INOVAGRI International Meeting | XXVIII CONIRD | I Simpósio Latino Americano de Salinidade

GROWTH, PHOTOSYNTHESIS AND FLUORESCENCE OF SORGHUM UNDER SALT CONDITION ARE AFFECTED BY NITROGEN SOURCE

Analya Roberta Fernandes Oliveira[1], Stelamaris de Oliveira Paula-Marinho[2], Gyedre dos Santos Araújo[3], Lineker de Sousa Lopes[4], Humberto Henrique de Carvalho[5], Enéas Gomes Filho[6]

ABSTRACT: The aim of the current study was to investigate the effectiveness of nitrogen source (NO_3^- or NH_4^+) in *Sorghum bicolor* L. subjected to salinity along time. The experiment was conducted in greenhouse, and the plants were cultivated in nutrient solution containing these forms of nitrogen (NO_3^- or NH_4^+) at 5.0 mM under salinity (75 mM NaCl). The experimental design was completely randomized with five replicates per treatment. The harvest occurred after 3, 6 and 12 days after salt (DAS) addition, when growth, gas exchanges and chlorophyll fluorescence parameters were measured. At 12 DAS, NH_4^+-fed plants showed lower leaf area and accumulation of biomass in the roots than NO_3^--fed, although they presented higher values of photosynthesis (*A*) and transpiration (*E*) rates, carboxylation efficiency of Rubisco (*A*/*Ci*) and electron transport rate (ETR) in this harvest time. Based on these results, NH_4^+ nutrition was not favorable to promote optimal growth under salinity in relation to NO_3^- nutrition, but further studies are necessary to clarify the influence of N form in the photosynthetic capacity of sorghum plants.
KEYWORDS: Ammonium, nitrate, salinity.

CRESCIMENTO, FOTOSSÍNTESE E FLUORESCÊNCIA DE SORGO EM CONDIÇÕES SALINAS SÃO AFETADOS PELA FONTE DE NITROGÊNIO

RESUMO: O presente estudo teve como objetivo investigar a eficácia da fonte de nitrogênio (NO_3^- ou NH_4^+) em plantas de *Sorghum bicolor* L. sujeitas à salinidade ao longo do tempo. O

[1] Mestre, Departamento de Bioquímica e Fisiologia Vegetal, UFC, Rua Campus do Pici, s/n., Fortaleza-CE, CEP 60440-554 Tel. +55 85 985071077 E-mail: analyaroberta_fernandes@hotmail.com
[2] Mestre, Departamento de Bioquímica e Fisiologia Vegetal, UFC, Tel. +55 85 996757622 E-mail: maris.biologa@gmail.com
[3] Mestre, Departamento de Bioquímica e Fisiologia Vegetal, UFC, Tel. +55 85 981092151 E-mail: gyedrearaujo@yahoo.com.br
[4] Doutor, Departamento de Bioquímica e Fisiologia Vegetal, UFC, Tel. +55 85 987845302 E-mail: linekerlk@gmail.com
[5] Prof. Doutor, Departamento de Bioquímica e Fisiologia Vegetal, UFC, Tel. +55 85 997881663 E-mail: humberto.carvalho@ufc.br
[6] Prof. Doutor, Departamento de Bioquímica e Fisiologia Vegetal, UFC, Tel. +55 85 999958572 E-mail: egomesf@ufc.br

experimento foi conduzido em casa de vegetação e as plantas foram cultivadas em solução nutritiva contendo essas formas de nitrogênio (NO_3^- or NH_4^+) a 5 mM sob salinidade (NaCl 75 mM). O delineamento experimental foi inteiramente casualizado com cinco repetições por tratamento. As coletas ocorreram depois de 3, 6 e 12 dias de adição do sal (DAS), quando parâmetros de crescimento, de trocas gasosas e de fluorescência da clorofila foram mensurados. Aos 12 DAS, plantas nutridas com NH_4^+ mostraram menor área foliar e menor acúmulo de biomassa nas raízes, em comparação com as nutridas com NO_3^-, apesar de apresentarem mais altos valores de taxas fotossintética (*A*) e transpiração (*E*), de eficiência de carboxilação da Rubisco (*A*/*Ci*) e de taxa de transporte de elétrons (ETR) neste tempo de coleta. Com base nisto, concluiu-se que a nutrição com NH_4^+ não foi favorável para promover um melhor crescimento, sob salinidade, em relação à nutrição com NO_3^-. Porém, mais estudos são necessários para esclarecer a influência da fonte de N na capacidade fotossintética de plantas de sorgo.
PALAVRAS-CHAVE: Amônio, nitrato, salinidade.

