

## Inoculation of *Andropogon gayanus* Kunth with *Glomus cubense* and *Azospirillum brasilense* in the presence of drought stress

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### Abstract

**Objective:** To evaluate the response of *Andropogon gayanus* Kunth to inoculation with *Glomus cubense* and *Azospirillum brasilense*, in the presence of drought stress.

**Materials and Methods:** In an established area of *A. gayanus*, an establishment cutting was performed after seed harvest and inoculation was done with EcoMic® (*Glomus cubense*) and INICA-8. The experimental design was a complete randomized block, with seven treatments and eight replicas. The treatments consisted of: EcoMic® immediately after cutting, INICA-8 immediately after cutting, INICA-8 + EcoMic® immediately after cutting, EcoMic® at cutting+ INICA-8 (15 days), INICA-8 at cutting + EcoMic® (15 days), absolute control and fertilized control (150 kg N/ha, NH<sub>3</sub>NO<sub>4</sub>). The dry weight of the aerial part, stem length, length of the flowering section of the stem, length of the inflorescence, dry weight of the racemes and flowering were evaluated. Variance analysis was performed. Differences among means were determined by Fisher's LSD.

**Results:** Area dry weight was higher ( $p < 0,0001$ ) in the fertilized control than in all treatments (12 036,1 kg/ha). The EcoMic® (9 612,5 kg/ha), EcoMic®+INICA-8 (8 475,0 kg/ha) and EcoMic® (cutting)+INICA-8 (15 days) treatments were superior to the absolute control (6 822,2 kg/ha), as well as to the other inoculated treatments. The EcoMic®+INICA-8 treatment showed statistical superiority in stem length (189,7 cm), raceme dry weight (0,071 g) and flowering (0,4462 %). EcoMic® (cutting)+INICA-8 (15 days) was higher in inflorescence length (16,39 cm) and in length of the flowering section of the stem (56,08 cm), although the fertilized control did not differ from these treatments.

**Conclusions:** The single application of INICA-8 had no higher effect on any of the studied variables. However, EcoMic®, at the time of cutting, alone or combined with *Azospirillum*, was determinant in most of the variables. These results prove the importance of the application of EcoMic® in this crop under drought stress conditions, as well as the synergy among the microorganisms that were inoculated, when combined with each other due to a higher effect on the plant.

**Key words:** Gramineae, microorganisms, synergism

### Introduction

Water deficit (drought) stress is one of the most common and with the highest impact on agriculture, because it generates, together with other abiotic stresses, decreases close to 50 % in the productivity of different crops of agro-economic interest (Sharma *et al.*, 2019; Ullah *et al.*, 2019). This environmental stressor can provoke a wide range of highly complicated morphological, anatomical, biochemical and molecular responses in plants, such as decreased plant water use efficiency, reduced transpiration and photosynthesis, production of reactive oxygen species (ROS), damaged cell membranes, and others, leading to a drop in crop yields (Takahashi *et al.*, 2020). One of the options to guarantee acceptable yields in grass during

the dry season is the use of species resistant to environmental stress. *Andropogon gayanus* (Kunth) is considered an important forage species for the tropics, since this grass is extensively cultivated in arid and semi-arid ecosystems in several countries, as it is able to develop and grow under drought stress and wet conditions (Funes *et al.*, 1998).

Among the strategies aimed at ensuring adequate crop nutrition is the management of mycorrhizal-arbuscular symbiosis, due to its potentialities to improve crop productivity and, at the same time, reduce the need for fertilizers, since plants yield to mycorrhizal-arbuscular fungi (AMF) carbonaceous compounds from photosynthesis; while they transfer to the plant mineral nutrients, especially the less accessible ones (Ezawa and Saito, 2018).

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AMF are considered the most abundant soil fungi in agricultural systems, where they can account for more than 30 % of their microbial mass. Because of that ubiquity, mycorrhizal symbiosis has been considered the most important of all those involving plants (Gutjahr and Parniske, 2017). Some studies indicate that inoculation with plant growth-promoting rhizobacteria (PGPR) can modulate key morphological and biochemical processes to mitigate drought stress in plants (Jochum *et al.*, 2019; Yaseen *et al.*, 2020). *Azospirillum brasilense* is proven to possess multiple plant growth-promoting properties, such as: N<sub>2</sub> fixation, 1-Amino-Cyclopropane-1-Carboxylate (ACC)-deaminase activity, P solubilization, and phytohormone production, among others (Osman *et al.*, 2020).

Therefore, the objective of this study was to evaluate the response of *A. gayanus* to inoculation with *Glomus cubense* and *A. brasilense*, in the presence of drought stress.

### Materials and Methods

*Location of the experiment.* The experiment was initiated on April 29, 2018, and evaluated on September 24 of the same year (148 days), at the Sancti Spiritus Pastures and Forages Research Station, located at 21° 53' 00" North latitude and 79° 21' 25" West longitude, altitude of 40 m.a.s.l.

*Basic agrochemical composition of the experimental soil.* The soil of the experimental area is classified as loose carbonate brown (Hernández *et al.*, 2015). The macronutrient content was very low in phosphorus and potassium and organic matter (table 1).

*Temperature and relative humidity.* The highest average temperatures during the experimental

period occurred between June and September (table 2); while there was a predominance of high relative humidity, especially in May, August and September.

*Rainfall.* Figure 1 shows the rainfall for 2018. In the experimental period, it very low in April (105,2 mm), June (84,6 mm) and September (102,3 mm). Except for April and May, in the other months it was below the historical average. In May, despite a high rainfall total, in the first two tens it was very low (Centro Meteorológico Provincial, 2019). In these first two months (April to the second ten of May), important phenological stages of the experimental crop occurred (from germination to the incipient development of the plant), which coincided with very low rainfall.

