Archivos de Zootecnia
Journal website: https://www.uco.es/ucopress/az/index.php/az/

Effects of associative diazotrophic bacteria on Marandu palisadegrass growth development

Silva Alves dos Santos, C.; Lima Guimarães, S.@; Bonfim-Silva, E.M.; Castilho Polizel de Souza, A.; Koetz, M.; da Silva Campos, D.T. and de Lima Canuto, E.

Universidade Federal de Rondonópolis-UFR, Rondonópolis, MT, Brazil.

SUMMARY

The objective of this study was to quantity Marandu palisadegrass (*Urochloa brizantha* cv. Marandu) growth responses to inoculation with associate diazotrophic bacteria found in Cerrado's dystrophic oxisol. A greenhouse study was conducted to evaluate Marandu palisadegrass responses to five treatments [three strains of nitrogen-fixing associative bacteria Y2 (*Nitrospirillum amazonense*), MTH2 (similar to *Herbaspirillum* spp.), MTB1 (similar to *Burkholderia* spp.), commercial inoculant (combination of Ab-V5 and Ab-V6 strains *(Azospirillum brasilense)*, and control (100 mg dm-3 N-ureia)]. Treatment were replicated five times in a completerandomized design. A 3 mL aliquota contain 109 mL-1 bacterial cells was applied close to root system on soil mix for each pot, except on control treatments. Three harvests were conducted at 30, 60 and 90-days afters sowing date. Response variables measured were canopy height, number of leaves per plant, and shoot and root system weight. Canopy height was greater (P<0,001) increased of 35 cm, from the first to the second grass cut. Number of leaves (P<0,001), shoot dry mass (P<0,001) and root were provided by treatment MTB1 the equivalent to 64.8, 85.42 and 47.3%, of the average values observed in the plants that received control treatment, with mineral nitrogen. There was a good development of marandu grass inoculated with diazotrophic bacteria, supplying part of the N necessary in the initial stages of its development, standing out among the studied inoculants the MTH2 and MTB1 treatments.

ADDITIONAL KEYWORDS
Biological nitrogen fixation.
Growth promoting bacteria.
Urochloa brizantha.

Efeito de bactérias diazotróficas associativas no desenvolvimento produtivo do capim Marandu

RESUMO

O objetivo deste estudo foi determinar o desenvolvimento do capim Marandu (*Urochloa brizantha* cv. Marandu) submetido à inoculação com bactérias diazotróficas associadas e cultivadas em Latossolo Vermelho do Cerrado. O experimento foi conduzido em casa de vegetação, com delineamento inteiramente casualizado e cinco tratamentos composto por três estirpes de bactérias associativas fixadoras de nitrogênio, Y2 (*Nitrospirillum amazonense*), MTH2 (similar à *Herbaspirillum* spp.), MTB1 (similar à Burkholderia spp.), um inoculante comercial formado pela combinação das estirpes Ab-V5 e Ab-V6 *(Azospirillum brasilense)*, um controle 100 mg dm-3 N-uréia e cinco repetições, foram inoculadas pela inserção de uma alíquota de 3 mL de caldo bacteriano contendo 109 mL-1 de células no solo próximo ao sistema radicular de cada planta. Foram realizados três cortes aos 30, 60 e 90 dias após a semeadura e avaliou-se altura da planta, número de folhas, matéria seca de parte aérea e da raiz do capim Marandu. A altura das plantas (P <0,001) aumentou em 35 cm, do primeiro para o segundo corte no C1. Para número de folhas (P <0,001), matéria seca da parte aérea (P <0,001) e raiz (P <0,001), o tratamento MTB1 forneceu o equivalente a 64,8, 85,42 e 47,3%, dos valores médios observados nas plantas que receberam tratamento controle com nitrogênio mineral. Houve bom desenvolvimento do capim Marandu inoculado com bactérias diazotróficas que supriu parte do N necessário ao seu desenvolvimento incial, destacamdo-se entre os inoculantes estudados os tratamentos MTH2 e MTB1.

PALAVRAS CHAVE ADICIONAIS
Bactérias promotoras de crescimento.
Fixação biológica de nitrogenio.
Urochloa brizantha.

