

EFEITOS DE PROTOCOLOS DE ADUBAÇÃO SOBRE AS CARACTERÍSTICAS MORFOLÓGICAS E PRODUTIVAS DA *Brachiaria Brizantha* CV. MARANDU

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RESUMO

Objetivou-se avaliar as características morfogênicas, produtivas e a eficiência no uso da água da *Brachiaria brizantha* cv. Marandu na presença e ausência de calagem, sob diferentes protocolos de adubação. O experimento foi realizado em casa de vegetação, na Universidade Estadual do Sudoeste da Bahia, Campus “Juvino Oliveira”, em Itapetinga, BA, entre março a junho de 2023, sendo organizado em delineamento inteiramente casualizado, com quatro repetições, em esquema fatorial 2 x 5, composto pela presença ou ausência da calagem e diferentes dosagens de NPK (0, 50, 100, 150 e 200%, sobre a recomendação de 100% da Quinta Aproximação - Recomendações para o uso de corretivos e fertilizantes em Minas Gerais), avaliados durante dois períodos de 28 dias. A adubação impulsionou o desenvolvimento das características morfogênicas e produtivas das plantas. A taxa de aparecimento foliar e o filocrono apresentaram comportamento quadrático, com pontos ótimos em 173,6% e 153,8% da dose, respectivamente. A taxa de alongamento foliar, o índice SPAD, o número de perfilhos e a produção de biomassa apresentaram respostas linear crescente à adubação, com aumentos proporcionais às doses aplicadas. A massa seca total e a massa seca de raiz também apresentaram incremento linear, enquanto o volume de raiz e a eficiência do uso da água mostraram comportamentos quadráticos, com pontos ótimos em 222,2% e 181,3% da adubação, respectivamente. Assim, doses superiores a 150% de NPK favorecem o desenvolvimento vegetal, mesmo sem calagem, otimizando a produtividade e a eficiência hídrica.

Palavras-chave: Eficiência no uso da água. Fertilização. Morfogênese. Pastagem. Produtividade.

1 INTRODUÇÃO

Na alimentação animal a pastagem é a forma mais econômica em relação ao uso de concentrados por possuir os nutrientes disponíveis de forma abundante na criação em sistema extensivo. Além disso, forragens bem manejadas promovem a sustentabilidade ambiental por auxiliarem na conservação do solo, combatendo a erosão e o surgimento de ervas daninhas, ciclagem de nutrientes, capturam carbono e combatendo as mudanças climáticas (MARINS et al., 2025).

A degradação das pastagens é um desafio enfrentado mundialmente principalmente devido ao uso ineficiente do solo para a implantação de culturas agricultáveis, ajuste inadequado de lotação por área, resultando-se também em perdas na produtividade dos animais. Esse cenário compromete o desenvolvimento das gramíneas devido ao déficit de minerais e aumenta a degradação das pastagens (DIAS FILHO, 2017).

Sendo assim, é fundamental a reposição dos nutrientes a partir das exigências para manter a cobertura do solo, desenvolvimento adequado da parte aérea, bem como promover o pleno desenvolvimento do sistema radicular (BECKER, 2025). Além disso, a crescente demanda por produtos de origem animal e a importância dos impactos ambientais na produção tornam a adubação uma estratégia eficiente no manejo de solos com déficit nutricional, pois estimula no desenvolvimento sustentável e auxilia na manutenção das pastagens para aumento da produtividade (LEITE & ARAÚJO, 2025).

Sendo assim, a adubação é uma prática indispensável para impulsionar o crescimento das plantas, pois reage rapidamente no solo, suprindo os nutrientes necessários para estimular o crescimento de forma eficiente (FLORENTINO et al., 2022).

O nitrogênio é primordial para auxiliar no crescimento, pois aumenta o desenvolvimento das folhas, sendo essencial no metabolismo das plantas por promover a síntese de proteínas e clorofilas (ALMEIDA et al., 2023).

Alexandrino et al. (2010) avaliaram pastagens de *Brachiaria brizantha* cv. Marandu e verificaram que a adubação nitrogenada aplicada desde o estabelecimento atuou como diferencial para o aumento do número de perfilhos e folhas.

Após o nitrogênio, o fósforo aumenta a expansão da parte aérea e das raízes, assim como para o metabolismo energético, respiração celular, melhorando a absorção de água e nutrientes (COSTA et al., 2023; OLIVEIRA et al., 2022).

Já o potássio regula a osmose realizada nas células vegetais, atuando como o principal cátion no turgor, manutenção da eletroneutralidade nas células e ativador das enzimas responsáveis pela respiração e fotossíntese (TAIZ & ZEIGER et al., 2013).

Conforme Touhami et al. (2022) a combinação de nitrogênio, fósforo e potássio (NPK) aumenta a reutilização de cálcio e fósforo no solo, atuando na evolução das raízes e na atividade dos microrganismos.

Além disso, a eficiência na adubação possui relação direta com a irrigação devido ao fornecimento de água proporcionar retenção de nutrientes como o nitrogênio, amplificação da atividade enzimática no solo, aumento na absorção, transporte dos nutrientes e sua utilização pelas plantas (HAN et al., 2025).

O objetivo deste estudo foi avaliar as características morfogênicas, produtivas e a eficiência no uso da água da *Brachiaria brizantha* cv. Marandu sob níveis crescentes de adubação, com ou sem aplicação de calagem, buscando maximizar a produtividade e melhorar a qualidade dessa gramínea.

2 METODOLOGIA

2.1 LOCAL E MONTAGEM DO EXPERIMENTO

O experimento foi conduzido em casa de vegetação, na Universidade Estadual do Sudoeste da Bahia, em Itapetinga-BA, entre março a junho de 2023.

O ensaio foi realizado em esquema fatorial 2x5, sem ou com calagem, com doses crescentes de NPK, sendo 0, 50, 100, 150 e 200% sobre a recomendação de 100% do 5ª aproximação, para avaliar o potencial de resposta da planta abaixo e acima dos níveis recomendados de adubação, que correspondeu a 341 kg de ureia/ha, 611 kg de super simples/ha e 103 kg de cloreto de potássio/ha (CANTARUTTI et al., 1999). O experimento foi organizado em delineamento inteiramente casualizado, com quatro repetições, totalizando 40 unidades experimentais.

O solo foi coletado na fazenda Bela Vista, município de Encruzilhada-BA, sendo adotada profundidade de 0 a 20 cm, e gleba foi classificada como latossolo vermelho escuro com textura Franco Argilo Arenosa.

De acordo com a análise química e física do solo, realizada pelo Laboratório de Solos do Departamento de Engenharia Agrícola e Solos da UESB-Vitória da Conquista (Tabelas 1 e 2), foram feitas as recomendações do “5ª aproximação”, desenvolvido pela Comissão de Fertilidade do Solo do Estado de Minas Gerais (ALVAREZ e RIBEIRO et al., 1999).

Tabela 1. Análise física do solo.

Composição granulométrica (g/kg)			
Areia	Silte	Argila	Classe textural
515	30	340	Franco Argilo Arenosa

Fonte: Laboratório de solos da UESB-Vitória da Conquista

Tabela 2. Análise química do solo.

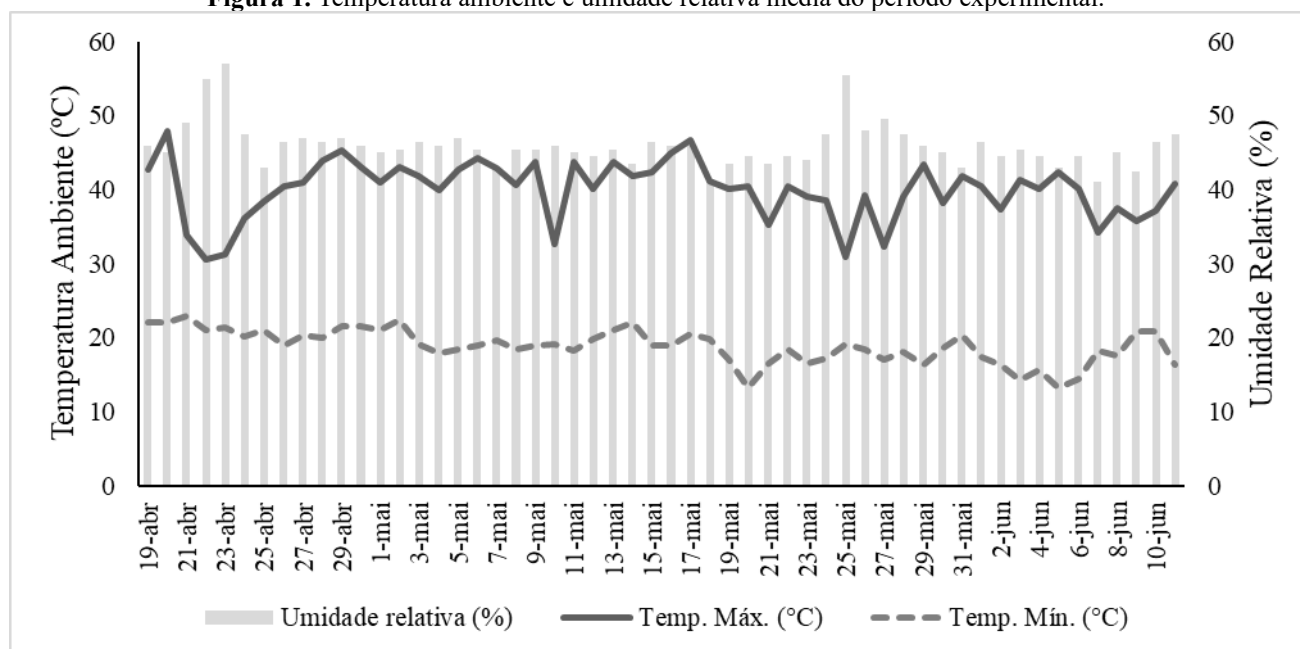
pH	*mg/dm ³		*cmolc/dm ³ de solo.....							%		
(H ₂ O)	P	K ⁺	Ca ²⁺	Mg ⁺	Al ³⁺	H ⁺	Na ⁺	SB ¹	t ²	T ³	V ⁴	m ⁵
4,5	5	0,13	0,6	0,4	1,1	3,6	-	1,1	2,2	5,8	19	49

¹Soma de Bases; ²CTC efetiva; ³CTC pH 7; ⁴Saturação de bases; ⁵Saturação por Al³⁺. Fonte: Laboratório de solos da UESB-Vitória da Conquista.

A calagem foi incorporada 30 dias antes do plantio, em vasos com capacidade de 12 litros, sendo cada um ocupado com 10 kg de solo seco e foi acrescentado 10,05 g de calcário dolomítico/vaso (correspondente a 1675 kg/ha) e com PRNT de 90%.

Foram feitas pesagens diárias, sempre mantendo os solos úmidos, com o registro pesos dos vasos durante o período experimental, assim como a umidade e temperaturas na casa de vegetação, obtidas por meio de um Termo-higrômetro Digital (Figuras 1).

Figura 1. Temperatura ambiente e umidade relativa média do período experimental.



A semeadura foi realizada em um canteiro de areia com 1 m x 0,80 cm, com o plantio de sementes de *Brachiaria brizantha* cv. Marandu. Posteriormente, aos 20 dias foi realizado o transplante, com a seleção de mudas uniformes e vigorosas, mantidas 4 plantas por vaso.

Um dia após o transplante foi realizada a adubação com ureia (fonte de N), Superfosfato simples (fonte de P) e Cloreto de potássio (fonte de K) para cada tratamento específico, diluídos na água da irrigação (quadro 1).

Quadro 1. Descrição dos tratamentos experimentais

Calagem	Dose NPK (%)	*Ureia		Superfosfato simples		Cloreto de potássio	
		(kg/ha)	(g/vaso)	(kg/ha)	(g/vaso)	(kg/ha)	(g/vaso)
Ausência / presença	0	---	---	---	---	---	---
	50	170,5	0,85	305,50	1,53	51,50	0,26
	100	341,0	1,71	611,0	3,05	103,0	0,52
	150	511,50	2,57	916,50	4,58	154,50	0,78
	200	682,0	3,41	1222,0	6,10	206,0	1,04

*Adubação fracionada em duas aplicações (transplante e primeiro corte). Fonte: Dados da pesquisa.

Transcorridos 48 dias do transplante, foi realizado o corte de uniformização, com resíduo de 15 cm do solo. Esse procedimento foi efetuado no primeiro e segundo corte. No estudo ocorreram dois períodos de 28 dias cada, totalizando 56 dias de avaliação.

2.2 ANÁLISES

2.2.1 Características morfológicas

Foram escolhidos dois perfilhos em cada vaso, homogêneos entre si e marcados com fitas, sendo realizadas medições no comprimento das folhas a cada três dias e no colmo ao final de cada período experimental.

Como parte das avaliações foram observados o aparecimento do ápice foliar, comprimento do colmo, número de folhas, segundo as instruções de Marcelino et al. (2006) foram calculados:

- Taxa de aparecimento foliar- TApF(folhas/dia/perfilho): divisão do número de folhas que emergiram nos perfilhos pelo período de rebrota.
- Filocrono = Inverso da TApF (dia/folha): $1 / TApF$
- Taxa de alongamento foliar- TAIF (cm/dia.perfilho): divisão da variação do comprimento das folhas pelo período de avaliação.
- Taxa de alongamento do colmo- TAIC (cm/perfilho.dia): diferença entre os comprimentos final e inicial do colmo dividida pelo intervalo de medidas.
- Duração de vida da folha- (DVF (dias): Tempo entre o aparecimento do ápice foliar e o primeiro sinal de senescência da lâmina.
- Número de perfilhos (NP) = contagem do número de perfilhos por vaso ao final de cada período.

2.2.2 Índice SPAD

A determinação da concentração de clorofila foi realizada de forma indireta, através do aparelho Chlorophyll meter SPAD-502 (Soil Plant Analysis Development) (MINOLTA, 1989). Os valores foram registrados no dia anterior a cada corte, a partir das 10 horas da manhã. Para realizar o

procedimento foram escolhidas, de modo aleatório, três folhas completamente expandidas por vaso e realizadas três leituras por folha, dentre elas um ponto em cada extremidade e um no centro da lâmina foliar, sempre com o sensor do aparelho na porção de um terço no meio da lâmina, evitando-se a nervura central.

2.2.3 Produção de massa fresca e seca

Após cada período experimental as amostras foram separadas e identificadas em sacos de papel. Em seguida, foram pesadas em balança analítica para obtenção da massa fresca. Em todos os períodos foram analisadas a produção da parte aérea (folha e colmo).

Já no segundo período também foram avaliadas a produção de resíduo e raiz. Após a pesagem, as amostras foram secas em estufa de ventilação forçada a 55°C por 72 horas. Após a secagem, foi realizada nova pesagem para determinar a massa seca. Com os dados obtidos em cada período, calculou-se a produção média de massa seca da parte aérea (considerando os dois períodos), bem como a produção de massa seca do resíduo e da raiz no segundo período.

2.2.4 Volume de raiz

Esse procedimento foi realizado somente no segundo corte, sendo utilizada uma proveta de 1000 mL (volume conhecido) para introduzir as raízes e com a diferença encontrada foi obtido o volume.

2.2.5 Uso e eficiência da água

Todos os vasos foram pesados para manter a umidade do solo, sendo registradas a quantidade de água repostas, e ao final de cada período foi calculada a quantidade de água utilizada.

Com a matéria seca produzida e a quantidade de água repostas durante o estudo foram efetuados cálculos para determinar a quantidade de água necessária para produzir 1 g de matéria seca com 1 L de água (gMS/L). Essa relação foi expressa em termos de eficiência de uso da água.

2.3 ANÁLISE ESTATÍSTICA

Os dados obtidos foram agrupados e analisados como a média dos dois cortes avaliativos e então submetidos à análise de variância (ANOVA), considerando como fontes de variação, os protocolos de adubação, calagem e sua interação, testados a 5% de probabilidade. A interação foi desdobrada, ou não, de acordo com a significância e o efeito dos protocolos de adubação foi avaliado

por análise de regressão enquanto o efeito calagem foi comparado pelo teste F, utilizando-se o programa estatístico SAEG Sistema para Análises Estatísticas (2007).

3 RESULTADOS E DISCUSSÃO

Não foi verificada interação entre calagem e adubação ($P>0,05$). Da mesma forma, as variáveis não foram influenciadas pela calagem ($P>0,05$), sendo que esta prática não contribuiu como o esperado para elevar a produtividade das plantas, o que pode ter sido resultado do ambiente controlado, o tempo de ação da calagem ter sido insuficiente, bem como a recomendação não ter sido eficiente para neutralizar a acidez do solo devido a sua textura arenosa.

Entretanto, houve efeito de adubação ($P<0,05$) para a taxa de aparecimento foliar (TApF), filocrono, taxa de alongamento foliar (TAIF), número de folhas vivas (NFV) índice SPAD (Tabela 3).

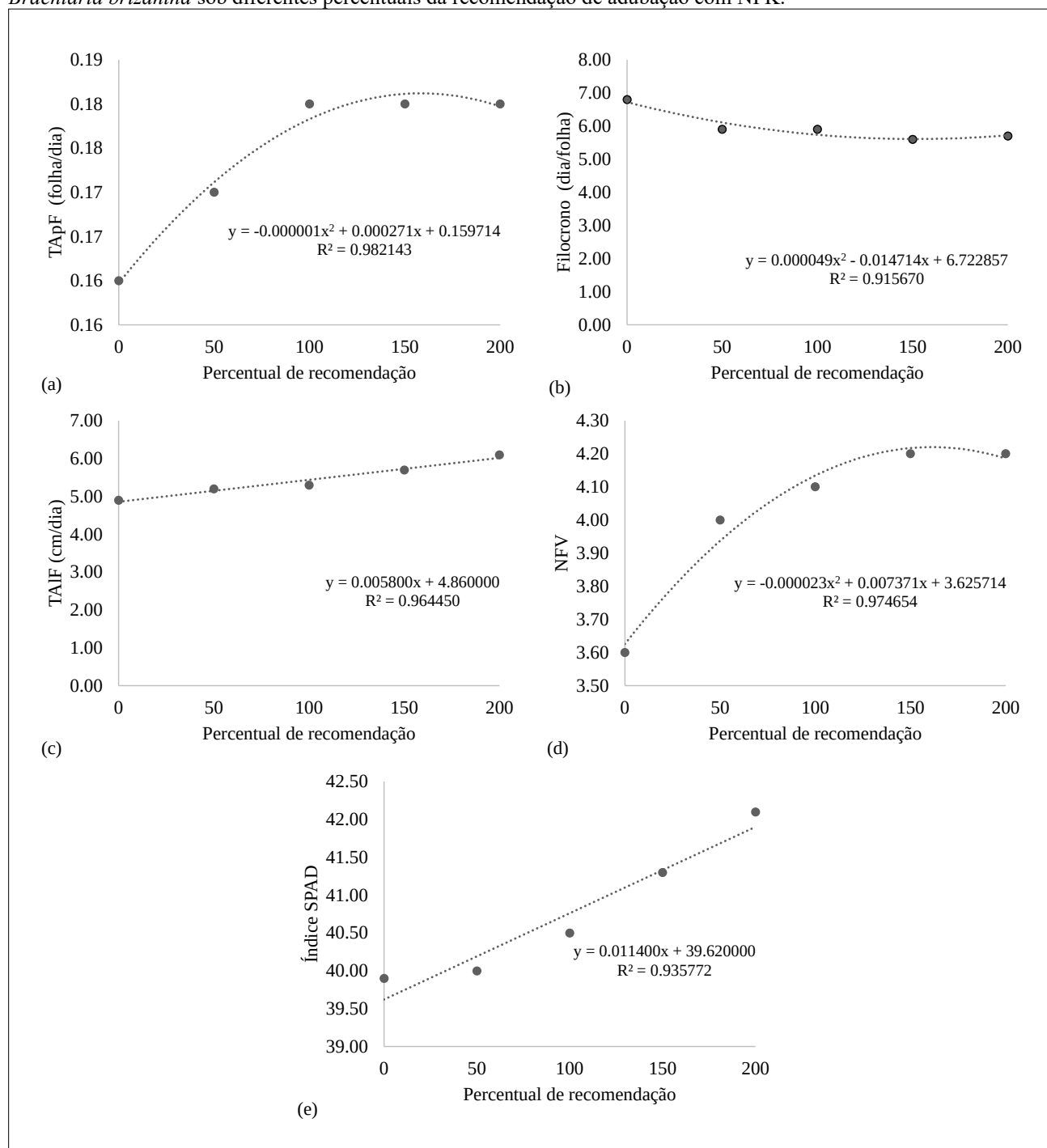
Tabela 2. Características morfogênicas e índice SPAD da *Brachiaria brizantha* sem ou com calagem sob diferentes percentuais da recomendação de adubação com NPK.

Variável	Calagem		Adubação (% da recomendação) ¹					EPM	Valor de P ²		
	Sem	Com	0	50	100	150	200		Cal	Adu	Cal*Adu
TApF (folha/dia)	0,17	0,18	0,16	0,17	0,18	0,18	0,18	0,0006	0,8245	0,0014	0,6745
Filocrono (dias/folha)	5,9	6,0	6,8	5,9	5,9	5,6	5,7	0,22	0,6173	0,0005	0,9018
TAIF (cm/dia)	5,5	5,6	4,9	5,2	5,3	5,7	6,1	0,28	0,8998	0,0013	0,1916
TAIC (cm/dia)	0,19	0,18	0,15	0,18	0,18	0,18	0,24	0,03	0,7613	0,0715	0,1816
DVF (dias)	23,0	22,9	22,7	23,0	23,0	23,0	23,0	0,16	0,32553	0,4229	0,4229
NFV (folhas/ perfilho)	4,0	4,0	3,6	4,0	4,1	4,2	4,2	0,12	0,8998	0,00015	0,7751
SPAD	41,0	40,5	39,9	40,0	40,5	41,3	42,1	0,59	0,1751	0,0282	0,5551

¹Adubação correspondente ao percentual da recomendação da 5ª aproximação. EPM=Erro padrão da média; Cal= calagem; Adu= adubo; CalxAdu= interação entre os fatores.

Para a TApF houve comportamento quadrático ($P<0,05$) com ponto de máxima em 173,6% da dose recomendada, o que correspondeu a 0,18 folhas/dia (Figura 2a). Essa variável impulsiona o surgimento e aumento da taxa fotossintética para auxiliar na recuperação da pastagem. Além disso, quanto maior é a TApF em relação ao filocrono ocorre o aparecimento das folhas em menores intervalos de tempo.

Figura 2. Taxa de aparecimento foliar, filocrono, taxa de alongamento foliar, número de folhas vivas e índice SPAD da *Brachiaria brizantha* sob diferentes percentuais da recomendação de adubação com NPK.



Fonte: Dados da pesquisa.

Martuscello et al. (2005) ao avaliarem a *Brachiaria brizantha* cv. Xaraés adubada com 0, 40, 80 e 120 mg/dm³ de N, adotando desfolha de três, quatro e cinco folhas expandidas, a TApF apresentou comportamento linear crescente em relação as doses de N, com 0,096 folhas/dia sem adubação nitrogenada e 0,121 folhas/dia com 120 mg/dm³ de N, o que demonstrou aumento de 25% em relação à ausência de nitrogênio. Já Silva et al. (2015) avaliaram a *Brachiaria brizantha* cv. Marandu em doses de 0, 250, 500, 750, e 1.000 kg/ha/ano de N e observaram elevação na TApF, que resultou em divisão e alongamento das células meristemáticas do perfilho.

O filocrono demonstrou comportamento quadrático com ponto de mínimo para o percentual de adubação de 153,8% da adubação que correspondeu a 5,6 dias em cada folha formada (Figura 2b). Para esta variável a medida em que os valores reduzem ocorre o aumento na produção de folhas em intervalos menores de tempo.

Em estudo desenvolvido por Martuscello et al. (2015) das características morfogênicas, estruturais e a produção de biomassa do *Panicum maximum* cv. Massai à campo, sob doses de 0, 80, 160 e 240 kg/ha/ano de N e encontraram valores de filocrono com 9,08 com a dosagem máxima.

Para TAIF houve comportamento linear crescente, com aumento de 0,0059 cm/dia para cada unidade percentual, correspondendo a 6,1 mm/dia (Figura 2c). Para esta variável quanto mais elevada a taxa mais rápido ocorre o surgimento de perfilhos, elevando a produção de folhas com a utilização de NPK, que também está relacionado com a TapF.

Lopes et al. (2013) afirma que a TAIF contribui com o fluxo de biomassa, impulsionando a maior proporção de folhas, contribuindo com o aumento na fotossíntese. Além disso, o nitrogênio aumenta a TAIF, número e tamanho das células sintetizadas na divisão celular, sendo que esse fenômeno comum em gramíneas cespitosas devido a competição por assimilados entre folhas e estolões (PEREIRA et al., 2011). De acordo com Martuscello et al. (2015) a adubação proporciona aumento linear da TAIF e TApF, reduzindo os valores de filocrono.

O NFV apresentou comportamento quadrático para o percentual de adubação de 168,5% que correspondeu a 4,24 folhas vivas/perfilho para atingir ponto de máxima (Figura 2d). Esta variável está relacionada com a TApF, pois a medida em que se elevaram os níveis de adubação houve aumento dessas variáveis, o que ocorreu devido a disponibilidade dos nutrientes promovidos pela adubação, irrigação e as condições favoráveis de temperatura e luminosidade da casa de vegetação. Além disso, geralmente essa variável também expressa relação com a DVF, o que não foi observado neste trabalho.

De acordo Costa et al. (2019) o número de folhas vivas/perfilho possui relação direta com a genética, mas podem ocorrer sofrer interferência do ambientais e manejo adotados. Em um estudo de Silva et al. (2016) em casa de vegetação para avaliar a *Brachiaria decumbens* cv. Basilisk, *brizantha*

cv. Marandu, Xaraés e *Panicum* cv. Mombaça e cv. Tanzânia, a cultivar Basilisk apresentou o melhor número de folhas vivas por perfilho (3,49 n° de folhas vivas/perfilho) dentre as *Brachiarias*.

De acordo Costa et al. (2019) o número de folhas vivas/perfilho é determinado pela genética, no entanto, podem sofrer interferência de fatores ambientais e manejo utilizados. Em um estudo desenvolvido por Bezerra et al. (2020) avaliou a influência de solos Cambissolo e Argissolo sobre a *Brachiaria* Piatã e Marandu e suas interações sobre as características produtivas, morfogênicas e estruturais, sendo encontrado para o Marandu foi encontrado 4,31 n° de folhas vivas/perfilho.

Para o Índice SPAD houve comportamento linear crescente, com aumento de 0,0115227 para cada unidade percentual, que correspondeu a 41,9 unidades SPAD (Figura 2e). Para avaliar a intensidade do verde das folhas é utilizado este índice pela sua correlação entre intensidade do verde e o teor de clorofila da folha (MARTUSCELLO et al., 2009).

Zanine et al. (2020) analisaram a *Brachiaria brizantha* cv. Piatã no antes e após o pastejo com uso de adubação nitrogenada durante as estações do ano, com doses de 0, 150, 300 e 450 kg/ha de N e encontraram 41,22 para o Índice SPAD na dose máxima, sendo encontrado com efeito linear crescente ($P < 0,05$) no pré-pastejo e valor do índice SPAD de 41,46 na Primavera. Esses resultados demonstraram que a adubação nitrogenada elevou a produção fotossintética, com maior desenvolvimento da parte aérea.

Para o número de perfilhos/ vaso, número de perfilhos/planta, massa fresca (g/vaso), massa seca de folha (g/ vaso), massa seca de colmo (g/ vaso), massa seca de raiz (g), massa seca total (g/ vaso), volume de raiz (mL) e eficiência no uso da água foram observadas diferenças apenas ($P < 0,05$) para adubação (Tabela 4).

Tabela 4. Crescimento e acúmulo de biomassa da *Brachiaria brizantha* com e sem calagem, sob percentuais da recomendação de adubação com NPK.

