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DIEGO DA SILVA CUNHA

Caracterização fisiológica, bioquímica e molecular da germinação de sementes de mamona (*Ricinus communis* L.) sob estresses salino e osmótico.

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Caracterização fisiológica, bioquímica e molecular da germinação de sementes de mamona (*Ricinus communis* L.) sob estresses salino e osmótico.

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“O cientista não é o homem que fornece as verdadeiras respostas; é quem faz as verdadeiras perguntas”.

Claude Lévi-Strauss

"Faça o teu melhor, na condição que você tem, enquanto não tem condições melhores, para fazer melhor ainda”.

(Mario Sergio Cortella)

CUNHA, Diego da Silva. Caracterização fisiológica, bioquímica e molecular da germinação de sementes de mamona (*Ricinus communis* L.) sob estresses salino e osmótico. 2023. Orientador: Renato Delmondez de Castro. 150 f. il. Tese (Doutorado em Biotecnologia - RENORBIO) - Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador, 2021

RESUMO

Ricinus communis L. (Euphorbiaceae) é uma espécie vegetal industrial, conhecida como mamona, a qual se destaca no cenário nacional e internacional pelo óleo produzido em suas sementes, e amplamente demandado pela chamada indústria ricinoquímica e de biocombustíveis. Além dos múltiplos usos comerciais e industriais, possui importância socioeconômica nas regiões semiáridas do Brasil, dentre outras localidades do mundo. Contudo, regiões semiáridas apresentam condições ambientais adversas de estresses abióticos, envolvendo baixa precipitação em períodos curtos de chuva e solos salinos, geralmente associados a temperaturas elevadas. As condições de restrição hídrica (seca) e salinidade são fatores que limitam a absorção de água e promovem subsequente estresse oxidativo resultante da geração de espécies reativas de oxigênio (ERO), tais como o ânion superóxido ($O_2^{\cdot-}$), radical hidroperoxila (HO_2^-), radical hidroxila (OH^-), peróxido de hidrogênio (H_2O_2) e oxigênio singleto, ($^{\cdot}O_2$), os quais em concentrações elevadas podem comprometer o metabolismo dos processos germinativos, e de crescimento e desenvolvimento de plântulas e plantas adultas, comprometendo a produtividade, podendo também ocasionar a morte das plantas. Os estádios de embebição e germinação das sementes e crescimento inicial de plântulas (mudas) constitui a fase mais crítica do ciclo de vida das plantas superiores durante os quais a disponibilidade hídrica é essencial. Ao passo que tem sido relatada a atividade de enzimas removedoras de ERO sob condições de restrição hídrica, tais como as enzimas superóxido dismutase (SOD), ascorbato peroxidase (APX), catalase (CAT) dentre outras, as quais atuam como eficientes mecanismos de desintoxicação de ERO, e que podem constituir marcadores moleculares para a elucidação de sobrevivência ou tolerância atrelados ao metabolismo germinativo e de crescimento de plântulas sob restrição hídrica. Diante disso, a presente proposta vislumbra a melhor compreensão acerca dos mecanismos envolvidos na resposta

da mamona aos estresses abióticos por restrição hídrica e estresse salino a nível fisiológico, relacionados a enzimas antioxidantes por estudos bioquímicos e moleculares. Foi desenvolvida uma **REVISÃO DE LITERATURA** onde foi identificado estudos sobre os avanços em pesquisas relacionadas ao cultivo de mamona sob estresses abióticos por restrição hídrica e estresse salino, germinação e estádios iniciais de desenvolvimento de plântulas e enzimas antioxidantes, destacando as enzimas superóxido dismutase (SOD), ascorbato peroxidase (APX) e catalase (CAT). No **CAPÍTULO 1** realizamos a caracterização biométrica de duas cultivares de *R. communis* (BRS Nordestina e BRS Paraguaçu), notamos que as sementes da Cultivar BRS Paraguaçu são maiores que as BRS Nordestina, o que pode estar relacionado com a maior absorção de água observada para as sementes dessa cultivar. Através dos estudos de embebição em restrição hídrica (PEG) e salina (NaCl) observamos que existe uma diferença na capacidade de restrição hídrica para essas duas soluções no mesmo potencial osmótico, onde para a embebição por PEG a -0.23 MPa houve uma drástica diminuição na absorção de água, inibindo o ciclo celular e o processo de germinação enquanto na embebição por NaCl ocorreu apenas um leve atraso na absorção de água comparado ao controle. A embebição em potenciais leves de NaCl (-0.23 MPa) pode estimular uma maior atividade da enzima antioxidante SOD, enquanto para a embebição em PEG devido a severa restrição hídrica a atividade da enzima SOD foi menor que o controle. No **CAPÍTULO 2** realizamos a caracterização da família de genes que codificam para a enzima Ascorbato peroxidase (APX) em mamona, foram encontrados 6 genes putativos *RcAPX*, através de análise filogenética identificamos os genes ortólogos em outras angiospermas onde classificamos os genes APX de acordo a localização intracelular (Citosol, plastídeo e peroxissomos). Observamos que os genes *RcAPX* possuem grande número de éxons/íntrons além de compartilharem motifs conservados. Foi observado um aumento da atividade total da enzima APX a partir de 48 h de embebição (pós-germinativo) em água (controle) e em NaCl -0.23 MPa (restrição osmótica salina) em ambas as cultivares avaliadas (Nordestina e Paraguaçu), enquanto a embebição em solução de PEG -0.23 MPa (restrição osmótica inerte) reprimiu a atividade da APX. No **CAPÍTULO 3** caracterizamos a família de gene Catalase (CAT), identificamos 2 genes putativos *RcCAT* preditos para a localização intracelular peroxissomo, a partir de comparação filogenética encontramos genes ortólogos em outras angiospermas onde observamos a classificação em três grupos. Encontramos diferenças na estrutura do gene e na ordem de motifs para o gene *RCAT2* comparado aos genes CAT de angiosperma. Através da análise de elementos regulatórios

na região promotora destes genes identificamos possíveis formas de regulação relacionadas a estresses bióticos e abióticos, assim como hormônios vegetais como ABA. Por fim, a atividade enzimática da catalase demonstrou ser modulada de acordo com o tempo de desenvolvimento, estresse durante a germinação e cultivar. Os resultados contribuem para o melhor entendimento dos efeitos dos estresses por restrição hídrica e salina em mamona, além de contribuir para caracterização de família de genes que codificam para enzimas antioxidantes importantes nas respostas das sementes a condições de estresses abióticos.

Palavras-chaves: *Ricinus* sp., Euphorbiaceae, Bioinformática, Restrição hídrica, Salinidade, Enzimas.

CUNHA, Diego da Silva. Physiological, biochemical, and molecular characterization of castor (*Ricinus communis* L.) seed germination under saline and osmotic stresses. 2021. Thesis advisor: Renato Delmondez de Castro. 150 s. ll. Thesis (Doctor in Biotechnology - RENORBIO) - Institute of Health Sciences, Federal University of Bahia, Salvador, 2021

ABSTRACT

Ricinus communis L. (Euphorbiaceae), known as castor, is a species that stands out in the national and international scenario for the oil extracted from its seeds which is widely demanded by by the global bioindustries. In addition to its multiple commercial and bioindustrial uses, it has socioeconomic importance in the semiarid regions of Brazil and global wide. However, semiarid regions have adverse environmental conditions such as short periods of rain and soils with saline levels. Conditions that limit water absorption and promote greater generation of reactive oxygen species (ROS), such as superoxide anion-O_2, hydroperoxyl-HO_2 radical, hydroxyl-OH radical, hydrogen peroxide-H_2O_2 and singlet oxygen, $^1O_2$, compromising the germinative processes, growth, and development of seedlings, reduce productivity and can lead to plant death. During the germination process, the activity of ROS removing enzymes has been reported, such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), among others, which constitute efficient detoxification mechanisms during imbibition, these enzymes can be used as molecular markers to elucidate the events that occur during the germination process. Therefore, this proposal provides a better understanding of the mechanisms involved in the response of castor beans to abiotic stresses by water restriction and saline conditions at the physiological level, related to antioxidant enzymes by means of biochemical and molecular studies. A **REVIEW** was developed where studies were identified on advances in research related to the cultivation of castor under abiotic stresses due to water restriction and salt stress, germination, and early stages of development of seedlings and antioxidant enzymes, shighlighting the enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). In **CHAPTER 1,** we performed the biometric characterization of two *R. communis* cultivars (BRS Nordestina and BRS Paraguaçu), we noticed that the seeds of Cultivar BRS Paraguaçu are larger than those of BRS Nordestina, which may be related to the higher water absorption observed for the

seeds of that cultivar. Through the imbibition studies in osmotic water restriction (PEG) and salinity (NaCl), we observed that there is a difference in the water restriction capacity for these two solutions at the same osmotic potential, where for the imbibition by PEG at -0.23 MPa there was a drastic decrease in water absorption, inhibiting the cell cycle and the germination process while in the same osmoticum by NaCl imbibition there was only a slight delay in water absorption compared to the control. The imbibition in light potentials of NaCl (-0.23 MPa) stimulated a greater activity of the antioxidant enzyme SOD, while for the imbibition in PEG due to severe water restriction, the activity of the SOD enzyme was lower than the control. In **CHAPTER 2,** we performed the characterization of the family of 6 putative *RcAPX* genes that code for the enzyme Ascorbate peroxidase (APX) in castor beans, , through phylogenetic analysis we identified the orthologous genes in other angiosperms where we classified the APX genes according to location intracellular (citosol, plastid and peroxisomes). We observed that the *RcAPX* genes have a large number of exons/introns in addition to sharing conserved motifs. An increase in the total activity of the APX enzyme was observed after 48 h of imbibition (post-germinative) in the imbibition of water (control) and in NaCl -0.23 MPa (saline restriction) in both cultivars evaluated, while that imbibition in a -0.23 MPa PEG repressed the activity of APX. In **CHAPTER 3,** we identified and characterized 2 putative *RcCAT* genes representing the Catalase (CAT) gene family in Castor beans, predicted for peroxisome intracellular localization, from phylogenetic comparison we found orthologous genes in other angiosperms where we observed the classification into three groups. We found differences in gene structure and motif order for the *RcCAT2* gene compared to the angiosperm CAT genes. Through the analysis of regulatory elements in the promoter region of these genes, we identified possible forms of regulation related to biotic and abiotic stresses, as well as plant hormones such as ABA. Finally, the enzymatic activity of catalase was shown to be modulated according to development time, stress during germination and respective genotype. The results herein contribute to a better understanding of the effects of water and saline restriction stresses in castor beans, in addition to the characterization of a family of genes that code for important antioxidant enzymes in seed responses to abiotic stress conditions.

Keywords: *Ricinus* sp., Euphorbiaceae, Bioinformatics, Water restriction, Salinity, Enzymes.

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1. INTRODUÇÃO GERAL

Ricinus communis L. é uma espécie vegetal xerófila e heliófila, conhecida como mamona e pertencente à família Euphorbiaceae, que se destaca no cenário nacional e internacional pelo óleo extraído de suas sementes (BIDIN et al., 2020). Trata-se de um óleo com características peculiares, sendo 90% constituído por ácido graxo ricinoléico, considerado matéria-prima base inúmeras aplicações na chamada 'indústria ricinoquímica' e de biocombustíveis (AZEVEDO; BELTRÃO; SEVERINO, 2007), tais como na produção de revestimentos protetores (tintas e vernizes), desinfetantes, germicidas, nylon, material plástico, vidros à prova de bala, cabos e fibra ótica, drogas farmacêuticas, e mais recentemente também na produção de biodiesel (MCKEON, 2016). A mamona, ainda fornece subprodutos como a torta da mamona, muito utilizado na agricultura como adubo orgânico, útil também na restauração de solos degradados ou esgotados nutricionalmente, sendo aplicado em cultivos de café, citros, cana-de-açúcar, hortaliças, frutíferas, entre outras (BAHIA et al., 2008; WITT et al., 2023).

Os principais países produtores de mamona no mundo são Índia, China, Brasil e Moçambique, nessa ordem (CONAB, 2023). A mamona apresenta elevado potencial socioeconômico em regiões semiáridas do globo, principalmente nos países subdesenvolvidos e em desenvolvimento, onde é comumente cultivada por pequenos agricultores familiares (DHARAJIYA et al., 2020; WANG et al., 2019), a exemplo do Brasil, onde a mamona é produzida essencialmente por agricultores familiares no semiárido nordestino (VASCONCELOS et al., 2017). Onde, contudo, predominam condições ambientais abióticas típicas, envolvendo escassez de chuvas e domínio de períodos longos de seca, associados à frequente salinidade dos solos e temperaturas elevadas (MUNNS et al., 2019; PINHEIRO et al., 2008; SÁ et al., 2016).

Mesmo sendo reconhecida por apresentar tolerância a estresses abióticos, ainda são escassos os programas de melhoramento genético visando a obtenção de genótipos superiores adaptados às condições ambientais adversas do semiárido (PULLARO et al., 2006; RIBEIRO et al., 2014). Ademais, os cultivares ou variedades comumente cultivadas apresentam baixa germinabilidade (germinação lenta e irregular) contribuindo para estandes de lavouras desuniformes (MENDES et al., 2009).

O ciclo de vida das plantas superiores compreende a germinação e desenvolvimento vegetativo pós-germinativo compreendendo o crescimento das plântulas (mudas) e desenvolvimento da fase adulta, seguido da fase reprodutiva com o florescimento e desenvolvimento de frutos e sementes (BEWLEY e BLACK, 1994). Sendo todas os referidos estádios marcados por eventos citológicos e morfofisiológicos associados à qualidade inicial das sementes, conforme também ocorre em mamona (DE CASTRO et al., 2000; VASCONCELOS et al., 2017). Do ponto de vista da produção agrícola, a mamona é bastante investigada, porém ainda são escassos estudos para um melhor entendimento dos aspectos citológicos e morfofisiológicos que governam o metabolismo das fases da germinação e formação inicial de plântulas, considerado o estádio mais crítico no ciclo de vida das plantas superiores (BRITO et al., 2016; TAIZ e ZEIGER, 2009; VASCONCELOS et al., 2017).

A seca e a salinidade podem ocorrer individualmente ou conjuntamente, impedindo a devida absorção de água, e com isso levar ao estresse oxidativo resultante da geração e acúmulo de ERO e possível efeitos fitotóxicos à planta, comprometendo o desenvolvimento, produção e produtividade das lavouras (CHOUDHURY et al., 2017; HASANUZZAMAN et al., 2020; MUNNS, 2002). Para evitar danos celulares e manter a homeostase em níveis satisfatórios, as plantas naturalmente utilizam de mecanismos enzimáticos antioxidantes que atuam para eliminar as ERO produzidas nas células. Dentre as quais constam as enzimas superóxido dismutase (SOD) ascorbato peroxidase (APX) e catalase (CAT). A SOD é a primeira enzima a ser ativada devido à presença de radical superóxido (O_2^-) no ambiente celular, sendo responsável pela dismutação do radical superóxido em peróxido de hidrogênio (H_2O_2), o qual por sua vez pode ser convertido em água (H_2O) e oxigênio (O_2) pelas enzimas CAT e APX (GILL e TUJETA, 2010; HASANUZZAMAN et al., 2020). A compreensão da atividade de cada enzima envolvida na resposta antioxidante, pode servir na busca de marcadores bioquímicos para a qualidade de sementes. Com isso, os resultados enzimáticos aliados aos fisiológicos, possibilitam uma visão geral do comportamento da semente sob condições de estresses abióticos (SANO et al., 2005).

O conhecimento acerca dos mecanismos de resposta da mamona aos estresses abióticos em nível fisiológico, bioquímico e molecular, tornam-se de extrema importância para o melhor entendimento do comportamento da espécie, possibilitando intervir ou melhorar a tolerância e o cultivo da mamona sob as condições de estresses como

presenciados no semiárido (RIBEIRO et al., 2015; TAIZ et al., 2017). A correlação entre estes aspectos possibilita ao pesquisador identificar marcadores bioquímicos e/ou moleculares passíveis de uso em programas de melhoramento para o desenvolvimento cultivares superiores, mais produtivas. Contribuindo, portanto, para melhor sucesso do cultivo por pequenos agricultores familiares em regiões semiáridas e no mundo.

2. OBJETIVOS

2.1. Objetivo Geral

Avaliar o perfil bioquímico e molecular do estresse hídrico e salino durante a germinação e crescimento inicial de plântulas de duas cultivares de mamona (BRS Nordestina e BRS Paraguaçu).

2.2. Objetivos Específicos

- Avaliar a germinação e crescimento inicial de plântulas de duas cultivares de mamona (BRS Nordestina e BRS Paraguaçu) submetidas à estresses por restrição hídrica (PEG e NaCl);
- Avaliar a reativação do ciclo celular por meio do acúmulo de tubulina e configurações do citoesqueleto microtubular durante a embebição e germinação de sementes sob estresses por restrição hídrica (PEG e NaCl) em duas cultivares de mamona (BRS Nordestina e BRS Paraguaçu).
- Avaliar o perfil de atividade antioxidante e modulação dos genes da enzima Superóxido Dismutase (SOD) durante a embebição e germinação de sementes de duas cultivares (BRS Nordestina e BRS Paraguaçu) sob estresses por restrição hídrica (PEG e NaCl).
- Caracterizar a família de genes e avaliar o perfil de expressão gênica da enzima antioxidante Ascorbato Peroxidase (APX) durante a embebição e germinação de duas cultivares (BRS Nordestina e BRS Paraguaçu) sob estresses por restrição hídrica (PEG e NaCl).
- Caracterizar a família de genes e avaliar o perfil de expressão gênica da enzima antioxidante Catalase (CAT) durante a embebição e germinação de sementes de duas cultivares (BRS Nordestina e BRS Paraguaçu) sob estresses por restrição hídrica (PEG e NaCl);

REVISÃO DE LITERATURA

1. A espécie *Ricinus communis* L. **(mamona)**

Ricinus communis L. é uma espécie vegetal oleaginosa pertencente à família Euphorbiaceae, popularmente conhecida como mamona, mamoneira, rícino, carrapateira, palma-de-cristo, bojureira, bafureira ou figueira-do-inferno. Na língua inglesa é chamada de "castor ou castor seed", e em espanhol como "higuerilla" ou "tartago" (KAUR e BHASKAR, 2020).

A mamona, foi classificada por Linnaeus em 1753 (ANJANI, 2012), pertence à subdivisão Fanerogamae ou Espermatophita; filo das Angiospermae; Classe Dicotiledônea; subclasse Archichlamydeae; ordem Geraniales; família Euphorbiaceae, gênero *Ricinus*, nome científico *Ricinus communis* L. (KAUR e BHASKAR, 2020). O nome Ricinus, é de origem latina, significa carrapato, devido à forma das suas sementes assemelharem ao ácaro *Ixodes ricinus* (LADDA; KAMTHANE, 2014; BIDIN et al, 2020). Os residentes espanhóis e portugueses, confundiram-na com uma planta diferente, batizando-a de *Vitex agnus castus*, e depois a chamaram de "agno casto". Devido a esta designação, os ingleses que comercializavam este óleo criaram a palavra "castor", e assim deu origem a este nome em todo o mundo anglófono (KAUR e BHASKAR, 2020).

Existem diversas opiniões sobre o centro de origem da mamona. Contudo, o maior número de evidências encontradas, sugerem que a sua origem tenha sido no continente africano, região da Etiópia (A-MAGID, 2014). A espécie foi introduzida no Brasil pelos portugueses durante o período de colonização, com interesse na extração de óleo das sementes para iluminação e lubrificação dos eixos das carroças (AZEVEDO; BELTRÃO; SEVERINO, 2007; MITRA et al., 2010). Devido a sua ampla adaptação às condições edafoclimáticas, hoje é encontrada praticamente em quase todo o território brasileiro, principalmente na região Nordeste (COSTA; HOESCHL, 2006; KAUR e BHASKAR, 2020; SEVERINO et al., 2012a).

A mamona é considerada uma planta xerófila e heliófila, caracterizada por um sistema radicular pivotante. Em situações de escassez de água, esse tipo de raiz favorece a busca por umidade em camadas mais profundas do solo (KAUR e BHASKAR, 2020). A raiz principal da mamona demonstra uma maior capacidade de penetração em comparação com solos úmidos, conforme observado por TÁVORA (1982). Esse aspecto possibilita a absorção de água em níveis mais profundos, permitindo que a planta tolere

condições de seca que poderiam causar danos graves a outras culturas (MCKEON, 2016). O caule é geniculado, espesso e ramificado, podendo apresentar variações quanto a coloração e a presença de cera. A haste principal cresce verticalmente sem ramificações, até o surgimento da primeira inflorescência. Os ramos laterais se desenvolvem a partir da axila da última folha, logo abaixo da inflorescência (KAUR e BHASKAR, 2020; SANTOS et al., 2007). As folhas apresentam filotaxia alternada, simples, grandes, podendo variar quanto à largura do limbo, cor, cerosidade, comprimento do pecíolo e na profundidade dos lóbulos (AZEVEDO; BELTRÃO; SEVERINO, 2007; KANCHAN; ATREYA; SHEKHAWAT, 2016).

A mamona é uma planta monóica, possuindo inflorescência do tipo panícula com presença de flores masculinas na parte inferior e flores femininas na parte superior, podendo ocorrer uma distribuição irregular ao longo do racemo (KANCHAN; ATREYA; SHEKHAWAT, 2016). A proporção de flores masculinas e femininas pode ser afetada pela temperatura, idade da planta, duração do dia e pela variedade utilizada, sendo que a deficiência hídrica e altas temperaturas induzem a formação de flores masculinas, enquanto condições climatológicas balanceadas induzem a formação de flores femininas. A flor feminina possui ovário tricarpelar, com um óvulo em cada carpelo, o qual originará a futura semente (SHEKHAWAT, 2016; KAUR e BHASKAR, 2020).

Os frutos de mamona são cápsulas globosas, também chamadas de bagas, medindo aproximadamente 2.4 cm, possui variação quanto à cor, cerosidade, forma, tamanho, deiscência, caducidade, e possuem acúleos (KAUR e BHASKAR, 2020; KANCHAN; ATREYA; SHEKHAWAT, 2016). Mesmo sendo considerada tolerante à seca, a mamoneira necessita de chuvas regulares no início do crescimento, e do período seco para a maturação dos frutos (WEISS, 2000).

As sementes variam muito em diferentes formas, cores, tamanhos e pesos (ZUCHI et al., 2010). São compostas de tegumento, rafe, micrópila, carúncula, endosperma, cotilédones e eixo embrionário e sua germinação é do tipo epígea (MARCOS-FILHO, 2015). As sementes da mamona possuem elevado teor de óleo, podendo variar de 35% a 55%, variedades selvagens, em geral, possuem sementes de tamanho pequeno e menos teor de óleo, existem cultivares comerciais no Brasil que possuem o teor de óleo variando entre 45% a 50%, sendo o principal componente do óleo, o ácido ricinoléico (MITRA et al., 2010; PRASAD e RAO, 2017).

A faixa ideal de temperatura para o seu desenvolvimento é de 20 a 30ºC, sendo 28°C a ótima. Temperaturas abaixo de 16ºC reduzem o teor de óleo e alteram suas características, além de reduzirem seu metabolismo, podendo paralisar o crescimento das plantas. Em temperaturas superiores a 35ºC ocorre a redução do teor de óleo e o comprometimento de proteínas nas sementes (AZEVEDO; BELTRÃO; SEVERINO, 2007).

A disseminação mundial da mamona como cultura agronômica deveu-se inicialmente ao seu alto teor de óleo nas sementes, originalmente usado na fabricação de lubrificantes, tintas e sabões e, mais recentemente, náilon, plásticos, adesivos, corantes, cosméticos e na produção de biodiesel (KAUR e BHASKAR, 2020). Demonstrando a ampla difusão e utilização da mamona que acentuam fortemente seu papel significativo e indispensável desde os tempos antigos (A-MAGID, 2014; ANJANI, 2012). Refletindo, também, que plantas não comestíveis, como a mamona, sempre foram importantes e continuarão sendo assim quanto às plantas alimentícias, portanto, enfatiza a necessidade de pesquisas de forma igual àquelas em plantas alimentícias (A-MAGID, 2014; XU et al., 2019).

2. Importância da mamona

Os principais países produtores de mamona são a Índia (74%), China (13%), o Brasil (6,1%) e Moçambique (2,5%) (FAO, 2021), os quais concentram 90% da produção global (BIDIN et al., 2020; PRASAD e RAO, 2017). A Índia lidera o comércio internacional, produzindo em torno de 85% da produção global de óleo extraído das sementes de mamona, assim como também é o maior exportador de óleo (FAO, 2021). Os Estados Unidos, União Europeia, Japão, Brasil e China respondem por até 84% da importação do óleo (PRASAD e RAO, 2017; WITT et al., 2023).

O óleo de rícino possui propriedades físico-químicas muito valorizadas, devido à presença de ácido ricinoléico em quantidades superiores a 87% (KANCHAN; ATREYA; SHEKHAWAT, 2016). Apresentando quatro funcionalidades: carboxilato, hidroxila, insaturação e hidrocarboneto de cadeia longa, presentes no ácido ricinoléico, tornaram essa molécula única no mundo químico (MCKEON, 2016; PRASAD e RAO, 2017).

Devido às suas características particulares, o óleo de mamona se tornou uma alternativa potencial aos produtos à base de petróleo e também um excelente candidato para exploração no modo de biorrefinaria, pois é totalmente biodegradável e matéria-prima renovável (PRASAD e RAO, 2017; WITT et al., 2023). O óleo de rícino possui uma variedade de aplicações, como adesivos, cosméticos e produtos de higiene pessoal, plastificantes, sabonetes especiais, substitutos de cera, surfactantes, tintas e revestimentos, perfumes, uma variedade de lubrificantes e graxas, bem como nas indústrias de alimentos, química fina e farmacêutica (BIDIN et al., 2020; MCKEON, 2016; PRASAD e RAO, 2017).

No Brasil a produção de mamona se destaca na região semiárida, onde são frequentes situações de secas ocasionadas pela escassez de chuva, tornando difícil a vida das populações locais, que se baseiam na agricultura familiar como principal fonte de sustento (Fig. 1) (PINHEIRO et al., 2008; SÁ et al., 2016). Além disso, a maior parte é cultivada em regiões onde o estresse salino está presente afetando a germinação e o crescimento das plantas de forma intensa (CASTRO; SANTOS, 2020; PINHEIRO et al., 2008; SÁ et al., 2016).

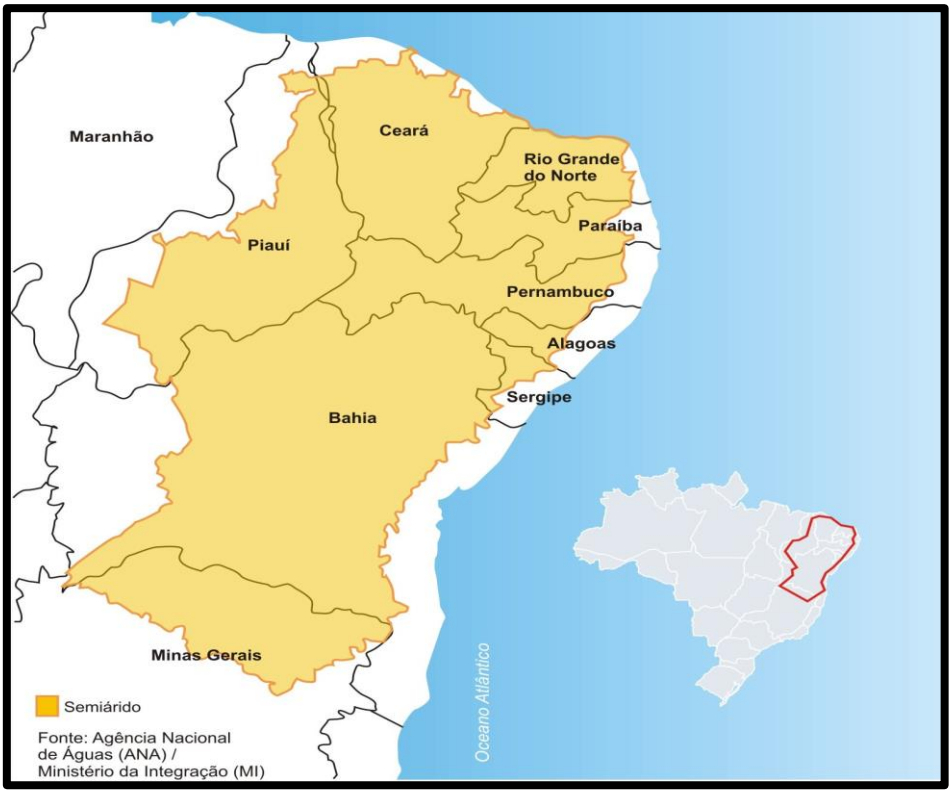

Figura 1. Mapa representando a região semiárida nordestina. Fonte: Agência Nacional de Água (ANA).

De acordo com os dados da Conab (2023), na safra 2022/2023 houve um aumento de 100% na produtividade, projetando um salto de produção de 42,8 mil para 91,4 mil toneladas, sendo o Estado da Bahia o maior produtor nacional. Tendo a mamona, um importante papel socioeconômico, servindo de fixadora de mão-de-obra, sendo consorciada com culturas alimentares como milho e feijão (BABITA et al., 2010; PINHEIRO et al., 2008; WITT et al., 2023), o que faz a mamona ser considerada um importante instrumento de desenvolvimento da agricultura nessa região (BRITO et al., 2016; SÁ et al., 2016; VASCONCELOS et al., 2017). Contudo, sua produtividade ainda se encontra longe do ideal, pois todo o sistema de produção, do plantio ao processamento, ainda ocorre de forma manual (SAVY FILHO, 2007). Principalmente o uso de variedades locais com ciclo longo e maturação de sementes desigual, pouco ou nenhum preparo do solo e fertilização (AZEVEDO; BELTRÃO; SEVERINO, 2007). Usando essa tecnologia, os agricultores têm baixa renda e a produção nacional de sementes de mamona é de 600 kg/ha. Esses valores são muito baixos para tornar a produção lucrativa.

Com a finalidade de melhorar a produção de mamona na região do semiárido a Embrapa Algodão vem ao longo dos anos desenvolvendo cultivares melhoradas para as áreas tradicionais de cultivo, como exemplo temos as cultivares BRS Nordestina e a BRS Paraguaçu, que apresentam produtividade média de 1500 kg/ha em sequeiro (ANDRADE; FREIRE, 2006).

A Cultivar BRS Nordestina foi obtida a partir de seleção individual com teste de progênies na variedade local Baianita, possui porte médio (1.9 m), caule de cor verde e coberto por cera, racemo cônico, frutos semideiscentes, suas sementes possuem coloração única preta, que sob estresse hídrico, podem apresentar pequenas pontuações brancas, sem padrão definido, pesando aproximadamente 0.68 g e com 49% de óleo (BAHIA et al., 2008; ZUCHI et al., 2010). Já a cultivar BRS Paraguaçu foi obtida por seleção em massa da variedade local Sangue de Boi, apresenta porte médio (1.6 m), caule roxo, coberto por cera, racemo oval, com frutos semideiscentes, semente pesando aproximadamente 0.71g e com 48% de óleo, assim como a BRS Nordestina, suas sementes são pretas e sob estresse hídrico pode apresentar manchas pequenas pontuações brancas não definidas (ANDRADE; FREIRE, 2006; BAHIA et al., 2008).

3. Germinação das sementes

No ciclo de vida das plantas, o objetivo biológico de uma semente é germinar e estabelecer uma nova planta quando em ambiente favorável (BEWLEY, 1997; DE CASTRO e HILHORST, 2006). O processo germinativo se inicia com a absorção de água (embebição), porém, há necessidade de que a semente alcance um nível adequado de hidratação o qual permita a reativação dos seus processos metabólicos (MARCOS-FILHOS, 2015).

Essa captação inicial de água é um processo físico impulsionado pelo potencial matricial dos constituintes da semente, que ocorre tanto em sementes vivas como mortas, pois consiste somente na ligação da água à matriz da semente, contanto que o material apresente sítios de ligação ou afinidade pela água (DE CASTRO e HILHORST, 2004). A água atua como um agente estimulador e controlador, uma vez que, além de promover o amolecimento do tegumento, favorecendo a penetração do oxigênio, e aumentar o volume do embrião e dos tecidos de reserva, ela estimula as atividades metabólicas básicas, favorecendo o crescimento do eixo embrionário (MARCOS-FILHOS, 2015).

Potenciais hídricos negativos, principalmente no início da embebição, inviabilizam a sequência dos eventos germinativos da semente durante a absorção de água (MARCOS-FILHOS, 2015). Por outro lado, o excesso de umidade ocasiona um decréscimo na germinação, visto que impede a penetração do oxigênio e reduz todo o processo metabólico resultante (BEWLEY e BLACK, 1994).

A absorção de água pelas sementes, consequentemente reativa o ciclo celular, que culmina na germinação propriamente dita (DE CASTRO e HILHORST, 2000). Os eventos celulares e moleculares que ocorrem durante a germinação de sementes estão organizados em uma sequência de ativação de processos que se inicia no núcleo celular. Estes processos são principalmente a síntese do DNA e a divisão celular e são reconhecidos como eventos do ciclo celular, que levam a padrões específicos de organogênese e morfogênese, no que diz respeito à diferenciação celular. Mais profundamente nos níveis citológico e molecular, o ciclo celular envolve um ciclo cromossômico em que a síntese para a replicação ocorre durante a interfase, e um ciclo mitótico que leva a célula a divisão (DE CASTRO e HILHORST, 2000; MARCOS-FILHOS, 2015).

A manutenção da integridade da molécula de DNA durante a expansão e divisão celular é de fundamental importância para a germinação de sementes, sendo esta integridade mantida por distintas matrizes de microtúbulos associados aos diferentes estágios do ciclo celular (WRIGHT et al., 2009). A rede do citoesqueleto, que é composto pelas matrizes de microtúbulos e filamentos de actina, coordena inúmeras atividades da célula, incluindo a divisão celular, mudança de forma da célula, transporte intracelular, diferenciação celular, organiza espacialmente as organelas, serve como uma estrutura de suporte para o seu movimento e, além disso, o citoesqueleto da planta desempenha um papel importante na mediação da resposta da célula a patógenos e simbiontes (FEDOROVA, 2007).

O citoesqueleto microtubular é redistribuído sob condições de estresse, tais como estresse hiperosmótico, por choque térmico e, temperatura baixa e dessecação, sendo o comportamento dos microtúbulos dependente do tipo de célula, diferenciação e estádio de desenvolvimento (VASCONCELOS et al., 2017). Apesar da forte correlação entre a natureza sensível dos microtúbulos e sua recuperação após o tratamento de estresse, ainda não está claro como células vivas regulam suas respostas aos estresses em intensidades diferentes (MÜLLER et al., 2009).

De acordo com Bewley e Black (1994), a germinação pode ser dividida em três fases, sendo a Fase I dada pela absorção de água de maneira física, devido a um gradiente de potencial hídrico, sendo a água direcionada à semente, esta fase é característica pelo aumento rápido do potencial hídrico do embrião, na Fase II acontece uma acentuada redução na absorção de água, estabelecendo um platô, onde inicia-se ativação do metabolismo, a Fase III, a absorção de água é retomada, aumentando consideravelmente a curva de embebição, porém desta vez associada ao crescimento do embrião, culminando na protrusão da raiz e alongamento das estruturas (Fig. 2) (BEWLEY e BLACK 1994).

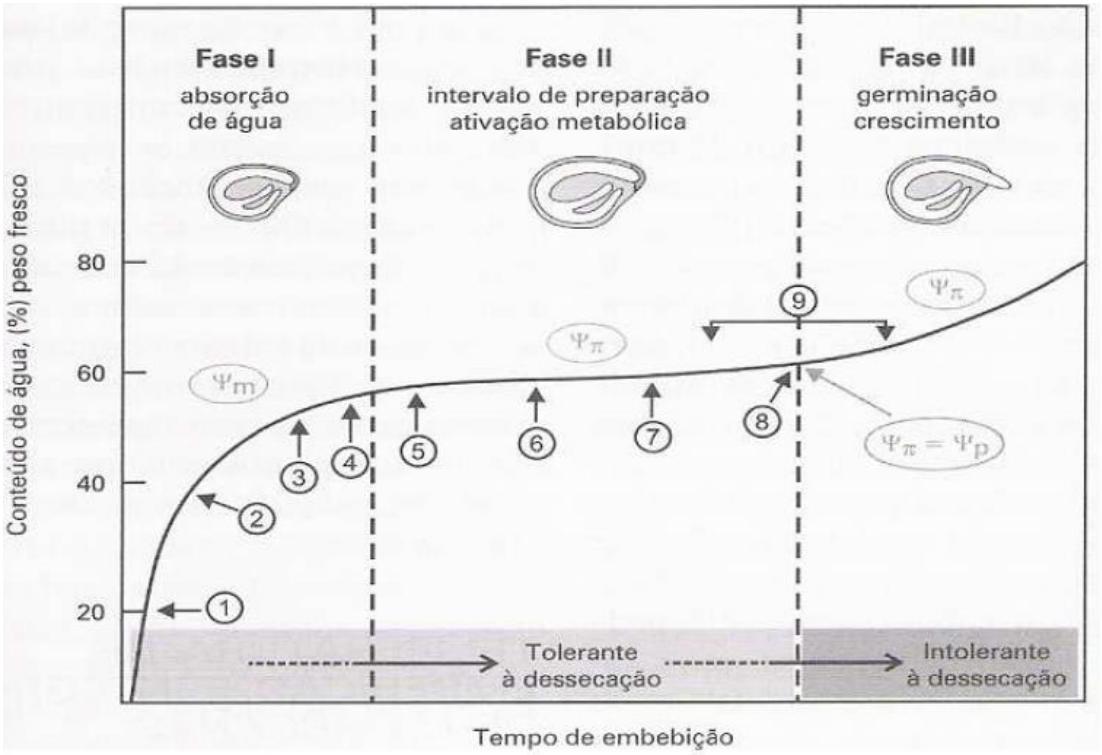

Figura 2. Representação esquemática do padrão trifásico de absorção de água durante a embebição de sementes, em relação aos conteúdos aproximados de água em que os diferentes eventos do processo germinativo são iniciados. (1) Respiração e acúmulo de ATP; (2) Síntese de mRNA e reparo de DNA; (3) Ativação de polissomos; (4) Síntese de proteínas a partir de mRNAs recentemente sintetizados; (5) Síntese e duplicação de DNA; (6) Início da degradação de reservas; (7) As células da radícula alongam-se; (8) Protrusão radicular; (9) Mitose. Potencial matricial (Ψm); potencial osmótico (Ψπ); potencial de pressão (Ψp). Adaptado de De Castro e Hilhorst (2004).

