analytical balance with an accuracy of 0.005 g.

For the evaluation of the root system, the experimental units were sent to the Laboratory of Roots Study, Department of Agronomy, State University of Londrina, placed in a plastic container with water and manually shaken.

The water and the suspended roots were poured into a one-millimeter mesh sieve. This operation was repeated until there was no more soil. After removal of soil, the length of the main root was measured. All roots retained in the sieve were collected and taken to the forced ventilation oven at 65ºC until constant mass was obtained. After drying, the roots were weighed with a precision scale and the results presented in milligrams of roots.

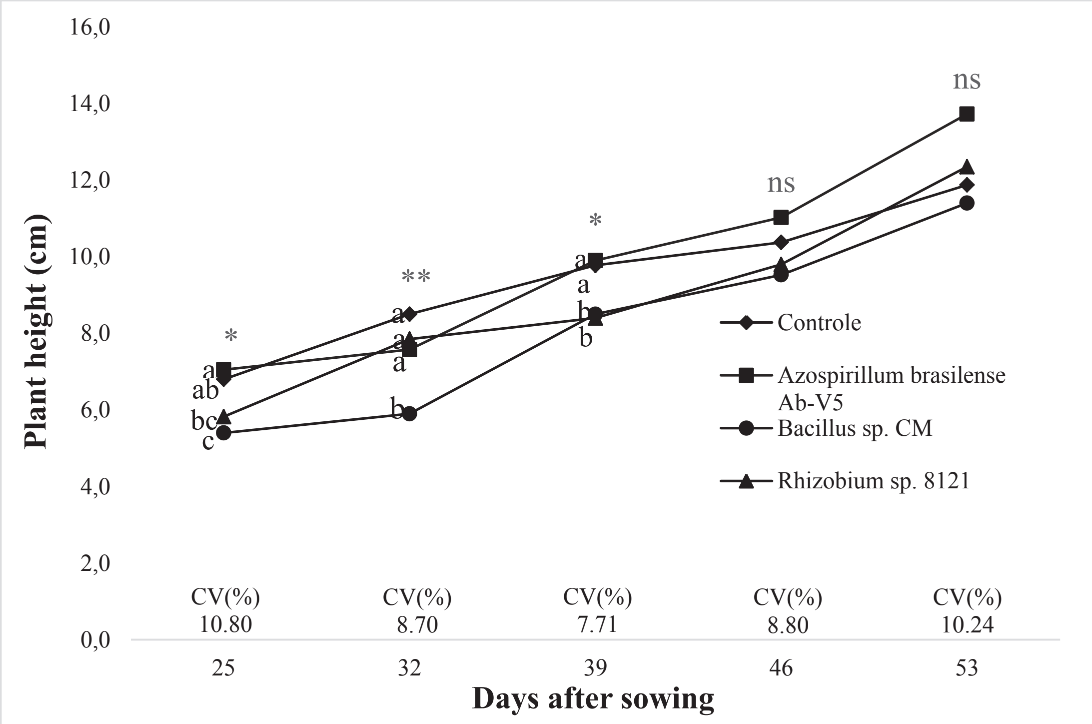
The assumptions of the analysis of variance were met and the results were submitted to analysis of variance and, when significant, the means were compared by the Duncan test, with the aid of the statistical program SASM-agri (CANTERI et al., 2001), with the significance level higher set at $p \leq 0.05$, except for root dry mass ($p \leq .10$).

