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CENTRO DE CIÊNCIAS AGRÁRIAS
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SABRINA KELLY DOS SANTOS

BIORREGULADORES NA MITIGAÇÃO DO ESTRESSE HÍDRICO E NO PERFIL DE ÓLEOS ESSENCIAIS EM COENTRO-MARANHÃO (*Eryngium foetidum* L.)

AREIA
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RESUMO GERAL

Com o objetivo de avaliar a ação de biorreguladres na morfofisiologia e o perfil de óleos essenciais em plantas de *Eryngium foetidum*, quatro experimentos foram conduzidos. No primeiro, duas concentrações de carnitina foram aplicadas em plantas de coentro-maranhão (100 µM e 10 mM), e após 72 dias, análises de crescimento, conteúdo fotossintético, parâmetros de trocas gasosas e fluorescência da clorofila *a*, foram mensuradas. No segundo experimento, 100 µM de carnitina foi aplicado nas plantas de coentro-maranhão em diferentes condições hídricas: bem-irrigado, seca e re-irrigação. No terceiro experimento 100 µM de ácido ascórbico foi aplicado nas plantas de coentro-maranhão sob seca. E no quarto experimento foi aplicado 100 µM de ácido salicílico nas plantas, também em diferentes condições hídricas (bem-irrigado, seca e re-irrigação). Após 100 dias, análises morfológicas, fisiológicas e perfil de óleos essenciais foram realizadas. A concentração 100 µM de carnitina aumentou o crescimento das plantas de coentro-maranhão em condições não-estressantes e 10 mM não alterou o crescimento. As condições hídricas e a aplicação de 100 µM de carnitina alteraram o perfil de óleos essenciais das plantas de coentro-maranhão. A produção de eríngio foi aumentada na seca, bem como na re-irrigação com a aplicação de carnitina, e a hidroquinona foi produzida apenas na combinação re-irrigação e carnitina. O ácido ascórbico, em condições sem estresse, restaurou a concentração de pigmentos, e na seca, modulou qualitativamente o perfil de óleos essenciais, sendo o dodecenal o composto que mais contribuiu para separação do perfil. O ácido salicílico não mitigou os efeitos da seca, mas em condições bem-irrigadas e re-irrigadas aumentou área foliar e pigmentos fotossintéticos. Além disto, a aplicação do ácido salicílico na seca modulou o perfil de óleos essenciais. Esses resultados servem como base para outros estudos sobre o uso exógeno dos biorreguladores carnitina, ácido ascórbico e ácido salicílico em plantas de coentro-maranhão, além de fornecer informações sobre a relação entre diferentes condições hídricas e os biorreguladores e fornecer novas perspectivas para explorar a produção de compostos de interesse industrial e econômico.

Palavras-chave: bioestimulante; estresse abiótico; metabólitos secundários; planta alimentícia não-convencional.

ABSTRACT

Aiming to evaluate the action of bioregulators on the morphophysiology and profile of essential oils in *Eryngium foetidum* plants, four experiments were conducted. In the first, two concentrations of carnitine were applied in culantro plants (100 µM and 10 mM), and after 72 days, analyzes of growth, photosynthetic content, gas exchange parameters and chlorophyll *a* fluorescence were measured. In the second experiment, 100 µM of carnitine was applied in culantro plants under different water conditions: well-watered, drought and re-watered. In the third experiment, 100 µM of ascorbic acid was applied in culantro plants under drought. In the fourth experiment, 100 µM of salicylic acid was applied in the plants, also in different water conditions (well-watered, drought and re-watered). After 100 days, morphological, physiological and essential oil profile analyzes were performed. 100 µM of carnitine increased growth of culantro plants under non-stressful conditions and 10 mM did not alter growth. Water conditions and application 100 µM of carnitine changed the essential oil profile of the culantro plants. Eryngial production was increased in drought as well as in re-watered with the application of carnitine, and hydroquinone was produced only in the combination of re-watering and carnitine. Ascorbic acid, under non-stress conditions, restored pigment concentration, and drought conditions, it modulated qualitatively the essential oil profile, with dodecenal being the compound that most contributed to this profile separation. Salicylic acid application did not mitigate the effects of drought, but under well-irrigated and re-irrigated conditions increased leaf area and photosynthetic pigments. Furthermore, the exogenous application of salicylic acid in drought modulated the profile of essential oils. These results in culantro can serve as a basis for other studies about use the bioregulators carnitine, ascorbic acid and salicylic acid in culantro plants, in addition to providing information on the relation between different water conditions and bioregulators, providing new perspectives to explore production the compounds of industrial and economic interest.

Keywords: biostimulant; abiotic stress; secondary metabolites; non-conventional food plant.

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1 INTRODUÇÃO GERAL

As plantas estão expostas a diversos estresses ambientais em condições naturais e agrícolas, sendo o estresse hídrico o que mais afeta o desenvolvimento e o crescimento, e consequentemente, a produtividade das plantas (Seleiman et al., 2021). Cerca de 80-95% da biomassa fresca vegetal é constituída por água, que desempenha importante papel em diversos processos fisiológicos (Brodersen et al., 2019). Assim, a seca além de ser considerada o principal estresse ambiental é também uma ameaça à segurança alimentar mundial (Okorie et al., 2019; Diatta et al., 2020).

Aproximadamente 50% das perdas das colheitas são consequência de estresses abióticos, dos quais 10% são atribuídos à seca (Kajla et al., 2015). Estudos apontam que a seca causará diminuição em mais de metade das terras aráveis até 2050 (Kasim et al., 2013; Billah et al., 2021), enquanto a população mundial precisará de um fornecimento contínuo de alimentos (Gatehouse et al., 2011; Zia et al., 2021). Portanto, é de suma importância soluções para cultivar plantas com água limitada (Gregersen et al., 2013).

Algumas técnicas são utilizadas para mitigar os efeitos do estresse hídrico, como a aplicação de biorreguladores (Silva et al., 2023). Os biorreguladores são moléculas orgânicas de origem natural ou sintética que, em baixas concentrações, agem no metabolismo das plantas estimulando ou inibindo enzimas ou sistemas enzimáticos específicos que auxiliam a regulação do metabolismo vegetal (Bisht et al., 2018; Small e Degenhardt, 2018). Nos últimos anos, vem crescendo a utilização de biorreguladores visando otimizar a quantidade e a qualidade de diversas culturas (Tormena et al., 2022; Jain et al., 2023).

Os biorreguladores influenciam processos como divisão, alongamento e diferenciação celular, floração, frutificação e amadurecimento de frutos, melhora o crescimento das plantas e translocação de nutrientes, induz a tolerância das plantas contra os estresses ambientais, já que desencadeiam uma cascata de eventos metabólicos, resultando em alterações no desenvolvimento intracelular e no crescimento vegetal. Também podem regular a produção de metabólitos secundários nas plantas (Ratnakumar et al., 2016; Jamwal et al., 2018; Oh et al., 2020; Zulfiqar e Ashraf, 2021; Tormena et al., 2022; Gill et al., 2023). Exemplos de moléculas biorreguladoras são giberelina, etileno (Tormena et al., 2022), citocininas, ácido ascórbico, ácido salicílico (Jain et al., 2023), bem como alguns aminoácidos, a exemplo da arginina (Nargesi et al. 2022), prolina (Ami et al. 2020) e carnitina (Turk et al. 2019).

O ácido salicílico (SA) é uma molécula sinalizadora, reguladora de processos fisiológicos e bioquímicos como termogênese, fechamento estomático e defesa das plantas a estresses biótico e abiótico, envolvida na resistência sistêmica adquirida em vários tecidos vegetais (Wani et al., 2017; Zhang e Li 2019). O ácido ascórbico (AsA) é uma molécula não-enzimática essencial na eliminação da produção de espécies reativas de oxigênio (EROs) como o peróxido de hidrogênio (H_2O_2), no processo fotossintético, atuando como fotoprotetor na dissipação do excesso de energia luminosa (Maruta, 2022; Celi et al., 2023). A carnitina está envolvida no metabolismo energético, na ação hormonal e na adaptação ao estresse abiótico, e tem um papel fundamental no transporte de ácidos graxos de cadeia longa do citosol para a membrana mitocondrial (Jacques et al., 2018; Oney-Birol, 2019).

Os metabólitos secundários, característicos de plantas medicinais, são a base de diversos medicamentos farmacêuticos comerciais e fitoterápicos (Li et al., 2020). Alguns óleos essenciais, normalmente produzidos por plantas aromáticas, são produtos do metabolismo secundário e definidos como substâncias complexas com alto valor agregado devido às suas aplicações na indústria farmacêutica (Leitão et al., 2020; Rodrigues et al., 2022). A espécie *Eryngium foetidum* L., conhecida popularmente no Brasil como chicória-do-pará, coentrão e coentro-maranhão, é utilizada como condimento e medicinal, tem despertado o interesse de pesquisadores e da indústria devido à presença de óleos essenciais e suas características farmacológicas antifúngica, antibacteriana e antileishmanicida (Thomas et al., 2017; Leitão et al., 2020).

Além de interferir no crescimento das plantas, os fatores ambientais e as práticas de cultivo, como a utilização exógena de biorreguladores, também interferem na biossíntese de metabólitos secundários (Nasiri et al. 2018; Ali, 2021; Santos et al., 2023; Jalili et al., 2023). Diante do exposto, para testar as hipóteses de que os biorreguladores podem atenuar os efeitos do estresse hídrico, bem como modular o perfil de óleos essenciais em coentro-maranhão, objetivou-se avaliar a ação do ácido salicílico, ácido ascórbico e carnitina no crescimento, morfofisiologia e perfil de óleos essenciais em plantas dessa espécie sob condições normais e estresse hídrico.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar o efeito de biorreguladores vegetais no crescimento, morfofisiologia, e perfil de óleos essenciais em plantas de coentro-maranhão submetidas a diferentes condições hídricas.

2.2 OBJETIVOS ESPECÍFICOS

- Compreender as modificações morfofisiológicas em plantas coentro-maranhão sob condições de estresse hídrico;
- Determinar o efeito de concentrações contrastantes do aminoácido carnitina aplicado exogenamente em plantas de coentro-maranhão;
- Analisar o efeito dos biorreguladores ácido ascórbico, ácido salicílico e carnitina no crescimento, particionamento de biomassa e capacidade fotossintética de plantas de coentro-maranhão submetidas a estresse hídrico;
- Avaliar a capacidade dos biorreguladores ácido ascórbico, ácido salicílico e carnitina em mitigar os danos morfofisiológicos causados pelo estresse hídrico em plantas de coentro-maranhão;
- Avaliar o perfil de óleos essenciais em plantas de coentro-maranhão submetidas a diferentes condições hídricas e a aplicação dos biorreguladores ácido ascórbico, ácido salicílico e carnitina.

3 REVISÃO DE LITERATURA

3.1 PROCESSOS AFETADOS PELO ESTRESSE HÍDRICO

A seca fisiológica ocorre quando a planta não consegue obter água suficiente (Malinowska et al., 2020), e isto afeta os aspectos morfológicos, fisiológicos e bioquímicos das plantas, como germinação, altura da planta, comprimento e diâmetro da raiz, biomassa total, abertura estomática, conteúdo relativo de água, conteúdo de carotenoides, estabilidade da membrana, além de aumentar a quantidade de espécies reativas de oxigênio (EROs) (Patil et al., 2021; Wahab et al., 2022).

A água é fundamental no processo germinativo das sementes e a sua falta inibe a fase de embebição e, consequentemente, dificulta a germinação (Islam et al., 2018), além de reduzir o vigor das mudas (Wahab et al., 2022). A altura das plantas é outra característica afetada pela restrição hídrica, já que reduz o crescimento e aumenta a taxa de abscisão foliar (Liang et al., 2018). A arquitetura e morfologia do sistema radicular também são afetadas, já que as raízes sofrem alterações para aumentar a capacidade de absorção de água e nutrientes em condições de estresse hídrico (Dinneny 2019; Ghafar et al., 2021).

A redução do conteúdo relativo de água é um dos primeiros sinais de resposta ao estresse hídrico (Hussain et al., 2018). O conteúdo relativo de água das folhas tem relação com a taxa de crescimento do tecido foliar e a taxa de transpiração (Kapoor et al., 2020), isto porque a redução do conteúdo relativo de água, reduz o potencial hídrico foliar, causando o fechamento dos estômatos (Wahab et al., 2022).

A restrição hídrica é um dos principais fatores ambientais que impedem a fotossíntese (Wahab et al., 2022). O estresse hídrico influencia o conteúdo de clorofila *a*, *b* e carotenoides, bem como afeta os fotossistemas I e II (Ashrafi et al., 2022; Wahab et al., 2022). A clorofila é um componente essencial na fotossíntese, e em condições de estresse é afetado drasticamente devido ao aumento do estresse oxidativo, degeneração ou foto-oxidação (Allakhverdiev, 2020).

O estresse hídrico pode provocar mudanças na concentração, composição e distribuição dos metabólitos primários e secundários, aumentando a capacidade de sobrevivência das plantas em condições de estresse (Mundim e Prigle, 2018). Os metabólitos secundários, são compostos naturais que desempenham diversas funções, envolvendo o crescimento e o desenvolvimento das plantas em condições normais, mas também em condições de estresse, como um mecanismo de defesa e tolerância, já que mudanças no ambiente acarretam em alterações no metabolismo das plantas (Ncube e Van Staden, 2015; Islam et al., 2017; Mundim e Pringle, 2018).

3.2 RESPOSTAS BIOQUÍMICAS DAS PLANTAS ÀS CONDIÇÕES DE ESTRESSE HÍDRICO

O primeiro sinal de resposta de uma planta ao estresse hídrico é o fechamento dos estômatos, que é controlado principalmente por sinais químicos, como a produção de ácido abscísico (Pirasteh-Anosheh et al., 2016; Hsu et al., 2021). O fechamento estomático está mais relacionado ao teor de umidade do solo do que ao estado da água nas folhas, e é uma das mais importantes reações da planta ao estresse hídrico, pois evita a perda de água via transpiração, em contrapartida, a absorção de CO_2 diminui e mais elétrons ficam disponíveis para produzir as EROs (Pirasteh-Anosheh et al., 2016; Yang et al., 2021). Quando o estresse se torna mais severo, os estômatos se fecham progressivamente, chegando a ficar totalmente fechados em condições extremas de estresse hídrico (Islam et al., 2020; Wahab et al., 2022).

Durante a escassez hídrica, a planta é submetida a estresse oxidativo, resultando em alterações fisiológicas e bioquímicas que causam distúrbios metabólicos (Bashir et al., 2021). No entanto, em resposta, a planta produz solutos ou osmólitos, como prolina, fenol, ácido ascórbico, glicina betaína, açúcares e poliaminas que se acumulam, principalmente no citoplasma (Chan et al., 2013; Islam et al., 2018). Essas moléculas têm a capacidade de eliminar as EROs, aumentar o potencial hídrico e proteger as moléculas biológicas da peroxidação lipídica, atenuando assim, os efeitos deletérios e mantendo as relações hídricas (Bashir et al., 2021; Wahab et al., 2022).

Sob estresse abiótico, as plantas produzem um conjunto de EROs, causando desequilíbrio e disfunção no sistema defensivo (Bilska et al., 2019). As EROs consistem em espécies radicais não-livre como o H_2O_2, e radicais livres, tais como oxigênio singlete (O_2-•) hidroxila (OH•) e superóxido (OH_2•), que são prejudiciais às células das plantas, já que causam danos oxidativos às proteínas, DNA e lipídios, além de degradar as estruturas celulares quando produzidas em excesso (Bi et al, 2016; Choudhury et al., 2017). No entanto, através de vias antioxidantes enzimáticas e não enzimáticas, as plantas garantem a homeostase celular e minimizam os efeitos negativos da superprodução das EROs para proteger os sistemas celulares (Bilska et al., 2019). O sistema antioxidante enzimático inclui o superóxido dismutase, catalase, glutationa redutase, ascorbato peroxidase, enquanto o sistema antioxidante não-enzimático inclui ao principalmente compostos de baixo peso molecular e solúveis em água, como ascorbato e glutationa (Islam et al., 2018; Bashir et al., 2021).

3.3 ÁCIDO SALICÍLICO

O ácido salicílico (AS) é um derivado fenólico encontrado no reino vegetal, conhecido como regulador de processos morfológicos, fisiológicos e bioquímicos como, desenvolvimento, floração, fechamento dos estômatos, produção de clorofila, germinação de mudas, crescimento celular, nodulação em leguminosas e defesa tanto local, quanto na resistência sistêmica adquirida em condições de estresses biótico e abiótico (Miura e Tada, 2014; Wani et al., 2017). O AS também influencia isoprenoides, como a-Tocoferol, carotenoides e monoterpenos, principalmente em condições de estresse hídrico (Arndt et al., 2001).

A biossíntese do AS ocorre via chiquimato por duas rotas metabólicas, via fenilalanina e via isocorismato (Mishra e Baek, 2021). A rota da fenilalanina, ocorre no citoplasma, em que, primeiramente, a fenilalanina transforma-se em ácido trans-cinâmico, para depois haver a formação do AS; já a rota do isocorismato ocorre no cloroplasto, em que o isocorismato pode ser diretamente transformado em AS (Rekhter et al., 2019; Sharma et al., 2020). Após ser produzido, o AS se liga a proteínas regulatórias da família NPR (NPR1, NPR3 e NPR4), se translocando para o núcleo, ligando-se a fatores de transcrição (TGAs) para transdução de sinal, aumentando assim a expressão dos genes relacionados às repostas fisiológicas deste hormônio (Zhang et al., 2006; Ding et al., 2018; Liu et al., 2020).

A aplicação exógena de AS mostrou-se eficaz em atenuar os efeitos deletérios do estresse hídrico em diferentes espécies, como *Triticum aestivum* (Ahmad et al., 2021), *Ocimum basilicum* (Damalas, 2019), *Oryza sativa* (Sohag et al., 2020), *Mentha pulegium* (Azad et al., 2021) e *Raphanus sativus* (Henschel et al., 2022).

3.4 ÁCIDO ASCÓRBICO

O ácido ascórbico (AsA) é um antioxidante de baixo peso molecular, conhecido como vitamica C, que funciona como um importante componente na divisão e expansão celular, na eliminação não-enzimática das EROs, no crescimento das plantas e na tolerância ao estresse (Akram et al., 2017). AsA é um cofator de enzimas que regula a biossíntese de hormônios, contribuindo para modulação do metabolismo em plantas, além de controlar e eliminar concentrações de H_2O_2 ao utilizar o ascorbato peroxidase (APX) (Lu e Guo, 2020; El-Beltagi et al. 2022).

O AsA serve como um doador de elétrons específico para o APX na conversão do H_2O_2 em H_2O (Caverzan et al., 2012; Mellidou et al., 2021). O H_2O_2 é produzido durante fotossíntese e respiração, mas em condições de estresses, há a produção em excesso, causando danos aos lipídios, ácidos nucléicos e proteínas, acarretando em danos oxidativos, em distúrbios fisiológicos e morte celular prematura (Wang et al., 2013; Liu et al., 2019). O acúmulo do AsA na forma oxidada, que ocorre no apoplasto, auxilia a lignificação da parede celular e regula o crescimento em condições de estresse (Fotopoulos et al., 2006; Mellidou et al., 2021). A propriedade redutora do AsA ocorre devido a porção entre os carbonos C2 e C3 emitirem dois prótons e elétrons, tornando-se um grupo dicetona do ácido desidroascórbico (DHA) (Bilska et al., 2019).

Nas plantas, o AsA pode ser formado através de várias vias, incluindo as vias D-glicose, L-galactose, ácido urônico, L-gulose e mio-inositol, embora a L-galactose seja a principal via de biossíntese (Bulley e Laing, 2016; Mellidou et al., 2021). A síntese e regeneração de ascorbato são influenciados pela qualidade e quantidade da luz, especialmente a luz vermelha, assim o *pool* do ascorbato tem interação direta com as cadeias de transporte de elétrons da fotossíntese e respiratória (Foyer e Noctor, 2011; Liao et al.; 2023). Estudos têm mostrado a importância da aplicação do AsA em plantas para mitigar os efeitos do estresse abiótico, tais como *Zea mays* (Ghassemi et al., 2020), *Capsicum annuum* (Khazaei et al., 2020), *Cicer arietinum* (El-Beltagi et al., 2022), *Raphanus sativus* (Henschel et al., 2023a) e *Cucumis sativus* (Zahra et al., 2021).

3.5 CARNITINA

A carnitina é um aminoácido sintetizado a partir da L-lisina e L-metionina, de ocorrência em animais, plantas, bactérias, leveduras e fungos (Bourdin et al. 2007; Dayanand et al. 2011; Rippa et al. 2012). Nas plantas, a carnitina é encontrada na forma livre e esterificada, associada às vias anabólicas do metabolismo lipídico (Masterson e Wood 2000; Nguyen et al. 2016). Este aminoácido participa dos processos de biossíntese da membrana, do armazenamento de lipídios, do metabolismo energético e hormonal, além de participar da exportação de ácidos graxos de cadeia longa do cloroplasto, como cadeia longa de acilcarnitina, para entrar na mitocôndria para sofrer β-oxidação e participar na tolerância ao estresse oxidativo (Masterson e Wood 2000; Charrier et al. 2011; Oney-Birol 2019).

A β-oxidação é o processo pelo qual os ácidos graxos são degradados em suas unidades de acetil constituintes (Masterson e Wood 2009). Nas plantas, o papel biológico da β-oxidação, que ocorre nos peroxissomos e glioxissomos, é fornecer precursores biossintéticos que se originam de lipídios armazenados (Pan et al. 2019). A β-oxidação também tem importância durante a fase do crescimento vegetativo e crescimento reprodutivo, como desenvolvimento e germinação de sementes (Goepfert e Poirier 2007), desenvolvimento do embrião e da flor, e na síntese do ácido jasmônico e ácido indolecético (Pan et al., 2019).

A utilização exógena da carnitina pode auxiliar na síntese de lipídios que atuam no ajuste de sistemas de membranas em condições de estresse (Santos et al., 2023). Estudos mostraram a importância da aplicação da carnitina para atenuar os estresses abióticos, tais como o estresse salino em plântulas de *Arabidopsis* (Charrier et al., 2011), estresse salino em sementes de *Hordeum vulgare (*Oney-Birol 2019) e estresse hídrico em *Eruca sativa* (Santos et al., 2022), *Raphanus sativus* (Henschel et al., 2023b) e *Eryngium foetidum* (Santos et al., 2023).

3.6 *Eryngium foetidum*

Eryngium foetidum L., pertencente à família Apiaceae, é uma planta alimentícia não convencional (PANC), popularmente conhecida no Brasil como chicória-do-pará, coentro-maranhão, coentrão e chicória-da-Amazônia (Thomas et al., 2017; Rosero-Gómez et al., 2020). Sua propagação é via sementes ou por reaproveitamento de touceiras (Rodrigues et al., 2021). As folhas de *E. foetidum* são utilizadas na culinária como condimento, por possuírem aroma e sabor característico, sendo o eríngio, um aldeído alifático, o responsável por esta característica (QuynH e KuboTa, 2012; Rodrigues et al., 2021).

Esta espécie também é utilizada na medicina popular para tratamento de enfermidades do trato gastrointestinal, febre, cólica, além de atuar como anti-inflamatório, anti-helmíntico, analgésico, antibactericida e antileishmanicida (Rojas-Silva et al., 2014; Shavandi et al., 2012; Lingaraju et al., 2016). Nesta perspectiva, alguns estudos têm mostrado o potencial farmacológico de *E. foetidum* ao analisar os compostos bioativos, antioxidantes e perfil de óleos essenciais nela contidos (Sumitha et al., 2014; Leitão et al., 2020; Santos et al., 2023).

Conhecer a composição dos bioativos nos vegetais é importante devido a importância que eles possuem, já que estão associados a melhorias na saúde, diminuição do risco de desenvolvimento de doenças, além da importância que possuem para a indústria farmacêutica (Rodrigues et al., 2021). Os compostos bioativos são importantes para a saúde humana devido os efeitos moduladores nas atividades fisiológicas ou celulares, pois uma das principais ações biológicas desses compostos está relacionada à sua capacidade antioxidante de eliminar as EROs e as espécies reativas de nitrogênio (Pokimica et al., 2019; Leitão et al., 2020).

Dentre os compostos bioativos encontrados em *E. foetidum*, os óleos essenciais, são substâncias abundantes, com alto valor agregado, pois possuem potencial na indústria farmacêutica (Rodrigues et al., 2021; Rodrigues et al., 2022; Campos et al., 2023). O perfil químico dos óleos essenciais desta espécie se caracteriza pela presença de aldeídos aromáticos e alifáticos, dentre os quais se destacam ácido dodecanoico, E-2-dodecenal, ácido trans-2-dodecanoico, tetradecanal e (E)-2-tridecenal, encontrados em maiores quantidades nas folhas dessa espécie (Rodrigues et al., 2021; Silalahi, 2021).

REFERÊNCIAS

AHMAD, A.; ASLAM, Z.; NAZ, M.; HUSSAIN, S.; JAVED, T.; ASLAM, S.; JAMAL, M. A. Exogenous salicylic acid-induced drought stress tolerance in wheat (*Triticum aestivum* L.) grown under hydroponic culture. **PloS one**, v. 6, p. e0260556, 2021.

AKRAM, N. A.; SHAFIQ, F.; ASHRAF, M. Ascorbic Acid-A potential oxidant scavenger and its role in plant development and abiotic stress tolerance. **Frontiers in Plant Science**, v. 8, p. 613, 2017.

ALLAKHVERDIEV, S. I. Optimising Photosynthesis for Environmental Fitness. **Functional Plant Biology**, v. 47, p. iii-vii, 2020.

ALI, BARKET. Salicylic acid: An efficient elicitor of secondary metabolite production in plants. **Biocatalysis and Agricultural Biotechnology**, v. 31, p. 101884, 2021.

AMI, K.; PLANCHAIS, S.; CABASSA, C.; GUIVARC'H, A.; VERY, A. A.; KHELIFI, M.; CAROL, P. Different proline responses of two *Algerian durum* wheat cultivars to in vitro salt stress. **Acta Physiologiae Plantarum**, v. 42, n. 2, p. 21, 2020

ARNDT, S. K.; CLIFFORD, S. C.; WANEK, W.; JONES, H. G.; POPP, M. Physiological and morphological adaptations of the fruit tree *Ziziphus rotundifolia* in response to progressive drought stress. **Tree Physiology**, v. 21, p. 705–715, 2001

ASHRAFI, M.; AZIMI-MOQADAM, M. R.; MOHSENIFARD, E.; SHEKARI, F.; JAFARY, H.; MORADI, P.; PUCCI, M.; ABATE, G.; MASTINU, A. Physiological and Molecular Aspects of Two Thymus Species Differently Sensitive to Drought Stress. **BioTech**, v. 11, n. 2, p. 8, 2022.

AZAD, N.; REZAYIAN, M.; HASSANPOUR, H.; NIKNAM, V.; EBRAHIMZADEH, H. Physiological Mechanism of Salicylic Acid in *Mentha pulegium* L. under salinity and drought stress. **Brazilian Journal of Botany**, v. 44, p. 359-369, 202.

BILLAH, M.; AKTAR, S.; BRESTIC, M.; ZIVCAK, M.; KHALDUN, A. B. M.; UDDIN, M. S.; HOSSAIN, A. Progressive genomic approaches to explore drought-and salt-induced oxidative stress responses in plants under changing climate. **Plants**, v. 10, p. 1910, 2021.

BASHIR, S. S.; HUSSAIN, A.; HUSSAIN, S. J.; WANI, O. A.; ZAHID NABI, S.; DAR, N. A.; MANSOOR, S. Plant drought stress tolerance: Understanding its physiological, biochemical and molecular mechanisms. **Biotechnology & Biotechnological Equipment**, v. 35, p. 1912-1925, 2021.

BI, A.; FAN, J.; HU, Z.; WANG, G.; AMOMBO, E.; FU, J.; HU, T. Differential acclimation of enzymatic antioxidant metabolism and photosystem II photochemistry in tall fescue under drought and heat and the combined stresses. **Frontiers in Plant Science**, v. 7, p. 453, 2016.

BILSKA, K.; WOJCIECHOWSKA, N.; ALIPOUR, S.; KALEMBA, E. M. Ascorbic acid-The little-known antioxidant in woody plants. **Antioxidants**, v. 8, p. 645, 2019.

BISHT, T. S.; RAWAT, L.; CHAKRABORTY, B.; Yadav, V. A Recent Advances in Use of Plant Growth Regulators (PGRs) in Fruit Crops - A Review. **International Journal of Current Microbiology and Applied Sciences**. v. 7, p. 1307–1336, 2018.

BOURDIN, B.; ADENIER, H.; PERRIN, Y. Carnitine is associated with fatty acid metabolism in plants. **Plant Physiology Biochemistry**, v. 45, p. 926-931, 2007.

BRODERSEN, C. R.; RODDY, A. B.; WASON, J. W.; MCELRONE, A. J. Functional status of xylem through time. **Annual Review of Plant Biology**, v. 70, p. 407-433, 2019.

BULLEY, S.; AND LAING, W. The regulation of ascorbate biosynthesis. **Current Opinion in Plant Biology**, v. 33, p. 15–22, 2016.

CAMPOS, R. A.; SEABRA JÚNIOR, S.; GONÇALVES, G. G.; PONCE, S. F., MACEDO, A. C.; LIN, C. M.; LIMA, G. P. Physiological responses and antioxidant properties of spiny coriander (*Eryngium foetidum* L.) under shading and nitrogen fertilization. **Acta Physiologiae Plantarum**, v. 45, p. 111, 2023.

CAVERZAN, A.; PASSAIA, G.; ROSA, S. B.; RIBEIRO, C. W.; LAZZAROTTO, F.; MARGIS-PINHEIRO, M. Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection. **Genetics and molecular biology**, v. 35, p. 1011-1019, 2012.

CELI, G. E. A.; GRATÃO, P. L.; LANZA, M. G. D. B.; REIS, A. R. Physiological and biochemical roles of ascorbic acid on mitigation of abiotic stresses in plants. **Plant Physiology and Biochemistry,** 107970, 2023.

CHAN, K. X.; WIRTZ, M.; PHUA, S. Y.; ESTAVILLO, G. M.; POGSON, B. J. Balancing metabolites in drought: The sulfur assimilation conundrum. **Trends in Plant Science**, v. 18, p. 18-29, 2013.

CHARRIER, A.; RIPPA, S.; YU, A.; NGUYEN, P. J.; RENOU, J. P.; PERRIN, Y. () The effect of carnitine on *Arabidopsis* development and recovery in salt stress conditions. **Planta,** v. 235, p. 123-135, 2011.

CHOUDHURY, F. K.; RIVERO, R. M.; BLUMWALD, E.; MITTLER, R. Reactive oxygen species, abiotic stress and stress combination. **The Plant Journal,** v. 90, p. 856-867, 2017.

DAMALAS, C. A. Improving drought tolerance in sweet basil (*Ocimum basilicum*) with salicylic acid. **Scientia Horticulturae**, v. 246, p. 360-365, 2019.

DAYANAND, C. D.; KRISHNAMURTHY, N.; ASHAKIRAN, S.; SHASHIDHAR, K. N. Carnitine: a novel health factor–an overview. International Journal of Pharmaceutical and Biomedical Research, v. 2, p. 79-89, 2011.

DIATTA, A. A.; FIKE, J. H.; BATTAGLIA, M. L.; GALBRAITH, J.; BAIG, M. B. Effects of biochar on soil fertility and crop productivity in arid regions: A review. **Arabian Journal of Geosciences**, v. 13, p. 595, 2020.

DING, Y.; SUN, T.; AO, K.; PENG, Y.; ZHANG, Y.; LI, X.; ZHANG, Y. Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity. **Cell**, v. 173, p. 1454-1467, 2018

DINNENY, J. R. Developmental responses to water and salinity in root systems. **Annual Review of Cell and Developmental Biology**, v. 35, p. 239-257, 2019.

