RESEARCH ARTICLE

Enhanced salt tolerance in maize plants induced by H_2O_2 leaf spraying is associated with improved gas exchange rather than with non-enzymatic antioxidant system

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ABSTRACT: Hydrogen peroxide (H_2O_2) is an essential signaling molecule that mediates plant responses against several biotic and abiotic stresses. H_2O_2 pretreatment has emerged as a signaling way, inducing salt stress acclimation in plants. Here, we analyzed the effects of H_2O_2 leaf pretreatment on the non-enzymatic defense system (ascorbate and glutathione), plant growth, relative water content (RWC), relative chlorophyll content, H_2O_2 content, and gas exchange in maize plants under NaCl stress. The results showed that salinity reduced the leaf area and shoot and root dry mass as compared to control, and the leaf spraying with H_2O_2 significantly improved the growth of salt stressed plants. Photosynthesis and transpiration, stomatal conductance and intercellular CO_2 concentration were strongly decreased by salinity after 7 and 14 days of salt exposure; however, the decrease was lower in plants sprayed with H_2O_2. The improved gas exchange in H_2O_2-sprayed stressed plants correlated positively with higher RWC and relative chlorophyll content and lower leaf H_2O_2 accumulation under NaCl stress conditions. Ascorbate and glutathione did not play any obvious effects as non-enzymatic antioxidants in the ROS scavenging. In conclusion, the salt tolerance induced by H_2O_2 leaf pretreatment is attributed to a reduction in the H_2O_2 content and maintenance of RWC and chlorophyll in maize leaves. These characteristics allow maize plants to maintain high rates of photosynthesis under salt stress and improve the growth.

KEYWORDS: ascorbate, glutathione, hydrogen peroxide, salinity, *Zea mays*.

INTRODUCTION

The production of reactive oxygen species (ROS) is a normal event of oxidative metabolism in plants but their generation is further enhanced in response to various biotic and abiotic stresses, such as salinity (Møller et al. 2007). Salinity is a limiting environmental factor, which impairs plant growth and development. It affects approximately 20% of the world's cultivated area and nearly half of the world's irrigated lands (Sairam and Tyagi 2004).

In excess, ROS can damage DNA, proteins, chlorophyll and membrane functions. The main ROS produced are hydrogen peroxide (H_2O_2), superoxide ($^{\bullet}O_2^-$) and hydroxyl ($^{\bullet}OH$) radicals (Azevedo Neto et al. 2008). Plants have evolved complex defense mechanisms to avoid an imbalance between generation and scavenging of ROS (Azevedo Neto et al. 2008, Gill and Tuteja 2010). ROS may be scavenged by both enzymatic and non-enzymatic pathways; nonetheless, the failure to control ROS may lead to oxidative stress (Wang et al. 2013). The enzymatic defense system includes superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), guaiacol peroxidase (GPX, EC 1.11.1.7) and ascorbate peroxidase (APX, EC 1.11.1.11) (Azevedo Neto et al. 2008,

Munns and Tester 2008). The non-enzymatic system is composed by lipophilic, such as tocopherols and carotenoids, and hydrophilic compounds, such as ascorbate and glutathione reduced, both scavengers of ROS (Azevedo Neto et al. 2008).

Ascorbate has an essential role in several physiological processes of plants, such as growth, differentiation and metabolism (Foyer 1993). Moreover, it plays a central role in photosynthetic protection, through the ROS scavenging in the Mehler peroxidase reaction as a reductant and the excess light energy dissipation in non-photochemical quenching (Huang et al. 2005). Green leaves usually have similar amounts of ascorbate and chlorophyll (Mckersie and Leshen 1994). Additionally, ascorbate may act as a reductant for many free radicals, thus minimizing the damage caused by oxidative stress and displaying an important role in protecting against salt stress (Mckersie and Leshen 1994). In an ascorbate-deficient *Arabidopsis* mutant, the hydrogen peroxide content increased dramatically and it resulted in lower contents of chlorophyll and low photosynthetic rate (Huang et al. 2005). These results were attributed to the lower activities of ascorbate-glutathione cycle enzymes, which induced a decrease in the reduced form of ascorbate.

The glutathione is a tripeptide (Glu-Cys-Gly) whose antioxidant function is assigned to the sulfhydryl group of cysteine (Renneberg 1982). This antioxidant can react with singlet oxygen, superoxide and hydroxyl radicals and thus functions directly as a free radical scavenger (Mckersie and Leshen 1994). Some studies have observed that the glutathione content increased in water-stressed sunflower plants (Sgherri and Navari-Izzo 1995) and in cell lines of salt-stressed groundnut (Jain et al. 2002).

Cell growth and photosynthesis are among the primary processes affected by salinity (Munns et al. 2006). Accordingly, the decline in productivity observed for many salt stressed plant species is often related to direct or indirect reductions in photosynthetic capacity (Steduto et al. 2000, Meloni et al. 2003). Direct effects include the decreased CO_2 availability because of diffusion limitations through the stomata and the mesophyll (Flexas et al. 2007) and/or the alterations of photosynthetic metabolism (Lawlor and Cornic 2002). Indirectly, photosynthetic rate can be reduced by the harmful effects of ROS on the photosynthetic machinery (Ort 2001, Chaves and Oliveira 2004).

