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Loisiana Feuser dos Santos

**Espectro de tolerância à dessecação de sementes em Lauraceas Tropicais é ampliado
pela *Ocotea pulchella* (Nees & Mart.) Mez.**

Florianópolis

2024

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Espectro de tolerância à dessecação de sementes em Lauraceas Tropicais é ampliado pela *Ocotea pulchella* (Nees & Mart.) Mez.

O presente trabalho em nível de Mestrado foi avaliado e aprovado, em 30 de outubro de 2023, pela banca examinadora composta pelos seguintes membros:

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Certificamos que esta é a versão original e final do trabalho de conclusão que foi julgado adequado para obtenção do título de Mestra em Ciências.

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Prof.(a) Dr.(a) Neusa Steiner
Orientador(a)

Florianópolis, 2024.

Dedico a minha filha Leticia Íria.
Que sempre saibas que a maternidade não é
um fim em si mesma e lembres sempre que
uma mulher pode e deve lutar pelo destino que
escolher.

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(Dioum, 1968, New Delhi.)

RESUMO

Sementes são a razão pela qual separamos o maior e mais moderno grupo de plantas em Angiospermas. Ainda assim, essas estruturas reprodutivas, são pouco estudadas quanto às suas especificidades entre espécies não comerciais. Em plantas a importância da família das Lauráceas tem sido amplamente relatada tanto em regiões de ecossistemas tropicais quanto subtropicais. Destacando a relevância das espécies desta família como componentes cruciais da estrutura de comunidades de plantas, fonte crucial de alimentos para diversas espécies da fauna, significância cultural, histórica econômica e de potencial de uso. Características morfofisiológicas, comportamento das sementes em relação à dessecação e perfil bioquímico são elementos chave para sobrevivência, estabelecimento e manutenção de espécies de Lauráceas e conseqüentemente dos ecossistemas nos quais estão inseridas. Sensibilidade à dessecação e a incapacidade de sobreviver ao armazenamento, em sementes, gera limitações tanto nas práticas de propagação quanto na preservação destes recursos genéticos. Neste estudo, sementes de *Ocotea pulchella* foram avaliadas, quanto ao conteúdo de água durante o desenvolvimento, morfometria, morfoanatomia, comportamento germinativo em diferentes conteúdos de água, perfil de carboidratos, poliaminas, armazenamento e introdução *in vitro*. Observou-se que o conteúdo de água apresenta oscilação durante o desenvolvimento das sementes, ocorrendo dessecação das sementes nos estágios finais observados. A resposta germinativa e o índice de velocidade de germinação foram significativamente influenciados pela dessecação, mostrando quedas significativas em relação à diminuição do conteúdo de água das sementes. O carboidrato mais abundantemente encontrado foi a arabinose, seguido da manose e sacarose, com predominância de monossacarídeos em relação a dissacarídeos. O perfil de poliaminas livres revelou que espermina apresenta maior abundância e diferença significativa em relação a quantidade de putrescina e espermidina. Os resultados indicam que *O. pulchella* pode apresentar comportamento intermediário em relação à sensibilidade à dessecação, sendo um promissor modelo, para estudos sobre tolerância à dessecação em sementes desta família.

Palavras-chave: dessecação; Lauraceas; conservação.

ABSTRACT

Seeds are the reason we separate the largest and most modern group of plants into Angiosperms. Even so, these reproductive structures are little studied regarding their specificities among non-commercial species. In plants, the importance of the Lauraceae family has been widely reported in both tropical and subtropical ecosystems. Highlighting the relevance of the species of this family as crucial components of the structure of plant communities, crucial source of food for several species of fauna, cultural significance, economic history, and potential use. Morphophysiological characteristics, seed behaviour in relation to desiccation and biochemical profile are key elements for the survival, establishment, and maintenance of Lauraceae species and consequently the ecosystems in which they are inserted. Sensitivity to desiccation and the inability to survive storage in seeds, generate limitations both in propagation practices and in the preservation of these genetic resources. In this study, seeds of *O. pulchella* were evaluated for water content during development, morphometry, morphoanatomy, germination behaviour in different water contents, carbohydrate profile, polyamines, storage, and in vitro introduction. It was observed that the water content oscillates during the development of the seeds, with desiccation of the seeds occurring in the final stages observed. The germination response and the germination speed index were significantly influenced by desiccation, showing significant decreases in relation to the decrease in seed water content. The most abundant carbohydrate found was arabinose, followed by mannose and sucrose, with a predominance of monosaccharides over disaccharides. The profile of free polyamines revealed that spermine has greater abundance and significant difference in relation to the amount of putrescine and spermidine. The results indicate that *O. pulchella* can present intermediate behaviour in relation to sensitivity to desiccation, being a promising model for studies on tolerance to desiccation in seeds of this family.

Keywords: seeds; desiccation; Lauraceae; conservation.

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RESUMO EXPANDIDO

Introdução

A pauta da restauração da Mata Atlântica tem sido central em muitos debates, já que estamos vivenciando os impactos de sua degradação (crise hídrica, alterações do microclima, erosão do solo) além do esgotamento dos serviços ecossistêmicos, paisagens altamente fragmentadas e extinção de espécies. Por conta deste cenário preocupante, os três setores da sociedade apresentam iniciativas de preservação, conservação e manejo das regiões de Mata Atlântica. Participando deste domínio fitogeográfico, existem atualmente 205 espécies de Lauraceae, sendo 128 delas endêmicas. A família Lauraceae compreende espécies de árvores e arbustos, altamente diversas e ecologicamente significativas, especialmente em florestas tropicais e subtropicais. Conservar espécies da família Lauraceae é essencial devido à sua importância ecológica, cultural e econômica. Iniciativas de reflorestamento e recuperação e conservação comumente desconsideram a fisiologia das sementes. Este cenário é especialmente preocupante quando consideramos as espécies de lauráceas. As sementes desta família apresentam perfil preocupante, destacando-se por baixas taxas de germinação, problemas para armazenagem em bancos de sementes convencionais e no solo. Este perfil desafiador, vem sendo ligado ao conteúdo de água das sementes pós dispersão, danos mecânicos e metabólicos ocasionados pela dessecação. *Ocotea pulchella* é uma espécie de árvore essencial para a conservação e restabelecimento da fitofisionomia da Mata Atlântica. A espécie apresenta comportamento primário e nucleador, ressaltando assim sua importância para a dinâmica de restabelecimento e reflorestamento em ambientes florestais que vão da restinga à floresta ombrófila densa estendendo-se por 15 estados brasileiros. Conhecer as particularidades morfofisiológicas, morfo anatômicas e bioquímicas auxilia não só na melhor compreensão das particularidades sobre as sementes destas espécies, como também contribui para uma melhor compreensão sobre o comportamento de sementes em relação à dessecação. Entender a dinâmica de dessecação das sementes ao longo do tempo, nos dá informações importantes sobre a possível dinâmica destas espécies na natureza e ainda embasa e orienta métodos de propagação, armazenamento e conservação. Neste sentido, o presente trabalho contribui para a construção do conhecimento necessário para desenvolver estudos mais robustos e estratégias de conservação mais eficazes.

Objetivos

O presente estudo teve como principal objetivo avaliar características morfofisiológicas de sementes e frutos de *O. pulchella*, bem como avaliar o comportamento germinativo de suas sementes, quando submetidas à dessecação. Com este intuito, buscou-se enriquecer o conhecimento científico sobre a espécie alvo, possibilitando e/ou facilitando a estruturação de protocolos para atividades técnicas e científicas de conservação, pesquisa e uso destas espécies.

Metodologia

Frutos de *O. pulchella* foram coletados na Unidade de Conservação Monumento Natural Municipal da Lagoa do Peri na cidade de Florianópolis, no estado de Santa Catarina (-27.727795 S, -48.509101 W), durante o período de outubro de 2021 a março de 2023. Quinze árvores foram demarcadas no local para coleta e acompanhamento de desenvolvimento dos frutos de *O. pulchella*. As árvores usadas no estudo encontravam-se em área de vegetação característica de Restinga. Para experimentos com sementes, frutos maduros de *O. pulchella*, foram despulpados manualmente e as sementes limpas superficialmente com papel toalha. O teor de água das sementes foi determinado através do método de estufa à 105°C por 24h. Procedimentos de dessecação foram realizados em potes plásticos herméticos contendo sílica gel. Para a curva de dessecação, sementes foram pesadas sistematicamente, a cada 15 minutos,

pelos primeiros 30 minutos, a cada hora, pelas primeiras 12 horas, a cada 12 horas por 5 dias e a cada 24 horas nos dias seguintes. Testes de germinação foram realizados em BOD, a $25 \pm 2^\circ\text{C}$ e 12h de fotoperíodo, sendo as germinações verificadas diariamente, por 60 dias. Sementes foram consideradas germinadas quando houve visualização de protrusão da radícula. A sobrevivência em relação ao armazenamento foi observada para 3 diferentes temperaturas (8°C , -18°C e -196°C) e 4 diferentes períodos a 8°C (0, 30, 40 e 50 dias). Para testes de tetrazólio, as sementes foram embebidas em solução de sal de tetrazólio 1% e mantidas no escuro em temperatura $30^\circ\text{C} \pm 2$ durante 2 horas. Análises histológicas foram realizadas através de cortes histológicos submetidos a microscopia de luz, com material infiltrado em historesina® (Leica Historesin, Heidelberg, Germany) seguido de aplicação dos corantes azul de toluidina (TB-O) e azul brilhante de comassie. Teores endógenos de poliaminas (PAs): putrescina, cadaverina, espermidina e espermina foram analisados com o auxílio de HPLC, utilizando-se 200 mg de matéria seca. Teores endógenos de carboidratos foram verificados com auxílio de HPLC, utilizando-se 400 mg de matéria seca. Explantes de sementes de *O. pulchella* foram introduzidos em meio de cultura e mantidos em BOD por 60 dias. Quando atingida a homocedasticidade dos dados obtidos, foi aplicado teste T e ANOVA, sendo as diferenças entre médias comparadas pelo teste Tukey ($p < 0,05$).

Resultados e Discussão

Sementes de *O. pulchella*, apresentaram redução do conteúdo de água ao final dos estágios de desenvolvimento dos frutos observados. A diminuição no conteúdo de água em sementes nos estágios finais do desenvolvimento é um traço mais comumente associado e observado em sementes que apresentam comportamento de tolerância à dessecação. Quando comparadas a outras espécies da família das Lauráceas, as sementes e eixos embrionários de *O. pulchella* podem ser representantes de menor tamanho. Tamanho e proporção do eixo embrionário, também são características ligadas a um comportamento de tolerância à dessecação em outras espécies. Tamanho afeta diretamente o raio entre superfície e área por volume. Sendo assim, sementes menores têm relativamente uma maior área de exposição ao ambiente externo e por isso tendem a perder água mais rapidamente. Além disso, cortes histológicos mostram que a composição do integumento, pode também favorecer a perda de água, uma vez que seus macroesclerídeos apresentam rupturas mesmo em sementes sem tratamento de dessecação. Além de favorecer a dessecação, um tegumento frágil e poroso também pode propiciar a germinação. De acordo com o observado em *O. pulchella*, após o contato com a água, as sementes demoram em torno de 7 dias para romper completamente o integumento e afastar os cotilédones, propiciando assim a protrusão da radícula que ocorre cerca de dois dias mais tarde e permite que o restante do desenvolvimento que resultará na plântula ocorra.

A resposta germinativa e o índice de velocidade de germinação foram significativamente influenciados pela dessecação nos dois anos observados (2022 e 2023), mostrando quedas significativas em relação à diminuição do conteúdo de água das sementes. Os diferentes anos não apresentaram diferença entre si quando submetidos ao teste F.

O carboidrato mais abundantemente encontrado foi a arabinose, seguido da manose e sacarose, com predominância de monossacarídeos em relação a dissacarídeos. O perfil de poliaminas livres revelou que espermina apresenta maior abundância e diferença significativa em relação a quantidade de putrescina e espermidina. Os resultados indicam que *O. pulchella* pode apresentar comportamento intermediário em relação à sensibilidade à dessecação, sendo um promissor modelo, para estudos sobre tolerância à dessecação em sementes desta família.

Considerações Finais

Estudos com a espécie *O. pulchella* apresentam significativa relevância quando consideramos que sua morfofisiologia e resposta à dessecação, apresenta comportamento marginal em relação à dessecação. Sendo assim, estudar de maneira mais aprofundada esta espécie pode auxiliar no desenvolvimento de técnicas para conservação dos demais representantes da família, bem como auxiliar a preencher as lacunas de conhecimento sobre resposta à dessecação para a família das Lauráceas.

Palavras-chaves: sementes; dessecação; Lauraceae; conservação.