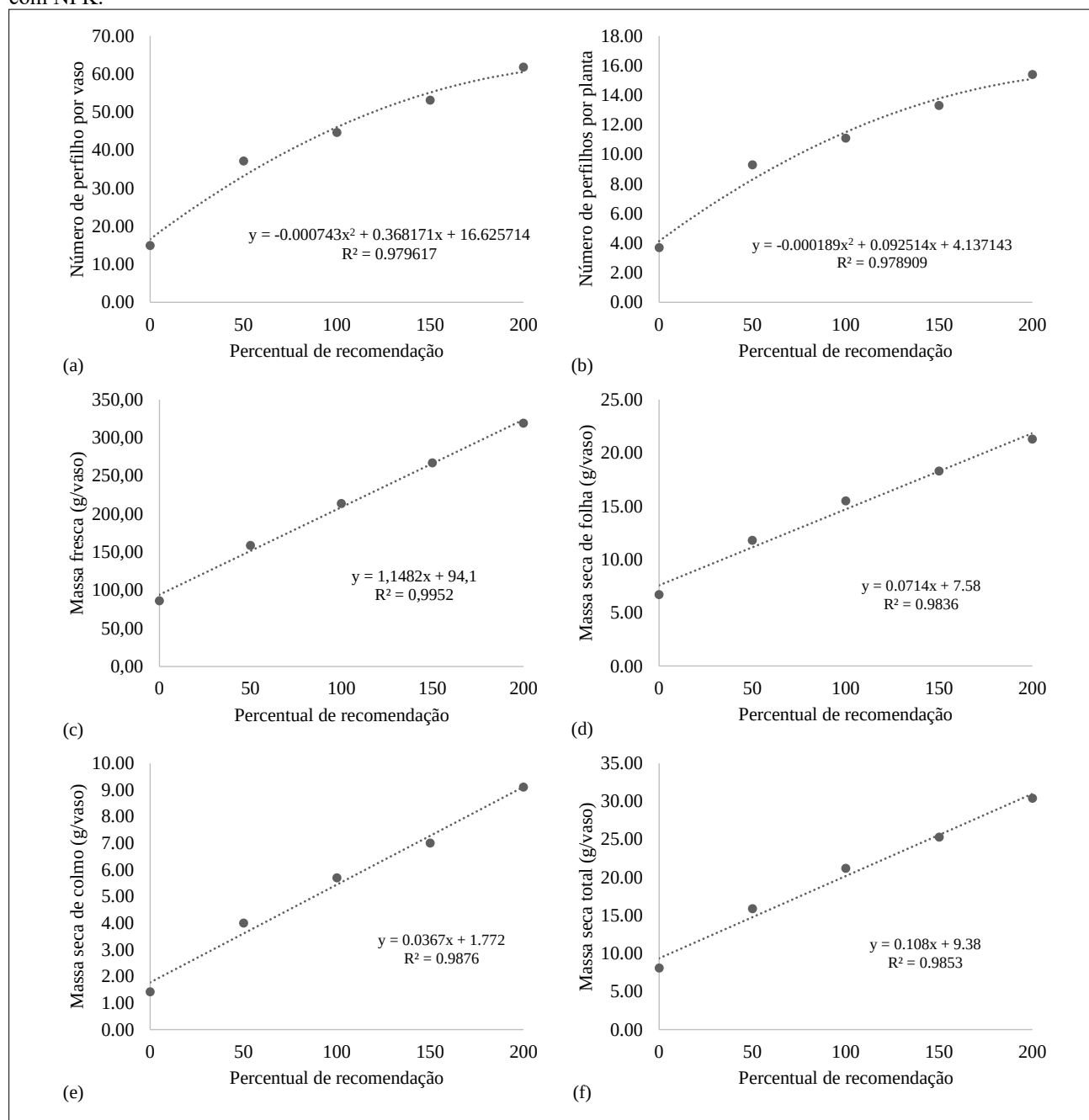
Variável	Calagem		Adubação (% da recomendação) ¹						Valor de P		
	Sem	Com	0	50	100	150	200	EPM	Cal	Adu	Cal*Adu
Número de perfilhos/vaso	40,9	43,7	14,9	37,1	44,6	53,1	61,8	3,58	0,2394	<0,0001	0,1972
Número de perfilhos por/planta	10,2	10,9	3,7	9,3	11,1	13,3	15,4	0,89	0,2405	<0,0001	0,1960
Massa fresca (g de MV/vaso)	207,8	210,1	86,2	158,6	213,8	266,9	319,1	7,74	0,6432	<0,0001	0,3048
Massa seca de folha (g/vaso)	14,7	14,7	6,7	11,8	15,5	18,3	21,3	0,50	0,8779	<0,0001	0,1105
Massa seca de colmo (g/vaso)	5,3	5,6	1,42	4,0	5,7	7,0	9,1	0,44	0,3237	<0,0001	0,1595
Massa seca total (g/vaso)	20,1	20,3	8,1	15,9	21,2	25,3	30,4	0,75	0,6299	<0,0001	0,0734

¹Adubação correspondente ao percentual da recomendação da 5ª aproximação. EPM= Erro padrão da média; Cal= calagem; Adu= adubo; CalxAdu= interação entre os fatores.

Para o número de perfilhos/vaso houve comportamento quadrático para o percentual de adubação de 246,9%, que correspondeu a 62,2 perfilhos por vaso para atingir o ponto de máxima (Figura 3a). Porém essa dosagem ultrapassa a recomendação máxima testada neste trabalho. O aumento do número de perfilhos promove o melhor desenvolvimento da pastagem, bem como a uniformidade, controlando o aparecimento de ervas daninhas, pragas e erosão do solo, resultando em vigor da pastagem, renovação rápida, persistência e maior produtividade.

Além disso, o surgimento de novos perfilhos possui relação direta com a TApF, demonstrando que os nutrientes foram compensados de forma eficiente pela adubação, contribuindo para o desenvolvimento da planta, que também se correlaciona com o aumento na produção de massa fresca. Por outro lado, a deficiência de nutrientes causada pela ausência de adubação reduz a produção de perfilhos e eleva o número de gemas dormentes nas pastagens.

Figura 3. Crescimento e acúmulo de biomassa da *Brachiaria brizantha* sob percentuais da recomendação de adubação com NPK.



Fonte: Dados da pesquisa.

Segundo Nunes et al. (2023) doses crescentes de fósforo aumentam o surgimento de novos perfilhos, impulsionam a capacidade fotossintética, crescimento e favorecendo a rebrota.

O número de perfilhos/planta demonstrou comportamento quadrático com ponto de máxima para o percentual de adubação de 246,7% que correspondeu a 15,5 perfilhos por planta (Figura 3b). Porém essa dosagem ultrapassa a recomendação máxima testada neste trabalho. O aumento na produção das plantas com a adubação incrementou a produção, auxiliou na recuperação e elevou o potencial de produção das gramíneas.

Em um estudo desenvolvido por Silva et al. (2016) na *Brachiaria decumbens* cv. Basilisk, *Brizantha* cv. Marandu, Xaraés e *Panicum* cv. Mombaça e cv. Tanzânia em casa de vegetação, verificaram que a Basilisk e Mombaça apresentaram maior número de perfilhos por planta (10,16 e 9,33), bem como a Marandu alcançou 4,58, demonstrando a alta capacidade de perfilhamento, na qual a adubação foi essencial, mesmo variando a produção de perfilhos de acordo com as cultivares.

A produção de massa fresca (g/vaso) apresentou comportamento linear crescente, com aumento de 1,14833 g para cada unidade percentual, o que correspondeu a 323,8 g (Figura 3c). Em pastagens a maior produção de massa fresca proporciona ampla oferta de forragem para o consumo dos animais e uso adubação eleva o crescimento da forragem, aumentando a produção das folhas.

A adubação promove o desenvolvimento completo da planta, sendo que a aplicação em de nitrogênio, fósforo e potássio juntos contribuem para a formação da parte aérea, ocorrendo como esperado a influência desta prática. Dentre os nutrientes mais importantes para a absorção nas plantas, o nitrogênio atua diretamente na fotossíntese, aumentando a produção de folhas diariamente e estimula o desenvolvimento estrutural.

Teixeira et al. (2018) avaliaram uma pastagem de *Brachiaria brizantha* cv. Marandu com 0, 100, 200 e 300 kg/ha de nitrogênio e verificaram que houve aumento linear crescente da massa fresca com aumento nas doses de fósforo e nitrogênio. Além disso, o fósforo na fase inicial contribui com o crescimento do sistema radicular e perfilhamento, promovendo aumento na produção em plantas adubadas.

Sendo assim, adubação nitrogenada estimula a produtividade a depender do nível adotado e a espécie, sendo possível elevar a capacidade de suporte da pastejo, acelerando a formação e o crescimento da parte aérea (SILVA et al., 2012).

Para Camargo et al. (2022), fatores como temperatura, irrigação e os nutrientes interferem diretamente sobre o índice de área foliar da parte aérea, influenciando na sua produção. Sendo assim, a adubação com o nitrogênio aumenta valor nutricional, capacidade de suporte e atua no funcionamento das células vegetais.

Para a massa seca de folha (g/vaso) houve comportamento linear crescente, com aumento de 0,0712854 para cada unidade percentual, que correspondeu a 21,8 g/vaso (Figura 3d).

O aumento no número de perfilhos e a TALF proporcionam maior produção de massa seca, elevando o potencial de fotossíntese, e como isso ocorre a expansão das folhas em menor tempo, levando ao aumento da produção de massa seca pela planta.

Em um estudo desenvolvido por Costa et al. (2009) para avaliarem a *Brachiaria brizantha* cultivares Marandu, Xaraés e MG-4, com 0, 50, 100 e 150 mg/dm³ de nitrogênio em casa de vegetação, houve aumento linear da massa seca para todas os cultivares com elevação desse nutriente, obtendo-se valores médios de 35,43; 36,80 e 39,98 g/vaso, com 26; 28 e 31% maiores do que sem adubação. Com base nesses resultados, os autores que adubação nitrogenada aumenta a densidade volumétrica da forragem e número de folhas no dossel, contribuindo com o crescimento e alongamento da parte aérea e maior produção de massa seca.

Rodrigues et al. (2008) avaliaram em casa de vegetação a *Brachiaria brizantha* cv. Xaraés com aplicação de 0, 75, 150 e 225 mg/dm³ de nitrogênio e 0, 50 e 100 mg/dm³ de potássio e perceberam que massa de folha apresentou comportamento quadrático para nitrogênio e potássio, com pontos de máxima nas doses de 177, 176 e 168 mg/dm³ para nitrogênio para todos os cortes e o potássio com 61 mg/dm³, demonstrando que estes nutrientes são essenciais para o crescimento da *Brachiaria brizantha* e afetam a produção de massa seca e o número de perfilhos.

A massa seca de colmo (g/vaso) apresentou comportamento linear crescente, com aumento de 0,0368863 g/vaso para cada unidade percentual, o que correspondeu a 9,2 g/vaso (Figura 3e).

Martuscello et al. (2009) avaliaram a produção de massa seca da *Brachiaria brizantha* cv. Xaraés e *Panicum maximum* cv. Massai em doses de 0, 40, 80 e 120 mg/dm³ de nitrogênio cultivados em casa de vegetação e verificaram que a massa do colmo apresentou comportamento linear positivo ($P < 0,05$) para as doses de N para ambas as forrageiras, o que já era esperado devido o nitrogênio contribuir com o acúmulo de massa seca na planta e promover o crescimento com doses crescentes de adubação.

Para massa seca total (g/vaso) houve comportamento linear crescente, com aumento de 0,108172 g para cada unidade percentual, que correspondeu a 31 g/vaso (Figura 3f). A associação dos nutrientes, principalmente o nitrogênio, acelerou a produção de massa seca das plantas, resultando em maior produtividade em comparação à ausência de adubação.

Alexandrino et al. (2003) ao avaliarem a *Brachiaria brizantha* cv. Marandu em casa de vegetação, utilizando doses de 0, 45, 90, 180 e 360 mg/dm³ de nitrogênio em frequências de corte entre 14 e 28 dias verificaram que a partir do primeiro corte houve reposta linear da produção de massa

seca total nas doses de 45, 90, 180 e 360 mg/dm³, com o incremento de 41,96; 73,21; 166,79; e 274,64% comparado às plantas que não receberam adubação.

Para massa seca de raiz (g), volume de raiz (mL) e eficiência no uso da água (g água/g MS) foram identificadas diferenças ($P < 0,05$) apenas com o uso da adubação (Tabela 5).

Tabela 5. Massa seca de raiz (g), volume de raiz (mL) e eficiência no uso da água (g água/g MS) da *Brachiaria brizantha* com e sem calagem, sob percentuais da recomendação de adubação com NPK.

Variável	Calagem		Adubação (% da recomendação) ¹						Valor de P		
	Sem	Com	0	50	100	150	200	EPM	Cal	Adu	Cal*Adu
Massa seca da raiz (g)	43,3	37,4	13,2	32,1	43,2	52,3	60,4	6,20	0,1451	<0,0001	0,6456
Volume de raiz (mL)	219,0	207,0	92,5	177,5	225,0	280,0	290,0	21,60	0,3869	<0,0001	0,7623
Eficiência no uso da água (g água/g MS)	97,2	95,1	141,1	102,5	87,0	78,5	71,6	4,52	0,5081	<0,0001	0,6731

¹Adubação correspondente ao percentual da recomendação da 5ª aproximação. EPM= Erro padrão da média; Cal= calagem; Adu= adubo; CalxAdu= interação entre os fatores.

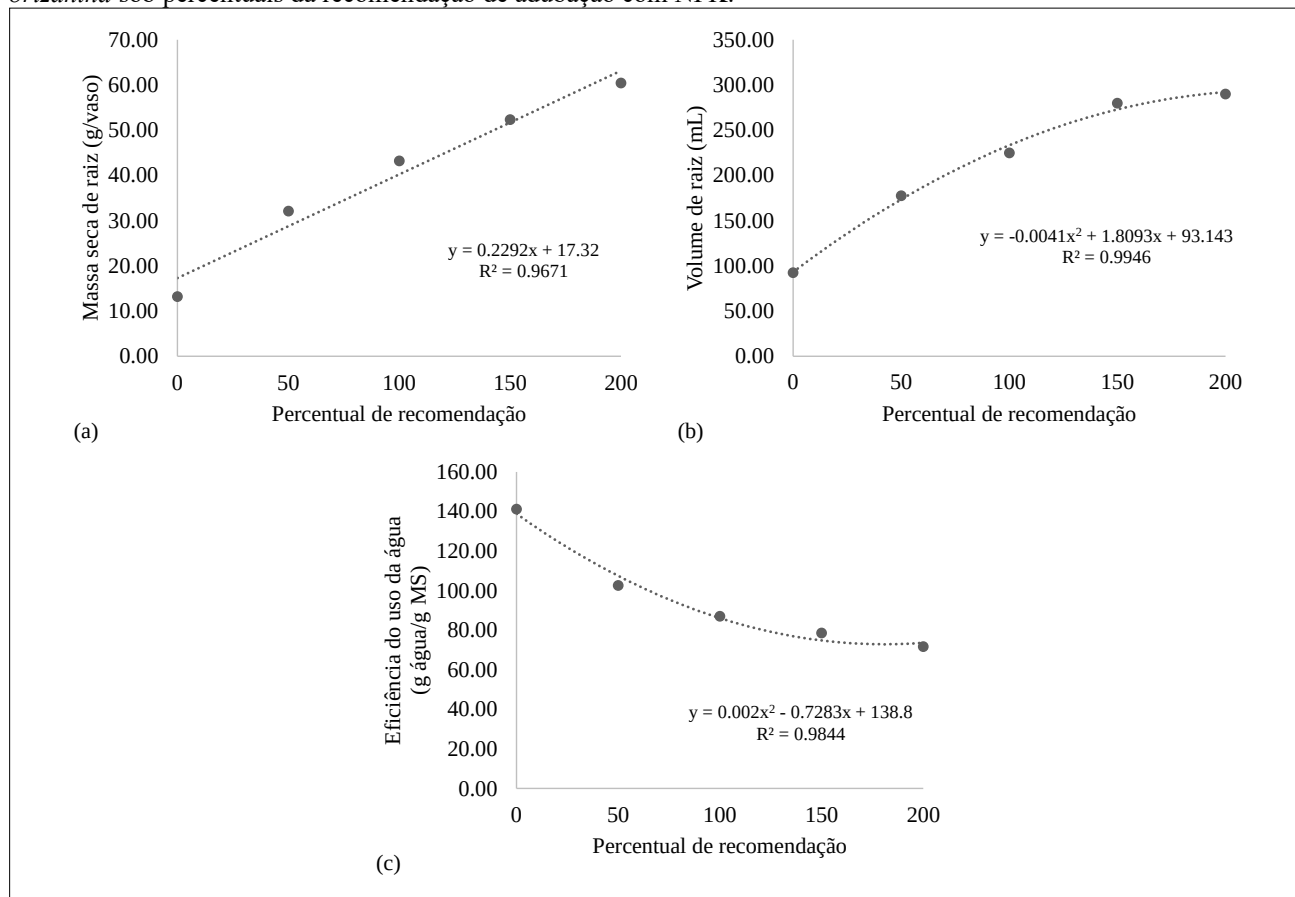
Para massa seca de raiz (g /vaso) houve comportamento linear crescente, com aumento de 0,230153 g/vaso para cada unidade percentual, o que correspondeu a 63,4 g/vaso (Figura 4a).

A produção desse componente apresentou desenvolvimento favorável com a adubação, pois a presença de NPK impulsionou o desenvolvimento do sistema radicular com doses crescentes de adubação, promovendo acelerado crescimento na planta.

Em um estudo desenvolvido por Fernandes et al. (2019) sobre a *Brachiaria brizantha* cv. Marandu e Massai (*Panicum maximum* x *P. infestum*) em casa de vegetação, utilizando solução nutritiva com 0%, 25%, 50%, 75% e 100% da concentração de fósforo em solução equivalente a 0 mg/dm³, 36,9 mg/dm³, 73,8 mg/dm³, 110,8 mg/dm³ e 147,6 mg/dm³ entre 0 e 60 dias após emergência, verificaram que a *Brachiaria* ao extrair e acumular fósforo após 60 dias de emergência houve melhor desenvolvimento e potencializou o máximo desenvolvimento do sistema radicular, pois esse nutriente favoreceu a absorção de água e nutrientes.

O volume de raiz apresentou comportamento quadrático, atingindo o ponto de máxima para o percentual de adubação de 222,2%, o que correspondeu a 294,2 cm³ (Figura 4b). Porém essa dosagem ultrapassa a recomendação máxima neste trabalho. O maior de volume de raiz promove um sistema radicular desenvolvido e profundo, aumenta a absorção de água e fósforo, que estimulam o crescimento, já o potássio auxilia na formação das raízes secundárias e o nitrogênio, melhora a fixação de carbono, estimulando o desenvolvimento das raízes.

Figura 4. Massa seca de raiz (g/vaso), volume de raiz (mL) e eficiência no uso da água (g água/ gMS) da *Brachiaria brizantha* sob percentuais da recomendação de adubação com NPK.



Fonte: Dados da pesquisa.

O maior volume de raiz contribuiu com maior produção de massa seca de raiz, promovendo o melhor desenvolvimento das plantas, o que poderia tornar maior a exploração do solo pelo sistema radicular em busca de maximização da absorção de água, principalmente nitrogênio e fósforo em condições de campo. Além disso, condições controladas favoreceram a absorção dos nutrientes, devido a reposição diária da água, o que favoreceu também a ação do potássio.

Segundo Fernandes et al. (2019) quando a necessidades de fósforo é suprida pela planta ocorre o surgimento acelerado nas raízes, aumenta eficiência na fotossíntese e desenvolvimento do sistema radicular.

De acordo com Silva et al. (2020) o maior volume de raiz possui relação com as propriedades físicas e quantidade de matéria orgânica presente no solo, o que facilita a penetração das raízes, maior retenção de umidade e absorção de nutrientes, especialmente nitrogênio e fósforo.

A eficiência do uso da água (água/ gMS) apresentou comportamento quadrático para o percentual de adubação de 181,3% que correspondeu a 72,9 g/L para atingir o ponto de mínima (Figura 4c).

A eficiência no uso da água promove redução nos custos com a irrigação, combatendo a escassez desse recurso, principalmente em locais com irregularidades de chuvas. A redução no uso desse recurso foi demonstrada pelos níveis de adubação crescentes, que proporcionaram aumento na massa seca e volume de raiz.

Além disso, a adubação com NPK auxiliando na absorção mais eficiente de água e também proporciona os nutrientes necessários ao solo.

Silva et al. (2007) avaliaram a recuperação da *Brachiaria decumbens* em casa de vegetação, utilizando doses de 0, 100, 200, 300 e 400 mg/dm³ nitrogênio e 0, 10, 20, 30 e 40 mg/dm³ de enxofre e observaram que a partir do segundo corte o nitrogênio desempenhou papel crucial na eficiência no uso da água, principalmente em 317 e 305 mg/dm³. Essa maior eficiência hídrica permitiu crescimento acelerado, recuperação das raízes e com direcionamento de mais energia para o desenvolvimento da parte aérea. Assim, a combinação de altas doses de nitrogênio com reposição diária da água promoveram um aumento significativo na produção de massa seca total, uma vez que a umidade do solo favoreceu a ação do nitrogênio no crescimento das plantas.

5 CONCLUSÃO

A adubação é uma prática favorável, pois demonstra melhorar as características morfológicas e produtivas, bem como a redução o uso da água, recurso que deve ser otimizado na produção, visando a sustentabilidade de todo ecossistema. Além disso, a utilização de NPK em doses a partir de 150% favorecem o desenvolvimento das plantas, mesmo sem a aplicação de calagem.

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Full Length Article

Water Availability and Microbiological Inoculation Alters Development and Yield of Buffel Grass (*Cenchrus ciliaris*)

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Abstract

This study examined the effect of inoculation with growth-promoting microorganisms and different levels of water availability on productive and morphogenetic traits of buffel grass (*Cenchrus ciliaris* L. cv. Aridus). The experiment was laid out in a randomized block design with a 4 × 2 factorial arrangement. The factors tested were microbial inoculation with *Azospirillum brasilense* + *Rhizopagus intraradices* or no inoculation (control) and four levels of water availability (20, 40, 60 and 80% of pot capacity). Five replicates were used, totaling 40 plastic pots. The water availability of 80% positively affected the morphogenetic traits, providing a 28% higher leaf appearance rate and a 50% higher leaf elongation rate; a 3-day shorter leaf lifespan; 19% larger leaves; and a 40% higher leaf area index compared with greater water restriction. Shoot, leaf, and root DM yields increased by 45, 49 and 61%, respectively, with 80% of water availability. Inoculation with plant growth-promoting microbes increased leaf elongation rate (by 25%), shoot DM yield (14%), and root dry matter (DM) yield (20%). Therefore, water stress affects the morphogenesis and productive development of buffel grass cv. Áridus. Inoculation with plant growth-promoting microbes can strengthen the root system and shoots of buffel grass, as well as increasing its resilience to adverse climatic conditions.

Keywords: *Cenchrus ciliaris*; Drought tolerance; Inoculation; Mycorrhizal fungus; Water restriction

Introduction

The pastoral environment must be regarded as a complex ecosystem where the main factors (animal, plant, climate, and soil) interact with and influence one another through the transfer of different levels of energy. The climate factor directly impacts plant and animal production in the tropics, especially in semi-arid regions, where rainfall occurs with irregular distribution as well as at low frequency and intensity (Gaur and Squires 2018).

These situations warrant the use of species capable of tolerating low water availability, such as buffel grass (*Cenchrus ciliaris* L.), a species originating in East Africa and Southeast Asia that was introduced in Brazil in the 1950 (Oliveira 1993). Buffel grass was established in semi-arid regions for ruminant feeding due to characteristics such as easy implementation, good nutritional value for these arid

regions (6–9% crude protein and 50% digestibility), and its well-known tolerance to drought conditions (Carrizo *et al.* 2021). However, the sparse rainfall in these regions is one of the main abiotic factors that cause serious losses in the yield, persistency, and nutritional value of the species (Tommasino *et al.* 2018; Maranhão *et al.* 2019).

The soil in these regions harbors enormous macro- and microbiological diversity, and in the zone of contact with plant roots (rhizosphere), microorganisms act through different processes of synergistic and antagonistic interaction according to the relationship between plant and microorganism and these interactions have been explored in recent years with the use of biological products based on plant growth-promoting bacteria and arbuscular mycorrhizal fungi (AMF) (Santoyo *et al.* 2021).

The synergism between these microorganisms is attributed to the production of compounds by some bacteria

that increase cell permeability and, therefore, the rate of root exudation, which stimulates hyphal growth and facilitates root penetration by the fungus (Jeffries *et al.* 2003). In addition, these bacteria produce phytohormones that stimulate root growth and increase mycorrhizal colonization (Villarreal *et al.* 2016).

Mycorrhizal fungi, on the other hand, provide a niche and/or habitat for bacteria, which can use their structures as intermediaries to reach the root tissue epidermis (Villarreal *et al.* 2016). Additionally, they provide nutrients for the bacteria that colonize the surfaces or interior of the spores, protecting against drying, radiation, predation, and salinity (Levy *et al.* 2009).

Nonetheless, several cases involve data heterogeneity and a certain degree of specificity between plants, bacterial species, and AMF isolates. For this reason, it is essential to investigate efficiency of the combined use of these inoculants in tropical forage plants, under water deficit conditions, to allow the elucidation and adoption of this biotechnology by rural producers. The objective of this study was to examine whether co-inoculation with *A. brasilense* and *R. intraradices* improves the morphogenetic and productive development of buffel grass plants under water stress.

Materials and Methods

Experimental details

The experiment was carried out on buffel grass (*Cenchrus ciliaris* L. cv. Aridus) from October to December 2020 in a greenhouse in the Forage Crops and Pasture section of the State University of Southwest Bahia, Juvino Oliveira campus, located at the coordinates: 15°38'46" of south latitude, 40°15'24" of west longitude and average altitude of 280 m, in the municipality of Itapetinga, Brazil. The minimum, maximum, and mean temperatures inside the greenhouse were recorded throughout the experimental period (Fig. 1).

A sandy-loam soil was used, according to a soil analysis protocol collected on the campus of UESB. The soil was collected from the arable layer (0 to 20 cm), crushed, passed through a 4 mm mesh sieve, and dried in air. Soil chemical analysis was carried out at the Department of Agricultural and Soil Engineering at UESB (Table 1).

According to the recommendations of the Soil Fertility Commission of Minas Gerais State (Alvarez *et al.* 1999), liming was not necessary, since the base saturation value in the collected soil layer was 79%. Only phosphorus (P) and nitrogen (N) correction was necessary. Therefore, after the uniformity cut, the area was top-dressed with 50 kg ha⁻¹ P₂O₅ in the form of single superphosphate (18% P₂O₅), corresponding to 1.39 g pot⁻¹; and 50 kg ha⁻¹ N in the form of urea (44% N), corresponding to 0.57 g pot⁻¹.

Evaluated treatments

The experiment was laid out in a 4 × 2 factorial arrangement

with four levels of water availability (20, 40, 60 and 80% of pot capacity) and two inoculation conditions (inoculation with *A. brasilense* and *R. intraradices*, or no inoculation), in a randomized block design. Five replicates were used, totaling 40 plastic pots with a capacity of 12 L and 706.5 cm² of area, which were filled with 10 dm³ of soil.

To determine pot capacity (PC), the pot with dried soil were weighed, soaked, fully drained, and weighed again. The maximum soil water-holding capacity (25%) was determined as the difference between wet (after draining) and dry weights, following the procedure described by Souza *et al.* (2000). The amount of water needed to restore each PC was calculated relative to this difference. To maintain the soil close to PC at the different levels of water availability, all pots were weighed twice daily, at 08:00 and 16:00 h.

Sowing and inoculation

Prior to planting, buffel grass, the seeds were inoculated following the instructions of the commercial product Azototal® (100 mL 50 kg⁻¹ seeds, which provides 2 × 10⁻⁸ CFU mL⁻¹ of the AbV5 and AbV6 strains). The seeds were then homogenized and kept in the shade for 30 min.

The seeds were planted in October 2020. Simultaneously to this stage, inoculation was carried out with the mycorrhizal fungus *R. intraradices* in a central location of the pot, following the instructions of the commercial product Rootella BR® of 120 g ha⁻¹, with 20,800 propagules g⁻¹ and taking into account the proportions of soil volume of the pot.

Experimental evaluations

Plant growth details: Upon showing approximately two fully expanded leaves, the plants were thinned to four plants per pot. During the initial establishment phase (23 days after germination), the soil in the pots was kept close to PC. After this initial period, the plants were cut for uniformity at a height of 10 cm, the nitrogen rate was applied, and re-inoculation was performed using the same rate of the bacterial inoculant.

These water conditions were maintained for 10 days and, later, the plants were subjected to water regimes of 20, 40, 60 and 80% of soil PC, in which they remained for 24 days. The end of the water restriction period was determined by the observation of curling and senescence of the leaves under 20% PC regime. At the end of the stress period, the plants were harvested, the pots were dismantled, and the evaluations were performed.

