SELECTION OF SUNFLOWER GENOTYPES WITH TOLERANCE TO SALINITY[1]

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ABSTRACT: This study aimed to identify sunflower genotypes with differential tolerance to salt stress. Six sunflower (*Helianthus annuus* L.) genotypes (Catissol, BRS 321, BRS 323, BRS 324, H360 and H251) were grown in a greenhouse-hydroponic culture in absence (control) and presence of NaCl at 100 mM (stress). Experimental design was completely randomized, in a 2 x 6 factorial scheme, consisting of two growth conditions (control and salt stress) and six sunflower genotypes, with five repetitions. Plant growth and CO_2 assimilation parameters were measured after ten days of salinity exposure. In general, salt stress severely decreased all analyzed plant growth parameters, irrespective of sunflower genotype; however, reductions in leaf area (LF), shoot (SDM), root (RDM) and total (TDM) dry mass were more aggressive in Catissol genotype (78, 72, 39 and 65%, respectively) and less pronounced in BRS 321 genotype (62, 57, 33 and 53%, respectively). Interestingly, better performance of BRS 321 genotype was attributed to superior photosynthetic rate under saline conditions. In conclusion, our data indicate that BRS 321 genotype is a good alternative for cultivating sunflower plants in saline and/or salinity-prone regions.

KEYWORDS: salt stress; photosynthesis; *Helianthus annuus* L.

SELEÇÃO DE GENÓTIPOS DE GIRASSOL COM TOLERANCIA À SALINIDADE

RESUMO: Esse estudo teve como objetivo identificar genótipos de girassol com tolerância diferencial ao estresse salino. Seis genótipos de girassol (*Helianthus annus L.*) (Catissol, BRS 321, BRS 323, BRS 324, H360, H251) foram cultivados em casa de vegetação, sob condições de cultivo hidropônico, na ausência (controle) e na presença de NaCl a 100 mM (estresse). O delineamento experimental foi inteiramente casualizado, em arranjo fatorial 2 x 6, consistindo de dois tratamentos de estresse (controle e estresse salino) e seis genótipos de girassol, com

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cinco repetições. Os parâmetros de crescimento e assimilação de CO_2 foram mensurados após 10 dias de exposição à salinidade. De modo geral, o estresse salino reduziu severamente todos os parâmetros de crescimento analisados, independente do genótipo de girassol; no entanto, as reduções na área foliar (AF) e massas secas da parte aérea (MSPA), das raízes (MSR) e total (MST) foram maiores no genótipo Catissol (78, 72, 39 e 65%, respectivamente) e menos pronunciadas no genótipo BRS 321 (62, 57, 33 e 53%, respectivamente). Curiosamente, o melhor desempenho do genótipo BRS 321 foi atribuído a maior taxa fotossintética sob condições de salinidade. Como conclusão, os resultados indicam que o genótipo BRS 321 é uma boa alternativa para cultivo de plantas de girassol em solos salinos.

PALAVRAS-CHAVE: estresse salino; fotossíntese; *Helianthus annus* L.

INTRODUCTION

Salinity is one of the main abiotic stresses that restrict crop productivity worldwide, affecting almost all the physiological, morphological, biochemical and molecular characteristics of plants (Gorai & Neffati, 2007; Shavrukov, 2013). Most areas affected by salinity occur naturally, however, a significant proportion of arable areas have become saline due to vegetation withdrawals and saline water irrigations (Munns & Tester, 2008). In Brazil, the soils affected by salts are around 20 to 25% of the arable land, mainly concentrated in the Northeast region (FAO, 2012).

During salt stress exposure, process like photosynthesis and cell growth can be directly affected by changes in photosynthetic machinery or indirectly by oxidative stress and toxic ions effects (Lawlor & Cornic, 2002; Munns *et al*., 2006; Chaves *et al*., 2011; Nebauer, 2013; Huang, 2014). In glycophytic C3 plants, the initial negative effects of salt stress on photosynthesis may arise from decrease in CO_2 availability as a consequence of limited stomatal conductance and mesophilic diffusion (Flexas *et al*., 2004). Later, salinity affects photosynthesis by non-stomatal mechanisms, including decreases in total chlorophyll content and electron transport in chloroplasts, thereby impairing the efficiency of photosystem II (Aragão *et al*., 2012; Yan *et al*., 2012).

Sunflower is an oilseed species that presents important agronomic characteristics. It has high content of oil in seeds and shows wide adaptation to different edaphoclimatic conditions (Morais *et al*., 2011), such as drought, cold and heat (Chambó *et al*., 2011); nevertheless, the sunflower seems to present poorer resistance to salinity. The climatic conditions in Northeast

of Brazil allow the agronomic exploitation of sunflower crop, however, the increase of soil salinization has impaired the productivity of numerous plant species and the use of arable lands. Therefore, the identification of salt tolerant sunflower cultivars can be a promising tool for cultivating in saline soils (Shahbaz *et al*., 2011).

