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Initial development of plants of wheat in response to association with diazotrophic bacteria and acid solution irrigator levels

Desenvolvimento inicial de plantas de trigo em resposta à associação com bactérias diazotróficas e níveis de irrigação por solução ácida

Desarrollo inicial de plantas de trigo en respuesta a la asociación con bacterias diazotróficas y niveles de irrigador en solución ácida

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Resumo

As espécies cultivadas possuem pH ideal do solo para absorção de nutrientes e desenvolvimento adequado, sendo necessário conhecer o desempenho das bactérias fixadoras de nitrogênio e promover o crescimento das plantas em diferentes níveis de acidez, a fim de aumentar os resultados para aumentar o rendimento das culturas com o uso de inoculação de tecnologia de sementes de gramíneas. Assim, o presente estudo teve como objetivo determinar o desenvolvimento inicial de mudas de trigo submetidas à inoculação com *Azospirillum brasilense* e *Herbaspirillum seropedicae* em diferentes níveis de solução de irrigação por acidez em substrato de areia autoclavado. Adotou-se o delineamento em blocos casualizados (RBD) em fatorial 3x4 com três níveis de acidez da solução (pH 4,5, 5,5 e 6,5) e o segundo fator [sem inoculação, sementes inoculadas no momento da semeadura com *A. brasilense* (AbV5), *H. seropedicae* (SmR1) e associação das duas cepas AbV5 + SmR1. O aumento do comprimento radicular foi detectado em pH 4,5, comparado a pH 5,5 e 6,5, independentemente ou não de *A. brasilense* e *H. seropedicae*. A inoculação com *A. brasilense* promove maior coleta de diâmetro, independentemente do pH e aumento do volume radicular para pH 6,5 sozinho ou associado a *H. seropedicae*.

Palavras-chave: *Azospirillum brasilense*; *Herbaspirillum seropedicae*; Bactérias promotoras de crescimento de plantas.

Abstract

The cultivated species have ideal soil pH for absorption of nutrients and proper development, it is necessary to know the performance of nitrogen-fixing bacteria and promoting plant growth (PGPBs) at different levels of acidity, in order to increase the results to elevate crop yields with the use of technology inoculation of seeds of gramineous. Thereby, the present study aimed to determine the initial development of wheat seedlings subjected to inoculation with *Azospirillum brasilense* and *Herbaspirillum seropedicae* at different levels of acidity irrigating solution in autoclaved sand substrate. Adopted the randomized block design (RBD) in a 3x4 factorial design with three levels of acidity of the solution (pH 4.5, 5.5 and 6.5) and the second factor [without inoculation, seeds inoculated at the time of seeding with *A. brasilense* (AbV5), *H. seropedicae* (SmR1) and association of the two strains AbV5+ SmR1. Increased root length was detected at pH 4.5 compared to pH 5.5 and 6.5 regardless or not with *A. brasilense* and *H. seropedicae*. Inoculation with *A. brasilense* promotes greater diameter collect, regardless of the pH and increase in root volume to pH 6.5 alone or associated with *H. seropedicae*.

Keywords: *Azospirillum brasilense*; *Herbaspirillum seropedicae*; Plant growth promoting bacteria.

Resumen

Las especies cultivadas tienen un pH ideal del suelo para la absorción de nutrientes y el desarrollo adecuado, es necesario conocer el rendimiento de las bacterias fijadoras de nitrógeno y promover el crecimiento de las plantas a diferentes niveles de acidez, con el fin de aumentar los resultados para aumentar los rendimientos de los cultivos con el uso de tecnología de inoculación de semillas de gramíneas. De este modo, el presente estudio tuvo como objetivo determinar el desarrollo inicial de las plántulas de trigo sometidas a inoculación con *Azospirillum brasilense* y *Herbaspirillum seropedicae* a diferentes niveles de solución de irrigación de acidez en sustrato de arena en autoclave. Adoptó el diseño de bloques al azar (RBD) en un diseño factorial 3x4 con tres niveles de acidez de la solución (pH 4.5, 5.5 y 6.5) y el segundo factor [sin inoculación, semillas inoculadas en el momento de la siembra con *A. brasilense* (AbV5), *H. seropedicae* (SmR1) y asociación de las dos cepas AbV5 + SmR1. Se detectó un aumento en la longitud de la raíz a pH 4.5 en comparación con pH 5.5 y 6.5 independientemente o no con *A. brasilense* y *H. seropedicae*. La inoculación con *A. brasilense* promueve una mayor recolección de diámetro, independientemente del pH y el aumento del volumen de la raíz a pH 6.5 solo o asociado con *H. seropedicae*.

Palabras clave: *Azospirillum brasilense*; *Herbaspirillum seropedicae*; Bactérias promotoras de crescimento de plantas.