LISTA DE ABREVIATURAS E SIGLAS

ANOVA	Analysis of Variance
BAP	6-benzilaminopurin
B1	Fruits of <i>O. pulchella</i> with < 8 mm and light brown colour
B2	Fruits of <i>O. pulchella</i> with < 8 mm and light medium colour
B3	Fruits of <i>O. pulchella</i> with < 8 mm and dark brown colour
CBB	Coomassie Brilliant Blue
DT	Desiccation Tolerant
DM	Dry Mass
DS	Desiccation Sensitive
ELSD	Evaporative light scattering detector
WC	Water Content
DW	Dry Weight
UFSC	Federal University of Santa Catarina
G	Number of germinated seeds
G1	Fruits of <i>O. pulchella</i> with 1 to 2 mm of height
G2	Fruits of <i>O. pulchella</i> with 3 to 4 mm of height
G3	Fruits of <i>O. pulchella</i> with 5 to 6 mm of height
G4	Fruits of <i>O. pulchella</i> with 7 to 8 mm of height
GSI	Germination Speed Index
LM	Light Microscopy
LS	Longitudinal Section
ROS	Reactive Oxygen Species
N	Number of days elapsed since sowing
PAs	Polyamines
PUT	Putrescine
SPD	Spermidine
SPM	Spermine
TB-O	Toluidine blue
TTC	% 2,3,5-triphenyltetrazolium chloride
HPLC	High-performance Liquid Chromatography
FS	Fresh Seeds
FW	Fresh weight
H2O	Water

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1 INTRODUÇÃO E JUSTIFICATIVA

A Mata Atlântica é o terceiro maior domínio vegetacional do Brasil, estendendo-se desde o litoral do Nordeste até o Rio Grande do Sul através de 15 estados, incluindo completa ou parcialmente todos os estados litorâneos do Brasil (Myers et al., 2000; Tabarelli et al., 2005; Lewinsohn; Prado, 2006; SOS Mata Atlântica, 2023). É considerada um dos *hotspots* de biodiversidade mais importantes do mundo e um dos dois mais importantes do Brasil, com aproximadamente 20 mil espécies de plantas, sendo 8 mil delas endêmicas (Mittermeier et al., 2011). A biodiversidade existente e mantida nesses locais tem sua relevância estendida para além da conservação da riqueza de espécies e seus endemismos. Sua área de abrangência abriga a maior densidade de população e lidera as atividades econômicas do Brasil (IBGE, 2020). As interações entre e dentro de espécies e o ambiente abiótico destas florestas, sustentam, regulam e fornecem serviços e benefícios culturais para as pessoas (Benayas et al., 2009). Os remanescentes atuais da Mata Atlântica desempenham papel fundamental no que concerne à segurança e proteção da água doce, regulação climática e produção de alimentos (Baptista; Rudel, 2006; Ribeiro et al., 2011). Restaurar as áreas deste domínio vegetacional, figura no topo de prioridades globais (Strassburg et al., 2019). Diante deste cenário, os três setores da sociedade brasileira e mundial, vêm desenvolvendo iniciativas com o intuito de proteger, conservar e restabelecer as áreas de Mata Atlântica (Grelle et al., 2021; Mohebalian et al., 2022). O Brasil é um dos 193 países que se comprometeram a tomar medidas ousadas e transformadoras para promover o desenvolvimento sustentável. Por conta dos objetivos de desenvolvimento sustentável (ODS), uma agenda foi adotada em 2015 pelas Nações Unidas (ONU), composta por 17 objetivos e 169 metas a serem atingidos de forma voluntária pelos países até 2030. Com isso, a recuperação do bioma Mata Atlântica tem uma interface direta com o ODS de número 15, intitulado como Ecossistemas Terrestres e Biodiversidade com a missão de: “Proteger, restaurar e promover o uso sustentável dos ecossistemas terrestres, gerir de forma sustentável as florestas, combater a desertificação, travar e reverter a degradação dos solos e travar a perda da biodiversidade”. Apesar disso, a área remanescente atual é de apenas 28% e estima-se que 1.544 espécies de plantas deste ambiente estejam ameaçadas, um equivalente a 60% de toda a lista de espécies ameaçadas no Brasil (Martinelli; Moraes, 2013; Rezende et al., 2018). Desta maneira, compreender como as espécies deste ecossistema responderão a novos regimes climáticos mostra-se urgente e prioritário (Wilson et al., 2021).

A família das Lauráceas compreende 2.500 a 3.000 espécies de árvores e arbustos, com exceção de *Cassytha*, sendo dividida em 50 gêneros (Nie et al., 2007). Espécies de Lauráceas tem grande representatividade e desempenham significativa relevância ecológica especialmente em florestas de regiões tropicais e subtropicais (Gentry, 1988; Burger, 1988). Na Mata Atlântica são atualmente encontradas 205 espécies de representantes dessa família, sendo 128 endêmicas (Quinet et al., 2017). Componente significativa da diversidade das florestas, no sul do Brasil, podem apresentar alto nível de endemismo (Brotto et al., 2019). Este cenário traz implicações significativas para a conservação e manejo destas espécies e seus ecossistemas. Espécies endêmicas podem trazer informações valiosas sobre a dinâmica de seus ecossistemas e evolução de plantas, além de sua relevância cultural e histórica. Apontada como uma das três famílias de plantas com maior potencial de uso sustentável não madeireiro das florestas de mata atlântica mista subtropical (Santos et al., 2016). Comumente espécies desta família são exploradas ostensivamente pelo seu valor medicinal, e diversas demonstram imenso potencial biotecnológico, pela presença de alta concentração de compostos potencialmente interessantes descritos na literatura (Laloo et al., 2006; Farias et al., 2023). O alto endemismo apresenta implicações pois aumenta sua vulnerabilidade diante de perturbações em seus habitats como, pragas, alta pressão antrópica e mudanças climáticas. A perda sistemática e/ou significativa destas espécies fragiliza todo o ecossistema dos quais participam, afetando a flora e fauna, gerando um ciclo alarmante de fragilização e perda de biodiversidade. Neste sentido alguns desafios cingem os estudos e esforços para conservação das espécies desta família. Espécies de Lauráceas apresentam indivíduos com características fenotípicas bastante semelhantes, sendo sua identificação reservada para especialistas, épocas do ano específicas (sazonais e inconstantes) como floração e frutificação ou testes genéticos (Brotto; Cervi; Santos, 2013; Yang et al., 2019). Adicionalmente a baixa germinação in situ e a falta de agentes dispersores tem se mostrado como fatores centrais para a perda significativa de diversidade genética das Lauráceas (Zhu et al., 2004). O gênero *Ocotea* Aubl., em sua atual organização, é o maior gênero entre Lauráceas Neotropicais, tendo entre 400 e 450 espécies reconhecidas (Trofimov; Moraes; Rohwer, 2019).

O. pulchella Mart. também conhecida em diferentes regiões como: Canela lajeana (SC), Caneleira, Canela-do-brejo, Canela Laranja, Canela Pimenta, Canela preta e Canelinha. A espécie tem ocorrência bem documentada nos estados do Espírito Santo, Minas Gerais, Mato Grosso do Sul até o Rio Grande do Sul. Ocorre nos Estados de Tocantins, Goiás, Distrito Federal, Minas Gerais, Espírito Santo, São Paulo, Rio de Janeiro, Paraná, Santa Catarina e Rio

Grande do Sul (Lorenzi, 1998). A espécie encontra-se amplamente distribuída em todas as formações vegetais, ambientes de floresta, restinga e no cerrado, tendo, porém, uma maior frequência nas sub-matas de pinhais, mas também ocorrendo na restinga litorânea e nos cerrados de São Paulo. A espécie mostra-se indiferente ao tipo de solo (Lopes; Gonçalves, 2006). Na Floresta Ombrófila Densa, no estado do Paraná, *O. pulchella* é a espécie do gênero *Ocotea*, que ocupa o maior número de ambientes (Brotto; Cervi; Santos, 2013). Avaliando os indivíduos que ocorriam no Estado de Santa Catarina, Reitz, Klein e Reis (1979) encontraram a espécie preferencialmente em florestas de pinhais adultos, mas também, nos capões com pinheiros jovens, e com abundante frequência na FOM (Floresta Ombrófila Mista) da região dos pinhais e na vegetação quaternária do litoral atlântico (Restinga), onde chamaram atenção a diferença destes indivíduos quanto ao porte. Sendo ainda igualmente encontrada com relativa frequência, no topo dos morros mais elevados, existentes na região da mata pluvial da encosta atlântica.

Depois da Imbuia, é sem dúvida, a *O. pulchella* (Canela-lageana), a Lauraceae de maior importância sociológica e de melhor expressão nos sub-bosques dos pinhais em Santa Catarina. Tornando-se em muitos locais a vicariante daquela (Klein, 1974, p. 14).

Além da ocorrência nas regiões Centro-Oeste, Sudeste e Sul do Brasil, é ainda encontrada na Argentina, Paraguai, Uruguai (Baitello; Marcovino, 2003). Estudos relativos à fenologia de *O. pulchella* apontam que as populações apresentam grande variação com relação à época e intensidade dos eventos de floração e frutificação (Reitz; Klein; Reis, 1979). Lorenzi descreveu florações para a espécie ocorrendo com maior intensidade nos meses de novembro a janeiro e frutificação predominante de maio a julho (Lorenzi, 1998). Um padrão muito semelhante ao observado por Lopes e Gonçalves (2006) em indivíduos da Floresta Ombrófila Mista, Floresta Ombrófila Densa e Campos sulinos (Campos + Estepe + Savana) do Rio Grande do Sul, apontando a época de floração para o período de novembro a fevereiro e frutificação de maio a julho. No estado do Paraná, Brotto, Cervi e Santos (2013) observou um período de floração de fevereiro a maio e setembro, frutificando de maio a janeiro e março. A espécie apresenta, potencialmente, papel crucial na sucessão ecológica necessária para regeneração principalmente nas florestas de araucárias, pois é produtora de grande quantidade de frutos, por vários meses de um mesmo ano (Carvalho et al., 2008). Frutos de *O. pulchella* constituem fonte de alimentos para uma ampla variedade de espécies de aves. O grande número de visitas sugere que seus frutos constituem um importante recurso alimentar para os dispersores de suas

sementes (Francisco; Galetti, 2002). A frugivoria é uma importante estratégia evolutiva de espécies de plantas, possibilitando a dispersão de seus descendentes e manutenção da sucessão florestal. A sobrevivência e perpetuação destas espécies, em cenários de alta ação antrópica, supressão de indivíduos produtores de frutos e fauna reduzida e ameaçada, torna-se altamente dependente de intervenção por meio de coleta e germinação de sementes os quais permitam a conservação e restabelecimento. Neste contexto, estudos que elucidem as características morfológicas de sementes, como estrutura do tegumento, embrião e endosperma desempenham importante papel na melhor compreensão destas sementes e sua dinâmica de germinação. Características morfofisiológicas vêm sendo intimamente ligadas às respostas à dessecação em sementes (Baskin; Baskin, 2014). Não obstante, estudos envolvendo a caracterização do processo de maturação em sementes, são essenciais para o sucesso de programas de conservação, propagação e utilização de espécies de plantas. Possibilitando conhecimento base para tomada de decisão em bancos de semente, armazenamento e utilização das sementes, contribuindo para a manutenção e restauração da biodiversidade.

Estima-se que 8% das atuais espécies de plantas tenham sementes sensíveis à dessecação, destas, 50% são importantes componentes de florestas tropicais (Wyse; Dickie, 2017). No Brasil, considerando estes dados de predição de ocorrência, estima-se que 52% das espécies da Mata Atlântica possuam sementes ditas recalcitrantes (Souza et al., 2015). Espécies de Lauráceas do gênero *Ocotea* apresentam baixo índice de germinação e tem sido classificadas como intolerantes à dessecação (Viana; Mantel, 1999; Carvalho et al., 2008; Dias et al., 2010; Pelegrini, 2013).

A germinação de sementes é o processo crucial de transformação que liberta todo o potencial de vida e diversidade genética de todas as plantas que investiram em sementes, como estratégia de propagação e perpetuação da espécie durante seu processo evolutivo (Donohue et al., 2010). Durante a germinação, atividades moleculares, hormonais e mecânicas, desenvolvem-se simultaneamente (Baskin; Baskin, 2014). Envolvendo a integração de sinais do ambiente, com este sistema complexo de comunicação entre células e compartimentos da semente (Holdsworth; Bentsink; Soppe, 2008). Embora muitos avanços no entendimento sobre esses mecanismos tenham sido alcançados nas últimas décadas, a variabilidade de especificidades entre espécies e ambientes, deixa ainda muitas perguntas a serem respondidas (Nonogaki, 2017).

Os processos envolvidos na aquisição da capacidade de manutenção da viabilidade em sementes, após longos períodos, é uma delas (Walters, 2015). Em 1970 com finalidade

majoritariamente agronômica, as sementes foram divididas em duas grandes categorias, com base na tolerância ou sensibilidade à dessecação e nas condições de armazenamento. Foram chamadas de ortodoxas todas as sementes que apresentavam algum limite de tolerância à dessecação, e que normalmente são dispersas pela planta mãe com baixo teor de água (aproximadamente $0,15 \text{ g H}_2\text{O/g MS}^{-1}$), podendo sobreviver à dessecação (Roberts, 1973). Foram consideradas sementes recalcitrantes, as que não apresentassem tolerância à dessecação, e que comumente são dispersas com alto teor de água (aproximadamente $0,40 \text{ g H}_2\text{O/g MS}^{-1}$), não sobrevivendo à secagem. Passaram-se quase duas décadas para que Ellis, Hong e Roberts (1990), estudando a dessecação e o armazenamento de quatro variedades de *Coffea arabica*, propusesse uma categoria intermediária, acomodando sementes capazes de sobreviver à limites de conteúdo de águas entre as duas categorias extremas, mas não tolerando o armazenamento em baixas temperaturas. A categoria intermediária inclui sementes que são capazes de tolerar maior perda de água que sementes sensíveis, mas não tão baixas quanto as sementes tolerantes, sementes que apresentam respostas anômalas a temperaturas entre -30 e 10° C e sementes que têm vida curta, independentemente de como são secas ou conservadas (Walters, 2015).

