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Inoculation of plant growth-promoting bacteria on *Pennisetum purpureum* Schumach cultivar BRS Capiaçu

Inoculação de bactérias promotoras de crescimento vegetal em rebolos de *Pennisetum purpureum* Schumach cultivar BRS Capiaçu

Natália Lima de Espíndola[1], *Brena Maíza de Siqueira Tavares*[1], *José Matheus Gonzaga Santos*[1], *Vitor Mineu Silva Barbosa*[1], *Isaneli Batista dos Santos*[1], *João Tiago Correia Oliveira*[1]

ABSTRACT

Pennisetum purpureum (Schumach) cultivar BRS Capiaçu stands out from other forage species for its high production capacity. In order to improve plant management in the field, it is necessary to standardize the germination/emergence of seeds or seedlings, as well as rapid plant development. The use of plant growth-promoting bacteria is, therefore, a viable and sustainable strategy, especially as it reduces the environmental damage caused by the trivial use of various agricultural inputs. This study aimed to evaluate the germination and morphological and physiological characteristics of BRS Capiaçu seedlings after inoculation of the stalk/seedlings with plant growth-promoting bacteria isolated from *Brachiaria decumbens* Stapf. and *Brachiaria humidicola* (Rendle.) Schweickerdt. The experiment was carried out in a completely randomized design, with two evaluations, on the 11th and 25th days after inoculation. Nineteen treatments were evaluated: 16 bacterial inoculants, two commercial inoculants (Biomais® and Biomaphos®), and one control without inoculation. All treatments were kept in a germination chamber at 25°C, standard deviation ±5°C under a 12-hour photoperiod. Bacterial inoculations promoted increases in germination and morphological and physiological characteristics of BRS Capiaçu seedlings on the 11th and 25th days after their inoculations, surpassing the control treatment and sometimes resembling or surpassing the commercial inoculums. Bacteria isolated from *B. decumbens* and with high indoleacetic acid production provided the greatest increases in seedling development. Finally, it can be concluded that bacterial inoculums, in addition to improving the establishment of Capiaçu plants, are an effective and sustainable alternative to the use of synthetic products, contributing to a more environmentally balanced agricultural ecosystem.

Keywords: biotechnology; forage; microorganism-plant; sustainability.

RESUMO

O *Pennisetum purpureum* (Schumach) cultivar BRS Capiaçu destaca-se das demais espécies forrageiras por apresentar elevada capacidade produtiva. Visando melhorar o manejo vegetal a campo, é necessário uniformizar a germinação/emergência de sementes ou mudas, bem como o rápido desenvolvimento vegetal. Assim, a utilização de bactérias promotoras de crescimento de planta enquadra-se como uma estratégia viável e sustentável, sobretudo por reduzir diversos danos ambientais causados pela prática trivial de diversos insumos agrícolas. Este estudo objetivou avaliar a germinação, as características morfológicas e fisiológicas de plântulas de BRS Capiaçu, após a inoculação do colmo/mudas com bactérias promotoras de crescimento de plantas isoladas de *Brachiaria decumbens* Stapf. e *Brachiaria humidicola* (Rendle.) Schweickerdt. O experimento foi realizado em delineamento inteiramente casualizado, com duas avaliações, ao 11º e 25º dias após as inoculações. Foram avaliados 19 tratamentos, sendo 16 inoculantes bacterianos, dois inoculantes comerciais (Biomais® e Biomaphos®) e um controle sem inoculação. Todos os tratamentos foram mantidos em câmara de germinação a 25°C, desvio padrão ±5°C sob 12h de fotoperíodo. As inoculações bacterianas promoveram aumentos na germinação e nas características morfológicas e fisiológicas de plântulas de BRS Capiaçu no 11º e 25º dias após suas inoculações, superando o tratamento controle e, por vezes, assemelhando ou superando os inóculos comerciais. As bactérias isoladas de *B. decumbens* e com alta produção de ácido indolacético proporcionaram os maiores incrementos no desenvolvimento das plântulas. Por fim, conclui-se que os inóculos bacterianos, além de melhorar o estabelecimento de plantas de Capiaçu, são uma alternativa eficaz e sustentável ao uso de produtos sintéticos, contribuindo com um ecossistema agrícola ambientalmente mais equilibrado.

Palavras-chave: biotecnologia; forrageira; microrganismo-planta; sustentabilidade.

[1]Federal University of Agreste of Pernambuco – Garanhuns (PE), Brazil.

Correspondence author: Natália Lima de Espíndola – Rua José Leitão, 565 – Boa Vista – CEP: 55291-150 – Garanhuns (PE), Brazil.
E-mail: agronataliaespindola@outlook.com

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Introduction

The use of areas for the production of Capiaçu is one of the most viable and efficient forms of land use for animal feed (Nascimento et al., 2024). For this purpose, the BRS Capiaçu cultivar stands out for having dense clumps, erect stalks that make harvesting easier, moderate tolerance to water stress, and high potential for biomass production (Siri-Prieto et al., 2020; Moreno et al., 2022).

The intensive use of agricultural practices aimed at the rapid production of pastures and grazing land, with heavy use of inputs, agrochemicals and mineral fertilizers, has a negative impact on biodiversity and the functioning of ecosystems (Deus et al., 2022; Wanga et al., 2024). These include pastoral environments, leading pasture and/or grassland areas to degradation, and more severely, to desertification (Frota et al., 2020). However, in recent years, public policies have emerged and are being encouraged at the national and international levels to ensure the conscious use of natural resources and agricultural practices that cause the least environmental impact with maximum productivity, thus ensuring environmental sustainability (Zerbe et al., 2022).

With these objectives in mind, the use of plant growth-promoting bacteria (PGPB) is gaining prominence as a low-cost environmental technology that enables the recovery and maintenance of agricultural systems (Deus et al., 2022), making it an excellent strategy to be used to promote plant germination, growth, and development (Oliveira et al., 2022). PGPB are microorganisms that are naturally present in the soil, in symbiosis and/or association with the plant, and can help the plant through direct and indirect mechanisms, including the production of phytohormones, such as indoleacetic acid (auxins), siderophores and extracellular enzymes, biological nitrogen fixation, as well as increasing resistance to pathogens (Oliveira et al., 2018; Lima et al., 2021; Elnahal et al., 2022). These bacteria, when multiplied and inoculated into seeds or plant tissues, colonize the inside (endophytic) and/or outside (epiphytic) of the plant, in addition to the rhizosphere, and thus contribute to plant development (Oliveira et al., 2022; Martins et al., 2023).

