Scientific Electronic Archives
Issue ID: Sci. Elec. Arch. Vol. 17 (1)
January/February 2024
DOI: http://dx.doi.org/10.36560/17120241841
Article link: https://sea.ufr.edu.br/SEA/article/view/1841

ISSN 2316-9281

Unraveling the potential: assessment of the effect of *Azospirillum brasilense* inoculated via seeds on the growth and production of Mombaça grass

Corresponding author
Carlos Luiz Vieira
Universidade do Estado de Mato Grosso - Campus Tangará da Serra
carlos.luiz_12@hotmail.com

Giulianna Zilocchi Miguel
Universidade do Estado de Mato Grosso – Campus Cáceres

Emerson de Oliveira Figueiredo
Instituto Federal de Mato Grosso - Campus Confresa

Gabriel Vinícius Batista da Silva
Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo

Rafael Rosa Rocha
Universidade do Estado de Mato Grosso - Campus Tangará da Serra

Altacis Junior de Oliveira
Universidade do Estado de Mato Grosso – Campus Cáceres

Abstract. Nitrogen fertilization in pastures is considered one of the primary limiting factors that significantly enhance forage biomass production. Traditional chemical fertilizers used in crop cultivation are derived from petrochemical and mining industries. These production chains have undergone continuous and consistent structural changes year after year, affecting the prices of these inputs for farmers, consequently escalating production costs and even rendering investment in livestock farming unfeasible. For this reason, research becomes crucial to evaluate alternative techniques that can complement or fulfill the nitrogen demand in forage crops. The bacterium *Azospirillum brasilense* has emerged as a viable alternative to reduce costs in pasture establishment and maintenance, serving as a nitrogen source when compared to traditional sources. The objective of this study was to assess the effect of *Azospirillum brasilense* inoculation on the growth and production of *Panicum maximum* cv. Mombaça. The experimental design employed was a randomized complete block design with four treatments and four replications. Each plot consisted of three plants, resulting in a total of 48 experimental units. The treatments comprised: T1 - natural soil (NS), T2 - *Azospirillum brasilense* (BAC.), T3 - nitrogen topdressing (N), T4 - *Azospirillum brasilense* and nitrogen topdressing (BAC+N). Evaluations were conducted at 60 and 90 days after sowing (DAS), assessing the following variables: plant height (PH), number of leaves (NL), number of tillers (NT), aboveground green biomass (AGGB), aboveground dry biomass (ADBB), root fresh biomass (RFB), and root dry biomass (RDB). Significant effects ($P<0.05$) were observed for inoculation and nitrogen topdressing on the assessed traits: plant height, number of leaves, number of tillers, aboveground green biomass, aboveground dry biomass, root fresh biomass, and root dry biomass. Notably, nitrogen topdressing either solely or in combination with seed inoculation of the forage yielded positive outcomes for all studied variables at both 60 and 90 DAS. A favorable impact on the development of Mombaça grass was evident across all parameters studied in samples receiving nitrogen topdressing and *Azospirillum* spp. inoculation.
Keywords: *Azospirillum brasilense*; Growth; Inoculation; *Panicum maximum* Mombaça; Production.

Introduction

Mombaça Grass (*Panicum maximum*) is a staple tropical forage renowned for its high productivity in the agricultural sector. However, in conditions of low soil fertility, its yield tends to dwindle, attributing to the species' heightened demands for soil fertility. When cultivated under optimal practices, Mombaça grass can yield approximately 33 t/ha of annual dry matter, accompanied by a satisfactory chemical composition. Thus, the consideration of investments in fertilizers becomes paramount, particularly in scenarios of intensified livestock production (EMBRAPA, 2014).

Crucial for pasture growth and development, nitrogen can be sourced through mineral fertilizers or the biological process of atmospheric nitrogen (N2) fixation (TAIZ; ZEIGER, 2009). As the consumption and costs of synthetic chemical fertilizers continue to rise, the quest for alternative nutrient sources that are ecologically sustainable and cost-effective becomes increasingly pertinent (SCHLINDWEIN et al., 2008).

