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CINTHIA SILVA DE QUEIROZ

HOMEOSTASE DO RETÍCULO ENDOPLASMÁTICO E SUA RELAÇÃO COM O MECANISMO DE TOLERÂNCIA AO ESTRESSE SALINO EM PLANTAS DE *Sorghum bicolor* (L.) Moench

FORTALEZA
2018

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Dissertação apresentada ao Mestrado Profissional em Bioquímica da Universidade Federal do Ceará, como requisito parcial à obtenção do título de Mestra. Área de concentração: Fisiologia, Bioquímica e Bioquímica Vegetal.

Orientador: Prof. Dr. Humberto Henrique de Carvalho.

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Aprovada em: 27 de julho de 2018

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RESUMO

Para evitar os efeitos prejudiciais do acumulo de sódio, as plantas desenvolveram mecanismos tais como a via de sinalização das proteínas carreadoras Na^+/H^+ (NHX) e a via SOS (salt overly sensitive). Além disso, o reticulo endoplasmático (RE) pode integrar a resposta das células vegetais. Portanto, propomos compreender os efeitos do comprometimento da homeostase (RE) e sua relação com o estresse salino durante os estágios iniciais do *Sorghum bicolor* CSF 20, uma variedade tolerante ao sal. Plântulas de três dias foram estimuladas com NaCl (0, 50, 75 e 100 mM), ditiotreitol (DTT) a 0, 2,5, 5,0 10,0 mM e os tratamentos combinados de NaCl e DTT. A tunicamicina (TUN) também foi usada como um segundo indutor de estresse do RE em uma PCR quantitativa, para corroborar com os resultados do DTT. Não houve mudanças nos parâmetros de crescimento nos tratamentos com NaCl. No entanto, o comprimento, a massa e o teor de sódio das plântulas diminuíram com o aumento da concentração de DTT. Com os tratamentos combinados de NaCl e DTT, o comprimento da parte aérea e as massas frescas e secas foram mantidos em níveis de controle. Por outro lado, os níveis de sódio diminuíram, em comparação ao tratamento com NaCl. Os genes analisados por qPCR revelaram que o NaCl foi capaz de induzir todos eles, exceto *SbbZIP60*, porém foi induzido com estresse combinados. Por fim, os resultados indicaram que as mudas de *S. bicolor* da variedade CSF 20 foram tolerantes ao sal e sensíveis ao estresse do RE. A combinação dos estresses restaurou a homeostase do RE estimulando uma diminuição do sódio via transportadores de membrana *SbNHX1*, *SbSOS1* e *SbPDI* RE-chaperone e o sensor *SbbZIP60* do RE.

Palavras-chave: *Sorghum bicolor.* Tolerância. Tunicamicina. UPR.

ABSTRACT

Plants have developed mechanisms to avoid harmful effects of Na^+ accumulation, such as the signaling pathway of carrier proteins Na^+/H^+ (NHX) and salt overly sensitive (SOS). Besides, endoplasmic reticulum (ER) could integrate plant cell response. Thus, we aimed to understand the effects of ER homeostasis impairment, and its relationship to salt stress during early stages of the *Sorghum bicolor* CSF 20 a salt-tolerant variety. Three days old seedlings were challenged with NaCl (0, 50, 75 and 100 mM), dithiothreitol (DTT) at 0, 2.5, 5.0 10.0 mM, and the combined NaCl and DTT treatments. Tunicamycin (TUN) was also used as a second inducer of ER stress in a quantitative PCR, to corroborate with DTT's results. There was no significant change in growth parameters under NaCl treatments. Nevertheless, seedling length, mass and Na^+ content were decreased as DTT concentration was increased. Under combined NaCl and DTT treatments, shoot length and fresh and dry masses were maintained at control levels. On the other hand, the levels of Na^+ were decreased, in comparison to NaCl treatment. Genes analyzed by qPCR revealed that NaCl was able to induce all of them, except for *SbbZIP60*, however it was induced under combined stresses. In conclusion, the results indicated that *S. bicolor* seedlings of CSF 20 variety were tolerant to salt and sensible to ER stress. The combination of both stresses restored the ER homeostasis promoting a decrease of Na^+ content via the membrane transporters *SbNHX1, SbSOS1*, and *SbPDI* ER-chaperone and the ER sensor *SbbZIP60*.

Keywords: *Sorghum bicolor.* Tolerance. Tunicamycin. UPR.

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1 INTRODUÇÃO

Nos últimos anos, o problema da salinidade no solo tem sido intensificado pela atividade humana, através das práticas de irrigação incompatíveis com as características físicas, químicas e mineralógicas do perfil do solo, além da má gestão agrícola, como: o desmatamento, a aplicação de fertilizantes de forma excessiva e o uso da água de irrigação de baixa qualidade (PLAUT; EDELSTEIN; BEN-HUR, 2013). O manejo inadequado da água e do solo, associados a baixa precipitação, a alta evapotranspiração, e as temperaturas extremas, que são características de regiões áridas e semiáridas, potencializam os efeitos da salinidade nessas regiões (AZEVEDO-NETO *et al*., 2004).

O estresse salino afeta diretamente e indiretamente o ambiente, uma vez que induz alterações significativas na cobertura vegetal e nas propriedades físicas e químicas do solo, gerando distúrbios aos ciclos biogeoquímicos e propiciando perda da biodiversidade (YADAV *et al.,* 2011). Nas plantas, a salinidade pode induzir diversas falhas nas funcionalidades fisiológicas, que variam dependendo do estádio de desenvolvimento da planta e do nível de salinidade (HOSSAIN *et al*., 2015). De modo geral, o excesso de sais altera a homeostase celular, prejudicando a fotossíntese, a síntese proteica, o metabolismo dos lipídios e a expressão gênica (AZEVEDO-NETO *et al*., 2004). Além disso, uma das primeiras respostas das plantas envolve a redução do crescimento vegetativo, seguida de inibição/cessação da expansão celular, que por sua vez, culmina na perda de produtividade das culturas (PARIDA; DAS, 2005). Deste modo diversas organelas são responsáveis pelo restabelecimento da homeostase celular e tolerância ao estresse.

Um mecanismo utilizado pelas plantas, a fim de evitar os efeitos danosos do acúmulo de Na^+ no citosol, é a exclusão ou compartimentação desse íon no vacúolo. Esse processo é realizado principalmente por proteínas transportadoras, chamadas de NHX (do inglês, Na^+/H^+*exchanger*) (MAATHUIS; AHMAD; PATISHTAN, 2014) e SOS (*Salt overly sensitive*) (JI, *et al*., 2013). O gene NHX é expresso nas raízes, folhas e tecidos florais e está localizado na membrana vacuolar, o tonoplasto (RODRÍGUEZ-ROSALES *et al*., 2008). A maioria das proteínas NHX estudadas medeiam o transporte eletroneutro de Na^+/H^+ e K^+/H^+, utilizando o gradiente de H^+ como força motriz (BASSIL; COKU; BLUMWALD, 2012). Algumas isoformas de NHX também estão envolvidas com a compartimentação de Na^+ em endossomos, sendo estas funcionalmente similares às NHX vacuolares (RODRÍGUEZ-ROSALES *et al*., 2009; BASSIL; COKU; BLUMWALD, 2012).

O transportador SOS1 é um transportador do tipo antiporte da membrana plasmática

que sob estresse salino reduz o nível do sódio pela exclusão do íon para o apoplasto das raízes e assim controlando o transporte para o xilema e consequentemente para as folhas (CUIM *et al*., 2011; BOSE, *et al*., 2014). Esse processo é dependente de energia e, portanto, acoplado à diferença eletroquímica de prótons geradas por três tipos de bombas transportadoras P-ATPase localizada na membrana plasmática e V-ATPase e PPase, localizadas no vacúolo (APSE; BLUMWALD, 2007).

Outro mecanismo que vem sendo investigado em plantas é a resposta do Retículo Endoplasmático (RE). A perturbação da homeostase do RE causada por estresse frequentemente promove a acumulação de proteínas mal dobradas no lúmen do RE, o qual dispara uma via de sinalização citoprotetora denominada UPR (*unfolded protein response*) que aumenta sua resposta (WALTER; RON, 2011). Em plantas, a via UPR é mediada por receptores IRE1 e homólogos dos receptores ATF6 (DENG; SRIVASTAVA; HOWELL, 2013; HOWELL, 2013), após perturbação da homeostase do RE esses dois receptores são ativados, resultando no aumento de chaperonas moleculares e ativação de mecanismos que aumentam a degradação proteica (ERAD). Em resposta ao estresse do RE IRE1 ativa o fator de transcrição bZiP60 que induz genes de resposta ao estresse (HOWELL, 2013; YANG *et al*., 2014). Além disso, dois ortólogos de ATF6, os fatores de transcrição bZIP28 e bZIP17 (DENG; SRIVASTAVA; HOWELL, 2013; HOWELL, 2013; YANG *et al*., 2014) são também ativados, sendo bZIP17 induzido por estresse salino (HENRIQUEZ-VALENCIA *et al*., 2015; LIU *et al*., 2007).

O sorgo (*Sorghum bicolor* (L.) Moench) é reconhecido por sua tolerância moderada aos estresses hídrico (TABOSA *et al*., 2002) e salino (TABOSA *et al*., 2007), podendo constituir em uma alternativa para cultivos sob tais condições. Alguns trabalhos têm demonstrado uma variedade tolerante (CSF20) ao estresse salino e se torna uma espécie promissora para esse tipo de estudo. Esses trabalhos relatam que a tolerância está associada à manutenção dos níveis de íons potássio e ainda à maior eficiência do sistema antioxidante, diminuindo o acumulo de ROS especialmente pela ação da superóxido dismutase SOD em plantas adultas (COSTA *et al*., 2005). Sob condições de estresse do RE, espécies reativas de oxigênio (ROS) são produzidas na organela de modo que as atividades de enovelamento proteico sejam restauradas (OZGUR, *et al*., 2014). Embora o mecanismo de resposta à ROS seja bem estabelecido durante a salinidade, a produção relacionada ao RE e sua sinalização ainda precisa ser detalhada, bem como o papel das ROS produzidas pelo citosol sobre as atividades do RE.

Diante destas evidências levantou-se a hipótese de que na variedade CSF20, consideradas tolerante ao estresse salino, a atividade dessas proteínas transportadoras é modulada pela homeostase do RE, controlando os níveis de ROS e assim melhorando a resposta

de plântulas durante as primeiras etapas de desenvolvimento. Na tentativa de responder essas perguntas foram realizados experimentos de indução do estresse salino e estresse do RE via DTT e Tunicamicina (um forte indutor do estresse do RE) e a investigação de parâmetros fisiológicos, bioquímicos e moleculares a fim de gerar novas informações para elucidar mecanismos que governam a tolerância aos indutores de estresse, e a relação com a homeostase do retículo endoplasmático em plantas de sorgo da variedade CSF20.

2 OBJETIVOS

2.1 Geral

Verificar se a tolerância ao estresse salino na cultivar de sorgo CSF20 é dependente da manutenção da homeostase do Retículo endoplasmático.

2.2 Específicos

a) Determinar se plântulas da variedade CSF 20 é tolerante ao estresse salino e do Retículo Endoplasmático;
b) Avaliar as alterações nos parâmetros de crescimento, avaliação dos íons Na^+ e K^+, quantificação de H_2O_2, MDA e enzimas antioxidativas da variedade de sorgo submetida ao estresse com DTT, NaCl e do estresse combinado (DTT+NaCl);
c) Verificar o comportamento do Retículo endoplasmático frente ao estresse salino por meio de expressão de genes por PCR em tempo real;
d) Avaliar a expressão dos sensores (bZIP60 e PDI) do Estresse do RE frente ao estresse salino na variedade CSF 20.

3 FUNDAMENTAÇÃO TEÓRICA

3.1 Salinidade

A salinidade do solo é classificada como um dos principais estresses ambientais que afetam a agricultura a nível mundial, causando prejuízos de bilhões de dólares em danos à safra todos os anos (JAMIL, 2011). Os sais no solo ocorrem como íons (formas eletricamente carregadas de átomos ou compostos), na região Nordeste os mais comuns são o sódio (Na^{+}), cálcio (Ca^{2+}), magnésio (Mg^{2+}), potássio (K^{+}), cloreto (Cl^{-}), sulfato (SO_4^{2-}), bicarbonato (HCO_3^{-}), carbonato (CO_3^{2-}), borato ($BO_3^{3-)}$ e nitrato (NO^{3-}) (FERREIRA *et al*., 2010) e quando a precipitação é insuficiente para lixiviar esses íons do solo, os mesmos se acumulam e as plantas absorvem esses nutrientes em excesso reduzindo o crescimento e desenvolvimento das mesmas (SHRIVASTAVA, 2015).

Segundo dados da FAO (*Food and Agriculture Organization of The United Nations*), os problemas de salinidade do solo estão cada vez maiores e assim diminuindo o potencial de produção a cada ano, com isso entre 0,3 e 1,5 milhões de hectares de terras agrícolas estão fora de produção e agravam os danos ambientais (FAO, 2015).

Vários fatores responsáveis tornam os solos salinos, dentre eles é possível citar o desmatamento, uso de fertilizantes químicos, altas temperaturas e baixos índices pluviométricos de algumas regiões que resultam em cultivos sob irrigação. Por sua vez quando feito de forma inadequada, utilizando água de baixa qualidade esse problema se agrava, afetando grande parte das terras irrigadas em todo o planeta (PLAUT; EDELSTEIN; BEN-HUR, 2013; SHRIVASTAVA, 2015).

Algumas técnicas de irrigação podem ser empregadas para a utilização de áreas salinas, na busca de condições adequadas para maximizar o crescimento das culturas (HUANG, 2018). Mesmo com métodos de irrigação, é preciso mais ações já que o problema com a salinidade é complexo e gera uma série de complicações, dentre essas ações para melhor uso do solo é possível citar: (1) lixiviação direta de sais; (2) domesticação de halófitas selvagens nativas para uso em sistemas agropastoris; (3) fitorremediação; (4) melhoria química e (5) plantio de variedades tolerantes ao sal (FAO, 2015). Para a obtenção de variedades tolerantes diversos grupos de pesquisa empregam técnicas de melhoramento genético a fim de criar variedades mais resistentes ao sal (WANG *et al*., 2012).

3.2 Estresse Salino em Plantas

Plantas sob estresse salino apresentam uma série de ajustamentos que são respostas a diversos fatores ambientais e hormonais e são regulados por genes relevantes (HUANG RUI-DONG, 2018). Os mecanismos bioquímicos e moleculares para a eliminação do excesso desses íons incluem: controle da absorção, acumulação seletiva, transporte para as folhas, compartimentalização, alterações no aparato fotossintético e na estrutura das membranas, indução de enzimas do sistema antioxidante e estimulação de fitohormônios (PARIDA; DAS, 2005; ESTEVES, 2008; TARI *et al*., 2013).

Vários genótipos de plantas tolerantes ao sal foram e estão sendo desenvolvidas, no entanto esses genótipos não são o suficiente para o problema em questão, já que apresentam algumas limitações quando expostos às condições no campo, sofrendo efeitos de múltiplos estresses ambientais, dificultando e tornando esse processo de seleção tão complexo que pode levar muito tempo para que os critérios de seleção sejam aplicados de forma a minimizar esses efeitos (JAMIL, 2011).

A respeito da tolerância a salinidade, as plantas são divididas em dois grupos: as glicófitas que são sensíveis na presença de determinadas quantidades de sais solúveis sobre o substrato e as plantas halófitas, que ao contrário das glicófitas conseguem tolerar elevadas quantidades de sais sem comprometer seu desenvolvimento (ACOSTA-MOTOS *et al*., 2017). Nas glicófitas o crescimento é inibido por concentrações entre 100-200 mM de NaCl, podendo resultar na morte das plantas, já as halófitas conseguem sobreviver na presença de altas concentrações de NaCl, cerca de 300-500 mM (MUNNS, 1986; PARIDA; DAS, 2005; FLOWERS, 2015).

No entanto, deve ser levado em consideração o estágio de desenvolvimento da planta, as condições de crescimento, fatores ambientais e o grau de tolerância da espécie (GÓMEZ-BELLOT, 2013).

Para o aumento da tolerância principalmente à seca e salinidade, as plantas acumulam solutos (osmólitos) para facilitar a maior absorção de água pelas raízes, hidratando a planta e ajudando na abertura estomática, dentre esses solutos destacam-se alguns carboidratos e compostos nitrogenados como glutamato, aspartato, glicina, prolina dentre outros (NOUNJAN *et al*., 2012). A prolina está entre um dos mais importantes osmólitos (TANG, 2015), além disso, possui propriedades antioxidantes que protegem algumas macromoléculas como as proteínas da desidratação ou proteínas de choque térmico (REDDY *et al*., 2015).

Para um solo ser considerado salino a sua condutividade elétrica (CE) em solução deve atingir valores de 4 dS m^{-1} (equivalente a 40 mM NaCl), gerando uma pressão osmótica de cerca de 0,2 MPa, esses valores podem causar toxicidade iônica, levando a clorose e necrose, devido ao acúmulo de Na^+ nas plantas, dependendo de cada espécie (ACOSTA-MOTOS *et al*., 2017).

