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MATHEUS BESSA DA SILVA

RESPOSTAS MORFOFISIOLÓGICAS À ADIÇÃO DE NUTRIENTES EM *PAVONIA VARIANS* MORIC. (MALVACEAE) E *MEGATHYRSUS MAXIMUS* (JACQ.) B.K. SIMON & S.W.L. JACOBS (POACEAE) SOB RESTRIÇÃO HÍDRICA.

RECIFE
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BANCA EXAMINADORA:

_____

Dr. Marcus Vinicius Loss Sperandio (Orientador)
Universidade Federal Rural de Pernambuco – UFRPE

_____

Dr. Diego Silva Batista (Titular)
Universidade Federal da Paraíba – UFPB

_____

Dra. Cláudia Ulisses de Carvalho Silva (Titular)
Universidade Federal Rural de Pernambuco – UFRPE

_____

Dr. Luiz Palhares Neto (Suplente)
Universidade Estadual do Sudoeste da Bahia – UESB

RECIFE
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RESUMO

A seca é um dos principais fatores limitantes ao crescimento e estabelecimento vegetal em ambientes como a Caatinga, e demanda das espécies um aparato bioquímico, fisiológico e morfológico que permitam superar os desafios encontrados em um ambiente semiárido de uma Floresta Tropical Sazonalmente Seca. As ações antrópicas têm impactado diretamente a estrutura vegetal dessa área, colaborando para intensificação da severidade das condições, como a disponibilidade e acesso nutricional pelas plantas. O trabalho objetivou analisar as respostas morfofisiológicas de *Pavonia varians* e *Megathyrsus maximus*, espécies presentes na Caatinga pernambucana, sob suplementação nutricional com Nitrogênio – N, Fósforo – P, e Potássio – K em regime de restrição hídrica e reidratação. O estudo foi desenvolvido em casa de vegetação com indivíduos jovens de *P. varians* e *M. maximus* coletadas no Parque Nacional do Catimbau em Pernambuco. A suplementação nutricional se deu através da aplicação de 4 doses (40ml/vaso) de uma solução de N (6mM), P (2mM) e K (6mM), a partir de 31 dias após o transplante (DAT) das plantas. Até os 51 DAT as plantas foram mantidas sobe rega mantendo-as a 70% de umidade da capacidade de pote (controle). Aos 52 DAT iniciou-se a suspensão de rega por 10 dias e procedido a primeira coleta e o restabelecimento de rega por mais 20 dias finalizando com uma segunda coleta das plantas. Através de análises de crescimento, fisiológicas, estresse oxidativo, metabólica e nutricional foi possível verificar a ação do NPK nas respostas a restrição hídrica e a reidratação em *P. varians* e *M. maximus*. A Prolina foi a principal resposta modulada pela suplementação nutricional com NPK a restrição hídrica em ambas as espécies. *P. varians* e *M. maximus* demonstram respostas ao NPK ofertado, contudo recrutando diferentes vias de defesa antioxidativa à restrição hídrica. *P. varians* mobilizou uma via não enzimática, com destaque para ação da prolina, conseguindo alcançar a redução de danos oxidativos. *M. maximus* por sua vez, mostrou-se beneficiada também através da atividade enzimática da APX, contudo não foi possível observar redução nos níveis de danos de membrana. O estudo mostrou que as espécies apresentaram comportamentos distintos em respostas a seca em decorrência da suspensão de rega, especialmente quando suplementadas com NPK. A suplementação nutricional favoreceu à um fortalecimento fisiológico das espécies, contribuindo no enfrentamento de maneira mais efetiva aos danos oxidativos causados pela seca, e sua consequente recuperação efetiva após reidratação, se mostrando uma ferramenta eficaz de combate ao déficit hídrico.

Palavras-chave: Estresse abiótico. Respostas bioquímicas. Nutrientes essenciais. Estresse oxidativo. Prolina.

ABSTRACT

Drought is one of the main factors limiting plant growth and establishment in environments such as the Caatinga, and demands from species a sufficient biochemical, physiological and morphological apparatus to enable them to overcome the challenges encountered in a semi-arid environment of a Seasonally Dry Tropical Forest. Anthropogenic actions have directly impacted the plant structure of this area, contributing to the intensification of the severity of conditions, such as nutritional availability and access by plants. The work aimed to analyze the morphophysiological responses of *Pavonia varians* and *Megathyrsus maximus*, species present in the Caatinga of Pernambuco, under nutritional supplementation with Nitrogen – N, Phosphorus – P, and Potassium – K under a regime of water restriction and rehydration. The study was carried out in a greenhouse with young individuals of *P. varians* and *M. maximus* collected in the Catimbau National Park in Pernambuco. Nutritional supplementation was carried out through the application of 4 doses (40ml/pot) of a solution of N (6mM), P (2mM) and K (6mM), starting 31 days after transplantation (DAT) of the plants. Until 51 DAT, the plants are maintained under watering, keeping them at 70% humidity of the pot capacity (control). 52 DAT, watering was suspended for 10 days and the first collection was carried out and irrigation was reestablished for another 20 days, ending with a second collection of plants. Through growth, physiological, oxidative, metabolic and nutritional stress analyses, it was possible to verify the action of NPK in the responses to water restriction and rehydration in *P. varians* and *M. maximus*. Proline was the main response modulated by nutritional supplementation with NPK to water restriction in both species. *P. varians* and *M. maximus* demonstrate responses to the NPK offered, however, recruiting different antioxidant defense pathways to water restriction. *P. varians* mobilized a non-enzymatic pathway, with emphasis on the action of proline, managing to reduce oxidative damage. *M. maximus*, in turn, also benefited through the enzymatic activity of APX, however it was not possible to observe a reduction in the levels of membrane damage. The study showed that the species demonstrate different behaviors in response to drought due to the suspension of irrigation, especially when supplemented with NPK. Nutritional supplementation favored the physiological strengthening of the species, contributing to more effective coping with oxidative damage caused by drought, and its consequent effective recovery after rehydration, proving to be an effective tool for combating water deficit.

Keywords: Abiotic stress. Biochemical responses. Essential nutrients. Oxidative stress. Proline.

1 INTRODUÇÃO

A disponibilidade hídrica, temperatura, salinidade, acesso à nutrientes e exposição solar, são fatores determinantes para distribuição e desenvolvimento da vida vegetal (GONG et al., 2020; HAAK et al., 2017; KOPECKÁ et al., 2023). Devido a sua condição séssil, as plantas estão sujeitas as alterações morfológicas, fisiológicas, bioquímicas e até genéticas em decorrência das condições ambientais. A seca ocasionada pela baixa disponibilidade hídrica é um cenário limitante para as plantas tendo em vista a importância da água para os organismos vegetais, interferindo no seu metabolismo e consequentemente em seu crescimento (KOPECKÁ et al., 2023; ZHANG et al., 2021).

Ambientes semiáridos como a Caatinga, uma Floresta Tropical Sazonalmente Seca, sofrem forte influência da seca em decorrência da baixa e irregular distribuição das chuvas e temperaturas elevadas. A Caatinga se apresenta como uma região semiárida de mais populosas do mundo (ARAUJO et al., 2023). A grande pressão antrópica existente nesse domínio morfoclimático é percebida através da modificação do ambiente por meio da alteração da estrutura vegetal e a consequente exposição do solo (ANTONGIOVANNI et al., 2020; SILVA et al., 2018).

Essa realidade somada as condições naturais severas da região (baixa precipitação, alta incidência solar, temperaturas elevadas), e agravadas pelo cenário de mudanças climáticas, pode impactar diretamente no acesso aos nutrientes alterando processos de mineralização e a consequente disponibilidade de nutrientes essenciais no solo afetando as espécies que estão estabelecidas em seu meio (OLIVEIRA, 2021; PINHEIRO et al., 2020). Dessa forma, a vegetação da Caatinga apresenta um conjunto de aparato adaptativo para o estabelecimento e desenvolvimento em condições adversas (BARBOSA et al., 2023; SILVA; BARBOSA, 2018).

O acesso a nutrientes, que trata-se de um fator essencial para o desenvolvimento vegetal (PRADO, 2021), é influenciada pela dinâmica hídrica no solo que possibilita a absorção de nutrientes da matriz do solo para os tecidos vegetais a partir das raízes (PRADO, 2021). Os nutrientes são elementos fundamentais pra o crescimento das plantas, destacando-se o Nitrogênio (N) Fósforo (P) e Potássio (K) que participam da construção de elementos moleculares, atividades metabólicas e desempenho fisiológico (MOREAU et al., 2019; PANDEY; MAHIWAL, 2020; WHITE; VENEKLAAS, 2012; XU; YAN; XIA, 2019).

Pavonia varians é uma espécie nativa da Caatinga utilizada pela população para tratamentos no sistema digestivo no combate a inflamações e infecções (LEAL et al., 2007). É uma espécie pouco estudada, retratada na literatura de forma indireta, sendo abordada, em sua grande maioria, em listagens botânicas e trabalhos que investigam a interação inseto-planta

(ATHIÊ-SOUZA et al., 2019; GIULIETTI et al., 2004; GOMES; RODAL; DE MELO, 2006; SCHLINDWEIN; PICK; MARTINS, 2012). *Megathyrsus maximus* é uma espécie de gramínea exótica de origem de países do continente africano (ROJAS-SANDOVAL; ACEVEDO-RODRÍGUEZ, 2022). Sua ampla distribuição mundial está relacionada ao seu uso forrageiro, assim como no Brasil, e nacionalmente já se apresenta como uma espécie naturalizada presente em todos os domínios fitogeográficos, incluindo a Caatinga (JANK et al., 2022).

Ambientes semiáridos são desafiadores para os organismos que nele se estabelecem, sobretudo quando sofrem com efeitos da ação humana de forma local e global. A melhora no status nutricional de plantas cultiváveis é uma estratégia investigada para superação dos efeitos deletérios da seca, uma vez que o status nutricional dos organismos reflete sua capacidade de resposta ao estabelecimento ao ambiente, favorecendo seu crescimento e desenvolvimento. Dessa forma o trabalho objetivou analisar as respostas morfofisiológicas de *P. varians* e *M. maximus*, espécies presentes na Caatinga pernambucana, sob suplementação nutricional com NPK prévia ao regime de restrição hídrica como ferramenta de superação dos danos causados pela seca.

2 OBJETIVOS

2.1 GERAL

Analisar as respostas morfofisiológicas em *Pavonia varians* (espécie nativa) e *Megathyrsus maximus* (espécie exótica) presentes na Caatinga pernambucana sob suplementação nutricional à restrição hídrica.

2.2 ESPECÍFICOS

- Compreender como *P. varians* e *M. maximus* submetidas a restrição hídrica responderão em sua morfologia (altura, peso seco de folhas, caule e raiz, e número de folhas) quando submetidas a suplementação nutricional com NPK.
- Investigar como se estabelecerá o ajustamento bioquímico (enzimático e metabólico) de *P. varians* e *M. maximus* quando suplementadas com dose nutricional de NPK e submetidas a restrição hídrica.
- Identificar as respostas fisiológicas (clorofila *a*, clorofila *b*, clorofila total, carotenoides, teor relativo de água) desencadeadas pela restrição hídrica, combinada com suplementação nutricional de NPK em *P. varians* e *M. maximus*.
- Avaliar os teores de nutrientes na parte aérea *P. varians* e *M. maximus* para compreender a relação do déficit hídrico e aplicação de NPK.

3. REFERENCIAL TEÓRICO

3.1 NUTRIENTES NA PLANTA

As plantas são organismos autótrofos, possuindo a capacidade de síntese de compostos orgânicos utilizados para seu desenvolvimento. Ainda que munidas dessa característica, assim como os demais seres vivos, as plantas necessitam ter acesso a um conjunto de elementos fundamentais para seu funcionamento, desenvolvimento e reprodução. Juntamente com a água, a disponibilidade e aquisição nutricional se apresentam como fatores importantes para a distribuição e estabelecimento de organismos vegetais.

O histórico da nutrição das plantas até aos conhecimentos modernos atuais percorre uma grande jornada, perpassando por contribuições de Aristóteles, postulações de Empédocles dos quatro elementos fundamentais (água, terra, fogo e ar), contribuições de J.B. von Helmont ao afirmar que as plantas eram compostas em sua totalidade por água, e a sustentação da teoria humista, pela qual as plantas eram providas organicamente do húmus. Estudos posteriores, por sua vez, desvendaram que a liberação de elementos a partir da decomposição do húmus e compostos inorgânicos e minerais, eram os responsáveis para o desenvolvimento das plantas: os nutrientes (BOARETTO et al., 2014).

Nutrientes são elementos químicos que podem ser acessados, assimilados e contribuem para o desenvolvimento vegetal (PRADO, 2021). Podem ser orgânicos ou minerais (não orgânicos) e apresentam diferentes demandas ao organismo (macro ou micronutrientes), especialmente essenciais para a composição, funcionamento e de caráter insubstituível no desenvolvimento vegetal (ARNON; STOUT, 1939; EPSTEIN, 1975): carbono (C), oxigênio (O) e hidrogênio (H), encontrados em compostos orgânicos, e o minerais nitrogênio (N), fósforo (P), enxofre (S), potássio (K), cálcio (Ca) e magnésio (Mg), classificados como macronutrientes, e ferro (Fe), manganês (Mn), zinco (Zn), cobre (Cu), boro (B) molibdênio (Mo) e cloro (Cl) classificados como micronutrientes.

3.1.1 Nitrogênio, Fósforo e Potássio nos Organismos Vegetais

Dentre os macronutrientes minerais, o N é um dos elementos de maior destaque para os organismos vivos, haja vista sua ampla participação como elemento constituinte de compostos nitrogenados, como exemplo, as bases nitrogenadas presentes nas unidades formadoras dos ácidos nucléicos (DNA e RNA), essencial na composição de aminoácidos, consequentemente, na síntese de proteínas (MOREAU et al., 2019).

Xu et al. (2019), observaram que o oferecimento de N às plantas, salvo a influência de fatores secundários (clima, quantidade, formas de aplicação...), favoreceu positivamente o crescimento vegetal, podendo ser relacionado como o nutriente comumente mais ligado ao desenvolvimento vegetal (MOREAU et al., 2019). Participa como elemento estruturante na molécula de clorofila, além de fitormônios como auxina e citocinina, atuando diretamente no crescimento vegetal e no incremento da fotossíntese, com consequente assimilação de compostos orgânicos essenciais para o desenvolvimento vegetal (MOREAU et al., 2019; XU et al., 2019).

K é um macronutriente que também desempenha papel de relevante nos organismos vegetais, quando em situação de estresse hídrico. Tem sua participação relacionada ao controle osmótico das células, tem como característica sua fácil mobilidade nos sistemas, sendo transportado a longas distâncias pelos vasos condutores, com participação significativa na diminuição do potencial osmótico celular. O potássio, em conjunto com seus transportadores, atua nas respostas a seca possibilitando maximização no uso da água, estabilidade das membranas biológicas, regulação osmótica e estomática e alongamento celular, favorecendo então um aprimoramento fisiológico para o enfrentamento à seca (PANDEY; MAHIWAL, 2020).

A presença do fósforo também apresenta incrementos aos organismos vegetais em situação de déficit hídrico. Estudos com culturas de interesse agrícola, mostram seu desempenho aprimorando taxas de eficiência do uso de água e fotossíntese líquida, além de mostrar aprimoramento na recuperação pós estresse, produção de perfilho e vigor de sementes (FÁTIMA et al., 2018; WHITE; VENEKLAAS, 2012). Nos organismos vegetais, o P tem importante papel na composição de ácidos nucléicos, carboidratos, moléculas como ATP, ADP e NADP, além de ser um componente essencial das membranas celulares através dos fosfolipídios.

3.2 ESTRESSE ABIÓTICO EM PLANTAS

As características e configurações ambientais são de suma importância no estabelecimento de espécies em seu contexto. Os estresses abióticos, de ordem química e física, desempenham importante papel ecológico e agrícola na distribuição e produção das espécies vegetais no ambiente, com seus distintos e diversos arranjos (ZHANG et al., 2021). Flutuações nas condições ótimas de acesso à água e nutrientes, salinidade, temperatura e luminosidade estão como os principais elementos que representam os estresses abióticos e influenciam no crescimento e desenvolvimento vegetal, em decorrência do não alcance do potencial genético

dos indivíduos e percebida na redução da biomassa, produção e reprodução vegetal (KOPECKÁ et al., 2023; ZHANG et al., 2021).

3.2.1 Mecanismos de Defesa: Caatinga em foco

Seca, calor, frio, alta salinidade, deficiências nutricionais são condições que alteram a homeostase das plantas, e somado ao fato de serem organismos sésseis, exigem que estas respondam de maneira a superar tais obstáculos, gerando então respostas ao estresse sofrido como ferramenta de superação à essas mudanças do ambiente (GONG et al., 2020; HAAK et al., 2017; MIRYEGANEH, 2021).

Duas grandes estratégias de defesa das plantas envolvem o escape e/ou a tolerância ao estresse. No primeiro caso, as plantas munidas de suas estratégias evitam as condições adversas, "fugindo" de seus efeitos, a exemplo as plantas de ciclo curto que são capazes de se desenvolverem vegetativa e reprodutivamente antes de períodos de estiagem (BARBOSA et al., 2023). No segundo caso, por sua vez, tais estratégias vegetais permitem que as plantas passem por situações de estresses de maneira a resistir seus efeitos negativos, opondo-se ao estresse, podendo haver ou não diminuição de seu potencial fisiológico (BARBOSA et al., 2023).

Para se aclimatar a diferentes estresses, as plantas usam diversas estratégias moleculares, fisiológicas, bioquímicas e morfológicas. Após a percepção do estresse (sinal), uma cascata de transdução de sinais envolvendo hormônios, $Ca2^{+}$ e espécies reativas de oxigênio (EROs) levam a reprogramação de milhares de genes e proteínas (YOU; CHAN, 2015). A resposta final da planta envolve entre outros, em nível bioquímico, alterações no bombeamento de prótons (H^{+}) promovido pela enzima de membrana plasmática PM H^{+}-ATPase (SPERANDIO et al., 2011), em nível fisiológico, alteração das taxas fotossintéticas, acúmulo de moléculas protetoras (prolina, açúcares e glicinabetaína), e em nível morfológico apresentam mudanças no crescimento de raiz e parte aérea (JUNIOR; LUCENA, 2022; SOUZA et al., 2020).

Um exemplo claro das respostas vegetais a situações de estresse, pode ser verificado na vegetação da Caatinga, pois trata-se de um domínio fitogeográfico em uma região semiárida presente, predominantemente no nordeste brasileiro, que apresenta longos períodos de estiagem em decorrência da baixa precipitação. Devido a essas características, a vegetação se classifica como Floresta Tropical Sazonalmente Seca e necessita de um conjunto de aparatos para lidar com a forte influência de altas temperaturas e irradiação e, sobretudo, a baixa e irregular distribuição na disponibilidade hídrica (SILVA; BARBOSA, 2018).

A Caatinga carrega em sua identidade uma das principais características de resistência ao estresse ambiental, de ordem natural, a qual está submetida. Do tupi-guarani, mata-branca reflete o caráter caducifólio da vegetação, deixando à mostra os troncos esbranquiçados nos períodos de estiagem (SENA, 2011). O caráter decíduo vem como uma estratégia de evitar a perda de água para o ambiente por meio da transpiração. O controle do movimento estomático, bem como sua densidade, também são características presentes como mecanismo de superação às altas temperaturas combinadas com a baixa disponibilidade de água (SOUZA et al., 2020). Comum para as plantas nativas da Caatinga, a presença de folhas pequenas e compostas, visam a diminuição de área foliar e otimização na dispersão do calor para a manutenção de um conforto térmico. É possível verificar também a presença de cera epicuticular nas folhas, tecidos de reserva de água e sistema radicular bem desenvolvidos, como ações que buscam a diminuição dos efeitos negativos em decorrência da baixa disponibilidade de água (SOUZA et al., 2020).