Diversos fatores influenciam a absorção de água pela semente, como: espécies, cultivares, características da própria semente como composição química, teor inicial de água e constituição do tegumento. Além de, fatores ambientais, como: umidade, oxigênio, temperatura, luz, salinidade, nutrientes são capazes de influenciar o processo germinativo e o desenvolvimento pós-germinativos de plântulas, destacando-se a disponibilidade de água (PIMENTEL, 1999; ZUCHI et al., 2012).

4. Estresse bióticos e abióticos afetam a germinação e formação de plântulas

As plantas durante seu ciclo de vida, seja em condições naturais ou agricultáveis, estão expostas a ambientes adversos, sendo, portanto, submetidas a vários tipos de estresse biótico e/ou abiótico (MUNNS et al., 2019; TAIZ et al., 2017). Do ponto de vista fisiológico, estresse é um fator externo que exerce uma influência desvantajosa sobre a planta, alterando seu equilíbrio (HAN et al., 2020; TAIZ et al., 2017). Nesse contexto, o conceito de estresse é atribuído àquela condição que ativa o metabolismo celular e incrementa a atividade fisiológica da planta, podendo também ser um fator positivo que impulsiona o crescimento vegetal ou uma condição desfavorável, que afeta negativamente o metabolismo, crescimento e desenvolvimento da planta, podendo ser de causas bióticas ou abióticas (ESMAEILI et al., 2019; TAIZ et al., 2017).

Os estresses bióticos são ocasionados por organismos vivos, como fungos, vírus, bactérias, nematoides, insetos etc. Esses agentes afetam a produtividade das culturas causando vários tipos de doenças, infecções e injúrias (GULL; LONE; WANI, 2019). Já os estresses abióticos afetam o crescimento, desenvolvimento, rendimento e qualidade, são considerados os principais limitadores da produtividade das culturas, como exemplo temos a seca (restrição hídrica), ou excesso de água, temperaturas extremas (frio, geada e calor), salinidade e toxicidade mineral (AHANGER et al., 2017; HAN et al., 2020; TAIZ et al., 2017).

A seca e a salinidade são consideradas os principais estresses abióticos limitantes para o estabelecimento, crescimento e produtividade das culturas (HAN et al., 2020; MANSOUR; SALAMA, 2019; MUNNS et al., 2019). Estudos mostraram que o estresse por seca e salinidade, isoladamente ou em combinação, causaram reduções significativas nos parâmetros de crescimento, fotossíntese e conteúdo de clorofila em culturas agrícolas, com a maior redução causada pela combinação desses dois estresses (DUGASA; CHALA; WU, 2019 IBRAHIM et al., 2019a; JIANG et al., 2019). Eles são os mais comuns que frequentemente ocorrem em regiões áridas e semiáridas (CASTRO; SANTOS, 2020; ZHU, 2016). Entender a tolerância das plantas a eles é de fundamental importância, constituindo, um dos principais tópicos das pesquisas atualmente (ACOSTA-MOTOS et al., 2017; ZELM; ZHANG; TESTERINK, 2020).

4.1. **Efeitos do estresse por restrição hídrica (seca)**

A seca é a ameaça mais substancial aos rendimentos das culturas em todo o mundo, a falta de água é um dos principais fatores ambientais que influenciam a germinação e o desenvolvimento de plântulas (SOLTANI; KAZEMITABAR; RANJBAR, 2023). A restrição hídrica surge pela falta de água, sendo uma situação muito comum, se tratando de regiões semiáridas onde se cultivam mamona (MUNNS, 2002; SELEIMAN et al., 2021).

A seca afeta a planta em diversos estádios de desenvolvimento, podendo comprometer seu crescimento e reduzir o seu rendimento (PANDEY et al., 2017). Na fase de germinação, altera a velocidade, uniformidade, viabilidade, vigor (BRITO et al., 2016; BRITO et al., 2015; RIBEIRO et al., 2015). Em fases posteriores a germinação, induz modificações morfológicas, fisiológicas e metabólicas em todos os órgãos, podendo ocasionar a morte do vegetal (LIU et al., 2022).

A germinação se inicia com o processo de embebição das sementes, e ao mesmo tempo depende do gradiente de potencial hídrico (tensão de água) existente entre a semente e o meio externo para que ocorra (MUNNS, 2002; TAIZ e ZEIGER, 2017). Cada espécie apresenta um valor de potencial hídrico no solo o qual abaixo dele a germinação não ocorre (MARCOS-FILHO, 2015; TAIZ e ZEIGER, 2017). A adaptabilidade e tolerância ao déficit hídrico são fatores intrínsecos de cada espécie vegetal, e os seus efeitos são diferentes durante o ciclo de vida do vegetal, pois os efeitos verificados em sementes nem sempre são observados em plantas (SELEIMAN et al., 2021). Diversos estudos têm sido conduzidos utilizando soluções aquosas com diferentes potenciais osmóticos para umedecer substratos e simular condições de baixa disponibilidade hídrica em condições controladas (FANTI e PEREZ, 2004). Entre as soluções, as mais utilizadas para simulação do déficit hídrico encontram-se o manitol e o polietilenoglicol (PEG), produtos inertes, atóxicos e não eletrolíticos (FANTI e PEREZ, 2004; MARCOS-FILHO, 2015).

O PEG vem sendo utilizado para simular condições de estresse hídrico, pois é um agente osmótico inerte cujas moléculas são muito grandes para penetrar na semente, evitando assim quaisquer efeitos tóxicos (FANTI e PEREZ, 2004). Como o PEG não entra no apoplasto, a água é retirada não apenas da célula, mas também da parede celular, simulando um solo seco (VERSLUES et al., 2006).

Gomes Neto et al. (2018), trabalhando com sementes de mamona, cv. Paraguaçu e EBDA MPA 34 a soluções de PEG, observaram limite da germinação a partir do potencial -0.23 MPa. Bertagnolli et al. (2003) ao avaliar o comportamento de sementes nuas e peletizadas de *Lactuca sativa* (alface), cv. Karla, sob diferentes potenciais hídricos e de temperaturas, verificaram uma redução da germinação a partir do potencial hídrico de -0.3 MPa de PEG. Em sementes de *Carthamus tinctorius* (cártamo), Dantas et al., (2011), verificaram limitação da germinação nos potenciais osmóticos -0.8 e -1.2 MPa em PEG. Em sementes de cenoura (*Daucus carota*), a germinação e o vigor foram afetados em potenciais abaixo de -0.3 MPa em PEG (SILVA et al., 2011). A germinação e o vigor de sementes de *Beta vulgaris* (beterraba) foram também prejudicadas em PEG sob potenciais inferiores a -0.4 MPa (MACIEL et al., 2015).

4.2. Estresse abiótico causado pela salinidade

O estresse salino afeta a produtividade agrícola em todo o mundo (MUCHATE et al., 2016; ZELM; ZHANG; TESTERINK, 2020). É uma condição do solo caracterizada por uma alta concentração de sais solúveis, podendo ocorrer em função de características naturais ou decorrente das atividades humanas (CASTRO; SANTOS, 2020; PEDROTTI et al., 2015). A maior parte das terras afetadas pela salinidade surgiram de causas naturais, devido ao acúmulo de sais por longos períodos (CASTRO; SANTOS, 2020; MUNNS et al., 2020), ocorrendo principalmente nas regiões áridas e semiáridas do mundo (CASTRO; SANTOS, 2020; PEDROTTI et al., 2015), regiões essas, em que se expandem os plantios de mamona (PINHEIRO et al., 2008).

As atividades humanas podem, também, provocar salinização dos solos, estão geralmente associadas: ao desmatamento, excesso de água de irrigação, a qualidade da água de irrigação abaixo do recomendado, uso de adubos químicos e sistemas de drenagem ineficientes (CASTRO; SANTOS, 2020; MINHAS et al., 2020; PEDROTTI et al., 2015; Sá et al., 2016). São atividades frequentemente observadas em regiões produtoras de mamona no semiárido.

A salinidade afeta o processo de embebição, devido ao aumento da concentração de sais, causando redução do potencial hídrico, que interfere no processo de absorção da água pelas sementes (MUNNS et al., 2019; SILVA et al., 2011). Além da influência da redução do potencial hídrico, as condições salinas prejudicam as sementes pelo efeito

tóxico dos íons, o que pode afetar algumas atividades enzimáticas em sementes, dificultando a absorção de outros nutrientes importantes, resultando em um tempo prolongado de germinação e consequente redução em alongamento de raiz e parte aérea (MUNNS; TESTER, 2008).

O excesso de sais reduz o potencial hídrico do meio, dificultando a absorção de água pela semente (MUNNS et al., 2020). Posteriormente, processos de divisão e alongamento celular podem ser afetados, assim como a mobilização das reservas indispensáveis para o processo de germinação (BRITO et al., 2016; NAWAZ et al., 2010; SILVA et al., 2011). A absorção excessiva de íons como Na^+ e Cl^- ocasiona redução da intensidade respiratória e da atividade de algumas enzimas envolvidas na germinação, restringindo a obtenção de energia no processo de divisão celular e no crescimento do eixo embrionário (NAWAZ et al., 2010).

5. Mecanismos de resistência aos estresses abióticos seca e salinidade

As respostas das plantas ao estresse pela falta de água ou excesso de sal, são muito comuns (MUNNS, 2002). A salinidade reduz a capacidade das plantas de absorver água, e isso rapidamente ocasionando reduções na taxa de crescimento, juntamente com um conjunto de alterações metabólicas idênticas às causadas pelo estresse hídrico promovido pela seca (GUO et al., 2019; MUCHATE et al., 2016, 2019).

Fisiologicamente, os efeitos adversos da seca e salinidade na germinação, crescimento e processos bioquímicos e moleculares da cultura podem ser resumidos como toxicidade iônica, estresse osmótico, deficiência mineral, desequilíbrio iônico, distúrbios fisiológicos, bioquímicos, moleculares ou suas combinações (GUO et al., 2019; MUCHATE et al., 2016). A nível celular, o ajuste osmótico é a principal adaptação das plantas para minimizar os efeitos do estresse por seca ou salinidade (MUCHATE et al., 2016). As células acumulam solutos orgânicos e inorgânicos para diminuir o potencial hídrico sem diminuir o conteúdo real de água, entre os principais osmólitos estão os açúcares solúveis, álcoois de açúcar, prolina, glicina betaína, ácidos orgânicos e trealose (TAIZ et al., 2017).

A maioria das espécies de plantas não pode tolerar secas prolongadas ou concentrações salinas acima de 200 mM de NaCl (FLOWERS; COLMER, 2008;

HASEGAWA; BRESSAN, 2000), pois essas condições provocam uma série de efeitos deletérios nas plantas que incluem distúrbio do equilíbrio osmótico celular, inibição da fotossíntese, inibição da atividade enzimática, e interferência na nutrição mineral, ocasionando um crescimento lento (MUNNS, 2002; MUNNS; TESTER, 2008). Além disso, esses e outros estresses abióticos ocasionam estresse oxidativo por meio do aumento da formação de espécies reativas de oxigênio (ERO), como superóxido (O_2^-), peróxido de hidrogênio H_2O_2 e radicais hidroxila $HO^{\cdot}$ que ocasionam estresse oxidativo (Fig. 3) (DUGASA; CHALA; WU, 2019; HUSSAIN et al., 2019). Os altos níveis de ERO na célula, são altamente tóxicos e danificam proteínas, lipídios, carboidratos e ácido desoxirribonucleico (LIU et al., 2022; ROSSATTO et al., 2017; YOU; CHAN, 2015).

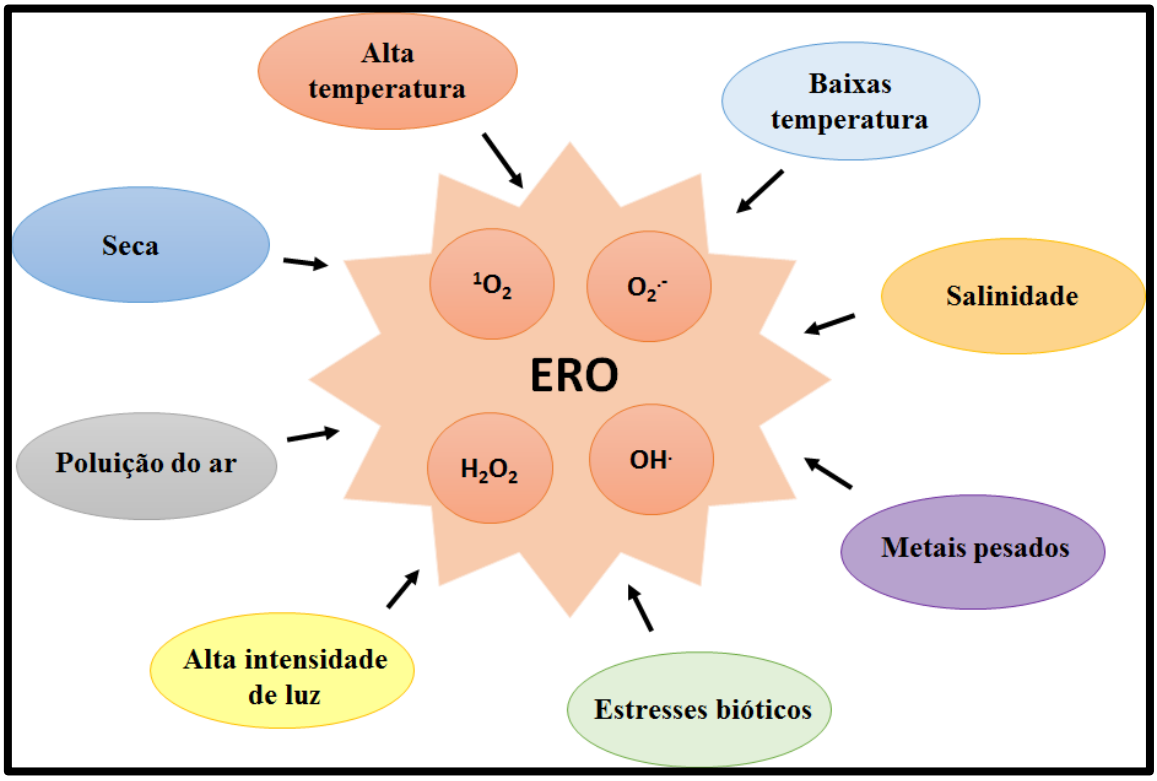

Figura 3. Várias causas responsáveis pela geração de ERO. Adaptado de Das e Roychoudhury (2014).

6. Função das ERO e a importância das enzimas antioxidantes na homeostase.

Em condições não estressantes, as ERO são produzidas em baixas concentrações, como subprodutos do metabolismo celular das plantas, para desempenhar papéis importantes como moléculas de sinalização envolvidas na germinação, crescimento,

desenvolvimento, gravitropismo, ação hormonal e muitos outros processos fisiológicos normais (CHOUDHURY et al., 2017; PANDEY et al., 2017). Quando em excesso, cada um deles é altamente citotóxico, devido à sua reatividade com vários componentes celulares principais (NOCTOR; MHAMDI, 2017; ROSSATTO et al., 2017).

As ERO podem ser formadas em diferentes compartimentos das células vegetais como mitocôndrias, cloroplastos, microssomas, glioxissomos, peroxissomos, apoplastos e no citosol (GILL et al., 2015; SCHIEBER; CHANDE, 2014; STEPHENIE et al., 2020). Embora todos os compartimentos da célula sejam locais possíveis para formação de ERO, os cloroplastos, mitocôndrias e peroxissomos são considerados os geradores mais importantes de (BAKER et al., 2023; STEPHENIE et al., 2020).

A presença de ERO resulta do metabolismo natural das células, podendo auxiliar na regulação celular, mas quando em excesso podem ocasionar danos levando à morte celular (SCHIEBER; CHANDE, 2014), A quantidade de ERO produzida e eliminada é controlada por vários fatores dentro do ambiente celular, que são conhecidos como antioxidantes e enzimas antioxidantes. Os antioxidantes incluem compostos como ácido ascórbico, glutationa, b-carotenos, flavonóides ou outros compostos fenólicos (GONG; RAO; YU, 2013; STEPHENIE et al., 2020).

As enzimas antioxidantes compreendem superóxido dismutase (SOD), catalase (CAT), ascorbato peroxidase (APX), glutationa peroxidase (GPX), peroxirredoxina (PrxR) entre outras, estão presentes em praticamente todos os compartimentos intracelulares. Normalmente, uma organela possui mais de uma enzima capaz de limpar um único tipo de ERO (IBRAHIM et al., 2019b; SOFO et al., 2015). A função básica das enzimas antioxidantes é facilitar o mecanismo de doação de elétrons dos antioxidantes e reciclar os antioxidantes oxidados de volta à sua forma reduzida como em uma reação reversa (GONG; RAO; YU, 2013; STEPHENIE et al., 2020).

Nos últimos anos, esforços substanciais foram feitos para compreender os mecanismos do sistema antioxidante em plantas. Havendo um crescente número de publicações abordando enzimas como SOD, CAT, APX, GPX entre outras. Isso demonstra a importância dessas enzimas para melhor compreender os processos biológicos que lidam com as respostas ao estresse oxidativo em plantas (CAVERZAN et al., 2012; SCHIEBER; CHANDE, 2014).

6.1. Características da Superóxido dismutase (SOD - EC 1.15.1.1)

As superóxidos dismutases constituem a primeira linha de defesa contra ERRO (JENA et al., 2023). É uma metaloenzima, localizada em diversos compartimentos celulares, desempenhando a função de catalisar a dismutação do O_2^- (radicais com tempo de vida curto – instáveis) que podem ocasionar dano celular, em O_2 e H_2O_2 que são moléculas mais estáveis (GOMES NETO et al., 2018; STEPHENIE et al., 2020).

As SODs são classificadas em três grupos com base no cofator metálico usado pela enzima, que são: SOD de ferro (Fe-SOD), SOD de manganês (Mn-SOD) e SOD de cobre-zinco (Cu/Zn- SOD), e esses SODs estão localizados em diferentes compartimentos da célula. Fe-SODs estão localizados no cloroplasto, Mn-SODs na mitocôndria e no peroxissomo, e Cu/Zn-SODs no cloroplasto, no citosol e, possivelmente, no espaço extracelular (Fig. 4) (ANDRADE et al., 2021; GILL et al., 2015).

As famílias de genes SODs foram já foram descritas em mamona (8 genes; GOMES NETO et al., 2018) e em outras espécies como *Arabidopsis thaliana* (7 genes; KLIEBENSTEIN et al., 1998), arroz (8 genes; NATH et al., 2014), sorgo (8 genes; FILIZ e TOMBULOĞLU, 2015), tomate (9 genes; FENG et al., 2016).

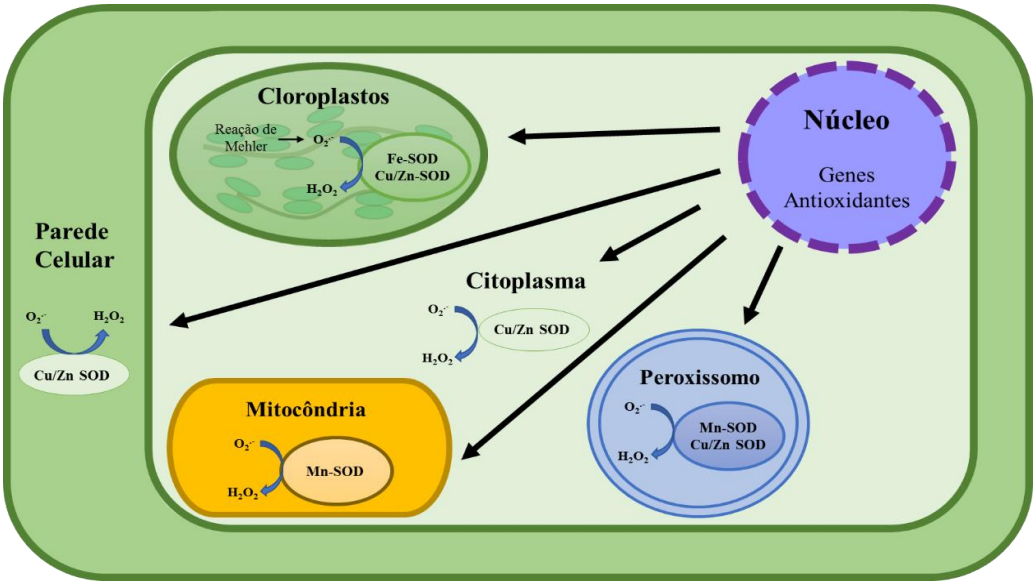

Figura 4. Localização das SODs em toda célula vegetal. Adaptado de Gill et al., (2015) e Hasanuzzaman et al. (2020).

6.2. Características da enzima Ascorbato peroxidase (APX - EC 1.11.1.1)

Ascorbato peroxidase é uma enzima chave no ciclo ascorbato-glutationa, que é o principal sistema de desintoxicação do H_2O_2 nos cloroplastos (HASANUZZAMAN et al., 2019; ZHANG et al., 2023). A APX utiliza o ascorbato como doador de elétrons para reduzir H_2O_2, produzindo dehidroascorbato (DHA) e água (CAVERZAN et al., 2012). Sua importância não se restringe aos cloroplastos, ela também desempenha um papel importante na eliminação de ERO no citosol, mitocôndrias e peroxissomos (Fig. 6) (NOCTOR E FOYER, 1998).

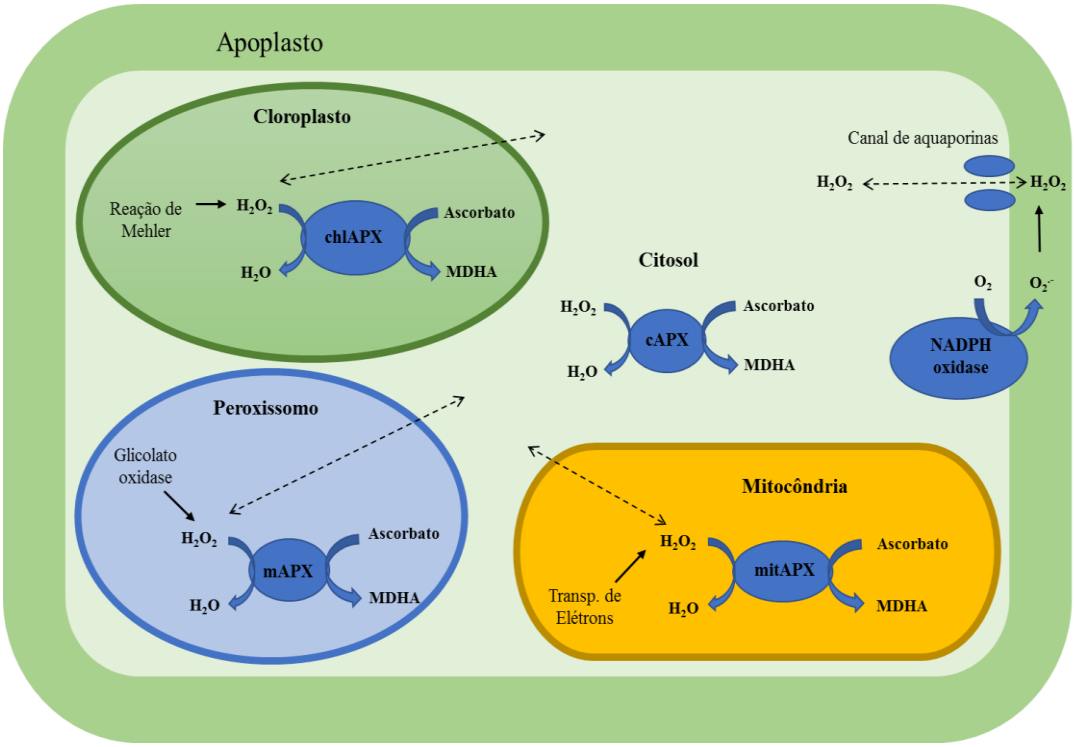

Figura 5. Eliminação do excesso de ERO em diferentes compartimentos intracelulares pelas enzimas APXs. A H_2O_2 é gerada no metabolismo normal por meio da reação de Mehler nos cloroplastos, transporte de elétrons na mitocôndria e fotorrespiração nos peroxissomos. Adaptado de Caverzan et al. (2012).

APX faz parte da classe I heme-peroxidases encontrada em algas vermelhas, clorófitas, membros do reino protista e nas plantas superiores (ZHANG et al., 2023). Nesses organismos as APXs são codificadas por pequenas famílias de genes (CAVERZAN et al., 2012). As diferentes isoformas são classificadas de acordo com sua localização subcelular. As isoformas solúveis são encontradas no citosol (cAPX),

mitocôndrias (mitAPX) e estroma do cloroplasto (sAPX), enquanto as isoformas ligadas à membrana são encontradas nos microrganismos (incluindo peroxissomo e glioxissoma) (mAPX) e tilacóides do cloroplasto (tAPX) (HASANUZZAMAN et al., 2019).

As APXs foram parcialmente caracterizadas em algumas espécies de plantas (CAVERNAZA et al., 2012). Em *Arabidopsis thaliana* foram descritos 9 genes (NARENDRA et al., 2006), 5 genes *Spinacia oleracea* (espinafre) (ISHIKAWA et al., 1998), 4 genes em *Vigna unguiculata* (feijão-caupi) (D'ARCY LAMETA et al., 2006), 6 genes em *Eucalyptus grandis* (TEIXEIRA et al., 2005), 7 genes em *Solanum lycopersicum* (tomate) (NAJAMI et al., 2008), 9 genes em *Oryza sativa* (arroz) (LAZZAROTTO et al., 2011). Em mamona foram relatados 6 genes, porém são necessários estudos aprofundados sobre a expressão desses genes em condições de estresse abióticos como seca e salinidade.

Nesse contexto, a presente tese teve como foco a caracterização fisiológica de sementes de mamona de duas cultivares (BRS Nordestina e BRS Paraguaçu), desenvolvidas para região semiárida, sob embebição em diferentes potenciais osmóticos em soluções de NaCl e PEG. O presente estudo buscou investigar à atividade de ciclo celular durante a embebição sob estresses por PEG e NaCl, através da detecção de tubulina, importante proteína na formação do citoesqueleto microtubulars que, durante a divisão celular, guiam a montagem e função do fuso mitótico. Além disso, buscou abordar informações sobre atividade enzimática das enzimas SOD, CAT e APX durante a germinação e formação inicial de plântulas, pois são importantes removedoras de espécies reativas de oxigênio (ERO) formados durante a reativação do metabolismo. Por fim, foi realizada a caracterização da família de genes que codificam para as enzimas CAT e APX por serem enzimas chaves na detoxificação do H_2O_2 e com isso estarem envolvidas na regulação da homeostase de processos de desenvolvimento e de resposta aos estresses abióticos como a seca e a salinidade. Através deste trabalho, esperamos contribuir com informações relevantes que podem ser utilizadas na compreensão dos mecanismos envolvidos na germinação e formação inicial de plântulas.

6.3. Características da Catalase (CAT - EC 1.11.1.6)

A catalase foi a primeira enzima antioxidante descoberta estando presente em todos os procariontes e eucariontes (MHAMDI et al., 2010). As diferentes isoformas

atuam na conversão de $2H_2O_2$ em O_2 e H_2O, evitando que as células sofram danos oxidativos. O H_2O_2 pode ser formado durante reações como dismutação de O_2^- por atividade da SOD, fotorrespiração, β-oxidação de ácidos graxos, decomposição induzida por prótons de O_2^- e na defesa contra patógenos (BAKER et al., 2023; RAZA et al., 2021).

As catalases estão presentes nos principais sítios de produção de H_2O_2, como peroxissomos, glioxissomos, mitocôndrias, citosol e cloroplastos (SHARMA e AHMAD, 2014; RAJPUT et al., 2021). Em sementes maduras, está localizado principalmente em glioxissomos (WILLEKENS et al., 1995). Esta enzima possui uma estrutura tetramérica em forma de haltere com quatro subunidades de monômero idênticas de 220.000 a 350.000 kD. O grupo prostético heme é o componente chave para a atividade enzimática. O grupo heme de cada unidade monomérica consiste em um átomo de ferro central ligado a um anel de protoporfirina, por possuir uma das maiores taxas de rotatividade entre todas as enzimas antioxidantes é considerada um removedor eficiente de ERO (Fig. 5) (JENA et al., 2023; SHARMA e AHMAD, 2014).

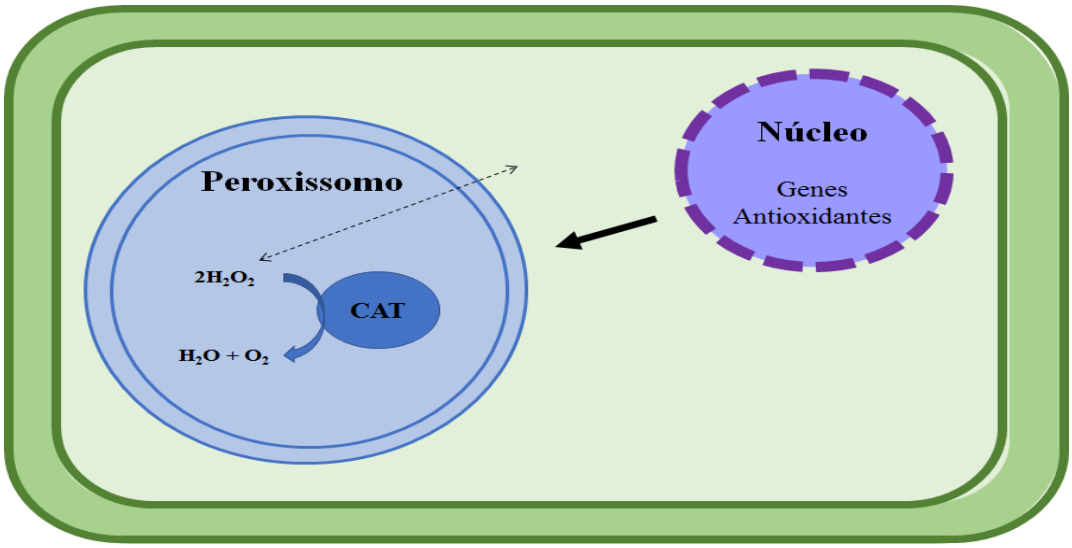

Figura 6. Atividade de Catalase em peroxissomo. Adaptado de Sharma e Ahmad, (2014).

A presença de múltiplas isoenzimas da catalase sugere sua versatilidade estrutural e funcional em uma variedade de espécies vegetais, sendo a expressão dos genes sujeita a vários fatores ambientais exógenos (SHARMA e AHMAD, 2014; VERMA et al., 2023). O aumento da atividade da CAT é considerado um traço adaptativo, que possivelmente ajuda a superar os danos no metabolismo dos tecidos, reduzindo os níveis tóxicos de H_2O_2 (RAZA et al., 2021).

Os genes CAT respondem diferencialmente a vários estresses bióticos e abióticos conhecidos que geram ERO (JENA et al., 2023; MHAMDI et al., 2010). Várias isoformas de CAT são encontradas nas plantas sendo expressas em tecidos fotossintéticos, vasculares e reprodutivos. Willekens et al., (1995) baseado na nomenclatura dos genes encontrado em tabaco, dividiu as catalases em três grupos: catalase monofuncional (CAT I), que são removedora de H_2O_2 produzido durante a fotorrespiração em tecidos fotossintéticos; As catalase peroxidase bifuncional (CAT II), que são produzidas em tecidos vasculares e podem exercer uma função de lignificação, contudo a sua função biológica permanece ainda desconhecida e; As binucleares que são catalase de manganês (Mn-catalase ou CAT III), estão presentes abundantemente em sementes de plantas jovens, cuja atividade está relacionada à remoção do H_2O_2 produzido durante a degradação dos ácidos graxos no glioxissoma (CHAKRAVARTY et al., 2015; MHAMDI et al., 2010).

Em sementes oleosas, o CAT é particularmente importante nos eventos iniciais do crescimento das mudas porque remove o H_2O_2 produzido durante a β-oxidação dos ácidos graxos (BEWLEY e BLACK, 1994). A CAT também é importante no ajuste do H_2O_2 celular e, em seguida, na modulação das vias de sinalização relacionadas (MITTLER, 2002).

Considerando as crescentes evidências sobre as contribuições da CAT em vários processos fisiológicos, é de grande importância a caracterização da mesma em mamona, espécie de interesse socioeconômico para semiárido, em que a seca e a salinidade afetam a produção.

7. Referências

ACOSTA-MOTOS, J. R.; ORTUÑO, M. F.; BERNAL-VICENTE, A.; DIAZ-VIVANCOS, P.; SANCHEZ-BLANCO, M. J.; HERNANDEZ, J. A. Plant responses to salt stress: adaptive mechanisms. **Agronomy**, v. 7, n. 1, p. 18, 2017.

AHANGER, M. A.; AKRAM, N. A.; ASHRAF, M.; ALYEMENI, M. N.; WIJAYA, L.; AHMAD, P. Plant responses to environmental stresses - from gene to biotechnology. **AoB Plants**, v. 9, n. 4, plx025, 2017

A-MAGID, A. Recovery of an Early Evidence of Castor Plant, *Ricinus communis* L. from the Central Sudan and its Positioning within a World-wide Context. **Journal of Arts and Social Sciences**, v. 5, n. 2, p. 45, 2014.

ANDRADE, F. P. DE; FREIRE, E. C. **Brs Nordestina**. p. 3–4, 2006.

ANDRADE, M. V. S.; DE CASTRO, R.D.; CUNHA, D. S.; GOMES NETO, V.; CAROSIO, M. G. A.; FERREIRA, A. G.; SOUZA-NETA, L. C.; FERNANDEZ, L. G.; RIBEIRO, P. R. *Stevia rebaudiana* (Bert.) Bertoni cultivated under different photoperiod conditions: Improving physiological and biochemical traits for industrial applications, **Industrial Crops and Products**, v. 168, 2021.

ANJANI, K. Castor genetic resources: A primary gene pool for exploitation. **Industrial Crops and Products**, v. 35, n. 1, p. 1–14, 2012.

AZEVEDO, D. M. P. DE et al. **Recomendações técnicas para o cultivo da mamoneira (***Ricinus communis* L.**) no Nordeste do Brasil.** Campina Grande: EMBRAPA-CNPA. Circular Técnica, 25, 1997.

AZEVEDO, D. M. P.; BELTRÃO, N. E. DE M.; SEVERINO, L. S. Manejo cultural. In: AZEVEDO, D. M. P. DE.; BELTRÃO, N. E. DE M. (Eds.). **O agronegócio da mamona no Brasil**. 2. ed. Brasília: Embrapa Informação Tecnológica, 2007. p. 223–253.

BABITA, M. MAHESWARI, M.; RAO, L. M.; SHANKER, A. K.; RAO, D. G. Osmotic adjustment, drought tolerance and yield in castor (*Ricinus communis* L.) hybrids. **Environmental and Experimental Botany**, v. 69, n. 3, p. 243–249, 2010.

BAHIA, H. F. et al. Divergência genética entre cinco cultivares de mamoneira. **Pesquisa**

Pecuária Brasileira, v. 43, n. 3, p. 357–362, 2008.

BAKER, A.; LIN, C.; LETT, C.; KARPINSKA, B.; WRIGHT, M. H.; FOYER, C. H. Catalase: A critical node in the regulation of cell fate. **Free Radical Biology and Medicine**. v.199, p.56-66. 2023. https://doi.org/10.1016/j.freeradbiomed.2023.02.009

BELTRÃO, N. E. DE M. A cadeia da mamona no Brasil, com ênfase para o segmento P&D: estado da arte, demandas de pesquisa e ações necessárias para o desenvolvimento. 1–20, 2004.

BERTAGNOLLI, C. M.; MENEZES, N. L. D.; STORCK, L.; SANTOS, O. S. D.; PASQUALLI, L. L. Performance of bare and pelleted lettuce (Lactuca sativa L.) seeds exposed to hydric and thermal stresses. **Revista Brasileira de sementes**, v. 25, p.7-13, 2003.

BEWLEY, J. D.; BLACK, M. **Seeds: physiology of development and germination**. Springer Science & Business Media. 1994.

BIDIN, C.; GOPINATH, S.; ARAVINDRAJ, R.; DEVARAJ, A.; KRISHNAN, S. G.; JEEVAANANTHAN, J. K. S. The production of biodiesel from castor oil as a potential feedstock and its usage in compression ignition Engine: A comprehensive review. **Mater Today Proc**. V. 33, p.84–92, 2020. https://doi.org/10.1016/j.matpr.2020.03.205

BRITO, C. D.; LOUREIRO, M. B.; RIBEIRO, P. R.; VASCONCELOS, P. C. T.; FERNANDEZ, L. G.; DE CASTRO, R. D. Osmoconditioning prevents the onset of microtubular cytoskeleton and activation of cell cycle and is detrimental for germination of *Jatropha curcas* L. seeds. **Plant Biol**, v. 18, p. 1053–1057, 2016. https://doi.org/10.1111/plb.12482

BRITO, K. Q. D.; SOUZA, F. G.; DANTAS JUNIOR, G. J.; BRITO, K. S. A.Efeito da salinidade na germinação e desenvolvimento inicial da mamona 'BRS energia.' **Rev Verde Agroecol e Desenvolv Sustentável**. V. 10, n. 4, p. 17-20, 2015. https://doi.org/10.18378/rvads.v10i4.3344

CARVALHO, J. M. F. C. et al. Melhoria da germinação das sementes de mamoneira (*Ricinus communis* L.) in vitro, através da quebra de dormência e desinfestação. **Comunicado técnico**, 125, p. 1–4, 2000.

CASTRO, F. C.; SANTOS, A. M. DOS. Salinidade do solo e risco de desertificação na

região semiárida. **Mercator**, v. 19, p. 1–13, 2020.

CAVERZAN, A. et al. Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. **Genetics and Molecular Biology**, v. 35, n. 4, p. 1011–1019, 2012.

CHOUDHURY, F. K. et al. Reactive oxygen species, abiotic stress and stress combination. **Plant Journal**, 2017.

COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB. Acompanhamento da Safra Brasileira. Grãos. Safra 2022/23. 12° Levantamento. 110 p.

COSTA, F. C.; HOESCHL, H. C. **Gestão do conhecimento na caia produtiva de biodiesel**. (A. B. das I. de P. T. (Abipti), M. da C. e T. (MCT), Eds.) CONGRESSO E BRASILEIRA TECNOLOGIA BIODIESEL. **Anais**. Brasília: 2006

D'ARCY-LAMETA, A., FERRARI-ILIOU, R., CONTOUR-ANSEL, D., PHAM-THI, A. T., & ZUILY-FODIL, Y. Isolation and characterization of four ascorbate peroxidase cDNAs responsive to water deficit in cowpea leaves. **Annals of Botany**, v. 97, n.1, p. 133-140, 2006.

