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Expressão de memória de estresse em sementes e mudas de *Cenostigma pyramidale* (Tul.) E. Gagnon & G.P. Lewis: influência da alta temperatura e da hidratação descontínua

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Orientador: Prof. Dra. Elizamar Ciríaco da Silva.

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Não tem importância porque, se com essa frase eu pelo menos cheguei a sugerir que a coisa é muito mais do que consegui dizer, então na verdade eu fiz muito: eu aludi!

- *Clarice Lispector*

RESUMO

Em ecossistemas secos, as sementes podem ser expostas a pulsos de alta temperatura e hidratação descontínua durante o processo germinativo. Esses eventos, além de estressores, podem atuar como sinais que induzem ajustes persistentes e modulam a germinação e a resiliência ao déficit hídrico. Essa dissertação teve como objetivo avaliar a influência da exposição de sementes a pulsos de alta temperatura e ciclos de hidratação e desidratação na expressão de memória de estresse em sementes e mudas de *Cenostigma pyramidale*, uma leguminosa lenhosa com ampla distribuição na Caatinga. Dessa forma, a dissertação foi estruturada em quatro artigos. No Artigo I, avaliou-se a influência de ciclos de hidratação e desidratação (HD) em diferentes estágios de embebição sobre a germinação e o crescimento inicial. Com isso, observou-se que ciclos aplicados em fases iniciais de hidratação incrementaram parâmetros germinativos e de crescimento, já em fases avançadas, reduziram o desempenho da germinação e do crescimento das plântulas. No Artigo II, sementes foram expostas a pulsos térmicos de 55, 65 e 75 °C por 30, 60 e 90 min. Os resultados evidenciaram resiliência germinativa, porém com mudanças na cinética e na sincronia conforme a intensidade e a duração dos pulsos. No Artigo III, testou-se a influência de tratamentos pré-germinativos (alta temperatura, ciclos de HD e combinação) na germinação sob déficit hídrico induzido com PEG 6000. Foi observado que as experiências prévias reorganizam a dinâmica temporal da germinação, compatível com memória de estresse. No Artigo IV, examinou-se a persistência desses sinais na fase de muda sob seca intermitente, no qual observou-se que a memória de estresse se expressa também na fase de muda, aumentando a resiliência e recuperação após reidratação através de ajustes fisiológicos. Conclui-se que *C. pyramidale* não responde ao semiárido apenas por resistência, mas também por plasticidade a partir de estímulos de calor e hidratação descontínua percebidos que se expressam na fase de semente e no crescimento inicial.

Palavras-chave: Rustificação de mudas; Semiárido; *Fabaceae;* Catingueira; Ecofisiologia.

ABSTRACT

In dry ecosystems, seeds may be exposed to pulses of high temperature and discontinuous hydration during germination. Beyond acting as stressors, these events can function as environmental cues that induce persistent adjustments and modulate germination as well as resilience to water deficit. This dissertation aimed to evaluate how exposure of seeds to high-temperature pulses and hydration–dehydration (HD) cycles influences the expression of stress memory in seeds and seedlings of *Cenostigma pyramidale*, a woody legume widely distributed in the Caatinga. Accordingly, the dissertation was structured into four articles. In Article I, we assessed the effects of HD cycles applied at different imbibition stages on germination and early growth. HD cycles applied during early hydration increased germination and growth-related parameters, whereas cycles imposed at later stages reduced germination performance and seedling growth. In Article II, seeds were subjected to thermal pulses of 55, 65, and 75 °C for 30, 60, and 90 min. The results indicated high germination resilience, although germination kinetics and synchrony varied with pulse intensity and duration. In Article III, we tested the influence of pre-germinative treatments (high temperature, HD cycles, and their combination) on germination under water deficit induced with PEG 6000. Prior experiences reorganized the temporal dynamics of germination, consistent with stress memory. In Article IV, we examined the persistence of these signals during the seedling stage under intermittent drought, showing that stress memory is also expressed in seedlings, enhancing resilience and recovery after rehydration through physiological adjustments. We conclude that *C. pyramidale* responds to semiarid conditions not only through resistance, but also through plasticity triggered by heat and discontinuous hydration cues, expressed during the seed phase and early establishment.

Keywords: seedling hardening; semiarid; *Fabaceae*; catingueira; ecophysiology.

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SUMÁRIO

1. APRESENTAÇÃO

Esta dissertação de mestrado investiga a expressão da memória de estresse em sementes e plântulas, considerando a influência da hidratação descontínua e da alta temperatura. A memória de estresse em plantas é um fenômeno fisiológico relevante para compreender como espécies vegetais percebem, armazenam e recuperam informações metabólicas associadas a eventos adversos, resultando em respostas mais rápidas e/ou mais eficientes diante de estresses subsequentes. Em um cenário de intensificação de extremos climáticos, seu estudo assume importância por contribuir para a compreensão de mecanismos adaptativos de sobrevivência e por oferecer subsídios a práticas voltadas à restauração em regiões semiáridas.

Em ambientes sazonalmente secos, como a Caatinga brasileira, a alta temperatura e a irregularidade das chuvas atuam como filtros ambientais centrais, modulando a regeneração e a persistência das espécies. Nesse contexto, sementes podem ser expostas a temperaturas elevadas na superfície do solo durante a estação seca e, posteriormente, a pulsos de hidratação descontínua no início da estação chuvosa. Essas flutuações, além de funcionarem como estressores, podem operar como sinais ambientais capazes de induzir ajustes fisiológicos persistentes, com potencial para influenciar o desempenho das plântulas sob déficit hídrico.

Diante disso, este trabalho foi conduzido a partir de uma sequência de perguntas norteadoras que orientou a execução dos experimentos e a organização dos artigos, tendo como espécie modelo *Cenostigma pyramidale* (catingueira), uma leguminosa lenhosa amplamente distribuída na Caatinga, com elevado valor ecológico e socioambiental.

Primeiramente, foi questionado se *a hidratação descontínua de sementes exerce influência significativa sobre o comportamento germinativo e o crescimento inicial de plântulas de catingueira?* Para responder a essa questão, desenvolveu-se o Artigo I, intitulado "A hidratação descontínua em distintos estágios de embebição modula a expressão da memória de hidratação em sementes e plântulas de *Cenostigma pyramidale* (Tul.) Gagnon & G.P. Lewis (*Fabaceae*)", a ser submetido ao periódico *Journal of Arid Environments*. Foi observado que a hidratação descontínua interage de forma significativa com parâmetros germinativos e de crescimento e que o estágio de embebição no momento da aplicação dos ciclos de hidratação e desidratação (ciclos de HD) é determinante para a resposta observada, sugerindo a atuação de mecanismos compatíveis com memória de estresse e possíveis *trade-offs* entre crescimento e defesa.

Em seguida, considerando que sementes na Caatinga podem estar naturalmente expostas a pulsos de alta temperatura na superfície do solo, formulou-se a segunda pergunta: *qual é a influência de pulsos de alta temperatura sobre a germinação de C. pyramidale?* Essa questão originou o Artigo II, intitulado: "Sementes de *Cenostigma pyramidale* (Tul.) Gagnon & G.P. Lewis (*Fabaceae*) exibem resiliência germinativa sob pulsos de alta temperatura", que foi submetido ao periódico *Arid Land Research and Management*. Nesse manuscrito, evidenciou-se elevada resiliência germinativa a exposições térmicas a 55ºC, 65ºC e 75ºC, reforçando a plasticidade da espécie nos estágios iniciais de desenvolvimento, ainda que determinados atributos germinativos sejam modulados conforme a intensidade e duração do pulso.

A partir dos resultados dos Experimentos I e II, emergiu a terceira pergunta: *se ciclos de HD e alta temperatura, isoladamente, alteram a germinação em condições ótimas, qual é o papel da combinação desses fatores quando as sementes são submetidas ao déficit hídrico? A exposição a esses fatores pode induzir a expressão de memória de estresse sob condições de estresse osmótico?* Para responder, conduziu-se o Experimento III, do qual resultou em um terceiro artigo a ser submetido ao periódico *Arid Land Research and Management*, intitulado "Mudando para sobreviver: a influência da alta temperatura e da hidratação descontínua no comportamento germinativo da espécie nativa da Caatinga *Cenostigma pyramidale*". Os resultados indicaram que a história pré-germinativa pode alterar o comportamento germinativo da espécie sob estresse hídrico e revelar padrões de plasticidade associados à memória de estresse.

Por fim, uma quarta questão tornou-se central para integrar a germinação ao desempenho pós-emergência: *os sinais percebidos na fase de semente persistem e se expressam na fase de plântula, modulando a resiliência à seca ao longo do desenvolvimento inicial por meio da expressão de memória de estresse?* Essa pergunta fundamentou o Artigo IV, intitulado "A experiência da semente modula a resiliência à seca em mudas de *Cenostigma pyramidale*: efeitos contrastantes dos ciclos de HD, pulsos térmicos e sua combinação", que avalia os efeitos de pulsos térmicos, ciclos de HD e sua combinação sobre atributos morfofisiológicos e bioquímicos de plântulas sob regimes hídricos contrastantes e déficit hídrico recorrente.

De modo geral, os resultados sustentam que a hidratação descontínua tende a favorecer um perfil funcional associado à manutenção fisiológica e à recuperação após eventos de seca, enquanto a combinação entre pulso térmico e ciclos de HD não necessariamente produz ganhos aditivos, sugerindo que múltiplos sinais de estresse podem impor custos fisiológicos capazes de limitar a expressão de benefícios sob seca prolongada e recorrente.

Assim, o conjunto de evidências apresentado nesta dissertação demonstra que a interação entre hidratação descontínua, alta temperatura e déficit hídrico desempenha papel central na modulação da germinação e do desempenho inicial de *Cenostigma pyramidale,* reforçando a memória de estresse como mecanismo ecofisiológico adaptativo em espécies de ambientes semiáridos. Esses resultados podem subsidiar estratégias aplicadas à produção de sementes e mudas mais tolerantes, contribuindo para ações de restauração e manejo em áreas degradadas sob crescente pressão climática

2. FUNDAMENTAÇÃO TEÓRICA

1.1 ENTRE FLORES E ESPINHOS: ECOLOGIA DA CAATINGA

A Caatinga é um bioma exclusivo do Brasil, conhecido por seu clima quente e semiárido, bem como por sua vegetação característica de Floresta Tropical Sazonalmente Seca (FTSS) (Queiroz, 2009). O bioma abrange uma área de 844.453 Km², o que representa aproximadamente 10% do território brasileiro. Além de ser encontrado em todos os estados da Região Nordeste do Brasil, também abrange o Norte do Estado de Minas Gerais. Em Sergipe, a vegetação da Caatinga corresponde a 3,8% dos 13% de cobertura vegetal nativa (IBGE, 2004; Queiroz, 2009; Brasil, 2012).

É importante ressaltar que as florestas tropicais sazonalmente secas (FTSS), como a Caatinga, estão no topo do ranking quando se trata de perda de cobertura vegetal, devido às atividades humanas (Powers *et al*., 2011; Paz et al., 2016). Entre as principais atividades que degradam o bioma Caatinga, destacam-se o desmatamento para fins agropecuários, como pastoreio, práticas agrícolas inadequadas e queimadas (Araújo *et al*., 2007; Fernandes *et al*., 2015; Melo, 2018). Em Sergipe, observa-se uma redução de aproximadamente 22,2% da área total da Caatinga entre os anos de 1992 e 2013. Estudos apontam que a degradação desse bioma está diretamente relacionada ao aumento das áreas destinadas à pecuária, cultivo agrícola e exposição do solo, de acordo com Fernandes *et al*. (2015).

As temperaturas nas áreas de Caatinga estão entre as mais altas quando comparadas com as médias de outros ecossistemas brasileiros, variando de 26ºC a 28ºC. A concentração pluviométrica é extremamente baixa, com uma média anual de 750mm. Além disso, as chuvas apresentam distribuição irregular e ocorrem em meses específicos (Velloso *et al.*, 2002; Queiroz, 2009; Andrade *et al.*, 2010). Devido às altas temperaturas, intensa radiação solar e ao solo ressecado devido à escassez de água, as espécies de fauna e flora que habitam esse bioma possuem características ecológicas, morfológicas e fisiológicas únicas, resultado de adaptações evolutivas (Prado, 2003; Trovão *et al.*, 2007; Queiroz, 2009; Andrade, 2017).

Quando observamos a vegetação da Caatinga, verifica-se características como uma floresta de porte baixo, predominando espécies arbóreas e arbustivas-arbóreas, com um dossel em sua maioria descontínuo (Nascimento; Machado; Dantas, 2015). Em adição a isso, as espécies vegetais, presentes nessa região, possuem adaptações foliares, como espinhos, acúleos, microfilia e características xeromórficas, possibilitando extrema resiliência e tolerância a diversos estresses ambientais (Queiroz, 2009). Os diásporos da Caatinga também apresentam características que lhes conferem maior tolerância as condições adversas e garantem o estabelecimento das espécies vegetais, como tegumentos mais rígidos, dormência tegumentar e resiliência frente a fatores como disponibilidade hídrica descontínua, altas temperaturas e salinidade (Meiado et al., 2013; Paz et al., 2016).

Esses dados evidenciam a riqueza biodiversa da Caatinga, no entanto, estudos apontam uma necessidade de maior implementação de projetos e estudos socioambientais que visem melhor compreender a ecologia desse bioma (Santos *et al.*, 2016) possibilitando, assim, o desenvolvimento de ações que visem a restauração do ecossistema da Caatinga, juntamente com o desenvolvimento de técnicas que englobem a conservação da fauna e flora singularmente peculiares dessa região (Silva *et al.*, 2016).

1.2 ECOS DA SOBREVIVÊNCIA: O ESTRESSE EM PLANTAS

O estresse em plantas é tido como um dos fenômenos de maior interesse e pesquisa nos estudos de vegetais (Demongeot *et al.*, 2019) e, apesar de haver divergências para uma definição completa e assertiva para esse termo aplicado às plantas, a maioria dos autores definem estresse como sendo uma influência desvantajosa exercida por fatores ambientais, sejam estes bióticos ou abióticos (calor, água, anoxia, parasitismo, infecções), que podem interferir na produtividade e bom desenvolvimento dos vegetais (Buchanan *et al.*, 2000; Smith *et al.*, 2010; Taiz; Zeiger, 2017).

Diante de tais descrições, Jansen e Potters (2017) alertam para um enviesamento agronômico que circunda as perspectivas anteriormente citas, voltadas para a produção vegetal e a segurança alimentar global, enfatizando os fatores ambientais como agentes estressores e se distanciando de uma análise acerca das respostas dos organismos vegetais à tais fatores. Urge, então, a necessidade de aplicar uma definição de estresse em plantas que leve em consideração os impactos espécies-específicos que esse fenômeno pode acarretar (Hopkins, 1999).

Nesse interim, discutem-se outras definições, como proposto por Larcher (1980), o qual refere-se ao estresse como um conjunto de mudanças fisiológicas que ocorre quando organismos vegetais experenciam condições desfavoráveis no ambiente ao qual está inserido. Já para Leclerc (2003), as condições ambientais desfavoráveis ocasionam um desvio do padrão normal de crescimento das plantas, tornando-se um fator estressor que consequentemente irá estimular uma resposta ao estresse do organismo vegetal, que pode ser a nível molecular, celular, genética ou fisiológica.

As mudanças fisiológicas direcionadas durante uma fase de estresse podem desencadear benefícios ou distúrbios para o desenvolvimento vegetal (Mahmood *et al*., 2020). Sendo assim, Selye (1964) e Lichtenthaler (1988) definem como *eustress* as respostas adaptativas a um fator estressor, que proporcionam incrementos metabólicos para o vegetal frente as novas condições ambientais. Geralmente o *eustress* ocorre quando o organismo está exposto a níveis poucos intensos do agente estressor. No entanto, em situações contrárias, ou seja, alta intensidade do estresse, pode ocorrer o *distress,* o qual se caracteriza por influenciar um padrão destrutivo para o metabolismo e bom funcionamento do vegetal. Ademais, as imposições ocasionadas entre o *eustress* e *distress* torna-se um fator determinante para a sobrevivência ou não de uma planta (Jansen; Potters, 2017).

Diante disso, o estresse em plantas pode ser resumido como um estado em que as condições impostas ao vegetal geram uma desestabilização das funções normais do indivíduo, podendo estimular respostas metabólicas que as guiarão para vias de normalização e tolerância, danos permanentes ou em casos mais extremos, à morte (Gaspar *et al*., 2002).

Não obstante, estudos mostram que a passagem por estresses recorrentes pode estimular a ativação da chamada memória de estresse, fenômeno que pode atuar na aquisição de maior tolerância e resistência no enfrentamento de condições adversas, às quais os organismos vegetais estão suscetíveis (Walter *et al*., 2013; Friedrich *et al*., 2019). Portanto, a compreensão detalhada das respostas das plantas ao estresse, especialmente em ambientes de clima mais extremo, como o semiárido, é crucial para o desenvolvimento de práticas ecológicas e de restauração.

1.3 MARCAS DE UMA VIDA: INFLUÊNCIA DOS ESTRESSES AMBIENTAIS NA AQUISIÇÃO DE MEMÓRIA EM PLANTAS

Durante as diversas fases da vida vegetal, esses organismos enfrentam constantes flutuações nas condições ambientais, sejam elas favoráveis ao seu desenvolvimento ou estressantes (Li; Liu, 2016; Kigel, 2017). Esses fatores impõem uma necessidade contínua de adaptação e ajustes fisiológicos para garantir sua sobrevivência e desempenho ecológico diante desses estresses recorrentes (Bruce *et al*., 2007; Wojtyla *et al*., 2020). Dessa forma, estudos observam que essa interação pode deixar marcas metabólicas que aumentam a tolerância e a resistência a estresses subsequentes e mais severos (Walter *et al*., 2013; Ramírez, 2015).

Estas marcas, por sua vez, possibilitam um fenômeno chamado de memória de estresse. Este conceito representa uma relação intrínseca entre a exposição à agentes estressores e as respostas direcionadas pelo organismo vegetal a esses eventos (Avramova, 2015; Sen e Puthur, 2020). Não obstante, a memória ao estresse pode envolver alterações em diferentes níveis, como morfológico, fisiológico, transcricional e epigenético (Ramírez *et al*., 2015; Sun *et al*., 2018; Demongeot *et al*., 2019).

Todavia, após a percepção de um estímulo de estresse, a planta pode seguir três vias até chegar a uma resposta final, sendo uma delas uma resposta direta que ocorre quase que imediatamente, não dependente do histórico de estímulos anteriores recebido pelo organismo; as demais vias envolvem o processo de memória, sendo a memória de aprendizado ou de armazenamento/resgate. No entanto, ainda não se sabe se as três vias de respostas ocorrem de maneira independente ou se há uma correlação entre elas (Trewavas, 2003; Demongeot *et al*., 2019).

Na memória por aprendizado, a repetição de um mesmo estímulo modula a intensidade da resposta da planta, e pode direcionar uma familiarização ao estresse, diminuindo a intensidade da resposta ou ativando uma sensibilização, na qual haverá uma resposta mais intensa (Knight *et al*., 1998). Já na forma de armazenamento e resgate, as alterações metabólicas decorrentes do direcionamento de resposta ao estresse são armazenadas. Diante disso, o organismo pode ficar em uma fase de alerta, o que permite uma resposta mais ágil, caso haja um estresse subsequente (Hilker; Schmulling, 2019; Jacques *et al*., 2021).

Não obstante, alguns estudos evidenciam um segundo mecanismo ocasionado pela memória ao estresse, definido como tolerância cruzada, no qual, ao experienciar um estresse primário, a planta desenvolve uma maior tolerância a um segundo agente estressor, diferente do primeiro (Zhang *et al.,* 2013; Li *et al*., 2014; Faralli *et al*., 2015). Dessa forma, a tolerância cruzada ocorre devido a ativação de múltiplas vias de sinalizações do estresse, durante a fase do estresse primário, e essa ativação direcionará vias sinergéticas ou antagônicas durante estresses subsequentes. Assim, um único estressor pode gerar tolerância para múltiplos estresses (Hossain *et al.*, 2018).

Nos últimos anos tem-se intensificado estudos que busquem compreender o papel da memória do estresse no desenvolvimento das plantas, além da necessidade de compreender as suas múltiplas formas de expressão em meio aos diferentes estágios da vida vegetal (Srivastava *et al.*, 2021), conforme discutido no tópico a seguir.

1.4 SOBREVIVENDO A EXTREMOS: A ALTA TEMPERATURA E HIDRATAÇÃO DESCONTÍNUA NA GERMINAÇÃO E CRESCIMENTO DE PLANTAS

O ciclo de vida das gimnospermas e angiospermas se caracteriza pelo desenvolvimento de uma semente, seu processo germinativo e por conseguinte a originação de um novo indivíduo (Ferreira; Borghtetti, 2004). A germinação, por sua vez, é definida como a retomada das atividades metabólicas e início da formação de uma nova plântula através do embrião resguardado pelas estruturas da semente (Bewley; Black, 1978; Taiz; Zeiger, 2017). Dessa forma, a performance de desenvolvimento e estabelecimento de uma planta está diretamente ligada ao sucesso obtido durante seu processo germinativo, sendo este influenciado por diferentes fatores internos e externos, tal como qualidade fisiológica da semente, disponibilidade hídrica e condições edafoclimáticas (Kigel, 2017).

Em ecossistemas áridos e semiáridos, como é o caso da Caatinga, é comum haver flutuações climáticas com a ocorrência de secas periódicas e altas temperaturas (Alves et al., 2009; Meiado et al., 2020). Sendo assim, sementes de espécies presentes nesses ambientes, quando dispersas nas camadas superficiais do solo, são naturalmente expostas à altas temperaturas e a descontinuidade da absorção de água durante o processo de germinação, principalmente em áreas abertas, devido à incidência de raios solares e altas taxas de evapotranspiração (Costa; Araújo, 2003; Santos, 2013; Meiado, 2014).

Trabalhos de monitoramento de campo já registraram temperatura média acima de 50 ºC na superfície do solo, apresentando teor de água no substrato em torno de 4% em áreas da Caatinga (Souto *et al.*, 2009; Almeida; Souto; Souto, 2013). Em outras regiões semiáridas do planeta, já foram registradas temperaturas da superfície do solo variando de 50 ºC (regiões temperadas) a 80 ºC (regiões desérticas) (Rizzini, 1976; Larcher, 2000; Fichino *et al.*, 2016).

Apesar da ocorrência desses fatores de forma conjunta, não foram encontrados estudos na literatura que buscassem avaliar a influência da exposição à alta temperatura e hidratação descontínua conjuntamente para o processo germinativo e desenvolvimento inicial de plântulas.

No entanto, separadamente, verifica-se que a hidratação descontínua, através dos ciclos de hidratação e desidratação (HD), e a pré-exposição de sementes à altas temperaturas podem agir de forma a incrementar ou reduzir parâmetros germinativos e de crescimento ou, em alguns casos, apresentar uma neutralidade para o organismo (Rito *et al*., 2009; Pedrero-López *et al*., 2016; Rafael *et al*., 2018; Freitas *et al*., 2021; Santos Júnior et al., 2022).

Os ciclos de hidratação e desidratação (HD) constituem uma técnica utilizada como tratamento condicionante (*priming*) em sementes, que busca simular, de forma controlada, a embebição hídrica descontínua que ocorre de forma natural no ambiente, antes da protrusão da radícula (Lima; Meiado, 2017; Santos Júnior *et al*., 2021). Além de permitir a análise da resposta ecofisiológica das espécies submetidas a esse fenômeno, essa técnica passou a ser bastante utilizada como uma forma de preparação dos vegetais para enfrentar alterações ambientais, uma vez que é capaz de estimular a ativação da memória de estresse em sementes, podendo interagir nos parâmetros germinativos, vigor e tolerância aos estresses ambientais em suas sementes e mudas (Dubrovsky, 1998; Rito *et al.,* 2009; Nascimento, 2016; Lima; Meiado, 2017; Freitas *et al*., 2021).

De maneira semelhante, alguns trabalhos abordam o uso da alta temperatura como técnica pré-germinativa (Paparella *et al.,* 2015; Farooq *et al.,* 2019). O termocondicionamento de sementes é aplicado utilizando-se diferentes amplitudes térmicas e tempos distintos (Farooq *et al.,* 2019). Estudos mostram que esse fenômeno pode auxiliar na superação da dormência tegumentar, uma vez que proporciona o surgimento de microfissuras no tegumento da semente, possibilitando assim o influxo de água e, consequentemente, a germinação (Rafael *et al.*, 2018; Santos Júnior *et al.,* 2022).

Não obstante, a alta temperatura também pode ocasionar a aceleração da atividade de enzimas e metabolismo celular (Carvalho; Nakagawa, 2012; Paparella *et al*., 2015), mudanças na conformação da parede celular e proteínas (Mittler; Finka; Golouubinoff, 2012). Sendo assim, tais alterações podem interagir com o processo germinativo, crescimento e desenvolvimento vegetal, como já observado em alguns trabalhos, nos quais, plantas oriundas de sementes condicionadas apresentaram melhor performance de germinação e crescimento (Mittler; Finka; Goloubinoff, 2012; Wang *et al*., 2016; Jespersen, 2020).

De acordo com Rizzini (1976), espécies que se beneficiam da passagem por altas temperaturas são denominadas de termófilas/pirófitas, possuindo assim melhor desempenho em ambientes quentes, secos ou suscetíveis a incêndios. Estas espécies possuem papel crucial para a restauração de bancos de sementes desses locais (FICHINO *et al.,* 2016). No entanto, determinadas espécies possuem menor tolerância a esse fator e, em alguns casos, a passagem pela alta temperatura pode causar danos ou até mesmo levar a morte do embrião (Fichino *et al*., 2012; Santana *et al*., 2019). Em adição a isso, nota-se uma escassez de estudos que busquem avaliar a influência desse fator, principalmente para espécies de regiões biodiversas e com maior suscetibilidade, como a Caatinga (Santos Júnior *et al*., 2022).

Portanto, considerando as singularidades da exposição de sementes a alta temperatura e hidratação descontínua, e diante da necessidade de uma maior compreensão da sua influência na germinação e crescimento de mudas, é importante analisar os efeitos desses fatores, não apenas separadamente, como também de forma conjunta. O maior conhecimento desses fenômenos permitirá o desenvolvimento e aprimoramento de práticas que visam a produção de mudas com melhor aclimatação para utilização em programas de recuperação de áreas degradadas, principalmente de regiões com clima semiárido, como a Caatinga brasileira.

1.5 VIVER É RESISTIR: CONHECENDO A BIOLOGIA E ECOLOGIA DE *Cenostigma pyramidale* (Tul.) E. Gagnon & G.P. Lewis

A família de angiospermas *Fabaceae* possui em torno de 727 gêneros e 19.327 espécies, apresentando distribuição cosmopolita e se destacando como uma das famílias com maior ocorrência na maioria dos ecossistemas do Brasil (Lima *et al*., 2013). No território nacional, é registrado a ocorrência de 212 gêneros nativos e cerca de 2.732 espécies (Lewis *et al*., 2005; Lima *et al*., 2013). Além disso, as leguminosas se apresentam como a família de maior representatividade no bioma Caatinga, local onde ocorrem 86 gêneros e 320 espécies, totalizando cerca de um terço da riqueza florística catingueira (Queiroz, 2009).

Nesse contexto, *Cenostigma pyramidale* (Tul.) E. Gagnon & G.P. Lewis se caracteriza como uma representante das leguminosas nativas da Caatinga, sendo popularmente conhecida como catingueira, catingueira-verdadeira, catingueira-de-porco, canela-de-velho ou pau-de-rato (Matias; Silva; Dantas, 2017). Possuindo ampla distribuição geográfica em toda Região Nordeste, a espécie pode ser encontrada em várzeas úmidas, no qual apresenta hábito arbóreo, podendo atingir até 12 metros de altura ou em regiões semiáridas, nas quais se reduz a arbustos com cerca de 2 metros (Maia, 2012; Carvalho, 2014).

C. pyramidale apresenta copa em formato de pirâmide invertida, folhas bipinadas e coriáceas, com comportamento decíduo durante períodos de seca, não apresentando espinhos em seus órgãos, (Carvalho, 2014). Além disso, suas flores são amarelas, distribuídas em racemos terminais e subterminais que dão origem a frutos do tipo legume, seco e deiscente, que apresentam média entre 6-10 centímetros, com coloração marrom-esverdeada e consistência coriácea, com dispersão balística (Silva; Matos, 1998; Maia, 2012; Carvalho, 2014). Já as suas sementes são ortodoxas, estenospérmicas e ovaladas, compridas lateralmente e com lados planos ou ligeiramente convexos. O tegumento é caracterizado por uma coloração castanho-escuro ou verde-escuro, liso, brilhante, delgado e coriáceo (Silva; Matos, 1998).

De maneira geral, as sementes de catingueira não apresentam dormência (Santos *et al*., 2012; Matias *et al*., 2013; Santos et al., 2014). No entanto, na literatura, recomenda-se o uso de tratamento pré-germinativo para possibilitar a homogeneidade na germinação (Oliveira *et al*., 2011). A temperatura ideal para o processo germinativo varia entre 25ºC a 35ºC graus e suas mudas conseguem manter um bom desempenho no crescimento em diferentes tipos de substratos, incluindo os mais pobres e pedregosos (Lima *et al*., 2011; Maia, 2012; Santos *et al*., 2014).

A catingueira possui também aspectos socioeconômicos devido ao seu uso para alimentação animal, produção de lenha e oferta de néctar, pólen e abrigo para abelhas silvestres nativas. Além disso, pode ser utilizada como recurso madeireiro e aplicações na fitofarmacologia (Maia, 2012; Carvalho, 2014).

Ademais, é reconhecido na literatura o alto potencial de tolerância que a espécie apresenta à estresses ambientais, tal como o estresse hídrico, salino e térmico (Antunes *et al*., 2011; Matias *et al*., 2013) sendo considerada espécie pioneira e com potencial para regeneração de áreas degradadas (Queiroz, 2009; Maia, 2012).

Entretanto, não foram encontrados na literatura estudos que buscaram avaliar conjuntamente a influência da alta temperatura e hidratação descontínua na germinação, crescimento e expressão de memória para a espécie citada. Portanto, tendo em vista a ocorrência desses dois fatores de forma simultânea no ambiente natural, compreender a sua dinâmica e interação com o desenvolvimento de *C. pyramidale* pode possibilitar dados capazes de sustentar técnicas para produção de mudas da espécie, bem como entender sua ecofisiologia em regiões de Caatinga.