A tolerância à dessecação é comumente definida como a capacidade das sementes de sobrevivência em limites de água abaixo de $0,1 \text{ g H}_2\text{O/g MS}^{-1}$ com subsequente reidratação sem que ocorra acúmulo de dano letal (Alpert, 2005; Leprince; Buitink, 2010). A capacidade de sobreviver à dessecação é adquirida majoritariamente durante as fases de embriogênese e maturação das sementes (Chatelain et al., 2012). Na embriogênese, ocorre a formação e diferenciação da estrutura básica da planta, após a sua conclusão, o desenvolvimento das sementes normalmente progride para a fase de maturação. Em sementes ditas tolerantes a dessecação a maturação pode ser dividida em maturação precoce e tardia. Na maturação precoce, as sementes acumulam compostos de armazenamento como proteínas, lipídeos e carboidratos (Dekkers et al., 2015). Já na maturação tardia as sementes secam enquanto ocorrem mudanças consideráveis nos níveis de transcriptoma e metaboloma (Fait et al., 2006; Angelovici et al., 2010). Nesta fase ao menos quatro tipos de mecanismos estão envolvidos de maneira sinérgica para mitigar os possíveis efeitos deletérios da dessecação: (i) prevenção de danos oxidativos através do acúmulo de compostos antioxidantes; (ii) bloqueamento do stress estrutural através da modificação da parede celular, com a reorganização das membranas intracelulares e do citoesqueleto, e ainda, condensação da cromatina; (iii) estabilização de membranas e proteínas por açúcares não redutores, proteínas abundantes em embriogênese tardia (LEA- *Late Embryogenesis Abundant*) e proteínas de choque térmico (HSP- *Heat Shock*

Protein) e (iv) desintoxicação e reparação de DNA e proteínas danificados durante a reidratação (Hoekstra; Golovina; Buitink, 2010; Koshland; Tapia, 2019). Assim como o contexto em que se desenvolve, a caracterização da sensibilidade ou tolerância à dessecação da semente mostra-se dependente de respostas multivariadas com a determinação do seu conteúdo crítico de água para a sobrevivência bem como o tempo decorrido para atingir este valor (Tweddle et al., 2003). Desta forma o desenvolvimento de sementes sensíveis à dessecação, se dá de maneira distinta e pode estar ligada principalmente a aparente supressão dos mecanismos protetores mencionados acima (Farrant et al., 1993; Francini et al., 2006; Delahaie et al., 2013). No entanto, o limiar, entre o comportamento tolerante e intolerante à dessecação, ainda não foi completamente esclarecido (Trusiak; Plitta- Michalak; Michalak, 2023), e cada vez mais tem sido observado um provável comportamento espectral em diversas espécies, principalmente dos neotrópicos, ainda escassos na literatura (Lima et al., 2020). Neste sentido, a diversidade de métodos muitas vezes ineficazes ou insuficientes, e o viés técnico, usados para classificação do comportamento fisiológico de sementes, aumentam a probabilidade de classificações equivocadas nos ainda poucos estudos que objetivam classificar espécies tropicais. Na busca por parâmetros que auxiliem a classificação de sementes quanto a sua capacidade de dessecação, o conteúdo de água inicial, após desprendimento da planta mãe, tem sido amplamente utilizado (Wyse; Dickie, 2017). Características morfológicas como tamanho e espessura do tegumento tem sido apontadas como preditoras de comportamento de sementes em relação a dessecação. Dados biométricos podem auxiliar na tomada de decisões para planejamento de pesquisas, bem como são importantes fomentos para estruturação de data bases, podendo ser utilizados para pesquisas com correlação de traços (Nonogaki, 2017; Brink et al., 2023). Este cenário apresenta obstáculos significativos principalmente quando consideramos o desenvolvimento de tecnologias para conservação de plantas. Bancos de sementes são promessas promissoras para a possibilidade de conservar a diversidade genética de plantas mundial (Chapman; Miles; Trivedi, 2019). Porém, sementes recalcitrantes apresentam um desafio ainda maior para os pesquisadores (Barbedo; Centeno; Ribeiro, 2013). Sementes denominadas recalcitrantes não sobrevivem a dessecação e não podem ser armazenadas a longo prazo em bancos de germoplasma convencionais (Walters; Pence, 2021).

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2 OBJETIVOS

2.1 OBJETIVO GERAL

Estudar a qualidade, morfologia e comportamento fisiológico e resposta a dessecação de sementes de *O. pulchella*.

2.2 OBJETIVOS ESPECÍFICOS E QUESTÕES NORTEADORAS DA PESQUISA

Questão norteadora

- Sementes de *O. pulchella* estão disponíveis para estudo?
1. Identificar e localizar plantas matrizes em fase reprodutiva e com disponibilidade de sementes da espécie *O. pulchella*;

Questão norteadora

- Como é a dinâmica do conteúdo de água em sementes de *O. pulchella* durante seu desenvolvimento?
2. Coletar sementes de *O. pulchella* em diferentes estágios de desenvolvimento e quantificar seu conteúdo de água.

Questão norteadora

- Quais os atributos anatômicos de sementes de *O. pulchella* e quais suas possíveis influências na resposta à dessecação e germinação?
3. Avaliar Parâmetros morfoanatômicos de sementes de *O. pulchella*

Questão norteadora

- Sementes de *O. pulchella* apresentam restrições para germinação?
4. Avaliar a viabilidade, vigor e qualidade fisiológica de sementes de *O. pulchella*;

Questão norteadora

- As sementes de *O. pulchella* são sensíveis à dessecação e essa sensibilidade pode variar de um ano para outro?
5. Estudar o limite de tolerância à dessecação de sementes de *O. pulchella*, por meio de teste de germinação em diferentes conteúdos de água das sementes por dois anos;

Questão norteadora

- É possível armazenar sementes de *O. pulchella*? Por quanto tempo?

6. Observar a viabilidade de sementes armazenadas em temperaturas e tempos distintos.

Questão norteadora

- Qual o conteúdo e variedade de poliaminas endógenas presentes em sementes de *O. pulchella*?
7. Analisar a presença e quantidade de poliaminas em sementes de *O. pulchella*.

Questão norteadora

- Quais os carboidratos e sua abundância encontrados em sementes de *O. pulchella*?
8. Quantificar e identificar carboidratos em sementes de *O. pulchella*.

Questão norteadora

- Eixos embrionários de sementes de *O. pulchella* se adaptam ao cultivo *in vitro*?
9. Estudar protocolo para introdução de eixos embrionários de *O. pulchella in vitro*.

CAPÍTULO ÚNICO

Seed desiccation tolerance spectrum in tropical Lauraceae is broaden by *Ocoteapulchella*

ABSTRACT

Lauracea family trees are a major component of tropical and Neotropical forests. Species within this family have significant ecological and economical relevance for society all over the globe. Seeds within these families are believed to be sensitive to desiccation, adding a major challenge and concern on conservation of biodiversity and reforestation of these important species. However, there are still just a few studies to support that statement, and not rarely they present more technical and superficial data. *O. pulchella* is one of the members of this family found both in coastal areas and dense forests. That range of occurrence presents the potential of the species for model studies on morphophysiological characteristic and response to desiccation for Lauracea. Aiming a better comprehension of how maturation unfolds for these species, we investigated the water content through the development of seeds and fruits for 111 days. Analysis of seeds germinative response towards desiccation, survival of stored seeds, histological analysis, in vitro assays, Pas and carbohydrate profile characterization were also conducted. Water content of whole fruits increased over 68 days, followed by a decline until day 111. Seeds displayed increasing water content until 53 days, then decreased notably, reaching lowest levels at 0.59 and 0.69 gH₂O.gDW⁻¹. Mature seeds took 21 days to stabilize water loss, reaching 0.50 in the first 6 hours of drying. Germination and GSI, decreased along with water loss, 0,33 gH₂O.gDW⁻¹ presented a crucial water content threshold that serves as a quantifiable benchmark for assessing *O. pulchella* seeds viability as they undergo desiccation. Storage conditions were found to negatively influence the seeds' germination potential. Spermine was the most abundant PAs observed, followed by Putrescine and Spermidine which did not significantly differ in abundance. Arabinoses was the major soluble carbohydrate found with 5,22 mg/ g FW⁻¹, followed by mannoses and sucrose with 2,31 mg/ g FW⁻¹ and xyloses 2,20 mg/ g FW⁻¹. Our data suggest that *O. pulchella* might give rich insights into the variability of seed desiccation response and biochemical wide profile of the Lauracea family.

Keywords: seeds maturation; Lauracea; desiccation; conservation.

1 INTRODUCTION

Lauraceae species have significant economic and ecological relevance, especially in forests of tropical and subtropical regions (Lima et al., 2020). Many species of this family are already extensively exploited for their medicinal and food value (Farias et al., 2023) in the Atlantic Forest, one of the globe's foremost biodiversity hotspots (Myers et al., 2000; Zachos; Habel, 2011). Seed physiology and germination of Lauraceae species are poorly understood and in Atlantic Forest many species are already rare or threatened. *Ocotea marcescens* and *Ocotea odorata* are present at IUCN (2002) red list as endangered, *Ocotea catharinensis* and *Ocotea porosa*, are present at the list as vulnerable. The survival and perpetuation of these species, in scenarios of high anthropic action and suppression of fruit-producing individuals, becomes highly dependent on intervention through the collection and germination of seeds, which allow conservation and restoration. However, few rare studies have dedicated to study the particularities of Lauraceae family seeds. Although *O. pulchella* is present at the same list as species of less concern, the specie has the potential to be a model case for study's concerning the same genera and family.

Seed physiological maturation is pivotal for germination and plant development. However, much remains unknown about seed longevity in biodiverse species (Daws et al., 2007; Pritchard, 2020), which is essential for preserving plant species and maintaining biodiversity. Seed longevity is influenced by factors like desiccation tolerance, crucial for effective seed conservation and ecosystem restoration.

Traditionally, seeds were categorized as 'recalcitrant' or 'orthodox' based on their desiccation and storage capabilities (Roberts, 1973). But posterior findings have identified 'intermediate' seeds that display characteristics of both categories (Ellis; Hong; Roberts, 1990). While these categories aid in general storage, they might not accurately reflect seed behavior (Barbedo, 2018). Comprehensive knowledge about seed desiccation responses is imperative for plant conservation and developing proper seed storage methods (Nadarajan et al., 2023). However, gaps in understanding the physiological and biochemical aspects still exist (Nonogaki, 2017; Pritchard et al., 2004; Vicré; Farrant; Driouich, 2004).

Biodiverse ecosystems often house seeds with high water content (WC), which display unique physiological traits. Notably, tropical species, which disperse in high humidity regions, tend to produce high WC seeds (Rodrigues et al., 2022). These seeds are more susceptible to deterioration during storage due to their metabolic activity (Pritchard, 2020), rendering long-

term conservation challenging. Research has consistently shown that such seeds face reduced germination and vigor post-desiccation (Viana et al., 2020; Rodrigues et al., 2022; Vieira et al., 2022).

Water is crucial for many biochemical processes within seeds. Seeds with high WC maintain metabolic activities and aid enzymatic reactions (Vicré; Farrant; Driouich, 2004; Umarani; Aadhavan; Faisal, 2015). However, elevated water content also makes seeds vulnerable to desiccation stress, with adverse impacts on membrane stability, enzyme activity, and protein integrity (Farrant et al., 2015). Issues arise due to excessive reactive oxygen species (ROS) in seeds, highlighting the importance of antioxidants in maintaining seed viability (Nishizawa; Yabuta; Shigeoka, 2008). Recent studies have also noted the conversion of hexoses into specific sugars (Obroucheva; Sinkevich; Lityagina, 2016) and the accumulation of certain proteins, suggesting these as defensive strategies against desiccation (Azarkovich, 2020; Ramtekey et al., 2022).

Storage is essential for preserving seed viability until optimal germination conditions are met (Walters; Pence, 2021). Modern techniques, such as refrigeration and freezing, effectively slow metabolic activities, thereby prolonging seed life (Nadarajan et al., 2023). While these methods are widely employed in seed banks and agriculture, high WC seeds pose challenges, particularly when exposed to freezing. The balance between cellular composition and water properties becomes critical. Excessive moisture can also promote microbial growth (Berjak; Pammenter, 2001), further complicating storage. Ultimately, understanding the limitations of desiccation tolerance in high WC seeds is crucial for enhancing their long-term storage potential (Ballesteros; Pritchard; Walters, 2020). Consequently, it becomes crucial to develop tailored conservation strategies that account for the unique physiological response of high-water content seeds to desiccation stress and address the challenges associated with their storage. This trade-off underscores the importance of efficient storage methods to preserve the viability of high-water content seeds, considering their limited desiccation tolerance (Walters; Pence, 2021).

