

**UNIVERSIDADE FEDERAL DE MINAS GERAIS**

**Escola de Veterinária**

**Programa de Pós-Graduação em Zootecnia**

Eduardo Moreira Barradas de Souza

**Avaliação e seleção de híbridos de *Megathyrus maximus* em relação a produtividade de forragem, valor nutritivo e tolerância ao estresse hídrico**

Belo Horizonte

2024

Eduardo Moreira Barradas de Souza

**Avaliação e seleção de híbridos de *Megathyrus maximus* em relação a produtividade de forragem, valor nutritivo e tolerância ao estresse hídrico**

Trabalho apresentado ao Programa de Pós-Graduação em Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais, como requisito parcial para obtenção do título de Doutor em Zootecnia.

Área de concentração: Produção Animal  
Orientador: Prof. Leandro Sâmia Lopes  
Coorientador: Domingos Sávio Campos Paciullo

Belo Horizonte

2024

S729a Souza, Eduardo Moreira Barradas de ,1994 -  
Avaliação e seleção de híbridos de *Megathyrus maximus* em relação a  
produtividade de forragem, valor nutritivo e tolerância ao estresse hídrico/  
Eduardo Moreira Barradas de Souza. -2024.  
114f: il.

Orientadora: Sâmia Leandro Lopes  
Coorientador: Domingos Sávio Campos Paciullo

Tese (Doutorado) apresentada à Faculdade de Medicina Veterinária da UFMG,  
como requisito parcial para obtenção do título de Doutor.  
Inclui Bibliografia.

1. Valor nutritivo - Teses - 2. Forragem - Teses - I. Lopes, Sâmia  
Leandro - II. Paciullo, Domingos Sávio Campos - III. Universidade  
Federal de Minas Gerais, Escola de Veterinária - IV. Título

CDD – 639.089

Bibliotecária responsável Cristiane Patrícia Gomes CRB 2569  
Biblioteca da Escola de Veterinária, UFMG.



Escola de Veterinária  
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## ATA DE DEFESA DE TESE DO ALUNO EDUARDO MOREIRA BARRADAS DE SOUZA

As 09:00 horas do dia 19 de fevereiro de 2024, reuniu-se, a Comissão Examinadora de Tese, aprovada por Ad referendum no dia 12/01/2024, para julgar, em exame final, a defesa da tese intitulada: Avaliação e seleção de híbridos de *M. maximus* em relação à produtividade de forragem, valor nutritivo e tolerância ao estresse hídrico, como requisito final para a obtenção do Grau de Doutor em Zootecnia, área de concentração Produção Animal.

Abrindo a sessão, o Presidente da Comissão, Prof. Leandro Sâmia Lopes, após dar a conhecer aos presentes o teor das Normas Regulamentares da Defesa de Tese, passou a palavra ao (a) candidato (a), para apresentação de seu trabalho. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do candidato (a). Logo após, a Comissão se reuniu, sem a presença do candidato e do público, para julgamento da tese, tendo sido atribuídas as seguintes indicações:

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Prof.(a)/Dr.(a) Mércia Regina Pereira de Figueiredo	X	<input type="checkbox"/>
Prof.(a)/Dr.(a) Carlos Augusto de Miranda Gomide	X	<input type="checkbox"/>

Pelas indicações, o (a) candidato (a) foi considerado (a):  Aprovado (a)  
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Para concluir o Doutorado, o(a) candidato(a) deverá entregar 03 volumes encadernados da versão final da tese acatando, se houver, as modificações sugeridas pela banca, e a comprovação de submissão de pelo menos um artigo científico em periódico recomendado pelo Colegiado dos Cursos. Para tanto terá o prazo máximo de 60 dias a contar da data defesa.

O resultado final, foi comunicado publicamente ao (a) candidato (a) pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora e encaminhada juntamente com um exemplar da tese apresentada para defesa.

Belo Horizonte, 19 de fevereiro de 2024.

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*A meus pais, Adailton e Eliuda.*

*Dedico*

## AGRADECIMENTOS

A conclusão desta tese representa não apenas um marco em minha vida acadêmica, mas também um momento de profunda gratidão a todos aqueles que tornaram esta jornada possível. Jornada esta que foi repleta de desafios, momentos de dúvida e obstáculos que testaram minha determinação. No entanto, essas dificuldades também foram os alicerces sobre os quais construí meu crescimento como pessoa e profissional. Cada obstáculo superado me fez amadurecer e me tornar mais resiliente, e estou profundamente grato por essas experiências que me moldaram.

Meus mais sinceros agradecimentos a Deus, cuja força e luz têm sido constantes em meu caminho. Sua orientação e apoio me deram a coragem necessária para enfrentar os momentos mais desafiadores desta jornada.

Agradeço aos meus pais Adailton e Eliuda, verdadeiras referências pessoais em minha vida. Eles são exemplos de trabalho árduo, dedicação e sacrifício, especialmente por terem se dedicado intensamente à lavoura de café para que eu pudesse dar continuidade aos meus estudos. Através deles, aprendi o valor do trabalho, da persistência e da resiliência. Eles me ensinaram que o sucesso não vem fácil, mas é conquistado através de esforço e dedicação. Por isso, tudo o que alcancei é uma homenagem ao amor e apoio que vocês me deram.

Agradeço aos meus avós Elzio, Leni e Adelina (*in memoriam*), que sempre estiveram ao meu lado com palavras de sabedoria e encorajamento. Sua presença e apoio foram essenciais em minha jornada. Às minhas irmãs, tias e a meu querido sobrinho, Bernardo, obrigado por estarem sempre presentes, oferecendo seu apoio incondicional e amor em todos os momentos.

Ao meu orientador professor Leandro Sâmia, sua orientação e ensinamentos foram cruciais para meu crescimento pessoal e profissional. Aos professores do Departamento de Zootecnia da Escola de Veterinária, professora Sandra, Ângela e professor Luciano, por todo conhecimento transmitido, oportunidades e por todos os ensinamentos profissionais e pessoais.

Aos secretários do colegiado da Pós-graduação em Zootecnia, Marcelo e Esther por toda atenção, disposição e paciência. Um agradecimento especial à Professora Hemilly que além de uma grande mentora se tornou uma grande amiga, agradeço a amizade, experiência, paciência e todos os conselhos em todos os momentos da minha caminhada.

À Embrapa Gado de leite, especialmente aos pesquisadores Domingos Paciullo, Carlos Augusto Gomide e Wadson Rocha pela oportunidade e ensinamentos. Um agradecimento ainda mais especial ao Domingos pela parceria e cumplicidade em todos os momentos. Aos outros incontáveis colaboradores, amigos e companheiros de jornada pelas inúmeras histórias e aprendizados que vou levar para sempre comigo.

Por fim, agradeço aos meus amigos Rafael, Juliana, Jaciara, Abias, Luiz, Mariana, Karen, Thamyres, Nathalia e Natalia que tornaram essa jornada mais leve e feliz. Suas risadas, conselhos e companhia foram um refúgio nos momentos de estresse acadêmico. Um agradecimento especial ao Hilton que me apoiou, incentivou e me orientando nas complexas decisões. Obrigado por fazer parte deste capítulo da minha vida.

Cada uma dessas pessoas desempenhou um papel vital em minha jornada, e esta tese é uma celebração não apenas do meu trabalho, mas também da generosidade, apoio e amor que recebi ao longo do caminho. Obrigado a todos por fazerem parte desta conquista!

## RESUMO

O objetivo deste trabalho foi avaliar e selecionar genótipos de *Megathyrsus maximus* quanto à produtividade de forragem, valor nutritivo e tolerância ao estresse hídrico. CAPÍTULO II: Este capítulo teve como objetivo avaliar e selecionar híbridos melhorados de *M. maximus* com potencial forrageiro. O experimento, iniciado em novembro de 2020, foi implantado sob delineamento experimental em blocos ao acaso com três repetições, em arranjo de parcelas subdivididas. As parcelas principais consistiam em dezoito híbridos melhorados e das cultivares testemunhas Mombaça e Massai, enquanto as subparcelas correspondiam às estações (chuvosa e seca). Características produtivas e de valor nutritivo foram avaliadas ao longo de dois anos (2021/2022). Utilizou-se o índice de seleção FAI-BLUP para identificar híbridos que combinassem características favoráveis nas épocas chuvosa e seca. O índice FAI-BLUP indicou os híbridos PM411, PM407, PM426 e PM412 para a época chuvosa, e os híbridos PM406, PM414, PM419 e a cultivar Mombaça para a época seca, demonstrando ganhos genéticos equilibrados e desejáveis para todas as características avaliadas. CAPÍTULO III: Este capítulo teve como objetivo avaliar a variabilidade e os mecanismos de resposta de híbridos de *Megathyrsus maximus* ao déficit hídrico e ao alagamento. O experimento foi conduzido em casa de vegetação, em delineamento experimental de blocos ao acaso, em arranjo fatorial. Os tratamentos consistiram na combinação de oito genótipos de *M. maximus*, três condições hídricas (controle, alagamento e déficit hídrico) e duas épocas de colheita (1ª colheita: após 20 dias de estresse hídrico; 2ª colheita: 12 dias após o período de recuperação). As características produtivas e morfológicas foram avaliadas ao final do período de estresse e após o período de recuperação. Todos os híbridos submetidos ao déficit hídrico apresentaram redução da massa aérea e radicular. Entretanto, após o período de estresse, observou-se uma resposta de sobrevivência ao déficit hídrico, com recuperação de aproximadamente 28,4% da parte aérea e 16,3% do sistema radicular em todos os genótipos. O híbrido PM409 destacou-se quanto à adaptação às condições de alagamento, apresentando uma maior produção de raízes (41,2%) em comparação à produção média dos demais genótipos. Além disso, não houve redução na produção de massa de folhas e caules em relação à condição controle. Os resultados indicaram que nenhum genótipo se mostrou tolerante ao déficit hídrico. Por outro lado, o híbrido PM409 apresentou características promissoras para tolerância ao estresse por alagamento.

**Palavras-chave:** estresse hídrico; índice de recuperação de raízes; índice de seleção fai-blup; massa de folhas; proteína bruta.

## ABSTRACT

The objective of this study was to evaluate and select genotypes of *Megathyrsus maximus* for forage productivity, nutritional value, and tolerance to water stress. CHAPTER II: This chapter aimed to evaluate and select improved hybrids of *M. maximus* with forage potential. The experiment, initiated in November 2020, was conducted under a randomized block design with three replications, arranged in split plots. The main plots consisted of eighteen improved hybrids and the control cultivars Mombaça and Massai, while the subplots corresponded to the seasons (rainy and dry). Productive and nutritional value characteristics were evaluated over two years (2021/2022). The FAI-BLUP selection index was used to identify hybrids that combined favorable characteristics in both the rainy and dry seasons. The FAI-BLUP index indicated the hybrids PM411, PM407, PM426, and PM412 for the rainy season, and the hybrids PM406, PM414, PM419, and the cultivar Mombaça for the dry season, demonstrating balanced and desirable genetic gains for all evaluated characteristics. CHAPTER III: This chapter aimed to evaluate the variability and response mechanisms of *Megathyrsus maximus* hybrids to water deficit and flooding. The experiment was conducted in a greenhouse under a randomized block design, in a factorial arrangement. The treatments consisted of a combination of eight genotypes of *M. maximus*, three water conditions (control, flooding, and water deficit), and two harvest times (1st harvest: after 20 days of water stress; 2nd harvest: 12 days after the recovery period). Productive and morphological characteristics were evaluated at the end of the stress period and after the recovery period. All hybrids subjected to water deficit showed a reduction in shoot and root mass. However, after the stress period, a survival response to water deficit was observed, with recovery of approximately 28.4% of the shoot and 16.3% of the root system in all genotypes. The hybrid PM409 stood out for its adaptation to flooding conditions, showing a higher root production (41.2%) compared to the average production of the other genotypes. Additionally, there was no reduction in leaf and stem mass production compared to the control condition. The results indicated that no genotype was tolerant to water deficit. On the other hand, the hybrid PM409 presented promising characteristics for tolerance to flooding stress.

**Keywords:** water stress; root recovery index; fai-blup selection index; leaf mass; crude protein.

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**LISTA DE TABELAS**

---

**3 CAPÍTULO II – Manuscript: Evaluation and selection of *Megathyrsus maximus* hybrids with forage potential**

**Table 1.** Conopy height (cm) and herbage mass (kg ha<sup>-1</sup>) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in two years (1<sup>st</sup> year - 2020/2021 and 2<sup>nd</sup> year - 2021/2022).....68

**Table 2.** Leaf (%), stem (%), and dead material (%) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça, two seasons (rainy and dry season) and in the 1<sup>st</sup> year.....73

**Table 3.** Leaf (%), and dead material (%) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça, two seasons (rainy and dry season) and in the 2<sup>nd</sup> year.....74

**Table 4.** Chemical composition, crude protein (%), and digestibility of organic matter (%) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça, two seasons (rainy and dry season), and in the 1<sup>st</sup> year.....75

**Table 5.** Chemical composition, crude protein (%), and digestibility of organic matter (%) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça, two seasons (rainy and dry season), and in the 2<sup>nd</sup> year.....76

**Table 6.** Chemical composition, neutral detergent fiber (%) and acid detergent fiber (%) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in two years (1<sup>st</sup> year - 2020/2021 and 2<sup>nd</sup> year - 2021/2022).....77

**Table 7.** Chemical composition, lignin (%) and cellulose (%) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça, two seasons (rainy and dry season), and in two years.....78

**4 CAPÍTULO III – Manuscript: Productive and morphological responses of *Megathyrsus maximus* hybrids submitted to water deficit and waterlogging**

**Table 1.** Productive characteristics (g pot<sup>-1</sup>) of *Megathyrsus maximus* genotypes, under three water conditions (control – waterlogging – water deficit).....106

**Table 2.** Morphological characteristics of *Megathyrsus maximus* genotypes, under three water conditions (control – waterlogging – water deficit).....112

**Table 3.** SPAD index of *Megathyrsus maximus* genotypes, during the stress imposition period with readings on days (0, 5, 10, 15 and 20) and 12 days after recovery from water stress.....113

---

---

**LISTA DE FIGURAS**

---

---

**3 CAPÍTULO II – Manuscript: Evaluation and selection of *Megathyrsus maximus* hybrids with forage potential**

**Figure 1.** Precipitation and average minimum and maximum temperatures during the experimental period. (Data from INMET, Station: Coronel Pacheco - A557).....67

**Figure 2.** (A) Leaf mass ( $\text{kg ha}^{-1}$ ) and (B) stem mass ( $\text{kg ha}^{-1}$ ) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in two seasons (rainy and dry season) in the 1<sup>st</sup> year; (C) Dead material mass ( $\text{kg ha}^{-1}$ ) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in dry season and in the 1<sup>st</sup> year.....69

**Figure 3.** (A) Leaf mass ( $\text{kg ha}^{-1}$ ) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in two seasons (rainy and dry season) in the 2<sup>nd</sup> year; (B) stem mass ( $\text{kg ha}^{-1}$ ) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in the 2<sup>nd</sup> year; (C) Dead material mass ( $\text{kg ha}^{-1}$ ) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in dry season and in the 2<sup>nd</sup> year.....71

**4 CAPÍTULO III – Manuscript: Productive and morphological responses of *Megathyrsus maximus* hybrids submitted to water deficit and waterlogging**

**Figure 1.** Soil water content (%) in pots containing genotypes of *Megathyrsus maximus* (PM407, PM411, PM409, PM408, PM415, PM427, PM414) and cv. Massai, under water deficit condition.....105

**Figure 2.** (A) Root to shoot ratio of *Megathyrsus maximus* genotypes, under three water conditions (control – waterlogging – water deficit); (B) Root to shoot ratio of *Megathyrsus maximus* genotypes, in two harvests (1<sup>st</sup> harvest – the end of water stress period and 2<sup>nd</sup> harvest – after recovery period); (C) Root to shoot ratio under three water conditions

(control – waterlogging – water deficit) and in two harvests (1st harvest – the end of water stress period and 2nd harvest – after recovery period).....107

**Figure 3.** (A) Root volume of *Megathyrsus maximus* genotypes, under three water conditions (control – waterlogging – water deficit); (B) Root volume under three water conditions (control – waterlogging – water deficit) and in two harvests (1st harvest – the end of water stress period and 2nd harvest – after recovery period).....108

**Figure 4.** (A) Root recovery index of *Megathyrsus maximus* genotypes; (B) Root recovery index under two harvests (1st harvest – the end of water stress period and 2nd harvest – after recovery period).....109

**Figure 5.** (A) Leaf area ( $\text{cm}^2 \text{ pot}^{-1}$ ) of *Megathyrsus maximus* genotypes, under three water conditions (control – waterlogging – water deficit); (B) leaf area ( $\text{cm}^2 \text{ pot}^{-1}$ ) under three water conditions (control – waterlogging – water deficit) in two harvests (1st harvest – the end of water stress period and 2nd harvest – after recovery period).....110

**Figure 6.** (A) Specific leaf area ( $\text{cm}^2 \text{ g}^{-1}$ ) of *Megathyrsus maximus* genotypes, under three water conditions (control – waterlogging – water deficit); (B) specific leaf area ( $\text{cm}^2 \text{ g}^{-1}$ ) under three water conditions (control – waterlogging – water deficit) in two harvests (1st harvest – the end of water stress period and 2nd harvest – after recovery period).....111

## SUMÁRIO

<b>1 INTRODUÇÃO GERAL</b> .....	14
<b>2 CAPÍTULO I – REVISÃO DE LITERATURA</b> .....	16
<b>2.1 Gramíneas do Gênero <i>Megathyrus maximus</i> (syn. <i>Panicum maximum</i>)</b> .....	16
<b>2.2 Melhoramento genético em forrageiras tropicais</b> .....	17
<b>2.3 Morfogênese: entendendo a dinâmica do desenvolvimento vegetal</b> .....	19
<b>2.4 Estresse hídrico em plantas forrageiras</b> .....	21
2.4.1 Efeitos do estresse por déficit hídrico no desenvolvimento e produção de plantas forrageiras.....	22
2.4.2 Efeitos do estresse hídrico por alagamento no desenvolvimento e produção de plantas forrageiras .....	24
<b>REFERÊNCIAS</b> .....	26
<b>3 CAPÍTULO II – Manuscript: Evaluation and selection of <i>Megathyrus maximus</i> hybrids with forage potential</b> .....	39
<b>4 CAPÍTULO III – Manuscript: Productive and morphological responses of <i>Megathyrus maximus</i> hybrids submitted to water deficit and waterlogging</b> .....	79
<b>5 CONSIDERAÇÕES FINAIS</b> .....	114

## 1 INTRODUÇÃO GERAL

A relevância da pesquisa de plantas forrageiras no Brasil é inquestionável, uma vez que a pecuária representa uma das principais atividades econômicas no país, com base em sistemas de criação a pasto. Esses sistemas estão associados ao menor custo de produção, maior sustentabilidade, quando bem manejados, maior qualidade de produto, bem como maior bem-estar animal (Pereira et al., 2016; Hanrahan et al., 2018; Merino et al., 2019). Apesar da participação significativa no PIB nacional (7,0%), o setor pecuário ainda possui um vasto potencial de crescimento (CEPEA, 2023).

Nos últimos anos diversas pesquisas têm sido desenvolvidas para aprimorar a pecuária nacional. Isso inclui técnicas avançadas de manejo de pastagem, como a reposição de nutrientes, correção de solo, intensificação do pastejo e a diversificação da pastagem com novas cultivares forrageiras (Euclides et al., 2009; Dias-Filho, 2014; Da Rocha Junior et al., 2017; Tesk et al., 2018). Essa evolução pode ser constatada com dados apresentados por De Oliveira Silva et al. (2021), que entre os anos de 2006 e 2017, demonstraram que houve um aumento de 6% na produtividade animal e uma redução de 2,5% nas áreas de pastagens no Brasil.

O lançamento de cultivares melhoradas, em particular, de *Megathyrsus maximus* Jacq. (syn. *Panicum maximum*), desempenha um papel crucial na evolução e sustentabilidade da produção animal (Jank et al., 2014; Torres et al., 2016). O programa de melhoramento na Embrapa Gado de Corte resultou no lançamento das cultivares Tanzânia e Mombaça, marcando um avanço significativo na pecuária nacional, com um aumento substancial na produtividade. De acordo com Jank et al. (2008), isso ocorreu devido estas cultivares serem cerca de 79% e 130%, respectivamente, mais produtivas que a cultivar comercial à época; o capim Colonião. Isso possibilitou a elevação significativa dos índices de produtividade da pecuária nacional. No entanto, algumas características contribuem para casos de insucesso na adoção destas cultivares, como a doença foliar causada pelo fungo *Bipolaris maydis*, na cultivar Tanzânia-1 (Jank et al., 2010; Paciullo e Gomide, 2016) e a dificuldade de manejo do pastejo das cultivares por conta do elevado alongamento de colmos demonstrado principalmente no período de florescimento (Barbosa et al., 2007).

A busca por forrageiras mais promissoras do ponto de vista de produtividade, valor nutritivo e adaptabilidade às condições edafoclimáticas e que sejam tolerantes a doenças e pragas de pastagem (e.g., *B. maydis* e cigarrinhas das pastagens), tem levado ao desenvolvimento de cultivares com características superiores, dentre elas citam-se a BRS Zuri, BRS Tamani e BRS Quênia. O desenvolvimento e lançamento de novas cultivares, leva ao

predomínio da utilização destes materiais a longo prazo nas diferentes regiões brasileiras, com maior responsabilidade técnica para a identificação e recomendação, com base na condição de cada sistema de produção (Paciullo e Gomide, 2016).

