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Effects of sowing depth and inoculation with *Pseudomonas fluorescens* on the initial growth of *Urochloa brizantha* (syn *Brachiaria brizantha*) cv. Marandú

Efeitos da profundidade de semeadura e inoculação com Pseudomonas fluorescens no crescimento inicial de Urochloa brizantha (syn Brachiaria brizantha) cv. Marandú

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ABSTRACT

The aim of the present study was to evaluate the effects of sowing depth and inoculation with *Pseudomonas fluorescens* on germination, emergence, shoot and root growth of *Urochloa brizantha*. A completely randomized design was used, in a 6 × 2 factorial arrangement, with 12 repetitions. The following sowing depths (SD) were evaluated: 0, 1, 2, 3, 6 and 12 cm; and the seed inoculation with *P. fluorescens* (I): with and without. Evaluations were carried out 25 days after plant emergence. No effects of the PS × I or I interaction were observed for all variables evaluated (P> 0.05). The germination and emergence percentages decreased linearly (P <0.05) as the SD increased. No plant emergence was observed at and at 12 cm depth. The morphometric characteristics of the plants (height, number of leaves, length of root and leaf blade width) decreased linearly with the increase of SD (P <0.05). Dry matter production of the aerial part and root were

not affected by SD (P> 0.05). However, an effect was observed on the shoot:root ratio, where plants sown more superficially had a greater relationship. The inoculation with *Pseudomonas fluorescens* in the seed, has no effect on the initial growth of Marandu grass. On the other hand, greater sowing depths affects negatively the initial growth.
Keywords: germination, pasture, plant growth, rhizobacteria, sustainability

RESUMO

Objetivou-se avaliar os efeitos de profundidades de semeadura e da inoculação com *Pseudomonas fluorescens* sobre a germinação, emergência, crescimento aéreo e radicular da *Urochloa brizantha*. O delineamento foi inteiramente casualizado, em arranjo fatorial 6 × 2, com 12 repetições. Foram avaliadas a profundidade de semeadura (PS): 0, 1, 2, 3, 6 e 12 cm; e a inoculação da semente com *P. fluorescens* (I): sem e com. As avaliações foram realizadas 25 dias após a emergência das plântulas. Não foram observados efeitos da interação PS × I nem de I para todas as variáveis avaliadas(P> 0.05). Os percentuais de germinação e emergência diminuíram linearmente (P< 0.05) em função do aumento da PS, sendo que na profundidade de 12 cm, não foi observada emergência de plantas. As características morfométricas das plantas (altura, número de folhas, comprimento de raiz e largura de lâmina foliar) diminuíram linearmente em função do aumento da PS (P< 0.05). As produções de massa seca de parte aérea e de raiz não foram afetadas pela PS (P> 0.05), porém, foi observada diminuição da relação parte aérea:raiz com o aumento da OS (P< 0.05). A inoculação com *Pseudomonas fluorescens* na semente não apresenta efeitos sobe o crescimento inicial do capim Marandú. Por outro lado, profundidades de semeadura maiores afetam negativamente o crescimento inicial.
Palavras-chave: crescimento vegetal, germinação, pastagem, rizobactéria, sustentabilidade

INTRODUCTION

The pasture establishment is considered one of the most important step, but it is commonly neglected in ruminant production systems, due to many factors. Soil preparation, liming, fertilization, seeding or initial grazing can affect the initial growth of the grass. In this context, integrated systems, as crop-livestock integration or crop-livestock-forest are suitable technologies to increase productivity and to recovery of degraded pastures (Silva et al., 2018).

During the grass sowing some problems may occur, especially with the seed depth. When the sowing depth is too high, the seed germinates but may not emerge to the surface due to the lack of energy reserves. On the other side, to the seeds can be eaten by birds, insects or carried by the rain when it is not incorporated to the soil (Santos et al., 2015). Therefore, the knowledge about the optimal depth can increase the number of plants that will emerge and affect the initial growth of the grass.

