

Lipid peroxidation and antioxidative enzymes in senescing tobacco leaves during post-flooding

Weei Pirng Hurng, Ching Huei Kao*

Department of Agronomy, National Taiwan University, Taipei, Taiwan, Republic of China

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Abstract

Role of lipid peroxidation and antioxidative enzymes (superoxide dismutase and catalase) in leaf senescence of tobacco plants during the recovery phase after 2 days of flooding was investigated. The senescence of leaves was followed by measuring the decrease in level of chlorophyll. An enhancement in leaf senescence was observed in flooded plants during the recovery phase. Increase in lipid peroxidation is closely correlated with senescence in leaves of flooded plants during recovery phase. Catalase and superoxide dismutase activities decreased in leaves of flooded plants during early and later phase of post-flooding, respectively. It seems that post-flooding-induced enhancement of leaf senescence is associated with an increased lipid peroxidation coupled with reduced catalase and superoxide dismutase activities.

Key words: Catalase; Flooding; *Nicotiana tabacum*; Senescence; Superoxide dismutase

1. Introduction

Reactive oxygen species, such as superoxide anion and hydrogen peroxide, can initiate lipid peroxidation [1]. Measurements of malondialdehyde (MDA) in tissues are frequently used to indicate lipid peroxidation and indirectly to indicate the function of oxygen species. Plants possess antioxidative enzymes, superoxide dismutase (SOD) and catalase, to remove superoxide anion and hydrogen peroxide, respectively [1–3].

It has been shown that postanoxic injury of soybean roots and in rhizomes of the anoxia-sensitive species, *Iris germanica*, after anoxic stress were associated with increased superoxide radical production capacity and lipid peroxidation coupled with a reduced SOD activity [4–6]. We have found that leaf senescence of tobacco was enhanced during the recovery phase after a short duration (2 days) of flooding. Leaf senescence has been shown to be associated with increasing levels of lipid peroxidation [3,7,8]. However, it is not known whether oxidative damage occurs in leaves during post-flooding. In the study now reported, activities of some enzymes associated with activated oxygen metabolism, as well as the process of lipid peroxidation were monitored in order to

* Corresponding author.

Abbreviations: EDTA, ethylenediamine tetraacetic acid; MDA, malondialdehyde; SOD, superoxide dismutase.

follow the pattern of senescence in leaves of tobacco plants which had been subjected to 2 previous days of flooding.

2. Materials and methods

Seedlings of *Nicotiana tabacum* (cv. Speight G-70) were selected 30 days after sowing the seeds in plastic trays and grown in a greenhouse thereafter, each in a small plastic bag (25 cm²). After 30 days, the seedlings were again selected for uniformity and transplanted to pots (5 dm²) containing sandy loam. Each pot contained a plant and received 30 g compound fertilizer (N-P₂O₅-K₂O, 7-21-21). Each treatment had 4 plants. Pots were then placed in the field.

Flooding treatment started at 40 days after transplanting when tobacco plants had 15–17 leaves. Tobacco plants were flooded for 2 days by maintaining the water level at 1 cm above the soil surface. Excess water was drained during a 6-day recovery period. Unflooded control plants were allowed to receive the optimum amount of water. At the times indicated, the lowest leaves were collected for chemical analysis and enzyme assays.

Chlorophyll levels were determined according to Wintermans and De Mots [9]. MDA was extracted with 5% (w/v) trichloroacetic acid and determined according to Heath and Packer [10]. For enzyme extraction, leaf tissues were homogenized in an ice bath with a mortar and pestle and 0.1 M sodium phosphate buffer (pH 6.8) containing 0.1% Triton X-100 and 0.05 g Polyclar and centrifuged at 12 000 × g, 4°C for 20 min. The supernant fractions were used for enzyme assays. Catalase activity was assayed by measuring the initial rate of disappearance of H₂O₂ [11]. Three ml of catalase assay reaction mixture contained 0.1 M sodium phosphate buffer (pH 7.0), 2 mM H₂O₂ and 0.1 ml enzyme extract. The decrease in H₂O₂ was followed as the decline in optical density at 240 nm, and activity was calculated using the extinction coefficient (40.0 mM⁻¹ cm⁻¹ at 240 nm) for H₂O₂ [11]. The activity was expressed in units, where one unit of catalase converts 1 nmol of H₂O₂ in 1 min. SOD activity was assayed by the method of Paoletti et al. [12]. The assay mixture contained 1.6 ml triethanolamine-diethanolamine

