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Exogenous phytohormones and growth-promoting microorganisms in Basilisk grass cultivation

E.B. Andrade[1,*], F.A. Teixeira[2], D.D. Fries[3], N.T. Cruz[4], R.R. Jardim[1], H.S. da Silva[1], B.E.F. dos Santos[1], T.M. Vieira[1], A.A. Seixas[1] and J.P. dos Santos[1]

[1]State University of Southwest Bahia, Postgraduate Program in Animal Science, BA 415 Highway, 45700-000, Itapetinga, Bahia, Brazil
[2]State University of Southwest Bahia, Department of Rural and Animal Technology, BA 415 Highway, 45700-000, Itapetinga, Bahia, Brazil
[3]State University of Southwest Bahia Department of Exact and Natural Sciences, BA 415 Highway, 45700-000, Itapetinga, Bahia, Brazil
[4]Federal University of Recôncavo da Bahia, Center for Agrarian, Environmental and Biological Sciences, Rui Barbosa Str., 170, 44380-000 Cruz das Almas, Bahia, Brazil
[*]Correspondence: elienezoo@gmail.com

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Abstract. The use of plant growth-promoting bio-inputs has been widely disseminated as a means to optimise pasture production processes. This study was conducted to evaluate the effects of applying exogenous phytohormones along with different microorganisms on the productive characteristics of Basilisk grass (*Urochloa decumbens*). The experiment was conducted in a 4×2 factorial design, in a completely randomised layout, evaluating four microorganism inoculations (no inoculation; *Azospirillum brasilense* + *Pseudomonas fluorescens*; *Rhizophagus intraradices*; *A. brasilense* + *P. fluorescens* + *R. intraradices*), combined or not with an exogenous phytohormone based on cytokinin, gibberellin and auxin. The results showed that inoculation with plant growth-promoting microorganisms stimulated an increase in root volume. In addition, the presence of the microorganisms increased the concentration of chlorophyll pigments, resulting in a 14% increase in the crude protein content of Basilisk grass compared with the control. The use of exogenous phytohormones also resulted in higher concentrations of total chlorophyll pigments and crude protein content, with increase in 25% and 9.7% respectively. The combined use of bacteria and mycorrhizal fungi, along with exogenous phytohormones, increased the accumulation of forage mass and leaf biomass. The combination enhanced carbohydrate accumulation in the leaves of Basilisk grass, thereby improving its nutritional quality. Therefore, considering the evidence found in this research, it becomes evident that the application of exogenous phytohormones, when combined with the inoculation of *A. brasilense*, *P. fluorescens*, and *R. intraradices*, represents a strategy to enhance the productivity of Basilisk grass.

Key words: diazotrophic bacteria, *Urochloa decumbens*, arbuscular mycorrhizal fungi, plant growth regulator.

INTRODUCTION

The high demand for animal-based foods for global consumption, particularly in the agricultural sector, requires Brazil to intensify technologies aimed at achieving sustainable production. However, according to Dias-Filho (2014), approximately 50% of Brazilian pastures are classified as degraded, and only 20% are in good condition for use. These factors, combined with inadequate soil management and low resource utilisation, are causing arable lands to increasingly lose their potential for nutrient cycling, microorganism activity, plant productivity and consequently, pasture supply for animals.

Thus, technologies that reduce environmental impact are increasingly being adopted. Microbiological inoculants, also known as biofertilizers, improve the development of forage species by increasing their biomass and nutritional characteristics. They also allow for the replacement of chemical fertilisers through the action of plant growth-promoting microorganisms (PGPMs) such as bacteria and fungi that act in the rhizosphere, providing benefits to the plant, such as the synthesis of phytohormones, nutrient mobilisation and phosphorus solubilisation (Hungria et al., 2010).

In this context, plant growth-promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) stand out as cost-effective production systems. Examples include *Azospirillum brasilense*, *Pseudomonas fluorescens*, and *Rhizophagus intraradices*, which together reduce the need for fertilisers, increase nitrogen use efficiency through biological fixation (Cunha et al., 2014; Hourani, 2023), enhance root system development (Licea-Herrera et al., 2020), produce plant hormones that aid in cell growth, reduce water stress and improve pathogen resistance (Nadeem et al., 2014).

Moreover, there are reports on exogenous phytohormones or plant growth regulators (PGR) that promote the inhibition or modification of plant morphophysiological processes, similar to endogenous phytohormones. Under potential stress conditions, these substances positively regulate genes, thereby modulating plant responses and significantly improving water use efficiency (Colebrook et al., 2014). Alternatives involving the use of plant growth-promoting microorganisms and exogenous phytohormones have proven to be promising in forage production, stimulating growth while reducing the environmental impacts of soil degradation and nutrient loss in a sustainable and economical manner.

Thus, the aim of this study was to evaluate the application of exogenous phytohormones associated with different plant growth-promoting microorganisms in the cultivation of *Urochloa decumbens* cv. Basilisk.

