EFFECTS OF H_2O_2 PRIMING IN CHLOROPLASTS ULTRASTRUCTURE OF MAIZE PLANTS UNDER SALINITY

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ABSTRACT: Priming with low concentrations of H_2O_2 has emerged as an important strategy to activate multiple acclimation responses that reinforce tolerance to abiotic stresses, including salt stress. This study aimed to investigate the role of H_2O_2 priming in the accumulation of reactive oxygen species (ROS) and its correlation with the structural chloroplasts integrity of maize plants under salinity. Maize plants cv. BR 5011 were sprayed with distilled water or 10 mM H_2O_2 solution and then subjected to salt treatments (0 and 80 mM NaCl). The experiments were carried in a randomized completely design, in a 2 x 2 factorial scheme, consisting of two growth conditions (absence or presence of salt stress) and two priming conditions with H_2O_2 (non-primed or H_2O_2-primed), with five replicates. The results suggest that H_2O_2 priming reduces salt damage effects by decreases ROS contents, as well as maintaining the structural integrity of chloroplasts.

KEYWORDS: Acclimation, salt stress, *Zea mays*

EFEITOS DO PRÉ-TRATAMENTO COM H_2O_2 NA ULTRAESTRUTURA DE CLOROPLASTOS DE PLANTAS DE MILHO SOB SALINIDADE

RESUMO: O pré-tratamento com baixas concentrações de H_2O_2 tem se mostrado uma importante estratégia para ativar múltiplas respostas de aclimatação, que atuam na tolerância à estresses abióticos, incluindo o estresse salino. O presente estudo teve como objetivo averiguar o papel do pré-tratamento com H_2O_2 no acúmulo de EROs e relacioná-las com a

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integridade estrutural dos cloroplastos de plantas de milho sob salinidade. Plantas de milho cv. BR 5011 foram pulverizadas com água ou solução de H_2O_2 a 10 mM e, em seguida, submetidas aos tratamentos salinos (0 e 80 mM de NaCl). Os experimentos foram realizados em um delineamento inteiramente casualizado, com arranjo fatorial 2 x 2, consistindo de duas condições de crescimento (ausência ou presença de estresse salino) e duas condições de pré-tratamento com H_2O_2 (ausência ou presença do pré-tratamento com H_2O_2), com cinco repetições. Os resultados obtidos sugerem que o pré-tratamento com H_2O_2 reduz os efeitos negativos da salinidade através de decréscimos nos teores de EROs, bem como na manutenção na integridade da ultraestrutura dos cloroplastos.

PALAVRAS-CHAVE: Aclimatação, estresse salino, *Zea mays*

INTRODUCTION

Salinity is one of the major abiotic stress restricting crop yield by impairing physiological, morphologic, biochemical and molecular processes in plants (Shavrukov, 2013), that causes ion toxicity and osmotic stress, leading to oxidative stress and a series of secondary stresses. The salt harmful effects directly redox homeostasis and altered photosynthetic machinery performance, such as chloroplasts ultrastructure; therefore, this phenomenon has led to research into salt tolerance with the aim of improving crop plants (Liang *et al*., 2018). An interesting alternative to induce the acclimatization to abiotic stress is the priming plants with organic, inorganic or growth-regulating compounds, which may be applied in the growth medium of roots or by spraying in leaves (González-Bosch *et al*., 2018). Recent studies have demonstrated that exogenous applications of H_2O_2 can confer tolerance to environmental stresses by modulating various physiological processes associated with higher antioxidant enzyme activities, as well as through the protection of cellular organelles indirectly (Hossain *et al*., 2015). Therefore, this study aimed to investigate the role of H_2O_2 priming on accumulating of reactive oxygen species (ROS) and structural integrity of chloroplasts of maize plants subjected to NaCl-stress.

MATERIAL AND METHODS

Seven days after sowing, maize seedlings (*Zea mays* L.) cv. BR 5011 (salt-sensitive), were transferred to a hydroponic system containing Hoagland's nutrient solution for five days, for an acclimation period. Then, the seedlings were sprayed with distilled water (control) or 10 mM H_2O_2 solution (priming). Forty-eight hours after priming beginning, the addition of NaCl was applied (80 mM NaCl). In each group of H_2O_2-primed plants, half of them remained in the absence of NaCl, thus constituting the control treatment (0 mM NaCl). The experiment was carried out under greenhouse conditions with the main temperature of 32.3 ± 6 °C, and the main relative humidity of 44.8 ± 10.9 %. The plants were harvested twelve days after the last salt dosage for the following analyses: H_2O_2 and $^{\bullet}O_2^-$ contents and chloroplasts ultrastructure. The determination of $^{\bullet}O_2^-$ contents was performed according to Klein *et al*. (2018) and H_2O_2 contents were determined by Fernando & Soysa (2015). The data were expressed in nmol.g^{-1} FM (fresh mass). The ultrastructure of chloroplasts was obtained by transmission electron microscope, according to Yamane *et al*. (2012). The experimental design was completely randomized following a 2 × 2 factorial scheme, composed of two growth conditions [with 80 mM NaCl(salt stress) and without NaCl (control)] and two priming treatments (non-primed or H_2O_2-primed). All analyses were performed using five repetitions per treatment. The data were submitted to two-way analysis of variance (ANOVA) and the main values were compared through Tukey's test ($p \leq 0.05$) using Sisvar® 5.3 program.