Until recently, H_2O_2 was seen as a toxic cellular metabolite (Azevedo Neto et al. 2005); nevertheless, it is toxic only at high concentrations (Uchida et al. 2002). According to Quan et al. (2008) and Szechynska-Hebda et al. (2012), the H_2O_2 is the most stable compound among ROS and the most feasible molecule for ROS-mediated signal transduction. H_2O_2 is produced in response to stress and mediates crosstalk between signalling pathways. Therefore, the H_2O_2 is a probable signaling molecule that contributes to the phenomenon of "cross-tolerance", whose exposure of plants to one stress (for instance, H_2O_2) may provide protection towards another stress (such as salinity) (Neill et al. 2002).

It has been reported that the exogenous application of H_2O_2 prior to salt exposition induced salinity tolerance in plants by activation of enzymatic antioxidant defense system (Uchida et al. 2002, Azevedo Neto et al. 2005, Gondim et al. 2010, 2012). Additionally, Gechev et al. (2002) and Gao et al. (2010) reported that H_2O_2 application improved the tolerance to oxidative and heat stress of tobacco and cumcumber plants, respectively. However, most of these researches are related to H_2O_2 application in seeds and root system, and there are few studies that examined its exogenous application through spraying leaves in plants under abiotic stress.

We tested the hypothesis that the non-enzymatic defense system confers an additional protection against oxidative damage in H_2O_2-induced salt tolerance in maize plants. In a previous study, the behavior of enzymatic defense pathway was examined (Gondim et al. 2012). Therefore, this study investigated the effects of H_2O_2 leaf spraying in maize plants under salt stress on the non-enzymatic defense system (ascorbate and glutathione) and its relationship with some physiological processes, such as plant growth, relative chlorophyll content, relative water content and gas exchanges.

MATERIALS AND METHODS

Plant Growth Conditions and Treatments: Maize seeds (*Zea mays* L.) of the triple hybrid BRS 3003 were surface-sterilized with 0.7% sodium hypochlorite solution and then washed several times in distilled water. Afterwards, they were sown in vermiculite moistened with distilled water and irrigated daily. Five days after sowing (DAS), the seedlings were transferred to plastic pots containing half-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) and were acclimated for 2 d. After 8 DAS, the seedlings were sprayed with distilled water (control) or 10 mM H_2O_2 in 0.025% Tween 20 at 6:30 am and again after 24 h. After 48 h of the first spraying, the seedlings were submitted to salinity of 80 mM NaCl in 2 stages by increasing the dosage by 40 mM NaCl per day to avoid osmotic shock.

Nutrient solutions were renewed weekly and harvests were performed seven and 14 d after the last salt addition. The experiment was carried out under greenhouse conditions, where the midday photosynthetic photon flux density was 1,500 μmol m^{-2} s^{-1}, the mean air temperature was 28.5°C

during the day and 25.4°C during the night, and a mean relative humidity was 63.9%.

In the harvests, the plants were separated into two groups. In the first one, leaf (the first fully expanded from the apex) and roots (one-third of apical portion) were frozen in liquid nitrogen and kept in a freezer (-80°C) for later biochemical analysis (H_2O_2 content, reduced and total ascorbate content and reduced and total glutathione content). In the second group, the plants were separated into shoot and roots and the growth parameters and relative water content were determinated.

Growth Parameters and Relative Water Content: In each harvest, leaf area was evaluated (LI-3100 Area Meter, Li-Cor., Inc, Lincoln, Nebraska, USA) and the plants were dried in a forced air circulation oven at 60°C for 48 h to provide the shoot dry mass (SDM) and root dry mass (RDM). Leaf relative water content (RWC) was measured using 10 leaf discs of 10 mm diameter. RWC was calculated according to the formula: RWC (%)=[(FW–DW)/(TW–DW)]×100, where: FW is the fresh weight, DW is the dry weight, after drying in the oven at 60°C for 48 h. Turgor weight (TW) was determined by subjecting leaves to rehydration for 6 h.

Gas Exchange Measurements and Relative Chlorophyll Content: Measurements of gas exchange were performed on the first fully expanded leaf using a portable photosynthesis system, infra-red gas analyser (IRGA, mod. LCi, ADC, Hoddesdon, UK). The assimilation rate (*A*), stomatal conductance (*gs*), transpiration rate (*E*) and intercellular CO_2 concentration (*Ci*) were measured under photosynthetic photon flux density of 1,200 μmol m^{-2} s^{-1}. The measurements were made between 8:00 and 10:00 am after 7 and 14 d of salt stress with 80 mM NaCl. Relative chlorophyll content (SPAD index) was also determined on the first fully expanded leaf, through three readings per leaf with a portable Minolta chlorophyll meter SPAD-502.

Evaluation of H_2O_2 and Antioxidants: Leaf and root tissues (0.5 g) were homogenized in 5 mL of 5% trichloroacetic acid (TCA). The homogenate was filtered and then centrifuged at 12,000 x *g* for 15 min. The supernatant fraction was used for determination of H_2O_2 contents and non-enzymatic antioxidants. All procedures were done at 4°C.

