PAB
Pesquisa Agropecuária Brasileira

Microbiology/ Scientific Notes

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Growth promotion in different *Urochloa* species inoculated with *Azospirillum baldaniorum*

Abstract – The objective of this work was to evaluate inoculation response to *Azospirillum baldaniorum* strain Sp245 in 14 *Urochloa* cultivars. The evaluated cultivars were: Marandú, Xaraés, Paiaguás, Piatã, Basilisk, B140, Comum, Tupi, H47, Llanero, Ruziziensis, Ipyporã, Mulato, and Convert HD364. Three experiments were conducted to measure root traits: two using soil and one a sterile substrate. Compared with the control, a single application of *A. baldaniorum* Sp245 increases dry matter accumulation and number of roots in cultivars Marandú, Llanero, Ruziziensis, and Convert HD364. However, after inoculation, cultivars Xaraés and Ipyporã show some root traits with lower values.

Index terms: *Brachiaria*, diazotrophic bacteria, inoculation, pasture.

Promoção de crescimento em diferentes espécies de *Urochloa* inoculadas com *Azospirillum baldaniorum*

Resumo – O objetivo deste trabalho foi avaliar a resposta à inoculação da estirpe Sp245 de *Azospirillum baldaniorum* em 14 cultivares de *Urochloa*. As cultivares avaliadas foram: Marandú, Xaraés, Paiaguás, Piatã, Basilisk, B140, Comum, Tupi, H47, Llanero, Ruziziensis, Ipyporã, Mulato e Convert HD364. Foram conduzidos três experimentos para mensuração de parâmetros radiculares: dois em solo e um em substrato estéril. Em comparação ao controle, a aplicação única de *A. baldaniorum* Sp245 aumenta o acúmulo de massa seca e a quantidade de raízes das cultivares Marandú, Llanero, Ruziziensis e Convert HD364. No entanto, após a inoculação, as cultivares Xaraés e Ipyporã apresentam alguns parâmetros radiculares com valores menores.

Termos para indexação: *Brachiaria*, bactéria diazotrófica, inoculação, pastagem.

Liliandra Barreto Emídio Gomes[1], Thamires Ferreira Rodrigues da Silva[1], Gabriela Cavalcanti Alves[1] and Veronica Massena Reis[2] ✉

[1] Universidade Federal Rural do Rio de Janeiro, Instituto de Agronomia, Departamento de Solos, BR-465, Km 07, Zona Rural, CEP 23890-000 Seropédica, RJ, Brazil.
E-mail: liliandrabarreto@hotmail.com, thami.dasilva@hotmail.com, gabcalves@gmail.com

[2] Embrapa Agrobiologia, BR-465, Km 07, Ecologia, CEP 23891-000 Seropédica, RJ, Brazil.
E-mail: veronica.massena@embrapa.br

✉ Corresponding author

Received
August 08, 2022

Accepted
March 14, 2023

How to cite
GOMES, L.B.E.; SILVA, T.F.R. da; ALVES, G.C.; REIS, V.M. Growth promotion in different *Urochloa* species inoculated with *Azospirillum baldaniorum*. **Pesquisa Agropecuária Brasileira**, v.58, e03079, 2023. DOI: https://doi.org/10.1590/S1678-3921.pab2023.v58.03079.

In Brazilian pastures, the most cultivated grass species are of the family Poaceae, genus *Urochloa* (Torres González & Morton, 2005), mainly *Urochloa decumbens* cultivar Basilisk, *Urochloa humidicola*, and *Urochloa brizantha* (Alvim et al., 2002). The interaction with bacteria can be beneficial for the growth of these plants, resulting in an increased biomass production (Carvalho et al., 2020), increased chlorophyll concentration and stomatal conductance, and improved biological nitrogen fixation (Cassán et al., 2014).

