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# Production and morphological characteristics of marandu grass under inoculation and levels of nitrogen fertilization

# Produção e características morfológicas de capim marandu sob inoculação e níveis de adubação nitrogenada

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## Highlights

Nitrogen fertilization enhances the production of the Marandu grass forage canopy.
High nitrogen dosage attenuates the action of diazotrophic microorganisms.
Conventional and coated urea must be used considering economic and climatic factors.

## Abstract

Forage production and quality are influenced by the supply of nitrogen, especially urea, which is highly subject to losses through volatilization. Alternatively, technology that reduces nitrogen release has emerged, restricting losses by coating the fertilizer with polymers. Furthermore, there is a combination of multifunctional soil microorganisms capable of capturing atmospheric nitrogen and making it available to forage plants as a possibility to reduce external dependence on nitrogen. Thus, this study aimed to evaluate seed inoculation and the use of coated urea in the production of shoot and root dry mass, and the morphological characteristics of Marandu grass (*Urochloa brizantha* cv. Marandu). The experiment conducted in pots was set up in a completely randomized design with four replications in a 5 x 4 x 4 factorial scheme. Five nitrogen fertilization doses (50, 100, 150, 200, and 250 kg N ha$^{-1}$) were applied in a single dose, using the synthetic fertilizer urea. The factors consisted of four techniques: fertilization with urea, fertilization with coated urea, Marandu grass seeds treated with commercial inoculant and fertilization with urea, and Marandu grass seeds treated with commercial inoculant and fertilization with coated urea. The observations (forage canopy height, number of tillers per pot, and shoot forage mass)

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were obtained every 21 days, totaling four sequential evaluation cuts. Root dry mass was obtained by a single measurement in the fourth cut, arranged in a 5 x 4 factorial scheme, with four replications. The dose of 50 kg N ha$^{-1}$, using conventional urea, characterizes economic and environmental provision to Marandu grass canopy height under adequate soil moisture conditions. The nitrogen dose up to 200 kg ha$^{-1}$ promotes an increase in the number of tillers, while equal and higher doses ensure maintenance of forage mass production potential for up to 2 cuts. Conventional urea enabled superior root mass production of Marandu grass at nitrogen doses between 150 and 250 kg ha$^{-1}$.
**Key words:** Urea. Urease. Tillers. Dose.

## Resumo

A produção e qualidade forrageira é influenciada pelo suprimento de nitrogênio, especialmente ureia, altamente sujeito a perdas por volatilização. Alternativamente, surge tecnologia que reduz a liberação de nitrogênio, restringindo as perdas pelo recobrimento do fertilizante com polímeros. Ainda, como possibilidade de reduzir a dependência externa de nitrogênio, decorre a combinação de microrganismos edáficos multifuncionais capazes de captar nitrogênio atmosférico e disponibilizá-lo às plantas forrageiras. Assim, objetivou-se avaliar inoculação de sementes e o uso de ureia revestida na produção de massa seca aérea e radicular e características morfológicas de capim Marandu (*Urochloa brizantha* cv. Marandu). Experimento em vasos foi organizado em delineamento inteiramente casualizado, com 4 repetições, em esquema fatorial 5 x 4 x 4. Foram empregadas 5 dosagens de adubação nitrogenada (50, 100, 150, 200 e 250 kg ha$^{-1}$ de N), aplicado em dose única, utilizando o fertilizante sintético ureia. Os fatores constituíram 4 técnicas: adubação com ureia; adubação com ureia revestida; sementes de capim Marandu tratadas com inoculante comercial e adubação com ureia; e, sementes de capim Marandu tratadas com inoculante comercial e adubação com ureia revestida. As observações (altura do dossel forrageiro; número de perfilhos por vaso; e, massa aérea de forragem) foram obtidas com frequência de 21 dias, totalizando quatro cortes avaliativos sequenciais. Massa seca de raiz foi obtida por medida única, no corte 4, organizado em esquema fatorial 5 x 4, com quatro repetições. Sob umidade adequada no solo, dosagem de 50 kg ha$^{-1}$ de nitrogênio, utilizando ureia convencional, caracteriza provisão econômica e ambiental à altura do dossel de capim Marandu. Dosagem de nitrogênio até 200 kg ha$^{-1}$ promove aumento no número de perfilhos e; dosagem igual e superior, asseguram manutenção do potencial de produção de massa de forragem por até 2 cortes. Ureia convencional viabilizou superior produção de massa de raízes de capim Marandu, nas dosagens de nitrogênio entre 150 e 250 kg ha$^{-1}$.
**Palavras-chave:** Ureia. Urease. Perfilhos. Dosagem.