Studies already carried out evaluating the effects of sowing depth have pointed that the seed needs ideal conditions to germinate, such as moisture, temperature and light, which are usually found at depths between 1 and 4 cm (Rezende et al, 2012; Castaldo et al. 2016; Schmoeller et al., 2019). According to Pacheco et al. (2010) and Rezende et al.

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(2012), depths greater than 5 cm, reduce emergence time, and shoot and root biomass.

In order to increase the efficiency during the establishment and growth of grasses, strategies such as the inoculation of *Urochloa* seeds with plant growth-promoting rhizobacteria (GPR) have been studied (Figueiredo et al., 2010; Brennecke et al., 2016). Heinrichs et al. (2020) observed an increase in shoot and root mass of Marandú grass when studying the seed with bacteria.

The GPR *Pseudomonas fluorescens* acts as a growth promoter, increasing the chlorophyll content, the photosynthesis rate, the absorption of nutrients and the production of biomass (Rêgo et al., 2014; Nascente et al., 2016). We believe that the inoculation can accelerate the growth of *Urochloa brizantha*, even when submitted to high sowing depths that are often used when the grass is intercropped with corn or soybean in integrated crop-livestock systems.

Thus, the objective was to evaluate the effect of sowing depth and *Pseudomonas fluorescens* bacteria on the initial growth of *Urochloa brizantha* cv. Marandú.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse at the University Federal Rural of Amazônia, Belém, Pará, Brazil. (Latitude 1º27'25" S; Longitude 48º26'36" W). The region's climate is of the Af type, characterized as humid tropical without a dry season. During the experimental period, from December 6th to December 30th, 2018, the minimum and maximum temperatures were averaged 32.2 and 22.7 ºC, respectively.

The tubes with a capacity of 0.276 dm^3 received soil samples corrected 70 days before planting with dolomitic limestone (PRNT 91%) and fertilized with 140 mg dm^{-3} of P_2O_5 (simple superphosphate) according to fertility analysis (pH in water=4.76; P=2.19 mg dm^{-3}; K= 0.14 $cmol_c\ dm^{-3}$; Ca^{2+}= 2.0 $cmol_c\ dm^{-3}$; Mg^{2+}= 1.0 $cmol_c\ dm^{-3}$; H + Al= 7 $cmol_c\ dm^{-3}$.

The germination test of 200 seeds was carried out in gerbox-type boxes with cotton, which were incubated for 7 days at 30 °C in a BOD oven, obtaining 41.5% of germination. Three days before sowing, *Pseudomonas fluorescens* (R55) were inoculated into seeds, based on the protocol by Kado & Heskett (1970).

The experiment was carried out in a completely randomized design, in a 6 × 2 factorial scheme with twelve replications. The factors studied were sowing depth (SD): 0, 1, 2, 3, 6 and 12 cm and inoculation with *P. fluorescens* (I): without and with inoculation in the seed.

The sowing of the seeds corresponding to the 0 cm treatment was done just placing them above the ground, the other seeds were sown with the help of sticks that marked the depths 1, 2, 3, 6 and 12 cm.

After sowing, using only the largest plants in each tube, daily observations were carried out to measure the number of emerged seedlings and the day of emergence, to obtain the percentage of germination (G%), percentage of emergence (E%) as a function of the number of seeds and the rate of emergence and accumulated emergence (AE), adding the number of seedlings emerged on the day with the day before. On the 21st day after sowing, the smallest plants were removed, leaving only 1 plant per tube.

The emergence velocity index (EVI) was calculated according to the equation proposed by Maguirre (1962):

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$$EVI = \frac{E1}{D1} + \frac{E2}{D2} + \cdots \frac{En}{Dn}$$

Where E1, E2, ... En is the number of normal seedlings computed in the first count, in the second count, until the last count; D1, D2, ...Dn is the number of days from sowing to first, second, until last count.

