NaCl-PRIMING MITIGATES OXIDATIVE DAMAGE AND Na$^+$ ACCUMULATION AND ENHANCES SALT TOLERANCE IN SORGHUM PLANTS

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ABSTRACT: In this study, we tested the hypothesis that priming with NaCl alleviates salt-induced damages by upregulating enzymatic antioxidant system and controlling ionic homeostasis in sorghum plants. After germination, uniform *Sorghum bicolor* seedlings were primed with NaCl at 0 (control), 10 (P10), 20 (P20) and 30 mM (P30) (pre-treatment), for seven days, and then subjected to 80 mM NaCl-stress. The most striking effects of NaCl-priming were registered in plants from P10-treatment. After five and ten days of salinity, lipid peroxidation and Na$^+$ accumulation was found to be drastically improved in roots and shoots; however, the increase was more severe in non-primed stressed plants. The lower oxidative damage in P10-stressed plants positively correlated with higher activity of catalase (CAT), dismutase superoxide (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (G-POD) enzymes under salinity. In general, CAT and SOD were the most responsive enzymes to NaCl priming, while APX and G-POD were responsive only after onset of salinity (five days) and/or in a single plant organ. In conclusion, NaCl priming enhances plant's capacity to retain overaccumulation of Na$^+$, and limit oxidative damage by stimulating effectively antioxidant system.

KEYWORDS: Antioxidant enzymes,ionic homeostasis,salt stress.

PRÉ-TRATAMENTO COM NaCl MINIMIZA OS DANOS OXIDATIVOS E A ACUMULAÇÃO DE Na$^+$ E AUMENTA A TOLERÂNCIA DE PLANTAS DE SORGO AO ESTRESSE SALINO

RESUMO: Neste estudo, foi verificado se o pré-tratamento com NaCl alivia os efeitos deletérios da salinidade através de mecanismos de controle da homeostase iônica e defesa antioxidativa. Plântulas de *Sorghum bicolor* foram cultivadas em solução nutritiva contendo NaCl a 0 (controle), 10 (P10), 20 (P20) e 30 (P30) mM (pré-tratamento), por sete dias. Em

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seguida, as plantas foram submetidas ao estresse com NaCl a 80 mM, sendo analisadas após 5 e 10 dias de estresse. Os efeitos mais marcantes do pré-tratamento foram registrados nas plantas estressadas da condição P10. A salinidade aumentou a peroxidação de lipídios e os teores de Na^+ nos órgãos analisados; contudo, os efeitos foram maiores nas plantas estressadas e não pré-tratadas, em comparação àquelas aclimatadas com NaCl a 10 mM. A redução dos danos oxidativos em plantas estressadas P10 foi devido à atividade das enzimas catalase (CAT), dismutase do superóxido (SOD), peroxidase do ascorbato (APX) e peroxidase do guaiacol (G-POD). As enzimas CAT e SOD foram positivamente responsivas ao pré-tratamento em ambos os órgãos e tempos analisados, enquanto que as demais enzimas foram moduladas somente após o estresse salino (5 dias) e/ou em um único órgão. Conclui-se que a aclimatação de plantas com baixas concentrações de NaCl é capaz de ativar repostas de tolerância à salinidade, como o sistema antioxidativo enzimático e o controle da homeostase iônica.

PALAVRAS-CHAVE: Enzimas antioxidantes; homeostase iônica; estresse salino.

INTRODUCTION

In some regions of world the plant production and crop yields are strongly limited due to abiotic stresses including soil salinity. The increased salinization process of agricultural land is expected to have global effects, causing a 30% land loss along to the next 25 years (Wang et al., 2003). Thus, efforts are currently being made to select more salt tolerant genotypes and/or increase salt tolerance of crop plants and hence achieve a sustainable agriculture.

Plant priming (pre-treatment of plants/seeds by previous or concomitant exposure to stressor or chemical compounds, making the plants more tolerant to future stress events) with some small molecules has received considerable attention as a powerfully tool to induce resistance against salt stress. Recent reports have shown that chloride sodium (NaCl), hydrogen peroxide (H_2O_2), nitric oxide (NO) and oligochitosan may act as a signaling molecule modulating multiple stress-responsive pathways increasing salt tolerance of numerous plant crops (Gondim et al., 2011; Ma et al., 2012; Pandolfi et al., 2016; Gadelha et al., 2017). In spite of the importance of plant priming, there is no information regarding the involvement of priming in salt responses of sorghum plants.

