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Effect of NaCl stress on H_2O_2 metabolism in rice leaves

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Abstract

The effect of NaCl stress on H_2O_2 metabolism in detached rice leaves was studied. NaCl (200 mM) treatment did not cause the accumulation of H_2O_2 and resulted in no increase in lipid peroxidation and membrane leakage of leaf tissues. The activities of peroxidase, ascorbate peroxidase, superoxide dismutase, and glutathione reductase were observed to be greater in NaCl-stressed rice leaves than in control leaves. However, glycolate oxidase was lower in NaCl-treated rice leaves than in the control leaves. There was no difference in catalase activity between NaCl and control treatments. These results suggest that some antioxidant enzymes can be activated in response to oxidative stress induced by NaCl.

Abbreviations: APOD – ascorbate peroxidase; CAT – catalase; GO – glycolate oxidase; GR – glutathione reductase; MDA – malondialdehyde; POD – peroxidase; SOD – superoxide dismutase

1. Introduction

Hydrogen-peroxide (H_2O_2) is a constituent of oxidative metabolism. It is a product of peroxisomal and chloroplastic oxidative reactions [8]. H_2O_2 itself is an active oxygen species. H_2O_2 and superoxide can inactivate various macromolecules directly, but it is their conversion to hydroxyl radicals by transition metals (i.e. the Haber-Weiss reaction) that accounts for their main toxicity [17]. Hydroxyl radicals reacts with proteins, lipids, and DNA, causing cell damage [3, 13].

The increase of active oxygen species seems to occur as a response to most, if not all, abiotic stresses including drought [33], salt [10–12, 15–16, 26, 31–32], extreme temperatures [23, 29], nutrient deficiency [18] and air pollution [4]. Plants possess a number of antioxidant molecules and enzymes that protect against oxidative damage. SOD catalyzes the dismutation of two molecules of superoxide into oxygen and H_2O_2. In the ascorbate-glutathione cycle, the enzymatic action of ascorbate peroxidase (APOD) reduces H_2O_2 using ascorbate as an electron donor [2]. Oxidized ascorbate is then reduced by reduced glutathione, generated from oxidized glutathione by glutathione reductase (GR) at the expense of NADPH [34]. Catalase (CAT) and peroxidase (POD) are implicated in removal of H_2O_2. The study of the role of the antioxidant system in salt stress is relatively recent. The results of most of the studies suggest that the resistance to salt stress is usually correlated with a more efficient antioxidative system [10–12, 26, 31–32]. The present study was conducted to examine the effect of NaCl on the activities of enzymes related to H_2O_2 metabolism in detached rice leaves.

2. Materials and methods

Rice (*Oryza sativa* L., cv. Taichung Native 1) was cultured as previously described [20]. The apical 3-cm segments excised from the third leaves of 12-day-old seedlings were used. A group of 10 segments was

floated in a Petri dish containing 10 mL of test solutions. Each treatment was replicated 4 times. Incubation was carried out at 27 °C in the light (40 μmol $m^{-2}s^{-1}$).

The H_2O_2 level was measured colorimetrically as described by Jana and Choudhuri [19]. H_2O_2 was extracted by homogenizing 50 mg leaf tissue with 3 mL of phosphate buffer (50 mM, pH 6.5). The homogenate was centrifuged at 6,000 *g* for 25 min. To determine H_2O_2 levels, 3 mL of extracted solution was mixed with 1 mL of 0.1% titanium sulphate in 20% (v/v) H_2SO_4 and the mixture was then centrifuged at 6,000 *g* for 15 min. The intensity of the yellow colour of the supernatant was measured at 410 nm. H_2O_2 level was calculated using the extinction coefficient 0.28 μmol^{-1}cm^{-1} and was expressed as μmol g^{-1} initial fresh weight.

Malondialdehyde (MDA) was extracted with 5% (w/v) trichloroacetic acid and determined according to Heath and Packer [14]. MDA level is routinely used as an index of lipid peroxidation and was expressed as nmol g^{-1} initial fresh weight. Conductivity of the medium containing leaf segments was determined directly using a conductivity meter (Suntex conductivity meter, Taipei, Taiwan). The conductivity of the medium containing no leaf segments was used for correction.