MATERIAL AND METHODS

Experimental details and tratament

The experiment was conducted in a greenhouse located at the University of Southwest Bahia, Juvino Oliveira Campus, situated at the following coordinates: 15°38'46" south latitude, 40°15'24" west longitude, and an average altitude of 280 m, in the municipality of Itapetinga-BA, during the period from May to August 2021. The climate of the municipality, according to the Köppen classification, is 'Cw' type, humid mesothermal, and hot subhumid. The average minimum and maximum temperatures

during the experimental period were 17 °C and 36 °C, respectively. The average minimum and maximum humidity values were 22% and 86%, respectively.

The soil used was collected from the 0–20 cm depth layer at the Itapetinga Campus of the State University of Southwest Bahia (UESB). The collected soil was broken up and passed through a sieve with a 4 mm mesh size. Subsequently, the pots were filled with the soil, and samples were collected for soil analysis, which was carried out at the Department of Agricultural Engineering and Soils of UESB (Table 1).

Table 1. Physical and chemical analysis of soil

Granulometric composition (g kg^{-1})												
Sand				Silt					Clay			
555				355					90			
Chemical analysis												
pH	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+	SB	t	T	V	m	OM
	mg dm^{-3}	----------			cmolc dm^{-3}	of soil	----------			%		g dm^{-3}
6.4	14	0.87	1.6	1.7	0.0	1.7	4.2	4.2	5.9	71	0	10

SB – Sum of bases; t – Effective cation exchange capacity; T – Cation exchange capacity at pH 7; V – Base saturation; m – Aluminium saturation; OM – Organic matter.

According to the recommendations of the Soil Fertility Commission of the State of Minas Gerais, there was no need for liming, and potassium fertilisation was not necessary as it was already adequately available (Alvarez & Ribeiro, 1999). However, phosphorus availability was low, but no phosphorus fertilisation was applied due to treatments involving phosphorus-solubilising microorganisms. Nitrogen fertilisation was applied, with 50 kg ha^{-1} of N in the form of urea (44% N) being applied, corresponding to 0.35 g per pot, applied as topdressing after the uniformization cut.

To determine the field capacity, all pots with dry soil were weighed, saturated with water, and weighed again after complete drainage of water. The final weight obtained was subtracted from the dry soil weight, thus corresponding to the field capacity (FC) value, which was used to replenish losses due to evapotranspiration. The experimental units were maintained at 70% of the FC value determined.

Experimental design

The experiment was conducted in a 4×2 factorial design, with four treatments involving microorganisms: 1 – control (non-inoculated); 2 – Bacteria (*Azospirillum brasilense* + *Pseudomonas fluorescens*), 3 – Arbuscular mycorrhizal fungi (*Rhizophagus intraradices*), 4 – Bacteria (*Azospirillum brasilense and Pseudomonas fluorescens*) + Fungus (*Rhizophagus intraradices*), associated or not with phytohormones (auxin + gibberellin + cytokinin). The design was completely randomised, with five replicates, totalling 40 plastic pots, each filled with 13 kg of soil.

Before sowing, seeds were inoculated with bacteria, and phytohormones were administered in accordance with the treatments and manufacturers' recommendations. After this step, the seeds were mixed thoroughly and kept in the shade for 30 min. The commercial products BioFree®, composed of *Azospirillum brasilense* AbV6 and *Pseudomonas fluorescens* CCTB03 bacteria (300 mL per 10 kg of seeds), and Stimulate®, containing the phytohormones auxin, gibberellin, and cytokinin (10 mL per 10 kg of seeds), were used. The sowing was conducted in May 2021, with

simultaneous inoculation of the mycorrhizal fungus (*Rhizophagus intraradices*) directly into the experimental units, following the recommendations of the commercial product Rootella BR® (120 g ha^{-1} with 20,800 propagules g^{-1}).

When the plants were 15 days old, thinning was performed, maintaining four plants per pot, based on the parameters of vigour and plant homogeneity. On the 30th day after planting, a uniform cut was performed, with an average residue height of 15 cm, marking the beginning of the evaluations. Immediately after the uniform cut, nitrogen fertilisation was applied, followed by foliar re-inoculation with bacteria (500 mL ha^{-1} of Biofree®) and foliar re-application of phytohormones (500 mL ha^{-1} of Stimulate®). Bacterial re-inoculation and phytohormone re-application were performed after each cut.

Analysis of morphogenic and structural characteristics

Two cycles were conducted to evaluate the morphogenetic and structural characteristics: one between the uniform cut and the first cut, and another between the first and second cuts. In each cycle, two tillers per pot were marked. Every 3 days, the following were determined: leaf apex appearance, leaf length and width, and stem length (distance from the ground to the ligule of the youngest leaf). Based on these measurements, the following parameters were calculated: leaf appearance rate (leaves^{-1} day^{-1}), phyllochron (days^{-1} leaf^{-1}), stem elongation rate (cm), leaf width (cm), and final leaf length (cm). Height measurements were taken using a graduated ruler without compressing the forage, considering the upper limit as the height of the leaf curvature around the ruler. At the end of each evaluation period, the number of tillers in each experimental unit was counted to determine the tiller population density.