Morphogenetic and structural traits: Once the levels of water availability were implemented, two tillers per pot were marked with colored ribbons (80 tillers in total) and evaluated every three days throughout the experimental period. The following variables were evaluated in each marked tiller: appearance of the leaf apex; leaf length, which was measured according to their stage of development. In

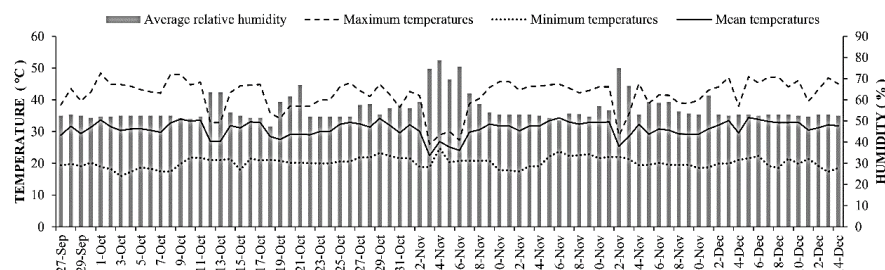


Fig. 1: Average relative humidity (%) and maximum, minimum, and mean temperatures (°C) during the experimental period

Table 1: Chemical characteristics of cultivated soil

Soil characteristics	Values
pH	6.30
Organic matter	7.00 g kg ⁻¹
Phosphorus	15.00 mg kg ⁻¹
Potassium	0.97 cmol kg ⁻¹
Calcium	1.50 cmol kg ⁻¹
Magnesium	1.60 cmol kg ⁻¹
Sum of bases	4.10 cmol kg ⁻¹
Cation exchange capacity	4.10 cmol kg ⁻¹
Base saturation	79.00 %

the fully expanded leaves, the length from the tip to the ligule was measured. In the case of expanding leaves, the same procedure was performed, only considering the ligule of the last fully expanded leaf as a measurement reference. For the senescent leaves, the difference between the final senescent length (yellowing and blackening) and the initial senescent length was observed. Pseudostem length was considered to be the distance from the ground to the last ligule. These data were then used to calculate the leaf appearance rate (leaves day⁻¹), phyllochron (days leaf⁻¹), leaf elongation rate (cm day⁻¹), stem elongation rate (cm), number of live leaves, leaf senescence rate, final leaf length (cm), and leaf lifespan (LLS = number of live leaves × phyllochron) (Lemaire and Chapman 1996). Plant height (cm) was measured on the day of dismantling, at the end of the period of water stress and rehydration, moments before the harvest for evaluations. This measurement was obtained with a graduated ruler, without compressing the forage and considering the height of the curvature of the leaves around the ruler as the upper limit. Tiller density was determined after measuring plant height, at the end of the water stress and rehydration period, by counting the number of tillers per pot. Vegetative tillers were considered those whose flower primordia was not externalized, whereas reproductive tillers were those that showed this characteristic.

Biomass production: At the end of the water stress period, two clumps were cut at 10 cm above ground level and the collected material was identified and separated into leaves, pseudostem (stem + sheaths), and dead material. To determine the pre-dried weight, the desiccated material was weighed fresh and after pre-drying in a forced-air oven at 65°C for 72 h. Another two clumps of the pots were used for the analysis of leaf area, which involved separating and

then scanning the leaves. The digitized images were evaluated in ImageJ software, which determines the area of the image covered by the leaves by contrast with the uncovered area. Leaf area was calculated as the sum of the area of the images referring to the two plants in each replicate. The values were summed to calculate total leaf area per pot (cm² pot⁻¹). Leaf area index (LAI) was determined by dividing the total leaf area by the total area of the pot filled with soil.

Root evaluation: The roots collected after the harvest were initially used for the measurement of length (cm). This variable was obtained using a graduated ruler fixed to a flat surface on which the root was placed. Subsequently, root volume (mL) was determined using a volumetric beaker containing a certain amount of water, where the fresh root was introduced and the root volume of each pot was calculated by difference. Next, the roots were weighed and dried in a forced-air oven at 65°C for 72 h to determine DM yield.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) adopting the following sources of variation: soil water availability, microbiological inoculation and the interactions among these factors. The mean values of the microbiological inoculation were compared by the Fisher's least significant difference using the and the significant variables were compared by the Tukey's HSD test at 1 and 5% significance level. Regression analysis was applied to the soil water availability, and equations were selected based on the coefficient of determination and the significance of the parameters using the SAS statistical package (2002).

Results

Morphogenetic and structural traits

The interaction between water availability and microbial inoculation of buffel grass did not affect its leaf appearance rate (LAR), phyllochron (PHY), leaf elongation rate (LER), pseudostem elongation rate (PER), or leaf senescence rate (LSR) (Table 2). Inoculation with plant growth-promoting microbes increased the rate of the appearance of successive leaves (LAR) by 11%. Additionally, it reduced the time

Table 2: Morphogenetic traits of buffel grass grown under different levels of water availability, with or without microbial inoculation

Variable	Water availability				Inoculation		P-value			CV (%)
	20	40	60	80	With	W/o	W×I	Water	Inoc	
LAR	0.18	0.19	0.22	0.23	0.21	0.19	0.49	0.01*	0.01*	10.67
PHY	5.73	5.24	4.47	4.43	4.67	5.32	0.41	0.01*	0.03	17.65
LER	1.68	2.06	2.42	2.52	2.41	1.93	0.36	0.01*	0.01*	11.53
PER	0.13	0.23	0.28	0.35	0.25	0.24	0.12	0.01*	0.85	26.80
LLS	29.20	30.58	26.52	25.99	26.77	29.36	0.56	0.013	0.02	11.91
FLL	15.92	17.96	17.91	18.94	17.75	17.63	0.27	0.01*	0.10	4.54
LSR	1.55	1.13	1.10	1.24	1.37	1.13	0.11	0.01*	0.01*	16.80
NLL	5.21	5.87	5.83	5.88	5.54	5.76	0.49	0.016	0.50	8.91
NRT	1.90	2.70	5.40	2.50	3.00	3.25	0.54	0.01*	0.69	11.55
NVT	50.80	48.50	50.20	47.00	45.70	52.55	0.01*	-	-	5.03
PH	19.50	20.40	25.90	24.90	23.65	21.70	0.06	0.01	0.01*	10.59
LAI	1.24	1.48	1.80	1.73	1.60	1.52	0.05	0.01*	0.50	24.24

Note: F values and significance for the variables inoculation (Inoc), water available (Water) and W×I interaction by Tukey's HSD test at 5% probability. LAR: leaf appearance rate (leaves tiller⁻¹ day⁻¹); PHY: phyllochron (days leaf⁻¹ tiller⁻¹); LER: leaf elongation rate (cm tiller⁻¹ day⁻¹); PER: pseudostem elongation rate (cm tiller⁻¹ day⁻¹); LLS: leaf lifespan (days); FLL: final leaf length (cm); LSR: leaf senescence rate (cm day⁻¹); NLL: number of live leaves; NRT: number of reproductive tillers; NVT: number of vegetative tillers; PH: plant height; LAI: leaf area index; * Values < 0.01

Table 3: Number of vegetative tillers of buffel grass grown under different levels of water availability, with or without inoculation with plant growth-promoting microorganisms

Inoculation	Water availability (%)				CV	Equation	R ²
	20	40	60	80			
With	42.2B	46.6B	47.0B	47.0A	5.12	1	0.96
Without	59.4A	50.4A	53.4A	48.0A	6.78	2	0.72

Means followed by the same lowercase letter in the column do not differ by Tukey's HSD test at 5% probability. Equation 1: $y = -0.00275X^2 + 0.349X + 36.5$; Equation 2: $y = 0.00163X^2 - 0.3335X + 64.35$

interval between the appearance of two consecutive leaves (phyllochron) by 14% and provided a 25% higher LER than control treatment (Table 2).

Higher soil water availability showed a positive linear response in LAR (Fig. 2a), LER (Fig. 2c), and PER (Fig. 2d), which increased by 28, 50 and 169%, respectively, and reduced phyllochron (Fig. 2b) by 1.3 days and LLS by three days (Fig. 2e). With the gradual increase in water availability decreased LSR (Fig. 2f) to a minimum estimated value of 1.06 cm day⁻¹ at 56.87% of PC, after which point it increased. Final leaf length responded linearly to the increase in water availability (Fig. 3a), increasing by 19% ($P < 0.01$), whereas number of live leaves (NLL) showed a quadratic response ($P < 0.05$), with a maximum estimated value of 5.95 leaves at 62.65% of PC (Fig. 3b).

There was a significant interaction effect between inoculation with plant growth-promoting microbes and water availability on the number of vegetative tillers (NVT) (Table 2). The inoculated treatment exhibited a quadratic polynomial curve with an estimated maximum value of 47 tillers at 63.45% of PC and an increase of 11%. Control treatment, however, showed a decreasing quadratic response to the increase in soil water availability, with a minimum number of 48 tillers at 80% of PC, showing a 24% decrease (Table 3).

Reproductive tillers began to appear at 21 days of regrowth. There was no significant interaction effect between water availability and microbial inoculation on the number of reproductive tillers of buffel grass ($P > 0.05$). This variable showed a significant response only to the water availability factor in isolation ($P < 0.01$; Fig. 3c), in a

quadratic function whose estimated maximum was 4.34 tillers at 54.92% of PC. The LAI of buffel grass fitted a quadratic model in response to increasing water availability ($P < 0.01$), with a maximum estimated index of 1.76 at 73% of PC, which represents a 40% growth (Fig. 3d). Plant height, in turn, had a positive linear response ($P < 0.01$), increasing 5.4 cm (Fig. 3e).

Biomass production

The interaction between inoculation with plant growth-promoting microbes and water availability was non-significant for the DM yield of the leaves, pseudostems, dead material, or shoots of buffel grass ($P > 0.05$). However, the isolated factors had a significant effect ($P < 0.01$) on all variables, except dead material. Inoculation with microbes influenced ($P < 0.05$) the DM yields of leaves, pseudostems, and shoots, which were 16, 18 and 14% higher, respectively than those obtained with the control treatment (Fig. 4).

Root evaluation

Shoot and leaf DM yields responded quadratically to increasing water availability (Fig. 5), with estimated maximum values of 11.80 g pot⁻¹ (shoots) at the water level of 75.21% of PC, and 5.56 g pot⁻¹ (leaves) at 68.34% of PC, which represents an increase of 49%. Pseudostem DM yield showed a positive linear response with a 65.5% higher value (Fig. 5). The interaction between water availability and microbial inoculation did not influence ($P > 0.05$) root DM yield, volume or length. However, these variables showed a

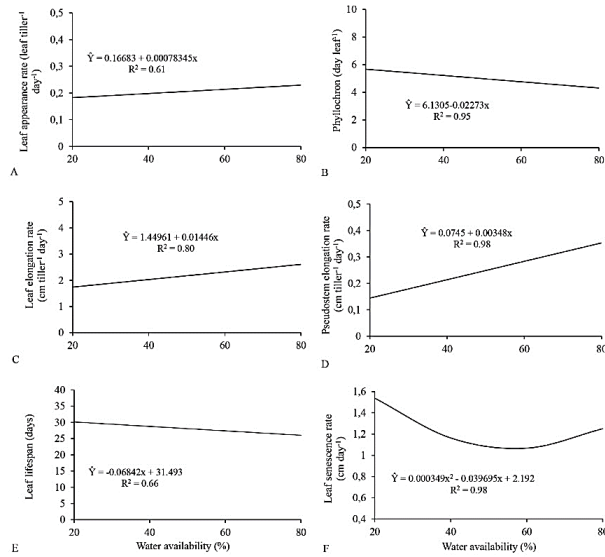


Fig. 2: Leaf appearance rate (A), phyllochron (B), leaf elongation rate (C), pseudostem elongation rate (D), leaf lifespan (E), and leaf senescence rate (F) of buffel grass plants under different levels of water availability

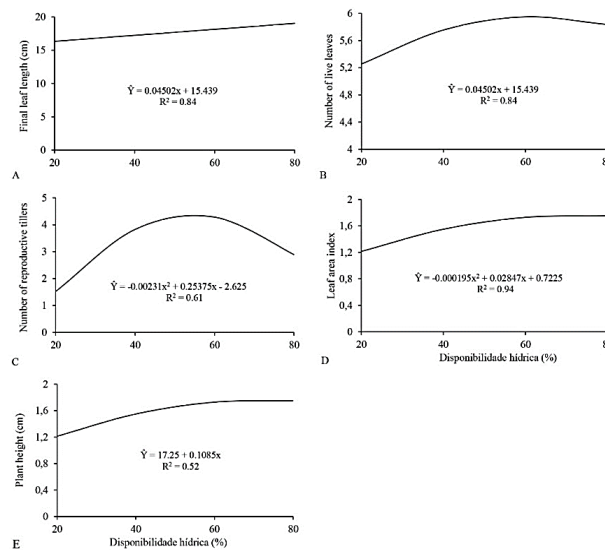


Fig. 3: Final leaf length (A), number of live leaves (B), number of reproductive tillers (C), leaf area index (D), and plant height (E) of buffel grass under different levels of water availability

significant response to the factors in isolation. Inoculating the buffel grass plants with *A. brasilense* and *R. intraradices* provided a 20% higher DM ($P < 0.01$) and 15% larger roots ($P < 0.01$) compared with the control set.

Root DM yield increased linearly, to a maximum of 61%, with increasing water availability (Fig. 6a). Root volume and length, on the other hand, showed a quadratic response (Fig. 6b, c), with estimated maximum values of 54 mL at 80% of PC and 42.55 cm at 61.01% of PC.

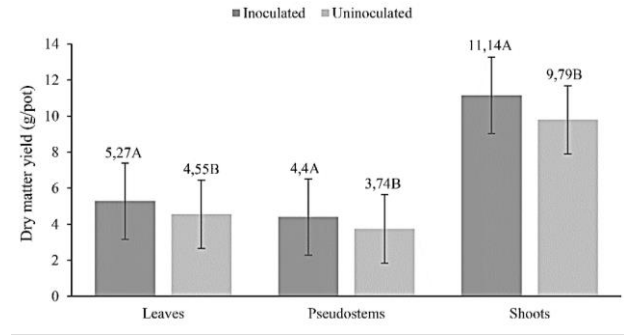


Fig. 4: Dry matter yield of leaves, pseudostems, and shoots in buffel grass inoculated or uninoculated with plant growth-promoting microorganisms. Means followed by the same letter do not differ from each other by Tukey's test at 5% probability

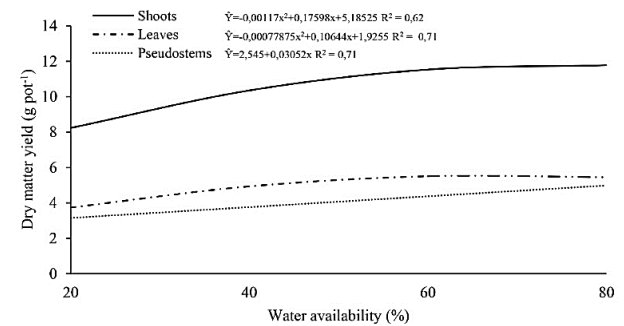


Fig. 5: Dry matter yields of shoots, leaves, and pseudostems in buffel grass under different levels of water availability

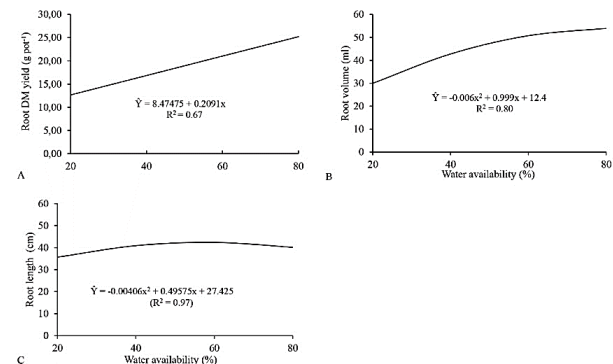


Fig. 6: Root DM yield (A), root volume (B), and root length (C) in buffel grass plants subjected to different levels of water availability

Discussion

In our study, by comparing the decreases in LER and LAR (50 and 28%, respectively) under lower water availability, we confirm the assertion of Lemaire *et al.* (2008) who stated that LAR is the last element to be penalized by water deficits, unlike LER, which is dependent on turgor pressure for cell elongation. As stated by Coussemment *et al.* (2021), the decreased turgor pressure of leaves and stems of grasses

caused by water stress results in an inhibition of cell expansion and differentiation, thereby reducing elongation rate.

Higher water availability influenced an increase in tissue turnover dynamics, as seen by the higher LAR and LER and shorter phyllochron, which caused a direct reduction in LLS. The LLS represents an important morphogenetic trait in the determination of tissue turnover, as it indicates the maximum forage yield potential (maximum amount of live material per area). Additionally, it is a parameter for determining grazing intensity (in continuous grazing) or the frequency between grazing events (in rotational grazing), thus ensuring the maintenance of LAI at values close to those necessary for greater efficiency in intercepting incident radiation and for maximum growth rates (Costa *et al.* 2013).

Both water stress and greater water availability resulted in higher LSR (Fig. 2f), mainly because leaf senescence is a common effect in plants nearing the stage of maturity and can also occur in situations of severe water deficit (Brevedan and Egli 2003). As the water deficit is prolonged, there is an acceleration in leaf senescence and death of established tillers, inhibition of tillering and branching, and retardation of plant growth and development (Oliveira *et al.* 2017).

The applied moisture levels affected the structural traits of buffel grass, which is a reflection of the influence of soil water level on physiological and morphogenetic variables of the plant. Final leaf length decreased under lower levels of water availability. This is also a reflection of the effect of water stress on LER and LAR, since the growth period of a leaf is a constant fraction of the interval between the appearance of consecutive leaves. Additionally, while increases in LER are directly correlated with a larger final leaf size, smaller leaves are associated with higher LAR (Cruz *et al.* 2021).

Water stress also influenced the NLL of buffel grass, which is a structural trait, determined genetically by the species (Paciullo *et al.* 2017), and which can be influenced by characteristics of the environment where the plant is grown. Upon reaching a certain NLL, there is a balance between LAR and the senescence of leaves that have exceeded their lifespan. In this situation, the emergence of a new leaf implies the senescence of the leaf that preceded it, maintaining NLL relatively constant (Lemaire *et al.* 2011). Under low water availability, forage grasses make adaptations in an attempt to reduce water losses, e.g., by reducing leaf area, decreasing leaf cell division and expansion, altering leaf shapes, and triggering leaf senescence and abscission (Taiz *et al.* 2017).

The tillering dynamics of tropical forage grasses has a rearrangement capacity, depending on the conditions imposed on the forage plant (water stress, inoculations, intensity and frequency of harvesting or grazing, etc.). Furthermore, depending on the growth condition, the species may alter the proportion between tiller density and

weight/height, in addition to the basal, aerial, and reproductive characteristics. Hence, the leaf area is able to maximize light absorption and help the plant to withstand imposed water stress condition imposed as noted in the present case.

A decreased number of reproductive tillers observed under greater water availability may imply that there was adequate growth condition, and buffel grass stopped prioritizing the tillers production rather invested its photoassimilates for foliar maintenance. It is important to note that factors such as the water regime can affect not only the vegetative development of forage grasses, but also the uniformity of inflorescence emission as well as seed filling, since this process requires a considerable amount of photoassimilates (Lima *et al.* 2020). Although the early flowering of buffel grass is known to be a strategy to perpetuate the species (Oliveira *et al.* 2023), its influence in reducing the nutritional value of the pasture makes this characteristic a target to be postponed in the area, which can be achieved mainly by shortening the grazing cycles. However, further investigations are needed to understand the behavior of the types of tillers that make up the pasture and how their combinations influence the structural arrangement of the available forage under water stress conditions and microbial inoculation.

The levels of water availability affected the plant height (Fig. 3e) and LAI (Fig. 3d) of buffel grass. The latter parameter constitutes the synthesis of the morphogenetic and structural traits of the grass and is a consequence of the balance between processes that determine the supply (photosynthesis) and demand (respiration and metabolism) of photoassimilates, which establish the pace of pasture growth (Pontes *et al.* 2010).

Inoculating the plants with the microorganisms *A. brasilense* and *R. intraradices* increased the biomass production of shoots, leaves, and pseudostems of buffel grass. Several studies have reported the effect of the mycorrhizal fungus *R. intraradices* in increasing biomass in several crops, e.g., *Eleusine coracana* (Tyagi *et al.* 2018), soybean (Jie *et al.* 2021) and maize (Stoffel *et al.* 2020). Its effects were correlated with greater nutrient uptake (mainly phosphorus), greater root system development, and improved photosynthetic efficiency in abiotic stress conditions.

Some studies describe the beneficial effects of co-inoculation between *A. brasilense* and AMF for both the inoculated plants and the microorganisms. Rousseaux *et al.* (2020) evaluated co-inoculation of *A. brasilense* and *Rhizoglossus irregulare* and N fertilization in *Brachiaria hybrida* cv. Mulato II and obtained similar values using a biofertilizer at a rate of 100 kg ha⁻¹, with a 43% increase in DM yield in comparison to the control plants (without inoculation or fertilization). In addition, the authors reported that the levels of mycorrhizal colonization were the result of the effectiveness of the fungus *R. irregulare* and the contribution of *A. brasilense* to the improvement of the

levels of root occupation by the fungus.

Decline in leaf and pseudostem yields in buffel grass and other tropical forage grasses under water deficits is widely reported (Maranhão *et al.* 2019; Souza *et al.* 2020). This result is mainly due to the key importance of water for the processes of maintenance of cell turgor and expansion, which explains the response of shoot biomass production to the different levels of water availability studied in the present trial.

Microbial inoculation increased root growth, biomass production and length (Fig. 6). Smith and Read (2008) described that the mycelium network of AMF connected to the roots is able to increase the root volume of plants and consequently improve their water and nutrient uptake efficiency. On the other hand, the colonization of the rhizosphere and root tissues by *A. brasilense* produces phytohormones that stimulate root growth as well as nitrogen uptake through biological nitrogen fixation, making this association of microorganisms particularly beneficial for structuring the root system of forage grasses.

Hungria *et al.* (2021) examined the root morphology of *Urochloa brizantha* cv. Marandu inoculated with *A. brasilense* and *Pseudomonas fluorescens* and found a 93% higher root DM weight, a 12% denser root system, 83% longer roots, and 33% more radicular branches when *U. brizantha* was inoculated with *A. brasilense*, demonstrating the effect of inoculation in strengthening the root system of the species. The response of the buffel grass root system to the different levels of water availability denotes the impact of water deficiency on this plant species, as evidenced by the traits of root volume, length, and DM (Fig. 6).

Conclusion

The ideal moisture range for the cultivation of buffel grass cv. Aridus is 55 to 70% of the soil's maximum water-holding capacity, as it maximizes the number of live leaves, leaf production, and leaf area index and minimizes leaf sensitivity and the number of fertile tillers. Inoculation improves the productive potential of shoots and root system of buffel grass, increasing its adaptability to semi-arid environments and making it more resilient to the current scenarios of climate change and global warming.

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Author Contributions

EMP: Planned the experiments, investigation, performed the analysis and writing original draft. FAT, DDF, CAAOF and KSA: Supervision, validation and review the text. HSS, ERTC, JPS, JRSF, FMJ and TMV: Investigation, collected

samples and experimental evaluations. All the authors have read and agreed to the submitted version of the manuscript.

Conflicts of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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Full Length Article

Growth and Development of *Megathyrsus maximus* cv. Mombasa is Improved by Inoculation of Plant Growth-Promoting Microorganisms

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Abstract

The present study aimed to assess the initial development of Mombasa grass inoculated with plant growth-promoting microorganisms. The experiment was conducted in a greenhouse using a completely randomized design. Mombasa grass was inoculated with *Azospirillum brasilense* and *Rhizophagus intraradices*, isolated and combined. Sole inoculation of *R. intraradices* or *A. brasilense* improved the morphogenic parameters of Mombasa grass, resulting in a higher leaf appearance and elongation rate, shorter time for the emergence of new leaves and a decrease of leaf senescence rate. The strategy of isolated inoculation with *A. brasilense* increased aboveground biomass production (19%). All the inoculations notably increased the SPAD index values and chlorophyll *a* concentration, with increments of 22 and 26%, respectively, reflecting an increase in mineral content and crude protein in plants, whilst *A. brasilense* inoculation displayed a much stronger effect on mineral content. These gains are attributed to the better root development of inoculated plants, which optimized nutrient absorption. Results suggested that sole inoculation of *A. brasilense* or *R. intraradices* improved initial growth of Mombasa grass; however, their combined application worked synergistically to stimulate root development and improved nutritional status. Therefore, the combined application of these microbial inoculants seemed an alternative capable of optimizing the initial growth of Mombasa grass for sustainable production. © 2024 Friends Science Publishers

Keywords: Bioinputs; Guinea grass; Microbial inoculation; Pasture; Sustainability

Introduction

Livestock production in pastures demonstrates the resilience of tropical forage grasses, however, their sustained exposure to stressful conditions compromises their physiology (Li *et al.* 2009), limiting productivity and reflecting in poor livestock management practices. The stress imposed on plants can be alleviated using sustainable tools, among which biological inputs play a significant role, improved the tolerance of plants to physiological disturbances caused by factors biotics and abiotic (Colla *et al.* 2015).

Among the bio inputs available in the market, microbiological inoculants composed of fungi or strains of rhizobacteria are utilized in diverse agriculture crops and frequently denominated as plant growth-promoting microorganisms (PGPM) (Brazil 2020). Their primary function is to enhance plant growth and provide protection

against biotic and abiotic stresses (Souza *et al.* 2015). These microorganisms can engage in mechanisms ranging from biological nitrogen fixation, phosphorus solubilization, resistance to soil pathogens, and an increase in root surface area (Bashan *et al.* 2012; Yasmin *et al.* 2016; Majeed *et al.* 2022), thereby providing plants with increased nutrient and water uptake.

Plant growth-promoting mechanisms, mediated by rhizobacteria and mycorrhizal fungi, demonstrate significant potential for optimizing plant development (Nadeem *et al.* 2014; Rousseaux *et al.* 2020; Dawood *et al.* 2023). These microorganisms can establish a synergistic relationship between themselves and between species of plants, maximizing their effectiveness in promoting the growth of host plants. Studies have shown that fungal structures function as a communication route for bacteria, facilitating their penetration into the epidermis of the root tissue, while

the production of phytohormones by these bacteria favors the colonization and mycelial growth of mycorrhiza (Ruíz-Sánchez *et al.* 2011; Villarreal *et al.* 2016). However, it is important to highlight that antagonistic interactions can arise due to competition for nutrients and other resources essential for the survival of these microorganisms in the rhizosphere. The success or failure of co-inoculation is intrinsically linked to the physiological stage of the host, the time of infection, and the divergent nutritional demands between fungi and rhizobacteria (Biró *et al.* 2000).

Considering the global panorama with climate and extreme events (Feller and Vaseva 2014), growing demand for food and the responsibility for sustainable pasture production (Guimarães *et al.* 2022); the use of microbial inoculants in grass species present in pastures, such as *Megathyrus maximus* (Jacq.) BK Simon and SWL Jacobs (syn. *Panicum maximus* Jacq.) emerge as a viable alternative to increase sustainability in the cultivation of agronomically important plants. However, studies are needed to clarify and validate the use of bioinoculants in pastures, aiding in the better adaptation of forage crops in challenging scenarios. The present study was aimed to evaluate the effect of single and dual inoculation with *R. intraradices* and *A. brasilense* on initial growth, root characteristics and chemical composition of aerial part of Mombasa grass.

Materials and Methods

Experimental details

Experiments were performed in the greenhouse of the Department of Rural and Animal Technology, State University of Southwest Bahia, Itapetinga, Bahia (15° 14' S, 40° 14' W), during October–January of the years 2020–2021. Weather data during the experimental period were obtained using a digital thermo-hygrometer. The average maximum and minimum temperature values were 39.4 °C and 20.4°C, respectively, and the maximum relative humidity was 84% and minimum was 20%.

The soil used in the experiment was collected at a depth of 0–20 cm and subjected to physical and chemical analysis at the Department of Agricultural and Soil Engineering, State University of Southwest Bahia. The soil analysis demonstrated the following result: Sandy-loam textured, clay 9%, silt 35.5%, sand 55.5%, pH (water) 6.3, phosphorus (ion-exchange resin extraction method) 15 mg.dm⁻³, potassium 0.97 cmolc.dm⁻³, calcium 1.5 cmolc.dm⁻³, magnesium 1.6 cmolc.dm⁻³, H + Al 1.1 cmolc.dm⁻³, sum of bases 4.1 cmolc.dm⁻³, effective cation exchange capacity 4.2 cmolc.dm⁻³, total cation exchange capacity 5.2 cmolc.dm⁻³, base saturation 79%, organic matter 7 g.dm⁻³.

Treatments and experimental design

Mombasa grass was evaluated in four different treatments consisting of (i) a non-inoculated group (Control), (ii)

inoculation with *Azospirillum brasilense*, (iii) inoculation with *Rhizophagus intraradices* and (iv) co-inoculation with *A. brasilense* and *R. intraradices*. The experiment was randomized according to completely randomized design with four replications, totaling 16 experimental units (plastic pots), with a capacity of 12 L and, which were filled with 10 kg of soil. To maintain the soil close to the water retention capacity in 30% (Souza *et al.* 2020), all pots were weighed every day and water was added as needed.

According to the recommendations of the Soil Fertility Commission of Minas Gerais State (Ribeiro *et al.* 1999), there was no need for liming. Only phosphorus and nitrogen were used after the uniformity cut, with basal fertilization being carried out for establishment with 50 kg ha⁻¹ of P₂O₅ and 50 kg ha⁻¹ of nitrogen in the form of urea and simple superphosphate respectively.

Application of PGPM and sowing of seeds

Before planting and inoculation, seeds were surface disinfected by immersion in 3% sodium hypochlorite for 3 min, followed by four consecutive rinses in water and air drying. For inoculation with *A. brasilense*, a commercial product containing Ab-V5 and Ab-V6 strains was used, with a guarantee of 2×10^8 CFU mL⁻¹, with a recommendation of 100 mL of inoculant for 5 kg of seeds, which were homogenized and dried in the shade for 30 min. For inoculation with *R. intraradices*, a commercial inoculant was used with a guarantee of 20,800 propagules g⁻¹, using 120 g ha⁻¹ added to the planting hole immediately after sowing; the co-inoculation treatment used a combination of the previously mentioned forms of inoculation. Five seeds were sown per unit experimental and 15 days after the emergence, seedlings were thinned to four plants per pot.

