Scientific Paper

Productivity of Tifton 85 bermudagrass, inoculated with *Bradyrhizobium* sp. and *Trichoderma harzianum*, subject to agricultural drought stress

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Abstract

A field trial was conducted, under stressing conditions of agricultural drought, in order to evaluate the effect of combined inoculation with *Bradyrhizobium* sp. and *Trichoderma harzianum* on agroproductive variables of the hybrid Tifton 85 (*Cynodon dactylon*). The experimental design was control plot, with five replicas and three treatments: control fertilized with NH_4NO_3 (150 kg of N/ha), absolute control and inoculated treatment. The dry weight of the aerial part (APDW), inoculation efficiency index based on the APDW (IEIAPDW), stem length (SL) and flowering (Flow.), were evaluated. In the APDW the statistical superiority of the inoculated treatment (326,8 g/m^2) over the absolute control (230,0 g/m^2), was observed, although these two treatments, in turn, were lower than the fertilized control (492,4 g/m^2). With regards to IEIAPDW, the inoculated treatment showed 42,1 % more biomass than the absolute control. In SL there were no statistical differences between the inoculated treatment (28,9 cm) and the absolute control (27,0 cm), while the fertilized control (36,8 cm) surpassed both. There were no differences among treatments in flowering. It is concluded that, although there was no effect of the microbial inoculants on the flowering status of the crop, or on the stem length, a productivity of aerial biomass was obtained in the inoculated treatment higher than that of the absolute control. In general, the efficiency of the microbial inoculants used was proven.

Keywords: *Cynodon dactylon*, flowering, inoculation.

Introduction

Tifton 85 [*Cynodon dactylon* (L.) Pers] is a hybrid resulting from the crossing of Tifton 68 with an accession of South African Bermudagrass that was introduced in the United States as pasture and for hay in the humid southern states, according to McNamee (2014), who also stated that the advantages of this grass, which include a high biomass potential, drought and insect tolerance and exceptional responses to fertilization with nitrogen, make it very popular in southern United States. In Cuba, just like other stoloniferous pasture grasses, this variety was successfully introduced as source of fiber and nutrients for cattle husbandry, and it is one of the pasture varieties recognized in the Official List of Commercial Varieties of Cuba (MINAG, 2016). Schwantes *et al*. (2017) state that this variety responds well to organic fertilization; while Ames *et al*. (2015) found that Tifton 85 was higher in dry matter and other indicators for hay production, compared with oat varieties.

Crop productivity is affected by diverse stressing environmental factors; and it is known that hydric stress, caused by drought, limits crop growth and productivity, especially in arid and semiarid areas (Yang *et al*., 2009). If it is also taken into consideration that the *Cynodon* genus is characterized by its capacity to extract large quantities of nutrients from the soil (Pant *et al*., 2004), then there is need to search for less costly ways of higher environmental impact than chemical fertilizers to guarantee adequate nutrition of this pasture.

Rhizobacteria can contribute to the plants tolerating better the effects of drought; among them, rhizobia occupy an important place in the induction of stress tolerance. Hussain *et al*. (2014) proved, in *in vitro* trials, that there were rhizobial isolates from drought tolerant grain legumes, due to their production of catalase. There are antecedents in Cuba of the positive effect of *Bradyrhizobium* when combining it with *Trichoderma harzianum* in pastures, such as buffel grass var. formidable (Bécquer *et al*., 2017b); and in cereals, such as triticale (Bécquer *et al*., 2016b) and corn (Bécquer *et al*., 2017a), crops that were subject to drought stress conditions.

On the other hand, the filamentous fungus *Trichoderma* induces defense mechanisms and stimulates plant growth (Woo *et al*., 2014). One of the factors that contribute to the beneficial biological activity of some *Trichoderma* species is related to a wide variety of secondary metabolites they produce (Vinale *et al*., 2014a, 2014b).

The objective of the study was to evaluate the effect of the combination of *Bradyrhizobium* sp. and *T. harzianum* on agroproductive variables of Bermuda grass Tifton 85, during continuous agricultural drought stress.