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3. ARTIGO 1

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Discontinuous hydration at distinct imbibition stages modulates the expression of hydration memory in seeds and seedlings of *Cenostigma pyramidale* (Tul.) Gagnon & G.P.Lewis (Fabaceae)

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Abstract

Plants in semi-arid ecosystems, such as those inhabiting the Caatinga, a Seasonally Dry Tropical Forest (SDTF) in Brazil, are frequently exposed to recurrent water stress events. Under such conditions, the activation of stress memory mechanisms can act as an adaptive strategy, enhancing the plant's ability to tolerate and respond more efficiently to subsequent stress episodes. This study investigated whether hydration–dehydration cycles (HD cycles) induce hydration memory in seeds and seedlings of *Cenostigma pyramidale*, a woody legume native to the Caatinga. Seeds were subjected to 0, 1, 2, or 3 HD cycles at two distinct stages of the imbibition curve: an early stage (½ of Phase I) and a later stage (¼ of Phase II), followed by germination under optimal conditions. Germinability, time to 50% germination, germination rate index, and synchrony were assessed. Seedlings were then cultivated in a greenhouse, and growth parameters were recorded. A significant interaction between hydration stage and number of HD cycles was observed. HD cycles applied at the early stage enhanced germination by increasing germinability and vigor while reducing germination time. In contrast, HD cycles at the later stage impaired germination and decreased the emergence rate index. Conversely, for seedlings, fewer HD cycles at the early stage and more cycles at the later stage reduced plant height and biomass. These results suggest that HD cycles differentially modulate hydration memory depending on developmental stage, potentially triggering both adaptive and sensitizing responses that may contribute to the ecological resilience and establishment of *C. pyramidale* in the Caatinga.

Keywords Seed priming · Stress memory · Hydration–dehydration cycles · Germination · Caatinga

Introduction

Plants, as sessile organisms, are continuously exposed to a range of biotic and abiotic environmental factors that can induce stress, requiring the activation of defense mechanisms (Galviz et al. 2022). Over the course of their evolutionary history, plant species have evolved a variety of adaptative strategies to ensure establishment and survival across diverse ecosystems (Hilker and Schmülling 2019).

Among these strategies, one that has increasingly attracted scientific interest is the expression of stress memory. Plant memory can be defined as the capacity to store physiological or metabolic information generated during a prior stress event and to retrieve these metabolic imprints upon subsequent exposure (Thellier and Lüttge 2013; Demongeot et al. 2019). Some studies have demonstrated that memory in plants can be expressed across multiple developmental stages. It can be established during early phases, such as the seed stage, and later recalled during germination or seedling growth (Freitas and Silva 2024; Silva et al. 2025).

These memory mechanisms are particularly relevant for species adapted to environments characterized by pronounced climatic fluctuations, such as semi-arid regions (Fleta-Soriano and Munne-Bosch 2016; Kambona et al. 2023).

In northeastern Brazil, the Caatinga ecosystem, classified as a seasonally dry tropical forest (SDTF) is defined by extreme climatic conditions, including high temperatures, irregular and poorly distributed rainfall, and well-defined dry seasons (Andrade-Lima 1981; Alves et al. 2009). Despite these challenging conditions, the Caatinga harbors remarkable floristic diversity and a wide array of adaptive mechanisms that support species survival (Fernandes and Queiroz 2018). Several studies have documented the expression of stress memory in seeds and plants native to this ecosystem, providing evidence that such mechanisms contribute to the ecological success of certain species (Silva et al. 2021; Freitas and Silva 2024; Lima and Meiado 2025; Silva et al. 2025).

A notable example is hydration memory expressed in seeds and seedlings from species native to the Caatinga (Lima and Meiado 2018). Given the irregular rainfall patterns, seeds within the soil seed bank are frequently subjected to discontinuous hydration during the germination process. This phenomenon can act as a priming stimulus, preparing the plant to cope with future stress conditions by inducing metabolic adjustments that activate the expression of stress memory (Pereira-da-Silva et al. 2024). As a result, enhanced germination performance (Santos et al. 2024), enhanced seedling growth, and increased tolerance to subsequent environmental stressors have been reported (Santos-Júnior et al. 2021).

Cenostigma pyramidale (Tul.) Gagnon & G.P. Lewis is a pioneer woody legume widely distributed throughout the Caatinga ecosystem (Ribeiro et al. 2021). Previous studies have shown that this species is tolerant to environmental stresses and adverse conditions (Pinho-Pessoa et al. 2018; Wright et al. 2023), and that discontinuous seed hydration can modulate the mobilization of seeds reserves (Pereira-da-Silva et al. 2024). Although studies in literature have investigated the influence of HD cycles on the germination and seedling growth of *C. pyramidale* under water stress (Silva et al. 2018; Oliveira et al. 2022), we did not find any work that evaluated the influence of hydration at different points of the species' imbibition curve.

Therefore, this study aimed to evaluate the effects of different numbers of hydration-dehydration (HD) cycles applied at distinct stages of seed imbibition on germination performance and early seedling growth in *C. pyramidale*. We tested three main hypotheses: (i) that the number of HD cycles influences seed germination behavior and seedling development; (ii) that the timing of HD application (½ of Phase I or ¼ of Phase II of imbibition) alters germination responses; and (iii) that there is an interaction between the number of HD cycles and the timing of their application, such that the physiological effects of HD cycles depend on the imbibition stage in which they occur.

Material and methods

Study area and seed collection

The experiment was conducted under semi-controlled conditions at the Laboratory of Plant Physiology and Ecophysiology and in a greenhouse belonging to the Department of Biology of the Federal University of Sergipe (UFS).

Cenostigma pyramidale seeds used in the experiment were donated by the Center for Ecology and Environmental Monitoring (NEMA) at the Federal University of Vale do São Francisco (UNIVASF). Seed lots were collected from native Caatinga areas in the Northeast region of Brazil.

Hydration and dehydration Cycles

The timing of the HD cycles was based on the hydration and dehydration curves of *C. pyramidale* seeds. To establish the hydration curve, five replicates of 20 seeds were initially weighed to obtain their pre-hydration mass. The seeds were then placed in 9 cm Petri dishes containing two layers of filter paper moistened with 10 mL of distilled water and kept at room temperature (25 °C). Weighing was performed at 60-minute intervals until radicle protrusion, which indicates the completion of germination. Based on the hydration curve, the time corresponding to half of Phase I (2 hours) and one-quarter of Phase II (9 hours) was selected as the standard hydration time, following the methodology of Lima et al. (2018).

The same experimental design used for hydration was employed to determine the dehydration curve. Two groups of five replicates, each with 20 seeds, were initially weighed to record their pre-hydration mass. The seeds were then placed in 9 cm Petri dishes containing two layers of filter paper moistened with 10 mL of distilled water and allowed to hydrate at room temperature (25 °C) for the periods corresponding to ½ of Phase I and ¼ of Phase II. After hydration, excess surface moisture was removed, and the replicates were weighed to obtain post-hydration mass. Subsequently, the seeds were transferred to a drying oven at 35 °C and weighed at 60-minute intervals until their mass returned to the original pre-hydration value.

The dehydration time was defined as the total time required for the seeds to return to their pre-hydration weight (Lima et al. 2018). This process took 2 hours for seeds hydrated at ½ of Phase I and 4 hours for those hydrated at ¼ of Phase II.

Following the determination of the hydration and dehydration curves, the experimental design was structured in a 2×4 factorial scheme. This included two hydration times (½ of Phase I and ¼ of Phase II), and four treatments: one (1C), two (2C), and three (3C) hydration–dehydration (HD) cycles, along with a control (0C) consisting of seeds not subjected to any HD cycles (Fig. 1).

Germination analysis

After the application of the hydration and dehydration cycles, seeds were placed in Petri dishes containing two layers of filter paper moistened with 10 mL of distilled water, which was replenished as needed (Fig. 1). Germination was monitored daily for 15 days, and seeds were considered germinated when radicle length reached ≥ 2 mm.

To evaluate the effects of HD cycles on the percentage of germinated seeds the germinability was calculated according to the equation:

$$\%G = \left(\sum_i n_i\right) \cdot N^{-1} \cdot 100$$

where $\sum n_i$ is the sum of germinated seeds in relation to the number of seeds available to germinate in the Petri dish (N); data are expressed as percentages (Ranal and Santana 2006).

To calculate the time required for 50% (T_{50}) of germination the T_{50} was calculated with the equation:

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right) \cdot \left(t_j - t_i\right)}{n_j - n_i}$$

where N represents the total number of germinated seeds, n_i is the cumulative number of germinated seeds at time t_i, and n_j is the cumulative number of germinated seeds at time t_j, when $n_i < \frac{N}{2} < n_j$ (Farooq et al. 2005).

The germination rate index (GRI) was calculated to evaluate how HD cycles influenced germination, using the following equation:

$$GRI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \cdots + \frac{G_n}{N_n}$$

Where G1, G2, … Gn represent the number of seedlings that germinated in the first, second, and up to the last observation, and N1, N2, … Nn correspond to the number of days after sowing (Maguire, 1962).

To check the uniformity of seed germination, synchrony index (SI) is given by:

$$SI = \sum_i \frac{C_{n_{i,2}}}{N}$$

where $C_{n_{i,2}} = n_i \frac{(n_i - 1)}{2}$ and $N = \sum n_i \left(\frac{\sum n_i - 1}{2}\right)$ is the combination of seeds germinated in the time i, in pairs, and n_i is the number of seeds germinated in the time (Primack, 1980).

c

Statistical analysis

Germination parameters were calculated using GerminaQuant 1.0 (Marques et al. 2015). Data normality was assessed using the Shapiro-Wilk test, and all variables met the assumption of residual normality (P > 0.05). Germination and growth variables were analyzed by two-way ANOVA, followed by Tukey's post hoc test for mean comparisons (P < 0.05). Statistical analyses were performed using PAST software (version 5.0), and graphical representations were generated with SigmaPlot (version 12.0).

Results

Discontinuous hydration of *C. pyramidale* seeds at different times and numbers of cycles interacted significantly with the germination parameters evaluated (Tab. 1).

Seeds subjected to one (1C), two (2C) and three (3C) HD cycles in an early stage of imbibition (½ of phase I) exhibited significantly higher germinability (%G) compared to the control (0C) (tab. 2). No statistical differences were observed in the time to 50% germination (T50) among treatments 0C, 1C, and 2C; however, 3C showed a significantly lower T50, indicating accelerated germination (tab. 2).

The germination rate index (GRI) increased progressively with the number of HD cycles, reaching the highest value in 3C (tab. 2). Conversely, the synchrony index (SI) was significantly lower in 3C compared to the other treatments, suggesting a more asynchronous germination process under repeated stress (tab. 2).

Under a later stage of hydration conditions (¼ of phase II), an opposite trend was observed (tab. 2). Increasing the number of HD cycles led to a reduction in %G, with treatment 1C showing the highest germinability, significantly different from both the control (0C) and 3C (tab. 2). For T50, all HD treatments showed significantly lower value than the control, reflecting a general acceleration of germination regardless of the number of cycles (tab. 2). The germination rate index (GRI) was also higher in all HD treatments than in the control, although no significant differences were found among the HD-treated groups (tab. 2). Notably, treatment 3C exhibited a reduction in GRI compared to its counterpart at ½ of Phase I. SI values did not differ significantly among treatments at this time point, but a significant decrease was observed in treatment 2C relative to its value at the earlier hydration stage (½ of phase I).

Discontinuous hydration of *C. pyramidale* seeds at different times and numbers of cycles interacted significantly with the seedling growth parameters evaluated (tab. 1).

In the seedling group derived from seeds hydrated at ½ of phase I of imbibition, plant height was significantly lower in the 1C treatment compared to the control, while the 2C and 3C treatments did not differ statistically from the other groups (fig. 2a). No significant differences were found among treatments for stem diameter and leaves number (fig. 2). Root length also did not differ significantly between treatments (fig. 2d); however, the 3C treatment showed a significant reduction when compared to the same treatment applied at ¼ of Phase II (fig. 2d), indicating a potential interaction between the number of hydration cycles and hydration timing.

Leaf, stem, and root dry biomass were also significantly reduced in the 1C treatment compared to the other treatments (fig. 3). Consequently, total dry biomass was lower in 1C, while the other treatments maintained similar values (fig. 3d).

For seedlings derived from seeds hydrated at ¼ of phase II, no significant differences were observed among treatments for plant height, number of leaves or root length (fig. 2). However, stem diameter decreased progressively with an increasing number of hydration–dehydration cycles, with the lowest value recorded in the 3C treatment (fig. 2b).

Regarding leaf dry biomass, treatments 1C, 2C, and 3C had significantly lower values compared to the control (fig. 3a). For stem dry biomass, treatments 1C and 3C exhibited significant reductions relative to the control, while 2C presented intermediate values, with no statistical difference from the other treatments (fig. 3b). Root dry biomass did not differ significantly among treatments (fig. 3c). Finally, total dry biomass was significantly reduced in treatments 1C and 3C when compared to the control (fig. 3d).

Discussion

Germination

The successful establishment of plant species in semi-arid environments like the Caatinga depends on adaptive mechanisms that confer tolerance to frequent environmental stress (Singh and Verma 2024). Discontinuous hydration (HD) has been proposed as one such strategy, although its effects remain complex and species-specific (Freitas and Silva 2024; Lima and Meiado 2025). While HD cycles may enhance germination and early seedling growth in some species (Freitas and Silva 2024; Santos et al. 2024), they can also reduce performance or produce neutral effects in others (Nicolau et al. 2020). Notably, HD can act as a trigger for stress memory, allowing seeds to retain metabolic information and respond more effectively to subsequent stress (Silva et al. 2025).

In *C. pyramidale*, germination was significantly influenced by both the timing and number of HD cycles. Seeds exposed to more cycles at an earlier stage of imbibition (½ of Phase I) exhibited higher vigor, as evidenced by increased germination percentage, reduced time to 50% germination (T50), and a higher germination rate index (GRI). Conversely, HD applied at a later stage (¼ of Phase II) improved T50 and GRI but negatively affected germinability (%G).

The increased germination speed and greater vigor observed after undergoing HD cycles indicate that *C. pyramidale* seeds are tolerant to dehydration and may play an important role in the successful establishment of propagules in their natural environment, where water availability is often transient. Thus, rapid and vigorous germination can enhance reproductive success and ensure species survival.

The results suggest that earlier HD cycles may activate **a** physiological priming effect, often referred to as “hydration memory” (Dubrovsky 1996, 1998). Priming is generally understood as a preparatory metabolic state induced by sublethal stress that enhances future stress responses (Chen and Arora 2013). In a study by Pereira-da-Silva et al. (2024), the authors demonstrated that discontinuous hydration during Phase I of imbibition alters the mobilization of reserves in *C. pyramidale* seeds, indicating that dehydration may trigger mechanisms aimed at optimizing energy use, thereby enhancing germination. These findings support the results of the present study, suggesting that the modulation of germinative behavior through hydration memory may be associated with adjustments in the reserve mobilization pattern, contributing to improved seed performance under stress conditions.

In contrast, the reduced germinability and vigor of seeds hydrated at ¼ of Phase II suggest that dehydration at more advanced metabolic stages may exceed the seeds' tolerance threshold, disrupting essential cellular processes. The decline in the synchrony index (SI) in some of these treatments supports this interpretation, as lower SI values may reflect greater heterogeneity in seed responses, potentially due to uneven recovery from stress or sublethal damage.

Phase I of imbibition is considered particularly favorable for priming due to reactivation of energy metabolism, DNA repair, antioxidant activity, and the priming of stress-response genes. In contrast, Phase II may be more involved in memory expression, with active protein synthesis from newly transcribed mRNAs (Chen and Arora 2013). These physiological processes likely underpin the differential responses that we observed.

Furthermore, since seeds only lose water to the environment during dehydration, the physiological processes initiated during hydration—such as reserve mobilization, biochemical synthesis, and DNA repair—are preserved and resume upon rehydration (Dubrovsky 1996, 1998).

This may explain the significant increase in germination parameters that we observed in seeds hydrated at the initial stage of Phase I as the number of HD cycles increased. Since the metabolic changes initiated during each hydration event are preserved and resume upon rehydration, these seeds may have been better able to repair potential damage to their metabolic machinery throughout the subsequent cycles, thereby ensuring improved germination performance.

Our germination results demonstrate a clear and distinct relationship between the species' germinative performance and the specific hydration phase during which hydration and dehydration (HD) cycles are applied. These findings not only contribute to a deeper understanding of the species' physiological responses under fluctuating environmental conditions but also offer valuable insights for developing seedling production techniques tailored for ecological restoration and reforestation initiatives in degraded areas.

Initial Growth

The seedling establishment phase is critical for plant success, and beyond environmental factors, strong germination performance enhances seedling establishment (Donohue et al. 2007). Tolerance to discontinuous hydration during germination may activate physiological stress memory mechanisms that enhance early seedling development and promote the emergence of vigorous, stress-resilient individuals, as reported for *Triplaris gardneriana* Wedd. (Polygonaceae) (Freitas et al. 2021) and *Sarcomphalus joazeiro* (Mart.) Hauenschild (Rhamnaceae) (Silva et al. 2025), both native to the Caatinga.

Additionally, the contrasting patterns that we observed between germination and seedling development suggest that hydration memory is transient and modulated in a spatial and time manner (Freitas et al. 2021).

As sessile organisms, plants rely on their ability to store information through stress memory to ensure faster and more effective responses to future environmental stressors, thereby improving ecological fitness (Jacques et al. 2021; Kambona et al. 2023). This memory can operate through short-term mechanisms (e.g., morphological, physiological, or metabolic changes) or long-term strategies such as epigenetic regulation (Crisp et al. 2016). However, maintaining such memory entails a metabolic cost that may compromise other vital processes such as growth and carbon allocation.

According to the growth–defense trade-off hypothesis (Herms and Mattson 1982; Huot et al. 2014), defense activation often comes at the expense of growth, as resources are reallocated away from photosynthesis and biomass accumulation (Crisp et al. 2016). This framework may help explain our findings, where seedlings from seeds hydrated early (½ of Phase I) and subjected to fewer HD cycles (1C) or hydrated later (¼ of Phase II) under more intense HD conditions (3C), showed lower growth and biomass production. These patterns may reflect a trade-off in which plants favor the maintenance of a heightened state of alert—potentially mediated by the retention of hydration memory acquired during the seed stage—over investment in growth.

While stress memory is often linked to enhanced performance, this relationship is not necessarily positive or linear. Depending on the context, repeated exposure to stress can result in either familiarization (a reduced response) or sensitization (an amplified response) (Conrath et al. 2006; Gagliano et al. 2014). Our findings suggest evidence of a sensitization of memory in *C. pyramidale* seedlings, as indicated by the observed reduction in growth and dry biomass accumulation. This may reflect a physiological reallocation toward more conservative developmental strategies. Although morpho-anatomical traits were not measured in our study, it is plausible that these patterns are associated with a trade-off between growth and defense, such as reductions in specific leaf area or limitations in cell elongation, as protective mechanisms against future fluctuations in water availability. Similar responses have been reported for this species in a study by Ribeiro et al. (2021).

Thus, remaining in a sensitized state may be particularly advantageous for species adapted to environments characterized by recurring stress events, such as drought and high temperatures, that define the Caatinga ecosystem, where *C. pyramidale* naturally occurs.

These results reinforce the adaptive value of priming-induced stress memory in early development stages of species from dry environments, as well as providing valuable insights for seedling production programs in nurseries aimed at ecological restoration of the Caatinga. Our findings highlight the delicate relationship between the hydration phase, the application of hydration–dehydration (HD) cycles, and the modulation of hydration memory expression.

Moreover, we propose that the careful application of HD cycles during early imbibition phases can be regarded as a form of physiological priming, capable of enhancing germinative performance, as evidenced in our germination data, and possibly activating mechanisms that confer greater tolerance to *C. pyramidale* seedlings during early growth. In this sense, practices based on controlled mild water stress simulations may be effectively integrated into pre-germinative management strategies for native species adapted to environments with water availability fluctuations.

However, since our observations were made under optimal conditions for germination and seedling development, further studies are needed to assess the germinative and early growth responses of *C. pyramidale* under abiotic stress conditions such as water deficit, salinity, and high temperatures. These investigations are essential to validate the applicability of hydration memory-based strategies under real-world stress scenarios typical of the Caatinga and others Seasonally Dry Tropical Forests.

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Statements and Declarations

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Author contributions All authors contributed to the study conception and design.

Table 1 Analysis of variance results for variables germinability (%G), time to 50% germination (T50), Germination rate index (GRI), and synchronization index (SI) of *Cenostigma pyramidale* (Tul.) E. Gagnon & G.P. Lewis (*Fabaceae*) seeds subjected to 0, 1, 2, and 3 cycles of hydration and dehydration (HD), with hydration performed either at ½ of Phase I or ¼ of Phase II.

Factors	**f**	**d.f**	**p-value**
Germinability			
HD cycles	10.5705	3	<.0001
Hydration Time	11.082	1	0.0027
HD cycles x Hydration Time	4.429	3	0.0129
T50			
HD cycles	12.7649	3	<.0001
Hydration Time	0.2568	1	0.6168
HD cycles x Hydration Time	1.3416	3	0.2843
GRI			
HD cycles	27.5906	3	<.0001
Hydration Time	4.4694	1	0.0451
HD cycles x Hydration Time	9.4964	3	0.0002
SI			
HD cycles	10.5481	3	<.0001
Hydration Time	16.1836	1	0.0004
HD cycles x Hydration Time	7.6415	3	0.0009
Vigor index			
HD cycles	10.5481	3	<.0001
Hydration Time	16.1836	1	0.0004
HD cycles x Hydration Time	7.6415	3	0.0009

The symbol × indicates interaction between the factors

Table 2 Germinability (%), time to 50% germination (T50), germination rate index (GRI), and synchronization index (SI) of *Cenostigma pyramidale* (Tul.) E. Gagnon & G.P. Lewis (*Fabaceae*) seeds subjected to 0, 1, 2, and 3 cycles of hydration and dehydration (HD), with hydration performed either at ½ of Phase I or ¼ of Phase II.

HD cycles	**Hydration time**	**%G**	**T50**	**GRI**	**SI**
0C	½ of Phase I	83bA	1.64aA	9.62cA	0.62aA
1C		94aA	1.46aA	13.12bA	0.64aA
2C		95aA	1.45abA	13.02bA	0.74aA
3C		92aA	1.22bA	16.14aA	0.47bA
0C	¼ of Phase II	83bA	1.64aA	9.62bA	0.62aA
1C		95aA	1.37bA	13.90aA	0.44bA
2C		87abB	1.35bA	13.32aA	0.55abB
3C		78bB	1.33bA	11.80aB	0.50abA

Lowercase letters indicate differences among HD cycle numbers within the same hydration time. Uppercase letters indicate differences for the same number of HD cycles between hydration times.

Table 3 Analysis of variance results for variables plant height (cm), stem diameter (mm), leaf number, root length (mm), leaf, stem, root and total dry biomass (g) of *Cenostigma pyramidale* (Tul.) E. Gagnon & G.P. Lewis (*Fabaceae*) seedlings subjected to 0, 1, 2, and 3 cycles of hydration and dehydration (HD), with hydration performed either at ½ of Phase I or ¼ of Phase II.

Factors	**f**	**d.f**	**p-value**
Plant height (cm)			
HD cycles	4.5146	3	0.0058
Hydration Time	1.8091	1	0.1827
HD cycles x Hydration Time	2.1594	3	0.1003
Stem diameter (mm)			
HD cycles	6.2254	3	0.0007
Hydration Time	0.0181	1	0.8931
HD cycles x Hydration Time	0.2765	3	0.2765
Leaf number			
HD cycles	1.1242	3	0.345
Hydration Time	0.1222	1	0.7275
HD cycles x Hydration Time	1.5149	3	0.218
Root length (mm)			
HD cycles	1.666	3	0.1819
Hydration Time	0.0636	1	0.8014
HD cycles x Hydration Time	4.6401	3	0.0050
Leaf dry biomass (g)			
HD cycles	10.180	3	<.0001
Hydration Time	0.8424	1	0.3616
HD cycles x Hydration Time	1.322	3	0.2738
Stem dry biomass (g)			
HD cycles	9.1063	3	<.0001
Hydration Time	3.2029	1	0.0776
HD cycles x Hydration Time	7.7877	3	0.0001
Root dry biomass (g)			
HD cycles	3.7382	3	0.0148
Hydration Time	0.7764	1	0.381
HD cycles x Hydration Time	1.2328	3	0.0404
Total dry biomass (g)			
HD cycles	21.4686	3	<.0001
Hydration Time	0.9787	1	0.3257
HD cycles x Hydration Time	2.6707	3	0.0539

The symbol × indicates interaction between the factors

List of Captions

Figure 1 Schematic overview of treatment with hydration and dehydration cycles in *Cenostigma pyramidale* (Tul.) E. Gagnon & G.P. Lewis (*Fabaceae*) seeds. Each HD cycle corresponds to hydration followed by dehydration at differents time of hydration.

Figure 2 Plant height (a), stem diameter (b), leaf number (c) and root length (d) of *Cenostigma pyramidale* (Tul.) E. Gagnon & G.P. Lewis (*Fabaceae*) seedlings subjected to 0, 1, 2, and 3 cycles of hydration and dehydration (HD), with hydration performed either at ½ of Phase I or ¼ of Phase II. Lowercase letters indicate differences among HD cycle numbers within the same hydration time. Uppercase letters indicate differences for the same number of HD cycles between hydration times.

Figure 3 Leaf (a), stem (b), root (c) and total (d) dry biomass of *Cenostigma pyramidale* (Tul.) E. Gagnon & G.P. Lewis (*Fabaceae*) seedlings subjected to 0 (control), 1, 2, and 3 cycles of hydration and dehydration (HD), with hydration performed either at ½ of Phase I or ¼ of Phase II. Lowercase letters indicate differences among HD cycle numbers within the same hydration time. Uppercase letters indicate differences for the same number of HD cycles between hydration times.

Figure 4 Overall responses of *Cenostigma pyramidale* (*Fabaceae*) seeds and seedlings treated with HD cycles at different times of hydration.

Figure 1

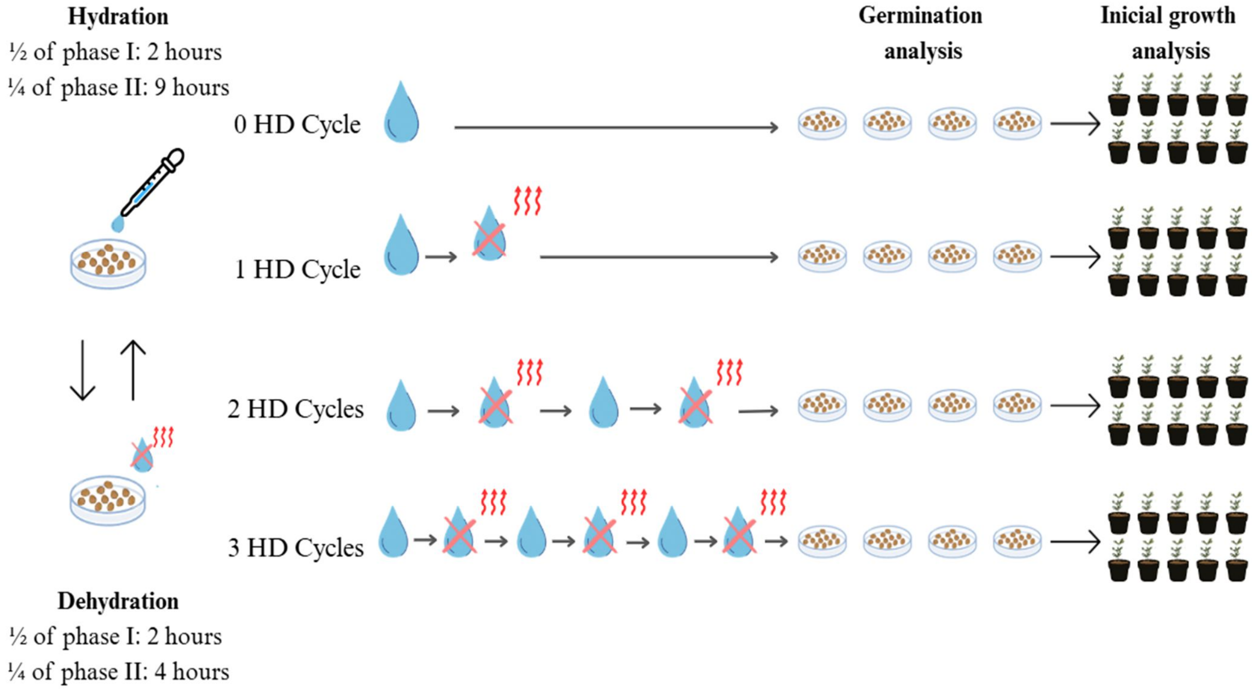

Hydration
½ of phase I: 2 hours
¼ of phase II: 9 hours
0 HD Cycle
1 HD Cycle
2 HD Cycles
3 HD Cycles
Dehydration
½ of phase I: 2 hours
¼ of phase II: 4 hours
Germination analysis
Inicial growth analysis

Figure 2

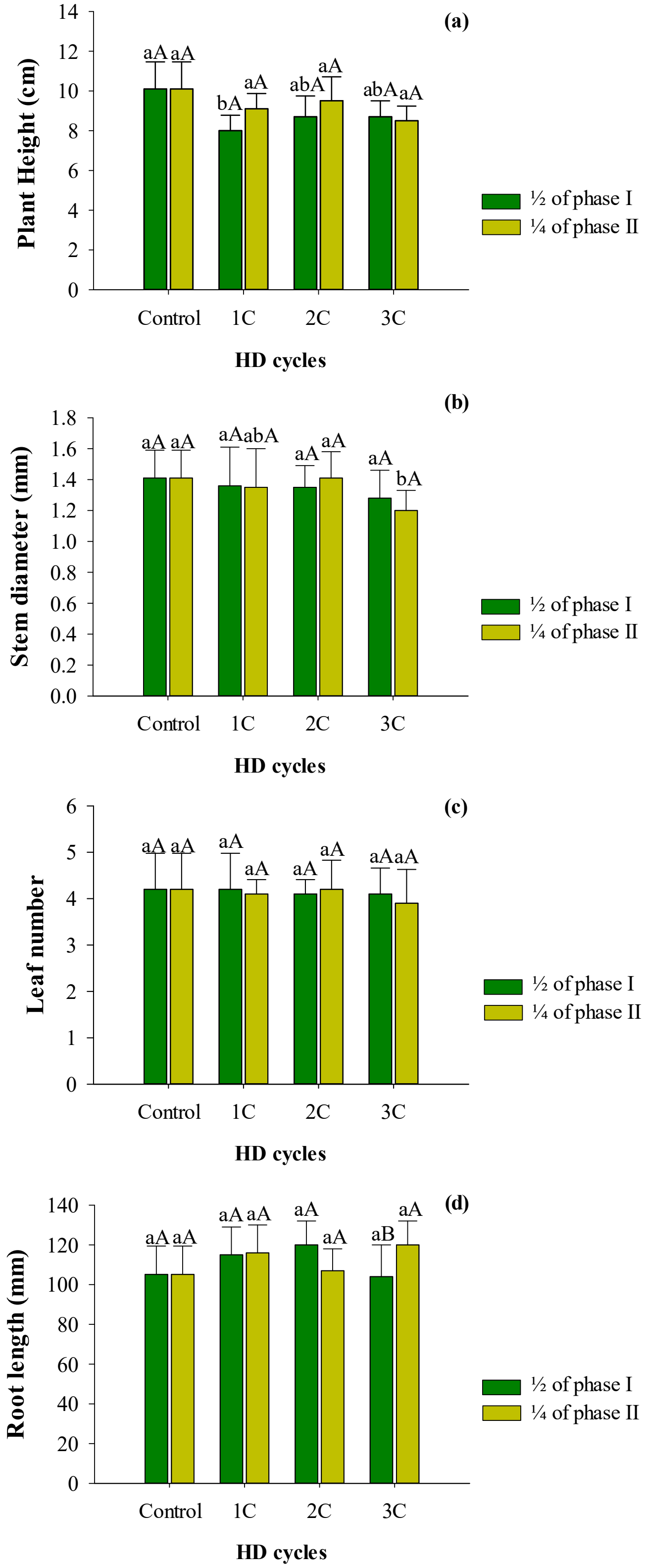

(a)
Plant Height (cm)
14
12
10
8
6
4
2
0
aA aA
bA aA
abA aA
abA aA
Control
1C
2C
3C
HD cycles
½ of phase I
¼ of phase II
(b)
Stem diameter (mm)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
aA aA
aA abA
aA aA
aA bA
Control
1C
2C
3C
HD cycles
½ of phase I
¼ of phase II
(c)
Leaf number
6
5
4
3
2
1
0
aA aA
aA aA
aA aA
aA aA
Control
1C
2C
3C
HD cycles
½ of phase I
¼ of phase II
(d)
Root length (mm)
140
120
100
80
60
40
20
0
aA aA
aA aA
aA aA
aB aA
Control
1C
2C
3C
HD cycles
½ of phase I
¼ of phase II