The microbial inoculants used in this study are registered with the Ministry of Agriculture, Livestock and Supply for commercialization in Brazilian territory. Commercial product containing *A. brasilense* strains registration number: 22902 10000-0; Commercial product containing *R. intraradices* registration number: PR-93923-10074-1.

Parameters analyzed

When the plants completed 30 days after emergence, a cut of uniformization was realized at a height of 20 cm, and fertilization with phosphorus and nitrogen was performed. After cutting to standardize the experimental units, monitoring of the regrowth of Mombasa grass began, with evaluation during two periods of 28 days.

For evaluation, two tillers per pot were marked with colored ribbons and evaluated every 3 days. The following were determined by measurements of individual leaves and tillers: leaf appearance rate (LAR, leaves tiller⁻¹ day⁻¹); phyllochron (PHY, leaves tiller⁻¹ day⁻¹); leaf elongation rate (LER, cm leaves tiller⁻¹ day⁻¹); leaf senescence rate (LSR,

cm tiller⁻¹ day⁻¹); number of living leaves per tiller (NLL), final leaf length (FLL, cm leaf⁻¹); tiller density (TD), and final plant height (FPH, cm).

At the end of each evaluation period, the SPAD (Soil Plant Analytical Division Value) index was read using a SPAD 502 Plus device at times of highest solar incidence, with readings taken in the middle third of two completely expanded leaves within each experimental unit. Then, the same leaves used to read the SPAD index were collected for extraction of photosynthetic pigments (Chlorophyll *a*, Chlorophyll *b*, Total chlorophyll and Carotenoids), which were cut, excluding the central vein, weighed 0.2 g, stored in 5 mL of dimethyl sulfoxide, and kept for 72 h in the dark. Afterwards, readings were taken on the spectrophotometer at wavelengths of 665, 649 and 480 nm and quantified according to Wellburn (1994), with results expressed in $\mu\text{g.g}^{-1}$ fresh mass.

After collecting the material for chlorophyll, a cut was made 20 cm from each experimental unit. The harvested material was identified, weighed, and taken to a forced air circulation oven at 65°C for pre-drying for 72 h, then weighed again, thus calculating the biomass production of the area (g.pot^{-1}). The material was then ground in a Willey-type knife mill with a 1 mm sieve to carry out bromatological composition, where the contents of dry matter (DM, method INCT-CA G-003/1), mineral matter (MM, INCT-CA method M-001/1), crude protein (CP, INCT-CA method N-001/1) and neutral detergent fiber (NDF, INCT-CA method F002/1) and acid detergent fiber (ADF, method INCT-CA F-004/1) according to methodologies described by Detmann *et al.* (2021), being carried out in the University's Bromatological Analysis Laboratory.

The experimental units were dismantled after the second cut, and the roots were removed and washed in running water. The volume of the root system was estimated using a cylindrical vessel with graduations, recording the water displacement after immersion of the roots. The root system was then dried in an oven at 65°C until constant weight and then weighed to obtain the root dry weight.

Statistical analysis

The data obtained were grouped and analyzed as the average of two experimental cutting. The dataset was analyzed by one-way analysis of variation (ANOVA), when significant differences were detected; means were compared with Tukey's test at the 5% level, by using SAS software OnDemand for Academics.

Results

Morphogenic and structural characteristics

Different inoculation treatments displayed diverse

responses on development and growth of Mombasa grass. LAR, LER, PHY and NLL ($P < 0.001$ for all) were greater with *A. brasilense* and *R. intraradices* inoculated single, whereas PHY and NLL ($P < 0.001$ for both) were higher in non-inoculated plants and co-inoculation. Compared to the non-inoculated plants, the plants treated by co-inoculation or single inoculation with *R. intraradices* and *A. brasilense* represents 83% significantly higher leaf senescence rate ($P < 0.001$). On the other hand, the variables FLL, TD and FPH were not significant ($P = 0.200$, $P = 0.082$ and $P = 0.080$ respectively), presenting averages of 39.44 cm leaf⁻¹, 16.62 tillers and 59.06 cm, respectively (Table 1).

Photosynthetic pigments

Chlorophyll *a* concentration and SPAD index value were influenced by the treatments tested ($P < 0.001$ for both) being 26 and 22%, respectively, greater when inoculated with microorganisms than in the non-inoculated plants. No differences were found for chlorophyll *b*, total chlorophyll, and carotenoids ($P = 0.090$, $P = 0.075$ and $P = 0.165$), with averages of 31.58 $\mu\text{g.g}^{-1}$ of fresh biomass, 211.19 $\mu\text{g.g}^{-1}$ of fresh biomass and 23.25 $\mu\text{g.g}^{-1}$ of fresh biomass, respectively (Table 2).

Productive parameters

The production of fresh and dry biomass (Fig. 1) was significantly greater ($P < 0.001$ for both) in the presence of *A. brasilense*, with respective values of 21.25 g. pot⁻¹ and 5.86 g.pot⁻¹. The other treatments were equivalent for these variables, not differing from each other or from the non-inoculated plants, with averages of 18.37 g.pot⁻¹ of fresh biomass ($P = 0.270$) and 5.05 g.pot⁻¹ of dry biomass ($P = 0.124$).

Bromatological characteristics

Inoculation with *A. brasilense* resulted in greater averages for MM and CP contents ($P = 0.001$ and $P = 0.010$) than the non-inoculated plants treatment, with percentage increases of 5.25 and 13.41%, respectively. Conversely, for variables DM, NDF and ADF no effects were observed for any inoculation ($P = 0.572$, $P = 0.165$ and $P = 0.362$ respectively), with an average of 27.55, 67.12 and 33.36% respectively (Table 3).

Root evaluation

Plants inoculated with *A. brasilense* and co-inoculated showed greater averages for root system volume (average of 74 mL) and root dry weight (average of 134.46 g) ($P < 0.001$), with a percentage increase of 29 and 18%, respectively, in relation to the non-inoculated plants (Table 4).

Table 1: Morphogenic and structural characteristics of Mombasa grass inoculated with plant growth-promoting microorganisms

Items	Control	<i>A. brasilense</i>	<i>R. intraradices</i>	Co-inoculation	CV%
LAR	0.23b	0.32a	0.36a	0.27b	8.75
PHY	4.32a	3.04b	3.01b	3.82a	9.73
LER	3.07c	4.72b	5.56ab	3.57cb	17.25
LSR	1.32a	0.92b	0.65b	0.60b	23.91
NLL	5.00b	7.15a	7.17a	5.66b	8.64
FLL	41.41	39.65	36.10	40.61	10.07
TD	16.25	15.25	17.75	17.25	17.92
FPH	59.50	59.75	60.25	56.75	8.66

LAR: Leaf appearance rate (tiller leaves⁻¹ day⁻¹); PHY: phyllochron (tiller leaves⁻¹ day⁻¹); LER: Leaf elongation rate (cm leaves tiller⁻¹ day⁻¹); LSR: leaf senescence rate (LSR, cm tiller⁻¹ day⁻¹); NLL: number of living leaves (per tiller); FLL: final length of leaves (cm.leaf⁻¹); DFV: leaf life span (days); NP: tiller density; FPH: final plant height (cm); CV: coefficient of variation; Means within lines followed by different letters differ by Tukey's test at 5% probability

Table 2: Concentration of chlorophylls, carotenoids (µg.g⁻¹ of fresh mass) and SPAD index of Mombasa grass inoculated with plant growth-promoting microorganisms

Items	Control	<i>A. brasilense</i>	<i>R. intraradices</i>	Co-inoculation	CV%
Chlorophyll <i>a</i>	152.66b	184.60ab	188.45ab	192.76a	9.69
Chlorophyll <i>b</i>	38.87	29.85	24.12	33.48	26.78
Total chlorophylls	191.51	214.45	212.58	226.24	8.33
Carotenoids	28.58	23.81	23.82	16.82	38.72
SPAD	16.17b	19.38a	19.00a	20.90a	5.98

CV: coefficient of variation; Means within lines followed by different letters differ by Tukey's test at 5% probability

Table 3: Chemical composition of Mombasa grass inoculated with plant growth-promoting microorganisms

Items	Control	<i>A. brasilense</i>	<i>R. intraradices</i>	Co-inoculation	CV%
DM	27.66	27.69	27.65	27.23	5.65
MM	7.81c	8.22a	7.86b	7.96b	7.74
NDF	65.54	68.96	68.45	65.54	4.39
ADF	32.11	34.58	34.54	32.23	8.66
CP	5.74b	6.51a	6.11ab	6.17ab	5.26

DM: dry matter; MM: mineral matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; CP: crude protein; CV: coefficient of variation. Means within lines followed by different letters differ by Tukey's test at 5% probability

Table 4: Root characteristics of Mombasa grass inoculated with plant growth-promoting microorganisms

Items	Control	<i>A. brasilense</i>	<i>R. intraradices</i>	Co-inoculation	CV%
Root volume	59.40b	77.00a	60.60b	71.00ba	13.44
Root dry weight	113.53b	130.84a	115.75b	138.08a	23.05

CV: coefficient of variation; Means within lines followed by different letters differ by Tukey's test at 5% probability

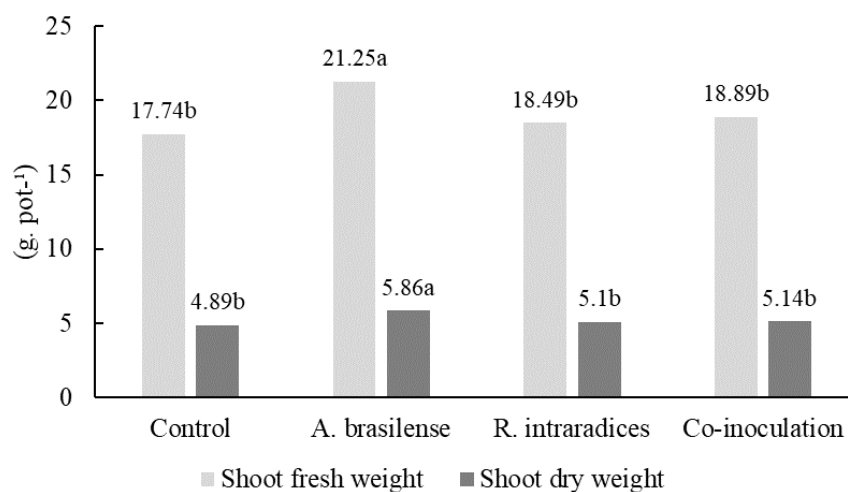


Fig. 1: Shoot fresh and dry weight of the aerial part of Mombasa grass inoculated with plant growth-promoting microorganisms. Means followed by different letters, for each items analyzed, differ by Tukey's test at 5% probability (Coefficient of variation for shoot fresh: 10.60%; Coefficient of variation for Shoot dry: 5.32%)

Discussion

The benefit of using plant growth-promoting microorganism has been reported by several authors, contributing positively to crop development with improvements in nutrient absorption, resistance to pathogens, and root development (Souza et al. 2011; Goswami et al. 2016; Fukami et al. 2018). In this study, we present results of morphogenic, bromatological, and biomass production characteristics in Mombasa grass, assessed under controlled conditions using commercial inoculants applied in seeds.

Seed inoculation with the microbial inoculant employed has improved various morphogenic parameters of Mombasa grass, leading to a greater leaf appearance rate and a lower phyllochron value, while also increasing the number of live leaves (Table 1). The ability of *A. brasilense* strains to produce and modulate the level of endogenous phytohormones like auxins, gibberellins and cytokinins (Cassán et al. 2020) and arbuscular mycorrhizal fungi *R. intraradices* to assist in water and nutrient absorption (Begum et al. 2019), can stimulate the proliferation and elongation of plant cells, root elongation and stimulating the differentiation of meristematic tissues (Glick 2014; Souza et al. 2017), reflected in the improvement of the morphogenic characteristics. This indicates that the inoculants used can activate mechanisms that influence the generation and development of new leaves, resulting in a greater flow of tissue, increased interception efficiency, and enhanced conversion of luminous energy by Mombasa grass.

Considering combined or isolated inoculation, remarkable results were observed for the SPAD index and chlorophyll *a* (Table 2), translating into greater photosynthetic efficiency and consequently increased biomass production. Our findings confirm the hypothesis that the use of plant growth-promoting microorganisms results in greater nitrogen assimilation by plants compared with the non-inoculated plants; this underscores the positive impact of using these microorganisms in enhancing plant growth and nutrient assimilation processes.

The strategy of isolated inoculation with *A. brasilense* increased the dry biomass production of the aboveground part of Mombasa grass, representing a percentage increase of approximately 19% compared with the non-inoculated plants. Microbial bioinoculants have numerous positive functions associated with the absorption of essential elements for plant development. *A. brasilense* has the ability to synthesize auxins that stimulate development and improve the root system (Hungria et al. 2021; Guimarães et al. 2023), optimizing nutrient absorption by adding improvements in photosynthetic activity through the efficient assimilation of these elements, which favors productive capacity.

This signifies potential benefits for productive systems that rely on pasture renewal techniques. Positive

gains in the biomass production of forage grasses associated with *A. brasilense* have been reported, as seen in species such as *U. ruziziensis* (Hungria et al. 2021), Mulato II grass (Rouseaux et al. 2020), Mombassa and Zuri grasses (Guimarães et al. 2023). These findings highlight the promising impact of microorganism-based strategies in enhancing biomass yield, which is particularly relevant for sustainable pasture management practices.

Improvement in forage quality is another crucial aspect of productive systems associated with microbial inoculant. The plant microorganism association can optimize fertilizer utilization through enhanced absorption of soil nutrient. The anticipation is that such advancements will contribute to more sustainable agricultural practices by promoting efficient nutrient use, thereby mitigating the environmental impacts associated with livestock production.

The results confirm the viability of using bioinoculants in the bromatological characteristics of Mombasa grass subjected to seed inoculation, as evidenced by the mineral content and crude protein values. This is particularly noticeable with the use of *A. brasilense*, which is characterized by a percentage increase of 5.25 and 13.41%, respectively, compared with non-inoculated plants. These improvements are attributed to the benefits of enhanced root growth, leading to increased nutrient absorption. The findings underscore the positive impact of seed inoculation with *A. brasilense* on the nutritional composition of Mombasa grass, highlighting its potential in optimizing the mineral and protein content of forage crops.

Strains of *A. brasilense* possess the capacity to synthesize phytohormones, primarily indole-3-acetic acid (IAA), which aids in root growth and optimizes the absorption of nutrients and water (Fukami et al. 2018; Cassán et al. 2020). Arbuscular mycorrhizal fungi, such as *R. intraradices*, can result in positive plant development. These fungi grow within the cortex cells and extend their hyphae into the soil, forming a mycelial network that acts as an extension of the roots, optimizing the absorption of water and nutrients (Smith and Read 2008). Therefore, plants with heavier roots and a larger root volume may indicate the potential for soil exploration, optimizing the absorption of essential elements for their development, which can contribute to the establishment and sustainability of the system.

Conclusion

These results suggested that single inoculation of *A. brasilense* or *R. intraradices* improved the initial growth of Mombasa grass. Data further revealed that dual inoculation with these microorganisms cooperated synergistically to stimulate root development and improved nutritional status. Therefore, the use of this microbial inoculant as an alternative is capable of optimizing the initial growth of Mombasa grass for sustainable production.

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Author Contributions

HSS: Formal analysis, Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft. TMV, NVS, ICD, BEFS, EMVP, JASJ and TPRS: Investigation, Methodology, Visualization. NTC: Writing – review and editing. DDF, RRJ and FAT: Supervision, Validation, Writing – review and editing. All the authors have read and agreed to the submitted version of the manuscript.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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Atributos produtivos e nutricionais da forragem hidropônica de milho, em diferentes estágios, cultivada em substratos de capim-elefante e serragem de madeira

Productive and nutritional attributes of hydroponic corn forage at different stages grown in elephant grass and wood sawdust substrates

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Resumo: O objetivo foi avaliar atributos produtivos e nutricionais da forragem hidropônica de milho, em diferentes estágios, cultivada em substratos de capim-elefante e serragem de madeira. Foram estabelecidos dois tratamentos (T), com diferentes substratos: T1 – capim-elefante picado e desidratado e T2 – serragem de madeira. No 13º e 20º dias após a semeadura, amostras da forragem foram coletadas para avaliação do desenvolvimento das plântulas, produção de biomassa e composição nutricional. O substrato capim-elefante promoveu melhor desenvolvimento das plântulas, maior produção de biomassa e concentração de matéria mineral, aos 13 dias, quando a serragem de madeira promoveu maior concentração de matéria seca, proteína bruta, lignina e carboidrato não fibrosos ($P < 0,05$). O substrato capim-elefante resultou em maior altura de plântula, produção de biomassa verde, concentração de matéria mineral, proteína bruta e extrato etéreo, aos 20 dias, quando a serragem de madeira promoveu maior comprimento de raiz, maior concentração de matéria seca, fibra em detergente ácido e lignina ($P < 0,05$). O substrato capim-elefante e a coleta aos 13 dias promoveram melhor resultado para a produção de forragem hidropônica, bem como para a sua composição nutricional.

Palavras-chave: biometria; *Pennisetum purpureum*; *Zea mays*

Abstract: The objective was to evaluate productive and nutritional attributes of hydroponic corn forage, at different stages, grown in substrates of elephant grass and wood sawdust. Two treatments (T) were established, with different substrates: T1 – chopped and dehydrated elephant grass and T2 – wood sawdust. On the 13th and 20th day after sowing, forage samples were collected to evaluate seedling development, biomass production and nutritional composition. The elephant grass substrate promoted better seedling development, greater biomass production and concentration of mineral matter, at 13 days, when wood sawdust promoted a greater concentration of dry matter, crude

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protein, lignin and non-fibrous carbohydrate ($P<0.05$). The elephant grass substrates resulted in greater seedling height, production of green biomass, concentration of mineral matter, crude protein and ether extract, at 20 days, when wood sawdust promoted greater root length and greater concentration of dry matter, acid detergent fiber and lignin ($P<0.05$). The elephant grass substrate and collection 13 days promoted better result to produce hydroponic forage and its nutritional composition.

Keywords: biometry; *Pennisetum purpureum*; *Zea mays*

1. Introdução

A forragem hidropônica é uma tecnologia voltada para a produção de biomassa vegetal, e consiste no cultivo de plantas com substratos naturais ou artificiais, ou mesmo sem substratos e sem solo. O cultivo pode ocorrer em bandejas no interior de estufas⁽¹⁾ ou em solo recoberto por lona^(2,3). Estudos tem aplicado a técnica para a produção de forragem de milho^(4,5), soja⁽⁴⁾, milheto⁽⁶⁾, aveia, cevada, trigo e sorgo⁽⁷⁾, com potencial para ser inserido na alimentação animal, especialmente nos períodos de escassez de alimento, dada a qualidade nutricional, principalmente quando considerada em sua totalidade.

A forragem hidropônica de milho tem ciclo curto e apresenta crescimento acelerado. O tempo médio de cultivo da forragem observado nos estudos é de 15 dias do plantio à colheita, colheitas precoces podem resultar em baixo rendimento por área, enquanto colheitas tardias podem causar competição entre plantas e redução da qualidade nutricional. A FAO (2001)⁽⁷⁾ recomenda que a colheita seja realizada entre 10 e 12 dias, pois, a partir desse período, se inicia o processo de perda da qualidade nutricional, o que foi observado e reportado por Almeida et al. (2021)⁽⁸⁾.

O uso de substratos na produção de forragem hidropônica contribui para o aumento do teor de massa seca, pode afetar o valor nutricional da forragem⁽⁹⁾ e permite a fixação do sistema radicular das plântulas, o que é importante para o bom desenvolvimento da forragem⁽¹⁰⁾. Estudos demonstram bons resultados com o aproveitamento de pastagens não utilizadas, para o cultivo de forragem hidropônica⁽⁵⁾, mas não há estudos relatando o uso da serragem de madeira como substrato para este fim.

A serragem de madeira é uma opção que apresenta qualidades desejáveis para substrato, a depender do tipo de madeira e do seu armazenamento⁽¹¹⁾. Em quase todas as cidades, desde as de pequeno porte, é comum encontrar serrarias ativas, cuja serragem de madeira considerada como resíduo muitas vezes não tem uso imediato, sendo doada em algumas situações ou comercializadas com valor acessível.

Dessa forma, o objetivo com o estudo foi avaliar atributos produtivos e nutricionais da forragem hidropônica de milho, em diferentes estágios, cultivada em substratos de capim-elefante e serragem de madeira.

2. Material e Métodos

O presente estudo foi executado no Setor Vegetal da Fazenda Experimental do Centro de Ciências Agrárias, Ambientais e Biológicas, na Universidade Federal do Recôncavo da Bahia, localizada a 12°40’0” de latitude Sul, 39°06’0” de longitude Oeste e 200 metros de altitude, cujo clima é classificado por Köppen como tropical quente e úmido, tipo Aw a Am.

O experimento foi montado em casa de vegetação para controle de aguação, com dois tratamentos (T), constituídos por diferentes substratos, para o cultivo da forragem hidropônica de milho (*Zea mays*, L.; variedade Al-Bandeirante; 93% de taxa de germinação), sendo: T1 (n = 5) – capim-elefante picado e desidratado; T2 (n = 5) – serragem de madeira. A unidade experimental (UE) foi representada por um canteiro (0,45m²) e para a distribuição dos tratamentos nas unidades experimentais foi utilizado o delineamento inteiramente casualizado.

O capim-elefante (*Pennisetum purpureum*) foi submetido ao processo de trituração em máquina forrageira (tamanho médio de 2cm), seguido da desidratação em temperatura ambiente, por quatro dias consecutivos, com revolvimento a cada duas horas. A serragem de madeira, com partículas predominantemente de 2,5mm, foi adquirida no comércio local. Ambos os substratos foram submetidos à análise da composição nutricional, em triplicatas (Tabela 1).

Tabela 1 Caracterização bromatológica, com base na matéria seca, dos substratos.

Variáveis (%)	Substrato capim-elefante	Substrato serragem de madeira
Matéria Seca	93,80	94,37
Matéria Mineral	7,67	2,16
Matéria Orgânica	92,33	97,84
Proteína Bruta	2,55	1,35
Extrato etéreo	8,96	9,70
Fibra em detergente neutro	71,66	82,41
Fibra em detergente ácido	37,92	58,21
Celulose	32,25	35,72
Lignina	4,25	21,14

A semente de milho foi desinfestada em solução de hipoclorito de sódio a 2% durante 10 minutos, sendo posteriormente lavada em água corrente e submetida ao condicionamento osmótico, que consistiu na imersão da semente em água, durante 24 horas, com posterior drenagem, para induzir a pré-germinação.

Os canteiros (0,45m²) foram montados sobre área de solo nivelado com lona dupla face de polietileno. Os substratos foram dispostos nas unidades experimentais em camadas de 0,03m de altura e umedecidos com água (1L.m⁻²). Em seguida, realizou-se a semeadura manual do milho, de forma homogênea, com densidade de 2,2 kg/m², e uma nova camada de substrato (0,02m) foi disposta, cobrindo totalmente as sementes, seguido do umedecimento (0,5L.m⁻²).

A partir do segundo dia após a semeadura até o 19º dia, a aguação foi realizada com auxílio de regador convencional, utilizando-se 2,0 litros de água/m²/dia, dividido igualmente em dois períodos. Nos dias chuvosos a aguação dos canteiros foi suspensa, pois os substratos mantinham a umidade da aguação do dia anterior.

Aos 13 e 20 dias, contados a partir da semeadura, realizou-se a colheita de uma amostra por canteiro, para avaliação da biometria (altura de plântula e comprimento de raiz), produção de biomassa verde e seca (kg.m⁻²) e composição nutricional da forragem (matéria seca - MS, matéria mineral - MM, proteína bruta - PB, extrato etéreo - EE, fibra em detergente neutro - FDN, fibra em detergente ácido - FDA, celulose, lignina, nutrientes digestíveis totais estimado - NDT e carboidrato não fibroso - CNF).

Para a avaliação da biometria, 10 plântulas de cada canteiro foram coletadas ao acaso e submetidas às mensurações da raiz e parte aérea com auxílio de paquímetro. Retirou-se então, uma amostra (0,09m²) de cada canteiro, e das amostras, o excesso de substrato foi removido por fricção, seguido da pesagem para determinação da produção de biomassa verde (PBV). A produção de biomassa seca (PBS) foi determinada a partir da PBV e do teor de MS de cada canteiro: $PBS/m^2 = (PBV \times MS)/100$.

Para a avaliação da composição nutricional, a amostra foi constituída pela parte aérea da forragem, raiz, sementes não germinadas e substrato remanescente. As amostras foram submetidas à pré-secagem em estufa de ventilação forçada, a 55 °C, por 72 horas, quando foram novamente pesadas, moídas em moinho tipo Willey, com peneira de 1mm, armazenadas para análise da MS, MM, PB, EE, FDN, FDA, celulose e lignina, de acordo com os protocolos descritos em Silva e Queiroz (2002) ⁽¹²⁾. O NDT foi estimado a partir da equação: $NDT = 83,79 - (0,4171 \times FDN)$ ⁽¹³⁾. O CNF foi calculado a partir da equação: $CNF = 100 - MM - EE - PB - FDN$, sendo todos os termos expressos como porcentagem da matéria seca ⁽¹⁴⁾.

Os dados obtidos foram avaliados quanto à normalidade pelo teste de Shapiro-Wilk. As variáveis apresentaram distribuição normal e os dados foram submetidos à análise de variância. Adotou-se o nível de significância de 5% para todas as avaliações.

3. Resultados e Discussão

O substrato capim-elefante promoveu melhor desenvolvimento das plântulas e maior produção de biomassa aos 13 dias, o que também foi observado aos 20 dias; a serragem de madeira resultou em maior comprimento de raiz aos 20 dias ($P < 0,05$). Ao avaliar a influência da idade de coleta, obteve-se maior PBV e PBS aos 13 dias e maior altura de plântula aos 20 dias para a forragem cultivada no substrato capim-elefante, quando também houve maior altura de plântula e comprimento de raiz para a forragem cultivada na serragem de madeira ($P < 0,05$). As demais variáveis não foram influenciadas pela idade de coleta ($P > 0,05$) (Tabela 2).

Tabela 2 Biometria de plântula e produção de biomassa verde (PBV) e seca (PBS) da forragem hidropônica de milho cultivada em diferentes substratos e colhida com diferentes idades.

Idade de coleta	Variáveis	Substrato capim-elefante	Substrato serragem de madeira	P valor
13 dias	Altura das plântulas (cm)	21,85 ± 4,62 ^{aB}	17,13 ± 3,94 ^{bB}	< 0,01
	Comprimento de raiz (cm)	17,09 ± 4,63 ^a	8,35 ± 2,82 ^{bB}	< 0,01
	PBV (kg.m ⁻²)	8,25 ± 0,91 ^{aA}	2,65 ± 2,25 ^b	0,004
	PBS (kg.m ⁻² MS)	2,17 ± 0,24 ^{aA}	0,88 ± 0,75 ^b	0,017
20 dias	Altura das plântulas (cm)	28,06 ± 5,00 ^{aA}	22,68 ± 5,68 ^{bA}	0,010
	Comprimento de raiz (cm)	15,02 ± 4,24 ^b	18,54 ± 5,32 ^{aA}	0,001
	PBV (kg.m ⁻²)	4,40 ± 0,92 ^{aB}	2,93 ± 0,49 ^b	0,050
	PBS (kg.m ⁻² MS)	1,20 ± 0,25 ^B	0,91 ± 0,15	0,133
P valor		< 0,01	< 0,01	

As variáveis apresentaram distribuição normal e os dados estão representados pela média ± desvio padrão. Letras minúsculas na linha indicam diferença entre os substratos. Letras maiúsculas na coluna indicam diferença entre as idades de coleta. Adotou-se a ANOVA ao nível de 5% de significância.

Os resultados obtidos para o desenvolvimento das plântulas e produção de biomassa podem ser justificados pela composição químico-bromatológica do substrato, uma vez que o capim-elefante desidratado possui maior teor de matéria mineral e proteína bruta. Possivelmente os nutrientes presentes foram disponibilizados para as plântulas, o que garantiu maior desenvolvimento e consequentemente maior produção de massa verde e seca.

As plântulas alcançaram valores que corroboram com o estudo da FAO (2001)⁽⁷⁾, no qual cita que a altura da forragem hidropônica, dependendo do período de crescimento, pode alcançar de 20 a 30 cm. Ndaru et al. (2020)⁽¹⁵⁾ obtiveram 27,33cm aos 12 dias e 37,33cm aos 20 dias. Os mesmos autores também reportaram produção de biomassa de 1,053kg e 1,233kg aos 12 e 20 dias, respectivamente. Santos et al. (2023)⁽⁵⁾ obtiveram, aos 13 dias pós-semeadura, altura de 13,35 cm e produção de 0,323kg.m⁻² de massa seca, ao utilizarem a serragem de madeira como substrato.

