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Research article

Catalase plays a key role in salt stress acclimation induced by hydrogen peroxide pretreatment in maize

Franklin Aragão Gondim [1], Enéas Gomes-Filho*, José Hélio Costa, Nara Lídia Mendes Alencar, José Tarquinio Prisco

Departamento de Bioquímica e Biologia Molecular and Instituto Nacional de Ciência e Tecnologia em Salinidade (INCTSal)/CNPq, Universidade Federal do Ceará, Caixa Postal 6039, 60440-970 Fortaleza, Ceará, Brazil

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ABSTRACT

Pretreatment in plants is recognized as a valuable strategy to stimulate plant defenses, leading to better plant development. This study evaluated the effects of H_2O_2 leaf spraying pretreatment on plant growth and investigated the antioxidative mechanisms involved in the response of maize plants to salt stress. It was found that salinity reduced maize seedling growth when compared to control conditions, and H_2O_2 foliar spraying was effective in minimizing this effect. Analysis of the antioxidative enzymes catalase (EC 1.11.1.6), guaiacol peroxidase (EC 1.11.1.7), ascorbate peroxidase (EC 1.11.1.1) and superoxide dismutase (EC 1.15.1.1) revealed that H_2O_2 spraying increased antioxidant enzyme activities. Catalase (CAT) was the most responsive of these enzymes to H_2O_2, with higher activity early (48 h) in the treatment, while guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) were responsive only at later stages (240 h) of treatment. Increased CAT activity appears linked to gene expression regulation. Lower malondialdehyde levels were detected in plants with higher CAT activity, which may result from the protective function of this enzyme. Overall, we can conclude that pretreatment with H_2O_2 leaf spraying was able to reduce the deleterious effects of salinity on seedling growth and lipid peroxidation. These responses could be attributed to the ability of H_2O_2 to induce antioxidant defenses, especially CAT activity.

1. Introduction

Salinity is a major abiotic stress that affects plant growth and limits crop productivity worldwide [1] and occurs on both irrigated and non-irrigated lands. High salt concentrations cause ion imbalance and hyperosmotic stress in plants [2]. The problem has been aggravated by agricultural practices such as irrigation that can cause water tables to rise and concentrate salts in the root zone [3]. Poor quality water for irrigation and poor drainage are the main reasons for increases in soil salinity in irrigated areas [4].

Reactive oxygen species (ROS) production is a normal biochemical event that occurs in plants. The ROS are generated during normal cellular metabolism, but there is evidence that ROS production is increased when plants are subjected to biotic or abiotic stresses [5]. The main ROS production sites are the chloroplasts, mitochondria and peroxisomes [6]. The most commonly produced ROS are singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and superoxide ($^\bullet O_2^-$) and hydroxyl ($^\bullet OH$) radicals [7,8]. H_2O_2 can also be produced in the cytosol, plasma membrane and extracellular matrix by various oxidases and cell wall peroxidases [9]. ROS, when in excess, can have detrimental effects on plant metabolism, causing oxidative damage to proteins, nucleic acids and lipids essential to membrane structure [5,10].

The harmful effects of ROS in plants can be reduced or eliminated by non-enzymatic and enzymatic defense systems [8]. The non-enzymatic system includes hydrophilic compounds (such as ascorbate and reduced glutathione) and lipophilic compounds (such as tocopherols and carotenoids) capable of quenching ROS [8]. The enzymatic defense system consists of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and other enzymes of the ascorbate–glutathione cycle [3,8,11].

The antioxidant enzyme system is recognized as the main mechanism of plant tolerance to environmental stress [12]. Many

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; DM, dry mass; FM, fresh mass; GPX, guaiacol peroxidase; HAS, hours after spraying; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; RDM, root dry mass; ROS, reactive oxygen species; RT-PCR, reverse transcription and polymerase chain reaction; SDM, shoot dry mass; SOD, superoxide dismutase.