Our investigative study aimed to investigate the influence of NaCl stress on the growth and CO_2 assimilation of six commercial genotypes of sunflower, in order to identify salt tolerant genotypes with potential to cultivate in saline environments.

MATERIAL AND METHODS

Seeds of six commercial sunflower genotypes (Catissol, BRS 321, BRS 323, BRS 324, H360 e H251) (*Helianthus annuns*), provided by Embrapa Soja, Brazil, were sterilized with 2% sodium hypochlorite for 5 min and then washed with distilled water. Thereafter, the seeds were sown in vermiculite moistened with distilled water. After seven days, the seedlings were transferred to a hydroponic system containing half-strength Hoagland's nutrient solution during five days, for an acclimation period. Then, the plants were subjected to salt stress with NaCl at 0 (control) and 100 mM NaCl (saline treatment) to nutrient solution, which was achieved by increasing the dosage by 25 mM per day in order to avoid osmotic shock. Nutrient solutions were renewed every 5 days and the plants were harvested ten days after the last salt addition. The experiment was carried out under greenhouse conditions, where the midday photosynthetic photon flux density (PPFD) at approximately 1,300 μmol m^{-2} s^{-1}, main temperature of 28.7 ± 3°C, and mean relative humidity of 64.3 ± 3.5 %.

After ten days of salt treatment, CO_2 assimilation rate was measured in the third fully expanded leaves between 10:00 and 11:00 a.m employing a portable photosynthetic system (LI-6400 XT, Li-Cor, Lincoln, NE, USA). Thereafter, five plants from control and salt stressed genotypes were individually harvested and separated in shoots (leaves + stems) and roots. The leaf area (LA) was measured using an area meter (LI-3100, LI-COR, Inc., Lincoln, NE, USA). Subsequently, fresh plant material was dried by lyophilization and the dry mass of shoots (DMS), roots (DMR) and total (DMT) was determined.

Experimental design was completely randomized following a 2 × 6 factorial scheme, composed of two growth conditions [nutrient solution with (salt stress) and without NaCl (control)] and six sunflower genotypes (Catissol, BRS 321, BRS 323, BRS 324, H360 and H251). All analyses were performed using five repetitions per treatment, it being an individual plant considered one repetition. The data were submitted to two-way analysis of variance

(ANOVA) and the main values were compared trough Tukey's test ($P \leq 0.05$) using Sisvar® 5.3 program.

RESULTS AND DISCUSSION

Salt stress severely limited the growth of sunflower plants, with the effects being more pronounced in the Catissol genotype (Figure 1). Under salinity, Catissol genotype presented a 72% reduction in shoot dry mass (SDM) in relation to respective control, while the genotypes BRS 321, BRS 323, BRS 324, H360 and H251 showed reductions of 57, 59, 55, 54 and 60%, respectively (Figure 1a). In roots, the highest decreases in dry mass were observed in stressed plants from genotypes Catissol, BRS 323, BRS 324 and H251, with values found be 39, 58, 53 and 40% lower than those of respective controls; whereas the lowest reductions (mean decrease of 33%) were detected in BRS 321 and H360 genotypes (Figure 1b). As a consequence, the total dry mass (TDM) from all sunflower genotypes was significantly reduced by salinity, it being more aggressive in plants from Catissol genotype (a 65% decrease).

In NaCl presence, in absolute terms, the highest values of total dry mass were registered in plants from BRS 321 genotype (5,20 g/plant), followed by ones from 360 (4,93 g/plant), 251 (4,87 g/plant), 323 (4,57 g/plant), Catissol (4,30 g/plant) and 324 (2,91 g/plant) genotypes (Figure 1c). In addition, salt stress promoted drastic decrease in leaf area of plants from all studied sunflower genotypes; nevertheless, the reductions were higher in Catissol (78%) and BRS 323 (68%) genotypes (Figure 1d). Surprisingly, under salt stress conditions, the values of SDM, RDM, TDM and LA of plants from BRS 321 genotype were found to be 129, 58, 105 and 131% higher than those from Catissol genotype, respectively.

