
**PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(BIOLOGIA VEGETAL)**

Da germinação à regeneração: o papel do alumínio (Al) e sua interação com os processos fisiológicos de espécies acumuladoras de Al do Cerrado

MATHEUS ARMELIN NOGUEIRA

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MATHEUS ARMELIN NOGUEIRA

Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Biologia Vegetal).

Orientador: Dr. Gustavo Habermann

Coorientador: Dr. Jean Carlos Cardoso

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AUTOR: MATHEUS ARMELIN NOGUEIRA

ORIENTADOR: GUSTAVO HABERMANN

COORIENTADOR: JEAN CARLOS CARDOSO

Aprovado como parte das exigências para obtenção do Título de Doutor em Ciências Biológicas (Biologia Vegetal), área: Biologia Vegetal pela Comissão Examinadora:

Prof. Dr. GUSTAVO HABERMANN (Participação Virtual)
Departamento de Biodiversidade / Instituto de Biociências de Rio Claro Unesp

Documento assinado digitalmente
 **GUSTAVO HABERMANN**
Data: 30/10/2023 16:06:03-0300
Verifique em <https://validar.iti.gov.br>

Prof^a. Dr^a. MARINA ALVES GAVASSI (Participação Virtual)
Departamento de Ciências Biológicas / UNESP / Bauru

Documento assinado digitalmente
 **MARINA ALVES GAVASSI**
Data: 27/10/2023 14:08:18-0300
Verifique em <https://validar.iti.gov.br>

Prof. Dr. CLEBERSON RIBEIRO (Participação Virtual)
Departamento de Biologia Geral / Universidade Federal de Viçosa

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 **CLEBERSON RIBEIRO**
Data: 27/10/2023 14:00:28-0300
Verifique em <https://validar.iti.gov.br>

Prof^a. Dr^a. ANNA CAROLINA GRESSLER BRESSAN (Participação Virtual)
Departamento de Ecologia / Universidade de São Paulo - Instituto de Biociências

Documento assinado digitalmente
 **ANNA CAROLINA GRESSLER BRESSAN**
Data: 27/10/2023 09:10:37-0300
Verifique em <https://validar.iti.gov.br>

Prof. Dr. LUIZ ALFREDO RODRIGUES PEREIRA (Participação por Parecer Circunstanciado)
Departamento de Botânica / Universidade de Brasília

Documento assinado digitalmente
 **LUIZ ALFREDO RODRIGUES PEREIRA**
Data: 29/10/2023 22:28:26-0300
Verifique em <https://validar.iti.gov.br>

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Resumo

O Al é o metal mais abundante na crosta terrestre e, quando presente em solos ácidos (pH < 5,0), torna-se solúvel e tóxico para a maioria das espécies de plantas. Por outro lado, existem plantas que conseguem prosperar em solos com alta disponibilidade de Al e são chamadas de acumuladoras. Essas plantas acumulam grandes quantidades de Al em seus tecidos sem apresentar sinais de toxicidade. No capítulo 1 utilizamos as espécies *Qualea grandiflora* e *Vochysia tucanorum* (Vochysiaceae), plantas acumuladoras de Al nativas do Cerrado, para testarmos diferentes hipóteses. Neste estudo, testamos a germinação de sementes em meio de cultura com concentrações crescentes de Al e sob a influência do etileno (Et). As sementes foram cultivadas *in vitro* em meio MS (Murashige e Skoog 1962) a ¼ de força contendo 0, 90, 180, 270 e 320 µM de Al. As sementes foram tratadas com água e Et para o cultivo. Aqui evidenciamos que o Al impacta significativamente a germinação e o crescimento de *Q. grandiflora* e *V. tucanorum* e que o Et induziu a germinação em sementes de *V. tucanorum* sem Al, sugerindo um efeito substitutivo. O capítulo 2 fornece insights valiosos sobre o papel do Al na organogênese e na produção de compostos fenólicos nessas espécies. O impacto do alumínio (Al) na organogênese e na produção de compostos fenólicos dessas espécies foi observado em Al a 320 µM e ausência de BAP (6-Benzilaminopurina), resultou na regeneração de raízes adventícias em ambas. No entanto, a adição de BAP sem Al não gerou respostas de regeneração. A regeneração e o desenvolvimento de brotos foram observados quando 0,44 e 0,88 µM de BAP foram adicionados ao meio de cultura com 320 µM de Al. Além disso, ambas as espécies exibiram um notável conteúdo fenólico, que foi ainda mais aprimorado pela adição de Al e/ou BAP. A capacidade antioxidante dos extratos também aumentou significativamente com a adição de Al e/ou BAP. Essas abordagens podem fornecer

perspectivas valiosas para estratégias de conservação e propagação dessas espécies do Cerrado.

Palavras-chave: Plantas acumuladoras, germinação de sementes, organogênese, espécies do Cerrado, micropropagação.

Abstract

Aluminum (Al) is the most abundant metal in the Earth's crust and, when present in acidic soils ($\text{pH} < 5.0$), becomes soluble and toxic to most plant species. Conversely, some plants thrive in soils with high Al availability and are termed accumulators. These plants accumulate large amounts of Al in their tissues without showing signs of toxicity. In Chapter 1, we employed the species *Qualea grandiflora* and *Vochysia tucanorum* (Vochysiaceae), Al-accumulating plants native to the Cerrado, to test different hypotheses. In this study, we examined seed germination in culture medium with increasing Al concentrations and under the influence of ethylene (Et). Seeds were cultured in vitro on 1/4-strength MS medium (Murashige and Skoog 1962) containing 0, 90, 180, 270, and 320 μM of Al. Seeds were treated with water and Et for cultivation. We highlighted that Al significantly impacts the germination and growth of *Q. grandiflora* and *V. tucanorum*, and Et induced germination in *V. tucanorum* seeds without Al, suggesting a substitutive effect. Chapter 2 provides valuable insights into the role of Al in organogenesis and phenolic compound production in these species. The impact of aluminum (Al) on organogenesis and phenolic compound production was observed at 320 μM Al and the absence of 6-Benzylaminopurine (BAP), resulting in the regeneration of adventitious roots in both species. However, the addition of BAP without Al did not generate regeneration responses. Regeneration and shoot development were observed when 0.44 and 0.88 μM BAP were added to the culture medium with 320 μM Al. Additionally, both species exhibited a notable phenolic content, further enhanced by the addition of Al and/or BAP. The antioxidant capacity of the extracts also significantly increased with the addition of Al and/or BAP. These approaches can provide valuable insights for conservation and propagation strategies for these Cerrado species.

Key words: Al-accumulating plants, seed germination, organogenesis, Cerrado species, micropropagation

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Introdução geral

A vegetação do Cerrado, amplamente conhecida como ‘savana brasileira’, apresenta entre 1000 e 2000 espécies por ha e se caracteriza por ser um dos *hotspots* mundiais de biodiversidade (COLLI; VIEIRA; DIANESE, 2020; MYERS *et al.*, 2000). Além disso, é o segundo maior bioma brasileiro (COUTINHO, 2002), sendo superado em área apenas pela Amazônia, ocupando 21% do território nacional (KLINK; MACHADO, 2005; RATTER; BRIDGEWATER; RIBEIRO, 2003).

O Cerrado é considerado um mosaico de fisionomias vegetais incluindo florestas, formações savânicas (campo sujo, campo cerrado) e formas campestres bem abertas (campo limpo), que juntas são referidas como Cerrado *sensu lato* (FERREIRA *et al.*, 2003). Espécies lenhosas são distribuídas em fisionomias mais densas, florestais, como é o caso do ‘Cerradão’. Já no cerrado *sensu strictu* (s. str.) as espécies constituem um tipo de fisionomia savânica, contendo uma vegetação mais arbustiva e árvores de sub-bosque, com alta irradiância ao nível do solo (KISSMANN *et al.*, 2012). Contudo, muitas espécies endêmicas têm se perdido nas últimas décadas devido principalmente à grande expansão da agricultura e intensa exploração local de produtos nativos (KLINK; MACHADO, 2005; SANO *et al.*, 2010). Essas atividades antrópicas têm provocado a degradação e a perda de habitats desse ecossistema, colocando em risco a sua conservação e o seu potencial biotecnológico (SANO *et al.*, 2019).

Cerca de metade dos 2 milhões de km² originais do Cerrado foram transformados em pastagens plantadas, culturas anuais e outros tipos de uso de solo (GOMES *et al.*, 2019). Além disso, estudos que contemplam espécies nativas do Cerrado ainda são incipientes se comparados aos de espécies de outros biomas brasileiros, intensificando a perda de material genético vegetal nativo (STRASSBURG *et al.*, 2017). Dessa forma, a conservação e preservação de tais espécies se torna essencial devido ao cenário atual.

Os solos sob o cerrado são geralmente pobres, ácidos ($\text{pH} < 5,0$), bem drenados, profundos e apresentam altos níveis de alumínio trocável (QUEIROZ NETO, 1982; REATTO; CORREIA; SPERA, 1998). O alumínio (Al) pode competir com outros elementos pelos mesmos sítios químicos nas partículas do solo, sendo o terceiro elemento químico mais abundante na crosta terrestre e, em solos ácidos, encontrado principalmente na forma trivalente (Al^{3+}), que é tóxico para a maioria das espécies de plantas (MATSUMOTO, 2000; VARDAR; ÜNAL, 2007). Os solos ácidos representam 30-40% das terras livres de gelo (KOCHIAN; HOEKENGA; PINEROS, 2004; VON UEXKÜLL; MUTERT, 1995) sendo, portanto, juntamente com o Al, ameaças potenciais à agricultura, com impactos negativos para os rendimentos.

O primeiro sintoma da toxicidade do Al é a diminuição do crescimento das raízes (HORST; WANG; ETICHA, 2010; SILVA *et al.*, 2019). Em espécies sensíveis, aproximadamente 80-90% do Al absorvido é retido no sistema radicular (SILVA *et al.*, 2023; VITORELLO; CAPALDI; STEFANUTO, 2005). Além dos efeitos diretos nas raízes, o Al pode causar efeitos ‘indiretos’ em órgãos acima do solo, como crescimento reduzido de brotos (JIANG *et al.*, 2009), baixa troca gasosa foliar e respostas fotoquímicas em *Coffea arabica* (KONRAD *et al.*, 2005), *Zea mays* (LIDON *et al.*, 1999) e *Citrus* spp. (BANHOS; DE SOUZA; HABERMANN, 2016; JIANG *et al.*, 2009; JIANG, 2008; SILVA *et al.*, 2018).

No entanto, apesar da toxicidade, a flora desta região prospera nestes solos ácidos (HABERMANN; BRESSAN, 2011; HARIDASAN, 2008; NOGUEIRA *et al.*, 2019; SOUZA, *et al.*, 2017). Algumas espécies possuem mecanismos de tolerância ao Al, exsudando ácidos orgânicos como citrato, malato, oxalato e succinato através das membranas celulares de suas raízes. Esses ácidos formam complexos não tóxicos com o Al, prevenindo sua absorção ainda na rizosfera (RYAN *et al.*, 2011). Paralelamente,

outras espécies conseguem se desenvolver em solos com alta concentração de Al (m%), acumulando Al em seus tecidos e órgãos sem apresentar danos ou sinais de toxicidade que possam interferir em seu metabolismo ou desenvolvimento (BRESSAN, *et al.*, 2021; DE ANDRADE *et al.*, 2011; DELHAIZE; RYAN, 1995; HARIDASAN, 1982). Exemplos dessas espécies incluem *Fagopyrum esculentum* Moench (Polygonaceae), *Hydrangea macrophylla* L. (Hydrangeaceae) e *Camellia sinensis* (L.) Kuntze (Theaceae), conforme relatado por MA *et al.*, 2001 e WATANABE e OSAKI, 2002. Plantas que armazenam mais de 1000 mg de alumínio por kg de massa foliar seca são chamadas de acumuladoras de Al (CHENERY, 1948). Algumas dessas espécies lenhosas acumuladoras de Al são nativas da vegetação do Cerrado na América do Sul (HARIDASAN, M., 1982) e pertencem às famílias Melastomataceae (gênero *Miconia*), Rubiaceae, Simplicaceae e Vochysiaceae (gêneros *Vochysia* e *Qualea*) (BRESSAN; COAN; HABERMANN, 2016; DE ANDRADE *et al.*, 2011; HARIDASAN, 1982; MALTA *et al.*, 2016; SOUZA, *et al.*, 2015). No entanto, nesta vegetação, essas espécies acumuladoras de Al podem apresentar entre 4.000 e até 20.000 mg Al por kg de massa seca (HARIDASAN, 1982; HARIDASAN, 2008; NOGUEIRA *et al.*, 2019), demonstrando uma grande variação na acumulação de Al. Além das folhas, essas plantas também podem reter Al nas sementes (HARIDASAN, 1982; SCALON; HARIDASAN; FRANCO, 2013).

Qualea grandiflora Mart. e *Vochysia tucanorum* Mart. são espécies de plantas nativas do Cerrado que acumulam Al em suas folhas e raízes (BRESSAN *et al.*, 2021; BRUNNER; SPERISEN, 2013; HARIDASAN, 1982; NOGUEIRA *et al.*, 2019; SILVA, *et al.*, 2023). Pesquisas anteriores mostraram que este metal pode apresentar uma resposta benigna neste grupo de plantas, aumentando a biomassa vegetal e o alongamento das raízes em *C. sinensis*, *Melastoma malabathricum* L. (Melastomataceae) e *Quercus*

serrata Murray (Fagaceae) (BOJÓRQUEZ-QUINTAL *et al.*, 2017). As relações específicas e o impacto potencial do Al nos parâmetros fisiológicos que explicam a influência positiva do alumínio no crescimento das plantas em espécies acumuladoras de alumínio são raramente documentadas ou não comumente relatadas. Outro padrão de distribuição entre essas plantas envolve a dependência do Al, como *Vochysia tucanorum* (Vochysiaceae). Esta espécie não cresce bem em solo calcário e apresenta folhas necróticas e raízes atrofiadas após 45 dias em comparação com um desenvolvimento perfeito em solo ácido rico em Al (SOUZA *et al.*, 2017). O mesmo padrão foi observado quando esta espécie perdeu suas folhas e apresentou raízes necróticas em solução nutritiva sem Al (BRESSAN *et al.*, 2021). *Qualea grandiflora* segue o mesmo comportamento em condições sem Al (CURY *et al.*, 2020). Apesar desses relatos, nenhum papel fisiológico foi atribuído ao Al nessas plantas ainda.

