EFFECTS OF EXOGENOUS SELENIUM APPLICATION ON THE GROWTH OF SENSITIVE AND TOLERANT SORGHUM PLANTS UNDER SALT STRESS

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ABSTRACT: Sorghum (*Sorghum bicolor*) is an important species for semiarid regions, such as Brazilian Northeast, where soil salinization and water quality is becoming a striking problem. Several research centers have evaluated the role of different compounds capable to induce changes in plant metabolism and improve salt tolerance. For some abiotic stress, selenium (Se) emerges as an alternative to activate plant defense responses; however, its involvement in tolerance to salt stress remain poorly understood. Thus, this work aimed to evaluate the effects of exogenous selenium application on the growth of sorghum plants under salt stress. Sorghum plants from CSF 20 (salt-tolerant) and CSF 18 (salt-sensitive) genotypes were grown in nutrient solutions containing sodium selenite (Na_2SeO_3) at 0 (control), 2, 4, 8 and 16 μM, and subjected to 75 mM NaCl-stress. The analyses were done after ten days of saline treatments. Overall, the benefits effects of Se were observed only in sorghum plants from salt-sensitive genotype, whereas no expressive modulation was registered in the salt tolerant one. Although leaf area and root dry mass were severely decreased by salt stress, the salt negative effects were almost completely abolished by 16 μM-Se supply in salt-sensitive genotype; nonetheless, the same responses were not observed for dry mass of leaves. In conclusion, selenium supply may constitute a strategy to overcome salt damage in *S. bicolor* growth depending on dose and plant genotype.

KEYWORDS: *Sorghum bicolor*, leaf area, salinity

EFEITOS DA APLICAÇÃO EXÓGENA DE SELÊNIO NO CRESCIMENTO DE PLANTAS DE SORGO SENSÍVEIS E TOLERANTES AO ESTRESSE SALINO

RESUMO: O sorgo (*Sorghum bicolor*) é uma espécie importante para regiões semiáridas, como a do Nordeste brasileiro, onde a salinização dos solos e a da água são problemas graves.

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Diversos centros de pesquisa têm avaliado o papel de diferentes compostos na indução de alterações no metabolismo das plantas e na melhora da tolerância ao estresse salino. Para alguns estresses abióticos, o selênio (Se) surge como uma alternativa que induz respostas de defesa de plantas, no entanto seu envolvimento na tolerância de plantas ao estresse salino permanece pobremente compreendido. Assim, este trabalho teve como objetivo avaliar os efeitos da aplicação exógena de Se no crescimento de plantas de sorgo sob estresse salino. Plantas de sorgo dos genótipos CSF 20 (tolerante a sal) e CSF 18 (sensível a sal) foram cultivadas em soluções nutritivas contendo selenito de sódio (Na_2SeO_3) a 0 (controle), 2, 4, 8 e 16 μM e submetidas a estresse salino com NaCl a 75 mM. As análises foram feitas após dez dias dos tratamentos salinos. Em geral, os efeitos benéficos do Se foram observados apenas em plantas de sorgo do genótipo sensível ao sal, enquanto que nenhuma modulação expressiva foi registrada no tolerante. Embora a área foliar e a massa seca da raiz tenham sido severamente diminuídas pelo estresse salino, os efeitos negativos do sal foram quase completamente eliminados pelo fornecimento de Se a 16 μM no genótipo sensível. Contudo, as mesmas respostas não foram observadas para massa seca de folhas. Desta forma, o fornecimento de selênio pode constituir uma estratégia para superar os danos causados pelo estresse salino no crescimento de S. bicolor, porém seus efeitos são dependentes da dose e do genótipo da planta.

PALAVRAS-CHAVE: *Sorghum bicolor*, área foliar, salinidade

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is a grass species with agronomic important for Northeast region of Brazil (Duarte, 2010). In this region, a large portion of arable areas is particularly exposed to high salt concentration in soil, which severely limits plants growth and productivity (Díaz-Lopes et al., 2012; Prisco & Gomes-Filho, 2008).