Para evitar a toxicidade causada pelo íon Na^+ ele deve ser mantido em baixas concentrações que por sua vez requer a capacidade de evitar o acúmulo de Na^+ através dos tecidos e para isso as plantas desenvolveram a capacidade de armazenar esse íon no vacúolo. Esse processo é realizado por proteínas transportadoras, chamadas de NHX, localizadas no tonoplasto que são responsáveis pelo transporte eletroneutro de Na^+/H^+ e K^+/H^+, utilizando um gradiente de prótons (H^+) como força motriz (MAATHUIS, 2014).

Outro mecanismo de tolerância ao sal foi a descoberta de um transportador (SOS1) Na^+/H^+ presente na membrana plasmática, esse transportador é capaz de transportar Na^+ para fora das células e é acoplado ao antiporte de H^+, gerando um gradiente eletroquímico constituído por três tipos de bombas transportadoras: (P-ATPase) presente na membrana plasmática, V-ATPase e PPase, encontradas no vacúolo (BOSE, *et al*., 2014).

As alterações no aparato fotossintético comprometem a abertura estomática, causam perturbações na cadeia de elétrons e a inibição de enzimas do ciclo de Calvin, como a Rubisco. As alterações afetam outras organelas além dos cloroplastos, e nesses locais ocorre formação de quantidades elevadas de espécies reativas de oxigênio como consequência do transporte de elétrons. Algumas espécies tolerantes mantêm ou aumentam o seu conteúdo de clorofila a fim de manter a homeostase do processo de fotossíntese, enquanto que nas espécies mais sensíveis ocorre diminuição, agravando ainda mais os efeitos do estresse na planta (ACOSTA-MOTOS *et al*., 2017).

As espécies reativas de oxigênio (EROs) são induzidas em diferentes compartimentos celulares como mitocôndrias e cloroplastos e são resultado do metabolismo celular, no entanto o aumento do estresse oxidativo pode causar a oxidação de moléculas importantes para a célula e peroxidação lipídica (KANGASJÄRVI, 2014). As espécies reativas são produzidas pela excitação do O_2, podendo formar o oxigênio singleto ($^1O_2$), o peróxido de hidrogênio (H_2O_2) e os radicais superóxido ($^{\bullet}O_2^-$) e hidroxil ($HO^{\bullet}$) que possuem alta instabilidade e poder de reagir com outras moléculas (PARIDA; DAS, 2005). Os níveis das enzimas do sistema antioxidante podem sofrer alteração estando em situação de estresse, podendo ocorrer um aumento dessas enzimas em plantas tolerantes e uma diminuição em plantas mais sensíveis (ACOSTA-MOTOS *et al*., 2017). Como as (EROS) desencadeiam vias

de resposta ao estresse, diz-se que elas agem como sinalizadoras importantes e por isso são consideradas um indicador celular de estresse desencadeando essa via de resposta (MITTLER, 2002).

3.3 Retículo Endoplasmático e via UPR em plantas

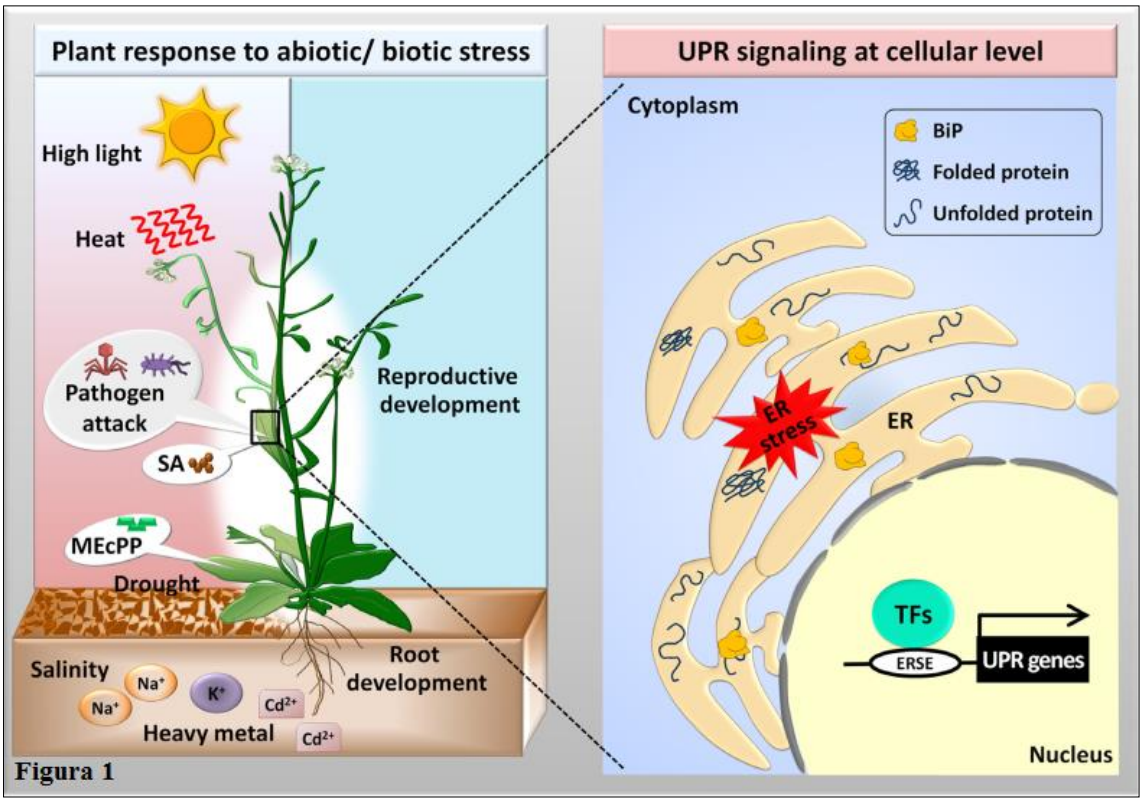
O sistema de endomembranas, incluindo o retículo endoplasmático (ER), o aparelho de Golgi, endossomas e outras organelas, é fundamental para a síntese, modificação, maturação e transporte de proteínas, constitui a via secretória das plantas, produzindo todas as moléculas que constituem membranas, parede celular ou participam dos mecanismos de defesa celular (NELSON & COX, 2014).

Plantas sob condições de estresse disparam vias de resposta as quais são importantes para a manutenção da homeostase e dessa forma restabelecer a atividade secretória, uma dessas vias (que é bem descrita em mamíferos) também é conhecida por reduzir danos causados pelo estresse e gerando respostas rápidas e eficientes, podendo dessa forma conferir diferentes níveis de tolerância às plantas (HOWELL, 2013).

Dentre essas fontes é possível citar os estresses de origem abiótica, como elevada intensidade luminosa, altas temperaturas, acúmulo de sais no solo (salinidade), metais pesados e baixa disponibilidade de água e por estresses bióticos causados por patógenos, por exemplo. UPR é ativada principalmente por proteínas mal dobradas que se acumulam no retículo endoplasmático (RE) resultando no aumento de chaperonas moleculares e ativação de mecanismos que aumentam a degradação proteica (ERAD) (WALTER; RON, 2011; HOWELL, 2013). Porém outros mecanismos que induzem a via UPR e a subsequente ativação de genes precisam ser investigados.

Essas respostas ativam os fatores de transcrição (TFs), que se ligam a elementos de resposta ao estresse do retículo endoplasmático (ERSEs) para que ocorra a regulação gênica para manter um equilíbrio homeostático (Figura 1). Importantes moléculas participam da sinalização para a ativação da via UPR, como 2-C-metil-D-eritritol-2,4-ciclopirofosfato (MEcPP) e ácido salicílico (SA), porém esses mecanismos ainda não foram elucidados (NAWKAR *et al*., 2018).

Figura 1 - Várias são as fontes que podem desencadear o estresse do retículo endoplasmático resultando na ativação da via UPR.

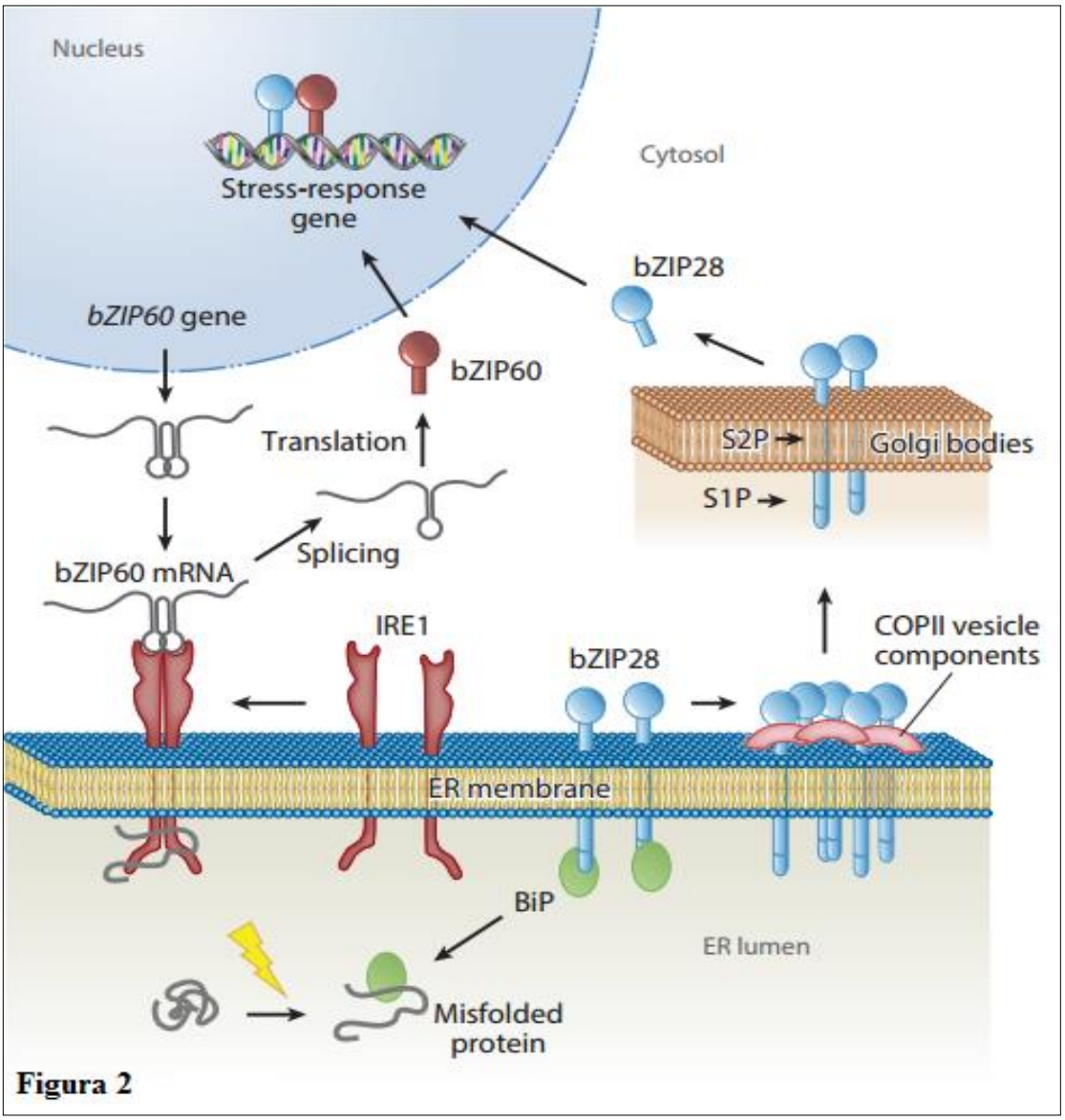


Fonte: (NAWKAR *et al*., 2018).

Em plantas, a via UPR é mediada por receptores de membrana IRE1 e homólogos dos receptores ATF6, IRE1 ativa o fator de transcrição (bZIP60) que induz genes de resposta ao estresse e ATF6 ativa os fatores de transcrição (bZIP17 e bZIP28) (HOWELL, 2013; YANG *et al*., 2014), sendo bZIP17 induzido por estresse salino (HENRIQUEZ-VALENCIA *et al*., 2015; LIU *et al*., 2007). A ativação desses sensores ocorre pelo acúmulo de proteínas mal dobradas no RE, disparando a via UPR.

O primeiro mecanismo envolve fatores de transcrição associados à membrana como bZIP28 e o outro é uma proteína quinase/ribonuclease (IRE1) que também está associada à membrana e ativa o fator de transcrição bZiP60. O bZIP28 é levado para corpos de Golgi onde são processados por S1P e S2P, que clivam o componente N-terminal do bZIP28 no citosol para que seja conduzido para o núcleo. IRE1 ativa mRNA que codifica bZIP60 direcionando-o também para o núcleo. Chegando ao núcleo o bZIP28 e bZIP60 podem sofrer heterodimerização e formar heterodímeros que podem atuar na regulação de genes de resposta ao estresse (HOWELL, 2013) (Figura 2).

Figura 2 - Mecanismo dos dois sensores da via de sinalização do RE encontrados em plantas.



Fonte: (HOWELL, 2013).

O mecanismo que envolve o dobramento de proteínas pode ser facilmente perturbado, servindo como meio pelo qual as plantas percebem e respondem ao estresse (LIU; HOWELL, 2010). O processo de dobramento é auxiliado por uma série de fatores presentes no retículo endoplasmático e quando essa demanda excede a capacidade do RE, sobrecarregando-o e causando acúmulo dessas proteínas, temos uma condição de estresse (VITALE, 2008).

A UPR tem sido descrita em plantas há alguns anos, principalmente em *Arabidopsis thaliana* (MARTINEZ; CHRISPEELS, 2003; KAMAUCHI *et al*., 2005) no entanto pouco se conhece a respeito dos mecanismos em outras espécies. Em mamíferos além dos dois sensores já descritos existe a presença de um terceiro sensor também associado à membrana que envolve uma proteína quinase que fosforila e inativa um fator de iniciação da tradução, eIF2a,

retardando a tradução (WALTER, 2011; HOWELL, 2013).

A UPR pode ser induzida por processos naturais como estresse térmico e salino (LIU; HOWELL, 2010), por agentes bióticos (HOWELL, 2013) ou por agentes como a tunicamicina, que interfere na glicosilação de proteínas, agentes redutores como o ditiotreitol (DTT) que impossibilita o dobramento adequado de proteínas contendo ligações dissulfeto já que as mesmas requerem um ambiente oxidante. Outro agente de estresse do RE é o ácido ciclopiazônico que inibe bombas de cálcio interferindo na função de chaperonas importantes para a dobra de proteínas no RE como a calnexina que é uma proteína de membrana, calreticulina, uma proteína presente no lúmen que são dependentes de cálcio e proteína de ligação luminal (BiP) (MICHALAK *et al*., 2009; NAWKAR *et al*., 2018).

O fator de ativação IRE1 é o considerado o mais antigo, porque é encontrado em leveduras, nematóides e mamíferos e não havia descrição desse fator em plantas, até a descoberta de dois genes que codificam IRE1 em *Arabidopsis* (KOIZUMI, 2001), e também através da descoberta do fator bZIP60 (associado a membrana) que era induzido por estresse e aumentava a expressão de genes da UPR (IWATA; KOIZUMI, 2012).

Ao passo em que as chaperonas moleculares são ativadas, o mecanismo de degradação protéica (ERAD) é aumentado a fim de manter a homeostase frente ao estresse do RE, o processo de degradação envolve etapas importantes, entre elas: reconhecimento (envolvendo ligases de ubiquitina), ubiquitinação, retrotranslocação e degradação (SMITH; PLOEGH; WEISSMAN, 2011; HOWELL, 2013).

3.4 Sorgo

O sorgo [*Sorghum bicolor* (L.) Moench] pertence à família Poaceae, e é o quinto cereal mais produzido no mundo, ficando atrás apenas do trigo, arroz, milho e cevada, tendo alcançado valores na produção mundial entre os períodos de 2013 a 2015 de 62,4 milhões de toneladas (FAO, 2017). Largamente cultivado em regiões tropicais áridas, semiáridas, subtropicais e regiões temperada sendo fonte importante na alimentação animal e em algumas regiões também é utilizado para o consumo humano e como matéria-prima para a fabricação de cerveja (HUANG, 2018).

O sorgo pode ser do tipo forrageiro, granífero, vassoura e sacarino, quando destinado à produção de forragem, produção de grãos, confecção de vassouras caseiras e produção de altos teores de açúcares que podem ser utilizados na fabricação de cervejas e outros processos, respectivamente (RAMATOULAYE *et al*., 2016).

É uma planta do tipo C4, típica de clima quente, de baixa exigência quanto à fertilidade de solo que apresenta características fisiológicas que lhe permitem sobreviver em condições adversas de temperaturas elevadas e ambientes secos, por exemplo, já que consegue reduzir sua atividade metabólica durante períodos de estresse e quando possível consegue restaurar esse equilíbrio (BONFIM-SILVA *et al*., 2012) e já foi comprovado o seu efeito na tolerância moderada ao estresse salino (TABOSA *et al.,* 2007; FREITAS, 2011) e hídrico (TABOSA *et al*., 2002) podendo sobreviver e crescer em condições adversas de temperatura e secas (SHAHBAZ, 2013) tornando-se uma boa espécie para estudos envolvendo salinidade.

