http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v47i1.71885

PASTURE AND FORAGE UTILIZATION

Initial growth of *Megathyrsus maximus* cv. Massai grass biostimulated with *Burkholderia pyrrocinia* at different sowing depths

Lorena Maués Moraes[1], Nauara Moura Lage Filho[2*], Victoria de Lima Martins[1], Daniel Sidney Araújo da Costa[1], Gisele Barata da Silva[1], Cristian Faturi[1], Aníbal Coutinho do Rêgo[2] and Thiago Carvalho da Silva[1]

[1]Universidade Federal Rural da Amazônia, Estr. Principal da Ufra, 2150, Curió-Utinga, 66095-780, Belém, Pará, Brazil. [2]Universidade Federal do Ceará, Fortaleza, Brazil. *Author for correspondence. E-mail: nauara.zootecnia@gmail.com

ABSTRACT. Sowing depth affects both germination and emergence of seeds. Thus, inoculation with *Burkholderia pyrrocinia*, a biostimulant, may have positive effects on grass establishment. The aim was to evaluate the effects of inoculation with *B. pyrrocinia* (BP; without and with) and sowing depth (SD; 0, 1, 2, 3, 6, and 12 cm) on the initial growth of *Megathyrsus maximus* cv. Massai. Germination, emergence, and initial shoot and root growth were assessed. The evaluations were performed 21 days after planting. The germination percentage (%G) was affected by the BP×SD interaction ($P<0.05$), with the highest %G occurring at 4.11 cm and 4.90 cm when the seed was inoculated and not inoculated, respectively. The emergence speed index (ESI) was also affected by the interaction of the factors ($p < 0.05$), following a quadratic effect when inoculation was performed, with the highest ESI at a depth of 4.17 cm, while the absence of inoculation resulted in a linear ESI across the depths. However, the initial forage mass was not influenced by the treatments ($p > 0.05$), with an average production of 0.065 g. Inoculation with *Burkholderia pyrrocinia* combined with sowing depth enhances the initial growth of *Megathyrsus maximus* cv. Massai by increasing the germination percentage and emergence speed.

Keywords: bacteria; germination; growth-promoting bacteria; grass; pasture; sustainable management.

Received on April 8, 2024.
Accepted on October 8, 2024.

Introduction

The degradation of pastures negatively affects animal performance, encouraging the search for alternatives for sustainable pasture intensification, with the aim of reducing the demand of new areas for animal production (Tilman et al., 2011). In this context, there are technologies aimed at promoting sustainable pasture intensification, meeting the demand for new areas while minimizing environmental degradation (French et al., 2015).

Improving pasture quality and productivity in a sustainable way is an important goal in agriculture. A promising approach to achieving it is the use of plant growth-promoting bacteria (PGPB) (Hungria et al., 2016). These bacteria play significant roles in the chemical, physical, and biological attributes of the soil and in the nutrient cycling, helping to promote a favorable environment for plant growth.

Costa et al. (2022) showed that the use of *Trichoderma asperellum* and *Bacillus subtilis* can both reduce the doses of fertilizers applied to *Urochloa brizantha* cv. Marandu grass and increase dry matter mass. Lopes et al. (2018), explored the effects of PGPB inoculation on grasses, specifically *Urochloa brizantha*, which resulted in improvements in nitrogen concentration, chlorophyll content, leaf area, net photosynthesis, and total biomass production.

Duarte et al. (2020), evaluating the effect of five species of PGPB on forage and root mass production of *Urochloa ruziziensis* under different levels of nitrogen fertilization, demonstrated that the application of PGPBs promoted increases in forage mass production, ranging from 11 to 69%. Additionally, the bacteria stimulated a 30% increase in root mass in plants compared to non-inoculated ones, resulting in a 25% increase in biomass production, potentially promoting tolerance to environmental stresses, such as droughts.

However, it is essential to consider that the efficacy of these bacteria can be influenced by environmental factors, such as the sowing depth of forage seeds. As mentioned by Rezende et al. (2012), deep sowing can hinder seedling emergence due to the high mechanical resistance of the soil over the seeds. On the other hand, shallow sowing can make seeds susceptible to excessive sun exposure and dehydration, which may affect the initial development of the seedlings.