Salt stress effects may arise primarily from decreased osmotic potential and toxic ion accumulation (Na^+ and Cl^-) (Ashraf, 2009), that cause disturbances in the major physiological and biochemical processes of plants (Hossain et al., 2015). Secondarily, water deficit and ionic toxicity can cause oxidative stress, characterized by the overproduction of reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•) (Demidchik, 2015). In excess, ROS can result in extensive damage to cell structures, carbohydrates, proteins, DNA and lipids, thereby causing degradation of chlorophyll, lipid peroxidation, membrane degradation, proteins and enzymes of the photosynthetic apparatus, enzymatic inhibition and, finally, cell death (Gill et al., 2013).

Considerable efforts have been made to enhance plant salt tolerance, including the use of signaling molecules as hydrogen peroxide (H_2O_2) (Gondim et al., 2012), abscisic acid (Li et al., 2010), compounds that release nitric oxide (NO) (Gadelha et al., 2017), polyamines (Kamiab et al., 2014), brassinosteroids (Sever Mutlu et al., 2017) and selenium (Jiang et al., 2017). These molecules were tested in order to evaluate their potential to mitigate the deleterious effects of salt stress in several crop species.

Selenium (Se) is an essential micronutrient for animals and usually considered a beneficial element for many plant species (Feng et al., 2013; Gupta & Gupta, 2017). Many studies have demonstrated that in low concentrations, Se may acts as a protective agent against several abiotic stresses, such as heavy metals (Chauhan et al., 2017), UV radiation (Golob et al., 2017), drought (Nawaz et al. 2016), high temperatures (Djanaguiraman et al., 2010) and salinity (Jiang et al., 2017). In plants subjected to abiotic stress, Se seems to act as an antioxidant, alleviating oxidative stress (Mozafariyan et al., 2016), as well as it may be involved in the efficiency of photosynthetic machinery, alleviating the chlorophyll degradation and preserving the chloroplast ultrastructure (Jiang et al., 2017). However, there is no information regarding on the role of exogenous Se supply in mitigating the harmful effects of salinity in sorghum plants. Therefore, this study aimed to evaluate the effects of exogenous Se application on plant growth of sorghum genotypes with contrasting tolerance to salt stress

MATERIALS AND METHODS

Experimental conditions and growth parameters

Seeds of sorghum (*Sorghum bicolor* L. Moench), CSF18 and CSF20 genotypes, previously stablished as salt-sensitive and salt-tolerant (Lacerda et al., 2003), respectively, were obtained from Instituto Agronômico de Pernambuco (IPA). The experiments were carried out in a greenhouse and the analyzes were measured at Plant Physiology Laboratory, of Department of Biochemistry and Molecular Biology, Federal University of Ceará (UFC).

Sorghum seeds were first sterilized with 2 % sodium hypochlorite, and subsequently sown in vermiculite moistened with distilled water. After seven days, the uniform seedlings were transferred to a tray containing 10.0 L Clark (1975) nutrient solution. After seven days, the plants were also selected by uniformity and transferred to 7 L buckets (two plants / bucket), containing nutrient solutions with different concentrations of Se (0, 2, 4, 8 and 16 µM Na_2SeO_3) and NaCl (0 and 75 mM). The studied treatments were the following:

→ ***Control*** - neither Se nor NaCl

→ ***Salt stress*** - no Se and NaCl treatment

→ ***Se 1*** **-** Treatment with 2 µM Se and NaCl stress;

→ ***Se 2*** **-** Treatment with 4 µM Se and NaCl stress;

→ ***Se 3*** **-** Treatment with 8 µM Se and NaCl stress;

→ ***Se 4*** **-** Treatment with 16 µM Se and NaCl stress;

The nutrient solutions were maintained under constant aeration and renewed every three days, and the pH values were maintained at approximately 6.0. Ten days after salt treatments, plants were individually harvested and the leaf area was determined using a LI-3000 leaf area meter (LI-COR, Inc. Lincoln, NE, USA). Subsequently, the plant material was dried in a forced-air circulation oven at 70°C and weighed to determine the dry mass (DM) of leaves and roots.