buffer (0.1 M, pH 7.4), 0.08 ml NADH (reduced form nicotinamide adenine dinucleotide, 7.5 mM), 0.05 ml EDTA/MnCl₂ (100 mM/50 mM, pH 7.0), 0.1 ml mercaptoethanol (10 mM) and 0.1 ml enzyme extract. The changes in optical density at 340 nm were recorded for 10 min. One unit of SOD was expressed as the amount of SOD capable of inhibiting by 50% the rate of NADH oxidation observed in the control.

3. Results and discussion

The senescence of tobacco leaves was followed by measuring the decrease in the level of chlorophyll. Fig. 1 shows the changes in the chlorophyll level in tobacco leaves during flooding and recovery compared with unstressed controls. In control leaves, the decrease of chlorophyll was evident at 2 days after the start of the experiment. The reported changes of the leaves by flooding and during recovery therefore may only apply to leaves which have already started the senescence process.

As can be seen from Fig. 1, the chlorophyll level was lower in leaves of tobacco plant flooded for 2 days than the corresponding controls during

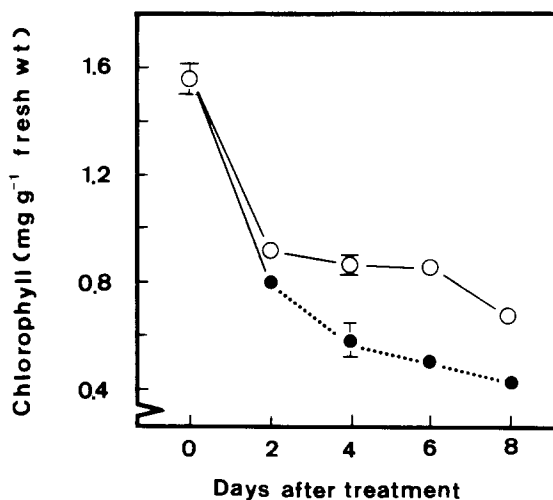


Fig. 1. Changes in chlorophyll levels in tobacco leaves during flooding and recovery (---) compared with unstressed controls. Vertical bars represent standard errors ($n = 4$). ○, Control; ●, flooded. Only those standard errors larger than the symbol size are shown.

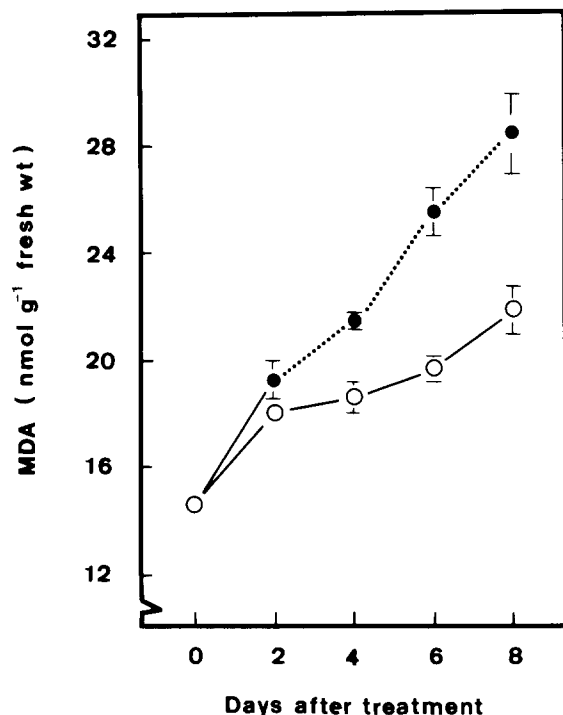


Fig. 2. Changes in malondialdehyde (MDA) levels in tobacco leaves during flooding and recovery (---) compared with unstressed controls. Vertical bars represent standard errors ($n = 4$). ○, Control; ●, flooded. Only those standard errors larger than the symbol size are shown.

flooding period and recovery period. The results also show that enhancement of chlorophyll reduction in leaves of flooded plants is greater during post-flooding than during flooding.