*State of the agricultural drought.* The intensity of agricultural drought during the time of the experiment (table 3) varied from severe (third ten of April, first and second ten of May), moderate (second and third ten of July and first ten of August), light (third ten of May, month of June, first ten of July and second ten of August), and the rest was considered very light. This data indicates that the experiment, in general, took place under conditions of agricultural drought, which was stressed in the above-mentioned tens of April, May, July and August (Centro Meteorológico Provincial, 2019).

The tens of higher drought intensity corresponded to the experimental set-up period, with the regrowth and initial development of the plants. Therefore, it is inferred that the grass, like the microorganisms that were inoculated, were subject to considerable water stress during that space of time.

Table 1. Basic soil characteristics of the experimental site.

Soil type	P <sub>2</sub> O <sub>5</sub> , mg/100g	K <sub>2</sub> O, mg/100g	OM, %	pH (KCl)
Loose carbonate brown	2,63	6,0	1,51	5,9

Table 2. Average temperature and relative humidity values.

Month	Average temperature, °C	Average humidity, %
April	24,3	78
May	24,6	86
June	26,4	79
July	27,2	77
August	26,7	80
September	26,3	83

Source: Centro Meteorológico Provincial (2019).

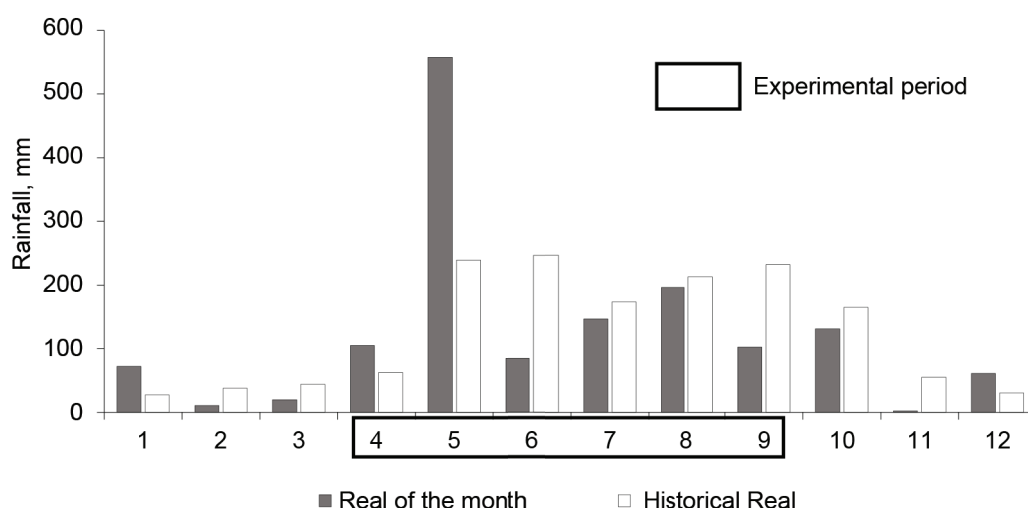


Figure 1. Rainfall occurred in 2018.

Source: Centro Meteorológico Provincial (2019). Sancti Spiritus Meteorological Station, located 500 m away from the experimental area.

Table 3. Category of the intensity of agricultural drought in the Sancti Spiritus Pastures and Forages Research Station.

Month/ten	Category of intensity of agricultural drought	Key
April 03	4	Severe drought
May 01	4	Severe drought
May 02	4	Severe drought
May 03	2	Slight drought
June 01	2	Slight drought
June 02	2	Slight drought
June 03	2	Slight drought
July 01	2	Slight drought
July 02	3	Moderate drought
July 03	3	Moderate drought
August 01	3	Moderate drought
August 02	2	Slight drought
August 03	1	Very slight drought
September 01	1	Very slight drought
September 02	1	Very slight drought
September 03	1	Very slight drought

Source: Centro Meteorológico Provincial (2019)

**Plant material.** *A. gayanus* Kunth (Poaceae), from the germplasm bank of the Sancti Spiritus Research Station, was evaluated.

**Experimental design.** A complete randomized block experimental design was applied (Melo *et al.*, 2020) with seven treatments and eight replicas (table 4). The plots measured 1 x 5 m (5 m<sup>2</sup>) and the distance between plots was 1 m. The net experimental area was 16 x 42 m (672 m<sup>2</sup>).

**Bacterial strain.** The strain INICA-8 of *A. brasilense*, provided by the Pastures and Forages Research Institute (IIPF, for its initials in Spanish), was used. According to the manufacturer's recommendations, the preparation was diluted in common water at a ratio of 1:10. Inoculation was carried out immediately after the establishment cutting (or 15 days after the cutting, as appropriate),

Table 4. Treatments used in the experiment.

No.	Treatment
T1	EcoMic® (immediately after cutting)
T2	INICA-8 (immediately after cutting)
T3	INICA-8+ EcoMic® (immediately after cutting)
T4	EcoMic® (immediately after cutting) + INICA-8 (15 days after cutting)
T5	INICA-8 (immediately after cutting) + EcoMic® (15 day after cutting)
T6	Absolute control (AC)
T7	Fertilized control (FC) with 150 kg N/ha (NH <sub>3</sub> NO <sub>4</sub> )

with an inoculant with a cell concentration of  $10^9$ - $10^{10}$  CFU/mL. A 1-L graduated burette was used, the contents of which were poured over the freshly cut tillers (or 15 days after cutting, so that, by regulating the jet, each tiller received about 125 mL of the liquid inoculant (40 L/ha).

