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CROP SCIENCE

Inoculation with plant-growth promoting bacteria improves seed germination and initial development of *Brachiaria decumbens*

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Abstract: The objective of this research was to evaluate the inoculation and co-inoculation of bacteria with biotechnological potential, isolated from *Brachiaria decumbens* Stapf. and *Brachiaria humidicola* (Rendle) Schweickerdt, under germination and seedling growth of *B. decumbens* cv. Basilisk, as well as, to verify the influence of the co-inoculum in the soil indigenous bacterial community. For this, two assays in a completely randomized design were set up. The experimental period was 21 days. In a germination chamber, 25 treatments were evaluated (24 bacterial inoculants and a control – without inoculation). In greenhouse assay, were used five co-inoculations (bacterial consortium). The bacterial consortium was obtained based on the bacterial strain performance in the germination test. In addition, the control and one treatment with mineral fertilizer (NPK) were tested. In germination test, the seed inoculation promoted increases of 61, 40, 144, 82, 6, 96, 91 and 52% in germination vigor, speed germination index, number of absorbent hairs, number of plumules, primary root length, hypocotyl length, total length, and dry matter of seedlings, respectively, when compared to control. The co-inoculation also increased the growth parameters of *B. decumbens* plants when compared to the control treatment. In addition, promoted changes in the soil bacterial community structure. Becoming an important strategy to increase the germination rate and germination speed of *B. decumbens* plants.

Key words: bacteria-plant association, forage grasses, microbial ecology, roots and rhizosphere bacterial.

INTRODUCTION

Forage grasses belonging to *Urochloa* genera, commonly known as *Brachiaria*, are cultivated worldwide (Cheruiyot et al. 2018). In Brazil alone, there are approximately 200 million hectares of pasture, which has a high degradation degree caused mainly by non-replenishment of soil nutrient, inadequate management and low seed germination rates under field conditions (Hungria et al. 2016, Bono et al. 2019).

The forage grass establishment in the field depends of the seed germination potential, being a crucial phase in determining pasture uniformity and yield potential (Silva et al. 2019a). Thus, the technologies that aim to increase, besides contributing to the plant-growth are essential for more efficient pasture management (Freitas et al. 2019). In this scenario, plant growth promoting bacteria (PGPB) can be an excellent strategy for improving seed germination rate and establishment of forage grasses. The PGPBs act as biological control agent against diseases,

producing phytohormones and hydrolytic enzymes necessary to seed germination and plant-growth, favoring the fast plant establishment (Andrade et al. 2019, Araújo et al. 2012, Kim et al. 2012, Lima et al. 2018, Oliveira et al. 2018, Sammauria et al. 2020, Souza et al. 2015, Terra et al. 2019).

Although the single inoculation (or the inoculum using only one microorganism specie), can promote the plant-growth, the co-inoculation with more than one microorganism species per inoculum, can increase the survival of the inoculated microbial population, increasing the probability of success due to the synergistic effect of different growth-promoting mechanisms (Sánchez et al. 2014, Sá et al. 2019).

The effect of PGPB inoculation depends on the plant genotype (Santos et al. 2019). This is because the plant species to be inoculated has a strong influence on the microbial community due to a wide variety of organic compounds that recruit the most distinct microbial groups to the rhizosphere soil. These microbial groups are often beneficial to the plant, contributing to nutrient acquisition, pathogen protection and resistance to environmental stress (Araújo et al. 2012, Wemheuer et al. 2016, Murphy et al. 2016, Santos et al. 2019). However, the inoculation of bacteria isolated from plant species and/or specific cultivars can facilitate colonization due to adaptation to biotic conditions, favoring the competition with native microbiota the plant and soil (Bashan et al. 2014, Sammauria et al. 2020).

Thus, our aim was to evaluate the inoculation and co-inoculation of *B. decumbens* cv. Basilisk seeds with growth-promoting bacteria isolated from *B. decumbens* Stapf. and *B. humidicola* (Rendle.) Schweickerdt on germination speed and seedling growth, as well as verifying the influence of co-inoculation on the native soil bacterial community.

MATERIALS AND METHODS

Two experiments were carried out. The first was assembled in the germination chamber (experiment 1) and, the second, was carried out in a greenhouse (experiment 2) at the Universidade Federal do Agreste de Pernambuco, Brazil (8°54'23.7"S 36°29'39.7"W). Were used commercial seeds of *B. decumbens* cv. Basilisk and 24 bacteria strains with different PGPB properties previously evaluated by Oliveira et al. (2018) and summarized in Supplementary Material - Table SI. The bacterial strains belonged to Microbial Genetics and Biotechnology Laboratory collection, located at Universidade Federal do Agreste de Pernambuco, Brazil. Twelve bacterial strains of each plant species were selected (*B. decumbens* Stapf and *B. humidicola* (Rendle.) Schweickerdt), being six from root (endophytic) and six from the rhizosphere (Table SI).

The experiment 1 was performed in a completely randomized design with 25 treatments, 24 inoculation and a control treatment (without inoculum), with four replicates, contained 50 seeds each. To inoculum were prepared from pure colonies, which were incubated in the TSB 10% (Trypticase Soy Broth) (1.7 g L^{-1} of tryptone – pancreatic digest of casein; 0.3 g L^{-1} of soytone – peptic digest of soybean; 0.25 g L^{-1} of glucose; 0.5 g L^{-1} of NaCl; 0.25 g L^{-1} of Na_2HPO_4; and pH = 7.3), this culture media was supplemented with 0.05% of tryptophan.

The cultures were diluted in a phosphate buffered saline solution (PBS) (8.0 g L^{-1} of NaCl. 200 mg L^{-1} of KCl; 1.44 g L^{-1} of Na_2HPO_4; 240 mg L^{-1} of KH_2PO_4; and pH = 7.4) and the optical density (OD) was adjusted in a spectrophotometer at 630 nm, corresponding to 10^6 CFU mL^{-1}. The seeds were disinfected using NaCl solution (1%) for 5 min, washed in distilled water and immersed in the inoculum for 30 min under gentle shaking.

The seeds were placed on a Germitest paper substrate moistened with distilled water 2.5 times its weight and maintained in a germination chamber at 25±5° C under a 12 h photoperiod, for 21 days. The germinated seed count was performed daily, from the seventh day after sowing, until the end of the experimental period. The germination rate was carried out following Brasil (2009) and Maguire (1962).

The plant-growth promotion evaluation was done at the 21° days after sowing. The parameters evaluated were: number of absorbent hairs (root hairs 3 mm in length); number of plumules per seedlings; length of primary root; distance between cotyledon and end of extended primary root; hypocotyl length; distance between the cotyledon and the base of the first extended seedling plumule, and the total length of the extended seedling. The dry mass of the seedlings was determined in the forced circulation greenhouse at 55° C for 72 h.

