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GEOVANE DAMASCENO NOBRE

PRIMING COM ÁCIDO SALICÍLICO COMO ESTRATÉGIA DE ATENUAÇÃO DOS EFEITOS DELETÉRIOS DA SALINIDADE EM PLÂNTULAS DE ARROZ

FORTALEZA

2024

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Dissertação apresentada ao programa de pós-graduação do Departamento de Bioquímica e Biologia Molecular da Universidade Federal do Ceará como requisito para a obtenção do grau de mestre em bioquímica. Área de concentração: Bioquímica Vegetal.

Orientador: Prof. Dr. Enéas Gomes Filho.

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“O Senhor o protegerá e preservará a sua vida; O Senhor o susterá em seu leito de enfermidade, e da doença o restaurará.” (Salmos 41:2-3).

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RESUMO

A salinidade é um estresse abiótico que prejudica fortemente o crescimento e desenvolvimento das plantas, principalmente em regiões áridas e semiáridas. No caso do arroz (*Oryza sativa* L.), o terceiro cereal mais produzido no mundo, essa forma de estresse pode comprometer gravemente os processos de germinação e estabelecimento das plântulas, o que justifica a necessidade de aprimoramento de técnicas que objetivem aumentar sua capacidade de aclimatação à salinidade. Como estudos têm mostrado que o condicionamento fisiológico (*priming*) pode tornar as culturas mais tolerantes aos estresses ambientais, no presente trabalho, testou-se a hipótese de que o *priming* com ácido salicílico (AS), pode atuar na mitigação dos efeitos deletérios gerados pela salinidade durante o estabelecimento de plântulas de arroz, cv SCSBRS 113, e que isso ocorre devido a alterações metabólicas capazes de favorecer a homeostase osmótica, iônica e redox. O trabalho foi conduzido em duas etapas. Na primeira, realizou-se um *screening* empregando-se AS nas concentrações de 0, 0,125, 0,25, 0,5, 1,0 e 2,0 mM como *priming* das sementes de arroz. Este experimento foi conduzido com cinco repetições de 50 sementes, que foram semeadas por dez dias em condições salinas (NaCl 80 mM), para que, por meio de análises dos parâmetros de germinação (primeira contagem de germinação, percentagem de sementes germinadas, índice de velocidade de germinação e tempo médio de germinação) e de crescimento das plântulas (comprimentos da parte aérea e raiz, massas frescas e secas da parte aérea e raízes), fosse determinada a concentração de AS mais adequada para o *priming* das sementes. Pelo fato da concentração de 2,0 mM de AS ter sido a que promoveu os maiores valores nas variáveis de germinação e, principalmente nas de crescimento das plântulas, essa concentração foi a selecionada para a etapa seguinte. Na segunda etapa, o experimento foi conduzido de maneira inteiramente casualizada, com quatro tratamentos, constando de duas concentrações de AS (0 e 2,0 mM) e duas de NaCl (0 e 80 mM) formando um fatorial 2 x 2. Os resultados apontaram que o estresse salino afetou negativamente o equilíbrio iônico e redox das plântulas, contudo, nas que tiveram suas sementes previamente submetidas ao *priming* com AS, houve uma melhoria na relação K^+/Na^+, na resposta antioxidante da enzima catalase e na atividade da enzima fenilalanina amônia liase. Nessas plântulas, também, foram menores o potencial osmótico, o extravasamento de eletrólitos e os conteúdos de malondialdeído e de peróxido de hidrogênio (H_2O_2). Por meio de GC/MS, determinou-se o conteúdo endógeno de AS, que foi significativamente aumentado nas plântulas provenientes de sementes submetidas ao *priming* com AS. Através de análises do perfil metabólico da parte aérea e raízes (metabolôma),

constatou-se que o *priming* com AS 2,0 mM favoreceu, por exemplo, o incremento nos teores de prolina e asparagina na parte aérea das plântulas de arroz, sugerindo uma possível melhora no ajustamento osmótico e na toxicidade gerada pela salinidade. Além disso, o *priming* com AS causou aumentos nos teores relativos de compostos fenólicos: ácido quínico, ácido caféico e quercitina, bem como em ácido ascórbico, metabólitos fundamentais para a regulação do estado redox das plantas. Por fim, o *priming* das sementes com AS 2,0 mM contribuiu para a mitigação dos danos desencadeados pela salinidade, provocando alterações metabólicas favoráveis à manutenção do equilíbrio iônico, osmótico e redox das plântulas de arroz.

Palavras-chave: ácido salicílico; estresse salino; germinação e estabelecimento da plântula; metaboloma; *Oryza sativa*; *priming* de sementes.

ABSTRACT

Salinity is an abiotic stress that affects plant growth and development, especially in arid and semi-arid regions. In the case of rice (*Oryza sativa* L.), the third most-produced cereal in the world, this condition can seriously compromise the germination and seedling establishment processes, which justifies the need to improve techniques aimed to increase its ability to acclimatize to salinity. As studies have shown that physiological conditioning can make crops more tolerant to environmental stresses. Hence, this study tested the hypothesis that seed priming with salicylic acid (SA) act to mitigate the harmful effects of salinity during the rice seedlings establishment by metabolic changes capable of favoring osmotic, ionic and redox homeostasis. The First, a screening evaluated SA at concentrations of 0, 0.125, 0.25, 0.5, 1.0, and 2.0 mM as a priming agent for rice seeds sown under saline conditions (80 mM NaCl). The treatments were composed by five replicates comprised by 50 seeds each. The germination parameters and seedling growth analysis selectedthe most suitable AS concentration for seed priming. The results showed that 2.0 mM concentration of SA promoted the highest values in the germination variables and, above all, in the seedling growth variables. Next, the second experiment consisted by four treatments comprising two concentrations of SA (0 and 2.0 mM) and two of NaCl (0 and 80 mM) arranged in a 2 x 2 factorial scheme conducted and a completely randomized design. Overall, salt stress negatively affected the ionic and redox balance of the seedlings. However, in those whose seeds had been previously SA-primed, there was an improvement in the K^+/Na^+ ratio, in antioxidant response though catalase enzyme, and SA path via phenylalanine ammonia-lyase enzyme. Additionally, osmotic potential, electrolyte leakage, and malondialdehyde and hydrogen peroxide (H_2O_2) contents were also lower in these seedlings. The GC/MS technique quantified the endogenous SA content, which significantly increased in the seedlings from SA-primed seeds. Through metabolic profile analysis of the shoot and roots (metabolome) of seedlings, SA-priming favored, for example, an increase in the levels of proline and asparagine in the shoots , suggesting a possible improvement in osmotic adjustment and the toxicity generated by salinity. In addition, priming with SA caused increases in the relative levels of phenolic compounds, including quinic acid, caffeic acid, and quercetin, as well as ascorbic acid, which corroborate the redox state of seedlings. Finally, priming the seeds with 2.0 mM SA helped to mitigate the toxicity caused by salinity, causing metabolic changes favorable to maintaining the ionic, osmotic, and redox balance of the rice seedlings.

Keywords: salicylic acid; salt stress; germination and seedling establishment; metabolome; *Oryza sativa*; seed *priming*.

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ANOVA	Análise de variância
AS / AS	Ácido salicílico
CAT	Catalase
CPA	Comprimento da parte aérea
CR	Comprimento da raiz
DM	Massa seca
EE	Extravasamento de eletrólitos
EROs / ROS	Espécies reativas de oxigênio
PAL	Fenilalanina amônia-liase
FM	Massa fresca
GC	Cromatografia em fase gasosa
IVEPA	Índice de velocidade de emissão de parte aérea
IVG	Índice de velocidade de germinação
MDA	Malondialdeído
MFPA	Massa fresca da parte aérea
MFR	Massa fresca da raiz
MS	Espectrometria de massa
MSPA	Massa seca da parte aérea
MSR	Massa seca da raiz
PCA	Análise de componentes principais
PCG	Primeira contagem de germinação
PPN	Percentagem de plântulas normais
PSG	Percentagem de sementes germinadas
RWC	Conteúdo relativo de água
TMG	Tempo médio de germinação

SUMÁRIO

1. JUSTIFICATIVA

As plantas estão constantemente expostas a diversos estresses, como calor, frio, déficit hídrico e salinidade. Contudo, o excesso de sais no solo ou na água de irrigação é considerado um dos fatores abióticos que mais prejudicam as funções fisiológicas das plantas, como o equilíbrio hídrico e nutricional, a síntese de proteínas e a expressão gênica. Além disso, a salinidade, geralmente, afeta negativamente o processo fotossintético, pois é capaz de reduzir a abertura dos estômatos e os teores de pigmentos fotossintéticos, causando danos aos fotossistemas, transporte de elétrons e estrutura dos cloroplastos e inibindo as enzimas envolvidas com a fixação de gás carbônico (NEGRÃO *et al*., 2017).

Essas alterações metabólicas, muitas vezes irreparáveis, são oriundas da redução do potencial hídrico no ambiente radicular (efeito osmótico) e/ou do acúmulo de íons (efeito iônico) potencialmente tóxicos aos tecidos das plantas. Tais efeitos produzem um ambiente oxirredutor instável, devido à grande produção de espécies reativas de oxigênio (EROs), que, em altas concentrações, causam danos aos componentes celulares (NATH *et al*., 2019). Vale ressaltar que plantas tolerantes conseguem prevenir ou minimizar esses efeitos deletérios, através de rearranjo do seu metabolismo, com o intuito de manter a homeostase celular. Esse fenômeno é conhecido como aclimatação e envolve os ajustes osmótico, redox e iônico (SHAHZAD *et al*., 2019).

O ajustamento osmótico, por exemplo, ocorre através da absorção de íons ou síntese de solutos orgânicos, como: açúcares solúveis, aminoácidos, proteínas que se acumulam no citosol e regulam o potencial osmótico e hídrico das raízes, mantendo elevado o teor relativo de água nos tecidos, e assim, evitando danos às trocas gasosas e ao alongamento celular (XU *et al*., 2016). Já para diminuir os danos causados pelas EROs, as plantas ativam complexas vias de defesa, que envolvem enzimas e/ou antioxidantes de baixo peso molecular (AHMAD *et al*., 2019). Por fim, a regulação do fluxo iônico, compreende estratégias como: o efluxo de Na^+ de volta para o meio de crescimento ou para o apoplasto; a compartimentação de Na^+ no vacúolo; o controle do carregamento do xilema; a retenção de Na^+ nas células do caule; a recirculação de Na^+ pelo floema; a alocação de sais para as folhas velhas; e/ou a excreção de Na^+ para a superfície das folhas (exclusivo de halófitas) (YAMAGUCHI *et al*., 2013).

Um dos cereais que vem sendo bastante estudado com relação aos efeitos deletérios causados pelo excesso de sais no solo ou na água de irrigação é o arroz (*Oryza sativa* L.). Trata-se de uma das espécies de gramíneas mais consumidas pela população mundial por ser

uma importante fonte de carboidratos, proteínas e vitaminas e que também possui elevada relevância econômica (FUKAGAWA; ZISKA, 2019). Por ser considerada uma cultura alimentar básica, diferentes métodos para atenuar os efeitos deletérios do estresse salino no cultivo de arroz têm sido desenvolvidos a fim de garantir uma produção sustentável. Afinal, a população humana está aumentando rapidamente o que tem exigido um incremento de 70 a 110% na produtividade das culturas agrícolas até 2050 (GANIE *et al.,* 2019).

Nos últimos anos, uma estratégia fisiológica que vem promovendo resultados positivos para aumentar a tolerância das plantas ao estresse (aclimatação) é a do *priming* ou do pré-tratamento de sementes ou plantas com agentes condicionantes, os quais são usados em baixas concentrações e que aclimatam as células vegetais para lidarem com situações desfavoráveis posteriores (HASAN *et al.,* 2016). Vale ressaltar que muitos tipos de compostos orgânicos e inorgânicos vêm sendo utilizados nesse processo de sensibilização das plantas com o intuito de potencializar mecanismos de defesa contra o estresse. Santos *et al.* (2019), por exemplo, evidenciaram a efetividade do ácido salicílico (AS) como uma molécula capaz de induzir respostas defensivas contra estresses bióticos e abióticos, além de auxiliar nos processos de fotossíntese, de regulação estomática, de absorção e transporte de nutrientes minerais, bem como no aumento da capacidade antioxidante em mudas pré-brotadas de cana de açúcar.

Diante disso, o presente trabalho buscou investigar se a aplicação exógena de AS em sementes de arroz (*Oryza sativa* L.) atua como um condicionante fisiológico ou efeito *priming* sendo capaz de atenuar os efeitos deletérios do estresse salino durante a germinação e o estabelecimento das plântulas, além de esclarecer se essa atenuação se deve a alterações favoráveis no metaboloma, com a produção de metabólitos-chaves relacionados a uma melhor resposta osmótica e de defesa antioxidante. Como o desenvolvimento de novas metodologias de cultivo depende de um olhar integrado que reúne dados fenotípicos, bioquímicos, fisiológicos e metabolômicos, os resultados obtidos nesta pesquisa poderão servir de subsídios para o aprimoramento da técnica de *priming* e ao uso de águas de má qualidade na irrigação em ambientes áridos e semiáridos.

2. HIPÓTESE

O NaCl afeta o desenvolvimento inicial das plantas, contudo o condicionamento fisiológico (*priming*) com ácido salicílico é capaz de tornar as plântulas de arroz (*Oryza sativa* L.) mais tolerantes à salinidade, por meio da síntese de solutos compatíveis que atuam na indução de mecanismos eficientes de ajustamento osmótico, do aumento da atividade de

enzimas antioxidantes e da ativação de vias metabólicas que ajudam a diminuir os efeitos prejudiciais dessa condição de estresse abiótico.

3. OBJETIVOS

3.1 Objetivo geral

Esclarecer os mecanismos responsáveis pelo aumento da tolerância à salinidade em plântulas de arroz condicionadas fisiologicamente com ácido salicílico, bem como identificar metabólitos fundamentais para minimizar os impactos gerados por essa forma de estresse abiótico.

3.2 Objetivos específicos

Utilizando-se sementes condicionadas com ácido salicílico ou não condicionadas e plântulas provenientes dessas sementes em presença ou ausência de salinidade, pretende-se:

1. Definir, por meio de uma triagem com diferentes concentrações de ácido salicílico, uma dose adequada para a realização do condicionamento fisiológico das sementes de arroz;
2. Determinar os indicadores ligados à germinação e crescimento: contagem de plântulas normais e de sementes germinadas, bem como a definição do percentual de germinação, do índice de velocidade de germinação, do índice de velocidade de emissão de parte aérea, do tempo médio de germinação, da determinação do comprimento e da massa fresca e seca da parte aérea e raízes;
3. Estimar o potencial osmótico e o teor relativo de água da parte aérea;
4. Verificar a resposta ao estresse oxidativo por meio da determinação dos indicadores de vazamento de eletrólitos e da peroxidação de lipídios de membrana nas partes aéreas e do conteúdo de peróxido de hidrogênio (H_2O_2) nas partes aéreas e raízes.
5. Quantificar os teores dos pigmentos fotossintéticos: clorofilas e carotenoides;
6. Quantificar os teores de solutos inorgânicos (Na^+e K^+) na parte aérea e raízes;
7. Mensurar a atividade antioxidante por meio da determinação da enzima catalase (CAT) e avaliar a atividade da enzima fenilalanina amônia-liase (PAL) com relação a síntese de compostos fenólicos na parte aérea e raízes;
8. Quantificar o conteúdo endógeno de ácido salicílico na parte aérea e raízes;
9. Avaliar o perfil metabólico da parte aérea e raízes.

4. FUNDAMENTAÇÃO TEÓRICA

4.1 O efeito do estresse salino em plantas

A salinidade é um dos principais fatores abióticos que limitam a produtividade agrícola em virtude de seus efeitos no crescimento e no desenvolvimento das plantas. Estima-se que em todo o mundo cerca de 424 milhões de hectares de solo superficial (0 - 30 cm de profundidade) e 833 milhões de hectares de subsolo (30 - 100 cm de profundiade) são afetados pelo sal (FAO, 2023). Tais áreas encontram-se, principalmente, em regiões áridas e semiáridas, que apresentam baixo índice pluviométrico, elevada taxa de evapotranspiração, atividade humana constituída por práticas de irrigação incompatíveis com as características físicas, químicas e mineralógicas do solo, bem como pela aplicação de fertilizantes de forma excessiva e pelo uso de água de irrigação de baixa qualidade, condições que favorecem o processo de salinização (BELTRÁN, 2016).

O estresse salino induz uma série de respostas morfológicas e fisiológicas nas plantas decorrentes de distúrbios metabólicos, que podem ser de natureza osmótica e/ou iônica. O fator osmótico é detectado imediatamente após exposição ao estresse, devido à redução do potencial osmótico da solução do solo, o que dificulta a absorção de água e nutrientes pelas raízes (DIAS *et al.,* 2016). Já o iônico depende de uma exposição prolongada ao estresse, o qual resulta na absorção excessiva de íons tóxicos, como, por exemplo, Na^+ e Cl^-, que ao se acumularem no citosol podem trazer graves prejuízos ao crescimento e desenvolvimento, podendo causar a morte das plantas (BERNSTEIN, 2019).

Vale ressaltar que, em adição aos efeitos diretos da salinidade sobre as plantas, existem muitos outros que ocorrem de forma secundária. Por exemplo, o estresse salino pode provocar um desbalanço no estado redox das células, gerando um estresse oxidativo, que produz EROs de maneira excessiva, tais como o peróxido de hidrogênio (H_2O_2), o oxigênio singleto ($^1O_2$) e os radicais livres superóxido ($\bullet O_2^-$) e hidroxil (HO•). Essas EROs são altamente reativas e podem alterar a homeostase através da oxidação de biomoléculas (DEMIDCHIK, 2015).

Outro problema diretamente relacionado com a salinidade é a inibição dos processos de fotossíntese e transpiração, que pode ocorrer devido ao fechamento dos estômatos (AMJAD *et al.,* 2014) ou ao acúmulo dos íons sódio e cloreto nos cloroplastos, que acabam afetando a integridade dos cloroplastos, causando redução no rendimento quântico do PSII e limitando a fixação do carbono (PÉREZ-LÓPEZ *et al.,* 2012). Além disso, a exposição à salinidade pode alterar os níveis dos pigmentos fotossintéticos e deformar a estrutura dos cloroplastos (BEJAOUI *et al.,* 2016).

Para lidar com os efeitos deletérios da salinidade, as plantas desenvolveram mecanismos fisiológicos e bioquímicos que visam atingir a homeostase e evitar a toxicidade celular. Dentre eles, podem-se citar: a exclusão de íons tóxicos dos tecidos, a compartimentação destes íons no vacúolo, e o acúmulo de solutos compatíveis (MIRANDA *et al.,* 2013; RANJIT; MANISH; PENNA, 2015), bem como os mecanismos antioxidantes enzimáticos, composto pelas enzimas dismutase do superóxido, catalase, peroxidase do ascorbato e peroxidase do guaiacol, dentre outras (AHMAD *et al.,* 2019) e os mecanismos não enzimáticos, que são formados por compostos de baixa massa molecular como o ascorbato reduzido, a glutationa reduzida, os tocoferois e os carotenoides (SHARMA *et al.,* 2012).

4.2 A cultura do Arroz

O arroz (*Oryza sativa* L.) é uma planta monocotiledônea existente desde 5000 a.C, mas que passou por domesticação apenas em 2000 a. C em áreas da China e do sul da Ásia. Pertence à família *Poaceae* (gramíneas), à ordem *Glumifloreae* e apresenta metabolismo fotossintético do tipo C3 (RANGAN; FURTADO; HENRY, 2016). É uma das culturas agrícolas mais importantes do mundo, pois além de ser consumida por cerca de metade da população mundial, fornece 20% do suprimento de energia alimentar, enquanto o trigo e o milho fornecem 19 e 5%, respectivamente (RAHMAN; ZHANG, 2023). Além disso, o arroz é considerado uma *commodity* estratégica, pois além de auxiliar na segurança alimentar global, está intimamente relacionado ao crescimento econômico, emprego, estabilidade social e paz regional (YADEV; KUMAR, 2018).

Os países asiáticos China, Índia, Indonésia, Bangladesh, Vietnã, Mianmar e Tailândia juntos são responsáveis por mais de 80% da produção global de arroz (RAHMAN; ZHANG, 2023) e, fora da Ásia, o Brasil é o país que mais produz e consome essa cultura, possuindo um número significativo de indústrias de processamentos que, além de agregar qualidade, torna o cereal brasileiro destaque no comércio internacional (BRAZILIAN RICE, 2023). Vale ressaltar que o estado do Rio Grande do Sul (RS) sustenta a maior produção do cereal no país, contribuindo, por exemplo, no período 2018/2019 com 42 mil toneladas de arroz beneficiado e 393 mil toneladas dele com casca. Quanto ao Nordeste brasileiro, o estado do Maranhão é o principal destaque, contribuindo com 1,25% da produção nacional, enquanto o estado do Ceará vem em quinto lugar, com 0,05%, sendo o município de Iguatu o maior produtor, seguido pela região de Baturité e do Baixo Jaguaribe (COMPANHIA NACIONAL DE ABASTECIMENTO, 2020).

Do ponto de vista nutricional, o arroz é constituído majoritariamente de carboidratos complexos, sendo o principal deles o amido, encontrando-se também outros constituintes, como os açúcares xilose, ramnose, maltose e glicose (RAO; MURALIKRISHNA, 2004). Além disso, representa importante fonte de proteínas, ricas em lisina e sete outros aminoácidos essenciais, de vitaminas do complexo B (B1, B2 e B3) e vitamina E (CHAUDHARI *et al.,* 2018), além de lipídios ricos em ácidos graxos insaturados (TONG; BAO, 2019) e fibras solúveis (em sua maioria, compostas por arabinoxilanos e β-D-glucanos) e insolúveis, as quais são ricas em celulose, hemicelulose e β-glucana (LAI *et al.,* 2007).

Com relação às condições de cultivo, o arroz é moderadamente sensível ao estresse salino e níveis de salinidade do solo acima do limite crítico (3,0 dS/m) podem afetar negativamente a produtividade de grãos. Lopes *et al.* (2020) mostraram, por exemplo, que plantas de arroz (cv. SCSBRS 113) cultivadas em presença de NaCl a 80 mM, apresentam redução média de 21% na capacidade de perfilhamento, de 53% na área foliar, de 37% na massa seca da parte aérea, de 40% na massa seca das folhas e de 32% na massa seca das raízes. Semelhantemente, Gadelha *et al.* (2021) detectaram que a salinidade (NaCl a 80 mM) prejudicou fortemente o crescimento das cultivares de arroz BRS Esmeralda e São Francisco, causando aumento nos teores de Na^+, diminuindo severamente suas áreas foliares e reduzindo suas capacidades fotossintéticas.

