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ORIGINAL RESEARCH

Effect of artificial acid rain and SO_2 on characteristics of delayed light emission

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ABSTRACT: The structure and function of chloroplast in plant leaves can be affected by acid rain and air pollution. The photosystem II in a plant is considered the primary site where light-induced delayed light emission (DLE) is produced. With the lamina of zijinghua (*Bauhinia variegata* L.) and soybean (*Glycine max* (L.) Merr.) as testing models, we studied the effects of artificial acid rain and SO_2 on characteristics of DLE by using a home-made weak luminescence detection system. The results show that the changes in DLE intensity of green plants can reflect the changes in chloroplast intactness and function. With proper calibration, DLE may provide an alternative means of evaluating environmental acid stress on plants. The changes in DLE intensity may provide a new approach for the detection of environmental pollution and its impact on the ecosystem. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: chloroplast; artificial acid rain; SO_2; delayed light emission; environmental stress

INTRODUCTION

With the global advancement of environmental protection to improve the quality of our atmosphere and to reduce pollution, instruments for environmental monitoring, especially those based on optical technology, have been rapidly developed. Most commercial instruments can only measure one type of gas at a time. They usually have low resolving power and are easily interfered with by other gases, hence limiting their applications.

Plants are sensitive to environmental stress (1). Poisonous gas usually enters a plant via its stomata in the foliage. Physiological and biochemical changes occur when the foliage is damaged. The changes of foliage reflect not only the presence of pollution but also its extent. Each plant has a different sensitivity to different pollutants and a plant can act as a warning system for a particular pollutant (2).

Delayed light emission (DLE) is a phenomenon of photon emission by a living system after its stimulation by visible radiation (3). For plants, DLE mainly comes from the inverse photochemistry reactions in the plant photosystem (PS) (4). The mechanism is as follows: when light illumination is interrupted, redox product generation from the PS II reaction centre (RC) can be reversed. The oxidized P_{680}^{+} recombines with the primary electron acceptor to produce excited P_{680}^{*}. DLE occurs during the excited P_{680} P_{680}^{*} to P_{680} transition. The process can be expressed as follows:

$$ZP_{680}Q \xrightarrow{h\upsilon} ZP_{680}^{+}Q^{-} \rightarrow ZP_{680}^{*}Q \rightarrow ZP_{680}Q^{+}\ h \cup_{DLE}$$

Where P_{680}^{+} and P_{680}^{*} are chlorophyll a (Chla) belonging to the reaction centre of PS at the oxidized and singlet excited states, respectively; Q is the primary electron acceptor; and Z is the first electron donor of the reaction centre. Because DLE is intrinsically related to the photosynthesis process and the emission time lasts longer than chlorophyll fluorescence, it can provide more valuable information about photosynthetic processes (5, 6). DLE has many practical applications (7–8). The chlorophyll content is directly related to the plant physiological state and function and there exists a linear correlation between the DLE intensity and chlorophyll content (within a limited range) (9). Also the interrelationships between DLE characteristics and leaf senescence (10), decrease in the thylakoid protein content and the photochemical efficiency of photosystem II (11, 12), inactivation of the oxygen evolution system (13), release of manganese (Mn) and degradation of D1 polypeptide have been reported (14). Accordingly, monitoring DLE provides an important method for the analysis of plant physiology.

When biotic or abiotic environmental factors change, they can affect the biochemical or biophysical process of a plant, e.g. inverse environmental factors can result in either reduced synthesis or the breakdown of

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photosynthetic pigment, or loss of water. This leads to a decrease in the photochemical efficiency of PS. DLE is an intrinsic fluorescence label of chemical efficiency in PS (15).

An ideal environmental monitoring system should have three functions: capability of monitoring physical, chemical and biological properties of the environment. Biological inspection is less exact compared with the traditional physical and chemical methods, but nevertheless, it can continuously monitor environmental changes, making real-time inspection possible.

At present, the chlorophyll fluorescence kinetic technique is widely used for monitoring environmental stress (16), but although it is a quick and non-invasive probe for studies of environmental pollution (17), some difficulties have been encountered (18). DLE could be a complementary technique for detecting environmental stress and monitoring the degree of environmental pollution.

