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Original Article

Growth promotion in wheat seedlings altered by conditions in the culture medium of *Azospirillum brasilense*

Promoção do crescimento em plântulas de trigo alteradas por condições do meio de cultura de *Azospirillum brasilense*

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Abstract

Agricultural management using technologies that help farmers increase productivity and reduce production costs must be promoted to ensure agricultural sustainability. The objective of the study was to achieve the pH effect of growth solution, chemical treatment, use of osmoprotector additive and mineral nitrate presence, on the activity of growth promoting bacteria, *Azospirillum brasilense*, and its effects on the physiological quality of seeds and wheat seedling growth. The first experiment evaluated the physiological quality of seeds and the second experiment was divided into four, evaluating the growth of wheat seedling in a hydroponic system. The experiments were prolonged in a very randomized design, with four replications. The physiological quality of the seeds was evaluated by germination tests, first germination count, length of the shoot and root and dry mass of the shoot and root. Initial growth was evaluated by quantifying the dry mass of the leaf shoot and root and the root system intervals. The pH of the solution and the presence of nitrogen did not influence the effects of inoculation of the *A. brasilense* bacteria. With the use of chemical treatment and osmoprotective additive, *A. brasilense* had no effect on the growth of wheat seedlings.

Keywords: hydroponics, root parameters, vigor.

Resumo

A gestão agrícola utilizando tecnologias que ajudem os agricultores a aumentar a produtividade e reduzir os custos de produção deve ser promovida para garantir a sustentabilidade agrícola. O objetivo desta pesquisa foi avaliar o efeito do pH da solução de imersão, tratamento químico, uso de aditivo osmoprotetor e presença de nitrogênio mineral, sobre a atividade da bactéria promotora de crescimento, *Azospirillum brasilense*, e seus efeitos sobre a qualidade fisiológica de sementes e crescimento de plântulas de trigo. O primeiro experimento avaliou a qualidade fisiológica de sementes e o segundo experimento foi dividido em quatro, avaliando o crescimento de mudas de trigo em sistema hidropônico. Os experimentos foram conduzidos em delineamento inteiramente casualizado, com quatro repetições. A qualidade fisiológica das sementes foi avaliada pelos testes de germinação, primeira contagem de germinação, comprimento da parte aérea e da raiz e massa seca da parte aérea e da raiz. O crescimento inicial foi avaliado pela quantificação da massa seca da parte foliar aérea e da raiz e parâmetros do sistema radicular. O pH da solução e a presença de nitrogênio não influenciaram os efeitos da inoculação da bactéria *A. brasilense*. Com o uso de tratamento químico e aditivo osmoprotetor, *A. brasilense* não teve efeito sobre o crescimento de plântulas de trigo.

Palavras-chave: hidroponia, parâmetros de raiz, vigor.

1. Introduction

Among the alternatives for obtaining wheat seedlings with rapid growth and development, homogeneous and vigorous stand, can be associated with seeds having growth-promoting bacteria (Ludwig et al., 2018). Positive results have been observed with inoculation of wheat crop using *Azospirillum brasilense* (Galindo et al., 2019; Munareto et al., 2019; Zaheer et al., 2019).

These bacteria alter the hormonal and structural processes of the seedlings, increasing their growth and initial development (Boleta et al., 2020). The main phytohormones produced are gibberellic and indoleacetic acid (Gul et al., 2023; Fukami et al., 2018; Jalal et al., 2022), who work in processes such as germination (mobilization of endosperm reserves) and initial seedling growth

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(cellular elongation). The number of roots and volume of explored soil increase (Gul et al., 2023), improving the efficiency of absorbing water and nutrients from the soil (Rana et al., 2022).