Vale ainda ressaltar que, pelo fato do arroz, em estádio de plântula, ser mais sensível ao estresse salino devido seus sistemas radiculares e mecanismos de regulação serem menos desenvolvidos do que em outros estádios, se faz necessário a implementação de mais estudos sobre o desenvolvimento inicial dessa planta sob tal condição de estresse abiótico, com o objetivo de ajudar na sua conservação em ecossistemas vulneráveis e de contribuir para seu incremento produtivo em virtude da crescente demanda mundial (ZHENG *et al.,* 2023).

4.3 O condicionamento fisiológico e o estresse em plantas

Para as plantas conseguirem se desenvolver na presença de algum agente causador de estresse, elas dependem de sua capacidade de aclimatação a tais condições adversas (FERRÃO *et al.,* 2016). Esse processo é variável entre as espécies e depende de certas alterações bioquímicas, fisiológicas e morfológicas (GONDIM *et al.,* 2010), que são fundamentais para que as plantas se tornem mais tolerantes às condições do meio a que estão acondicionadas. Nesse sentido, a técnica que faz uso de compostos atenuadores de estresses em plantas, chamada de *priming* ou pré-tratamento é, em muitos casos, eficaz na redução das perdas provocadas por fatores abióticos e bióticos estressantes, pois contribui para aumento da

tolerância a distintas condições ambientais (MANONMANI; BEGUNI; JAYANTHI, 2014). Essa melhoria se deve a ativação de importantes mecanismos que contribuem para o processo germinativo como, por exemplo, a respiração, o enfraquecimento do endosperma e a transcrição e tradução de genes (CHEN; ARORA, 2013).

O condicionamento fisiológico é uma técnica que visa hidratar parcialmente as sementes sob determinada temperatura e tempo, permitindo a ativação de processos metabólicos essenciais para a germinação, contudo, sem que ocorra a emissão da raiz primária (PAPARELLA *et al.,* 2015). Ele pode ser efetuado principalmente mediante o osmocondicionamento, o hidrocondicionamento e o matricondicionamento. No osmocondicionamento, utiliza-se um soluto não permeável como o polietilenoglicol (PEG) de alto pelo molecular (PEG 6000, e.g.) em um potencial osmótico conhecido, a fim de controlar a embebição das sementes; no hidrocondicionamento ocorre a adição lenta de água às sementes até que atinjam um conteúdo de água específico; enquanto no matricondicionamento o controle da embebição é realizado por meio de um material inerte (MARCOS FILHO, 2005). Após o condicionamento, as sementes são submetidas ou não à um processo de secagem (MCDONALD, 1999).

É importante lembrar que também existem muitos relatos na literatura sobre a eficiência de moléculas sinalizadoras, que podem ser usadas como *primings* em sementes a fim de aumentar a tolerância ao estresse. Dentre elas, podem ser citadas: a quitosana (CHOUHAN; MANDAL, 2021), o ácido indolacético (ASHRAF *et al.,* 2006), a melatonina (AKBARI *et al.,* 2020), o peróxido de hidrogênio (SADAK, 2022), o nitroprussiato de sódio (PIRES *et al.,* 2016) e até mesmo fitormônios como o ácido abscísico, giberelinas, jasmonatos, salicilatos e auxinas (ASHRAF *et al.,* 2008).

4.4 O ácido salicílico e o condicionamento fisiológico

A aplicação exógena de reguladores de crescimento, que atuam sobre os processos de germinação, tem potencializado o aumento de produtividade de culturas de grande importância agrícola, como o algodão, o arroz, o feijão, o milho e a soja. Dentre esses reguladores, destaca-se o ácido salicílico (AS), um composto fenólico e metabólito secundário, que além de influenciar muitos processos fisiológicos, como a permeabilidade iônica e a fotossíntese (ANWAR *et al.,* 2013), desempenha importante papel na defesa das plantas contra vários estresses abióticos e bióticos (JINI; JOSEPH, 2017). Na figura 1, é mostrado a estrutura do AS e de alguns compostos fenólicos que são precursores de sua síntese.

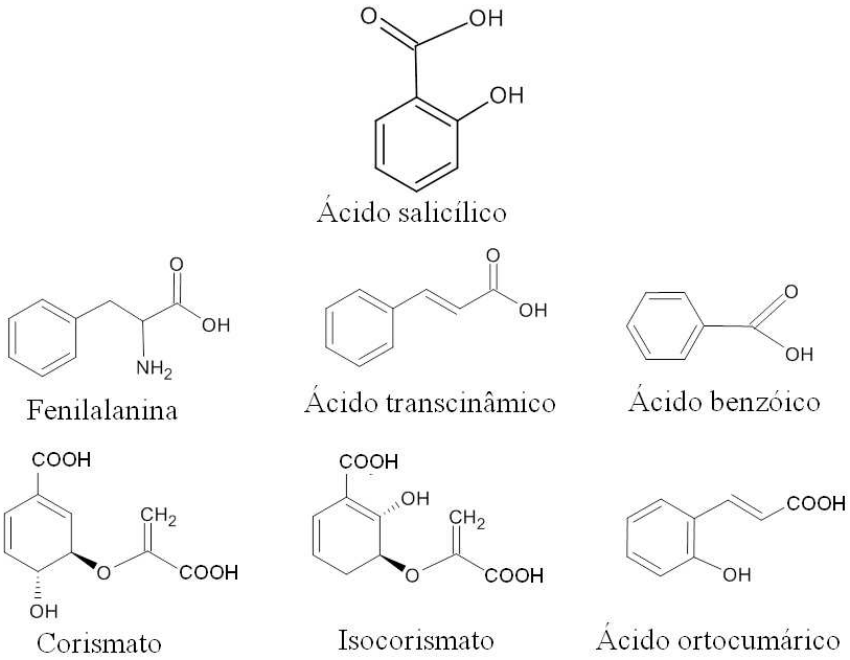


Figura 1. Estruturas químicas do ácido salicílico e dos compostos que participam de sua biossíntese (JAYAKANNAN *et al.,* 2015; RASKIN, 1992).

O AS pode ser sintetizado a partir de duas vias: a via da fenilalanina, que ocorre no citosol e a do isocorismato, que ocorre nos cloroplastos (Figura 2). Na primeira, o ácido transcinâmico é produzido a partir da fenilalanina pela ação da enzima fenilalanina amônia-liase (PAL). Em seguida, o ácido transcinâmico é convertido em ácido benzóico que, após ser catalisado pela enzima ácido benzóico-2-hidroxilase (AB2H), é convertido em AS (MUSTAFA *et al.,* 2009). Cabe destacar que, por meio de uma reação de orto-hidroxilação, o ácido transcinâmico também pode ser convertido em ácido ortocumárico que, ao ser oxidado, é convertido em AS (RASKIN, 1992). Já a via do isocorismato ocorre a partir de duas etapas, em que primeiramente o corismato é convertido em isocorismato pela ação da isocorismato-sintase (ICS) e, em seguida, o isocorismato é catalisado pela enzima isocorismato piruvato-liase (IPL), que é responsável pela síntese de AS (STRAWN *et al.,* 2007).

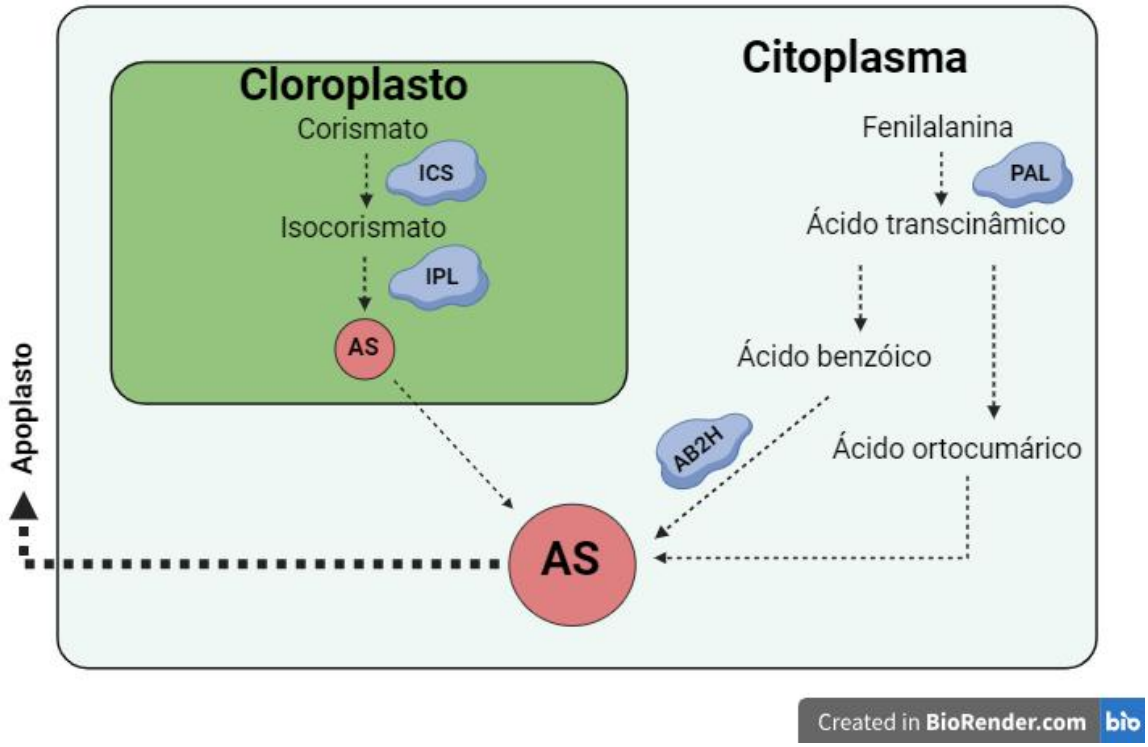


Figura 2. Biossíntese de ácido salicílico (AS); fenilalanina amônia-liase (PAL), ácido benzóico-2-hidroxilase (AB2H), isocorismato-sintase (ICS) e isocorismato piruvato-liase (IPL) (JAYAKANNAN *et al.,* 2015; RASKIN, 1992). Fonte: elaborada pelo autor

Vale ressaltar, que durante o estresse salino em mudas de arroz, por exemplo, a via da fenilalanina é a principal responsável pela elevação endógena de AS (SAWADA; SHIM; USUI, 2006). Por outro lado, estudos feitos em *Arabidopsis* apontam que durante a exposição a patógenos a maior parte do AS é sintetizado a partir da atividade da ICS (VLOT *et al.,* 2009). Diante de condições estressantes, independentemente do mecanismo de biossíntese envolvido, o ácido salicilico é entregue ao local de ação através do apoplasto (KAYA *et al.,* 2023), a fim de desempenhar importante papel no crescimento das plantas, na indução de flores, na absorção de íons, no movimento estomático, na reversão dos efeitos do ABA na abscisão foliar, no aumento dos níveis de pigmentos fotossintéticos (YUSUF *et al.,* 2013), bem como na eliminação de espécies reativas de oxigênio (EROs) (SALEEM *et al.,* 2021).

Alguns estudos têm mostrado, por exemplo, que o condicionamento fisiológico de sementes com AS pode auxiliar na atenuação dos efeitos da salinidade, aumentando significativamente a porcentagem de germinação de algumas culturas (ALAMRI *et al.,* 2018), promovendo o acúmulo de osmorreguladores (MAIA JÚNIOR *et al.,* 2020) e melhorando os pesos fresco e seco de mudas de interesse (JAYAKANNAN *et al.,* 2013). Loutfy *et al.*

(2020), por exemplo, demonstraram que plantas de trigo cultivadas sob salinidade (NaCl a 150 mM) e que tiveram suas sementes previamente embebidas com AS a 0,5 mM não só recuperaram a perda de massa fresca das folhas e raízes causada pela salinidade, como aumentaram a atividade de enzimas antioxidantes, melhoraram a acumulação de osmoprotetores importantes, como a prolina, aumentaram os teores de açúcares solúveis e ácidos orgânicos, bem como diminuíram os teores de Na^+ nas plantas. Já Shaki *et al.* (2017), verificaram que a aplicação exógena de AS a 1,0 mM, em folhas de plantas de cártamo cultivadas sob NaCl a 200 mM, contribuiu para um incremento na atividade da enzima PAL e, consequentemente, um aumento na quantidade de compostos fenólicos fundamentais para a redução secundária de EROs e aumento da tolerância à salinidade.

Em um estudo com plantas de quinoa pré-condicionadas com AS a 1,5 mM e cultivadas sob diferentes concentrações salinas equivalentes a 0, 7, 14 e 21 dS m^{-1}, Mohammadi *et al.* (2022), observaram nas plantas condicionadas uma redução de 22,14% nos níveis de Cl^- em comparação às plantas não pré-tratadas, mas cultivadas nas mesmas condições salinas. Eles também perceberam que o AS teve um efeito positivo na absorção de íons Ca^{2+} e Mg^{2+}, o que contribuiu para aumentar o crescimento radicular dessas plantas.

Em virtude desses e de outros efeitos benéficos do AS, muitos estudos têm sido elaborados para elucidar seu papel na proteção das culturas agrícolas contra os efeitos deletérios de estressores abióticos, os quais vêm se intensificando devido ao aquecimento global (JINI; JOSEPH, 2017). Apesar disso, ainda se constata a existência de várias lacunas sobre suas interações com outros metabólitos vegetais envolvidos no processo de aclimatação. Diante disso, este trabalho, por exemplo, buscou investigar quais mecanismos bioquímicos, fisiológicos e metabolômicos são alterados em plântulas de arroz que tiveram suas sementes condicionadas com AS e suas respostas após cultivo em meio salino.

5. MATERIAIS E MÉTODOS

5.1 Experimento 1: Determinação da concentração de ácido salicílico mais adequada para o *priming* das sementes de arroz

Este experimento teve por objetivo determinar a melhor concentração (ou a mais adequada) de AS para ser utilizada no Experimento 2, em que se procurará testar a hipótese central deste trabalho, ou seja, de que o condicionamento fisiológico ou *priming* com AS é capaz de mitigar a toxicidade gerada pelo sal durante o estabelecimento de plântulas de arroz (*Oriza sativa* L.), cv SCSBRS 113, e que isso se deve a alterações metabólicas favoráveis à uma melhor homeostase osmótica, iônica e redox.

5.1.1 *Sementes, local e condução do experimento*

Este experimento foi realizado utilizando-se sementes de arroz (*Oryza sativa* L.), cv SCSBRS 113*,* obtidas junto à empresa brasileira AgroGiust – SC. Com a finalidade de definir a melhor concentração de AS a ser usada no *priming* das sementes de arroz com AS, alguns parâmetros de germinação e de estabelecimento das plântulas foram avaliados do 3º ao 10º dia após a semeadura, sob condições de salinidade (NaCl a 80 mM) e em função de seis concentrações de AS: 0, 0,125, 0,25, 0,5, 1,0 e 2,0 mM.

Tais concentrações foram selecionadas a partir de dados da literatura que, em geral utilizam o AS no máximo a 2,0 mM. Como exemplos, temos os seguintes trabalhos: Baninasab e Baghbanha (2013), que testaram o impacto da aplicação de várias concentrações de AS (0, 0,25, 0,50 ou 1,0 mM) em sementes de pepino (*Cucumis sativus* L.) semeadas sob diferentes soluções de NaCl (0, 50, 100 e 150 mM); Bagheri (2014), que investigou os efeitos do *priming* com AS nas concentrações de 1,0 e 2,0 mM, em sementes de milho semeadas sob estresse salino; e Yücel e Heybet (2016), que examinaram as respostas de plântulas de trigo provenientes de sementes condicionadas previamente com 0,5 mM de AS e sob estresse salino.

Vale destacar que concentrações de AS como *priming* mais elevadas não foram usadas nesse experimento de triagem, em virtude de pesquisas terem sinalizado que concentrações mais elevadas desse fitormônio, podem ser prejudiciais ao desenvolvimento inicial de mudas. Soliman *et al.* (2016), por exemplo, mostraram efeitos inibitórios em parâmetros de germinação e crescimento de sementes de fava em virtude do condicionamento com 3,0 mM de AS. Por fim, resultados semelhantes foram relatados para a germinação de embriões de milho, devido o uso de doses de AS na faixa de 3 a 5 mM (GUAN *et al.,* 2007).

5.1.2 *Condicionamento fisiológico das sementes de arroz e semeadura*

Inicialmente, as sementes de arroz foram selecionadas pelo tamanho, desinfetadas superficialmente com hipoclorito de sódio a 1% (v/v), sendo as sementes deixadas nesta solução por 5 min sob agitação. Posteriormente, as sementes foram lavadas exaustivamente com água destilada para retirada do cloro residual.

Para o condicionamento fisiológico, as sementes de arroz foram deixadas embeber na presença de AS nas concentrações de 0, 0,125, 0,25, 0,5, 1,0 e 2,0 mM, por 24 h à temperatura de 25 ºC. Posteriormente, para completar o tratamento de *priming*, as sementes

foram transferidas e deixadas sobre folhas de papel germitest onde ficaram por 24 h ao ar livre a fim de perderem a umidade absorvida que podia fazê-las germinar precocemente.

Em seguida, as sementes condicionadas (em número de 50) de cada concentração de AS utilizada no *priming* foram semeadas sobre duas folhas de papel germitest (28 x 38 cm), as quais foram cobertas por mais uma folha, todas previamente autoclavadas a 120 °C e umedecidas com 40 mL de solução de NaCl a 80 mM. As folhas de papel contendo as sementes foram enroladas em formato de canudo, armazenadas em recipientes de plásticos contendo 20 mL da solução salina, cobertas com sacos plásticos (para manter a umidade) e mantidas em câmara BOD, com fotoperíodo de 12 h e temperatura média de 25 ºC durante o dia e a noite. Vale destacar que nenhuma reposição da solução salina, durante os dias de cultivo, foi necessária. O experimento foi conduzido com cinco repetições de 50 sementes cada.

Os seguintes parâmetros de germinação e medidas de crescimento das plântulas de arroz foram determinados como descritos a seguir.

5.1.3 *Primeira contagem de germinação, porcentagem de sementes germinadas e porcentagem de plântulas normais*

A contagem de sementes germinadas foi realizada diariamente durante dez dias de cultivo e a primeira contagem de germinação consistiu no registro das porcentagens de sementes que apresentaram radículas emitidas no terceiro dia. A porcentagem de sementes germinadas foi calculada no décimo dia, a partir da contabilização das plântulas geradas. Já a porcentagem de plântulas normais foi calculada por meio da quantificação de plântulas que apresentaram raízes e partes aéreas emitidas sem nenhuma deformação ao término do processo de cultivo.

5.1.4 *Índice de velocidade de germinação e de emissão da parte aérea*

O índice de velocidade de germinação (IVG) foi calculado pelo somatório do número de sementes germinadas a cada dia (sementes com radículas emitidas), dividido pelo número de dias decorridos entre a semeadura e a germinação, de acordo com a fórmula definida por Maguire (1962) a seguir:

$$\text{IVG} = \sum_{i=1}^{n} \left(\frac{\text{Gi}}{\text{Ni}}\right), \text{ onde:}$$

Gi = número de sementes germinadas a cada dia de contagem;

Ni = número de dias decorridos da semeadura;
n = n-ézimo tempo de contagem.

O índice de velocidade de emissão da parte aérea (IVEPA) foi calculado de forma análoga à do (IVG), sendo calculado pelo somatório do número de plântulas com a parte aérea emergida, dividido pelo número de dias decorridos entre a semeadura e o início da emissão da parte aérea.

5.1.5 *Tempo médio de germinação*

O tempo médio de germinação (TMG) foi calculado pelo somatório do produto do número de sementes germinadas pelo número de dias decorridos entre a semeadura e a germinação, dividido pelo somatório do número das sementes germinadas, a cada dia, segundo fórmula desenvolvida por Labouriau (1983) a seguir:

$\text{TMG} = \sum_{i=1}^{n}(\text{Gi} * \text{Ni}) / \sum \text{Gn}$, onde:

Gi = número de sementes germinadas a cada dia de contagem;
Ni = tempo decorrido entre o início da germinação e a n-ésima contagem;
n = n-ézimo tempo de contagem.

5.1.6 *Crescimento das plântulas*

Para análise do crescimento da parte aérea e raízes, aos 10 dias após a semeadura, as plântulas foram fotografadas e as imagens carregadas em um software de processamento e análise de imagens (ImageJ) para que os comprimentos da parte aérea e das raízes fossem aferidos. Em seguida, o material vegetal foi dividido em parte aérea e raízes, pesado em balança eletrônica para determinação da massa fresca (MF) e, posteriormente, levados a estufa a 60 ºC por 72 h, para obtenção da massa seca (MS).

5.1.7 *Desenho experimental e análise estatística*

O experimento foi conduzido a partir de seis tratamentos de *priming* com AS (0, 0,125, 0,25, 0,5, 1,0 e 2,0 mM) em sementes de arroz semeadas na presença de NaCl a 80 mM, com cinco repetições de 50 sementes, em que as análises de germinação e de crescimento foram realizadas a fim de que fosse determinada a concentração de AS mais adequada para o *priming*. Os dados obtidos foram submetidos a análise de variância (ANOVA) usando o software Sisvar (FERREIRA, 2011). O desdobramento desta análise se deu por meio de curvas de regressão polinomiais lineares, quadráticas e cúbicas, em nível de 5% de probabilidade, ajustadas para todas as variáveis.

5.2 Experimento 2: Ação do *priming* de sementes de arroz com ácido salicílico em minimizar os efeitos deletérios da salinidade nas homeostases osmótica, iônica e redox e as alterações no metaboloma

Este experimento teve por finalidade testar a hipótese de que o *priming* com AS minora os efeitos da salinidade nas homeostases osmótica, iônica e redox, com alterações favoráveis no perfil metabólico das plântulas de arroz. Para tal, as sementes de arroz previamente condicionadas com AS 2,0 mM e água destilada (de certa forma, um controle) foram semeadas em presença e ausência de salinidade (NaCl a 80 mM), formando um fatorial 2 x 2, sendo o experimento conduzido com quatro repetições. Neste estudo, foram determinados: o conteúdo relativo de água, o potencial osmótico, os danos de membrana (vazamentos de eletrólitos e peroxidadação lipídica), os teores dos íons Na^+ e K^+, as atividades das enzimas catalase e fenilamônia-liase, e o metaboloma, cujas metodologias, bem como os resultados, estão apresentados na forma de um artigo completo a ser submetido (ver item 7).

6. RESULTADOS

6.1 Resultados do Experimento 1

6.1.2 *Screening da concentração de ácido salicílico para o condicionamento fisiológico das sementes e germinação*

Neste estudo, o condicionamento fisiológico das sementes de arroz com AS exógeno, ou *priming*, nas várias concentrações, afetou positivamente todas as variáveis relativas à germinação das sementes sob condições de salinidade (NaCl a 80 mM), exceto o tempo médio de germinação (TMG), que não foi afetado significativamente pelo tratamento de *priming* com AS (Tabela 1), indicando importante papel desse fitormônio na minimização dos danos gerados pela salinidade. As variáveis primeira contagem de germinação (PCG), percentual de sementes germinadas (PSG), índice de velocidade de germinação (IVG) e índice de emissão de parte aérea (IVEPA) foram significativamente afetadas pelo *priming*.