INTRODUCTION

The soil salinization affects the plant growth and production, which is more prominent in the arid and semi-arid regions due to the low rainfall, high evapotranspiration and the use of low-quality irrigation water (Ribeiro, 2010). In plants, the salinity provoke disturbs in water, ionic and biochemical balance caused by osmotic and ionic effects. The first impact is associated with reduction of water potential of the soil, which restricts the growth plant by interfering with water and nutrient uptake by roots (Dias & Blanco, 2010). On the other hand, an ionic effect is related to toxic level of ion (Na^+ and Cl^-) accumulation in tissues, becoming harmful to the physiological and biochemical process of the plant, including photosynthesis (Horie *et al.*, 2012). In addition, recent studies in salinity tolerance field suggest that the nitrogen source provided in the growth medium can influence the salinity responses (Iqbal *et al.*, 2015). However, those results have been quite controversial, ever since some researchers report NO_3^- is determinant for salt stress tolerance, and others show that NH_4^+ nutrition or combination of two sources is favorable for plant growth under salinity. The sorghum (*Sorghum bicolor* L.) is the fifth cereal most cultivated in the world, been a relevant crop and one of the most versatile and efficient, both from the photosynthetic point of view (Yan *et al.*, 2012) and its applicability, including nutrition, human feeding and ethanol production

(Moraes *et al.*, 2012). Thus, this study was conducted to verify the role of the nitrogen source in the growth and development of sorghum plants under different salt stress time.

MATERIALS AND METHODS

The experiment was conducted in greenhouse of the laboratory of Plant Physiology, Department of Biochemistry and Molecular Biology, Federal University of Ceará (UFC) in Fortaleza-CE. Sorghum seeds (*Sorghum bicolor* L. Moench.) of the genotype CSF 20, provided by Instituto Agronômico de Pernambuco (IPA), Brazil, were sown in vermiculite. After four days the seedlings were transferred to plastic pots containing Hoagland's nutrient solution (Hoagland & Arnon 1950) formulated to contain nitrogen form at 5.0 mM, as NO_3^- or NH_4^+. After twelve days of the acclimation, the seedlings were subjected to saline condition with 75 mM NaCl, then they were harvested after 3, 6 and 12 days after salt (DAS) addition.

The experiment was carried out under greenhouse conditions, where the mean air temperatures were the day and night, respectively, 33.8 ± 2 °C and 27.4 ± 2 °C, and the mean relative humidity was 61.0 ± 15%. In each harvest time, leaf area (LA) was evaluated (LI-3100 Area Meter, Li-Cor., Inc, Lincoln, Nebraska, USA), and after they were separated into shoots (stems + leaves) and roots, the material was dried in oven at 60 °C for 72 h to provide the shoot dry mass (SDM) and root dry mass (RDM). Measurements of gas exchange and chlorophyll fluorescence parameters were performed between 9:00 and 11:00 am after each harvest using an infrared gas analyzer (IRGA) (LCi, ADC, Hoddesdon, UK) with a fluorometer (6400-40, LI-COR, USA) coupled under photosynthetic photon flux density of 1,200 $\mu mol\ m^{-2}\ s^{-1}$.

The experimental design was completely randomized, where the treatments were two nitrogen forms (NO_3^- and NH_4^+) under salinity (75 mM NaCl), with five replicates per treatment. The data were subjected to the analysis of variance (ANOVA F-test) using the SISVAR program.

RESULTS AND DISCUSSION

There were no significant differences in the growth parameters by different nitrogen sources, until the application of NaCl (data not presented). Under salinity, the effects of nitrogen nutrition were observed in LA, RDM and SDM of plants at 3, 6 and 12 days after salt addition (75 mM NaCl) (Table 1).

Table 1 Growth parameters of *Sorghum bicolor* by different nitrogen source (NO_3^- or NH_4^+) under salinity stress (75 mM NaCl) at 3, 6 and 12 days after salt (DAS) addition

Harvest time (DAS)	Treatment	Leaf area	Root dry mass	Shoot dry mass
		cm^2 $plant^{-1}$	g. $plant^{-1}$	g. $plant^{-1}$
3	NO_3^-	47.5 ± 1.9 a	0.078 ± 0.01 a	0.150 ± 0.01 a
	NH_4^+	36.2 ± 1.3 a	0.078 ± 0.005 a	0.147 ± 0.01 a
6	NO_3^-	116.4 ± 2.6 a	0.202 ± 0.01 a	0.460 ± 0.02 a
	NH_4^+	119.9 ± 7.2 a	0.208 ± 0.01 a	0.534 ± 0.01 a
12	NO_3^-	315.0 ± 16.1 a	0.583 ± 0.03 a	1.57 ± 0.04 a
	NH_4^+	262.1 ± 11.9 b	0.499 ± 0.05 b	1.60 ± 0.07 a

Data indicate mean ± SE (n=5). Different letters in the same harvest time indicate a significant difference.