1. Introduction

Diazotrophic bacteria can be used to partially replace nitrogen fertilizers (Novakowiski et al., 2011; Piccinin et al., 2013), because they promote increase in root length, number of roots and radicial roots, being called plant growth promoting bacteria (PGPBs), due the production and estimulaton of hormone that potentiate the plant development (Egamberdieva, 2012). Several studies have been carried out to investigate the synergism between (PGPBs) and cultivated species, corn, wheat, *Brachiaria* sp. Some bacteria were isolated and are currently studied, such as the species *Azospirillum brasilense* and *Herbaspirillum seropedicae*. Kuss et al. (2007) and Vogel et al. (2013) reported the occurrence and activity of these bacteria in soil and plant, that favorable environmental conditions are strongly influenced by physical, chemical and biological stresses.

According to Rufini et al., (2011), the pH of the solution affects the action of the BNFs, increasing the pH stimulates the symbiosis between bacteria and plants. The sensitivity of the interaction of diazotrophic bacteria with the plant species is strictly related to abiotic factors, such as acidity, which in turn affects the microbiota, limiting its survival, persistence and multiplication (Ferreira et al., 2014). According to Novais et al. (2007) the ideal soil pH range for agriculture is between 5.5 and 6.5. The activity of the PGPBs grow at an ideal pH range of 6.0 to 7.0 and few grow well at pH below 5.0 (Rodrigues et al., 2006; Ali et al., 2009).

In this context, it is appropriate to know the relationship between plant and bacteria, because the cultivated species lack an ideal soil pH for adequate nutrient absorption and adequate development. Thus, it is necessary to know the performance of PGPBs in different levels of acidity, to potentialize utilization in order to increase crop productivity. Thus, the objective of this work was to verify the initial development of wheat seedlings submitted to inoculation of *A. brasilense* and *H. seropedicae* at different levels of acidity of the irrigating solution in autoclaved sand substrate.

2. Methodology

The experiment was conducted at the Station of Horticulture and Protected Cultivation "Prof. Dr. Mário César Lopes "belonging to the State University of the West of Paraná, Campus of Marechal Cândido Rondon - PR, under coordinates 54º 22 'W, 24º 46' S and altitude around of 420 m.

The experimental design was a randomized blocks (CRB), in a 3x4 factorial scheme, with four replications totaling 48 experimental plots, formed by three acid levels of the irrigating solution in autoclaved sand substrate (pH 4.5, 5.5 and 6.5) And four treatments of seed inoculation, being characterized as witness treatment (absence of seed inoculation); Inoculation of wheat seeds with *Azospirillum brasilense* (AbV5); Inoculation with *Herbaspirillum seropedicae* (SmR1) and inoculation with the association of the two bacterial species (AbV5 + SmR1).

The strains of *A. brasilense* and *H. seropedicae* were supplied by the laboratory of Biochemistry and Molecular Biology of the UFPR of Curitiba. The volume of the inoculating solution from 4 mL to 1000 seeds was used, with a population of 10^7 colony forming units mL^{-1} (CFU mL^{-1}). The seeds of wheat cultivar CD 150 were inoculated in a flow chamber with the aid of plastic bags, homogenizing the distribution of the inoculant, and after one hour sowing was carried out.

Polyethylene trays were used with sand sterilized in an autoclave (20 minutes at 120°C and 1 atm). The experimental plots were organized in trays of 9.46 dm^3, with dimensions of 43.00 x 27.50 x 8.00 cm. 25 seeds were seeded per plot, then irrigated with solution and kept in a germination chamber type BOD at 25°C and photoperiod of 12 hours. Irrigations were performed daily, with standard solution with distilled water at different pH (4.5, 5.5 and 6.5) adjusted with hydrochloric acid (0.1N HCl) and sodium hydroxide (0.1N NaOH).

The seedlings emerged were evaluated per seven days, folowing the Seed Analysis Rules (Brasil, 2009), determining the Emergence Velocity Index (EVI), the first counting of emerged seedlings (%) and percentage of Emerged Plants (%).

After eight days of conduction, ten wheat seedlings from each experimental plot were submitted to shoot length (SL), root (RL), stem diameter (SD) (mm) and root number (NR). With the aid of a graduated cylinder, root volume (cm^3) (RV) was quantified through the technique of displacement of the water column, from the complete immersion of the roots in the same. The seedlings were separated in shoot and root, being forced to the oven of forced circulation of air for drying to 65 ± 2ºC; After 72 hours the samples were weighed, determining the shoot dry matter mass (SM) and root (RM).

The data were tabulated and analyzed in the Sisvar 5.1 Build 72 program (Ferreira, 2019). Variance analysis was performed and Tukey's test was applied at 5% error probability for the means provided for both different pH levels of the irrigating solution and seed inoculation.

3. Results and discussion

According to data from the analysis of variance shown in Table 1, both percentage of emerged plants, first count, root length, stem diameter, root volume and root dry matter mass were statistically different in at least one of the sources of variation analyzed (pH levels and / or inoculation of seeds with bacteria). For shoot length, root number and shoot dry mass, no significant difference was identified.