Essa tolerância também é consequência de características morfológicas do sistema radicular e da parte aérea, as raízes são ramificadas e finas, permitindo que a planta consiga absorver mais água e na parte aérea existe uma camada espessa de cera que recobre a epiderme das folhas impedindo a perda excessiva de água por transpiração tornando mais difícil a desidratação (VIEIRA,2006; SILVA, 2003).

4 ARTIGO

Combined NaCl and DTT diminish harmful ER-stress effects in the sorghum seedlings CSF 20 variety

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Abstract

Plants have developed mechanisms to avoid harmful effects of Na^+ accumulation, such as the signaling pathway of carrier proteins Na^+/H^+ (NHX) and salt overly sensitive (SOS). Besides, endoplasmic reticulum (ER) could integrate plant cell response. Thus, we aimed to understand the effects of ER homeostasis impairment, and its relationship to salt stress during early stages of the *Sorghum bicolor* CSF 20 a salt-tolerant variety. Three days old seedlings were challenged with NaCl (0, 50, 75 and 100 mM), dithiothreitol (DTT) at 0, 2.5, 5.0 10.0 mM, and the combined NaCl and DTT treatments. Tunicamycin (TUN) was also used as a second inducer of ER stress in a quantitative PCR, to corroborate with DTT's results. There was no significant change in growth parameters under NaCl treatments. Nevertheless, seedling length, mass and Na^+ content were decreased as DTT concentration was increased. Under combined NaCl and DTT treatments, shoot length and fresh and dry masses were maintained at control levels. On the other hand, the levels of Na^+ were decreased, in comparison to NaCl treatment. Genes analyzed by qPCR revealed that NaCl was able to induce all of them, except for *SbbZIP60*, however it was induced under combined stresses. In conclusion, the results indicated that *S. bicolor* seedlings of CSF 20 variety were tolerant to salt and sensible to ER stress. The combination of both stresses restored the ER homeostasis promoting a decrease of Na^+ content via the membrane transporters *SbNHX1, SbSOS1*, and *SbPDI* ER-chaperone and the ER sensor *SbbZIP60*.

Keywords: Endoplasmic reticulum, **s**alinity, salt stress tolerance, tunicamycin, UPR.

Abbreviations

APX, ascorbate peroxidase;

bZIP60, basic leucine zipper transcription factor 60;

CAT, catalase;

DTT, dithiothreitol;

ER, endoplasmic reticulum;

GPX, guaiacol peroxidase;

NHX, Na^+/H^+ exchangers

PDI, protein disulfide isomerase;

ROS, reactive oxygen species;

Sb, *Sorghum bicolor*

SOD, superoxide dismutase;

SOS, salt overly sensitive

TUN, tunicamycin;

UPR, unfolded protein response;

VHA2, vacuolar H^+-ATPase 2.

1. Introduction

Salinity is described as an accumulation of soluble ions in soils that cause impacts on ecosystems, crop yield, and the economy (QADIR et al., 2014; ZÖRB; GEILFUS; DIETZ, 2019). Inadequate agricultural management practices, such as deforestation, excessive use of fertilizers, and the management of low-quality irrigation water associated with low precipitation, high evapotranspiration, and severe temperatures which are typical of arid and semi-arid areas make the salinity impacts more evident in these regions (ABUELGASIM; AMMAD, 2019; CABRAL JÚNIOR et al., 2019). Regarding the impact of salinity on soil, several disturbances in plant physiology are induced by it, in which the extent depends on the development stage, salinity level, or time of exposure (ISAYENKOV; MAATHUIS, 2019; SAFDAR et al., 2019). The responses occur within minutes to days, being related to Na^+ sensing and signaling (KÖSTER et al., 2019; WU, 2018). Salinity alters the plant cell homeostasis, impacting essential processes like photosynthesis, protein synthesis, lipid metabolism, and gene expression, culminating in crop productivity losses (AZEVEDO NETO et al., 2006; KEUTGEN; PAWELZIK, 2009; RADANIELSON et al., 2018).

Plants employ an efficient mechanism to avoid the damaging effects of Na^+ accumulation. This process is mainly carried out by a group of a plant antiporters, called Na^+/H^+ *exchanger* (NHX), as a major player in the vacuolar cation movement (MAATHUIS; AHMAD; PATISHTAN, 2014), and salt overly sensitive (SOS) signaling pathway, that include SOS1, SOS2, and SOS3 proteins carriers, responsible to the exclusion of cytosolic Na^+ to apoplast (JI et al., 2013). The *NHX* gene is expressed in the roots, leaves, and floral tissues, its protein is located in the vacuolar membrane, the tonoplast (RODRÍGUEZ-ROSALES et al., 2009). Most of the NHX proteins studied mediate the transport of Na^+/H^+ e K^+/H^+, using the H^+ gradient as driving force (BASSIL; COKU; BLUMWALD, 2012). NHX isoforms are also involved in the Na^+ partitioning in endosomes, however, they are functionally similar to the vacuolar NHX (RODRÍGUEZ-ROSALES et al., 2009). The SOS1 protein is a plasma membrane antiporter which, under salt stress, reduces the sodium level by excluding the ion to the root apoplast, thus controlling the transport to xylem and consequently to the leaves (BOSE et al., 2014; YATOO et al., 2018). This process is energy-dependent regulated by SOS2 and SOS3 cytosolic proteins. Therefore, coupled to the electrochemical difference of protons generated by three types of transport pump: P-ATPase placed on the plasma membrane, and V-ATPase and PPase, located in the vacuole (APSE; BLUMWALD, 2007).

Several biotic and abiotic stresses as salinity also impair the endoplasmic reticulum

(ER) homeostasis by an accumulation of unfolded or misfolded proteins in the lumen (BAO; HOWELL, 2017; PARK; PARK, 2019). It promotes cell death when ER stress is severe, or it triggers a cytoprotective signaling pathway called unfolded protein response (UPR), to communicate ER and nucleus via down or upregulation gene expressions, thus enhancing its response (NAWKAR et al., 2018). In yeasts and metazoans, UPR consists of three transmembrane ER-resident conserved sensors, activating transcription factor 6 (ATF6), inositol-requiring protein 1 (IRE1), and protein kinase RNA (PKR)-like ER kinase (PERK) (WALTER; RON, 2011). In *Arabidopsis thaliana*, only two main types of ER stress sensors were found. First, the transmembrane protein IRE1 activates the basic leucine zipper transcription factor 60 (bZIP60), second, another ER arm is activated composed by bZIP28, and bZIP17 inducing downstream genes (DENG; SRIVASTAVA; HOWELL, 2013; HOWELL, 2013; YANG et al., 2014). The UPR has been clearly implicated in plant development and defense (BAO; HOWELL, 2017; KIM; YAMAGUCHI-SHINOZAKI; SHINOZAKI, 2018), as well as in responses to heat (NEILL et al., 2019), drought (CARVALHO et al., 2014), and salt stresses (GUAN et al., 2018; HENRIQUEZ-VALENCIA et al., 2015; LIU et al., 2007). Under normal conditions, the luminal domains of these transmembrane stress sensors are kept inactive through ER-resident chaperones (DENG; SRIVASTAVA; HOWELL, 2013; HOWELL, 2013; WAN; JIANG, 2016). Since these receptors are activated, the result is an increase of molecular chaperones, and activation of UPR or ER-associated protein degradation (ERAD), therefore protein quality control is guaranteed (KOENIG; PLOEGH, 2014). Mostly, UPR has been studied using interference of the post-translational protein changes by chemicals. For example, tunicamycin (TUN) promotes inhibition of N-glycosylation, dithiothreitol (DTT) acts by interruption to disulfide bonds, and azetidine-2-carboxylic acid (AZC) inhibits in the formation of native protein structures (HOWELL, 2013). Thus, in plants models, ER response brought new insights on crop yield improvement and putative mechanisms to deal with environmental stresses.

A C4 metabolism grass *Sorghum bicolor* (L.) Moench belongs to the POACEAE family, it is known for its mild tolerance, and the ability to withstand several types of pressures, especially to water, saline, and heat stresses (OLIVEIRA; GOMES-FILHO, 2009; PENNISI, 2009; TABOSA et al., 2007). Thus, it is a promising species for crop improvement since several varieties have shown different behavior when irrigated with salinized waters (GUIMARÃES et al., 2018; KAUSAR; GULL, 2019). The CSF 20 variety stands out as a relatively salt-tolerant variety when compared to other varieties. Such salt response has been associated with the maintenance of potassium ion levels and the higher efficiency of the antioxidant system,

decreasing reactive oxygen species (ROS) accumulation, especially by the action of superoxide dismutase (SOD), and improvement of photosynthetic apparatus under salinity (COELHO et al., 2018; FREITAS et al., 2019; SILVA et al., 2003). Besides, under ER stress ROS are also produced to restore protein folding activities (OZGUR et al., 2014). Although the mechanism of ROS response is well established during salinity, its production in relation to ER and signaling still needs to be detailed, as well as the role of ROS produced by the cytosol on ER activities.

Based on that, we aimed to understand if in the CSF 20 variety of *Sorghum bicolor* the restoration of ER homeostasis is related to the activation of Na^+ transporters, improving the salt responses during the early stages of development. In the attempt to address such question, we performed experiments to induce the ER and salt stresses via different concentrations of NaCl and, DTT and TUN (two stronger inducers of ER stress). Additionally, the investigation of physiological, biochemical, and molecular mechanisms provides new information to elucidate mechanisms that govern salt tolerance as well as the relation ER homeostasis as fundamental for a satisfactory response, especially in sorghum plants of the variety CSF 20.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of sorghum [*Sorghum bicolor* (L.) Moench] variety CFS20, obtained from the Instituto Agronômico de Pernambuco (IPA), Brazil, were peeled and superficially treated with 2% sodium hypochlorite under shaking, then washed several times in distilled water. For each treatment 20 seeds were sown between two layers of one folded sheet of autoclaved germitest-type paper-towns (28 cm x 38 cm), previously moistened with 20 mL of distilled water (2.5 times the dry mass of paper). They were rolled up and arranged in glass bottles plus 5 ml of distilled water in the bottom, and then covered to a plastic bag and kept to BOD (Biochemical Oxygen Demand) chamber at constant temperature of 30 °C at day, and 26 °C at night, relative humidity of 90%, and photoperiod of 12 h. After three days of sowing, the seedlings were selected by uniformity, vigor, and sanity, then transferred to a new autoclaved germitest-type paper-towns moistened with 20 mL of Clark nutrient solution, pH 6.0 in half-strength to provide macronutrients [$(NH_4)_2SO_4$; KH_2PO_4; KNO_3; KCl; $Ca(NO_3)_2$; $MgSO_4$], micronutrients and Fe-EDTA (CLARK, 1975). Then, each treatment was arranged as described before and returned to the BOD chamber. After 4 days of treatments, 10 plants composed a repetition and used as fresh or dried material, or they were frozen in nitrogen and kept at -80 °C

for further analysis.

2.2. Experimental design and treatments

The experiments were randomized in BOD chamber as described before. In all of them, three-day-old seedlings were submitted to treatments for four days. The first round was composed by four concentrations of NaCl [0 (control), 50 mM, 75 mM, and 100 mM]. The second round of experiment was composed by four concentrations of dithiothreitol (DTT) [0 (control), 2.5 mM, 5.0 mM, and 10.0 mM], the values were normalized by control and used to construct a radar plot. Based on these results, we carried out a third experiment, in which seedlings were submitted to isolated NaCl 75 mM and DTT 10 mM treatments, and a combination of both chemicals. For qPCR assay, a fourth experiment was performed using 2.5µg/mL tunicamycin (TUN) as a single treatment or combined with 75 mM NaCl, totalizing six treatments. For all experiments we considered control formed by seedlings growing in the absence of chemicals. Experimental conditions were composed by four or five replicates, been 10 different biological plants per replicate. Tunicamycin was purchased from Sigma-Aldrich and diluted in dimethyl sulfoxide, then in distilled water.

2.3. Growth measurements

After each treatment, the length of each shoot and root were obtained using ImageJ software (RASBAND, 2016). Fresh mass of shoots (SFM) and roots (RFM) were measured using an electronic balance. Then, they were stored in paper bags, kept oven-dried at 60 °C for 72 h, after that shoots and roots dry masses (SDM and RDM, respectively) were taken, as well as the ratio of tissues (SDM/RDM). The heights were expressed in cm plant^{-1}, and fresh or dry mass were expressed in mg plant^{-1}.

2.4. Leaf and root ion content

Inorganic Na^+ and K^+ ions measurements were performed homogenizing 50 mg of dried leaf or root tissues ground with a mortar and pestle, added to 1 mL of deionized water (CATALDO et al., 1975). Then, the homogenate was maintained in a water bath at 40 °C for 1 h, shaking it every 20 min, then 15 min centrifuged at 3,000 g. The clear supernatant was used for ions quantification by flame photometry [Micronal®, model B462 (São Paulo, São Paulo, Brazil)] and expressed as µmol g^{-1} de dry mass (DM) (MALAVOLTA; VITTI; OLIVEIRA, 1989).

2.5. Lipid peroxidation and hydrogen peroxide evaluation

Lipid peroxidation was evaluated by TBARS, thiobarbituric acid reactive substances (CAKMAK; HORST, 1991). A fresh 0.5 g of tissue was crushed and homogenized in 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 x *g* for 20 min at 4 °C, and the supernatant was transferred to a new 2.0 ml tube. 100 µl of clear supernatant was added in 1.0 ml of 20% TCA, containing 0.5% thiobarbituric acid (TBA) and 0.1 M, pH 7.0 phosphate buffer. The mixture was heated at 90°C for 20 min and quickly cooled in an ice-bath. TBARS quantification was performed from the non-specific (600 nm) specific (535 nm) absorbance readings performed on a microplate spectrophotometer (Synergy ™ Mx Model, BioTek). malondialdehyde-reactive concentration was calculated from the molar extinction coefficient (ε = 155 mM^{-1} cm^{-1}) using the Beer-Lambert equation. Hydrogen peroxide (H_2O_2) content was extracted according to described above, and determined by monitoring the absorbance of potassium iodide at 390 nm (MUHAMMAD, 2016). The H_2O_2 content of leaves and roots were quantified by spectrophotometric readings at 390 nm by reference to a standard curve prepared with H_2O_2 solutions (INGRAM, 1976). The values were expressed as mmol g^{-1} FM.

2.6. Antioxidant enzymes assay

Crude protein extracts were prepared by homogenizing 0.1 g of fresh frozen shoots and roots added to 5.0 mL of extraction buffer (100 mM potassium phosphate, pH 7.0, containing 0.1 mM EDTA) in a cold mortar (KANG et al., 2011). The homogenate was filtered through a nylon cloth and centrifuged at 12,000x*g* for 15 min at 4 °C. The clear supernatant was saved and used for total proteins and the enzymatic activity assays described next. Catalase (CAT; EC 1.11.1.6) enzyme activity was measured by monitoring H_2O_2 breakdown at 240 nm (ε = 36 M^{-1} cm^{-1}) (BEERS; SIZER, 1952). Guaiacol peroxidase (GPX, EC 1.11.1.7) was performed by monitoring the increase in absorbance at 470 nm (ε = 26.6 mM^{-1} cm^{-1}) due to tetraguaiacol formation, which one mol of tetraguaicol correspond to four moles of H_2O_2 consumed (PLEWA; SMITH; WAGNER, 1991). For ascorbate peroxidase (APX,1.11.1.11) activity determination, 2.0 mM ascorbic acid (AsA) was added to the extraction buffer (NAKANO; ASADA, 1981), then it was measured by monitoring absorbance decreasing at 290 nm (ε = mM^{-1} cm^{-1}). CAT, GPX, and activities were both expressed as µmol H_2O_2 min^{-1} mg^{-1} protein. The activity of superoxide dismutase (SOD, EC 1.15.1.1) enzyme was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride

(NBT) at 560 nm (GIANNOPOLITIS; RIES, 1977). One SOD activity was expressed in unit (U) mg^{-1} protein, which was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction rate per minute.

2.7. Expression analysis by qPCR

Total RNA was isolated from each root and shoot tissues using an SV Total RNA Isolation System - Promega Corporation, according to the manufacturer's protocol. The RNA extracted was quantified (NanoDrop 2000 spectrophotometer, Thermo Scientific TM, Waltham, USA) and integrity checked after electrophoresis in 1.5% (m/v) agarose gel done in a Pharmacia Biotec electrophoresis system, at 50 mA, 100 V. cDNA libraries were carried out using M-MLV reverse transcriptase in accordance with the manufacturer. The procedure employed for the reverse transcription-polymerase chain reaction (RT-PCR) was divided into two steps. The first step consisted of RNA, RNase-free water, and oligo(dT)s incubation at 70 ºC for 5 min, following by chilling at 4 ºC for 5 min. The second step consisted of adding the RNase inhibitor, Reverse Transcription Mix and oligo(dT)s and annealing at 25 ºC for 5 min, following by elongation at 42 ºC for 60 min, and enzyme denaturation at 70 ºC for 15 min. The synthesized cDNA was kept under -20 ºC until used.