Tabela 1 – Resumo do quadro de ANOVA para as variáveis: primeira contagem de germinação (PCG), percentagem de sementes germinadas (PSG), percentagem de plântulas normais (PPN), índice de velocidade de germinação (IVG), índice de velocidade de emissão de parte aérea (IVEPA) e tempo médio de germinação (TMG) de sementes de arroz (*Oryza sativa* L.), cv. SCSBRS 113, condicionadas com ácido salicílico (AS) e semeadas em condições de estresse salino (NaCl a 80 mM).

Fonte de variação	G.L.	Quadrado Médio					
		PCG	PSG	PPN	IVG	IVEPA	TMG
Tratamento	5	70,61**	18,69**	45,97*	0,034**	0,059**	$0,005^{ns}$
Erro	24	15,93	3,66	16,33	0,005	0,013	0,010
C.V (%)		4,36	1,93	4,26	2,37	3,82	3,26

* Significativo com p < 0,05; ** significativo com p < 0,01; não significativo (ns) com p> 0,05.

A PCG foi realizada no terceiro dia após a semeadura, no qual foi possível observar a emissão da radícula. De acordo com a análise de regressão, essa variável apresentou comportamento quadrático em função da concentração de AS, mostrando-se crescente com o aumento da concentração até o valor de 1,33 mM, quando apresentou valor máximo estimado de 95,94% para a PCG (Figura 3A). Por outro lado, a PSG apresentou um comportamento cúbico, com máximo absoluto em torno de 0,6 mM de AS (PSG = 100%), quando a partir daí teve leve decréscimo até cerca da concentração de 1,6 mM, e a partir daí, começou a crescer chegando na concentração de 2,0 mM de AS ao valor estimado pela regressão, e que foi igual ao experimental, de 99,9% (Figura 3B).

Os índices IVG e IVEPA, apresentaram curvas de regressão cúbicas semelhantes à da PSG (Figura 3B, C, D), porém com menores variações em função da concentração de AS, sendo os valores de IVG e IVEPA, praticamente os mesmos e iguais a aproximadamente 3, independentemente da concentração de AS utilizada no *priming*. Em relação ao percentual de plântulas normais (PPN) não foi evidenciada nenhuma regressão polinomial válida, logo sua variação significativa (p < 0,05) mostrada no resumo do quadro de ANOVA (Tabela 1), deve-se exclusivamente ao termo independente da equação de regressão gerada ($Y=10,323X^{3ns} - 30,104X^{2ns} +22,397X^{ns} +91,483^{**}$).

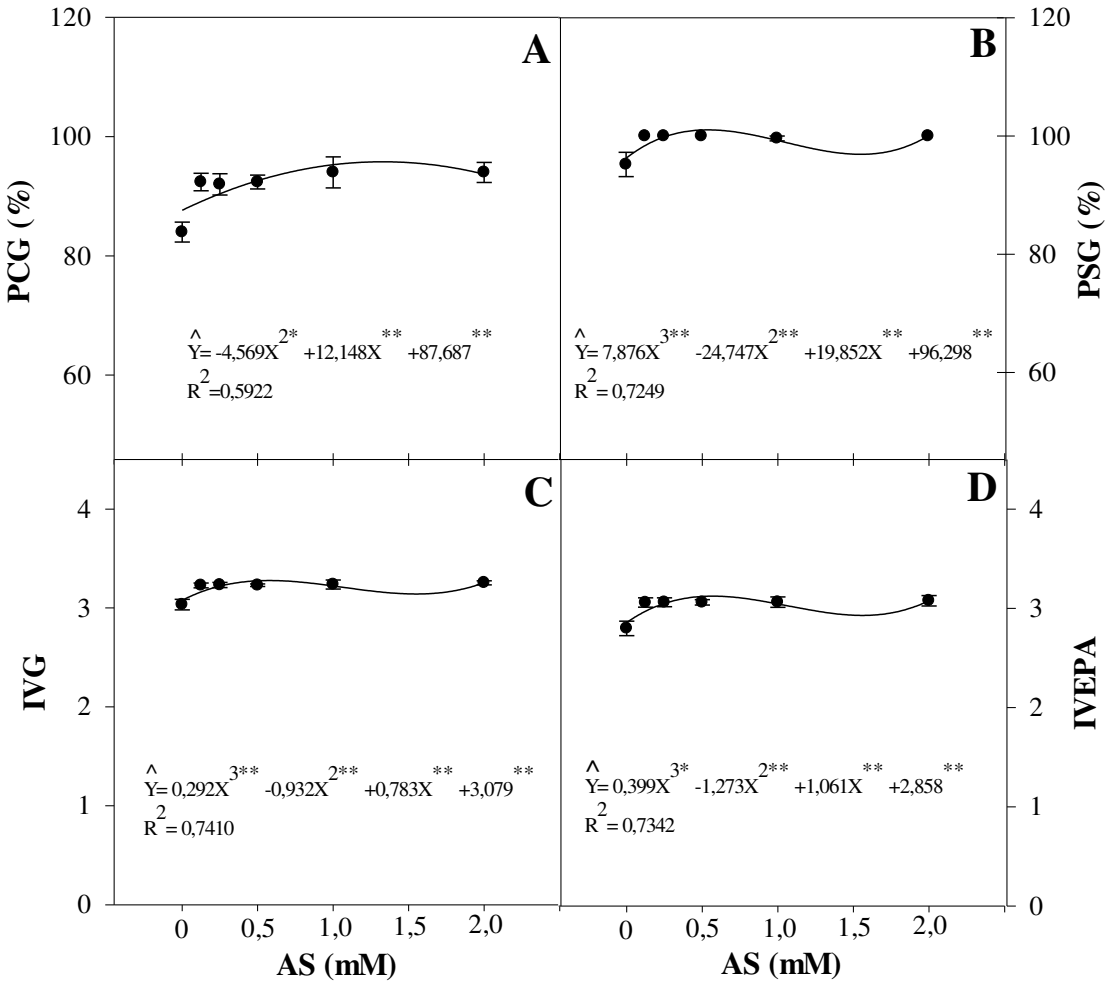


Figura 3 – Primeira contagem de germinação (PCG, **A**), percentagem de sementes germinadas (PSG, **B**), índice de velocidade de germinação (IVG, **C**) e índice de velocidade de emissão de parte aérea (IVEPA, **D**) de sementes de arroz (*Oryza sativa* L.), cv. SCSBRS 113, condicionadas com diferentes concentrações de ácido salicílico (AS) e semeadas em condições de estresse salino (NaCl a 80 mM). Os pontos das regressões representam as médias de cinco repetições ± erro padrão; já as análises estatísticas foram representadas por * significativo com $p < 0,05$ e ** significativo com $p < 0,01$.

Com base nas análises dos parâmetros de crescimento, foi observado que, ao décimo dia após a semeadura em NaCl a 80 mM, as plântulas provenientes de sementes condicionadas com concentrações crescentes de AS apresentaram diferenças estatísticas significativas, a nível de 1% ($p < 0,01$), para todas as variáveis examinadas (Tabela 2).

Tabela 2 – Resumo do quadro de ANOVA para as variáveis de crescimento: massa fresca da parte aérea (MFPA), massa seca da parte aérea (MSPA), massa fresca das raízes (MFR), massa seca das raízes (MSR); comprimento da parte aérea (CPA) e comprimento da raiz principal (CR) de plântulas oriundas de sementes de arroz (*Oryza sativa* L.), cv. SCSBRS 113, condicionadas com concentrações crescentes de ácido salicílico e semeadas em condições de estresse salino por 10 dias.

		Quadrado Médio					
Fonte de variação	G.L.	MFPA	MSPA	MFR	MSR	CPA	CR
Tratamento	5	0,024**	0,0004**	0,038**	0,0006**	0,764**	8,40**
Erro	20	0,004	0,0001	0,005	0,00005	0,11	1,08
C.V (%)		9,54	9,88	17,14	7,21	6,75	8,89

** Significativo com p < 0,01.

De acordo com a análise de regressão, a variável massa fresca da parte aérea (MFPA) apresentou comportamento quadrático, onde até o valor de 1,35 mM de AS, mostrou-se crescente, apresentando valor máximo estimado pela regressão de 0,78 g (Figura 4A). Por outro lado, os comportamentos das massas seca da parte aérea (MSPA) e fresca das raízes (MFR), foram mais bem explicados por meio de regressões lineares, que mostraram que cada acréscimo de uma unidade de concentração (mM) de AS gera adições nessas massas de 0,011 g e 0,109 g, respectivamente, evidenciado pelas respectivas tangentes das retas de regressão (Figuras 4B, C). A variável massa seca das raízes (MSR) apresentou curva de regressão cúbica, com comportamento crescente até aproximadamente 0,6 mM de AS (em que MSR = 0,10 g), quando a partir daí decresceu até cerca de 1,4 mM, para a partir daí começar a crescer, chegando na concentração de 2,0 mM de AS ao valor estimado pela regressão de 0,12 g (Figura 4D).

Semelhantemente, a variável comprimento da parte aérea (CPA) também apresentou um comportamento cúbico, sendo crescente até aproximadamente 0,7 mM de AS (CPA = 5,2 cm), seguido de leve decréscimo até cerca da concentração de 1,5 mM de AS, quando começou a crescer chegando na concentração de 2,0 mM de AS ao valor estimado (e que foi igual ao experimental) de 5,5 cm (Figura 4E). Já o comprimento da raiz principal (CR) apresentou curva de regressão linear, em que cada acréscimo de uma unidade de concentração (mM) de AS, gera o acréscimo de 1,6 cm no comprimento da raiz (Figura 4F).

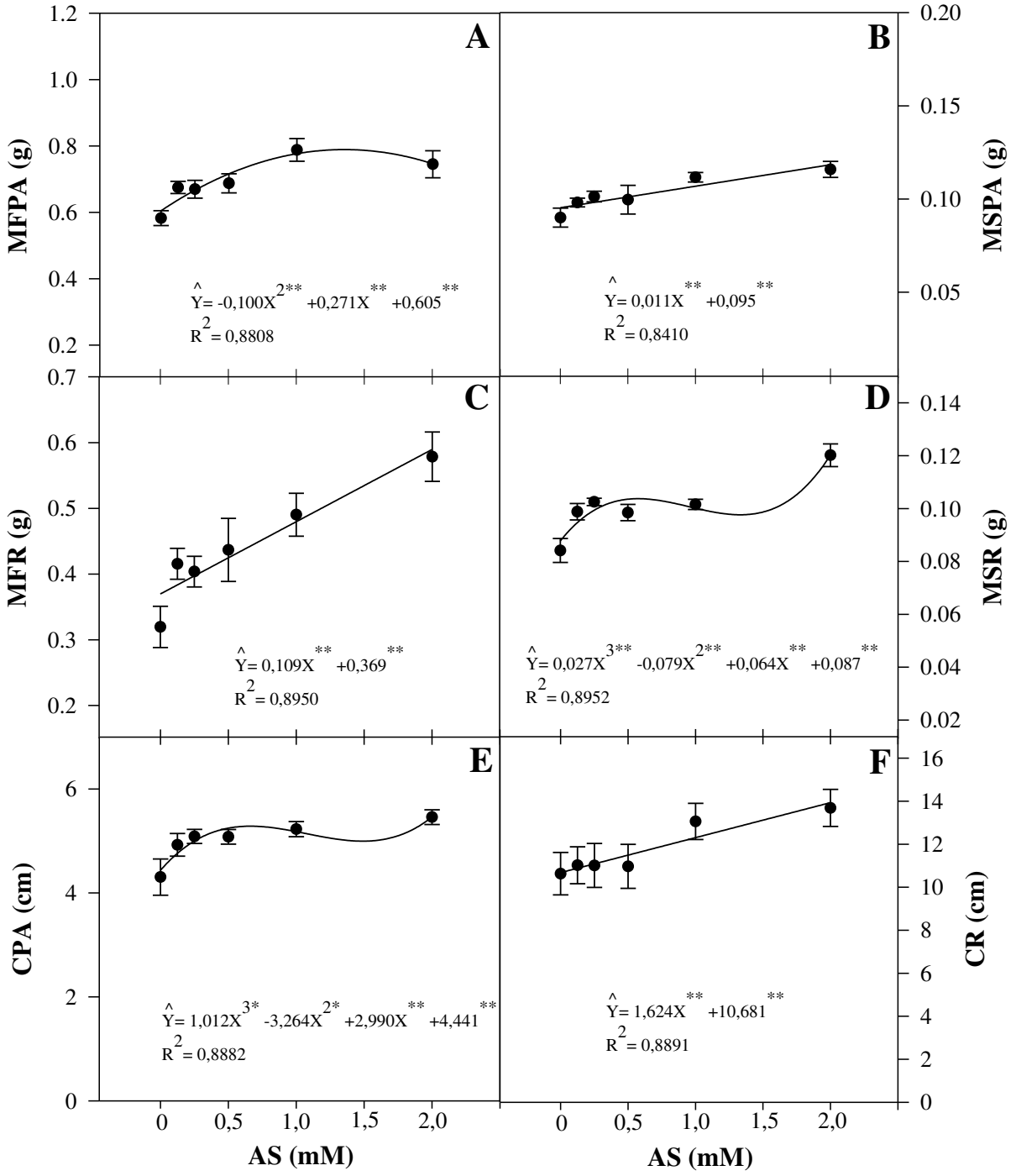


Figura 4 – Massa fresca da parte aérea (**A**), massa seca da parte aérea (**B**), massa fresca das raízes (**C**), massa seca das raízes (**D**), comprimento da parte aérea (**E**) e comprimento da raiz principal (**F**) de plântulas de arroz (*Oryza sativa* L.), cv. SCSBRS 113, oriundas de sementes condicionadas com diferentes concentrações de ácido salicílico (AS) e semeadas em condições de estresse salino (NaCl a 80 mM). Os pontos das regressões representam as médias de cinco repetições ± erro padrão; já as análises estatísticas foram representadas por * significativo com $p < 0,05$ e ** significativo com $p < 0,01$.

Em resumo, o priming com AS foi capaz de atenuar efeitos deletérios gerados pela salinidade, principalmente em maiores concentrações, a ponto de permitir que houvesse um aumento nos comprimentos da parte aérea e da raiz das plântulas de arroz, como pode ser visto na foto da figura 5.

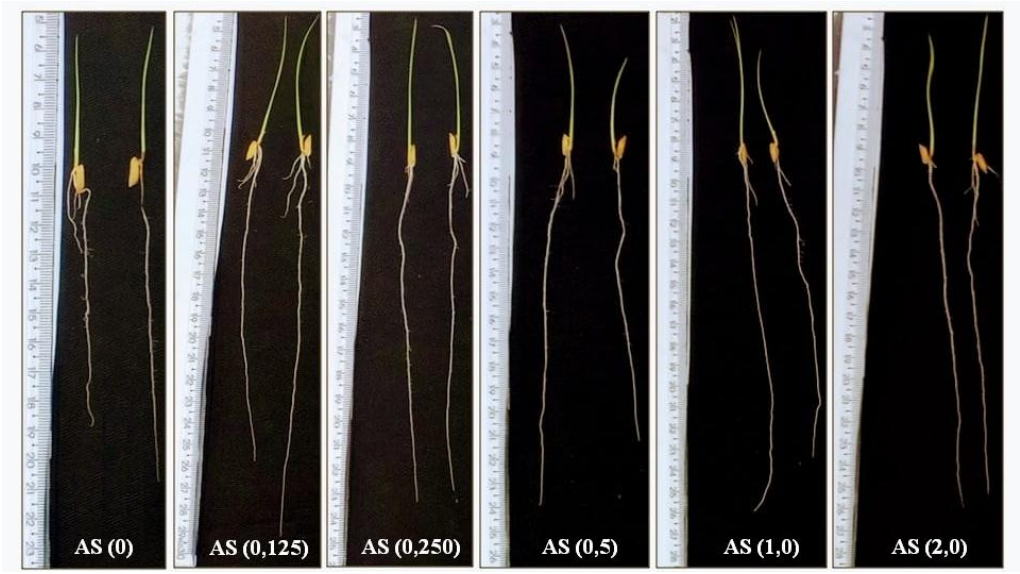


Figura 5 – Plântulas de arroz (*Oryza sativa* L.), cv. SCSBRS 113, provenientes de sementes submetidas ao condicionamento fisiológico com seis concentrações de ácido salicílico (AS): 0; 0,125; 0,250; 0,5; 1,0 e 2,0 mM e cultivadas em presença de NaCl a 80 mM.

6.2 Discussão

Os resultados do *screening* realizado com as seis concentrações de AS (0, 0,125, 0,25, 0,5, 1,0 e 2,0 mM), empregadas como *priming* das sementes de arroz, mostraram, com relação aos parâmetros de germinação, efeito positivo (exceto para o TMG), porém os aumentos causados por essas concentrações em cada uma das variáveis analisadas foram relativamente pequenos (Figura 3). Das concentrações testadas de AS, no *priming* das sementes de arroz, a que promoveu efeito pouco melhor que as outras nos parâmetros de germinação (exceção da PCG) foi a de 2,0 mM. No caso da PCG, o valor máximo previsto pela equação de regressão se dá a 1,33 mM de AS (Figura 3A), o que corresponde ao valor de 95,9% para essa variável, portanto, apenas um pouco superior ao valor estimado de 93,7% na concentração de 2,0 mM de AS.

O *priming* de sementes com AS tem sido usado com sucesso, promovendo melhor germinação em algumas espécies, tais como o trigo (ALAMRI *et al.,* 2018), o bálsamo (SHAIKH-ABOL-HASANI; ROSHANDEL, 2019), a cevada (EL-TAYEB, 2005), o tomate (JAVAHERI *et al.,* 2012) e o arroz (JINI; JOSEPH, 2017; SHETEIWY *et al.,* 2018), sob

condições de salinidade. Essa melhoria pode estar relacionada à ativação da síntese de enzimas antioxidantes e de proteínas de armazenamento das sementes (HONGNAA *et al.,* 2021), associada à expressão de genes-chave envolvidos na biossíntese de giberelina (GA) e na regulação negativa daqueles envolvidos na biossíntese de ácido abscísico (ABA), resultando em um equilíbrio hormonal favorável entre GA e ABA (Liu *et al.,* 2019).

Com relação aos parâmetros morfológicos, os *primings* com AS nas diversas concentrações promoveram aumentos nos comprimentos da parte aérea e da raíz principal, bem como nos valores de massas secas e frescas da parte aérea e raízes (Figura 4). Esses resultados são corroborados com dados obtidos por Jayakannan *et al.* (2013), que relataram que o tratamento com AS também aumentou os pesos fresco e seco de plântulas de *Arabidopsis* sob condições de salinidade. Com relação a esses parâmetros de crescimento (Figura 4), novamente a dose de 2,0 mM se destacou como a mais adequada entre as doses testadas para aumento da tolerância ao estresse salino, exceto para o caso da MFPA, cujo valor máximo estimado, pela curva de regressão dos dados, se dá na concentração de 1,35 mM de AS. Ressalte-se, no entanto, o valor de MFPA nesse ponto é ligeiramente superior ao encontrado na dose de 2,0 mM.

Esse aumento da tolerância pelo priming das sementes pode estar relacionado à regulação de processos fisiológicos importantes, como o metabolismo do nitrogênio e o da prolina (SHETEIWY *et al.,* 2019), ao acúmulo de outros osmorreguladores, como a glicina, as betaínas e os carboidratos solúveis (MAIA JÚNIOR *et al.,* 2020), bem como à síntese induzida de proteínas quinases, que desempenham papéis importantes na regulação da divisão celular, diferenciação e morfogênese (DOLATABADIAN *et al.,* 2009).

6.3 Conclusões

Com base nos resultados de germinação e de desempenho geral das plântulas de arroz cultivadas sob salinidade (NaCl a 80 mM), foi possível concluir que o condicionamento fisiológico das sementes com AS nas várias concentrações (selecionadas com bases na literatura), melhorou os principais parâmetros ligados à germinação e ao crescimento. Contudo, a atenuação dos efeitos deletérios da salinidade ocorreu de forma mais evidente nas plântulas provenientes de sementes submetidas aos *primings* com as maiores concentrações de AS testadas. Pelo fato da concentração de 2,0 mM de AS ter sido a que promoveu os maiores valores nas variáveis estudadas, principalmente nas de crescimento das plântulas, essa concentração foi a selecionada para as próximas etapas propostas nesta pesquisa.

7. EXPERIMENT II: SALYCILIC ACID SEED PRIMING MITIGATES THE EFFECTS OF SALT STRESS ON RICE SEEDLINGS BY MODULATING METABOLITES AND PREVENTING IONIC, OSMOTIC AND OXIDATIVE DAMAGE

ABSTRACT

Salinity is one of the abiotic stresses that most affect the metabolism and development of plants, especially in the germination, seedling establishment, and growth stages. One of the crops directly affected by salt excess in the soil or irrigation water is rice, one of the most consumed grasses in the world, which justifies the need to improve techniques to increase its tolerance. Based on the literature, which points to salicylic acid (SA) as an inducer of stress tolerance in various crops, this study tested the hypothesis that seed priming with SA is capable of mitigating salt toxicity during the establishment of rice seedlings (*Oryza sativa* L.), cv SCSBRS 113, and that this is due to metabolic changes favorable to adjustments in osmotic, ionic and redox homeostasis. The experiment was conducted in a completely randomized design with four treatments, consisting of two concentrations of SA (0 and 2.0 mM) and two of NaCl (0 and 80 mM). The results showed that salinity increased ionic and redox toxicity; however, the seedlings that had their seeds primed with SA increased the K^+/Na^+ ratio, the antioxidant response of the enzyme catalase, and the activity of the enzyme phenylalanine ammonia-lyase. On the other hand, osmotic potential, electrolyte leakage, and malondialdehyde and hydrogen peroxide (H_2O_2) content were lower in these seedlings. Moreover, the endogenous content of SA, determined by GC/MS, increased in seedlings preconditioned with SA. On the other hand, metabolic profile analysis revealed that SA primed seeds contributed to an increase in amino acids, soluble sugars, organic acids, and other chemical classes of metabolites in the seedlings. The increased contents of proline and asparagine may suggest a potential role osmotic adjustment and in the toxicity generated by salinity. There was also evidence of induction in the phenylpropanoids such as quinic acid, caffeic acid, and quercetin, as well as in ascorbic acid, important regulators of the redox state of plants. Taken together, the results suggest that SA-primed seed was effective in mitigating the deleterious effects of salt stress, inducing favorable metabolic modulations, and improved ionic, osmotic, and redox homeostasis in rice seedlings.

Keywords: salt stress; germination; priming, salicylic acid, metabolomics, *Oryza sativa* L.