Research, such as the study by Zuffo et al. (2014), observed that sowing depth plays a crucial role in the emergence of different grass species. For example, in *Urochloa dictyoneura* cv. Llanero, shallow sowing and a sowing depth of 6.0 to 8.0 cm drastically reduced emergence speed. Additionally, the study by Arruda et al. (2023) highlighted that the best results for emergence, growth, and dry mass productivity of roots and shoots of *Urochloa ruziziensis* were obtained at a sowing depth between 1 and 3 cm. From 5 cm onwards, a low emergence rate was observed, and a depth of 8 cm becomes unfeasible for the studied crop.

Therefore, when adopting the use of PGPB to improve pasture growth, it is necessary to carefully consider the sowing depth as part of an integrated management system. The choice of appropriate depth can influence seedling emergence and, consequently, the success of bacterial inoculation and pasture productivity, thus contributing to a more sustainable management system (Guimarães et al., 2022).

Considering this, it is believed that the use of PGPB in pasture establishment can enable plant growth at greater depths, potentially accelerating the initial growth of grasses, as they can play an essential role in plant growth, increasing productivity, and providing resistance to pathogens and abiotic stresses (Duarte et al., 2020).

Thus, inoculation with *B. pyrrocinia* and sowing depth may influence the initial growth of *M. maximum,* improving and/or enhancing this phase, even at greater sowing depths. Therefore, the aim was to evaluate the effect of *B. pyrrocinia* and sowing depths on the initial growth of *M. maximum* cv. Massai seedlings.

Material and methods

The experiment was conducted in a greenhouse at the Federal Rural University of the Amazon, Belém, Pará State (01°27'25''S, 48°26'36''W). The region has an Af-type climate, characterized as tropical humid, with average temperatures ranging between 22.7°C to 32.2 °C (Alvares et al., 2013). In order to prepare the soil, samples were collected from a depth of 0 to 20 cm, dried at room temperature, and sieved through a 1 mm mesh. Then, the soil was amended with dolomitic limestone (PRNT 91%) and fertilized with 140 mg dm^{-3} of P_2O_5 (single superphosphate), based on soil analysis, which indicated the following characteristics: pH in water = 4.76, P = 2.19 mg dm^{-3}, K = 0.14 cmolc dm^{-3}, Ca^{2+} = 2.0 cmolc dm^{-3}, Mg^{2+} = 1.0 cmolc dm^{-3}, H + Al = 7 cmolc dm^{-3}.

The germination test was conducted with 200 seeds of *Megathyrsus maximus* cv. Massai. The seeds were placed in acrylic boxes (gerbox), sown on germination paper (germitest), and incubated in BOD (Biochemical Oxygen Demand) chambers at 30°C for 7 days, resulting in a germination rate of 50%.

The experiment was conducted in a completely randomized design with a 2×6 factorial scheme, resulting in 12 treatments with 6 replicates each. The factors analyzed were sowing depth (SD - 0, 1, 2, 3, 6, and 12 cm) and the inoculation with *B. pyrrocinia* (BP), with two conditions: without inoculation and with seed inoculation.

The seeds were commercially acquired from a 2018 batch, with a cultural value of 50%. Sowing was carried out in seedling trays. The day before sowing, inoculation with *B. pyrrocinia* (BRM-32113) (Lopes et al., 2018) was performed on the seeds according to the protocol proposed by Kado and Heskett (1970). The sowing of seeds at a depth of 0 cm was done by placing the seeds on the soil surface of the trays. For the depths of 1, 2, 3, 6, and 12 cm, the seeds were planted using a graduated tube, pushing the soil down to the treatment depth, inserting the seed into the soil, and then covering it. Moisture maintenance throughout seed germination and initial seedling growth was ensured by replenishing moisture based on soil field capacity calculation, providing adequate conditions for all treatments, both with and without the application of the bioinoculant.

