# SORGHUM SEEDLINGS RESPONSES TO SALT AND ENDOPLASMIC RETICULUM STRESSES

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**ABSTRACT**: Endoplasmic reticulum (ER) response to abiotic and biotic stresses has been investigated, but barely associated with salinity. Thus, we tested seedlings of sorghum cv. CSF20 in the early development by increasing NaCl concentration to evaluate such tolerance in this stage, and if seedlings could be tolerant or sensitive to disruption of ER homeostasis. The experimental design was completely randomized composed by 4 treatments of NaCl (0, 50, 75, and 100 mM); or dithiothreitol (DTT - 0, 2.5, 5.0 and 10.0 mM) for 4 days. Length and weight of shoots and roots were measured as well as inorganic ions, lipid peroxidation and $H_2O_2$. NaCl did not change fresh and dry mass from the lower to the highest level of salinity (0-100 mM), as well as hydrogen peroxide and lipid peroxidation under the lower concentration. On the other hand, DTT promoted a decrease of seedling length, regardless of the concentration used. DTT also changed the ion portioning between shoots and roots, increased $K^+$ and reduced $Na^+$ contents, especially in the roots. In conclusion, the sorghum cv. CSF20 was also salt-tolerant in the early seedling stage. Further, until 2.5 mM DTT, the seedlings may tolerate ER stress, and above 5mM it started to exhibit symptoms.

**KEYWORDS**: Dithiothreitol, sodium chloride, homeostasis.

# RESPOSTA DE PLÂNTULAS DE SORGO AOS ESTRESSES SALINO E DO RETÍCULO ENDOPLASMÁTICO

**RESUMO**: A resposta do retículo endoplasmático (RE) a estresses bióticos e abióticos tem sido investigada nos últimos anos, mas pouco associada à salinidade. Assim, plântulas de

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sorgo cv. CSF20 nos estádios iniciais de desenvolvimento foram testadas com o aumento da concentração de NaCl para avaliar a tolerância nesse estágio, e se elas também podem ser tolerantes ou sensíveis a perturbação da homeostase do RE. O desenho experimental foi inteiramente casualizado com 4 tratamentos de NaCl (0, 50, 75 e 100 mM); ou ditiothreitol (DTT - 0, 2.5, 5.0 e 10.0 mM) por 4 dias. Foram medidos o comprimento, biomassa, íons inorgânicos, peroxidação de lipídeos e $H_2O_2$. Massa fresca e seca também não foram alterados pela presença do NaCl, bem como peroxidação de lipídios e $H_2O_2$. Por outro lado, DTT promoveu diminuição do comprimento da plântula independente da concentração usada. DTT também alterou a relação de íons entre raízes e parte aérea, aumentando os conteúdos de $K^+$ e reduzindo $Na^+$ especialmente nas raízes. Como conclusão, o sorgo cv. CSF20 também foi tolerante no estádio inicial de desenvolvimento. Além disso, até 2.5 mM de DTT as plântulas puderam tolerar o estresse do RE, e acima de 5mM começaram a exibir sintomas.
**PALAVRAS-CHAVE**: Ditiotreitol, cloreto de sódio, homeostase

## INTRODUCTION

Salt stress is an example of abiotic stress, which directly impacts plant growth (Zörb *et al*., 2019). Sorghum [*Sorghum bicolor* (L.) Moench] is more salt-sensitive until 24 days old than older plants (Oliveira *et al*., 2013). It is a complex issue that includes osmotic stress, ion toxicity, nutritional deficiencies, and formation of reactive oxygen species (Parihar *et al*., 2015). The response of endoplasmic reticulum (ER) to abiotic and biotic stresses has been also investigated (Park; Park, 2019), but barely associated with salinity. Mostly, ER-stress has been performed by dithiothreitol (DTT), a reducing agent disturbing of protein disulfide bonds (Bao *et al*., 2019). Thus, we tested seedlings of sorghum cv. CSF20 in the early development by increasing NaCl concentration to evaluate such tolerance in this stage, and if cv. CSF20 could be tolerant or sensitive to disruption of ER homeostasis.