Materials and Methods

Location. The trial was set up during the last ten days of December, 2016, in a seed production plot of the Pastures and Forages Research Station of Sancti Spiritus, Cuba; located at 21º 53′ 00″ North latitude and 79º 21′ 25″ West longitude, and at a height of 40 m.a.s.l.

Rhizobium strain. The strain Ho5 was applied, belonging to the *Bradyrhizobium* sp. genus, microsymbiont of *Desmodium triflorum*, legume from an arid animal husbandry ecosystem of Holguín, Cuba (Bécquer *et al*., 2016b).

Trichoderma strain. The product TRICOSAVE 34 (LABIOFAM, S.A.), was used, composed by a substrate of rice hull and heads inoculated with sporulated mycelia of *T. harzianum* A-34.

Plant material. The hybrid Tifton 85 [*C. dactylon* (L.) Pers] was evaluated.

Preparation of the inoculants:

Trichoderma. This product, following the technical recommendation made by the manufacturer, was added to tap water, at a rate of 35 g/L; and it was filtered with gauze, before inoculating the plants (1-2 x 10^9 conidia/g). The final titer of the suspension (10^6-10^8 conidia/mL) was in correspondence with the recommendations made by Wolffhechell and Jensen (1992).

Bradyrhizobium. The strain grew on solid yeast-manitol medium and was re-suspended in liquid medium until achieving a cell concentration of 10^6-10^8 CFU/mL, which was tested by counting the viable cells (Vincent, 1970). For the inoculation of the plants, the inoculant was diluted in 1:10 proportion in 0,9 % saline solution.

Agricultural management of the experiment. The experiment was set up on a previously established plot, 34 x 16 m, aimed at the production of seed from Tifton 85 Bermudagrass. An establishment cutting was made in order to inoculate, and no irrigation was applied during the experimental period. Harvest took place 132 days after that cutting.

Inoculation of the plants:

- With *Trichoderma*. The inoculation was performed immediately after the establishment cutting, with a manual sprayer of 20-L capacity whose content was poured on the recently-cut stems, so that when the spray was regulated 3 440 mL of liquid inoculant were inoculated in the area of the corresponding treatment (dose equivalent to 250 L/ha of final suspension, according to recommendations made by the manufacturer).
- With *Bradyrhizobium*. The inoculation was made 15 days after the initial application with *Trichoderma*, with a manual sprayer of 20-L capacity whose content was poured on the plant regrowths, for which 3 440 mL of liquid inoculant (final suspension) were inoculated in the area of the corresponding treatment (dose of 20 L/ha of initial inoculant).

The treatment absolute control received 3 440 mL of tap water at the beginning and 3 440 mL after 15 days. The fertilized control received 3,2 kg of NO_3NH_4, equivalent to 150 kg of N/ha, and received 3 440 mL of tap water when the fertilizer was applied and 3 440 mL after 15 days.

Evaluation of the climate variables. The temperature, rainfall, relative humidity and wind data, as well as their analysis, were collected at the Meteorological Station of Sancti Spiritus.

Determination of the status of agricultural drought. The status of agricultural drought was determined through the index of aridity or index of agricultural drought (Solano *et al*., 2004), which was used to test whether the trial was conducted under hydric stress conditions:

IE = ETR / ETP, where:

ETR: estimated actual evapotranspiration, depending on the moisture status of the soil.

ETP: estimated potential evapotranspiration, depending on the atmospheric conditions.

When ETR = ETP, the soil water supply is adequate. When ETR < ETP, there is water insufficiency.

Basic agrochemical composition of the experimental soil. The soil of the experimental area corresponds to the loosen Fluvisol type (Hernández-Jiménez *et al*., 2015); it shows a very low content of P_2O_5 (13,2 mg/100 g of soil.- Oniani) and K_2O (14,5 mg/100 g of soil.-Oniani), as well as of organic matter (2,25 %.- Walkley-Black), pH-4,8 and a cation exchange capacity of 18 meq/100 g (Mehlich).