Quantitative PCR (qPCR) was performed on a RealPlex 4S thermocycler (Eppendorf®) by detecting fluorescence levels. All qPCR amplifications of target and reference genes were carried out in biological triplicates in a total volume of 20 µL according to the manufacturer's instructions of GoTaq qPCR Master Mix (Promega). The amplification reactions were carried out through 40 thermal cycles, composed of 15 s at 95 °C, followed by 15 s at a specific annealing temperature for each primer (Supplemental table S1) and finally at 20 s at 60 °C. Initial denaturation was performed at 95 °C for 2 min. Primer efficiency was determined by the dilution method, where were obtained efficiency between 95-100%. Melting curves were performed in order to verify the absence of unspecific products and dimer formation. The levels of relative expression of *SbbZIP60*, *SbPDI*, *SbNHX1*, *SbSOS1*, SbVHA2 of roots and shoots were determined using the mean Ct (Cycle threshold) values. The Housekeeping genes were previously evaluated (MIRANDA et al., 2017), thus SbUBC 18 was used as the reference to normalize the amount of cDNA in each reaction. The relative quantification of transcripts was done using the $2^{-\Delta\Delta CT}$ method (LIVAK; SCHMITTGEN, 2001), the primer sequences used in this work were also provided (Supplemental table S1).

2.8. Statistical analysis

The data were subjected to analysis of variance (ANOVA, F-test), and the significant difference between the means was performed using Tukey test ($p \leq 0.05$) and SISVAR software (FERREIRA, 2011).

3. Results

3.1. Growth parameters of sorghum seedlings are not changed by increasing NaCl concentrations

In order to evaluate sorghum salt sensibility at the beginning of development, three days old seedlings were submitted to increasing concentrations of NaCl [0 (control), 50, 75 and 100 mM] for four days, totalizing seven days of plant development. From the lowest (0 mM) to the highest (100 mM) concentration of NaCl, both shoots and roots showed similar growth patterns (Figure 1A and Figure S1). A detailed Tukey's significance test ($p \leq 0.05$) is also provided in supplemental table S2. None of the growth parameters measured had significant changes, such as length, fresh and dry mass, and their respective ratios. The only exception was the fresh mass of shoots, in which both 75 and 100 mM NaCl promoted a significant decrease in the mean value.

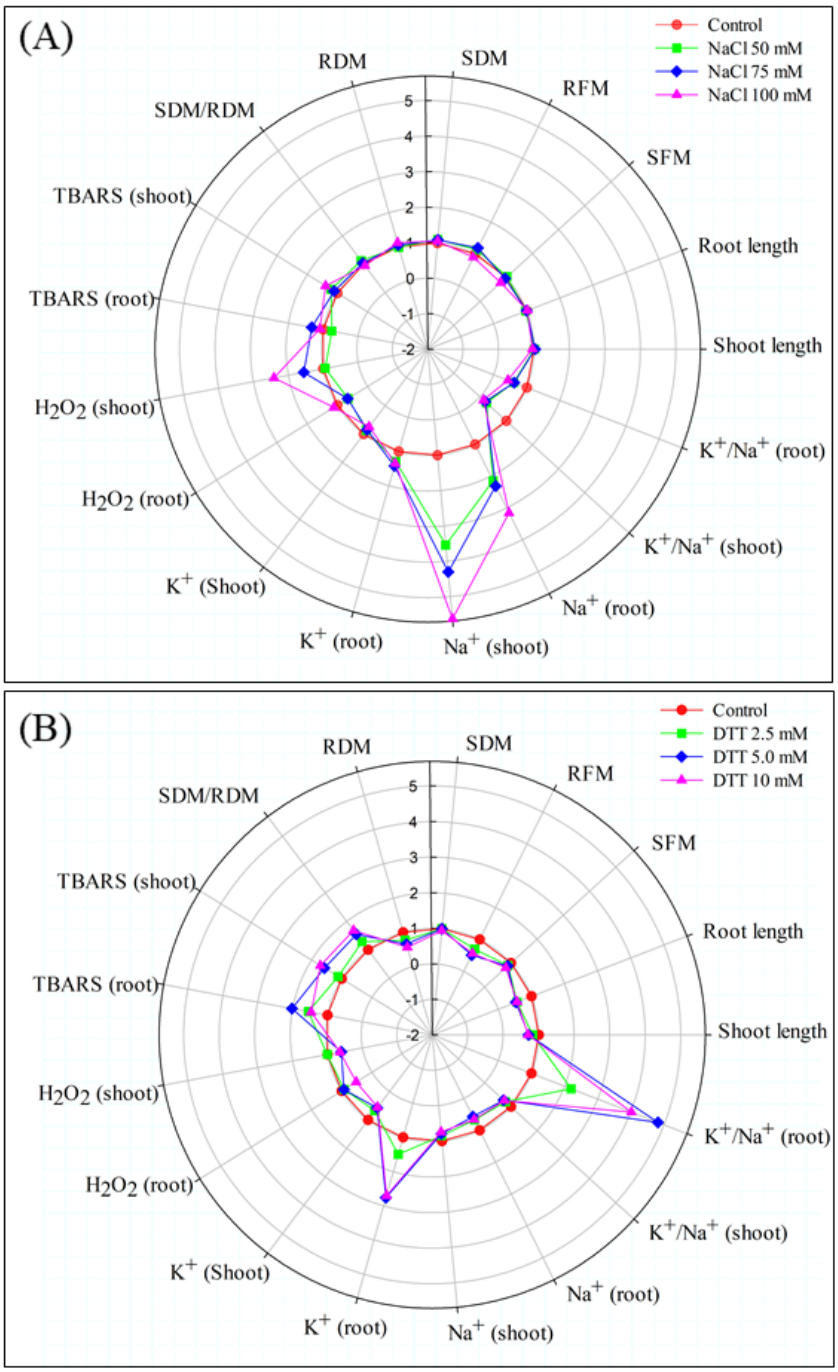

Figure 1. Radar plot representation of *Sorghum bicolor* seedlings responses. Seedlings after three days of sowing and submitted for four days to an increasing concentration of NaCl (A) or DTT (B). The control treatment was growing in the absence of NaCl or DTT, then it was taking as a normalized value (1.0). Meaning abbreviations are represented by root and shoot dry masses (RDM and SDM), respectively; root and shoot fresh masses (RFM and SFM), respectively; the ratio of tissues dry masses (SDM/RDM); thiobarbituric acid reactive substances (TBARS). Data are relative to three biological replicates, a detailed Tukey's

significance test ($p \leq 0.05$) was done before normalization and it is provided in supplementary table S2 and S3.

3.2. Ions contents and reactive oxygen species were changed by increasing NaCl concentrations

The increase of salt concentration also changed the content of both Na^+ and K^+ ions (Figure 1A and supplemental table S2). The K^+ content was decreased in shoots and increased in roots after increasing NaCl concentrations. On the other hand, both shoots and roots exhibited an over-accumulation of Na^+, following the increase of NaCl concentration. The K^+/Na^+ ratios in shoots and roots were also significantly reduced by salinity exposure. However, the increasing NaCl concentration from 50 mM did not aggravate such unbalance of these two ions. Additionally, in shoots, salinity promoted a significant accumulation of both TBARS and H_2O_2 contents only at NaCl 100 mM. However, in the roots, TBARS and H_2O_2 contents were not changed by salinity.

3.3. Growth parameters of sorghum seedlings are impaired by increasing DTT concentrations

As performed during NaCl treatments, three days old seedlings were submitted to different concentrations of DTT [0 (control), 2.5, 5.0 and 10.0 mM) for four days, totalizing seven days of plant development. The presence of DTT from 2.5 mM negatively affected both shoot and root length, as well as fresh and dry masses, however, the shoots/roots ratios of dry mass were significantly increased at 5.0 mM and 10 mM (Figure 1B, and Figure S2). A detailed Tukey's significance test ($p \leq 0.05$) is provided in supplemental table S3.

3.4. Ions contents and reactive oxygen species were changed by increasing DTT concentrations

The presence of different DTT concentrations affected the accumulation of both Na^+ and K^+ ions in different ways (Figure 1B, and Supplemental table S3). In shoots, the K^+ and Na^+ contents were decreased, including K^+/Na^+ ratios, when it was compared to control seedlings. In roots, the K^+ content was increased, as well as K^+/Na^+ ratio, whilst Na^+ content was decreased in all DTT doses. Also, the presence of DTT promoted a significative accumulation of TBARS in shoots from 5 mM, and in roots from 2.5 mM. On the other hand, H_2O_2 contents were decreased in shoots from 5 mM, and in roots at 10 mM.

3.5. Combined NaCl and DTT treatments recover sorghum seedlings growth and decrease Na^+ content in roots

Based on our previous results, to combine both treatments we took NaCl 75 mM, which the plant responses were close to control seedlings, and the highest concentration of DTT (10 mM), which the responses were different from the verified in control seedlings. Furthermore, recent works have reported that 75 mM NaCl is considered a high concentration to induce responses in sorghum leaves (FREITAS et al., 2019; MIRANDA et al., 2017). Then, we carried out a set of experiments to evaluate whether sorghum salt tolerance in the first stage of seedling development is affected by DTT. Moreover, if the sorghum-sensibility to DTT is ameliorated or aggravated by the presence of salt. Three days old seedlings were submitted to isolated NaCl 75 mM and DTT 10 mM treatments, as presented above, followed by a combination of both chemicals for 4 days, totalizing 7 days of seedling development.

The combined NaCl and DTT mM treatments did not promote significant changes in growth parameters if compared to control seedlings, as observed by length, fresh and dry masses (Figures 2, and Figure S3), except by SFM.

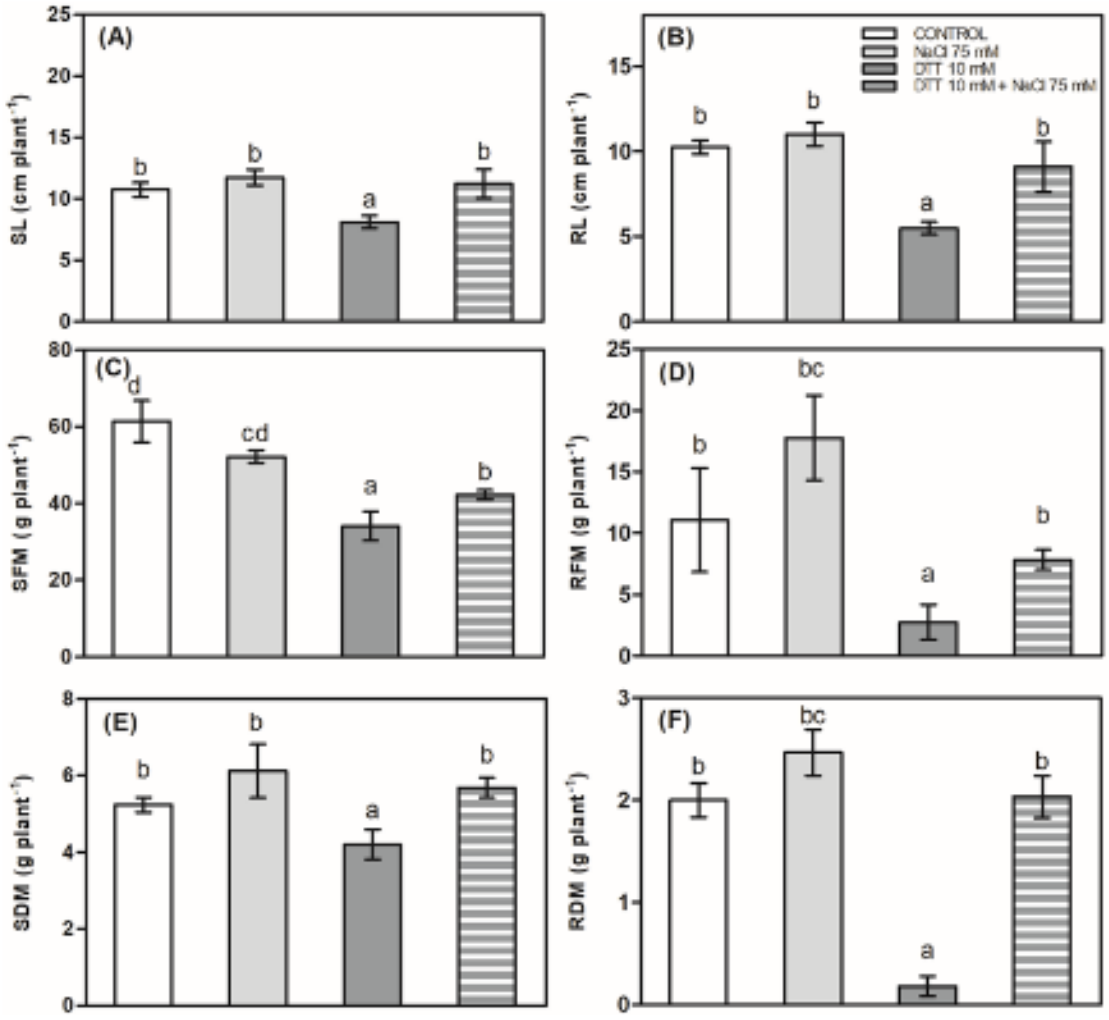

Figure 2. Growth measurements of *Sorghum bicolor* seedlings after three days of sowing and submitted for four days to 75 mM NaCl, 10 mM dithiothreitol (DTT), and the combination of both. The control treatment was growing in the absence of NaCl or DTT. Meaning abbreviations are represented by shoot and root length (SL and RL), respectively (A and B); shoot and root fresh masses (SFM and RFM), respectively (C and D); shoot and root dry masses (SDM and RDM), respectively (E and F). Different lowercase letters indicate significant differences among treatments according to Tukey's significance test ($p \leq 0.05$). Columns indicate means of three biological replicates and bars indicate standard deviation (±).

However, if it was compared to single DTT, the seedling length and masses were all increased. The K^+ contents of combined treatment were decreased in shoots, as well as shoot K^+/Na^+ ratio when compared to control plants, but in roots the content of K^+ was increased (Figure 3A, B, and E). In contrast, the Na^+ content was increased only in shoots (Figure 3C). When compared to single NaCl treatment, the levels Na^+ of combined treatment were decreased in both shoot and roots (Figures 3C, D), while the K^+/Na^+ ratio was not change (Figure 3F). Moreover, when compared to single DTT treatment, the combined treatment decreased the K^+ content in roots (Figure 3B) and increased the level of Na^+ in both roots and shoots (Figure 3C, D).

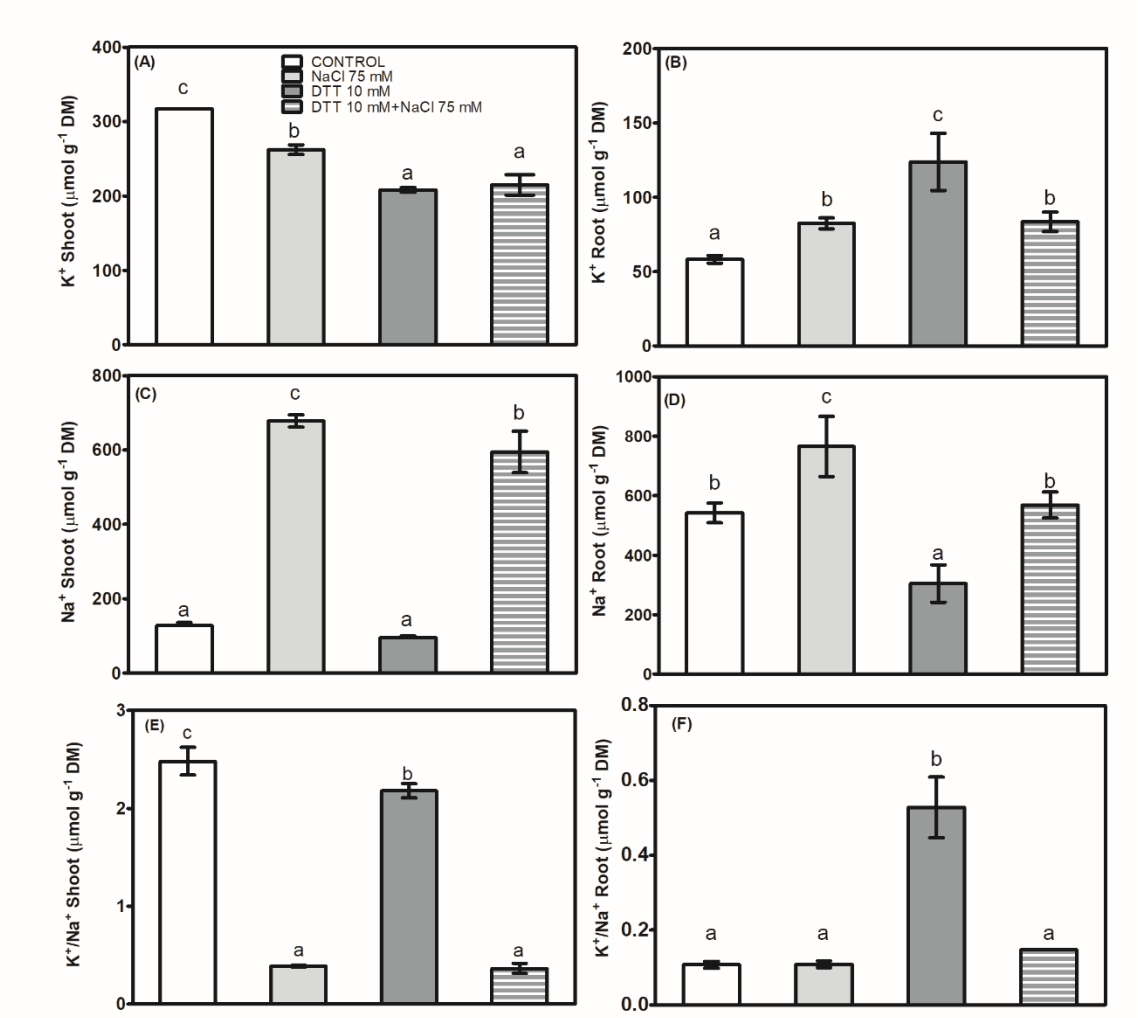

Figure 3. Ion contents of *Sorghum bicolor* seedlings after three days of sowing and submitted for four days to 75 mM NaCl, 10 mM dithiothreitol (DTT), and the combination of both. The control treatment was growing in the absence of NaCl or DTT. Na^+, K^+ contents and its ratios were measured in the shoots (A, C and E) and roots (B, D and F). Different lowercase letters indicate significant differences among treatments according to Tukey's significance test ($p \leq 0.05$). Columns indicate means of three biological replicates and bars indicate standard deviation (±).

