RESEARCH ARTICLE

Pretreatment with H_2O_2 in maize seeds: effects on germination and seedling acclimation to salt stress

Franklin A. Gondim[1,2], Enéas Gomes-Filho[1*], Claudivan F. Lacerda[3], José Tarquinio Prisco[1], André D. Azevedo Neto[4] and Elton C. Marques[1]

[1] Departamento de Bioquímica e Biologia Molecular and Instituto Nacional de Ciência e Tecnologia em Salinidade (INCTSal)/CNPq, Universidade Federal do Ceará, Caixa Postal 6039, 60455-970, Fortaleza, Ceará, Brazil.

[2] Present address: Instituto Federal de Educação, Ciência e Tecnologia do Ceará, Caixa Postal 95, 63900-000, Quixadá, Ceará, Brazil.

[3] Departamento de Engenharia Agrícola and Instituto Nacional de Ciência e Tecnologia em Salinidade (INCTSal)/CNPq, Centro de Ciências Agrárias, Universidade Federal do Ceará, 60455-900, Fortaleza, Ceará, Brazil.

[4] Centro de Ciências Exatas e Tecnológicas, Universidade Federal do Recôncavo da Bahia, 44380-000, Cruz das Almas, Bahia, Brazil.

[*] Corresponding author. Tel.: +55 85 3366 9405; fax: +55 85 3366 9829 e-mail address: egomesf@ufc.br
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ABSTRACT

The aim of this study was to evaluate the effects of H_2O_2 on germination and acclimation of maize plants subject to salt stress. Three experiments using BRS3003 seeds, a triple hybrid of maize, were carried out in a growth room and in greenhouse. In the first experiment, H_2O_2 accelerated the germination percentage of seeds at 100 mM, but not at 500 mM. In the second experiment, the pretreatment of seeds was observed to induce a pronounced increase in ascorbate peroxidase (APX) and catalase (CAT) enzyme activity after 30 h of soaking in H_2O_2. It was also observed that guaiacol peroxidase (GPX) activity was smaller in the seeds soaked in H_2O_2 for 12, 24, 30, 36 and 42 h, in relation to those soaked in distilled water. The superoxide dismutase (SOD) activity was not affected by the pretreatment of seeds, except for the 24 h treatment. Only one CAT isoform was detected. In the third experiment, seeds were pretreated with 36 h soaking in 100 mM H_2O_2 solution or in distilled water and later cultivated in Hoagland's nutrient solution or nutrient solution with 80 mM NaCl. The results showed the pretreatment of seeds with H_2O_2 induced acclimation of the plants to salinity. It decreased the deleterious effects of salt stress on the growth of maize. In addition, the differences in antioxidative enzyme activities may explain the increased tolerance to salt stress of plants originated from H_2O_2 pretreated seeds.

Key words: antioxidant enzymes, hydrogen peroxide, oxidative stress, salinity stress, *Zea mays*

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; DM, dry mass; FM, fresh mass; GPX, guaiacol peroxidase; H_2O_2, hydrogen peroxide; LA, leaf area; RDM, root dry mass; ROS, reactive oxygen species; SDM, shoot dry mass; SOD, superoxide dismutase; TDM, total dry mass.

INTRODUCTION

Soil salinity is one of the major abiotic stresses affecting the productivity and quality of crops (Chinnusamy et al., 2005). FAO (2005) data shows that 20% of irrigated land and 2.1% of dry-land agriculture are salt-affected. The problem of soil salinity is increasing due to poor soil and water management in irrigated areas.

Salt stress, like other abiotic stresses, can also lead to oxidative stress due to increased production of reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide anion

($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\bullet OH$) (Bray et al., 2000). These ROS are highly reactive and can alter normal cellular metabolism with the oxidative damage to carbohydrates, proteins and nucleic acids, and cause peroxidation of membrane lipids (Azevedo Neto et al., 2008).

Plants have a complex antioxidative system to prevent the oxidative damage of ROS that involves both non-enzymatic and enzymatic antioxidant defenses (Asada, 1999; Azevedo Neto et al., 2008). Non-enzymatic defenses include antioxidants such as ascorbate, glutathione, α-tocopherol and carotenoids. Enzymatic defenses include superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and other ascorbate-glutathione cycle enzymes (Chinnusamy et al., 2005; Azevedo Neto et al., 2008).

H_2O_2 is a vital cellular component with various functions in the development, metabolism and homeostasis of aerobic organisms (Bienert et al., 2006). In plants, hydrogen peroxide is one of the major and the most stable ROS and regulates basic processes, such as acclimation, defense and development (Ślesak et al., 2007). Contrary to superoxide, H_2O_2 belongs to non-radical ROS and is a molecule that carries no net charge (Halliwell, 2006). Due to its relatively stability and diffusibility through membranes, hydrogen peroxide is more likely a long-distance signaling molecule (Vranová et al., 2002), acting as a translocating second messenger triggering Ca^{2+} fluxes, protein modifications and gene expression (Bienert et al., 2006). In addition, Agarwal et al. (2005) hypothesized that the H_2O_2 produced as a result of the treatment with various signaling molecules could in turn induce the synthesis or activate various transcription factors, which are associated with the induction of antioxidative enzymes.