INFORMATION
Cronología del artículo.
Recibido/Received: 02/07/2020
Aceptado/Accepted: 24.09.2020
On-line: 15/01/2021
Correspondencia a los autores/Contact e-mail:
slguimaraes@hotmail.com

INTRODUCTION

In Brazil, grasslands occupy approximately 180 million hectares, and around half of that has *Urochloa* genus plants (Boddey et al., 2006 and Rufin et al., 2015). Althought, estimates show that around 70 mi hectares of pastures are in different stages of land degradation (Hungria, 2016). Inadequate management and lack of nutrient replacement are two of the main causes of soil degradation. The use of diazotrophic bacteria can supply part of the nitrogen required by grasses (Ribeiro et al., 2020) and together with the proper edaphic management they can provide better vegetation cover and, consequently, favor the conservation of soil water and the better use of plants for the other nutrients present in soil solution (Bonfim-Silva et al., 2011).

Among the factors that influence pasture degradation, the deficiency of nitrogen is one of the most important (Oliveira et al., 1997). The use of nitrogen fertilizer increases production costs and can compromised systems sustainability. There is been recent efforts on better understanding the potential of biological nitrogen fixation (BNF) in tropical grasses, as an economical and sustainable alternative of supplying nitrogen to plants (Souto, 1982; Bashan et al., 2004 and Silva et al., 2010). Research efforts have focused on the use of associative diazotrophic bacteria as *Azospirillum*, *Herbaspirillum*, *Burkholderia* (Bosa et al., 2016). These genuses can fix N through biological nitrogen fixation (BNF) and produce plant hormones, promoting an increase in the growth of different plants species with emphasis on the use of forage grasses (Silva & Reis, 2009 and Marini et al., 2015).

The objective of this study was to quantify the growth development of marandu palisadegrass under inoculation of associative diazotrophic bacteria which was cultivated in Cerrado's dystrophic Oxisol.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Federal University of Mato Grosso (16º28', 50º34', 284 m altitude). Soil used was collected and is classified as dystrophic Red Oxisol (Donagema et al., 2011). Soil was collected to 0.20 m depth. Soil chemical and physical characteristics are: P and K were 1.4 and 66 mg dm^{-3}; Organic Matter (OM) = 18.8 g dm^{-3}; pH=4.6 (0.01 mol L^{-1} $CaCl_2$); Al; Ca and Mg = 0.5, 1.1 and 0.4 $cmol_c$ dm^{-3}, respectively; and clay, silt and sand = 525, 150 and 325 g kg^{-1} soil, respectively. The base saturation of the soil was raised up to 50% with the incorporation of dolomitic limestone, with Real Power of Total Neutralization (RPTN = 80.3%), reacting for a period of 30 days to correct acidity for 5.5. During the merging between the limestone and the soil, moisture was maintained by the gravimetric method at 60% of maximum water retention, determined in laboratory in pots of the same volume used in the experiment, in three replicates according to the methodology described by (Santos et al., 2013). After the incubation initial application of P, K and sulfur was conducted on a rate of, respectively, 200, 150 and 40 mg dm^{-3}(Bonfim-silva et al., 2011). Fertilizer sources used were triple superphosphate, potassium chloride and calcium sulfate. Micronutrient fertilization was done with boric acid, copper chloride, zinc chloride and sodium molybdate at the doses of 1.5 mg dm^{-3}; 2.5 mg dm^{-3}; 2.0 mg dm^{-3} and 0.25 mg dm^{-3}, respectively (Bonfim-Silva et al., 2007).

Treatments were Y2 (*Nitrospirillum amazonense*), MTH2 (similar to *Herbaspirillum* spp.), MTB1 (similar to *Burkholderia* spp.), an inoculant composed of the *Azospirillum* Ab-V5 and Ab-V6 strains and a nitrogen control (100 mg dm^{-3} N-urea). The experimental design was complete randomized with five replicates (8 dm^3 each pot), totalling 25 plots. The seeding rate consisted in fifteen seeds per plot, and seven days after germination, thinning was conducted to keep five plants per pot.

The contrasts applied to analyze treatments within each cut consisted of: (C1) = nitrogen fertilization vs. strains of diazotrophic bacteria; (C2) = *Azospirillum brasilense* vs. other diazotrophic strains (MTH2, Y2 and MTB1); (C3) = MTH2 vs. Y2 and MTB1; (C4) = Y2 vs MTB1. The contrasts applied for analysis of the cuts in each of the treatments consisted of: (C1) = first cut vs. second and third cuts; (C2) = second cut vs. third cut.

