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Delay of senescence of detached cucumber cotyledons by triadimefon

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Abstract

Changes in contents of reactive oxygen species (O_2^- and H_2O_2) and non-enzymatic antioxidants, activities of antioxidant enzymes and lipid peroxidation were investigated during senescence of detached cucumber cotyledons dipped in water (control) and 20 mg dm^{-3} triadimefon (TDM). O_2^- and H_2O_2 accumulation and lipid peroxidation were observed during senescence of cucumber cotyledons, which coincided with a drop in the contents of carotenoids (Car) and ascorbic acid (AsA), and the activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), and an increase in the activity of peroxidase (POD). However, TDM could significantly inhibit the accumulation of O_2^- and H_2O_2, and lipid peroxidation by preventing the decrease of CAT, APX, Car and AsA and the increase of POD, while TDM had little effect on SOD activity during the senescence. Therefore we can draw a conclusion that TDM protects the membrane system and retards the senescence of detached cucumber cotyledons.

Additional key words: antioxidants, antioxidant enzymes, *Cucumis sativus*, lipid peroxidation, reactive oxygen species

Introduction

Senescence of leaves is an active process that allows redistribution of their nutrients as the plant reaches final stages of its development. On the other hand, senescence involves an oxidative process due to the overproduction of reactive oxygen species (ROS) such as H_2O_2, superoxide (O_2^-) radicals, hydroxyl radicals ($^{\cdot}OH$), and singlet oxygen ($^1O_2$) (Piquery *et al.* 2000, Scebba *et al.* 2001). Cellular damage caused by ROS, via lipid peroxidation, is generally considered to be a major contributor to the senescence syndrome (Dhindsa *et al.* 1981). However, the damage might be reduced or prevented by enzymatic and non-enzymatic antioxidant defense systems. Superoxide dismutase (SOD) catalyzes the dismutation of O_2^- into oxygen and H_2O_2, thus playing a key role in this defense mechanism. Ascorbate peroxidase (APX) reduces H_2O_2 to water, with ascorbate as electron donor. Catalase (CAT) and in a way peroxidase (POD) are implicated in removal of H_2O_2. In addition, plant tissues contain substantial amounts of carotenoids (Car) that serve as non-enzymatic oxygen radical scavengers (Young and Britton 1990), and ascorbic acid (AsA) that can act directly as a free-radical scavenger (Larson 1988).

Plant growth regulators are playing important roles in crop production. Triadimefon [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone], a triazole derivative, has both fungicidal and plant growth regulatory properties. Its plant growth regulating properties are mediated by interference with the isoprenoid pathway and subsequent shift in balance of important plant hormones, including gibberellin, abscisic acid (ABA) and cytokinins (Fletcher and Hofstra 1985). Some evidence shows that TDM could protect plants from several types of abiotic stresses, including cold, heat (Asare-Boamah and Fletcher 1986), drought (Fletcher and Nath 1984, Asare-Boamah *et al.* 1986, Guo *et al.* 1997) and salt (Muthukumarasamy and Panneerselvam 1997, Muthukumarasamy *et al.* 2000) in various plants such as cucumber, barley, rice, peanut and rape. In recent years, it has been reported that TDM could retard the senescence of wheat and barley leaves, and of cut rose flowers (Buchenauer and Grossman 1977, Zhou and Xu

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Abbreviations: ABA - abscisic acid; APX - ascorbate peroxidase; AsA - ascorbic acid; Car - carotenoids; CAT - catalase; CK - control; MDA - malondialdehyde; NBT - nitroblue tetrazolium; POD - peroxidase; REC - relative electrical conductivity; ROS - reactive oxygen species; SOD - superoxide dismutase; TDM - triadimefon.
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2000). However, the influence of triadimefon on generation and scavenge of ROS, and lipid peroxidation during the senescence of plants is little known.