## MATERIAL AND METHODS

Seeds of sorghum cv. CFS20 were germinated between two layers of paper in glass bottles with 5,0 ml of distilled water in the bottom, and then covered to a plastic bag and kept to BOD chamber at constant temperature of 30° at day and 26° at night, relative humidity of

90% and 12-h photoperiod. The experimental design was completely randomized and two independent rounds were performed. Experiment I: three days of sowing (DAS), seedlings were transferred to a new paper moistened with 20 mL of NaCl solution composed by for treatments (0, 50, 75, and 100 mM NaCl) and 4 repetitions, each repetition was composed by 10 different seedlings. Each treatment was arranged as described before and returned to the BOD chamber. After 4 days (7 days of DAS) length, fresh mass of shoots and roots were measured as well as inorganic $Na^+$ and $K^+$ ions, lipid peroxidation and $H_2O_2$. Experiment I: For the ER stress experiment, we used 20 mL of dithiothreitol (DTT - Sigma Aldrich) solution composed by for treatments (0, 2.5, 5.0 and 10.0 mM) and 4 repetitions, each repetition was composed by 10 different seedlings.

The data of independent experiments were subjected to the analysis of variance (ANOVA F-test) and submitted to regression test ($p \leq 0.05$) to comparation using the SISVAR program (Data not showed). Then, the values were normalized to control treatment and summarized in a radar plot (SigmaPlot).

## RESULTS AND DISCUSSION

At seven days of development, we did not find any change in the growth of shoots or roots from the lower to the highest level of salinity (0-100 mM). Despite the increment of $Na^+$ in shoots and roots, the maintenance of growth seedling may be linked to the ability to keep $K^+$, especially in roots, which led to low reactive oxygen species (ROS). Indeed, we found low lipid peroxidation and hydrogen peroxide (Figures 1A). Conversely, the growth of sorghum plants has been reported to be negatively impaired by salinity, decrease of the dry mass of leaves and roots, as well as foliar area, are usually observed in different genotypes (Freitas *et al*., 2011). Also, the increased of ROS, lipid peroxidation (Costa *et al*., 2005) and the ability to avoid toxic $Na^+$ and, or to maintain levels of $K^+$ (Lacerda *et al*., 2001) have been reported as an indicator of oxidative stress in plants under salinity. It supports that the cv. CSF20 may be also tolerant to early seedling stage.

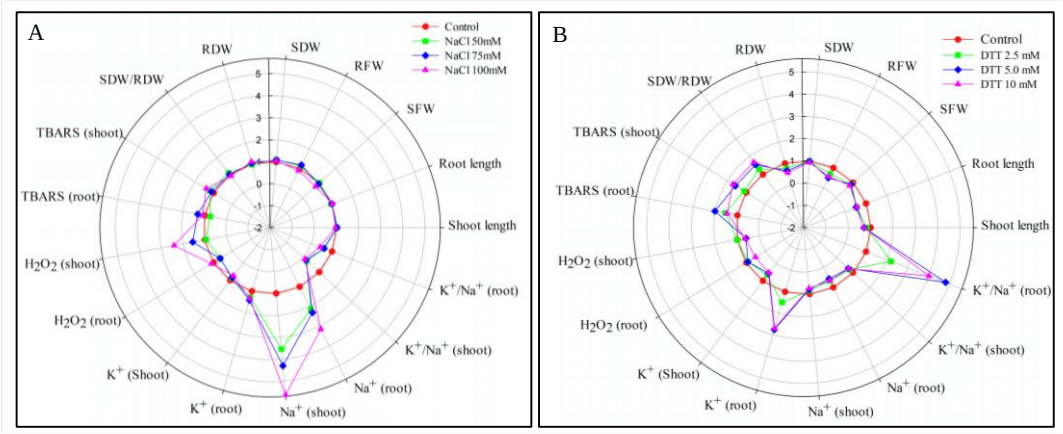


**Figure 1.** Radar plots of sorghum seedlings: (A) exposed to salt stress obtained at 3 days of sowing; and (B) endoplasmic reticulum stress exposed 3 to 7 days of sowing. Shoot fresh mass (SFW), shoot dry mass (SDW), root fresh mass (RFW), root dry mass (RDW), and SDW/RDW ratio. Data are the means of 4 repetitions (each repetition composed of 10 different seedlings).