Table 1 - Analysis of variance for variables measured in the initial development of wheat seedlings up to eight days after sowing, submitted to inoculation of *A. brasilense* and *H. seropedicae* at different levels of acidity of the irrigating solution of the autoclaved sand substrate.

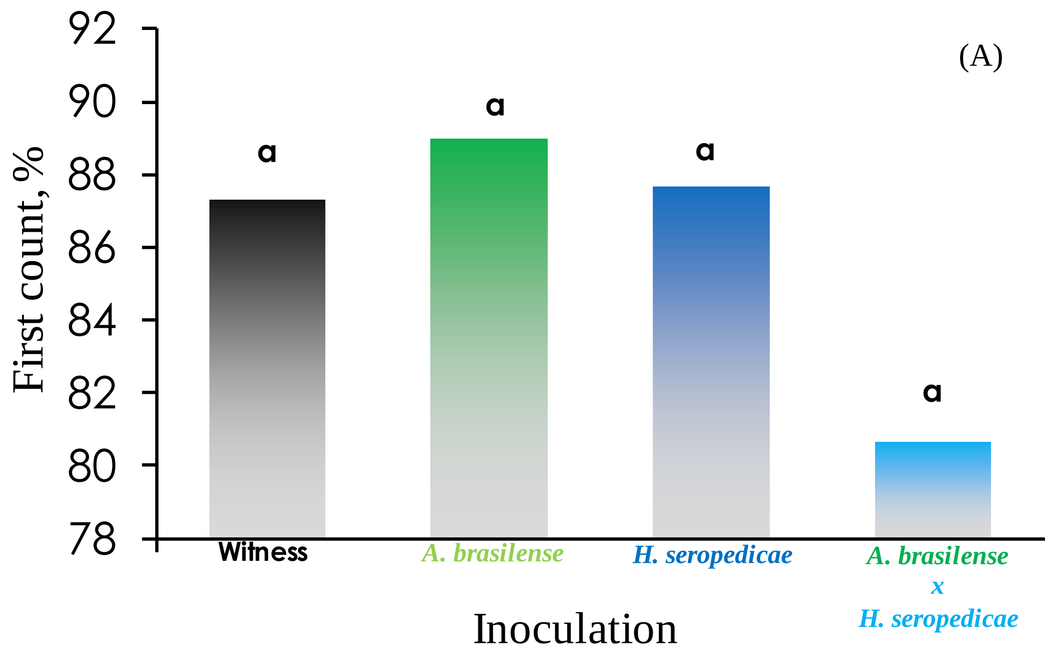
F.V.	% PE	% FC	SL	RL	SD	RV	RN	SM	M
					Valores de F				
Inoculation(I)	8.11*	5.48*	2.57^{ns}	1.34^{ns}	4.99*	1.88^{ns}	2.06^{ns}	1.49^{ns}	0.18^{ns}
pH	2.16^{ns}	1.45^{ns}	4.74^{ns}	13.93**	1.10^{ns}	3.26^{ns}	2.46^{ns}	1.32^{ns}	1.09^{ns}
I x pH	0.42^{ns}	0.49^{ns}	1.66^{ns}	1.85^{ns}	1.66^{ns}	6.21**	0.86^{ns}	1.61^{ns}	3.53*
Average	88.75	86.17	13.14	20.33	1.64	0.84	4.49	9.11	8.50
CV(%)	7.34	9.17	3.61	6.87	3.41	20.46	5.13	12.53	8.19

**significativo a 5% pelo teste F; **significativo a 1% pelo teste F; ^{ns} não significativo pelo teste F. [(1)]PE: emergency percentage; FC: First Count; SL: shoot lenght; RL: Root lenght; SD: Stem diameter; RV: Root volume; RN: Root number; SDMA: Shoot dry mass of aerial; RM: Root dry mass.Source: Authors.

The first count of the emergence during the combined inoculation of *A. brasilense* and *H. seropedicae* was observed to be -7.63% and -9.37% reduction when compared to the witness and the isolated inoculation of *A. brasilense*, respectively (Figure 1A). The percentage of germination was affected only by the inoculation methods, so that the combined inoculation of the bacteria presented germination of 7.06% lower than the witness and of -8.42% when compared to the isolated inoculation of the bacteria (Figure 1B).

The inoculation of two species, nitrogen fixation and / or plant growth promoters with differentiated action characteristics, can maximize the effects of plant growth promotion. However, it has been reported that combined use may initially lead to competition between bacteria for binding and colonization sites, and inhibition of plant development can be observed (Plazinski & Rolfe, 1985). This condition is likely to be observed when using the combination with bacteria in which at least one is required to survive on living plant cells such as *H. seropedicae*.

Figure 1 - Percentage of seedlings emerged at the first count (A) and percentage of emergence (B) of wheat seedlings at 8 days after sowing in the average of the treatments submitted to the inoculation of *A. brasilense* and *H. seropedicae*, regardless of the acidity level of the irrigant solution in sand substrate autoclaved.