The generation of H_2O_2 is increased due to a wide variety of stresses, and some authors have suggested that H_2O_2 is a key factor mediating the phenomena of acclimation and cross-tolerance (Neill et al., 2002). Thus, endogenous H_2O_2 production has been shown to increase as a result of the chilling stress in maize seedlings, and exogenously applied H_2O_2 increased chilling stress tolerance (Prasad et al., 1994). Exogenous H_2O_2 treatments simultaneously enhanced multi-resistance to heat, chilling, drought and salt stresses in maize seedlings (Gong et al., 2001). Uchida et al. (2002) and Azevedo Neto et al. (2005) demonstrated that the pretreatment with H_2O_2 in nutrient solution induces acclimation to salt stress in rice and maize seedlings, respectively. However, there is no information if the pretreatment of maize seeds with H_2O_2 is also capable to induce acclimation of the plants to salt stress.

In seeds, the stimulating effect of hydrogen peroxide on germination and subsequent seedling growth has been noted in a few number of plant species. The first evidence of this effect was the stimulation of *Pseudotsuga menziesii* seed germination by soaking seeds in 1% H_2O_2 (Ching, 1959). Similarly, H_2O_2 seed germination stimulation was also obtained in *Zinnia elegans* (Ogawa and Iwabuchi, 2001), *Panicum virgatum*, *Andropogon gerardii* and *Sorghastrum nutans* (Sarath et al., 2007). Recently, the pretreatment of seeds with H_2O_2 induced salt tolerance of wheat seedlings (Wahid et al., 2007).

In recent years, the role of H_2O_2 in plant acclimation has increasingly become a matter of interest. However, the role of H_2O_2 as maize seed pretreatment to induce salt tolerance is still unclear. Thus, the aim of this study was to evaluate the effects of hydrogen peroxide pretreatment of seeds on germination and acclimation of maize seedlings to salt stress, to better understand the physiological and biochemical mechanisms involved.

MATERIALS AND METHODS

Experiments, growth condition and treatments: Three experiments using BRS3003 seeds, a triple hybrid of maize (*Zea mays* L.), were carried out in a growth room and in greenhouse. In all experiments, seeds were selected for size and shape, had their surface sterilized with 1% sodium hypochlorite for 5 min, and rinsed with distilled water.

The first experiment analyzed the effects of H_2O_2 pretreatment of seeds on germination. Seeds were sown in Gerbox plastic boxes and soaked in distilled water (control) or H_2O_2 solutions at 100 or 500 mM, in the dark. After 36 h sowing, germinated seeds were recorded at intervals of 1 to 2 hours. Seeds with radicle emergence of 1 mm or more were counted as germinated. The experiment was carried out under growth room conditions, and the temperature and relative air humidity were 24.3°C and 71.7%, respectively. The experimental design was completely randomized, with three H_2O_2 levels (0, 100 and 500 mM), five replicates, each with

a group of 20 seeds per treatment. Percentage data were submitted to arcsin transformation prior to statistical analysis. The values obtained were submitted to the analysis of variance (ANOVA), and the means were compared through Tukey's test ($P \leq 0.05$). The values were compared applying the same time after sowing.

The second experiment studied the effects of H_2O_2 pretreatment of seeds on the activities of antioxidative enzymes and catalase (CAT) isoenzymes. Seeds were sown in Gerbox plastic boxes and soaked in distilled water (control) or 100 mM H_2O_2 solution, in the dark, for 12, 24, 30, 36, 42, and 48 h. After that, the seeds were collected, weighed and stored at -25°C for further analyses. The experiment was carried out under the same environmental conditions as described for the first experiment. The experimental design was completely randomized, with two treatments (seeds soaked in distilled water or 100 mM H_2O_2), four replicates, each with a group of 20 seeds. For each extract, enzyme activities were determined in duplicate assays. The data were submitted to the analysis of variance (ANOVA), and the means were compared through the Student's t-test ($P \leq 0.05$). The values were compared applying the same soaking time.

The third experiment assessed the effects of H_2O_2 pretreatment of seeds on salt stress acclimation. Seeds were soaked in distilled water or 100 mM H_2O_2 solution, in the dark, for 36 h. After soaking, the seeds of each treatment were sown on filter paper moistened with half-strength Hoagland's nutrient solution or nutrient solution with 80 mM NaCl, in a growth room. Six days after sowing, the seedlings were transferred to trays containing half-strength Hoagland's nutrient solution or nutrient solution with NaCl at 80 mM under greenhouse conditions. Then, the plants were submitted to four treatments: water/control (seeds not pretreated with H_2O_2 and not salt-stressed), H_2O_2/control (seeds pretreated with H_2O_2 and not salt-stressed), water/salt-stressed (seeds not pretreated with H_2O_2 and salt-stressed) and H_2O_2/salt-stressed (seeds pretreated with H_2O_2 and salt-stressed). The mean values of temperature, relative air humidity and photosynthetic active radiation were, respectively, 24.3°C, 71.7% and 90 μmol m^{-2} s^{-1} in the growth room and 28.5°C, 63.9% and 1500 μmol m^{-2} s^{-1} (at noon) in the greenhouse. Plants were harvested 6, 11 and 16 days after sowing. Plants from each treatment were separated into shoot and roots for dry mass (DM) determinations and leaf area (LA) measurements, as described in Azevedo Neto et al. (2004). Samples of leaves and roots were frozen in liquid nitrogen, lyophilized, ground to a powder and kept in freezer (-25°C) for further biochemical analyses. The experimental design was completely randomized, following a factorial arrangement (two NaCl levels and two H_2O_2 levels), with four treatments and five replicates. The data were submitted to the analysis of variance (ANOVA), and the means were compared through Tukey's test ($P \leq 0.05$). The values were compared applying the same harvest time. For each extract, enzyme activities were determined in duplicate assays.