However, responses are associated with favorable edaphoclimatic conditions for bacterial survival processes, association and stimulation of phytohormone production (Ludwig et al., 2022). Among the factors that can affect the interaction between plant and *A. brasilense* are the pH of the soaking solution, mixture with chemical treatments, the use of osmoprotective additives and the presence of mineral nitrogen. Bacteria activity increases in soils with a pH between 6 to 6.5 (Pinto et al., 2017). In general, tropical soils are acidic, causing phytotoxicity due to the action of the elements iron and aluminum, in addition, they reduce the availability of nutrients for plants, being harmful to the development and establishment of microorganisms.

Chemical seed treatment (TS) is performed to protect the seedlings and ensure uniformity in the initial development of the crop (Freiberg et al., 2017). The use of TS together with biological products can reduce the effectiveness of non-target microorganisms (Vogel et al., 2015; Munareto et al., 2018).

As an alternative to protect the bacteria, there is the use of osmoprotective additives with the inoculant, in order to form a film and prevent their direct contact with the chemical (Fipke et al., 2019). However, the use of a layer around the seed can harm the process of water imbibition and oxygen entry, hindering the initial germination process.

A. brasilense provides numerous benefits for the promotion of plant growth such as nitrogen fixation (Fukami et al., 2018). However, the efficiency of the bacteria can be reduced with the presence of high levels of nitrogen fertilizers (Fukami et al., 2016).

Thus, considering the potential of *A. brasilense* in promoting wheat growth, the objective of this research was to evaluate the effect of the pH of the soaking solution, chemical treatment of seeds, use of an osmoprotective additive and the presence of mineral nitrogen, on the activity of *A. brasilense* and the yours effects on the physiological quality of seeds and seedling growth.

2. Material and Methods

To characterize the response to seed inoculation with *A. brasilense* on the physiological quality and growth of wheat seedlings, the research was divided into two studies. The first study evaluated the physiological quality of seeds in response to inoculation with the bacteria and the second study was divided into four experiments evaluating the growth of wheat seedlings.

2.1. Physiological quality of seeds

The experiment was conducted in the first half of 2018. Seeds of the cultivar TBio Toruk (Biotrigo Genética Ltda) were used, which presented 13.4% and 41.3 g, respectively, for water content and the mass of 1,000 seeds (Brasil, 2009). The experimental design was completely randomized and the treatments distributed in a 2×8 bifactorial, with four replications. The first factor consisted of the pH levels of the soaking solution (4.8 and 6.5) and the second, the forms of inoculation, namely: control (test), inoculation with *A. brasilense* (A), chemical treatment (TS), osmoprotective additive (O), A+O, A+TS, O+TS and A+O+TS.

In preparing the soaking solution, HCl was used to adjust the pH value of 4.8 and NaOH to a value of 6.5 (value considered ideal for bacterial growth). As chemical treatment, *Imidacloprid* + *Thiodicarb* (Cropstar®) and *Triazol* (Baytan®) were used, both at a dose of 3 mL kg^{-1} of seed. The application of the TS was carried out the day before the installation of the experiment, from the homogenization of products and seeds inside plastic packaging.

A product containing strains Ab-V_5 and Ab-V_6 of the bacterium *A. brasilense* (2 x 10^8 UFC mL^{-1}) was used as inoculant, at a dose of 100 mL for 40 kg of seed (Azototal® liquid). The osmoprotective additive, composed of encapsulating osmoprotective biopolymers and a complex of sugars, was applied at a dosage of 100 mL for 40 kg of seed (Protege TS®). The application of the inoculant and the additive was carried out on the day of installation of the experiment, separately or mixed according to the treatment, from the homogenization of the products and the seeds inside a plastic package.