Figure 3

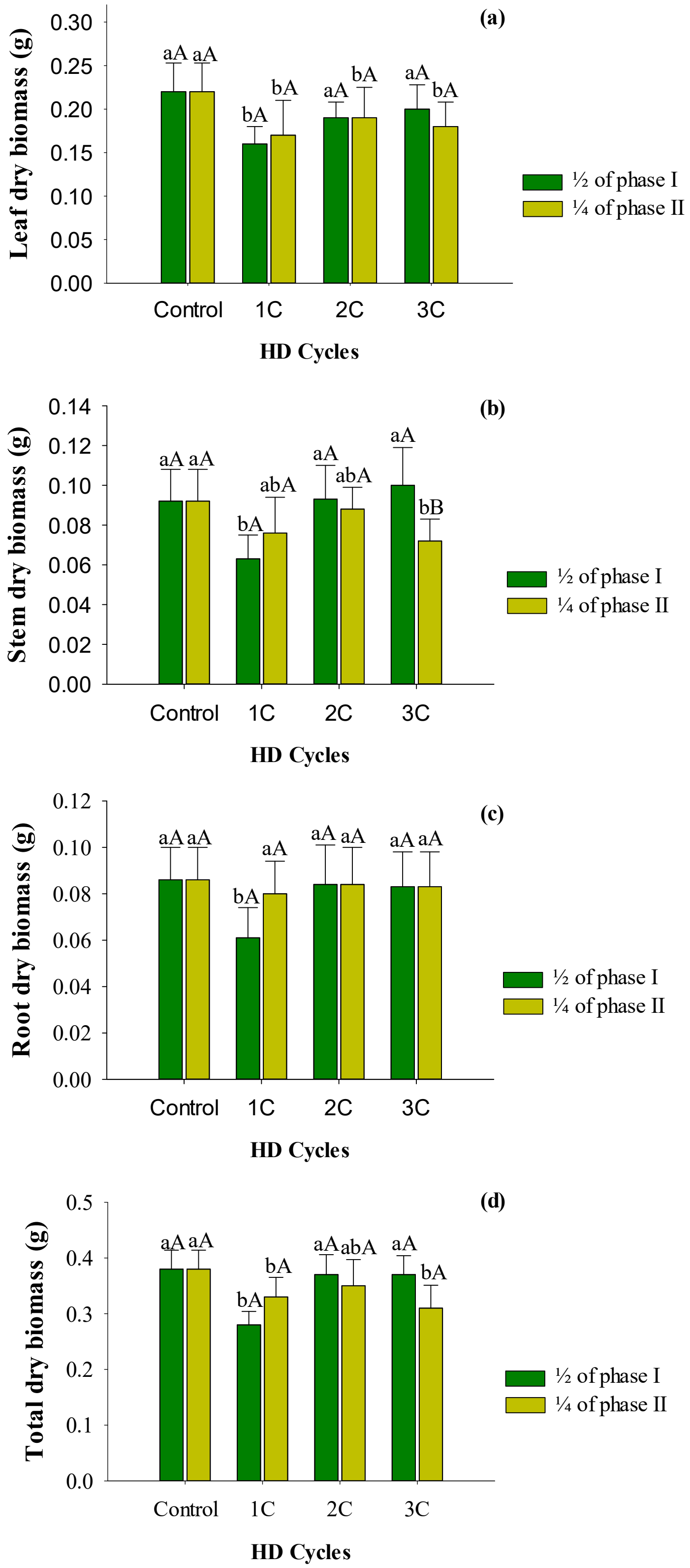
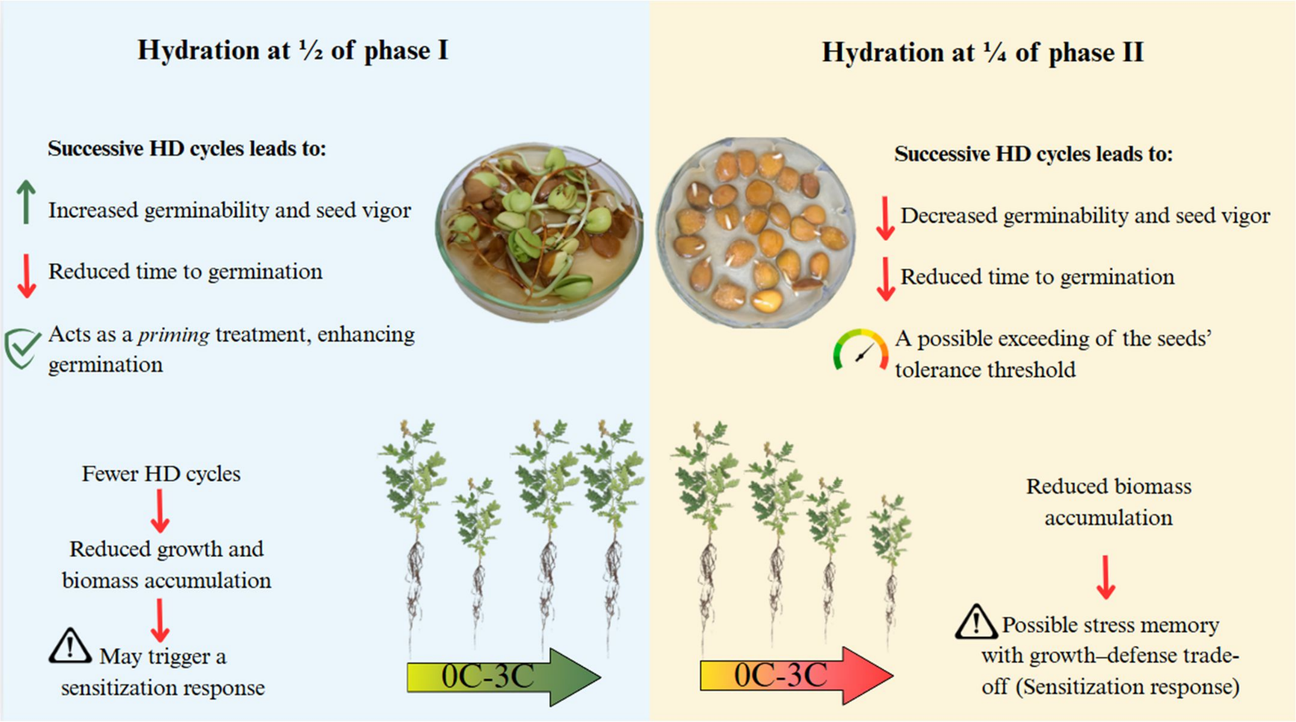

(a)
Leaf dry biomass (g)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
aA aA
bA bA
aA bA
aA bA
Control
1C
2C
3C
HD Cycles
½ of phase I
¼ of phase II
(b)
Stem dry biomass (g)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
aA aA
bA abA
aA abA
aA bB
Control
1C
2C
3C
HD Cycles
½ of phase I
¼ of phase II
(c)
Root dry biomass (g)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
aA aA
bA aA
aA aA
aA aA
Control
1C
2C
3C
HD Cycles
½ of phase I
¼ of phase II
(d)
Total dry biomass (g)
0.5
0.4
0.3
0.2
0.1
0.0
aA aA
bA bA
aA abA
aA bA
Control
1C
2C
3C
HD Cycles
½ of phase I
¼ of phase II

Figure 4


Hydration at ½ of phase I
Successive HD cycles leads to:
↑ Increased germinability and seed vigor
↓ Reduced time to germination
✓ Acts as a *priming* treatment, enhancing germination
Fewer HD cycles
↓
Reduced growth and biomass accumulation
↓
⚠ May trigger a sensitization response
0C-3C
Hydration at ¼ of phase II
Successive HD cycles leads to:
↓ Decreased germinability and seed vigor
↓ Reduced time to germination
A possible exceeding of the seeds' tolerance threshold
0C-3C
Reduced biomass accumulation
↓
⚠ Possible stress memory with growth–defense trade-off (Sensitization response)

4. ARTIGO 2

Submetido ao periódico *Arid Land Research and Management*

Seeds of *Cenostigma pyramidale* (Tul.) Gagnon & G.P. Lewis (Fabaceae) exhibit germinative resilience under high-temperature pulses

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Abstract

Plant reproductive success is closely linked to the germination performance of dispersed seeds, which are frequently subjected to harsh environmental conditions after release from the maternal plant. In Seasonally Dry Tropical Forests (SDTF), such as the Brazilian Caatinga, seeds are commonly exposed to intense thermal pulses at the soil surface, where temperatures can exceed 50 °C. Despite the ecological importance of this phenomenon, the effects of high-temperature pulses exposure on seed germination and early seedling growth of native Caatinga species remain poorly understood, particularly under current climate change scenarios. This study investigated the effects of high-temperature pulses on the germination and initial growth of *Cenostigma pyramidale*, a widely distributed native species of the Caatinga. Seeds were exposed to temperatures of 55, 65 and 75 °C for 30, 60 and 90 minutes, followed by assessments of germination and seedling initial growth. The results revealed a high intrinsic tolerance of *C. pyramidale* seeds to high-temperature pulses exposure. However, prolonged thermal pulses and higher temperatures significantly reduced germinability, mean germination rate and synchrony, while increasing germination time. Seedlings from seeds exposed to 65 °C for 30 and 60 minutes showed increased height, whereas no significant effects were detected for other growth traits or biomass accumulation. These findings indicate that *C. pyramidale* exhibits resilience to transient thermal stress and physiological plasticity during early development, highlighting adaptive traits that may contribute to persistence in seasonally dry tropical ecosystems under future warming scenarios.

Key-words: Thermal stress; Caatinga; Seed germination; post-germinative growth; Heat pulses.

Introduction

The natural environment is shaped by a complex interplay of climatic and environmental factors that operate across specific spatial and temporal scales. These conditions compel organisms to respond rapidly and efficiently to external stimuli, ensuring their survival (Souza et al., 2024; Becklin et al., 2016). Environmental pressures are particularly intense for sessile organisms such as plants, which rely on precise physiological and morphological mechanisms to establish and reproduce successfully (Galviz et al., 2022). Throughout their evolutionary history, plants have developed a broad range of structural and functional adaptations that enable them to withstand adverse conditions and achieve ecological success (Hilker and Schmülling 2019).

Seasonally Dry Tropical Forests (SDTFs) are among the ecosystems that best exemplify this adaptive capacity. In Brazil, the Caatinga is the main SDTF, covering approximately 11% of the national territory (Meiado et al., 2020). This ecosystem is characterized by a semi-arid climate with high temperatures, irregular and poorly distributed rainfall, and prolonged dry seasons (Alves et al., 2009; Meiado et al., 2020). Despite these constraints, the Caatinga hosts a rich and diverse flora composed of species highly adapted and tolerant to multiple environmental stresses (Fernandes & Queiroz, 2018).

Seed germination represents a critical stage in the plant life cycle, and environmental conditions before, during, and after germination can significantly affect this process, either confer physiological benefits or cause damage (Baskin and Baskin, 1998; Gagliano et al., 2014). In semi-arid regions such as the Caatinga, seeds are often exposed to high temperatures from dispersal until the onset of germination near the soil surface (Meiado et al., 2012; Dantas et al., 2024). In a study conducted by Dantas and co-authors (2020), it has been demonstrated that some Caatinga species can germinate under elevated temperatures, typically between 35 and 45 °C.

However, soil surface temperatures in the Caatinga can exceed 50 °C (Martins et al. 2024), reaching daily peaks between 12:00 and 14:00 h when solar radiation is highest (Souto et al. 2009). Seeds lying on the soil surface may therefore experience recurrent thermal pulses throughout the day. Santos-Júnior et al. (2023) reported that seeds of *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae) tolerate prolonged exposure to 70 °C, which enhanced germination and early seedling growth, suggesting a possible *thermal priming* effect.

Nevertheless, few studies have investigated the effects of thermal pulses on seed viability and germination in Caatinga species. Most available data concern species from fire-prone ecosystems such as the Cerrado (Rizzini, 1976; Santana et al., 2019) or focus on heat exposure as a mechanism for overcoming physical dormancy (Zomer et al. 2022; Cuena Lombraña et al. 2024).

Given current projections of increasing temperature and the higher frequency of extreme climatic events (Marengo et al. 2017), understanding the effects of thermal exposure on seeds is crucial for predicting regeneration dynamics in the Caatinga. Especially considering the ecological dynamics of the environment, as well as the phenology of native species.

Thus, the present study aimed to evaluate the effects of exposing *Cenostigma pyramidale* (Tul.) Gagnon & G.P.Lewis (Fabaceae) seeds to high temperatures (55, 65, and 75 °C) and different exposure times (0, 30, 60, and 90 min) on germination and early seedling growth. This species was selected because it is a widely distributed woody legume in the Caatinga, known for its tolerance to environmental stress (Dantas et al., 2020; Wright et al. 2023). Its seeds are autocorically dispersed at the end of the dry season, a period marked by high temperatures and low soil moisture, which may expose them to short-term heat pulses (Silva et al., 2024).

We tested three hypotheses: (i) *C. pyramidale* seeds are tolerant to high temperatures pulses; (ii) longer heat pulses cause physiological damage and reduce germinability; and (iii) thermal exposure influences the seedling phase, producing more vigorous individuals from seeds exposed to moderate heat.

Materials and methods

Study area and seed collection

The experiment was conducted under semi-controlled conditions at the Laboratory of Plant Physiology and Ecophysiology and in a greenhouse belonging to the Department of Biology of the Federal University of Sergipe (UFS).

Cenostigma pyramidale seeds used in the experiment were donated by the Center for Ecology and Environmental Monitoring (NEMA) at the Federal University of Vale do São Francisco (UNIVASF). Seed lots were collected from native Caatinga areas in the Northeast region of Brazil.

Exposure to high temperatures and seed germination

Seeds of *C. pyramidale* were first disinfected in a 3% sodium hypochlorite (NaClO) solution for three minutes. They were then rinsed with distilled water to remove any residual chemicals from the seed coat. After washing, seeds were dried with absorbent paper and placed in plastic trays lined with paper towels, where they remained at room temperature (25 °C) for 24 h.

Subsequently, the seeds were transferred to Petri dishes lined with two layers of sterilized filter paper and subjected to heat-exposure treatments in a drying oven (model WHL-25AB) at three temperatures (55, 65, and 75 °C) for 0 (control), 30, 60, and 90 min. The temperature levels were selected based on previous studies (Santos-Júnior et al., 2023; Santos-Júnior & Silva, 2024) and on the maximum soil-surface temperatures recorded in Caatinga ecosystems (Souto et al., 2009; Martins et al., 2024). Exposure times were designed to simulate short-term thermal pulses that naturally occur in the semi-arid environment throughout the day.

After exposure to high temperatures, seeds were weighed to evaluate the effects of the treatments on seed water dynamics. The percentage of relative water loss between exposures (PRWL) and the relative water content (RWC) in relation to total water percentage were calculated using the following equation:

$\mathrm{PRWL}\ (\%) = \frac{\mathrm{M_i} - \mathrm{M_s}}{\mathrm{M_i}} \times 100$ where M_i is the seed mass before thermal exposure and M_s is the seed mass after exposure.

For total relative water content, the following equation was applied:

$\mathrm{RWC}(\%) = \frac{M_f - M_d}{M_i - M_d} \times 100$ where M_i is the initial seed mass before exposure, M_f is the final seed mass after exposure to heat pulses, and M_d is the dry mass obtained after 24 h of drying in an oven.

Germination analysis

After exposure to high temperatures, seeds were hydrated with 10 mL of distilled water and kept on a laboratory bench at 25 °C. The moisture of the filter paper was maintained by replenishing distilled water whenever necessary.

Each treatment consisted of five replicates of 20 seeds ($n = 100$ per treatment). Germination was recorded daily and considered complete when no new germination events occurred for seven consecutive days, totaling a 15-day period after sowing.

Seeds were considered germinated when the radicle protrusion reached a length of ≥ 2 mm.

To evaluate the effect of thermal exposure on seed germination, germinability (%) was calculated using the following equation:

$$\mathrm{G} = \frac{\sum n_i}{N} \cdot 100, \quad 0 \leq G \leq 100$$

where $\sum n_i$ represents the total number of seeds germinated during the experimental period and N is the total number of seeds placed to germinate in the Petri dishes (Ranal & Santana, 2006).

The time required for 50% seed germination (T_{50}) was calculated according to Farooq *et al.* (2005) using the following equation:

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right) \cdot (t_j - t_i)}{n_j - n_i}$$

where N is the total number of germinated seeds, n_i is the cumulative number of seeds germinated at time t_i, and $n_j$is the cumulative number of seeds germinated at time t_j, when $n_i < \frac{N}{2} < n_j$.

The mean germination rate (MGR) was expressed as the average daily germination rate ($\bar{v}$; day^{-1}), calculated according to Ranal and Santana (2006) using the following equation:

$$\overline{v} = \frac{1}{\bar{t}}, \quad 0 < \overline{v} \leq 1.$$

where $\bar{t}$represents the mean germination time (MGT) according to Labouriau (1983).

The uniformity of germination was evaluated by the coefficient of variation of germination time (CV_t; %), calculated using the following expression:

$$\mathrm{CV_t} = \frac{s_t}{\bar{t}} \cdot 100, \quad 0 < \mathrm{CV_t} <$$

where $s_t$is the standard deviation of germination time, expressed by the following equation: $s_t = \sqrt{\frac{\sum_{i=1}^{k} n_i (t_i - \bar{t})^2}{\left(\sum_{i=1}^{k} n_i - 1\right)^{-1}}}$ and $\bar{t}$ is the mean germination time (MGT) (Ranal and Santana 2006).

To determine germination synchrony, the **s**ynchrony index (SI) was calculated according to Ranal and Santana (2006), using the following equation:

$$SI = \sum_{i} \frac{C_{n_{i},2}}{N}$$

Where $C_{n_{i,2}} = n_i \frac{(n_i - 1)}{2}$ e $N = \sum n_i \left(\frac{\sum n_i - 1}{2}\right)$ where $n_i$is the number of seeds germinated at time i, $C_{n_i,2}$represents the number of possible pairs of seeds germinated at the same time i, and $N$is the total number of possible seed pairs throughout the entire germination period.

Finally, the predictability of germination events was evaluated using the germination uncertainty index (U; bit), calculated according to Labouriau and Valadares (1976), using the following expression:

$$\mathrm{U} = -\sum_{i=1}^{k} f_i \log_2 f_i, \quad \text{with } f_i = \frac{n_i}{\sum_{i=1}^{k} n_i}, \quad 0 \le U \le \log_2 n$$

where $f_i$is the relative frequency of germination at time i, $n_i$is the number of seeds germinated on day i, and $k$is the last day of observation (Ranal & Santana, 2006).

Plant growth analysis

After germination, 10 seedlings from each pre-germination treatment were transplanted into 300 g plastic containers filled with topsoil and maintained in an agricultural greenhouse under daily irrigation for 30 days (Fig. 1).

At the end of this period, morphometric parameters, including plant height, stem diameter, number of leaves, and root length, were recorded. Subsequently, seedlings were harvested, separated into leaves, stems, and roots, placed in paper bags, and dried in a forced-air oven at 70 °C to determine leaf, stem, root, and total dry biomass.

Statistical analysis

Germination parameters were calculated using the germinationmetrics package in R software (version 4.4.0). Prior to statistical analyses, data were evaluated for distributional adequacy and model assumptions. Generalized Linear Models (GLMs) were fitted considering temperature, exposure time, and their interaction as fixed factors. Continuous positive variables were modeled using a Gamma error distribution with a log link function, while count data were analyzed using a Poisson distribution. When model singularities occurred due to missing factor combinations, Type II likelihood-ratio tests were applied.

Model significance was assessed through analysis of deviance, and results were expressed as degrees of freedom, test statistics, and p-values. When significant effects were detected, pairwise comparisons were performed using estimated marginal means (EMMs) with Tukey-adjusted post hoc tests, comparing temperatures within each exposure time and exposure times within each temperature level. Multivariate patterns were explored using heatmaps with hierarchical clustering, principal component analysis (PCA) biplots, and radar charts. All statistical analyses and graphical procedures were conducted in the R software environment (Version 4.0.0).

Results

Germination

Exposure of *C. pyramidale* seeds to different temperatures and exposure times significantly affected the germination parameters evaluated (Tab. 1).

In general, exposure to short thermal pulses (30 and 60 min) did not reduce seed germinability at 55 °C and 65 °C, showing no significant difference from the control group (Fig. 2A). However, a progressive decline in germination percentage was observed in seeds exposed to 75 °C for 60 and 90 min (Fig. 2A). Seeds exposed to 55 °C showed no reduction in germinability at any exposure time, whereas exposure at 65 °C reduced germination only at the longest duration (90 min) (Fig. 2A).

Regardless of temperature, exposure to high thermal pulses increased the time required for 50% of seeds to germinate (T_{50}%) (Fig. 2B). Accordingly, longer exposure times resulted in higher T_{50} values, with the highest means recorded at 75 °C for 60 and 90 min (Fig. 2B).

The mean germination rate (MGR) and synchrony index (SI) exhibited similar patterns. Both parameters decreased under longer exposure durations (60 and 90 min), particularly at 75 °C. At the longest exposure (90 min), both MGR and SI showed significant reductions across all temperature treatments (Fig. 2C and 2D).

For the coefficient of variation of germination time (CV_t), significant differences were detected only in seeds exposed to 75 °C for 90 min, which differed from all other treatments for both temperature and exposure time factors (Fig. 2E).

Similarly, the germination uncertainty index (U) increased with longer exposure times to high temperatures. However, seeds exposed to 75 °C for 90 min exhibited a lower germination uncertainty than the other treatments, likely reflecting the smaller number of seeds that germinated under this condition, which may have influenced the index calculation (Fig. 2F).

Multivariate analyses consistently demonstrated that treatment effects were structured along gradients of temperature and exposure time. The combined use of heatmap, PCA, and radar plots revealed coherent treatment-specific patterns without overlapping between the most extreme thermal treatments and the moderate ones (Fig. 3).

The heatmap revealed clear differences in the standardized responses (z-score) of germination-related variables across temperature and exposure time treatments (Fig. 3A). Treatments at 75 °C, particularly at 60 and 90 min, showed higher standardized values for seed water loss (%WaterLost) and T50, while treatments at 55 °C and 65 °C clustered closer together, showing comparatively higher values of germination percentage (%G) and mean germination rate (MGR). Hierarchical clustering separated high-temperature treatments from moderate-temperature treatments, indicating distinct multivariate response patterns (Fig. 3A).

Principal Component Analysis explained a large proportion of the total variance in the dataset. The first principal component (PC1) accounted for 72.8% of the variation, while the second component (PC2) explained 18.2%, together representing 91.0% of the total variance (Fig. 3B). The PCA biplot showed a clear spatial separation of treatments according to temperature and exposure time. Treatments at 75 °C were consistently positioned on the positive side of PC1, whereas treatments at 55 °C and 65 °C were positioned predominantly on the negative side. The variables %WaterLost, T50, and Germination Uncertainty were strongly associated with the positive direction of PC1, while %G, MGR, and SI were more closely aligned with the negative direction of this axis (Fig. 3B)

Radar plots highlighted distinct physiological profiles among treatments (Fig. 3C). Seeds exposed to 55 °C (30, 60, and 90 min) showed relatively uniform polygon shapes, characterized by higher values of %G and lower values of T50 and %WaterLost (Fig. 3C). Treatments at 65 °C exhibited intermediate profiles, with moderate expansion of the polygons towards T50 and %WaterLost, while maintaining relatively stable values for %G and MGR (Fig. 3C). At 75 °C, particularly under 60- and 90-min exposures, radar plots showed marked expansion towards %WaterLost and T50, with a concomitant contraction towards %G and SI, revealing increased dispersion in the multivariate profiles across these treatments (Fig. 3C).

Inicial Growth

Regarding the initial growth parameters, statistically significant differences were observed only for plant height (Tab. 2).

When comparing temperatures within the same exposure time, plants derived from seeds exposed to 65 °C for 30 and 60 min showed greater height, significantly differing from those exposed to 55 °C and 75 °C (Tab. 3). Thus, exposure of seeds to 65 °C for a short or moderate period promoted greater shoot elongation but reduced stem thickness (Tab. 3).

No significant differences were found for number of leaves, root length (Tab. 3), or leaf, stem, root, and total dry biomass (Tab. 4).

Discussion

Seed Germination

Seed germination is a crucial stage in the plant life cycle and directly influences reproductive success, recruitment patterns, and population persistence (Gagliano et al., 2014). For species inhabiting environments with strong climatic unpredictability, such as the Caatinga dry forest, the production of vigorous and stress-resilient seeds is essential for maintaining long-term population stability (Donohue et al., 2007). However, the germination stage is physiologically sensitive and can be strongly affected by fluctuations in temperature and water availability, two environmental factors that vary widely across both spatial and temporal scales in the Caatinga ecosystem (Dantas et al., 2020).

Our results demonstrate that *Cenostigma pyramidale* seeds exhibit remarkable tolerance to thermal pulses, even under extreme temperatures (65–75 °C). This high level of thermal resilience is ecologically relevant, as surface soil temperatures in the Caatinga commonly exceed 60 °C during the dry season (Souto et al., 2009; Martins et al., 2024). Such tolerance may therefore represent an adaptive trait that enhances the persistence and broad distribution of *C. pyramidale* across different microhabitats within the ecossystem.

From a phenological perspective, seed dispersal in *C. pyramidale* occurs through ballistic dehiscence and is concentrated during the transition from the dry season to the onset of the rainy season (Leite and Machado, 2009; Lima et al., 2018). In this type of dispersal syndrome, seeds may land in shaded areas or in fully exposed microsites where soil temperatures are substantially higher. Consequently, seeds experience a complex spatial–temporal mosaic of environmental conditions, including high evaporative demand, rapid water loss, and exposure to thermal pulses (Poppinga et al., 2019). The ability to withstand such conditions is therefore critical for the maintenance of both transient and persistent soil seed banks (De Paula et al., 2023).

Thermal exposure caused a progressive reduction in seed water content, which was most pronounced at higher temperatures and longer exposure times. High temperatures can accelerate physiological aging, promote membrane destabilization, alter lipid fluidity, and induce microfissures in the seed coat, all factors known to impair seed viability in other species (Cancino et al., 1993; Daws et al., 2007). Despite these potential stressors, *C. pyramidale* seeds maintained relatively high germinability and rapid germination rates, indicating robust physiological resilience.

C. pyramidale produces orthodox seeds that are dispersed with low moisture content, approximately 10% water (Oliveira et al., 2011). In addition, the seed coat features a palisade layer of macrosclereids and associated tissues that form a strongly protective structure, a trait widely found in Fabaceae (Melo-Pinna et al., 1999). Physiologically, the seeds present a suite of protective mechanisms, including increased antioxidant activity and accumulation of soluble sugars, proteins, and amino acids (Matias, 2019). This set of mechanisms, together with other protective factors associated with desiccation and heat tolerance, such as the synthesis of heat-shock proteins (sHSPs) (Wehmeyer and Vierling, 2000), LEA proteins, and antioxidant defense pathways (Xing et al., 2025), may be strongly related to the high thermal tolerance observed in the species.

The observed increase in T50 under severe thermal treatments suggests a direct effect of water loss on germination speed. Although seeds with lower moisture content typically exhibit a higher water potential gradient and therefore absorb water more rapidly (Martel et al., 2018; Lombrãna et al., 2024), studies also indicate that exposure to high temperatures can increase abscisic acid (ABA) synthesis and reduce the production of hydrolytic enzymes essential for initiating the biochemical cascades that drive germination (Martel et al., 2018; Suriyasak et al., 2020; Jatana et al., 2024).

Although such physiological parameters were not analyzed in the present study, our findings may indicate two possible explanations based on evidence in the literature:

(i) Thermal drying may induce secondary coat-imposed dormancy, thereby slowing water uptake and delaying germination (Jaganathan et al., 2019). This mechanism may act as a bet-hedging strategy widely observed in species from unpredictable environments, in which staggering germination events over time—as indicated by the increased T50 in our study—helps populations avoid synchronous failure under unfavorable climatic conditions (Abley et al., 2024). Given that *C. pyramidale* seeds are dispersed between the late dry season and the onset of the rainy season (Leite and Machado, 2009; Lima et al., 2018), the activation of a bet-hedging mechanism may ensure higher chances of germination only when environmental conditions become more stable, thereby improving seedling establishment and survival.

(ii) Alternatively, exposure to high temperatures may cause sublethal damage to internal tissues, including partial loss of membrane integrity, cytoplasmic leakage, DNA damage, and reduced levels of hydrolytic enzymes (Martel et al., 2018; Suriyasak et al., 2020). In such cases, seeds may need to allocate resources to repair these physiological injuries before completing germination, resulting in slower germination dynamics (Mácová et al., 2022; Kumar et al., 2023). Thus, the delayed germination and reduced germinability observed in the treatments with longer exposure to thermal pulses may be related to physiological and mechanical damage occurring in the seed tissues during thermal stress.

Finally, considering the projected increase in the frequency and intensity of heat waves under climate-change scenarios, the thermal resilience exhibited by *C. pyramidale* seeds may confer a significant adaptive advantage. However, the decline in germination performance at the most extreme treatments also highlights the importance of identifying the species' thermal thresholds to anticipate potential impacts on regeneration dynamics in a warming climate.

Inicial Growth

In addition to the germination responses, thermal exposure also influenced early seedling development. Significant differences were detected only for plant height, suggesting that the thermal effects observed at the seed stage produced limited carry-over effects on later developmental traits. Seeds exposed to 65 °C for 30 and 60 min produced taller seedlings compared with those exposed to 55 °C and 75 °C, indicating that moderate thermal stress may induce a compensatory elongation response during early growth. Similar results have been reported for *Enterolobium contortisiliquum* (Vell.) Morong (Fabaceae) (Santos-Júnior et al., 2023) and *Ceiba glaziovii* (Kuntze) K. Schum. (Malvaceae) (Santos-Júnior & Silva, 2024), both native Caatinga species that exhibited enhanced seedling growth following exposure of seeds to thermal pulses at 70 °C.

However, this increase in height may reflect a resource-allocation trade-off, in which seedlings prioritize shoot elongation to optimize light capture after overcoming the initial stress encountered during imbibition and germination (Huot & Montgomery, 2014).

