Cultivos Tropicales, 2020, vol. 41, no. 3, e03
ISSN impreso: 0258-5936
ISSN digital: 1819-4087
http://ediciones.inca.edu.cu
julio-septiembre
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas

Original article

Biofertilization with *Rhizoglomus irregularulare* and *Azospirillum brasilense* in *Brachiaria* hybrid cv. Mulato II

Pedro J. González-Cañizares[1*]

Reynerio Reyes-Rouseaux[2]

Delio Fernández-Milanés[2]

Ana Nelis San Juan-Rodríguez[3]

Daisy Dopico-Ramirez[3]

Juan F. Ramírez-Pedroso[2]

Luis R. Fundora-Sánchez[1]

[1]Instituto Nacional de Ciencias Agrícolas. Gaveta Postal No. 1 San José de las Lajas, Mayabeque. Cuba

[2]Instituto de Investigaciones de Pastos y Forrajes. Avenida Independencia Km 8 ½ Boyeros, La Habana, Cuba

[3]Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar

[*]Author for correspondence: pgonzalez@inca.edu.cu

ABSTRACT

An experiment was carried out with the objective of evaluating the effect of biofertilization with the plant growth promoting bacteria *Azospirillum brasilense* and the arbuscular mycorrhizal fungus *Rhizoglomus irregulare* in *Brachiaria* hybrid grass cv. Mulato II cultivated in a Mollic Carbonated Cambisol. It included four treatments formed by the inoculation, alone and in combination, of both microorganisms plus a control without inoculation, in a Latin Square design. The inoculum with *R. irregulare* had a concentration of 30 g^{-1}spores and was applied by the method of coating the seeds, while *A. Brasilense*, with a concentration of 10^9 CFU mL^{-1}, was applied by embedding the seeds at the time of planting, and at a rate of 20 L ha^{-1}after the first cut. Four cuts were made, at intervals of 60 and 90 days in the rainy and dry season, respectively. *R. irregulare* increased the frequency and intensity of mycorrhizal colonization, as well as the number of spores in the rhizosphere, although the greatest results were achieved when applied with *A. brasilense*. Biofertilizers separately increased the growth of the aerial and radical part of the plants, the nitrogen concentrations in the aerial biomass and the dry mass yield of the grass, but the higher values of these variables, as well as the greater contribution of the biofertilization in Nitrogen grass nutrition was obtained with its joint application. The effect of biofertilization was maintained for one year.

Key words: rhizobacteria, arbuscular mycorrhiza, growth, nutritional status, forage yields

INTRODUCTION

Pastures and forages are the cheapest sources of food for cattle, so obtaining high volumes of biomass of sufficient quality to meet the nutritional requirements of animals continues to be a first-rate need for the livestock sector.

The fertilization of pastures and fodder crops contributes to increasing the biomass supply per unit area and time, and consequently, animal production. In this way, not only are the nutrients extracted from the soil restored through the feed consumed by livestock, but their nutritional value and persistence are also improved [(1,2)].

Studies on pasture fertilization, until a few years ago, focused mainly on increasing biomass production per unit area. However, the increasing increase in fertilizer prices, together with the need for environmentally friendly technologies, imposes the design of fertilization strategies that guarantee adequate nutrition for these crops, decrease the use of external inputs, and at the same time, ensure the protection of natural resources [(3,4)].

Such strategies include biofertilization, due to its potential to increase the yields of agricultural crops, improve the biological properties of the soil and reduce the use of mineral fertilizers [(5,6)]; therefore, the obtaining and use of biofertilizers to increase the efficiency of the use of nutrients and promote the growth of plants continues to be studied.

The cultivation of forage species of the *Brachiaria* genus, specifically *B. hybrid* cv. Mulato II, has been spreading in tropical areas due to its high potential for biomass production and nutritional value for livestock feed; however, their fertilizer requirements to achieve high yields are usually high [(7)].

The use of associative bacteria of the genus *Azospirillum* has been shown to contribute to improving the performance of forage Poaceae. Not only for their ability to fix atmospheric nitrogen and their contribution to reducing the need for a supply of this nutrient via fertilization, but also for other benefits such as the production of phytohormones, the solubilization of phosphates, the biocontrol of pathogens and the protection of plants against abiotic stresses [8-10].