Extract preparation: Seed extracts for enzyme assays were prepared by grinding ten seeds in 10 mL of ice-cold extraction buffer (100 mM potassium phosphate buffer, pH 7.0, 0.1 mM EDTA). For the estimation of APX, extraction buffer was further supplemented with 2 mM ascorbic acid. Leaf and root tissue extracts were prepared by homogenizing lyophilized leaves (0.20 g) and roots (0.15 g) in 4 mL of ice-cold extraction buffer. In both cases, the homogenate was filtered through muslin cloth and centrifuged at 12,000 *g* for 15 min. The supernatant fraction was used as crude extract for enzyme activities and CAT gel activity assay. All operations were performed at 4°C.

Enzyme assays: Assays for antioxidative enzymes were conducted according to methods reported previously (Azevedo Neto et al., 2005). Total SOD (EC 1.15.1.1) activity was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT), as described by Giannopolitis and Ries (1977). One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction rate and the results expressed as U g^{-1} of fresh mass (FM) for seeds or dry mass (DM) for leaves and roots. Total CAT (EC 1.11.1.6) activity was measured according to the method proposed by Beers and Sizer (1952), with minor modifications. Total APX (EC 1.11.1.1) activity was assayed according to Nakano and Asada (1981). Total GPX (EC 1.11.1.7) activity was determined as described by Urbanek et al. (1991). For CAT, APX and GPX the results were expressed as μmol H_2O_2 min^{-1} g^{-1} FM (for seeds) or DM (for leaves and roots).

Native polyacrylamide gel electrophoresis and CAT gel activity assays in seeds: Native polyacrylamide gel electrophoresis (PAGE) was performed in 7%

polyacrylamide, according to Davis (1964), on vertical slab gel using the miniVE electrophoresis system (Amersham Biosciences, Sweden) at 4°C and 150 V for approximately 2 h. Seed protein extracts, 400 μg of each sample were loaded into each gel well. CAT activity was detected following the procedure of Woodbury et al. (1971). Gels were then soaked in 0.01% H_2O_2 for 15 min, rinsed in water and stained in a solution containing 1% potassium ferricyanide and 1% ferric chloride, for 3 minutes.

RESULTS

Effect of H_2O_2 pretreatment of seeds on germination: The time course of germination percentage of seeds soaked in distilled water or H_2O_2 solutions at 100 or 500 mM is provided in Figure 1. Until 42 h after sowing, seeds soaked in 100 mM H_2O_2 showed increased germination percentage, when compared with other treatments; and 36 h after sowing, the germination percentage of seeds soaked in 100 mM H_2O_2 was 92%, while in both, 500 mM H_2O_2 or distilled water was only 22.5%.

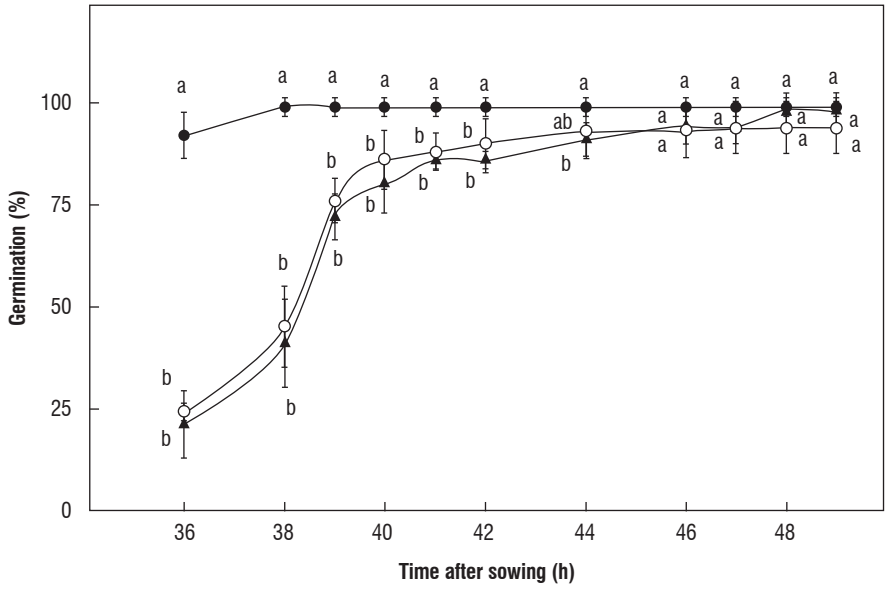

Figure 1. Time course of germination percentage of maize seeds soaked in distilled water (○) or H_2O_2 solutions at 100 mM (●) or 500 mM (▲). Values indicate the mean ± SD. Values with the same letter in the same time after sowing are not significantly different, according to Tukey's test ($P \leq 0.05$).

