Forage Mass, Tillering, Nutritive Value and Root System of Ruzigrass Inoculated with Plant Growth Promoting Bacteria Associated with Doses of N-Fertilizer

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Abstract

The aim of this study was evaluating the effect of the inoculation of plant growth promoting bacteria (PGPB) in forage mass, tillering, nutritive value and root system of ruzigrass (Urochloa ruziziensis (R. Germ. & Evrard) Crins (syn. of Brachiaria ruziziensis) associated with doses of N-fertilizer. The bacteria inoculated were Azospirillum brasilense Ab-V5, Pseudomonas fluorescens CCTB03 and Pantoea ananatis AMG 521, plus the control treatment (non-inoculated), associated with doses of N-fertilizer (0, 50 and 100 kg N ha^{-1}). The experiment was performed in a randomized block design, in a 4x3 factorial scheme, with four replicates, totaling 48 plots (12 m^2). There were no effects of the PGPB and the use of N-fertilizer on the leaf blade, stem+sheath, forage mass, daily and yearly accumulation of forage mass. The PGPB did not have influence on the density of tillers. The doses of 50 and 100 kg of N ha^{-1} increased the amount of tillers. The AMG 521 strain associated with N-fertilizer provided heavier tillers. There was no effect of the PGPB on crude protein (CP), neutral detergent fiber (NDF), as well as acid detergent fiber (ADF), and in vitro digestibility of the dry matter (IVDDM). The use of 100 kg of N ha^{-1} contributed to an increase in CP and a decrease in NDF. The AMG 521 strain contributed to a smaller diameter of the root. Strains CCTB03 and AMG 521 demonstrated a smaller area, length and root density when associated with the dose of 50kg of N ha^{-1}. In general, the PGPB were not efficient in promoting productive increments in ruzigrass.

Keywords: growth promotion, plant biomass, plant growth hormones.

1. INTRODUCTION

Nitrogen (N) is one of the most required nutrients by plants (Dobbelaere and Okon, 2007). When it is applied, plants have positive responses in terms of production and nutritive value of forage mass (Palmer et al, 2014). However, its use in pasture management leads to an increase in production costs (Guimarães et al., 2011), besides damaging the environment due to the fact that, out of the total of N-fertilizer applied, plants' assimilation barely exceeds 50% (Freitas e Rodrigues, 2010). As for the rest, it ends up being wasted in the system (Van Groenigen et al., 2015).

For that reason, it is indispensable to use more efficient and sustainable agricultural technologies capable of reducing dependence on the use of N-fertilizer. An alternative to mitigate the negative impact of conventional practices for pasture fertilization is the use of plant growth promoting bacteria (PGPB) associated with grasses. PGPB have the ability of N_2 fixation (Sarathambal et al., 2015), and they can promote plants growth by synthesizing hormones, solubilizing phosphate and producing siderophores (Kavamura et al., 2013).

Studies with PGPB associated with tropical grasses started in the 50's, with the isolation and description of *Azospirillum* sp. in sugar cane rhizosphere, by Döbereiner and Ruschel (1958). Since then, other bacterial genera have been isolated and studied with the same potential of bringing benefits to tropical grasses.

Nowadays, it is possible to find studies that present the positive effects of inoculation in grasses, such as the increment of forage mass and the reduction in the need for the support of N-fertilizer in pastures of *Urochloa* sp. (Hungria et al., 2016; Leite et al., 2018; Lopes et al., 2018; Duarte et al., 2020) and *Cynodon* sp. (Aguirre et al., 2018).

Studies on the behavior of PGPB in association with N-fertilizer have been demonstrating that, for bacteria such as *A. brasilense*, the use of high doses of N-fertilizer reduces its effects on grasses productive parameters (Cassán e Diaz-Zorita, 2016), or completely inhibits the plant's response to the inoculation (Ozturk et al., 2003). Yet, in sites where N limits production, the effects of the inoculation are more evident, or when moderate doses of N-fertilizer are used in association with PGPB, we can see the complementary effect of such association with increments mainly in biomass production, as reported by Cassán and Diaz-Zorita (2016).

Nonetheless, since we are talking about studies whose some aspects must be elucidated, the results are still inconclusive, and more data are necessary to support the indication of inoculant tests as a commercial product for tropical pastures.

The development of alternative methods capable of modernizing and enabling pasture production systems, besides studying their effects at different stages of pasture growth, allows understanding the biological responses of forages when associated with PGPB, and serves as guidelines for scientists' decision-making, and further transference of such technology to producers (Mamédio et al., 2020).