EL-BELTAGI, H. S.; SHAH, S.; ULLAH, S.; SULAIMAN, MANSOUR, A. T., & SHALABY, T. A. Impacts of ascorbic acid and alpha-tocopherol on Chickpea (*Cicer arietinum* L.) grown in water deficit regimes for sustainable production. **Sustainability**, v. 14, p. 8861, 2022.

FOTOPOULOS, V.; SANMARTIN, M.; KANELLIS, A.K. Effect of ascorbate oxidase over-expression on ascorbate recycling gene expression in response to agents imposing oxidative stress. **Journal of Experimental Botany**, v. 57, p. 3933–3943, 2006.

FOYER, C. H.; NOCTOR, G. Ascorbate and glutathione: The heart of the redox Hub. **Plant Physiology**, v. 155, p. 2–18, 2011.

GATEHOUSE, A.; FERRY, N.; EDWARDS, M.; Bell, H. Insect-resistant biotech crops and their impacts on beneficial arthropods Philos. Philosophical Transactions of the Royal Society B: Biological Sciences, v. 366, p. 1438-1452, 2011.

GHAFAR, M. A.; AKRAM, N. A.; SALEEM, M. H.; WANG, J.; WIJAYA, L.; ALYEMENI, M. N. Ecotypic Morphological and Physio-Biochemical Responses of Two Differentially Adapted Forage Grasses, *Cenchrus ciliaris* L. and *Cyperus arenarius* Retz. to Drought Stress. **Sustainability**, v. 13, p. 8069, 2021

GHASSEMI, A.; FARZANEH, S.; MOHARRAMNEJAD, S. Impact of ascorbic acid on seed yield and its components in sweet corn (*Zea mays* L.) under drought stress. **Journal of Plant Physiology and Breeding**, v. 10, p. 41-49, 2020

GILL, K.; KUMAR, P.; NEGI, S.; SHARMA, R.; JOSHI, A. K.; SUPRUN, I. I.; AL-NAKIB, E. A. Physiological perspective of plant growth regulators in flowering, fruit setting and ripening process in citrus. **Scientia Horticulturae**, v. 309, p. 111628, 2023.

GOEPFERT, S.; POIRIER, Y. β-Oxidation in fatty acid degradation and beyond. **Current Opinion in Plant Biology**, v. 10, p. 245–251, 2007

GREGERSEN, I. B.; SØRUP, H. J. D.; MADSEN H.; ROSBJERG D.; MIKKELSEN P. S.; ARNBJERG- NIELSEN, K. Assessing future climatic changes of rainfall extremes at small spatio-temporal scales. **Climatic change**, v. 118, p. 783-797, 2013

HENSCHEL, J. M.; DANTAS, E. F. O.; SOARES, V. A.; SANTOS, S. K.; SANTOS, L. W. O.; DIAS, T. J.; BATISTA, D. S. Salicylic acid mitigates the effects of mild drought stress on radish (*Raphanus sativus*) growth. **Functional Plant Biology**, v. 49, p. 822-831, 2022.

HENSCHEL, J. M.; DANTAS, E. F. O.; SOARES, V. A.; SANTOS, S. K.; GOMES, D. S.; FERREIRA, L. M., LOPES, A. S.; DIAS, T. J.; BATISTA, D. S. Drought stress mitigation by foliar application of L-carnitine and its effect on radish morphophysiology. **Physiology and Molecular Biology of Plants**, v. 29, p. 579-590, 2023b.

HENSCHEL, J. M.; SOARES, V. A.; FIGUEIREDO, M. C.; SANTOS, S. K.; DIAS, T. J.; BATISTA, D. S. Radish (*Raphanus sativus* L.) growth and gas exchange responses to exogenous ascorbic acid and irrigation levels. Vegetos, v. 36, p. 566-574, 2023a.

HSU, P. K.; DUBEAUX, G.; TAKAHASHI, Y.; SCHROEDER, J. I. Signaling mechanisms in abscisic acid-mediated stomatal closure. **The Plant Journal**, v. 105, p. 307-321. 2021.

HUSSAIN, S.; KHALID, M. F.; SAQIB, M.; AHMAD, S.; ZAFAR, W.; RAO, M. J.; MORILLON, R.; ANJUM, M. A. Drought Tolerance in Citrus Rootstocks Is Associated with Better Antioxidant Defense Mechanism. **Acta Physiologiae Plantarum**, v. 40, p. 135, 2018.

ISLAM, M. J.; HASSAN, M. K.; SARKER, S. R.; RAHMAN, A. B.; FAKIR, M. S. A. Light and temperature effects on sprout yield and its proximate composition and vitamin C content in lignosus and mung beans. **Journal of the Bangladesh Agricultural University**, v. 15, p. 248–254. 2017.

ISLAM, M. J.; KIM, J. W.; BEGUM, M. K.; SOHEL, M. A. T.; LIM, Y. S. Physiological and Biochemical Changes in Sugar Beet Seedlings to Confer Stress Adaptability under Drought Condition. **Plants**, v. 9, p. 1511, 2020.

ISLAM, M. M.; KAYESH, E.; ZAMAN, E.; URMI, T. A.; HAQUE, M. M. Evaluation of Rice (*Oryza sativa* L.) Genotypes for Drought Tolerance at Germination and Early Seedling Stage. **Agriculturists**, v. 16, p. 44-54, 2018

JAIN, S.; NIDHI, N.; KALE, S.; RATHOD, M.; DHURVE, L.; MEHARA, H.; BAIDYA, B. K. A Comprehensive Review on Role of Bio-Regulators in the Growth and Development of Fruit and Vegetable Crops. **International Journal of Environment and Climate Change**, v. 13, p. 2879-2892, 2023.

JALILI, I.; EBADI, A.; ASKARI, M. A.; KALATEHJARI, S.; AAZAMI, M. A. Foliar application of putrescine, salicylic acid, and ascorbic acid mitigates frost stress damage in *Vitis vinifera* cv. 'Giziluzum'. **BMC Plant Biology**, v. 23, p. 1-15, 2023.

JAMWAL, K.; BHATTACHARYA, S.; PURI, S.; Plant growth regulator mediated consequences of secondary metabolites in medicinal plants, **Journal of Applied Research on Medicinal and Aromatic Plants** v. 9, p. 26-38, 2018.

KAJLA, M., YADAV V.K.; KHOKHAR, J.; SINGH, S.; CHHOKAR, R.; Meena, R. P.; SHARMA, R. K.; Increase in wheat production through management of abiotic stresses: a review. **Journal of Applied and Natural Science**, v. 7, p. 1070-1080, 2015.

KAPOOR, D.; BHARDWAJ, S.; LANDI, M.; SHARMA, A.; RAMAKRISHNAN, M.; SHARMA, A. The Impact of Drought in Plant Metabolism: How to Exploit Tolerance Mechanisms to Increase Crop Production. **Applied Sciences**, v. 10, p. 5692, 2020.

KASIM, W. A.; OSMAN, M. E.; OMAR, M. N.; ABD EL-DAIM, I. A.; BEJAI, S.; MEIJER, J. Control of drought stress in wheat using plant-growth-promoting bacteria. **Journal of plant growth regulation**, v. 32, p. 122-130, 2013.

KHAZAEI, Z.; ESMAIELPOUR, B.; ESTAJI, A. Ameliorative effects of ascorbic acid on tolerance to drought stress on pepper (*Capsicum annuum* L) plants. **Physiology and Molecular Biology of Plants**, v. 26, p. 1649-1662, 2020.

LEITÃO, D. D. S. T. C.; SIQUEIRA, F. C.; SOUSA, S. H. B.; MERCADANTE, A. Z.; CHISTE, R. C.; LOPES, A. S. Amazonian *Eryngium foetidum* leaves exhibited very high contents of bioactive compounds and high singlet oxygen quenching capacity. **International Journal of Food Properties**, v. 23, p. 1452-1464, 2020.

LI, Y.; KONG, D.; FU, Y.; SUSSMAN, M. R.; WU, H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. **Plant Physiology and Biochemistry**, v. 148, p. 80-89, 2020.

LIAO, G.; XU, Q.; ALLAN, A. C.; XU, X. L-Ascorbic acid metabolism and regulation in fruit crops. **Plant Physiology**, v. 192, p. 1684-1695, 2023.

LIANG, B.; GAO, T.; ZHAO, Q.; MA, C.; CHEN, Q.; WEI, Z.; LI, C.; LI, C.; MA, F. Effects of Exogenous Dopamine on the Uptake, Transport, and Resorption of Apple Ionome under Moderate Drought. **Frontiers in Plant Science**, v. 9, p. 755, 2018.

LINGARAJU, D. P.; SUDARSHANA, M. S.; MAHENDRA, C.; RAO, K.P.; Phytochemical screening and antimicrobial activity of leaf extracts of *Eryngium foetidum* L. (Apiaceae). **Indo American Journal of Pharmaceutical Research**, v. 6, p. 4339-4344, 2016

LIU, G.; HUANG, Y.; ZHAI, L. Impact of nutritional and environmental factors on inflammation, oxidative stress, and the microbiome. **BioMed Research International**, v. 2018, 2018.

LIU, Y.; SUN, T.; SUN, Y.; ZHANG, Y.; RADOJIČIĆ, A.; DING, Y.; TIAN, H.; HUANG, X.; LAN, J.; CHEN, S.; ORDUNA, A. R.; ZHANG, K.; LI, X.; ZHANG, Y. Diverse roles of the salicylic acid receptors NPR1 and NPR3/NPR4 in plant immunity. **The Plant Cell**, v, 32, 4002-4016, 2020.

LU, Y.; GUO, X. The effect of light in vitamin C metabolism regulation and accumulation in mung bean (*Vigna radiata*) germination. **Plant Foods for Human Nutrition**, v. 75, p. 24-29, 2020.

MALINOWSKA, M.; DONNISON, I.; ROBSON, P. Morphological and Physiological Traits That Explain Yield Response to Drought Stress in Miscanthus. **Agronomy**, v. 10, p. 1194, 2020.

MARUTA, T. How does light acilitate vitamin C biosynthesis in leaves? **Bioscience, Biotechnology and Biochemistry**, v. 86, p. 1173-1182, 2022.

MASTERSON, C, WOOD, C. Influence of mitochondrial β-oxidation on early pea seedling development. **New Phytologist**, v. 181, p. 832-84, 2009.

MASTERSON, C.; WOOD C. Mitochondrial oxidation of fatty acids in higher plants. **Journal of Plant Physiology**, v. 109, p. 217-224, 2000.

MELLIDOU, I.; KOUKOUNARAS, A.; KOSTAS, S.; PATELOU, E.; KANELLIS, A. K. Regulation of vitamin C accumulation for improved tomato fruit quality and alleviation of abiotic stress. **Genes**, v. 12, p. 694, 2021.

MISHRA, A. K.; BAEK, K. H. Salicylic acid biosynthesis and metabolism: a divergent pathway for plants and bacteria. **Biomolecules**, v. 11, p. 705. 2021.

MIURA, K.; TADA, Y. Regulation of water, salinity, and cold stress responses by salicylic acid. **Frontiers in Plant Science**, v. 5, p. 70455, 2014.

MUNDIM, F. M.; PRINGLE, E. G. Whole-plant metabolic allocation under water stress. **Frontiers in Plant Science,** v. 9, p. 368590, 2018.

NARGESI, M.; SEDAGHATHOOR, S.M.; HASHEMABADI, D. S. Effect of arginine, glutamine, humic acid, and fulvic acid spraying on olive cultivars in saline conditions. **Plant Physiology Reports**, v. 27, p. 295-307, 2022.

NASIRI, Y.; ZANDI, H.; MORSHEDLOO, M. R. Effect of salicylic acid and ascorbic acid on essential oil content and composition of dragonhead (*Dracocephalum moldavica* L.) under organic farming. **Journal of Essential Oil Bearing Plants**, v. 21, p. 362-373, 2018.

NCUBE, B.; VAN STADEN, J. Tilting plant metabolism for improved metabolite biosynthesis and enhanced human benefit. **Molecules** v. 20, p. 12698-12731, 2015.

NGUYEN, P. J.; RIPPA, S.; ROSSEZ, Y.; PERRIN, Y. Acylcarnitines participate in developmental processes associated to lipid metabolism in plants. **Planta**, v. 243, p. 1011-1022, 2016.

OH, M. H.; HONEY, S. H.; TAX, F. E. The control of cell expansion, cell division, and vascular development by brassinosteroids: a historical perspective. **International Journal of Molecular Sciences**, v. 21, p. 1743, 2020.

OKORIE, V. O.; MPHAMBUKELI, T. N.; AMUSAN, S. O. Exploring the political economy of water and food security nexus in BRICS. **Africa Insight,** v. 48, p. 21–38, 2019.

PAN, R.; LIU, J.; HU, J. Peroxisomes in plant reproduction and seed-related development. **Journal of Integrative Plant Biology**, v. 61, 784-802, 2019.

PATIL, S. V.; PATIL, C. D.; MOHITE, B. V. Isolation and screening of ACC deaminase-producing microbes for drought stress management in crops. **Practical Handbook on Agricultural Microbiology**, p. 361-367, 2021.

PIRASTEH-ANOSHEH, H.; SAED-MOUCHESHI, A.; PAKNIYAT, H.; PESSARAKLI, M. Stomatal responses to drought stress. **Water stress and crop plants: A sustainable approach**, v. 1, p. 24-40, 2016.

POKIMICA, B.; GARCÍA-CONESA, M. T. Critical Evaluation of Gene Expression Changes in Human Tissues in Response to Supplementation with Dietary Bioactive Compounds: Moving Towards Better-quality Studies. **Nutrients,** v. 10, p. 807, 2018.

QUYNH, C. T. T.; KUBOTA, K. Aroma constituents and enzyme activities of Japanese long coriander leaves (Culantro, *Eryngium foetidum* L.). **Food Science and Technology Research,** v. 18, p. 287-294, 2012.

RATNAKUMAR, P.; KHAN, M. I. R.; MINHAS, P. S.; FAROOQ, M. A.; SULTANA, R.; PER, T. S.; DEOKATE, P. P.; KHAN, N. A.; SINGH, Y.; RANE, J. Can plant bio-regulators minimize crop productivity losses caused by drought, salinity and heat stress? An integrated review. **Journal of Applied Botany and Food Quality**, v. 89, p. 113-125 2016.

REKHTER, D.; LÜDKE, D.; DING, Y.; FEUSSNER, K.; ZIENKIEWICZ, K.; LIPKA, V.; WIERMER, M.; ZHANG, Y.; FEUSSNER, I. Isochorismate-derived biosynthesis of the plant stress hormone salicylic acid. **Science** v. 365, p. 498-502, 2019.

RIPPA, S.; ZHAO, Y.; MERLIER, F.; CHARRIER, A.; PERRIN, Y. The carnitine biosynthetic pathway in Arabidopsis thaliana shares similar features with the pathway of mammals and fungi. **Plant Physiology and Biochemistry**, v. 60, p. 109-114, 2012.

RODRIGUES, T. L. M.; CASTRO, G. L. S.; VIANA, R. G.; GURGEL, E. S. C.; SILVA, S. G.; OLIVEIRA, M. S.; ANDRADE, E. H. D. A. Physiological performance and chemical compositions of the *Eryngium foetidum* L. (Apiaceae) essential oil cultivated with different fertilizer sources. **Natural Product Research**, v. 35, p. 5544-5548, 2021.

RODRIGUES, T. L.; SILVA, M. E.; GURGEL, E. S.; OLIVEIRA, M. S.; LUCAS, F. C. *Eryngium foetidum* L. (Apiaceae): A literature review of traditional uses, chemical composition, and pharmacological activities. **Evidence-Based Complementary and Alternative Medicine**, v. 2022, 2022.

ROJAS-SILVA, P.; GRAZIOSE, R.; VESELY, B.; POULEV, A.; MBEUNKUI, F.; GRACE, M. H.; KYLE, D. E.; LILA, M. A.; RASKIN, I. Leishmanicidal activity of a daucane sesquiterpene isolated from *Eryngium foetidum*. **Pharmaceutical Biology**, v. 52, p. 398-401, 2014.

ROSERO-GÓMEZ, C. A.; ZAMBRANO, M. L.; GARCÍA, K. E.; VIRACOCHA, L. A. Nomenclatura y usos del culantro de monte (*Eryngium foetidum* L.) en la comunidad San Antonio de Padua, cantón Quinsaloma, Provincia de Los Ríos–Ecuador. **Boletín Latinoamericano y del Caribe de plantas medicinales y arómaticas**, v. 19, p. 334-343, 2020.

SANTOS, S. K.; SILVA GOMES, D.; OLIVEIRA, A. F. P.; SILVA, A. M. O.; MOURA, V. S.; GUSMÃO, M. H. A.; MATOS E. M.; VICCINI L. F.; GRAZUL, R. M.; HENSCHEL, J. M.; BATISTA, D. S. Water stress and exogenous carnitine on growth and essential oil profile of *Eryngium foetidum* L. 3 **Biotech**, v. 13, 328, 2023.

SANTOS, S. K.; SILVA GOMES, D.; SANTOS, L. W. O.; AZEVEDO SOARES, V.; DANTAS, E. F. O.; HENSCHEL, J. M.; BATISTA, D. S. Exogenous carnitine mitigates the deleterious effects of mild-water stress on arugula by modulating morphophysiological responses. **Journal of Plant Growth Regulation**, v. 42, p. 4073-4082, 2023.

SANTOS, S. K.; SILVA GOMES, D.; SANTOS, L. W. O.; SOARES, V. A.; DANTAS, E. F. O.; HENSCHEL, J. M.; BATISTA, D. S. Exogenous carnitine mitigates the deleterious effects of mild-water stress on arugula by modulating morphophysiological responses. **Journal of Plant Growth Regulation**, v. 42, 4073-4082, 2022

SELEIMAN, M. F.; AL-SUHAIBANI, N.; ALI, N.; AKMAL, M.; ALOTAIBI, M.; REFAY, Y.; DINDAROGLU, T.; ABDUL-WAJID, H. H.; BATTAGLIA, M. L. Drought stress impacts on plants and different approaches to alleviate its adverse effects. **Plants**, v. 10, p. 259, 2021.

SHARMA, A.; SIDHU, G. P. S.; ARANITI, F.; BALI, A. S.; SHAHZAD, B.; TRIPATHI, D. K.; BRESTIC, M.; SKALICKY, M.; LANDI, M. The role of salicylic acid in plants exposed to heavy metals. **Molecules**, v. 25, p. 540, 2020.

SHAVANDI, M. A.; HADDADIAN, Z.; ISMAIL, M. H. S. Eryngium foetidum L. Coriandrum sativum and *Persicaria odorata* L.: a review. **Journal of Asian Scientific Research**, v. 2, p. 410, 2012.

SILALAHI, M. Essential oils and uses of *Eryngium foetidum* L**. GSC Biological and Pharmaceutical Sciences**, v. 15, p. 289-294, 2021.

SILVA, A. L. J.; FERREIRA, V. M.; DOS SANTOS, C. M.; DOS SANTOS, J. V.; DE MOURA BARROS, J. M. T.; DA SILVA BARBOSA, W. S.; BARROZO, M. A. S.; JUSTINO, G. C. 24-Epibrassinolide promotes activation of physiological compensation mechanisms in response to drought stress and rehydration and improves yield in soybean**. Journal of Agronomy and Crop Science**, v. 209, p. 355-370, 2023.

SMALL, C. C.; DEGENHARDT, D. Plant growth regulators for enhancing revegetation success in reclamation: A review. **Ecological engineering**, v. 118, p. 43-51, 2018.

SOHAG A. A. M.; TAHJIB-UL-ARIF M.; BRESTIČ M.; AFRIN S.; SAKIL M. A.; HOSSAIN M. T.; HOSSAIN M. A.; HOSSAIN M. A. Exogenous salicylic acid and hydrogen peroxide attenuates drought stress in rice. **Plant Soil Environ**, v. 66, p. 7–13, 2020.

SUMITHA, K. V.; PRAJITHA, V.; SANDHYA, V. N.; ANJANA, S.; THOPPIL, J. E. Potential larvicidal principles in *Eryngium foetidum* L. (Apiaceae), an omnipresent weed, effective against Aedes albopictus skuse. **Journal of Essential Oil Bearing Plants**, v. 17, p. 1279-1286, 2014.

THOMAS, P.S.; ESSIEN, E.E.; NTUK, S.J.; CHOUDHARY, M.I. *Eryngium foetidum* L. essential oils: Chemical composition and antioxidant capacity. **Medicines** v. 4, p. 24, 2017.

TORMENA, C. D.; RUTLEDGE, D. N.; RAKOCEVIC, M.; BRUNS, R. E.; SCARMINIO, I. S.; MARCHEAFAVE, G. G.; PAULI, E. D. Exogenous application of bioregulators in *Coffea arabica* beans during ripening: Investigation of UV–Visible and NIR mixture design-fingerprints using AComDim-ICA. **Microchemical Journal**, v. 181, p. 107702, 2022.

TURK, H.; ERDAL, S.; DUMLUPINAR, R. Carnitine-induced physio-biochemical and molecular alterations in maize seedlings in response to cold stress. **Archives of Agronomy and Soil Science**, v. 66, p. 925-941, 2019.

WAHAB, A.; ABDI, G.; SALEEM, M. H.; ALI, B.; ULLAH, S.; SHAH, W.; MUMTAZ, S.; YASIN, G.; MURESAN, C. C.; MARC, R. A. Plants' physio-biochemical and phyto-hormonal responses to alleviate the adverse effects of drought stress: A comprehensive review. **Plants**, v. 11, p. 1620, 2022.

WANG, J.; ZHANG, Z.; HUANG, R. Regulation of ascorbic acid synthesis in plants. **Plant Signaling & Behavior**, v. 8, p. e24536, 2013.

WANI, A. B.; CHADAR, H.; WANI, A. H.; SINGH, S.; UPADHYAY, N. Salicylic acid to decrease plant stress. **Environmental Chemistry Letters**, v. 15, p. 101-123, 2017.

YANG, Y. J.; BI, M. H.; NIE, Z. F.; JIANG, H.; LIU, X. D.; FANG, X. W.; BRODRIBB, T. J. Evolution of stomatal closure to optimize water-use efficiency in response to dehydration in ferns and seed plants. **New Phytologist**, v. 230, p. 2001-2010, 2021.

ZAHRA, G.; MARJAN, M.; TAHER, B.; RANJBAR, M. E. Foliar application of ascorbic acid and gamma aminobutyric acid can improve important properties of deficit irrigated cucumber plants (*Cucumis sativus* cv. Us). **Gesunde Pflanzen**, v. 73, p. 77-84, 2021.

ZHANG, Y.; CHENG, Y. T.; QU, N.; ZHAO, Q.; BI, D.; LI, X. Negative regulation of defense responses in *Arabidopsis* by two NPR1 paralogs. **The Plant Journal**, v. 48, p. 647-656, 2006.

ZHANG, Y.; LI, X. Salicylic acid: biosynthesis, perception, and contributions to plant immunity. **Current Opinion in Plant Biology**, v. 50, p. 29-36, 2019.

ZIA, R.; NAWAZ, M. S.; SIDDIQUE, M. J.; HAKIM, S.; IMRAN, A. Plant survival under drought stress: Implications, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. **Microbiological research**, v. 242, p. 126626, 2021.

ZULFIQAR, F.; ASHRAF, M. Bioregulators: unlocking their potential role in regulation of the plant oxidative defense system. **Plant Molecular Biology**, v. 105, p. 11-41, 2021.

4 CAPÍTULO I: EXOGENOUS CARNITINE APPLICATION ENHANCES THE GROWTH OF CULANTRO (Eryngium foetidum) PLANTS

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Abstract

Carnitine is an amino acid that is common to all living organisms and is involved in energy metabolism and stress tolerance in plants. In this context, the exogenous supply of carnitine may affect lipid metabolism, and consequently, plant growth. *Eryngium foetidum* L. (culantro) is a perennial herb with culinary and traditional medicinal uses owing to its chemical composition, which is rich in bioactive compounds. Considering the importance of *E. foetidum* and the potential of carnitine to modulate energy metabolism in plants, we investigated if exogenous application of carnitine can modulate the morphophysiological development of culantro plants. Plants (57-d-old) were leaf sprayed with water (control), 100 µM carnitine, and 10 mM carnitine. At 72 days after sowing, growth, photosynthetic pigment content, gas exchange parameters, and chlorophyll *a* fluorescence were measured. Plants treated with 100 µM carnitine were taller, had greater leaf area, and higher biomass accumulation and allocation to roots compared to the control. Carnitine reduced the maximum fluorescence and quantum efficiency of PSII, but did not affect gas exchange parameters. Here, 100 µM carnitine improved plant development and increased culantro yield. These results suggest that carnitine has potential as a growth regulator in culantro and other crops. This study will be fundamental to support future experiments on the practical use of carnitine as a bioregulator in plants as well as further studies on the biochemical and molecular mechanisms involved in this regulation.

Keywords: aromatic plant; bioregulators; culantro; carnitine; spiny coriander.

4.1 INTRODUCTION

Carnitine (β-hydroxy-γ-trimethyl ammonium butyrate) is an amino acid synthesized from L-lysine and L-methionine, naturally occurring in animals, plants, bacteria, yeast, and fungi (Bourdin et al. 2007; Dayanand et al. 2011; Rippa et al. 2012), It is involved in energy and hormone metabolism, as well as participates in oxidative stress tolerance and in the transport of long-chain fatty acids from the cytosol to the mitochondrial membrane (Bourdin et al. 2007;

Charrier et al. 2012; Oney-Birol 2019). Carnitine was initially identified in animals (Dayanand et al. 2011); however, its presence has been observed in several plant species as well, including cereals and legumes (Wood et al. 1992; Bourdin et al. 2007; Masterson and Wood 2009). Although carnitine levels are 100 times lower in plants than in animals, studies have shown a link between carnitine levels and lipid metabolism in plants (Bourdin et al. 2007; Oney-Birol 2019).

In animals, carnitine is responsible for lipid catabolism and acts as a cofactor in the transport of activated fatty acids between peroxisomes and mitochondria for β-oxidation (Nguyen et al. 2016). This fatty acid trafficking process is known as the carnitine transport system and involves carnitine transferase and translocase activities (Steiber et al. 2004). Very long-chain, branched-chain fatty acids, and medium-chain dicarboxylic acids are partially oxidized to medium-chain fatty acids in peroxisomes and then exported to the mitochondria to complete β-oxidation (Nguyen et al. 2016). In plants, carnitine is present in free and esterified forms during membrane biosynthesis and lipid storage processes and is associated with the anabolic pathways of lipid metabolism (Nguyen et al. 2016). In addition, it participates in the transport of long-chain fatty acids from the chloroplast, such as long-chain acylcarnitine, to the mitochondria, where they undergo β-oxidation (Masterson and Wood 2000).

Considering the existence of medium and long-chain acylcarnitines, which confirms the link between carnitine and fatty acid metabolism in vegetables (Nguyen et al. 2016), its exogenous supply can help in the synthesis of lipids that help in the adjustment of membrane systems under stress conditions. Charrier et al. (2012) applied carnitine to Arabidopsis thaliana seedlings, which conferred a possible osmolytic action as well as a possible metabolic implication in the recovery from salt stress. Exogenous application of carnitine reduces oxidative damage to DNA and induces plant tolerance to abiotic stresses (Oney-Birol 2019). In addition, carnitine acts by antagonistically modulating the abscisic acid (ABA) pathway because the intracellular accumulation of carnitine leads to the modulation of ABA-responsive genes (Charrier et al. 2012).

Culantro (*Eryngium foetidum* L.), also known as spiny coriander, belongs to the Apiaceae family and is a perennial herb native to Central America. It is extensively used as a condiment and medicinal and aromatic plant in Vietnam, India, Mexico, China, and the Amazon region (Shavandi et al. 2012; Singh et al. 2013). Among its compounds, (E)- 2-Dodecenal is the main compound responsible for the characteristic flavor of the species, and daucane is a sesquiterpene with proven leishmanicidal activity (Rojas-Silva et al. 2014; Chandrika et al 2015). Culantro is one of the main non-conventional vegetables grown in Brazil (Brazil 2010). This plant is commonly used in folk medicine to treat burns, earaches, fever, hypertension, constipation, seizures, asthma, stomach pain, worms, snake bites, diarrhea, cramps, and to stimulate appetite (Shavandi et al. 2012; Singh et al. 2013). Despite the culinary and medicinal importance of this species and the potential of carnitine to modulate energy metabolism in these plants, studies on both are still scarce. The objective of this study was to evaluate the effects of exogenous carnitine application on the morphophysiological development of *E. foetidum* plants.

4.2 MATERIALS AND METHODS

4.2.1 Experimental location and plant material

The experiment was conducted between September and December in a greenhouse covered with a transparent film, with sunlight penetrating the experimental area of the Seedling Production Laboratory of the Center for Human, Social and Agrarian Sciences, Federal University of Paraíba, Bananeiras, Paraíba, Brazil (6° 45′ S, 35° 38′ W, elevation of 526 m), at an average temperature of 26.7 °C (20.1–33.7 °C) and relative humidity of 86% (AGRITEMPO 2022). Culantro seeds (*E. foetidum*) were donated by growers from local rural communities in the municipality of Areia, Paraíba, Brazil (6° 57′ S, 35° 41′ W, elevation of 623 m).

4.2.2 Plant growth and carnitine treatments

Seeds were sown in polyethylene bags (17 × 21 cm) containing a commercial substrate (Mecplant®, Telêmaco Borba, Brazil). The plants were transplanted into polyethylene bags (22 × 28 cm) and fertilized with NPK (4–14-8) (≈ 2 g per bag) 35 days after sowing (DAS). At 57 DAS, the plants were subjected to treatment with water (control), 100 µM carnitine, and 10 mM of carnitine using hand sprayers every 2 days (57, 59, 61, 63, 65, 67, 69, and 71 DAS) until both sides of each leaf were completely wet. The carnitine solution was prepared by dissolving it in distilled water and adding the surfactant polysorbate 80 (0.03% (v/v); Tween-80®) to increase adhesion.

4.2.3 Morphophysiological analysis

At 72 DAS, the growth parameters, photosynthetic pigment content, gas exchange parameters, and chlorophyll a fluorescence were measured. To determine plant growth, shoot height was measured with a graduated ruler and 10 plants were collected and separated into shoots and roots using a scalpel and weighed to determine the fresh mass. The leaf area and number of leaves were determined by image analysis using the ImageJ software. After morphological measurements, shoots and roots of the culantro plants were oven-dried at 65 °C until a constant weight was reached to determine their dry weight. The shoot to root ratio was calculated as the dry weight of the shoots divided by the dry weight of the roots.

The levels of photosynthetic pigments were determined according to Wellburn (1994) with some modifications. Five discs (1 cm^2 each) from fully expanded leaves of five plants per treatment were incubated for 48 h in the dark with 5 mL of dimethyl sulfoxide (Santos et al. 2008). The absorbance of the extract was then measured at 480, 649, and 665 nm using a spectrophotometer (GTA-96 UV–VIS, Global Trade Technology, São Paulo, Brazil). The levels of chlorophyll a and b, chlorophyll a/b ratio, total chlorophyll content, and total carotenoids were determined.

Gas exchange parameters of the fully expanded leaves of five plants per treatment were measured using an open-flow gas exchange infrared gas analyzer (LCpro-SD Portable Photosynthesis Measurement System, ADC BioScientific, Hod- desdon, UK) between 08:00 and 09:00 h. The conditions in the leaf chamber consisted of a reference CO^2 concentration of 400 μmol CO2 mol^{-1} and photosynthetically active radiation of 1000 μmol m^{-2} s^{-1} with 10% blue light. Additionally, net carbon assimilation rate (A) and stomatal conductance (gS) were determined.

Chlorophyll a fluorescence of the fully expanded leaves of five plants per treatment was determined using a portable fluorometer (OS-30p +; Opti-Sciences, Hudson, NY, USA) between 08:00 and 09:00 h. Leaves were dark-adapted for 30 min, and then the minimum chlorophyll a fluorescence in the dark-adapted state (F_0), maximum chlorophyll a fluorescence in the dark-adapted state (F_M), and the maximum quantum yield of PSII (F_V/F_M) were measured.