Among the nitrogen-fixing bacteria associated with the genus *Urochloa*, those from the genus *Azospirillum* stand out. Of the 22

species of *Azospirillum* spp., *Azospirillum brasilense* is the most known and used worldwide (Cassán & Diaz-Zorita, 2016; Santos et al., 2021; Cassán et al., 2020). Recently, this lineage was renamed *Azospirillum baldaniorum* (Ferreira et al., 2020) and is currently used as an inoculant in Latin America (Cassan & Diaz-Zorita 2016; Santos et al., 2021). Of the *A. baldaniorum* strains, Sp245 is the most studied and, therefore, its genome has been sequenced by several research groups, preceded by that of Wisniewski-Dyé et al. (2011). This well-characterized strain produces growth regulators, especially from the auxin class (Cassán et al., 2014), which has led it to become a model strain for the study of the plant-bacteria interaction.

Most studies on inoculation, however, only analyze one species of *Urochloa* (Hungria et al., 2016), although several are grown in Brazilian pastures. This shows the importance of evaluating how different species of this grass behave when inoculated with diazotrophic growth-promoting bacteria.

The objective of this work was to evaluate inoculation response to *Azospirillum baldaniorum* strain Sp245 in 14 *Urochloa* cultivars.

The seeds of the 14 studied *Urochloa* cultivars were provided by Embrapa Gado de Corte, located in the municipality of Campo Grande, in the state of Mato Grosso, Brazil. *Azospirillum baldaniorum* Sp245 (strain BR11005) was obtained from Centro de Recursos Biológicos Johanna Döbereiner, the bacterial culture collection of Embrapa Agrobiologia. A seed coating of 250 g peat-based inoculant, at a concentration of 7.2×10^8 cells per gram of peat, was applied to each kilogram of *Urochloa* seeds. A 10% sucrose solution was used as an adhesive solution. After inoculation, the seeds were maintained at 25°C to dry the peat before sowing. The most probable number method (Baldani et al., 2014) was used to estimate the initial population present in 1.0 g of seeds at sowing time. The lowest and highest populations were 2.1×10^4 and 3.5×10^5 cells per gram of seeds, found for cultivars Piatã and Ipyporã, respectively.

The soil used in the experiments, characterized as an Argissolo (Santos et al., 2018), i.e., a Typic Hapludult (Soil Survey Staff, 2014), was collected from the experimental station of Embrapa Agrobiologia. Soil chemical analysis showed: 1.19% C, 0.12% total N (Kjeldahl), 0.07 $cmol_c\ dm^{-3}$ Al, 1.65 $cmol_c\ dm^{-3}$ Ca, 0.87 $cmol_c\ dm^{-3}$ Mg, 34.3 mg dm^{-3} available exchangeable K, 1.22 mg dm^{-3} available P (Mehlich 1), and pH (in H_2O) 5.5. To each 10 kg of soil, 9.2 g single superphosphate (P), 1.93 g potassium sulphate (K), and 0.5 g fritted trace elements (FTE-BR12, consisting of 1.8% B, 0.8% Cu, 3.0% Fe, 2.0% Mn, 0.1% Mo) were added. Nitrogen fertilizer and liming were not applied since there were no toxic levels of Al in the soil and pH was near the ideal for the crop. Plastic boxes (35x40x15 cm) containing 12 kg of the homogenized and fertilized soil were transferred to a greenhouse and used for seed sowing. A small polyethylene plastic screen was used to standardize seed distribution.

Three experiments were conducted in the municipality of Seropédica, in the state of Rio de Janeiro, Brazil. The first and second experiments were carried out in a greenhouse under controlled temperature and humidity, using a completely randomized design with four replicates. The third experiment was performed on a sterile substrate to evaluate the root traits of the cultivars used in the first and second experiments, in a completely randomized design with three replicates.