Postanoxic injury of soybean roots and in rhizomes of anoxia-sensitive *Iris germanica* has been shown to be associated with lipid peroxidation [4–6]. Free radical-induced lipid peroxidation is considered to be an important mechanism of leaf senescence [3,7,8,13]. Thus, lipid peroxidation, as judged by the level of MDA, in leaves of flooded plants during post-flooding is expected to be higher than the corresponding controls. As indicated in Fig. 2, this is indeed the case. The increase of the MDA level in leaves of flooded plants is closely associated with the decrease of the chlorophyll level during post-flooding.

The striking increase in lipid peroxidation seen

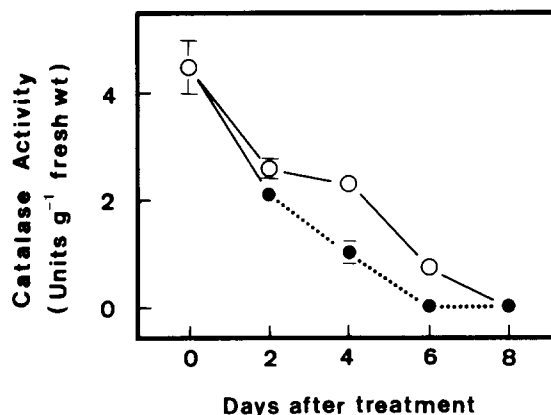


Fig. 3. Changes in catalase activity in tobacco leaves during flooding and recovery (---) compared with unstressed controls. Vertical bars represent standard errors ($n = 4$). ○, Control; ●, flooded. Only those standard errors larger than the symbol size are shown.

in leaves of flooded plants during post-flooding may be a reflection of the decline of protective enzymes. Likely candidates for these enzymes are SOD and catalase since these dismutate superoxide and destroy hydrogen peroxide, respectively,

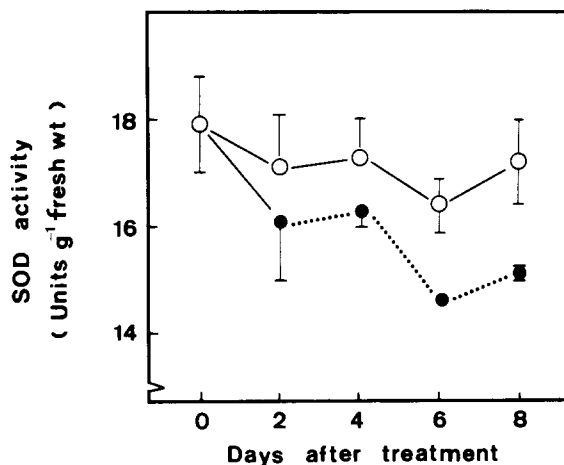


Fig. 4. Changes in superoxide dismutase (SOD) activity in tobacco leaves during flooding and recovery (---) compared with unstressed controls. Vertical bars represent standard errors ($n = 4$). ○, Control; ●, flooded. Only those standard errors larger than the symbol size are shown.

which are known to be involved in the evolution of the highly reactive and toxic hydroxyl radicals, a potent initiator of lipid peroxidation [14]. Figs. 3 and 4 show the changes of catalase and SOD activities in leaves of tobacco plants during flooding and recovery compared with unstressed controls during the first 4 days of post-flooding. SOD activity in leaves of flooded plants was similar to that in the unstressed controls during the first 2 days of post-flooding, but was lower thereafter. The relationship between SOD and catalase activities and lipid peroxidation has been demonstrated under drought, post-anoxia and salinity conditions [5,6,15–17]. Increased lipid peroxidation coupled with reduced SOD and catalase activities in leaves during post-flooding has not previously been demonstrated. In the present investigation, we provide the evidence that oxidative damage occurs during post-flooding.

In conclusion, we found that post-flooding induced enhancement of leaf senescence coincided with an elevated lipid peroxidation and reduced SOD and catalase activities in tobacco leaves.

4. References

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