On day 25 after sowing, measurements of the morphometric characteristics of the plants were performed: plant height (PH) – cm; leaf blade length (LBL) – cm; leaf blade width (LBW) – cm; number of leaves (NL), number of fully expanded leaves (NFEL), root length (RL) - cm and leaf area (LA) - cm^2.

After the measurements, destructive analyzes of the plants were carried out to evaluate the morphological components of the shoot and root. The leaf blades were separated to determine the leaf area, with the aid of the LI-COR LI-3100C Area Meter equipment. The separation of the aerial part from the root was done close to the ground, the roots were removed from the tubes, removing the soil with water, then the roots were measured and placed in a ventilated area to remove excess moisture. The aerial part and root samples were weighed, placed in paper bags and kept in an oven with forced air circulation (55 °C until reaching constant weight) to determine the aerial part dry mass (APDM) and root dry mass (RDM).

The shoot and root dry mass were added to find the total dry weight (TDW) - g, the percentage of shoot and root dry mass was found by the equation:

$$APDM, RDM(\%) = \frac{APDM(g) \times 100}{TDW(g)}$$

The Shoot:Root ratio (S:R) was found based on the APDM divided by the RDM.

Data were submitted to the normality test through Shapiro-Wilk analysis and submitted to analysis of variance. The sowing depths were submitted to regression analysis, selecting the equations by the coefficient of determination (R^2) and by the significance of 0.05. For inoculation, the data were submitted to the t test, adopting a significance level of 0.05. Statistical analyzes were performed using the statistical program SISVAR®.

RESULTS AND DISCUSSION

No effects of PS × I or I interaction were observed for all variables evaluated ($P > 0.05$). However, significance ($P < 0.05$) was observed for sowing depth in the variables germination, emergence, emergence speed index, plant height, leaf blade width, root length, number of leaves and total number of leaves expanded (table 1).

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Table 1: Germinative characteristics and morphometric characteristics of the cultivar *Urochloa brizantha* cv. Marandú as a function of sowing depth without and with inoculation of *Pseudomona fluorescens*.

Variables	***P. fluorescens***		**Sowing depth (cm)**						**EPM**	**P-valor**		
	without	with	0	1	2	3	6	12		I	SD	I*SD
G (%)	27.08	28.47	45.83	43.75	27.08	27.08	18.75	4.16	3.56	0.78	0.00	0.48
E (%)	26.38	27.78	45.83	43.75	27.08	27.08	18.75	0.00	3.49	0.78	0.00	0.46
EVI	1.15	1.30	1.76	1.82	0.96	1.01	0.57	***	0.16	0.51	0.00	0.45
ET (days)	2.82	2.83	3.75	2.72	2.33	2.79	2.50	***	0.40	0.98	0.56	0.20
PH (cm)	12.16	14.57	16.08	19.99	11.25	13.35	7.15	***	1.75	0.33	0.04	0.62
LBL (cm)	4.88	5.96	5.94	7.81	4.58	5.64	3.13	***	0.71	0.29	0.06	0.62
LBW (cm)	0.27	0.32	0.40	0.41	0.22	0.28	0.16	***	0.04	0.33	0.01	0.59
RL (cm)	9.86	11.48	15.41	16.14	8.07	9.00	4.71	***	1.30	0.38	0.00	0.82
LA (cm²)	7.01	8.20	8.65	11.11	6.38	8.06	3.82	***	1.20	0.48	0.09	0.51
NL	2.23	2.60	3.33	3.29	1.83	2.20	1.42	***	0.29	0.37	0.01	0.59
NFEL	1.73	2.03	2.62	2.58	1.42	1.70	1.08	***	0.23	0.36	0.01	0.61

Note: I= Inoculation with *Pseudomonas fluorescens*; SD = Sowing depth; G = Germination; E = Emergency; EVI = emergence velocity index; ET = Emergency time; PH = plant height; LBL = leaf blade length; LBW = leaf blade width; RL = Root length; LA = leaf area; NL = number of leaves; NFEL = number of fully expanded leaves.
***= No data recorded.

