

Effects of simulated acid precipitation on photosynthesis, chlorophyll fluorescence, and antioxidative enzymes in *Cucumis sativus* L.

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Abstract

The effects of simulated acid rain on gas exchange, chlorophyll fluorescence, and anti-oxidative enzyme activity in cucumber seedlings (*Cucumis sativus* L. cv. Jingchun No. 4) were investigated. Acid rain significantly reduced net photosynthetic rate and mainly non-stomatal factors contributed to the decrease of photosynthesis during the experimental period. The reduced photosynthesis was associated with a decreased maximal photochemical efficiency (F_v/F_m) and the average quantum yield of the photosystem 2 (PS2) reaction centres (Φ_{PS2}). Meanwhile, acid rain significantly increased the activities of guaiacol peroxidase (GPX) and superoxide dismutase (SOD), but decreased the activity of catalase (CAT) together with an increased content of malonyldialdehyde (MDA). Hence the changes in photosynthesis in acid rain treatment might be a secondary effect of acidity damage probably due to lipid peroxidation of lipids and proteins in thylakoid membrane rather than direct effect on PS2 reaction centre.

Additional key words: acid rain; catalase; chlorophyll fluorescence; guaiacol peroxidase; lipid peroxidation; malonyldialdehyde; net photosynthetic rate; photosystem 2; superoxide dismutase.

Introduction

The increase of acid substances in air is one of the dramatic changes in atmospheric environment in the last century and has been one of the most serious environmental problems. As one of the detrimental effects, acid deposition may cause stress in agricultural plants (Amthor 1984, Ashenden and Bell 1989). The effects of acid rain on plants can be determined at several levels, from changes in biochemical and physiological processes through organ and whole-plant level response, including visible symptoms of injury (chlorosis and/or necrosis), and invisible effects such as reduced photosynthesis, nutrient loss from leaves, altered water balance, variation of several enzyme activities (Ferenbaugh 1976, Lee *et al.* 1981, Evans 1982, Ashenden *et al.* 1989, Darrall 1989). Usually, the effects of pollution on biochemical processes may be detected much earlier than visible injuries and noticeable changes in growth and yield since the later become apparent only after exposure to relatively long periods (Porter *et al.* 1987, Takemoto 1987).

Shan *et al.* (1996) reported that chlorophyll (Chl) contents of Armand pine increased but net photosynthetic rate (P_N) on a Chl content basis decreased with increasing

acidity of rain. Similarly, photosynthetic CO_2 fixation and photochemical activity of bean plants were sharply decreased in the first hours of the treatment by acid rain at pH 1.8 (Velikova *et al.* 1999). Several authors suggested that acid rain might induce peroxidation of the cell membrane (Chen *et al.* 1991, Velikova *et al.* 2000). Increased superoxide dismutase (SOD) and peroxidase (POD) activities were frequently observed in plants after acid rain treatment; these antioxidative enzyme activities have been suggested as indicators of pollution stress (Endress *et al.* 1980, Markolla *et al.* 1984, Castillo *et al.* 1987, Chen *et al.* 1991, Bowler *et al.* 1992).

The detrimental effects of acid rain greatly depend on the acidity of the rain. Amthor (1984) suggested that rain more acidic than pH 3.0 can cause significant damage to plants. In China, acid rain monitoring data from the late 1970's demonstrate that rain is acidic in most places, with pH ranging from 2.3 to 4.47 (Liu *et al.* 1988). Culturing plants under plastic-film shelter greatly improved plant growth in commercial production, suggesting the detrimental effects of natural rain on plants (Yu *et al.* unpublished).

Received 20 May 2002, accepted 25 July 2002.

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Acknowledgements: The National Natural Science Foundation of China, 863 Plan of China, National Young Teacher Award of China, and Natural Science Foundation of Zhejiang Province have supported this work as a special project.

Cucumber plants are relatively susceptible to the environmental stress. Hence, research on the effects of acid rain has practical significance for cucumber production. The aim of the present paper was to investigate the effects of simulated acid rain on photosynthesis and lipid peroxi-

Materials and methods

Plants: Cucumber (*Cucumis sativus* L. cv. Jinchun No. 4) seeds were germinated at 28 °C in rockwool. Seven days later, the seedlings were transplanted in a plastic vessel (13 000 cm³) filled with 1 000 cm³ of a continuously aerated nutrient solution (Yu and Matsui 1997). The vessel was placed in the growth chamber with a 12 h photoperiod, temperature of 23–25 °C, and photosynthetic photon flux density (PPFD) of 600 µmol m⁻² s⁻¹. Each vessel was covered with a spume, which did not permit the acid rain to contaminate the nutrient solution.

