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Full Length Article

Growth and Development of *Megathyrsus maximus* cv. Mombasa is Improved by Inoculation of Plant Growth-Promoting Microorganisms

Hackson Santos da Silva[1*], Thatiane Mota Vieira[1], Brenda Emilly Ferreira dos Santos[1], Ingridy de Carvalho Dutra[1], Nadjane Vieira da Silva[1], Natan Teles Cruz[1], Renata Rodrigues Jardim[1], Jair Amorim Sousa Junior[2], Tiago Pereira Ribeiro dos Santos[2], Edson Marcos Viana Porto[3], Daniela Deitos Fries[4] and Fábio Andrade Teixeira[5]

[1]*Postgraduate Program in Animal Science, State University of Southwest Bahia, 45700-000, Itapetinga, Bahia, Brazil*
[2]*Undergraduate student, State University of Southwest Bahia, 45700-000, Itapetinga, Bahia, Brazil*
[3]*Department of Agricultural Sciences, State University of Montes Claros, 39401-089, Montes Claros, Minas Gerais, Brazil*
[4]*Department of Exact and Natural Sciences, State University of Southwest Bahia, 45700-000, Itapetinga, Bahia, Brazil*
[5]*Department of Rural and Animal Technology, State University of Southwest Bahia, 45700-000, Itapetinga, Bahia, Brazil*
[*]For correspondence: hackkson@gmail.com
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Abstract

The present study aimed to assess the initial development of Mombasa grass inoculated with plant growth-promoting microorganisms. The experiment was conducted in a greenhouse using a completely randomized design. Mombasa grass was inoculated with *Azospirillum brasilense* and *Rhizophagus intraradices*, isolated and combined. Sole inoculation of *R. intraradices* or *A. brasiliense* improved the morphogenic parameters of Mombasa grass, resulting in a higher leaf appearance and elongation rate, shorter time for the emergence of new leaves and a decrease of leaf senescence rate. The strategy of isolated inoculation with *A. brasilense* increased aboveground biomass production (19%). All the inoculations notably increased the SPAD index values and chlorophyll *a* concentration, with increments of 22 and 26%, respectively, reflecting an increase in mineral content and crude protein in plants, whilst *A. brasilense* inoculation displayed a much stronger effect on mineral content. These gains are attributed to the better root development of inoculated plants, which optimized nutrient absorption. Results suggested that sole inoculation of *A. brasilense* or *R. intraradices* improved initial growth of Mombasa grass; however, their combined application worked synergistically to stimulate root development and improved nutritional status. Therefore, the combined application of these microbial inoculants seemed an alternative capable of optimizing the initial growth of Mombasa grass for sustainable production.

Keywords: Bioinputs; Guinea grass; Microbial inoculation; Pasture; Sustainability

Introduction

Livestock production in pastures demonstrates the resilience of tropical forage grasses, however, their sustained exposure to stressful conditions compromises their physiology (Li *et al.* 2009), limiting productivity and reflecting in poor livestock management practices. The stress imposed on plants can be alleviated using sustainable tools, among which biological inputs play a significant role, improved the tolerance of plants to physiological disturbances caused by factors biotics and abiotic (Colla *et al.* 2015).

Among the bio inputs available in the market, microbiological inoculants composed of fungi or strains of rhizobacteria are utilized in diverse agriculture crops and frequently denominated as plant growth-promoting microorganisms (PGPM) (Brazil 2020). Their primary function is to enhance plant growth and provide protection against biotic and abiotic stresses (Souza *et al.* 2015). These microorganisms can engage in mechanisms ranging from biological nitrogen fixation, phosphorus solubilization, resistance to soil pathogens, and an increase in root surface area (Bashan *et al.* 2012; Yasmin *et al.* 2016; Majeed *et al.* 2022), thereby providing plants with increased nutrient and water uptake.