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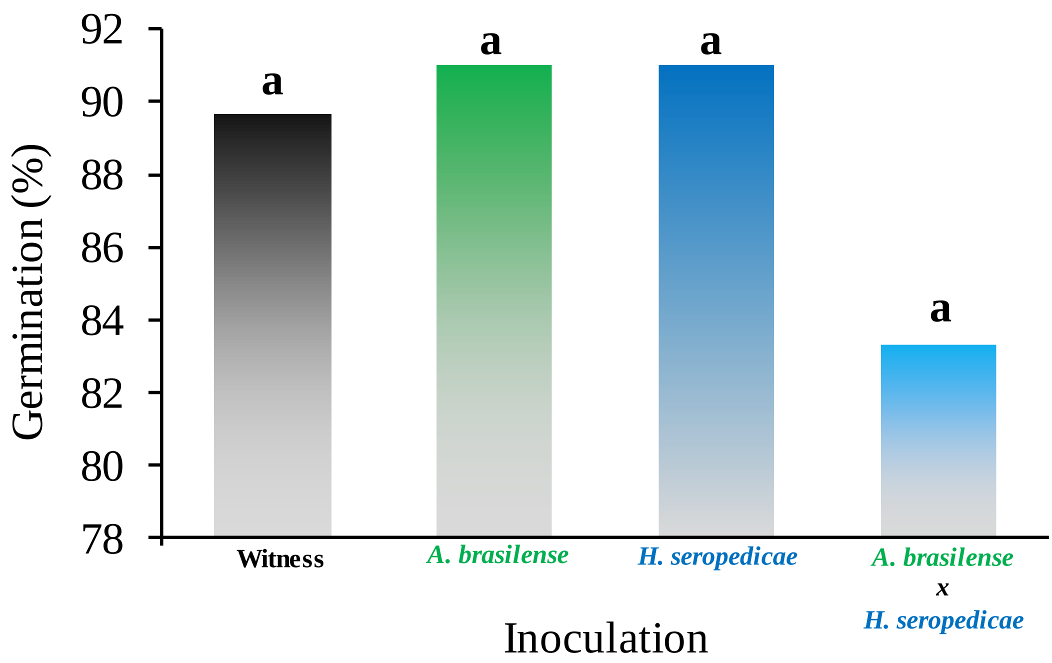
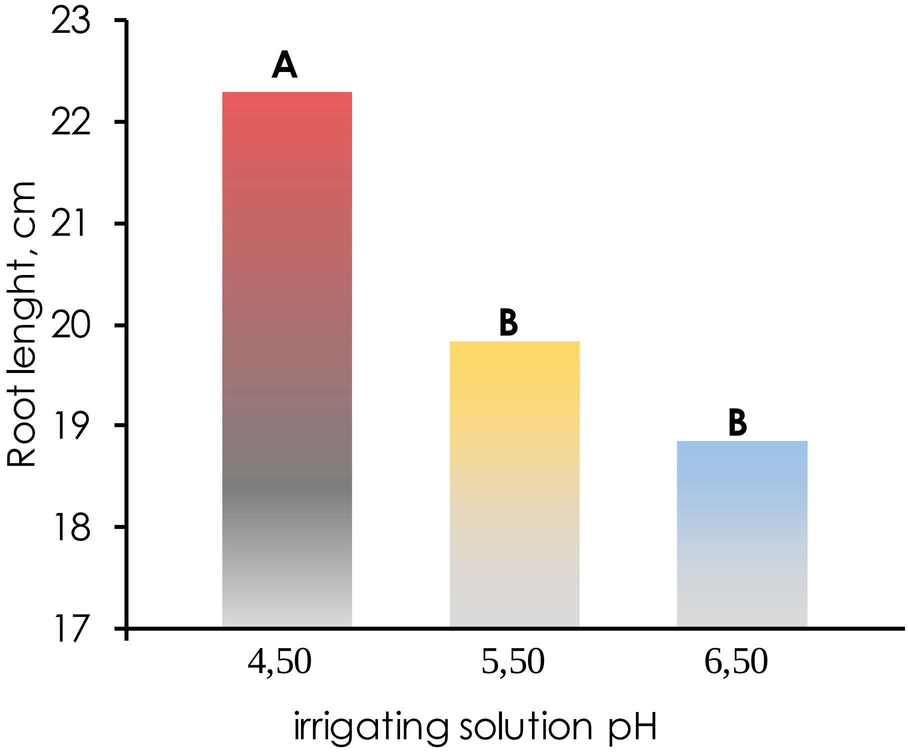
(B)



Source: Authors.

For root length variable there was a significant difference for the substrate pH factors (Figure 2). Seedlings irrigated with pH 4.5 irrigated solution had longer roots than the other pH levels (5.5 and 6.5), independent of seed inoculation. In this way, the use of irrigating solution with pH of 4.5 did not affect the development of wheat seeds until eight days after sowing (DAS), maintaining root development. The increase in root length presented in the solution at pH 4.5 is related to a higher root colonization of the bacteria in a more acidic condition (Dobereiner et al., 1976).