The Y2 strain was multiplied in LGI liquid medium (Döbereiner et al., 1995) and lineage MTH2 and MTB1 were multiplied in DYGS liquid medium (Rodrigues Neto, 1986) under agitation at 100 rpm for 24 hours. An aliquot containing 3 mL of bacterial broth (10^9 cells mL^{-1}) was applied to the soil around the root system area of each plant. Three harvests were conducted after 30, 60 and 90-d after sowing to a 5-cm stubble height in the three cuts, respectively. After each harvest, nitrogen fertilizer was applied only to control treatments (100 mg dm^{-3} as urea) and applied potassium fertilization (200 mg dm^{-3} as potassium chloride) in all treatments, inoculated and nitrogen control, respectively. In addition, 3 mL of bacterial mix was applied to inoculation treatments.

The response variables measured were canopy height, number of leaves, and shoot and root dry weight. The canopy height was measured using a ruler. After each harvest, plant parts were separated and samples were placed in an oven with forced air circulation at 65 ºC until constant weight, and weighed. In the third cut, the roots were sieved washed over a 4 mm mesh. Similarly, root samples were dryed, then, weighed.

Statistical analysis was performed using the statistical program SISVAR 4.2 (Ferreira, 2011) submitted to analysis of variance and the comparison of means was conducted by orthogonal contrasts and F-test, P-value≤0.5.

RESULTS

There were no differences among treatments due to canopy height, except on the thid harvest. On, C1 plants inoculated with diazotrophic bacteria results a higher height of 6.4 cm compared with the treatment that received nitrogen fertilizer in the first cut. Considering that the maximum height of the Marandu grass plants observed corresponds to the treatment with nitrogen fertilization (100%), the other treatments presented height between 62 and 65% of the maximum production (13.9 cm) in the third cut, for C1 (P<0,001), when compared to nitrogen treatment (**Table I**).

For height between the cuts of the Marandu grass, there were significant differences for the contrasts C1 (P<0,001) and C2 (P<0,001), respectively. The highest height of the grass was observed in C1 for the treatment MTH2, with an increase of 35 cm. In C2, observed higher height for Marandu grass in all treatments, and MTH2 treatment, which generated an increase of 30.4 cm (**Table II**).

There was a significant difference in C1 for the first (P=0,011), second (P<0,001) and third (P<0,001) cuts, and based on the C1 estimates there was an increase in the number of leaves along the cuts of marandu grass 44.55; 156.4 and 275.5 leaves pot $^{-1}$, respectively (**Table III**).

There was a significant difference in the contrasts C1 (P<0.000) and C2 (P<0.000) for the nitrogen control treatment, and in the third cut the number of leaves was 48% higher compared to the first cut of the grass, characterizing an increase in the number of leaves along the cuts of the plants that received nitrogen fertilization. There was also a significant difference in C2 (P=0.048) for the treatment that received inoculation with *Azospirillum brasilense*. However, it was observed that in the third cut there was a reduction of 25% in comparison with the number of leaves produced in the first cut of the grass, showing a tendency of decrease in the number of leaves along the cuts, for the plants that were treated with *Azospirillum brasilense* (**Table IV**).

For shoot dry mass there was a significant difference of the treatments in the C1 (P<0.000) for the three cuts, as well as in the C2 (P=0.001), C3 (P<0.000) and C4 (P<0.000) in the second cut, respectively. However, in C1 there was an increase in shoot dry matter over the three cuts with 37.26; 16.11 and 48.70g pot^{-1}, in relation to the inoculated treatments (Table V). However, in the present study, result an increase in the treatments inoculated in the second cut, when the inoculated plants reached between 72.48 and 85.41% of the maximum dry matter yield compared to the plants that received nitrogen fertilization.

Table I. Treatment averages, F values and estimates of orthogonal contrasts of Marandu grass height inoculated with associative diazotrophic bacteria (Médias de tratamento, valores F e estimativas de contrastes ortogonais da altura do capim Marandu inoculado com bactérias diazotróficas associativas).