Plant growth-promoting mechanisms, mediated by rhizobacteria and mycorrhizal fungi, demonstrate significant potential for optimizing plant development (Nadeem *et al.* 2014; Rouseaux *et al.* 2020; Dawood *et al.* 2023). These microorganisms can establish a synergistic relationship between themselves and between species of plants, maximizing their effectiveness in promoting the growth of host plants. Studies have shown that fungal structures function as a communication route for bacteria, facilitating their penetration into the epidermis of the root tissue, while

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the production of phytohormones by these bacteria favors the colonization and mycelial growth of mycorrhiza (Ruíz-Sánches *et al.* 2011; Villarreal *et al.* 2016). However, it is important to highlight that antagonistic interactions can arise due to competition for nutrients and other resources essential for the survival of these microorganisms in the rhizosphere. The success or failure of co-inoculation is intrinsically linked to the physiological stage of the host, the time of infection, and the divergent nutritional demands between fungi and rhizobacteria (Biró *et al.* 2000).

Considering the global panorama with climate and extreme events (Feller and Vaseva 2014), growing demand for food and the responsibility for sustainable pasture production (Guimarães *et al.* 2022); the use of microbial inoculants in grass species present in pastures, such as *Megathyrsus maximus* (Jacq.) BK Simon and SWL Jacobs (syn. *Panicum maximus* Jacq.) emerge as a viable alternative to increase sustainability in the cultivation of agronomically important plants. However, studies are needed to clarify and validate the use of bioinoculants in pastures, aiding in the better adaptation of forage crops in challenging scenarios. The present study was aimed to evaluate the effect of single and dual inoculation with *R. intraradices* and *A. brasilense* on initial growth, root characteristics and chemical composition of aerial part of Mombasa grass.

Materials and Methods

Experimental details

Experiments were performed in the greenhouse of the Department of Rural and Animal Technology, State University of Southwest Bahia, Itapetinga, Bahia (15º 14' S, 40º 14' W), during October–January of the years 2020–2021. Weather data during the experimental period were obtained using a digital thermo-hygrometer. The average maximum and minimum temperature values were 39.4 ºC and 20.4ºC, respectively, and the maximum relative humidity was 84% and minimum was 20%.

The soil used in the experiment was collected at a depth of 0–20 cm and subjected to physical and chemical analysis at the Department of Agricultural and Soil Engineering, State University of Southwest Bahia. The soil analysis demonstrated the following result: Sandy-loam textured, clay 9%, silt 35.5%, sand 55.5%, pH (water) 6.3, phosphorus (ion-exchange resin extraction method) 15 mg.dm^{-3}, potassium 0.97 cmolc.dm^{-3}, calcium 1.5 cmolc.dm^{-3}, magnesium 1.6 cmolc.dm^{-3}, H + Al 1.1 cmolc.dm^{-3}, sum of bases 4.1 cmolc.dm^{-3}, effective cation exchange capacity 4.2 cmolc.dm^{-3}, total cation exchange capacity 5.2 cmolc.dm^{-3}, base saturation 79%, organic matter 7 g.dm^{-3}.

Treatments and experimental design

Mombasa grass was evaluated in four different treatments consisting of (i) a non-inoculated group (Control), (ii) inoculation with *Azospirillum brasilense*, (iii) inoculation with *Rhizophagus intraradices* and (iv) co-inoculation with *A. brasilense* and *R. intraradices*. The experiment was randomized according to completely randomized design with four replications, totaling 16 experimental units (plastic pots), with a capacity of 12 L and, which were filled with 10 kg of soil. To maintain the soil close to the water retention capacity in 30% (Souza *et al.* 2020), all pots were weighed every day and water was added as needed.

According to the recommendations of the Soil Fertility Commission of Minas Gerais State (Ribeiro *et al.* 1999), there was no need for liming. Only phosphorus and nitrogen were used after the uniformity cut, with basal fertilization being carried out for establishment with 50 kg ha^{-1} of P_2O_5 and 50 kg ha^{-1} of nitrogen in the form of urea and simple superphosphate respectively.