Experimental design and statistical analysis. An experimental design of control plot, with three treatments and five replicas, was applied. The treatments were: 1) Inoculated: *Trichoderma* upon cutting + *Bradyrizobium* sp. 15 days after cutting (Trich.+Ho 13.15d.); 2) Absolute control (AC); Control fertilized with 150 kg of N/ha (FC). The area was divided into three strips 4 m wide and 34 m long (136 m^2), with 2 m of space between rows. Each strip was considered a treatment, and the replicas were taken in each plot. An ANOVA analysis was carried out. The differences among means were determined through Fisher's LSD. The percentage data were transformed by $arcsin\sqrt{P}$ and the statistical program StatGraphics Centurion XV was used.

As variables, the following were evaluated: the aerial part dry weight (APDW, g/m^2), with a frame of 0,25 m^2; stem length (SL, m) and inflorescence (flower, %). In addition, the inoculation efficiency index was calculated based on the APDW (IE-IAPDW, %), according to the formula proposed by Santillana *et al.* (2012):

IEI: [(Inoculated treatment – absolute control) / absolute control] x 100

Results and Discussion

Agricultural drought status

November ended with a regular IE, and December with insufficient IE (table 1). The agricultural drought was stressed in the period from January to April, and the IE was maintained with critical category in each of the months and highly critical in March.

The main indicators of water balance for the Pastures and Forages Research Station of Sancti Spiritus –based on the data of the pluviometer placed in the periphery of the study area–, are shown in table 1. The loss of soil moisture occurred progressively in the experimental period and the evapotranspiration was poor, especially in February and March, 2017.

Aerial part dry weight and inoculation efficiency index based on the APDW

Statistical difference was found of the inoculated treatment with regards to the absolute control, although they were both lower than the fertilized control (table 2). This result is not surprising, because as N is the nutrient required in higher quantity in forage production systems (Snyder and Leep, 2007), logically the plant takes the element with higher availability and assimilation in the soil, process that microorganisms cannot facilitate equally. Nevertheless, the positive effect of the microbial inoculants with this same treatment with regards to the absolute control was noted.

In the IEI, the inoculated treatment showed 42,1 % more aerial biomass than the absolute control. Bécquer *et al.* (2017b) found higher results in this indicator in *Cenchrus ciliaris* L., when subjecting such grass to drought stress. Alwhibi *et al.* (2017) reported that tomato plants inoculated with *T. harzianum* showed increase in the foliage, roots and chlorophyll, compared with the non-inoculated controls. In an experiment with vine, Pascale *et al.* (2017) found that two *T. harzianum* strains were capable of improving yield, polyphenol contents and antioxidant activity of the plants. On the other hand, it is known that strains of *Rhizobium sullae*, isolated in the semiarid region of Tunisia and moderately drought tolerant, significantly increased the

Table 1. Main indicators of the water balance and of the drought category.

Period	Initial moisture (%)	Final moisture (%)	ETP	ETR	Runoff	IE
November/2016	35,5	31,1	4,40	2,70	8,5	R
December/2016	31,1	24,9	4,28	0,52	2,3	I
January/2017	24,9	16,6	3,99	0,91	1,3	C
February/2017	16,6	12,4	4,54	0,12	0,1	C
March/2017	12,4	6,9	5,99	0,11	0	HC
April/2017	6,9	8,2	4,86	2,21	2,4	C

ETP: estimated potential evapotranspiration, ETR: estimated actual evapotranspiration.
R: regular, I: insufficient, C: critical, HC: highly critical.

Table 2. Aerial part dry weight and inoculation efficiency index.

Treatment	APDW (g/m²)	IEI (%)	Extrapolated APDW (kg/ha)
Absolute control	230,0^{c}	-	2 300,0^{c}
Inoculated treatment	326,8^{b}	42,1	3 268,0^{b}
Fertilized control	492,4^{a}	-	4 924,0^{a}
SE ±	28,069***		280,69***

APDW: aerial part dry weight ; IEI: inoculation efficiency index

*** p < 0,001

dry aerial biomass of plants inoculated in this type of environmental stress (Fitouri *et al*., 2012).

