ENVIRONMENTAL SCIENCES Vol. 1, No. 2, 87~91
Bulletin of the Korean Environmental Sciences Society

Changes in MDA and Ascorbic Acid Contents, and SOD Activity in Paraquat-Treated Spinach Leaf Discs under Light

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(Manuscript received on 5 September, 1997)

Leaf discs were excised from spinach leaves (*Spinacia oleracea* L.) and floated in phosphate buffer (pH 6.8) containing paraquat solutions (0, 0.1, 1.0, 10, 20, 50 and 100 ppm), and incubated in the growth chamber under 5,500 lux illumination at 25℃ for 24 hr.

Treatment with paraquat caused the formation of malondialdehyde (MDA), an indicator of lipid peroxidation in leaf discs. When 1.0, 10, 20, 50 and 100 ppm of paraquat solutions were applied to leaf discs, the contents of MDA were increased by 63, 86, 100, 140 and 150% of the level without paraquat treatment, respectively.

1.0, 10, 20, 50 and 100 ppm of paraquat treatments reduced the amounts of ascorbic acid in leaf discs by 23, 35, 38, 42 and 56% of the level without paraquat treatment, respectively.

Activities of superoxide dismutase (SOD) in leaf discs of 1.0, 10, 20, 50 and 100 ppm of paraquat treatments were decreased by 23, 42, 48, 61 and 70% of the level of SOD in non-treated group, respectively.

The results suggest that paraquat may cause peroxidation of membrane lipid in spinach leaves as a result of paraquat-induced destruction of physiological defense against oxygen phytotoxicity.

Key words: paraquat. ascorbic acid, malondialdehyde, superoxide dismutase, lipid peroxidation

1. Introduction

Paraquat (1,1'-dimethyl-4,4'-bipyridylium ion) is a non-selective contact herbicide which rapidly kills weeds. This herbicide is bound so strongly by clay mineral by means of base exchange that it is considered to be essentially biological inactive in most soils (Ashton and Crafts, 1981; Bergmeyer,1986). Paraquat undergoes a cyclic reduction-oxidation process in plants, which is initiated by an electron excited by light quanta through photochemical systems in the photosynthetic pigments. As a result of the cyclic reduction-oxidation process, free radical formed can react rapidly with molecular oxygen to generate the superoxide anion (Youngman and Dodge, 1979). Thus, superoxide is important to phytotoxicity and has the capacity to produce large amounts of reactive oxygen species such as singlet oxygen. These oxygen radicals have been shown to attack biomolecules and initiate lipid peroxidation (Bus, 1976).

Paraquat damages cellular membrane systems as a result of lipid peroxidation and causes the formation of MDA, an indicator of lipid peroxidation in plant cells (Dalton, 1992). Peter *et al.* (1992) reported that paraquat was able to cause lipid peroxidation beyond a certain threshold. Only when the amount of reactive oxygen species produced by paraquat exceeds the cellular capacity to dissipate them, peroxidative damage to membrane lipids occurs. Sakaki *et al.* (1983)

reported that the contents of MDA were considerably increased in spinach leaves by toxic oxygen species. On the basis of these results they suggested that toxic oxygens such as superoxide radical and singlet oxygen generated by paraquat induced the lipid peroxidation leading to membrane damage.

Ascorbic acid reacts rapidly with most of the reactive oxygen species such as superoxide, hydrogen radical, hydrogen peroxide and singlet oxygen to give rise to dehydroascorbic acid (Halliwell and Gutteridge, 1985). Protection against phytotoxic peroxidation is achieved by the antioxidant such as the ascorbic acid (Tappel 1980). Ascorbic acid is particularly effective antioxidant against toxic superoxide and is present in the chloroplast stroma (Halliwell 1982). Law *et al.* (1983) reported that addition of paraquat to illuminated chloroplasts caused a rapid oxidation of ascorbic acid. Also, paraquat leads to a rapid loss of ascorbic acid in spruce sapling (Westphal, 1992). These results suggest that ascorbic acid disappears in the presence of toxic oxygen species induced by paraquat.

It is generally accepted that paraquat destroys plants by photosynthetically generated superoxide radical and by other harmful compounds such as hydrogen peroxide and hydroxyl radical (Farrington, 1973; Rabinowitch and Fridovich, 1983). The superoxide dismutase (SOD, EC 1.15.1.1) in the detoxifying process converts superoxide radicals to hydrogen peroxide. Most abundant enzyme in plants is Cu, Zn-SOD which is characterized by a broad pH-optimum between pH 7 and 10 (Forman and Fridovich ,1973). Dalton (1992) reported that SOD activity was 29.2 units per mg protein in the paraquat-treated pants compared to 11.4 units per mg protein in the control group after treatment with 1 mM of paraquat. It seems likely that the treatment of spinach leaf discs with 1 mM of paraquat causes a marked increase in SOD activity which results in an increase in their resistance to the toxic effect of superoxide radicals.