For the evaluation of the physiological quality of the seeds, the following tests were carried out: germination, considering eight subsamples of 50 seeds per treatment, placed in paper rolls, moistened with distilled water in an amount corresponding to 2.5 times the dry paper mass. The rolls were placed in closed plastic bags and kept in BOD regulated at a temperature of 30 °C for 8 days. The evaluation was carried out eight days after the installation of the test and the results expressed as percentage of normal seedlings (Brasil, 2009); first germination count, was performed together with the germination test, to evaluate the number of normal seedlings on the fifth day after the beginning of the test. The results were expressed as percentage of normal seedlings (Brasil, 2009); shoot and radicle length were conducted together with the first germination count test, measuring 10 seedlings per treatment. The average lengths of shoots and roots were obtained by dividing the sum of the measurements observed in the subsamples by the number of seedlings, and the results were expressed in cm seedling^{-1} and, seedling dry mass, was quantified in the seedlings used in the determination of shoot and root length. Each repetition was placed in paper bags and taken to an oven with forced air circulation, kept at a temperature of 55 °C for 72 hours. Afterwards, each repetition was weighed on a precision scale, with the results expressed in g seedling^{-1}.

2.2. Seedling growth

In order to isolate the effects of the studied factors on the initial growth of seedlings, the second study was divided into four substudies, carried out in a completely randomized design, with treatments distributed in a 2×2 bifactorial, with four replications (Table 1).

The experiments were carried out in a hydroponic system in a greenhouse. Seeds of the cultivar TBio Toruk (Biotrigo Genética Ltda) were used. Initially, the seeds were placed on paper moistened with distilled water in

Table 1. Description of treatments according to subdivision on initial seedling growth.

Subdivisions	Trat. Fitos.*	*A. brasilense*	Additive	Nitrogen	pH solution
[1]Experiment 1	Presense	Presence	-	-	6.5
	Absence	Absence			
[2]Experiment 2	Present	Presençe	Presençe	-	6.5
		Absence	Absence		
[3]Experiment 3	Present	Presençe	-	-	4.8
		Absence			6.5
[4]Experiment 4	-	Presençe	-	Presençe	6.5
		Absence		Absence	

*Trat Fitos.; chemical treatment, *A. brasilense*: inoculation with *A. brasilense*, Additive: osmoprotective additive, Nitrogen: nitrogen in the nutrient solution, pH: pH of the hydroponic solution; [1]In order to evaluate the influence of chemical treatment of seeds on the activity of *A. brasilense* and the effects of the bacteria on the initial growth of seedlings; [2]In order to evaluate the use of the osmoprotective additive as a technology to reduce the harmful effect caused by chemical treatment on the activity of *A. brasilense*; [3]In order to evaluate at which pH value of the hydroponic solution, the plants show the greatest growth and the bacteria the best effect on the initial growth of seedlings; [4]Aiming to evaluate the influence of nitrogen in the nutrient solution on the activity of *A. brasilense* and the effects of the bacteria on the initial growth of seedlings.

an amount corresponding to 2.5 times the mass of the dry paper, and the paper rolls were placed in plastic bags and kept in BOD regulated at a temperature of 25 °C for nine days.

On the tenth day after sowing, uniform and representative seedlings of the set were selected, the seeds were detached and transferred to the hydroponic system. The nutrient solution was composed of: $CaCl_2$: 221.96 mg L^{-1}; $MgSO_4$: 246.40 mg L^{-1}; KH_2PO_4: 544.40 mg L^{-1}; $FeSO_4$/Na-EDTA: 13.34 mg L^{-1} and complete micronutrient solution: Mo: 0.09 mg L^{-1}; B: 1.54 mg L^{-1}; Cu: 0.11 mg L^{-1}; Mn: 0.39 mg L^{-1}; Zn: 0.57 mg L^{-1} and Ni: 0.04 mg L^{-1}. Based on the initial pH of the solution, HCl was used to adjust the pH to 4.8 and NaOH to 6.5. As chemical treatment, osmoprotective additive and inoculant, the same products and application methods of the first study were used.

The hydroponic system was composed of trays and each comprised a plot where ten plants were grown for 18 days after transplantation. The replacement of the nutrient solution was performed every six days. On the eighteenth day the plants were collected, shoot and root were separated and the following were determined: leaf area, dry mass of the aerial part and roots and parameters of the root system.