*Arbuscular mycorrhizal fungi strain.* The product EcoMic®, based on *G. cubense*, provided by LABIOFAM<sup>1</sup> Sancti Spiritus, containing 30 spores/g of substrate, was used. According to the manufacturer's recommendations, the preparation was diluted in common water, in a ratio of 1:10. It was inoculated by applying the aqueous dilution on the freshly cut tillers (or 15 days after cutting, depending on the treatment), at a rate of 125 mL/tiller, with a dose equivalent to 50 kg/ha.

*Management of the experiment.* The experiment was carried out in a previously established area of 20 x 46 m (920 m<sup>2</sup>), intended for the production of *Andropogon* seed. A cutting and cultivation with oxen was carried out after seed harvest, to proceed immediately to inoculation, and no irrigation was applied during the experimental period.

*Determination of agricultural drought status.* The agricultural drought status was determined through the aridity index or agricultural drought index (IE) (Solano and Vázquez, 1999), which was used to check whether the experiment was carried out under water stress conditions:

$$IE = ETR / ETP$$

where:

ETR-Estimated actual evapotranspiration, dependent on soil moisture status. ETP- Estimated potential evapotranspiration, dependent on atmospheric conditions.

When ETR=ETP, soil water supply is adequate.

When ETR<ETP, there is insufficient water.

*Variables.* Aerial part dry weight (APDW, kg/ha; extrapolated). The aerial biomass, composed of leaves, stems, reproductive organs and senescent material, was weighed. It was previously dried at 60 °C for 48 hours in an oven. Stem length (SL, cm) was measured from the soil surface to the insertion of the first inflorescence. The length of the flowering section of the stem (LFSS, cm) was measured from the insertion of the first inflorescence to the insertion of the terminal inflorescence. For inflorescence length (LInfl., cm), the length of the peduncle was measured together with the length of the spikelets. For raceme dry weight (DWRAC, g), bunches with mature spikelets were weighed and dried at 60 °C for 48 hours in an oven and flowering (flower, %). The number of flowering stems was counted and the percent of the total was calculated.

Inoculation efficiency index (IEI, %), according to the formula proposed by Santillana *et al.* (2012):

$$IEI: [(inoculated\ treatment - absolute\ control) / absolute\ control] \times 100.$$

*Statistical analysis.* Variance analysis was performed. Differences among means were determined by Fisher's LSD. Percentage data were transformed by  $\sqrt{\arcsin(P)}$  to ensure fulfillment of assumptions (López and González, 2016). The statistical program StatGraphics Centurion XV (STATGRAPHICS Centurion, 2007) was used.

## Results and Discussion

*Dry weight of the aerial part.* This variable (fig. 2) was characterized by the superiority ( $p < 0,0001$ ) of the values of the fertilized control over all treatments (12 036,1 kg/ha). The EcoMic® (9 612,5 kg/ha), EcoMic®+INICA-8 (8 475,0 kg/ha) and EcoMic®(cutting)+INICA-8 (15 days) treatments were higher than the absolute control (6 822,2 kg/ha), as well as than the other inoculated treatments.

<sup>1</sup> Biopharmaceuticals and Chemicals Production Business Group.

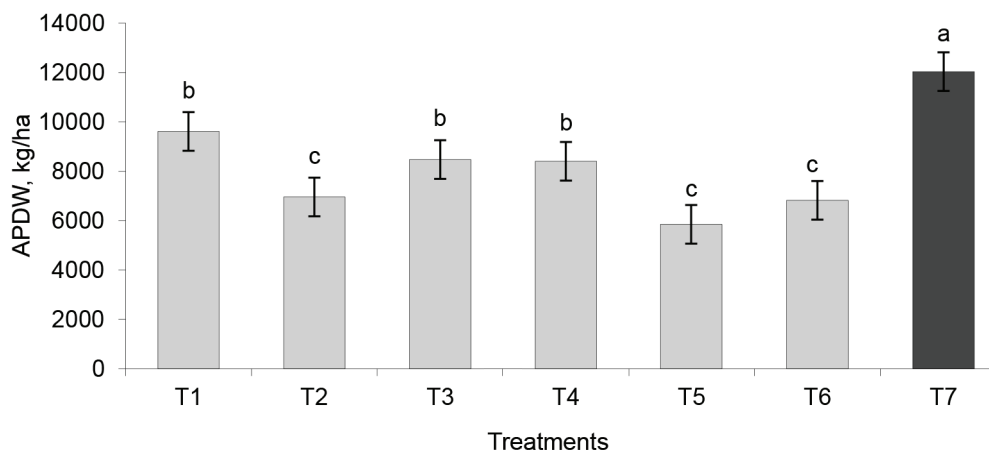


Figure 2. Effect of treatments on aerial part dry weight (APDW) of *A. gayanus*.

Data from one cutting (extrapolated data).

a, b and c: values with different superscripts differ at  $p < 0,0001$

T1-EcoMic® (immediately after cutting), T2-INICA-8 (immediately after cutting), T3-INICA-8+ EcoMic® (immediately after cutting), T4-EcoMic® (immediately after cutting) + INICA-8 (15 days after cutting), T5-INICA-8 (immediately after cutting) + EcoMic® (15 days after cutting), T6-Absolute control and T7-Control fertilized with 150 kg N/ha ( $\text{NH}_3\text{NO}_3$ ).

The total values in APDW were higher than those obtained by Reyes-Pérez *et al.* (2018), for *A. gayanus* in 75 days of cultivation, but similar to those reported by Argel *et al.* (2007), for *Brachiaria hibrido* cv Mulato II, in 105 days of cultivation. According to the results for this variable, since the experiment was conducted under agricultural drought conditions, it cannot be ruled out that one of the factors that favored plant tolerance to water stress was the use of *Azospirillum* in the corresponding microbial combinations.