For extraction of enzymes, leaf tissues were homogenized with 0.1 M sodium phosphate buffer (pH 6.8) in a chilled pestle and mortar. The homogenate was centrifuged at 12,000 *g* for 20 min and the resulting supernatant was used for determination of enzyme activity. The whole extraction procedure was carried out at 4 °C. GO was determined according to the method of Brennan et al. [7]. CAT activity was assayed by measuring the initial rate of disappearance of H_2O_2 [21]. The decrease in H_2O_2 was followed as the decline in optical density at 240 nm, and activity was calculated using the extinction coefficient (40 mM^{-1}cm^{-1} at 240 nm) for H_2O_2 [21]. POD activity was measured using a modification of the procedure of MacAdam et al. [23]. Activity was calculated using the extinction coefficient (22.6 mM^{-1}cm^{-1} at 470 nm) for tetraguaiacol. SOD was determined according to Paoletti et al. [27]. APOD was determined according to Nakano and Asada [25]. The decrease in ascorbate concentration was followed as a decline in optical density at 290 nm and activity was calculated using the extinction coefficient (2.8 mM^{-1}cm^{-1} at 290 nm) for ascorbate. GR was determined by the method of Foster and Hess [9]. Enzyme activity was expressed on the basis of mg protein. The method of Bradford [6] was used to determine protein content.

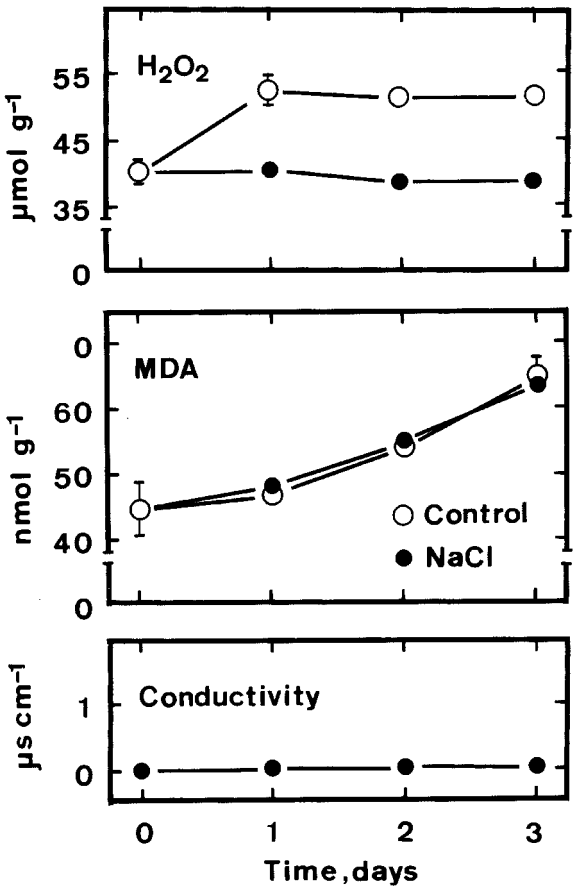

Figure 1. Changes in conductivity in the medium and levels of H_2O_2 and MDA in detached rice leaves incubated in 5 mM sodium phosphate buffer at pH 7.0 (○) or in 200 mM NaCl dissolved in the buffer (●). Vertical bars represents standard errors (n = 4).

3. Results and discussion

Singha and Choudhuri [32] proposed that H_2O_2 could play an important role in the mechanism of salt injury and the same has also been proposed for drought-stress damage [24]. The accumulation of H_2O_2 due to salt stress has been reported in *Vigna catjang*, rice and pea plants [16]. To see if H_2O_2 is important in regulating salt injury in our detached rice leaf system, we first determined the changes of H_2O_2 level under NaCl stress condition. The results are shown in Figure 1. The H_2O_2 level remained unchanged during 3 days of NaCl (200 mM) incubation. However, there was

