UNIVERSIDADE FEDERAL DO CEARÁ
CENTRO DE CIÊNCIAS
DEPARTAMENTO DE BIOQUÍMICA E BIOLOGIA MOLECULAR
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA

LYNDEFANIA MELO DE SOUSA

MECANISMOS MOLECULARES E BIOQUÍMICOS ENVOLVIDOS NA GERMINAÇÃO DE SEMENTES DE FEIJÃO-CAUPI (*Vigna unguiculata* L.) TRATADAS COM INIBIDOR DA ENZIMA OXIDASE ALTERNATIVA (AOX)

FORTALEZA
2024

LYNDEFANIA MELO DE SOUSA

MECANISMOS MOLECULARES E BIOQUÍMICOS ENVOLVIDOS NA GERMINAÇÃO DE SEMENTES DE FEIJÃO-CAUPI (*Vigna unguiculata* L.) TRATADAS COM INIBIDOR DA ENZIMA OXIDASE ALTERNATIVA (AOX)

Tese apresentada ao Programa de Pós-Graduação em Bioquímica da Universidade Federal do Ceará, como requisito parcial à obtenção do título de doutora em Bioquímica.
Área de concentração: Bioquímica Vegetal

Orientador: Prof. Dr. José Hélio Costa

FORTALEZA
2024

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Sistema de Bibliotecas
Gerada automaticamente pelo módulo Catalog, mediante os dados fornecidos pelo(a) autor(a)

S697m Sousa, Lyndefania Melo de.
Mecanismos moleculares e bioquímicos envolvidos na germinação de sementes de feijão-caupi (Vigna unguiculata L.) tratadas com inibidor da enzima oxidase alternativa (AOX) / Lyndefania Melo de Sousa. – 2024.
85 f. : il. color.

Tese (doutorado) – Universidade Federal do Ceará, Centro de Ciências, Programa de Pós-Graduação em Bioquímica , Fortaleza, 2024.
Orientação: Prof. Dr. José Helio Costa.

1. EROS. 2. N-propil-galato. 3. Homeostase redox. I. Título.

CDD 572

LYNDEFANIA MELO DE SOUSA

MECANISMOS MOLECULARES E BIOQUÍMICOS ENVOLVIDOS NA GERMINAÇÃO DE SEMENTES DE FEIJÃO-CAUPI (*Vigna unguiculata* L.) TRATADAS COM INIBIDOR DA ENZIMA OXIDASE ALTERNATIVA (AOX)

Tese apresentada ao Programa de Pós-Graduação em Bioquímica da Universidade Federal do Ceará, como requisito parcial à obtenção do título de doutora em Bioquímica. Área de concentração: Bioquímica Vegetal

Aprovada em: 30/07/2024.

BANCA EXAMINADORA

_____

Prof. Dr. José Hélio Costa (Orientador)

Universidade Federal do Ceará (UFC)

_____

Prof. Dr. Clesivan Pereira dos Santos

Universidade Federal do Maranhão (UFMA)

_____

Prof. Dr. Rafael de Souza Miranda

Universidade Federal do Piauí (UFPI)

_____

Dra. Thaís Andrade Germano

Universidade Federal do Ceará (UFC)

_____

Dra. Josiane Celerino de Carvalho

Instituto Nacional de Pesquisas da Amazônia (INPA)

A Deus.

Aos meus pais, Liduina e Assis.

A minha filha Camilla

AGRADECIMENTOS

A Deus por ter me dado saúde e força para realização do presente trabalho.

A minha filha Camilla por ser minha fonte de inspiração e principal conforto nos momentos de dificuldade. Aos meus pais Liduína e Assis por todo amor, dedicação e incentivo que foram essenciais durante a realização do presente trabalho. Ao meu companheiro Gerôncio por todo o cuidado, carinho e compreensão, além da preciosa ajuda nos momentos de dificuldade. A todos os demais membros da minha família que sempre me incentivaram e ajudaram.

A Universidade Federal do Ceará e ao Programa de Pós-Graduação em Bioquímica pelo apoio institucional.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Código de Financiamento 001), juntamente com o apoio financeiro da Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) e do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Ao Prof. Dr. José Hélio Costa pela orientação e valiosas contribuições durante todo o desenvolvimento do presente trabalho, além dos ensinamentos, apoio, amizade e momentos de descontração.

A Dra. Thais Andrade Germano por toda a ajuda durante todo o projeto, pelas orientações, correções e por todo apoio e amizade, além de gentilmente ter aceitado participar da banca examinadora.

Ao Dr. Shahid Aziz por sua boa disponibilidade em ajudar durante a realização do presente projeto, além todas as contribuições durante a escrita do artigo, e pela amizade e bom convívio.

Ao Prof. Dr. Rafael de Souza Miranda, ao Dr. Clesivan Pereira dos Santos e a Dra. Josiane Celerino de Carvalho, pela amizade por gentilmente aceitarem participar da minha banca examinadora.

A Giovana e ao Jonatas pelo auxílio durante as análises bioquímicas e fisiológicas, além da amizade, apoio e incentivo.

Ao Matheus pela ajuda nas análises transcriptômica e expressão dos genes. Aos meus demais colegas e ex-colegas de Laboratório: Rachel, Mathias, Adriele, Andresiane, Adriana, Edna, Débora Evelyn e Débora pela ajuda durante a realização do trabalho, pelo bom convívio, amizade e momentos de descontração.

Aos laboratórios de Biocristalografia, Fisiologia Vegetal (LabFIVE) e ao de Fisiologia vegetal e pós-colheita de frutos, pela ajuda durante o desenvolvimento do presente projeto.

Aos demais colegas do programa de Pós-Graduação em Bioquímica pelo bom convívio e amizade. E a todos que direta ou indiretamente fizeram parte da minha formação, o meu muito obrigado.

“Se eu vi mais longe, foi por estar sobre ombros de gigantes.”
Isaac Newton.

RESUMO

As plantas apresentam mecanismos que as possibilitam crescer e se desenvolver em condições adversas. Entre eles, destaca-se a via alternativa da respiração mitocondrial mediada pela oxidase alternativa (AOX), que é uma enzima vista como um ponto chave de reprogramação celular e no controle da formação de espécies reativas de oxigênio (EROS) sob condições de estresse. A germinação ocorre com intensa respiração mitocondrial que pode levar ao aumento de EROS, os quais devem ser controlados a fim de manter a homeostase redox celular. O modelo de estudo foi o feijão-caupi (*Vigna unguiculata* L.), cultura de grande importância para a alimentação humana e animal. Desse modo, o presente estudo tem como objetivo analisar o papel da AOX durante o processo de germinação das sementes de feijão-caupi, relacionando os processos bioquímicos, fisiológicos e moleculares. Para este fim, sementes de feijão-caupi foram submetidas a tratamento com concentrações variáveis (1, 2.5 e 5 mM) de n-propil-galato (PG) e analisadas nos períodos de 16, 32 e 48 horas após o início da germinação. Em seguida, foram realizadas análises do comportamento germinativo, metabolismo antioxidante e análise transcriptômica, a fim de identificar os genes diferencialmente expressos e relacionados com as principais rotas metabólicas do processo germinativo. Os resultados revelaram que a concentração de 5 mM PG causou a inibição da germinação às 16h, seguida de uma redução da germinação em 2.5 e 5 mM às 32h e posterior recuperação as 48h. Desse modo, escolheu-se a concentração de 2.5 mM, que se mostrou como uma concentração limítrofe capaz de alterar o processo germinativo, para as análises do metabolismo antioxidante e transcriptômica. Ademais, a redução da germinação por PG foi associada aos níveis iniciais mais baixos de H_2O_2 e a maior atividade antioxidante, enquanto a recuperação as 48h foi intimamente relacionada com a regulação positiva e consistente de AOX, principalmente AOX1, juntamente com genes relacionados a desintoxicação de xenobiontes e ao do metabolismo do NAD(P)H. Desse modo, conclui-se que a inibição da AOX por PG causou uma aumento na atividade redox celular que foi associada com a redução da germinação durante as horas iniciais, enquanto a regulação positiva da AOX foi intimamente relacionado com a posterior recuperação da germinação às 48h. Desse modo, as informações obtidas no presente estudo, fornecem informação de base sobre o efeito do PG durante a germinação de sementes e o perfil de expressão diferencial dos principais genes envolvido no processo germinativo.

Palavras-chave: EROS; N-propil-galato; Homeostase redox.

ABSTRACT

Plants have mechanisms that enable them to grow and develop under adverse conditions. Among them, the alternative pathway of mitochondrial respiration mediated by alternative oxidase (AOX) stands out, which is an enzyme seen as a key point of cellular reprogramming and in the control of the formation of reactive oxygen species (ROS) under stress conditions. Germination occurs with intense mitochondrial respiration that can lead to an increase in ROS, which must be controlled to maintain cellular redox homeostasis. The study model was the cowpea (Vigna unguiculata L.), a crop of great importance for human and animal nutrition. Thus, the present study aims to analyze the role of AOX during the germination process of cowpea seeds, relating the biochemical, physiological and molecular processes. For this purpose, cowpea seeds were treated with variable concentrations (1, 2.5 and 5 mM) of n-propyl gallate (PG) and analyzed at 16, 32 and 48 h after the beginning of germination. Then, analyses of germination behavior, antioxidant metabolism and transcriptomic analysis were performed to identify differentially expressed genes related to the main metabolic pathways of the germination process. The results revealed that the 5 mM PG concentration caused germination inhibition at 16 h, followed by a reduction in germination at 2.5 and 5 mM at 32 h and subsequent recovery at 48 h. Thus, the 2.5 mM concentration was chosen, which proved to be a borderline concentration capable of altering the germination process, for the analyses of antioxidant metabolism and transcriptomics. Furthermore, the reduction of germination by PG was associated with lower initial H_2O_2 levels and higher antioxidant activity, while recovery at 48 h was closely related to the consistent upregulation of AOX, mainly AOX1, together with genes related to xenobiotic detoxification and NAD(P)H metabolism. Thus, it is concluded that the inhibition of AOX by PG caused an increase in cellular redox activity that was associated with the reduction of germination during the initial hours, while the upregulation of AOX was closely related to the subsequent recovery of germination at 48 h. Thus, the information obtained in the present study provides basic information on the effect of PG during seed germination and the differential expression profile of the main genes involved in the germination process.

Keywords: ROS; N-propyl gallate; Redox homeostasis

LISTA DE FIGURAS

SUMÁRIO

1 INTRODUÇÃO

A germinação das sementes é uma etapa crucial no ciclo de vida das plantas, sendo responsável pela sobrevivência, manutenção das espécies e aumento da produtividade agrícola. No entanto, para que esse processo ocorra de modo eficiente é necessário que a semente encontre condições ambientais favoráveis, tais como a disponibilidade de água, luz, oxigênio, nutrientes e temperatura em quantidades adequadas, além dos fatores internos como a sinalização hormonal (principalmente os hormônios giberelina e ácido abscísico) que auxiliam na quebra da dormência e início da germinação (CARRERA-CASTAÑO et al., 2020).

O processo germinativo inicia-se com a absorção de água pela semente seca e termina com a protusão da radícula. Durante esse processo, ocorre a reativação metabólica dos componentes da semente e um aumento na atividade respiratória mitocondrial, garantindo fornecimento adequado de energia. No entanto, uma das principais consequências é o aumento da formação de espécies reativas de oxigênio (EROS), que são consideradas reguladores chave da germinação e do desenvolvimento da plântula (FAROOQ et al., 2021; Bailly, 2023; JHANJI et al., 2024). Contudo, estudos demonstraram que o acúmulo de EROS durante a germinação pode desencadear um estresse oxidativo, que pode levar ao retardo ou até mesmo a inibição do processo germinativo. Desse modo, o controle da quantidade de EROS no ambiente celular está intimamente associado com a eficiência germinativa (Bailly,2019; Bailly,2023).

Entre os mecanismos apresentados pelas plantas para reduzir os efeitos tóxicos do acúmulo de radicais livres, tem-se a presença de um eficiente sistema antioxidante, composto por enzimas antioxidantes como a catalase, a superóxido desmutase (SOD) e as peroxidases juntamente com a presença de antioxidantes não enzimáticos como o ascorbato, a glutationa e os compostos fenólicos (Laxa et al., 2019). Além disso, os vegetais contam com a presença da via alternativa da respiração mitocondrial que tem como principal enzima a Oxidase alternativa (AOX) que é uma oxidase terminal responsável pela transferência de elétrons do ubiquinol para o oxigênio, gerando água. Contudo, esse fenômeno ocorre com reduzido (ou sem) o translocamento de prótons pela membrana para produzir ATP. Esse processo auxilia no equilíbrio redox da célula, evitando o excesso de EROS (Garmash, 2021).

A AOX é fundamental para que diferentes processos durante o desenvolvimento vegetal ocorram de modo eficiente, entre eles se tem a sinalização hormonal, a atividade

fotossintética, a tolerância a estresses bióticos e abióticos, bem como a germinação de semente e a embriogênese somática (Selinski et al., 2018; Mohanapriya et al., 2019; Bailly; Merendino, 2021). Trabalhos anteriores realizados com cenoura, ervilha e arroz, revelaram que a inibição da AOX foi acompanhada de uma redução da taxa germinativa e diminuição do comprimento radicular (Arnholdt-schmitt et al., 2018; Mohanapriya et al., 2019; Kumari et al., 2021; Rodrigues etal., 2023).

O modelo de estudo utilizado é o feijão-caupi (*Vigna unguiculata*) que é uma eudicotiledônea de origem africana amplamente cultivada nas regiões áridas e semiáridas do mundo. No Brasil, ele é o segundo principal tipo de feijão cultivado, com uma produtividade estimada em 643,1 mil toneladas. Sua produção é concentrada nas regiões Norte e Nordeste, com uma expansão para a região Centro-Oeste e Sudeste, nos estados de Mato Grosso e Minas Gerais, respectivamente (CONAB, 2023). Essa cultura tem importante papel socioeconômico, sendo um alimento rico em nutrientes essenciais para a dieta humana, tais como, proteínas, carboidratos, minerais e fibras (Jayathilake et al., 2018), além de sua utilização na alimentação animal.

Apesar de ser uma espécie bem adaptada às regiões áridas e semiáridas sua produção de grãos é impactada por estresses ambientais que podem afetar negativamente o processo germinativo e o desenvolvimento das plantas (Boukar et al., 2019). Nesse contexto, a análise de dados referentes aos mecanismos moleculares e bioquímicos relacionados à germinação e inibição da AOX, uma enzima chave de reprogramação celular sob condições de estresse, fornecerão conhecimentos sobre a fisiologia do processo de germinativo, o perfil de expressão de genes, as principais vias metabólicas bem como os diferentes processos bioquímicos envolvidos durante a germinação de sementes de feijão-caupi submetidos a tratamento com n-propil-galato.

2 REVISÃO DE LITERATURA

2.1 A Germinação de sementes

As plantas superiores ou espermatófitas são caracterizadas pela presença das sementes, que são estruturas especializadas em dispersão, possibilitando às plantas um maior sucesso reprodutivo e independência da água. As sementes desenvolvem-se a partir dos rudimentos seminais (óvulos), e após a fecundação ocorre a formação do embrião que servirá como unidade dispersora permitindo a manutenção da biodiversidade de diversas espécies vegetais (Taiz; Zeiger, 2017).

O processo de germinação finaliza o processo de dispersão da semente, influenciando na localização e crescimento da planta (Penfield, 2017). Desse modo, esse fenômeno é responsável por diversos eventos bioquímicos, fisiológicos e moleculares que são responsáveis pela quebra da dormência da semente e crescimento do embrião. Assim, a germinação inicia-se com a absorção de água pela semente madura, seguida pelo alongamento do eixo embrionário, que na maioria das vezes, culmina com a ruptura da camada de cobertura e emergência da radícula (Steinbrecher; Leubner-Metzger, 2017).

Durante a germinação são evidenciadas duas fases principais, a primeira é marcada pela embebição com uma rápida absorção de água pela semente juntamente com a ativação do metabolismo celular garantindo o fornecimento de energia, ativação do ciclo celular, retomada da transcrição do RNAm e síntese proteica. A segunda fase é caracterizada pela redução na absorção de água, intensa mobilização de reservas energéticas, tais como carboidratos, lipídeos e proteínas e aumento dos processos de divisão e elongação celular que permitem a protrusão da radícula (Bareke, 2018)

Diversos fatores influenciam o processo de germinação, tanto extrínsecos como intrínsecos. Entre os extrínsecos têm-se as condições edafoclimáticas do ambiente e a disponibilidade de água, temperatura, oxigênio e luminosidade. Já com relação às intrínsecas têm-se as diferenças entre as espécies vegetais, bem como seus diferentes genótipos, além da ação hormonal que exerce um papel central durante o processo de germinação (Gong, 2022). Os hormônios etileno e giberelina (GA), através de uma complexa rede de sinalização, atuam no processo de quebra da dormência e início da germinação. Ademais, o ácido abscísico (ABA) é essencial para a indução e manutenção da dormência (Ishibashi; Yuasa, Iwaya-Inoue, 2018).

Outro importante evento que ocorre durante a germinação é o aumento da atividade mitocondrial que é responsável pela produção de energia para suprir reações metabólicas que são essenciais para o desenvolvimento do embrião. Uma das consequências da respiração mitocondrial é a produção das espécies reativas de oxigênio (EROS) (Considine et al., 2017). O papel das EROS durante a germinação ainda não está completamente esclarecido. No entanto, estudos sugerem que a concentração do EROS no ambiente celular pode favorecer ou inibir a germinação. Em concentrações controladas as EROS atuam como sinalizadores celulares e são essenciais para a elongação da radícula. Entretanto, em níveis elevados podem inibir a germinação, ou até mesmo levar à morte celular (Bailly, 2019)

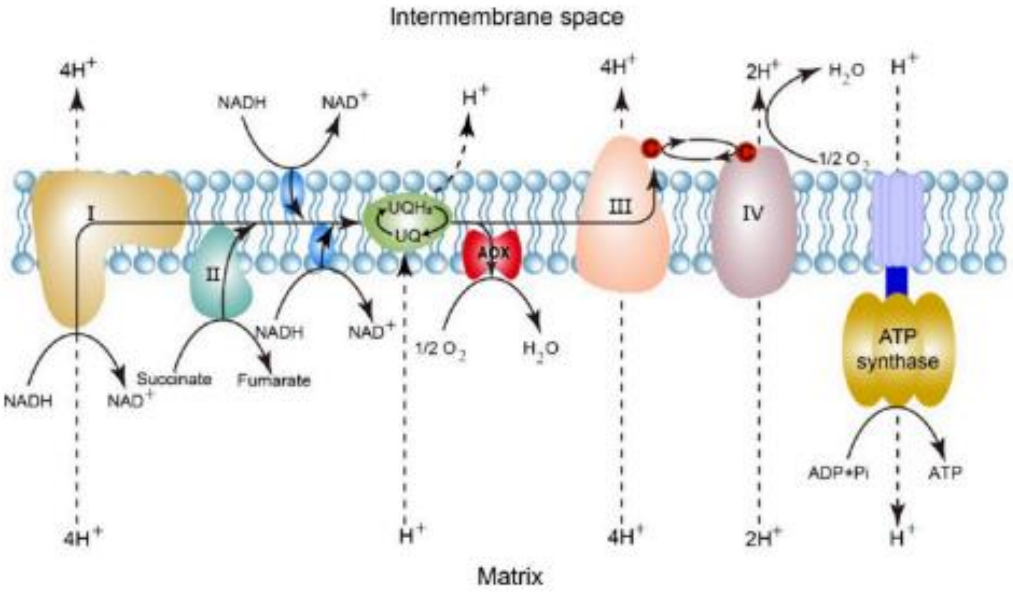
Os mecanismos epigenômicos também estão associados com a regulação da germinação, pois se revelaram capazes de modular a expressão de genes relacionados à dormência, maturação e germinação. Demonstrou-se que modificadores e remodeladores de cromatina específicos promovem a dormência ou germinação das sementes, aumentando e/ou reprimindo a expressão de subconjuntos de genes específicos (Carrera-Castaño et al., 2020). Além disso, estudos transcriptômicos em Arabidopsis mostraram alterações na quantidade de transcritos durante a germinação, permitindo a identificação de "*splicing alternativos*" associados com os mecanismos de regulação (Narsai et al., 2017).

Diferentes estratégias têm sido utilizadas para aumentar a eficiência do processo de germinação e consequentemente a produtividade vegetal. Dentre eles podemos destacar a adição artificial de etileno e GA, a estratificação e escarificação dos grãos reduzindo o período de dormência, além da irrigação, temperatura, aeração e luz adequada, tendo em vista que determinadas sementes necessitam de luz para promover a germinação, enquanto para outras não é necessário. Ademais é muito frequente o uso de fertilizantes e pesticidas que podem causar dissimilaridades genéticas, danos ambientais e redução da segurança alimentar (Rifna; Ramanan; Mahendran, 2019). Pode-se também destacar que a busca por sementes com maior eficiência germinativa é uma condição essencial para o desenvolvimento vegetal e manutenção da espécie. Nesse contexto, é crescente a busca por genótipos com maior tolerância às condições de estresse através da identificação e análise do perfil de expressão de genes alvos, auxiliando na compreensão do seu mecanismo de ação nas principais vias metabólicas associadas ao processo de germinação (Nunes et al., 2019; Zaman et al., 2019, Zhang et al., 2024.).

2.2 A oxidase alternativa (AOX)

A oxidase alternativa (AOX) é uma oxidase terminal encontrada na membrana interna das mitocôndrias de plantas, algas e de alguns fungos e protistas, exercendo uma função central na via alternativa da respiração mitocondrial, auxiliando no metabolismo energético das plantas, especialmente em condições de estresse. Ao contrário da cadeia principal de transporte de elétrons (Figura 1), que passa pelos complexos I, II, III e IV, a AOX permite que ocorra a transferência dos elétrons do ubiquinol diretamente para o oxigênio, formando água sem gerar ATP (Selinski et al., 2018). Além disso, diferente da via citocromo oxidase (principal), a AOX é insensível ao cianeto, mas tem sua ação inibida pelo ácido salicil-hidroxâmico (SHAM), n-octil-galato e n-propil-galato (Romero-Aguilar et al., 2020; Rosell-Hidalgo et al., 2020).

Figura 1. Estrutura da cadeia transportadora de elétrons na respiração mitocondrial.



Fonte: (Li et al., 2024)

A AOX é uma proteína integral de membrana, normalmente, encontrada na forma de dímero com aproximadamente 36 KDa, apresentando um grau muito elevado de conservação do núcleo catalítico das AOXs, apresentando as principais diferenças na região N-terminal como demonstrado em estudos com Arabidopsis (Pennisi et al., 2016). A AOX é codificada por uma pequena família multigênica contendo de um a seis membros gênicos, com uma alta conservação na estrutura éxon-íntron, apresentando quatro éxons interrompidos

por três íntrons (Considine, 2002), característica essa posteriormente evidenciada nos estudos com *Vigna unguiculata* (Costa et al., 2010).