3.2.2 Estresse por Restrição Hídrica

A água é o principal recurso para o desenvolvimento vegetal, sendo a seca o principal fator limitante em seu desenvolvimento e produção (GEILFUS, 2019). A água nos organismos vegetais atua como mantenedora da organização homeostática dos organismos, favorecendo reações necessárias à fisiologia, transporte de nutrientes e metabólitos ao longo de toda a estrutura vegetal, sendo responsável pela composição de cerca de 95% dos tecidos não lenhosos vegetais (GEILFUS, 2019; LAMBERS; OLIVEIRA, 2019).

O estresse por restrição hídrica, ou déficit hídrico nas plantas, pode ser definido como a insuficiência na captação de água do solo para suprir a necessidade fisiológica do organismo vegetal. O processo de transpiração é uma das principais vias de perda de água da planta para atmosfera, gerando estresse hídrico quando não há equilíbrio no balanço entre obtenção de água e perda por transpiração. Dessa forma, o déficit hídrico pode ocorrer especialmente nos períodos de estiagem por redução na precipitação, ou por retenção de água nos poros no solo (CAMPOS et al., 2021; GEILFUS, 2019).

A severidade da restrição hídrica pode causar diferentes danos as plantas, sejam mais leves e de caráter reversível, como inibição da fotossíntese, diminuição da expansão celular e menor crescimento, podendo chegar até mesmo a causar a morte do organismo vegetal em situações de estresse mais severo (CAMPOS et al., 2021).

A falta de água implicará de modo direto no crescimento, desenvolvimento e produção vegetal. A redução na condutância estomática é diretamente afetada, desencadeando

desregulação no processo fotossintético por limitação nos principais elementos atuantes neste processo (água e CO_2) (CAMPOS et al., 2021). A dinâmica das Espécies Reativas de Oxigênio (EROs) também é afetada pela redução da condutância estomática. Ainda que desempenhem papel importante na sinalização celular, em estado de estresse hídrico ocorrerá um acúmulo de EROs em decorrência do desbalanço do estado redox celular devido a diminuição da oxidação do NADPH e a consequente redução do oxigênio do estroma em $O_2 \cdot -$ (ânion superóxido), causando danos às membranas lipídicas, DNA e ao aparato fotossintético por meio da oxidação dessas estruturas (BARBOSA et al., 2023; SOUZA et al., 2020).

Os efeitos da falta de água no metabolismo vegetal têm implicações significativas em todo o ciclo de vida das plantas. Por exemplo, nas sementes ela influenciará negativamente sua germinação, uma vez que nesse processo, o acesso a água é fundamental, seja pela quebra da dormência e/ou hidratação da semente para então haver o *start* da germinação. O processo reprodutivo também poderá ser afetado pela perda de turgescência das peças florais, o que poderá causar limitações de acesso na atividade de polinização, ou em um grau mais severo ação de abortamento dos aparelhos reprodutivos e até mesmo dos frutos (CAMPOS et al., 2021). Assim, fica claro que os efeitos do estresse hídrico no metabolismo vegetal têm consequências que se estendem por todo o ciclo de vida das plantas, desde a germinação até a reprodução.

Como ferramenta de combate ao estresse hídrico, as plantas podem apresentar estratégias morfológicas, fisiológicas e moleculares. As respostas morfológicas envolvem redução de área foliar, aumento do crescimento radicular, alterações na dinâmica de relação raiz e parte aérea e perda de folhas, atividades comuns visualizadas em plantas da Caatinga, indicando ajustamentos ontogenéticos que favoreçam a superação do estresse (CAMPOS et al., 2021; HINOJOSA et al., 2018).

Estratégias fisiológicas envolvem principalmente regulação osmótica e defesa antioxidante. O ajuste osmótico favorece o controle do movimento estomático, que em decorrência do armazenamento intracelular de solutos, possibilita a manutenção da transpiração, fotossíntese e ainda a expansão foliar (JUNIOR; LUCENA, 2022). Atuando na osmorregulação celular favorecendo a turgescência das células, esses solutos podem ser íons inorgânicos (potássio e cloro), açucares (sacarose), aminoácidos (prolina) e proteínas (JUNIOR; LUCENA, 2022; SOUZA et al., 2020). Alterações nos teores de pigmentos (clorofila e carotenoide) também são uma via de proteção celular contra o déficit hídrico (SOUZA et al., 2020).

A atividade antioxidante ocorre em decorrência da presença natural de EROs, como aníon superóxido ($O_2 \cdot -$) e peróxido de hidrogênio (H_2O_2), mas que em condições de estresse

é favorecido seu acúmulo tornando-se uma problemática. O mecanismo de defesa antioxidante nas células pode ocorrer de maneira enzimática e não enzimática. São exemplos do sistema não enzimático os carotenoides, ascorbato e glutationa, enquanto se enquadram no sistema enzimático as enzimas superóxido dismutase (SOD), catalase (CAT) e ascorbato peroxidase (APX) (SOUZA et al., 2020). Ambos os sistemas atuam para a promoção de uma otimização funcional celular.

A terceira via, molecular, atua através da ativação de rotas e funcionamento de proteínas do estresse, atuando como osmoproterores, como as aquaporinas (HINOJOSA et al., 2018; JUNIOR; LUCENA, 2022). As proteínas aquaporinas atuam na manutenção homeostática do fluxo da água em tecidos vegetais quando em condições de variação de disponibilidade hídrica, demonstrando diferentes vias de abertura do poro como EROs, fitormônios, pH, etc. Atuam bioquimicamente no combate aos estresses causados pela restrição hídrica por melhorar o conteúdo de água e a atividade enzimática, reduzindo os danos à membrana e radicais de oxigênio. Sua expressão no sistema radicular promove aprimoramento substancial no desenvolvimento de raízes, desempenhando papel na tolerância à seca. Por transportarem água, mas também CO_2, beneficiam na fisiologia vegetal ao reduzir a suscetibilidade aos efeitos do estresse por melhorarem taxas de fotossíntese, condutância estomática e transpiração (PATEL; MISHRA, 2021).

3.3 DUAS ESPÉCIES: *PAVONIA VARIANS* E *MEGATHYRSUS MAXIMUS*

3.3.1 *Pavonia varians* Moric (Malvaceae)

Pavonia varians Moric. é uma espécie vegetal pertencente à família botânica Malvaceae. Conhecida popularmente como malva-cabeça-de-veado, malva-grande ou malvão, é um táxon endêmico brasileiro com distribuição registrada para a região nordeste, presente nos estados do Ceará, Bahia, Pernambuco, Piauí e Rio Grande do Norte, sendo relatada para regiões de Restinga e Caatinga (ATHIÊ-SOUZA et al., 2019; FERNANDO et al., 2020; LEAL et al., 2007; SANTOS-FILHO et al., 2011).

P. varians é uma espécie que pode ser encontrada apresentando diferentes formas de vida (hábito herbáceo, subarbustivo e arbustivo). Possuindo caule ereto no início do desenvolvimento, com a maturidade, podendo alcançar comprimento de 1-2,5m, apresenta ramos cilíndricos que se desenvolvem radialmente de forma quase paralela ao solo, apresentando como indumento tricomas estrelados, em maior frequência, ou simples. Apresenta inflorescência uniflora, originando-se da axila foliar, de cor amarelada com manchas vináceas

na base de suas pétalas (ESTEVES, 2015; MOREIRA; BRAGANÇA, 2011). Suas folhas palmadas se apresentam com 3 a 7 lobos, marginalmente onduladas, crenadas-serrilhadas, de caráter pubescente, demonstram em suas faces (adaxial e abaxial) tricomas estrelados e poucos estrelados como indumento, conferindo às folhas aspecto velutino verde acinzentado. Com ciclo de vida perene, desenvolve-se em substrato arenoso representativamente às margens do Rio São Francisco (ESTEVES, 2015; MOREIRA; BRAGANÇA, 2011).

Talvez por sua distribuição, registrada unicamente à região Nordeste brasileira, estudos que investiguem diretamente *P. varians* sejam pouco desenvolvidos. Os estudos que contemplam a espécie, no geral, referem-se à listagens botânicas que buscaram descrever a flora de determinados ambientes (ATHIÊ-SOUZA et al., 2019; GIULIETTI et al., 2004; GOMES; RODAL; DE MELO, 2006; SANTOS-FILHO et al., 2011) ou, indiretamente, dentro de trabalhos que contemplam a relação animal-planta (MENEZES et al., 2021; NOBRE, 2007; SCHLINDWEIN et al., 2012, 2009).

Alguns autores investigaram de forma mais específica a espécie, como nos trabalhos de Leal et al. (2007) e Fernando et al. (2020). Na medicina tradicional é relatado que as folhas de *P. varians* são utilizadas como agente anti-inflamatório para complicações na boca e garganta e no combate a infecções no sistema digestivo (LEAL et al., 2007). Fernando et al. (2020) através de seu estudo realizaram a descrição do perfil fitoquímico de *P. varians*, possibilitando o conhecimento da presença de de esteróides e triterpenos, taninos, cumarinas e, destacadamente, flavonóides e alcalóides que apresentam ação antioxidante. Com sua análise *in sílico* foi possível verificar ação hipoglicemiante através da interação tirilosídeos e a enzima α-amilase de *P. varians* (FERNANDO et al., 2020). *P. varians* foi descrita como elemento dentro da rede trófica da abelha *Ptilothrix plumata* Smith, sendo uma das principais fontes de provento no estágio larval da espécie de abelha nativa (SCHLINDWEIN et al., 2012, 2009).

Percebe-se que há ainda muito a se conhecer sobre a espécie. Estudos direcionados objetivamente a compreensão da ecologia e fisiologia da espécie se fazem necessários. Ao considerar a distribuição da espécie a uma região altamente antropizada como a Caatinga, sua presença relatada a áreas agricultáveis, e não obstante, os efeitos das mudanças climáticas, é uma espécie que necessita de mais esforços para desvendá-la e abrir espaço para tomada de medidas para sua conservação e consequentemente para a manutenção da estrutura ambiental.

3.3.2 *Megathyrsus maximus* B.K.Simon & S.W.L.Jacobs (Poaceae)

Megathyrsus maximus (Jacq.) B.K.Simon & S.W.L.Jacobs é uma espécie vegetal pertencente à família botânica Poaceae. Devido a suas diferentes variedades designadas, pode

ser popularmente conhecida como capim-sempre-verde, capim-colonião, capim-mombaça ou capim-guiné, tendo sido outrora classificado com outras nomenclaturas, tendo como sinonímias *Panicum maximum* ou *Urochloa máxima* (SILVA, 2020).

De hábito herbáceo, *M. maximus* se apresenta em touceiras geralmente com 2 m de altura, mas sendo possível se desenvolver até alcançar 4,5 m de altura. Possui caule ereto, do tipo colmo, e rígido com a presença de estrias de componente ceroso na região dos entrenós e pilosidade como na região dos nós como indumento. Apesar de se desenvolver em densas touceiras, apresenta curtos rizomas e colmos raramente ramificados, contudo, um profundo sistema radicular (ROJAS-SANDOVAL; ACEVEDO-RODRÍGUEZ, 2022; SILVA, 2020). Aparato foliar incompleto, mostrando ausência de pecíolo. A lâmina foliar pode se apresentar em diferentes tons de verde (claro, escuro, verde azulado), é estruturalmente lineares-lanceoladas, base estreita, ápice acuminado, sem indumentos (lisas) ou apresentado indumentos da forma de pilosidade esparsa ou pubescente. Apresenta inflorescência do tipo racemosa na forma de panícula, com espiguetas solitárias com coloração esverdeada antes da maturação, onde passa assumir coloração avermelhada (ROJAS-SANDOVAL; ACEVEDO-RODRÍGUEZ, 2022; SILVA, 2020).

No Brasil, *M. maximus* é amplamente distribuída em território nacional. Ainda que se apresente com status de provável ocorrência para o estado de Rondônia (SILVA, 2020), Rojas-Sandoval e Acevedo-Rodríguez (2022) demonstram sua presença para todo o território nacional, tendo abrangência sobre todas as regiões com ocorrência para todos os domínios fitogeográficos presentes no país.

É possível encontrar, em menor ou maior grau, 13 variedades distribuídas em território nacional. Desde o tradicional cultivar Colonião, sendo o primeiro esforço da EMBRAPA no melhoramento da espécie, como também, Vencedor, Tanzânia-1, Mombaça, Massai, BRS Zuri, BRS Tamani, BRS Quênia. IAC Tobaiatã, IAC Centenário e IAC Centauro são outras cultivares presentes no Brasil como resultado dos esforços do Instituto Agronômico de Campinas no melhoramento de *M. maximus*. Como resultados das pesquisas do Instituto de Zootecnia de São Paulo, é possível encontrar indivíduos das cultivares IZ-1 e Aruana (IZ-5) (JANK, 2003; JANK et al., 2014, 2017; JANK et al., 2022).

Sua distribuição no globo se estabelece de forma ainda mais ampla. Originária do continente Africano, atualmente é descrita como espécie Pantropical. Teve sua introdução estabelecida para o continente Americano, com destaque especial para a porção Sul do continente com exitoso estabelecimento e distribuição, assim como Oceania, e em menor proporção para os continentes Europeu e Asiático (ROJAS-SANDOVAL; ACEVEDO-RODRÍGUEZ, 2022).

Sua grande distribuição está relacionada ao forte potencial forrageiro, sendo utilizada amplamente para pasto em diversas partes do mundo. Sua dispersão está associada diretamente a facilidade de colonização e ampla distribuição. Ainda que uma das principais formas de multiplicação da espécie seja por propagação de rizomas ou repicagem de touceiras, é através da anemocoria sua principal forma de dispersão (ROJAS-SANDOVAL; ACEVEDO-RODRÍGUEZ, 2022). Suas sementes tem o vento como principal dispersor. Levando em consideração que sua presença está situada em campos abertos para o forrageio animal, fica evidente a facilidade nesse deslocamento de sementes a partir da ação do vento.

Frente às características de manejo, estratégias ecológicas e a contínua e crescente demanda ao setor agropecuário para provisão alimentar global é plausível voltar os olhos para uma temática de importante relevância: invasões biológicas. De forma geral, invasão biológica é um processo que ocorre através da introdução de espécies em um ambiente outro que não o de sua ocorrência natural, e dessa forma a espécie não-nativa se estabelece ao ponto de superar os indivíduos nativos do ambiente (SILVA; FABRICANTE, 2019). Vale a ressalva que a invasão biológica não se esvazia apenas em critérios biogeográficos ou de mensuração de impacto, sendo necessária a uma percepção integrativa. Dessa forma não apenas espécies exóticas, mas espécies nativas também podem apresentar essa potencialidade caso encontre condições no ambiente que a favoreça na competição por recursos (VALÉRY et al., 2008).

No Brasil, *M. maximus* é considerada como exótica naturalizada de acordo com (SILVA, 2020), de fato sendo uma espécie, que até pelo tempo de introdução, está eficientemente estabelecida as condições encontradas, possibilitando a automanutenção das populações. *M. maximus* ao apresentar características semelhantes a outras espécies tidas como invasoras (RHODES et al., 2021), desperta atenção sobre sua atuação invisível, principalmente tendo em vista que ela não se restringe apenas às áreas às quais é cultivável para provisão animal, mas presente de forma ampla para outros ambientes como áreas degradadas, antropizadas, terrenos baldios e beira de estradas (SIBBR, 2022).

De acordo com o Sistema de Informação sobre a Biodiversidade Brasileira, *M. maximus* assume um status de espécie exótica invasora, tendo entrada na Lista de Espécies Exóticas Invasoras do país (SIBBR, 2022). Além do status da espécie para o país, ainda é relatado outros países que compartilham o status de invasibilidade para a espécie, como Estados Unidos (Havaí e Guam), Equador (Ilhas Galápagos), Austrália, China, Japão Coréia entre outros (SIBBR, 2022).

Exceto para o Nordeste, todas as regiões apresentam estados onde *M. maximus* é considerada como espécie exótica invasora: Pará, Rondônia e Amazonas (Norte), Santa Catarina (Sul), Minas Gerais e São Paulo (Sudeste), e Mato Grosso, Mato Grosso do Sul e

Distrito Federal (Centro-Oeste) (SIBBR, 2022b). Contudo estudos como de Fabricante et al. (2021) e Reis et al. (2022a, 2022b) fazem apontamentos da existência de *M. maximus* em áreas além de pastos para provisionamento das culturas pecuárias, estando presentes em áreas protegidas como Parque Nacional Serra de Itabaiana ou na Área de Proteção Morro do Urubu (Sergipe).

Para a Caatinga, Silva e Fabricante (2019) registraram a presença da espécie no Parque Nacional do Catimbau, uma área de proteção à biodiversidade presente no estado nordestino de Pernambuco. Dadas as características da espécie fica evidente a necessária investigação de seu comportamento em comparação a espécies nativas e sua capacidade de superação da flora nativa, como observado por Targino (2023) em seu estudo do impacto de *M. maximus* para uma área de Mata Atlântica.

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CAPÍTULO I

Supplementation with NPK previous to water restriction confers different biochemical and growth modulations in a native and an exotic species present in a Brazilian semi-arid region

Matheus Bessa da Silva, Marcus Vinícius Loss Sperandio

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Title: Supplementation with NPK previous to water restriction confers different biochemical and growth modulations in a native and an exotic species present in a Brazilian semi-arid region

Authors: Matheus Bessa da Silva[a]*, Marcus Vinícius Loss Sperandio[ab]

Affiliation: [a]Graduate Program in Biodiversity, Federal Rural University of Pernambuco, R. Dom Manuel de Medeiros, Dois Irmãos, CEP 52171-900, Recife, Pernambuco, Brazil. [b]Federal Rural University of Pernambuco, Department of Biology, R. Dom Manuel de Medeiros, Dois Irmãos, CEP 52171-900, Recife, Pernambuco, Brazil

*** Corresponding author:** matheus.bessa@ufrpe.br

ORCID:

Matheus Bessa da Silva (0000-0002-1926-567X), Marcus Vinicius Loss Sperandio (0000-0001-7289-3336)

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Abstract

Drought and altered nutrient dynamics are being aggravated by anthropic actions, especially in semiarid environments such as the Brazilian Seasonally Dry Tropical Forests (Caatinga). However, it is not known how nutrients levels can affect growth and biochemical responses in plants present in Caatinga to drought. The aim of this study was to evaluate morphological and biochemical responses in *Pavonia varians* (native specie with medicinal potential) and *Megathyrsus maximus* (invasive exotic specie used in cattle feeding) present in Caatinga with application of Nitrogen, Phosphorus and Potassium (NPK) under water restriction and rehydration. The experiment was conducted in a greenhouse with seedlings collected in Catimbau National Park (Pernambuco state-Brazil) in a factorial with NPK application and water regimes. Plant harvest was performed after water restriction and then after rehydration. NPK application improved *P. varians* responses to water deficit, increasing leaves dry weight, total N, total K, and proline content. Additionally, MDA and H_2O_2 decreased in *P. varians* under water restriction with NPK application. In contrast, *M. maximus* without NPK application under water restriction increased SOD and CAT activity and decreased MDA levels. After rehydration, both species exhibited growth recovery, but NPK application in *P. varians* increased total K and total soluble carbohydrates, meanwhile *M. maximus* increased number of leaves and root dry weight. Species have different mechanisms for combating drought stress, especially non-enzymatically through proline. Nutritional treatment with pre-stress NPK showed an effective alternative against oxidative damage, especially for the native species *P. varians*.

Keywords: Caatinga. Oxidative stress. *Pavonia varians*. *Megathyrsus maximus*.

1. Introduction

Drought is one of the most common stress challenging plant growth, affecting plant distribution and survival in tropical dry forests (Zhang et al. 2021; Kopecká et al. 2023). Plants in the Brazilian Seasonally Dry Tropical Forests (Caatinga) are frequently exposed to water deficits due to lower precipitation and high temperatures, in addition to shallow soils (Santos et al. 2014). In addition, the Caatinga biome presents other challenges, such as high population density and anthropic disturbances in the environment, affecting native plant population, introduction of exotic species to cattle feeding, and soil nutrients loss (Silva et al. 2018; Oliveira et al. 2021). Lower nutrient availability may decrease plant growth and affect plant distribution, especially in recovering areas (Pinheiro et al. 2020; Leite et al. 2022).