Hydrogen peroxide content was measured according to Sergiev et al. (1997), after reaction with potassium iodide (KI). The reaction mixture consisted of leaf extract, 2.5 mM K-phosphate buffer (pH 7.0) and 0.5 M KI. The reaction mixture was kept in the dark for 60 min. The amount of H_2O_2 was determined spectrophotometrically at 390 nm by reference to a standard curve prepared with H_2O_2 solutions.

Measurements of reduced (AsA) and total ascorbate [AsA+oxidized ascorbate (DHA)] contents were done according to Kampfenkel et al. (1995). The AsA content was determined upon the addition of leaf extract to a mixture containing 30 mM potassium phosphate buffer (pH 7.4), 2.5% TCA, 8.4% H_2PO_4, 0.8% bipyridyl, 0.3% $FeCl_3$. The reaction was performed at 40°C for 30 min, and the absorbance was read at 525 nm. The content of AsA+DHA was determined by adding the leaf extract to a mixture containing 0.5 mM DTT and 30 mM potassium phosphate buffer (pH 7.4). The reaction mixture was maintained at 40°C during 15 min. Thereafter, 0.025% N-ethylmaleimide (w/v, in water), 2.5% TCA, 8.4% H_2PO_4, 0.8% bipyridyl and 0.3% $FeCl_3$ were added to the reaction medium. The absorbance was measured at 525 nm and the contents of AsA+DHA and AsA were estimated using L-ascorbate as the standard. The ascorbate redox state of was calculated according to the formula: Ascorbate redox state (%)=[AsA/(AsA+DHA)×100].

Reduced (GSH) and total glutathione [GSH+oxidized glutathione (GSSG)] contents were determined according to Griffith (1980). The GSH content was determined in reaction mixture containing leaf extract, 130 mM sodium phosphate buffer (pH 7.4) and 7 mM sodium phosphate buffer (pH 6,8) containing 6 mM 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB). The reaction mixture was maintained at 30°C for 10 min and the absorbance was read at 412 nm.

Total glutathione was measured after reduction of GSSG to GSH. The GSSG reduction was performed by adding the leaf extract to a mixture consisting of 130 mM sodium phosphate buffer (pH 7.4) containing one unit of glutathione reductase. The reaction mixture was maintained at 30°C for 10 min. Thereafter, NADPH at 50 mM was added and the mixture was maintained at 30°C for 10 min. Finally, sodium phosphate buffer at 7 mM (pH 6.8) containing 6 mM of DTNB were added and the reaction mixture was kept at 30°C for 10 min. The absorbance was measured at 412 nm and the contents of GSH+GSSG and GSH were estimated using GSH as a standard. The glutathione redox state was calculated as follows: Glutathione redox state (%)=[(GSH)/(GSH+GSSG)×100].

Experimental Design and Data Analysis: The experimental design was completely randomized, following a factorial scheme composed by two salinity levels (0 and 80 mM NaCl) and two H_2O_2 levels (0 and 10 mM). Analyses were performed using five plants (replications) per treatment. The results were subjected to a two-way analysis of variance (ANOVA). When a difference was significant ($p \leq 0.05$), the values were compared through Tukey's test.

RESULTS

Growth, Chlorophyll and Relative Water Content: Salinity markedly decreased the SDM and RDM in both times of analysis (Table 1). However, the H_2O_2 spraying was able to reduce the adverse effects of stress imposed by NaCl, except in the SDM at the 14th day of treatment. Moreover, the SDM and RDM from H_2O_2-sprayed and stressed plants were higher than the ones from water-sprayed stressed plants. Salt stress promoted reductions in LA of maize plants in both evaluation times; nonetheless, LA of H_2O_2-sprayed plants was less affected by salt stress than the one of water-sprayed plants (Table 1).

Regardless the salt condition, plants sprayed with H_2O_2 displayed higher relative content chlorophyll (SPAD index) when compared to water sprayed ones (Figure 1A). At the 7th day of treatment, the relative chlorophyll content was significantly increased by salinity in both water- and H_2O_2-sprayed plants. Conversely, the relative chlorophyll content was reduced by salinity when compared to control after 14 d of treatment, whereas it remained unaltered in H_2O_2-sprayed plants. When salinity was absent, RWC was not significantly altered by H_2O_2 spraying during the experiment (Figure 1B). Under salt stress conditions, the RWC was reduced only in water-sprayed plants as compared to control ones in all evaluations.

Gas Exchange: After seven days of treatment, the H_2O_2-sprayed plants showed higher photosynthesis, transpiration and stomatal conductance than the water-sprayed plants under control conditions (Figures 2A-2C). On the other hand, these parameters were not significantly altered by the H_2O_2 spraying after 14 d of treatment. Although the photosynthesis, transpiration, stomatal conductance and intercellular CO_2 concentration were strongly decreased by salinity after 7 and 14 d of treatment, plants sprayed with H_2O_2 were less affected than the ones water-sprayed, except for stomatal conductance after 7 d (Figure 2).