In this context, this study aimed to evaluate the effect of the inoculation of PGPB on the production of forage mass, tillering, nutritive value and root system of ruzigrass associated with doses of N-fertilizer.

2. MATERIAL AND METHODS

2.1. Site and experimental design

The experiment was carried out on Iguatemi Experimental Farm, which belongs to the State University of Maringá (UEM), in Iguatemi, city of Maringá, state of Paraná – Brazil (23°25'S, 51°57'W; 550 m a.m.s.l.), from October 2016 to September 2018.

The soil of the experimental area is classified as Dystropheric Red Latosol (Santos et al., 2018). It was collected from the 0-0.2 m depth layer, and presented the following chemical characteristics: hydrogen potential (pH in H_2O) - 5,6; SMP index - =6,6; phosphorus (P-Mehlich) - 10,75 mg dm^{-3}; potassium (K^+) - 0,12 $cmol_c dm^{-3}$; aluminum (Al^{3+}) - 0,00 $cmol_c dm^{-3}$; calcium (Ca^{2+}) - 1,36 $cmol_c dm^{-3}$; magnesium (Mg^{2+}) - 0,59 $cmol_c dm^{-3}$ and base saturation (V) - 39,4%; cation exchange capacity (CTC pH 7.0) - 5,25; organic matter (MO) - 11,8 g dm^{-3}; sand - 830 g kg^{-1}; silt - 30 g kg^{-1} and clay - 140 g kg^{-1}. Acidity correction of the soil was performed with dolomitic limestone, with an increase in base saturation to 50% and incorporation of dolomitic limestone with RPTN = 91% (real power of total neutralization).

The grass species used was ruzigrass (*Urochloa ruziziensis* (R. Germ. & Evrard) Crins (syn. of *Brachiaria ruziziensis*). The experiment was conducted in randomized blocks, arranged in a 4x3 factorial scheme, with four replicates, totaling 48 plots, with 4 x 3 m each (12 m^2).

The bacteria inoculated were *Azospirillum brasilense* Ab-V5 (=CNPSo 2083), *Pseudomonas fluorescens* CCTB03 (=CNPSo 2719) and *Pantoea ananatis* AMG 521 (=CNPSo 2798), plus the control treatment (with no bacteria), associated with three doses of N-fertilizer (0, 50 and 100 kg N ha^{-1}).

The strains are deposited in the *Embrapa Soja* Multifunctional Microorganisms Collection: Diazotrophic and Plant Growth Promoting Bacteria (World Federation Culture Colletion-WFCC#1213, World Data Centre for Microorganisms-WDCM#1054). The bacteria derive from selection programs of PGPB of Embrapa Soja: *Azospirillum brasilense* Ab-V5, selected in Brazil, initially for the culture of corn (*Zea mays*) and wheat (*Triticum aestivum*) (Hungria et al., 2010); *Pseudomonas fluorescens* CCTB03 from the company Total Biotecnologia, and *Pantoea ananatis* AMG 521, isolated in Spain (Megías et al., 2016).

For the inoculants preparation, the strains were cultivated in DYGS media (Fukami et al., 2018) and their concentration was adjusted to 10^8 cells mL^{-1}, obtained from the correlation of growth curves previously obtained by the Culture Collection for each strain and the corresponding optical densities. For inoculation, 15 mL of each inoculum were used per Kg of seeds before sowing. The seeds were dried for approximately 30 minutes at a cool place and protected from the sun. Then we sowed the amount of 10 kg ha^{-1} (culture value of the non-treated seeds of 50%).

One week before sowing, fertilization was performed with the application of 84 kg P_2O_5 ha^{-1} (simple superphosphate 18% P_2O_5), 42,5 kg K_2O ha^{-1} (potassium chloride 60% de K_2O). After seedling emergence, the basal dose of 20 kgN ha^{-1} (urea 45 % of N) was applied in all experimental plots. The incorporation of limestone and fertilizers was done with the use of light harrowing.

When the ruzigrass reached, on average, 35-40 cm of height, it was uniformly cut at 15 cm. The heights were measured with the help of a 1-meter millimetered ruler. After that, the plots received N-fertilizer, according to the treatments (0, 50 and 100 kg N ha^{-1}). The amount of 100 kg N ha^{-1} was divided into two applications, with a 15-day interval.

During the experimental period, climate conditions were monitored, with an average record of rainfall (119 mm), relative humidity (83%) and maximum average temperatures (27°C) and minimum (17°C), as shown in Figure 1.