Determination of chlorophyll content and SPAD index

For the determination of the SPAD (Soil Plant Analytical Division) index, the SPAD 502 Plus device was used around 10 a.m., with readings taken on the middle third of three randomly chosen fully expanded leaves in each experimental unit. At the end of each evaluation period, two fully expanded leaves were collected from each replicate, always around 10:00 a.m., and placed in aluminium foil envelopes, which were immediately stored in ice and taken to the laboratory for determination of chlorophyll a, chlorophyll b according to the methodology of Hiscox & Israelstam (1979).

For this, 0.03 g of the fresh leaf mass collected was placed in a glass vial containing 5 mL of dimethyl sulfoxide and wrapped in aluminium foil for 72 h. The readings were taken on a spectrophotometer at absorbance wavelengths of 665, 649, and 480 nm. For the quantification of chlorophylls, the formulas defined by Wellburn (1994) were used: Chlorophyll a = (12.19×A665) - (3.45×A649); Chlorophyll b = (21.99×A649) - (5.32×A665); The values adjusted to mg g^{-1} of fresh matter. The total chlorophyll content was calculated by summing chlorophyll a and b.

Determination of productive and morphological characteristics

Two cuts were made with a residual height of 15 cm to determine dry matter production (number of days between cuts = 28). In each cut, two subsamples (two plants per subsample) were obtained from each experimental unit. The first subsample was separated into leaf, sheath + stem, and dead material. The material was weighed and placed in a forced-air oven at 55 °C for 72 h. Subsequently, the samples were weighed

again to determine dry mass, and the following characteristics were calculated: total plant dry matter production, leaf dry matter production, stem dry matter production, percentage of leaf blades, percentage of stems, and leaf-to-stem ratio.

Chemical-bromatological composition

The second sample (whole plant) was also weighed and placed in a forced-air oven at 55°C for 72 h, then weighed, ground in a knife mill with a 1-mm sieve, and subjected to chemical-bromatological analyses to determine the levels of dry matter (DM, INCT-CA method G-003/1), crude protein (CP, INCT-CA method N-001/1), mineral matter (MM, INCT-CA method M-001/1), aether extract (EE, INCT-CA method G-004/1), neutral detergent fibre (NDF, INCT-CA method F-002/1), acid detergent fibre (ADF, INCT-CA method F-004/1), hemicellulose, and lignin (INCT-CA method F-005/1), according to the techniques described by Detmann et al. (2021).

For the determination of non-fibre carbohydrates corrected for ash and protein (NFC) and total carbohydrates (TCHOT) in the samples, the equations proposed by Sniffen et al. (1992) were used. The total digestible nutrient (TDN) content of the forage was calculated using the equation proposed by Cappelle et al. (2001), and dry matter digestibility (DMD) was calculated using the equation proposed by Rodrigues (2010).

After harvesting, the collected roots were used to determine their length using a ruler graduated in centimetres. Root volume was determined using a volumetric flask containing a specific quantity of water in which fresh roots were immersed. The difference in volume before and after the immersion of the roots allowed the calculation of root volume. Subsequently, the roots were weighed and placed in a forced-air circulation oven at 55 °C for 72 h to determine the dry mass.

Statistical analysis

The data were subjected to analysis of variance using the statistical programme SAS On Demand for Academics, considering microorganisms (M), phytohormones (F), and the interaction M×F as sources of variation. The interaction was split or not when means were compared using Tukey's test at 5% significance level.

RESULTS

Morphogenetic characteristics of *Urochloa decumbens* cv. Basilisk showed no interaction effect ($P > 0.05$) between inoculation with microorganisms and the presence of phytohormones. The leaf appearance rate (LAR), phyllochron, final leaf length and width (FLL and FWL), tiller population density (TPD), and height did not show ($P > 0.05$) differences among the evaluated treatments, whereas the stem elongation rate (SER) was higher for Basilisk grass plants that received phytohormones (Table 2).

The total leaf mass and the stem percentage showed a significant interaction ($P < 0.05$) between microbial inoculation and the presence of phytohormones. However, for stem mass, leaf percentage, and the leaf-to-stem ratio, the interaction was not significant ($P > 0.05$) (Table 3).

Table 2. Morphogenic characteristics of Basilisk grass cultivated with or without microbial inoculation in the presence or absence of plant growth regulators

Item	Microorganisms				PGR		*P value*			CV
	Cont	Bac	Fun	Bac+Fun	With	Without	M*PGR	PGR	M	(%)
TApF	0.16	0.16	0.17	0.15	0.16	0.16	0.434	0.368	0.120	6.9
Phyllo	6.35	6.62	6.40	6.78	6.39	6.68	0.971	0.078	0.204	7.5
TAlC	1.14	1.12	1.16	1.11	1.15 a	1.11 b	0.892	0.032	0.363	6.0
FLL	20.62	21.11	21.16	22.06	21.49	20.98	0.110	0.345	0.306	7.9
FLW	1.51	1.53	1.56	1.63	1.54	1.58	0.574	0.359	0.164	8.0
TPD	10.32	10.57	10.07	10.82	10.5	10.3	0.257	0.474	0.27	8.3
HP	39.57	38.8	39.85	39.3	39.8	38.9	0.910	0.207	0.703	5.2