Além de sua habilidade de acumular Al e se adaptar às condições adversas, algumas plantas típicas do Cerrado também têm demonstrado a capacidade de produzir compostos fenólicos (ARRUDA; ARAÚJO; JUNIOR, 2022) como forma de adaptação para resistir ao estresse oxidativo causado por condições ambientais extremas (FARIAS *et al.*, 2013; MOREIRA-ARAÚJO *et al.*, 2019). Os ácidos fenólicos são os metabólitos secundários mais abundantes nas plantas e desempenham papéis importantes em diversos processos fisiológicos (ZHANG *et al.*, 2022). Eles desempenham um papel importante nos mecanismos de defesa das plantas, sendo depositados nas paredes celulares após a infecção por patógenos (KUMAR, Santosh *et al.*, 2020). Quando são excretados pelos sistemas radiculares das plantas, podem inibir o crescimento na rizosfera adjacente e afetar a flora bacteriana do solo (CHOWDHARY *et al.*, 2021). Eles também podem atuar como moduladores do desenvolvimento das plantas, regulando o catabolismo do ácido indol acético (IAA). Eles são eficazes na regulação do crescimento das plantas,

diferenciação celular e organogênese (KUMAR *et al.*, 2020; KUMAR *et al.*, 2023). Eles são conhecidos por suas propriedades antioxidantes (FARIAS *et al.*, 2013), e além de sustentarem a sobrevivência dessas plantas em um ambiente hostil, também despertaram o interesse na indústria farmacêutica e alimentícia devido aos benefícios potenciais para a saúde humana (ALBUQUERQUE *et al.*, 2021; SOARES, 2002). A interação complexa entre o ambiente do Cerrado, incluindo a presença de alumínio, pode influenciar a presença de compostos fenólicos e sua atividade antioxidante (NOGUEIRA *et al.*, 2023).

Investigar os impactos do Al nessas plantas pode ser um desafio, pois propagá-las e cultivá-las em condições *ex situ* que isolam os efeitos do Al de outros elementos é uma tarefa complexa (CARDOSO; DA SILVA, 2013; LEITE *et al.*, 2021; LIMA *et al.*, 2022). A cultura de tecidos pode ser uma técnica valiosa para conservar, cultivar e propagar espécies do Cerrado sob condições controladas, permitindo o isolamento e teste de fatores, como o Al, no meio de cultura. Ela é utilizada para a propagação de plantas quando a regeneração através da germinação de sementes é desafiadora. Técnicas de propagação *in vitro* vem sendo utilizadas como alternativas para propagação de espécies nativas do Cerrado, especialmente para fins econômicos e de conservação (FRANÇA *et al.*, 1995; PEREIRA *et al.*, 2005; REZENDE *et al.*, 2019). Elas oferecem diversas possibilidades para a propagação de plantas podendo se utilizar, por exemplo, de materiais vegetativos para a indução de organogênese e regeneração sob condições assépticas e controladas (CARDOSO; SHENG GERALD; TEIXEIRA DA SILVA, 2018).

Entre as técnicas normalmente utilizadas na cultura de tecidos vegetais, a micropropagação tem sido amplamente utilizada para propagar rapidamente várias espécies de interesse econômico, tais como *Eucalyptus* spp., *Citrus* spp., (*Caryocar brasiliense*), o cajuzinho-do-cerrado (*Anacardium othonianum* Rizz.), o araticum

(*Annona crassiflora*) e o baru (*Dipteryx alata* Vogel.), (AYALA *et al.*, 2019; DANIELLE SOUSA *et al.*, 2017; RIBEIRO *et al.*, 2009; SANTOS *et al.*, 2006; SILVEIRA; DA SILVA; SIBOV, 2022). A micropropagação consiste em técnicas de propagação vegetativa com o objetivo de obter multiplicação vegetal através: a) embriogênese somática, b) indução direta ou indireta de calos para formação de partes aéreas, e c) indução do desenvolvimento de botões apicais/axilares (AL-MAYAH, 2019). Em comparação com os métodos tradicionais de propagação vegetativa de plantas, ela apresenta diversas vantagens, como a obtenção de plantas geneticamente uniformes, livres de pragas e doenças, com alto vigor e qualidade, em um curto espaço de tempo e em um pequeno espaço físico (CARDOSO; SHENG GERALD; TEIXEIRA DA SILVA, 2018; MURASHIGE; SKOOG, 1962). Essa técnica pode contribuir para o estudo da fisiologia, da bioquímica e da genética das plantas do cerrado, bem como para o seu uso sustentável e a sua valorização econômica (NIEMEYER, 2017; SANTOS *et al.*, 2006). Além disso, as técnicas de micropropagação não dependem das condições climáticas e são especialmente úteis em espécies que apresentam sementes recalcitrantes, que perdem rapidamente a sua viabilidade; sendo o caso de várias espécies perenes tropicais (LITZ; JAISWAL, 1991). A micropropagação é também importante para os esforços de conservação a longo prazo para preservar a biodiversidade de espécies e ecossistemas ameaçados (CHOKHELI *et al.*, 2020). Apesar dessas vantagens, a micropropagação de plantas do Cerrado também enfrenta alguns desafios como a escassez de informações sobre as exigências nutricionais e fisiológicas das espécies, a baixa taxa de multiplicação e enraizamento *in vitro*, a ocorrência de anormalidades morfológicas e fisiológicas nas plântulas, a dificuldade de aclimatização e a falta de protocolos padronizados para cada espécie.

A germinação de plantas do Cerrado apresenta características distintas, refletindo a resiliência e a adaptabilidade dessas espécies ao seu ambiente único (FRANCO; HARIDASAN; FERREIRA, 2008; ZAIDAN; CARREIRA, 2008). Existem sementes de certas espécies (ou até mesmo dentro da mesma espécie) que não conseguem germinar ou encontram dificuldades para fazê-lo, mesmo quando incubadas em condições que pareçam ser favoráveis. Essas sementes são classificadas como dormentes e não são capazes de germinar nas mesmas condições (BEWLEY, 1997; CORBINEAU *et al.*, 1995). A maioria das sementes de espécies arbóreas do Cerrado exibe um estado de dormência, com a dormência física sendo a mais prevalente (BASKIN; BASKIN, 2001). Estratégias de regeneração dessas espécies envolvem germinação, no entanto, a dormência de sementes é um fenômeno comum na maioria dessas espécies (ZAIDAN; CARREIRA, 2008). Especificamente, o etileno (Et) regula a germinação e a dormência de várias espécies por meio de uma complexa rede de sinalização hormonal (ARC *et al.*, 2013; MATILLA; MATILLA-VÁZQUEZ, 2008). No contexto das sementes de *V. tucanorum*, foi demonstrado que o Et tem a capacidade de interromper a dormência das sementes e promover a germinação (PEREIRA *et al.*, 2011), embora as justificativas fisiológicas para este fenômeno ainda não estejam completamente esclarecidas.

Considerando esse cenário, o presente trabalho utilizou-se de duas espécies nativas do Cerrado acumuladoras de Al: *Q. grandiflora* e *V. tucanorum*. No capítulo 1, considerando a dificuldade em obter mudas de espécies lenhosas do Cerrado que acumulam Al, e uma possível interação entre o Et e a dormência das sementes dessas espécies, foram realizados testes de germinação em meio de cultura com concentrações crescentes de Al sob a influência de Et, avaliando-se parâmetros de crescimento e performance germinativa. A hipótese é que seus embriões dependem do Al para retomar o crescimento e desenvolvimento e que o Et poderia melhorar o desempenho da

germinação. No capítulo 2, testamos a hipótese da relevância do Al e sua possível interação com a citocinina 6-benzilaminopurina (BAP) na promoção da organogênese de brotos e raízes adventícias dessas espécies. Além disso, investigou-se se a presença de Al e BAP tem uma relação com o conteúdo total de ácidos fenólicos e a atividade antioxidante em extratos dessas duas espécies do Cerrado. Essa abordagem integrada permite uma compreensão mais profunda das interações complexas entre essas espécies do Cerrado, o Al, o BAP e o Et. Dado que não há estudos existentes que empregam o Al em meio de cultura para plantas lenhosas nativas do Cerrado, a importância deste estudo reside em sua capacidade de abrir portas para novas linhas de pesquisa futuras sobre o assunto. Ele oferece uma nova perspectiva e um ponto de partida valioso para explorar ainda mais o potencial e as aplicações do Al na cultura de tecidos de plantas nativas do Cerrado.

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CAPÍTULO I

Is aluminum (Al) essential for embryos germination of *Qualea grandiflora* and

***Vochysia tucanorum*, Al-accumulating species?**

Matheus Armelin Nogueira¹, Jean Carlos Cardoso²,

Gustavo Habermann³

¹Programa de Pós-Graduação em Biologia Vegetal, Departamento de Biodiversidade, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brazil;

²Laboratório de Fisiologia Vegetal e Cultura de Tecidos, Departamento de Biotecnologia, Produção de Plantas e Animais, Centro de Ciências Agrárias, Universidade Federal de São Carlos, Rodovia Anhanguera, km 174, CEP 13600-970 Araras, SP, Brazil

³Departamento de Biodiversidade, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brazil.

Abstract

Qualea grandiflora and *Vochysia tucanorum* (Vochysiaceae) are Aluminum-accumulating woody species from the Cerrado vegetation in South America and retain aluminum (Al) in their seeds. Studies suggest Al dependency for these species, but no physiological role has been ascribed to Al in these plants. Due to difficulties in obtaining seedlings of these species (ethylene (Et) seems to enhance their germination), we germinated their seeds in culture medium with increasing Al concentrations and under ethylene (Et) influence to see how important is their seed Al content to germination. Despite showing more than 8,000 (*Q. grandiflora*) and 32,000 (*V. tucanorum*) mg Al kg⁻¹ seeds, both species, but mainly *V. tucanorum*, germinated better in Al-enriched medium. Et induced germination of *V. tucanorum* seeds when Al was not present in the medium, suggesting Et could replace Al during germination of this Al-accumulating species. Further research is proposed on the physiological roles of Al and Et in these Al-accumulating species.

Key-words: Al³⁺, Brazilian savanna, culture medium, ethylene, Vochysiaceae.

Introduction

Aluminum (Al) is the third most abundant element in the Earth's crust, and in acidic soils (pH < 5.0) it is found mainly in the trivalent form (Al^{3+}), which is toxic to most plant species (Matsumoto 2000; Vardar and Ünal 2007). Acidic soils account for 30–40 % of ice-free land (Von Uexküll and Mutert 1995; Kochian et al. 2004). Therefore, acidic soils and Al are potential threats to agriculture with negative impacts for yields.

The first symptom of Al toxicity is the inhibition of root growth (Horst et al. 2010; Silva et al. 2019). In sensitive species, approximately 80–90 % absorbed Al is retained in the root system (Vitorello et al. 2005; Silva et al. 2019, 2023). Besides direct effects on the roots, Al can cause ‘*indirect*’ effects on aboveground organs, such as reduced shoot growth (Jiang et al. 2009), low gas exchange rates and photochemical performance in *Coffea arabica* (Konrad et al. 2005), *Zea mays* (Lidon et al. 1999) and *Citrus* spp. (Jiang et al., 2008, 2009; Banhos et al., 2016; Silva et al., 2018).

Some species are tolerant to Al and exude organic acids (OA) (citrate, malate, oxalate, and succinate) through the root cell membranes forming non-toxic Al-OA complexes, avoiding Al uptake in the rhizosphere (Ryan et al. 2011). Other species grow well on acidic soils with high Al saturation (m%) showing no toxicity symptoms (Haridasan 1982; Ma et al. 2001; Bressan et al. 2016). These plants absorb and accumulate Al in their roots (Zaia et al. 2022) and different organs (Timpone and Habermann 2022) but mainly in leaves (Haridasan 1982; Brunner and Sperisen 2013; Bressan et al. 2016; Nogueira et al. 2019). These are considered Al-accumulating species when their leaves show at least 1000 mg Al per kg dry mass (Chenery 1948; Jansen et al. 2002).

Some of these Al-accumulating woody species are native to the Cerrado vegetation in South America (Haridasan, 1982) and belong to Melastomataceae (genus

Miconia), Rubiaceae, Simplicaceae, and Vochysiaceae (genera *Vochysia* and *Qualea*) (Haridasan, 1982; Andrade et al. 2011; Souza et al. 2015; Bressan et al. 2016; Malta et al. 2016). Nevertheless, Cerrado Al-accumulating species show between 4,000 and up to 20,000 mg Al kg⁻¹ dry leaf (Haridasan 1982; Haridasan and De Araújo 1988; Nogueira et al. 2019), demonstrating a large range in Al accumulation. Besides leaves, these plants can also retain Al in seeds (Haridasan et al. 1986; Scalón et al. 2013).

Another distribution pattern between these plants involves dependency on Al, as in *Vochysia tucanorum* (Vochysiaceae). This species does not grow well in calcareous soil, shows necrotic leaves and stunted roots after 45 days in comparison to a perfect development in acidic soil rich in Al (Souza et al. 2017). The same pattern was observed when this species shed their leaves and showed necrotic roots in nutrient solution without Al (Bressan et al. 2021). *Qualea grandiflora* follows the same response in conditions without Al (Cury et al. 2020). Despite these reports, no physiological role has been ascribed to Al in Al-accumulating and -depending species.

Regeneration strategies of Cerrado woody plants involves germination, but most of these woody species show some type of seed dormancy (Zaidan and Carreira 2008). In the case of *V. tucanorum* seeds, ethylene (Et) was evidenced to break their seed dormancy and induce germination (Pereira et al. 2011), although the physiological reasons for this dormancy break remained unclear.

Plant tissue culture is a technique used for plant propagation when regeneration through seed germination is challenging. Given the difficulty in obtaining seedlings of Al-accumulating Cerrado woody species, like *Q. grandiflora* and *V. tucanorum* and a possible interaction between these seeds with Et and seed dormancy, we tested germination of seeds of these species in a culture medium with increasing concentrations

of Al and under the influence of Et. We hypothesized that their embryos depend on Al to resume growth and development and that Et could enhance the germination performance.

Material and methods

Study area and plant material

The seeds were obtained from fruits collected in two different areas: (i) a forest-type Cerrado vegetation called ‘Cerradão’, which is a more densely treed savanna, whose name is the augmentative of ‘Cerrado’ in Portuguese, and (ii) a remnant of a savanna-type physiognomy named Cerrado *sensu stricto* (*s. str.*) broadly known as ‘Brazilian savanna’. The ‘Cerradão’ area was located at the “São José da Conquista” farm (22°15' S, 47°42' W; 770 m of altitude), in the municipality of Itirapina, São Paulo state, southeastern Brazil, and the Cerrado *s. str.* remnant was located at “Campininha” farm, at the Mogi Guaçu Biological reserve (22°15' S, 47° 09' W; 680 m of altitude), an area that has been preserved in its natural conditions since 1950.