In the first experiment, 14 *Urochloa* cultivars were studied: Marandú, Xaraés, Paiaguás, Piatã, and B140 of *U. brizantha*; Basilisk of *U. decumbens*; Comum, Tupi, H47, and Llanero of *U. humidicola*; Ruziziensis of *U. ruziziensis*; and three hybrids, Ipyporã, Mulato, and Convert HD364. Two treatments were applied: with or without inoculation with *A. baldaniorum* Sp245. A total of 30 seeds of each cultivar were sown in July and harvested 60 days later in late winter, at a cutting height set at 5.0 cm above ground level.

In the second experiment, 7 cultivars were selected for evaluation based on the results of the first one. Compared with the control, the chosen cultivars were: Marandú and Llanero, due to their better response in terms of shoot dry mass (SDM) and total N accumulation; Convert HD364, based on its higher dry mass accumulation; B140 and Paiaguás, because of their lowest SDM response; and Ruziziensis and Basilisk, due to their neutral SDM response (Table 1). Using the same soil and box setup of the first experiment, the selected cultivars were sown again in October to obtain 7 plants per plastic box to be evaluated 30 days later in spring/summer. For the analysis, plant samples were divided into shoots and roots. After being weighed, the parts were dried using the MSM 510/480/CR forced-air circulation oven (M.S. Mistura Equipamentos para Laboratório, Rio de Janeiro, RJ, Brazil), at 65°C, until

reaching constant weight. The variables SDM and root dry mass (RDM) were evaluated. The total SDM of 7 plants in each tray was considered one replicate of each treatment. The samples were ground in the STAR FT-50 equipment (Fortinox, Piracicaba, SP, Brazil) and analyzed to determine N concentration, expressed in percentage. The total nitrogen of the samples was determined using the Kjeldahl method (Bremner & Mulvaney, 1982). To obtain nutrient accumulation in milligram per tray, nutrient concentration (grams of nutrient per kilogram of dry mass) was multiplied by the corresponding plant dry mass (grams).

In the third experiment, 13 cultivars were evaluated. In this case, cultivar Mulato was not studied due to the lack of viable seeds. Five seeds of the other cultivars were sown per pot filled with 0.5 kg sterile substrate of sand and vermiculite at 2:1 v/v. Thinning was carried out five days after emergence, leaving two plants per pot. After ten days, 25 mL Hoagland nutrient solution without N and with pH 5.1 (Hoagland & Arnon, 1950) were added to each pot; this was then repeated every five days. For inoculation, *A. baldaniorum* Sp245 was grown until reaching $O.D._{600}$ 1 equivalent to 10^9 cells per millimeter. An aliquot of 100 µL of the bacterial suspension was dropped on each seed at sowing, except on those of the control treatment. The experiment was harvested 20 days after seed emergence. The root system of the seedlings was carefully separated from the shoots and washed in running water until totally free of substrate particles. The washed roots were then stored in flasks containing 50% ethanol solution in order to preserve the root structures.

The WinRHIZO Pro 2016 system (Regent Instruments Inc., Quebec, Canada), coupled with the

Table 1. Dry mass production and nitrogen accumulation of 14 *Urochloa* cultivars inoculated or not with *Azospirillum baldaniorum* strain Sp245 in two experiments at 60 and 30 days after planting, respectively[(1)].