Sorghum bicolor, an annual C4-grass crop, is not only commonly consumed as human food and livestock feed, but it is also widely employed as a suitable feedstock for a variety of biological processes, including ethanol production (Whitfield et al., 2012). Sorghum crop is often grown in areas of stressful environmental conditions, such as drought and soil salinity. Nevertheless, although sorghum has widely known as a moderately salt tolerant crop (Lacerda

et al., 2003), its cultivation in several agricultural areas has becoming a serious problem due to gradual salt accumulation in soils.

Our working hypothesis was that NaCl priming mitigates salt-induced damages in sorghum plants by activating mechanisms for ionic homeostasis control and cellular detoxification. To test this hypothesis, we first exposed sorghum plants to low levels of NaCl and then subjected to 80 mM NaCl-stress for ten days. Growth and biochemical stress indicators were investigated to determine the relationship between NaCl priming and salinity tolerance.

MATERIAL AND METHODS

Seed of sorghum [*S. bicolor* (L.) Moench] of genotype CSF20 [(obtained from Instituto Agronômico de Pernambuco (IPA), Recife, Pernambuco, Brazil] were sown in vermiculite moistened with distilled water. For days after the sowing, the uniform seedlings were transferred to plastic trays (10.0 L) containing one-half strength Hoagland's nutrient solutions (Hoagland & Arnon, 1950) and the NaCl priming treatments by adding NaCl at 10, 20 and 30 mM to nutrient solution. A plant group remained in nutrient solution without NaCl addition (no priming). After eight days of acclimation period, the salt treatments were administered by adding 80 mM NaCl in two doses of 40 mM per day. Nutrient solutions were renewed every three days and the harvests were performed five and ten days after the last salt addition. The experiments were carried out in a greenhouse, where the midday photosynthetic photon flux density (PPFD) was 1,300 μmol m^{-2} s^{-1}, the mean air temperature was 29.4°C during the day and 26.7°C at night, and the main air relative humidity was 65.2%.

In each harvest time, five plants from each treatment were individually harvested. Firstly, leaf area was evaluated through a LI-3000 leaf area meter (LI-COR, Inc. Lincoln, Nebraska, USA). Then, plants were separated in shoots (leaves + stems) and roots, frozen in liquid nitrogen, and after dried by lyophilization, the dry mass was measured.

For ion content, 30 mg lyophilized samples from the shoot and root were homogenized with deionized water at 45°C for 1 h. After centrifuged at 3000 $\times$ g for 15 min at room temperature, the supernatant was collected and used to determine K$^+$ e Na$^+$ and Cl$^-$ contents. The K$^+$ and Na$^+$ concentrations were measured by flame photometry, while the Cl$^-$ content was spectrophotometrically determined according to Gaines et al. (1984), based on the absorbance reading at 460 nm with NaCl as a standard. Lipid peroxidation was estimated as thiobarbituric acid reactive substances (TBARS) according to method of Heath & Packer (1968). The concentration of TBARS was measured at 532 nm and the value for non-specific turbidity at 600 nm was subtracted. The TBARS values were estimated using its extinction coefficient of 155 mM^{-1} cm^{-1}.

For enzyme assays, fresh samples were ground in a mortar with liquid nitrogen and then homogenized with extraction buffer (100 mM potassium phosphate, pH 7.0, containing 0.1 mM EDTA) at 4ºC. The homogenate was centrifuged at $15,000 \times g$ for 15 min at 4ºC and supernatant used for assays of the activities of superoxide dismutase (SOD), guaiacol peroxidase (G-POX), catalase (CAT) and ascorbate peroxidase (APX). SOD activity was measured by evaluating its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), as previously described by Beauchamp & Fricovich (1971). One SOD activity unit (AU) was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction reaction. Catalase and guaiacol peroxidase were assayed according to the methods of Havir & McHale (1987) and Kar & Mishra (1976), by monitoring the absorbance at 240 nm and 420 nm, respectively. CAT and G-POD activities were estimated using the molar extinction coefficient of, respectively, 36 M^{-1} cm^{-1} and 26.6 mM^{-1} cm^{-1}. Ascorbate peroxidase activity was measured by the decrease in absorbance readings at 290 nm, using the molar extinction coefficient of 2.8 mM^{-1} cm^{-1} (Nakano & Asada, 1981).