Figure 2 - Root length of wheat seedlings at 8 days after sowing on the average of the treatments submitted to the acid levels of the irrigating solution, independent of seed inoculation, on autoclaved sand substrate.

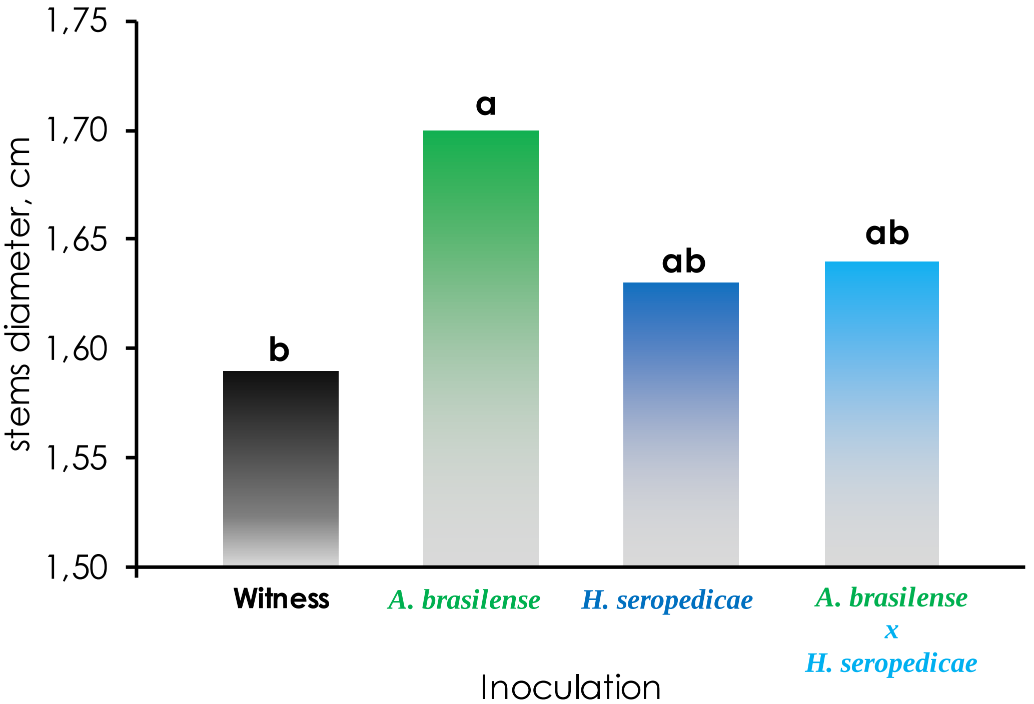


Source: Authors.

Thus, the proximity of the bacteria to the root surface allows the excreted growth promoters to be rapidly assimilated by the plants and consequently stimulating root elongation. It should be noted that rhizosphere is able to change its pH according to plant nutrition (Brachini et al., 2000). The same authors also report that the rhizosphere pH of coffee plants was lower by up to 0.3 points in relation to soil pH. This condition can have a lower pH in the rhizosphere, intensifying the action of bacteria in the rhizosphere, and interfering favorably in the root development. In fact, Dobereiner et al. (1976) demonstrated that in a more acidic substrate, the distribution of diazotrophic bacteria can be exclusively in the rhizosphere of plants. At high concentrations, rhizosphere bacteria synthesize auxin as secondary metabolites due to rich sources of root exudates from plants, providing auxin to plants in the rhizosphere (Egamberdieva, 2012). Bashan et al. (2005) demonstraram que após a remoção das plantas do solo, os materiais residuais remanescentes no solo sustentaram *A. brasilense* por algumas semanas, a partir daí, quando esses nutrientes presumivelmente se exauriram, outros parâmetros abióticos do solo, determinaram a taxa de sobrevivência da bactéria. Quando há elevada rizosfera, *A. brasilense* tem sobrevivência independente da aridez do solo, sobretudo perturbações do solo como percolação de água ou remoção de plantas afetam direta e rapidamente os níveis populacionais de *A. brasilense*.

However, when the stem diameter was observed, only a significant effect of the inoculations was obtained, with higher mean values in the inoculated seedlings with *A. brasilense* presenting a thicker stem diameter than the witness, with an increase of 6.47%, irrespective of the pH of the irrigating solution (Figure 3). However, the inoculation with *H. seropedicae* and the combined use of the *A. brasilense* and *H. seropedicae* strains, the stem diameter was similar to the witness regardless of the acid level of the irrigating solution.

Figure 3 - Corn stem diameter of wheat seedlings at 8 days after sowing, submitted to inoculation of *A. brasilense* and *H. seropedicae*, regardless of the acidity level of the irrigating solution of the autoclaved sand substrate.