In this context, nitrogen-fixing bacteria emerge as a promising ally, offering an alternative approach to conventional fertilization. These bacteria confer numerous benefits to plants and boast a more economical profile when compared to conventional fertilizers, all while preserving the environment (SCHLINDWEIN et al., 2008). The biological nitrogen fixation process is orchestrated by a consortium of diazotrophic bacteria, which absorb atmospheric nitrogen (N2) and convert it into ammonium (NH4) through a symbiotic relationship. A classic illustration of this association exists between grasses and bacteria of the genus *Azospirillum* spp.

Going beyond nitrogen fixation, *Azospirillum* spp. bacteria establish themselves in the tissues of select grass species without inducing any harm. These bacteria act as growth promoters, producing hormones like auxins and gibberellins, which expedite extensive plant development, particularly in root systems. Furthermore, they enhance assimilate translocation and water absorption, contributing to robust growth (PEOPLES et al., 1995).

These advantages have garnered attention through a plethora of studies involving *Azospirillum brasilense*. Notably, when these bacteria are harnessed as inoculants across various crops like wheat, oats, sugarcane, brachiaria grasses, and maize, they consistently exhibit a capacity to induce significant variations in growth parameters. These encompass the spectrum from biomass accumulation and tissue nitrogen content to plant height, leaf area, tillering,
root length, and even root volume (DALLA SANTA et al., 2008; MONTAGNER et al., 2014; CHAVES et al., 2015; SALANTUR et al., 2006).

Given the above premises, the core objective of this study is to scrutinize the effects of utilizing Azospirillum brasilense bacteria, inoculated via seeds, on the developmental trajectory and overall production of Mombaça grass.

Materials and methods

The research was conducted in the experimental area of the State University of Mato Grosso, located in the municipality of Cáceres/MT, at latitude 16°04'33"S and longitude 57°39'10"W. The region is characterized by two well-defined seasons (dry in winter and wet in summer), with an average annual temperature of 25°C, which can sometimes reach up to 40°C, and an average precipitation of 1,396 mm per year. The period from January to March corresponds to the wettest season, while the period between September and October has the highest solar radiation and the lowest precipitation rate (SILVA et al., 2008).

The seeds were obtained from a reputable company, and the planting was carried out in an open environment. Plastic buckets of 5 liters (21.2 x 20.7 cm) and subsoil soil were used for planting.

The experimental design used was a randomized complete block design with four treatments and four replications. Each plot consisted of three plants, totaling 48 experimental units. The treatments consisted of: T1 - natural soil (SN), T2 - *Azospirillum brasilense* (BAC.), T3 - nitrogen fertilization as a topdressing (N), T4 - *Azospirillum brasilense* and nitrogen fertilization as a topdressing (BAC+N).

The soil used to fill the pots was classified as dystrophic red-yellow latosol (SANTOS et al., 2013). The soil was sieved and subjected to chemical and physical analysis, and the necessary base fertilizations were carried out following the guidelines of (DONAGEMA et al., 2011). The soil exhibited the following attributes: 5.23 mg dm-3 of P; 120.60 mg dm-3 of K; 6.26 cmolc dm-3 of Ca2+; 3.38 cmolc dm-3 of Mg2+; 0 cmolc dm-3 of Al3+; 23.98 g kg-1 of organic matter; SB: 9.95 cmolc dm-3; CTC: 12.7 cmolc dm-3; pH in water of 6.4; Sand content of 568.8 g kg-1; Silt content of 174.5 g kg-1; and Clay content of 256.7 g kg-1.

The pasture cultivar used was Mombaça (*Panicum maximum*), which was directly sown into the soil. After 30 days after sowing (DAS), thinning was carried out, leaving only three plants per bucket.

The quantity of *Azospirillum brasilense* inoculated into the seeds was calculated according to the manufacturer's recommendation (100 ml of the liquid product AZOTROP per 50 kg of seeds), and nitrogen topdressing was applied following the recommendation of Costa et al. (2006). The topdressing fertilization was performed at 30 days after sowing (DAS).