Five mature plants of *Qualea grandiflora* Mart. and *Vochysia tucanorum* Mart. (Vochysiaceae) were randomly selected in both areas between August and September (2020 - 2021), during fruit phase. Soil samples were collected next to the trees at 20–40 cm of depth. Fertility parameters, such as pH, cation exchange capacity (CEC), soil Al saturation (m%) and macro- and micronutrient availabilities were measured (Supplementary material 1). The plants were divided into four quadrants (N, S, E, W), and mature fully expanded green leaves and fruits were collected from each quadrant.

Aluminum concentration in donor plants

The samples (leaf, fruit, and seed) were taken to a plant nutrition laboratory at the Agronomic Institute of Campinas (IAC, Campinas, Brazil), ground, and digested in a

solution of nitric:perchloric acids (3:1, v/v). The concentration of Al was assessed by the inductively coupled plasma optical emission spectroscopy (ICP-OES) method and expressed as mg Al per kg dry mass (Table 1).

Experimental strategy

Seeds of both species were separated from their fruits, washed, and stored at 25 ± 2 °C. The seeds were immersed for 24 hours in deionized water 240 g L^{-1} 2-chloroethyl phosphonic acid (Ethrel®), an ethylene (Et) -releasing compound. Then, water-treated, and Et-treated seeds had their tegument removed and the embryos were put to germinate (“seeded”) *in vitro* with culture medium containing 0, 90, 180, 270, and $320 \text{ }\mu\text{M}$ Al. The percentage of germination (%G), mean germination time, germination speed index, root length and biomass, shoot length and biomass were assessed over time.

To check Et accumulation in the embryos and Et x Al interaction, two groups of seeds (Et-treated and water-treated) were inoculated *in vitro* with culture medium containing 0, 180, and $320 \text{ }\mu\text{M}$ Al and then, Et evolution (production) was quantified in the embryos 14 days after inoculation.

Culture medium

A MS (Murashige and Skoog 1962) medium at $\frac{1}{4}$ strength (25% of macronutrients) was used. This reduction in nutrients was necessary to approximate the medium nutrient status to the limited nutrient availability found in Cerrado soils where donor plants grew (Table 1). The medium contained 30 g L^{-1} sucrose, 0.1 g L^{-1} myo-inositol, 0.5 mg L^{-1} 6-benzylaminopurine (BAP), and 2 mg L^{-1} gibberellic acid (GA_3) (Duchefa, Haarlem, Netherlands), and it was solidified with 2.7 g L^{-1} Gelrite®. This medium has been used to successfully germinate seeds of other Cerrado species (Cardoso

and da Silva 2013). The medium pH was adjusted to 4.5 ± 0.05 , and it was sterilized (with an autoclave) at $121\text{ }^{\circ}\text{C}$ for 25 min. The Al concentrations in the medium (0, 90, 180, 270, and $320\text{ }\mu\text{M}$ Al) were selected in accordance with other studies that tested Al toxicity in *Picea glauca* (Nosko et al. 1988), *Triticum aestivum* (Lima and Copeland, 1990), *Oryza sativa* (Kikui et al. 2005), and *Nicotiana tabacum* (Vardar et al. 2006). However, there is only one work involving the use of Al *in vitro* in culture media for Cerrado species (Nogueira et al. 2023).

In vitro seed germination essays

The seeds were immersed in 70 % ethanol (v/v) for 3 min, followed by a 50 % sodium hypochlorite (containing 1.0–1.25% of active chlorine) solution for 20 min, and rinsed three times with sterilized deionized water. After cleansing, the mature zygotic embryos (MZE) were retrieved under a laminar flow chamber and inoculated (seeded) in borosilicate test tubes (20 cm high $\times$ 2.1 cm wide) containing 65 mL of the culture media and sealed with parafilm[®].

Test tubes were set in racks under $26 \pm 1\text{ }^{\circ}\text{C}$ and $25\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$ provided with 60W fluorescent bulbs (Phillips, Netherlands) under a 16 h photoperiod.

Embryo germination parameters

The percentage of germination (%), and root and shoot lengths (cm) were measured at 7, 14, 21, 28, and 35 days after *in vitro* seeding. Germination speed index (GSI) was calculated according to the method described by Maguire (1962) using the following equation: $\text{GSI} = G_1/N_1 + G_2/N_2 + \dots + G_5/N_5$, where G_n/N_n is the number of germinated seeds per number of days in the first (7 days), second (14 days), third (21 days), fourth (28 days), and fifth (35 days) evaluations.

We calculated germination time (GT) using the following equation:

$GT = \sum (Gi \times i) / \sum Gi$, where “ i ”, is the number of days between seed inoculation (day 7) and seed germination; Gi , the number of seeds germinated on day i . GT corresponds to the mean germination time of the fraction of seeds that germinated, and does not factor in seeds that failed to germinate (Ellis and Roberts 1980).

Ethylene measurements

On the 14th day of inoculation, 20 mL of air from test tubes was taken with a gas tithe syringe and analyzed in a gas chromatograph (TRACE 1300 Series, Thermo Fisher Scientific, USA) equipped with a flame ionization detector and 1.5 m column Poropak-Q (80–100 mesh). Chromatograms were generated, Et concentrations calculated and expressed as $\mu\text{L Kg}^{-1} \text{ h}^{-1}$ fresh mass, based on the volume of air in the flasks formed between the culture medium and the sealing, and incubation (for Et production) time.

Data analysis

The experiments were conducted in randomized blocks, and 10 embryos (replicates) were used per treatment. Each replicate consisted of one testing tube containing one embryo. The embryo germination experiments were repeated twice.

Aluminum (0, 90, 180, 270, and 320 μM Al) and Et were studied as the causes of variation. The germination performance (%G), germination time and germination speed index) as well as shoot and root length and biomass were evaluated. The data were submitted to a two-way analysis of variance (two-way ANOVA) to test the Al and Et factors. Tukey test ($p < 0.05$) was used to compare mean values between treatments.

Results

Aluminum concentration in leaves, fruits, and seeds

Aluminum concentration was significantly higher in the organs of *V. tucanorum* in comparison to *Q. grandiflora*. Leaf, fruit, and seed Al concentration was five-, 11- and four-fold higher in *V. tucanorum* than *Q. grandiflora* (Table 1).

Aluminum and ethylene effect on seed germination

Embryos from water-treated seeds of *Q. grandiflora* germinated well (%G > 70%) (Fig. 1A) when compared with *V. tucanorum* (%G < 60%) (Fig. 1B), and Al improved its %G only at 180 and 270 μ M Al. Ethylene-treated seeds improved %G only at 90 and 320 μ M Al (Fig. 1A). Embryos from water-treated seeds of *V. tucanorum*, however, did not germinate without Al, and only embryos from Et-treated seeds germinated under no Al (Fig. 1B). In the medium with Al, embryos from Et-treated seeds showed increased (+30%) %G (Fig. 1B). Therefore, treating *V. tucanorum* seeds with Et induced embryo germination when Al was not present in the medium, while *Q. grandiflora* embryos germinated independent of Al presence in the medium and treating their seeds with Et did not significantly influence embryo germination.

Embryos from water-treated seeds of *Q. grandiflora* showed the highest GSI at 180 μ M Al (Fig. 1C), while embryos from water-treated seeds of *V. tucanorum* showed the highest GSI at 270 μ M Al (Fig. 1D). Ethylene-treated seeds improved GSI of *Q. grandiflora* embryos only when cultivated with Al (Fig. 1C), while *V. tucanorum* embryos, which did not germinate without Al, showed significantly higher GSI when their seeds were treated with Et and their embryos seeded in media with Al (Fig. 1D).

Germination time of embryos from water-treated *Q. grandiflora* seeds was maximum at 90 μM Al and it was reduced with the increasing Al concentrations in the medium (Fig. 1E). Ethylene-treated seeds did not positively influence *Q. grandiflora* (Fig. 1E) or *V. tucanorum* (Fig. 1F) embryo germination. Germination time of embryos from water-treated *V. tucanorum* seeds was maximum at 90 μM Al (Fig. 1F).

Aluminum and ethylene effect on root and shoot growth

The root length of germinated embryos from water-treated seeds of *Q. grandiflora* was the same between Al treatments on each evaluation date (7, 14, 21, 28, and 35 days) (Fig. 2A, B). This parameter did not differ between Al treatments when embryos came from Et-treated seeds (Fig. 2C, D). *Vochysia tucanorum* embryos from water-treated seeds did not germinate and as expected, did not show root length (Fig. 3A, B). The root length of germinated embryos from water-treated *V. tucanorum* seeds grew similarly between Al treatments (Fig. 3A, B). Germinated embryos from ethylene-treated *V. tucanorum* seeds, however, showed higher root length at 90, 180, 270, and 320 μM Al, from 21 days (Fig. 3C, D). Therefore, differences in the root length between Al treatments were only observed in germinated embryos from *V. tucanorum* Et-treated seeds.

Shoot biomass of germinated embryos from Et- and water-treated seeds of *Q. grandiflora* (Fig. 4A) and *V. tucanorum* (Fig. 4B) varied considerably between Al treatments. The root biomass of germinated embryos of *Q. grandiflora* Et-treated seeds was significantly higher than those from water-treated seeds at 0, 90, 180, and 270 μM Al (Fig. 4C). This parameter measured in germinated embryos of *V. tucanorum* Et-treated seeds was higher than those from water-treated seeds only at 90 μM Al (Fig. 4D).

Therefore, the root rather than shoot biomass was improved in the presence of Al, especially for *Q. grandiflora*.

Ethylene production in vitro

Although Et was ‘detected’ in germinated embryos from water- and Et-treated seeds of *Q. grandiflora* and *V. tucanorum* in media with 0, 180 and 320 μM Al, no significant differences were observed between most treatments. ‘Non-germinated’ embryos from water-treated *V. tucanorum* seeds produced no Et when compared with germinated embryos from water-treated seeds with 180 and 320 μM Al in the media (Table 2). Therefore, Et was produced in germinated embryos from both species regardless if their seeds were treated with Et or water.

Discussion

In the present study, we tested the germination of *Q. grandiflora* and *V. tucanorum* embryos in culture medium with increasing concentrations of Al under the influence of Et. Embryos from water-treated seeds did not germinate in medium without Al (Fig. 1B). This is intriguing because *V. tucanorum* seeds showed more than 32,000 mg Al per kg seed (Table 1), meaning that the Al present in their seed, somehow, does not produce the same effect on germination as if provided by the medium in contact with the embryo. The Al concentration in the hypocotyl of germinated embryos from Et-treated *V. tucanorum* seeds was approximately 20,000 mg kg^{-1} (data not shown). These results suggest that *V. tucanorum* seeds require Al in the germination medium to germinate. Soils from the Cerrado, where seeds of these species supposedly lay on (although there is leaf litter before seeds get in contact with the soil) with wind — these are anemochoric seeds

(Kawasaki 2007; Ishara and Maimoni-Rodella 2011; Kuhlmann and Ribeiro 2016; Gottsberger and Silberbauer-Gottsberger 2018) — show low pH and m% higher than 70% (Haridasan 1982; Souza et al. 2015; Bressan et al. 2016; Nogueira et al. 2019).

On the other hand, treating *V. tucanorum* seeds with Et induced embryo germination when Al was not present in the medium. This suggests that ethylene may exert a similar effect to Al in promoting germination in this specie. A previous study had already demonstrated Et breaks seed dormancy and improve germination of seeds of *V. tucanorum* (Pereira et al. 2011), but no further physiological explanation was given by these authors. In Al-sensitive species, it is demonstrated that Al induces overproduction of Et (Massot et al. 2002; Sun et al. 2007), which seems to act as a signal to change auxin distribution in the roots, disrupting AUX1- and PIN2-mediated auxin polar transport, leading to arrest of root elongation (Sun et al. 2010). There are not such refined studies in roots or seeds of Cerrado woody species, but it could be possible that in the case of Al-accumulating species such as *V. tucanorum*, the Al induces auxin action/transport in these seeds, promoting germination, and Et is able to substitute the Al in this process. Nevertheless, this hypothesis remains to be further studied in seeds of these Cerrado species.

Contrary to what is observed in Al-sensitive species, where Al stunts root growth (Horst et al. 2010; Silva et al. 2019, 2023), in the case of Al-accumulating species, but especially *V. tucanorum*, Al in the medium is critical for seed germination and initial root growth (Fig. 3A, B). In addition, the presence of Et (Et-treated seeds) enhanced even more the root growth (Fig. 3C, D), suggesting a synergistic effect of Et and Al on root growth in this species.

In contrast, *Q. grandiflora* embryos germinated independent of Al presence in the

medium and treating their seeds with Et did not significantly influence embryo germination (Fig. 1A) or root elongation (Fig. 2A, B). It was unexpected to observe that Al did not exert a significant effect on these parameters of *Q. grandiflora* because this is an Al-accumulating species (Haridasan 1982; Bressan et al. 2016; Nogueira et al. 2019) and some studies point out beneficial Al effects on its growth. Saplings of *Q. grandiflora* supplemented with 150 μM Al in the substrate had healthier appearance, larger shoots, and roots with greater root biomass in comparison with plants not supplemented with Al (Cury et al. 2020). In another study, *Q. grandiflora* saplings cultivated without Al in nutrient solution show compromised root integrity, reducing the leaf hydration and the Rubisco (Ribulose 1,5-biphosphate carboxylase/oxygenase) *in vivo* performance, when compared to plants grown with Al (Silva et al. 2023). These studies, however, analyzed saplings (mature plants with some months of age), and it is possible the beneficial Al effects on this species may only become apparent later in development. Our findings suggest that, during *in vitro* culture, the presence of both Al and ethylene had no significant impact on either germination or initial root growth in seedlings of *Q. grandiflora* (Fig. 1A; Fig. 2) contrasting with what was observed for *V. tucanorum* (Fig. 1B; Fig. 3).

Despite that, the contrasting response to Al and Et between the two species is also reflected in their root growth. In *Q. grandiflora*, the root length of germinated embryos remained constant across different Al treatments (Fig. 2), indicating a limited influence of Al on root growth. The root biomass, however, of *Q. grandiflora* from Et-treated seeds was significantly higher than those from water-treated seeds up to 270 μM Al (Fig. 4C), suggesting a positive interaction between Et and Al in promoting root growth. Root length of *V. tucanorum*, on the other hand, was higher when seeds were treated with Et and cultivated in media with Al (Fig. 3), but the positive effect of Et was only observed up to

90 μM Al in the culture medium (Fig. 4D), indicating the increase in biomass for both species shows a threshold of Al influence. Thus, root elongation and carbon incorporation into biomass are distinct responses and should be evaluated with care.