On the other hand, arbuscular mycorrhizal fungi (AMF) are integral components of the rhizosphere of grasses, whose plants remain closely associated through a network of interconnected hyphae that increase the volume of soil explored by the roots and facilitate the absorption of the nutrients and water [11,12]. In addition, to other services, such as increased tolerance to water stress, protection against pathogens, and restoration of photosynthetic tissues after defoliation [13]. In fact, the management of biofertilization with these edaphic microorganisms has also yielded positive results in increasing yields and improving nutrient absorption from pastures [14,15].

However, studies on the joint management of biofertilization based on associative bacteria and AMF in pastures and forage crops are not abundant, despite the possible advantages of the introduction of both microorganisms in the nutrient supply schemes of these crops [16,17].

Based on such premises, the present work was carried out, with the objective of evaluating the response of *Brachiaria hybrid* cv. 36087 (Mulato II) to the simple and combined application of biofertilizers based on the rhizobacterium *Azospirillum brasilense* and the species of AMF *Rhizoglomus irregulare*.

MATERIALS AND METHODS

An experiment was carried out in the Typical Vaquería 23 of the Basic Unit of Cooperative Production Juan Oramas, located in Guanabacoa municipality, Havana province, at 2º 08 'north latitude and 82º 11' west longitude, on a Brown soil Fluffy Carbonate [18], whose main chemical characteristics are presented in Table 1.

Table 1. Chemical characteristics of the soil (depth: 0-20 cm)

pH	OM	P_2O_5	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CIB
H_2O	(%)	(mg 100 g^{-1})	(cmol$_c$ kg^{-1})				
7.7	3.85	122	50.8	5.0	0.32	0.99	59.09
(0.1)	(0.20)	(18)	(2.4)	(0.9)	(0.09)	(0.12)	(3.75)

OM: organic material. CIB: base Exchange capacity. Values in parentheses indicate confidence intervals (α=0.05)

The soil had alkaline pH, medium and high contents of organic matter (OM) and assimilable phosphorus, respectively, as well as high base exchange capacity (CIB), highlighting within these, the high values of interchangeable Ca and K [(19)].

The behavior of rainfall and temperature during the period in which the experiment was conducted are shown in Figure 1.

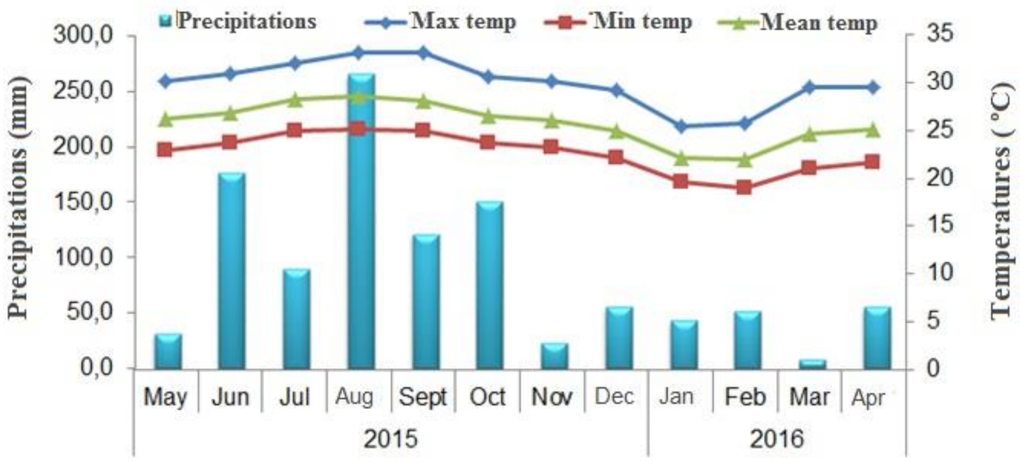


Figure 1. Behavior of rainfall and temperature during the run period of the experiment

During the time the experiment was conducted (May 2015-April 2016), the total precipitation was 1187 mm, of which 75 % occurred during the rainy period (May and October 2015) and the rest, between November 2015 and April 2016 [(20)].

Four treatments were evaluated, consisting of a control without biofertilization, and the single and combined applications of biofertilizers based on the rhizobacteria *A. brasilense* and the species of *R. irregulare* AMF, in a Latin square design. The plots constituted the experimental unit, and had a total area of 21 m^2 and a calculation area of 14 m^2.