MATERIALS AND METHODS

Sampling of plant leaves

The leaves of zijinghua (*Bauhinia variegata* L.) that grow naturally on the campus of South China Normal University (Guangzhou, China) were collected immediately before each measurement. The experiments were processed in the autumn, when the average high temperature is 30°C and the average low temperature is 20°C.

DLE-sensing instrumentation

The DLE detection system was custom-built in our laboratory, based on an intensified CCD ICCD-576-S/1 (−40°C; Princeton Instruments, USA). A diagram of the system is shown in Fig. 1. The entire set-up was housed in a light-tight chamber. Samples were stabilized inside the chamber for 10 min before starting each measurement. The irradiated light intensity was 230 µmol/m^2/s from a 50 W tungsten lamp (Reporter L50H; Sachtler Corp., USA) and the illumination time was 4 s. The data collection started at 0.5 s upon the completion of the light irradiation, and lasted for 5 s. DLE from a sample, after passing a band filter (685IL24.4, Spectro-Film, USA), was collected by a Nikon photographic lens (50 mm, F/1.8; Nikon, Japan) and projected onto a micro-channel plate (MCP) and a cooled CCD assembly. The output signal was collected by a PC and LabVIEW (National Instrument, Version 6.1) via a ST-130 (Princeton Instruments, USA) controller. Registered data is expressed as counts/s (cps).

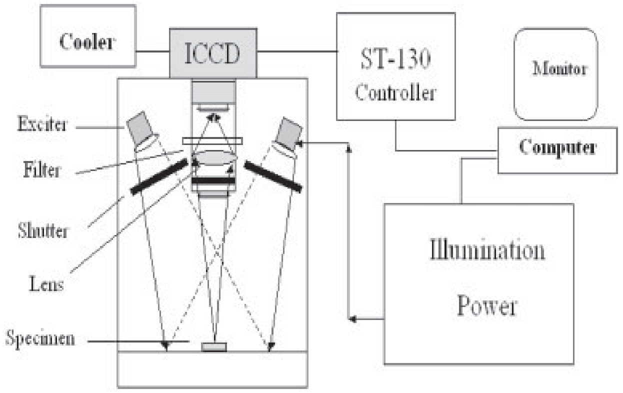

Figure 1. A schematic diagram of the experimental set-up.

The preparation of chloroplasts

The chloroplasts were prepared according to a previously published procedure (19). The main vein was removed and the clean leaves crushed with buffer (0.2 mol/L NaCl, 0.1 mol/L sucrose, 0.05 mol/L phosphate, pH 7.4). The homogenate was filtered through eight layers of cheesecloth. The filtrate was centrifuged for 5 min at $600 \times g$. After removing the precipitate, the supernatant was further centrifuged for 10 min at $500 \times g$. The precipitate (chloroplasts) was dissolved in isotonic solutions (0.05 mol/L NaCl, 0.3 mol/L sucrose, 0.05 mol/L phosphate, pH 6.9). The entire process was conducted at 4°C in a dark environment. The isolated chloroplasts were stored in liquid N_2.

Measurement of the chloroplasts' intactness

A portable dissolution oxygen analyser (JPB-607; Exact Science Apparatus Ltd, Shanghai, China) was used to measure the intactness of the chloroplasts. According to the Hill reaction theory, only chloroplasts with a broken membrane can be photo-oxidized by potassium ferricyanide under the condition of equivalent osmotic pressure. The Hill reaction velocity was measured by an oxygen electrode. Comparing the Hill reaction velocity of ruptured and intact chloroplasts can assess the percentage intactness of chloroplasts (20). The formula is as follows, where B and A are the rate of oxygen evolutions of ruptured and intact chloroplasts, respectively:

$$\frac{B - A}{B} \times 100\% = \%\ \text{intact chloroplasts}$$

Measurement of the chloroplasts' oxygen evolution rate

The thin film oxygen electrode was used to detect the CO_2 dependence of oxygen evolution. The oxygen electrode was calibrated for each experiment, as described previously (21). The reaction medium of the oxygen electrode was the same as the diluted medium used for the experiments with the chloroplasts. 5 mmol/L $NaHCO_3$ and 500 μmol Superoxide dismutase (SOD) were added while detecting the CO_2-dependent oxygen evolution velocity (22).