## Introduction

Urea, highly susceptible to loss due to nitrogen (N) volatilization, is the most used fertilizer, as it has a high concentration in the amide form, that is, 45% N. Controlled release urea or coated with a urease chemical reaction inhibitor, featuring higher agronomic efficiency compared to conventional pearly urea, has been available on the market as an option to mitigate losses due to volatilization (Almeida, 2016).

Brazilian agriculture is the main supplier of food to the world and has advanced in association with environmental sustainability

(Instituto de Pesquisa Econômica Aplicada [IPEA], 2022). In the 20th century, Brazilian research found an alternative to reduce dependence on fertilizers. Seed treatment with microorganisms beneficial to cultivation, called inoculation, is a common practice in soybean production and does not require the use of nitrogen fertilizer in the cultivation. Some soil microorganisms, such as atmospheric nitrogen-fixing bacteria (diazotrophic bacteria), plant growth-promoting bacteria, and mycorrhizal fungi, have strategic use to ensure productivity at low cost and less dependence on imported inputs, such as fertilizers.

Seed and leaf inoculation in forage crops has been investigated to increase pasture mass production. The combination of microorganisms with multifunctional properties, *Azospirillum brasilense* and *Pseudomonas fluorescens,* allows to increase the leaf nitrogen content and productivity in brachiaria pasture by more than 20% (Hungria et al., 2016; Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2023). The technology promotes the synthesis of phytohormones, root growth, biological nitrogen fixation, resistance to environmental adversities, efficiency in water and nutrient absorption, reduction in production costs, and less need for nitrogen fertilization (Hungria, 2011). In this sense, this study aimed to evaluate seed inoculation and the use of coated urea in the shoot and root dry mass production and morphological characteristics of Marandu grass (*Urochloa brizantha* cv. Marandu).

## Material and Methods

The experiment was conducted from September 2022 to January 2023 in a protected environment (greenhouse) at the Federal Institute of Education, Science, and Technology of Rondônia (IFRO), Campus of Ariquemes. Daily temperature and relative humidity data in the greenhouse were recorded during the evaluation period (Figure 1).