The evaluations were conducted at 60 and 90 DAS, with the following variables being assessed: plant height, number of leaves, dry and fresh weight of both aboveground and root parts. For these assessments, a graduated ruler, digital caliper, direct leaf inspection, and analytical balance were utilized.

To determine the dry weight of both the aboveground and root parts, the material was cut and subjected to drying in an air-circulating oven at 60°C for 72 hours (GUIMARÃES, 2011).

The mean values obtained for each evaluated parameter underwent analysis of variance and were compared using the Scott Knot test at a 5% probability level through the AgroEstat software (BARBOSA & MALDONADO, 2015).

Results and discussions

From the results obtained in the analysis of variance, significant effects (P<0.05) were observed for both inoculation and nitrogen topdressing concerning the evaluated characteristics: plant height (PH), number of leaves (NL), number of tillers (NT), aboveground fresh weight (AFW), aboveground dry weight (ADW), root fresh weight (RFW), and root dry weight (RDW). It is evident that nitrogen topdressing, whether applied alone or in combination with seed inoculation, yielded positive outcomes for all variables examined at 60 and 90 days after sowing (Table 1 and 2).

Table 1. Summary of analysis of variance for the variables: plant height (PH), number of leaves (NL), number of tillers (NT), aboveground fresh weight (AFW), aboveground dry weight (ADW), at 60 days after sowing, in relation to the use of *Azospirillum brasilense* bacteria inoculated via seeds in the development and production of Mombaça grass.

Treatments	PH (cm)	NL (nº)	NT (nº)	AFW (g)	ADW (g)
Natural Soil	81,27 b	8,79 c	2,91 c	3,57 b	1,16 b
Bacteria	85,51 b	18,94 bc	7,02 b	13,27 a	3,90 a
Urea	95,56 a	31,33 a	12,16 a	18,02 a	6,02 a
Bacteria + Urea	89,53 ab	22,43 ab	9,33 b	13,04 a	4,13 a

Means followed by different letters vertically differ significantly from each other according to the Scott Knot test at a 5% probability level.

Table 2. Summary of variance analysis for the variables: plant height (PH), number of leaves (NL), number of tillers (NT), aboveground fresh weight (AFW), aboveground dry weight (ADW), fresh root weight (FRW), dry root weight (DRW), at 90 days after sowing, in relation to the use of *Azospirillum brasilense* bacteria inoculated via seeds in the development and production of Mombaça grass.

Treatments	PH (cm)	NL (nº)	NT (nº)	AFW (g)	ADW (g)	FRW (g)	DRW (g)
Natural Soil	69,8 c	16,5 b	4,90 c	6,34 c	1,54 c	27,0 b	5,13 b
Bacteria	69,3 c	15,9 b	5,83 b	7,38 c	1,73 c	29,2 b	4,94 b
Urea	89,6 a	27,9 a	7,80 a	26,6 a	7,07 a	53,5 a	11,2 a
Bacteria + Urea	77,2 b	22,5 a	6,47 b	18,2 b	5,21 b	59,1 a	11,3 a

Means followed by different letters vertically differ significantly from each other according to the Scott Knot test at a 5% probability level.

In terms of plant height and number of leaves, the treatments that stood out the most were those that received only nitrogen topdressing and those that were inoculated with Azospirillum brasilense via seeds. However, when the inoculation treatments were compared to the absolute control, it was observed that *Azospirillum brasilense* led to a 10% increase in the number of leaves and taller plant height (Table 02 and 03).

Considering the maximum number of tillers found (Table 02 and 03), the treatment that stood out was the one that received nitrogen topdressing. However, the treatment that solely had the bacteria inoculated, when compared to the control, exhibited superior development.

This indicates that irrespective of inoculation, nitrogen has the ability to stimulate the growth points of the forage, allowing for greater tillering. Under vegetative conditions, forage grasses display a high rate of leaf appearance, resulting in a dense tiller population. Each leaf insertion possesses a bud that, based on available conditions, can transform into a tiller (ALEXANDRINO et al., 2010).

According to Nabinger (1996), nitrogen scarcity available to plants increases the number of dormant buds, negatively impacting tillering in forage plants. Conversely, adequate nitrogen supply allows for maximal tiller production.