Estudos anteriores classificaram a AOX em duas subfamílias, as AOX1 e AOX2 (Considine et al., 2002), que nas angiospermas estão distribuídas em quatro grupos filogenéticos AOX1a–c/1e, AOX1d, AOX2a–c e AOX2d (Costa et al., 2014, 2017). Adicionalmente, também foi identificado que no feijão-caupi a AOX é codificada por 3 genes (AOX1, AOX2a e 2d) (Costa et al., 2004, 2010). A análise do perfil de expressão da AOX demonstra que as AOX1 estão frequentemente associadas à exposição das plantas a condições de estresse. No entanto, as AOX2 são expressas constitutivamente durante diferentes fases do desenvolvimento, em órgãos ou tecidos específicos, tais como os fotossintéticos. Desse modo, acredita-se que essas subfamílias podem estar relacionadas a funções específicas desde a germinação ao desenvolvimento vegetal (Selinski et. al., 2018). No feijão-caupi, foi demonstrado que além do gene AOX1, o AOX2d também é responsivo a estresses revelando um padrão diferenciado em relação à soja, uma das principais leguminosas estudadas (Costa et al., 2010).

A expressão da AOX é estimulada por diversos fatores, tais como a disfunção dos complexos I, III e IV da cadeia transportadora de elétrons (CTE) e o acúmulo de citrato proveniente do ciclo do ácido tricarboxílico. A luminosidade também induz a expressão da AOX, para que esta mantenha o equilíbrio redox em mitocôndrias e cloroplastos. As condições de estresse promovem a indução da expressão da AOX, uma vez que situações estressantes podem levar a um aumento na quantidade de espécies reativas de oxigênio (EROS), e a AOX atuaria para manter a homeostase celular (Saha; Borosvskii; Panda, 2016). Além disso, a expressão diferencial da AOX também está intimamente associada à diversidade genética e fenotípica da célula, possibilitando uma maior plasticidade do metabolismo das células vegetais, que estão relacionados com a função de dissipação de energia das mitocôndrias (Garmash, 2022).

Portanto, a AOX tem um papel preponderante na manutenção da homeostase redox celular. Ela atua na regulação do nível de ubiquinona reduzida na CTE e na manutenção do equilíbrio redox na célula, limitando a produção EROS (Selinski et al., 2018). Essa enzima também é responsável pela proteção do aparelho fotossintético dissipando excesso de redutores, otimizando o '*status*' energético (ATP e NADPH) e gerenciando os reservatórios de carboidratos celulares em resposta às mudanças nas taxas de fixação e demanda de carbono

para crescimento e manutenção (Jianget al., 2019; Vanlerberghe et al., 2020). Desse modo, a AOX, é uma enzima fundamental durante o desenvolvimento das plantas, especialmente em fases críticas como a germinação das sementes, crescimento das raízes e senescência das folhas, contribuindo assim para a regulação do balanço energético (Arnholdt-Schmitt; Patil, 2017; Liu et al., 2021; Wen; Zhang, 2022).

Alguns estudos demonstram que a AOX tem papel relevante nos mecanismos de tolerância das plantas às condições de estresse, ao facilitar a manutenção do metabolismo energético e modular os níveis de EROS, evitando danos oxidativos que são comuns em diferentes tipos de estresse (Demircan; Cucun; Uzilday, 2020; Challabathula et al., 2022). Desse modo, observou-se nos estresses bióticos que a AOX foi responsiva a agentes patogênicos como fungos, bactérias e vírus, através da utilização de substâncias elicitoras, como o ácido salicílico, que desencadeiam reações de hipersensibilidade limitando o desenvolvimento do patógeno (Saha; Borosvskii; Panda, 2016). Nos estresses abióticos, a AOX também apresentou um padrão de expressão diferencial quando submetido às condições de seca, intensa luminosidade, frio e salinidade (Dahal; Vanlerberghe, 2017; Jiang et al., 2019; Pham et al., 2018; Turk 2019). Estudos prévios também relataram que a AOX pode ter um efeito direto na sinalização de estresse, suprimindo a produção de EROS, alterando indiretamente o status energético da célula provocando cascatas específicas de transdução de sinal (Van Aken et al., 2009). Levando em consideração os dados expostos acima, destaca-se a importância da AOX com um dos principais componentes envolvidos nas respostas de defesa da planta, auxiliando no equilíbrio redox celular, que é fundamental para a sobrevivência das plantas em condições adversas.

2.2.1 O papel da AOX durante o processo de germinação

A AOX desempenha uma função essencial durante a germinação de sementes, sendo responsável por modular os níveis de EROS condição vital para um eficiente e bem sucedido processo germinativo, garantido que as sementes possam iniciar o crescimento de modo eficaz e superar os obstáculos associados a transição de um estado dormente para um estado ativo (Bailly; Merendino, 2021). Desse modo, a atividade da AOX está associada com diversos fenômenos que ocorrem durante o processo de germinação. Estudos realizados durante a germinação do Amaranto (*Amaranthus* spp.) demonstrou que a AOX apresentou uma importante atividade antioxidante controlando a produção de EROs, além de estar

relacionada com a liberação de compostos fenólicos na parede celular (Sandoval-Sicairos et al., 2020). Ela também foi responsável pela promoção da homeostase celular sob as grandes alterações metabólicas que ocorrem durante o desenvolvimento da germinação e pós germinação (Velada et al., 2016).

Estudos conduzidos com plantas parasitas, arroz e ervilha evidenciaram que a inibição da AOX por SHAM, causou uma significativa redução, ou até mesmo uma inibição, do processo germinativo (Nun et al., 2003; Kumari et al., 2022; Rodrigues et al., 2023). Trabalhos com cenoura demonstraram que a AOX atua como sensor na percepção de estresses ambientais e reprogramação induzida por auxina durante o processo de germinação, como indicado pelo aumento na expressão da AOX. No entanto, a inibição da AOX por SHAM levou a uma redução na taxa de germinação e micorrizas, além de afetar o crescimento e emergência da radícula. Dessa forma, pode-se inferir que o aumento da expressão da AOX durante a germinação está relacionado ao maior vigor da semente e robustez da planta (Mohanapriya et al., 2019). Análises "*in silico*" demonstraram que os genes da AOX foram diferencialmente expressos durante o período da germinação, sendo regulados positiva ou negativamente a depender da espécie estudada e da subfamília (AOX1/ AOX2). Estudo sobre a expressão da AOX durante a germinação e estresse por salinidade em Arabidopsis revelou uma alta expressão da AOX2 durante o início da germinação das sementes (Ruan et al., 2024). Resultado semelhante também foi encontrado em estudos comparando dois cultivares de ervilha, onde ambos apresentam alta expressão da AOX2 nas primeiras 16 horas de germinação (Rodrigues et al., 2023). Esses achados podem indicar que os genes da AOX poderiam atuar como promissores candidatos a marcadores funcionais durante as etapas da germinação (Arnholdt-Schmitt et al., 2018).

Estudos prévios relacionando a AOX com o feijão-caupi demonstraram que ambos os genes AOX1 e AOX2d foram induzidos e co-expressos durante a germinação (Costa et al., 2010), diferente do que é observado em Arabidopsis onde os transcritos da AOX2 apresentam-se em grande quantidade na semente seca, com posterior redução, enquanto os da AOX1 apresentam uma redução a zero horas, seguido de um aumento após 48 horas do início da embebição (Saisho et al., 2001). Trabalhos anteriores com PG inibiu em 100% a via alternativa de elétrons em ensaio in vitro com mitocôndrias purificadas de feijão-caupi (Costa et al., 2007). Contudo, estudos realizados com *Vigna radiata* demonstraram que a concentração de 1 mM de PG, não afetou o processo de germinação, enquanto a concentração de 10 mM causou a sua inibição (Chaudhuri; Kar, 2008).

A atividade da AOX também é fundamental para um fornecimento adequado de energia durante a germinação das sementes, um processo que requer a ativação de diferentes vias metabólicas para garantir o crescimento do embrião e formação dos eixos embrionários (Pu et al., 2015). Desse modo, a AOX é responsável por fornecer uma rota alternativa para a transferência de elétrons, evitando a sobrecarga da CTE (via clássica), além disso a AOX ajuda otimizar a oxidação dos principais substratos envolvidos no metabolismo de carboidratos, lipídios e proteínas, garantindo uma eficiente produção de ATP durante a germinação (Li et al., 2024). Nesse contexto, a combinação das abordagens bioquímicas e moleculares, são de grande relevância para compreensão das principais alterações no perfil de expressão das principais enzimas envolvidas no metabolismo energético, durante a germinação de sementes de feijão submetidas ao tratamento com PG.

2.3 O feijão-caupi

O feijão-caupi (*Vigna ungiculata* L.), também conhecido como feijão de corda, fradinho ou massacar, é uma leguminosa de origem africana, amplamente cultivada nas regiões tropicais e subtropicais. É uma planta herbácea, anual, que apresenta grande variabilidade fenotípica e morfológica a depender do fotoperíodo e das condições de crescimento (Freire Filho, 2011). Essa planta pertence à divisão Magnoliophyta, classe Magnoliopsida, ordem Fabales, família Leguminosae, tribo Phaseoleae e gênero *Vigna*, apresentando cromossomos diplóides com 2n=22, além de um genoma nuclear de tamanho relativamente curto com aproximadamente 640,6 Mpb (Carvalho et al., 2017).

O cultivo do feijão-caupi está entre as principais atividades agroeconômicas do Brasil, apresentando grande destaque social, econômico e alimentar devido ao seu baixo custo e alto valor nutritivo, sendo cultivado principalmente para à cultura de subsistência e à comercialização no mercado interno. Entre as características que favorecem o plantio desse vegetal está a sua tolerância a condições adversas, incluindo ambientes quentes, secos e solos pobres em nutrientes e com faixa variáveis de pH (Boukar et al., 2019). Essas condições edafoclimáticas estão frequentemente relacionadas com as regiões áridas e semiáridas do Brasil. Suas principais formas de cultivo são a sequeiro, que tem predominância na região Nordeste, em áreas de agricultura de subsistência e pequenos agricultores. Outro modo é o cultivo irrigado, comum na região centro oeste, apresentando uma maior produtividade e mais de um ciclo de cultivo durante o ano (Freire Filho, 2011).

A região nordeste concentra as principais regiões áridas e semiáridas do Brasil, e apresenta-se como a pioneira no cultivo de feijão-caupi, que por volta do ano de 2006 começou a expandir para a região Centro Oeste (Freire-Filho, 2020). As principais áreas de cultivo estão nos estados da Bahia, Ceará, Piauí, Pernambuco, Maranhão, Mato Grosso e Goiás. De acordo com o levantamento de grãos das safras 2022/2023, os estados da região Nordeste com maior destaque foram: Bahia, Ceará e Piauí. Os dois primeiros apresentaram uma maior representatividade na primeira safra com uma produção de 122,9, 119,3 e 74,5 mil toneladas, respectivamente (CONAB, 2023). A comercialização do feijão-caupi ocorre principalmente na forma de grãos, os quais são ricos em proteínas e carboidratos, contendo, respectivamente, de 23 a 32% e 50 a 60% do seu peso seco, o que os tornam importantes fontes nutricionais para alimentação humana e combate à desnutrição em países em desenvolvimento. Entretanto, em algumas partes do mundo, as folhas e flores também são consideradas comestíveis. Alguns estudos têm demonstrado que o consumo de feijão-caupi na dieta alimentar também está relacionado com a prevenção de doenças como o câncer, o diabetes, a hipertensão, além de ter sido associado com a redução do colesterol (Jayathilake et al., 2018).

Os estresses ambientais, tanto de origem biótica como abiótica, são fatores limitantes da produção de feijão-caupi afetando negativamente a germinação de sementes, o crescimento, o desenvolvimento e produtividade das plantas. Entre os de origem biótica destacam-se os insetos, parasitas, microrganismos e as ervas daninhas (Togola et al., 2017; Tauseef et al., 2021; Tenório et al., 2019, Phiri et al., 2018). Quanto aos de origem abiótica destacam-se a seca, a salinidade, as altas temperaturas, que podem se apresentar de forma isolada ou combinada (Chiulele, 2003; Barros et al., 2021; Jayawardhane, 2022). Embora o feijão-caupi seja uma cultura considerada tolerante à seca, sua produção é significativamente reduzida quando submetida à seca severa. Outros fatores limitantes são o excesso de calor no período noturno que reduz o tamanho das vagens e os solos pobres em fósforo que limitam a fixação de nitrogênio nos nódulos radiculares (Boukar et al., 2016).

Aperfeiçoar cultivo e a germinação do feijão-caupi requer uma abordagem integrada com a combinação de práticas agronômicas adequadas e eficiente manejo de recursos, além de tecnologias inovadoras para garantir a sustentabilidade e a eficiência da produção. A crescente busca por tecnologias que melhorem a qualidade e produtividade do feijão-caupi é uma das prioridades dos programas de melhoramento genético, buscando o desenvolvimento de variedades resistentes aos diferentes tipos de estresses, o que favorece a

maior produção dos grãos, além de fornecer maior segurança alimentar (Qaim, 2020; Sallam et al., 2019; Hatzig et al., 2018).

3 OBJETIVOS

3.1 Objetivo geral

Analisar os efeitos bioquímicos, fisiológicos e moleculares de sementes de feijão durante a germinação e inibição da AOX com n-propil-galato

3.2 Objetivos específicos

- Avaliar o efeito da inibição da AOX na germinação de sementes de feijão-caupi.
- Avaliar alterações na homeostase iônica celular de sementes de feijão-caupi.
- Identificar os genes diferencialmente expressos (GDEs) via análise transcriptômica
- (RNA-seq) das sementes de feijão-caupi durante a germinação e inibição da AOX.
- Identificar as vias metabólicas dos GDEs nas sementes de feijão-caupi durante a germinação e inibição da AOX.

4 ARTIGO

Article submitted to Journal Plant Physiology and Biochemistry

Exploring Molecular and Biochemical Mechanisms in Germination of Cowpea (*Vigna unguiculata* L.) Seeds Under Inhibition of Alternative oxidase (AOX) by Propyl Gallate

Exploring Molecular and Biochemical Mechanisms in Germination of Cowpea (*Vigna unguiculata* L.) Seeds Under Inhibition of Alternative oxidase (AOX) by Propyl Gallate

Lyndefania Melo de Sousa[1], Thais Andrade Germano[1,2], Shahid Aziz[1,2], Matheus Finger Ramos de Oliveira[1], Giovanna Magalhães Bastos Salvador[1], Rafael de Souza Miranda[3], Birgit Arnholdt-Schmitt[1,2], Jose Helio Costa[1,2] *

[1] Functional Genomics and Bioinformatics, Department of Biochemistry and Molecular Biology, Federal University of Ceara, Fortaleza 60451-970, Ceara, Brazil
[2] Non-Institutional Competence Focus (NICFocus) 'Functional Cell Reprogramming and Organism Plasticity' (FunCROP) (Coordinated from Foros de Vale de Figueira), 7050-704 Alentejo, Portugal
[3] Department of Plant Science, Postgraduate Program in Agricultural Sciences, Federal University of Piauí, Teresina, Piauí, Brazil
*Correspondence: JHC (helio.costa@ufc.br)

ABSTRACT

Alternative oxidase (AOX) is crucial to maintain the redox balance in the cell, a key point for efficient and successful germination. Here, we performed comprehensive physiological and biochemical assessments followed by in-depth transcriptomic analyses on cowpea seed germination at 16, 32 and 48h under propyl gallate (PG) treatment, an AOX inhibitor. Analyses of different PG concentrations (1, 2.5 and 5 mM) initially revealed that 2.5 and 5 mM PG caused inhibitory effects in seed germination at 16h. However, the inhibition induced by 2.5 mM PG was recovered at 48h regarding all physiological parameters proving to be a threshold level to study the molecular and biochemical mechanisms involving AOX function. Inhibitory PG effects were linked to early lower H_2O_2 levels and higher antioxidant activity with consistent AOX upregulation (mainly *AOX1*) accompanying the germination recovery at 48h. In addition, application of AOX inhibitor PG reduced transcripts level for important metabolic pathways as cell cycle progress, energy status and krebs cycle. However, glycolysis and fermentation linked genes were upregulated at 16 and 48h indicating the vital role of these pathways in seed germination as well as a critical AOX involvement in redirecting their metabolic flux. Overall, our data put in evidence an extensive cross-talk between AOX and the general metabolism for seed germination in cowpea.

Keywords:
Rouxinol; Differential expression; ROS control; Fermentation; NAD(P)H/NAD(P)$^+$ ratio.

1. Introduction

Seed germination is a critical stage in higher plants' life cycles, ensuring the species' establishment and survival. Seeds germinate when they encounter favorable conditions, including adequate water, light, oxygen, nutrients, and temperature, which allow them to break dormancy (Klupczyńska & Pawłowski, 2021). Generally, the germination begins with the imbibition of the seeds and the metabolic activation of cellular components, ending with the emergence of the embryonic axes, mainly the radicle, and the seedling establishment. During the imbibition phase, intense mitochondrial activity provides the necessary energy for successful germination. However, this process also leads to reactive oxygen species (ROS) formation. The role of ROS during germination is not yet completely understood, but studies show that the concentration of ROS in the cellular environment can either favor or inhibit germination. ROS act as cellular signaling molecules at controlled concentrations essential to maintaining cellular homeostasis during radicle elongation. Conversely, at high levels, ROS can inhibit germination or even lead to cell death (Bailly, 2019). Therefore, germination efficiency is closely associated with the cellular ROS content (Bailly, 2023).

Plants employ several strategies to maintain cellular redox homeostasis. Among these, the enzymatic and non-enzymatic antioxidant systems are particularly important. The key enzymatic antioxidants include catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), glutathione peroxidases (GPX), and ascorbate peroxidases (APX). Notable non-enzymatic antioxidants comprise glutathione, ascorbate, tocopherols, and phenolic compounds, which are crucial in eliminating superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) (Sachdev; Ansari; Ansari, 2023). Besides the antioxidant systems, another crucial strategy involves the mitochondrial alternative respiration pathway mediated by Alternative Oxidase (AOX). This enzyme transfers electrons from ubiquinol to oxygen, generating water. Nevertheless, this process takes place with minimal or no proton translocation across the membrane to generate ATP, which favours the cellular redox balance and controls ROS production (Garmash, 2021), both of which are necessary for seed germination. Thus, AOX acts to promote cellular homeostasis under the major metabolic changes that occur during germination and post-germination development (Velada et al., 2016).

By acting as a bypass in the electron transport chain (ETC), AOX prevents over-reduction of the ubiquinone pool and production of ROS, thereby facilitating the maintenance of redox homeostasis when the ETC is impaired or overloaded (Jayawardhane et al., 2020). AOX regulates the NADH/NAD^+ ratio, which is crucial for metabolic processes such as glycolysis and the Krebs cycle (Fedotova et al., 2023). Furthermore, the accumulated NAD^+ serves as a coenzyme in the oxidation of ethanol to acetaldehyde, which is catalyzed by the cytoplasmic enzyme Aldehyde Dehydrogenases (ALDHs). Then, the acetate can be used for the acetylation of molecules like DNA and proteins or converting them into acetyl-CoA by acetyl-CoA synthetase. Acetyl-CoA can enter the Krebs cycle for energy production or can be used for carbon biosynthesis (Jiang et al., 2020).

Biochemical and molecular analyses of cowpeas revealed novel aspects of protein regulation as well as the identification and expression of the AOX multigene family. Lima Jr. et al. (2000) observed that AOX from Cowpea is regulated by pH and pyruvate, with optimum activity at pH 7.25. AOX capacity was also differently regulated under stress in cultivars differing under drought and salt tolerance (Costa et al., 2007). Finally, Costa et al. (2004, 2007, 2010) found that Cowpea AOX is encoded by a small gene family consisting of AOX1, 2a and 2b (renamed to Aox2d by Costa et al. 2014) differentially expressed during

plant development and in response to variable stress conditions. Generally, a coexpression of Aox1 and 2d was observed under stress (Costa et al., 2010), contrasting with the previous statement obtained from data in other species that Aox1 was stress-induced and Aox2 was linked to a 'housekeeping' function (Considine et al., 2002, Clifton et al., 2005). Cowpea Aox1 and 2d co-expression was also stimulated in imbibed seeds compared to dry seeds (Costa et al., 2010) indicating an important involvement of AOX during germination. In this sense, Arnholdt-Schmitt et al. (2018) stated that germination efficiency and greater plant robustness are closely associated with the AOX expression profile.

The function of AOX during seed germination has been investigated in parasitic plants (Nun et al., 2003), carrots (Mohanapriya et al., 2019), and peas (Rodrigues et al., 2023) using AOX inhibitors such as n-propyl gallate, octyl gallate, and SHAM. These studies revealed negative effects of AOX inhibitors byreducing germination rate, as well as radicle emergence and growth. However, these investigations were limited mainly to physiological observations, not exploring the biochemical and molecular mechanisms involved with AOX inhibition. In this context, the present study aims to analyze the role of AOX during the germination process of cowpea seeds, by relating specific biochemical and physiological parameters with transcriptome analyses of seeds under AOX inhibition by propyl gallate. In addition, target genes of the main metabolic pathways associated with seed germination were explored to gain insight into the specific molecular mechanisms linked to AOX inhibition.

2. Materials and methods

2.1 Seed germination and treatment conditions.

Cowpea seeds (BRS-Rouxinol, lineage TE 90-180-10E), were obtained from the germplasm bank of the Department of Phytotechnics at the Federal University of Ceará, Brazil. Firstly, the best seeds were selected based on uniformity and intact seed coats. Subsequently, the seeds were disinfected with 70% alcohol for 1 min, followed by soaking in a 2.5% sodium hypochlorite (NaClO) solution for 10 min. Immediately afterward, the seeds were thoroughly rinsed with distilled water.

The germination process was conducted using germitest paper soaked in water (control) and solutions containing 1 mM, 2.5 mM, or 5 mM of the AOX inhibitor n-propyl gallate (PG) in 50 mL of water and 500 µL of ethanol. The experiment took place in a Biochemical Oxygen Demand (B.O.D.) germination chamber, maintained in the dark at a temperature of 25°C. The effect of AOX inhibition on the germination of cowpea seeds was evaluated at 16, 32, and 48 hours after the start of germination. Seeds were considered germinated if their radicles were at least 2 mm long. Water absorption by the seeds was also measured during the germination process, indicated by the increase in fresh mass after imbibition, as well as root length at 16, 32, and 48 hours. Each treatment included three replicates, with 30 seeds per replicate.

2.2 Electrical conductivity (ds/m)

The electrical conductivity test was determined with whole seeds. For this, 25 seeds were used in 75 mL of deionized water for 24 hours at 25ºC. Electrical conductivity was checked using the electrical conductivity meter (Mp513, Sanxin®) and the results were expressed in $\mu S.cm^{-1}.g^{-1}$.

2.3 Germination behavior

Germination behavior was measured by determining the germination percentage (%G), the germination speed index (GSI), and the mean germination time (MGT). The %G was evaluated by counting the number of germinated seeds within each period analyzed and expressing this as a percentage (Labouriau, 1983). The GSI was calculated by summing the number of seeds germinated each day and dividing by the number of days elapsed from the beginning of germination, which represents the number of seeds germinated per day (Maguire, 1962). The MGT was defined as the sum of the number of germinated seeds multiplied by the incubation time in days, divided by the total number of germinated seeds per day (Labouriau, 1983).

2.4 Hydrogen peroxide content

The content of hydrogen peroxide (H_2O_2), was determined as described by Velikova et al. (2000). Approximately 0.1 g of seeds were homogenized in an ice bath with 1 mL 5 % TCA (w/v) and centrifuged at 12,000 x g for 20 min at 4 °C. The supernatant (250 µL) was collected and added to 250 µL of potassium phosphate buffer (10 mM, pH 7.0) and 500 µL of KI (1 M). Then, the absorbance was measured at 390 nm in the spectrophotometer and H_2O_2 content was given on a standard curve expressed as µmol of $H_2O_2.g^{-1}$ of fresh matter.