DANTAS, C. V. S.; SILVA, I. B.; PEREIRA, G. M.; MAIA, J. M.; LIMA, J. P. M. S.; MACEDO, C. E. C. D. Influência da sanidade e deficit hídrico na germinação de sementes de *Carthamus tinctorius* L. **Revista Brasileira de Sementes**, v. 33, p. 574-582, 2011.

DAS, K.; ROYCHOUDHURY, A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. **Frontiers in Environmental Science**, v. 2, n. DEC, p. 1–13, 2014.

DE CASTRO, R. D.; VAN LAMMEREN, A. A. M.; GROOT, S. P. C.; BINO, R. J.; HILHORST, H. W. M. Cell division and subsequent radicle protrusion in tomato seeds are inhibited by osmotic stress but DNA synthesis and formation of microtubular cytoskeleton are not. **Plant Physiol**, v. 122, p. 327–335, 2000. https://doi.org/10.1104/pp.122.2.327

DE CASTRO, R. D.; BRADFORD, K. J.; HILHORST, H. W. Desenvolvimento de sementes e conteúdo de água. ***Germinação: Do básico ao aplicado*** *(AG Ferreira & F. Borghetti, eds.). Artmed, Porto Alegre*, p. 51-68. 2004

DE CASTRO, R. D.; HILHORST, H. W. Hormonal control of seed development in GA-

and ABA-deficient tomato (*Lycopersicon esculentum* Mill. cv. *Moneymaker*) mutants. **Plant Science**, v. 170, n. 3, p. 462-470, 2006.

DHARAJIYA, D. T.; SHAH, A.; GALVADIYA, B. P.; PATEL, M. P.; SRIVASTAVA, R.; PAGI, N. K.; SOLANKI, S. D.; PARIDA, S. K.; TIWARI, K. K. Genome-wide microsatellite markers in castor (*Ricinus communis* L.): Identification, development, characterization, and transferability in Euphorbiaceae, **Industrial Crops and Products**. v. 151, p. 112461, 2020. https://doi.org/10.1016/j.indcrop.2020.112461

DUGASA, M. T.; CHALA, I. G.; WU, F. Genotypic difference in secondary metabolism-related enzyme activities and their relative gene expression patterns, osmolyte and plant hormones in wheat. **Physiologia Plantarum**, v. 168, n. 4, p. 921-933, 2020.

ESMAEILI, N. et al. Co-overexpression of AVP1 and OsSIZ1 in *Arabidopsis* substantially enhances plant tolerance to drought, salt, and heat stresses. **Scientific Reports**, v. 9, n. 1, p. 1–15, 2019.

FANTI, S. C.; PEREZ, S. C. J. G. D. A. Seed germination of paineira under water and salt stress. **Pesquisa Agropecuária Brasileira**, v. 39, p. 903-909, 2004.

FEDOROVA, E. E.; DE FELIPE, M. R.; PUEYO, J. J.; LUCAS, M. M. Conformation of cytoskeletal elements during the division of infected Lupinus albus L. nodule cells. **J. Exp. Bot**. v. 58, p. 2225–2236, 2007.

FENG, K.; YU, J.; CHENG, Y.; RUAN, M.; WANG, R.; YE, Q.; WAN, H. The SOD gene family in tomato: identification, phylogenetic relationships, and expression patterns. **Frontiers in Plant Science**, v. *7*, p. 1279. 2016

FILIZ, E.; KOC, I.; OZYIGIT, I. I. Comparative analysis and modeling of superoxide dismutases (SODs) in *Brachypodium distachyon* L. **Applied biochemistry and biotechnology**, v.173, p. 1183-1196, 2014.

GILL, S. SINGH et al. Superoxide dismutase - mentor of abiotic stress tolerance in crop plants. **Environ sci. Pollut Res.**, v. 22, p. 10375–10394, 2015.

GOMES NETO, V. et al. Characterization of the superoxide dismutase gene family in seeds of two *Ricinus communis* L. genotypes submitted to germination under water restriction conditions. **Environmental and Experimental Botany**, v. 155, p. 453–463, 1 nov. 2018.

GONÇALVES, N. P. Colheita de armazenamento da mamona. **Informe Agropecuário,** v. 7, n. 82, p. 44–45, 1981.

GONG, Y.; RAO, L.; YU, D. We are IntechOpen, the world' s leading publisher of Open Access books Built by scientists, for scientists TOP 1%. In: STOYTCHEVA, M.; ZLATEV, R. (Eds.). **Agricultural Chemistry**. Intechopen, 2013. v. 32p. 113–152.

GULL, A.; LONE, A. A.; WANI, N. U. I. Biotic and Abiotic Stresses in Plants. In: **Intechopen**. 2019.

GUO, X. et al. Effects of calcium on emergence and seedling growth of castor bean under salinity stress. **Current Science**, v. 116, n. 12, p. 2028–2035, 2019.

HAN, B. et al. Changes and associations of genomic transcriptions and histone methylations with salt stress in castor bean. **Plant and Cell Physiology**, v. 114, n. June, p. e00146, 2020.

HASANUZZAMAN, M.; BHUYAN M. H. M. B, ZULFIQAR F., et al. Reactive Oxygen Species and Antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. antioxidants (Basel). v.9, n.8, p. 681-29, 2020. https://doi:10.3390/antiox9080681

HASANUZZAMAN, M.; BHUYAN, M. H. M. B.; ANEE, T. I.; PARVIN, K.; NAHAR, K.; MAHMUD, J. A.; FUJITA, M. Regulation of Ascorbate-Glutathione pathway in mitigating oxidative damage in plants under abiotic stress. antioxidants (Basel, Switzerland), v. 8, n. 9, p. 384, 2019. https://doi.org/10.3390/antiox8090384

HASSAN, M. AL et al. Antioxidant responses under salinity and drought in three closely related wild monocots with different ecological optima. **AoB PLANTS**, v. 9, n. 2, 2017.

HUSSAIN, S. et al. Salinity Stress in Arid and Semi-Arid Climates: Effects and Management in Field Crops. In: HUSSAIN, S. (Ed.). **Climate Change and Agriculture**. Intechopen, 2019.

IBRAHIM, W. et al. Genotypic differences in leaf secondary metabolism, plant hormones and yield under alone and combined stress of drought and salinity in cotton genotypes. **Physiologia Plantarum**, v. 165, n. 2, p. 343–355, 2019a.

IBRAHIM, W. et al. Comparative physiological analysis in the tolerance to salinity and drought individual and combination in two cotton genotypes with contrasting salt

tolerance. **Physiologia Plantarum**, v. 165, n. 2, p. 155–168, 2019b.

ISHIKAWA, T., YOSHIMURA, K., SAKAI, K., TAMOI, M., TAKEDA, T., SHIGEOKA, S. Molecular characterization and physiological role of a glyoxysome-bound ascorbate peroxidase from spinach. **Plant and cell physiology**, v. 39, n. 1, p. 23-34, 1998.

JENA, A. B.; SAMAL, R. R.; BHOL, N. K.; DUTTAROY, A. K. Cellular Red-Ox system in health and disease: The latest update. **Biomed Pharmacother**, v. 162, p. 114606, 2023. https://doi:10.1016/j.biopha.2023.114606

JIANG, Z. et al. Plant cell-surface GIPC sphingolipids sense salt to trigger Ca2+ influx. **Nature**, v. 572, n. 7769, p. 341–346, 2019.

KANCHAN, T.; ATREYA, A.; SHEKHAWAT, R. S. Cathartic turned bioweapon: *Ricinus communis*, the castor bean. **Wilderness & Environmental Medicine**, v. 27, n. 3, p. 444-445. 2016.

KAUR, R.; BHASKAR, T. Potential of castor plant (*Ricinus communis*) for production of biofuels, chemicals, and value-added products. In **Waste biorefinery**. Elsevier, 2020. pp. 269-310.

KLIEBENSTEIN, D. J.; MONDE, R. A.; LAST, R. L. Superoxide dismutase in *Arabidopsis*: an eclectic enzyme family with disparate regulation and protein localization. **Plant physiology**, v. 118, n. 2, p. 637-650, 1998.

LADDA, P. L.; KAMTHANE, R. B. *Ricinus communis* (Castor): An Overview. **Int. J. of Res. in Pharmacology & Pharmacotherapeutics**, v. 3, n. 2, p. 136–144, 2014.

LAGO, A. A. et al. Dormência em sementes de três cultivares de mamona. **Bragantia**, v. 38, n. 1, p. 41–44, 1979.

LAKSHMAMMA, P. et al. Growth and yield of diff erent castor genotypes varying in drought tolerance. **Ann. Arid Zone**, v. 48, p. 35–39, 2009.

LAZZAROTTO, F.; TEIXEIRA, F. K.; ROSA, S. B. et al. Ascorbate peroxidase-related (APx-R) is a new heme-containing protein functionally associated with ascorbate peroxidase but evolutionarily divergent. **New Phytol**. V. 191, p. 234–250, 2011. https://doi.org/10.1111/j.1469-8137.2011.03659.x

LIU, Z.; MA, C.; HOU, L.; WU, X.; WANG, D.; ZHANG, L.; LIU, P. Exogenous SA affects rice seed germination under salt stress by regulating Na+/K+ balance and endogenous GAs and ABA homeostasis. **International Journal of Molecular Sciences**, v. 23, n. 6, p. 3293, 2022.

LIU, H. et al. Genome-wide identification of and functional insights into the late embryogenesis abundant (LEA) gene family in bread wheat (*Triticum aestivum*). **Scientific Reports**, v. 9, n. 1, p. 1–11, 2019.

MACIEL, K. S.; LOPES, J. C.; MORAES, C. E.; FARIAS, C. C. M.; LIMA, P. A. M. Germinação de sementes de beterraba em condições de estresse hídrico e salino. **Nucleus**, v. 12, n.2, p. 189-200, 2015.

MANSOUR, M. M. F.; SALAMA, K. H. A. Cellular Mechanisms of Plant Salt Tolerance. In: GIRI, B.; VARMA, A. (Eds.). **Microorganisms in Saline Environments: Strategies and Functions**. Springer Nature Switzerland, 2019. p. 169–210.

MARCOS-FILHO, J. (2015). **Fisiologia de sementes de plantas cultivadas**. Londrina: [s.n.].

MCKEON, T. A. Chapter 4 - Castor (*Ricinus communis* L.). In: MCKEON, T. A. et al. (Eds.). **Industrial Oil Crops**. AOCS Press, 2016. p. 75–112.

MENDES, R. DE C. et al. Tratamentos pré-germinativos em sementes de mamona (*Ricinus communis* L.). **Revista Brasileira de Sementes**, v. 31, n. 1, p. 187–194, 2009.

MHAMDI, A. et al. Catalase function in plants: A focus on Arabidopsis mutants as stress-mimic models. **Journal of Experimental Botany**, v. 61, n. 15, p. 4197–4220, 2010.

MINHAS, P. S. et al. Coping with salinity in irrigated agriculture: Crop evapotranspiration and water management issues. **Agricultural Water Management**, v. 227, n. May 2019, p. 105832, 2020.

MITRA, J. et al. A review on the challenges for increased production of castor. **Industrial Crops and Products**, v. 33, n. 1, p. 1–14, 2010.

MOSHKIN, V. A. **Castor**. New Delhi: Amerind Publishing, 1986. 315 p.

MUCHATE, N. S. et al. NaCl induced salt adaptive changes and enhanced accumulation of 20-hydroxyecdysone in the in vitro shoot cultures of *Spinacia oleracea* (L.). **Scientific**

Reports, v. 9, n. 1, p. 1–10, 2019.

MUCHATE, N. S. et al. Plant Salt Stress: Adaptive Responses, Tolerance Mechanism and Bioengineering for Salt Tolerance. **Botanical Review**, v. 82, n. 4, 2016.

MÜLLER, S.; WRIGHT, A. J.; SMITH, L. G. Division plane control in plants: new players in the band. **Trends Cell Biol**, v. 19, p. 180–188, 2009.

MUNNS, R. Comparative physiology of salt and water stress. **Plant, Cell and Environment**, v. 25, n. 2, p. 239–250, 2002.

MUNNS, R. et al. Energy costs of salt tolerance in crop plants. **New Phytologist**, 2019.

MUNNS, R. et al. Osmotic adjustment and energy limitations to plant growth in saline soil. **New Phytologist**, v. 225, n. 3, p. 1091–1096, 2020.

MUNNS, R.; TESTER, M. Mechanisms of Salinity Tolerance. **Annual Review of Plant Biology**, v. 59, n. 1, p. 651–681, 2008.

NARENDRA, S.; VENKATARAMANI, S.; SHEN, G.; WANG, J.; PASAPULA, V.; LIN, Y.; ZHANG, H. The Arabidopsis ascorbate peroxidase 3 is a peroxisomal membrane-bound antioxidant enzyme and is dispensable for Arabidopsis growth and development. **Journal of Experimental Botany**, v. 57, n.12, p. 3033-3042, 2006.

NAWAZ, K.; HUSSAIN, K.; MAJEED, A.; KHAN, F.; AFGHAN, S.; ALI, K. Fatality of salt stress to plants: Morphological, physiological and biochemical aspects. **African Journal of Biotechnology**, v. 9, n. 34, 2010.

NOBRE, R. G. et al. Emergência, crescimento e produção da mamoneira sob estresse salino e adubação nitrogenada. **Revista Ciência Agronômica,** v. 44, n. 1, p. 76–85, 2013.

NOCTOR, G.; FOYER, C. H. Ascorbate and glutathione: keeping active oxygen under control. Annual review of plant biology, v. 49, n. 1, p. 249-279, 1998.

NOCTOR, G.; MHAMDI, A. Climate Change, CO_2, and Defense: The Metabolic, Redox, and Signaling Perspectives. **Trends in Plant Science**, v. 22, n. 10, p. 857–870, 2017.

OGUNNIYI, D. S. Castor oil: A vital industrial raw material. **Bioresource Technology**, v. 97, p. 1086–1091, 2006.

PALACIOS, C.; SERRA, D.; TORRES, P. Papel ecológico dos metabólitos secundários frente ao estresse abiótico. **Botânica no Inverno 2013**, n. April, p. 52–59, 2013.

PANDEY, S. FARTYAL, D.; AGARWAL, A.; SHUKLA, T.; JAMES, D.; KAUL, T.; NEGI, Y. K.; ARORA, S.; REDDY, M. K. Abiotic stress tolerance in plants: Myriad roles of ascorbate peroxidase. **Frontiers in Plant Science**. v. 8, n. 581. 2017.

PEDROTTI, A. et al. Causas e consequências do processo de salinização dos solos. **Revista Eletrônica em Gestão, Educação e Tecnologia Ambiental**, v. 19, n. 2, p. 1308–1324, 2015.

PIMENTEL, C. Relações hídricas em dois híbridos de milho sob dois ciclos de deficiência hídrica. **Pesquisa Agropecuária Brasileira**, v. 34, n. 11, p. 2021–2027, 1999.

PINHEIRO, H. A. et al. Leaf gas exchange, chloroplastic pigments and dry matter accumulation in castor bean (*Ricinus communis* L) seedlings subjected to salt stress conditions. **Industrial Crops and Products**, v. 27, n. 3, p. 385–392, 2008.

PRASAD, R. B.; RAO, B. V. Chemical derivatization of castor oil and their industrial utilization. In Fatty Acids. AOCS Press. p. 279-303. 2017.

PULLARO, T. C. et al. Effects of killed cover crop mulch on weeds, weed seeds, and herbivores. **Agriculture, Ecosystems and Environment**, v. 115, n. 4, p. 97–104, 2006.

RAJPUT, V. D.; HARISH, SINGH, R. K.; VERMA, K. K.; SHARMA, L.; QUIROZ-FIGUEROA, F. R.; MEENA, M.; GOUR, V. S.; MINKINA, T.; SUSHKOVA, S.; MANDZHIEVA, S. Recent Developments in Enzymatic Antioxidant Defence Mechanism in Plants with Special Reference to Abiotic Stress. **Biology**, v. 10, n. 4, p. 267, 2021. https://doi.org/10.3390/biology10040267

RAZA, A; SU, W.; GAO, A.; MEHMOOD, S.S.; HUSSAIN, M.A.; NIE, W.; LV, Y.; ZOU, X.; ZHANG, X. Catalase (CAT) Gene Family in Rapeseed (*Brassica napus* L.): Genome-Wide Analysis, Identification, and Expression Pattern in Response to Multiple Hormones and Abiotic Stress Conditions. **International Journal of Molecular Sciences**. v. 22, n.8, p.4281. 2021. https://doi.org/10.3390/ijms22084281

RIBEIRO, P. R. et al. Effect of temperature on biomass allocation in seedlings of two contrasting genotypes of the oilseed crop *Ricinus communis*. **Journal of Plant Physiology**, v. 185, p. 31–39, 2015.

RIBEIRO, P. R. et al. Physiological and biochemical responses of *Ricinus communis* seedlings to different temperatures: A metabolomics approach. **BMC Plant Biology**, v.

14, n. 1, p. 1–14, 2014.

RIBEIRO, P. R.; DE CASTRO, R. D.; FERNANDEZ, L. G. Chemical constituents of the oilseed crop *Ricinus communis* and their pharmacological activities: A review. **Industrial Crops and Products**, v. 91, p. 358–376, 2016.

ROSSATTO, T. et al. Gene expression and activity of antioxidant enzymes in rice plants, cv. BRS AG, under saline stress. **Physiology and Molecular Biology of Plants**, v. 23, p. 865-875, 2017.

SÁ, F. V. D. S. et al. Tolerance of castor bean cultivars under salt stress. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 20, n. 6, p. 557–563, 2016.

SANO, S., TAO, S., ENDO, Y., INABA, T., HOSSAIN, M. A., MIYAKE, C., MATSUO, M., AOKI, H., ASADA, K., Saito, K. Purification and cDNA cloning of chloroplastic monodehydroascorbate reductase from spinach. **Bioscience, biotechnology, and biochemistry**, v. 69, p. 762-772, 2005.

SANTOS, H. O. **Conservação de sementes de mamona (***Ricinus communis* L.**)**. [s.l.] Universidade Federal de Lavras, 2010.

SANTOS, R. F. et al. Aspectos econômicos do agronegócio da mamona. In: AZEVEDO, D. M. P.; BELTRÃO, N. E. M. (Eds.). **O agronegócio da mamona no Brasil**. Campina Grande: EMBRAPA ALGODÃO, 2007. p. 21–41.

SAVY FILHO, A. et al. Novas Cultivares IAC-2028: nova cultivar de mamona. **Pesquisa Agropecuária Brasileira**, v. 42, n. 3, p. 449–452, 2007.

SCHIEBER, M.; CHANDE, N. S. ROS Function in Redox Signaling and Oxidative Stress.pdf. **Current Biology**, v. 24, p. 453–462, 2014.

SELEIMAN, M. F.; AL-SUHAIBANI, N.; ALI, N.; AKMAL, M.; ALOTAIBI, M.; REFAY, Y.; DINDAROGLU, T.; ABDUL-WAJID, H. H.; BATTAGLIA, M. L. Drought stress impacts on plants and different approaches to alleviate its adverse effects. plants (Basel, Switzerland), v. 10, n. 2, p.259, 2021. https://doi.org/10.3390/plants10020259

SEVERINO, L. S. et al. A review on the challenges for increased production of castor. **Agronomy Journal**, v. 104, n. 4, p. 853–880, 2012.

SEVERINO, L. S. et al. Crescimento e produtividade da mamoneira adubada com

macronutrientes e micronutrientes. **Pesquisa Agropecuária Brasileira**, v. 41, n. 4, p. 563–568, 2006.

SEVERINO, L. S.; MENDES, B. S. S.; LIMA, G. S. Seed coat specific weight and endosperm composition define the oil content of castor seed. **Industrial Crops and Products**, v. 75, p. 14–19, 2015.

SHARMA, I.; AHMAD, P. Chapter 4 - Catalase: A Versatile Antioxidant in Plants, Editor(s): Ahmad, P. Oxidative Damage to Plants, Academic Press, 2014, Pages 131-148, https://doi.org/10.1016/B978-0-12-799963-0.00004-6

SILVA, G. F.; SANTOS OLIVEIRA, G.; NASCIMENTO, J. J. V. R.; PEREIRA, R. G.; PAIVA, M. R. D. F. C. Germinação e crescimento inicial de mamoneiras irrigadas com água salina em diferentes volumes de substrato. **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v. 6, n. 2, p. 27, 2011.

SOFO, A. et al. Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. **International Journal of Molecular Sciences**, v. 16, n. 6, p. 13561–13578, 2015.

SOLTANI, S. S. R.; GHOLAM, A. K.; SEYED, K. P.; ALI, P. Z.; HAMID, N. Evaluation of photosynthetic pigment, antioxidant and non-antioxidant activity and some morphological traits changes under drought stress in castor plant (*Ricinus communis* L.), **Journal of Crop Breeding**, p. 0-0, 2022.

STEPHENIE, S. et al. An insight on superoxide dismutase (SOD) from plants for mammalian health enhancement. **Journal of Functional Foods**, v. 68, n. March, p. 103917, 2020.

TAIZ, L.; ZEIGER, E.; MOLLER, I.; MURPHY, A. **Fisiologia e desenvolvimento vegetal**. 6.ed. Porto Alegre: Artmed, 2017. 888 p.

TÁVORA, F. J. A. **A cultura da mamona**. Fortaleza: EPACE, 1982. 112 p.

TEIXEIRA, F. K.; MENEZES-BENAVENTE, L.; GALVÃO, V. C.; MARGIS-PINHEIRO, M. Multigene families encode the major enzymes of antioxidant metabolism in Eucalyptus grandis L. **Genetics and Molecular Biology**, v. 28, p. 529-538, 2005.

VASCONCELOS, P. DE C. T. et al. New insights into the mechanism underlying *Ricinus communis* L. tolerance to drought stress during germination. **Industrial Crops and**

Products, v. 103, p. 99–106, 2017.

VERMA, D.; KAUSHAL, N.; BALHARA, R.; SINGH, K.; Genome-wide analysis of Catalase gene family reveal insights into abiotic stress response mechanism in *Brassica juncea* and *B. rapa*. **Plant Science**. v. 330, p.111620. 2023. https://doi.org/10.1016/j.plantsci.2023.111620.

VERSLUES, P. E.; AGARWAL, M.; KATIYAR-AGARWAL, S.; ZHU, J.; ZHU, J. K. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. **The Plant Journal**, v. 45, n. 4, p. 523-539, 2006.

WANG, X. et al. Quantitative proteomic analysis of castor (*Ricinus communis* L.) seeds during early imbibition provided novel insights into cold stress response. **International Journal of Molecular Sciences**, v. 20, n. 2, 2019.

WEISS, E. A. Castor. In: **Oilseed Crops (World Agriculture Series)**. 2. ed. Oxford: Blackwell Science Ltd, 2000. p. 13–51.

WEISS, E. A. **Oilseed Crops**. London: Longman, 1983.

WILLEKENS, H.; INZÉ, D.; VAN MONTAGU, M.; VAN CAMP, W. Catalases in plants. **Molecular Breeding**, v. 1, p. 207-228, 1995.

WITT, T. W.; FLYNN, K. C.; ZOZ, T.; LEE, T. O.; MONTEIRO, J. E. B. A. A site suitability analysis for castor (Ricinus communis L.) production during Brazil's second harvest incorporating disease prediction. **Heliyon**, v. 9, n. 8, p. e18981, 2023. https://doi.org/10.1016/j.heliyon.2023.e18981

WRIGHT, A. J.; GALLAGHER, K.; SMITH, L. G. Discordia1 and alternative discordia1 function redundantly at the cortical division site to promote preprophase band formation and orient division planes in maize. **The Plant Cell**, v.21, n. 1, p. 234-247, 2009.

XU, W. et al. Genomic analysis reveals rich genetic variation and potential targets of selection during domestication of castor bean from perennial woody tree to annual semi-woody crop. **Plant Direct**, v. 3, n. 10, p. 1–16, 2019.

YOU, J.; CHAN, Z. ROS regulation during abiotic stress responses in crop plants. **Frontiers in Plant Science**, v. 6, n. DEC, p. 1–15, 2015.

ZELM, E. VAN; ZHANG, Y.; TESTERINK, C. Salt Tolerance Mechanisms of Plants.

Annual Review of Plant Biology, v. 6, p. 1–31, 2020.

ZHANG, B.; LEWIS, J. A.; KOVACS, F.; SATTLER, S. E.; SARATH, G.; KANG, C. Activity of Cytosolic Ascorbate Peroxidase (APX) from *Panicum virgatum* against Ascorbate and Phenylpropanoids. **Int J Mol Sci**. v. 24, n. 2, p. 1778, 2023. https://doi:10.3390/ijms24021778

ZHANG, Z.; HE, K.; ZHANG, T.; TANG, D.; LI, R.; JIA, S. Physiological responses of Goji berry (*Lycium barbarum* L.) to saline-alkaline soil from Qinghai region, China. **Scientific Reports**, v. 9, n. 1, p. 12057, 2019.

ZHU, J. K. Abiotic stress signaling and responses in plants. **Cell**, v. 167, n.2, p 313-324. 2016.

ZUCHI, J.; PANOZZO, L. E.; HEBERLE, E.; ARAUJO, E. F. Curva de embebição e condutividade elétrica de sementes de mamona classificadas por tamanho. **Revista brasileira de sementes**, v. 34, p. 504-509, 2012.

ZUCHI, J.; BEVILAQUA, G. A. P.; SEDIYAMA, C. S.; ZANUNCIO, J. C. Variation on castor seed quality and size by sowing time and floral order. **Revista Ciência Agronômica**, v. 41, n. 3, p. 403–408, 2010.

CAPÍTULO 1*. Castor (*Ricinus communis* L.) differential cell cycle and metabolism reactivation, germinability, and seedling performance under NaCl and PEG osmoticum: stress tolerance related to genotype-preestablished superoxide dismutase activity.

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Castor (*Ricinus communis* L.) differential cell cycle and metabolism reactivation, germinability, and seedling performance under NaCl and PEG osmoticum: stress tolerance related to genotype-preestablished superoxide dismutase activity.

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ABSTRACT

Castor (*Ricinus communis*) is a relevant industrial oilseed feedstock for many industrial applications, being globally mainly cultivated by smallholder farmers in semiarid areas, where abiotic stresses predominate. Therefore, susceptible to generating reactive oxygen species (ROS) and subsequent oxidative stress, compromising cell metabolism upon seed imbibition and germination, seedling and crop establishment, and yield. The present study evaluated the consequences of water restriction by Polyethylene glycol (PEG) and Sodium chloride (NaCl) on cell cycle and metabolism reactivation on germinability, seedling growth, and vigor parameters in 2 commercial castor genotypes (Nordestina and

Paraguaçu). PEG water restriction inhibited germination completely at -0.23 MPa or lower, presumably due to reduced oxygen availability. The restrictive effects of NaCl saline stress on germination were observed only from -0.46 MPa onwards, affecting dry mass accumulation and the development of normal seedlings. In general, superoxide dismutase (SOD) activity increased in NaCl -0.23 MPa, whereas its modulation during imbibition (24h) seemed to depend on its initial levels in dry seeds in a genotype-specific manner, therefore, resulting in the higher stress tolerance of Nordestina compared to Paraguaçu. Overall, results show that Castor germination and seedling development are more sensitive to the restrictive effects of PEG than NaCl at similar osmotic potentials, contributing to a better understanding of the responses to water restriction stresses by different Castor genotypes. Ultimately, SOD may constitute a potential marker for characterizing castor genotypes in stressful situations during germination, early seedling, and crop establishment, and a target for breeding for Castor-improved stress tolerance.

Keywords: *Ricinus* sp, Metabolism reactivation, Salinity, Polyethylene glycol, Water restriction.

1. INTRODUCTION

Ricinus communis L. (Euphorbiaceae) is commonly known as ‘castor’ and composes a globally relevant non-edible industrial crop due to its biochemical composition and the multiple industrial applicability of the oil extracted from its seeds (Rahbari et al. 2021; Su et al. 2020). It is a source of an attractive and versatile feedstock for high-quality lubricants, biofuels, cosmetics, and medical, among other numerous chemical industries (Bidin et al. 2020; Kaur and Bhaskar 2020). As in other parts of the world, in Brazil, it is mainly cultivated by small holder farmers in the Northeastern semi-arid region, where it can be intercropped with food crops, such as corn and beans (Marengo et al. 2022; Oliveira Filho et al. 2016; Vasconcelos et al. 2017).

Water restriction is commonly imposed in semiarid areas by drought and/or soil salinity, which can be coupled with high temperatures, affecting crop establishment and development (Cavalcante et al. 2021; Marengo et al. 2022; Melo et al. 2022). Water restriction is generally attributed to a reduction in water potential, i.e., the more negative the potential values, the less free water is available (Ievinsh 2023). On the other hand, salinity also causes water restriction, which may be coupled with phytotoxic effects due to the absorption of ions by the seeds and plants (Gong 2021; Tavares et al. 2021; Zhao

et al. 2021). Thus, water restriction influences the water uptake by the seeds (imbibition), and subsequent reactivation of metabolism, germination, and subsequent seedling and crop establishment (Pereira et al. 2017; Song et al. 2021).

Water uptake is essential for the reactivation of metabolism and germination in orthodox seeds, like castor (Pereira et al. 2017), as upon hydration, the embryo's cells reactivate its metabolism in which various molecular, biochemical, and physiological events occur (Brito et al. 2016; Pereira et al. 2017; Zhao et al. 2021), among which the reactivation of the cell cycle that induces the formation of microtubules through the polymerization of α- and β-tubulin protein dimers that are vital components involved in the maintenance of the cell cytoskeleton associated with cell metabolism and expansion, and further cell proliferation through mitotic events (De Castro et al. 2000; Hashimoto 2015; Hsiao and Huang 2023; Oracz et al. 2007). The detection of tubulin accumulation contributes to the investigation of cell cycle reactivation processes, being considered a marker for metabolism and cell cycle reactivation in developmental events, including seed germination, seedling growth, and plant development through the growth of vegetative (branches, leaves, roots) and reproductive (flowers, fruits, and seed formation) shoots (Brito et al. 2016; De Castro et al. 2000; Vasconcelos et al. 2017).

On the other hand, water restriction stresses can induce the production and accumulation of reactive oxygen species (ROS), causing subsequent oxidative stress. ROS are unstable and highly reactive molecules that may damage proteins proteins, carbohydrates, lipids, and nucleic acids in cells (Lanza et al. 2021; Silva et al. 2020; Stephenie et al. 2020). Plants have developed efficient antioxidant machinery to eliminate ROS in many cellular compartments (Gomes Neto et al. 2018; Soltani et al. 2022). The superoxide dismutase (SOD, EC 1.15.1.1) enzyme is considered the important of abiotic stress responses in plants (Rajput et al. 2021), being the first in the line of the antioxidant enzymes system, catalyzing the dismutation of $O^{2-\cdot}$ forming H_2O_2 and O^2, found in all subcellular compartments, such as apoplasts, cytoplasm, chloroplasts, mitochondria, nuclei, and peroxisomes (Gomes Neto et al. 2018; Rajput et al. 2021). SOD enzymes are classified according to the presence of cofactor (Cu/Zn, Mn, or Fe) in plants (Ismaiel and Piercey-Normore 2023; Su et al. 2020; Yu et al. 2022).

Although there are reports on the effects and behavior of castor seedlings under drought and salinity conditions (Babita et al. 2010; Sá et al. 2016; Wang 2019), there are, in general, still few studies on the biochemical and molecular effects that such stresses promote during the initial stages of imbibition, germination, and seedling establishment.

The knowledge behind the concept of the antioxidant enzymes system has been a source of potential markers for plant breeding programs and, therefore, can contribute to the development of superior castor genotypes better adapted to the common stressful conditions in semiarid regions, especially when family subsistence farming systems carry out castor cultivation as in the Northeastern Brazilian and other global semiarid areas (Brito et al. 2020; Ekbic et al. 2017; Gomes Neto et al. 2018; Vasconcelos et al. 2017).

Hence, the objectives of this study were to compare two different Brazilian castor cultivars developed as suitable for semiarid regions by evaluating the restrictive effects imposed by PEG and NaCl solutions on water uptake, cell cycle reactivation, and SOD activity upon germination and seedling establishment performance between both genotypes.

2. MATERIALS AND METHODS

2.1 Seed morphometry, water content, and weight

Both seed batches of castor cv. BRS Nordestina and of cv. BRS Paraguaçu were kindly provided by the Brazilian Agricultural Research Corporation (EMBRAPA) in Campina Grande, Brazil. Seeds were received at the Laboratory of Biochemistry, Biotechnology, and Bioproducts (LBBB) at the Federal University of Bahia (UFBA), where they were immediately stored at room temperature within containers with silica gel for low relative humidity (RH%). After one week of storage, the seeds were measured. Water content on a fresh weight basis was determined by confronting the initial fresh weights of 4 replicates of 10g of seeds against their dry weights after drying at 105±3°C for 24h. Morphometry was based on 4 replicates of 50 seeds using a digital caliper (Marathon) with an accuracy of 0.01mm for measuring the length (from the hilum to the opposite edge), width, and thickness (median regions). The weights of 1000 seeds were based on 8 replicates of 100 seeds weighed on an analytical scale (Shimadzu, Mod. AUW220D) (Brasil 2009).

2.2 Germinability

Seeds were incubated for imbibition and germination in water (0.0 MPa) as control and at different osmotic potentials (-0.23, -0.46, -0.69 MPa) in aqueous solutions of PEG (polyethylene glycol 8000, Sigma-Aldrich, cod. P2139) and NaCl (Sigma-Aldrich, cod. 71379). Seeds of both genotypes initially had the caruncles removed with forceps, followed by surface disinfestation (5 min immersion in 0.5% hypochlorite) plus 3 x 5 min washings with double distilled water, and subsequently scarified with the aid of sandpaper no. 120 to avoid the possible influence of physical hard-tegument dormancy. Seeds were then sown over moistened sterile germination paper placed in plastic trays (5 x 20 seeds) followed by incubation in germination chambers at 30±1°C, continuously dark, for 144h (7 days). Seeds were considered germinated with 2 cm radicle protrusion (Gomes Neto et al. 2018). The germinability parameters evaluated were percentage of maximum germination (G_{max}); germination speed measured as the time to reach 50% germination of viable seeds (T_{50}); germination uniformity obtained in the time interval between 84 and 16% of viable seeds (U_{84-16}); and the overall germination performance index measured as the "area under the curve" after 144h of imbibition (AUC_{144}) representing the integration of all germinability parameters, using the GERMINATOR software package (Joosen et al. 2010).

2.3 Seedling performance

Seven days old seedlings were evaluated for their growth performance based on the percentage of normal seedlings (PNS), root length (RL), epicotyl length (EL), root dry mass (RDM), epicotyl dry mass (EDM), root dry mass/length ratio (RDM/RL), and epicotyl dry mass/length ratio (EDM/EL).

2.4 Seed imbibition curves

Seeds imbibed in water, PEG, or NaCl were weighed every 3h until (72h) and water uptake was expressed as a percentage of the initial dry weight of the seeds based on the average weight of 3 replicates of 10 seeds for each treatment. The initial water content and seed dry weight were determined at 105°C ± 3°C for 24h (Brasil 2009; Gomes Neto et al. 2018).

2.5 Cell cycle reactivation

Reactivation of the cell cycle during imbibition was based on the immunohistochemical analysis of β-tubulin accumulation and microtubular cytoskeleton configurations in castor seed embryonic cells (Vasconcelos et al. 2017). Immunodetection was on at least 5 embryonic radicles (ca. 1 mm) isolated from dry seeds (0h), and seeds imbibed for 24h at 0.0 MPa in water (control) and -0.23, -0.46, and -0.69 MPa water restriction conditions by PEG and saline NaCl solutions. Isolated embryonic radicle samples were then fixed in 4% paraformaldehyde (JT. Baker, Mexico), dehydrated in a series of increasing ethanol concentrations (JT. Baker, Mexico), followed by a series of ethanol substitutions, incorporated in a butyl methyl methacrylate (BMM) mixture (Sigma-Aldrich, USA), and polymerized in UV at -20°C for 24h (Baskin et al. 1992). Samples of ultramicrotome longitudinal sections (ca. 3 µm thick) (Leica, mod. EM UC7) were fixed on slides, followed by immersion of slides in 100% acetone for removal of BMM followed by immediate rinsing (3 x 5 min) with phosphate-buffered saline (PBS). Sections on slides were then blocked with hydroxyl tetramonium chloride (Sigma-Aldrich, USA) and acetylated bovine serum albumin (BSA-c, Aurion, Netherlands) (Faria et al. 2005), followed by slides with sections by incubation in 1% v/v primary antibody solution (DM1A monoclonal anti-tubulin clone, Sigma-Aldrich, USA, cod. T9026), and subsequent incubation in 1% v/v secondary antibody solution (Alexa Fluor®488 goat anti-mouse IgG (H + L), Molecular Probes/Life Technologies, USA, cod. A-11001). The omission of the first antibody in all slides and sections was used as a negative control for all treatments. Tubulin or microtubules were observed under fluorescence microscopy (Olympus QC Color 3, USA).

2.6 Protein extraction, quantification, and SOD activity.

Total protein extraction was measured following the methods employer for castor tissues (Gomes Neto et al. 2018), in which embryonic axes were isolated from dry seeds (0h), and seeds imbibed for 24, 48, and 72h at 30°C continuously dark, at 0.0 MPa in water (control), and under water restriction conditions at -0.23 and -0.46 MPa in PEG and NaCl solutions. The samples of each treatment were composed of 50 embryonic axes isolated and stored frozen at -80°C until further use. Samples of 5 mg of each frozen sample was ground in the presence of 125 uL of 0.1 M potassium phosphate buffer (pH

7.8). Samples were gently stirred on a shaker table for 1h on ice-cooled condition and then centrifuged at 14,000 rpm for 30 min at 4°C.

The supernatant was then transferred to new microtubes and stored at -20°C or immediately used for further analysis. Total protein quantification was performed using the method described by Bradford (1976) with modifications. An analytical curve was built using points from 0 to 1.4 $mg.mL^{-1}$ of bovine serum albumin (BSA). Protein quantification was performed using 5 µl of total protein extract plus 250 µl of Bradford reagent (Sigma-Aldrich B6916). Absorbance measurements at 595 nm were performed after 15 minutes in a microplate spectrophotometer (Varian, Cary 50 MPR, United States), as well as the measurements of total SOD activity of the enzymatic extracts which were produced according to previously adjusted protocols (Gomes Neto et al. 2018; Andrade et al. 2021), in which samples consisted of 175 µL of the reaction mixture (50 mM PPB, pH 7.8), 13 mM methionine, 75 mM NBT, 2 mM riboflavin, 0.1 mM EDTA) plus15 µL of the respective enzyme extracts. The reaction started when the reaction mixture samples were exposed to a 27W fluorescent lamp at 25°C for 15min.