Leaf area and root system parameters: mean diameter of roots, total root length, length of axial roots, length of lateral roots, total volume, volume of axial roots, volume of lateral roots and surface area were obtained through analysis of images using WinRhizo Pro 2013 software, coupled to an EPSON Expression 11000 scanner equipped with additional light (TPU), at 600 dpi definition, with values expressed per plant.

The length, volume and root area of the axial and lateral roots were extracted from the total diameter classes obtained by WinRhizo, being considered axial roots those with a diameter greater than 0.3 mm and lateral roots those with a diameter less than 0.3 mm. For this classification, a previous test was performed, separating lateral roots from the main ones, obtaining these values as mean values. After the evaluations, the material was placed in paper bags and dried in an oven with forced air circulation at 40 °C, the dry mass of the shoot and root was determined, and the values were expressed in g $plant^{-1}$.

2.3. Statistical analysis

The data collected for both studies were submitted to variance analysis (ANOVA), and when a significant difference was observed between treatments, the complementary Scott-Knott mean separation test was performed at 5% probability of error, with the aid of the Sisvar program (Ferreira, 2011).

3. Results and Discussion

3.1. Physiological quality of seeds

The percentage of normal seedlings in the germination test was not influenced by the pH of the solution or treatments applied via seed. This is probably because in germination the embryonic axis uses resources acquired during the formation and maturation of seeds (Cotrim et al., 2016). In addition, *A. brasilense* associates with seedlings only after radicle emission, not interfering with seed germination (Rampim et al., 2012).

In the first germination count, seeds that were only inoculated had the seedling vigor preserved, with values similar to the control (Figure 1A). The seeds that received chemical treatment or osmoprotective additive, even in the presence of *A. brasilense*, presented lower values to

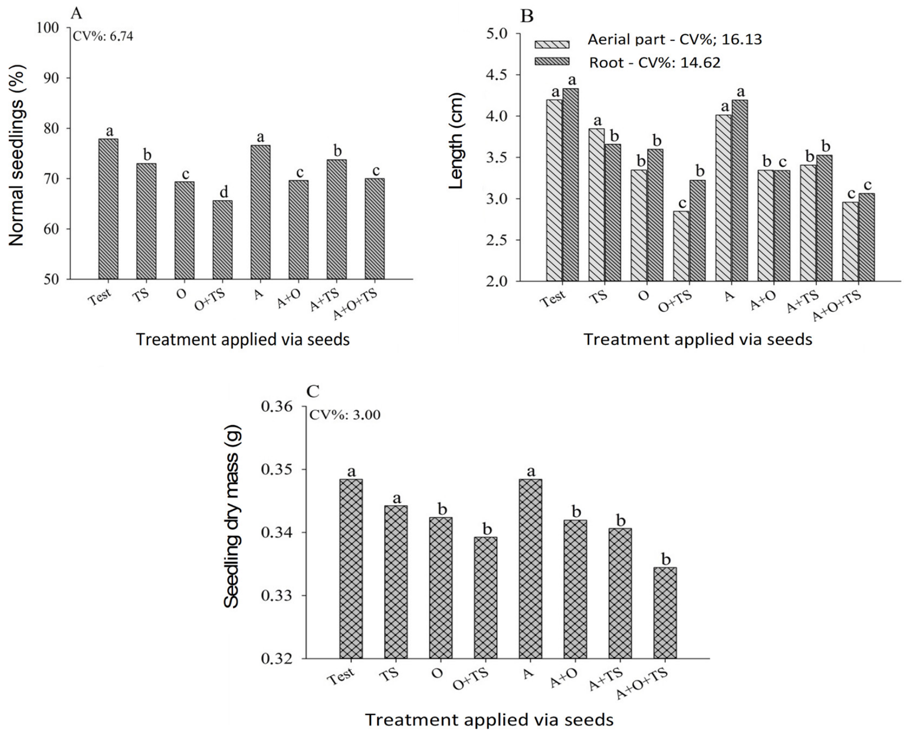

Figure 1. Effect of treatments applied to seeds on the first germination count (A), shoot and root length (B) and seedlings dry mass (C) of wheat. Control (test), inoculation with *A. brasilense* (A), Chemical treatment of seed (TS), osmoprotective additive (O); Bars with the same letter do not differ statistically from each other by the Scott-Knott test (α≤0.05).