The effect of PGPB inoculation depends on the plant genotype, that is, the plant species to be inoculated has an influence on the microbial community, due to a variety of organic compounds released into the soil (phytohormones, flavonoids, genes, among others) that affect the most distinct microbial groups in the soil, rhizosphere, and in direct contact with the plant (Taiz et al., 2017; Oliveira et al., 2022). Several studies have proven for decades that these microorganisms, closely associated with plants, are key parts in promoting sustainable plant growth and can be used to formulate bioproducts (Kuklinsky-Sobral et al., 2004). In forage plants, inoculation is successful with sugar cane, *Saccharum officinarum* (Lima et al., 2021); elephant grass, *Pennisetum purpureum* (Schumach) cv. BRS Kurumi (Rodrigues et al., 2016); and *Brachiaria* grass, *Brachiaria decumbens* cv. Brasilisk (Oliveira et al., 2022). However, the effect of PGPB inoculation on the germination and development of the elephant grass cultivar BRS Capiaçu is still scarce.

Despite all the efforts to bioprospect and formulate inoculants capable of promoting plant growth, little was elucidated, as a huge microbial diversity with unknown functions is still being described (Pascutti et al., 2024). This lack of knowledge, especially regarding microbial interactions in an agroecosystem, has led to scientific inconsistency in the production and use of bioinoculants (Trabelsi et al., 2020). Thus, work on bioprospecting and inoculation of beneficial microorganisms is of crucial importance (Figueredo et al., 2023).

Advancing knowledge in the area of bioprospecting and applying beneficial microorganisms to plants is a tool with a strong impact on sustainable agriculture, reducing environmental damage (Oliveira et al., 2018). With a view to increasing the germination speed index and improving initial development, combined with the sustainable production of weeds with low environmental impact, this work is based on the hypothesis that inoculation with PGPB is a critical factor in ensuring good initial development of BRS Capiaçu. Therefore, the main objective of this work was to evaluate the effect of inoculating PGPB isolated from *B. decumbens* Stapf. and *Brachiaria humidicola* (Rendle.) Schweickerdt on germination and morphophysiological characteristics during the initial development of the BRS Capiaçu plant.

Materials and Methods

Plant material

The experiment was carried out using plant material (seedlings) from cowsheds grown in the municipality of Bom Conselho, Pernambuco, Brazil, on partner properties of the Brazilian Agricultural Research Corporation (EMBRAPA) and released for sale.

Bacterial strains

Sixteen bacterial strains isolated from *B. decumbens* Stapf. and *B. humidicola* (Rendle.) Schweickerdt were used, eight from the root (endophytic) and eight from the rhizosphere (epiphytic), all with biotechnological potential and previously evaluated by Oliveira et al. (2018). The bacterial strains belonged to the Júlia Kuklinsky-Sobral bacterial culture collection at the Forage and Biotechnology Center of the Federal University of Agreste of Pernambuco, Brazil.

Experimental design

The trial was conducted in a completely randomized design with 19 treatments: 16 bacterial inoculations, two commercial inoculums (Biomais® and Biomaphos®), and one control (no inoculum), each treatment with four replicates, containing 20 seedlings. The seedlings were represented by a lateral bud/node, with three centimeters of internode on each side of the node, following the recommendation of Barreto et al. (2001).

Making the seedlings

Initially, the BRS Capiaçu plants were screened in the field, eliminating those that showed symptoms of deficiency and/or were affected by phytopathogens. Subsequently, the plants were cut and the stalks were cleaned by removing the leaves and leaf sheaths. To standardize the seedlings, the first three lateral buds near the neck of the plant (the boundary between the stem and the root) were discarded via cutting, besides the first three lateral buds with a fully expanded leaf near the apical meristem, considering fully expanded leaves to be those with the ligule exposed.

Preparation of the inoculum and bacterial inoculation

The individual bacterial inoculums were prepared from pure colonies, incubated in tryptone soy broth and then the cultures were diluted in phosphate buffered saline, with the optical density adjusted in a spectrophotometer at 630 nm, corresponding to 10^6 colony forming unit (mL^{-1}). Prior to inoculation, the seedlings were disinfected with NaClO solution (1%) for 5 minutes, washed in distilled water, and then immersed in the inoculum for 30 minutes under gentle manual shaking, according to Lima et al. (2021).

The seedlings were placed in a germitest paper substrate moistened with water, 2.5 times the weight of the paper, and kept in a germination chamber at 25°C, standard deviation ±5°C under a 12-hour photoperiod for 25 days, as recommended by Oliveira et al. (2022). For the treatments with commercial inoculums, the seedlings were inoculated according to the manufacturer's recommendations. In the control treatment (without inoculum), the seedlings were immersed in phosphate buffered saline for 30 minutes under gentle manual agitation.

Variables analyzed

Germinated seedlings were counted daily, starting on the third day after inoculation and ending on the tenth day, according to Brasil (2009). The germination speed index (GSI) was determined as proposed by Maguire (1962). Evaluations of plant growth promotion took place on the 11th and 25th days after inoculations, evaluating ten seedlings from each repetition, totaling 760 seedlings at each evaluation time.

The variables analyzed were the number of plumules (NP); length of the aerial part of the seedling (LP), considering the distance between the neck and the first extended seedling; width of the coleoptile (WC), at a distance of one centimeter from the seedling; concentration of chlorophyll a and b, measuring the middle third of the first seedling, using the SPAD-502 chlorophyll meter; number of roots (NR), counting the roots that emerged from the neck; and length of the largest root (LR), evaluating the distance between the neck and the root meristem of the largest extended root; as well as the green weight (GW) and dry weight (DW) of the seedlings.

To determine GW and DW, the LP was separated from the root system by a cross-section at the neck, then the samples were weighed on a semi-analytical balance and dried in a forced-air circulation oven at 55°C for 72 hours.

Statistical analysis

In the statistical analyses, the differences between the groups were compared by orthogonal contrast using the Student's *t*-test at 5 and 1% probability for the variables collected on the 11th and 25th days after inoculating the seedlings. Next, the means of all the treatments by variable on the 11th and 25th days after inoculation were compared with the control employing the Dunnett test at 5%. The treatments that stood out were compared by applying the Scott-Knott test at 5%, using the statistical software Sisvar®, version 5.6 (Ferreira, 2007). The treatment groups were evaluated by principal coordinate analysis (PCoA), according to the Bray-Curtis similarity matrix, with Past® statistical software, version 4.0 (Hammer et al., 2001).