Dentre as cultivares de *M. maximus* lançadas recentemente, pode-se destacar a cultivar BRS Zuri, que é reconhecida por apresentar alta produtividade, alto valor nutritivo e resistência a pragas e doenças (cigarrinhas das pastagens e *B. maydis*) (Jank et al., 2014). Por outro lado, ressalta-se que a cultivar BRS Quênia apesar de apresentar menor porte em relação à BRS Zuri e as cultivares convencionais, como Mombaça e Tanzânia, possui melhor valor nutritivo devido à alta proporção de folhas, influenciando na digestibilidade (Jank et al., 2017). Além disso, esta é uma gramínea que possui porte médio, com touceiras com maior densidade de perfilhos e folhas, com menores proporções de colmos e material morto (Cavalli, 2016), mas não tolera encharcamento (Jank et al., 2017). A cultivar BRS Tamani, apresenta características similares a BRS Quênia, por outro lado, é um material que apresenta maior resistência a condições de baixas temperaturas. Porém, não é uma opção para utilização em solos mal drenados.

Apesar da disponibilidade de diversas cultivares de *M. maximus* altamente produtivas e com alta qualidade nutricional, a oferta de materiais forrageiros adaptadas aos diversos ecossistemas a pastoris do Brasil ainda é limitada, além de haver uma carência de materiais resistentes aos fatores bióticos e abióticos que frequentemente afetam essas cultivares (Jank, 2019). Nesse contexto, o programa de melhoramento de *M. maximus* tem avançado significativamente, resultando no desenvolvimento de vários híbridos. No entanto, resta uma lacuna de conhecimento sobre a produtividade e o valor nutritivo desses novos materiais, além de sua capacidade de responder a estresses ambientais comumente encontrados em regiões tropicais.

O progresso contínuo no melhoramento genético não se limita apenas à busca por maior produtividade e qualidade nutricional. Atualmente, há um foco crescente na obtenção de materiais mais adaptados ao período seco e às condições de alagamento do solo, aspectos cruciais para o sucesso da cultura em diversas regiões do país. Este avanço reflete uma abordagem holística que visa não apenas maximizar a produção, mas também garantir a tolerância das culturas frente às adversidades ambientais.

A hipótese do presente estudo é que os genótipos de *M. maximus* apresentem variabilidade em características morfológicas e estruturais que irão influenciar em índices produtivos e qualitativos contrastantes, bem como avaliar respostas a estresse por déficit hídrico e alagamento. Portanto, objetivou-se avaliar e selecionar híbridos de *M. maximus* com potencial forrageiro com base na produtividade forrageira e no valor nutritivo, além de avaliar os

impactos do estresse por déficit hídrico e alagamento sobre as características produtivas e morfológicas, assim como sua capacidade de recuperação em condições adequadas de água no solo.

## 2 CAPÍTULO I – REVISÃO DE LITERATURA

### 2.1 Gramíneas do Gênero *Megathyrsus maximus* (syn. *Panicum maximum*)

O Brasil apresenta um extenso território, com condições edafoclimáticas que favorecem o desenvolvimento vegetativo de gramíneas forrageiras tropicais, o que possibilita que a maior parte do rebanho bovino nacional seja criado a pasto. Dessa forma, a pecuária baseada em pastagens se direciona a processos de intensificação, com o objetivo de aumentar a produtividade de carne e leite (Moura et al., 2017; Ferraz e Felício, 2010).

Dentre as gramíneas tropicais, destaca-se a espécie *Megathyrsus maximus* que pertence à família Poaceae, subfamília Panicoideae e tribo Paniceae. Esta espécie é reconhecida nacionalmente por sua produtividade forrageira, propagação por sementes e por possuir produção elevada de folhas. Geralmente de grande porte, adaptada a diversos tipos de solo e clima, porém, exigente em fertilidade do solo. Além disso, apresenta alta aceitabilidade pelos animais, tornando-se uma escolha comum para formação de pastagens (Jank et al., 2013).

As cultivares de *M. maximus*, em sua maioria são plantas perenes de hábito cespitoso, variam em altura de 0,5 e 4,5 m, com colmos eretos contendo de três a 15 nós, podendo ou não apresentar pelos. As folhas, lanceoladas, variam de 15 a 100 cm de comprimento e 3,5 cm de largura. Suas inflorescências possuem forma de panícula aberta, elípticas, com cerca de 2 mm de comprimento (Alves et al., 2008).

O gênero *Megathyrsus* possui complexo agâmico formado por três espécies: *M. infestum*, *M. trichocladum* e o próprio *M. maximus*. Originário da África Tropical, foi introduzido no continente americano por volta do século XVII, possivelmente associado ao uso como cama para escravos (Chase, 1944). Embora sua introdução no Brasil remonte a esse período, o melhoramento genético de *M. maximus* no país é relativamente recente, iniciando-se em 1982 com a implementação do banco de germoplasma da Embrapa Gado de Corte, a partir de acessos coletados no Quênia e Tanzânia entre 1967 e 1969 (Combes e Pernès, 1970; Savidan et al., 1989).

A partir desse banco de germoplasma, seguido de avaliações agronômicas e morfológicas, foram realizados os primeiros cruzamentos e selecionados materiais que resultaram nas cultivares Tanzânia-1 (1990), Mombaça (1993), Massai (2001), BRS Zuri

(2014) e, mais recentemente, as cultivares híbridas BRS Tamani (2015) e BRS Quênia (2017) (Jank et al., 2017).

No Brasil, as cultivares de *M. maximus* mais amplamente plantadas são Massai, Tanzânia e Mombaça, especialmente em sistemas de produção de leite, conforme destacado por Paciullo e Gomide, (2016). Apesar disso, ainda existem áreas de pastagens remanescentes com outras cultivares do gênero, incluindo a cultivar Colonião (Jank et al., 2013).

Apesar da diversidade de materiais forrageiros da espécie *M. maximus* no mercado, as áreas de pastagens são ocupadas basicamente por apenas duas cultivares. Nesse cenário, diante da baixa diversidade das pastagens e a necessidade de materiais forrageiros mais produtivos, de maior qualidade, resistentes a patógenos e que façam o melhor uso dos recursos disponíveis de forma sustentável (Braz et al., 2017), há uma carência da diversidade dos genótipos forrageiros nos sistemas de produção.

Nesse contexto, o desenvolvimento de novos materiais forrageiros, seja a partir da variabilidade natural do banco de germoplasma ou por meio de hibridação, visa obter genótipos que sejam produtivos, tenham produção de sementes viáveis, respondam de forma eficiente ao uso de fertilizantes e tolerantes a fatores bióticos e abióticos (Valle et al., 2013; Torres et al., 2016).

## 2.2 Melhoramento genético em forrageiras tropicais

A pecuária desempenha um papel vital na economia global, atendendo a crescente demanda por carne e produtos lácteos. Nas regiões tropicais e subtropicais, onde o clima é caracterizado por altas temperaturas e pluviosidade variável, as gramíneas forrageiras desempenham um papel fundamental na alimentação de ruminantes, fornecendo a principal fonte de alimento. A busca por garantir produção de carne e leite de forma sustentável nessas regiões tem gerado um foco significativo para com o desenvolvimento de plantas forrageiras melhoradas.

O melhoramento genético surge como uma abordagem estratégica para otimizar a produção de plantas forrageiras. Conforme apontado por Jank et al. (2011), o melhoramento visa desenvolver variedades de forragens que sejam resistentes a pragas, doenças e estresses ambientais, além de apresentar qualidade nutricional, produtividade e adaptabilidade a diferentes ambientes tropicais.

No Brasil, o interesse pela genética de plantas forrageiras teve início na década de 1980, com o intuito de introduzir novos genes, selecionar características associadas a maior produção, qualidade e adaptação, e, por fim, manipular esses genes por meio de cruzamentos (Jank et al.,

2005). Aproximadamente 40% das gramíneas forrageiras se reproduzem de maneira sexuada, na qual os gametas masculinos fertilizam os gametas femininos, gerando híbridos. No entanto, a outra parcela destas gramíneas se reproduzem de forma assexuada, por meio da apomixia, um processo na qual os gametas masculinos não fertilizam os femininos, resultando em sementes que são clones da planta-mãe (Jank et al., 2011).

Entre as gramíneas  $C_4$  utilizadas em pastagens, a maioria se reproduz por apomixia, incluindo a espécie *M. maximus*, que é considerada um modelo nesse aspecto. A apomixia é um mecanismo de propagação vegetativa por sementes, no qual o embrião não surge de um processo de fecundação. Nessa espécie, assim como em outras gramíneas forrageiras tropicais, a apomixia envolve uma combinação de aposporia, ou seja, não ocorre a redução dos gametas seguida da partenogênese que é a ausência de fecundação (Warmke, 1954; Combes, 1975; Savidan, 1982; Jank et al., 2008). Dessa forma, o cruzamento entre plantas sexuais e apomíticas de *M. maximus* constitui alternativa viável para obtenção de híbridos superiores quanto a produção vegetal e, por conseguinte, de produção animal (Martuscello et al., 2007).

No processo de seleção de plantas com vistas ao desenvolvimento de novas variedades ou a escolha de genitores superiores para cruzamentos, é de extrema importância garantir a superioridade genética. A análise características em um grupo de indivíduos ao longo do tempo é uma prática comum no melhoramento genético de plantas perenes (Cruz et al., 2004). Os híbridos resultantes são submetidos a diversas fases de avaliações antes de serem lançados comercialmente.

No processo inicial, os híbridos resultantes do cruzamento entre plantas sexuais e apomíticas são submetidos à avaliação em pequenas parcelas de dois metros quadrados, com repetições. Normalmente, nesta fase, muitos materiais são submetidos a análise. Nesses ensaios experimentais, diversas características são avaliadas, tais como, produção de forragem, componentes morfológicos, produção de sementes, capacidade de rebrota após corte, intensidade e período de florescimento, bem como incidência de insetos e doenças, além da qualidade da forragem. Além disso, é realizada uma caracterização morfológica dos materiais em estudos, isso inclui mensuração de altura e porte das plantas e folhas, pilosidade de folhas, colmos e hastes e o tipo de inflorescência, entre outros aspectos (Jank et al., 2005).

A etapa subsequente consiste na seleção dos genótipos ou híbridos promissores que são então submetidos a avaliação em uma rede nacional de testes em locais diversos, ainda em parcelas só que ligeiramente maiores do que na fase anterior. Esta fase, tem como propósito testar a adaptabilidade das plantas a diferentes tipos de solos e condições climáticas. As mesmas características avaliadas na fase anterior são observadas novamente. A partir desse ensaio, até

oito genótipos podem ser selecionados para próxima etapa, na qual são feitas avaliações em piquetes de aproximadamente 1.000 m<sup>2</sup>, submetidos ao pastejo animal. Isso permite analisar o impacto do pisoteio e pastejo dos animais sobre as plantas forrageiras. Em uma fase posterior, os genótipos mais promissores são avaliados em relação ao desempenho animal sob pastejo, medindo a produção de carne ou leite, em área substancialmente maiores, podendo chegar a até 10 hectares (Jank et al., 2005).

É importante salientar que o principal objetivo dos programas de melhoramento genético é, inicialmente, aprimorar a produção forrageira, o que é fundamental para aumentar a produtividade das pastagens e, conseqüentemente, da pecuária. A busca por cultivares de maior qualidade, resistentes a patógenos ou que atendam a demandas específicas torna-se prioritária somente quando o mercado atinge um estado de saturação em relação às opções de cultivares de alta produtividade de uma determinada espécie ou gênero (Jank et al., 2008).

### **2.3 Morfogênese: entendendo a dinâmica do desenvolvimento vegetal**

O êxito do manejo de pastagens vai além da simples disponibilidade de nutrientes no solo e da escolha da espécie forrageira no sistema produtivo. O sucesso é também intrinsecamente ligado à compreensão dos mecanismos morfofisiológicos e a forma como interagem com o ambiente (Fagundes et al., 2006). Nesse contexto, a pesquisa sobre as características morfofisiológicas em gramíneas forrageiras assume uma posição central e insubstituível.

A morfogênese, área do conhecimento que se dedica ao estudo da origem e desenvolvimento dos diversos órgãos da planta, assim como as transformações que moldam sua produção e as modificações na forma e estrutura da planta ao longo do tempo, desempenha um papel crucial (Chapman e Lemaire, 1993; Silveira, 2006). Essa abordagem possibilita determinar características morfofisiológicas e estruturais que impactam diretamente o índice de área foliar (IAF), fornecendo ferramentas valiosas para o melhoramento genético de plantas forrageiras.

Em sistemas de produção a pasto, as folhas e perfilhos são as unidades elementares consumidas pelos animais, tornando essencial compreender a dinâmica de crescimento e desenvolvimento dessas partes da planta. As características morfofisiológicas, enraizadas no genótipo, são influenciadas pelas condições ambientais, como temperatura, luz, água e nutrientes (Porto, 2009). Por sua vez, as características estruturais são diretamente afetadas pelas morfofisiológicas.

Apesar de não haver evidências sólidas sobre a relação benéfica entre morfogênese e melhoramento genético de plantas forrageiras, existe um vasto potencial para tal associação. O estudo da morfogênese permite acompanhar o desenvolvimento das plantas e sua interação com o ambiente, informação crucial na seleção de genótipos.

A morfogênese de uma planta forrageira durante seu desenvolvimento vegetativo pode ser caracterizada por diversos fatores, como o aparecimento e alongamento de colmos e lâminas foliares, além da longevidade das folhas (Chapman e Lemaire, 1993). A partir dessas informações, podem ser determinadas taxas como a de aparecimento (TxApfo), alongamento (TxAlfo) e senescência foliar (TxSefo). Além disso, taxas de alongamento de colmo (TxAlco), filocrono, tempo de vida das folhas (TVF), comprimento de folha (Cfo) e número de folhas totais (NFT) e vivas (NFV) por perfilho.

A taxa de aparecimento de folhas (TxApfo), é expressa em número médio de folhas surgidas por perfilho, em determinado período, representada normalmente em número de folha/dia/perfilho. O inverso da TxApfo é o filocrono, que é basicamente determinado pelo número de dias entre o aparecimento de duas folhas sucessivas (Porto, 2009). A taxa de alongamento foliar (TxAlfo), é uma medida de grande importância para o fluxo de tecidos das plantas, que normalmente é expressa em mm/dia, é uma resposta que é altamente dependente das condições do meio como temperatura, luz e disponibilidade de nutrientes. A TxAlfo se correlaciona positivamente com o rendimento de forragem, mas se correlaciona negativamente com o número de perfilho por planta (Horst et al., 1978). Diante disso, esta é uma variável importante quando se faz uma abordagem da morfogênese relacionada ao processo de melhoramento e seleção de plantas forrageiras (Silveira, 2006).

A taxa de alongamento de colmos (TxAlco), é um componente de grande relevância e que interfere na estrutura do pasto, assim como, no equilíbrio do processo de competição por luz (Sbrissia e Silva, 2001). O desenvolvimento de colmos pode favorecer o aumento da produção de massa seca, porém, pode apresentar efeitos negativos sobre o aproveitamento e a qualidade da forragem produzida, alterando o comportamento ingestivo dos animais e, conseqüentemente, o consumo de forragem, devido à redução na relação lâmina/colmo (Ferreira, 2017).

O tempo de vida das folhas (TVF) é o período no qual as folhas permanecem intactas no perfilho, sem apresentar sinais de senescência. Essa característica é determinante para o número máximo de folhas vivas que um perfilho pode manter e é influenciada pela temperatura, da mesma maneira que a taxa de aparecimento de folhas (TxApfo). A compreensão do tempo de vida das folhas é de fundamental importância para a gestão do pastejo, uma vez que ela

fornece informações valiosas sobre o potencial de produção da espécie, ou seja, a quantidade máxima de material vegetativo disponível por unidade de área. Nesse contexto, estabelece-se uma conexão relevante entre a TVF e a avaliação de cultivares forrageiros, uma vez que essa característica pode ser usada para determinar a frequência ideal de desfolhação das plantas (Silveira, 2006). A taxa de senescência de folhas (TxSefo), é processo pelo qual ocorre a redução da atividade metabólica da folha. A folha é considerada morta quando 50% do seu comprimento se apresenta em processo de senescência.

O comprimento das folhas (Cfo) é um atributo influenciado por dois fatores principais: a taxa de alongamento de foliar (TxAlfo) e a taxa de aparecimento foliar (TxApfo). Para um determinado genótipo, o período de alongamento da folha mantém uma proporção constante ao longo da sequência de folhas (Dale, 1982). Consequentemente, as variações nas TxAlfo e TxApfo, seja por meio de práticas de manejo (como intensidade de pastejo, frequência de desfolhação e adubação) ou devido às flutuações climáticas, podem resultar em variações no comprimento final das folhas (Porto, 2009).

O comprimento da lâmina foliar é uma característica flexível da planta que responde à intensidade de desfolha. O Cfo pode ser considerado como uma estratégia morfológica de adaptação das plantas às pressões do pastejo (Lemaire e Chapman, 1996). Como resultado dessa estratégia, observa-se uma redução no comprimento das lâminas em pastagens que sofrem desfolha mais intensa (Porto, 2009). O número de folhas vivas (NFV) sofre influência direta de TxApfo e do TVF, por isso, qualquer mudança em uma destas características morfogênicas afetará o número de folhas vivas por perfilho (Nabinger e Pontes, 2001). O NFV é semelhante para cada espécie, no qual em um determinado momento em que, para cada folha que entra em senescência, surge uma nova folha. Esse mecanismo existe em decorrência do tempo limitado de vida da folha, que é determinado por características genéticas e influenciado por condições climáticas e de manejo (Hodgson, 1990).

Em suma, o estudo da morfogênese é essencial para entender a dinâmica do desenvolvimento vegetal e fornecer insights valiosos para o manejo eficaz de pastagens, contribuindo para o sucesso da pecuária.

#### **2.4 Estresse hídrico em plantas forrageiras**

As gramíneas desempenham um papel significativo na alimentação animal (Pereira et al., 2009), especialmente no Brasil, onde a produção de bovinos é predominantemente baseada em sistemas de criação a pasto, com apenas 17,19% dos animais abatidos provenientes de confinamento (Abiec, 2022). No entanto, esses sistemas enfrentam desafios decorrentes da

estacionalidade climática, que é caracterizado por períodos que as plantas forrageiras podem ser submetidas ao estresse por déficit hídrico e, a depender do relevo e o solo, podem estar sujeitas a condições de alagamento. Com isso, o desenvolvimento de materiais forrageiros que se desenvolvam sob ambientes adversos é essencial (Santos et al., 2013).

Nesse contexto, torna-se indispensável avaliar o desempenho de plantas forrageiras em condições de déficit hídrico e alagamento. Isso nos permite compreender amplamente os efeitos desses estresses na produção e nas adaptações das gramíneas a essas condições, contribuindo para minimizar os impactos causados pelo estresse hídrico.

#### 2.4.1 Efeitos do estresse por déficit hídrico no desenvolvimento e produção de plantas forrageiras

O estresse por déficit hídrico é um importante fator abiótico que influencia negativamente no desenvolvimento e a produtividade de diversas culturas em todo o mundo (Kramer, 1983). Esse estresse ocorre quando o potencial hídrico e o turgor das plantas são reduzidos a ponto de afetar a morfologia, fisiologia e o metabolismo (Hsiao, 1973). No entanto, a severidade desses efeitos está relacionada à intensidade e duração do estresse, bem como ao estágio de desenvolvimento e ao genótipo da planta (Kramer, 1983).

As plantas forrageiras podem responder ao déficit hídrico de várias maneiras, incluindo sobrevivência à seca, escape da seca e tolerância à seca. A sobrevivência à seca envolve a capacidade da planta de reduzir ou interromper temporariamente seu crescimento durante períodos secos e depois retomá-lo quando as condições são favoráveis (Lelièvre et al., 2011). O escape da seca refere-se à capacidade da planta de completar seu ciclo reprodutivo antes que o déficit hídrico ocorra, enquanto a tolerância à seca envolve adaptações fisiológicas, como acúmulo de solutos, para manter o turgor celular sob condições de déficit hídrico (Nilsen e Orcutt, 1996; Levitt, 1980).

Além disso, a tolerância à desidratação pode ser potencializada com maximização da captura de água do solo, enquanto desvia a maior parte da umidade para o processo de transpiração estomática (Blum, 2009). O desenvolvimento de um sistema radicular com alta densidade e que atinge estratos mais profundos do solo, são características importantes para sustentar a produtividade em ambientes com condições limitantes de água (Wasson et al., 2012; White e Snow, 2012). De acordo com Lilley e Kirkegaard (2011) as principais estratégias que as plantas possuem para melhorar o desempenho em condições de déficit hídrico estão relacionadas ao desenvolvimento de raiz. Resultados semelhantes foram observados por Huang et al. (2014), em que constataram que a produção de um sistema radicular mais profundo é mais importante para evitar a desidratação do que o comprimento total de raiz e a biomassa de raiz.

O sistema radicular desempenha um papel importante na extração de recursos, que normalmente são translocados para parte aérea da planta, o que mostra a importância de se avaliar a relação de biomassa de raiz: biomassa da parte aérea (Comas et al., 2013).

De modo geral, em condições limitantes de água o crescimento radicular é mantido por mais tempo em relação ao crescimento da parte aérea (Hanslin et al., 2019; Volaire et al., 2014), porém, a alocação de biomassa na relação raiz: parte aérea é dependente de diversos fatores como, os recursos disponíveis, intensidade do estresse e o material vegetal (Padilla et al. 2013; Rosas et al. 2013; Valladares et al. 2007). Normalmente, o aumento da razão raiz: parte aérea se deve a redução do crescimento da parte aérea e, em contrapartida, a manutenção simultânea do desenvolvimento radicular, principalmente em condições de limitação hídrica menos severas (Voltaire et al., 2014). Isso se deve ao fato de que em condições de déficit hídrico a morfologia da parte aérea é um dos componentes mais afetados (Kavar et al., 2008). Espécies de plantas forrageiras que evitam o processo de desidratação, em geral suspendem o desenvolvimento da parte aérea da planta, com o processo de senescência das folhas, além disso, normalmente verifica-se o fechamento de estômatos, como um possível mecanismo de reduzir o consumo de água e equilibrar as relações hídricas na condição de déficit hídrico (Santos et al., 2013; Munné-Bosch e Alegre, 2004; Verslues et al., 2006; Voltaire e Lelièvre, 2001).