In relation to LA, there was a significant change only in 12 DAS, in which NH_4^+-fed plants showed lower LA than NO_3^--fed plants. A similar response was exhibited in relation to biomass accumulation of roots in 12 DAS. It has been reported that under conditions of salt stress, the plants produced less biomass, being this effect more noticeable when NH_4^+ is added in growth medium (Meng *et al*., 2016). The growth inhibition under NH_4^+ nutrition can be associated with a toxic effect of the ion that varies depending on species and different cultivars (Esteban *et al.*, 2016).

The salt stress affects the plant growth mainly for impairing the photosynthetic capacity. Then, in order to investigate the correlation between the N form with differences in carbon assimilation, gas exchanges parameters were determined in sorghum plants under salinity (Table 2).

Table 2 Photosynthetic rate (*A*); transpiration rate (*E*); carboxylation efficiency of Rubisco (*A/Ci*) of *Sorghum bicolor* by different nitrogen source (NO_3^- or NH_4^+) under salinity stress (75 mM NaCl) at 3, 6 and 12 days after salt (DAS) addition

Harvest time	Treatment	*A*	*E*	*A/Ci*
		mmol CO_2 m^{-2} s^{-1}	mmolm^{-2} s^{-1}	-
3 DAS	NO_3^-	26.0 ± 1.0 b	5.6 ± 0.2 b	0.21 ± 0.01 b
	NH_4^+	31.3 ± 1.6 a	7.5 ± 0.4 a	0.35 ± 0.02 a
6 DAS	NO_3^-	27.2 ± 0.7 b	6.3 ± 0.1 b	0.20 ± 0.01 b
	NH_4^+	33.7 ±1.7 a	7.5 ± 0.2 a	0.43 ± 0.03 a
12 DAS	NO_3^-	21.0 ± 0.8 b	6.4 ± 0.2 b	0.10 ± 0.003 b
	NH_4^+	31.3 ± 0.8 a	8.3 ± 0.5 a	0.25 ± 0.02 a

Data indicate mean ± SE (n=5). Different letters in the same harvest time indicate a significant difference.

The photosynthetic (*A*) and transpiration (*E*) rates and carboxylation efficiency of Rubisco (*A*/*Ci*) significantly varied depending on the nitrogen source, were significantly higher when plants were supplied with NH_4^+. However, the high value of *A* did not promote greater growth in NH_4^+-fed plants, in relation to those NO_3^--fed. After three days of salt exposure, it observed a higher *A*, g_s and *E* in the NH_4^+-fed plants than those that were supplied with NO_3^- (Miranda *et al.*, 2013). Gao *et al.* (2016) reported that canola (NO_3^-- preferring) showed growth inhibition, and lower A and g_s when supplied with NH_4^+ to NO_3^- under salt condition, whereas rice (NH_4^+- preferring) had no significant differences caused to the nitrogen forms.

The influence of the nitrogen form in chlorophyll fluorescence parameters differed during salt stress exposure, as showed in table 3. In three days of salt exposure, NO_3^--fed plants presented superior maximum quantum yield of photosystem II (Fv/Fm) and relative energy excess at the PSII level (EXC), also the energy excess was dissipated, as denoted by the high non-photochemical quenching (NPQ); whereas, the NH_4^+ nutrition was more efficient than NO_3^- nutrition in 6 DAS. However, these differences did not persist to 12 days of salt stress in which there was a significant difference only to electron transport rate (ETR) between the N source. According to Miranda *et al.* (2014), favorable electron transport (ETR) allows the generation of reducing power to carbon assimilation and minimizing the production of reactive oxygen species.

Table 3 Maximum quantum yield of photosystem II (Fv/Fm); electron transport rate (ETR); non-photochemical quenching (NPQ) and relative energy excess at the PSII level (EXC) of *Sorghum bicolor* by different nitrogen source (NO_3^- or NH_4^+) under salinity stress (75 mM NaCl) at 3, 6 and 12 days after salt (DAS) addition

Harvest time (DAS)	Treatment	Fv/Fm	ETR	NPQ	EXC
3	NO_3^-	0.78 ± 0.003 a	212.0 ± 4.4 a	1.09 ± 0.1 a	0.26 ± 0.01 a
	NH_4^+	0.76 ± 0.001 b	216.9 ± 7.4 a	0.83 ± 0.05 b	0.20 ± 0.01 b
6	NO_3^-	0.74 ± 0.004 b	213.9 ± 1.5 a	0.87 ± 0.02 a	0.20 ± 0.01 b
	NH_4^+	0.77 ± 0.002 a	207.2 ± 3.3 a	0.88 ± 0.06 a	0.25 ± 0.01 a
12	NO_3^-	0.76 ± 0.01 a	229.4 ± 1.9 b	0.74 ± 0.02 a	0.18 ± 0.01 a
	NH_4^+	0.75 ± 0.002 a	237.8 ± 2.7 a	0.78 ± 0.05 a	0.15 ± 0.01 a

Data indicate mean ± SE (n=5). Different letters in the same harvest time indicate a significant difference.