Tiwari *et al.* (2018) stated that many rhizobacteria contain the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which splits the ethylene precursor ACC into  $\alpha$ -ketobutyrate and ammonium. It therefore reduces ethylene levels in drought-stressed plants. This allows the root system to develop without the inhibition of this compound, which favors higher nutrient absorption by the plant and, therefore, its higher development.

There was a superior effect in the single application of *G. cubense*, as well as in its inoculation immediately after the establishment cutting, followed by inoculation with *Azospirillum*. It is possible that the formation of mycorrhizae in the plant roots through inoculation with arbuscular mycorrhizal fungi (AMF) facilitated the subsequent entry of the bacteria into the root tissue, where the production of their metabolites would have a higher effect on the plant.

Bécquer *et al.* (2019) obtained higher values of aerial biomass of *Cenchrus ciliaris* L., compared with the absolute control, by inoculating the rhizobacterium *Bradyrhizobium* sp., and *Funneliformis mosseae*, under water stress conditions. Djonova *et al.* (2016) found that the combination of AMF and *A. brasilense* led to higher values of aerial biomass in the grasses *Festuca arundinacea* (Schreber) and *Phleum pretense* L.

When calculating the IEI on the basis of PSPA (fig. 3), it was observed that the treatment inoculated with EcoMic® was the most efficient, followed by EcoMic®+INICA-8 and EcoMic® (cutting)+INICA-8(15 days); while it was insignificant in INICA-8, and null in INICA-8(cutting) + EcoMic® (15 days).

**Stem length.** The treatment inoculated with EcoMic® was higher ( $p < 0.001$ ) than the absolute control, than INICA-8, than EcoMic® (cutting)+INICA-8 (15 days after cutting) and than INICA-8 (cutting)+EcoMic® (15 days after cutting), although it did not differ from the fertilized control and EcoMic®+INICA-8 (fig. 4).

These results agree with those obtained by Díaz-Franco *et al.* (2008), who observed that the simple application of *Glomus intraradices* on sorghum had a higher effect on plant height than the application of *A. brasilense*.

Castillo-Pacheco *et al.* (2018), in experiments with tomato, observed that stem length increased

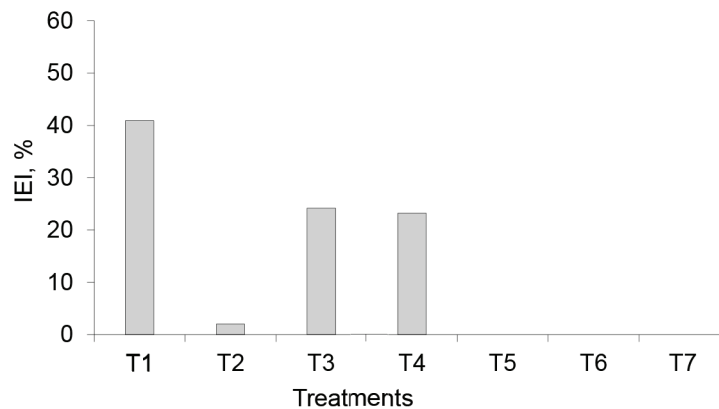


Figure 3. Inoculation efficiency index based on APDW in *A. gayanus*, in one cutting.

T1-EcoMic® (immediately after cutting), T2-INICA-8 (immediately after cutting), T3-INICA-8+ EcoMic® (immediately after cutting), T4-EcoMic® (immediately after cutting) + INICA-8 (15 days after cutting), T5-INICA-8 (immediately after cutting) + EcoMic® (15 days after cutting), T6-Absolute control and T7-Control fertilized with 150 kg N/ha ( $\text{NH}_3\text{NO}_4$ ).

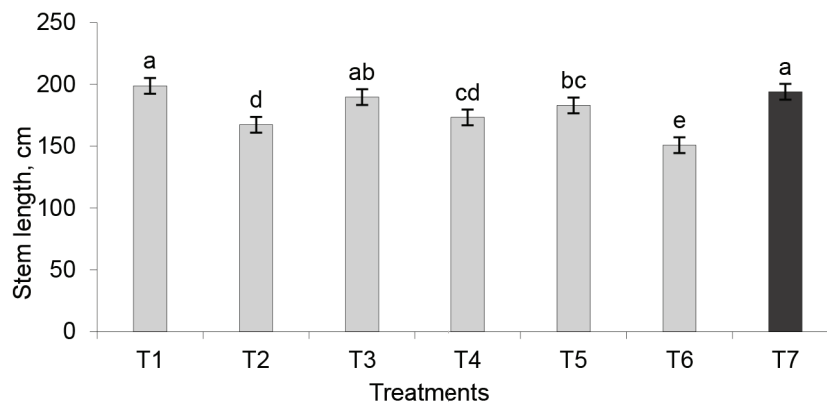


Figure 4. Effect of *A. gayanus* inoculation on stem length.

a, b and c: values with different superscripts differ at  $p < 0,001$

T1-EcoMic® (immediately after cutting), T2-INICA-8 (immediately after cutting), T3-INICA-8 + EcoMic® (immediately after cutting), T4-EcoMic® (immediately after cutting) + INICA-8 (15 days after cutting), T5-INICA-8 (immediately after cutting) + EcoMic® (15 days after cutting), T6-Absolute control and T7-Control fertilized with 150 kg N/ha ( $\text{NH}_3\text{NO}_4$ ).

more with the application of *G. cubense*, than with other species of that genus.

Sanclemente-Reyes *et al.* (2018) consider that through the symbiosis established between AMF and plants, the absorption of some nutrients of difficult mobility in the soil (P, Fe, among others) is increased, due to the action of the external mycelium of AMF, attached to the root systems of plants, higher absorption of water, N, K and some micronutrients.