Results and Discussion

The bacterial inoculums promoted the germination and development of BRS Capiaçu seedlings at both times evaluated, surpassing the control treatment and sometimes resembling or surpassing the commercial inoculums (Table 1). The inoculated microbiota promoted percentage increases of more than 100% in several variables, such as NP, LP, and NR on the 11th day, and NP and SPADa on the 25th day after inoculation (Table 2). This led to a distancing between these groups, regardless of the evaluation time, at the 11th, 25th, or total (combination of the two evaluation times) (Figures 1A, 1B, and 1C).These results suggest that the inoculated microbiota was able to interact or colonize the plant's tissues and in this mutualistic interaction, the microorganisms, through direct and indirect mechanisms (Oliveira et al., 2018; Elnahal et al., 2022), promoted the plant's development. Meanwhile, the plant provides carbonaceous compounds to sustain the synthetic bacterial community (Azevedo et al., 2000) and thus both develop.

Similar to what was found in this study, promising results in the germination, morphophysiological characteristics, and production of plants inoculated with PGPBs were found in other species of forage grasses, such as elephant grass cv. BRS Kurumi (Rodrigues et al., 2016), sugarcane (Lima et al., 2021), Brasilisk grass (Oliveira et al., 2022), and Marandu grass (Da Costa et al., 2022). Such studies that bioprospect methodologies to understand the microorganism-plant interaction and highlight the potential of new PGPB, using them in favor of plant and productive development with less impact on ecosystems, strongly contribute to environmental sustainability (Ramakrishna et al., 2019; Guimarães et al., 2023).

When considering the germination results, it can be seen that the inoculations promoted increases in GSI of over 42% compared to the commercial inoculums and the control treatment (Tables 1 and 2).

Table 1 – Comparison between groups of means by orthogonal contrasts for the characteristics of germination and initial development of elephant grass seedlings (Pennisetum purpureum Schum.) cultivar BRS Capiaçu, evaluated at the 11th and up to the 25th day in a germination chamber at 25±5°C under a 12-hour photoperiod after inoculation with potentially plant growth-promoting bacteria.

Groups	11th day after inoculation with growth-promoting bacteria								25th day after inoculation with growth-promoting bacteria							
	GSI	NP	LP	WC	NR	LR	GW	DW	NP	LP	WC	SPAD		NR	LR	GW	DW
	%		cm	mm		cm	------ g ------			cm	mm	a	b		cm	------ g ------	
TI	1,460	0,810	36,660	5,896	8,094	58,161	16,643	4,210	1,884	63,171	8,242	13,430	3,751	20,583	94,600	19,561	4,242
BI	1,520	0,863	39,262	6,760	8,630	59,471	16,831	4,221	2,034	65,372	8,471	14,088	3,866	21,400	94,691	20,254	4,235
CI	1,012	0,351	15,891	2,482	3,790	30,600	15,090	4,122	0,740	45,650	6,400	6,490	2,838	13,977	93,991	14,061	4,334
Control	1,027	0,410	23,120	4,411	6,211	33,030	14,590	3,331	1,441	22,890	9,419	3,594	1,700	9,634	54,782	14,090	3,481
BDI	1,691	1,005	43,515	6,352	9,330	59,850	18,000	4,462	2,044	65,131	8,452	14,041	3,771	20,790	94,027	20,546	4,280
BHI	1,355	0,721	35,010	6,255	7,925	58,335	15,661	3,975	2,100	64,145	8,413	14,732	3,882	21,000	93,278	20,632	4,321
EI	1,540	0,865	40,635	6,325	8,495	59,520	16,321	4,200	2,061	65,322	8,452	14,081	3,867	20,910	95,338	20,562	4,280
RI	1,505	0,862	37,890	6,280	8,760	58,665	17,342	4,235	2,070	63,957	8,411	14,691	3,787	20,882	91,961	20,619	4,292
General	1,244	0,610	29,890	5,154	7,153	45,596	15,617	3,771	1,663	43,031	8,831	8,512	2,726	15,109	74,691	16,826	3,862
TI vs. control																	
Student's t-test	4,279**	4,239**	8,963**	6,831**	2,180*	4,529**	1,738ns	2,565ns	4,871**	20,777**	-5,683**	11,684**	4,300**	17,630**	4,295**	6,165**	3,872**
BI vs. Control																	
Student's t-test	4,810**	4,832**	10,646**	8,537**	2,794**	5,138**	1,896*	2,590*	6,408**	21,835**	-4,558**	12,672**	4,524**	18,898**	4,290**	6,918**	3,802**
BI vs. CI																	
Student's t-test	6,741**	7,513**	21,179**	21,371**	7,677**	7,712**	2,029ns	0,406ns	19,213**	13,930**	13,680**	12,701**	2,954**	16,392**	0,117ns	9,556**	-0,713ns
BDI vs. BHI																	
Student's t-test	5,006**	4,798**	4,014**	4,916**	2,422*	3,688**	3,202**	2,418*	-0,481ns	-0,017ns	2,527*	-2943**	-0,419ns	1,757ns	0,719ns	-0,078ns	-0,258ns
EI vs. RI																	
Student's t-test	0,500ns	0,080ns	1,296ns	-1,891ns	-0,458ns	-0,063ns	-1,394ns	-0,203ns	-1,444ns	0,726ns	1,242ns	-0,420ns	0,735ns	1,352ns	1,862ns	0,346ns	1,016ns

TI: total inoculum (sum of bacterial inoculants plus commercial inoculants); BI: bacterial inoculants; CI: commercial inoculants (Biomais® and Biomaphos®); Control: no inoculum; BDI: *Brachiaria decumbens* isolates; BHI: *Brachiaria humidicola isolates;* EI: endophytic isolates; RI: rhizosphere isolates; General: overall average; GSI: germination speed index; NP: number of plumules; LP: aerial part length; WC: coleoptile width; NR: root number; LR: length of largest root; GW: green weight; DW: dry weight; SPAD: measurement of the green intensity of the primary plumule; ns: not significant; * and **: significant at 5% and 1% probability by the t-test, respectively.