Apesar da altura das plântulas ter aumentado com a idade, tal crescimento não foi suficiente para garantir maior PBV e PBS, principalmente com o substrato capim-elefante, que reduziu pela metade em comparação à primeira colheita. Tal ocorrência corrobora o estudo da FAO (2001)⁽⁷⁾, no qual sugere que colheita realizada entre 7 e 10 dias garante bons índices de produtividade, diferente da colheita da forragem com idade mais avançada, que pode resultar em redução de biomassa seca e da qualidade nutricional. Müller et al. (2006)⁽⁶⁾ relataram redução de 2,28kg.m⁻² para 1,62kg.m⁻² na produção de massa seca da forragem hidropônica de milheto com colheita aos 10 e 20 dias, respectivamente.

A serragem de madeira promoveu, aos 13 dias, maior concentração de MS e de PB, quando o substrato capim-elefante resultou em maior concentração de MM (P<0,05) e o EE foi semelhante entre os tratamentos (P>0,05). A serragem de madeira promoveu, aos 20 dias, maior concentração de MS e com o capim-elefante obteve-se maior concentração de MM, PB e EE (P<0,05). A idade de coleta influenciou na composição bromatológica da forragem. Obteve-se maior concentração de MM e PB aos 20 dias em ambos os substratos; e para a forragem cultivada em serragem de madeira, obteve-se maior teor de EE aos 13 dias (P<0,05). As demais variáveis não foram influenciadas pela idade de coleta (P>0,05) (Tabela 3).

Tabela 3 Composição bromatológica da forragem hidropônica de milho cultivada em diferentes substratos e colhida com diferentes idades.

Idade de coleta	Variáveis (%)	Substrato capim-elefante	Substrato serragem de madeira	P valor
13 dias	Matéria seca	26,96 ± 2,50 ^b	31,70 ± 2,73 ^a	< 0,01
	Matéria mineral	3,99 ± 0,63 ^{aB}	1,10 ± 0,52 ^{bB}	< 0,01
	Proteína bruta	10,21 ± 0,54 ^{bB}	10,85 ± 0,27 ^{aB}	0,010
	Extrato etéreo	18,13 ± 2,46	18,49 ± 0,21 ^A	0,685
20 dias	Matéria seca	27,29 ± 1,10 ^b	30,93 ± 1,15 ^a	< 0,01
	Matéria mineral	6,50 ± 0,92 ^{aA}	3,45 ± 0,34 ^{bA}	< 0,01
	Proteína bruta	12,72 ± 0,41 ^{aA}	11,83 ± 0,32 ^{bA}	0,001
	Extrato etéreo	18,66 ± 0,50 ^a	17,60 ± 0,27 ^{bB}	0,001
P valor		< 0,01	< 0,01	

As variáveis apresentaram distribuição normal e os dados estão representados pela média ± desvio padrão. Letras minúsculas na linha indicam diferença entre os substratos. Letras maiúsculas na coluna indicam diferença entre as idades de coleta. Adotou-se a ANOVA ao nível de 5% de significância.

A composição bromatológica do material produzido em hidroponia é variável, como descrito por Shit (2019)⁽¹⁶⁾ e *vários fatores podem influenciar* essa composição. Acredita-se que, neste estudo, a composição bromatológica da forragem tenha sido influenciada também pela composição do próprio substrato, uma vez que fez parte da amostra utilizada para análise, *a plântula inteira, grãos de milho não germinados e substrato remanescente*.

A MS foi superior na forragem cultivada em serragem de madeira, cujo substrato também apresentou teor de MS (94,37%) superior ao capim-elefante (93,80%), o que provavelmente justifica tal resultado. Semelhante a este estudo, Campêlo et al. (2007)⁽⁴⁾ obtiveram teor de MS superior no substrato casca de arroz em relação ao capim-elefante e, ao avaliar o teor de MS da raiz das plântulas + substratos, observaram menor teor para o capim-elefante (19,62%) em relação à casca de arroz (41,12%).

O maior teor de MM foi obtido na forragem cultivada em capim-elefante, o qual também apresentou maior teor de cinzas em sua composição em relação à serragem de madeira

(Tabela 1). Tal resultado corresponde ao desenvolvimento das plântulas, visto que alguns minerais têm a função de promover o desenvolvimento destas. O sistema radicular bem desenvolvido proporciona maior captação de nutrientes e estes ficam retidos na forragem. Fonseca et al. (2021)⁽⁹⁾ obtiveram 3,98% de MM na forragem hidropônica de milho cultivada em feno de Tifton moído e sugeriram que a concentração de MM é um indicador de nutrição das plantas.

A influência do substrato sobre o teor de PB também é reportada em outros estudos. Neste estudo, apesar de ter sido avaliado o substrato + plântulas, e do capim-elefante ter resultado em maior teor de PB, os teores obtidos em ambos os tratamentos são satisfatórios para atender de forma complementar às exigências nutricionais de animais ruminantes e de herbívoros não ruminantes. Na produção de forragem hidropônica de milho em substrato de capim-elefante, Santos et al. (2023)⁽⁵⁾ relataram concentração de PB de 11,48% aos 13 dias.

O capim-elefante promoveu maior concentração de EE aos 20 dias de idade ($P < 0,0$). O teor de EE presente neste estudo é superior ao encontrado na literatura para a forragem hidropônica de milho. Segundo Shit (2019)⁽¹⁶⁾, é esperado que o conteúdo de EE aumente na planta devido ao aumento de estruturas lipídicas e clorofila, conforme a planta cresce. Tal fato foi observado no estudo de Naik et al. (2012)⁽¹⁷⁾, que descreveram diferença no teor de EE no 5º dia (2,27%) em comparação ao 7º dia (3,49%). Holanda et al. (2021)⁽⁴⁾ relataram 1,4% de EE em forragem de milho cultivada em substrato de casca de arroz.

No presente estudo, logo após a germinação das sementes, notou-se a proliferação de fungos nos canteiros e amostras das sementes de milho e dos substratos foram analisadas. Identificou-se os fungos dos gêneros *Rhizopus* spp. e *Aspergillus* spp., ambos encontrados nas sementes de milho utilizadas, sendo o primeiro também encontrado na serragem de madeira, os quais se proliferaram nas forragens produzidas. Tal fato pode justificar o teor elevado de EE no presente estudo. Segundo Murphy (2001)⁽¹⁸⁾, microrganismos como fungos, acumulam cerca de 30% a 80% de lipídios em sua biomassa.

A concentração de MM e de PB aumentou com a idade de coleta, fato já reportado em outros estudos. Almeida et al. (2021)⁽⁸⁾ testaram diferentes idades de coleta e observaram que a PB reduziu do 10º (10,38%) até o 20º dia (8,78%), mas aumentou no 25º dia (11,37%); e o EE reduziu do 10º (2,10%) até o 20º dia (1,45%) de coleta, assim como ocorreu no presente estudo. Ndaru et al. (2020)⁽¹⁵⁾ observaram que a MM (2,37 X 2,60%) e a PB (14,91 X 18,43%) aumentou do 12º para o 20º dia. Manhães et al. (2011)⁽¹⁹⁾ encontraram para milho forrageiro colhido aos 10 e 17 dias, 10,25% e 15,00% de PB, respectivamente.

Ao avaliar a influência dos substratos, obteve-se maior concentração de lignina na forragem cultivada na serragem de madeira ($P < 0,05$), aos 13 dias, quando a FDN, FDA e celulose foram semelhantes entre os tratamentos ($P > 0,05$). A serragem de madeira também promoveu maior concentração de FDA e de lignina ($P < 0,05$), aos 20 dias, quando a concentração FDN e de celulose foi semelhante entre os tratamentos ($P > 0,05$). Ao avaliar a idade de coleta, apenas o conteúdo de celulose foi influenciado, sendo maior na forragem cultivada em capim-elefante aos 20 dias ($P < 0,05$). Para a forragem cultivada na serragem de madeira, nenhuma das variáveis foi influenciada pela idade de coleta ($P > 0,05$) (Tabela 4).

Rocha et al. (2014)⁽²⁰⁾ encontraram, aos 15 dias, valor superior tanto para FDN (78,32%) como para FDA (59,85%) e justificaram que o alto teor de FDN no substrato (86,67%) pode ter promovido maior teor de FDN na forragem. Müller et al. (2005)⁽²¹⁾ sugeriram que à medida que aumenta a densidade da sementeira ocorre redução no teor de FDN da forragem de milho. O menor diâmetro do caule das plantas, devido à competição por espaço quando a densidade de sementeira é elevada, pode resultar em menor espaçamento da parede celular e, conseqüentemente, diminuir o teor de fibra. Tal fato foi observado nos estudos de Almeida et al. (2021)⁽⁸⁾ e de Rocha et al. (2014)⁽²⁰⁾ para FDN e FDA, que obtiveram redução dessas frações com o aumento da densidade de colheita.

Tabela 4 Conteúdo de fibra da forragem hidropônica de milho cultivada em diferentes substratos e colhida com diferentes idades.

Idade de coleta	Variáveis (%)	Substrato capim-elefante	Substrato serragem de madeira	P valor
13 dias	Fibra em detergente neutro	43,73 ± 5,14	41,57 ± 4,07	0,370
	Fibra em detergente ácido	18,68 ± 3,16	18,83 ± 2,43	0,918
	Celulose	15,82 ± 2,49 ^a	14,05 ± 1,88	0,131
	Lignina	2,22 ± 0,83 ^b	4,32 ± 0,75 ^a	< 0,01
20 dias	Fibra em detergente neutro	41,07 ± 1,78	42,31 ± 4,71	0,501
	Fibra em detergente ácido	16,31 ± 1,10 ^b	19,33 ± 3,49 ^a	0,038
	Celulose	13,77 ± 0,77 ^B	14,12 ± 2,17	0,674
	Lignina	1,51 ± 0,64 ^b	4,68 ± 2,11 ^a	< 0,01
P valor		< 0,01	> 0,05	

As variáveis apresentaram distribuição normal e os dados estão representados pela média ± desvio padrão. Letras minúsculas na linha indicam diferença entre os substratos. Letras maiúsculas na coluna indicam diferença entre as idades de coleta. Adotou-se a ANOVA ao nível de 5% de significância.

O teor de lignina da forragem cultivada em substrato de serragem foi superior em relação ao capim-elefante, independentemente da idade de coleta, o que provavelmente foi influenciado pelo conteúdo de lignina do próprio substrato. Entretanto é importante ressaltar que o teor de lignina obtido em ambos os tratamentos está abaixo da quantidade de lignina reportada por Valadares Filho et al. (2020)⁽²²⁾ em outras forragens verdes comumente fornecidas para ruminantes, como a *Brachiaria decumbens* (5,35%), capim-elefante (6,88%) e alfafa (7,35%).

Aos 13 dias após a sementeira, houve maior concentração de CNF para a forragem cultivada em serragem de madeira ($P < 0,05$), quando o NDT foi semelhante entre os tratamentos ($P > 0,05$). Aos 20 dias não houve interferência dos substratos para nenhuma das variáveis ($P > 0,05$). Houve redução, com o aumento da idade de coleta, na concentração de CNF da forragem produzida no capim-elefante ($P < 0,05$) (Figura 1).

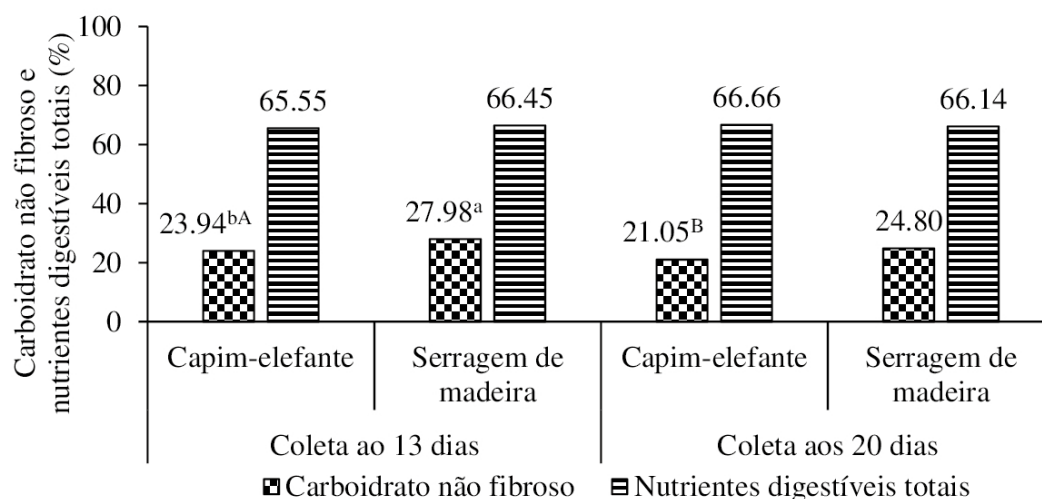


Figura 1 Carbohidrato não fibroso e nutrientes digestíveis totais da forragem hidropônica de milho cultivada em diferentes substratos e colhida com diferentes idades. Letras minúsculas indicam diferença entre os substratos e letras maiúsculas indicam diferença entre as idades de coleta, pela ANOVA ao nível de 5% de significância.

O menor teor de CNF observado na forragem cultivada em capim-elefante pode ser justificado pelos teores mais elevados de FDN e MM, uma vez que, quanto maior forem os teores de PB, EE, MM e FDN, menor será a quantidade de CNF. O CNF de um alimento compreende um grupo de polissacarídeo não amiláceo hidrossolúvel, os quais são constituídos pelas frações resistentes às enzimas digestivas de mamíferos e não recuperadas no resíduo de FDN. Holanda et al. (2021) ⁽⁴⁾ obtiveram CNF inferior (14,6%), isso porque os seus teores de cinzas (10,9%) e FDN (66,4%) foram muito superiores, o que inevitavelmente influenciou o teor de CNF.

4. Conclusão

O substrato capim-elefante e a coleta aos 13 dias pós-semeadura promoveram melhor resultado para a produção de forragem hidropônica, bem como para a sua composição nutricional.

Declaração De Conflito De Interesses

Não temos nenhum conflito de interesses a declarar.

Contribuições dos autores

Investigação e redação (esboço original): TB da Cruz-Neiva; JC Cerqueira; ESC dos Santos; RAL Almeida; EL Candeias-Oliveira; HS Silva; ALA Santana. *Recursos:* LEC Andrade Sobrinho; *Análise formal dos dados:* KN de Oliveira; ALA Santana. *Metodologia, Gerenciamento do projeto, Redação (revisão e edição):* ALA Santana

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Exogenous phytohormones and growth-promoting microorganisms in Basilisk grass cultivation

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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 treatments

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		<i>P value</i>			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
TAIC	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 ($\text{leaf}^{-1} \text{ tiller}^{-1} \text{ day}^{-1}$); Phyllo – phyllochron ($\text{day}^{-1} \text{ leaf}^{-1} \text{ tiller}^{-1}$); TAIC – stem elongation rate ($\text{cm}^{-1} \text{ tiller}^{-1} \text{ day}^{-1}$); 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		<i>P value</i>			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		<i>P value</i>			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; Fun – 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; Fun – 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		<i>P value</i>			CV (%)
	Cont	Bac	Fun	Bac+Fun	With	Without	M*PGR	PGR	M	
CLa	0.48	0.53	0.54	0.53	0.55	0.49	0.01	<0.01	0.00	8.39
CLb	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; Fun – 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. brasiliense* 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 <i>a</i>	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; CLa – 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		<i>P value</i>			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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Yield and morphology of *Nopalea cochenillifera* under N fertilization and biological inoculation

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Abstract. The objective was to evaluate the effect of inoculation with *Azospirillum brasilense* and levels of nitrogen fertilization on the productive aspects of the spineless cactus (*Nopalea cochenillifera*). The experiment was carried in Ribeirão do Largo, Southwest region of the state of Bahia, from April 2019 to April 2020. The experiment was carried out in a 2×4 factorial, in a randomized block design with eight treatments and four replications. The treatments consisted of the absence or inoculation with *Azospirillum brasilense* and nitrogen fertilization levels 0, 50, 100 and 150 kg⁻¹ of N ha⁻¹. There was a positive effect for the use of the bacteria on cladode area index, height, number of secondary, tertiary and total cladodes, total weight for cladodes of all orders and also for final weight per ha⁻¹. There was a significant effect of the use of the inoculant for the levels of total soluble sugars and starch, where there was a decrease in the values for the treatments submitted to the use of the bacteria. There was a quadratic effect for the weight of the tertiary cladodes, number of tertiary cladodes and total of spineless cactus when submitted to nitrogen fertilization levels. There was a linear test for cladodes area index when nitrogen fertilization was used. The use of *Azospirillum brasilense* is positive for the cultivation of spineless cactus, improving development and increasing crop productivity.

Key words: *Azospirillum brasilense*, cladodes, microorganisms, morphometric characteristics, spineless forage cactus.

INTRODUCTION

Pasture growth in the Brazilian semiarid region is limited by the low water availability in the region, which means that pasture production does not meet the needs of the herd during the drought, directly impacting zootechnical indexes, requiring an alternative to meet this demand for food.

The spineless cactus is an alternative for cultivation in drier regions, because the plant can produce even water restriction situation, besides having a high roughness and the ability to produce forage (Silva et al., 2014). Higher spineless cactus productivity can be achieved when the crop is subjected to nitrogen fertilization, a fact that can be justified by the increase in the emission of cladodes, which in turn reflects directly on the production (Souza & Fernandes, 2006; Cunha et al., 2012).

With the growing demand for nitrogen fertilizers, an alternative has been sought to reduce costs and meet the nitrogen demands of plants, which can be achieved with the use of growth-promoting bacteria, which biologically fix atmospheric nitrogen, making it available for the culture with which it is associated. In addition to biological nitrogen fixation, these bacteria can stimulate plant growth by releasing phytohormones (Hungria, 2011).

Among the growth promoting bacteria we have *Azospirillum brasilense*, which has been bringing positive results in the agricultural scenario, being used in the cultivation of corn, wheat and pastures, increasing productivity and reducing production costs of these crops (Guimarães et al., 2022).

In order to characterize bacterial isolates in giant and spineless cactus (*Opuntia* spp. and *Nopalea* spp.), using a partial gene, Silva et al. (2015), managed to isolate twelve types of bacteria, among them the *Azospirillum* genus. Bacteria of the genus *Azospirillum* are free living organisms, considered as growth promoters of plants, capable of fixing atmospheric nitrogen in a usable form for plants, these organisms can live in a way associated with the rhizosphere of plants internally or externally (Okon & Lacerda Gonzales, 1994).

The mode of action of the bacterium is associated with nitrogen fixation and also the secretion of phytohormones, which interfere with the development of the plant, acting mainly on the root system, making it have a greater capacity for absorbing nutrients, the result being better development of the plant, the combination of factors (Bashan & Levanony, 1990; Okon & Itzigsohn, 1995).

The nitrogen fixing diazotrophic bacteria, a group to which the genus *Azospirillum* spp. belongs, can perform biological nitrogen fixation, fixing N₂ in NH₃, with FBN being the main form of entry of atmospheric nitrogen into natural systems (Hungria, 2011; Freitas et al., 2015). These bacteria can be used as an alternative to supply nitrogen to the spineless cactus and provide for its growth, however, no work has addressed this subject.

These bacteria can be used as an alternative to supply nitrogen to the spineless cactus and provide for its growth Almeida et al. (2012), report that the practice of nitrogen fertilization in palm guarantees a significant increase in productivity, regardless of the cultivar, showing positive responses to nitrogen fertilization in conjunction with other nutrients.

The objective of this work was to evaluate the effect of inoculation with *Azospirillum brasilense* and levels of nitrogen fertilization on the productive aspects of the spineless cactus.

MATERIALS AND METHODS

The experiment was conducted on a farm in the district of Rio do Curral, Encruzilhada, BA, the geographical coordinates in which the experimental area is located are 15° 33'35" south latitude, 40° 43'39" west longitude, and with an approximate altitude of 890 m. The region is characterized by a tropical climate, with an average annual rainfall of 777 mm, an average annual temperature of 22.2 °C according to the Köppen classification.

The treatments were carried out in a randomized block design, composed of 8 treatments arranged in a 2×4 factorial scheme, with four replications, totaling thirty-two experimental units. The first factor was constituted by the presence or absence of the bacteria (*Azospirillum brasilense*) in the dosage of 1 L ha⁻¹, with the administration of the amount divided in two moments, the second factor was constituted in levels of nitrogen fertilization (0, 50, 100 e 150 kg ha⁻¹ of N) so that it can be verified whether there will be a linear increase in productivity. The experimental plot had its stipulated dimensions of 8 m × 4 m, and a useful area of 6 m × 3 m.

With the defined area, 5 soil samples were collected at a depth of 0 to 20 cm, which after being mixed were sent for analysis at the Laboratory of the Department of Agricultural and Soil Engineering at UESB, Vitória da Conquista Campus, the chemical analysis of the soil revealed the following characteristics: pH (water) = 4.8; $p = 1.0 \text{ mg dm}^{-3}$; $\text{K}^+ = 0.10 \text{ cmolc dm}^{-3}$; $\text{Ca}^{2+} = 1.3 \text{ cmolc dm}^{-3}$; $\text{Mg}^{2+} = 1.0 \text{ cmolc dm}^{-3}$; $\text{Al}^{3+} = 0.7 \text{ cmolc dm}^{-3}$; $\text{H}^+ = 6.5 \text{ cmolc dm}^{-3}$; and organic matter (OM) = 18.0 g dm⁻³. The recommendation for fertilizing and liming was carried out according to Ribeiro et al. (1999), with a calculated dose of 4.09 ton ha⁻¹ of limestone, after ninety days of the application of the limestone, phosphorus was applied in the amount of 181,8 kg ha⁻¹ of P₂O₅, using the simple superphosphate as the source of the nutrient, and potassium in the amount of 282,48 kg ha⁻¹ of K₂O using potassium chloride as the nutrient source. The planting furrows were made, with the aid of a furrower, for planting spineless cactus. The seedlings obtained were removed from the mother plants, selected in the field, and after this process, they were left to rest in the shade for a period of fifteen days for the wilt and healing of the wounds arising from the harvest and selection process.

The crop was implanted at 0.10 m × 2 m spacing, aiming at a population of 50,000 plants ha⁻¹. The cladodes were deposited in the planting furrows on April 29, 2019, burying them to a depth of up to one-third the length of the cladode. The first application of the bacteria (*Azospirillum brasilense*) was carried out shortly after planting the cladodes, on April 29, at a dose of 500 mL ha⁻¹, or 1.6 mL per plot, the strains used come from selection research carried out in Brazil by Embrapa (Brazilian Agricultural Research Corporation), with strains AbV5 (CNPSO 2083) and AbV6 (CNPSO 2084) which are currently used as inoculants for agricultural crops such as corn (*Zea mays* L.) for pastures planted with *Urochloa* spp. (*Brachiaria* spp.) and can also be used in coinoculation of soybean (*Glycine max* L.) (Hungria et al., 2010; Hungria et al., 2013; Hungria et al., 2016). At the beginning of the water season, on October 29, the second dose of the bacteria (500 mL ha⁻¹, or 1.6 mL in each experimental unit) was applied. The microorganism was applied to the plant cladodes in a sprayed manner, diluting 1.6 mL of the solution with *A. brasilense* in 500 mL of distilled water. At the beginning of the water period, the treatments submitted to nitrogen fertilization received the first fertilization dose, which was divided into 50 kg ha⁻¹ of N or 355 g of urea per experimental unit, for each month, during the months of October, November and December, respecting the pre-established quantities for each treatment, which were applied in installments. Nitrogen fertilization in cover was carried out by hand. Weed control was performed through manual weeding, without using phytosanitary products.

In April 2020, quarterly collections of morphometric data were carried out as a monthly increase in the width and length of the cladodes of the plants. The final data collection took place on April 29, 2020, where morphological data were collected, such as width, length and thickness of all cladodes of spineless cactus plants, with the aid of

a caliper and an invar basimeter, also the height and number of cladodes data. In possession of these values, the cladodes area indices were calculated according to the methodology proposed by Pinheiro et al. (2015). The cladodes collected from the plots were weighed in the field, with a precision scale to determine the production of green mass. The cladode samples were pre-dried in a forced ventilation oven at 55 °C for 72 hours, then they were weighed again to determine the dry mass production. Total soluble sugars and starch were quantified using the Antrona method (Dische, 1962).

The results were subjected to analysis of variance and, when significant, determined whether the regression to nitrogen levels and the F Test to compare the means of treatment without and with inoculation T adopted the 5% level of probability and used the statistical software SAS.

RESULTS AND DISCUSSION

The variables enlargement rate of secondary cladodes, elongation rate of primary cladodes and elongation rate of secondary cladodes (Enlar C2, Elong C1 and Elong C2), were influenced by inoculation ($P < 0.05$), with higher rates being demonstrated when use of bacteria, showing that inoculated plants have a higher rate of increase in the dimensions of cladodes (Table 1). It can also be observed the quadratic behavior for the Elong C2 variable, with maximum value 138.9 kg ha⁻¹ of N.

Table 1. Enlargement and elongation rate of cladode the spineless cactus (cm month⁻¹) depending on the application of *Azospirillum brasilense* and nitrogen fertilization

Items	BAC		Nitrogen (kg ha ⁻¹)				CV	P-Value		
	With	Without	0	50	100	150		BAC	N	BACxN
Enlar C1	0.77A	0.76A	0.73	0.74	0.81	0.79	10.57	0.671	0.173	0.347
Enlar C2	0.77A	0.72B	0.71	0.72	0.76	0.79	7.43	0.010	0.055	0.068
Elong C1	2.04A	1.86B	1.96	1.87	2.00	1.98	10.38	0.027	0.562	0.424
Elong C2	2.11A	2.04B	$\hat{Y} = 1.96 + 0.002x - 0.000009x^2$; $R^2 = 0.99$				3.66	0.022	< 0.001	0.714

Enlar C1: Primary cladode enlargement rate; Enlar C2: secondary cladode enlargement rate; Elong C1: primary cladode elongation rate; Elong C2: secondary cladode elongation rate; BAC: bacteria; N: nitrogen. Means followed by the same capital letter, for bacteria, do not differ by the *F* test ($P > 0.05$).

For the total soluble sugars and starch, the effect of inoculation was verified ($P < 0.05$), with lower levels for the inoculated plants (Table 2). Such behavior is related to productivity, in the situation that plants in constant growth accumulate less reserve and sugars, because they are in constant use, as can be seen, inoculated plants obtained greater growth and presented lower levels of total soluble sugars and starch.

Table 2. Total soluble sugars and starch of the spineless cactus cladodes (mg g⁻¹) depending on the application of *Azospirillum brasilense* and nitrogen fertilization

Items	BAC		Nitrogen (kg ha ⁻¹)				CV5	P-Value		
	With	Without	0	50	100	150		BAC	N	BACxN
TSS	80.09B	103.74A	85.16	85.70	93.38	103.41	23.02	0.004	0.300	0.952
STARCH	13.47B	17.29A	13.86	15.58	15.55	16.54	25.23	0.011	0.588	0.658

TSS: Total soluble sugars; STARCH: Starch Content; BAC: bacteria; N: nitrogen; Means followed by the same capital letter, for bacteria, do not differ by the *F* test ($P > 0.05$).

Low levels of TSS and starch in plants with higher growth are justified, because plants in full vegetative growth, constantly consume soluble sugars for their development, and only store these carbohydrates when they slow their growth (Watt et al., 2013).

The inoculation factor had a significant influence ($P < 0.05$) for the variables of primary, secondary cladodes and average total length (LPC, LSC and LTTC) and also for the variables of secondary and tertiary cladodes, average total width (WSC, WTC, WTTC) (Table 3), where there were higher values for the variables when the inoculation was used. For the variable mean total thickness of cladodes, there was no difference between the factors studied ($P > 0.05$).

Table 3. Dimensions of the spineless cactus cladodes (cm) depending on the application of *Azospirillum brasilense* and nitrogen fertilization.

Items	BAC		Nitrogen (kg ha ⁻¹)				CV	P-Value		
	With	Without	0	50	100	150		BAC	N	BACxN
LPC	24.55A	22.43B	23.62	22.45	24.08	23.81	10.38	0.022	0.562	0.424
LSC	25.32A	24.52B	$\hat{Y} = 23.64 + 0.029x - 0.0001x^2$; $R^2=0.99$				3.66	0.020	< 0.001	0.710
LTC	23.32A	20.45A	19.29	23.22	21.26	23.76	23.53	0.129	0.317	0.659
LTTC	24.42A	23.11B	23.24	23.43	23.91	24.96	6.01	0.017	0.346	0.324
WPC	9.35A	9.20A	8.87	8.89	9.57	9.78	10.57	0.671	0.173	0.347
WSC	9.30A	8.64B	8.58	8.64	9.49	9.18	7.43	0.010	0.055	0.068
WTC	8.61A	7.25B	7.37	8.34	7.82	8.20	20.00	0.024	0.624	0.803
WTTC	9.05A	8.56B	8.53	8.49	9.23	8.98	6.34	0.020	0.051	0.506
TTC	1.68	1.43	1.63	1.41	1.66	1.52	30.71	0.165	0.707	0.351
PHEI	65.97A	60.04B	58.77	62.91	63.84	66.49	12.71	0.049	0.310	0.528

SPC: Length of primary cladodes; LSC: length of secondary cladodes; LTC: length of tertiary cladodes; LTTC: length of total cladodes; WPC: width of primary cladodes; WSC: width of secondary cladodes; WTC: width of tertiary cladodes; WTTC: width of total cladodes; TTC: thickness of total cladodes; PHEI: plant height; BAC: bacteria; N: nitrogen. Means followed by the same capital letter, for bacteria, do not differ by the F test ($P > 0.05$).