Application of PGPM and sowing of seeds

Before planting and inoculation, seeds were surface disinfected by immersion in 3% sodium hypochlorite for 3 min, followed by four consecutive rinses in water and air drying. For inoculation with *A. brasilense*, a commercial product containing Ab-V5 and Ab-V6 strains was used, with a guarantee of 2 x 10^{8} CFU mL^{-1}, with a recommendation of 100 mL of inoculant for 5 kg of seeds, which were homogenized and dried in the shade for 30 min. For inoculation with *R. intraradices*, a commercial inoculant was used with a guarantee of 20,800 propagules g^{-1}, using 120 g ha^{-1} added to the planting hole immediately after sowing; the co-inoculation treatment used a combination of the previously mentioned forms of inoculation. Five seeds were sown per unit experimental and 15 days after the emergence, seedlings were thinned to four plants per pot.

The microbial inoculants used in this study are registered with the Ministry of Agriculture, Livestock and Supply for commercialization in Brazilian territory. Commercial product containing *A. brasilense* strains registration number: 22902 10000-0; Commercial product containing *R. intraradices* registration number: PR-93923-10074-1.

Parameters analyzed

When the plants completed 30 days after emergence, a cut of uniformization was realized at a height of 20 cm, and fertilization with phosphorus and nitrogen was performed. After cutting to standardize the experimental units, monitoring of the regrowth of Mombasa grass began, with evaluation during two periods of 28 days.

For evaluation, two tillers per pot were marked with colored ribbons and evaluated every 3 days. The following were determined by measurements of individual leaves and tillers: leaf appearance rate (LAR, leaves tiller^{-1} day^{-1}); phyllochron (PHY, leaves tiller^{-1} day^{-1}); leaf elongation rate (LER, cm leaves tiller^{-1} day^{-1}); leaf senescence rate (LSR, cm tiller^{-1} day^{-1}); number of living leaves per tiller (NLL), final leaf length (FLL, cm leaf^{-1}); tiller density (TD), and final plant height (FPH, cm).

At the end of each evaluation period, the SPAD (Soil Plant Analytical Division Value) index was read using a SPAD 502 Plus device at times of highest solar incidence, with readings taken in the middle third of two completely expanded leaves within each experimental unit. Then, the same leaves used to read the SPAD index were collected for extraction of photosynthetic pigments (Chlorophyll *a*, Chlorophyll *b*, Total chlorophyll and Carotenoids), which were cut, excluding the central vein, weighed 0.2 g, stored in 5 mL of dimethyl sulfoxide, and kept for 72 h in the dark. Afterwards, readings were taken on the spectrophotometer at wavelengths of 665, 649 and 480 nm and quantified according to Wellburn (1994), with results expressed in μg.g^{-1} fresh mass.

After collecting the material for chlorophyll, a cut was made 20 cm from each experimental unit. The harvested material was identified, weighed, and taken to a forced air circulation oven at 65°C for pre-drying for 72 h, then weighed again, thus calculating the biomass production of the area (g.pot^{-1}). The material was then ground in a Willey-type knife mill with a 1 mm sieve to carry out bromatological composition, where the contents of dry matter (DM, method INCT-CA G-003/1), mineral matter (MM, INCT-CA method M-001/1), crude protein (CP, INCT-CA method N-001/1) and neutral detergent fiber (NDF, INCT-CA method F002/1) and acid detergent fiber (ADF, method INCT-CA F-004/1) according to methodologies described by Detmann *et al.* (2021), being carried out in the University's Bromatological Analysis Laboratory.

The experimental units were dismantled after the second cut, and the roots were removed and washed in running water. The volume of the root system was estimated using a cylindrical vessel with graduations, recording the water displacement after immersion of the roots. The root system was then dried in an oven at 65°C until constant weight and then weighed to obtain the root dry weight.

Statistical analysis

The data obtained were grouped and analyzed as the average of two experimental cutting. The dataset was analyzed by one-way analysis of variation (ANOVA), when significant differences were detected; means were compared with Tukey's test at the 5% level, by using SAS software OnDemand for Academics.