2.5 Activity of antioxidant enzymes

The specific activity of antioxidant enzymes was assessed using 1 g of seeds, which were homogenized in 5 mL of potassium phosphate buffer (0.1 M, pH 7.0) containing EDTA (0.1 mM) for 10 min. The homogenate was centrifuged (12,000 x g for 15 min at 4°C) and, then, the supernatant was collected and used to measure enzyme activity. The protein concentration in the crude extract was determined using the Bradford method (1976), with bovine serum albumin as the standard. The results, expressed in $g.Kg^{-1}$ of fresh matter, were used to calculate the specific enzymatic activity.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined through the photochemical reduction of p-nitrotetrazolium blue (NBT), following the methods of Beauchamp and Fridovich (1971) and Giannopolitis and Ries (1977), with absorbance readings at 560 nm. One activity unit (AU) was defined as the amount of enzyme required to inhibit 50% of NBT photoreduction, and SOD activity was expressed as UA mg^{-1} protein. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured according to Nakano and Asada (1981) by the decrease in absorbance at 290 nm due to ascorbate oxidation. APX activity was determined using a molar extinction coefficient of 2.8 mM^{-1} cm^{-1} and expressed as µmol H_2O_2 $min^{-1}mg^{-1}$ protein. Catalase (CAT, EC 1.11.1.6) activity was measured according to the method of Beers and Sizer (1952), with modifications. CAT activity was determined by the consumption of H_2O_2, evaluated by the decrease in absorbance at 240 nm, and results were expressed as µmol H_2O_2 min^{-1} g^{-1} protein. Guaiacol peroxidase (G-POD, EC 1.11.1.9) activity was measured according to Urbanek et al. (1991), by the increase in absorbance at 470 nm due to the formation of tetraguaiacol from the oxidation of guaiacol. Enzyme activity was calculated using the molar extinction coefficient of tetraguaiacol (26.66 mM^{-1} cm^{-1}), considering the formation of 1 mole of tetraguaiacol per consumption of 4 moles of H_2O_2, with results expressed as µmol H_2O_2 min^{-1} mg^{-1} protein.

2.6 Extraction, quantification, purity and integrity of total RNA

Total RNA extraction was conducted using 0.1g of sample powdered in liquid nitrogen with the RNeasy® Plant Mini Kit (Qiagen, Hilden, Germany), following the

manufacturer's instructions. One step was included to remove genomic DNA contamination using RNase-Free DNase Set (Qiagen, Hilden, Germany). Quantification of total RNA samples was performed using spectrophotometric readings at 260 nm with the Nanodrop 2000 device (Thermo Scientific). Purity analysis included measuring the $A_{260/A280}$ ratio to detect protein contamination (ideal values between 1.8 and 2) and the $A_{260/A230}$ ratio to assess polysaccharide contamination (values greater than 2 indicate insignificant contamination). The integrity of total RNA was assessed using a 1.5% agarose gel stained with ethidium bromide for 10 minutes. Visualization of the ribosomal RNA bands (18S and 28S) was achieved by exposing the gel to UV light using a transilluminator. Images were captured using the Mini BIS Pro system (BioImaging Systems) with Gel CaptureTM software. Quantification and quality assessment of RNA for sequencing were performed using the Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA), which calculates RNA Integrity Numbers (RIN). RIN values range from 1 to 10, with values above seven indicating high integrity of total RNA (Schroeder et al., 2006).

2.8 RNA sequencing, Reads quality check and mapping to the reference genome

The RNA-seq data was generated by the commercially available sequencing company, Macrogen Korea. The obtained sequence reads were qualitatively assessed using the FastQC program (Andrews, 2010). Sequences with low-quality nucleotide bases (Phred score < 20) were filtered out using the Trimmomatic program (Bolger, Lohse, & Usadel, 2014). The STAR tool version 2.7.11b (Dobin et al., 2013) was then used to align the high-quality reads to the reference genome of *Vigna unguiculata* (NCBI, ID:ASM411807v2).

2.9 Differential expression analysis and functional annotation of genes

The number of filtered reads mapped to each gene was calculated using HTSeq v. 2.0.5 (Putri et al., 2022), guided by gene annotations using the GFF (General Feature Format) files available in the NCBI database. The read counts were then normalized using the edgeR package (Robinson et al., 2010). For differential expression analysis, only genes with a count per million (CPM) ≥ 2 in at least three samples were selected. The differential expression analysis and principal component analysis (PCA) were performed using the DESeq2 package v.1.30.0 (Love et al., 2014), following the normalization of the filtered reads. Genes with a p-value ≥ 0.05 and a log2 fold-change ≤ -1 or ≥ 1 were considered differentially expressed (DEGs). Functional enrichment analysis of DEGs was performed using the GOseq R package v. 1.54.0 (Young et al., 2010) 2021).

In particular, the expression of genes related to the main metabolic pathways linked to seed germination was investigated (Suppl. Table 1). Firstly, Magic Blast was performed to map the genes (Boratyn et al., 2019). Then, mapped reads were quantified by HTseq v. 2.0.5 and normalized using the RPKM (Reads per kilobase of transcript per million of mapped reads) method (Mortazavi et al., 2008).

2.10 Experimental design and statistical analysis

The experimental design was randomized and employed a 4x3 factorial scheme, consisting of four PG concentrations (0, 1, 2.5 and 5 mM PG) at three time points (16, 32, and 48 hours after treatment). The data obtained from the physiological and biochemical analyses were statistically analyzed using GraphPad Prism 5.0 software. Analysis of variance (ANOVA) was performed using a two-way test, followed by the Bonferroni test. Differences

were considered significant at $p < 0.05$. Results were expressed as the mean ± standard deviation of three biological replicates.

3 Results

3.1 Germination behavior

The germination in control condition and under different concentrations (1 mM, 2.5 mM, and 5 mM) of n-propyl gallate (PG) was investigated in cowpea seeds. Initially, the vigor of seeds used in this work was analyzed by the electrical conductivity test, which showed an average of 44.04 $\mu S.cm^{-1}.g^{-1}$ and indicated a seed germination viability. Data on the percentage of germination (%G), germination speed index (GSI), and mean germination time (MGT) after 48h are presented in Table 1. GSI analysis demonstrated that the lowest values ($p < 0.05$), 21.40 and 20.22 seeds/day were observed at 2.5 mM and 5 mM PG concentrations, respectively. Additionally, no significant difference was observed in the %G and MGT among the different concentrations at 48h. However, at 32h, significant reduction in %G was noted in 2.5 mM and 5 mM PG treatments (Figure 1).

Regarding radicle length (RL), it was observed that at 16 hours after starting the germination, the 5 mM PG concentration did not show any germinated seed (Figure 1, Table 2). Comparing the other concentrations, it was identified that the 2.5 mM PG concentration exhibited shorter radicle lengths than the others at 16 hours. In addition, at 32-hour and 48-hour time points, the 5 mM PG concentration significantly reduced the radicle growth ($p < 0.05$).

3.2 H_2O_2 content and antioxidant enzymes

Figure 2 shows the content of H_2O_2 and antioxidant enzymes such as SOD, APX, CAT and G-POD during the germination of cowpea seeds under control and PG treatments (2.5 mM). Quantification of H_2O_2 in cowpea seeds during germination revealed significant reductions in H_2O_2 ($p < 0.05$) at 16 and 32h (Figure 2A). Regarding antioxidant enzymes, SOD activities increased significantly ($p < 0.05$) in treatment with PG at all times (Figure 2B), while APX increased activity ($p < 0.05$) only at 16h and 48h (Figure 2C). CAT showed no statistical difference at any of the times analyzed (Figure 2D) and G-POD activity was not detected in cowpea seeds.

3.3. RNA-seq Analysis and Gene annotation

Based on the biochemical and physiological results, cowpea beans germinated under control and a single PG concentration (2.5 mM) at 16, 32, and 48h of treatment (biological duplicates) was used to transcriptional profile investigation. A total of 571 million paired-end raw reads with fragments of 151 base pairs (bp) were generated from the RNA-seq of 12 samples. The average Q30 and CG content presented values of approximately 93% and 47%, respectively. After removing adapters and low-quality reads, 251 million filtered reads were obtained, of which 219 million were mapped with high quality to the reference genome of *Vigna unguiculata* (NCBI-ASM411807v2). The uniformity check of the 12 samples by PCA is shown in Figure 3.

3.4. Expression and enrichment analyses of differentially expressed genes (DEGs)

The expression analysis revealed a total of 1876 differentially expressed genes (DEGs) identified at 16, 32, and 48h under both control and PG (2.5 mM). Of these, 1049 were up-regulated and 827 were down-regulated (Figure 4). Comparative analysis between the control and treated groups showed a total of 437 up-regulated and 297 down-regulated DEGs at 16h; 41 up-regulated and 81 down-regulated DEGs at 32h; and 743 up-regulated and 464 down-regulated DEGs at 48h (Figure 4A). Furthermore, some DEGs were highly upregulated in response to PG treatment, and this response occurred mostly at all times analyzed (Figure 4B, Suppl. Table 2). These genes were: two mannitol dehydrogenases (ManDH), glutathione S-transferase (GST), Jasmonic acid carboxyl methyltransferase (JMT), cytochrome P450 (CYP736A12-like), transcription factor ORG2-like, ethylene-responsive transcription factor (ERF107-like) and 1-aminocyclopropane-1-carboxylate oxidase 1-like (ACO1).

Subsequent Gene Ontology (GO) enrichment analysis categorized the identified DEGs into three GO classes: biological process (BP), cellular component (CC), and molecular function (MF) (Suppl. Table 3). This analysis revealed that pathways related to BPs exhibited the highest enrichment (Figure 5). Notably, the glutathione metabolic process (GO:0006749) was enriched at both 16h and 48h when comparing the control and treated groups. The glycolytic process (GO:0006096) and the lignin biosynthetic process (GO:0009809) showed significant enrichment only at 16h (Figure 5A). At 32h, notable enrichments included one-dimensional cell growth (GO:0009826), dephosphorylation of peptidyl threonine (GO:0035970), and floral development (GO:0009908) (Figure 5B). Additionally, the stress response (GO:006950) and the activated ethylene signaling pathway (GO:0009873) were enriched at 48h (Figure 5C).

3.5. Regulation of AOX genes and selected metabolic pathways

The transcriptional profile of AOX and genes associated with critical metabolic processes during germination were evaluated in response to PG treatments. These metabolic pathways included the electron transport chain (ETC), glycolysis, fermentation, aldehyde detoxification (acetate synthesis), pyruvate oxidation (via mitochondria), the tricarboxylic acid (TCA) or Krebs cycle, beta oxidation of lipids, gluconeogenesis, antioxidant enzymes, cell cycle, cell energy status, and structural regulation.

Firstly, we evaluated the expression levels of AOX and electron transport chain (ETC) genes (Figure 6, Suppl. Table 4). The total expression of AOX gene showed a significant increase at all time points, especially at 48h (Figure 6). Regarding the expression of individual gene members of the AOX family (AOX1, 2a and 2d), it was possible to observe that the increased expression was mainly associated with AOX1, despite a significant increase in AOX2a mRNA level at 48 h. The transcript level of AOX2d also increased at all times (nonsignificant) (Suppl. Figure 1, Suppl. Table 5). The other genes directly linked to alternative respiration in mitochondria, such as the alternative NAD(P)H dehydrogenases (NDA and NDB), were connected to AOX expression only at 48h since transcript reduction or stability was observed at 16 and 32h (Figure 6, Suppl. Table 4). Most cytochrome ETC-related genes (components of complex I and IV) showed a stable expression profile, while transcription of uncoupling protein (UCP) mRNAs reduced significantly at 16h. In addition, the gene expression of plastid terminal oxidase (PTOX), a chloroplastic homologous protein associated with AOX, showed downregulation at 16 and 32h, and upregulation at 48 h in response to PG (Figure 6, Suppl. Table 4).

Regarding the genes involved in glycolysis and fermentation, it was evident that hexokinase (HXK) genes were negatively regulated at all the time points analyzed (Figure 7A, Suppl. Table 6). However, a significant increase in the transcripts of phosphofructokinase (PFK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), pyruvate kinase (PK), pyruvate dehydrogenase kinase (PDCK), and alcohol dehydrogenase (ADH) was identified at 16 hours. At 32h, only PK showed a reduction in transcripts. In 48h, PFK, PK, and PDC genes were upregulated, which were connected with the aldehyde dehydrogenase (ALDH) gene upregulation from cytosol and other cell compartments (peroxisome and nucleus), contrary to observed at 16h when ALDH genes remained stable or were downregulated (Figure 7B, Suppl. Table 7). For lactate dehydrogenase (LacDH), significant reductions in transcripts were identified at 32 and 48h (Figure 7A, Suppl. Table 6).

The expression analysis of genes linked to the pyruvate dehydrogenase (PDH) complex was investigated to gain insight into the metabolic flux of pyruvate to mitochondria (Figure 8A, Suppl. Table 8). The PDH genes showed a reduced transcript number at 16h, with subsequent recovery reaching similar levels to those of the control at 32 and 48h. Other genes encoding proteins which are linked to phosphorylation/dephosphorylation of PDH, reinforced the inhibition of pyruvate flux to mitochondria with a reduced transcript of Pyruvate dehydrogenase phosphatase 2C (PDP-2c) at 16 and 32h. This phenomenon is known to be involved in PDH activation, while transcripts of pyruvate dehydrogenase kinase (PDK), involved in PDH inactivation, remained stable throughout the course of the experiment (Figure 8A, Suppl. Table 8). As observed in the case of PDH, different genes involved in the regulation of Krebs cycle such as citrate synthase (CS), alpha-ketoglutarate dehydrogenase (α-keto-GDH), succinate dehydrogenase (SucDH), and fumarase, also showed early downregulation (16h) in response to PG with subsequent recovery at 32 and 48h ((Figure 8B, Suppl. Table 9).

Expression analyses of genes linked to lipid metabolism (beta oxidation) [acyl-coenzyme A oxidase (ACX), 3-ketoacyl-CoA thiolase (Thiolase)], glyoxylate cycle (malate synthase) and gluconeogenesis [phosphoenolpyruvate carboxykinase (PEPCK), pyruvate orthophosphate dikinase (PPDK)] are shown in Figure 9 (Suppl. Table 10). The genes linked to beta oxidation revealed stable expression compared to control conditions, with the exception of a ACX significantly high expressed at 48h. On the other hand, malate synthase transcripts increased at 32 and 48h, while genes related to gluconeogenesis, showed early downregulation at 16 (PEPCK) and 32h (PPDK) followed by upregulation at 32 (PEPCK) and 48h (PPDK) (Figure 9, Suppl. Table 10).

The effect of PG treatment was also evaluated on genes linked to antioxidant enzymes (APX, GR, SOD, CAT, GPX and G-POD) during cowpea germination (Figure 10, Suppl. Table 11). Among these genes, G-POD was barely detected. The expression of total genes from each enzyme showed variation along the time. At 16h, APX and GR transcripts decreased, while SOD mRNA increased significantly. GPX was upregulated at 16 (non significant) and 32h, and decreased at 48h. The majority of genes presented a similar expression profile compared to the control at 32h. At 48h, despite the increased transcript level of GR, a significant decrease was observed for APX, SOD, and CAT mRNA (Figure 10, Suppl. Table 11).

The transcript level analyses of genes involved in the cell cycle progression (E2F and gamma-tubulin), energy status (SNF1 and TOR) and cell structure (alpha and beta-tubulin) revealed that the presence of PG inhibited these metabolic processes mainly at 16h (except beta-tubulin) (Figure 11, Suppl. Table 12). After this time point, the inhibition of gene

expression was milder, showing stability or upregulation at 32 and/or 48h of treatment. An exception was observed in the case of structural genes (alpha and beta-tubulin) with upregulation occurring at 32h followed by downregulation at 48h (Figure 11, Suppl. Table 12).

4. Discussion

This study investigated the impact of propyl gallate (PG), an inhibitor of alternative oxidase (AOX), on cowpea seed germination. To elucidate the molecular level changes induced by PG treatment, comprehensive physiological and biochemical assessments were performed, followed by in-depth transcriptomic analyses. AOX is a vital plant enzyme that provides an alternative electron route when the ETC is inhibited, allowing for flexible electron transport and maintaining cellular redox balance (Vanlerberghe, 2013). It also reduces ROS production and is involved in thermogenesis. AOX regulation is complex and influenced by environmental factors, highlighting its importance in plant adaptation. In addition, considering that the germination progress may be closely related to the internal ROS level, which is regulated by ROS-scavenging systems (Gomes and Garcia, 2013; Farooq et al., 2021), and lowering ROS formation systems by alternative respiration mediated by AOX (Maxwell et al., 1999). We investigated the consequences of AOX inhibition in germinating cowpea seeds by monitoring the main physiological parameters (%G, GSI, MGT and radicle length), H_2O_2 content, and antioxidant enzyme activities to support the transcriptomic analysis in order to gain insights into the identification of biochemical and molecular mechanisms associated with AOX function during cowpea germination.

Initially, the electrical conductivity test of seeds exhibited low leachate (44.04 $\mu S.cm^{-1}.g^{-1}$) indicating the high quality and vigorousness of the seeds used in this study. Similar results were reported by Dutra et al. (2006) when evaluating the physiological potential of cowpea seeds. Furthermore, concerning PG treatment, previous data in *Vigna radiata*, a very closely related species to cowpea (*V. unguiculata*), showed that the germination was not affected by 1 mM PG, while 10 mM caused a very strong inhibition after 48h (Chaudhuri; Kar, 2008). Thus, we decided to use the concentrations of 1, 2.5 and 5 mM PG to explore primarily the physiological and biochemical changes before advancing in molecular analyses.

Corroborating with the previous findings on *V. radiata* data (Chaudhuri; Kar, 2008), our study demonstrated that 1 mM PG had no effect on cowpea germination (Figure 1, Table 1). Whereas 2.5 and 5 mM PG inhibited %G and GSI at 16 and 32h, but this inhibitory effect was reversed at 48h evidenced by resumed germination (Figure 1, Table 1). Despite a similar germination pattern, 5 mM PG induced inhibitory effect on radicle length (RL) along all time points compared to 2.5 mM PG concentration (Table 2), which showed lower RL only at 16h. These data align with studies on carrot and rice seed germination, which revealed a reduction in RL under 5 to 20 mM salicylhydroxamic acid (SHAM), another AOX inhibitor (Mohanapriya et al., 2019; Kumari et al., 2022). Thus, considering that 2.5 mM PG was the single treatment with inhibitory effects followed by recovery in the different physiological parameters examined here, it seemed an ideal condition to explore the transcriptome responses associated with the AOX function during cowpea seed germination.

Interestingly, the total AOX expression was upregulated under PG treatment across all the time points, reaching a higher intensity at 48h (Figure 6, Suppl. Table 4). The response was mainly attributed to *AOX1*, with a considerable contribution of *AOX2a* at 48h (Suppl. Figure 1, Suppl. Table 5). In fact, *AOX1* has been reported as a stress responsive gene

in several species (Considine et al., 2002), and the PG treatment can be considered a stress condition. In addition, most recently, Ruan et al. (2024) reported a critical role of *AOX2* in Arabidopsis seed germination using mutants. Curiously, the cowpea *AOX2a* response occurred following the recovery of physiological parameters and intensification of germination at 48h after the imbibition (Figure 1, Table 1, Table 2, Suppl. Figure 1) suggesting a similar function compared to Arabidopsis *AOX2*.

It is important to highlight that the 2.5 mM PG appeared to be a limitrophe concentration to affect the cowpea germination, suggesting that the germinating seeds could reverse the AOX inhibition by increasing AOX gene expression until 48h. Furthermore, evidence given by the alternative NAD(P)H dehydrogenases (*NDA* and *NDB*) expression shows an early disconnection with AOX expression at 16 and 32h, but presents a similar expression stimulation pattern at 48h (Figure 6, Table 4), indicating that the alternative respiration pathway was restored at 48h after exposure to the inhibitor. In Arabidopsis, it has been observed that *AOX1a* and *NDA,* as well as *AOX1a* and *NDB* co-expression stimulate alternative respiration, providing NAD(P)H oxidation from the mitochondrial matrix and cytosol, respectively (Rasmusson et al., 2004; Elhafez et al., 2006, Smith et al., 2011, Sweetman et al., 2019). In addition, extensive and positive changes affecting secondary metabolism, photosynthesis, and oxidative stress were observed only when both *AOX1a* and *NDB2* were overexpressed together (Sweetman et al., 2019; 2022).

Regarding cellular redox status, the inverse relation was observed between the reduced H_2O_2 content and the increased gene expression/enzymatic activity of antioxidant enzymes during initial PG treatment (Figure 2, Figure 10). Besides this, the germination recovery can also be evidenced by the increased H_2O_2 content in PG treatment at 48h which corroborates with the %G recovery in the same period (Figure 1, Figure 2). This finding highlights a critical point impairing cowpea seed germination since it is well known that the germination success depends on internal ROS contents in the seeds (Gomes and Garcia, 2013; Farooq et al., 2021). Here, we can hypothesise that AOX inhibition would cause a cellular imbalance in redox status, generally increasing NAD(P)H/$NAD(P)^+$ ratio and most of these antioxidant enzymes use the reducing power of NAD(P)H for their activity (Costa et al., 2021; Decros et al., 2023). In Arabidopsis, *AOX1a* and *NDB2* silencing/overexpression confirmed the essential role of alternative respiration pathways regulating the cytosolic NAD(P)H/$NAD(P)^+$ ratio (Vishwakarma et al., 2014; 2015; 2016; Sweetman et al., 2019; Jethva et al., 2023).

Among the different metabolic pathways regulated by cytosolic $NAD(P)^+$, gene ontology revealed a crucial involvement of glycolysis at 16h in response to PG (Figure 5A). In effect, with exception of HXK, significant transcript increases of PFK, GAPDH and PK indicate that glycolysis was stimulated at 16h (Figure 7, Suppl Table 6). In this context, cytosolic HXK is a versatile enzyme acting in many metabolic pathways, besides glycolysis (Kim et al., 2013, Aguilera-Alvarado and Sánchez-Nieto, 2017, Wu et al., 2023), thus, this fact corroborates with the extensive early downregulation of several important metabolic pathways in our study (Figure 8, Suppl Table 8, Suppl Table 9). In addition, our analysis suggests that the stimulated metabolic flux of glycolytic pathway was conducted for ethanol fermentation (PDC and ADH), instead of lactic fermentation or pyruvate oxidation in mitochondria via krebs cycle (Figure 7, Figure 8, Suppl Table 8, Suppl Table 9). In this sense, Bharadwaj et al. (2021) observed that enhanced fermentation is temporarily necessary in early seed germination.

A central role has been attributed to AOX regulating the CoV-MAC-TED trait under stress. This trait links ROS/RNS balancing mediated by AOX, aerobic fermentation, cell restructuration, and cell cycle progression as critical metabolic pathways for early cell reprogramming (Arnholdt-Schmitt et al., 2021; 2022a; 2022b; 2024; Aziz et al., 2022; Costa

et al., 2021a; 2021b). Here, analyses of different genes associated with these pathways showed that despite early increased transcript stimulation of glycolysis and alcohol fermentation, inhibition of genes linked to energy status and cell cycle generally occurred at 16 and 32h with recovery at 48h (Figure 7A, Figure 11). Therefore, these findings reinforce the early (at 16 and 32h) AOX inability to exert its function. Early inhibition extended also to gluconeogenesis-associated genes such as PEPCK (at 16h) and PPDK (at 32h) (Figure 9).