The experiment 2 was assembled following completely randomized design with seven treatments, being five bacterial strain (called MIX), a treatment using only mineral fertilization, and a control treatment without inoculum and mineral fertilization (Table I). The microcosm was assembled in pots containing 7.5 L of soil and sown with 15 seeds per pot, resulting in a total of 30 replicates. The bacterial MIX was composed by bacterial strains isolated from roots and rhizosphere, except for MIX 1, which was formulated according to the bacterial strain performance in the experiment 1. Fifteen co-inoculated seeds were sown in each microcosm. The seed emergency was evaluated at 7 days after planting (DAP). Plant-growth characteristics were analyzed in the 21 DAP.

The soil used in all treatments was classified as Yellow Latosol (EMBRAPA 2006), presented pH (H_2O): 5.22; P: 5.22 mg dm^{-3}; K^+: 0.24 $cmol_c$ dm^{-3}; Ca^{2+}: 4.70 $cmol_c$ dm^{-3}; Mg^{2+}: 2.80 $cmol_c$ dm^{-3}; Al^{3+}: 0.30 $cmol_c$ dm^{-3}; H+Al: 0.65 $cmol_c$ dm^{-3}; effective cation exchange capacity (CEC effective): 8.04 and potential cation exchange capacity (CEC potential): 8.39. The NPK formulation (30-60-30) was applied in the treatment managed with mineral fertilization to according Cavalcanti (2008).

The bacterial cultivation, individual inoculum preparation and seed superficial disinfection were carried out in a similar way to experiment 1. The preparation of the co-inoculant formulations was performed by mixing and homogenizing individual cultures according to Table I. The seeds were immersed in the co-inoculant for 30 min under light agitation (50 rpm). The sowing depth was 2 cm. After seed coverage, 50 mL of co-inoculant was applied to the soil.

For emergency and initial growth of seedlings, the first count of emerged seedlings occurred on the 7 DAP, considering as emergence the seedlings that exposed the first plumule. The speed index was determined as proposed by Maguire (1962), and on the 21 DAP, the percentage of emerged seedlings was calculated, accounting the number of seedlings per pot, based on the number of seeds deposited per pot. Finally, we counted the number of plumules per seedlings; seedling height using graduated ruler, considering the distance between the soil to the tip of the highest plumule not extended; and the pseudoculm diameter at 5 cm from the ground.

The leaf area of the primary plumules was estimated according to Bianco et al. (2000), considering the dimensional parameters, width and plumule length. The green plumules intensity was evaluated using the portable meter SPAD-502 (Soil Plant Analysis Development), chlorophyll meter, measuring the primary plumules in the intermediate portion. The dry mass of the seedlings was made by cutting at

Table I. Bacterial isolates for formulation of co-inoculant (MIX) used on seeds *Brachiaria decumbens* cv. Basilisk under greenhouse conditions.

Co-inoculants	Bacterial isolates	Origin of the bacterial strain
MIX 1	Differentiated isolates *	------
MIX 2	UAGB 10 – UAGB 39 – UAGB 60 UAGB 94 – UAGB 106 – UAGB 132	*Brachiaria humidicola* (Rendle.) Schweickerdt.
MIX 3	UAGB 68 – UAGB 71 – UAGB 80 UAGB 96 – UAGB 147 – UAGB 167	*B. decumbens* Stapf.
MIX 4	UAGB 01 – UAGB 93 – UAGB 105 UAGB 110 – UAGB 119 – UAGB 128	*Brachiaria humidicola* (Rendle.) Schweickerdt
MIX 5	UAGB 69 – UAGB 70 – UAGB 139 UAGB 150 – UAGB 154 – UAGB 156	*B. decumbens* Stapf.

*** Bacterial isolates from Experiment 1.**

the base of the plants, weighing and drying the plant material in the greenhouse at 55° C for 72 h.

The rhizospheric bacterial community was evaluated at 21 days after planting. For this, soil rhizospheric samples were collected from each plot. Then, every 10 plots, the samples were homogenized to form a composite sample, which totaled 3 composite samples per treatment. Three soil samples were collected before the experiment was implemented (initial control). The soil DNA total was extracted with Power Soil DNA kit (MoBio; USA), followed by the integrity evaluation by 1.2% agarose gel electrophoresis in TAE 1x buffer (40 mM of Tris-acetate; 1 MM of EDTA).

The 16S rDNA gene was amplified using the primers set 027F (5'-GAGAGTTTGATCCTGGCTCAG-3') and 1387R (5'-CGGTGTGTACAAGGCCCGGAACG-3'), and the amplification reaction follow the Heuer et al. (1997) recommendations. The PCR product was evaluated in 1.2% agarose gel electrophoresis in TAE 1x buffer and stained with blue green loading. For the restriction fragment length polymorphism analysis (RFLP), the PCR products of the 16s rDNA gene were digested with restriction enzymes *Hind*III, *Hae*III and *Mbo*I. For each enzyme, individual blends were prepared containing 7 µL of the PCR product, 10 µL of the specific buffer for each enzyme; 2 U of restriction enzyme and 2.7 µL ultrapure water. The digestions were performed at 37° C for 10 h. The digestion products were separated by 2.5% agarose gel electrophoresis in TAE 1x buffer and stained with blue green loading.

Statistical analysis, in experiment 1, the differences between groups were evaluated by orthogonal contrast using the t-test at 5% of probability. Subsequently, the averages of all treatments were compared in relation to the control by the Dunnett test at 5%, the treatments that stood out were compared for each variable by the Tukey test at 5%. In the experiment 2, the differences between groups were analyzed by orthogonal contrast using the 5% t test, followed by the Tukey test at 5% between treatments, using the statistical software SISVAR® version 5.7 (Ferreira 2007). The groups of the rhizospheric bacterial community was evaluated by Principal Coordinates Analysis (PCoA) and the significance between groups was tested by the ANOSIM test, using the using the Bray-Curtis similarity matrix (Ramette 2007). Both analyses were performed in the statistical software PAST® version 4.0 (Hammer et al. 2001).

RESULTS

The bacteria inoculation promoted a significant increase in germination and seedling growth of *B. decumbens*, showing the largest increases in the vigor and germination speed index, as well as number of absorbent hairs, primary root length, hypocotyl and total seedling, confirming our initial hypotheses (Table II).

In general, the inoculation of *Brachiaria* seeds increased the germination vigor (GV) and germination speed index (GSI) by 61 and 40% in relation to the control, respectively (Table III). For these two variables, only 5 bacterial strains performed better than the control treatment, named UAGC10, UAGC71, UAGC150, UAGC 154 and UAGC167 (Table IV) by the Dunnett test at 5% probability.

Also, the inoculations with *Klebsiella* (UAGB 154) and *Rhizobium* (UAGB 167), giving the largest increases as compared to the control, with 108% and 80%, respectively. For number of absorbent hairs (NAH) and plumules (NPL), only 29% (7 strains) of the bacterial inoculum differed from the control (Table IV). The inoculants increasing the NAH and NPL at 144 and 82% (Table III). The *Klebsiella* (UAGB 156) strain promoted an increase of 300% in the NAH when compared to control.