A redução da produtividade influenciada pelo déficit hídrico, também pode ocorrer por outros fatores, como a redução da taxa de fotossintética (Saud et al., 2017), desordens no processo de absorção de assimilados e aumento na taxa de transpiração (Gulías et al., 2003). Isso se deve, provavelmente a ação do ácido abscísico (ABA). O ácido abscísico é um dos mais importantes compostos relacionados à sinalização do estresse. Este composto é sintetizado nas raízes de plantas sob condições de déficit hídrico. A translocação desse composto para a parte aérea causa redução na transpiração pelo fechamento dos estômatos e inibe o processo de desenvolvimento da planta. Além disso, influencia na manutenção do desenvolvimento da raiz, o que resulta no aumento da sua condutividade hidráulica (Kang e Zhang, 2004).

Perlikowskia et al. (2019) ao avaliar o efeito de condições limitantes de água em forrageiras de *Lolium multiflorum* e *Festuca arundinacea*, observaram que mesmo em condições de déficit hídrico as plantas foram capazes de manter alto teor de água foliar e absorção de água. Os autores salientam que essa resposta se deve provavelmente a uma combinação de adaptações fisiológicas, incluindo a redução da taxa de transpiração e adaptações morfológicas, como a redução da parte aérea e em contrapartida com um aumento no desenvolvimento de raízes.

De acordo com Hanslin et al. (2019), o fechamento de estômatos e a redução da produção de biomassa aérea foram as principais estratégias para evitar o processo de desidratação em gramíneas forrageiras temperadas em condições hídricas limitantes. Além disso, tem sido relatado que o fechamento estomático pode resultar em reduções acentuadas nas taxas fotossintéticas de plantas sob déficit hídrico (AbdElgawad et al., 2015; Xu et al., 2013; Chen, et al. , 2014; Lawlor e Corninc, 2002). Isto é devido ao sistema de troca de CO<sub>2</sub> que é reduzida diante do fechamento dos estômatos (Santos et al., 2013). Por outro lado, decréscimos na fotossíntese estão intimamente associados à redução no teor de clorofila de gramíneas em condições severas de estresse por déficit hídrico (Farooq et al., 2009). Ebrahimiyan et al. (2013) observaram aumento significativo no teor de clorofila de genótipos de *Festuca arundinacea* em condição de seca leve. No entanto, sob déficit hídrico severo houve uma redução substancial no teor de clorofila. Esses resultados são semelhantes aos relatados Souza et al. (2021), que também constataram que houve redução do índice relativo de clorofila em genótipos de *Urochloa* spp. sob condição severa de estresse por déficit hídrico.

A recuperação ao déficit hídrico tem sido caracterizada como a capacidade da planta em retomar o metabolismo e o desenvolvimento após a suspensão do estresse (Luo, 2010). As plantas possuem diversas estratégias para evitar a desidratação de folhas (Volaire et al., 1998). Dentre estas estratégias, o processo de senescência das folhas com a severidade do déficit hídrico, ajuda na proteção dos tecidos meristemáticos e dessa forma, auxilia na recuperação da planta no período após a interrupção do estresse (Bewley, 1995; West et al., 1990), visto que, os meristemas estão protegidos. Beloni et al. (2017), observaram alta taxa de sobrevivência de perfilhos do acesso BGP 289 de *Paspalum malacophyllum* após o período de recuperação ao estresse por déficit hídrico, em que, este material foi caracterizado por apresentar tolerância à desidratação. Por outro lado, o acesso BGP 397 de *Paspalum regnellii* apresentou maior taxa de recuperação do sistema radicular. De acordo com os autores, isso ocorreu devido a proteção dos meristemas radiculares no período de limitação hídrica que permitiu uma rápida recuperação das raízes. Com isso, propiciou o aparecimento de novos perfilhos e o crescimento da parte aérea no período pós-estresse. Perlikowskia et al. (2019) também verificaram que o híbrido INT-40 (*Lolium multiflorum* x *Festuca arundinacea*) apresentou rápida recuperação no desenvolvimento do sistema radicular após o período de reidratação.

#### 2.4.2 Efeitos do estresse hídrico por alagamento no desenvolvimento e produção de plantas forrageiras

Em sistemas de produção agrícola as inundações, sejam de curto ou longo prazo, podem ser ocasionadas por chuvas intensas, drenagem inadequada do solo ou por transbordamento de

curtos de água. Além disso, as áreas de pastagens geralmente estão localizadas em regiões marginais, que não são aptas para a agricultura (Dias-Filho e Carvalho, 2000), de modo que são áreas que podem ser regularmente sujeitas a inundações ou alagamento.

O alagamento causa um impacto negativo em todo ecossistema pastoril, devido à redução imediata na troca de gases entre as plantas e o ambiente (Armstrong et al., 1994). Na medida em que todo oxigênio disponível no solo é utilizado pelos organismos microbianos do solo e pelo processo de respiração das raízes das plantas (Sasidharan et al., 2017), se estabelece a condição anoxia, que obriga as plantas a mudarem suas estratégias de sobrevivência.

Diante disso, o uso de cultivares tolerantes ao alagamento pode possibilitar a mitigação dos riscos climáticos e dessa forma, reduzir a suscetibilidade dos sistemas de produção animal a pasto em regiões tropicais (Dias-Filho, 2005; Dias-Filho & Andrade, 2005). Os mecanismos de tolerância ao alagamento são baseados em diversas características e estratégias adaptativas, que melhoram as trocas gasosas e mantêm a produção de energia na planta (Armstrong et al., 1994).

Em geral, as condições anóxicas do solo influenciam as raízes a mudarem suas estratégias para o metabolismo anaeróbico com o objetivo de manter a produção de energia e as funções da planta (Loka et al., 2018; Colmer, 2003). Desse modo, as plantas impreterivelmente entram em processo de quiescência (Bailey-Serres et al., 2012), que em suma é caracterizado como uma resposta de rápido crescimento da parte aérea para garantir a manutenção da fotossíntese e a produção de aerênquima e raízes adventícias para possibilitar a transferência efetiva de oxigênio para as raízes (Sasidharan e Voesenek, 2015; Armstrong et al., 1994).

A troca gasosa entre a parte aérea da planta e o sistema radicular é facilitada com a formação do aerênquima, tecido esponjoso que cria grandes espaços intracelulares que possibilita a troca de gases (Jackson e Armstrong, 1999). Ao avaliar as respostas de gramíneas forrageiras ao alagamento Ploschuk et al. (2017) verificaram aumento da produção de raízes de *Phalaris aquatica*. Os autores salientaram que este resultado se deve ao aumento de produção de aerênquima nas raízes, que possibilitou a manutenção da parte aérea da planta. Jiménez et al. (2015), relataram que a capacidade de desenvolvimento de aerênquima é uma importante adaptação anatômica em forrageiras, permitindo o desenvolvimento da planta em condições limitantes de oxigênio no solo.

A formação de raízes adventícias está diretamente relacionada ao aumento da concentração de etileno na planta (Yin et al., 2009). O etileno também é responsável por outras estratégias adaptativas a nível molecular, fisiológico e morfológico na planta, e dessa forma,

possibilita auxiliar no aumento da tolerância ao estresse por alagamento (Yamauchi et al., 2018; Sairam et al., 2008).

Além dos efeitos do alagamento no sistema radicular, a fisiologia e a morfologia da parte aérea de gramíneas também apresentam alterações diante de estresse hídrico por alagamento (Mollard et al. 2010; Striker et al. 2011). Plantas submetidas ao alagamento podem apresentar fechamento de estômatos, redução na transpiração e, conseqüentemente, redução na taxa fotossintética (Ploschuk et al., 2017; Voesenek e Bailey-Serres, 2015), redução de número de perfilhos, número de folhas verdes por perfilho, comprimento de lâmina foliar, aumento da senescência foliar e, por conseqüência, a redução da área foliar funcional (Sasidharan et al. 2017; Cardoso et al. 2013; Mollard et al. 2010; Striker et al. 2008; Malik et al. 2002).

Ploschuk et al. (2017) verificaram que *Dactylis glomerata* e *Bromus catharticus* são materiais forrageiros que apresentam sensibilidade ao alagamento, o que desencadeou no aumento da senescência foliar precoce, fechamento progressivo dos estômatos, seguido pela queda na fotossíntese líquida. Por outro lado, *Phalaris aquatica* apresentou ser tolerante ao alagamento, visto que, evidenciou aumento tanto no desenvolvimento de raiz quanto da parte aérea, com isso não houve impacto nas respostas fisiológicas foliares durante o período de estresse. Resultados semelhantes foram verificados por Dias-Filho e Carvalho (2000), ao avaliar respostas de gramíneas de *Brachiaria* spp. ao alagamento. O estresse por alagamento influenciou na redução da área foliar de todos os materiais estudados. No entanto, a condutância estomática, a fotossíntese líquida, o teor de clorofila e a taxa de alongamento foliar foram reduzidas somente na cultivar Marandu (*Brachiaria brizantha*).

De acordo com Striker (2012) avaliar a tolerância ao alagamento é tão importante quanto verificar a capacidade de recuperação das plantas ao estresse, uma vez que a manutenção da parte aérea e um aparato fotossintético eficiente durante o alagamento pode resultar em maiores quantidades de foto assimilados mobilizados para as raízes após o fim do período de estresse. Isso pode resultar em uma recuperação mais rápida de um sistema radicular que foi drasticamente reduzido em condições de alagamento. Desse modo, níveis mais altos de antioxidantes, combinados com a ausência de danos à parte aérea durante o alagamento, podem resultar em taxas de recuperação mais rápidas após o fim do estresse (Jiménez et al., 2015).

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### **3 CAPÍTULO II - Manuscript: Evaluation and selection of *Megathyrsus maximus* hybrids with forage potential**

Este artigo foi elaborado conforme as normas do periódico "*Revista Brasileira de Zootecnia*".

## Evaluation and selection of *Megathyrus maximus* hybrids with forage potential

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**ABSTRACT-** This study aimed to evaluate and select improved hybrids of *M. maximus* with forage potential. The experiment was implemented in November 2020, under a randomized block design with three replications in a split-plot arrangement. The main plots consisted of eighteen improved hybrids and the control cultivars Mombaça and Massai, while the subplots corresponded to seasons (rainy and dry). Productive and nutritional value characteristics were evaluated over two years (2021/2022). Additionally, the FAI-BLUP Selection Index was used to identify hybrids that simultaneously met favorable characteristics in both the rainy and dry seasons. The FAI-BLUP index indicated the hybrids PM411, PM407, PM426, and PM412 for the rainy season, and the hybrids PM406, PM414, PM419, and the cultivar Mombaça for the dry season, providing balanced and desirable genetic gains for all evaluated characteristics.

**Keywords:** crude protein, FAI-BLUP selection index, forage production, nutritional value

### 1. Introduction

In the tropics, grasses of the species *Megathyrus maximus* (syn. *Panicum maximum*) are widely used in pasture-based production systems, standing out for their forage productivity and nutritional quality, which enhance animal performance in these systems (Wilkinson et al., 2020; Euclides et al., 2015; Dia-Filho et al., 2014). Despite the

diversity of forage materials of the species *M. maximus* on the market, pastures are predominantly occupied by only two cultivars of the species, Mombaça and Tanzânia. This limited diversity represents a significant risk, as these traditional cultivars may succumb to diseases or pests, such as *Bipolaris maydis*, which has already caused significant losses in the Tanzânia cultivar (Braz et al., 2015).

In addition to these challenges, it is crucial to consider the impact of climatic conditions on forage characteristics, as variations in temperature, rainfall, and light intensity directly influence growth rate, nutritional composition, and plant resilience to biotic and abiotic stresses (Sustek-Sánchez et al., 2023; Moyo and Nsahlai, 2021; Chapman, 2016; Giridhar and Samireddypalle, 2015; Lemaire et al., 2009). In tropical regions, where climate changes can intensify periods of drought or excessive rainfall, the ability of forages to maintain productivity and quality under different climatic scenarios is critical. Therefore, selecting cultivars that meet the nutritional needs of herds and are resilient and adaptable to environmental changes becomes a priority in breeding programs. Recent studies indicate that genotypes with better adaptation to climatic variations have greater potential to sustain food production in the face of challenges imposed by climate change (Courtney et al., 2023; Cushman et al., 2022; Vermeulen et al., 2012; Hatfield et al., 2011).

In this context, continuous research is essential to develop, evaluate, and select forage materials that are productive, of high quality, resistant to pathogens, and use resources sustainably. This approach aims to increase pasture diversification in livestock production systems, reducing the risks associated with reliance on a few cultivars (Braz et al., 2017; Valle et al., 2017; Braz et al., 2013). Tropical forage breeding programs have played a fundamental role in developing new cultivars, especially through hybridization techniques. In the selection process of superior materials, it is important to evaluate the

performance of genotypes under different environmental conditions (season/year) to ensure experimental precision. This evaluation is fundamental due to the significant impact of the environment on the expression of forage productivity and nutritional value characteristics (Sollenberger et al., 2019; Euclides et al., 2016a).

The use of selection indices can improve the efficiency of this process, addressing multiple characteristics simultaneously to achieve genotypes that closely match the ideotype (Woyann et al., 2020). An ideotype is defined by a set of desirable characteristics that result in efficient adaptation of the plant material to its environment (Debaeke and Quilot-Turion, 2014). For forage plants, productive and nutritional value characteristics, such as height, forage mass, leaf production, crude protein, and in vitro organic matter digestibility, are essential. Several indices have been proposed to select multiple characteristics, including the Factor Analysis and Genotype-Ideotype Distance Selection Index (FAI-BLUP) (Rocha et al., 2018), which uses a mixed-model approach.

The FAI-BLUP index integrates factor analysis and genotype-ideotype design for multiple characteristic selection. The advantages of this method include the correlation structure that considers the data and the direction indicated by the breeder to select genotypes closer to the ideotype (Rocha et al., 2018). The FAI-BLUP methodology is used to predict genetic values from phenotypic data, eliminating the effects of experimental error present in these data (Resende et al., 2014).

Thus, this study aimed to evaluate and select *M. maximus* hybrids with forage potential, considering productive and nutritional value characteristics, during two different climatic seasons, using a mixed-model selection index.

## **2. Materials and methods**

### *2.1. Plant material*

Eighteen genotypes of *Megathyrsus maximus* (PM406, PM407, PM408, PM409, PM411, PM412, PM414, PM415, PM416, PM419, PM420, PM421, PM422, PM425, PM426, PM427, PM428, PM429) from the *M. maximus* breeding program coordinated by Embrapa Gado de Corte were evaluated. Additionally, the cultivars Mombaça and Massai were included as controls due to their widespread adoption in livestock production systems in Brazil (Paciullo and Gomide, 2016). These cultivars are recognized for their agronomic and nutritional characteristics, making them reliable standards for evaluating new forage varieties of the genus *Megathyrsus*.

## 2.2. Experimental design and plant measurements

The experiment was conducted at the Embrapa Dairy Cattle experimental field in Coronel Pacheco, Minas Gerais, Brazil (21°33'22"S and 43°06'15"W, altitude 410 m). The region has a climate classified as Cwa (mesothermal) according to the Köppen-Geiger classification (Peel et al., 2007). Climatic data were collected from the automatic weather station of the National Institute of Meteorology (INMET), located 800 m from the experimental area (Fig. 1). The soil in the experimental area is a dystrophic Red-Yellow Latosol with predominantly clayey texture (Santos et al., 2018). Soil samples were collected from the 0-20 cm layer for chemical characterization before the experiment establishment, presenting the following results: pH (H<sub>2</sub>O), 4.70; Ca, 0.97 cmol<sub>c</sub> dm<sup>-3</sup>; P, 21.3 mg dm<sup>-3</sup>; K, 90.0 mg dm<sup>-3</sup>; Mg, 0.46 cmol<sub>c</sub> dm<sup>-3</sup>; H + Al, 5.28 cmol<sub>c</sub> dm<sup>-3</sup>; Al, 0.4 cmol<sub>c</sub> dm<sup>-3</sup>; effective cation exchange capacity 2.06 cmol<sub>c</sub> dm<sup>-3</sup>; total cation exchange capacity 6.94 cmol<sub>c</sub> dm<sup>-3</sup>; base saturation 23.9%; aluminum saturation 19.4%; base sum 1.66 cmol<sub>c</sub> dm<sup>-3</sup>.

In November 2020, after soil preparation through plowing and harrowing, the genotypes were planted with seeds, together with phosphate fertilization (100 kg ha<sup>-1</sup> of

P<sub>2</sub>O<sub>5</sub>, single superphosphate). The experimental plots, spaced one meter apart, consisted of four rows of four meters with 0.5 m spacing between rows (6.0 m<sup>2</sup>). The useful area of each plot comprised the two central rows, excluding a 0.5 m border strip at the ends. In December 2020, a uniformity cut was made on the plots at a height of 20 cm. In subsequent cuts during the rainy season, all plots were fertilized with 50 kg ha<sup>-1</sup> of N and K<sub>2</sub>O (20-00-20, N-P-K).

The experimental design was a randomized block design with three replications in a split-plot arrangement, where genotypes composed the main plots and the season (rainy and dry) the subplots, totaling 60 experimental plots. The experimental analyses were conducted over two consecutive years (2021/2022). Collections were carried out every four to five weeks during the rainy season and every seven to eight weeks during the dry season. The average canopy height was measured with a graduated ruler in centimeters, in triplicate within the useful area of each plot, choosing random points. The height was measured from the ground level to the curvature of fully expanded leaves. Forage mass was estimated with the aid of a 1.0 x 0.5 m metal frame at one point per plot. The material contained within each square was cut 20 cm from ground level and taken to the laboratory for weighing.

For the evaluation of forage morphological components, a subsample of approximately 400 grams was taken to determine the total forage mass. This subsample was separated into leaf blade, stem (stem + sheath), and dead material fractions, which were weighed and dried in a forced-air circulation oven at 55°C for 72 hours. Forage mass values were converted to kg ha<sup>-1</sup> of dry matter (DM), and morphological components were expressed as a proportion (%) of the total forage mass. Whole plant samples, after drying, were ground and subjected to analyses to determine crude protein, neutral detergent

fiber, acid detergent fiber, lignin, and cellulose contents, as well as in vitro organic matter digestibility, using near-infrared spectroscopy (NIRS) according to Marten et al. (1985).

### 2.3. Statistical analysis

Statistical analyses were performed using R Software version 4.3.3 (R Core Team, 2024), and the data were initially tested for mathematical assumptions with the Shapiro–Wilk and Bartlett tests. The statistical model used was:

$$Y_{ijk} = \mu + b_k + G_i + \theta_{ik} + S_j + (G*S)_{ij} + e_{ijk}$$

in which  $Y_{ijk}$  represents the observation in the  $k$ -th block,  $i$ -th genotype, and  $j$ -th season;  $\mu$  = general mean;  $b_k$  = block effect  $k$ ;  $G_i$  = genotype effect  $i$ ;  $\theta_{ik}$  = random error associated with each block  $k$  in plot  $i$ ;  $S_j$  = fixed season effect  $j$ ;  $(G*S)_{ij}$  = interaction of genotype  $i$  and season  $j$ ;  $e_{ijk}$  = random error assigned to subplot  $Y_{ijk}$ .

Data were subjected to analysis of variance ( $p \leq 0.05$ ) followed by the Skott-Knott mean clustering method using the *SkottKnott* package. To assist in the selection of hybrids that simultaneously present favorable characteristics, the FAI-BLUP Selection Index (Rocha et al., 2018) based on factor analysis was used. This index allows selecting genotypes with the shortest distance to a target ideotype, presented by the formula:

$$P_{ij} = \frac{\frac{1}{d_{ij}}}{\sum_{i=1; j=1}^{i=n; j=m} \frac{1}{d_{ij}}},$$

where  $P_{ij}$  is the probability of the  $i$ -th genotype ( $i = 1, 2, \dots, n$ ) being similar to the  $j$ -th ideotype ( $j = 1, 2, \dots, m$ );  $d_{ij}$  is the standardized mean Euclidean distance between the  $i$ -th genotype and the  $j$ -th ideotype. Principal component analysis, factor analysis, and genotype-ideotype design were performed using the FAI-BLUP index routine developed by Rocha et al. (2018) through R software version 4.3.3 (R Core Team, 2024).

### 3. Results

#### 3.1. Productive and structural characteristics

There was no significant difference for the interaction between genotypes and seasons for canopy height ( $p = 0.653$ ) during the two years evaluated. However, there was a significant genotype effect in the first ( $p < 0.001$ ) and second years ( $p < 0.001$ ) (Table 1). Canopy height also varied with the season of the year ( $p < 0.001$ ), regardless of the evaluation year, with higher heights in the rainy season compared to the dry season (105.9 vs. 85.8 cm and 71.3 vs. 56.0 cm for the first and second years, respectively). Regarding forage mass, there was a significant genotype effect in the first year ( $p < 0.001$ ), but this effect was not observed in the second year ( $p = 0.204$ ) (Table 1). Forage mass was higher in the rainy season (3,802 kg ha<sup>-1</sup>) than in the dry season (2,107 kg ha<sup>-1</sup>) in the first year ( $p < 0.001$ ). In the second year, the same pattern was observed, with higher forage mass in the rainy season (2,747 kg ha<sup>-1</sup>) compared to the dry season (1,160 kg ha<sup>-1</sup>) ( $p < 0.001$ ).