The cultivation of soybean

Soybean is sensitive to environmental SO_2 (23). It is cultivated according to the procedure of Kim *et al.* (24). Chinese Soybean Seeds (*Glycine max* (L.) Merr.) were submerged in 6% H_2O_2 for 20 min, then allowed to germinate at 25°C. The germinated soybeans were cultivated in 400 cm^3 pots (one plant per pot) in a climate chamber (LRH-250-GSb; Guangdong, China) at a photon flux density (PFD) of 270 $\mu mol/m^2/s$ provided by high-frequency fluorescent strip lights supplemented with tungsten lights, on a 12 h light/12 h dark cycle. The daytime temperature was 28°C and the night-time temperature 16°C. The grown soybean plants used for the experiments were 4 weeks old, when the first trifoliate leaves were fully expanded, at which time they were moved into an open culturing box and divided into two groups. One group was cultured in regular room conditions as a control. The other was cultured in the same room conditions but also exposed to SO_2 gas (1 μl/L), 5 h/day (9:00–14:00 h), and measured after 2 days of treatment. Net photosynthesis rate (*Pn*) and DLE were then measured.

Measurement of net photosynthesis rate (*Pn*)

The *Pn* was measured directly by a commercially available system (LI-6200; LI-COR, USA, courtesy of the Photosynthesis Center of the Life Science Institute, South China Normal University), in a custom-built climate chamber. The climate chamber was equipped with a dew point producer (LI-610; LI-COR, USA) for relative humidity, and a modulated tungsten lamp (max intensity, 900 $\mu mol/m^2/s$) for artificial illumination. The chamber was operated at a stable environmental temperature (28°C). The CO_2 concentration within the chamber was monitored using a CO_2 concentration gauge and was between 320–330 ppm throughout the study.

RESULTS AND DISCUSSION

The effect of artificial acid rain on plant DLE

In China, acid rain is mainly caused by the oxidation reaction of SO_2 with H_2O_2 and O_3 in water droplets (25). Leaves of equal size, with close initial light-induced DLE intensity and weight, were measured simultaneously, after having been submerged in the sulphuric acid (CAS #7664-93-9; JuHua Corporation, China) solution of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} or 10^{-6} mol/L and water for 45 min, respectively (the corresponding pH values are 1.7, 2.7, 3.7, 4.7, 5.7, 7.0). The measurements of DLE intensity are shown in Fig. 2.

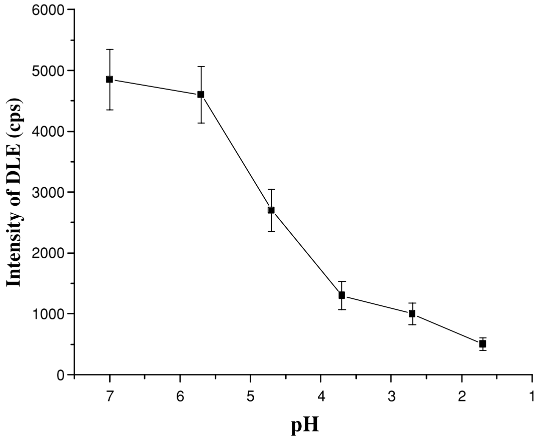

Figure 2. The relationship between the pH value of artificial acid rain and the intensity of DLE. Each data point represents average values ± SD from five samples with three repeated measurements.

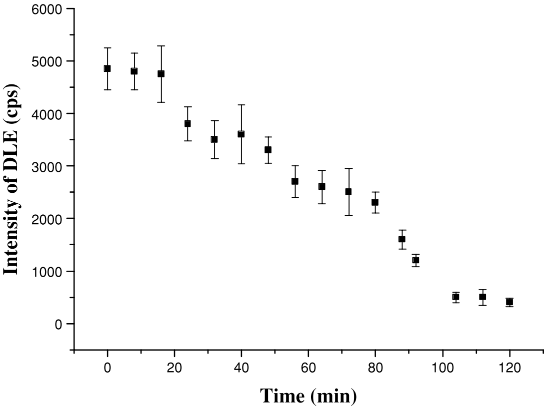

Figure 3. Effect of artificial acid rain on the temporal profile of DLE. Each data point represents average values ± SD from five samples with three repeated measurements.