As representatives of the biodiversity within the Atlantic Forest, seeds of *O. pulchella* might provide critical insights into preserving and rehabilitating this ecosystem. Given the evolving global environmental challenges, it is essential to understand the distinct physiological and biochemical reactions of high-water content seeds to desiccation stresses. In this context, our study focuses on a comprehensive assessment of *O. pulchella* fruit and seed maturation processes. Additionally, we aim to understand the seeds' desiccation responses and analyze their

metabolic profile. The objective is not just to enhance the existing knowledge on Lauraceae species but also to inform and refine possible conservation and restoration strategies for such important species.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

Fruits of *O. pulchella* were collected from a conservation unit at Florianópolis in Santa Catarina state, Brazil (-27.727795 South and -48.509101 West), directly from 15 matrix trees from December 2021 to February 2023. Seeds were individualized from the fruit by manually removing pericarp and then gently paper cleaned.

2.2 FRUIT AND SEEDS DEVELOPMENT BIOMETRY

The replicates of twenty fresh fruits and seeds were used for biometry measurements (length, weight) using caliper and precision scale (Mettler, Florianópolis, Brazil). Fruits and seeds development were categorized by size and color. Images were obtained with digital camera (12MP) and by stereomicroscope (Olympus® - SZH10, Tokyo, Japan) equipped with image capture system (Olympus® - DP71) and Controller software.

2.3 FRUITS AND SEEDS WATER CONTENT AND DESICCATION

Water content (WC) was evaluated for fruits and seeds. After the measurement of the initial fresh weight (FW), samples were kept in the oven at 105 ± 2 °C during 24 h (Brazil, 2013) and weighed again using precision scale (Hong; Ellis, 1996; Black; Hugh, 2002).

Seed desiccation curve was performed using ten randomly sampled from different fruits, fresh seeds. Fresh seeds were weighted, and then accommodated on grids in a hermetically sealed plastic box with silica gel in a ratio of 2:1, at a temperature of 21 ± 3 °C (Zhang et al., 2015). Seeds were wheightened every 15 min for the first 30 min, every hour for the first 12 h; every 12 hours for the next 5 days and then every 24 hours for 11 days. Desiccation tolerance experiments were performed in two different years using six WC (Year 1: 0.58, 0.50, 0.38, 0.32,

0.22 and 0.12 gH₂O.gDW⁻¹ and Year 2: 0.6, 0.54, 0.39, 0.31, 0.26 and 0.18 gH₂O.gDW⁻¹). Desiccated seeds viability was evaluated by the germination and tetrazolium test.

2.4 SEED GERMINATION

For germination tests, seeds were disinfected with 70% ethanol for one minute, followed by hypochlorite solution (2%, v/v) for 10 minutes and then rinsed three times with sterilized distilled water. Seeds were accommodated onto petri dishes with filter paper moistened with 2.5 g of sterilized distilled water per gram of paper, and kept in biochemical oxygen demand germination chamber (BOD) (Eletrolab©, Sao Paulo, Brazil) at 27 ± 1 °C and 12h photoperiod (Barbedo et al., 2002; Pires et al., 2009). Seeds were considered germinated when showing 2 mm of radicle protrusion and were daily evaluated for 60 days. Data was expressed in germination rate (%) and germination speed index (GSI) (Maguire, 1962). GSI was calculated as the sum of the ratio between the number of germinated seeds per day and the respective day of observation: $GSI = G1/N1 + G2/N2... + Gn/Nn$, where G is the number of germinated seeds on the day of observation, and N, the number of days elapsed since sowing (Maguire, 1962). Seedling morphometry was evaluated at the end of experiments (60 days) using a calypter.

2.5 LIGHT MICROSCOPY

Seeds were processed for light microscopy (LM) analysis following Steiner et al. (2015) with some adjustments. The samples were first fixed in a solution of paraformaldehyde (2.5%) and phosphate buffer (0.1 M) at 8°C for 7 days. Then, they were dehydrated in a series of ethanol and infiltrated with Histo-resin (Leica® Histo-resin, Heidelberg, Germany) under vacuum for 31 days. Semi-thin sections (4 µm) were cut with a hand rotary microtome (Leica RM 2145, Florianopolis, Brazil). Samples were stained with toluidine blue (TB-O) to visualize acid polysaccharides and phenolic compounds (O'Brien et al., 1964). Additionally, Coomassie Brilliant Blue (CBB) were applied to detect proteins (Ventrella et al., 2013) and neutral polysaccharides (O'Brien; McCully, 1981), respectively. The sections were examined using an Olympus BX 41 microscope with the Olympus DP 40 image capture system and Q-capture pro Software® (version 5.1).

2.6 TETRAZOLIUM TEST

Three replicates of 15 seeds were manually sliced in two pieces, then the cotyledons and embryo axis were immersed in 1% 2,3,5-triphenyl tetrazolium chloride (TTC) solution (1%, v/v), followed by incubation in darkness at 30 °C for two hours (Brasil, 2013). The embryos were classified as viable or unviable by staining intensity, according to (Brasil, 2013).

2.7 SEED STORAGE ASSAY

Seeds were stored at three different temperatures (8 ± 2 °C, -18 ± 1 °C and -196 ± 4 °C). A total of twelve replicates of 15 fresh fruits in hermetically sealed plastic bags were stored during 0, 30, 40 e 50 days at 8 °C (3 reps/times). Twenty replicates of seeds were wrapped in aluminum foil and in hermetically sealed plastic bags and then storage for a week at -18° C and -180° C. Seeds viability was evaluated by tetrazolium tests.

2.8 *IN VITRO* ASSAY

Seeds from mature fruits were previously washed with water and commercial detergent (2%, v/v) for 5 minutes and rinsed three times with distilled water. Clean seeds were disinfected with ethanol (70% v/v) for 1 minute, followed by immersion in hypochlorite solution (2%, v/v) for 5 minutes and then rinsed three times with sterilized distilled water. In the laminar airflow cabinet, tip of seeds (0.4 cm) including embryo axis and partial cotyledons were placed in Woody Plant Medium (WPM) ($2,41 \text{ g L}^{-1}$) supplemented with 6-benzilaminopurin BAP ($4.0 \mu\text{M L}^{-1}$), activated charcoal 2.0 g L^{-1} and phytagel 2.0 g L^{-1} according to Santa-Catarina et al. (2006) with modifications. After inoculation, petri dishes were kept in a germination chamber (BOD) at 27 ± 2 °C and 12 hours photoperiod ($43 \mu\text{mol m}^{-2} \text{ s}^{-1}$) for 60 days. Seedling development, root and shoot length were evaluated from 4 replicates of 4 tip seeds.

2.9 POLYAMINE ANALYSIS

Polyamines (PAs) quantification and qualification were performed using three biological replicates of 200 mg dry mass (DM) from a mix of 5 mature seeds. Samples were grounded using 1,5 mL of 5% (v/v) perchloric acid. The free PAs were extracted, dansylated

and quantified as described in Steiner et al. (2007). The HPLC (Shimadzu, Nakagyo-Ku, Kyoto, Japan) examination was carried out on the supernatant using a 5 μm C18 reverse-phase column (Shimadzu Shin-pack CLC ODS). The gradient of absolute acetonitrile was set to 65% for the first 11 minutes, 65–100% from 11 to 25 min, 100% from 25 to 35 min, then adjusted to 65% from 35 to 44 min, with flow rate of 1.0 mL min⁻¹ at 40 °C. The determination of PAs was made using a fluorescence detector at 340 nm (excitation) and 510 nm (emission). The peak areas and retention times were compared for the standard PAs, putrescine (Put), cadaverine (Cad), spermidine (Spd) and spermine (Spm). The final concentration of PAs was expressed in mmol mg⁻¹ DW. PAs ratio was calculated as: (PUT/SPD+SPM). The internal standard 1,7-diaminoheptane (DAH) was used. Statistical analyses were performed using the R software, version 4.2.2 143 (Missouri, USA), and $P < 0.05$ ($\alpha = 0.05$) was statistically considered significant (R Core Team, 2023).

2.10 CARBOHYDRATE ANALYSIS

Soluble carbohydrates were determined as described in (Aragão et al., 2016) using three biological replicates of 300 mg lyophilized mass. Fresh seeds samples were lyophilized and 300 mg DW was macerated in extraction solution containing 80 % ethanol (Merck® 22), 3 % polyvinylpolypyrrolidone (Sigma-Aldrich®) and 1 % ascorbic acid (Sigma-Aldrich®) at 4 °C. Extracts were incubated for 90 min at 70 °C. Followed by centrifugation at 20.000×g for 10 min, supernatant was collected, and pellets were re-extracted. Soluble carbohydrates were separated by HPLC (Shimadzu, Kyoto, Japan) using a Prevail Carbohydrate ES (Alltech Associates, Deerfield, IL, USA) column (5 μm - 250 × 4.6 mm) and a pre-column (5 μm - 7.5 × 4.6 mm). Gradient was achieved by decreasing proportions of absolute acetonitrile (Merck® 28) with water. Then acetonitrile gradient was maintained at 80 % during the first 16 min, 80–70 % between 16 and 23 min and 70 % from 23 to 30 min, with flow rate of 1 mL min⁻¹ at 25 °C. Carbohydrates were detected using an evaporative light scattering detector ELSD-LT II (Shimadzu) at 40 °C with nitrogen gas pressure maintained at 350 kPa. Soluble carbohydrates were identified and quantified by comparisons of retention times and peak areas (>1%) with known quantities of carbohydrate standards for arabinose, fructose, glucose, maltose, and sucrose (Sigma-Aldrich® 33).

2.11 STATISTICAL ANALYSES

Experiments were carried out in a completely randomized design. Data homoscedasticity was verified by Bartlett test and normality by Shapiro-Wilk test. Statistical analyses were performed from data submission to analysis of variance (ANOVA), and the means were compared by Tukey's test ($p \leq 0.05$). For PAs data means were compared using Scott-Knott test at 5% significance for qualitative factors, and polynomial regression analysis for quantitative ones. Statistical software R was employed for analysis (R Core Team, 2019).

3 RESULTS AND DISCUSSION

3.1 MORPHOMETRY OF FRUIT AND SEED DEVELOPMENT

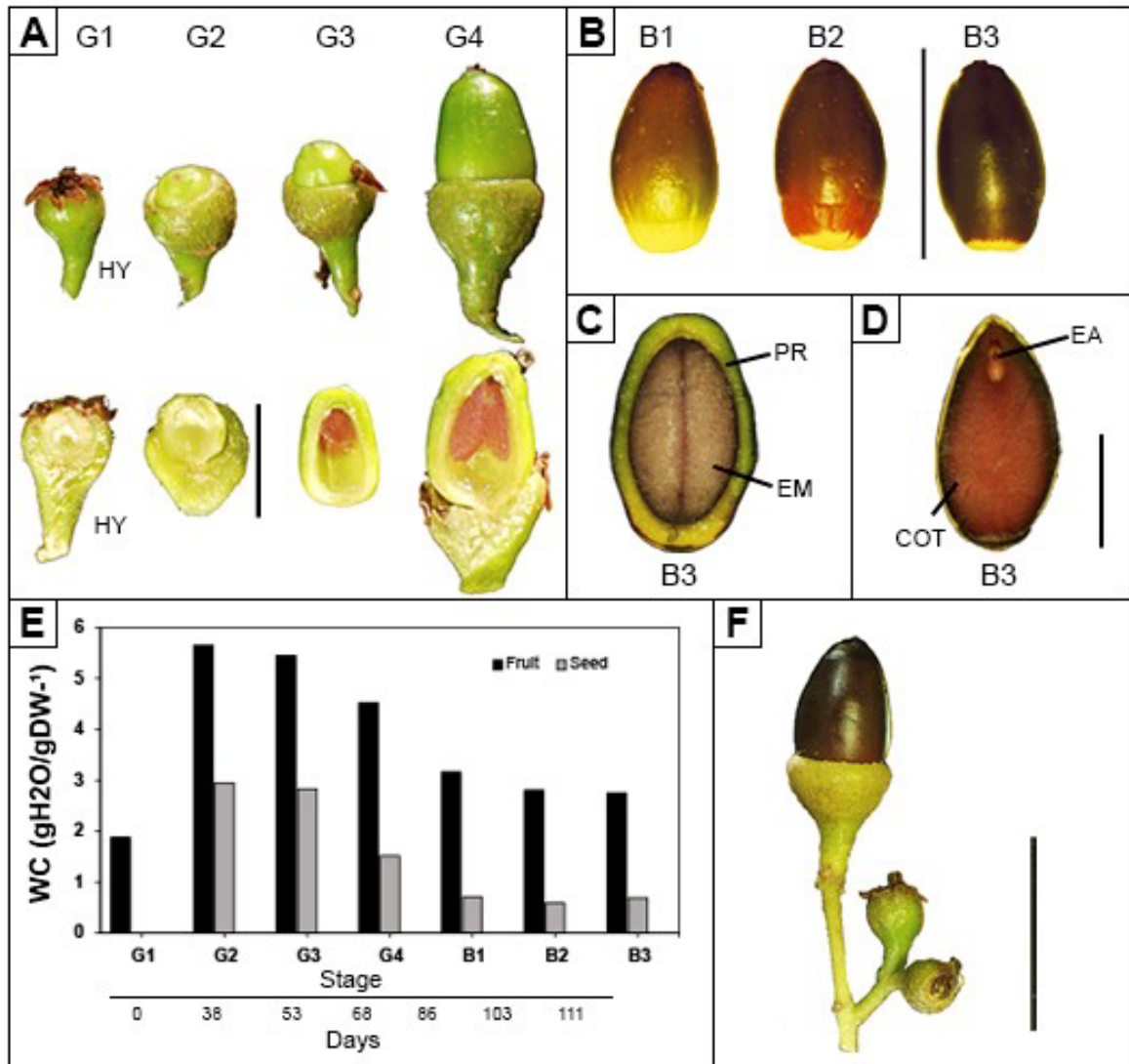
O. pulchella fruits are green and visible at the tree at stage G1 (1 to 2 mm) taking 68 days to grow to G4 (7-8 mm) (Fig. 1 A, B, Table 1). Fruit maturation stages B1 to B3 (8-10mm) occurs at 86 to 111 days, respectively and, do not increase size, but change exocarp color from green to dark brown (Fig. 1 B). Longitudinal section (LS) shows the presence of endosperm at G1 and G2 fruit stage, globular embryo at G3 (5 to 7 mm) and heart embryo at G4 stage (Fig. 1A). Pericarp and cotyledonary embryos were identified in fruit stage B3 when they are shed from the tree (Fig. 1 C). *O. pulchella* seed hold two cotyledons, as the major embryo portion, and a small embryonal axis (Fig. 1 C e D). The seed and embryonal axis fresh weigh was on average 0,07 grams and 0,04 milligrams respectively, and this means the axis represent only 0,06% of the seed. Fruit development was asynchronous as more than one stage was observed at the same time in all the trees (Fig 1 E).