The greatest increases occurred in treatments with the strains *Enterobacter kobei* CPI 105566 (UAGB69), *Rhizobium cauense* CCBAU 101002 (UAGB150), and *Sinomonas atrocyanea* DSM 20127 (UAGB71), with the latter standing out with an 84% increase compared to the control. *S. atrocyanea* has an *in vitro* production of indolic compounds of more than 110.0 µg mL^{-1} (Appendix), which possibly led to a greater increase in GSI than in the control treatment (Tables 3 and 4).According to Taiz et al. (2017), indolic compounds and auxin precursors act both on cell elongation and development and on root primordia and embryo stimuli, facilitating seed and seedling germination. Among the biotechnological potential of the PGPB used to develop inoculums, the ability to produce auxin-like compounds stands out in terms of increasing germination and initial plant development (Miljakovi'c et al., 2022; Oliveira et al., 2022). In the other treatments, the microorganism-plant interaction probably played a fundamental role in the positive results.

For the morphophysiological variables, in percentage terms, the inoculated microbiota enabled an increase compared to the commercial inoculums, ranging from 2% for DW to 138% for WC on the 11th day after inoculations, and from 0% for DW to 153% for NP on the 25th day after inoculations. As regards the control treatment, increases ranged from 14% for GW to 97% for NP on the 11th day after inoculations, and from 0% for WC to 273% for SPADa on the 25th day after inoculations (Table 2). The number of strains was higher than the control treatment in the variables LP, WC, and CR on the 11th day after inoculations, and LP, SPADa, NR, and GW on the 25th day after inoculations (Table 3). Factors such as the high percentage of increase and number of strains with plant growth promotion potential, as observed in this work, became an important target for manipulating the plant microbiome in favor of agricultural production (Ramakrishna et al., 2019; Rilling et al., 2019).

For WC, inoculations with the strains *R. cauense* CCBAU 101002 (UAGB150) and *Burkholderia cenocepacia* AU 1054 (UAGB139) stood out on the 11th and 25th day after inoculations, promoting increases of 113 and 10% compared to the control treatment, respectively (Table 4). The development of coleoptile in plants inoculated with PGPB was observed by Ünüvar et al. (2022) in the wheat crop, where they empha-

Table 2 – Performance of bacterial strains in relation to commercial inoculums and the control (no inoculum) in the germination test, morphological and physiological characteristics of elephant grass seedlings (Pennisetum purpureum Schum.) cultivar BRS Capiaçu grown at the 11th and up to the 25th day in a germination chamber at 25±5°C under a 12-hour photoperiod after inoculation with potentially plant growth-promoting bacteria.

Treatments	11th day after inoculation with growth-promoting bacteria								25th day after inoculation with growth-promoting bacteria								
	GSI	NP	LP	WC	NR	LR	GW	DW	NP	LP	WC	SPAD		NR	LR	GW	DW
	%		cm	mm		cm	------ g ------			cm	mm	a	B		cm	------ g ------	
All bacterial isolates (n=16)																	
MBI	1,460	0,810	36,660	5,896	8,094	58,161	16,643	4,210	1,884	63,171	8,242	13,430	3,751	20,583	94,600	19,561	4,242
MCI	1,005	0,347	15,894	2,476	3,787	30,597	15,086	4,116	0,743	45,646	6,404	6,486	2,838	13,969	93,897	14,059	4,330
MC	1,027	0,410	23,120	4,411	6,211	33,030	14,590	3,331	1,441	22,890	9,419	3,594	1,700	9,634	54,782	14,09	3,481
PSB1 (%)	45,27	133,43	130,65	138,13	133,73	90,09	10,32	2,28	153,56	38,39	28,70	107,15	32,17	47,35	0,75	39,14	0
PSB2 (%)	42,16	97,56	58,56	33,66	30,32	76,09	14,07	26,39	30,74	175,98	0	273,68	120,64	113,65	76,68	38,83	21,86
Isolates from roots of Brachiaria humidicola (Rendle.) Schweickerdt (n=4)																	
MBI	1,446	0,733	36,686	5,226	72,860	52,831	15,515	4,004	2,140	76,724	8,776	14,522	4,231	21,062	104,452	20,063	4,371
MCI	1,005	0,347	15,894	2,476	3,787	30,597	15,086	4,116	0,743	45,646	6,404	6,486	2,838	13,969	93,897	14,059	4,330
MC	1,027	0,410	23,120	4,411	6,211	33,030	14,590	3,331	1,441	22,890	9,419	3,594	1,700	9,634	54,782	14,090	3,481
PSB1 (%)	43,88	111,24	130,82	111,07	1781,61	72,67	2,84	2,72	188,02	68,33	37,04	124,56	36,27	54,69	11,24	42,71	0,95
PSB2 (%)	40,79	78,78	58,67	18,47	1073,08	59,94	6,34	20,20	48,51	235,18	-	304,06	127,47	118,62	90,67	42,39	25,56
Isolates from the rhizosphere of Brachiaria humidicola (Rendle.) Schweickerdt (n=4)																	
MBI	1,259	0,713	33,327	5,837	8,560	58,598	15,799	3,940	1,981	64,062	7,522	17,738	3,695	20,061	81,422	20,510	4,139
MCI	1,005	0,347	15,894	2,476	3,787	30,597	15,086	4,116	0,743	45,646	6,404	6,486	2,838	13,969	93,897	14,059	4,330
MC	1,027	0,410	23,120	4,411	6,211	33,030	14,590	3,331	1,441	22,890	9,419	3,594	1,700	9,634	54,782	14,090	3,481
PSB1 (%)	25,27	105,48	109,68	135,74	126,03	91,52	4,73	-	166,62	40,35	17,93	173,48	30,20	43,61	0	45,88	0
PSB2 (%)	22,59	73,90	44,15	132,32	37,82	77,40	8,29	18,28	37,47	179,86	0	393,54	117,35	108,23	48,62	45,56	18,90
Isolates from Brachiaria decumbens Stapf. roots (n=4)																	
MBI	1,628	1,001	44,419	6,808	9,704	65,837	17,123	4,387	1,556	66,507	8,447	12,720	3,841	20,447	94,007	20,774	4,300
MCI	1,005	0,347	15,894	2,476	3,787	30,597	15,086	4,116	0,743	45,646	6,404	6,486	2,838	13,969	93,897	14,059	4,330
MC	1,027	0,410	23,120	4,411	6,211	33,030	14,590	3,331	1,441	22,890	9,419	3,594	1,700	9,634	54,782	14,090	3,481
PSB1 (%)	61,68	188,47	179,47	174,96	156,24	115,17	13,50	6,58	109,42	45,70	31,90	96,11	35,34	46,37	0,12	47,76	0
PSB2 (%)	58,52	144,14	92,12	54,34	56,24	99,32	17,36	31,70	7,98	190,55	0	253,92	125,94	112,24	71,60	47,44	23,53
Isolates from the rhizosphere of Brachiaria decumbens (Rendle.) Schweickerdt (n=4)																	
MBI	1,747	1,035	42,444	7,412	8,963	69,111	18,883	4,534	2,281	64,166	9,074	11,372	3,700	24,031	98,878	19,644	4,101
MCI	1,005	0,347	15,894	2,476	3,787	30,597	15,086	4,116	0,743	45,646	6,404	6,486	2,838	13,969	93,897	14,059	4,330
MC	1,027	0,410	23,120	4,411	6,211	33,030	14,590	3,331	1,441	22,890	9,419	3,594	1,700	9,634	54,782	14,090	3,481
PSB1 (%)	73,83	198,27	148,17	199,35	136,68	125,87	25,17	10,16	243,34	40,57	41,69	75,33	30,37	72,03	5,30	39,76	0
PSB2 (%)	70,11	218,29	83,58	68,03	44,30	109,24	29,42	36,12	58,29	380,32	0	216,42	117,64	149,44	80,49	39,42	17,81