Simulated acid rain treatment: Experiments were carried out with 18-d-old cucumber seedlings. After the emergence of the three primary leaves, each seedling was sprayed with simulated acid rain (pH of 2.0, 3.5, 5.0) or re-distilled water (pH 7.0). Each seedling was sprayed three times and each time with 4 cm³ of solution. The solution for realising the acid rain was prepared according to Seufert *et al.* (1990) and contained [kg m⁻³]: NH₄NO₃ 1.3, MgSO₄×7 H₂O 3.0, Na₂SO₄ 2.5, KHCO₃ 1.3, and CaCl₂×2 H₂O 3.1. After diluting the initial solution 1 : 100, pH was adjusted to 2.0, 3.5, and 5.0 with 0.2 M H₂SO₄. Tween 80 (0.5 %, v/v) was used as surfactant. All measurements were done with the 2nd primary leaves.

Gas exchange and Chl fluorescence were measured at 3, 12, 24, 36, and 60 h after the termination of acid rain treatment. The photon-saturated P_N (P_{Nsat}), stomatal conductance (g_s), and intracellular CO₂ concentration (C_i) were measured at 800 µmol m⁻² s⁻¹, 350±5.0 µmol mol⁻¹ and 25 °C, using a portable photosynthesis apparatus (Ciras-1, PP-Systems, UK). Saturation kinetics was recorded with irradiances from 10 to 1 200 µmol m⁻² s⁻¹. Chl fluorescence was measured using a portable pulse-modulated fluorometer (PMS-2, Hansatech, UK), and was performed in the dark-adaptation clips. Before each measurement, leaves were darkened for at least 15 min. The minimal fluorescence level in the dark-adapted state

(F_0) was determined by the measuring modulated radiation (<0.05 µmol m⁻² s⁻¹). The maximal fluorescence levels in the dark-adapted (F_m) and light-adapted (F_m') states were determined before or after addition of the actinic radiation (100 µmol m⁻² s⁻¹) by 0.8 s saturating pulse modulation radiation (12 000 µmol m⁻² s⁻¹). Using both light and dark fluorescence parameters, F_v/F_m (the maximum efficiency of PS2 photochemistry in the dark-adapted) and Φ_{PS2} (the average quantum yield of the PS2 reaction centres) were calculated (Krause and Weis 1991, Yu *et al.* 2002).

Activities of antioxidant enzymes and malonyldialdehyde (MDA) content: To obtain the enzyme extract, 500 mg of leaves was homogenised in 3 cm³ of cold potassium phosphate buffer (0.1 M, pH 7.0) containing 4 % (m/v) polyvinylpyrrolidone (m.m. 25 000). The homogenate was centrifuged for 20 min at 12 000×g and the obtained supernatant was used as enzyme extract. All steps in the preparation of the enzyme extract were carried out at 0–4 °C. An aliquot of the extract was used to determine its protein content by the method of Bradford (1976) utilising bovine serum albumin as the standard. The activity of guaiacol peroxidase (GPX, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6) were assayed according to Cakmak (1991). Superoxide dismutase (SOD, EC 1.1.1.15.1) was assayed by monitoring the reduction of nitroblue tetrazolium by superoxides generated by a xanthine oxidase system at 560 nm. One unit of the enzyme activity inhibits the rate of reduction of nitroblue tetrazolium by 50 % (Beauchamp and Fridovich 1971). The thiobarbituric acid (TBA) test, which determines MDA as an end product of lipid peroxidation, was used for the measurement of lipid peroxidation in leaves. MDA was determined according to Gossett *et al.* (1994).

Statistical analyses: The values presented are average ± standard error of at least 3 replications.

Results

Effect of acid rain on gas exchange and Chl fluorescence parameters: P_{Nsat} expressed on leaf area basis (Fig. 1) was significantly reduced over the range of the acidities examined. The reduction increased with increasing acidity of the simulated rain, and plants exposed to pH 2.0, 3.5, and 5.0 had significantly lower P_{Nsat} than

plants exposed to pH 7.0. P_{Nsat} reduction by acid rain was most apparent after 6 h of acidic rain treatment. For example, P_{Nsat} for pH 2.0 was reduced by 25, 41, 33, and 31 % after 3, 6, 12, and 24 h of the treatment, respectively. No reduction in P_{Nsat} , however, was observed after 60 h, indicating that the temporary injury of simulated

acid rain on cucumber did not cause irreversible damage. Meanwhile, decreases in g_s were observed in acidic rain treatments in the first several hours after treatments, although the differences were not significant at most times. The decreases of g_s were accompanied with increases in C_i .