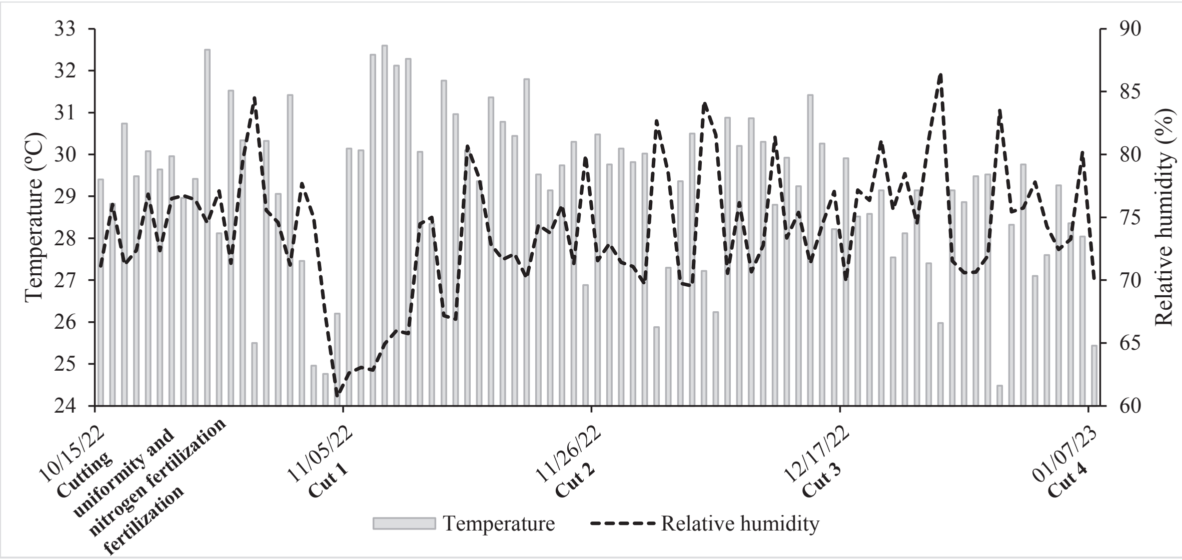


**Figure 1.** Temperature and relative humidity during the experimental period (84 days).

The experiment was conducted in a completely randomized design in a 5x4x4 factorial scheme, with four replications, totaling 320 observations. Five doses of nitrogen fertilization (50, 100, 150, 200, and 250 kg N $ha^{-1}$) were applied in a single dose using the synthetic fertilizer urea. The factors consisted of four techniques: fertilization with urea (U), fertilization with coated urea (C), Marandu grass seeds treated with commercial inoculant and fertilization with urea (I + U), and seed inoculation associated with fertilization with coated urea (I + C). Observations were obtained every 21 days, totaling four sequential evaluative collections.

The experiment was set up on September 6, 2022. A commercial biological product consisting of four bacteria (*Azospirillum brasiliense, Rhizobium tropici, Bacillus subtilis* and *Pseudomonas fluorescens*) with minimum guaranteed levels at a concentration of $10^8$ colony-forming units per milliliter (mL) was used for inoculation. As recommended by the manufacturer, 10 mL of the inoculant was homogenized in 500 g of Marandu grass seeds obtained from a commercial lot of the 2020/2021 growing season, with a pure live seed of 80%. Subsequently, the seeds dried for 30 minutes in a place protected from heat and solar radiation, followed by sowing in the experimental plots (pots).

The substrate for sowing and performance of the experiment consisted of the homogenization of 60, 25, and 15% of ravine soil (Oxisol), washed sand, and composting, respectively. The chemical characterization of the substrate presented the following results: pH ($H_2O$) = 5.7; $Al^{3+}$, $Ca^{2+}$, $Mg^{2+}$, $H+Al^{3+}$ = 0.00, 2.00, 1.30, and 2.32 $cmol_c$ $dm^{-3}$, respectively; P, K (Mehlich), Fe, and Zn = 30.8, 61, 57.00, and 5.8 mg $dm^{-3}$, respectively; organic matter = 17 mg $dm^{-3}$; V = 60%; and CEC = 5.78 cmolc dm-3. The substrate texture consisted of a proportion of 377, 123, and 500 g $kg^{-1}$ of clay, silt, and sand, respectively. Pots with a 7 $dm^3$ capacity (surface area of 0.0314 $m^2$) were filled with the substrate, considering a mean density of 1.1 kg $m^{-3}$ and daily moisture maintenance by gravimetry of around 60% of the field capacity.

Thinning was carried out 13 days after sowing (DAS), leaving five grass plants per pot. The plants were cut at 39 DAS (October 15, 2022) aiming their standardization at 0.15 m high from the substrate in the experimental plots (Gobbi et al., 2018). At this time, nitrogen fertilizer was applied, including the techniques in which fertilizer was coated (C and I + C). For the C technique, a commercial product was dosed with polymeric materials to produce controlled-release fertilizer at the rate of 3 g per 1,000 g of urea, homogenized until the product completely adhered to the fertilizer. On that occasion, the crop was inoculated via leaf in the I + U and I + C techniques.