For the primary root length (PRL), only three bacterial inoculants differed statistically from the control (Table IV), with the average of all inoculants providing an increase of PRL at 6% (Table III). The largest increases compared to the control were observed with the *Klebsiella* (UAGB 154) strain at primary root length and total seedling length, with increases of 39% and 129%, respectively, and with *Sinomonas* (UAGB 71) in the hypocotyl length, with an increase of 134% (Table V).

The five strains *Klebsiella* (UAGB 60, UAGB 156 and UAGB 154), *Rhizobium* (UAGB 167) and *Sinomonas* (UAGB 71) that obtained the highest percentages of increase against the control in

Table II. Comparison between averages groups by orthogonal contrasts for germination and initial seedling growth characteristics of *Brachiaria decumbens* cv. Basilisk with 21 days in germination chamber at 25±5° C under 12 h photoperiod after inoculation in seeds of potentially growth promoting plant growth bacteria.

Groups	GV	SGI	NAH	NPL	PRL	HYL	TLS	DMS
	-- % --	----------			---------- cm ----------			g seedlings^{-1}
Inoculant	36.229	2.760	7.041	1.285	4.140	7.330	11.031	0.009
IBD	38.916	2.939	7.135	1.279	4.336	7.376	11.368	0.006
IBH	33.541	2.580	6.948	1.291	3.944	7.285	10.694	0.011
Control	22.500	1.971	2.885	0.706	3.878	3.728	5.767	0.006
General	35.680	2.728	6.875	1.262	4.129	7.186	10.820	0.009
Inoculant *vs.* Control								
Test T	2.575*	2.079*	4.191*	4.452*	0.677*	5.796*	4.267*	0.527*
IBD *vs.* IBH								
Test T	2.497*	2.370*	0.475*	-0.164^{ns}	2.104*	0.366*	1.318*	-2.810^{ns}

GV: Germination vigor; SGI: Speed germination index; NAH: Number of absorbent hairs; NPL: Number of plumules; PRL: Primary root length; HYL: Hypocotyl length; TLS: Total length of seedling; DMS: Dry matter of seedlings; IBD: Mean of the isolates inoculated of *B. decumbens* Stapf. IBH: Mean of isolates inoculated of *B. humidicola* (Rendle.) Schweickerdt; ns: Not significant, and *: Significant at 5% probability by t-test.

Table III. Performance of bacterial strains in the germination test in relation to control (without inoculant).

Treatments	GV	SGI	NAH	NPL	PRL	HYL	TLS	DMS
	-- % --	----------			-------- cm --------			g seedlings $^{-1}$
Isolates from *B. humidicola* (Rendle.) Schweickerdt roots (n = 6)								
MBI	29.833	2.255	7.989	1.183	4.060	7.289	10.465	0.016
MC	22.500	1.971	2.885	0.706	3.878	3.728	5.767	0.006
BSP (%)	32	14	176	67	4	95	81	164
Isolates from *B. humidicola* (Rendle.) Schweickerdt rhizosphere (n = 6)								
MBI	37.250	2.906	5.907	1.400	3.828	7.281	10.924	0.0072
MC	22.500	1.971	2.885	0.706	3.878	3.728	5.767	0.0060
BSP (%)	65	47	104	98	0	95	89	19
Isolates from *B. decumbens* Stapf. roots (n = 6)								
MBI	38.833	2.938	7.815	1.256	4.343	7.257	11.557	0.0068
MC	22.500	1.971	2.885	0.706	3.878	3.728	5.767	0.0060
BSP (%)	72	49	170	77	12	94	100	13
Isolates from *B. decumbens* (Rendle.) Schweickerdt rhizosphere (n = 6)								
MBI	39.000	2.942	6.457	1.302	4.330	7.497	11.180	0.0065
MC	22.500	1.971	2.885	0.706	3.878	3.728	5.767	0.0060
BSP (%)	73	49	123	84	11	101	93	8
All bacterial isolates (n = 24)								
MBI	36.229	2.760	7.042	1.285	4.140	7.331	11.031	0.0090
MC	22.500	1.971	2.885	0.706	3.878	3.728	5.767	0.0060
BSP (%)	61	40	144	82	6	96	91	51

MBI: mean of bacterial isolates; MC: mean of control; BSP: bacterial strains performance - percentage increase of MBI in relation to MC; GV: Germination vigor; SGI: Speed germination index; NAH: Number of absorbent hairs; NPL: Number of plumules; PRL: Primary root length; HYL: Hypocotyl length; TLS: Total length of seedling; DMS: Dry matter of seedlings.

experiment 1, being used to compose the MIX 1 of experiment 2, of these, only *Klebsiella* (UAGB 60) presents as its origin plant *B. humidicola* (Rendle.) Schweickerdt (Table SI). In general, bacterial inoculum obtained from *B. decumbens* plants showed higher and better performance in the germination test than isolated strains of *B. humidicola* (Rendle.) Schweickerdt (Table IV and V). Considering the niche, root bacterial strains promoted better performance of NAH, while rhizospheric bacterial inoculum had better performance in GV, SGI and NPL (Table IV and V).

Under greenhouse conditions, the co-inoculation group promoted increase as compared to the control and were inferior to chemical fertilization treatment. Among the bacterial MIXs, the inoculated *Brachiaria* species promoted greater emergence and initial plant development, leaf area of primary plumule, the green intensity of primary plumule and dry mass of seedling aerial part (Table VI). MIX 1, although the strains stand out under germination chamber conditions, did not promote increments in greenhouse conditions compared to other MIX treatments (Table VI).

Table IV. Germination characteristics and initial growth of *Brachiaria decumbens* cv. Basilisk seedlings after seed inoculation of potentially plant growth promoting bacteria, with 21 days in germination chamber at 25±5° C under 12 h photoperiod.