Means followed by different lowercase letters for phytohormone presence differ significantly according to Tukey's test ($P < 0.05$). TapF – leaf appearance rate (leaf^{-1} tiller^{-1} day^{-1}); Phyllo – phyllochron (day^{-1} leaf^{-1} tiller^{-1}); TAlC – stem elongation rate (cm^{-1} tiller^{-1} day^{-1}); FLL – final leaf length (cm); FLW – final leaf width (cm); TPD – tiller population density; HP – plant height (cm); Cont – control; Bac – Bacteria; Fun – Fungus; M*PGR – interaction between microorganisms (M) and plant growth regulators (PGR); CV – coefficient of variation.

Table 3. Agronomic characteristics of Basilisk grass cultivated with or without microbial inoculation in the presence or absence of plant growth regulators

Item	Microorganisms				PGR		*P value*			CV
	Cont	Bac	Fun	Bac+Fun	With	Without	M*PGR	PGR	M	(%)
TDB	9.8	9.8	11.0	11.0	10.1	10.7	0.003	0.073	0.009	10.2
LDB	6.26	6.39	6.87	6.96	6.4	6.9	< 0.001	0.07	0.084	10.6
SDB	3.7BC	3.5 C	4.1AB	4.2 A	3.8	3.9	0.257	0.608	0.001	16.5
Leaf	64.22	64.85	62.9	63.92	63.7	64.1	0.195	0.763	0.698	6.1
Stem	37.48	37.92	37.07	37.73	38.2	36.9	0.025	0.278	0.968	10.6
L/S	1.94	1.90	1.78	1.82	1.8	1.9	0.116	0.559	0.591	16.0

Means followed by different uppercase letters for microorganisms and lowercase letters for the presence of phytohormones differ significantly according to Tukey's test ($P < 0.05$). TDB – total dry biomass (g pot^{-1}); LDB – leaf dry biomass (g pot^{-1}); SDB – stem dry biomass (g pot^{-1}); leaf (%); stem (%); L/S – leaf to stem ratio; Cont – control; Bac – Bacteria; Fung – Fungus; M*PGR – interaction between microorganisms (M) and plant growth regulators (PGR); CV – coefficient of variation.

The highest accumulation of total dry biomass, leaf dry biomass, and stem percentage was achieved with co-inoculation of bacteria and fungi combined with the presence of PGR (Table 4).

Table 4. Unfolding of agronomic variables: significant interactions between microorganisms and plant growth regulators in Basilisk grass

Item	PGR	Microorganisms			
		Cont	Bac	Fun	Bac+Fun
TDB	Without	10.19Aa	10.93Aa	11.70Aa	9.92Ba
	With	9.25Ab	8.63Bb	10.29Aab	12.06Aa
LDB	Without	6.47Aa	7.39Aa	7.32Aa	6.15Ba
	With	6.05Ab	5.40Bb	6.43Aab	7.78Aa
Stem	Without	38.37Aa	35.41Aa	38.86Aa	34.80Ba
	With	36.59Aa	40.44Aa	35.30Aa	40.66Aa

Means followed by uppercase letters in the column and lowercase letters in the row differ significantly according to Tukey's test ($P < 0.05$). PGR – plant growth regulators; TDB – total dry biomass (g pot^{-1}); LDB – leaf dry biomass (g pot^{-1}); stem (%).

Root mass and volume were influenced by the interaction ($P < 0.05$) between microbial inoculation and phytohormones. Root length was influenced ($P < 0.05$) by the presence of phytohormones and was greater in plants that received this input (Table 5). The use of PGR results in a 5.96% increase in the final root length of basilisk grass.

Table 5. Root characteristics of Basilisk grass cultivated with or without microbial inoculation under the presence or absence of phytohormones

Item	Microorganisms				PGR		*P value*			CV
	Cont	Bac	Fun	Bac+Fun	Com	Sem	M*PGR	PGR	M	(%)
RB	15.5	19.0	16.0	17.1	16.1	17.4	0.002	0.004	< 0.001	8.1
RV	162.0	190.0	168.0	188.0	178	176	< 0.001	0.631	< 0.001	7.4
RL	46.4	46.8	46.2	47.1	48.0a	45.3b	0.545	0.003	0.874	5.9

Means followed by different uppercase letters for microorganisms and lowercase letters for the presence of phytohormones differ significantly according to Tukey's test ($P < 0.05$). RB – root biomass (g pot^{-1}); RV – root volume (mL); RL – root length (cm). Cont – control; Bac – Bacteria; Fung – Fungus; M*PGR – interaction between microorganisms (M) and plant growth regulators (PGR); CV – coefficient of variation.