Antioxidative responses of sorghum seedlings during combining treatments were also evaluated. In both roots and shoot, the content of TBARS of combined treatment was slightly higher than control treatment (Figure 4A, B). Although it was lower than single NaCl or DTT treatments in shoots and lower than single DTT in roots. Combined treatment also kept the same H_2O_2 content as that of control treatment (Figure 4C and D). In contrast, it was increased when compared to DTT treatment.

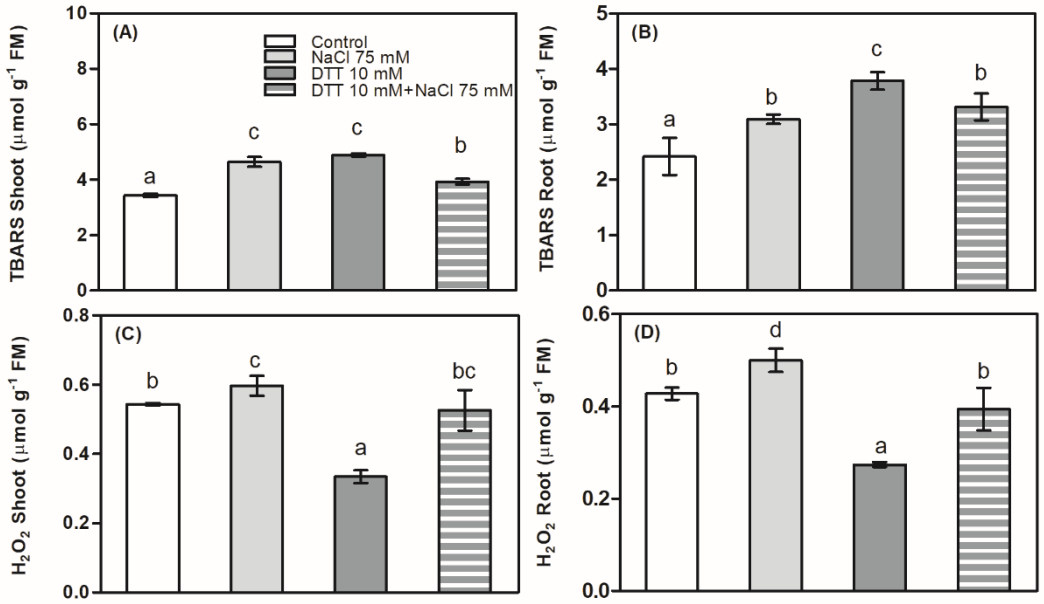

Figure 4. Thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide (H_2O_2) contents of *Sorghum bicolor* seedlings after three days of sowing and submitted for four days to 75 mM NaCl, 10 mM dithiothreitol (DTT), and the combination of both. The control seedlings were growing in the absence of NaCl or DTT. The TBARS and H_2O_2 contents were determinate in shoots (A and C) and roots (B and D). Different lowercase letters indicate significant differences among treatments according to Tukey's significance test ($p \leq 0.05$). Columns indicate means of three biological replicates and bars indicate standard deviation (±).

Antioxidant enzymes activity of single and combined treatments were evaluated and compared to control treatment as well. No significative changes in CAT, APX, GPX, and SOD activities were found in shoots of all treatments (Figures 5A, C, E, G). However, in roots, CAT activity was decreased by DTT and combined treatments (Figure 5B), both APX and GPX activities were reduced by NaCl, DTT, or combined treatment of these two chemicals compared to control (Figures 5D, F), although during combined treatments the activities of these enzymes presented reduction compared to single DTT treatment, being similar to single NaCl treatment. In opposite, SOD activity was decreased only in the single DTT treatment, and under combined treatments the values were kept at control values (Figure 5H).

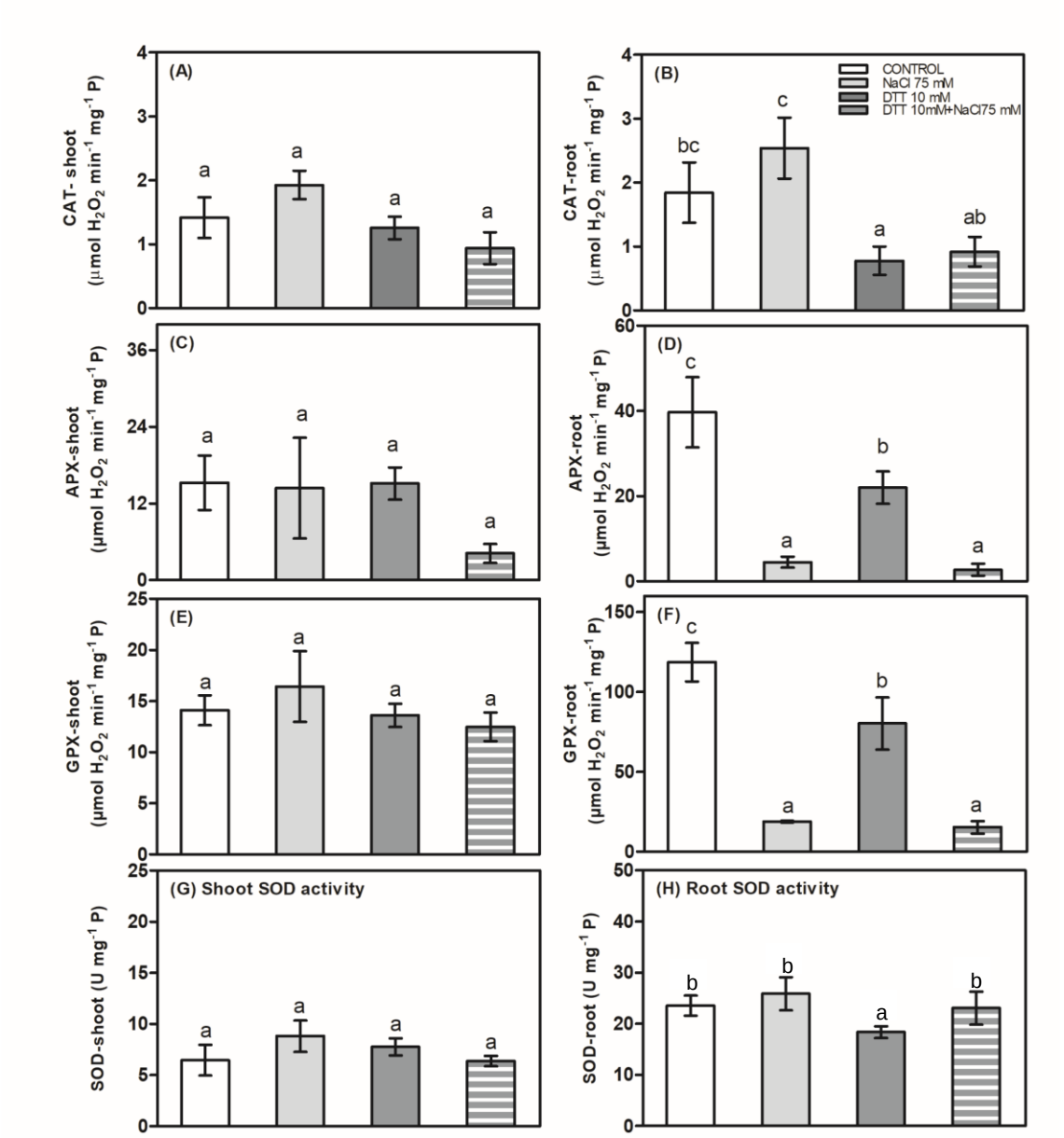

Figure 5. Antioxidant enzymes activity of *Sorghum bicolor* seedlings after three days of sowing and submitted for four days to 75 mM NaCl, 10 mM dithiothreitol (DTT), and the combination of both. The control seedlings were growing in the absence of NaCl or DTT. The activities were determinate in shoots (A, C, and E) and roots (B, D, F). Meaning abbreviations are represented by catalase (CAT); ascorbate peroxidase (APX); guaiacol peroxidase (GPX); superoxide dismutase (SOD). Different lowercase letters indicate significant differences among treatments according to Tukey's significance test ($p \leq 0.05$). Columns indicate means of three biological replicates and bars indicate standard deviation (±).

3.6. ER-related genes are up-regulated during DTT and TUN treatments

Aiming to evaluate whether single NaCl treatment or the combination with DTT or

tunicamycin treatments can induce ER responses, the relative expression of the *S. bicolor* disulfide isomerase (*SbPDI*) and basic leucine zipper transcription factor 60 (*SbbZIP60*) ortholog genes were analyzed in shoots and roots. NaCl induced the expression of SbPDI in both tissues (Figure 6A and B), meanwhile *SbbZIP60* didn't have its induced expression (Figure 6C and 6D). Under single DTT or TUN treatments, both genes presented up-regulation. The combined NaCl and DTT or NaCl and TUN treatments promoted a down-regulation *SbPDI* expression in shoots to the same level that was verified using only NaCl, although the expression values were still higher than control treatment (Figure 6A). In roots, under combined NaCl and DTT treatments, the level of *SbPDI* was the same as verified using single DTT or TUN treatments, and under combined NaCl and TUN treatments the level of *SbPDI* was the same verified using only NaCl (Figure 6B). Additionally, the expression of *SbbZIP60* was not induced by single NaCl treatment (Figure 6C and D). However, it was induced by DTT and TUN single treatments, as well as under combined NaCl and DTT treatments, and combined NaCl and TUN treatments. Otherwise, the expression of *SbbZIP60* in roots under TUN was lower than under DTT.

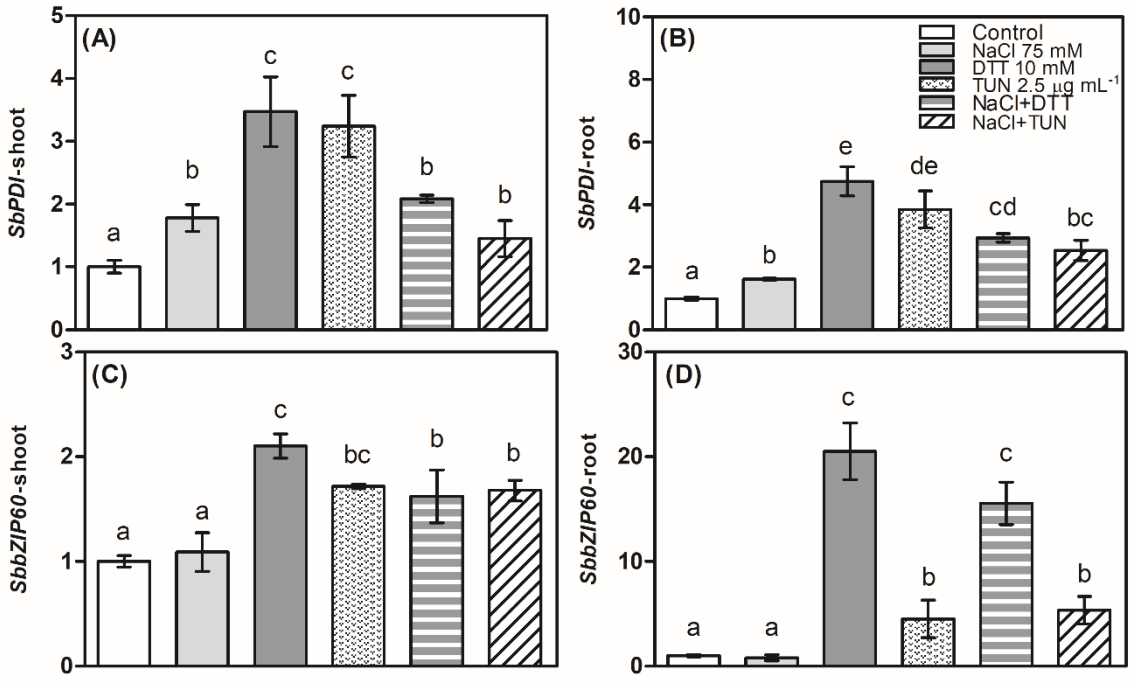

Figure 6. Relative expression profile of endoplasmic reticulum genes *SbPDI* and *SbbZIP60*, of *Sorghum bicolor* seedlings after three days of sowing and submitted for four days to 75 mM NaCl, 10 mM dithiothreitol (DTT), 2.5 µg ml^{-1} tunicamycin (TUN), NaCl, and to combinations of NaCl and DTT, and NaCl and TUN. The control seedlings were growing in the absence of NaCl or DTT. The expression profiles were determinate in shoots (A and C) and roots (B and

D). Gene expression was normalized using as reference gene *SbUBC18*. Different lowercase letters indicate significant differences among treatments according to Tukey's significance test ($p \leq 0.05$). Columns indicate means of three biological replicates and bars indicate standard deviation (±).

3.7. Expression of salt related genes are upregulated during NaCl and combined treatments

We also evaluated the effect of single or combined treatments in the salt-responsive genes vacuolar Na^+/H^+ antiporter (*SbNHX1*), salt overly sensitive Na^+/H^+ plasma membrane antiporter (*SbSOS1*), and vacuolar H^+-ATPase (*SbVHA2*). Salinity increased the expression levels of *SbNHX1*, *SbSOS1*, and *SbVHA2* genes in both shoots and roots tissues (Figure 7A-E). In addition, the expression of *SbNHX1* and *SbSOS1* were not induced during DTT or TUN treatments. Under combined treatments, NaCl and DTT and NaCl and TUN, the expressions of *SbNHX1* were upregulated, but in shoots they were lower than under single NaCl treatment (Figure 7A), and in roots they were like the verified using only NaCl (Figure 7B). Also, the expressions of *SbSOS1* under both combined treatments were similar to NaCl treatment in roots and shoots (Figure 7C, D). The expression of *SbVHA2* was increased in all treatments performed in both shoots and roots, but in roots it was higher under NaCl treatment, while in shoot they increased equally (Figure 7E, F).