After sowing, daily observations were made to determine the number of emerging seedlings and the day of their emergence. The goal was to calculate the germination percentage (G, %) and the emergence percentage (E, %), considering the initial number of seeds sown and the seeds that germinated but did not emerge. On the 14th day after planting, thinning was performed and only one plant was kept per tray.

The emergence speed index (ESI) was calculated according to the formula proposed by Maguire (1962): $ESI = \frac{E1}{D1} + \frac{E2}{D2} + \cdots \frac{En}{Dn}$, where E1, E2, ..., En represent the number of normal seedlings observed in the first count, second count, and successively until the last count. D_1, D_2, ..., D_n represent the number of days from sowing to the first, second, and subsequent counts, until the last one.

After the 21st day of planting, morphological characteristics of the plants were measured, including plant height (PH), leaf blade length (LBL), and leaf blade width (LBW) in centimeters (cm), number of leaves (NL), and number of expanded leaves (NEL). Then, the plants were subjected to destructive analysis, with both aerial parts and roots being separated at the soil surface. Leaf blades were separated to determine leaf area (LA, cm^2) using a leaf area meter (Li-3100c, Li-cor, Lincoln, NE, USA). Roots were removed from the tubes, washed with water, air-dried, and measured to obtain root length (RL, cm).

After cutting the plants, the aerial parts and roots were weighed and dried in a forced air oven at 60°C for 72 hours. Then, the samples were removed and weighed anew to determine the shoot dry mass (SDM, g per tray) and root dry mass (RDM, g per tray). With these data, it was possible to calculate the SDM/RDM ratio, percentage of shoot (%S), and percentage of roots (%R). Therefore, the estimates of %S and %R were based on the dry mass values measured after drying, ensuring an accurate assessment of plant biomass.

The collected data were subjected to normality tests using the Shapiro-Wilk analysis, followed by analysis of variance (ANOVA). In the statistical model, the fixed effects of SD, BP, the interaction of SD×BP, and the random effect of experimental error were considered. Due to the low emergence of plants at the 12 cm depth, this depth was excluded from the morphological parameters model evaluated in the plants.

The response variable data related to SD were fitted to models using regression analysis, with the polynomial degree equations selected based on both coefficient of determination (R^2) and significance of the equation parameters. The factor related to *B. pyrrocinia* inoculation (BP) was compared using the t-test. All statistical analyses were performed using the SISVAR® statistical program, with 0.05 as the significance level for type I error.

Results and discussion

It was observed that the interaction between BP and SD influenced ($p < 0.05$) the germination percentage (G, %), emergence percentage (E, %), and the emergence speed index (ESI). Additionally, there was an effect of BP ($p < 0.05$) on the time to emergence (TE) and the leaf area (LA), with a reduction in TE by an average of 1.38 days in inoculated plants and an increase in LA by an average of 5.72 cm^2 compared to non-inoculated plants. An effect of SD ($p < 0.05$) was also observed on plant height (PH), leaf blade length (LBL), leaf blade width (LBW), LA, and root length (RL, Table 1).

The use of PGPB can play an important role in plant growth, as these microorganisms can synthesize siderophores and antioxidants, assist in nutrient acquisition from the soil, and contribute to resistance responses to different types of abiotic and biotic stresses (Kumar & Verma, 2018). Additionally, it is important to note that sowing depth can influence the germination and emergence of different species (Rezende et al., 2012). The combination of PGPB use and the appropriate choice of sowing depth (SD) may have created ideal conditions for the successful germination and emergence of *M. maximum* cv. Massai, due to the ability of the microorganisms to synthesize substances that promote faster emergence.

Table 1. Germination and morphological characteristics of *Megathyrsus maximus* cv. Massai as a function of sowing depth with and without *Burkholder a pyrrocinia* inoculation.