Cultivar	Total N (mg)		Shoot dry mass (g per box)		Root dry mass (g per box)	
	Control	Sp245	Control	Sp245	Control	Sp245
			Experiment I (60 days after planting)			
B140	332.8	224.8	21.9a	14.5b	-	-
Piatã	237.5	227.7	26.6	29.2	-	-
Xaraés	217.3	228.4	23.4	21.8	-	-
Paiaguás	242.9	154.4	22.3	20.5	-	-
Marandú	186.8	292.6	21.5b	32.0a	-	-
Comum	188.4	189.6	16.0	20.1	-	-
Llanero	146.9b	305.4a	17.6b	25.2a	-	-
H47	135.5	119.5	16.1	14.9	-	-
Tupi	115.3	127.3	9.3	12.3	-	-
Ruziziensis	199.8	208.0	21.5	23.8	-	-
Basilisk	157.7	177.2	17.4	13.4	-	-
Ipyporã	236.9	275.0	25.7	21.1	-	-
Mulato	278.2	205.9	22.5	16.0	-	-
Convert HD364	169.3	279.9	16.1b	26.8a	-	-
B140	332.8	224.8	21.9	14.5	-	-
Piatã	237.5	227.7	26.6	29.2	-	-
			Experiment II (30 days after planting)[(2)]			
B140	154.6b	281.9a	4.1b	8.2a	3.2	3.9
Paiáguas	314.7	316.0	11.4	13.1	6.2	6.6
Marandú	160.3	187.9	3.9	5.2	2.8b	4.3a
Ruziziensis	102.5b	295.4a	5.4b	9.1a	3.1b	6.2a
Llanero	349.3	309.1	12.2	14.3	5.7 b	8.7a
Basilisk	383.5	312.3	15.8	15.1	10.5	12.5
Convert HD364	366.7	381.5	13.6b	16.8 a	14.0b	16.1a

[(1)]Means (n=5) followed by different letters, in the lines for each parameter, differ by Tukey's test, at 5% probability. [(2)]Experiment II was conducted with 7 cultivars that were selected based on the results of experiment I.

EPSON Expression 11000XL professional flatbed scanner (EPSON, Nagano, Japan), was used for the analysis of root traits at a 400 dpi resolution. To obtain the images, the roots were laid in a 20x30 cm acrylic tray filled with water in order to avoid their overlapping. Four plants of each genotype were evaluated for root length, root volume, root diameter, root area, number of tips, number of crossings, and number of forks.

The data were subjected to the analysis of variance (ANOVA) using the F-test, at 5% probability. When significant, the mean values of the inoculation treatments were compared by Tukey's test, also at 5% probability. The Rbio statistical software was used (Bhering, 2017), and the assumptions for ANOVA were checked using the R, version 4.2.2, software (R Core Team, 2022). The tests of Durbin-Watson, Bartlett, and Shapiro-Wilk, at 5% probability, were used to check for independence of errors, normality, and homoscedasticity, respectively. All assumptions were met.

In the first experiment, inoculated cultivars Convert HD364, Llanero, and Marandú produced 38, 35, and 29% more SDM than the non-inoculated ones and also showed the highest total N accumulation. Inoculated cultivar B140 presented an inhibited seed development, producing 31% less SDM than the control (Table 1). Moreover, cultivars Xaraés and Marandú presented similar SDM and RDM values of 23.4 and 21.5 grams per box, respectively, when not inoculated, but different values of 21.7 and 32 grams per box when inoculated.

In the second experiment, the overall mean response to inoculation of the 7 evaluated genotypes was a 21 and 35% increase in SDM and RDM, respectively, 30 days after sowing when compared with the control. Cultivar B140 responded positively to inoculation when planted in spring due to the greater exposure to natural light in this season, which can directly interfere in plant development. Compared with the control, the inoculated cultivar Ruziziensis showed a gain of approximately 40% in SDM, whereas Convert HD364 had a gain of 11.5% in SDM and 21% in RDM after 30 days of sowing. Marandú presented 34.4 grams per box of SDM when inoculated and 24.49 grams per box when not inoculated, contrasting with the result of the first experiment. The same trend was observed for Convert HD364, which presented 28.95 and 18.01 grams per box, respectively, with and without inoculation.

In the third experiment, the obtained results suggest that inoculation modified root architecture in terms of length, area, number of tips, number of branches, and number of crossings (Table 2). The results of the Sp245 inoculation treatments were compared with those of the control. Root length decreased in inoculated cultivar B140, but increased in Marandú, Llanero, Tupi, Ruziziensis, and Convert HD340. In addition, root area decreased in inoculated

Table 2. Root parameters of 13 *Urochloa* cultivars inoculated or not with *Azospirillum baldaniorum* strain Sp245 20 days after seed emergence[(1)].