Experimental design was completely randomized. For the growth and ion accumulation assays, the experiment was divided into five treatments, including a control (neither NaCl-priming nor NaCl-stress), salt stressed (no NaCl-priming and NaCl-stress), P10 (10 mM NaCl-priming and NaCl-stress), P20 (20 mM-NaCl priming and NaCl-stress) and P30 (30 mM-NaCl priming and NaCl-stress). For the lipid peroxidation and enzymatic antioxidant analyses, the experiment was arranged in a 2×2 factorial scheme composed of two salinity levels (0 and 80 mM NaCl) and two NaCl priming treatments [no NaCl-priming and P10 NaCl-priming (the level of NaCl-priming that improve salt resistance of *S. bicolor* plants)]. All analyses were performed using five plants (repetitions) per treatment. The data were subjected to analysis of variance (ANOVA) and, when a difference was significant ($p \leq 0.05$), the mean values were compared using Tukey's test.

RESULTS AND DISCUSSION

Salt stress is widely known to impair plant growth and productivity by disturbing numerous physiological and biochemical processes like ion homeostasis and ion activities (Gomes-Filho et al., 2008; Miranda et al., 2017). Concordantly, in this study, the dry mass of shoot (DMS), root (DMR) and total (DMT) was drastically decreased by salinity, irrespective of NaCl priming (Fig. 1); however, the effects were less pronounced in P10-acclimated plants. Under 80 mM NaCl-stress, P10-acclimated plants displayed main values of DMS, DMR and DMT 146% higher than those of stressed plants only (Fig. 1A and 1B). In a similar way, salinity

significantly reduced the leaf area (LA) of plants from all stress treatments, but LA values of P10-acclimated plants were higher than those of stressed (Fig. 1C).

Our data clearly evidence that NaCl-priming was effective to reduce salt deleterious effects on growth of sorghum plants (Fig. 1), which was closely related to better ionic homeostasis. Herein, salinity promoted severe reductions in K^+ content and an over accumulation of Na^+ in both shoot and root tissues of sorghum plants (Fig. 1A and 1B). In shoot, under salt stress, the lowest Na^+ accumulation was observed in P10-acclimated plants (Fig. 1A). Yet, in root, the Na^+ content was higher in plants from P30 treatment, followed by those from other stress and NaCl-priming treatments (Fig. 1B). As a consequence, although salt stress significantly decreased the K^+/Na^+ ratio in both plant tissues, a greater K^+/Na^+ ratio was established in the shoot of P10-acclimated plants in comparison with other salt treatments (Fig. 2A and 2B). Consistently, a low Na^+ accumulation together with elevated K^+/Na^+ ratio has been considered as an important mechanism for salt tolerance in plant species (Yamaguchi et al., 2013; Miranda et al., 2017). In fact, our findings demonstrated that the most salt tolerant sorghum plants (P10-acclimated) showed a lower content of toxic Na^+ ions coupled to greater K^+/Na^+ ratio in leaves (Fig. 2A and 2C).

In order to evaluate if NaCl-priming alleviates salt-induced oxidative damage, the lipid peroxidation and enzymatic antioxidants were measured. For all analyzed time-points (5 and 10 days of salt treatments), salt stress dramatically increased the lipid peroxidation in non-acclimated plants, it being more aggressive in root tissues. On the other hand, a slight increase in lipid peroxidation by salinity in P10-acclimated plants was observed only after ten days of salt treatment (Fig. 3). In addition, under salinity, P10-acclimated plants displayed values of lipid peroxidation lower than those of non-acclimated plants, especially in photosynthetic tissues. These data suggest that salinity could cause injuries to cellular components like lipids, proteins and nucleic acids, as previously reported in *Jatropha curcas* (Gadelha et al., 2017) and other plant species (Demidchik, 2015), but NaCl-priming can partially prevent the oxidative damage normally caused by salt stress.

To avoid the salt-induced oxidative damage, plant cells have developed an elegant defense system, including enzymatic and non-enzymatic components. Recent reports have shown that plant species employing highly efficient antioxidant systems had improved salt stress tolerance (Noctor & Foyer, 2016; Gadelha et al., 2017). This idea is supported by the facts that antioxidant enzyme activities significantly increased in P10-acclimated stressed sorghum plants, whereas the lipid peroxidation significantly decreased in comparison to non-acclimated stressed plants (Fig. 3 and 4).