Effects of H_2O_2 pretreatment of seeds on the activities of antioxidative enzymes and isoenzymes of catalase: No change in total CAT activity was observed in control treatment, except for a transient decrease (61%) at 24 h (Figure 2A). However, soaking seeds with H_2O_2 induced a marked increase in CAT activity. Thus, H_2O_2-soaking-induced increases in CAT activity were 48, 122, 176, and 195% at 30, 36, 42, and 48 h, respectively, when compared to control.

Total APX activity was not detected in control seeds, but in H_2O_2 soaked seeds, APX activity was detected starting at 36 h and significantly increased up to 48 h soaking (Figure 2B).

Soaking in H_2O_2 reduced total GPX activity in most soaking times, and this effect was more conspicuous at 12 and 24 h (Figure 2C). However, in H_2O_2 soaked seeds, GPX activity increased with soaking time.

When comparing CAT, APX and GPX activities in H_2O_2 soaked seeds for 48 h, CAT activity was 286- and 180-fold greater than APX and GPX activities, respectively. There was no significant difference in total SOD activity between treatments, except for a transient decrease (56%) in enzyme activity in seeds soaked in H_2O_2 for 24 h (Figure 2D).

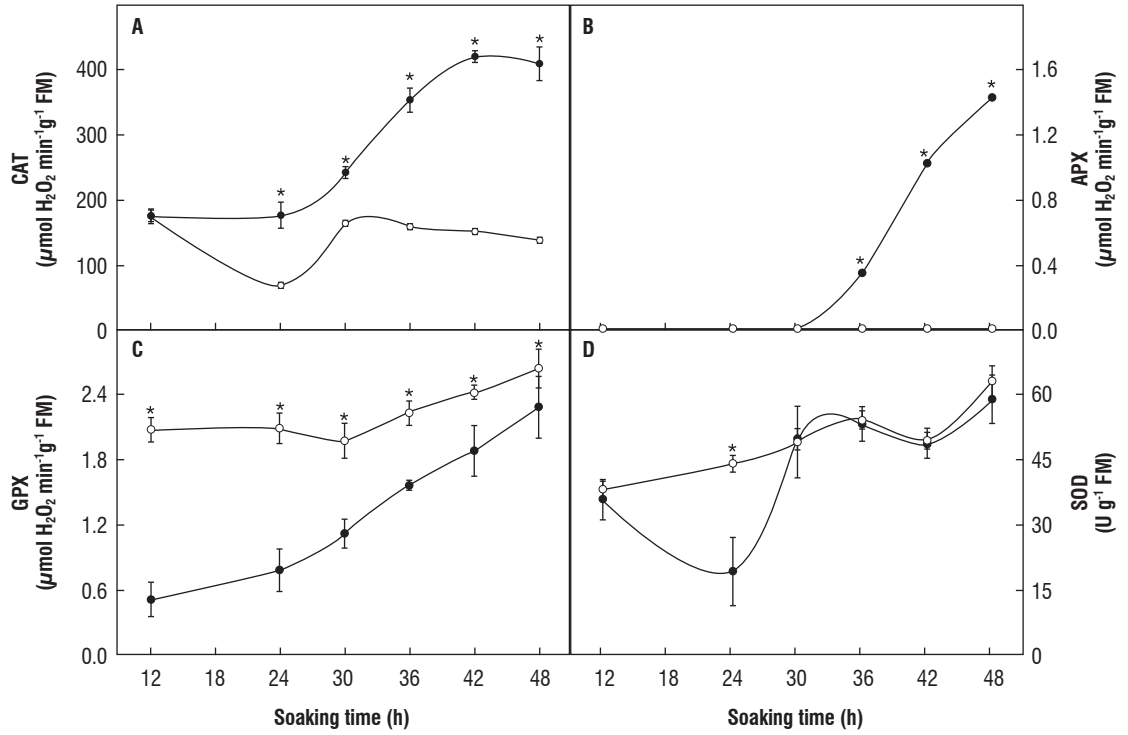

Figure 2. Enzyme activities of CAT (A), APX (B), GPX (C) and SOD (D) in maize seeds soaked in 100 mM H_2O_2 solution (●) or distilled water (○) for 12, 24, 30, 36, 42 and 48 h in darkness. Values indicate the mean ± SD. The symbol (*) indicates that the values in the same soaking time are significantly different, according to Student's t-test ($P \leq 0.05$).

CAT activity staining gel only exhibited one prominent isoform, whose intensity was higher in seeds soaked in H_2O_2 for 36 and 48 h (Figure 3).

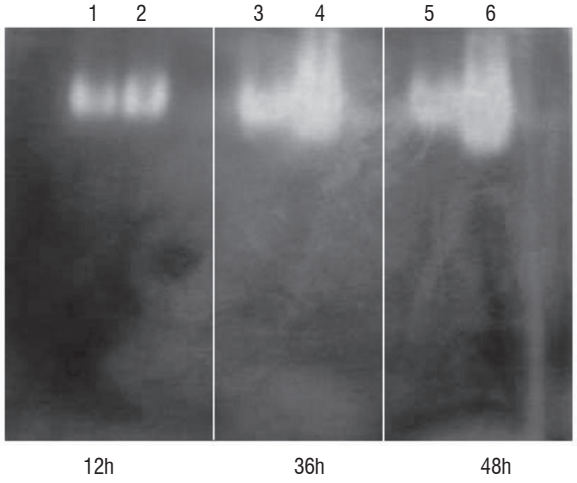

Figure 3. CAT gel activity analysis in maize seeds soaked in distilled water (lines 1, 3, and 5) or 100 mM H_2O_2 solution (lines 2, 4, and 6) for 12, 36, and 48 h.