Drought decreases plant growth by water loss, stomata closure, lower photosynthesis, reduced shoot growth, Reactive Oxygen Species (ROS) and oxidative stress (Souza et al. 2020; Campos et al. 2021; Barbosa et al. 2023). Among the biochemical responses to drought, osmotic adjustment with proline, soluble sugars and Potassium (K) aid plants maintain cell turgor by decreasing water potential (Ψw) (Souza et al. 2020; Junior and Lucena 2022). In addition, drought induces the production of ROS, increasing superoxide (O_2·-) and hydrogen peroxide (H2O2) which can cause membrane and photosystems damage (Sun et al. 2020). The enzymes Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX) and Catalase (CAT) catalyze the detoxification of ROS and can lower cellular damage under drought conditions (Sun et al. 2020). In addition, nutrients such as Nitrogen (N), phosphorus (P) and Potassium (K) can affect plant responses to water deficit, representing an important environmental resource to plant growth and water deficit response.

Nitrogen (N) improves photosynthesis and plant growth by participating in chlorophyll structure and protein synthesis (Xu et al. 2019). Additionally, N can affect drought responses, and vice-versa, with an interaction in plant responses to drought and N supply (Ding et al.

2018). Drought affect enzymes activity related to N assimilation (Nitrate Reductase, nitrite reductase, glutamine synthetase (GS)/glutamine oxoglutarate aminotransferase/glutamate synthase), nitrate transporter expression (*NRT1.1*) in sweet potato (Xia et al. 2020). Drought and heat decreased N assimilation related enzymes, however, N supply increased SOD, APX, CAT, Proline, soluble sugar, and soluble protein in winter wheat (Ru et al., 2023). On the other hand, Meng et al. (2021) reported that increased N through agriculture fertilization may influence grassland community relations between drought, N and plant production. P and K improve plant responses to drought by increasing antioxidant capacity and compatible solutes (Zhu et al. 2020; Anokye et al. 2021; Khan et al. 2023). Altogether, N, P and K are important to plant responses to drought, however, little is known about the biochemical responses to water deficit and nutrients supply of important species present in dry lands in Caatinga, such as *Pavonia varians* (native specie present in regeneration areas and used in traditional medicine with antioxidant potential) and *Megathyrsus maximus* (invasive exotic specie used in cattle feeding).

Pavonia varians Moric. is a plant species with a subshrub/shrub habit native to Brazil, its distribution covers caatinga and restinga environments, restricted to the Brazilian northeast in the states of Ceará, Bahia, Pernambuco and Piauí (Leal et al. 2007; Santos-Filho et al. 2011; Athiê-Souza et al. 2019; Fernando et al. 2020). In general, it is included indirectly in studies of botanical lists or studies that address insect-plant interactions. (Kiill 2001; Giulietti et al. 2004; Gomes et al. 2006; Schlindwein et al. 2009, 2012; Santos-Filho et al. 2011; Manhães 2015; Athiê-Souza et al. 2019). In folk medicine, *P. varians* leaves are used to combat infections in the digestive system and as an anti-inflammatory agent for the mouth and throat (Leal et al. 2007). In their study, Fernando et al. (2020) investigated its phytochemical profile describing the presence of steroids and triterpenes, tannins, coumarins and, notably, flavonoids and alkaloids. Investigations show the *in vitro* antioxidant capacity of *P. varians* extracts (Leal et

al. 2007), as well as their hypoglycemic action *in silico* (Fernando et al. 2020). It is a species present in the interaction network of native bee fauna, being one of the main sources of income in the larval development of the solitary bee *Ptilothrix plumata* Smith (Schlindwein et al. 2009, 2012).

In turn, *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L.Jacobs is a species of grass originated from tropical and subtropical environments on the African continent, but which currently has a wide distribution throughout the Americas, Asia, Europe and Oceania (Rojas-Sandoval and Acevedo-Rodríguez 2022; Soti and Thomas 2022). For Brazil, the species is registered for all phytogeographical domains, being widely distributed in the national territory and can be categorized as naturalized status (Silva 2020), although other studies point out its invasive nature, which in turn implies impacts on the ecosystem as economic (Rojas-Sandoval and Acevedo-Rodríguez 2022; Soti and Thomas 2022). Its wide distribution is related to its use in cattle feed, being used to prepare hay and silage, or even *in natura*, making it possible to achieve high productivity rates in tropical regions such as Brazil (Viciedo et al. 2021; Soti and Thomas 2022). It is a species that shows good performance especially when exposed to the sun, presenting optimal growth in the range of 30° and 40°C, and in addition to the fact that it is known for having high protein levels, tolerance to grazing and resistance to environmental stress. like water deficit, makes *M. maximus* one of the main species in the world used in pasture for agriculture (Viciedo et al. 2021; Soti and Thomas 2022).

To survive water deficit, plants present biochemical and physiological adaptations, responding to oxidative stress and water status (Haak et al. 2017; Gong et al. 2020; Miryeganeh 2021; Barbosa et al. 2023). Additionally to the ecological and/or economic importance of *P. varians* and *M. maximus*, studying their growth and biochemical responses to water deficit and rehydration with NPK supplementation will increase the knowledge of a native and exotic species of dry lands, which may be beneficial to future studies in plant communities and

regeneration (Santos et al. 2014; Leite et al. 2022). The aim of this study was to evaluate the growth and biochemical responses of *P. varians* and *M. maximus* to water deficit and rehydration after nutrient supplementation (Nitrogen, Phosphorus and Potassium - NPK). Thus, our hypothesis was that NPK supplementation improves *P. varians* and *M. maximus* responses to water deficit by reducing oxidative stress.

2. Materials and Methods

2.1 Collection of Material in the Field

Individuals of the native species *Pavonia varians* Moric (Malvaceae) and the exotic species, with naturalized status (Esteves 2015; Soti and Thomas 2022), *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L.Jacobs (Poaceae) were collected. The collection was carried out in a Caatinga environment, in the Catimbau National Park (PARNA Catimbau – 8° 34' 0.43"S 37° 14' 22.78"W) in Pernambuco/BR, a semi-arid region with a Seasonally Dry Tropical Climate.

Young healthy individuals with 10-20 cm of aerial part, free of diseases or signs of predation, were considered for the native species *P. varians.* For *M. maximus*, given its characteristic of propagation by tillering, small clumps were collected in the field for subsequent treatment and separation of individuals in a greenhouse, of which tillers with phytosanitary conditions similar to the native species and with leaf blade length were considered. 10-15 cm. The material was transported in containers (trays, boxes, buckets) with a little moistened soil to the place where the experiment was installed.

2.2 Experiment Location and Conditions

The experiment was carried out in a greenhouse at the Federal Rural University of Pernambuco, in Recife, in the state of Pernambuco/BR (8°00' 45.6"S 34°57' 03.8"W).

The individuals were individually transplanted, the day following collection, into low-density polyethylene bags with holes for water drainage with a capacity of 1.7 L. A mixture of

washed sand, plant substrate (BIOPLANT PLUS©, compost by MM 14-16-18, agricultural limestone, Sphagnum peat, coconut fiber, rice husk and pine bark) and commercial substrate (TERRA NOVA MAXXI©, containing organic compost, limestone, processed and decomposed bark and vermiculite, expanded), respectively, in the following proportion 3.5:1.5:1, leaving 2 cm of unfilled edge in each bag. Each bag with one individual was considered an experimental unit.

The soil chemical analysis recorded the following values: 1.49 g kg^{-1} of N, 3.80 $cmol_c$ dm^{-3} of Ca, 1.30 $cmol_c$ dm^{-3} of Mg, 1.02 $cmol_c$ dm^{-3} of K, 0.05 $cmol_c$ dm^{-3} of Al, 114.44 mg dm^{-3} of P and pH of 6.21.

The plants were left in the process of establishment and acclimatization, being watered every other day with the aid of a watering can for a period of 30 days after transplanting (DAT). From 31 DAT onwards, monitoring began to standardize the humidity of the experimental units to 70%.

Given the semi-arid characteristic of the species' origin, a standard soil humidity of 70% was chosen since 100% humidity could become a stress agent due to lack of oxygen to the roots. Soil moisture and its determination to maintain 70% were carried out using the portable soil moisture sensor HydroSense II (Campbell Scientific) on alternate days.

During the application of water restriction, which occurred at 53-62 DAT, a maximum average temperature of 32.55 ºC was recorded. An average daily relative humidity of 59.28% was recorded for the same interval, making it possible to record an average minimum relative humidity of 37% in the greenhouse environment. The Vapor Pressure Deficit Index – DPV recorded an average of 3.35 kPa for the same period, reaching maximum values of 4.40 kPa and 4.17 kPa.

2.3 Experimental Conduct and Design

The experiment followed a Completely Randomized Design (CRD), with 5 replications, in two stages: Water Restriction and Rehydration, both in a 2×2 scheme with two water levels (maintenance of irrigation at 70% soil moisture and suspension of irrigation/restoration of irrigation at 70% humidity) and two nutritional levels (1- with NPK supplementation and 2- without NPK supplementation).

Nutritional treatment began on 31 DAT by applying the first dose of supplementation with Nitrogen (N), Phosphorus (P) and Potassium (K), having as sources potassium nitrate (KNO_3), monobasic potassium phosphate (KH_2PO_4 and dibasic potassium phosphate (K_2HPO_4), marking the beginning of the experiment. Four doses (40ml) of NPK solution were applied with a final concentration of each of the nutrients: N: 6 mM; P: 2 mM; K: 6 mM. The other half of the experimental units remained without NPK solution supplementation. Nutritional treatment was considered until 51 DAT. This configuration of the nutritional test was common for both stages of the experiment.

After the end of the nutritional treatment, at 52 DAT, in a group of plants, containing plants without and with NPK supplementation, water restriction treatment was started by suspending irrigation, while the other experimental units remained irrigated. The suspension was maintained until the plants showed signs of stress through wilting or curling of the leaves, and collection was then carried out, marking the end of the stress experiment at 62 DAT.

After collecting the water restriction phase, the rehydration stage began with the remaining experimental units not collected. All plants that were under suspension had their watering restored at 63 DAT, returning soil moisture maintenance to 70%. Rehydration treatment was considered for 20 days after reestablishing irrigation. At 83 DAT, the end of the rehydration experiment was marked by the collection of experimental units.

2.4. Growth Analysis and Water Relative Content

The growth of the species was evaluated based on the Number of Leaves fully expanded and photosynthetically active during the experiment, Dry Mass of leaves, roots and stems and Root/Leaves ratio. The dry mass of the stem was determined after drying in an oven at 70°C for five days in paper bags. Root samples were dried in an oven at 70°C for one week in paper envelopes, and through the relationship of fresh mass and dry mass, the root dry mass of the individuals was proportionally determined.

The Relative Water Content was determined from the relationship between the fresh, turgid and dry masses of individual leaf discs (Barrs and Weatherley 1962; Gomes Junior et al. 2021). From the TRA of the leaves, it was possible to determine the proportional dry mass of the leaves.

2.5. Biochemical Analysis

Fresh plant material, leaf and root, was used to carry out biochemical analyses. The collected material was subjected to rapid freezing in liquid nitrogen and kept refrigerated in a freezer at -20°C. With the aid of a pestle and mortar, the frozen material was macerated again with liquid nitrogen, remaining at low temperature until analysis was carried out.

The quantification of chlorophyll (a, b and total) and carotenoids was determined through an extract using 80% acetone in the leaves of the species studied (Lichtenthaler 1987; Bezerra Neto and Barreto 2011). The Chlorophyll content was determined non-destructively (*in vivo*), after supplementation with NPK, using the portable chlorophyll meter SPAD-502 (Konika Minolta) (Online resource 3).

The extracts for the analysis of Total Protein and oxidative stress enzymes (Superoxide Dismutase – SOD, Catalase – CAT, and Ascorbate Peroxidase – APX) were obtained using 100mM Potassium Phosphate Buffer pH 7.5, added with Ethylenediamine Tetra-Acetic Acid (EDTA) 1 mM and Dithiothreitol (DTT) 3 mM (Azevedo et al. 1998). Polyvinylpolypyrrolidone (PVPP) was used in the extraction process as an antioxidant agent.

The entire extraction procedure was carried out on ice. Determination of Total Protein content was made according to Bradford (1976), using Bovine Serum Albumin (BSA) as standard.

SOD activity was evaluated by the enzyme's ability to inhibit the photoreduction of Nitrotetrazolium Blue (NBT) (Giannopolitis and Ries 1977). CAT activity was estimated according to Havir and McHale (1987) with adaptations from Azevedo et al. (1998), determined by the decrease in absorbance during 1 minute (first and last reading). APX activity was carried out according to Nakano and Asada (1981), monitoring the ascorbate oxidation rate in a spectrophotometer for 1 minute. During the reaction process, the extracts were kept at low temperature.

To determine the oxidative markers Malondialdehyde (MDA) and Hydrogen Peroxide (H_2O_2), the extract was prepared, under low temperature, using 0.1% Trichloroacetic Acid (TCA) as an extracting agent. PVPP was also used during extraction. Lipid peroxidation was analyzed by determining the MDA content by heating in a water bath at 95°C (Heath and Packer 1968). The determination of H_2O_2 in the samples was carried out using 1M potassium iodide, under low temperature and in the absence of light (Alexieva et al. 2001; Loreto and Velikova 2001).

From 80% alcoholic extract it was possible to analyze Total Soluble Carbohydrates, Nitrate and Free Amino Acids (Bezerra Neto and Barreto 2011). The content of Total Soluble Carbohydrates was given using the Antrona spectrophotometric method (Yemm and Willis 1954; Bezerra Neto and Barreto 2011). Nitrate determination was carried out using the Salicylic Acid colorimetric method by nitrifying its carbon 5 (Cataldo et al. 1975; Bezerra Neto and Barreto 2011). The Ninhydrin method was used to determine Free Amino Acids, through the decarboxylation of amino acids via heating (Yemm et al. 1955; Bezerra Neto and Barreto 2011).

Free Proline was determined by the Acid Ninhydrin method by heating and reacting with toluene the extract obtained from 3% Sulfosalicylic Acid (Bates et al. 1973; Bezerra Neto and Barreto 2011).

The plant extracts of Nitrogen, Phosphorus and Potassium were obtained wet via acid digestion for macronutrients with Sulfuric Acid in a digester block on leaf material dried in an oven at 70°C (Tedesco 1982; Detmann et al. 2021). The total Nitrogen content was determined by the Kjeldahl method and titrated with 0.02N HCl (Tedesco 1982; Detmann et al. 2021). The total phosphorus content was determined by the Molybdate-Vanadate colorimetric method (Bataglia et al. 1983; Miyazawa et al. 2009; Bezerra Neto and Barreto 2011). The total potassium content was determined by flame photometry (Miyazawa et al. 2009; Bezerra Neto and Barreto 2011).

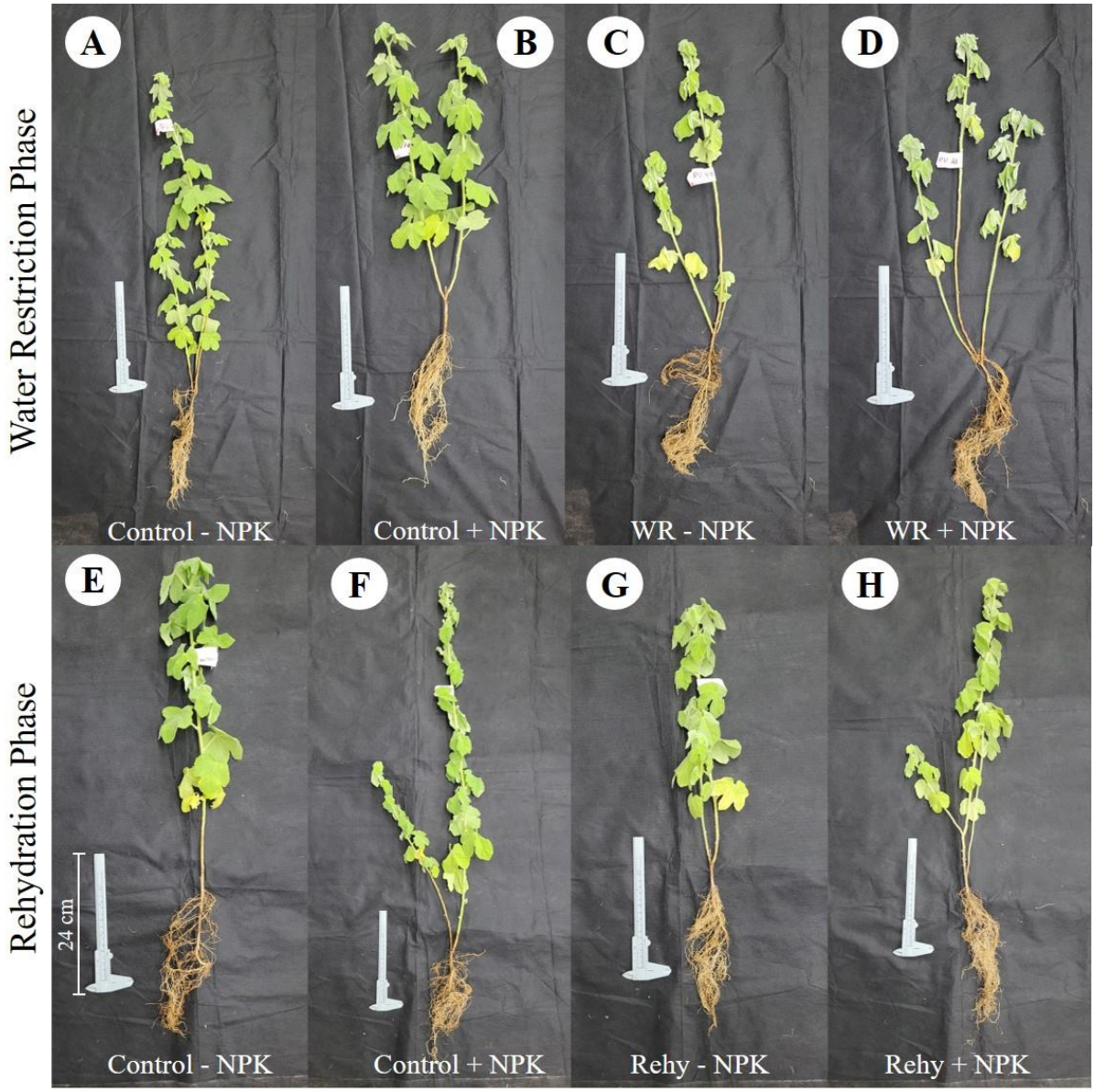
2.6. Statistical Analysis

The results of the analyzes were tested for the normality of the distribution of residues and submitted to analysis of variance (ANOVA) when the assumption of normality was met. When with non-normal distribution, data were transformed via logarithm or square root. The interaction was tested using the LSD test at $p < 0.05$. All statistical analyses were performed using the RStudio program (R Core Team 2023), including Hierarchical Cluster Analysis (HCA) Principal Component Analysis (PCA).

3. Results

Plants of *P. varians* and *M. maximus* after water deficit and rehydration are shown in Figure 1 and Figure 2, respectively. Ten days with water restriction, both species showed leaves wilting. The analysis of variance (Online resource 1 and Online resource 2) has shown that water restriction (WR), NPK supplementation (NPK) and their interaction differentially affected the growth (shoot and root), oxidative stress and biochemical parameters in *P. varians* and *M.*

267 *maximus* ($p < 0.05$). However, *P. varians* after rehydration only carbohydrates, nitrogen and
268 potassium were significantly affected by the treatments (Online resource 1). On the other hand,
269 *M. maximus* after rehydration were affected by the water and nutrients treatment on growth and
270 biochemical parameters (Online resource 2). The detailed responses of *P. varians* and *M.*
271 *maximus* in our study is presented below.