A better understanding of the fruit and seed development can be achieved by WC dynamic which has been associated with seed maturation and physiological behaviour (Bareke, 2018). Seeds with heart stage embryos of *O. ira* (Heo; Van der Werff; Tobe, 1998) and *O. catharinensis* seeds (Santa-Catarina et al., 2006) has been reported, but no WC or sequential development were study in tropical Lauraceae. Seed development involves a sequence of changes in structures, biochemistry, and WC within the fertilized egg until it reaches the physiological maturity (Jimenez-Lopez et al., 2022). During early seed development, cell division and expansion occurs, and the presence of water enable metabolic activity, supporting the seed growth (Lee et al., 2023). Orthodox seeds acquire the ability to survive desiccation

during the end of the embryogenic program (Leprince et al., 2017). Typically they are characterized by maturation drying and accumulation of late embryogenesis protein (LEA), thermostable heat shock (HSPs) and prion-like protein named FLOE1 before they are shed (Dorone et al., 2021; Sripathy; Groot, 2023). Lauraceae seeds have been classified as desiccation-sensitive based on taxonomic relatedness, for sampling bias and estimate the proportion of seed plants with desiccation-sensitive seeds (Wyse; Dickie, 2017). Additionally, previous studies report *Ocotea* genus seeds as recalcitrant, based mainly on storage response and WC shedding time (Carvalho; Carvalho; Davide, 2009; Pires et al., 2009).

O. pulchella seeds show a desiccation stage before shed (B3- 0.69 gH₂O.gDW⁻¹) which might have implications in desiccation tolerance which was not observed before in tropical Lauraceae species.

Figure 1 – Morphology and water content (WC) *O. pulchella* fruits development. Whole fruit development and longitudinal section (LS) of stages denominated G1, G2, G3, G4 (hy hypanthium) (A); Fruits development and maturation at stages, B1, B2, B3; note the color change at the different stages (B); Fruit LS at stage B3 indicating (pr) pericarp and (e) embryo (C); and stage B3 indicating (ea) embrional axis; (cot) cotyledon (D); WC during whole fruit development (0-111days) and individual structures (fruit and seed) (E); Detail of a branch indicate asynchronous fruits development (F).



*Scale bars: a, c 5 mm; b, e 1 cm
Source: Elaborated by the author (2024).

Table 1 – Timeline characteristics of *O. pulchella* fruit and seed development. Days of observation and stages were nominated by combination of color and size of fruit. N =15 plants

<i>Ocotea pulchella</i> fruit/seed development							
Fruit/seed stage	Green				Light brown	Brown	Dark brown
	G1	G2	G3	G4	B1	B2	B3
Days after fruit was visible	0	38	53	68	86	103	111
Fruit WC (g)	1,88 ± 0,18	5,65 ± 0,89	5,45 ± 0,73	4,52 ± 1,24	3,16 ± 0,49	2,81 ± 0,18	2,75 ± 0,63
Seed WC (g)	-	2,95 ± 0,7	2,83 ± 0,49	1,52 ± 0,7	0,71 ± 0,08	0,59 ± 0,02	0,69 ± 0,07
Fruit size (mm)	1-2	3-4	5-7	7-8	8-10	8-10	8-10
Embryo stage	nv	nv	globular	heart	Cot	Cot	Cot

*nv – not visible; Cot – cotyledonary; FW – fresh weight.

The seed WC at dispersion moment from mother plant, is one first characteristic to be considered in regards to studying seeds desiccation response, but it is not sufficient to predict the overall outcome (Ievinsh, 2023). Seed size play a crucial role in desiccation responses since directly affects the surface area-to-volume ratio of a seed (Pritchard et al., 2004). Smaller seeds generally have a higher surface area-to-volume ratio compared to larger seeds. This higher ratio means that smaller seeds have a relatively larger surface area exposed to the environment. Consequently, smaller seeds tend to lose water more rapidly through evaporation, making them more susceptible to desiccation, but not necessarily desiccation tolerant. It has been reported that the proportion of the embryonic axis within the seed may affect its desiccation tolerance (Xia et al., 2014). In our study the embryonic axis is relatively small, compared to the overall seed size (Figure 1D; Table 1) and positioned near the micropyle side as has been observed in numerous Lauraceae species (Heo; Van der Werff; Tobe, 1998). Also, the Lauraceae family is known by the variety of fruit sizes and weights and *Persea americana* (305 g) might be the heaviest example compared to other members of this family (Kaiser; Wolstenholme, 1994). Considering the data for the Lauracea family until now, *O. pulchella* has small fruits and seeds (Anexo A – Table S1).

3.2 MORPHOHISTOLOGY OF SEED STRUCTURES

O. pulchella mature seeds have the multicellular tegument structure covering an embryo. Like in most Lauraceae, *O. pulchella*, drupes fruits hold a seed covered by a pericarp compost of an exocarp, mesocarp and endocarp. Anatomical analysis indicates three distinct tissues, the outermost region shows the presence of a cuticle (Ct) followed by the first tissue layer of macrosclereids cells (Ma), then a one-layered cell middle tissue over multi sized cells with thickened lignified walls (Fig. 2 A). The inner tissue contains a vascular region with irregular small fattened thin-walled cells. The spaces in macrosclereids cells (Fig. 2 B) can indicate breakage of the structure, suggesting permeability of the tegument in this species (Acosta Fernández et al., 2023). Tegument affects the dynamics of water loss and enhances the complexities of determining desiccation tolerance especially, but not exclusively, in the Lauracea family (Jaganathan et al., 2019). It acts as a modulator of seed-environment relationships including safeguarding the embryo integrity, insects and pathogens attacks and regulation gaseous and water exchange (Daws et al., 2007). In terms of desiccation dynamics, it plays a crucial role as a barrier against water loss and absorption, affecting the time and rate

of the embryo water content as well germination (Jaganathan et al., 2019). A permeable tegument allows water absorption which promote a physiological signal cascade in the embryos towards to germination (Ma et al., 2004; Munz et al., 2017). *O. pulchella* seed tegument could be classified as permeable but play a barrier role in seed desiccation dynamic as we further discuss in this study.

O. pulchella embryos are surrounded by a protoderm of small rectangular cell with small nucleus (Fig. 2C). Cotyledons cells are sizes variable, vacuolated with a peripheral nucleus and small cells with a dense cytoplasm and, some fully differentiated as vascular cells (Fig. 2C). Proteins bodies and the presence of phenolic compounds were indicated by positive reaction to CBB and TBO, respectively (Fig. 2C, D). Embryonal axis (EA) is composed by the plumule (2 primary structures), shoot apical meristem (SAM), hypocotyl and root apical meristem (RAM) (Fig 2 E, F). Embryo RAM is surrounded by abundant cells that stain green by the metachromatic reaction to TBO (Fig. 2 G, E) indicating the presence of polyphenols (O'Brien et al., 1964; Rogge-Renner et al., 2013). *O. pulchella* seed anatomical characteristics observed in this study (Fig. 2), agree with those observed in other Lauraceae seeds species (Sastri, 1958; Heo; Van der Werff; Tobe, 1998). The presence of membrane-bound organelles such as vacuoles play roles in storage, waste management, and maintenance of turgor pressure (Jiang et al., 2021). Peripheric cell nucleus could be related to maximizing available cytoplasmic space, especially for storage functions (Ingram, 2010). The presence of phenolic compounds here observed, might be related to protecting the seed from environmental stressors, but also related internal oxidative stress (Corso et al., 2020). Phenolic compounds might help protect cellular components from damage caused by reactive oxygen species generated during embryo desiccation and rehydration processes (Mierziak; Kostyn; Kulma, 2014). They could also play a role in maintaining cell membrane integrity and stability during germination (Azimian; Roshandel, 2015). The presence of phenolic compounds in the cotyledonary region of seeds could be a part of the seed's overall defense and survival strategy.

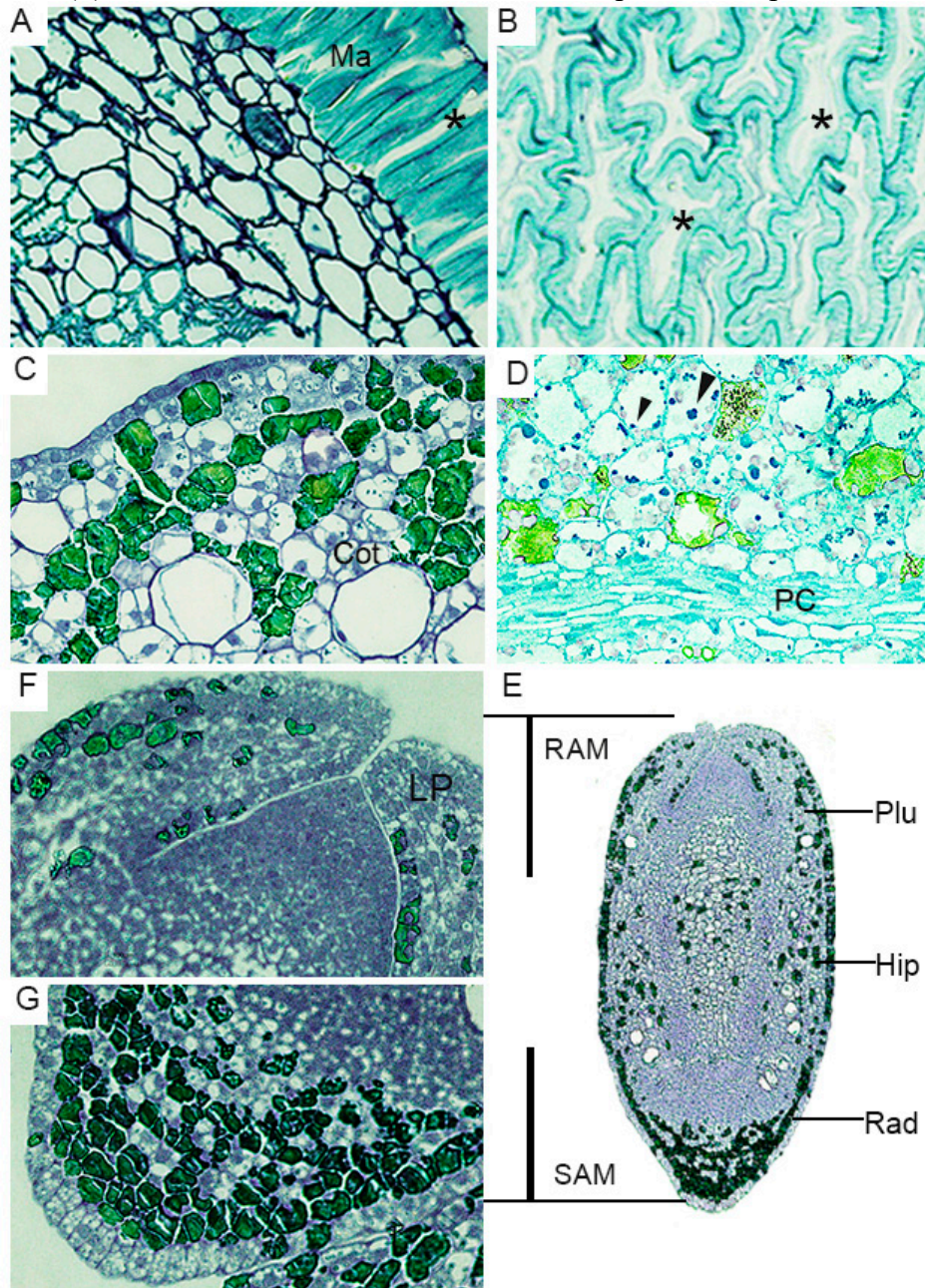
Another critical aspect of seeds anatomical implications to desiccation, is intricately linked to the activity of their two essential meristems. SAM and RAM play pivotal roles in determining the post-germination fate of the plant (Murray et al., 2012; Yamoune et al., 2021). Recent studies have highlighted the involvement of specific genes and molecular signaling pathways, such as those related to auxins and cytokinins, in preserving SAM integrity during drying conditions (Li et al., 2018; Jogawat et al., 2021).