Effects of H_2O_2 pretreatment of seeds on acclimation of plants to salt stress: Data of changes in shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM) and leaf area (LA) are provided in Figure 4. It should be noted that the pretreatment of seeds with H_2O_2 (H_2O_2/control treatment) by themselves little affected the plant growth in relation to water/control treatment. In addition, it should be observed that stressed plants had their SDM, RDM, TDM and LA reduced when compared to control treatments. However, salt-induced growth inhibition decreased when the seeds were soaked in 100 mM H_2O_2. The comparison of H_2O_2/salt-stressed with water/salt-stressed plants showed increased SDM, RDM, TDM and LA, 46, 71, 52 and 61%, respectively, at day 16. At day 11, RDM, TDM and LA in H_2O_2/salt-stressed plants was 52, 29 and 46%, respectively, higher than in water/salt-stressed plants. At day 6, RDM, TDM and LA in H_2O_2/salt-stressed plants was 10, 8 and 21%, respectively, higher than in water/salt-stressed plants.

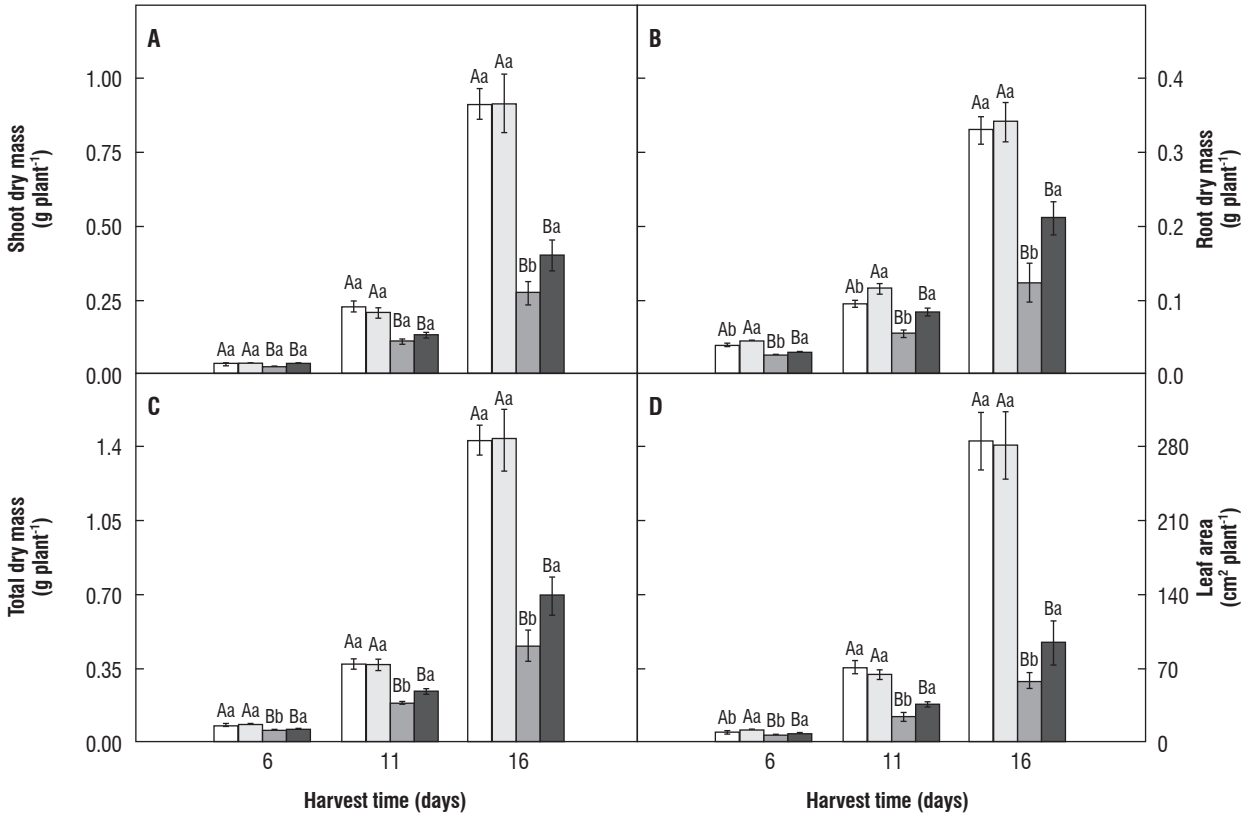

Figure 4. Shoot (A), root (B), total (C) dry masses and leaf area (D) of maize plants. The plants were submitted to four treatments: water/control (□); H_2O_2/control (□); water/salt-stressed (■); and H_2O_2/salt-stressed (■). Plants were harvested at 6, 11 and 16 days after sowing. In the same harvest time values followed by the same capital letter or small letter are not statistically different for NaCl and H_2O_2 treatments, respectively, according to Tukey's test ($P \leq 0.05$).