Cultivar	Length (m)		Projected area (m^2)		Surface area (m^2)		Tips (unit)		Forks (unit)		Crossings (unit)	
	Control	Sp245	Control	Sp245	Control	Sp245	Control	Sp245	Control	Sp245	Control	Sp245
B140	14.810a	12.062b	3.37	3.08	10.59	9.67	285	286	502	562	100	85
Basilisk	10.821	9.383	2.62	2.76	8.23	8.67	268a	156b	399	358	62	70
H47	7.730	8.442	1.90b	2.92a	5.97	6.83	125	126	212	232	49	46
Humidicola	13.216	14.753	2.81	3.07	8.83	9.64	248	281	328	404	90	104
Llanero	17.564b	22.732a	3.71b	4.73a	11.66b	14.85a	240b	373a	556b	704a	155b	179a
Marandú	11.813b	16.573a	2.73b	3.62a	8.58b	11.37a	192	278	358b	516a	88b	134a
Convert HD364	11.204b	14.321a	2.32	3.01	7.29b	9.44a	223	277	315b	457a	90b	124a
Paiaguás	14.257	16.141	3.89	4.28	12.22	13.44	222	241	449	453	87	90
Piatã	12.873	14.996	3.41	3.98	10.71	12.51	245	238	442	465	84	99
Ruziziensis	17.730b	22.231a	3.70	4.84	1.63b	15.20a	368	403	561	665	140b	177a
Tupi	10.045b	13.333a	2.59	2.61	6.21b	8.19a	270	267	324b	473a	71b	128a
Xaraés	16.289	15.612	4.15a	2.66a	13.02a	8.37b	247a	161b	561a	320b	112a	63b
Ipyporã	18.311	18.634	4.89	5.48	15.37a	13.01a	238	217	498a	388b	108a	79b

[(1)]Means followed by different letters, in the lines for each root parameter, differ by Tukey's test, at 5% probability.

cultivar Xaraés, but increased in Marandú, Llanero, Tupi, Ruziziensis, and Convert HD364. The number of crossings, tips, and forks decreased in the Xaraés and Ipyporã inoculated cultivars, but increased in Marandú, Tupi, Ruziziensis, Convert HD340, and Llanero. Therefore, root diameters differ between cultivars; however, inoculation 30 days after sowing was not related to these differences.

The growth promoted in *Urochloa* cultivars due to the inoculation with *A. baldaniorum* Sp245 can be explained by several mechanisms, such as biological nitrogen fixation, production of plant growth regulators (auxin class compounds and gibberellins), beneficial effects of siderophores, phosphate solubilization, and pathogen biocontrol (Guimarães et al., 2011; Cassán et al., 2014; 2020; Cassán & Diaz-Zorita, 2016; Kaushal, 2019). Whatever the mechanism, root growth promotion is pointed out as one of the main reasons for inoculated plants to overcome several abiotic stresses as waterlogged areas or soil salinity (Santos et al., 2021).

The obtained results indicate that the response to inoculation differs among the tested cultivars, as also observed by Soares et al. (2021). When studying root and shoot samples of three *Urochloa* cultivars inoculated with *A. baldaniorum* Sp245 using the qPCR method, these authors found that the populations of the bacteria were higher in the roots and shoots of Piatã and Basilisk, but lower in those of Paiaguás.

A single application of *A. baldaniorum* Sp245 results in a higher dry matter accumulation and number of roots in cultivars Marandú, Llanero, Ruziziensis, and Convert HD364. However, after inoculation, cultivars Xaraés and Ipyporã show some root traits with lower values.

Acknowledgments

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for financing, in part, this study (Finance Code 001); to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for fellowships; and to Institutos Nacionais de Ciência e Tecnologia (INCT) of CNPq, for grant (number 465133/2014-2).

References

ALVIM, M.J.; BOTREL, M. de A.; XAVIER, D.F. **As principais espécies de *Brachiaria* utilizadas no País**. Juiz de Fora: Embrapa Gado de Leite, 2002. 4p. (Embrapa Gado de Leite. Comunicado técnico, 22).