These findings underscore the SAM's role in buffering against the potential loss of meristematic activity due to desiccation.

Contrasting the SAM, the RAM is located at the tip of the root and is responsible for root growth and development (Fujinami; Toshihiro; Ryoko, 2020). When it comes to seed desiccation, the response of RAM is equally crucial. While root growth might halt during desiccation, the RAM's structural and molecular characteristics determine the seed's capacity to re-establish root growth after germination (Kka; Rookes; Cahill, 2018). Studies have illuminated the role of specific transcription factors and hormone signaling pathways, like abscisic acid (ABA), in preserving RAM integrity during dehydration, thus ensuring successful post-germination root development (Godwin; Farrona, 2020). The dynamic interplay between SAM and RAM during seed desiccation plays a significant role in the plant's capacity to rebound after rehydration during germination (Wolny; Braszewska-Zalewska; Hasterok, 2014). Although seed morpho-anatomical features and WC are interesting and complementary proxies, relying solely on common functional traits may not fully elucidate intricate physiological processes within a species (Daws; Garwood; Pritchard, 2005). It is necessary to supplement these traits with other physiologically relevant measurements to obtain a more comprehensive understanding of seed physiology (Hill; Edwards; Franks, 2012). When we consider desiccation tolerance or sensitivity in seeds, this necessity becomes especially relevant.

Figure 2 – Light microscopy analyses of *O. pulchella* seed stained with Toluidine Blue-O (TB-O). Tegument transversal section (TS)/ tegument showing cuticle over three tissue layers: macrosclereid outermost layer, one-layered cell middle tissue over multi sized cells layer (A).

Details of breakage macrosclerids tissue (B). Cotyledonary region shows vacuolated cells with peripheral nucleus and small dense cytoplasm cells with nucleus, as well the presence of phenolic compounds indicated by TB-O reaction (C). Detail of cotyledons cells with presence of storage proteins bodies (arrows) reactive to Coomassie Brilliant Blue (CBB) (D). Whole embryonal axis structure stained with TB-O showing plumule, shoot apical meristem (SAM), hypocotyl region and root apical meristem (RAM) (E). Detail of SAM region covered by the plumule (F). Details of RAM indicate the abundant presence of phenolic compounds (G)



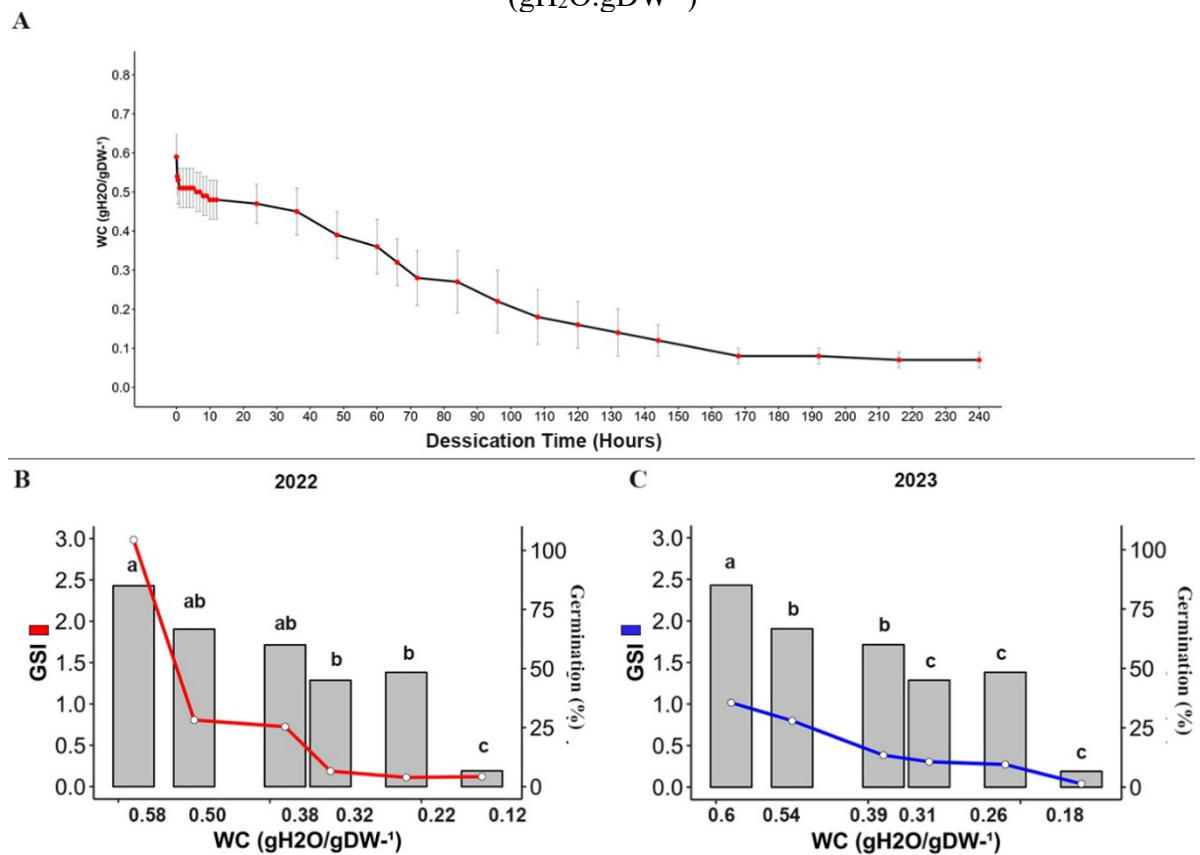
Ma macrosclerids, *pc* procambium, *cot* cotyledons, *plu* plumule, *hyp* hypocotyl, *rad* radicle, Scale: A, B 2.5 mm; C 50 μ m; E 1000 μ m; D, F, G 4000 μ m.

3.3 SEED GERMINATION UNDER DESICCATION

O. pulchella fresh seeds WC was $0.69 \text{ gH}_2\text{O.gDW}^{-1}$ and took around 180 hours of desiccation to reach the stable value of WC $0.12 \text{ gH}_2\text{O.gDW}^{-1}$ (Fig 3 A). The first major drop in seed WC occurred within the first 6 hours with $0.50 \text{ gH}_2\text{O.gDW}^{-1}$ (Fig. 3 A, Supl Tab. 1) and this might be related to the loosening of the tegument. After 20 hours, the WC was $0.47 \text{ gH}_2\text{O.gDW}^{-1}$ a more gradual reduction started and after 66 hours seeds WC reached $0.32 \text{ gH}_2\text{O.gDW}^{-1}$ (year I) and $0.31 \text{ gH}_2\text{O.gDW}^{-1}$ (year II). Fresh seeds show 85% and 93,33 % of germination, in the year I and II, respectively (Fig. 3B, D). It was observed a systematically seed germination decrease as the seed WC dropped in the two years experiments. The seed critical WC was $0.33 \text{ gH}_2\text{O.gDW}^{-1}$, when the germination was less than 50% and this took more than 66 h of desiccation (Fig 1. A, B). At 144 h when seed WC was 0.12 (year I) and 0.18 (year II) $\text{gH}_2\text{O.gDW}^{-1}$, when germination was 6,67% and 15,56 %, respectively (Fig. 3B, D). The loss of more than 50% of initial WC ($0.31 \text{ (year II) gH}_2\text{O.gDW}^{-1}$) significantly impacted the germination (33%) and was accompanied by a decrease in seed vigor with an GSI value of (0,19) (Fig. 3C, E). The seed germination was affected by desiccation, but the data was not affected by the year of the seed collection and experiments according to the *t* test (Supplementary table). The WC of $0.33 \text{ gH}_2\text{O.gDW}^{-1}$ at which germination rates dropped below 50% serves as an important threshold for determining *O. pulchella* seed viability during desiccation. Similar critical seed WC have been reported in tropical Myrtaceae and Clusiaceae species which have been classified as desiccation sensitive (Viana et al., 2020; Rodrigues et al., 2022; Vieira et al., 2022). The classification between desiccation tolerant and sensitive is based in a WC window of $0.05 \text{ gH}_2\text{O.gDW}^{-1}$ to $0.25 \text{ gH}_2\text{O.gDW}^{-1}$ (Walters, 2015). For Lauraceae species, most studies on seeds germination response use water percentage as mass-based measures for tissue water content. Water percentage is an imprecise measure of WC in seeds since do not shows a linear expression of water content in tissues, because fresh weight appears in both the numerator term and the denominator term of the equation. The critical WC observed by Carvalho (2008) for *Ocotea corimbosa*, was 11,6 % of moisture and for *O. pulchella* 11,6 %. Based on Hong and Elis (1996) methodology for seeds classification, the species were classified as recalcitrant. More than two decades passed, until Wyse and Dickie (2017), estimated that 50% of the seeds of tropical species are recalcitrant. Although, this has been largely confirmed in Lauraceae family and *O. pulchella* seeds, this study pushes the desiccation spectrum value towards to intermediatry classification. Intermediary seeds have been

described as the ones that die when WC is below $0,15 \text{ gH}_2\text{O.gDW}^{-1}$ (Walters, 2015). Overall, it worth to mention that although *O. pulchella* seeds shows an intermediary critical WC, this opens a new spectrum of WC seed data to work with Lauraceae seed storage and species conservation.

Figure 3 – Desiccation effects on *O. pulchella* seeds germination. (A) Water content (WC) ($\text{gH}_2\text{O.gDW}^{-1}$) of seeds over desiccation time (hours). (B) Germination speed index (GSI) over germination rate (%) for different WC in the year I. WC observed: 0.58, 0.5, 0.38, 0.32, 0.22 and 0.12 ($\text{gH}_2\text{O.gDW}^{-1}$) (C) Germination speed index (GSI) over germination rate (%) for different WC in the year II. WC observed: 0.6, 0.54, 0.39, 0.31, 0.26 and 0.18 ($\text{gH}_2\text{O.gDW}^{-1}$)

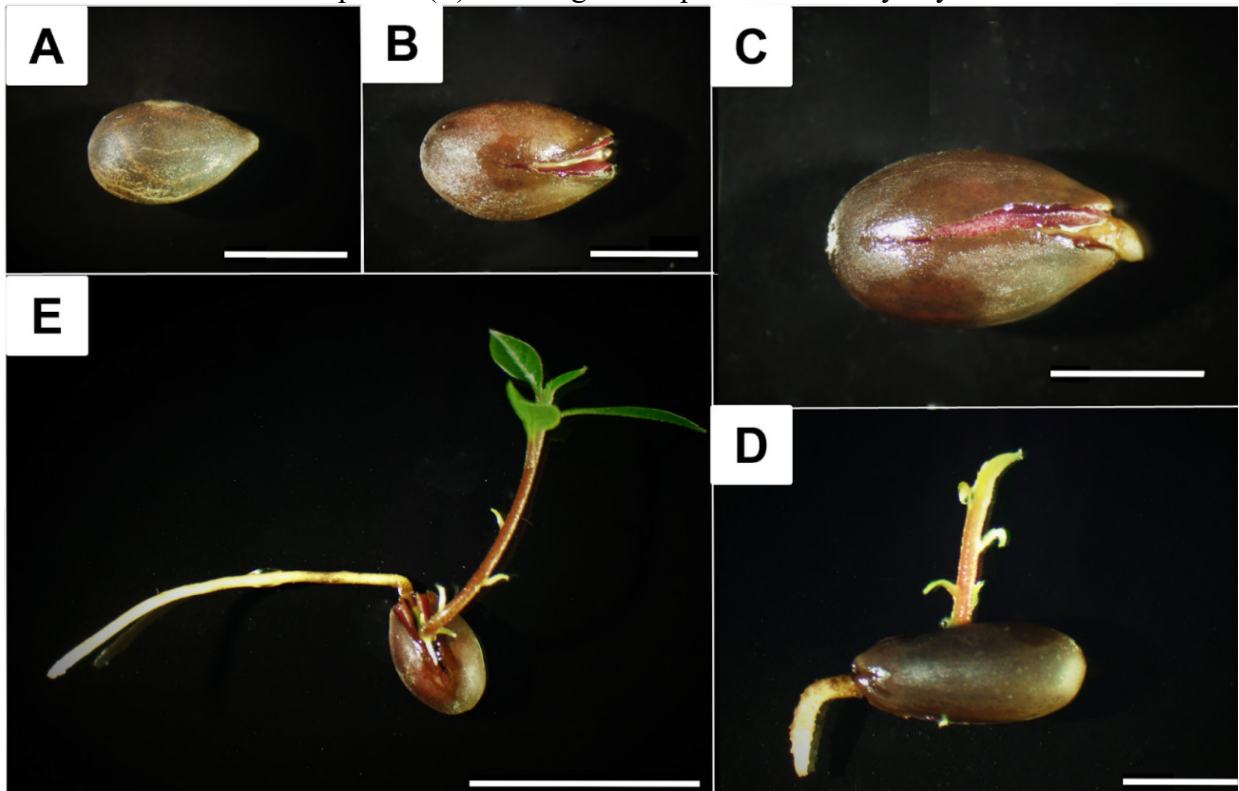


* Germination was assessed for 8 weeks. Values are means of replicates. Different letters represent a significant difference by the Tukey test at 5% probability.