Variables	Inoculation		Sowing Depth (cm)						SEM	P-value		
	Without	With	0	1	2	3	6	12		BP	SD	BP×SD
G (%)	61.46	51.04	39.58	61.46	67.71	79.17	73.96	15,63	3.82	0.90	<0.01	0.02
E (%)	55.90	47.92	37.50	59.38	67.71	75.00	71.88	0,00	3.82	0.90	<0.01	0.02
ESI	1.01	1.20	0.85	1.05	1.06	1.40	1.17	ND	0.08	0.16	<0.01	0.02
TE (days)	6.18 b	4.80 a	5.42	5.17	6.50	5.54	4.83	ND	0.34	<0.01	0.26	0.33
PH (cm)	28.00	26.21	19.43	26.31	36.01	27.84	25.91	ND	1.86	0.50	<0.01	0.46
LBL (cm)	10.76	9.97	7.52	9.43	13.67	11.56	9.65	ND	0.65	0.39	<0.01	0.41
LBW (cm)	0.38	0.34	0.27	0.33	0.45	0.39	0.34	ND	0.06	0.21	0.01	0.33
LA (cm^2)	15.97 a	10.25 b	6.91	12.09	16.63	16.67	13.26	ND	1.62	0.01	0.05	0.87
NL	6.10 a	4.95 b	4.13	5.17	6.42	6.04	6.39	ND	0.41	0.05	0.11	0.77
NTLFE	4.00	4.80	3.29	4.08	5.25	4.79	4.58	ND	0.31	0.07	0.07	0.71
RL (cm)	16.51	16.90	13.74	15.18	20.93	18.68	15.02	ND	1.09	0.80	0.02	0.12

Notes: G = germination; E = emergence; ESI = emergence speed index; TE = time to emergence; PH = plant height; LBL = leaf blade length; LBW = leaf blade width; RL = root length; LA = leaf area; NL= number of leaves; NFL = total number of leaves; SEM = standard error of the mean; I = inoculation with *B. pyrrocinia*, SD = sowing depth; ND = not determined.

A quadratic behavior was observed for germination as a function of sowing depth for both inoculated and non-inoculated seeds with BP. However, germination with BP inoculation reached a maximum value of 77.75% at an SD of 4.19 cm, while germination without the presence of the bacterium reached a maximum value of 80.38% at an SD of 4.47 cm (Figure 1).

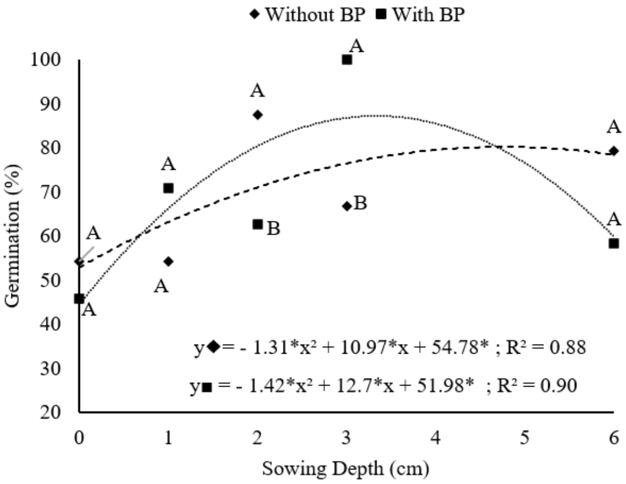

Figure 1. Germination (%) of *Megathyrsus maximus* cv. Massai 21 days after sowing as a function of *B. pyrrocinia* and sowing depth.

The highest germination values were observed at intermediate depths, excluding the extremes, regardless of BP inoculation. The lowest germination percentages observed at both shallower depths and those close to 5.0 cm can be explained by unfavorable edaphoclimatic conditions at these depths, which may impair the seed germination process. These conditions include factors such as nutrient availability, soil oxygenation, and temperature, which can vary significantly depending on the sowing depth, thus affecting seed germination (Rezende et al., 2012; Teixeira et al., 2018).

The presence of *B. pyrrocinia* (BP) resulted in a quadratic response for the emergence speed index (ESI), with the highest index (1.35) at a depth of 4.17 cm ($p < 0.05$). On the other hand, in the absence of BP, a linear increase ($p < 0.05$) in ESI was observed as sowing depth increased (Figure 2).