Treatments	Cuts 1º	2º	3º
		Average	
Control	65,40	56,20	38,80
A.brasilense	71,40	54,00	24,60
MTH2	75,20	55,40	25,00
MTB1	71,20	57,60	25,60
Y2	69,40	52,20	24,20
Contrasts		F_c	
C1	0,054^{ns}	0,669^{ns}	0,000***
C2	0,875^{ns}	0,752^{ns}	0,921^{ns}
C3	0,175^{ns}	0,889^{ns}	0,978^{ns}
C4	0,664^{ns}	0,195^{ns}	0,735^{ns}
		Estimate	
C1	-6,400	1,400	13,950
C2	-0,533	-1,066	-0,333
C3	4,900	0,500	0,100
C4	1,800	5,400	1,400

C1 = Control vs. Strains of diazotrophic bacteria; C2 = A. brasilense vs other strains of diazotrophic bacteria; C3 = MTH2 vs Y2 and MTB1; C4 = MTB1 vs. Y2, ns, *** - Not significant, significant 0.1%, respectively, by the test F.

For cuts within treatments, there was a significant difference in C1 (P<0.000) and C2 (P<0.000), except for C1 within the control treatment (**Table VI**).

A significant difference was observed in C1 (P<0.000) with higher root dry mass in plants that received nitrogen fertilization, followed by MTB1 treatment that produced the equivalent of (47.34%) of the maximum yield. There was no significant difference for the C2, C3 and C4 contrasts, respectively (**Table VII**).

DISCUSSION

In the present study the results indicate that, in both the first and second cuts, the nitrogen supplied by the bacteria to Marandu grass was sufficient to maintain the energy demand that plant needed for completing their initial and intermediate development. This result may be related to the rapid availability of nitrogen to plants, acting as a structural component of macromolecules and enzymes involved in the vegetative development process (Malavolta, 2006). Similar results were observed for height of *Urochloa brizantha* cv. Insurgente, submitted to inoculation with diazotrophic bacteria combined with fungi, which result in height equivalent to that of the nitrogen-fertilizer treatment (Lozano-Contreras et al., 2013).

Marini et al. (2015) studies with maize inoculated based on *Azospirillum brasilense*, asociated with different levels of nitrogen fertilization, observed that there was no significant difference in corn height compared with inoculated treatment and control without mineral nitrogen.

Guimarães et al.(2011), in studies realized with *Urochloa brizantha* cv. Marandu inoculated with *Azospirillum spp*. observed that in the variable plants height,

Table II. Treatment averages, F values and estimates of orthogonal contrasts of Marandu grass height inoculated with associative diazotrophic bacteria (Médias de tratamento, valores F e estimativas de contrastes ortogonais da altura do capim Marandu inoculado com bactérias diazotróficas associativas).

Cuts	Treatments Control	brasilense	MTH2	MTB1	Y2
		Average			
1º	65,40	71,40	75,20	71,20	69,40
2º	56,20	54,00	55,40	57,60	52,20
3º	38,80	24,60	25,00	25,60	24,20
Contrasts		F_c			
C1	0,000***	0,000***	0,000***	0,000***	0,000***
C2	0,000***	0,000***	0,000***	0,000***	0,000***
		Estimate			
C1	17.900	32,1	35	29,6	31,2
C2	17,400	29,4	30,4	32	28

C1: First cut vs. second and third cuts; C2: Second court vs. third cut. *** Significant at 0.1%, by the test of F.

the highest values were obtained in the treatment with inoculated plants, when compared to the absolute control (without nitrogen and without inoculation), as well as values close to those obtained with nitrogen fertilization.

In C1 there was an increase in number of leaves along the cuts of Marandu grass. According to Sá Medica et al. (2017), the induction of tillering certainly arises as a function of the cut, resulting in a counterpart in the increase in the number of leaves per plant, which in the presence of nitrogen occurs at a higher rate due to the origin of new tillers. Sales et al. (2014) observed a linear increase in the number of leaves of Marandu grass under increasing doses of nitrogen. Among the contrasts involving the bacterial strains, the C4 was highlighted, with a higher number of leaves compared to the other treatments inoculated.

Alexandrino et al. (2010) observed an increase in the number of leaves of *Urochloa brizantha* cv. Marandu, having received complete fertilization during the establishment of the plants, also attributing that the population density and the weight of tillers are the two components that define the main indices in the increases of the dry matter production of the plant.