Fresh *O. pulchella* seeds germination revealed a consistent pattern of tegument breakage in the first hours after harvest and subsequent exposure of the delicate embryo structures. Cellular elongation and division appear to occur mainly at the embryonal axis (EA) region. This generates an internal pressure within the seed and force the gradual disruption of the tegument, and after 7 days allows radicle protrusion as well the further embryo development (Fig. 4 A-C). Seed germination was followed by elongation of root, development of embryonic axis, and

apical shoot development (Fig. 4 D). After sixty days the seedlings with developed root and shoot were observed (Fig. 4 E). This data gives a precise and interesting timeline *O. pulchella* seed germination. This is appropriate standard protocol for been use in seed viability test as well in seedling production system of Lauracee species.

Figure 4 – Morphology of *O. pulchella* seed germination. (A) Fresh seed, (B) Tegument rupture, (C) radicle protrusion through tegument, (D) Seed germination with shoot and root development (E) Seedling development after sixty days



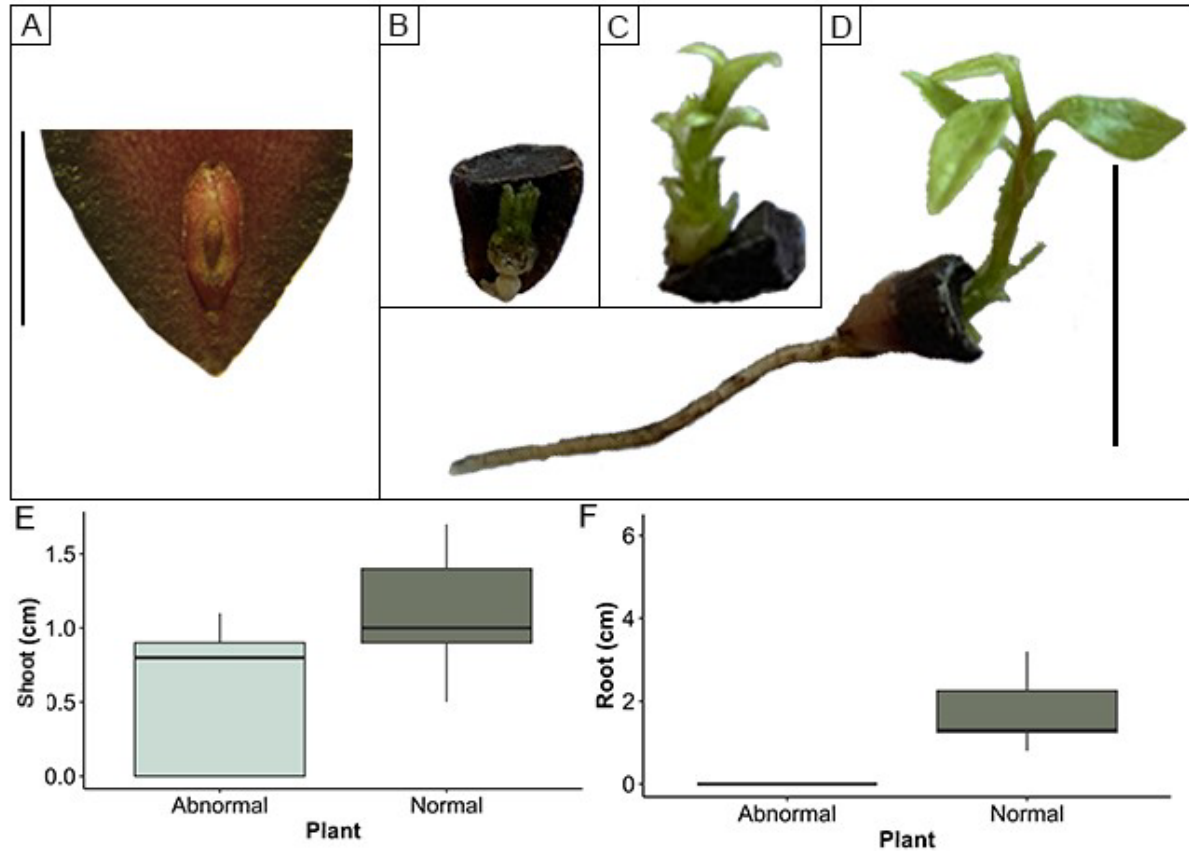
* Scale bars: A, B, D, E 5 mm; e C 1cm.

3.4 IN VITRO ASSAY

Seeds species that are desiccation intermediary or sensitive pose significant challenges for ex situ germplasm conservation. *O. pulchella* seed tips with embryo axis were successfully in vitro germinated with 89% of seedlings surviving (Fig. 6 A, D). Explants were considered germinated when the radicle had 2 mm of development (Fig. 6 B). Germination development was observed after 7 days in culture medium supplemented with hormones and vitamins. An in vitro asynchronous shoot and root development were observed from the seed tips after 60 days (Fig. 6). Anormal development was observed when seed tips showed only root or only shoot development (Fig. 6C). Biotechnological tools such as *in vitro* germination can offer a

promising alternative by providing a controlled environment conducive to germination and healthy plantlet development (Salgotra; Chauhan, 2023). In vitro germination can be combined to embryo cryopreservation protocols and used as ex situ conservation strategy program (Steiner et al., 2020; Goeten et al., 2023). Morphological and physiological studies have already been conducted on zygotic and somatic embryos from *Ocotea* genus toward to somatic embryos protocols development (Pelegriani et al., 2011; Santa-Catarina; Maciel; Pedrotti, 2001; Santa-Catarina et al., 2006). Somatic embryogenesis has been induced in other Lauraceae species such as *Laurus nobilis* (Canhoto; Lopes; Cruz, 1999), *Persea americana* Mill. (Witjaksono; Litz; Pliego-Alfaro, 1999), *O. catharinensis*, and *O. porosa* and *O. odorifera* (Santa-Catarina; Maciel; Pedrotti, 2001; Kowalski; Van Staden, 2001; Santa-Catarina et al., 2006; Pelegriani et al., 2011) assessed the impact of various pre-treatments on the in vitro establishment of *O. bullata*, a species native to South Africa (Kowalski; Van Staden, 2001). Although, the studies of somatic embryos have been helpful to address in vitro zygotic embryo germination, the somaclonal variation and abnormal somatic embryos are a concern for conservation program. In this sense, the *O. pulchella* seed desiccation tolerance threshold as well the in vitro germination protocol could be a new approach and the first step for ex situ conservation of tropical Lauraceae species.

Figure 5 – *O. pulchella* *in vitro* seed germination. (A) Seed tip embryo (explants). (B) Seed tip embryo 07 days. (C) Abnormal development (no roots). (D) Normal seedling development with shoot and root development after 60 days. N=4 Boxplot of shoot length in abnormal and normal plants (E) Boxplot of root length in abnormal and normal plants (F)



3.5 SEED STORAGE ASSAYS

O. pulchella fresh seeds showed 93,33% of viability through to 1% 2,3,5-triphenyltetrazolium chloride (TTC) test (Fig. 5). Seeds stored at 8 °C, for 30 days decrease the positive reaction to Tetrazolium to 37,78%, in comparison to fresh seeds and after 40 days to 24,44% (Fig. 5 A). At 50 and 60 days of storage only 6,67% and 4.44% of seeds reacting positively to TTC (Fig. 5 A, B). Fresh seeds stored at -18 °C and - 195,8 °C had no positive reaction to the TTC test. Seed WC and storage temperature are two main factors influencing seed longevity (Nasehzadeh; Ellis, 2017).

As observed by Pires et al. (2009) and in our observations, *O. pulchella* seeds exhibit a high-water content by time of dispersion of the mother plant. This trait anticipates unique challenges during storage due to physical properties of water and heightened metabolic activity (Walters, 2015; Nadarajan et al., 2023).

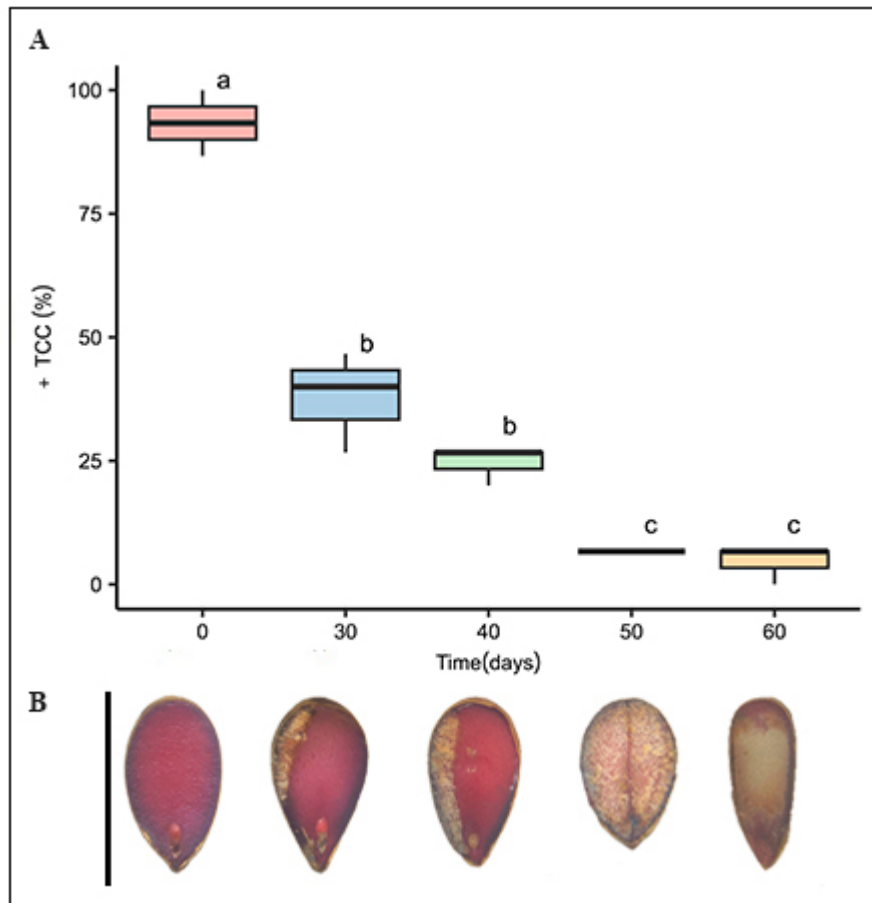
The delicate balance between water's physical qualities and cellular structure can lead to injury or death in subzero temperatures (Wesley-Smith et al., 2014). When high-water seeds freeze, cellular damage and dehydration are major difficulties. Ice crystals can mechanically puncture cell walls and membranes, causing irreparable seed structural damage (Walters; Pence, 2021). Dehydration can denature proteins, disrupt metabolic pathways, and change cellular osmotic balance, reducing seed viability and growth after thawing.

Molecular stresses can result from freezing and thawing, causing osmotic imbalances and cellular injury by creating high-solute pockets (Nadarajan et al., 2023). Seeds with high water content are more susceptible to freezing, while seeds with lower water content may crystallize well. The high-water content of seeds and 8 °C temperature can affect water flow within cells, highlighting the need for careful storage parameter control to sustain seed life (Farrant; Hilhorst, 2021).

Extreme cold kills the chemical that promotes growth and metabolism, emphasizing the significance of precise preservation measures, careful temperature management, and a deep understanding of each seed type's physiological needs and biochemical profile while conserving and storing. Desiccation sensitivity can be mitigated by controlled drying methods and proper storage (Pritchard, 2020).

Storage at 8 °C can cause physiological reactions in seeds, balancing metabolic activity and preservation (Ellis; Hong; Roberts, 1991). High metabolic activity increases oxygen and carbon dioxide consumption and output, depleting important energy supplies faster. Temperature affects enzymes and the plant's ability to fight oxidative stress, damaging cellular components and potentially impacting seed viability.

Figure 6 – *O. pulchella* seeds viability during storage at 8°C. (A) Box plot data distribution of positive reactions to 1% 2,3,5-triphenyltetrazolium chloride (TTC), with seeds of different storage time (days). (B) Effect of storage on the Tetrazolium salt reaction with the seed tissues. N= 15



Scale bar: 1cm. Means followed by the same letters do not show significant differences according to the Tukey test at 5% probability.