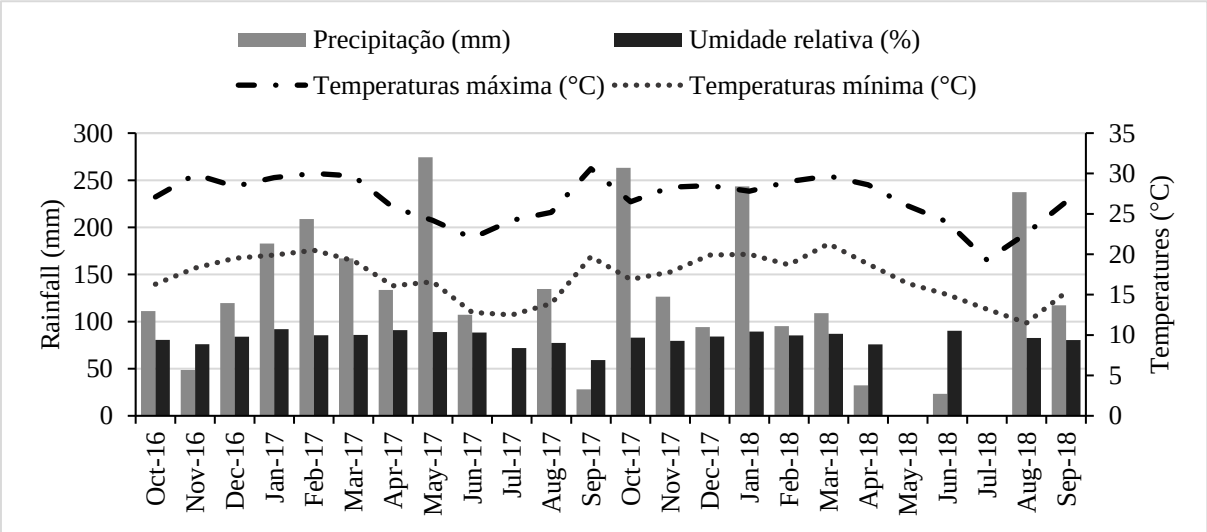

Figure 1. Climatic conditions (rainfall, relative humidity, maximum and minimum temperatures) registered at the Automatic Meteorological Station (FEI-UEM) over the experimental period (October to December 2017 and January to September 2018).

2.2. Measurement of the studied parameters

Cutting management was done based on a light interception (LI) of 95 %. LI was measured with the AccuPAR equipment, PAR/LAI ceptometer®) model LP-80 (Decagon Devices Inc, Pullman, United States). The monitoring of the LI focusing on the forage canopy was performed through the scanning of six random spots per plot every seven days.

After the plot mean reached the expected LI, we randomly collected 10 tillers representing the plot. They were weighed and dried in a stove with forced air circulation at 55 °C for 72 hours and weighed again for determination of dry mass. Afterwards, we randomly collected two samples of forage mass, with cuts at 15 cm of stubble height, by using a STIHL® pruner, model HS 82 R (STIHL, Rio Grande do Sul, Brazil), with the following measures: 0,5x0,5 m (0,25 m²). After obtaining forage mass, we performed uniformity cutting at the same stubble height, in the entire experimental plot.

After harvesting, the material was identified, weighed, and turned into two sub-samples: one for separating the morphological components (leaf blade, stem+sheath and senescent material), and the other for determining dried forage mass. The samples were conditioned in paper bags, weighed and dried in a stove with forced air circulation at 55 °C for 72 hours. Then, it was all weighed again for determination of dry mass and, after that, milled with a stationary "Thomas Wiley" mill adapted with a 2 mm sieve.

Crude protein (CP, g kg^{-1}), neutral detergent fiber (NDF g kg^{-1}), acid detergent fiber (ADF g kg^{-1}) and *in vitro* digestibility of the dry matter (IVDDM g kg^{-1}) were quantified in the leaf blade by Near Infrared Reflectance Spectroscopy – NIRS), (Foss NIRSystems, XDS Rapid Content Analyzer, Denmark).

For the NIRS scanning, we constructed a calibration curve from the laboratorial analytical data of 110 samples, which were analyzed for N using the micro-Kjeldahl method (Tecator, Sweden), and

converted into protein by using the 6.25 factor, according to the methodology described by the AOAC (1990). NDF and ADF were determined in accordance with Van Soest et al. (1991), LIG according to Goering and Van Soest (1970) and IVDDM was obtained in compliance with techniques (traditional methodology) described by Tilley and Terry (1963) and Holden (1999), using a DAISYII artificial rumen (ANKOM™ Technology Corp., Fairport, NY).

For the elaboration of the calibration curve, the samples spectra were scanned by the software ISIScan and exported to the software WinISI III Project Manager 1.50e (Infrasoft International, LLC, 2000, Port Matilda, PA, USA). Reflectance data were stored with log 1/R, with intervals of 2 nm between 700 and 2500 nm.