Forage canopy height (HGT, in cm) and the number of tillers per pot (NTP, No. tillers $pot^{-1}$) were recorded at 60 (cut 1), 81 (cut 2), 102 (cut 3), and 123 DAS (cut 4), with a 21-day defoliation frequency. Shoot forage mass (DMP) was determined with a defoliation intensity of 0.15 m using leaves and pseudostem, which were dried in a forced-air ventilation oven at 55 °C until constant mass and expressed in g $pot^{-1}$.

The root dry mass portion (RDM) was obtained by a single measurement in cut 4. At that time, the roots were removed from the pots and washed with running water. Subsequently, the root volume of the experimental plots was dried in a forced-air circulation oven at 55 °C until constant mass and expressed as root dry mass per pot (g $pot^{-1}$). RDM followed a completely randomized design analysis in a 5 x 4 factorial scheme, with four replications, totaling 80 observations.

The data were subjected to analysis of variance using the F-test and, when a significant effect was found for the interactions, the means of the quantitative factors were subjected to regression analysis to test linear, quadratic, and cubic mathematical models. The best model was chosen considering the coefficient of determination ($R^2$). The means for the qualitative factors were compared using the Tukey test at a 5% significance level, using the SISVAR software (Ferreira, 2019).

## Results and Discussion

The analysis of variance identified a significant effect for forage canopy height (HGT), number of tillers (NTP), and forage production (DMP) of Marandu grass (Table 1). HGT showed an interaction between nitrogen doses (D) and cuts (C), while NTP showed an interaction between Techniques (T) and D. On the other hand, DMP presented an interaction between T, D, and C, showing interdependence between factors and levels.

**Table 1**
**Summary of analysis of variance for forage canopy height (HGT), tiller number (NTP) and forage production (DMP) of Marandu grass subjected to inoculation of seeds and fertilizers (T), under doses of nitrogen (D), in four evaluative cuts (C)**

| Source of variation | ----------------------- Variables ----------------------- | | | |
|---|---|---|---|---|
| | DF(1) | HGT (cm) | NTP (No. tillers $pot^{-1}$) | DMP (g $pot^{-1}$) |
| Techniques (T) | 3 | 0,309 ns | 3,422 * | 3,602 * |
| Nitrogen of doses (D) | 4 | 3,446 ** | 22,660 ** | 17,512 ** |
| Cuts (C) | 3 | 288,768 ** | 44,327 ** | 743,810 ** |
| T x D | 12 | 1,188 ns | 2,869 ** | 2,197 * |
| T x C | 9 | 1,630 ns | 0,279 ns | 4,873 ** |
| D x C | 12 | 5,885 ** | 0,607 ns | 10,885 ** |
| T x D x C | 36 | 0,865 ns | 0,211 ns | 4,646 ** |
| Residual | 240 | -- | -- | -- |
| Coefficient of variation (%) | | 9,36 | 16,93 | 17,30 |
| Mean | | 46,82 | 27,35 | 5,17 |

(1) Degree of freedom. **, * and ns, significant a 1%, 5% and not significant, respectively, by the Test F

Except for the 150 kg N $ha^{-1}$ dose, the initial cut differed significantly from the following cuts, mainly cut 4 (Table 2), with a mean decrease in HGT of 34.06% over cut 1. The application of nitrogen fertilizer in a single dose (in the standardization cut) compromised the HGT of the forage canopy in the experimental period. This situation is due to a momentary critical concentration of nitrogen (N) essential for cultivated plants (Irving, 2015). The high activity of the photosynthetic mechanism of grasses (C4 metabolism) leads to N remobilization to the new leaves when there is no instant N availability in the soil for plant growth. Therefore, nitrogen fertilization splitting must be adjusted to maintain height, thus favoring a higher proportion of leaves on the plant during the forage production cycle.