3. RESULTS AND DISCUSSION

3.1. Seed morphometry, water content, and dry matter

Seeds of cv. Paraguaçu were larger than cv. Nordestina, as the seed length was 15.55 and 15.40mm, the width was 12.30 and 11.43mm, and the thickness was 7.20 and 6.72mm, respectively. The weight of 1000 seeds showed that cv. Paraguaçu was also heavier than cv. Nordestina at similar water content, 75.37 and 62.0g, respectively (Table 1). Seed phenotype is a key discriminator parameter for genetic variability among the genotypes of the same species (Rukhsar et al. 2017; Senthilvel et al. 2016), whereas castor is known for its significant phenotypical variability among genotypes. Castor seed biometry can vary among cultivars and within racemes. In contrast, the seed size and density can influence germinability and seedling vigor and, therefore, the tolerance of seeds and seedlings to environmental conditions (Rukhsar et al. 2017; Senthilvel et al. 2016; Severino and Auld 2013).

Tab 1. Biometry of castor seeds cv. Nordestina and cv. Paraguaçu. Height, width, and thickness (mm), water content (%), and weight of 1000 seeds (g). Lo wercase le t - ters indicate si gni ficant trea tment di ffe rence s (Scott -Knot t test, $P < 0.05$).

Cultivar	Height	Width	Thickness	Water content (%)	Weight 1000 seeds (g)
	(mm)				
Nordestina	15.40±0,02 b	11.43±0.03 b	6.72±0,03 b	3.25±0.23 a	62.0±1.00 b
Paraguaçu	15.55±0,05 a	12.30±0.22 a	7.20±0,12 a	3.57±0.16 a	75.4±3.19 a

The size and quality of castor seeds can be influenced by the genotype, sowing time, plant growth and development, number of racemes, and position of the seed/fruit within the raceme (Severino et al. 2013). Castor seeds generally reach physiological maturity at around 22% of water content. This occurs at the later stages of fruit/seed development, i.e., during maturation drying, when fruits are still connected to the mother plant, and when seeds generally reach good germinability levels. This is typically the case in castor cv. Nordestina and cv. Paraguaçu, which are indehiscent genotypes. On the other hand, in castor dehiscent genotypes, the maturation drying may be completed after seed dispersal. Therefore, germinability levels may also depend on the duration, microflora, and the environmental conditions that seeds are exposed to while dispersed in the soil (Bewley et al. 2013; Vallejos et al. 2011).

It is recommended that castor seeds be stored with water content below 10%, which is generally reachable after harvest during storage at low relative humidity (RH%) conditions (Drumond 2020). The initial water content of cv. Nordestina and cv. Paraguaçu seeds were 3.25, and 3.57%, respectively (Table 1). A previous report found that the water content of 6.7% in freshly harvested castor seeds also decreased to 3.5% when stored at low RH% within a container supplied with silica gel (Vasconcelos et al. 2017).

Our results corroborate the differences in seed morphometry and dry mass weight, with similar water content, between castor cultivars developed by the EMBRAPA breeding program specifically for the semiarid region in Brazil. While we intended to investigate deeper and characterize better the physiological, cytological, and biochemical performances of both cultivars under the restrictive effects by different osmotic conditions of PEG and NaCl during germination and seedling establishment, which

together compose the re-start and the most critical phase within the plant's life cycle, and crucial for proper crop establishment (Meng et al. 2017; Micco et al. 2013).

3.2. Differential restrictive effects by PEG and NaCl on germinability parameters

The seeds of both cultivars had maximum germination (100%) when imbibed in water (0.0 MPa). However, germination was fully inhibited in both cultivars when imbibed in all PEG treatments. In contrast, germination in seeds of both cultivars did not occur by NaCl only at -0.69 MPa (Fig. 1a-c). The NaCl ions can penetrate the cell, whereas PEG is a highly inert molecular weight molecule not uptaken by the cell. While having a high affinity for water, it can also reduce oxygen availability due to its high viscosity (Awan et al. 2021; Ensing et al. 2019).

Therefore, the difference between germination inhibition in NaCl and PEG can be due to PEG's combined water and oxygen restriction capacity compared to NaCl, which would substantially restrict the reactivation of the overall seed metabolism. The effect of similar restrictive effects by PEG has been reported to occur also at -0.2 MPa in two other Castor cultivars (EBDA-MPA34 and Paraguaçu) (Vasconcelos et al. 2017), corroborating the results for PEG in the present study. Studies with *R. communis* cultivars by Song et al. (2021) demonstrate that both salt (NaCl) and water stress (PEG-6000) caused similar reductions in germination percentage, biomass accumulation, foliar gas exchange, and plant chlorophyll pigments, while PEG induced more severe harmful effects compared to salt stress. There are other reports in which Castor showed high sensitivity to the lack of oxygen (Else et al. 2001; van Dongen et al. 2003).

On the other hand, imbibition in NaCl solution allows seed uptake of water and the Na^+ and Cl^- ions providing a decrease in the intracellular osmotic potential and consequently regulating the hydration and cell turgor due to the maintenance of the water potential between the seed and the substrate (Ievinsh 2023), whereas large number of Na^+ and provided more energy to help the Castor seedlings cope with salt stress in its transition from the cotyledonary to true leaves stage (Wang et al. 2019).

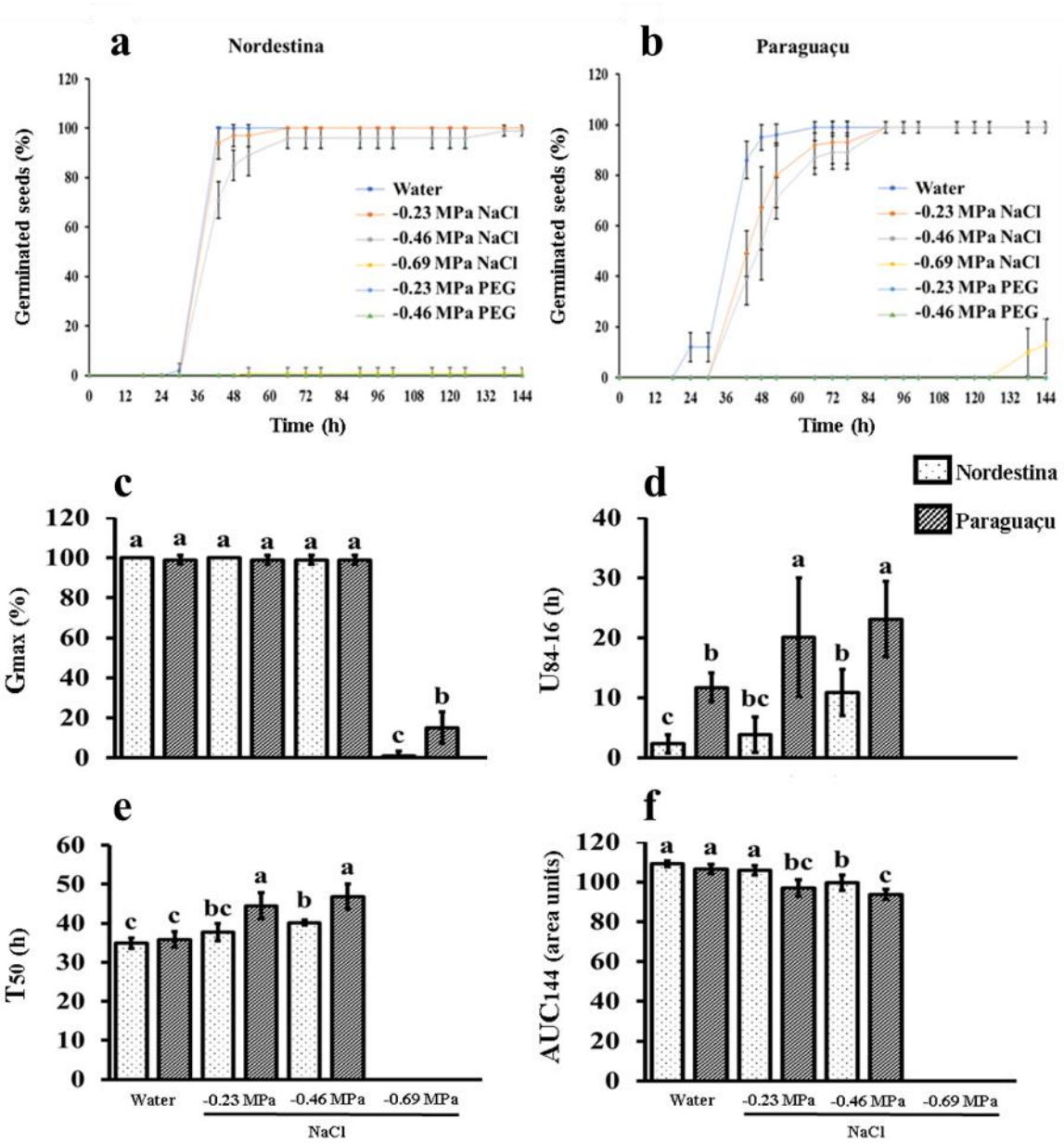

Fig 1. Germinability of *R. communis* cv. Nordestina and cv. Paraguaçu seeds in different NaCl and PEG osmotic potentials. **a-b:** Germination curves; **c:** Maximum germination (Gmax, %); **d:** Germination uniformity, i.e., the time interval between 84 and 16% of viable seeds germinated (U_{84-16}); **e:** Germination speed, as the time to reach 50% germination (T_{50}); **f:** Overall germination performance, as the Area Under the Curve index between 0 and 144h imbibition (AUC_{144}). Lowercase letters above the bars indicate significant treatment differences (Skott-Knott test, $p<0.05$)

Although both genotypes were able to germinate fully in water (0.0 MPa) (Fig. 1a-c), the germination uniformity between 84 and 16% germination in time (U_{84-16}) was higher for cv. Nordestina seeds (between 24 and 36h) as compared to cv. Paraguaçu seeds (between 18 and 66h) (Fig. 1a-b-d), whereas the germination speed (T_{50}) of both genotypes was the highest when germinating in water (Fig. 1e). Although the difference in uniformity, the overall high germination percentage (G_{max}) and speed (T_{50}) seemed to have mainly contributed to the similar high overall water control (0.0 MPa) germinability indexes (AUC) between both genotypes (Fig. 1f).

There were also no differences for G_{max} between both genotypes in NaCl at -0.23 and -0.46 MPa (Fig. 1a-c), while cv. Nordestina showed better germination uniformity (U_{84-16}) and speed (T_{50}). Therefore, a better overall germination performance index (AUC) than cv. Paraguaçu (Fig. 1d-f). These results corroborate previous studies in which Castor cv. Energia and cv. IAC 226 seeds also imbibed in NaCl at -0.23 and -0.46 MPa did not affect maximum germination but affected germination uniformity and speed (Brito et al. 2015; Moraes et al. 2015). A similar effect could also be observed in physic nut (*Jatropha curcas* L.) seeds where maximum germination was unaffected. Still, germination was delayed and affected the capacity for developing normal seedlings from seeds subjected to comparable levels of NaCl saline stress (Andréo-Souza et al. 2010).

Seeds are especially vulnerable to water restriction and salinity, initially observing a decrease in water uptake, consequently modifying the imbibition process (Deng et al. 2023; Soltani et al. 2022). Cell division and elongation processes are also affected, as well as the metabolism involving the mobilization of essential reserves for the germination process to occur (Nawaz et al. 2010; Song et al. 2021). Seed physiological processes are genetically characterized during their formation, and the performance of seeds during germination may vary between species and cultivars (Ievinsh 2023). In our study, cv. Nordestina showed better performance in the germination parameters in saline NaCl solutions, whereas cv. Paraguaçu presented minimum germination (15%±8) in NaCl -0.69 MPa (Fig. 1a-f).

3.3. Differential dry mass accumulation in seedlings submitted to restrictive NaCl osmotic conditions.

The effects of different osmotic potentials were also evaluated for the production, growth, and vigor of seedlings originating from germinated seeds of both cultivars in water and NaCl at -0.23 and -0.46 MPa. In contrast, seeds did not germinate in NaCl at -0.69 MPa and in PEG at all osmotic potentials. Therefore, it could not generate any seedlings.

The percentages of normal and abnormal seedlings were evaluated to assess seedling production and quality. In contrast, growth and vigor were assessed by the lengths and dry mass accumulation of roots and epicotyls and by the relative growth index as measured by the ratios between dry mass and length of roots and epicotyls.

Seeds of cv. Nordestina and cv. Paraguaçu produced normal seedlings (NoS) and abnormal seedlings (AbS) in NaCl at -0.23 and -0.46 MPa at similar rates as the in water (control, at 0.0 MPa). However, a significant reduction in the number of normal seedlings was observed for both cultivars in NaCl at -0.69 MPa (Fig. S1). A comparable profile has been observed in a study with *Jatropha curcas* (also a Euphorbiaceae), which was observed in a delay in germination and the growth of the initial seedlings as the water potential of NaCl solutions increased (Andréo-Souza et al. 2010).

Overall, the seedlings of cv. Nordestina presented larger RL compared to cv. Paraguaçu when grown in water (control) and all NaCl potentials, whereas the RL was not affected in both cultivars imbibed in NaCl compared to controls in water, but cv. Paraguaçu was more sensitive than cv. Nordestina in NaCl -0.23 and -0.46 MPa (Fig. 2a). On the other hand, seedlings of both cultivars, in general, presented a reduction in their EL when imbibed in NaCl at -0.23 MPa or higher as compared to the control in water, being the epicotyls of cv. Paraguaçu more sensitive than those of cv. Nordestina (Fig. 2b). However, the accumulation of RDM contrasted between cultivars, in which cv. Paraguaçu seedlings accumulated more RDM than cv. Nordestina when in NaCl, whereas in water, the accumulation of RDM in cv. Nordestina was higher than in cv. Paraguaçu (Fig. 2c). The cv. Nordestina accumulated more EDM than cv. Paraguaçu in the water control condition, whereas the accumulation of EDM was lower in water control and without differences among cultivars in NaCl -0.23 and -0.46 MPa treatments (Fig. 2d). The NaCl osmotic potentials did not affect the cv. Nordestina RDM/RL ratio compared to the control. In contrast, the cv. Paraguaçu RDM/RL ratio increased, suggesting a better

adaptation of the root system of cv. Paraguaçu seedlings imbibed in NaCl (Fig. 2e). Conversely, the EDM/EL ratios were reduced in both cultivars, suggesting a compensating adaptability behavior of the shoot systems of both cultivars under imbibition in NaCl (Fig. 2f).

Water restriction acts by reducing the speed of physiological and biochemical processes. Therefore, affects seed germinability and seedling development. Lack of proper water amounts may interfere with the reactivation of seed metabolism, such as reductions in the breakdown and translocation of seed reserves to the different parts of the new seedling (Deng et al. 2023; Soltani et al. 2022), which may then present smaller lengths and less dry matter mass accumulation (Cao et al. 2018). The effect of water restriction by NaCl saline solutions in castor seedlings can be observed by the reduction in height resulting from a progressive increase in salinity, (Devora-Isiordia et al. 2018; Lima et al. 2014) as observed in the present study.

Cellular responses to water restriction and salinity include membrane system adjustments, changes in cell wall architecture, in cell cycle and cell division, and the reactivation of metabolism in the case of (orthodox) seeds upon imbibition and germination (Deng et al. 2023; Devora-Isiordia et al. 2018). In addition, plants alter metabolism in some ways, including producing compatible solutes such as proline, raffinose, and glycine betaine, which can stabilize proteins and cell structures and maintain cell turgor by osmotic adjustment. Besides, activating redox metabolism removes excess levels of ROS and restores cellular redox balance (Munns and Tester 2008; Valliyodan and Nguyen 2006).

Salinity and drought are the principal abiotic stresses that limit plant growth and crop productivity (Ma et al. 2020). Both salt and water stress (PEG) caused similar reductions in germination percentage, biomass accumulation, leaf gas exchange, and chlorophyll pigments, while more severe detrimental effects were induced by water stress compared to salt stress (Rahbari 2021; Soltani et al. 2021). Plants respond to these stresses differently through morphological, physiological, and biochemical changes and by regulating numerous genes, which can be expressed differently across cultures (Song et al. 2021; Wang et al. 2019). Castor seedlings can accumulate osmotic regulators (eg, soluble sugars and proline) and phytohormones in their cotyledons and roots to improve their salt tolerance. Furthermore, specific changes in endoplasmic reticulum protein processing were observed in cotyledons, and roots could increase lignin synthesis to

reduce membrane damage in this species under salt stress (Deng et al. 2023). Salinity affected the lipids and carbon reserves from cotyledonary leaves of *Jatropha curcas* seedlings. Protein and amino acid accumulation were also observed in roots and associated with the saline stress response (Lira et al. 2021).

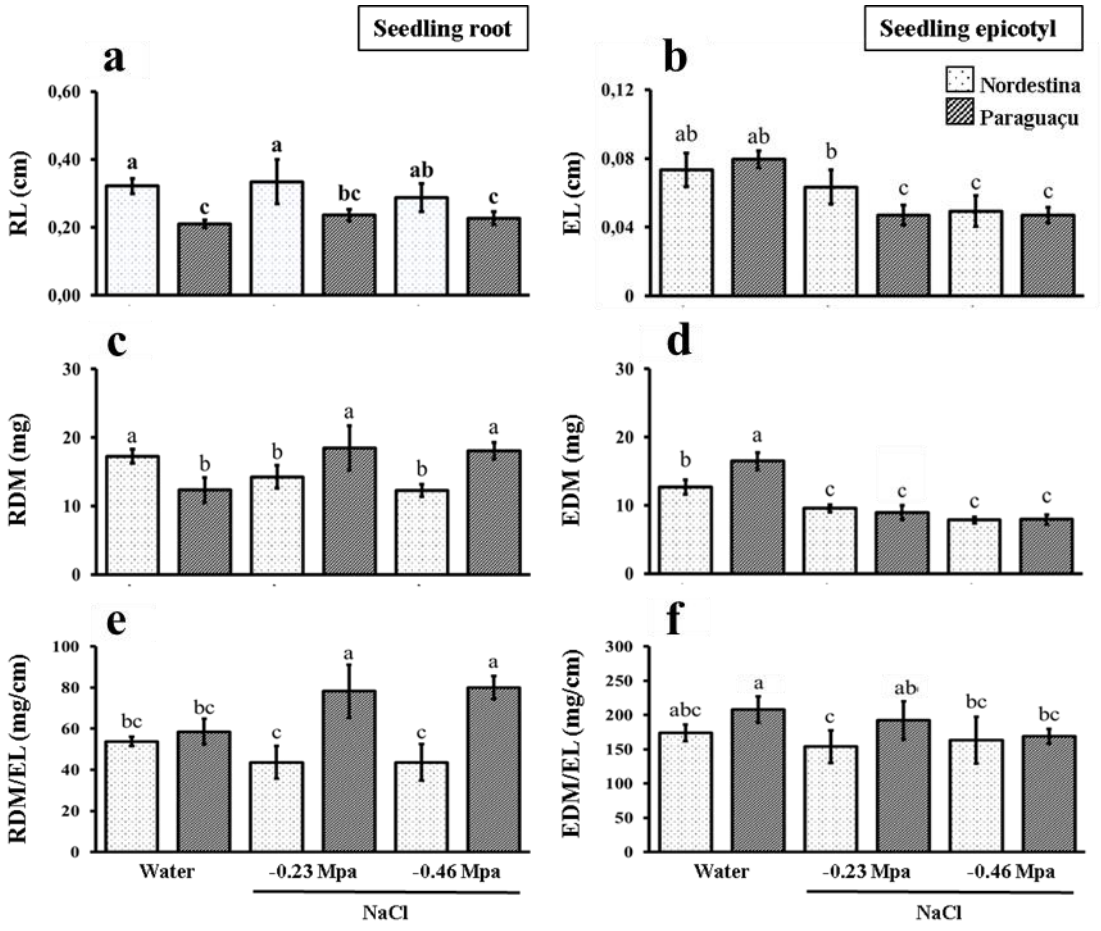

Fig 2. Seedling root and epicotyl of *R. communis* of seeds germinated under water restriction by NaCl and PEG solutions. **a:** Root length (RL); **b:** Epicotyl length (EL); **c:** Root dry mass (RDM); **d**: Epicotyl dry mass (EDM); **e:** Root dry mass per length ratio (RDM/RL); **f:** Epicotyl dry mass per length (EDM/EL). Different lowercase letters above the bars indicate significant differences between treatments, Scott-Knott test ($P<0.05$)

3.4. Patterns of seed water uptake under water restriction by NaCl and PEG

Both cultivars presented similar triphasic water uptake patterns (Fig. 3) as typical of orthodox seeds (El-Maarouf-Bouteau 2022), in which a rapid water uptake was observed in both cultivars up to 12h of imbibition (Phase-I) in which the larger seeds of

cv. Paraguaçu (larger seeds) absorbed more water (ca. 20%) than the smaller seeds of the cv. Nordestina (Fig. 3a-b). Both cultivars reached 20% of water content in water (0.0 MPa) and NaCl solution (-0.23 MPa), while in PEG solution, the seed water uptake reached only 12.34% for cv. Nordestina and 15.46% for cv. Paraguaçu (Fig 3a-b). A similar pattern was also observed between cv. Paraguaçu and the smaller seeds of cv. EBDA-MPA34 (Gomes Neto et al. 2018).

During the imbibition process, the metabolism reactivates gradually with water absorption. Cellular respiration is one of the processes described for being activated to produce energy in the form of ATP after the seed absorbs around 20% of water content, where processes such as glycolysis, Krebs cycle, and electron transport chain are necessary (Bewley et al. 2013; El-Maarouf-Bouteau 2022). In Phase-I, the first activities associated with the repair of damage accumulated during drying and the storage period of the seeds occur, such as the repair of membranes and DNA (Bewley et al. 2013). The formation of polysomes from free ribosomes also happens early during imbibition to create the machinery for translating messenger RNAs (mRNAs) into proteins (Nonogaki, 2017).

From 24 to 36h of imbibition, a decrease in water uptake by the seeds was observed (Phase-II). In control (0.0 MPa), Phase-II was considered for both genotypes starting around 24h, where the cv. Nordestina absorbed approximately 27.39% in water content and the cv. Paraguaçu absorbed around 33.52% of water (Fig. 3b). There was a slight delay at the beginning of Phase-II for seeds soaked in NaCl compared with control, occurring after 30h, having absorbed 27.84% of water uptake in seeds of the cv. Nordestina and 31.98% in seeds of cv. Paraguaçu. In PEG for both cultivars, Phase-II was observed from 48h on, with 21.52% of water uptake in cv. Nordestina and 29.29% of water uptake in cv. Paraguaçu (Fig. 3a-b). The osmotic PEG solution (-0.23 MPa) did not allow germination (Phase-III), which is marked by an increase in seed water content associated with the initiation of cell division and, consequently, embryonic elongation and differentiation (Bewley et al., 2013), which in fact, cannot be observed in seeds imbibed in PEG solution, as they did not absorb enough water (Fig. 3a-b) to proceed with the activation of metabolism and cell cycle activities (Fig. 4).

Phase-III (radicle protrusion) in water (control, 0.0 MPa) was observed from 30h on at cv. Nordestina (30.33% of water uptake), Paraguaçu started from 33h (37.48% of water uptake) (Fig. 3a-b). The water restriction effects imposed by the imbibition in NaCl

(-0.23 MPa) were able to slightly delay the water uptake compared to the imbibition in control (0.0 MPa), being observed at the beginning of Phase-III (radicle protrusion) with 33h cv. Nordestina (29.06% water uptake) and 48h cv. Paraguaçu (44.43% water uptake) (Fig. 3A). While the imbibition in PEG (-0.23 MPa) promoted a drastic restriction in the water absorption, being observed water absorption after 72h of imbibition around 21% for cv. Nordestina and 31% for cv. Paraguaçu. The water restriction imposed by the imbibition in PEG (-0.23 MPa) was enough to inhibit the germination process, i.e., Phase-III was not initiated (Fig. 3b).

Another study with *R. communis* showed that Phase-II initiated ca. 24h and completed ca. 40h (Zuchi et al. 2012). Differences in the percentage of water uptake between Castor genotypes may indicate phenotypic variability related to morphometry parameters. In contrast, the seed lot quality can also cause variability in seed imbibition patterns (Rodrigues et al. 2010). Furthermore, seed water uptake, in general, can be affected depending on the species, genotype, environmental factors, and characteristics of the seed itself, such as size, weight, chemical composition, initial water content, and the constitution of the seed coat (El-Maarouf-Bouteau 2022; Gomes Neto et al. 2018; Zuchi et al. 2012).

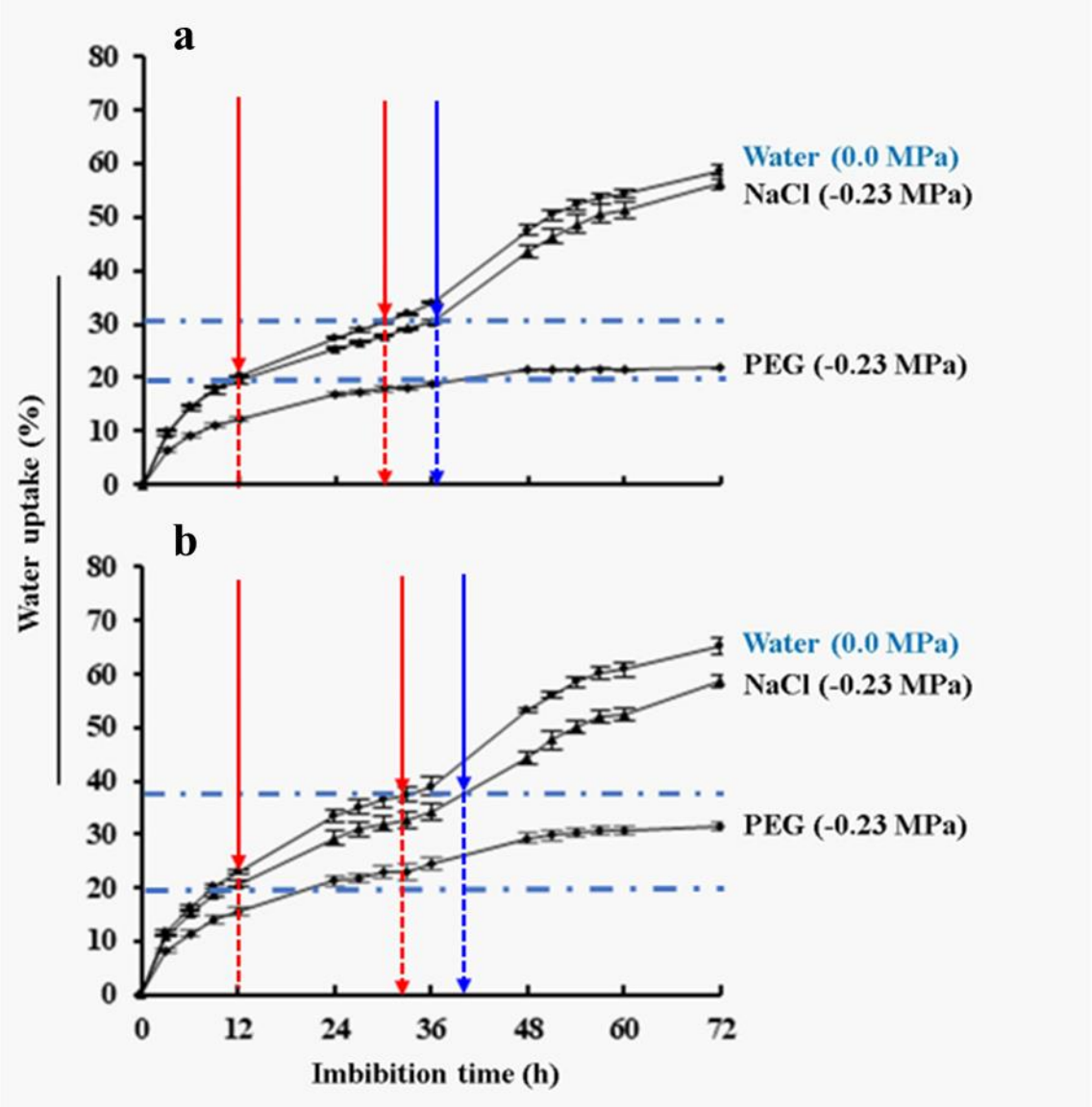

Fig. 3 Water uptake of *R. communis* seeds imbibed in NaCl and PEG Solution was measured as the percentage of the dry weight of seeds imbibed up to 72h. **a:** cv. Nordestina; **b:** cv. Paraguaçu. First red arrows identify the transitions from Phase-I to Phase-II and Phase-II to Phase-III in water (control). Blue arrows identify the transition from Phase-II to Phase-III in NaCl. Seeds imbibed in PEG did not reach Phase-III (no germination). Mean values with standard deviations are shown for 3 biological replicates of 10 seeds.

3.5. Water restriction by PEG entirely prevent cell cycle reactivation and germinative processes

The evaluation of cell cycle reactivation through the immunocytochemical detection of the cytoskeletal β-tubulin allowed a better understanding of the effects of water restriction by NaCl and PEG during germination on *R. communis* seeds, i.e., performed in embryo tissues at times 0 (dry seed) and 24h, both in water (0.0 MPa) and under water restriction in NaCl and PEG (-0.23 and -0.46 MPa).

B-tubulin granules dispersed in embryonic cytoplasm indicated a typical cell division arrest in dry seeds (Fig. 4a-b) (De Castro et al. 2000a, b; Hashimoto 2015; Hsiao and Huang 2023). The abundance of the cortical microtubular cytoskeleton and some mitotic configurations indicated reactivation of the cell cycle in embryos of non-germinated seeds imbibed for 24h in water (0.0 MPa) (Fig. 4c-d). The same was observed in embryos of both cultivars imbibed for 24h at NaCl -0.23 MPa (Fig. 4e-f) and -0.46 MPa (Fig. 4g-h) solutions in PEG -but none in PEG solutions (Fig. 4i-l), apart from only some dispersed β-tubulin granules, like that found in dry seed cells. Seeds did not germinate when imbibed in PEG -0.23 MPa throughout the 144h imbibition period.

Generally, the preparation for cell division occurs well before root protrusion, as it requires cell cycle initiation (Bewley et al. 2013). To initiate the cell cycle activity, the seeds need to absorb water content to reach Phase-II, which in the present study occurred from 24h onwards in seeds of both cultivars in water control (0.0 MPa) and NaCl (-0.23 MPa) conditions, where was observed the accumulation of β-tubulin and presence of mitotic spindles (Fig. 4c-f). However, mitotic spindles were not observed in embryos of seeds imbibed in -0.46 MPa NaCl, nor in both PEG osmotic potentials, indicating a more substantial water restriction stress and inhibition of the germination process by PEG (Fig. 4g-l).

Germination occurs initially by imbibition, followed by elongation and cell division, depending on the species (Bewley et al. 2013). The dynamics of microtubular cytoskeleton configurations during germination may vary depending on the species (De Castro et al. 2000). In contrast, in other *R. communis* cultivars, cortical and mitotic microtubules were observed up to 48h imbibition in water (Vasconcelos et al. 2017), similarly as observed with the other genotypes in the present study. Also, cortical and mitotic cytoskeletons were observed during *Solanum lycopersicum* (Tomato) seed

imbibition, i.e., before radicle protrusion or before germination *per se* (De Castro et al. 2000), while mitotic microtubules were not observed before germination in and *Jatropha curcas* (Brito et al. 2016).

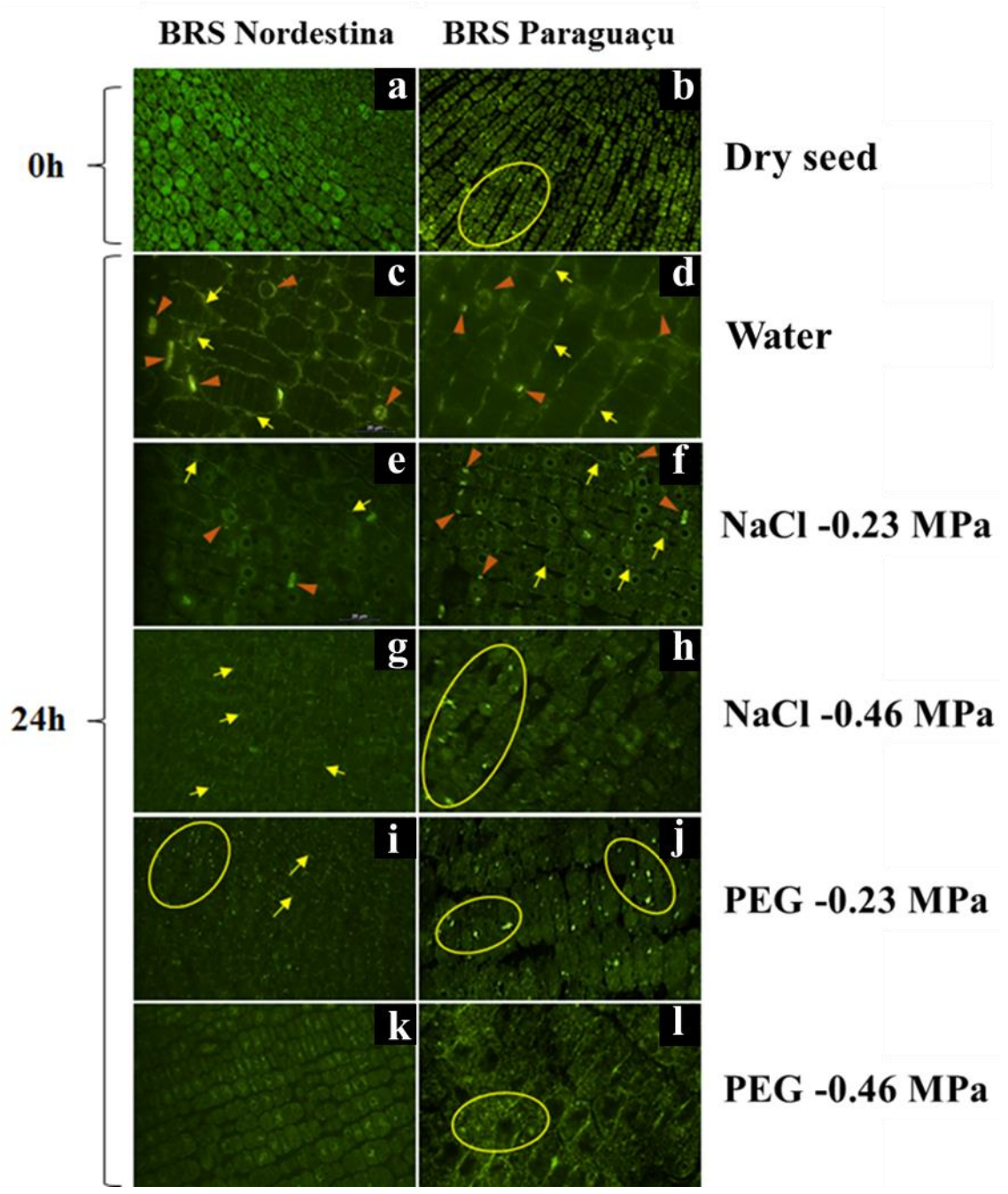

Fig. 4 Cell cycle activity resulting from α-tubulin accumulation and formation of microtubular cytoskeleton configurations in seeds of two cultivars of *R. communis* submitted to water restriction imbibition. **a-b:** Dry seeds; **c-d:** Water imbibition (Control); **e-h:** NaCl imbibition; **i-l:** PEG imbibition. Circles demonstrate the presence of fluorescent β-tubulin granules dispersed in the cytoplasm of the cells. Arrows

demonstrate the accumulation of microtubular cytoskeleton in cortical configurations. Red head arrows indicate cells with mitotic configurations.

In orthodox seeds, such as *R. communis*, the reactivation of the cell cycle and induction of mitosis during germination occurs after a period of imbibition, with DNA synthesis preceding mitosis because most meristem cells of mature seeds have 2C DNA content (Kozeko and Troyan 2000). The latency period before DNA replication is species-specific and constitutes a complex pre-replicative period that concludes the transition from the quiescence state (G0) to the G1 phase of cell cycle reactivation (Costas et al. 2011; Herrick 2011; Kozeko and Troyan 2000). Overall, the reactivation of the cell cycle, as analyzed by the accumulation of tubulin and formation of microtubular cytoskeleton configurations, was not restricted when seeds were imbibed in water (control) but delayed in NaCl depending on the osmotic potential and was fully restricted in PEG irrespective of the osmotic potential. The genotype Paraguaçu showed a somewhat differentiated pattern in which some cortical cytoskeleton accumulated higher contents than Nordestina. These results corroborate the imbibition and germinative pattern progression among both genotypes and the different water restriction conditions.

3.6 Higher stress tolerance related to preestablished SOD activity in a cultivar-specific manner and its enhancement under NaCl-restrictive conditions.

SOD activity showed distinct patterns between both cultivars, in which higher SOD activity occurred in dry seeds of cv. Nordestina than of cv. Paraguaçu, 11.11 against 3.21 U SOD.mg^{-1} of protein, respectively (Fig. 5a-b). After 24h of imbibition, the cv. Nordestina showed no variation in SOD activity in control seeds imbibed in water and under NaCl stress as compared to dry seeds but showed a decrease in SOD activity under PEG treatment, while cv. Paraguaçu increased SOD activity in all treatments compared to dry seeds. Overall, SOD activity was higher in NaCl -0.23 MPa and -0.46 MPa, and in PEG -0.23 MPa than in control seeds imbibed in water and PEG -0.46 MPa (Fig. 5a-b). In 48h of imbibition, the cv. Nordestina and cv. Paraguaçu showed no variation in SOD activity. In cv. Nordestina, SOD activity decreased in PEG -0.23 MPa and -0.46 MPa (Fig 5c-d).