Treatments	GV	SGI	NAH	NPL	PRL	HYL	TLS	DMS
	-- % --	----------			---------- cm ----------			g seedlings $^{-1}$
UAGB 01	33.000^{ns}	2.549^{ns}	9.389*	1.022^{ns}	3.948^{ns}	7.335^{ns}	11.264^{ns}	0.056^{ns}
UAGB 93	33.500^{ns}	2.462^{ns}	8.700*	1.150^{ns}	5.333*	7.537^{ns}	10.725^{ns}	0.007^{ns}
UAGB 94	19.500^{ns}	1.412^{ns}	7.961^{ns}	0.997^{ns}	4.132^{ns}	7.209^{ns}	10.025^{ns}	0.008^{ns}
UAGB 105	41.500^{ns}	3.112^{ns}	7.225^{ns}	1.175^{ns}	3.755^{ns}	7.517^{ns}	11.272^{ns}	0.008^{ns}
UAGB 106	30.000^{ns}	2.224^{ns}	6.575^{ns}	1.275^{ns}	4.017^{ns}	6.687^{ns}	10.367^{ns}	0.009^{ns}
UAGB 110	21.500^{ns}	1.769^{ns}	8.082*	1.477*	3.177^{ns}	7.448^{ns}	9.135^{ns}	0.007^{ns}
UAGB 10	39.500^{ns}	3.509*	6.522^{ns}	1.130^{ns}	3.843^{ns}	7.214^{ns}	11.090^{ns}	0.007^{ns}
UAGB 39	39.500^{ns}	3.054^{ns}	4.939^{ns}	1.287^{ns}	3.762^{ns}	8.575*	12.304*	0.006^{ns}
UAGB 60	38.500^{ns}	2.881^{ns}	5.725^{ns}	1.625*	3.205^{ns}	7.203^{ns}	10.237^{ns}	0.008^{ns}
UAGB 119	33.500^{ns}	2.593^{ns}	5.650^{ns}	1.425*	4.262^{ns}	5.762^{ns}	10.025^{ns}	0.008^{ns}
UAGB 128	37.500^{ns}	2.773^{ns}	4.633^{ns}	1.333^{ns}	3.969^{ns}	7.833*	10.862^{ns}	0.007^{ns}
UAGB 132	35.000^{ns}	2.625^{ns}	7.975^{ns}	1.600*	3.927^{ns}	7.100^{ns}	11.027^{ns}	0.007^{ns}
UAGB 68	41.500^{ns}	3.197^{ns}	5.525^{ns}	1.400*	4.250^{ns}	7.447^{ns}	11.697^{ns}	0.009^{ns}
UAGB 69	34.500^{ns}	2.593^{ns}	5.800^{ns}	1.300^{ns}	3.535^{ns}	6.687^{ns}	10.222^{ns}	0.007^{ns}
UAGB 70	37.500^{ns}	2.725^{ns}	5.812^{ns}	1.081^{ns}	3.426^{ns}	7.200^{ns}	10.067^{ns}	0.008^{ns}
UAGB 154	47.000*	3.532*	8.400*	1.005^{ns}	5.420*	7.792*	13.212*	0.005^{ns}
UAGB 156	26.500^{ns}	2.025^{ns}	11.550*	1.300^{ns}	4.740^{ns}	7.082^{ns}	11.822*	0.006^{ns}
UAGB 167	46.000*	3.558*	9.800*	1.450*	4.685^{ns}	7.335^{ns}	12.320*	0.006^{ns}
UAGB 71	41.000^{ns}	3.209*	5.400^{ns}	1.425*	4.585^{ns}	8.732*	13.117*	0.006^{ns}
UAGB 80	42.000^{ns}	3.152^{ns}	6.705^{ns}	1.283^{ns}	5.229*	8.708*	12.205*	0.004^{ns}
UAGB 96	32.500^{ns}	2.446^{ns}	8.087*	1.281^{ns}	4.160^{ns}	7.177^{ns}	10.905^{ns}	0.008^{ns}
UAGB 139	36.000^{ns}	2.734^{ns}	4.950^{ns}	1.150^{ns}	4.415^{ns}	6.497^{ns}	10.912^{ns}	0.007^{ns}
UAGB 147	38.500^{ns}	2.903^{ns}	5.875^{ns}	1.325^{ns}	4.645^{ns}	6.030^{ns}	9.160^{ns}	0.007^{ns}
UAGB 150	44.000^{ns}	3.205*	7.725^{ns}	1.350^{ns}	2.945^{ns}	7.835*	10.780^{ns}	0.007^{ns}
Control	22.500	1.971	2.885	0.706	3.878	3.728	5.767	0.006
CV (%)	29.6	27.4	27.8	20.4	19.8	17.6	22.3	25.1

GV: Germination vigor; SGI: Speed germination index; NAH: Number of absorbent hairs; NPL: Number of plumules; PRL: Primary root length; HYL: Hypocotyl length; TLS: Total length of seedling; DMS: Dry matter of seedlings; ^{ns}: Not significant, and *: Significant, higher than control, by Dunnett test, at a 5% probability level.

Similar to observed under germination chamber conditions, the MIX 3, bacteria isolated from *B. decumbens*, presented the best results, resembling the mineral fertilization and significantly superior to the control, except for the percentage of emerged seedlings (Table VII). In relation to the control, there was an increase in emergency speed index of 18% and 33%, with MIXs 3 and 5, and in the percentage of emerged seedlings of 33% and 18%, with MIXs 2 and 3, respectively (Table VII).

Table V. Germination characteristics and initial growth of *Brachiaria decumbens* cv. Basilisk seedlings after seed inoculation of potentially plant-growth promoting bacteria, with 21 days in germination chamber at 25± 5° under 12 h photoperiod of the treatment's superior to the control, pre-selected by the Dunnett test.

Treatments	GV	SGI	NAH	NPL	PRL	HYL	TLS
	-- % --	----------			---------- cm ----------		
UAGB 01	*	*	9.389b	*	*	*	*
UAGB 93	*	*	8.700b	*	5.333a	*	*
UAGB 110	*	*	8.082b	1.477a	*	*	*
UAGB 10	*	3.509a	*	*	*	*	*
UAGB 39	*	*	*	*	*	8.575a	12.304a
UAGB 60	*	*	*	1.625a	*	*	*
UAGB 119	*	*	*	1.425a	*	*	*
UAGB 128	*	*	*	*	*	7.833a	*
UAGB 132	*	*	*	1.600a	*	*	*
UAGB 68	*	*	*	1.400a	*	*	*
UAGB 154	47.000a	3.532a	8.400b	*	5.420a	7.792a	13.212a
UAGB 156	*	*	11.550a	*	*	*	11.822a
UAGB 167	46.000a	3.558a	9.800b	1.450a	*	*	12.320a
UAGB 71	*	3.209a	*	1.425a	*	8.732a	13.117a
UAGB 80	*	*	*	*	5.229a	8.708a	12.205a
UAGB 96	*	*	8.087b	*	*	*	*
UAGB 150	*	3.205a	*	*	*	7.835a	*
CV (%)	9.7	7.0	13.4	17.4	10.3	15.5	18.9

GV: Stamina of germination, SGI: Speed germination index, NAH: Number of absorbent hairs, NPL: Number of plumules, PRL: Primary root length, HYL: Hypocotyl length, TLS: Total length of seedling, and the means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability.

All MIXs contributed positively to the increase plumes number per seedling. For seedling height, MIX 3 increased by 10%. In the pseudoculm diameter, the MIX 2, 3 and 4 increased by 7% and MIXs 2 for the leaf area of the primary leaflet. Also, MIX 3, 4 and 5 provided an increase greater than 28% as compared to control. The green intensity of the primary plumes, MIXs 3 and 5 were highlighted, giving rise to 4% and 5%, respectively, in photosynthetic pigment levels. For the dry mass of the seedling aerial part, MIX 3 provided an increment of 50% as compared to control (Table VII).

Regarding the rhizosphere bacteria community, co-inoculations and chemical fertilization promoted changes in the bacterial community structure (Figure 1). The MIX 2, 3 and 4 showed overlapping, demonstrated by the ANOSIM analysis a greater similarity of the communities among the inoculated soils ($R<0.111$) (Table VIII). It should be noted that only the germination of the seeds provided alteration in the bacterial community.