In this study, we demonstrate that Al has a significant effect on the germination and growth of *Q. grandiflora* and *V. tucanorum*. Treating *V. tucanorum* seeds with Et induced embryo germination when Al was not present in the medium, suggesting a substitutive relationship between these compounds. Embryos of *Q. grandiflora* germinated independent of Al in the medium and Et did not influence their germination. We propose to further investigate the physiological role of Al and Et in the germination as well as seedling and sapling growth of these species.

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Tables

Table 1. Aluminum concentration in leaves, fruit, and seeds of *Qualea grandiflora* Mart. and *Vochysia tucanorum* Mart. collected in a cerrado *sensu stricto* remnant, in the municipalities of Itirapina and Mogi Guaçu, São Paulo state, southeastern Brazil.

Species	Organ	mg Al kg ⁻¹ dry mass
<i>Q. grandiflora</i>	Leaf	8,053.66 ± 323.71
	Fruit	1,305.88 ± 523.62
	Seed	8,406.0 ± 236.66
<i>V. tucanorum</i>	Leaf	40,536.66 ± 1,844.59
	Fruit	11,036.67 ± 1,294.69
	Seed	32,250.0 ± 964.36

Mean values (n = 5) ± standard deviation

Table 2. Ethylene concentration ($\mu\text{L}/\text{Kg}^{-1} \text{h}^{-1}$) in germinated embryos from ethylene- and water-treated seeds of *Qualea grandiflora* Mart. and *Vochysia tucanorum* Mart., grown for 14 days with 0, 180 and 320 μM Al.

Species	Et	μM Al	$\mu\text{L kg}^{-1} \text{h}^{-1}$
<i>Q. grandiflora</i>	-	0	0.002 ± 0.00 a
		180	0.006 ± 0.10 a
		320	0.003 ± 0.02 a
<i>Q. grandiflora</i>	+	0	0.001 ± 0.01 a
		180	0.002 ± 0.03 a
		320	0.002 ± 0.01 a
<i>V. tucanorum</i>	-	0	0 ± 0.00 b
		180	0.006 ± 0.04 a
		320	0.008 ± 0.02 a
<i>V. tucanorum</i>	+	0	0.007 ± 0.00 a
		180	0.010 ± 0.01 a
		320	0.007 ± 0.04 a

Mean values ($n = 3$) $\pm$ standard deviation

(-) = water-treated seeds

(+) = Ethylene-treated seeds

For each Et- or water-treated seeds of the same species, distinct letters represent significant differences between Al treatments by Tukey test ($P < 0.05$)

Figures

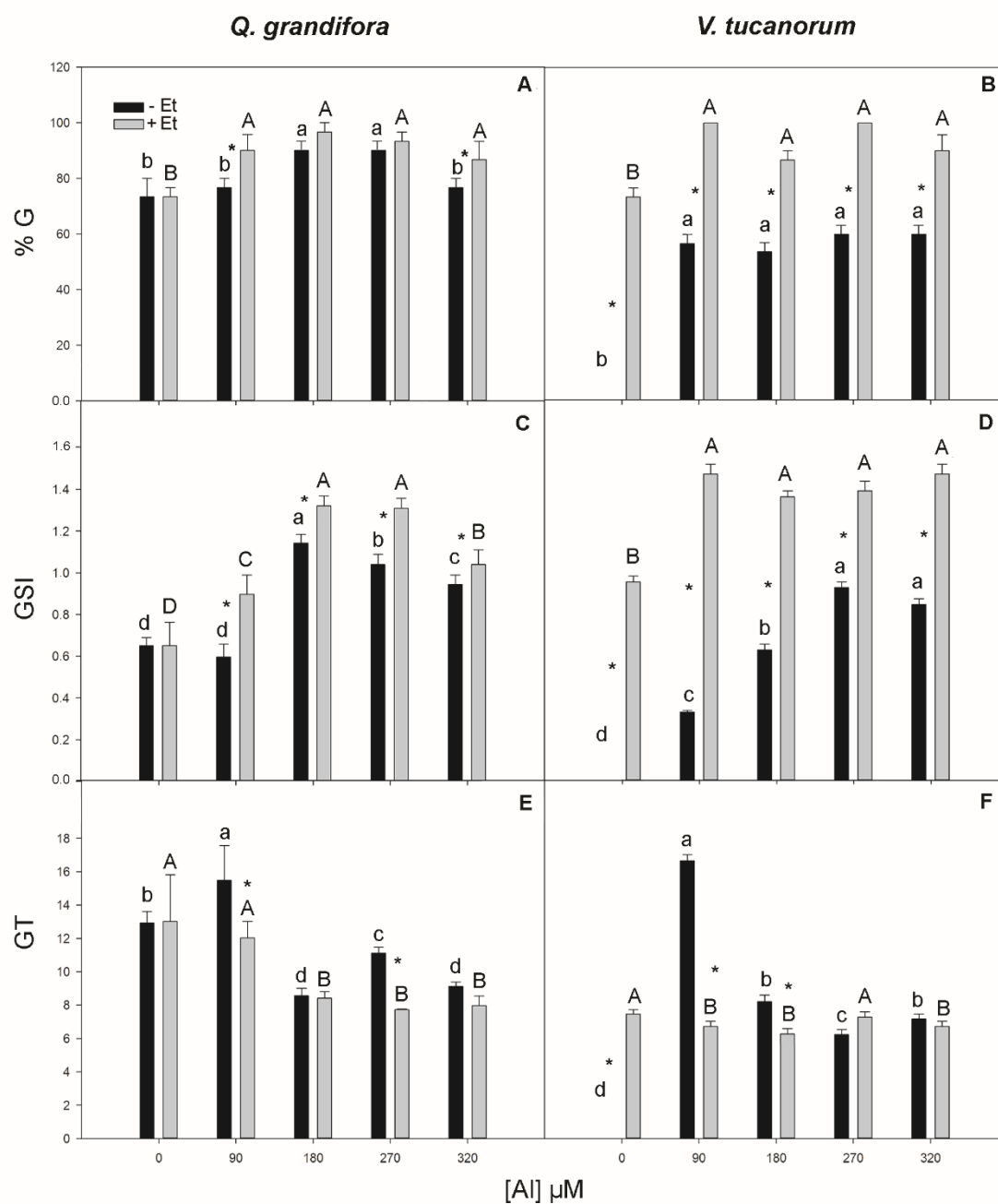


Figure 1. Mean values ($n = 20$) of germination percentage (G%) (A, B), time (GT) (C, D), and speed index (GSI) (E, F) of mature zygotic embryos of *Q. grandiflora* (A, C, E) and *V. tucanorum* (B, D, F) rescued from water-treated seeds (-Et) and ethylene-treated seeds (+Et) grown on culture

medium containing 0, 90, 180, 270 and 320 μM Al. Different lower case and upper case letters indicate significant differences by Tukey test ($P < 0.05$) between Al-treatments in water-treated and Et-treated seeds, respectively. For each Al treatment, asterisks indicate significant differences by Student t-test ($P < 0.05$) between water- and Et-treated seeds. Bars = s.e. Significant differences indicated by Tukey test.

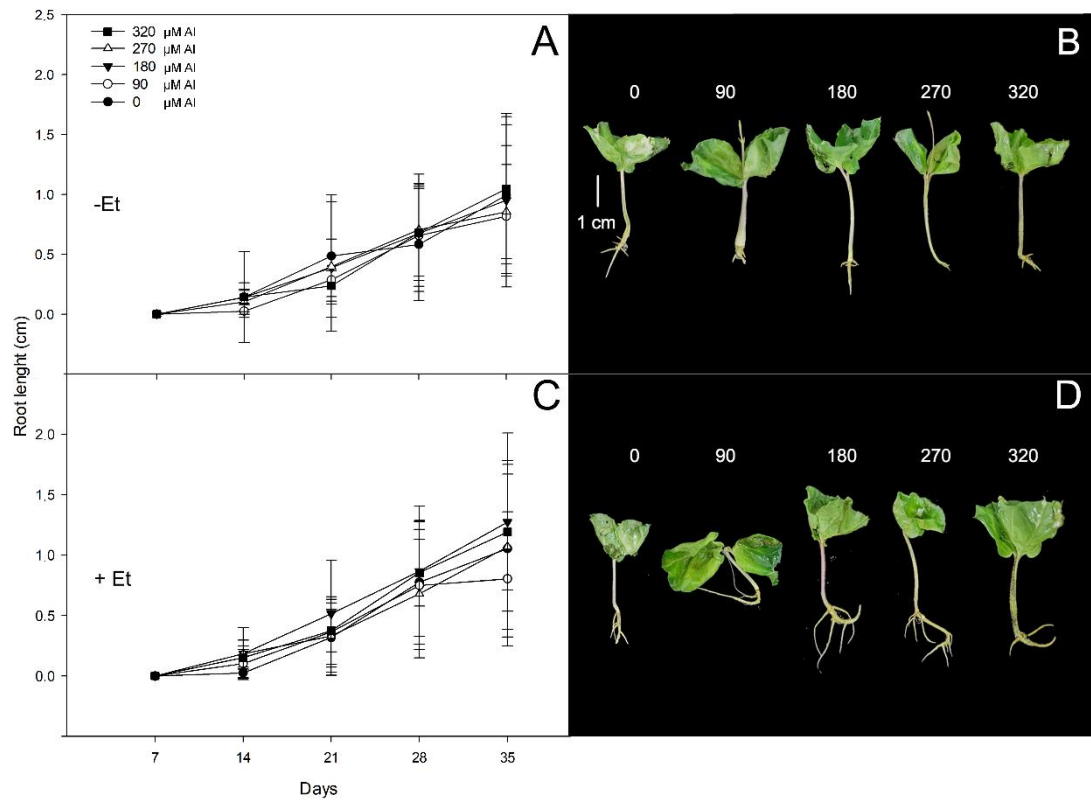


Figure 2. Mean value ($n = 20$) of root of mature zygotic embryos of *Q. grandiflora* rescued from water-treated seeds (-Et) (A) and ethylene-treated seeds (+Et) (C) grown on culture medium containing 0, 90, 180, 270, and 320 μM Al at 14, 21, 28, and 35 days after inoculation. Morphological details of water-treated (B) and Et-treated (D) seedlings at the end of the 35-day evaluation period. No significant differences were detected between treatments as determined by the Tukey and Student's tests ($p > 0.05$). Bars = s.e.

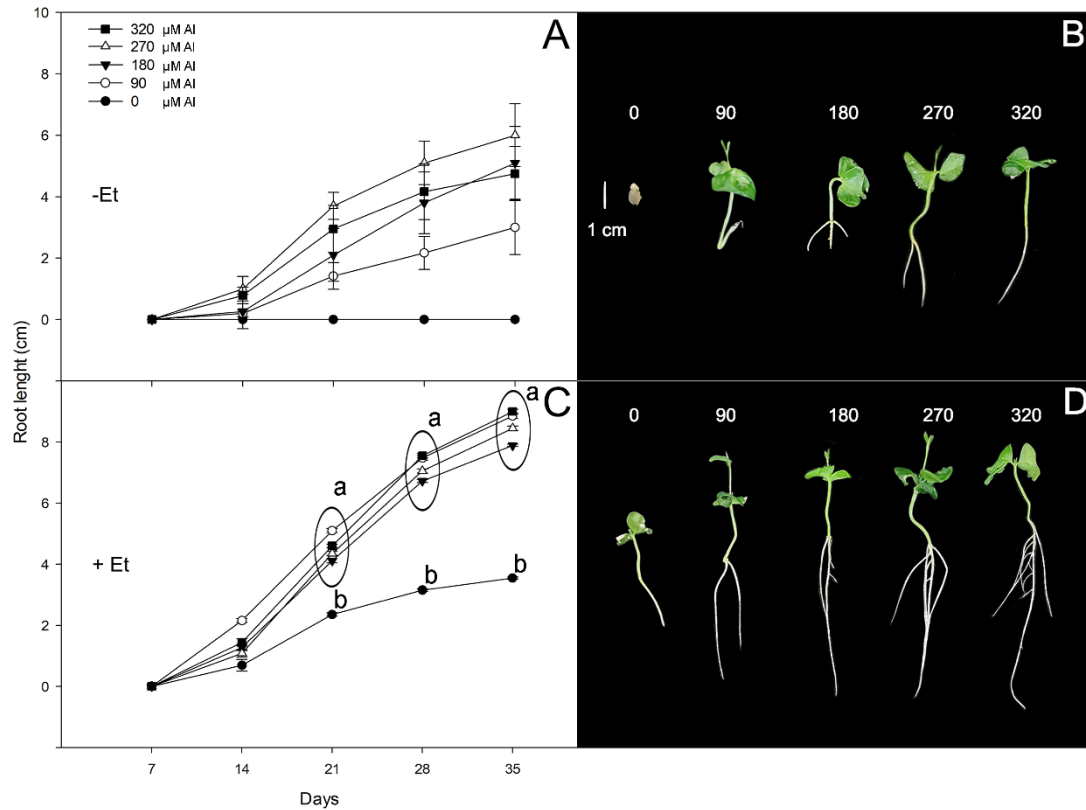


Figure 3. Mean value ($n = 20$) of root of mature zygotic embryos of *V. tucanorum* rescued from water-treated seeds (-Et) (A) and ethylene-treated seeds (+Et) (C) grown on culture medium containing 0, 90, 180, 270, and 320 μM Al at 14, 21, 28, and 35 days after inoculation. Morphological details of water-treated (B) and Et-treated (D) seedlings at the end of the 35-day evaluation period. Different letters indicate significant differences by Tukey test ($P < 0.05$) between Al-treatments in Et-treated seeds. For each Al treatment, the significant differences between the water- and Et-treated seeds were determined by Student's t-test ($P < 0.05$). Bars = s.e. Significant differences indicated by Tukey test. Ellipses indicate statistically similar treatments.