272
273 **Figure 1.** Plants of *P. varians* harvested 10 days after water restriction (WR) (A-D) and 20
274 days after rehydration (Rehy) (E-H). A: no application of NPK without water restriction; B)
275 application of NPK without water restriction; C: no application of NPK with water restriction;
276 D: application of NPK with water restriction; E: no application of NPK without water
277 restriction; F: application of NPK without water restriction; G: no application of NPK with
278 rehydration after water restriction; H: application of NPK with rehydration after water
279 restriction. Scale (A-H): 24cm.

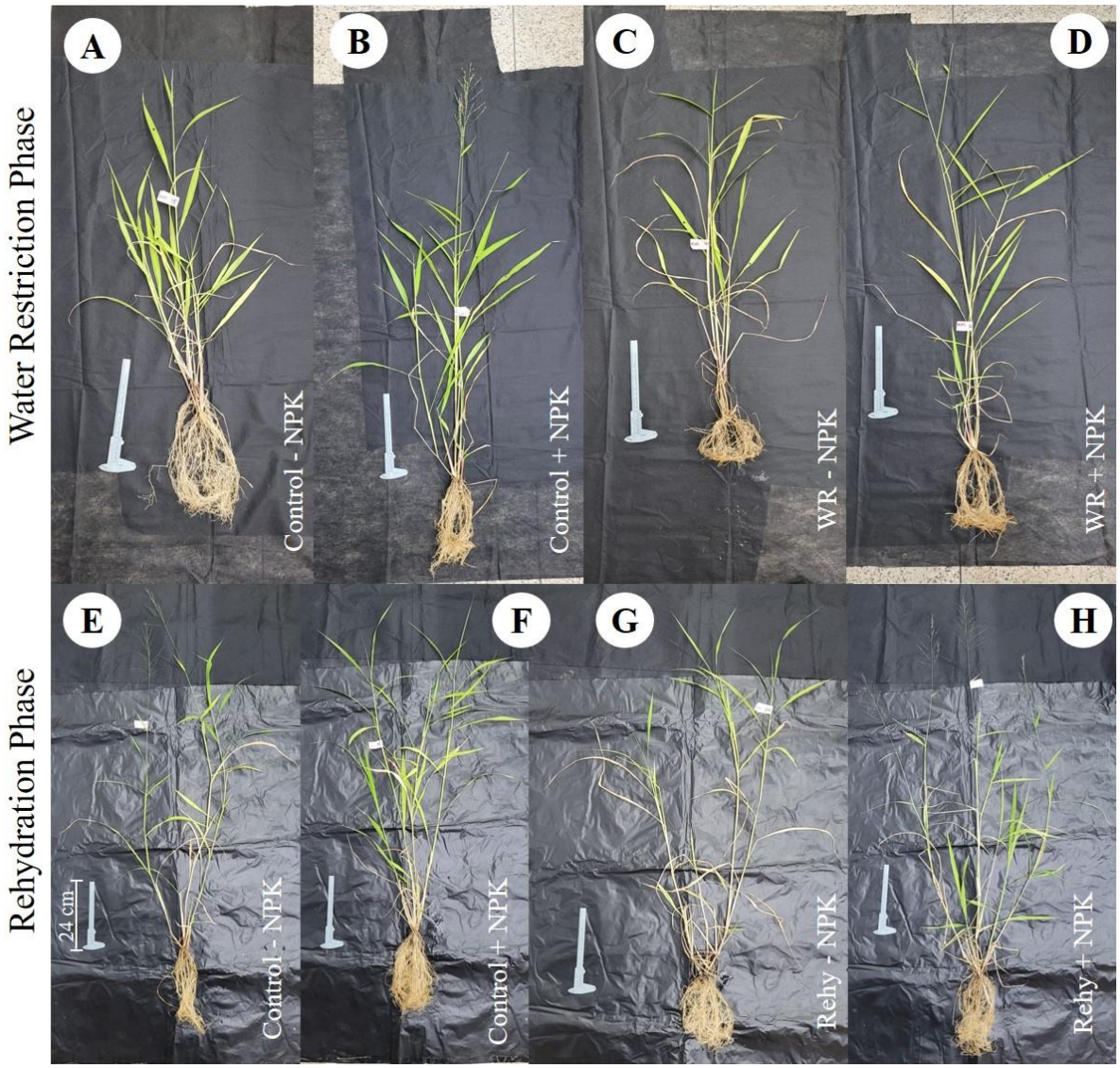


Figure 2. Plants of *M. maximus* harvested 10 days after water restriction (WR) (A-D) and 20 days after rehydration (Rehy) (E-H). A: no application of NPK without water restriction; B) application of NPK without water restriction; C: no application of NPK with water restriction; D: application of NPK with water restriction; E: no application of NPK without water restriction; F: application of NPK without water restriction; G: no application of NPK with rehydration after water restriction; H: application of NPK with rehydration after water restriction. Scale (A-H): 24cm.

3.1. Effect of NPK application on growth parameters under water deficit

Water restriction for 10 days did not affect the weight of *P. varians* (leaves, stem and root) (Figure 3 and Online resource 1), however, NPK application increased leaves dry weight by 89.4% and 81.5% under control (70% pot capacity) and water restriction, respectively. Consequently, root/leave ratio decreased with NPK application (Figure 3E). Water Relative

Content (WRC) was only affected by NPK application under control (70% pot capacity) (Online resource 1). *P. varians* with water restriction decreased WRC approximately 56.50% compared to control (Figure 3F). After rehydration, growth parameters of *P. varians* did not differ statistically ($p < 0.05$) (Figure 3 and Online resource 1), showing full recovery after water deficit for 10 days.

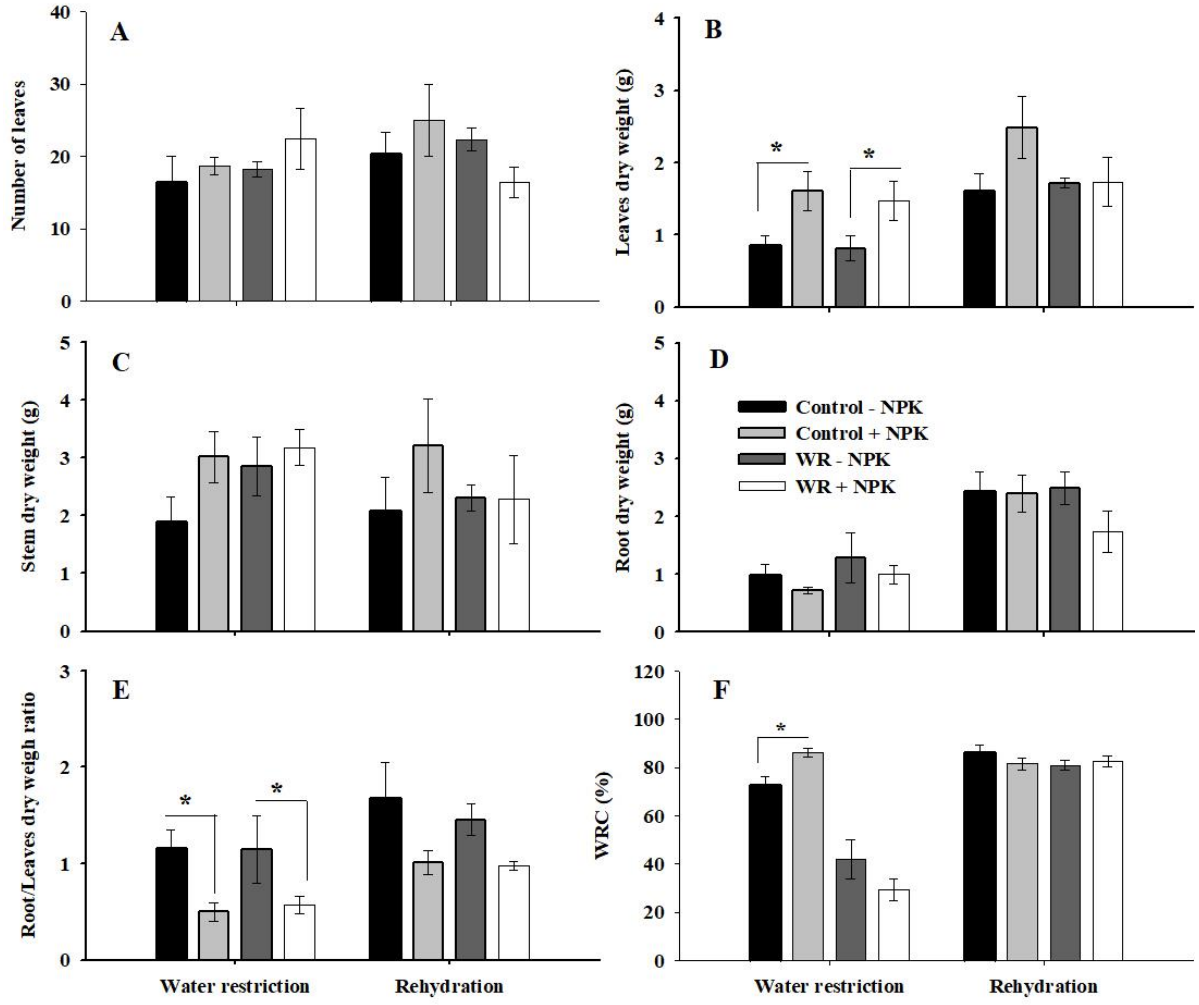


Figure 3. Growth parameters in *P. varians* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. WRC - Water relative content. * Represent the influence of NPK application on growth responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).

Growth of *M. maximus* was affected by water restriction and nutrients (Figure 4 and Online resource 2). *M. maximus* subjected to water restriction and NPK application increased number of leaves (50.7%) and stem dry weight (47%) compared to plants *M. maximus*

subjected to water restriction without NPK application. Leaves dry weight increased with NPK application only in control conditions (56.4%). Water restriction for 10 days significantly affected WRC ($p < 0.05$), however, WRC was not significantly affected by NPK application (Online resource 2). Rehydration affected growth parameters in *M. maximus* with NPK application (Figure 4 and Online resource 2), increasing number of leaves (67%), stem dry weight (220%), and root dry weight (137%) compared to plants with water restriction without NPK application.

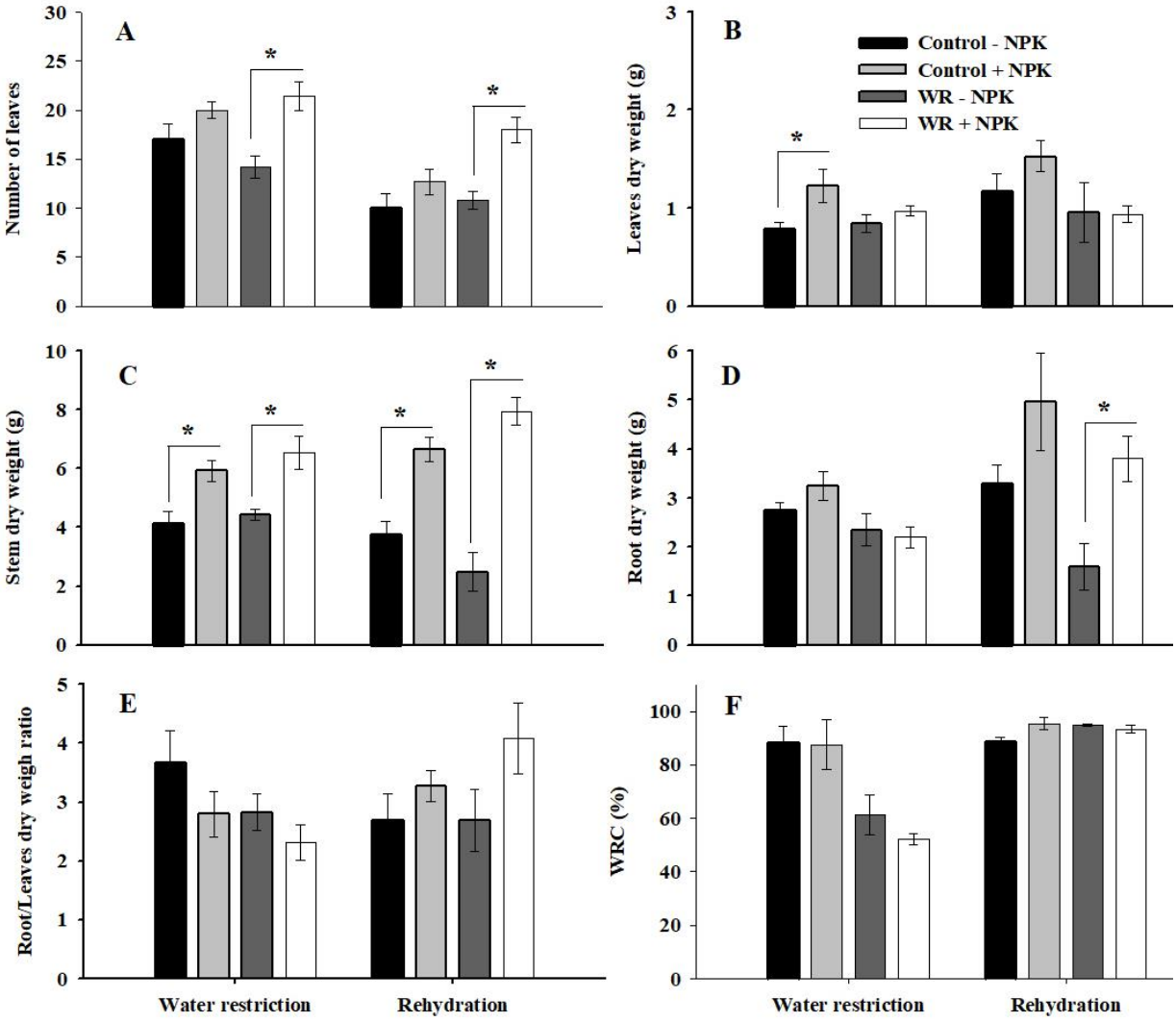


Figure 4. Growth parameters in *M. maximus* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. WRC - Water relative content. * Represent the influence of NPK application on growth responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).

3.2. NPK application modulated oxidative stress under water deficit

NPK application modulated CAT and APX activity in *P. varians* without water restriction (control), decreasing CAT activity (76.5%) and increasing APX activity (239%) compared to plants without water restriction (control) and no NPK application (Figure 5). Conversely, NPK application did not alter CAT and APX activity in *Pavonia varians* with water restriction for 10 days, compared to plants without NPK application. SOD activity was not significantly affected by water restriction or NPK application ($p < 0.05$). NPK application increased protein content in leaves (Figure 5D) and roots (Online resource 4) of *P. varians* in control conditions, compared to plants without water restriction and no NPK application. After 20 days of rehydration, there was no statistically significant change in SOD, CAT, APX activity, as well as protein content.

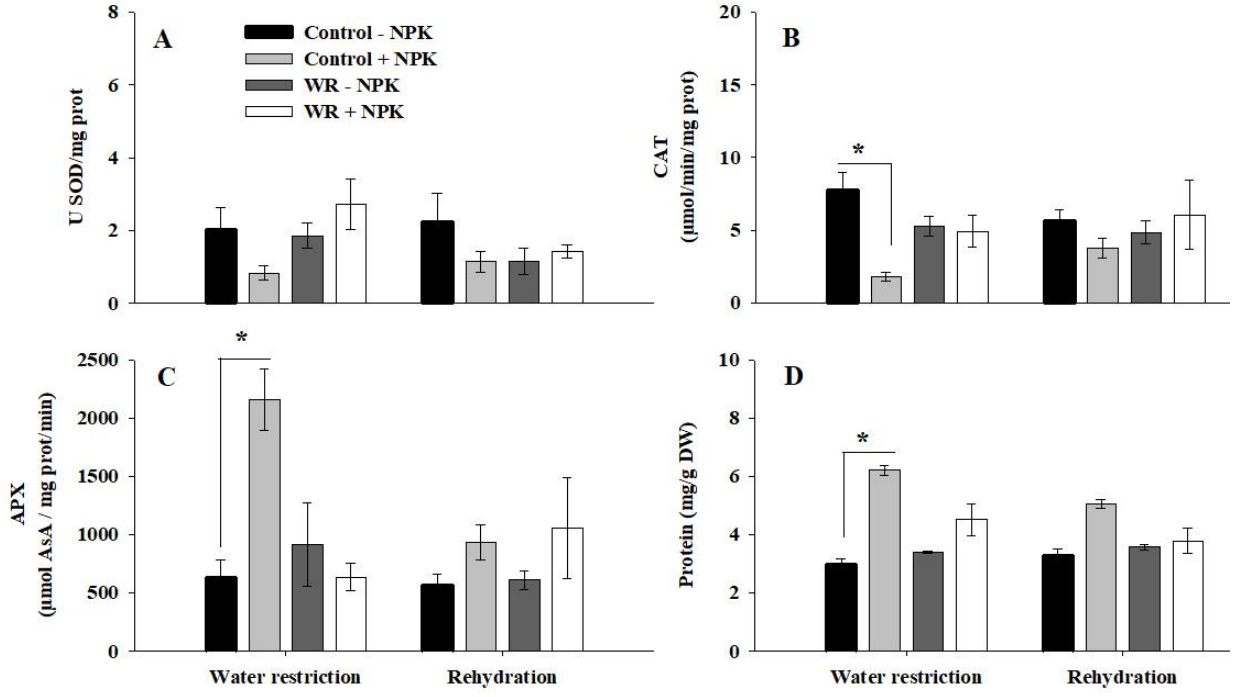


Figure 5. Superoxide Dismutase activity (SOD) (A), Catalase activity (CAT) (B), Ascorbate Peroxidase activity (APX) (C) and protein content (D) in leaves of *P. varians* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).

Plants of *M. maximus* with NPK application modulated SOD, CAT and APX activity under water restriction (Figure 6), decreasing SOD (36.7%) and CAT (65.1%) activity compared to plants under water restriction without NPK application. Conversely, APX activity increased (333.4%) with water restriction and NPK application compared to plants under water restriction without NPK application. Additionally, NPK application increased APX activity (102%) without water restriction (control) and after rehydration (163.6%), compared with control plants without NPK application. The protein content was higher in plants with NPK application compared to plants without NPK application (163%) under water restriction. The protein content in roots was not affected by water restriction and NPK application, however, after rehydration the protein content was higher with NPK application (Online resource 5).

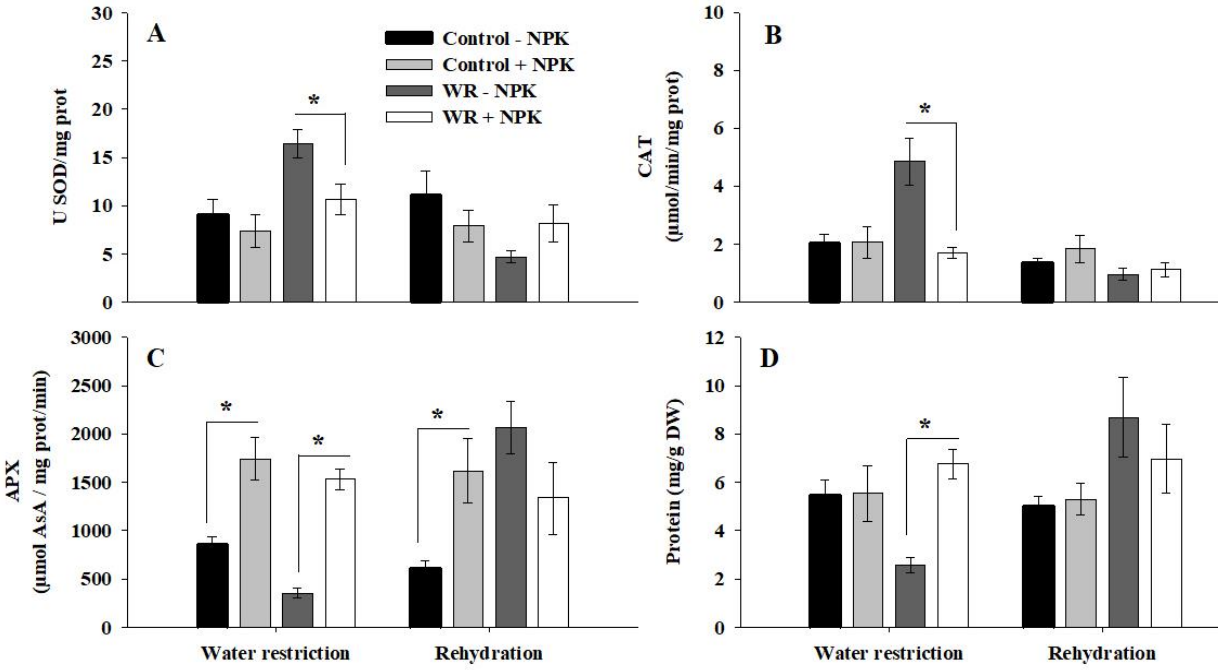


Figure 6. Superoxide Dismutase activity (SOD) (A), Catalase activity (CAT) (B), Ascorbate Peroxidase activity (APX) (C) and protein content (D) in leaves of *M. maximus* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).