Table VI. Comparison between groups of means by orthogonal contrasts for emergence characteristics and promotion of seedling growth at 21 days in the greenhouse after co-inoculation (MIX) of potentially plant-growth promoting bacteria in seeds *Brachiaria decumbens* cv. Basilisk.

Group average	ESI	PSE	NPL	SEH	PSD	LAP	SPAD	SSDM
	----	%	----	---- cm ----		cm^2	----	$g\ seedlings^{-1}$
ACI	8.913	59.556	3.004	11.997	0.144	4.442	25.913	0.164
MF	10.667	72.000	3.000	12.603	0.156	4.153	26.587	0.289
Control	8.333	55.556	2.867	11.647	0.138	3.471	25.553	0.161
CIBD	10.483	59.666	3.005	12.211	0.145	5.023	26.820	0.193
CIBH	7.883	63.111	3.005	12.040	0.150	4.520	25.748	0.165
MIX 1	7.833	52.222	3.000	11.480	0.126	3.124	24.427	0.104
CIBDH	9.183	61.388	3.005	12.126	0.148	4.7715	26.284	0.179
General	9.304	62.370	2.957	12.082	0.146	4.022	26.018	0.205
	ACI *vs.* MF							
Test T	-2.773*	-19.677*	0.007^{ns}	-0.958^{ns}	-1.961*	0.457^{ns}	-1.066*	-19.733*
	CI *vs.* Control							
Test T	0.917ns	6.324*	0.217^{ns}	0.553^{ns}	0.885^{ns}	1.535*	0.569ns	0.506^{ns}
	CIBD *vs.* CIBH							
Test T	4.504*	-5.966*	0.000^{ns}	0.296^{ns}	0.866^{ns}	0.871*	1.856*	4.763*
	MIX 1 *vs.* CIBDH							
Test T	-2.092*	-14.021*	-0.009^{ns}	-1.001^{ns}	-3.408*	-2.552*	-2.877*	-11.658*

ESI: Emergency speed index; PSE: Percentage of seedlings emerged; NPL: Number of plumules; SEH: Seedling height; PSD: Pseudoculm diameter; LAP: Leaf area of the primary plumule; SPAD: Measurement of the green intensity of primary plumule; SSDM: Seedling shoot dry mass; ACI: Averages of all co-inoculant (MIX 1, 2, 3, 4, and 5); MF: Mineral fertilization; CIBD: Mean of co-inoculant (MIX 3 and 5) formulated with bacteria isolated from *B. decumbens* Stapf. CIBH: Mean of co-inoculant (MIX 2 and 4) formulated with bacteria isolated from *B. humidicola* (Rendle.) Schweickerdt; MIX 1: Co-inoculant formulated with the five best bacteria strains used in the germination and growth promotion of *B. decumbens* cv. Basilisk under germinating chamber conditions; CIBHD: Mean of co-inoculant (MIX 2, 3, 4 and 5) formulated with bacteria isolated from *B. decumbens* Starf and *B. humidicola* (Rendle.) Schweickerdt; ^{ns}: Not significant, and *: Significant at 5% probability by t-test.

DISCUSSION

As observed in this study with *B. decumbens* and in other observations using grass seeds as *Panicum virgatum* (Kim et al. 2012), *P. maximum* Jacq. (Caro et al. 2014), *P. maximum* cv. Mombaça (Silva et al. 2019b), *Zea mays* (Cecatto Júnior et al. 2019), and *Oryza sativa* (Verma et al. 2018) the inoculation of plant-growth promoting bacteria has provided the increase in germination under controlled conditions. Reis et al. (2013) emphasize the importance of rapid germination seeds as a way to obtain a greater uniformity of pasture. Vigorous seedlings can compete more efficiently under stress conditions for light, nutrients and water, influencing the establishment of the plant population in the pasture, thus reducing the number of invasive plants, contributing to lower water losses and soil erosion, reducing waiting time for entry of animals into pasture (Araújo et al. 2010, Zuffo et al. 2014).

The microorganisms capable of producing auxin-like compounds can exert beneficial effects on the plant development, mainly in

Table VII. Emergence characteristics and promotion of seedling growth at 21 days of greenhouse cultivation after co-inoculation (MIX) of plant-growth promoting bacteria in *Brachiaria decumbens* cv. Basilisk seeds.

Treatments	ESI	PSE	NPL	SEH	PSD	LAP	SPAD	SSDM
	----	%	----	---- cm ----		cm^2	----	g seedlings^{-1}
MIX 1	7.833b	52.225b	3.000a	11.480b	0.126c	3.123b	24.426b	0.103b
MIX 2	7.933b	74.000a	3.000a	11.968b	0.155a	4.592a	25.553b	0.148b
MIX 3	9.866a	65.780a	3.000a	12.843a	0.153a	5.172a	26.643a	0.237a
MIX 4	7.833b	52.225b	3.011a	12,.113b	0.145a	4.445a	25.941b	0.182b
MIX 5	11.100a	53.555b	3.011a	11.580b	0.138b	4.873a	26.996a	0.147b
MF	10.666a	72.000a	3.000a	12.603a	0.156a	4.152a	26.586a	0.289a
Control	8.333b	55.555b	2.864b	11.647b	0.138b	3.470b	25.555b	0.160b
CV (%)	19.6	24.0	3.6	14.1	15.7	23.8	11.2	23.2

ESI: Emergency speed index; PSE: Percentage of seedlings emerged; NPL: Number of plumules; SEH: Seedling height; PSD: Pseudoculm diameter; LAP: Leaf area of the primary plumule; SPAD: Measurement of the green intensity of primary plumule; SSDM: Seedling shoot dry mass; MIX 1: Co-inoculant formulated with the five best bacteria strains used in the germination and growth promotion of *B. decumbens* cv. Basilisk test under germinating chamber conditions; MIX 2 and 4: Co-inoculant formulated with bacterial strains isolated from *B. humidicola* (Rendle.) Schweickerdt; MIX 3 and 5: Co-inoculant formulated with bacterial strains isolated in *B. decumbens* Stapf and MF: Mineral fertilization. Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability.

Table VIII. Analysis of similarity (ANOSIM) of the rhizosphere bacterial groups presents on the seedlings *Brachiaria decumbens* cv. Basilisk at 21 days after co-inoculation with plant-growth promoting bacteria.

Treatments	MIX 1	MIX 2	MIX 3	MIX 4	MIX 5	MF	CON
MIX 2	0.370						
MIX 3	0.500	0.037					
MIX 4	0.481	0.074	0.111				
MIX 5	0.796	0.296	0.592	0.020			
MF	1.000	0.981	1.000	0.592	0.722		
CON	1.000	0.889	1.000	0.815	0.852	1.000	
SZ	1.000	1.000	1.000	0.778	0.833	1.000	0.574

MIX 1: Co-inoculum formulated with the five best bacteria strains in the germination and growth promotion of *B. decumbens* cv. Basilisk test under germinating chamber conditions; MIX 2 and 4: Co-inoculant formulated with bacterial strains isolated from *B. humidicola* (Rendle.) Schweickerdt; MIX 3 and 5: Co-inoculum formulated with bacterial strains isolated in *B. decumbens* Stapf; MF: Mineral fertilization; CON: Control and SZ: Soil zero, before the experimental period.

the root system, with improvements in the development and architecture of the root system, besides the increase of germination and emergence (Araújo et al. 2012, Mia et al. 2012). Plants infected by growth promoting bacteria that produce auxin-like compounds increase the water and nutrient uptake capacity and, consequently, can potentiate its development and the chances of establishing the crop (Jochum et al. 2019). However, plant growth may not interfere with dry mass, due to the action of this phytohormone on cell stretching and vacuolar turgor (Conceição et al. 2008), as observed in this study under conditions of germination chamber and greenhouse.