BALDANI, J.I.; REIS, V.M.; VIDEIRA, S.S.; BODDEY, L.H.; BALDANI, V.L.D. The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: a practical guide for microbiologists. **Plant and Soil**, v.384, p.413-431, 2014. DOI: https://doi.org/10.1007/s11104-014-2186-6.

BHERING, L.L. Rbio: a tool for biometric and statistical analysis using the R platform. **Crop Breeding and Applied Biotechnology**, v.17, p.187-190, 2017. DOI: https://doi.org/10.1590/1984-70332017v17n2s29.

BREMNER, J.; MULVANEY, C. Nitrogen-total. In: PAGE, A.L. (Ed.). **Methods of soil analysis**: part 2: chemical and microbiological properties. 2nd ed. Madison: American Society of Agronomy; Soil Science Society of America, 1982. p.595-624. DOI: https://doi.org/10.2134/agronmonogr9.2.2ed.c31.

CARVALHO, C.L.M. de; DUARTE, A.N.M.; HUNGRIA, M.; NOGUEIRA, M.A.; MOREIRA, A.; SOARES FILHO, C.V. Nitrogen in shoots, number of tillers, biomass yield and nutritive value of Zuri Guinea grass inoculated with plant-growth promoting bacteria. **International Journal for Innovation Education and Research**, v.8, p.437-463, 2020. DOI: https://doi.org/10.31686/ijier.vol8.iss5.2360.

CASSÁN, F.; CONIGLIO, A.; LÓPEZ, G.; MOLINA, R.; NIEVAS, S.; CARLAN, C. Le N. de; DONADIO, F.; TORRES, D.; ROSAS, S.; PEDROSA, F.O.; SOUZA, E. de; DIÁZ ZORITA, M.; de-BASHAN, L.; MORA, V. Everything you must know about *Azospirillum* and its impact on agriculture and beyond. **Biology and Fertility of Soils**, v.56, p.461-479, 2020. DOI: https://doi.org/10.1007/s00374-020-01463-y.

CASSÁN, F.; DIAZ-ZORITA, M. *Azospirillum* sp. in current agriculture: from the laboratory to the field. **Soil Biology and Biochemistry**, v.103, p.117-130, 2016. DOI: https://doi.org/10.1016/j.soilbio.2016.08.020.

CASSÁN, F.; VANDERLEYDEN, J.; SPAEPEN, S. Physiological and agronomical aspects of phytohormone production by model plant-growth-promoting rhizobacteria (PGPR) belonging to the Genus *Azospirillum*. **Journal of Plant Growth Regulation**, v.33, p.440-459, 2014. DOI: https://doi.org/10.1007/s00344-013-9362-4.

FERREIRA, N. dos S.; SANT'ANNA, F.H.; REIS, V.M.; AMBROSINI, A.; VOLPIANO, C.G.; ROTHBALLER, M.; SCHWAB, S.; BAURA, V.A.; BALSANELLI, E.; PEDROSA, F. de O.; PASSAGLIA, L.M.P.; SOUZA, E.M. de; HARTMANN, A.; CASSAN, F.; ZILLI, J.E. Genome-based reclassification of *Azospirillum brasilense* Sp245 as the type strain of *Azospirillum baldaniorum* sp. nov. **International Journal of Systematic and Evolutionary Microbiology**, v.70, p.6203-6212, 2020. DOI: https://doi.org/10.1099/ijsem.0.004517.

GUIMARÃES, S.L.; BONFIM-SILVA, E.M.; POLIZEL, A.C.; CAMPOS, D.T. da S. Produção de capim-marandu inoculado com *Azospirillum* spp. **Enciclopédia Biosfera**, v.7, p.816-825, 2011.

HOAGLAND, D.R.; ARNOLD, D.I. **The water-culture method for growing plants without soil**. Berkley: University of California, 1950. (California Agricultural Experiment Station. Circular, 347).