The soil was prepared by mowing (plowing), harrowing, crossing (plowing) and harrowing, at intervals of approximately 25 days between each one. The sowing of the grass was carried out in May 2015, in rows 50 cm apart and by dripping, with a dose of 10 kg of total seed ha^{-1} (1 kg of pure germinable seed ha^{-1}) and at a depth of 1.5 cm. Before sowing, 10 soil samples were taken with an auger by the zigzag method, at a depth of 0-20 cm, to which the pH in H_2O was determined (potentiometry, soil-water ratio 1: 2.5). The contents of organic matter (Walkley and Black), assimilable P (extraction with H_2SO_4 0.05 mol L^{-1} and colorimetric determination), interchangeable cations [extraction with NH_4Ac 1 mol L^{-1} pH 7 and determination by complexometry (Ca and Mg) were determined. As well as, flame photometry (Na and K)], and the CIB (sum of interchangeable bases) were determined according to the analytical techniques established in the soil and plant laboratory of the National Institute of Agricultural Sciences (INCA) [21].

For biofertilization with *A. brasilense,* the commercial product Nitrofix®, from the Cuban Institute for Research on Sugar Cane Derivatives (ICIDCA) was used, which contained the 8I strain, with a concentration of 109 CFU ml^{-1}. A solution composed of a mixture of biofertilizer and water in a 1:10 ratio was prepared, in which the seeds were immersed for 15 minutes. Subsequently they were extracted and dried in the shade, and planting was carried out. 30 days after sowing, a mixture of the biofertilizer and water was prepared in the proportion already described, and it was applied to the soil, very close to the furrows, using a manual backpack at a rate of 20 L ha^{-1} of Nitrofix®.

For the application of the mycorrhizal biofertilizer, the INCAM-11 strain of the AMF species *Rhizoglomus irregulare* [22] from the INCA collection was used. The inoculum was multiplied in a clay substrate autoclaved at 120 °C for one hour for 3 days, with the use of *Brachiaria decumbens* cv. Basilisk as a host plant, and contained 30 spores per gram of substrate, as well as abundant fragments of rootlets and hyphae of the fungus.

The inoculation was carried out by the seed coating method, for which the seeds were immersed in a fluid paste, made by mixing a quantity of solid inoculum equivalent to 10 % of the weight of the seeds and water, in a proportion of 60 mL of water for every 100 g of inoculum. The seeds were after drying in the shade, sown. In the treatment where both biofertilizers applied together, the product containing *A. brasilense* was added to the water used to prepare the mycorrhizal inoculant for planting, maintaining the same inoculum-water ratio. Fertilizers were not applied in the experiment.

Four cuts were at a height of 10 cm made from the soil surface, the first two at 90 and 150 days after planting, both in the rainy season, and the others at 90-day intervals, coinciding with the least period rainy. In each cut, the fresh mass of the aerial part of the grass that occupied the calculation area of the plots was weighed and samples of 200 g were taken. These were taken to an air circulation stove at 70 ºC for 72 hours, to determine the percentage of dry mass, estimate the yield of dry mass, and the concentrations of N, P, K in the biomass [21].

At the time of each cut, at five points in the calculation area of each plot, the height of the canopy was measured using a graduated ruler, and the reading point was considered, the point where most of the leaves lost their vertical position. In the second and fourth cut, framed in the rainy and dry season, respectively, three sub-samples of roots and soil were taken from the rhizosphere at a depth of 0–20 cm, using a cylinder metallic 5 cm in diameter and 20 cm high. The sampling points were equidistant distributed and separated 10 cm from the grooves.

The subsamples were homogenized to form a sample composed of a plot, from which 1 g of rootlets was extracted for staining and clarification [(23)]. The frequency of mycorrhizal colonization was using the intercepts method evaluated [(24)], the visual density or intensity of colonization [(25)], and the number of spores in the rhizosphere, from the sieving and decantation of these structures by the wet route and its observation under a microscope [(26)].

Root density was determined just before the second cut; to do this, at three equidistant points located in the calculation area of each plot and 10 cm from the furrow, a vertical cut of the soil was made to a depth of 25 cm, and a metal cylinder 5 cm in diameter and 20 cm in diameter length was inserted. The soil contained in each cylinder was deposited in a glass container, running water was added and manually stirred until the coarse aggregates were dissolved, and then it was passed through a 0.5 mm sieve. The roots collected in the sieve were washed again to remove soil remains, air-dried and placed in a stove at 70 ºC until a constant mass was obtained.

Statistical data processing was performed using analysis of variance and Duncan's multiple range test [(27)] at $p<0.05$. In the variables corresponding to the chemical characterization of the soil, as well as in those whose results are shown in graphs, the confidence interval of the means ($\alpha=0.05$) was used as a dispersion statistic or as a criterion for comparison [(28)]. In all cases, the statistical program SPSS 25 was used [(29)].