The purpose of this experiment was to investigate the herbicidal effects of paraquat on lipid peroxidation and ascorbic acid content, and SOD activity in spinach leaves under light.

2. Materials and Methods

2.1. Plant materials

Fresh leaves of spinach (*Spinacia oleracea* 'L.) were obtained from supermarket. Leaf discs (1.5 cm in diameter) were excised from spinach leaves with corkborer and rinsed with distilled water and floated in phosphate buffer (pH 6.8) containing various concentrations of paraquat solutions and incubated in the growth chamber under 5,500 lux illumination (INS. DX-100) at 25 ℃ for 24 hr.

2.2. Paraquat treatments

Leaf discs were incubated in petri dishes with 50 mM phosphate medium supplemented with various concentrations of paraquat solutions (0, 0.1, 1.0, 10, 20, 50 and 100 ppm) for 24 hr at 25℃.

2.3. Measurement of MDA contents

MDA content was measured using the procedure of Heath and Packer (1968) and an extinction coefficient of 155 $mM^{-1}cm^{-1}$ to determine the amount of lipid peroxidation. 500 mg leaf discs obtained from control group (0 ppm) and from experimental groups (0.1, 1.0, 10, 20, 50 and 100 ppm) were homgenized in 3 ml of 1% TCA with motar and pestle. The homogenates were mixed with thiobarbituric acid and trichloro-

acetic acid at the final concentrations of 0.3 and 12.5% (w/v), respectively, and then incubated in a boiling water for 30 min. The mixture was rapidly cooled in an ice bath and then centrifuged at 18,000g for 8 min. MDA contents in the supernatants were determined from the absorbances at 532 nm.

2.4. Measurement of ascorbic acid contents

400 mg of leaf discs obtained from control group and from experimental groups were homogenized in 4 ml of 5% metaphosphoric acid with the prechilled mortar and pestle and then homogenates were centrifuged at 18,000g for 30 min. 1 ml of citrate / acetate buffer (pH 4.15) and 1 ml of 0.2 mM dichlorophenolindophenol were added to each supernatant. After 30 sec, the absorbance was determined at 520 nm.

2.5. Measurement of SOD activities

For the determination of SOD activities, 800 mg of leaf discs were homogenized with the prechilled mortar and pestle in 1 ml of phosphate buffer (pH 7.8). The homogenates were centrifuged at 15,000g for 30 min at 4℃. The supernatants were dialyzed overnight against 10 mM phosphate buffer (pH 7.8). After centrifugation of the dialyzed solutions at 15,000g for 30 min at 4℃, the supernatants were used for SOD activities. The estimation of SOD activities was based on the inhibition of cytochrome c reduction caused by O_2^- (Tanaka and Sugahara 1980). The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.1 mM xanthine and 50 μg xanthine oxidase in a total volume of 1.0 ml. After the addition of xanthine oxidase, the increase in absorbance at 550 nm was followed at 25 °C.

3. Results and Discussion

Paraquat damages cellular membrane synthesis as a result of lipid peroxidation and carries out the formation of MDA, an indicator of lipid peroxidation in plant cells (Dalton, 1992). MDA is formed from fatty acid because of singlet oxygen generated from superoxide radical (Peter, 1992). Sakaki *et al.* (1983) reported that toxic oxygen species increased the contents of MDA in spinach leaves.

In this experiment, leaf discs of spinach were prepared from control group without paraquat and from experimental groups with various concentrations of paraquat solutions under illumination and measured for their amounts of MDA. Fig. 1 shows the change in the contents of MDA in leaf discs.

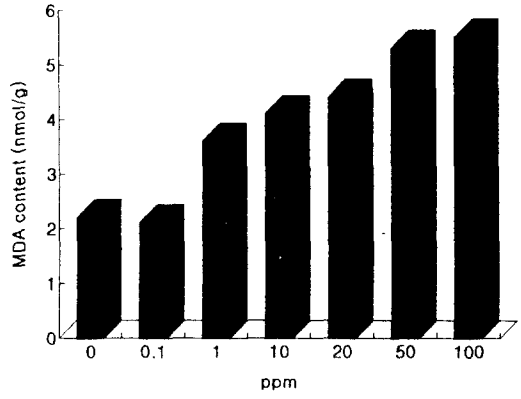

Fig. 1. Changes in MDA contents in leaf discs of spinach incubated for 24 hr with various concentrations of paraquat solutions under light.

MDA content was a little decreased at 0.1 ppm-treated group as compared with control group, and followed by a subsequent drastic rise. When 1.0, 10, 20, 50 and 100 ppm of paraquat solutions were applied to leaf discs, the contents

of MDA were increased by 63, 86, 100, 140 and 150% of the level of control group, respectively. Based on this result the toxic oxygens produced from paraquat under light may cause the lipid peroxidation following by the increase of MDA content.