both the control and the inoculation of the bacterium in isolation. The use of TS + osmoprotective additive reduced the vigor in relation to the control by 15.73%.

The results obtained in the present experiment reinforce the results reported by Ludwig et al. (2018) and Munareto et al. (2018) indicating the phytotoxic effect of chemical treatment on seedling germination and growth. The germination and vigor of seeds are reduced with the addition of products, live or synthetic, on the seeds (Fipke et al., 2019). In soil this phytotoxicity may not occur, because part of the product is subject to adsorption in soil colloids (Baćmaga et al., 2019) or is leached or diluted by rainfall (Fagundes et al., 2017).

In the treatment with the osmoprotective additive, it is assumed that the increase in the number of physical barriers around the seeds may have caused a reduction in the imbibition speed and the oxygen flow into the seed, decreasing the speed of the metabolic reactions involved with the germination (Melo et al., 2015). Furthermore, the increase in the number of physical barriers that the embryonic axis had to break may have caused a reduction in germination speed.

The length of the shoot and root, similar to that observed in the first germination count, was lower with the application of the osmoprotective additive and chemical treatment (Figure 1B). In the aerial part (shoot), the reduction in relation to the control was 32 and 29%, respectively, with the use of osmoprotective additive + TS and *A. brasilense* + osmoprotective additive + TS. In the root system, the reductions were 16 and 29%, in the use of osmoprotective additive + TS and *A. brasilense* + osmoprotective additive. These treatments germinated later, with less time for seedling growth (Camargo et al., 2022).

The reduction in seedling vigor was also observed in the seedling dry mass. As the number of products applied on the seeds increased, there was a gradual reduction in the seedling dry mass (Figure 1C). Possibly the absence of increase in seedling vigor by inoculation is due to the fact that the association between the bacterium and the plant takes some time to be verified and, therefore, benefits are observed.

3.2. Seedling growth

3.2.1. Experiment 1

The inoculation increased by 18% the dry mass of roots and 15% the total volume of roots in relation to the non-inoculated. The greater volume of axial roots in the inoculated seedlings (22%) was possibly responsible for the increase in the total volume and dry mass of roots, since the volume of laterals was not altered (Figure 2).

By changing the morphology of the roots, the bacteria make it possible to explore a greater volume of soil, increasing the assimilation capacity of the nitrate available in the soil (Galindo et al., 2018) and biological fixation of atmospheric nitrogen (Martins et al., 2018). Among these mechanisms the increase of the root system may be the most important, since it allows greater absorption of minerals and water (Galindo et al., 2018).

The production of phytohormones by bacteria may be the factor responsible for the effect observed in root growth. One of the hormones produced by strains of *A. brasilense* is 3-indoleacetic acid (AIA) (Jalal et al., 2022; Fukami et al., 2018), root growth being more sensitive to the addition of auxin than shoot cells. In the chemical treatment of seeds, it was observed that seedlings from treated seeds presented a 35% reduction in leaf area, 28% in shoot dry mass, 30% in root dry mass, 6% in mean root diameter, 42% in length of roots, 32% in the volume of roots and 40% in the surface area in relation to the untreated ones (Figure 3).

The fact that seedlings from seeds without chemical treatment were superior to those from treated seeds can be explained by a possible phytotoxic effect of the products on plant development. In the studies by Ludwig et al. (2018), seed treatment with triadimenol fungicide impaired wheat seedling emergence and germination.