There is an intrinsic relationship between the germination process, the post-germination phase, and early seedling establishment. Thus, the environmental events experienced by seeds can leave "physiological marks" that manifest during early growth (Donohue et al., 2010). In some cases, these marks can enhance growth parameters or increase tolerance to environmental stressors (Crisp et al., 2016), whereas in other cases they may cause injuries and physiological damage that result in increased seedling fragility (Hussain et al., 2016).

The absence of significant differences in leaf number, stem and root length, and biomass components suggests that, despite alterations in seedling architecture, overall seedling vigor and biomass accumulation were not substantially impaired. These responses reinforce the idea that *C. pyramidale* seedlings possess a degree of developmental plasticity, adjusting their morphology in ways that partially buffer the effects of thermal stress experienced by the seeds. This mechanism reinforces the resilience of the species to thermal pulses across differents exposure times and temperatures and may contribute to its ecological success in the highly adverse conditions of the Caatinga.

Taken together, our findings demonstrate that *C. pyramidale* seeds possess a high degree of thermal tolerance, which appears to be a key adaptive trait for persistence in the extreme and heterogeneous environments of the Caatinga. The capacity to maintain germinability and seedling performance under short and moderate thermal pulses suggests an efficient combination of structural and physiological protection mechanisms, while the sensitivity observed under more severe exposures highlights the existence of physiological thresholds beyond which performance declines.

The limited carry-over effects on early growth, coupled with evidence of developmental plasticity, indicate that thermal stress experienced during the seed phase can be partially buffered during seedling establishment (Fig. 4). These results emphasize the importance of seed-level thermal resilience in shaping recruitment dynamics and reinforce the relevance of understanding species-specific thermal limits in the context of increasing heat-wave frequency under future climate-change scenarios.

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Author contributions All authors contributed to the study conception and design.

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Table 1 Results of generalized linear models (GLMs) and analysis of deviance for germinability (%G), time to 50% germination (T50), mean germination rate (MGR), synchronization index (SI), coefficient of variation of germination time (CVt), and germination uncertainty of *Cenostigma pyramidale* seeds subjected to high-temperature pulses of 55, 65, and 75 °C for 30, 60, and 90 min.

Factors	**X^2**	**d.f**	**p-value**
Germinability			
Temperature	0.853	3	0.8366
Exposure Time	0.710	2	0.7013
Temperature x Exposure Time	284.64	6	<.0001
T50%			
Temperature	32.965	3	<.0001
Exposure Time	5.533	2	0.0629
Temperature x Exposure Time	156.45	6	<.0001
MGR			
Temperature	28.269	3	<.0001
Exposure Time	0.822	2	0.663
Temperature x Exposure Time	267.83	6	<.0001
SI			
Temperature	12.052	3	0.0071
Exposure Time	0.758	2	0.6845
Temperature x Exposure Time	116.97	6	<.0001
CVt			
Temperature	1.460	3	0.6917
Exposure Time	1.070	2	0.5869
Temperature x Exposure Time	752.70	6	<.0001
Uncertainty			
Temperature	17.800	3	<.0001
Exposure Time	1.400	2	0.4974
Temperature x Exposure Time	3398.2	6	<.0001

The symbol × indicates interaction between the factors

Table 2 Results of generalized linear models (GLMs) and analysis of deviance for plant height (cm), stem diameter (mm), leaf number, root length (mm), leaf, stem, root and total dry biomass (g) of *Cenostigma pyramidale* seeds subjected to high-temperature pulses of 55, 65, and 75 °C for 30, 60, and 90 min.

Factors	X^2	d.f	p-value
Plant height (cm)			
Temperature	8.453	2	0.0146
Exposure Time	3.832	3	0.2802
Temperature x Exposure Time	6.310	4	0.1771
Stem diameter (mm)			
Temperature	3.896	2	0.1425
Exposure Time	3.356	3	0.3398
Temperature x Exposure Time	3.789	4	0.4365
Leaf number			
Temperature	0.016	2	0.9919
Exposure Time	0.111	3	0.9905
Temperature x Exposure Time	0.1764	4	0.9963
Root length (mm)			
Temperature	1.003	2	0.6056
Exposure Time	6.8853	3	0.1609
Temperature x Exposure Time	3.8074	4	0.4327
Leaf dry biomass (g)			
Temperature	0.590	2	0.7442
Exposure Time	3.734	3	0.2916
Temperature x Exposure Time	1.457	4	0.8342
Stem dry biomass (g)			
Temperature	2.857	2	0.2396
Exposure Time	3.048	3	0.3842
Temperature x Exposure Time	2.686	4	0.2396
Root dry biomass (g)			
Temperature	2.691	2	0.2604
Exposure Time	4.082	3	0.2527
Temperature x Exposure Time	4.881	4	0.2996
Total dry biomass (g)			
Temperature	1.959	2	0.3754
Exposure Time	4.163	3	0.2444
Temperature x Exposure Time	2.970	4	0.5628

The symbol × indicates interaction between the factors.

Table 3. Plant height (a), stem diameter (b), leaf number (c) and root length (d) of *Cenostigma pyramidale* seeds subjected to high-temperature pulses of 55, 65, and 75 °C for 30, 60, and 90 minutes. Lowercase letters compare means within the same temperature, while uppercase letters compare means within the same exposure time.

Temperature	Time	Plant Height (cm)	Stem diameter (mm)	Number of leaves	Root length (cm)
55°C	C	11.48±0.67Aa	1.54±0.19Aa	4±0.0Aa	12.1±0.82Aa
	30	**11.5±0.86Aab**	1.54±0.25Aa	4±0.70Aa	12.5±1.00Aa
	60	**11±0.61Ab**	1.54±0.18Aa	4.4±0.54Aa	12.5±0.61Aa
	90	11.3±0.27Aa	1.58±0.14Aa	3.88±0.52Aa	12.6±0.41Aa
65°C	C	11.48±0.67Aa	1.54±0.19Aa	4±0.0Aa	12.1±0.82Aa
	30	**13.5±1.27Aa**	1.5±0.35Aa	3.8±0.44Aa	12.6±0.65Aa
	60	**13.1±1.01Aa**	1.24±0.18Aa	4±0.0Aa	11.9±0.54Aa
	90	11.4±2.58Aa	1.38±0.24Aa	4.2±0.44Aa	13±0.79Aa
75°C	C	11.48±0.67Aa	1.54±0.19Aa	4±0.0Aa	12.1±0.82Aa
	30	**11.5±1.00Ab**	1.52±0.16Aa	4±0.00Aa	13.2±0.96Aa
	60	-	-	-	-
	90	-	-	-	-

Table 4. Leaf (a), stem (b), root (c) and total (d) dry biomass (g) of *Cenostigma pyramidale* seeds subjected to high-temperature pulses of 55, 65, and 75 °C for 30, 60, and 90 min. Lowercase letters compare means within the same temperature, while uppercase letters compare means within the same exposure time.

Temperature	Time	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Total dry weight (g)
55°C	C	0.21±0.05Aa	0.11±0.03Aa	0.09±0.04Aa	0.41±0.09Aa
	30	0.18±0.07Aa	0.09±0.03Aa	0.06±0.02Aa	0.33±0.12Aa
	60	0.18±0.04Aa	0.12±0.06Aa	0.07±0.04Aa	0.38±0.12Aa
	90	0.17±0.06Aa	0.10±0.01Aa	0.08±0.01Aa	0.36±0.06Aa
65°C	C	0.21±0.05Aa	0.11±0.03Aa	0.09±0.04Aa	0.41±0.09Aa
	30	0.15±0.04Aa	0.11±0.04Aa	0.05±0.01Aa	0.28±0.07Aa
	60	0.20±0.08Aa	0.11±0.05Aa	0.09±0.03Aa	0.41±0.16Aa
	90	0.16±0.06Aa	0.10±0.01Aa	0.06±0.03Aa	0.31±0.13Aa
75°C	C	0.21±0.05Aa	0.11±0.03Aa	0.09±0.04Aa	0.41±0.09Aa
	30	0.19±0.03Aa	0.12±0.02Aa	0.10±0.03Aa	0.41±0.09Aa
	60	-	-	-	-
	90	-	-	-	-

List of Captions

Fig. 1. Schematic overview of the experimental design showing the application of high-temperature pulses and the evaluation of germination and early seedling growth in *Cenostigma pyramidale*.

Fig. 2. Germinability (%) (a), time to 50% germination (T50) (b), mean germination rate (c), germination synchrony (d), coefficient of variation of germination time (e), and germination uncertainty (f) of *Cenostigma pyramidale* seeds subjected to high-temperature pulses of 55, 65, and 75 °C for 30, 60, and 90 min. Lowercase letters compare means within the same temperature, while uppercase letters compare means within the same exposure time.

Fig. 3. Heatmap with hierarchical clustering showing the relationships among high-temperature pulses, exposure time, germination parameters, and seed water content after thermal treatments. The color scale indicates response intensity, with blue indicating higher responses and red indicating lower responses (a). Principal component analysis (PCA) showing the distribution of samples, with ellipses representing temperatures and symbols indicating exposure times (b), and a radar chart illustrating the individual effects of temperature and exposure time on germination parameters and seed water loss in *Cenostigma pyramidale* (c). Treatments are indicated as temperature_exposure time.

Fig. 4. Overall responses of *Cenostigma pyramidale* seeds and seedlings subjected to thermal pulses of 55, 65, and 75 °C for 30, 60, and 90 min.

477 **Figure 1**

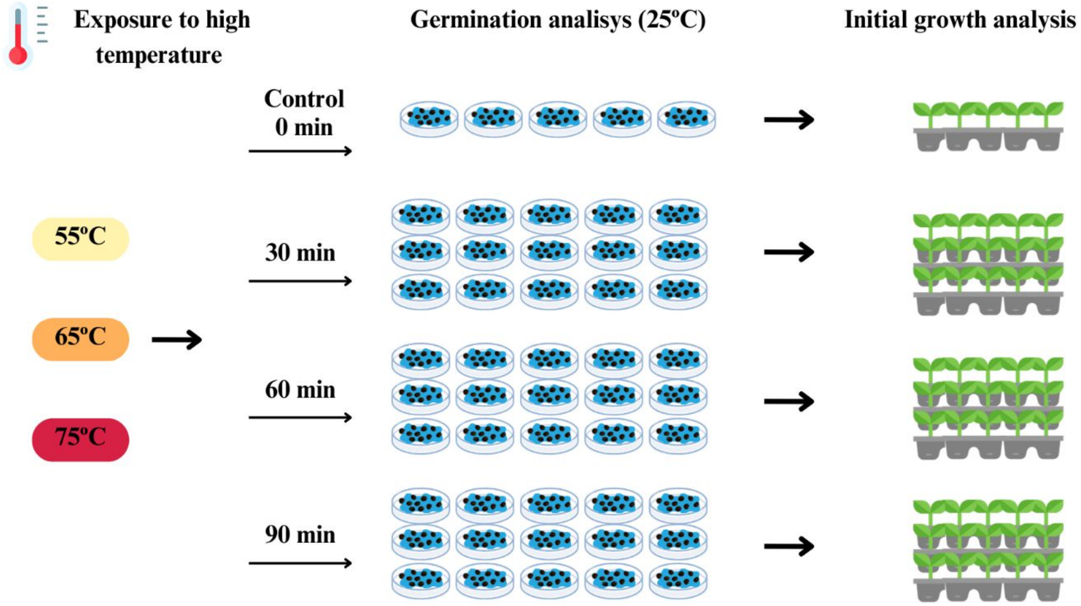

Exposure to high temperature
Germination analisys (25ºC)
Initial growth analysis
Control
0 min
55ºC
65ºC
75ºC
30 min
60 min
90 min

Figure 2

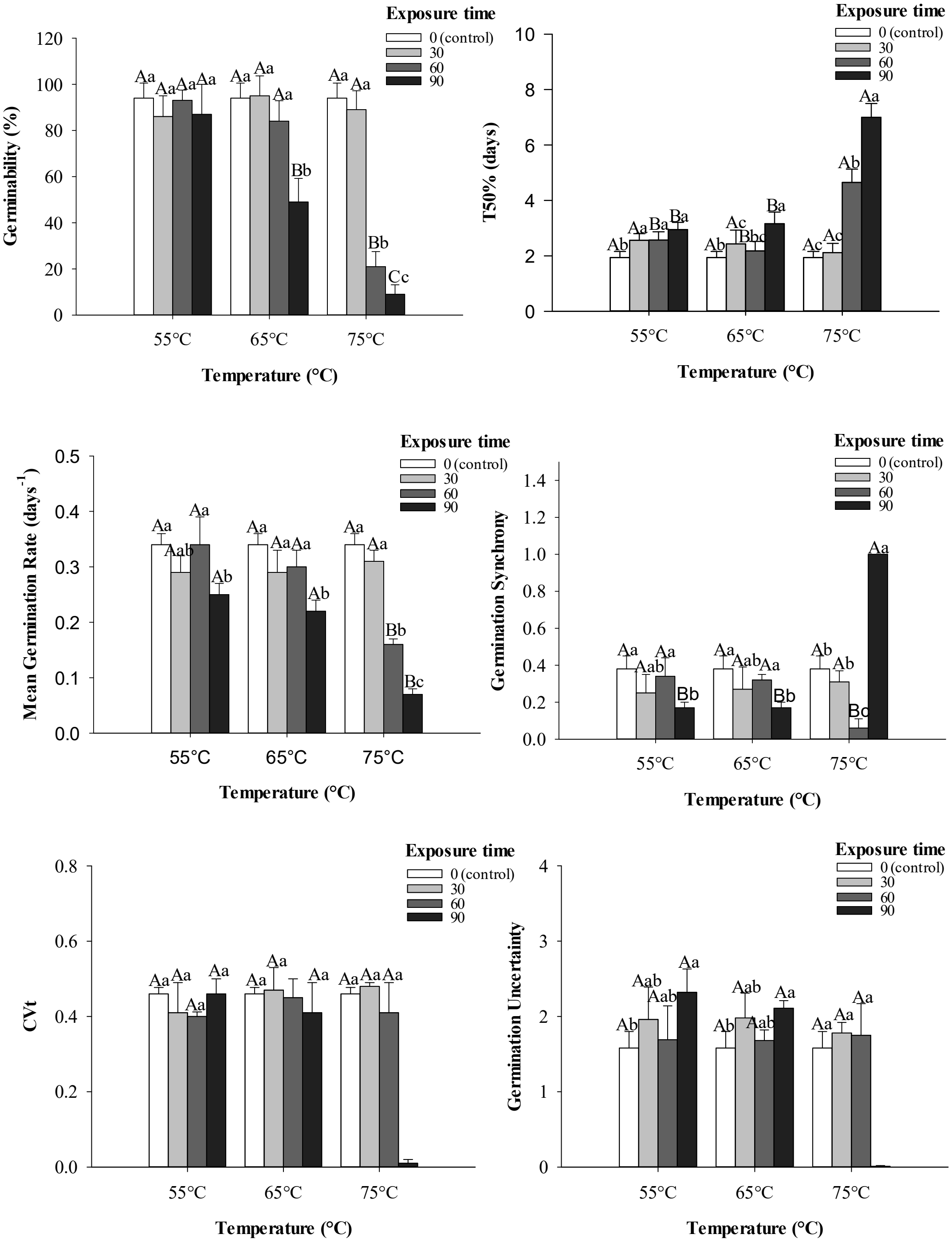

Exposure time
0 (control)
30
60
90
Germinability (%)
120
100
80
60
40
20
0
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Bb
Aa
Aa
Bb
Cc
55°C
65°C
75°C
Temperature (°C)
Exposure time
0 (control)
30
60
90
T50% (days)
10
8
6
4
2
0
Ab
Aa
Ba
Ba
Ab
Ac
Bbc
Ba
Ac
Ac
Ab
Aa
55°C
65°C
75°C
Temperature (°C)
Exposure time
0 (control)
30
60
90
Mean Germination Rate (days^{-1})
0.5
0.4
0.3
0.2
0.1
0.0
Aa
Aab
Aa
Ab
Aa
Aa
Aa
Ab
Aa
Aa
Bb
Bc
55°C
65°C
75°C
Temperature (°C)
Exposure time
0 (control)
30
60
90
Germination Synchrony
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Aa
Aab
Aa
Bb
Aa
Aab
Aa
Bb
Ab
Ab
Bc
Aa
55°C
65°C
75°C
Temperature (°C)
Exposure time
0 (control)
30
60
90
CVt
0.8
0.6
0.4
0.2
0.0
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
55°C
65°C
75°C
Temperature (°C)
Exposure time
0 (control)
30
60
90
Germination Uncertainty
4
3
2
1
0
Ab
Aab
Aab
Aa
Ab
Aab
Aab
Aa
Aa
Aa
Aa
55°C
65°C
75°C
Temperature (°C)

Figure 3

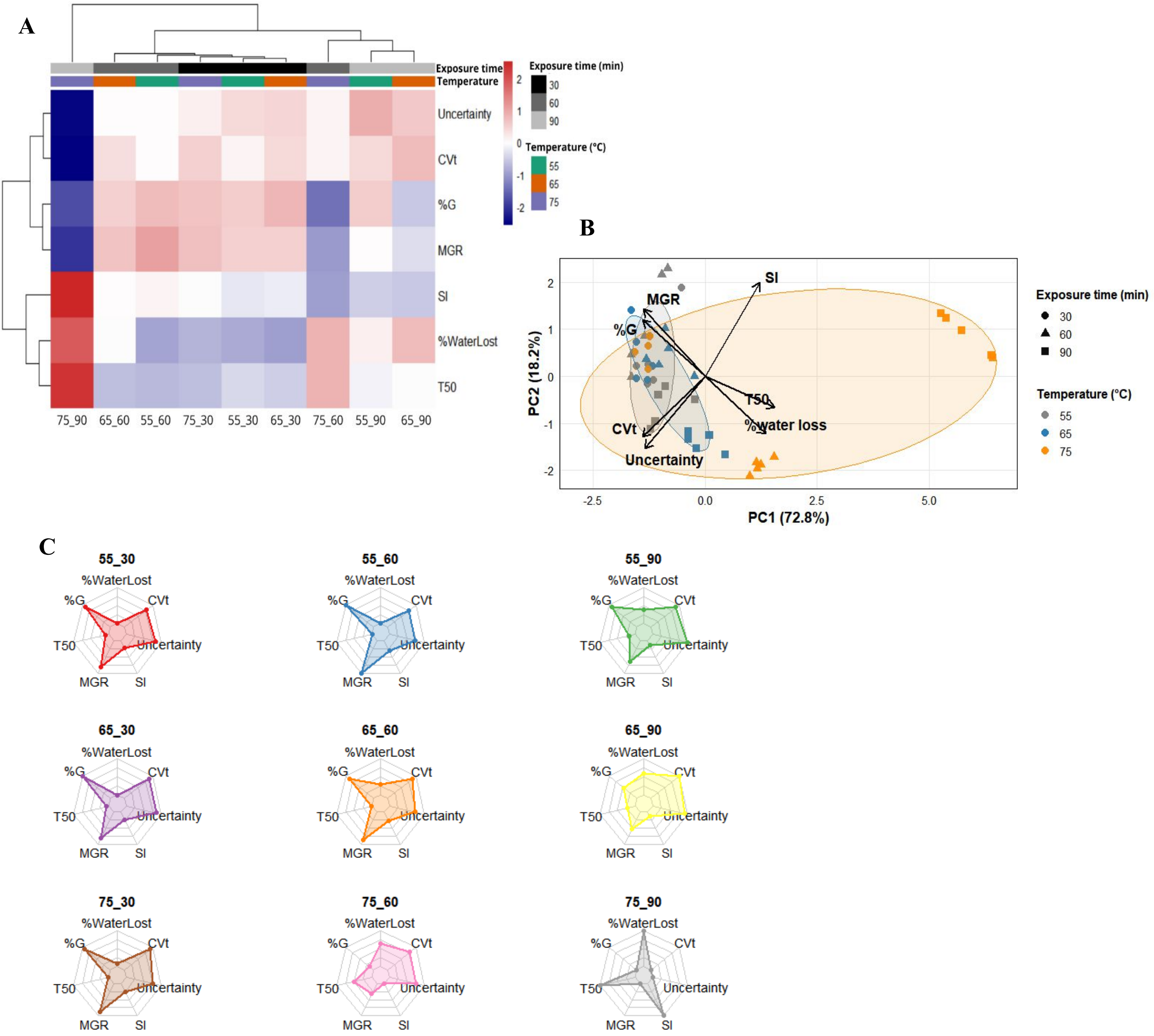
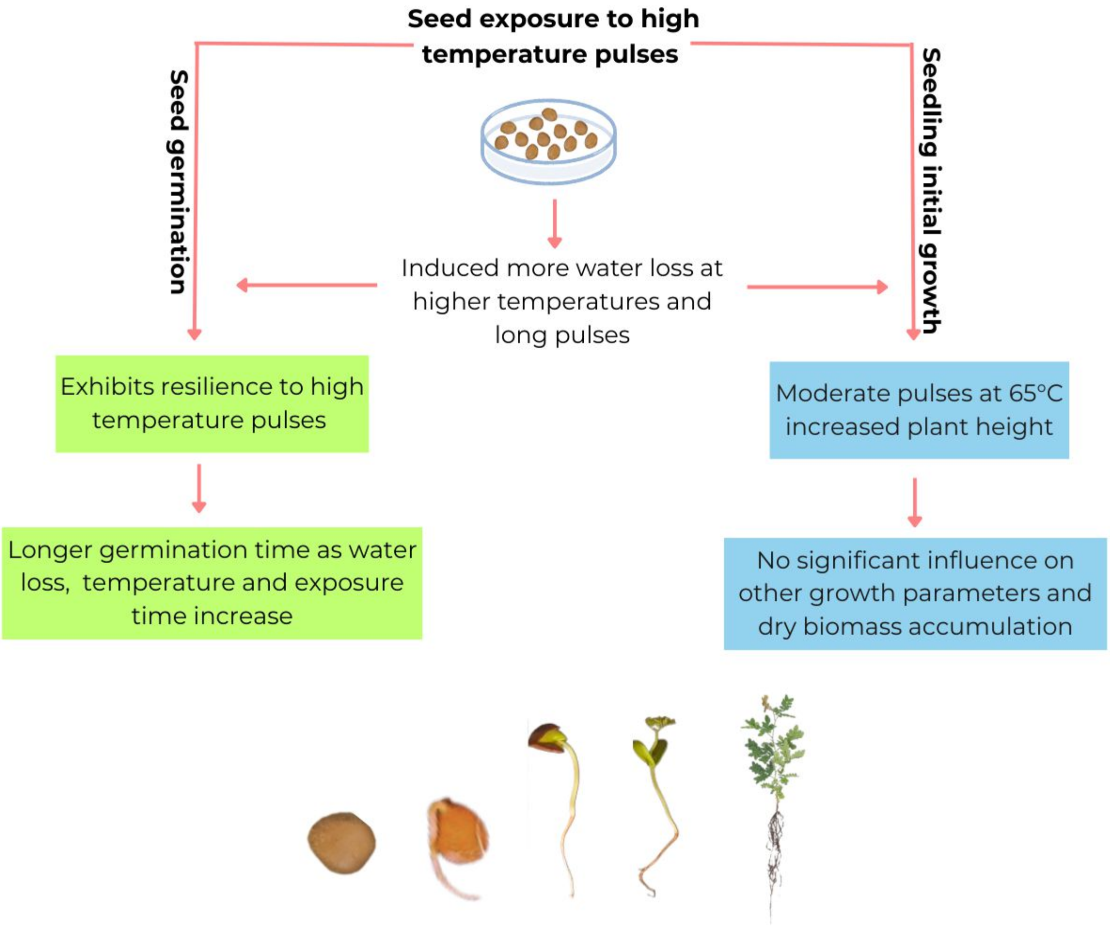

A
Exposure time
Temperature
Uncertainty
CVt
%G
MGR
SI
%WaterLost
T50
75_90
65_60
55_60
75_30
55_30
65_30
75_60
55_90
65_90
2
1
0
-1
-2
Exposure time (min)
30
60
90
Temperature (°C)
55
65
75
B
SI
MGR
%G
T50
%water loss
CVt
Uncertainty
PC1 (72.8%)
PC2 (18.2%)
-2.5
0.0
2.5
5.0
2
1
0
-1
-2
Exposure time (min)
30
60
90
Temperature (°C)
55
65
75
C
55_30
55_60
55_90
65_30
65_60
65_90
75_30
75_60
75_90
%WaterLost
CVt
Uncertainty
SI
MGR
T50
%G

Figure 4


Seed exposure to high temperature pulses
Seed germination
Seedling initial growth
Induced more water loss at higher temperatures and long pulses
Exhibits resilience to high temperature pulses
Longer germination time as water loss, temperature and exposure time increase
Moderate pulses at 65°C increased plant height
No significant influence on other growth parameters and dry biomass accumulation

5. ARTIGO 3

A ser submetido para o periódico *Plant Ecology*

Changing to Survive: the influence of high temperature and discontinuous hydration on the germination behavior of the Caatinga-native tree *Cenostigma pyramidale*

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Abstract

Understanding how seeds respond to abiotic stress is essential to unravel adaptive germination strategies in semiarid environments. This study evaluated the effects of pre-germinative treatments—high temperature (T), hydration–dehydration cycles (HD), and their combination (T-HD)—on the germination of *Cenostigma pyramidale* under varying osmotic potentials simulated with PEG 6000. All germination parameters were significantly influenced by osmotic stress, pre-germinative treatment, and their interaction ($p < 0.05$). Germinability decreased with lower osmotic potentials, although HD and T-HD treatments maintained higher percentages under moderate and severe water deficit (–0.6 and –0.9 MPa). Mean germination time increased with water stress, with HD promoting faster germination under non-stress conditions. T-HD-treated seeds exhibited slower and less synchronized germination, particularly under optimal water availability. The combination of HD and T also led to increased variability and uncertainty in germination timing. These patterns suggest distinct adaptive strategies: HD treatment favored a best-bet strategy with rapid and uniform germination, while T-HD treatment activated a bet-hedging mechanism, delaying and spreading germination over time—possibly due to heat-induced ABA accumulation. Such plasticity in germination behavior may enhance ecological success in the Caatinga, where moisture is intermittent and soil temperatures are extreme. Our findings highlight the role of environmental priming in shaping seed germination strategies and the importance of physiological memory in coping with recurrent stress.

1. Introduction

Throughout the evolutionary history of plants, the acquisition of diverse adaptive mechanisms has been crucial for their ecological success and for enabling the colonization of a wide range of environments (Hilker and Schmülling 2019). Seed production stands out as a key strategy that ensures species dispersal and propagation (Howe and Smallwood 1982; Fenner and Thompson 2005). Once dispersed, seeds must encounter favorable conditions to initiate germination, a process that involves sequential stages of water uptake (imbibition), metabolic activation, and radicle protrusion (Rosental et al., 2014; Bareke 2018). However, abiotic environmental factors such as water availability, temperature, light, and substrate conditions may limit or even prevent germination, causing damage to embryonic tissues or, under extreme conditions, leading to embryo death (Ferreira and Borghetti 2004; Fenner and Thompson 2005).

In arid and semi-arid environments, where climatic fluctuations are intense, seed germination becomes particularly susceptible to abiotic constraints (Baskin and Baskin 2014). This is evident in the Caatinga, a Seasonally Dry Tropical Forest located in Northeastern Brazil, which is characterized by irregular and uneven rainfall, high temperatures, and prolonged dry periods (Andrade-Lima 1981; Alves et al. 2009). These conditions strongly influence the reproductive cycles of plant species, as well as their germination processes (Leite and Machado 2009; 2010).

Seeds in the Caatinga soil seed bank are often exposed to high temperatures during dry seasons (Texeira, 2010) and are subjected to hydration and dehydration (HD) cycles due to the intermittent availability of water in surface soil layers (Meiado, 2013; Lima and Meiado 2017; 2018). Despite these harsh conditions, many species display tolerance, completing germination and producing new individuals (Dantas et al., 2020; Silva et al., 2025). This reflects eco-physiological adaptation mechanisms that have long intrigued researchers seeking to understand how plants acquire tolerance to environmental pressures and secure their ecological success (Galviz et al. 2022; Kambona et al., 2023).

Recent studies have shown that plants can store information from prior environmental stress exposures, enabling them to adjust their responses to future stresses. This phenomenon, known as stress memory, has been documented in seeds and plants at different developmental stages (Thellier and Lüttge 2013; Demongeot et al. 2019). In Caatinga species, exposure to high temperatures has been reported to enhance germination and promote the development of more vigorous seedlings (Rafael et al., 2018; Jespersen, 2020; Santos-Júnior et al., 2022;). Likewise, hydration and dehydration cycles have been associated with the activation of hydration memory, which increases tolerance to environmental stresses such as soil salinity, water deficit, and high temperatures, resulting in more robust seedlings (Lima and Meiado 2018; Santos-Júnior et al. 2021; Pereira-da-Silva et al., 2024; Santos et al. 2024).

Understanding these responses is essential for uncovering the mechanisms of tolerance and resilience of species inhabiting semi-arid ecosystems. However, stress memory responses are often species-specific or even population-dependent (Lima and Meiado 2017). Furthermore, there is a lack of studies addressing the combined influence of high temperature and discontinuous hydration on seed germination, even though these factors may act independently or synergistically. Therefore, the objective of this study was to analyze the effects of seed exposure to high temperature (65 °C) and hydration/dehydration cycles on germination under water deficit conditions induced by PEG 6000 solutions. We selected *Cenostigma pyramidale* (Tul.) E. Gagnon & G.P. Lewis (Fabaceae) as a model species, a woody legume widely distributed in the Caatinga, known for its high tolerance to environmental stresses and its socio-environmental importance (Pinho-Pessoa et al. 2018; Ribeiro et al., 2021; Wright et al. 2023)

We worked with the following hypotheses: i) pre-germination treatments exert distinct effects on germination parameters; ii) the reduction of osmotic potential negatively influences variables such as germinability and germination speed; iii) and there is an interaction between these two factors, so that the germination performance of the treatments varies according to the osmotic potential. Thus, we expect that hydration and dehydration cycles, as well as their combination with high temperature, will enhance germination performance, especially under more negative osmotic potentials, due to the induction of stress memory.

2. Materials and methods

2.1 Study area and seed collect

The experiment was conducted under semi-controlled conditions at the Plant Physiology and Ecophysiology Laboratory, Federal University of Sergipe (UFS), Brazil. Seeds of *Cenostigma pyramidale* were collected from a Caatinga area in Northeastern Brazil and provided by the Center for

Ecology and Environmental Monitoring (NEMA) of the Federal University of the São Francisco Valley (UNIVASF).

2.2 Hydration and dehydration curves

To determine the hydration and dehydration durations for applying HD cycles, imbibition and drying curves were constructed using the same seed batch. The imbibition curve was established with four replicates of 25 seeds each (n = 100), previously weighed to determine their initial mass. Seeds were placed in Petri dishes containing two layers of filter paper moistened with 10 mL of distilled water. They were weighed every 60 minutes until the beginning of Phase III of imbibition, defined by radicle protrusion ≥ 2 mm (Lima and Meiado, 2018). The time corresponding to ½ of Phase I was defined as the standard hydration period (4 hours).

The drying curve followed the same experimental setup. After hydration, the seeds were transferred to a drying oven at 35 °C and weighed every 60 minutes until their initial mass was restored (Lima and Meiado 2018), which occurred after approximately 5 hours. This period was used as the standard dehydration time for the HD cycles.