Under control conditions, CAT, SOD and G-POD activity of P10-acclimated plants was bigger than non-acclimated ones, in both plant organs (Fig. 4). In general, under salt stress,

CAT, SOD, G-POD and APX activities were increased in roots and shoots of plants from all treatments; nevertheless, P10-acclimated stressed plants displayed higher CAT, SOD, G-POD and APX activity values than those of non-acclimated stressed plants, except for APX in roots and G-POD in shoot at the second time-point. Our data demonstrated that, when sorghum plants were acclimated with 10 mM NaCl, a higher operation of enzymatic antioxidants under 80 mM-NaCl stressis observed.

CONCLUSION

Our findings reveal that priming of seedlings with NaCl triggers important responses against salt stress and improves plant's salt tolerance of *S. bicolor*. The role of NaCl priming to overcome salinity harmful effects seems to rely partly on its particular properties to induce mechanisms for regulating cellular ion homeostasis and antioxidant components thereby alleviating oxidative damage. Thus, NaCl priming emerges as a plausible cultivation technique to prevent severe growth losses due to salinity and might have significant practical application for cultivating *S. bicolor* plants in saline environments.

ACKNOWLEDGMENTS

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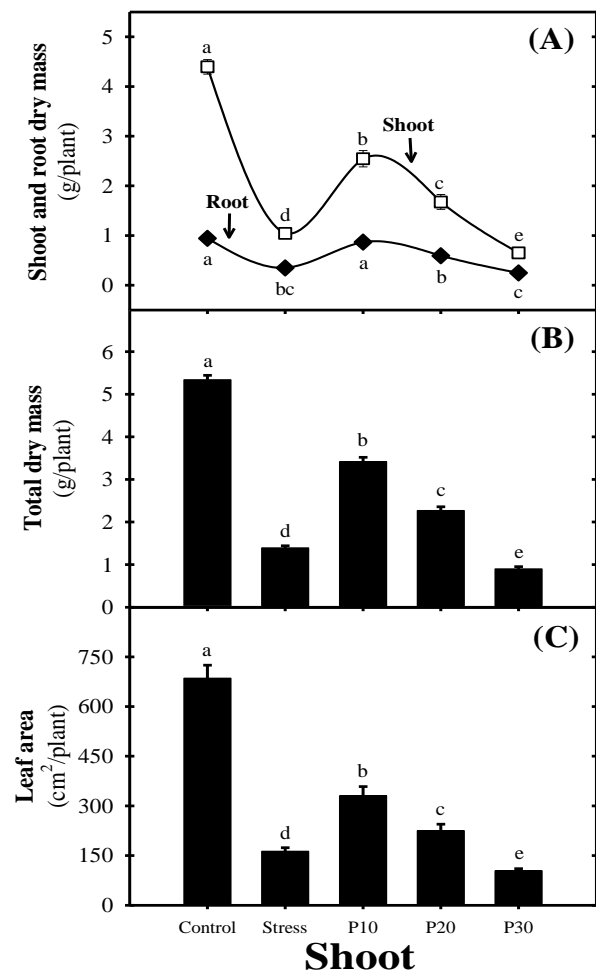
Figure 1. Shoot and root (**A**) and total (**B**) dry mass, and leaf area (**C**) of *Sorghum bicolor* plants in absence (control) and presence of 80 mM-NaCl stress (Stress, P10, P20 and P30). The plants were primed with NaCl at 0 (stress, negative control), 10 (P10), 20 (P20) and 30 mM for eight days prior to imposition of salt treatments. The measurements were obtained at ten days after salinity imposition. Values represent the means of five repetitions + standard error. Different lowercase letters represent significant differences due to salt stress using Tukey's test ($p \leq 0.05$).
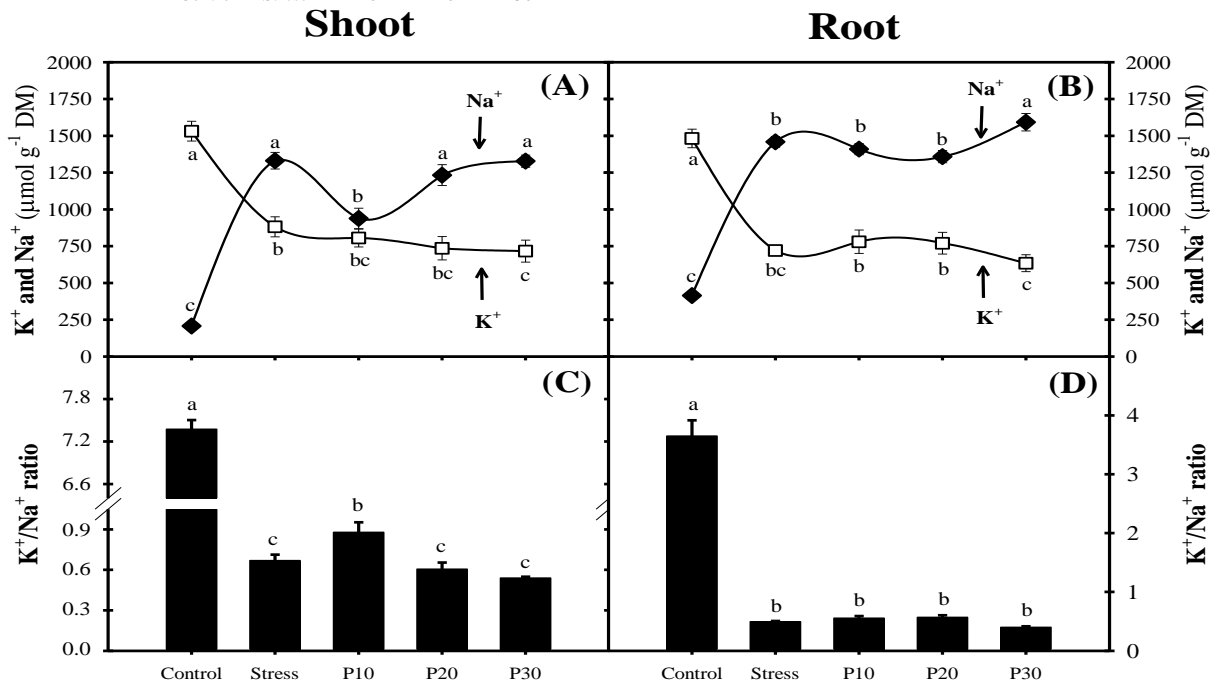