**Table 2**

**Means and regression equations for forage canopy height (HGT) of Marandu grass, under doses of nitrogen (N), in four evaluative cuts (C)**

| Cut | Doses of nitrogen (kg N $ha^{-1}$) | | | | | Adjusted regression equation | $R^2$ |
|---|---|---|---|---|---|---|---|
| | 50 | 100 | 150 | 200 | 250 | | |
| | (HGT, cm) | | | | | | |
| 1 | 60,56 a[1] | 57,03 a | 54,56 a | 53,81 a | 56,31 a | $\hat{y} = 3{,}94\times10^{-4}x^2 - 1{,}41563\times10^{-1}x + 66{,}8625$ | 0,9824 |
| 2 | 51,91 b | 49,25 b | 53,25 a | 47,88 b | 48,19 b | ns[2] | |
| 3 | 39,75 c | 41,82 c | 44,06 b | 44,44 b | 47,81 b | $\hat{y} = 3{,}75\times10^{-2}x + 37{,}94875$ | 0,9587 |
| 4 | 36,66 c | 34,39 d | 39,61 c | 37,25 c | 37,74 c | ns | |

(1) Means followed by different letters in the column differ significantly from each other ($p<0.05$) using the Tukey test.
(2) not significant.

The data obtained for cuts 1 and 3 characterized quadratic and linear models in the regression analysis, respectively (Table 2). The model for cut 1 indicated maximum HGT under the dose of 50 kg N $ha^{-1}$, corresponding to 60.77 cm. Cut 3 showed an increase directly proportional to the tested doses, in which the increase of 50 kg N $ha^{-1}$ corresponded to 1.88 cm in height of the forage canopy. Similarly, Silva et al. (2013) found a daily increase equivalent to 6.24 cm. However, the authors split the urea fertilization into three applications within an interval of 30 days. Thus, the efficient use of fertilizer depends on adequate pasture management, regardless of the applied N dose (Gimenes et al., 2011). The progression of N doses did not ensure increases in the forage canopy height, except for cut 3 (Table 2), predisposing the fertilizer to losses, such as leaching, due to daily irrigations. Therefore, consisting of the nutrient that most influences pasture productivity and quality, the dose of 50 kg N $ha^{-1}$ is sufficient to express forage potential in height, reducing environmental and economic losses caused by the production system.

Exclusively the dose of 100 kg N $ha^{-1}$ characterized a significant difference in NTP between treatments (Table 3). The inoculation treatment (I + U) and dose of 100 kg N $ha^{-1}$ expressed maximum NTP, differing significantly from the coated urea treatment (C) and dose of 100 kg N $ha^{-1}$. Urea (U) and the treatment inoculation and coated urea (I + C) under 100 kg N $ha^{-1}$ had a similar effect, with a mean NTP equivalent to 25.34 tillers per pot. The other tested doses showed no significant differences (Table 3). Therefore, U did not differ from C, I + U, or I + C regarding NTP. According to Sampaio et al. (2021), nitrogen fertilization is essential to promote forage development, specifically tillering, regardless of inoculation. Nitrogen affects tiller density, as it is effective in activating the base buds, and stimulating tillering, as observed in this study (Alexandrino et al., 2010). Split fertilization is essential for maintaining the grass tillering potential (Cabral et al., 2012). Furthermore, according to the authors, tillering in grasses is influenced by combinations of nutritional, climate, and management factors, such as cutting intensity and frequency.

**Table 3**

**Means and regression equations of tiller number (NTP) of Marandu grass subjected to inoculation of seeds and fertilizers (T), under doses of nitrogen (D)**