Principal component analysis (PCA) was carried out before the calibration curves were elaborated by using the partial least squares (PLS) regression model. Then we performed the crossed validation with the software confronting the data from the laboratorial analysis with those estimated by the calibration curve of the NIRS.

After the elimination of the outliers, we used 77 samples for CP, 79 for NDF, 79 for ADF and 71 for IVDDM. For CP, NDF, ADF and IVDDM, respectively, we obtained the following parameters of curve validation: determination coefficient (R^2; 0.99, 0.92, 0.94 and 0.86), cross validation error (SECV; 0.48, 1.43, 0.66 and 4.62%), R^2 corrected by the cross validation error (1-VR; 0.99, 0.88, 0.91 and 0.76), prediction error (SEP; 0.34, 1.03, 0.50 and 3.28) and mean ± standard deviation (X±SD; 12.65±3.33, 51.39±3.73, 24.72±2.12 e 68.54±8.68).

Tillers population density (TPD, tillers.m²) was estimated every 28 days by manually counting the tillers of the plots, considering all the live tillers observed inside the metal frame of 0,5x0,15 m (0,075 m^2), according to the methodology proposed by Sbrissia and Silva (2008). Two representative points were marked inside each plot with a pipe, and the measurements were taken by placing the rectangle at the marked point.

The daily forage mass accumulation rate (dFMA, kg of DM ha^{-1} day) was obtained by dividing the accumulated forage mass by the number of days referring to the harvests interval. The yearly forage mass accumulation rate (yFMA, kg of DM ha^{-1} year) was calculated through the sum of all partial harvests of the experimental period (Barbosa et al., 2007).

Root dry mass (RM, kg ha^{-1}) was determined at the end of each season of the year (autumn, winter, spring and summer), based on the collection of two soil samples with roots in the depth of 0 to 20 cm in each experimental plot, with a steel probe (10 cm of diameter and 50 cm of length), with an opening in the middle to make sample stratification easier. The samples were conditioned in plastic bags previously identified, washed in running water for total soil removal (Soares Filho et al., 2013), weighed and dried in a stove with forced air circulation at 55 °C for 72 hours.

For roots geometry, we withdrew 1 g of roots from the samples after drying for determining root diameter (RDi, mm), root area (RA, mm².dm³), root length (RL, mm) and root density (RDe, mm.cm³) by digitalizing the roots with an HP 3400 Scanner, and the images scanning was done by using the software DELTA T SCAN®.

2.3. Statistical Analysis

We used the PROC GLIMMIX from SAS University (Sas Institute Inc. Cary, CA) in all statistical analyses. The variables were initially tested regarding normality (Shapiro–Wilk test). The data were analyzed in a factorial scheme of four treatments, namely control (non-inoculated), *Azospirillum brasilense* Ab-V5, *Pseudomonas fluorescens* CCTB03 and *Pantoea ananatis* AMG 521) x three doses of N-fertilizer, zero, 50 and 100 kg N/ha. We also considered the data from each season as measures repeated in time, and the random effects of the block and year of analysis. The choice of covariance matrix was made by using the Information Criteria by Akaike (Wolfinger, 1993). The means were estimated by using the "LSMEANS", and the comparison was made through the difference probability (PDIFF), using the Tukey test at 5% of significance.

3. RESULTS

There was no effect of interaction between the PGPB and the doses of N-fertilizer for the leaf blade (LB, kg de MS ha^{-1}), stem+sheath (SS, kg of DM ha^{-1}), production of forage mass (FM, kg of DM ha^{-1}), daily accumulation of forage mass (dAFM, kg of DM ha^{-1} day^{-1}) and yearly accumulation of forage mass (yAFM, kg of DM ha^{-1} $year^{-1}$) (Figure 2).