Oxidative Damage and Antioxidant Defense System: Under control conditions, the H_2O_2 content in leaves and roots did not vary significantly due to H_2O_2 spraying (Figure 3). Salt stress induced an increase in leaf H_2O_2 content after 7 and 14 d of NaCl exposure, and the average increase was 47.5% when compared to control (Figure 3A). On the other hand, the H_2O_2

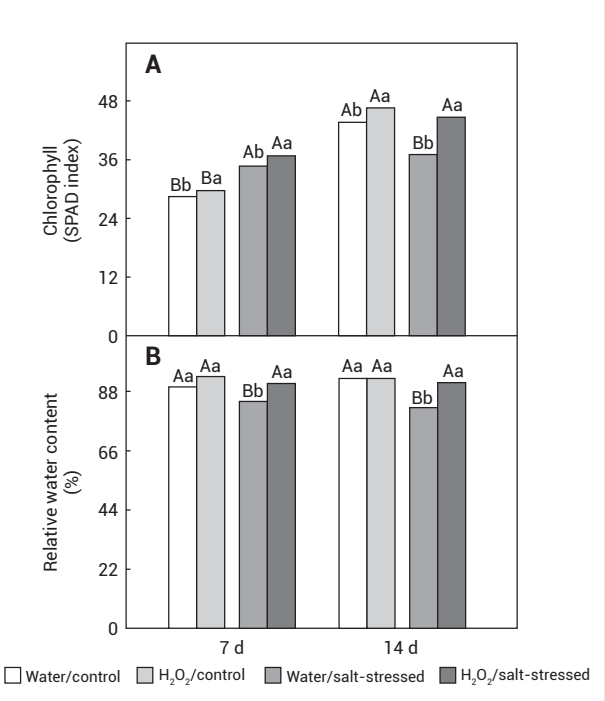

Figure 1. Relative chlorophyll content (SPAD index, A) and relative water content (B) of maize plants pretreated with water or H_2O_2 and in the absence or presence of 80 mM NaCl after seven and 14 d of salt exposure. Values represent the mean of five replicates. In each time of analysis, different capital letters indicate significant differences due to salt stress (water/control×water/salt-stressed or H_2O_2/control×H_2O_2/salt stressed), whereas different small letters indicate significant differences due to H_2O_2 pretreatment (water/control×H_2O_2/control or water/salt-stressed×H_2O_2/salt stressed), according to Tukey's test ($p \leq 0.05$).

Table 1. Shoot dry mass, root dry mass and leaf area of maize plants in absence of salinity and sprayed with distilled water (water/control) or H_2O_2 (H_2O_2/control) and in the presence of 80 mM NaCl and sprayed with distilled water (water/salt-stressed) or H_2O_2 (H_2O_2/salt-stressed)

Treatment	SDM (g plant^{-1})		RDM (g plant^{-1})		LA (cm^2 plant^{-1})	
	7 d	14 d	7 d	14 d	7 d	14 d
Water/control	1.01 Aa	4.20 Aa	0.29 Aa	0.64 Aa	374.7 Aa	959.0 Aa
H_2O_2/control	1.07 Aa	4.49 Aa	0.29 Aa	0.63 Aa	361.7 Aa	820.7 Ab
Water/salt-stressed	0.54 Bb	1.37 Bb	0.24 Bb	0.44 Bb	118.3 Bb	191.5 Bb
H_2O_2/salt-stressed	1.04 Aa	2.40 Ba	0.35 Aa	0.74 Aa	291.5 Ba	355.0 Ba

Data are means of five repetitions. In each time of analysis, different capital letters indicate significant differences due to salt stress (water/control×water/salt-stressed or H_2O_2/control×H_2O_2/salt stressed), whereas different small letters indicate significant differences due to H_2O_2 pretreatment (water/control×H_2O_2/control or water/salt-stressed×H_2O_2/salt stressed), according to Tukey's test ($p \leq 0.05$).
SDM: shoot dry mass; RDM: root dry mass; LA: leaf area.

content of H_2O_2-sprayed plants was slight increased (5%) by salinity only after 7 d of NaCl exposure. Additionally, the leaf H_2O_2 contents in water-sprayed and stressed plants were 31 and 30% higher than the ones in H_2O_2-sprayed and stressed plants after 7 and 14 d of treatment, respectively (Figure 3A). In roots, salt stress promoted an increase in the H_2O_2 content in both water and H_2O_2 treatments after 7 d of treatment. However, the increase was also less pronounced in the plants sprayed with H_2O_2 (Figure 3B).

Under control conditions, the AsA and AsA/(AsA+DHA) in leaves and roots were not affected by H_2O_2 spraying (Figure 4). Although salinity had increased the leaf AsA content after 7 and 14 d of treatment, it did not promote any significant alteration in AsA/(AsA+DHA) (Figures 4A and 4B). In roots, the AsA content was decreased by salinity in both water and H_2O_2 sprayed plants in both evaluations (Figure 4C); however, plants treated with H_2O_2 were less affected by salinity after 7 d of NaCl stress. Surprisingly, the ascorbate redox state was reduced by salinity only after 7 d of treatment, with stressed plants sprayed with water presenting lower ascorbate redox state (Figure 4D).