This reduction in seedling vigor can be explained by the toxic effects exerted by these chemicals (Vogel et al., 2015). In addition, the absence of interaction between the factors led to the assumption that the chemical treatment is toxic to bacteria, since there was no difference between the presence or absence of bacteria when using the chemical treatment, these values being lower than those observed for seedlings only with inoculation (Figure 2).

3.2.2. Experiment 2

There was no influence of the use of the osmoprotective additive on the responses presented by inoculation with *A. brasiliense* on seedling growth. However, there was a reduction in shoot dry mass and length, volume and root surface area with the use of the additive (Figure 4). Possibly, the additive increased the retention of the chemical treatment on the surface of the seeds, increasing its phytotoxic effects on the seedlings (Fipke et al., 2019). Furthermore, the increase in the number of physical

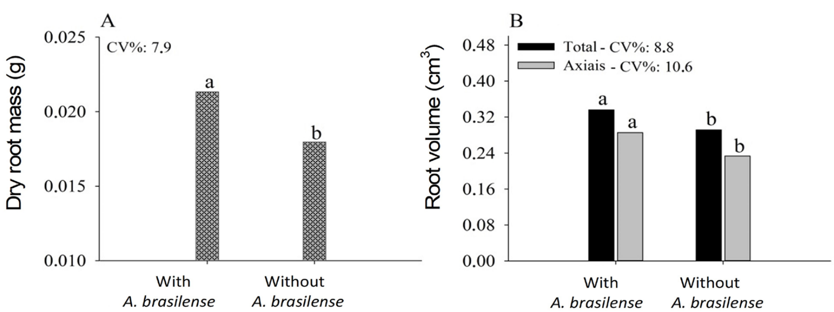

Figure 2. Response of inoculation with *A. brasilense* on the dry mass of roots (A) and root volume (B.) of wheat seedlings at 28 days after sowing. Bars with the same letter do not differ statistically from each other by the Scott-Knott test (α≤0.05). CV: Coeficient of variation

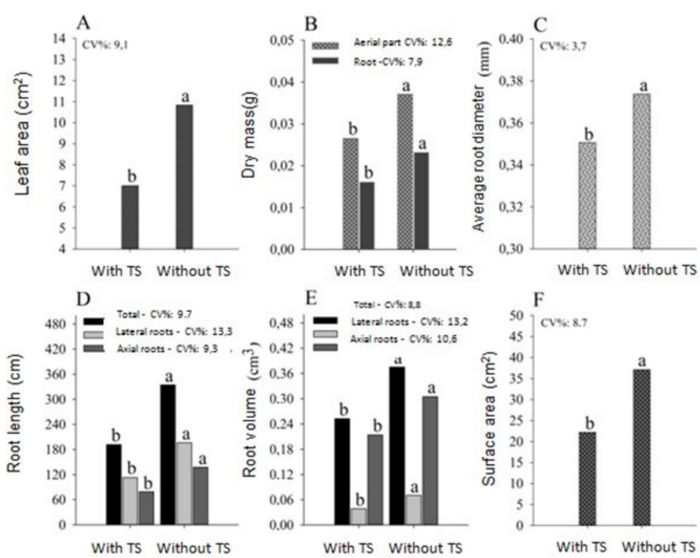

Figure 3. Effect of the chemical treatment of seeds on the leaf area (A), aerial part dry mass and roots (B.), mean root diameter (C), root length (D), root volume (E) surface area (F) of wheat seedlings at 28 days after sowing. Bars with the same le CV: Coeficient of variation do not differ statistically from each other by the Scott-Knott test (α≤0.05). CV: Coeficient of variation

barriers that the embryonic axis had to break may have caused a reduction in the speed of the germination process. These seeds germinated later, leaving less time for seedlings to grow.

Similar to what was observed in substudy 1, when *A. brasilense* was used, an increase of 21% was observed in shoot dry mass, 44% in total root length, 18% in lateral root volume and 38% in surface area (Figure 5).