7.1 Introduction

Plants are constantly subjected to various environmental stresses. One of the main ones being soil or irrigation water salinity, which occurs mainly in arid and semi-arid regions (SULEYMANOV *et al.,* 2023). It is estimated that more than 800 million hectares worldwide are affected by salinity, but it is predicted that this condition will increase by 2050 and that, for example, more than half of the land used to grow arable crops will also be affected (PINCAY; CANTOS, 2024). In regions such as the Brazilian Northeast, due to the occurrence of high temperatures and the irregular rainfall distribution associated with intense evaporation throughout the year, it is common for toxic ions (Na^+ and Cl^-) to accumulate in the soil solution, which impairs the productive performance of plants (LIMA *et al.,* 2023).

Among abiotic stresses, an excess of salts in the soil is one of the most damaging, as it lowers the soil's osmotic potential, causing a reduction in water absorption by the roots, which makes it difficult for plants to absorb water (DIAS *et al.,* 2016; OUAHZIZI *et al.,* 2023; PACHEPSKY *et al.,* 2024). This osmotic effect can also decrease the relative water content of plants, mainly due to the reduction in cytoplasmic volume and the loss of cell turgor as a consequence of the outflow of intracellular water (JUMPA *et al.,* 2023). In addition, when ions are absorbed in excess by plants (mainly Na^+ and Cl^- ions), they cause toxicity and impair the absorption of other ions, generating a nutritional imbalance (DWININGSIH *et al.,* 2021; CHAUDHARY *et al.,* 2024). In addition, they damage cell membranes, causing electrolyte leakage (KAYA *et al.,* 2024), thereby compromising fundamental metabolic processes such as protein and lipid synthesis (KHARE *et al.,* 2020; MISRA *et al.,* 2021). They also generate an accumulation of reactive oxygen species (ROS), such as the superoxide radical ($^{\bullet}O_2$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$), inducing secondary oxidative stress (AZEEM *et al.,* 2023). Finally, an excess of absorbed ions compromises the photosynthetic process, as they not only reduce stomata opening and pigment levels but also cause severe damage to photosystems, electron transport, and the structure of chloroplasts (STEFANOV *et al.,* 2023).

It is noteworthy that plants higher salinity tolerance manage to minimize the harmful effects of excess ions, especially sodium ions, through diverse mechanisms, including efflux of Na^+ back into the growth medium or into the apoplast; the compartmentalization of Na^+ in the vacuole; xylem loading control; the retention of Na^+ in stem cells; the recirculation of Na^+ through the phloem; the allocation of salts to older leaves; the excretion of Na^+ onto the leaf

surface (YAMAGUCHI *et al*., 2013). Moreover, the synthesis of osmoprotectants, such as soluble sugars, polyols, and amino acids and its accumulation in the cytosol leads to the regulation of the osmotic and water potential of the roots, maintaining the water content of the tissues and preventing damage to gas exchange (FAN *et al.,* 2016). Additionally, these plants fight oxidative damage generated by ROS mainly using an internal defense system made up of enzymatic antioxidants, such as the enzymes superoxide dismutase, catalase, ascorbate peroxidase, among others, as well as non-enzymatic antioxidants, such as ascorbate, glutathione, phenolic acids, flavonoids, carotenoids and α-tocopherol (RAI *et al.,* 2023; CHAUDHARY *et al.,* 2024).

One of the significant grain crops that have been affected by excess salts in the soil or irrigation water is rice (*Oryza sativa* L.), a cereal that is part of the diet of more than half of the world's population (SINGH *et al.,* 2019), and is rich in carbohydrates, proteins, fibers, minerals, and vitamins (GUPTA *et al.,* 2018). Due to its high sensitivity to salinity, several studies have been carried out to develop techniques capable of mitigating the deleterious effects of this form of stress on rice cultivation, ensuring its productivity in a sustainable manner (GANIE *et al.,* 2019).

Among these techniques, seed priming consists of previous exposure to conditioning agents, employed in low concentrations to induce mild stress capable of acclimatizing plant cells to handle subsequent unfavorable situations (HASAN *et al.,* 2016). This conditioning is responsible for the pre-occurrence of metabolic events and the activation of a stress-responsive system capable of improving germination and seedling development (EL-HAWARY; HASHEM; HASANUZZAMAN 2023). It is feasible because plants can create a "stress memory" or "stress imprinting" that helps boost defense mechanisms (WILLADINO *et al.,* 2016).

Alternatively, diverse organic compounds have been reported as priming agents to induce defensive responses in plants against biotic or abiotic stresses. It is the case, for example, with SA, a well-known mediator of processes such as photosynthesis, regulation of stomatal opening, absorption and transportation of mineral nutrients (AMANY; IBRAHIM, 2015), and can also act to increase the antioxidant capacity of cells, reducing the damage caused by ROS (KHAN *et al.,* 2015).

In order to investigate whether SA seed priming is able to mitigate the harmful effects of salinity on rice seedlings (*Oryza sativa*), some studies focusing on germination and growth processes (JINI; JOSEPH, 2017), as well as gas exchange and chlorophyll fluorescence, as well as on antioxidant activities (SHETEIWY *et al.,* 2018), have been carried out so far. On

the other hand, our work sought not only to clarify the biochemical and physiological parameters linked previous exposition of exogenous SA in seeds but mainly to relate them to the metabolic profile analysis of rice seedlings grown under salinity to following a better understand of their osmotic, ionic and redox homeostasis maintenance process.

7.2 Materials and Methods

7.2.1 *Seeds and place of experiment*

The experiment used rice seeds (*Oryza sativa* L.), cv SCSBRS 113, supplied by the Brazilian Company AgroGiust – SC. The analyses were carried out at the Plant Physiology Laboratory of the Biochemistry and Molecular Biology Department of the Federal University of Ceará (UFC), Fortaleza, Ceará, Brazil.

7.2.2 *Treatments and sowing*

The rice seeds were sanitized with 1% (v/v) sodium hypochlorite and then primed with 2.0 mM SA or distilled water. The seeds were soaked in a 2.0 mM AS solution or in distilled water for 24 h at 25 ºC, then transferred to a dry clean paper where they were left for 24 hours in the open air to lose the absorbed moisture that could cause them to germinate (emit the radicle) during priming. The AS concentration of 2.0 mM was selected from a preliminary screening experiment, conducted with six SA concentrations.

Subsequently, 50 seeds from each priming treatment were sown on two sheets of germitest paper (28 x 38 cm) and covered with another sheet, both previously autoclaved at 120 °C and moistened with 40 mL of distilled water or 40 mL of 80 mM NaCl solution. The paper sheets with the seeds were rolled into straw shapes and stored in plastic containers (5 rolls/container) containing 20 mL of distilled water or 20 mL of 80 mM NaCl solution, which were covered with plastic bags to maintain humidity. The plastic containers containing the rolls of paper with the seeds were then kept in a BOD chamber during the day and night, with a 12 h photoperiod and an average temperature of 25ºC. After 10 d of cultivation, the seedlings were collected, immediately used or frozen in liquid nitrogen and stored at -80 °C until processing. The experiment was conducted with five replicates composed by 50 seeds each.

7.2.3 *Relative water content and osmotic potential*

Freshly collected samples of the shoot of rice seedlings from seeds pre-treated with distilled water or 2.0 mM SA after 10 d after sowing under control or salt stress conditions (80 mM NaCl) were taken and weighed to determine the fresh mass (FM). The samples were immersed in containers of deionized water at room temperature, in which they remained until completely hydrated, so that a turgid mass (TM) was obtained. They were then dried in an oven at 60 °C for 72 h to determine their dry mass (DM). The relative water content (RWC) of the shoot was determined according to Čatský (1960), based on the formula: RWC (%) = $[(FM - DM) (TM - DM)^{-1}] \times 100$.

To determine the osmotic potential (Ψs), samples of the shoot (about 0.1 g) were weighed, then frozen in liquid nitrogen and stored at -25 °C until analysis. Measurements were made on an osmometer (VAPRO – Vapor Pressure Osmometer, mod. 5600, Wescor, Utah, USA), using clear leaf juice obtained by pressing the tissues with an injection plunger and then centrifuging at 6,000 × g for 5 min at 4 ºC. The Ψs values were estimated using the Van't Hoff equation: Ψs = - R T C, where R is the universal gas constant (0.00831 kg MPa mol^{-1} K^{-1}), T is the absolute temperature (298 K) and C is the solute concentration, in mol kg^{-1}, obtained using an osmometer (BAO *et al.*, 2014). The results were expressed in MPa.

7.2.4 *Membrane damage: electrolyte leakage and lipid peroxidation*

Electrolyte leakage was assessed after incubating freshly collected tissue from the shoot of rice seedlings in deionized water at 25 °C for 6 h. After this period, the initial electrical conductivity (EC1) of the medium was measured using a conductivity meter (Micronal, model AJX-515), and then the samples were incubated in a water bath at 100 °C for 30 min. After reaching room temperature again, the final electrical conductivity (EC2) was measured. Electrolyte leakage was estimated using the following equation: Electrolyte leakage (%) = (EC1/EC2) × 100 (SINGH *et al.*, 2007).

Membrane lipid peroxidation was determined using the thiobarbituric acid reactive substances (TBARS) test, according to Heath and Packer (1968). The extract used in this determination was obtained after pulverizing the fresh tissue of the shoot of the rice seedlings with liquid nitrogen, followed by maceration at 4 °C with 5% trichloroacetic acid. The homogenate was then centrifuged at 12,000 × g for 15 min at 4 °C. The supernatant was then homogenized in a solution composed of 0.5% thiobarbituric acid and 20% trichloacetic acid. After the mixture was kept in a water bath at 100 °C for 30 min and cooled in an ice bath, the

TBARS content was estimated by subtracting the absorbance readings at 532 nm and 600 nm, based on the molar extinction coefficient of malondialdehyde (MDA, ε = 155 mM^{-1} cm^{-1})**.** The results were expressed in nmol g^{-1} MDA FM.

7.2.5 *Photosynthetic pigments: Chlorophylls and carotenoids*

Freshly collected tissue samples (around 50 mg) from the shoot of rice seedlings 10 d after sowing were immersed in 2.0 mL of a solution of dimethyl sulfoxide (DMSO) saturated with calcium carbonate ($CaCO_3$) in tubes lined with aluminum foil for 48 h. The samples were then incubated in a water bath at 65 °C for 45 min. From the pigment extract, the absorbance readings at 665, 649, and 480 nm were determined and the pigment content was estimated according to Wellburn (1994) and the results were expressed in mg g^{-1} FM.

7.2.6 *Inorganic solute content*

Inorganic ions (Na^+ and K^+) were extracted from root and shoot tissues, dried in an oven at 60 ºC for 72 h, according to Cataldo *et al.* (1975), with minor adaptations. Samples of 50 mg of the dried plant material were incubated with distilled water in a water bath at 45 °C for 1 h. The suspended material was centrifuged at 5.000 × g for 15 min and the supernatant was filtered using filter paper. Na^+ and K^+ ions were determined by flame photometry (Micronal®, model B462, Brazil), according to Malavolta *et al.* (1989). The results were expressed in µmol Na^+, K^+ g^{-1} DM.

7.2.7 *Hydrogen peroxide content*

The extracts for the determination of hydrogen peroxide (H_2O_2) were prepared according to the methodology described by Cheeseman (2006), with modifications. Initially, about 0.5 g of fresh shoot tissue was frozen in liquid nitrogen, pulverized and homogenized with 100 mM potassium phosphate buffer solution, pH 6.3, at 4 ºC. The homogenate was then centrifuged at 12,000 × g for 15 min at 4 °C, the supernatant was collected and the H_2O_2 content was determined as described by Fernando and Soysa (2015), with some modifications. The reaction medium for H_2O_2 determination consisted of an aliquot of plant extract, phenol at 12 mM, 4-amino antipyrine at 0.5 mM, potassium phosphate buffer at 84 mM (pH 7.0), and HRP (horseradish peroxidase) at 1 U mL^{-1}. The mixture was stirred and placed in a water bath at 37 ^oC for 30 min. The H_2O_2 content was determined through absorbance readings at 505 nm, using a standard curve obtained from increasing concentrations of H_2O_2 as a reference. The results were expressed in µmol g^{-1} FM.

7.2.8 *Catalase and phenylalanine ammonia-lyase enzyme activity*

The extract for determining the activity of the enzyme catalase (CAT, EC 1.11.1.6) was obtained by macerating root and shoot tissues (frozen at -80 ^{o}C) with 100 mM potassium phosphate buffer solution, pH 7.0, containing 0.1 mM EDTA. CAT activity was determined according to Havir and McHale (1987), through the decrease in absorbance at 240 nm, due to the consumption of H_2O_2, and using its molar extinction coefficient (ε = 36 M^{-1} cm^{-1}). The results were expressed in µmol min^{-1} mg^{-1} of protein.

To determine phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) enzyme activity, the methodology proposed by Mehta and Bhavnarayana (1981) was used, with minor adaptations. For extraction, 100 mg of shoot material and roots (frozen -80 ºC) were macerated with 2 mL of 50 mM sodium phosphate buffer, pH 6.5. The homogenate was then centrifuged at 12,000 g at 4 ºC for 20 min and the supernatant was used as an enzyme extract. PAL activity was determined by adding 150 µL of the extract to 1 mL of the reaction medium (25 mM Tris-HCl, pH 8.8, containing 25 mM L-phenylalanine). The reaction took place for 1 h at 37 ºC and was stopped by adding 60 µL of 6 N HCl. The reaction product (trans-cinnamic acid) was determined by reading the absorbance at 290 nm. The molar extinction coefficient of trans-cinnamic acid (104 mM^{-1} cm^{-1}) was used to calculate the activity. The results were expressed in µmol min^{-1} mg^{-1} of protein.

7.2.9 *Metabolic profile analysis*

The metabolites were extracted and derivatized according to the methodology described by Lisec *et al.* (2006). Samples of shoots and roots, frozen in liquid nitrogen, were ground separately and converted into powder. The extracts were prepared from 50 mg of the powder of these samples, which were homogenized in methanol at 70 °C for 15 min with constant stirring. In each sample, 0.2 mg mL^{-1} of ribitol was added as an internal standard. After stirring in a Thermomix for 15 min at 70 ºC (350 rpm), the homogenate was centrifuged at 12,000 × g for 10 min at 4 ºC. A chloroform/water solution (1:2 v/v) was added to the supernatant, followed by stirring in a vortex for 15 s. After centrifugation at 3.000 × g for 15 min, 150 µL aliquots of the upper phase (polar phase) were collected and dried in SpeedVac overnight. The derivatization process of the dry extract was initiated by reaction with methoxyamine (20 mg mL^{-1} in pyridine) at 37 °C for 2 h, followed by 35µL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) at 37 °C for 30 min.

The metabolites were separated and detected according to Roessner *et al.* (2001) with modifications, using a gas chromatograph coupled to a mass spectrometer (GC/MS, QP-PLUS 2010, Shimadzu, Japão), in which 1.0 µL of the derivatized material was injected in split mode 5. Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1}. The RTX-5MS capillary column (30 m × 0.25 mm × 0.25 µm) was used to separate the metabolites. The chromatographic run was set up as follows: initial temperature of 80 °C for 2 min, followed by an increase at a rate of 10 °C min^{-1} until the final temperature of 315 °C was reached, which was maintained for 8 min. The injection, interface and ion source temperatures were 230, 250 and 200 °C, respectively.

The mass spectrometer was operated at 70 eV (EI), with a mass fragment detection range of 40-700 (m/z), starting after a time of 3 min. The chromatograms and mass spectra were analyzed using the XcaliburTM 2.1 program (Thermo Fisher Scientific, Waltham, MA, USA). The metabolites were identified on the basis of retention time and fragmentation spectrum and by comparison with an internal library constructed from a mixture of standards with their mass spectra previously identified in the Golm Metabolome Database (GMD). The relative concentration of each metabolite was determined and normalized based on ribitol, with subsequent correction for the mass of the sample used during extraction.

7.2.10 *Determination of endogenous salicylic acid*

The extraction and derivatization of SA in tissue samples from the shoot and roots of rice seedlings was carried out in the same way as described in section 7.2.9, according to the methodology described by Lisec *et al.* (2006). On this occasion, 1.0 µL samples of the derivatized extract were injected into the SIM (Selected Ion Monitoring) mode of the gas chromatograph coupled to mass spectrometry (GC/MS, QP-PLUS 2010, Shimadzu, Japan) so that the SA mass fragments, previously identified in the Golm Metabolome Database (GMD), could be monitored and their relative intensities calculated. The endogenous SA content of the samples was quantified according to the methodology described by Nehela *et al.* (2016) with minor adaptations. A standard curve was made using 150 µL aliquots of SA standards containing 25, 20, 10, 5, 2.5, 1.0, 0.5, 0.4, 0.2 and 0.1 ppm, which were dried in a SpeedVac, derivatized and injected into the SIM mode of the GC/MS. From the areas of the peaks obtained and the preparation of the standard curve with these ten SA concentrations, it was possible to determine the endogenous SA content in the samples, which was expressed in ng g^{-1} FM.

7.2.11 *Experimental design and data analysis*

The experiment was conducted in a completely randomized design, in the form of a 2 x 2 factorial, with two priming treatments (distilled water and SA at 2.0 mM) and two sowing conditions (in the absence or presence of 80 mM NaCl), with four replicates. As a result of this factorial, four treatments were established: 1. seeds conditioned with distilled water and seedlings grown in the absence of NaCl; 2. seeds conditioned with distilled water and seedlings grown in the presence of 80 mM NaCl; 3. seeds conditioned with SA at 2.0 mM and seedlings grown in the absence of NaCl; and 4. seeds conditioned with SA at 2.0 mM and seedlings grown in the presence of 80 mM NaCl. The results were submitted to analysis of variance (ANOVA) and, in the event of significant differences between the stress levels, the means were compared using the Tukey test ($p \leq 0.05$). Statistical analyses were carried out using SISVAR 5.3 software (FERREIRA, 2011). In order to analyze the metabolic profiles, the relative concentrations were processed in MetaboAnalyst 5.0. Principal Component Analysis (PCA) was carried out to identify the differences in metabolic composition between the four treatments. The relative values of the metabolites were also compared using the Tukey test ($p \leq 0.05$).

7.3 Results

7.3.1 *Relative water content and osmotic potential*

The relative water content (RWC) was not affected by salinity or the seed *priming* treatment; however, the interaction between these two factors was highly significant ($p < 0.01$) (Table S1). Salinity reduced the RWC of the shoot of the seedlings whose seeds were primed with distilled water by 13.5%, but in the seedlings from seeds pretreated with SA, the RWC was higher in those grown under salinity (an increase of 10.3%) (Figure 6A).

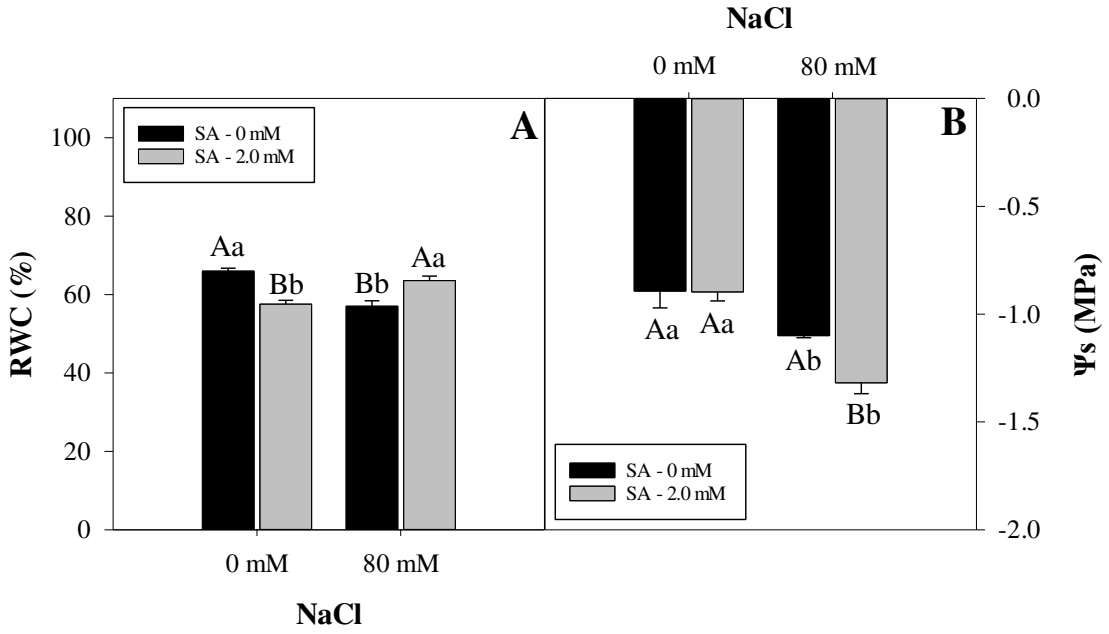


Figure 6 – Relative water content (RWC, **A**) and osmotic potential (Ψs, **B**) in the shoot of rice seedlings (Oryza sativa L.), cv SCSBRS 113, from seeds submitted to conditioning with distilled water (SA 0 mM) or salicylic acid (SA) at 2.0 mM and grown in the absence (NaCl 0 mM) or presence of NaCl at 80 mM. Capital letters compare the mean values between the priming treatments for the same saline condition and lowercase letters compare the means of the saline treatments within the same *priming*. Averages with different letters differ statistically by Tukey's test with $p < 0.05$. The values represent the averages of four repetitions + standard error.

Ψs was significantly affected by both salinity and seed priming (both $p < 0.01$) and there was an interaction between the factors ($p < 0.05$) (Table S1). Considering seedlings grown in the absence of salinity, regardless of priming, whether with distilled water (SA 0 mM) or SA 2 mM, Ψs the values were similar. However, in seedlings grown under salinity, Ψs was lower (19.8% reduction) in those from seeds pretreated with SA 2.0 mM (Figure 6B). Considering seeds pretreated with distilled water (SA 0 mM), Ψs was lower in seedlings under salinity. Similarly, in seedlings from seeds primed with SA 2.0 mM, Ψs was lower (46.9% reduction) in seedlings grown in the presence of NaCl 80 mM (Figure 6B).

7.3.2 *Membrane damage: electrolyte leakage and lipid peroxidation*

According to the analysis of variance, membrane damage in the shoot tissues of rice seedlings, estimated by electrolyte leakage and MDA levels, was significantly affected by the salinity and seed pretreatment treatments, and there were highly significant interactions between the factors for both variables ($p < 0.01$) (Table S1).