After 72h of imbibition (post-germinative phase), and intense increase in SOD activity was observed in both cultivars under NaCl water restriction (-0.23 and -0.46 MPa)

compared to dry seeds, while cv. Nordestina showed higher SOD activity at NaCl -0.23 than the control, whereas cv. Paraguaçu had less SOD activity in dry seeds than in cv. Nordestina showed a significant increase in SOD activity in water control and NaCl -0.23 MPa compared to dry seeds (Fig. 5e-f). The results suggest that the high level of SOD activity in dry seeds can support the modulation of SOD activity during imbibition and germination (cv. Nordestina 24h of imbibition), while the lower SOD activity in dry seeds, as in cv. Paraguaçu seems to demand the modulation of SOD activity to support the balance of ERO. The water restriction conditions imposed by PEG inhibited germination, cell division, and SOD activity. The decrease in seed germination subjected to water stress is attributed to the reduction of enzymatic activity, which promotes lesser meristematic development, and for each species, there is a critical water potential value below which germination does not occur (Soltani et al. 2022; Yu et al. 2022; Zheng et al. 2021). The high concentrations of Na^+ and Cl^- ions cause protoplasmic swelling, affecting the activity of enzymes, and causing quantitative and qualitative changes in metabolism, resulting in low energy production, disturbances in nitrogen assimilation, alterations in the pattern of amino acids, and the metabolism of proteins. The excess of Na^+ and Cl^- in the protoplasm promotes disturbances in the ionic balance of K^+ and Ca^{2+}, as well as in the specific effect of the ions on enzymes and membranes (Lira et al. 2021).

Different castor bean varieties may have a greater capacity to deal with abiotic stress, showing less damage to growth and more significant activity of the enzyme superoxide dismutase (SOD) and accumulation of proteins, phenols, and flavonoids (Gomes Neto et al. 2018; Rajput et al. 2021; Soltani et al. 2022).

In addition, salt stress increases malondialdehyde (MDA) and proline content while decreasing dry weight and soluble sugar content. Transcriptome analysis of castor bean tissues revealed differential expression of genes involved in protein processing in the endoplasmic reticulum in the cotyledons and the biosynthesis of phenylpropanoids in roots. Furthermore, genes related to plant hormone signal transduction, starch and sucrose metabolism, and arginine and proline metabolism were induced in both tissues. These different responses and their synergistic relationship contribute to increasing castor bean tolerance to salinity (Deng et al. 2023; Soltani et al. 2022).

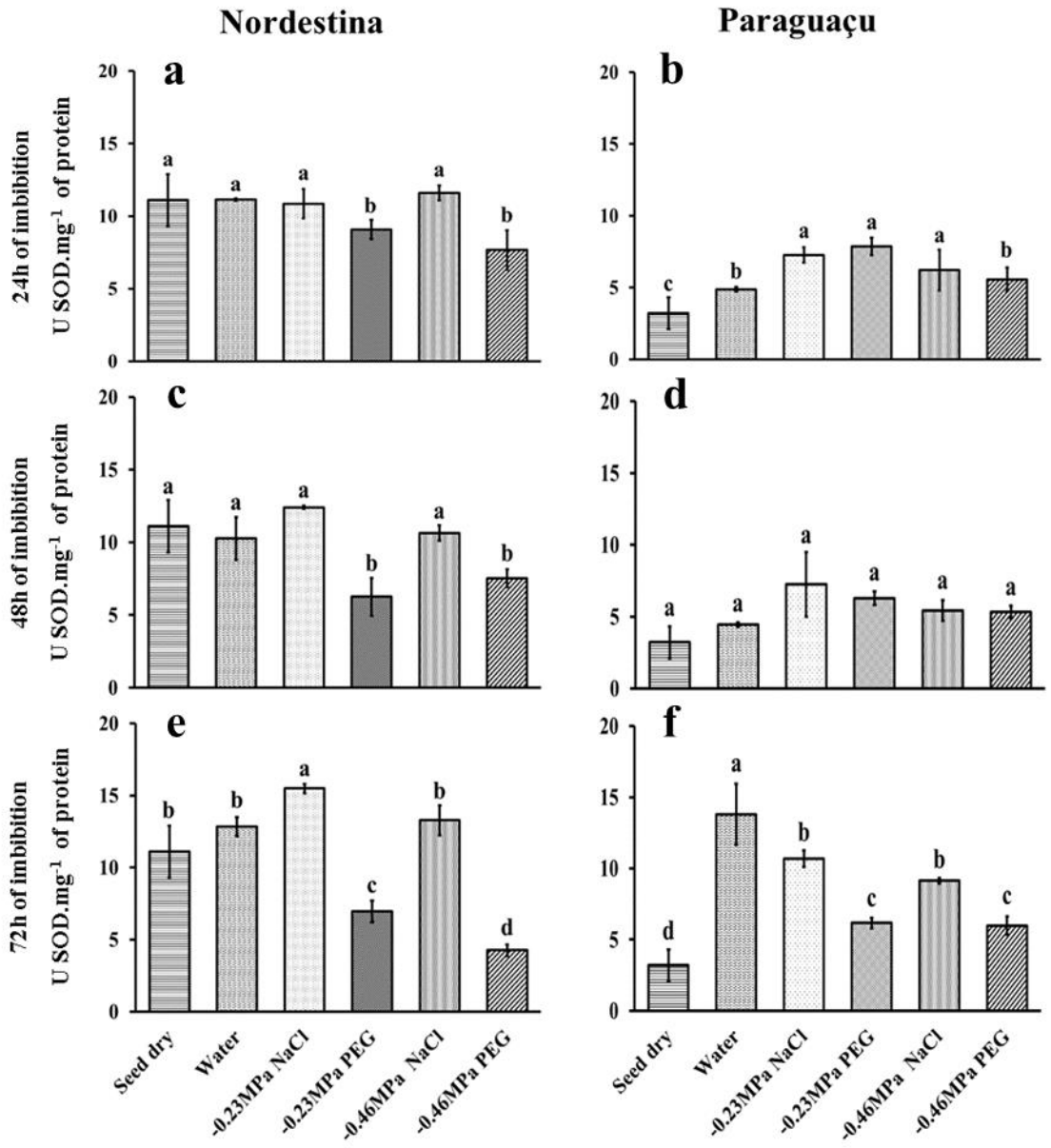

Fig. 5 Superoxide dismutase activity during germination and post-germinated seeds of *R. communis*. In dry seeds, imbibed in water (Control) and NaCl and PEG solution (-0.23 and -0.46 MPa) at 24, 48, and 72h. **a, c, e:** SOD activity in cv. Nordestina; **b, d, f:** SOD activity in cv. Paraguaçu. Different lowercase letters above the bars indicate significant differences between treatments, Scott-Knott test ($P < 0.05$)

4. CONCLUSIONS

The difference in seed morphometry of *R. communis* cultivars influenced water uptake, the reactivation of the cell cycle, SOD activity, and overall germinative metabolism under NaCl restrictive osmotic conditions while demonstrating higher sensitivity to the restrictive effects of PEG since the lowest water potential tested at -0.23 MPa, as it may also reduce oxygen availability. The restrictive effects of NaCl saline stress on germination were observed only from -0.46 MPa onwards, affecting dry mass accumulation and the production of normal seedlings. In general, SOD activity increased in NaCl -0.23 MPa, whereas its modulation during the onset of imbibition (24h) seemed to depend on its initial levels in dry seeds in a genotype-specific manner, therefore, resulting in the higher stress tolerance of cv. Nordestina compared to cv. Paraguaçu. Overall, the results show that Castor seed germination and seedling development are more sensitive to the restrictive effects of PEG than NaCl at similar osmotic potentials, contributing to a better understanding of the responses to water restriction stresses by different *R. communis* genotypes. Ultimately, SOD appears to be a potential marker for characterizing castor genotypes in stressful situations during germination and early seedling and crop establishment and a target for breeding aimed at Castor-improved stress tolerance.

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5. REFERENCES

Andréo-Souza Y, Pereira AL, Silva FFS, Riebeiro-Reis RC, Evangelista MRV, De Castro RD, Danta BF (2010) Effect of salinity on physic nut (*Jatropha Curcas* L.) seed germination and seedling initial growth. Rev Bras Sementes 32:83–92. https://doi.org/10.1590/s0101-31222010000200010

Babita M, Maheswari M, Rao LM, Shanker AK, Rao DG (2010) Osmotic adjustment, drought tolerance and yield in castor (*Ricinus communis* L.) hybrids. Environ Exp Bot 69:243–249. https://doi.org/10.1016/j.envexpbot.2010.05.006

Baskin TI, Busby CH, Fowke LC, Sammut M, Gubler F (1992) Improvements in immunostaining samples embedded in methacrylate: localization of microtubules and other antigens throughout developing organs in plants of diverse taxa. Planta 187:405–413. https://doi.org/10.1007/BF00195665

Basu S, Groot, SPC (2023) Seed vigour and invigoration. In: Dadlani, M., Yadava, D. K. (Eds.), Seed Science and Technology. Springer, Singapore. https://doi.org/10.1007/978-981-19-5888-5_4.

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds – Physiology of development, germination and dormancy. New York, USA. https://doi.org/10.1007/978-1-4614-4693-4

Bidin C, Gopinath S, Aravindraj R, Devaraj A, Krishnan SG, Jeevaananthan JKS (2020) The production of biodiesel from castor oil as a potential feedstock and its usage in compression ignition Engine: A comprehensive review. Mater Today Proc 33:84–92. https://doi.org/10.1016/j.matpr.2020.03.205

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/https://doi.org/10.1016/0003-2697(76)90527-3

Brasil (2009) Regra para análises de sementes, Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: Mapa/ACS. 399 p

Brito CD, Loureiro MB, Ribeiro PR, Vasconcelos PCT, Fernandez LG, De Castro RD (2016) Osmoconditioning prevents the onset of microtubular cytoskeleton and activation of cell cycle and is detrimental for germination of *Jatropha curcas* L. seeds. Plant Biol

18:1053–1057. https://doi.org/10.1111/plb.12482

Brito VC, Almeida CP, Barbosa RR, Carosio MGA, Ferreira AG, Fernandez LG, De Castro RD, Hilhorst H, Ligterink W, Ribeiro PR (2020) Overexpression of *Ricinus communis* L. malate synthase enhances seed tolerance to abiotic stress during germination. Ind Crops Prod 145:1–8. https://doi.org/10.1016/j.indcrop.2020.112110

Cao X, Zhong C, Zhu C, Zhu L, Zhang J, Wu L, Jin Q (2018) Ammonium uptake and metabolism alleviat e PEG-induced water stress in rice seedling s. Plan t Physiol. Biochem. 132, 128–137. https://doi.org/10.1016/j.plaphy.2018.08.041.

Chhajro MA, Hu H, Kubar KA, Kalhoro SA, Narejo MN, Sarfaraz Q, Koondhar N, Magsi S (2022) Physiological and growth responses of castor (Ricinus Communis L) under cadmium stressed environment. Joarps 3(2) ,242–247. https://joarps.org /index.php/ojs/article/view /74.

Costas C, Sanchez MDLP, Sequeira-Mendes J, Gutierrez C (2011) Progress in understanding DNA replication control. Plant Science 181:203-209. https://doi.org/10.1016/j.plantsci.2011.04.020

De Castro RD, Van Lammeren AAM, Groot SPC, Bino RJ, Hilhorst HWM (2000) Cell division and subsequent radicle protrusion in tomato seeds are inhibited by osmotic stress but DNA synthesis and formation of microtubular cytoskeleton are not. Plant Physiol 122:327–335. https://doi.org/10.1104/pp.122.2.327

De Micco V, De Pascale S, Paradiso R, Aronne G (2013) Microgravity effects on different stages of higher plant life cycle and completion of the seed-to-seed cycle. Plant Biology 16:31–38. https://doi.org/10.1111/plb.12098

Deng X, Ma Y, Cheng S, Jin Z, Shi C, Liu J, Lin J, Yan X (2023) Adaptação da mamona ao estresse de salinidade durante o estágio inicial de muda por métodos fisiológicos e transcriptômicos. Agronomia 13(3):693. https://doi.org/10.3390/agronomy13030693

Devora-Isiordia G, Valdez -Torres L, Granillo-Moreno K (2018) Evaluation of the effect of the salinity of irrigation water on the yield of castor plant hybrids (*Ricinus communis* L.) in Mexico. Int. J. Hydrol. 2(5), 61 3 –61 6. https://doi.org/10.15406 / ijh.2018.02.0013 3.

Díaz AS, Cruz YC, Duarte VP, Castro EM, Magalhães PC, Pereira FJ (2021) The role of reactive oxygen species and nitricoxide in the formation of root cortical aerenchyma

under cadmium contamination. Physiol. Plantarum 173, 2323–2333. https://doi.org/10.1111/ppl.13582.

El-Maarouf-Bouteau H (2022) The Seed and the Metabolism Regulation. Biology 11(2):168. https://doi.org/10.3390/biology11020168

Else MA, Coupland D, Dutton L, Jackson MB (2001) Decreased root hydraulic conductivity reduces leaf water potential, initiates stomatal closure and slows leaf expansion in flooded plants of castor oil (*Ricinus communis*) despite diminished delivery of ABA from the roots to shoots in xylem sap.-Physiol. Plant 111: 46–54. https://doi.org/10.1034/j.1399-3054.2001.1110107.x

Ensing B, Tiwari A, Tros M, Hunger J, Domingos SR, Pérez C, Smits G, Bonn M, Bonn D, Woutersen S (2019) Sobre a origem das solubilidades extremamente diferentes dos poliéteres em água. Nat Commun 10:2893. https://doi.org/10.1038/s41467-019-10783-z

Faria JMR, Buitink J, Van Lammeren AAM, Hilhorst HWM (2005) Changes in DNA and microtubules during loss and re-establishment of desiccation tolerance in germinating *Medicago truncatula* seeds. J Exp Bot 56:2119–2130. https://doi.org/10.1093/jxb/eri210

Farooq M, Usman M, Nadeem F, urRehman H, Wahid A, Basra SM, Siddique KH (2019) Seed priming in field crops: potentia l benefits, adoption and challenges. Crop Pasture Sci. 70 (9), 731–771. https://doi.org/10.1071/CP18604.

Ferreira DF (2011) SISVAR: computer statistica l analysis system. Cienc. e Agrotecnol 35 (6), 1039–1042. https://doi.org/10.1590/S141 3 -7054201100060000 1 .

Gill SS, Anjum NA, Gill T, Yadav S, Hassanuzzaman M, Fujita M, Mishra P, Sabat SC, Tuteja N (2015) Superoxide dismutase - mentor of abiotic stress tolerance in crop plants. Env sci Pollut Res 22:10375–10394. https://doi.org/10.1007/s11356-015-4532-5

Gomes Neto V, Ribeiro PR, Del-Bem LE, Bernal DT, Cunha Lima ST, Ligterink W, Fernandez LG, De Castro R (2018) Characterization of the superoxide dismutase gene family in seeds of two *Ricinus communis* L. genotypes submitted to germination under water restriction conditions. Environ Exp Bot 155:453–463. https://doi.org/10.1016/J.ENVEXPBOT.2018.08.001

Gong H, Li Y, Li S (2021) Effects of the interaction between biochar and nutrients on soil organic carbon sequestration in soda saline-alkali grassland: a review. Glob. Ecol. Conserv. 26, e01449. https://doi.org/10.1016/j.gecco.2020.e01449.

Gong Y, Wang W, Tian D, Chen g B, Yang T (2023) Salinity distribution pattern and its induced adaptability of tomato roots. Sci. Hortic. 313, 111905. https://doi.org/ 10.1016/j.scienta.2023.111905.

Herrick J (2011) Genetic variation and DNA replication timing, or why is there late replicating dna?. Evolution. 65(11):3031–3047. https://doi.org/10.1111/j.1558-5646.2011.01407.x

Hsiao AS, Huang JY (2023) Microtubule Regulation in Plants: From Morphological Development to Stress Adaptation. Biomolecules. 13(4):627. https://doi.org/10.3390/biom13040627

Ievinsh G, Landorfa-Svalbe Z, Andersone-Ozola U, Karlsons A, Osvalde A. (2022) Salinity and heavy metal tolerance, and phytoextraction potential of ranunculus sceleratus plants from a sandy coastal beach. Life (Basel). 23(12):1959. https://doi:10.3390/life12121959.

Ismaiel MMS, Piercey-Normore MD (2023) Antioxidant enzymes of *Pseudochlorella pringsheimii* under two stressors: variation of SOD Isoforms activity. J Plant Res. https://doi.org/10.1007/s10265-023-01473-5

Joosen RVL, Kodde J, Willems LAJ, Ligterink W, Van Der Plas LHW, Hilhorst HWM (2010) Germinator: A software package for high-throughput scoring and curve fitting of Arabidopsis seed germination. Plant J 62:148–159. https://doi.org/10.1111/j.1365-313X.2009.04116.x

Kallamadi PR, Nadigatla VPRGR, Mulpuri S (2015) Molecular diversity in castor (*Ricinus communis* L.). Ind Crops Prod 66:271–281. https://doi.org/10.1016/j.indcrop.2014.12.061

Kaur R, Bhaskar T (2020) Potential of castor plant (*Ricinus communis*) for production of biofuels, chemicals, and value-added products In: Bhaskar T, Pandey A, Rene ER, Tsang DCW (Eds) Waste Biorefinery. Elsevier p. 269–310. https://doi.org/10.1016/b978-0-12-818228-4.00011-3

Kozeko LE, Troyan VM (2000) The relationship between the mitotic activity and moisture content of recalcitrant seeds of *Acer saccharinum* (L.) during maturation, post-maturation drying and germination. Seed Sci Res 10:225–232. https://doi.org/10.1017/s0960258500000258

Landoni M, Bertagnon G, Ghidoli M, Cassani E, Adani F, Pilu R (2023) Opportunities and challenges of castor bean (*Ricinus communis* L.) genetic improvement. Agronomy. 13(8):2076. https://doi.org/10.3390/agronomy13082076

Lira E, Souza J, Galdino L, Macêdo C, Silva A, Melo Y, Santos I, Arriel N, Meneses C, Maia J (2021) Changes in reserve mobilization caused by salinity could interfere in the initial growthof *Jatropha curcas*. Sustainability 13:7446. https://doi.org/10.3390/su13137446

Lopes LS, Prisco JT, Gomes-Filho E (2018) Inducing salt tolerance in castor bean through seed priming. Aust J Crop Sci 12:943–953. https://doi.org/10.21475/ajcs.18.12.06.PNE1011

Ma Y, Dias MC, Freitas H (2020) Drought and Salinity Stress Responses and Microbe-Induced Tolerance in Plants. Frontiers in Plant Science, 11 https://doi.org/10.3389/fpls.2020.591911

Marengo JÁ, Galdos MV, Challinor A, Cunha AP, Marin FR, Vianna MS, Alvala RCS, Alves LM, Moraes OL, Bender F (2022) Drought in Northeast Brazil: A review of agricultural and policy adaptation options for food security. Climate Resil Sustain1: e17. https://doi.org/10.1002/cli2.17

Meng Y, Shuai H, Luo X, Chen F, Zhou W, Yang W, Shu K. Karrikins (2017) Regulators involved in phytohormone signaling networks during seed germination and seedling development. Front Plant Sci. 7:2021. https://doi.org/10.3389/fpls.2016.02021

Michel BE (1983) Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. Plant Physiol. 72(1):66–70. https://doi.org/10.1104/pp.72.1.66.

Moraes PF, Laat DM, Santos MEAHP, Colombo CA, Kiihl T (2015) Expressão gênica diferencial em genótipos de mamona (*Ricinus communis* L.) submetidos a déficit hídrico induzido por PEG. Bragantia 74:25–32. https://doi.org/10.1590/1678-4499.0207

Munns R, Gilliham M (2015) Salinity tolerance of crops – what is the cost? New Phytol. 208:668–673. https://doi.org/10.1111/nph.13519

Munns R, James RA, Sirault XRR, Furbank RT, Jones HG (2010) New phenotyping methods for screening wheat and barley for beneficial responses to water deficit. J Exp Bot 61:3499–3507. https://doi.org/10.1093/jxb/erq199

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Nawaz K, Hussain K, Majeed A, Khan F, Afghan S, Ali K (2010) Fatality of salt stress to plants: Morphological, physiological and biochemical aspects. African J Biotechnol 9:5475–5480. https://doi.org/10.4314/ajb.v9i34

Nonogaki H (2017) Seed biology updates – highlights and new discoveries in seed dormancy and germination research. Front Plant Sci, 11. https://doi.org/10.3389/fpls.2017.00524

Oracz K, Bouteau HEM, Farrant JM, Cooper K, Belghazi M, Job C, Job D, Corbineau F, Bailly C (2007) ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. Plant J 50:452–465. https://doi.org/10.1111/j.1365-313X.2007.03063.x

Pereira WVS, Faria JMR, José AC, Tonetti OAO, Ligterink W, Hilhorst HWM (2017) Is the loss of desiccation tolerance in orthodox seeds affected by provenance? South African J Bot 112:296–302. https://doi.org/10.1016/j.sajb.2017.06.008

Rahbari A, Masoud Sinaki, J, Damavandi A, Rezvan S (2021) Castor bean (*Ricinus communis* L.) responses to drought stress and foliar application of Zn-nano fertilizer and humic acid: grain yield, oil content, antioxidant activity, and photosynthetic pigments. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 49(4):12003. https://doi.org/10.15835/nbha49412003

Rajput VD, Harish, Singh RK, Verma KK, Sharma L, Quiroz-Figueroa FR, Meena M, Gour VS, Minkina T, Sushkova S, Mandzhieva S (2021) Recent Developments in Enzymatic Antioxidant Defence Mechanism in Plants with Special Reference to Abiotic Stress. Biology (Basel)10(4):267. https://doi.org/10.3390/biology10040267.

Rukhsar, Patel MP, Parmar DJ, Kalola AD, Kumar S (2017) Morphological and molecular diversity patterns in castor germplasm accessions. Industrial Crops and Products 97:316-323. https://doi.org/10.1016/j.indcrop.2016.12.036

Senthilvel S, Shaik M, Anjani K, Shaw RK, Kumari P, Sarada C, Kiran BU (2017) Genetic variability and population structure in a collection of inbred lines derived from a core germplasm of castor. Journal Plant Biochem Biotechnol 26:27–34. https://doi.org/10.1007/s13562-016-0356-8

Severino LS, Auld DL (2013) A framework for the study of the growth and development of castor plant. Industrial Crops and Products 46:25-38. https://doi.org/10.1016/j.indcrop.2013.01.006

Silva MMA, Willadino L, Santos DYAC, Oliveira AFM, Camara TR (2015) Response of *Ricinus communis* L. to in vitro water stress induced by polyethylene glycol. Plant Growth Regul 78:195–204. https://doi.org/10.1007/s10725-015-0085-3

Silva VM, Tavanti RF, Gratão PL, Alcock TD, Reis AR (2020) Selenate and selenite affect photosynthetic pigments and ROS scavenging through distinct mechanisms in cowpea (*Vigna unguiculata* (L.) walp) plants. Ecotoxicol Environ Saf 201:110777. https://doi.org/10.1016/j.ecoenv.2020.110777

Soltani SS, Ranjbar GA, Kazemitabar SK, Parizi AP, Zarini HN (2022) Evaluation of photosynthetic pigment, antioxidant and non-antioxidant activity and some morphological traits changes under drought stress in castor plant (*Ricinus communis* L.). Journal of Crop Breedingvol 14:119-130. https://doi.org/10.52547/jcb.14.44.119

Song X, Zhou G, Shi L, Ahmad I, Shi X, Zhu G, Jiao X (2021) Comparative effects of salinity and drought on seed germination, seedling growth, photosynthetic productivity, pigments content and antioxidan t enzyme s of castor bean (*Ricinus communis*). Crop Sci. 72, 541–550. https://doi.org/10.107/CP20495.

Stephenie S, Chang YP, Gnanasekaran A, Esa NM, Gnanaraj C (2020) An insight on superoxide dismutase (SOD) from plants for mammalian health enhancement. J Funct Foods 68:1–10. https://doi.org/10.1016/j.jff.2020.103917

Su B, Lin W, Lin L, Huang C, Chuang W, Wu D, Shih C, Lee T (2020) Laying Diet Supplementation with *Ricinus communis* L. leaves and Evaluation of Productive Performance and Potential Modulation of Antioxidative Status. Journal of Poultry Science 57(4): 259-269. https://doi.org/10.2141/jpsa.0190077.

Tavares DS, Fernandes TEK, Rita YL, Rocha DC, Sant'Anna-Santos BF, Gomes M P (2021) Germinativ e metabolism an d seedling growth of cowpea (*Vigna unguiculata*) under salt and osmotic stress. South Afr. J. Bot. 139:399–408. https:// doi.org/10.1016/j.sajb.2021.03.019.

Vallejos M, Rondanini D, Wassnera DF (2011) Water relationships of castor bean (*Ricinus communis* L.) seeds related to final seed dry weight and physiological maturity.

Eur J Agron 35:93–101. https://doi.org/10.1016/j.eja.2011.04.003

Valliyodan B, Nguyen HT (2006) Understanding regulatory networks and engineering for enhanced drought tolerance in plants. Curr Opin Plant Biol. 9(2):189-95. https://doi:10.1016/j.pbi.2006.01.019.

Van Dongen JT, Schurr U, Pfister M, Geigenberger P (2003) Phloem metabolism and function have to cope with low internal oxygen. Plant Physiology. 131(4):1529–1543. https://doi.org/10.1104/pp.102.017202

Vasconcelos PCT, Loureiro MB, Lima AMMF, Ribeiro PR, Bernal DT, Moreno MLV, Fernandez LG, De Castro RD (2017) New insights into the mechanism underlying *Ricinus communis* L. tolerance to drought stress during germination. Ind Crops Prod 103:99–106. https://doi.org/10.1016/j.indcrop.2017.03.038

Wang Y, Peng X, Salvato F, Wang Y, Yan X, Zhou Z, Lin J (2019) Salt-adaptive strategies in oil seed crop *Ricinus communis* early seedlings (cotyledon vs. true leaf) revealed from proteomics analysis. Ecotoxicology and Environmental Safety, 171:12-25. https://doi.org/10.1016/j.ecoenv.2018.12.046

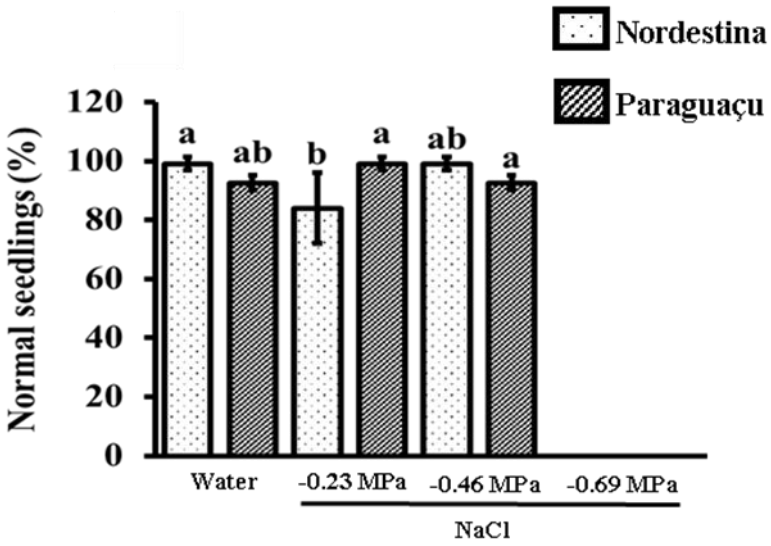
Yu W, Kong G, Chao J, Yin T, Tian H, Ya H, He L, Zhang H (2022) Genome-wide identification of the rubber tree superoxide dismutase (SOD) gene family and analysis of its expression under abiotic stress. PeerJ 10:e14251. https://doi.org/10.7717/peerj.14251

Zhao S, Zhang Q, Liu M, Zhou H, Ma C, Wang P (2021) Regulation of Plant Responses to Salt Stress. International Journal of Molecular Sciences. 22(9):4609. https://doi.org/10.3390/ijms22094609

Zheng J, Suhono GBF, Li Y, Jiang MY, Chen Y, Siddique KHM (2021) Salt-tolerance in castor bean (*Ricinus communis* L.) is associated with thicker roots and better tissue K+/Na+ distribution. Agricultura 11(9): 821. https://doi.org/10.3390/agriculture11090821

Zuchi J, Panozzo LE, Heberle E, Araujo EF (2012) Imbibition and electrical conductivity of castor bean seeds classified by size. Rev Bras Sementes 34:504–509. https://doi.org/10.1590/S0101-31222012000300019

6. SUPPLEMENTARY



Supplementary Fig. 1 Normal seedlings (NoS) of *R. communis* cv. Nordestina and cv. Paraguaçu seeds in different NaCl osmotic potentials

CAPÍTULO 2. Ascorbate peroxidase gene family in *Ricinus communis* L.**: new insights in redox homeostasis maintenance during germination and post-germination stages under abiotic stresses**

Chapter to be submitted for publication in 2024.

Ascorbate peroxidase gene family in *Ricinus communis* L.: new insights in redox homeostasis maintenance during germination and post-germination stages under abiotic stresses

ABSTRACT

Ricinus communis L. is an oilseed of socioeconomic importance for small holder farming in the Brazilian semiarid region. The major problem of *R. communis* growth in Brazil is the abiotic stress condition that affects seed production. The abiotic stress enhances the Reactive Oxygen Species (ROS) level causing damage to cells, causing poor germination and decrease in seedling vigor. Ascorbate peroxidase (APX) is a key antioxidant enzyme that plays a key role in scavenging H_2O_2. However, the characteristics and functions of APX in *R. communis* remain unclear at the moment. In this study, the roles of the APX enzyme in *Ricinus communis* were investigated using *in silico, molecular and biochemistry* analysis. We identified 6 *RcAPX* genes predicted to peroxisome, chloroplast, and cytosol that presented orthologs in *Arabidopsis thaliana*, *Populus trichocarpa*, *Oryza sativa,* and *Sorghum bicolor* by a phylogenetic tree. The *RcAPX* genes showed distribution in different chromosomes location and related abiotic stresses responsive elements (ABRE, ARE, MeJARE, GARE, AUXIN, MYB, and MYC). Besides that, the protein analysis of the *RcAPX* shows conserved heme and cation binding domains (HBD and CBD). The total APX enzymatic activity shows a different pattern in the two cultivars analyzed, showing the enhancement of activity from 48h (post-germination) of imbibition in control and saline stresses, but not in water restriction. The *RcAPX* genes presented different patterns of expression, showing induced during germination (24 h of imbibition) and post-germination 48h and 72 h (post-germination) of imbibition stages under control, saline stress and water restriction compared to dry seeds. The *RcAPX3* and *RcAPX5* genes show saline stress response by cultivar dependent while the *RcAPX1* and *RcAPX2* genes show water restriction response by cultivar dependent and according time of the development. The *RcAPX1, RcAPX2,* and *RcAPX4* genes presented heat response in specific time of the development. Our study provides valuable information to understand the potential role of the APX enzyme in *R. communis* in redox homeostasis maintenance during germination and post-germination stages under abiotic stresses.

Keywords: Ascorbate peroxidase, antioxidant enzymes, Castor bean.

1. INTRODUCTION

Ricinus communis L. (Euphorbiaceae) is a very important inedible oilseed species for the Brazilian semiarid region, is planted mainly by family farmers, and is a source of income (Mai, Xue, Azeem 2023; Rahbari et al. 2021). The oil extracted from its seeds has a unique composition (ricinoleic acid), is attractive for chemical and cosmetic industries (Bidin et al. 2020; Alsubeie, 2023), and is considered an ideal raw material for the production of biodiesel (Rivas et al. 2023). However, in semi-arid regions, in addition to water restriction due to drought and soil salinity, which are the main abiotic stresses (Marengo et al. 2022; Alsubeie 2023; Sofo et al. 2015), high temperatures also occur (Ribeiro et al. 2015). These abiotic stresses can occur individually or together, causing oxidative stress because of increased formation of reactive oxygen species (ROS) (Akbudak et al. 2018; Wang et al. 2019a; Munns et al. 2020) and because of climate change, that caused the aggravation of these abiotic stresses, it has become a major threat to sustainable agricultural production (Munns et al. 2019; Hasanuzzaman et al. 2020).

The main sites of cellular ROS generation are chloroplasts, mitochondria, peroxisomes, apoplasts, and plasma membranes (Raja et al. 2017). Although ROS in small concentrations can act as signaling molecules to activate stress responses, high concentrations of ROS can damage various cellular components, leading to cell death (Lanza et al. 2021; Silva et al. 2020; Stephenie et al. 2020). Plants have developed enzymatic and non-enzymatic mechanisms to eliminate ROS, such as non-enzymatic and enzymatic antioxidants, to protect cells from uncontrolled oxidative damage Gomes Neto et al. 2018; Soltani et al. 2022). The enzymatic mechanisms are formed by several enzymes, such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), glutathione peroxidase (GPX, EC 1.11.1.9), mono dehydro ascorbate reductase (MDHAR, EC 1.6.5.4), dehydro ascorbate reductase (DHAR, EC 1.6.5.4), glutathione reductase (GR, EC 1.8.1.7) (Hasanuzzaman et al. 2020; Kaur and Bhaskar 2020; Ismaiel and Piercey-Normore 2023; Yu et al. 2022).

APX enzyme forms a family of type I heme-containing peroxidases that catalyze H_2O_2 in water using ascorbate as a specific electron donor, functioning in the maintenance

of cellular reduction/oxidation (redox) homeostasis by scavenging ROS (Caverzan et al. 2012; Jardim-Messeder et al. 2022). APX enzymes can be encoded by small multigene families in higher plants and are classified into different groups according to their subcellular localization (Lazzarotto et al. 2011; Li et al. 2020). The main detoxification system for hydrogen peroxide in plant chloroplasts is the ascorbate-glutathione cycle, in which APX is a key enzyme (Caverzan et al. 2012; Jardim-Messeder et al. 2022). The importance of APX and the ascorbate-glutathione cycle is not restricted to chloroplasts; it also plays a role in eliminating ROS in the cytosol, mitochondria, and peroxisomes (Noctor and Foyer 1998; Caverzan et al. 2012).

The important role of the APX gene family in antioxidant stress has now been demonstrated in a variety of plants (Ma et al. 2022). The APX genes have already been characterized in several plant species, such as *Arabidopsis thaliana* (8 genes) (Narendra et al., 2006; Santos et al., 1996), *Gossypium hirsutum* (26 genes) (Tao et al. 2018), *Oryza sativa* (8 genes) (Teixeira et al., 2006; Wu and Wang, 2019), *Populus trichocarpa* (11 genes) (Leng et al. 2021), *Solanum lycopersicum* (7 genes) (Najami et al. 2008) and *Triticum aestivum* (21 genes) (Tyagi et al. 2020). Considering the high demand for *R. communis* oil by industries, a great effort is needed for the development of cultivars tolerant to abiotic stresses and widely adapted by traditional techniques of genetic improvement and genetic engineering (Wang et al. 2021). It is necessary to reveal the molecular mechanism underlying the beaver's resistance to inferior growth conditions.

In this study, we characterize the family APX enzyme in *R. communis* through in silico analysis, the activity enzyme, and the gene expression of the APX, during the germination and early seedling stage of two cultivars (BRS Nordestina and BRS Paraguaçu) subjected to abiotic stresses.

2. MATERIALS AND METHODS

2.1 Identification of *RcAPX* Genes and Physicochemical Properties

To identify the APX sequences in *R. communis*, the amino acid sequences of Ascorbate peroxidase (APX) from *Arabidopsis thaliana* (HU et al., 2016) were used as a query against the *R. communis* genome from Phytozome database (https://phytozome-next.jgi.doe.gov/) using as a cut-off point an e-value of 1e-10. Sequences obtained were

submitted to the Pfam database to confirm the APX domains (PF00141). The molecular weight, isoelectric point (pI), length, and Grand Average of Hydropathicity (GRAVY) value of proteins were determined using the ProtParam (https://web.expasy.org/protparam/). The subcellular localization was analyzed using the Plant-mSubP http://bioinfo.usu.edu/Plant-mSubP/). The presence of transmembrane helices was analyzed using the TMHMM v2.0 server (https://services.healthtech.dtu.dk/service.php? TMHMM-2.0).

2.2 Gene Structure and Proteins Motif Analysis of *RcAPX*

The exon–intron structure was analyzed using the Gene Structure Display Server - GSDS 2. (http://gsds.gao-lab.org/). The conserved motifs were identified using the Multiple EM for Motif Elicitation (MEME) Suite 5.1.1 (https://meme-suite.org/meme/tools/meme). The primary structure, conserved amino-acid residues, catalytic site, active site, metal-binding sites, and signature motifs were analyzed using the Multalin (http://multalin.toulouse.inra.fr/multalin/multalin.html) to examine the primary structure, conserved amino-acid residues, catalytic site, active site, metal-binding sites, and signature motifs (Corpet, 1988; Tyagi, et al., 2021). The secondary structure of the *RcAPX* proteins was analyzed using the NPSA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html). The tertiary structure of *RcAPX* proteins was predicted using the SWISS-MODEL database (https://swissmodel.expasy.org).

2.2 Phylogenetic Tree and Chromosomal Positions

To investigate the phylogenetic relationships of APX genes in *R. communis* compared with other angiosperms, we constructed a phylogenetic tree using, *R. communis* (This work), *Arabidopsis thaliana* (Hu et al. 2016), *Oryza sativa* (WU and WANG, 2019), *Populus trichocarpa* (Leng et al. 2021), and *Sorghum bicolor* (Akbudak et al. 2018). The APX sequences were aligned on the online multiple-sequence comparison by log expectation (MUSCLE) platform and phylogenetic analysis was performed using Molecular Evolutionary Genetics Analysis (MEGA 7.0) software (https://www.megasoftware.net/) (Tamura et al. 2007) using the Neighbor-joining (NJ) method with the following parameters: p-distance, pair exclusion and bootstrap (1,000

replicates). The chromosomal location of *RcAPX* genes was accessed by (Yu et al. (2019). *RcAPX* genes that were not present in any chromosomal were represented in the scaffold position.

2.1. Promoter cis-element Analysis

The Phytozome database (https://phytozome.jgi.doe.gov/pz/portal.html) was used to obtain 1000 bp upstream from the start of transcription (ATG) of the *RcAPX* genes. The promoter region analysis was performed on the Plant Care database website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) (Leng et al. 2021) TBTools (https://bio.tools/tbtools) was used to visualize the results.

2.2. Plant Samples and Growth Conditions

Assays were carried out with the cultivars BRS Nordestina and BRS Paraguaçu in trays containing germination paper, moistened with solutions: water (Control), NaCl, and PEG-8000 at potentials -0.23 and -0.46 MPa, and conditioned in BOD (Eletrolab – EL402/150) at 30ºC, the collections were performed at times 0h (Dry seeds), 24h (germination), 48h and 72h (post-germination) of imbibition. The experimental design was completely randomized using 3 replications of 20 seeds, the embryos were collected and stored in a freezer at -80°C.