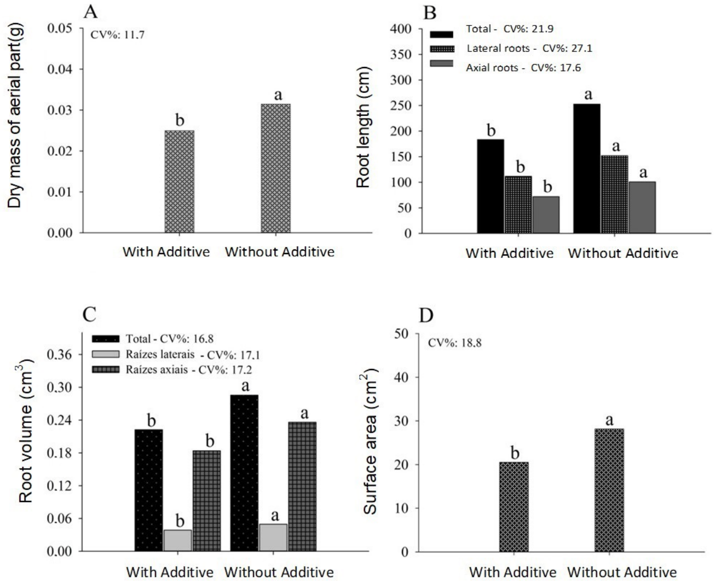

Figure 4. Effect of the use of osmoprotectant additive on the aerial part dry mass (A), root length (B), root volume (C) and surface area (D) of wheat seedlings at 28 days after sowing. Bars with the same letter do not differ statistically from each other by the Scott-Knott test (α≤0.05). CV: Coeficient of variation.

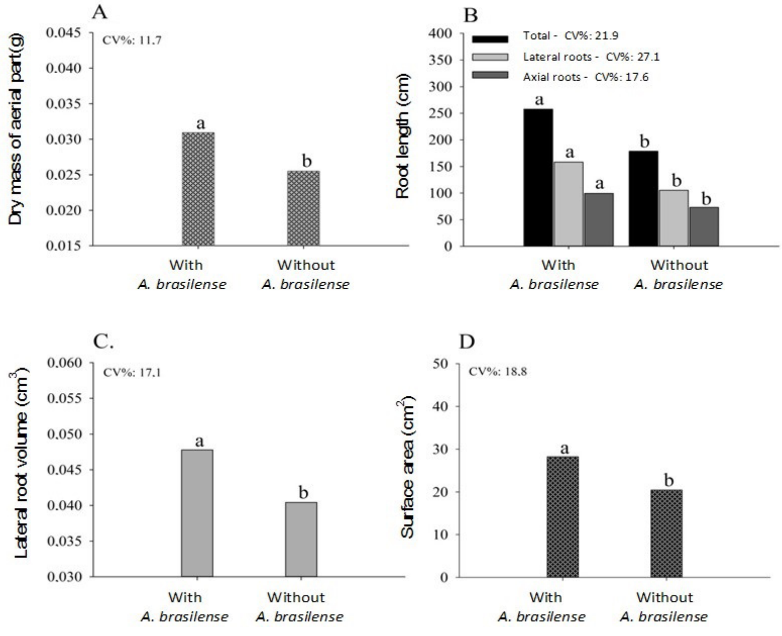

Figure 5. Effect of seed inoculation with *A. brasilense* on aerial part dry mass (A), root length (B), lateral root volume (C) and root surface area (D) of wheat seedlings at 28 days after sowing. Bars with the same letter do not differ statistically from each other by the Scott-Knott test (α≤0.05). CV: Coeficient of variation