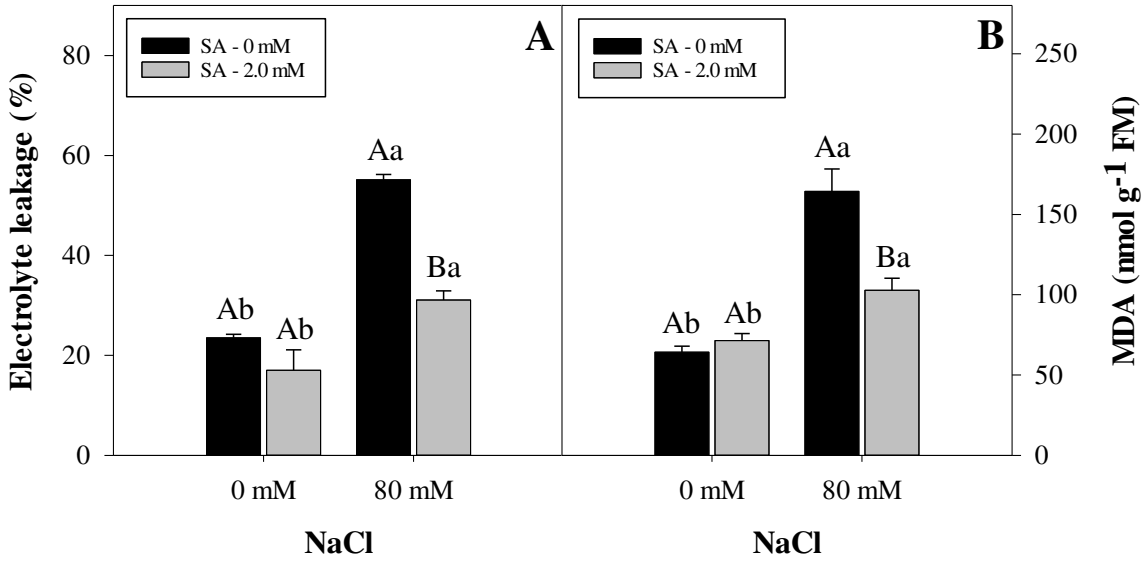


Figure 7 – Percentage of electrolyte leakage (**A**) and malondialdehyde levels (MDA, **B**) in the shoot of rice seedlings (*Oryza sativa* L.), cv SCSBRS 113, from seeds subjected to physiological conditioning with distilled water (SA 0 mM) or salicylic acid (SA) at 2.0 mM and grown in the absence (NaCl 0 mM) or presence of NaCl 80 mM. Capital letters compare the mean values between the priming treatments for the same saline condition and lowercase letters compare the means of the saline treatments within the same priming. Averages with different letters differ statistically by Tukey's test with $p < 0.05$. The values represent the averages of four repetitions + standard error.

Salinity significantly increased the percentage of electrolytes released by the shoot tissues. When comparing seedlings from primed seeds with distilled water, electrolyte leakage in the shoot of those grown under saline stress was 134.7% greater than those grown in the absence of salinity (Figure 7A). Electrolyte leakage was also higher in plants from seeds primed with SA that were grown under salinity compared to those grown in the absence of salinity. However, it is worth noting that the electrolyte leakage values of seedlings from seeds pretreated with distilled water or SA and sown in the absence of salinity did not differ from each other. Seedlings grown under salinity from seeds pretreated with SA 2.0 mM had a 43.6% reduction in electrolyte leakage compared to those from seeds primed with distilled water (Figure 7A).

Salinity also significantly increased the MDA levels, possibly due to a process of membrane lipid peroxidation, whose behavior about the treatments was similar to that of electrolyte leakage (Figure 7). Priming with SA contributed to a 37.4% reduction in the MDA levels of seedlings grown under saline stress conditions compared to those of seeds pretreated with distilled water and grown under salinity (Figure 7B). With regard to the seedlings grown in the absence of salinity, the MDA levels did not differ, regardless of the physiological conditioning of the seeds, whether with distilled water or SA.

7.3.3 *Photosynthetic pigments: Chlorophylls and carotenoids*

Chlorophyll a and carotenoid contents were affected by both salinity and seed priming treatment and there was a significant interaction between the factors at the 1% probability level (Table S1). Conversely, chlorophyll *b* levels were not affected by any of the factors (average value of 6.5 mg g^{-1} FM), and there was no interaction between them, while total chlorophyll levels, although not influenced by any of the factors, showed a highly significant interaction between them ($p < 0.01$). Highlighting the action of priming seeds with SA in promoting an increase in pigment levels under saline conditions, the chlorophyll *a* and *total* chlorophyll contents of seedlings from seeds conditioned with this phytohormone and grown under 80 mM NaCl showed increases of 45.2% and 31.0%, respectively, compared to seedlings whose seeds primed with distilled water and which were also grown under salinity (Figure 8A, B). Under non-saline conditions, priming with SA did not affect *total* chlorophyll content, but contributed to a 14.2% reduction in chlorophyll *a* content. Carotenoids, on the other hand, behaved similarly to chlorophyll *a* and *total*, with their levels in seedlings pretreated with SA and grown under salt stress being 65.8% higher than those in seedlings from the same salt treatment but from pretreated seeds with distilled water (Figure 8C).

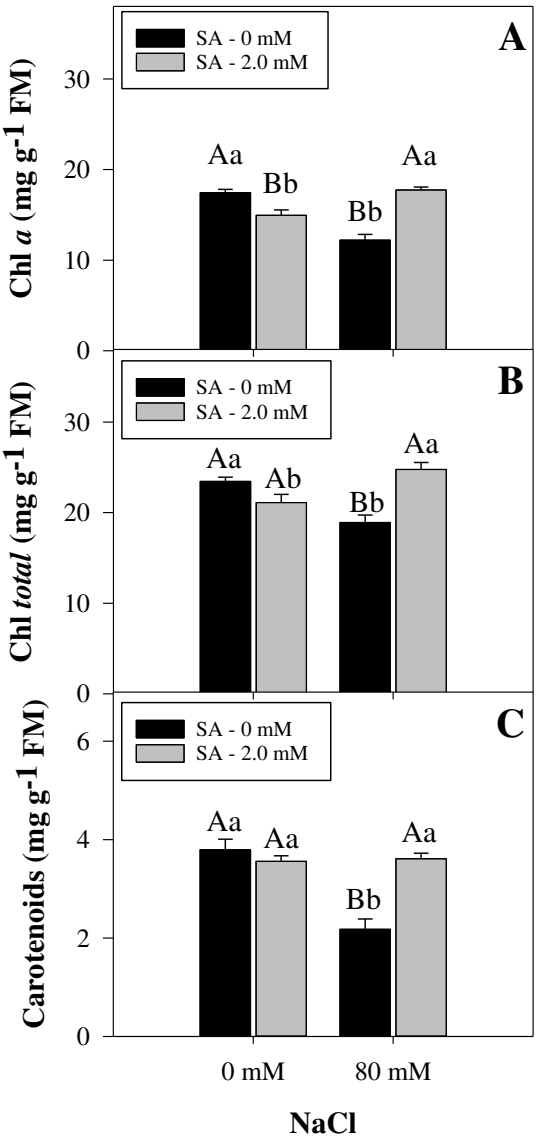


Figure 8 – Chlorophyll *a* (Chl *a*, **A**), total chlorophyll (Chl *total*, **B**) and carotenoid (C) contents of rice seedlings (*Oryza sativa* L.), cv SCSBRS 113, from seeds subjected to physiological conditioning with distilled water (SA 0 mM) or salicylic acid (SA) at 2.0 mM and grown in the absence (NaCl 0 mM) or presence of NaCl 80 mM. Capital letters compare the mean values between the priming treatments for the same saline condition and lowercase letters compare the means of the saline treatments within the same priming. Averages with different letters differ statistically by Tukey's test with $p < 0.05$. The values represent the averages of four repetitions + standard error.

7.3.4 *Inorganic solute content*

The Na^+ ion levels in the shoot and root tissues were significantly affected by the salinity and seed pretreatment treatments (in both tissues $p < 0.01$) and there was an interaction between the factors at the 5% level for the shoot tissues and at the 1% level for the roots (Table S1). The saline treatment strongly stimulated the increase in sodium ion levels, both in the shoot and in the roots (Figure 9). Considering only rice seedlings from seeds

pretreated with distilled water, the Na^+ contents of the shoot (Figure 9A) and roots (Figure 9B) were increased by 379.3% and 162.8%, respectively, in seedlings growing in the presence of 80 mM NaCl. It is worth noting that in the roots of seedlings from seeds grown in the absence of salinity, pretreatment with SA 2.0 mM also contributed to a reduction in the content of this ion compared to seedlings from seeds pretreated with distilled water. However, when comparing only seedlings grown under salt stress, priming the seeds with AS reduced the Na^+ content by 33.2% in the shoot and 42% in the roots (Figure 9).

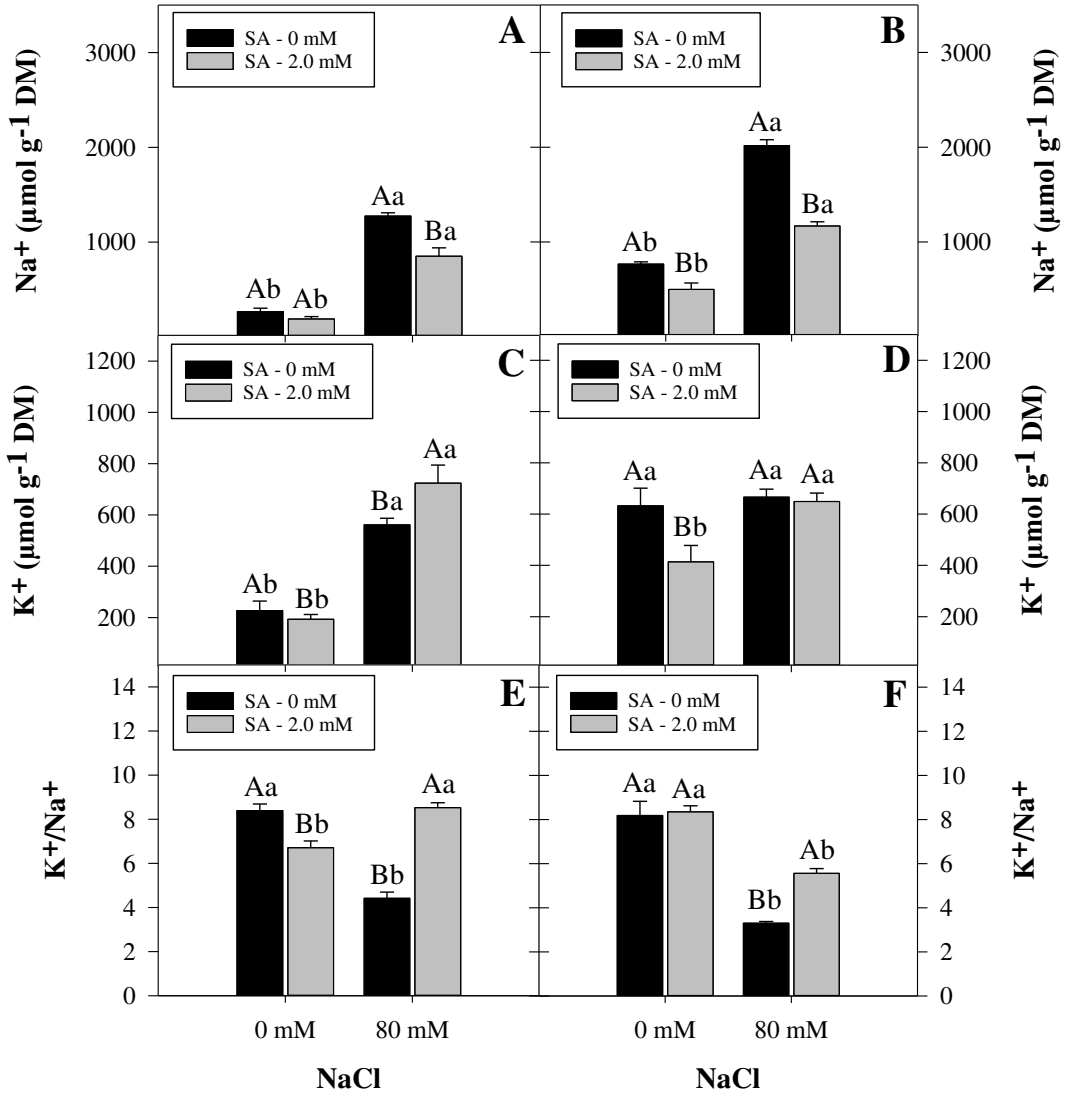


Figure 9 - Sodium ion (Na^+) content in the shoot (**A**) and roots (**B**); Potassium ion (K^+) content in the shoot (**C**) and roots (**D**) and K^+/Na^+ ratio in the shoot (**E**) and roots (**F**) of rice seedlings (*Oryza sativa* L.), cv SCSBRS 113, from seeds subjected to physiological conditioning with distilled water (SA 0 mM) or salicylic acid (SA) at 2.0 mM and grown in the absence (NaCl 0 mM) or presence of NaCl 80 mM. Capital letters compare the mean values between the priming treatments for the same saline condition and lowercase letters compare the means of the saline treatments within the same priming. Averages with different letters differ statistically by Tukey's test with $p< 0.05$. The values represent the averages of four repetitions + standard error.

In the shoot, K^+ content was affected by salinity ($p < 0.01$), but was not affected by seed priming, although there was a highly significant interaction between the variation factors (Table S1). In the roots, however, the levels of this ion were altered only by salinity ($p < 0.01$), but not by seed priming and there was no interaction between the two factors. In the seedlings grown in the presence of salinity, priming the seeds with distilled water and 2.0 mM SA resulted in an increase in K^+ content in the shoot of 147% and 275% compared to the respective control treatments (Figure 9C). As for the seedlings under no salt stress, the potassium content in the shoot was reduced by 15.0% when the seeds were pretreated with 2.0 mM SA compared to those pretreated with distilled water. In the roots, the pre-treatment of the seeds did not affect the potassium content of the seedlings under salinity, but in those grown in the absence of salinity, the K^+ content was 34.5% lower in those from seeds pretreated with SA 2.0 mM (Figure 9D).

In both the aerial and root tissues, the K^+/Na^+ ratio was significantly affected by salinity and seed pre-treatment ($p < 0.01$). In addition, there was an interaction between the factors at the 1% level in both tissues (Table S1). In the shoot of seedlings grown in the absence of salt stress, pretreatment with SA 2.0 mM was responsible for a 20% reduction in the K^+/Na^+ ratio compared to seedlings primed with distilled water (Figure 9E, F). On the other hand, priming these same tissues with SA contributed to a significantly higher K^+/Na^+ ratio (93.1% increase) under saline conditions compared to seedlings previously conditioned with distilled water only (Figure 9E). In the roots, the highest K^+/Na^+ ratios were found in seedlings not subjected to salinity (Figure 9F), although there was no statistical difference between the priming treatments in this growing condition. However, taking only salinity into account, it can be seen that the exogenous use of SA in the seed pre-treatment made a positive contribution to improving ionic adjustment by increasing the K^+/Na^+ ratio in the roots by 68.6% (Figure 9F).

7.3.5 *Hydrogen peroxide (H_2O_2) content*

The analysis of variance showed that the H_2O_2 content of the shoot tissues of rice seedlings was significantly affected by salinity and seed pre-treatment, and there was a highly significant interaction ($p < 0.01$) between these two factors (Table S1). However, in the roots, the content of this reactive oxygen species was altered only by salinity ($p < 0.01$), but not by seed priming and there was no interaction between the two factors. Considering seedlings grown in the absence of salinity, regardless of whether they were primed with distilled water or 2.0 mM SA, the H_2O_2 content was similar in the shoot and the roots (Figures 10A, B).

However, this content was considerably increased in the seedlings exposed to salinity, with an increase of 100.4% of this reactive oxygen species in the shoot and 104.6% in the roots, compared to the seedlings pretreated with distilled water alone. It is worth noting that this salinity-induced increase was reduced by 36.6% in the shoot of the seedlings previously primed with AS (Figure 10A).

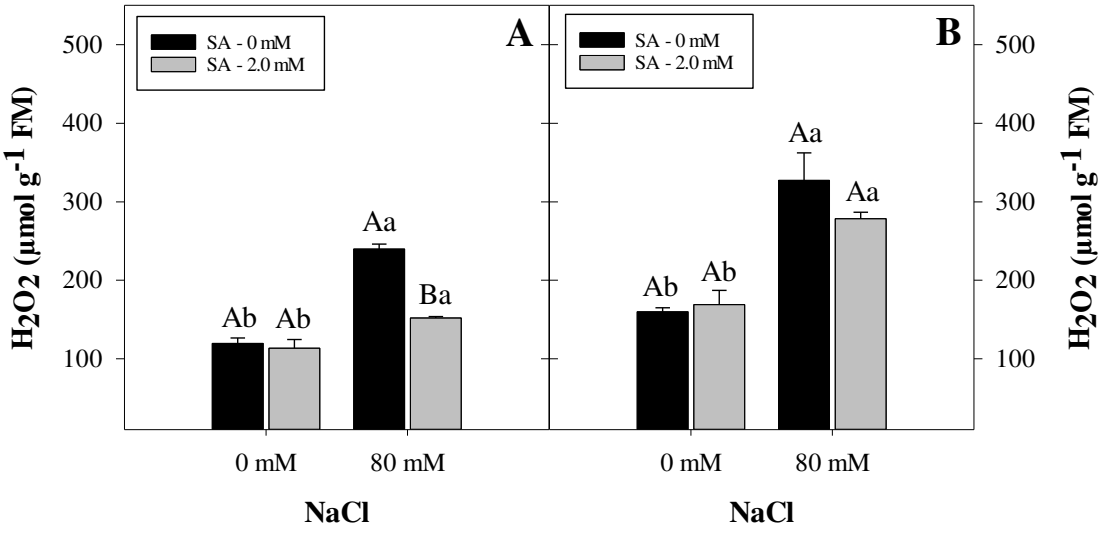


Figure 10 - Hydrogen peroxide (H_2O_2) content in the shoot (**A**) and roots (**B**) of rice seedlings (*Oryza sativa* L.), cv SCSBRS 113, from seeds subjected to physiological conditioning with distilled water (SA 0 mM) or salicylic acid (SA) at 2.0 mM and grown in the absence (NaCl 0 mM) or presence of NaCl 80 mM. Capital letters compare the mean values between the priming treatments for the same saline condition and lowercase letters compare the means of the saline treatments within the same priming. Averages with different letters differ statistically by Tukey's test with $p < 0.05$. The values represent the averages of four repetitions + standard error.

7.3.6 *Catalase and phenylalanine ammonia-lyase enzyme activity*

The activity of catalase (CAT) in the tissues of the shoot was significantly affected by the salinity treatments and the pretreatment of the seeds with SA ($p < 0.01$), but there was no interaction between the two factors (Table S1). Regarding the roots, there was a difference at the 1% level considering only the salt stress condition, but CAT activity was not influenced by the priming treatment nor there was any interaction between the factors. In the shoot of rice seedlings from primed seeds with 2.0 mM SA and grown under 80 mM NaCl, there was a 49.6% increase in catalase activity compared to seedlings primed with distilled water and also grown under salinity. In non-saline conditions, this increase was approximately the same (47.5%) (Figure 11A). In the roots, priming the seeds with SA 2.0 mM did not influence CAT activity compared to priming with distilled water, regardless of the growing medium, i.e.

whether in the presence or absence of salinity; however, CAT activity values were much higher under saline conditions (on average 86.3%) (Figure 11B).

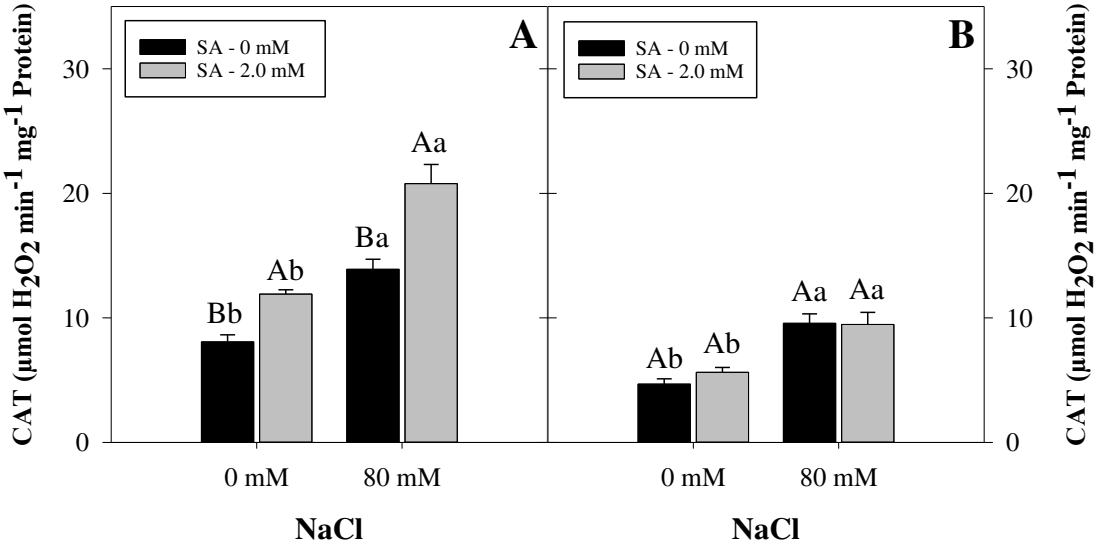


Figure 11 - Catalase (CAT) activity in the shoot (**A**) and roots (**B**) of rice seedlings (*Oryza sativa* L.), cv SCSBRS 113, from seeds subjected to physiological conditioning with distilled water (SA 0 mM) or salicylic acid (SA) at 2.0 mM and grown in the absence (NaCl 0 mM) or presence of NaCl 80 mM. Capital letters compare the mean values between the priming treatments for the same saline condition and lower case letters compare the means of the saline treatments within the same priming. Averages with different letters differ statistically by Tukey's test with $p < 0.05$. The values represent the averages of four repetitions + standard error.

The analysis of variance also showed that, just like the activity of CAT in the shoot, which was affected by the variation factors, salinity and seed priming, both at 1% probability without, however, any interaction between the factors, the activity of the enzyme phenylalanine ammonia-lyase (PAL) showed similar behavior. However, unlike what happened with CAT activity in the roots, no statistical significance could be seen in these tissues about the PAL enzyme. Under conditions of salt stress, it was possible to see that, in the shoot, seedlings from seeds pretreated with SA 2.0 mM showed higher values of PAL activity (27.4% increase) compared to seedlings primed with distilled water. Similarly, priming with SA caused a 39.1% increase in this activity under non-saline conditions (Figure 12).