In the presence of phytohormones, the control group plants, along with those inoculated with fungi, showed lower root mass and volume (Table 6). The use of phytohormones associated with bacteria resulted in a 37.14% increase in basilisk grass root biomass. In the absence of exogenous phytohormones, plants that were inoculated with microorganisms showed a 21% increase in root volume compared to plants in the control group (Table 6). Root volume was greater in plants without phytohormones when inoculated with microorganisms. When combined with phytohormones, bacteria had a more significant effect on root volume than fungi.

Table 6. Unfolding of the interaction between microorganisms and phytohormone on root mass and volume of Basilisk grass

Item	PGR	Microorganisms			
		Cont	Bac	Fun	Bac+Fun
RB	Without	16.62Aa	18.65Aa	17.50Aa	16.72Aa
	With	14.35Ab	19.68Aa	13.76Bb	17.50Aa
RV	Without	152Ab	188Aa	184Aa	180Aa
	With	172Aab	192Aa	152Bb	196Aa

Means followed by uppercase letters in the column and lowercase letters in the row differ significantly according to Tukey's test ($P < 0.05$). PGR – plant growth regulators; RB – root biomass (g pot^{-1}); RV – root volume (mL); Cont – control; Bac – Bacteria; Fung – Fungus.

The interaction between microbial inoculation and the presence of phytohormones was significant ($P < 0.05$) for chlorophyll a concentration, total chlorophyll, and SPAD index (Table 7).

The control group and bacteria + fungus, which were associated with the presence of phytohormones, showed higher concentrations of chlorophyll a and total chlorophyll when compared with the same groups in the absence of phytohormones (Table 8). With the addition of phytohormones, there were no differences between the inoculations with microorganisms. The combined use of phytohormones and microorganisms

(bacteria and fungi) resulted in a 20% increase in total chlorophyll. Additionally, a 25% increase was observed with the use of phytohormones alone.

Table 7. Concentration of chlorophylls and SPAD index of Basilisk grass cultivated with or without microbial inoculation in the presence or absence of phytohormone

Item	Microorganisms				PGR		*P value*			CV
	Cont	Bac	Fun	Bac+Fun	With	Without	M*PGR	PGR	M	(%)
CL*a*	0.48	0.53	0.54	0.53	0.55	0.49	0.01	<0.01	0.00	8.39
CL*b*	0.13	0.13	0.14	0.15	0.14	0.13	0.28	0.10	0.06	14.5
CLT	0.61	0.67	0.70	0.67	0.70	0.63	<0.01	<0.01	<0.01	7.2
SPD	22.76	22.85	23.38	22.71	22.95	22.91	<0.01	0.92	0.80	7.46

Means followed by different uppercase letters for microorganism differ from each other by Tukey's test ($P < 0.05$). CLa – chlorophyll *a*; CLb – chlorophyll *b*; CLT – total chlorophylls; SPAD index; Cont – control; Bac – Bacteria; Fung – fungus; M*PGR – interaction between microorganisms (M) and plant growth regulators (PGR); CV – coefficient of variation.

However, in the absence of phytohormones, higher levels of chlorophyll a and total chlorophyll were observed in the group inoculated with bacteria and isolated fungi. Basilisk grass plants exhibited a lower SPAD index when inoculated with *A. brasilense* and phytohormones. There was no difference in the SPAD index between plants that received phytohormones and those that did not, regardless of the microorganism inoculation.

Table 8. Unfolding of chlorophyll concentrations with significant interaction between microorganisms and phytohormones in Basilisk grass

Item	PGR	Microorganisms			
		Cont	Bac	Fun	Bac+Fun
CL *a*	Without	0.426Bb	0.552Aa	0.523Aa	0.496Bab
	With	0.534Aa	0.525Aa	0.575Aa	0.571Aa
CLT	Without	0.545Bb	0.690Aa	0.688Aa	0.615Bab
	With	0.682Aa	0.660Aa	0.723Aa	0.740Aa
SPAD	Without	22.3Aa	24.6Aa	22.7Aa	22.0Aa
	With	23.3Aa	21.1Ba	24.0Aa	23.5Aa

Means followed by uppercase letters in the column and lowercase letters in the row differ from each other by Tukey's test ($P < 0.05$). PGR – plant growth regulators; CL*a* – chlorophyll *a* (mg.g); CLT – Total Chlorophylls; SPAD index.

The contents of dry matter (DM), crude protein (CP), aether extract (EE), ash (ASH), total carbohydrates (TC), and non-fibrous carbohydrates (NFC) showed an interaction effect ($P < 0.05$) between inoculations and the presence of phytohormones (Table 9). The neutral detergent fibre (NDF) and lignin (LIG) contents were not affected ($P > 0.05$) by the factors evaluated in this experiment.

The levels of acid detergent fibre (ADF), hemicellulose (HEMI) and dry matter digestibility (DMD) were affected by phytohormones. Although plants that did not receive phytohormones had higher ADF content, plants that received phytohormones showed higher levels of HEMI and DMD.