As the strain Ho5 (*Bradyrhizobium* sp.) came from an arid animal husbandry ecosystem of Cuba (Bécquer *et al*., 2016b) the statement expressed by Timmusk *et al*. (2014) regarding the fact that extreme environments can be the source of rhizobacteria with high tolerance potential, which can be transferred to the plants, is corroborated.

When the data were extrapolated (table 2), the inoculated treatment showed a yield of 3 268 kg/ha (statistically higher than the absolute control), for which the positive effect of these microorganisms on the pasture that was subject to agricultural drought stress can be inferred.

On the other hand, the yield of the fertilized control was 4 924 kg/ha. McNamee (2014), in the second year of establishment of a plot of Tifton 85 Bermudagrass, with 168 kg N/ha and under more favorable edaphoclimatic conditions than the ones in this trial, obtained yields between 4 251 and 6 260 kg/ha. If it is taken into consideration that in this experiment a lower dose of fertilizer was used, besides the severe environmental limitations, the high potential of this variety under stressing environmental conditions of Cuba is inferred, when fertilizing it with biological products, as well as with industrial nitrogen carriers.

Stem length and inoculation efficiency index based on the stem length

There were no statistical differences between the inoculated treatment (28,9 cm) and the absolute control (27,0 cm), in stem length; while the fertilized control (36,8 cm) exceeded both treatments. Nevertheless, when calculating the IEI, a 7 % difference was observed with regards to the absolute control (table 3).

Table 3. Stem length and inoculation efficiency index based on SL.

Treatment	SL (cm)	IEI (%)
Absolute control	27,0^{b}	-
Inoculated treatment	28,9^{b}	7,0
Fertilized control	36,8^{a}	-
SE ±	1,81985***	

SL: Stem length; IEI: inoculation efficiency index

*** $p < 0,001$

These results do not coincide with the ones obtained by Bécquer *et al.* (2016a), who inoculated triticale with a *Bradyrhizobium* sp. strain and *T. harzianum* at the moment of planting, treatment that propitiated higher stem elongation in the plants subject to agricultural drought; as well as with the results obtained by Bécquer *et al.* (2017b) in *C. ciliaris*, when inoculating with *T. harzianum* and *Bradyrhizobium* sp., also under drought conditions. It is possible that the quantity of phytohormones in charge of cell elongation in the stem was not sufficient for the forage species that was used, in spite of their positive effect on total biomass. In addition, the hydric stress to which the crop was subject should have negatively influenced this process.

Flowering

There were no differences among the treatments (table 4) regarding flowering. Sánchez-López *et al.* (2012) stated that the inoculation of tomato with diverse rhizosphere bacteria significantly increased plant flowering, perhaps due to the capacity of these bacteria to produce indoles and siderophores and to solubilize phosphorus, mechanisms that promote plant growth. However, the results that were obtained in this trial contradict such statement, which indicates that drought stress could have affected the flowering stimulating capacity in the microorganisms that were inoculated.

Table 4. Effect of the treatments on flowering.

Treatment	Flowering (%)	
	Transformed data	Original data
Absolute control	1,5708	50,0
Inoculated treatment	1,5254	47,8
Fertilized control	1,6412	53,2
SE ±	0,122723	

Another interpretation could be based on the role of ethylene as responsible for flowering in many plant species (Reid, 1995); and, as during drought stress the ethylene biosynthesis is enhanced (Ali *et al*., 2014), it is possible that the microorganisms that were applied to the crop were not capable of eliminating the primer of such substance (1-aminocyclopropane, 1 carboxylic acid-ACC) through ACC-deaminase (Saleem *et al*., 2007), typical of them, which could have influenced this result for the inoculated treatment.

It is concluded that, although there was no effect of the microbial inoculants on the flowering status of the crop, or stem length, a productivity of the aerial biomass was obtained in the inoculated treatment higher than that of the absolute control. In general, the efficiency of the microbial inoculants that were used was proven, in spite of the drought stress suffered by the crop.

Likewise, it is recommended to evaluate the application of the microbial inoculants in more extensive pasture areas, under agricultural drought conditions.

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