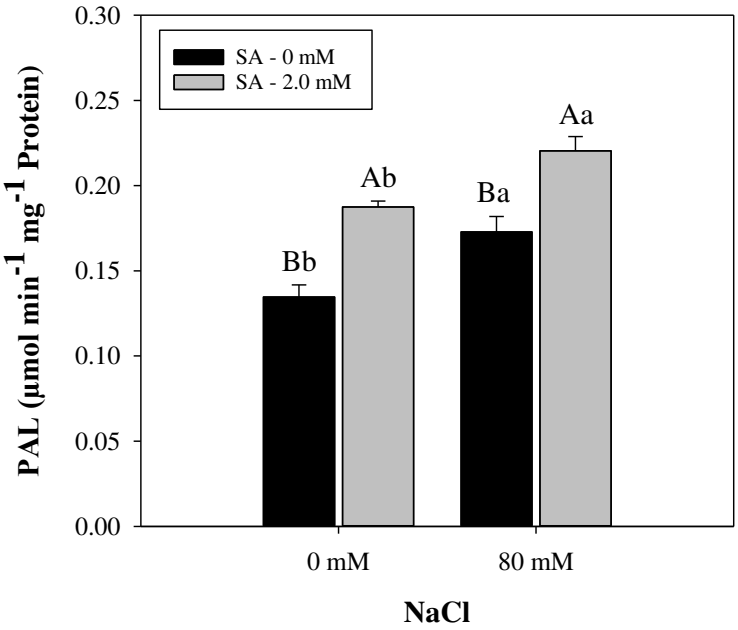


Figura 12 - Phenylalanine ammonia-lyase (PAL) activity in the shoot of rice seedlings (*Oryza sativa* L.), cv SCSBRS 113, from seeds subjected to physiological conditioning with distilled water (SA 0 mM) or salicylic acid (SA at 2.0 mM and grown in the absence (NaCl 0 mM) or presence of NaCl 80 mM. Capital letters compare the mean values between the priming treatments for the same saline condition and lowercase letters compare the means of the saline treatments within the same priming. Averages with different letters differ statistically by Tukey's test with $p < 0.05$. The values represent the averages of four repetitions + standard error.

7.3.7 *Quantification of endogenous salicylic acid content*

The SA content of aerial and root tissues was significantly affected by salinity treatment and seed priming (in both tissues, $p < 0.01$), but there was no interaction between these factors (Table S1). In seedlings from seeds primed with distilled water (SA 0 mM), cultivation in the presence of 80 mM NaCl caused an increase of 11.8% in the SA content of the shoot (Figure 13A) and 15.5% of the roots (Figure 13B). In addition, seedlings from seeds primed with SA and sown under saline stress showed higher SA levels compared to those pretreated exclusively with distilled water. This increase was 22.2% in the shoot and 13.4% in the roots. With regard to seedlings grown in the absence of salt stress, conditioning with SA contributed to a 21.1% increase in the shoot compared to seedlings conditioned with distilled water. It is worth noting that for this last analysis criterion, there was no significant difference in the roots of the seedlings.

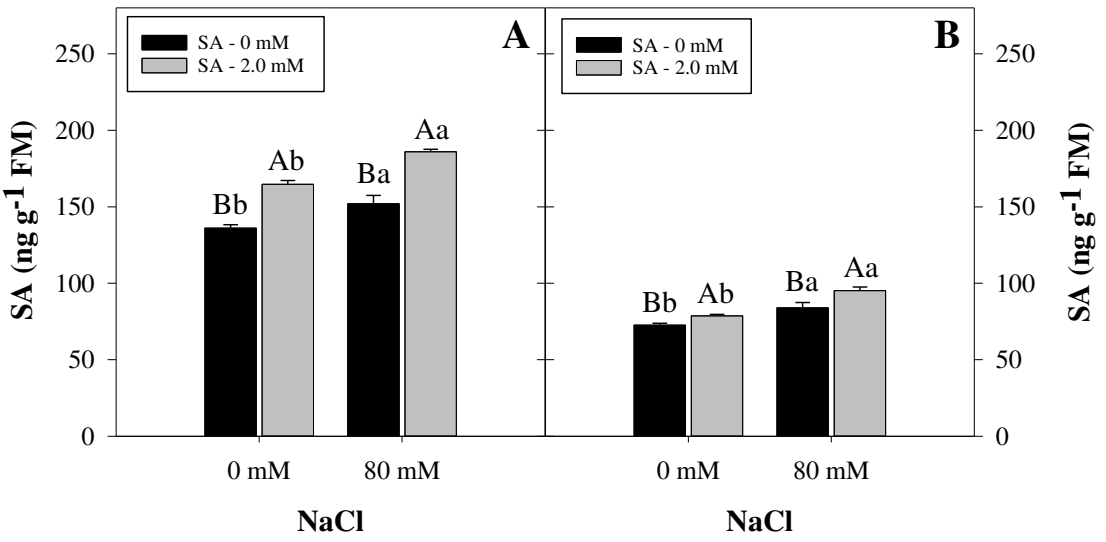


Figure 13 – Salicylic acid (SA) content in the shoot (**A**) and roots (**B**) of rice seedlings (*Oryza sativa* L.), cv SCSBRS 113, from seeds subjected to physiological conditioning with distilled water (SA 0 mM) or with salicylic acid (SA at 2.0 mM) and grown in the absence (NaCl 0 mM) or presence of NaCl 80 mM. Capital letters compare the mean values between the priming treatments for the same saline condition and lowercase letters compare the means of the saline treatments within the same priming. Averages with different letters differ statistically by Tukey's test with $p < 0.05$. The values represent the averages of four repetitions + standard error.

7.3.8 *Profile of metabolic responses in shoots and roots of rice to priming with salicylic acid*

As a result of the metabolic profile analyses (metabolome), 46 metabolites were identified in the shoot and 44 in the roots of the rice seedlings (Oryza sativa L.), cv. SCSBRS 113 submitted to the four treatments. In the shoot, 13 amino acids, 14 sugars and derivatives, 12 organic acids, three phenolic compounds, one nitrogenous compound, one amine, one flavonoid and one vitamin were detected. It is worth noting that the same metabolites found in the shoot were identified in the roots, except for ascorbic acid and quercetin (Table S2).

According to the principal component analysis (PC1 and PC2) of the metabolic profile of the shoot of the rice seedlings, there was a clear separation between the four treatments, with variances of 30.1% for PC1 and 21.1% for PC2 (Figure 14A). With regard to the roots, a separation was observed in which PC1 accounted for 45% of the total variance, while PC2 accounted for 18.2% (Figure 14B); however, it should be noted that there was an overlap between the non-saline treatments, and the most obvious separation in the roots was between

the groups of seedlings grown under saline stress and from seeds conditioned with distilled water and pretreated with 2.0 mM SA, influenced mainly by the axis corresponding to PC2.

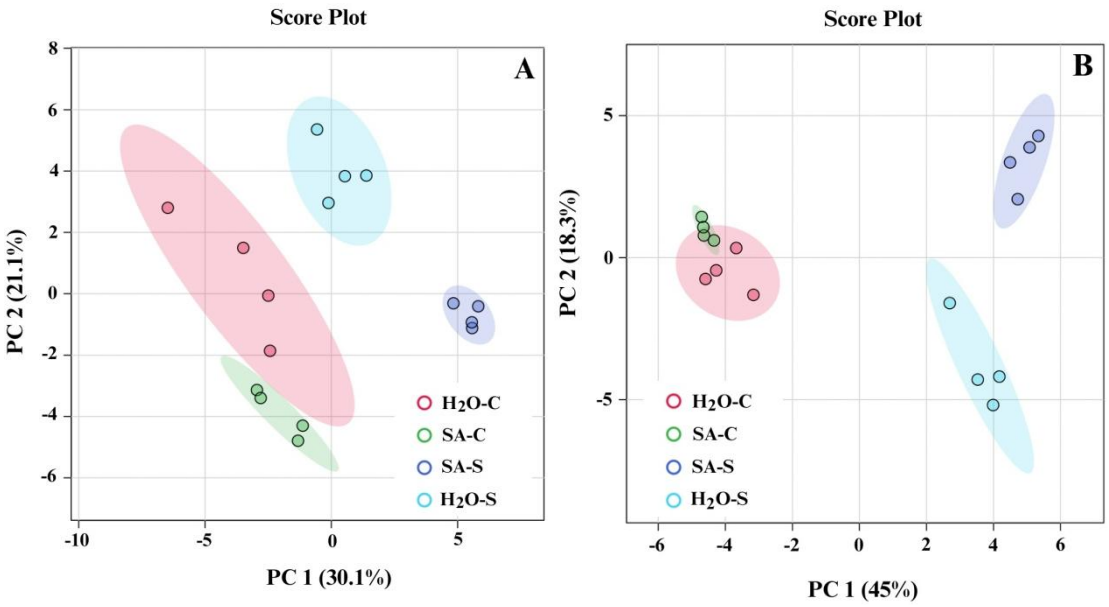


Figure 14 - Principal component analysis (PC1 and PC2) of the metabolic profile with scores plot of the shoot (**A**) and roots (**B**) of rice seedlings (*Oryza sativa* L.), cv. SCSBRS 113, from seeds subjected to physiological conditioning with distilled water (SA 0 mM) and grown in the absence (**H_2O-C**) or presence of NaCl 80 mM (**H_2O-S**) and subjected to physiological conditioning with salicylic acid (SA) 2.0 mM and grown in the absence (**SA-C**) or presence of NaCl 80 mM (**SA-S**). Colored circles represent each of the four replicates of the different treatments, while ellipses indicate a 95% confidence interval.

The loading graphs for each PCA showed the influence of the metabolites on the separation of the four treatments. In the shoot (Figure 15A; Table S3), the metabolites present in PC1 that contributed most to the separation were galactonic acid, alanine, glucose-6-phosphate, butyric acid and trehalose. In addition, for PC2, the most influential metabolites were glutamic acid, melezitose, citric acid, glycolic acid and threonine. In the roots (Figure 15B; Table S3) the metabolites that contributed positively to PC1 were aspartic acid, maltotriose, valine, threonine and serine. For PC2, quinic acid, threonic acid, caffeic acid, putrescine and pyruvic acid stood out.

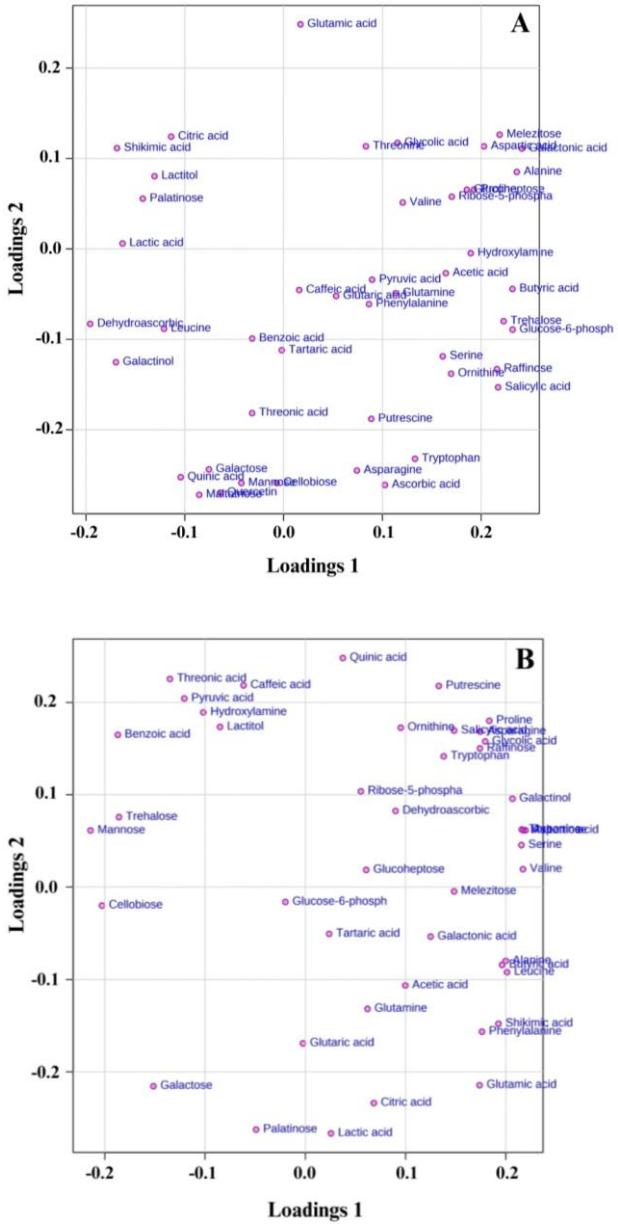


Figure 15 - Loading graph of the metabolites that most contributed to the separation of components 1 and 2 for the treatments in the shoot (**A**) and roots (**B**) of rice seedlings (*Oryza sativa* L.), cv. SCSBRS 113, from seeds subjected to physiological conditioning with distilled water (SA 0 mM) and grown in the absence or presence of NaCl 80 mM and subjected to physiological conditioning with salicylic acid (SA) 2.0 mM and grown in the absence or presence of NaCl 80 mM.

To evaluate the metabolic dynamics triggered by the physiological conditioning induced by SA in rice seedlings (*Oryza sativa* L.), cv. SCSBRS 113, grown under saline and non-saline conditions, the relative intensities each metabolite detected were statistically analyzed (Tables S4 and S5) and their relative abundances were represented using heatmaps (Figure 16).

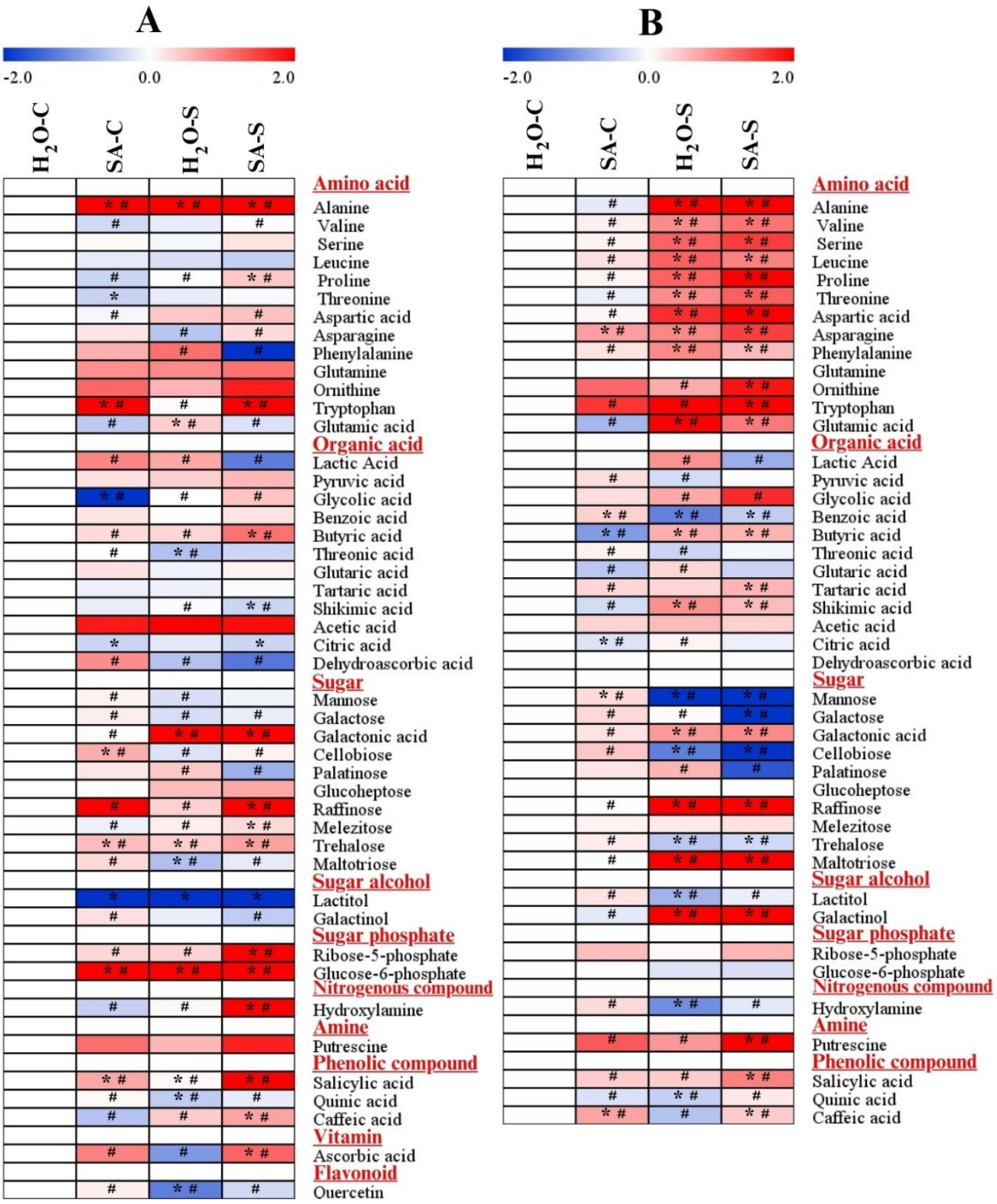


Figure 16. Heat map of the relative intensities of metabolites in the shoot (**A**) and roots (**B**) of rice seedlings (*Oryza sativa* L.), cv. SCSBRS 113, from seeds subjected to physiological conditioning with distilled water (SA 0 mM) and grown in the absence (**H_2O-C**) or presence of NaCl 80 mM (**H_2O-S**) and subjected to physiological conditioning with salicylic acid (SA) 2.0 mM and grown in the absence (**SA-C**) or presence of NaCl 80 mM (**SA-S**). The heat map shows an increasing (red scale) or decreasing (blue scale) scale of relative metabolite intensities. Statistically significant differences between the control seedlings (**H_2O-C**) and those of the other treatments are represented by (*). Significant differences between the **H_2O-S**, **SA-C** and **SA-S** treatments are represented by (#).

In the shoot, under non-saline conditions, priming the seeds with SA 2.0 mM promoted a significant increase in the relative amount of the metabolites alanine, tryptophan, cellobiose, trehalose and glucose-6-phosphate compared to the control and a reduction in the metabolites threonine, glycolic acid, citric acid and lactitol (Table S4; Figure 16A). In the shoot of the seedlings under conditions of salt stress (NaCl 80 mM), the physiological conditioning of the seeds with SA 2.0 mM significantly increased the relative amount of the metabolites alanine, proline, asparagine, tryptophan, butyric acid, galactonic acid, raffinose, melezitose, trehalose, ribose-5-phosphate, glucose-6-phosphate, hydroxylamine, quinic acid, caffeic acid and ascorbic acid and reduced that of the metabolites phenylalanine, glutamic acid, lactic acid, shikimic acid and palatinose. It is worth noting that priming with SA altered the metabolic profile of the shoot to the point of significantly increasing the relative intensity of the phenolic compound SA in saline and non-saline conditions (Table S4; Figure 16A).

With regard to the roots of seedlings grown in the absence of salinity, physiological conditioning with SA 2.0 mM was responsible for a significant increase in four metabolites - asparagine, benzoic acid, mannose and caffeic acid - and a decrease in the relative amount of butyric acid. On the other hand, under salinity, priming with SA contributed to a significant increase in the relative amounts of the metabolites serine, proline, threonine, asparagine, ornithine, tryptophan, glycolic acid, benzoic acid, aspartic acid, maltotriose, lactitol, galactinol, hydroxylamine, putrescine, quinic acid and caffeic acid and a reduction in the metabolites phenylalanine, glutamic acid, lactic acid, shikimic acid, galactose, cellobiose and palatinose) (Table S5; Figure 16B). Finally, as was observed in the shoot of the rice seedlings, there was also a significant increase in the relative intensity of the phenolic compound salicylic acid in the roots of the seedlings pretreated with SA and grown under salinity; however, this behavior was not evident in the seedlings primed with SA under the absence of salinity.

7.4 Discussion

Salinity is one of the environmental stresses that most compromise the growth and productivity of crops, as it inhibits the absorption of nutrients and water by the roots, induces osmotic and ionic stress in plants (DWININGSIH *et al.,* 2021) and causes overproduction of reactive oxygen species (ROS), which affect processes such as photosynthesis (AZEEM *et al.,* 2023). In order to mitigate these harmful effects, many studies have sought to develop techniques capable of increasing salinity tolerance and crop productivity. The use of seed

priming, for example, has proved to be a promising technique, as it improves germination and seedling development by accelerating the pre-occurrence of favorable metabolic events capable of overcoming adverse conditions through the activation of a stress defense system (EL-HAWARY; HASHEM; HASANUZZAMAN 2023). This paper delivers evidence that priming with salicylic acid (SA) alleviates the damage caused by salt stress in rice seedlings (*Oryza sativa* L.), cv. SCSBRS 113, as well as important information on the mechanisms involved.

7.4.1 *Salicylic acid positively affects relative water content and reduces osmotic potential*

Priming seeds with SA has been used by some authors successfully, promoting better germination under saline conditions (BANINASAB; BAGHBANHA, 2013; BAGHERI, 2014; YÜCEL; HEYBET, 2016). However, the concentrations of SA used vary depending on the species and genotype. Initially, preliminary experiments carried out by us also proved the beneficial effect of priming rice seeds with SA on germination and seedling establishment under salinity (80 mM NaCl), with the concentration of 2.0 mM provided the best results (results not shown) and which we considered the most suitable for conducting the experiments carried out here.

There is strong evidence that SA helped prevent a decrease in the turgidity of the shoot tissues; while salinity reduced the RWC of the seedlings, priming the seeds with SA 2.0 mM led to an increase to the point where it was equal to that of the control seedlings (Figure 6A). Furthermore, while salinity decreased the Ψs of the shoot of the seedlings, the physiological conditioning by rice seeds SA pretreatment helped to maintain it at an even lower level (Figure 6B). These results were in line with those of other authors who used seed priming with SA (LOUTFY *et al.,* 2020) or spraying the plants with this phytohormone (PAI; SHARMA, 2022) in order to mitigate the adverse effects of salinity on the tissues, and who also found an increase in RWC. The reduction in Ψs decreased the cellular water potential and favors better water absorption by seedling roots and is due to the ability of seedlings to accumulate osmolytes (or compatible solutes) in their tissues, which are partly responsible for lowering water potential (BERNSTEIN, 2019). It is also feasible that inorganic ions play a more substantial role, as they may be are absorbed by plant tissues and stored in large quantities in subcellular compartments such as vacuoles and endocytic vesicles. This processes potentially enables the restoration of the water status of seedlings (WANG *et al.,* 2019).

7.4.2 *Salicylic acid helps reduce membrane damage*

In general, our results showed that seed priming with SA 2.0 mM had a positive impact, reducing the percentage of electrolytes released in the shoot (Figure 7A), as well as reducing the content of MDA (Figure 7B), a significant sign of the lipid peroxidation process in plants under stress (CHEN *et al.,* 2024). It is possibly associated with the activation of antioxidant responses, which were able to reduce the impacts caused by the accumulation of ROS (AL-WHAIBI *et al.,* 2012, BATISTA *et al.,* 2019, LIU et al., 2022), which was evidenced by a 36.6% reduction in H_2O_2 content, which increased considerably in both the roots and the shoot of seedlings from seeds not conditioned with SA 2.0 mM and which were under salinity (Figure 10). This reduction in oxidative damage may have occurred due to the 49.6% increase in the activity of the enzyme catalase (CAT) (Figure 11), which was possibly induced by the exogenous application of SA (LIU *et al.,* 2022; PAI; SHARMA, 2022).