Table 9. Bromatological characteristics of Basilisk grass cultivated with or without microbial inoculation under presence or absence of phytohormone

Item	Microorganisms				PGR		*P value*			CV
	Cont	Bac	Fung	Bac+Fun	With	Without	M*PGR	PGR	M	(%)
DM	19.4	20.7	18.9	20.7	20.6	19.3	0.024	0.092	0.226	11.9
CP	9.7	10.2	10.2	10.7	10.2	10.1	0.048	0.746	0.035	7.0
NDF	71.3	71.2	71.1	71.3	71.4	71.1	0.426	0.353	0.986	1.7
ADF	46.5	46.1	45.8	41.9	43.5b	46.7a	0.822	0.027	0.064	9.2
HEM	24.7	29.0	25.5	25.1	27.4 a	24.7b	0.934	0.038	0.082	15.4
LIG	4.8	5.6	5.2	5.0	5.2	5.1	0.385	0.745	0.313	19.1
ASH	7.5	8.1	8.2	8.1	8.3	7.9	0.010	< 0.001	0.217	3.3
TDN	54.1	53.1	54.1	54.0	54.0	54.1	0.426	0.353	0.988	0.9
TC	79.0	78.3	78.5	78.2	78.3	78.7	0.004	0.212	0.337	1.4
NFC	7.7	7.3	7.1	6.9	6.9	7.6	0.003	0.098	0.621	20.1
DMD	52.6	56.2	53.2	53.0	55.0a	52.5b	0.822	0.020	0.064	5.9

Means followed by different lowercase letters for phytohormone presence differ from each other by Tukey's test ($P < 0.05$). DM – dry matter; CP – crude protein; NDF – neutral detergent fibre; ADF – acid detergent fibre; HEM – hemicellulose; LIG – lignin; EE – ether extract; ASH – ash; TDN – total digestible nutrients; TC – total carbohydrates; NFC – non-fibrous carbohydrates; DMD – dry matter digestibility; Cont – control; Bac – Bacteria; Fung – fungus; M*PGR – interaction between microorganisms (M) and plant growth regulators (PGR); CV – coefficient of variation.

With co-inoculation, Basilisk grass plants exhibited higher dry matter content when phytohormones were added. In the absence of phytohormones, the dry matter content did not differ between the inoculations with microorganisms, whereas in the presence of phytohormones, the highest dry matter content was recorded for plants inoculated with bacteria and co-inoculated (Bac + Fung) (Table 10).

Table 10. Unfolding of bromatological characteristics with significant interaction between microorganisms and phytohormones of basilisk grass

Item	PGR	Microorganisms			
		Control	Bacteria	Fungus	Bac+Fung
DM	Without	20.5Aa	19.7Aa	18.6Aa	18.4Ba
	With	18.4Ab	21.7Aa	19.1Aab	24.1Aa
CP	Without	9.2Bb	9.9Aab	10.5Aab	11.0Aa
	With	10.1Aa	10.4Aa	9.9Aa	10.4Aa
ASH	Without	7.7Ba	7.9Ba	7.9Ba	8.2Aa
	With	8.2Aa	8.3Aa	8.5Aa	8.0Aa
TC	Without	80.0Aa	78.8Aab	78.7Aab	77.3Bb
	With	78.0Ba	78.2Aa	77.8Aa	79.1Aa
NFC	Without	8.9Aa	7.8Aab	8.2Aab	5.7Bb
	With	6.6Ba	6.7Aa	6.1Bb	8.1Aa

Means followed by uppercase letters in the column and lowercase letters in the row differ from each other by Tukey's test ($P < 0.05$). PGR – plant growth regulators; DM – dry matter; CP – crude protein; ASH – ash; TC – total carbohydrates; NFC – non-fibrous carbohydrates; Bac – Bacteria; Fung – fungus.

The control group, in the presence of phytohormones, showed a 9.7% increase in CP content compared to the same group that did not receive phytohormones. The highest crude protein (CP) content, when no phytohormones were added, was found in plants

that were co-inoculated; however, in the presence of the phytohormone-based product, this content did not vary with the application of microorganisms.

Plants from the control group, inoculated with bacteria and fungus individually, showed higher ash content when phytohormones were added to the system (Table 10). With or without phytohormones, the evaluated inoculations did not present differences in the mean ash content.

In the control group, Basilisk grass showed lower total carbohydrates (TC) and non-fibrous carbohydrates (NFC) content when evaluated alongside phytohormones. Conversely, plants that received co-inoculation exhibited higher TC and NFC contents when evaluated alongside phytohormones. Plants that did not receive phytohormone application showed higher total and non-fibrous carbohydrate content when they did not receive inoculation or were inoculated with bacteria and fungi individually (Table 10). In the presence of phytohormones, there was no difference between the inoculations with microorganisms.