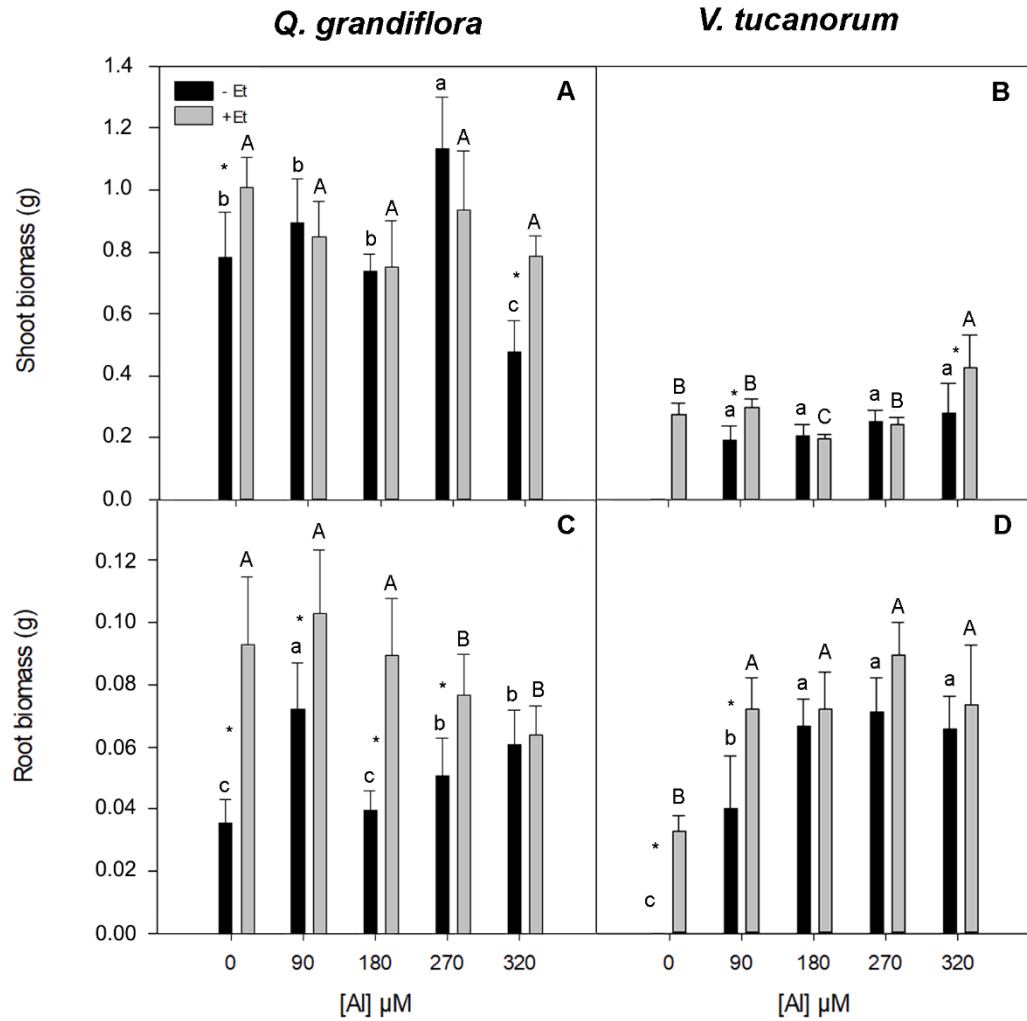


Figure 4. Mean values ($n = 20$) of shoot biomass (A, B) and root biomass (C, D) of mature zygotic embryos of *Q. grandiflora* (A, C) and *V. tucanorum* (B, D), rescued from water-treated seeds (-Et) and ethylene-treated seeds (+Et) grown on culture medium containing 0; 90; 180; 270, and 320 μM of Al. Different lower case and upper case letters indicate significant differences by Tukey test ($P < 0.05$) between Al-treatments in water-treated and Et-treated seeds, respectively. For each Al treatment, asterisks indicate significant differences by Student t-test ($P < 0.05$) between water- and Et-treated seeds. Bars = s.e. Significant differences indicated by Tukey test.

Supplementary material

Supplementary material 1. Macro and micronutrient concentrations and fertility parameters in a cerrado *sensu stricto* remnant, in the municipalities of Itirapina and Mogi Guaçu, São Paulo state, southeastern Brazil.

Soil Type	pH	OM	P resin	Al ³⁺	H+Al ³⁺	K ⁺	Ca ²⁺	Mg ²⁺	BS	CEC
	(CaCl ₂)	(g dm ⁻³)	(mg dm ⁻³)	-----mmol charges dm ⁻³ -----						
Itirapina	3.8 ± 0.1 a	9.4 ± 1.9 b	1.8 ± 0.4 a	15.8 ± 2.6 a	41.6 ± 5.7 b	0.4 ± 0.2 a	2.4 ± 0.9 a	1 ± 0.0 a	3.8 ± 1.0 a	45.4 ± 5.3 a
Mogi Guaçu	3.8 ± 0.5 a	14.74 ± 3.4 a	0.7 ± 0.0 b	12.6 ± 0.8 a	50.16 ± 6.3 a	0.3± 0.5 b	0.24 ± 0.1 b	0.48 ± 0.04 b	1.04 ± 0.15 b	51.2 ± 6.4 a

V%	m%	B	Cu	Fe	Mn	Zn
(%)	(%)	-----mg dm ⁻³ -----				
8.6 ± 2.8 a	80.4 ± 6.8 b	0.27 ± 0.0 a	0.28 ± 0.0 b	55.2 ± 17.2 a	1.4 ± 0.6 b	0.1 ± 0.0 b
2.0 ± 0.0 b	92.2 ± 0.4 a	0.18 ± 0.0 b	0.34 ± 0.1 a	56.1 ± 15.1 a	1.92 ± 0.5 a	0.2 ± 0.0 a

OM = Organic matter; BS = Base saturation ($K^+ + Ca^{2+} + Mg^{2+}$); CEC = $K^+ + Ca^{2+} + Mg^{2+} + H^+ + Al^{3+}$
; V % = Fertility rate [$V = 100 (K^+ + Ca^{2+} + Mg^{2+}) / CEC - 1$], %;

Soil Al saturation (m%) = $(100 \times Al^{3+}) / (BS + Al^{3+})$

Mean values (n = 5) ± standard deviation

Different letters represent significant difference between soil types by Student t-test

CAPÍTULO II

***In vitro* organogenesis, content phenols and antioxidant capacity of two aluminum accumulator plants species from the Cerrado region, Brazil**

Matheus Armelin Nogueira^{1*}, Vitor Rodrigues Marin², Gustavo Habermann³, Jean Carlos Cardoso⁴

¹*Programa de Pós-Graduação em Biologia Vegetal, Departamento de Biodiversidade, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Av. 24-A, 1515, 13506-900, Rio Claro, SP, Brazil*

²*Departamento de Biologia Geral e Aplicada, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Av. 24-A, 1515, 13506-900 Rio Claro, SP, Brazil*

³*Departamento de Biodiversidade, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Av. 24-A, 1515, 13506-900, Rio Claro, SP, Brazil*

⁴ *Laboratório de Fisiologia Vegetal e Cultura de Tecidos, Departamento de Biotecnologia, Produção de Plantas e Animais, Centro de Ciências Agrárias, Universidade Federal de São Carlos, Rodovia Anhanguera, km 174, CEP 13600-970 Araras, SP, Brazil*

* Corresponding author email: matheus.armelin@unesp.br

Abstract

Aluminum (Al) is the most plentiful metal present in the Earth's crust, and when present in acidic soils ($\text{pH} < 5.0$), it becomes soluble and toxic to most plant species. Species from acidic and aluminum-rich soil regions, such as Brazilian Cerrado, developed mechanisms allowing for their growth in these conditions. Some can accumulate Al in their tissues, especially in the leaves. However, the possible functions of Al in these plants are unknown. This study investigated the impact of Al on the organogenesis and production of phenolic compounds in extracts from cotyledonary leaf segments of two Al-accumulator species, *Qualea grandiflora* Mart. and *Vochysia tucanorum* Mart. (Vochysiaceae). The addition of Al at 320 μM in the absence of BAP resulted in the regeneration of both species' adventitious roots in cotyledonary leaf segments. In contrast, adding BAP without Al did not generate regeneration responses. However, shoot regeneration and development occurred when 0.44 and 0.88 μM BAP was added to the culture medium with 320 μM Al. Both species exhibited a noteworthy phenolic content, further enhanced by adding Al and, or, BAP. The antioxidant capacity of extracts also demonstrated a significant increase from the addition of Al and, or, BAP in both species. These findings have important significance for the cultivation and propagation of these species and demonstrate a close relationship between Al and the evolution of these plants. This study is the first to relate Al with phenolic content and antioxidant activity in these two Cerrado plant species, filling a gap in existing research.

Keywords: Aluminum, adventitious shoots, cytokinins, organogenesis, Plant species

Introduction

The Cerrado biome in Brazil is a diverse and endangered area, covering about 20% of the country and containing high levels of endemism (Colli *et al.* 2020). It is considered a vital center of plant diversity with approximately 10,000 plant species, many of which are unique to this biome (Ratter *et al.* 2003; Klink and Machado 2005). The Cerrado soil is acidic ($\text{pH} < 5.0$), dystrophic, has low nutrient availability, and it also contains high levels of available aluminum ($\text{m\%} > 70\%$) [$\text{m\%} = (100 \times \text{Al})/(\text{BS} + \text{Al})$, where BS is the base saturation] (Vitorello *et al.* 2005; Singh *et al.* 2017). In acidic soils ($\text{pH} < 5.0$), aluminum minerals undergo solubilization, resulting in the formation of Al^{3+} ions as well as related oxides and hydroxides. These forms of aluminum are considered toxic to numerous plant species (Kochian *et al.* 2015).

However, despite the toxicity, the flora in this region thrives in these acidic soils (Haridasan 2008; Habermann and Bressan 2011; de Souza *et al.* 2017; Nogueira *et al.* 2019). Although some species are capable of growing in soils with high Al saturation ($\text{m\%}$), others also developed the capacity to accumulate Al in different tissues and organs without any damage or sign of toxicity that could affect their metabolism or development (Haridasan 1982; Delhaize and Ryan 1995; de Andrade *et al.* 2011; Bressan *et al.* 2021). Examples of such species include *Fagopyrum esculentum* Moench (Polygonaceae), *Hydrangea macrophylla* L. (Hydrangeaceae), and *Camellia sinensis* (L.) Kuntze (Theaceae) as reported by Ma *et al.* (2001) and Watanabe and Osaki (2002).

Plants that store more than 1 g of aluminum per kg of dry leaf mass are referred to as Al-accumulators (Chenery 1948). *Qualea grandiflora* Mart. and *Vochysia tucanorum* Mart. are native plant species from the Cerrado that accumulate Al in their leaves and roots (Haridasan 1982; Brunner and Sperisen 2013; Nogueira *et al.* 2019; Bressan *et al.* 2021;

Silva *et al.* 2023). Previous research has shown that this metal might exhibit a benign response in this group of plants, increasing plant biomass and root elongation in *C. sinensis*, *Melastoma malabathricum* L. (Melastomataceae) and *Quercus serrata* Murray (Fagaceae) (Bojórquez-Quintal *et al.* 2017). The specific relationships and potential impact of Al on physiological parameters that explain the positive influence of aluminum on plant growth in aluminum-accumulating species are seldom documented or not commonly reported.

In the Brazilian Cerrado biome, many plant species produce phenolic compounds and have developed adaptations to resist the oxidative stress caused by extreme environments (Farias *et al.* 2013; Moreira-Araújo *et al.* 2019). These adaptations include a higher expression and activity of antioxidant enzymes and an increased synthesis of phytochemicals, particularly phenolic compounds (Arruda *et al.* 2022).

Phenolic acids are the most abundant secondary metabolites in plants and play important roles in diverse physiological processes (Zhang *et al.* 2022). They play an important role in plant defense mechanisms by being deposited in cell walls after pathogen infection. When they are excreted from plant root systems, they can inhibit growth within the adjacent rhizosphere and affect bacterial flora of the soil. Phenolics can also act as modulators of plant development by regulating indole acetic acid (IAA) catabolism. They are effective in regulating plant growth, cell differentiation and organogenesis (Kumar *et al.* 2020, 2023).

In addition to the adaptations that many plant species in the Brazilian Cerrado biome have developed to resist oxidative stress, such as the production of phenolic compounds, these species also face other challenges, such as the presence of aluminum (Al) in the soil. Studying the effects of Al on these plants can be difficult due to the

challenges of propagating and growing them in *ex situ* conditions that isolate the effects of Al from other elements (Cardoso and Silva 2013; Leite *et al.* 2021; Lima *et al.* 2022). *In vitro* propagation can be a valuable technique for conserving, cultivating, and propagating Cerrado species under controlled conditions, allowing for the isolation and testing of factors, such as Al, in the culture media.

This study aimed to test the hypothesis regarding the significance of aluminum (Al) and its potential interaction with the cytokinin 6-benzylaminopurine (BAP) in promoting adventitious shoot and root organogenesis in *Q. grandiflora* and *V. tucanorum*. Additionally, this study investigated if the presence of Al and BAP has a relationship between the total phenolics content and antioxidant activity in extracts of these two Cerrado species.

Material and Methods

Plant Material

Seeds of *Qualea grandiflora* Mart. and *Vochysia tucanorum* Mart. were obtained from fruits collected during the dehiscence season (between August and December 2021) in reminiscent areas of Cerrado at the municipalities of Itirapina and Mogi Guaçu, São Paulo State, Brazil. The fruits were opened, and the seeds were removed and stored at 25 °C $\pm$ 2 in an acclimatized room until their use for *in vitro* seedling germination. The experiment was conducted at the Center for Agricultural Sciences at the Federal University of São Carlos (CCA/UFSCar) in Araras, SP, Brazil.

Aluminum concentration

The samples (seeds and cotyledonary leaves) were taken to the Plant Nutrition Laboratory of the Agronomic Institute of Campinas (IAC, Campinas, Brazil), ground, and digested in a solution of sulfuric, nitric, and perchloric acids (1:10:2, v/v/v). After digestion, Al concentrations were measured using atomic absorption spectroscopy (Sarruge and Haag 1974). The values were expressed as mg Al per kg of dry mass (Supplementary material 1).