The length of secondary cladodes variable showed quadratic behavior when the nitrogen fertilization levels were evaluated, presenting the highest length cladode (25.8 cm) when the plot was subjected to application of 147 kg ha⁻¹ of N. The width of primary cladodes variable did not show any difference for no factor.

The relationships between the morphological characteristics of the plant, such as width, length and height, are closely linked to the area index of cladodes, and consequently to the yield of the plant (Neder et al., 2013; Pinheiro et al., 2014).

Factors such as cladodes area index and height indicate the quality of fertilizer management on the crop, as they will directly correspond to productivity, in addition to estimates of forage accumulation (Costa et al., 2012; Litke et al., 2018; Hourani, 2023).

The interaction between inoculation with the bacterium *Azospirillum brasilense* and nitrogen fertilization was not significant ($P > 0.05$) for the cladodes area index (CAI), however, there was an isolated effect of the factors studied (Fig. 1) for CAI, obtaining higher values when they were inoculated with the bacteria.

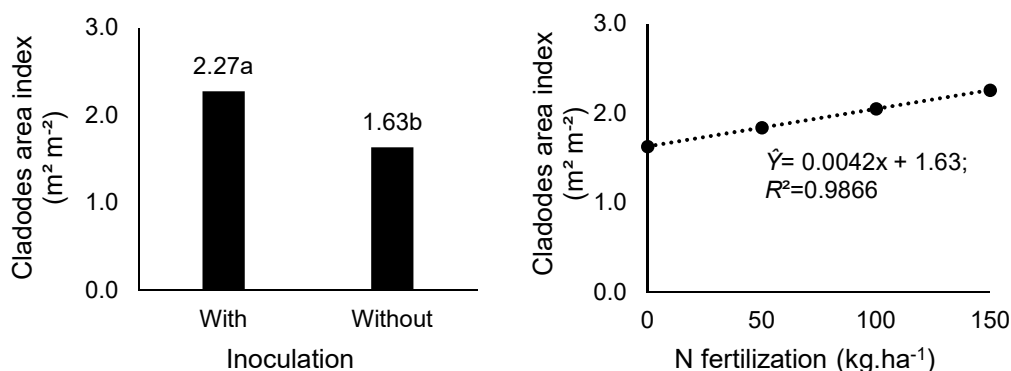


Figure 1. Cladodes area index of spineless cactus depending on the application of *Azospirillum brasilense* and nitrogen fertilization.

Averages followed by the same capital letter, for bacteria, do not differ by the *F* test ($P > 0.05$).

The variable CAI showed an increasing linear response to nitrogen fertilization levels, and it can be observed that as the nitrogen fertilization dose increases, the cladode area index has a positive response, presenting 2.29 when submitted to a dose of 150 kg ha⁻¹ of N. Dubeux Júnior & Santos (2005) present in their work results that the spineless cactus extracts 9 kg of nitrogen for each ton of dry matter produced, emphasizing the importance of nitrogen fertilization for culture.

Lemaire & Chapman (1996), show that the appearance and development of leaves has a fundamental role in morphogenesis, and directly influences the structure of the pasture. As with the monthly increment values, the elongation and enlargement rates had significant increases when the plants were subjected to inoculation, which directly reflected in the height and CAI variables, also showing higher values with the inoculation, making the use of the same be beneficial for the development and growth of the spineless cactus.

Significant increments ($P < 0.05$) were obtained for the variables total average weight of primary cladodes, total average weight of secondary cladodes, total average weight of tertiary cladodes, total weight of green mass of cladodes and production of matter dry (TWPC, TWSC, TWTC, TWE and DMP). The plants that did not receive inoculation with the bacteria were the least productive (Table 4).

Table 4. Total weight of spineless cactus (kg ha⁻¹) depending on the application of *Azospirillum brasilense* and nitrogen fertilization

Items	BAC		Nitrogen (kg ha ⁻¹)				CV	P-Value		
	With	Without	0	50	100	150		BAC	N	BACxN
TWPC	108,638A	85,572B	93,154	94,133	96,752	104,379	21.47	0.005	0.702	0.103
TWSC	131,087A	85,626B	90,656	109,027	113,294	120,450	20.86	0.001	0.085	0.576
TWTC	50,3800A	28,103B	$\hat{Y} = 32,58 - 66.06x + 1.32x^2$; $R^2=0.99$				34.74	0.001	0.025	0.303
TW	292,480A	200,453B	217,960	237,067	251,437	279,402	18.01	0.001	0.070	0.181
DMP	23,813A	16,695B	18,537	19,301	20,971	22,203	18.97	0.001	0.249	0.229

TWPC: Total weight of primary cladodes; TWSC: total weight of secondary cladodes; TWTC: total weight of tertiary cladodes; TW: total weight of cladodes; DMP: dry matter production; Averages followed by the same capital letter, for bacteria, do not differ by the *F* test ($P > 0.05$).

For the variable total weight of tertiary cladodes (TWTC), when submitted to nitrogen fertilization levels, it presented a quadratic behavior, where the lowest weight of cladodes, 32,585 kg, can be identified, when nitrogen fertilization with values of 24.9 kg ha⁻¹ of N was used.

Plants inoculated with *Azospirillum brasilense*, show greater development and production, as this bacterium can fix atmospheric nitrogen and produce phytohormones, inhabiting the rhizosphere of the plants, favoring the development of the same (Okon & Labandera Gonzalez, 1994; Bashan et al., 2004; Fiori et al., 2011).

Phytohormones such as cytokinin and auxin act directly on the growth and development of the plant, being transported to all parts of the plants through the xylem (Hungria et al., 2011). Cytokinin stimulates cell division, which can be called cytokinesis and, together with the auxin that regulates growth, providing greater vegetative development (Radwan et al., 2004; Moreira et al., 2010; Taiz & Zeiger, 2017).

Such development may be justified by the direct link with the production of phytohormones and biological nitrogen fixation, since they are growth promoters and act in different physiological processes, both in the root system and in the aerial part of the plant, therefore, justifying the largest length and width of the spineless cactus for treatments submitted to inoculation with *Azospirillum brasilense* (Dobbelaere et al., 2002; Hungria, 2011).

The auxins (indolacetic acid, indolbutyric acid and naphthalene acetic acid) and cytokinins (benzylaminopurine, isopentenyladenine and zeatin), will act on organs and tissues, being transported in the plant, where it is possible to identify the cytokinin in greater quantity, as the main responsible for favor the development of the aerial part of plants, which together with auxins will regulate plant growth (Moubayidin et al., 2009; Hungria et al., 2011; Nakhooda et al., 2012).

Greater development, production and vegetative growth were observed in inoculated plants, as can be seen by the value of total weight of cladodes (TWE), which reflect the result of the variables that indicate a better growth and development of spineless cactus plants that received the application of *Azospirillum brasilense*, attributing the bacterium the results.

CONCLUSIONS

The use of *Azospirillum brasilense* contributes positively to the development and productivity of the spineless cactus.

The use of nitrogen fertilization did not promote significant effects on crop productivity in the doses used.

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Productive and biochemical responses of Marandu grass under fertilization protocols

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Abstract. The aim of this study was to evaluate the effects of liming and chemical fertilization protocols, containing different combinations of nitrogen (N), phosphorus (P) and potassium (K), on the photosynthetic pigments, productive characteristics and carbohydrate concentration of *Urochloa brizantha* cv. Marandu. Five fertilization protocols were evaluated (PK fertilizer combination; NP fertilizer combination; NK fertilizer combination; NPK fertilizer combination; and without fertilizer - control), associated or not with soil correction via liming, in a completely randomized design, with four replicates. The experimental units consisted of plastic pots with a capacity of 12 liters, which were filled with 10 dm³ of sandy clay loam soil. Seeds of *Urochloa brizantha* cv. Marandu, with 80% cultural value were used. With the corrected soil, the NP protocol provided greater production of dry mass of residue (PMSRE), root volume, leaf area, leaf area index, total sugar content (AST) and starch in the root, compared to the other protocols. Without liming, the NP protocol provided greater production of aerial part dry mass (PMSPA), root volume, leaf aerial volume, leaf aerial index and AST content in leaves, roots and residue. The NPK protocol, with liming, was 73% higher in chlorophyll a content, 50% in carotenoid content, 90% in PMSPA, 78% in leaf area and 76.2% in leaf area index, compared to the soil without correction. The use of NP fertilization with liming is recommended as it provides positive responses on the photosynthetic pigments, productive characteristics and total sugar content of Marandu grass.

Key words: starch, *Brachiaria brizantha*, limestone, chlorophyll, phosphorus, nitrogen.

INTRODUCTION

In Brazil, approximately 80% of pastures are composed of the genus *Urochloa*, with emphasis on the species *Urochloa brizantha* cv. Marandu, widely spread and used throughout the country, representing approximately 50% of the pasture area (Cardoso et al., 2016). Its use in different production systems is justified by its characteristics, resistance to drought and pasture leafhoppers and high dry matter productivity, average of 20 ton ha year⁻¹, in addition to its high crude protein content (Valle et al., 2010).

However, the species has not expressed its maximum productive potential on many properties due to the degradation of cultivated areas. According to data from MapBiomass (2022), around 52% of the areas designated for pasture in Brazil face some level of degradation, which is associated with compaction and gradual decrease in soil fertility, especially due to NPK deficiency (Paciullo et al., 2007; Vendrame et al., 2010), which has resulted in low production and quality of grasses and consequently in lower animal performance and profitability.

The application of agricultural correctives, as liming and fertilization is crucial to avoid degradation and to be able to maintain the productivity of forage species, in addition, the stability of the system is ensured as the balance between the nutrients that are extracted from the soil and those that are supplied. Liming aims to raise the pH to values between 5.5 and 6.0, which is the range in which most nutrients are absorbed by plants (Rossetto et al., 2005). Furthermore, it increases the concentration of magnesium and calcium, promoting their binding with elements that are harmful to plants, such as aluminium, making the region more suitable for plant development.

Nitrogen, phosphate and potassium fertilizations are intended to maintain and promote the growth of forage grasses, being responsible for the constitution of vital organic compounds, plant development, in addition to influencing plant metabolism, efficient use of water, translocation of carbohydrates, growth and development of tillers, as well as the size and number of leaves and stems (De Morais et al., 2016).

Currently, research on grass development has focused on understanding biochemical parameters and their relationship with biomass production and root development (De Oliveira et al., 2018; Cruz et al., 2023; Seixas et al., 2023). Carbohydrates are synthesized in leaves through the photosynthetic process and serve as a source of energy for plant growth and development, in addition to being stored in the roots and base of the stem, functioning as a reserve carbohydrate that will be used according to the plant's needs (Taiz et al., 2017). Understanding the biochemical processes in forage grasses is essential, due to agronomic practices commonly carried out in the establishment of pastures, such as liming and fertilization, to propose pasture fertilization protocols that are appropriate to the possibility of adopting or not cultural treatments during planting.

Therefore, this study aimed to evaluate the productive characteristics and quantify photosynthetic pigments, Soluble and reserve carbohydrates of *U. brizantha* cv. Marandu as a function of liming and chemical fertilization protocols during two growth cycles. We hope to identify which combination of fertilizer protocol and soil correction can improve the biochemical and productive parameters evaluated in *U. brizantha* cv. Marandu.

MATERIALS AND METHODS

Experimental details and treatments

The experiment was conducted in a greenhouse located at the Southwest Bahia State University, Juvino Oliveira Campus, located at the following coordinates: 15°38'46'' south latitude, 40°15'24'' west longitude, average longitude of 280 m, in the municipality of Itapetinga-BA, from April to June 2019, with a maximum temperature of 41.4 °C and a minimum temperature of 20.8 °C throughout the experimental period. The research was conducted in a completely randomized design, in a 5×2 factorial design, five

fertilization protocols (Control: absence of fertilizers; PK fertilizer combination; NP fertilizer combination; NK fertilizer combination; NPK fertilizer combination), associated or not with soil correction via liming, with four replicates. The experimental units consisted of plastic pots with a capacity of 12 liters, filled with 10 dm³ of soil with a sandy-clay loam texture. The soil was collected at a depth of 0 to 20 cm and its chemical characteristics are described in Table 1.

Table 1. Soil chemical analysis

pH	mg dm ⁻³	Cmol _c dm ⁻³ of soil								%	
(H ₂ O)	P	K ⁺	Ca ²⁺	Mg ²⁺	Al ³⁺	H ⁺	S.B	t	T	V	m
4.7	1	0.1	0.6	0.4	1.3	5.9	1.1	2.4	8.3	13	54

S.B: Sum of bases; t: effective cation exchange capacity; T: pH cation exchange capacity; V: base saturation; m: Aluminium saturation.

The pots subjected to the soil correction factor had limestone applied 30 days before planting, with the application of 18.6 g of calcitic limestone with PRNT 82% per pot, determined by the exchangeable Al and Ca + mg method (Alvarez Vênegas & Ribeiro, 1999).

$$NC \text{ (t ha}^{-1}\text{)} = Y * [Al^{3+} + (mt.t/100)] + [X - (Ca^2 + Mg^2)] * 100/PRNT \quad (1)$$

where NC = limestone requirement; Y = factor that varies with the soil's acidity buffering capacity and can be defined according to the texture; mt = maximum aluminum saturation tolerated by the crop; t = effective cation exchange capacity; X = calcium and magnesium requirement of the crop; PRNT = relative total neutralization power of the limestone to be applied

For the chemical fertilization factor, the P and K sources were applied at the time of planting, with 110 kg ha⁻¹ of P₂O₅, (3.06 g of triple superphosphate per pot) and 60 kg ha⁻¹ of K₂O (0.52 g of potassium chloride per pot), respectively. The N source (urea) occurred with the application of 150 kg ha⁻¹ in a split form, in two applications, the first application being after the first standardization cut and the second application after the second standardization cut (0.84 g pot⁻¹).

Seeds of *Urochloa brizantha* cv. Marandu, with 80% cultural value were used. Twenty days after emergence (DAE), non-vigorous or non-homogeneous plants were removed, keeping only four plants in each experimental unit (repetitions). At 40 DAE, the first uniformity cut was made at a height of 10 cm at ground level, followed by the first application of N fertilization and at 68 DAE the second standardization cut was carried out with application of the second dose of N fertilization. After the distribution of the fertilization protocols, monitoring and evaluation of the plants began during two cycles growth period of 28 days each, when the grass reached an average height of 30 cm.

To determine the water holding capacity of soil, three pots containing dry soil were initially weighed and then saturated with water for three days. After draining excess water, the pots were weighed again and the difference in weight between the wet and dry soil afterwards was determined as the maximum water holding capacity of the soil, which was 80%. This value was then used as a reference to maintain the water levels of the experimental units close to the retention capacity, with the buckets being weighed daily and water replaced when necessary.

Plant analysis

At the end of each growth cycle, two fully expanded leaves were collected on each experimental unit and packaged in envelopes made of aluminum foil and immediately stored on ice, which were taken to the laboratory to determine the levels of chlorophyll *a*, chlorophyll *b* and carotenoids, where 0.03 g of the fresh mass (MF) of the collected leaf was placed in a glass bottle containing 5 mL of dimethyl sulfoxide and wrapped in aluminum foil for 72 hours. Then, a reading was taken on the spectrophotometer for the quantification of photosynthetic pigments according to Wellburn (1994) and values expressed in mg g^{-1} MF.

Next, the aerial part of the plants was cut at 10 cm above the ground and separated into stem/pseudo-stem and leaf blade, then weighed in natural matter. All leaf blades were scanned using an HP G2710 photo scanner at 300 dpi resolution and subsequently analyzed with the aid of the ImageJ® program, with the leaf area (LA) calculated and its value expressed in $\text{cm}^2 \text{ pot}^{-1}$. Based on the LA data, the leaf area index (LAI) was calculated according to the equation defined by Cairo et al. (2008).

The morphological fractions were grouped and dried in a forced circulation oven at 55 °C for 72 h, they were then weighed to obtain the dry mass production values of the aerial part (PMSPA). The residue, corresponding to 0–10 cm of soil, was also weighed to determine the dry mass production values of the residue (PMSRE). The pots were disassembled and then the root volume (RV) of each experimental unit was determined, using a graduated cylinder with a known volume of water, where the root was introduced and through the difference, the VR was obtained, then the roots were taken to the greenhouse forced circulation at 55 °C for 72 h to determine root dry biomass production (PMSR).

For quantification of total soluble sugars (AST), 0.3 g of leaf, residue and root samples were weighed and ground in a ball mill and subjected to extraction in distilled water, with maceration followed by centrifugation (9,000 g for 20 minutes). This process was repeated twice more and the supernatant was collected and used to quantify water-soluble carbohydrates by the Anthrone method (Dische, 1962). The pellet obtained after the extraction of carbohydrates from the residue and root fractions was again suspended in 5 mL of 200 mM potassium acetate buffer solution (pH 4.8) and heated in a water bath at 100 °C for 5 min under constant stirring. The mixture was then cooled to approximately 50 °C, at which point the enzyme solution containing 0.08 mL of the amyloglucosidase enzyme was added. The mixture was kept in a water bath at 50 °C for two hours, under stirring. After incubation, centrifugation was performed at 9,000 g for 20 minutes, the supernatant was collected and the volume completed to 5 mL with the same buffer, and then the starch content (AMD) was quantified by the Anthrone method (Dische, 1962).

Statistical analysis

Data were subjected to analysis of variance using the Statistical Analysis System (SAS) OnDemand for Academics program (SAS Institute Inc., Cary, NC), considering the fertilization, liming protocols and the interaction as sources of variation. Significant interactions were unfolded and the means compared by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

The interaction between liming and fertilization protocols was significant ($P < 0.05$) for the concentration of chlorophyll *a* and carotenoids (Table 2). For chlorophyll *b*, there was only an effect of the liming factor, in which, in its absence, the plants showed a reduction of 13.33% in relation to the plants subjected to liming, which presented an average of 1.5 mg g⁻¹ MF.

This accessory pigment collaborates with chlorophyll *a* in absorbing light for photosynthesis, even in circumstances of low sunlight. This increase in photosynthetic efficiency allows plants to capture a greater diversity of wavelengths of sunlight and transform it into chemical energy. This highlights the importance of adequately correcting the soil to promote the development of plants with higher levels of photosynthetic pigments.

When liming was used, higher ($P < 0.05$) concentrations of chlorophyll *a* (mg g⁻¹ MF) and carotenoids (mg g⁻¹ MF) were recorded in the NK and NPK protocols. On the other hand, when the soil was not amended, a higher ($P < 0.05$) concentration of chlorophyll *a* (mg g⁻¹ MF) was observed in the PK protocol compared to the NPK protocol. Without liming, the concentration of carotenoids (mg g⁻¹ MF) was higher ($P < 0.05$) using the PK protocol (Table 2).

Table 2. Chlorophyll *a* and carotenoid content of *Urochloa brizantha* cv. Marandu in five chemical fertilization protocols, with and without liming

Liming	Chemical fertilization protocols				
	Without	PK ¹	NP ²	NK ³	NPK ⁴
	Chlorophyll <i>a</i> (mg g ⁻¹ MF)				
Without	2.1Aab	2.4Aa	2.1Aab	1.9Bbc	1.5Bc
With	1.6Bb	1.1Bc	1.7Ab	2.7Aa	2.6Aa
Without	3.2Aab	3.5Aab	3.7Aa	3.2Bab	2.8Bb
With	3.0Ab	2.5Bb	3.1Ab	4.2Aa	4.3Aa
	Carotenoids (mg g ⁻¹ MF)				
Without	0.3Ab	0.4Aa	0.3Ab	0.2Bc	0.2Bc
With	0.1Bb	0.1Bb	0.2Bb	0.4Aa	0.3Aa

Averages followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by the Tukey test ($P > 0.05$). ¹Phosphorus + potassium; ²Nitrogen + phosphorus; ³Nitrogen + potassium; ⁴Nitrogen + phosphorus + potassium; MF: fresh matter.

When examining the effect of fertilization protocols on liming, the NK and NPK protocols with liming provided higher ($P < 0.05$) concentrations of chlorophyll *a* (mg g⁻¹ MF) and carotenoids (mg g⁻¹ MF), compared to unamended soil. The PK protocol, without liming, provided higher ($P < 0.05$) concentrations of chlorophyll *a* (mg g⁻¹ MF) and carotenoids (mg g⁻¹ MF) compared to the amended soil (Table 2).

Greater nutrient availability can enable better vegetative growth of plants, resulting in greater production of green biomass. According to Taiz et al. (2017), an increase in the synthesis of chlorophyll *a* enhances the production of ATP in chloroplasts, since this pigment increases the absorption of light, which is then converted into energy used during the photophosphorylation process. Therefore, carotenoids play a role as accessory pigments in the absorption and transfer of radiant energy, in addition to acting as protectors of chlorophyll against photodegradation caused by high irradiation (Taiz et al., 2017).

The interaction between liming and fertilization protocols was significant ($P < 0.05$) for production of dry mass of aerial part (PMSPA) and dry mass of residue (PMSRE) of *Urochloa brizantha* cv. Marandu (Table 3).

Table 3. Production of dry mass of aerial part (PMSPA) and production of dry mass of residue (PMSRE) of *Urochloa brizantha* cv. Marandu in five chemical fertilization protocols, with and without liming

Liming	Chemical fertilization protocols				
	Sem	PK ¹	NP ²	NK ³	NPK ⁴
	PMSPA (g pot ⁻¹)				
Without	3.1Ad	4.0Acd	16.2Aa	5.3Bc	8.2Bb
With	4.2Ac	5.0Ac	17.3Aa	8.6Ab	15.6Aa
	PMSRE (g pot ⁻¹)				
Without	4.6Ab	5.1Ab	18.1Aa	6.7Bb	18.8Aa
With	5.1Ad	6.8Acd	18.6Aa	9.5Ab	8.6Bbc

Averages followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by the Tukey test ($P > 0.05$). ¹Phosphorus + potassium; ²Nitrogen + phosphorus; ³Nitrogen + potassium; ⁴Nitrogen + phosphorus + potassium.

With the amended soil, higher ($P < 0.05$) PMSPA yields (g pot⁻¹) were observed with the NP and NPK protocols, while, in the absence of liming, the NP protocol provided higher ($P < 0.05$) PMSPA (g pot⁻¹). The use of the NK and NPK protocols, in the presence of liming, resulted in higher ($P < 0.05$) PMSPA (g pot⁻¹) (Table 3). This may have occurred due to the possibility that liming provided an increase in the pH, possibly within the recommended range (5.5–6.0), of the soil to a range where the availability of N and P were enhanced, thus contributing to greater plant growth and PMSPA. According to FERLIN et al. (1999), the presence of nitrogen is one of the main facilitators of the growth and development of grasses, since this nutrient participates directly in the plant's metabolism, contributing to the formation of nucleic acids, proteins, membranes and chlorophyll molecules.

It is important to highlight that the expressive results of the NP protocol, in PMSPA and PMSRE indicate that, in the possibility of not being able to carry out soil correction, in the short term, in sandy clay loam soils, with 1 mg dm⁻³ of P, pH in H₂O of 4.7 and base saturation of 13% (Table 1), Marandu grass planting can be carried out using NP fertilization, as it responds positively to the production of aerial part mass and residue. For PMSRE, higher ($P < 0.05$) yields were observed in the absence of liming when NP and NPK were applied and, in the presence of liming, when only NP was used (Table 3). When analyzing the effects of different chemical fertilization protocols on PMSRE, it is highlighted that the combination of NP, regardless of liming, and the use of NPK in the absence of liming resulted in higher averages for this variable (Table 3).

These results highlight the importance of the positive contributions of the protocols, NP, even in the absence of liming and the NPK protocol, not only in PMSPA and PMSRE, as well as in the concentrations of chlorophyll *a* (mg g⁻¹ MF), carotenoids, respectively (Table 2). The results obtained demonstrate the positive influence of the nutrients N and P on the productive processes of Marandu grass, highlighting especially the importance of P when associated with liming, with crucial participation in the development of the root system and in the increase of photosynthetic pigments of

Marandu grass. Even in unamended soils, the presence of adequate nutrients can result in greater residue production, which can benefit the tillering process.

In the presence of liming, higher ($P < 0.05$) values of root dry mass production (PMSRA) were recorded in the NP and NK protocols and root volume in the NP fertilization protocol, while, without liming, greater ($P < 0.05$) PMSRA was observed in the NP and NPK protocols and root volume in the NP protocol (Table 4).

When examining the effect of fertilization protocols, in liming, on PMSRA and root volume, the NP and NPK protocols, without liming, provided better responses when compared to the amended soil (Table 4), indicating that even without the possibility of liming, the combination of NP and NPK can contribute positively to stimulating root production, since the soil used in the experiment had a low P content (Table 1). The increase in availability, mainly of P and N, regardless of the correction, enabled satisfactory performance of the plants in these protocols, reflecting in the increase of their photosynthetic rate and, consequently, in the increase in the production of photoassimilates, thus promoting greater development of both the aerial part and the root system of the plants (Table 4).

Table 4. Root dry mass production (PMSRA) and root volume of *Urochloa brizantha* cv. Marandu in five chemical fertilization protocols, with and without liming

Liming	Chemical fertilization protocols				
	Sem	PK ¹	NP ²	NK ³	NPK ⁴
	PMSRA (g pot ⁻¹)				
Without	11.6Ab	19.4Ab	35.8Aa	14.5Ab	31.9Aa
With	10.8Ac	14.6Abc	26.1Ba	22.4Aab	16.5Bbc
	Volume de raiz (mL)				
Without	0.08Ad	0.11Ac	0.24Aa	0.09Bcd	0.20Ab
With	0.07Ac	0.11Abc	0.18Ba	0.13Ab	0.11Bb

Averages followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by the Tukey test ($P > 0.05$). ¹Phosphorus + potassium; ²Nitrogen + phosphorus; ³Nitrogen + potassium; ⁴Nitrogen + phosphorus + potassium.

For the variables, leaf area and leaf area index, a significant interaction ($P < 0.05$) was observed between liming and fertilization protocols (Table 5). Regardless of liming, the NP fertilization protocol provided greater ($P < 0.05$) leaf area and leaf area index. When examining the effect of fertilization protocols on liming, the NK and NPK protocols with liming provided greater ($P < 0.05$) leaf area and leaf area index compared to unamended soil (Table 5). The importance of the leaf area index in a crop is well recognized as an indicator of productivity, since the photosynthetic process depends on the interception of light energy and its conversion into chemical energy by the leaves.

When examining the impact of liming on total soluble sugars (TSS) present in leaves, a higher content of this sugar was found in the absence of liming when the fertilizer contained NP, and in the presence of liming when NPK was used. Regarding fertilization, a higher AST content in the leaves was observed in the absence of fertilization, with PK and NP without the application of liming, and with NPK associated with liming.

Table 5. Leaf area and leaf area index of *Urochloa brizantha* cv. Marandu with and without liming and subjected to different fertilizations

Liming	Chemical fertilization protocols				
	Sem	PK ¹	NP ²	NK ³	NPK ⁴
	Leaf area (cm ² pot ⁻¹)				
Without	453.0Ad	559.0Ad	2,869.1Aa	942.9Bc	1,461.9Bb
With	627.9Ad	732.5Ad	2,991.4Aa	1,472.4Ac	2,607.6Ab
	Leaf area index				
Without	0.6Ad	0.8Ad	4.1Aa	1.3Bc	2.1Bb
With	0.9Ad	1.0Ad	4.2Aa	2.1Ac	3.7Ab

Averages followed by the same lowercase letter in the row and uppercase letter in the column do not differ from each other by the Tukey test ($P > 0.05$). ¹Phosphorus + potassium; ²Nitrogen + phosphorus; ³Nitrogen + potassium; ⁴Nitrogen + phosphorus + potassium.

With the corrected soil, the NPK protocol contributed to higher ($P < 0.05$) total soluble sugar (AST) levels, in the leaves and residue. The NP protocol contributed to higher ($P < 0.05$) AST contents in the root and starch in the root, while in the absence of liming, the NP protocol provided higher ($P < 0.05$) AST contents in the leaves, residue and root, and the PK protocol provided higher ($P < .05$) starch contents in the residue (Table 6).