The absence of the effect of *Pseudomonas fluorescens* on the factors can be explained by the plant's allelopathic processes, which activate its autoimmune system to release substances that inhibit microbial growth (Noguchi et al. 2014; Lopes et al., 2018). The germinated plants managed to emerge on the surface, with the exception of the 12 cm sowing depth where only germination occurred, which can be explained by the high tolerance of *Urochloa* to increased sowing depth (Derré et al., 2017). The germination, emergence and emergence speed index were higher at more superficial depths (Figure 1 and 2) due to the fact that during germination the seedling can break the physical barriers of the soil more easily and quickly, in addition to having a greater input of moisture, light and temperature. (Rezende et al., 2012; Zuffo et al. 2014). Ikeda et al. (2013) point out that seed vigor is related to its emergence speed, the higher the vigor, the higher the index. Our data corroborate the results found by Schmoeller et al. (2019), which observed high and low plant emergence at 0 cm and 10 cm, respectively, spowing when evaluating different cultivars of *Urochloa* and *Megathyrsus* submitted to different depths.

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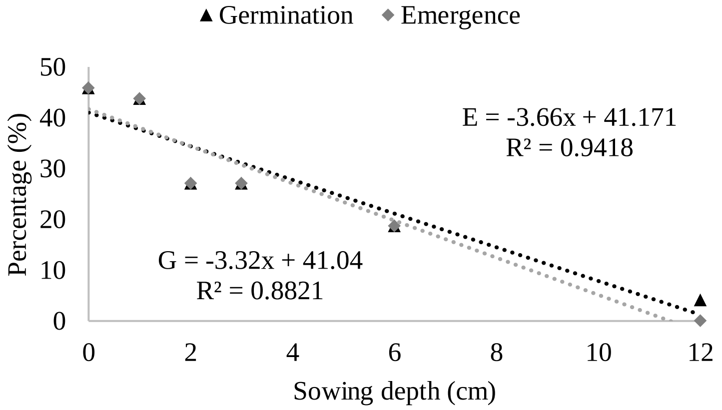

Figure 1: Germination (G, %) and emergence (E, %) of *Urochloa brizantha* cv. Marandú 25 days after sowing as a function of sowing depth.

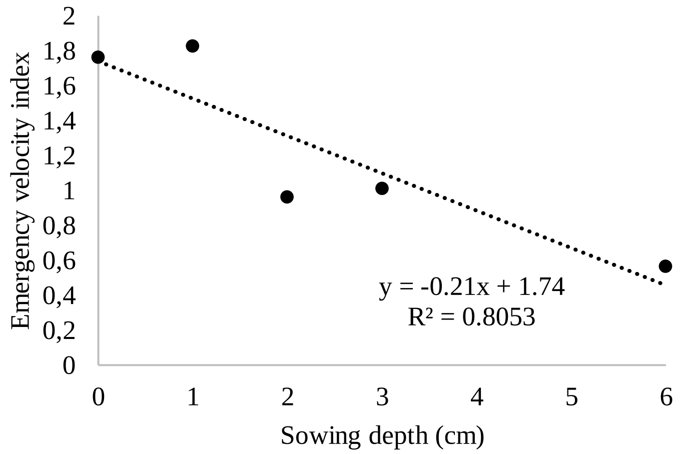

Figure 2: Emergency velocity index (EVI) of *Urochloa brizantha* cv. Marandú 25 days after sowing as a function of sowing depth.

The EVI of 12 cm was not calculates because the 12 cm depth did not show emergence. According to Zuffo et al. (2014), sowing depths greater than 6 cm result in a decrease in EVI due to the distance and the mechanical soil barrier, requiring a larger area of soil to be broken to be able to emerge.