Figure 2. Concentrations of potassium (K^+) and sodium (Na^+) (**A,B**) and K^+/Na^+ ratio (**C,D**) in shoot and roots of *Sorghum bicolor* plants in absence (control) and presence of 80 mM-NaCl stress (Stress, P10, P20 and P30). More details as Fig. 1.
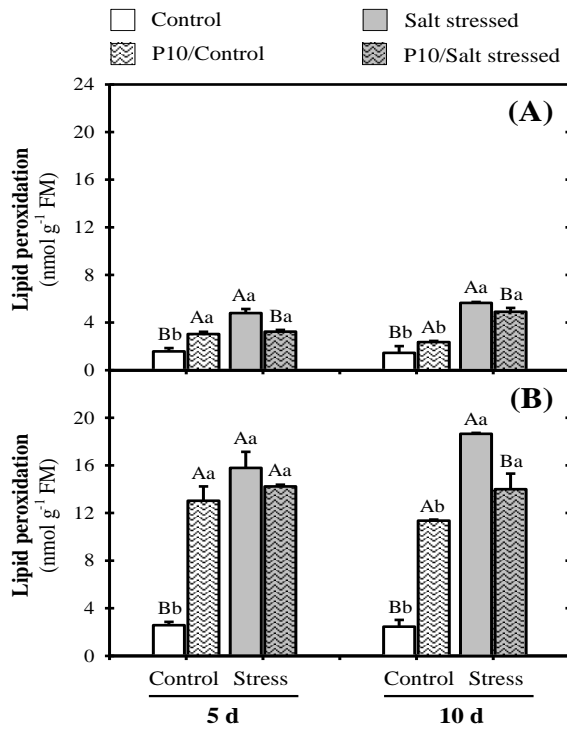
Figure 3. Lipid peroxidation in shoot (**A**) and root (**B**) tissues of *Sorghum bicolor* plants in absence (control) and presence of 80 mM NaCl-stress (salt stressed) and primed with NaCl at 0 and 10 mM. Values are given as the mean of five biological repetitions + standard error. At each time point, significant differences due to NaCl-priming at the same salt