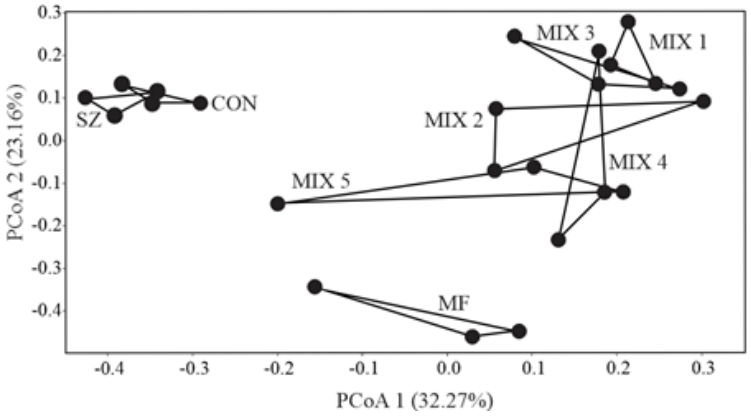

Figure 1. Principal coordinates analysis (PCoA) of the rhizospheric bacterial community seedlings of *Brachiaria decumbens* cv. Basilisk at 21 days after co-inoculation PGPB. MIX 1: Co-inoculant formulated with the five best bacteria strains in the germination and growth promotion of *B. decumbens* cv. Basilisk test under germinating chamber conditions; MIX 2 and 4: Co-inoculant formulated with bacterial strains isolated from *B. humidicola* (Rendle.) Schweickerdt; MIX 3 and 5: Co-inoculant formulated with bacterial strains isolated in *B. decumbens* Stapf. MF: Mineral fertilization; CON: Control, and SZ: Soil zero, before the experimental period.

The highest increases in the germination were observed by strains *Klebsiella* (UAGB 60, UAGB 156 and UAGB 154), *Rhizobium* (UAGB 167) and *Sinomonas* (UAGB 71). The latter three showed *in vitro* indol auxin-like compounds production higher than 100.08 μg mL^{-1} (Table SI). Possibly, favoring the variables increased vigor and germination speed index, besides the characteristics of primary root length, hypocotyl and total length of seedlings. In the other treatments, the production of indol acetic acid varied from 4.98 to 67.17 μg mL^{-1} (Table SI), probably the interaction of plant microorganism assumed a fundamental role in the positive biological results of this interaction.

Among the co-inoculations, MIX 1, formed by the five best strains under germination chamber, did not favor the emergence of the initial seedling's development in a greenhouse environment (Table VI and VII). The low performance of bacterial inoculants has been observed in several studies in greenhouse and field conditions. This is due to the lower competitiveness of the bacterial strains that make up the inoculant compared to the soil native microbial community (Ramakrishna et al. 2019, Rilling et al. 2019), the physical and chemical conditions of the soil or even the efficiency of application of the inoculant (Santos et al. 2019, Souza et al. 2015).

Although almost all plant tissues are capable of producing low levels of indole acetic acid, apical meristems of stems and young leaves are the main synthesis sites of these phytohormones. In leaf primordia, the auxin accumulates at the apex, as the leaves develop, accumulations of these phytohormones can be detected on the leaf margins (Taiz et al. 2017). Thus, bacteria that produce auxin-like compounds may exert a positive effect on the dimensional parameters of the leaves (Mutai et al. 2017).

Because nitrogen is one of the constituents of chlorophyll, the content of this pigment can be used as an indicator of the nitrogen level in the leaves (Ghimire et al. 2017, Pedreira et al. 2017). Plant-associated diazotrophic bacteria can supply nitrogen more effectively, raising their levels and consequently raising chlorophyll levels (Kelemu et al. 2011, Sammauria et al. 2020). Similar results were observed by Chauhan et al. (2013) to co-inoculate different bacterial strains in sugarcane (*Saccharum officinarum* L.).

The productivity increase in plants inoculated with diazotrophic bacteria possibly occurred due to the increase of the available

nitrogen (Sammauria et al. 2020, Shahverdi et al. 2014). This nutrient supplied through biological fixation, besides stimulating plant production, facilitates the production of phytohormones by bacteria, which in turn favor the development of roots and consequently the absorption of nitrogen, phosphorus and other nutrient indispensable to plant development (Lima et al. 2018, Tripathi et al. 2013).

According to Salamone et al. (2012), the inoculation of plant-growth-promoting bacteria alters the indigenous community of the soil, favoring the heterogeneity of the bacterial community (Trabelsi et al. 2011), as observed in this study. The MIXs 2, 3 and 4 showed overlapping, demonstrated by the ANOSIM analysis a greater similarity of the communities among the inoculated soils (R<0.111) (Table VIII). The ANOSIM analysis observes significant differences based on algorithms of mean distances between groups generating R correlation. Values R>0.75 indicates that the groups are well separated, for groups 0.25>R<0.75, but overlapping groups exist, and R<0.25 values there is no separation between the groups (Clarke & Gorley 2001, Ramette 2007).

Rare are the studies evaluating the inoculation of different strains of bacteria with the potential to promote plant growth in *B. decumbens* seeds, evidencing the importance of the present study, with unpublished and quite promising results. In both inoculation and co-inoculation of plant growth promoting, bacteria in seeds of *B. decumbens* cv. Basilisk promoted higher germination, emergence and initial development of seedlings, having of *B. decumbens* homologous bacteria and with a high yield of indole acetic acid providing higher growths in the vegetable.

The co-inoculation and chemical fertilization processes alter the bacterial community in the soil. However, it is important to emphasize the importance of studies that evaluate the entire life cycle of the plant, as well as the stress caused by cutting the plant, and/or grazing. Assuming that the use of inoculums and co-inoculants of plant growth promoting bacteria in forage programs makes it possible to glimpse the reduction of production costs, as well as the increase of pasture productivity.

CONCLUSIONS

The inoculation and co-inoculation of plant growth promoting bacteria in seeds of *B. decumbens* cv. Basilisk provided an increase in the vigor of germinated seeds, germination speed index and the promotion of seedling growth at 21 days. The bacteria isolated from *B. decumbens* with high production of indole acetic acid providing greater increments to the development and growth of seedlings. The processes of co-inoculation and mineral fertilization change the bacterial community in the soil. Thus, we provided evidence that co-inoculation promotes an increase in seed performance in grass pasture in controlled conditions.