The NPK application affected the Malondialdehyde (MDA) and Hydrogen peroxide (H_2O_2) differently in *P. varians* (Figure 7) and *M. maximus* (Figure 9). *Pavonia varians* subjected to water restriction decreased MDA (57.9%) and H_2O_2 (53.2%) levels when supplemented with NPK, compared to plants with water restriction and without NPK application (Figure 7). Twenty days after rehydration, MDA and H_2O_2 levels did not differ statistically ($p < 0.05$) (Figure 7 and Online resource 1). The content of chlorophyll *a*, chlorophyll *b* and total chlorophyll were not significantly affected by water restriction or NPK application at harvest after water restriction and rehydration (Online resource 1 and Online resource 6). However, *P. varians* exhibited higher SPAD index 11 days (43.5%) and 21 days after (16.3%) NPK application, compared to plants without NPK application (Online resource 3).

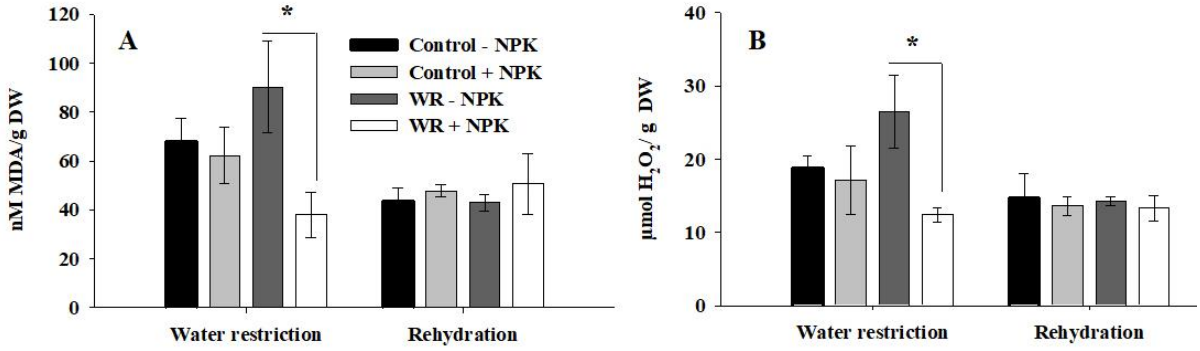


Figure 7. Malondialdehyde (MDA) (A) and Hydrogen peroxide (H_2O_2) (B) levels in leaves of *P. varians* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).

Conversely to *P. varians*, *M. maximus* without NPK application presented lower MDA content under restriction compared to plants with NPK application under restriction (Figure 8). H_2O_2 level was affected by water treatments (Online resource 2), where plants under restriction showed reduced levels (or lower content) compared to plants maintained in control at 70% soil humidity (Figure 8B). In turn, nutritional treatments, without or with supplementary

application of NPK, showed no difference in their action to reduce H_2O_2 levels in *M. maximus* (Figure 8B, Online resource 2). The content of chlorophyll *a*, chlorophyll *b* and total chlorophyll were affected by water restriction in *M. maximus* at harvest after water restriction and rehydration (Online resource2), however, NPK application did not affect chlorophyll content (Online resource 7). The SPAD index after 11 days and 21 days after NPK application were 78.2% and 43.8% higher compared to plants without NPK application (Online resource 3).

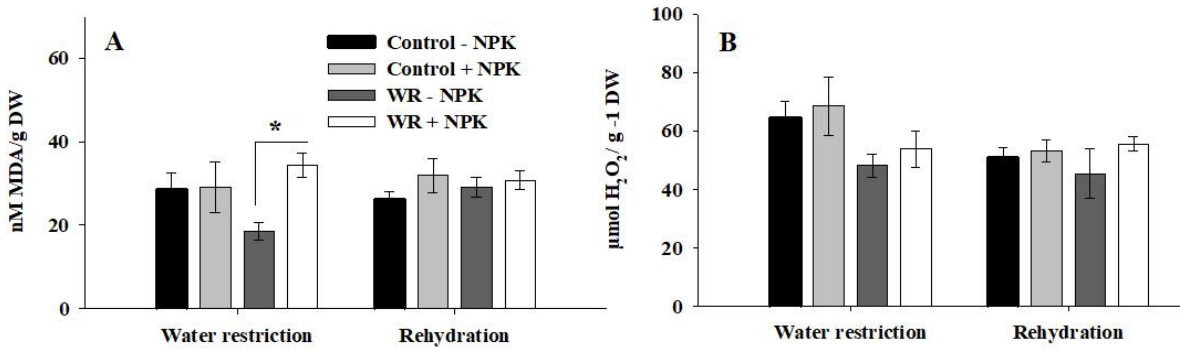


Figure 8. Malondialdehyde (MDA) (A) and Hydrogen peroxide (H_2O_2) (B) levels in leaves of *M. maximus* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).

3.3. Effects on proline, amino acids, total soluble carbohydrates and nitrate

The levels of Proline in *P. varians* plants responded significantly to NPK application when subjected to water restriction, showing an increase of 256% and 366% in concentrations in leaves and roots (Figure 9). Amino acids and Total Soluble Carbohydrates did not respond significantly to the NPK application when subjected to water restriction, however, control treatment with NPK application increased amino acids and Total Soluble Carbohydrates concentrations in the leaves of *P. varians* 65.4% and 139.5%, respectively, when compared to plants without nutrients also in a water control situation (Online resource 8). In roots, there were no significant differences in NPK application in either plants under water restriction or

control (Online resource 9). Nitrate did not show a significant difference to nutritional supplementation in the control and water suspension treatments in the leaves of *P. varians*, but it showed a 71.8% decrease in its concentration in the roots supplemented with NPK in the water control compared to control plants without nutritional supplementation (Online resource 9). Upon rehydration, it was possible to observe a 95% increase in the concentration of Total Soluble Carbohydrates only in the leaves of *P. varians* plants with NPK application when compared to non-supplemented plants (Figures S5 and S6). Proline, Amino Acids and Nitrate showed no significant difference in their concentrations upon rehydration to nutritional treatment with or without NPK supplementation in leaves and roots (Figure 9, Online resource 8 and Online resource 9).

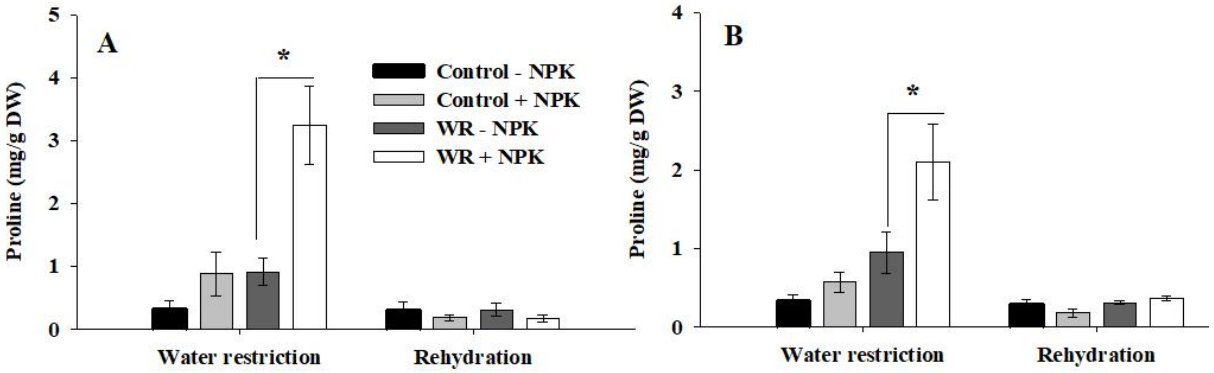


Figure 9. Proline content in leaves (A) and roots (B) of *P. varians* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).

Proline in *M. maximus* plants also responded significantly to NPK application when subjected to water restriction, showing an increase in 1368% and 461.5% concentration in leaves and roots, respectively (Figure 10). In turn, *M. maximus*, despite showing an increase in the concentration of Total Soluble Carbohydrates in the leaf tissue in plants with NPK application in the water control treatment (104.3%), did not show any difference to the NPK applied in the irrigation suspension (Online resource 10). There was no statistical difference

between NPK application in the levels of amino acids and nitrate in *M. maximus* leaves, whether for control or water restriction (Online resource 10). In the roots of *M. maximus*, amino acids, Total Soluble Carbohydrates and nitrate showed no significant difference in their concentrations upon NPK supplementation in both water treatments in the water restriction phase (Online resource 11). After rehydration, it was not possible to observe any difference in the concentrations of Proline, amino acids, Total Soluble Carbohydrates and nitrate in the leaves of *M. maximus* plants with NPK application in relation to non-supplemented plants (Online resource 10). In the roots, after rehydration, an increase in the concentrations of Total Soluble Carbohydrates (167.3%) and nitrate (176.9%) was observed in response to nutritional supplementation with NPK in water control plants (Online resource 11). The concentrations of proline and amino acids in the roots of *M. maximus* did not show a significant difference in the addition of NPK in the rehydration phase (Figure 10, Online resource 11).

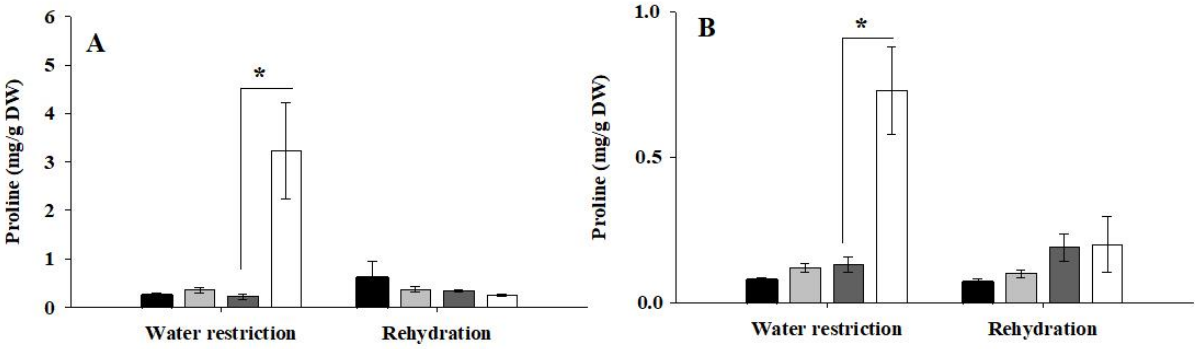


Figure 10. Proline content in leaves (A) and roots (B) of *M. maximus* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).

3.4. Effects on nitrogen, phosphorus and potassium levels

P. varians leaves presented an increase in total Nitrogen and Potassium contents, 61.3% and 30.9% respectively, in response to NPK application in plants under irrigation suspension when compared to plants without NPK supplementation (Figure 11A, 11C). In the rehydration phase, *P. varians* plants showed no differences between individuals that underwent irrigation

suspension and were after rehydrated with NPK application. In turn, a significant increase in the potassium content in the leaves of *P. varians* with NPK application that went through water suspension and were rehydrated (Figure 11C). There was no significant difference in phosphorus levels when supplemented with NPK, either in the water restriction phase or in the rehydration phase (Figure 11B).

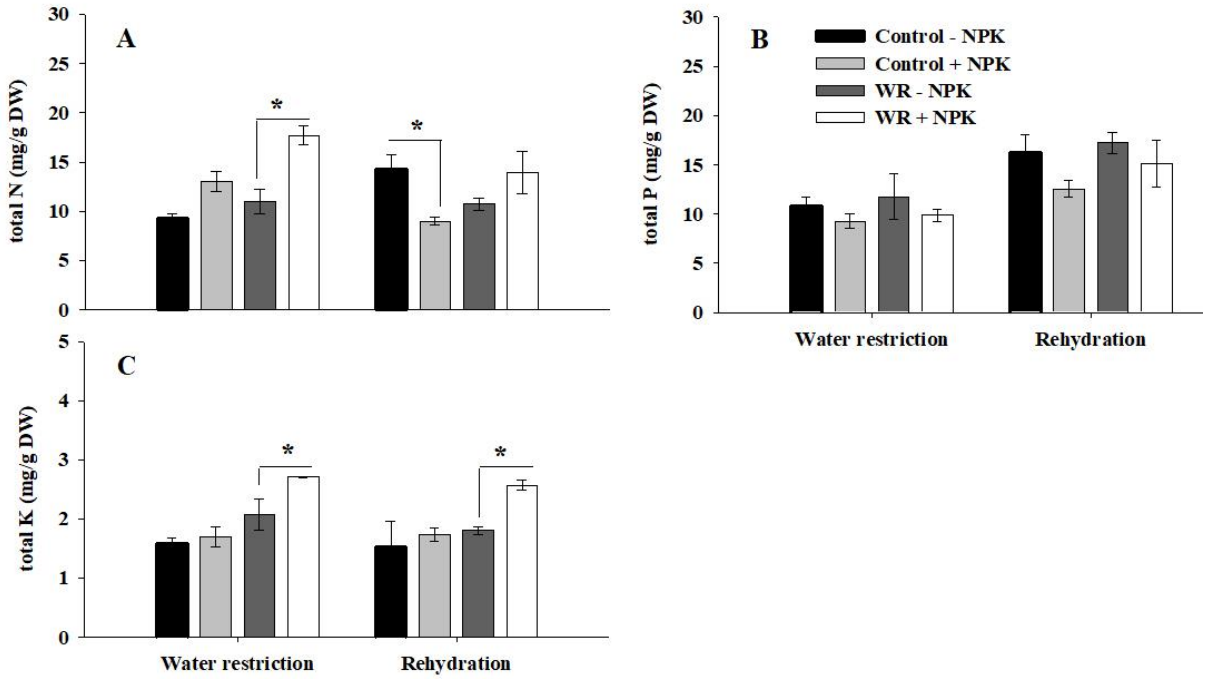


Figure 11. Nitrogen (A), Phosphorus (B) and Potassium (C) in *P. varians* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).

In the water restriction phase, there was no significant difference in the total levels of Nitrogen, Phosphorus and Potassium in response to the NPK application on *M. maximus* leaves, whether in the control treatment or suspension of irrigation (Figure 12). This scenario is maintained for Nitrogen and Potassium, without significant changes in their levels in the rehydration phase (Figure 12A, 12C). However, *M. maximus* plants with NPK application showed a reduction in total foliar phosphorus levels in the rehydration phase, whether for plants

with water irrigation (control) or for those rehydrated after a period of water restriction (Figure 12B).

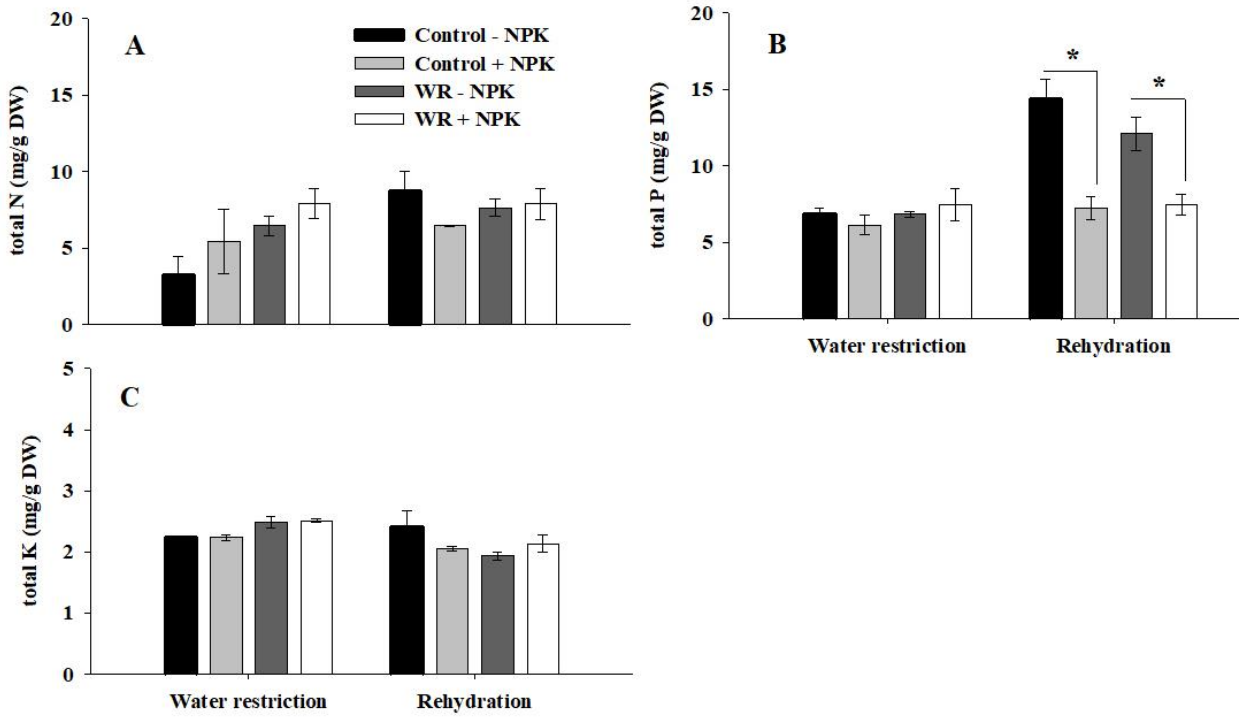


Figure 12. Nitrogen (A), Phosphorus (P) and Potassium (K) in *M. maxximus* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).

3.5. Multivariate analysis of *P. varians* and *M. maximus*

Through Hierarchical Cluster Analysis (HCA) it was possible to verify the performance of nutritional supplementation with NPK for the species *P. varians* and *M. maximus* in response to water restriction (Figures 13 and 14).

In *P. varians*, the cluster groups were determined according to the NPK application (with or without NPK application) (Figure 13). Evidencing similarity in responses, the additional supply of NPK in *P. varians* enabled similar behavior of plants under water restriction compared to plants under control (pot capacity at 70%) also supplemented with NPK. Consequently, *P. varians* not supplemented with NPK were grouped together, despite water restriction or maintaining pot capacity at 70%. After rehydration, the treatments control

and water restriction without NPK application grouped, meanwhile, treatment control with NPK application did not group with water restriction with NPK (Online resource 122).

The Principal Component Analysis (PCA) of *P. varians* harvested after water restriction represented 84.2% of total variation (Online resource 13). *P. varians* subjected to water restriction with NPK application grouped with proline (leaves and roots), number of leaves, N and K levels, highlighting the influence of N and K levels on *P. varians* growth. Meanwhile, *P. varians* with water and NPK application grouped with leaves total soluble carbohydrates, APX and protein in roots. The PCA of *P. varians* after rehydration represented 83.4% of total variation, highlighting the influence of NPK application modulating growth and biochemical responses.