In vitro seed germination and seedling development

The modified Murashige and Skoog (MS; Murashige and Skoog 1962) was the basal culture medium, reducing the original salt concentration to $\frac{1}{4}$ of macronutrients. The medium ($\frac{1}{4}$ MS) was supplemented with 30.0 g L⁻¹ of sucrose (Synth, Diadema, Brazil), and 0.1 g L⁻¹ of myo-inositol (Synth) and solidified with 2.7 g L⁻¹ of Gelrite® Gellan gum (Duchefa, Haarlem, Netherlands). Also, plant growth regulators were added to the culture medium: 0.44 μ M 6-benzylaminopurine (BAP), 5.7 μ M gibberellic acid (GA₃) (Duchefa), and 90.0 μ M of aluminum chloride AlCl₃ · 6H₂O (Synth). This culture medium was successfully used to germinate other Cerrado species (Cardoso and da Silva 2013). The pH of the culture medium was adjusted to 4.5 ± 0.05 , and it was sterilized by autoclave at 121 °C for 25 min. The Al range concentration in the medium (0 and 320.0 μ M of Al) were selected in accordance with other studies that tested Al toxicity in *Picea glauca* (Nosko *et al.* 1988), *Triticum aestivum* (Lima and Copeland 1990), *Oryza sativa* (Kikui *et al.* 2005), and *Nicotiana tabacum* (Vardar *et al.* 2006).

The asepsis of seeds was realized by immersion of the seeds in 70% ethanol (Synth) (v/v) for 3 min, followed by a solution containing 50% sodium hypochlorite

(Candura, Piracicaba, Brazil) (1.0 to 1.25% of active chlorine) for 20 min, and, at last, rinsed three times with sterilized distilled water for 3 min each. After this superficial disinfection, the mature zygotic embryos (MZE) were excised under a laminar flow chamber and inoculated in test tubes containing 65 mL of the culture media. After, inoculated seeds were incubated for 35 d in $26\pm 1^{\circ}\text{C}$ and maintained under a 16-hr photoperiod (60 W fluorescent lamps; Phillips, Amsterdam, Netherlands).

Organogenesis Induction from Cotyledonary Leaves Segments of Germinated Seedlings

Mature and healthy cotyledonary leaves from 35-d-old *in vitro* Vochysiaceae species seedlings were uniformly cut into 1 cm² segments and used as explants to induce organogenesis. This study tested the different concentrations of 6-benzylaminopurine (BAP at 0; 0.44 and 0.88 μM) and Aluminum (0 and 320.0 μM) on cotyledonary leaf segments organogenesis of the two Al-accumulator species, *V. tucanorum* and *Q. grandiflora* (Vochysiaceae). These treatments were added to the modified $\frac{1}{4}$ MS culture medium described above.

Biometric Parameters

Some regeneration parameters were adopted to evaluate the effects of BAP and Al added to the culture media. These included the evaluation of the percentage of leaf explants with shoot and root induction, the number of shoots regenerated per leaf segment, the number of leaves per leaf segment, the mean shoot length (cm), and the number of roots per leaf segment.

Total Phenolics and Antioxidant Activity

Extract preparation: Dried cotyledonary leaf segments used to induce the organogenesis in the two Al-accumulator species were crushed and macerated using 70% ethanol at the temperature of 50°C. Each sample consisted of individual cotyledonary leaf segments in triplicates (three separate extraction processes). After filtration with a Buchner funnel, the extracts were concentrated at 40°C under reduced pressure using a rotary evaporator. The concentrated extracts were then stored overnight at -40°C and subsequently subjected to freeze-drying (L101, Liobras, São Carlos, Brazil). The dried extracts were standardized at 2.0 mg mL⁻¹ in methanol and stored at -4°C until used.

Determination of Total Phenolic Content: To determine the total phenolic content in leaf segment samples, a modified spectrophotometric method was used based on the Folin-Ciocalteu assay described by Kang *et al.* (2011). Aliquots of 0.2 mL of the stored extracts (2.0 mg mL⁻¹) were added to 2.5 mL of Folin-Ciocalteu's reagent (Dinâmica, Indaiatuba, Brazil) (10 % v/v). Samples were kept in the dark for 2 minutes at room temperature. Following incubation, 2.0 mL of Na₂CO₃ (Dinâmica) (7.5% v/v) were added to samples and kept under the same conditions for 1 hr.

The samples were transferred to plastic cuvettes for absorbance reading in a spectrophotometer with UV-vis detector Biochrom Libra S50 UV/Vis (Biochrom, Cambridge, United Kingdom). The mixture was measured for its absorbance at 765 nm using a blank solution with identical constituents in which the extract was substituted with distilled water. To prepare the calibration curve, pyrocatechol (Dinâmica) was utilized as a standard. The experiment was realized in triplicates. The total phenolic content in the extracts was expressed as mg Galic acid equivalents (GAE) g⁻¹ extract.

DPPH Radical Scavenging Assay: The antioxidant activity of the extracts obtained was assessed using the DPPH radical scavenging method. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable organic free radical that can be neutralized or eliminated by receiving electrons or hydrogen from a compound with antioxidant capacity (Oliveira 2015).

The DPPH radical capture reaction causes a color change in the test solution from violet to light violet or yellow depending on how much of the radical is captured (Oliveira, 2015). This color modification can be measured in a UV-vis spectrophotometer at a wavelength between 515 to 517 nm to quantify how much DPPH was inhibited and define the antioxidant capacity of the tested sample (Oliveira, 2015).

The evaluation of antioxidant activity followed the protocols of Rufino *et al.* (2007) and Tavares *et al.* (2018) with modifications. Initially, a 0.06 μM DPPH (Sigma-Aldrich) solution was prepared by diluting 2.4 mg of the reagent in 100 mL of methanol (Synth). The extracts (2.0 mg mL^{-1}) were diluted in methanol to a concentration of $500.0 \mu\text{g mL}^{-1}$.

To perform the DPPH radical scavenging assay, $900.0 \mu\text{L}$ of the DPPH solution was transferred to microtubes followed by $100.0 \mu\text{L}$ of the diluted extracts. As a positive control, a solution of ascorbic acid (Synth) in methanol at the same concentrations as the samples was used. For the negative control, $100.0 \mu\text{L}$ of methanol was added instead of the samples. The microtubes were homogenized on a benchtop vortex mixer and kept in the dark for 3 hr to allow for the reaction to occur.

After 3 hr, samples were transferred to cell cuvettes for absorbance reading in a spectrophotometer with UV-vis detector Biochrom Libra S50 UV/Vis (Biochrom,

Cambridge, United Kingdom). Methanol was used to calibrate the spectrophotometer. Using the absorbance values obtained, the percentage of DPPH inhibition was calculated using the following formula:

$$\% \text{ inhibition of DPPH radical activity} = \frac{(A \text{ Control} - A \text{ Sample})}{A \text{ Control}} \times 100$$

In this context, 'A Control' refers to the absorbance value obtained from the control group (DPPH solution and methanol), and 'A Sample' represents the absorbance value obtained from the experimental group (DPPH solution and sample).

Statistical Analysis

The experiment contained six treatments for shoot organogenesis induction with three concentrations of BAP (0, 0.44, and 0.88 μM) and two concentrations of Al (0 and 320.0 μM). Each repetition consisted of a single cotyledonary leaf segment per flask and 10 flasks per treatment (repetitions).

The data analysis and visualization were carried out by employing the Sigma Plot 12.0 (2011, Systat Software, Inc., San Jose, CA) application. Each treatment that examined the total phenolic content and DPPH radical scavenging assay employed three separate cotyledonary leaf segments. The outcomes were presented as the mean value with the standard error of the mean (SE) whereas the difference's significance was assessed through one-way ANOVA and Tukey post-test of multiple comparisons. Pearson's correlations were calculated between the total phenolic content, DPPH radical scavenging, and the regenerations parameters. The results were deemed significant if the p-value was less than 0.05.

Results

In Vitro Seed Germination

A preliminary germination assay was conducted to assess the viability of *Q. grandiflora* and *V. tucanorum* seeds in the presence of Al. The success of the germination of the seeds and development of the seedlings with the expansion of cotyledonary leaves for both Al-accumulator species was only obtained when 90.0 μM of Al were previously added to the *in vitro* culture media used (data not presented) (Fig. 1).

Effects of BAP and Al Treatments on Shoot and Root Proliferation

Table 1 shows the effects of BAP and Al treatments added to the culture media on shoot and root organogenesis from cotyledonary leaf segments of *Q. grandiflora* and *V. tucanorum* at 30 d. For *Q. grandiflora*, the control (0 μM of BAP and 0 μM of Al) did not result in any shoot or root regeneration, and the dark brown color of the cotyledonary leaves indicated tissue senescence and lack of regeneration (Table 1) [Supplementary material 2]. However, adding Al at 320.0 μM in the absence of BAP in the culture media resulted in the regeneration of both species' adventitious roots in cotyledonary leaf segments (Fig. 2 b, f). Also, the addition of BAP in the absence of Al in the culture media did not result in any regeneration responses of the tissue (Fig. 2 a, e). However, the culture media containing BAP (0.44 and 0.88 μM) and Al (320.0 μM) resulted in shoot regeneration and development with intermediary callus phase at 30 d (Table 1; Fig. 2 c, d, g, h).

Total Phenolic Contents of Essential Extracts

For *Q. grandiflora*, the control treatment had the lowest phenolic content with a mean value of $0.53 \mu\text{g GAE g}^{-1}$ (Fig. 3). The addition of BAP resulted in a significant increase in phenolic content compared to the control treatment, and both $0.44 \mu\text{M}$ of BAP and $0.88 \mu\text{M}$ of BAP treatments showed similar phenolic contents with mean values of 0.82 and $0.77 \mu\text{g GAE g}^{-1}$, respectively. However, the Al treatment showed a significantly higher phenolic content with a mean value of $1.05 \mu\text{g GAE g}^{-1}$ further increasing to $1.21 \mu\text{g GAE g}^{-1}$ and $1.34 \mu\text{g GAE g}^{-1}$ for Al combined with $0.44 \mu\text{M}$ BAP and Al combined with $0.88 \mu\text{M}$ of BAP, respectively (Fig. 3).

For *V. tucanorum*, the control treatment had a higher phenolic content than *Q. grandiflora* with a mean value of $1.06 \mu\text{g GAE g}^{-1}$ (Fig. 4). The addition of BAP resulted in a slight increase in phenolic content with the highest phenolic content observed for the $0.88 \mu\text{M}$ of BAP treatment with a mean value of $2.88 \mu\text{g GAE g}^{-1}$, which was significantly different from all other treatments except Al combined with $0.88 \mu\text{M}$ of BAP treatment ($5.65 \mu\text{g GAE g}^{-1}$) (Fig. 4). These results show that both species have significant phenolic content, which can be further increased by adding Al and/or BAP.

DPPH Radical Scavenging Assay of Extracts

Figures 5 and 6 show the results of the assays for different treatments, including control, 0.44 , and $0.88 \mu\text{M}$ of BAP, $320 \mu\text{M}$ of Al, and $320.0 \mu\text{M}$ of Al combined with 0.44 or $0.88 \mu\text{M}$ of BAP. The percentage values of DPPH indicate the antioxidant capacity of each treatment.

For *Q. grandiflora*, all treatments showed similar antioxidant capacities, with mean values of 45.11% and 45.86%, respectively (Fig. 5). For *V. tucanorum*, the control treatment had the lowest antioxidant capacity with a mean value of 33.58% (Fig. 6). In contrast, all other treatments showed much higher antioxidant capacities ranging from 73.68% for Al treatment to 99.25% for Al combined with 0.88 μ M of BAP treatment.

Discussion

This study aimed to investigate the effects of Al and BAP for the induction of *in vitro* organogenesis regeneration of *Q. grandiflora* and *V. tucanorum*. Additionally, the hypothesis tested the necessity of higher concentrations of Al in the culture media, aiming for the success of *in vitro* regeneration of these Al-accumulator species. The results showed that both species require both Al and BAP, leading to callus induction and shoot regeneration in leaf cotyledonary explants. The isolated addition of Al in culture media also resulted in adventitious root formation in these explants, which was not observed under control conditions or by the addition of only BAP in culture media (Table 1).

The process of plant regeneration through direct organogenesis is multifaceted and necessitates the optimization of several factors, such as the type and concentration of plant growth regulators (PGRs). The use of cytokinins alone, or in conjunction with auxins, is critical for regenerating numerous species of woody trees (Gaba 2005; Phillips and Garda 2019; Verma *et al.* 2021). This study reported the first instance of organogenesis induction through the addition of Al in the culture media for these two species. Adventitious shoot induction was observed in the presence of BAP while root induction was observed in its absence. Furthermore, the results showed that the

combination of Al and BAP had a synergistic effect on sprout and shoot proliferation in both species tested (Table 1; Fig. 2 *c, d, g, h*).

Interestingly, a remarkable result was that root growth was only observed when plants were treated with Al alone without adding BAP (Table 1; Fig. 2 *b, f*). This effect was consistent across both species, indicating that Al may be a critical factor in promoting root growth. These results have important implications for Al-accumulator plant propagation, highlighting the potential benefits of its use as a growth-promoting agent in plant tissue culture.

Al is considered beneficial for some tolerant plant species that grow in regions with acidic soils ($\text{pH} < 5.0$) as it stimulates their growth and development (Bojórquez-Quintal *et al.* 2017). This metal promotes the uptake of essential nutrients, such as nitrogen, phosphorus, potassium, and magnesium, increases the photosynthetic rate, and enhances root elongation and biomass production in the aerial part (Hajiboland *et al.* 2013; Guilherme Pereira *et al.* 2018; Silambarasan *et al.* 2022). In addition, Al can boost the activity of enzymes that protect against oxidative stress and improve tolerance to herbivores and pathogens (Kaur *et al.* 2016). Several Cerrado plant species, including *Eugenia dysenterica* DC, *Miconia albicans* (Sw.) Steud, *Vochysia thyrsoidea* Pohl, *V. tucanorum*, and *Q. grandiflora*, which are known for their ability to accumulate metals, require Al for regular and good development (Haridasan 2008; Almeida Rodrigues *et al.* 2019; Cury *et al.* 2020; Bressan *et al.* 2021). Despite its importance in these species, the function of Al has not been fully elucidated.

Some studies have reported the use of Al in *in vitro* culture medium. For example, one study tested the effects of Al on the antioxidant capacity and profile of phenolic compounds in *Vaccinium corymbosum* L. (blueberries) grown *in vitro*. The authors

observed that during the exposure period of blueberry seedlings to different doses of Al, there was a decrease in lipid peroxidation and an increase in antioxidant capacity of the extracts on DPPH assays, which could be due to the increase in the total content of phenolic compounds (Manquian *et al.* 2013).

Another study with palm (*Phoenix dactylifera* L.) cultivar Um-Aldehin, the authors observed that the addition of aluminum to the culture medium had the most significant effect on shoot regeneration and increased the number of shoots of this species. Also, the antioxidant capacity of date palm shoots varies with the Al treatment applied. The non-enzymatic antioxidant defense mechanism is differentially activated according to Al concentration, indicating that the effective antioxidant response occurs at high concentrations of Al (Al-Mayahi 2019). It has been suggested that the antioxidant capacity of phenolic compounds increases the tolerance of plants to Al by detoxifying it through the formation of stable Al complexes (Tolrà *et al.* 2009).