Here, we hypothesize that alternative pathways contribute to the efficiency of the fermentation process by producing acetaldehyde and acetate instead of ethanol. At 16h, the metabolic flux seems to be directed to ethanol formation, since the reverse conversion of ethanol to acetaldehyde would be impaired by limited cytosolic NAD^+ due to AOX inhibition. High transcript levels of ADH with concomitant reduced ALDH mRNA, an enzyme that converts acetaldehyde to acetate in a NAD^+ dependent reaction, also corroborate this observation. However, at 48h, with seed germination recovery, we observed significant increases in ALDH transcript levels and stable ADH mRNA (Figure 7B, Suppl Table 7) indicating that metabolic flux was conducted to acetate synthesis. In Arabidopsis, acetate synthesis via glycolysis and fermentation, has been linked to a survival strategy under stress (Kim et al., 2017), which acetate acts as a versatile molecule for phytohormone signaling and epigenetic regulation as well as energy production, metabolite biosynthesis, and protein defense regulation (Jardine and McDowell, 2023).

Some genes involved in detoxification processes, NAD(P)H metabolism, or essential phytohormones for seed germination were highly upregulated in all timepoints (Suppl Table 2). In this regard, continuous upregulation (16 to 48h) of mannitol dehydrogenase (ManDH) and NADPH-cytochrome P450 oxidoreductase (CYP) genes coding predicted cytosolic and endoplasmic reticulum enzymes, respectively, could contribute to increased cytosolic $NAD(P)^+$ (Suppl Table 2). High expression of ManDH is closely associated with increased efficiency in NAD^+ uptake and improved stress tolerance (Patel et al., 2015), while CYP is well known to be involved in xenobiotic detoxification (i.e., propyl gallate in this study), among several other functions, using the reducing power of NAD(P)H (Pandian et al., 2020). Another highly upregulated gene with a primary role in xenobiotic detoxification was glutathione S-transferase (GST) (Hayes et al., 2005). In addition, GST is also involved in the maintenance of antioxidant efficiency (Kumar et al., 2021). Some genes appear to be linked to germination success, such as the transcription factor ORG2, which is induced in Arabidopsis seeds with a higher germination rate under chromium stress (Colzi et al., 2023). Similarly, the ethylene-responsive transcription factor (ERF107) and ACO1 are associated with elevated ethylene, a phytohormone known to enhance seed germination (Franzoni et al., 2019; Wang et al., 2020). Finally, the jasmonic acid carboxyl methyltransferase (JMT) gene is involved in jasmonic acid and methyl jasmonate biosynthesis, phytohormones involved in the regulation of seed germination (Pan et al., 2023, Sohn et al., 2011). Thus, all of these upregulated genes appear to work together to supply AOX impairment and restore seed germination.

In conclusion, our findings demonstrate that a 2.5 mM PG concentration was a threshold level for the germination of cowpea seeds with a physiological inhibitory effect at 16h. However, these effects were temporary, as the germination process returned to its normal state within 48 hours, which coincided with a significant up-regulation of AOX expression. This inhibitory effect was also linked to reduced H_2O_2 levels compared to control conditions, highlighting a disruption in antioxidant system efficiency that may involve a cell redox status imbalance caused by AOX inhibition. Additionally, it is noteworthy that the significant upregulation of genes linked to the detoxification of xenobiotics (such as propyl gallate) and the regulation of NAD(P)H metabolism, indicates their potential involvement in reversing the inhibition of AOX.

Author contributions:

The manuscript is a part of PhD study of **L.M.S** that together with **J.H.C** conceptualized, designed, and wrote the final manuscript. **G.M.B.S** helped in physiological and biochemical analysis. **M.F.R.O** performed transcriptomic analysis. **T.A.G** and **S.A** helped with the interpretation of the results, and developed the primary manuscript. **B.A.S**, and **R.S.M** contributed to the final organization and reviewed the manuscript. All authors read and agreed to the publication of the manuscript.

Acknowledgements: S.A. and T.A.G. are grateful to CNPq and FUNCAP for the post-doctoral fellowship. J.H.C. is grateful to CNPq for the researcher fellowship (CNPq grant 313899/2021-5) and to FUNCAP, CNPq and CAPES for continuous scientific support.

Funding: This work was supported by Funcap [grant number: Proc. PS1-00186-00419.01.00/21].

Figure and Table caption:

Table 1. Germination parameters of cowpea seed under control and propyl gallate treatments (1, 2.5 and 5 mM) are presented. The data are shown as means and standard deviation. Germination percentage (%G), germination speed index (GSI), and mean germination time (MGT). Significant differences from the controls are indicated by * at $p < 0.05$.

Table 2. Radicle length (RL) of cowpea seed under control and propyl gallate treatments (1, 2.5 and 5 mM) at 16, 32 and 48h. Values are expressed as means and standard deviation. Significant differences from the controls are indicated by * at $p < 0.05$.

Figure 1. Germination percentage (%G) in cowpea seeds under control and propyl gallate treatments (1, 2.5 and 5 mM) at 16, 32 and 48h. Vertical bars represent standard deviation of the mean (n=3). Significant differences from the controls are indicated by * at $p < 0.05$.

Figure 2. Hydrogen peroxide contents and antioxidant enzymes activities in the cowpea seed under control and PG treatments are presented. (**A**) H_2O_2 contents, (**B**) Superoxide dismutase - SOD, (**C**) Ascorbate peroxidase - APX, (**D**) catalase - CAT. Vertical bars represent standard

deviation of the means (n=3). Significant differences from the controls are indicated by * at p < 0.05.

Figure 3. Principal component analysis (PCA) plot based on the regularized log transformation. Colored points inside the clusters represent biological replicates from each treatment. Values indicated on the axis of the factor map correspond to the percentage of total variance explained by each axis (PC1 and PC2). C and T refers to control and propyl gallate treatment, respectively, at three time points (16, 32 and 48h).

Figure 4. The numbers of differentially expressed genes (DEGs) in the cowpea seed under control and PG treatments. (**A**) Numbers of DEGs in pairwise comparisons between control and PG-treated cowpeas at each time point. (**B**) Venn diagram shows the number of commonly and uniquely DEGs during cowpea germination. Green and red numbers into graph represent down and up-regulated DEGs, respectively. C and T refers to control and propyl gallate treatment, respectively, at three time points (16, 32 and 48h).

Figure 5. GO enrichment circle plots for the top 10 most significant GO categories for each comparison. GO analysis of DEGs between (**A**) T16h vs C16h, (**B**) T32h vs C32h and (**C**) T48h vs C48h. The associated tables present the GO term ID and function. The outer circle shows the relative fold change for each gene: red and blue points showing up and down-regulated genes. The inner quadrants are colored based on the z-score and their size based on the enrichment p-value: larger surface, lower the p-value and vice-versa.

Figure 6. Relative expression of genes linked to electron transport chain (ETC) in Cowpea/bean seeds germination under control and Propyl gallate (PG)-stressed conditions. The relative gene expression was calculated between PG treated and control for each time point (16, 32 and 48h). Significant differences from the controls are indicated by * at p < 0.05.

Figure 7. Relative expression of genes linked to (**A**) glycolysis and fermentation and (**B**) aldehyde detoxification in Cowpea/bean seeds germination under control and Propyl gallate (PG)-stressed conditions. The relative gene expression was calculated between PG treated and control for each time point (16, 32 and 48h). Significant differences from the controls are indicated by * at $p < 0.05$.

Figure 8. Relative expression of genes linked to (**A**) pyruvate oxidation and (**B**) Krebs cycle in Cowpea/bean seeds germination under control and Propyl gallate (PG)-stressed conditions. The relative gene expression was calculated between PG treated and control for each time point (16, 32 and 48h). Significant differences from the controls are indicated by * at $p < 0.05$.

Figure 9. Relative expression of genes linked to lipid metabolism, glyoxylate cycle and gluconeogenesis in Cowpea/bean seeds germination under control and Propyl gallate (PG)-stressed conditions. The relative gene expression was calculated between PG treated and control for each time point (16, 32 and 48h). Significant differences from the controls are indicated by * at $p < 0.05$.

Figure 10. Relative expression of genes linked to antioxidant enzymes in Cowpea/bean seeds germination under control and Propyl gallate (PG)-stressed conditions. The relative gene expression was calculated between PG treated and control for each time point (16, 32 and 48h). Significant differences from the controls are indicated by * at $p < 0.05$.

Figure 11. Relative expression of genes linked to cell cycle progression, energy status and cell structure in Cowpea/bean seeds germination under control and Propyl gallate (PG)-stressed conditions. The relative gene expression was calculated between PG treated and control for each time point (16, 32 and 48h). Significant differences from the controls are indicated by * at $p < 0.05$.

Supplementary Figure 1 Relative expression of alternative oxidase (AOX) genes in Cowpea/bean seeds germination under control and Propyl gallate (PG)-stressed conditions. The relative gene expression was calculated between PG treated and control for each time point (16, 32 and 48h). Significant differences from the controls are indicated by * at $p < 0.05$.

Supplementary Table 1. List of *Vigna unguiculata* genes analyzed in germination under propyl gallate treatments.

Supplementary Table 2. Supplementary Table 2. Common up-regulated genes during germination of cowpea/cowpea seeds under control and propyl gallate (PG) stress conditions. These genes were found in the interception of all comparisons. The log2 fold-change value was calculated for PG treated versus control for each time point (16, 32 and 48h). Up-regulated genes present a log2 fold-change ≥1 and the value of adjusted $p< 0.05$.

Supplementary Table 3. GO enrichment analysis during Cowpea/bean seeds germination under propyl gallate treatments. The table consists of the name and ID of the GO term, besides its gene ontology classes: biological process (BP), cellular component (CC) and molecular function (MF). Over and under represented *P*-value calculated to each GO term based on information present in fourth and fifth columns.

Supplementary Table 4. Means of RPKM values ± SE (standard error) of gene expression during electron transport chain (ETC) in Cowpea/bean seeds germination under control and Propyl gallate-stressed conditions. Statistical analysis using a t-test was applied concerning the control at each time point. Upregulated and downregulated genes are indicated in green and red, respectively. Significant differences from the controls are indicated by * at $p < 0.05$.

Supplementary Table 5. Means of RPKM values ± SE (standard error) of alternative oxidase (AOX) gene expression level in Cowpea/bean seeds germination under control and Propyl gallate-stressed conditions. Statistical analysis using a t-test was applied concerning the

control at each time point. Upregulated and downregulated genes are indicated in green and red, respectively. Significant differences from the controls are indicated by * at $p < 0.05$.

Supplementary Table 6. Means of RPKM values ± SE (standard error) of gene expression during glycolysis and fermentation in Cowpea/bean seeds germination under control and Propyl gallate-stressed conditions. Statistical analysis using a t-test was applied concerning the control at each time point. Upregulated and downregulated genes are indicated in green and red, respectively. Significant differences from the controls are indicated by * at $p < 0.05$.

Supplementary Table 7. Means of RPKM values ± SE (standard error) of aldehyde detoxification gene expression in Cowpea/bean seeds germination under control and Propyl gallate-stressed conditions. Statistical analysis using a t-test was applied concerning the control at each time point. Upregulated and downregulated genes are indicated in green and red, respectively. Significant differences from the controls are indicated by * at $p < 0.05$.

Supplementary Table 8. Means of RPKM values ± SE (standard error) of pyruvate oxidation linked genes expression level in Cowpea/bean seeds germination under control and Propyl gallate-stressed conditions. Statistical analysis using a t-test was applied concerning the control at each time point. Upregulated and downregulated genes are indicated in green and red, respectively. Significant differences from the controls are indicated by * at $p < 0.05$.

Supplementary Table 9. Means of RPKM values ± SE (standard error) of Krebs cycle linked genes expression level in Cowpea/bean seeds germination under control and Propyl gallate-stressed conditions. Statistical analysis using a t-test was applied concerning the control at each time point. Upregulated and downregulated genes are indicated in green and red, respectively. Significant differences from the controls are indicated by * at $p < 0.05$.

Supplementary Table 10. Means of RPKM values ± SE (standard error) of gene expression during β oxidation and gluconeogenesis in Cowpea/bean seeds germination under control and Propyl gallate-stressed conditions. Statistical analysis using a t-test was applied concerning the control at each time point. Upregulated and downregulated genes are indicated in green and red, respectively. Significant differences from the controls are indicated by * at $p < 0.05$.

Supplementary Table 11. Means of RPKM values ± SE (standard error) of expression of antioxidant enzymes coding genes in Cowpea/bean seeds germination under control and Propyl gallate-stressed conditions. Statistical analysis using a t-test was applied concerning the control at each time point. Upregulated and downregulated genes are indicated in green and red, respectively. Significant differences from the controls are indicated by * at $p < 0.05$.

Supplementary Table 12. Means of RPKM values ± SE (standard error) of cell cycle energy status genes expression level in Cowpea/bean seeds germination under control and Propyl gallate-stressed conditions. Statistical analysis using a t-test was applied concerning the control at each time point. Upregulated and downregulated genes are indicated in green and red, respectively. Significant differences from the controls are indicated by * at $p < 0.05$.

References

Aguilera-Alvarado, G. P., & Sánchez-Nieto, S. (2017). Plant hexokinases are multifaceted proteins. *Plant and Cell Physiology*, *58*(7), 1151-1160. https://doi.org/10.1093/pcp/pcx062

Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Arnholdt-Schmitt, B., Mohanapriya, G., Sathishkumar, R., Macedo, E. S., & Costa, J. H. (2018). Predicting biomass production from plant robustness and germination efficiency by calorespirometry. *Biofuels: Greenhouse Gas Mitigation and Global Warming: Next Generation Biofuels and Role of Biotechnology*, 81-94. https://doi.org/10.1007/978-81-322-3763-1_5

Arnholdt-Schmitt, B., Aziz, S., & Costa, J. H. (2022a). Efficient Rebalancing of ROS Levels in Plants Links to Temporarily Enhanced Aerobic Fermentation, Distinct Cell Restructuration, and Resilience in Field. *Innovations in Digital Health, Diagnostics, and Biomarkers*, *2*(2022), 60-63. https://doi.org/10.36401/IDDB-22-02

Arnholdt-Schmitt, B., Aziz, S., & Costa, J. H. (2022b). Diversity Control in Early Transcribed ROS/RNS-balancing Genes: A Common Mechanism for Healthy Resilience?. *Innovations in Digital Health, Diagnostics, and Biomarkers*, *2*(2022), 56-59. https://doi.org/10.36401/IDDB-22-03

Arnholdt-Schmitt, B., Sircar, D., Aziz, S., Germano, T. A., Thiers, K. L. L., Noceda, C., Bharadwaj, R., Mohanapriya,G. & Costa, J. H. (2024). Transcriptome Analyses in Adult Olive Trees Indicate Acetaldehyde Release and Cyanide-Mediated Respiration Traits as Critical for Tolerance against Xylella fastidiosa and Suggest AOX Gene Family as Marker for Multiple-Resilience. *Pathogens*, *13*(3), 227. https://doi.org/10.3390/pathogens13030227

Aziz, S., Germano, T. A., Thiers, K. L. L., Batista, M. C., de Souza Miranda, R., Arnholdt-Schmitt, B., & Costa, J. H. (2022). Transcriptome analyses in a selected gene set indicate alternative oxidase (AOX) and early enhanced fermentation as critical for salinity tolerance in rice. *Plants*, *11*(16), 2145. https://doi.org/10.3390/plants11162145

Bailly, C. (2019). The signalling role of ROS in the regulation of seed germination and dormancy. *Biochemical journal*, *476*(20), 3019-3032. https://doi.org/10.1042/BCJ20190159

Bailly, C. (2023). ROS in seed germination. In *Advances in Botanical Research* (Vol. 105, pp. 177-204). Academic Press. https://doi.org/10.1016/bs.abr.2022.10.003

Beers, R. F., & Sizer, I. W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol chem*, *195*(1), 133-140. ttps://doi.org/10.1016/S0021-9258(19)50881-Xh

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry*, *44*(1), 276-287. https://doi.org/10.1016/0003-2697(71)90370-8

Bharadwaj, R., Noceda, C., Mohanapriya, G., Kumar, S. R., Thiers, K. L. L., Costa, J. H., Macedo, E.S., Kumari, A., Gupta, K.J., Srivastava, S., Adholeya, A., Oliveira, M., Velada, I., Sircar, D., Sathishkumar, R. & Arnholdt-Schmitt, B. (2021). Adaptive reprogramming during early seed germination requires temporarily enhanced fermentation-A critical role for alternative oxidase regulation that concerns also microbiota effectiveness. *Frontiers in Plant Science*, *12*, 686274. https://doi.org/10.3389/fpls.2021.686274

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114-2120. https://doi.org/10.1093/bioinformatics/btu170

Boratyn, G. M., Thierry-Mieg, J., Thierry-Mieg, D., Busby, B., & Madden, T. L. (2019). Magic-BLAST, an accurate RNA-seq aligner for long and short reads. *BMC bioinformatics*, *20*, 1-19. https://doi.org/10.1093/bioinformatics/btu170

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Chaudhuri, A., & Kar, R. K. (2008). Inhibition of seed germination by propyl gallate, a free radical scavenger and recovery of germination by hydrogen peroxide and ethylene in Vigna Radiata. *World J. Agric. Sci*, *4*, 914-921. http://www.idosi.org/wjas/wjas4(s)/19.pdf

Colzi, I., Gonnelli, C., Vergata, C., Golia, G., Coppi, A., Castellani, M. B., Giovino, A., Buti, M., Sabato, T., Capuana, M., Aprile, A., De Bellis, L., Cicatelli, A., Guarino, F., Castiglione, S., Ioannou, A.G., Fotopoulos, V., & Martinelli, F. (2023). Transgenerational effects of chromium stress at the phenotypic and molecular level in Arabidopsis thaliana. *Journal of hazardous materials*, *442*, 130092. https://doi.org/10.1016/j.jhazmat.2022.130092

Considine, M. J., Holtzapffel, R. C., Day, D. A., Whelan, J., & Millar, A. H. (2002). Molecular distinction between alternative oxidase from monocots and dicots. *Plant Physiology*, *129*(3), 949-953. https://doi.org/10.1104/pp.004150

Costa, J. H., Hasenfratz-Sauder, M. P., Pham-Thi, A. T., Lima, M. D. G. S., Dizengremel, P., Jolivet, Y., & de Melo, D. F. (2004). Identification in Vigna unguiculata (L.) Walp. of two cDNAs encoding mitochondrial alternative oxidase orthologous to soybean alternative oxidase genes 2a and 2b. *Plant Science*, *167*(2), 233-239. https://doi.org/10.1016/j.plantsci.2004.03.016

Costa, J. H., Jolivet, Y., Hasenfratz-Sauder, M. P., Orellano, E. G., Lima, M. D. G. S., Dizengremel, P., & de Melo, D. F. (2007). Alternative oxidase regulation in roots of Vigna unguiculata cultivars differing in drought/salt tolerance. *Journal of plant physiology*, *164*(6), 718-727. https://doi.org/10.1016/j.jplph.2006.04.001

Costa, J. H., Mota, E. F., Cambursano, M. V., Lauxmann, M. A., De Oliveira, L. M. N., Lima, M. D. G. S., Orellano, E.G. & De Melo, D. F. (2010). Stress-induced co-expression of two alternative oxidase (VuAox1 and 2b) genes in Vigna unguiculata. *Journal of plant physiology*, *167*(7), 561-570. https://doi.org/10.1016/j.jplph.2009.11.001

Costa, J. H., McDonald, A. E., Arnholdt-Schmitt, B., & de Melo, D. F. (2014). A classification scheme for alternative oxidases reveals the taxonomic distribution and evolutionary history of the enzyme in angiosperms. *Mitochondrion*, *19*, 172-183. https://doi.org/10.1016/j.mito.2014.04.007

Costa, J. H., Aziz, S., Noceda, C., & Arnholdt-Schmitt, B. (2021a). Transcriptome data from human nasal epithelial cells infected by H3N2 influenza virus indicate early unbalanced ROS/RNA levels, temporarily increased aerobic fermentation linked to enhanced α-tubulin and rapid energy-dependent IRF9-marked immunization. *bioRxiv*, 2021-10. https://doi.org/10.1101/2021.10.18.464828

Costa, J. H., Mohanapriya, G., Bharadwaj, R., Noceda, C., Thiers, K. L. L., Aziz, S., Srivastava, S., Oliveira, M., Gupta, K. J., Kumari, A., Sircar, D., Kumar, S.R., Achra, A., Sathishkumar, R., Adholeya, A. & Arnholdt-Schmitt, B. (2021b). ROS/RNS balancing, aerobic fermentation regulation and cell cycle control–a complex early trait ('CoV-MAC-TED') for combating SARS-CoV-2-induced cell reprogramming. *Frontiers in Immunology*, *12*, 673692. https://doi.org/10.3389/fimmu.2021.673692

Costa, J. H., Roque, A. L. M., Aziz, S., Dos Santos, C. P., Germano, T. A., Batista, M. C., Thiers, K. L. L., Saraiva, K. D. C., & Arnholdt-Schmitt, B. (2021c). Genome-wide identification of ascorbate-glutathione cycle gene families in soybean (Glycine max) reveals gene duplication events and specificity of gene members linked to development and stress conditions. *International Journal of Biological Macromolecules*, *187*, 528-543. https://doi.org/10.1016/j.ijbiomac.2021.07.103

Decros, G., Dussarrat, T., Baldet, P., Cassan, C., Cabasson, C., Dieuaide-Noubhani, M., Destailleur, A., Flandin, A., Prigent, S., Mori, K., Colombié, S., Jorly, J., Gibon, Y., Beauvoit, B., & Pétriacq, P. (2023). Enzyme-based kinetic modelling of ASC–GSH cycle during tomato fruit development reveals the importance of reducing power and ROS availability. *New Phytologist*, *240*(1), 242-257. https://doi.org/10.1111/nph.19160

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S.Batut, P., Chaisson, M., & Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, *29*(1), 15-21. https://doi.org/10.1093/bioinformatics/bts635

Dutra, A. S., Medeiros Filho, S., & Teófilo, E. M. (2006). Condutividade elétrica em sementes de feijão caupi. *Revista Ciência Agronômica*, *37*(2), 166-170. ISSN 0045-6888

Elhafez, D., Murcha, M. W., Clifton, R., Soole, K. L., Day, D. A., & Whelan, J. (2006). Characterization of mitochondrial alternative NAD (P) H dehydrogenases in Arabidopsis: intraorganelle location and expression. *Plant and Cell Physiology*, *47*(1), 43-54. https://doi.org/10.1093/pcp/pci221

Farooq, M. A., Zhang, X., Zafar, M. M., Ma, W., & Zhao, J. (2021). Roles of reactive oxygen species and mitochondria in seed germination. *Frontiers in plant science*, *12*, 781734. https://doi.org/10.3389/fpls.2021.781734

Fedotova, O. A., Polyakova, E. A., & Grabelnych, O. I. (2023). Ca2+-dependent oxidation of exogenous NADH and NADPH by the mitochondria of spring wheat and its relation with AOX capacity and ROS content at high temperatures. *Journal of Plant Physiology*, *283*, 153943. https://doi.org/10.1016/j.jplph.2023.153943

Franzoni, G., Cocetta, G., Trivellini, A., & Ferrante, A. (2019). Transcriptional regulation in rocket leaves as affected by salinity. *Plants*, *9*(1), 20. https://doi.org/10.3390/plants9010020