MBI: mean of bacterial isolates; MCI: mean of commercial inoculates (Biomais® and Biomaphos®); MC: mean of control; PBS1: performance of bacterial strains - percentage increase in MBI in relation to commercial inoculates; PSB2: performance of bacterial strains - percentage increase in MBI in relation to control; GSI: germination speed index; NP: number of plumules; LP: aerial part length; WC: coleoptile width; NR: root number; LR: length of largest root; GW: green weight; DW: dry weight; SPAD: measurement of the green intensity of the primary plumule ● treatment with bacterial inoculums (of which: ▪ treatment with bacterial inoculums isolated from *Brachiaria decumbens* Stapf., and + treatment with bacterial inoculums isolated from *Brachiaria humidicola* (Rendle) Schweickerdt); × treatment with commercial inoculums (Biomais® and Biomaphos®); and ▲ control treatment.

sized the need for this structure to fully develop in order for the plants to emerge properly, corroborating Rebetzke et al. (2007) statement that seedling development depends on the coleoptile, an important factor in crop establishment.

In relation to the NR and LR variables, *Klebsiella variicola* F2R9 (UAGB154) provided the greatest increase in the control treatment, 86% in the NR variable, and *S. atrocyanea* DSM 20127 (UAGB71), with 129% for LR (Table 4) on the 11th day after inoculation. On the 25th day after inoculation, the bacteria that provided the greatest increases were *Sphingomonas paucimobilis* DSM 30198 (UAGB80) with a 194% increase in NR and *Burkholderia territorii* LMG 28158 (UAGB105) with a 124% increase in LR (Table 4).

The increase in the morphology of the root system, with the variables NR and LR, provided by the genera *Burkholderia*, *Klebsiella*, and *Sphingomonas*, can be explained by Li et al. (2016). According to the authors, most strains belonging to these genera, when associated with the plant, are capable of producing biofilm on the surface of the roots, in addition to phytohormones that promote a significant increase in the thickness of these structures. Better root development enables efficient absorption of both water and nutrients, also

Table 3 – Germination characteristics and initial development of elephant grass seedlings (*Pennisetum purpureum* Schum.) cultivar BRS Capiaçu grown at the 11th and up to the 25th day in a germination chamber at 25±5°C under a 12-hour photoperiod after inoculation with potentially plant growth-promoting bacteria.

Treatments	11th day after inoculation with growth-promoting bacteria								25th day after inoculation of growth-promoting bacteria							
	GSI	NP	LP	WC	NR	LR	GW	DW	NP	LP	WC	SPAD		NR	LR	GW	DW
	%		cm	mm		cm	------ g ------			cm	mm	a	b		cm	------ g ------	
UAGB167	1,610*	1,045*	44,937*	6,352*	8,035ns	68,000*	13,670ns	3,272ns	2,000*	78,252*	8,100ns	24,325*	3,640ns	24,792*	94,562*	15,417ns	3,530ns
UAGB105	1,605*	1,070*	44,080*	5,980*	5,700ns	67,810*	16,240ns	3,955ns	3,125*	57,755*	7,645ns	12,000*	3,700*	20,437*	123,172*	24,152*	4,612*
UAGB150	1,797*	1,122*	54,015*	9,415*	5,535ns	62,305*	17,762ns	4,037ns	2,562*	86,685*	8,147ns	12,432*	2,550ns	23,062*	98,707*	17,965*	3,517ns
UAGB154	1,595*	1,220*	45,927*	6,292*	11,565*	65,722*	19,302ns	5,000*	1,675ns	66,752*	8,355ns	5,887ns	3,050ns	21,187*	85,785ns	25,882*	5,840*
UAGB69	1,692*	0,970*	44,515*	6,897*	9,907*	67,107*	20,012*	5,492*	1,625ns	76,045*	8,770ns	11,900*	3,550ns	21,500*	84,777ns	23,862*	4,355*
UAGB128	1,620*	1,002*	35,350*	6,685*	10,725*	65,965*	17,115ns	4,150ns	1,562ns	83,637*	7,520ns	11,750*	2,332ns	22,870*	84,855ns	21,277*	4,465*
UAGB60	1,350ns	0,680ns	33,082*	6,750*	7,357ns	64,407*	14,785ns	3,742ns	2,375*	42,522*	6,850ns	15,562*	5,125*	15,000*	86,390ns	17,082ns	3,532ns
UAGB10	1,327ns	0,702ns	27,655ns	5,415ns	6,002ns	63,635*	17,115ns	4,402ns	1,000ns	64,995*	9,407ns	16,092*	3,925*	19,375*	69,937ns	23,622*	5,175*
UAGB139	1,660*	0,752ns	33,780*	6,275*	10,595*	70,205*	22,987*	5,150*	2,500*	62,625*	10,377*	12,275*	4,100*	27,000*	117,615*	25,325*	4,912*
UAGB71	1,892*	1,032*	35,102*	7,512*	9,690ns	75,755*	17,805ns	4,647ns	2,562*	63,767*	9,287ns	11,762*	3,850*	17,687*	107,700*	16,807ns	4,425*
UAGB119	0,740ns	0,470ns	37,222*	4,497ns	10,157*	40,385ns	14,180ns	3,467ns	2,987*	65,097*	6,512ns	27,550*	3,400ns	23,000*	84,507ns	20,060*	3,385ns
UAGB110	1,242ns	0,440ns	14,910ns	2,440ns	6,407ns	40,180ns	15,995ns	4,027ns	1,950*	67,125*	8,447ns	14,975*	4,350*	17,375*	98,960*	23,172*	5,367*
UAGB1	1,620*	0,652ns	43,792*	6,470*	8,940ns	61,982*	14,192ns	3,370ns	2,000*	65,717*	9,655ns	14,927*	3,575ns	21,437*	95,550*	15,242ns	3,517ns
UAGB156	1,617*	0,770ns	42,927*	7,692*	9,310ns	62,472*	15,510ns	3,785ns	1,500ns	44,980*	8,562ns	12,050*	5,125*	14,312*	110,905*	17,937*	3,475ns
UAGB80	1,642*	1,137*	46,882*	6,447*	10,035*	68,160*	16,980ns	4,302ns	1,500ns	43,587*	8,485ns	9,022*	4,300*	28,375*	71,490ns	18,480*	3,552ns
UAGB106	1,317ns	0,770ns	43,960*	6,012*	8,097ns	41,352ns	15,632ns	4,665ns	1,487ns	76,302*	9,357ns	16,187*	5,300*	25,000*	100,127*	17,687*	3,990ns
Biomais*	1,157ns	0,410ns	14,300ns	2,427ns	3,885ns	29,262ns	14,672ns	3,627ns	1,255ns	53,192*	6,542ns	8,947*	3,875*	21,125*	102,527*	15,470ns	4,385*
Biomaphos*	0,852ns	0,285ns	17,487ns	2,525ns	3,690ns	31,932ns	15,500ns	4,605ns	0,230ns	38,100*	6,265ns	4,025ns	1,800ns	6,812ns	85,267ns	12,647ns	4,275ns