| Techniques | Doses of nitrogen (kg N $ha^{-1}$) | | | | | Adjusted regression equation | $R^2$ |
|---|---|---|---|---|---|---|---|
| | 50 | 100 | 150 | 200 | 250 | | |
| | (NTP) | | | | | | |
| Urea (U) | 25,75 | 25,88 ab(1) | 28,25 | 30,81 | 28,63 | $\hat{y}$ = - 5×$10^{-6}x^3$ + 1,973×$10^{-3}x^2$ - 0,21592x + 32,2375 | 0,9934 |
| Coated urea (C) | 25,19 | 23,75 b | 30,25 | 33,94 | 27,81 | $\hat{y}$ = - 1,2×$10^{-5}x^3$ + 4,977×$10^{-3}x^2$ - 0,5628x + 42,3125 | 0,9970 |
| Inoculation (I + U) | 21,88 | 29,81 a | 26,82 | 29,34 | 28,88 | ns(2) | |
| I + R | 21,19 | 24,81 ab | 26,56 | 29,44 | 28,06 | $\hat{y}$ = - 2,54×$10^{-4}x^2$ + 0,1128x + 16,0625 | 0,9618 |

(1) Means followed by different letters in the column differ significantly from each other (p<0.05) using the Tukey test.
(2) not significant.

Still for NTP, the regression analysis for the factors U and C adapted to the cubic model (Table 3). In this case, the maximum NTP values, that is, 28.16 and 32.84 tillers per pot, are obtained with the doses 185.44 and 197.23 kg N $ha^{-1}$, respectively. U presented 25.88 tillers per pot under 100 kg N $ha^{-1}$, similar to what was found by Sampaio et al. (2021) with the same dose. In contrast, the quadratic model fitted the data for the I + C factor ($R^2$ = 0.9618), and the prediction equation indicated a maximum NTP (28.59 tillers per pot) with a dose of 222.09 kg N $ha^{-1}$ (Table 3). Therefore, nitrogen fertilization evidenced the number of tillers and, consequently, an increase in DMP (Table 4). However, fertilization above that recommended by the mathematical model showed a decrease in NTP, in addition to the economic and environmental losses caused by the excess fertilizer available in the production system.

The evaluated N doses showed a mean reduction in DMP equivalent to 31.72% when comparing cut 4 with cut 1 (Table 4). It indicates the need to split fertilization for constant production between cuts (Martins et al., 2014; Viçosi et al., 2020; Teixeira et al., 2022), as the plants are not efficient in retaining the available nutrient and may lose it via volatilization or leaching (Neves et al., 2019). In fact, frequent harvests deplete carbohydrates from the stem's basal reserve (Silva et al., 2012), reducing the potential for forage production in cropping cycles (Table 4). Bono et al. (2019) assessed nitrogen sources in the dry mass production of Marandu grass in three evaluation cuts and found no reduction in DMP between evaluations in which the splitting of fertilization was adopted. However, the authors observed that N doses applied to the soil (30, 60, 120, and 240 kg $ha^{-1}$) influenced DMP. In the present study, the dose of 250 kg N $ha^{-1}$ (Table 4) showed a significant difference from cut 3. Therefore, the residual effect of the fertilizer for higher doses lasted for up to two cuts.

A significant effect was observed for treatments at a dose of 100 kg N $ha^{-1}$ only for cut 1, while I + U, with maximum DMP (Table 4), differed significantly from the other treatments. Treatment C with 150 kg N $ha^{-1}$ characterized a reduced DMP in cut 1, while cut 2 showed maximum DMP, differing significantly from the other cuts (Table 4). Furthermore, cuts 3 and 4 showed no effect between treatments. The absence of significant results for C over U occurred because the experiment was conducted under favorable and constant soil moisture conditions. Martins et al. (2014) and Viçosi et al. (2020) worked under different irrigation conditions and, therefore, with different soil moisture conditions, and also found no differences between conventional and coated fertilizers.