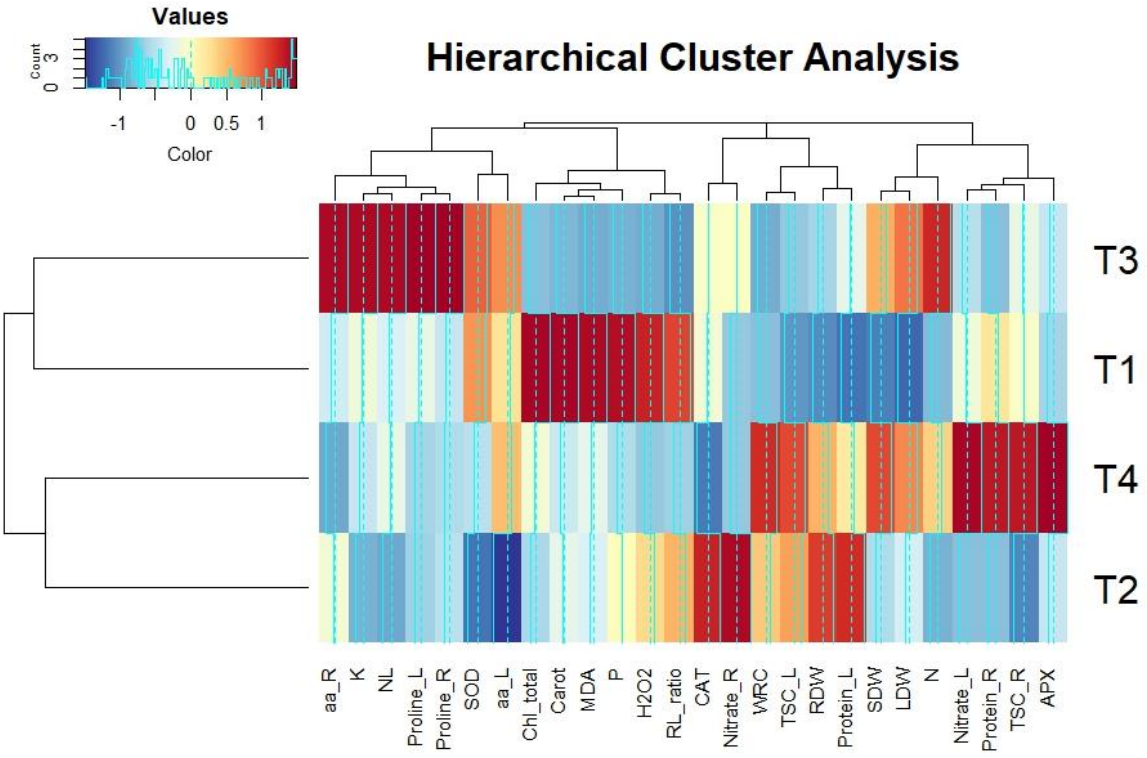


Figure 13. Hierarchical cluster analysis (HCA) of growth, physiological and biochemical parameters of *P. varians* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction. Treatments keys on the right are as follows: T1 - water restriction + NPK; T2 - water restriction - NPK; T3 - control + NPK; T4 - control - NPK.

The Principal Component Analysis (PCA) of *P. varians* harvested after water restriction represented 84.2% of total variation (Online resource 13). *P. varians* subjected to water restriction with NPK application grouped with proline (leaves and roots), number of leaves, N and K levels, highlighting the influence of N and K levels on *P. varians* growth. Meanwhile, *P. varians* with water and NPK application grouped with leaves total soluble carbohydrates, APX and protein in roots. The PCA of *P. varians* after rehydration represented 83.4% of total variation, highlighting the influence of NPK application modulating growth and biochemical responses.

On the other hand, *M. maximus* did not present a cluster related to nutritional treatment as observed in *P. varians*, however, NPK supplementation was still relevant in responses to water restriction (Figure 14). *M. maximus* plants in a situation of water suppression, through the NPK application, demonstrated similar behavior to plants maintained at 70% of pot capacity and without nutritional supplementation. However, plants under water restriction, but without additional NPK addition, grouped with individuals maintained at 70% of pot capacity and nutritionally supplemented. After rehydration, *M. maximus* responses are grouped according to the NPK application (Online resource 15).

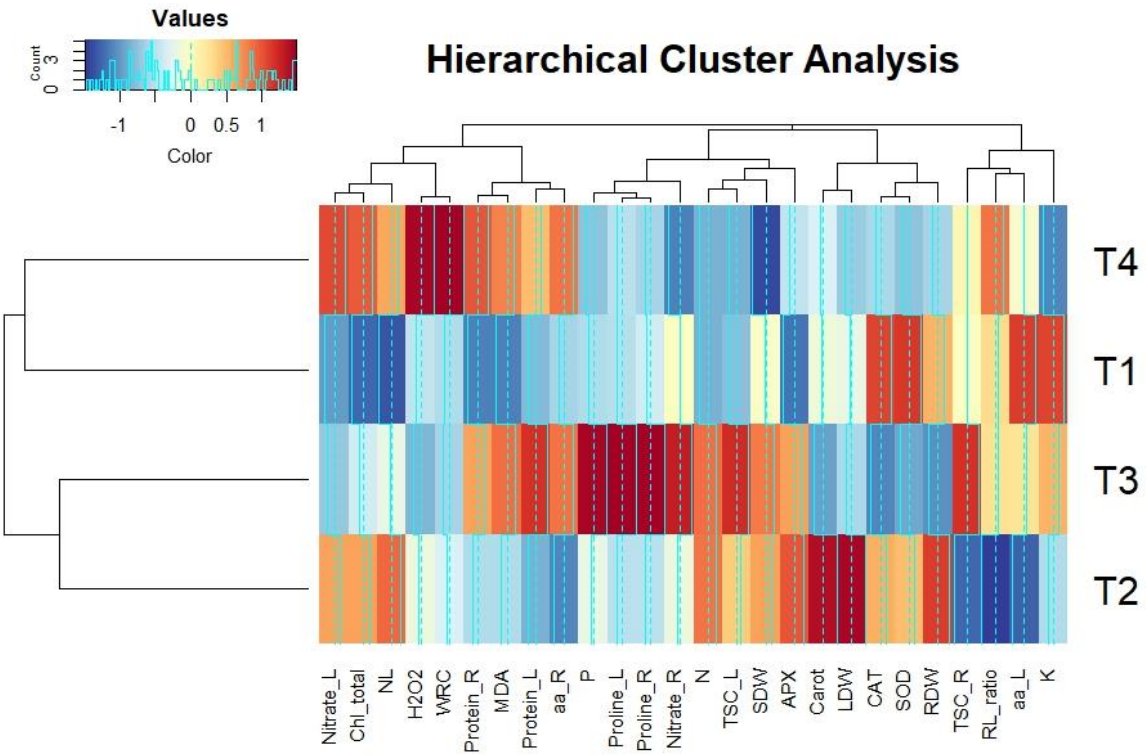


Figure 14. Hierarchical cluster analysis (HCA) of growth, physiological and biochemical parameters of *M. maximus* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction. Treatment keys on the right are as follows: 1 - water restriction + NPK; 2 - water restriction - NPK; 3 - control + NPK; 4 - control - NPK.

The PCA of *M. maximus* demonstrates the effects of water availability and NPK application on growth, biochemical and physiological responses. At the harvest after water restriction, PCA represented 85.8% of total variation (Online resource 16). The treatment of water restriction with NPK application is grouped primarily with parameters related to N, such as proline (root and leaves), amino acids (root and leaves) and total N levels. On the other hand, the treatment with NPK application and control (70% pot capacity) grouped total chlorophyll, nitrate (leaves), H_2O_2, root dry weight and water relative content. At the harvest after rehydration, PCA represented 87.8% of total variation (Online resource 17). The treatments with NPK application and water restriction were close with NPK application and control (70% pot capacity), grouping with nitrate (root), amino acids (leave), MDA, number of leaves and stem dry weight.

4. Discussion

Pavonia varians is a species native to the Caatinga, an environment characteristically marked by its semi-arid climate, as it is a seasonally dry forest, with low and irregular precipitation, high temperatures and high solar incidence (Marques et al. 2020; Santos et al. 2020). To all this is added the anthropic factor as an agent that transforms the environment (Antongiovanni et al. 2020; Araujo et al. 2023). To this end, it is expected that the species will demonstrate an efficient apparatus that allows it to live in these challenging environments (Marques et al. 2020). Through this study, it was possible to demonstrate the good adaptation of *P. varians* to water restriction (10 days), even though it presents a reduction in relative water content. This was noted in the maintenance of parameters that are notably affected by reduced water availability, such as growth (number of leaves, dry mass of leaves, stems and roots) and primary metabolites (proteins, enzymes, carbohydrates), mobilizing different strategies to ensure their good and efficient development. Furthermore, it proved to be responsive to NPK, which helped to improve parameters of growth, possibly related to greater photosynthetic activity and increased carbohydrate content in response to the increase in chlorophylls, in addition to favoring an increase in relative water content. on the leaves.

The study leads to the understanding that oxidative control in *P. varias* occurs non-enzymatically through the supplementary application of NPK in response to drought and its impacts. Through the application of NPK it was possible to obtain less oxidative damage despite there being no significant changes in the enzymatic activity of SOD, CAT and APX. Whether via the enzymatic or non-enzymatic route, both pathways aim to reduce oxidative data caused by ROS in cells (Hussain et al. 2019b). In turn, in *P. varians* response to NPK supplementation, it prominently demonstrated an increase in proline levels, both in leaves and roots. Proline is an amino acid well described in the literature that acts against abiotic stress such as drought (Hayat et al. 2012; Ahmad et al. 2019; Yang et al. 2021). It is a molecule that

571 acts in cellular oxidative defense by combating ROS. It contributes to osmotic regulation,
572 favoring an improvement in cellular water status, protection of molecules and stimulating
573 enzymatic activity (Furlan et al., 2020; Moreno-Galván et al., 2020; Saddique et al., 2020).

574 Among the macronutrients, Nitrogen has a prominent role as it participates as an
575 important element in the structure of molecules essential for plant development (ATP, NADP,
576 NADPH, chlorophylls, proteins, etc) (Song et al. 2019). Through NPK supplementation,
577 investment in amino acid synthesis was directed towards proline synthesis, acting in oxidative
578 protection. The maintenance in the chlorophylls content, reflects the reduction of oxidative
579 damage to cellular membranes and molecules.

580 It is documented that drought has negative effects on nutrient uptake, reducing nutrient
581 mobility in the soil and affecting plant mechanisms related to nutrient uptake, as well as
582 reducing nutrient levels (Waraich et al. 2011; Bista et al. 2018). However, *P. varians* plants
583 even with water restriction were able to increase N and K levels when supplemented with NPK,
584 which may be related to increases in Proline levels. Additionally, drought can decrease protein
585 levels by inhibiting N metabolism or by oxidative damage (Hernández et al. 2012; Bista et al.
586 2018). The results indicate *P. varians* maintain protein levels in root and leaves even with water
587 restriction, as well as total K levels, which may be beneficial to maintain plant growth. On the
588 other hand, *M. maximus* present decreases in protein content in leaves under water deficit
589 without NPK application, although the total N and K was not affected by NPK application.

590 The importance of NPK supply to *P. varians* responses under water restriction and
591 maintaining N metabolism active was highlighted by the PCA analysis. The content of
592 molecules derived from N metabolism are grouped with the treatment of water restriction with
593 NPK application, such as Proline (root and leaves), total N, amino acids (root and leaves) and
594 number of leaves. The NPK application with 70% pot capacity favored *P. varians* growth,
595 increasing total soluble carbohydrates (leaves), protein (root and leaves). We hypothesize that

596 the adaptation of *P. varians* to arid environments do not require modulating SOD, CAT and
597 APX activities, since it was able to maintain cellular homeostasis with water restriction,
598 evidenced by maintaining protein levels, total soluble carbohydrates (leaves and root) and
599 amino-N levels (leaves), regardless of N supply. However, N supply is also important to
600 increase Proline levels in *P. varians* under water restriction. The resilience of *P. varians* to
601 water restriction was evidenced by the harvest after rehydration, which presented full recovery.
602 The HCA analysis showed *P. varians* responses grouped according to nutrient supply (with or
603 without NPK application), evidencing its resilience to water restriction and responses to NPK
604 supply.

605 Drought can reduce Nitrogen and Phosphorus content in grasses, such as *Zea mays*,
606 *Hordeum vulgare* and *Andropogon gerardii*) as reported by Bista et al. (2018). In our study
607 with *M. maximus*, NPK content did not decrease with water restriction. Additionally, NPK
608 application increased the number of leaves and stem dry weight under water restriction
609 compared to non-supplemented plants under water restriction, indicating the beneficial impacts
610 of NPK supplementation under water restriction. Although *M. maximus* under water restriction
611 without NPK application, showed lower MDA content, higher SOD and CAT activity, the
612 increased proline levels in root and leaves with NPK application seems to play a vital role in
613 *M. maximus* maintaining growth under water restriction. Besides acting as compatible
614 osmolyte and stabilizing proteins under drought conditions, proline also mitigates ROS impacts
615 and may be an important marker to drought tolerance (Furlan et al., 2020; Saddique et al., 2020;
616 Moreno-Galván et al., 2020). PCA analysis showed proline levels in root and leaves grouped
617 with the treatment of water restriction with NPK application, highlighting the positive effect
618 of N supply on *M. maximus* responses. The growth analysis of *M. maximus* after rehydration
619 showed that NPK application improved its responses despite the previous water deficit, as

evidenced by increasing number of leaves and stem dry weight compared to plants without NPK application.

Pavonia varians and *M. maximus* present in dry regions of Caatinga may compete for water and nutrients to expand their coverage area. The nutrients present in the soil by organic matter mineralization or added by fertilizing nearby agricultural fields can affect plant responses to water restriction. Oxidative damage can be expressed as a result of different stressors (temperature, salinity, light, etc) in addition to the period and intensity of exposure to such factors (Ahmad et al, 2019; Hussain et al, 2019 a, b; Khayatnezhad and Gholamin, 2021). The increase in temperatures due to climate change may reduce the impact of drought on *M. maximus* (Viciedo et al., 2021). Information on the response and impact of other stressors for native species of the Caatinga, represented here by *P. varians*, and overcoming strategies are still progressing at a slow pace. Given the ecological impact of *M. maximus* and its invasive potential on the environment (Soti and Thomas, 2022), increased NPK supply mitigated the effects of water restriction, notably through the action of proline, which could confer advantages in competition in detriment of native vegetation.

5. Conclusion

Water restriction is a reality for plants present in semi-arid areas such as the Caatinga, bringing numerous challenges to their physiological/biochemical stability and, consequently, their growth. Our objective was achieved, and it was possible to observe that supplementation with NPK favored the responses of both species in combating drought symptoms and their full recovery after rehydration. Supplementation proved to be a way of strengthening the species, especially for the native species that showed marked increases before being subjected to restriction, contributing to good performance when going through drought with low levels of MDA and H_2O_2. Our hypothesis was confirmed, showing that there was a reduction in

oxidative stress as a result of NPK supplementation in plants when in a state of water restriction, particularly through the participation of Proline, contributing to the reduction of oxidative damage caused by drought. The study showed the participation of the non-enzymatic system in combating water stress, especially for *P. varians*, highlighting the importance of this antioxidant defense system. Therefore, it is necessary to develop future research that elucidates the role of this device in more detail in combating oxidative damage caused by drought. Nutritional supplementation with NPK before exposure to water deficit proved to be a viable tool to strengthen individuals to face drought, whether for cultivation or opening horizons as a strategy for the success of recovery processes in areas of the Caatinga affected by human action.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Author contribution

Silva MB: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft, Visualization**.**

Sperandio MVL: Conceptualization, Writing - Original Draft, Formal analysis.

Data Availability

The datasets used in this study are available from the corresponding author on reasonable request.

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Journal of Plant Growth Regulation

Electronic supplementary material

Title: Supplementation with NPK previous to water restriction confers different biochemical and growth modulations in a native and an exotic species present in a Brazilian semi-arid region.

Authors: Matheus Bessa da Silva, Marcus Vinícius Loss Sperandio

Water restriction				Rehydration			
Variables	**W**	**NS**	**W*NS**	**Variables**	**W**	**NS**	**W*NS**
LDW	ns	**	ns	LDW	ns	ns	ns
SDW	ns	ns	ns	SDW	ns	ns	ns
RDW	ns	ns	ns	RDW	ns	ns	ns
R/L ratio	ns	**	ns	R/L ratio	ns	ns	ns
NL	ns	ns	ns	NL	ns	ns	ns
WRC	***	ns	**	WRC	ns	ns	ns
CHL *a*	ns	ns	ns	CHL *a*	ns	ns	ns
CHL *b*	ns	ns	ns	CHL *b*	ns	ns	ns
CHL total	ns	ns	ns	CHL total	ns	ns	ns
CAR	ns	ns	ns	CAR	ns	ns	ns
PTN L	ns	*	ns	PTN L	ns	ns	ns
SOD	ns	ns	ns	SOD	ns	ns	ns
CAT	ns	**	**	CAT	ns	ns	ns
APX	*	*	**	APX	ns	ns	ns
MDA	ns	*	ns	MDA	ns	ns	ns
H2O2	ns	*	ns	H2O2	ns	ns	ns
TSC L	*	*	**	TSC L	ns	ns	*
AA L	***	ns	ns	AA L	ns	ns	ns
PRO L	***	***	*	PRO L	ns	ns	ns
NO3 L	*	ns	ns	NO3 L	ns	ns	ns
PTN R	**	*	***	PTN R	ns	ns	ns
TSC R	ns	ns	ns	TSC R	ns	ns	ns
AA R	ns	*	ns	AA R	ns	ns	ns

NO3 R	ns	ns	*	NO3 R	ns	ns	ns
PRO R	**	*	ns	PRO R	ns	ns	ns
N	*	***	ns	N	ns	ns	*
P	ns	ns	ns	P	ns	ns	ns
K	**	ns	ns	K	*	ns	ns

Online resource 1. Analysis of variance (ANOVA) of *P. varians* variables, collected 10 days after suspension of irrigation in the water restriction phase and 20 days after reestablishment of watering in the rehydration phase, in relation to water treatments (W), water treatments nutritional supplementation (NS) and the interaction between water treatments and nutritional supplementation (W*NS). Water treatments: 70% of pot capacity, suspension of watering and re-establishment of watering. Nutritional supplementation treatments: without supplementary addition of Nitrogen, Phosphorus and Potassium (NPK) and with supplementary addition of NPK. * significance different at $p < 0.05$; ** significance different at $p < 0.01$; *** significance different at $p < 0.001$; ns = no significance. LDW: leaves dry weight; SDW: stem dry weight; RDW: root dry weight; R/L ratio: dry weight ratio of root and leaves; NL: number of leaves; WRC: water relative content; CHL *a*: chlorophyll *a*; CHL *b*: chlorophyll *b*; CHL total: total chlorophyll; CAR: carotenoids; PTN L: protein in leaves; SOD: Superoxide dismutase activity; CAT: Catalase activity; APX: Ascorbate peroxidase activity; MDA: malondialdehyde; H2O2: hydrogen peroxide; TSC L: total soluble carbohydrates in leaves; AA L: amino acids in leaves; PRO L: proline in leaves; NO3 L: nitrate in leaves; PTN R: protein in roots; TSC R: total soluble protein in roots; AA R: amino acids in roots; NO3 R: nitrate in roots; PRO R: proline in roots; N: total Nitrogen content; P: total phosphorus content; K: total potassium content.