In the first year, leaf and stem masses were influenced by the interaction between genotypes and season ( $p < 0.001$ ). For leaf mass, genotypes PM407, PM408, PM411, PM412, PM414, PM420, PM427, PM429, and the cultivar Mombaça presented higher values than the other genotypes in the rainy season. In the dry season, the materials were divided into two mean groups, and only genotypes PM414 and PM425 did not show differences in leaf mass between seasons (Fig. 2A).

Regarding stem mass, in the first year, the highest value was observed in the dry season for genotypes PM407, PM411, PM415, PM427, and PM429. Stem mass was approximately 4.39 times higher in the dry season compared to the rainy season (Fig. 2B). In the rainy season, both in the first and second years, none of the genotypes showed dead

material in their morphological composition. In the dry season, there was a significant genotype effect ( $p < 0.002$ ) for dead material mass (Fig. 2C).

In the second year, the interactions between genotypes and season were significant for leaf mass ( $p < 0.001$ ). Leaf mass was higher in the rainy season for all genotypes compared to the dry season. Among the genotypes that showed higher leaf mass production, only PM409, PM411, PM412, PM414, PM415, PM416, PM425, PM426, PM427, and the cultivar Mombaça maintained higher leaf mass in both seasons (Fig. 3A). For stem mass, there was a significant genotype effect ( $p < 0.003$ ) (Fig. 3B). Stem mass was higher in the rainy season (274.37 kg ha<sup>-1</sup>), with a significant reduction in the dry season (56.89 kg ha<sup>-1</sup>) of the second year ( $p < 0.004$ ). Regarding dead material mass, there was a significant genotype effect ( $p < 0.001$ ) in the dry season (Fig. 3C).

Leaf percentage showed a significant interaction between genotypes and season in the first year ( $p < 0.001$ ) (Table 2). In the rainy season, the cultivar Massai, along with genotypes PM406, PM407, PM408, PM409, PM412, PM421, PM422, PM425, and PM429, showed the highest leaf percentages. In the dry season, the genotypes were divided into four mean groups, with PM422, PM425, and the cultivar Mombaça showing the highest leaf percentages. Genotypes PM406, PM414, PM419, PM420, PM422, PM425, and the cultivar Mombaça did not show a significant reduction in leaf percentage between seasons.

The interaction between genotype and season ( $p < 0.001$ ) was also significant for stem percentage in the first year (Table 2). In the rainy season, genotypes PM411, PM414, PM415, PM416, PM419, PM420, PM426, PM427, PM428, and the cultivar Mombaça showed approximately 46.0% more stems on average than the group with the lowest percentage of this component. For genotypes PM407, PM408, PM412, PM415, PM427, PM428, PM429, and the cultivar Massai, the average stem percentage was higher in the

dry season (24.84%) compared to the rainy season (8.30%). For the other genotypes, there was no significant difference between seasons (Table 2).

In the first year, the dead material percentage varied with the genotype in the dry season, with genotypes PM407, PM416, PM421, PM426, and the cultivar Massai showing approximately twice as much dead material as the other materials evaluated ( $p < 0.008$ ) (Table 2).

In the second year, the leaf percentage was influenced by the interaction between genotype and season ( $p < 0.04$ ). In the rainy season, the genotypes did not show significant differences in leaf percentage. However, in the dry season, the materials were divided into three mean groups, with genotypes PM426 and the cultivar Massai being the most negatively affected by the dry season during the second year. Additionally, it is relevant to mention that besides PM426 and the cultivar Massai, PM408, PM409, and PM415 were the only genotypes that showed a significant reduction in leaf percentage between seasons in the second year (Table 3).

Regarding stem percentage, there was no significant effect of the interaction between genotype and season ( $p = 0.09$ ). However, there was a significant effect for genotype ( $p < 0.004$ ), with PM406, PM408, PM409, PM411, PM414, PM415, PM416, PM426, PM427, PM428, and the cultivars Massai and Mombaça showing higher stem percentages (12.2%) compared to the other genotypes with (6.6%). Regarding the season factor, there was no significant difference in stem percentage between the rainy season (9.9%) and the dry season (9.9%) ( $p = 0.16$ ).

The dead material percentage had a significant effect for genotype ( $p < 0.04$ ) in the dry season. The genotypes were divided into two mean groups, with the group with the highest dead material percentage showing, on average, approximately 22.0%, while the

group formed by genotypes PM407, PM409, PM411, PM412, PM414, PM416, PM419, PM425, PM427, PM428, and the cultivar Mombaça showed 9.2% dead material (Table 3).

### *3.2. Chemical composition*

There was a significant interaction between genotype and season for crude protein content ( $p < 0.006$ ) and organic matter digestibility ( $p < 0.01$ ) in the first year (Table 4). In the rainy season, there were no significant differences between genotypes for both variables. However, during the dry season, the genotypes were divided into two mean groups. The genotypes in the first group showed, on average, 11.9% crude protein and 61.0% organic matter digestibility, while those in the second group had averages of 9.7% and 55.4%, respectively. The dry season negatively affected crude protein content, with a reduction from 15.7% in the rainy season to 10.8%, on average, in the dry season. The only exception was genotype PM406, which did not show a significant difference in crude protein content between seasons. Organic matter digestibility decreased in the dry season only for genotypes PM408, PM411, PM420, PM421, PM422, PM425, and PM429, with an average reduction of 9.0% between seasons. On the other hand, genotype PM414 showed an opposite effect, with higher organic matter digestibility in the dry season and a significant reduction in the rainy season.

In the second year, there was also a significant interaction between genotypes and season for crude protein ( $p < 0.002$ ) and organic matter digestibility ( $p < 0.01$ ) (Table 5). For crude protein content, there were no significant differences between genotypes in the rainy season. In this season, the highest crude protein contents were observed compared to the dry season, except for genotype PM429, which did not show a significant difference. In the dry season, the genotypes were again divided into two groups, with averages of 9.4% and 7.8%. Organic matter digestibility in the second year also showed a significant

reduction in the dry season compared to the rainy season. In the rainy season, the genotypes were divided into two mean groups; a group with lower digestibility, including genotypes PM421 and PM429, which also showed the lowest digestibilities in the dry season, along with genotype PM408.

There was no significant interaction effect between genotype and season for neutral detergent fiber (NDF) content in the first ( $p = 0.99$ ) and second years ( $p = 0.438$ ). However, there was a significant genotype effect in both years ( $p < 0.002$  in the first year and  $p < 0.005$  in the second year) (Table 6). In both years, the genotypes were divided into two mean groups. In the first year, the means were 74.2% and 71.6%, and in the second year, they were 78.5% and 75.8%. The NDF content also showed a significant season effect in the first year ( $p < 0.001$ ), with a reduction in NDF content in the dry season (71.8%) compared to the rainy season (73.8%). In the second year, there was a reversal ( $p < 0.001$ ), with lower NDF content in the rainy season (76.1%) and an increase in the dry season (77.7%).

Regarding acid detergent fiber (ADF) content, there was no significant interaction effect between genotype and season ( $p = 0.564$ ) in both years (Table 6). However, in the second year, the season factor showed a significant effect ( $p < 0.001$ ), with an increase in ADF percentage in the rainy season (41.1%) compared to the dry season (38.7%). In the first year, the ADF content between seasons did not show a significant difference ( $p = 0.121$ ).

Regarding lignin percentage, a significant interaction was observed between genotype and season, both in the first ( $p < 0.001$ ) and second years ( $p < 0.008$ ) (Table 7). In the first year, both in the rainy and dry seasons, the lignin percentage was divided into two mean groups. The groups showed averages of 4.2% and 3.9% in the rainy season and 3.5% and 3.1% in the dry season. The dry season negatively influenced the lignin

percentage, resulting in a considerable reduction of this component in all genotypes. The averages decreased from 4.1% in the rainy season to 3.4% in the dry season.

In the second year, there were no significant differences between genotypes for lignin percentage in the rainy season. However, in the dry season, the genotypes were classified into three mean groups. Unlike the first year, in the second year, there were no significant differences between seasons for most genotypes. The genotypes that showed a reduction in lignin percentage in the dry season were PM407, PM409, PM412, PM414, PM419, PM421, and PM425.

There was no significant interaction effect between genotype and season for cellulose content in the first year ( $p = 0.054$ ). However, there was a significant genotype effect ( $p < 0.001$ ). The genotypes were divided into two mean groups, with the group with the highest cellulose percentage showing, on average, approximately 34.7%, while the group formed by genotypes PM406, PM407, PM411, PM419, PM420, PM426, PM428, and the cultivar Mombaça showed, on average, 33.0% cellulose. Cellulose content also showed a significant season effect in the first year ( $p < 0.001$ ), with a reduction in cellulose content in the dry season (33.5%) compared to the rainy season (34.1%). In the second year, there was a significant interaction between genotypes and season for cellulose content ( $p < 0.03$ ). In the rainy season, the materials were divided into three mean groups, with genotype PM429 showing the highest cellulose content. However, in the dry season, the genotypes did not show significant differences. Additionally, it is important to mention that the dry season influenced the reduction of cellulose content in all materials compared to the rainy season (Table 7).

### 3.3. FAI-BLUP index

In the exploratory factor analysis, it was observed that during the rainy season, the first four principal components had eigenvalues greater than one. The total variability

explained by these components reached 87%, indicating that four factors are sufficient to represent most of the observed variability. In the dry season, the first three principal components also had eigenvalues greater than one, explaining more than 78% of the total variability.

Using the FAI-BLUP index, it was possible to classify the genotypes based on multiple characteristics, minimizing multicollinearity and considering both positive and negative genetic correlations between characteristics within each factor. This approach allowed maintaining the relationships between characteristics and enabling the adoption of biological meaning for the ideotypes. During the rainy season, four main factors were identified, while in the dry season, there were three. In the rainy season, the factors were defined as follows: FA1 - Crude Protein, ADF, Organic Matter Digestibility, Lignin, and Cellulose; FA2 - Forage Mass and Leaf Mass; FA3 - Height; FA4 - Stem Mass, NDF, and Leaf and Stem Percentage. In the dry season, FA1 included Crude Protein, NDF, ADF, Organic Matter Digestibility, and Lignin; FA2 - Forage Mass, Stem Mass, Lignin, and Leaf and Stem Percentage; and FA3 - Height and Leaf Mass.

The ideotypes formed based on the defined factors totaled 16 in the rainy season and 8 in the dry season. The predicted genetic gain for the first factor in the rainy season, considering the FAI-BLUP index, allowed obtaining gains in the desired direction for the characteristics crude protein (1.29%) and organic matter digestibility (1.21%). The predicted gains for the second and third factors were in the desired direction for all characteristics: forage mass (1.41%), leaf mass (7.02%), and height (0.61%), with the highest genetic gains observed for leaf mass. For the fourth factor, the index predicted a favorable gain only for leaf percentage (0.79%).

In the dry season, the predicted genetic gain for the first factor allowed positive gains for crude protein (10.61%) and organic matter digestibility (4.39%). For the second

factor, the gains in the favorable direction were for the characteristics forage mass (2.77%), leaf percentage (4.16%), and lignin (0.39%). For the third factor, the index predicted a favorable gain for both characteristics, height (6.0%) and leaf mass (24.77%).

Finally, from the genotype-ideotype distance, among the 20 materials evaluated, four stood out in each period: PM411, PM407, PM426, and PM412 during the rainy season, and PM406, PM414, PM419, and the cultivar Mombaça in the dry season. The index values for these genotypes were respectively 0.0672, 0.0629, 0.0577, and 0.0534 in the rainy season, and 0.1850, 0.1846, 0.1454, and 0.1592 in the dry season, demonstrating favorable phenotypes for the characteristics of interest.

#### **4. Discussion**

In our study, the use of the FAI-BLUP selection index through ideotype design allowed ranking genotypes considering multiple characteristics simultaneously, enabling the identification of genotypes with better performance, stability, and expected gain with selection.

By employing the concept of factor analysis to analyze the performance of genotypes, selection was conducted based on predicted genetic values and spatial probability based on genotype-ideotype distance (Rocha et al., 2018). This allowed classifying the best genotypes in relation to the ideotype designed based on productive and nutritional value characteristics necessary for higher productivity and forage quality. Desirable characteristics included greater height, forage mass, leaf mass, crude protein content, and organic matter digestibility.

Selecting genotypes close to the ideotype was crucial to define more productive and higher-quality forage materials in different seasons. The results showed that during the dry season, all genotypes exhibited a reduction in both canopy height and forage mass compared to the rainy season. The observed seasonal variation, with significantly higher

values in the rainy season, aligns with the typical response of tropical forage grasses to seasonal climatic patterns (Sbrissia et al., 2020; Da Silva et al., 2015; Gastal and Lemaire, 2015; Bircham and Hodgson, 1983).

It is important to highlight that these results indicate a compensation between lower canopy height and higher forage mass (Table 1), especially for genotype PM407, selected by the FAI-BLUP index in the rainy season. This response pattern may seem contradictory compared to expectations based on previous studies (Sbrissia et al., 2018; Berone et al., 2007). However, there is a compensation relationship between tiller size and density, where canopies with smaller tillers may exhibit higher density, resulting in high productivities due to the greater population of tillers in the area (Sbrissia et al., 2003).

Since the forages were subjected to the same edaphoclimatic and management conditions, the most likely explanation for the lower canopy height of some genotypes is related to the genetic characteristic that defines the more limited height growth of these materials. The analysis of forage mass production revealed that despite the apparent lower height, these genotypes managed to optimize production efficiency. This suggests a growth strategy characterized by the priority allocation of photoassimilates to the aerial part, especially for the formation of new tillers.

Additionally, it is important to consider aspects such as tiller density, number of leaves per tiller, and leaf size, which are determinants in the leaf area index (LAI). In forage plants, leaf size is mainly influenced by blade length, while the number of leaves per tiller remains relatively stable, making tiller population density the component most susceptible to changes in LAI (Matthew et al., 2000). Tiller density results from the balance between tiller emergence and death, influencing tiller population stability and consequently modifying pasture structure and leaf area photosynthetic efficiency

(Bahmani et al., 2003; Da Silva et al., 2008). The results found are similar to those reported by Fernandes et al. (2014), who observed high forage mass production in the Massai cultivar (18.9 tons ha<sup>-1</sup> year<sup>-1</sup>), despite its small size.

The results in the two seasons revealed distinct patterns of leaf, stem, and dead material mass production among the forage genotypes, providing important information for the careful selection of these materials under different environmental conditions. These responses influenced the selection index, which indicated different materials for the two seasons. Additionally, it is important to highlight that the highest favorable genetic gain was obtained for leaf mass, emphasizing the importance of this characteristic, as forage materials with a higher proportion of leaf mass compared to stem and dead material are desirable due to the nutritional importance of leaves in ruminant diets (Souza et al., 2021; Martuscello et al., 2007).

It is important to note that materials that maintain leaf mass production in the dry season at the expense of other morphological components may be associated with specific adaptive strategies of these materials to face adverse conditions of the dry season, directing resources for plant survival and maintenance (Verslues et al., 2006; Santos et al., 2013). Additionally, these results highlight the importance of considering not only the total forage mass produced but also the morphological composition, especially in terms of leaves and stems. The choice of genotypes that maintain substantial leaf mass production even during the dry season can contribute to ensuring a consistent supply of quality forage, contributing to the food security of animals in pasture-based production systems (Voltonini et al., 2010; Fonseca et al., 2013; Silva et al., 2019).

The variation in morphological composition over the two years, in both seasons, highlights the plasticity of these genotypes and cultivars in response to environmental variations (De Kroon et al., 2005). In the rainy season, materials with a higher percentage

of leaves and lower percentage of stems indicate a more efficient adaptation to maximize leaf biomass production during favorable conditions. In the dry season, materials exhibiting a predominance of leaves reinforce the consistency of these genotypes in adaptation strategies, especially to water stress. This consistency is notable, particularly in the second year, when there was low rainfall, with only 25.56 mm during the period in question (Fig. 1). Additionally, this adaptation is supported by morphological and physiological characteristics that favor the survival and performance of these genotypes in diverse environments (Buxton and Fales, 1994).

The results obtained in this study corroborate and expand the scientific literature's observations regarding the morphological composition of *M. maximus* forage materials. According to Jank et al. (2010), genotypes of this species generally exhibit a high percentage of leaves, with values consistently above 80%. This characteristic is crucial for the nutritional quality of forage, as leaves, as highlighted by Martuscello et al. (2007) and Benvenuti et al. (2008), offer better bromatological composition and higher digestibility compared to the stem component.

In this context, the genotypes selected by the FAI-BLUP index showed remarkable results, converging with the observations of Jank et al. (2010) and Fernandes et al. (2014) regarding the leaf percentage in their morphological composition. Over the two years of evaluation and in both seasons, the genotypes exhibited high leaf percentages. Except for genotypes PM406 and PM414, which showed leaf percentages below 80%, it is important to note that even under atypical environmental conditions (Fig. 1), there was consistency in the leaf proportion, highlighting the robustness of these genotypes in maintaining a favorable morphological profile regardless of seasonal variations.

The analysis of crude protein content of the materials throughout the experimental period revealed distinct patterns according to the season. In the rainy season of both

years, there were no significant differences between genotypes (Tables 4 and 5). However, in the dry season, crude protein contents varied among genotypes in both years. Genotypes PM406, PM407, PM415, PM416, PM419, PM426, PM427, PM428, along with the cultivars Massai and Mombaça, showed higher crude protein contents during the dry season in both experimental years. This differential response highlights the genetic influence on the ability to maintain higher crude protein levels under challenging conditions such as the dry season. Additionally, a general reduction in crude protein percentage was observed between seasons, with this decrease being particularly significant during the dry season. This phenomenon can be attributed to several factors, including the increase in NDF, which contributes to the dilution of crude protein concerning total dry matter (Euclides et al., 2016b).

The pattern of protein reduction in the dry season compared to the rainy season is corroborated by the results of Da Silva et al. (2020), who also observed a difference in crude protein contents in the Mombaça cultivar depending on the season. This reveals the strong influence of seasonal conditions on the responses of these forages regarding crude protein contents.

The results obtained for organic matter digestibility provide important insights into the genotypes' response to seasonal variations and their interactions with other nutritional parameters. The absence of a significant difference between genotypes in the rainy season of the first year suggests relative stability in organic matter digestibility under more favorable environmental conditions (Table 4). However, the reduction observed in the second year for PM429 and PM421 may be related to annual variations in climatic conditions, such as the reduction in average temperature and precipitation (Fig. 1).

Additionally, there was a significant positive correlation between organic matter digestibility and crude protein in both years, mainly in the dry season (1st year: 0.831;  $p < 0.0001$ ; 2nd year: 0.501;  $p < 0.0001$ ), reinforcing the interdependence of these two key parameters in forage plant nutritional quality. It was expected that organic matter digestibility would show a negative correlation with the fibrous fractions of the plant (NDF, ADF, lignin, and cellulose), and the results corroborate those of Fernandes et al. (2014) when evaluating the nutritional value of *M. maximus* genotypes. In the first year, a high and negative correlation was observed with NDF (-0.786,  $p < 0.001$ ), ADF (-0.839,  $p < 0.001$ ), and lignin (-0.843,  $p < 0.001$ ), as well as with cellulose (-0.611,  $p < 0.001$ ). In the second year, this relationship persisted, with the following correlations: NDF (-0.637,  $p < 0.001$ ), ADF (-0.572,  $p < 0.001$ ), lignin (-0.798,  $p < 0.001$ ), and cellulose (-0.158,  $p < 0.001$ ). These results indicate that genotypes with higher fiber contents show a reduction in digestibility, compromising the efficiency of nutrient utilization by ruminal microorganisms (Akin, 1989; Wilson and Mertens, 1995).

The correlation between high nutritional value and low dry matter yield, evidenced by the negative correlation with fibrous fractions, highlights the complexity of the interactions between nutritional quality and forage production of *M. maximus*. This relationship shows that genotypes with higher digestibility tend to have lower fiber content, contributing to the forage's nutritional quality. However, exploring the correlation between organic matter digestibility and forage mass production revealed a moderate and positive correlation (0.381;  $p < 0.002$ ). This suggests that although there is a general tendency for genotypes with higher digestibility to have lower foliar yield, this relationship is not directly proportional.

This complexity in adaptive responses is crucial when considering genotype selection to meet specific pasture system demands, indicating that it is possible to select

forages with higher organic matter digestibility without compromising forage mass yield (Stabile et al., 2012). This strategic approach in genotype selection highlights the importance of considering not only nutritional quality but also forage productivity.

The four genotypes selected for the seasons among the 20 evaluated materials showed satisfactory selection gains for all characteristics based on the combination of desirable traits. Thus, the selection index was able to select more productive and higher-quality genotypes in different environmental conditions. Using the selection index allows shortening the time required for efficient genotype selection, enabling accurate selection through measurements on each plant and prediction of their genotypic values. The highest predicted genetic gains were for leaf mass, a characteristic that influences both forage productivity and material quality.

## **5. Conclusion**

The FAI-BLUP index indicated genotypes PM411, PM407, PM426, and PM412 for the rainy season, and genotypes PM406, PM414, PM419, and the cultivar Mombaça for the dry season, these being genotypes with higher forage potential based on multiple characteristics, without weight assignment, free from multicollinearity, and with balanced genetic gains observed in the desirable direction for all evaluated characteristics.

## **Acknowledgments**

The authors thanks the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, Brazil) for the financial support. The Embrapa Gado de Leite (Minas Gerais, Brazil) for providing the necessary infrastructure to carry out this project.