Leaf GSH and GSH/(GSH+GSSG) were incresead by salinity after 7 and 14 d of treatment, regardless the H_2O_2

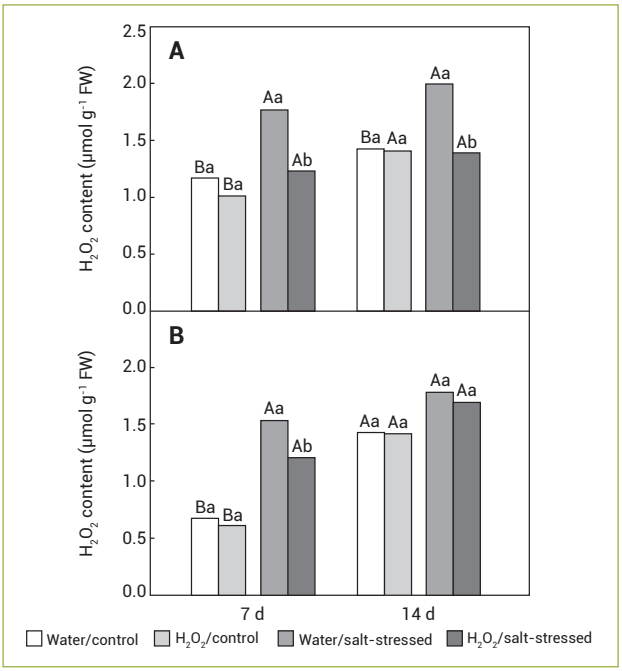

Figure 3. H_2O_2 content in leaves (A) and roots (B) of maize plants pretreated with water or H_2O_2 and in the absence or presence of 80 mM NaCl after 7 and 14 d of salt exposure. Statistical details as in Figure 1.

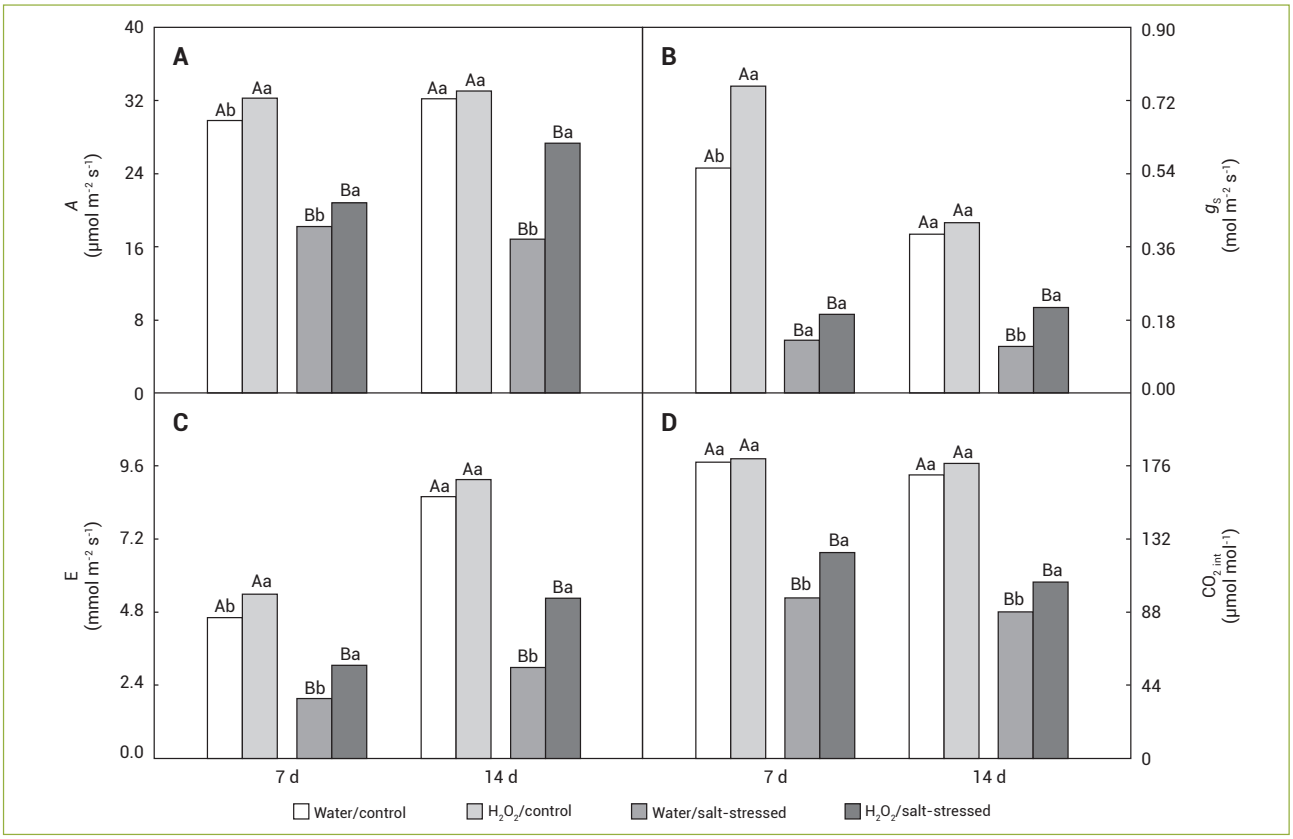

Figure 2. Photosynthetic rate (A), stomatal conductance (B), transpiration (C) and intercellular CO_2 concentration (D) of maize plants pretreated with water or H_2O_2 and in the absence or presence of 80 mM NaCl after 7 and 14 d of salt exposure. Statistical details as in Figure 1.