This work was undertaken to make a further study on the protection roles of TDM on senescent plant cell membrane system. So we investigated the effect of TDM on changes in antioxidant contents and antioxidant enzyme activities, reactive oxygen species and lipid peroxidation of detached cucumber cotyledons.

Materials and methods

Cucumber (*Cucumis sativus* L. cv. Xintai Mici) was used in the experiment. A 15 % water-dispersible powder of TDM was provided by the *United Chemical Factory* of Zhangjiegang City, Jiangsu Province of China.

The seeds were surface sterilized with 0.1 % $HgCl_2$ solution for 10 min and thoroughly washed with deionized water. The surface sterilized seeds were imbibed in distilled water for 10 h and then sown in to trays of vermiculite with Hogland nutrient solution. The plants were cultivated in a growth chamber at 14-h photoperiod, irradiance of 40 μmol m^{-2} s^{-1}, day/night temperature of 28/20 °C and relative humidity of 80 ± 5 %. 2-cm hypocotyl segments with cotyledons cut from 7-d-old seedlings were dipped in a Petri dish containing 10 cm^3 of 20 mg dm^{-3} TDM or water for 4 d. The concentration of 20 mg dm^{-3} triadimefon was selected according to our previous investigations (Feng *et al.* 2000). The cultivation conditions were similar to those before detaching. Samples for physiological and biochemical analyses were taken on day 0 (pre-treatment), 1st, 2nd, 3rd and 4th day during senescence in random. Five measurements of each parameter were made. For each parameter, a mean and standard error of mean were calculated and the statistical significance of differences between control and treated plants were evaluated by Student's *t*-test.

The H_2O_2 content was determined by the method of Mukherjee and Choudhuri (1983). Isolation was made from 1 g leaf tissue in ice-cold acetone. By addition of 5 % (m/v) titanylsulphate and conc. NH_4OH solution, the peroxide-titanium complex was precipitated and this sediment was dissolved in 2 M H_2SO_4. The absorbance was read at 415 nm (*Hitachi* spectrophotometer *U-3010* Tokyo, Japan) against water. The H_2O_2 content was calculated from a standard curve prepared in a similar way. O_2^- level was extracted and determined according to Chaitanya and Naithani (1994). 0.5 g leaves were homogenized in cold sodium phosphate buffer (0.2 M, pH 7.2) containing diethyldithiocarbamate (10^{-3} M) to inhibit SOD activity. The homogenate was immediately centrifuged for 10 min at 10 000 *g*. In the supernatant, superoxide anion (O_2^-) was measured by its capacity to reduce nitroblue tetrazolium (2.5×10^{-4} M). The absorbance of the end product was measured at 540 nm.

Malondialdehyde (MDA), a decomposition product from the peroxidation of polyunsaturated fatty acids, was measured as thiobarbituric acid reactive substances from centrifuged leaf extracts in 10 % trichloroacetic acid (Li and Mei 1989). The absorbance of the extract was read at 532 nm and the values were corrected by subtracting the absorbance at 600 nm. The concentration of MDA was calculated using coefficient of absorbance of 155 $mmol^{-1}$ cm^{-1}. Membrane permeability of leaves was measured by relative electrical conductivity (REC) (Zhou and Leul 1998). 0.5 g leaf tissue was placed in a 25 cm^3 test tube containing 15 cm^3 deionized water. The leaf sample was immersed and vibrated for 30 min, and then the conductivity of the solution was measured using a conductivity meter (*DDS-11A, Shanghai Instrument Co.*, Shanghai, China). After boiling the samples for 15 min, their conductivity was measured again when the solution was cooled to room temperature. $REC = C_1/C_2 \times 100$ (where C_1 and C_2 are the electrolyte conductivities measured before and after boiling, respectively).