2.3. Determination of Ascorbate Peroxidase Enzyme Activity (APX -EC 1.11.1.11)

To estimate the activity of the APX enzyme, 20 mg were ground in liquid nitrogen and homogenized in 0.1 mM potassium phosphate buffer (pH 7.8). Samples were centrifuged at 14,000 g for 10 min at 4°C. The supernatant was collected and stored at -80°C. Protein was quantified according to the method Bradford, (1976) and bovine serum albumin (BSA) was used as a standard. The absorbances were read in a spectrophotometer (Varian, model Cary 100, USA) at 950 nm and compared with the standard curve of bovine serum albumin (0.3 to 2.5 mg. mL^{-1}). The reaction medium consisted of 125 mM (pH 7.0) potassium phosphate buffer; 12.5 mM ascorbic acid; 0.1 mM ethylenediamine tetraacetic acid (EDTA), 75 µL Milli-Q water, 12.5 µL protein extract and 25 µL H_2O_2,

for a total reaction volume of 250 µL. The reaction was carried out at 25ºC with an absorbance reading at 290 nm in a spectrophotometer (Varian, model Cary 100, USA), for 7 minutes with reading intervals of 10 seconds. The reaction was started with the addition of 12.5 µL of enzymatic extract (sample), with the reaction medium free of H_2O_2 and the sample blank. The ascorbate oxidation rate was determined by the decrease in absorbance at 290 nm (2.8 mM.cm-1). APX activity was expressed as mmol ASA consumed min-1 µg-1 proteins, ie APX (mmol ASA min-¹ µg-¹ Prot) (García-Limones et al. 2002).

2.4. Gene Expression of *RcAPX* by qRT-PCR

The primers for *RcAPX* genes were designed according to the following parameters: melting temperatures of 58–62°C, lengths of 19–23 bp, and amplicon lengths of 67–224 bp by Primer3 software (v.0.4.0) (https://bioinfo.ut.ee/primer3-0.4.0/) (Supplementary Table 1). Amplification efficiency was evaluated based on a serial two-fold dilution series of a pooled cDNA. The specificity of the primer was verified by separating the products on a 2% agarose gel and analyzing the melting curve.

To analyze the gene expression of *RcAPX* genes total RNA was extracted using 5 mg of lyophilized and ground samples using the hot borate method with modifications (WAN and WILKINS, 1994). First strand cDNA was synthesized with 1 µg of total RNA using the GoScript™ Reverse Transcriptase Promega™ cDNA synthesis kit according to the manufacturer's instructions. qRT-PCR was performed in a total volume of 20 µL containing 5 µL of cDNA (20x diluted), 1 µL of primers (10 µM), 10 µL of IQ SYBR Green Supermix (Applied), and 4 µL of ultrapure water (Tab. 2). The serine/threonine phosphatase 2A (PP2AA1) and Actin (ACT) were used as reference genes (Ribeiro et al. 2014; Gomes Neto et al. 2018, 2020).

2.5. The Transcriptional Profiling of *RcAPX* Genes Under Heat Stress

The transcriptional profiling of the *RcAPX* genes was retrieved from microarray analysis (Ribeiro et al. 2018). The *R. communis* samples were collected in different development stages during germination and seedling stages (Dry seed, early imbibition - EI, radicle protrusion – RP (germination per se), and young seedlings with 2 cm root -

R2) at three different temperatures (20, 25 and 35°C). The analysis was performed on three biological replicates of 20 seeds each.

3. RESULTS AND DISCUSSION

3.1. Identification, and characterization of APX genes in *R. communis* (*RcAPX*)

In this study, we identified 6 predicted *RcAPX* genes. The prediction of the subcellular localization indicates two cytosolic isoforms, two peroxisomal, and two chloroplast isoforms. (Table 1). Similar predictions for cytosolic, peroxisomal, and chloroplast localization of APX proteins have been reported for plants; *A. thaliana*, *B. juncea*, *B. rapa*, *G. hirsutum*, *P. trichocarpa* and *S. lycopersicum* (Gupta et al. 2019; Leng et al., 2021; Najami et al. 2008; Tao et al. 2018; Verma et al., 2021).

The length of *RcAPX* ranged from 223 to 379 amino acid residues (aa) (Table 1, File S2), a similar trend in the average length of amino acids in APX proteins from *A. thaliana* (250-426), *C. sativus* (249-446), *G. max* (223-451), and *V. vinifera* (250-372), *B. juncea* (250-863), *B. rapa* (250-440) have been reported (Ozyigit et al. 2016; Panchuk et al. 2005; Verma et al., 2021). The average molecular weight (MW) of the cytoplasm group proteins *RcAPX1* and *RcAPX2* (24.55, 28.78), peroxisomes *RcAPX3* and *RcAPX4* (32.06, 25.88), plastid 1 *RcAPX5* (40.84) and plastid 2 and *RcAPX6* (36.03 kDa) (Tabel. 1). The isoelectric point (pI) ranged from 5.56 to 8.22, suggesting that the enzymes reach a balance between their negative and positive charges in alkaline pHs, with the exception of *RcAPX*, present in the cytosol, which presented a pI of 5.56, suggesting prefer acid pH. Furthermore, *RcAPX* sequences showed low overall mean hydropathicity index (GRAVY) values from -0.202 to -0.431, suggesting better interactions with water due to its hydrophilic nature (Chang and Yang, 2013) (Tabel. 1).

Table 1. Characterization of APX genes found in *R. communis* (*RcAPX*).

Locus ID	Name	P. Length (a.a)	Iso. Point (pI)	Mol. Weight (kDa)	(GRAVY)	Índ. instability	Subcellular Localization
29781.m000013	*RcAPX1*	223	6.71	24,55	-0.431	33.50	Cytosol
29805.m001492	*RcAPX2*	259	5.56	28,78	-0.202	92.66	Cytosol
29602.m000217	*RcAPX3*	288	7.13	32,06	-0.422	41.63	Peroxisome
29656.m000477	*RcAPX4*	235	8.22	25,88	-0.338	34.61	Peroxisome
29648.m002024	*RcAPX5*	379	7.69	40,84	-0.380	49.33	Plastid
29848.m004624	*RcAPX6*	328	8.74	36,03	-0.207	36.81	Plastid

3.2. Phylogenetic analysis and evaluation of chromosomal location

The gene groups in the phylogenetic tree were classified according to *A. thaliana* (*AtAPX*), being divided into four groups with intracellular location (PANCHUK et al. 2005). Group I, consist of cytoplasmic APX, group II includes peroxisome APX, and groups III and IV contain all APX present in plastids.

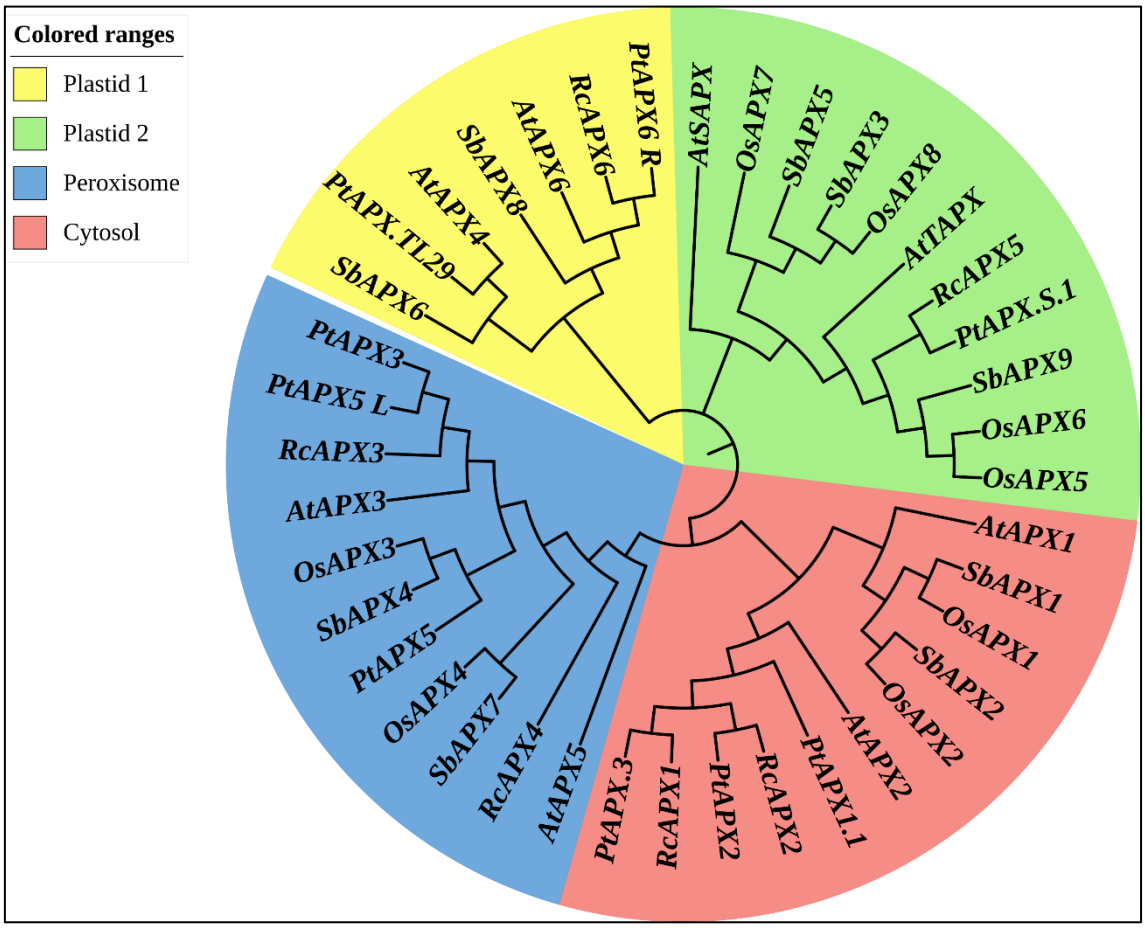


Figure 1. Phylogenetic relationship of the *RcAPX* family in selected angiosperms. The groups were classified according to the 4 clades formed concerning their subcellular localization. Group Plastids 1 (yellow), group Plastids 2 (green), group peroxisomes (blue), and group Cytosol (red). The phylogenetic tree was built in the MEGA 7.0.26 software (https://www.megasoftware.net/) using the Neighbor-joining (NJ) method with 1000 bootstraps.

The number of APX genes in each group varied slightly between different species. Group I range between two to four genes, group II presented conserved 2 genes, group

III ranged between 1 to 4 genes, and group IV ranged between one to two genes. The *RcAPX* genes were found in each group (Fig 1).

The chromosomal location of the *RcAPX* genes was physically mapped onto the 10 chromosomes of *R. communis* (Yu et al., 2019). It was possible to locate 3 *RcAPX* genes (*RcAPX4*, *RcAPX2*, and *RcAPX3*) that were distributed in three different chromosomes, 6, 7, and 10 respectively (Fig. 2-A). The genes *RcAPX1*, *RcAPX5*, and *RcAPX6* could not be identified in the chromosomes, but it was possible to predict them in the scaffolds being located respectively in the scaffolds Sca-26781; Sca-29648 and Sca-29848 (Fig. 2-B).

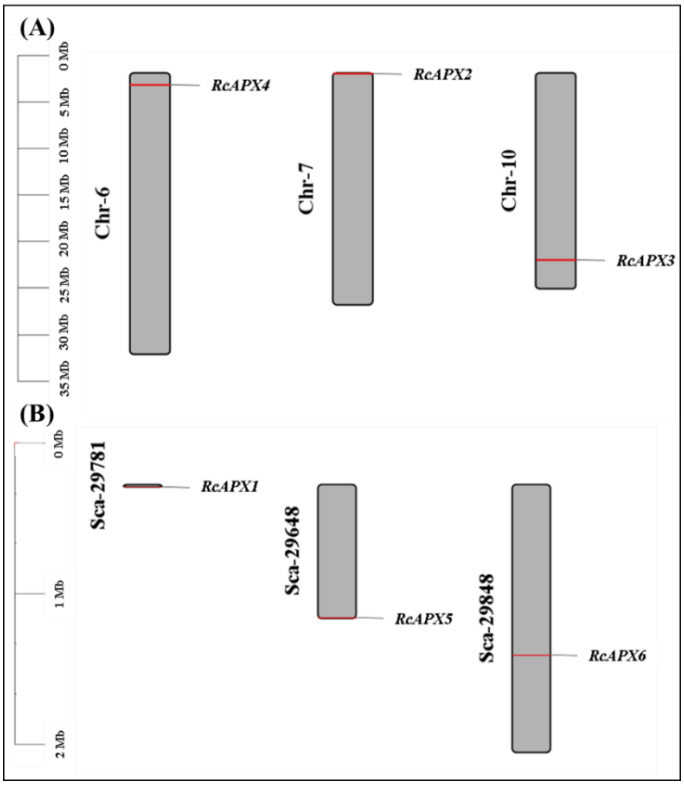


Fig 2. Chromosomal location of *RcAPX* genes. (A) The genes *RcAPX2*, *RcAPX3* and *RcAPX4* were distributed on three chromosomes (Chr-7, Chr-6 and Chr-10). Scale bar = 5Mb. (B) The genes *RcAPX1*, *RcAPX5* and *RcAPX6* were distributed on three Scarffold (Sca-29781, Sca-29648 and Sca-29848). Scale bar = 1Mb.

3.3. Gene structure and conserved protein motifs pattern of the *RcAPX*

The *RcAPX* genes presented distinct numbers of exons and introns. The number of introns varied between 6 to 10. Six introns were found in the *RcAPX1* and *RcAPX4* genes, eight were found in the *RcAPX2* and *RcAPX3* genes, ten were found in the *RcAPX5* gene and nine were found in the *RcAPX6* gene (Fig. 3-A). The difference in chromosomal

distribution, gene structure, and subcellular localization suggest a difference in the evolution of the genes.

A similar variation in exon/intron was also observed in *P. trichocarpa* (LENG et al., 2021). The *RcAPX1* and *RcAPX2* (cytoplasmic group) presented six to eight introns, and the *RcAPX3* and *RcAPX4* (peroxisome group) presented six to eight, however, a long intron region was observed in the *RcAPX3* gene, which may be related to mutations in the improvement of the recombination frequency (Alam and Grosh, 2017). The *RcAPX5* (plastid 1) presented 10 introns, while the *RcAPX6* (plastid group 2) presented 9 introns. The peroxisome genes presented a major number of introns compared to the cytosol and plastid genes. The total length of the *RcAPX1*, *RcAPX2,* and *RcAPX4* genes is less than 2 kb, while *RcAPX3*, *RcAPX5*, and *RcAPX6* are up than 5 kb (Figure 3-A).

The exon/intron gain/loss results in structural variability in various genes (Filiz and Tombuloğlu, 2015). Our results suggest that several evolutionary gene events accumulated in these genes and led to an increase or decrease in the number of introns, and may have resulted in functional differences, this phenomenon is observed even between homologous genes (Leng et al., 2021; Filiz and Tombuloğlu, 2015).

The MEME database was used to analyze conserved motifs in the *RcAPXs* genes, and a total of ten conserved motifs were found, with lengths ranging from 6 to 50 aa (Fig. 2-C, Tab. Sup. 2). This analysis identified 10 distinct conserved motifs. Motifs 1, 2, and 6 were found in all genes *RcAPXs*. Motif 3 was found in *RcAPX2*, *RcAPX3*, *RcAPX4*, *RcAPX5* and *RcAPX6*. Motif 4 was found *RcAPX1*, *RcAPX3*, *RcAPX5* and *RcAPX6*. Motif 5 was found in *RcAPX1*, *RcAPX3*, *RcAPX4* and *RcAPX5*. Motif 7 was found only in the genes of the cytosol (*RcAPX1* and *RcAPX2*) and plastid (*RcAPX3* and *RcAPX4*) groups. Motif 8 was found in *RcAPX1*, *RcAPX2*, *RcAPX3* and *RcAPX5*. Motif 9 was found in the *RcAPX3* gene of the cytosol group and the *RcAPX4* gene of the peroxisome group. Motif 10 is exclusive of the cytosol group (*RcAPX1* and *RcAPX2*), with a small amino acid sequence MGKNYP in the N-terminal of the sequence, an N-terminal extension was found, which corresponds to the chloroplast/mitochondrial transit peptide (Figure 3; Table sup. 4).

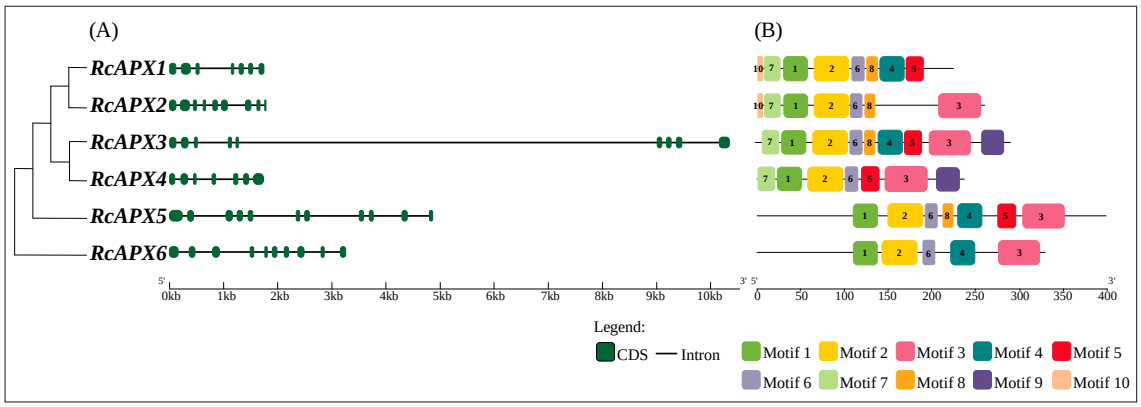


Fig 3. Gene structure, and conserved motif analysis of the *RcAPX* genes. (A) Intron-exon length is represented for each *RcAPX* genes. (B) Motif analysis showing the organization of ten conserved motifs represented by boxes of different colors.

Analysis of the aa residues of motifs 1, 4, and 5 showed that they represent the active site domain (ASD), heme-binding domain (HBD), and cation-binding domain (CBD), respectively (Fig. 4). The *RcAPX* proteins showed a conserved pattern of several aa residues involved in the HBD of ligands such as Lys, Cys, Arg, Trp, His, etc. However, in the case of ASD and CBD, most of these residues, such as Cys-32, Arg-172, Trp-179, etc. were absent or replaced by the other aa residues. Variations in protein groups were also observed in *T. aestivum* L. (Tyagi et al., 2020).

BLAST sequence search of other motifs revealed the presence of the heme-dependent peroxidase domain (Tyagi et al., 2020). In addition, two signature reasons were preserved; chloroplastic domain 1 (K-N-I-[EKTP]-E-W-P) near ASD and chloroplastic domain 2 (E-T-K-Y-T-[KE]-[TDNE]-G-P-G-[AE]-[PA]-G-G-Q-S) near CBD were also detected in *RcAPX5* proteins (Fig. 4), similar results found by (Tyagi et al., 2020)

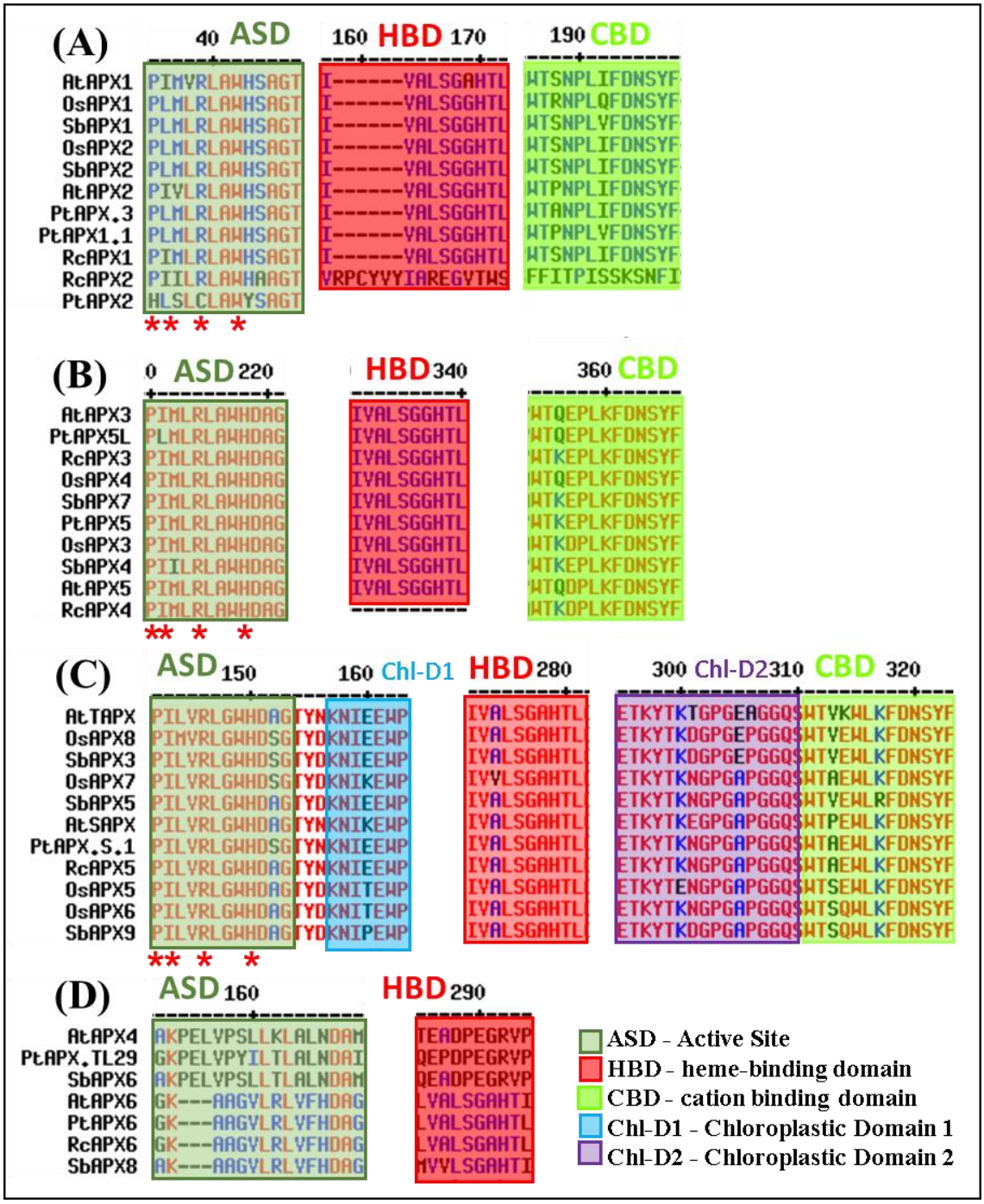


Fig 4. Protein sequence and structure analysis of *RcAPXs*. **A)** Multiple sequence alignments of cytosolic *RcAPX1* and *RcAPX2* protein sequences with *AtAPX1* and *AtAPX2*. **B)** peroxisomal *RcAPX3* and *RcAPX4* proteins with *AtAPX3* and *AtAPX5*. **C)** chloroplastic *RcAPX5* proteins with *AtTAPX* and *AtSAPXS*. **D)** *RcAPX6* protein with *AtAPX4* and *AtAPX6*. Green, red, and yellow shaded boxes represent the active site domain (ASD), heme-binding domain (HBD), and cation-binding domain (CBD) present in all the groups but substituted with new sets of residues in *RcAPX* group proteins. Two additional regions chloroplastic domains 1 and 2 are highlighted with blue and purple

shaded boxes and present only in chloroplastic APX proteins. Red asterisks and green marks represent the amino-acid residues involved in heme-binding and ascorbate binding (Lys, Cys, and Arg), respectively. The residues important for cytoplasmic and peroxisomal localization are underlined with thick green and black lines, respectively.

Protein is a basic unit of life activities, and its structure determines its function, the study of protein structure is useful to get a complete understanding of its function (Leng et al., 2021). The secondary structure of the *RcAPX* proteíns was classified into four structure patterns: α-helix, β-turn, extended strand, and random coil. In the *RcAPX* proteins, the random coil contained the highest number of amino acids, followed by the a-helix (Supplementary Tab. 6). Similar results were found by Leng et al., (2021). For the tertiary structure, a variety of structures were envisioned, and suggestions as to the functions of *RcAPX* proteins play a number of roles. The PDB templates and files for each *RcAPX* protein are provided in Supplementary Fig 1.

3.4. Regulatory elements on the promoter region of genes *RcAPX*

The cis-promoter elements play an important role in transcriptional regulation in response to abiotic stress and phytohormone treatment, they function as transcription factor binding sites (Gomes Neto et al., 2021; Yang et al., 2019; Leng et al., 2021). In our results, different elements associated with different responses to stress, such as regulatory hormones including abscisic acid, gibberellins, and auxin, defense, stress, endosperm, anaerobic induction, light, MYB, MYC, and metabolism regulation were found in *RcAPX* proteins (Fig 5). Regulatory elements of expression in the endosperm were identified in 2 genes (*RcAPX3* and *RcAPX6*), only one gene related to metabolism regulation (*RcAPX2*), one related to stress defense (*RcAPX3*), regulation by anaerobic induction were found 3 genes (*RcAPX2*, *RcAPX4,* and *RcAPX6*). The regulation by the MYC transcription factor was found in 3 genes (*RcAPX1*, *RcAPX2,* and *RcAPX5*), while the regulation for the MYB transcription factor response was found in 5 genes (*RcAPX1*, *RcAPX2*, *RcAPX3*, *RcAPX4,* and *RcAPX5*), MYB proteins play a crucial role in plant growth and development and stress responses, furthermore, MYB transcription factors were closely associated with a variety of abiotic stress-related proteins with MAC-complex and SKIP (XIE et al., 2022).

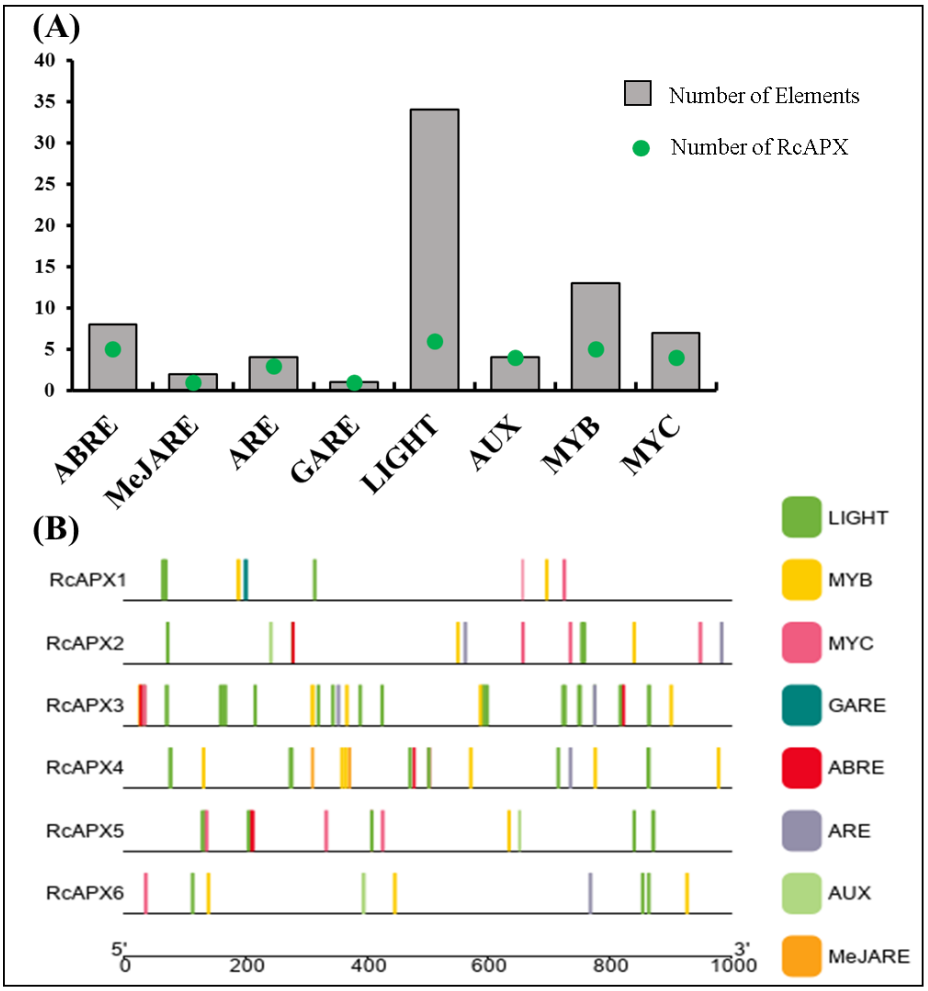


Fig 5. Analysis of the promoter region of the *RcAPX* genes. Cis elements were identified using the PlantCare database using the 1 kb promoter region upstream of the start codon of the *RcAPX* genes.

The light-responsive elements were found in all *RcAPX* genes, *RcAPX3* being the highest amount. The site of regulation by gibberellin was found only in one gene (*RcAPX1*), for auxin regulation it was found in 4 genes (*RcAPX2*, *RcAPX3*, *RcAPX5*, and *RcAPX6*). The site of regulation by abscisic acid (ABA) was found in 4 genes (*RcAPX2*, *RcAPX3*, *RcAPX4*, and *RcAPX5*). ABA is a key regulator of plant adaptation, response, and tolerance to abiotic stresses, regulating the expression of many genes under water, saline and pathogen attack (Cheng et al., 2017, 2018; Kaur et al., 2017). Komarnytsky and Borisjuk, (2003) report the importance of elements such as abscisic acid in response to oxidative damage. Normally, abscisic acid is responsible for the plant's defense against stress, its levels increase in the presence of stresses such as drought, salinity, cold, and

heat (Lata and Prasad, 2011; Zhang et al., 2019). Each *RcAPX* gene contained at least one cis-element related to abiotic stress, which indicated that *RcAPX* genes play important roles in responses to abiotic stresses. For example, *RcAPX3* contained 4 and two cis-elements related to abiotic stress and phytohormone in the promoter, respectively. Many cis-related MYB and MIC elements were found in the promoter sequences of the genes, they are involved in various abiotic stress responses (Gomes Neto et al., 2021). Also, light-related elements were detected in all genes, being in greater quantity in the *RcAPX3* gene with 15 elements. Thus, we speculate that *RcAPXs* may be related to the light stress response.

3.5. Ascorbate peroxidase (APX) enzyme activity enhances cultivar dependent from 48h of imbibition in control and saline stress but not in water stress

The profile of APX enzyme activity was performed in different conditions: distilled water (control), water restriction by PEG, and saline by NaCl at potentials (-0.23 and -0.46 MPa), during seed imbibition in the following times: 0 (dry seeds), 24, 48 and 72h). In general, the enzymatic activity of the APX enzyme increased after 48h of imbibition in the control and NaCl, while the imbibition in PEG did not increase in the activity of the APX enzyme between the cultivars. The Nordestina cultivar showed a great enhancement of APX activity compared with dry seed in water and saline stress while the Paraguaçu cultivar showed no statistical difference.

The profile of APX activity was different between cultivars with stress and cv. BRS Nordestina was able to maintain higher levels of APX enzyme activity in saline imbibition -0.23 and -0.46 MPa, at imbibition times of 48 and 72h compared to cv. BRS Paraguaçu (**Fig. 6 A-B**). Among enzymes, APX may play a specific role in H_2O_2 due to its high affinity for hydrogen peroxide (QIN; HU; ZHU 2008). APX encompasses different isoenzymes, which are encoded by a multigene family and found in many cellular compartments. Plants have an elaborate defense system against environmental adversities that can increase ROS levels (WU and WANG 2019). However, it is suggested that APX is activated soon after the end of the germination process in *R. communis*, since it is possible to observe the increase in APX activity in the control and aqueous NaCl solutions soon after germination (root protrusion) at 48 hours of imbibition.

Lin and Pu (2010) studied changes in enzymes involved in ROS elimination in salinity-tolerant and sensitive sweet potatoes, commenting that after exposure to salinity (450 mM NaCl), APX activity increased in plants at 24 and 48h, and this response was greater in a salt-stress-tolerant genotype than in salt-sensitive ones.

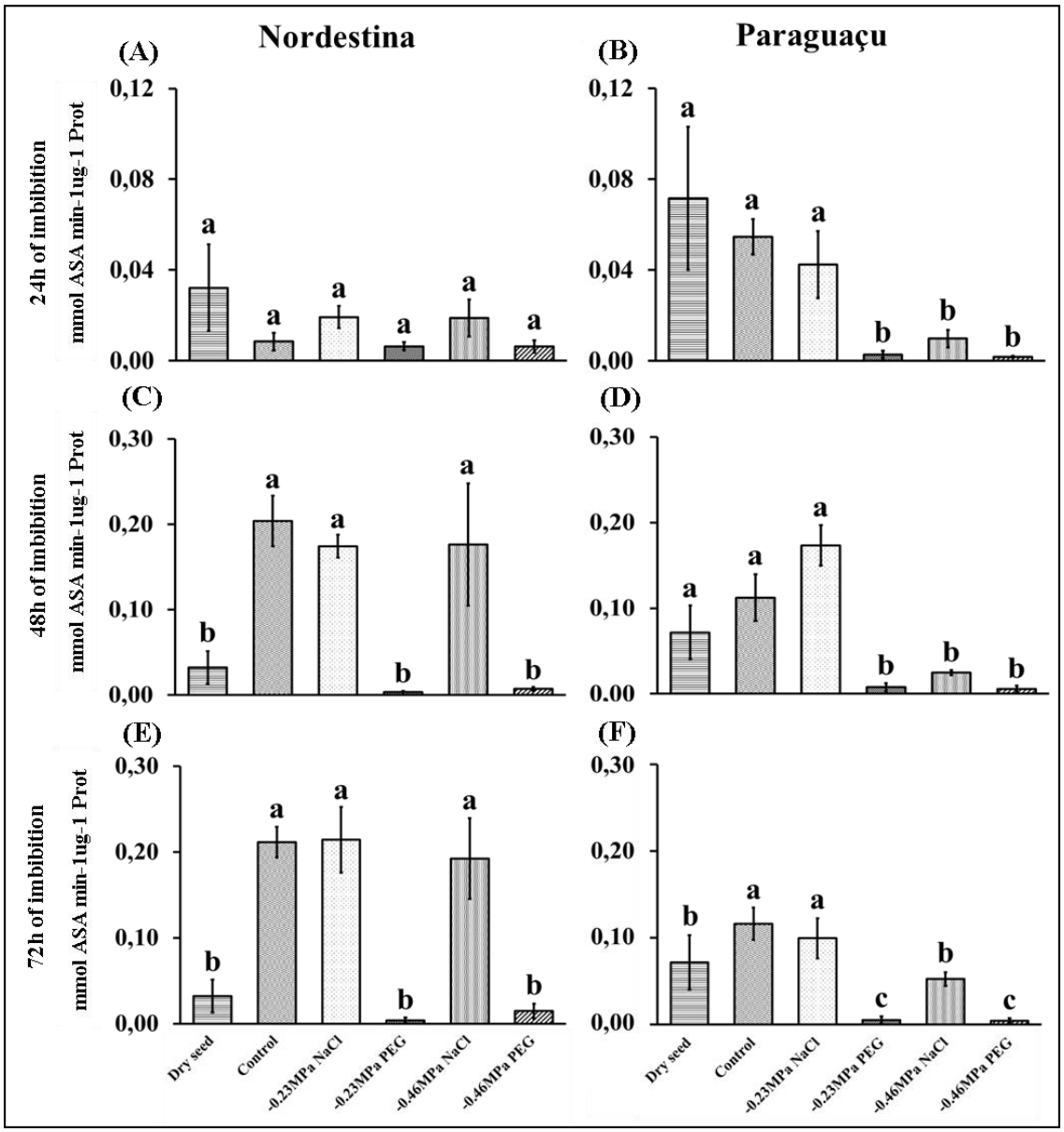


Fig 6. Ascorbate peroxidase (APX) activity in the cotyledon axis of two cultivars of R. communis, Nordestina, and Paraguaçu. In dry seed, soaked in water (Control) and NaCl and PEG solution (-0.23 and -0.46 MPa), at 24, 48, and 72h. A, C, and E APX activity in cv. Northeastern. B, D, and F) APX activity in cv. Paraguay. Different lowercase letters above the bars indicate significant differences between treatments, Scott-Knott test ($P < 0.05$).

Regarding the reduction in the activity of the APX enzyme in the times of 48 and 72h when in water restriction by PEG (-0.23 and -0.46 MPa), it may be related to the water content absorbed by the seeds, it is delaying the metabolic activity and the germination processes, in addition, other enzymes such as SOD, CAT or other peroxidases are more active in this initial phase of germination.

3.6. Modulation of *RcAPX* genes under saline stress and water restriction

The gene expression of *RcAPX* genes was observed in dry seeds, 24h of imbibition (Germination time), 48h and 72h of imbibition (pos-germinated seeds) in control (water), saline stress (NaCl solution) and water restriction (PEG solution).

The *RcAPX1* gene was highly expressed in all treatments compared to dry seeds in both cultivars. In 48h of the imbibition presented the high expressed in water restriction and saline stress treatments compared to control in Nordestina cultivar. In general, the pattern of *RcAPX1* expression in control and saline stress were similar (Fig 5 A-F). The *RcAPX2* gene presented repression in all treatments compared to dry seeds for both cultivars (Fig 5 G-L). In 72h of the imbibition was observed the induction of the *RcAPX2* gene in water restriction treatment compared to the control in both cultivars (Fig 5 I-L). The *RcAPX3* gene presented high expressed in all treatments compared to dry seed in both cultivars. The induction by saline stress was observed in 48h and 72h of imbibition by cultivar dependent (Nordestina) while was observed repression by water stress treatment in 48h of imbibition compared to control in both cultivars (Fig 5 M-R). The *RcAPX5* gene presented high expressed in all treatment compared to dry seeds in both cultivars, except in 72h of imbibition that water restriction presented the same level of expression than dry seeds. The saline stress induction was observed in 24 and 72h of imbibition compared to control in Nordestina cultivar (S-X).

The *RcAPX* genes presented different pattern of expression. The specific *RcAPX* demonstrated to be responsive according to time of development, stress treatment and cultivar dependent. The cytosolic genes (*RcAPX1* and *RcAPX2*) responded to water restriction while the cytosolic, peroxisome and plastid genes (*RcAPX1*, *RcAPX3* and *RcAPX5*) responded only to saline stress.

Our results suggest the importance of *RcAPX* genes in response to water restriction and saline stress during germination and imbibition in early stage of seedling development. However, the cytosolic genes demonstrated to be water restriction reponse while the peroxisome and plastid genes demonstrated to be saline stress response by cultivar dependent.