GSI: germination speed index; NP: number of plumules; LP: aerial part length; WC: coleoptile width; NR: root number; LR: length of largest root; GW: green weight; DW: dry weight; SPAD: measurement of the green intensity of the primary plumule; ns : not significant; *: significant, superior to the control, by Dunnett's test, up to a probability level of 5%.

increasing biomass production capacity, and consequently, tolerance to biotic stresses such as cutting and/or animal consumption, and abiotic stresses such as drought (Reis et al., 2013; Souza et al., 2017; Fukami et al., 2018).

For GW and DW on the 11th day after inoculations, two and three bacterial inoculums differed from the control treatment and were superior to the commercial inoculums (Table 3), with the strains *B. cenocepacia* AU 1054 (UAGB139) and *E. kobei* CPI 105566 (UAGB69) providing the greatest increases, with 57 and 64%, respectively (Table 4). On the 25th day after inoculation, 12 bacterial inoculums differed from the control for GW and eight for DW (Table 3). In both variables, *K. variicola* F2R9 (UAGB154) provided the greatest increases compared to the control treatment, with 83% for GW and 67% for DW (Table 4).

Similar to this study, various authors observed the improvements in the morphological characteristics of plants through the inoculation of PGPB. Rodrigues et al. (2016) identified increases in corn plants inoculated in a controlled environment with endophytic and rhizospheric microorganisms isolated from sugarcane plants, with a large contribution from the *Klebsiella* and *Enterobacter* genera, associating the performance of both with the ability to produce indoleacetic acid. Li et al. (2016), using a co-inoculum composed of the genera *Enterobacter*, *Sphingomonas*, *Pantoea*, and *Bacillus* isolated from elephant grass, obtained increases of 116 and 81% for GW and DW, respectively, in an elephant grass hybrid (*P. americanum* × *P. purpureum* Schumach) when compared to uninoculated plants. The maximization of these variables by the co-inoculum was explained by the high production of phytohormones and siderophores by the strains used. The authors, therefore, suggest that the use of PGPB in elephant grass is effective in improving plant development and, consequently, the biomass yield of this forage crop.

For SPAD a and b levels, inoculations with *E. kobei* CIP 105566 (UAGB119) and *S. paucimobilis* DSM 30198 (UAGB80) led to increases of 666 and 211%, respectively, when compared to the control treatment (Table 4). These results can possibly be explained by the fact that both

Table 4 – Germination characteristics and initial development of elephant grass seedlings (*Pennisetum purpureum* Schum.) cultivar BRS Capiaçu grown at the 11th and up to 25th day in a germination chamber at 25±5°C under a 12-hour photoperiod after inoculation with potentially plant growth-promoting bacteria pre-selected by the Dunnet test.