Results and Discussion

PGPR influenced the development of aerial parts of the canola plants. Canola showed a linear increase in height (Figure 1) from 25 to 53 DAS (days after sowing). Inoculation with *A. brasilense* and the control obtained higher plant height at 25, 32 and 39 DAS (means 6.93, 8.22 and 9.90 cm, respectively) in relation to the other treatments. *Bacillus* sp. (strain CM) negatively influenced the growth of the plants, reducing the height at 25, 32, and 39 DAS (means 5.40, 5.90 and 8.40 cm, respectively). Ahmadi-Rad et al. (2016) observed at the end of the cycle that inoculation with *Azotobacter* decreased the height of canola plants, indicating that not all associations with PGPR are beneficial. Plants with a more developed root system, as well as aerial part and shoot tissues, present more conditions and competitive advantages for survival under stress than less developed plants (GONÇALVES; LYNCH, 2014). At 46 and 53 DAS, although not statistically different, *Azospirillum b*. presented a tendency toward greater height (11.03 and 13.73 cm, respectively) than the control (10.38 and 11.88 cm, respectively) (Figure 1). These results are in agreement with El-Howeity and Asfour (2012), who obtained superior results in the parameters of plant height (124 cm) and number of silique per plant (518.8) after inoculation of canola with *Azospirillum b.* planted in newly recovered desert. In the initial development of the plants, shoot dry matter values were similar between the control and PGPR (Figure 2). However, these results may be due to development stage, as early in development growth in height is prioritized over the accumulation of vegetal mass.

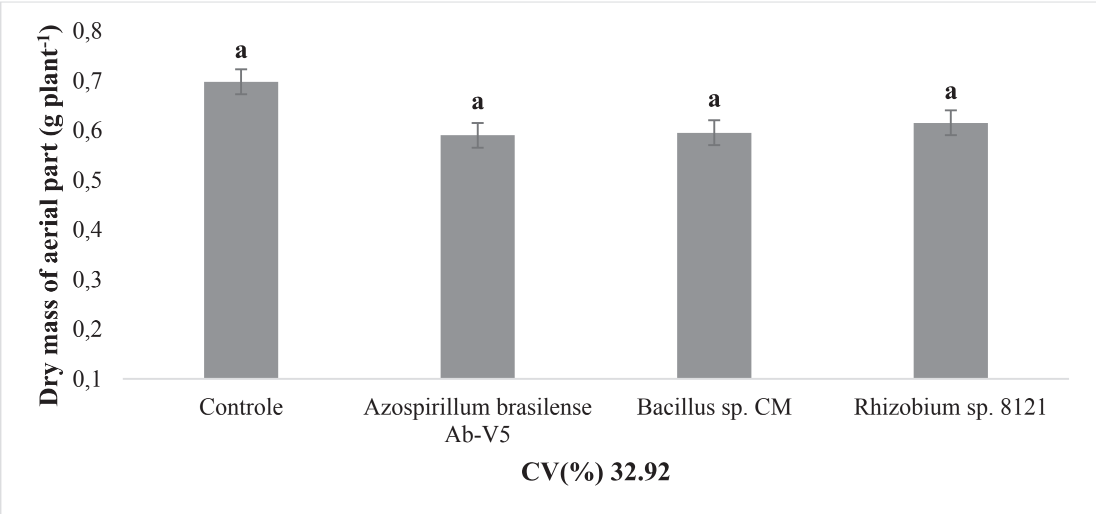
Inoculation with PGPR promoted greater development of the canola root system (Figure 3). *Azospirillum b.* provided higher dry matter increment roots (0.74 g plant-1) compared to the control (0.28 g plant-1), which resulted in 2.64 times more root mass. A higher volume of roots may give the plant greater tolerance to abiotic stress and greater capacity for nutrient absorption, consequently promoting higher quality and productivity. Although to a lesser extent, *Bacillus* sp. and *Rhizobium* sp. also provided a greater weight of dry matter of the root system when compared to treatment without inoculation. However, *Bacillus* sp. presented lower plant height than all other treatments. Aquino et al. (2018) found that *Rhizobium* sp. and other rhizobacteria (*M. komagatae*, *Azomonas sp*.) promoted a significant increase in the root area of *Crambe abyssinica* (*Brassicaceae*), emphasizing the importance of studies with growth promoting bacteria in different production environments.

Figure 1. Height of canola plants in five periods (25, 32, 39,46 and 53 days after sowing), under the effect of different plant growth promoting bacteria.