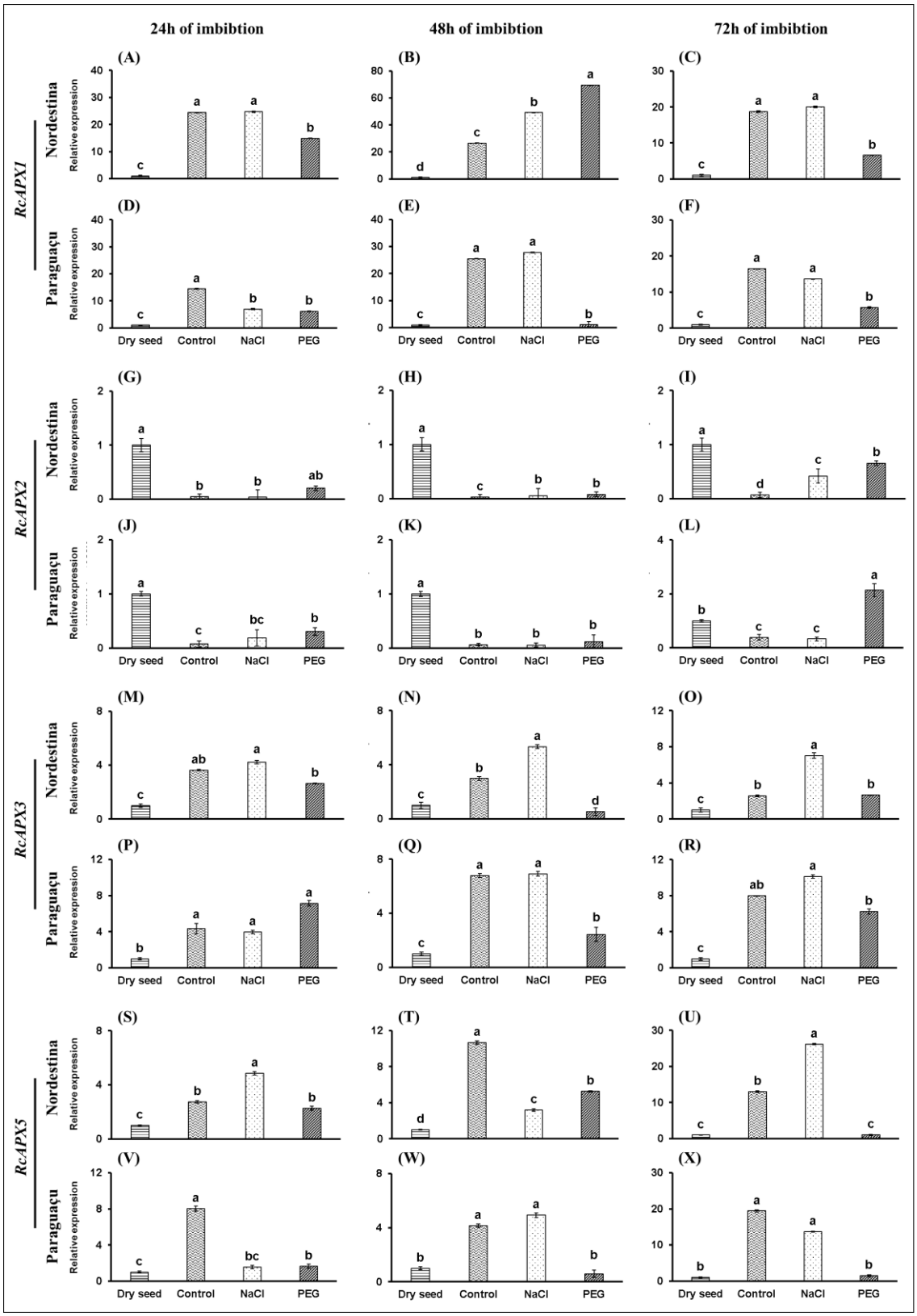


Fig 5. The relative expression level of *RcAPX* genes under salt and water stress was estimated by RT-qPCR. Relative expression of *RcAPX1* in Nordestina (**A, B, C**) and Paraguaçu (**D, E, F**), *RcAPX2* in Nordestina (**G, H, I**) and Paraguaçu (**J, K, L**), relative expression of *RcAPX3* in Nordestina (**M, N, O**) and Paraguaçu (**P, Q, R**) and relative

expression of *RcAPX5* in Nordestina (**S, T, U**) and Paraguaçu (**V, W, X**), in the periods of 24, 48 and 72h. Error bars represent the standard deviations of three biological replicates, and expression levels were normalized using the *ACT* and *PP2AA1* reference genes. Columns on the x-axis represent different treatments (dry seed, Control - water, NaCl, and PEG. The letters above the bars indicate significant differences between different samples by Tukey's HSD ($p<0.05$).

Plants protect cells from oxidative damage by increasing the expression level of genes that encode antioxidant enzymes (Yang et al., 2020). The APX is described to plays an important role in the physiology of water stress (Liu et al., 2012). In sorghum, the APX genes demonstrated different response to water stress, in roots the APX presented downregulated while in leaves was upregulated (Akbudak et al., 2018). In sweet potato, the salt-stress-tolerant presented greater APX activity compared with salt-sensitive genotypes, and APX isoforms demonstrated different level of expression according to tissue and duration of stress (Lin and Pu, 2010). In rice, the cytosolic APX genes (*OsAPX2* and *OsAPX7*) demonstrated to be induced by salt stress (Najami et al., 2008; Teixeira et al., 2006). Besides that, the overexpression of APX gene improved drought resistance and salt tolerance during vegetative stage (Li et al., 2009; Leng et al., 2021).

3.7. Profiling of the *RcAPX* genes by different temperatures during germination and early seedling development

A heat map plot was constructed using log-transformed transcriptome microarray data to illustrate the expression profile of *RcAPX* genes during germination and early seedling development at different temperatures (Fig. 8). Expression of the *RcAPX* gene appears to be modulated by temperature and is dependent on the development stages. In general, the *RcAPX* genes demonstrated lower levels of expression in dry seed and Early Imbibition Sample (EI) compared to Root Protrusion Sample (RP) and young seedlings with 2 cm root (R2).

The *RcAPX2* and *RcAPX6* genes presented high expressed in dry seeds compared to other *RcAPX* genes. In the early imbibition stage (6h of imbibition - EI) samples, the *RcAPX2* and *RcAPX4* genes were highly expressed under heat stress (35°C) compared to control (25°C) while the *RcAPX1*, *RcAPX3* and *RcAPX5* no change in expression. In the root protrusion stage (RP) the *RcAPX1* and *RcAPX4* genes were highly expressed under

heat stress (35°C) while the *RcAPX3*, *RcAPX5*, and *RcAPX6* presented less expression, and the *RcAPX2* no changed. In the root of 2 cm of the seedling stage (R2) samples, only the *RcAPX2* genes were highly expressed under heat stress (35°C) compared to the control (25°C). The *RcAPX1*, *RcAPX2*, and *RcAPX4* genes presented heat response during germination, radicle protrusion moment and early seedling stages in *R. communis*. The *RcAPX1* and *RcAPX2* were predicted to be localized in the cytosol while the *RcAPX4* was peroxisome, indicating the possible role of *RcAPX* genes in these compartments. In general, all *RcAPX* genes presented high expression in low temperature in young seedlings with 2 cm root (R2) samples.

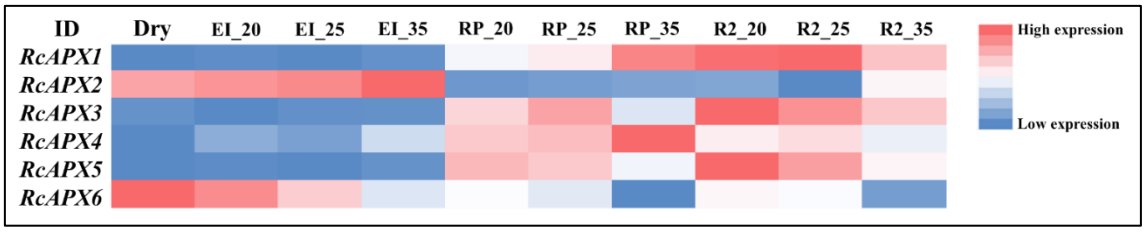


Fig 8. Modulation of *RcAPX* genes under heat stress during germination and early seedling growth. Different colors indicate the difference in gene-level expression (Red-high, white-middle, blue-low). EI (Early imbibition at 20, 25, and 35°C), RP (Radicle protrusion at 20, 25, and 35°C), and R2 (Seedling of 2 cm root at 20, 25, and 35°C).

Enhanced APX activity in plants has been demonstrated under various abiotic stresses. It was observed that eight genes *APX* genes under salt, cold, heat, and high light stresses in ten arabidopsis ecotypes formed a total of 320 patterns of different expression, which 149 conditions (47%) were up-regulated, while 171 (53%) were down-regulated (Caverzan et al., 2012; Filiz et al., 2018). Drought resistant sugarcane plants exhibited high antioxidant capacity due to their higher SOD and APX activity, this response maintained the plant's full recovery of photosynthesis and growth under drought at low temperatures (Sales et al., 2013). APX activity was higher in barley cultivars acclimated to cold than in plants not acclimated to cold temperature (DAI et al., 2009). Our results suggest the response of specific *RcAPX* genes to heat stress (35°C) during germination, radicle protrusion moment and early seedling stage in *R. communis*, as also by low temperature in early seedling stage samples.

4. CONCLUSION

The *R. communis* genome has 6 predicted genes that encode the APX enzyme (*RcAPX*). The activity of the APX enzyme shows an increase in after 48h of imbibition. The regulatory of cis-elements, subcellular localization, enzyme activity and gene expression show the different role of APX in *R. communis* response to different abiotic stresses. The present findings contribute to a better understanding the APX function in *R. communis* and present a framework for a more detailed analysis of *RcAPX* gene family. The relevant information contributes to future improvement of the *R. communis* cultivars to semi-arid region, providing socioeconomic support for family farmers in these regions.

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5. REFERENCES

Akbudak MA, Filiz E, Vatansever R, Kontbay K (2018) Genome-Wide Identification and Expression Profiling of Ascorbate Peroxidase (APX) and Glutathione Peroxidase (GPX) Genes Under Drought Stress in Sorghum (Sorghum bicolor L.). J Plant Growth Regul 37:925–936. https://doi.org/10.1007/s00344-018-9788-9

Akram R, Fahad S, Masood N, et al (2019) Plant Growth and Morphological Changes in Rice Under Abiotic Stress. Elsevier Inc.

Bidin C, Gopinath S, Aravindraj R, et al (2020) The production of biodiesel from castor oil as a potential feedstock and its usage in compression ignition Engine: A comprehensive review. Mater Today Proc 33:84–92. https://doi.org/10.1016/j.matpr.2020.03.205

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/https://doi.org/10.1016/0003-2697(76)90527-3

Caverzan A, Passaia G, Rosa SB, et al (2012) Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. Genet Mol Biol 35:1011–1019

Chin DC, Senthil Kumar R, Suen CS, et al (2019) Plant Cytosolic Ascorbate Peroxidase with Dual Catalytic Activity Modulates Abiotic Stress Tolerances. iScience 16:31–49. https://doi.org/10.1016/j.isci.2019.05.014

Das K, Roychoudhury A (2014) Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Front Environ Sci 2:1–13. https://doi.org/10.3389/fenvs.2014.00053

García-Limones C, Hervás A, Navas-Cortés JA, et al (2002) Induction of an antioxidant enzyme system and other oxidative stress markers associated with compatible and incompatible interactions between chickpea (Cicer arietinum L.) and Fusarium oxysporum f. sp. ciceris. Physiol Mol Plant Pathol 61:325–337. https://doi.org/10.1006/pmpp.2003.0445

Gill S singh, Anjum NA, Gill T, et al (2015) Superoxide dismutase - mentor of abiotic stress tolerance in crop plants.pdf. Env sci Pollut Res 22:10375–10394. https://doi.org/10.1007/s11356-015-4532-5

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gomes Neto V, Barbosa RR, Carosio MGA, et al (2020) Sequence analysis of Ricinus communis small heat-shock protein (sHSP) subfamily and its role in abiotic stress responses. Ind Crops Prod 152:112541. https://doi.org/10.1016/j.indcrop.2020.112541

Gomes Neto V, Ribeiro PR, Del-Bem LE, et al (2018) Characterization of the superoxide dismutase gene family in seeds of two Ricinus communis L. genotypes submitted to germination under water restriction conditions. Environ Exp Bot 155:453–463. https://doi.org/10.1016/J.ENVEXPBOT.2018.08.001

Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, et al (2020) Reactive Oxygen Species and Antioxidant Defense inPlants under Abioti.pdf. Antioxidants 9 (681):1–52

Hassan M Al, Chaura J, Donat-Torres MP, et al (2017) Antioxidant responses under salinity and drought in three closely related wild monocots with different ecological optima. AoB Plants 9:. https://doi.org/10.1093/aobpla/plx009

Hu L, Yang Y, Jiang L, Liu S (2016) The catalase gene family in cucumber : genome-wide identification and organization. 415:408–415

Jardim-Messeder D, Caverzan A, Bastos GA, et al (2022) Genome-wide, evolutionary, and functional analyses of ascorbate peroxidase (APX) family in Poaceae species. Genet Mol Biol 46:. https://doi.org/10.1590/1678-4685-GMB-2022-0153

Kaur R, Bhaskar T (2020) Potential of castor plant (Ricinus communis) for production of biofuels, chemicals, and value-added products. In: Bhaskar T, Pandey A, Rene ER, Tsang DCW (eds) Waste Biorefinery. Elsevier B.V., pp 269–310

Koussevitzky S, Suzuki N, Huntington S, et al (2008) Ascorbate peroxidase 1 plays a key role in the response of Arabidopsis thaliana to stress combination. J Biol Chem 283:34197–34203. https://doi.org/10.1074/jbc.M806337200

Lazzarotto F, Teixeira FK, Rosa SB, et al (2011) Ascorbate peroxidase-related (APx-R) is a new heme-containing protein functionally associated with ascorbate peroxidase but evolutionarily divergent. New Phytol 191:234–250. https://doi.org/10.1111/j.1469-8137.2011.03659.x

Leng X, Wang H, Zhang S, et al (2021) Identification and characterization of the apx gene family and its expression pattern under phytohormone treatment and abiotic stress in populus trichocarpa. Genes (Basel) 12:1–22. https://doi.org/10.3390/genes12030334

Li H, Liu JX, Wang Y, Zhuang J (2020) The ascorbate peroxidase 1 regulates ascorbic acid metabolism in fresh-cut leaves of tea plant during postharvest storage under light/dark conditions. Plant Sci 296:110500. https://doi.org/10.1016/j.plantsci.2020.110500

Ma L, Qi W, Bai J, et al (2022) Genome-Wide Identification and Analysis of the Ascorbate Peroxidase (APX) Gene Family of Winter Rapeseed (Brassica rapa L.) Under Abiotic Stress. Front Genet 12:1–17. https://doi.org/10.3389/fgene.2021.753624

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405–410

Munns R, Day DA, Fricke W, et al (2019) Energy costs of salt tolerance in crop plants. New Phytol. https://doi.org/10.1111/nph.15864

Munns R, Passioura JB, Colmer TD, Byrt CS (2020) Osmotic adjustment and energy limitations to plant growth in saline soil. New Phytol 225:1091–1096. https://doi.org/10.1111/nph.15862

Munns R, Tester M (2008) Mechanisms of Salinity Tolerance. Annu Rev Plant Biol 59:651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Najami N, Janda T, Barriah W, et al (2008) Ascorbate peroxidase gene family in tomato: Its identification and characterization. Mol Genet Genomics 279:171–182. https://doi.org/10.1007/s00438-007-0305-2

Noctor G, Foyer CH (1998) ASCORBATE AND GLUTATHIONE: Keeping Active Oxygen Under Control. Annu Rev Plant Physiol Plant Mol Biol 49:249–279. https://doi.org/10.1146/annurev.arplant.49.1.249

Pandey S, Fartyal D, Agarwal A, et al (2017) Abiotic stress tolerance in plants: Myriad roles of ascorbate peroxidase. Front. Plant Sci. 8

Raja V, Majeed U, Kang H, et al (2017) Abiotic stress: Interplay between ROS, hormones and MAPKs. Environ Exp Bot 137:142–157. https://doi.org/10.1016/j.envexpbot.2017.02.010

Ribeiro PR, Fernandez LG, de Castro RD, et al (2014) Physiological and biochemical responses of Ricinus communis seedlings to different temperatures: A metabolomics approach. BMC Plant Biol 14:1–14. https://doi.org/10.1186/s12870-014-0223-5

Ribeiro PR, Willems LAJ, Silva AT, et al (2018) Transcriptome profiling of Ricinus communis L. provides new insights underlying the mechanisms towards thermotolerance during seed imbibition and germination. Ind Crops Prod 126:380–393. https://doi.org/10.1016/j.indcrop.2018.10.024

Ribeiro PR, Zanotti RF, Deflers C, et al (2015) Effect of temperature on biomass allocation in seedlings of two contrasting genotypes of the oilseed crop Ricinus communis. J Plant Physiol 185:31–39. https://doi.org/10.1016/j.jplph.2015.07.005

Scholz V, da Silva JN (2008) Prospects and risks of the use of castor oil as a fuel. Biomass and Bioenergy 32:95–100. https://doi.org/10.1016/j.biombioe.2007.08.004

Sofo A, Scopa A, Nuzzaci M, Vitti A (2015) Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. Int J Mol Sci 16:13561–13578. https://doi.org/10.3390/ijms160613561

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Tao C, Jin X, Zhu L, et al (2018) Genome-wide investigation and expression profiling of APX gene family in Gossypium hirsutum provide new insights in redox homeostasis maintenance during different fiber development stages. Mol Genet Genomics 293:685–697. https://doi.org/10.1007/s00438-017-1413-2

Tyagi S, Shumayla, Verma PC, et al (2020) Molecular characterization of ascorbate peroxidase (APX) and APX-related (APX-R) genes in Triticum aestivum L. Genomics 112:4208–4223. https://doi.org/10.1016/j.ygeno.2020.07.023

Vasconcelos P de CT, Loureiro MB, Lima ÁMMF, et al (2017) New insights into the mechanism underlying Ricinus communis L. tolerance to drought stress during germination. Ind Crops Prod 103:99–106. https://doi.org/10.1016/j.indcrop.2017.03.038

Wang W, Cheng Y, Chen D, et al (2019a) The Catalase Gene Family in Cotton: Genome-Wide Characterization and Bioinformatics Analysis. Cells 8:86. https://doi.org/10.3390/cells8020086

Wang X, Li M, Liu X, et al (2019b) Quantitative proteomic analysis of castor (Ricinus communis L.) seeds during early imbibition provided novel insights into cold stress response. Int J Mol Sci 20:. https://doi.org/10.3390/ijms20020355

Wang X, Liu X, An Y qiang C, et al (2021) Identification of Glutathione Peroxidase Gene Family in Ricinus communis and Functional Characterization of RcGPX4 in Cold Tolerance. Front Plant Sci 12:. https://doi.org/10.3389/fpls.2021.707127

You Z, Zhang Q, Peng Z, Miao X (2019) Lipid Droplets Mediate Salt Stress Tolerance in Parachlorella kessleri. Plant Physiol 181:510–526. https://doi.org/10.1104/pp.19.00666

7. SUPPLEMENTARY

Supplementary tab 1. Primes used *RcAPX*

Name	Gene ID	Fw	Rv	Amplicon (pb)
RcAPX1	29781.m000013	TTGACCCCATTAAGGAGCAG	TCCCTCAAGTGACCAGAACC	187
RcAPX2	29805.m001492	AGGGAAGGTGTCACATGGAG	CCTCATCGGCAGCATATTTT	223
RcAPX3	29602.m000217	CTTGCAGGTGTTGTTGCAGT	ACAGACCCATTCGGTGAAAG	157
RcAPX5	29648.m002024	GCCAAATTTGATCCTCCTGA	TACTTGGCTGCCACAAACTG	105

Supplementary Tab 3. Predicted genes from *RcAPX* orthologs in selected angiosperms.

Name	**Grup**	***A. thaliana***	***O. sativa***	***P. trichocarpa***	***S. bicolor***
RcAPX1	I	*AtAPX1*, *AtAPX2*	*OsAPX1*, *OsAPX2*	*PtAPX1.1*, *PtAPX1.2*, *PtAPX2*, PtAPX.3	*SbAPX1*, *SbAPX2*
RcAPX2	I	*AtAPX1*, *AtAPX2*	*OsAPX1*, *OsAPX2*	*PtAPX1.1*, *PtAPX1.2*, *PtAPX2*, PtAPX.3	*SbAPX1*, *SbAPX2*
RcAPX3	II	*AtAPX3*, *AtAPX5*	*OsAPX3*, *OsAPX4*	*PtAPX3*, *PtAPX5*, *PtAPX5.like*	*SbAPX4*, *SbAPX7*
RcAPX4	II	*AtAPX3*, *AtAPX5*	*OsAPX3*, *OsAPX4*	*PtAPX3*, *PtAPX5*, *PtAPX5.like*	*SbAPX4*, *SbAPX7*
RcAPX5	III	*AtSAPX*, AtTAPX,	*OsAPX5*, *OsAPX6*, *OsAPX7*, *OsAPX8*	*PtAPX.S.a*, *PtAPX.S.2*	*SbAPX3*, *SbAPX5*, *SbAPX6*, *SbAPX9*
RcAPX6	IV	*AtAPX4*, *AtAPX6*	-	*PtAPX6 related*, *PtAPX.TL29*	*SbAPX6*, *SbAPX8*

Supplementary Tab 4. Sequence logos for the conserved motifs of *RcAPXs* proteins. The logos were identified by MEME software. The character and size of each logo represent the proportion of an amino acid at the specific site. The statistical analysis indicating the probability of obtaining the same alignment score in a random database of the same size and the same amino acid composition is indicated, as well as the frequency of the motifs in the set of proteins analyzed (sites) and the size of the motif (width).

Motif logo		**E-Value**	**Sites**	**Width**
1	KNCAPIMLRLAWHDAGTYDVNTKTGGPNG	7.9e-056	6	29
2	ELAHGANNGLDIALDLLEPIKEKHPIITYADLYQLAGVVAV	9.3e-050	5	41
3	DEGLJKLPTDKALLEDPEFRPYVEKYAEDEDAFFKDYAEAHKKLSELGF	1.6e-044	5	49

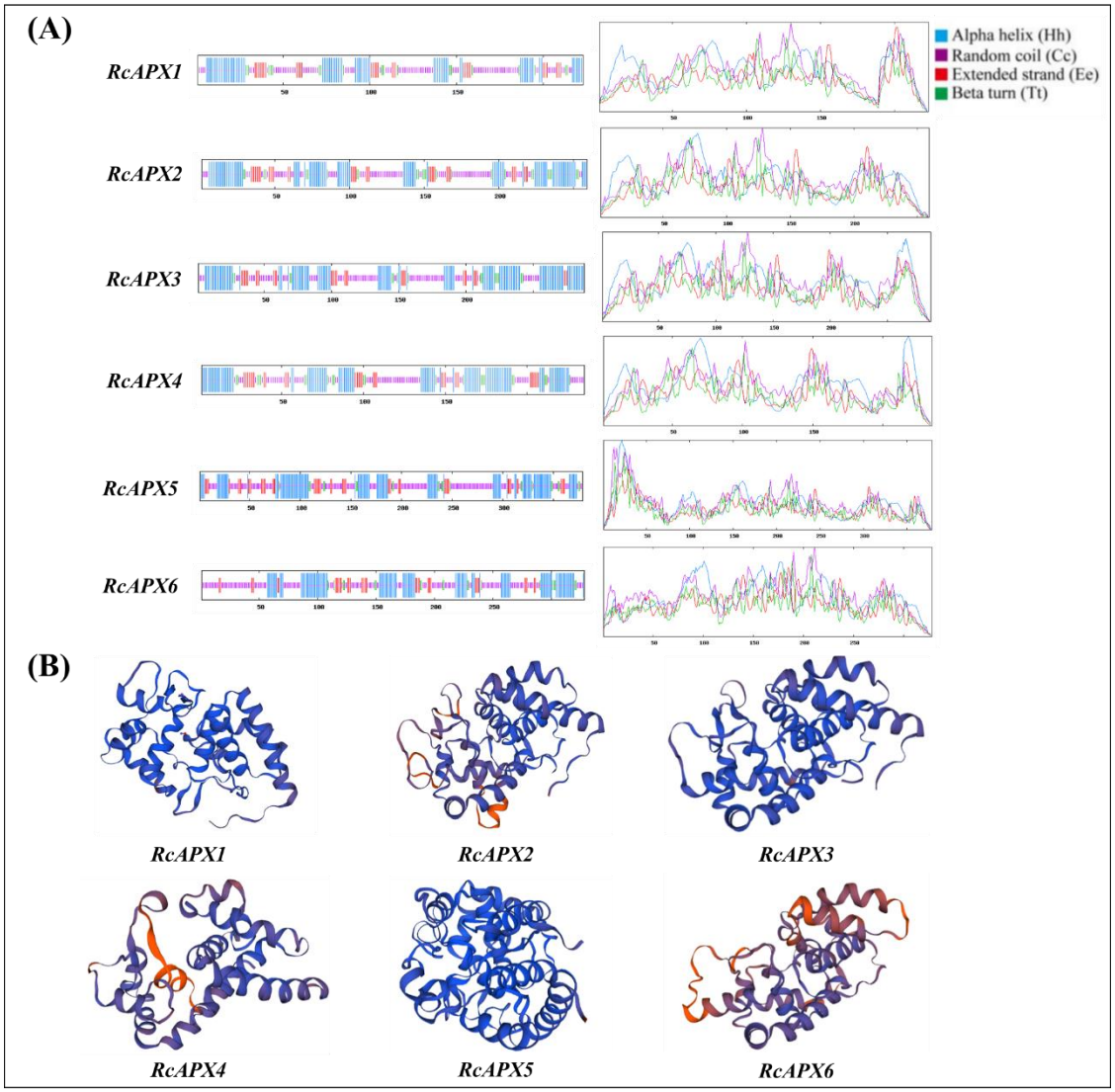
4	HLRDVFHRMGLSDKDIVALSGGHTLGRAH	4.8e-024	4	29
				
5	ERSGFEGPWTKEPLKFDNSYF	2.4e-019	4	21
				
6	TGGPEIPFVPGRKDK	6.0e-015	6	15
				
7	VDEEYQKEIDKARRDLRGLI	6.9e-011	4	20
				
8	PPPEGRLPDATKG	1.1e-002	4	13
				
9	KCCVJFAQGAVGVALAAALVIFGYFYE	1.1e+002	2	27
				
10	MGKNYP	2.8e+002	2	6
				

Supplementary Tab 5. Number of stress-related regulatory elements in APX gene promoters in *R. communis*

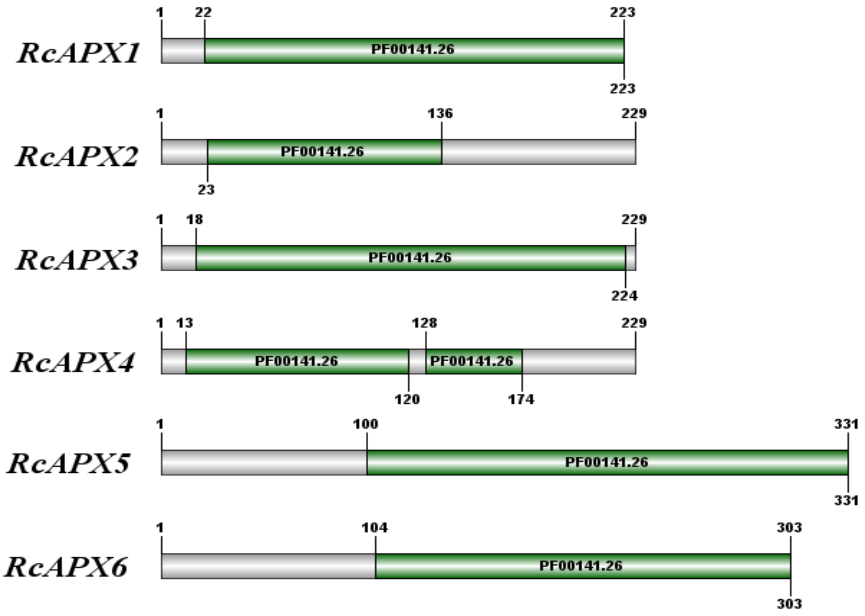
	Ácido abscísico	Auxina	Defesa e estresse	Endosperma	Giberelina	Indução anaeróbica	Luz	MYB	MYC	Reg. do metabolismo	Total
RcAPX1					1		3	3	2		9
RcAPX2	2	1				2	2	2	3	1	13
RcAPX3	1	1	1	1			7	4			15
RcAPX4	3					1	4	3			11
RcAPX5	2	1					3	1	3		10
RcAPX6		1		1		1	2				5
Total	8	4	1	2	1	4	21	13	8	1	

Supplementary Tab 6. Secondary structures of *RcAPX* proteins.

	Alpha helix (Hh)	**Extended strand (Ee)**	**Beta turn (Tt)**	**Random coil (Cc)**
RcAPX1	71 (31,84%)	31 (13,90%)	18 (8,07%)	103 (46,19%)
RcAPX2	96 (37,07%)	34 (13,13%)	25 (9,65%)	104 (40,15%)
RcAPX3	126 (43,75%)	33 (11,46%)	15 (5,21%)	114 (39,58%)
RcAPX4	97 (41,28%)	32 (13,62%)	22 (9,36%)	84 (35,74%)
RcAPX5	130 (34,30%)	52 (13,72%)	23 (6,07%)	174 (45,91%)
RcAPX6	110 (33,54%)	37 (11,28%)	19 (5,79%)	162 (49,39%)



Supplementary Fig 1. Secondary and tertiary structures of *RcAPX* proteins. **(A)** Secondary structure of *RcAPX* proteins. Motifs are represented by different colors: blue, a-helix; purple, random coil; red, extended strand; green, b-turn. **(B)** Tertiary structure of *RcAPX* proteins.



Supplementary Fig 2. Genes whose expression was analyzed are underlined. Functional domains are represented by colored boxes and classified according to PFam database (http://pfam.xfam.org/). Protein schemes were drawn using IBS 1.0.3 software (http://ibs.biocuckoo.org/).

CAPÍTULO 3. Expression profile of the Catalase gene family in castor bean (*Ricinus communis* L.) **provide new insights into the maintenance of redox homeostasis during different germination stages under water restriction and salt stress.**

Chapter to be submitted for publication in 2024.

Expression profile of the Catalase gene family in castor bean (*Ricinus communis* L.) provide new insights into the maintenance of redox homeostasis during germination under water and salt stress.

ABSTRACT

Castor bean (*Ricinus communis* L. - Euphorbiaceae) is an inedible oilseed, recognized for the quality and unique properties of the oil extracted from its seeds, highly valued by industries. The species is mainly cultivated in the Brazilian semiarid region by farmers. In this region, is limited by abiotic stresses such as drought (water restriction) and salinity present in soils, which may limit water absorption and cause phytotoxic effects by generating reactive oxygen species (ROS), compromising productivity. To eliminate ROS and maintain satisfactory levels of homeostasis, compromising the productivity plants use antioxidant enzymes. Catalase (CAT) is one of the main antioxidant enzymes that converts the H_2O_2 generated during photorespiration into H_2O and O_2. Thus, the objective was to characterize the catalase genes and the catalase antioxidant biochemical profiles during germination in 2 castor bean cultivars (BRS Nordestina and BRS Paraguaçu) under the same osmotic potential (0.0, -0.23, -0,46, -0.69 MPa) of water restriction stresses by imbibition in aqueous solutions of NaCl and polyethylene glycol (PEG-8000). We performed the characterization of the catalase gene family in castor bean through bioinformatics, enzymatic activity and gene expression analysis. Two predicted genes for CAT were identified in the castor bean genome. This number was lower than that found in other angiosperms such as *A. thaliana* (3 genes), *O. sativa* (3 genes) and *P. trichocarpa* (3 genes). The *RcCAT2* gene has a different gene structure, conserved motif patterns, and protein size than CAT in angiosperms. Through the analysis of the promotion of the region, it was possible to identify regulatory elements by related hormones and transcription factors related to the plant's response to abiotic and biotic stresses. In general, the CAT activity was increased for the two cultivars in the control from the time of 72h. An increase in CAT activity was also observed for the treatment with NaCl in both cultivars compared to the previous times of imbibition studied. Also, cv. BRS Nordestina has higher CAT activity in water restriction by NaCl (-0.23 MPa) compared to control while cv. BRS Paraguaçu presented greater CAT activity in the control. It was demonstrated that the enzyme that the enzyme catalase acts during

germination and plays an important role in the response to abiotic stresses, where cvs. BRS Nordestina and BRS Paraguaçu demonstrated different profiles in abiotic stress situations. Gene expression studies indicate the modulation of *RcCAT* genes in situations of abiotic stress.

Keywords: *Ricinus*, Euphorbiaceae, water restriction, catalase

1. INTRODUCTION

Abiotic stresses such as heat, cold, drought and salinity negatively impact plant growth, reproduction and survival, limiting the productivity and yield of agricultural crops (Ghorbel et al. 2023; Munns et al. 2019). Among all of them, water restriction caused by drought and soil salinity are the main ones, which often occur causing large reductions in agricultural production, mainly in semi-arid and arid climate regions (Guo et al. 2018; Hussain et al. 2019; Ibrahim et al. 2019).

In the semiarid region of Brazil, castor bean is a crop that stands out, being planted mostly by family farmers, being an important labor fixation crop and source of basic income for these families (Vasconcelos et al. 2017; Nascimento et al. 2022). However, even though castor bean is a rustic plant, its production is limited in semiarid regions, as drought and salinity reduce water absorption, which can cause phytotoxic effects by generating reactive oxygen species (ROS), compromising development and productivity. of plants (Hassan et al. 2017; Nascimento et al. 2022).

ROS include superoxide ($O_2^{\cdot -}$), hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Ibrahim et al. 2019; Mittler 2017). Under non-stressful conditions, small amounts of ROS are normally produced by cellular metabolism, being formed in vital processes such as photosynthesis and respiration (Baker et al. 2023; Yang et al. 2023). They play important roles in cells, as signaling molecules involved in growth, development, gravitropism, hormonal action and many other physiological processes (Ibrahim et al. 2019; Isah 2019; Mittler 2017). However, when in excess, they cause major disturbances in intracellular ionic homeostasis, affect the integrity of the cell membrane, inhibit enzymatic activities and disrupt the function of the photosynthetic apparatus and DNA (Demidchik et al. 2010; You; Chan, 2015). The inefficient elimination of ROS can

inhibit plant growth and reduce productivity, more serious effects can lead to plant death (Ibrahim et al. 2019; Mittler 2017; Munns et al. 2019).

Germination can induce ROS production, traditionally considered a negative effect, as seeds rapidly increase oxygen uptake and oxidative phosphorylation in order to support energy for the germination process (Baker et al. 2023; Mittler 2017; Yang et al. 2023). The production of H_2O_2 was demonstrated during the initial soaking stage of sunflower seeds, soybeans and tomatoes (Bailly et al. 2002; Morohashi 2002; Puntarulo et al. 1991). If the excessive generation of ROS is not strictly controlled, they can cause harmful effects during the early stages of growth and development of embryos and seedlings (Choudhury et al. 2017; Leymarie et al. 2012). Therefore, antioxidant mechanisms have been considered of particular importance for successful germination (De Gara et al. 1997; Tommasi et al. 2001).

Defense mechanisms against biotic and abiotic stresses are categorized into non-enzymatic and enzymatic antioxidants (Isah 2019). The non-enzymatic ones are composed of metabolites such as: ascorbic acid, glutathione, b-carotenes, flavonoids, phenolic compounds, among others (Ali; Elozeiri 2017; Hassan et al. 2017; Hussain et al. 2016; Ibrahim et al. 2019). The enzymatic systems are formed by several enzymes, among them: superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11) and other peroxidases. They are important for dealing with biotic and abiotic stress disorders in adult plants, as well as for successful germination (Baker et al. 2023; Verma et al. 2023; Yang et al. 2023).

Catalase is a ubiquitous tetrameric enzyme that plays a key role in preventing oxidative cell damage by producing water and oxygen from two H_2O_2 molecules with high efficiency (Baker et al. 2023; Mhamdi et al. 2010). It is highly active and does not require cellular reducers, as they mainly catalyze the dismutase reaction (Yang et al. 2023). In plants, CAT eliminates H_2O_2 generated during mitochondrial electron transport, fatty acid oxidation, and photorespiratory oxidation under normal and stressed conditions (Verma et al. 2023; Zandalinas et al. 2020).

CAT plays a critical role in the development, defense and senescence of plants (Mhamdi et al. 2010; Verma et al. 2023), being an integral part of the plant antioxidant system, found in almost all living organisms, preferentially existing in peroxisomes, but is also detected in cytosol, mitochondria and chloroplasts (Hu et al. 2016; Baker et al.

2023; Wang et al. 2019). Due to their efficient catalytic and regulatory properties among all antioxidant enzymes, many plant CATs have been extensively studied at the genetic, biochemical and molecular levels (Sharma et al. 2012; Wang et al. 2019).

Several studies have demonstrated the importance of CAT in the response of plants to abiotic and biotic factors (Callegari et al. 2021; Verma et al. 2023). Generally, catalase is encoded by a small family of genes (Ghorbel et al. 2023; Wu et al. 2023). In *Arabidopsis thaliana*, *Nicotiana tabacum* (Tobacco), *Oryza sativa* (rice), three genes coding for catalase were found and only two in *Hordeum vulgare* (Barley) (Alam; Ghosh, 2018; Janmohammadi; Abbasi; Sabaghnia, 2011; Mhamdi et al. 2010; Wang et al. 2019). In castor bean, information about the genes that code for catalase is scarce in the literature and the association of modulation of these genes in abiotic stress environments due to water restriction or salinity is of fundamental importance to improve the performance of this crop in semiarid regions. Therefore, the present study will address the identification and characterization of CAT genes in castor beans through bioinformatics analysis, enzymatic activity and the gene expression profile of the genes found predicted for catalase during germination under abiotic stresses due to water restriction and saline stress.

CAT plays a critical role in the development, defense and senescence of plants (Ghorbel et al. 2023; Mhandi et al. 2010;), being an integral part of the antioxidant system of plants, commonly found in almost all living organisms, existing preferentially in peroxisomes, but is also detected in the cytosol, mitochondria and chloroplasts (Hu et al. 2016; Wang et al. 2019). Due to their efficient catalytic and regulatory properties among all antioxidant enzymes, many plant CATs have been extensively studied at the genetic, biochemical and molecular levels (Ghorbel et al. 2023; Wang et al. 2019).