Statistical analyses

The experimental design was completely randomized, with five replicates. Each experimental unit (repetition) consisting of two plants. The data were subjected to a two-way analysis of variance (ANOVA), and when a difference was significant ($p \leq 0.05$), the mean values were compared by Tukey's test.

RESULTS AND DISCUSSION

Salt stress has been extensively shown to severely reduce plant growth and limit crop productivity. Herein, the sorghum growth was reduced by presence of 75 mM NaCl stress; however, the level of damage clearly depended on genotype. As shown in table 1, the salt stress severely decreased the leaf and root dry mass and leaf area. In salt-sensitive genotype (CSF18), the values of shoot and root dry mass and leaf area were reduced by 60, 35 and 52%, respectively, as compared to control; while in salt-tolerant genotype (CSF20), these reductions were only 53, 23 and 49%. Our data are in accordance with those of Lacerda et al. (2003), which showed that the salt deleterious effects in sorghum growth were less severe in salt-tolerant genotype (CSF20) in comparison with salt-sensitive genotype (CSF18) (Table 1).

In this study, the effectiveness of selenium (Se) supply in ameliorating the salt damage on the growth of sorghum genotypes was examined. Interestingly, in salt-stressed CSF18 plants, the leaf dry mass was unaltered by Se supply (Fig 1 A), whereas the root dry mass and leaf area were increased by 52 and 35% under 16 µM Se treatment (Fig 1 B,C). Conversely, in CSF20 plants, no obvious differences in the growth parameters (leaf and root dry mass and leaf area) were observed between the Se treatments and only salt stressed (0 µM Se).

Previous studies have shown that Se improves plant salt tolerance (Jiang et al., 2017; Mozafariyan et al., 2016; Diao et al 2014). Most these studies have suggested that, under stressful conditions, Se seems to act as an antioxidant, alleviating oxidative stress. In addition, this element may be involved in photosynthesis and ionic homeostasis regulation. Jiang et al (2017) showed that the 1 µM Se supply significantly increased the shoot and root dry mass of salt stressed maize plants. Similarly, Diao et al. (2014) demonstrated that exogenous Se improved the growth of tomato seedlings under salinity in both salt-sensitive and salt-tolerant genotypes. On the other hand, we did not observe any beneficial effects of Se in sorghum plants from salt-tolerant genotype (Fig 1D, E, F), but a slight benefic influence was registered in leaf area and root dry mass in salt-sensitive plants (Fig 1 A, B, C). Our findings suggest that the beneficial effects of Se supply are dose and genotype dependent (Jiang et al. 2017 and Lehotai et al., 2012).

CONCLUSION

Our data indicate that the Se supply may constitute a strategy to overcome salt damage in *S. bicolor* growth depending on dose and plant genotype. Nonetheless, further studies must be carried out to understanding the precise role of Se in increasing salt tolerance of *S. bicolor* plants.

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Table 1. Dry mass of leaves and roots and leaf area of Sorghum bicolor after ten days in absence (Control) or presence of 75 mM NaCl (salt stress). Values are given as the mean of five biological replications + standard error. Significant differences are indicated by different lowercase letters, using Tukey's test ($p \leq 0.05$).

Genotypes	**Growth measurements**		**Control**	**Salt Stress**
CSF18	Dry Mass	Leaves	0,898 ±0,040 a	0,356 ± 0,018 b
		Roots	0,282 ± 0,008 a	0,183 ± 0,009 b
	Leaf area		209,127 ± 14,969 a	100,140 ± 1,776 b
CSF20	Dry Mass	Leaves	1,025 ± 0,074 a	0,480 ± 0,014 b
		Roots	0,307 ± 0,019 a	0,236 ± 0,006 b
	Leaf area		243,780 ± 10,186 a	123,090 ± 2,388 b