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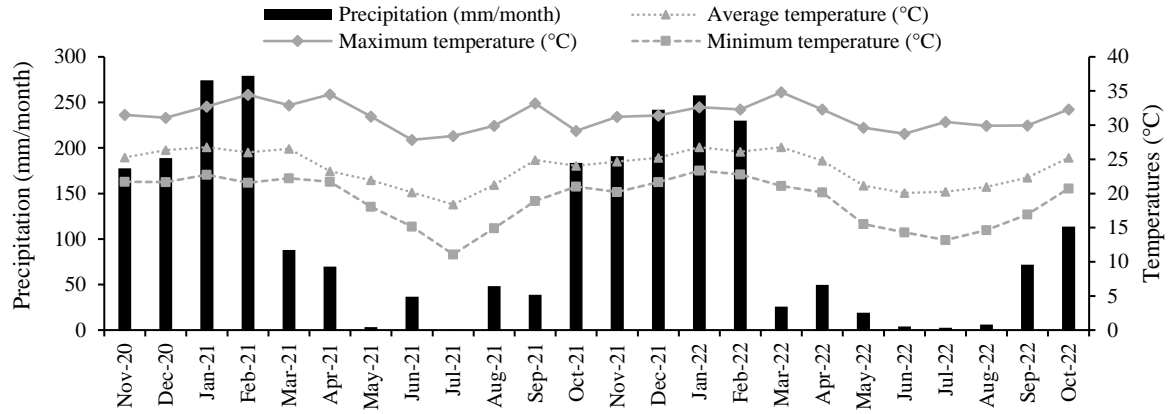
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## Tables and figures

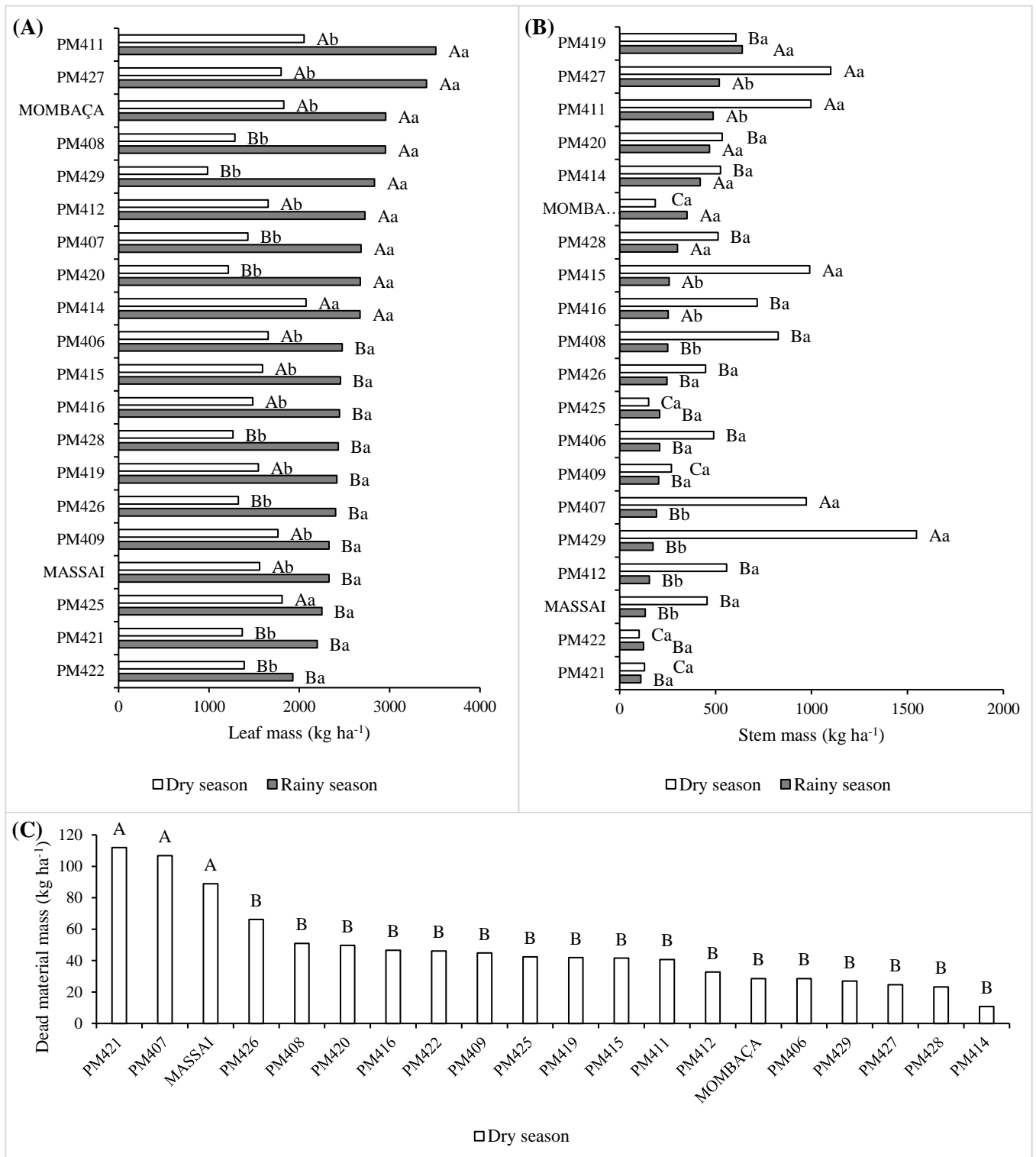


**Figure 1.** Precipitation and average minimum and maximum temperatures during the experimental period. (Data from INMET, Station: Coronel Pacheco - A557).

**Table 1.** Conopy height (cm) and herbage mass (kg ha<sup>-1</sup>) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in two years (1<sup>st</sup> year - 2020/2021 and 2<sup>nd</sup> year - 2021/2022)

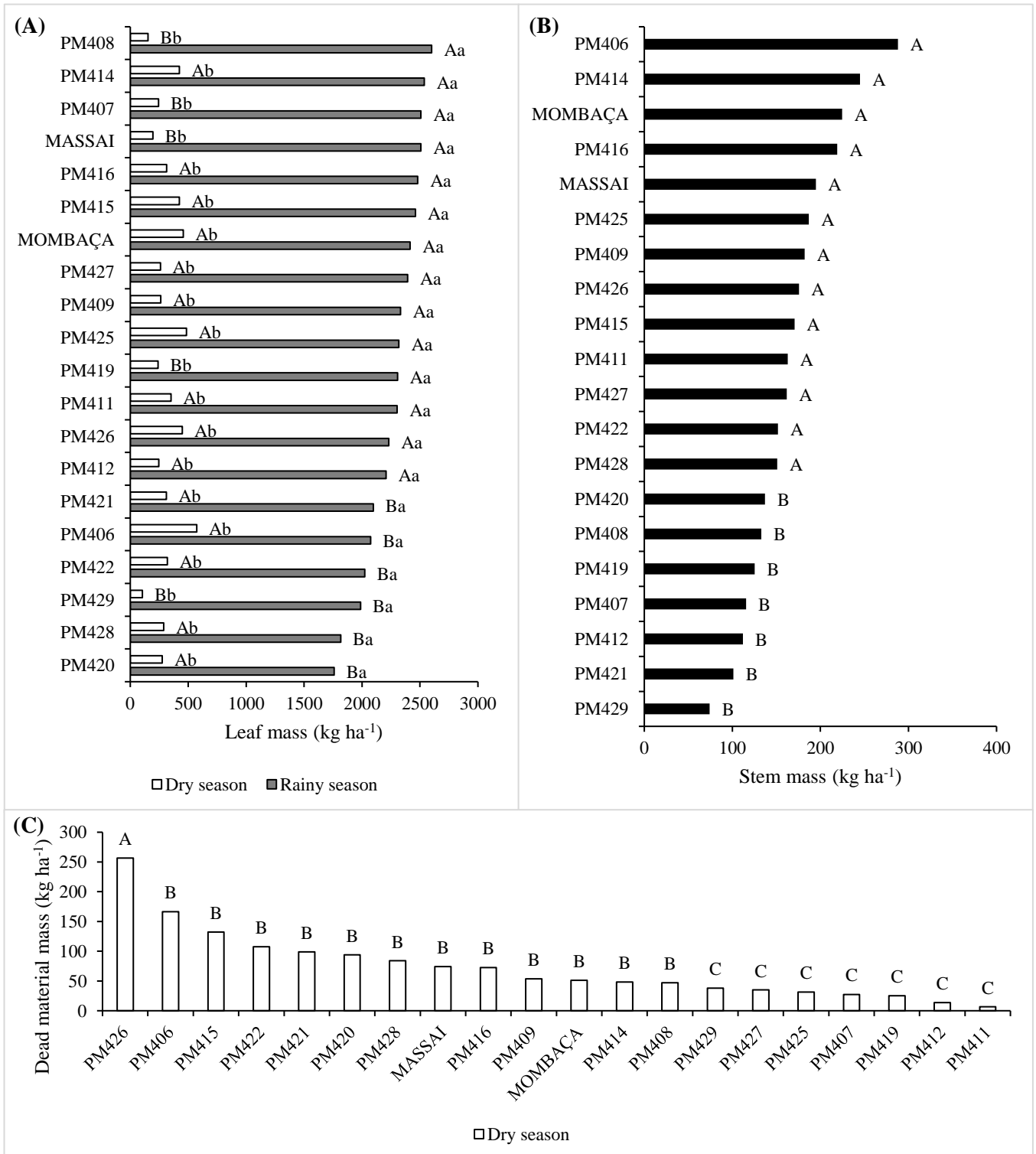
Genotypes	Conopy height		Herbage mass	
	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year
MASSAI	85.5 B	71.1 B	2950 A	2276
MOMBAÇA	94.7 A	76.5 A	2760 B	1949
PM406	92.7 A	75.5 A	2909 B	1979
PM407	81.6 B	68.8 B	3075 A	2154
PM408	78.6 C	62.8 B	3142 A	2091
PM409	91.4 A	70.6 B	3111 A	1950
PM411	99.2 A	66.8 B	3511 A	1853
PM412	95.5 A	78.4 A	2780 B	1966
PM414	91.9 A	74.6 A	3280 A	2312
PM415	95.0 A	73.3 A	3305 A	1987
PM416	85.5 B	73.6 A	2861 B	2188
PM419	89.4 A	68.3 B	2958 A	1770
PM420	75.5 C	60.6 B	2640 B	1572
PM421	85.0 B	66.9 B	2701 B	1823
PM422	83.6 B	68.0 B	2597 B	1684
PM425	97.7 A	79.2 A	2908 B	1835
PM426	84.4 B	69.6 B	2704 B	2016
PM427	99.1 A	76.3 A	3564 A	2044
PM428	81.9 B	70.4 B	2542 B	1720
PM429	83.8 B	66.7 B	2793 B	1898

Different letters in the column indicate difference by Scott Knott's cluster test ( $p < 0.05$ ).



**Figure 2.** (A) Leaf mass (kg ha<sup>-1</sup>) and (B) stem mass (kg ha<sup>-1</sup>) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in two seasons (rainy and dry season) in the 1<sup>st</sup> year; (C) Dead material mass (kg ha<sup>-1</sup>) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in dry season and in the 1<sup>st</sup> year.

(A) and (B) Uppercase letters compare genotypes and lowercase letters compare seasons using the Skott Knott cluster test ( $p < 0.05$ ); (C) Uppercase letters compare genotypes using the Skott Knott cluster test ( $p < 0.05$ ).



**Figure 3.** (A) Leaf mass (kg ha<sup>-1</sup>) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in two seasons (rainy and dry season) in the 2<sup>nd</sup> year; (B) stem mass (kg ha<sup>-1</sup>) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in the 2<sup>nd</sup> year; (C) Dead material mass (kg ha<sup>-1</sup>) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça in dry season and in the 2<sup>nd</sup> year.

(A) Uppercase letters compare genotypes and lowercase letters compare seasons using the Skott Knott cluster test ( $p < 0.05$ ); (B) and (C) Uppercase letters compare genotypes using the Skott Knott cluster test ( $p < 0.05$ ).

**Table 2.** Leaf (%), stem (%), and dead material (%) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça, two seasons (rainy and dry season), and in the 1<sup>st</sup> year

Genotypes	Leaf (%)		Stem (%)		Dead material (%)
	Rainy season	Dry season	Rainy season	Dry season	Dry season
MASSAI	94.7 Aa	76.7 Cb	5.3 Bb	18.6 Ba	4.7 A
MOMBAÇA	89.5 Ba	90.7 Aa	10.4 Aa	6.4 Ca	2.9 B
PM406	92.0 Aa	85.3 Ba	7.9 Ba	12.4 Ba	2.2 B
PM407	93.2 Aa	64.9 Db	6.8 Bb	26.9 Aa	8.1 A
PM408	92.3 Aa	68.7 Db	7.7 Bb	28.3 Aa	2.9 B
PM409	91.9 Aa	84.3 Bb	8.1 Ba	11.8 Ba	3.6 B
PM411	88.7 Ba	79.1 Cb	11.2 Aa	18.6 Ba	2.4 B
PM412	94.6 Aa	80.8 Bb	5.4 Bb	16.5 Ba	2.7 B
PM414	86.5 Ba	83.9 Ba	13.5 Aa	15.5 Ba	0.5 B
PM415	90.4 Ba	72.8 Cb	9.6 Ab	23.6 Aa	3.6 B
PM416	90.3 Ba	77.3 Cb	9.7 Aa	17.8 Ba	4.9 A
PM419	79.5 Ba	81.4 Ba	20.5 Aa	15.7 Ba	2.9 B
PM420	85.6 Ba	77.9 Ca	14.4 Aa	18.2 Ba	3.9 B
PM421	95.4 Aa	87.7 Bb	4.6 Ba	5.6 Ca	6.7 A
PM422	93.8 Aa	91.7 Aa	6.2 Ba	4.8 Ca	3.4 B
PM425	91.6 Aa	92.2 Aa	8.4 Ba	5.5 Ca	2.3 B
PM426	90.6 Ba	77.9 Cb	9.4 Aa	16.7 Ba	5.4 A
PM427	87.6 Ba	74.7 Cb	12.4 Ab	23.6 Aa	1.6 B
PM428	89.2 Ba	79.0 Cb	10.8 Ab	19.2 Ba	1.8 B
PM429	94.5 Aa	60.7 Db	5.5 Bb	35.7 Aa	3.6 B

Means followed by a different uppercase letter in the column and lowercase letter in the row differ by Scott Knott's cluster test ( $p < 0.05$ ).

**Table 3.** Leaf (%), and dead material (%) of *Megathyrsus maximus* genotypes and cvs. Massai and Mombaça, two seasons (rainy and dry season), and in the 2<sup>nd</sup> year

Genotypes	Leaf (%)		Dead material (%)
	Rainy season	Dry season	Dry season
MASSAI	88.8 Aa	55.5 Cb	22.1 A
MOMBAÇA	87.2 Aa	82.9 Aa	8.8 B
PM406	85.1 Aa	71.2 Ba	15.9 A
PM407	91.6 Aa	87.6 Aa	9.4 B
PM408	91.6 Aa	70.4 Bb	21.8 A
PM409	89.8 Aa	68.9 Bb	12.9 B
PM411	90.3 Aa	85.9 Aa	1.6 B
PM412	91.3 Aa	90.7 Aa	5.6 B
PM414	87.5 Aa	76.0 Ba	8.4 B
PM415	90.5 Aa	71.1 Bb	21.5 A
PM416	89.3 Aa	79.7 Aa	13.3 B
PM419	91.5 Aa	89.9 Aa	9.4 B
PM420	87.1 Aa	72.5 Ba	24.1 A
PM421	91.4 Aa	77.7 Ba	20.3 A
PM422	88.5 Aa	77.0 Ba	20.3 A
PM425	87.3 Aa	92.5 Aa	5.8 B
PM426	90.4 Aa	59.5 Cb	30.4 A
PM427	90.5 Aa	82.4 Aa	10.8 B
PM428	87.6 Aa	83.4 Aa	15.2 B
PM429	94.5 Aa	74.0 Bb	21.7 A

Means followed by a different uppercase letter in the column and lowercase letter in the row differ by Scott Knott's cluster test ( $p < 0.05$ ).

**Table 4.** Chemical composition, crude protein (%), and digestibility of organic matter (%) of *Megathyrus maximus* genotypes and cvs. Massai and Mombaça, two seasons (rainy and dry season), and in the 1<sup>st</sup> year

Genotypes	Crude Protein		Digestibility of OM	
	Rainy season	Dry season	Rainy season	Dry season
MASSAI	15.0 Aa	12.4 Ab	60.2 Aa	60.4 Aa
MOMBAÇA	16.5 Aa	11.7 Ab	61.1 Aa	59.9 Aa
PM406	15.3 Aa	13.9 Aa	62.5 Aa	63.9 Aa
PM407	16.1 Aa	12.8 Ab	61.5 Aa	61.1 Aa
PM408	15.9 Aa	10.7 Bb	60.9 Aa	57.3 Bb
PM409	15.4 Aa	9.6 Bb	60.7 Aa	60.3 Aa
PM411	15.1 Aa	9.7 Bb	61.5 Aa	50.1 Bb
PM412	14.8 Aa	9.3 Bb	60.2 Aa	57.8 Ba
PM414	15.5 Aa	12.2 Ab	59.1 Ab	62.9 Aa
PM415	15.3 Aa	11.1 Ab	60.8 Aa	61.2 Aa
PM416	15.2 Aa	9.7 Ab	61.3 Aa	59.9 Aa
PM419	16.6 Aa	12.6 Ab	61.3 Aa	62.8 Aa
PM420	16.9 Aa	10.1 Bb	62.5 Aa	58.9 Ab
PM421	15.8 Aa	10.1 Bb	60.1 Aa	55.4 Bb
PM422	16.4 Aa	9.6 Bb	64.4 Aa	57.2 Bb
PM425	15.9 Aa	9.6 Bb	63.5 Aa	59.4 Ab
PM426	16.9 Aa	11.7 Ab	61.9 Aa	61.8 Aa
PM427	15.0 Aa	11.8 Ab	60.9 Aa	59.8 Aa
PM428	16.1 Aa	11.4 Ab	60.3 Aa	59.9 Aa
PM429	14.6 Aa	9.0 Bb	58.9 Aa	54.5 Bb

Means followed by a different uppercase letter in the column and lowercase letter in the row differ by Skott Knott cluster test ( $p < 0.05$ ).

**Table 5.** Chemical composition, crude protein (%), and digestibility of organic matter (%) of *Megathyrus maximus* genotypes and cvs. Massai and Mombaça, two seasons (rainy and dry season), and in the 2<sup>nd</sup> year

Genotypes	Crude Protein		Digestibility of OM	
	Rainy season	Dry season	Rainy season	Dry season
MASSAI	12.7 Aa	9.5 Ab	59.8 Aa	47.0 Cb
MOMBAÇA	12.9 Aa	9.2 Ab	61.6 Aa	49.8 Bb
PM406	13.7 Aa	10.2 Ab	62.7 Aa	52.5 Ab
PM407	13.8 Aa	10.7 Ab	60.6 Aa	48.9 Bb
PM408	12.9 Aa	8.2 Bb	60.3 Aa	42.3 Db
PM409	11.1 Aa	7.5 Bb	59.4 Aa	49.0 Bb
PM411	10.6 Aa	6.5 Bb	60.8 Aa	47.9 Cb
PM412	12.0 Aa	9.5 Ab	60.8 Aa	48.9 Bb
PM414	12.5 Aa	8.3 Bb	60.6 Aa	49.5 Bb
PM415	12.8 Aa	9.4 Ab	62.0 Aa	49.9 Bb
PM416	12.4 Aa	8.8 Ab	61.2 Aa	49.9 Bb
PM419	13.2 Aa	8.8 Ab	61.2 Aa	49.2 Bb
PM420	12.8 Aa	8.2 Bb	60.0 Aa	46.4 Cb
PM421	13.2 Aa	9.0 Ab	53.9 Ba	44.7 Db
PM422	13.2 Aa	7.5 Bb	61.8 Aa	45.9 Cb
PM425	12.8 Aa	7.6 Bb	61.4 Aa	54.2 Ab
PM426	13.8 Aa	9.3 Ab	63.3 Aa	52.1 Ab
PM427	12.2 Aa	9.0 Ab	59.2 Aa	47.7 Cb
PM428	13.6 Aa	8.9 Ab	61.4 Aa	47.6 Cb
PM429	10.9 Aa	9.9 Aa	56.0 Ba	44.0 Db

Means followed by a different uppercase letter in the column and lowercase letter in the row differ by Skott Knott cluster test ( $p < 0.05$ ).

**Table 6.** Chemical composition, neutral detergent fiber (%) and acid detergent fiber (%) of *Megathyrus maximus* genotypes and cvs. Massai and Mombaça in two years (1<sup>st</sup> year - 2020/2021 and 2<sup>nd</sup> year - 2021/2022)

Genotypes	NDF		ADF	
	1 <sup>st</sup> year	2 <sup>nd</sup> year	1 <sup>st</sup> year	2 <sup>nd</sup> year
MASSAI	74.3 A	77.4 A	38.0	38.9
MOMBAÇA	71.3 B	75.7 B	38.8	40.6
PM406	70.8 B	75.4 B	37.9	39.9
PM407	73.2 A	77.0 B	37.4	38.0
PM408	74.3 A	78.1 A	38.5	40.3
PM409	73.3 A	77.9 A	39.5	40.9
PM411	71.3 B	77.5 A	38.9	39.7
PM412	73.9 A	75.9 B	40.4	40.7
PM414	71.9 B	75.8 B	38.4	39.9
PM415	72.3 B	75.4 B	39.6	40.9
PM416	72.7 B	75.7 B	38.8	40.7
PM419	71.8 B	76.6 B	36.5	38.4
PM420	71.4 B	75.7 B	37.6	39.2
PM421	73.7 A	78.7 A	39.4	40.1
PM422	74.6 A	79.0 A	38.7	39.7
PM425	74.5 A	78.9 A	39.4	39.9
PM426	70.7 B	74.1 B	37.5	38.6
PM427	71.9 B	75.6 B	38.7	40.1
PM428	71.7 B	76.4 B	38.6	39.9
PM429	76.2 A	80.6 A	40.0	41.1

Different letters in the column indicate difference by Scott Knott's cluster test ( $p < 0.05$ ).