Treatments	11th day after inoculation with growth-promoting bacteria								25th day after inoculation with growth-promoting bacteria							
	GSI	NP	LP	WC	NR	LR	GW	DW	NP	LP	WC	SPAD		NR	LR	GW	DW
	%		cm	mm		cm	------ g ------			cm	mm	a	b		cm	------ g ------	
UAGB167	1,610c	1,045c	44,937c	6,352c	-	68,000b	-	-	2,000d	78,252b	-	24,325b	-	24,792b	94,562c	-	-
UAGB105	1,605c	1,070c	44,080c	5,980c	-	67,810c	-	-	3,125a	57,755d	-	12,000d	3,700c	20,437d	123,172a	24,152a	4,612c
UAGB150	1,797b	1,122b	54,015a	9,415a	-	62,305c	-	-	2,562b	86,685a	-	12,432d	-	23,062c	98,707c	17,965c	-
UAGB154	1,595c	1,220a	45,927b	6,292c	11,565a	65,722c	-	5,000a	-	66,752c	-	-	-	21,187d	-	25,882a	5,840a
UAGB69	1,692c	0,970c	44,515c	6,897c	9,907a	67,107c	20,012a	5,492a	-	76,045b	-	11,900d	-	21,500d	-	23,862a	4,355c
UAGB128	1,620c	1,002c	35,350d	6,685c	10,725a	65,965c	-	-	-	83,637a	-	11,750d	-	22,870c	-	21,277b	4,465c
UAGB60	-	-	33,082d	6,750c	-	64,407c	-	-	2,375c	42,522e	-	15,562c	5,125a	15,000f	-	-	-
UAGB10	-	-	-	-	-	63,635c	-	-	-	64,995c	-	16,092c	3,925c	19,375d	-	23,622a	5,175b
UAGB139	1,660c	-	33,780d	6,275c	10,595a	70,205a	22,987a	5,150a	2,500b	62,625c	10,377a	12,275d	4,100b	27,000a	117,615a	25,325a	4,912b
UAGB71	1,892a	1,032c	35,102d	7,512b	-	75,755a	-	-	2,562b	63,767c	-	11,762d	3,850c	17,687e	107,700b	-	4,425c
UAGB119	-	-	37,222d	-	10,157a	-	-	-	2,987a	65,097c	-	27,550a	-	23,000c	-	20,060b	-
UAGB110	-	-	-	-	-	-	-	-	1,950d	67,125c	-	14,975c	4,350b	17,375e	98,960c	23,172a	5,367b
UAGB1	1,620c	-	43,792c	6,470c	-	61,982c	-	-	2,000d	65,717c	-	14,927c	-	21,437d	95,550c	-	-
UAGB156	1,617c	-	42,927c	7,692b	-	62,472c	-	-	-	44,980e	-	12,050d	5,125a	14,312f	110,905b	17,937c	-
UAGB80	1,642c	1,137b	46,882b	6,447c	10,035a	68,160b	-	-	-	43,587e	-	9,022e	4,300b	28,375a	-	18,480c	-
UAGB106	-	-	43,960c	6,012c	-	-	-	-	-	76,302b	-	16,187c	5,300a	25,000b	100,127b	17,687c	-
Biomais®	-	-	-	-	-	-	-	-	-	53,192d	-	8,947e	3,875c	21,125d	102,527b	-	4,385c
Biomaphos®	-	-	-	-	-	-	-	-	-	38,100e	-	-	-	-	-	-	-

GSI: germination speed index; NP: number of plumules; LP: aerial part length; WC: coleoptile width; NR: root number; LR: length of largest root; GW: green weight; DW: dry weight; SPAD: measurement of the green intensity of the primary plumule. Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability

bacteria are diazotrophic and interact more with BRS Capiaçu plants. Mutumba et al. (2019), by inoculating *Enterobacter* and other strains into wheat plants, observed significant elevations in the SPAD index. The authors believe that it is because these strains produce beneficial effects on the plant's physiological parameters due to biological nitrogen fixation. Teodoro et al. (2024) noted increases in the SPAD index in *Megathyrsus maximus* grass plants inoculated with PGPB and different doses of fertilizer, and stressed the importance of inoculation in pastures, given the benefits of the microorganism-plant interaction for its development.

The poor performance of the commercial inoculums in relation to the bacterial inoculations evaluated and the control treatment may have been due to both products not being registered for elephant grass, and therefore, do not have specificity for BRS Capiaçu, and/or may have been a reflection of the inoculation method used, which was directly on the seedling. When the recommendation by manufacturers is via seed in the corn crop, this condition may influence its efficiency. According to Ecco et al. (2022), variations in the response of inoculants in the field or controlled environments depend on their correct contact with the plant and inoculation routes.

The five strains *E. kobei* CPI 105566 (UAGB69), *S. atrocyanea* DSM 20127 (UAGB71), *B. cenocepacia* AU 1054 (UAGB139), *R. cauense* CCBAU 101002 (UAGB150), and *K. variicola* F2R9 (UAGB154) obtained the highest percentages of increase in relation to the control treatment on the 11th day after inoculations (Table 4), all were isolated from *B. decumbens*. The isolated strains of *B. decumbens* group together and distance themselves from the other treatments (Figure 1D). They differ from the *B. humidicola* isolates (Table 1), providing the highest percentages of increase regardless of the isolation niche (Table 2).

This was not observed on the 25th day after inoculation, where there was a change in the strains highlighted, with the maintenance of *B. cenocepacia* AU 1054 (UAGB139), *R. cauense* CCBAU 101002 (UAGB150), and *K. variicola* F2R9 (UAGB158) isolat-

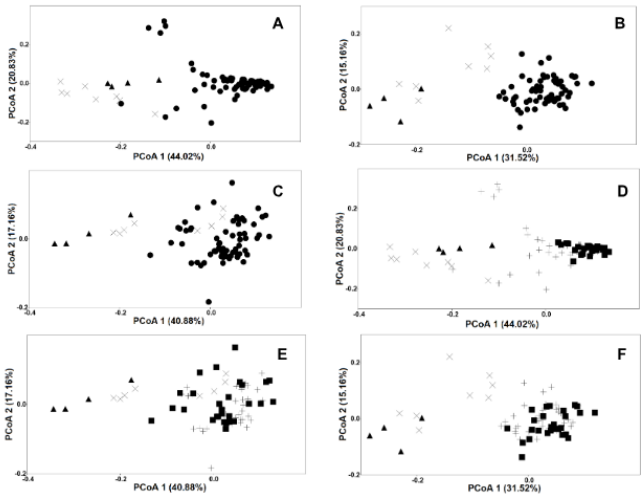

Figure 1 – Principal coordinates analysis of germination variables and morphological and physiological characteristics of elephant grass (Pennisetum purpureum Schumach) cultivar BRS Capiaçu grown at the 11th and 25th days in a germination chamber at 25±5°C under a 12-hour photoperiod after inoculation with potentially plant growth-promoting bacteria. Grouping of treatments: (A) On the 11th day after inoculations; (B) On the 25th day after inoculations; (C) In total, data from the 11th and 25th day after inoculations; (D) Differentiating the plant species from which the bacteria were isolated, on the 11th day after inoculations; (E) Differentiating the plant species from which the bacteria were isolated, on the 25th day after inoculations; (F) Differentiating the plant species from which the bacteria were isolated, in total data from the 11th and 25th day after inoculations.

ed from *B. decumbens*, and the incorporation of *S. paucimobilis* DSM 3018 (UAGB80) isolated from *B. decumbens*, and *B. territorii* LMG 28158 *(*UAGB105) and *E. kobei* CIP 105566 (UAGB119) isolated from *B. humidicola* (Table 1 and Appendix), providing a greater interaction between the groups of isolates by plant species (Figures 1E and 1F). It should be noted that there was no influence of the isolate niches of the strains on their ability to favor the germination and initial development of BRS Capiaçu seedlings (Table 1).