For enzyme extraction, 1 g of fresh mass was ground with a pestle in an ice-cold mortar with 10 cm^3 of 0.05 M sodium phosphate buffer (pH 7.0). The homogenates were filtered through 4 layers of cheese cloth and then centrifuged at 4 °C for 20 min at 15 000 *g*. The supernatants were used for the assays of enzyme activities. SOD activity, the basis of which is its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT), was determined according to the method of Dhindsa *et al.* (1981). One unit of SOD was defined as the amount of enzyme resulting in 50 % inhibition of the rate of NBT chloride reduction at 560 nm. POD activity was assayed adopting the method of Kar and Choudhuri (1987). Activity was calculated using the coefficient of absorbance (22.6 $mmol^{-1}$ cm^{-1} at 470 nm) for tetraguaiacol. CAT activity was measured by the H_2O_2 reduction method (Aebi 1984). Activity was calculated using the coefficient (40 $mmol^{-1}$ cm^{-1} at 240 nm) for H_2O_2. APX was determined according to Nakano and Asada (1981). The decrease in ascorbate concentration was followed as a decline in absorbance at 290 nm and activity was calculated using the coefficient (2.8 $mmol^{-1}$ cm^{-1} at 290 nm) for ascorbate. For CAT, POD and APX, one unit of enzymes was defined as the amount necessary to hydrolyze 1 μmol(substrate) min^{-1} at 25 °C.

Protein content in the enzyme extracts was determined according to Bradford (1976), using bovine serum albumin as a standard. Total carotenoids were extracted with 80 % acetone and estimated spectrophotometrically at 480 nm as described by Evans (1988).

Ascorbic acid (AsA) content was determined using the method described by Kraus and Austin-Fletcher (1994). An extract was prepared by homogenizing leaves in 6 % (m/v) trichloroacetic acid on ice and the supernatant was obtained by centrifuging the homogenate at 4 000 *g* for 10 min. A standard curve in the range of 0 - 450 nmol ascorbate was used for calibration.

Results

The senescence of detached cucumber cotyledons was followed by measuring the decrease of protein content. The noticeable effect of TDM on the retardation of protein loss in detached cucumber cotyledons was evident at 2nd day after incubation ($P < 0.05$). On the 4th day, the protein content in TDM was 67 % higher than that in control (Fig. 1*A*).

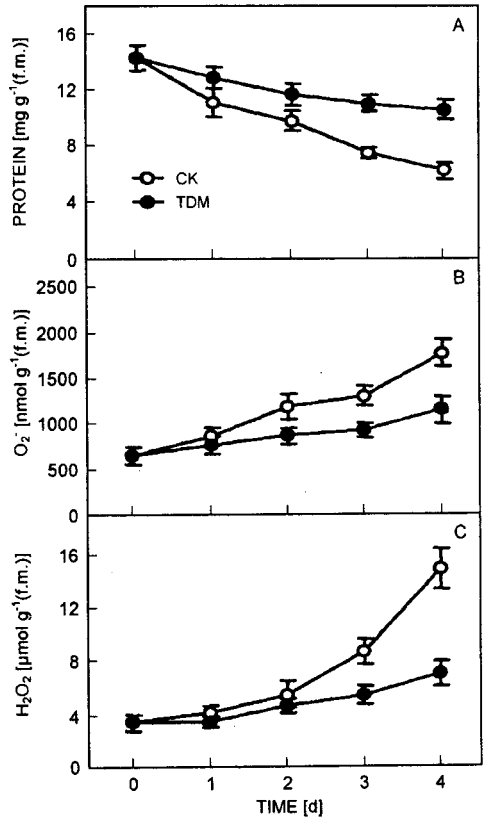

Fig. 1. Changes in the protein, O_2^- and H_2O_2 contents in detached cucumber cotyledons treated with TDM or water. *Vertical bars* represent SE ($n = 5$).

O_2^- and H_2O_2 contents were increased significantly with the processing of senescence, but the accumulation was slowed in cotyledons treated with TDM, especially at a later stage (Fig. 1*B*,*C*). On the 1st and 2nd days there is no significant difference between TDM-treated and control plants, whereas on the 4th day, O_2^- and H_2O_2 contents in TDM-treated cotyledons were 30.1 and 52.7 % lower than those in controls ($P < 0.01$), respectively.

