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BRIEF COMMUNICATION

Involvement of lipid peroxidation in water stress-promoted senescence of detached rice leaves

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Abstract

Role of lipid peroxidation and antioxidative enzymes (catalase, peroxidase, superoxide dismutase, ascorbate peroxidase and glutathione reductase) in water stress-promoted senescence of detached rice leaves was investigated. The senescence was followed by measuring the decrease in protein content. Increased lipid peroxidation was closely correlated with senescence in water stressed leaves. Decrease in superoxide dismutase activity was evident 8 h after beginning of water stress. However, decreased catalase, peroxidase, and ascorbate peroxidase activity was observed only when senescence was observerd. Glutathione reductase was not affected by water stress. Free radical scavengers retarded water stress-enhanced senescence.

Additional key words: antioxidative enzymes, *Oryza sativa*.

Lipid peroxidation is considered to be an important mechanism of leaf senescence (Dhindsa *et al.* 1981, Kunnert and Ederer 1985, Strother 1988, Thompson *et al.* 1987). Free radicals can initiate lipid peroxidation (Kellogg and Fridovich 1975). Water stress is known to promote leaf senescence (Chen and Kao 1990, Dwivedi *et al.* 1979, Lin and Kao 1998, Mukherjee and Choudhuri 1981). During water stress leaf stomata close limiting water loss and the influx of CO_2. Lowered CO_2 influx leads to a decrease in carbon reduction by the Calvin cycle and to a decrease in oxidized $NADP^+$ to serve as an electron-acceptor in photosynthesis. As a result, electrons flow to the alternative electron acceptor, O_2, producing superoxide radical and consequently other reactive oxygen species, the most damaging of which is the hydroxyl radical (Scandalios 1993). Recently, Sairam *et al.* (1998) reported that drought imposed at two stages after anthesis of wheat resulted in an increase in lipid peroxidation. It appears that lipid peroxidation may also be associated with water stress-promoted senescence of leaves. In this study, the possible involvement of lipid peroxidation in the regulation of water stress-promoted senescence of detached rice leaves was investigated.

Rice (*Oryza sativa* cv. Taichung Native 1) was cultured on a stainless net floating on half-strength Johnson's modified nutrient solution (pH 4.2) in a 500-cm^3 beaker (Kao 1980). The nutrient solution was replaced every three days. Rice plants were grown for 12 d in a greenhouse, under natural light and the day/night temperature of 30/25 °C. The apical 3 cm of the third leaf of 12-d-old seedling was used for the experiment. A group of 10 segments floated in a Petri dish containing 10 cm^3 of distilled water served as controls. For induction of mild water stress, leaf segments were exposed to the vapour above a solution of 0.5 M NaCl to decrease air humidity to 98.5 % (Chen and Kao 1990). All samples were kept at temperature at 27 °C and irradiance of 40 μmol m^{-2} s^{-1} for 8, 24, 48, and 72 h.

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Abbreviations: APOD - ascorbate peroxidase; ASC - ascorbate; GSH - reduced glutathione; GR - glutathione reductase; MDA - malondialdehyde; SB - sodium benzoate; SOD - superoxide dismutase; TU - thiourea.

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The senescence of detached rice leaves was followed by measuring the decrease of protein content. Leaf segments were homogenized in 50 mM sodium phosphate buffer (pH 7.5). The extracts were centrifuged at 17 600 *g* for 20 min, and the supernatants were used for determination of protein by the method of Bradford (1976). Malondialdehyde (MDA) was extracted with 5 % (m/v) trichloroacetic acid and determined according to Heath and Packer (1968). MDA level is routinely used as an index of lipid peroxidation. Protein and MDA contents were expressed on the basis of initial fresh mass (f.m.). For the experient of the effect of free radical scavengers on protein content, detached rice were pretreated with either water or 1 mM ascorbate, 5 mM glutathione (GSH), 5 mM sodium benzoate (SB) or 5 mM thiourea (TU) for 12 h and then treated with water stress for 24 h in the light.

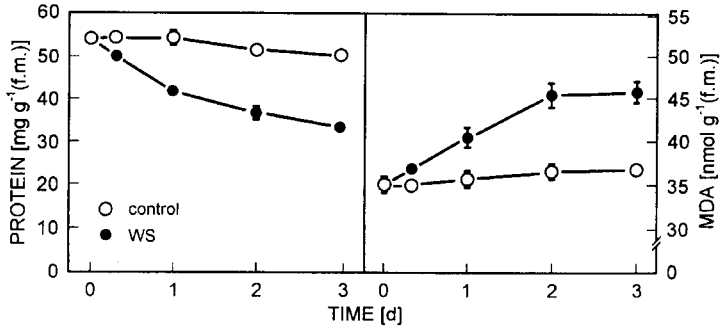

Fig. 1. Changes in protein and malondialdehyde (MDA) content in control and water-stressed (WS) detached rice leaves. Means ± SE ($n = 4$).