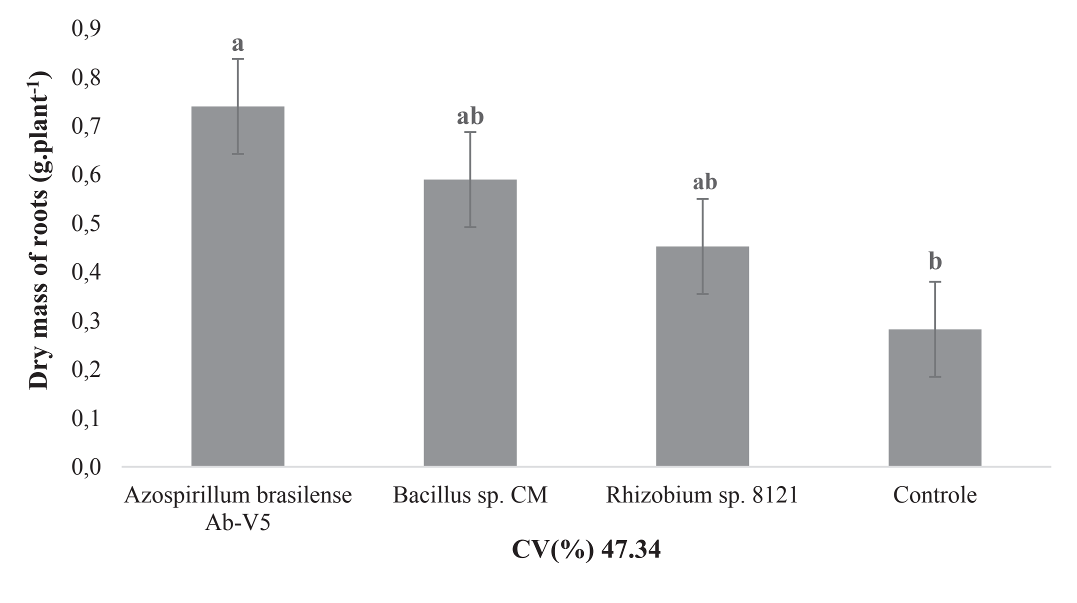
Means followed by the same letter in the column do not differ significantly from each other by the Duncan Test * Significant at 5%. ** Significant at 1% probability level. CV: coefficient of variation.

Figure 2. Dry mass of the aerial part of canola (g plant-1) at 53 days after sowing, under the effect of different bacteria promoting growth of plants.



Means followed by the same letter in the column do not differ significantly from each other by the Duncan Test at the 5% probability level. CV: coefficient of variation.

Figure 3. Dry mass of root of canola plant (g plant-1) at 53 days after sowing, under the effect of different plant growth promoting bacteria.



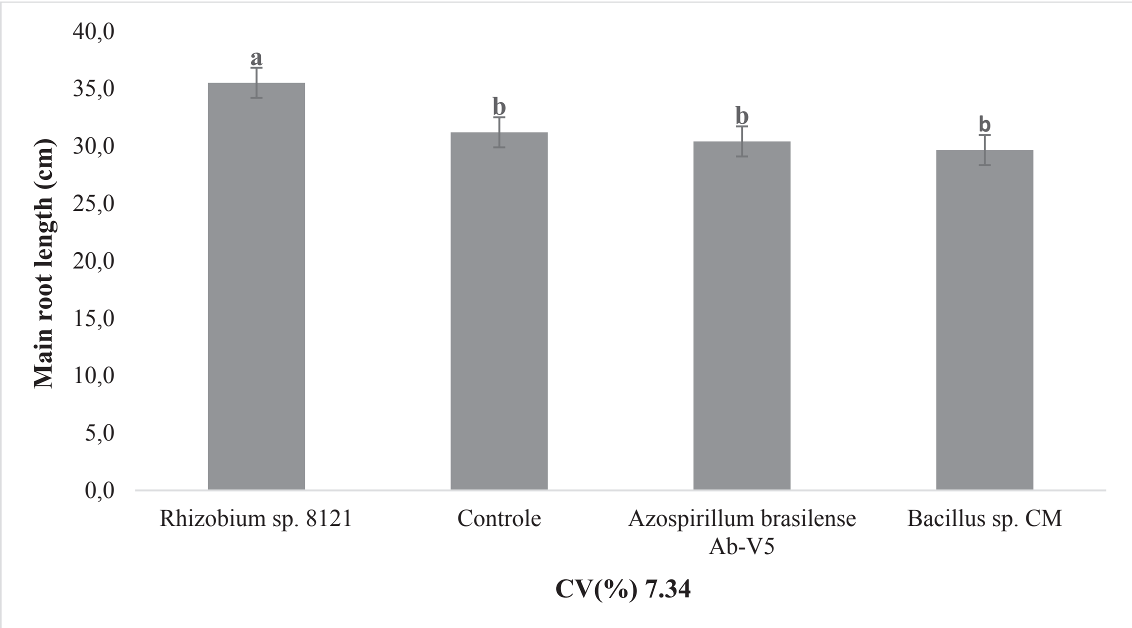
Means followed by the same letter in the column do not differ significantly from each other by the Duncan Test at the 10% probability level. CV: coefficient of variation.