DISCUSSION

The action of plant growth-promoting microorganisms (PGPM), such as the bacteria *Azospirillum brasilense*, *Pseudomonas fluorescens*, and the mycorrhizal fungus *Rhizophagus intraradices*, involves a symbiotic mechanism in which the host plant provides energy for the microorganism's growth while they assist the plant in its development. Among the benefits these microorganisms bring to forage plants, one can highlight the increase in water and nutrient absorption, synthesis and release of phytohormones, and activation of plant protection mechanisms (Dowarah et al., 2021; Guimarães et al., 2022; Liao et al., 2023). In this sense, the microorganism-plant interaction can alter the physiology involving mechanisms of plant tissue formation, thus resulting in modifications in the structure of morphological components of a forage plant.

The morphogenetic characteristics of forage plants are affected by a combination of factors involving their genetics and the ecological factors of the environment in which the plant is being cultivated (Cruz et al., 2022). In this study, the inoculation with microorganisms was not sufficient to alter the morphogenetic characteristics of Basilisk grass (Table 2). It is likely that the amount of nitrogen fertilisation (50 kg of N ha^{-1}) performed in all plants inferred the same pattern of morphogenetic characteristics of Basilisk grass, regardless of the use of inoculation with bio-inputs. According to Duarte et al. (2020), bacterial strains act differently depending on the forage plant species that are inserted in the pasture ecosystem. These authors reported that inoculation with different species of microorganisms did not promote changes in the phyllochron, leaf appearance rates, stem elongation, and tiller density of Xaraés (*Urochloa brizantha* cv. Xaraés) and Ruziziensis (*Urochloa ruziziensis*) grasses.

The use of plant growth-promoting bacteria (PGPB) and arbuscular mycorrhizal fungi in forage grasses has been widely advocated as a means of mitigating plant stresses (Goswami & Deka, 2020; Marro et al., 2022; Zhang et al., 2022). Thus, beyond reducing stress caused by factors existing in the pasture ecosystem, the action of these microorganisms can also aid in increasing the productivity of forage plants.

In this study, in the absence of exogenous phytohormones, inoculation with PGPB and AMF, either individually or co-inoculated, resulted in a 21% increase in root volume (Table 6) and a 14% increase in crude protein content (Table 10) compared with non-inoculated plants. These increases reveal the positive effect of the symbiotic interaction between the microorganisms and the forage plant used in this study. In support of our findings, in *Urochloa* syn. *Brachiaria* species, inoculation with *A. brasilense* (Hungria et al., 2021) and *P. fluorescens* (Lopes et al., 2018, 2021) promoted significant gains in root characteristics and chlorophyll pigment concentration of the forage plant.

Arbuscular mycorrhizal fungi can facilitate the absorption of nitrogen and phosphorus by plants with the assistance of specific proton pumps, such as H+-ATPases, which stimulate the absorption process of these nutrients (Lanfranco et al., 2018; Liu et al., 2020; Li et al., 2022). According to Hungria et al. (2021), inoculation with bacteria of the species *A. brasilense* provides nitrogen increment in pasture cultivation, thus serving as an ally for supplying the nitrogen inputs necessary for the production of forage grasses. In addition, bacteria of the species *P. fluorescens* can solubilise phosphorus present in the soil, facilitating the absorption of this nutrient by plant roots (Guimarães et al., 2022). In the bacteria-plant relationship, the facilitation mechanisms for nitrogen absorption involve physiological processes resulting from the presence of the nitrogenase enzyme complex (Kour et al., 2019), as well as the ACC-deaminase synthesis route (Danish et al., 2020), while the solubilisation and availability of phosphorus are carried out through acidification, chelation, and exchange reactions, which increase the availability and absorption of this nutrient for the plant (Senthil Kumar et al., 2018; Barin et al., 2022).

Inoculation with PGPM can promote the release of phytohormones (auxins, gibberellins, cytokinins, jasmonates, etc.) that act as regulators of plant growth (Fukami et al., 2017). Endogenous release of phytohormones is related not only to plant development and growth but also to plant survival mechanisms against stresses caused by biotic and abiotic factors (Iqbal et al., 2022), with each hormone playing a different role in the physiological maintenance of forage plants (Jogawat et al., 2021; Hussain et al., 2024). In this regard, the use of plant growth regulator (PGR) can assist in providing the hormonal boost that the plant needs for its development, thus increasing its forage production.

In light of the benefits that the association of plant growth-promoting microorganisms brings to the plant, it is possible to identify, in the data presented in this research, that the effect of co-inoculation can be enhanced with the use of PGR. Co-inoculation + phytohormones resulted in percentage increases of 21% for total mass and 26% for leaf mass compared with the absence of PGR (Table 4). This combination also benefited the percentage of grass stems, with a recorded increase of 16% compared with the control, where such gain is necessary to ensure plant support and structure. Previous studies have reported significant increases in forage production in the presence of PGR (Rocha et al., 2020) and rhizosphere microorganisms (Hungria et al., 2021; Guimarães et al., 2022).

The use of plant growth regulators as a form of bioinputs, aimed at contributing to the regrowth of Basilisk grass, could be a viable alternative, given that plants receiving the PGR showed higher rates of stem elongation (Table 2). Such a pattern can be identified in the literature where Oliveira et al. (2019) reported higher rates of stem

accumulation in Marandu grass (*Urochloa brizantha* cv. Marandu) when PGR was used, a result of the synergistic action of exogenous phytohormone utilisation. Furthermore, the beneficial effects of using plant growth regulators for Basilisk grass are evidenced by the increase in the root length (Table 5) and leaf digestibility (Table 9) of Basilisk grass.