Several studies have considered the accumulation of biomass as an essential index to estimate the level of plant's tolerance to abiotic stresses (Hajlaoui *et al*., 2010; Janmohammadi *et al*., 2012; Zörb *et al*., 2013). In concordance with, sunflower genotypes displayed differential biomass accumulation as affected by salt stress. Interestingly, a higher tolerance to salinity was revealed by plants from BRS 321 genotype, as proved by less conspicuous salt effects on leaf area and shoot dry mass (Figure 1a, d). Zahra *et al*. (2014) studying wheat cultivars with differential salt tolerance observed that the shoot and root growth was severely reduced by salt stress (150 and 300 mM NaCl), but the reductions was less drastic in tolerant genotype.

In recent years, photosynthesis has been selected as a biochemical indicator of salinity tolerance in various plant crops (Qiu *et al*., 2011; Zahra *et al*., 2014). In general, photosynthesis can be negatively affected by salt stress due to the decrease of CO_2 availability for Rubisco

activity (Hura *et al*., 2007); as well as decreases in the efficiency of biochemical processes, such as the low electron transport through the electron transport chain of the chloroplast and the inhibition of the activity of several enzymes related to CO_2 assimilation (Wise *et al*., 2004; Thiagarajan *et al*., 2007). Herein, saline stress significantly reduced the photosynthetic rate of Catissol, BRS 323, BRS 324, H360 and H25 plants, with the most strongly effects in Catissol genotype, whereas no significant changes were registered in the BRS 321 genotype (Figure 2). Our data clearly demonstrated that higher salt sensitivity of Catissol plants was correlated with the lower assimilation of CO_2 during entire experiment (Figures 1 and 2). Similarly, in studies with species that exhibit contrasting responses to salinity, *Ricinus communis* (tolerant) and *Jatropha curcas* (sensitive), Lima Neto *et al*. (2014) showed that photosynthesis decreased intensely in both species, but the CO_2 assimilation reduction was more pronounced in the salt sensitive one.

CONCLUSION

Sunflower plants from Catissol genotype have poorer capacity to withstand salt harmful effects; horever, plants from BRS 321 genotype show high performance of photosynthetic efficiency and elevated growth under salt stress among all studied genotypes; constituting a plausible alternative for cultivating sunflower plants in saline and/or salinity-prone areas;

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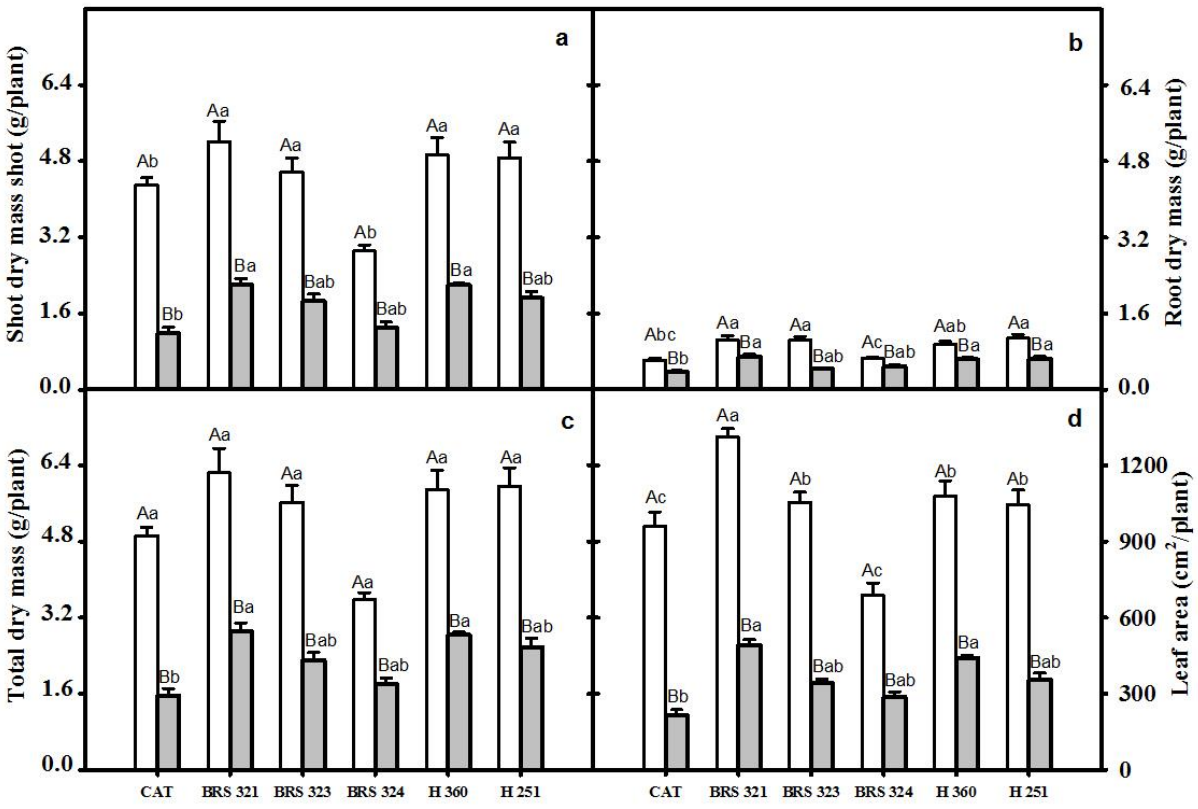

