Running title: Improving the development of Mombasa grass using bioinputs

**Growth and development of *Megathyrsus maximus* cv. Mombasa affected by inoculation of plant growth-promoting microorganisms**

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**Novelty statement**

The use of plant growth-promoting microorganisms influences the development of Mombasa grass; Greater aboveground biomass production was attributed to inoculation with *A. brasilense*; Positive effects on the roots of Mombasa grass were identified when inoculated with *A. brasilense* isolated or in combination with *R. intraradices*; Non-inoculated plants exhibited reduced levels of total minerals and crude protein;

**Abstract**

*Megathyrsus maximus* holds significant importance in animal protein production in tropical regions, however inefficient pasture management remains a primary concern for the agricultural sector. In this context, the use of beneficial microorganisms for plants has emerged as a sustainable technology with the potential to modulate plant growth, ensuring the species remains in the grazing system and optimize the productivity. 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. The use of microorganisms affected the morphogenic parameters of Mombasa grass, resulting in a higher leaf appearance rate and a shorter time for the emergence of new leaves. The strategy of isolated inoculation with *A. brasilense* increased aboveground biomass production, representing a percentage increase of approximately 19% compared with non-inoculated plants. The presence of microorganisms 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. These gains are attributed to the root development of inoculated plants, which optimized nutrient and water absorption. *Azospirillum brasilense* and *Rhizophagus intraradices* positively contributed to the development of Mombasa grass, resulting in increased biomass production and improved nutritional status.

**Key words:** 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 poor livestock management practices.

The stress imposed on plants can be alleviated using sustainable tools, among which biological inputs or bioinputs play a significant role. These inputs improve and enhance the tolerance of plants to physiological disturbances caused by various stresses. Furthermore, the global market is becoming increasingly demanding, seeking efficient livestock production that employs sustainable technologies without harming the environment.

Among the bioinputs 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; Nehra; Yasmin et al., 2016), thereby providing plants with increased nutrient and water uptake.

The use of microbiological inoculants has proven promising in various crops, sparking interest in their application in pastures, particularly with grasses of the genus *Megathyrsus maximus* (Jacq.) B.K. Simon and S.W.L. Jacobs (syn. *Panicum maximum* Jacq.), as it represents one of the most cultivated genus (Veras et al., 2020). However, studies are needed to clarify and validate the use of bioinputs in pastures, aiding in the better adaptation of forage crops in challenging scenarios.

Therefore, the objective of this study was to evaluate the effect of isolated and combined inoculation of *Azospirillum brasilense* and *Rhizophagus intraradices* on Mombasa grass.

**Materials and methods**

**Experimental Details**

The study was carried out in a greenhouse at Forage and Pasture Department at State University of Southwest Bahia, in Itapetinga, Bahia (15º 14' S, 40º 14' W). During the period from October 2020 to January 2021. Weather data during the experimental period were obtained using a digital thermo-hygrometer. The average maximum and minimum temperature values were 39.4ºC 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 *Azospirillum brasilense* and *Rhizophagus intraradices*, in a completely randomized design with four treatments and four replications, totaling 16 experimental units (plastic pots), with a capacity of 12 L and, which were filled with 10 dm3 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 P2O5 in the form of simple superphosphate and 50 kg ha-1 of nitrogen in the form of urea.

**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 108 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. 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.

**Parameters analyzed**

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. (2012), 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 an 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**

Data from the evaluation periods were analyzed by to analysis of variance, rejecting the null hypothesis, the means were compared using Tukey’s test at a significance level with α = 0.05 with SAS software OnDemand for Academics programme.

**Results**

**Morphogenic and structural characteristics**

LAR, PHY, LER, LSR, and NLL were affected by the treatments tested (P < 0.001 for all). LAR, LER, and NLL were greater with *A. brasilense* and *R. intraradices* inoculated alone or in combination, whereas PHY and NLL were greater in control and co-inoculation. Conversely, 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 values were affected by the treatments tested (P < 0.001 for both), being 26% and 22% greater when inoculated with microorganisms than in the control. 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 (figure 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 control, 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 control treatment, with percentage increases of 5.25% and 13.41%, respectively. Conversely, the variables DM, NDF and ADF were not affected by the treatments tested (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 control (table 4).

**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 bioinputs employed has affected 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. The ability of *Azospirillum brasilense* strains to produce phytohormones (Cassán et al., 2020) and arbuscular mycorrhizal fungi *Rhizophagus intraradices* to assist in water and nutrient absorption (Begum et al., 2019) is reflected in the improvement of the morphogenic characteristics evaluated in this study. This indicates that the inoculants used can exert activities 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 control, 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 control. This signifies potential benefits for productive systems that rely on pasture renewal techniques. Positive gains in the biomass production of forage grasses associated with microorganisms have been reported, as seen in species such as *U. ruziziensis* (Hungria et al., 2021) and Mulato II grass (Rouseaux et al., 2020). 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 bioinputs. 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 bioinputs 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 (Cassán et al., 2020; Fukami et al., 2018). Arbuscular mycorrhizal fungi (AMF), 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**

This study demonstrates that the use of microbiological inoculants alters most of the morphogenic characteristics of Mombasa grass during its initial development, resulting in increased biomass production and improved nutritional status. These findings represent a promising alternative capable of optimizing production systems animal on pasture with reduced environmental impacts.

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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, LAV and TPRS:** Investigation, Methodology, Visualization. **NTC:** Conceptualization, Data curation, Methodology, 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 Interests**

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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**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.

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**Figure 1.** Fresh and shoot dry 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.

**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.