4.2.4 Experimental design and statistical analysis

The experimental design was completely randomized with three treatments (control, 100 µM carnitine, and 10 mM carnitine) and 13 replicates, and each experimental unit was composed of one bag with one plant. The data were tested for normality and homogeneity by subjecting it to analysis of variance, and the means were compared by Duncan's test ($P \leq 0.05$) using Genes software (Cruz 2016). The distance between the treatments was determined using canonical discriminant analysis in a biplot graph. The treatments were separated into different groups using the Tocher optimization method and generalized squared interpoint distance of Mahalanobis (D2). The grouping quality was evaluated using the co-optical correlation coefficient (r). The relative contribution of each variable to discriminate treatments was quantified using Singh's (1981) criterion.

4.3 RESULTS

Analysis of variance revealed a significant effect of carnitine treatment on leaf area, plant height, root fresh mass, leaf fresh mass, root dry mass, leaf dry mass, shoot/root ratio, F_M, and F_V/F_M (Table 1).

Plants treated with 100 µM carnitine were taller and had greater leaf area than the control and plants treated with 10 mM carnitine, which did not differ from each other (Figs. 1 and 2A, B). The accumulation of fresh and dry mass in the roots and shoots was significantly higher in plants treated with 100 µM carnitine than in those under other treatments (Figure 2C–F). This result was confirmed by the fact that 100 µM carnitine increased the root mass by 147%, while the leaf mass increased only by 108%. In contrast, 10 mM carnitine did not alter biomass allocation compared to the control. However, both carnitine concentrations. significantly reduced the maximum fluorescence (FM) and quantum efficiency of PSII (F_V/F_M) compared to the control, but did not differ from each other (Figure 2H, I).

Canonical discriminant analysis showed that the first two canonical variables explained 100% of the variability among the treatments, allowing for a two-dimensional scatter plot representation (Figure 3B). The treatments were separated into two groups: group 1 (blue circle), corresponding to 100 µM carnitine treatment, and group 2 (red circle), corresponding to the control and 10 mM carnitine treatment. The relative contributions of the 18 original variables showed that leaf fresh mass, root fresh mass, root dry mass, and leaf dry mass contributed the most, accounting for 31.9%, 18.8%, 16.6%, and 9.1% of the total variance, respectively (Figure 3C).

Table 1 Summary of the analysis of variance of morphophysiological variables in culantro (*Eryngium foetidum* L.) plants after 72 days of treatment with different carnitine concentrations: 0 (control), 100 µM, and 10 mM

Variable	*p*-value	Coefficient of variation (%)
Leaf area	4.29*	52.51
Number of leaves	100.00 ns	20.94
Plant height	0.53**	16.92
Root length	12.72 ns	23.19
Root fresh mass	2.31*	63.49
Leaf fresh mass	4.76*	58.48
Root dry mass	2.24*	57.19
Leaf dry mass	4.98*	54.91
Shoot/root ratio	4.17*	11.75
F_0	18.05 ns	5.76
F_M	3.10*	12.68
F_V/F_M	2.36*	1.35
A	100.00 ns	13.46
g_s	31.85 ns	12.11
Chlorophyll *a*	100.00 ns	11.54
Chlorophyll *b*	5.47 ns	11.80
Total chlorophylls	24.20 ns	11.28
Total carotenoids	100.00 ns	10.59

^{ns} = Non-significant at a 5% probability; * and ** imply significance at 5% and 1% probability levels, respectively, based on the F test

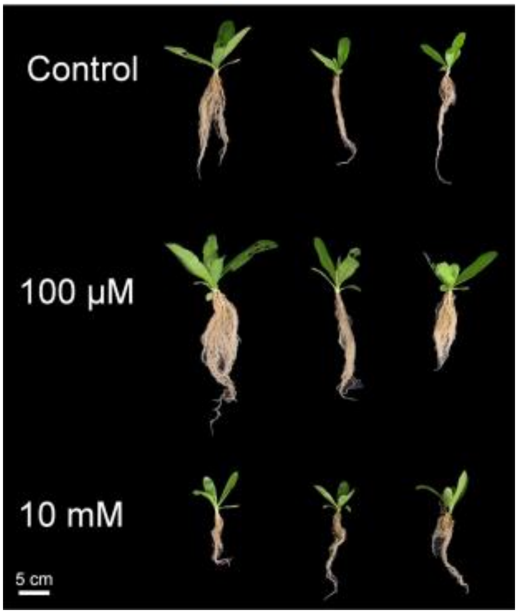

Figure 1 Growth of culantro (*Eryngium foetidum* L.) plants after 72 days of treatment with different carnitine concentrations: 0 (control), 100 μM, and 10 mM

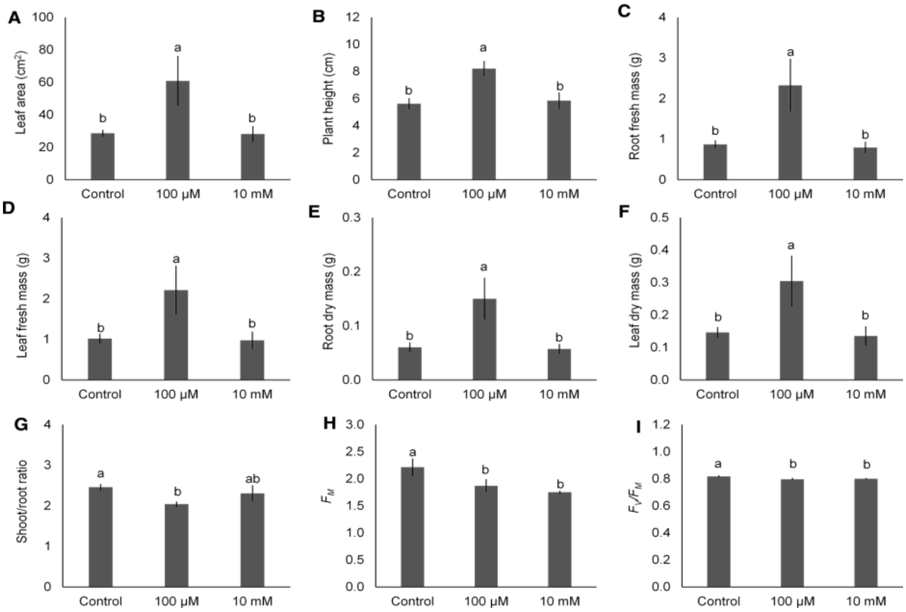

Figure 2 Morphophysiological parameters in 72-d-old culantro (*Eryngium foetidum* L.) plants treated with different carnitine concentrations: 0 (control), 100 μM, and 10 mM (FM: maximum fluorescence; F_V/F_M: PSII maximum quantum yield). Values represent means ± standard error (n = 5). Values with same letters do not differ significantly (Duncan's Multiple Range Test; $P \geq 0.05$).

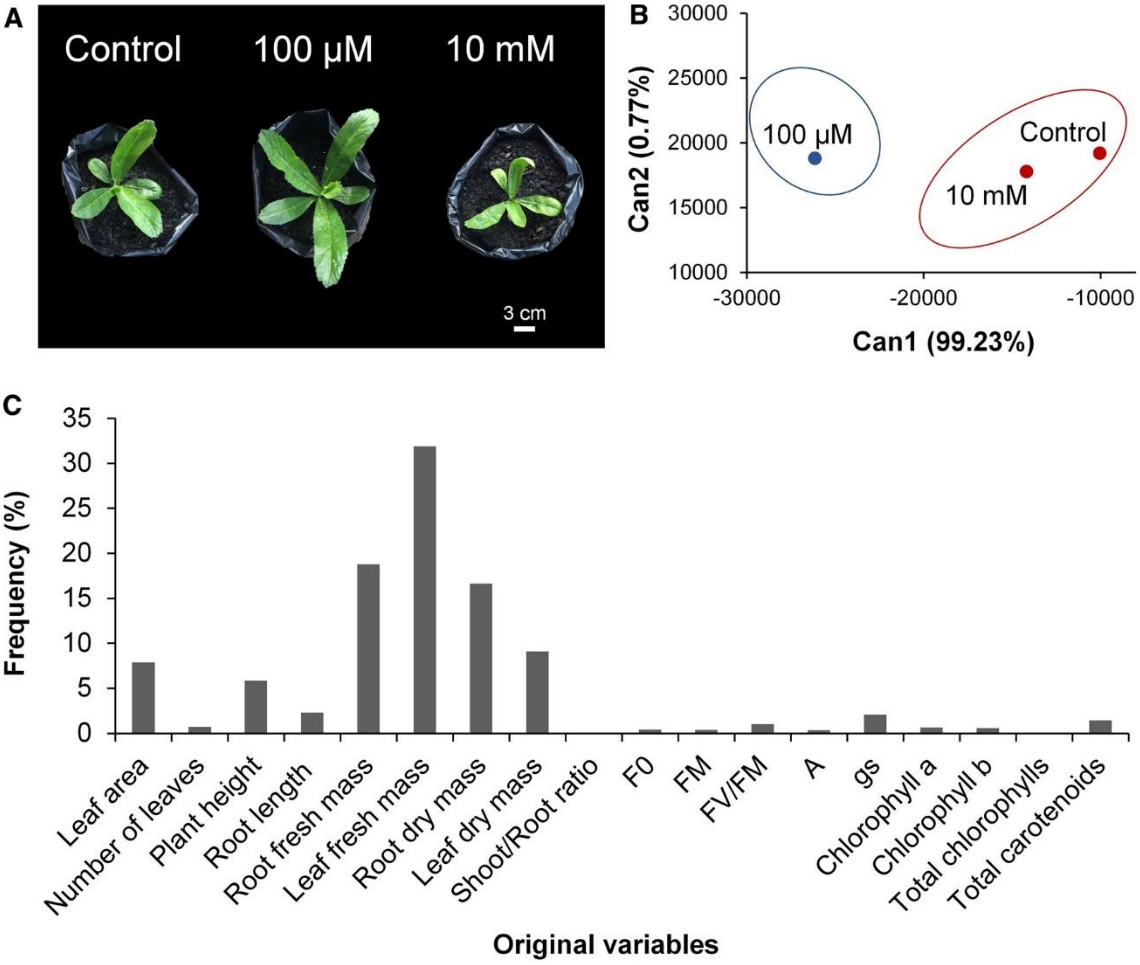

Figure 3 Growth and canonical variables obtained from original variables in 72-d-old culantro (*Eryngium foetidum* L.) plants treated with different carnitine concentrations: 0 (control), 100 µM, and 10 mM. A Top view of the plants; B Biplot showing the mean scores for the carnitine treatments. Axes represent the first two canonical variables—Can1 and Can2. The ellipses represent the groups formed using Singh's (1981) method; C Relative contributions of original variables to the canonical variable.

4.4 DISCUSSION

Although carnitine has been recently identified in the plant kingdom (Wood et al. 1992), little data about its role in plant growth have been reported to date, and data about its role in culantro is not available. Thus, we tested the effect of carnitine at a low (100 µM) and a high concentration (10 mM), in order to verify whether the effects of this compound would be signaling as a growth regulator, or nutritional by providing the amino acid. Here, 100 µM carnitine treatment improved the development of culantro plants and increased the biomass production of both shoots and roots. Similar results were found for maize seedlings. in which 25 µM carnitine treatment increased the over- all growth (Turk et al. 2020). In addition to the overall increase in culantro growth, 100 µM carnitine altered biomass allocation, with more biomass being allocated to roots instead of leaves. These responses may occur due to the stimulatory effect of carnitine on root growth (Turk et al. 2020).

Culantro leaves are consumed because of their flavor conferred by the essential oils; hence, the aerial part of this species is of interest. Thus, as the application of 100 µM carnitine increased the leaf area and mass, it can be assumed that this treatment increased the culantro yield. In maize seedlings, carnitine treatment increases plant growth and leaf area owing to higher photosynthetic capacity (Turk et al. 2020). A similar result was found in this study, where 100 µM carnitine increased leaf area by 113%. However, no difference was found in carbon assimilation, suggesting that the increased plant growth was due to another mechanism, such as increased mitochondrial respiration and nitrogen assimilation (Turk et al. 2020).

Chlorophyll fluorescence measurements represent the activity of photosystems and are widely utilized to assess the physiological status of plants (Kalaji et al. 2014). When dark-adapted leaves are exposed to a saturating light pulse, fluorescence increases from a minimum (Fo) to a maximum level (F_M), with the Fv/F_M ratio providing an estimate of the maximum quantum yield of the primary photochemistry of PSII (Schansker et al. 2014; Tsimilli-Michael 2020). Thus, the reductions in F_M and Fv/F_M caused by carnitine treatment suggest a low photochemical capacity, either due to thereduced pool of quinone A or the inability of PSII acceptors to be fully reduced (Goltsev et al. 2016).

Carnitine treatment increases unsaturated fatty acid levels and decreases saturated fatty acid levels, thereby altering plasma membrane fluidity (Los et al. 2013; Turk et al. 2019). Considering that carnitine acts as a cofactor in the transfer of fatty acids between peroxisomes and mitochondria (Nguyen et al. 2016), it is possible that the higher concentration of carnitine used in this study altered membrane integrity, and consequently, the functionality of chloroplasts and energy metabolism, resulting in reduced growth and chlorosis and shrinking of the leaves (Figure 3A). This was further supported by the separation of treatments into two groups (Figure 3B), indicating that carnitine application at low concentration (group 1) enhanced culantro growth; however, plants treated with 10 mM carnitine did not differ from the control (group 2), showing that the high concentration was not effective to nutritionally supply culantro plants.

Considering the role of carnitine in plant defense, mainly through the induction of osmoregulation and antioxidant defense (Charrier et al. 2012; Jacques et al. 2018; Oney- Birol 2019; Turk et al. 2020), lower culantro growth under high carnitine levels may be a result of the inherent trade-off between growth and plant defense, in which the induction of defense mechanisms requires high energy input that could otherwise be used to enhance growth (Kudo et al. 2019). In addition, carnitine is linked to fatty acid metabolic pathways (Bourdin et al. 2007; Oney-Birol 2019), which are essential for energy storage, cell structure, and signal transduction (Frank et al. 2015). In this context, carnitine presents high potential as a growth regulator in culantro, with the possibility of being applied to other species as well.

4.5 CONCLUSIONS

Carnitine has been reported as a modulator of plant metabolism and defense against abiotic stresses. Here, 100 µM carnitine was effective in enhancing culantro growth under non-stressful conditions, mainly through the modulation of root growth. In addition, the effect of carnitine was dose-dependent, as its high concentration (10 mM) did not alter growth. This indicates that carnitine is a potential growth regulator in culantro and probably in other crops too. Further studies are still required to understand the biochemical and molecular mechanisms involved in carnitine regulation in plants, but our results in culantro can serve as a basis for other studies on the practical use of carnitine as a bioregulator.

REFERENCES

AGRITEMPO. **Dados meteorológicos**. Disponível em <https://www.agritempo.gov.br/agritempo/jsp/PesquisaClima/index.jsp?siglaUF=PB>. Accessed 29 Jan 2022. Bananeiras.

BOURDIN, B.; ADENIER, H.; PERRIN, Y. Carnitine is associated with fatty acid metabolism in plants. **Plant Physiology Biochemistry** v. 45, n. 12, p. 926-931, 2007.

MAPA. 2010. **Manual de hortaliças não-convencionais**. Brasília: Mapa/ACS, 92 p.

CHANDRIKA, R.; SARASWATHI, K. J. T.; MALLAVARAPU, G. R. Constituents of the essential oils of the leaf and root of *Eryngium foetidum* L. from two locations in India. **Journal of Essential Oil Bearing Plants**, v. 18, n. 2, p. 349-358, 2015.

CHARRIER, A.; RIPPA, S.; YU, A.; NGUYEN, P. J.; RENOU, J. P.; PERRIN, Y. The effect of carnitine on Arabidopsis development and recovery in salt stress conditions. **Planta**, v. 235, p. 123-135, 2012.

CRUZ, C. D. Genes Software-extended and integrated with the R, MATLAB and Selegen. **Acta Scientiarum. Agronomy**, v. 38, n. 4, p. 547-552, 2016.

DAYANAND, C. D.; KRISHNAMURTHY, N.; ASHAKIRAN, S.; SHASHIDHAR, K. N. Carnitine: a novel health factor–an overview. **International Journal of Pharmacy & Biomedical Research**, v. 2, n. 2, p. 79-89, 2011.

FRANK, J. A.; MORONI, M.; MOSHOURAB, R.; SUMSER, M.; LEWIN, G. R.; TRAUNER, D. Photoswitchable fatty acids enable optical control of TRPV1. **Nature communications**, v. 6, n. 1, p. 7118, 2015.

W. Bąba, T., J. Mojski, H. Kociel & S. I. Allakhverdiev

GOLTSEV, V. N.; KALAJI, H. M.; PAUNOV, M.; BABA, W.; HORACZEK, T.; MOJSKI J.; KOCIEL, H.; ALLAKHVERDIEV S. I. Variable chlorophyll fluorescence and its use for assessing physiological condition of plant photosynthetic apparatus. **Russian journal of plant physiology**, v. 63, p. 869-893, 2016.

JACQUES, F.; RIPPA, S.; PERRIN, Y. Physiology of L-carnitine in plants in light of the knowledge in animals and microorganisms. **Plant Science**, v. 274, p. 432-440, 2018.

KALAJI, H. M.; SCHANSKER, G.; LADLE, R. J. Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. **Photosynthesis research**, v. 122, p. 121-158, 2014

KUDO, M.; KIDOKORO, S.; YOSHIDA, T. A gene-stacking approach to overcome the trade-off between drought stress tolerance and growth in Arabidopsis. **The Plant Journal**, v. 97, n. 2, p. 240-256, 2019

LELANDAIS-BRIÈRE, C.; JOVANOVIC, M.; TORRES, G. A. M.; PERRIN, Y.; LEMOINE, R.; CORRE-MENGUY, F.; HARTMANN, C. Disruption of AtOCT1, an organic cation transporter gene, affects root development and carnitine-related responses in Arabidopsis. **The Plant Journal**, v. 51, n. 2, p. 154-164, 2007.

LOS, D. A.; MIRONOV, K. S.; ALLAKHVERDIEV, S. I. Regulatory role of membrane fluidity in gene expression and physiological functions. **Photosynthesis research**, v. 116, p. 489-509, 2013.

MASTERSON, C.; WOOD, C. Mitochondrial β-oxidation of fatty acids in higher plants. **Physiologia Plantarum**, v. 109, n. 3, p. 217-224, 2000

MASTERSON, C.; WOOD, C. Influence of mitochondrial β-oxidation on early pea seedling development. **New Phytologist**, v. 181, n. 4, p. 832-842, 2009.

NGUYEN, P. J.; RIPPA, S.; ROSSEZ, Y.; PERRIN, Y. Acylcarnitines participate in developmental processes associated to lipid metabolism in plants. **Planta**, v. 243, p. 1011-1022, 2016.

ONEY-BIROL, S. Exogenous L-carnitine promotes plant growth and cell division by mitigating genotoxic damage of salt stress. **Scientific reports**, v. 9, n. 1, p. 17229, 2019.

RIPPA, S.; ZHAO, Y.; MERLIER, F.; CHARRIER, A.; PERRIN, Y. The carnitine biosynthetic pathway in *Arabidopsis thaliana* shares similar features with the pathway of mammals and fungi. Plant Physiol Bio- chem 60:109–114. https://doi.org/10.1016/j.plaphy.2012.08.001

ROJAS-SILVA, P.; GRAZIOSE, R.; VESELY, B. Leishmanicidal activity of a daucane sesquiterpene isolated from *Eryngium foetidum*. **Pharmaceutical Biology**, v. 52, n. 3, p. 398-401, 2014.

SANTOS, R. P.; CRUZ, A. C. F.; IAREMA, L.; KUKI, K.N.; OTONI, W. C. Protocolo para extração de pigmentos foliares em porta-enxertos de videira micropropagados. **Ceres** v. 55, p. 356–364, 2008.

SCHANSKER, G.; TÓTH, S. Z.; HOLZWARTH, A. R.; GARAB, G. Chlorophyll a fluorescence: beyond the limits of the QA model. **Photosynthesis research**, v. 120, p. 43-58, 2014.

SHAVANDI, M. A.; HADDADIAN, Z.; ISMAIL, M. H. S. *Eryngium foetidum* L., *Coriandrum sativum* and *Persicaria ordorata* L.: a review. **Journal of Asian Scientific Research**, v. 2, n. 8, p. 410, 2012.

SINGH, D. The relative importance of characters affecting genetic divergence. **Indian Journal of Genetics and Plant Breeding,** v. 41, n. 2, p. 237-245, 1981.

SINGH, S.; SINGH, D. R.; BANU, S.; SALIM, K. M. Determination of bioactives and antioxidant activity in *Eryngium foetidum* L.: a traditional culinary and medicinal herb. **Proceedings of the National Academy of Sciences, India Section B: Biological Sciences**, v. 83, p. 453-460, 2013.

STEIBER, A.; KERNER, J.; HOPPEL, C. L. Carnitine: a nutritional, biosynthetic, and functional perspective. **Molecular aspects of medicine**, v. 25, n. 5-6, p. 455-473, 2004.

TSIMILLI-MICHAEL, M. Special issue in honour of Prof. Reto J. Strasser–Revisiting JIP-test: an educative review on concepts, assumptions, approximations, definitions and terminology. **Photosynthetica**, v. 58, n. SI, p. 275-292, 2020.

TURK, H.; ERDAL, S.; DUMLUPINAR, R. Exogenous carnitine application augments transport of fatty acids into mitochondria and stimulates mitochondrial respiration in maize seedlings grown under normal and cold conditions. **Cryobiology**, v. 91, p. 97-103, 2019.

TURK, H.; ERDAL, S.; DUMLUPINAR, R. Carnitine-induced physiobiochemical and molecular alterations in maize seedlings in response to cold stress. **Archives of Agronomy and Soil Science**, v. 66, n. 7, p. 925-941, 2020.

WELLBURN, A. R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J**ournal of plant physiology**, v. 144, n. 3, p. 307-313, 1994.

WOOD, C.; MASTERSON, C.; THOMAS, D. R. The role of carnitine in plant cell metabolism. In: Tobin AK (ed) **Plant organelles, Society for Experimental Biology seminar series**. Cambridge University Press, Cambridge, 1992, pp 229–263

5 CAPÍTULO II: WATER STRESS AND EXOGENOUS CARNITINE ON GROWTH AND ESSENTIAL OIL PROFILE OF *ERYNGIUM FOETIDUM* L.

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Abstract

Water stress influences plant growth and metabolism. Carnitine, an amino acid involved in lipid metabolism, has been related to responses of plants to abiotic stresses, also modulating their metabolites. Culantro (*Eryngium foetidum* L.) is a perennial herb, rich in essential oils, native to Latin America, commonly used due to its culinary and medicinal properties. Here, we investigated the effect of exogenous carnitine on morphophysiology and the essential oil profile of culantro plants under water stress. For this, plants were grown under three water conditions: well-watered, drought stress, and re-watered; and sprayed with exogenous carnitine (100 µM) or water (control). Culantro growth was impaired by drought and enhanced by re-watering. Carnitine, in turn, did not reverse drought effects on growth, and impaired the growth of re-watered plants, also improving photosynthetic pigment content. Water conditions and carnitine application changed the essential oil profile of the plants. Drought and re-watering improved the production of eryngial, which was even increased with exogenous carnitine in re-watered plants. In addition, hydroquinone was only produced with the combination of re-watering and carnitine application. The application of exogenous carnitine can be a strategy to induce the production of essential oil compounds with cosmetic and pharmaceutical importance in culantro.

Keywords: culantro; drought stress, medicinal plants; re-watering; secondary metabolites profile; sesquiterpenes.

5.1 INTRODUCTION

Water stress is a main environmental factor affecting the morphophysiology and production of plants (Liang et al. 2019; Soares et al. 2022). Thus, plants have strategies to prevent water loss in order to maintain optimal water balance, such as stomatal closure, osmoregulation, reactive oxygen species (ROS) scavenging, and production of secondary metabolites (Gupta et al. 2020), the latter are organic molecules produced by plants, classified into alkaloids, phenolics, and terpenoids (Takshak and Agrawal 2019). These compounds act as chemical regulators and messengers in plants, playing a fundamental role in fertilization, defense against pathogens and herbivores, and tolerance to abiotic stresses (Böttger et al. 2018). Due to their aromatic and therapeu tic properties, they are used for dyes, medicines, artificial flavoring products, nutraceuticals, perfumes, among others (Wink 2015; Kulak et al. 2019).

Culantro (*Eryngium foetidum* L.) belongs to the Apiaceae family and is a perennial herb native to Central America, with autogamous characteristics and propagation mainly through seeds (Singh et al. 2014; Rodrigues et al. 2022). This species is commonly used in folk medicine to treat burns, earaches, fever, hypertension, constipation, asthma, stomach pain, worms, diarrhea, rheumatism, cramps, and to stimulate appetite (Shavandi et al. 2012; Singh et al. 2013; Rodrigues et al. 2022). Culantro is widely used as a flavoring condiment in foods, having great relevance for the Amazonian food culture (Rodrigues et al. 2022), also having impact on human health, inhibiting the growth of pathogenic bacteria, such as *Helicobacter pylori*, showing anti-leishmanial activity against *Leishmania tarentolae* and *L. donovani*, and being effective against trypanosomes, nematodes, and fungi (Paul et al. 2010; Jaramillo et al. 2011; Rojas-Silva et al. 2014; Mabeku et al 2016). Furthermore, culantro is rich in essential oils stored on its secretory ducts (Bhavana et al. 2013; Rodrigues et al. 2020, 2022), with a characteristic flavor and aroma due an aliphatic aldehyde called eryngial ((E)-2-dodecenal), which is present mostly on the leaves (Quynh and Kubota 2012; Rodrigues et al. 2020).

Mitigation strategies can be used to attenuate water stress in plants (Sai et al. 2016). The application of bioregulators, such as amino acids like proline (Ami et al. 2020; Santos et al 2022a), arginine (Nargesi et al. 2022), and carnitine (Charrier et al. 2012; Turk et al. 2019a, b; Santos et al. 2022b), has been studied. Carnitine is a quaternary ammonium compound involved in metabolic functions like energy metabolism and stress tolerance (Charrier et al. 2012). This amino acid participates in the degradation of triglycerides to fatty acids and their transportation into mitochondria, stimulating respiration, being essential for energy storage, cell structure, and signal transduction (Bourdin et al. 2007; Frank et al. 2015; Oney-Birol 2019; Turk et al. 2019a, b). In addition, exogenous carnitine has been pointed as a bioregulator, modulating plant growth under non-stressful conditions (Charrier et al. 2012; Santos et al. 2022c) and mitigating moderate water stress in plant species, such as arugula (Santos et al. 2022b) and radish (Henschel et al. 2023). This way, the objective of this study was to evaluate the action of carnitine on growth, morphophysiology and the essential oil profile of culantro plants under water stress.

5.2 MATERIALS AND METHODS

5.2.1 Experimental location and plant material

The experiment was conducted between January and May 2022, in a greenhouse covered with transparent film, located in the experimental area of the Seedling Production Laboratory of the Center for Human, Social and Agrarian Sciences/Federal University of Paraiba (CCHSA/UFPB), in Bananeiras, Paraiba, Brazil (6° 45′ S, 35° 38′ W, elevation of 526 m). Culantro seeds (E. foetidum) were, donated by growers from local rural communities in the municipality of Areia, Paraíba, Brazil (6° 57′ S, 35° 41′ W, elevation of 623 m).

5.2.2 Irrigation and bioregulators treatments

The culantro seeds were sown in trays containing 200 cells. At 35 days after sowing (DAS), when plants presented four fully expanded leaves, they were transplanted to polyethylene bags (22 × 28 cm) containing commercial substrate (Mecplant®, Telêmaco Borba, Brazil). The bags were irrigated until 100% bag capacity (BC) for fifteen days. 50 DAS, the plants were subjected to the treatments: well-watered (80% BC), drought (40% BC), and re-watered (12 days without irrigation with subsequent irrigation with 80% BC, with the application of water (control) or 100 µM carnitine (L-carnitine, Growth Supplements, Tijucas, Brazil) (Santos et al. 2022c), using hand sprayers every 6 days (50, 56, 62, 68, 74, 80, 86, 92, and 98 DAS). The duration of water restriction of 12 days was established based on previous survival tests. Carnitine solution was dissolved in distilled water with addition of the surfactant polysorbate 80 (Tween-80®, 0.03%) (v/v) to increase adhesion to the leaves.

5.2.3 Morphophysiological analysis

At 100 DAS, photosynthetic pigments content, gas exchange parameters, and chlorophyll a fluorescence were measured, and 102 DAS growth parameters were measured. The leaf area, number of leaves, specific leaf area, and root length were determined through image analysis using the software ImageJ (Abramoff et al. 2004). Five plants were collected and separated into shoots and roots using a scalpel and weighed for the determination of the fresh mass. After morphologic measurements, shoots and roots of culantro plants were oven-dried at 65 °C until a constant weight to determine their dry weight. The shoot/ root ratio was determined as the dry weight of shoots divided by the dry weight of roots. The total biomass was determined as the sum of dry weight of shoots and roots.

Gas exchange measurements were performed using an open-flow gas exchange system infrared gas analyzer (IRGA, LCpro-SD Portable Photosynthesis System, ADC BioScientific, Hoddesdon, UK). The analyses were made on fully expanded leaves of five plants per treatment between 8 and 11 h a.m. The conditions in the leaf chamber consisted of an ambient temperature and reference CO2 and artificial photosynthetically active radiation of 1000 µmol m^{-2} s^{-1} with 10% blue light. The net carbon assimilation rate (A, µmol CO_2 m^{-2} s^{-1}), stomatal con ductance (g_S, mol H_2O m^{-2} s^{-1}), internal CO_2 concentration (Ci, mmol CO_2 mol^{-1} air) and leaf transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}), and water use efficiency (*A/E*), carboxylation efficiency (*A/Ci*) were determined. Light response curves of photosynthesis were studied by varying photosynthetic photon flux density (PPFD) from 0 to 1800 mol m^{-2} s^{-1}. Light response curve measurements were made on fully expanded leaves of three plants per treatment between 8 and 11 h a.m. From light response curves, were calculated the following parameters: dark respiration (R_{dark}) (µmol m^{-2} s^{-1}), apparent quantum yield (mol/mol), light compensation point (LCP) (µmol m m^{-2} s^{-1}), maximum gross assimilation rate (A_{max}) (µmol m^{-2} s^{-1}), and light saturation point (LSP) (µmol m^{-2} s^{-1}).

Levels of photosynthetic pigments were determined according to Santos et al. (2008), with modifications, following the equation of Wellburn (1994). For this, four disks (1 cm2) from fully expanded leaves of four plants per treatment were incubated for 48 h in dark conditions with 7 mL dimethyl sulfoxide (Santos et al. 2008). Then, the extract was read at 480, 649, and 665 nm using a spectrophotometer (GTA-96 UV–Vis, Global Trade Technology, São Paulo, Brazil). The levels of chlorophyll a, chlorophyll b, chlorophyll a/b ratio, total chlorophylls, and total carotenoids were determined. The relative water content (RWC) was determined according to Barrs and Weatherley (1962), with modifications. Ten leaf disks (1 cm^2) were collected from fully expanded leaves of five plants per treatment and immediately weighed (fresh mass, FM). The disks were incubated for 6 h in distilled water, weighed for turgid mass (TM), and oven-dried at 65 °C for 24 h for dry mass (DM) determination. RWC was calculated as [(FM-DM)/(TM-DM)] × 100, and expressed as a percentage (%).

5.2.4 Microextraction of essential oils

Approximately 500 mg of leaves were collected and stored at -18 °C in test tubes with a screw cap, following Castro et al. (2020), with modifications. After freezing, 1 mL methanol was added to each sample. To accelerate the extraction process, samples were immersed in an ultrasonic bath (Ultra Cleaner 800, UNIQUE) at 40 kHz and room temperature for 10 min. Subsequently, the supernatant was filtered through a sterile cotton wick. Resulting samples of 1 μL clear solution containing the extracted oils were analyzed by gas chromatography.