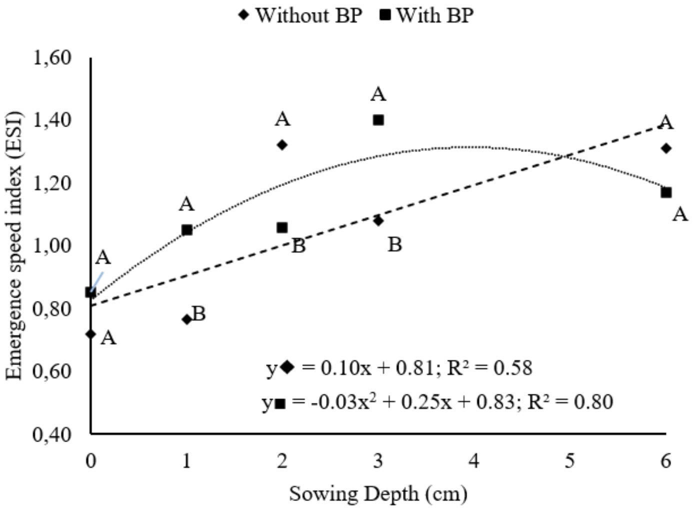

Figure 2. Emergence speed index (ESI) of *Megathyrsus maximus* cv. Massai 21 days after sowing as a function of *B. pyrrocinia* and sowing depth.

The presence of PGPB may have facilitated the acceleration of plant emergence, as PGPB can enhance nutrient cycling, absorption, and translocation, thereby reducing the stress to which plants are subjected. Additionally, PGPB are known for their ability to produce plant growth phytohormones, which stimulate plant development (Vimal et al., 2017, Kumar & Verma, 2018).

However, the observation of a higher ESI in the absence of *B. pyrrocinia* inoculation can possibly be explained by factors such as specific soil conditions, plant genetic variability, environmental conditions, and the influence of stress from a specific SD (Foloni et al., 2009). Thus, the effect of PGPB on germination and emergence of seeds may depend on a complex set of factors that vary according to circumstances, leading to distinct responses as evidenced in the experiment with *M. maximum* cv. Massai. Additionally, it is important to consider the role of ethylene, which acts in breaking seed dormancy and plays a crucial role in accelerating seedling emergence (Glick et al., 2012).

It was not possible to calculate the ESI at a depth of 12 cm, as no plant emergence was observed at this depth. Therefore, sowing at greater depths, starting from 10 cm, may result in a reduction of the ESI due to the increased distance and mechanical resistance of the soil, as well as decreased light and oxygen availability in the soil, as highlighted by Ikeda et al. (2013). This may explain the absence of plant emergence at a depth of 12 cm and suggests that sowing depth plays a crucial role in germination and emergence of seeds.

The use of *B. pyrrocinia* resulted in a shorter time to emergence (TE) (4.80 days) compared to the absence of PGPB, which had a TE of 6.18 days. This demonstrates that the use of PGPB aids in early plant growth, decreasing the time needed for seedlings to break through the soil's mechanical barrier and begin their growth process and leaf expansion. This effect can be attributed to the beneficial interactions between PGPB and plants, which promote faster germination and seedling emergence (Ikeda et al., 2013).

Higher averages for leaf area (LA) and number of leaves (NL) were observed (Table 1) in the absence of *B. pyrrocinia*.

This may have occurred due to the fact that, after seedling germination and emergence, a series of metabolic processes begins, including the synthesis of growth-regulating substances that influence cell division and elongation in plants. Thus, the seeds may have exhibited similar cell division between treatments, as growth depends not only on environmental conditions but also on the plant's morphogenetic information (Arruda et al., 2023).

Plant height (PH) showed a quadratic effect with increasing depth, reaching a maximum height of 32.70 cm at a depth of 3.40 cm (Figure 3a), followed by a decrease in growth at greater depths, as also observed by Martins et al. (2022). Shallow sowing may leave seeds more susceptible to environmental variations such as high temperatures and water deficiency, potentially reducing seedling moisture for growth, resulting in lower plant populations (Proctor & Sullivan, 2013). On the other hand, deeper sowing may lead to greater energy consumption during germination, as the seed expends its energy reserves to break through the physical barrier of the soil.