7.4.3 *Salicylic acid increases the levels of photosynthetic pigments*

In saline conditions, the high levels of ROS generated by the strong absorption of toxic ions, such as Na^+ and Cl^-, caused damage to cell membranes and strongly reduced the levels of chlorophyll *a*, chlorophyll *b* and carotenoids (LOUTFY, 2020; HAMANI *et al.,* 2020; MAHDAVIAN, 2023). Although the content of chlorophyll *b* was not affected by any of the treatments (Table S1), the contents of chlorophyll *a*, total chlorophyll and carotenoids were significantly reduced by salt stress in seedlings from pretreated seeds with distilled water (Figure 8A, B, C). However, remarkably, in the seedlings from seeds conditioned with SA 2.0 mM, and under salinity, the levels of these pigments remained the same as in the respective control seedlings from seeds conditioned with distilled water and sown in the absence of salinity. The high levels of chlorophyll in the seedlings of seeds primed with SA 2.0 mM are possibly due to a reduction in the activity of chlorophyllases, enzymes that degrade this pigment (HUNDARE; JOSHI; JOSHI, 2022). On the other hand, carotenoid levels may have increased in order to provide better protection for chloroplast membranes against oxidative damage, as they can release absorbed energy in the form of heat through photosystems I and II (MAHDAVIAN, 2023).

7.4.4 *Salicylic acid contributes to ion homeostasis*

When Na^+ and Cl^- ions are absorbed by plants under saline conditions, they exert a toxic ionic effect, strongly affecting the K^+/Na^+ ratio and impairing plant growth and development (DWININGSIH *et al.,* 2021; CHAUDHARY *et al.,* 2024). The large increase in

Na^+ content observed in rice seedlings from pretreated seeds with distilled water and subjected to salinity (379.3% and 162.8%, respectively, in the shoot and roots) was largely reduced by priming the seeds with SA 2.0 mM (Figure 9). This reduction in Na^+ content in both tissues may have occurred due to an efflux of this ion back into the growth medium or into the apoplast (YAMAGUCHI *et al.,* 2013). Priming with SA 2.0 mM also contributed to a higher K^+/Na^+ ratio, both in the shoot and in the roots, under saline conditions (Figure 9E, F), which is an important indication that there was an increase in tolerance or acclimatization to salinity in the seedlings originating from seeds pretreated with SA 2.0 mM. This ion homeostasis maintenance may have occurred due to a positive interference on the ion transporter channels so that the plant's water status was improved (AL-GHUMAIZ; ABD-ELMONIEM; MOTAWEI, 2017; RUBIO *et al.,* 2020). It is worth noting that this behavior of reducing ionic toxicity induced by the exogenous application of SA has also been identified in other research with different crops (JINI; JOSEPH, 2017; LOUTFY *et al*., 2020).

7.4.5 *Salicylic acid contributes to redox homeostasis*

H_2O_2 is a molecule that in low concentrations plays an important metabolic role, acting as a stress signal; however, when in large quantities, it acts as a ROS, capable of altering cellular and molecular constituents, for example, oxidizing DNA, proteins, carbohydrates, lipids, and enzymes, and can even cause programmed cell death (KESAWAR *et al.,* 2023). Under abiotic stress conditions such as salinity, plants produce large quantities of this reactive oxygen species, making it necessary to neutralize it using enzymatic or non-enzymatic antioxidants (DUMONT; RIVOAL, 2023).

It is important to note that the reduction in the accumulation of H_2O_2 in the shoot of rice seedlings from seeds conditioned with SA 2.0 mM and grown under salt stress (Figure 10A) was accompanied by an increase in CAT activity in the shoot of the seedlings from this same treatment (Figure 11A), which may have been fundamental in maintaining the homeostasis of the redox system (AHMAD *et al.,* 2019; CHENG *et al.,* 2020; LIU *et al.,* 2022). It is possible to infer that this increase in CAT activity is largely responsible for the reduction in membrane damage (Figure 7) and the possible elimination of other ROS, such as the superoxide radical ($^{\bullet}O_2$) which is converted into H_2O_2 by the action of SOD and eliminated by CAT, producing O_2 and H_2O (HE *et al.,* 2023). It is interesting to note that, in the root tissues of the seedlings from the saline treatment (NaCl 80 mM), regardless of whether the seeds were primed with distilled water or SA 2.0 mM, the H_2O_2 content was the same (Figure 10B), just as CAT activity was the same in the roots of the seedlings under

salinity, regardless of the priming the seeds were subjected to (Figure 11B). This fact demonstrates the coordination between H_2O_2 levels and CAT activity, with the increase in activity of this enzyme being justified by the need to quickly eliminate the H_2O_2 produced (FERRUZZI; FAGAN, 2023).

7.4.6 *Priming with salicylic acid increases the activity of phenylalanine ammonia-lyase and alters the levels of phenolic metabolites*

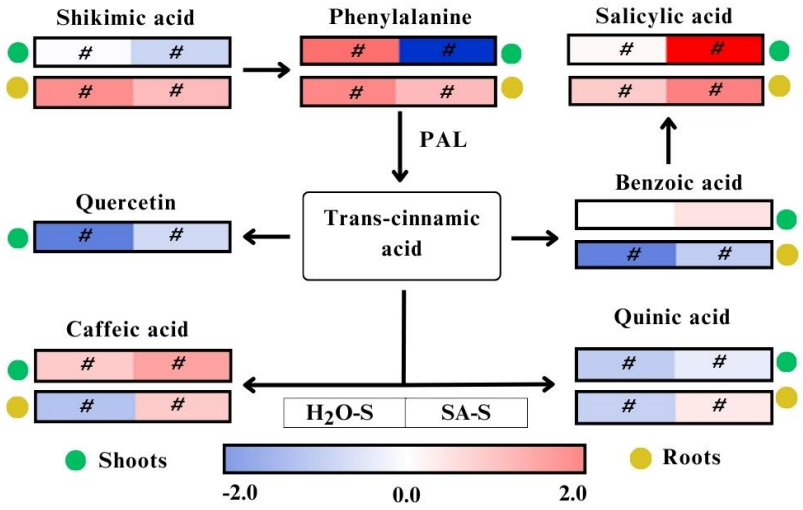
Salicylic acid is a phenolic compound and a secondary metabolite that can influence various physiological processes, including photosynthesis. It is also capable to affect membrane permeability (ANWAR *et al.,* 2013) and enhance plant defense against various abiotic and biotic stresses (JINI; JOSEPH, 2017).

Under saline conditions, endogenous SA levels were higher in both the shoot and the roots of rice seedlings from seeds conditioned with 2.0 mM SA (Figure 13). This result corroborates the hypothesis that the endogenous SA content tends to increase in plants under salt stress due to the crucial role of this phytohormone as a signaling molecule in the activation of antiporters and/or proton pumps that cause Na^+ to accumulate in the vacuoles and reduce salt toxicity in the upper sections of plants (ABDOLI *et al.,* 2020). It is also possible that these higher levels of endogenous SA may be related to the activation of genes and pathways related to plant defense to mitigate the deleterious effects of salinity (KIM *et al.,* 2017; PAI; SHARMA, 2022; PAI; SHARMA, 2024).

Altogether, the increase in endogenous SA content in the shoot of rice seedlings from seeds conditioned with 2.0 mM SA (Figure 13A) may be related to the corresponding increase in PAL activity in these same organs and conditioning treatment with SA (Figure 12). This idea is based on the fact that PAL catalyzes the conversion of phenylalanine into trans-cinnamic acid. This compound is subsequently converted into benzoic acid which, in turn, can be converted into SA by the enzyme benzoic acid-2-hydroxylase (MUSTAFA *et al.,* 2009; JAYAKANNAN *et al.,* 2015). Increases in the activity of this enzyme, induced by the exogenous application of SA, were also identified in studies with leaves of rice and wheat seedlings that were sown under high salinity (YÜCEL; HEYBET, 2016; SHETEIWY *et al.,* 2019). Therefore, it is important to note that the increase in PAL activity induced by priming with SA (Figure 12) may contribute to the biosynthesis of phenylpropanoids and hence to that of different phenolic compounds, and may represent an important defense against the effects of salinity, since such compounds can contribute to the elimination of ROS, acting, for

example, in the elimination of singlet oxygen ($^1O_2$) or as hydrogen donors (KOVACIK *et al.,* 2009; FARHADI; GHASSEMI-GOLEZANI 2020).

The relationship between priming with SA, phenylpropanoid biosynthesis, and PAL activity can be better analyzed based on the results of the metabolic profile, which showed that in saline conditions, conditioning the seeds with 2.0 mM SA alters an important pathway for the synthesis of this phytohormone and other phenolic compounds, such as quinic acid, caffeic acid and quercetin (Figure 17). These metabolites are known to contribute to osmoprotection, redox homeostasis and plant root growth in response to salt stress (ZHAN *et al.,* 2017; LEVY; SARKISSIAN; SCRIVER, 2018; SINGH *et al.,* 2021; VIANA *et al.,* 2023; GE *et al.,* 2023). In the shoot of the seedlings, for example, we observed that AS induced an increase in these compounds and a decrease in the levels of shikimic acid and phenylalanine (Figure 17), which may have occurred due to the increase in PAL activity (Figure 12) (FARHADI; GHASSEMI-GOLEZANI, 2020). In general, in the roots, we observed similar changes to those found in the metabolic profile of the shoot, however, in addition to the fact that the metabolite quercetin was not identified in their tissues, no statistical significance could be seen in the roots about the PAL enzyme (Table S1).



Source: prepared by the author

Figure 17 – Metabolic pathway of phenolic compounds from phenylalanine, with indication of changes in metabolites in the shoot and roots of rice seedlings (*Oryza sativa* L.), cv. SCSBRS 113, from seeds subjected to physiological conditioning with distilled water and grown in the presence of 80 mM NaCl (H_2O-S; left half of the horizontal bars) and from seeds subjected to physiological conditioning with 2.0 mM salicylic acid (SA) and grown in the presence of 80 mM NaCl (SA-S; right half of the horizontal bars). The heat map shows an increasing (red scale) or decreasing (blue scale) scale of relative metabolite intensities. Significant changes by Tukey's test ($p < 0.05$) between (H_2O-S) and (SA-S) seedlings are represented by (#).

7.4.7 *Priming with salicylic acid favorably alters the metabolic profile*

Under salt stress, various metabolic responses tend to occur in the leaves and roots of plants in order to favor an increase in tolerance to this abiotic condition. It is known that salt-tolerant plants accumulate higher concentrations of metabolites that are essential for eliminating ROS and for cellular homeostasis (CHANG *et al.,* 2019; RAJKUMARI *et al.,* 2023).

In this context, we investigated the metabolic changes caused by priming the seeds with 2.0 mM AS. In the seedlings grown only under salinity, the metabolic profile analysis showed that priming caused the accumulation of four amino acids in the aerial part, alanine, proline, asparagine, and tryptophan, and six in the roots, proline, threonine, aspartic acid, asparagine, ornithine and tryptophan. The increase in proline, which was 36.3% in the shoot and 165.2% in the roots (Figure 16; Tables S4 and S5) may be related to the improvement observed in the processes of osmoregulation of the shoot (reduction in osmotic potential, Figure 6B) and the reduction of oxidative effects on cell membranes (Figure 7). Proline is an amino acid that tends to accumulate in plants in response to various stress conditions, offering them a wide range of protective functions, including osmotic adjustment and stabilization of cell structure (NOUNJANA; NGHIA; THEERAKULPISUT, 2012; ZULFIQAR; AKRAM; ASHRAF, 2020; GUO *et al.,* 2023). Corroborating these data, Pai and Sharma (2022) also detected an increase in proline accumulation after SA application. The increase in asparagine of 79.4% in the aerial part and 48% in the roots (Figure 16; Tables S4 and S5) may be related to the transport and storage of nitrogen, for the synthesis and storage of proteins and for the detoxification of excess ammonia in the tissues (AKIN; KAYA, 2024; AVDOULI *et al.,* 2021).

The greater accumulation of aspartic acid in the roots, on the other hand, is a strong indication of increased tolerance (or acclimatization) to salinity since this metabolite not only acts by supplying nitrogen to the plants but is also a precursor of other nitrogen-containing molecules. It can also accumulate, together with other amino acids such as proline, to provide better osmotic adjustment and greater membrane stability (SADAK *et al.,* 2022).

Another important change in the metabolome stimulated by priming the seeds with SA was the accumulation observed in some sugars and derivatives (Figure 16; Tables S4 and S5). Under abiotic stress conditions, these metabolites can contribute to reducing osmotic stress, as well as regulating cell metabolism, growth, senescence and signaling pathways (SAMI *et al.,* 2016; RAJKUMARI *et al.,* 2023). In the seedlings under salinity, our study showed that

priming the seeds with SA 2.0 mM led to increases in the relative intensities of the following sugars and derivatives in the shoot: galactonic acid, raffinose, melizitose, trehalose, ribose-5-phosphate, and glucose-6-phosphate; while in the roots, increases were observed in maltotriose, lactitol, and galactinol. About the other treatments, few changes were detected. The accumulation of trehalose, raffinose and galactinol possibly contributed to improved membrane protection, since they increase the capacity to eliminate ROS under abiotic stress conditions (LIUA *et al.,* 2021). On the other hand, according to some authors, increases in melizitose, trehalose, glucose-6-phosphate and lactitol due to salt stress positively interfere with osmoprotection (CHOURASIA *et al.,* 2021; PAUL; DAS, 2023; WANG *et al.,* 2023).

Also, priming the seeds with SA 2.0 mM significantly affected the content of putrescine in the roots of seedlings grown under salinity, suggesting a possible interference in the metabolism of arginine, an important precursor of polyamine biosynthesis (ESTEBAN *et al.,* 2016). The observed increase in this metabolite was 173.3% (Figure 16B; Table S5), which may have improved the tolerance of rice seedlings to salinity, as this metabolite is known to trigger ethylene synthesis, stimulate cell elongation, stabilize biomembranes and neutralize the accumulation of sodium and chloride ions and the efflux of potassium from cells (RAJKUMARI *et al.,* 2023).

Finally, priming the seeds with SA 2.0 mM caused a significant 366.6% accumulation of the metabolite ascorbic acid in the shoot of the seedlings under stress (Figure 16A; Table S4), an important low molecular weight vitamin and antioxidant that can act as an enzyme cofactor, contributing to the elimination of ROS (EL-BELTAGI; MOHAMED; SOFY, 2020).

7.5 Conclusions

The salinity of the growth medium, with 80 mM NaCl, caused deleterious changes to the metabolism of rice seedlings, cv SCSBRS 113; however, priming the seeds with 2.0 mM salicylic acid reversed most of these changes. Comparing seedlings originating from seeds pretreated with salicylic acid with those not pretreated with this phytohormone, and both being in saline conditions, it was shown that in the first seedlings: 1. there was a reduction in ionic toxicity, with a higher and more favorable K^+/Na^+ ratio; 2. redox homeostasis was more favorable, with less membrane damage and higher activities of the antioxidant enzymes studied - catalase and phenylalanine ammonia-lyase; and 3. osmotic homeostasis was more favorable, with a greater reduction in the osmotic potential of the shoot. The results of the metabolic profile analysis of the aerial part and roots of these same seedlings showed

favorable changes in the relative levels of metabolites: proline, asparagine and aspartic acid (amino acids); maltotriose, lactitol and galactinol (sugar derivatives), ascorbic acid (vitamin), putrescine (polyamine) and salicylic acid (phenol), which were probably important in alleviating the deleterious effects of salts and increasing the tolerance to salinity of rice seedlings originating from seeds conditioned with salicylic acid.

8. CONSIDERAÇÕES FINAIS E CONCLUSÕES

Este trabalho mostrou, por meios de parâmetros bioquímicos, fisiológicos e metabolômicos, que o condicionamento fisiológico (ou *priming*) de sementes de arroz, cv SCSBRS 113, com ácido salicílico a 2.0 mM afetou positivamente a germinação e o desenvolvimento inicial das plântulas sob salinidade (NaCl 80 mM). O *priming* melhorou os principais parâmetros ligados à germinação (PCG, PSC, IVG e IVEPA) e ao crescimento (CPA, CR, MFR, MFPA, MSR, MSPA) em praticamente todas as concentrações testadas, porém a de 2.0 mM de ácido salicílico foi a que apresentou melhores resultados e foi escolhida para os estudos subsequentes.

Os resultados aqui apresentados mostraram que, sob salinidade, o *priming* das sementes com ácido salicílico diminuiu o acúmulo de Na^+ e aumentou o de K^+ de tal forma que a relação K^+/Na^+ aumentou nos tecidos da parte aérea, o que deve ter contribuído para a melhoria da homeostase iônica. O priming, da mesma forma, também interferiu positivamente na homeostase redox das plântulas sob estresse, desde que induziu um aumento de atividade das enzimas CAT e PAL, ao mesmo tempo que reduziu o acúmulo de H_2O_2, o vazamento e eletrólitos e a pexoxidação lipídica. A homeostase osmótica, por sua vez, também foi mais favorável, sendo observada maior redução no potencial osmótico da parte aérea das plântulas sob salinidade.

A análise metabolômica revelou que, em resposta ao estresse salino, o ácido salicílico modulou positivamente compostos como aminoácidos, açúcares e derivados e os metabólitos putrescina e ácido ascórbico, contribuindo assim para aclimatação e aumento de tolerância a essa condição abiótica de estresse. Os resultados da análise do perfil metabólico da parte aérea e raízes dessas mesmas plântulas, apontaram alterações favoráveis nos teores relativos dos metabólitos: prolina, asparagina e ácido aspártico (aminoácidos); maltotriose, lactitol e galactinol (derivados de açúcares), ácido ascórbico (vitamina), putrescina (poliamina) e ácido salicílico (fenol).

Os resultados, portanto, forneceram informações importantes sobre a influência do *priming* com moléculas orgânicas sinalizadoras, em particular do ácido salicílico, na aclimatação de plântulas de arroz à salinidade, auxiliando na compreensão do metabolismo das plantas sob estresse salino e no desenvolvimento ou aprimoramento de técnicas relacionadas ao aumento de tolerância das culturas à essa condição abiótica de cultivo.

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APPENDICE A – ANALYSIS OF VARIANCE FOR THE BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS

Table S1. Summary of the analysis of variance (ANOVA) table for the biochemical and physiological parameters of the shoot and roots of rice seedlings (*Oryza sativa* L.), cv. SCSBRS 113, from seeds submitted to physiological conditioning (*priming*) with salicylic acid and distilled water and grown under salinity conditions.

Variables	Source of variation		
	Salinity (S)	Salicylic acid (SA)	S x SA
RWC - Relative water content (%)	8.8^{ns}	3.43^{ns}	222.7^{**}
Ψs - Osmotic potential (MPa)	0.39^{**}	0.04^{**}	0.04^{*}
Electrolyte leakage (%)	2089.85^{**}	935.06^{**}	309.49^{**}
MDA (nmol g^{-1} FM)	17287.36^{**}	2962.85^{*}	47.26^{**}
Chlorophyll *a* (mg g^{-1} FM)	5.83^{*}	9.23^{*}	64.29^{**}
Chlorophyll *b* (mg g^{-1} FM)	0.012^{ns}	0.51^{ns}	2.46^{ns}
Total chlorophyll (mg g^{-1} FM)	0.76^{ns}	12.46^{ns}	67.14^{**}
Carotenoids (mg g^{-1} FM)	2.43^{**}	1.43^{**}	2.78^{**}
Na^+ - Shoots (µmol g^{-1} DM)	2964141.95^{**}	303609.51^{**}	88051.99^{*}
Na^+ - Roots (µmol g^{-1} DM)	3681152.29^{**}	1244867.08^{**}	338511.70^{**}
K^+ - Shoots (µmol g^{-1} DM)	930909.91^{**}	819.66^{ns}	88140.45^{**}
K^+ - Roots (µmol g^{-1} DM)	72384.81^{*}	55432.12^{ns}	40896.21^{ns}
K^+/Na^+ - Shoots	4.65^{**}	5.89^{**}	33.60^{**}
K^+/Na^+ - Roots	58.91^{**}	5.86^{**}	4.40^{**}
H_2O_2 - Shoots (µmol g^{-1} FM)	25167.64^{**}	8796.57^{**}	6690.07^{**}
H_2O_2 - Roots (µmol g^{-1} FM)	76598.31^{**}	1575.93^{ns}	3358.13^{ns}
CAT - Shoots (µmol H_2O_2 min^{-1} mg^{-1} Prot)	216.25^{**}	114.87^{**}	9.31^{ns}
CAT - Roots (µmol H_2O_2 min^{-1} mg^{-1} Prot)	76.13^{**}	0.74^{ns}	1.07^{ns}
PAL - Shoots (µmol min^{-1} mg^{-1} Prot)	0.005^{**}	0.01^{**}	0.00^{ns}
PAL - Roots (µmol min^{-1} mg^{-1} Prot)	0.00^{ns}	0.01^{ns}	0.007^{ns}
SA - Shoots (ng g^{-1} FM)	1391.68^{**}	3921.13^{**}	26.97^{ns}
SA - Roots (ng g^{-1} FM)	770.96^{**}	299.94^{**}	27.31^{ns}

* Significant at $p < 0.05$; ** significant at $p < 0.01$; not significant (ns) at $p > 0.05$.

APPENDICE B - MAIN CHARACTERISTICS OF THE METABOLITES IDENTIFIED IN THE SHOOT AND ROOTS OF RICE SEEDLINGS

Table S2 - List of metabolites identified in the shoot and roots of rice seedlings (*Oryza sativa* L.), cv. SCSBRS 113, submitted to the four treatments and main characteristics: chemical classification, retention time, mass fragment and identifier number in the Kyoto Encyclopedia of Genes and Genomes (KEGG ID).