level (control × P10/control or salt stressed × P10/salt stressed) are denoted by different capital letters, whereas significant differences due to salt stress in the same NaCl-priming (control × salt stressed or P10/control × P10/salt stressed) are indicated by different lowercase letters, using Tukey's test (p ≤ 0.05).
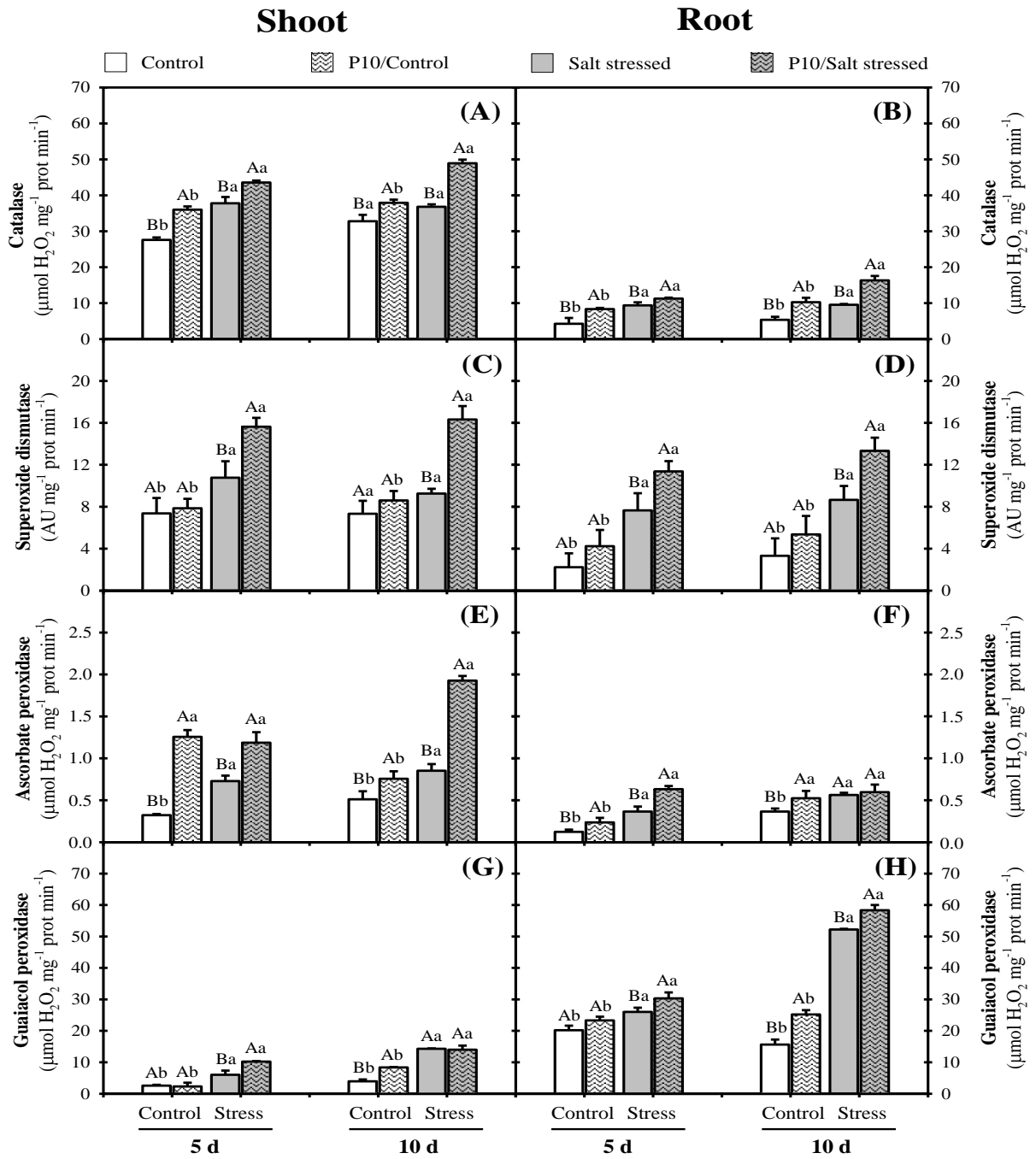
Figure 4. Catalase (A, B), superoxide dismutase (C, D), ascorbate peroxidase (E, F) and guaiacol peroxidase (G, H) activity in shoot and roots of Sorghum bicolor plants in absence (control) and presence of 80 mM NaCl-stress (salt stressed) and primed with NaCl at 0 and 10 mM. More details as Fig. 3.