However, it is noteworthy that this grass, without nitrogen application but with diazotrophic bacterial inoculation, produced more tillers than the control (without N application and without inoculation) (Table 02 and 03). These results suggest that inoculation is a sustainable alternative for increasing tillering and forage production under conditions of low nitrogen availability.

Studies conducted by Oliveira et al., (2007) working with grasses yielded positive results in treatments without nitrogen application but with diazotrophic bacterial inoculation, displaying higher forage production than the control (without N application and without inoculation). This is emphasized by the authors as a sustainable alternative for increasing forage production.

In the first and second cuts, treatments using *Azospirillum* spp. demonstrated higher aboveground fresh and dry weight production than the Control (without N application and without inoculation) (Table 02 and 03).

This highlights the efficiency of biological fixation carried out by these bacteria and their nitrogen supply to plants. Similar results to this study were obtained by Oliveira (2019) and Caleffi (2021), who observed the contribution of *Azospirillum brasilense* in terms of nitrogen supply in ryegrass and irrigated rice development, respectively. According to

Matos (2019), Azospirillum brasilense has the capacity to fix up to 30 kg N ha-1 when used in a single application.

However, the treatment that only received nitrogen topdressing also showed positive results compared to all treatments that received Azospirillum brasilense inoculation.

The lower total fresh and dry weight production in the absence of *Azospirillum brasilense* and urea use (T1) can be attributed to lower tillering in this treatment compared to the other treatments. According to Alexandrino et al., (2005), the dry weight production of stems and sheaths present in tillers is a relevant component for forage production, as stems and sheaths are storage organs of organic substances in grasses, which can interfere with the regrowth capacity of grasses.

The best results in terms of fresh and dry root weight were obtained in treatments that received urea topdressing (T3; T4). However, the combination of the bacteria with urea resulted in better outcomes, demonstrating the efficacy of bacteria inoculation in seeds, as well as topdressing (Table 03).

In addition to the biological nitrogen fixation, *Azospirillum* bacteria contribute to the expansion of the root surface area of plants, resulting in a greater amount of explored substrate. According to Reis Junior et al., (2004), these bacteria can produce phytohormones, growth-promoting and regulating substances such as auxins, cytokinins, and gibberellins, which go beyond mere rootlet increase and lead to larger diameters of lateral and adventitious roots. This phenomenon was observed in this study.

These assertions are corroborated by the findings of this study, as treatments with isolated bacteria demonstrated a greater increase in root mass, surpassing the absolute control (Table 03).

Conclusion

In summary, the results obtained in this study emphasize that inoculation with *Azospirillum brasilense*, either alone or in conjunction with nitrogen fertilization, emerges as a highly effective approach to enhance the growth and production of Mombaça grass. The ability of this bacterium to fix nitrogen and its beneficiais influence on root expansion emerge as cruciais factors contributing to these positive effects. However, it is essential to acknowledge the complexity of the system and the interconnection among various factors for a comprehensive and complete understanding of the results.

References

ALEXANDRINO, E.; VAZ, R. G. M. V.; SANTOS, A. C. Características da *Brachiaria brizantha* cv. Marandu durante o seu estabelecimento submetida a diferentes doses de nitrogênio. Bioscience Journal, Uberlândia v. 26, n. 6, p. 886-893, 2010.

BARBOSA, J. Z.; CONSALTER, R.; MOTTA, A. C. V. Fixação biológica de nitrogênio em Poaceae. Evidência, Joaçaba, 2012, 12.1: 7-18.

BARBOSA, J.; MALDONADO, J. Experimentação agronômica e AgroEstat: sistema para análises estatísticas de ensaios agronômicos. 2, Jaboticabal: Departamento de Ciências Exatas–Universidade Estadual Paulista (UNESP). 2015.