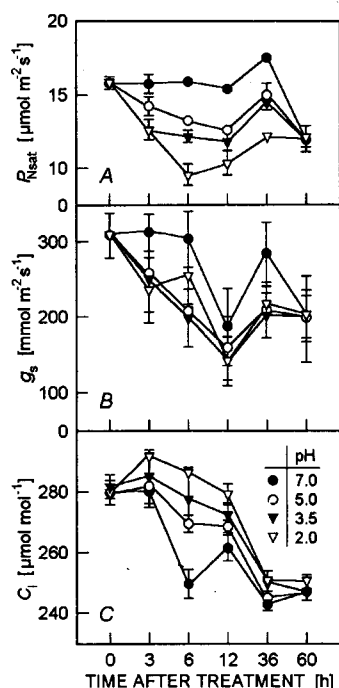


Fig. 1. Effects of simulated acid rain at different pH on the photon-saturated net photosynthetic rate (P_{Nsat}), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) of cucumber leaves. Means of three measurements (three seedlings) with standard deviations shown by vertical bars.

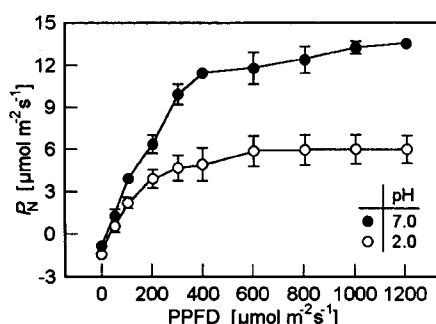


Fig. 2. Changes in irradiance response curves of net photosynthetic rate (P_N) in cucumber leaves. Means of three measurements (three seedlings) with standard deviations shown by vertical bars. Measurements were made 6 h after simulated acid rain treatment.

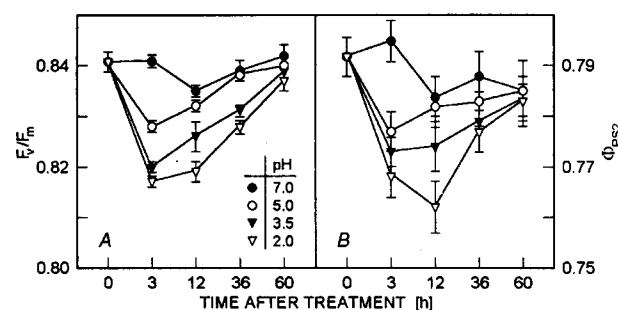


Fig. 3. Effects of simulated acid rain at pH 2.0 on F_v/F_m (A) and Φ_{PS2} (B) in cucumber leaves. Means of three measurements (three seedlings) with standard deviations shown by vertical bars.

P_N gradually increased with the increasing PPFD from 50 to $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 2). P_N for pH 2.0 treatment, however, was significantly lower than that of control at all

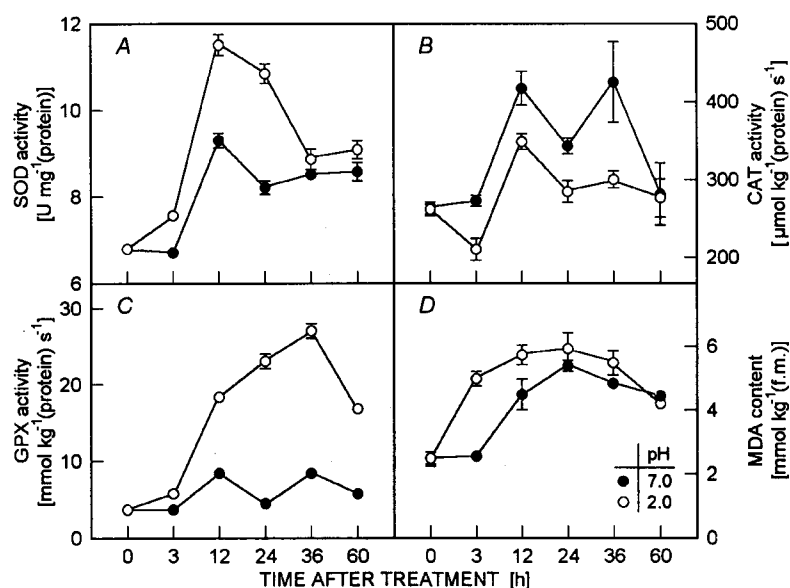


Fig. 4. Effects of simulated acid rain on lipid peroxidation (MDA) and activities of guaiacol peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT) in cucumber leaves.

irradiance. P_N of treated plants was reduced to 44–56 % of the control values at the PPFD tested.

A slight but significant decrease in F_v/F_m ratio, which characterises the maximal quantum yield of the primary photochemical reactions in dark-adapted leaves, was observed after 3 h of treatment (Fig. 3). There were no significant differences in F_v/F_m ratio between control and acid rain treatments after 6 h of treatment. Φ_{PS2} followed a similar trend to that of F_v/F_m . There were no differences in Φ_{PS2} among the four treatments after 60 h of acidification, indicating that PS2 system was not the target of acid rain injury.