From Fig. 2, it is seen that the DLE of leaves decreased with pH values of 5.7–3.7. There was no visible damage caused by the acid on the leaf surface. When the pH value was lower than 3.7, small yellow spots started to appear on the leaf surface, and the DLE intensity decreased. Below pH 2.7, most leaves lost their green colour and the same time the DLE almost disappeared.

In order to record the temporal profile of DLE caused by artificial acid rain erosion, leaf samples were submerged in a beaker with 10 mL water. After the baseline DLE intensity was measured, the laminae were subjected to an acid solution of 10^{-5} mol/L (pH 4.7) and DLE values were recorded every 8 min for a total of 2 h. The results (Fig. 3) show that for the first 18 min,

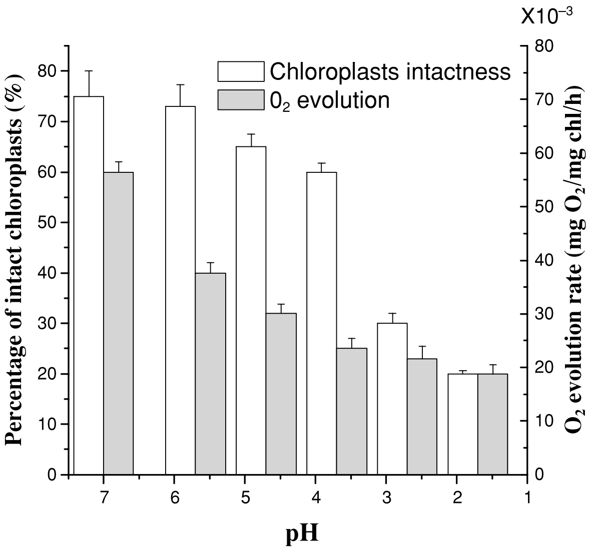

Figure 4. Changes in the intactness of chloroplasts and their function after 2 h erosion of artificial acid rain. Each data point represents average values ± SD from five leaf samples with three repeated measurements.

the action of artificial acid rain (pH 4.7) did not evidently change the DLE intensity. With extended acid action, the intensity of DLE started to decrease. After 2 h of action, the leaves became yellow, and the accompanying DLE disappeared almost completely. Considering that the acid effect may vary for different parts in chloroplast, the intactness and oxygen evolution ability of chloroplasts were evaluated after 2 h of exposure to acids. The results (Fig. 4) show that the chloroplast intactness was nearly identical when leaves were subjected to water (pH 7.0) or weak acid (pH 5.7), yet the oxygen evolution of the chloroplasts was evidently depressed with the acid treatment. This means that although the intactness of the chloroplasts did not change, the oxygen evolution based on CO$_2$ was lower than the minimum value of A type chloroplasts depicted by Hall (50 µg O$_2$/mg chl/h) (26). The results suggest that, with weak acid, the decrease of DLE intensity was due to the loss of the chloroplasts' function, rather than their intactness. Strong acid, however, can destroy both the structure and the function of chloroplasts. There is no reverse action of the photochemistry in the PSII, thus the DLE disappears completely.

The effect of SO$_2$ on plant DLE

Leaf samples were subjected to SO$_2$. Using an ICCD system, the difference in DLE between the control and the experimental groups was evaluated. The results are shown in Fig. 5(A). The photosynthesis rates of the two groups were also measured using a commercial system (Model: LI-6200 LI-COR, USA) for the purposes of comparison. The results are shown in Fig. 5(B).