Garmash, E. V. (2021). Role of mitochondrial alternative oxidase in the regulation of cellular homeostasis during development of photosynthetic function in greening leaves. *Plant Biology*, *23*(2), 221-228. https://doi.org/10.1111/plb.13217

Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant physiology*, *59*(2), 309-314. https://doi.org/10.1104/pp.59.2.309

Gomes, M. P., & Garcia, Q. S. (2013). Reactive oxygen species and seed germination. *Biologia*, *68*, 351-357. https://doi.org/10.2478/s11756-013-0161-y

Hayes, J. D., Flanagan, J. U., & Jowsey, I. R. (2005). Glutathione transferases. *Annu. Rev. Pharmacol. Toxicol.*, *45*(1), 51-88. https://doi.org/10.1146/annurev.pharmtox.45.120403.095857

Jardine, K. J., & McDowell, N. (2023). Fermentation-mediated growth, signaling, and defense in plants. *New Phytologist*, *239*(3), 839-851.Fermentation-mediated growth, signaling, and defense in plants. https://doi.org/10.1111/nph.19015

Jayawardhane, J., Cochrane, D. W., Vyas, P., Bykova, N. V., Vanlerberghe, G. C., & Igamberdiev, A. U. (2020). Roles for plant mitochondrial alternative oxidase under normoxia, hypoxia, and reoxygenation conditions. *Frontiers in Plant Science*, *11*, 566. https://doi.org/10.3389/fpls.2020.00566

Jethva, J., Lichtenauer, S., Schmidt-Schippers, R., Steffen-Heins, A., Poschet, G., Wirtz, M., Van Dongen, J.T., Eirich, J., Finkemeier, I., Bilger, W. , Schwarzländer M., & Sauter, M. (2023). Mitochondrial alternative NADH dehydrogenases NDA1 and NDA2 promote survival of reoxygenation stress in Arabidopsis by safeguarding photosynthesis and limiting ROS generation. *New Phytologist*, *238*(1), 96-112. https://doi.org/10.1111/nph.18657

KIM, Y. M., Heinzel, N., GIESE, J. O., Koeber, J., Melzer, M., Rutten, T., VON WIRÉN, N., SONNEWALD, U., & HAJIREZAEI, M. R. (2013). A dual role of tobacco hexokinase 1 in primary metabolism and sugar sensing. *Plant, cell & environment*, *36*(7), 1311-1327. https://doi.org/10.1111/pce.12060

Kim, J. M., To, T. K., Matsui, A., Tanoi, K., Kobayashi, N. I., Matsuda, F., Habu, Y., Ogawa, D., Sakamoto, T., Matsunaga, S., Bashir, K., Rasheed, S., Ando, M., Takeda, H., Kawaura, K., Kusano, M., Fukushima, A., Endo, T. A., Kuromori, t., Ishida, J., Morosawa, T., Tanaka, M., Torii, C., Takebayashi, Y., Sakakibara, H., Ogihara, Y., Saito, K., Shinozaki, K., Devoto, A. & Seki, M. (2017). Acetate-mediated novel survival strategy against drought in plants. *Nature Plants*, *3*(7), 1-7. https://doi.org/10.1038/nplants.2017.97

Klupczyńska, E. A., & Pawłowski, T. A. (2021). Regulation of seed dormancy and germination mechanisms in a changing environment. *International journal of molecular sciences*, *22*(3), 1357. https://doi.org/10.3390/ijms22031357

Kumar, A., Kumar, V., Dubey, A. K., Ansari, M. A., Narayan, S., Meenakshi, Kumar, S., Pandey, V., Pande, V. & Sanyal, I. (2021). Chickpea glutaredoxin (CaGrx) gene mitigates drought and salinity stress by modulating the physiological performance and antioxidant defense mechanisms. *Physiology and Molecular Biology of Plants*, *27*(5), 923-944. https://doi.org/10.1007/s12298-021-00999-z

Kumari, A., Singh, P., Kaladhar, V. C., Manbir, Paul, D., Pathak, P. K., & Gupta, K. J. (2022). Phytoglobin-NO cycle and AOX pathway play a role in anaerobic germination and growth of deepwater rice. *Plant, Cell & Environment*, *45*(1), 178-190. https://doi.org/10.1111/pce.14198

Labouriau, G. (1983). A germinação das sementes. ISBN: 0827017669,9780827017665

Lima-Júnior, A., de Melo, D. F., Costa, J. H., Orellano, E. G., Jolivet, Y., Jarmuszkiewicz, W., Sluse, F., Dizengremel, P., & Lima, M. S. (2000). Effect of pH on CN-resistant respiratory activity and regulation on Vigna uniguiculata mitochondria. *Plant Physiology and Biochemistry*, *38*(10), 765-771. https://doi.org/10.1016/S0981-9428(00)01184-0

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology*, *15*, 1-21. https://doi.org/10.1186/s13059-014-0550-8

Maguire, J. D. (1962). Speed of germination-aid in selection and evaluation for seedling emergence and vigor. https://doi.org/10.2135/cropsci1962.0011183X000200020033x

Maxwell, D. P., Wang, Y., & McIntosh, L. (1999). The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. *Proceedings of the National Academy of Sciences*, *96*(14), 8271-8276. https://doi.org/10.1073/pnas.96.14.8271

Mohanapriya, G., Bharadwaj, R., Noceda, C., Costa, J. H., Kumar, S. R., Sathishkumar, R., Thiers, K.L.L., Macedo, E. S., Silva, S. Annicchiarico, P., Groot, S. P.C., Kodde, J., Kumari, A., K. J., Gupta & Arnholdt-Schmitt, B. (2019). Alternative oxidase (AOX) senses stress levels to coordinate auxin-induced reprogramming from seed germination to somatic embryogenesis—A role relevant for seed vigor prediction and plant robustness. *Frontiers in Plant Science*, *10*, 1134. https://doi.org/10.3389/fpls.2019.01134

Mortazavi, A., Williams, B. A., McCue, K., Schaeffer, L., & Wold, B. (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature methods*, *5*(7), 621-628. https://doi.org/10.1038/nmeth.1226

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and cell physiology*, *22*(5), 867-880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Nun, N. B., Plakhine, D., Joel, D. M., & Mayer, A. M. (2003). Changes in the activity of the alternative oxidase in Orobanche seeds during conditioning and their possible physiological function. *Phytochemistry*, *64*(1), 235-241. https://doi.org/10.1016/S0031-9422(03)00165-1

Patel, T. K., Krasnyanski, S. F., Allen, G. C., Louws, F. J., Panthee, D. R., & Williamson, J. D. (2015). Tomato plants overexpressing a celery mannitol dehydrogenase (MTD) have decreased susceptibility to Botrytis cinerea. *American Journal of Plant Sciences*, *6*(08), 1116. http://dx.doi.org/10.4236/ajps.2015.68116

Pan, J., Wang, H., You, Q., Cao, R., Sun, G., & Yu, D. (2023). Jasmonate-regulated seed germination and crosstalk with other phytohormones. *Journal of experimental botany*, *74*(4), 1162-1175. https://doi.org/10.1093/jxb/erac440

Pandian, B. A., Sathishraj, R., Djanaguiraman, M., Prasad, P. V., & Jugulam, M. (2020). Role of cytochrome P450 enzymes in plant stress response. *Antioxidants*, *9*(5), 454 .https://doi.org/10.3390/antiox9050454

Putri, G. H., Anders, S., Pyl, P. T., Pimanda, J. E., & Zanini, F. (2022). Analysing high-throughput sequencing data in Python with HTSeq 2.0. *Bioinformatics*, *38*(10), 2943-2945. https://doi.org/10.1093/bioinformatics/btac166

Rasmusson, A. G., Soole, K. L., & Elthon, T. E. (2004). Alternative NAD (P) H dehydrogenases of plant mitochondria. *Annu. Rev. Plant Biol.*, *55*(1), 23-39. https://doi.org/10.1146/annurev.arplant.55.031903.141720

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *bioinformatics*, *26*(1), 139-140. https://doi.org/10.1093/bioinformatics/btp616

Rodrigues, L., Nogales, A., Nunes, J., Rodrigues, L., Hansen, L. D., & Cardoso, H. (2023). Germination of Pisum sativum L. Seeds Is Associated with the Alternative Respiratory Pathway. *Biology*, *12*(10), 1318. https://doi.org/10.3390/biology12101318

Ruan, M., He, W., He, R., Wang, X., Wei, J., Zhu, Y., Li, R., Jiang, Z., Na X., Wang, X., & Bi, Y. (2024). Alternative oxidase 2 influences Arabidopsis seed germination under salt stress by modulating ABA signalling and ROS homeostasis. *Environmental and Experimental Botany*, *217*, 105568. https://doi.org/10.1016/j.envexpbot.2023.105568

Sachdev, S., Ansari, S. A., & Ansari, M. I. (2023). Antioxidant defensive mechanisms to regulate cellular redox homeostatic balance. In *Reactive Oxygen Species in Plants: The Right Balance* (pp. 143-172). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-19-9884-3_9

Schroeder, A., Mueller, O., Stocker, S., Salowsky, R., Leiber, M., Gassmann, M., ... & Ragg, T. (2006). The RIN: an RNA integrity number for assigning integrity values to RNA measurements. *BMC molecular biology*, *7*, 1-14. https://doi.org/10.1186/1471-2199-7-3

Smith, C., Barthet, M., Melino, V., Smith, P., Day, D., & Soole, K. (2011). Alterations in the mitochondrial alternative NAD (P) H dehydrogenase NDB4 lead to changes in mitochondrial electron transport chain composition, plant growth and response to oxidative stress. *Plant and Cell Physiology*, *52*(7), 1222-1237. https://doi.org/10.1093/pcp/pcr073

Sohn, H. B., Lee, H. Y., Seo, J. S., Jung, C., Jeon, J. H., Kim, J. H., Lee, W. Y., Lee, J. S., Cheong, J.J. & Choi, Y. D. (2011). Overexpression of jasmonic acid carboxyl methyltransferase increases tuber yield and size in transgenic potato. *Plant Biotechnology Reports*, *5*, 27-34. https://doi.org/10.1007/s11816-010-0153-0

Sweetman, C., Waterman, C. D., Rainbird, B. M., Smith, P. M., Jenkins, C. D., Day, D. A., & Soole, K. L. (2019). AtNDB2 is the main external NADH dehydrogenase in mitochondria and is important for tolerance to environmental stress. *Plant Physiology*, *181*(2), 774-788.https://doi.org/10.1104/pp.19.00877

Sweetman, C., Waterman, C. D., Wong, D. C., Day, D. A., Jenkins, C. L., & Soole, K. L. (2022). Altering the balance between AOX1A and NDB2 expression affects a common set of transcripts in Arabidopsis. *Frontiers in plant science*, *13*, 876843. https://doi.org/10.3389/fpls.2022.876843

Urbanek, H., Kuźniak-Gębarowska, E., & Herka, K. (1991). Elicitation of defence responses in bean leaves by Botrytis cinerea polygalacturonase. ISSN : 1861-1664

Vanlerberghe, G. C. (2013). Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. *International journal of molecular sciences*, *14*(4), 6805-6847. https://doi.org/10.3390/ijms14046805

Velada, I., Cardoso, H. G., Ragonezi, C., Nogales, A., Ferreira, A., Valadas, V., & Arnholdt-Schmitt, B. (2016). Alternative oxidase gene family in Hypericum perforatum L.: characterization and expression at the post-germinative phase. *Frontiers in Plant Science*, *7*, 1043. https://doi.org/10.3389/fpls.2016.01043

Velikova, V., Yordanov, I., & Edreva, A. J. P. S. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant science*, *151*(1), 59-66. https://doi.org/10.1016/S0168-9452(99)00197-1

Vishwakarma, A., Bashyam, L., Senthilkumaran, B., Scheibe, R., & Padmasree, K. (2014). Physiological role of AOX1a in photosynthesis and maintenance of cellular redox homeostasis under high light in Arabidopsis thaliana. *Plant Physiology and Biochemistry*, *81*, 44-53.https://doi.org/10.1016/j.plaphy.2014.01.019

Vishwakarma, A., Tetali, S. D., Selinski, J., Scheibe, R., & Padmasree, K. (2015). Importance of the alternative oxidase (AOX) pathway in regulating cellular redox and ROS homeostasis to optimize photosynthesis during restriction of the cytochrome oxidase pathway in Arabidopsis thaliana. *Annals of botany*, *116*(4), 555-569. https://doi.org/10.1093/aob/mcv122

Vishwakarma, A., Dalal, A., Tetali, S. D., Kirti, P. B., & Padmasree, K. (2016). Genetic engineering of AtAOX1a in Saccharomyces cerevisiae prevents oxidative damage and maintains redox homeostasis. *FEBS Open bio*, *6*(2), 135-146. https://doi.org/10.1002/2211-5463.12028

Wang, Y., Diao, P., Kong, L., Yu, R., Zhang, M., Zuo, T., Fan, Y., Niu, Y., Yan, F., & Wuriyanghan, H. (2020). Ethylene enhances seed germination and seedling growth under salinity by reducing oxidative stress and promoting chlorophyll content via ETR2 pathway. *Frontiers in plant science*, *11*, 1066. https://doi.org/10.3389/fpls.2020.01066

Wu, R., Lin, X., He, J., Min, A., Pang, L., Wang, Y., Lin, Y., Zhang, Y., He, W., Li, M., Zhang, Y., Luo, Y., Wang, X., Tang, H., & Chen, Q. (2023). Hexokinase1: a glucose sensor involved in drought stress response and sugar metabolism depending on its kinase activity in strawberry. *Frontiers in Plant Science*, *14*, 1069830. https://doi.org/10.3389/fpls.2023.1069830

Young, M. D., Wakefield, M. J., Smyth, G. K., & Oshlack, A. (2010). Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome biology*, *11*, 1-12..https://doi.org/10.1186/gb-2010-11-2-r14

Highlights

- PG treatment (2.5 mM) reduces initial germination followed by recovery at 48h;
- Physiological parameters and H_2O_2 content evidence germination recovery;
- Alternative oxidase genes (AOX1 and 2a) is involved in germination recovery;
- Glycolysis and ethanol fermentation play a vital role in PG-treated seed germination;
- AOX is linked to redirection of fermentation products in PG-treated seed germination.

Figure 1.

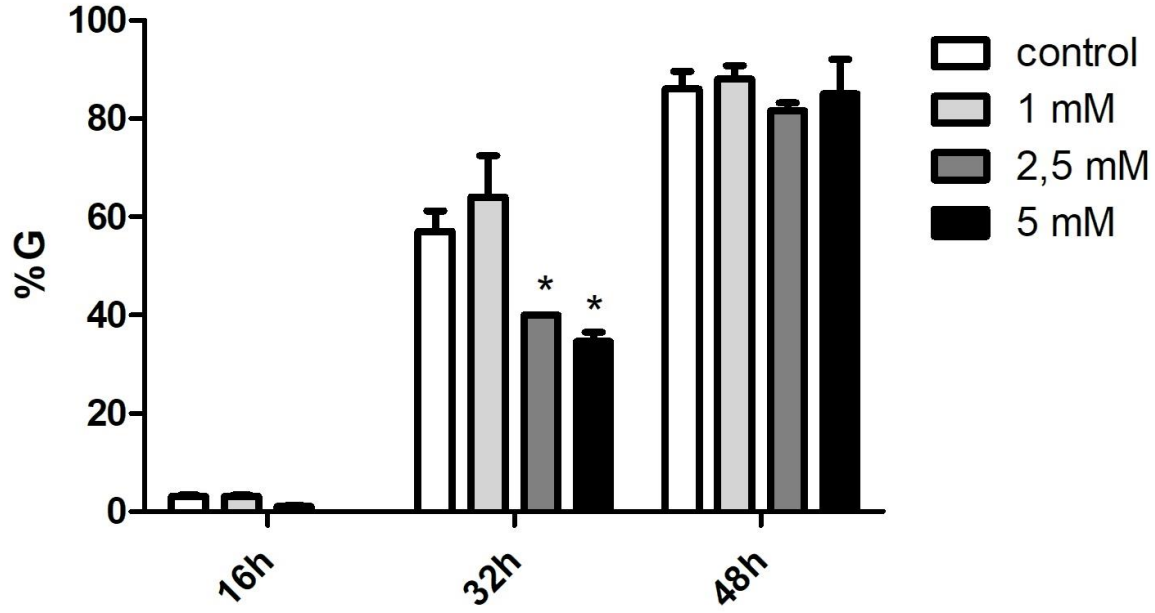


Figure 2.

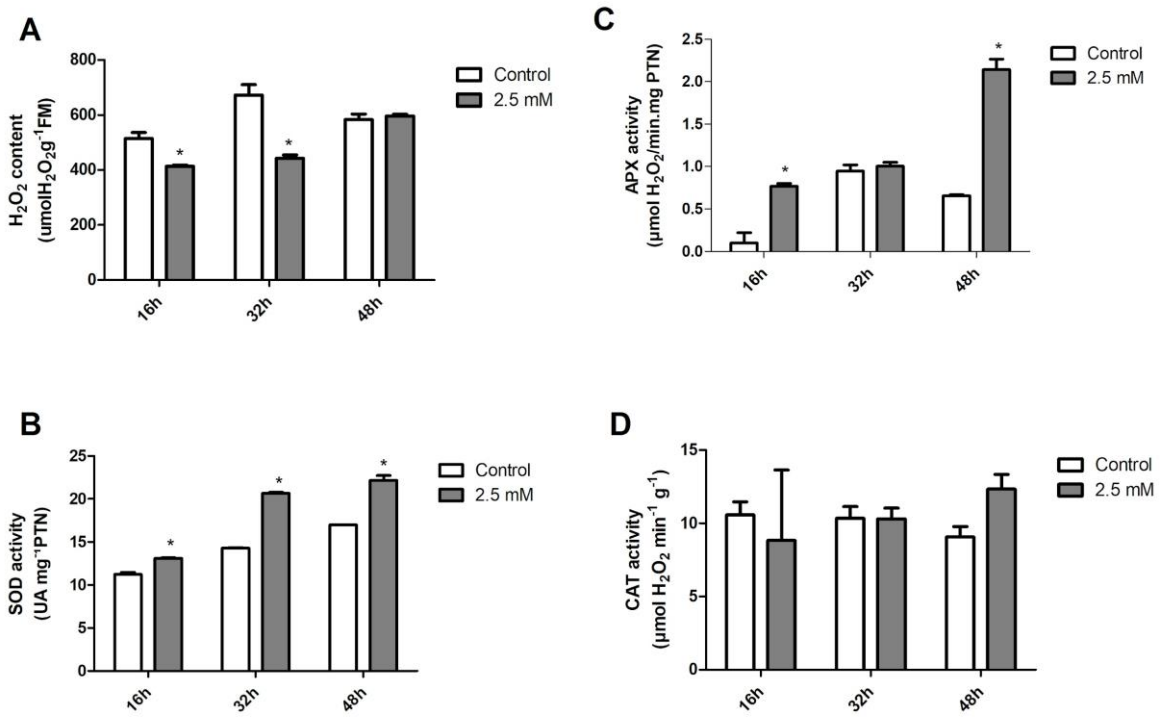

A
H_2O_2 content
(umolH_2O_2g^{-1}FM)
800
600
400
200
0
16h
32h
48h
Control
2.5 mM
*
B
SOD activity
(UA mg^{-1}PTN)
25
20
15
10
5
0
16h
32h
48h
Control
2.5 mM
C
APX activity
(µmol H_2O_2/min.mg PTN)
2.5
2.0
1.5
1.0
0.5
0.0
16h
32h
48h
Control
2.5 mM
D
CAT activity
(µmol H_2O_2 min^{-1} g^{-1})
15
10
5
0
16h
32h
48h
Control
2.5 mM

Figure 3.

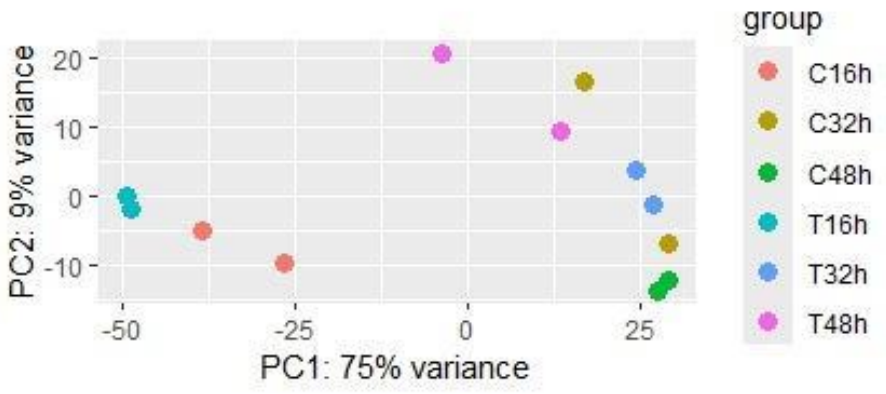

group
C16h
C32h
C48h
T16h
T32h
T48h
PC2: 9% variance
20
10
0
-10
-50
-25
0
25
PC1: 75% variance

Figure 4.

A

Comparisons	Total	Upregulated	Downregulated
T16h vs C16h	734	437	297
T32h vs C32h	122	41	81
T48h vs C48h	1207	743	464

B

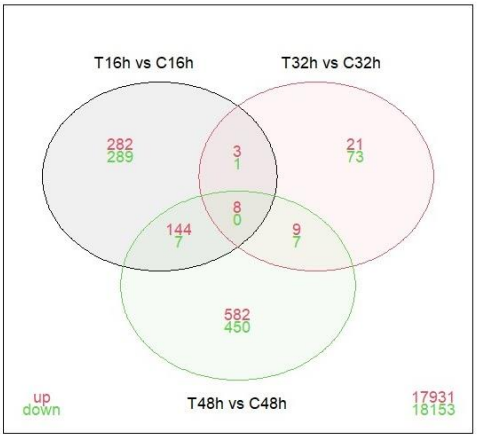
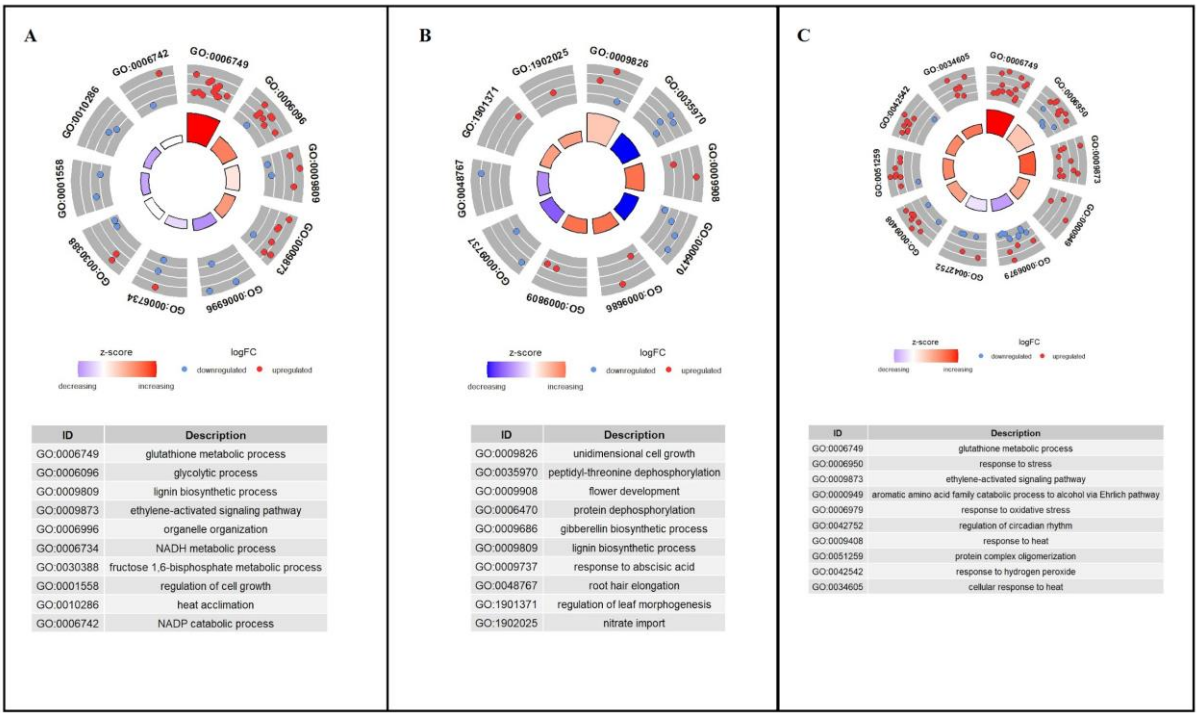


Figure 5.