Table 6. Total soluble sugar (TSS) content in the leaf, residue and root and starch content in the residue and root of *Urochloa brizantha* cv. Marandu with and without liming and subjected to different fertilizations

Liming	Chemical fertilization protocols				
	Sem	PK	NP	NK	NPK
	AST Leaf (mg g ⁻¹ DM)				
Without	66.4Ac	72.4Ab	93.4Aa	55.2Ad	69.8Bbc
With	54.9Bc	53.1Bc	84.6Bb	56.3Ac	104.2Aa
	AST residue (mg g ⁻¹ DM)				
Without	59.3Bb	60.4Ab	68.9Ba	43.7Ac	46.0Bc
With	66.5Ac	58.2Ad	82.4Ab	46.5Ae	87.8Aa
Without	33.8Ab	25.1Bc	38.8Ba	16.9Bd	23.2Bc
With	28.3Bd	34.6Ac	66.5Aa	25.9Ad	52.8Ab
	Starch residue (mg g ⁻¹ DM)				
Without	67.6Bb	72.0Aa	55.4Bc	44.4Ad	63.7Ac
With	73.3Aa	69.4Aa	60.6Ab	42.2Ac	53.7Bb
Without	93.7Aa	81.4Ab	81.4Bb	73.4Ac	71.5Bc
With	88.1Bb	72.9Bc	96.2Aa	71.1Ac	86.2Ab

Means followed by the same capital letter in the row and lowercase letter in the column do not differ from each other according to the Tukey test ($P > 0.05$). ¹Phosphorus + potassium; ²Nitrogen + phosphorus; ³Nitrogen + potassium; ⁴Nitrogen + phosphorus + potassium.

When examining the effect of fertilization protocols on liming, the NPK protocol, with liming provided higher ($P < 0.05$) AST content in the leaves, in the residue and starch content in the root, compared to the unamended soil. The NP protocol provided a higher ($P < 0.05$) AST content in the root and residue, and starch content in the residue and root with the corrected soil (Table 6).

Carbohydrates play a fundamental role in the respiration required for the formation of new tissues, and are therefore crucial during the regrowth process (Avice et al., 1996). The expressive results of the NP and NPK protocols with liming indicate the positive contribution in the allocation of photoassimilates, mainly in the residue and in the root, with the purpose of accumulating essential energy reserves for the initial regrowth process. When there is a greater supply of N to plants, their photosynthetic capacity is maximized due to the potentiation of photoassimilates, resulting in greater production of sugars, which are end products of photosynthesis. This explains the results of this study, where higher AST levels were observed in the presence of N, especially when associated with phosphate fertilization.

CONCLUSIONS

The combination of NP fertilization and liming favors the increase in plant mass of *Urochloa brizantha* cv. Marandu grass, in addition to promoting positive effects on pigments related to photosynthesis, production characteristics and total sugar levels. Promoting plant growth and development after cutting, facilitating the understanding of resource allocation and adaptation strategies of forage plants, especially with regard to carbohydrate metabolism, highlighting significant sustainable plant management practices and the rational use of fertilizers.

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Plant growth-promoting microorganisms as mitigators of water stress in pastures: a narrative review

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ABSTRACT

Water stress is a reality present in pastoral areas throughout Brazil, and more challengingly in semi-arid regions. These climatic conditions test the tolerance to water stress of the forage species used, which despite their rusticity, markedly decrease their performance in low water availability, which limits the expression of all their productive potential. In this sense, it is important to search for sustainable technologies that help a greater resilience of animal production on pasture and that are beneficial to the environment, such as the use of microorganisms that promote plant growth, which can potentially help these species to tolerate the deleterious effects of water stress. Therefore, the objective of this review was to compile information on the use of plant growth-promoting bacteria, arbuscular mycorrhizal fungi and their co-inoculation with water stress mitigating agents in forage plants. In view of data obtained from research platforms, the effects of inoculations with beneficial soil microorganisms on morphophysiological and productive characteristics of forage plants were addressed and how this symbiosis can potentially help plants to tolerate water stress, in addition to describing the specifics of the tripartite relationship between fungi, bacteria and forage plants. With this review, it was found that the use of microbiological inputs as modulators of species tolerance has potential use, requiring a greater volume of studies to consolidate the technique in a way that strengthens it with a sustainable and resilient alternative of livestock to pasture.

Keywords: Bacteria, Fungi, Mycorrhiza, Co-inoculation, Resilience.

1 INTRODUCTION

The conditions of pasture animal production in Brazil, predominantly in dryland, have water deficiency (common in semiarid regions) and low natural fertility of the areas, situations that put to the test the adaptation of tropical forage species to stressful growth conditions. These systems face long periods of drought, mainly due to the incidence of dry periods during the rainy season (summer), which, depending on the region, can occur at different times of the year, regardless of the season.

Such environments can cause a negative impact on forage plants, decreasing their productivity by

physiological, nutritional and hormonal imbalances, limiting the productive potential of these areas and negatively reflecting on the zootechnical indices of the animals explored in this system.

In view of this situation, it is necessary to search for solutions with low environmental impact, which can optimize animal production without compromising natural resources and that provide resilience characteristics to the sector, such as reducing dependence on external inputs and increasing the efficiency of these productive systems.

Among the sustainable alternatives, the use of biological inputs based on beneficial soil microorganisms, such as plant growth-promoting bacteria (BPCP) and arbuscular mycorrhizal fungi (AMF) are among the most promising technologies to achieve the sustainability of agricultural systems, because they provide improvements ranging from characteristics to promote growth of shoots and plant roots, even improving the enzymatic protection to the conditions of biotic and abiotic stresses inherent to agricultural and livestock activity nowadays.

Plants evolved in a sophisticated way with soil microorganisms to overcome water stress and their adverse effects within cells, strengthening the intrinsic mechanisms of tolerance to plant stress, producing exopolysaccharides, phytohormones, ACC deaminase, osolites and volatile compounds (Shaffiqueet al., 2022).

The use of this technology has great potential to become a reality in the formation and persistence of pastures, due to the great interest on the part of cattle ranchers, mainly because it is an advantageous alternative for cattle grazing, soil management and environmental quality, due to its low cost, and also for responding to society's demands for a more sustainable livestock (Mamédio et al., 2020).

According to Jiang et al. (2021), AMF are characterized by a mutualistic relationship with plant roots and, because they are mandatory biotrophic, receive carbon compounds from plants and produce an extensive network of hyphae that has access to nutrient stains outside the rhizosphere, favoring the absorption of unmobile elements in the soil such as phosphorus, seeking water in pores of the soil not accessible to the root hair.

Bpcp, as described by Fracasso et al. (2020), act through mechanisms that stimulate plant development, among which we can mention biological nitrogen fixation (BNF), amino acid and phytomonium production, as well as improvement of the availability of nutrients such as phosphorus, through phosphate solubilization processes.

Thus, in theory, the tolerance of tropical forage grasses to these negative drought events can be enhanced and/or aided by beneficial natural microorganisms, including bacteria and fungi, which through symbiotic or associative relationships, provide mitigation of biochemical and physiological disturbances caused by this condition and providing greater resilience to sustainable soil use.

However, according to Nadeem et al. (2014), although microbial inoculants are being widely used to improve plant growth under controlled field and natural conditions, the results obtained with these studies did not reach a reasonable degree of efficacy and consistency necessary for their large-scale

commercialization, due to the complexity of interactions between the various factors present in these ecological relationships.

Therefore, it is important to compile information that helps better understanding of this biological process so that it can be widely used in grazing activities, providing greater resilience, food security and less environmental impact to this productive activity. In this sense, the present work aims to analyze and understand the various relationships between beneficial soil microorganisms and their ability to mitigate the deleterious effects of water stress on forage plants.

2 METHODOLOGY

This is a narrative literature review on the mitigation of water stress in tropical forage plants by the action of microorganisms that promoter plant growth.

Narrative review consists of mapping, categorization and analysis of the literature, allowing the updating of knowledge on a theme and the discussion of a given subject from a theoretical or contextual point of view, as well as the identification of approaches, gaps and perspectives for future studies (Vosgerau & Romanowski, 2014).

The searches were based on the research question: Are microorganisms promoting plant growth capable of mitigating the negative effects of water stress on pastures? The bibliographic search was performed in the Databases Scielo, Google Scholar, Scopus, Science Direct and Web of Sciences and the following descriptors were used: abiotic stress, water stress in pastures, arbuscular mycorrhizal fungi, plant growth-promoting bacteria, mitigation of water stress by beneficial soil microorganisms, tolerance to water stress, as well as their respective descriptors in the English language: abiotic stress, water stress in pastures, arbuscular mycorrhizal fungi, plant growth-promoting bacteria, of water stress by beneficial soil microorganisms, tolerance to water stress.

The inclusion criteria defined for the selection of articles were: articles published in Portuguese, English and Spanish, which in full or partially portrayed the theme of the study and indexed in these databases from 1980 to 2022. Initially, 308 articles were found, and later, through the exclusion criteria (duplicity, non-fulfillment of the objective of this review and escape from the theme) selected 114 articles that met the eligibility criteria described and composed from the initial conceptualization of terms and concepts, to the analysis and discussion of the present review.

3 RESULTS AND DISCUSSION

3.1 ABIOTIC STRESSES IN FORAGE PLANTS

Brazilian livestock farming is characterized by animal feed to pasture, extensive areas with cultivated pastures and favorable climatic conditions, which provide competitive advantages of our farms compared to other producing countries. However, climatic variations, especially those related to water availability are common in several regions and seasons, imposing a seasonal character on forage and animal

production, because despite the rusticity and adaptation of tropical forage grasses, these critical conditions throughout the year affect their productivity.

According to estimates of the Intergovernmental Panel on Climate Change (Stocker, 2014) Brazil will have an increase of 2 to 7 °C in its temperature by the year 2100, showing greater variability in precipitation scenarios, with regionalized trends of increase in maximum and minimum temperature extremes.

This situation refers to the challenges that Brazilian livestock companies face and will continue to experience in the near future, demanding the use of production systems less sensitive to these conditions, aiming at the mitigation of greenhouse gases and the adoption of animal and forage components adapted to climate change.

In this context, ecophysiology as a tool for the study of forage responses to the abiotic and biotic conditions of the pasture ecosystem can generate information that contributes to a better balance between the productive demand of the system and biological sustainability of its main factors.

The dynamics of individual plants and the pasture edby community can be altered by the conditions of the environment in which they are subject, directly affecting the structure of the lawn, as well as the adaptive mechanisms of the plant to the stress imposed by the biotic and abiotic factors of the pasture ecosystem (Lemaire, 2001).

Thus, stress can be defined as any environmental condition that prevents the plant from reaching its full genetic potential (Taiz et al., 2017), and may come from the most diverse sources of environmental variations, such as those derived from temperature (high temperatures and cold), water availability (drought and flooding), radiation (Light and UV), chemical stress (mineral salts, pollutants and toxic gases) and mechanical stress (wind, soil movement and submersion), but especially those linked to the context of climate change, being able to highlight the effects caused by the increase in temperature and water deficit that generate physiological and biochemical disorders to plants (Table 1).

Table 1. Physiological and biochemical disorders in plants caused by abiotic stress.

Environmental factor	Primary effects	Side effects
Water deficit	Reduction of water potential (U) Cellular dehydration Hydraulic resistance	Reduction of cellular/leaf expansion Reduction of cellular and metabolic activities Stomatic closure Photosynthetic inhibition Leaf abscission Change in carbon partition Cytorrise Cavitation Destabilization of membranes and proteins Production of EROs Ionic cytotoxicity Cell death
High temperature	Destabilization of membranes and proteins	Photosynthetic and respiratory inhibition Production of EROs Cell death

Source: Adapted from Taiz et al. (2017).

Table 1 presents the various physiological effects presented by plants submitted to the two main factors of abiotic stress (water and high temperature) and which are related to climate changes caused by the effects of global warming.

The impact of these physiological disturbances on commercial crops or livestock activities compromises the supply of food and consequently food security, especially in regions of challenging agricultural and livestock activities, such as regions with arid and semi-arid climate. This concern is not an exclusivity of agricultural systems composed of annual crops, which are comparatively more affected by climatic extremes.

Tropical forage grasses, Plants of C4 metabolism, with high efficiency in the use of natural resources with light and temperature, also present physiological disturbances to this stress conditions, and have developed throughout their evolution some morphophysiological adaptations that help them to tolerate these conditions to some degree of disturbance.

In a review by Pimentel et al. (2016) the main factors of abiotic and biotic stress and their respective adaptive characteristics are listed (Chart 1).

Table 1. Stress caused by biotic and abiotic factors and adaptive response of tropical forage grasses.

Factors	Stress to the plant	Plant response
Compaction	Impairs root growth	Issue of lateral roots Thickening of roots
Flooding	Low ATP availability Reactive oxygen species Toxic levels of the elements Lower stomatic conductance	Formation of Adventitious roots Reduction of root biomass
Drought	Reactive oxygen species Lower carbon assimilation	Decreased stomatic opening Reduction of stomata diameter Osmotic cell adjustment Deepening of roots
Grazing	Decrease of photosynthetic appenditis	Source/Drain Ratio Changes Change in the population of childchildren
Shadow	Decrease in solar radiation	Increase in shoot/root ratio Decrease in peresoning Increase in leaf-specific surface Increase in chlorophyll content
Nutrient deficiency	Lack of essential elements	Accelerates root growth Increases surface and root length

Source: Pimentel et al. (2016).

Chart 1 gathered information on stress caused in forage plants by several factors in the pastoral environment and their respective adaptive response, which demonstrates that forage grasses evolved to adapt through physiological and morphological adjustments in an attempt to minimize the effect of these stress situations.

Water stress in forage plants

Water deficit is a situation common to the production of many crops worldwide, a phenomenon that is associated with factors such as occurrence and distribution of rainfall, evaporative demand and capacity

to store soil water (Pimentel et al., 2016).

According to Naing & Kim, (2021) drought stress is considered one of the most severe abiotic stresses to crop yield, impairing normal plant growth by disturbance of cell division, leaf surface and stem growth, and root cell proliferation negatively affecting plant growth.

Water stress can be described as all water content of a tissue or cell that is below the maximum water content displayed when the plant is in the state of highest hydration, and this water deficit (insufficient water availability) occurs in most natural or agricultural habitats and is mainly caused by intermittent periods until continuous without precipitation, drought is the meteorological term for a period of insufficient precipitation that results in water deficit for the plant (Taiz et al., 2017).

The main consequences of drought in cultivated plants are the reduced rate of cell division and expansion, leaf size, stem elongation, root proliferation, stomatic oscillations, change in water and nutrient relationships in plants, providing lower crop productivity, and inefficiency in water use (Li et al. 2009).

Severe water restrictions promote stoppage of the growth and death of the aerial part of the plant and limit animal production, both due to low quality and forage availability. On the other hand, mild water deficiencies reduce the growth speed of plants, slowing the formation of stems and result in plants with higher leaf proportions and potentially digestible nutrient content (Santos et al., 2011).

In addition to all the effects on crop productivity in general, drought decreases the access of plants to available and essential resources for their growth, such as nutrients present in the rhizosphere that need water to be absorbed. With limited water supply, the absorption of nutrients by the roots decreases because a decline in soil water potential slows the rate of nutrient diffusion between the soil matrix and the root surface (Farooq et al., 2009).

Another important aspect is the effects of lower water availability of plants on their CO₂ assimilation and, consequently, on their photosynthetic rate. Reductions in leaf area (size and number) and stomatic closure, impaired activities of carboxylation enzymes and ATP synthesis and destruction of photosynthetic devices are among the main factors that decrease carbon fixation in drought (Yamance et al., 2003).

Severe drought limits photosynthesis due to a decline in ribulose-1 activities, 5-bisphosphate carboxylase / oxygenase (Rubisco), Phosphoenolpyruvate carboxylase enzyme (PEPCase), NADP-Mallica enzyme (NADP-ME), fructose-1, 6-bisphosphatase (FBPase) and pyruvate phosphate dikinase (PPDK), and limits the photosynthetic area due to reduced leaf expansion and number of leaves. In addition, the transport of non-cyclic electrons corresponds to the reduced requirements of NADPH production and thus reduces the synthesis of ATP (Farroq et al., 2012).

The condition of low water availability for plants disturbs the balance between production of Reactive Oxygen Species (REOs) and antioxidant defense causing accumulation of OU, which induces oxidative stress. With stomatic closure, the CO₂ influx and its internal reduction in leaves not only directly reduces carboxylation, but also directs more electrons to form OU And promotes photorespiration.

In tropical forage plants there are several effects of water stress reported in the scientific literature of the area, which can mention the work carried out by Carrizo et al. (2021) evaluating the effects of water stress and recovery in two lines of buffel grass (*Cenchrus ciliaris*), which had the water supply interrupted until the soil reached 20% of the soil moisture content. The authors found a reversible decrease in leaf water ratio and damage to photosystem II, leading to an increase in the generation of reactive oxygen species, lipid peroxidation and the additional development of lignified tissues and bulliform cells and a greater thickness of the axial epidermis.

Maranhão et al. (2019) studying the effects of water restriction (30, 60, 90 and 120% of reference evapotranspiration – Eto) on buffel grass cv. Gayndah obtained a lower photosynthetic foliar rate, stomatic conductance, leaf transpiration rate and a higher leaf temperature in the water slide correcting at 30% of Eto, which demonstrates the impact of water stress even in a forage considered as drought tolerant.

Siddique et al. (2016) studying the physiological responses of water stress on two species (*Halopyrum mucronatum* and *Cenchrus ciliaris*), observed in conditions of low water availability, reductions in chlorophyll, carotenoid, stomatic conductance and increase in free proline content in the two studied species.

Tommasino et al. (2018) evaluating the simultaneous stress of low water availability (30% of soil water content) and high temperatures (45 °C) on two buffel grass genotypes, found oxidative damage caused by reduced activity of antioxidant enzymes, directly impacting the growth of the cultivars studied.

Therefore, there are several physiological disturbances caused by water stress in tropical forage grasses, including in a species considered tolerant to water stress, such as buffel grass.

Mechanisms for adapting plants to drought

Plants throughout their evolution have developed several morphological and biochemical adaptations to tolerate events of low water availability, as a way to mitigate or escape from disturbances caused by the lack of available water.

According to Farooq et al. (2009), plants throughout their evolution developed several mechanisms that allowed them to live with water stress, including morphological adaptations, osmotic adjustment, optimization of water resources, antioxidant systems that reduce the effects of reactive oxygen species (ROS) linked to drought, and the induction of a variety of genes and proteins that respond to stress.

Drought tolerance is a complex phenomenon associated with cuticle thickness, stomatic regulation, root system, hormonal balances, antioxidant defense system, osmotic adjustment and maintenance of water content in tissue, etc. (Farooq et al., 2012).

In general, the mechanisms of drought resistance by plants can be divided into physiological, morphological and aspects adaptations related to molecular mechanisms.

Among the adaptations to drought we can mention the ability of some species to "escape" from the period of lower water availability, either by the short life cycle (corresponding to the rainy season) or going

into dormancy before scarcity, as several species of plants of the Caatinga biome do to survive.

Another form of adaptation is the prevention of drought by phenotypic flexibility, when the plant has the ability to maintain high water status or cellular hydration under water deficit, performing this mechanism either by capturing more water from the soil or minimizing water loss through transpiration (Blum, 2005).

As for morphological aspects, Wang & Yamauchi (2006) describe root plasticity as an important aspect to avoid drought, through a greater depth of rooting, root proliferation and root length density.

Regarding physiological adaptations, several are the metabolic modifications made by plants to face the low level of water in their tissues, being able to mention the most important, with osmotic adjustment, synthesis of plant growth substances (plant hormones) and antioxidant defenses.

Accumulation of organic and inorganic solutes under drought and/or salinity, which help decrease water potential without decreasing water content, is referred to as osotic adjustment or osmoregulation (Serraj & Sinclair, 2002). However, these solutes do not cause harmful effects on membranes, enzymes and other macromolecules, even at higher concentrations, so they are also called compatible solutes (Kiani et al., 2007).

Biosynthesis and accumulation of compatible solutes, such as the amino acid proline, occur in the vacuola or cytosol, with osmoprotective function, which maintains the water balance and preserves the cellular integrity of proteins, enzymes and membranes, for the continuity of vital activities, and is one of the adaptive strategies of vegetables to the multiple effects caused by stresses (Abdul Jaleel et al., 2007).

These compatible solutes include soluble sugars, sugar alcohols, proline, glycinebetaine (GB), organic acids, trehalose, etc. These compatible solutes not only help maintain turgor pressure, but also to protect enzymes and cell macromolecules from the harmful effects of OS (Farooq et al. 2009).

Osmotic adjustment is the main defense mechanism of the plant capable of reducing cellular water potential (Guirra et al. (2022). It is performed by accumulating biomolecules (sugars, amino acids, proline, etc.) in the cytosol without poisoning the cell (Butt et al., 2017).

Another physiological aspect in the adaptation of plants to drought is the action of plant hormones, which in low concentrations can inhibit, modify or promote howtoand qualitatively plant growth.

Auxins, Gibberellins, Cytokinins, Ethylene, and Abscissic Acid (ABA) are the most studied phytohormones in higher plants. Of these, Gibberellins and Cytokinins promote plant growth (growth promoters), while Ethylene and ABA have inhibitory effects (growth retarders) (Taiz et al., 2017). Water stress alters the endogenous synthesis of these growth substances. Generally, under stress conditions, the concentration of growth retarders increases at the expense of growth promoters to regulate the water budget of plants (Farooq et al., 2009).

According to Taiz et al. (2017), under water deficit conditions there is the accumulation of abscissic acid (ABA) in the roots, inducing the leaves to decrease the opening of the stomata, reducing gas exchange, inhibiting photosynthesis, and thus producing reactive oxygen species (ROS) that can promote damage to

DNA and cell membranes.

Limited water supply promotes oxidative stress with overproduction of REOs. Influx of CO₂ declined with stomatic closure or impaired activities of enzymes and photosynthetic devices damaged under water stress deregulate photosynthesis, leading to the generation of a variety of OU, such as O₂⁻, ¹O₂, H₂O₂, HO₂⁻ and HO⁻ (Lawlor & Cornic, 2002).

According to Naing & Kim (2021), these EROS are highly reactive and toxic, and their overproduction causes oxidative damage to membranes (e.g., lipid peroxidation), proteins, RNA, and DNA and other macromolecules in the absence of any protective mechanism, which ultimately lead to plant death.

The plants developed enzymatic and non-enzymatic defense mechanisms capable of neutralizing the cytotoxicity of THE (Barbosa et al., 2014). The antioxidant defense cell system begins with an enzymatic cascade, but also involves non-enzymatic components, among which are ascorbate (AsA), glutathione (GSH), β-carotene and α-tocopherol. Such antioxidants can prevent the formation of free radicals, kidnap them or promote their degradation, preventing the occurrence of damage to plant cells (Serkedjieva, 2011).

Of the enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione reductase (GR) and ascorbate peroxidase (APX), non-enzymatic antioxidants, ascorbic acid, α-tocopherol, reduced glutathione, β-carotene salicylates, solutes compatible as proline accumulate in higher plants under water stress to avoid oxidative damage (Ozkur et al., 2009).

3.2 BENEFICIAL SOIL MICROORGANISMS AS MODULATORS OF TOLERANCE TO WATER STRESS IN PLANTS

One of the alternatives to help plants cope with low water availability is the use of microbial communities associated with plants, such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (BPCP), which optimize plant growth and development under different types of abiotic and biotic stresses.

The application of microorganisms such as BPCP and FMA are useful for and improving the sustainability of agriculture and environmental stability (Kumar & Verma, 2018).

Plant Growth Promoting Bacteria (BPCP)

The definition of plant growth-promoting bacteria (BPCV), plant growth-promoting bacteria (BPCP) or even plant growth-promoting rhizobacteria (PCR) encompasses groups of free-living or epiphytic microorganisms with the ability to establish symbiotic, associative or non-associative relationships with plants (Glick, 2012).

The study of the interactions between plant and microorganisms has intensified in recent years in order to understand the various factors involved for the selection of strains of bacteria efficient in promoting growth of large crops (Ferreira et al., 2014).

PCBPs have been found in association with a large number of forage cereal and grass species, with a wide range of genera described, including *Pseudomonas*, *Burkholderia*, *Bacillus*, *Bradyrhizobium*, *Rhizobium*, *Gluconacetobacter*, *Herbaspirillum* and *Azospirillum* (Hungary et al., 2010; Vine et al., 2012).

The mechanisms by which bacteria can influence plant growth differ between species and strains, so there is no single mechanism to promote plant growth. Studies have been conducted in relation to the abilities of various bacteria to promote plant growth, including endophytic bacteria (Souza et al., 2015).

Endophytes are conventionally defined as bacteria or fungi that colonize the internal tissues of plants, can be isolated from the plant after surface disinfection and do not cause negative effects on plant growth (Gaiero et al., 2013).

According to Souza et al. (2015), BPCP has been reported for their improvement in plant development because they provide a number of benefits through several mechanisms such as: biological nitrogen fixation, production of indole compounds, production of iron-chelant siderophores that at high concentrations can inhibit the growth of phytopathogens, ACC deaminase activity and solubilization of organic and inorganic phosphates.

Many beneficial microorganisms, including bacteria and fungi that live in the soil and those associated with plant roots, are able to solubilize insoluble phosphorus from the soil (Bechtaoui et al., 2020).

Bacteria often solubilize mineral phosphorus (P) (inorganic) by synthesizing low molecular weight organic acids, such as gluconic acid and citric acid (Rodriguez et al., 2004). Phosphate solubilizing bacteria solubilize inorganic forms of phosphates, such as $\text{Ca}_3(\text{PO}_4)_2$, FePO_4 and AlPO_4 , through the production of organic acids, siderophores and hydroxyls. Phosphate binds with hydroxyl groups and chelating carboxyls and thereby releases cations that induce soil acidification, resulting in the release of soluble phosphate (Kpombrekou & Tabatabai, 1994).

Iron, despite being one of the most abundant elements in the earth's crust, in the soil, is often in a form not available for direct assimilation by plants and microorganisms, due to the low solubility of the iron ion (Fe^{3+}) (predominant form in nature). To improve the absorption of this nutrient, plants and some bacteria produce low molecular weight compounds that bind and sequester to available forms of soil iron. Bacteria synthesize low molecular weight siderophores, molecules with a very high affinity for Fe^{3+} and membrane receptors that can bind to the Fe-siderophore complex, thus allowing the absorption of iron by bacteria (Neilsen, 1981).

The siderophores produced by plants bind with a lower affinity to iron when compared to the siderophores produced by bacteria, and bacterial ferro-siderophore complexes can be absorbed and used by plants (Wang et al., 1993).

In addition to these aspects of promoting growth in plants under adequate conditions of cultivation, these microorganisms present several mechanisms that can help plants in their tolerance to the impact caused by water deficit.

According to Kaushal & Wani (2016), the application of growth-promoting bacteria (BPC)

mitigates the negative effects of drought on plants through various physiological and biochemical processes in plants, including changes in phytohormone levels, activation of the antioxidant defense system and the accumulation of various compatible organic solutes such as sugars, amino acids and polyamines. Potential mechanisms include (1) production of phytohormones such as Indoleacetic Acid (IAA), Cytokinins and Abscissic Acid (ABA), (2) exopolysaccharides, (3) ACC deaminase and (4) induction of systemic tolerance (Kumar & Verma, 2018).

Goswami et al. (2015) report the importance of IAA, an auxin that regulates the differentiation of vascular tissue, adventitious and lateral root, cell division and bud growth during water stress. ABA is an important growth regulator during water stress. When the seed or plant is inoculated with BPCP, ABA concentration increases and regulates plant physiology to tolerate water stress. This phytohormone mitigates water stress by regulating the transcription of the gene related to drought and hydraulic conductivity of the root (Jiang et al., 2013).

Hormones such as auxins, gibberelins, kinetin and ethylene are produced specifically by these microorganisms and play an important role in root strengthening (Ahmad & Kibret, 2014).

According to Bal et al. (2013), the organisms that produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, responsible for the degradation of ACC (ethylene precursor) in ammonia and α -ketobutyrate, are able to promote plant growth by reducing ethylene levels in the plant.

By facilitating the formation of long roots and the protection of plants under stress, due to the deleterious effects of the presence of ethylene, these bacteria can increase survival and protect the yield of a plant variety, especially during the first days after sowing, where plants are generally more vulnerable to damage by various environmental stresses (Glick, 2004).

The presence of bacteria can also lead to the production of osmolytic substances by the plant and thus act synergistically, mediating changes in the elasticity of root cell walls, contributing to drought tolerance (Dimkpa et al., 2009).

And among the wide range of species and strains of plant growth-promoting bacteria, those of the genus *Azospirillum* have been widely tested in forage grasses, with satisfactory results in the nutrition of host plants, such as those observed by Hungary et al. (2021) and abiotic stress mitigators, as contacted by Bulegon et al. (2017).

Azospirillum are the most studied BPCP, mainly because of their ability to produce growth regulatory compounds, such as indole-3-acetic acid (IAA) and gibberelins, and to exert growth-promoting effects on a diverse set of hosts (Cortés-Patiño et al., 2021).

Bulegon et al. (2016) studying the effects of water stress on *Brachiaria ruziziensis* associated with *A. brasilense*, noted that the bacterium favors the maintenance of chlorophyll content in leaves, helping plants to tolerate this abiotic stress.

The bacterium *A. brasilense* can minimize oxidative damage caused by water stress by increasing the contents of chlorophylls and carotenoids in inoculated plants, under water deficit (Bulegon et al., 2016)

and by synthesizing 1-carboxylic amino cyclopropane acid (ACC) deaminase that degrades ACC, precursor substrate of ethylene synthesis (Glick, 2014).

Leite et al. (2019) evaluating the performance of Capim Marandu under different doses of N and inoculations with *Azospirillum brasilense* in different seasons, observed a 20% reduction in the need for nitrogen addition and a higher performance in various productive parameters at dry season of the year with microbiological inoculation.