Total SOD, CAT, GPX and APX activities in leaves are provided in Figure 5. SOD activity in H_2O_2/control plants was 17% higher than in water/control plants at day 6 (Figure 5A). No significant differences were observed in SOD activity between water/salt-stressed plants and H_2O_2/salt-stressed plants at day 6. However, SOD activity in salt-stressed plants had the average increase of 29% in relation to water/control plants. At days 11 and 16, SOD activity in H_2O_2/salt-stressed plants was 34 and 23% higher than in water/salt-stressed plants, respectively. It should be observed that CAT activity in water/salt-stressed plants was reduced by 65 and 39% when compared to controls (plants originated from H_2O_2 or water-pretreated seeds) at days 6 and 11, respectively (Figure 5B). On the other hand, in H_2O_2/salt-stressed plants, CAT activity did not differ from H_2O_2/control plants at days 6 and 11, and was 229 and 61% higher than in water/salt-stressed plants, respectively. At day 16, no significant differences were observed in CAT activity between water/salt-stressed plants and H_2O_2/salt-stressed plants. However, CAT activity in these treatments had the average increase of 117% in relation to water/control plants. GPX activity was significantly increased as a result of seed pretreatment with H_2O_2 at days 6 and 16 (Figure 5C). Thus, GPX activity in H_2O_2/control plants was, respectively, 93 and 59% higher than in water/control plants. Additionally, at day 6 and 16, GPX activity in H_2O_2/salt-stressed plants was, respectively, 75 and 68% higher than in water/salt-stressed plants. APX activity was induced by salt stress at day 6 (Figure 5D). At day 16, the H_2O_2/control plants showed APX activity 41% higher than in water/control plants, and H_2O_2/salt-stressed plants showed the highest activity, which was 50% higher than in water/salt-stressed plants.

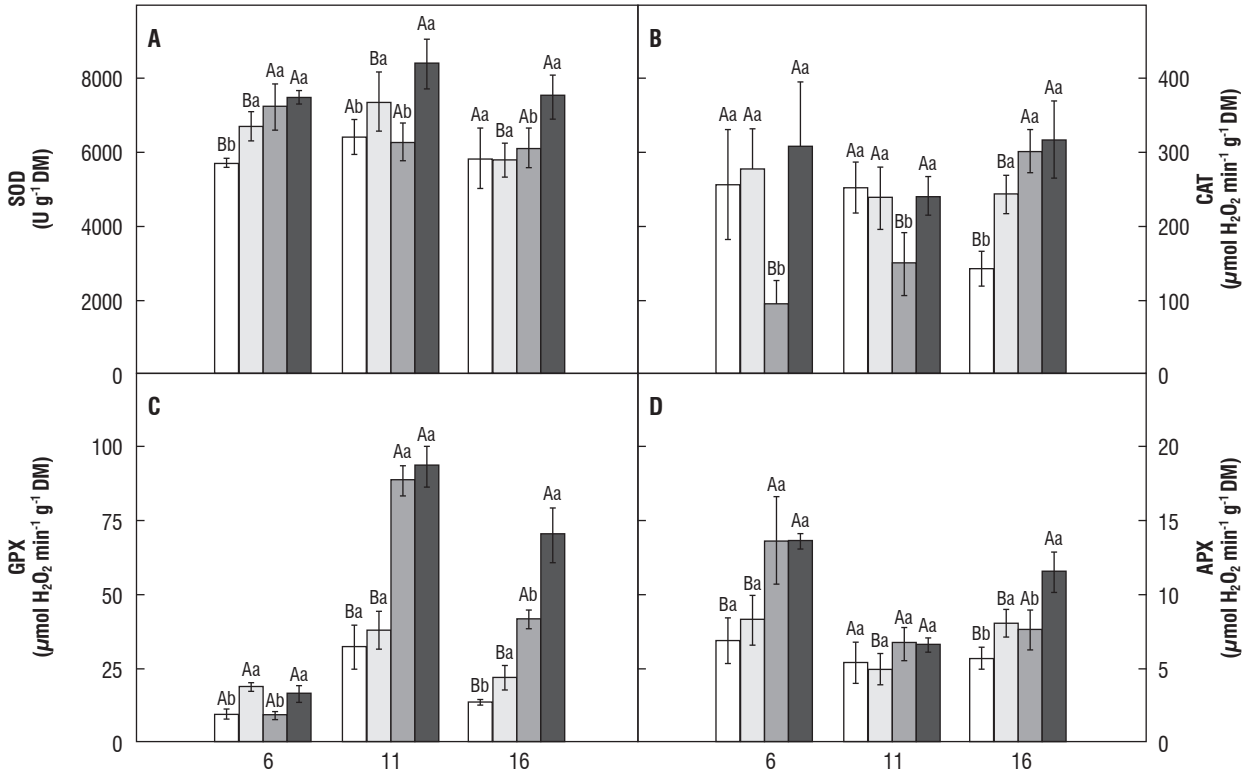

Figure 5. Total SOD, CAT, GPX, and APX activity in maize leaves. Additional details as in Figure 4.

Total SOD, CAT, GPX and APX activities in roots are illustrated in Figure 6. SOD activity in H_2O_2/salt-stressed plants was significantly higher than water/salt-stressed plants by 63, 40, 38%, at days 6, 11 and 16, respectively (Figure 6A). At day 6, CAT activity was not detected in roots (Figure 6B). However, at day 11, CAT activity was induced by H_2O_2 pretreatment, so the values in H_2O_2/control and H_2O_2/salt-stressed plants were 207 and 112% higher than in water/control and water/salt-stressed plants, respectively. At day 16, CAT activity in water/control plants was not detected, and no differences were observed when comparing the other treatments. No significant differences were observed in GPX activity when comparing the treatments (Figure 6C). In roots, APX activity increased at 6, 11 and 16 days in plants subjected to salt stress (Figure 6D).