The plant height showed a linear reduction with the increase in sowing depth (Figure 3), once again showing the difficulty of the plant to overcome the physical soil barrier (Pacheco et al., 2010), in addition to the fact that water stress at greater depths affects seed germination (Castaldo et al., 2016). As the emergence speed was lower from 6 cm onwards, as a consequence, it took longer for the seedling to break through the soil and start its growth and leaf elongation process (Teixeira et al., 2018). Greater depths have a greater compaction of the soil, providing greater difficulty for the plant to emerge and seek light for its development (Labegalini et al., 2016).

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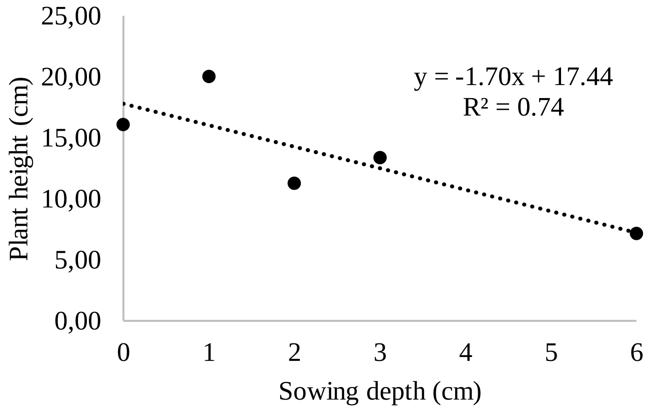

Figure 3: Height of *Urochloa brizantha* cv. Marandú 25 days after sowing as a function of sowing depth.

Planting at low depths, such as 0 and 1 cm, favored wider leaves (Figure 4), even though a linear decrease was observed. According to Cabral et al. (2012) and Botin & Carvalho (2015), this may be related to the fact that the seedlings were able to break the physical barrier of the soil quickly and during its development and a longer time performing photosynthesis, which accelerates the plant cell division process (Lacube et al., 2016).

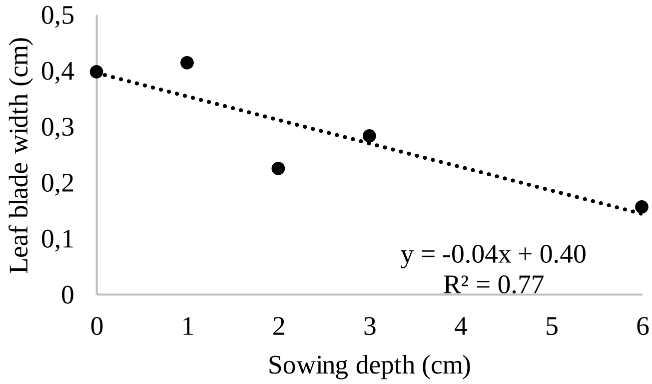

Figure 4: Leaf blade width (cm) of *Urochloa brizantha* cv. Marandú 25 days after sowing as a function of sowing depth. It was possible to observe a linear effect of decreasing root length as a function of increasing sowing depth (Figure 5) in which the root length at depths 0 and 1 cm had higher values with 15.4 and 16.1 cm respectively, when compared to other observed depths.

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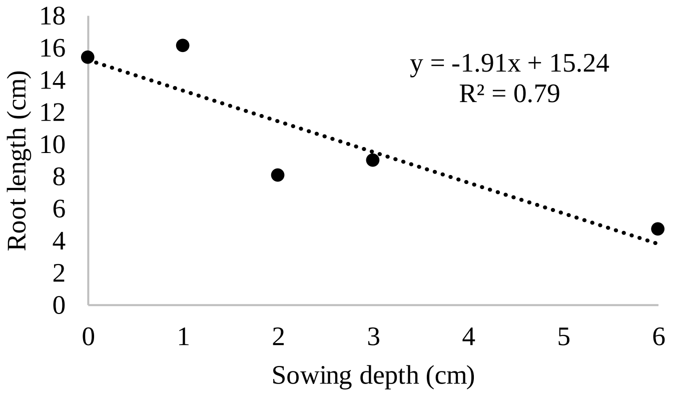

Figure 5: Root length (cm) of *Urochloa brizantha* cv. Marandú 25 days after sowing as a function of sowing depth.