Bulegon et al. (2019) evaluating the effect of inoculation of *the bacterium Azospirillum brasilense* in *urochoa ruziziensis* grass under water stress, observed the increase in the relative water content, under water deficit conditions, with less reduction in the rate of liquid assimilation of CO₂ and reduction of damage to cell membranes. This process is mediated by stimulating the production and release of compounds such as plant hormones, mainly auxin and abscisic acid, but also compounds such as betaine osolites, proline and amino acids.

And also Fukami et al. (2018) evaluating mechanisms of stress resistance, concluded that *the bacterium Azospirillum* provides protection to corn plants by increasing their antioxidant defense, through the activities of catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) in leaves, and ascorbate peroxidase (APX) in the roots.

Mamédio et al. (2020) in a literature review on the influence of BPCP on the persistence of tropical pastures in water deficit concluded the use of these biological inputs in tropical grasses is an alternative for the maintenance of pasture growth and development, even when the soil nutritional profile does not meet the needs of grasses and environmental conditions are adverse. However, there are not many studies testing inoculants in tropical grasses under water deficit conditions and reaffirmed the need for studies with the most exploited grass species in animal production, as well as more detailed analyses of the efficiency of this technology, in order to better employ these products in grasses in a context of water deficit.

Arbuscular mycorrhais fungi (AMFs)

Arbuscular mycorrhizal fungi (AMF) form one of the most common associations in nature, arbuscular mycorrhiza (MA), a symbiotic relationship formed between these fungi and the roots of 82% of plant species, including crops of economic importance exerting great influence on various agroecological and ecosystem processes (Silvana et al., 2020).

This interaction is mainly based on the carbon exchange of the host plant and nutrients from fungi to the host plant in a symbiotic system (Atul-Nayyar et al., 2009).

Mycorrhizas can be ectomycorrhizas, in which the fungus covers the root, but does not penetrate it or arbuscular mycorrhizas, in which the hyphae of the fungus enter the root and penetrate the cell wall of the root cells forming arbuscular structures, inside the cell wall, but outside the plasma membrane (Sadava et al., 2009).

Recently, it was observed that the symbiotic interaction of plants with AMF, besides being important

from the agricultural and ecological point of view (Yang et al., 2008), could be a sustainable mitigation practice for water deficit (Aroca, 2012).

According to Gamalero et al. (2009), the main mechanisms used by arbuscular mycorrhizas to help plants overcome the effects of drought include: (A) Better water absorption; Arbuscular mycorrhiza effectively extend the roots of plants providing a more efficient water catchment. (B) Better mineral nutrition, especially phosphorus, as a consequence of the effective extension of plant roots. In fact, arbuscular mycorrhizae can provide the main mechanism for phosphorus absorption in many plants, with CVBP possibly acting as a secondary or adjuvant mechanism of mycorrhizas in terms of absorption of P. (C) Changes in root architecture. (D) Modification of some physiological and enzymatic activities, especially those involved in antioxidant responses of the plant. (E) induction of the plant hormone Abscissic Acid (ABA), which may play an important role in mediating some plant responses to different stresses including drought (Danneberg et al., 1992).

It has been reported that the effect of AMF increases with the intensity of water deficit (Miransari et al., 2007); however, the effect is not predictable and the magnitude and type of response depend on AMF and plant species and the degree of stress prevalent in water deficit (Zhongqun et al., 2007).

One of the possible mechanisms for improving the tolerance of mycorrhizal plants to water deficit may be related to increased hydraulic conductivity of the roots (Augé et al., 2008).

Live hyphae that are involved in the transport of water (Allen, 2009) have a diameter between 2 μm and 5 μm and can penetrate the smaller pores of the soil that are inaccessible to the lateral root (10 μm to 20 μm in diameter) and thus absorb water that is not available for non-mycorrhizal plants (Marulanda et al., 2003). In addition, AMF demonstrated beneficial effect on soil structures, specifically generating aggregates due to the production of a glycoprotein known as glomalin (Wu et al., 2008).

Glomalin is a glycoprotein that is released by the arbuscular mycorrhizal fungus after its death, containing 2-5% Fe, 4-6% O, 0.03–0.1% P, 36–59% C, 33–49% H and 3–5% N (Wright et al., 1999). Due to its high adhesivity and hydrophobicity, it plays an important role as a cementing material with soil particles and acts as a highly stable form of organic carbon storage, which represents an important fraction of the total organic matter present in the soil (Bhale et al., 2018).

According to Fernández-Lizarazo & Moreno-Fonseca (2016) regarding aid in antioxidant defense, two mechanisms were proposed to explain the low oxidative damage in plants submitted to water deficits and inoculated with AMF. The first consists of the direct absorption of water by the hyphae and their transfer to the host plant, increasing the water content and decreasing the generation of reactive oxygen species (REOs). The second mechanism implies an increase in the production of enzymatic and non-enzymatic antioxidants induced by symbiosis with AMF (Abbaspour et al., 2012), especially in water deficit conditions (Amiri et al., 2015).

Under conditions of water and saline stresses, plants accumulate some organic solutes (proline, soluble sugars, betaine glycine, among others) and inorganic ions to maintain greater osmotic adjustment

(Yang et al., 2009).

As for the improvement of nutrient absorption by the symbiotic relationship between mycorrhizal fungi and plants, much is due to the expansion of the root contact area, due to the greater coverage provided by fungal hyphae. As colonization develops within the roots, fungal hyphae grow abroad at distances that can extend up to about 25 cm, depending on the genotype of fungi and soil conditions (Jakobsen et al., 1992).

As phosphorus is less mobile in soils with little water, an increase in its acquisition as a consequence of association with AMF is important for improving the nutrition of host plants (Augé, 2004).

The main function of AMF is to provide phosphorus to plant roots through phosphate conveyors present in the hyfal membrane. Extra-radical and filamentous hyphae networks of AMF assist in the absorption of freely available phosphates. This symbiotic interaction results in the hydrolysis of organic phosphates that are present in the soil and provide soluble phosphates to the host plant through hyphae (Bagyaraj et al., 2015).

Ruiz-Lozano et al. (2012) emphasize the existence of clear evidence that the symbiosis of the plants to water stress, by altering various physiological effects or ecological processes, which are summarized in Chart 2, including the improvement of soil water retention properties and the ability of hyphae to absorb water from inaccessible sources to roots and the transfer of this water to the host plant, with contributions of up to 20% more in the total absorption of water by plants.

There are several studies that report the effects of the use of mycorrhizas as mitigating water stress in plants, presenting effects of decreased oxidative stress (Chen et al., 2020), increase in proline levels and chlorophyll content of leaves (Tyagi et al., 2017), water potential and steamatic conduction of leaves (Li et al., 2014) and rate of liquid photosynthesis (Fracasso et al., 2020).

Table 2. Representation of the integral physiological processes by which FM A can improve the tolerance of the host plant to drought.

Physiological effects	Processes involved
Protection against oxidative damage	Antioxidant production
Maintains gas exchange and plant growth	Stomatic conductance and hormonal ballast
Reduction of osmotic potential in tissues. Avoiding water loss	Osmotic adjustment
Search for water in sources inaccessible to the roots	Water retention capacity and water absorption by hyphae
Water absorption and maintenance of the hydraulic properties of the roots	Regulation of aquaporin

Source: Adapted from Cheng et al. (2021).

Odokonyero et al., (2017), for example, evaluating the effects of endophytic fungus, *Acremonium implicatum*, in the growth and physiological responses of five brachiaria cultivars (Basilisk, Tully, Marandu, Cayman and Mulato II) in well-irrigated and drought-stressed conditions (irrigation suspended for 21 days), verified that plants in symbiosis with endophyte fungus significantly increased the stomatic conductance of the leaf and reduced the xylem diameter. However, the smallest leaf area was found in plants inoculated in three cultivars, compared to the control and both in well-irrigated conditions and in

water stress, which indicates a cost of infection of endophytes for host cultivars.

Grasses, in general, have a high level of association with AMF, which is clear when the rate of mycorrhizal colonization in their fine roots is quantified (Guimarães et al., 2022). Table 3 shows significant rates of mycorrhizal colonization in roots of different tropical forage grasses, which demonstrates their high symbiotic affinity with these species, and the need for further research that explores all this potential with the selection of species/strains to make up biological inoculants.

Table 3. Mycorrhizal colonization rate identified in different tropical forage grasses and in different localities in Brazil.

Tropical forage grass	Mycorrhizal colonization rate	Locality	Reference
<i>Megathyrsus maximus</i> , <i>Urochloa brizantha</i> and <i>Paspalum notatum</i>	90%	Greenhouse	Zangaro et al., (2018)
<i>Urochloa Decumbens</i>	44%	Cerrado biome	Kanno et al., (2006)
<i>Humidicola Urochloa</i>	60%	Cerrado biome	Ramos et al., (2012)
<i>Sorghum sp. and Pennisetum purpureum</i>	80%	Atlantic forest	Rondina et al., (2014)
<i>Ruziziensis</i>	60%	Cerrado biome	Morais et al., (2019)
<i>Cynodon sp.</i>	75%	Pantanal Biome	Zangaro et al., (2012)

Adapted from Guimarães et al., (2022).

Although the potential of arbuscular mycorrhazic fungi naturally colonize forage plants, as presented in Chart 3, and increase their productivity, decreasing the degree of soil degradation and improving the resilience of forage plants to water stress, no study on the application of commercial inoculants containing AMF in tropical forage grasses has been developed in Brazil to date (Guimarães et al., 2022).

Co-inoculation of BPCP and FMA

The rhizosphere is a highly dynamic ecosystem that differs in biological composition depending on the physical and chemical conditions of the soil, native microorganisms and plants that are inserted in different biomes. Some of the important interactions include plant-plant interactions, root-microorganism interactions, and microbe-microbe interactions (Lau & Lennon, 2011). Synergism and antagonism in response to these interactions depend on the nature of the microbial strains involved in these interactions, as well as on plant species.

The growth promotion mechanisms provided by BPCP and mycorrhazas can be very useful for improving plant growth using these microbial populations together, particularly under stressful environments (Nadeem et al., 2014).

Figure 1 describes several plant growth promotion mechanisms used by arbuscular mycorrhizal fungi (AMF), plant growth promoting bacteria (BPCP) and example of their synergism. It is important to highlight that plant growth-promoting microorganisms act in different ways and their combinations and their results will depend on the host plant, compatibility between microorganisms and abiotic conditions.

Bacteria can produce compounds to increase cell permeability so as to increase the rate of root

exudation, which stimulates hifal growth and facilitates the penetration of roots by the fungus (Jeffries et al., 2003). On the other hand, mycorrhazas help the plant to resist against abiotic stress, increasing the surface area of the roots for nutrient acquisition or through more specific mechanisms (Sikes, 2010). In addition, BPCP improves the development of microsymbionts and facilitates the colonization of plant roots by AMF (Jaderlund et al., 2008). The presence of BPCP supports the establishment of mycorrhazas and improves the ability to perform various functions properly.

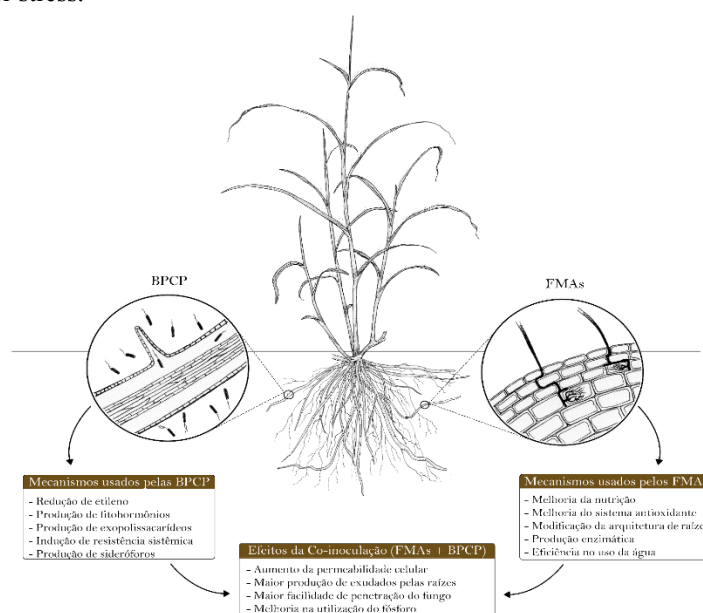
In the case of AMF the interaction with bacteria of the *genus Azospirillum* occurs directly, providing a niche and / or habitat or indirectly modifying the morphophysiology of the host plant. This communication in the soil can be beneficial for both microorganisms and the host plant (Domínguez-Núñez et al., 2015).

Colonization generated by both microbial groups stimulates the hydrolysis of cell wall hemicellulose and generates oligosaccharides that will be used in the nutrition process. The enzymes generated during this process are responsible for morphological changes that begin on the surface and end in the inner regions of the cortex. These results suggest that bacteria associated with fungi use metabolites produced by plant cells and microsymbionts that interact with them (Domínguez-Núñez et al., 2015).

Miyauchi et al. (2008) showed that mycorrhizal colonization increased colonization of diazotrophic bacteria in corn roots. The bacterium did not affect mycorrhizal morphology and colonization. This result suggests a beneficial effect of the action of AMF in helping diazotrophic bacteria penetrate and colonize plant roots (Raimam et al., 2007).

Fungal structures have been shown to be used by bacteria as intermediates to reach the root tissue epidermis, and that the production of phytomoniums by them stimulates root growth and, in fact, mycorrhizal colonization (Villarreal et al., 2016).

Figure 1. Mechanisms used to promote plant growth, provided by the association between rhizobacteria and arbuscular mycorrhizal fungi under water stress.



Source: Adapted from Nadeem et al. (2014); Revillini et al. (2016).

The entry of diazotrophic bacteria into plants can occur through AMF spores (Bhowmik & Singh, 2004) or bacterial colonization in extra-root hyphae of AMF (Toljander et al., 2006). THE AMF, in addition to providing nutrients for the bacteria that colonize the surfaces or inside the spores, provide protection against desiccation, radiation, predation and salinity (Levy et al., 2009). During the process of penetration of infective hyphae, there is greater exudation of nutrients by the plant, accelerating the growth of bacteria (Paula et al., 1991).

However, antagonistic interactions may also occur due to competition for nutrients and other essential resources for the life of these microorganisms.

Bauer et al. (2012) found no interactions between FMAs and N₂ fixers in *Panicum* communities or monocultures, indicating that in the short term the effects of these microbial functional groups would not be additive.

The interactions of microorganisms can be more than just competitive. Bellone & Carrizo de Bellone (2012) described the process of *colonization by Azospirillum brasilense* and *Glomus intraradices* in sugarcane roots, mainly in young roots and its subsequent introduction into xylem and phloem, favored by the endodermal disorganization of the cell that occurs during the growth of future lateral roots.

Also according to the authors, in the root sites with maximum colonization, fungi strongly decrease the cell wall, which allows better exchanges of metabolites between bacteria and fungi. In the lumen of xylem vessels, bacteria randomly colonize and the presence of intracellular hyphae increases the colonization of bacteria, which occupy the intercellular spaces generated by fungi.

Associative diazotrophs, on the other hand, are not separated from mycelium from endomycorrhizal fungi. These nitrogen fasteners can function both inside and outside the lateral structures of AMFs. This can result in a competition for nutrients between the two microsymbionts. The success and failure of co-inoculation of *Azospirillum* and AMF may therefore depend on the physiological stage of the host, the time of infections or the nutrient demands of the microsymbiont partners (Biró et al., 2000).

According to Ferrera-Cerrato & Alarcón (2004), there are numerous factors that can influence the symbiotic efficacy of microorganisms in plants, such as the strain used, the host plant and the edaphical conditions. It is important to understand this when dealing with such factors in order to optimize plant growth.

Behrooz et al. (2019), evaluating the effect of co-inoculation of arbuscular mycorrhizal fungi (*Glomus mosseae*, *G. etunicatum* and a mixture of these) and plant growth-promoting bacteria (*Azotobacter chroococcum* + *Azospirillum lipoferum*) as relievers of the effect of water stress on Walnut seedlings, observed the reduction of growth (plant height, root length, number of leaves and fresh weight), leaf nutrient content (N, P and Zn) and in contrast, increase in proline values, total soluble sugar, activity of the starch peroxidase enzyme and total phenolic content of the leaves under this stress.

In forage plant, Ronseaux et al. (2020) evaluating the co-inoculation of *Azospirillum brasilenses* and *R. intraradices* and fertilization with N in Mulatto II grass, observed with biofertilization (without N) values

similar to the dose of 100 kg/ha.

And Ruiz-Sanches et al. (2011) evaluating microbiological inoculations (*Azospirillum brasilenses*, *Glomus intraradices*, *A. brasilenses* + *G. intraradices* and control without inoculation) in rice plants under water stress, observed positive effect of co-inoculation of microorganisms on the colonization of plant roots by the fungus mycorrhizal, indicating beneficial effect of the bacterium on propagule germination and the micellar growth of mycorrhizal. Also on these results, the authors observed that plants under water stress (two weeks with 50% wc and two weeks with 25%) and inoculated with mycorrhizal and co-inoculated had higher photosynthetic efficiency and stomatic conductance compared to the other treatments, as well as effects on higher shoot and root production.

In general, there are few studies evaluating the effectiveness of the joint use of microorganisms and forage grasses, thus requiring trials that generate information to understand this tripartite relationship between fungi, bacteria and host plants and the use of their benefits in pastoral environments, especially in conditions of water deficiency.

3.4 EXAMPLES OF PASTURE USE SITUATIONS OR STEPS THAT CAN BENEFIT FROM INOCULATIONS OF MICROBIOLOGICAL INPUTS TO ATTENUATE WATER STRESS

Despite little information on the possibilities of using these biological inputs pastoral environments, in addition to those already conceptualized, as growth promoters and in place of chemical fertilization, it is possible to infer about the potentialities of actions and modulation effect of microbiological inoculations on forage plants in various stages of pasture use, and can mention:

Phase of implantation or formation of pasture

The planting window in dryland crops is concentrated in a short period of time depending on the operationalization and planning of the activity. In this time window, the seed comes into contact with soil moisture and the process of imbibition and subsequent germination occurs, which depend on the relationship between the water potential of the seed and the soil so that there is adequate transfer of water from soil to seed.

Particularly in tropical forage grasses, which most plantations are carried out to toss, the little contact of the soil with the seed can delay the imbibition, tinting and emergence of seedlings, and added to this, this phase can be preceded by water scarcity, which can impair the formation of an initial plant stand per square meter, compromising the initial use of pasture;

Water stress soon after sowing is one of the abiotic factors that most influence the germination process, directly interfering in the enzymatic activities of the plant minimizing productive yield, mainly due to the low recovery capacity after abiotic stress (Francisco et al., 2016). This low recovery capacity can be mitigated by inoculating with plant growth-promoting microorganisms, which can aid the mechanisms of tolerance to water stress imposed on seedlings.

Use of pasture in semiarid regions

Extreme weather events due to climate change are already causing disturbances in animal production systems and the projections for the near future are of aggravation of these disturbances, especially those associated with increased air temperature, concentration of rains, longer dry season and higher frequency of summers (Ambrosio et al., 2018).

The semiarid and arid regions are the most vulnerable to suffer from the impacts of global and regional climate change (Marengo et al., 2016), mainly because they are characterized by rainfall irregularities and low rainfall rates, compromising animal feed and persistence of forage species in the long term.

Livestock, developed predominantly in pastures in these regions, faces marked seasonality of pasture production, concentrating greater availability of forage in the rainy season and scarcity in the dry season, which compromises the support capacity throughout the year and hinders the stable annual dimensioning of the herd, which can lead to supergrazing at the time of lower forage supply and, therefore, compromising the perenity of pastures (Porto et al., 2022).

Water stress mitigation technologies are very important for semiarid regions, which naturally present a water balance between evapotranspiration and annual precipitation, which limit the persistence and plant sustainability of many species in these regions. Thus, these interventions provided that they are proven effective have a potential for resilience of agricultural activities in these challenging areas for the durability of many forage species.

Occurrence of "summer" periods

Rainfall irregularity in pasture areas in Brazil is a common situation faced every year by grazing livestock activity, imposing a seasonal character in the food supply of animals, generating challenges in planning throughout the year and added to this, the phenomena of summer (Dry Spells) often occur, which are defined as a period of five or more consecutive days without rain within the rainy season of a given region (Magalhães et al., 2020). Veranicos can compromise the stocking rates of these areas at a time, theoretically of greater water security.

The water stress mitigation characteristics discussed in the present review can improve the tolerance system of forage plants at this time (unpredictable) of low water supply in the soil, through improvement of morphophysiological adaptations of plants at a time that usually presents adequate temperature and luminosity for pasture growth.

Use of pasture in periods of dry water transition:

Forage production concentrates its growth between 70 and 80% in the water period and 20 to 30% in the dry season (Reis et al., 2011), for this reason throughout the year, in addition to the water and drought periods, it can also be subdivided into two transition periods: dry water transition and dry-water transition

(Roth et al., 2017). Such transitions allow better to understand the changes that the pasture undergoes and consequently the supply of fodder to the animals, facilitating the management of grazing and correction of its deficiencies via nutritional supplementation.

Specifically, the transition waters - drought is the period of the year situated between the rainy and dry season, is an intermediate period of growth and nutritional value of plants, which presents progressive decrease in rainfall, temperatures and photoperiod, which alters the pattern of pasture growth and compromises forage production and progressively decreases its nutritional value and digestibility for animals.

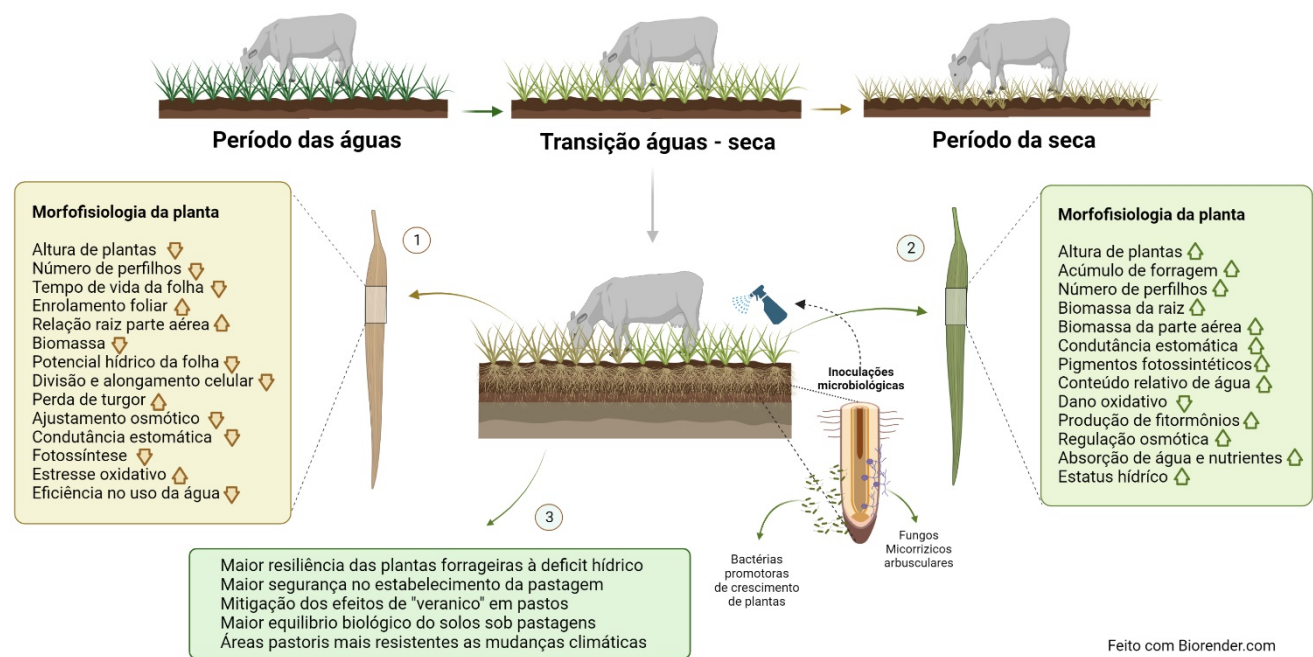
Pasture in early autumn presents good quality and a lot of mass and as the season progresses, due to the reduction of rains and temperatures, the fodder begins to senescence and present low nutritional quality, especially due to the aging of plant tissues. Another marked change in canopy structure is the accumulation of stem and senescent material, causing a decrease in the quality of forage and, consequently, a reduction in the consumption and performance of animals (Roth et al., 2017).

Lima et al. (2012) describe that, with the advance in the transition period and, consequently, advance in the physiological age of the plants, there is a decrease in cp content and dry matter digestibility (DM), an increase in neutral detergent fiber (NDF) and acid detergent fiber (ADF).

Bioinput technology could help grazing management in the transition period, in theory by slowing the morphophysiological seasonality of the species with the decrease in water availability and would add to other grazing management strategies in this period, such as the deferred use of pasture. The deferred use of pasture consists of sealing an aisle of the pasture in the final third of the water period, for use in the dry period of the year, providing greater accumulation of fodder during the autumn period (in central Brazil) and would maintain the physiological activities of the plants, such as the content of photosynthetically active pigments for example, which would help a characteristic "stay green", where the plant would remain longer green and maintaining its photosynthetic metabolism for longer as the dry season progresses.

This possibility could decrease the production and quality seasonality that occurs in most tropical forage grass species. Figure 2 describes the effects of water stress on tropical forage grasses and the improvement of the tolerance of these species with inoculation with plant growth-promoting microorganisms that may contribute to greater resilience of pastures in conditions of decreased rainfall in the period known as the water-dry transition.

Figure 2. Main morphophysiological responses of forage plants in water stress condition (1), effects of mitigation of this stress modulated by plant growth-promoting microorganisms (2) and potential benefits of microbiological inoculation of pastures under water deficit conditions (3).



Adapted from Mamédio et al. (2020); Bulegon et al. (2017 and 2019) and Odokonyero et al. (2017).

All these situations could benefit from the relationships between microorganisms and forage plants, enhancing the characteristics of tolerance to water stress of these species and providing greater resilience of this productive system with low environmental impact. According to Walker et al. (2004), resilient pastures are those that incur a smaller reduction in production and recover more rapidly after a stress event such as drought/flood, pest pressure/disease.

Despite the several microbiological mechanisms described in the present review, which may in theory improve the tolerance of tropical forage species to water deficit events, scientific studies in the literature that address the behavior of these inoculated plants under water stress conditions or throughout the seasons are incipient. Chart 4 describes the scientific articles selected to make up the corpus of the review and that specifically address forage species inoculated in conditions of water stress and the characteristics that helped mitigate water stress in these pastures.

Table 4. Articles selected to make up the Research Corpus of this review that specifically address the mitigation of the effects of water stress by the use of microbiological inoculants in tropical forage grass species.

Tropical Forage Grass	Microorganism (Species)	Water stress mitigation characteristic	Reference
<i>Ruziziensis</i>	<i>Azospirillum</i>	Increased activity of antioxidant enzymes and protection of photosynthesizing pigments	Bulegon et al. (2016)
<i>Cultivars of Urochloa</i> (Basilisk, Tully, Marandu, Cayman and Mulato II)	<i>Acremonium implicatum</i> (FMA)	Maintenance of stomatic conductance of the sheet and reduced xylem diameter	Odokoniero et al., (2017)

<i>Urochloa brizantha</i> cv. Marandu	<i>Azospirillum</i>	Greater tillering and root biomass	Leite et al., (2019)
<i>Ruziziensis</i>	<i>Azospirillum</i>	Maintenance of the relative content of water, net assimilation rate of CO ₂ , and absolute integrity of the cell membrane membranes;	Bulegon et al., (2019)

It is necessary to advance research that addresses forage plants in field conditions under situations of multiple stress and with a greater competition between microorganisms inoculated with the native microbiota of these areas, to improve the effectiveness of these biological inputs in pastoral environments.

According to Guimarães et al., (2022) despite the increased commercialization of microbial inoculants in Brazil, their use in pastures is still modest, representing less than 0.1% of the doses sold annually, this demonstrates the long way to go to realize the adoption of this technology in areas that are traditionally more lacking in the use of agricultural inputs.

4 FINAL CONSIDERATIONS

In view of the research conducted in this study, it is observed that there is great potential to use the benefits of symbiotic relationships between forage plants and beneficial soil microorganisms to improve the tolerance to water stress of tropical forage grasses.

However, there are few studies with the use of microorganisms in forage plants mainly under field conditions (under different conditions of abiotic stress), to advance the knowledge of the interrelationships between inoculations (isolated or with a polymicrobial character), forage plants and grazing animals.

Although known microbial interventions are promising, their dose, frequency and time still require practical validation.

Based on this information, new studies with selection of species/strains of microorganisms and their combinations, which have the potential to modulate tolerances of tropical forage grass species should be conducted, in addition to the need for studies addressing the effectiveness of biological inputs in different forage species and in practical situations of pasture use cited in this review, such as the implantation phase, management in semiarid regions with incidence of dry spells and strategic use of pasture in times of dry water transition.

It is also necessary to understand the characteristics of competition or mutual assistance among microorganisms that can potentially be used together, since each of them has different characteristics of growth promotion and improvement of tolerance to water stress in forage plants.

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