The present authors of this study found a positive correlation between total phenol content and the percentage of regeneration for *Q. grandiflora* ($R = 0,7389$; $p < 0.05$) and *V. tucanorum* ($R = 0,8401$; $p < 0.05$). Also, this study found that both species had significantly higher total phenol content when treated with Al and 0.88 μM of BAP compared to the control group for both species. Phenolic compounds have been shown to play a crucial role in plant defense mechanisms against biotic and abiotic stress (Tuladhar *et al.* 2021), as well as potential applications as natural antioxidants and antimicrobial agents for human health (Tungmunthum *et al.* 2018). In addition, the total phenolics content are receiving considerable attention due to the presence of compounds with antioxidant activity that can capture single oxygen species. Furthermore, phenolic compounds show a crucial function in the oxido-reduction properties of substances, as well as in the adsorption and/or neutralization of lipid peroxidation and free radicals

(Casassa *et al.* 2016; Dias *et al.* 2019). They function as antioxidants, in both primary and secondary roles, while also playing a significant role as chelating agents (Tosun *et al.* 2009; Casassa *et al.* 2016; Dias *et al.* 2019).

The findings are consistent with prior research on the effects of PGRs on the *in vitro* production of secondary metabolites, as demonstrated in *Aloe arborescens* Mill (Amoo *et al.* 2012). Plant growth regulators (PGRs) are vitally important in plant tissue culture, playing a crucial role in both the regulation of secondary metabolite production and the control of fundamental growth and developmental processes in plants (Dörnenburg and Knorr 1995). Studies have demonstrated that cytokinins are involved in regulating the expression of genes associated with secondary metabolite pathways. There have been numerous investigations into the relationship between cytokinins and auxins and secondary metabolite production in plant tissue culture (Meyer and Van Staden 1995; Miura *et al.* 1998; Luczkiewicz and Cisowski 2001; Coste *et al.* 2011).

Phenols are essential plant constituents due to their hydroxyl groups, which provide them with a scavenging ability on free radicals (Tosun *et al.* 2009). As such, the phenolic content of plants may directly contribute to their antioxidant activity (Tosun *et al.* 2009). It is, therefore, crucial to note the importance of phenols in plant extracts to enhance their antioxidant potential. Nevertheless, *Q. grandiflora* did not present a significant difference between the treatments regarding the antioxidant activity. For *V. tucanorum*, the addition of BAP and, or, Al in the culture medium resulted in an increase of the antioxidant activity when compared with the control treatment.

Liang and Kitts (2014) noted that the DPPH method can detect two mechanisms of free radical reduction. Using this method, the present authors of this study were able to determine the antioxidant and potential workings of ethanolic extracts obtained from

Q. grandiflora and *V. tucanorum* (as shown in Fig. 5 and 6). The results of this study suggest that the ethanolic extracts from these Vochysiaceae species exhibit an antioxidant capacity achieved through a scavenger and quenching process, which is likely due to the presence of phenols.

The capacity of these extracts to eliminate DPPH radicals can mainly be attributed to the existence of phenolic hydroxyl groups. Nevertheless, various approaches have been proposed to explain the antioxidant qualities of these substances. These approaches consist of stopping the initiation of radical chains, binding to catalysts that contain transition metal ions, breaking down peroxides, obstructing continued hydrogen abstraction, and removing free radicals (Diplock 1997).

Comparative studies of Al-accumulating plant species from the Cerrado under varying Al availabilities are scarce, likely due to the challenges associated with domesticating these plants. This study aimed to overcome this limitation by initiating the development of a protocol to induce indirect organogenesis and proliferation of shoots in these species. Through this research, the present authors hope to shed light on the mechanisms underlying Al accumulation and tolerance in these plants and identify potential sources of bioactive compounds in Cerrado plant species. This approach may assist in future research about cultivating Al-accumulating plant species from the Cerrado, providing a steady supply of plant material for future comparative studies.

Conclusions

The main conclusions of the actual study suggested that adding Al in culture media provides biochemical, physiological, and development switches in the isolated leaf tissues that lead to adventitious shoot (+BAP) or root regeneration (-BAP) under *in vitro*

culture for *Q. grandiflora* and *V. tucanorum*. The presence of Al also enhanced the phenolic content and antioxidant capacity of isolated leaf tissues for both Al-accumulator species. These findings have important implications for the *ex situ* cultivation and propagation of Vochysiaceae species and demonstrate that Al has a close relationship with the evolution and development of these species. Furthermore, no research was found correlating Al with metabolite production and tissue regeneration since this is the first report in the literature to relate Al with phenolic content and antioxidant activity.

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Tables

Table 1. Effects of 6-benzylaminopurine (BAP) and Al treatments on shoot and root proliferation of *Qualea grandiflora* Mart. and *Vochysia tucanorum* Mart. leaf explants derived shoots cultured on ¼ Murashige and Skoog multiplication medium after 30 d.

Specie	Plant Growth Regulators (PGR µM)		Number of shoots per explant	Number of leafs per explant	Shoot length (cm)	Number of roots per explant	Percentage shoot induction
<i>Q. grandiflora</i>	<u>BAP</u>	<u>Al</u>					
	0	0	-	-	-	-	-
	0	320	-	-	-	0.5 ± 0.52 ^a	-
	0.44	0	-	-	-	-	-
	0.44	320	1.4 ± 0.54 ^a	7.5 ± 1.97 ^a	4.3 ± 0.54 ^b	-	48.22 ± 4.0 ^a
	0.88	0	-	-	-	-	-
	0.88	320	3.2 ± 3.16 ^a	7.25 ± 3.20 ^a	4.5 ± 0.83 ^a	-	56.41 ± 2.6 ^a
<i>V. tucanorum</i>	0	0	-	-	-	-	-
	0	320	-	-	-	4.1 ± 2.18 ^a	-
	0.44	0	-	-	-	-	-
	0.44	320	1.3 ± 0.78 ^b	1.2 ± 0.23 ^b	0.5 ± 0.89 ^b	-	32.26 ± 6.1 ^b
	0.88	0	-	-	-	-	-
	0.88	320	3.8 ± 1.21 ^a	4.54 ± 1.78 ^a	3.5 ± 0.24 ^a	-	66.02 ± 1.0 ^a

Means are followed by ± SE (standard error). Different letters indicate significant differences between columns ($p < 0.05$). “-” means

no proliferation of leaf explants derived shoots on multiplication MS ¼.

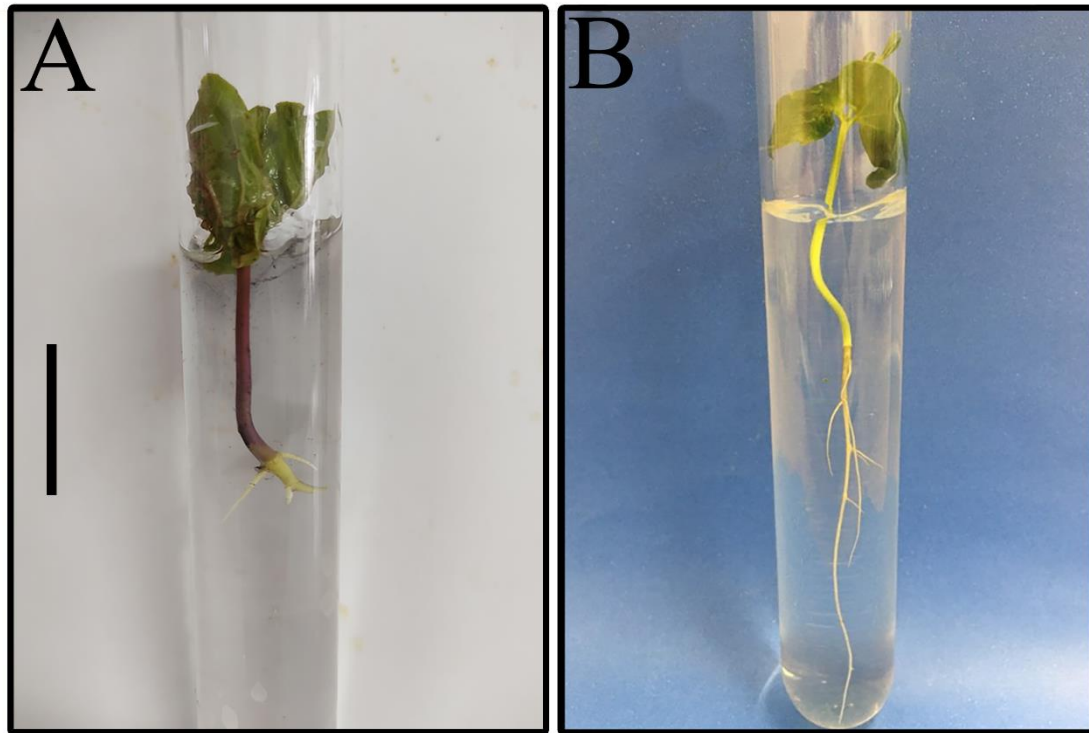
Figures

Figure 1. Explants (35 d) provided from seeds of (A) *Qualea grandiflora* Mart. and (B) *Vochysia tucanorum* Mart. in a $\frac{1}{4}$ Murashige and Skoog (MS) medium supplemented with 0.44 μM 6-benzylaminopurine (BAP), 5.7 μM gibberellic acid (GA_3) and 90.0 μM of Al. (Scale bar = 5 cm).

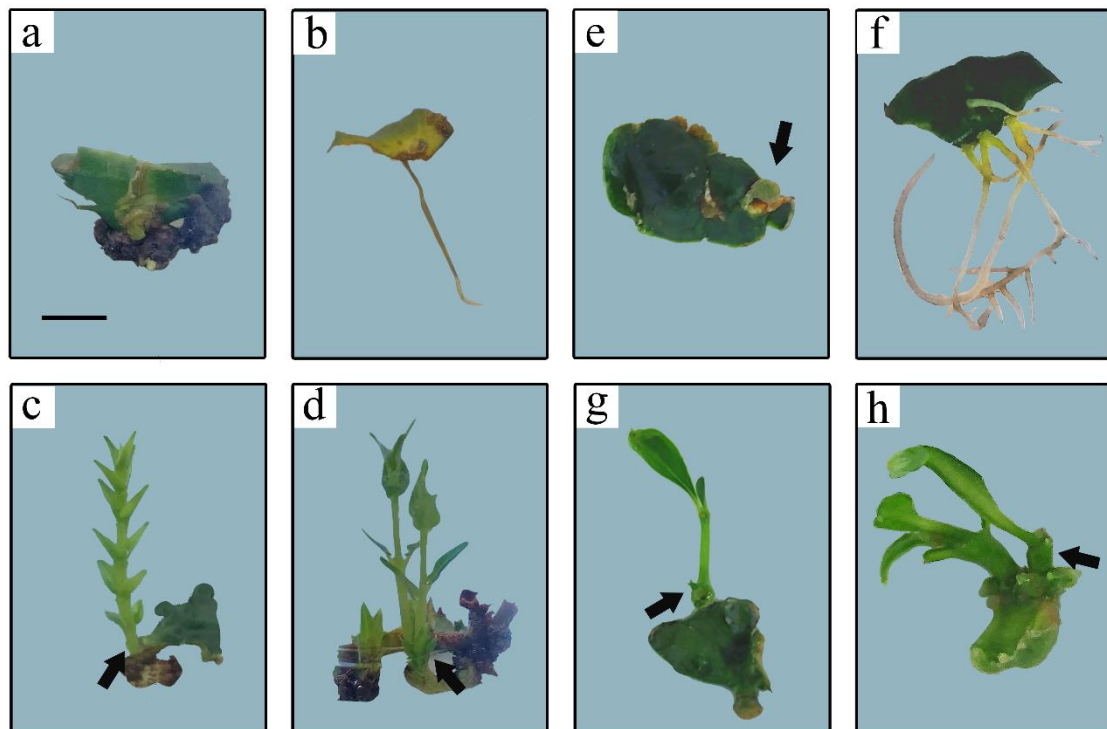


Figure 2. Effects of various 6-benzylaminopurine (BAP) and Al combinations on *Qualea grandiflora* Mart. (a-d) and *Vochysia tucanorum* Mart. (e-h) cotyledonary leaf segments organogenesis after 30 d; (a, e) Al free medium, no organogenesis; (b, f) 320 µM of Al, root induction; (c, g) 0.44 µM of BAP and 320.0 µM of Al, callus and shoot induction; and (d, h) 0.88 µM of BAP and 320.0 µM of Al, callus and shoot induction. Arrows indicate callus formation. (Scale bar = 1 cm).

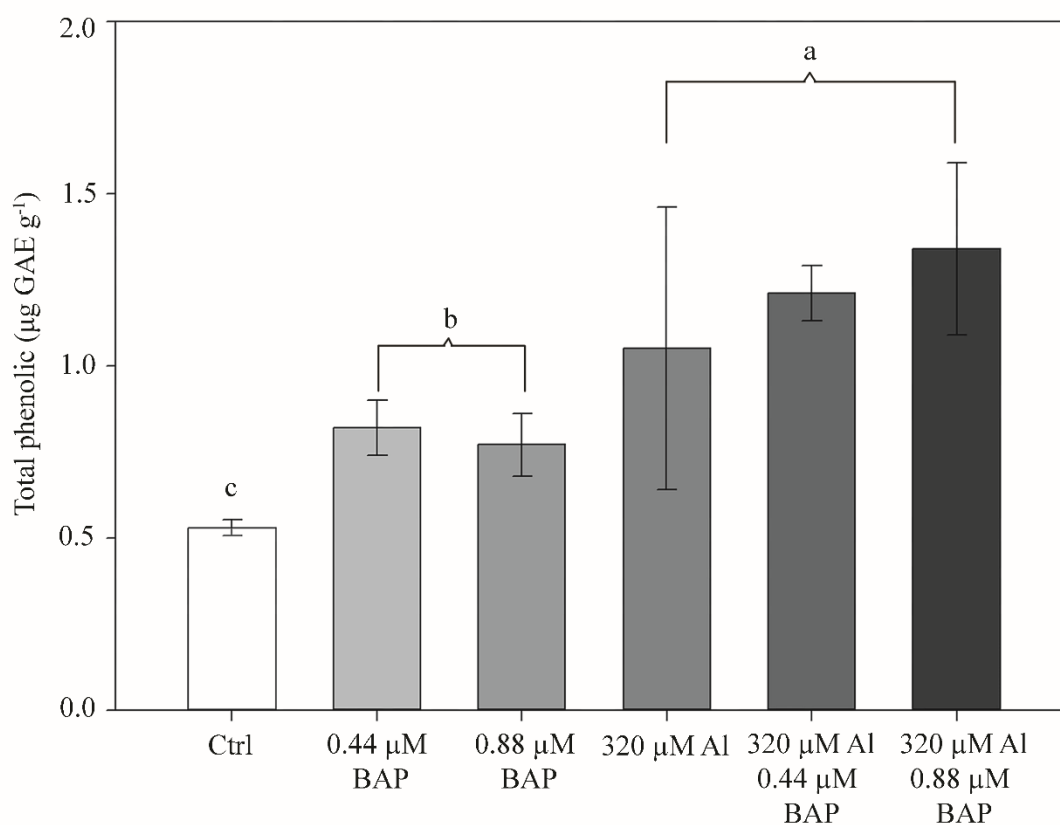


Figure 3. Total phenolic content in *Qualea grandiflora* Mart. cotyledonary leaf segments at different concentrations of 6-benzylaminopurine (BAP) (0; 0.44 and 0.88 µM) and Al (0 and 320.0 µM). Data are mean ± SE (n = 3). * represents treatment groups by statistical similarity according to Tukey post-test of multiple comparisons (P < 0.05).