ID	Description
GO:0006749	glutathione metabolic process
GO:0006096	glycolytic process
GO:0009809	lignin biosynthetic process
GO:0009873	ethylene-activated signaling pathway
GO:0006996	organelle organization
GO:0006734	NADH metabolic process
GO:0030388	fructose 1,6-bisphosphate metabolic process
GO:0001558	regulation of cell growth
GO:0010286	heat acclimation
GO:0006742	NADP catabolic process

ID	Description
GO:0009826	unidimensional cell growth
GO:0035970	peptidyl-threonine dephosphorylation
GO:0009908	flower development
GO:0006470	protein dephosphorylation
GO:0009686	gibberellin biosynthetic process
GO:0009809	lignin biosynthetic process
GO:0009737	response to abscisic acid
GO:0048767	root hair elongation
GO:1901371	regulation of leaf morphogenesis
GO:1902025	nitrate import

ID	Description
GO:0006749	glutathione metabolic process
GO:0006950	response to stress
GO:0009873	ethylene-activated signaling pathway
GO:0000949	aromatic amino acid family catabolic process to alcohol via Ehrlich pathway
GO:0006979	response to oxidative stress
GO:0042752	regulation of circadian rhythm
GO:0009408	response to heat
GO:0051259	protein complex oligomerization
GO:0042542	response to hydrogen peroxide
GO:0034605	cellular response to heat

Figure 6.

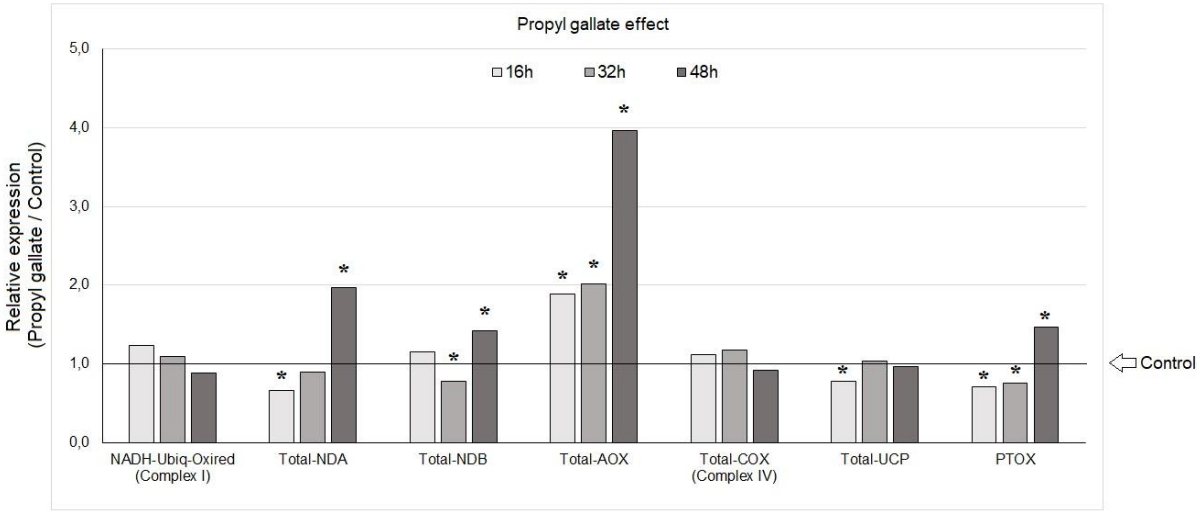

Propyl gallate effect
16h
32h
48h
Relative expression
(Propyl gallate / Control)
5,0
4,0
3,0
2,0
1,0
0,0
Control
NADH-Ubiq-Oxired
(Complex I)
Total-NDA
Total-NDB
Total-AOX
Total-COX
(Complex IV)
Total-UCP
PTOX

Figure 7

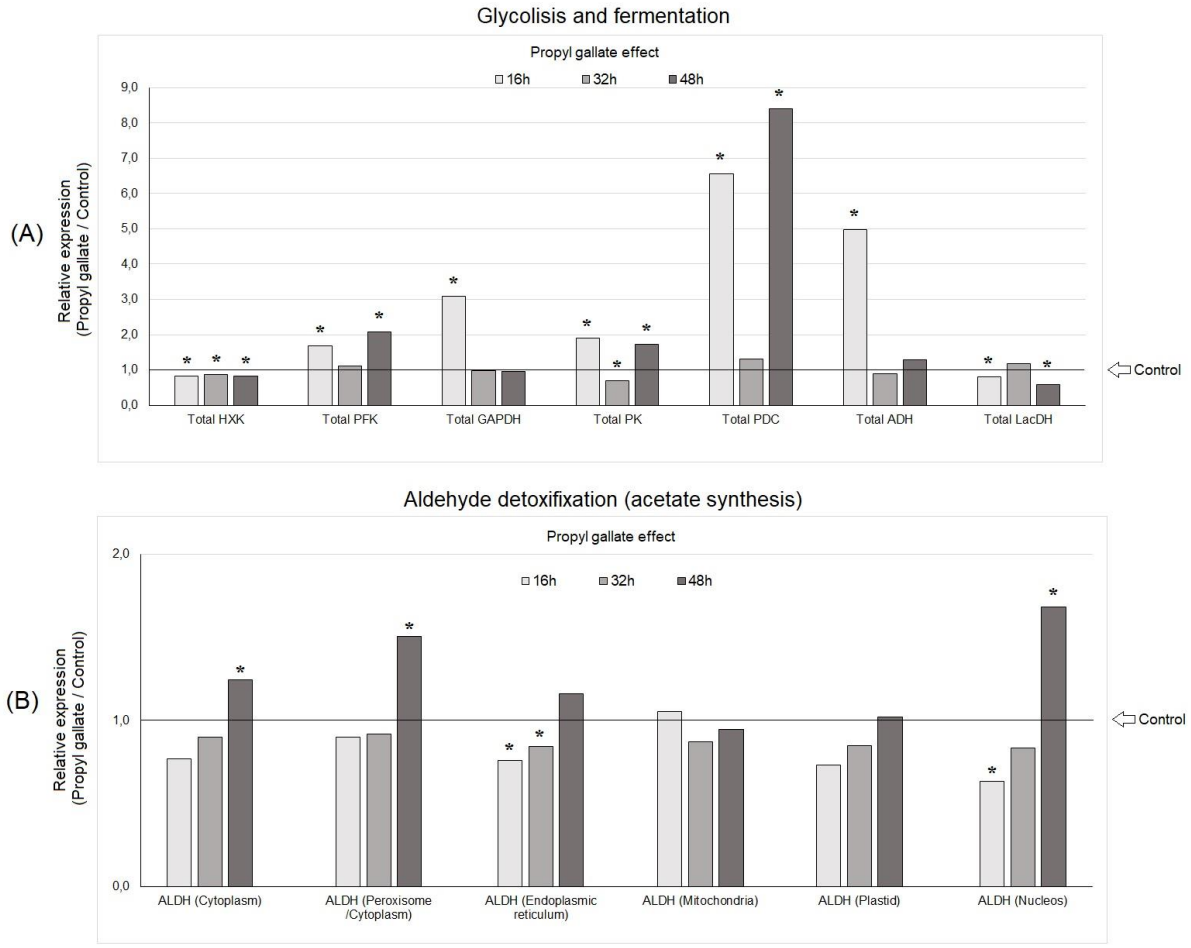

(A)
Glycolisis and fermentation
Propyl gallate effect
16h
32h
48h
Relative expression (Propyl gallate / Control)
9,0
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Total HXK
Total PFK
Total GAPDH
Total PK
Total PDC
Total ADH
Total LacDH
Control
(B)
Aldehyde detoxifixation (acetate synthesis)
Propyl gallate effect
16h
32h
48h
Relative expression (Propyl gallate / Control)
2,0
1,0
0,0
ALDH (Cytoplasm)
ALDH (Peroxisome /Cytoplasm)
ALDH (Endoplasmic reticulum)
ALDH (Mitochondria)
ALDH (Plastid)
ALDH (Nucleos)
Control

Figure 8.

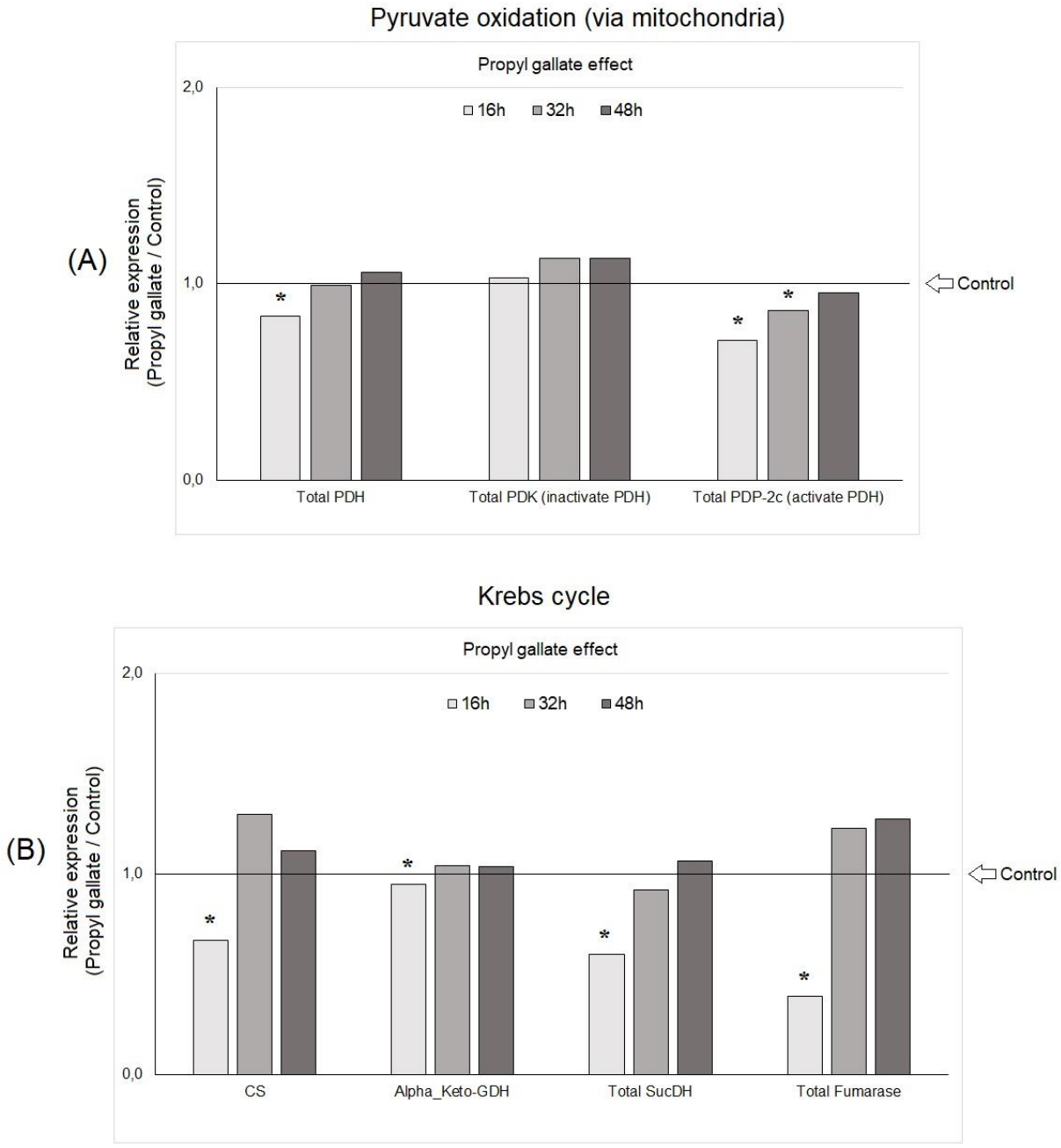

Pyruvate oxidation (via mitochondria)
Propyl gallate effect
16h
32h
48h
(A)
Relative expression
(Propyl gallate / Control)
2,0
1,0
0,0
Control
Total PDH
Total PDK (inactivate PDH)
Total PDP-2c (activate PDH)
Krebs cycle
Propyl gallate effect
16h
32h
48h
(B)
Relative expression
(Propyl gallate / Control)
2,0
1,0
0,0
Control
CS
Alpha_Keto-GDH
Total SucDH
Total Fumarase

Figure 9

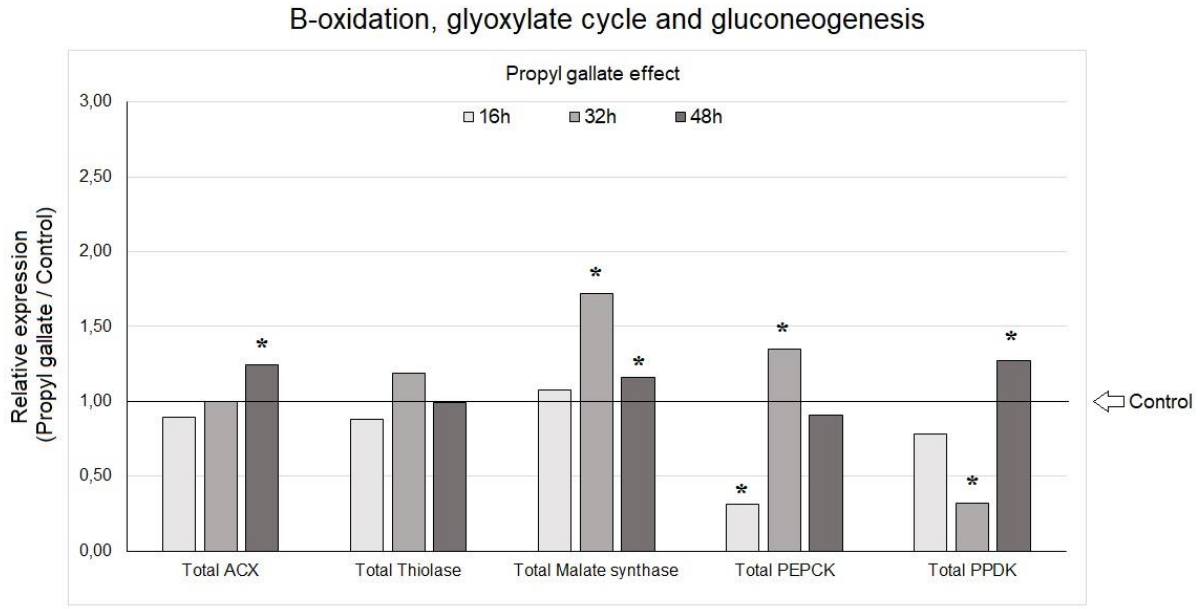

B-oxidation, glyoxylate cycle and gluconeogenesis
Propyl gallate effect
16h
32h
48h
Relative expression
(Propyl gallate / Control)
3,00
2,50
2,00
1,50
1,00
0,50
0,00
Control
Total ACX
Total Thiolase
Total Malate synthase
Total PEPCK
Total PPDK

Figure 10.

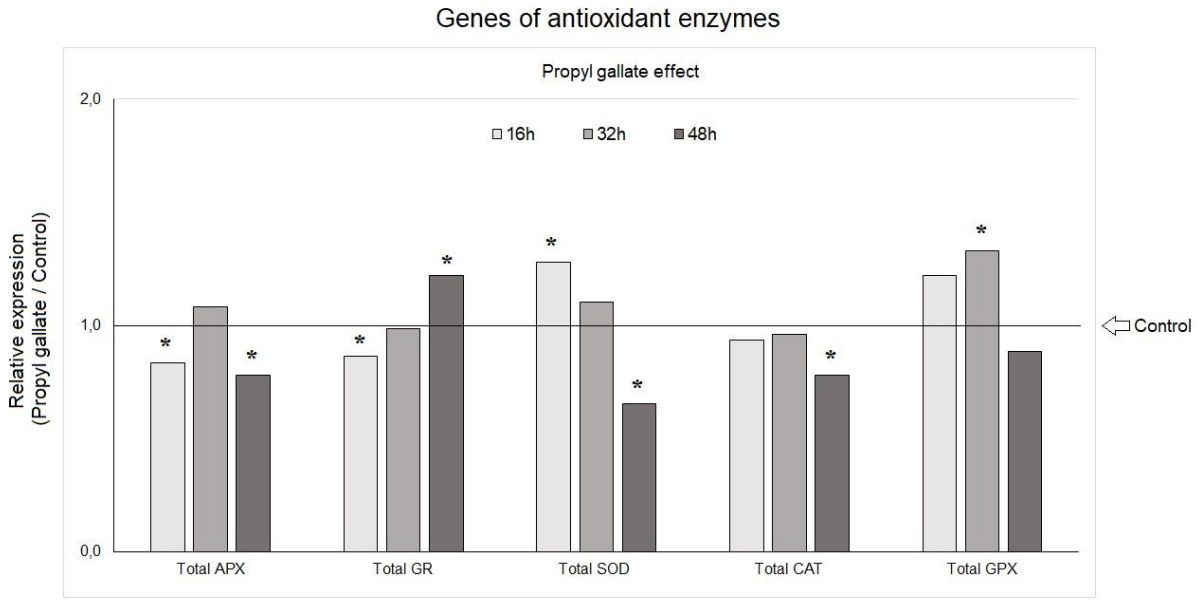
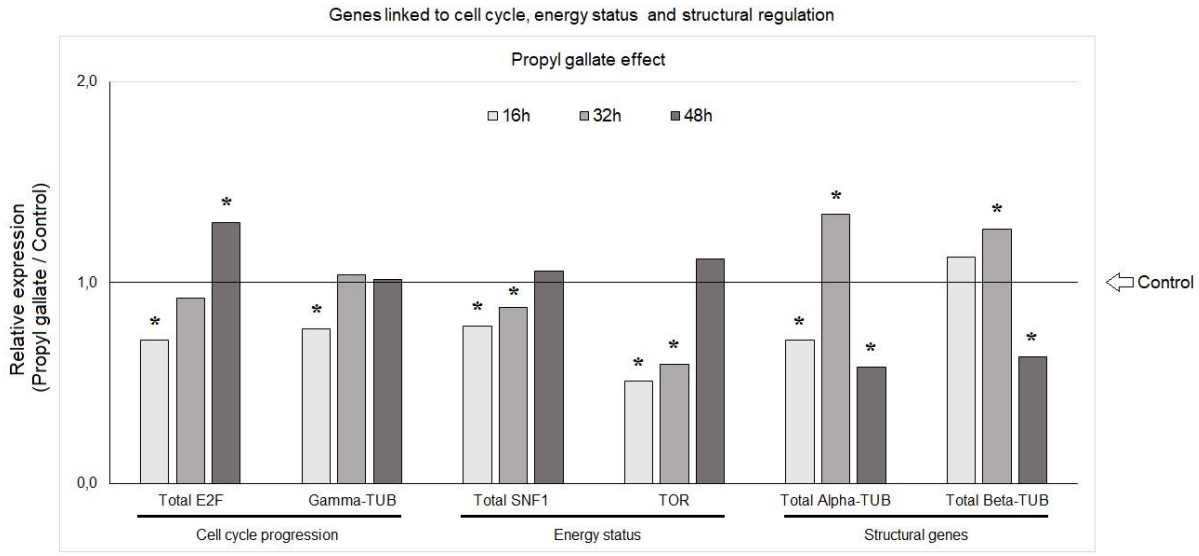

Genes of antioxidant enzymes
Propyl gallate effect
16h
32h
48h
2,0
1,0
0,0
Relative expression
(Propyl gallate / Control)
Control
Total APX
Total GR
Total SOD
Total CAT
Total GPX

Figure 11


Genes linked to cell cycle, energy status and structural regulation
Propyl gallate effect
16h
32h
48h
Relative expression
(Propyl gallate / Control)
2,0
1,0
0,0
Control
Total E2F
Gamma-TUB
Total SNF1
TOR
Total Alpha-TUB
Total Beta-TUB
Cell cycle progression
Energy status
Structural genes

Supplementary figure 1.

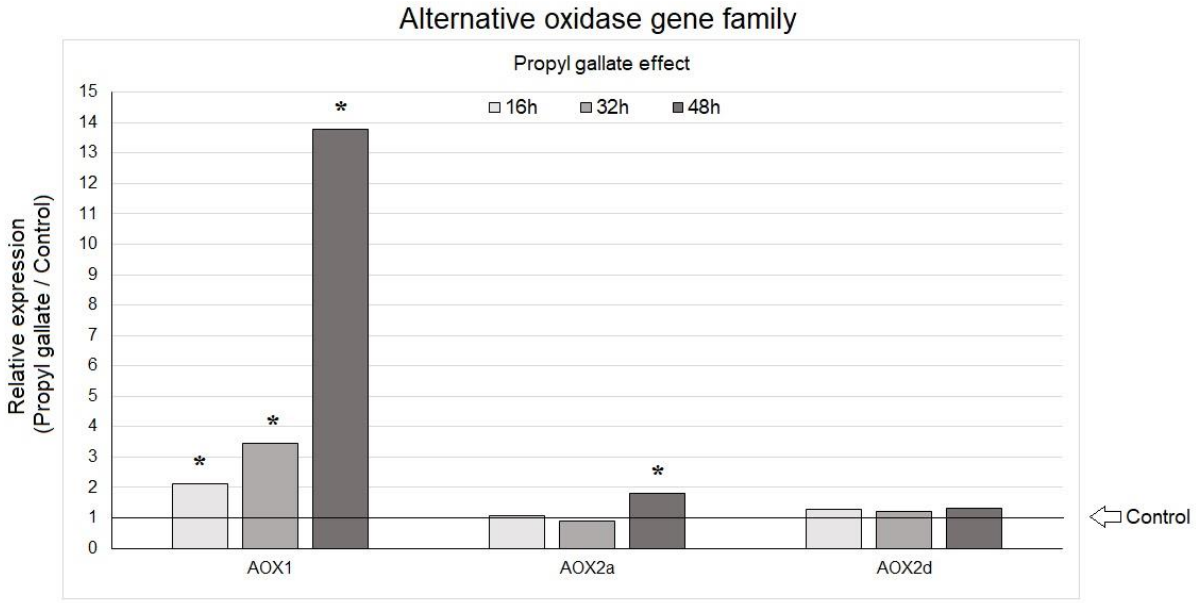


Table 1.