Figure 1. Shoot dry mass (**a**), root (**b**) and total (**c**) and leaf area (**d**) of sunflower plants from genotypes Catissol (CAT), BRS 321, BRS 323, BRS 324, H360 and H251, grown in nutrient solutions in absence (control, *open square*) and presence of 100 mM NaCl-stress (salt stress, *filled square*). Measurements were done ten days after salt treatments. Values represent the means of five repetitions + standard error. Means followed by the same lowercase letters at the same salinity level, or by equal capital letters in the same genotype, are not statistically different according to Tukey's test ($p \geq 0.05$).

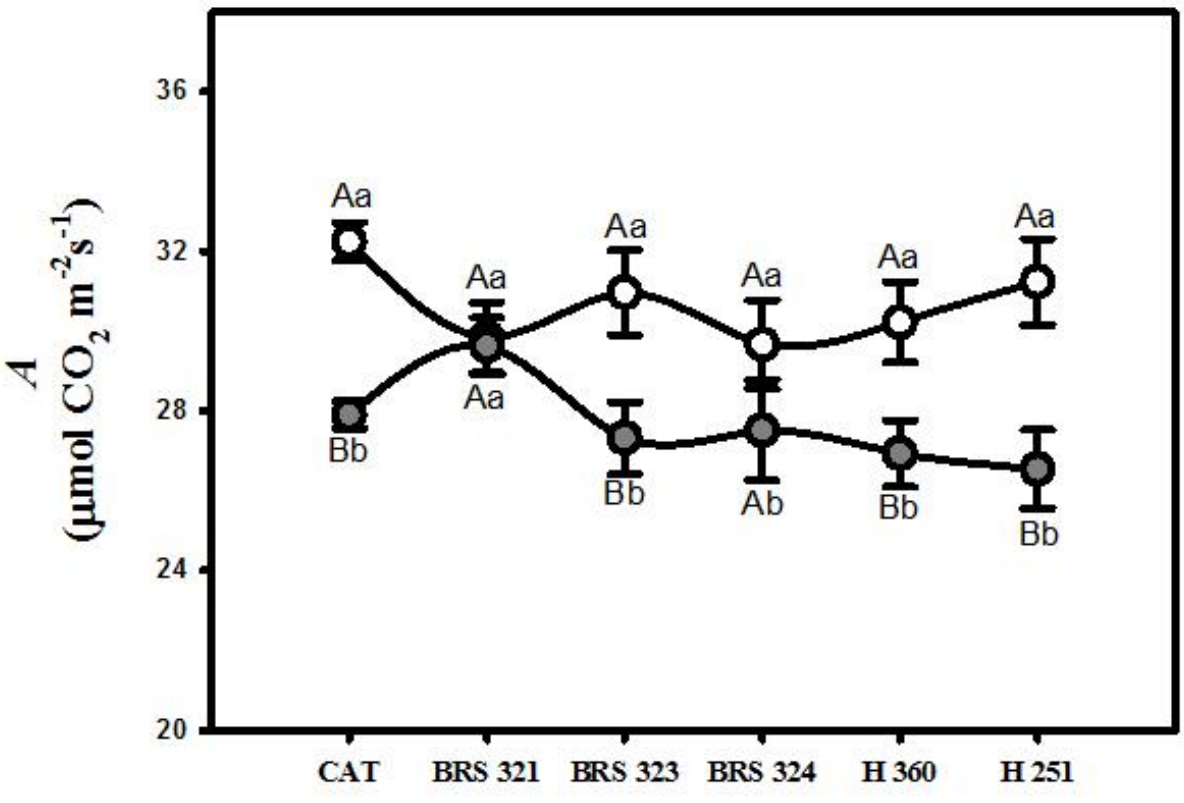

Figure 2. CO_2 assimilation (***A***) of sunflower plants from Catissol, BRS 321, BRS 323, BRS 324, H360 and H251 genotypes, grown in nutrient solutions in absence (control, *open square*) and presence of 100 mM NaCl-stress (salt stress, *filled square*). Measurements were done ten days after salt treatments. Values represent the means of five repetitions ± standard error. Means followed by the same lowercase letters at the same salinity level, or by equal capital letters in the same genotype, are not statistically different according to Tukey's test ($p \geq 0.05$).