Source: Authors.

This result is in agreement with that found by Dartora et al. (2013), in which they used AbV5 (A. brasilense) and SmR1 (*H. seropedicae*) strains, obtained a larger stem diameter in the vegetative and reproductive phases of corn, both in the absence and in the presence of nitrogen. The increase of stem diameter leads to a greater accumulation of soluble solids which, in turn, aid in the formation of grains.

The highest mean root length in 8 DAS for isolated inoculation of *A. brasilense* is related to higher AIA production in relation to *H. seropedicae*, in a similar concentration of bacteria, promoting greater growth, as evidenced by the increase of stem diameter (Perrig Et al., 2007). On the other hand, the joint use of the strains of *A. brasilense* and *H. seropedicae* results in a complex interaction, the interaction between a bacteria and a plant is already complex, with different infectious processes, being positive or negative. Souza et al., (2011) verified a lower total concentration of bacteria in the plant in the interaction between *H. seropedicae* + *A. brasilense* + Leifsonia xyli subsp. Xyli, compare to the isolated use.

Perin et al. (2003) reported better colonization of plants when using the interaction between *A. brasilense* (Sp7) and *H. seropedicae* (Z67) in maize and rice plants. As these authors use in plants with larger seeds such as corn, colonization is favored and increases in plant characteristics are more evident when using combined bacteria, but when using small seeds such as rice, this response is reduced, probably due to lower intensity of excretion of exudates from smaller seeds, which stimulate bacterial colonization. In this way, the combined inoculation in wheat seeds, that is, small seeds, leads to late colonization, when compared to isolated inoculation, reflecting in slower responses. Consequently, the isolated use of *H. seropedicae* due to its action internally in the plant, after colonization in 8 DAS, led to a lower supply of phytohormones during the initial development of wheat, with no difference in stem diameter.

Unlike root length and stem diameter, for root volume there was interaction between solution pH and seed inoculation, so that the pH of 4.5 and 5.5 did not interfere with the seed treatments with *A. Brasilense*, *H. seropedicae* and their association. When the substrate was irrigated with pH 6.5 solution, the treatments that received *A. brasilense* presented higher averages (Table 2).

Such response may comprise elongated and less robust roots (smaller diameter) at pH 4.5 and 5.5. At these pH values of the irrigating solution, the bacteria tend to concentrate in the vicinity of the roots, thus having high concentrations of growth promoters present that stimulate root growth (Table 2), not reflecting differences in root volume. Because in the rhizosphere, they synthesize auxin as secondary metabolites due to the rich sources of root exudates of the plants, providing auxin to the plants in the rhizosphere, thus stimulating root growth (Egamberdieva, 2012). At pH 6.5 the witness and *H. seropedicae* presented roots with lower volume. It was observed that inoculation of seeds with the *H. seropedicae* strain had an unfavorable effect on the root volume, related to the time required for the infectious process during germination (Juge et al., 2012) and the lower production of Phytorials in the establishment (Perrig et al., 2007).

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Table 2 - Volume and dry matter of seedlings of wheat at 8 days after sowing, submitted to inoculation of *A. brasilense* and *H. seropedicae* at different levels of acidity of the irrigating solution of the autoclaved sand substrate.

Inoculation	Root volume (cm³)			Root dry mass (g)		
	pH			pH		
	4,5	5,5	6,5	4,5	5,5	6,5
Witness	0.65Aa	0.58Aa	0.60Ab	0.09Aa	0.08Bb	0.08Aba
Azo	0.80Ba	0.55Ba	1.38Aa	0.08Aa	0.09Aa	0.09Aa
Herb	0.75Aa	0.88Aa	0.90Ab	0.09Aa	0.09ABab	0.08Ba
Azo x Herb	0.85Ba	0.80Ba	1.38Aa	0.09Aa	0.08Aab	0.08Aa

Azo: *Azospirillum brasilense*; Herb: *Herbaspirillum seropedicae* Averages followed by lowercase letters refers to columns and averages with capital letters referring to the rows. Source: Authors.

In the present work, the species *A. brasilense* endophytic with preference for colonization in the rhizosphere, indicated improvements in root volume especially at pH 6.5, ideal for its development in the plants (Döbereiner et al., 1995). This response promotes greater root development with *A. brasilense* (Gitti et al., 2012), which in addition to root elongation promotes the development of secondary lateral roots, allowing greater association with beneficial microorganisms and greater absorption of water and nutrients (Dimpka et al. 2009)

For root dry matter mass, treatments with higher volumes remained higher, related to the positive correlation between these variables. The plants irrigated with solution of pH 5.5 obtained superiority in the inoculation of *A. brasilense* in relation to the witness, but with an average similar to the other treatments of inoculation. There was also no significant difference when inoculated with *A. brasilense* and *H. seropedicae*.