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**Table 4**
**Means of forage production (DMP) of Marandu grass subjected to inoculation of seeds and fertilizers (T), under doses of nitrogen (D), in four evaluative cuts (C)**

| Techniques | Cut | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | (NTP) | | | |
| Urea (U) | 8,53 A[(1)] | 4,15 B | 2,80 B | 2,65 B |
| Coated urea (C) | 8,78 A | 5,55 B | 2,78 C | 2,90 C |
| Inoculation (I + U) | 7,55 A | 4,93 B | 2,43 C | 2,43 C |
| I + R | 7,70 A | 4,30 B | 2,40 B | 2,35 B |
| | 100 kg N $ha^{-1}$ | | | |
| Urea (U) | 9,40 Aab | 6,22 B | 2,60 C | 2,40 C |
| Coated urea (C) | 8,65 Abc | 5,08 B | 2,08 C | 2,65 C |
| Inoculation (I + U) | 11,18 Aa | 6,45 B | 2,70 C | 2,70 C |
| I + R | 6,58 Ac | 5,48 A | 2,63 B | 2,98 B |
| | 150 kg N $ha^{-1}$ | | | |
| Urea (U) | 9,45 Aa | 5,73 Bb | 2,85 C | 2,38 C |
| Coated urea (C) | 4,75 Bb | 10,75 Aa | 4,18 BC | 2,53 C |
| Inoculation (I + U) | 8,70 Aa | 6,25 Bb | 3,35 C | 2,68 C |
| I + R | 8,13 Aa | 6,38 Ab | 3,13 C | 2,23 C |
| | 200 kg N $ha^{-1}$ | | | |
| Urea (U) | 9,08 A | 7,20 A | 3,95 B | 2,43 B |
| Coated urea (C) | 9,08 A | 6,58 B | 3,93 C | 3,20 C |
| Inoculation (I + U) | 9,10 A | 7,43 A | 2,95 B | 2,45 B |
| I + R | 8,63 A | 6,70 A | 4,40 B | 2,70 B |
| | 250 kg N $ha^{-1}$ | | | |
| Urea (U) | 8,20 A | 7,45 A | 3,80 B | 2,33 B |
| Coated urea (C) | 8,20 A | 8,43 A | 4,73 B | 2,85 B |
| Inoculation (I + U) | 7,48 A | 8,15 A | 4,00 B | 2,20 B |
| I + R | 7,63 A | 7,65 A | 3,80 B | 2,48 B |

(1) Means followed by different letters, uppercase in the line and lowercase in the column, differ statistically from each other ($p<0.05$) using the Tukey test.

These data do not demonstrate what has been found in the literature about the productive benefits of inoculation in forage grasses. Plants show variability in their response to the inoculation of microorganisms, which can be controlled by genetic factors, physiological variations in endophytes, and infection mechanisms, with specificity even at the level of varieties (Montañez, 2005; Hanisch et al., 2017). Inoculation and fertilization with N applied in a single dose do not influence DMP up to a dose of 150 kg N $ha^{-1}$ in tropical soils with a high organic matter content (Hanisch et al., 2017). Sampaio et al. (2021) analyzed nitrogen fertilization associated with inoculation and co-inoculation in Marandu grass and observed that the potential for N fixation by diazotrophic bacteria for doses higher than 100 kg N $ha^{-1}$ can be reduced or even inhibited in the presence of high concentrations of the fertilizer in the soil, as it reduces the activity of the nitrogenase enzyme, responsible for transforming atmospheric N into ammonia.

The DMP obtained in the U factor (Table 5) characterized a quadratic model in cut 1 and linear models directly proportional to the evolution of N doses in cuts 2 and 3. The tested models did not characterize the data from cut 4. Only cut 3 showed a linear model directly proportional to the progression of N doses for C (Table 5). In contrast, only cuts 2 and 3 showed a positive linear model for DMP in I + U. For the I + R factor, the cubic model represented cut 1 and the linear model was fitted for cuts 2 and 3.

