NaCl-INDUCED DAMAGES ON PHOTOSYNTHESIS AND CHLOROPLAST ULTRASTRUCTURE OF SORGHUM PLANTS

Sávio Justino Da Silva[1], Stelamaris De Oliveira Paula-Marinho[2], Rafael De Souza Miranda[3], Gyedre Dos Santos Araújo[4], Lineker De Sousa Lopes[5], Enéas Gomes-Filho[6]

ABSTRACT: In this study, we investigated the NaCl impact in the growth and photosynthetic apparatus of sorghum plants, highlighting the oxidative damages on photosynthesis. After germination, uniform *Sorghum bicolor* seedlings were transferred to Hoagland's nutrient solution and treated with NaCl at 0 (control) and 75 mM (salt stress) for twelve days. Sorghum growth was severely decreased by salinity, which was associated with reducing photosynthesis performance and photochemical efficiency. In parallel, there was high ROS production and increased MDA content in leaves and injury in chloroplast structure under salt stress. This was related to impairment on biochemical (reduced CO_2 assimilation rate) and photochemical efficiency (reducing photochemical quenching and increasing energy excess at the PSII). Then, our results suggested that NaCl-induced oxidative stress promoted disruption of chloroplast integrity and impairment of the photochemical process, which resulted in reduced photosynthesis and biomass accumulation of stressed-sorghum plant.

KEYWORDS: Salt stress, oxidative stress, chloroplast integrity

DANOS INDUZIDOS PELO NaCl À FOTOSSÍNTESE E ULTRAESTRUTURA DE CLOROPLASTOS EM PLANTAS DE SORGO

RESUMO: Neste estudo, investigou-se o impacto do NaCl no crescimento e aparato fotossintético de plantas de sorgo, com destaque aos danos oxidativos na fotossíntese. Após germinação, plântulas uniformes de *Sorghum bicolor* foram cultivadas em solução nutritiva de Hoagland e tratadas com NaCl a 0 (controle) e 75 mM (estresse salino) por doze dias. A salinidade reduziu severamente o crescimento, a atividade fotossintética e eficiência

---

[1] Graduando, Departamento de Bioquímica e Biologia Molecular, UFC, Bloco 907, Sala 2015, CEP 60440-554, Fortaleza, CE. Fone (85) 98548-5644. E-mail: saviojustino.silva@gmail.com
[2] Doutora, Departamento de Bioquímica e Biologia Molecular, UFC, Fortaleza, CE.
[3] Prof. Doutor, Programa de Pós-graduação em Ciências Agrárias, UFPI, Bom Jesus, PI
[4] Doutora, Departamento de Bioquímica e Biologia Molecular, UFC, Fortaleza, CE.
[5] Doutor, Departamento de Bioquímica e Biologia Molecular, UFC, Fortaleza, CE.
[6] Prof. Doutor, Departamento de Bioquímica e Biologia Molecular, UFC, Fortaleza, CE.

fotoquímica de plantas de sorgo. Além disso, foi observado em folhas de plantas estressadas um aumento significativo na produção de ROS e conteúdo de MDA, bem como danos à estrutura dos cloroplastos. Estes efeitos foram relacionados com reduções na eficiência bioquímica (taxa de assimilação de CO_2 reduzida) e fotoquímica (redução no quenching fotoquímico e aumento no excesso de energia no PSII). Os resultados sugerem que o estresse oxidativo induzido pelo NaCl promoveu danos a integridade dos cloroplastos e diminuição do processo fotoquímico, levando a redução na fotossíntese e acúmulo de biomassa em plântulas de sorgo sob salinidade.

PALAVRAS-CHAVE: Estresse salino, estresse oxidativo, integridade dos cloroplastos

INTRODUCTION

The soil salinization has affected plant growth and production, which is a consequence of the low rainfall, high evapotranspiration, and the use of low-quality irrigation water (BELTRÁN, 2016). In plants, the salinity provokes water, ionic and biochemical unbalance caused by osmotic and ionic effects. The rupture on the physiological, biochemical, morphological, and molecular processes of the plant induces secondary effects, including the overgeneration of reactive oxygen species (ROS), resulting from oxidative stress (AHMAD et al., 2019). Salinity-induced oxidative stress in photosynthetic tissues can provoke severe damage to photosynthetic machinery associated with a reduction in photosynthetic pigments content, impairment in photochemical efficiency of photosystem II and damages in the ultrastructure of chloroplasts (OMOTO et al., 2009; ARAÚJO et al., 2018). Thus, efforts are currently being made to comprehend the salinity impact in plant metabolism, such as photosynthesis, to investigate target mechanisms to improve plant tolerance to salt stress. *Sorghum bicolor* is considered the fifth most important cereal globally, which had high value nutritional to human and animal feed and to ethanol production (WHITFIELD et al., 2012). Although sorghum is widely known as a moderately salt-tolerant crop (LACERDA et al., 2003), its cultivation in several agricultural areas has become a severe problem due to gradual salt accumulation in soils. This study was conducted to verify the NaCl impact in growth and photosynthesis of sorghum plants, mainly focusing on oxidative damages on photosynthetic tissues.