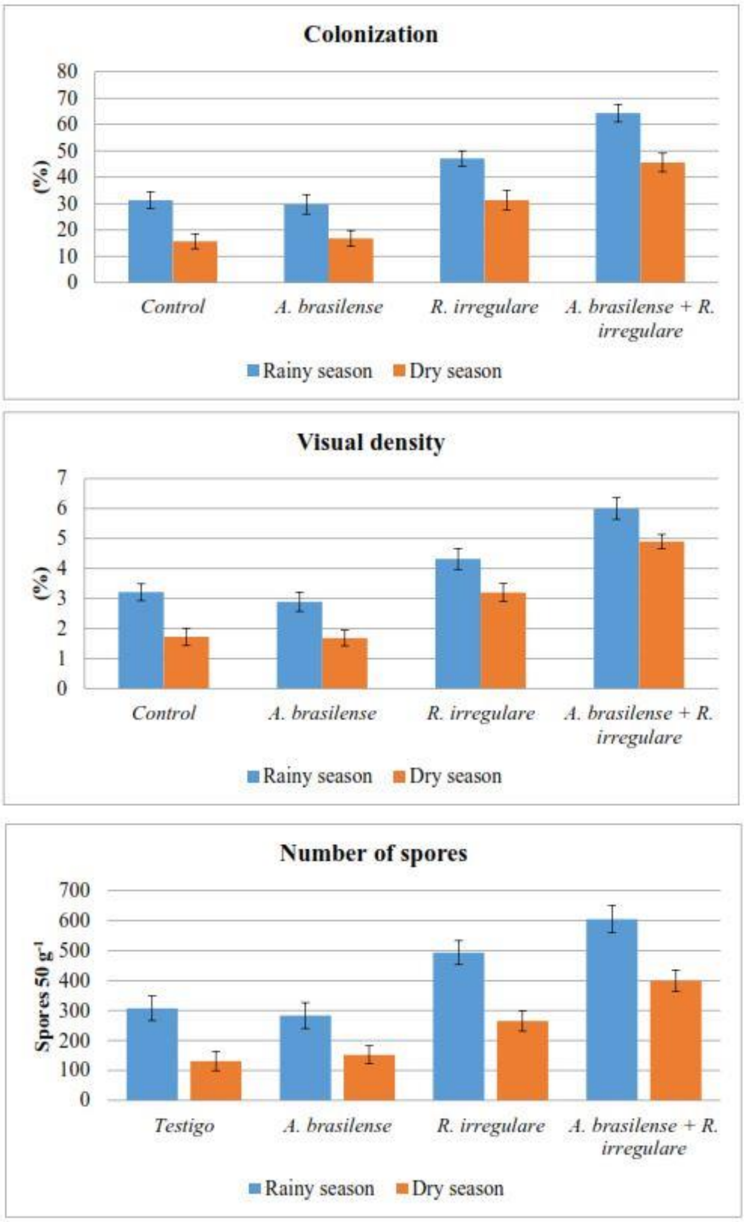
RESULTS AND DISCUSSION

Figure 2 shows the influence of treatments on fungal variables. Both in the rainy and in the rainy period, with the inoculation of *R. irregulare*, a significant increase in the frequency and intensity of mycorrhizal colonization (visual density) was found, as well as the number of spores in the rhizosphere. However, the greatest effects were observed with the coinoculation of *A. brasilense* and *R. irregular*. It can be attributed to a positive effect of the growth-stimulating substances produced by *A. brasilense* in increasing the length and branching of the roots and consequently, in the increase of colonization sites,

Cultivos Tropicales, 2020, vol. 41, no. 3, e03
ISSN impreso: 0258-5936
ISSN digital: 1819-4087
http://ediciones.inca.edu.cu
julio-septiembre
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas

as well as the stimulation of these substances in the formation and multiplication of mycorrhizal structures [16,30].

The values of the fungal variables were higher in the rainy period than in the dry period, probably due to the seasonality of the biomass production of the grass. During the rainy season, a rapid growth of the pastures occurs due to the higher levels of rainfall and temperature, as shown in Figure 1. Both factors, together with the increase in luminosity [31,32], lead to the absorption of a greater quantity of nutrients for the formation of biomass and consequently, the formation of greater amounts of mycorrhizal structures to guarantee the access of the plants to the soil resources [33,34].