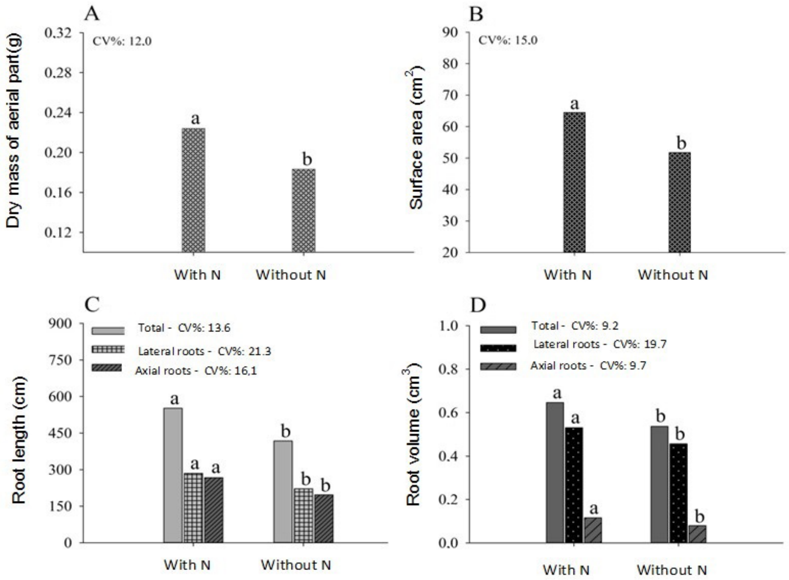

Figure 6. Effect of nitrogen on nutrient solution on shoot dry mass (A), surface area (B), root length (C) and root volume (D) of wheat seedlings at 28 days after sowing. Bars with the same letter do not differ statistically from each other by the Scott-Knott test. CV: Coeficient of variation

3.2.3. Experiment 3

There was no difference in seedling growth as a function of nutrient solution pH, results that agree with those mentioned by Pinto et al. (2017). These authors stated that the high H^+ activity in the solution (pH values equal to or greater than 4) is not a limiting factor for normal plant growth, if there is an adequate supply of all essential nutrients, without the presence of toxic elements. Furthermore, no change in the response to inoculation was observed as a function of the pH of the solution. The concentration of H^+ ions appeared not to have any effect on *A. brasilense.* However, in soil, the indirect influence of soil solution pH on the availability of nutrients and toxic compounds to plants and microbial cells can restrict root growth and reduce the activity of *A. brasilense*.

Similar to what was observed in the previous substudies, there was an effect of inoculation with *A. brasilense* on seedling growth, resulting in an increase of 37% in leaf area, 44% in shoot dry mass, 38% root dry mass, 55% in surface area, 57% in total root length and 49% in total root volume with the use of the bacteria.

3.2.4. Experiment 4

There was no effect of the interaction between inoculation with *A. brasilense* and use of N in the composition of the nutrient solution. However, similar to what was observed in the previous substudies, there was an increase in seedling growth with the inoculation of the bacteria. Possibly, in the presence of nitrogen, *A. brasilense* could induce greater initial plant growth, since the $NH_4H_2PO_4$ and NH_4NO_3 sources stimulate the production of indoles, when in low concentrations as in this study condition (Pham et al., 2022), which was not observed in this study.

The presence of N in the nutrient solution resulted in an increase of 22% in shoot dry mass, 32% in root length, 20% in root volume and 24% in surface area (Figure 6). N is related to photosynthesis, respiration, growth and root activity, in addition to the ionic absorption of other nutrients. Thus, nitrogen is one of the nutrients that promote greater morphophysiological changes in the plant (Nakao et al., 2018).

4. Conclusions

The number of normal seedlings is not influenced by the pH of the soaking solution and by the treatments applied via seed; however, there is a reduction in seedling vigor with the addition of chemical treatment or osmoprotective additive.

The pH of the solution and the presence of nitrogen, in the tested values, did not influence the effects observed with the inoculation of *A. brasilense.*

With the use of chemical treatment and osmoprotective additive, there was no effect of *A. brasilense* on wheat seedling growth.

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