These results differ from those found by Ndeddy Aka and Babalola (2016) and Turan et al. (2014), which verified higher root length and dry matter of *Brassica juncea* plants inoculated with *Bacillus subtilis*. On the other hand, Aung et al. (2015), in work with *Brassica rapa* and *Brassica juncea,* did not find increases in biomass production in plants inoculated with *Bacillus pumilus*. Thus, it is evident that the associations between *Brassica* plants and *Bacillus* bacteria may also imply neutrality or even antagonistic effects. Therefore, the identification of PGPR that carry out associations that promote benefits to the development of the plants is of paramount importance. Under the conditions in this study, *Bacillus* sp. negatively impacted the height of canola plants.

Bacteria of the genus *Rhizobium*, with capacity for the solubilization of phosphates, were found naturally inhabiting the rhizosphere and roots of canola plants (FARINA et al., 2012). Although treatment with Rhizobium sp. produced a small increase (4 cm) in the length of the main root compared to the control (35 and 31 cm, respectively - Figure 4), this was not sufficient to promote further development of the root system, resulting in 39% less root mass than *Azospirillum b.* (Figure 3).

The results are in agreement with previous studies that verified beneficial effects of the inoculation of diazotrophic bacteria of the genus *Azospirillum* in *Brassica napus*. Baniaghil et al. (2013) observed higher dry matter weight of roots of canola plants subjected to saline stress and, under water stress, higher root surface area when inoculated with *Azospirillum lipoferum* was observed by Saeed et al. (2016). In addition to the root system, positive effects of Azospirillum sp. on the production and quality of the canola grains were also verified (NOSHEEN et al., 2011; YASARI et al., 2016; AHMADI-RAD et al., 2016). These results confirm that PGPR has high potential for application in agriculture, since they can improve plant growth through the production of phytohormones (IAA, GA), solubilization of mineral phosphate, and antagonism of plant pathogens under limiting or stressing conditions (VESSEY, 2003; DOBBELAERA et al., 2003, AQUINO et al., 2018).

Figure 4. Main root length canola plants 53 days after sowing, under the influence of different bacteria promoting plant growth.



Means followed by the same letter in the column do not differ significantly from each other by the Duncan Test at the 5% probability level. CV: coefficient of variation.

However, these soil-plant-microbe interactions are complex, and the results may differ depending on the environment conditions, soil type, location, influencing plant health and productivity. The identification of strains of PGPR that associate and promote the best development of brassicas such as canola and crambe are of great importance for the production of biodiesel achieving international standards of quality with low cost of production, and also allow their cultivation in less favored areas in which the soils are less fertile or with scarce resources for the acquisition of inputs, mainly nitrogen fertilizers (AQUINO et al., 2018). In addition to the aforementioned aspects, it is also important to emphasize the importance of environmental issues, since the negative impacts of chemical fertilizers on the soil, water courses and groundwater would be mitigated. In this context, the importance of research involving vegetable biostimulants for the sustainability of agriculture at the global level is highlighted.

Conclusion

The rhizobacterium *Azospirillum brasilense* strain Ab-V5 presented as a growth promoter in canola plants, providing 2.6 times greater root development and increased plant height. The rhizobacterium *Bacillus* spp. of the CM strain, however, causes less development of canola plants in the early stages.

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