Several studies have shown that communities of various bacteria associated with AMF enhance

mycorrhization and plant growth (Agnolucci *et al.*, 2015), which also applies to the present experiment, being the combination of *G. cubense* with *A. brasilense* at the time of cutting, one of the treatments that showed statistical superiority.

Mohamed and Massoud (2017), when inoculating a combination of *Azotobacter* and AMF on orange, observed a synergism that was evident in plant growth, N, P and K extraction in leaves, and yield. Bécquer *et al.* (2021) found that the combined inoculation of *G. cubense* and *A. brasilense* (strain INICA-8) exerted a superior effect on the studied



variables, especially on stem length and number of branches. However, Sánchez-de-la-Cruz *et al.* (2008) found that, in greenhouse experiments, single inoculation of *G. intraradices* as well as *A. brasilense* increased plant height in wheat, but the combination of these microorganisms had no detectable effect on the studied variables.

*Length of the flowering section of the stem.* In this variable, the effect on the plant of the combined application of the biofertilizers was corroborated, although only the combined treatment EcoMic® (cutting) + INICA-8 (15 days after cutting) was superior ( $p < 0,01$ ) to the single application of EcoMic®, to INICA-8 and to the absolute control. EcoMic® (cutting) + INICA-8 (15 days after cutting) did not differ from INICA-8 (immediately after cutting) + EcoMic® (15 days after cutting), with EcoMic+INICA-8, as well as with the fertilized control. These last three treatments, in turn, were statistically similar to the absolute control (fig. 5).

The initial application of AMF and subsequent application of *Azospirillum* through the product INICA-8 influenced the positive effect of this treatment. AMF are known to be integral components of the rhizosphere, whose plants remain closely associated through a network of interconnected hyphae that increase the volume of soil explored by roots, improve its structure, and facilitate nutrient and water uptake, among other functions (Motta *et al.*, 2017; Busso and Fernandez,

2018). Omar *et al.* (2017) reported that *A. brasilense* increases the availability of nutrients to the plant and modulates the level of hormones in plants, because it synthesizes indoleacetic acid (IAA), gibberellic acid (GA3) and abscisic acid (ABA). It also decreases acetylene production in plants via the ACC-desaminase pathway (Renoud *et al.*, 2022). Tiwari *et al.* (2019) claim that *Azospirillum* is a rhizobacterium capable of counteracting the effect of drought on plants through its plant growth promotion mechanisms.

The AMF-bacteria interaction increases the colonization by both microorganisms in the host. On the one hand, germination and hyphal growth of endophytes is increased and, on the other hand, the bacterial population in the plant rhizosphere is increased in the presence of AMF (Long *et al.*, 2017).

Reyes-Rouseaux *et al.* (2020), by combined inoculation of *Azospirillum* and AMF in *Urochloa hibrido* cv. Mulato II, without nitrogen application, observed that the agroproductive studied indicators reached similar values to those recorded with the addition of 100 kg N/ha.

*Inflorescence length.* The treatments EcoMic® (cutting) + INICA-8 (15 days) (16,39 cm) and INICA-8 (cutting)+ EcoMic® (15 days) were higher ( $p < 0,05$ ) than the absolute control, EcoMic® and INICA-8. In addition, they were similar to the fertilized control and EcoMic®+INICA-8 (fig. 6).

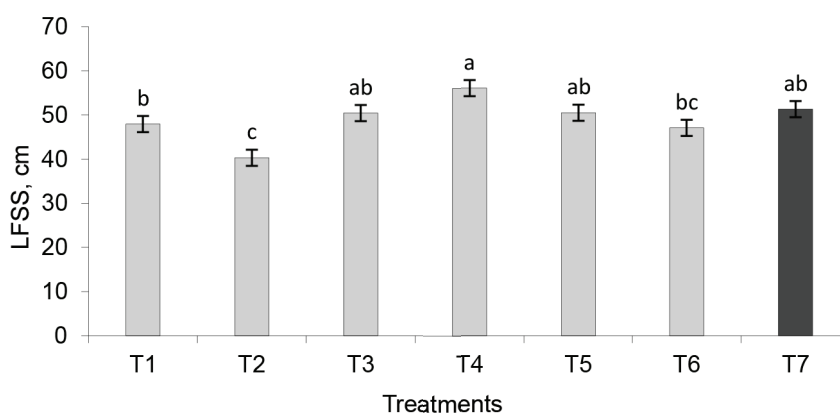


Figure 5. Effect of *A. gayanus* inoculation on the length of the flowering stem section (LFSS).

a, b and c: values with different superscripts differ at  $p < 0,01$

T1-EcoMic® (immediately after cutting), T2-INICA-8 (immediately after cutting), T3-INICA-8+ EcoMic® (immediately after cutting), T4-EcoMic® (immediately after cutting) + INICA-8 (15 days after cutting), T5-INICA-8 (immediately after cutting) + EcoMic® (15 days after cutting), T6-Absolute control (AC) and T7-Control fertilized (FC) with 150 kg N/ha ( $\text{NH}_3\text{NO}_3$ ).