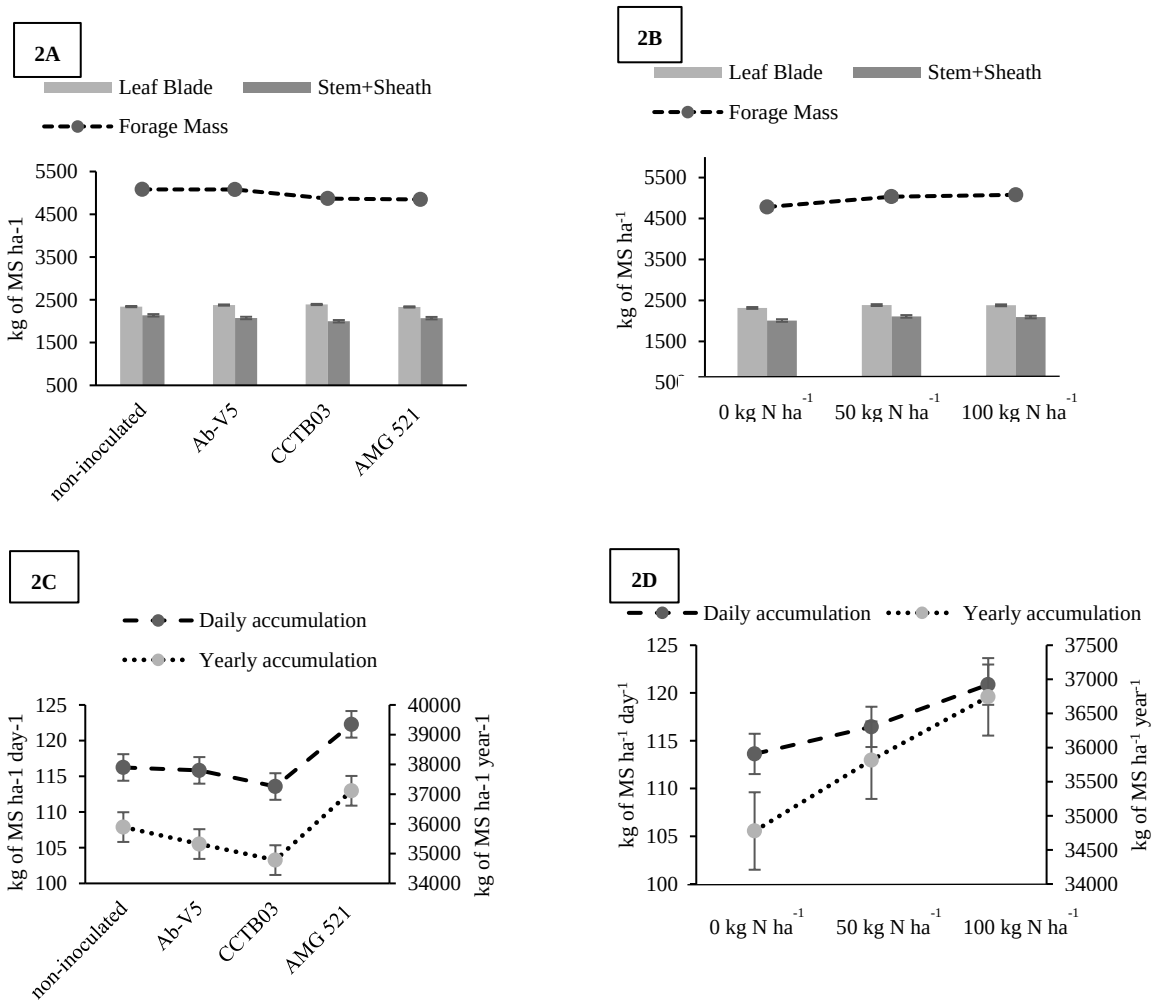

Figure 2. leaf blade, stem+sheath, forage mass (2A and 2B), daily forage mass accumulation and yearly forage mass accumulation (2C e 2D) of ruzigrass inoculated with plant growth-promoting bacteria and N-fertilizer doses.

We did not observe any effects of the inoculation of PGPB strains on these parameters, presenting on average 2367,04 kg of DM ha^{-1} of LB, 2048,15 kg of DM ha^{-1} of SS, 4930,84 kg of DM ha^{-1} of FM, 116,98 kg of DM ha^{-1} day^{-1} of dAFM, 35778,00 kg of DM ha^{-1} $year^{-1}$ of yAFM (Figures 2A and 2C).

Regarding the use of doses of N-fertilizer, there were no effects either, presenting on average 2360,57 kg of DM ha^{-1} of LB, 2070,47 kg of DM ha^{-1} of SS, 4964,61 kg of DM ha^{-1} of FM, 116,98 kg of DM ha^{-1} day^{-1} of dAFM, 35778,00 kg of DM ha^{-1} $year^{-1}$ of yAFM (Figures 2B and 2D).

The interaction between PGPB and the doses of N-fertilizer had no effects on tiller population density (TPD; tillers.m²) of ruzigrass (Table 1). The inoculation of the PGPB strains had no effects on the TPD results, with an average of 1009 tillers.m². Yet, the doses of N-fertilizer presented effects on TPD, with a greater number of tillers ($p = 0,0427$) at the doses of 50 and 100 kg of N ha^{-1}.