In this experiment, the total mass and leaf mass in co-inoculated plants (PGPB + AMF) in the presence of PGR were correlated with the increase in photosynthetic activity promoted by the elevation of chlorophyll a and total (Table 8). The increase in chlorophyll content enhances the photosynthesis of the forage plant through the absorption of light and carbon, which will be used for plant growth and maintenance (Taiz et al., 2017). Thus, the elevation of photosynthetic pigments in Basilisk grass plants contributes to the increase in forage mass observed in plants inoculated with PGPB + AMF + PGR (Table 4). Furthermore, the effect of enhancing photosynthesis through the elevation of photosynthetic pigments was observed when the leaves of co-inoculated plants (PGPB + AMF) in the presence of PGR resulted in higher concentrations of carbohydrates (Table 10). This physiological increase in photosynthesis may provide greater energy reserves to be used for growth, productivity and mobilized during periods of stress experienced by the plant (Vendruscolo et al., 2021; Cruz et al., 2023).

Regardless of the use of microorganisms, plants showed longer roots with the use of phytohormones (Table 5). However, plants inoculated with fungi showed lower root mass and volume when associated with phytohormones (Table 7), but not enough to affect nutrient absorption. The presence of phytohormones can stimulate the development of root hairs in plants inoculated with AMF, thereby increasing branching and/or density. Liu et al. (2016) consider that studies reveal an increase in root hairs, especially in situations where cultivation is carried out in soil with low fertility.

Reinforcing the previously mentioned assertion, the data presented in this research infer efficient nutrient absorption by plants associated with *R. intraradices* in the presence of PGR, which obtained a 7.5% increase in mineral content in the aerial part (Table 10), surpassing plants grown without PGR, indicating a positive association between fungi and PGR on the nutritional status of the evaluated grass species. Moreover, inoculation with the association between PGPB and PGR increased the mineral content of Basilisk grass leaves by 5% compared with plants that received PGPB without growth regulators (Table 10). This indicates that the combined action of bacteria and PGR has the capacity to optimise mineral absorption and positively contribute to mineral fixation in Basilisk grass leaves.

It is still possible to identify the beneficial effect of using PGR on the nutritional quality of the forage plant when, in the data presented in this research, we identified that plants in the control group, with the addition of exogenous phytohormones, showed an increase of 9.8% and 6.4% for crude protein and mineral content (Table 10), respectively, compared with plants untreated with PGR. These results support the findings of Pezenti et al. (2022), who reported higher levels of crude protein in Napier grass (*Pennisetum purpureum* cv. Napier) when treated with plant growth regulators.

The increase in crude protein in plants treated with plant growth regulators may be related to the increase in total chlorophyll found in this study. Overall, chlorophylls are nitrogenous compounds related to the nitrogen content in the leaf, which is associated with biomass yield and improvements in the quality of this morphological component (López-Calderón et al., 2020; Kaspary et al., 2020).

Plants not inoculated with microorganisms (PGPB and AMF isolated or combined) and not treated with PGR showed lower levels of total chlorophyll, whereas plants inoculated with PGPB and AMF isolated or co-inoculated promoted an increase in chlorophyll a and total chlorophyll of 23% and 22%, respectively, for chlorophyll a and total concentration (Table 8), as well as lower levels of crude protein. Among the factors that can stimulate the reduction of photosynthetic pigments in leaves, leaf senescence promotes the degradation of chlorophylls, which are translocated to organs during growth. Thus, according to Huang et al. (2022), maintaining chlorophyll concentrations is of fundamental importance to keep the leaf in a vegetative state, delaying its senescence, which is a mechanism involving the synthesis and accumulation of ethylene that stimulates cell death, resulting in a decrease in leaf nutrients (Taiz et al., 2017; Yu et al., 2021). However, interactions between hormones, such as auxin and cytokinin, can reduce gene expression that activates leaf senescence, thereby delaying its death (Chen & Huang, 2022).

In this experiment, the PGR used comprised kinetin (which acts similarly to cytokinin), 4-indole-3-butyric acid (a form of synthetic auxin), and gibberellic acid (such as GA3). Thus, it is possible that the use of PGR delayed the process of leaf senescence, maintaining higher levels of chlorophyll and crude protein concentration compared with plants that were not treated with the growth regulator (Table 8 and 10).

CONCLUSIONS

The combination of plant growth regulators with *A. brasilense*, *P. fluorescens*, and *R. intraradices* contributes to the increase in Basilisk grass biomass. Furthermore, the use of a plant growth regulator leads to increases in crude protein content in non-inoculated plants. Inoculation with microorganisms improves the nutritional quality through the accumulation of chlorophyll pigments, resulting in the elevation of Basilisk grass's crude protein content.

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