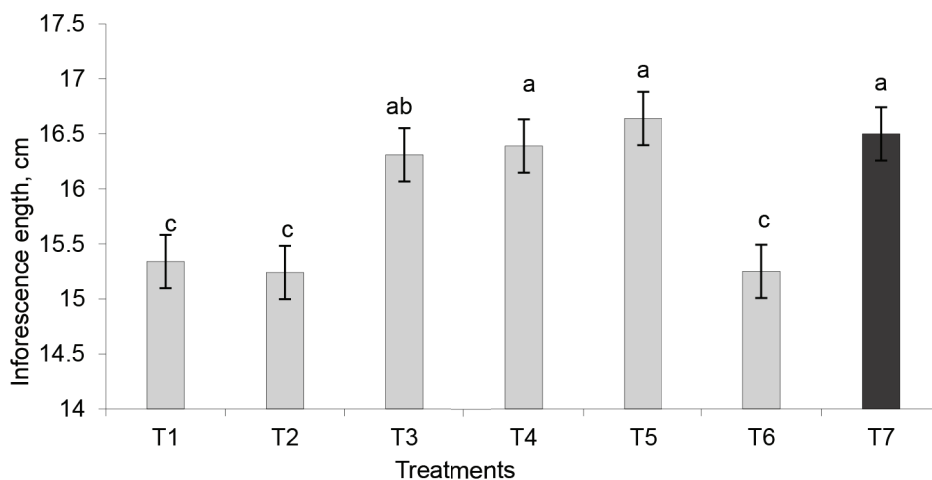


Figure 6. Effect of *A. gayanus* inoculation on inflorescence length.

a, b and c: values with non-common superscripts differ at  $p < 0,05$

T1-EcoMic® (immediately after cutting), T2-INICA-8 (immediately after cutting), T3-INICA-8+ EcoMic® (immediately after cutting), T4-EcoMic® (immediately after cutting) + INICA-8 (15 days after cutting), T5-INICA-8 (immediately after cutting) + EcoMic® (15 days after cutting), T6-Absolute control (AC) and T7-Contro fertilized (FC) with 150 kg N/ha ( $\text{NH}_3\text{NO}_3$ ).

The experiment was under agricultural drought conditions at certain times of its development, so it is not excluded that *Azospirillum* influenced plant tolerance to water stress. According to Pereyra *et al.* (2012), wheat seedlings inoculated with *Azospirillum* strains benefit from an improved water level during salt stress and osmotic stress, because xylem ducts widen, and coleoptile water conductance is improved. *Azospirillum*, like other rhizobacteria, is able to induce the synthesis of antioxidant enzymes in plants, which reduces the destructive effect of reactive oxygen species (Fukami *et al.*, 2018).

Mycorrhizal symbiosis is known to have a marked effect on plant and soil water relations under stress conditions, as it positively modifies stomatal conductance, photosynthetic rate, leaf water potential, osmolyte concentration, water use efficiency and nutrient assimilation; while fungal exudates promote soil particle cohesion and increase water retention (van der Heijden *et al.*, 2017). Also, in this variable, the combination of these two microorganisms significantly influenced the plant. Meenakshisundaram *et al.* (2011) found beneficial effect on growth, biomass and on nitrogen and chlorophyll content, when inoculating *Delonix regia* with an *Azospirillum* strain, alone or in different combinations with AMF.

*Dry weight of racemes.* The EcoMic®+INICA-8 treatment (fig. 7) was higher ( $p < 0,05$ ) than the

absolute control, EcoMic®, INICA-8 (0,060 g each), as well as INICA-8 (cutting)+EcoMic (15d) (0,061 g), but was statistically similar to the fertilized control (0,074 g) and EcoMic® (cutting)+INICA-8 (15d) (0,065 g). The IEI was higher for EcoMic®+INICA-8 (18,3 %). Although it cannot be considered high, it had the greatest impact on this variable (figure 8).

As in the previous variables, the single application of INICA-8 did not exert a superior effect. This could be ascribed to the fact that several biotic and abiotic factors affected the behavior of the bacteria and produced inconsistent responses to biofertilization. Among these factors are the physical-chemical conditions of the soil, genotype of the host and ability of the bacteria to become established and compete with the native microflora (Pecina-Quintero *et al.*, 2005). Drought stress, which could have affected both the plants and the rhizobacteria that were inoculated, should also be added to these factors.

However, it was observed that the combination of both biofertilizers in the same period of time was superior in terms of its effect on the plants compared with their single application. Therefore, it is inferred that there was a positive synergistic effect of the microorganisms that make up these products.

Bécquer *et al.* (2019b) reported that the combination of *Bradyrhizobium* sp. with *G. cubense*



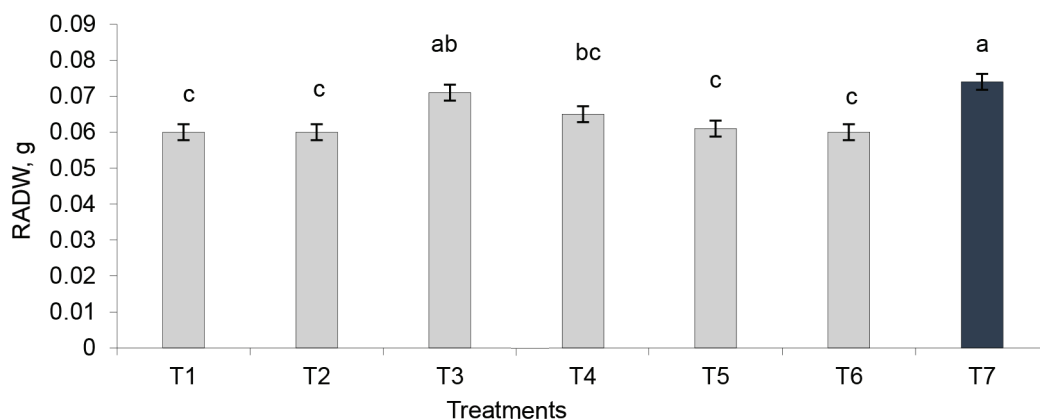


Figure 7. Effect of treatments on raceme dry weight (BWSW) of *A. gayanus*.

a, b and c: values with different superscripts differ at  $p < 0,001$ . Standard error: 0,001

T1-EcoMic® (immediately after cutting), T2-INICA-8 (immediately after cutting), T3-INICA-8+ EcoMic® (immediately after cutting), T4-EcoMic® (immediately after cutting) + INICA-8 (15 days after cutting), T5-INICA-8 (immediately after cutting) + EcoMic® (15 days after cutting), T6-Absolute control (AC) and T7-Control fertilized (FC) with 150 kg N/ha ( $\text{NH}_3\text{NO}_4$ ).