Treatment	%G	GSI	MTG
Control	86 ± 2,08	26,07 ± 0,27	1,76 ± 0,08
1 mM	92 ± 4,16	28,61 ± 0,54	1,74 ± 0,09
2,5 mM	82 ± 0,88	21,40 ± 0,17*	1,65 ± 0,02
5 mM	80 ± 55,7	20,22 ± 0,53*	1,71 ± 0,003

Table 2.

Treatment	**RL (16h)**	**RL (32h)**	**RL (48h)**
Control	0.5 ± 0.05	0.98 ± 0.12	1.48 ± 0.11
1 mM	0.35 ± 0.05	1.01 ± 0.10	1,49 ± 0.10
2.5 mM	0.2 ± 0.05 *	0.79 ± 0.07	1.38 ± 0.07
5 mM	0 *	0.64 ± 0.04 *	1.00 ± 0.06 *

Supplementary Table 1.

Function	Gene	Gene members	Accession number (mRNA)
B-oxidation	acyl-coenzyme A oxidase (step 1)	Vu_ACX-1a	XM_028063480.1 (LOC114177891)
		Vu_ACX-1b	XM_028058596.1 (LOC114173920)
		Vu_ACX-2	XM_028065526.1 (LOC114179259)
		Vu_ACX-3	XM_028055069.1 (LOC114169770)
	3-ketoacyl-CoA thiolase (step 4)	Vu_thiolase-1a	XM_028060622.1 (LOC114175812)
		Vu_thiolase-1b	XM_028084366.1 (LOC114194242)
		Vu_thiolase-2a	XM_028082482.1 (LOC114192697)
		Vu_thiolase-2b	XM_028050924.1 (LOC114166224)
Glyoxylate cycle	malate synthase	Vu_Mls-1a	XM_028061615.1 (LOC114176542)
		Vu_Mls-1b	XM_028062841.1 (LOC114177473)
Gluconeogenesis	phosphoenolpyruvate carboxykinase (PEPCK)	Vu_PEPCK-1	XM_028062228.1 (LOC114177011),
		Vu_PEPCK-2	XM_028059044.1 (LOC114174245)
	pyruvate orthophosphate dikinase (PPDK)	Vu_PPDK-1	XM_028060788.1 (LOC114175936)
		Vu_PPDK-2	XM_028060791.1 (LOC114175937)
Glycolysis	Hexokinase (Total HXK)	Vu_HXK-1a	XM_028063707.1 (LOC114178033)
		Vu_HXK-1b	XM_028069172.1 (LOC114182319)
		Vu_HXK-2	XM_028050233.1 (LOC114165663)
	Phosphofructokinase (PFK) (Total PFK)	Vu_PFK-1	XM_028068772.1 (LOC114182031)
		Vu_PFK-2	XM_028061414.1 (LOC114176374)
		Vu_PFK-3	XM_028062331.1 (LOC114177095)
		Vu_PFK-6	XM_028048145.1 (LOC114163808)
	Pyruvate kinase (Total PK)	Vu_PK-1a	XM_028047959.1 (LOC114163662)
		Vu_PK-1b	XM_028081428.1 (LOC114191953)
		Vu_PK-1c	XM_028078242.1 (LOC114189621)
		Vu_PK-1d	XM_028081720.1 (LOC114192117)

		Vu_PK-5	XM_028068289.1 (LOC114181745)
		Vu_PK-6	XM_028063062.1 (LOC114177636)
		Vu_PK-7	XM_028068328.1 (LOC114181778)
Fermentation	Pyruvate decarboxylase (Total PDC)	Vu_PDC-1a	XM_028077776.1 (LOC114189071)
		Vu_PDC-1b	XM_028077738.1 (LOC114189040)
		Vu_PDC-2a	XM_028051292.1 (LOC114166544)
		Vu_PDC-2b	XM_028072584.1 (LOC114185083)
	Alcohol dehydrogenase (Total ADH)	Vu_ADH-1a	XM_028049295.1 (LOC114164576)
		Vu_ADH-1b	XM_028049550.1 (LOC114164787)
		Vu_ADH-1c	XM_028083103.1 (LOC114193351)
		Vu_ADH-1d	XM_028085065.1 (LOC114194697)
		Vu_ADH-3a	XM_028079486.1 (LOC114190551)
		Vu_ADH-3b	XM_028046928.1 (LOC114162918)
	Lactate dehydrogenase (Total LacDH)	Vu_LacDH-1a	XM_028078734.1 (LOC114189998)
		Vu_LacDH-1b	XM_028051221.1 (LOC114166484)
Pyruvate oxidation via mitochondria	Complex of the pyruvate dehydrogenase (Total PDH)	Vu_PDH-E1A1	XM_028058497.1 (LOC114173848)
		Vu_PDH-E1A2	XM_028065072.1 (LOC114178926)
		Vu_PDH-E1B	XM_028073272.1 (LOC114185513)
		Vu_PDH-E2.1	XM_028081038.1 (LOC114191691)
		Vu_PDH-E2.2	XM_028085291.1 (LOC114194883)
		Vu_PDH-E2.3	XM_028050612.1 (LOC114165967)
	Pyruvate dehydrogenase kinase (PD Kinase)	Vu_PDK-1a	XM_028049255.1 (LOC114164544)
		Vu_PDK-1b	XM_028062439.1 (LOC114177182)
	Pyruvate dehydrogenase phosphatase 2C (PDC 2c)	Vu_PDP-2c1	XM_028072271.1 (LOC114184888)
		Vu_PDP-2c2	XM_028074323. (LOC114186265)
Krebs cycle	Citrate synthase (Total CS)	Vu_CS	XM_028082598.1 (LOC114192780)
	Alpha-ketoglutarate dehydrogenase (Total Alpha_keto-GDH)	Vu_Alpha_Keto-GDH-1	XM_028084521.1 (LOC114194355)
	Succinate dehydrogenase (Total	Vu_SucDH-1	XM_028061581.1

	SucDH)		(LOC114176517)
		Vu_SucDH-2	XM_028057623.1 (LOC114173323)
	Fumarase	Vu_Fumarase-1a	XM_028082566.1 (LOC114192758)
		Vu_Fumarase-1b	XM_028082202.1 (LOC114192469)
ROS formation control	Alternative oxidase (Total AOX)	Vu_AOX1	XM_028055801.1 (LOC114170318)
		Vu_AOX2a	XM_028062079.1 (LOC114176891)
		Vu_AOX2d	XM_028066450.1 (LOC114179937)
	Total UCP	Vu_UCP1a	XM_028050698.1 (LOC114166047)
		Vu_UCP1b	XM_028071081.1 (LOC114183904)
		Vu_UCP2	XM_028076161.1 (LOC114187792)
	PTOX	Vu_PTOX	XM_028074643.1 (LOC114186666)
ROS scavenger	cytosolic APX	Vu_APX1	XM_028054667.1 (LOC114169494)
	peroxisomal APX	Vu_APX3	XM_028055281.1 (LOC114169897)
		Vu_APX5	XM_028057776.1 (LOC114173407)
	plastid APX	Vu_APX4	XM_028083367.1 (LOC114193534)
	plastid.mito APX	Vu_stAPX	XM_028048755.1 (LOC114164188)
		Vu_APX6	XM_028046378.1 (LOC114162470)
	cyto.peroxi GR	Vu_GR-1	XM_028068533.1 (LOC114181890)
	plastid.mito GR	Vu_GR-2	XM_028081336.1 (LOC114191891)
	cytosolic SOD	Vu_Cu/Zn-SOD1	XM_028076317.1 (LOC114187910)
	peroxisomal SOD	Vu_Cu/Zn-SOD3	XM_028067824.1 (LOC114181385)
	plastidial SOD	Vu_Cu/Zn-SOD2	XM_028055564.1 (LOC114170083)
		Vu_Fe-SOD1	XM_028081587.1 (LOC114192036)
		Vu_Fe-SOD2	XM_028079044.1 (LOC114190239)
		Vu_Fe-SOD3	XM_028056565.1 (LOC114171688)
		Vu_Cu/Chap-SOD1	XM_028064610.1 (LOC114178604)
	mitochondrial SOD	Vu_Mn-SOD1	XM_028048111.1 (LOC114163789)
	cytosolic GPX	Vu_GPX8a	XM_028056842.1 (LOC114172250)
		Vu_GPX8b	XM_028061393.1

			(LOC114176364)
	plastidial GPX	Vu_GPX1	XM_028085774.1 (LOC114195346)
	mitochondrial GPX	Vu_GPX6	XM_028065067.1 (LOC114178919)
	RE/Golgi GPX	Vu_GPX3	XM_028059687.1 (LOC114174952)
	Total CAT	Vu_CAT1	XM_028047190.1 (LOC114163102)
		Vu_CAT3	XM_028084938.1 (LOC114194602)
Cell respiration	Total-NDA (NAD(P)H dehydrogenase A)	Vu_NDA1	XM_028077841.1 (LOC114189132)
		Vu_NDA2.1	XM_028085161.1 (LOC114194763)
		Vu_NDA2.2	XM_028063003.1 (LOC114177594)
	Total-NDB (NAD(P)H dehydrogenase B)	Vu_NDB1	XM_028055259.1 (LOC114169881)
		Vu_NDB2.1	XM_028075386.1 (LOC114187206)
		Vu_NDB2.2	XM_028065088.1 (LOC114178936)
		Vu_NDB3	XM_028055507.1 (LOC114170040)
	NADH: ubiquinone oxidoreductase (complex I)	Vu_NADH-Ubiq-Oxired	XM_028053216.1 (LOC114168419)
	Cytochrome C oxidase (Total COX – complex IV)	Vu_COX-2.1	NM_001376035.1 (LOC114163077)
		Vu_COX-2.2	XM_028046434.1 (LOC114162517)
		Vu_COX-5b1	XM_028051595.1 (LOC114166776)
		Vu_COX-5b2	XM_028070361.1 (LOC114183357)
		Vu_COX-5c1	XM_028066134.1 (LOC114179430)
		Vu_COX-5c2	XM_028078119.1 (LOC114189531)
		Vu_COX-6a	XM_028061166.1 (LOC114176209)
		Vu_COX-6b	XM_028058997.1 (LOC114174208)
		Vu_COX-6b1	XM_028086012.1 (LOC114195513)
		Vu_COX-6b2	XM_028061247.1 (LOC114176265)
		Vu_COX-A	XM_028054993.1 (LOC114169726)
		Vu_COX-B	XM_028074202.1 (LOC114186175)
		Vu_COX-C	XM_028062676.1 (LOC114177367)
Acetate synthesis	Cytosolic Aldehyde dehydrogenase (ALDH)	Vu_ALDH22A1-RE1	XM_028063094.1 (LOC114177656)
		Vu_ALDH22A1-RE2	XM_028077685.1

			(LOC114188989)
		Vu_ALDH10A8-cit_pero1	XM_028065277.1 (LOC114179073)
		Vu_ALDH10A8-cit_pero2	XM_028049635.1 (LOC114164863)
		Vu_ALDH7B4-cyt1	XM_028047530.1 (LOC114163305)
		Vu_ALDH2C4-cyt1	XM_028065686.1 (LOC114179363
		Vu_ALDH2C4-cyt2	XM_028068936.1 (LOC114182153)
		Vu_ALDH2C4-cyt3	XM_028066035.1 (LOC114179619)
		Vu_ALDH2C4-cyt4	XM_028067492.1 (LOC114181137)
		Vu_ALDH3F1-RE1	XM_028071531.1 (LOC114184247)
		Vu_ALDH3F1-RE2	XM_028062413.1 (LOC114177158)
		Vu_ALDH3F1_RE3	XM_028053505.1 (LOC114168619)
		Vu_ALDH3H1-cyt1	XM_028046199.1 (LOC114162343)
		Vu_ALDH3H1-RE1	XM_028084736.1 (LOC114194483)
	Mitochondrial Aldehyde dehydrogenase (ALDH)	Vu_ALDH12A1-Mit1	XM_028059707.1 (LOC114174972)
		Vu_ALDH6B2-Mit	XM_028063639.1 (LOC114177993)
		Vu_ALDH6B2-Nucleos	XM_028080987.1 (LOC114191663)
		Vu_ALDH5F1-Mit	XM_028060439.1 (LOC114175660)
		Vu_ALDH2B4-Mit1	XM_028065328.1 (LOC114179110)
		Vu_ALDH2B7-Mit1	XM_028057386.1 (LOC114173144)
		Vu_ALDH2B7-Mit2	XM_028064403.1 (LOC114178474)
		Vu_ALDH2B7-Mit3	XM_028067457.1 (LOC114181107)
	Plastidic Aldehyde dehydrogenase (ALDH)	Vu_ALDH3H1-Plast1	XM_028065345.1 (LOC114179122)
Cell cycle regulation	E2F transcription factor (Total E2F)	Vu_ E2F-A	XM_028078513.1 (LOC114189813)
		Vu_ E2F-B	XM_028054674.1 (LOC114169500)
		Vu_ E2F-C	XM_028046680.1 (LOC114162719)
	Gamma-tubulin (Gamma TUB)	Vu_Gamma-TUB	XM_028074485.1 (LOC114186550)
Energy status regulation	SNF1 kinase (Total SNF1)	Vu_SNF1-1	XM_028072361.1 (LOC114184942
		Vu_SNF1-2	XM_028068856.1 (LOC114182100)
		Vu_SNF1-3	XM_028074104.1

			(LOC114186086)
	Target of rapamycin (TOR)	Vu_TOR	XM_028057512.1 (LOC114173244)
Structural cell reorganization	Alpha tubulin (Alpha TUB)	Vu_Alpha-TUB1	XM_028067332.1 (LOC114181014)
		Vu_Alpha-TUB2	XM_028060055.1 (LOC114175274)
		Vu_Alpha-TUB3	XM_028068016.1 (LOC114181541)
		Vu_Alpha-TUB4	XM_028048180.1 (LOC114163828)
		Vu_Alpha-TUB5	XM_028081366.1 (LOC114191912)
		Vu_Alpha-TUB6	XM_028054573.1 (LOC114169430)
		Vu_Alpha-TUB7	XM_028063140.1 (LOC114177683)
	Beta tubulin (Beta TUB)	Vu_Beta-TUB1	XM_028086905.1 (LOC114196303)
		Vu_Beta-TUB2	XM_028060987.1 (LOC114176077)
		Vu_Beta-TUB3	XM_028085170.1 (LOC114194769)
		Vu_Beta-TUB4	XM_028080185.1 (LOC114191038)
		Vu_Beta-TUB5	XM_028075722.1 (LOC114187461)
		Vu_Beta-TUB6	XM_028063438.1 (LOC114177857)
		Vu_Beta-TUB7	XM_028051785.1 (LOC114166910)
		Vu_Beta-TUB8	XM_028060038.1 (LOC114175259)

Supplementary table 2

Name of DEGS	ID	log2FC_16h	log2FC_32h	log2FC_48h
Probable mannitol dehydrogenase	LOC114173915	6,08252817	3,766584283	5,868942512
Probable glutathione S-transferase	LOC114183990	2,877651086	1,621872006	3,622364636
Jasmonic acid carboxyl methyltransferase	LOC114164464	4,474315226	2,984486685	3,988784868
Cytochrome P450 CYP736A12-like	LOC114179959	1,819159009	1,319661783	1,153913627
Transcription factor ORG2-like	LOC114178106	5,954439666	4,801466549	5,776209011
Ethylene-responsive transcription factor ERF107-like	LOC114191052	1,850641543	1,80767952	2,050052624
1-aminocyclopropane-1-carboxylate oxidase 1-like	LOC114179298	2,235931985	2,266922036	3,123877762
Probable mannitol dehydrogenase	LOC114173303	3,94196333	3,813630879	8,146935446

Supplementary table 3

	category	over_represe	under_repre	numDEInCat	numInCat	term
1227	GO:000674	2,91E+05	#########	13	42	glutathione r
986	GO:000609	6,28E+08	#########	9	39	glycolytic pr
1694	GO:000980	2,57E+09	#########	5	11	lignin biosyn
1711	GO:000987	7,98E+09	#########	7	29	ethylene-act
1289	GO:000699	#########	#########	3	5	organelle or
1220	GO:000673	#########	#########	3	5	NADH metal
2404	GO:003038	#########	#########	4	11	fructose 1,6-
244	GO:000155	#########	1	2	2	regulation of
1832	GO:001028	#########	1	2	2	heat acclim

Supplementary table 4

	NADH-Ubiq-Oxired	Total-NDA	Total-NDB	Total-AOX	Total-COX	Total-UCP	PTOX
Control 16h	68,22 ± 5,69	18,71 ± 0,75	38,33 ± 1,54	256,78 ± 12,78	906,11 ± 24,99	11,73 ± 0,84	28,78 ± 2,53
Control 32h	115,81 ± 4,78	35,14 ± 1,07	29,66 ± 1,72	49,32 ± 9,97	1370,89 ± 79,42	27,95 ± 1,33	33,31 ± 2,39
Control 48h	130,78 ± 4,59	15,3 ± 0,36	21,92 ± 1,42	28,37 ± 0,4	1424,25 ± 47,83	28,12 ± 3,52	20,58 ± 0,72
Propyl gallate 16h	84,46 ± 8,79	12,45 ± 1,08*	44,12 ± 6,51	483,62 ± 14,18*	1006,61 ± 33,55	9,11 ± 0,37*	20,44 ± 2,87*
Propyl gallate 32h	126,62 ± 10,98	31,21 ± 4,28	23,1 ± 1,58*	99,51 ± 4,2*	1610,87 ± 96,67	28,78 ± 1,87	24,97 ± 1,92*
Propyl gallate 48h	114,93 ± 17,47	30,19 ± 1,56*	31 ± 1,91*	112,3 ± 9,27*	1300,1 ± 51,32	27,08 ± 2,09	30,18 ± 4,69*

Supplementary table 5

	AOX1	**AOX2a**	**AOX2d**
Control 16h	192,39 ± 1,23	43,34 ± 10,02	21,06 ± 6,8
Control 32h	19,36 ± 9,06	11,59 ± 1,88	18,38 ± 2,19
Control 48h	5,79 ± 0,54	5,99 ± 0,2	16,6 ± 0,78
Propyl gallate 16h	410,65 ± 11,55*	45,89 ± 3,35	27,09 ± 2,46
Propyl gallate 32h	66,87 ± 3,89*	10,4 ± 1,34	22,24 ± 2,33
Propyl gallate 48h	79,78 ± 12,92*	10,75 ± 1,07*	21,77 ± 1,84

Supplementary table 6

	Total HXK	Total PFK	Total PK	Total PDC	Total ADH	Total LacDH
Control 16h	110,56 ± 1,52	44,35 ± 3,31	290,28 ± 14,47	164,71 ± 38,49	152,59 ± 12,04	84,75 ± 2,22
Control 32h	409,86 ± 11,12	52,28 ± 2,21	499,13 ± 42,94	135,81 ± 8,52	497,35 ± 20,25	432,53 ± 14,07
Control 48h	433,91 ± 7,69	45,4 ± 2,14	301,67 ± 20,93	53,49 ± 1,25	467,72 ± 25,35	656,22 ± 12,11
Propyl gallate 16h	90,95 ± 3,18*	74,14 ± 1,87*	549,75 ± 17,79*	1078,53 ± 45,92*	760,46 ± 29,11*	68,81 ± 0,72*
Propyl gallate 32h	355,18 ± 14,13*	58,36 ± 2,38	350,05 ± 16,32*	178,84 ± 13,81	448,12 ± 31,37	510,89 ± 34,42
Propyl gallate 48h	360,43 ± 12,78*	94,19 ± 3,01*	522,63 ± 38,86*	449,77 ± 1,84*	603,66 ± 62	380,47 ± 28,31*

Supplementary table 7

	ALDH (Cytoplasm)	ALDH (Peroxisome /Cytoplasm)	ALDH (Endoplasmic reticulum)	ALDH (Mitochondria)	ALDH (Plastid)	ALDH (Nucleus)
Control 16h	493,11 ± 67,56	559,25 ± 29,57	262,29 ± 16,56	1021,08 ± 19,87	6,55 ± 0,73	3,37 ± 0,23
Control 32h	776,7 ± 20,2	852,76 ± 12	345,33 ± 11,99	965,92 ± 59,99	19,64 ± 2,72	2,66 ± 0,08
Control 48h	634,77 ± 26,36	524,3 ± 17,82	272,43 ± 19,65	986,59 ± 47,58	14,72 ± 0,69	2,44 ± 0,19
Propyl gallate 16h	378,57 ± 2,85	502,09 ± 3,51	199,14 ± 3,23*	1075,89 ± 4,25	4,79 ± 0,98	2,13 ± 0,18*
Propyl gallate 32h	698,19 ± 35,59	783,38 ± 48,45	291,06 ± 12,96*	839,27 ± 38,06	16,61 ± 2,51	2,23 ± 0,36
Propyl gallate 48h	788,66 ± 8,31*	789,64 ± 57,14*	316,26 ± 12,59	935,41 ± 53,16	15,02 ± 2,01	4,11 ± 0,49*

Supplementary table 8

	Total PDH	Total PDK	Total PDC 2c
Control 16h	335,1 ± 10,88	24,54 ± 0,88	59,34 ± 6,39
Control 32h	467,25 ± 38,74	97,75 ± 6,35	60,84 ± 2,14
Control 48h	410,81 ± 19,44	84,76 ± 7,03	48,89 ± 3,19
Propyl gallate 16h	279,52 ± 7,72*	25,31 ± 2,42	42,25 ± 0,39*
Propyl gallate 32h	462,7 ± 15,26	110,62 ± 2,02	52,44 ± 0,89*
Propyl gallate 48h	435,13 ± 9,74	95,75 ± 8,24	46,55 ± 5,01

Supplementary table 9

	CS	Alpha_Keto-GDH	Total SucDH	Total Fumarase
Control 16h	51 ± 2,71	125,71 ± 1,23	63,45 ± 4,17	13,21 ± 3,81
Control 32h	62,67 ± 2,09	160,29 ± 8,58	125,1 ± 8,01	35,88 ± 8,18
Control 48h	63,84 ± 1,42	136,33 ± 9,24	108,87 ± 6,6	30,38 ± 0,73
Propyl gallate 16h	34,17 ± 0,53*	119,6 ± 0,29*	37,99 ± 1,12*	5,17 ± 0,1*
Propyl gallate 32h	81,29 ± 14,24	167,25 ± 13,56	115,49 ± 5,07	44,06 ± 3,7
Propyl gallate 48h	71,34 ± 4,94	141,61 ± 10,6	115,97 ± 7,23	38,77 ± 1,93*

Supplementary table 10

	Total ACX	Total Thiolase	Total Malate synthase	Total PEPCK	Total PPDK
Control 16h	120,89 ± 6,48	278,32 ± 32,72	587,97 ± 17,68	60,02 ± 4,84	3,17 ± 0,3
Control 32h	155,85 ± 7,49	451,95 ± 7,78	270,82 ± 19,94	419,06 ± 47,57	4,79 ± 0,13
Control 48h	135,34 ± 9,38	451,78 ± 16,14	183,46 ± 5,83	523,8 ± 7,08	2,75 ± 0,13
Propyl gallate 16h	108,18 ± 2,05	245,29 ± 8,15	641,17 ± 23,15	18,56 ± 0,54*	2,47 ± 0,12
Propyl gallate 32h	155,9 ± 10,08	536,02 ± 37,77	466,45 ± 3,73*	563,73 ± 6,72*	1,51 ± 0,11*
Propyl gallate 48h	168,43 ± 2,59*	446,94 ± 21,12	212,26 ± 7,94*	475,03 ± 58,73	3,49 ± 0,2*

Supplementary table 11

	Total APX	Total GR	Total SOD	Total CAT	Total GPX
Control 16h	469,73 ± 2,05	96,66 ± 5,25	372,39 ± 11,81	606,54 ± 28,91	521,64 ± 30,81
Control 32h	1294,83 ± 57,39	158,44 ± 23,5	936,75 ± 94,2	657,91 ± 7,81	732,14 ± 24,81
Control 48h	1511,73 ± 55,44	110,55 ± 5,1	1510,44 ± 114,72	576,07 ± 12,49	908,21 ± 63,73
Propyl gallate 16h	391,76 ± 19,39*	83,66 ± 3,03*	475,7 ± 4,11*	567,89 ± 1,43	636,51 ± 44,89
Propyl gallate 32h	1399,87 ± 66,11	156,17 ± 4,24	1031,45 ± 94,3	633,04 ± 6,16	973,97 ± 20,29*
Propyl gallate 48h	1181,33 ± 15,79*	134,8 ± 7,56*	990,32 ± 61,18*	448,36 ± 8,05*	802,3 ± 26,33

Supplementary table 12

	Total E2F	Gamma-TUB	Total SNF1	TOR	Total Alpha-TUB	Total Beta-TUB
Control 16h	90,57 ± 3,57	7,52 ± 0,22	113,72 ± 2,88	19,37 ± 1,47	697,38 ± 43,62	525 ± 36,01
Control 32h	46,66 ± 4,52	15,58 ± 1,36	122,58 ± 1,88	15,61 ± 1,21	1264,3 ± 41,62	1108,95 ± 34,61
Control 48h	33,18 ± 1,92	15,67 ± 1,65	114,42 ± 7,41	13,05 ± 0,47	1954,53 ± 55,37	1557,69 ± 23,62
Propyl gallate 16h	64,55 ± 4,51*	5,77 ± 0,71*	89,29 ± 2,08*	9,9 ± 0,27*	496,61 ± 6,91*	591,54 ± 17,33
Propyl gallate 32h	42,95 ± 1,19	16,22 ± 2,23	107,63 ± 1,45*	9,26 ± 0,4*	1697,58 ± 137,93*	1403,25 ± 32,24*
Propyl gallate 48h	43,18 ± 2,63*	15,93 ± 0,74	120,91 ± 4,59	14,6 ± 3,03	1136,01 ± 73,51*	978 ± 5,97*

REFERÊNCIAS

ARNHOLDT-SCHMITT, Birgit; MOHANAPRIYA, Gunasekaran; SATHISHKUMAR, Ramalingam; MACEDO, Elisete Santos; COSTA, José Hélio. Predicting Biomass Production from Plant Robustness and Germination Efficiency by Calorespirometry. **Biofuels**: Greenhouse Gas Mitigation and Global Warming, [*s.l.*], p. 81-94, 2018.