The plants inoculated in the present study in the second cut achieved an approximate Shoot dry mass yield in comparison to the plants that received nitrogen fertilization. According to Miranda et al. (2018), in studies with *Urochloa brizanta* cv. Marandu using strains of diazotrophic bacteria (UNIFENAS 100-13 and UNIFENAS 100-94), it was observed that the plants that received inoculation produced shoot dry mass of aerial part equivalent to the plants that were treated with mineral nitrogen.

Bosa et al. (2016) in studies realized with Xaraés grass inoculated with associative diazotrophic bacteria, state that there was a significant increase (3.48g $vase^{-1}$) shoot dry mass of the aerial part with the treatments inoculated in the first of the three cuts, when compared with the nitrogen fertilization treatments and absolute control.

According to Silva et al. (2016), were observed higher values of shoot dry mass of *Urochloa* inoculated from the second cut, attributing such positive effect to the presence of the inoculation with *Azospirillum* on the development and yield of grass biomass. According to Costa (2007), the most suitable cutting age for Marandu grass, aiming to reconcile the highest yield and best crude protein content, is between 56 and 70 days of vegetative growth. In the present study, the highest values of shoot dry mass were observed at 60 days of Marandu grass cultivation.

In the present study, there was partial equivalence of the root dry mass in the inoculated treatments in relation to the control treatment, which leads us to understand these results and those observed in studies with *Urochloa brizantha* cv Paiaguás, carried out by Rocha & Costa (2018), noting that in general, the performance of the plants inoculated with *Azospirillum* brasilense was similar or superior to the plants that

Table III. Treatment averages, F values and estimates of orthogonal contrasts of the number of leaves of Marandu grass inoculated with associative diazotrophic bacteria (Médias de tratamento, valores F e estimativas de contrastes ortogonais do número de folhas do capim Marandu inoculado com bactérias diazotróficas associativas**).**

Treatments	Cuts		
	1º	2º	3º
		Average	
Control	214,20	306,60	413,40
A.brasilense	167,20	134,80	124,40
MTH2	161,60	133,80	133,00
MTB1	183,80	156,20	172,40
Y2	166,00	148,40	122,20
Contrasts		F_c	
C1	0,011*	0,000***	0,000***
C2	0,853ns	0,248 ns	0,310 ns
C3	0,479 ns	0,088 ns	0,453 ns
C4	0,412 ns	0,105 ns	0,024*
		Estimate	
C1	44,550	156,400	275,500
C2	-3,266	-20,533	-18,000
C3	-13,300	-32,300	-14,100
C4	17,800	35,400	49,800

C1 = Control vs. Strains of diazotrophic bacteria; C2 = *A. brasilense* vs other strains of diazotrophic bacteria; C3 = MTH2 vs Y2 and MTB1; C4 = MTB1 vs. Y2, ns, * and *** - Not significant, significant 5 and 0.1%, respectively, by the test F.

Table IV. **Treatment means, F values and estimates of orthogonal contrasts of the number of leaves of Marandu grass inoculated with associative diazotrophic bacteria** (O tratamento significa, valores F e estimativas de contrastes ortogonais do número de folhas do capim Marandu inoculadas com bactérias diazotróficas associativas).

		Treatments			
Cuts	Control	Brasilense	MTH2	MTB1	Y2
		Average			
1º	214,20	167,20	161,60	183,80	166,00
2º	306,60	134,80	133,80	156,20	148,40
3º	413,40	124,40	133,00	172,40	122,20
Contrasts		Fc			
C1	0,000***	0,048*	0,136ns	0,753ns	0,105ns
C2	0,000***	0,631ns	0,970ns	0,586ns	0,229ns
		Estimate			
C1	-145,800	37,600	347,848	5,900	30,700
C2	-106,800	10,400	0,800	11,800	26,200

C1: First cut vs. second and third cuts; C2: Second court vs. third cut.ns, * and *** -Not significant, significant at 5% and 0.1%, respectively, by the F. test.

Table V. **Treatment averages, F values and estimates of orthogonal contrasts of the dry mass of aerial part (g pot^{-1}) of the *Urochloa brizantha* cv. Marandu for treatments inside cuts** (Médias de tratamento, valores F e estimativas de contrastes ortogonais da massa seca de parte aérea (g pot-1) do currículo *Urochloa brizantha* cv. Marandu para tratamentos dentro de cortes).