Means with confidence intervals that overlap each other do not differ significantly (α=0.05)

Figure 2. Effect of treatments on fungal variables. The vertical bars show the confidence interval

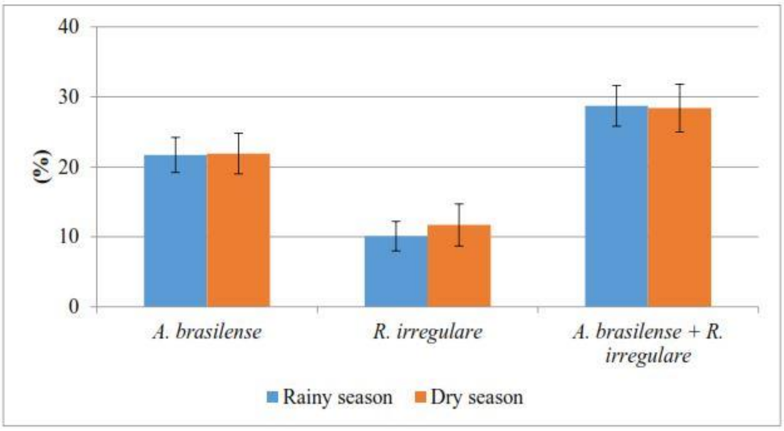
Table 2 shows the effect of biofertilization on macronutrient concentrations (N, P and K) in the pasture biomass of the pasture. Both in the rainy and in the rainy period, the separate application of both biofertilizers increased the concentrations of N in relation to the non-inoculated treatment (control), although between both treatments, the highest values corresponded to *A. brasilense* but the greatest effects were found with the joint application of biofertilizers.

Table 2. Effect of treatments on the concentrations (g kg^{-1}MS) of K, P and K in the aerial biomass

Treatments	Rainy season			Little rainy season		
	N	**P**	**K**	**N**	**P**	**K**
Control	11.2 d	2.3	17.3	12.1 d	2.6	18.5
A. brasilense	14.3 b	2.4	18.5	15.5 b	2.5	19.3
R. irregulare	12.7 c	2.3	17.4	13.7 c	2.4	18.7
A. brasilense + *R. irregulare*	15.7 a	2.5	18.2	16.9 a	2.5	19.5
ES	0.3**	0.1	0.2	0.3**	0.1	0.4

A. brasilense: inoculation with *Azospirillum brasilense; R. irregulare:* inoculation with *Rhizoglomus irregulare.* Averages with different letters in the same column differ significantly at p<0.05 (Duncan, 1955)

When evaluating the participation of biofertilizers in the increase of nitrogen concentrations in biomass (Figure 3), a similar result was observed; that is, the application of one or the other biofertilizer alone improved grass nutrition with this nutrient during both periods, although the highest values corresponded to treatment with *A. brasilense*. However, the greatest participation was achieved with the joint application of both biofertilizers.



Participation (%)=[(N concentration (g kg^{-1}) in the aerial biomass of the biofertilized treatment - N concentration (g kg^{-1}) of the control aerial biomass)/ N concentration (g kg^{-1}) of the above-ground biomass of the inoculated treatment] x 100

The vertical bars show the confidence interval. Means with confidence intervals that overlap each other do not differ significantly (α=0.05)

Figure 3. Participation of biofertilizers in increasing N concentrations in biomass

Forage poaceae require significant amounts of N to improve their yield and nutritional value [7,35], and judging by the organic matter content, the concentrations of N in the soil where the experiment was carried out were not sufficient to satisfy the demand of the crop. ; Furthermore, no nitrogen fertilizer was applied in the experiment. Furthermore, AMF has been shown to actively participate in the transfer of N from the soil to the host plant [36]. From these premises, a positive effect of inoculation with *R. irregulare* on the absorption of N, on the increase in the concentrations of this element in the above-ground biomass, as observed in Figure 3, could be expected, and consequently , an improvement of the nitrogen nutrition of the grass.

With respect to inoculation with *A. brasilense*, it is known that this microorganism can fix significant amounts of N in grass species of the *Brachiaria* genus [8,37], so that this could explain its greater effect in increasing concentrations of N in biomass and its greatest contribution to nitrogen nutrition, in relation to *R. irregulare*. However, the fact that both variables have reached the highest values with the treatment where the microorganisms were applied jointly reflects a complementary effect of the same in improving the nitrogen nutrition of the grass.