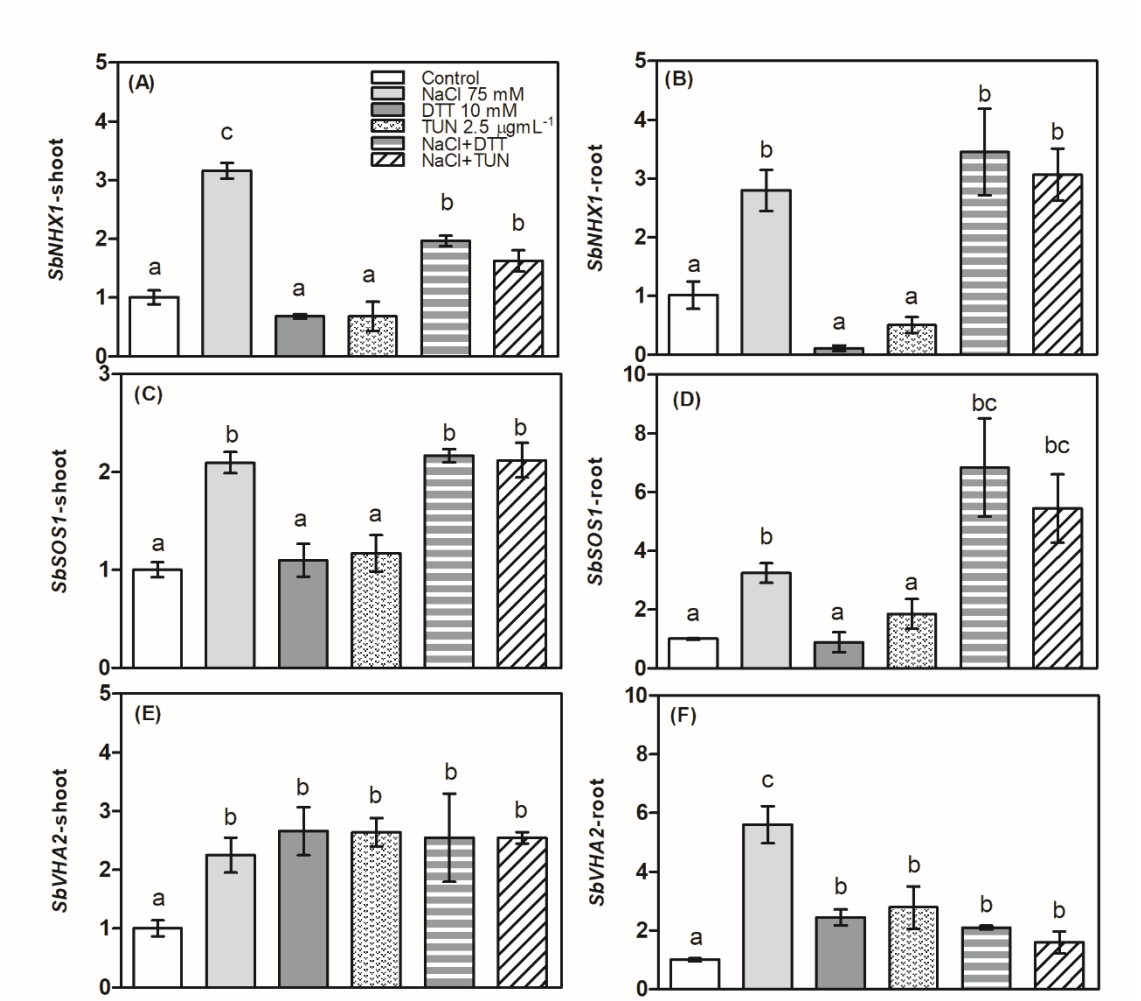

Figure 7. Relative expression profiles of sodium related genes, *SbNHX1*, *SbSOS1* and *SbVHA2*, of Sorghum *bicolor* seedlings after three days of sowing and submitted for four days to 75 mM NaCl, 10 mM dithiothreitol (DTT), 2.5 µg ml^{-1} tunicamycin (TUN), and to combinations of NaCl and DTT, and NaCl and TUN. The control seedlings were growing in the absence of NaCl or DTT. The expression profiles were determinate in shoots (A and C) and roots (B and D). Gene expression was normalized using as reference gene *SbUBC18*. Different lowercase letters indicate significant differences among treatments according to Tukey's significance test ($p \leq 0.05$). Columns indicate means of three biological replicates and bars indicate standard deviation (±).

4. Discussion

The development of tolerant plant genotypes to abiotic stresses has been one of the purposes of plant breeding programs, aiming the improvement of crop productivity, quality, and yield, to sustain the global food security (RAZA et al., 2019; REDDY, 2019). Salt stress stands as one of the most important abiotic stress in environments around the world, directly impacting plant growth and development (ZÖRB; GEILFUS; DIETZ, 2019). Such stress involves a complex network that includes osmotic stress, ion toxicity, nutritional deficiencies, and generation of reactive oxygen species, and induction of key metabolites (BATISTA et al., 2019; PARIHAR et al., 2015). Previous work reported that sorghum is more salt-sensitive until 24 days of development than in older stages (OLIVEIRA et al., 2013). Thus, some genotypes standouts against others due to their moderate tolerance to drought and saline stress, particularly at mature stages (COSTA et al., 2005; LACERDA et al., 2005, 2003).

Here, we challenged seedlings of variety CSF 20 in the early development stage by increasing NaCl concentration to verify its sensibility in this stage and to define a dose for further analysis. After 4 days of treatments, we did not find changes in the growth of shoots or roots from the lower to the highest level of salinity (0-100 mM). One interesting finding in this study is the fact that sorghum seedlings maintained lower contents of Na^+ in roots than in leaves, and it did not promote negative effects on plant growth (Figure 1A, Figure S2 and supplemental table S2), our results indicate that it was linked to an up-regulation of *SbNHX1*, *SbSOS1*, and *SbVHA2* under NaCl treatment (Figure 7), which had been reported as a response to saline conditions (CHUAMNAKTHONG; NAMPEI; UEDA, 2019). In fact, lower Na^+ accumulation in shoots is associated with the activity of Na^+/H^+ antiporters and proton pumps in the plasma membrane and vacuoles (MIRANDA et al., 2017). Moreover, the *Sb*SOS1 transporter is also able to extrude back some Na^+ from the xylem into the root stele and regulate the long-distance Na^+ movement, by controlling Na^+ loading in the xylem vessels, although in high concentrations of NaCl, or long times of exposure, it was not capable to totally avoid such accumulation (CUIN et al., 2011; SHI et al., 2002). Considering that part of Na^+ is still moved to the shoots, the up-regulation of *SbNHX1* (Figure 7) may act as a relevant response to salinity avoiding Na^+ toxicity by vacuolar compartmentalization. Despite the increment of Na^+, the maintenance of seedling's growth may be also linked to the capacity to keep levels of K^+ stable, which helps to handle reactive oxygen species (ROS) levels (COSTA et al., 2005). Indeed, the decrease of Na^+ content was more evident in roots than shoots, following by increase of K^+ (Figure 1A, Supplemental table S2). Also, NaCl was able to induce ER stress, since the

expression of *SbPDI* was induced in both shoot and roots (Figure 6), although UPR was not induced after four days.

Here, despite the size of seedlings have been increased, hydrogen peroxide content was more increased in shoots than roots, however, it was not followed by changes in the dismutase activity (Figure 4A, 4B, and 5H). Conversely, the growth of sorghum plants has been reported to be negatively impaired by salinity, the decrease of dry mass of leaves and roots, as well as foliar area, are usually observed in different cultivars (COELHO et al., 2018; FREITAS et al., 2011; SILVA et al., 2019). Also, the increase of ROS, lipid peroxidation (COSTA et al., 2005), and the ability to avoid the increase of toxic Na^+ and, or to maintain levels of K^+ (LACERDA et al., 2001) have been all reported as an indicator of oxidative stress in plants under salinity. It supports that the CSF 20 variety was able to keep the early seedling development stage and four days of NaCl exposure by an efficient system of Na^+/H^+ antiporters and proton pumps.

Since endoplasmic reticulum (ER) respond to both abiotic and biotic stresses (PARK; PARK, 2019) and the seedlings were capable to stand with to salinity, we evaluated whether the seedlings would be affected by disruption of ER homeostasis, using DTT as a reducing agent disturbing protein disulfide bonds (BAO; BASSHAM; HOWELL, 2019; HOWELL, 2013), affecting several developmental and physiological processes (YU et al., 2019). Indeed, DTT was able to induce ER-stress as we found the up-regulation of the ER-genes *SbPDI* and *SbbZIP60*, in both shoots and roots (Figure 6). DTT treatment also promoted a significant decrease of seedling length and masses, regardless of the concentration used (Figure 1B, and supplemental table S3). In agreement, in wheat under 7.5 mM DTT treatment, there was an inhibition of growth after two days due to ER-stress, with accelerated cell death (YU et al., 2019). Moreover, lipid peroxidation was increased under any concentration. Here, in shoots from 5 mM, and roots under 10 mM, DTT treatment may contribute to hydrogen peroxide scavenger. In fact, the decrease of H_2O_2 also has been reported to promote a decrease of lignin accumulation in chamomile roots (KOVÁČIK et al., 2010), which it is necessary for regular root establishment (FOREMAN et al., 2003). Lateral roots were also strongly affected by DTT treatment, as increasing concentrations of DTT, a fewer number of lateral roots are perceived (Figure S2). Besides, the reduced growth may also be linked to lipid peroxidation induced by other ROS, which contributes to membrane cell instability and increased electrolyte leakage rate (YU et al., 2019) associated to disrupted selective portioning of K^+ over Na^+ by membrane transporters (WANG et al., 2019). Thus, the presence of high DTT concentrations may promote an ionic imbalance between shoots and roots, then it promoted a huge reduction

of Na^+ content, especially in the roots. However, it was not associated with an up-regulation of *SbNHX1*, *SbSOS1* genes (Figure 7). Therefore, our results suggest that under 2.5 mM the CSF 20 variety may be less sensitive to this reducing agent, and above 5 mM the plant started to exhibit deleterious symptoms (Figure S2), which was perceived at ER levels, since both *Sb*PDI *and Atb*ZIP60 were accumulated. In fact, ER chaperones such as the ER binding protein (BiP) and PDI are commonly used as markers for ER stress (SRIVASTAVA et al., 2018).

As the impairment of Na^+ accumulation was observed by increasing the concentration of DTT, in both root and shoots, we decided to evaluate the effect of DTT combined to NaCl stress in the seedlings at the same development. As discussed before, NaCl did not change seedlings development, whilst the shoot and root length were decreased by DTT (Figure 2). The combined NaCl and DTT treatments were able to increase the shoot length to control values, as well as the root fresh and dry masses. In part, our results are supported by a previous study that the maintenance of masses and recovery of paprika pepper roots under salinity by the treatment with 5 mM of DTT, was a result of increasing water channels activity (CARVAJAL; MARTÍNEZ; ALCARAZ, 1999). The use of DTT as an ER stressor can be positive sometimes, it promotes an alteration of global cellular thiol-disulfide status, which could lead to an increased metabolic flux into respiratory pathways, starch, cell wall, and amino acid synthesis (KOLBE et al., 2006), contributing to keeping plant development. Additionally, DTT has been used previously against others stresses as an efficient scavenger, for example, toxic Hg^{2+} ions were removed from roots after 5.0 mM DTT treatment (CABAÑERO; CARVAJAL, 2007; CARVAJAL; MARTÍNEZ; ALCARAZ, 1999), likewise it may works to toxic Na^+. Indeed, the combined DTT and NaCl treatments were not able to increase the K^+ contents, but surprisingly decreased the levels of toxic Na^+ in both shoots and roots (Figure 3). Such decrease of Na^+ was also followed by keeping the expression of *Sb*NHX1, *Sb*SOS1, and *Sb*VHA2 in both shoots and roots (Figure 7). Both *Sb*NHX1 and *Sb*SOS1 presented the same pattern of expression in both shoots and roots (Figure 7), with an increase of expression following NaCl and DTT or NaCl and TUN treatments. Moreover, under combined stresses, either caused by NaCl and DTT or NaCl and TUN, the expression of *SbSOS1* was higher in roots (Figure 7D), which may contribute to sodium exclusion. Such control of uptake and internal Na^+ fluxes associated with the up-regulation of ER-chaperones like *SbPDI*, it is vital for plant metabolic functioning, growth, and re-establishment of cellular ionic homeostasis in order to deal with the saline environment (MAATHUIS; AHMAD; PATISHTAN, 2014; NAZAR et al., 2011), minimizing the deleterious effects promoted by ER stress in plants under combined DTT and NaCl treatments (Figure 3). In fact, ER chaperones such PDI evaluated

here, and others as ER binding protein (BiP), calnexin, and calreticulin are commonly used as markers for ER stress (SRIVASTAVA et al., 2018), indicating a positive mechanism to deal with accumulation of unfolded proteins.

The activation of certain enzymes may be dependent of thioredoxins or reductant agents as DTT, acting in oxidative stress response (LEMAIRE et al., 2004). Indeed, in both shoots and roots, lipid peroxidation was not increased by combined stresses (Figure 4). Although, single DTT treatment had decreased hydrogen peroxide, which it is in agreement to hydrogen peroxide scavenger function attributed to DTT (KOVÁČIK et al., 2010), the combination with NaCl promoted a recover of it to control levels. Since the level of TBARS and H_2O_2 was not changed by combined treatments, no significative changes were found in shoot-CAT, -APX, -GPX, and -SOD activities (Figure 5). However, in roots CAT activity was decreased by DTT and combined stresses, APX and GPX activities were reduced by all treatments, and the activity of SOD was decreased by single DTT and recovered by combined stresses. In agreement, the presence of DTT has been reported to promote a reduction state of cysteine residues, which led to decreased CAT activity in unicellular photosynthetic eukaryote and barley (LEMAIRE et al., 2004; MAEDA; FINNIE; SVENSSON, 2004). |The decrease of peroxidase activity of APX and GPX by DTT treatment in roots, which has been to the decreased of H_2O_2 in roots (KOVÁČIK et al., 2010). Thus, such detoxifying enzymes activity decreasing is also necessary for the maintenance of H_2O_2 levels to signaling and response to changes in the environment (SHAO et al., 2008).

Researchers concerning ER stress in plants are described mainly for Arabidopsis (ANGELOS et al., 2017; KIM; YAMAGUCHI-SHINOZAKI; SHINOZAKI, 2018; OZGUR et al., 2014). Many biotic and abiotic stresses, such as high temperatures and salinity, have impaired protein folding in the ER (KØRNER et al., 2015; MORENO et al., 2012; NAWKAR et al., 2018). Here, the relative expression of *Sb*PDI an ER related gene was accumulated in both shoots and roots (Figure 6), indicating that NaCl was able to induce ER stress after 4 days of stress. We could find differences in the ER stresses combined to NaCl, indicating a connection between these two stresses, but not in single NaCl stress regarding unfolded protein response (UPR), a cytoprotective signaling pathway (NAWKAR et al., 2018; WALTER; RON, 2011). In fact, PDI is an abundant oxidoreductase enzyme induced by ER stress to mitigate the harmful effects of misfolded proteins or reductant agents (ANGELOS et al., 2017; HOWELL, 2013; VERGHESE et al., 2012; WANG; KAUFMAN, 2016). PDI proteins have been considered a marker of salt response, its accumulation was reported in salt-tolerant rice seedlings (GHAFFARI et al., 2014), and barley roots (MOSTEK et al., 2015). Thus, PDI

associated with others ER-residents, as BiP, calnexin, and calreticulin help the maintenance of protein conformation of sorghum seedlings under salt stress, as well as ER stress (CARVALHO et al., 2014; SILVA et al., 2015). Further, there is some evidence that in Arabidopsis *Atb*ZIP60 respond to abiotic stress to increase the expression of ER-chaperones, it could take a few hours (ZHANG et al., 2017), or could be by a signaling pathway that is different to that triggered by the unfolded protein response (HENRIQUEZ-VALENCIA et al., 2015), in agreement *Atb*ZIP60 was accumulated under combined stresses. Thus, our results indicate that the increase of chaperones after four days of stresses was due to *Sb*bZIP60 only in combined stresses, although in single NaCl it could be earlier or it was due to the other arm of UPR, like bZIP17/28, and different times of responses still need to be evaluated in the future, .

Here we have addressed the effect of ER inducers on salt-tolerant CSF 20 variety in presence or absence of NaCl, thus we considered it a starting and new insights are raised regarding new varieties analysis. Therefore, sorghum seedlings were able to stand with NaCl and sensible to high doses of DTT. Moreover, under combined treatments, the presence of NaCl diminished deleterious effects of DTT, growth and the level of ROS, which is important to cell signaling, then decreasing the content of toxic Na^+ ion via membrane transporters *Sb*NHX1 and *Sb*SOS1, and the ER related gene *Sb*PDI and *Atb*ZIP60 restoring ER homeostasis. Based on our results the integrative response of ER and abiotic stresses can be useful to the development of new strategies to improve plant tolerance against several others environmental conditions, such as flooding, heat, drought, salinity, among others, even to pathogen response.

Conflicts of interest

The authors declare that there is no conflict of interest.

Author contributions

C.S.Q. and I.M.C.P carried out the research and data acquisition, K.R.P.L. and R.S.C.B. carried out qPCR analysis, M.S.A. and E.G-F revised the manuscript, H.H.C. designed the research concept and wrote the manuscript; all authors discussed the results and contributed to the final manuscript.

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Supporting Information

Table S1. Reference and target gene primer sequences.

Table S2. Response of *Sorghum bicolor* seedlings submitted to different concentrations of NaCl for four days. The data refer to root length, shoot and root fresh mass (SFW and RFW, respectively), shoot and root dry mass (SDW and RDW, respectively), ion contents (K^+ and Na^+ shoots and roots), hydrogen peroxide (H_2O_2 in roots and shoots), thiobarbituric acid reactive substances (TBARS in shoots and roots). At each treatment concentration, different lowercase letters indicate significant differences due to increasing of saline stress (0 mM, 50 mM, 75 mM and 100 mM of NaCl). According to the Tukey test ($p \leq 0.05$), data are the means of 5 repetitions ± standard deviation.

Table S3. Response of *Sorghum bicolor* seedlings submitted to different concentrations of DTT for four days. The data refer to root length, shoot and root fresh mass (SFW and RFW, respectively), shoot and root dry mass (SDW and RDW, respectively), ion contents (K^+ and Na^+ shoots and roots), hydrogen peroxide (H_2O_2 in roots and shoots), thiobarbituric acid reactive substances (TBARS in shoots and roots). At each treatment concentration, different lowercase letters indicate significant differences due to increasing of saline stress (0 mM, 50 mM, 75 mM and 100 mM of NaCl). According to the Tukey test ($p \leq 0.05$), data are the means of 5 repetitions ± standard deviation.