* Corresponding author. Tel.: +55 85 3366 9405; fax: +55 85 3366 9829.
E-mail address: egomesf@ufc.br (E. Gomes-Filho).
[1] Instituto Federal de Educação, Ciência e Tecnologia do Ceará, Av. Contorno Norte, 10 Parque Industrial, CEP: 61925-315, Maracanaú, Ceará, Brazil.

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studies have shown a correlation between salt tolerance and efficient antioxidant systems in tolerant genotypes ([13], in *Triticum aestivum* L. [1,14], in *Oriza sativa* L. [12], in *Beta vulgaris*; and [15], in *Zea mays*). Moreover, even in sensitive plants, salinity tolerance has been achieved by pretreatment with H_2O_2, as demonstrated in monocotyledonous species such as rice [16] and maize [17]. In both studies, H_2O_2, applied to the root system, was capable of stimulating the antioxidant enzyme system.

Studies have shown that ROS, especially H_2O_2, play an important role as signaling molecules and are produced and controlled by metabolism, being beneficial at low concentrations and harmful when produced in excess [18,19]. Experiments conducted at low H_2O_2 cell concentrations in which the plant is challenged by an environmental change have shown that H_2O_2 influences plant growth and development [19].

There are few studies that examine spraying leaves with H_2O_2. In *Nicotiana tabacum* plants, increased tolerance to oxidative stress generated by high light intensities or catalase inhibitor (aminotriazole) was observed by spraying them with H_2O_2 [20]. In addition, it was proposed that H_2O_2 foliar application contributed to increased antioxidant enzyme activity, decreased lipid peroxidation and chloroplast ultrastructure protection in *Cucumis sativus* [21]. Other strategies of H_2O_2 application have been used such as in seeds [22] and in root system [16,17], however these methods appear less suitable than leaf spraying. In seeds, H_2O_2 would be applied a single time before germination, whilst application to the root system requires the plants to be grown hydroponically and thus is not suitable for plants cultivated in soil. In this context, leaf spraying is a simpler system of H_2O_2 application, useful in different stages of plant development, and is inexpensive and easy to use by farmers. Therefore, the hypothesis of this study is that H_2O_2 leaf spray is an effective method to induce the antioxidative enzymatic system and minimizes the deleterious effects of salinity on maize plant growth.

In order to gain insight on H_2O_2 leaf spraying and its relationship with salt tolerance, the aim of this study was to evaluate the effects of H_2O_2 leaf spraying on growth, antioxidative enzyme activities and lipid peroxidation in maize plants under salt stress.

2. Results

2.1. Effect of H_2O_2 leaf spraying on plant growth under salt stress

The effect of 10 mM H_2O_2 leaf spraying on plant growth under salt stress was investigated through shoot and root dry mass analyses in the following plant treatments: water/control, H_2O_2/control, water/salt-stressed and H_2O_2/salt-stressed (Figs. 1 and 2). In shoot dry mass (SDM) analyses (Fig. 1A), at 48 h after spraying (HAS), the SDM in the H_2O_2/control plants was *ca.* 23% higher than in the water/control plants. At 96 HAS no significant differences were observed in SDM between treatments. However, at 240 HAS, SDM was *ca.* 21% higher in the H_2O_2/control plants compared to the water/control plants, while in the water/salt-stressed and H_2O_2/salt-stressed plants, the SDM was *ca.* 61% and 31% lower than that of the water/control plants, respectively. At 240 HAS, SDM in H_2O_2/salt-stressed plants was *ca.* 22% higher than in water/salt-stressed plants. In the root dry mass (RDM) (Fig. 1B), it was found that major differences also occurred at 240 HAS, but a peculiar behavior, compared to SDM, was observed. The RDM was similar in the H_2O_2/control, water/control and H_2O_2/salt-stressed plants, but it was *ca.* 36% lower in the water/salt-stressed plants in relation to the water/control plants. Moreover, at 240 HAS, RDM in H_2O_2/salt-stressed plants was *ca.* 69% higher than in water/salt-stressed plants.