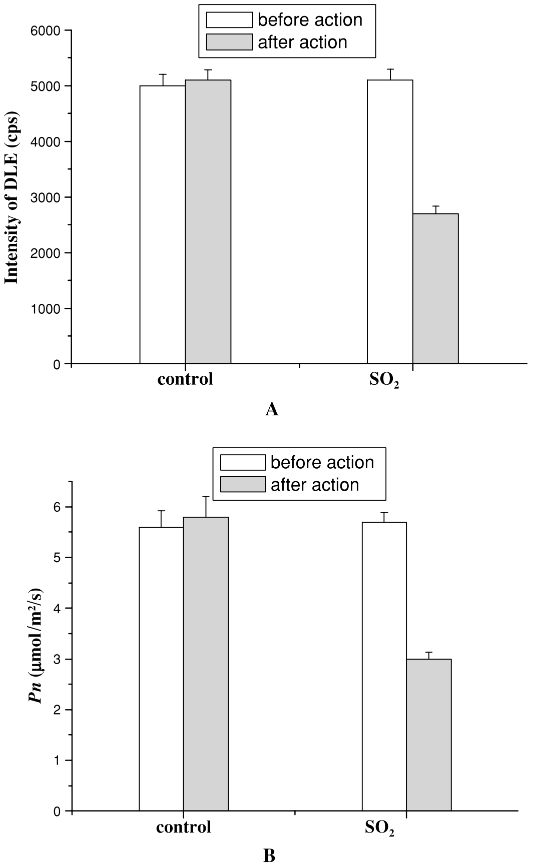

Figure 5. The change of the intensity of DLE (A) and *Pn* (B) in leaves before and after SO$_2$ treatment. Each data point represents average values ± SD from five leaf samples. Fumigated SO$_2$ concentration was 1 µl/L for 2 days.

The statistics showed that the DLE intensities of the control and experimental groups were near to 2:1, identical to that for the photosynthesis rates measured by the commercial system. Both DLE and photosynthesis rate, after the leaves were exposed to SO$_2$, decreased to nearly half of the initial values. Visually, the leaves showed dark green flecks at the beginning, followed by loss of colour. The results suggest that SO$_2$ entered the lamina through the stomata, penetrated though the cell walls, and HSO$_3^-$, SO$_3^{2-}$ and H$^+$ were produced. Then the cell was damaged by the ions, resulting in reduced DLE, mainly through the following three potential mechanisms.

The pH value of the cell was reduced by H$^+$, which caused the stomata to close and a partial transformation of chlorophyll into Ph (phaeophytin). This affected the absorption of both CO$_2$ and light energy, and therefore depressed the ability for photosynthesis and the intensity of DLE (23). Additionally, pH imbalances may alter enzyme conformation and carboxylation reactions and increase mitochondrial respiration (27), all of which would contribute to a lower net photosynthesis (28–29). Second, SO$_3^{2-}$ and HSO$_3^-$ caused direct damage to the

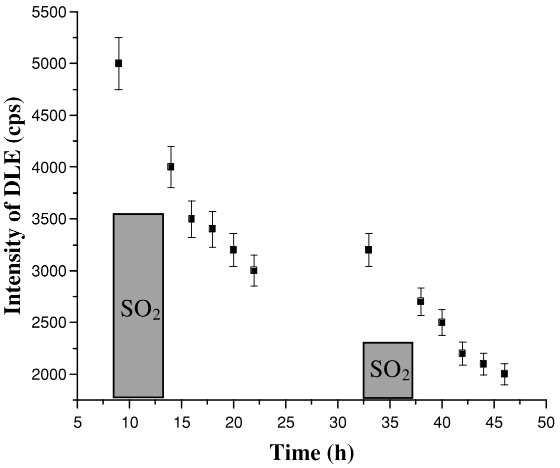

Figure 6. Temporal profile of DLE intensity with SO_2 treatment. Fumigated SO_2 concentration was 1 µl/L. Each data point represents average values ± SD from five leaf samples.