Ascorbic acid reacts rapidly with most of the reactive oxygen species to give rise to dehydroascorbic acid (Halliwell and Gutteridge, 1985). Ascorbic acid protects against phytotoxic peroxidation (Tappel, 1980). Law *et al.* (1983) reported that addition of paraquat to illuminated chloroplasts caused a rapid oxidation of ascorbic acid. Also, paraquat led to a rapid loss of ascorbic acid in spruce sapling (Westphal, 1992).

In the present work, leaf discs of spinach were analyzed for their contents of ascorbic acid by method described above. The changes in the amounts of ascorbic acid in leaf discs were seen in the Fig. 2.

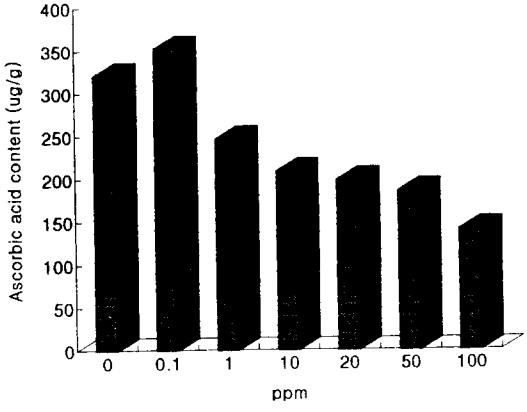

Fig. 2. Changes in ascorbic acid contents in leaf discs of spinach incubated for 24 hr with various concentrations of paraquat solutions under light.

As shown in Fig. 2 at 0.1 ppm of paraquat-treated group, the percent amount of ascorbic acid was increased by 10% as compared with control group, but at above 1.0 ppm of paraquat-treated groups, the percentage of amount of ascorbic acid were decreased with increasing paraquat solution. When 1.0, 10, 20, 50 and 100 ppm of paraquat solutions were applied to leaf discs, ascorbic acid contents were reduced by 23, 35, 38, 42 and 56%, respectively as compared with control group. These results suggest that the increase in ascorbic acid content at lower concentration of paraquat solution is due to the defensive system in plants leading to scavenging superoxide generated by paraquat. Namely, ascorbic acid is necessary to toxic hydrogen peroxide in chloroplasts via the ascorbic acid defense system (Law, 1983). But at higher concentrations of paraquat solutions, the decrease in ascorbic acid content may be the result of the oxidation of ascorbic acid to dehydroascorbic acid by a lot of superoxide produced by paraquat.

SOD converts superoxide radicals into hydrogen peroxide and oxygen. In chloroplast, Cu,Zn-SOD enzymes are sensitive to hydrogen peroxide and therefore, the lack of SOD enzyme activity induction in the *Conyza* sp. plants may be due to the inactivation of Cu,Zn-SOD enzymes by paraquat-induced formation of hydrogen peroxide (Forman and Fridovich, 1973). Sakaki *et al.* (1980) reported that the activity of SOD in spinach leaves treated with 2% DDTC under light was decreased by 65% after 2 hr, thereafter gradually diminished to 77% after 22 hr.

Leaf discs were analyzed for their activities of SOD by the inhibition of cytochrome c reduction as described in method materials. One unit of SOD activity was defined as the amount of enzyme required to cause inhibition rate (50%) of cytochrome c reduction at 550 nm. Fig. 3 shows the changes in SOD activities of spinach.

As shown in Fig. 3 at only 0,1 ppm of paraquat, activity of SOD was increased by 30% as compared with control group. But the concentrations of paraquat solutions above 0.1 ppm

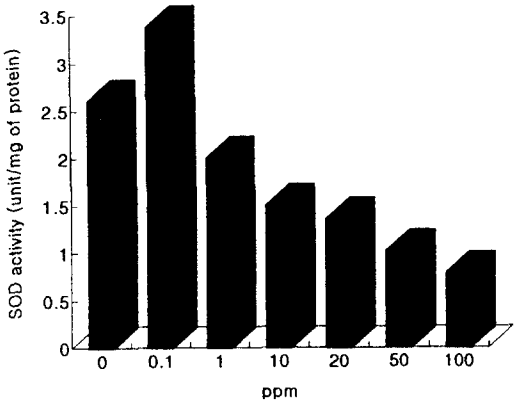

Fig. 3. Changes of SOD activities in leaf discs of spinach incubated for 24 hr with various concentrations of paraquat solutions under light.

caused the decrease in activities of SOD. When 1.0, 10, 20, 50 and 100 ppm of paraquat solutions were applied to leaf discs, SOD activities were decreased by 23, 42, 48, 61 and 70%, respectively as compared with control group. Based on these results the activity of SOD is increased to dismutate superoxide radicals to the hydrogen peroxide and oxygen at the lower concentration of paraquat. But at higher concentrations of paraquat, the reduction of SOD activities may be due to the destruction of chloroplasts by reactive oxygens.

As results of this experiment, it is suggest that paraquat may cause peroxidation of membrane lipid in spinach leaves as a result of paraquat-induced destruction of physiological defense against oxygen phytotoxicity. Paraquat has inhibitory effect on the process and pathway of plant metabolism.

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