When comparing the enzyme activities in leaves and roots, it should be noted that CAT activity in roots was on average 80% lower than in leaves. By contrast, GPX and APX activities in roots were 9.5- and 4.7-fold greater than in leaves. When comparing H_2O_2-scavenging enzyme activity, CAT and GPX were the most important H_2O_2-scavenging enzymes in leaves and roots, respectively.

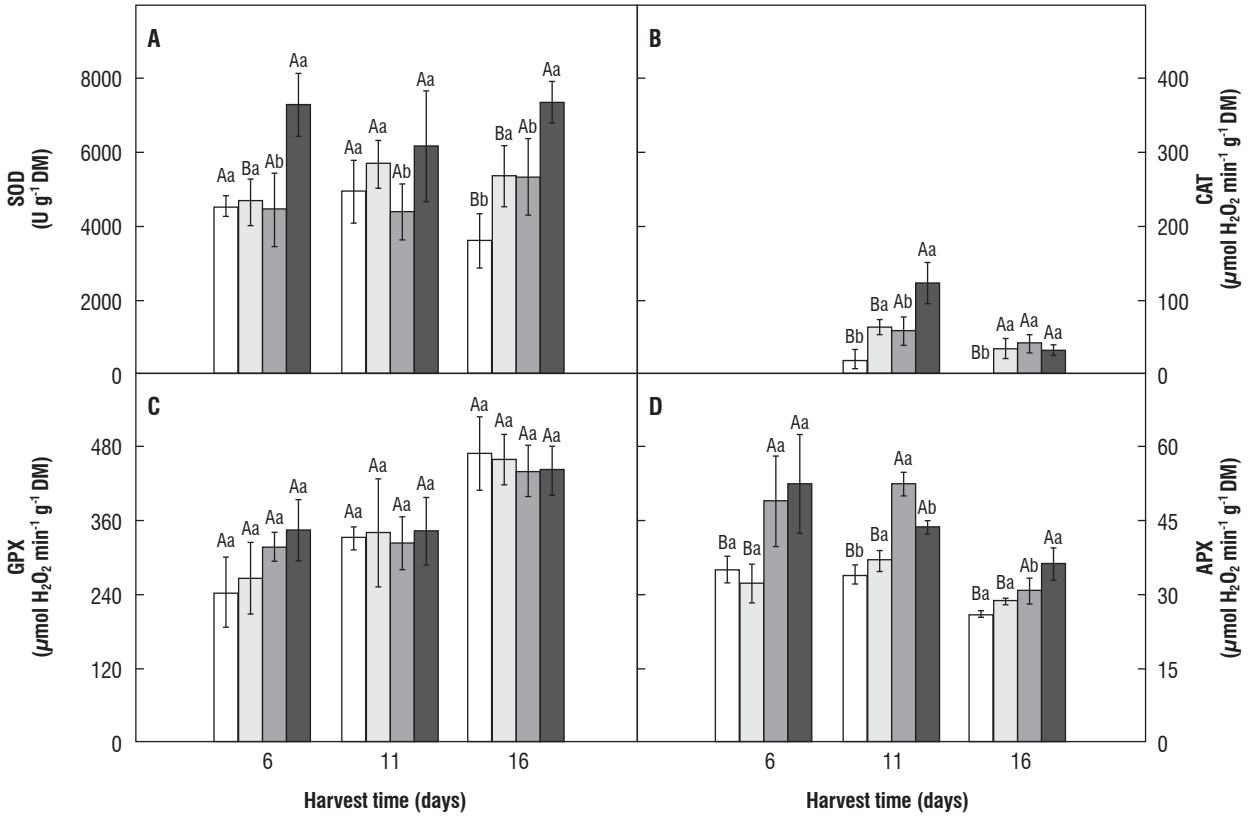

Figure 6. Total SOD, CAT, GPX, and APX activity in maize roots. Additional details as in Figure 4.

DISCUSSION

This study showed that H_2O_2 pretreatment accelerated the germination of seeds (Figure 1). As in our study, some authors have also observed increased germination of other species by soaking seeds in H_2O_2 (Ching, 1959; Ogawa and Iwabuchi, 2001; Sarath et al., 2007). Ching (1959) showed that O_2 and H_2O uptake is substantially increased in H_2O_2 soaked *Pseudotsuga menziesii* seeds than in the control, suggesting an enhanced conversion rate of reserve lipids to carbohydrates and, consequently, increased synthesis of cellular components (Ching, 1959). In addition, it is possible that the oxidation of germination inhibitors present in pericarp by H_2O_2 promotes seed germination (Ogawa and Iwabuchi, 2001).