CALEFFI, H.V.; GONCALVES, G.K.; SCHULLER, M.; RODRIGUES, M.A.; ROSA, E.V.C.; RODRIGUES, P.E.B.; GONCALVES, D.R.N.; GALARZA, R.M.de. 2021. Utilização do *Azospirillum brasilense* e composto orgânico na produção de arroz irrigado. Em: Desafios e Perspectivas nas ciências agrárias, v.1. (Catapan, E.A). Local de Publicação: Editora Brazilian Journals. 319 a 334p.

CHAVES, V. A.; SANTOS, S. G.; SCHULTZ, N.; PEREIRA, W.; SOUSA, J. S.; MONTEIRO, R. C.; REIS, V. M. Desenvolvimento inicial de duas variedades de cana-de-açúcar inoculadas com bactérias diazotróficas. Revista Brasileira de Ciências do Solo, v. 39, p. 1595-1602, 2015.

COELHO, R. M. Efeitos da concentração de matéria seca e do uso de inoculante bacteriano-enzimático, na silagem de tifton 85 (*Cynodon* spp.), sobre a digestão de nutrientes, parâmetros ruminais e comportamento ingestivo em novilhos de corte em crescimento. 2002. 143 f. Dissertação (Mestrado) - Curso de Agronomia, Ciência Animal e Pastagens, Universidade de São Paulo, Piracicaba, Sp, 2002.

COSTA, K. D. P.; OLIVEIRA, I. P.; FAQUIN, V. Adubação nitrogenada para pastagens do gênero Brachiaria em solos do Cerrado. Documentos 192. Embrapa Arroz e Feijão, 2006. 60p.

DALLA SANTA, O. R.; DALLA SANTA, H. S.; FERNÁNDES, R.; MICHELENA, G., JUNIOR, P. R.; SOCCOL, C. R. Influence of *Azospirillum* sp. inoculation in wheat, barley an doats. Ambiência, v. 4, n. 2, p. 197-207, 2008.

DIAS, V. P.; FERNANDES, E. Fertilizantes: uma visão global sintética. BNDES Setorial, n. 24, p. 97-138, 2006.

DONAGEMA, G. K.; CAMPOS, D. D.; CALDERANO, S. B.; TEIXEIRA, W. G.; VIANA, J. H. M. Manual de métodos de análise do solo. Embrapa Solos, Documentos, 132. 2º edição revista. Dados eletrônicos. Rio de Janeiro, Embrapa Solos, 2011. 225p

GUIMARÃES, S.; ONFIM-SILVA, E. M.; POLIZEL, A.; CAMPOS, D. PRODUÇÃO DE CAPIM-MARANDU INOCULADO COM *Azospirillum* spp. Enciclopédia Biosfera, 2011, 7.13.

HARTMANN, A; BALDAM, J.I. The genus Azospirillum. In: DWORKIN, M. (eds.) The Prokaryotes. New York: Springer, 2006. p.115-140.

HUNGRIA. M.; CAMPO, R. J.; SOUZA, E. M.; PEDROSA, F. O. Inoculation with selected strains of Azospirillum brasilense and A. lipoferum improves yields of maize and wheat in Brazil. Plant and Soil. v.331, n.1, pp.413-425, 2010.

MANEJO DO CAPIM-MOMBAÇA PARA PERÍODOS DE ÁGUAS E SECA. EMBRAPA, 2014. Disponível em: < https://www.embrapa.br/busca-de-noticias/-/noticia/2118000/artigo-manejo-do-capim-mombaca-para-periodos-de-aguas-e-seca >. Acesso em: 11, abril 2021.

MONTAGNER SOUZA, T.; MATEUS PRANDO, A.; REIKA TAKABAYASHI, C.; SIFUENTES DOS SANTOS, J.; TIEME ISHIKAWA, A.; DE SOUZA MADUREIRA FELÍCIO, A. L.; YOKO HIROOKA, E. Composição química e desoxinivalenol em trigo da região Centro-Sul do Paraná: adubação nitrogenada em cobertura associada com Azospirillum brasilense. Semina: Ciências Agrárias, v. 35, n. 1, 2014.