References

ABUELGASIM, A.; AMMAD, R. Mapping soil salinity in arid and semi-arid regions using Landsat 8 OLI satellite data. **Remote Sensing Applications: Society and Environment**, v. 13, p. 415–425, jan. 2019.

ANGELOS, E. et al. Maintaining the factory: the roles of the unfolded protein response in cellular homeostasis in plants. **The Plant Journal**, v. 90, n. 4, p. 671–682, maio 2017.

APSE, M. P.; BLUMWALD, E. Na + transport in plants. **FEBS Letters**, v. 581, n. 12, p. 2247–2254, 25 maio 2007.

AZEVEDO NETO, A. D. DE et al. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. **Environmental and Experimental Botany**, v. 56, n. 1, p. 87–94, maio 2006.

BAO, Y.; BASSHAM, D. C.; HOWELL, S. H. A Functional Unfolded Protein Response Is Required for Normal Vegetative Development. **Plant Physiology**, v. 179, n. 4, p. 1834–1843, abr. 2019.

BAO, Y.; HOWELL, S. H. The Unfolded Protein Response Supports Plant Development and Defense as well as Responses to Abiotic Stress. **Frontiers in Plant Science**, v. 8, n. March, p. 1–6, 2017.

BASSIL, E.; COKU, A.; BLUMWALD, E. Cellular ion homeostasis: emerging roles of intracellular NHX Na+/H+ antiporters in plant growth and development. **Journal of Experimental Botany**, v. 63, n. 16, p. 5727–5740, 1 out. 2012.

BATISTA, V. C. V. et al. Salicylic acid modulates primary and volatile metabolites to alleviate salt stress-induced photosynthesis impairment on medicinal plant Egletes viscosa. **Environmental and Experimental Botany**, v. 167, n. June, p. 103870, nov. 2019.

BEERS, R. F.; SIZER, I. W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. **The Journal of biological chemistry**, v. 195, n. 1, p. 133–40, mar. 1952.

BOSE, J. et al. Kinetics of xylem loading, membrane potential maintenance, and sensitivity of K+ -permeable channels to reactive oxygen species: physiological traits that differentiate salinity tolerance between pea and barley. **Plant, Cell & Environment**, v. 37, n. 3, p. 589–600, mar. 2014.

CABAÑERO, F. J.; CARVAJAL, M. Different cation stresses affect specifically osmotic root hydraulic conductance, involving aquaporins, ATPase and xylem loading of ions in Capsicum annuum, L. plants. **Journal of Plant Physiology**, v. 164, n. 10, p. 1300–1310, out. 2007.

CABRAL JÚNIOR, J. B. et al. Detecting linear trend of reference evapotranspiration in irrigated farming areas in Brazil's semiarid region. **Theoretical and Applied Climatology**, p. 1–11, 1 mar. 2019.

CAKMAK, I.; HORST, W. J. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). **Physiologia Plantarum**, v. 83, n. 3, p. 463–468, nov. 1991.

CARVAJAL, M.; MARTÍNEZ, V.; ALCARAZ, C. F. Physiological function of water channels as affected by salinity in roots of paprika pepper. **Physiologia Plantarum**, v. 105, n. 1, p. 95–101, 4 jan. 1999.

CARVALHO, H. H. et al. The Endoplasmic Reticulum Binding Protein BiP Displays Dual Function in Modulating Cell Death Events. **Plant Physiology**, v. 164, n. 2, p. 654–670, 1 fev. 2014.

CATALDO, D. A. et al. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. **Communications in Soil Science and Plant Analysis**, v. 6, n. 1, p. 71–80, 11 jan. 1975.

CHUAMNAKTHONG, S.; NAMPEI, M.; UEDA, A. Characterization of Na+ exclusion mechanism in rice under saline-alkaline stress conditions. **Plant Science**, v. 287, p. 110171, out. 2019.

CLARK, R. B. Characterization of phosphatase of intact maize roots. **Journal of Agricultural and Food Chemistry**, v. 23, n. 3, p. 458–460, maio 1975.

COELHO, D. S. et al. Growth and foliar contents of Na+and Cl-in genotypes of forage sorghum irrigated with salinized waters. **Irriga**, v. 23, n. 1, p. 108–120, 2018.

COSTA, P. H. A. DA et al. Antioxidant-enzymatic system of two sorghum genotypes differing in salt tolerance. **Brazilian Journal of Plant Physiology**, v. 17, n. 4, p. 353–361, 2005.

CUIN, T. A. et al. Assessing the role of root plasma membrane and tonoplast Na + /H + exchangers in salinity tolerance in wheat: in planta quantification methods. **Plant, Cell & Environment**, v. 34, n. 6, p. 947–961, jun. 2011.

DENG, Y.; SRIVASTAVA, R.; HOWELL, S. Endoplasmic Reticulum (ER) Stress Response and Its Physiological Roles in Plants. **International Journal of Molecular Sciences**, v. 14, n. 4, p. 8188–8212, 15 abr. 2013.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia**, v. 35, n. 6, p. 1039–1042, dez. 2011.

FOREMAN, J. et al. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. **Nature**, 2003.

FREITAS, P. A. F. et al. Salt acclimation in sorghum plants by exogenous proline: physiological and biochemical changes and regulation of proline metabolism. **Plant Cell Reports**, v. 38, n. 3, p. 403–416, 25 mar. 2019.

FREITAS, V. S. et al. Changes in physiological and biochemical indicators associated with salt tolerance in cotton, sorghum and cowpea. **African Journal of Biochemistry Research**, v. 5, n. 8, p. 264–271, 2011.

GHAFFARI, A. et al. Physiology and proteome responses of two contrasting rice mutants and their wild type parent under salt stress conditions at the vegetative stage. **Journal of Plant Physiology**, v. 171, n. 1, p. 31–44, jan. 2014.

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide Dismutases. **Plant Physiology**, v. 59, n. 2, p. 309–314, 1 fev. 1977.

GUAN, P. et al. Sensitive to salt1, an endoplasmic reticulum-localized chaperone, positively regulates salt resistance. **Plant Physiology**, v. 178, n. 3, p. 1390–1405, nov. 2018.

GUIMARÃES, M. J. M. et al. Antioxidant defenses of irrigated forage sorghum with saline aquaculture effluent. **Revista Caatinga**, v. 31, n. 1, p. 135–142, mar. 2018.

HENRIQUEZ-VALENCIA, C. et al. bZIP17 and bZIP60 Regulate the Expression of BiP3 and Other Salt Stress Responsive Genes in an UPR-Independent Manner in Arabidopsis thaliana. **Journal of cellular biochemistry**, v. 116, n. 8, p. 1638–45, ago. 2015.

HOWELL, S. H. Endoplasmic Reticulum Stress Responses in Plants. **Annual Review of Plant Biology**, v. 64, n. 1, p. 477–499, 29 abr. 2013.

INGRAM, M. The Tom Gibson memorial lecture. The microbiological role of nitrite in meat products. **Society for Applied Bacteriology symposium series**, v. 4, n. 1, p. 1–18, fev. 1976.

ISAYENKOV, S. V.; MAATHUIS, F. J. M. Plant salinity stress: many unanswered questions remain. **Frontiers in Plant Science**, v. 10, p. 1–11, 15 fev. 2019.

JI, H. et al. The Salt Overly Sensitive (SOS) Pathway: Established and Emerging Roles. **Molecular Plant**, v. 6, n. 2, p. 275–286, mar. 2013.

KANG, W. et al. The effect of NaCl on proline metabolism in Saussurea amara seedlings. **African Journal of Biotechnology**, v. 10, n. 15, p. 2886–2893, 11 abr. 2011.

KAUSAR, A.; GULL, M. Influence of salinity stress on the uptake of magnesium, phosphorus, and yield of salt susceptible and tolerant sorghum cultivars (Sorghum bicolor L.). **Journal of Applied Biology & Biotechnology**, v. 7, n. 3, p. 53–58, 2019.

KEUTGEN, A. J.; PAWELZIK, E. Impacts of NaCl stress on plant growth and mineral nutrient assimilation in two cultivars of strawberry. **Environmental and Experimental Botany**, v. 65, n. 2–3, p. 170–176, mar. 2009.

KIM, J.-S.; YAMAGUCHI-SHINOZAKI, K.; SHINOZAKI, K. ER-Anchored Transcription Factors bZIP17 and bZIP28 Regulate Root Elongation. **Plant Physiology**, v. 176, n. 3, p. 2221–2230, mar. 2018.

KOENIG, P.-A.; PLOEGH, H. L. Protein quality control in the endoplasmic reticulum. **F1000Prime Reports**, v. 6, n. 69, p. 147–172, 8 jul. 2014.

KOLBE, A. et al. Combined Transcript and Metabolite Profiling of Arabidopsis Leaves Reveals Fundamental Effects of the Thiol-Disulfide Status on Plant Metabolism. **Plant Physiology**, v. 141, n. 2, p. 412–422, jun. 2006.

KØRNER, C. et al. Endoplasmic Reticulum Stress Signaling in Plant Immunity—At the Crossroad of Life and Death. **International Journal of Molecular Sciences**, v. 16, n. 11, p. 26582–26598, 5 nov. 2015.

KÖSTER, P. et al. The battle of two ions: Ca 2+ signalling against Na + stress. **Plant Biology**, v. 21, n. S1, p. 39–48, 8 jan. 2019.

KOVÁČIK, J. et al. Lignification and related parameters in copper-exposed Matricaria chamomilla roots: Role of H2O2 and NO in this process. **Plant Science**, 2010.

LACERDA, C. F. et al. Changes in growth and in solute concentrations in sorghum leaves and roots during salt stress recovery. **Environmental and Experimental Botany**, v. 54, n. 1, p. 69–76, ago. 2005.

LACERDA, C. F. DE et al. Plant growth and solute accumulation and distribution in two sorghum genotypes, under NaCl stress. **Revista Brasileira de Fisiologia Vegetal**, v. 13, n. 3, p. 270–284, 2001.

LACERDA, C. F. DE et al. Osmotic adjustment in roots and leaves of two sorghum genotypes under NaCl stress. **Brasilian Journal of Plant Physiology**, v. 15, n. 2, p. 113–118, 2003.

LEMAIRE, S. D. et al. New thioredoxin targets in the unicellular photosynthetic eukaryote Chlamydomonas reinhardtii. **Proceedings of the National Academy of Sciences**, v. 101, n. 19, p. 7475–7480, 11 maio 2004.

LIU, J.-X. et al. Salt stress responses in Arabidopsis utilize a signal transduction pathway related to endoplasmic reticulum stress signaling. **The Plant Journal**, v. 51, n. 5, p. 897–909, 2007.

LIVAK, K. J.; SCHMITTGEN, T. D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. **Methods**, v. 25, n. 4, p. 402–408, dez. 2001.

MAATHUIS, F. J. M.; AHMAD, I.; PATISHTAN, J. Regulation of Na+ fluxes in plants. **Frontiers in Plant Science**, v. 5, n. 467, p. 1–9, 16 set. 2014.

MAEDA, K.; FINNIE, C.; SVENSSON, B. Cy5 maleimide labelling for sensitive detection of free thiols in native protein extracts: identification of seed proteins targeted by barley thioredoxin h isoforms. **Biochemical Journal**, v. 378, n. 2, p. 497–507, 1 mar. 2004.

MALAVOLTA, E.; VITTI, G. C.; OLIVEIRA, S. A. **Avaliação do Estado Nutricional das Plantas. Princípios e Aplicações.** [s.l: s.n.].

MIRANDA, R. DE S. et al. Integrative Control Between Proton Pumps and SOS1 Antiporters in Roots is Crucial for Maintaining Low Na+ Accumulation and Salt Tolerance in Ammonium-Supplied Sorghum bicolor. **Plant and Cell Physiology**, v. 58, n. 3, p. 522–536, mar. 2017.

MORENO, A. A. et al. IRE1/bZIP60-mediated unfolded protein response plays distinct roles in plant immunity and abiotic stress responses. **PLoS ONE**, v. 7, n. 2, 2012.

MOSTEK, A. et al. Alterations in root proteome of salt-sensitive and tolerant barley lines under salt stress conditions. **Journal of Plant Physiology**, v. 174, p. 166–176, fev. 2015.

MUHAMMAD, T. Influence of nursery sowing dates, seedling age and nitrogen levels on bulb quality and marketable yield of onion (Allium cepa L.). **Pure and Applied Biology**, v. 51, n. 2, p. 121–124, 10 jun. 2016.

NAKANO, Y.; ASADA, K. Hydrogen peroxide is scavenged by ascorbato specific peroxidase in spinach chloroplasts. **Plant Cell Physiol**, 1981.

NAWKAR, G. M. et al. Activation of the Transducers of Unfolded Protein Response in Plants. **Frontiers in Plant Science**, v. 9, n. February, p. 1–10, 20 fev. 2018.

NAZAR, R. et al. Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. **Journal of Plant Physiology**, v. 168, n. 8, p. 807–815, maio 2011.

NEILL, E. M. et al. Plant growth regulators interact with elevated temperature to alter heat stress signaling via the Unfolded Protein Response. **bioRxiv**, p. 1–13, 2019.

OLIVEIRA, A. B. DE; GOMES-FILHO, E. Germinação e vigor de sementes de sorgo forrageiro sob estresse hídrico e salino. **Revista Brasileira de Sementes**, v. 31, n. 3, p. 48–56, 2009.

OLIVEIRA, V. P. DE et al. Physiological and biochemical characteristics of Sorghum bicolor and Sorghum sudanense subjected to salt stress in two stages of development. **African Journal of Agricultural Research**, v. 8, n. 8, p. 660–670, 2013.

OZGUR, R. et al. Endoplasmic reticulum stress triggers ROS signalling, changes the redox state, and regulates the antioxidant defence of Arabidopsis thaliana. **Journal of Experimental Botany**, v. 65, n. 5, p. 1377–1390, mar. 2014.

PARIHAR, P. et al. Effect of salinity stress on plants and its tolerance strategies: a review. **Environmental Science and Pollution Research**, v. 22, n. 6, p. 4056–4075, 16 mar. 2015.

PARK, C.-J.; PARK, J. M. Endoplasmic Reticulum Plays a Critical Role in Integrating Signals Generated by Both Biotic and Abiotic Stress in Plants. **Frontiers in Plant Science**, v. 10, n. April, p. 1–8, 4 abr. 2019.

PENNISI, E. Plant genetics: How sorghum withstands heat and drought. **Science**, v. 323, n. 5914, p. 573–573, 30 jan. 2009.

PLEWA, M. J.; SMITH, S. R.; WAGNER, E. D. Diethyldithiocarbamate suppresses the plant activation of aromatic amines into mutagens by inhibiting tobacco cell peroxidase. **Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis**, v. 247, n. 1, p. 57–64, mar. 1991.

QADIR, M. et al. Economics of salt-induced land degradation and restoration. **Natural Resources Forum**, v. 38, n. 4, p. 282–295, nov. 2014.

RADANIELSON, A. M. et al. Describing the physiological responses of different rice genotypes to salt stress using sigmoid and piecewise linear functions. **Field Crops Research**, v. 220, p. 46–56, 2018.

RASBAND, W. **ImageJU. S. National Institutes of Health, Bethesda, Maryland, USA**, 2016.

RAZA, A. et al. Impact of Climate Change on Crops Adaptation and Strategies to Tackle Its Outcome: A Review. **Plants**, 2019.

REDDY, P. S. Breeding for Abiotic Stress Resistance in Sorghum. In: **Breeding Sorghum for Diverse End Uses**. [s.l.] Elsevier, 2019. p. 325–340.

RODRÍGUEZ-ROSALES, M. P. et al. Plant NHX cation/proton antiporters. **Plant Signaling & Behavior**, v. 4, n. 4, p. 265–276, abr. 2009.

SAFDAR, H. et al. A review: Impact of salinity on plant growth. v. 17, n. 1, p. 34–40, 2019.

SHAO, N. et al. Photosynthetic electron flow affects H2O2 signaling by inactivation of catalase in Chlamydomonas reinhardtii. **Planta**, 2008.

SHI, H. et al. The Putative Plasma Membrane Na + /H + Antiporter SOS1 Controls Long-Distance Na + Transport in Plants. **The Plant Cell**, v. 14, n. 2, p. 465–477, fev. 2002.

SILVA, J. V. et al. Crescimento e osmorregulação em dois genótipos de sorgo submetidos a estresse salino 1 Growth and osmorregulation in two sorghum genotypes under salt stress Material e Métodos. **Biologia**, v. 34, n. 2, p. 125–131, 2003.

SILVA, M. L. D. S. et al. Growth and photosynthetic parameters of saccharine sorghum plants subjected to salinity. **Acta Scientiarum. Agronomy**, v. 41, n. 1, p. 42607, 13 mar. 2019.

SILVA, P. A. et al. Comprehensive analysis of the endoplasmic reticulum stress response in the soybean genome: Conserved and plant-specific features. **BMC Genomics**, v. 16, n. 1, p. 1–20, 2015.