RESULTS AND DISCUSSION

In general, salt stress significantly increased the ROS of maize plants; however, the H_2O_2 priming was effective in minimizing the damage of salinity. Under control conditions, the H_2O_2 priming increased the leaf $^{\bullet}O_2^-$ content. Salt stress also caused a significant increase these contents; however, the H_2O_2-priming under salinity reduced the negative effect of NaCl as compared to non-primed plants (Figure 1a).

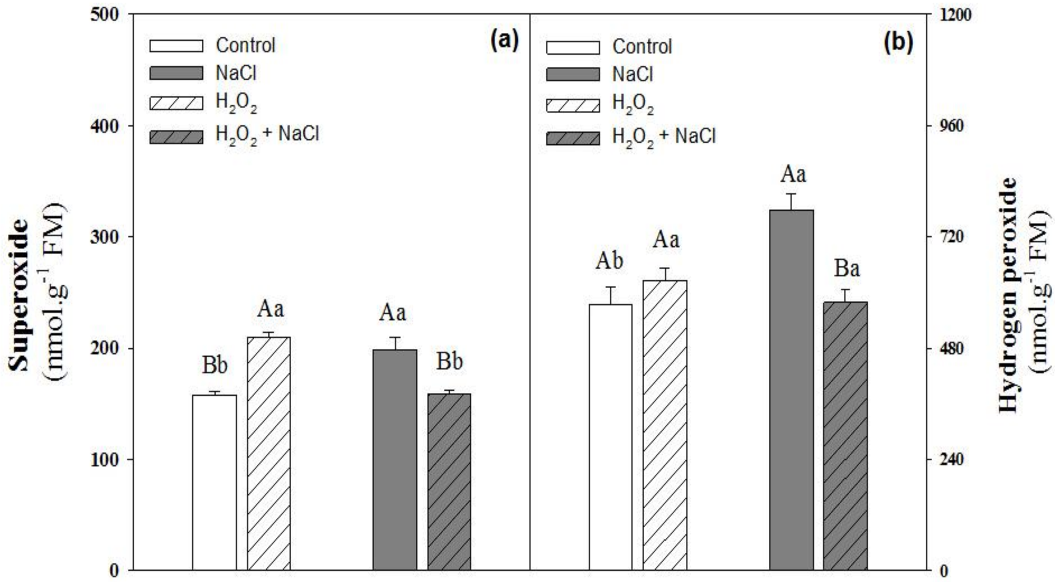

Figure 1. Superoxide (**a**) and hydrogen peroxide (**b**) in leaves of maize var. 5011 at twelve days after salt treatments. Values represent the means of five repetitions + standard error. Means followed by the same lowercase letters at the same H_2O_2 pre-treatment, or by equal capital letters in the same salinity level, are not statistically different ($p \leq 0.05$).

Under salinity, H_2O_2-primed plants displayed values of leaf H_2O_2 content higher than those of non-primed plants (Figure 1b). Some studies also observed evidence that H_2O_2 priming may act as an inducer of abiotic stress tolerance. Sathiyaraj *et al*. (2014) found that H_2O_2-primed *Panax ginseng* seedling decreased the concentration of both endogenous H_2O_2 and $^{\bullet}O_2^-$ which represents less damage to the cells and it occurs due to increases in ROS scavengers enzymes. Similarly, Hasanuzzaman *et al*. (2017) observed that H_2O_2-primed seedlings of *Brassica napus* resulted in decreasing the endogenous contents of ROS and in decreasing the oxidative damage under abiotic stress. Analysis of chloroplast ultrastructure has been useful with a reliable stress marker during abiotic stress (Zechmann *et al*., 2019). In our study, salinity caused notably disorganization on chloroplasts and H_2O_2 priming alleviated the structural changes induced by salt stress (Figures. 2 and 3).