The treatments had no effect on the concentrations of P and K in the above-ground biomass, which was in correspondence with the high contents of both elements in the soil (Table 1); in fact, this was a criterion for deciding not to apply phosphoric or potassium fertilizer in the experiment.

Table 3 shows the effect of the treatments on some indicators of growth and dry mass DM yield of the aerial part of the grass. As in the variables previously analyzed, a significant effect of biofertilization was found on canopy height and DM yield, both in the rainy season and in the least rainy season, as well as on the grass root density. When comparing the results obtained with the application of these microorganisms separately, the inoculation with *A. brasilense* showed higher values than those achieved with *R. irregulare* in these variables, although the greatest effects were obtained with the joint application of both biofertilizers.

The effect of the treatments on the growth and yield of the pasture seems to be related to the influence of biofertilizers on the concentrations of N in the biomass of the aerial part of the plants. It is known that, of all the mineral nutrients, this element is, quantitatively, the most important for forage crops [7,38]. Thus, the improvement of nitrogen nutrition caused by the application of biofertilizers, as observed in the previous table could have favorably affected canopy height and root growth, and consequently, grass yield.

Table 3. Effect of treatments on canopy height, root density and dry mass (DM) yield of grass

Treatments	**Rainy season**			**Little rainy season**	
	Height (cm)	**Density of roots ($g\ m^{-2}$)**	**DM ($t\ ha^{-1}$)**	**Height (cm)**	**DM ($t\ ha^{-1}$)**
Control	42.3 d	522.7 d	9.02 d	19.9 d	3.13 d
A. brasilense	89.7 b	781.3 b	11.58 b	45.7 b	5.52 b
R. irregulare	64.5 c	653.5 c	10.23 c	32.2 c	4.34 c
A. brasilense + *R. irregulare*	110.5 a	927.8 a	12.74 a	59.3 a	6.33 a
ES	4.9**	27.1**	0.39**	2.0**	0.25**

A brasilense: inoculation with *Azospirillum brasilense; R. irregularulare:* inoculation with *R. irregularulare.* ES: standard error. Averages with different letters in the same column differ significantly at $p<0.05$ (Duncan´s test)

In the case of *A. brasilense*, in addition to its contribution to the biological fixation of N previously mentioned, this microorganism also exerts different growth stimulation mechanisms through the production of phytohormones, mainly indolacetic acid and gibberellins, which modifies the metabolism of plants and leads to better uptake of water and minerals [8,39]. This could also explain its greater influence on grass growth, in relation to *R. irregulare*.

With regard to AMF, several authors have found a positive effect on N concentrations in the biomass of the aerial part of pastures inoculated with efficient strains. It is from an increase in the use of this element, given both by the increase of the radical surface of plants; such as by the formation of mycorrhizal structures that allow access to this and other soil resources [36], which could be influencing the results, obtained.

It is interesting to note that the adding of *R. irregulare* has been effective in improving the concentrations of N in the biomass of the aerial part, the growth and the yield of the pasture, despite the high concentrations of P in the soil. Some works report that the effect of mycorrhizal inoculation can be in the presence of high contents of this element inhibited [40]. However, others have found that plants inoculated with efficient AMF strains can achieve effective mycorrhizal function even in soils with high concentrations of P, if other nutrients limit their growth and development [41].

CONCLUSIONS

- Biofertilization with *A. brasilense* and *R. irregulare* constitutes an effective alternative to improve nitrogen nutrition and yield of B. hybrid cv.
- Mulato II, at least during the first year of its application. The best benefits are obtained with the joint application of both microorganisms.
- It is suggested to carry out studies to evaluate its contribution to the reduction of the application of fertilizers, mainly nitrogenous ones.

BIBLIOGRAPHY

1. Vieira Teixeira RN, Eduardo Pereira C, Kikuti H, Borges Deminicis B. *Brachiaria brizantha* (Syn. *Uroclhoa brizantha*) cv. Marandu sob diferentes doses de nitrogênio e fósforo em Humaitá-AM, Brasil. Brazilian Journal of Applied Technology for Agricultural Science/Revista Brasileira de Tecnologia Aplicada nas Ciências Agrárias. 2018;11(2):35–41.
2. de Faria DA, Avelino ACD, Cabral CEA, de Abreu JG, de Barros LV, Cabral CHA, et al. Investigating the Optimal Day for Nitrogen Fertilization on Piatã palisadegrass and Quênia guineagrass after Defoliation. Journal of Experimental Agriculture International. 2019;34(6):1–11.
3. Richardson AE, Kirkby CA, Banerjee S, Kirkegaard JA. The inorganic nutrient cost of building soil carbon. Carbon Management. 2014;5(3):265–8.
4. Gupta G, Parihar SS, Ahirwar NK, Snehi SK, Singh V. Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J Microb Biochem Technol. 2015;7(2):96–102.