5.2.5 Qualitative analysis of essential oils

Qualitative analysis of essential oils was carried out on a gas chromatographer coupled to a mass spectrometer (GCMS- QP2010 Plus; Shimadzu, Kyoto, Japan) and an Rtx-5MS® column (Restek, Bellefonte, PA, USA) of 30 m × 0.25 mm, with three technical replicates. The initial oven temperature was 50 °C, where it was maintained for 3 min, followed by an increase of 6 °C min−1 to 240 °C. The injector was operated in split mode (1:10) at 240 °C, and the interface and mass detector were operated at 250 °C.

Helium was used as the carrier gas, with a flow of 1.69 mL min^{-1}. The constituents were identified by com paring the obtained mass spectra with those of the NIST 9.0 database (correlation > 95%) and confirmed by the corresponding retention index (Kováts Index) compared to published data.

5.2.6 Experimental design and statistical analysis

The experiment was in a completely randomized design, in a 2 × 3 factorial scheme (carnitine application × water condition) with two simultaneously repetitions, ten replicates for each combination of water level and carnitine treatment, and the experimental unit composed by one bag with one plant each. For all the analyses, sampling was done through a draw to ensure randomization. The model used for the analysis of variance was: $Yijk = \mu + Wi + Cj + (WC)\ ij + Eijk$; where: $Yijk$ = are the observations of the dependent variables, μ = the overall mean effect, Wi = effect of the level ith of water factor, Cj = effect of the level *ith* of carnitine factor, (WC) ij = effect of the interaction *ijth* water level × carnitine, and $Eijk$ = random error component. The data were tested for normality and homogeneity using the Shapiro–Wilk and Bartlett tests, respectively, subjected to analysis of variance, and the means compared by Tukey's test ($P \leq 0.05$) using the Genes software (Cruz 2016).

Volatiles profile data were submitted to multivariate analysis. The distance between the treatments was determined using canonical discriminant analysis in a three-dimensional scatter plot. The treatments were separated into different groups using the Tocher optimization method and generalized squared interpoint distance of Mahalanobis (D2). The grouping quality was evaluated using the co-optical correlation coefficient (r). The relative contribution of each variable to discriminate treatments was quantified using Singh (1981) criterion.

5.3 RESULTS

5.3.1 Drought and exogenous carnitine modulate morphophysiology of culantro plants

Water levels and carnitine application affected most mor- phophysiological parameters (Online Resource 1, Supplementary Table 1), with drought stress affecting overall plant growth (Figure 1a). Re-watered plants doubled leaf dry and fresh mass, root dry and fresh mass, and total biomass compared to well-watered plants (Figure 1b–f). Carnitine application increased the shoot/root ratio of re-watered compared to well-watered and drought plants. Similarly, in control plants, the highest shoot/root ratio occurred upon re-watering (Figure 1g).

Drought increased root length and decreased the leaf area and number of leaves compared to the well-watered and re- watered plants (Figure 2a–c). Re-watered treatment doubled the number of leaves and leaf area compared to well-watered treatment; however, these increases were reversed by carnitine application. There was no difference among treatments for specific leaf area (Figure 2d).

The application of carnitine increased the content of chlorophyll a, total chlorophylls, and total carotenoid in the well-watered plants, and chlorophyll a and total carotenoids in re-watered plants. In contrast, carnitine reduced chlorophyll a in drought stressed plants compared to the control (Figure 3a–d). There was no difference among the treatments for carbon assimilation rate, stomatal conductance, internal CO_2 concentration, and carboxylation efficiency (Figure 4a–d). Carnitine application increased respiration in the dark in re-watered treatment, but not in well-watered and drought (Figure 4e). Similarly, carnitine increased the LCP in re-watered and drought, but not in well-watered plants (Figure 4f).

5.3.2 The essential oil profile of culantro is modified by drought and applying carnitine

The water levels and the addition of carnitine significantly altered two of the main compounds detected: hydroquinone and eryngial (Online Resource 1, Supplementary Table 1). The first three canonical variables explained 94.1% of the variability among the treatments based on the essential oils profile, allowing for a three-dimensional scatter plot representation

(Figure 5a). The treatments were separated into four groups: group 1 (green circle), well-watered plants; group 2 (red circle), well-watered and drought control plants; group 3 (blue circle), drought plants with carnitine application; and group 4 (yellow circle) re-watered plants with carnitine application.

The relative contributions of the original variables showed that eryngial and hydroquinone were the most prominent compounds, contributing 38.13% and 24.81, respectively, to the total variance (Figure 5b). Drought and re-watering augmented eryngial content, compared to well-watered plants. In the re-watered, the application of carnitine even increased this compound (Figure 5c). Hydroquinone,in turn, was only detected in re-watered plants treated with carnitine (Figure 5d).

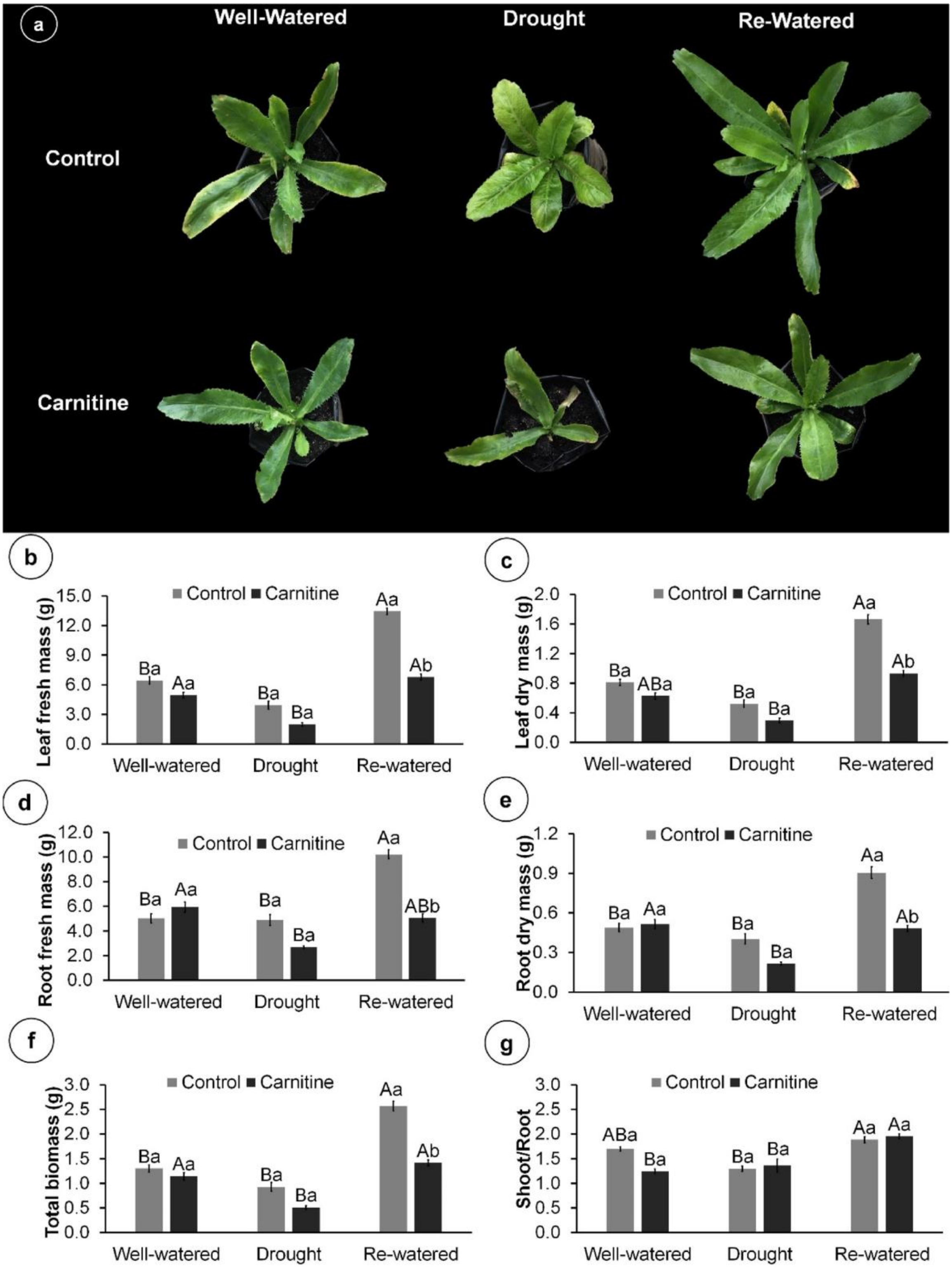

Figure 1 Growth of 102-day-old *Eryngium foetidum* L. plants sprayed with water or carnitine, and grown under different water conditions. Representative plants of each condition (a); Leaf fresh mass (b); Leaf dry mass (c); Root fresh mass (d); Root dry mass (e); Total biomass (f); and Shoot/root ratio (g). Columns represent the mean

of five replicates, and bars represent the standard error. Means followed by the same letter do not differ by Tukey's test ($P \leq 0.05$). Capital letters compare water conditions within carnitine levels, and lowercase letters compare between carnitine and control within each water condition.

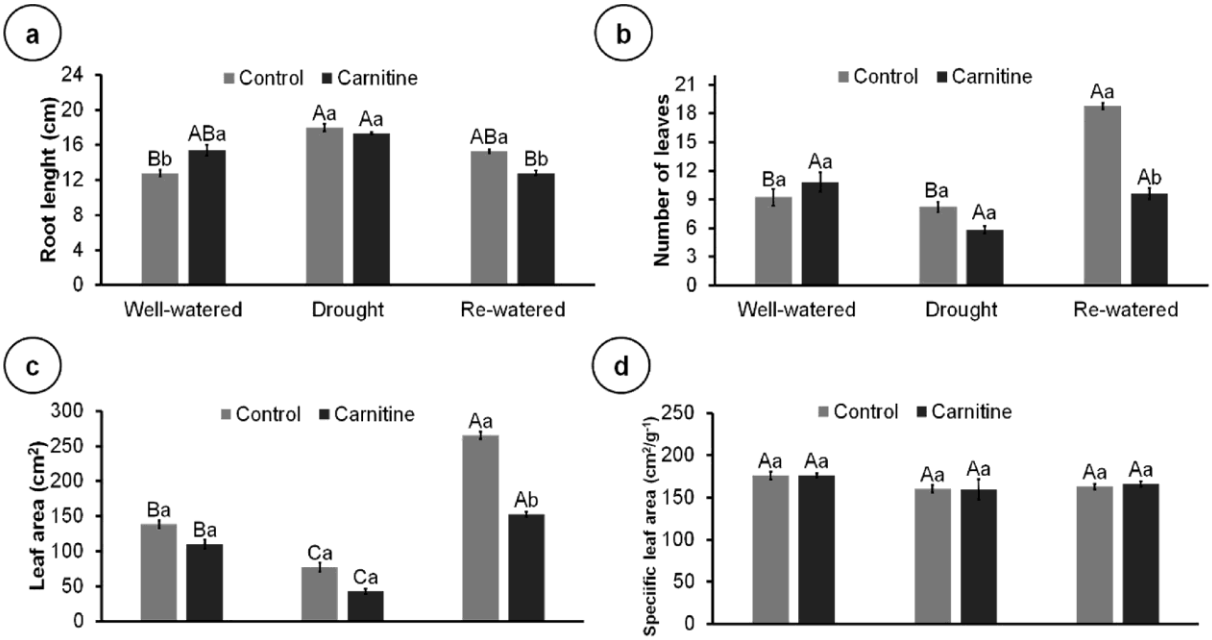

Figure 2 Growth parameters of 102-day-old *Eryngium foetidum* L. plants sprayed with water or carnitine, and grown under different water conditions. Root length (a); number of leaves (b); leaf area (c); and specific leaf area (d). Columns represent the mean of five replicates, and bars represent the standard error. Means followed by the same letter do not differ by Tukey's test ($P \leq 0.05$). Capital letters compare water conditions within carnitine levels, and lowercase letters compare between carnitine and control within each water condition.

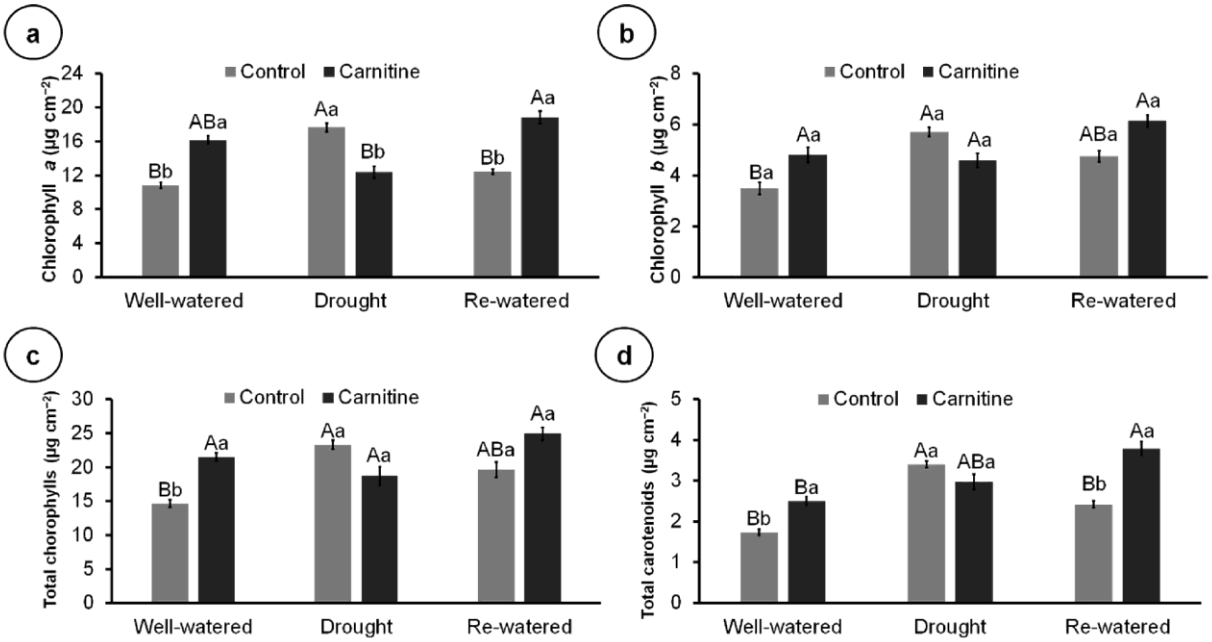

Figure 3 Contents of photosynthetic pigments of 100-day-old *Eryngium foetidum* L. plants sprayed with water or carnitine, and grown under different water conditions. Chlorophyll *a* (**a**); Chlorophyll *b* (**b**); Totalchlorophylls (**c**); and total carotenoids (**d**). Columns represent the mean of four replicates, and bars represent the standard error. Means followed by the same letter do not differ by Tukey's test ($P \leq 0.05$). Capital letters compare water conditions within carnitine levels, and lowercase letters compare between carnitine and control within each water condition

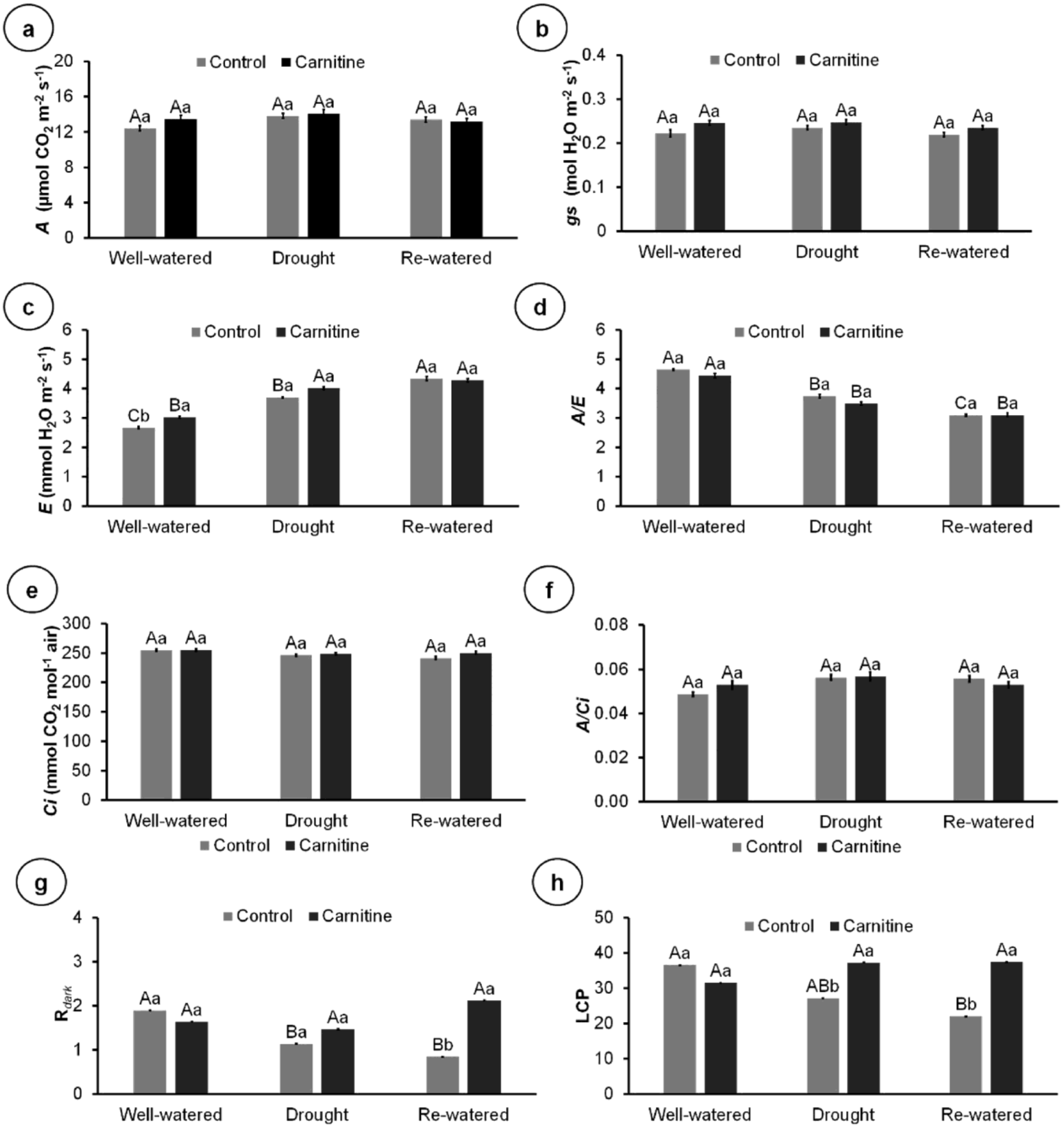

Figure 4 Gas exchange parameters of 100-day-old *Eryngium foetidum* L. plants sprayed with water or carnitine, and grown under different water conditions. *A*, net carbon assimilation rate (**a**); gs, stomatal conductance (**b**); *E*, evapotranspiration rate (**c**); *A*/*E*, water use efficiency(**d**); Ci, internal CO_2 concentration (**e**); *A*/Ci, carboxylation efficiency(**f**); R_{dark}, dark respiration (**g**); and LCP, light compensation point (**h**). Columns represent the mean of four replicates, and bars representthe standard error. Means followed by the same letter do not differby Tukey's test ($P \leq 0.05$). Capital letters compare water conditions within carnitine levels, and lowercase letters compare between carnitine and control within each water condition.

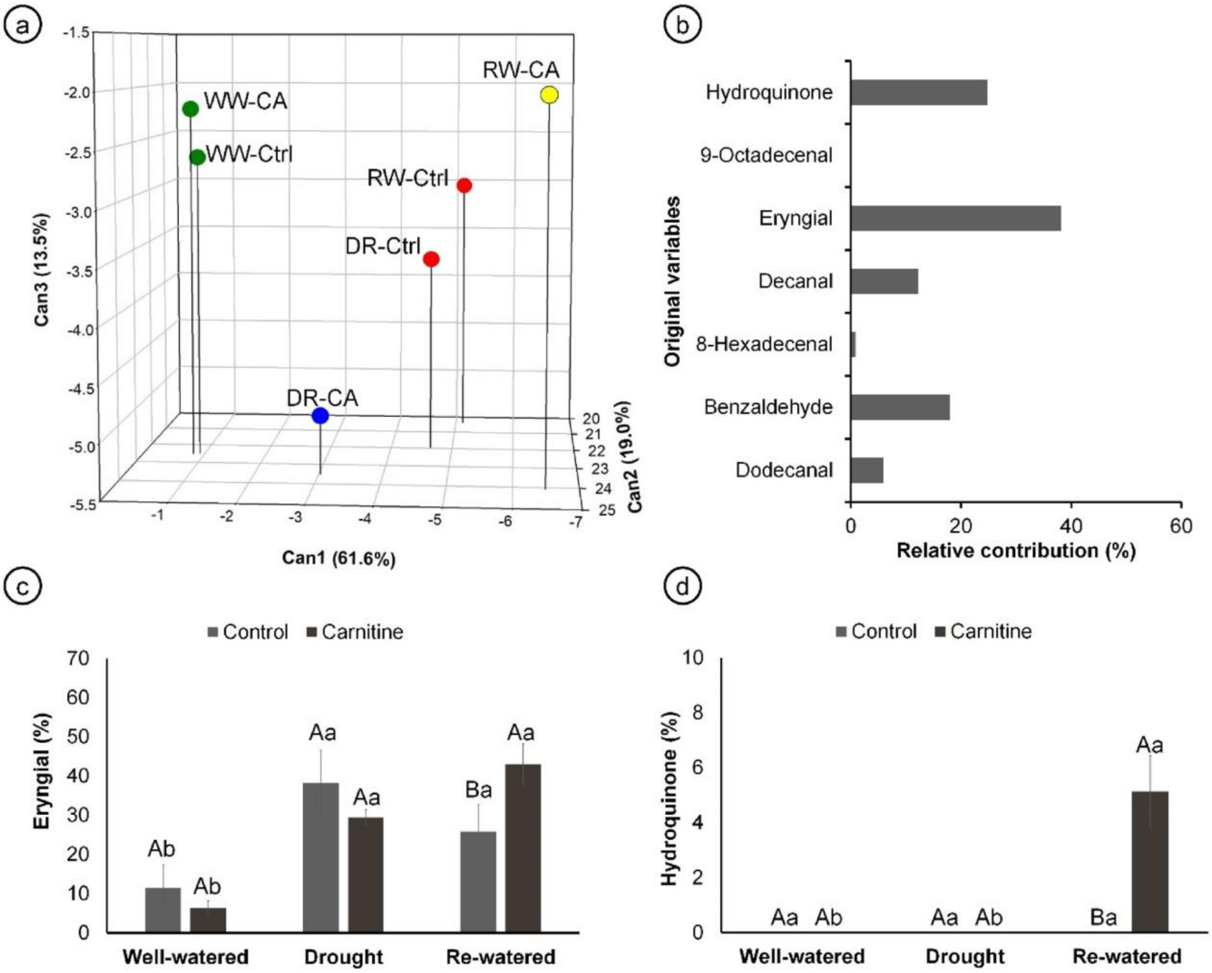

Figure 5. Essential oil profile of 102-day-old *Eryngium foetidum* L. plants sprayed with water or carnitine, and grown under different water conditions. 3D scatter-plot of the first three canonical variables (% total variance explained by each canonical component is indicated in parentheses; treatments indicated by the same color were assembled into the same group by the Tocher optimization method and the generalized squared interpoint distance of Mahalanobis; WW- Ctrl: well-watered + water application, WW-CA: well-watered + carnitine application, DR-Ctrl: drought + water application, DR-CA: drought + carnitine application, RW-Ctrl: re-watered + water application, RW-CA: re-watered + carnitine application) (**a**); Relative contributions of the original variables, calculated using the Singh method, to the canonical variables (**b**); production of eryngial (**c**); and production of hydroquinone (**d**). Means followed by the same letter do not differ by Tukey's test ($P \leq 0.05$). Capital letters compare among water conditions, and lowercase letters compare between carnitine and control within each water condition

5.4 DISCUSSION

Water is important for plant growth and food production; thus, it is essential to comprehend plant responses under drought conditions to increase crop production (Henschel et al. 2022). Here, the aerial part growth of culantro plants was impaired by drought and increased by re-watering, while root growth was increased by drought (Figure 1). Drought induced reductions in cell division and turgor are responsible for the decrease in leaf area (Tardieu et al. 2014); however, even under water stress, plants can maximize water uptake from the soil by increasing the root growth, which is one of the main survival strategies of plants under drought conditions. During periods of water scarcity, the root system undergoes morphological changes to enhance its ability to absorb water and nutrients, such us deeper roots due increased root growth rate and greater lateral root growth, and these modifications can be attributed to coordinated cell division, elongation, and differentiation events in the root apex (Lynch et al. 2018; Dinneny 2019; Gupta et al. 2020). Here, there probably were changes in cell division and differentiation, as evidenced by the increase in root length.

Re-watering increased the number of leaves and leaf area, which increased the photosynthetic capacity of plants, increasing the fresh and dry mass (Figures. 1, 2, 4). This increase in plant growth after a period of water stress followed by re-watering is an important plant survival strategy, since enhances its adaptation to drought (Sun et al. 2016; Gupta et al. 2020). Here, carnitine not increased root mass (Figure 1), unlike reported by Lelandais-Brière et al. (2007) and Santos et al. (2022c), in which carnitine application increased root density and growth in Arabidopsis and culantro, respectively. Re-watering altered the shoot/root ratio of the plants compared to drought, leading to more biomass allocated to shoots than roots (Figure 1). Rapid leaf growth after re-watering is essential to maximize light capture and, consequently, biomass accumulation (Xu and Zhou 2006; Toscano et al. 2014).

The fact that drought reduce leaf growth, resulting in a higher cell density per area (Ren et al. 2019), can explain the increase in pigment levels under this condition. Carnitine application increased the photosynthetic pigments content in well-watered and re-watered (Figure 3), showing that this compound can act in the signaling of the synthesis pathways of these pigments within the chloroplast. Increased dark respiration in re-watered + carnitine (Figure 4g) may have occurred due the transport of acyl-CoA to mitochondria during gluconeogenesis, which is mediated by carnitine. This process increases plant respiration, which can contribute to the recovery of growth and regeneration (Steiber et al. 2004). This higher respiration caused by carnitine application also increased LCP, indicating lower light use efficiency (Song et al. 2015).

Secondary metabolites are responsible for plant adaptation and survival, especially under unfavorable conditions (Takshak and Agrawal 2019). Plants modulate their secondary metabolism in response to stress factors, which can improve the quality of medicinal and aromatic plants (Szabó et al. 2017; Costa et al. 2020). Culantro is recognized by its aroma, given by the essential oils (Singh et al. 2014). Water stress induces changes in the profile of essential oils in culantro, and the application of carnitine in these plants under drought induced further qualitative changes in the composition of volatiles (Figure 5a, b). The compounds that contributed the most to the difference among these profiles were eryngial and hydroquinone (Figure 5b–d). Amino acids have been reported to alter the profile of secondary metabolites in plants, as demonstrated by Talaat et al. (2014), where the application of tyrosine and phenylalanine resulted in qualitative differences in the essential oils in *Ammi visnaga*. Amino acids participate in the synthesis of other compounds, such as proteins, vitamins, enzymes, and terpenoids. The canonical discriminant analysis showed that watering treatment and carnitine application interact to determine the essential oil profiles of the culantro plants (Figure 5a). Martins et al. (2003) and Chandrika et al. (2015) found different profiles of essential oils in culantro depending on the geographic location where it was collected, showing that ambient conditions influence the composition of these compounds. Rodrigues et al. (2020) also reported that mineral fertilizer composition can change the essential oil composition in the leaves and roots of culantro.

Here, drought and re-watering increased the production of eryngial, which is the major component of culantro essential oil (Darriet et al. 2014; Thomas et al. 2017; Rodrigues et al. 2020). Eryngial is an aliphatic and aromatic aldehyde with antibacterial activity and responsible for the flavor and aroma of culantro and other plants (Abiko et al. 2020; Kara- kaya et al. 2020). In addition, it has anthelmintic, antibacterial, and anti-inflammatory activity, being characterized as a yellowish oil with a pungent odor (Paul et al. 2011; Forbes et al. 2014). Considering that aldehydes, such as eryngial, are derived from α- or β-oxidation of fatty acids (Bridgemohan et al. 2021), and that carnitine is involved in fatty acids catabolism (Bourdin et al. 2007), it may explain the increase in eryngial upon carnitine application found here.

Besides, in re-watered plants, carnitine induced the pro- duction of hydroquinone, which is an aromatic phenolic compound derivative from benzene (Cabrera-Alonso et al. 2019; Sun et al. 2021). Hydroquinone is used in the production of antioxidants, agrochemicals, and photographic paper (Jeyanthi et al. 2021). In the cosmetic industry, it is also used as a skin lightener and to treat hyperpigmentation, such as melasma, freckles, senile lentigines, and chloasma (Elferjani et al. 2017). Furthermore, it also shows antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa (Ma et al. 2019; Jeyanthi et al. 2021). Hydroquinone has been found in some plant species, such as Majorana hortensis, *Arctostaphylos uva-ursi*, *Vaccinium vitis idaea*, *Pyrus communis*, and *Ecdysanthera rosea* (Zhu et al. 2010; Rychlinska and Nowak 2012). However, here we report the presence of hydroquinone in culantro for the first time. It is noteworthy that this compound only appeared with the specific combination of re-watering with carnitine application. The carnitine metabolism in plants and its relation with secondary metabolites are still unclear, but here the application of this amino acid increased the production of eryngial and promoted the production of hydroquinone, indicating that this compound may be related to secondary metabolites pathways.

5.5 CONCLUSION

Culantro plants increase biomass when subjected to a period of drought followed by rehydration. Foliar application of carnitine leads to changes in the synthesis of photosynthetic pigments and essential oil profile, including the induction of hydroquinone production when this application is combined with re-watering. The application of exogenous carnitine can be a strategy to induce the production of essential oil compounds of cosmetic and pharmaceutical importance in culantro. However, more research is needed to understand the action of carnitine in the production of secondary metabolites in plants.

REFERENCES

ABIKO, Y.; OKADA, M.; AOKI, H.; MIZOKAWA, M.; KUMAGAI, Y. A strategy for repression of arsenic toxicity through nuclear factor E2 related factor 2 activation mediated by the (E)-2-alkenals in Coriandrum sativum L. leaf extract. **Food and Chemical Toxicology**, v. 145, p. 111706, 2020.

ABRAMOFF, M. D.; MAGELHAES, P. J.; RAM, S. J. Image processing with ImageJ. **Biophotonics international**, v. 11, n. 7, p. 36-42, 2004.

AMI, K.; PLANCHAIS, S.; CABASSA, C.: GUIVARC’H, A.; VERY, A. A.; KHELIFI, M.; DJEBBAR, R.; ABROUS-BELBACHIR, O.; CAROL, P. Different proline responses of two Algerian durum wheat cultivars to in vitro salt stress. **Acta physiologiae plantarum**, v. 42, n. 2, p. 21, 2020.

Barr HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for BARR, H. D.; WEATHERLEY, P. E. A re-examination of the relative turgidity technique for estimating water deficits in leaves. **Australian journal of biological sciences**, v. 15, n. 3, p. 413-428, 1962.

BHAVANA, G. P.; CHANDRIKA, R.; THARA SARASWATHI, K. J. Quantitative determination of secondary compounds in populations of *Eryngium foetidum* L. from India. **Int J Curr Sci**, p. 24-28, 2013.

BÖTTGER, A.; VOTHKNECHT, U.; BOLLE, C.; WOLF, A. Plant secondary metabolites and their general function in plants. **Lessons on Caffeine, Cannabis & Co: Plant-derived drugs and their interaction with human receptors**, p. 3-17, 2018.

BOURDIN, B.; ADENIER, H.; PERRIN, Y. Carnitine is associated with fatty acid metabolism in plants. **Plant Physiology and Biochemistry**, v. 45, n. 12, p. 926-931, 2007.