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2. MATERIALS AND METHODS

2.1. Identification of genes coding for CAT in castor bean (*RcCAT*)

The amino acid sequences of the genes that encode the CAT enzyme from *Arabidopsis thaliana* (Mhamdi et al. 2010) were used as a query and confronted against the castor bean genome through the BLASTP tool submitted to the Phytozome platform (https://phytozome.jgi.doe.gov/pz/portal.html). The presence of the CAT enzyme domain was confirmed in the Pfam database (http://pfam.xfam.org). Sequences with CAT domain was considered putative genes for catalase enzyme in castor bean (*RcCAT*).

2.2. Phylogenetic analysis of CAT genes in angiosperms

To investigate the phylogenetic relationships of CAT genes in castor bean with other angiosperms, we constructed a phylogenetic tree along with CAT amino acid sequences: *A. thaliana* (Mhamdi et al. 2010), *O. sativa* (Joo; Lee; Song, 2014), *P. trichocarpa* (Hu et al. 2016). The identified CAT sequences were aligned on the online multiple sequence comparison platform by log expectation (MUSCLE) and the phylogenetic analysis was performed using the Molecular Evolutionary Genetics Analysis (MEGA 7.0) software (http://www.megasoftware.net) (TAMURA et. al. 2007) using the Neighbor-joining (NJ) method with the following parameters: p-distance, pair exclusion and bootstrap (1,000 replicates).

2.3. Motif analysis, prediction of intracellular localization and physicochemical characterization of CAT genes in angiosperms

Motif analysis and intracellular localization was performed using the amino acid sequence of CAT proteins in angiosperms. Motifs were identified using the online program Multiple EM for Motif Elicitation (MEME) 5.0.2 (https://meme-suite.org/meme). Prediction of intracellular location was performed by the Cello platform (http://cello.life.nctu.edu.tw), while molecular weight and predicted isoelectric point (pI) were performed by the ProtParam platform (https://web.expasy.org/protparam).

2.4. Regulatory elements in angiosperm CAT genes

We used 1000 bp upstream of the start of transcription (ATG) of the promoter region of CAT genes in angiosperms, obtained from the Phytozome database (https://phytozome.jgi.doe.gov/pz/portal.html). The promoter region analysis was performed on the Plant care database website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) (Lescot et al. 2002).

2.5. Plant Material

2.5.1 Sampling for osmotic and saline stress during germination

The tests were carried out with BRS Paraguaçu and BRS Nordestina cultivars in trays containing germination paper, moistened three times the weight of the paper with aqueous solutions: water (control), NaCl and PEG-8000 at potentials 0, -0.23 and -0.46 MPa, and conditioned in BOD at 30ºC, the collections were carried out at times 0 (dry seeds), 24, 48 and 72h of imbibition. The experimental design was completely randomized using 5 replications with 20 seeds, the embryos were collected and stored in freezer -80°C.

2.6. Extraction and quantification of total proteins

For protein extraction, approximately 5 mg of frozen embryonic axis were weighed in microtubes using an analytical balance, then ground in liquid nitrogen using glass rods and homogenized in 125 µL of 0.1 potassium phosphate buffer M (pH 7.8). The homogenate was centrifuged at 14,000 g for 10 min at 4°C. The supernatant was

collected, and aliquots were made, later stored at -80°C. Protein quantification was performed using the Bradford Sigma-Aldrich reagent (Steinheim, Germany). Absorbances were read in a spectrophotometer (Varian, model Cary 100, United States) at 950 nm and compared with the standard curve of bovine serum albumin (0.3 to 2.5 $mg.mL^{-1}$).

2.7. Determination of CAT activity in castor bean embryo samples

CAT activity was determined according to the method by Beers Jr and Sizer (1952). The reaction was performed in 200 μL containing 200 mM phosphate buffer (pH 7.0), 12.5 mM H_2O_2 and 1 μL of enzymatic extract. The decomposition of H_2O_2 was recorded as a decrease in absorbance at 240 nm for a period of 3 minutes at intervals of 10 seconds, using a spectrophotometer (Varian, model Cary 100, USA). The specific activity of the enzyme was expressed as μmol H_2O_2 reduced/min mg of total protein.

3. RESULTS AND DISCUSSION

3.1. Identification and characterization of CAT genes in angiosperm species

Two predicted genes for the CAT enzyme in castor bean (*RcCAT1* and *RcCAT2*) were identified. The characterization of *RcCAT* genes was carried out together with CAT genes in angiosperms, seeking to verify similarities and differences between the genes.

The total length of the amino acid sequence of angiosperm CAT proteins in general proved to be quite similar, ranging from 475 to 492 aa, respectively, except for the RcCAT2 protein, which was 974 aa long. Similarly, the overall molecular weight ranged from 54.75 to 57.01 kDa except for the *RcCAT* protein which demonstrated a length of 112.35 kDa. The pI of the CAT proteins proved to be close to neutral pH, varying between 6.31 and 7.38 respectively. CAT genes in angiosperms have been shown to predict intracellular localization for the peroxisome cell compartment. In peroxisomes, H_2O_2 is formed during the glyoxylate photorespiratory cycle and by xanthine oxidase with the superoxide dismutase enzymatic system (Corpas et al. 2008; Corpas; Barroso; Rio 2001; Foyer et al. 2009). The CAT enzyme can also be found in other cellular compartments such as the cytoplasm, lumen of chloroplasts and in the cell mitochondria

of photosynthetic organisms (Callegari et al., 2021; Sharma; Ahmad 2014; Spanou et al. 2012; Gu et al. 2013).

Tab 2. Physical-chemical characterization and intracellular localization of CAT proteins in angiosperms.

Locus ID	Name	P. Length (a.a)	Mol. Weight (kDa)	Iso. Point (pI)	Subcellular Localization
AT1G20630.1	*AtCAT1*	492	56,76	6.95	Peroxisome
AT4G35090.1	*AtCAT2*	492	56,93	6.63	Peroxisome
AT1G20620.1	*AtCAT3*	492	56,69	7.31	Peroxisome
29830.m001438	*RcCAT1*	492	57,01	7.38	Peroxisome
30170.m014364	*RcCAT2*	974	112,35	6.84	Peroxisome
Potri.002G009800	*PtCAT1*	492	56,64	6.76	Peroxisome
Potri.005G100400	*PtCAT2*	492	56,94	7.09	Peroxisome
Potri.005G251600	*PtCAT3*	492	56,69	6.68	Peroxisome
Os02g02400.1	*OsCATA*	492	56,70	6.52	Peroxisome
Os06g51150.2	*OsCATB*	475	54,75	6.31	Peroxisome
Os03g03910.1	*OsCATC*	492	56,76	6.93	Peroxisome

3.2. Phylogenetic Analysis of the CAT Gene Family

Amino acid sequences were used to build the phylogenetic tree of predicted *RcCAT* and with other angiosperms such as *A. thaliana* (Mhamdi et al. 2010), *O. sativa* (Joo et al. 2014), *P. trichocarpa* (Hu et al. 2016) (Fig. 12).

The number of CAT genes in castor bean (2 genes) is smaller in *A. thaliana*, *O. sativa* and *P. trichocarpa*, which presented a similar number of CAT genes. A closer relationship was observed between castor bean and *P. trichocarpa* genes in most clades, despite the difference in the number of genes (2, 3 respectively), which is related to the fact that they are species of the same botanical family (Euphorbiaceae).

CAT genes in angiosperms can be divided into three classes (I, II and III) according to the classification in *A. thaliana* Mhamdi et al. (2010). The *RcCAT1* gene was grouped in class I while the *RcCAT2* gene in class II. *The RcCAT1* gene presented as orthologous genes *AtCAT2*, *PtCAT1*, *PtCAT2* and *PtCAT3*, while *RcCAT2* presented as

orthologous genes *AtCAT1*, *OsCATB* and *OsCATC*. All three *P. trichocarpa* CAT genes (*PtCAT1-PtCAT3*) were presented in the form of an exclusive cluster belonging to class I. Furthermore, castor bean as well as *P. trichocarpa* did not demonstrate to have genes in class III.

In *A. thaliana*, the CAT genes are shown to be highly conserved, consisting of three genes: *AtCAT1* (AT1G20630.1), *AtCAT2* (AT4G35090.1) and *AtCAT3* (AT1G20620.1). CAT genes are an extremely efficient mechanism for removing H_2O_2, as it decomposes H_2O_2 without consuming the cell reducing equivalents (Callegari et al. 2021; Mhamdi et al. 2010). In addition, *AtCAT* genes are shown to have specific regulation, varying with developmental time, specific tissue and depending on abiotic stress. The *AtCAT1* gene is expressed mainly in reproductive tissues and seeds, while the *AtCAT2* gene is strongly expressed in photosynthetic tissue and the *AtCAT3* gene is especially in roots and young leaves (Zimmermann et al. 2006; Du et al. 2008; Mhamdi et al. 2010).

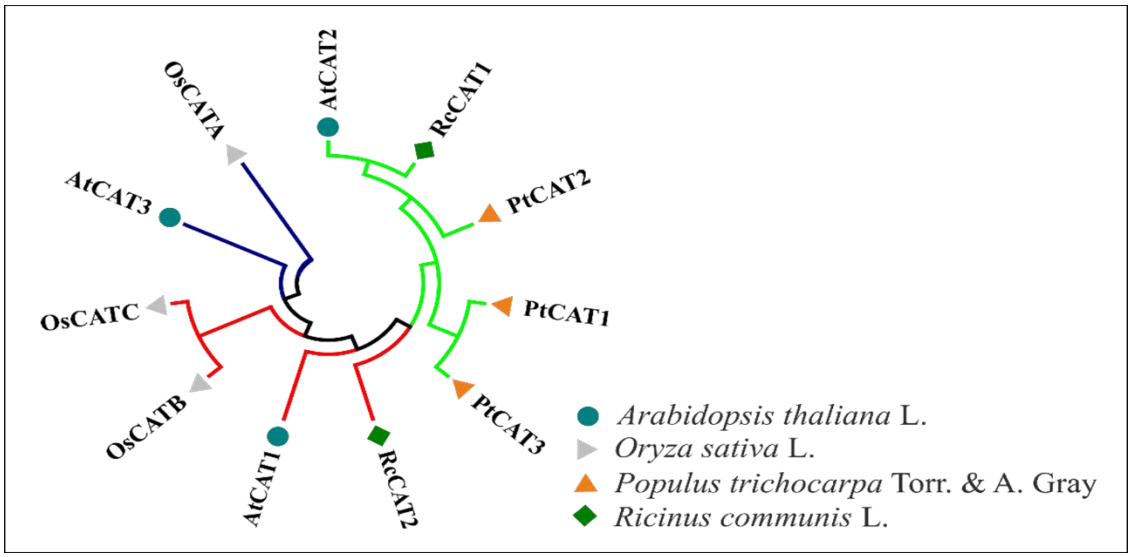


Fig 12. Phylogenetic relationship of the CAT family in selected angiosperms. Genes from different species are shown in different colors. *A. thaliana* (blue-green), *O. sativa* (gray), *P. trichocarpa* (brown) and *R. communis* (castor - green).

3.3. Analysis of conserved motifs and gene structure

The conserved motifs were identified using the Multiple Expectation Maximization for Motif Elicitation (MEME) platform, ten conserved motifs were

identified for the angiosperm CAT proteins of this study, with lengths ranging from 29 to 50 aa (Fig. 13 Tab. Sup. 1) . CAT proteins in angiosperms showed great similarity in the number of motifs found and the order in which they are found in the amino acid sequence. However, the *RcCAT2* protein showed a different motif organization pattern compared to other angiosperm CAT proteins, demonstrating a possible difference in the protein's primary sequence pattern, which may affect secondary and tertiary structures (Fig. 13 A).

The structure of CAT genes was evaluated using Gene Structure Display Server (GSDS) software. In general, the CAT genes in angiosperms in this study were smaller than 4 kb, except for *OsCATB*, *RcCAT2* and *PtCAT1*, which exceed 4 kb. Difference in the number of introns between 3 and 12 was observed (Fig. 13 B). the *OsCATA* gene had three introns, the *OsCATC* gene had five introns, the *AtCAT3*, *AtCAT1*, *PtCAT3*, *PtCAT2* genes had six introns, the *OsCATB*, *PtCAT1*, *RcCAT1*, *AtCAT2* genes had seven introns and the *RcCAT2* gene had twelve introns. Alam and Grosh (2017) observed in transcripts of *AtCAT* and *OsCAT*, the presence of large introns and that could improve the frequency of recombination, as well as maintain the balance of mutation bias, suggesting that the difference found in the pattern of motifs and structure of the gene that occurs in *RcCAT2* may be due to the presence of a large intronic region.

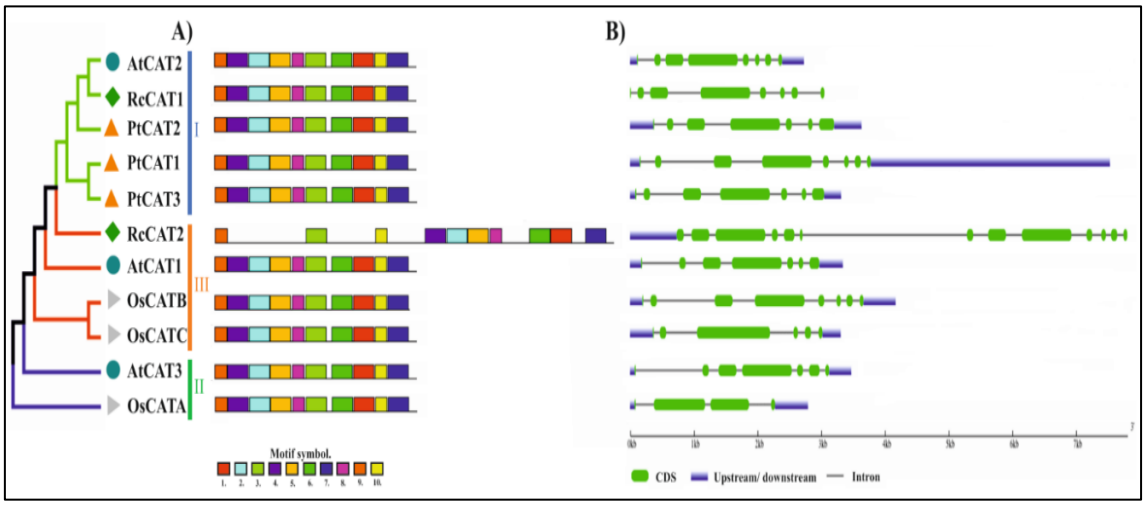


Fig 13. Compositions of conserved domains and structures of CAT genes in angiosperms. (A) Conserved motifs of *RcCAT* proteins and other angiosperms. (B) Exon-intron structures.

3.4. *In silico* analysis of regulatory elements of CAT genes in angiosperms

The analysis of regulatory elements of CAT genes was analyzed in order to understand how these genes can be modulated by abiotic stresses. The CAT family genes presented a series of regulatory elements related to abiotic stress, main regulatory elements were grouped in responsive elements related to meristem expression, salicylic acid, low temperature, defense and stress, anaerobic induction, MIC transcription factor, MYB transcript, light, MeJa (methyl jasmonate), gibberellin, auxin and ABA (abscisic acid) (Fig. 14).

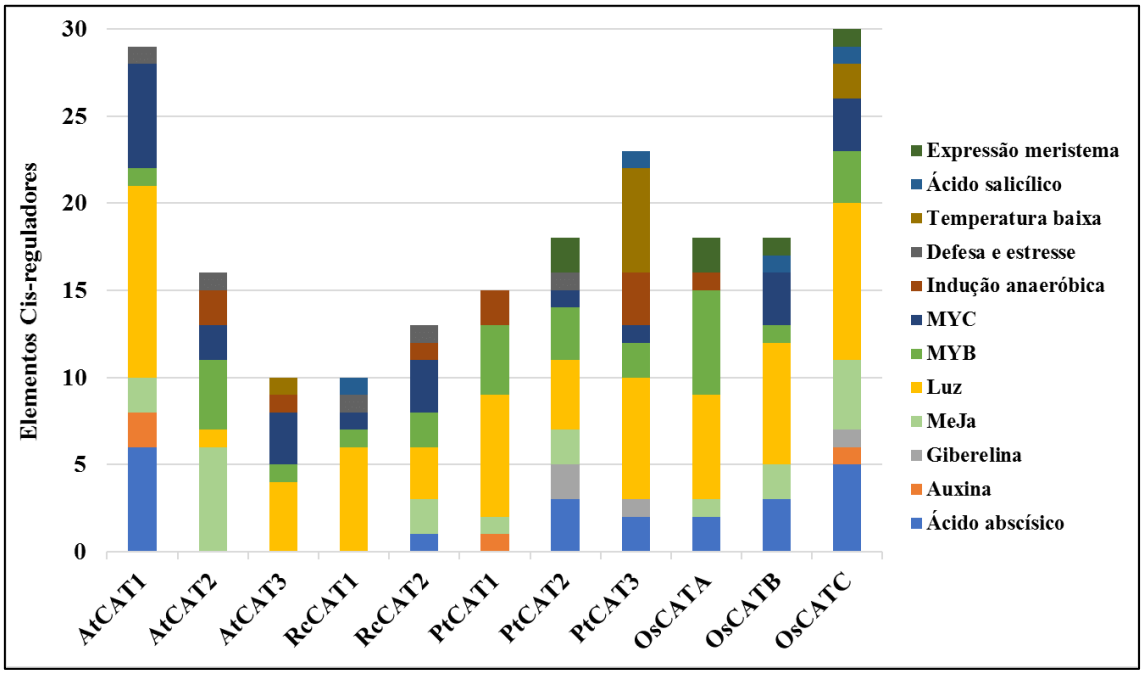
Light responsive elements are present in the promoter region of all CAT genes, anaerobic induction response was found in 6 genes (*AtCAT2*, *AtCAT3*, *RcCAT2*, *PtCAT1*, *PtCAT3* and *OsCATA*), low temperature response elements were found in 3 genes (*AtCAT3*, *PtCAT3* and *OsCATC*) being absent in *RcCAT1* and *RcCAT2*. The defense and stress response element were found in 4 genes (*AtCAT2*, *RcCAT1*, *RcCAT2* and *PtCAT2*).

Regulatory elements for MYC and MYB transcription factors were also found, these transcription factors are related to plant response to abiotic stresses (Li et al., 2019). Regulatory elements for the MYC transcription factor were found in 9 genes (*AtCAT1*, *AtCAT2*, *AtCAT3*, *RcCAT1*, *RcCAT2*, *PtCAT2*, *PtCAT3*, *OsCATB* and *OsCATC*), while regulatory elements for the MYB transcription factor were found in all 13 genes.

A great diversity of regulations by different plant hormones was found. Elements of salicylic acid response were found in 4 genes (*RcCAT1*, *PtCAT3*, *OsCATB* and *OsCATC*). MeJa response elements were found in 8 genes (*AtCAT1*, *AtCAT2*, *RcCAT2*, *PtCAT1*, *PtCAT2*, *OsCATA*, *OsCATB* and *OsCATC*), auxin was found in 3 genes (*AtCAT1*, *PtCAT1* and *OsCATC*), gibberellin was found in 3 genes (*PtCAT2*, *PtCAT3* and *OsCATC*) and abscisic acid response was found in 7 genes (*AtCAT1*, *RcCAT2*, *PtCAT2*, *PtCAT3*, *OsCATA*, *OsCATB* and *OsCATC*).

In Arabidopsis, *AtCAT1* is an important scavenger of H_2O_2 generated under various abiotic stresses. *AtCAT2* and *AtCAT3* are key H_2O_2 scavengers contributing to ROS homeostasis in light or dark, respectively. Furthermore, *AtCAT2* and *AtCAT3* are the main isoforms in Arabidopsis rosette tissue (Mhamdi et al., 2010). Also, *AtCAT2* is induced under cold and drought stresses, and *AtCAT3* is activated mainly by abscisic acid and oxidative treatments, as well as in the senescence phase (Du et al., 2008). Our results suggest a greater complexity in the regulation of *RcCAT* genes, involving not only

regulation by abiotic and ABA stresses, but also by other plant hormones and transcription factors MYB and MYC.



Figu 14. Analysis of the promoter region of the angiosperm genes of the present study, castor bean *RcCAT1* and *RcCAT2*.

3.5. Catalase enzymatic activity under saline and osmotic stress

CAT activity showed no change over time from imbibition time in the control treatment until 72h of imbibition for cv. BRS Nordestina while for cv. BRS Paraguaçu showed a decrease in CAT activity at 48 and 72 hours compared to dry seed and 24 hours of imbibition. In general, within 24 hours of imbibition, cv. BRS Nordestina showed CAT activity levels in water, NaCl and PEG lower than the dry seed while the cv. BRS Paraguaçu showed the same levels of CAT activity in water and osmotic stress by NaCl and PEG independent of osmotic potential compared to dry seed. In 48 hours of imbibition, cv. BRS Nordestina showed higher levels of CAT activity in NaCl restriction (-0.46 MPa) compared to control and other treatments at the same imbibition time while cv. BRS Paraguaçu showed lower CAT activity in the control compared to 24h soaking and also higher CAT activity in all PEG and NaCl water restriction treatments compared to the control. At 72h of imbibition, no difference was observed in CAT activity between treatments for cv. BRS Nordestina, while for cv. BRS Paraguaçu showed greater CAT

activity in the NaCl and PEG treatments with greater water restriction (-0.46 MPa). Potentials with less water restriction (-0.23 MPa) showed greater CAT activity than the control (water) (Fig. 15).

The activity of the catalase enzyme showed a higher level in the NaCl and PEG treatments, in terms of cultivar and soaking time. The activity of CAT and other antioxidant enzymes are described to be initially low in seed germination and increase during development (Cakmak; Strbac; Marschner, 1993; Hite; Auh; Scandalios, 1999). In our study, we verified that the cultivars showed different patterns of CAT activity in 24h of imbibition, which can be explained by the fact that cv. BRS Nordestina showed higher CAT activity already in dry seed compared to cv. BRS Paraguacu. In general, water restriction by NaCl and PEG were able to induce an increase in CAT activity in castor bean seeds.

Dry seeds have a stock of catalase, which during the germination process, there is a decrease in this reserve due to the formation of respiration products, which lead to the formation of H_2O_2 and this leads the plant to increase production in a short period of time. of catalase, and the decrease in metabolism activity leads to a decrease in the expression of enzymes (Bailly et al., 2001; Verma et al. 2023).

In our study, CAT activity levels remained constant or decreased with imbibition time in the control treatment (water). However, if a stressor occurs, catalase levels tend to increase as observed under germination conditions under NaCl and PEG solutions. The water restriction imposed by NaCl and PEG was able to induce greater CAT activity in both cultivars. Catalase activity in seeds may indicate germination capacity under abiotic stress conditions. The catalase activity in seeds can serve as a parameter that indicates the germination capacity under abiotic stress conditions (Prodanovic et al., 2007). And the measurement of its activity can be a parameter to determine the viability and germination of the seed (Ak; Yucel; Ayan, 2012).

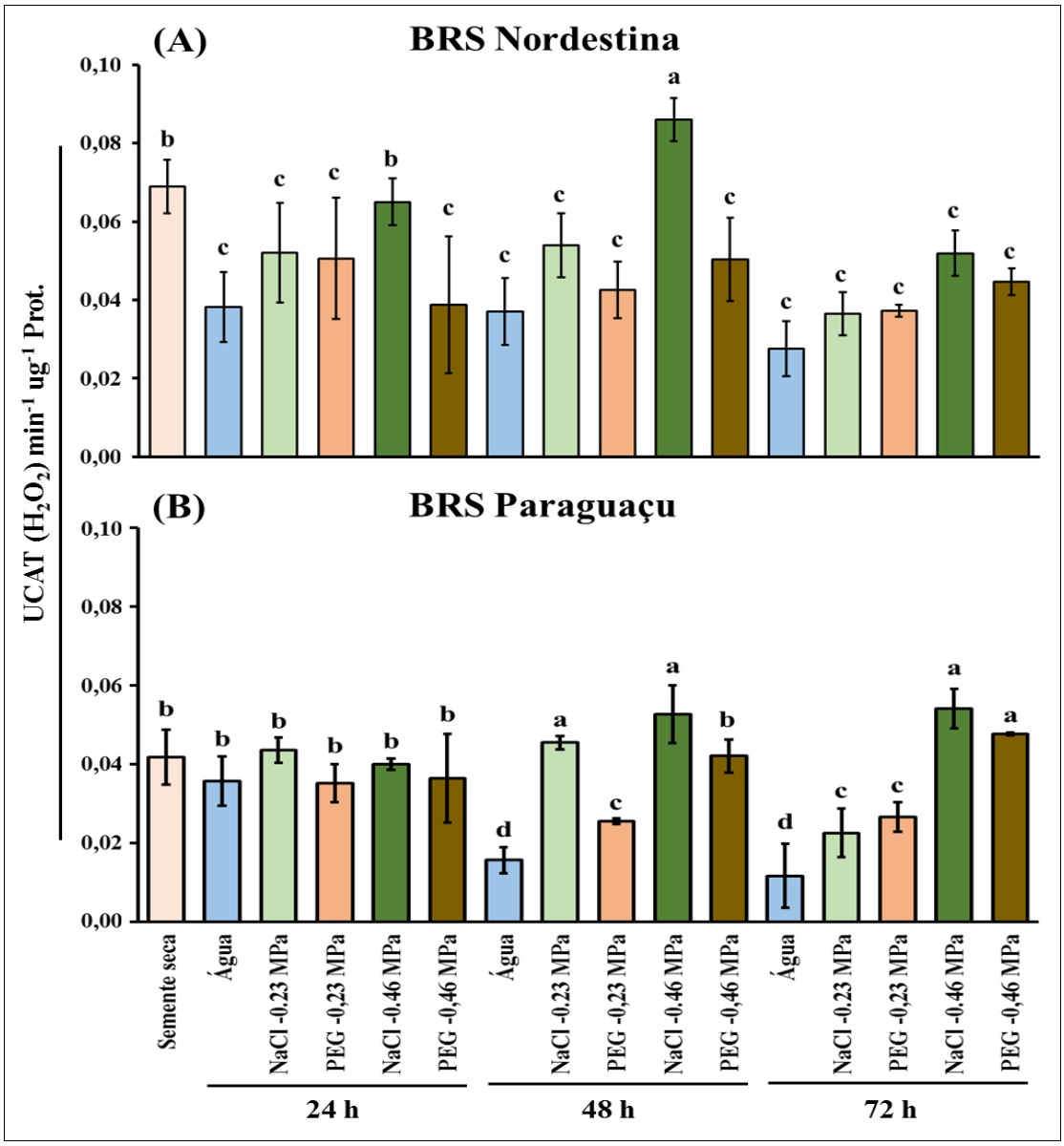


Fig 15. Catalase activity (CAT) in embryos of two castor bean cultivars under water restriction in NaCl and PEG-8000. **A)** BRS Paraguaçu; **B)** BRS Nordestina. Scott-knott test at 5%.

The effects of NaCl on H_2O_2 content and CAT activity have been studied in several groups of plants and in unicellular algae that have a high tolerance for NaCl. And a significant accumulation of H_2O_2 was verified in all plants tested after their exposure to high levels of NaCl, with an increase in CAT activity being observed in response to treatment with NaCl (Wang et al. 2013). In rice, plants sensitive to salt stress, demonstrated a more pronounced decrease in CAT activity with increasing salinity compared to more tolerant genotypes (Kibria et al. 2017).

4. CONCLUSIONS

The castor genome presents 2 genes predicted for the catalase enzyme, located in the peroxisomes. The structure of the *RcCAT* genes in castor bean show differences in order and size compared to other angiosperms present in the study. Regulatory elements in the promoter region of the genes indicate that possible regulation via the ABA pathway and plant hormones related to the response to abiotic and biotic stresses. The activity of the catalase enzyme is modulated according to development time, stress during germination and cultivar, demonstrating that they are induced under conditions of more severe water restriction by PEG and NaCl. Our data suggest that CAT is an important enzyme in seed response during germination under water restriction in castor beans.

5. REFERENCES

Alam NB, Ghosh A (2018) Comprehensive analysis and transcript profiling of Arabidopsis thaliana and *Oryza sativa* catalase gene family suggests their specific roles in development and stress responses. Plant Physiology and Biochemistry. 123:54–64.

Ali AS, Elozeiri AA (2017) Metabolic processes during seed germination. Advances in Seed Biology. 141–166.

Baker A, Lin C, Lett C, Karpinska B, Wright MH, Foyer CH (2023) Catalase: A critical node in the regulation of cell fate. Free Radical Biology and Medicine. 199:56-66. https://doi.org/10.1016/j.freeradbiomed.2023.02.009.

Choudhury FK, Rivero RM, Blumwald E, Mittler R (2017) Reactive oxygen species, abiotic stress and stress combination. Plant J. 90(5):856-867. doi:10.1111/tpj.13299

Demidchik V et al. (2010) Arabidopsis root K+-efflux conductance activated by hydroxyl radicals: Single-channel properties, genetic basis and involvement in stress-induced cell death. Journal of Cell Science. 123(9):1468–1479.

Ghorbel M, Zribi I, Besbes M, Bouali N, Brini F (2023) Catalase Gene Family in Durum Wheat: Genome-wide analysis and expression profiling in response to multiple abiotic stress conditions. Plants. 12(14):2720. https://doi.org/10.3390/plants12142720

Gopavajhula V R, Chaitanya KV, Khan PAA, Shaik JP, Reddy PN, Alanazi M (2013) Modeling and analysis of soybean (*Glycine max*. L) Cu/Zn, Mn and Fe superoxide dismutases. Genetics and Molecular Biology. 36:225–236.

GUO R, et al. Metabolic responses to drought stress in the tissues of drought-tolerant and drought-sensitive wheat genotype seedlings. AoB PLANTS, 10(2):1–13.

Hassan MAL et al. (2017) Antioxidant responses under salinity and drought in three closely related wild monocots with different ecological optima. AoB PLANTS. Feb 21;9(2):plx009. doi:10.1093/aobpla/plx009.

Hu L, Yang Y, Jiang L, Liu S. (2016) The catalase gene family in cucumber: genome-wide identification and organization. Genet Mol Biol. 39(3):408-415. doi:10.1590/1678-4685-GMB-2015-0192

Hussain S, Shaukat M, Ashraf M, Zhu C, Jin Q, Zhang J (2019) Salinity Stress in Arid and Semi-Arid Climates: Effects and Management in Field Crops [Internet]. Climate Change and Agriculture. IntechOpen. Available from: http://dx.doi.org/10.5772/intechopen.87982

Ibrahim W, Qiu CW, Zhang C, Cao F, Shuijin Z, Wu F (2019) Comparative physiological analysis in the tolerance to salinity and drought individual and combination in two cotton genotypes with contrasting salt tolerance. Physiol Plant.165(2):155-168. doi:10.1111/ppl.12791

Isah T (2019) Stress and defense responses in plant secondary metabolites production. Biol Res. 52:39. https://doi.org/10.1186/s40659-019-0246-3

Janmohammadi M, Abbasi A, Sabaghnia N (2011) Influence of NaCl treatments on growth and biochemical parameters of castor bean (*Ricinus communis* L.). Acta Agricultura Slovenica. 99(1):31–40.

Joo J, Lee YH, Song SI (2014) Rice CatA, CatB, and CatC are involved in environmental stress response, root growth, and photorespiration, respectively. Journal of Plant Biology. 57(6):375–382.

Mansour MMF, ALI EF (2017) Evaluation of proline functions in saline conditions. Phytochemistry. 140:52–68.

Mhamdi A, Queval G, Chaouch S, Vanderauwera S, Van Breusegem F, Noctor G. (2010) Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models. J Exp Bot. 61(15):4197-4220. doi:10.1093/jxb/erq282

Mittler R (2017) ROS Are Good.Trends in Plant Science. 22(1):11-19. https://doi.org/10.1016/j.tplants.2016.08.002.

Moraes, P. F. et al. (2015) Expressão gênica diferencial em genótipos de mamona (*Ricinus communis* L.) submetidos a déficit hídrico induzido por PEG. Bragantia. 74(1):25–32.

Munns, R. (2002) Comparative physiology of salt and water stress. Plant, Cell and Environment. 25(2):239–250.

Munns R, et al. (2019) Energy costs of salt tolerance in crop plants. New Phytologist. 225: 1047–1048.

Munns R, Gilliham, M. (2015) Tansley insight Salinity tolerance of crops – what is the cost?. **New Phytologist**, 208(C):668–673.

Munns R, Tester M (2008) Mechanisms of Salinity Tolerance. Annual review of plant biology. 59(1);651–681.

Nascimento DA, Brito AS, Silva LMN, Peixouto LS, Cotrim VF (2022) Water use efficiency of castor bean under semiarid conditions of Brazil. Agricultural Water Management, 260:107278. https://doi.org/10.1016/j.agwat.2021.107278.

Ribeiro PR, De Castro RD, Fernandez LG (2016) Chemical constituents of the oilseed crop *Ricinus communis* and their pharmacological activities: A review. Industrial Crops and Products. 91:358–376.

Vasconcelos PCT et al. (2017) New insights into the mechanism underlying *Ricinus communis* L. tolerance to drought stress during germination. Industrial Crops and Products. 103:99–106.

Verma D, Kaushal N, Balhara R, Singh K (2023) Genome-wide analysis of Catalase gene family reveal insights into abiotic stress response mechanism in Brassica juncea and B. rapa. Plant Science. 330:111620. https://doi.org/10.1016/j.plantsci.2023.111620.

Wan CY, Wilkins TA (1994) A modified hot borate method significantly enhances the yield of high-quality RNA from cotton (*Gossypium hirsutum* L.). Anal Biochem. 223(1):7-12. doi: 10.1006/abio.1994.1538.

Wang T, Lv J, Xu J, Wang X, Zhu X, Guo L (2023) The catalase-peroxidase PiCP1 plays a critical role in abiotic stress resistance, pathogenicity and asexual structure development in Phytophthora infestans. Enviromental microbiology. 25:532-547

WANG, W. et al. The Catalase Gene Family in Cotton: Genome-Wide Characterization and Bioinformatics Analysis. **Cells**, v. 8, n. 2, p. 86, 2019.

Wu Q, Chen Y, Zou W, Pan Y, Lin P, Xu L, Grisham MP, Ding Q, Su Y, Que Y, (2023) Genome-wide characterization of sugarcane catalase gene family identifies a ScCAT1 gene associated disease resistance. International Journal of Biological Macromolecules. 232:12339. https://doi.org/10.1016/j.ijbiomac.2023.123398.

Yang F, Liu Y, Zhang X, Liu X, Wang G, Jing X, Wang X, Zhang Z, Hao G, Zhang S, You C (2023) Oxidative post-translational modification of catalase confers salt stress

acclimatization by regulating H2O2 homeostasis in Malus hupehensis. Journal of Plant Physiology. 287:154037, https://doi.org/10.1016/j.jplph.2023.154037.

You J, Chan Z (2015) ROS regulation during abiotic stress responses in crop plants. Frontiers in Plant Science. 6:1–15.

Zandalinas SI, Fichman Y, Devireddy AR, Sengupta S, Azad RK, Mittler R (2020) Systemic signaling during abiotic stress combination in plants. Proc. Natl. Acad. Sci. USA, 117(24):13810-13820. doi: 10.1073/pnas.2005077117

6. SUPPLEMENTARY

Tabela suplementar 1. Tabela com sequência dos Motif das angiospermas presentes em nosso estudo.

MOTIF	SEQUENCE
1	KLLQTRIFSYADTQRHRLGPNYLQLPVNAPKCAHHNNHHEGFMNFMHRDE
2	TCADFLRAPGVQTPVIVRFSTVIHERGSPETLRDPRGFAVKFYTREGNFD
3	KFHWKPTCGVKCLLDDEAIKVGGANHSHATQDLYDSIAAGNYPEWKLFIQ
4	LTVGSRGPILLEDYHLVEKLANFDRERIPERVVHARGASAKGFFEVTHDI
5	VGNNFPVFFIRDGMKFPDMVHALKPNPKSHIQENWRILDFFSHHPESLHM
6	LDVTKTWPEDILPLQPVGRLVLNKNIDNFFAENEQLAFCPAIVVPGIYYS
7	KENNFKQPGERYRSWAPDRQERFIRRWVDALSDPRVTHEIRSIWISYWSQ
8	FDDIGIPQDYRHMEGSGVNTYTLINKAGK
9	MDPYKYRPSSAYNSPFWTTNSGAPVWNNN
10	YFPSRYDPVRHAEKYPIPPAVCSGKREKC

GENERAL CONCLUSION

This research provides a comprehensive insight into the physiological, biochemical and molecular responses of *R. communis* genotypes to water restriction stresses, with a focus on the osmotic restrictive effects of PEG and NaCl. Seed morphometry significantly influenced germinative development, revealing varying sensitivities among cultivars. The increased SOD enzyme activity under specific stress conditions suggests its potential as a tolerance marker. Additionally, the analysis of the *RcAPX* gene shed valuable light on the regulation and role of APX in response to abiotic stresses. The presence of genes for the CAT enzyme, their unique structure, and regulation via ABA and plant hormones underscore their importance in responding to water restriction. These findings contribute to understanding and potentially enhancing *R. communis* genotypes, fostering advancements in adaptation to semiarid conditions and providing socio-economic support for small scale Castor bean farming in these regions.

PRODUÇÃO TÉCNICA E CIENTÍFICA

Resumos

CUNHA, D. S.; GOMES NETO, V.; ANDRADE, M. V. S.; SANTOS, I. D.; LOUREIRO, M. B.; BERNAL, D. T.; FERNANDEZ, L. G.; ALVES, H. D.; OLIVEIRA JUNIOR, J. L.; RIBEIRO, P. R.; De CASTRO, R. Resposta fisiológica e antioxidante durante a germinação de sementes de *Ricinus communis* l. submetidas à estresses hídrico e salino. In**: 15 Anos de Renorbio - III Encontro de Biotecnologia do Nordeste**, Salvador, 2021.

CUNHA, D. S.; GOMES NETO, V.; ANDRADE, M. V. S.; SANTOS, I. D.; RIBEIRO, P. R.; FERNANDEZ, L. G.; BERNAL, D. T.; LOUREIRO, M. B.; De CASTRO, R. Antioxidant responses to water restriction and saline stresses during germination in *Ricinus Communis* L. In: **Seed Innovation Systems for the 21st Century**, 2021.

CUNHA, D. S.; ANDRADE, M. V. S.; BRITO, C. D.; ALVES, H. D.; CABRAL, V. B. S.; BERNAL, D. T.; LOUREIRO, M. B.; FERNANDEZ, L. G.; De CASTRO, R. Efeito do estresse abiótico na germinação de sementes de mamona (*Ricinus communis* L.). In: **I Forum de Integração e Pesquisa do ICS**, 2019.

Artigo

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