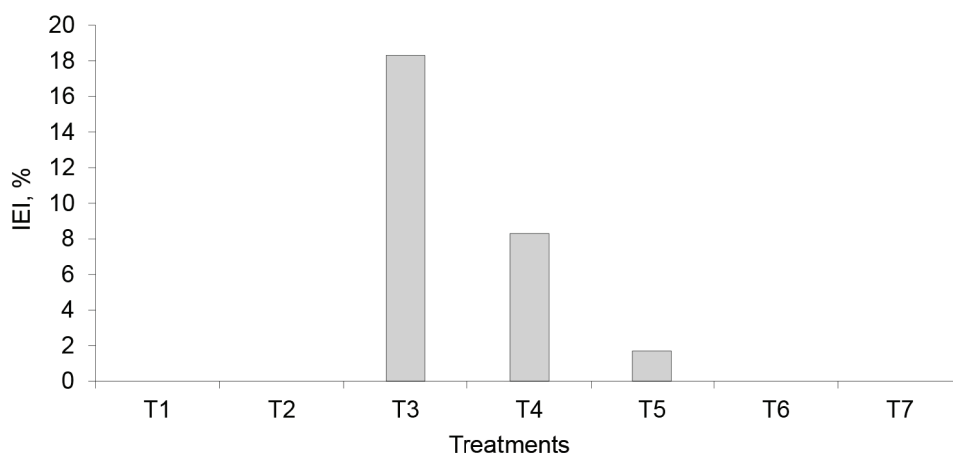


Figure 8. Inoculation efficiency index (IEI) in the inoculated treatments of *A. gayanus*, based on raceme dry weight.

T1-EcoMic® (immediately after cutting), T2-INICA-8 (immediately after cutting), T3-INICA-8+ EcoMic® (immediately after cutting), T4-EcoMic® (immediately after cutting) + INICA-8 (15 days after cutting), T5-INICA-8 (immediately after cutting) + EcoMic® (15 days after cutting), T6-Absolute control and T7-Control fertilized with 150 kg N/ha ( $\text{NH}_3\text{NO}_4$ ).

influenced the results of all agroproductive and biochemical variables evaluated in Mulato II grass.

According to Olalde-Portugal and Serratos (2008), the simultaneous combination of growth-promoting rhizobacteria and arbuscular mycorrhizal fungi induces synergism, which is expressed in increased growth, plant phosphorus content and yield, compared with those inoculated separately. Bona *et al.* (2017) consider that the result of the tripartite mutualistic symbiosis among AMF-

bacteria-host is the increase in plant development, because its capacity to absorb from the soil more water and nutrients such as nitrogen, phosphorus, potassium and microelements is increased.

**Flowering.** Treatments inoculated with EcoMic®+INICA-8 and EcoMic®(cutting)+INICA-8 (115 days after cutting) were superior ( $p < 0,05$ ) to the absolute control. EcoMic®+INICA-8 also had higher values than INICA-8 (0,4462), but did not differ from the fertilized control (0,7129), EcoMic®

(cutting)+INICA-8 (15 days after cutting), INICA-8 (cutting)+ EcoMic® (15 days after cutting) and EcoMic® (figure 9). The IEI (fig. 10) was higher for EcoMic+INICA-8 (84,19 %), followed by EcoMic® (cutting)+INICA-8(15 d) (61,97 %).

Although the amount of phosphorus in the plant was not quantified in this study, it is known that among the multiple functions of this macroelement

is that of positively influencing flower development (Ávila, 2005).

The marked difference in the number of flowering stems between the mycorrhizal treatments at the time of cutting and those combined with *Azospirillum* and the control could be due to the amount of P absorbed by the plants through the action of AMF, which were applied from the first

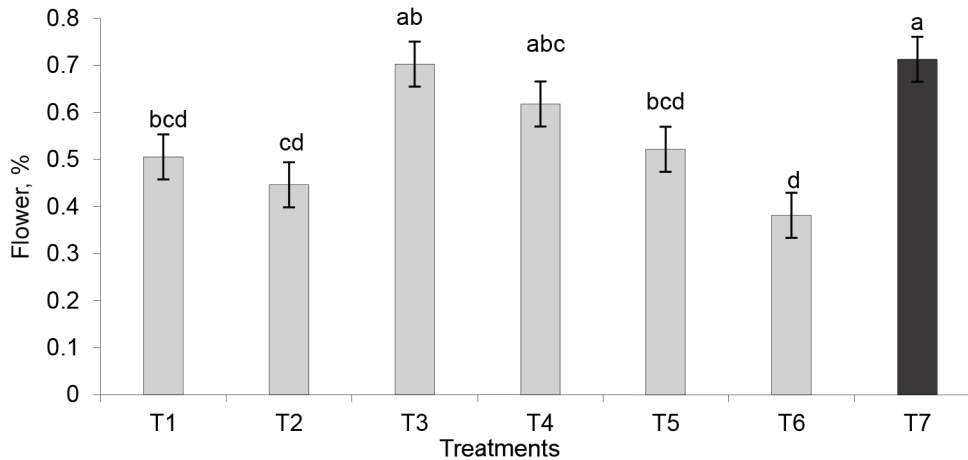


Figure 9. Effect of *A. gayanus* inoculation on flowering. Transformed values are shown with  $2\arcsin\sqrt{P}$  on the y-axis, and original values inside the bars.

a, b and c: values with different superscripts differ at  $p < 0,05$

T1-EcoMic® (immediately after cutting), T2-INICA-8 (immediately after cutting), T3-INICA-8+ EcoMic® (immediately after cutting), T4-EcoMic® (immediately after cutting) + INICA-8 (15 days after cutting), T5-INICA-8 (immediately after cutting) + EcoMic® (15 days after cutting), T6-Absolute control (AC) and T7-Control fertilized (FC) with 150 kg N/ha ( $\text{NH}_3\text{NO}_4$ ).