NABINGER, C. Aspectos ecofisiológicos do manejo de pastagens e utilização de modelos como ferramenta de diagnóstico e indicação de necessidades de pesquisa. In: REUNIÃO DO GRUPO TÉCNICO REGIONAL DO CONE SUL (ZONA CAMPOS) EM MELHORAMENTO E UTILIZAÇÃO DE RECURSOS FORRAGEIROS DAS ÁREAS TROPICAL E SUBTROPICAL, 1996, Porto Alegre. Anais... Porto Alegre: UFRGS, 1996. p.17-62.

OKON, Y.; LABANDERA-GONZALES, C.A. Agronomic applications of Azospirillum: na evaluation of 20 years worldwide field inoculation. Soil Biology and Biochemistry, v.26, p.1591-1601, 1994.

OLIVEIRA, A. I.; BIRCK, V.; DE MORAES GALARZA, R.; CALEFFI, H. V.; SARTURI, J. C.; GONÇALVES, G. K. UTILIZAÇÃO DE INSUMOS ALTERNATIVOS E SEUS EFEITOS NA PRODUÇÃO DO AZEVÉM (*Lollium multiflorum*) DESTINADO À COBERTURA VERDE. In: IX SIEPEX-IX Salão Integrado de Ensino, Pesquisa e Extensão. 2019.

OLIVEIRA, P. P. A.; OLIVEIRA, W. S.; BARIONI, W. J.; Produção de forragem e qualidade de *Brachiaria brizantha* cv. Marandu com *Azospirillum brasilense* e fertilizada com nitrogênio. Embrapa pecuária sudeste, São Carlos, SP, 2007

PEOPLES, M. B.; HERRIDGE, D. F.; LADHA, J. K. Biological nitrogen fixation: na efficient source of nitrogen for sustainable agricultural production? Plant and soil, v.174, n. 1-2, p. 3-28, 1995.

REIS JUNIOR, F. B.; SILVA, M. F.; TEIXEIRA, K. R. S.; URQUIAGA, S.; REIS, V. M.Identificação de isolados de *Azospirillum* amazonense associados a Brachiaria spp.,em diferentes épocas e condições de cultivo e produção de fitormônio pela bactéria.Revista Brasileira de Ciência do Solo, v. 28, p. 103-113, 2004.

REIS, V.M.; BALDINI, J.I.; BALDINI, V.L.D.; DOBEREINER, J. Biological dinitrogen fixation in gramineae and palm Trees. CRC Criticai Review in Plant Science, v.19, p.227-247, 2000.

SALANTUR, A.; OZTURK, A.; AKTEN, S. Grow thand yield response of spring wheat (Triticuma estivum L.) to inoculation with rhizobacteria. Plant Soil and Environment, v. 52, n. 3, p. 111, 2006.

SANTOS, H.G. dos; JACOMINE, P.K.T.; ANJOS, L.H.C. dos; OLIVEIRA, V.A. de; LUMBRERAS, J.F.; COELHO, M.R.; ALMEIDA, J.A. de; CUNHA, T.J.F.; OLIVEIRA, J.B. de. Sistema brasileiro de classificação de solos. 3.ed. rev. e ampl. Brasília: Embrapa, 2013. 353p

SCHLINDWEIN, G.; VARGAS, L.K.; LISBOA, B.B.; AZAMBUJA, A.C.; GRANADA, C.E.; GABIATTI, N.C.; PRATES, F.; STUMPF, R. Influência da inoculação de rizóbios sobre a germinação e o vigor de plântulas de alface. Ciência Rural, Santa Maria, v.38, n.3, p. 658-664,2008.

SILVA, A.; DE SOUZA F.; EDVARD E.; DA CUNHA, S. B. Padrões de canal do rio Paraguai na região de Cáceres (MT). Revista Brasileira de Geociências, v. 38, n. 1, p. 167-177, 2008.

SILVEIRA, E. L. Inoculações de bactérias promotoras de crescimento no cultivo de arroz em solução nutritiva. 2008, 99p. Tese (Doutorado) – Ciências Agrárias e Veterinárias. Universidade Estadual Paulista. Jaboticabal, 2008.

TAIZ, L.; ZEIGER, E. Fisiologia vegetal. 4. ed. Porto Alegre: Artmed. p.819, 2008.