**Table 7.** Chemical composition, lignin (%) and cellulose (%) of *Megathyrus maximus* genotypes and cvs. Massai and Mombaça, two seasons (rainy and dry season), and in two years

Genotypes	Lignin*		Lignin**		Cellulose**	
	Rainy season	Dry season	Rainy season	Dry season	Rainy season	Dry season
MASSAI	4.06 Ba	3.46 Ab	4.23 Aa	4.13 Aa	37.03 Ba	32.16 Ab
MOMBAÇA	4.13 Aa	3.46 Ab	4.13 Aa	3.80 Aa	36.80 Ca	33.13 Ab
PM406	4.00 Ba	3.46 Ab	4.10 Aa	3.86 Aa	36.06 Ca	33.86 Ab
PM407	4.00 Ba	3.33 Ab	4.23 Aa	3.56 Bb	36.16 Ca	31.66 Ab
PM408	4.13 Aa	3.80 Ab	4.10 Aa	4.50 Aa	36.66 Ca	34.40 Ab
PM409	4.13 Aa	3.13 Bb	4.20 Aa	3.60 Bb	37.86 Ba	33.96 Ab
PM411	4.00 Ba	3.23 Bb	3.83 Aa	3.70 Ba	36.93 Ba	33.86 Ab
PM412	4.30 Aa	3.50 Ab	4.20 Aa	3.63 Bb	37.40 Ba	32.43 Ab
PM414	4.33 Aa	3.23 Bb	4.33 Aa	3.66 Bb	37.73 Ba	33.66 Ab
PM415	4.30 Aa	3.36 Ab	4.33 Aa	4.00 Aa	37.36 Ba	33.56 Ab
PM416	4.16 Aa	3.13 Bb	4.30 Aa	3.90 Aa	37.36 Ba	33.70 Ab
PM419	4.00 Ba	2.86 Bb	4.10 Aa	3.30 Cb	36.10 Ca	30.86 Ab
PM420	3.90 Ba	3.16 Bb	4.00 Aa	3.73 Ba	35.00 Ca	31.76 Ab
PM421	4.23 Aa	3.60 Ab	4.50 Aa	3.96 Ab	36.63 Ca	33.06 Ab
PM422	3.83 Ba	3.36 Ab	4.00 Aa	3.83 Aa	37.00 Ba	34.36 Ab
PM425	3.93 Ba	3.53 Ab	4.20 Aa	3.03 Cb	37.23 Ba	33.53 Ab
PM426	4.06 Ba	3.10 Bb	3.86 Aa	3.50 Ba	35.90 Ca	32.16 Ab
PM427	4.06 Ba	3.50 Ab	4.26 Aa	3.93 Aa	37.23 Ba	32.76 Ab
PM428	4.20 Aa	3.26 Bb	4.10 Aa	3.83 Aa	36.13 Ca	33.16 Ab
PM429	4.16 Aa	3.60 Ab	4.46 Aa	4.06 Aa	38.13 Aa	33.00 Ab

Means followed by a different uppercase letter in the column and lowercase letter in the row differ by Skott Knott cluster test ( $p < 0.05$ ).

\*1<sup>st</sup> year - 2020/2021; \*\*2<sup>nd</sup> year - 2021/2022.

**4CAPÍTULO III - Manuscript: Productive and morphological responses of *Megathyrus maximus* hybrids submitted to water deficit and waterlogging**

Este artigo foi elaborado conforme as normas do periódico "*Grass and Forage Science*".

## **Productive and morphological responses of *Megathyrus maximus* hybrids submitted to water deficit and waterlogging**

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**ABSTRACT** - This study aimed to assess the variability and response mechanisms of hybrids of *Megathyrus maximus* to water deficit and waterlogging. The experiment was carried out in a greenhouse using a randomized block design with an 8×3×2 factorial arrangement. Treatments were the combination of eight genotypes of *M. maximus*, three water conditions (control, waterlogging, and water deficit), and two harvest times (1st harvest: after 20 days of water stress; 2nd harvest: 12 days after recovery period). Productive and morphological traits were evaluated at the end of the stress period and after the recovery period. All hybrids submitted to water deficit showed reduced aerial and root mass. However, after the stress period, a survival response to the water deficit was observed, with the recovery of approximately 28.4% of the aerial part and 16.3% of the root system in all genotypes. The hybrid PM409 showed an adaptive potential to waterlogging conditions, as suggested by the greater root production (41.2%) observed in this hybrid compared to the average production of other genotypes. In addition, there was no reduction on leaf and stem mass production compared to the control condition. The results suggest that no material showed to be tolerant to water deficit. On the other hand, the hybrid PM409 showed promising traits for waterlogging stress tolerance.

**Keywords:** biomass production, root recovery index, root to shoot ratio, root volume

## 1. INTRODUCTION

The increasing concern about climate change projections has raised a growing awareness within the scientific community, mainly due the impacts that such changes can impose on various productive sectors, with emphasis on agriculture and livestock (IPCC, 2014). Among the extreme events predicted, the occurrence of intense droughts and heavy rains is expected to become more frequent, representing a major challenge for the agricultural sector in Brazil (OCHA, 2020; IPCC, 2018).

In cattle production systems, where pastures play an important role, vulnerability to climate risks is particularly evident (Lesk et al., 2016). In a study addressing different scenarios related to climate change, Pezzopane et al. (2017) highlight the increasing seasonality in the production of *Megathyrus maximus* cv. Tanzania, with a focus on the challenges associated with soil water availability.

Given this context, the search for strategies that mitigate the impacts of water stress on pastures is highlighted. Forage cultivars tolerant to water deficit and waterlogging emerge as a promising approach to minimize adverse effects on forage production and the performance of animals kept on pasture (Euclides et al., 2016). The importance of this approach is even more evident considering that degraded pastures, one of the main obstacles to pasture-based production systems in the tropics, are often associated with abiotic stresses (Dias-Filho, 2005; Dias-Filho and Andrade, 2005; Teixeira Neto et al., 2000).

However, besides immediate tolerance to stress, the plant's capacity for recovery after the stress period is still poorly characterized. The persistence of pastures under water deficit or waterlogging conditions significantly depends on plant survival mechanisms and their recovery ability (Dias-Filho and Andrade, 2005).

The effects of water deficit and waterlogging stress on forage plants are diverse and impact both physiology and morphology, consequently affecting productive characteristics.

Faced with water deficit, plants can adopt strategies such as drought tolerance, drought survival, and drought escape. Drought tolerance involves physiological adaptations, such as solute accumulation and the development of a deeper root system, to maintain cell turgor and sustain productivity (Wasson et al., 2012; Blum, 2009). Drought survival is associated with the plant's ability to reduce or temporarily halt its growth during dry periods, linked to a decrease in photosynthetic rate and changes in assimilate absorption, resuming when conditions become favorable (Saud et al., 2017; Lelièvre et al., 2011; Gulías et al., 2003). Drought escape refers to the plant's ability to complete its reproductive cycle before the onset of water deficit (Nilsen and Orcutt, 1996; Levitt, 1980).

On the other hand, waterlogging poses distinct challenges to forage plants, leading them to adopt adaptive strategies that improve gas exchange between roots and the environment and maintain energy production in the plant (Armstrong et al., 1994). Plants develop tolerance mechanisms, including anaerobic metabolism and the formation of adventitious roots and aerenchyma, to ensure survival under soil anoxic conditions (Loka et al., 2018; Sasidharan and Voesenek, 2015; Colmer, 2003). Additionally, waterlogging results in stomatal closure, reduced photosynthetic rate, and decreased functional leaf area, directly affecting aerial mass production (Ploschuk et al., 2017; Sasidharan et al., 2017; Voesenek and Bailey-Serres, 2015; Cardoso et al., 2013).

In this context, understanding the responses of forage plants to water stresses and waterlogging, as well as their recovery capacity, is fundamental for the development of more efficient management strategies in pasture-based animal production systems in the tropics. However, despite advances in previous research, significant gaps still exist in the understanding of these processes, especially concerning *Megathyrsus maximus* hybrids. This study aims to fill this gap by characterizing response mechanisms and evaluating the genetic variability of these hybrids in resistance to water deficit and waterlogging.

## 2. MATERIAL AND METHODS

### 2.1. Plant material

A total eight genotypes of *Megathyrus maximus*, seven from the breeding program (PM407, PM409, PM408, PM411, PM414, PM415, PM427) coordinated by Embrapa beef cattle, and the Massai cultivar were used. These forage materials were selected based on their productive potential determined in a preliminary field study. The Massai cultivar was used as a control due to its good productive capacity, relative tolerance to water deficit and waterlogging stress (Veras et al., 2020; Ribeiro-Júnior et al., 2017; Volpe et al., 2008), and wide use in Brazilian pastures.

### 2.2. Experimental design and plant measurements

The experiment was conducted in a greenhouse in Juiz de Fora, Minas Gerais state, Brazil (21°46'56.4"S and 43°22'12.7"W; 882 m asl). The experimental design was randomized blocks with factorial arrangement 8×3×2 (eight genotypes, three water conditions, and two harvest times) using three replications. The water conditions were as follows: (I) control - sufficient water (maintained at 100% of the field capacity), this condition was provided as previously reported by Bernardo et al. (2019); (II) water deficit - irrigation interrupted on day 0, and (III) waterlogging - provided via watering the soil until saturation (set as 3 cm of water above the ground) from day 0. The waterlogging condition was assured via the double pot system with a plastic bag to prevent water leakage. The water lost via evapotranspiration was replaced daily. The first harvest was performed after 20 days of water stress and the second after 12 days of recovery.

Polyethylene pots (4 dm<sup>3</sup>) filled with soil (4 kg) collected from the arable layer (first 20 cm) were used. Before filling the pots, the soil was crushed to pass through a 5-mm screen sieve and then air-dried.

The soil had a sandy clay loam texture, with 29% clay, 13% silt, and 58% sand (analyzed via pipette method). The soil had the following chemical traits:  $\text{pH}_{(\text{H}_2\text{O})}$  5.4; organic matter of 2.18 dag  $\text{kg}^{-1}$ ; Ca 1.5 cmolc  $\text{dm}^{-3}$ ; P 2.5 mg  $\text{dm}^{-3}$ ; K 51 mg  $\text{dm}^{-3}$ ; Mg 0.8 cmolc  $\text{dm}^{-3}$ ; H + Al 3.14 cmolc  $\text{dm}^{-3}$ ; Al 0.3 cmolc  $\text{dm}^{-3}$ ; effective cation exchange capacity 2.5 cmolc  $\text{dm}^{-3}$ ; total cation exchange capacity 5.6 cmolc  $\text{dm}^{-3}$ ; base saturation 44%; aluminum saturation 4%; and total exchangeable bases 2.4 cmolc  $\text{dm}^{-3}$ . Based on the soil chemical analysis, 0.496 g  $\text{kg}^{-1}$  of limestone, 0.333 g  $\text{kg}^{-1}$  of single superphosphate, and 0.014 g  $\text{kg}^{-1}$  of potassium chloride were added according to the recommendations for using correctives and fertilizers in Minas Gerais - 5th approximation (Cantarutti et al., 1999).

The seeds were planted directly in the pots, and a thinning was performed after 30 days, leaving two plants per pot. After the plant reestablishment, a cut (at 20 cm from the soil) and a top-dressing fertilization (0.085 g  $\text{kg}^{-1}$  of N and 0.042 g  $\text{kg}^{-1}$  of K per pot) diluted in water was performed. During the establishment phase, the soil in the pots was kept close to the field capacity to ensure proper plant development.

Water conditions were applied ten days after the standardization cut. After treatment implementation, the pots under water deficit were weighed on days 0, 5, 10, 15, and 20 to monitor the water content in the soil via the gravimetric method (Fig. 1).

The experimental period was from March 17 to April 18, 2022. At the end of the stress period, plants from half of the pots were harvested to assess productive and structural traits. The other half of the pots were kept under 100% of the field capacity to assess plant recovery after the water stress period.

On the day plants were submitted to water conditions, one tiller was identified per pot to assess morphogenic traits. Measurements were performed twice a week during the stress and recovery period. In the selected tillers, information on the appearance and length of the leaf blade and stems was recorded as previously described by Sbrissia et al. (2020). From these

records, the leaf appearance rate (LAR), leaf elongation rate (LER), and leaf senescence rate (LSR) were calculated. In addition, the stem elongation rate (SER), phyllochron, leaf lifespan (LLS), leaf length (LL), total leaf number (TLN), and live leaf number (LLN) per tiller were determined.

The SPAD index (relative chlorophyll content determined based on the intensity of the green color of the leaves) was measured in three youngest fully expanded leaves per pot using a portable chlorophyll meter (SPAD-502, Konica-Minolta, Osaka, Japan). Readings were performed on days 0, 5, 10, 15, 20 and after 12 days of recovery.

The aerial plant components were cut to assess aerial mass at 20 cm from the soil. Samples were separated into leaf blades and stem, then dried in a forced-air oven at  $55^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for 72 hours. Subsequently, samples were weighed to determine the leaf and stem (stems + sheath) mass.

Roots were placed on sieves and washed in running water to withdraw all soil particles completely after the water stress and the recovery period. The root volume was estimated by measuring the displacement of water in a graduated cylinder, that is, by the difference in the known volume of water. The direct response of the root volume was obtained using unit equivalence ( $1\text{ml} = 1\text{cm}^3$ ). Roots were dried at  $55^{\circ}\text{C} \pm 5^{\circ}\text{C}$  in a forced-air oven for 72 hours and then weighed to determine dried mass.

Before each cut, the number of tillers was counted. The height of the plants was measured from the ground level to the end of the highest leaf (i.e., the fully extended plant) using a graduated ruler.

After collecting materials, the leaf area was determined in a leaf area integrating unit (LI-COR Model LI-3100). The specific leaf area was calculated by dividing the leaf area by the leaf dry mass (Radford, 1967).

In addition, information on the leaf dry mass to stem dry mass ratio, the root dry mass to aerial dry mass ratio, and the root recovery index (root dry mass after recovery/root mass after water stress) were measured.

### 2.3. Statistical analysis

Statistical analyses were performed using R Software version 3.6.1 (R Core Team, 2019), and the data were initially tested for the mathematical assumptions with Shapiro–Wilk test and Bartlett tests. The statistical model used was:

$$Y_{ijkl} = \mu + b_k + G_i + W_j + H_l + (G*W)_{ij} + (G*H)_{il} + (W*H)_{jl} + (G*W*H)_{ijl} + e_{ijkl}$$

in which  $Y_{ijkl}$  represents the observation in  $k^{\text{th}}$  block, in  $i^{\text{th}}$  genotype, in  $j^{\text{th}}$  water conditions and in  $l^{\text{th}}$  harvest times;  $\mu$  = the overall mean;  $b_k$  = the block effect  $k$ ;  $G_i$  = fixed effect of the genotype  $i$ ;  $W_j$  = fixed effect of water conditions  $j$ ;  $H_l$  = fixed effect of harvest times  $l$ ;  $(G*W)_{ij}$  = interaction of the effects of genotype  $i$  on water conditions  $j$ ;  $(G*H)_{il}$  = interaction of the effects of genotype  $i$  on harvest times  $l$ ;  $(W*H)_{jl}$  = interaction of the effects of water conditions  $j$  on harvest times  $l$ ;  $(G*W*H)_{ijl}$  = interaction of the effects of genotype  $i$  on water conditions  $j$  at harvest times  $l$  and  $e_{ijkl}$  = random error associated to  $Y_{ijkl}$  [ $e_{ijkl} \sim \text{NID}(0, \sigma^2)$ ].

Data were analyzed as repeated measurements using a randomized block design with an  $8 \times 3 \times 2$  factorial arrangement. Comparisons among means were performed according to the Tukey test and significant differences were set at  $p \leq 0.05$ .

## 3. RESULTS

### 3.1. Productive traits

An interaction between water condition and genotype was observed ( $p < 0.0001$ ) for leaf dry mass production. Water deficit reduced leaf mass in all genotypes. In the same way, except for PM409, the waterlogging reduced the leaf mass production (Table 1). In addition, a harvest time effect ( $p < 0.0001$ ) on leaf mass was observed. Plants showed a greater leaf mass (10.70 g

pot<sup>-1</sup>) in the second harvest (i.e., after the recovery period) than in the harvest performed after a period of 20 days of stress period (8.33 g pot<sup>-1</sup>).

An interaction between water stress and genotype was observed ( $p < 0.003$ ) for the stem dry mass (Table 1). A reduction of stem dry mass was observed in all plants submitted to water deficit, to the point there were no stems in the samples above the cut-off height (20 cm). The waterlogging condition also impaired stem dry mass production. However, PM409 and PM411 genotypes showed similar results to the control group. An interaction between harvest time and genotype ( $p < 0.0005$ ) was observed for the stem dry mass. Greater stem yield after the stress recovery period (second harvest time) was observed in the genotypes, except for PM411 and PM414 genotypes that had similar values to that observed in the cut after the stress period. In addition, an interaction between water condition and harvest time was observed for stem dry mass ( $p < 0.007$ ). Plants submitted to waterlogging showed greater stem mass yield in the second harvest season (3.84 g pot<sup>-1</sup>) than in the first harvest (1.86 g pot<sup>-1</sup>).

There was an interaction between water condition and genotype for leaf to stem ratio ( $p < 0.0005$ ). The waterlogging increased the leaf to stem ratio in the PM408 and PM414 genotypes, while the other genotypes showed no difference compared to the control condition (Table 1).

Root dry mass ( $p < 0.009$ ) was negatively influenced by water deficit but had no effect of waterlogging. It is important to highlight that waterlogging benefited root production in the PM409 genotype, as it presented a higher root mass value than in the control condition (Table 1). Also, an interaction between water condition and harvest time was observed ( $p < 0.0001$ ) for root mass. In the second harvest, a greater root dry mass (35.32 g pot<sup>-1</sup>) was observed in plants submitted to waterlogging compared to the first harvest (15.81 g pot<sup>-1</sup>). However, no difference was observed between the first (7.83 g pot<sup>-1</sup>) and the second harvest (9.11 g pot<sup>-1</sup>) in plants submitted to water deficit.

Interactions were observed between water condition and genotype ( $p < 0.05$ ), harvest time and genotype ( $p < 0.04$ ), and water condition and harvest time ( $p < 0.0001$ ) for the root to shoot ratio (Fig. 2). The water deficit increased the root to shoot ratio in the PM415 genotype. In contrast, no difference was observed in PM408, PM409, PM411, PM414, and PM427 genotypes. Waterlogging increased root to shoot ratio compared to water deficit in PM409 genotype; however, no difference was observed between the two water conditions (Fig. 2A). The root to shoot ratio was increased after the stress recovery period. However, PM408, PM411, and PM415 genotypes showed lower recovery potential resulting in non-significant difference between harvest times (Fig. 2B). In control and waterlogging conditions, the root to shoot ratio was greater in the second harvest. On the other hand, this ratio was lower in the second harvest time in plants submitted to water deficit (Fig. 2C).

Interactions between water condition and genotype ( $p < 0.01$ ) and harvest time and water condition ( $p < 0.0001$ ) were observed for root volume (Fig. 3). The water deficit reduced the root volume in all genotypes (Fig. 3A). However, in waterlogging condition, the PM409 genotype showed greater root volume. In the second harvest (after the recovery period), greater root volume was observed in all stress conditions (Fig. 3B).

The root recovery index was greater in the PM409 genotype, regardless of the nature of water stress. It is important to highlight that the PM415 genotype showed the lowest root recovery rate ( $< 1.0$ ) (Fig. 4A). Regarding water conditions, the root recovery index was approximately 51.0% greater in plants submitted to waterlogging than in those under water deficit (Fig. 4B).

### **3.2. Morphological and structural traits**

An interaction ( $p < 0.003$ ) between water condition and genotype was observed for leaf area (Fig. 5A). All genotypes showed lower leaf area when submitted to water deficit compared to waterlogging and control. On the other hand, two response patterns were observed under

waterlogging: (I) lower leaf area in PM407, PM408, PM414, PM415, and PM427 hybrids and Massai cultivar; and (II) no modification of leaf area in PM409 and PM411 hybrids. Furthermore, an interaction ( $p < 0.0001$ ) between harvesting time and water condition was observed for leaf area (Fig. 5B). Plants under water deficit showed a five times greater potential for leaf area recovering between the first and second harvest times than plants under waterlogging (0.08-fold).

Interactions between water condition and genotype ( $p < 0.001$ ) and water condition and harvest time ( $p < 0.0001$ ) were observed for specific leaf area (Fig. 6). The water deficit reduced the specific leaf area in all genotypes (Fig. 6A). However, it was more pronounced in the PM407 genotype. Nevertheless, waterlogging did not affect the specific leaf area compared to the control conditions. A lower specific leaf area due to water deficit was observed in the first harvest. However, in the second harvest, after the recovery period, plants under water deficit showed, on average, a specific leaf area 20% higher than plants submitted to waterlogging and control (Fig. 6B).