The highest challenge for the use of bacterial inoculants in pastures is: 1) lack of consistent results on the use of inoculants formulated with PGBPs in field conditions; 2) absence of adequate inoculation technologies for pasture areas; 3) absence of monitoring of strains bacterial which compose the inoculant to verify their survival and permanence over time and space variations. In front of the above-mentioned challenges, our research can be a promising strategy for the management and recovery of degraded pastures through the selection of PGPBs efficient in field trials and subsequent formulation of a bio-product (inoculant).

REFERENCES

ANDRADE FM, PEREIRA TA, SOUZA TP, GUIMARÃES PHS, MARTINS AD, SCHWAN RF, PASQUAL M & DÓRIA J. 2019. Beneficial effects of inoculation of growth-promoting bacteria in strawberry. Microbiol Res 223: 120-128.

ARAÚJO AES, ROSSETTO CAV, BALDAN VLD & BALDANI JI. 2010. Germinação e vigor de sementes de arroz inoculadas com bactérias diazotróficas. Cienc Agrotec 34: 932-939.

ARAÚJO FF, GUABERTO LM & SILVA IF. 2012. Bioprospecção de rizobactérias promotoras de crescimento em *Brachiaria brizantha*. R Bras Zootec 41: 521-527.

BASHAN Y, DE-BASHAN LE, PRABHU SR & HERMANDEZ JP. 2014. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perpectives (1998-2013). Plant Soil 378: 1-33.

BIANCO S, BRENDOLAN RA, ALVES PLCA & PITELLI RA. Estimativa de área Foliar de plantas daninhas: *Brachiaria decumbens* Stapf. e *Brachiaria brizantha* (Hochst.) Stapf. R Planta Daninha 18: 79-84.

BRASIL. 2009. Regras para análise de sementes, 1ª ed., Brasília: Ministério da Agricultura, Pecuária e Abastecimento. Mapa/ACS, 398 p.

BONO JAM, RUFINO RS & GONÇALVES RC. 2019. Fertilizante nitrogenados em cobertura para pastagem Marandu (*Brachiaria brizantha*) no Mato Grasso do Sul. Uniciência 32: 127-132.

CARO DMC, RUBIANO MFG, FANDIÑO BAR & BUITRAGO RRB. 2014. Inoculación con *Azospirillum* spp. y *Enterobacter aglomerans* en pasto Guinea (*Panicum maximum* Jacq.) en el Departamento de Cesar (Colombia). Rev Fac Nac Agron Medellin 67: 7271-7280.

CAVALCANTI FJA. 2008. Recomendações de adubação para o Estado de Pernambuco, 3ª ed., Recife: Instituto Agronômico de Pernambuco, 212 p.

CECATTO JÚNIOR R, GUIMARÃES VF, BELEGON LG, SUSS AD, BAZEI GL, BRITO TS & INIGAKI AM. 2019. Inoculation of maize seeds with *Azospirillum* and magnesium through foliar application to enhance productive performance. J Agric Sci 11: 225-233.

CHAUHAN H, BAGYARAJ DJ & SHARMA A. 2013. Plant growth-promoting bacterial endophytes from sugarcane and their potential in promoting growth of the host under field conditions. Expert Agricul 49: 43-52.

CHERUIYOT D, MIDEGA CAO, UECKERMANN EA, BERG JV, PICKETT JA & KHAN ZR. 2018. Genotypic response of *Brachiaria* (*Urochloa* spp.) to spider mite (*Oligonychus trichardti*) (Acari: Tetranychidae) and adaptability to different environments. Field Crops Res 225: 163-169.

CLARKE KR & GORLEY RN. 2001. PRIMER v5: User manual/tutorial, 1ª ed., United Kingdon: PRIMER-E.Plymouth, 91 p.

CONCEIÇÃO PM, VIEIRA HD, CANELLAS LP, MARQUES JÚNIOR RB & OLIVARES FL. 2008. Recobrimento de sementes de milho com ácidos húmicos e bactérias diazotróficas endofíticas. Pesq Agropec Bras 43: 545-548.

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. 2006. Sistema brasileiro de classificação de solos. 2ª ed., Rio de Janeiro: Embrapa Solos/Centro Nacional de Pesquisas de Solos, 206 p.

FERREIRA DF. 2007. SISVAR: um programa para análises e ensino de estatística. Rev Symp 6: 36-41.

FREITAS PVDX, TOMAZELLO DA, ISMAR MG, BRAW TT, ROMUALDO AF, MACIEL AALP, FIRMINO AE, SILVA NETO CM & FRANÇA AFS. 2019. Produção de gramíneas forrageiras inoculadas com *Azospirillum brasilense* associada a adubação nitrogenada. Rev Cient Rural 21: 31-46.

HAMMER Ø, HARPER DAT & RYAN PD. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis–Palaeontol. Electron 4: 1-9.

HEUER H, KRSEK M, BAKER P, SMALLA K & WELLINGTON EMH. 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rDNA and gel-electrophoretic separation in denaturing gradients. Appl Environ Microbiol 63: 3233-3241.

HUNGRIA M, NOGUEIRA MA & ARAUJO RS. 2016. 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. Agr Ecosyst Environ 221: 125-131.

JOCHUM MD, McWILLIAMS KL. BORREGO EJ, KOLOMIETS MV, NIU G. PIERSON EA & JO YK. 2019. Bioprospecting plant growth-promoting rhizobacteria that mitigate drought stress in grasses. Front Microbiol 10: 1-9.

KELEMU S, FORY P, ZULETA C, RICAURTE J, RAO I & LASCANO C. 2011. Detecting bacterial endophytes in tropical grasses of the *Brachiaria* genus and determining their role in improving plant growth. Afr J Biotechnol 10: 965-976.

KIM S, LOWMAN S, HOU G, NOWAK J, FLINN B & MEI C. 2012. Growth promotion and colonization of switch grass (*Panicum virgatum*) cv. Alamo by bacterial endophyte *Burkholderia phytofirmans* strain PsJN. Biotechnol Biofuels 5: 1-10.

LIMA DRM, SANTOS IB, OLIVEIRA JTC, BARBOSA JG, DINIZ WPS, FARIAS ARB, FREIRE FJ & KUKLISNSKY-SOBRAL J. 2018.

Tolerance of potentially diazotrophic bacteria to adverse environmental conditions and plant growth-promotion in sugarcane. Arch Agron Soil Sci 64: 1534-1548.

MAGUIRE JD. 1962. Speed of germination-aid in selection and evaluation for seedlings emergence and vigor. Crop Sci 2: 176-177.

MIA MAB, SHAMSUDDIN ZH & MAHMOOD M. 2012. Effects of rhizobia and plant growth promoting bacteria inoculation on germination and seedling vigor of lowland rice. Afr J Biotechnol 11: 3758-3765.

MURPHY CA, FOSTER BL & GAO C. 2016. Temporal dynamics in rhizosphere bacterial communities os three perennial grassland species. Agronomy 6: 01-17.