The witness treatment and the inoculation of *H. seropedicae* showed higher averages at pH 4.5, differing significantly from 5.5 and 6.5, respectively. These results can be related to the lower energy expenditure in these conditions, since in pH 4.5 there was a greater development of the root system, as well as for the inoculation with A. brasilense, in a way that reduced the accumulation of dry matter, since the activity Metabolic system of the root system was directed to the initial development of the seedlings and not to the accumulation of dry matter mass.

In the present study, it is possible to observe the increase in the dry mass of roots in the initial stages of crops in different crops, such as soybeans (Cássan et al., 2009; Bulegon et al., 2014), beans (Costa et al., 2013), And wheat (Dartora et al., 2013, Lemos et al., 2013), due to the growth promotion provided by A. brasilense.

4. Considerações Finais

There is a longer root length at pH 4.5 in relation to pH 5.5 and 6.5, regardless of the use or not of *A. brasilense* and *H. seropedicae*.

The inoculation with *Azospirillum brasilense* promotes an increase in the stem diameter independent of pH and raises the root volume when submitted to pH 6.5, isolated or associated with *H. seropedicae*.

Irrigation with solution pH 4.5 and inoculation of *A. brasilense* isolated or associated to *H. seropedicae* promoted longer roots, increasing its contact surface with the substrate.

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Referências

Ali, S. F., Rawat, L. S., Meghvansi, M. K., Mahna, S. K. (2009). Selection of stress-tolerant rhizobial isolates of wild legumes growing in dry regions of Rajasthan, India. *Journal of Agricultural and Biological Science*, 4,13-18.

Bashan, Y., Puente, M. E., Rodriguez-Mendoza, M. N., Toledo, G., Holguin, G., Ferrera-Cerrato R., Pedrin, S. (1995). Survival of *Azospirillum brasilense* in the Bulk Soil and Rhizosphere of 23 Soil Types. *Applied and Environmental Microbiology*, 1938–1945. Retrieved from https://aem.asm.org/content/61/5/1938.short

Braccini, M. C. L., Martinez, H. E. P., Lucca, E., Braccini, A., Mendonça, S. M. (2000). Avaliação do pH da rizosfera de genótipos de café em resposta a toxidez de alumínio no solo. *Bragantia*, 59, 83-88. https://doi.org/10.1590/S0006-87052000000100013.

Brasil. (2009). Regras para análise de sementes. Ministério da Agricultura, Pecuária e Abastecimento. *Secretaria de Defesa Agropecuária. Brasília*, DF: Mapa, 395 p.

Bulegon, L. G., Klein, J., Rampim, L., Guimarães, V. F., Battistus, A. G., & Kestring, D. (2014). Desenvolvimento inicial de plântulas de soja inoculadas e co-inoculadas com Azospirilllum brasilense e Bradyrhizobium japonicum. *Jornal of Agronomic Sciences*, 3,26–37.

Cassán, F., Perriga, P., Sgroya, V., Masciarellia, O., Pennab, C., Lunaa, V. (2009). Azospirillum brasilense Az39 and Bradyrhizobium japonicum E109, inoculated singly or in combinati promote seed germination and early seedling gron corn (*Zea mays* L.) and soybean (Glycine max L.). *European Journal of Soil Biology*, 45, 28–35. https://doi.org/10.1016/j.ejsobi.2008.08.005

Costa, E. M., Nóbrega, R. S. A., Carvalho, F., Trochmann, A., Ferreira, L. D. V. M., & Moreira, F. M. (2013). Promoção do crescimento vegetal e diversidade genética de bactérias isoladas de nódulos de feijão-caupi. *Pesquisa Agropecuária Brasileira*, 48, 1275–1284. https://doi.org/10.1590/S0100-204X2013000900012.

Dartora, J., Guimarães, V. F., Marini, D., Pinto Júnior, A. S., Cruz, L. M., & Mensch, R. (2013). Influência do tratamento de sementes no desenvolvimento inicial de plântulas de milho e trigo inoculados com *Azospirillum brasilense*. *Scientia Agraria Paranaensis*, 12, 175-181.

Dimkpa, C., Weinand, T., & Asch, F. (2009). Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant, Cell and Environment*, 32, 1682-1694. https://doi.org/10.1111/j.1365-3040.2009.02028.x

Döbereiner, J., Baldani, V. L. D., & Baldani, J. I. (1995). Como isolar e identificar BPCP de plantas não-leguminosas. Itaguaí: Embrapa - CNPAB, 60p.

Döbereiner, J., Marriel, I. E., & Nery, M. (1976). Ecological distribution of Spirillum lipoferum *Beijerinckia* spp. *Canadian Journal of Microbiology*, 22, 1464-1473. https://doi.org/10.1139/m76-217

Egamberdieva, D. (2012). Indole-acetic acid production by root associated bacteria and its role in plant growth and development. In: Keller, A. H., & Fallon, M. D. (Ed). Auxins: Structure, Biosynthesis and Functions. Hauppauge: *Nova Science Publishers, Incorporated,* 103-122.