Table 1. Tillers population density (TPD, tillers.m²) of ruzigrass inoculated with plant growth-promoting bacteria and N-fertilizer doses

Parameter	Plant Growth-Promoting Bacteria				SEM
	non-inoculated	Ab-V5	CCTB03	AMG 521	
TPD (tillers.m²)	1005	1027	989	1015	79
Parameter	N-fertilizer Doses (kg of N ha^{-1})				SEM
	0	50		100	
TPD (tillers.m²)	973 b	1005 ab		1050 a	78,13

Azospirillum brasilense Ab-V5; *Pseudomonas fluorescens* CCTB03; *Pantoea ananatis* AMG 521. SEM = standard error of mean. Means followed by different lowercase letters in each line are significantly different ($P < 0.05$ Tukey test).

The interaction between the PGPB and the doses of N-fertilizer for tiller weight (TW, $p = 0,0004$) is presented in Table 2. The results showed that, for the treatment group that received only doses of N-fertilizer without the inoculation of PGPB, the tillers were heavier when receiving N-fertilizer.

Table 2. Effect of interaction between plant-growth promoting bacteria (PGPB) and x N-fertilizer doses in tiller weight (TW; g) of ruzigrass

Parameter	non-inoculated	Ab-V5	CCTB03	AMG 521	SEM
0 kg of N ha^{-1}	1,158 B	1,643 A	1,704 A	1,183 B	0,143
50 kg of N ha^{-1}	1,489 A	1,656 A	1,276 A	1,510 A	0,143
100 kg of N ha^{-1}	1,510 A	1,399 A	1,570 A	1,526 A	0,143

Azospirillum brasilense Ab-V5; *Pseudomonas fluorescens* CCTB03; *Pantoea ananatis* AMG 521. SEM = standard error of mean. Means followed by different uppercase letters in each line are significantly different ($P < 0.05$ Tukey test).

The TW values were similar between the Ab-V5 and CCTB03 strains and the doses of N-fertilizer, with an average weight of 1,54 g. For AMG521 strain, the association with the doses of N-fertilizer led to a greater tiller weight, 1,52 on average, in relation to 1,18 provided by the inoculation in the zero dose of N-fertilizer.

Crude protein contractions (CP; g kg^{-1}), neutral detergent fiber (NDF; g kg^{-1}), acid detergent fiber (ADF; g kg^{-1}) and *in vitro* digestibility of the dry matter (IVDDM; g kg^{-1}) are shown in Table 3. There were no interaction effects between the PGPB and the doses of N-fertilizer regarding these parameters.

Table 3. Crude protein (CP; g kg^{-1}), neutral detergent fiber (NDF; g kg^{-1}), acid detergent fiber (ADF; g kg^{-1}) and *in vitro* digestibility of dry matter (IVDDM; g kg^{-1}) of ruzigrass inoculated with plant growth-promoting bacteria and N-fertilizer doses

Parameter	Plant Growth-Promoting Bacteria				SEM
	non-inoculated	Ab-V5	CCTB03	AMG 521	
CP (g kg^{-1})	126,44	128,62	128,9	127,33	3,1
NDF (g kg^{-1})	548,76	548,83	552,27	549,82	16,3
ADF (g kg^{-1})	268,4	271,2	270,2	270,6	2,9
IVDDM (g kg^{-1})	742,3	743,8	742,5	742,5	15,3
Parameter	N-fertilizer Doses (kg of N ha^{-1})				SEM
	0	50	100		
CP (g kg^{-1})	122,39 b	127,22 b	133,85 a		2,60
NDF (g kg^{-1})	554,05 a	548,93 ab	546,79 b		16,30
ADF (g kg^{-1})	271,00	269,60	269,70		2,80
IVDDM (g kg^{-1})	740,70	743,80	743,90		15,30

Azospirillum brasilense Ab-V5; *Pseudomonas fluorescens* CCTB03; *Pantoea ananatis* AMG 521. SEM = standard error of mean. Means followed by different lowercase letters in each line are significantly different ($P < 0.05$ Tukey test).

The inoculation of the PGPB strains had no effects on the results of CP, NDF, ADF and IVDDM, with an average of 127,82, 549,92, 270,10 and 742,78 g kg^{-1}, respectively. However, the doses of N-fertilizer had effects on the aforementioned parameters (Table 3), with a greater concentration of CP and smaller concentration of NDF (p = 0,0001 and 0,0085, respectively) in the dose of 100 kg of N ha^{-1}. ADF and IVDDM were not influenced by N-fertilizer.

Root mass (RM; kg ha^{-1}) and root diameter (RDi; mm) are presented in Table 4. There was no effect of the interaction between the PGPB and the doses of N-fertilizer for these parameters.