BRIDGEMOHAN, P.; BRIDGEMOHAN, R. S.; MOHAMMED, M.; DEITCH, M.; SARRAN, H. Comparative analysis of the odorants of cilantro (*Coriandrum sativum* L.) and culantro (*Eryngium foetidum* L.,) as aromatic crop mimics of family: Apiaceae. **Journal of Horticulture and Postharvest Research**, v. 4, n. 4, p. 479-496, 2021.

CABRERA-ALONSO, R.; GUEVARA, E.; RAMÍREZ-ELÍAS, M. G.; MONCADA, B.; GONZÁLEZ, F. J. Surface-enhanced Raman scattering of hydroquinone assisted by gold nanorods. **Journal of Nanophotonics**, v. 13, n. 3, p. 036006-036006, 2019.

CASTRO, K. M.; BATISTA, D. S.; SILVA, T. D.; FORTINI, E. A.; FELIPE, S. H. S.; FERNANDES, A. M.; OTONI, W. C. Water deficit modulates growth, morphology, and the essential oil profile in *Lippia alba* L.(Verbenaceae) grown in vitro. **Plant Cell, Tissue and Organ Culture (PCTOC)**, v. 141, p. 55-65, 2020.

CHANDRIKA, R.; SARASWATHI, K. J. T.; MALLAVARAPU, G. R. Constituents of the essential oils of the leaf and root of *Eryngium foetidum* L. from two locations in India. **Journal of Essential Oil Bearing Plants**, v. 18, n. 2, p. 349-358, 2015.

CHARRIER, A.; RIPPA, S.; YU, A.; NGUYEN, P. J.; RENOU, J. P.; PERRIN, Y. The effect of carnitine on Arabidopsis development and recovery in salt stress conditions. **Planta**, v. 235, p. 123-135, 2012.

CRUZ, C. D. Genes Software-extended and integrated with the R, Matlab and Selegen. **Acta Scientiarum. Agronomy**, v. 38, n. 4, p. 547-552, 2016.

DARRIET, F.; ANDREANI, S.; CIAN, M. C.; COSTA, J.; MUSELLI, A. Chemical variability and antioxidant activity of *Eryngium maritimum* L. essential oils from Corsica and Sardinia. **Flavour and Fragrance Journal**, v. 29, n. 1, p. 3-13, 2014.

DINNENY, J. R. Developmental responses to water and salinity in root systems. **Annual Review of Cell and Developmental Biology**, v. 35, n. 1, p. 239-257, 2019.

ELFERJANI, H. S.; AHMIDA, N. H.; AHMIDA, A. Determination of hydroquinone in some pharmaceutical and cosmetic preparations by spectrophotometric method. **International Journal of Science and Research**, v. 6, p. 2219-2224, 2017.

FORBES, W. M.; GALLIMORE, W. A.; MANSINGH, A.; REESE, P. B.; ROBINSON, R. D. Eryngial (trans-2-dodecenal), a bioactive compound from *Eryngium foetidum*: its identification, chemical isolation, characterization and comparison with ivermectin in vitro. **Parasitology**, v. 141, n. 2, p. 269-278, 2014.

FRANK, J. A.; MORONI, M.; MOSHOURAB, R.; SUMSER, M.; LEWIN, G. R.; TRAUNER, D. Photoswitchable fatty acids enable optical control of TRPV1. **Nature communications**, v. 6, n. 1, p. 7118, 2015.

GUPTA, A.; RICO-MEDINA, A.; CAÑO-DELGADO, A. I. The physiology of plant responses to drought. **Science**, v. 368, n. 6488, p. 266-269, 2020.

HENSCHEL, J. M.; DANTAS, E. F. O.; DE AZEVEDO SOARES, V.; SANTOS, S. K.; SANTOS, L. W. O.; DIAS, T. J.; BATISTA, D. S. Salicylic acid mitigates the effects of mild drought stress on radish (*Raphanus sativus*) growth. **Functional Plant Biology**, v. 49, n. 9, p. 822-831, 2022.

HENSCHEL, J. M.; DANTAS, E. F. O.; DE AZEVEDO SOARES, V.; SANTOS, S. K.; GOMES, D. S.; FERREIRA, L. M.; BATISTA, D. S. Drought stress mitigation by foliar application of L-carnitine and its effect on radish morphophysiology. **Physiology and Molecular Biology of Plants**, v. 29, n. 4, p. 579-590, 2023.

JARAMILLO, B. E.; DUARTE, E.; MARTELO, I. Volatile chemical composition of the essential oil from Colombian *Eryngium foetidum* L. and determination of its antioxidant activity. **Revista Cubana de Plantas Medicinales**, v. 16, n. 2, p. 140-150, 2011.

JEYANTHI, V.; VELUSAMY, P.; KUMAR, G. V.; KIRUBA, K. Effect of naturally isolated hydroquinone in disturbing the cell membrane integrity of Pseudomonas aeruginosa MTCC 741 and Staphylococcus aureus MTCC 740. **Heliyon**, v. 7, n. 5, 2021.

KARAKAYA, S.; ÖZDEMIR, Ö.; KOCA, M.; DEMIRCI, B.; AKSAKAL, Ö.; TURKEZ, H.; BASER, K. H. C. Cytotoxic effect and molecular docking studies of essential oils of *Cymbocarpum erythraeum* (DC.) Boiss.(Apiaceae) as potential inhibitors of cholinesterase. **Journal of Essential Oil Research**, v. 32, n. 5, p. 436-448, 2020.

KULAK, M.; OZKAN, A.; BINDAK, R. A bibliometric analysis of the essential oil-bearing plants exposed to the water stress: How long way we have come and how much further?. **Scientia horticulturae**, v. 246, p. 418-436, 2019.

LELANDAIS-BRIÈRE, C.; JOVANOVIC, M.; TORRES, G. A.; PERRIN, Y.; LEMOINE, R.; CORRE-MENGUY, F.; HARTMANN, C. Disruption of AtOCT1, an organic cation transporter gene, affects root development and carnitine-related responses in Arabidopsis. **The Plant Journal**, v. 51, n. 2, p. 154-164, 2007.

LIANG, G.; BU, J.; ZHANG, S.; JING, G.; ZHANG, G.; LIU, X. Effects of drought stress on the photosynthetic physiological parameters of Populus× euramericana “Neva”. **Journal of Forestry Research**, v. 30, p. 409-416, 2019.

LYNCH, J. P. Rightsizing root phenotypes for drought resistance. **Journal of Experimental Botany**, v. 69, n. 13, p. 3279-3292, 2018.

MA, C.; HE, N.; ZHAO, Y.; XIA, D.; WEI, J.; KANG, W. Antimicrobial mechanism of hydroquinone. **Applied Biochemistry and Biotechnology**, v. 189, p. 1291-1303, 2019.

KOUITCHEU MABEKU, L. B.; EYOUM BILLE, B.; NGUEPI, E. In vitro and In vivo anti-Helicobacter activities of *Eryngium foetidum* (Apiaceae), *Bidens pilosa* (Asteraceae), and *Galinsoga ciliata* (Asteraceae) against Helicobacter pylori. **BioMed Research International**, v. 2016, n. 1, p. 2171032, 2016.

MARTINS, A. P.; SALGUEIRO, L. R.; DA CUNHA, A. P.; VILA, R.; CAÑIGUERAL, S.; TOMI, F.; CASANOVA, J. Essential oil composition of *Eryngium foetidum* from S. Tome e Principe. **Journal of essential oil research**, v. 15, n. 2, p. 93-95, 2003.

NARGESI, M.; SEDAGHATHOOR, S. M.; HASHEMABADI, D. S. Effect of arginine, glutamine, humic acid, and fulvic acid spraying on olive cultivars in saline conditions. **Plant Physiology Reports**, v. 27, n. 2, p. 295-307, 2022.

ONEY-BIROL, S. Exogenous L-carnitine promotes plant growth and cell division by mitigating genotoxic damage of salt stress. **Scientific reports**, v. 9, n. 1, p. 17229, 2019.

PAUL, J. H.; SEAFORTH, C. E.; TIKASINGH, T. *Eryngium foetidum* L.: A review. **Fitoterapia**, v. 82, n. 3, p. 302-308, 2011.

QUYNH, C. T. T.; KUBOTA, K. Aroma constituents and enzyme activities of Japanese long coriander leaves (Culantro, *Eryngium foetidum* L.). **Food science and technology research**, v. 18, n. 2, p. 287-294, 2012.

REN, T.; WERADUWAGE, S. M.; SHARKEY, T. D. Prospects for enhancing leaf photosynthetic capacity by manipulating mesophyll cell morphology. **Journal of Experimental Botany**, v. 70, n. 4, p. 1153-1165, 2019.

RODRIGUES, T. L. M.; CASTRO, G. L. S.; VIANA, R. G.; GURGEL, E. S. C.; SILVA, S. G.; DE OLIVEIRA, M. S.; ANDRADE, E. H. D. A. Physiological performance and chemical compositions of the *Eryngium foetidum* L. (Apiaceae) essential oil cultivated with different fertilizer sources. **Natural Product Research**, v. 35, n. 23, p. 5544-5548, 2021.

RODRIGUES, T. L.; SILVA, M. E.; GURGEL, E. S.; OLIVEIRA, M. S.; LUCAS, F. C. *Eryngium foetidum* L.(Apiaceae): A literature review of traditional uses, chemical composition, and pharmacological activities. **Evidence-Based Complementary and Alternative Medicine**, v. 2022, n. 1, p. 2896895, 2022.

ROJAS-SILVA, P.; GRAZIOSE, R.; VESELY, B.; POULEV, A.; MBEUNKUI, F.; GRACE,

M. H.; RASKIN, I. Leishmanicidal activity of a daucane sesquiterpene isolated from *Eryngium foetidum*. **Pharmaceutical Biology**, v. 52, n. 3, p. 398-401, 2014.

RYCHLINSKA, I.; NOWAK, S. Quantitative determination of arbutin and hydroquinone in different plant materials by HPLC. **Notulae Botanicae Horti Agrobotanici Cluj-Napoca**, v. 40, n. 2, p. 109-113, 2012.

SAI, S. K. P. V.; SANDHYA, V.; SHRIVASTAVA, M.; SKZ, A. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. **Microbiological research**, v. 184, p. 13-24, 2016.

SANTOS, R. P.; CRUZ, A. C. F. D.; IAREMA, L.; KUKI, K. N.; OTONI, W. C. Protocolo para extração de pigmentos foliares em porta-enxertos de videira micropropagados. Rev Ceres 55:356–364, 2008.

SANTOS, A. R.; MELO, Y. L.; OLIVEIRA, L. F.; CAVALCANTE, I. E.; SOUZA FERRAZ, R. L.; SILVA SÁ, F. V.; MELO, A. S. Exogenous silicon and proline modulate osmoprotection and antioxidant activity in cowpea under drought stress. **Journal of Soil Science and Plant Nutrition**, v. 22, n. 2, p. 1692-1699, 2022.

SANTOS, S. K.; GOMES, D. S.; SANTOS, L. W. O.; AZEVEDO SOARES, V.; DANTAS, E. F. O.; HENSCHEL, J. M.; BATISTA, D. S. Exogenous carnitine mitigates the deleterious effects of mild-water stress on arugula by modulating morphophysiological responses. **Journal of Plant Growth Regulation**, v. 42, n. 7, p. 4073-4082, 2023.

SANTOS, S. K.; AZEVEDO SOARES, V.; DANTAS, E. F. O.; SANTOS, L. W. O.; GOMES, D. S.; HENSCHEL, J. M.; BATISTA, D. S. Exogenous carnitine application enhances the growth of culantro (*Eryngium foetidum*) plants. **Vegetos**, v. 36, n. 2, p. 393-399, 2023.

SHAVANDI, M. A.; HADDADIAN, Z.; ISMAIL, M. H. S. (2012) *Eryngium foetidum L. Coriandrum sativum and Persicaria odorata L.: a review.* **Journal of Asian Scientific Research**, v. 2, n. 8, p. 410, 2012.

SINGH, D. The relative importance of characters affecting genetic divergence. **Indian Journal of Genetics and Plant Breeding (The)**, v. 41, n. 2, p. 237-245, 1981.

SINGH, S.; SINGH, D. R.; BANU, S.; SALIM, K. M. Determination of bioactives and antioxidant activity in *Eryngium foetidum* L.: a traditional culinary and medicinal herb. **Proceedings of the National Academy of Sciences, India Section B: Biological Sciences**, v. 83, p. 453-460, 2013.

SINGH, B. K.; RAMAKRISHNA, Y.; NGACHAN, S. V. Spiny coriander (*Eryngium foetidum* L.): a commonly used, neglected spicing-culinary herb of Mizoram, India. **Genetic Resources and Crop Evolution**, v. 61, n. 6, p. 1085-1090, 2014.

SOARES, V. A.; DANTAS, E. F. O.; SANTOS, S. K.; SANTOS, L. W. O.; DIAS, T. J.; HENSCHEL, J. M.; BATISTA, D. S. Effect of salicylic acid on the growth and biomass partitioning in water-stressed radish plants. **Vegetos**, v. 35, n. 3, p. 585-591, 2022.

SONG, L.; ZHANG, Y. J.; CHEN, X.; LI, S.; LU, H. Z.; WU, C. S.; SHI, X. M. Water relations and gas exchange of fan bryophytes and their adaptations to microhabitats in an Asian subtropical montane cloud forest. **Journal of Plant Research**, v. 128, p. 573-584, 2015.

STEIBER, A.; KERNER, J.; HOPPEL, C. L. Carnitine: a nutritional, biosynthetic, and functional perspective. **Molecular aspects of medicine**, v. 25, n. 5-6, p. 455-473, 2004.

SUN, C.; GAO, X.; CHEN, X.; FU, J.; ZHANG, Y. Metabolic and growth responses of maize to successive drought and re-watering cycles. **Agricultural water management**, v. 172, p. 62-73, 2016.

SUN, X.; KOLLING, D. R.; SMYTHERS, A. L.; DEAL, R. A. Investigations of the photochemical charge-transfer reduction of uranyl UO22+ (VI) to uranyl UO2+ (V) by benzene-1, 4-diol (1, 4-C6H4 (OH) 2) and oxalate (C2O42−) by UV–Vis, electron paramagnetic resonance, and luminescence spectroscopies. **Inorganica Chimica Acta**, v. 525, p. 120451, 2021.

SZABÓ, K.; RADÁCSI, P.; RAJHÁRT, P.; LADÁNYI, M.; NÉMETH, É. Stress-induced changes of growth, yield and bioactive compounds in lemon balm cultivars. **Plant Physiology and Biochemistry**, v. 119, p. 170-177, 2017.

TAKSHAK, S.; AGRAWAL, S. B. Defense potential of secondary metabolites in medicinal plants under UV-B stress. **Journal of Photochemistry and Photobiology B: Biology**, v. 193, p. 51-88, 2019.

TALAAT, I. M.; KHATTAB, H. I.; AHMED, A. M. Changes in growth, hormones levels and essential oil content of Ammi visnaga L. plants treated with some bioregulators. **Saudi Journal of Biological Sciences**, v. 21, n. 4, p. 355-365, 2014.

TARDIEU, F.; PARENT, B.; CALDEIRA, C. F.; WELCKER, C. Genetic and physiological controls of growth under water deficit. **Plant physiology**, v. 164, n. 4, p. 1628-1635, 2014.

THOMAS, P. S.; ESSIEN, E. E.; NTUK, S. J.; CHOUDHARY, M. I.; THOMAS, P. S.; ESSIEN, E. E.; NTUK, S. J.; CHOUDHARY, M. I. *Eryngium foetidum* L. essential oils: chemical composition and antioxidant capacity. **Medicines**, v. 4, n. 2, p. 24, 2017.

TOSCANO, S.; SCUDERI, D.; GIUFFRIDA, F.; ROMANO, D. Responses of Mediterranean ornamental shrubs to drought stress and recovery. **Scientia Horticulturae**, v. 178, p. 145-153, 2014.

TURK, H.; ERDAL, S.; DUMLUPINAR, R. Exogenous carnitine application augments transport of fatty acids into mitochondria and stimulates mitochondrial respiration in maize seedlings grown under normal and cold conditions. **Cryobiology**, v. 91, p. 97-103, 2019.

TURK, H.; ERDAL, S.; DUMLUPINAR, R. Carnitine-induced physio-biochemical and molecular alterations in maize seedlings in response to cold stress. **Archives of Agronomy and Soil Science**, v. 66, n. 7, p. 925-941, 2020.

WELLBURN, A. R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. **Journal of plant physiology**, v. 144, n. 3, p. 307-313, 1994.

WINK, M. (2015) Modes of action of herbal medicines and plant secondary metabolites. **Medicines**, v. 2, n. 3, p. 251-286, 2015.

XU, Z. Z.; ZHOU, G. S. Nitrogen metabolism and photosynthesis in Leymus chinensis in response to long-term soil drought. **Journal of Plant Growth Regulation**, v. 25, p. 252-266, 2006.

ZHU, X.; ZHANG, Q.; KONG, L.; WANG, F.; LUO, S. New hydroquinone diglycoside acyl esters and sesquiterpene and apocarotenoid from Ecdysanthera rosea. **Fitoterapia**, v. 81, n. 7, p. 906-909, 2010.

6 CAPÍTULO III: ASCORBIC ACID MEDIATED MITIGATION OF DROUGHT EFFECTS ON GROWTH, PHYSIOLOGY, AND ESSENTIAL OIL PROFILE IN CULANTRO (Eryngium *foetidum* L.)

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Abstract

The negative effects of drought can be reduced by the application of ascorbic acid (AsA), an important non-enzymatic antioxidant in plants. Culantro (*Eryngium foetidum* L.) is a perennial leafy herb rich in essential oils, used in both culinary and traditional medicine. In this study, we assessed the action of AsA on the growth, morphophysiology, and essential oil profile of culantro plants under water stress. For this purpose, plants were cultivated under well-irrigated and drought conditions, and sprayed with AsA (100 µM) or water (control). Drought reduced growth and *F/FM*, and increased transpiration and pigment concentration. AsA, on the other hand, restored pigment concentration to non-stress conditions. Furthermore, drought and exogenous AsA led to changes in the essential oil profile. The application of AsA and drought modulated qualitatively the essential oil profile in culantro, with dodecanal being the compound that most contributed to this profile separation. Thus, the application of 100 µM AsA is a possible strategy for modifying the production of compounds in culantro. This is the first report that exogenous AsA and drought affect the growth and essential oil composition of culantro. These results offer new insights and contribute to understanding the effects of AsA on drought mitigation, as well as its modulation of the essential oil profile in aromatic plant species.

Keywords: antioxidant; aromatic plant; bioregulator; dodecanal; medicinal species.

6.1 INTRODUCTION

Drought, the most common climatic constraint, is characterized by water scarcity, high temperatures, and elevated evaporation rates (Raza et al. 2019; Bandurska 2022). In agriculture, drought’s impact intensifies due to water scarcity and increasing food demand driven by population growth (Seleiman et al. 2021). Drought reduces cellular water content, negatively impacting plant physiology, morphology, and biochemistry. It triggers stomatal closure, diminishing CO2 absorption, disrupting photosynthesis, and elevating reactive oxygen species (ROS) concentrations (Claeys 2013; Muthoni and Kabira 2016; Nasir and Toth 2022). Plants employ mechanisms, including enhanced root development and the prowduction of antioxidants, to mitigate water stress damage (Bandurska 2022; Nasir and Toth 2022). Techniques like exogenous application of biostimulants, such as ascorbic acid, can be employed to alleviate drought effects (Henschel et al. 2023).

Ascorbic acid (AsA) is a water-soluble, non-enzymatic antioxidant molecule capable of controlling and eliminating hydrogen peroxide (H_2O_2) concentrations using ascorbate peroxidase

(APX) (Malik et al. 2015; El-Beltagi et al. 2022). AsA serves as a cofactor for enzymes that regulate hormone biosynthesis, contributing to the modulation of plant metabolism (Oliveira et al. 2020). Thus, the negative impacts of drought can be reduced through the exogenous application of AsA (Khazaei and Estaji 2020; Naz et al. 2022).

Some plants are rich in phytochemicals such as essential oils, flavonoids, and coumarins, as exemplified by *Eryngium foetidum* L. (Paul et al. 2011). This is a leafy plant, known as culantro or wild coriander, belonging to the Apiaceae family and native to Central America (Singh et al. 2013). In traditional medicine, *E. foetidum* has been used to treat flu, hypertension, constipation, diarrhea, and malaria (Paul et al. 2011). Due to its aromatic similarity to *Coriandrum sativum*, its leaves are often used in cooking (Thomas et al. 2017). *E. foetidum* is rich in essential oils, composed of compounds effective against parasitic trypanosomes, nematodes, fungi, and bacteria in humans and mammals (Rodrigues et al. 2021).

Water conditions and cultivation practices, such as the application of plant regulators, lead to changes in essential oil profiles (Said-Al Ahl et al. 2014; Hazzoumi et al. 2015; Nasiri et al. 2018; Moreno-Pizani et al. 2020). AsA application has been shown to affect the essential oil profile in *Coriandrum sativum* (Said-Al Ah et al. 2014) and *Dracocephalum moldavica* (Nasiri et al. 2018), while water stress increased the concentration of essential oils in *Cymbopogon winterianus* (Costa et al. 2020). Therefore, to test the hypothesis that AsA can mitigate water stress and modulate the essential oil profile in *E. foetidum*, the objective of this study was to evaluate the effect of AsA on the growth, morphophysiology, and essential oil profile in plants of this species under water stress.

6.2 MATERIALS AND METHODS

6.2.1 Plant material and experimental localization

The study took place from January to May in a greenhouse at the Seedling Production Laboratory of the Federal University of Paraiba, in Bananeiras, Brazil. The greenhouse was covered with transparent film and located at coordinates 6°45′S, 35°38′W, with an elevation of 526 m. Culantro seeds (*E. foetidum* L.) were provided by local farmers from rural communities in Areia, Brazil, situated at 6°57′S, 35°41′W, with an elevation of 623 m.

6.2.2 Ascorbic acid and drought treatments

Culantro seeds were initially planted in 200-cell polyethylene trays with commercial substrate (Mecplant®, Telêmaco Borba, Brazil), composed by bioactivated pine bark and vermiculite, containing limestone, macro and micro- nutrients. After 35 days, when the plants had four fully-expanded leaves, they were transplanted into polyethylene bags (22 × 28 cm) with the same substrate. Irrigation was performed until 100% bag capacity for fifteen days. At 50 days after sowing, the plants underwent different irrigation levels: well-watered (80% bag capacity), drought (40% bag capacity), and re-watered (twelve days without irrigation followed by irrigation with 80% bag capacity). Addition- ally, the plants were leaf-sprayed with either water (control) or 100 µM ascorbic acid using hand sprayers every six days, with 12 mL per plant until completely wet. The twelve-day water restriction period was determined based on previous survival tests. The ascorbic acid solution included polysorbate 80 (Tween-80®, 0.03%) to enhance leaf adhesion.

6.2.3 Morphophysiological parameters

At 100 days after sowing (DAS), we examined photosynthetic pigments, gas exchange parameters, and chlorophyll a fluorescence. Concurrently, growth parameters, including leaf area, number of leaves, specific leaf area, and root length, were measured at 102 DAS. ImageJ software was used to access leaf area. After separating the plants into shoots and roots, their fresh mass was determined. Following morphological measurements, culantro plant shoots and roots were oven-dried at 65 °C until a constant weight was achieved to determine their dry weight. The shoot/root ratio was calculated as the dry weight of shoots divided by the dry weight of roots. Total biomass was determined by summing the dry weight of shoots and roots.

Gas exchange measurements were performed using an open-flow gas exchange system infrared gas analyzer (IRGA, LCpro-SD Portable Photosynthesis System, ADC BioScientific, Hoddesdon, UK), between 8:00 h and 10:00 h a.m, following the conditions described by Santos et al. (2023). The net carbon assimilation rate (A, µmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (g_S, mol H_2O $m^{-2}s^{-1}$), internal CO_2 concentration (Ci, mmol CO_2 mol^{-1} air), leaf transpiration rate (E, mmol H_2O $m^{-2}s^{-1}$), water use efficient (A/E), carboxylation efficiency (A/Ci), and dark respiration (µmol $m^{-2}s^{-1}$) were determined. The chlorophyll a fluorescence parameters: minimum chlorophyll a fluorescence in the dark-adapted state (F_0), maximum chlorophyll a fluorescence in the dark-adapted state (F_M), variable fluorescence (F_V), variable to minimum fluorescence ratio (F_V/F_0), and maximum quantum yield of PSII (F_V/F_M), were determined with a portable fluorometer (OS-30p+, Opti-Sciences, Hud- son, NY, USA), as described by Santos et al. (2023).

Photosynthetic pigment levels were assessed following Wellburn's method (1994) with modifications. Four 1 cm^2 leaf discs from each treatment underwent a 48-hour incubation in dark conditions with 7 mL dimethyl sulfoxide (Santos et al. 2008). The resulting extract was spectrophotometrically analyzed at 480, 649, and 665 nm (GTA-96 UV-VIS, Global Trade Technology, São Paulo, Brazil), determining chlorophyll a, chlorophyll b, chlorophyll a/b ratio, total chlorophylls, and total carotenoids.

6.2.4 Microextraction and qualitative analysis of essential oils

About 500 mg of leaves were gathered and stored at -18 °C in test tubes with screw caps, following Castro et al. (2020) with modifications. Following freezing, 1 mL of methanol was added to each sample. To expedite the extraction, samples were subjected to 10 min of ultrasonic bath treatment (Ultra Cleaner 800, UNIQUE) at 40 kHz and 25 °C. The resulting supernatant was then filtered through a sterile cotton wick. Samples containing 1 µL of the clear solution with the extracted oils were subjected to gas chromatography for analysis. Qualitative assessment of essential oils was performed using a gas chromatograph coupled to a mass spectrometer (GCMS-QP2010 Plus; Shimadzu, Kyoto, Japan) and an Rtx-5MS® column (Restek, Bellefonte, PA, USA) with dimensions of 30 m × 0.25 mm, utilizing three technical replicates. The initial oven temperature was set at 50 °C, maintained for 3 min, followed by an increase of 6 °C min^{-1} to 240 °C. The injector operated in split mode (1:10) at 240 °C, and the interface and mass detector were operated at 250 °C. Helium served as the carrier gas at a flow rate of 1.69 mL min^{-1}. Identification of constituents was done by comparing obtained mass spectra with the NIST 9.0 data- base (correlation > 95%) and confirmed by the corresponding retention index (Kováts Index) compared to published data.

6.2.5 Experimental design and statistical analyzes

The experimental design was entirely randomized, in a 2 × 2 factorial scheme (ascorbic acid application × water condition) with ten replicates, with the experimental unit composed by one bag with one plant each. The data used were obtained from three time-shifted repeated experiments. Data underwent normality and homogeneity tests using the Shapiro-Wilk and Bartlett tests, respectively, and subjected to analysis of variance by F test ($P \leq 0.05$) using the Genes software (Cruz 2016). Volatiles profile date was submitted to multivariate analysis. The distance between the treatments was determined using principal components analysis in a two-dimensional scatter plot. The treatments were separated into different groups using the Tocher optimization method and generalized squared interpoint distance of Mahalanobis (D2). The grouping quality was evaluated using the co-optical correlation coefficient (r). The relative contribution of each variable to discriminate treatments was quantified using Singh's (1981) criterion.

6.3 RESULTS AND DISCUSSION

The variables showing a significant interaction among the analyzed factors were chlorophyll a and carotenoids (Table 1). For the variables root fresh mass, number of leaves, Fv/F_0, *Ci*, g_S, *A*, *A/Ci*, and dark respiration, there was no significant effect of drought, AsA application, or their interaction (Table 1). Drought significantly constrained the growth of culantro plants (Figure 1a), evidenced by a 52.14% and 52.35% reduction in the fresh and dry masses of the shoot, respectively, compared to the control (Figure 1b-c). Water plays a crucial role in plant development; thus, comprehending plant responses to water stress and devising strategies to mitigate its detrimental effects on growth is imperative (Santos et al. 2022; Khalid et al. 2023; El Boukhari et al. 2023). Water deficit conditions result in turgor loss, restricting cell expansion and adversely affecting plant growth (Ozturk et al. 2021), possibly explaining the observed reduction in culantro plant growth under drought conditions. Additionally, Santos et al. (2023) also reported similar growth reductions in culantro plants due to drought. Under drought conditions, there was a significant reduction in leaf area, root dry mass, and water use efficiency by 45.48%, 36.47%, and 24.52%, respectively, compared to the control (Figure 1d, e, g). Concurrently, transpiration increased by 22.90% under drought (Figure 1f). Water use efficiency, defined as the ratio of CO_2 diffusion in the leaf to water loss, experiences reduction due to factors such as altered CO_2 concentration at the Rubisco site or reduced carboxylation during stress (Way et al. 2014; Gholinezhad 2020). Similar effects of water stress, leading to reduced biomass and water use efficiency, have been observed in *Calendula officinalis* plants (Gholinezhad 2020).

Culantro leaves exhibited altered levels of chlorophyll *a* and carotenoids due to water stress and AsA application. Under drought conditions, there was an increase in chlorophyll a and carotenoid content compared to non-stressed control plants (Figure 2a, b). However, AsA application led to a decrease in chlorophyll *a* and leaf carotenoids in culantro plants exposed to drought, while increasing carotenoid content under non-stress conditions (Figure 2a, b). Drought also resulted in a 44.23% increase in chlorophyll b content (Figure 2c) and *a* decrease in the F_V/F_M ratio (Figure 2d).

Leaves, crucial for efficient photosynthesis, exhibit varied architectures under environmental constraints to aid acclimatization (Ren et al. 2019). In response to stress, culantro plants adapt by reducing leaf area and increasing chlorophylls. Drought-induced plants show heightened pigment concentration, potentially aiding photosynthesis recovery (Chen et al. 2016). Application of Ascorbic Acid (AsA) restores conditions akin to non-stress, highlighting its ameliorative effects. The modulation of chlorophyll and carotenoid responses is influenced by plant sensitivity (Rustioni and Bianchi 2021). Elevated carotenoid levels in culantro under water stress serve as photoprotective agents, dissipating excess energy and preventing overexcitation of photosystem II (Zhang et al. 2021). Furthermore, they act as antioxidants, reinforcing thylakoid and cell membrane integrity, contributing to plant acclimatization to stress (Young and Lowe 2018; Ahluwalia et al. 2021).

The F_V/F_M ratio, a marker of maximum PSII photochemistry efficiency, is widely employed to identify PSII disturbances induced by plant stress (Sharma et al. 2015; Badr and Brüggemann 2020). Here, drought-induced a decrease in F_V/F_M, signaling reduced PSII efficiency and disruption in the photosynthetic apparatus (Glynn et al. 2003). Values below 0.83, as observed here, are commonly associated with stressed plants, indicating diminished photochemical energy capacity within PSII (Kalaji et al. 2016; Badr and Brüggemann 2020).

Principal components, showing 98.19% of treatment variability (Figure 2e), separated treatments into two groups: group 1 (red circle) comprising non-stressed control plants (water), and group 2 (purple circle) consisting of non- stressed plants with Ascorbic Acid (AsA) application, along with control plants with AsA under drought (Figure 2e), which allowed demonstrating that both drought and exogenous application of AsA altered the essential oil profile. Essential oil components in culantro plants exhibited relative contributions between 15 and 20% (Figure 2f), with the dodecanal being the compound that contributed most to the separation between treatments in PCA. Genetic properties influence variations in essential oil content, but environmental conditions and growth-promoting substances' application can amplify secondary metabolite production, including essential oils (Gomes et al. 2013; Hemmati et al. 2018; Aftab et al. 2019). Carnitine foliar application induced changes in culantro essential oil profile, triggering hydroquinone syn- thesis (Santos et al. 2023).