Similar recent studies support our results, with strains belonging to the genera *Burkholderia*, *Enterobacter*, and *Klebsiella* showing the highest percentages of increase compared to the control during the evaluations (Table 4), essentially characterized by producing auxin-like compounds in culture medium with and without the addition of the amino acid precursor L-Tryptophan and being diazotrophic (Appendix).

Inoculation with multifunctional microorganisms, such as those belonging to the genera *Burkholderia*, *Enterobacter*, *Azospirillum*, and *Klebsiella*, favors germination and plant development, and consequently, significant plant production (Oliveira et al., 2022; Battisti and Vendruscolo, 2023). Therefore, corroborating the entire hypothesis of the work, even knowing the microbial potential in aiding the germination and initial development of forage plants, further research is needed detailing the molecular aspects that relate to the taxonomy-function binomial to precisely identify which microorganisms perform key communication functions and growth promotion functions within this synthetic community (Mendes et al., 2011).

Finally, it is proposed that it is crucial to elucidate or bioprospect new microorganisms capable of promoting the increase of physiological and morphological characteristics of forage plants *in vivo* (Abdelaal et al., 2021). In general, these concepts support this study's hypothesis of using PGPB to improve the germination and initial development of BRS Capiaçu seedlings, thus, guaranteeing a lower proportion of synthetic inputs applied in weeding areas, and greater productivity and environmental sustainability.

Conclusion

The bacterial inoculations promoted improvements in germination and in the morphological and physiological characteristics of BRS Capiaçu seedlings on the 11th and 25th days after inoculations, surpassing the control treatment and sometimes resembling or surpassing the commercial inoculums (Biomais® and Biomaphos®). Strains isolated from *B. decumbens* and with high production of indolic compounds stood out. The genera *Burkholderia*, *Enterobacter*, and *Klebsiella* promoted greater morphophysiological development of BRS Capiaçu seedlings.

Given the small number of studies relating the use of such microorganisms to the BRS Capiaçu cultivar, this work makes a promising contribution to the scientific community. The use of PGPB with high phytohormone production, besides helping to develop cultivars, also favors environmental sustainability by reducing the impact caused by chemical products.

Authors' Contributions

ESPÍNDOLA, N.L.: conceptualization, data curation, formal analysis, investigation, methodology, project and resource administration, software, validation, visualization, writing – original draft, writing – review and editing; TAVARES, B.M.S.: writing – original draft; SANTOS, J.M.G.: writing – original draft; BARBOSA, V.M.S.: writing – original draft; SANTOS, I.B.: writing – original draft; OLIVEIRA, J.T.C.: data curation, formal analysis, acquisition, methodology, project administration, resources, software, supervision, validation, visualization, writing – review and editing.

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Appendix

Appendix 1 - Characterization of origin and biotechnological potential of diazotrophic bacterial isolates from *Brachiaria decumbens* Stapf. and *Brachiaria humidicola* (Rendle) Schweick

Isolate code	Species identified*	QS	Enzimas (EI)			IPS	IAA (µg mL^{-1})	
			CE	P-5	P-8	AM	CLT	SLT
		Brachiaria decumbens Stapf. isolates in the Root Endophytic niche						
UAGB69	*Enterobacter kobei* CPI 105566	-	0.000	0.000	4.736	0.000	0.000	162.100	0.000
UAGB154	*Klebsiella variicola* F2R9	-	0.000	0.000	1.092	0.000	2.056	100.080	14.644
UAGB156	*Klebsiella variicola* AT-22	+	0.000	0.000	1.177	0.000	1.374	67.177	20.800
UAGB167	*Rhizobium hainanense* I66	+	0.000	0.000	0.000	0.000	0.000	138.641	15.940
Brachiaria decumbens Stapf. isolates in the Root Endophytic niche									
UAGB71	*Sinomonas atrocyanea* DSM 20127	-	0.000	4.003	3.764	2.765	0.000	113.670	0.000
UAGB80	*Sphingomonas paucimobilis* DSM 30198	-	0.000	0.000	1.869	1.622	2.037	5.844	0.000
UAGB139	*Burkholderia cenocepacia* AU 1054	+	0.000	0.000	2.988	0.000	5.484	4.722	2.533
UAGB150	*Rhizobium cauense* CCBAU 101002	-	1.038	6.275	4.394	2.477	3.155	5.033	3.999
Isolates of *B. humidicola* (Rendle) Schweick. in the Root Endophytic niche									
UAGB01	*Pantoea* sp.	+	0.000	0.000	4.256	1.807	3.827	35.521	5.469
UAGB105	*Burkholderia territorii* LMG 28158	+	1.685	0.000	0.000	0.000	3.050	51.785	23.877
UAGB106	*Burkholderia lata* 383	+	1.653	0.000	0.000	0.000	3.493	49.231	26.333
UAGB110	*Enterobacter* sp.	+	6.715	0.000	0.000	0.000	3.733	2.090	0.855
Isolates of *B. humidicola* (Rendle) Schweick. in the Rhizosphere niche									
UAGB10	*Bacillus anthracis* str. Ames	+	1.200	0.000	4.864	1.090	0.000	13.482	9.211
UAGB60	*Klebsiella* sp.	+	0.000	0.000	2.299	2.204	0.000	4.982	0.000
UAGB119	*Enterobacter kobei* CIP 105566	+	1.238	0.000	0.000	0.000	2.793	3.733	15.600
UAGB128	*Ralstonia pickettii* 12J	-	0.000	0.000	0.000	0.000	3.050	10.980	8.877

+ Positive for the evaluated characteristic; - Negative for evaluated characteristic; *QS* Production of the quorum sensing molecule; *EI* Enzymatic Index (relation between the diameter of the hydrolysis halo and the diameter of the bacterial colony); *CE* Cellulase production; *P-5* Pectinase production at pH 5.0; *P-8* Pectinase production at pH 8.0; *AM* Amylase production; *IPS* Inorganic phosphate solubilization index; *IAA* of indole acetic acid; *CLT* IAA production in culture medium with supply of the precursor amino acid L-Tryptophan; *SLT* IAA production without L-Tryptophan. Source: Oliveira et al (2018).