treatments; however, water-sprayed and stressed plants showed values of GSH/(GSH+GSSG) higher than the stressed plants sprayed with H_2O_2 after 14 d of NaCl exposure (Figures 5A and 5B). Root GSH and GSH/(GSH+GSSG) were significantly detected in control plants after 14 d of treatment and they were higher in H_2O_2-sprayed plants than in water-sprayed plants (Figures 5C and 5D)

DISCUSSION

Several studies have shown the beneficial effects of H_2O_2 pretreatement on salt tolerance in monocotyledonous plants (Uchida et al. 2002, Azevedo Neto et al. 2005, Wahid et al. 2007). In our previous study (Gondim et al. 2012), we also demonstrated that H_2O_2 pretreatment could reverse the harmful effects of salinity on growth by alleviating salinity-induced membrane damage, which was associated to the ability of H_2O_2 to induce antioxidant enzymatic defenses, especially catalase activity. Herein, we described that the leaf H_2O_2 spraying was effective to reduce the salinity deleterious effects on growth and gas exchange of maize plants, which was not closely related to a better non-enzymatic antioxidant system.

Salinity stress has been shown to reduce the overall growth and productivity of plants by disturbing several physiological and biochemical processes like photosynthesis, ion homeostasis and enzyme activities (James et al. 2006, Gomes-Filho et al. 2008, Hasegawa 2013). In fact, our results are in agreement with those previously reported for different crops such as cowpea (Costa et al. 2003, Freitas et al. 2011), sorghum (Lacerda et al. 2003, Freitas et al. 2011), maize (Azevedo Neto et al. 2005), cotton (Freitas et al. 2011) and pea (Noreen and Ashraf 2009).

In this study, the chlorophyll content was negatively affected by salinity only in water sprayed plants after 14 d of treatment (Figure 1A). According to Singh and Dubey (1995), the loss of chlorophyll content acts as a cellular marker of salt stress, and it could be related to photoinhibition or ROS formation. Therefore, the pretreatment with H_2O_2 was effective to reduce the detrimental effects of salinity in chlorophyll content (Figure 1A). The prevented chlorophyll degradation