3.6 SEED POLYMINES AND CARBOHYDRATES CONTENT

Fresh seeds of *O. pulchella* exhibit an intriguing polyamine profile, with a notable total free PAs content of $0.029 \mu\text{mol. g}^{-1} \text{DW}$ (Fig. 7a). The distribution of PAs reveals Spermine (Spm) as the dominant polyamine ($0.014 \mu\text{mol. g}^{-1} \text{DW}$), trailed closely by Putrescine (Put) ($0.010 \mu\text{mol. g}^{-1} \text{DW}$) and Spermidine (Spd), with $0.005557 \mu\text{mol. g}^{-1} \text{DW}$ and no significant variance in abundance among the latter two (Fig. 7a). PAs are recognized for their pivotal roles in a spectrum of physiological processes, cellular metabolism, and stress response (Arun et al., 2016; El-Tarabily et al., 2021). However, the intricate dynamics of endogenous polyamines, particularly in response to seed desiccation under drought stress, remain an ongoing scientific exploration (Pál; Szalai; Janda, 2015). The abundance of polyamines has been closely linked to seed desiccation tolerance. This linkage hinges on their multifaceted functions, which

encompass the preservation of membrane integrity, safeguarding cellular constituents, maintaining DNA integrity, orchestrating gene expression, and facilitating reparative processes post-desiccation (Liu et al., 2016). Notably, the pattern found in *O. pulchella* seeds underscores a pivotal role for Spermine, emerges as a key player in stimulating gene transcription linked to antioxidant enzymes, thereby bolstering the seeds' defense against oxidative stress (Pál; Szalai; Janda, 2015). Beyond their roles in gene expression, polyamines actively partake in stabilizing membrane phospholipids within plant tissues, acting as signaling molecules to regulate stress-responsive pathways, and mitigating oxidative stress, as well-documented (Minocha; Majumdar; Minocha, 2014). Spermidine, on the other hand, has its prominence being attributed to its association with the ABA signaling pathway, consequently influencing gene expression (Pál; Szalai; Janda, 2015; Steiner et al., 2017). PUT is essential precursor for SPD and SPM. However, each polyamine can revert to its former state by shedding one amine group when needed (Minocha; Majumdar; Minocha, 2014; Singh et al., 2018). PUT has been shown to modulate ion channels, stabilize membranes, and influence DNA and protein synthesis (Lechowska et al., 2022). Specifically, in relation to seeds desiccation response, putrescine might act as a protective agent against cellular damage (Minocha; Majumdar; Minocha, 2014).

The interplay between various polyamines and other protective mechanisms collectively might help to unfold the remarkable capacity of seeds to endure desiccation and surmount the associated challenges (Minocha; Majumdar; Minocha, 2014). The accumulation of polyamines has been empirically observed under diverse abiotic stresses, encompassing the rigors of seeds desiccation (Rodrigues et al., 2022; Vieira et al., 2022). However, there is no patterns for seed desiccation and a better comprehension of their precise roles and implications necessitates further scientific inquiry (Nandy et al., 2022). In this regard, the quantification of endogenous polyamine, in tandem with a comprehensive assessment of seed desiccation-induced germinative responses, stands to substantially data base concerning these intricate interactions and their broader implications. This approach offers an avenue for unravelling the interplay of polyamines in seeds defense against desiccation detrimental damage, potentially uncovering novel strategies for enhancing plant resilience in the face of environmental stresses.

Furthermore, of plant resilience, it is worth considering another crucial facet of seed fortitude: the content of monosaccharides and disaccharides. These carbohydrate compounds, intricately intertwined with polyamines and other protective mechanisms, contribute significantly to a seed ability to withstand desiccation and environmental challenges. Emerging studies suggest that the levels of monosaccharides, such as glucoses and fructose, as well as

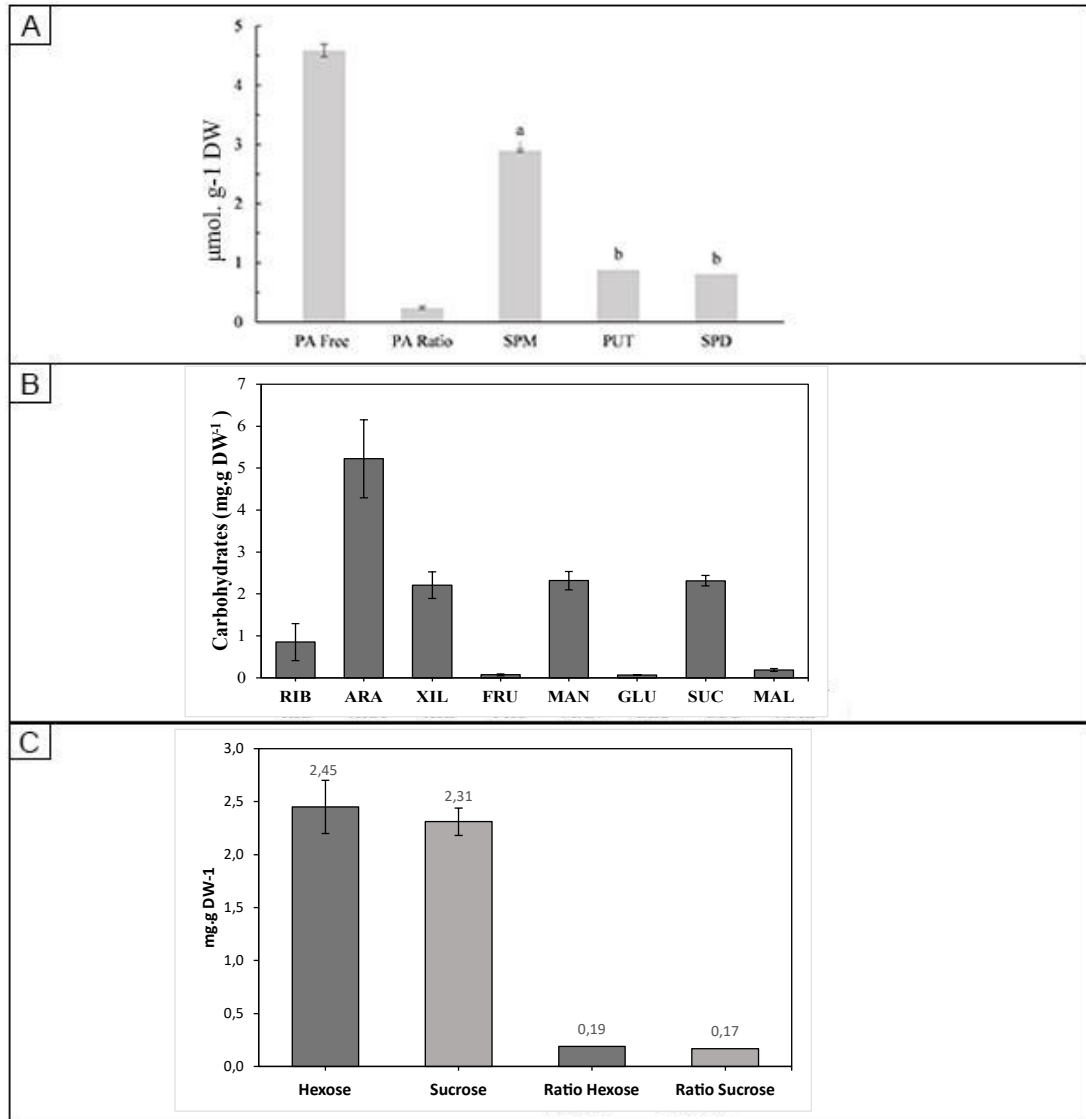
disaccharides like sucrose, play a pivotal role in bolstering seed desiccation tolerance. Monosaccharides such as hexoses not only act as compatible solutes, safeguarding cellular structures by osmotic regulation during desiccation stress, but they also serve as precursors for biosynthetic pathways associated with stress-responsive metabolites (Wang; Yong-Ling, 2013; Minocha; Majumdar; Minocha, 2014). The *O. pulchella* profile for soluble carbohydrates showed greater presence of monosaccharides (RIB, ARA, XIL, FRU, MAN, GLU) in comparison with disaccharides (MAL, SUC) (Fig. 7 B). Arabinoses was the major soluble carbohydrate found with 5,22 mg/ g FW⁻¹, followed by mannoses and sucrose with 2,31 mg/ g FW⁻¹ and xyloses 2,20 mg/ g FW⁻¹ (Fig. 7 B).

Carbohydrates, intricately intertwined with polyamines and other protective mechanisms, contribute significantly to a seed's ability to withstand desiccation and environmental challenges. Beyond the role of energy source, carbohydrates, exhibits multifaceted effects on cellular signaling pathways. Sucrose emerges in seed biology, orchestrating a myriad of processes and responses, having a central player as a signaling molecule, influencing gene expression, hormonal regulation, and physiological pathways. Sucrose metabolism undergoes dynamic changes, being a crucial regulator, impacting various aspects of plant growth, development, and stress responses (Sakr et al., 2018). Abiotic stimuli activate enzymes involved in sucrose breakdown, such as invertases and sucrose synthase, leading to the generation of fructose and glucose. These sugar derivatives can act as signaling molecules themselves. Trehalose, a disaccharide synthesized from glucose, interacts closely with sucrose metabolism. Trehalose acts as an osmolyte, protecting cellular structures and maintaining membrane integrity under osmotic stresses (Saddhe; Mishra; Kumar, 2021). It also participates in protein protection and reactive oxygen species (ROS) scavenging, enhancing stress tolerance. Fructans, synthesized by fructosyltransferase (Fts) in the vacuole, interact with membrane lipids, maintaining lipid phase transitions and fluidity (Zulfiqar et al., 2020). Overall, this interaction enhances desiccation tolerance, safeguarding cellular structures under adverse conditions. Unveiling metabolic profiles associated with abiotic stress responses provides valuable data into plant physiology and offers potential avenues for conservation purposes and stress resilience improvements.

The soluble carbohydrate profile of *O. pulchella* fresh seeds reveals a fascinating glimpse into its potential desiccation resistance. Their prominent presence of monosaccharides such as arabinose, mannose, fructose, and glucose, which are abundantly present, suggests the seed's inherent strategy to counteract the cellular disruptions arising from desiccation. On the

other hand, the detection of sucrose, a versatile disaccharide, adds another layer to this resilience. Beyond its role as an energy source, sucrose can influence gene expression, hormonal regulation, and a multitude of physiological pathways, equipping the seed with tools to navigate the stresses of desiccation. The rich and diverse carbohydrate profile indicates a robust metabolic reservoir that the seed can tap into during stress. This not only provides energy but also serves as precursors for vital stress-responsive metabolites. The notably high concentration of arabinose, in particular, hints at a possible specialized adaptation of *O. pulchella* seeds, though its specific role in desiccation resistance warrants further investigation. In sum, *O. pulchella* carbohydrate composition paints a picture of a seed more prepared for resilience, using an arsenal of sugars to protect and persevere in the face of environmental challenges, such as desiccation.

Figure 7 – Endogenous PAs and Carbohydrate content in *O. pulchella* fresh seeds. Free PAs total, PA ratio (PUT/SPD+SPM), Spermine (SPM), Putrescine (PUT), Spermidine (SPD). Values are the mean of three replicates and vertical bars represent \pm SD. Letters represent significance over the Tukey test with 5% probability (A). Soluble carbohydrate profile for *O. pulchella* mature seeds. Ribose (RIB), Arabinose (ARA), Xylose (XYL), Fructose (FRU), Mannose (MAN), Glucose (GLU), Sucrose (SUC), Maltose (MAL)



Values are means of three replicates and vertical bars represent \pm SD (B). Total hexose, total sucrose, ratio of Hexoses (Hexoses/Total Carbohydrates), ratio (Sucrose/Total Carbohydrates) per mg.g DW⁻¹ (C).

4 CONCLUSIONS

In our observations *O. pulchella* seeds took 111 days until maturation.

Our results indicate that *O. pulchella* seeds have a period of desiccation within the fruits, before dispersion from the mother plant.

Seeds from fresh fruits had 85% and 93,33 % of germination, in the year I and II, respectively.

A desiccation threshold for the species, appears to be near $0.30 \text{ gH}_2\text{O.gDW}^{-1}$, where germination drops below 50%.

Temperatures of $-18 \text{ }^\circ\text{C}$ and $-195,8 \text{ }^\circ\text{C}$ are not suitable as an alternative for whole seed storage, of *O. pulchella*.

The structure of seed tips can be successfully introduced in culture medium for in vitro cultivation, purposes.

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ANEXO A – MATERIAL SUPLEMENTAR

Table S1. *O. pulchella* seeds, WC treatments, germination percentage and germination speed index. Values are the mean of three replicates \pm SD. Distinct letters indicate means that are statistically different according to the Tukey test ($p < 0.05$).

Year	WC	Time	Germination (%)	Tukey (5%)	GSI
2022	0,58	0	85,00 \pm 8,66	a	1,02 \pm 0,12
	0,5	6	66,67 \pm 0,80	ab	0,80 \pm 0,24
	0,38	48	60,00 \pm 0,38	ab	0,38 \pm 0,07
	0,32	66	45,00 \pm 0,31	b	0,31 \pm 0,08
	0,22	96	48,33 \pm 0,27	b	0,27 \pm 0,07
	0,12	144	6,67 \pm 0,04	c	0,04 \pm 0,07
2023	0,6	0	93,33 \pm 3,33	a	2,98 \pm 0,21
	0,54	6	65,00 \pm 5,00	b	0,81 \pm 0,14
	0,39	48	63,33 \pm 7,64	b	0,73 \pm 0,06
	0,31	66	33,33 \pm 11,55	c	0,19 \pm 0,06
	0,26	96	22,22 \pm 3,85	c	0,11 \pm 0,02
	0,18	144	15,56 \pm 10,18	c	0,12 \pm 0,13