Results

Morphogenic and structural characteristics

Different inoculation treatments displayed diverse responses on development and growth of Mombasa grass. LAR, LER, PHY and NLL ($P < 0.001$ for all) were greater with *A. brasilense* and *R. intraradices* inoculated single, whereas PHY and NLL ($P < 0.001$ for both) were higher in non-inoculated plants and co-inoculation. Compared to the non-inoculated plants, the plants treated by co-inoculation or single inoculation with *R. intraradices* and *A. brasilense* represents 83% significantly higher leaf senescence rate ($P < 0.001$). On the other hand, the variables FLL, TD and FPH were not significant ($P = 0.200$, $P = 0.082$ and $P = 0.080$ respectively), presenting averages of 39.44 cm leaf^{-1}, 16.62 tillers and 59.06 cm, respectively (Table 1).

Photosynthetic pigments

Chlorophyll a concentration and SPAD index value were influenced by the treatments tested ($P < 0.001$ for both) being 26 and 22%, respectively, greater when inoculated with microorganisms than in the non-inoculated plants. No differences were found for chlorophyll *b*, total chlorophyll, and carotenoids ($P = 0.090$, $P = 0.075$ and $P = 0.165$), with averages of 31.58 μg.g^{-1} of fresh biomass, 211.19 μg.g^{-1} of fresh biomass and 23.25 μg.g^{-1} of fresh biomass, respectively (Table 2).

Productive parameters

The production of fresh and dry biomass (Fig. 1) was significantly greater ($P < 0.001$ for both) in the presence of *A. brasilense*, with respective values of 21.25 g. pot^{-1} and 5.86 g.pot^{-1}. The other treatments were equivalent for these variables, not differing from each other or from the non-inoculated plants, with averages of 18.37 g.pot^{-1} of fresh biomass ($P = 0.270$) and 5.05 g.pot^{-1} of dry biomass ($P = 0.124$).

Bromatological characteristics

Inoculation with *A. brasilense* resulted in greater averages for MM and CP contents ($P = 0.001$ and $P = 0.010$) than the non-inoculated plants treatment, with percentage increases of 5.25 and 13.41%, respectively. Conversely, for variables DM, NDF and ADF no effects were observed for any inoculation ($P = 0.572$, $P = 0.165$ and $P = 0.362$ respectively), with an average of 27.55, 67.12 and 33.36% respectively (Table 3).

Root evaluation

Plants inoculated with *A. brasilense* and co-inoculated showed greater averages for root system volume (average of 74 mL) and root dry weight (average of 134.46 g) ($P < 0.001$), with a percentage increase of 29 and 18%, respectively, in relation to the non-inoculated plants (Table 4).

Table 1: Morphogenic and structural characteristics of Mombasa grass inoculated with plant growth-promoting microorganisms

Items	Control	*A. brasilense*	*R. intraradices*	Co-inoculation	CV%
LAR	0.23b	0.32a	0.36a	0.27b	8.75
PHY	4.32a	3.04b	3.01b	3.82a	9.73
LER	3.07c	4.72b	5.56ab	3.57cb	17.25
LSR	1.32a	0.92b	0.65b	0.60b	23.91
NLL	5.00b	7.15a	7.17a	5.66b	8.64
FLL	41.41	39.65	36.10	40.61	10.07
TD	16.25	15.25	17.75	17.25	17.92
FPH	59.50	59.75	60.25	56.75	8.66

LAR: Leaf appearance rate (tiller leaves^{-1} day^{-1}); PHY: phyllochron (tiller leaves^{-1} day^{-1}); LER: Leaf elongation rate (cm leaves tiller^{-1} day^{-1}); LSR: leaf senescence rate (LSR, cm tiller^{-1} day^{-1}); NLL: number of living leaves (per tiller); FLL: final length of leaves (cm.leaf^{-1}); DFV: leaf life span (days); NP: tiller density; FPH: final plant height (cm); CV: coefficient of variation; Means within lines followed by different letters differ by Tukey's test at 5% probability

Table 2: Concentration of chlorophylls, carotenoids (µg.g^{-1} of fresh mass) and SPAD index of Mombasa grass inoculated with plant growth-promoting microorganisms

Items	Control	*A. brasilense*	*R. intraradices*	Co-inoculation	CV%
Chlorophyll *a*	152.66b	184.60ab	188.45ab	192.76a	9.69
Chlorophyll *b*	38.87	29.85	24.12	33.48	26.78
Total chlorophylls	191.51	214.45	212.58	226.24	8.33
Carotenoids	28.58	23.81	23.82	16.82	38.72
SPAD	16.17b	19.38a	19.00a	20.90a	5.98