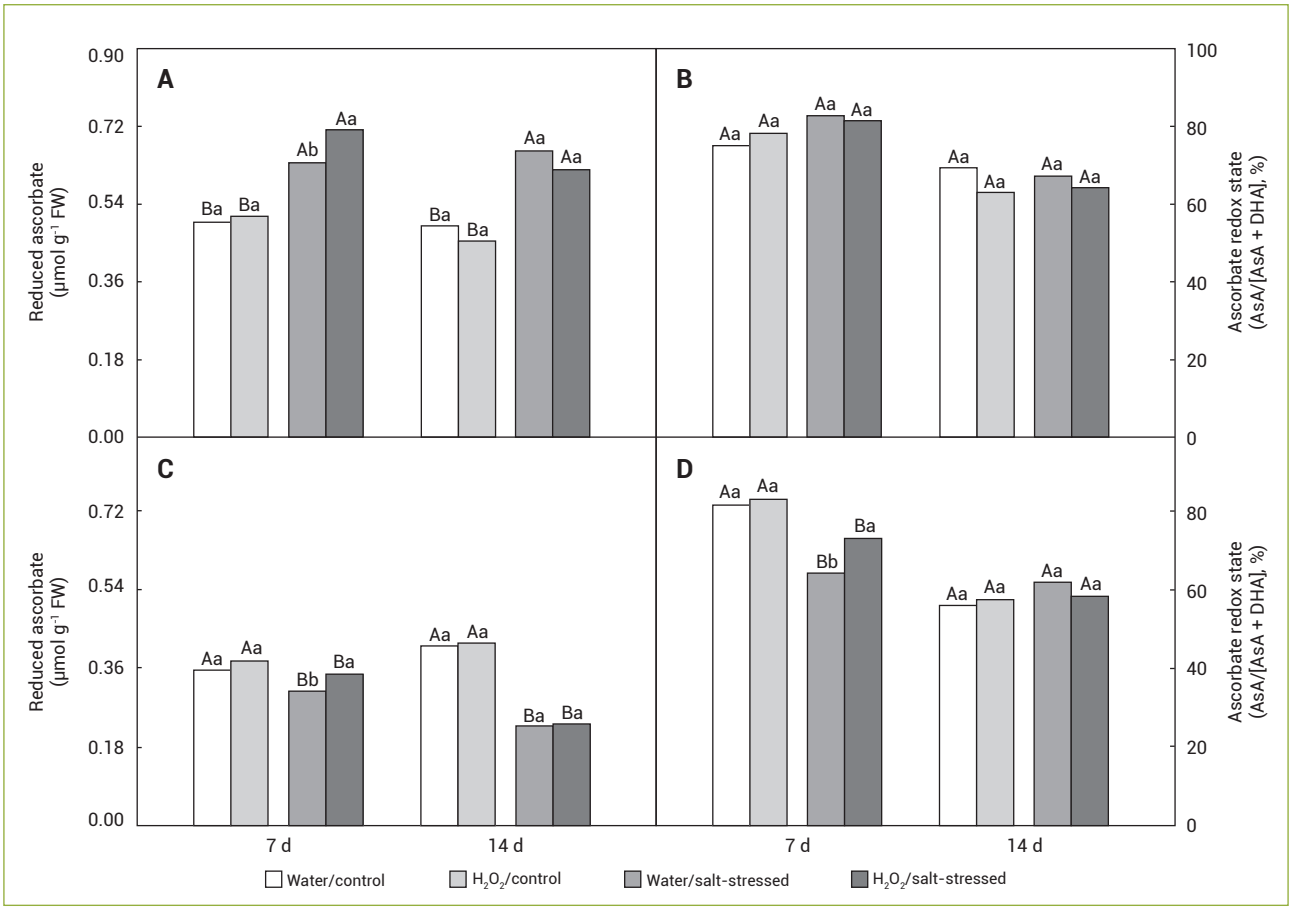

Figure 4. Reduced ascorbate content and ascorbate redox state in the leaves (A and B) and roots (C and D) of maize plants pretreated with water or H_2O_2 and in the absence or presence of 80 mM NaCl after 7 and 14 d of salt exposure. Statistical details as in Figure 1.

due to H_2O_2 supplying may be assigned to maintain higher RWC and lower hydrogen peroxide content in leaves under salt stress (Figures 1B and 3A) (Chakraborty et al. 2012). Additionaly, the higher RWC in H_2O_2-sprayed stressed plants can be explained by the ability of H_2O_2 to induce mechanisms that allow the plant to obtain and preserve high water content, as well as accumulate high contents of ions and compatible solutes under saline conditions, which resulted in better growth of stressed plants (Table 1 and Figure 1B).

The most common response to salt stress is a decrease in photosynthesis, and it may be caused by salt-induced changes like ROS formation, water status alteration and reduction in chlorophyll content and CO_2 diffusion through stomatal guard cells (Chaves and Oliveira 2004, Munns and Tester 2008). It has been reported that reduction in photosynthesis by low stomatal conductance, which causes CO_2 availability, occurs during early exposure to salt stress, while biochemical limitations arise due to long-term NaCl exposure (Silva et al. 2011). Thus, the reduction of photosynthesis in maize plants was caused by stomatal closure, decreasing the intercellular CO_2 concentration for Rubisco activity (Figures 2A, 2B and 2D) (Shahbaz et al. 2010).

Some studies correlate the maintenance of gas exchange with salt tolerance in plants (James et al. 2006, Munns and Tester 2008). In this work, it should be emphasized that all gas exchange parameters were less affected by salinity in plants previously treated with H_2O_2 (Figure 2). Therefore, our data indicate that H_2O_2 supplying increased *gs*, which enabled high photosynthetic rate and improved shoot dry mass (Figures 2A and 2B, Table 1). In addition, the lower leaf H_2O_2 accumulation induced by the H_2O_2 pretreatment in NaCl stressed plants is an evidence that plants were able to control oxidative damages caused by ROS in the photosynthetic machinery and maintain leaf gas exchange (Figures 2 and 3). Similarly, Wahid et al. (2007) observed that the H_2O_2-pretreatment in wheat seeds caused increases in *A*, *E* and *gs* in plants subjected to salinity when compared to non-treated seedlings.

AsA is a soluble compound ubiquitously present in photosynthetic organisms, acting as an important antioxidant in plant cells (Nakano and Asada 1981). According to