5. Mahanty T, Bhattacharjee S, Goswami M, Bhattacharyya P, Das B, Ghosh A, et al. Biofertilizers: a potential approach for sustainable agriculture development. Environmental Science and Pollution Research. 2017;24(4):3315–35.

6. Finkel OM, Castrillo G, Paredes SH, González IS, Dangl JL. Understanding and exploiting plant beneficial microbes. Current opinion in plant biology. 2017;38:155–63.

7. Marques DL, de Souza França AF, Oliveira LG, Arnhold E, Ferreira RN, Correa DS, et al. Production and chemical composition of hybrid *Brachiaria* cv. Mulato II under a system of cuts and nitrogen fertilization. Bioscience Journal. 2017;33(3):685–96.

8. Cassán F, Diaz-Zorita M. *Azospirillum sp.* in current agriculture: From the laboratory to the field. Soil Biology and Biochemistry. 2016;103:117–30.

9. Hungria M, Nogueira MA, Araujo RS. Inoculation of *Brachiaria* spp. with the plant growth-promoting bacterium *Azospirillum brasilense*: an environment-friendly component in the reclamation of degraded pastures in the tropics. Agriculture, Ecosystems & Environment. 2016;221:125–31.

10. Basu S, Rabara R, Negi S. Towards a better greener future-an alternative strategy using biofertilizers. I: Plant growth promoting bacteria. Plant Gene. 2017;12:43–9.

11. Motta PEF da, Siqueira JO, Ribeiro BT, Silva SHG, Poggere GC, Curi N. *Urochloa decumbens* growth and P uptake as affected by long-term phosphate fertilization, mycorrhizal inoculation and historical land use in contrasting Oxisols of the Brazilian Cerrado. Ciência e Agrotecnologia. 2017;41(2):209–19.

12. Jach-Smith LC, Jackson RD. N addition undermines N supplied by arbuscular mycorrhizal fungi to native perennial grasses. Soil Biology and Biochemistry. 2018;116:148–57.

13. Ambrosino ML, Busso CA, Cabello MN, Velázquez MS, Torres YA, Ithurrart LS, et al. Total and structure colonization by arbuscular mycorrhizal fungi in native, perennial grasses of different forage quality exposed to defoliation. Journal of King Saud University-Science. 2018;

14. Koziol L, Bever JD. The missing link in grassland restoration: arbuscular mycorrhizal fungi inoculation increases plant diversity and accelerates succession. Journal of Applied Ecology. 2017;54(5):1301–9.

15. Teutscherova N, Vazquez E, Arevalo A, Pulleman M, Rao I, Arango J. Differences in arbuscular mycorrhizal colonization and P acquisition between genotypes of the tropical *Brachiaria* grasses: is there a relation with BNI activity? Biology and Fertility of Soils. 2019;55(4):325–37.

16. Carina VT, Emilia MM, Miguel US, Ortiz DR, Manjunatha B, Thangaswamy S, et al. Effect of Arbuscular mycorrhizal fungi (AMF) and *Azospirillum* on growth and nutrition of banana plantlets during acclimatization phase. Journal of Applied Pharmaceutical Science. 2016;6(06):131–8.

17. Fukami J, Cerezini P, Hungria M. *Azospirillum*: benefits that go far beyond biological nitrogen fixation. AMB Express. 2018;8(1):73.

18. Hernández JA, Pérez JJM, Bosch ID, Castro SN. Clasificación de los suelos de Cuba 2015. Mayabeque, Cuba: Ediciones INCA. 2015;93:91.

19. Paneque VM, Calaña JM. La fertilización de los cultivos. Aspectos teórico-prácticos para su recomendación. Folleto impreso. Curso de postgrado. Instituto Nacional de Ciencias Agrícolas. Departamento de biofertilizantes y nutrición de las plantas. San José de las Lajas, La Habana. 2001;28.

20. INSMET. Boletín Agrometeorológico. 2018.

21. Paneque-Pérez VM, Calaña JM, Calderón M, Borges Y, Hernández T, Caruncho M. Manual de técnicas analíticas para análisis de suelo, foliar, abonos orgánicos y fertilizantes químicos. Ediciones INCA; 2010.