MDA content and REC were increased significantly in senescent cucumber cotyledons treated with TDM or water (Fig. 2). However, MDA content and REC were lower in cotyledons dipped in TDM ($P < 0.01$).

The decline in SOD, CAT and APX activities in either TDM or CK cotyledons was observed during the senescence of detached cucumber cotyledons (Fig. 3*A*,*B*,*D*). However, TDM significantly inhibited decreases of CAT and APX activities, and had little effect on SOD activity. POD activity was increased remarkably in cotyledons treated by TDM and water (Fig. 3*C*), and the increase of POD activity was greater in control than in TDM-treated cotyledons, especially at later stages of detaching ($P < 0.05$).

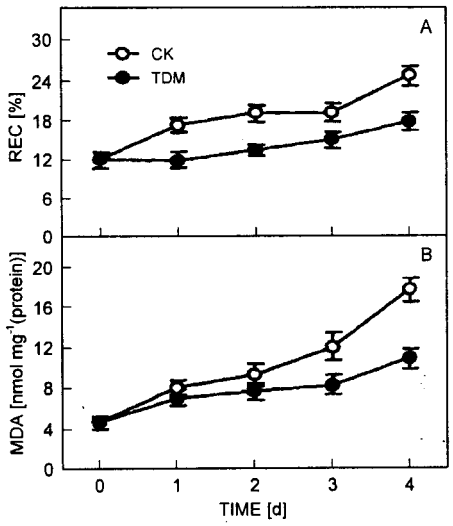

Fig. 2. Changes in the REC and MDA in detached cucumber cotyledons treated with TDM or water. *Vertical bars* represent SE ($n = 5$).

The Car content in cotyledons gradually declined with increasing time of senescence (Fig. 3*E*), indicating that senescence resulted in Car degradation. Trend in changes of AsA content in detached cotyledons was similar to that of Car (Fig. 3*F*). On the 4th day, the Car and AsA contents in cotyledons treated with TDM were 33.8, 32.6 % higher than those in control, respectively.

Discussion

Susceptibility of plants to oxidative stress depends on the overall balance between factors that increase oxidant generation and those cellular components that exhibit an antioxidant capability (Foyer *et al.* 1994). Oxidative damage in plant tissues is especially important during senescence and is characterized by a notable increase of ROS (Thompson *et al.* 1987). The present results also showed that O_2^- and H_2O_2 were accumulated during senescence (Fig. 1), whereas TDM could effectively inhibit the accumulation of ROS. H_2O_2 itself is a constituent of oxidative metabolism, and which reacts with O_2^- to form hydroxyl radicals. Hydroxyl radicals, in turn, initiate peroxidation of membrane lipids and destruction of protein (Halliwell 1987). From the results (Fig. 1), it can be inferred that TDM maybe inhibit the production of hydroxyl radicals. At the same time, senescence induced a decrease in non-enzymatic antioxidants (Car and AsA) and antioxidative enzyme activities (SOD, CAT and APX) (Fig. 3). However, TDM significantly prevented decreases of Car and AsA contents, and CAT and APX activities during the senescence, which produced declined O_2^- and H_2O_2 levels in detached cotyledons. The role of POD in plants is complicated and in present investigation, POD activity was increased in the two treatments, suggesting that POD catalyzed the generation of ROS (Thompson *et al.* 1987). Therefore, the present results were consistent with previous hypothesis that triazole-induced stress tolerance in plants may be caused, at least in part, by increased antioxidant activities, which in turn reduce stress-related oxidative damage to cell membranes (Fletcher and Hofstra 1988).