Our results showed that H_2O_2 pretreatment induces a substantial increase in CAT and APX activities in seeds (Figure 2). Recent studies trying to understand antioxidative system function in germination showed a substantial increase in CAT and SOD activities of maize seeds, as a result of the ROS-induced antioxidant gene expression (Mylona et al., 2007). In *Chenopodium rubrum*, the highest CAT and SOD activities were found prior to radicle protrusion, while GPX activity was maximal at the time of radicle protrusion and seedling development (Ducic et al., 2003). In wheat, increased antioxidative enzyme activity was observed during germination, suggesting that germination is associated with enhanced cellular capacity to detoxify H_2O_2 (Cakmak et al., 1993).

SOD activity in H_2O_2 soaked seeds at 24 h was lower than in control seeds (Figure 2D). SOD catalyzes the dismutation of superoxide into hydrogen peroxide and molecular oxygen. Its activity modulates the relative amounts of $^{\bullet}O_2^-$ and H_2O_2, the two Haber–Weiss reaction substrates and reduces the risk of

•OH formation (Azevedo Neto et al., 2008). SOD isozymes present different sensitivity to KCN and H_2O_2 inhibitors. While Mn-SOD is resistant to these inhibitors, Cu/Zn-SOD is sensitive to both of them, and Fe-SOD is resistant to KCN and sensitive to H_2O_2 (McKersie and Leshem, 1994). Therefore, it is possible that, at 24 h, the excess of H_2O_2 in the soaking solution (100 mM), combined with low CAT activity, might have induced the decrease in SOD activity.

Data of CAT activity staining gel show only one prominent isoform, which was clearly stimulated by H_2O_2 (Figure 3). The results also show an increase in isoform intensity in H_2O_2 soaked seeds at 36 and 48 h. In maize seeds treated with ROS-generating xenobiotics, two CAT isoforms were found, and intensity was increased with the elevated xenobiotic concentration (Mylona et al., 2007).

Plant growth (SDM, RDM, TDM and LA) was inhibited by salinity (Figure 4). However, pretreatment with H_2O_2 reduced the deleterious effects of salt stress on the growth of seedlings. Similarly, Hu et al. (2009) showed that plant growth was significantly repressed by cadmium exposure. However, pretreatment with 100 μM H_2O_2 for 1d mitigated cadmium stress by inducing antioxidative enzyme activities. Our data suggest that soaking seeds in 100 mM H_2O_2 solution for 36 h before germination in salinity conditions led to a salt stress acclimation process. Although acclimation is considered a complex phenomenon, our results indicated that, while salt stress can be detrimental to plants originated from seeds pretreated with water, the previous application of mild oxidative stress in seeds could lead to reduced deleterious effects of salinity on plant growth.

SOD is the major $^{\bullet}O_2^-$ scavenger and plays a key role in cellular defense mechanisms against ROS (Van Breusegem et al., 2001). Our study showed that leaf and root SOD activities in H_2O_2/salt-stressed plants was higher than in water/salt-stressed plants (FIGURES 5 and 6), suggesting that plants originating from H_2O_2 pretreated seeds had better $^{\bullet}O_2^-$ radical scavenging ability.

SOD initiates detoxification of $^{\bullet}O_2^-$ by forming H_2O_2, which is also toxic and must be eliminated by converting into H_2O in subsequent reactions (Azevedo Neto et al., 2005). In plants, H_2O_2 is scavenged by a number of enzymes, but catalases and peroxidases are considered the major H_2O_2 detoxifying enzymes in plants (Vaidyanathan et al., 2003). Salt stress significantly reduced CAT activity in water/salt-stressed plants, but this effect was not observed in H_2O_2/salt-stressed plants. Therefore, it could be assumed that the reduced deleterious effects of salt in H_2O_2/salt-stressed plants can be attributed, at least in part, to higher CAT activity in these plants.

GPX activity data in leaves (Figure 5) suggest that the enzyme activity was differently induced by H_2O_2 pretreatment and salt stress throughout the experimental period. Therefore, at day 6, leaf GPX activity seemed to result from H_2O_2-induced acclimation, while high leaf GPX activity, at day 11, seemed to be induced by salt stress. On the other hand, enhanced leaf GPX activity, at day 16, seemed to be correlated with both salt stress and H_2O_2-induced acclimation.

APX is an important enzyme of the antioxidative system that uses ascorbate as the electron donor for the reduction of H_2O_2 to water (Asada, 1992). Some studies on the response of APX expression to some stress conditions and pathogen attacks have highlighted the importance of APX activity in controlling H_2O_2 concentration in intracellular signaling (Shigeoka et al., 2002). Additionally, studies on the regulation of APX gene expression through H_2O_2 have also been made. The treatment of cultured soybean cells with exogenous H_2O_2 resulted in alteration to cytosolic APX transcription levels (Lee et al., 1999). This study supports the idea that cytosolic APX gene expression is up-regulated in response to cellular H_2O_2 levels (Shigeoka et al., 2002). Our results show that, in the short term, H_2O_2 pretreatment of seeds did not induce APX activity. However, at the end of the experimental period, H_2O_2 pretreatment increased APX activity in leaves and roots of both H_2O_2/control and H_2O_2/salt-stressed plants (FIGURES 5 and 6).

In summary, our study concluded that 100 mM H_2O_2 are enough to accelerate germination of maize seeds and this effect may be related, at least in part, to increased CAT and APX activities, which were the main H_2O_2-scavenging enzyme in seeds. In addition, differences in antioxidative enzyme activity may explain the increased tolerance to salt stress of plants originated from H_2O_2 pretreated seeds.

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