Ascorbic Acid (AsA) demonstrated an enhancement in the essential oil yield of *Calendula oficinalis* plants under both stress and non-stress conditions (Hemmati et al. 2018). Its positive impact on essential oil quality is attributed to its role as a growth regulator, participation in the regeneration of metabolites like tocopherols, and functioning as an enzymatic cofactor for ascorbate peroxidase, eliminating hydrogen peroxide and singlet oxygen. AsA also safeguards enzyme catalytic sites, like hydroxylases, from irreversible oxidation by reactive oxygen species (Caverzan et al. 2012; Locato et al. 2013). Possessing redox properties, AsA acts as a cofactor for enzymes involved in hormonal biosynthesis, including cytokinins synthesized through mevalonate and methylerythritol phosphate pathways (Said-Al et al. 2014). Terpenes, widespread secondary metabolites, are formed by terpene synthase enzyme action converting isoprenoids into terpenes (Jiang et al. 2019; Camas-Reyes et al. 2022). AsA application likely increased terpenoid production, altering the essential oil profile of culantro plants. Moreover, secondary metabolites produced in response to drought and AsA application differ from those under non-stress conditions. Environmental conditions and growth regulators critically influence plant growth, development, and essential oil quality and quantity in aromatic plants (Khan et al. 2015).

In conclusion, drought reduces the growth of culantro plants. Under drought conditions, exogenous application of AsA restores pigments to non-stress conditions and alters the essential oil profile in culantro. These results provide new insights into the action of AsA in mitigating water stress and modulating the essential oil profile in aromatic plants, and they may assist in future studies aimed at obtaining specific compounds.

Table 1 Results (significance) of the ANOVA for the effects of water stress conditions, ascorbic acid treatment, and their interactions on the morphophysiological parameters of 102-day-old culantro (*Eryngium foetidum* L.) plants sprayed with ascorbic acid (100 µM) or water (0 µM), and grown under 80% (well-watered) or 15% (drought stress) of water holding capacity.

Variable	Stress	Ascorbic acid	Stress × Ascorbic acid	CV (%)
Shoot fresh mass	0.04**	15.10^{ns}	35.89^{ns}	35.43
Root fresh mass	21.07^{ns}	18.93^{ns}	47.81^{ns}	39.48
Shoot dry mass	0.12**	20.52^{ns}	45.24^{ns}	40.3
Root dry mass	2.73*	20.95^{ns}	43.32^{ns}	41.05
Number of leaves	69.00^{ns}	69.00^{ns}	86.40^{ns}	42.58
Leaf area	0.15**	13.93^{ns}	30.90^{ns}	34.56
Fv/Fm	2.38*	5.62^{ns}	9.94^{ns}	4.16
Fv/Fo	7.25^{ns}	6.25^{ns}	9.21^{ns}	13.39
Ci	89.47^{ns}	34.75^{ns}	95.78^{ns}	6.75
E	0.06**	12.10^{ns}	5.48^{ns}	10.8
gS	48.49^{ns}	78.68^{ns}	8.83^{ns}	19.04
A	47.96^{ns}	85.12^{ns}	14.32^{ns}	19.06
WUE	0.01**	9.49^{ns}	69.31^{ns}	10.93
A/Ci	60.08^{ns}	72.19^{ns}	18.45^{ns}	22.62
Dark respiration	33.91^{ns}	69.22^{ns}	38.70^{ns}	47.22
Chlorophyll *a*	1.06*	38.75^{ns}	1.31*	19.72
Chlorophyll *b*	0.07**	15.10^{ns}	8.94^{ns}	16.14
Carotenoids	0.01**	9.60^{ns}	0.01**	12.3

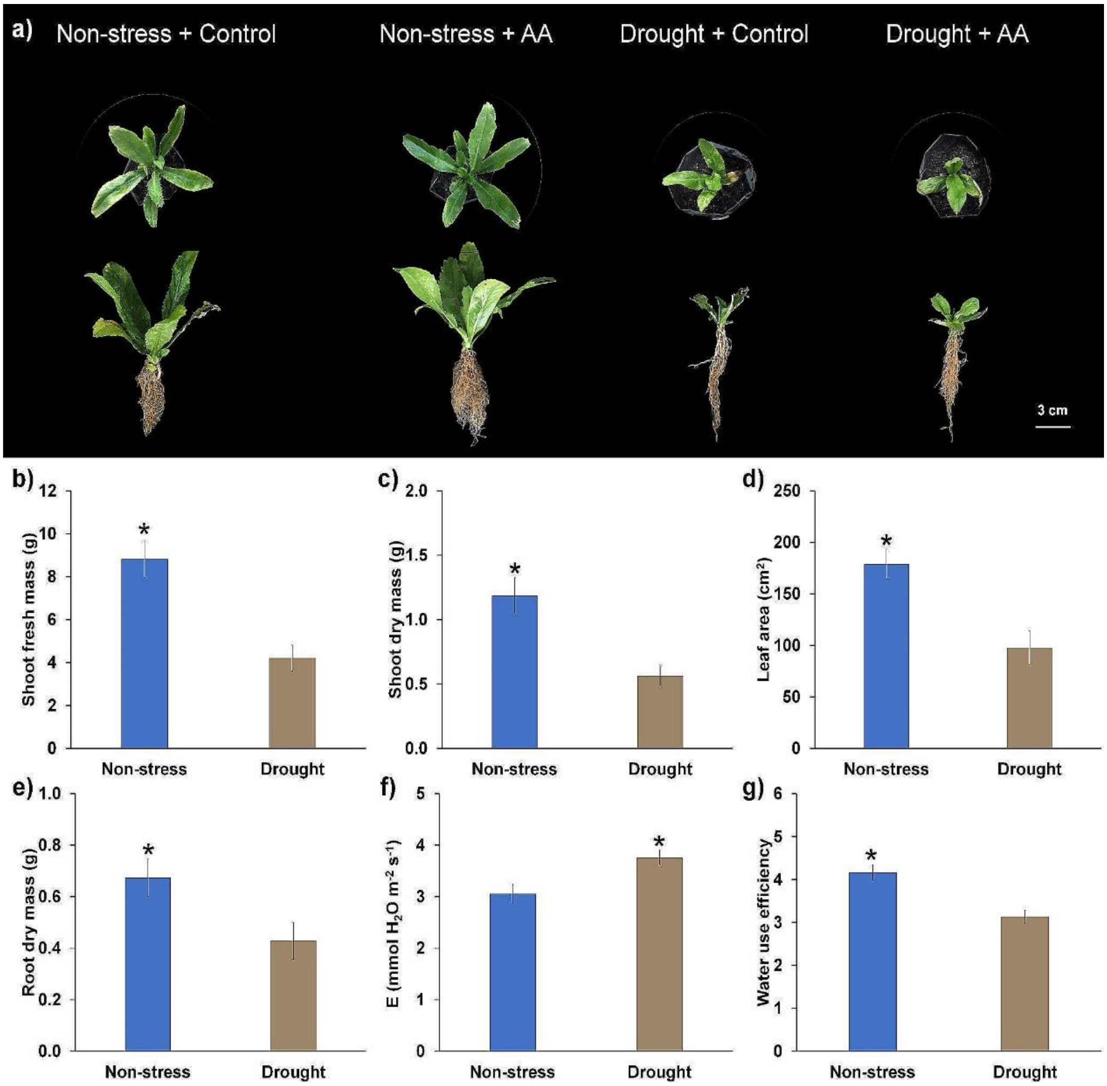

Figure 1 *Eryngium foetidum* L. plants subjected to drought and application of 100 µM ascorbic acid. Plant phenotype after 102 days of cultivation (**a**); Shoot fresh mass (**b**); Shoot dry mass (**c**); Leaf area (**d**); Root dry mass (**e**); leaf transpiration rate (**f**); and Water use efficiency (**g**). Asterisks indicate significant differences by the F-test ($P \leq 0.05$).

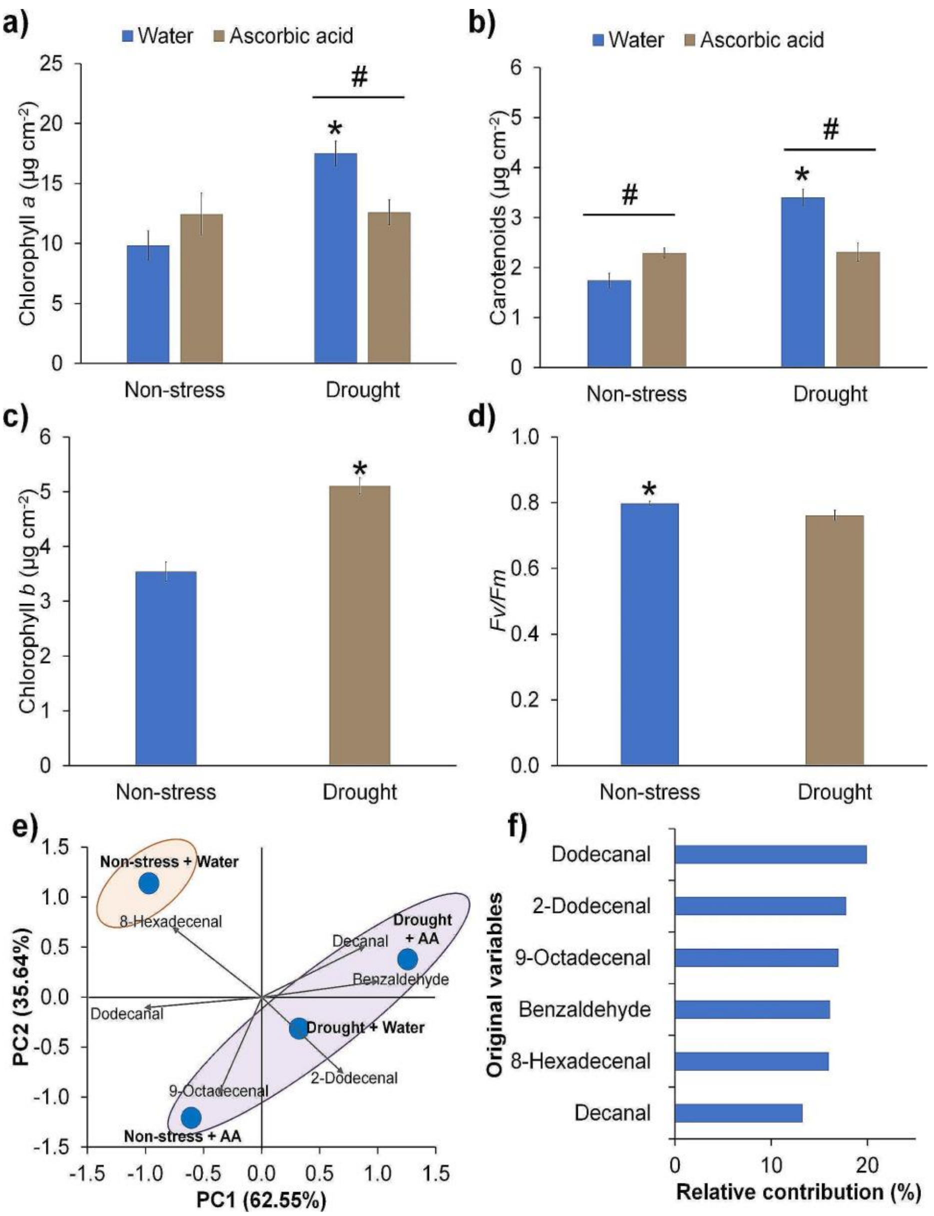

Figure 2 Chlorophyll a (a); carotenoids (b); and chlorophyll b contents (c); maximum quantum efficiency (F_V/F_M) (d); 2D scatter plot of the two principal components (% of total variance explained by each principal component is indicated in parentheses; treatments indicated by the same color were grouped into the same cluster by the Tocher optimization method and Mahalanobis generalized squared distance between points; non-stress + water; non-stress +AA (ascorbic acid); drought + water; drought +AA (e); Relative contribution of the original variables, calculated by the Singh method (f). Asterisks indicate significant differences between non-stressed and drought plants, and hashtags indicate significant differences between ascorbic acid and control (water).

REFERENCES

AHLUWALIA, O.; SINGH, P. C.; BHATIA, R. A review on drought stress in plants: implications, mitigation and the role of plant growth promoting rhizobacteria. **Resources, Environment and Sustainability**, v. 5, p. 100032, 2021

BADR, A.; BRÜGGEMANN, W. Comparative analysis of drought stress response of maize genotypes using chlorophyll fluorescence measurements and leaf relative water content. **Photosynthetica**, v. 58, n. 2, p. 38-645, 2020.

BANDURSKA, H. Drought stress responses: coping Strategy and Resistance. **Plants**, v. 11, n. 7, p. 922, 2022

CAMAS-REYES, A.; VUELVAS-NOLASCO, R.; CABRERA-PONCE, J. L. Effect of different cytokinins on shoot outgrowth and bioactive compounds profile of lemograss essential oil. **International Journal of Plant Biology**, v. 13, n. 3, p. 298-314, 2022.

CAVERZAN, A.; PASSAIA, G.; ROSA, S. B. Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection. **Genetics and molecular biology**, v. 35, p. 1011-1019, 2012.

CHEN, D.; WANG, S.; CAO, B. Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. **Frontiers in Plant Science**, v. 6, p. 1241, 2016.

CLAEYS, H.; INZÉ, D. The agony of choice: how plants balance growth and survival under water-limiting conditions. **Plant physiology**, v. 162, n. 4, p. 1768-1779, 2013.

COSTA, A. S. V.; HOTT, M. C.; HORN, A. H. Management of citronella (*Cymbopogon winterianus Jowitt ex Bor*) for the production of essential oils. **SN Applied Sciences**, v. 2, n. 12, p. 2132, 2020.

CRUZ, C. D. Genes software-extended and integrated with the R, matlab and selegen. **Acta Scientiarum. Agronomy**, v. 38, n. 4, p. 547-552, 2016.

EL-BELTAGI, H. S.; SULAIMAN.; MOHAMED, M. E. M.; ULLAH, S.; SHAH, S. Effects of ascorbic acid and/or α-Tocopherol on agronomic and physio-biochemical traits of oat (*Avena sativa* L.) under Drought Condition. **Agronomy**, v. 12, n. 10, p. 2296, 2022.

EL-BOUKHARI, M. E. M.; BARAKATE, M.; DRISSI, B.; BOUHIA, Y.; LYAMLOULI, K. Seaweed extract biostimulants differentially act in Mitigating Drought stress on Faba Bean (*Vicia faba* L). **Journal of Plant Growth Regulation**, v. 42, n. 9, p. 5642-5652, 2023.

GHOLINEZHAD, E. Impact of drought stress and stress modifiers on water use efficiency, membrane lipidation indices, and water relationship indices of pot marigold (*Calendula oficinalis* L). **Brazilian Journal of Botany**, v. 43, n. 4, p. 747-759, 2020.

GLYNN, P.; FRASER, C.; GILLIAN, A. Foliar salt tolerance of Acer genotypes using chlorophyll fluorescence. **Journal of Arboriculture**, v. 29, n. 2, p. 61-65, 2003.

GOMES, F.; TAHARA, E. B.; BUSSO, C.; KOWALTOWSKI, A. J.; BARROS, M. H. Nde1 deletion improves mitochondrial DNA maintenance in Saccharomyces cerevisiae coenzyme Q mutants. **Biochemical Journal**, v. 449, n. 3, p. 595-603, 2013

HAZZOUMI, Z.; MOUSTAKIME, Y.; ELHARCHLI, E.; JOUTEI, K. A. Effect of arbuscular mycorrhizal fungi (AMF) and water stress on growth, phenolic compounds, glandular hairs, and yield of essential oil in basil (*Ocimum gratissimum* L). **Chemical and Biological Technologies in Agriculture**, v. 2, p. 1-11, 2015.

HEMMATI, K.; EBADI, A.; KHOMARI, S.; SEDGHI, M. Influence of ascorbic acid and 24-epibrassinolide on physiological characteristics of pot marigold under water-stress condition. **Journal of Plant Interactions**, v. 13, n. 1, p. 364-372, 2018.

HENSCHEL, J. M.; SOARES, V. A.; FIGUEIREDO, M. C.; SANTOS, S. K.; DIAS, T. J.; BATISTA, D. S. Radish (*Raphanus sativus* L.) growth and gas exchange responses to exogenous ascorbic acid and irrigation levels. **Vegetos**, v. 36, n. 2, p. 566-574, 2023.

JIANG, S. Y.; JIN, J.; SAROJAM, R.; RAMACHANDRAN, S. A comprehensive survey on the terpene synthase gene family provides new insight into its evolutionary patterns. **Genome biology and evolution**, v. 11, n. 8, p. 2078-2098, 2019.

KALAJI, H. M.; JAJOO, A.; OUKARROUM, A.; BRESTIC, M.; ZIVCAK, M.; SAMBORSKA, I. A.; LADLE, R. J. Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. **Acta physiologiae plantarum**, v. 38, p. 1-11, 2016.

KHALID, M. F.; HUDA, S.; YONG, M.; LI, L.; LI, L.; CHEN, Z. H.; AHMED, T. Alleviation of drought and salt stress in vegetables: crop responses and mitigation strategies. **Plant Growth Regulation**, v. 99, n. 2, p. 177-194, 2023.

KHAN, A. F.; MUJEEB, F.; AHA, F.; FAROOQI, A. Effect of plant growth regulators on growth and essential oil content in palmarosa (*Cymbopogon martini*). **Asian J. Pharm. Clin. Res**, v. 8, n. 2, p. 373-376, 2015.

KHAZAEI, Z.; ESTAJI, A. Effect of foliar application of ascorbic acid on sweet pepper (*Capsicum annuum*) plants under drought stress. **Acta Physiologiae Plantarum**, v. 42, n. 7, p. 118, 2020.

LOCATO, V.; CIMINI, S.; GARA, L. D. Strategies to increase vitamin C in plants: from plant defense perspective to food biofortification. **Frontiers in plant science**, v. 4, p. 152, 2013.

MALIK, S.; ASHRAF, M.; ARSHAD, M.; MALIK, T. A. Effect of ascorbic acid application on physiology of wheat under drought stress. **Pakistan Journal of Agricultural Sciences**, v. 52, n. 1, 2015

MORENO-PIZANI, M. A.; PAREDES-TREJO, F. J.; FARIAS-RAMIREZ, A. J.; SANTOS, H. T.; PRADO MASSARIOLI, A.; MARIN, F. R., ALENCAR, S. M. (2020) Essential oil content of Baccharis Crispa Spreng. Regulated by water stress and Seasonal Variation. **AgriEngineering**, v. 2, n. 3, p. 458-470, 2020.

MUTHONI, J.; KABIRA, J. N. Potato production under Drought conditions: identification of adaptive traits. **International Journal of Horticulture**, v. 6, 2016.

NASIR, M. W.; TOTH, Z. Effect of Drought stress on Potato production: a review. **Agronomy**,

v. 12, n. 3, p. 635, 2022.

NASIRI, Y.; ZANDI, H.; MORSHEDLOO, M. R. Effect of salicylic acid and ascorbic acid on essential oil content and composition of drag- onhead (*Dracocephalum moldavica* L.) under organic farming. **Journal of Essential Oil Bearing Plants**, v. 21, n. 2, p. 362-373, 2018.

NAZ, S.; MUSHTAQ, A.; ALI, S.; MUHAMMAD, H. M. D.; SADDIQ, B.; AHMAD, R.; ALTAF, M. A. Foliar application of ascorbic acid enhances growth and yield of lettuce (Lactuca sativa) under saline conditions by improving antioxidant defence mechanism. **Functional Plant Biology**, 2022.

OZTURK, M.; TURKYILMAZ UNAL, B.; GARCÍA-CAPARRÓS, P.; KHURSHEED, A.; GUL, A.; HASANUZZAMAN, M. Osmoregulation and its actions during the drought stress in plants. **Physiologia plantarum**, v. 172, n. 2, p. 1321-1335, 2021.9

PAUL, J. H. A.; SEAFORTH, C. E.; TIKASINGH, T. *Eryngium foetidum* L.: a review. **Fitoterapia**, v. 82, n. 3, p. 302-308, 2011.

RAZA, A.; RAZZAQ, A.; MEHMOOD, S. S.; ZOU, X.; ZHANG, X.; LV, Y.; XU, J. Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. **Plants**, v. 8, n. 2, p. 34, 2019.

REN, T.; WERADUWAGE, S. M.; SHARKEY, T. D. Prospects for enhancing leaf photosynthetic capacity by manipulating mesophyll cell morphology. **Journal of Experimental Botany**, v. 70, n. 4, p. 1153-1165, 2019

RODRIGUES, T. L. M.; CASTRO, G. L. S.; VIANA, R. G.; GURGEL, E. S. C.; SILVA, S. G.; OLIVEIRA, M. S.; ANDRADE, E. H. D. A. Physiological performance and chemical compositions of the *Eryngium foetidum* L. (Apiaceae) essential oil cultivated with different fertilizer sources. **Natural Product Research**, v. 35, n. 23, p. 5544-5548, 2021

RUSTIONI, L.; BIANCHI, D. Drought increases chlorophyll content in stems of Vitis interspecific hybrids. **Theoretical and experimental plant physiology**, v. 33, n. 1, p. 69-78, 2021.

SAID-AL, A. H. L.; HAH, E. L.; GENDY, A. G.; OMER, E. A. Effect of ascorbic acid, salicylic acid on coriander productivity and essential oil cultivated in two different locations. **Advances in Environmental Biology**, v. 8, n. 7, p. 2236-2250, 2014.

SANTOS, S. K.; GOMES, D. S.; SANTOS, L. W. O.; SOARES, V. A.; DANTAS, E. F. O.; HENSCHEL, J. M.; BATISTA, D. S. Exogenous carnitine mitigates the deleterious effects of mild-water stress on Arugula by modulating morphophysiological responses. **Journal of Plant Growth Regulation**, v. 42, n. 7, p. 4073-4082, 2023.

SANTOS, S. K.; GOMES, D. S.; OLIVEIRA, A. F. P.; SILVA, A. M. O.; MOURA, V. S., GUSMÃO, M. H. A.; MATOS, E. M.; VICCINI, L. F.; GRAZUL, R. M.; HENSCHEL, J.M.; BATISTA, D. S. Water stress and exogenous carnitine on growth and essential oil profile of *Eryngium foetidum* L. **Biotech**, v. 13, n. 10, p. 328, 2023.

Seleiman, M. F.; Al-Suhaibani, N.; Ali, N.; Akmal, M.; Alotaibi, M.; Refay, Y.; Battaglia, M. L. Drought stress impacts on plants and different approaches to alleviate its adverse effects. **Plants**, v. 10, n. 2, p. 259, 2021.

SHARMA, D. K.; ANDERSEN, S. B.; OTTOSEN, C. O.; ROSENQVIST, E. Wheat cultivars selected for high F_V/F_M under heat stress maintain high photosynthesis, total chlorophyll, stomatal conductance, transpiration and dry matter. **Physiologia plantarum**, v. 153, n. 2, p. 284-298, 2015.

SINGH, S.; SINGH, D. R.; BANU, S.; SALIM, K. M. Determination of Bioactives and antioxidant activity in *Eryngium foetidum* L.: A Traditional Culinary and Medicinal Herb. **Proceedings of the National Academy of Sciences, India Section B: Biological Sciences**, v. 83, p. 453-460, 2013.

SINGH D. The relative importance of characters affecting genetic divergence. **Indian Journal of Genetics and Plant Breeding**, v. 41, n. 2, p. 237-245, 1981.

THOMAS, P. S.; ESSIEN, E. E.; NTUK, S. J.; CHOUDHARY, M. I. *Eryngium foetidum* L. essential oils: Chemical composition and anti- oxidant capacity. **Medicines**, v. 4, n. 2, p. 24, 2017.

WAY, D. A.; KATUL, G. G.; MANZONI, S.; VICO, G. Increasing water use efficiency along the C3 to C4 evolutionary pathway: a stomatal optimization perspective. **Journal of Experimental Botany**, v. 65, n. 13, p. 3683-3693, 2014.

YOUNG, A. J.; LOWE, G. L. Carotenoids-antioxidant properties. **Antioxidants**, v. 7, n. 2, p. 28, 2018.

ZHANG, R. R.; WANG, Y. H.; LI, T.; TAN, G. F.; TAO, J. P.; SU, X. J.; XIONG, A. S. Effects of simulated drought stress on carotenoid contents and expression of related genes in carrot taproots. **Protoplasma**, v. 258, p. 379-390, 2021.

7 CAPÍTULO IV: SALICYLIC ACID AND WATER STRESS: EFFECTS ON MORPHOPHYSIOLOGY AND ESSENTIAL OIL PROFILE OF *Eryngium foetidum*

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Abstract

The exogenous application of bioregulators, such as salicylic acid (SA), has exhibited promising outcomes in alleviating drought stress. Nevertheless, its impact on culantro (*Eryngium foetidum* L.) remains unexplored. Thus, the aim of this study was to assess how SA impacts the growth, morpho- physiology, and essential oil composition of culantro when subjected to drought. To achieve this, culantro plants were grown under three different watering regimes: well-watered, drought-stressed, and re-watered. Additionally, they were either treated with SA (100 µM) or left untreated, with water serving as the control. SA application did not mitigate the effects of drought in biomass production but increased biomass, leaf number, leaf area, and photosynthetic pigments under well-irrigated and re-watered conditions. After a drought period followed by re-watering, plants recovered membrane integrity independently of SA application. Water stress and the exogenous application of SA also modulated the profile of essential oils. This is the first report about SA and drought affecting growth and essential oil composition in culantro.

Keywords: abiotic stress; bioregulator; *Eryngium foetidum* L.; secondary metabolites; wild coriander.

7.1 INTRODUCTION

The exponential growth of world population requires an increase in agricultural production to meet food needs; however, one of the great challenges faced by agriculture are the production losses caused by abiotic stresses, such as drought, salinity, temperature and heavy metals (Bhardwaj et al. 2022). Moreover, considering the climate change predictions, the impact of these abiotic stresses on agriculture are expected to be greatly worsened in the future (Zulfiqar and Ashraf 2021). Drought stress, for example, negatively affects plant growth and productivity, also inducing metabolic, physiological and biochemical changes (Saud et al. 2014). For instance, drought decreases cell elongation and division, affects photoassimilates production and partitioning, and induces oxidative damages due to the overproduction of reactive oxygen species (ROS) (Sikder et al. 2016; Tang et al. 2018).

Plants can induce defense mechanisms, such as stomatal closure and osmotic adjustment, to prevent the harmful effects of water stress (Agurla et al. 2018; Al-Yasi et al.

2020). Under drought stress, plants close their stomata to prevent water loss, which consequently reduces the CO2 uptake, directly affecting the photosynthetic rate (Kato et al. 2023). This impairment in gas exchanges induces changes in leaf metabolism, inhibiting photosynthesis (Chaves et al. 2002; Sikder et al. 2016). Moreover, under drought stress, plants can induce the production and accumulation of osmoprotective molecules, such as amino acids, carbohydrates, proteins and inorganic ions, to maintain cell turgor (Jangra et al. 2022).

Considering the strong impacts of drought in crop productivity, it is imperative to develop strategies to overcome these negative effects. In this context, plant breeding, optimal crop management, and the use of plant bioregulators have been extensively used (Zulfiqar and Ashraf 2021). Bioregulators are natural or synthetic plant growth regulators, essential for plant development and metabolism under normal and stress conditions (Gaion et al. 2018; Zulfiqar et al. 2019). Plant bioregulators include auxin, gibberellin, abscisic acid, cytokinin, salicylic acid, nitric oxide, methyl jasmonate, paclobutrazol, ethephon, among others (Trolinder 2020; Zulfiqar and Ashraf 2021; Tormena et al. 2022).

Salicylic acid (SA) is a phenolic compound naturally synthesized by plants, which can also be applied exogenously via foliar or seed pretreatment (Asghari and Aghdam 2010; Dawood et al. 2012; Janda et al. 2020). SA regulates plant growth and development, and plays an important role in defense against abiotic stresses, inducing local and systemic acquired resistance (Zhang et al. 2010; Henschel et al. 2022). Application of SA regulates endogenous hormone levels (El-Mergawi and Abd El-Wahed 2020), modulates growth, activity of antioxidant enzymes such as peroxidase, catalase, ascorbate peroxidase, and the production of secondary metabolites such as phenols and flavonoids (Brito et al. 2019; Serna-Ecolano et al. 2021; Soares et al. 2022). In addition, SA increases carbon dioxide assimilation and chlorophyll content (Yildirim et al. 2008).

Eryngium foetidum L., popularly known as culantro and wild coriander, is a perennial herbaceous species, native to Central America, belonging to Apiaceae family (Santos et al. 2022). Culantro resembles coriander (*Coriandrum sativum*) for its strong aroma, however, it has a more resistant nature and a longer shelf life (Singh et al. 2014). It is an aromatic plant, being used as medicine in China, India, Vietnam, Mexico, and the Amazon region (Paul et al. 2011; Rodrigues et al. 2022). In addition, secondary metabolites produced by culantro can be used against *Leishmania tarentolae*, *L. donovani*, fungi, and bacteria such as *Bacillus cereus* and *Staphylococcus aureus* (Rojas-Silva et al. 2014; Panda et al. 2016; Borah et al. 2020). The essential oil of culantro is rich in aromatic and aliphatic aldehydes, with (2E)-2-dodecenal, also known as eryngial, being the predominant constituent, which is responsible for the aroma and flavor of this species (Thomas et al. 2017; Rodrigues et al. 2020). To test the hypotheses that SA can modulate the profile of essential oils in *E. foetidum*, and that its exogenous application can mitigate water stress in this species, the aim this study was to evaluate the effects of salicylic acid on growth, morphophysiology, and essential oil profile of culantro plants under water stress.

7.2 MATERIALS AND METHODS

7.2.1 Experimental location.

The experiment was conducted from January to May 2022 in a greenhouse covered with transparent film, situated in the experimental area of the Seedling Production Laboratory at the Center for Human, Social, and Agrarian Sciences of the Federal University of Paraiba (CCHSA/UFPB) in Bananeiras, PB, Brazil. The coordinates of the location are 6◦45′ S, 35◦38′ W, with an elevation of 526 m. The methodology employed in this study is derived from the previous work of our research group (Santos et al., 2023).

7.2.2 Irrigation and salicylic acid treatments.

Culantro (*E. foetidum* L.) plants were grown and, 50 days after sowing (DAS), subjected to these irrigation levels: 80% bag capacity (BC)-well-watered, and 40% BC-drought. Then, they were re-watered after twelve days of drought, according to Santos et al (2023). Moreover, plants were leaf-sprayed with water (control) or 100 µM SA using hand sprayers (Bestfer, Duque de Caxias, Brazil) every six days. Leaves were sprayed until completely wet (12 mL per plant).

7.2.3 Morphophysiological analysis.

At 102 DAS, growth, photosynthetic pigments, gas exchanges, and chlorophyll a fluorescence were measured. Leaf area, number of leaves, specific leaf area, and root length were quantified by image analysis utilizing the soft- ware ImageJ version 1.53k (Santos et la., 2022; Santos et al., 2023). Shoots and roots were oven-dried at 65 ◦C until they reached a constant weight to determine their dry weight, shoot/root ratio, and total biomass. Gas-exchange and light-response curves of photosynthesis were measured with an open-flow gas-exchange infrared gas analyzer (IRGA, LCpro-SD Portable Photosynthe- sis System, ADC BioScientific, Hoddesdon, UK) on fully expanded leaves of five plants per treatment between 8:00 a.m. and 10:00 a.m., with the conditions previously defined by Santos et al (2023). The net photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_S, mol H_2O m^{-2} s^{-1}), internal CO_2 concentration (Ci, mmol CO_2 mol^{-1} air), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), water use efficiency (*A/E*), carboxylation efficiency (*A/Ci*), dark respiration (R_{dark}, µmol m^{-2} s^{-1}), apparent quantum yield (mol mol^{-1}), light compensation point (LCP, µmol m^{-2} s^{-1}), maximum gross assimilation rate (A_{max}, µmol m^{-2} s^{-1}), and light saturation point (LSP, µmol m^{-2} s^{-1}) were determined. Photosynthetic pigment concentration (chlorophyll *a*, chlorophyll *b*, and total carotenoids) was determined according to Wellburn (1994), with modifications proposed by Santos et al. [33]. Electrolyte leakage was quantified according to Bajji et al. (2002) and Santos et al. (2023), to assess tissue death in response to drought stress.