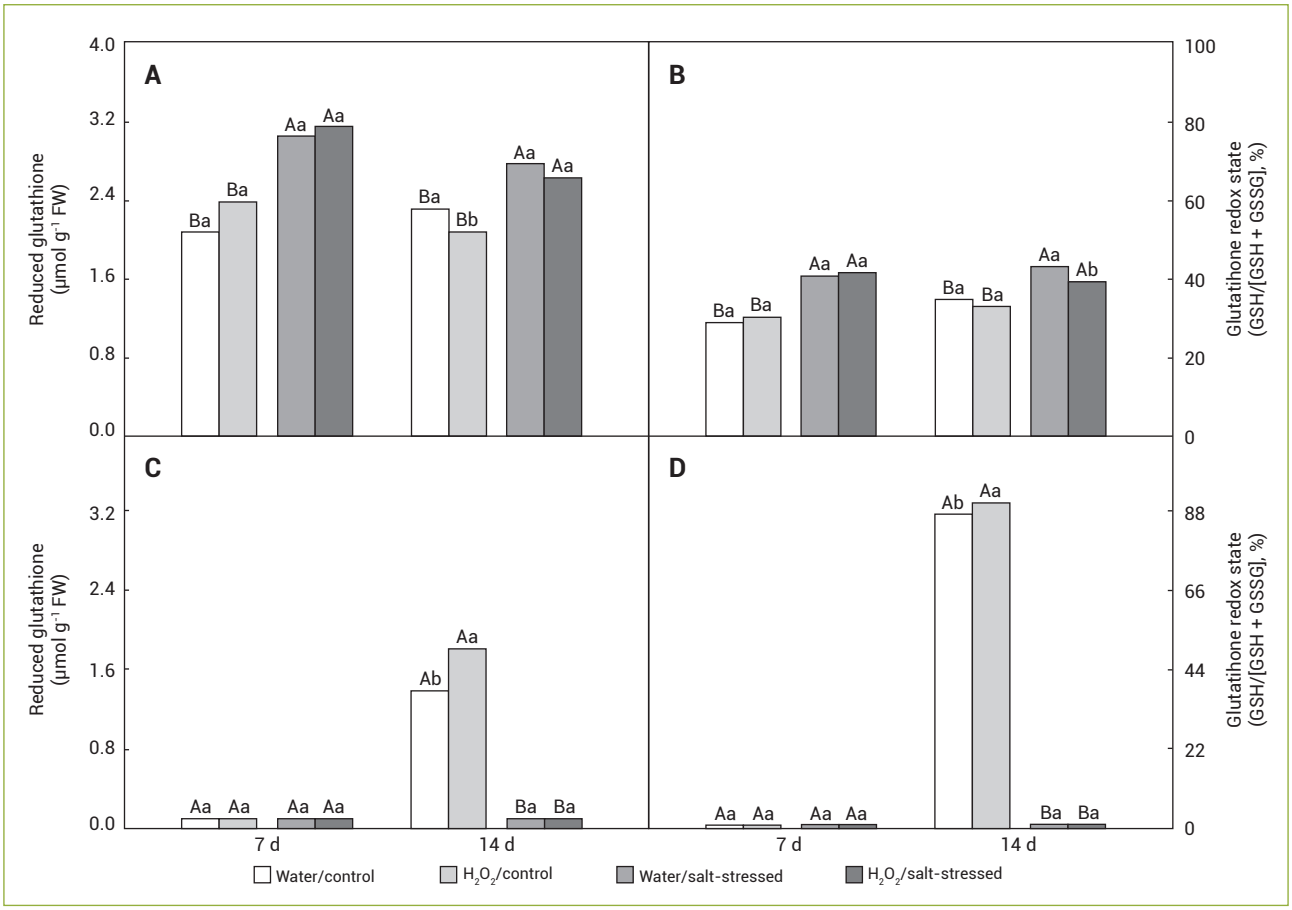

Figure 5. Reduced glutathione content and glutathione redox state in the leaves (A and B) and roots (C and D) of maize plants pretreated with water or H_2O_2 and in the absence or presence of 80 mM NaCl after 7 and 14 d of salt exposure. Statistical details as in Figure 1.

Noctor and Foyer (1998), the ascorbate could react directly with hydroxyl radicals, superoxide, and singlet oxygen and thus promote oxidative protection against several stresses. Moreover, it has been reported that the AsA and the changes in ascorbate redox state are directly correlated with stress tolerance in plant species (Noctor and Foyer 1998, Wang et al. 2010, Xu et al. 2011). Conversely, the leaf amount of AsA and ascorbate redox state did not act in the ROS scavenging and salt stress acclimation herein (Figures 4A and 4C).

Reduced glutathione (GSH) plays an essential role in the antioxidative defense system, acting in the AsA regeneration as well as in the scavenging of ROS such as singlet oxygen and hydroxil radical (Asada 1994). In this study, the leaf contents of GSH and AsA were increased by salinity regardless the H_2O_2 pretreatment, whereas the g_s and A were decreased (Figures 2A and 2B, 4A and 5A). The increased ascorbate and glutathione contents in salt stressed plants may be explained by a link between gas exchange and biosynthesis of these compounds. The NaCl-induced reduction in g_s might have negatively affected the Calvin cycle, which in turn increases the NADPH/$NADP^+$ ratio and limits the photochemical reactions due to $NADP^+$ shortage (Rizhsky et al. 2003). Probably, the ascorbate-glutathione cycle enzymes involved in the GSH and AsA synthesis (such as monodehydroascorbate reductase and glutathione reductase, which oxidate NADPH to $NADP^+$) had their activities increased, providing $NADP^+$ for the photosynthetic process.

In summary, H_2O_2-pretreatment of maize plants improved salinity tolerance, which was associated with (i) a reduction in leaf H_2O_2 content and (ii) maintenance of leaf RWC and chlorophyll content. These responses caused better performance of gas exchange and plant growth under saline conditions. Nevertheless, ascorbate and glutathione did not have any obvious effects as non-enzymatic antioxidants. In general, the leaf H_2O_2 spraying may be an alternative for maize plants growing under salinized soils, since it has a low cost and could be applied in different stages of plant development.

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