SRIVASTAVA, R. et al. Response to Persistent ER Stress in Plants: A Multiphasic Process That Transitions Cells from Prosurvival Activities to Cell Death. **The Plant Cell**, v. 30, n. 6, p. 1220–1242, jun. 2018.

TABOSA, J. N. et al. Sorghum Genotypes Evaluation Under Salinity Levels and Gamma Ray Doses. **Revista Brasileira de Milho e Sorgo**, v. 6, n. 3, p. 339–350, 30 dez. 2007.

VERGHESE, J. et al. Biology of the Heat Shock Response and Protein Chaperones: Budding Yeast (Saccharomyces cerevisiae) as a Model System. **Microbiology and Molecular Biology Reviews**, v. 76, n. 2, p. 115–158, 1 jun. 2012.

WALTER, P.; RON, D. The Unfolded Protein Response: From Stress Pathway to Homeostatic Regulation. **Science**, v. 334, n. 6059, p. 1081–1086, 25 nov. 2011.

WAN, S.; JIANG, L. Endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) in plants. **Protoplasma**, v. 253, n. 3, p. 753–764, 2016.

WANG, M.; KAUFMAN, R. J. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. **Nature**, v. 529, n. 7586, p. 326–335, 21 jan. 2016.

WANG, Q. et al. The Effect of AtHKT1;1 or AtSOS1 Mutation on the Expressions of Na+ or K+ Transporter Genes and Ion Homeostasis in Arabidopsis thaliana under Salt Stress. **International Journal of Molecular Sciences**, 2019.

WU, H. Plant salt tolerance and Na + sensing and transport. **The Crop Journal**, v. 6, n. 3, p. 215–225, jun. 2018.

YANG, Z.-T. et al. A plasma membrane-tethered transcription factor, NAC062/ANAC062/NTL6, mediates the unfolded protein response in Arabidopsis. **The Plant Journal**, v. 79, n. 6, p. 1033–1043, set. 2014.

YATOO, M. I. et al. Anti-inflammatory drugs and herbs with special emphasis on herbal medicines for countering inflammatory diseases and disorders - a review. **Recent Patents on Inflammation & Allergy Drug Discovery**, v. 12, n. 1, p. 39–58, 21 ago. 2018.

YU, X. et al. Transcriptome and physiological analyses for revealing genes involved in wheat response to endoplasmic reticulum stress. **BMC Plant Biology**, v. 19, n. 1, p. 193, 9 dez. 2019.

ZHANG, L. et al. Osmotic Stress Induced Cell Death in Wheat Is Alleviated by Tauroursodeoxycholic Acid and Involves Endoplasmic Reticulum Stress–Related Gene Expression. **Frontiers in Plant Science**, v. 8, p. 1–14, 3 maio 2017.

ZÖRB, C.; GEILFUS, C. -M.; DIETZ, K. -J. Salinity and crop yield. **Plant Biology**, v. 21, n. S1, p. 31–38, 5 jan. 2019.

REFERÊNCIAS

ACOSTA-MOTOS, J.R.; ORTUÑO, M.F.; BERNAL-VICENTE,A. DIAZ-VIVANCOS, P.; SANCHEZ-BLANCO, M.J.; HERNANDEZ, J.A. Plant Responses to Salt Stress: Adaptive Mechanisms. **Agronomy,** v. 7, n. 18, 2017.

APSE, M. P.; BLUMWALD, E. Na+ transport in plants. **FEBS Letters,** v. 581, n. 12, p. 2247-2254, maio 2007.

AZEVEDO-NETO, A.D; PRISCO, J.T; ENÉAS-FILHO, J; LACERDA, C.F; SILVA, J.V; COSTA, P.H.A; GOMES-FILHO, E. Effects of salt stress on plant growth, stomatal response and solute accumulation of different maize genotypes. **Braz. J. Plant Physiol,** Londrina, v. 16, n. 1, jan./abr. 2004.

BASSIL, E.; COKU, A.; BLUMWALD, E. "Cellular ion homeostasis: emerging roles of intracellular NHX Na+/H+ antiporters in plant growth and development." **Journal of experimental botany,** v. 63, n. 16, p. 5727-5740, 2012.

BONFIM-SILVA, E. M.; KROTH, B. E.; SILVA, T. J. A.; FREITAS, D. C.; Disponibilidades Hídricas no Desenvolvimento Inicial de Sorgo e pH do Solo. **Centro Científico Conhecer,** Goiânia, v.8, n.14, p397-407, 2012.

BOSE, J.; SHABALA, L.; POTTOSIN, I.; ZENG, F;, VELARDE-BUENDÍA, A.M.; MASSART, A.et al. Kinetics of xylem loading, membrane potential maintenance, and sensitivity of K+-permeable channels to reactive oxygen species: physiological traits that differentiate salinity tolerance between pea and barley. **PlantCell Environ.,** v. 37, p. 589–600, 2014.

COSTA, P. H. A. da; AZEVEDO NETO, A. D. de; BEZERRA, M. A.; PRISCO, J. T.; GOMES-FILHO, E. Antioxidant-enzymatic system of two sorghum genotypes differing in salt tolerance. **Brazilian Journal of Plant Physiology,** v. 17, n. 4, p. 353-362, 2005.

CUINT, A.; BOSE, J.; STEFANO, G.; JHAD.; TESTER, M.; MANCUSOS, et al. Assessing the role of root plasma membrane and tonoplast Na+/H+ exchangers in salinity tolerance in wheat: in planta quantification methods. PlantCell Environ., v. 34, p. 947–961, 2011.

DENG, Y; SRIVASTAVA, R; HOWELL, S.H. Endoplasmic reticulum (RE) stress response and its physiological roles in plants. **Int J Mol Sci.,** v. 14, p. 8188–212, 2013.

ESTEVES, B.S; SUZUKI, M.S**.** Efeito da salinidade sobre as plantas. **Oecol. Bras**., v. 12, n. 4, p. 662-679, 2008.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. **Status of the World's Soil Resources**. Rome: FAO, 2015. 648 p.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. **FAO:** and Agriculture Organization of the United Nations. Food Outlook: Biannual report on global food markets. 2017. 152 p.

FERREIRA, P. A.; J. B. L. SILVA; H. A. RUIZ. **Manejo da Salinidade na Agricultura:** Estudos Básicos e Aplicados. Fortaleza: Aspectos físicos e químicos de solos em regiões áridas e semiáridas, 2010.

FLOWERS, T.J.; COLMER, T.D. Plant salt tolerance: Adaptations in halophytes. **Ann. Bot**., v. 115, p. 327–331, 2015.

FREITAS, V. S.; ALENCAR, N. L. M.; LACERDA, C. F.; PRISCO, J. T.; GOMES FILHO, E. Changes in physiological and biochemical indicators associated with salt tolerance in cotton, sorghum and cowpea. **African Journal of Biochemistry Research**., v. 5, n. 8, p. 264-271, 2011.

GÓMEZ-BELLOT, M.J.; ÁLVAREZ, S.; CASTILLO, M.; BAÑÓN, S.; ORTUÑO, M.F.; SÁNCHEZ-BLANCO, M.J. Water relations, nutrient content and developmental responses of Euonymus plants irrigated with water of different degrees of salinity and quality. **J. Plant Res**., v. 126, p. 567–576, 2013.

HENRIQUEZ-VALENCIA, C; MORENO, A.A; SANDOVAL-IBAÑEZ, O; MITINA, I; BLANCO-HERRERA, F; CIFUENTES-ESQUIVEL, N, *et al*. bZIP17 and bZIP60 regulate the expression of BiP3 and other salt stress responsive genes in an UPR independent manner in Arabidopsis thaliana. **J Cell Biochem.,** n. 8, 2015.

HOSSAIN, M; RAHMAN, S.N; BRATTACHARYA, P; JACKS, G; SAHA, R; RAHMAN, M. Sustainability of arsenic mitigation interventions - an evaluation of different alternative safe drinking water options provided in Matlab, an arsenic hot spot in Bangladesh. **Frontiers in Environmental Science,** v. 3, n. 30, maio 2015.

HOWELL, S.H. Endoplasmic reticulum stress responses in plants. **Annu Ver Plant Biol.,** v. 64, p. 477–499, 2013.

HUANG, R. D. Research progress on plant tolerance to soil salinity and alkalinity in sorghum. **Journal of Integrative Agriculture,** v. 17, n. 4, p. 739–746, 2018.

JAMIL, A.; RIAZ, S.; ASHRAF, M.; FOOLAD, M. R. Gene expression profiling of plants under salt stress. **Crit. Rev. Plant Sci.,** v. 30, n. 5, p. 435–458, 2011.

KANGASJÄRVI, S.; KANGASJÄRVI, J. Towards understanding extracellular ROS sensory and signaling systems in plants. **Advances in Botany**, v. 2014, n. 1, p. 1-10, 2014.

LIU, J.X; SRIVASTAVA, R; CHE, P; HOWELL, S.H. Salt stress responses in Arabidopsis utilize a signal transduction pathway related to endoplasmic reticulum stress signaling. **Plant J,** v. 51, p. 897–909, 2007.

LIU, J.-X.; HOWELL, S.H. Endoplasmic Reticulum Protein Quality Control and Its Relationship to Environmental Stress Responses in Plants. **Plant Cell,** 22, 1–13, 2010.

KAMAUCHI, S.; NAKATANI, H.; NAKANO, C.; URADE, R. Gene expression in response to endoplasmic reticulum stress in *Arabidopsis thaliana*. **FEBS J.,** v. 272, p. 3461–76, 2005

KOIZUMI, N.; MARTINEZ, I.M.; KIMATA, Y.; KOHNO, K.; SANO, H.; CHRISPEELS, M.J. Molecular characterization of two Arabidopsis Ire1 homologs, endoplasmic reticulum-located transmembrane protein kinases. **Plant Physiol***., v.* 127, p. 949–62, 2001

MAATHUIS, F.J; AHMAD, I; PATISHTAN, J. Regulation of Na+ fluxes in plants. **Frontiers in Plant Science,** v. 5, p. 1-9, 2014.

MARTINEZ, I.M.; CHRISPEELS, M.J. Genomic analysis of the unfolded protein response in Arabidopsis shows its connection to important cellular processes. **Plant Cell., v.** 15, n. 561, 2003.

MICHALAK, M.; GROENENDYK, J.; SZABO, E.; GOLD, L.I.; OPAS, M. Calreticulin, a multi-process calcium buffering chaperone of the endoplasmic reticulum. **Biochem. J***., v.* 417, p. 651–666, 2009.

MITTLER, R. Oxidative stress, antioxidants and stress tolerance. **Trends Plant Sci.,** v. 7, n.9, p. 405-410. 2002.

MUNNS, R.; TERMAAT, A. Whole plant response to salinity. Aust. **J. Plant Physiol**., v. 13, p. 143–160, 1986,

NAWKAR, G.M; LEE, E.S; SHELAKE, R.M; PARK, J.H; RYU, S.W; KANG, C.H; LEE, S.Y. Activation of the Transducers of Unfolded Protein Response in Plants. **Frontiers in Plant Science,** v. 9, n. 214, 2018.

NELSON, D. L.; COX, M. M. **Lehninger Principles of Biochemistry**. 6 th ed. NY: W. H. Freeman & Co, 2014.

NOUNJAN, N; NGHIA, P.T.; THEERAKULPISUT, P. Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. **Journal of Plant Physiology**, v. 169, n. 6, p. 596-604, 2012.

OZGUR, R. et al. Endoplasmic reticulum stress triggers ROS signalling, changes the redox state, and regulates the antioxidant defence of Arabidopsis thaliana. **Journal of Experimental Botany,** v. 65, n. 5, p. 1377-1390, mar. 2014.

PARIDA, A.K; DAS, A.B. Salt tolerance and salinity effects on plants: a review. **Ecotoxicology and Environmental Safety,** v. 60, p. 324-349, 2005.

PLAUT, Z; EDELSTEIN, M. BEN-HUR, M. Overcoming salinity barriers to crop production using traditional methods. **Crit. Rev. Plan Sci**., v. 32, p. 250-291, 2013.

RAMATOULAYE, F.; MADY, C.; FALLOU, S.; AMADOU, K.; CYRIL, D. Production and use Sorghum: A literature review. **J. Nutrition Health Food Sci**., v. 4, p. 1-4, 2016

REDDY, P. Surender et al. Proline over-accumulation alleviates salt stress and protects photosynthetic and antioxidant enzyme activities in transgenic sorghum [*Sorghum bicolor* (L.) Moench]. **Plant Physiology and Biochemistry**, v. 94, n. 1, p. 104-113, 2015.

RODRÍGUEZ-ROSALES, M. P; GÁLVEZ, F. J; HUERTAS, R; ARANDA, M. N; BAGHOUR, M; CAGNAC, O; VENEMA, K. Plant NHX cation/próton antiporters. **Plant signaling & behavior,** v. 4, n. 4, p. 265-276, 2009.

RODRÍGUEZ-ROSALES; JIANG, X.; GÁLVEZ, F.J.; ARANDA. M.N.; CUBERO, B.; VENEMA K."Overexpression of the tomato K^+/H^+ antiporter LeNHX2 confers salt tolerance by improving potassium compartmentalization."**NewPhytologist,** v. 179, n. 2, p. 366-377, 2008.

SHAHBAZ, M.; ASHRAF, M. Improving salinity tolerance in cereals. **Critical Reviews in Plant Sciences,** v. 32, p. 237–249, 2013.

SHRIVASTAVA, P.; KUMAR, R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. **Saudi Journal of Biological Sciences**. v. 22, p. 123–131, 2015.

SILVA, J.V; LACERDA, C.F; AZEVEDO NETO, A.D; COSTA, P.H. A; ENÉAS FILHO, J; PRISCO, J.T; GOMES FILHO, E. Crescimento e osmorregulação em dois genótipos de sorgo submetidos a estresse salino. **Revista Ciência Agronômica**, v.34, n.2, p.125-131, 2003.

SMITH, M.H.; PLOEGH, H.L.; WEISSMAN, J.S. Road to ruin: targeting proteins for degradation in the endoplasmic reticulum. **Science,** v. 334, p. 1086–1090, 2011

TABOSA, J.N; COLAÇO, W; REIS, O.V; SIMPLÍCIO, J.B; CARVALHO, H.W.L; DIAS, F.M. Sorghum genotypes evaluation under alinity level sand gammaray. **Revista Brasileira de Milho e Sorgo,** v. 6, n. 3, p.339-350, 2007.

TABOSA, J.N; REIS, O.V; BRITO, A.R.M.B; MONTEIRO, M.C.D; SIMPLÍCIO, J.B; OLIVEIRA, J.A.C; SILVA, F.G; AZEVEDO NETO, A.D; DIAS, F.M; LIRA, M.A; TAVARES FILHO, J. J; NASCIMENTO, M. M. A; LIMA, L. E; CARVALHO, H. W. L; OLIVEIRA, L. R. Comportamento de cultivares de sorgo forrageiro em diferentes ambientes agroecológicos dos Estados de Pernambuco e Alagoas. **Revista Brasileira de Milho e Sorgo**, v.1, n. 2, p.47-58, 2002.

TARI, I.; LASKAY, G.; TAKÁCS, Z.; POÓR, P. Response of Sorghum to Abiotic Stresses: A Review. **JAgro Crop Sci**., v. 199, p. 264–274, 2013.

VITALE, A.; BOSTON, R.S. Endoplasmic reticulum quality control and the unfolded protein response:insights from plants. **Traffic.,** v. 9, p. 1581–1588, 2008.

TANG, X.; MU, X.; SHAO, H.; WANG, H.; BRESTIC, M. Global plant-responding mechanisms to salt stress: Physiological and molecular levels and implications in biotechnology. **Crit. Rev. Biotechnol**., v. 35, p. 425– 437, 2015.

VIEIRA, M. R. **Produtividade, composição químico-bromatológica e nutrição mineral de plantas de sorgo forrageiro irrigadas com águas salinas. Fortaleza.** 2006. 97f. Dissertação. (Mestrado em Irrigação e Drenagem) – Universidade Federal do Ceará, Fortaleza, 2006.

WALTER, P; RON, D. The unfolded protein response: from stress pathway to homeostatic regulation. **Science,** v. 334, p. 1081–1086, 2011.

WANG, Y.; REN, X.; YU, Z. J.; CAI, Q. A.; LIN, X. X.; MA, R. Advances in the transformation of salt tolerance gene into maize. **Journal of Anhui Agricultural Science**. v. 40, p. 3908–3911, 2012.

YADAV, N. M; BAGDI, D.L; KARRALYA, B.L. "Effect of salt stress on physiological, biochemical, growth and yield variables of wheat (Triticuma estivum L.)."**Agricultural Science Digest,** v. 31, n. 4, 2011.

YANG, Z.T; LU, S.J; WANG, M.J; BI, D.L; SUN, L; ZHOU, S.F.; SONG, Z.T.; LIU, J.X. A plasma membrane-tethered transcription factor, NAC062/ANAC062/NTL6, mediates the unfolded protein response in Arabidopsis. **Plant J.,** v. 79, p. 1033–1043, 2014.