		Cuts	
Treatments	1º	2º	3º
		Average	
Control	54,45	48,48	64,26
A.brasilense	15,74	37,26	15,46
MTH2	15,93	36,92	15,41
MTB1	20,12	41,41	17,27
Y2	16,96	35,14	14,07
Contrasts		F_c	
C1	0,000***	0,000***	0,000***
C2	0,325ns	0,001***	0,949 ns
C3	0,210 ns	0,000***	0,902 ns
C4	0,188 ns	0,000***	0,184 ns
		Estimate	
C1	37,263	16,113	48,708
C2	-1,926	6,522	-0,124
C3	-2,611	9,276	-0,256
C4	3,166	-15,040	3,200

C1 = Control vs. Strains of diazotrophic bacteria; C2 = *A. brasilense* vs other strains of diazotrophic bacteria; C3 = MTH2 vs Y2 and MTB1; C4 = MTB1 vs. Y2, ns, *** - Not significant, significant 0.1%, respectively, by the test F.

Table VI. **Treatment means, F values and estimates of orthogonal contrasts of the dry mass of shoot Marandu grass inoculated with associative diazotrophic bacteria** (Tratamento significa, valores F e estimativas de contrastes ortogonais da massa seca de parte aérea do capim marandu inoculado com bactérias diazotróficas associativas.).

		Treatments			
Cuts	Control	*A. brasilense*	MTH2	MTB1	Y2
		Médias			
1º	54,45	15,74	15,93	20,12	16,96
2º	48,48	37,26	36,92	41,41	35,14
3º	64,26	15,46	15,41	17,27	14,07
Contrasts		Fc			
C1	0,355^{ns}	0,000***	0,000***	0,000***	0,000***
C2	0,000***	0,000***	0,000***	0,000***	0,000***
		Estimate			
C1	-1,920	-10,616	-10,238	1,426	-7,660
C2	-15,784	21,796	21,504	2,852	21,092

C1: First cut vs. second and third cuts; C2: Second court vs. third cut. ns Not significant and significant *** 0.1% by the test of F.

Table VII. **Treatment averages, F values and estimates of orthogonal contrasts of root dry mass (g pot^{-1}) of Marandu grass inoculated with associative diazotrophic bacteria** (Médias de tratamento, valores F e estimativas de contrastes ortogonais de massa seca radicular (g pot-1) do capim marandu inoculado com bactérias diazotróficas associativas).

	Treatments Averages				Contrasts	Estimate	F_c
	Controle	Brasilense	MTH2	MTB1	Y2		
	124,5	33,3	41,48	58,94	39,89		
			Coeficiente Contraste				
C1	4	-1	-1	-1	-1	81,08	0.000***
C2	0	3	-1	-1	-1	-2,55	0.788^{ns}
C3	0	0	2	-1	-1	-16,11	0.121^{ns}
C4	0	0	0	1	-1	19,052	0.113^{ns}

C1 = Control vs. Strains of diazotrophic bacteria; C2 = *A. brasilense* vs other strains of diazotrophic bacteria; C3 = MTH2 vs Y2 and MTB1; C4 = MTB1 vs. Y2, ns, *** - Not significant, significant 0.1%, respectively, by the test F.

received nitrogen fertilization, emphasizing that, in this case, the bacteria supplied nitrogen to plants in equivalence to nitrogen fertilizer. However Guimarães et al. (2011), in studies with *Urochloa decumbens* inoculated with *Azospirillum spp.* observed that there was no statistical difference between the treatments with inoculation and the nitrogen fertilizer for the root dry mass variable.

For Galeano et al. (2019), inoculation of maize plants with commercial inoculant (AbV5 and AbV6) resulted in a higher root mean dry mass (0.188 g) followed by treatment with the strains MAY1 and BR11001, compared to the control without nitrogen.

CONCLUSION

The inoculation of *Urochloa brizantha* cv. Marandu with the associative diazotrophic bacteria MTH2 and MTB1 provides part of the nitrogen necessary for the development of the grass, contributing positively to its production.

BIBLIOGRAPHY

Alexandrino, E, Vaz, RGMV & Santos, AC 2010, 'Characteristics of *B. brizantha* cv. Marandu during its establishment submitted to different doses of Nitrogen'. *Bioscience. J. Uberlândia*, v. 26, n. 6, pp. 886-893.