Table 4. Root mass (RM; kg ha^{-1}) and root diameter (RDi; mm) of ruzigrass inoculated with plant growth-promoting bacteria and N-fertilizer doses

Parameter	Plant Growth-Promoting Bacteria				SEM
	non-inoculated	Ab-V5	CCTB03	AMG 521	
RM (ton ha^{-1})	11,147	11,77	11,398	11,305	0,476
RDi (mm)	0,20 a	0,19 ab	0,18 ab	0,17 b	0,010

Parameter	N-fertilizer Doses (kg of N ha^{-1})			SEM
	0	50	100	
RM (ton ha^{-1})	10,971	11,144	12,1	0,412
RDi (mm)	0,18	0,18	0,19	0,010

Azospirillum brasilense Ab-V5; *Pseudomonas fluorescens* CCTB03; *Pantoea ananatis* AMG 521. SEM = standard error of mean. Means followed by different lowercase letters in each line are significantly different ($P < 0.05$ Tukey test).

The inoculation of the PGPB strains had no effect on the RM results, with an average of 11.41-ton ha^{-1}. Yet, regarding RDi, the AMG 521 strain was the one that presented the best result with the smallest root diameter (0,17 mm) of ruzigrass. With regard to the use of N-fertilizer, there was no effect on RM and RDi (Table 4), with an average of 11.41-ton ha^{-1} and 0,18 mm, respectively.

The interaction between PGPB and doses of N-fertilizer for root area (RA, p = 0,0230), root length (RL, p = 0,0420) and root density (RDe, p = 0,0117) are presented in Table 5.

Table 5. Effect of interaction between plant-growth promoting bacteria (PGPB) and x N-fertilizer doses in root area (RA, mm².dm³), root length (RL, mm) and root density (RDe, mm.cm³) of ruzigrass

Parameter	non-inoculated	Ab-V5	CCTB03	AMG 521	SEM
	---------- Root Area (mm².dm³)----------				
0 kg of N ha^{-1}	67,34 A	84,18 A	70,27 A	66,83 A	7,280
50 kg of N ha^{-1}	83,52 A	66,07 AB	50,12 B	39,09 B	7,280
100 kg of N ha^{-1}	75,08 A	70,01 A	44,67 A	62,60 A	7,280
	---------- Root Length (mm)----------				
0 kg of N ha^{-1}	343,20 A	362,62 A	361,47 A	365,62 A	24,820
50 kg of N ha^{-1}	358,33 A	352,51 AB	254,63 B	239,10 B	24,820
100 kg of N ha^{-1}	289,69 A	275,12 A	257,91 A	243,02 A	24,820
	---------- Root Density (mm.cm³)----------				
0 kg of N ha^{-1}	0,42 A	0,46 A	0,46 A	0,47 A	0,030
50 kg of N ha^{-1}	0,47 A	0,45 AB	0,32 B	0,30 B	0,030
100 kg of N ha^{-1}	0,36 A	0,34 A	0,33 A	0,31 A	0,030

Azospirillum brasilense Ab-V5; *Pseudomonas fluorescens* CCTB03; *Pantoea ananatis* AMG 521. SEM = standard error of mean. Means followed by different uppercase letters in each line are significantly different ($P < 0.05$ Tukey test).

For RA, RL and RDe, the results showed that the control treatment that received only doses of N-fertilizer without the inoculation of PGPB, and the treatment with the inoculation of the Ab-V5 strain, had similar results, with an average of 75,31 and 73,42 mm².dm³, 330,41 and 330,08 mm, 0,42 and 0,42 mm.cm³, respectively. The CCTB03 and AMG 521 strains demonstrated smaller RA, RL and RDe when associated with the dose of 50 kg of N ha^{-1}.

4. DISCUSSION

Even though the influence of the inoculation of PGPB and doses of N-fertilizer on the production and accumulation of forage mass (Figure 2) was not verified in this study, the literature shows positive results based on the isolated action of PGPB or N-fertilizer, or the association between them.

Results demonstrating an increase in biomass as the use of N-fertilizer in tropical grasses is reduced have been reported in studies conducted by Hungria et al. (2016) in *U. brizantha* cv. Marandu and *U. ruziziensis*, Aguirre et al. (2018) in *Cynodon dactylon* (L.) Pers. cv. Coastcross-1 and Leite et al. (2018) in *U. brizantha* cv. Marandu. However, these results are still inconclusive, since further research is necessary for provide a more solid and replicable database in a way that this technology can be transformed, in fact, into a commercial product to be used in tropical pastures.