2.3 Exposure to high temperature and hydration–dehydration cycles

Four pre-germinative treatments were applied: T – exposure of seeds to high temperature (65 °C for 60 min); HD – application of three hydration–dehydration cycles; T-HD – combination of treatments T and HD; and C – control (untreated seeds).

For the high-temperature treatment, seeds were placed in Petri dishes containing two layers of filter paper and incubated in a drying oven at 65 °C for 60 minutes. The HD cycles were applied using the durations determined in the previous step (Section 2.2). Seeds in the HD group underwent three successive hydration–dehydration cycles, while seeds in the T-HD group were first exposed to high temperature (65 °C for 60 min) and then subjected to three hydration–dehydration cycles.

2.4 Germination under water deficit and seed recovery

Following the pre-germination treatments, seeds were subjected to germination under different osmotic potentials: 0.0 (distilled water), –0.1, –0.3, –0.6, and –0.9 MPa. These potentials were generated using polyethylene glycol (PEG 6000) solutions (Vilella et al., 1991). For each osmotic potential, four replicates of 25 seeds were used per treatment group (C, T, HD, and T-HD). Seeds were placed in Petri dishes lined with two layers of filter paper moistened with 15 mL of the respective PEG solution.

Germination was monitored daily and considered complete when no new germination events were observed for seven consecutive days, totaling 16 days after sowing.

After 16 days of incubation in PEG 6000 solutions (osmotic potentials), seeds that did not show radicle protrusion were removed from the solutions and transferred to new Petri dishes containing filter paper moistened with distilled water, and then incubated under the same environmental conditions adopted during germination under PEG (i.e., same temperature, photoperiod, and incubation/conditioning conditions). This procedure was used to quantify the capacity for germination recovery after the removal of osmotic stress, following the approach proposed by Sena et al. (2023). From this step, two metrics were calculated:

Recovery germination (RG), which estimates the proportion of seeds that failed to germinate under PEG but germinated after transfer to distilled water:

$$RG(\%) = \left[\frac{(a-b)}{(c-b)}\right] \times 100$$

And final germination (FG), which represents total germination at the end of the experiment by summing seeds that germinated during PEG exposure and those that germinated during the recovery phase in distilled water:

$$FG(\%) = \left(\frac{a}{c}\right) \times 100$$

Where: a = total number of germinated seeds at the end of the assay (germinated in PEG + germinated after transfer to distilled water); b = total number of seeds germinated in the PEG solutions; c = total number of seeds tested. As defined by Sena et al. (2023), when RG = 0, final germination reflects only germination that occurred under stress (i.e., FG = b/c × 100), and may also indicate lack of recovery (including non-viable seeds, depending on the treatment).

2.5 Seedling length, dry biomass, and seedling vigor index

Seedling growth was assessed using 20 seedlings per treatment, considering only normal seedlings obtained from the pre-germinative treatments C, T, HD, and T–HD that germinated under PEG 6000 conditions. For each seedling, hypocotyl length was measured from the cotyledonary node to the hypocotyl–radicle junction, and radicle length was measured from the hypocotyl–radicle junction to the root tip. Measurements were performed with a digital caliper (Digimess), and lengths were expressed in millimeters (mm).

After length measurements, the same seedlings were used to determine seedling dry biomass. Seedlings were placed in paper bags and dried in a forced-air oven at 70 °C until constant mass. Dry mass was recorded on an analytical balance and expressed as mg seedling^{-1}.

Seedling vigor was quantified using the Seedling Vigor Index (SVI) according to Maroufi et al. (2011), calculated as:

$$\mathrm{SVI} = \mathrm{GP} \times \mathrm{SDW}$$

where GP is the germination percentage (%) for each treatment and osmotic potential, and SDW is the mean seedling dry weight.

2.6 Germination parameters and statistical analysis

To evaluate the effects of pre-germinative treatments on the percentage of germinated seeds, we calculated germinability (%G) according to the equation:

$$G = \frac{\sum n_i}{N} \cdot 100, \quad 0 \leq G \leq 100$$

where $\sum n_i$ is the sum of germinated seeds in relation to the number of seeds available to germinate in the Petri dish (N); data are expressed as percentages with values range from 0 to 100 (Ranal and Santana 2006).

To calculate the mean germination time (MGT), we used the following equation:

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

Where t_i is the period from the start of the experiment to the ith observation, n_i is the number of seeds germinated at time i and k is the last germination time (Labouriau 1983).

To represent the germination rate, we used the mean germination rate ($\bar{v}; \mathrm{day}^{-1}$) which was calculated using the equation:

$$\bar{v} = \frac{1}{\bar{t}}, \quad 0 < \bar{v} \leq 1.$$

Where $\bar{t}$ corresponds to the mean germination time (MGT) (Labouriau, 1983).

We determined germination uniformity by calculating the coefficient of variation of the time (CVt; %) using the expression:

$$\mathrm{CV_t} = \frac{s_t}{\bar{t}} \cdot 100, \quad 0 < \mathrm{CV_t} <$$

where s_t is the standard deviation of the germination time, expressed by:

$s_t = \sqrt{\frac{\sum_{i=1}^{k} n_i (t_i - \bar{t})^2}{\left(\sum_{i=1}^{k} n_i - 1\right)^{-1}}}$ and $\bar{t}$ is the mean germination time (Ranal & Santana 2006).

We assessed germination synchrony based on the synchrony index (SI), which is given by the formula:

$$SI = \sum_{i} \frac{C_{n_{i},2}}{N}$$

where $C_{n_{i,2}} = n_i \frac{(n_i - 1)}{2}$ and $N = \sum n_i \left(\frac{\sum n_i - 1}{2}\right)$ is the combination of seeds germinated in the time *i*, in pairs, and n_i is the number of seeds germinated in the time (Ranal and Santana 2006).

Furthermore, we checked the germination uncertainty, represented by Uncertainty (U; bit), using the following expression:

$$U = -\sum_{i=1}^{k} f_i \log_2 f_i, \quad \text{with } f_i = \frac{n_i}{\sum_{i=1}^{k} n_i}, \quad 0 \leq U \leq \log_2 n$$

where f_i is the relative frequency of germination at time *i*, n_i is the number of seeds germinated on day *i*, and *k* is the last day of observation (Ranal and Santana **2006**).

2.7 *Statistical analyses*

Germination metrics were computed using the germinationmetrics package in R (v4.4.0). Germination metrics were analyzed using Generalized Linear Models (GLM), assuming binomial distribution for proportional variables and gamma distribution for continuous variables, with treatment, osmotic potential, and their interaction as fixed effects. When significant effects were detected, estimated marginal means were compared using the Sidak adjustment ($\alpha = 0.05$) via the emmeans package in R.

In addition, hypocotyl length, radicle length, seedling dry biomass, SVI, and FG were also evaluated by two-way ANOVA, considering pre-germinative treatment (C, T, HD, T–HD) and osmotic potential as factors. When ANOVA indicated significant effects ($p < 0.05$), means were compared using Tukey's HSD test ($\alpha = 0.05$) to evaluate differences among treatments within the same osmotic potential and among osmotic potentials within the same treatment.

3. Results

3.1 Germination performance and temporal dynamics

The osmotic potential, the pre-germinative treatments, and the interaction between both significantly influenced all the evaluated germination parameters ($p < 0.05$) (Fig. 2). In addition, the cumulative germination curves evidenced marked shifts in the onset, steepness, and final plateaus of germination across osmotic potentials and treatments (Fig. 1), reinforcing that water deficit progressively delayed and constrained germination dynamics.

Germinability remained high under non-water-stress conditions (0.0 MPa), with all treatments showing values above 65%. The HD treatment showed a slightly superior performance, while C, T, and T-HD remained statistically similar (Fig. 2A). As the osmotic potential decreased, there was a gradual reduction in germinability for all treatments, with T-HD maintaining relatively more stable values at moderate potentials such as –0.1 and –0.3 MPa. At –0.6 MPa, HD and T-HD presented higher means than the control, but it did not differ from treatment T. Under the most negative osmotic potential (–0.9 MPa), T-HD was statistically different from the other treatments, showing the highest germination percentage (Fig. 2A). Consistently, under non-stress conditions HD reached the maximum accumulation earlier than the other treatments, whereas at –0.6 and –0.9 MPa germination remained low overall but T-HD maintained the highest final accumulation among treatments (Fig. 1).

Recovery germination (%RG) showed peaks at –0.1 MPa for T (79%) and at –0.3 MPa for C and HD (61.8% each), whereas T-HD showed lower recovery at –0.3 MPa (26.1%) and intermediate values under –0.6 MPa (49.5%) and –0.9 MPa (41%) (Tab. 2).

When total germination was considered (%FG = germination under PEG + recovery), no differences were detected among treatments within each osmotic potential (Tab. 2).

In contrast, within-treatment comparisons across osmotic potentials revealed clear declines in %FG with increasing osmotic potencials. In C, %FG remained stable from 0.0 to –0.3 MPa and decreased at –0.6 and –0.9 MPa. In T, the highest %FG occurred at –0.1 MPa, values at 0.0, –0.3, and –0.6 MPa were intermediate, and the lowest %FG was recorded at –0.9 MPa. In HD, %FG was maintained from 0.0 to –0.3 MPa, declined to an intermediate level at –0.6 MPa, and reached the lowest group at –0.9 MPa. In T-HD, %FG was highest at –0.1 MPa, remained intermediate at 0.0, –0.3, and –0.6 MPa, and decreased at –0.9 MPa (Tab. 2).

Overall, the mean germination time (MGT) increased with the intensification of water deficit, reflecting greater difficulty in the initiation of the germination process under more negative osmotic potentials (Fig. 2D). At 0.0 MPa, HD showed faster germination, with lower MGT values compared to the other treatments. Interestingly, seed exposure to high temperature (T) resulted in the longest mean germination time under optimal water availability conditions. The control group (C) and the combination (T-HD) did not differ from each other in this same condition. However, under moderate and severe osmotic potentials, HD and T-HD exhibited lower MGT, while C and T showed slower and more prolonged germination, and the exception was for the –0.3 MPa, where no differences were observed between treatments (Fig. 2D). These differences in MGT were reflected in the temporal displacement of the curves, with delayed initiation and more gradual accumulation as osmotic potential became more negative, particularly for C and T (Fig. 1).

The mean germination rate (MGR) followed a proportional trend to MGT, showing also a decrease in means as osmotic potential became more negative. Under non-stress conditions, HD presented the highest rate, followed by C and T-HD, while T showed the lowest rate. Treatments HD and T-HD maintained higher rates compared to C and T at –0.1 and –0.6 MPa potentials, with no significant statistical differences observed among treatments at –0.3 and –0.9 MPa (Fig. 2B).

3.2 *Germination patterning: synchrony and temporal dispersion*

Germination synchrony was significantly influenced by pre-germinative treatments and osmotic potential ($p < 0.05$) (Fig. 2E). Under non-water-stress conditions (0.0 MPa), HD showed the highest synchrony, while T exhibited the lowest values. Under mild water deficit (–0.1 MPa), no statistical differences among treatments were observed, all presenting low synchrony. At intermediate potentials (–0.3 MPa), T stood out with higher values, surpassing C and T-HD. Under more negative potentials (–0.6 and –0.9 MPa), T-HD showed the highest synchrony, followed by HD and T, while C maintained the lowest values (Fig. 2E).

The coefficient of variation of germination time (CVt) indicated that T-HD showed greater dispersion of times under non-water-stress conditions, whereas HD showed lower values, representing greater uniformity. At intermediate potentials, such as –0.3 MPa, T-HD and HD maintained higher CVt, suggesting a broader temporal distribution of germination. At severe potentials, the dispersion of germination events generally decreased, although HD and T-HD still showed higher values (Fig. 2C).

Germination uncertainty (GU) complemented the temporal variability analyses, confirming that T-HD exhibited less predictable germination at 0.0 MPa, while HD stood out for its greater concentration and predictability. As osmotic potential decreased, germination uncertainty also reduced, especially at –0.6 and –0.9 MPa, where germination events were more limited and concentrated, with no marked differences among treatments (Fig. 2F).

3.3 *Seedling vigor under osmotic stress*

Seedling performance metrics were also affected by osmotic potential and pre-germinative treatments (Fig. 3). Hypocotyl length decreased with increasing water deficit, and under –0.3 MPa T-HD maintained higher values relative to the other treatments, indicating better preservation of seedling elongation under stronger osmotic constraint (Fig. 3A).

Seedling dry biomass showed comparatively modest variation at 0.0 MPa, but differences emerged under stress, with treatment-dependent shifts in median biomass values across –0.1 and –0.3 MPa (Fig. 3A). Radicle length was generally high at 0.0 MPa for C, T, and HD but consistently lower for T-HD, while under –0.1 and –0.3 MPa treatment effects remained evident (Fig. 3B).

The vigor index declined with increasing water deficit; nevertheless, T-HD showed comparatively higher vigor under –0.3 MPa, aligning with its more stable germination performance under moderate-to-severe osmotic potentials (Fig. 3B).

Discussion

The interaction between seeds and abiotic factors plays a decisive role in activating physiological and metabolic adjustments that can ensure a more vigorous germination process or the acquisition of tolerance to severe and recurrent environmental stresses, such as water deficit (Chen and Arora, 2013). These factors may act as priming agents, preparing seeds to respond more efficiently to unfavorable conditions and thereby increasing their ecological success (Srivastava et al., 2021). In semiarid environments such as the Caatinga, seeds are frequently exposed to high temperatures and intermittent water pulses—conditions that can induce hydration–dehydration (HD) cycles with complex physiological effects (Dubrovsky, 1996; Meiado, 2013; Pereira-da-Silva et al., 2024).

Our results showed that the germination of *Cenostigma pyramidale* was significantly modulated by the interaction among high temperature, HD cycles (applied alone or in combination), and induced osmotic potentials. This interaction was expressed not only in germination percentages, but also in the kinetics and temporal organization of germination, as shown by the cumulative curves (Fig. 1) and by shifts in MGT, MGR, synchrony, CVt, and uncertainty (Fig. 2).

The use of PEG 6000, widely adopted to simulate water deficit by lowering the osmotic potential of the solution without penetrating seed tissues (Villela et al., 1991), revealed that as the osmotic potential became more negative, germinability and vigor decreased, while the mean germination time (MGT) increased. This can be explained by the fact that impaired imbibition may compromise enzymatic reactivation, reserve mobilization, and cell expansion in the embryonic axis (Ferreira and Borghetti, 2004; Baskin and Baskin, 2014). Consistent with this interpretation, the cumulative germination curves became progressively less steep and reached lower plateaus as osmotic potential decreased, evidencing slower and more limited germination under –0.6 and –0.9 MPa (Fig. 1).

Seed recovery tests demonstrated that low germination under severe osmotic stress (–0.6 and –0.9 MPa) resulted solely from water restriction rather than loss of viability (Tab. 2), a pattern previously observed in species from seasonal environments (Bhatt et al., 2022). Notably, HD and especially T-HD treatments showed better performance in parameters such as germinability, MGT, and synchrony under more negative osmotic potentials. This advantage is visible at the level of germinability and synchrony under severe stress (Fig. 1a,e) and is further supported by the higher germination accumulation maintained by T-HD at –0.6 and –0.9 MPa (Fig. 1).

Importantly, the recovery germination (%RG) data reinforce that PEG imposed a predominantly osmotic constraint rather than a lethal effect, because a substantial fraction of ungerminated seeds resumed germination after release, even at the most negative potentials. This post-stress response was treatment-dependent: thermal exposure (T) tended to maximize recovery germination under mild-to-severe deficit (–0.1 to –0.6 MPa), whereas T-HD displayed comparatively lower recovery at intermediate deficit (–0.3 MPa), indicating that the same final germination can arise from distinct temporal routes (germination during stress vs. germination after rehydration).

These results suggest that HD cycles induced stress memory, enhancing drought tolerance. This effect is associated with the partial and reversible activation of metabolism during hydration, followed by dehydration without loss of the induced biochemical changes (Dubrovsky, 1998). Moreover, HD cycles likely stimulated the accumulation of osmoprotectants such as proline and soluble sugars, which stabilize membranes and proteins and reduce cellular water potential, thereby creating a favorable gradient for water uptake once water becomes available again (Aswathi et al., 2021; Dias et al., 2024). Because proline and soluble sugars were not directly quantified here, these biochemical pathways should be interpreted as plausible mechanisms that may underlie the improved performance of HD/T-HD under water deficit rather than as demonstrated causal drivers.

In contrast, exposure to high temperature (T) alone did not improve germination speed or synchrony, indicating that heat did not act as a positive factor for breaking seed coat dormancy in *C. pyramidale* or enhancing water uptake—effects already reported for other species (Martel et al., 2018; Lombrãna et al., 2024). However, the higher recovery germination observed in T under several osmotic potentials suggests that heat may have shifted part of the germination response toward the post-stress window, favoring germination once water availability was restored rather than accelerating germination under PEG. When combined with HD cycles (T-HD), high temperature altered the germination dynamics, leading to increased MGT, lower synchrony, and greater temporal variability (CVt), even under conditions of adequate water availability. This pattern is particularly evident under 0.0 MPa, where T-HD exhibited greater temporal dispersion (CVt) and uncertainty than HD (Fig. 1C, F), despite comparable germinability (Fig. 1A), indicating that the combination affected the timing structure of germination rather than only the final proportion.

Studies indicate that thermal exposure may increase abscisic acid (ABA) synthesis and reduce the activity of hydrolytic enzymes (Martel et al., 2018; Suriyasak et al., 2020), which could explain the slower germination in T-HD. Additionally, reduced water content in seeds during heat treatment may intensify ABA signaling, inhibiting or delaying metabolic activities required for the completion of germination (Abley et al., 2024). Although ABA content was not evaluated in this study, since C. pyramidale seeds are orthodox and naturally have low water content, the additional reduction in seed water content after exposure to high temperature could induce an increase in ABA levels, acting as a mechanism to delay the germination process. Given that ABA was not measured, this explanation should be viewed as a testable hypothesis for future work, ideally integrating hormone profiling and reserve mobilization assays across pre-germinative treatments.

Nevertheless, the patterns we observed suggest that the different environmental stimuli simulated in our study influenced the expression of distinct ecological strategies in the germination behavior of *C. pyramidale*. HD treatment promoted rapid and synchronized germination, indicating a strategy known as best-bet, in which seeds take advantage of favorable, even if short-lived, environmental pulses and invest in fast and uniform germination, forming a cohort of propagules (Pausas et al., 2022). This may be advantageous when seeds encounter sustained moisture availability. On the other hand, the T-HD treatment resulted in slower and staggered germination, a pattern typical of bet-hedging strategies, where the temporal spread of germination events reduces risk in unpredictable environments (Abley et al., 2024).

This duality in germination responses may reflect an adaptive mechanism of *C. pyramidale*, allowing different fractions of the seed bank to respond differently to environmental cues. Importantly, the "best-bet" versus "bet-hedging" interpretation is supported here by convergent evidence from synchrony, CVt, and uncertainty (Fig. 1E; F) together with the visual displacement of germination trajectories over time (Fig. 2), strengthening the ecological inference beyond any single metric. The partitioning of germination into "during-stress" and "post-stress" components (as captured by %FG and %RG) further supports this framework, because treatments differed not only in how many seeds germinated, but also in *when* germination was expressed relative to osmotic constraint.

In the Caatinga context where soil temperatures can exceed 50 °C and soil moisture varies rapidly (Souto et al., 2009; Almeida; Souto; Souto, 2013) the staggered germination observed in T-HD may provide a survival advantage, as not all seeds germinate during potentially adverse climatic windows (Fajardo et al., 2013; Lopez-Iglesias et al., 2014). Phenological studies indicate that seed dispersal in *C. pyramidale* occurs during the transition from the rainy to the dry season (Gonçalves et al., 2021), a period of high microclimatic variability. Thus, the activation of bet-hedging mechanisms, as suggested by our results, may represent an essential adaptive strategy for the species' ecological success.

Therefore, the activation of a mechanism that allows temporal variation in individual seed germination events—such as that observed in T-HD treatment—may ensure that a larger number of seeds remain viable and ready to germinate during more regular periods of water availability. In contrast, the behavior observed in seeds subjected only to discontinuous hydration suggests the opposite strategy, favoring a more synchronous and uniform germination process. This may benefit the species during milder climatic conditions with longer-lasting water pulses. In this sense, the higher final germination of T-HD at the most negative potential, coupled with measurable recovery germination, is consistent with a conservative strategy in which part of the seed lot "waits out" the osmotic restriction and completes germination after stress relaxation, without implying a gain in germination speed under stress.

Beyond germination, our seedling results indicate that treatment effects extended into early establishment traits under water limitation (Fig. 3). Under –0.3 MPa T-HD tended to maintain comparatively higher hypocotyl length and vigor index relative to the other treatments (Fig. 3A, B), suggesting that priming may translate into improved post-germinative performance under moderate osmotic stress. Because recruitment in semiarid systems depends on both successful germination and rapid establishment during brief moisture windows, the combined evidence from germination dynamics (Figs. 1–2) and seedling performance (Fig. 3) supports the view that HD-based priming—especially when combined with thermal exposure—can shape multiple stages of the regeneration niche in *C. pyramidale.*

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Author contributions All authors contributed to the study conception and design.

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Table 1. Values for total water content (TWC), relative water loss (RWL), and dry biomass of *Cenostigma pyramidale* seeds after exposure to high temperature (65°C).

Tratement	**TWC (%)**	**RWL (%)**	**Biomass (g)**
Exposure to 65°C for 60 minutes	7,5	3,89	2,23

Table 2. Recovery germination (%RG) and final germination (%FG) of *Cenostigma pyramidale* seeds subjected to different osmotic potentials (MPa) under PEG 6000 and four pre-germinative treatments: control (C), thermal pulse (T), hydration–dehydration cycles (HD), and the combined treatment (T-HD). %RG represents germination after transferring from PEG solution to water (recovery phase), whereas %FG represents total germination (germinated under PEG + germinated during recovery).

Osmotic (MPa)	**C**	**T**	**HD**	**THD**
		%RG (recovery germination)		
0	0	0	0	0
-0.1	49.7	79	4.5	49
-0.3	61.8	71.4	61.8	26.1
-0.6	37.6	56.4	45.4	49.5
-0.9	45.3	47	42.3	41
		%FG (final germination)		
0	79aA	60aBC	80aA	70aAB
-0.1	76aA	89aA	76aA	82aA
-0.3	73aA	80aBA	73aA	72aAB
-0.6	44aB	65aBC	59aAB	64aAB
-0.9	49aB	51aC	49aB	55aB

Lowercase letters indicate comparisons among treatments within the same osmotic concentration, while uppercase letters indicate comparisons of the same treatment across different osmotic concentrations. Means followed by the same letter do not differ significantly according to the Tukey test (p = 0.05).

List of captions

Figure 1. Cumulative germination of *Cenostigma pyramidale* seeds subjected to different pre-germinative treatments—C (control), T (high temperature), HD (hydration–dehydration cycles), and T-HD (high temperature + hydration–dehydration)—under decreasing osmotic potentials (0.0, –0.1, –0.3, –0.6, and –0.9 MPa), simulated with PEG 6000. Curves represent the temporal progression of germination, highlighting shifts in onset, rate of accumulation, and final germination plateau across treatments and water-deficit intensities.

Figure 2. Germinability (a), mean germination rate (b), coefficient of variation of germination time (c), mean germination time (d), germination synchrony (e), and germination uncertainty (f) of *Cenostigma pyramidale* seeds subjected to high temperature (T), discontinuous hydration (HD), the combination of high temperature and discontinuous hydration (T-HD), and control (C), with germination evaluated under different osmotic potentials. Lowercase letters indicate comparisons among treatments within the same osmotic concentration, while uppercase letters indicate comparisons of the same treatment across different osmotic concentrations. Means followed by the same letter do not differ significantly according to the Sidak test ($\alpha = 0.05$).

Figure 3. Seedling performance of *Cenostigma pyramidale* as affected by pre-germinative treatments (C, T, HD, T-HD) under osmotic potentials of 0.0, –0.1, and –0.3 MPa (PEG 6000). (A) Hypocotyl length and seedling dry biomass. (B) Radicle length and vigor index. Boxplots show the median (central line), interquartile range (box), and minimum–maximum values (whiskers); points represent individual observations. Different letters indicate significant differences among pre-germinative treatments within each osmotic potential ($p < 0.05$) based on post hoc comparisons.

Figure 1.

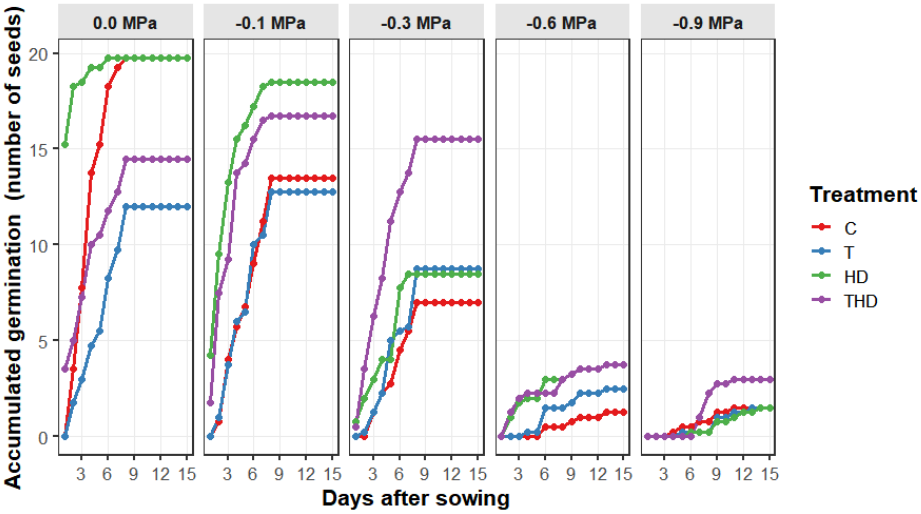

0.0 MPa
-0.1 MPa
-0.3 MPa
-0.6 MPa
-0.9 MPa
Accumulated germination (number of seeds)
0
5
10
15
20
3
6
9
12
15
Days after sowing
Treatment
C
T
HD
THD

Figure 2.

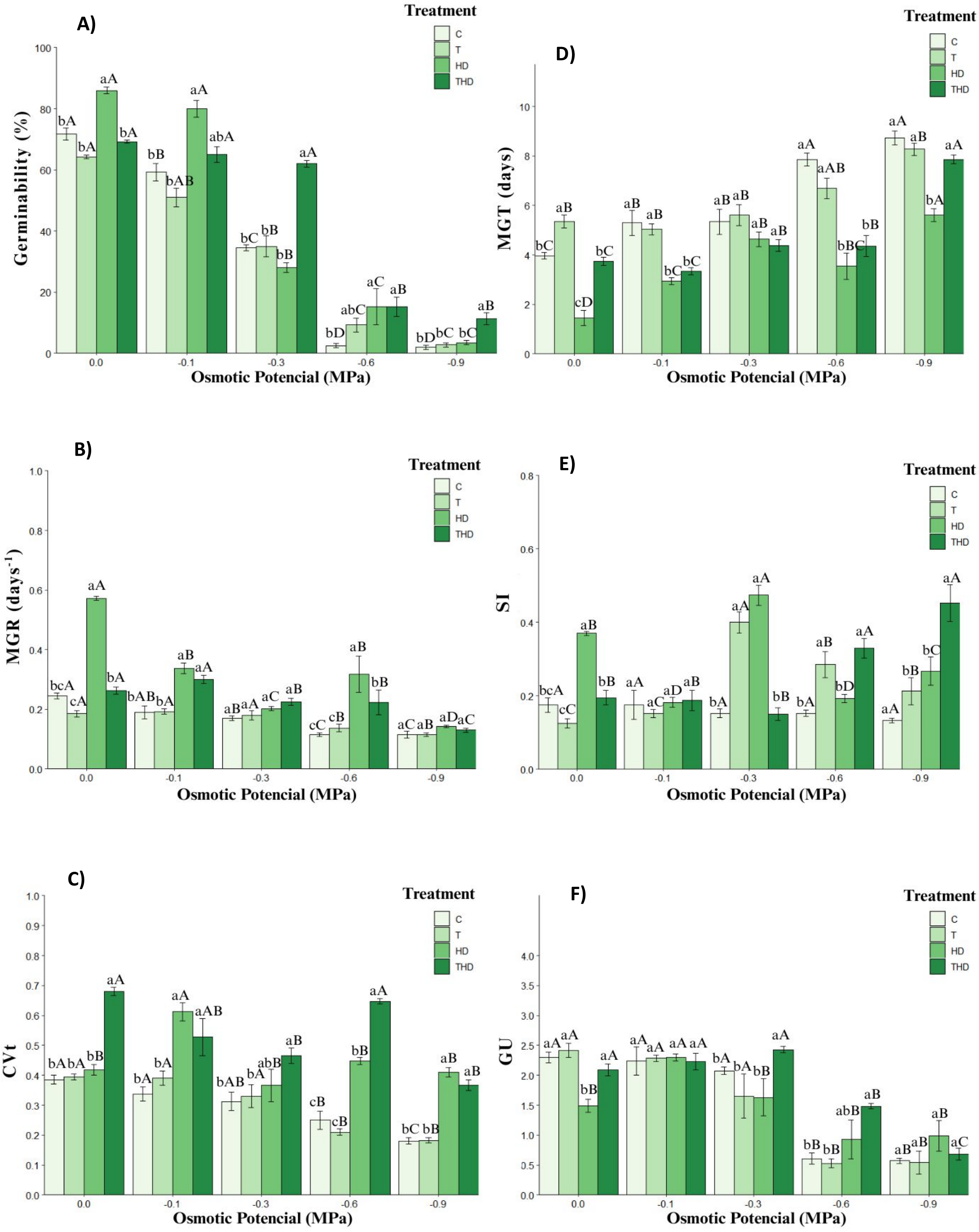
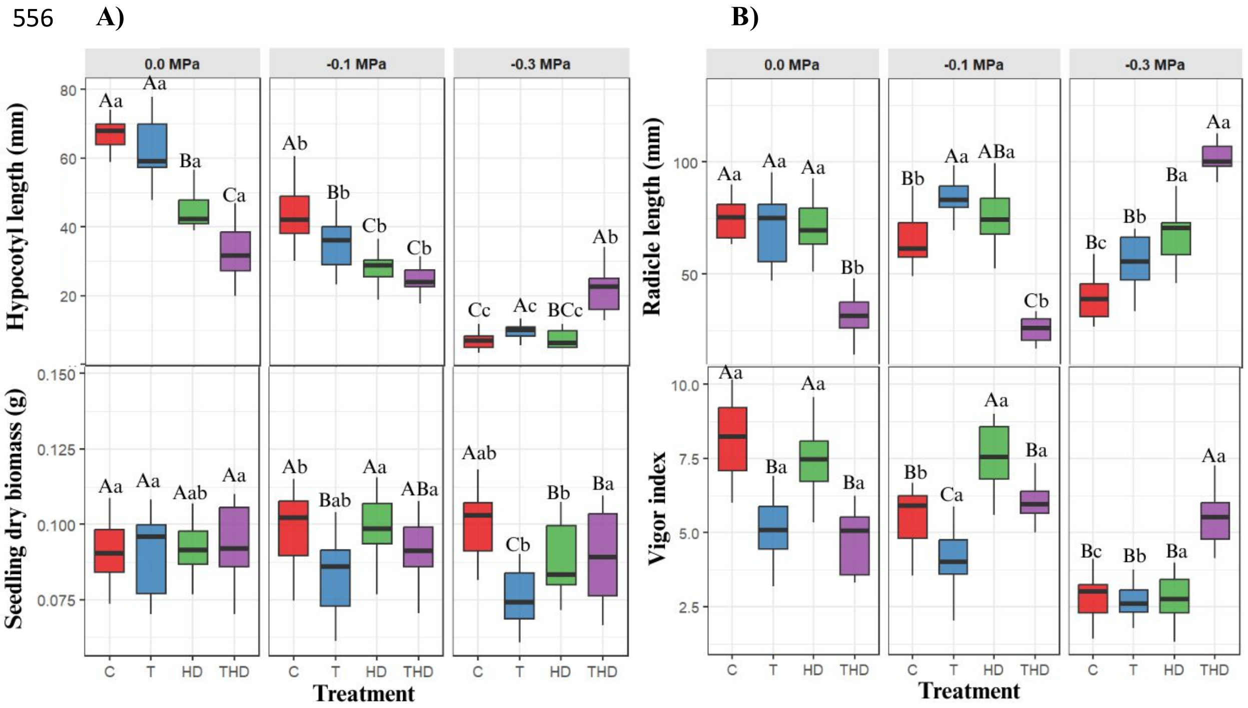

A)
Treatment
C
T
HD
THD
Germinability (%)
100
80
60
40
20
0
bA
bA
aA
bA
bB
bAB
aA
abA
bC
bB
bB
aA
bD
abC
aC
aB
bD
bC
bC
aB
0.0
-0.1
-0.3
-0.6
-0.9
Osmotic Potencial (MPa)
D)
Treatment
C
T
HD
THD
MGT (days)
10
8
6
4
2
0
bC
aB
cD
bC
aB
aB
bC
bC
aB
aB
aB
aB
aA
aAB
bBC
bB
aA
aB
bA
aA
0.0
-0.1
-0.3
-0.6
-0.9
Osmotic Potencial (MPa)
B)
Treatment
C
T
HD
THD
MGR (days^{-1})
1.0
0.8
0.6
0.4
0.2
0.0
bcA
cA
aA
bA
bAB
bA
aB
aA
aB
aA
aC
aB
cC
cB
aB
bB
aC
aB
aD
aC
0.0
-0.1
-0.3
-0.6
-0.9
Osmotic Potencial (MPa)
E)
Treatment
C
T
HD
THD
SI
0.8
0.6
0.4
0.2
0.0
bcA
cC
aB
bB
aA
aC
aD
aB
bA
aA
aA
bB
bA
aB
bD
aA
aA
bB
bC
aA
0.0
-0.1
-0.3
-0.6
-0.9
Osmotic Potencial (MPa)
C)
Treatment
C
T
HD
THD
CVt
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
bA
bA
bB
aA
bA
bA
aA
aAB
bAB
bA
abB
aB
cB
cB
bB
aA
bC
bB
aB
aB
0.0
-0.1
-0.3
-0.6
-0.9
Osmotic Potencial (MPa)
F)
Treatment
C
T
HD
THD
GU
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
aA
aA
bB
aA
aA
aA
aA
aA
bA
bA
bB
aA
bB
bB
abB
aB
aB
aB
aB
aC
0.0
-0.1
-0.3
-0.6
-0.9
Osmotic Potencial (MPa)

Figure 3.