Bashan, Y, Holguin, G, & De-Bashan, LE 2004, '*Azospirillum*-plant relationships: physiological, molecular, agricultural and environmental advances'. *Canadian Journal of Microbiology*, v.50, pp. 521-577.

Boddey, RM, Jantalia, CP, Macedo, MO, Oliveira, OC , Resende, AS , Alves, BJR & Urquiaga, S 2006, 'Potential of carbon Atlantic forest region Brazil'. In: Lal, R, Cerri, CC, Bernoux, M, Etchevers, J. Cerri, E. (Eds.). Carbon sequestetration in soil of Latin American. The Haworth press Bubghamton. New York. pp. 305-347.

Bonfim-Silva, EM, Monteiro, FA & Silva, T J A 2007, 'Nitrogen and sulfur in the production and use of water by degraded brachiaria grass'. *Brazilian Journal of Soil Science*, v.31, pp.309-317.

Bonfim-Silva, EM, Silva, TJA, Cabral, CEA, Kroth, BE & Rezende, D 2011, 'Desenvolvimento Inicial de gramíneas submetidas ao estresse hídrico'. *Revista Caatinga*, Mossóro, v.24, pp.180-186.

Bosa, CK, Guimarães, SL, Polizel, AC, Bonfim-Silva, EM & Canuto, EL 2016, 'Productive and nutritional characteristics of Xaraean grass inoculated with associative diazotrophic bacteria'. *Arq. Bras. med. vet. Zootec.*, v.68 n.5, pp.1360-1368.

Costa, KDP, Oliveira, IP, Faquin, V, Neves, BP, Rodrigues, C & Sampaio, F 2007, 'Cutting interval in dry mass production and chemical-bromatological composition of *Urochloa brizantha* cv. MG-5'. *Agrotechnical science*, Lavras, v. 31, n. 4, pp. 1197-1202.

Döbereiner, J, Baldani, VLD & Baldani JI 1995, '*Como isolar e identificar bactérias diazotróficas de plantas não-leguminosas'*. Embrapa-SPI, Brasília. Available in: https://www.bdpa.cnptia.embrapa.br/consulta/busca?b=ad&id=622470&biblioteca=CNPAB&busca=autoria:%22BALDANI,%20V.L.D.%22&qFacets=autoria:%22BALDANI,%20V.L.D.%22&sort=&paginacao=t&paginaAtual=1. Access in: May 5, 2020.

Donagema, GK, Campos, DV, Calderano, SB, Teixeira, WG, Viana, JM 2011, '*Manual de métodos de análise de solos'*. Embrapa- SPI, Rio de Janeiro 2 ed. (Eds.): Embrapa Solos. ISSN: 1517-2627. Available in: https://www.infoteca.cnptia.embrapa.br/bitstream/doc/990374/1/ManualdeMtodosdeAnilisedeSolo.pdf. Access in: May 10, 2020.

Ferreira, DF 2011, '*Sisvar: a program for analysis and teaching of statistics'*. Symposium Journal. Lavras. v.3, pp.317-345.

Galeano, RMS, Campelo, APS, Mackert, A & Brazil, MS 2019, 'Initial development and quantification of maize proteins after inoculation with new strains of Azospirillum brasilense'. *Journal of Neotropical Agriculture*, Cassilândia-MS, v. 6, n. 2, pp. 95-99.

Guimarães, SL, Bonfim-Silva, EM, Polizel, AC & Campos, TS 2011, 'Production of Marandu grass inoculated with azospirillum spp.' *Encyclopedia Biosphere*, Centro Científico Conhecer - v.7, pp.819.

Hungria, M, Nogueira, MA & Araujo, RS 2016, 'Inoculation of Urochloa spp. with the plant growth-promoting bacterium *Azospirillum brasilense*: An environment-friendly component in the reclamation of degraded pastures in the tropics'. *Agric. Ecosyst. Environ.* V. 221, pp.125-131.

Lozano-Contreras, MG, Rivas-Pantoja, F & Castillo-Huchim, JE 2013, 'Growth of Urochloa brizantha seedlings in response to the application of mycorrhizal fungi and diazotrophic bacteria'. *Pastures and Forages*, v. 36, n. 2, pp.227-232.