HUNGRIA, M.; NOGUEIRA, M.A.; ARAUJO, R.S. Inoculation of *Brachiaria* spp. with the plant growth-promoting bacterium *Azospirillum brasilense*: an environment-friendly component in the reclamation of degraded pastures in the tropics. **Agriculture, Ecosystems and Environment**, v.221, p.125-131, 2016. DOI: https://doi.org/10.1016/j.agee.2016.01.024.

KAUSHAL, M. Portraying rhizobacterial mechanisms in drought tolerance: a way forward toward sustainable agriculture. In: SINGH, A.K.; KUMAR, A.; SINGH, P.K. (Ed.). **PGPR amelioration in sustainable agriculture**: Food Security and Environmental Management. [S.l.]: Woodland Publishing, 2019. p.195-216. DOI: https://doi.org/10.1016/B978-0-12-815879-1.00010-0.

R CORE TEAM. **R**: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2022.

SANTOS, H.G. dos; JACOMINE, P.K.T.; ANJOS, L.H.C. dos; OLIVEIRA, V.Á. de; LUMBRERAS, J.F.; COELHO, M.R.; ALMEIDA, J.A. de; ARAÚJO FILHO, J.C. de; OLIVEIRA, J.B. de; CUNHA, T.J.F. **Sistema brasileiro de classificação de solos**. 5.ed. rev. e ampl. Brasília: Embrapa, 2018. 356p. Available at: <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/199517/1/SiBCS-2018-ISBN-9788570358004.pdf>. Accessed on: May 22 2020.

SANTOS, M.S.; NOGUEIRA, M.A.; HUNGRIA, M. Outstanding impact of *Azospirillum brasilense* strains Ab-V5 and Ab-V6 on the Brazilian agriculture: lessons that farmers are receptive to adopt new microbial inoculants. **Revista Brasileira de Ciência do Solo**, v.45, e0200128, 2021. DOI: https://doi.org/10.36783/18069657rbcs20200128.

SOARES, I.C.; PACHECO, R.S.; SILVA, C.G.N. da; SANTOS, R.S.; BALDANI, J.I.; URQUIAGA, S.; VIDAL, M.S.; SIMOES-ARAUJO, J. Real-time PCR method to quantify Sp245 strain of *Azospirillum baldaniorum* on *Brachiaria* grasses under field conditions. **Plant and Soil**, v.468, p.525-538, 2021. DOI: https://doi.org/10.1007/s11104-021-05137-y.

SOIL SURVEY STAFF. **Keys to soil taxonomy**. 12th ed. Washington: USDA, 2014. 360p.

TORRES GONZÁLEZ, A.M.; MORTON, C.M. Molecular and morphological phylogenetic analysis of *Brachiaria* and *Urochloa* (Poaceae). **Molecular Plylogenetics and Evolution**, v.37, p.36-44, 2005. DOI: https://doi.org/10.1016/j.ympev.2005.06.003.

WISNIEWSKI-DYÉ, F.; BORZIAK, K.; KHALSA-MOYERS, G.; ALEXANDRE, G.; SUKHARNIKOV, L.O.; WUICHET, K.; HURST, G.B.; MCDONALD, W.H.; ROBERTSON, J.S.; BARBE, V.; CALTEAU, A.; ROUY, Z.; MANGENOT, S.; PRIGENT-COMBARET, C.; NORMAND, P.; BOYER, M.; SIGUIER, P.; DESSAUX, Y.; ELMERICH, C.; CONDEMINE, G.; KRISHNEN, G.; KENNEDY, I.; PATERSON, A.H.; GONZÁLEZ, V.; MAVINGUI, P.; ZHULIN, I.B. A*zospirillum* genomes reveal transition of bacteria from aquatic to terrestrial environments. **PLoS Genetics**, v.7, e1002430, 2011. DOI: https://doi.org/10.1371/journal.pgen.1002430.