Ferreira, D. F. (2019). Sisvar: a computer analysis system to fixed effects split plot type designs. Revista Brasileira de Biometria, 37, 529-535. http://dx.doi.org/1010.28951/rbb.v37i4.45

Ferreira, E. P. B., Knupp, A. M., & Martin-Didonet, C. C. G. (2014). Crescimento de cultivares de arroz (*Oryza sativa* L.) influenciado pela inoculação com bactérias promotoras de crescimento de plantas. *Bioscience Journal*, 30, 655–665.

Gitti, D. C., Arf, O., Kaneko, F. H., Rodrigues, R. A. F., Buzetti, S., Portugal, J. R., & Corsini, D C. D. C. (2012). Inoculação de Azospirillum brasilense em cultivares de feijões cultivados no inverno. *Revista Agrarian*, 5, 36-46. http://dx.doi.org/10.30612/agrarian.v5i15.1297

Juge, C., Prévost, D., Bertrand, A., Bipfubusa, M., & Chalifour, F. P. (2012). Growth and biochemical responses of soybean to double and triple microbial associations with *Bradyrhizobium*, *Azospirillum* and arbuscular mycorrhizae. *Appliled Soil Ecology*, 61, 147–157. https://doi.org/10.1016/j.apsoil.2012.05.006

Kuss, A. V., Kuss, V. V., Lovato, T., & Flôres, M. L. (2007). Fixação de nitrogênio e produção de ácido indolacético in vitro por bactérias diazotróficas endofíticas. *Pesquisa Agropecuária Brasileira*, 42:1459-1456. https://doi.org/10.1590/S0100-204X2007001000013

Novais, R. F., Alvarez V., V. H., Barros, N. F., Fontes, R. L. F., Cantarutti, R. B., & Neves, J. C. L. (2007). Fertilidade do solo. *Sociedade Brasileira de Ciências do Solo*, 1017.

Lemos, J. M., Guimarães, V. F., Vendrusculo, E. C. G., Santos, M. F., & Offemann, L. C. (2013). Resposta de cultivares de trigo à inoculação de sementes com *Azospirillum brasilense*, e à adubação nitrogenada em cobertura. *Científica*, 41, 189-198. http://dx.doi.org/10.15361/1984-5529.2013v41n2p189-198

Novakowiski, J. H., Sandini, I. E., Falbo, M. K., Moraes, A., Novakowiski, J. H., & Cheng, N. C. (2011). Efeito residual da adubação nitrogenada e inoculação de Azospirillum brasilense na cultura do milho. *Semina: Ciências Agrárias*, 32, 1687–1698. http://dx.doi.org/10.5433/1679-0359.2011v32Suplp1687

Perin, L., Silva, M. F., Ferreira, J. S., Canuto, E. L., Medeiros, A. F. A., Olivares, F. L., & Reis, V. M. (2003) Avaliação da capacidade de estabelecimento endofítico de estirpes de *Azospirillum* e *Herbaspirillum* em milho e arroz. *Agronomia*, 37, 47-53.

Perrig, D., Boiero, M. L., Masciarelli, O. A., Penna, C., Ruiz, O. A., Cassán, F. D., & Luna, M.V. (2007). Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation. *Applied microbiology and Biotechnology*, 75, 1143–1150. http://dx.doi.org/10.1007/s00253-007-0909-9.

Piccinin, G. G., Braccini, A. L., Gomes, L., Dan, D. M., Loli, G., Hossa, K. R., & Ponce, R. M. (2013). Rendimento e desempenho agronônico da cultura do trigo em manejo com *Azospirillum brasilense*. *Revista Agrarian*, 6, 393–401.

Rodrigues, C. S., Laranjo, M., & Oliveira, S. (2006). Effect of heat and pH stress in the growth of chickpea mesorhizobia. *Current Microbiology*, 53, 1-7. http://dx.doi.org/10.1007/s00284-005-4515-8

Rufini, M., Ferreira, P. A. A., Soares, B. L., Oliveira, D. P., Andrade, M. J. B., & Moreira, F. M. S. (2011). Simbiose de bactérias fixadoras de nitrogênio com feijoeiro-comum. *Pesquisa Agropecuária Brasileira*, 46, 81-88. http://dx.doi.org/10.1590/S0100-204X2011000100011

Souza, S. R., Daros, E., Fragoso, R. B., & Ruaro, L. (2011). Efeito de bactérias diazotroficas nos componentes de produção de cana-de-açúcar infectadas pelo raquitismo das soqueiras. *Journal of Biotchnology and Biodiversity*, 2, 21-27. https://doi.org/10.20873/jbb.uft.cemaf.v2n4.souza.

Vogel, G. F., Bichel, A., Martonkoski, L., & Martins, P. J. (2013). Desempenho agronômico de *Azospirillum brasilense* na cultura do arroz: uma revisão. *Revista em Agronegócio e Meio Ambiente*, 6, 567-578.

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