A)
B)
0.0 MPa
-0.1 MPa
-0.3 MPa
Hypocotyl length (mm)
80
60
40
20
Aa
Aa
Ba
Ca
Ab
Bb
Cb
Cb
Cc
Ac
BCc
Ab
Seedling dry biomass (g)
0.150
0.125
0.100
0.075
Aa
Aa
Aab
Aa
Ab
Bab
Aa
ABa
Aab
Cb
Bb
Ba
C
T
HD
THD
Treatment
Radicle length (mm)
100
50
Aa
Aa
Aa
Bb
Bb
Aa
ABa
Cb
Bc
Bb
Ba
Aa
Vigor index
10.0
7.5
5.0
2.5
Aa
Ba
Aa
Ba
Bb
Ca
Aa
Ba
Bc
Bb
Ba
Aa

6. ARTIGO 4

A ser submetido para o periódico *New Forests*

Seed experience shapes drought resilience in *Cenostigma pyramidale* seedlings: contrasting effects of HD cycles, thermal pulses, and their combination

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Abstract

Seasonally Dry Tropical Forests are increasingly exposed to intensified drought and high-temperature events, making the early life stages of woody species particularly vulnerable. In the Caatinga, seeds can experience thermal pulses and discontinuous hydration before germination, yet the combined effects of these cues on the expression of stress memory during seedling development remain poorly understood. In our study, seeds were subjected to four pre-germinative treatments: control, thermal pulse (65ºC), hydration–dehydration cycles, and the combined treatment. Seedlings were grown under daily irrigation or intermittent irrigation composed of two drought cycles separated by rehydration. Growth traits, biomass partitioning, relative water content, photosynthetic pigments, total soluble sugars, and proline were evaluated at key developmental stages, and multivariate analyses were used to integrate trait responses over time. Water regime was the main driver of growth and biomass accumulation, with intermittent irrigation reducing shoot growth and total biomass across treatments. Pre-germinative treatments mainly influenced physiological and biochemical adjustments under water deficit. Seedlings originating from hydration–dehydration cycles exhibited increased root length and coordinated maintenance of water status, photosynthetic pigments, and osmolyte accumulation under recurrent drought. In contrast, the combined thermal and hydration treatment did not confer structural or integrative advantages under intermittent irrigation, indicating potential metabolic costs associated with multiple stress cues. Overall, the results indicate that stress memory in *C. pyramidale* is primarily expressed through physiological resilience rather than enhanced growth, supporting survival and functional stability under recurrent drought while revealing limits to memory effectiveness when thermal and hydric cues are combined.

Keywords: Caatinga; hydration memory; thermal pulse; physiological resilience; Catingueira.

1. Introduction

Recent evidence indicates that tropical regions, including Seasonally Dry Tropical Forests (SDTFs), are already experiencing an intensification of climatic extremes, marked by increases in the frequency and severity of droughts and by rising temperatures (Campo et al., 2023). These trends reflect an ongoing process rather than a distant projection, particularly in semi-arid ecosystems (Marengo et al., 2017).

The Caatinga, a Brazilian SDTF located in Northeastern Brazil, is characterized by high temperatures and prolonged dry periods that act as a major environmental filter, shaping vegetation dynamics, regeneration patterns, and species persistence (Alves et al., 2009; Meiado et al., 2020). Over recent decades, observational and ecological evidence has indicated increasing aridity in this biome, with direct effects on key ecological processes (Marengo et al., 2017).

In Caatinga-like environments, the seedling stage represents a critical bottleneck for the establishment of woody species, because early mortality is high and strongly dependent on water availability (Bandaru et al., 2026). During this phase, survival depends on a delicate balance between rapid resource acquisition and the ability to modulate physiological activity in response to fluctuating environmental conditions (Espinosa et al., 2024). Therefore, mechanisms that promote both stress tolerance and functional recovery after drought events become crucial for recruitment success (Santos-Júnior et al., 2021; Santos et al., 2024).

Seeds in the Caatinga soil seed bank are naturally exposed to extreme abiotic conditions prior to germination. During the dry season, surface-level temperatures can exceed 60 °C (Teixeira, 2010), whereas the onset of the rainy season often produces discontinuous hydration pulses that are insufficient to trigger immediate germination but are sufficient to activate metabolic processes (Meiado, 2013; Lima and Meiado, 2017). In this context, these pre-germinative fluctuations are not merely stressors but also environmental cues capable of inducing physiological adjustments that persist beyond germination and influence seedling performance (Freitas et al., 2021; Santos-Júnior et al., 2021).

The ability of plants to perceive, store, and later retrieve metabolic information associated with stressful events has been widely discussed within the framework of stress memory (Thellier and Lüttge, 2013). Stress memory refers to the capacity of plants to retain metabolic, physiological, or molecular information generated during a previous stress event and to retrieve it upon subsequent exposure, resulting in faster and/or more effective responses (Bruce et al., 2007; Galviz et al., 2022). This mechanism has been proposed as a central component of adaptive plasticity in environments characterized by high environmental unpredictability (Jacques et al., 2021).

Hydration–dehydration (HD) cycles applied to seeds of Caatinga species, for example, are known to promote metabolic repair, synchronize germination, and improve seedling performance under moderate stress, through the activation of antioxidant systems and osmotic adjustment pathways (Pereira-da-Silva et al., 2024; Santos et al., 2024; Silva et al., 2025). Similarly, exposing seeds to short pulses of high temperature can induce protective responses, including the activation of heat-shock proteins (HSPs), which are frequently linked to cellular stability and increased germination vigor, and can also enhance early seedling growth (Santos-Júnior et al., 2023; Santos Júnior et al., 2026). However, it remains poorly understood whether combining thermal and hydration-related cues during the seed phase produces beneficial priming or instead imposes physiological costs that constrain the expression of stress memory under severe and recurrent drought.

It is important to note that the functional expression of stress memory may involve trade-offs between structural growth and physiological maintenance (Hilker et al., 2016). Although memory-induced adjustments may favor osmoregulation, protection of the photosynthetic apparatus, and cellular stability (Galviz et al., 2025; Silva et al., 2025), these responses do not necessarily translate into greater biomass accumulation, particularly under severe or recurrent stress (Galviz et al., 2025; Heck et al., 2025). In semi-arid environments, persistence may depend less on maximizing growth and more on conserving physiological function and rapidly recovering during transient windows of water availability.

In this study, we evaluated the effects of pre-germinative exposure to high-temperature pulses (65 °C), to hydration–dehydration cycles, and to their combination on the acquisition and expression of stress memory in seedlings of *Cenostigma pyramidale* (Tul.) E. Gagnon & G.P. Lewis, a widely distributed and ecologically relevant Caatinga species. Although previous work has shown that HD cycles can induce stress memory in seedlings of this species under water deficit (Oliveira, 2021), investigations addressing the role of high temperature, alone or combined with discontinuous hydration, in modulating this memory remain scarce.

Accordingly, we tested the hypotheses that: (i) pre-germinative stress induces a persistent physiological memory capable of altering morphophysiological traits throughout early development; (ii) seedlings originating from seeds exposed only to HD cycles exhibit higher drought tolerance and faster physiological recovery after drought events, through the activation of osmoregulatory and cell-protection mechanisms; and (iii) combined exposure to thermal stress and HD cycles, rather than producing synergistic effects, imposes metabolic costs that limit seedling resilience under severe and recurrent water déficit.

2. Material and Methods

2.1. Study Area and Seed Source

The experiment was conducted under semi-controlled environmental conditions at the Plant Physiology and Ecophysiology Laboratory of the Federal University of Sergipe (UFS). Seeds of *Cenostigma pyramidale* were collected from a Caatinga fragment in Northeastern Brazil and supplied by the Center for Ecology and Environmental Monitoring (NEMA) of the Federal University of the Vale do São Francisco (UNIVASF).

2.2. Pre-germinative treatments

The experimental design comprised four pre-germinative treatments randomly applied to the seeds: Control (C), consisting of intact seeds without prior treatment; Thermal pulse (T), in which seeds were exposed to high temperature (65 °C) for 60 min; Hydration–dehydration cycles (HD), where seeds were subjected to three successive hydration–dehydration cycles; and Combined treatment (T-HD), in which seeds were first exposed to a thermal pulse (65 °C for 60 min) and subsequently subjected to the three hydration–dehydration cycles.

2.2.1. Application of thermal pulses and hydration–dehydration cycles

Prior to the application of pre-germinative treatments, seeds of *Cenostigma pyramidale* were disinfected in a 3% sodium hypochlorite (NaClO) solution for 3 min, rinsed thoroughly with distilled water, and dried on absorbent paper. Seeds were then kept at room temperature (25 °C) for 24 h.

To define the duration of hydration and dehydration phases, imbibition and drying curves were constructed using the same seed lot. For the imbibition curve, four replicates of 25 seeds (n = 100) were weighed to obtain initial mass and placed in Petri dishes lined with two sheets of filter paper moistened with 10 mL of distilled water. Seed mass was recorded at 60-min intervals until the onset of Phase III of imbibition, defined by radicle protrusion < 2 mm (Lima and Meiado, 2018). Based on these kinetics, the hydration period was set at 4 h, corresponding to half of Phase I duration.

For the dehydration curve, hydrated seeds were transferred to a forced-air oven at 35 °C and weighed hourly until they returned to their initial dry mass, which occurred after approximately 5 h (Lima and Meiado, 2018). This interval was adopted as the standard dehydration period for each HD cycle.

Thermal pulse treatments were applied by placing seeds in a drying oven (model WHL-25AB) at 65 °C for 60 min. This temperature and exposure time were selected based on previous studies (Santos-Júnior et al., 2023; Santos-Júnior and Silva, 2024) and on soil surface temperatures recorded in Caatinga ecosystems during periods of high solar irradiance (Souto et al., 2009; Martins et al., 2024).

Following the application of pre-germinative treatments, seeds were placed in Petri dishes lined with filter paper moistened with distilled water to germinate.

2.3. Experimental Design and Growth Conditions

After radicle protrusion and initial seedling development, seedlings were transplanted into 15 cm diameter pots containing approximately 2 kg of local soil substrate. Plants were maintained under daily irrigation for a 30-day acclimatization period to ensure uniform establishment. During the experimental period, mean air temperature and relative humidity in the greenhouse were 30 °C and 72%, respectively.

After acclimatization, the experiment was conducted using a completely randomized design in a 4 × 2 factorial scheme, consisting of four pre-germinative treatments (C, T, HD, T-HD) combined with two irrigation regimes: (i) Daily Irrigation (DI), in which soil moisture was maintained at 80% of field capacity; and (ii) Intermittent Irrigation (IR), characterized by cycles of water withholding and rehydration. The soil field capacity was determined gravimetrically prior to the experiment. Each treatment consisted of five repetitions per irrigation regime.

The intermittent irrigation regime consisted of two drought cycles separated by a recovery period. Irrigation was suspended for 25 days (first drought cycle), followed by 25 days of rehydration (recovery), and then suspended again for an additional 25 days (second drought cycle). Soil moisture was monitored daily using a Falker HFM2010 digital moisture meter to ensure consistent stress application.

Physiological and biochemical evaluations were performed at three key time points: 55 days after transplanting (DAT), corresponding to the end of the first drought cycle; 80 DAT, at the end of the recovery period; and 105 DAT, at the end of the second drought cycle. Air temperature, relative humidity, and soil moisture values recorded at each sampling time are presented in Table 1.

2.4. Growth, biomass partitioning and vigor index

Plant growth was monitored a cada 15 dias by measuring plant height (cm) and stem diameter (mm) using a digital caliper with 0.01 mm precision (Digimess, China), as well as by counting the number of fully expanded leaves.

At harvest (105 DAT), plants were separated into leaves, stems, and roots. The plant material was oven-dried at 70 °C until constant weight to determine leaf dry mass (LDM), stem dry mass (SDM), and root dry mass (RDM) and total dry mass (TDM) (Benicasa 2003). Root system length was measured as the length of the main root.

Seedling quality was quantified using the Dickson Quality Index (DQI), calculated according to the formulation proposed by Dickson et al. (1960). This index integrates morphological and biomass attributes, including total dry mass (TDM), plant height (H), stem diameter (SD), and the distribution of biomass between shoot (SDM) and root systems (RDM). Because it combines indicators of robustness and biomass allocation, DQI is widely used as an integrative measure of seedling quality, with higher values indicating more vigorous and better-structured seedlings. DQI was calculated by the equation:

$$\mathrm{DQI} = \frac{TDM}{\left(\left(\frac{H}{SD}\right) + \left(\frac{SDM}{RDM}\right)\right)}$$

Plant performance under water deficit was further evaluated using the Drought Tolerance Efficiency (DTE), calculated following Fischer et al. (1982). This index estimates the capacity of plants to maintain biomass production under drought conditions relative to well-watered controls, serving as an indicator of tolerance to water limitation. Higher DTE values reflect greater efficiency in sustaining growth under drought stress and thus indicate superior adaptation to water-limited environments. DTE was calculated as:

$$\mathrm{DTE} = \frac{\mathrm{DM_d}}{\mathrm{DM_c}}$$

where $\mathrm{DM_d}$ represents the total dry mass of plants subjected to drought stress (under intermittent irrigation), and $\mathrm{DM_c}$ corresponds to the total dry mass of plants grown under daily irrigation (control condition).

In addition, the Drought Tolerance Index (DTI) was determined according to the equation proposed by Abenavoli et al. (2016). This index is conceptually similar to DTE but incorporates normalization based on mean biomass values across all treatments, allowing a more integrative comparison of drought responses. Higher DTI values indicate greater drought tolerance. DTI was calculated by the equation:

$$DTI = \frac{\left(\frac{DM_d}{DM_c}\right)}{\left(\frac{\bar{X}DM_s}{\bar{X}DM_c}\right)}$$

In this calculation, $\bar{\mathrm{DM}}_s$represents the mean total dry mass of all plants subjected to drought stress, while $\bar{\mathrm{DM}}_c$corresponds to the mean total dry mass of all control plants, irrespective of pre-germinative treatments.

Finally, drought sensitivity was assessed using the Drought Sensitivity Index (SDI), calculated according to Ali and El-Sadek (2016), based on the same biomass parameters described above. This index quantifies the relative reduction in growth caused by water deficit when compared to control conditions. By definition, control plants present an SDI value equal to zero, serving as the reference condition. Lower SDI values indicate reduced sensitivity to water deficit.

$$SDI = \frac{DM_c - DM_d}{DM_c}$$

2.6. Physiological Analyses

2.6.1. Relative Water Content (RWC)

Leaf water status was assessed by determining the relative water content (RWC). Five leaf discs (1 cm² each) were collected from fully expanded leaves between 11:00 and 12:00 h. The discs were immediately weighed using an analytical balance (Shimadzu AUY220) to obtain fresh weight (FW). Subsequently, the discs were placed in Petri dishes containing distilled water and maintained in the dark at room temperature (25 °C) for 24 h to achieve full turgidity. After this period, the turgid weight (TW) was recorded. The discs were then oven-dried at 70 °C until constant weight to determine dry weight (DW).

Relative water content was calculated according to Weatherley (1950) using the following equation:

$$\text{RWC (\%)} = \frac{FW - DW}{TW - DW} \times 100$$

2.6.2. Photosynthetic Pigments

Chlorophyll a, chlorophyll b, and carotenoid contents were quantified by extracting pigments from 100 mg of fresh leaf tissue in 5 mL of 96% ethanol. Samples were incubated in the dark for 24 h to ensure complete pigment extraction. Absorbance readings were obtained at 665, 649, and 470 nm using a spectrophotometer. Pigment concentrations were calculated according to the equations proposed by Lichtenthaler and Buschmann (2001) and expressed on a fresh weight basis (mg g^{-1} FW).

2.7. Biochemical Assays

To determine the concentrations of osmotically active organic solutes, 0.5 g of fresh leaf tissue was sampled. The tissue was homogenized in a mortar with 5 mL of 0.1 M sodium phosphate buffer (pH 7.0) supplemented with 0.01 M EDTA. The homogenate was filtered and subsequently centrifuged at 4000 × g for 10 min, and the resulting supernatant was collected and stored for subsequent analyses.

Total soluble carbohydrate content was quantified using the phenol–sulfuric acid assay, with D-(+)-glucose employed as the calibration standard, and results were expressed as µg g^{-1} FW (Dubois et al., 1956). Free proline concentration was determined by the acid ninhydrin method, using L-proline as the standard, and values were expressed as µmol g^{-1} FW (Bates et al., 1973).

2.8. Statistical Analysis

Data were subjected to Shapiro-Wilk and Levene tests to verify normality and homogeneity of variance, respectively. When assumptions were not met, data were log-transformed prior to analysis. Results were analyzed using a two-way Analysis of Variance (ANOVA), considering pre-germinative treatments (C, T, HD, and T-HD) and irrigation regimes [daily irrigation (DI) and intermittent irrigation (IR)] as factors. When significant differences were detected ($P < 0.05$), means were compared using the Tukey test ($P < 0.05$) using R software (version 4.4.0). Analyses were conducted within each DAT when variables were measured at multiple time points (55, 80, and 105 DAT).

Growth traits (plant height, stem diameter, and number of leaves) and destructive measurements (biomass partitioning and root length) were analyzed at 105 DAT. To explore multivariate patterns, heatmaps were built using z-score standardized variables, and PCA was performed on standardized data with 95% confidence ellipses for treatments.

3. **Results**

3.1. Shoot growth responses to water regime

At the end of the experiment (105 DAT), shoot growth variables responded predominantly to the water regime, with trait-specific effects of pre-germinative treatments (Tab. 2; Fig. 1).

Overall, plant height, stem diameter, and leaf number were higher under daily irrigation (DI) than under intermittent irrigation (IR) across all treatments (Fig. 1). Thus, water availability was the main driver of shoot growth.

When treatments were compared within the same water regime, distinct patterns emerged. For plant height, no differences among pre-germinative treatments were detected under DI (Fig. 1A), indicating strong overlap under favorable water supply. Under IR, seedlings from the T treatment were shorter than those from HD, whereas C and T-HD showed intermediate values (Fig. 1B).

For stem diameter, under DI, seedlings from T exhibited a larger stem diameter than HD, while C and T-HD did not differ from the other treatments (Fig. 1C). Therefore, under DI, differences among treatments were limited and trait dependent. Under IR, T-HD showed the lowest stem diameter, differing from C, T, and HD, which did not differ from each other (Fig. 1D).

Leaf number decreased under IR in all treatments, with no differences among C, T, HD, and T-HD within each water regime, consistent with the absence of a treatment effect for this variable (Tab. 2; Fig. 1E; 1F).

3.2. Plant water status (RWC) over time

Differences in RWC were observed between water regimes within the same treatment and among treatments within the same water regime, depending on the sampling time (DAT) (Tab. 3; Fig. 1G–H).

At 55 DAT, at the end of the first drought cycle under IR, T-HD showed the highest RWC, exceeding C and T, which had the lowest values, whereas HD was intermediate (Fig. 1H). Under DI, T exhibited the lowest RWC, differing from the other treatments (Fig. 1G). When comparing water regimes within each treatment, T and T-HD displayed higher RWC under IR than under DI, whereas C and HD did not differ between regimes at this time point (Fig. 1G–H).

At 80 DAT, at the end of the recovery period under IR, no differences among treatments were detected (Fig. 1H), indicating convergence in water status following rehydration. Under DI, RWC was lower in HD than in C and T-HD, with T showing intermediate values (Fig. 1G). Between regimes, only the control (C) differed, with lower RWC under DI than under IR, whereas T, HD, and T-HD did not differ between regimes (Fig. 1G–H).

At 105 DAT, at the end of the second drought cycle, under IR, T showed the highest RWC, differing from T-HD, which had the lowest value, whereas C and HD were intermediate (Fig. 1H). Under DI, C had the highest RWC, differing from HD, which had the lowest mean, while T and T-HD were intermediate (Fig. 1G). When comparing regimes within each treatment, C, T, and T-HD showed higher RWC under DI than under IR, whereas HD did not differ between regimes (Fig. 1G–H).

3.3. Photosynthetic pigments

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents varied according to water regime, pre-germinative treatment, and sampling time (DAT) (Tab. 3; Fig. 2).

At 55 DAT, differences among treatments were detected mainly under DI, with T-HD showing the lowest chlorophyll contents, whereas C, T, and HD displayed similar values. Under IR, treatments did not differ in chlorophyll levels at this time point (Fig. 2E, F, and H). Carotenoids showed trait- and treatment-specific variation under DI and broad overlaps among treatments under IR (Fig. 2G).

At 80 DAT, corresponding to the end of the recovery period under IR, clear treatment differences emerged under IR, with HD showing higher chlorophyll and total chlorophyll than C, T, and T-HD, which were similar to each other (Fig. 2E-H). Under DI, treatments did not differ significantly for these pigments (Fig. 2A-D). Notably, for HD, chlorophyll levels under IR became comparable to those under DI, suggesting recovery of the photosynthetic apparatus during rehydration (Fig. 2D and H).

At 105 DAT, at the end of the second drought cycle, chlorophyll a, total chlorophyll, and carotenoids were generally higher under DI than under IR (Fig. 2A, C and D). Under IR, HD maintained the highest chlorophyll contents, whereas C showed the lowest values, with T and T-HD presenting intermediate levels (Fig. 2H). Under DI, differences among treatments were less pronounced and depended on the pigment evaluated (Fig. 2D). For chlorophyll b, responses were more variable among treatments, with no consistent pattern across water regimes (Fig. 2B and F).

Overall, treatment effects on photosynthetic pigments were more evident under IR, particularly at the time points associated with rehydration (80 DAT) and recurrent drought (105 DAT) (Fig. 2).

3.4. Osmolytes: total soluble sugars (TSS) and proline

Leaf TSS varied as a function of treatment and sampling time (Tab. 3; Fig. 3A; 3D). At 55 DAT, under DI, HD exhibited the highest TSS, whereas the control (C) had the lowest values, with T and T-HD intermediate. Under IR at 55 DAT, HD and T-HD showed higher TSS than C and T (Fig. 3A; 3D).

At 80 DAT, there was broad overlap among treatments, with no significant differences under either DI or IR (Fig. 3A; 3D). At 105 DAT, under DI, T-HD showed the highest TSS, whereas C had the lowest values, with no significant differences between T and HD. Under IR, HD maintained the highest TSS, differing from C, T, and T-HD, which showed lower values (Fig. 3A; 3D).

Leaf proline varied according to water regime, treatment, and DAT (Fig. 3B; 3E). At 55 DAT, proline levels were higher under IR than under DI across all treatments. Under DI, the control exhibited the highest values, differing from T, HD, and T-HD, which showed lower levels. Under IR, treatments did not separate statistically, indicating overlapping responses at this time point.

At 80 DAT, under IR, T-HD had the highest leaf proline, differing from T and HD, whereas the control did not differ from the other treatments. Under DI, treatments did not differ statistically (Fig. 3B; 3E).

At 105 DAT, under DI, T and T-HD showed the highest proline levels, differing from the control, whereas HD did not differ from the other treatments. Under IR, HD exhibited the highest proline, differing from C and T-HD, while T had intermediate values and did not differ significantly from the other treatments (Fig. 3B; Fig. 3E).

At 105 DAT, root TSS and root proline differed among treatments and water regimes (Tab. 3; Fig. 3C; 3F). For root TSS, under DI, T-HD showed the highest values, differing from the control, while T and HD were intermediate. Under IR, HD exhibited the highest root TSS, whereas C, T, and T-HD had lower values (Fig. 3C).

For root proline, under DI, T showed the highest content, C and HD were intermediate, and T-HD had the lowest concentration. Under IR, C and HD showed the highest values, differing from T-HD, whereas T did not differ from the other treatments (Fig. 3F).

3.5. Biomass production and partitioning

Biomass production was strongly influenced by water regime, regardless of the pre-germinative treatment (Tab. 2; Fig. 4).

Leaf, stem, root, and total dry biomass were consistently higher under DI than under IR across all treatments (Fig. 4). There was substantial overlap among treatments within each water regime, as comparisons among treatments revealed no significant differences for any biomass trait under either DI or IR (Fig. 4).

These results indicate that, although recurrent water deficit reduced total biomass accumulation, pre-germinative treatments did not alter the final pattern of biomass allocation among plant organs.

3.6. Root architecture

In contrast to biomass traits, root length responded mainly to the pre-germinative treatment, independently of the water regime (Tab. 2; Fig. 4D). Under IR, seedlings originating from seeds subjected to hydration–dehydration cycles (HD) exhibited greater root length than C, T, and T-HD (Fig. 4E).

This effect was not observed under DI, where no differences among treatments were detected. Moreover, comparisons between water regimes within each treatment showed no significant differences, indicating that root length was not affected by the final water regime (Fig. 4D).

3.7. Integrative indices of seedling quality and drought tolerance

For the Dickson Quality Index (DQI), significant differences between water regimes were observed only in C, T, and HD, with higher values under DI than under IR. For T-HD, no significant difference between regimes was detected (Fig. 5A).

When comparing treatments within the same water regime, differences were detected only under IR, where HD had a lower value than T-HD, while C and T did not differ from the other treatments (Fig. 5A).

DTE, SDI, and DTI did not differ significantly among treatments, with C, T, HD, and T-HD showing statistically similar values (Fig. 5B-D).

3.8. Multivariate analyses: heatmap and principal component analysis (PCA)

The multivariate integration of morphological, physiological, and biochemical variables was assessed using a heatmap and principal component analysis (PCA), considering treatments, water regimes, and sampling times (Fig. 6–7).

Multivariate patterns over time (heatmap and PCA)

Multivariate analyses indicated a consistent temporal trajectory in seedling responses modulated by water regime and pre-germinative treatments (Fig. 6-7). Overall, three phases were evident across the experiment: an initial response to the first drought cycle (55 DAT), a convergence phase associated with rehydration (80 DAT), and a more pronounced divergence among treatments after the second drought cycle (105 DAT).

55 DAT — Initial response to the first drought cycle

At 55 DAT under DI, the heatmap revealed high heterogeneity among treatments (Fig. 6). T showed a higher relative contribution of TSS and structural growth (height and leaf number), whereas C was characterized by higher relative values of total chlorophyll, carotenoids, and proline, associated with lower growth. T-HD exhibited low relative values of RWC and growth, while HD showed an intermediate profile with a higher contribution of stem diameter. Consistently, PCA indicated broad overlap among the four treatments under DI, with no clear separation in multivariate space; PC1 was primarily associated with photosynthetic pigments, whereas PC2 related to growth and proline (Fig. 7A).

Under IR at 55 DAT (end of the first drought cycle), treatments showed more contrasting responses (Fig. 6).T- HD displayed a higher relative contribution of photosynthetic pigments, whereas T stood out for high TSS and plant height. C was strongly associated with proline and stem diameter but had low relative values of RWC and leaf number, while HD maintained high RWC but low pigment and proline values. Accordingly, PCA revealed functional separation among treatments along PC1, which separated variables related to water status and growth from those related to pigments; in this configuration, HD clustered near RWC and growth, T-HD and C aligned with photosynthetic pigments, and T occupied an intermediate position (Fig. 7B).

80 DAT — Physiological convergence during rehydration

At 80 DAT under DI, profiles became more homogeneous among treatments (Fig. 6). T-HD showed a higher relative contribution of structural growth (height and diameter), whereas C showed greater association with RWC, proline, and TSS, accompanied by lower relative pigment values. T maintained a high contribution of photosynthetic pigments, associated with reduced RWC and proline. PCA confirmed this pattern, showing strong overlap among treatments and no distinct clustering; PC1 was associated with structural growth and RWC, while PC2 related mainly to leaf number and proline (Fig. 7C).

Under IR at 80 DAT (end of the recovery period), differentiated patterns among treatments emerged (Fig. 6). T-HD showed higher relative contributions of RWC, TSS, and leaf number, whereas T exhibited high relative values of total chlorophyll and carotenoids. HD remained strongly associated with proline and TSS, while C showed a higher relative contribution of structural growth but low pigment values. PCA indicated partial functional separation among treatments, with T-HD associated with RWC and leaf number, HD with photosynthetic pigments, and HD shifted toward TSS; the control partially overlapped with the other treatments (Fig. 7D).