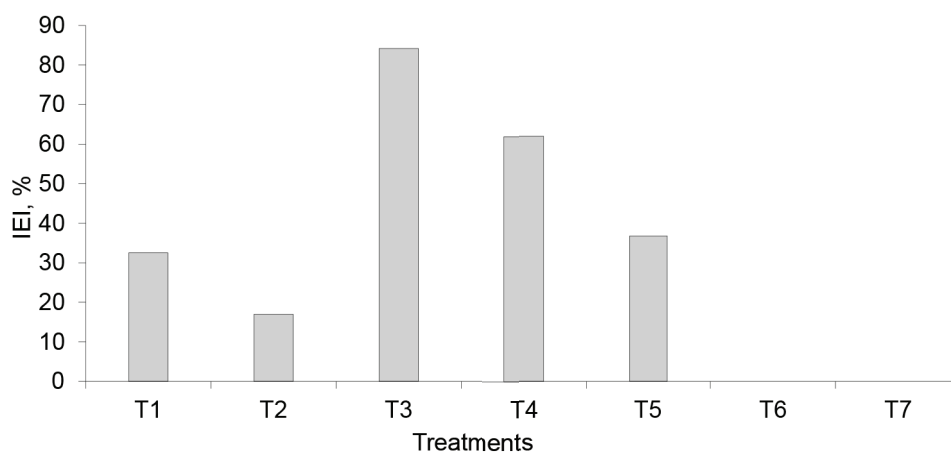


Figure 10. Inoculation efficiency index (IEI) in the inoculated treatments of *A. gayanus*, based on flowering.

T1-EcoMic® (immediately after cutting), T2-INICA-8 (immediately after cutting), T3-INICA-8+ EcoMic® (immediately after cutting), T4-EcoMic® (immediately after cutting) + INICA-8 (15 days after cutting), T5-INICA-8 (immediately after cutting) + EcoMic® (15 days after cutting), T6-Absolute control (AC) and T7-Test fertilized (TF) with 150 kg N/ha ( $\text{NH}_3\text{NO}_4$ ).

moment. This hypothesis is supported by different authors who corroborate that P is one of the nutrients whose absorption is favored by the action of mycorrhizae, because in addition to its low mobility, especially in tropical regions, between 95 and 99 % of the amounts of this element are in forms unavailable to plants (Zhang *et al.*, 2018). These benefits are due, in part, to the exploration of hyphae in the soil in search of nutrients and water, which expands the capabilities of roots alone. Also Omar *et al.* (2017) and Osman *et al.* (2020) reported the ability of *A. brasilense* to solubilize phosphates. On the other hand, there is evidence that the combination of these microorganisms can play a higher effect on plants (Bona *et al.*, 2017).

In this experiment, no increase in flowering was observed when inoculating the crop with *Azospirillum*, or AMF separately, but, similar to the other studied variables, only the combination of these microorganisms, in the same space of time, as well as fractionally, efficiently influenced this variable. However, Pérez *et al.* (2018) found that single inoculation with AMF in *Plukonetia volubilis* favored flower formation, due to the influence of phytohormones produced by these fungi, which affect growth and flowering. It is possible that the taxonomic location of the plants influences their response to this stimulus.

### Conclusions

The single application of INICA-8 had no effect on any of the studied variables. However, EcoMic® at the time of cutting, simply or in combination with *Azospirillum*, was determinant in most of the variables. These results prove the importance of the application of EcoMic® in this crop, under drought stress conditions, as well as the synergy between the microorganisms that were inoculated, due to a higher effect on the plant, when combined with each other.

### Recommendations

To evaluate the selected treatments in other field trials with *Andropogon*, under different soil and climatic conditions and in several cuts, is recommended.

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constitutes a significant contribution in qualified human resources.

### Conflicts of interest

No conflicts of interest are declared.

### Authors' contribution

- Carlos José Bécquer-Granados. Participated in 30 % of the total time. Responsible for the research, elaborated the protocol, led and participated in the set-up and evaluation of the experiment, performed the corresponding statistical analyses and wrote the manuscript.
- Rance Rojas Perez. Undergraduate student (fourth year of Agronomic Engineering) at the José Martí Pérez University of Sancti Spíritus. Participated in 20 % of the total time. Contributed to the setting up, maintenance and evaluation of the experiment, as well as the corresponding statistical analysis.
- Adelaida Benita Puentes Pérez. 15 % of participation. Participated in the setting up and evaluation of the experiment.
- Urbano Ávila Cordoví†. 15 % of participation in the setting up and evaluation of the field experiments. Contributed his knowledge as a soil and fertilizer specialist.
- José Antonio Nápoles Gómez. 15 % participation in planting, fertilization and evaluation of the experiment. Advised on the experimental design and statistical analysis.
- Fernando Medinilla Nápoles. 5 % participation. Contributed with the analysis of the state of agricultural drought in the experimental area and advised the work team with his knowledge on agrometeorology.

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