The extension of the root system will depend on the plant's genetic potential and environmental factors (Razuk, 2002). During the initial period of growth of the root and other components of the seedling, the energy reserves end up being converted and mobilized to the root and coleoptile so that it can emerge from the soil, and thus expose its first leaf before the energy reserve is exhausted (Graham, 2008). Dependence on reserves for long periods after germination can lead to depletion and make emergence impossible (Taiz & Zeiger, 2006). In this case, root length was shown to be related to the speed at which the plant manages to emerge and at a depth of 12 cm, it was impossible for the root to grow because the seed energy reserves were exhausted before emerging from the soil.

The number of leaves had a negative linear effect, with a low number of leaves when sowed at greater depths (Figure 6). This can be explained by the time of emergence of the plants, those with greater sowing depth took longer to emerge from the soil, with that the phenological stage was different, being in the earlier stages than plants from shallow depths that emerged more quickly and began their growth and leaf development (Nandi et al. 2018).

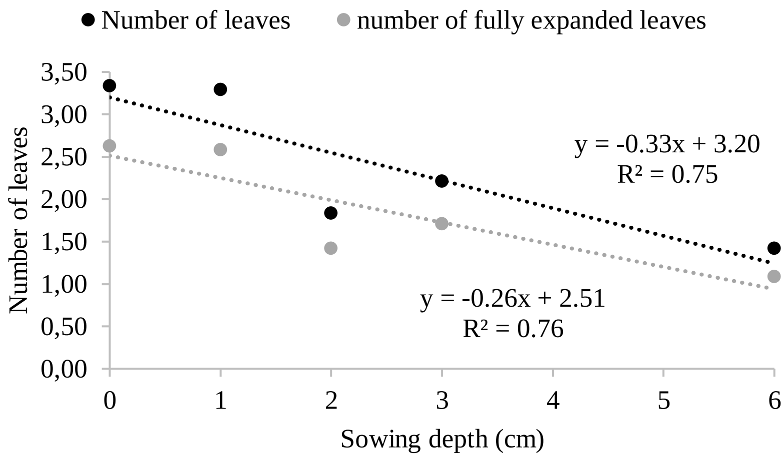

Figure 6: Number of leaves and number of fully expanded leaves of *Urochloa brizantha* cv. Marandú 25 days after sowing as a function of sowing depth.

The shoot and root fresh and dry masses were not influenced by the use of *Pseudomonas fluorescens* (P> 0.05) and by the depth at which they were sown (P> 0.05; Table 2). Pacheco et al. (2010) observed a reduction in the natural and dry mass of shoot with the increase in sowing depth. The SFM and SDM are linked to morphometric characteristics such as plant height, leaf blade length, leaf blade width and number of leaves. It can be explained by the fact that the leaf area was not influenced by the sowing depth, in view of that, the aerial part mass did not present variations according to the depth increase, also in the absence or presence of the bacteria.

Table 2: Aerial and root production of *Urochloa brizantha* cv. Marandú as a function of sowing depth without and with inoculation of *Pseudomonas fluorescens*.