Fig. 2 shows photographs of plants at 240 HAS. The water/salt-stressed plants presented reduced growth compared to the

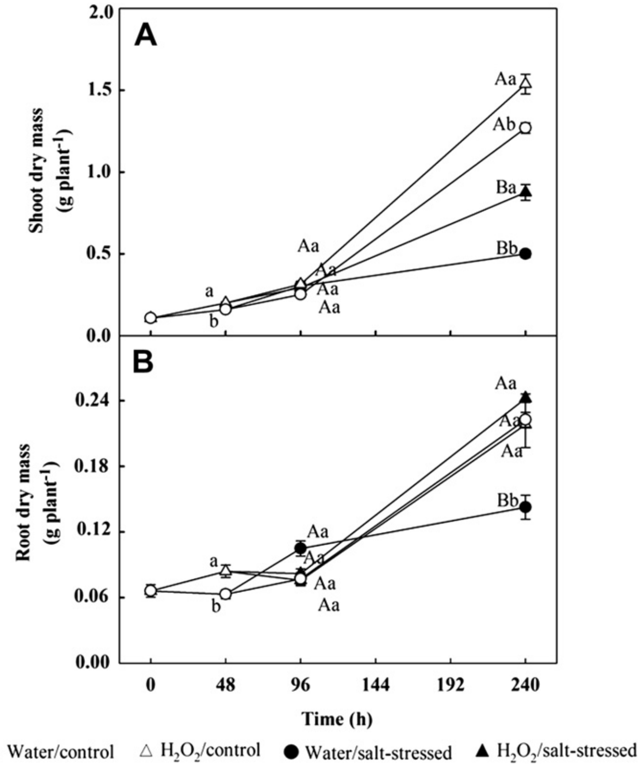

Fig. 1. Shoot (A) and root (B) dry masses of maize plants at 0 (initial time of plant harvest – just before spraying), 48, 96 and 240 h after leaf spraying. The plants were submitted to four treatments: 1. sprayed with distilled water and not salt-stressed (water/control), 2. sprayed with H_2O_2 solution and not salt-stressed (H_2O_2/control), 3. sprayed with distilled water and salt-stressed (water/salt-stressed), and 4. sprayed with H_2O_2 solution and salt-stressed (H_2O_2/salt-stressed). The leaf spraying was carried out after the first harvest (0 h) and repeated after 24 h. Salt was added at 48 and 72 h after spraying (HAS). Each value corresponds to the mean ± standard error of five replicates. 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 ($P \leq 0.05$).

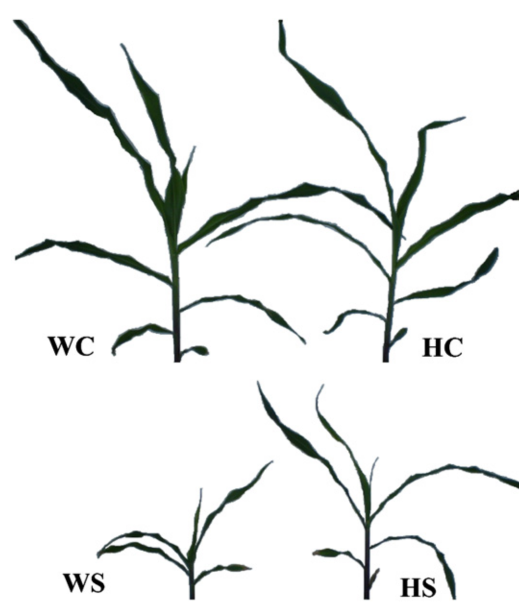

Fig. 2. Maize plants under different treatments at 240 h after leaf spraying: water/control plants (WC); H_2O_2/control plants (HC); water/salt-stressed plants (WS); and H_2O_2/salt-stressed plants (HS).