The use of PGPB, for instance, demonstrates the capacity to contribute to the growth and development of forage, allowing increments of up to 60 % in forage mass, as reported by Megías et al. (2017) and Leite et al. (2018). Some studies have attributed the increments in terms of forage mass to hormones, such as cytokinins, gibberellins and auxins, synthesized by PGPB (Fukami et al., 2017 and Gouda et al., 2018).

Regarding research on the use of fertilizers in pasture, it is known that all nutrients are necessary and important for the growth and development of pasture. That way, a lack of any nutrient can, at some stages of forage maturity, hinder the expression of its productive potential.

Among the nutrients, nitrogen (N) is considered one of the most required by plants (Dobbelaere e Okon, 2007). Therefore, it is one of the most efficient nutrients when it comes to enabling grasses to have a greater accumulation of mass production and a better nutritive value (Palmer et al, 2014).

Although the use of N-fertilizer increases mass production, it also leads to more costs in pasture management (Guimarães et al., 2011). That is why the use of PGPB is so interesting for the modernization of animal production on pasture, thus, minimizing the external dependency on the use of N due to the compensation by the increment of N in the system through the biological fixation of it. Besides, PGPB improve the availability of other nutrients, such as phosphorus.

In this study, the use of greater doses of N-fertilizer (100 kg of N ha^{-1}) in ruzigrass contributed to a larger amount of tillers in the forage canopy (Table 1), as verified by Lima et al. (2016). We also found out that the inoculation of PGPB associated with doses of N-fertilizer contributed to the emergence of heavier tillers. The production of heavier tillers (Table 2), and in greater amount, allows increments in the production of forage mass and an increase in pasture support capacity, with a positive impact on animal production (Cecato et al., 2011).

The greater concentration of CP and the smaller concentration of NDF, associated with the greater dose of N-fertilizer (kg de N ha^{-1}) was possibly due to the increase in nitrogen compounds in the plant and, as a consequence, a decrease in fibrous compounds, as mentioned by Van Soest (1975), since they are inversely proportional. N composes part of the structure of nucleic acids and proteins and, for that reason, its supply is directly related to the raise in CP (Malavolta, 2006).

The smaller RDi verified with the inoculation of the AMG 521 strain (Table 4) is considered as the best result, due to the fact that thinner root hairs are more efficient, enabling greater exploration of the soil

and better absorption of water and nutrients, as demonstrated in studies by Do Vale et al., (2013), and Verbon and Liberman (2016). In their turn, roots of greater diameter are related to the plant's energetic supply (Ribeiro et al., 2011; Sanches et al., 2017). Greater root volume, besides improving absorptivity and exploring a greater area of the soil, also prevents soil compactation since the most superficial layers to the deepest ones (Cecato et al., 2006).

This change in morphology and more expressive root volume can affect the plant's efficiency in the use of nutrients available in the soil (Sureshbabu et al., 2016; Verbon and Liberman, 2016). This alteration is possibly related to bacterial action, which possibly potentializes the activity of hormones commonly synthesized by the plant, as described by Spaepen (2015) and Mamédio et al. (2020).

PGPB play two roles that have a direct effect in terms of enhancing efficiency in the use of N. One of them is the production of hormones, and the other one is the capacity of biologically fixing nitrogen (Rodolem et al., 2017).

The literature clearly proves that bacteria depend on the availability of a certain amount of N in the system, so that their metabolic activities occur. The absence of N in the soil may inhibit microbial activity, leaving the bacteria dormant (Marschner et al., 2006). Likewise, the excess of N has the same effects on these microorganisms (Zhu et al., 2016).

The use of N-fertilizer has had its efficiency proven in tissues renovation, thus, considerably increasing the production of forage mass. Nevertheless, its indiscriminate use can not only compromise the microbiota, but also be a serious pollution agent against the ecosystem. For that reason, the use of PGPB has been considered a sustainable alternative for reducing the amount of N-fertilizer required by forage, so that it can achieve its productive potential.

For their action, these bacteria have been described as plants growth promoters from the synthesis of substances that contribute to a higher interaction of roots with nutrients cycling (Moreira et. al., 2010).

The absence of effects verified in the association between PGPB and tropical grasses can be attributed to an inadequate combination of them, since not all bacteria are responsive to all grass species (Mamédio et al., 2020).

5. CONCLUSION

Although plant growth promoting bacteria were not efficient in the production and accumulation of forage mass, of both the aerial part and the root area of ruzigrass, they still represent a sustainable alternative, which is viable when it comes to livestock on pasture. It is due to the fact that their use potentializes the action of N-fertilizer in pasture, reducing the amount used and, thus, leading to a decrease in the production costs.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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