Effect of acid rain on activity of anti-oxidative enzymes

Discussion

Acid rain may affect photosynthesis through altered leaf chemistry and morphology, cellular pH balance, activities of ribulose-1,5-bisphosphate carboxylase/oxygenase and nitrate reductase, carbon partitioning, mitochondrial respiration, chloroplast membrane integrity, and stomatal and mesophyll conductance (Neufeld *et al.* 1985, Muthuchelian *et al.* 1993, Shumijko *et al.* 1996, Turunen *et al.* 1997). We found that pH levels of 2.0, 3.5, and 5.0 reduced P_{Nsat} of cucumber seedlings. The reduction increased with increasing acidity, especially in the first hours of acid rain treatment. P_{Nsat} , however, gradually recovered at all treatments in 6 to 60 h after the onset of acidification, suggesting that the inhibition is reversible. This is generally in agreement with the study of Velikova *et al.* (1999) who stated that both stomatal and non-stomatal factors contributed to a decreased P_N in bean plants. We found that the reduction in g_s was accompanied by elevated C_i in all treated plants, suggesting that non-stomatal factors are involved in reduction of CO_2 utilisation, resulting in the accumulation of intracellular CO_2 . In addition, decreased P_N may be due to an increased intracellular accumulation of H^+ contained in acid rain; this probably leads to uncoupled electron transport and insufficient accumulation of ATP and NADPH (Velikova *et al.* 1999). This is confirmed by Chl fluorescence analysis which showed that acid rain treatment induced a significant decrease in F_v/F_m (maximal photochemical efficiency of PS2) and electron transport rate in PS2 (Φ_{PS2}). It is also clear from Fig. 2 that the initial slope at low irradiance did not greatly change after acid rain treatment, implying that the photochemical quantum yield was not decreased. Hence, acid rain would not affect the photochemical steps, but the downstream metabolism, which is the limiting step at high irradiance.

In our experiment, P_{Nsat} recovered gradually after 6 h of acidic rain treatment. There were almost no differences in P_{Nsat} , g_s , C_i , and F_v/F_m in 60 h after treatment. This observation implied the involvement of some defence

systems: SOD activity significantly increased after acid rain treatment. The effects lasted as long as 36 h. Acid rain treatment also greatly increased GPX activity and the effect was much more apparent than that on SOD activity. It increased to 215 % of the control after 12 h of treatment and after 36 h it was more than 5-fold higher than in the control. Increase in GPX activity was observed even 60 h after the treatment. In sharp contrast with GPX and SOD activities, CAT activity significantly decreased after acid rain treatment and the effects lasted at least for 60 h. It was 82 and 67 % of the control in 24 and 36 h after treatment, respectively. Generally, acid rain treatment increased MDA content (Fig. 4). Highest MDA content was observed after 3 h of the acid rain treatment.

Plants have developed anti-oxidative system to cope with stress. When plants are subjected to stress, the balance between the production of active oxygen species (AOS) and the quenching activity of antioxidants may be upset and oxidative damage may be the result (Elstner *et al.* 1988). In our experiment, significant increases in SOD and GPX activities were observed after 3 h of acid rain treatment, suggesting increased production of active oxygen species such as superoxide and H_2O_2 . The overproduction of active oxygen species may bring about peroxidation of membrane lipids, which lead to membrane damage (Gossett *et al.* 1994). In fact, increased lipid peroxidation (MDA content) was observed in acid-rain-treated seedlings, indicating that acid rain induced membrane damage in cucumber leaves. Therefore, the damage to chloroplast membranes seems to be, at least partly, responsible for the decrease in P_N (Neufeld *et al.* 1985, Šiffel *et al.* 1996).

Damage to photosynthesis system is reflected in the depression of maximal photochemical efficiency F_v/F_m . As shown in Fig. 3, F_v/F_m values decreased slightly for all treatments as compared with the controls. The lower F_v/F_m observed was mostly a result of decline in F_m (values not shown), which indicates an impaired capacity for Q_A reduction and increased non-radioactive energy dissipation upon exposure to acidic rain. Acid rain also decreased Φ_{PS2} , suggesting that electron transport rate in PS2 was inhibited. Cucumber plant is very sensitive to environmental stress: increased MDA contents and decreased photosynthetic activity were observed in the control, suggesting that the control leaves underwent a senescence process or were also subjected to stress during the experiment.

In summary, our results suggest that acid rain had a temporary negative effect on the photosynthesis of cucumber seedlings. The changes in F_v/F_m and Φ_{PS2} in acid rain treatment may be a secondary effect of damage by acidity, probably resulting from lipid peroxidation (accu-

mulation of MDA by acid rain) rather than direct effect on PS2 reaction centre. Acid rain treatment induced oxi-

dative stress related to membrane damage but did not cause irreversible changes.

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