An interaction between water condition and genotype was observed (Table 2) for leaf elongation rate ( $p < 0.001$ ), stem elongation rate ( $p < 0.0003$ ), leaf lifespan ( $p < 0.003$ ), and the number of live leaves ( $p < 0.02$ ). Water deficit impaired leaf elongation rate in all genotypes evaluated. However, except for the PM415 genotype, no reduction was observed in plants submitted to waterlogging compared to the control. The stem elongation rate was reduced in plants submitted to water deficit. However, for genotypes, PM407, PM411 and PM414, there was no significant reduction in relation to the control condition.

A similar effect was observed for leaf lifespan and live leaf number. Indeed, plants submitted to water deficit showed lower values than those on waterlogging and control conditions. The PM411 genotype showed the highest live leaf number per tiller when submitted to waterlogging (Table 2).

Leaf senescence rate was affected ( $p < 0.0001$ ) by water conditions. Higher values were observed in plants submitted to water deficit ( $2.85 \text{ cm tiller}^{-1} \text{ day}^{-1}$ ) than the control ( $1.88 \text{ cm tiller}^{-1} \text{ day}^{-1}$ ) and waterlogging ( $1.13 \text{ cm tiller}^{-1} \text{ day}^{-1}$ ) groups, which did not differ from each other.

Leaf appearance rate ( $\text{leaf tiller}^{-1} \text{ day}^{-1}$ ) and the total leaf number per tiller were also affected by water conditions ( $p < 0.0001$ ). The leaf appearance rate was lower in plants submitted to water deficit ( $0.05 \text{ leaf tiller}^{-1} \text{ day}^{-1}$ ) than in those on waterlogging and control ( $0.09 \text{ leaf tiller}^{-1} \text{ day}^{-1}$ ) treatments. Water deficit reduced the total leaf number per tiller (2.44). However, waterlogging and control showed a similar total leaf number per tiller (4.36 and 4.48, respectively).

Phyllochron was higher ( $p < 0.02$ ) in the Massai cultivar, while no difference was observed in PM407, PM408, PM409, PM414, and PM415 genotypes. Plants submitted to water deficit showed greater phyllochron ( $19.39 \text{ days leaf}^{-1} \text{ tiller}^{-1}$ ) ( $p < 0.0001$ ). Plants on waterlogging and control treatments showed a lower number of days to the appearance of a leaf in the tiller with values of 10.97 and 11.73 days, respectively. There was no significant interaction between water condition and genotype for leaf length ( $p < 0.0001$ ). However, in relation to genotypes ( $p < 0.0001$ ) the lowest leaf length was in the PM408 genotype but did not differ from the Massai cultivar. Water deficit reduced leaf length (40.91 cm) ( $p < 0.0001$ ) in plants. However, this effect was not observed in plants submitted to waterlogging (58.95 cm), which did not differ from the control group (64.88 cm).

The average number of tillers per pot ( $p < 0.0001$ ) was greater in PM407 and PM408 genotypes and the Massai cultivar. The water deficit reduced the average tiller number ( $p < 0.0001$ ) in relation to the control. On the other hand, waterlogging did not influence the tiller number compared to the control.

There was an effect genotypes on the plant height ( $p < 0.0001$ ). Plant height was higher for the PM409 genotype but did not differ between PM414, PM415, PM427, and Massai cultivar. Plant height ( $p < 0.0001$ ) was reduced in both water stress conditions, but in a more intense way under water deficit (81.22 cm) than waterlogging (121.57 cm).

### 3.3. SPAD Index

Interactions between water condition and genotype ( $p < 0.0001$ ), genotype and readings ( $p < 0.02$ ) (Table 3), and water condition and readings ( $p < 0.0001$ ) for the SPAD index were observed.

When submitted to waterlogging conditions, a greater SPAD index was observed in the Massai cultivar, while no difference was observed in PM408, PM409, and PM411 genotypes. In plants submitted to water deficit, a greater SPAD index was observed in the PM408 genotype, while no difference was observed in the Massai cultivar and PM409, PM411, PM414, and PM415 genotypes. Regarding genotypes, the SPAD index was lower for all materials in the waterlogging conditions except for PM411 and PM407 genotypes. The PM411 genotype showed no difference when submitted to the water conditions. In contrast, the PM407 genotype showed a similar reduction of the SPAD when submitted to the two water stress conditions.

Regardless of the water condition, the highest SPAD index was observed in the reading performed on day 5 in all genotypes, except for the PM415 genotype which showed the highest SPAD index on day 10. In general, the SPAD index was reduced in all genotypes over time. The lowest result was observed in the last reading, performed after the recovery period of water stress (Table 3).

The SPAD index generally decreased in all treatments over time, suggesting an overall reduction in chlorophyll content. Plants submitted to water deficit showed a lower SPAD index than those in the control conditions from the fifth reading. This observation suggests a reduced chlorophyll content due to water deficit, which was recovered after the rehydration

period. Similar responses were observed when plants were submitted to waterlogging conditions. However, a lower SPAD index was observed when compared to the other water conditions.

## **4. DISCUSSION**

### **4.1. Water deficit survival**

Our results showed that all genotypes reduced the leaf area development (Fig. 5A) and the mass production of leaves and stems (Table 1) when submitted to water deficit. These results corroborate previous findings by Verslues et al. (2006), who demonstrated a decrease in development and growth of aboveground components in plants subjected to water deficit conditions. This reduction is one of the plants' first responses to reduced water availability in the soil, resulting in severe physiological water deficit. This deficit reduced water potential and plant turgor, which led to reduction in aerial mass production, and increasing leaf senescence rate (Table 2). These effects represent possible mechanisms for reducing transpiration and, consequently, reducing water loss by the plant, characterizing a drought survival process (Saud et al., 2017; Lelièvre et al., 2011; Munne-Bosch and Alegre, 2004; Volaire and Lelièvre, 2001).

Plants that are tolerant to water deficit depend on dehydration resistance mechanisms to sustain growth. This involves the accumulation of metabolites, which helps maintain cell turgor (Chaves, 1991), favoring cell expansion and leaf elongation. However, despite these mechanisms, observed responses such as reduced leaf elongation rate (Table 2), reduced tiller number, and increased senescence rate suggest that these mechanisms were not sufficient to sustain plant development.

The root system is one of the components most affected by water deficit, as observed by Kavar et al. (2008). Water deficit impaired the development of root mass (Table 1), showing an average reduction of 51.2% compared to the control conditions after 20 days. This effect could

have been enhanced by the physical limitation of the pots, which may have influenced the development in root depth and hence affected the velocity and severity of the water deficit.

It is important to highlight the importance of studying the root system and the root mass to shoot mass ratio, as roots play an important role in water and nutrient allocation to the aerial tissue part (Comas et al., 2013). Thus, these characteristics are important for mitigating the limiting effects of stress (Lilley and Kirkegaard, 2011). Increases in root to shoot mass ratio in response to water deficit reflect the interruption of aerial tissue growth and the simultaneous maintenance of root development, especially in the initial phase of stress (Fig. 2A), as also highlighted by Hodge et al. (2009) and observed in Napier grass (*Pennisetum purpureum*) and Mulato grass (*Brachiaria ruziziensis* x *Brachiaria brizantha*) cultivars (Cardoso et al., 2015).

The reduction in the root to shoot ratio during the recovery period (i.e., second harvest season) (Fig. 2C) coupled with the lowest root recovery index (Fig. 4B) suggest a preferential mobilization of reserves for leaf area production (Fig. 5B and Fig. 6B), aiming for a faster photosynthesis restoration (Beloni et al., 2017). The root recovery index (Fig. 4) reflects the degree of preservation of meristematic tissues and the integrity of root system membranes, showing that even under severe water deficit conditions, the protection of root meristems allowed rapid recovery of root volume (Fig. 3B), promoting the development of the aboveground part, with an increase in leaf area (Fig. 5B), specific leaf area (Fig. 6B), leaf, and stem mass.

It is important to emphasize that a reduced root system and a low recovery rate should not be interpreted as negative aspects of the plant, as they may be associated with a resource conservation strategy. This is due to the fact that a deep root system requires a higher energy investment for root synthesis, maintenance, and absorption (Adiku et al., 2000).

Chlorophyll content assessments have been widely reported in plants under conditions of limited soil water availability. Ebrahimiyan et al. (2013) observed an increased chlorophyll

content in *Festuca arundinacea* Schreb. genotypes submitted to mild dry condition; however, the authors observed a substantial reduction in chlorophyll content when submitted to severe water deficit conditions. Similar results were observed in Ryegrass (AbdElgawad et al., 2015) and eleven *Festuca arundinacea* Schreb. cultivars (Sarmast et al., 2015). In the moderate drought condition (onset of water deficit), there was a slight increase in the SPAD index. However, with the worsening of the water deficit (Fig. 1), a decrease (compared to the control conditions) in the SPAD index was observed after the five days of water stress, coinciding with the drastic drop in soil water content (Fig. 1). This outcome is mainly due to the water deficit effects on leaves that inhibit photosynthesis (Farooq et al., 2009).

#### **4.2. Mechanisms of tolerance to excess water**

Forage plants that demonstrate greater tolerance to waterlogging are generally grasses capable of developing adaptations for transport oxygen to water-logged tissues (Dias-Filho, 2013). These adaptations may vary among species or even within the same species, influenced by factors such as intensity, duration, species, growth stage, and plant acclimatization capacity (Striker and Colmer, 2016; Bailey-Serres et al., 2012; Setter et al., 2009).

In general, the development of adventitious roots above the soil level was observed in all genotypes, suggesting that this may have favored better absorption of water, oxygen, and nutrients (Dias-Filho, 2006; Armstrong et al., 1994). These morpho-anatomical changes in genotypes influence rapid growth of the shoot, resulting in increased stem weight and plant height (Beloni et al., 2017; Bailey-Serres et al., 2012). However, contrary to this expectation, this study observed a reduction in canopy height in all genotypes compared to the control, as well as a decrease in stem dry mass, except for hybrids PM409 and PM411, which showed no reduction compared to the control condition (Table 1).

Waterlogging often tends to reduce the total plant mass (Lopez and Kusar, 1999; Yamamoto et al., 1995), especially root production, as plants typically reallocate root reserves

to aerial tissues (Dias-Filho and Carvalho, 2000). However, our results showed that all genotypes submitted to waterlogging did not reduce the production of root dry mass (Table 1) and root volume (Fig. 3A) compared to the control condition. It is important to highlight that the PM409 genotype showed greater (37.2%) root mass production under waterlogging conditions compared to the control treatment. This effect is possibly a result of intense adventitious root production and root aerenchyma formation (Cardoso et al., 2013; Colmer and Voesenek, 2009). This response of the PM409 genotype influenced the leaf area (Fig. 5A) and specific leaf area maintenance (Fig. 6A), the leaf and stem dry mass production (Table 1), and the main morphogenic variables (Table 2), reflecting in the root to shoot ratio (Fig. 2A), indicating that for genotype PM409 under waterlogging stress, there was no preferential reallocation of mass from the root system to the aerial tissues, as observed in other studies (Dias-Filho and Carvalho, 2000; Yamamoto et al., 1995).

In the present study, the genotypes of *Megathyrsus maximus* submitted to waterlogging showed the lowest SPAD indexes (Table 3) compared to water deficit and control conditions, differently from the results obtained by Cardoso et al. (2013) and Beloni et al. (2017), where higher SPAD indexes were found when *Brachiaria* hybrids and *Paspalum* sp. accessions were subjected to waterlogging, these forages being considered tolerant to this type of stress. Possibly, the waterlogging caused a denitrification process in the soil that increased chlorosis occurrence in plant leaves. This effect could have reduced the nitrogen absorption by plants (Adams and Akhtar, 1994). Sharp reductions in chlorophyll content were also reported in the studies of Bailey-Serres et al. (2012) and Liu and Jiang (2015) when plants were submitted to conditions of excess water.

In the period after waterlogging stress, unlike water deficit, genotypes prioritized root recovery over aerial tissues (Fig. 2C). Furthermore, plants submitted to waterlogging showed a higher root recovery rate (Fig. 4B). According to Kato et al. (2014) this effect suggests an

effective antioxidant system to cope with oxygen re-exposure during the stress recovery period properly. A similar result was obtained by Puyang et al. (2015), who assessed Kentucky bluegrass cultivars (*Poa pratensis* L.) tolerant to waterlogging.

#### **4.3. Responses of *Megathyrsus maximus* hybrids to water stress**

Assessing the responses of improved forage materials to water deficit and waterlogging is important to determine the tolerance capacity, which allows the classification and selection of certain genotypes. Regarding the water deficit stress condition, no genotype showed to be tolerant. However, we emphasize the existence of a survival mechanism with root and aerial tissues recovery after the stress recovery period (Fig. 2C, Fig. 3C and Fig. 5B). Given the productive, morphological, and structural responses, the PM409 genotype is a material that showed adaptive characteristics to waterlogging conditions. However, this is not the most productive genotype under ideal soil moisture conditions. In addition, the PM411 genotype also revealed a resource conservation potential when submitted to waterlogging conditions (Tables 1 and 2; Fig. 5A).

The results were important for identifying genotypes with promising traits to endure water stress. However, further studies are needed under field conditions to prove the responses of *M. maximus* genotypes to water deficit and waterlogging.

## **5. CONCLUSION**

Water deficit affects the productive and morphological traits of *M. maximus* genotypes more than waterlogging.

Only the PM409 genotype showed stress tolerance traits among the materials assessed under waterlogging. On the other hand, none of the materials showed tolerance to water deficit.

Thus, searching for improved materials of *M. maximus* that are tolerant to waterlogging is a promising strategy, considering the differential impact on genotypes under water stress conditions.

## **ACKNOWLEDGMENTS**

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, Brazil) for the financial support. The Embrapa Gado de Leite (Minas Gerais, Brazil) for providing the necessary infrastructure to carry out this project.

## **CONFLICT OF INTEREST STATEMENT**

The authors declare that there is no conflict of interest.

## **DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available on request from the corresponding author.

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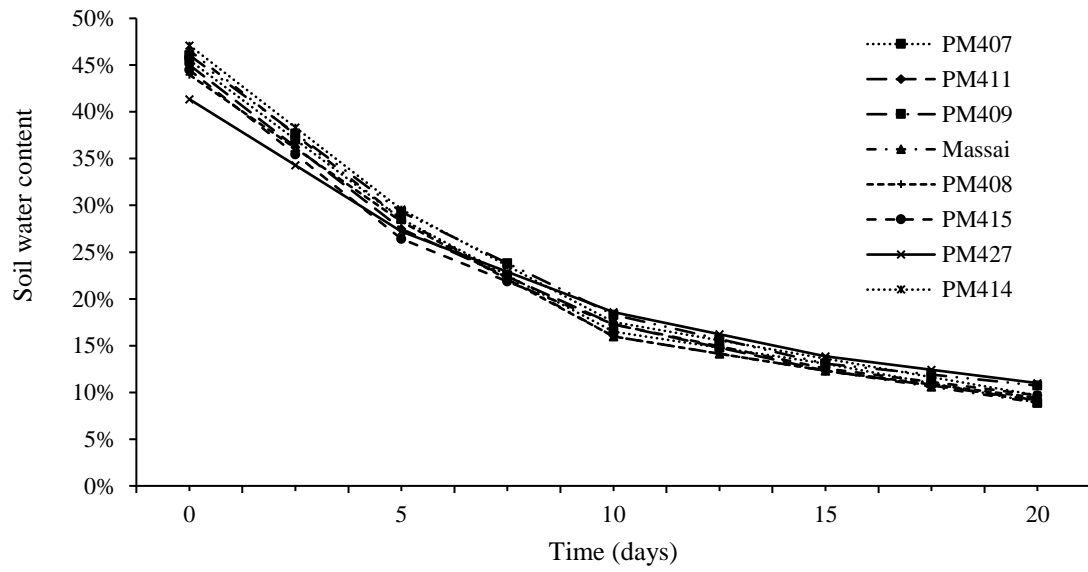
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### Tables and figures

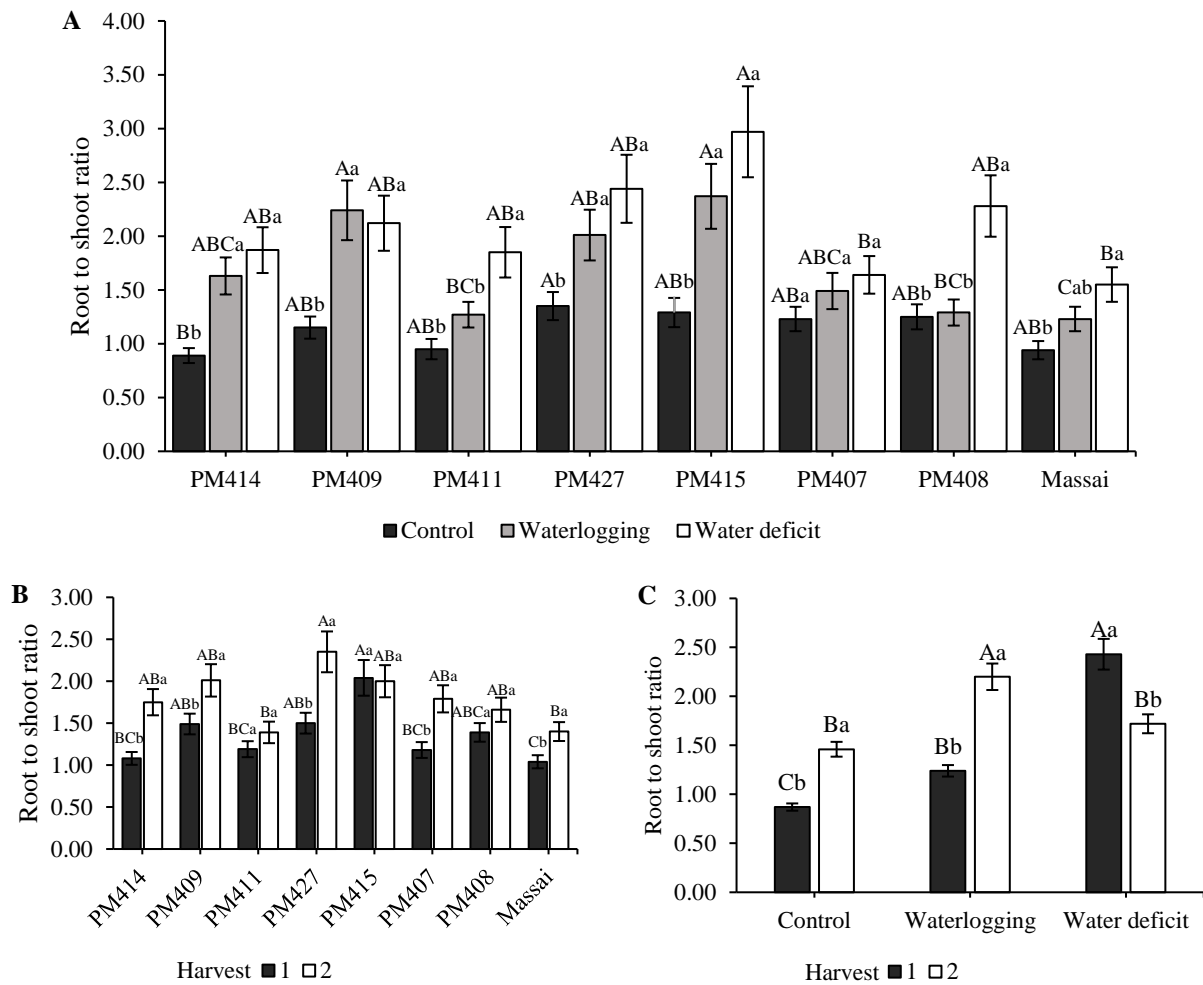


**Figure 1.** Soil water content (%) in pots containing genotypes of *Megathyrsus maximus* (PM407, PM411, PM409, PM408, PM415, PM427, PM414) and cv. Massai, under water deficit condition.