photosynthesis apparatus and resulted in a reduction in redox action products, including deoxidized Q, which also leads to a decrease in DLE (30). The indirect damage caused by SO_3^{2-} and HSO_3^- may be more serious than the direct damage (23). The HSO_3^- reacted with ketone compounds or aldehydes and chemically converted into α-hydrosyl suphonate. This could reduce the activity of glycolic acid oxidase. It also restrained the degree of opening of the stomata and fixation of CO_2 and photosynthetic phosphorylation. Third, damage was generated by the formation of free radicals. O^{2-} was generated in the thylakoid membrane after illumination (31). This led to the oxidation of SO_3^{2-} and HSO_3^- and generated more free radicals (such as HSO_3^-, OH^-, O^{2-}), which decomposed some macromolecule compounds (chlorophyll, DNA, NADH-NADPH). The lipoid could be bleached by peroxide, which resulted in damage to the thylakoid membrane structure (32), hence the reduction of photosynthesis and the subsequent decrease in DLE.

The temporal profile of DLE from samples subjected to SO_2 was obtained by recording DLE intensity every 2 h for a total of 2 days. The samples were treated with SO_2 (1 µl/L) for 5 h (9:00–14:00 h) every day (Fig. 6). The results show that DLE intensity continued to decrease for several hours after the completion of SO_2 treatment. There may be an accumulation of the effect caused by SO_2, similar to that discussed earlier. The minor recovery in DLE intensity in the long term (19 h) may be attributed to the plants intrinsic defence system (33).

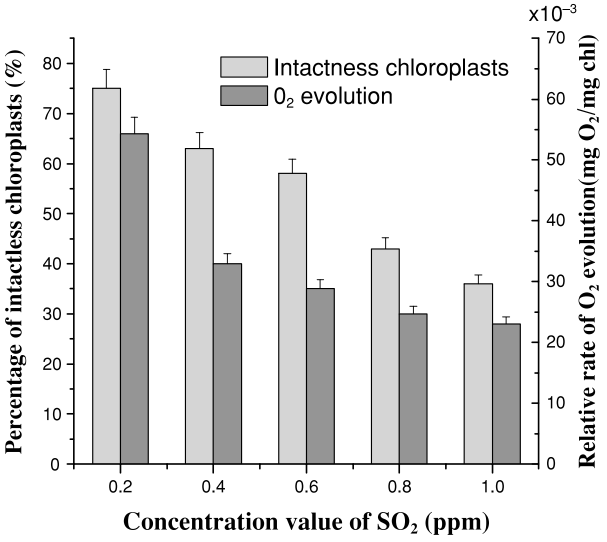

Figure 7. Effects of SO_2 on the intactness and function of chloroplasts. Each data point represents average values ± SD from five leaf samples.

The effects of SO_2 on soybean leaves were further investigated by evaluating the intactness and oxygen evolution ability of the chloroplasts upon exposure to SO_2. The results are shown in Fig. 7. Similar to the findings with acidity, a light exposure to SO_2 had little effect on the intactness of the chloroplasts but could cause a significant change in their oxygen evolution ability. Heavy exposure to SO_2 resulted in severe losses of both intactness and oxygen evolution ability of chloroplasts.

With the actions of acid rain and SO_2, the chlorophyll protein complexes in the thylakoid membrane are oxidized and decompose. This causes the abilities of P_{680} in PSII for charge separation and transport to produce Q^- ($Q_A^-Q_B^{=}$) to decrease, e.g. the total amount of P_{680}^* formed in recombination of P_{680}^+ and Q^- decrease. DLE intensity, directly related to the concentration of Q^- generated in the RC of PSII during light illumination (34), thus, decreases. Comparison of Figs 4 and 7 shows that the environmental impact on a plant first reduces its cellular function, and this is followed by structural and morphological changes. The above experimental results suggest that the changes of DLE intensity can reflect the effects of acid environmental stress on plants. Hopefully, light-induced DLE may provide a new powerful method for the detection of environment pollutants.

CONCLUSION

With the laminae of zijinghua and soybean as a testing model, we have studied the effects of artificial acid rain and SO_2 on characteristics of DLE, using a custom-built weak luminescence detection system. The results show that the changes in DLE intensity of green plants reflect changes in the intactness and functions of chloroplasts. The effects of acid pollutants can be detected quantitatively by means of DLE. The technique may be more practical for field application than the chlorophyll fluorescence technique for evaluating environment stress.

Acknowledgements

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