MATERIAL AND METHODS

Sorghum seeds [*Sorgum bicolor* (L.) Moench.] of genotype CSF 20, provided by Instituto Agronômico de Pernambuco (IPA), Brazil, were sown in vermiculite for four days. After the seedlings were transferred to plastic pots containing Hoagland's nutrient solution (HOAGLAND & ARNON, 1950) formulated to contain nitrogen form at 5.0 mM. After eight days of acclimation, the seedlings were subjected to saline conditions with 75 mM NaCl, and they were harvested after 12 days after salt addition. The experiments were carried out in a greenhouse with the midday photosynthetic photon flux density (PPFD) of 1,200 μmol m^{-2} s^{-1}. Also, the mean air temperature was 32.2 °C during the day and 25.9 ºC at night and the average air relative humidity was 63.4%. Measurements of gas exchange and chlorophyll fluorescence parameters were performed between 9:00 to 11:00 am after each harvest using an infrared gas analyzer (IRGA) with a fluorometer (6400-40, LI-COR, USA) coupled under PPFD of 1,200 μmol m^{-2} s^{-1}. The plants were separated at shoots (leaves + stems) and roots, frozen in liquid nitrogen, and after dried by lyophilization, the dry mass was measured. The content of photosynthetic pigments also was measured with extraction in dimethyl sulfoxide (DMSO) solution saturated with $CaCO_3$, as described by Wellburn (1994). In leaves, the mesophyll chloroplast ultrastructure was investigated from fully-expanded leaf sections (1-2 mm^2) by transmission electron microscopy (OMOTO et al., 2009), while ROS production was examined by confocal laser scanning microscopy (CLSM), using the probe 2',7'-dichlorofluorescein diacetate (DCFH-DA). The superoxide radical ($\bullet O_2^-$) content (ELSTNER & HEUPEL, 1976), H_2O_2 concentration (FERNANDO & SOUZA, 2015), and lipid peroxidation (HEATH & PACKER, 1968) were determined in leaves of sorghum plants. The data were subjected to ANOVA, and the means were compared using Tukey's test ($p<0.05$).

RESULTS AND DISCUSSION

Salt stress is widely known to impair plant growth and productivity by disturbing numerous physiological and biochemical processes like ion and oxidative homeostasis (NEGRÃO et al., 2017). Concordantly, in this study, the biomass accumulation of sorghum plants was drastically reduced by salt stress, and shoot tissues suffered the most harmful effect with 46% reduction compared with control conditions (Table 1). In salinity presence, the CO_2 assimilation rate (*A*), ΦPSII, and qP parameters were significantly decreased, well as photosynthetic pigments (Table 1). Consequently, the low PSII efficiency under salt stress was associated with a higher non-photochemical quenching (NPQ) compared with control conditions. These results suggest that a part of absorbed energy was not driven to reaction centers, which could be dissipated via thermal processes, such as heat dissipation or xanthophyll cycle, displaying NPQ raise (ALLEL et al., 2018; ARAÚJO et al., 2018).

Table 1. Plant dry mass (shoot and roots), photosynthetic parameters, and photosynthetic pigments contents of sorghum plants cv. CSF 20 grown under salinity stress (75 mM NaCl) for 12 days.