ARNHOLDT-SCHMITT, Birgit; COSTA, José H.; MELO, Dirce Fernandes de. AOX – a functional marker for efficient cell reprogramming under stress? **Trends In Plant Science**, [*s.l.*], v. 11, n. 6, p. 281-287, 2006.

ARNHOLDT-SCHMITT, Birgit; PATIL, Vinod Kumar. Studying Individual Plant AOX Gene Functionality in Early Growth Regulation: a new approach. **Methods In Molecular Biology**, [*s.l.*], p. 235-244, 2017.

BAILLY, Christophe. ROS in seed germination. **Advances In Botanical Research**, [*s.l.*], p. 177-204, 2023.

BAILLY, Christophe. The signalling role of ROS in the regulation of seed germination and dormancy. **Biochemical Journal**, [*s.l.*], v. 476, n. 20, p. 3019-3032, 2019

BAILLY, Christophe; MERENDINO, Livia. Oxidative signalling in seed germination and early seedling growth: an emerging role for ros trafficking and inter-organelle communication. **Biochemical Journal**, [*s.l.*], v. 478, n. 10, p. 1977-1984, 2021.

BAREKE, T. Biology of seed development and germination physiology. **Adv. Plants Agric. Res**, [*s.l.*], v. 8, n. 4, p. 336-46, 2018.

BARROS, Juliane Rafaele Alves; GUIMARÃES, Miguel Julio Machado; SILVA, Rodrigo Moura e; RÊGO, Maydara Thaylla Cavalcanti; MELO, Natoniel Franklin de; CHAVES, Agnaldo Rodrigues de Melo; ANGELOTTI, Francislene. Selection of cowpea cultivars for high temperature tolerance: physiological, biochemical and yield aspects. **Physiology And Molecular Biology Of Plants**, [*s.l.*], v. 27, n. 1, p. 29-38, 2021.

BOUKAR, Ousmane; BELKO, Nouhoun; CHAMARTHI, Siva; TOGOLA, Abou; BATIENO, Joseph; OWUSU, Emmanuel; HARUNA, Mohammed; DIALLO, Sory; UMAR, Muhammed Lawan; OLUFAJO, Olusoji. Cowpea (Vigna unguiculata): genetics, genomics and breeding. **Plant Breeding**, [*s.l.*], v. 138, n. 4, p. 415-424, 2018.

BOUKAR, Ousmane et al. Genomic tools in cowpea breeding programs: status and perspectives. **Frontiers in plant science**, [*s.l.*], v. 7, p. 757, 2016.

CARVALHO, Márcia; LINO-NETO, Teresa; ROSA, Eduardo; CARNIDE, Valdemar. Cowpea: a legume crop for a challenging environment. **Journal Of The Science Of Food And Agriculture**, [*s.l.*], v. 97, n. 13, p. 4273-4284, 2017.

CHALLABATHULA, Dinakar; ANALIN, Benedict; MOHANAN, Akhil; BAKKA, Kavya. Differential modulation of photosynthesis, ROS and antioxidant enzyme activities in stress-sensitive and -tolerant rice cultivars during salinity and drought upon restriction of COX and AOX pathways of mitochondrial oxidative electron transport. **Journal Of Plant Physiology**, [*s.l.*], v. 268, p. 153583, 2022.

CHAUDHURI, Abira; KAR, Rup Kumar. Inhibition of seed germination by propyl gallate, a free radical scavenger and recovery of germination by hydrogen peroxide and ethylene in Vigna Radiata. **World J. Agric. Sci**, [*s.l.*], v. 4, p. 914-921, 2008.

CHIULELE, Rogério Marcos. **Morphological and Physiological responses of Cowpea (Vigna unguiculata (L) Walp.) Cultivars to induced water stress and phosphorus nutrition**. 2003. Tese de Doutorado. Stellenbosch: Stellenbosch University.

CONAB - COMPANHIA NACIONAL DE ABASTECIMENTO. Acompanhamento da Safra Brasileira de Grãos, Brasília, DF, v. 11, safra 2023/24, n. 8 oitavo levantamento, maio 2024.

CONSIDINE, Michael J.; DIAZ-VIVANCOS, Pedro; KERCHEV, Pavel; SIGNORELLI, Santiago; AGUDELO-ROMERO, Patricia; GIBBS, Daniel J.; FOYER, Christine H. Learning To Breathe: developmental phase transitions in oxygen status. **Trends In Plant Science**, [*s.l.*], v. 22, n. 2, p. 140-153, 2017.

CONSIDINE, Michael James; HOLTZAPFFEL, Ruth C.; DAY, David A.; WHELAN, James; MILLAR, A. Harvey. Molecular Distinction between Alternative Oxidase from Monocots and Dicots. **Plant Physiology**, [*s.l.*], v. 129, n. 3, p. 949-953, 2002.

COSTA, José Hélio; MCDONALD, Allison E.; ARNHOLDT-SCHMITT, Birgit; MELO, Dirce Fernandes de. A classification scheme for alternative oxidases reveals the taxonomic distribution and evolutionary history of the enzyme in angiosperms. **Mitochondrion**, [*s.l.*], v. 19, p. 172-183, 2014.

COSTA, José Hélio; JOLIVET, Yves; HASENFRATZ-SAUDER, Marie-Paule; ORELLANO, Elena Graciela; LIMA, Maria da Guia Silva; DIZENGREMEL, Pierre; MELO, Dirce Fernandes de. Alternative oxidase regulation in roots of Vigna unguiculata cultivars differing in drought/salt tolerance. **Journal Of Plant Physiology**, [*s.l.*], v. 164, n. 6, p. 718-727, 2007.

COSTA, José Hélio; HASENFRATZ-SAUDER, Marie-Paule; PHAM-THI, Anh Thu; LIMA, Maria da Guia Silva; DIZENGREMEL, Pierre; JOLIVET, Yves; MELO, Dirce Fernandes de. Identification in Vigna unguiculata (L.) Walp. of two cDNAs encoding mitochondrial alternative oxidase orthologous to soybean alternative oxidase genes 2a and 2b. **Plant Science**, [*s.l.*], v. 167, n. 2, p. 233-239, 2004.

COSTA, José Hélio; SANTOS, Clesivan Pereira dos; LIMA, Beatriz de Sousa e; NETTO, Anthônio Nunes Moreira; SARAIVA, Kátia Daniella da Cruz; ARNHOLDT-SCHMITT, Birgit. In silico identification of alternative oxidase 2 (AOX2) in monocots: a new evolutionary scenario. **Journal Of Plant Physiology**, [*s.l.*], v. 210, p. 58-63, 2017

COSTA, José Hélio; MOTA, Erika Freitas; CAMBURSANO, Mariana Virginia; LAUXMANN, Martin Alexander; OLIVEIRA, Luciana Maia Nogueira de; LIMA, Maria da Guia Silva; ORELLANO, Elena Graciela; MELO, Dirce Fernandes de. Stress-induced co-expression of two alternative oxidase (VuAox1 and 2b) genes in Vigna unguiculata. **Journal Of Plant Physiology**, [*s.l.*], v. 167, n. 7, p. 561-570, 2010.

DAHAL, Keshav; VANLERBERGHE, Greg C. Alternative oxidase respiration maintains both mitochondrial and chloroplast function during drought. **New Phytologist**, [*s.l.*], v. 213, n. 2, p. 560-571, 2016.

TENÓRIO, Dyana A. de; MEDEIROS, Erika Valente de; LIMA, Cristiano S.; SILVA, Jéssica M. da; BARROS, Jamilly A. de; NEVES, Rejane P.; LARANJEIRA, Delson. Biological control of Rhizoctonia solani in cowpea plants using yeast. **Tropical Plant Pathology**, [*s.l.*], v. 44, n. 2, p. 113-119, 2019.

DEMIRCAN, Nil; CUCUN, Gokhan; UZILDAY, Baris. Mitochondrial alternative oxidase (AOX1a) is required for the mitigation of arsenic-induced oxidative stress in Arabidopsis thaliana. **Plant Biotechnology Reports**, [*s.l.*], v. 14, n. 2, p. 235-245, 2020.

FAROOQ, Muhammad Awais et al. Roles of reactive oxygen species and mitochondria in seed germination. **Frontiers in plant science**, [*s.l.*], v. 12, p. 781734, 2021.

FREIRE FILHO, F. R.; COSTA, A. F. Feijão-caupi: Classificação botânica e importância. **Cadernos do Semiárido riquezas & oportunidades**, [*s.l.*], v. 17, p. 17-20, 2020.

FREIRE FILHO, Francisco Rodrigues. Feijão-caupi no Brasil: produção, melhoramento genético, avanços e desafios. **Embrapa Meio-Norte-Livro científico** (ALICE), 2011

GARMASH, E. V. Role of mitochondrial alternative oxidase in the regulation of cellular homeostasis during development of photosynthetic function in greening leaves. **Plant Biology**, [*s.l.*], v. 23, n. 2, p. 221-228,2021.

GARMASH, E. V. Signal pathways for regulation of plant alternative oxidase genes' expression. **Russian Journal of Plant Physiology**, [*s.l.*], v. 69, n. 1, p. 1, 2022.

GONG, Diankai; HE, Fei; LIU, Jingyan; ZHANG, Cheng; WANG, Yanrong; TIAN, Shujun; SUN, Chi; ZHANG, Xue. Understanding of Hormonal Regulation in Rice Seed Germination. **Life**, [S.L.], v. 12, n. 7, p. 1021, 2022.

HATZIG, Sarah et al. Hidden effects of seed quality breeding on germination in oilseed rape (Brassica napus L.). **Frontiers in plant science**, [*s.l.*], v. 9, p. 419, 2018.

ISHIBASHI, Yushi; YUASA, Takashi; IWAYA-INOUE, Mari. Mechanisms of Maturation and Germination in Crop Seeds Exposed to Environmental Stresses with a Focus on Nutrients, Water Status, and Reactive Oxygen Species. **Advances In Experimental Medicine And Biology**, [*s.l.*], p. 233-257, 2018.

JHANJI, Shalini; GOYAL, Eena; CHUMBER, Manisha; KAUR, Gurpreet. Exploring fine tuning between phytohormones and ROS signaling cascade in regulation of seed dormancy, germination and seedling development. **Plant Physiology And Biochemistry**, [*s.l.*], v. 207, p. 108352, 2024.

JAYATHILAKE, Chathuni; VISVANATHAN, Rizliya; DEEN, Afka; BANGAMUWAGE, Ruksheela; JAYAWARDANA, Barana C; NAMMI, Srinivas; LIYANAGE, Ruvini. Cowpea: an overview on its nutritional facts and health benefits. **Journal Of The Science Of Food And Agriculture**, [*s.l.*], v. 98, n. 13, p. 4793-4806, 2018.

JAYAWARDHANE, Jayamini; GOYALI, Juran C.; ZAFARI, Somaieh; IGAMBERDIEV, Abir U. The Response of Cowpea (Vigna unguiculata) Plants to Three Abiotic Stresses Applied with Increasing Intensity: hypoxia, salinity, and water deficit. **Metabolites**, [S.L.], v. 12, n. 1, p. 38, 2022.

JIANG, Zhenxiang; WATANABE, Chihiro K. A.; MIYAGI, Atsuko; KAWAI-YAMADA, Maki; TERASHIMA, Ichiro; NOGUCHI, Ko. Mitochondrial AOX Supports Redox Balance of Photosynthetic Electron Transport, Primary Metabolite Balance, and Growth in Arabidopsis thaliana under High Light. **International Journal Of Molecular Sciences**, [*s.l.*], v. 20, n. 12, p. 3067, 2019.

KUMARI, Aprajita; SINGH, Pooja; KALADHAR, Vemula Chandra; MANBIR; PAUL, Debarati; PATHAK, Pradeep Kumar; GUPTA, Kapuganti Jagadis. Phytoglobin-NO cycle and AOX pathway play a role in anaerobic germination and growth of deepwater rice. **Plant, Cell & Environment**, [S.L.], v. 45, n. 1, p. 178-190, 2021.

LAXA, Miriam; LIEBTHAL, Michael; TELMAN, Wilena; CHIBANI, Kamel; DIETZ, Karl-Josef. The Role of the Plant Antioxidant System in Drought Tolerance. **Antioxidants**, [*s.l.*], v. 8, n. 4, p. 94, 2019.

LI, Jiye et al. Alternative oxidase: from molecule and function to future inhibitors. **ACS omega**, [*s.l.*], v. 9, n. 11, p. 12478-12499, 2024.

LI, Jing; ZHANG, Litao; YU, Wenjie; ZHANG, Mengjie; CHEN, Feng; LIU, Jianguo. Mitochondrial alternative oxidase pathway accelerates non-motile cell germination by enhancing respiratory carbon metabolism and maintaining redox poise in Haematococcus pluvialis. **Bioresource Technology**, [*s.l.*], v. 402, p. 130729, 2024.

LIU, Yang et al. Alternative oxidase inhibition impairs tobacco root development and root hair formation. **Frontiers in Plant Science**, [*s.l.*], v. 12, p. 664792, 2021.

MOHANAPRIYA, Gunasekaran et al. Alternative oxidase (AOX) senses stress levels to coordinate auxin-induced reprogramming from seed germination to somatic embryogenesis—A role relevant for seed vigor prediction and plant robustness. **Frontiers in Plant Science**, [*s.l.*], v. 10, p. 1134, 2019.

NARSAI, Reena et al. Extensive transcriptomic and epigenomic remodelling occurs during Arabidopsis thaliana germination. **Genome biology**, [S.L.] [*s.l.*], v. 18, n. 1, p. 1-18, 2017.

NUN, Nurit Bar; PLAKHINE, Dina; JOEL, Daniel M.; MAYER, Alfred M. Changes in the activity of the alternative oxidase in Orobanche seeds during conditioning and their possible physiological function. **Phytochemistry**, [*s.l.*], v. 64, n. 1, p. 235-241, 2003

NUNES, Luma Rayane de Lima; PINHEIRO, Paloma Rayane; PINHEIRO, Charles Lobo; LIMA, Kelly Andressa Peres; DUTRA, Alek Sandro. GERMINATION AND VIGOUR IN SEEDS OF THE COWPEA IN RESPONSE TO SALT AND HEAT STRESS. **Revista Caatinga**, [*s.l.*], v. 32, n. 1, p. 143-151, 2019.

PENFIELD, Steven. Seed dormancy and germination. **Current Biology**, [*s.l.*], v. 27, n. 17, p. R874-R878, 2017.

PENNISI, Rosa; SALVI, Daniele; BRANDI, Valentina; ANGELINI, Riccardo; ASCENZI, Paolo; POLTICELLI, Fabio. Molecular Evolution of Alternative Oxidase Proteins: a phylogenetic and structure modeling approach. **Journal Of Molecular Evolution**, [*s.l.*], v. 82, n. 4-5, p. 207-218, 2016.

PHAM, Hanh M.; KEBEDE, Hirut; RITCHIE, Glen; TROLINDER, Norma; WRIGHT, Robert J. Alternative oxidase (AOX) over-expression improves cell expansion and elongation in cotton seedling exposed to cool temperatures. **Theoretical And Applied Genetics**, [*s.l.*], v. 131, n. 11, p. 2287-2298, 2018.

PHIRI, C. K. et al. Screening for resistance mechanisms in cowpea genotypes on Alectra vogelii. **American Journal of Plant Sciences**, [*s.l.*], v. 9, n. 6, p. 1362-1379, 2018.

PU, Xiaojun et al. Roles of mitochondrial energy dissipation systems in plant development and acclimation to stress. **Annals of Botany**, [*s.l.*], v. 116, n. 4, p. 583-600, 2015.

QAIM, Matin. Role of new plant breeding technologies for food security and sustainable agricultural development. **Applied Economic Perspectives and Policy**, [*s.l.*], v. 42, n. 2, p. 129-150, 2020.

RIFNA, E. J.; RAMANAN, K. Ratish; MAHENDRAN, R. Emerging technology applications for improving seed germination. **Trends in Food Science & Technology**, [*s.l.*], v. 86, p. 95-108, 2019.

RODRIGUES, Lénia et al. Germination of Pisum sativum L. Seeds Is Associated with the Alternative Respiratory Pathway. **Biology**, [*s.l.*], v. 12, n. 10, p. 1318, 2023.

ROGOV, A. G. et al. Alternative oxidase: distribution, induction, properties, structure, regulation, and functions. **Biochemistry (Moscow)**, [*s.l.*], v. 79, n. 13, p. 1615-1634, 2014.

ROMERO-AGUILAR, Lucero et al. On the use of n-octyl gallate and salicylhydroxamic acid to study the alternative oxidase role. **Archives of Biochemistry and Biophysics**, [*s.l.*], v. 694, p. 108603, 2020.

ROSELL-HIDALGO, Alicia et al. QSAR and molecular docking for the search of AOX inhibitors: a rational drug discovery approach. **Journal of Computer-Aided Molecular Design**, [*s.l.*], p. 1-16, 2020.

RUAN, Mengjiao et al. Alternative oxidase 2 influences Arabidopsis seed germination under salt stress by modulating ABA signalling and ROS homeostasis. **Environmental and Experimental Botany**, [*s.l.*], v. 217, p. 105568, 2024.

SAHA, Bedabrata; BOROVSKII, Gennadii; PANDA, Sanjib Kumar. Alternative oxidase and plant stress tolerance. **Plant signaling & behavior**, [*s.l.*], v. 11, n. 12, p. e1256530, 2016.

SAISHO, Daisuke et al. The gene for alternative oxidase-2 (AOX2) from Arabidopsis thaliana consists of five exons unlike other AOX genes and is transcribed at an early stage during germination. **Genes & genetic systems**, [*s.l.*], v. 76, n. 2, p. 89-97, 2001.

SALLAM, Ahmed et al. Drought stress tolerance in wheat and barley: advances in physiology, breeding and genetics research. **International journal of molecular sciences**, [*s.l.*], v. 20, n. 13, p. 3137, 2019.

SANDOVAL-SICAIROS, Eslim Sugey et al. Phytochemical compounds and antioxidant activity modified by germination and hydrolysis in Mexican amaranth. **Plant Foods for Human Nutrition**, [*s.l.*], p. 1-8, 2020.

SELINSKI, Jennifer et al. Alternative oxidase is positive for plant performance. **Trends in plant science**, [*s.l.*], v. 23, n. 7, p. 588-597, 2018.

STEINBRECHER, Tina; LEUBNER-METZGER, Gerhard. The biomechanics of seed germination. **Journal of Experimental Botany**, [*s.l.*], v. 68, n. 4, p. 765-783, 2017.

TAIZ, L.; ZEIGER, E.; MOLLER, I. M.; MURPHY, A. **Fisiologia e desenvolvimento vegetal**. 6 ed. Porto Alegre: Artmed Editora, 2017.

TAUSEEF, Atirah et al. Differential response of cowpea towards the CuO nanoparticles under Meloidogyne incognita stress. **South African Journal of Botany**, [*s.l.*], v. 139, p. 175-182, 2021.

TOGOLA, A. et al. Host plant resistance to insect pests of cowpea (Vigna unguiculata L. Walp.): achievements and future prospects. **Euphytica**, [*s.l.*], v. 213, n. 11, p. 1-16, 2017.

TURK, Hulya. Chitosan-induced enhanced expression and activation of alternative oxidase confer tolerance to salt stress in maize seedlings. **Plant Physiology and Biochemistry**, [*s.l.*], v. 141, p. 415-422, 2019.

VAN AKEN, Olivier et al. Alternative oxidase: a target and regulator of stress responses. **Physiologia plantarum**, [*s.l.*], v. 137, n. 4, p. 354-361, 2009.

VELADA, Isabel et al. Alternative oxidase gene family in Hypericum perforatum L.: characterization and expression at the post-germinative phase. **Frontiers in Plant Science**, [*s.l.*], v. 7, p. 1043, 2016.

WEN, Yu; ZHANG, Dawei. RETRACTED: Alternative oxidase is involved in leaf senescence via regulation of Salicylic acid accumulation in tomato. 2022.

ZAMAN, Md Shahin Uz et al. Changes in gene expression during germination reveal pea genotypes with either “quiescence” or “escape” mechanisms of waterlogging tolerance. **Plant, cell & environment**, [*s.l.*], v. 42, n. 1, p. 245-258, 2019.

ZHANG, Zhao et al. Comparative Phenotypic and Transcriptomic Analyses Provide Novel Insights into the Molecular Mechanism of Seed Germination in Response to Low Temperature Stress in Alfalfa. **International Journal of Molecular Sciences**, [*s.l.*], v. 25, n. 13, p. 7244, 2024.