7.2.4 Microextraction and qualitative analysis of essential oils.

Approximately 500 mg of leaves were collected and stored at -18 ◦C in test tubes with a screw cap, following Castro et al. (2020), and the microextraction was performed following Santos et al. (2023). The qualitative analysis of essential oils was carried out on a gas chromatographer coupled to a mass spectrometer (GCMS-QP2010 Plus; Shimadzu, Kyoto, Japan) and an Rtx-5MS® column (Restek, Bellefonte, PA, USA) of 30 m x 0.25 mm, with three technical replicates and running conditions according to Santos et al. (2023). The compounds were identified by comparing mass spectra to the NIST 9.0 database (correlation 95%) and confirmed with the Kováts retention index.

7.2.5 Experimental design and statistical analysis.

The experimental design was completely randomized, using a 2 x 3 factorial scheme (SA application x water condition) with ten replicates, each consisting of one bag containing one plant. Data underwent normality and homogeneity tests (Shapiro–Wilk and Bartlett, respectively), followed by an analysis of variance and Tukey's test ($p>0.05$), using Genes software version 2015.5.0 (Cruz, 2016). Growth parameters and essential oil profiles underwent multivariate analyses. Treatment distances were assessed through canonical discriminant analysis in a three-dimensional scatter plot. Treatments were grouped using the Tocher optimization method and Mahalanobis' generalized squared interpoint distance (D2). Variable contributions to treatment discrimination were quantified using the Singh (1981) criterion.

7.3 RESULTS

The highest plant growth was obtained under re-watered and well-watered conditions, while drought limited culantro development (Figure 1a). Without SA application, re-watering doubled the leaf fresh mass, root fresh mass, and total biomass, with increments of 108.61%, 111.38%, and 127.89%, respectively, compared to well-watered plants (Figure 1b,d,f). Re-watering also increased the leaf dry mass and root dry mass of control plants compared to the well-watered condition (Figure 1c,e). In well-watered plants, SA application increased leaf fresh mass, root fresh mass, leaf dry mass, root dry mass, and total biomass by 102.98%, 99.37%, 105.51%, 72.04%, and 103.79%, respectively. Similarly, SA treatment increased the root fresh and dry mass and total biomass compared to the control in re-watered plants. By contrast, SA decreased the leaf dry mass and total biomass of culantro compared to control plants. Water conditions did not affect the biomass allocation of control plants; however, compared to the control, SA increased the shoot/root ratio of well-watered plants (Figure 1g).

Drought decreased the leaf area of control plants compared to well-watered and re-watered conditions (Figure 2a,b). On the other hand, SA increased the number of leaves by 136.67% and the leaf area by 66.08% compared to control plants under well-watered conditions (Figure 2a,b). Similarly, SA increased the leaf area of re-watered plants by 28.75% compared to the control. Water conditions did not affect the specific leaf area of control plants; however, in SA-treated plants, re-watering increased the specific leaf area compared to well-watered conditions (Figure 2c). Intriguingly, drought reduced the electrolyte leakage of control plants compared to well-watered conditions (Figure 2d). On the other hand, SA reduced electrolyte leakage in well-watered plants but increased it in drought-stressed plants. In turn, re-watering reduced electrolyte leakage independently of SA application (Figure 2d).

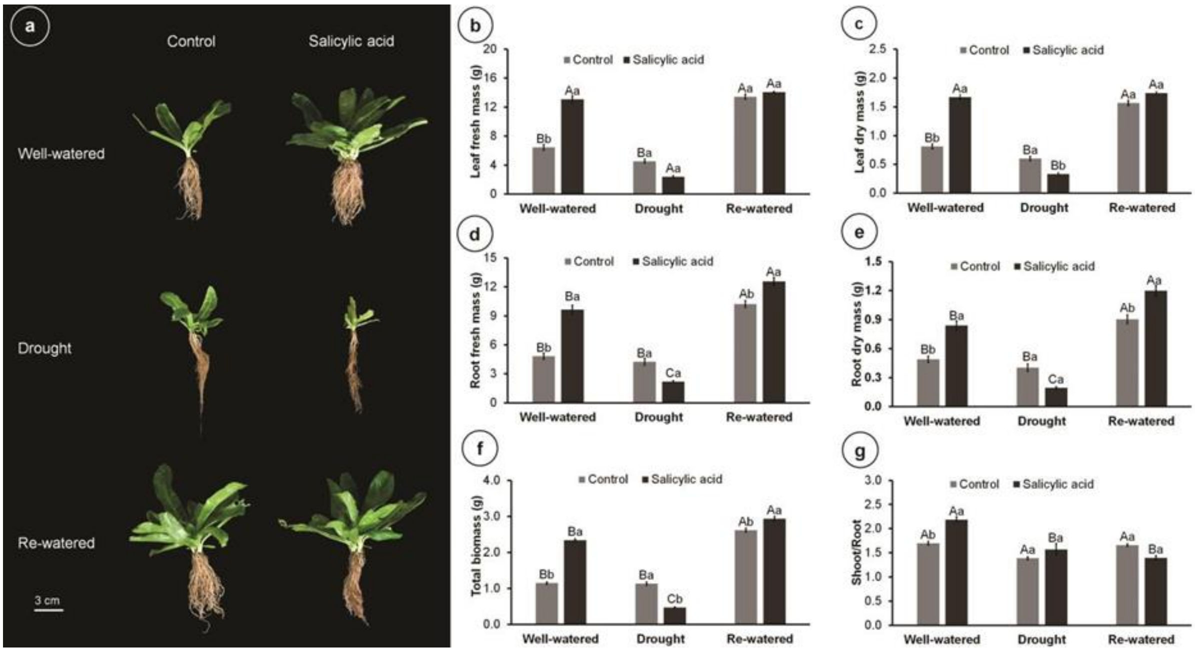

Figure 1. Biomass production and partitioning of 102-day-old culantro plants treated with water (control) or salicylic acid and grown under different water levels (80% BC-well-watered; 40% BC-drought; and re-watered after 12 days of water restriction). Values represent means ± standard error (n = 6). Capital letters compare water levels within each salicylic acid treatment, and lowercase letters compare control and salicylic acid treatment

within each water level (Tukey's test; $p \leq 0.05$). (a) plant phenotype; (b) leaf fresh mass; (c) leaf dry mass; (d) root fresh mass; (e) root dry mass; (f) total biomass; (g) shoot/root ratio.

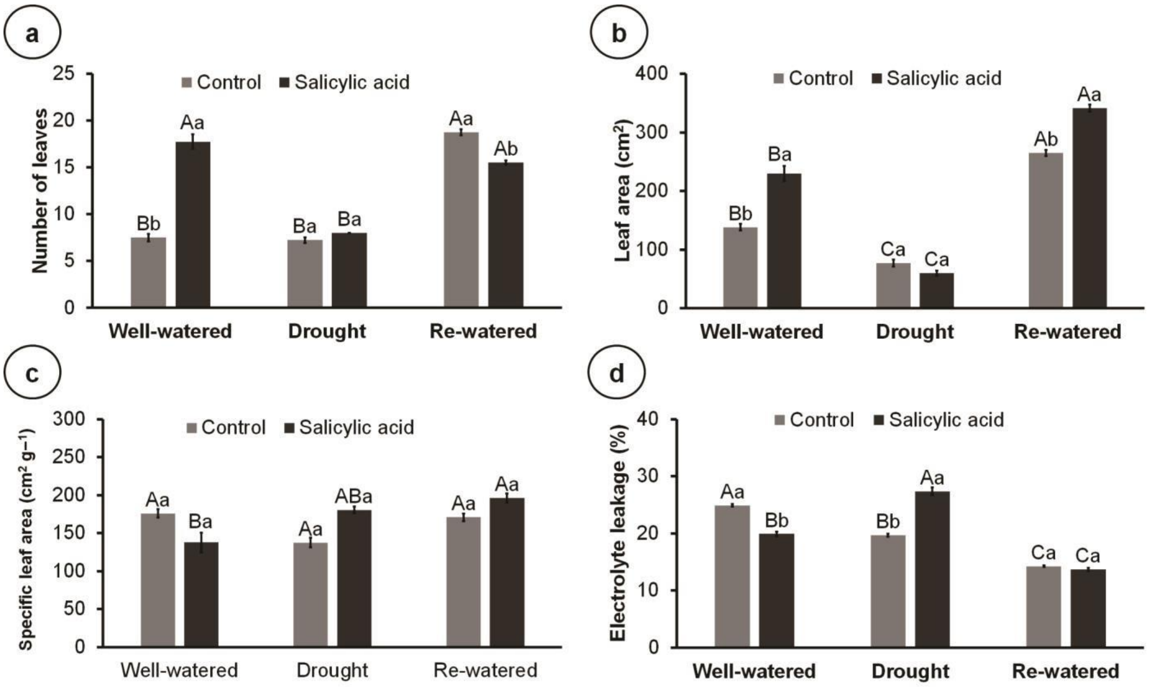

Figure 2. Number of leaves, leaf area, specific leaf area, and electrolyte leakage of 102-day-old culantro plants treated with water (control) or salicylic acid and grown under different water levels (80% BC-well-watered; 40% BC-drought; and re-watered after 12 days of water restriction). Values represent means ± standard error ($n = 6$). Capital letters compare water levels within each salicylic acid treatment, and lowercase letters compare control and salicylic acid treatment within each water level (Tukey's test; $p \leq 0.05$). (a) number of leaves; (b) leaf area; (c) specific leaf area; (d) electrolyte leakage.

Drought stress increased the concentration of chlorophyll *a*, chlorophyll *b*, and carotenoids compared to the well-watered condition; however, SA application reversed this effect, reducing the concentration of pigments compared to the control (Figure 3a,b,d). By contrast, SA increased the concentration of chlorophyll a, chlorophyll b, and carotenoids compared to the control in re-watered plants (Figure 3a,b,d). Moreover, within SA-treated plants, re-watering resulted in the highest concentration of pigments compared to well-watered and drought conditions. Re-watering reduced the chlorophyll *a/b* ratio in control plants compared to well-watered plants (Figure 3c). In turn, SA reduced the chlorophyll *a/b* ratio in well-watered plants but increased that of re-watered plants, compared to the control (Figure 3c).

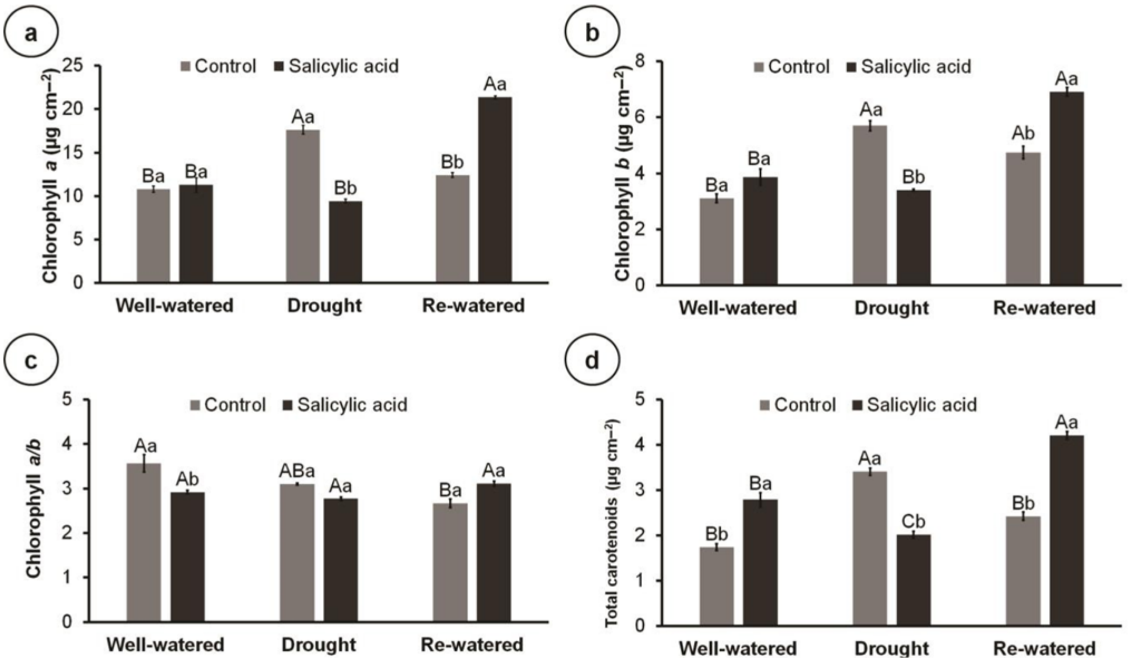

Figure 3. Photosynthetic pigments of 100-day-old culantro plants treated with water (control) or salicylic acid, and grown under different water levels (80% BC-well-watered; 40% BC-drought; and re-watered after 12 days of water restriction). Values represent means ± standard error (n = 6). Capital letters compare water levels within each salicylic acid treatment, and lowercase letters compare control and salicylic acid treatment within each water level (Tukey's test; $p \leq 0.05$). (a) chlorophyll *a*; (b) chlorophyll *b*; (c) chlorophyll *a/b*; (d) total carotenoids.

Within control plants, *A*, g_S, and *Ci* were not affected by water conditions; however, re-watered plants had higher *E*, and lower *A*/*E* and *A*/*Ci* than well-watered ones (Figure 4a–f). On the other hand, SA application reduced A and g_S compared to control plants under well- watered and drought conditions (Figure 4a,b). Similarly, SA reduced *A*/*E* and *A*/*Ci* under well-watered conditions (Figure 4e,f). Interestingly, *E* was not affected by SA treatment, while *Ci* was not affected by either the water condition or SA treatment (Figure 4c,d).

Within control plants, *A*, g_S, and *Ci* were not affected by water conditions; however, re-watered plants had higher *E*, and lower *A/E* and *A/Ci* than well-watered ones (Figure 4a–f). On the other hand, SA application reduced *A* and g_S compared to control plants under well- watered and drought conditions (Figure 4a,b). Similarly, SA reduced *A/E* and *A/Ci* under well-watered conditions (Figure 4e,f). Interestingly, *E* was not affected by SA treatment, while *Ci* was not affected by either the water condition or SA treatment (Figure 4c,d).

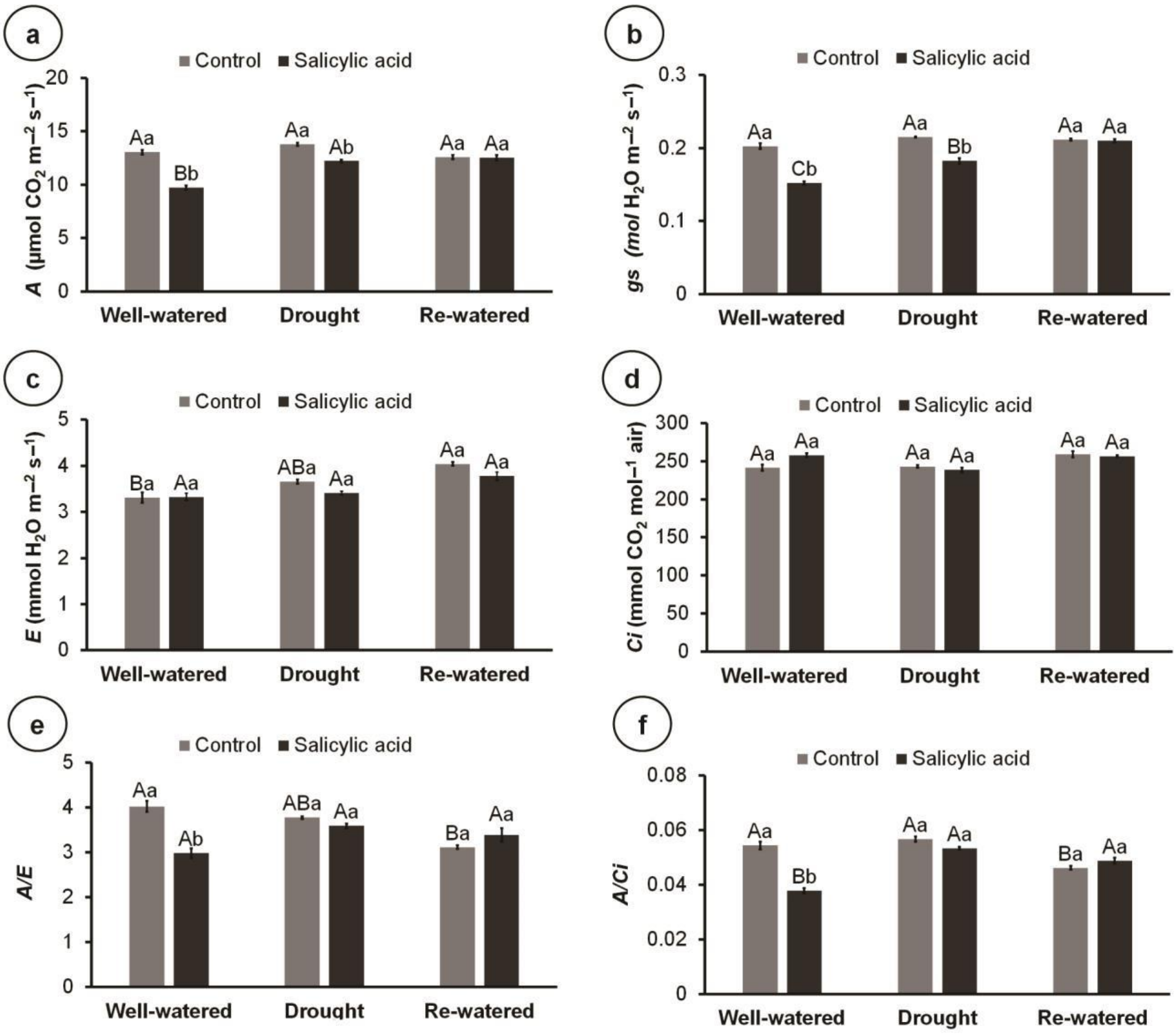

Figure 4. Gas exchange parameters of 100-day-old culantro plants treated with water (control) or salicylic acid and grown under different water levels (80% BC-well-watered; 40% BC-drought; and re-watered after 12 days of water restriction). Values represent means ± standard error (n = 6). Capital letters compare water levels within each salicylic acid treatment, and lowercase letters compare control and salicylic acid treatment within each water level (Tukey's test; $p \leq 0.05$). (a) Net carbon assimilation rate (*A*); (b) stomatal conductance (g_S); (c) leaf transpiration rate (E); (d) internal CO_2 concentration (*Ci*); (e) water use efficiency (*A/E*); (f) instantaneous carboxylation efficiency (*A/Ci*).

There was a reduction in the R_{dark} and LCP of control plants under drought stress; however, SA reversed this effect, increasing R_{dark} and LCP under drought (Figure 5a,c). Similarly, re-watering reduced the R_{dark} and LCP of control plants compared to the well-watered condition; however, SA did not affect these variables under the re-watered condition (Figure 5a,c). On the other hand, drought increased the A_{max} of control plants compared to well-watered and re-watered conditions (Figure 5b). Moreover, A_{max} was reduced by SA under drought compared to control plants. Within control plants, re-watering resulted in the lowest LSP, while no differences between water conditions were found within SA-treated plants

(Figure 5d). Furthermore, SA reduced LSP under well-watered and drought conditions compared to control plants, while under the re-watered condition, SA increased LSP (Figure 5d).

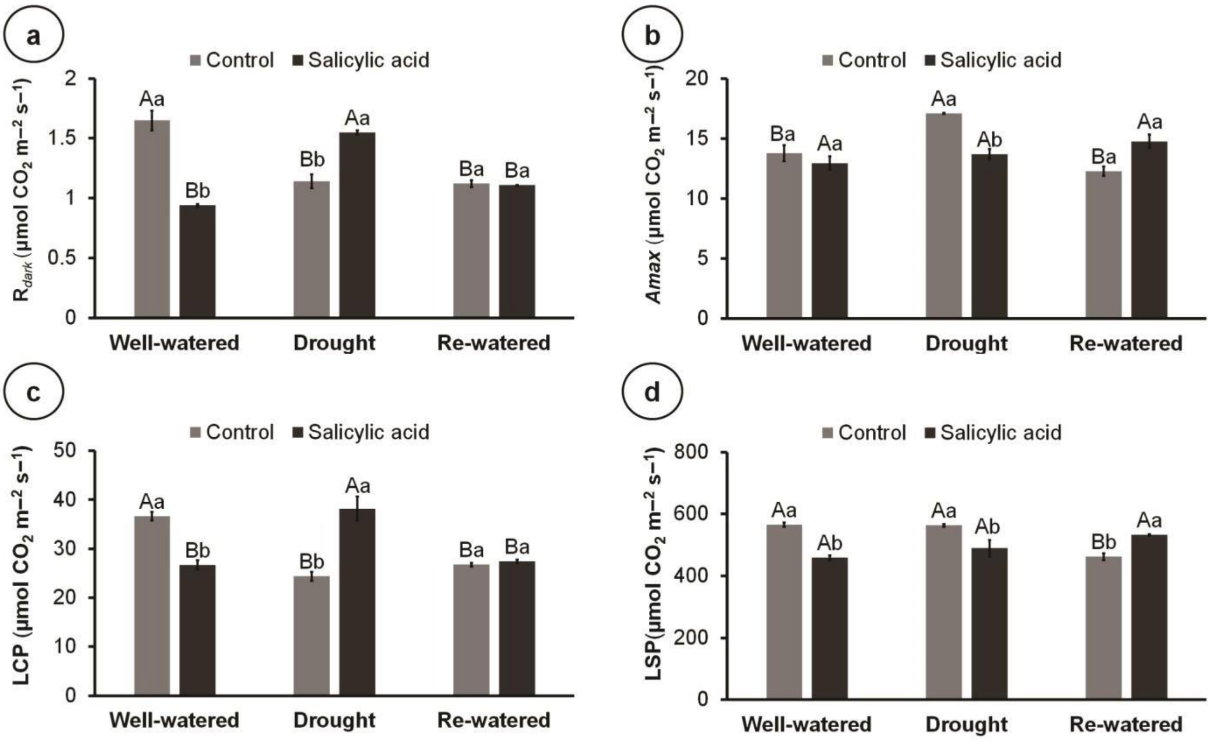

Figure 5. Light-curve parameters of 100-day-old culantro plants treated with water (control) or salicylic acid and grown under different water levels (80% BC-well-watered; 40% BC-drought; and re-watered after 12 days of water restriction). Values represent means ± standard error (n = 6). Capital letters compare water levels within each salicylic acid treatment, and lowercase letters compare control and salicylic acid treatment within each water level (Tukey's test; $p \leq 0.05$). (a) Dark respiration (R_{dark}); (b) maximum gross assimilation rate (A_{max}); (c) light compensation point (LCP); (d) light saturation point (LSP).

The first three canonical variables explained 99.56% of the variability among the treatments for morphophysiological parameters, allowing for a three-dimensional scatter plot representation (Figure 6a). The treatments were separated into four groups: group 1 (blue circle), comprising control and SA-treated plants under drought (DR-Ctrl and DR- SA); group 2 (green circle), comprising control and SA-treated plants under re-watered condition (RW-Ctrl and RW-SA); group 3 (red circle), comprising well-watered control plants (WW-Ctrl); and group 4 (yellow circle), comprising well-watered plants treated with SA (WW-SA). The relative contributions of the original variables showed that leaf area (15.4%), total biomass (15.4%), and shoot dry mass (13%) were the variables that most contributed to the total variance (Figure 6b).

Regarding the essential oil profile, the first three canonical variables explained 92.92% of the variability among treatments (Figure 6c). Treatments were separated into three groups: group 1 (red circle), corresponding to well-watered control plants (WW-Ctrl); group 2 (yellow circle), corresponding to well-watered plants treated with SA; and group 3 (blue circle), comprising control and SA-treated plants under drought stress and re-watered conditions (DR-Ctrl, DR-SA, RW-Ctrl, and RW-SA). The relative contributions of the original variables showed that 8-hexadecenal was the compound that most contributed to the total variance (60.3%) (Figure 6d).

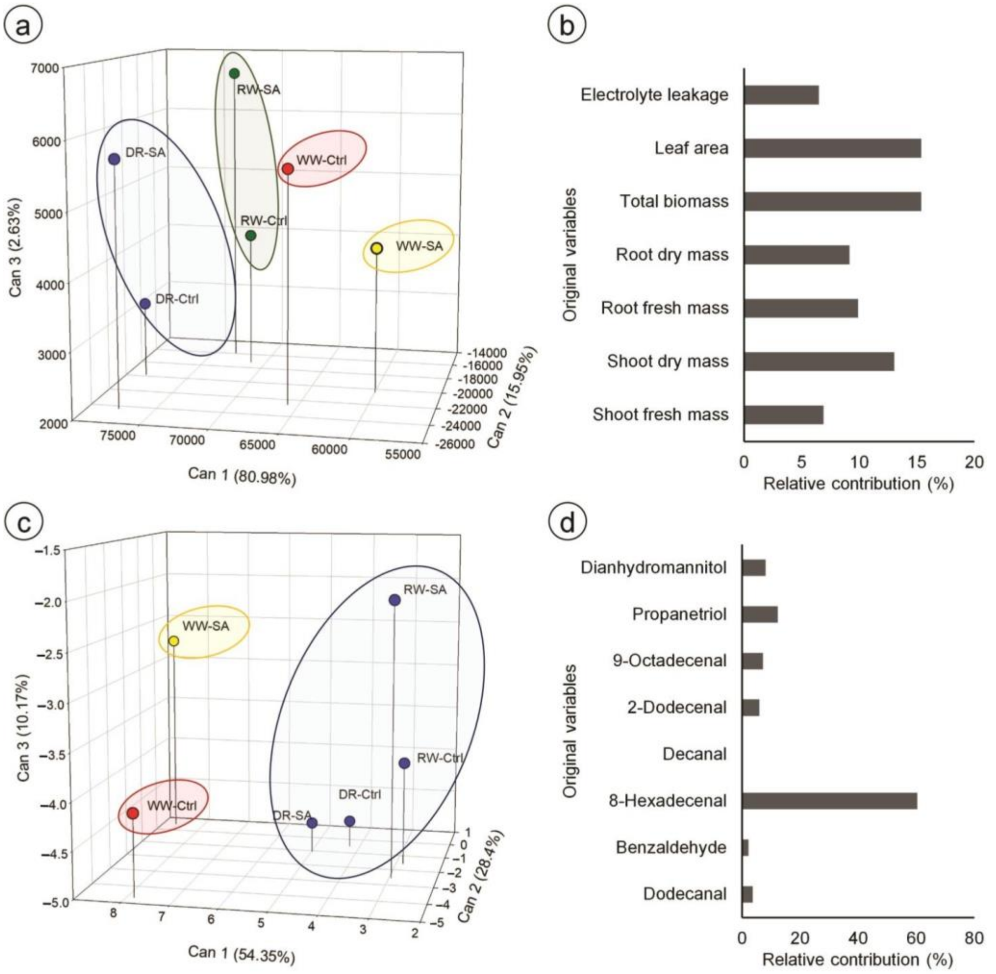

Figure 6. Canonical variables from original parameters in culantro plants treated with water (control) or salicylic acid and grown under different water levels (80% BC-well-watered; 40% BC-drought; and re-watered after 12 days of water restriction). (a,c) 3D scatter plots of the first three canonical components obtained from the morphophysiological variables and essential oil profile, respectively; (b,d) relative contribution of original variables to the canonical variables, calculated using the Singh method. The percentage of total variance explained by each canonical component is indicated in parentheses; treatments into the same ellipses were grouped by the Tocher optimization method and the generalized distance of Mahalanobis.

7.4 DISCUSSION

Water is essential for plant growth, and drought stress represents a major challenge for agriculture. Drought stress impact crop production by affecting plant metabolism, physiology, and biochemistry (Fadiji et al. 2022; Henschel et al. 2022). Our results showed that drought stress reduced the leaf area of culantro plants, but did not decrease biomass production and number of leaves. Drought is known to reduce cell turgor pressure, inhibiting cell expansion and, consequently, plant growth (Zhao et al. 2006; Kudo et al. 2019; Khaleghnezhad et al. 2021). This may explain the reduction in leaf area of culantro under drought stress; however, no reduction in biomass production was found under drought, suggesting that culantro might be tolerant to irrigation with 40% of BC. Interestingly, re-watering of plants after 12 of water restriction was the condition that resulted in the highest leaf and root biomass, number of leaves, and leaf area. These results suggest that water restriction might have primed culantro plants, inducing defense responses that resulted in better performance after re-watering. In fact, studies have shown that drought stress priming may improve drought tolerance in plants due to “stress memory” mechanisms, such as the maintenance of water status, osmotic adjustment, and expression of stress related genes (Blum 2017; Jacques et al. 2021; Sintaha et al. 2022).

Under drought stress plants generally direct more biomass to the roots, increasing their ability to absorb water and nutrients (Eziz et al. 2017; Dinneny 2019; Gupta et al. 2020). Here, water conditions did not alter biomass partitioning of control plants, but SA treatment increased biomass allocation to shoots under well-watered condition, as shown by the higher shoot/root ratio. Moreover, SA treatment strongly increased leaf and root biomass under well-watered condition, indicating the role of this plant hormone in plant growth regulation. Indeed, SA have been related not only to stress responses, but also to growth regulation (Rivas-San Vicente et al. 2011; Zulfiqar et al. 2021b). Considering that the organ of commercial interest for culantro is the leaf (Rodrigues et al. 2020), our results indicate that SA treatment has the potential to increase the commercial production of culantro under well-watered conditions. In the same way, re-watering increased overall culantro growth independently of SA treatment, suggesting that drought priming is a promising technique to increase biomass production in culantro.

The specific leaf area is an estimative of leaf thickness that plays an important role in ecological characteristic of plants, explaining variations in photosynthetic and respiratory rates per unit of leaf dry mass and in light interception (Reich et al. 1998; Sugiyama 2005; Koester et al. 2014). Here, water conditions did not affect specific leaf area in control plants; however, in SA-treated plants, re-watering resulted in the highest specific leaf area, indicating that this condition resulted in thinner leaves. As increased specific leaf area upon SA treatment have been related to the role of this hormone in nutrient uptake and to the crosstalk with ethylene (Leslie and Romani, 1986; Farhangi-Abriz and Ghassemi-Golezani, 2018; Ghassemi-Golezani et al. 2018), this could help to explain the higher specific leaf area under this condition.

Electrolyte leakage is an indicator of cell membrane integrity, with increases in electrolyte leakage values indicating higher leakage of ions due to loss of membrane integrity (Khoshbakht and Asgharei, 2015). In plant cells, electrolyte leakage can be detected almost instantly after the exposition to a stress factor due to K^+ efflux and production of ROS, which can cause oxidative damages to cell membranes and impair plant development (Demidchik et al. 2014; Niu et al. 2021). SA application did not reduce and even increased electrolyte leakage in drought-stressed culantro plants, indicating reduced membrane stability. Considering that the effectiveness of SA in stress mitigation is highly dependent of its concentration and that high levels of SA can induce oxidative damages (Borsani et al. 2001; Prakash et al. 2021; Soares et al. 2022), our results suggest that SA treatment, combined with drought, may have induced oxidative damages in culantro. By contrast, re-watering resulted in the lowest electrolyte leakage independently of SA addition, indicating higher membrane integrity. Considering that plants induce antioxidant defenses in response to drought, reducing oxidative damages, and increasing membrane stability (Siddiqui et al. 2010; Xi et al. 2018; Gonzaléz-Orenga et al. 2019), our results suggest that drought priming may have induced such responses in culantro plants, explaining the reduction in electrolyte leakage upon re-watering observed here. Recovery after a period of stress is a key mechanism in drought tolerance and plant survival, especially in regions where plants are exposed to repeated cycles of drought and irrigation (Chen et al. 2016). Thus, our results further confirm the beneficial effect of drought priming in culantro plants.