Parameters	Control (C)	Salt Stress (S)	C x S
Shoot dry mass (g plant^{-1})	3.53 ± 0.08	1.91 ± 0.12	*
Root dry mass (g plant^{-1})	0.75 ± 0.03	0.50 ± 0.02	*
CO_2 assimilation rate [A] (μmol CO_2 m^{-2} s^{-1})	31.63 ± 1.85	22.73 ± 1.79	*
Effective quantum yield of PSII [ΦPSII]	0.47 ± 0.01	0.41 ± 0.02	*
Photochemical quenching [qP]	0.76 ± 0.01	0.73 ± 0.01	*
Non-photochemical quenching [NPQ]	0.49 ± 0.02	0.74 ± 0.05	*
Relative energy excess at the PSII level [EXC]	0.14 ± 0.01	0.22 ± 0.02	*
Chlorophyll total (mg g^{-1} dry mass)	30.02 ± 1.94	21.00 ± 1.26	*
Carotenoids (mg g^{-1} dry mass)	3.97 ± 0.25	3.09 ± 0.18	*

Data indicate mean ± SE (n=5). The asterisk indicates a significant difference ($p<0.05$).

Although stressed plants employed mechanisms to mitigate damage to the photosynthetic apparatus, the unsatisfactory functioning of PSII may be associated with over reduction of electron transport chain (↑EXC) (Table 1), inducing oxidative damage due to enhanced reactive oxygen species production in NaCl-stressed plants (ABDELGAWAD et al., 2016). Consistently, salt-induced EXC increasing was associated with overgeneration and accumulation of ROS ($\bullet O_2^-$ and H_2O_2), high lipid peroxidation (MDA content), and chloroplast structure damage in sorghum plants under salt stress (Fig. 1).

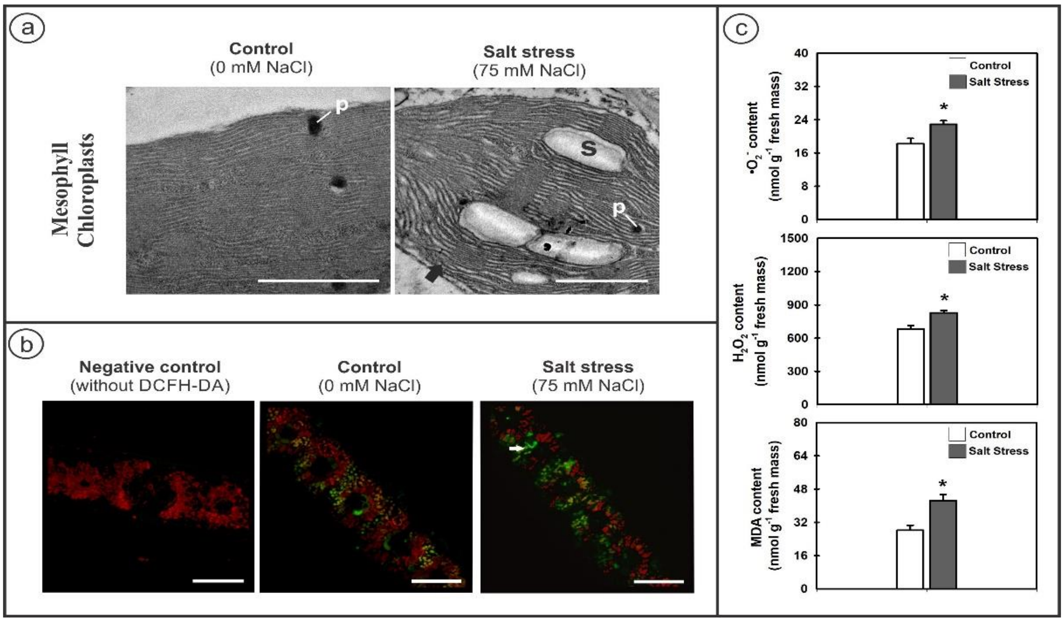

Figure 1. (**a**) Transmission electron micrograph of mesophyll chloroplasts of sorghum plants. Black arrow – wavy thylakoids, p – plastoglobules, S – starch grain. Bar= 1 µm. (**b**) The ROS production (in vivo – green fluorescence) in leaves of sorghum plants after incubation with 2’,7’-dichlorofluorescein diacetate (DCFH-DA). White arrow – ROS production in bundle sheath cells, Red color = chlorophyll *b* autofluorescence. Bar= 100 µm. (**c**) The superoxide radical ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) content in leaves of sorghum plants cv. CSF 20 grown with 0 mM (control) and 75 mM NaCl (salt stress).

Salinity-induced oxidative stress can promote severe consequences to photosynthetic machinery, which can lead to growth inhibition and reduced CO_2 assimilation rate, well as the damage to chloroplast structure and the accumulation of ROS, as previously reported in *Dianthus superbus* (MA et al., 2017). In C4 plants, NaCl induced severe damages to chloroplasts, such as swelling of thylakoids; moreover, the chloroplasts of mesophyll cells show more sensitivity to salinity than those of bundle sheath cells (OMOTO et al., 2009). Our findings demonstrated that salt-induced oxidative stress leads to the unstacking of grana and swelling of thylakoids, contributing to reduced photosynthesis and biomass accumulation.