Variables	***P. fluorescens***		**sowing depth (cm)**						**EPM**	**P-valor**		
	without	with	0	1	2	3	6	12		I	SD	I*SD
SFM (g)	0.18	0.22	0.23	0.28	0.17	0.22	0.08	***	0.03	0.39	0.10	0.37
SDM (g)	0.04	0.04	0.05	0.06	0.03	0.05	0.02	***	0.01	0.43	0.07	0.43
RFM (g)	0.35	0.38	0.37	0.54	0.37	0.41	0.13	***	0.07	0.77	0.14	0.47
RDM (g)	0.08	0.09	0.09	0.12	0.08	0.12	0.03	***	0.02	0.50	0.16	0.68
S:R	0.37	0.34	0.65	0.44	0.18	0.28	0.22	***	0.07	0.81	0.02	0.77
TDM (g)	0.21	0.15	0.12	0.16	0.25	0.21	0.16	***	0.02	0.09	0.19	0.92

Note: I= Inoculation with *Pseudomonas fluorescens*; SD = Sowing Depth; SFM = shoot fresh mass; SDM = shoot dry mass; RFM = root fresh mass; RDM = root dry mass; S:R = shoot:root ratio; TDW = Total dry mass.
***= No data recorded.

The RFM and RDM were not influenced by the sowing depth (P< 0.05). By analyzing the root system Mota et al. (2014) noted that DRM decreased with sowing depth increase, due to the energy reserves present in the seed. Cultivars of the *Urochloa* genus have the characteristic of their roots being fasciculate, even though the root length decreases at greater depths, the root mass is linked to the set length, diameter and volume of the root, thus the root mass was similar between 0 to 6 cm sowing depth.

Root mass was also not influenced by the inoculation of *Pseudomonas fluorescens*. Silva et al. (2013) observed that the biomass of *Urochloa brizantha*, when inoculated with associative bacteria did not show differentiation, noting that this may be related to the inoculum and the methodology used for inoculation of bacterial suspension in *Urochloa*. Furthermore, *U. brizantha* may have synthesized substances that inhibited microbial growth, not allowing this microorganism to become an auxiliary in the increase in root mass.

The relation of shoot dry mass and root dry mass was affected by sowing depth with a quadratic adjustment (P< 0.05; Figure 7). The minimum shoot/root ratio was observed with 0 cm of sowing depth and the smallest relation with 4.1 cm of depth.

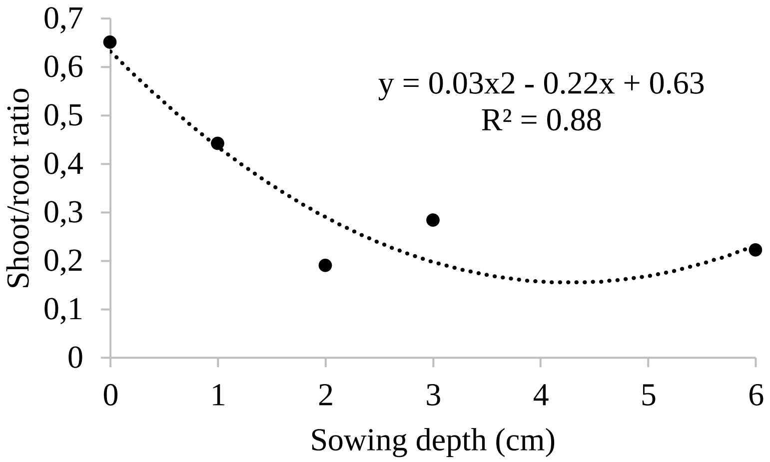

Figure 7: Relation of shoot dry mass with root dry mass of *Urochloa brizantha* cv. Marandú 25 days after sowing as a function of sowing depth.

Sowing between 2 to 6 cm intervals caused a considerable reduction in the area-to-root ratio. At low depths, both shoot and roots are able to establish itself quickly, no longer depending on the energy reserve from the seed and thus begin their cell growth and division more quickly (Cabral et al., 2012).

CONCLUSION

The use of sowing depths 0 and 2 cm favor germination, emergence, aerial and root growth of *Urochloa brizantha* cv. Marandú. However, it is recommended to use seeding depths between 1 and 3 cm, as a depth of 0 cm can bring environmental risks. The use of *Pseudomonas fluorescens* rhizobacteria through seed inoculation does not increase the growth of *Urochloa brizantha* cv. Marandú.

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