**Table 5**
**Regression equation of forage production (DMP) of Marandu grass subjected to inoculation of seeds and fertilizers (T), in four evaluative cuts (C)**

| Techniques | ---------- Cut ---------- | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Urea (U) | $\hat{y} = 1,12\times10^{-4}x^2 - 3,1693\times10^{-2}x + 7,26$<br>$R^2 = 0,9886$ | $\hat{y} = 1,515\times10^{-2}x + 3,8775$<br>$R^2 = 0,8222$ | $\hat{y} = 6,7\times10^{-3}x + 2,195$<br>$R^2 = 0,7171$ | ns(1) |
| Coated urea (C) | ns | ns | $\hat{y} = 1,35\times10^{-2}x + 1,41$<br>$R^2 = 0,8068$ | ns |
| Inoculation (I + U) | ns | $\hat{y} = 1,485\times10^{-2}x + 4,4125$<br>$R^2 = 0,9149$ | $\hat{y} = 6,8\times10^{-3}x + 2,065$<br>$R^2 = 0,7658$ | ns |
| I + R | $\hat{y} = - 3,0\times10^{-6}x^3 + 1,23\times10^{-3}x^2 - 0,15356 + 12,605$<br>$R^2 = 0,9335$ | $\hat{y} = 1,585\times10^{-2}x + 3,7225$<br>$R^2 = 0,9709$ | $\hat{y} = 9,15\times10^{-3}x + 1,8975$<br>$R^2 = 0,7606$ | ns |

(1) not significant.

The analysis of variance identified an interaction between T and D for root dry mass (RDM) (Table 6). Exclusively the doses 50 and 100 kg N ha$^{-1}$ did not characterize an effect between treatments (Table 7). However, U with 150 kg N ha$^{-1}$ and U with 250 kg N ha$^{-1}$ showed significant RDM over the other treatments. The treatment I + U with 200 kg N ha$^{-1}$ had a similar effect to U with 200 kg N ha$^{-1}$, differing from those with coated urea (C and I + C). Hungria et al. (2021) evaluated the root system of BRS Piatã grasses (U. brizantha) grown in pots, formed by seed inoculation with *A. brasilense,* and recorded inoculation severity for dry mass and root length. As established by Hanisch et al. (2017), the adequate availability of other nutrients in the soil, in addition to N, may have discouraged bacteria from protecting the grass root system and, consequently, reduced the effect of inoculation on plant performance in this experiment.

**Table 6**
**Summary of analysis of variance for root dry mass (RDM) of Marandu grass subjected to inoculation of seeds and fertilizers (T)**

| Source of variation | DF(1) | RDM --- (g pot$^{-1}$) --- |
|---|---|---|
| Techniques (T) | 3 | 44,700 ** |
| Nitrogen of doses (D) | 4 | 18,451 ** |
| T x D | 12 | 14,855 ** |
| Residual | 60 | -- |
| Coefficient of variation (%) | -- | 13,99 |
| Mean | -- | 16,31 |

(1) Degree of freedom. ** significant a 1%, by the Test F.

**Table 7**
**Means of root dry mass (MSR) of Marandu grass subjected to inoculation of seeds and fertilizers (T), under doses of nitrogen (D)**

| Techniques | Doses of nitrogen (kg N ha$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 50 | 100 | 150 | 200 | 250 |
| | ---------------------------- (MSR, g pot$^{-1}$) ---------------------------- | | | | |
| Urea (U) | 14,45 | 16,23 | 31,60 a(1) | 18,78 ab | 23,03 a |
| Coated urea (C) | 13,85 | 13,50 | 21,05 b | 16,68 b | 12,40 b |
| Inoculation (I + U) | 13,85 | 16,00 | 13,98 bc | 24,35 a | 13,75 b |
| I + R | 14,95 | 11,78 | 11,63 c | 13,45 b | 11,05 b |

(1) Means followed by different letters in the column differ significantly from each other (p<0.05) using the Tukey test.

## Conclusion

The dose of 50 kg ha$^{-1}$ of nitrogen with conventional urea characterized economic and environmental provision at the canopy height of the Marandu grass under adequate soil moisture conditions.

Nitrogen doses up to 200 kg ha$^{-1}$ promoted an increase in the number of tillers, while equal and higher doses ensure maintenance of forage mass production potential for up to two cuts.

Conventional urea enabled superior root mass production of Marandu grass at nitrogen doses between 150 and 250 kg ha$^{-1}$.

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