22. Sieverding E, da Silva GA, Berndt R, Oehl F. *Rhizoglomus*, a new genus of the Glomeraceae. Mycotaxon. 2014;129(2):373–86.

23. Rodríguez Yon Jy, Arias Pérez L, Medina Carmona A, Mujica Pérez Y, Medina García LR, Fernández Suárez K, et al. Alternativa de la técnica de tinción para determinar la colonización micorrízica. Cultivos Tropicales. 2015;36(2):18–21.

24. Giovannetti M, Mosse B. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New phytologist. 1980;489–500.

25. Trouvelot A, Kough JL, Gianinazzi-Pearson V. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthode d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson V, Gianinazzi S, Eds, Physiological and Genetical Aspects of Mycorrhizae. INRA: Paris, 1986; 217-221.

26. Herrera RA, Ferrer RL, Furrazola E, Orozco MO. Estrategia de funcionamiento de las micorrizas VA en un bosque tropical. Biodiversidad en Iberoamérica. Ecosistemas, evolución y procesos sociales. Programa Iberoamericano de Ciencia y Tecnología para el desarrollo. Subprograma, Diversidad Biológica. Mérida. 1995.

27. Duncan DB. Multiple Ranges and Multiple F. test Biometrics. Biometrics. 1955;11:1.

28. Payton ME, Miller AE, Raun WR. Testing statistical hypotheses using standard error bars and confidence intervals. Communications in Soil Science and Plant Analysis. 2000;31(5–6):547–51.

29. SPSS. Statistical software, version 25. SPSS. 2017.

30. Domínguez-Núñez JA, Berrocal-Lobo M, Albanesi AS. Interaction of *Azospirillum* and mycorrhiza. In: Handbook for *Azospirillum*. Springer; 2015. p. 419–32.

31. Araujo LC de, Santos PM, Rodriguez D, Pezzopane JRM. Key factors that influence for seasonal production of Guinea grass. Scientia Agricola. 2018;75(3):191–6.

32. Pezzopane JRM, Santos PM, da Cruz PG, Bosi C, Sentelhas PC. An integrated agrometeorological model to simulate Marandu palisade grass productivity. Field Crops Research. 2018;224:13–21.

33. Soudzilovskaia NA, Douma JC, Akhmetzhanova AA, van Bodegom PM, Cornwell WK, Moens EJ, et al. Global patterns of plant root colonization intensity by mycorrhizal fungi explained by climate and soil chemistry. Global Ecology and Biogeography. 2015;24(3):371–82.

34. Adil S, Muneer MA, Imran M, Munir MZ. Seasonality of arbuscular mycorrhiza and dark septate endophytes in some grasses under arid climatic conditions. J. Agric. Res. 2017;55(4):601–10.

35. Machado SLM, de Sales ECJ, Reis ST, Mesquita VG, Carvalho ZG, Monção FP, et al. Forage accumulation, tillering and bromatological characteristics of *Brachiaria* grass under nitrogen fertilization. Científica. 2017;45(2):197–203.

36. Bücking H, Kafle A. Role of arbuscular mycorrhizal fungi in the nitrogen uptake of plants: current knowledge and research gaps. Agronomy. 2015;5(4):587–612.

37. Parreira LH, Martins ME, Ribeiro MM, Sena-Júnior J. Efeito da bactéria *Azospirillum brasilense* na adubação química e orgânica em pastagens constituídas de *Brachiaria brizantha* cv. Marandu. Em: Enciclopedia Biosfera. 2015;11(21):838.

38. Franco MY, Neves MF, Sobrinho CDJ, Santos SR, Pereira ML, Santos JMA. Adubação nitrogenada em pastagem de *Urochoa brizantha* cv. Paiaguás: Recuperação de nitrogênio e eficiencia na produção de forragem. 2015.

39. Karimi N, Zarea MJ, Mehnaz S. Endophytic *Azospirillum* for enhancement of growth and yield of wheat. Environmental Sustainability. 2018;1(2):149–58.

40. Ji B, Bever JD. Plant preferential allocation and fungal reward decline with soil phosphorus: implications for mycorrhizal mutualism. Ecosphere. 2016;7(5):e01256.

41. Mei L, Yang X, Zhang S, Zhang T, Guo J. Arbuscular mycorrhizal fungi alleviate phosphorus limitation by reducing plant N: P ratios under warming and nitrogen addition in a temperate meadow ecosystem. Science of the total environment. 2019;686:1129–39.