Malavolta, E 2006, '*Manual of mineral nutrition of plants'*. Agronomica Ceres. São Paulo, p.638.

Malavolta, E, Vitti, GC & Oliveira, SA 1997, *Evaluation of the Nutritional State of the Plants: Principles and Application.* Brazilian Association for Research on Potassium and Phosphate. 2nd. Potafos, Piracicaba, SP, Brazil. p. 319.

Marini, D, Guimarães, VF, Dartora, J, Lana, MDC & Pinto Júnior, A S 2015, 'Growth and yield of corn hybrids in response to association with *Azospirillum brasilense* and nitrogen fertilization'. *Revista Ceres*, v.62, n.1, pp.117-123.

Miranda, CC, Florentino, LA, Rezende, AVD, Nogueira, DA, Leite, RF & Naves, LP 2018, 'Development of *Urochloa brizantha* fertilized with phonolite and inoculated with potassium solubilizing diazotrophic bacteria'. *Journal of Agricultural Sciences*, v.41, n.3, pp. 41-50.

Oliveira, OC, Oliveira, IP, Ferreira, E, Alves, BJR, Cadisch, G, Miranda, CHB, Vilela, L, Boddey, RM & Urquiaga, S 1997,' *Low soil nutrient availability as a potential cause of pasture degradation in the Brazilian cerrado*.' In: National Symposium on the Recovery of Degraded Areas.

Ribeiro, NVS, Vidal, MS, Barrios, SCL, Baldani, VLD, & Baldani, JI 2020, 'Genetic diversity and growth promoting characteristics of diazotrophic bacteria isolated from 20 genotypes of Brachiaria spp.' *Plant and Soil*, v. 451, n. 1, pp. 187-205.

Rocha, RAFS & Costa, RRGF 2018, 'Performance of *Urochloa brizantha* cv paiaguás inoculated with *Azospirillum brasilense* and different doses of nitrogen'. *Global Science and Technology*, v. 11, n. 3, pp. 177-186.

Rodrigues Neto, J, Malavolta Junior, VA & Victor, O 1986, 'Simple medium for the isolation and cultivation of *xanthomonas campestris PV. Citri type B.' Summa Phytopathologica*, v.12, p. 60.

Rufin, P, Müller, H, Pflugmacher, D, Hostert, P 2015, 'Land use intensity trajectories on Amazonian pastures derived from Landsat time series'. *International Journal of Applied Earth Observation and Geoinformatio,* v. 41, pp. 1–10.

Sá Medica, JA, Reis, NS & Santos, MER 2017,'Morphological characterization in pastures of Marandu grass submitted to frequency of defoliation and levels of fertilization'. *Brazilian Animal Science*, v.18. pp. 1-13.

Sales, EC J, Reis, ST, Monção, FP, Antunes, AB, Alves, DD, Aguiar, ACR & Mota, VAC 2014, 'Forage production, structural characteristics and efficiency of nitrogen utilization in Marandu grass'. *Agrarian*, v. 7, n. 25, pp. 434-446.

Santos, CS, Silva, PF, Santos, JC, Silva, CH & Barros AC 2013, 'Estimativa da umidade na capacidade de campo em vasos e em laboratório'. *Revista Verde*. v. 8, n. 2, pp. 151-160.

Silva, JAN, Ceccon, G, Rocha, EC & Souza, CM 2016,'Cowpea and brachiaria productivity with inoculation in the seeds, in single and intercropping cultivation'. *Agrarian*, v. 9, n. 31, pp. 44-46.

Silva, LLGG, Alves, G, Ribeiro, J, Urquiaga, S, Souto, S, Figueiredo, MVB & Burity, HA 2010,'Biological fixation of nitrogen in pastures with different cutting intensities'. Animal Husbandry Records, v.59, n.225, pp. 21-30.

Silva, MF & Reis, VM 2009,'Production, characterization and application of polyclonal antibody against *Azospirillum amazonense* strain AM 15'. *Revista de Ciências Agronômicas*, v. 68, n. 1, pp. 1-11.

Souto, SM 1982, 'Seasonal variation of N_2 fixation and denitrification in tropical forage grasses'. Dissertation. Federal Rural University of Rio de Janeiro. Seropédica.