CV: coefficient of variation; Means within lines followed by different letters differ by Tukey's test at 5% probability

Table 3: Chemical composition of Mombasa grass inoculated with plant growth-promoting microorganisms

Items	Control	*A. brasilense*	*R. intraradices*	Co-inoculation	CV%
DM	27.66	27.69	27.65	27.23	5.65
MM	7.81c	8.22a	7.86b	7.96b	7.74
NDF	65.54	68.96	68.45	65.54	4.39
ADF	32.11	34.58	34.54	32.23	8.66
CP	5.74b	6.51a	6.11ab	6.17ab	5.26

DM: dry matter; MM: mineral matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; CP: crude protein; CV: coefficient of variation. Means within lines followed by different letters differ by Tukey's test at 5% probability

Table 4: Root characteristics of Mombasa grass inoculated with plant growth-promoting microorganisms

Items	Control	*A. brasilense*	*R. intraradices*	Co-inoculation	CV%
Root volume	59.40b	77.00a	60.60b	71.00ba	13.44
Root dry weight	113.53b	130.84a	115.75b	138.08a	23.05

CV: coefficient of variation; Means within lines followed by different letters differ by Tukey's test at 5% probability

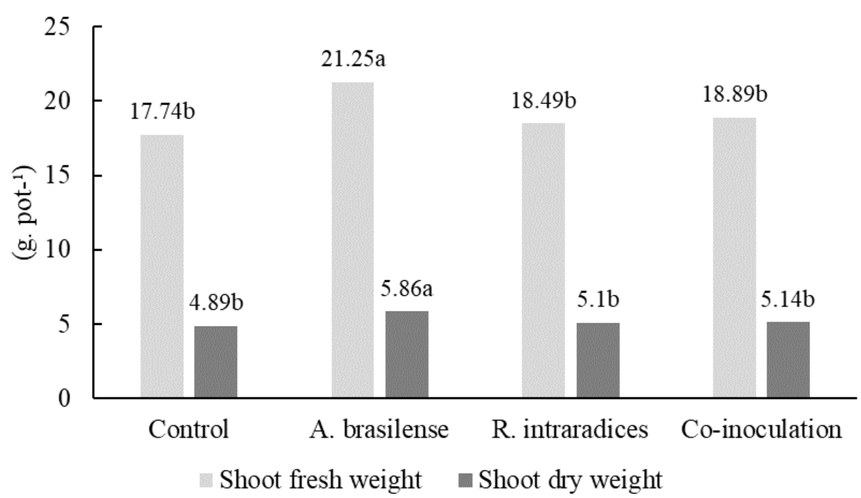

Fig. 1: Shoot fresh and dry weight of the aerial part of Mombasa grass inoculated with plant growth-promoting microorganisms. Means followed by different letters, for each items analyzed, differ by Tukey's test at 5% probability (Coefficient of variation for shoot fresh: 10.60%; Coefficient of variation for Shoot dry: 5.32%)

Discussion

The benefit of using plant growth-promoting microorganism has been reported by several authors, contributing positively to crop development with improvements in nutrient absorption, resistance to pathogens, and root development (Souza *et al.* 2011; Goswami *et al.* 2016; Fukami *et al.* 2018). In this study, we present results of morphogenic, bromatological, and biomass production characteristics in Mombasa grass, assessed under controlled conditions using commercial inoculants applied in seeds.

Seed inoculation with the microbial inoculant employed has improved various morphogenic parameters of Mombasa grass, leading to a greater leaf appearance rate and a lower phyllochron value, while also increasing the number of live leaves (Table 1). The ability of *A. brasilense* strains to produce and modulate the level of endogenous phytohormones like auxins, gibberellins and cytokinins (Cassán *et al.* 2020) and arbuscular mycorrhizal fungi *R. intraradices* to assist in water and nutrient absorption (Begum *et al.* 2019), can stimulate the proliferation and elongation of plant cells, root elongation and stimulating the differentiation of meristematic tissues (Glick 2014; Souza *et al.* 2017), reflected in the improvement of the morphogenic characteristics. This indicates that the inoculants used can activate mechanisms that influence the generation and development of new leaves, resulting in a greater flow of tissue, increased interception efficiency, and enhanced conversion of luminous energy by Mombasa grass.