CONCLUSION

Our results suggest that sorghum plants exposure to salt for a long time (12 DAS) showed lower growth in NH4+-medium, despite it was not associated with a reduction in gas exchanges parameters. Thus, further biochemical studies are necessary to elucidate the mechanisms associated with tolerance of the photosynthetic process in salt-stressed sorghum in NH4+-medium.

ACKNOWLEDGMENTS

The authors would like to thank the CNPq, FUNCAP, INCTSal, IPA and UFC.

REFERENCES

DIAS, N.D.; BLANCO, F.F. Efeitos dos sais no solo e na planta. In: GHEYI, H. R.; DIAS, N. S.; LACERDA, C. F. **Manejo da salinidade na agricultura: Estudos básicos e aplicados.** Fortaleza: Instituto Nacional de Ciência e Tecnologia em Salinidade. p. 129-140, 2010.

ESTEBAN, R.; ARIZ, I.; CRUZ, C.; MORAN, J.F. Review: Mechanisms of ammonium toxicity and the quest for tolerance. **Plant Science**, v. 248, p. 92-101, 2016.

GAO, L.; LIU, M.; WANG, M.; SHEN, Q.; GUO, S. Enhanced salt tolerance under nitrate nutrition is associated with apoplast Na^+ content in canola (*Brassica napus* L.) and rice (*Oryza sativa* L.) plants. **Plant and Cell Physiology**, v. 57(11), p. 2323-2333, 2016.

HOAGLAND, D.R.; ARNON, D.I. **The water culture method for growing plants without soil.** California. Agr. Exp. STA. Cir, 347p., 1950.

HORIE, T.; KARAHARA, I.; KATSUHARA, M. Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants. **Rice**, v. 5, p. 11, 2012.

IQBAL, N.; UMAR, S.; KHAN, N. Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (*Brassica juncea*). **Journal of Plant Physiology**, v. 178, p. 84 – 91, 2015.

MENG, S.; SU, L.; LI, Y.; WANG, Y.; ZHANG, C.; ZHAO, Z. Nitrate and ammonium contribute to the distinct nitrogen metabolism of *Populus simonii* during moderate salt stress. **Plos One**, v. 11, e0150354, 2016.

MIRANDA, R.S.; ALVAREZ-PIZARRO, J.C.; ARAÚJO, C.M.S.; PRISCO, J.T.; GOMES-FILHO, E. Influence of inorganic nitrogen sources on K^+/ Na^+ homeostasis and salt tolerance in sorghum plants. **Acta Physiologiae Plantarum**, v. 35, p. 841–852, 2013.

MIRANDA, R.S.; MESQUITA, R.O.; FREITAS, N.S.; PRISCO, J.T.; GOMES-FILHO, E. Nitrate: ammonium nutrition alleviates detrimental effects of salinity by enhancing photosystem II efficiency in sorghum plants. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v. 18, p. S8–S12, 2014.

MORAES, É.A.; QUEIROZ, V.A.V.; SHAFFERT, R.E.; COSTA, N.M.B.; NELSON, J.D.; RIBEIRO, S.M.R.; MARTINO, H.S.D. In vivo protein quality of new sorghum genotypes for human consumption. **Food Chemistry**, v. 134, n. 3, p. 1549-1555, 2012.

RIBEIRO, M.R.; RIBEIRO FILHO, M.R.; JACOMINE, P.K.T. Origem e classificação dos solos afetados por sais In: GHEYI, H. R.; DIAS, N. S.; LACERDA, C. F. de; GOMES FILHO, E. (Ed.) **Manejo da salinidade na agricultura: Estudos básicos e aplicados.** Fortaleza: Instituto Nacional de Ciência e Tecnologia em Salinidade, 2 ed., p. 9-16, 2016.

YAN, K.; CHEN, P.; SHAO, H.; ZHAO, S.; ZHANG, L.; ZHANG, L.; XU, G.; SUN, J. Responses of photosynthesis and photosystem II to higher temperature and salt stress in sorghum. **Journal of Agronomy and Crop Science**, v. 198 n. 3, p. 218-225, 2012.