105 DAT — Divergence after the second drought cycle

At 105 DAT under DI, differences among treatments remained evident (Fig. 6). T-HD showed a higher relative contribution of structural growth and photosynthetic pigments, whereas HD stood out for high TSS. T showed greater association with proline and total chlorophyll, accompanied by reduced RWC, while C exhibited a higher relative contribution of RWC but low values of growth and photosynthetic metabolism. PCA reflected this pattern, with T-HD clustering in the quadrant associated with growth and pigments, whereas HD and T were more strongly associated with TSS and proline; the control occupied an intermediate position with no clear separation from the other treatments (Fig. 7E).

Under IR at 105 DAT (end of the second drought cycle), contrasts among treatments were more pronounced (Fig. 6). T-HD exhibited a high relative contribution simultaneously for RWC, total chlorophyll, carotenoids, proline, and TSS, along with positive values for leaf number. T was associated mainly with structural growth and carotenoids, whereas C and HD showed predominantly low contributions for most variables. Consistently, PCA showed clear separation among treatments along PC1, dominated by biochemical and photosynthetic variables, with T-HD positioned at the positive extreme, T more closely associated with structural growth variables, and C and HD grouped in the opposite quadrant, indicating lower contributions of these variables under recurrent drought (Fig. 7F).

4. Discussion

Semi-arid environments such as the Caatinga impose recurrent challenges on plants due to the unpredictability of water availability, making morphophysiological plasticity and the ability to anticipate responses to stress events key determinants of ecological success (Arshad et al., 2023). In this context, stress memory has been recognized as a relevant adaptive mechanism, enabling the modulation of responses to subsequent stresses based on prior experiences (Bruce et al., 2007; Hilker et al., 2016). However, evidence indicates that the effectiveness of this memory depends on the intensity, duration, and combination of the stresses involved, as well as the ontogenetic stage at which they are perceived (Galviz et al., 2025; Heck et al., 2025; Silva et al., 2025).

Cenostigma pyramidale shows a high capacity for responses based on prior environmental cues under thermal fluctuations and water availability (Pinho-Pessoa et al., 2018; Oliveira, 2022). Our results support this capacity, while also revealing clear limits between signal perception and the functional expression of stress memory under severe and recurrent water deficit.

The water regime was the main factor modulating plant growth and overall performance throughout the experiment. Intermittent irrigation, characterized by two prolonged cycles of water withholding, promoted consistent reductions in shoot growth, biomass production, and relative water content, confirming that the imposed stress was sufficiently intense to constrain fundamental physiological processes (Kambona et al., 2023). Under prolonged water deficit, reduced turgor pressure limits cell expansion, whereas tissue dehydration and hormonal imbalance tend to restrict mitotic activity in meristems, resulting in smaller plants and lower biomass accumulation (Silva et al., 2013; Seleiman et al., 2021). This pattern reinforces the role of water deficit as a dominant ecological filter in semi-arid environments, exerting strong control over plant structural performance, particularly when water limitation is prolonged and recurrent (Arshad et al., 2023).

Nevertheless, the observed patterns likely reflect not only the physiological constraints imposed by water deficit, but also metabolic costs associated with maintenance and survival strategies triggered by specific pre-germinative treatments. In particular, the combination of high temperature and hydration–dehydration cycles appear to have imposed an additional metabolic burden on seeds, leading to persistent penalties during plant development. In our dataset, this interpretation is consistent with the reduced stem diameter of T-HD plants under intermittent irrigation and the absence of improvement in seedling-quality metrics relative to HD under stress. Similar patterns have been reported for other species, in which the integration of multiple stress cues at the seed stage can amplify physiological costs, limiting the functional expression of memory and revealing trade-offs between structural growth and physiological maintenance under recurrent stress (Zandalinas and Mittler, 2022; Sato et al., 2023).

The expression of stress memory in plants originating from seeds subjected to hydration–dehydration cycles was integrated across structural, physiological, and biochemical levels. Under water deficit, this group invested more in structures associated with water acquisition, as indicated by increased root length, combined with the maintenance of photosynthetic pigments and the accumulation of compatible solutes such as total soluble sugars (TSS) and proline. The accumulation of TSS and proline under intermittent irrigation may reflect both active osmotic responses (Silva et al., 2013) and concentration effects associated with reduced tissue water content (Ozturk et al., 2021). However, treatment differentiation during rehydration and the partial decoupling between RWC and osmolytes at specific sampling points suggest metabolic regulation beyond a purely physical concentration effect.

Similar responses have been observed in other species, such as *Annona squamosa* (Freitas and Silva, 2024) and *Sarcomphalus joazeiro* (Costa Oliveira et al., 2024). Taken together, these adjustments indicate that stress memory promoted a functional strategy oriented toward exploiting available water resources, enhancing cellular protection, and sustaining metabolism under adverse conditions (Silva et al., 2013; Jacques et al., 2021).

Although these mechanisms did not translate into consistent increases in growth or biomass accumulation, they may contribute to maintaining physiological homeostasis, since TSS and proline play central roles in osmoregulation, membrane stabilization, and the mitigation of oxidative damage (Hayat et al., 2012; Kambona et al., 2023). Thus, even when memory does not maximize growth under severe stress, it may reduce the risk of physiological collapse and preserve functional capacity, increasing the likelihood of renewed development when water availability is restored (Galviz et al., 2025; Jacques et al., 2021).

In contrast to the performance of plants originating from the HD treatment, the results for plants derived from the combined high-temperature pulse and hydration–dehydration cycles indicate that stronger prior stress does not necessarily translate into greater memory-related benefits (Crisp et al., 2016). Although some studies report improvements in germination parameters and early growth following exposure of seeds to high temperature (Santos-Júnior et al., 2023; Santos-Júnior and Silva, 2024) and discontinuous hydration (Freitas and Silva, 2024; Santos et al., 2024), these factors are also recognized as capable of inducing cellular damage and embryo injury when they exceed specific thresholds (Hasanuzzaman et al., 2013; Paparella et al., 2015).

Despite the scarcity of studies evaluating the joint action of these two factors, evidence from other stress combinations indicates that integrating multiple adverse signals can impose high metabolic costs by redirecting resources toward homeostasis and defense (Suzuki et al., 2014; Zandalinas et al., 2020). Under such conditions, potential memory benefits may be partially offset by the costs of maintaining stress-protective states, resulting in neutral or even negative effects on performance (Crisp et al., 2016). In our study, this overlap of costs, combined with recurrent water deficit, likely limited the performance of T-HD plants, which, despite maintaining survival under severe stress, did not show structural or functional advantages relative to HD.

Plant water status and recovery capacity after rehydration provided supporting evidence for the persistence of stress-related functional adjustments, particularly in HD treatment. This pattern highlights water status as a central component of physiological performance during and after stress events, indicating that memory not only mitigates immediate impacts of water deficit but can also favor a more efficient return to functional conditions after rehydration (Kambona et al., 2023). Similar outcomes were reported by Heck et al. (2025) for *Acanthostyles buniifolius*, reinforcing the relevance of this mechanism under recurrent water stress.

In Caatinga species, this type of response is ecologically meaningful because, in environments characterized by irregular and unpredictable rainfall pulses, the ability to recover rapidly during short windows of water availability can be decisive for survival and ecological success (Meiado et al., 2020).

The application of integrative indices of vigor and drought tolerance revealed important limitations in the ability of these metrics to capture the functional complexity associated with stress memory. The Dickson Quality Index (DQI) showed an overall reduction under intermittent irrigation in C, T, and HD, reflecting the structural penalty imposed by recurrent water deficit. Even so, the HD treatment exhibited higher DQI values than the control under stress, indicating that the physiological and morphological adjustments induced by discontinuous hydration contributed to the relative maintenance of structural vigor. An increase in DQI following hydration–dehydration cycles has also been reported for *Annona muricata* (Freitas et al., 2024). In contrast, T-HD did not differ between water regimes, suggesting that metabolic costs associated with the combined pre-germinative stresses constrained the expression of vigor gains even under favorable water availability.

However, the lack of differences among treatments in drought tolerance indices (SDI, DTE, and DTI) indicates that when drought tolerance is evaluated using metrics strongly dependent on growth and biomass accumulation, the functional benefits of stress memory may be underestimated (Tardieu, 2012). These indices share a direct dependence on total biomass and structural performance, which may marginalize adaptive responses based on physiological maintenance, osmoregulation, and metabolic stability (Hilker et al., 2016; Freitas et al., 2024). In our experiment, this is consistent with the observation that HD plants maintained higher pigment and osmolyte levels under intermittent irrigation without a corresponding increase in total biomass, thereby challenging classical interpretations of vigor based solely on growth.

Partially distinct results were reported by Oliveira (2021) for *C. pyramidale*, in which seedlings originating from seeds exposed to hydration–dehydration cycles also accumulated proline and soluble sugars but, unlike in the present study, exhibited increased root biomass. Although much of the literature associates stress memory acquisition with structural gains such as increased growth and biomass accumulation (Bruce et al., 2007; Hilker et al., 2016), in species adapted to semi-arid environments this relationship is not necessarily linear, since greater biomass simultaneously implies higher water demand and higher tissue maintenance costs (Poorter et al., 2012; Volaire, 2018).

In this context, it is important to emphasize that the stress regime imposed here was more intense and recurrent, comprising two prolonged periods of water withholding, which may explain the absence of clear biomass gains among treatments. Under these conditions, our results suggest that, for *C. pyramidale*, stress memory may prioritize the maintenance of physiological functionality over structural growth, reflecting an adaptive adjustment consistent with environments marked by high hydrological unpredictability (Volaire, 2017).

The integration of multivariate analyses indicates that stress memory in *C. pyramidale* operates as a dynamic and reversible process, strongly dependent on water context, involving phases of physiological imprinting, functional latency, and reactivation under recurrent stress (Crisp et al., 2016). Plants from the HD treatment showed consistent reorganization in multivariate space, particularly under intermittent irrigation at the moments of greatest water restriction (55 and 105 DAT), reflecting coordination among water status, osmotic metabolism, and maintenance of the photosynthetic apparatus. During rehydration (80 DAT), a temporary convergence among treatments was observed, evidenced by overlap in the PCA and homogenization in the heatmap profiles, indicating a recovery state in which memory remains functionally latent (Galviz et al., 2022).

Subsequent divergence after the second drought cycle demonstrates that this latency does not represent memory erasure, but rather rapid reactivation upon stress recurrence, consistent with a stress-recall pattern (Thellier and Lüttge, 2013; Hilker and Schmülling, 2019). These results reinforce that, in semi-arid species, stress memory may preferentially support physiological functionality and metabolic resilience rather than maximize structural performance, constituting an adaptive strategy under highly unpredictable water availability (Volaire, 2017; Pinho-Pessoa et al., 2018).

Finally, integrating our findings with previous studies on *C. pyramidale* supports the view that drought-related memory is real but context-dependent. Studies focused on the seed stage indicate that discontinuous hydration can reprogram metabolism and reserve mobilization, favoring germination and early establishment. The present study extends this understanding by showing that such memory can persist and be expressed at later developmental stages, modulating physiological, biochemical, and structural responses under recurrent water deficit. However, the addition of thermal stress revealed clear limits to memory effectiveness, suggesting that metabolic overload associated with multiple stress cues can impose substantial structural costs.

In summary, our results indicate that stress memory in *C. pyramidale* operates primarily along the axis of physiological resilience, promoting functional adjustments that favor survival and recovery under adverse hydric conditions, but not necessarily maximal growth. This strategy may be particularly advantageous in semi-arid environments, where hydrological unpredictability selects organisms capable of balancing functional efficiency and resource economy. By highlighting both the benefits and the limits of drought-related memory, this study contributes to a more realistic and ecologically meaningful understanding of adaptive strategies in Caatinga native species.

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Table 1. Mean soil moisture (SU%), air temperature (AT, °C), and relative air humidity (AU%) recorded at 55, 80, and 105 days after transplanting (DAT) during the experimental period with *Cenostigma pyramidale*. Measurements were obtained under daily irrigation (DI) and intermittent irrigation (IR) regimes for each pre-germinative treatment: Control (C), Thermal pulse (T), Discontinuous hydration (HD), and combined treatment (T-HD). Air temperature and relative humidity correspond to mean values recorded in the greenhouse at each sampling date.

DAT	Treatment	Hidric regime	SU%	AT (ºC)	AU%
55	C	DI	15.5	34.2	69.6
		IR	9.2		
	T	DI	15		
		IR	9.6		
	HD	DI	14.9		
		IR	8.9		
	T-HD	DI	15.2		
		IR	9.8		
80	C	DI	14.9	32.5	65.8
		IR	14.4		
	T	DI	15.3		
		IR	15.5		
	HD	DI	14.6		
		IR	15.1		
	T-HD	DI	15.3		
		IR	15.6		
105	C	DI	14.3	33.9	67.6
		IR	5.3		
	T	DI	15.0		
		IR	4.9		
	HD	DI	15.5		
		IR	5		
	T-HD	DI	14.9		
		IR	5.1		

Table 2. Results of two-way analysis of variance (ANOVA) testing the effects of pre-germinative treatments (Control – C, Thermal pulse – T, Discontinuous hydration – HD, and combined treatment – T-HD), water regime (daily irrigation and intermittent irrigation), and their interaction on growth traits and biomass partitioning of *Cenostigma pyramidale* plants. Variables include plant height, stem diameter, number of leaves, leaf, stem, root and total dry biomass, and root length. F values, degrees of freedom (d.f.), and associated P-values are shown.

Factors	**F**	**d.f**	**p-value**
Plant height (cm)			
Treatment	1.82	3	0.1639
Water regime	390.04	1	<.0001
Treatment x Water regime	4.05	3	0.0151
Stem diameter (mm)			
Treatment	5.98	3	0.0023
Water regime	280.78	1	<.0001
Treatment x Water regime	5.44	3	0.0039
Number of leaves			
Treatment	2.16	3	0.1125
Water regime	91.90	1	<.0001
Treatment x Water regime	0.17	3	0.9186
Leaf dry biomass (g)			
Treatment	1.74	3	0.1788
Water regime	105.30	1	<.0001
Treatment x Water regime	1.73	3	0.1744
Stem dry biomass (g)			
Treatment	0.58	3	0.6345
Water regime	52.84	1	<.0001
Treatment x Water regime	1.39	3	0.2636
Root dry biomass (g)			
Treatment	2.59	3	0.0699
Water regime	100.10	1	<.0001
Treatment x Water regime	0.57	3	0.6379
Total dry biomass (g)			
HD cycles	0.63	3	0.6013
Hydration Time	121.53	1	<.0001
HD cycles x Hydration Time	1.36	3	0.2734
Root length (mm)			
Treatment	8.33	3	<.0001

Water regime	0.38	1	0.8014
Treatment x Water regime	4.58	3	<.0001

The symbol × indicates interaction between the factors.

Table 3. Results of two-way analysis of variance (ANOVA) testing the effects of pre-germinative treatments (Control – C, Thermal pulse – T, Discontinuous hydration – HD, and combined treatment – T-HD), water regime (daily irrigation and intermittent irrigation), and their interaction on physiological, biochemical, and integrative drought-response variables of *Cenostigma pyramidale*. Relative water content (RWC), photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids), leaf and root total soluble sugars (TSS), leaf and root proline, Dickson Quality Index (DQI), Drought Tolerance Efficiency (DTE), Sensitivity Drought Index (SDI), and Drought Tolerance Index (DTI) were evaluated at 55, 80, and 105 days after transplanting (DAT), when applicable. F values and corresponding P-values are presented.

Variable	**DAT**	**Treatment**		**Water regime**		**Treatment X Water regime**	
		F	P	F	P	F	P
RWC	55	6.80	0.0011	19.74	<.0001	6.54	0.0014
	80	3.42	0.0288	0.32	0.5735	3.07	0.0416
	105	6.87	0.0011	69.78	<.0001	4.76	0.0075
Chlorophyll a	55	3.64	0.230	25.56	<.0001	1.34	0.2799
	80	7.10	0.0009	0.05	0.8311	1.84	0.1591
	105	3.95	0.0168	47.14	<.0001	0.35	0.7882
Chlorophyll b	55	4.87	0.006	16.93	<.0001	2.19	0.1084
	80	4.45	0.0101	0.13	0.7723	2.70	0.0618
	105	8.20	<.0001	40.38	<.0001	1.27	0.3025
Total Chlorophyll	55	4.13	0.0139	23.22	<.0001	1.64	0.2001
	80	6.09	0.0021	0.00	0.9739	2.07	0.1233
	105	7.78	0.0005	65.68	<.0001	0.61	0.6107
Carotenoids	55	4.91	0.0064	11.12	0.0022	0.97	0.4189
	80	2.35	0.0914	0.23	0.6342	3.54	0.0253
	105	4.01	0.0158	34.58	<.0001	0.22	0.8797
Leaf Proline	55	7.81	0.005	567.17	<.0001	2.37	0.0892
	80	6.39	0.0016	7.02	0.0124	1.85	0.1574
	105	4.84	0.0069	27.48	<.0001	5.53	0.0036
Leaf TSS	55	59.90	<.0001	2.72	0.1087	2.83	0.0538
	80	0.19	0.9056	0.00	0.9519	1.20	0.3254
	105	9.45	<.0001	10.72	0.0025	15.11	<.0001
Root TSS	-	2.48	0.0787	10.43	0.0029	16.72	<.0001
Root proline	-	13.81	<.0001	27.76	<.0001	11.47	<.0001
DQI	-	3.23	0.035	53.85	0.001	1.82	0.163

DTE	-	3.18	0.053	-	-	-	-
SDI	-	3.18	0.053	-	-	-	-
DTI	-	3.18	0.053	-	-	-	-

The symbol × indicates interaction between the factors

List of captions

Figure 1. Plant height (A-B), stem diameter (C-D), number of leaves (E-F), and relative water content (RWC) (G-H) of *Cenostigma pyramidale* plants derived from seeds subjected to different pre-germinative treatments (C, T, HD and T-HD). Measurements were performed at different days after transplanting (DAT) under daily and intermittent irrigation regimes. Uppercase letters indicate comparisons among treatments within the same irrigation regime and DAT, whereas lowercase letters indicate comparisons between irrigation regimes for the same treatment within the same DAT. Means followed by the same letter do not differ significantly according to the **Tukey** test ($P > 0.05$).

Figure 2. Chlorophyll *a (A; E)*, chlorophyll *b (B; F)*, carotenoids (C; G), and total chlorophyll (D; H) contents of *Cenostigma pyramidale* plants originating from seeds subjected to different pre-germinative treatments (C, T, HD, T-HD), Measurements were performed at different days after transplanting (DAT) under daily and intermittent irrigation regimes. Uppercase letters indicate comparisons among treatments within the same irrigation regime and DAT, whereas lowercase letters indicate comparisons between irrigation regimes for the same treatment within the same DAT. Means followed by the same letter do not differ significantly according to the **Tukey** test ($P > 0.05$).

Figure 3. Total soluble sugars (TSS) (A; D) and proline (B; E) contents in leaves at 55, 80, and 105 days after transplanting (DAT), and in roots (C; F) at 105 DAT, of *Cenostigma pyramidale* plants derived from seeds subjected to different pre-germinative treatments (C, T, HD, T-HD) under daily and intermittent irrigation regimes. Measurements were performed at different days after transplanting (DAT) under daily and intermittent irrigation regimes. Uppercase letters indicate comparisons among treatments within the same irrigation regime and DAT, whereas lowercase letters indicate comparisons between irrigation regimes for the same treatment within the same DAT. Means followed by the same letter do not differ significantly according to the **Tukey** test ($P > 0.05$).

Figure 4. Leaf dry mass (A), stem dry mass (B), root dry mass (C), total dry mass (D), and root length (E) of *Cenostigma pyramidale* plants derived from seeds subjected to different pre-germinative treatments (C, T, HD, T-HD), evaluated under daily (DI) and intermittent (IR) irrigation regimes.

Uppercase letters indicate comparisons among treatments within the same irrigation regime, while lowercase letters indicate comparisons of the same treatment across different irrigation regimes. Means followed by the same letter do not differ significantly according to the Tukey test (P > 0.05).

Figure 5. Dickson Quality Index (DQI; A), Drought Tolerance Efficiency (DTE; B), Sensitivity Drought Index (SDI; C), and Drought Tolerance Index (DTI; D) of *Cenostigma pyramidale* plants derived from seeds subjected to different pre-germinative treatments (C, T, HD, T-HD), grown under daily irrigation (DI) and intermittent irrigation (IR) regimes. Uppercase letters indicate comparisons among treatments within the same irrigation regime, while lowercase letters indicate comparisons between irrigation regimes within the same treatment. Means followed by the same letter do not differ significantly according to the Tukey test (P > 0.05). Symbols represent means ± standard error.

Figure 6. Heatmap representation of standardized (z-score) morphophysiological and biochemical variables of *Cenostigma pyramidale* plants originating from seeds subjected to control (C), high temperature (T), discontinuous hydration (HD), and combined treatment (T-HD), evaluated at 55, 80, and 105 days after transplanting (DAT) under daily irrigation (DI; A, C, E) and intermittent irrigation (IR; B, D, F) regimes. Red and blue colors indicate positive and negative deviations from the mean, respectively. The heatmaps highlight treatment-specific patterns and temporal shifts in physiological coordination associated with stress memory, recovery, and recall phases.

Figure 7. Principal Component Analysis (PCA) of morphophysiological and biochemical traits of *Cenostigma pyramidale* plants originating from seeds subjected to control (C), high temperature (T), discontinuous hydration (HD), and combined treatment (T-HD), evaluated at 55, 80, and 105 days after transplanting (DAT) under daily irrigation (DI; A, C, E) and intermittent irrigation (IR; B, D, F) regimes. Ellipses represent 95% confidence intervals for each treatment. Vectors indicate the contribution of variables to the principal components. Percentages on the axes correspond to the variance explained by each component.

Figure 1.

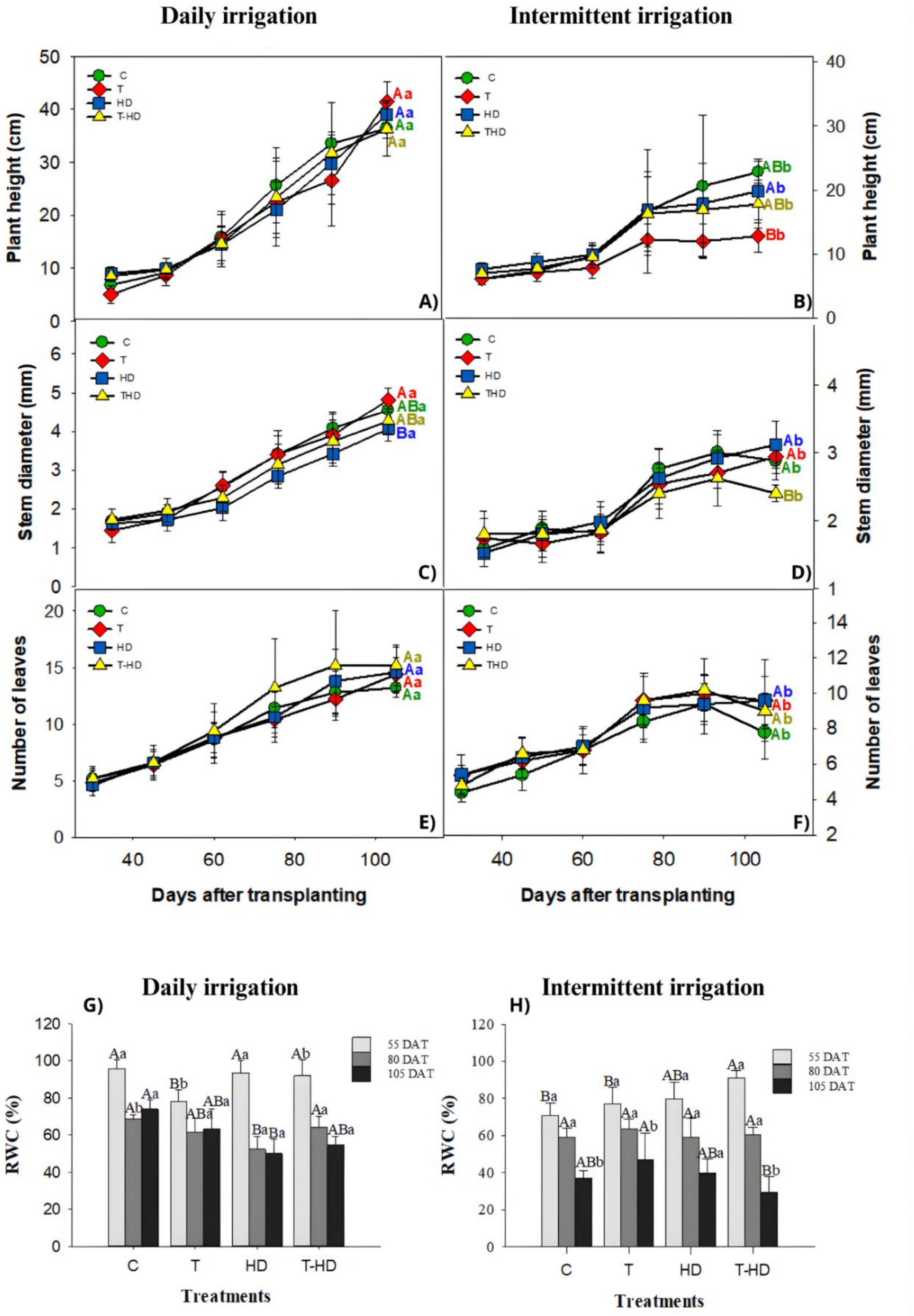

Daily irrigation
Intermittent irrigation
C
T
HD
T-HD
THD
Plant height (cm)
A)
B)
Aa
Aa
Aa
Aa
ABb
Ab
ABb
Bb
Stem diameter (mm)
C)
D)
Aa
ABa
ABa
Ba
Ab
Ab
Ab
Bb
Number of leaves
E)
F)
Aa
Aa
Aa
Aa
Ab
Ab
Ab
Ab
Days after transplanting
40
60
80
100
G)
H)
RWC (%)
55 DAT
80 DAT
105 DAT
Aa
Ab
Aa
Bb
ABa
ABa
Aa
Ba
Ba
Ab
Aa
ABa
Ba
Aa
ABb
Ba
Aa
Ab
ABa
Aa
ABa
Aa
Aa
Bb
Treatments
0
20
40
60
80
100
120

Figure 2.

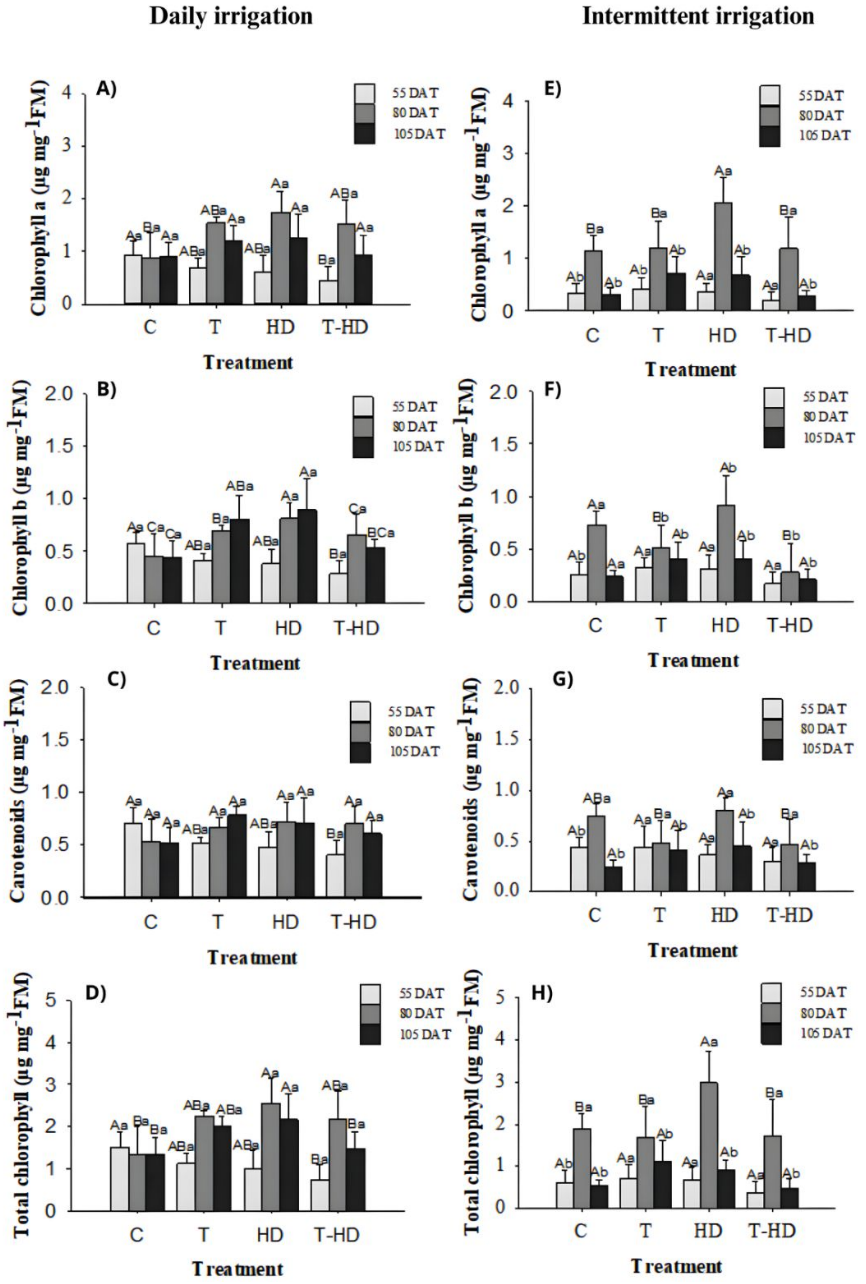

Daily irrigation
Intermittent irrigation
A)
Chlorophyll a (μg mg-1FM)
0
1
2
3
4
55 DAT
80 DAT
105 DAT
Aa
Ba
Aa
ABa
ABa
Aa
ABa
Aa
Aa
Ba
ABa
Aa
C
T
HD
T-HD
Treatment
E)
Chlorophyll a (μg mg-1FM)
Ab
Ba
Ab
Ab
Ba
Ab
Ab
Aa
Ab
Aa
Ba
Ab
B)
Chlorophyll b (μg mg-1FM)
0.0
0.5
1.0
1.5
2.0
Aa
Ca
Ca
ABa
Ba
ABa
ABa
Aa
Aa
Ba
Ca
BCa
F)
Chlorophyll b (μg mg-1FM)
Ab
Aa
Aa
Aa
Bb
Ab
Aa
Ab
Ab
Aa
Bb
Ab
C)
Carotenoids (μg mg-1FM)
Aa
Aa
Aa
ABa
Aa
Aa
ABa
Aa
Aa
Ba
Aa
Aa
G)
Carotenoids (μg mg-1FM)
Ab
ABa
Ab
Aa
Ba
Ab
Ab
Aa
Ab
Aa
Ba
Ab
D)
Total chlorophyll (μg mg-1FM)
0
1
2
3
4
5
Aa
Ba
Ba
ABa
ABa
ABa
ABa
Aa
Aa
Ba
ABa
Ba
H)
Total chlorophyll (μg mg-1FM)
Ab
Ba
Ab
Aa
Ba
Ab
Aa
Aa
Ab
Aa
Ba
Ab

Figure 3.