SA have been related to increases in the content of chlorophylls and carotenoids and reductions in chlorophyll catabolism (Costa et al. 2005; Luo et al. 2012; Eismann et al. 2020). Here, SA increased the content of chlorophyll *a* and *b*, and carotenoids in re-watered plants and reduced them in drought-stressed plants. These results, together with electrolyte leakage results, suggest that the combination between drought stress and SA treatment may have triggered oxidative damages, degrading photosynthetic membranes and pigments. By contrast, in re-watered plants, drought priming may have induced antioxidant defenses, which in combination with SA, even increased pigment contents and chloroplast membrane stability. In fact, SA have been related to changes in the monogalactosyldiacylglycerol/digalactosyldiacylglycerol ratio in chloroplast membranes, increasing the stability of photosystem complexes and chloroplasts

(Belkadhi et al. 2015; Rocha et al. 2018). Accordingly, SA treatment has also shown to reduce electrolyte leakage and increase chlorophyll content in other species, such as *Linum usitatissimum*, under abiotic stresses (Belkhadi et al. 2010).

Exogenous SA has shown to affect photosynthetic capacity of plants, depending on factors such as plant species, duration of treatment, form of application, and environmental conditions, with high SA concentrations negatively affecting photosynthesis (Janda et al. 2012; Wu et al. 2013; Janda et al. 2014). This may explain the reduction in photosynthetic capacity of plants treated with SA under well-watered and drought conditions. Furthermore, SA treatment decreased g_S of well-watered and drought-stressed plants; however, *Ci* was maintained high under these conditions, indicating that the lower *A* was not related to the CO_2 availability (Aires et al. 2022). In turn, the lower carboxylation efficiency (*A/Ci*) of well-watered plants treated with SA suggest that this treatment may have affected biochemical reactions. In fact, SA have been related to changes in biochemical reactions, and in the composition and volume of thylakoid membranes, decreasing photochemical efficiency and photosynthetic rates (Uzunova and Popova 2000; Grigorova et al. 2013; Aires et al. 2022).

Dark respiration is a redox process, where the amount of CO_2 released by respiration is greater than the CO_2 fixed in photosynthesis (Tan et al. 2013). Here, drought stress and re-watering reduced R_{dark} and LCP in control plants, and SA reversed this effect under drought, increasing R_{dark} and LCP. These results indicate that SA-treated plants had higher energy consumption and, thus, required higher light quantities to compensate the levels of CO_2 up taken and released by plants through respiration and photosynthetic processes. Similar results were observed in *Arabidopsis* mutants with high SA content, which also exhibited increased dark respiration (Janda et al. 2014). SA also reversed the increased *Amax* in drought plants and reduced LSP in drought and well-watered plants, suggesting that SA may be inducing photoinhibition under these conditions. Considering that photoinhibition is associated with oxidative damages in chloroplast membranes (He et al. 2021), these results further confirm the hypothesis that SA treatment may have induced oxidative stress, dismantling membranes and increasing electrolyte leakage.

Canonical analysis showed that SA altered morphophysiology of well-watered plants; however, under drought and re-watering conditions, the morphophysiological responses of control were grouped together with those of SA-treated plants. This indicates that the water condition was the main factor involved in the range of morphophysiological responses. Regarding the essential oil profile of culantro, our results indicated that water stress and SA application affected the essential oil profile. Due to its action in plant defense responses, SA is largely known as an elicitor of secondary metabolite production in plants (Golkar et al. 2019; Ali 2021). Accordingly, our results showed that SA treatment altered the essential oil profile of well-watered plants; however, drought and re-watering also altered the essential oil profile of culantro independently of SA. These results indicate that the secondary metabolites produced in response to water stress are different from those produced under well-watered or upon SA treatment. In fact, it is known that the synthesis of secondary metabolites is highly affected by environmental factors, especially water stress, altering metabolite composition and yield (Bettaieb et al. 2009). Among the compounds found here, 8-hexadecenal, also known as trogodermal, was the compound that most contributed to the total variance. This compound was also identified as one the main constituents of essential oils in *Foeniculum vulgare* (Islam et al. 2021), and is characterized as a highly active sexual pheromone of *Trogoderma*, leading them to a mating behavior (Cross et al. 1976; Mori, 2009; Morrison et al. 2020). These findings contribute to future studies aiming at increasing the production of compounds of interest.

7.5 CONCLUSIONS

Drought reduced the leaf area, which was recovered upon rehydration. Moreover, re-watering caused a drought-priming effect that resulted in increased growth and membrane stability. The exogenous application of salicylic acid enhances growth and modulates the essential oil profile in well-watered culantro. The composition of essential oils was also modulated by water stress, whether followed by rehydration or not. Understanding the regulation of the profile of essential oils in culantro may enable the production of compounds of economic and industrial interest. Thus, our results generate new perspectives to explore the production of compounds of interest in culantro essential oils.

REFERENCES

AGURLA, S.; GAHIR, S.; MUNEMASA, S.; MURATA, Y.; RAGHAVENDRA, A. S. (2018). **Mechanism of Stomatal Closure in Plants Exposed to Drought and Cold Stress.** In: IWAYA-INOUE, M.; SAKURAI, M.; UEMURA, M. (eds) Survival Strategies in Extreme Cold and Desiccation. Advances in Experimental Medicine and Biology, v. 1081. Springer, Singapore.

AIRES, E. S.; FERRAZ, A. K. L.; CARVALHO, B. L.; TEIXEIRA, F. P.; PUTTI, F. F.; SOUZA, E. P.; RODRIGUES, J. D.; ONO, E. O. Foliar application of salicylic acid to mitigate water stress in tomato. **Plants**, v. 11, n. 13, p. 1775, 2022.

ALI, B. Salicylic acid: An efficient elicitor of secondary metabolite production in plants.

Biocatalysis and Agricultural Biotechnology, v. 31, p. 101884, 2021

AL-YASI, H.; ATTIA, H.; ALAMER, K.; HASSAN, F.; ALI, E.; ELSHAZL, S.; KADAMBOT, H.M.; HESSINI, K. Impact of drought on growth, photosynthesis, osmotic adjustment, and cell wall elasticity in Damask rose. **Plant Physiology and Biochemistry**, v. 150, p. 133-139, 2020.

ASGHARI, M.; AGHDAM, M. S. Impact of salicylic acid on post-harvest physiology of horticultural crops. **Trends in Food Science & Technology**, v. 21, n. 10, p. 502-509, 2010.

BAJJI, M.; KINET, J. M.; LUTTS, S. The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. **Plant growth regulation**, v. 36, p. 61-70, 2002.

BELKADHI, A.; DE-HARO, A.; OBREGON, S.; CHAÏBI, W.; DJEBALI, W. Exogenous salicylic acid protects phospholipids against cadmium stress in flax (*Linum usitatissimum* L.). **Ecotoxicology and Environmental Safety**, v. 120, p. 102-109, 2015

BELKHADI, L. A.; HEDIJI, H.; ABBES, Z.; NOUAIRI, I.; BARHOUMI, Z.; ZARROUK, M.; CHAIBI, W.; DJEBALI, W. Effects of exogenous salicylic acid pre-treatment on cadmium toxicity and leaf lipid content in *Linum usitatissimum*. **Ecotoxicology and Environmental Safety**, v. 73, n. 5, p. 1004-1011, 2010.

BETTAIEB, I.; ZAKHAMA, N.; WANNES, W. A.; KCHOUK, M. E.; MARZOUK, B. Water deficit effects on Salvia officinalis fatty acids and essential oils composition. **Scientia horticulturae**, v. 120, n. 2, p. 271-275, 2009.

BHARDWAJ, K.; RAINA, M.; SANFRATELLO, G.M.; PANDEY, P.; SINGH, A.; RAJWANSHI, R.; NEGI, N.P.; RUSTAGI, A.; KUMA, K.D. Exogenous Melatonin Counteracts Salinity and Cadmium Stress via Photosynthetic Machinery and Antioxidant Modulation in *Solanum lycopersicum* L. **Journal of Plant Growth Regulation**, v. 42, n. 10, p. 6332-6348, 2023

BORAH, J.; SINGH, K. L.; MONDAL, S.; DUTTA, S. P. Phytochemical analysis and synergistic interaction of plants extracts on bacterial and fungal isolates. **International Journal of Recent Academic Research**, v. 2, n. 4, p. 618-623, 2020.

CASTRO, K. M.; BATISTA, D. S.; SILVA, T. D.; FORTINI, E. A.; FELIPE, S. H. S.; FERNANDES, A. M.; SOUSA, R. M. J.; NASCIMENTO, L. S. Q.; CAMPOS, V. R.; GRAZUL, R. M. Water deficit modulates growth, morphology, and the essential oil profile in

Lippia alba L. (Verbenaceae) grown in vitro. **Plant Cell, Tissue and Organ Culture (PCTOC)**, v. 141, p. 55-65, 2020.

CHAVES, M.M.; PEREIRA, J.S.; MAROCO, J.; RODRIGUES, M.L.; RICARDO, C.P.P.; OSÓRIO, M.L.; CARVALHO, I.; FARIA, T.; PINHEIRO, C. How plants cope with water stress in the field photosynthesis and growth. **Annals of botany**, v. 89, n. 7, p. 907, 2002.

CHEN, S. D.; WANG, B.; CAO, D.; CAO, G.; LENG, H.; LI, L.; YIN, L.; SHAN, X.; DENG, X. Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in Maize seedlings. **Frontiers in Plant Science**, v. 6, p. 1241, 2016.

CROSS, J.H.; BYLER, R.C.; CASSIDY, R.F.; SILVERSTEIN, R.M.; GREENBLATT, R.E.; BURKHOLDER, W.E.; LEVINSON, A.R.; LEVINSON, H.Z. Porapak- Q collection of pheromone components and isolation of (Z)-and (E)-14-methyl-8-hexadecenal, sex pheromone components, from the females of four species of Trogoderma (*Coleoptera: Dermestidae*). **Journal Chemical Ecology.** v. 2, p. 457-468, 1976.

CRUZ, C.D. Genes Software-extended and integrated with the R, MATLAB and Selegen. **Acta Scientiarum. Agronomy**, v. 38, n. 4, p. 547-552, 2016.

DAWOOD, M.G.; SADAK, M.S.; HOZAYEN, M. Physiological role of salicylic acid in improving performance, yield and some biochemical aspects of sunflower plant grown under newly reclaimed sandy soil. **Australian Journal of Basic and Applied Sciences**, v. 6, n. 4, p. 82-89, 2012.

DEMIDCHIK, V.; STRALTSOVA, D.; MEDVEDEV, S. S.; POZHVANOV, G. A.; SOKOLIK, A.; YURIN, V. Stress-induced electrolyte leakage: The role of K^+-permeable channels and involvement in programmed cell death and metabolic adjustment. **Journal of experimental botany**, v. 65, n. 5, p. 1259-1270, 2014.

DINNENY, J. R. Developmental Responses to Water and Salinity in Root Systems. **Annual Review of Cell and Developmental Biology**, v. 35, n. 1, p. 239-257, 2019.

EISMANN, A. I.; REIS, R. P.; SILVA, A. F.; CAVALCANTI, D. N. *Ulva spp*. carotenoids: Responses to environmental conditions. **Algal research**, v. 48, p. 101916, 2020.

EL-MERGAWI, R.A.; ABD EL-WAHED, M.S.A. Effect of exogenous salicylic acid or indole acetic acid on their endogenous levels, germination, and growth in maize. **Bulletin of the National Research Centre**, v. 44, p. 1-8, 2020.

FADIJI, A. E.; SANTOYO, G.; YADAV, A. N.; BABALOLA, O. O. Efforts towards overcoming drought stress in crops: Revisiting the mechanisms employed by plant growth-promoting bacteria. **Frontiers in Microbiology**, v. 13, p. 962427, 2022.

GAION, L.A.; MONTEIRO, C.C.; CRUZ, F.J.R.; ROSSATTO, D.R.; LOPEZ-DIAZ, I.; CARRERA, E.; LIMA, J.E.; PERES, L.E.P.; CARVALHO, R.F. Constitutive gibberellin response in grafted tomato modulates root-to-shoot signaling under drought stress. **Journal of Plant Physiology**, v. 221, p. 11-21, 2018.

GHASSEMI-GOLEZANI, K.; FARHANGI-ABRIZ, S.; BANDEHAGH, A. Salicylic acid and jasmonic acid alter physiological performance, assimilate mobilization and seed filling of soybean under salt stress. **Acta Agriculturae Slovenica**, v. 111, n. 3, p. 597-607, 2018.

GOLKAR, P.; TAGHIZADEH, M.; YOUSEFIAN, Z. The effects of chitosan and salicylic acid on elicitation of secondary metabolites and antioxidant activity of safflower under in vitro salinity stress. **Plant Cell, Tissue and Organ Culture**, v. 137, p. 575-585, 2019.

GRIGOROVA, B.; VASSILEVA, V.; KLIMCHUK, D.; VASEVA, I.; DEMIREVSKA, K.; FELLER, U. Drought, High Temperature, and Their Combination Affect Ultrastructure of Chloroplasts and Mitochondria in Wheat (*Triticum aestivum* L.) Leaves. **Journal of Plant Interactions**, v. 7, n. 3, p. 204-213, 2012.

GUPTA, A.; RICO-MEDINA, A.; CAÑO-DELGADO, A. I. The physiology of plant responses to drought. **Science**, v. 368, n. 6488, p. 266-269, 2020.

HE, W.; YAN, K.; ZHANG, Y.; BIAN, L.; MEI, H.; HAN, G. Contrasting photosynthesis, photoinhibition and oxidative damage in honeysuckle (*Lonicera japonica* Thunb.) under iso-osmotic salt and drought stresses. **Environmental and Experimental Botany**, v. 182, p. 104313, 2021

HENSCHEL, J.M.; DANTAS, E.F.O.; SOARES, V.A.; SANTOS, S.K.; SANTOS, L.W.O.; DIAS, T.J.; BATISTA, D.S. Salicylic acid mitigates the effects of mild drought stress on radish (*Raphanus sativus*) growth. **Functional Plant Biology**, v. 49, n. 9, p. 822-831, 2022.

ISLAM, M.; SRIVASTAVA, A.; KUMAR, S.; VERMA, N. K. Chemical and pharmacological properties of *Foeniculum vulgare* Mill: A review. **World Journal of Pharmaceutical and Life Sciences** v. 7, p. 48-56, 2022.

JACQUES, C.; SALON, C.; BARNARD, R. L.; VERNOUD, V.; PRUDENT, M. Drought stress memory at the plant cycle level: A review. **Plants**, v. 10, n. 9, p. 1873, 2021.

JANDA, T.; GONDOR, O. K.; YORDANOVA, R.; SZALAI, G.; PÁL, M. Salicylic acid and photosynthesis: Signaling and effects. **Acta physiologiae plantarum**, v. 36, p. 2537-2546, 2014.

JANDA, T.; SZALAI, G.; PÁL, M. **International Journal of Molecular Sciences**, v. 21, n. 7, p. 2655, 2020.

JANGRA, M.; DEVI, S.; SATPAL-KUMAR, N.; GOYAL, V.; MEHROTRA, S. Amelioration Effect of Salicylic Acid Under Salt Stress in *Sorghum bicolor* L. **Applied biochemistry and biotechnology**, v. 194, n. 10, p. 4400-4423, 2022.

KATO, Y.; OI, T.; TANIGUCHI, M. Aggregative movement of C4 mesophyll chloroplasts is promoted by low CO_2 under high intensity blue light. **Plant Biology**, v. 25, n. 4, p. 563-570, 2023.

KHALEGHNEZHAD, V.; YOUSEFI, A. R.; TAVAKOLI, A.; FARAJMAND, B.; MASTINU, A. Concentrations-dependent effect of exogenous abscisic acid on photosynthesis, growth and phenolic content of *Dracocephalum moldavica* L. under drought stress. **Planta**, v. 253, n. 6, p. 127, 2021.

KHOSHBAKHT, D.; ASGHAREI, M. R. Influence of foliar-applied salicylic acid on growth, gas-exchange characteristics, and chlorophyll fluorescence in citrus under saline conditions. **Photosynthetica**, v. 53, p. 410-418, 2015.

KOESTER, R. P.; SKONECZKA, J. A.; CARY, T. R.; DIERS, B. W.; AINSWORTH, E. A. Historical gains in soybean (*Glycine max* Merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. **Journal of experimental botany**, v. 65, n. 12, p. 3311-3321, 2014.

KUDO, M.; KIDOKORO, S.; YOSHIDA, T.; MIZOI, J.; KOJIMA, M.; TAKEBAYASHI, Y.; SAKAKIBARA, H.; FERNIE, A. R.; SHINOZAKI, K.; YAMAGUCHI- SHINOZAKI, K. A gene-stacking approach to overcome the trade-off between drought stress tolerance and growth in Arabidopsis. **The Plant Journal**, v. 97, n. 2, p. 240-256, 2019

LESLIE, C. A.; ROMANI, R. J. Salicylic acid: A new inhibitor of ethylene biosynthesis. **Plant Cell Reports**, v. 5, p. 144-146, 1986.

LUO, Z.; WU, X.; XIE, Y.; CHEN, C. Alleviation of chilling injury and browning of postharvest bamboo shoot by salicylic acid treatment. **Food Chemistry**, v. 131, n. 2, p. 456-461, 2012.

MORI, K. Pheromone synthesis. Part 240: Cross-metathesis with Grubbs I (but not Grubbs II) catalyst for the synthesis of (R)-trogodermal (14-methyl-8-hexadecenal) to study the optical rotatory powers of compounds with a terminal sec-butyl group. **Tetrahedron**, v. 65, n. 19, p. 3900-3909, 2009.

MORRISON, W.R.; GROSDIDIER, R.F.; ARTHUR, F.H.; MYERS, S.W.; DOMINGUE, M.J. Attraction, arrestment, and preference by immature *Trogoderma variabile* and *Trogoderma granarium* to food and pheromonal stimuli. **Journal of pest science**, v. 93, p. 135-147, 2020.

NIU, T.; ZHANG, T.; QIAO, Y.; WEN, P.; ZHAI, G.; LIU, E.; AL-BAKRE, D. A.; AL-HARBI, M.S.; GAO, X.; YANG, X. Glycine betaine mitigates drought stress-induced oxidative damage in pears. **Plos one**, v. 16, n. 11, p. e0251389, 2021.

PANDA, S. K.; MOHANTA, Y. K.; PADHI, L.; PARK, Y. H.; MOHANTA, T. P.; BAE, H. Large scale screening of ethnomedicinal plants for identification of potential antibacterial compounds. **Molecules**, v. 21, n. 3, p. 293, 2016

PRAKASH, V.; SINGH, V. P.; TRIPATHI, D. K.; SHARMA, S.; CORPAS, F. J. Nitric oxide (NO) and salicylic acid (SA): A framework for their relationship in plant development under abiotic stress. **Plant Biology**, v. 23, p. 39-49, 2021.

REICH, P.B.; WALTERS, M.B.; ELSWORTH, D.S.; VOSE, J.M.; VOLIN, J.C.; GRESHAM, C.; BOWMAN, D. Relationships of leaf dark respiration to leaf nitrogen, specific leaf area and leaf life-span: A test across biomes and functional groups. **Oecologia**, v. 114, p. 471-482, 1998.

RIVAS-SAN-VICENTE, M.; PLASENCIA, J. Salicylic acid beyond defence: Its role in plant growth and development. **Journal of experimental botany**, v. 62, n. 10, p. 3321-3338, 2011.

ROCHA, J.; NITENBERG, M.; GIRARD-EGROT, A.; JOUHET, J.; MARÉCHAL, E.; BLOCK, M. A.; BRETON, C. Do galactolipid synthases play a key role in the biogenesis of chloroplast membranes of higher plants? **Frontiers in Plant Science**, v. 9, p. 126, 2018.

RODRIGUES, T. L. M.; SILVA, M. E.; GURGEL, E. S.; OLIVEIRA, M. S.; LUCAS, F. C. *Eryngium Foetidum* L. (Apiaceae): A literature review of traditional uses, chemical composition, and pharmacological activities. **Evidence-Based Complementary and Alternative Medicine**, v. 2022, n. 1, p. 2896895, 2022.

ROJAS-SILVA, P.; GRAZIOSE, R.; VESELY, B.; POULEV, A.; MBEUNKUI, F.; GRACE, M.H.; KYLE, D. E.; LILA, M. A.; RASKIN, I. Leishmanicidal activity of a daucane sesquiterpene isolated from *Eryngium foetidum*. **Pharmaceutical Biology**, v. 52, n. 3, p. 398-

401, 2014.

SANTOS, R. P.; CRUZ, A. C. F.; IAREMA, L.; KUKI, K. N.; OTONI, W. C. Protocolo para extração de pigmentos foliares em porta-enxertos de videira micropropagados**. Ceres**, v. 55, P. 356–364.

SANTOS, S. K.; GOMES, D. S.; OLIVEIRA, A. F. P.; SILVA, A. M. O.; MOURA, V. S.; GUSMÃO, M. H. A.; MATOS, E. M.; VICCINI, L. F.; GRAZUL, R. M.; HENSCHEL, J. M.; BATISTA, D. S. Water stress and exogenous carnitine on growth and essential oil profile of *Eryngium foetidum* L. **3 Biotech**, v. 13, n. 10, p. 328, 2023

SANTOS, S.K.; GOMES, D. S.; SOARES, V. A.; DANTAS, E.F.O.; OLIVEIRA, A.F.P.; GUSMÃO, M.H.A.; MATOS, E.M.; VICCINI, L.F.; GRAZUL, R.M.; HENSCHEL, J.M.; BATISTA, D. S. Ascorbic acid mediated mitigation of drought effects on growth, physiology, and essential oil profile in culantro (*Eryngium foetidum* L.). **Vegetos**, p. 1-8, 2024

SANTOS, S.K.; SOARES, V.A.; DANTAS, E.F.O.; SANTOS, L.W.O.; GOMES, D.S.; HENSCHEL, J.M.; BATISTA, D.S. Exogenous carnitine application enhances the growth of culantro (*Eryngium foetidum*) plants. **Vegetos**, v. 36, n. 2, p. 393-399, 2023

SAUD, S.; LI, X.; CHEN, Y.; ZHANG, L.; FAHAD, S.; HUSSAIN, S.; SADIQ, A.; CHEN, Y. Silicon application increases drought tolerance of Kentucky bluegrass by improving plant water relations and morphophysiological functions. T**he Scientific World Journal**, v. 2014, n. 1, p. 368694, 2014.

SERNA-ESCOLANO, V.; MARTÍNEZ-ROMERO, D.; GIMÉNEZ, M.J.; SERRANO, M.; GARCÍA-MARTÍNEZ, S.; VALERO, D.; VALVERDE, J.M.; ZAPATA, P.J. Enhancing antioxidant systems by preharvest treatments with methyl jasmonate and salicylic acid leads to maintain lemon quality during cold storage. **Food Chemistry**, v. 338, p. 128044, 2021

SIKDER, S.; QIAO, Y.; BAODI, D.; SHI, C.; LIU, M. Effect of water stress on leaf level gas exchange capacity and water-use efficiency of wheat cultivars. **Indian Journal of Plant Physiology**, v. 21, p. 300-305, 2016.

SINGH, B. K.; RAMAKRISHNA, Y.; NGACHAN, S. V. Spiny coriander (*Eryngium foetidum* L.): A commonly used, neglected spicing-culinary herb of Mizoram, India. **Genetic Resources and Crop Evolution**, v. 61, n. 6, p. 1085-1090, 2014.

SINGH, D. The relative importance of characters affecting genetic divergence. **Indian Journal of Genetics and Plant Breeding** v. 41, n. 2, p. 237-245, 1981

SINTAHA, M.; MAN, C. K.; YUNG, W. S.; DUAN, S.; LI, M. W.; LAM, H. M. Drought stress priming improved the drought tolerance of soybean. **Plants**, v. 11, p. 2954.

SOARES, V.A.; DANTAS, E.F.; SANTOS, S.K.; SANTOS, L.W.; DIAS, T.J.; HENSCHEL, J.M.; BATISTA, D.S. Effect of salicylic acid on the growth and biomass partitioning in water-stressed radish plants. **Vegetos**, v. 35, n. 3, p. 585-591, 2022.

SUGIYAMA, S. Developmental basis of interspecific differences in leaf size and specific leaf area among C3 grass species. **Functional Ecology**, v. 19, n. 6, p. 916-924, 2005.

TAN, K. Y.; ZHOU, G. S.; REN, S. X. Response of leaf dark respiration of winter wheat to changes in CO_2 concentration and temperature. **Chinese Science Bulletin**, v. 58, p. 1795-1800, 2013.

TANG, Y.Y.; YUAN, Y.H.; SHU, S.; GUO, S.R. Regulatory mechanism of NaCl stress on photosynthesis and antioxidant capacity mediated by transglutaminase in cucumber (*Cucumis sativus* L.) seedlings. **Scientia Horticulturae**, v. 235, p. 294-306, 2018.

THOMAS, P. S.; ESSIEN, E. E.; NTUK, S. J.; CHOUDHARY, M. I. *Eryngium foetidum* L. Essential Oils: Chemical Composition and Antioxidant Capacity. **Medicines**, v. 4, n. 2, p. 24, 2017.

TORMENA, C. D.; RUTLEDGE, D. N.; RAKOCEVIC, M.; BRUNS, R. E.; SCARMINIO, I. S.; MARCHEAFAVE, G. G.; PAULI, E. D. Exogenous application of bioregulators in *Coffea arabica* beans during ripening: Investigation of UV–Visible and NIR mixture design-fingerprints using AComDim-ICA. **Microchemical Journal**, v. 181, p. 107702, 2022.

TROLINDER, N. L. Use of plant bioregulators in tissue culture. In Plant Biochemical Regulators, 1st ed.; GAUSMAN, H.M. Ed.; CRC Press: Boca Raton, FL, USA, 2020; pp. 99–112.

WELLBURN, A. R. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. **Journal of plant physiology**, v. 144, n. 3, p. 307-313, 1994.

XI, J. J.; CHEN, H. Y.; BAI, W. P.; YANG, R. C.; YANG, P. Z.; CHEN, R. J.; HU, T. M.; WANG, S. M. Sodium-related adaptations to drought: New insights from the xerophyte plant *Zygophyllum xanthoxylum*. **Frontiers in Plant Science**, v. 9, p. 1678, 2018.

YILDIRIM, E.; TURAN, M.; GUVENC, I. Effect of foliar salicylic acid applications on growth, chlorophyll and mineral content of cucumber (*Cucumis sativus* L.) grown under salt stress. J. Plant Nutr. 2008, 31, 593–612. [CrossRef]

ZHANG, Y.; XU, S.; DING, P.; WANG, D.; CHENG, Y.T.; HE, J.; GAO, M.; XU, F.; LI, Y.; ZHU, Z. Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. **Proceedings of the National Academy of Sciences**, v. 107, n. 42, p. 18220-18225, 2010.

ZULFIQAR, F.; CHEN, J.; FINNEGAN, P. M.; YOUNIS, A.; NAFEES, M.; ZORRIG, W.; HAMED, K. B. Application of trehalose and salicylic acid mitigates drought stress in sweet basil and improves plant growth. **Plants**, v. 10, n. 6, p. 1078, 2021.

ZULFIQAR, F.; YOUNIS, A.; ABIDEEN, Z.; FRANCINI, A.; FERRANTE, A. Bioregulators can improve biomass production, photosynthetic efficiency, and ornamental quality of *Gazania rigens* L. **Agronomy**, v. 9, n. 11, p. 773, 2019.

8 CONCLUSÃO GERAL

A aplicação de 100 µM de carnitina aumenta o crescimento do coentro-maranhão em condições não estressantes, sobretudo através da modulação do crescimento da raiz. Após período de seca com posterior irrigação, as plantas de coentro-maranhão aumentam a biomassa. A aplicação exógena da carnitina, ácido salicílico e ácido ascórbico não mitigaram o estresse hídrico, mas modularam o perfil de óleo essencial das plantas de coentro-maranhão

ANEXO A - Supplementary Table 1 - CAPÍTULO II

Supplementary Table 1 Results (significance) of the ANOVA for the effects of water condition (WC), carnitine (SA), and their interactions, on the morphophysiological parameters and essential oil compounds of 102-day-old *Eryngium foetidum* L. plants sprayed with water or carnitine (100 µM), and grown under different water conditions

Parameter	**Water condition (WC)**	**Carnitine (CA)**	**WC × CA**
Leaf fresh mass (g)	3.97 *	0.08 **	0.11 **
Leaf dry mass (g)	2.88 *	1.01 *	0.14 **
Root fresh mass (g)	4.33 *	2.22 *	2.06 *
Root dry mass (g)	4.33 *	1.89 *	4.62 *
Total biomass (g)	4.44 *	4.02 *	2.47 *
Shoot/root ratio	2.22 *	8.88 ^{ns}	4.44 *
Root length (cm)	4.88 *	3.21 *	4.82 *
Number of leaves	3.08 *	0.08 **	2.01 *
Leaf area (cm^2)	4.66 *	3.44 *	3.09 *
Specific leaf area (cm^2 g^{-1})	65.23 ^{ns}	77.90 ^{ns}	100.00 ^{ns}
Chlorophyll *a* (µg cm^{-2})	33.33 ^{ns}	2.04 *	1.18 *
Chlorophyll *b* (µg cm^{-2})	4.44 *	0.04 **	0.92 **
Total chlorophylls (µg cm^{-2})	2.84 *	3.22 *	1.11 *
Total carotenoids (µg cm^{-2})	0.92 **	0.00 **	0.25 **
A (µmol CO$_2$ m^{-2} s^{-1})	66.67 ^{ns}	16.67 ^{ns}	85.55 ^{ns}
g_s (mol H$_2$O m^{-2} s^{-1})	77.10 ^{ns}	100.00 ^{ns}	100.00 ^{ns}
E (mmol H$_2$O m^{-2} s^{-1})	0.21 **	1.11 *	0.80 **
A/E	0.74 **	66.66 ^{ns}	4.22 *
Ci (mmol CO$_2$ mol^{-1} air)	100.00 ^{ns}	58.84 ^{ns}	8.99 ^{ns}
A/Ci	100.00 ^{ns}	100.00 ^{ns}	44.38 ^{ns}
R$_{dark}$	0.92 **	3.12 *	1.88 *
Light compensation point	1.15 *	1.15 *	3.33 *
F_0	33.33 ^{ns}	67.33 ^{ns}	100.00 ^{ns}
F_M	12.91 ^{ns}	20.45 ^{ns}	33.33 ^{ns}
F_V	8.16 ^{ns}	7.90 ^{ns}	88.17 ^{ns}
F_V/F_M	3.21 *	10.11 ^{ns}	72.14 ^{ns}
F_V/F_0	67.77 ^{ns}	100.00 ^{ns}	100.00 ^{ns}
O	100.00 ^{ns}	100.00 ^{ns}	100.00 ^{ns}
J	82.39 ^{ns}	100.00 ^{ns}	62.88 ^{ns}
I	100.00 ^{ns}	100.00 ^{ns}	100.00 ^{ns}
P	50.00 ^{ns}	91.10 ^{ns}	100.00 ^{ns}
S	53.33 ^{ns}	7.10 ^{ns}	12.12 ^{ns}
A	6.21 ^{ns}	5.04 ^{ns}	9.36 ^{ns}
T$_M$	3.33 *	0.72 **	1.22 **
Hydroquinone	0.07 **	0.00 **	2.17 *
9-Octadecenal	100.00 ^{ns}	100.00 ^{ns}	67.67 ^{ns}
Eryngial	0.23 **	0.00 **	0.11 **
Decanal	6.28 ^{ns}	42.92 ^{ns}	100.00 ^{ns}

8-Hexadecenal	13.31 ^{ns}	100.00 ^{ns}	100.00 ^{ns}
Benzaldehyde	54.82 ^{ns}	21.15 ^{ns}	83.33 ^{ns}
Dodecanal	5.64 ^{ns}	83.33 ^{ns}	7.21 ^{ns}

^{ns} = Non-significant at a 5% probability; * and ** significant at a level of 5 and 1% probability, respectively, by the F test.