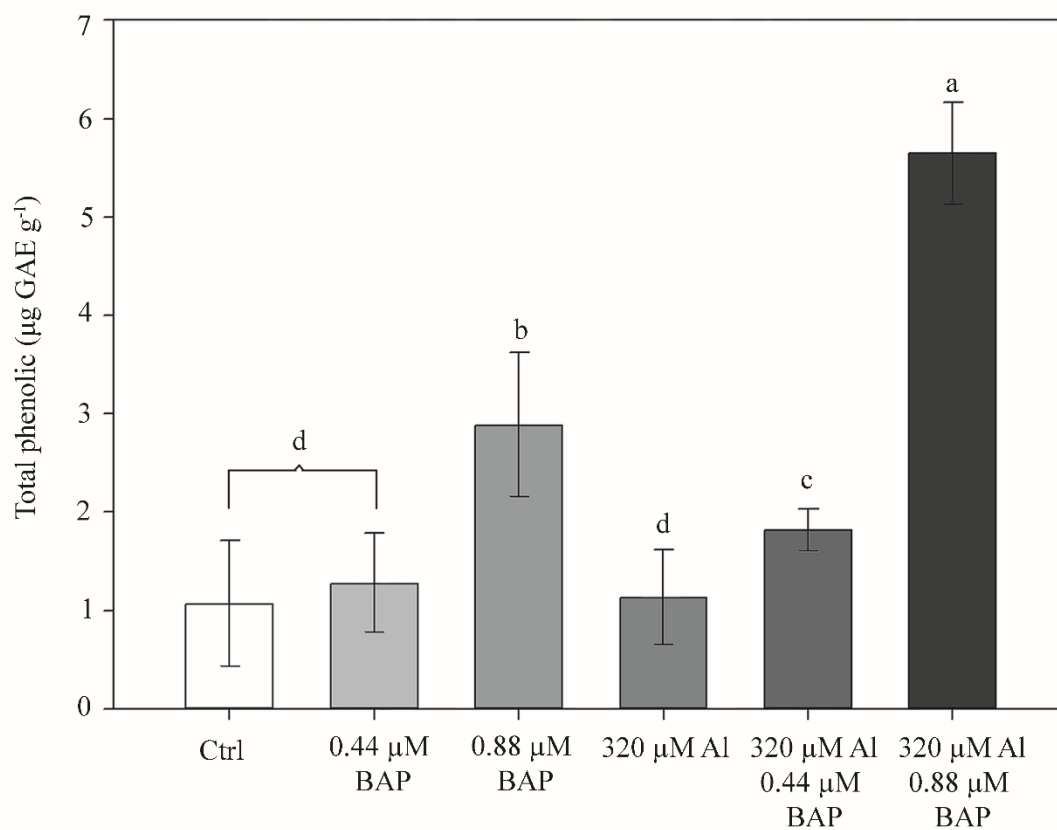


Figure 4. Total phenolic content in *Vochysia tucanorum* Mart. cotyledonary leaf segments at different concentrations of 6-benzylaminopurine (BAP) (0; 0.44 and 0.88 µM) and Al (0 and 320.0 µM). Data are mean $\pm$ SE (n = 3). * represents treatment groups by statistical similarity according to Tukey post-test of multiple comparisons ($P < 0.05$).

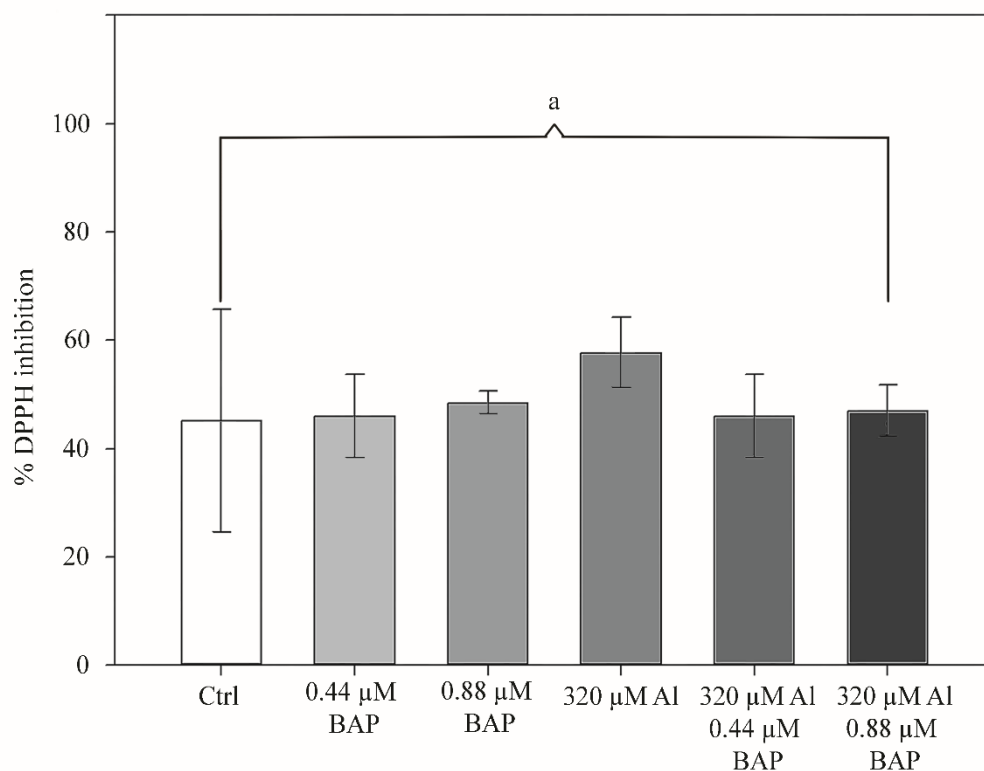


Figure 5. DPPH radical scavenging assay of *Qualea grandiflora* Mart. cotyledonary leaf segments at different concentrations of 6-benzylaminopurine (BAP) (0; 0.44 and 0.88 μM) and Al (0 and 320.0 μM). Data are mean $\pm$ SE ($n = 3$). * represents treatment groups by statistical similarity according to Tukey post-test of multiple comparisons ($P < 0.05$).

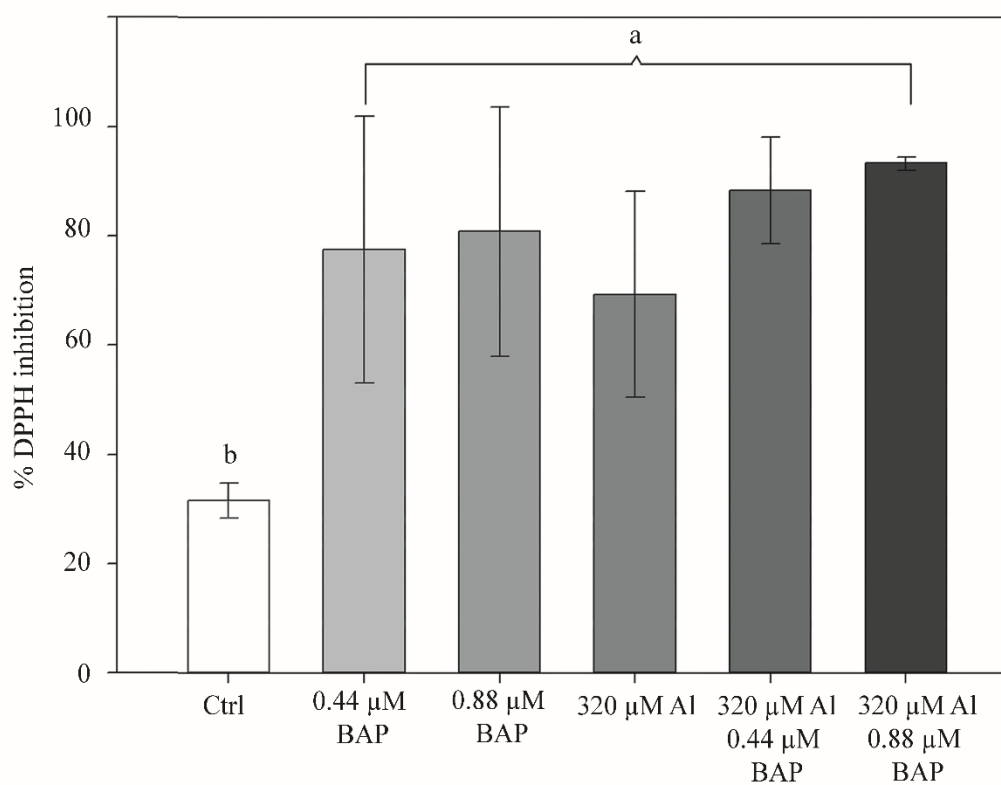


Figure 6. DPPH radical scavenging assay of *Vochysia tucanorum* Mart. cotyledonary leaf segments at different concentrations of 6-benzylaminopurine (BAP) (0; 0.44 and 0.88 μM) and Al (0 and 320.0 μM). Data are mean $\pm$ SE ($n = 3$). * represents treatment groups by statistical similarity according to Tukey post-test of multiple comparisons ($P < 0.05$).

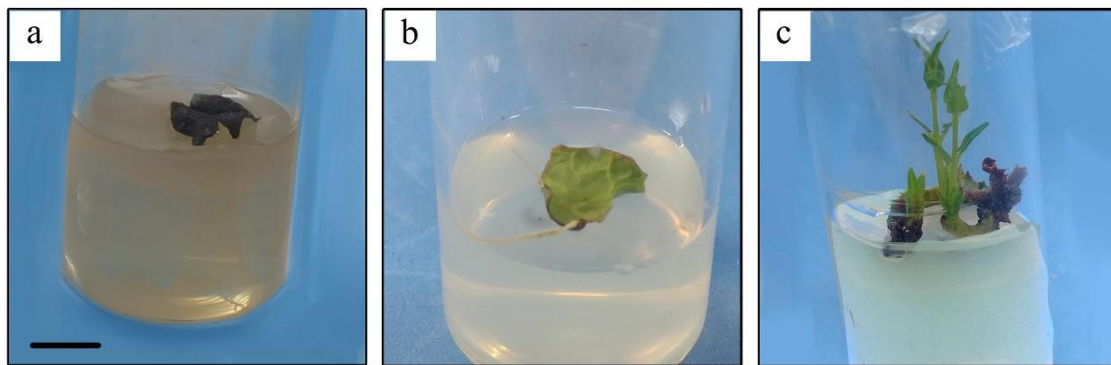
Supplementary material

Supplementary material 1. Aluminum concentration in seeds and cotyledonary leaves of *Qualea grandiflora* Mart. and *Vochysia tucanorum* Mart. grown on culture medium containing 90.0 μM of aluminum.

Species	Organ	mg Al kg ⁻¹ dry mass
<i>Q. grandiflora</i>	cotyledonary leaf	4179.0
	seed	7870.0 $\pm$ 444.1
<i>V. tucanorum</i>	cotyledonary leaf	28937.2
	seed	31150.0 $\pm$ 212.1

Mean values (n = 3) $\pm$ standard deviation

(*) The results represent the dry mass of the cotyledonary leaves evaluated.

Supplementary material 2.

Supplementary material 2. Comparison of leaf tissue in different treatments on *Qualea grandiflora* Mart. (a) Medium with absence of aluminum, the leaf tissue turned brown, indicating senescence. (b) 320.0 μM of Al, root induction and (c) 0.88 μM of 6-benzylaminopurine (BAP) and 320.0 μM of Al, callus and shoot induction. The treatments with Al and, or, BAP did not show oxidation. (Scale bar = 1 cm).

Considerações finais

Neste estudo, demonstramos que o Al tem um efeito significativo na germinação e crescimento de *Q. grandiflora* e *V. tucanorum*. O tratamento das sementes de *V. tucanorum* com Et induziu a germinação do embrião quando o Al não estava presente no meio, sugerindo uma relação substitutiva entre esses compostos. Os embriões de *Q. grandiflora* germinaram independentemente do Al no meio e o Et não influenciou sua germinação. Além disso, os resultados sugerem que a adição de Al em meios de cultura proporciona mudanças bioquímicas, fisiológicas e de desenvolvimento nos tecidos foliares isolados que levam à regeneração de brotos adventícios (+BAP) ou raízes (-BAP) sob cultura *in vitro* para essas espécies. A presença de Al também aumentou o conteúdo fenólico e a capacidade antioxidante dos tecidos foliares isolados para ambas as espécies. Essas descobertas têm implicações importantes para o cultivo e propagação *ex situ* das espécies de Vochysiaceae e demonstram que o Al tem uma relação estreita com sua evolução e o seu desenvolvimento. Além disso, nenhuma pesquisa foi encontrada correlacionando Al com a produção de metabólitos e a regeneração de tecidos, uma vez que este é o primeiro relato na literatura a relacionar Al com o conteúdo fenólico e a atividade antioxidante. Diante desses resultados, sugerimos uma investigação mais detalhada sobre o papel fisiológico do alumínio (Al) e do etileno (Et) na germinação e no desenvolvimento de plântulas e mudas dessas espécies. Além disso, é importante explorar aspectos relacionados à produção de compostos secundários e entender como eles influenciam a regeneração de tecidos nessas espécies. Essa abordagem aprofundada pode fornecer perspectivas valiosas para aprimorar estratégias de conservação e propagação dessas espécies do Cerrado.