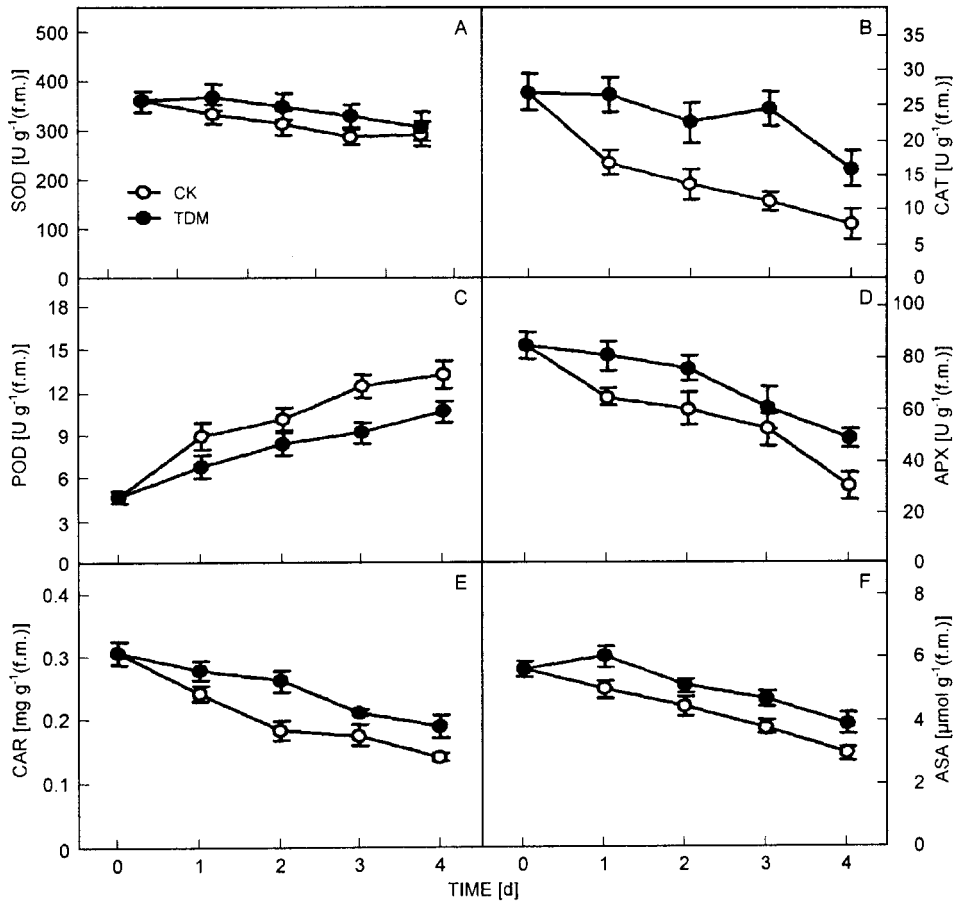

Fig. 3. Effects of TDM on activities of SOD, CAT, POD and APX and on contents of Car and AsA during senescence of detached cucumber cotyledons. *Vertical bars* represent SE (n = 5).

During senescence, electrolyte leakage (as indicated by REC) and MDA content in detached cucumber cotyledons were dramatically increased. However, TDM could inhibit electrolyte leakage and MDA accumulation, and consequently decreased senescence-induced lipid peroxidation of cucumber cotyledons, which was correlated with declined O_2^- and H_2O_2 contents. Enhanced electrolyte leakage was considered a symptom of stress-induced membrane damage and deterioration (Simon 1974). MDA, a product of lipid peroxidation, damages

enzymes and plant membranes (Jian 1992). Hence, the data indicated that TDM retarded the senescence of detached cucumber cotyledons. The results were in accordance with the decreased lipid peroxidation reported in soybean leaves treated with paclobutrazol (Upadhyaya *et al.* 1985) and oat leaves by 2,4-dichlorophenoxyacetic acid (2,4-D), gibberellin and N^6-benzyladenine (Li and Mei 1989).

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