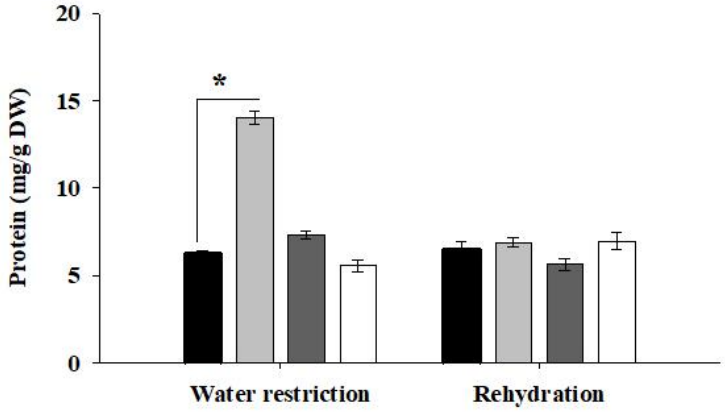
Water restriction				**Rehydration**			
Variables	**WR**	**NS**	**WR*NS**	**Variables**	**WR**	**NS**	**WR*NS**
LDW	ns	**	ns	LDW	ns	ns	ns
SDW	ns	***	ns	SDW	ns	***	*
RDW	*	ns	ns	RDW	*	**	ns
R/L ratio	ns	*	ns	R/L ratio	ns	*	ns
NL	ns	**	ns	NL	*	***	ns
WRC	***	ns	ns	WRC	*	ns	ns
CHL *a*	*	ns	ns	CHL *a*	ns	ns	ns
CHL *b*	**	ns	ns	CHL *b*	ns	ns	ns
CHL total	**	ns	ns	CHL total	ns	ns	ns
CAR	ns	*	ns	CAR	ns	ns	ns
PTN L	ns	*	*	PTN L	*	ns	ns
SOD	**	*	ns	SOD	ns	ns	ns
CAT	*	**	*	CAT	ns	ns	ns
APX	*	***	ns	APX	ns	ns	**

MDA	ns	*	*	MDA	ns	ns	ns
H2O2	*	ns	ns	H2O2	ns	ns	ns
TSC L	ns	**	ns	TSC L	ns	ns	ns
AA L	ns	ns	ns	AA L	ns	*	ns
PRO L	**	***	***	PRO L	ns	ns	ns
NO3 L	*	ns	*	NO3 L	ns	ns	ns
PTN R	ns	ns	ns	PTN R	ns	**	ns
TSC R	ns	ns	ns	TSC R	ns	**	ns
AA R	ns	ns	ns	AA R	ns	ns	ns
NO3 R	**	ns	ns	NO3 R	ns	**	ns
PRO R	***	***	**	PRO R	ns	ns	ns
N	ns	ns	ns	N	ns	ns	ns
P	ns	ns	ns	P	ns	***	ns
K	**	ns	ns	K	ns	ns	ns

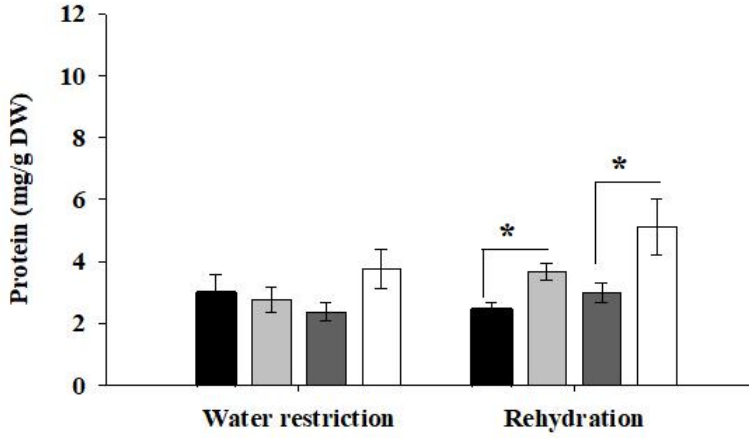
Online resource 2. Analysis of variance (ANOVA) of *M. maximus* variables, collected 10 days after suspension of irrigation in the water restriction phase and 20 days after reestablishment of watering in the rehydration phase, in relation to water treatments (W), water treatments nutritional supplementation (NS) and the interaction between water treatments and nutritional supplementation (W*NS). Water treatments: 70% of pot capacity, suspension of watering and re-establishment of watering. Nutritional supplementation treatments: without supplementary addition of Nitrogen, Phosphorus and Potassium (NPK) and with supplementary addition of NPK. * significance different at $p < 0.05$; ** significance different at $p < 0.01$; *** significance different at $p < 0.001$; ns = no significance. LDW: leaves dry weight; SDW: stem dry weight; RDW: root dry weight; R/L ratio: dry weight ratio of root and leaves; NL: number of leaves; WRC: water relative content; CHL *a*: chlorophyll *a*; CHL *b*: chlorophyll *b*; CHL total: total chlorophyll; CAR: carotenoids; PTN L: protein in leaves; SOD: Superoxide dismutase activity; CAT: Catalase activity; APX: Ascorbate peroxidase activity; MDA: malondialdehyde; H2O2: hydrogen peroxide; TSC L: total soluble carbohydrates in leaves; AA L: amino acids in leaves; PRO L: proline in leaves; NO3 L: nitrate in leaves; PTN R: protein in roots; TSC R: total soluble protein in roots; AA R: amino acids in roots; NO3 R: nitrate in roots; PRO R: proline in roots; N: total Nitrogen content; P: total phosphorus content; K: total potassium content.

	Pavonia varians		*Megathyrsus maximus*	
	S.I 1	**S.I 2**	**S.I 1**	**S.I 2**
Without NPK	28.5 ±2.05	29.56 ±1.83	22.5 ±0.67	19.4 ±2.24
With NPK	40.94 ±4.40*	34.4 ± 0,81*	40.1 ±1.56***	27.9 ±0.62**

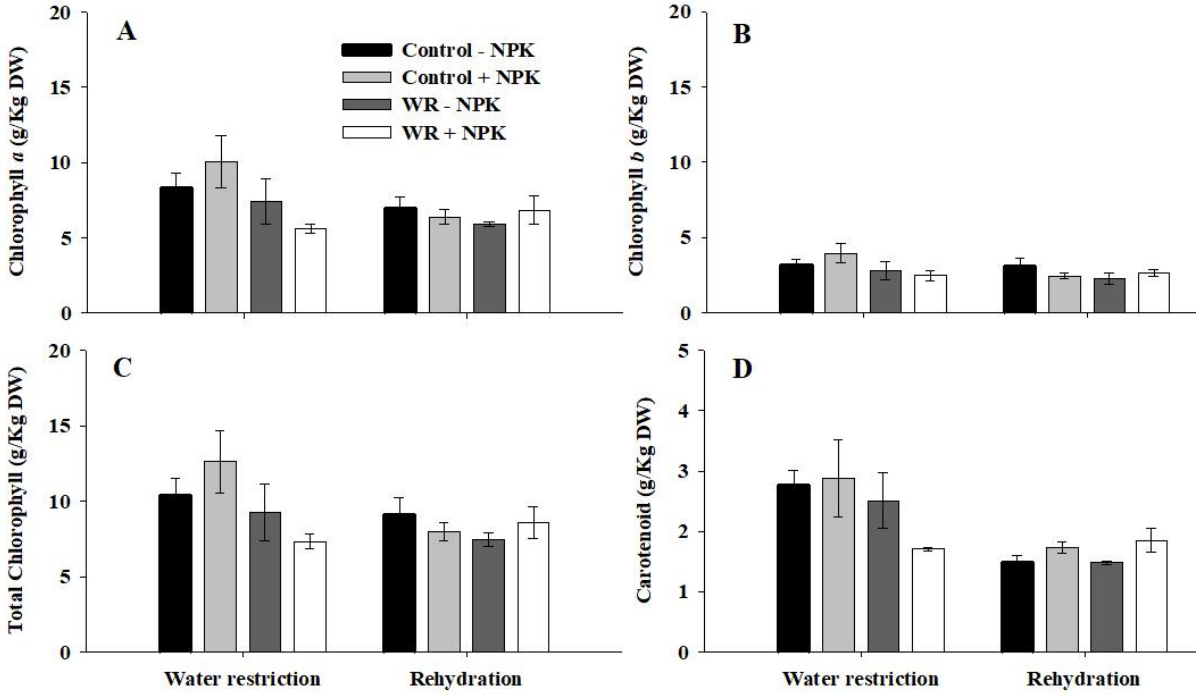
Online resource 3. SPAD index of *P. varians* and *M. maximus* after nutritional supplementation (N.S) with NPK measured before applying water restriction. Analysis of Variance (ANOVA): * significant difference at $p < 0.05$; ** significant difference $p < 0.01$; *** significant difference $p < 0.001$; ns = no significance. S.I 1: SPAD Index 11 days after supplementation. S.I 2: SPAD Index 21 days after supplementation.



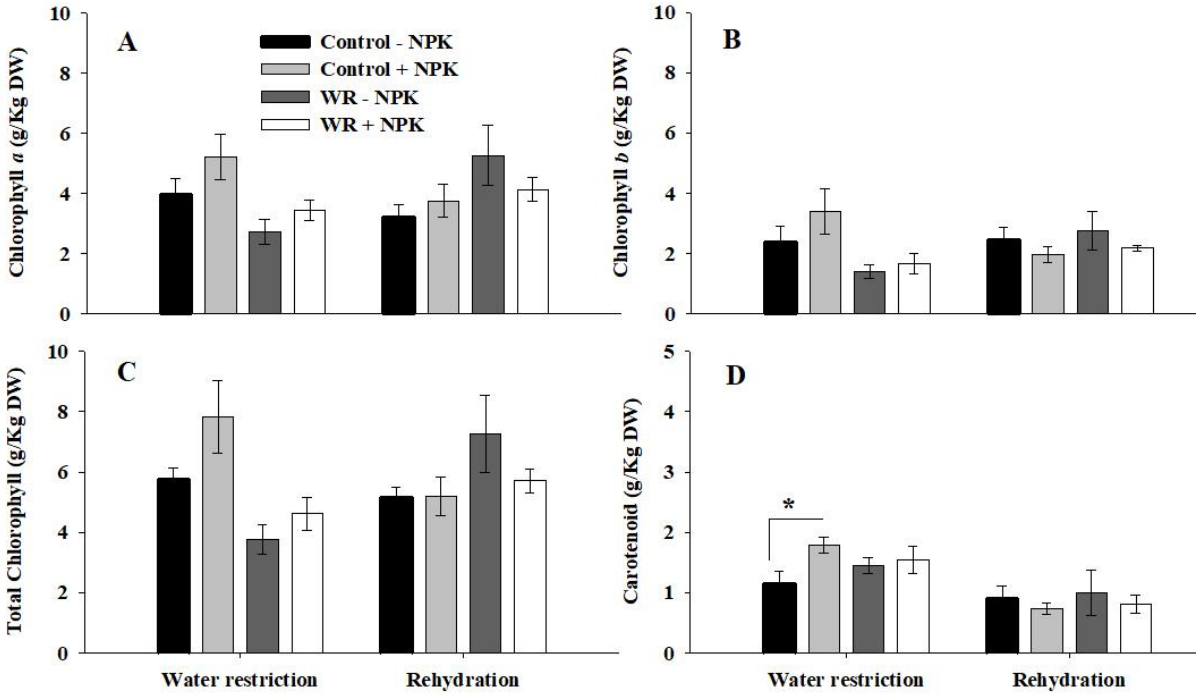
Online resource 4. Protein content in roots of *P. varians* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. DW - dry weight. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).



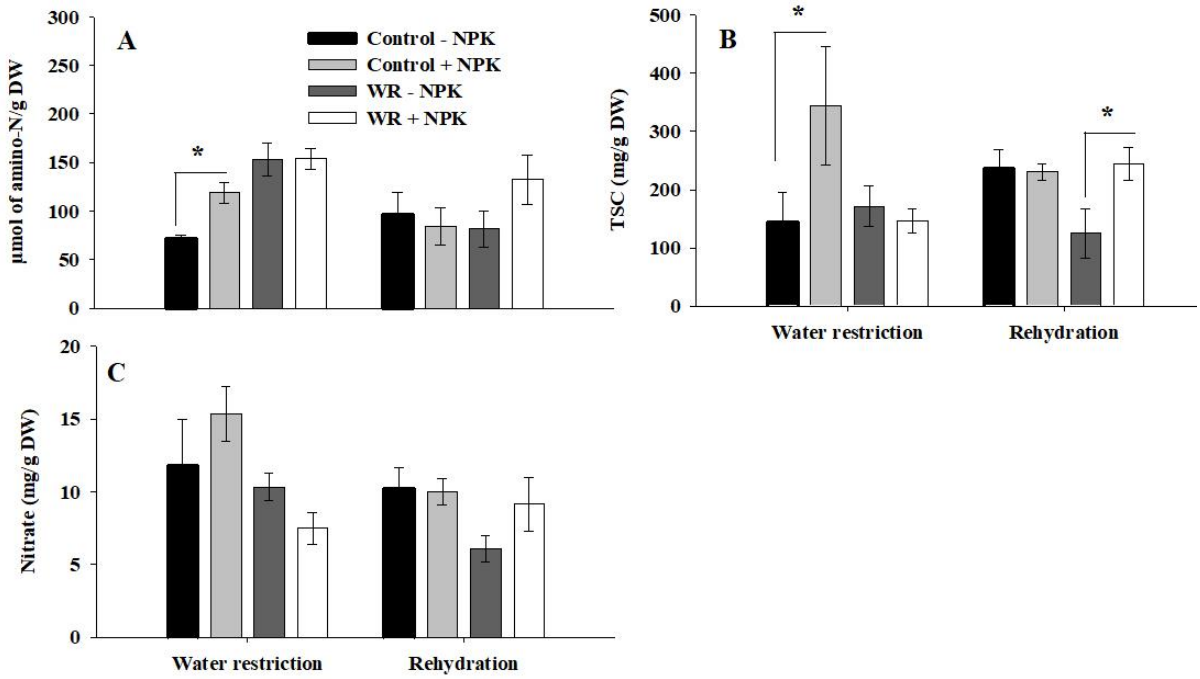
Online resource 5. Protein content in roots of *M. maximus* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. DW - dry weight. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).



Online resource 6. Chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids content in leaves of *P. varians* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. DW - dry weight. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).

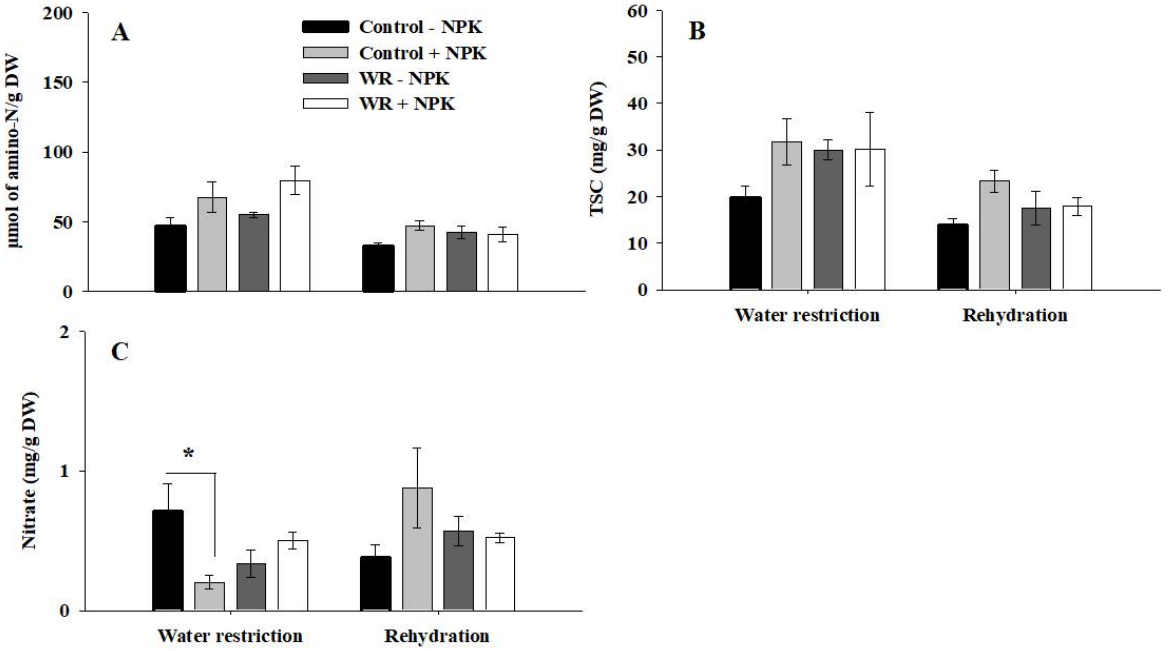


Online resource 7. Chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids content in leaves of *M. maximus* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. DW - dry weight. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).

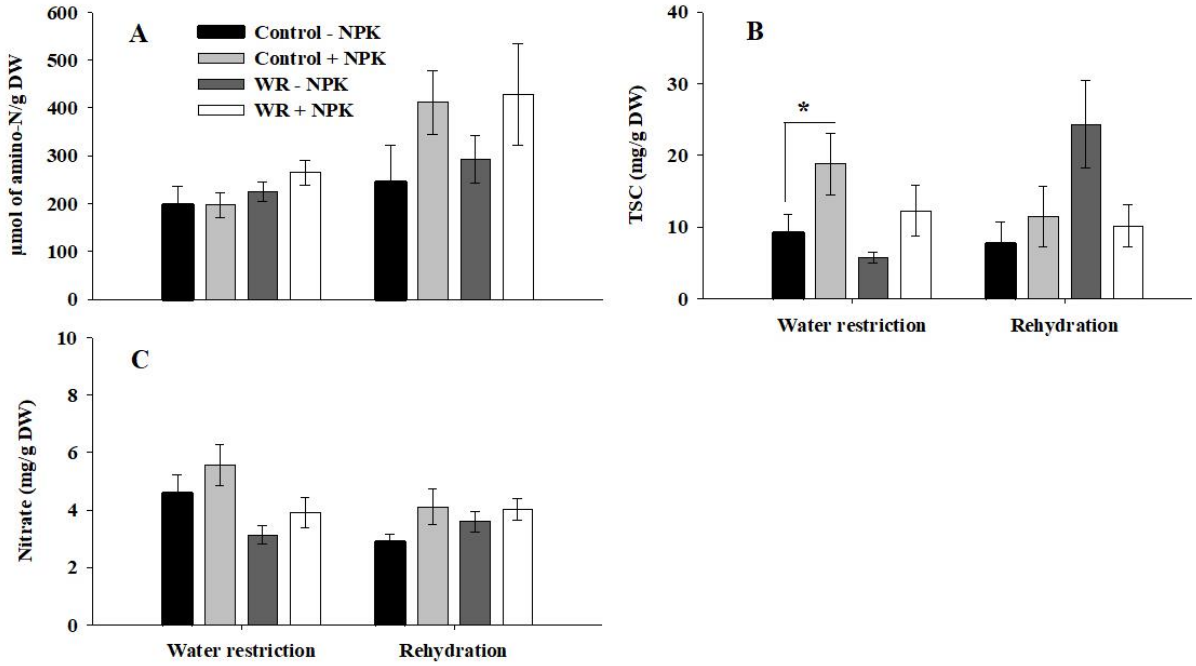


Online resource 8. Amino-N (A), total soluble carbohydrates - TSC (B) and nitrate (C) content in leaves of *P. varians* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested

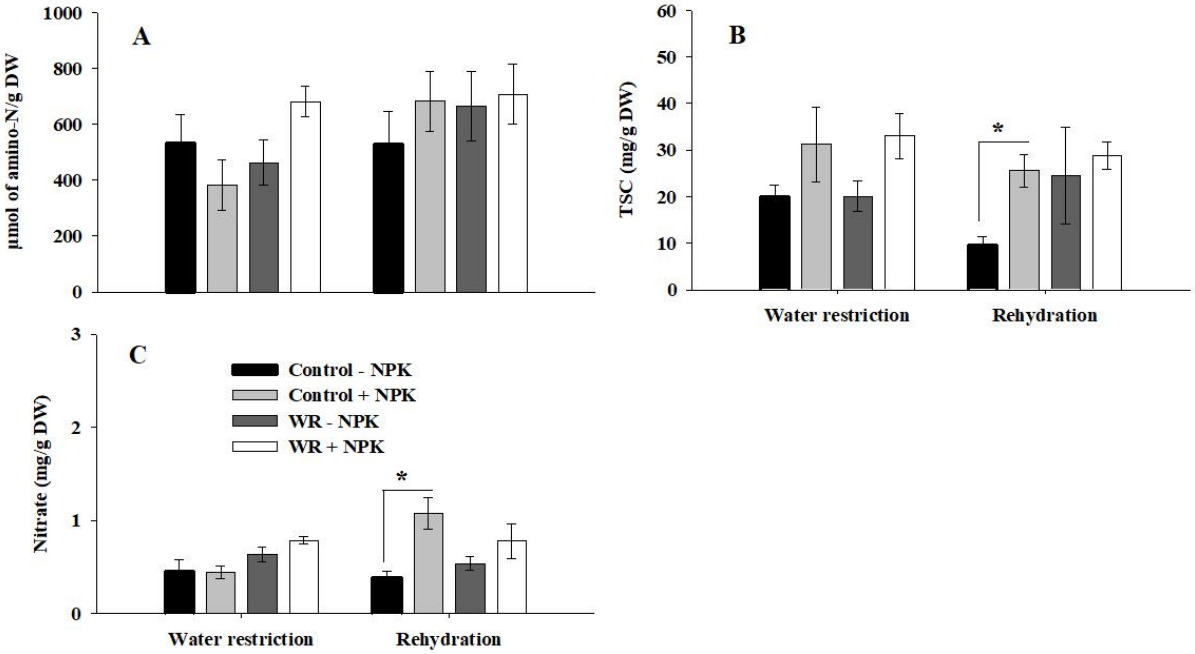
after 10 days of water restriction and 20 days after rehydration. DW - dry weight. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).