Name of metabolite	Chemical classification	Retention time (min)	Mass fragment (m/z)	Kegg Compound
1. Hydroxylamine	Nitrogenous compound	5.55	133	C00192
2. Lactic Acid	Organic acid	6.88	117	C00186
3. Pyruvic acid	Organic acid	7.12	174	C00022
4. Glycolic acid	Organic acid	7.64	177	C00160
5. Alanine	Amino acid	8.18	116	C00041
6. Valine	Amino acid	10.38	144	C00183
7. Serine	Amino acid	11.11	116	C00065
8. Leucine	Amino acid	11.69	158	C00123
9. Proline	Amino acid	11.74	142	C00148
10. Threonine	Amino acid	13.13	117	C00188
11. Benzoic acid	Organic acid	14.49	179	C00108
12. Salicylic acid	Phenolic compound	14.78	267	C00805
13. Aspartic acid	Amino acid	14.89	232	C00049
14. Butyric acid	Organic acid	14.98	174	C00073
15. Threonic acid	Organic acid	15.47	292	C01620
16. Glutaric acid	Organic acid	15.57	198	C00026
17. Putrescine	Amine	15.66	142	C00134
18. Tartaric acid	Organic acid	15.93	292	C00898
19. Asparagine	Amino acid	16.00	188	C00152
20. Glutamic acid	Amino acid	16.08	246	C00025
21. Phenylalanine	Amino acid	16.22	192	C00079
22. Glutamine	Amino acid	17.82	156	C00064
23. Acetic acid	Organic acid	17.84	292	C00033
24. Shikimic acid	Organic acid	18.22	204	C00493
25. Citric acid	Organic acid	18.38	273	C00158
26. Ornithine	Amino acid	18.48	142	C00077
27. Dehydroascorbic acid	Organic acid	18.74	316	C00425
28. Quinic acid	Phenolic compound	18.88	345	C06746
29. Mannose	Sugar	19.31	319	C00159
30. Galactose	Sugar	19.49	319	C00984

Table S2 – Continued

Name of metabolites	Chemical classification	Retention time (min)	Mass fragment (m/z)	Kegg Compound
31. Ascorbic acid*	Vitamin	19.74	449	C00072
32. Lactitol	Sugar alcohol	20.06	204	C13542
33. Cellobiose	Sugar	20.55	204	C06422
34. Galactonic acid	Sugar	20.75	333	C00880
35. Caffeic acid	Phenolic compound	21.39	219	C01481
36. Palatinose	Sugar	21.95	204	C01742
37. Ribose-5-phosphate	Sugar phosphate	23.13	315	C00199
38. Glucose-6-phosphate	Sugar phosphate	23.23	387	C00668
39. Glucoheptose	Sugar	24.13	160	C21042
40. Raffinose	Sugar	24.96	361	C00492
41. Melezitose	Sugar	25.77	361	C08243
42. Trehalose	Sugar	26.53	361	C01083
43. Tryptophan	Amino acid	26.90	361	C00078
44. Galactinol	Sugar alcohol	28.43	204	C01697
45. Quercetin*	Flavonoid	28.93	559	C00389
46. Maltotriose	Sugar	29.60	204	C01835

* Unidentified metabolites in the roots of rice seedlings

APPENDICE C – LOADING PLOT OF DETECTED METABOLITES

Table S3: Loading plot of the metabolites detected that most contributed to the separation of components 1 and 2 in the shoot and roots of rice seedlings (*Oryza sativa* L.), cv. SCSBRS 113, from seeds subjected to physiological conditioning with distilled water or salicylic acid and grown in the absence or presence of 80 mM NaCl.

Shoots				**Roots**			
Metabolites	**PC 1**	**Metabolites**	**PC 2**	**Metabolites**	**PC 1**	**Metabolites**	**PC 2**
Galactonic acid	0.241	Glutamic acid	0.248	Aspartic acid	0.219	Quinic acid	0.248
Alanine	0.236	Melezitose	0.126	Maltotriose	0.217	Threonic acid	0.225
Glucose-6-phosphate	0.231	Citric acid	0.124	Valine	0.217	Caffeic acid	0.218
Butyric acid	0.231	Glycolic acid	0.117	Threonine	0.215	Putrescine	0.217
Trehalose	0.222	Threonine	0.113	Serine	0.215	Pyruvic acid	0.204
Melezitose	0.218	Aspartic acid	0.113	Galactinol	0.206	Hydroxylamine	0.189
Salicylic acid	0.217	Shikimic acid	0.111	Leucine	0.201	Proline	0.18
Raffinose	0.216	Galactonic acid	0.11	Alanine	0.199	Lactitol	0.173
Aspartic acid	0.202	Alanine	0.085	Butyric acid	0.196	Ornithine	0.172
Proline	0.192	Lactitol	0.08	Shikimic acid	0.192	Salicylic acid	0.169
Hydroxylamine	0.189	Proline	0.065	Proline	0.183	Asparagine	0.168
Glucoheptose	0.185	Glucoheptose	0.065	Glycolic acid	0.179	Benzoic acid	0.164
Ribose-5-phosphate	0.17	Ribose-5-phosphate	0.057	Phenylalanine	0.176	Glycolic acid	0.157
Ornithine	0.169	Palatinose	0.055	Asparagine	0.174	Raffinose	0.15
Acetic acid	0.164	Valine	0.051	Raffinose	0.174	Tryptophan	0.141
Serine	0.161	Lactic acid	0.005	Glutamic acid	0.173	Ribose-5-phosphate	0.103
Tryptophan	0.133	Hydroxylamine	-0.004	Salicylic acid	0.148	Galactinol	0.095
Valine	0.12	Acetic acid	-0.026	Melezitose	0.148	Dehydroascorbic acid	0.082
Glycolic acid	0.115	Pyruvic acid	-0.034	Tryptophan	0.138	Trehalose	0.075
Glutamine	0.113	Butyric acid	-0.044	Putrescine	0.133	Threonine	0.062
Ascorbic acid	0.102	Caffeic acid	-0.045	Galactonic acid	0.124	Aspartic acid	0.061
Pyruvic acid	0.089	Glutamine	-0.049	Acetic acid	0.099	Maltotriose	0.061
Putrescine	0.088	Glutaric acid	-0.052	Ornithine	0.095	Mannose	0.061
Phenylalanine	0.086	Phenylalanine	-0.061	Dehydroascorbic acid	0.089	Serine	0.045
Threonine	0.083	Trehalose	-0.079	Citric acid	0.068	Valine	0.019
Asparagine	0.074	Dehydroascorbic acid	-0.083	Glutamine	0.062	Glucoheptose	0.018
Glutaric acid	0.053	Leucine	-0.088	Glucoheptose	0.06	Melezitose	-0.004
Glutamic acid	0.017	Glucose-6-phosphate	-0.089	Ribose-5-phosphate	0.055	Glucose-6-phosphate	-0.016

Table S3 – Continued

Shoots				Roots			
Metabolites	**PC 1**	**Metabolites**	**PC 2**	**Metabolites**	**PC 1**	**Metabolites**	**PC 2**
Caffeic acid	0.015	Benzoic acid	-0.099	Quinic acid	0.037	Cellobiose	-0.019
Tartaric acid	-0.001	Tartaric acid	-0.112	Lactic acid	0.025	Tartaric acid	-0.05
Cellobiose	-0.007	Serine	-0.118	Tartaric acid	0.023	Galactonic acid	-0.053
Benzoic acid	-0.031	Galactinol	-0.125	Glutaric acid	-0.002	Alanine	-0.079
Threonic acid	-0.031	Raffinose	-0.133	Glucose-6-phosphate	-0.019	Butyric acid	-0.084
Mannose	-0.042	Ornithine	-0.138	Palatinose	-0.049	Leucine	-0.092
Quercetin	-0.064	Salicylic acid	-0.153	Caffeic acid	-0.061	Acetic acid	-0.106
Galactose	-0.075	Threonic acid	-0.181	Lactitol	-0.085	Glutamine	-0.131
Maltotriose	-0.085	Putrescine	-0.188	Hydroxylamine	-0.101	Shikimic acid	-0.148
Quinic acid	-0.104	Tryptophan	-0.232	Pyruvic acid	-0.12	Phenylalanine	-0.156
Citric acid	-0.113	Galactose	-0.244	Threonic acid	-0.134	Glutaric acid	-0.169
Leucine	-0.121	Asparagine	-0.245	Galactose	-0.151	Glutamic acid	-0.214
Lactitol	-0.13	Quinic acid	-0.252	Trehalose	-0.185	Galactose	-0.215
Palatinose	-0.142	Cellobiose	-0.258	Benzoic acid	-0.186	Citric acid	-0.233
Lactic acid	-0.163	Mannose	-0.259	Cellobiose	-0.202	Palatinose	-0.262
Shikimic acid	-0.168	Ascorbic acid	-0.261	Mannose	-0.214	Lactic acid	-0.266
Galactinol	-0.169	Quercetin	-0.269				
Dehydroascorbic acid	-0.195	Maltotriose	-0.272				

APPENDICE D – RELATIVE INTENSITIES OF METABOLITES IN THE SHOOT OF RICE PLANTS (*Oryza sativa* L.), cv. SCSBRS 113, FROM SEEDS OF THE FOUR TREATMENTS

Table S4 – Relative intensities of the metabolites identified in the shoot of rice seedlings (*Oryza sativ*a L.), cv. SCSBRS 113, from seeds physiologically conditioned with distilled water and grown in the absence (**H_2O-C**) or presence of 80 mM NaCl (**H_2O-S**) and from seeds physiologically conditioned with 2.0 mM salicylic acid and grown in the absence (**SA-C**) or presence of 80 mM NaCl (**SA-S**). The values represent the means of four replicates ± standard error. Statistical comparisons between the four treatments were made using the Tukey test ($P < 0.05$).

Metabolites	Relative intensity											
	H_2O-C			SA-C			H_2O-S			SA-S		
Amino acid												
Alanine *	0.9	±0.03	**d**	16.9	±2.00	**c**	42	±1.66	**b**	57	±3.72	**a**
Valine *	355	±25.7	**ba**	277	±8.56	**b**	315	±7.60	**ba**	362	±13.9	**a**
Serine	130	±9.47	**a**	134	±6.83	**a**	124	±5.63	**a**	151	±2.17	**a**
Leucine	233	±29.4	**a**	204	±4.35	**a**	189	±9.12	**a**	169	±5.44	**a**
Proline *	88	±6.84	**b**	66.4	±6.36	**b**	87	±5.03	**b**	119	±6.24	**a**
Threonine *	92	±6.04	**a**	68.4	±2.30	**b**	82	±3.91	**ba**	89	±3.03	**ba**
Aspartic acid *	119	±7.58	**ba**	113	±13.3	**b**	161	±11.9	**ba**	167	±8.26	**a**
Asparagine *	40	±5.55	**ba**	46.6	±1.34	**ba**	27	±2.82	**b**	49	±6.85	**a**
Phenylalanine *	5.4	±2.80	**ba**	8.39	±2.57	**ba**	12	±0.60	**a**	0.8	±0.04	**b**
Glutamine	19	±6.64	**a**	34.4	±8.08	**a**	36	±8.87	**a**	41	±12.97	**a**
Ornithine	5.3	±2.71	**a**	12	±3.58	**a**	7.9	±2.36	**a**	18	±2.35	**a**
Tryptophan *	0.7	±0.08	**b**	11.3	±3.78	**a**	0.8	±0.04	**b**	13	±1.21	**a**
Glutamic acid *	186	±9.59	**ba**	131	±6.63	**b**	245	±10.4	**a**	154	±17.7	**b**
Organic acid												
Lactic Acid *	180	±26.5	**ba**	351	±50.2	**a**	289	±41.7	**a**	75	±18.3	**b**
Pyruvic acid *	0.2	±0.01	**b**	0.27	±0.01	**ba**	0.3	±0.00	**ba**	0.3	±0.02	**a**
Glycolic acid *	0.1	±0.01	**a**	0.01	±0.00	**b**	0.1	±0.00	**a**	0.1	±0.00	**a**
Benzoic acid	0.1	±0.01	**a**	0.14	±0.02	**a**	0.1	±0.01	**a**	0.1	±0.01	**a**
Butyric acid *	63	±4.62	**b**	76.9	±6.83	**b**	79	±7.12	**b**	137	±11.5	**a**
Threonic acid *	0.5	±0.02	**a**	0.5	±0.04	**a**	0.3	±0.01	**b**	0.4	±0.03	**ba**
Glutaric acid	0.3	±0.01	**a**	0.32	±0.03	**a**	0.3	±0.01	**a**	0.3	±0.02	**a**
Tartaric acid	0.5	±0.02	**a**	0.42	±0.02	**a**	0.4	±0.02	**a**	0.5	±0.02	**a**
Shikimic acid *	89	±1.26	**a**	79.1	±2.88	**ba**	88	±4.27	**a**	67	±2.41	**b**
Acetic acid	0.2	±0.19	**a**	0.74	±0.06	**a**	2.7	±2.38	**a**	0.8	±0.05	**a**
Citric acid *	128	±5.94	**a**	96.6	±4.21	**b**	112	±2.81	**ba**	98	±3.41	**b**
Dehydroascorbic acid *	0.2	±0.03	**ba**	0.28	±0.04	**a**	0.1	±0.01	**b**	0.1	±0.01	**b**

* Significant with $p < 0.05$; not significant (ns) with $p > 0.05$.

Table S4 – Continued

Metabolites	Relative intensity											
	H_2O-C			SA-C			H_2O-S			SA-S		
Sugar												
Mannose *	609	±44.5	**ba**	655	±12.8	**a**	494	±29.6	**b**	567	±13.16	**ba**
Galactose *	212	±14.1	**ba**	234	±4.75	**a**	170	±11.8	**b**	189	±9.03	**b**
Galactonic acid *	0.4	±0.05	**c**	0.44	±0.06	**c**	13	±0.94	**b**	19	±1.06	**a**
Cellobiose *	48	±7.57	**b**	75.3	±3.22	**a**	40	±2.57	**b**	52	±4.39	**b**
Palatinose*	172	±21.4	**ba**	198	±9.34	**ba**	232	±41.5	**a**	101	±11.1	**b**
Glucoheptose	8.2	±2.47	**a**	8.29	±2.47	**a**	12	±0.22	**a**	13	±0.70	**a**
Raffinose *	0.6	±0.08	**b**	3.29	±2.94	**b**	0.8	±0.02	**b**	13	±1.16	**a**
Melezitose *	979	±15.1	**cb**	897	±20.8	**c**	1077.1	±9.86	**b**	1193.5	±36.5	**a**
Trehalose *	24	±0.67	**c**	34.4	±1.2	**b**	31	±1.23	**b**	40	±2.03	**a**
Maltotriose *	42	±1.56	**ba**	51.2	±3.38	**a**	27	±1.74	**c**	36	±1.40	**cb**
Sugar alcohol												
Lactitol *	139	±43.5	**a**	2.59	±2.32	**b**	0.7	±0.03	**b**	0.9	±0.06	**b**
Galactinol *	43	±2.60	**ba**	51	±3.85	**a**	39	±1.01	**ba**	31	±1.94	**b**
Sugar phosphate												
Ribose-5-phosphate *	0.6	±0.07	**b**	0.68	±0.10	**b**	0.7	±0.05	**b**	13	±1.04	**a**
Glucose-6-phosphate *	0.7	±0.12	**c**	11.3	±3.43	**b**	11	±3.23	**b**	22	±1.61	**a**
Nitrogenous compound												
Hydroxylamine *	0.5	±0.07	**b**	0.38	±0.12	**b**	0.5	±0.06	**b**	7.8	±2.33	**a**
Amine												
Putrescine	5.3	±2.75	**a**	11.7	±3.49	**a**	7.9	±2.34	**a**	18	±2.17	**a**
Phenolic compound												
Salicylic acid *	0.5	±0.04	**c**	0.73	±0.07	**b**	0.5	±0.03	**b**	13	±0.31	**a**
Quinic acid *	126	±5.76	**ba**	131	±3.69	**a**	90	±2.63	**c**	111	±1.24	**b**
Caffeic acid *	0	±0.01	**cb**	0.02	±0.00	**c**	0	±0.00	**b**	0.1	±0.00	**a**
Vitamin												
Ascorbic acid *	0.1	±0.01	**cb**	0.12	±0.01	**ba**	0	±0.00	**c**	0.1	±0.02	**a**
Flavonoid												
Quercetin *	0.5	±0.03	**ba**	0.55	±0.04	**a**	0.2	±0.02	**c**	0.4	±0.02	**b**

* Significant with $p < 0.05$; not significant (ns) with $p > 0.05$.

APPENDICE E – RELATIVE INTENSITIES OF METABOLITES IN THE ROOT PLANTS OF RICE (*Oryza sativa* L.), cv SCSBRS 113, FROM SEEDS OF THE FOUR TREATMENTS

Table S5 – Relative intensities of the metabolites identified in the roots of rice seedlings (*Oryza sativa*, L.), cv SCSBRS 113, from seeds physiologically conditioned with distilled water and grown in the absence (**H_2O-C**) or presence of 80 mM NaCl (**H_2O-S**) and from seeds physiologically conditioned with 2.0 mM salicylic acid and grown in the absence (**SA-C**) or presence of 80 mM NaCl (**SA-S**). The values represent the means of four replicates ± standard error. Statistical comparisons between the four treatments were made using the Tukey test ($P < 0.05$).

METABOLITES	RELATIVE INTENSITY											
	H_2O-C			SA-C			H_2O-S			SA-S		
Amino acid												
Alanine *	0.9	±0.05	**b**	0.74	±0.03	**b**	17	±1.24	**a**	15	±0.37	**a**
Valine *	60	±5.13	**b**	65.5	±3.12	**b**	114	±4.77	**a**	129	±2.77	**a**
Serine *	29	±1.96	**c**	31.1	±1.40	**c**	68	±3.00	**b**	84	±4.10	**a**
Leucine *	37	±3.96	**b**	44.1	±3.47	**b**	89	±2.91	**a**	74	±1.44	**a**
Proline *	12	±3.85	**c**	12.6	±0.89	**c**	28	±2.87	**b**	70	±2.69	**a**
Threonine *	13	±1.02	**c**	12.2	±0.49	**c**	25	±1.15	**b**	32	±1.13	**a**
Aspartic acid *	22	±1.46	**c**	23.1	±1.44	**c**	69	±1.10	**b**	92	±2.05	**a**
Asparagine *	0.1	±0.01	**c**	0.22	±0.01	**b**	0.3	±0.02	**b**	0.4	±0.01	**a**
Phenylalanine *	0.2	±0.01	**c**	0.28	±0.01	**c**	0.5	±0.01	**a**	0.4	±0.02	**b**
Glutamine	0	±0.00	**a**	0	±0.00	**a**	0	±0.01	**a**	0	±0.01	**a**
Ornithine *	0.2	±0.02	**b**	0.43	±0.09	**ba**	0.3	±0.03	**b**	0.7	±0.07	**a**
Tryptophan *	0	±0.00	**b**	0.03	±0.01	**b**	0	±0.01	**b**	0.1	±0.01	**a**
Glutamic acid *	33	±3.94	**c**	20.4	±1.11	**c**	142	±6.53	**a**	69	±1.25	**b**
Organic acid												
Lactic Acid *	25	±4.67	**ba**	24.2	±3.23	**ba**	45	±8.84	**a**	15	±3.26	**b**
Pyruvic acid *	0.3	±0.02	**ba**	0.35	±0.02	**a**	0.2	±0.02	**b**	0.3	±0.01	**ba**
Glycolic acid *	0.1	±0.00	**ba**	0.06	±0.00	**ba**	0.1	±0.00	**b**	0.2	±0.02	**a**
Benzoic acid *	0.1	±0.01	**b**	0.18	±0.01	**a**	0.1	±0.01	**d**	0.1	±0.00	**c**
Butyric acid *	50	±2.83	**b**	25.6	±1.72	**c**	78	±8.12	**a**	77	±2.13	**a**
Threonic acid *	79	±2.03	**ba**	86.5	±1.80	**a**	60	±6.90	**b**	75	±2.82	**ba**
Glutaric acid *	0.2	±0.02	**ba**	0.11	±0.00	**b**	0.2	±0.02	**a**	0.1	±0.01	**ba**
Tartaric acid *	0	±0.00	**b**	0.05	±0.01	**b**	0.1	±0.01	**ba**	0.1	±0.00	**a**
Shikimic acid *	68	±4.17	**c**	53.8	±2.39	**c**	126	±3.58	**a**	99	±4.40	**b**
Acetic acid	11	±0.18	**a**	14.2	±0.46	**a**	16	±1.72	**a**	15	±1.19	**a**
Citric acid *	272	±11.3	**ba**	228	±12.7	**c**	292	±9.09	**a**	247	±2.57	**cb**
Dehydroascorbic acid	0	±0.00	**a**	0.01	±0.01	**a**	0	±0.01	**a**	0	±0.00	**a**

* Significant with $p < 0.05$; not significant (ns) with $p > 0.05$.

Table S5 – Continued

METABOLITES	RELATIVE INTENSITY											
	H_2O-C			SA-C			H_2O-S			SA-S		
Sugar												
Mannose *	325	±18.2	**b**	396	±14.1	**a**	29	±5.05	**c**	70	±7.73	**c**
Galactose *	88	±10.6	a	109	±3.00	**a**	90	±4.73	**a**	17	±1.46	b
Galactonic acid *	0.1	±0.01	b	0.14	±0.01	**b**	0.2	±0.01	**a**	0.2	±0.01	a
Cellobiose *	21	±2.51	**a**	29	±0.99	**a**	9	±2.68	**b**	0.7	±0.02	**c**
Palatinose*	201	±28.08	**ba**	232	±18.9	**ba**	301	±66.6	**a**	65	±8.17	**b**
Glucoheptose	0	±0.00	**a**	0	±0.00	**a**	0	±0.00	**a**	0	±0.00	**a**
Raffinose *	0	±0.01	**b**	0	±0.00	**b**	0.1	±0.01	**a**	0.1	±0.01	**a**
Melezitose	679	±38.04	**a**	728	±10.06	**a**	773	±25.6	**a**	796	±17.7	**a**
Trehalose *	48	±1.74	**a**	53.4	±1.65	**a**	33	±3.09	**b**	37	±1.78	**b**
Maltotriose *	0.6	±0.06	**c**	0.62	±0.04	**c**	17	±0.79	**b**	23	±0.69	**a**
Sugar alcohol												
Lactitol *	0.4	±0.01	**ba**	0.49	±0.03	**a**	0.3	±0.01	**c**	0.4	±0.01	**b**
Galactinol *	0.8	±0.06	**c**	0.66	±0.05	**c**	16	±1.05	**b**	24	±0.96	**a**
Sugar phosphate												
Ribose-5-phosphate	0.3	±0.02	**a**	0.37	±0.01	**a**	0.3	±0.06	**a**	0.4	±0.03	**a**
Glucose-6-phosphate	0.4	±0.04	**a**	0.37	±0.04	**a**	0.3	±0.03	**a**	0.3	±0.01	**a**
Nitrogenous compound												
Hydroxylamine *	0.8	±0.07	**ba**	0.92	±0.01	**a**	0.4	±0.01	**c**	0.7	±0.02	**b**
Amine												
Putrescine *	0.2	±0.02	**b**	0.42	±0.09	**b**	0.3	±0.03	**b**	0.8	±0.07	**a**
Phenolic compound												
Salicylic acid *	0	±0.00	**b**	0.04	±0.00	**b**	0	±0.00	**b**	0.1	±0.01	**a**
Quinic acid *	140	±7.89	**ba**	115	±7.26	**cb**	103	±3.06	**c**	158	±6.31	**a**
Caffeic acid *	0	±0.00	**b**	0.05	±0.01	**a**	0	±0.00	**b**	0	±0.00	**a**

* Significant with $p < 0.05$; not significant (ns) with $p > 0.05$.