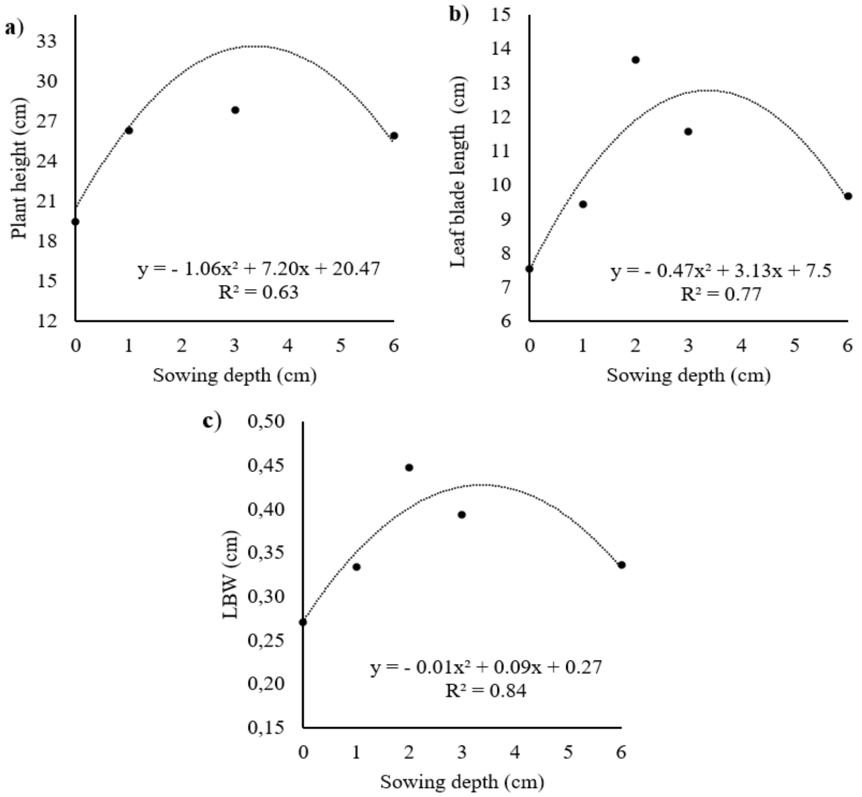

Figure 3. Plant height (a), leaf blade length (b) and leaf blade width (c) of *Megathyrsus maximus* cv. Massai after 21 days after sowing as a function of sowing depth.

Additionally, at greater depths, a higher emergence speed index (ESI) is observed, which may indicate that the seed takes longer to emerge at the surface, potentially delaying the start of vegetative development. Plants that emerge later may have a shorter period to fully develop before maturation or before facing adverse environmental conditions, such as low oxygenation, limited light, and reduced water and nutrient availability for plant growth (Zuffo et al., 2014).

As depth increased, it took longer for the plant to reach the surface, causing the seedling to grow in search of sunlight for photosynthesis. Thus, the greatest leaf length (12.71 cm) was obtained with seeds sown at a depth of 3.33 cm (Figure 3b). Also, placing seeds closer to the surface allowed seedlings with leaves at the surface to perform photosynthesis more intensely than those at greater depths (Alves et al., 2014).

Leaf blade width (LBW) showed a quadratic effect with increasing depth, reaching a maximum width of 0.47 cm at a depth of 4.50 cm (Figure 3c). The increase in depth up to 4.50 cm resulted in wider leaves, followed by a decrease as sowing depth increased. This behavior may be due to the fact that seedlings at shallower depths had greater access to sunlight and, consequently, higher photosynthetic intensity, which promoted greater leaf growth (Glick et al., 2012).