MUTAI C, NJUGUNA J & GHIMIRE S. 2017. Brachiaria grasses (*Brachiaria* spp.) harbor a diverse bacterial community with multiple attributes beneficial to plant growth and development. Open Microbiol J 6: 1-11.

OLIVEIRA JTC, SILVA GT, DINIZ WPS, FIGUEREDO EF, SANTOS IB, LIMA DRM, VERDI MCQ, KUKLINSKY-SOBRAL J & FREIRE FJ. 2018. Diazotrophic bactéria isolated from *Brachiaria* spp.: genetic and physiological diversity. Cienc e Investig Agrar 45: 277-289.

PEDREIRA BC, BARBOSA PL, PEREIRA LET, MOMBACH MA, DOMICIANO LF, PEREIRA DH & FERREIRA A. 2017. Tiller density and tillering na *Brachiaria brizantha* cv. Marandu pastures inoculated with *Azospirillum brasilense*. Arq Bras Med Vet Zootec 69: 1039-1046.

RAMAKRISHNA W, YADAV R & LI K. 2019. Plant growth promoting bacteria in agriculture: Two sides of a coin. Appl Soil Ecol 138:10-18.

REIS RA, BERNARDES TF & SIQUEIRA GR. 2013. Forragicultura: ciência, tecnologia e gestão dos recursos forrageiros, 1ª ed., Jaboticabal: Funep, 714 p.

RAMETTE A. 2007. Multivariate analyses in microbial ecology. FEMS Microbiol Ecol 62: 142-160.

RILLING JI, ACUÑA JJ, NANNIPIERI P, CASSAN F, MARUYAMA F & JORQUERA MA. 2019. Current opinion and perspectives on the methods for tracking and monitoring plant growth–promoting bacteria. Soil Biol Biochem 130: 205-219.

SÁ GCR, CARVALHO CLM, MOREIRA A, HUNGRIA M, NOGUEIRA MA, HEINRICHS R & SOARES FILHO CV. 2019. Biomass yield, nitrogen accumulation and nutritive value of Mavuno grass inoculated with plant growth-promoting bacteria. Commu Soil Sci Plant 50: 1931-1942.

SALAMONE IEG, FUNES JM, SALVO LPD, ORTEGA JSE, D'AURIA F, FERRANDO L & SCAVINO AF. 2012. Inoculation of paddy rice with *Azospirillum brasilense* and *Pseudomonas fluorescens*: Impacto f plant genotypes on rhizosphere microbial communities and field crop production. Appl Soil Ecol 61: 196-204.

SAMMAURIA R, KUMAWAT S, KUMAWAT P, SINGH J & JATWA TK. 2020. Microbial inoculants: potential tool for sustainability of agricultural production systems. Arch Micribiol doi: 10.1007/s00203-019-01795-w.

SÁNCHEZ AC, GUTIÉRREZ RT, SANTANA RC, URRUTIA AR, FAUVART M, MICHIELS J & VANDERLEYDEN J. 2014. Effects of co-inoculation of native *Rhizobium* and *Pseudomonas strains* on growth parameters and yield of two contrasting *Phaseolus vulgaris* L. genotypes under Cuban soil conditions. Eur J Soil Biol 62: 105-112.

SANTOS MS, NOGUEIRA MA & HUNGRIA M. 2019. Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of benefcial bacteria in agriculture. AMB ExprESS 9: 1-22.

SHAHVERDI M, MIRSHEKARI B, RAHMANI HA, RASHIDI V & ARDAKANI MR. 2014. Response of forage quality in Persian clover upon co-inoculation with native *Rhizobium leguminosarum symbiovar* (sv.) *trifoli* RTB_3 and plant-growth promoting *Pseudomonas florescence* 11168 under diferente levels of chemical fertilizers. Afr J Microbiol Res 8: 155-161.

SILVA GZ, MARTINS CC, CRUZ JO, JEROMINI TS & MACHADO CG. 2019a. Production regions and physical quality of *Urochloa decumbens* cv. Basilisk seeds. Biosci J 35: 236-243.

SILVA KP, SILVA GOA, OLIVEIRA TE, REZENDE AV & FLORENTINO LA. 2019b. Growth promotion of Guinea grass by diazotrophic bacteria. Pesq Agropec Trop 49: 1-8.

SOUZA R, AMBROSINIM A & PASSAGLIA LMP. 2015. Plant growth-promoting bacteria as inoculantes in agricultural soils. J Genet Mol Biol 38: 401-419.

TAIZ L, ZEIGER E, MØLLER IM & MURPHY A. 2017. Fisiologia e desenvolvimento vegetal, 6ª ed., Porto Alegre: Artmed, 858 p.

TERRA ABC, SOUZA FRC, MANTOVANI JR, REZENDE AV & FLORENTINO LA. 2019. Physiological characterization of diazotrophic bacteria isolated from *Brachiaria brizantha* rhizosphere. Rev Caatinga 32: 658-666.

TRABELSI D. MENGONI A, AMMAR HB & MHAMDI R. 2011. Effect of on-field inoculation of *Phaseolus vulgaris* with rhizobia on soil bacterial communities. FEMS Microbial Lett 77: 211-222.

TRIPATHI J, SINGH AK, TIWARI P & MENAKA YM. 2013. Comparative effectiveness of different isolates of *Azospirillum* on nitrogen fixation and yield and yield

attributing characters of tomato in Chhattisgarh. Afr J Microbiol Res 7: 3615-3620.

VERMA SK, KINGSLEY K, BERGEN M, ENGLISH C, ELMORE M, KHARWAR RN & WHITE JF. 2018. Bacterial endophytes from rice cut grass (*Leersia oryzoides* L.) increase growth, promote root gravitropic response, stimulate root hair formation, and protect rice seedlings from disease. Plant Soil 422: 223-238.

WEMHEUER F, WEMHEUER B, KRETZSCHMAR D, PFEIFFER B, HERZOG S, DANIEL R & VIDAL S. 2016. Impact of grassland management regimes on bacterial endophyte diversity differs with grass species. Lett Appl Microbiol 62: 323-329.

ZUFFO AM, ANDRADE FR, SILVA LMA, MENEZES KO, SILVA RL & PIAUILINO AC. 2014. Profundidade de semeadura e superação de dormência no crescimento inicial de sementes de *Brachiaria dictyoneura* (Fig. & De Not.) Stapf (1919) cv. Llanero. Rev Ceres 61: 948-955.

SUPPLEMENTARY MATERIAL

Table SI. Properties of plant-growth promotion for the 24 diazotrophic bacteria associated with *Brachiaria humidicola* (Rendle.) Schweickerdt. and *Brachiaria decumbens* Stapf isolated from root (endophytic) and rhizosphere niche.

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Authors contribution

JTCO, JKS, FJF, MVFS, MAL: Substantial contribution to acquisition of data. JTCO, APAP, AJS, FJK, MAL: Substantial contribution to analysis and interpretation of data. JTCO, APAP, AJS, FJK: Critically revising the article for important intellectual content. All authors contributed with Substantial contribution to conception and design, Drafting the article and final approval of the version to be published.