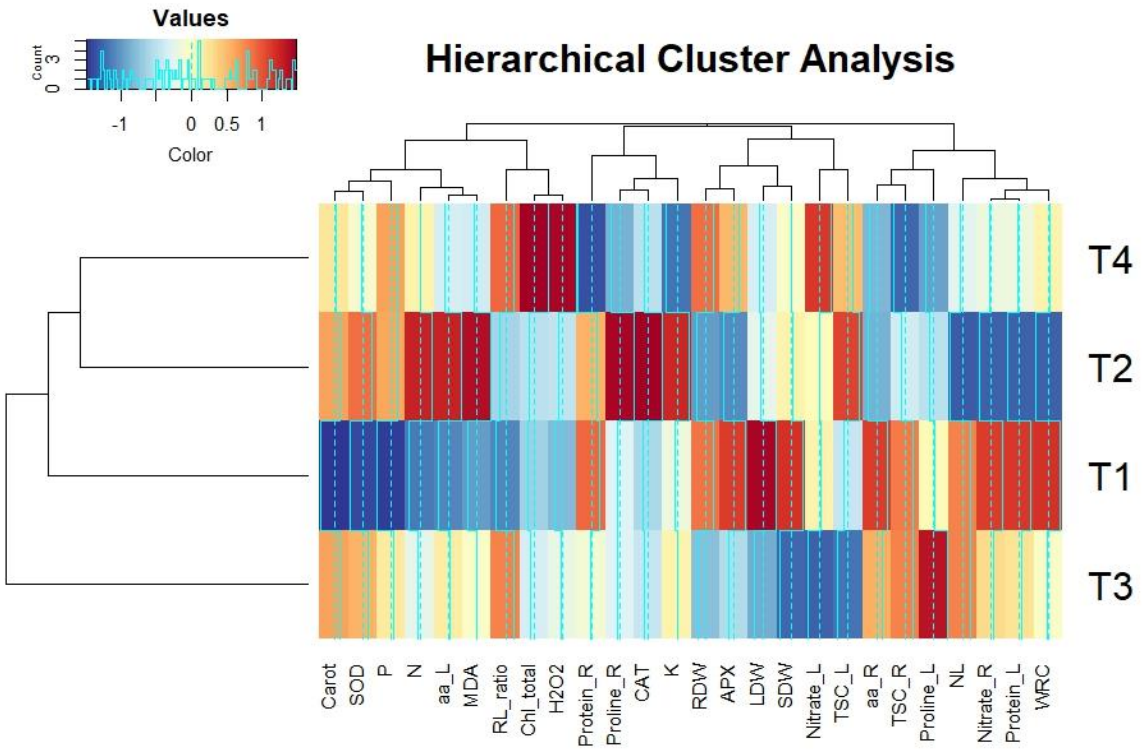
Online resource 9. Amino-N (A), total soluble carbohydrates - TSC (B) and nitrate (C) content in roots of *P. varians* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. DW - dry weight. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).



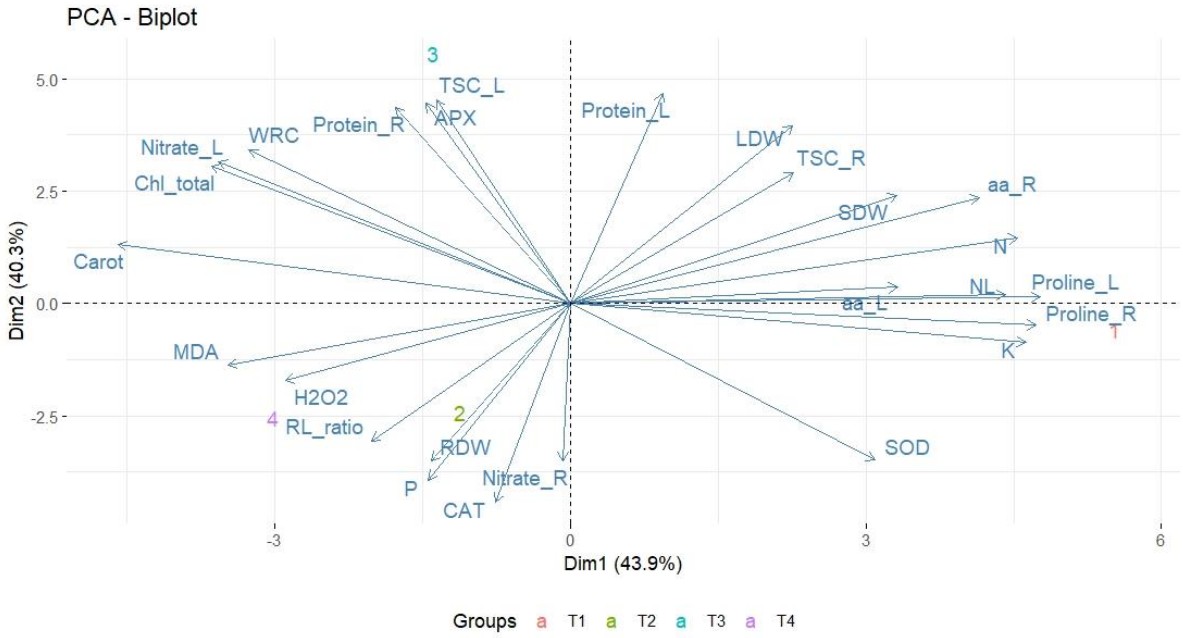
Online resource 10. Amino-N (A), total soluble carbohydrates - TSC (B) and nitrate (C) content in leaves of *M. maximus* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. DW - dry weight. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).



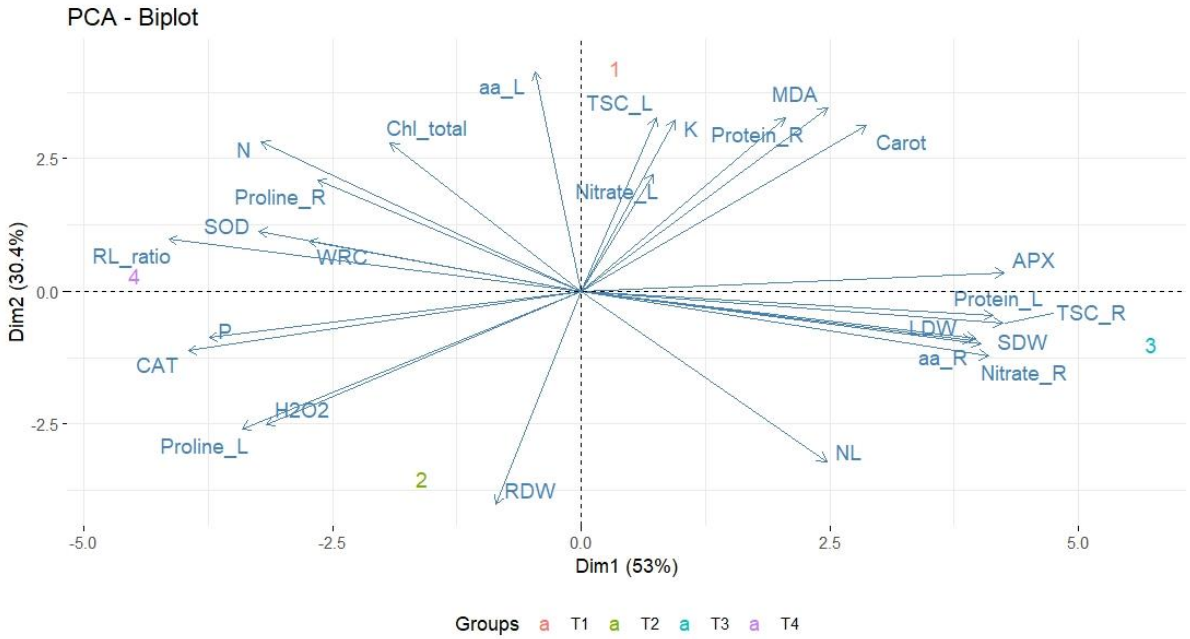
Online resource 11. Amino-N (A), total soluble carbohydrates - TSC (B) and nitrate (C) content in roots of *M. maximus* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction and 20 days after rehydration. DW - dry weight. * Represent the influence of NPK application on plant responses under control or water deficit conditions and after rehydration by LSD test ($p < 0.05$).



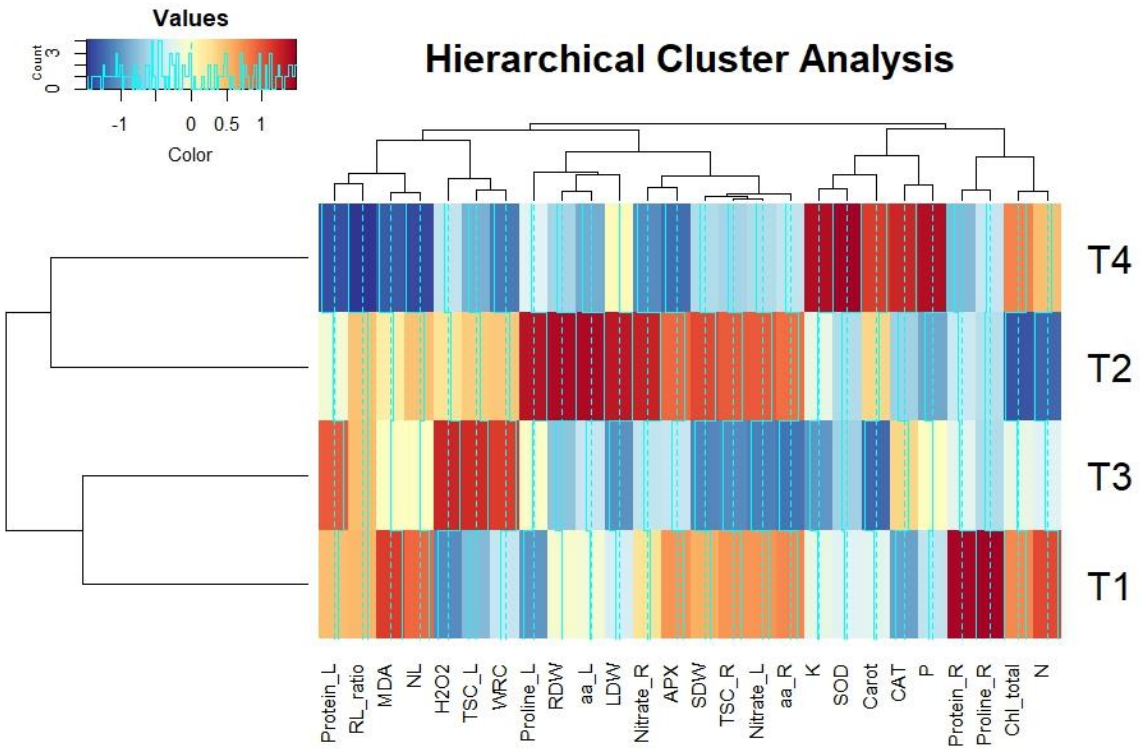
Online resource 12. Hierarchical cluster analysis (HCA) of growth, physiological and biochemical parameters of *P. varians* harvested after 20 days with rehydration after water restriction treatment (70% pot capacity (control) or water deficit by withholding water (WR)) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK). Treatments keys on the right are as follows: T1 - water restriction + NPK; T2 - water restriction - NPK; T3 - control + NPK; T4 - control - NPK.



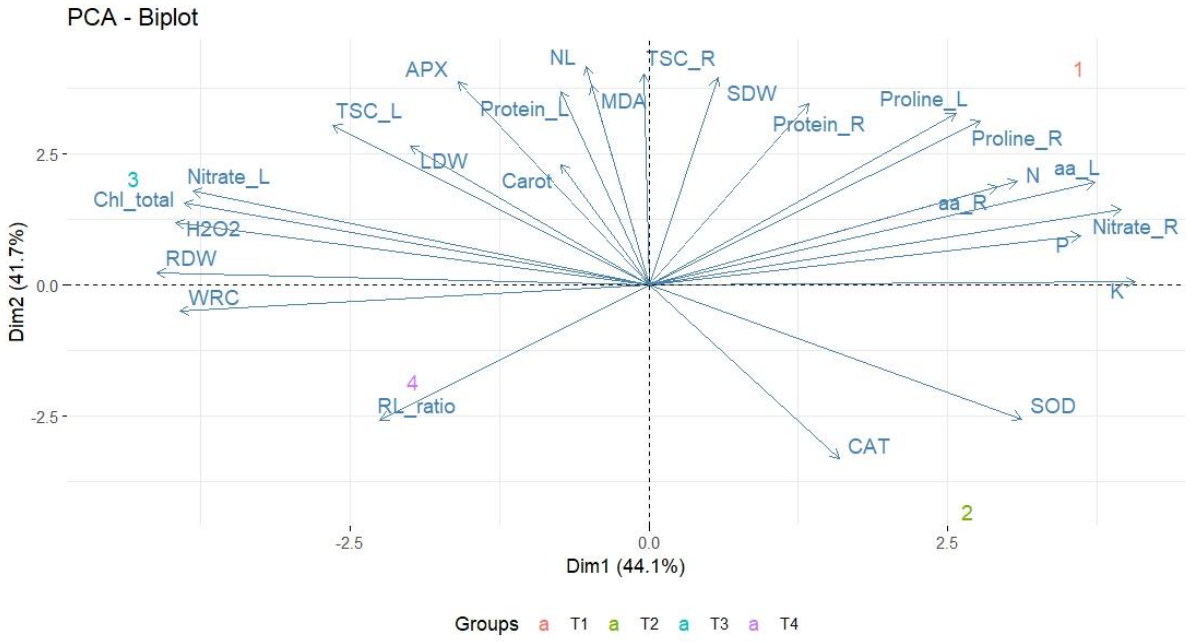
Online resource 13. Principal Component Analysis (PCA) of growth, physiological and biochemical parameters of *P. varians* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK), harvested after 10 days of water restriction. Treatment keys are as follows: 1 (T1)- water restriction + NPK; 2 (T2) - water restriction - NPK; 3 (T3) - control + NPK; 4 (T4) - control - NPK.



Online resource 14. Principal Component Analysis (PCA) of growth, physiological and biochemical parameters of *P. varians* harvested after 20 days with rehydration after water restriction treatment (70% pot capacity (control) or water deficit by withholding water (WR)) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK). Treatment keys are as follows: 1 (T1)- water restriction + NPK; 2 (T2) - water restriction - NPK; 3 (T3) - control + NPK; 4 (T4) - control - NPK.

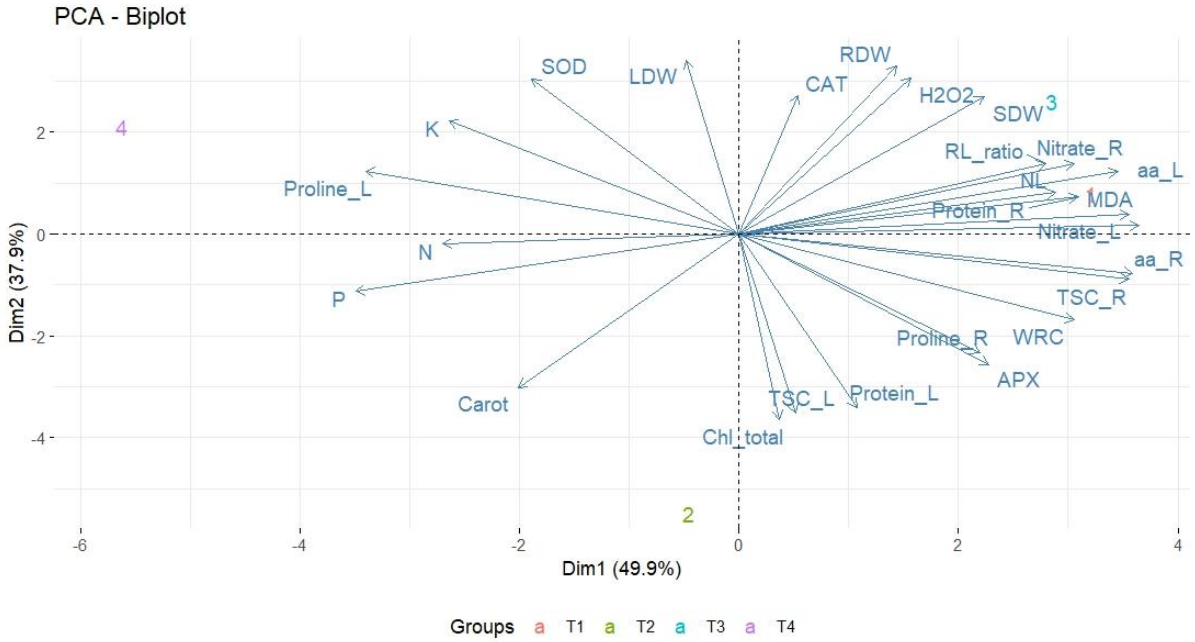


Online resource 15. Hierarchical cluster analysis (HCA) of growth, physiological and biochemical parameters of *M. maximus* harvested after 20 days with rehydration after water restriction treatment (70% pot capacity (control) or water deficit by withholding water (WR)) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK). Treatments keys on the right are as follows: T1 - water restriction + NPK; T2 - water restriction - NPK; T3 - control + NPK; T4 - control - NPK.



Online resource 16. Principal Component Analysis (PCA) of growth, physiological and biochemical parameters of *M. maximus* with 70% pot capacity (control) or water deficit by withholding water (WR) without or with Nitrogen, Phosphorus and Potassium supplementation

(NPK), harvested after 10 days of water restriction. Treatment keys are as follows: 1 (T1)- water restriction + NPK; 2 (T2) - water restriction - NPK; 3 (T3) - control + NPK; 4 (T4) - control - NPK.



Online resource 17. Principal Component Analysis (PCA) of growth, physiological and biochemical parameters of *M. maximus* harvested after 20 days with rehydration after water restriction treatment (70% pot capacity (control) or water deficit by withholding water (WR)) without or with Nitrogen, Phosphorus and Potassium supplementation (NPK). Treatment keys are as follows: 1 (T1)- water restriction + NPK; 2 (T2) - water restriction - NPK; 3 (T3) - control + NPK; 4 (T4) - control - NPK.