CONCLUSIONS

Our findings reveal that NaCl stress promoted oxidative damage by increasing ROS production, which results in disruption of chloroplast integrity and impairment of the photochemical process in sorghum plants. However, further biochemical studies are necessary to elucidate the mechanisms associated with response to salt stress in sorghum plants.

BIBLIOGRAPHIC REFERENCES

ABDELGAWAD, H.; ZINTA, G.; HEGAB, M. M.; PANDEY, R.; ASARD, H.; ABUELSOUD, W. High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. **Frontier in Plant Science**, v. 7, p. 276, 2016.

AHMAD, R.; HUSSAIN, S.; ANJUM, M. A.; KHALID, M. F.; SAQIB, M.; ZAKIR, I.; HASSAN, A.; FAHAD, S.; AHMAD, S. **Oxidative stress and antioxidant defense mechanisms in plants under salt stress**. In: Hasanuzzaman M., Hakeem K., Nahar K., Alharby H. (eds) Plant Abiotic Stress Tolerance. Springer, Cham, 2019.

ALLEL, D.; BEN-AMAR, A.; ABDELLY, C. Leaf photosynthesis, chlorophyll fluorescence and ion content of barley (Hordeum vulgare) in response to salinity. **Journal of Plant Nutrition**, v. 41, p. 497-508, 2018.

ARAÚJO, G. S.; MIRANDA, R. S.; MESQUITA, R. O.; PAULA, S. O.; PRISCO, J. T.; GOMES-Filho, E. Nitrogen assimilation pathways and ionic homeostasis are crucial for photosynthetic apparatus efficiency in salt-tolerant sunflower genotypes. **Plant Growth Regulation**, v. 86, p. 375, 2018.

BELTRÁN, J. M. **Integrated approach to address salinity problems in irrigated agriculture**. IN: GREYI, H.R.; DIAS, N.S.; LACERDA, C.F.; GOMES-FILHO, E. (Ed.) Manejo da salinidade na agricultura: Estudo básico e aplicado. Fortaleza, INCTSal, 2016.

ELSTNER, E. F.; HEUPEL, A. Inhibition of nitrite formation from hydroxyl ammonium chloride: a simple assay for superoxide dismutase. **Anal Biochem**, v. 70, p. 616-620, 1976.

FERNANDO, C. D.; SOUZA, P. Optimized enzymatic colorimetric assay for determination of hydrogen peroxide (H_2O_2) scavenging activity of plant extracts. **Methods X**, p. 283-291, 2015.

HEATH, R. L.; PACKER, L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. **Archives of Biochemistry and Biophysics**, v. 125, p. 385-395, 1968.

HOAGLAND, D. R.; ARNON, D. I.The water culture method for growing plants without soil. **California Agricultural Experiment Station**, v. 347, p. 1-39, 1950.

LACERDA, C. F.; CAMBRAIA, J.; OLIVA, M. A.; RUIZ, H. A. Osmotic adjustment in roots and leaves of two sorghum genotypes under NaCl stress. **Brazilian Journal of Plant Physiology**, v. 15, p. 113-118, 2003.

MA, X.; ZENG, J.; ZHANG, X.; HU, Q.; QIAN, R. Salicylic acid alleviates the adverse effects of salt stress on *Dianthus superbus* (Caryophyllaceae) by activating photosynthesis, protecting morphological structure, and enhancing the antioxidant system. **Frontier in Plant Science**, v. 8, p. 600, 2017.

NEGRÃO, S.; SCHMÖCKEL, S. M.; TESTER, M. Evaluating physiological responses of plants to salinity stress. **Annals of Botany**, p. 119:1-11, 2017.

OMOTO, E.; KAWASAKI, M.; TANIGUCHI, M.; MIYAKE, H. Salinity induces granal development in bundle sheath chloroplasts of NADP-malic enzyme type C4 plants. **Plant Production Science**, v. 12, p. 199-207, 2009.

WELLBURN, A. R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. **Journal Plant Physiology**, v. 144, p. 307-313, 1994.

WHITFIELD, M. B.; CHINN, M. S.; VEAL, M. W. Processing of materials derived from sweet sorghum for biobased products. **Industrial Crops and Products**, v. 37, p. 362-375, 2012.