Considering combined or isolated inoculation, remarkable results were observed for the SPAD index and chlorophyll *a* (Table 2), translating into greater photosynthetic efficiency and consequently increased biomass production. Our findings confirm the hypothesis that the use of plant growth-promoting microorganisms results in greater nitrogen assimilation by plants compared with the non-inoculated plants; this underscores the positive impact of using these microorganisms in enhancing plant growth and nutrient assimilation processes.

The strategy of isolated inoculation with *A. brasilense* increased the dry biomass production of the aboveground part of Mombasa grass, representing a percentage increase of approximately 19% compared with the non-inoculated plants. Microbial bioinoculants have numerous positive functions associated with the absorption of essential elements for plant development. *A. brasilense* has the ability to synthesize auxins that stimulate development and improve the root system (Hungria *et al.* 2021; Guimarães *et al.* 2023), optimizing nutrient absorption by adding improvements in photosynthetic activity through the efficient assimilation of these elements, which favors productive capacity.

This signifies potential benefits for productive systems that rely on pasture renewal techniques. Positive gains in the biomass production of forage grasses associated with *A. brasilense* have been reported, as seen in species such as *U. ruziziensis* (Hungria *et al.* 2021), Mulato II grass (Rouseaux *et al.* 2020), Mombassa and Zuri grasses (Guimarães *et al.* 2023). These findings highlight the promising impact of microorganism-based strategies in enhancing biomass yield, which is particularly relevant for sustainable pasture management practices.

Improvement in forage quality is another crucial aspect of productive systems associated with microbial inoculant. The plant microorganism association can optimize fertilizer utilization through enhanced absorption de soil nutrient. The anticipation is that such advancements will contribute to more sustainable agricultural practices by promoting efficient nutrient use, thereby mitigating the environmental impacts associated with livestock production.

The results confirm the viability of using bioinoculants in the bromatological characteristics of Mombasa grass subjected to seed inoculation, as evidenced by the mineral content and crude protein values. This is particularly noticeable with the use of *A. brasilense*, which is characterized by a percentage increase of 5.25 and 13.41%, respectively, compared with non-inoculated plants. These improvements are attributed to the benefits of enhanced root growth, leading to increased nutrient absorption. The findings underscore the positive impact of seed inoculation with *A. brasilense* on the nutritional composition of Mombasa grass, highlighting its potential in optimizing the mineral and protein content of forage crops.

Strains of *A. brasilense* possess the capacity to synthesize phytohormones, primarily indole-3-acetic acid (IAA), which aids in root growth and optimizes the absorption of nutrients and water (Fukami *et al.* 2018; Cassán *et al.* 2020). Arbuscular mycorrhizal fungi, such as *R. intraradices*, can result in positive plant development. These fungi grow within the cortex cells and extend their hyphae into the soil, forming a mycelial network that acts as an extension of the roots, optimizing the absorption of water and nutrients (Smith and Read 2008). Therefore, plants with heavier roots and a larger root volume may indicate the potential for soil exploration, optimizing the absorption of essential elements for their development, which can contribute to the establishment and sustainability of the system.

Conclusion

These results suggested that single inoculation of *A. brasilense* or *R. intraradices* improved the initial growth of Mombasa grass. Data further revealed that dual inoculation with these microorganisms cooperated synergistically to stimulate root development and improved nutritional status. Therefore, the use of this microbial inoculant as an alternative is capable of optimizing the initial growth of Mombasa grass for sustainable production.

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Author Contributions

HSS: Formal analysis, Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft. TMV, NVS, ICD, BEFS, EMVP, JASJ and TPRS: Investigation, Methodology, Visualization. NTC: Writing – review and editing. DDF, RRJ and FAT: Supervision, Validation, Writing – review and editing. All the authors have read and agreed to the submitted version of the manuscript.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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