Since the sorghum seedlings were tolerant to salt, we evaluated if cv. CSF20 could be tolerant or sensitive to disruption of ER homeostasis. In this current work, fresh and dry mass were not changed by the presence of DTT, as well as hydrogen peroxide and lipid peroxidation, which means satisfactory plant health and low cell damage of seedlings under the lower concentration (Figure 1B). On the other hand, it promoted a decrease of seedling length, regardless of the concentration used. In agreement, 7.5 mM DTT treatment inhibited the height and weight of wheat plants after two days (Yu *et al*., 2019). DTT may act as a hydrogen peroxide scavenger in shoots from 5 mM, and in roots under 10 mM, the decrease of $H_2O_2$ promotes a decrease of lignin accumulation (Kováčik *et al*., 2010) which are necessary for regular root growth (Foreman *et al*., 2003). Besides, the reduced growth may also be linked to lipid peroxidation induced by other ROS, which contributes to membrane cell instability, associated to the selective portioning of $K^+$ over $Na^+$ by membrane transporters (Wang *et al*., 2019). In fact, the presence of higher concentrations of DTT promoted an ionic imbalance, it changed the $K^+$ portioning between shoots and roots, increasing $K^+$ and reducing $Na^+$ contents, especially in the roots.

## CONCLUSIONS

The sorghum cv. CSF20 is NaCl tolerant in the early seedling stage. Further, our results suggest that until 2.5 mM DTT the seedlings may tolerate this reducing agent, and above 5mM the plant started to exhibit deleterious symptoms.

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## REFERENCES

Bao, Y., Bassham, D.C., Howell, S.H., A Functional Unfolded Protein Response Is Required for Normal Vegetative Development. **Plant Physiol.** v. 179, p. 1834–1843, 2019.

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 Plant Physioly.** v. 17, p. 353–361, 2005.

Foreman, J., Demidchik, V., Bothwell, J.H.F., Mylona, P., Miedema, H., Angel Torres, M., Linstead, P., Costa, S., Brownlee, C., Jones, J.D.G., Davies, J.M., Dolan, L., Reactive oxygen species produced by NADPH oxidase regulate plant growth. **Nature.** v.27, p. 422-426, 2003.

Freitas, V.S., Lídia, N., Alencar, M., Feitosa De Lacerda, C., Prisco, J.T., Gomes-Filho, E., Changes in physiological and biochemical indicators associated with salt tolerance in cotton, sorghum and cowpea. **African Journal Biochemestry.** v. 5, p. 264–271, 2011.

Kováčik, J., Grúz, J., Klejdus, B., Štork, F., Marchiosi, R., Ferrarese-Filho, O. Lignification and related parameters in copper-exposed Matricaria chamomilla roots: Role of $H_2O_2$ and NO in this process. **Plant Science.** v. 119, p. 383-389. 2010.

Lacerda, C.F. De, Cambraia, J., Cano, M.A.O., Ruiz, H.A. Plant growth and solute accumulation and distribution in two sorghum genotypes, under NaCl stress. **Revista Brasileira de Fisiologia Vegetal.** v. 13, p. 270–284, 2001.

Oliveira, V.P. De, Marques, E.C., Lacerda, C.F. De, Prisco, J.T., Gomes-filho, E. Physiological and biochemical characteristics of *Sorghum bicolor* and *Sorghum sudanense* subjected to salt stress in two stages of development. **African Journal Agricultural Research.** v. 8, p. 660–670, 2013.

Parihar, P., Singh, S., Singh, R., Singh, V.P., Prasad, S.M. Effect of salinity stress on plants and its tolerance strategies: a review. **Environmental Scince Pollution Research.** v. 22, p.

4056–4075, 2015.

Park, C.-J., Park, J.M. ER Plays a Critical Role in Integrating Signals Generated by Both Biotic and Abiotic Stress in Plants. **Frontiers in Plant Science.** v. 10, p. 1–8. 2019.

Wang, Q., Guan, C., Wang, P., Ma, Q., Bao, A.-K., Zhang, J.-L., Wang, S.-M. The Effect of *At*HKT1;1 or *At*SOS1 Mutation on the Expressions of $Na^+$ or $K^+$ Transporter Genes and Ion Homeostasis in Arabidopsis thaliana under Salt Stress. **International Journal Molecular Science.** v. 20, p. 1–15.2019.

Yu, X., Wang, T., Zhu, M., Zhang, L., Zhang, F., Jing, E., Ren, Y., Wang, Z., Xin, Z., Lin, T., Transcriptome and physiological analyses for revealing genes involved in wheat response to endoplasmic reticulum stress. BMC Plant Biol. v. 19, p. 193, 2019.

Zörb, C., Geilfus, C. -M., Dietz, K. -J., Salinity and crop yield. **Plant Biology.** v. 21, p. 31–38, 2019.