In Figure 4a, it was observed that depth influenced root length (RL), with the greatest length (19.71 cm) at a depth of 3.20 cm. According to Maan et al. (2023), plant roots tend to extend deeper into the soil when sown at greater depths, possibly as an adaptation strategy to seek essential resources such as water and nutrients. Such behavior can be interpreted as a response to the growth environment, where deeper soil may offer more stable conditions regarding moisture and temperature, encouraging more extensive root growth. However, the reduction in RL at depths greater than 3.20 cm may have been due to the soil's decreased oxygen availability, which is necessary for root system respiration. Thus, it is possible to infer that sowing at the correct depth benefits seedling root development, allowing the growth of more vigorous plants with greater growth potential (Alves et al., 2014).

A quadratic effect was observed with increasing depth, with a maximum leaf area (LA) of 17.79 cm² at a depth of 3.62 cm (Figure 4b). The increase in LA with sowing depth may be due to better access to water and nutrients, an adaptive response to favorable soil conditions, less competition with weeds, and ideal microclimatic conditions, promoting greater leaf growth (Lee et al., 2017). No effect was observed for *Burkholderia pyrrocinia* inoculation ($p > 0.05$) or sowing depth ($p > 0.05$) on the productive variables SDM, RDM, DMS, %S, and %R, as shown in Table 2. The lack of observed effect on productive characteristics may be due to the metabolic and genetic traits of the forage species.

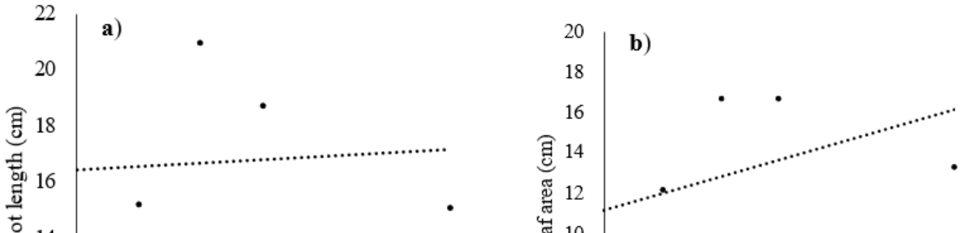

Figure 4. Root length (a) and leaf area (b) of *Megathyrsus maximus* cv. Massai 21 days after sowing as a function of sowing depth.

Table 2. Mean values of forage mass variables for shoot and root evaluated in *M. maximus* cv. Massai seedlings, as a function of *Burkholderia pyrrocinia* inoculation and sowing depth.

Variables	*B. pyrrocinia*		Sowing depth (cm)					EPM	P-value		
	Without	With	0	1	2	3	6		BP	SD	BS×SD
SDM (g)	0.07	0.06	0.06	0.07	0.07	0.07	0.06	0.01	0.56	0.99	0.99
RDM (g)	0.04	0.04	0.06	0.05	0.06	0.05	0.04	0.01	0.85	0.30	0.91
SDM/RDM	1.82	1.52	1.31	1.79	1.71	2.11	1.43	0.13	0.27	0.39	0.51
%S	57.62	53.31	52.13	58.47	53.17	57.50	56.06	1.08	0.06	0.35	0.73
%R	42.38	46.69	47.87	41.53	46.83	42.50	43.94	1.08	0.06	0.35	0.73

SDM - Shoot dry mass, RDM - Root dry mass, SDM/RDM - Shoot dry mass to root dry mass ratio, S (%) - Shoot percentage, R (%) - Root percentage, SEM - Standard error of the mean, BP – Inoculation with *Burkholderia pyrrocinia*, SD - Sowing depth.

Conclusion

The inoculation of *Burkholderia pyrrocinia* appears to positively affect the initial growth of *Megathyrsus maximus* cv. Massai by increasing the germination percentage and emergence speed. However, further studies are necessary to confirm this hypothesis. Sowing *Megathyrsus maximus* cv. Massai at depths between 2.0 and 4.0 cm results in more vigorous, taller, and longer plants.

Acknowledgments

The authors thank the Federal Rural University of the Amazon (UFRA), the Study Group on Ruminants and Forage in the Amazon (GERFAM; www.gerfam.com.br), and the Plant Protection Laboratory (LPP) for their support. We would also like to thank the reviewers of the LETRARE project from the Federal University of Ceará, whose dedication and commitment were essential for the completion of this work.

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