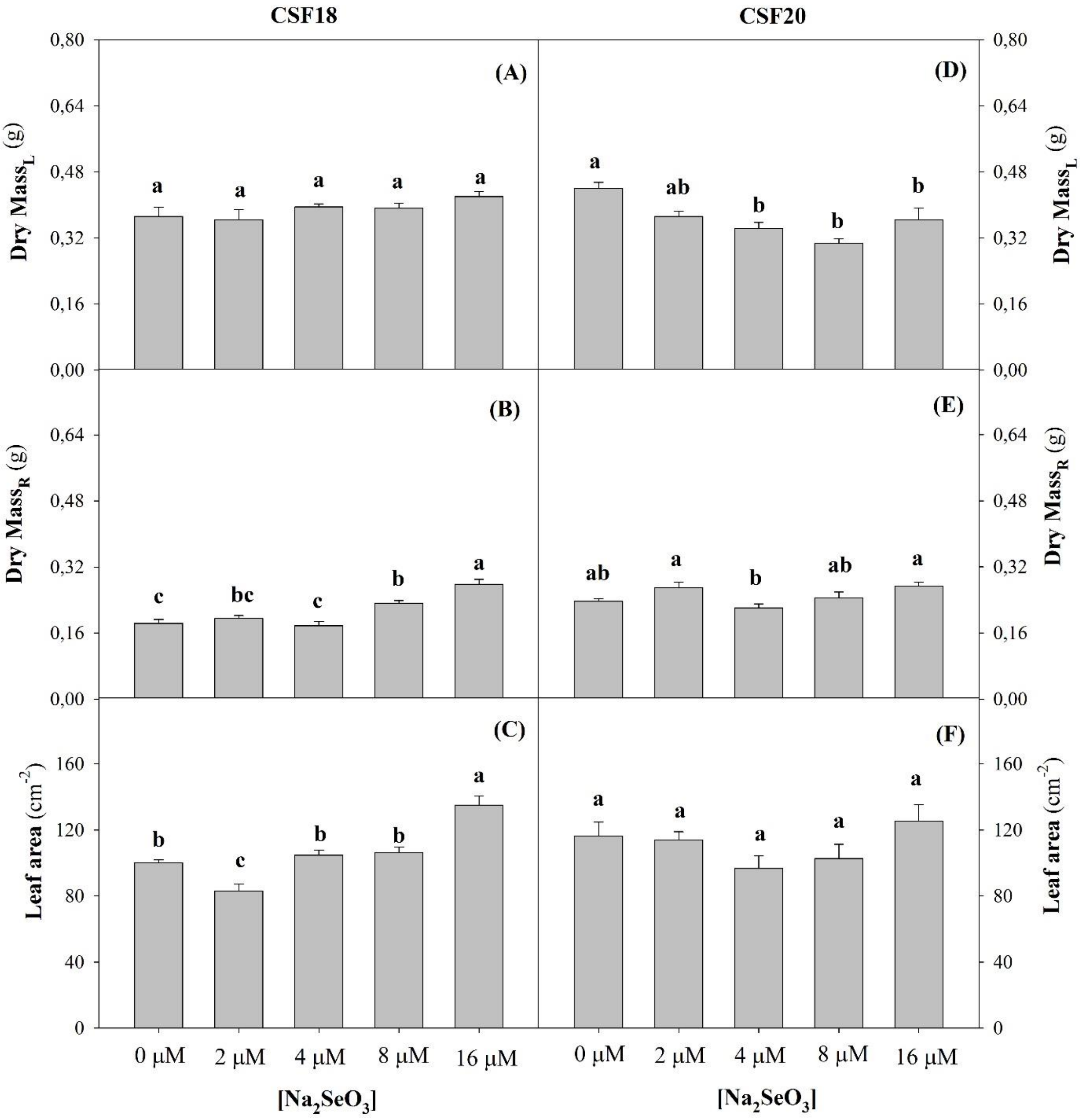

Figure 1. Dry mass of leaves (A, D) and roots (B, E) and leaf area (B, D) of Sorghum bicolor after ten days in presence of 75 mM NaCl (salt stressed) and 0, 2, 4, 8 or 16 µM Na_2SeO_3. Values are given as the mean of five biological replications + standard error. Significant differences due to selenium supply are indicated by different lowercase letters, using Tukey's test ($p \leq 0.05$).