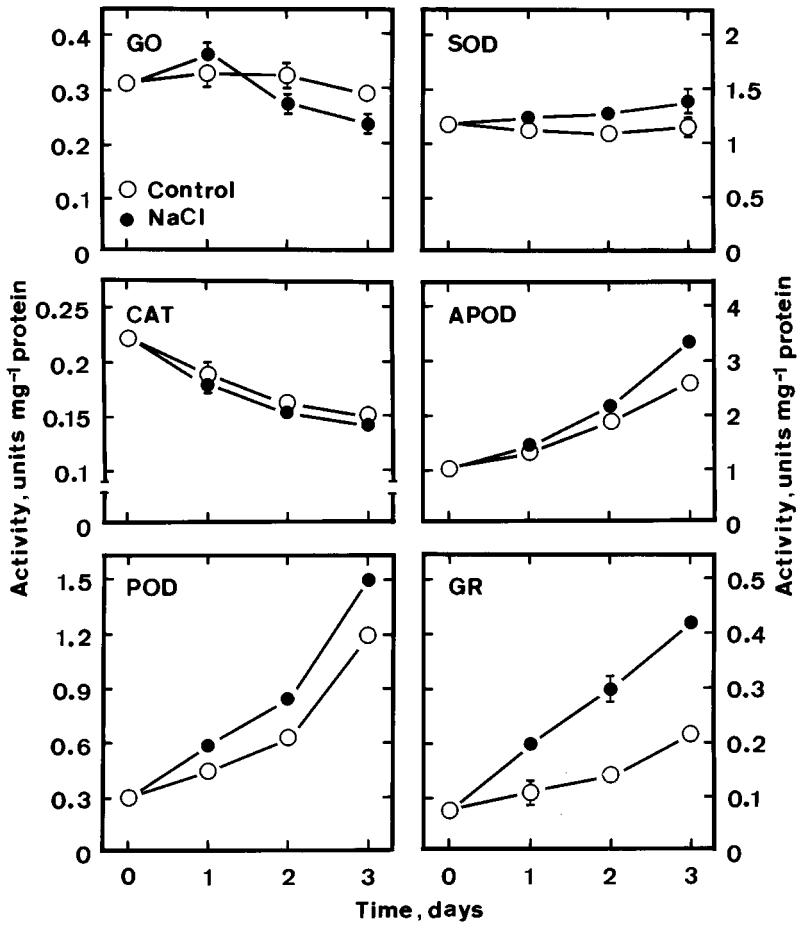

Figure 2. Changes in activities of GO, CAT, POD, SOD, APOD and GR in detached rice leaves incubated in 5 mM sodium phosphate buffer at pH 7.0 (○) or in 200 mM NaCl dissolved in the buffer (●). Vertical bars represents standard errors (n = 4).

an accumulation of H_2O_2 level in control leaves. It is clear that the H_2O_2 level did not increase in detached rice leaves treated with NaCl. Figure 1 also demonstrated that NaCl treatment resulted in no increase in lipid peroxidation, measured as MDA content, and membrane leakage of leaf tissue.

Figure 2 shows the effect of NaCl on the activities of enzymes related to H_2O_2 metabolism. The activity of GO, the enzyme which catalyzes the synthesis of H_2O_2 in the peroxisome, remained unchanged in control leaves. NaCl treatment resulted in a lower activity as compared with the control leaves. However, there was no difference in the activity of CAT, the enzyme responsible for eliminating H_2O_2 in control and treated leaves. POD, APOD, and GR activities increased significantly in detached rice leaves in the presence or absence of NaCl. However, the effect was greater in the presence of NaCl than in the control. Figure 2 also shows that SOD activity was found to be higher in NaCl-treated leaves than in the control leaves.

It has been reported that SOD and POD or APOD activities may have an important role in the mechanism of salt tolerance of pea cells and leaves [15, 16, 26]. The results of Gossett et al. [10–12] suggest that protection from oxidative damage by a more reactive ascorbate-glutathione cycle and a higher level of antioxidants like CAT, POD and α-tocopherol are involved in the development of salt tolerance in cotton plants. In the present investigation, we were able show that POD, APOD, SOD and GR were higher and GO was lower in NaCl-stressed leaves than the control leaves. This would explain why NaCl did not result in the accumulation of H_2O_2 and there was no increase in lipid peroxidation and membrane leakage of leaf tissues.

Table 1. Effect of NaCl pretreatment on levels of protein in detached rice leaves treated with paraquat

Pretreatment	Treatment	Protein level, mg g^{-1} fresh weight
Control	H_2O	46.50 ± 0.60
	Paraquat	3.97 ± 0.87
NaCl	H_2O	41.79 ± 1.22
	Paraquat	17.62 ± 2.06

Ten detached rice were pretreated with 5 mM sodium phosphate buffer at pH 7.0 (control) or 200 mM NaCl dissolved in the buffer for 2 days and then transferred to either water or paraquat (10 μM) for 24 h in the light. Each treatment (10 detached rice leaves) was replicated 4 times.

It has been demonstrated that transgenic plants overexpressing SOD, APOD, and GR had increased resistance to paraquat-mediated oxidative stress [1, 5, 28, 30]. Thus, we were interested to study whether NaCl-treated detached rice leaves were more resistant to paraquat than the control leaves. To test this, detached rice leaves were pretreated with either sodium phosphate buffer (pH 7.0) or NaCl dissolved in the buffer for 2 days and then transferred to either water or paraquat (10 μM) for 24 h in the light. It is evident that NaCl-pretreatment reduced the toxicity of paraquat, judged by the changes in protein levels (Table 1).

Acknowledgements

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