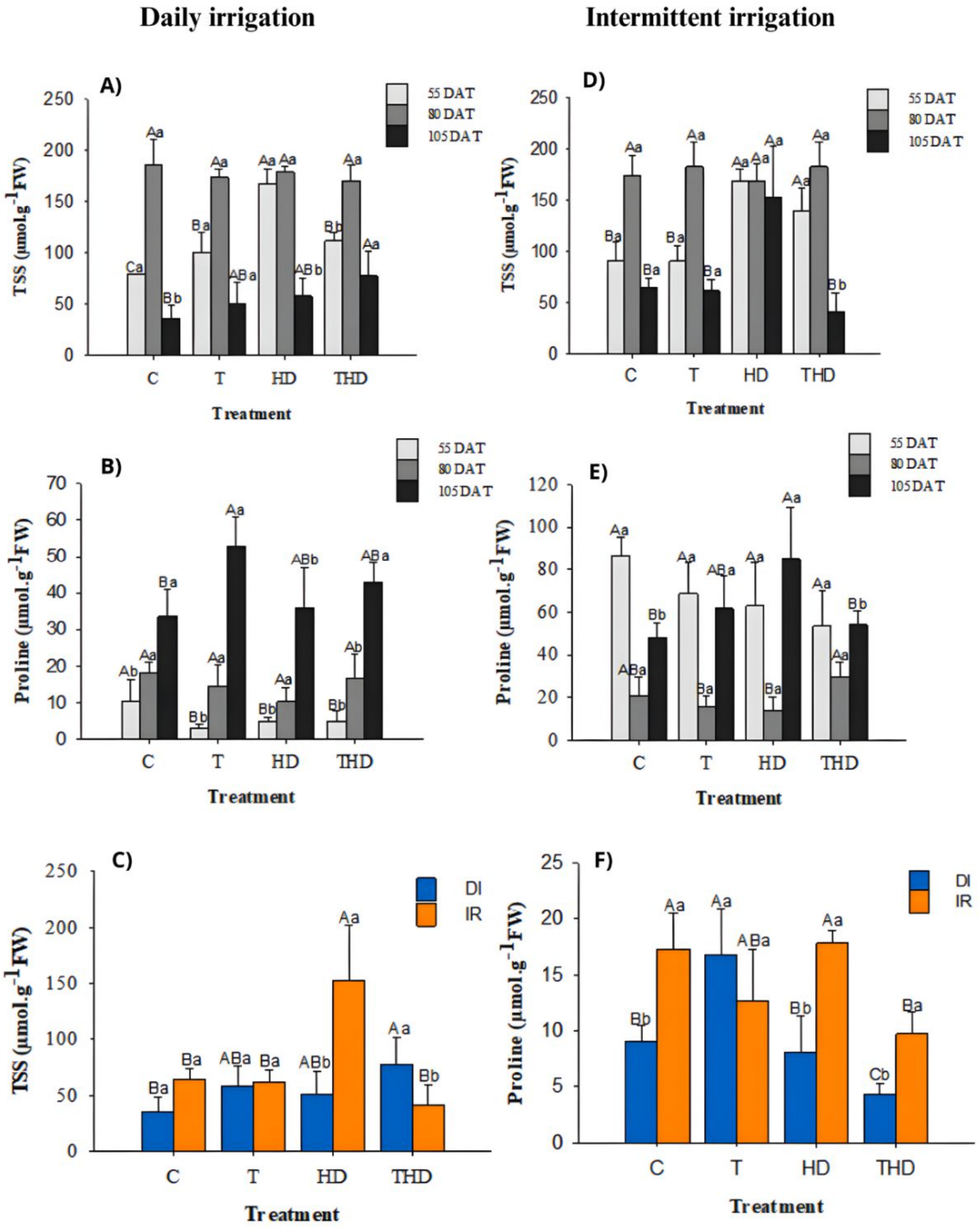

Daily irrigation
Intermittent irrigation
A)
TSS (µmol.g-1FW)
0
50
100
150
200
250
55 DAT
80 DAT
105 DAT
Ca
Aa
Bb
Ba
Aa
ABa
Aa
Aa
ABb
Bb
Aa
Aa
C
T
HD
THD
Treatment
D)
TSS (µmol.g-1FW)
Ba
Aa
Ba
Ba
Aa
Ba
Aa
Aa
Aa
Aa
Aa
Bb
C
T
HD
THD
Treatment
B)
Proline (µmol.g-1FW)
0
10
20
30
40
50
60
70
Ab
Aa
Ba
Bb
Aa
Aa
Bb
Aa
ABb
Bb
Ab
ABa
C
T
HD
THD
Treatment
E)
Proline (µmol.g-1FW)
0
20
40
60
80
100
120
Aa
ABa
Bb
Aa
Ba
ABa
Aa
Ba
Aa
Aa
Aa
Bb
C
T
HD
THD
Treatment
C)
TSS (µmol.g-1FW)
0
50
100
150
200
250
DI
IR
Ba
Ba
ABa
Ba
ABb
Aa
Aa
Bb
C
T
HD
THD
Treatment
F)
Proline (µmol.g-1FW)
0
5
10
15
20
25
DI
IR
Bb
Aa
Aa
ABa
Bb
Aa
Cb
Ba
C
T
HD
THD
Treatment

Figure 4.

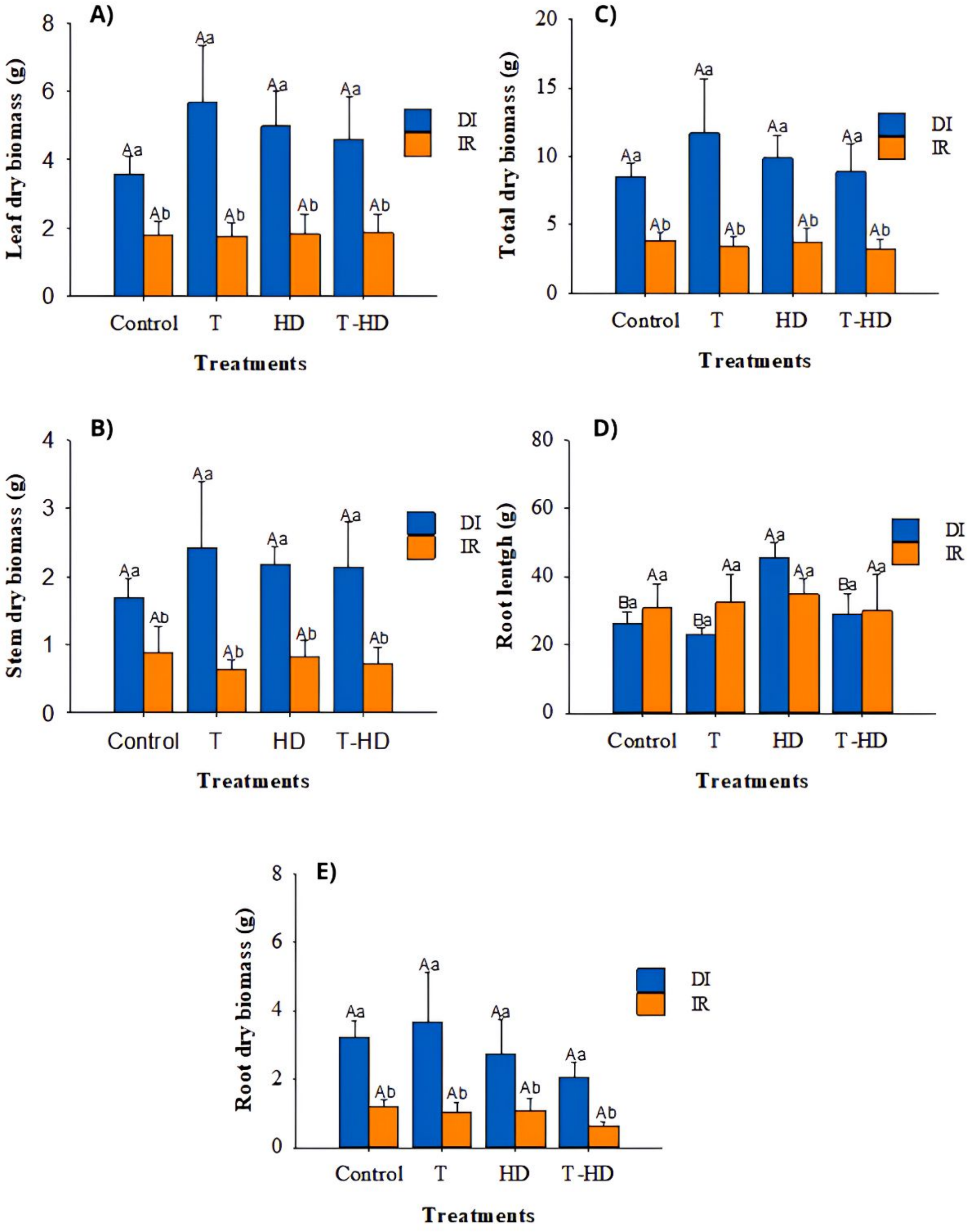

A)
Leaf dry biomass (g)
0
2
4
6
8
Aa
Ab
Control
T
HD
T-HD
Treatments
DI
IR
C)
Total dry biomass (g)
0
5
10
15
20
Aa
Ab
Control
T
HD
T-HD
Treatments
DI
IR
B)
Stem dry biomass (g)
0
1
2
3
4
Aa
Ab
Control
T
HD
T-HD
Treatments
DI
IR
D)
Root length (g)
0
20
40
60
80
Ba
Aa
Control
T
HD
T-HD
Treatments
DI
IR
E)
Root dry biomass (g)
0
2
4
6
8
Aa
Ab
Control
T
HD
T-HD
Treatments
DI
IR

Figure 5.

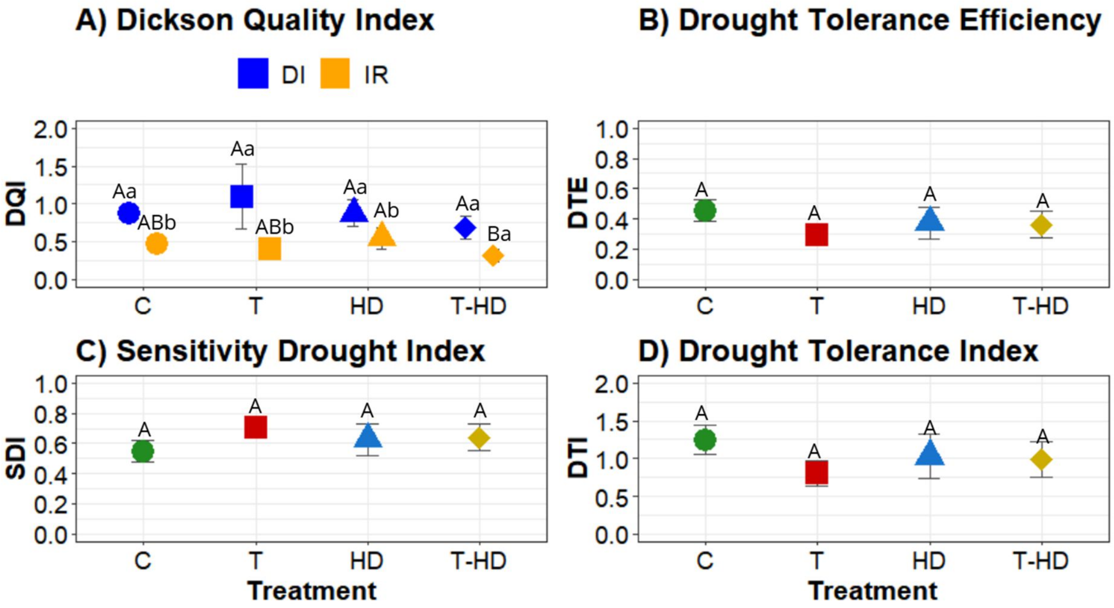

A) Dickson Quality Index
DI
IR
DQI
2.0
1.5
1.0
0.5
0.0
Aa
ABb
Aa
ABb
Aa
Ab
Aa
Ba
C
T
HD
T-HD
B) Drought Tolerance Efficiency
DTE
1.0
0.8
0.6
0.4
0.2
0.0
A
A
A
A
C
T
HD
T-HD
C) Sensitivity Drought Index
SDI
1.0
0.8
0.6
0.4
0.2
0.0
A
A
A
A
C
T
HD
T-HD
Treatment
D) Drought Tolerance Index
DTI
2.0
1.5
1.0
0.5
0.0
A
A
A
A
C
T
HD
T-HD
Treatment

Figure 6.

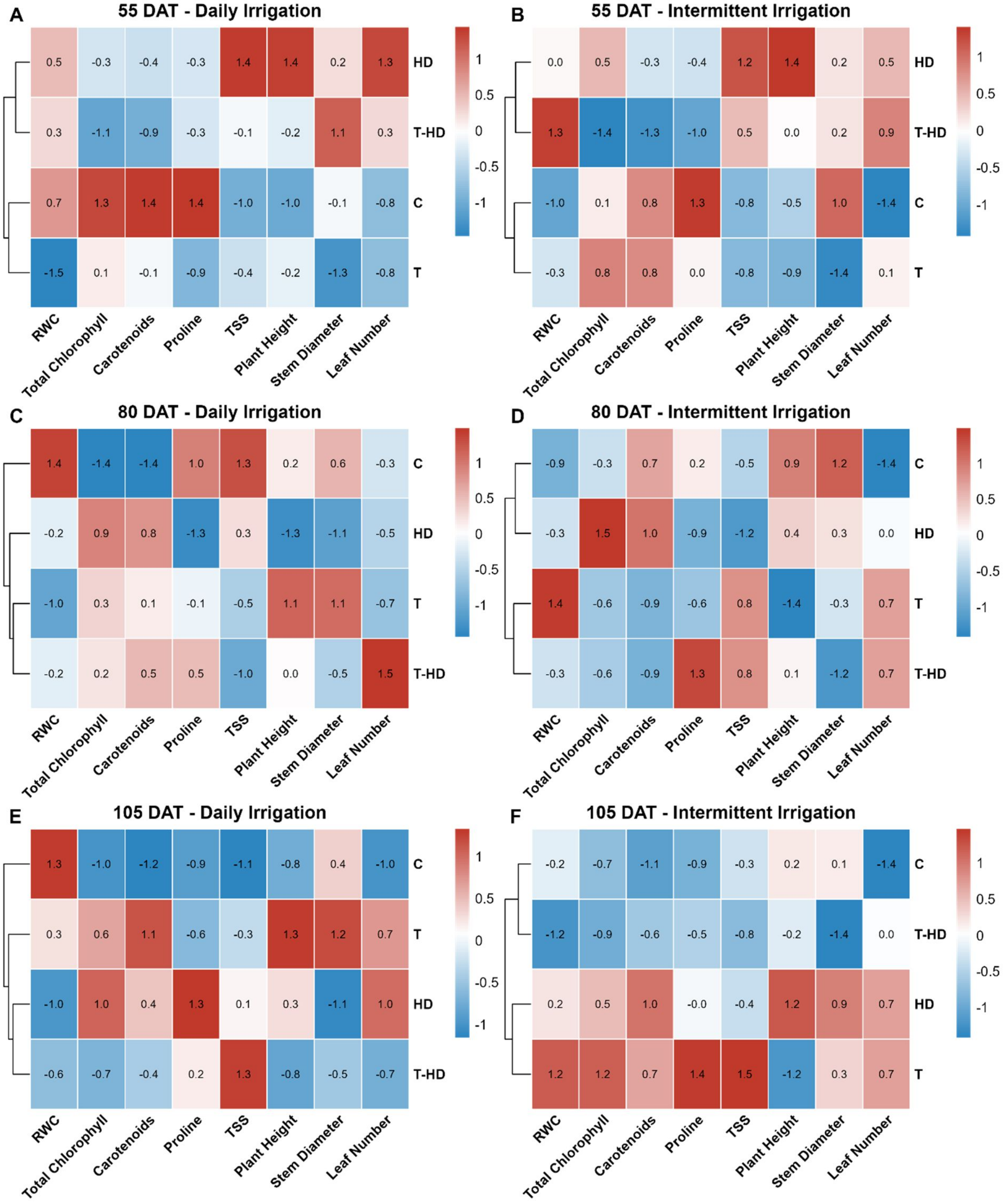
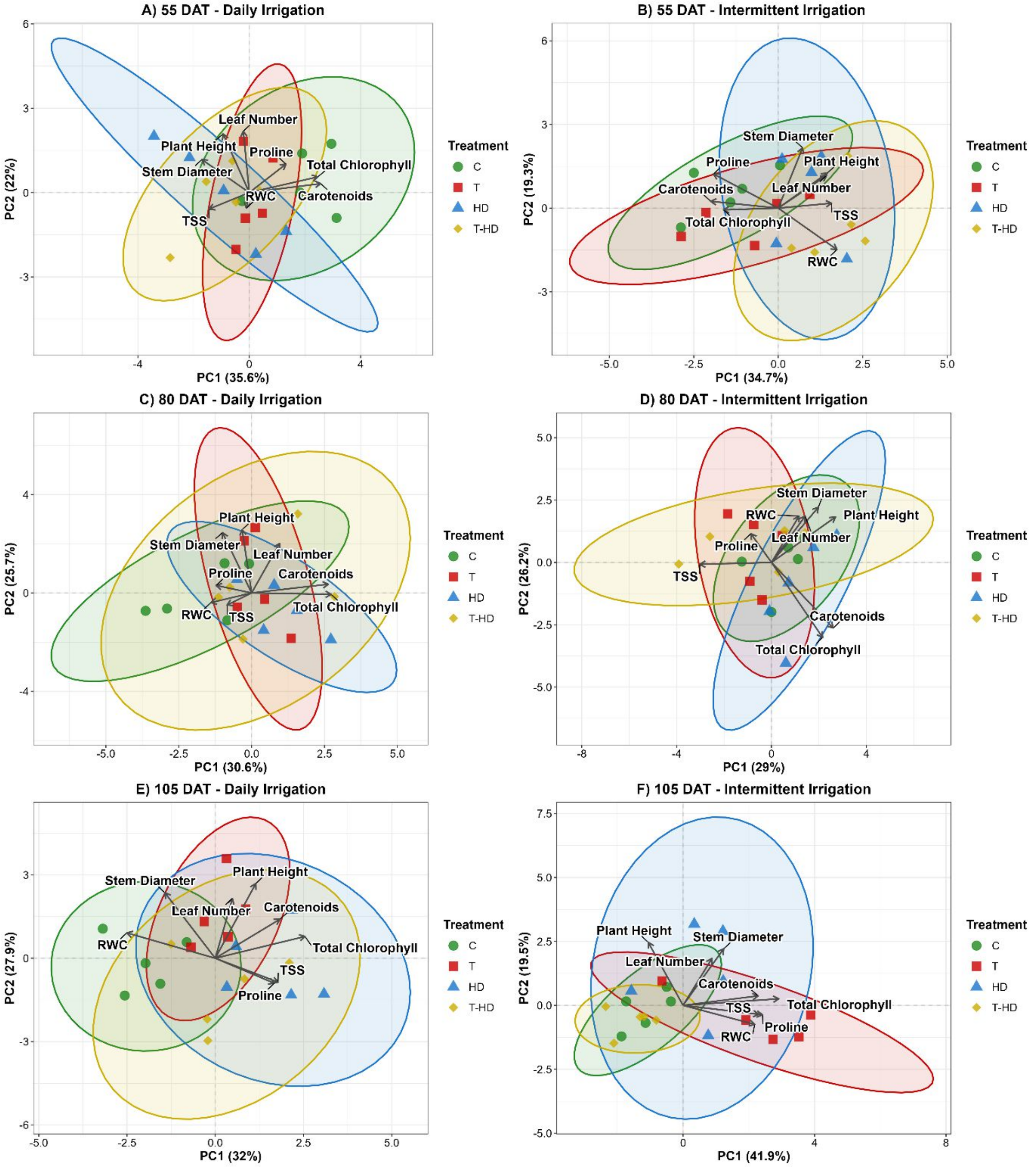

A
55 DAT - Daily Irrigation
HD 0.5 -0.3 -0.4 -0.3 1.4 1.4 0.2 1.3
T-HD 0.3 -1.1 -0.9 -0.3 -0.1 -0.2 1.1 0.3
C 0.7 1.3 1.4 1.4 -1.0 -1.0 -0.1 -0.8
T -1.5 0.1 -0.1 -0.9 -0.4 -0.2 -1.3 -0.8
1
0.5
0
-0.5
-1
RWC
Total Chlorophyll
Carotenoids
Proline
TSS
Plant Height
Stem Diameter
Leaf Number
B
55 DAT - Intermittent Irrigation
HD 0.0 0.5 -0.3 -0.4 1.2 1.4 0.2 0.5
T-HD 1.3 -1.4 -1.3 -1.0 0.5 0.0 0.2 0.9
C -1.0 0.1 0.8 1.3 -0.8 -0.5 1.0 -1.4
T -0.3 0.8 0.8 0.0 -0.8 -0.9 -1.4 0.1
1
0.5
0
-0.5
-1
RWC
Total Chlorophyll
Carotenoids
Proline
TSS
Plant Height
Stem Diameter
Leaf Number
C
80 DAT - Daily Irrigation
C 1.4 -1.4 -1.4 1.0 1.3 0.2 0.6 -0.3
HD -0.2 0.9 0.8 -1.3 0.3 -1.3 -1.1 -0.5
T -1.0 0.3 0.1 -0.1 -0.5 1.1 1.1 -0.7
T-HD -0.2 0.2 0.5 0.5 -1.0 0.0 -0.5 1.5
1
0.5
0
-0.5
-1
RWC
Total Chlorophyll
Carotenoids
Proline
TSS
Plant Height
Stem Diameter
Leaf Number
D
80 DAT - Intermittent Irrigation
C -0.9 -0.3 0.7 0.2 -0.5 0.9 1.2 -1.4
HD -0.3 1.5 1.0 -0.9 -1.2 0.4 0.3 0.0
T 1.4 -0.6 -0.9 -0.6 0.8 -1.4 -0.3 0.7
T-HD -0.3 -0.6 -0.9 1.3 0.8 0.1 -1.2 0.7
1
0.5
0
-0.5
-1
RWC
Total Chlorophyll
Carotenoids
Proline
TSS
Plant Height
Stem Diameter
Leaf Number
E
105 DAT - Daily Irrigation
C 1.3 -1.0 -1.2 -0.9 -1.1 -0.8 0.4 -1.0
T 0.3 0.6 1.1 -0.6 -0.3 1.3 1.2 0.7
HD -1.0 1.0 0.4 1.3 0.1 0.3 -1.1 1.0
T-HD -0.6 -0.7 -0.4 0.2 1.3 -0.8 -0.5 -0.7
1
0.5
0
-0.5
-1
RWC
Total Chlorophyll
Carotenoids
Proline
TSS
Plant Height
Stem Diameter
Leaf Number
F
105 DAT - Intermittent Irrigation
C -0.2 -0.7 -1.1 -0.9 -0.3 0.2 0.1 -1.4
T-HD -1.2 -0.9 -0.6 -0.5 -0.8 -0.2 -1.4 0.0
HD 0.2 0.5 1.0 -0.0 -0.4 1.2 0.9 0.7
T 1.2 1.2 0.7 1.4 1.5 -1.2 0.3 0.7
1
0.5
0
-0.5
-1
RWC
Total Chlorophyll
Carotenoids
Proline
TSS
Plant Height
Stem Diameter
Leaf Number

Figure 7.


A) 55 DAT - Daily Irrigation
PC1 (35.6%)
PC2 (22%)
Leaf Number
Plant Height
Proline
Total Chlorophyll
Stem Diameter
RWC
Carotenoids
TSS
B) 55 DAT - Intermittent Irrigation
PC1 (34.7%)
PC2 (19.3%)
Stem Diameter
Proline
Plant Height
Carotenoids
Leaf Number
TSS
Total Chlorophyll
RWC
C) 80 DAT - Daily Irrigation
PC1 (30.6%)
PC2 (25.7%)
Plant Height
Stem Diameter
Leaf Number
Proline
Carotenoids
Total Chlorophyll
RWC
TSS
D) 80 DAT - Intermittent Irrigation
PC1 (29%)
PC2 (26.2%)
Stem Diameter
RWC
Plant Height
Leaf Number
Proline
TSS
Carotenoids
Total Chlorophyll
E) 105 DAT - Daily Irrigation
PC1 (32%)
PC2 (27.9%)
Stem Diameter
Plant Height
Leaf Number
Carotenoids
RWC
Total Chlorophyll
TSS
Proline
F) 105 DAT - Intermittent Irrigation
PC1 (41.9%)
PC2 (19.5%)
Plant Height
Stem Diameter
Leaf Number
Carotenoids
TSS
Total Chlorophyll
Proline
RWC
Treatment
C
T
HD
T-HD

7. CONSIDERAÇÕES FINAIS

Em ambientes sazonalmente secos como a Caatinga, o início da vida vegetal é atravessado por sinais ambientais intermitentes e, muitas vezes, contraditórios: disponibilidade hídrica descontínua e pulsos de altas temperaturas na superfície do solo. Ao investigar a espécie *Cenostigma pyramidale* (Catingueira) ao longo do contínuo semente–plântula, esta dissertação evidencia que a resposta da espécie a esses sinais não se resume a "tolerar" ou "não tolerar" o estresse. O que se observa é a atuação de uma plasticidade fina, na qual o momento e a combinação das experiências pré-germinativas modulam a forma como a planta organiza sua germinação, seu crescimento inicial e, sobretudo, sua manutenção funcional diante da seca.

O conjunto dos resultados sustenta que a hidratação descontínua pode operar como um gatilho de ajustes persistentes, mas dependente do estágio fisiológico da semente. Quando os ciclos ocorrem em fase inicial de embebição, predominam respostas de incrementos germinativos como aumento da germinabilidade e aceleração do processo, ainda que, em alguns cenários, com perda de sincronia. Quando a desidratação ocorre em fases mais avançadas, o mesmo tipo de estímulo passa a carregar maior custo, com redução de desempenho germinativo e redução de componentes do crescimento inicial, sugerindo que há janelas fisiológicas em que o sinal é informativo e janelas em que ele pode atuar provocando maior sensibilização do organismo. Assim, a memória de estresse associada à hidratação descontínua não é um efeito universal, podendo ser condicionada pelo timing e pela intensidade.

Em paralelo, a exposição a pulsos de alta temperatura revelou uma característica ecologicamente importante: as sementes apresentam elevada resiliência térmica, preservando a capacidade de germinar, mesmo após passar por condições extremas. Ainda assim, esse calor não é neutro, pois possui influência sobre a cinética germinativa, retardando a progressão temporal e reduzindo taxa e sincronia quando a intensidade/duração se eleva, além de indicar a existência de limiares além dos quais a performance declina. Esses resultados contribuem para compreender por que a espécie é capaz de persistir em mosaicos microclimáticos extremos, sem que isso signifique ausência de custo fisiológico em situações mais severas.

Quando essas experiências prévias são colocadas "à prova" sob restrição hídrica, emerge um ponto central da dissertação: o estresse antecedente não altera apenas quanto germina, mas como germina, e essa organização temporal pode ter valor adaptativo em sistemas imprevisíveis. A hidratação descontínua, em diferentes cenários, esteve associada a germinação mais rápida e concentrada; já a combinação entre calor e hidratação descontínua, em determinadas condições, favoreceu maior espalhamento temporal da germinação e manutenção relativa do desempenho sob potenciais osmóticos mais negativos. Essa dualidade é relevante porque sugere que *C. pyramidale* pode expressar, conforme os sinais percebidos e nas condições experimentais, estratégias distintas: em alguns contextos, "aproveitar" rapidamente janelas de umidade; em outros, reduzir risco distribuindo eventos germinativos no tempo.

Na fase de plântulas, sob seca prolongada e recorrente, a mensagem integradora torna-se ainda mais clara: o regime hídrico domina crescimento e biomassa, e os ganhos associados à memória de estresse nem sempre se traduzem em maior acúmulo estrutural. Em vez disso, os resultados apontam que a expressão mais consistente da memória ocorre ao longo do eixo da resiliência fisiológica, por meio da manutenção e recuperação do status hídrico em momentos-chave, preservação do aparato fotossintético, acúmulo de solutos compatíveis e ajustes em traços relacionados à aquisição de água (como maior comprimento radicular em condições de estresse). Esses sinais convergem para a interpretação de que, sob seca severa e repetida, *C. pyramidale* tende a priorizar a estabilidade funcional e a capacidade de retomada quando a água retorna, mais do que maximizar crescimento. Isso corrobora a ideia de que, em ambientes semiáridos, "tolerância" não é sinônimo de produzir mais biomassa, mas de evitar colapso fisiológico e manter margem de recuperação.

Ao mesmo tempo, o conjunto dos dados também delimita limites, pois a combinação de múltiplos sinais pré-germinativos podem impor custos. Em situações específicas, a exposição conjunta a calor e hidratação descontínua não ampliou vantagens e esteve associada a penalidades estruturais sob estresse recorrente, sugerindo que a integração de estímulos não é necessariamente sinérgica. Essa evidência é valiosa porque evita uma conclusão simplificadora do tipo "quanto mais estresse, melhor", e reforça uma visão mais realista, na qual a memória de estresse é um recurso adaptativo, mas também exige custos a depender do contexto.

Do ponto de vista científico, esta dissertação contribui ao demonstrar, de forma integrada, que a resposta de *C. pyramidale* aos filtros ambientais da Caatinga envolve (i) sensibilidade ao momento em que a hidratação descontínua, (ii) robustez térmica com limiares fisiológicos detectáveis, e (iii) expressão de memória mais evidente em atributos funcionais do que em métricas clássicas baseadas apenas em crescimento. Do ponto de vista aplicado, os resultados dão suporte ao uso criterioso de tratamentos pré-germinativos como ferramenta de manejo fisiológico na produção de sementes e mudas para restauração, ao demonstrar que simular estresses brandos pode modular desempenho, mas a definição de intensidade, ordem e janela fisiológica é determinante para evitar custos desnecessários.

Como perspectivas futuras, faz-se necessário a agenda conectar padrão e mecanismo, por meio de análises hormonais, marcadores de estresse oxidativo, integridade de membranas, rotas de osmoproteção, proteínas associadas ao calor e possíveis assinaturas epigenéticas. Além disso, testar a estabilidade dessas respostas em condições mais próximas do campo, com variação térmica diária, solos reais, pulsos de chuva irregulares e múltiplos estresses combinados. Dessa forma, será possível traduzir, com maior precisão, essa plasticidade em recomendações robustas para conservação e restauração.

Em síntese, os resultados aqui reunidos mostram que *Cenostigama pyramidale* não responde ao semiárido apenas pela resistência; responde também pela capacidade de ajustar ritmo, prioridade e estratégia. Essas características explicam a sua alta distribuição na Caatinga, demonstrando que em um ambiente onde a disponibilidade hídrica é estacional e o calor é constante, sobreviver pode depender menos de crescer sempre e mais de estar fisiologicamente pronto para recomeçar quando a oportunidade aparece.