**Table 1.** Productive characteristics (g pot<sup>-1</sup>) of *Megathyrsus maximus* genotypes, under three water conditions (control – waterlogging – water deficit)

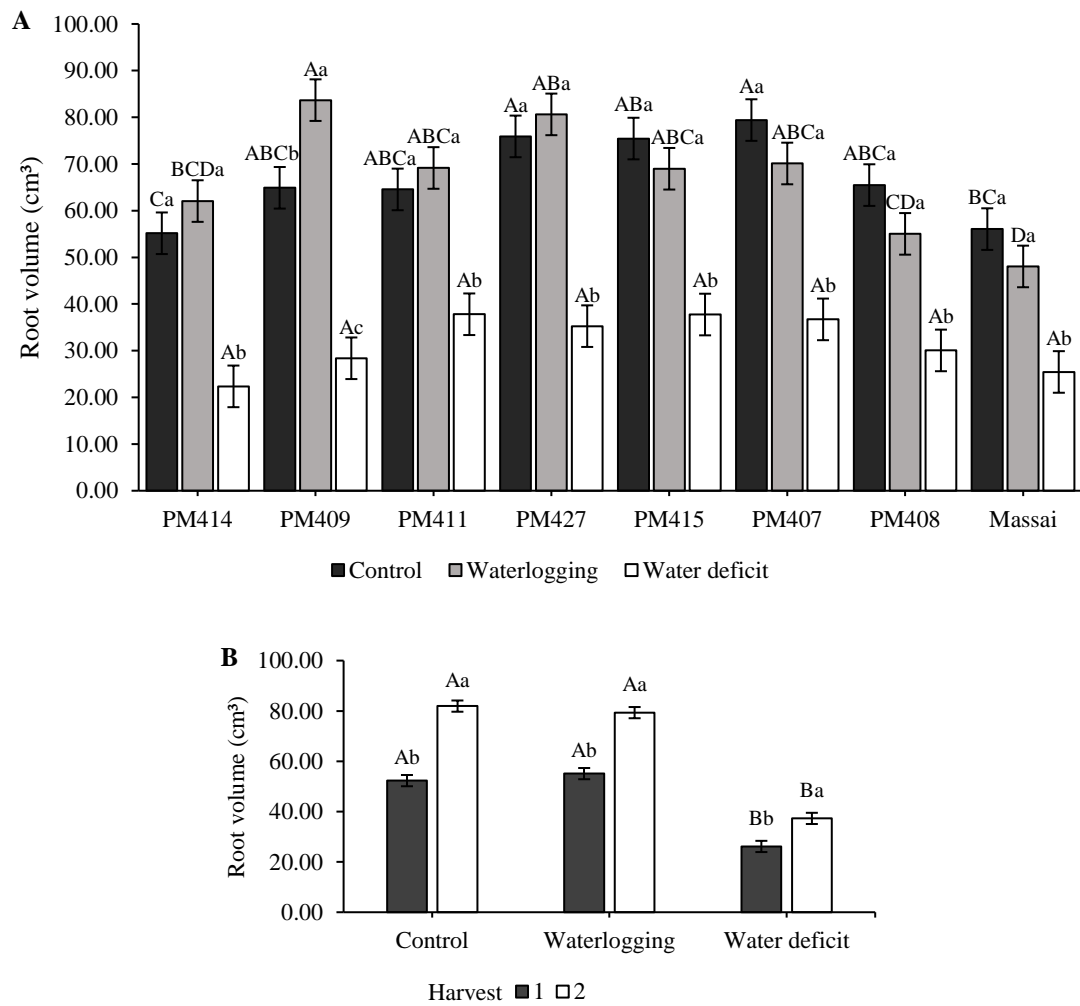
Water conditions	Genotypes								SEM
	PM414	PM409	PM411	PM427	PM415	PM407	PM408	Massai	
Leaf dry mass									
Control	17.68 Aa	13.87 Abc	17.39 Aab	17.40 Aab	14.67 Aabc	14.31 Aabc	12.56 Ac	14.90 Aabc	
Waterlogging	12.81 Bab	12.13 Aabc	13.02 Ba	13.19 Ba	9.91 Bbc	10.77 Babc	9.33 Bc	9.51 Bc	0.39
Water deficit	2.90 Cb	3.39 Bb	4.05 Cab	3.62 Cb	3.31 Cb	5.57 Ca	4.04 Cab	4.32 Cab	
Stem dry mass									
Control	3.52 Acd	3.38 Acd	2.57 Ad	5.33 Aabc	4.39 Abcd	4.85 Abcd	7.09 Aa	6.44 Aab	
Waterlogging	1.47 Ba	3.63 Aa	2.34 Aa	3.72 Ba	2.13 Ba	2.89 Ba	3.60 Ba	3.06 Ba	0.51
Water deficit	-	-	-	-	-	-	-	-	
leaf dry mass to stem dry mass ration									
Control	4.28 Bab	4.25 Aab	6.87 Aa	3.54 Aab	3.61 Aab	3.12 Abc	1.92 Bc	2.53 Abc	
Waterlogging	9.11 Aa	4.06 Aabc	6.25 Aab	3.66 Abc	5.10 Aabc	4.11 Aabc	2.92 Ac	3.51 Abc	0.25
Water deficit	-	-	-	-	-	-	-	-	
Root dry mass									
Control	16.72 Ab	20.63 Bab	25.79 Aab	30.90 Aa	24.33 Aab	24.6 Aab	24.13 Aab	23.29 Aab	
Waterlogging	24.10 Aabc	32.87 Aa	20.23 Aabc	32.67 Aa	28.19 Aab	25.39 Aabc	17.04 Abc	15.25 Ac	1.07
Water deficit	5.69 Bb	7.65 Cab	10.83 Ba	8.69 Bab	10.39 Ba	9.55 Bab	9.26 Bab	6.88 Bab	

Means followed by a different uppercase letter in the column and lowercase letter in the row differ by Tukey test ( $p < 0.05$ ); (SEM) standard error of the mean.



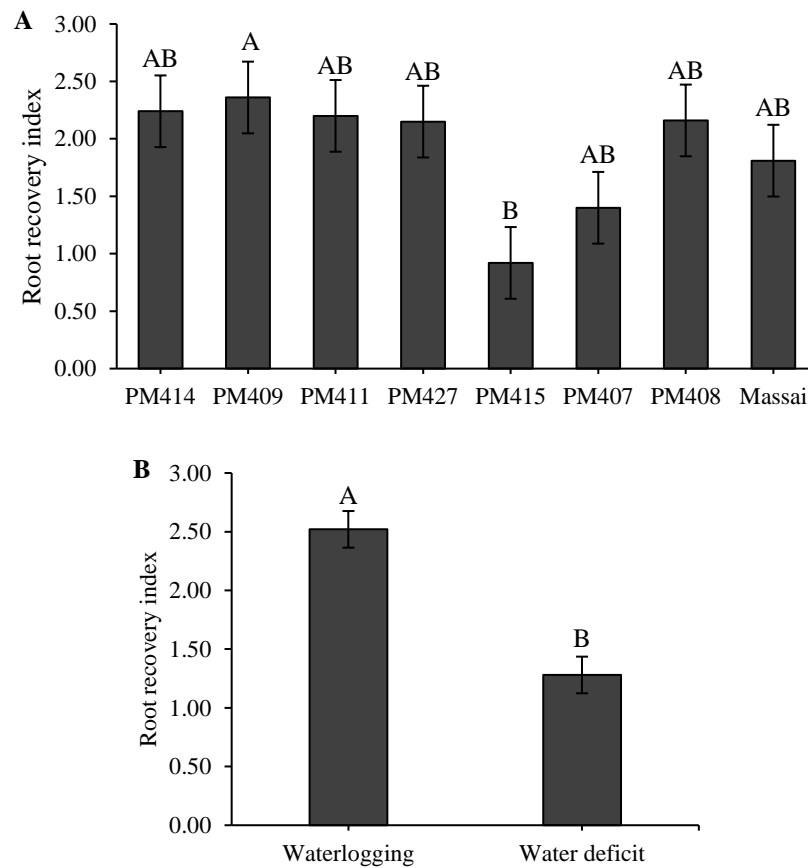
**Figure 2.** (A) Root to shoot ratio of *Megathyrsus maximus* genotypes, under three water conditions (control – waterlogging – water deficit); (B) Root to shoot ratio of *Megathyrsus maximus* genotypes, in two harvests (1st harvest – the end of water stress period and 2nd harvest – after recovery period); (C) Root to shoot ratio under three water conditions (control – waterlogging – water deficit) and in two harvests (1st harvest – the end of water stress period and 2nd harvest – after recovery period).

(A) Uppercase letters compare genotypes and lowercase letters compare water condition by Tukey test ( $p < 0.05$ ), values are the means of eight genotypes and three replicates  $\pm$  standard error of the means; (B) Uppercase letters compare genotypes and lowercase letters compare harvests by Tukey test ( $p < 0.05$ ), values are the means of eight genotypes and three replicates  $\pm$  standard error of the means; (C) Uppercase letters compare water condition and lowercase letters compare harvests by Tukey test ( $p < 0.05$ ), values are the means of eight genotypes and three replicates  $\pm$  standard error of the means.



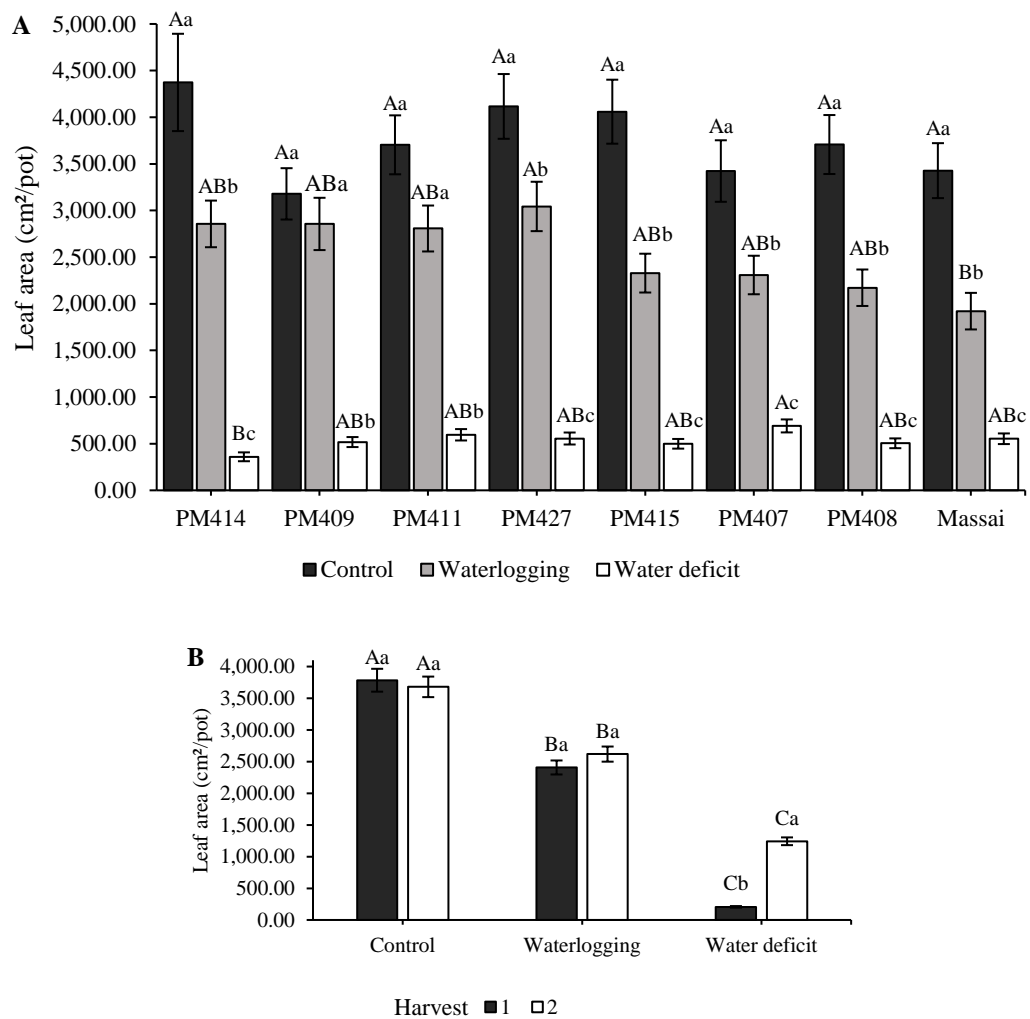
**Figure 3.** (A) Root volume of *Megathyrsus maximus* genotypes, under three water conditions (control – waterlogging – water deficit); (B) Root volume under three water conditions (control – waterlogging – water deficit) and in two harvests (1st harvest – the end of water stress period and 2nd harvest – after recovery period).

(A) Uppercase letters compare genotypes and lowercase letters compare water condition by Tukey test ( $p < 0.05$ ), values are the means of eight genotypes and three replicates  $\pm$  standard error of the means; (B) Uppercase letters compare water condition and lowercase letters compare harvests by Tukey test ( $p < 0.05$ ), values are the means of eight genotypes and three replicates  $\pm$  standard error of the means.



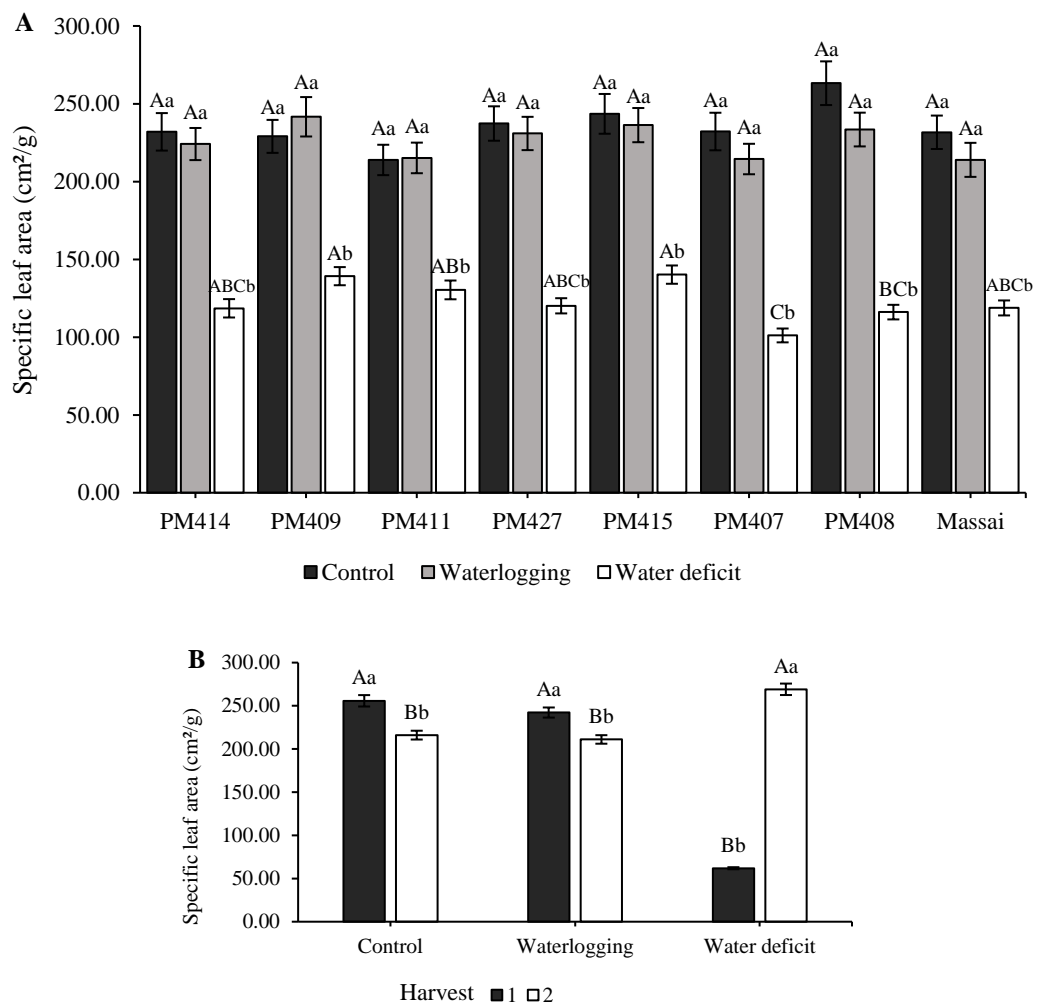
**Figure 4.** (A) Root recovery index of *Megathyrsus maximus* genotypes; (B) Root recovery index under two harvests (1st harvest – the end of water stress period and 2nd harvest – after recovery period).

Different letters in row indicate difference by Tukey test ( $p < 0.05$ ); (A and B) Values are the means of eight genotypes and three replicates  $\pm$  standard error of the means.



**Figure 5.** (A) Leaf area (cm<sup>2</sup> pot<sup>-1</sup>) of *Megathyrsus maximus* genotypes, under three water conditions (control – waterlogging – water deficit); (B) leaf area (cm<sup>2</sup> pot<sup>-1</sup>) under three water conditions (control – waterlogging – water deficit) in two harvests (1st harvest – the end of water stress period and 2nd harvest – after recovery period).

(A) Uppercase letters compare genotypes and lowercase letters compare water condition by Tukey test ( $p < 0.05$ ), values are the means of eight genotypes and three replicates  $\pm$  standard error of the means; (B) Uppercase letters compare water condition and lowercase letters compare harvests by Tukey test ( $p < 0.05$ ), values are the means of eight genotypes and three replicates  $\pm$  standard error of the means.



**Figure 6.** (A) Specific leaf area ( $\text{cm}^2 \text{g}^{-1}$ ) of *Megathyrsus maximus* genotypes, under three water conditions (control – waterlogging – water deficit); (B) specific leaf area ( $\text{cm}^2 \text{g}^{-1}$ ) under three water conditions (control – waterlogging – water deficit) in two harvests (1st harvest – the end of water stress period and 2nd harvest – after recovery period).

(A) Uppercase letters compare genotypes and lowercase letters compare water condition by Tukey test ( $p < 0.05$ ), values are the means of eight genotypes and three replicates  $\pm$  standard error of the means; (B) Uppercase letters compare water condition and lowercase letters compare harvests by Tukey test ( $p < 0.05$ ), values are the means of eight genotypes and three replicates  $\pm$  standard error of the means.

**Table 2.** Morphological characteristics of *Megathyrsus maximus* genotypes, under three water conditions (control – waterlogging – water deficit)

Water conditions	Genotypes								SEM
	PM414	PM409	PM411	PM427	PM415	PM407	PM408	Massai	
Leaf elongation rate (cm tiller <sup>-1</sup> day <sup>-1</sup> )									
Control	7.00 Abc	8.19 Ab	4.94 ABcde	6.19 Abcd	11.91 Aa	4.79 Acde	3.59 Ade	3.08 ABe	
waterlogging	6.93 Aa	6.41 Aab	5.76 Aab	5.61 Aab	4.86 Bab	4.71 Aab	3.15 ABb	4.34 Aab	0.64
Water deficit	1.67 Ba	1.89 Ba	2.97 Ba	2.26 Ba	1.69 Ca	2.32 Ba	1.14 Ba	1.92 Ba	
Stem elongation rate (cm tiller <sup>-1</sup> day <sup>-1</sup> )									
Control	0.50 Ac	0.50 ABc	0.38 Ac	0.88 Abc	0.76 Ac	0.17 Ac	1.80 Aa	1.65 Aab	
waterlogging	0.40 Ab	0.79 Aab	0.51 Ab	0.71 Aab	0.61 Aab	0.26 Ab	1.29 Aa	0.77 Bab	0.16
Water deficit	0.07 Aa	0.13 Ba	0.04 Aa	0.05 Ba	0.04 Ba	0.10 Aa	0.02 Ba	0.02 Ca	
Leaf lifespan (days)									
Control	36.66 Aa	41.25 Aa	35.75 Aa	36.66 Aa	36.11 Aa	36.66 Aa	38.50 Aa	42.16 Aa	
waterlogging	33.00 ABa	35.75 Aa	42.53 Aa	39.41 Aa	44.91 Aa	42.16 ABa	36.66 Aa	40.33 ABa	3.63
Water deficit	21.02 Bb	17.30 Bb	21.00 Bb	21.33 Bb	21.03 Bb	49.50 Ba	23.00 Bb	26.69 Bb	
Live leaf number									
Control	3.33 Aab	5.55 Aa	3.00 Bb	3.66 Aab	3.29 Aab	3.00 Ab	3.33 Aab	3.00 Ab	
waterlogging	3.33 Aa	3.66 Ba	4.66 Aa	4.00 Aa	4.00 Aa	3.33 Aa	3.66 Aa	3.66 Aa	0.46
Water deficit	1.00 Ba	1.66 Ca	1.00 Ca	1.33 Ba	1.00 Ba	3.00 Aa	1.00 Ba	1.33 Ba	

Means followed by a different uppercase letter in the column and lowercase letter in the row differ by Tukey test ( $p < 0.05$ ); (SEM) standard error of the mean.

**Table 3.** SPAD index of *Megathyrus maximus* genotypes, during the stress imposition period with readings on days (0, 5, 10, 15 and 20) and 12 days after recovery from water stress

Genotypes	Readings (days)					
	0	5	10	15	20	32
PM414	37.40 Ab	41.24 ABa	39.78 Aab	37.34 ABb	36.91 Ab	24.33 Dc
PM409	38.65 Aab	42.15 ABa	40.45 Aab	39.64 ABab	37.90 Ab	29.41 Cc
PM411	39.08 Aab	42.42 ABa	41.29 Aab	38.12 ABbc	34.48 Ac	27.70 CDd
PM427	37.46 Aab	40.43 Ba	39.49 Aab	36.48 ABb	36.17 Ab	27.36 CDc
PM415	39.53 Aab	40.85 ABab	41.75 Aa	40.05 Aab	37.06 Ab	29.72 BCc
PM407	37.83 Aab	41.22 ABa	38.86 Aab	36.03 Bb	35.84 Ab	28.88 Cc
PM408	40.08 Ab	44.51 Aa	41.19 Aab	37.63 ABb	38.32 Ab	33.46 ABc
Massai	39.96 Aab	42.81 ABa	40.94 Aab	38.58 ABb	38.39 Ab	33.94 Ac

Means followed by a different uppercase letter in the column and lowercase letter in the row differ by Tukey test ( $p < 0.05$ ).

## 5 CONSIDERAÇÕES FINAIS

Os resultados deste estudo fornecem contribuições substanciais para o entendimento da resposta de genótipos de *Megathyrsus maximus* as condições específicas das estações do ano e em condições de estresse hídrico. No primeiro artigo pelo índice de seleção FAI-BLUP indicou os híbridos PM411, PM407, PM426 e PM412 para a época chuvosa, e os híbridos PM406, PM414, PM419 e a cultivar Mombaça para a época seca, demonstrando ganhos genéticos equilibrados e desejáveis para todas as características avaliadas. Já no segundo artigo, o híbrido PM409 mostrou-se tolerante ao alagamento, com maior produção de raízes e com a manutenção do desenvolvimento dos componentes aéreos. Com relação, ao estresse por déficit hídrico nenhum material demonstrou tolerância, porém, foi observada uma recuperação significativa após o período de estresse. Embora o experimento com avaliação dos genótipos em estresse por déficit hídrico não tenha revelado tolerância a esse estresse, os genótipos identificados como promissores na época seca pelo índice de seleção FAI-BLUP no primeiro artigo, demonstrou respostas promissoras em ambientes desafiadores em estudos a campo. É importante destacar, no entanto, que uma limitação do segundo estudo foi a impossibilidade de inclusão de todos os genótipos na avaliação de estresse hídrico. Portanto, seria recomendável realizar uma avaliação adicional desses materiais em condições controladas de estresse hídrico para confirmar os resultados obtidos.