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. Catalase (EC 1.11.1.6), peroxidase (EC 1.11.1.7), superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APOD, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2) were assayed as described previously (Chang and Kao 1998). All data were expressed on the basis of initial f.m. U for catalase, peroxidase, SOD, APOD and GR was defined as the amount of enzyme which decomposes 1 μmol of H_2O_2 per min, causes the formation of 1 μmol tetraguaiacol per min, inhibits 50 % the rate of NADH oxidation

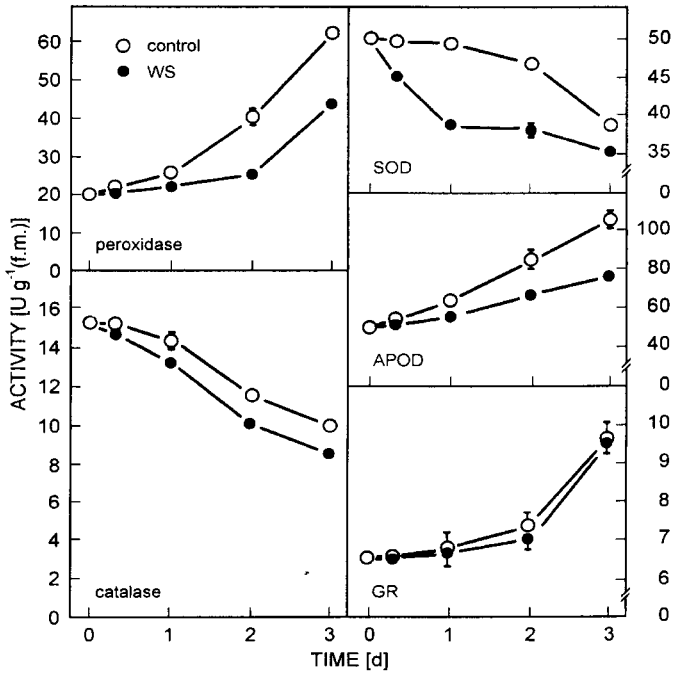

Fig. 2. Changes in peroxidase, catalase, superoxide dismutase (SOD), ascorbate peroxidase (APOD), and glutathione reductase (GR) activities of control and water-stressed (WS) detached rice leaves. Means ± SE ($n = 4$).

observed in the control, oxidizes 1 μmol of ascorbate per min, and decrease in A_{340} per min, respectively. All experiments were repeated three times; within each experiment, treatments were replicated 4 times. Similar results and identical trends were obtained in all experiments. The data reported here are from a single experiment. The progression of senescence of detached rice leaves in the light (promotion of protein degradation) started 8 h after water stress treatment and continued during further 72 h. MDA content in water-stressed detached rice leaves was observed to be higher than that in controls throughout the entire period. This showed that promotion of senescence of detached rice leaves by water stress was linked to lipid peroxidation.

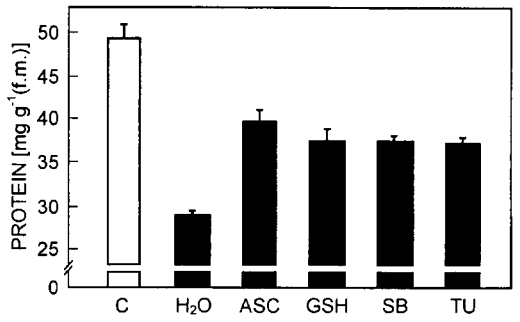

Fig. 3. Effect of free radical scavengers on protein content in of control (C) and water-stressed detached rice leaves pretreated with either water, 1 mM ascorbate (ASC), 5 mM reduced glutathione (GSH), 5 mM sodium benzoate (SB), or 5 mM thiourea (TU) for 12 h. Means ± SE (n = 4).

It is generally considered that lipid peroxidation is induced by free radicals (Kellogg and Fridovich 1975). Evidence has been provided to show that the capacity of leaves for active oxygen generation is increased by water stress (Smirnoff 1993). The increase in lipid peroxidation seen in water-stressed detached rice leaves might be a reflection of the decline of antioxidative enzyme activities. Water-stressed detached rice leaves had lower activities in catalase, peroxidase, and APOD than the controls (Fig. 2). However, these effects were not evident until 24 h after start of water stress treatment. Since the increase in lipid peroxidation was observed already 8 h after water stress (Fig. 1), catalase, peroxidase, and ASC peroxidase may play minor role if any in regulating lipid peroxidation in water-stressed detached rice leaves. GR in detached rice leaves does not seem to be affected by water stress (Fig. 2).

Plant tissues can generate superoxide radicals which are removed by SOD and as a consequence H_2O_2 is produced. The decline in SOD activity induced by water stress (Fig. 2) is coincided with the increase in lipid peroxidation or senescence (Fig. 1). Thus, SOD may play an important role in regulating lipid peroxidation in water-stressed detached rice leaves. If lipid peroxidation is indeed induced by free radicals as we speculated and plays an important role in water stress-promoted rice leaf senescence, then we would expect an inhibition of water stress-promoted senescence in detached rice leaves by the addition of free radical scavengers such as ascorbate (ASC), glutathione (GSH), sodium benzoate (SB) and thiourea (TU) and this was confirmed (Fig. 3).

In conclusion, we found that water stress-induced enhancement of leaf senescence coincided with an elevated lipid peroxidation and reduced SOD activity in detached rice leaves.

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