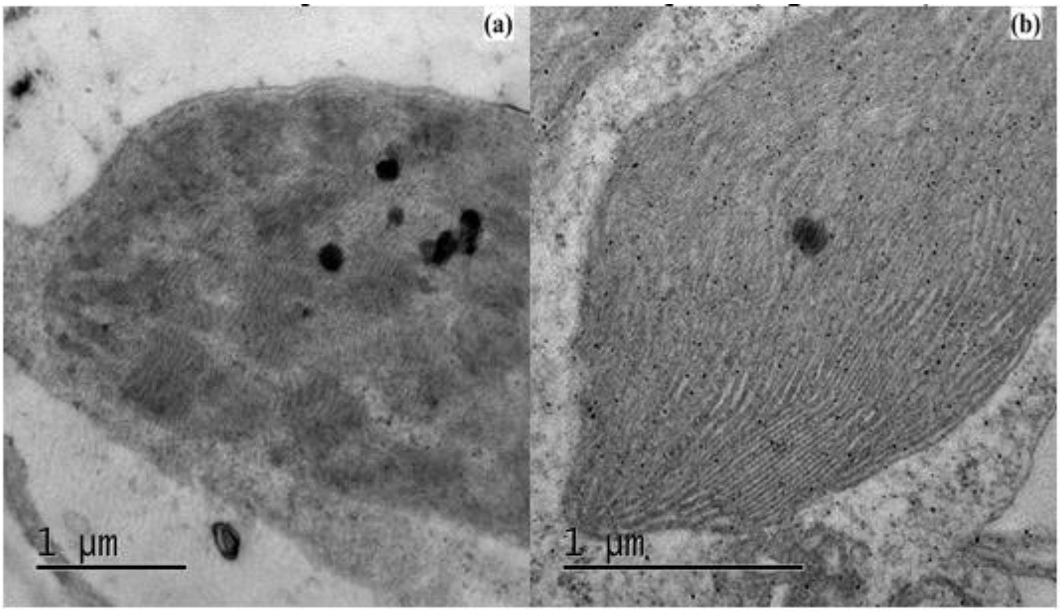

Figure 2. Chloroplast ultrastructure in leaves of maize plants under (a) control conditions and (b) H_2O_2-primed. Measurements were done twelve days after salt treatments in the transmission electron microscopy.

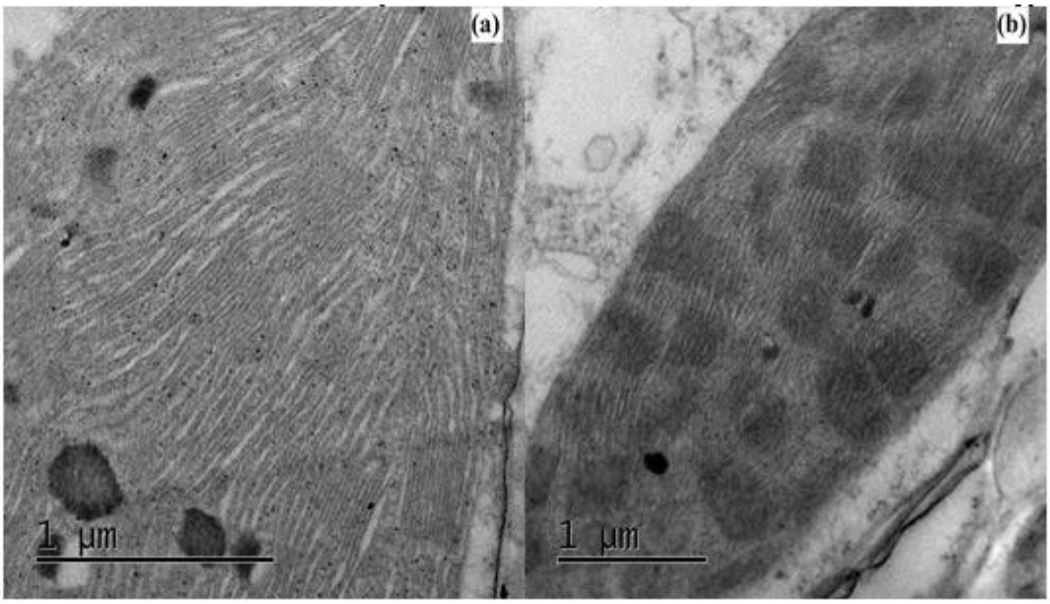

Figure 3. Chloroplast ultrastructure in leaves of maize plants under (a) salt stress and (b) H_2O_2-primed under salinity. Measurements were done twelve days after salt treatments in the transmission electron microscopy.

The ultrastructure of chloroplasts from maize plants, under control conditions, were characterized by well-stacked thylakoids and well-developed granal. In addition, the effect of priming H_2O_2 subtly altered in the structure of chloroplasts. However, under salinity, was observed a structural damage in chloroplasts, such as rupturing stacking and rippling in thylakoid membranes with reducing stacking granal. In contrast, in primed H_2O_2 plants, under salinity, it was similar to that in control treatment. Similarly, Cao *et al*. (2015) observed that silicon priming protected the structure of the chloroplast in tomato leaves (*Solanum* *lycopersicum* L.) from severe oxidative damage, such as the distortion of the grana lamellae. Recently, Shen *et al*. (2019) reported that exogenous putrescine could stabilize the structure of the thylakoid membrane of cucumber plants (*Cucumis sativus L*) and alleviate the damage to the structure of photosynthetic organs caused by salt stress.

CONCLUSIONS

Our findings suggest that the H_2O_2 priming alleviates the negative effects of salinity by reducing of ROS overproduction and maintain the ultrastructure integrity of chloroplasts of maize plants under salt stress.

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