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Effects of Simulated Acid Rain Stress on Chlorophyll Fluorescence Characteristics and Growth in Leaves of *Lithocarpus glaber* and *Schima superba* Seedlings

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Acid rain is a serious environmental problem worldwide. *Lithocarpus glaber* and *Schima superba* are distributed over large areas in southern China. In this study, the effect of simulated acid rain (SAR) (heavy, pH = 2.5; moderate, pH = 4.0; and control pH = 5.6) on growth, chlorophyll content and leaf chlorophyll fluorescence characteristics of *L. glaber* and *S. superba* were investigated. In *L. glaber*, the leaf relative chlorophyll content (SPAD), the net photosynthetic rate (P_n), the PSII maximum photochemical efficiency (Fv/Fm), actual PSII photochemical quantum yield (ΦPSII), photochemical quenching (qP), plant height and stem diameter all decreased, when acidity of simulated acid rain increased. Regarding non-photochemical quenching (qN), *L. glaber* exposed to simulated acid rain had higher value than control plants. However, when exposed to simulated acid rain, SPAD, P_n, Fv/Fm, ΦPSII, qP, plant height and stem diameter in *S. superba* seedlings were all in the order of moderate acid rain > control > heavy acid rain, suggesting that moderate acid rain promoted photosynthesis and growth of *S. superba* to some extent. These results suggest that *L. glaber* was more sensitive to simulated acid rain than *S. superba* and provide an insight into the possible mechanisms of the action of acid rain on these two important species in China.

Keywords: *Lithocarpus glaber*, *Schima superba*, Acid rain, Photosynthesis, Fv/Fm, ΦPSII.

INTRODUCTION

Rain water in natural state is slightly acidic with a pH value ranging from 5.6 to 7.0 due to the presence of carbon dioxide in the atmosphere. The acid rain means the pH value is lower than 5.6 levels. Nowadays, acid rain has become a global environmental issue, due to inadvertent human interference, such as combustion of fossil fuels and industrial processes[1]. Acid rain stress is also a serious problem in China in recent years. Acid rain monitoring data from the late 1970's demonstrate that rain is acidic in most places throughout China, with pH ranging from 2.3 to 4.47[2]. As one of the detrimental effects, acid rain may result in various toxicity symptoms in plants, including visible effects of injury (chlorosis and:or necrosis) and invisible effects such as reduced photosynthesis, nutrient loss from leaves, altered water balance and variation of several enzyme activities[3-5]. Previous data indicated that acid rain treatment decreased photosynthetic CO_2 fixation and photochemical activity of bean plants. Lipid peroxidation and increased level of H_2O_2 were induced in acid rain-treated bean leaves[6]. Shan *et al.*[7] reported that chlorolhyll content of Armand pine increased but net photosynthetic rate (P_n) on a chlorolhyll content basis decreased with increasing acidity of rain. In addition, acid rain significantly increased the activities of guaiacol peroxidase (GPX) and superoxide dismutase (SOD), but decreased the activity of catalase (CAT) in cucumber seedlings[8].

So far, there have been a number of reports concerning the forest decline in Europe, North America and East Asia[9-10]. For example, trees such as *Pinus armandi* Franch., *Pinus massoniana* Lamb. and *Abies fabri* Craib declined in southwestern China[11]. Although several studies suggested that acid rain decreased germination, seedling growth and chlorophyll content in hardwood tree species[12-13], it is still less understanding to physiological and biochemical responses of woody tree species to acid rain. Both *Lithocarpus glaber* and *Schima superba* are heliophilous and dominant species in forest ecosystems in southern China. They are widespread and useful for firewood and reforestation. The serious damage symptoms of *S. superba* affected by acid rain have been reported in some forests in China[14]. However, the knowledge of the mechanisms by which acid rain affects *S. superba* is limited. There are also few studies on the effect of acid rain on *L. glaber*. Is acid rain one of the reasons for the decrease of the population size of *L. glaber* and *S. superba* in southern China? Therefore, it is necessary to clarify the effect of simulated acid rain (SAR) on the growth and chlorophyll fluorescence characteristics of them in further detail. And also the response of these two wood species to simulated acid rain is compared in this study.

EXPERIMENTAL

The experiment was carried out at the field base of Zhejiang Agriculture and Forest College in Hangzhou, China (119° 44' E, 30° 16'N). The climate here is humid subtropical monsoon weather. The total solar radiation is 1847.3 h annually. The mean annual temperature is 16.4 °C with a maximum of 40.4 °C in July and a minimum of -9.2 °C in January. The average annual precipitation is 1628.6 mm. The mean relative humidity is 80 %. Two tree species (*L. glaber* and *S. superba*) were used in this study. 2-year-old seedlings of *L. glaber* and *S. superba* were collected from broad-leaf and coniferous forests and then transplanted into pots (one plant per pot). The pots were filled with soil collected in the same forests as the plants and placed in an open area. Simulated acid rain (SAR) was prepared by adding both H_2SO_4 and HNO_3 to a base solution, with a 1:1 mol ratio of H_2SO_4 and HNO_3. Young trees were sprayed with a simulated acid rain with a pH 2.5, 4.0 and 5.6 every day.

The response of photosynthetic rate to simulated acid rain was studied in leaves of *L. glaber* and *S. superba* in 2011 (from May to November). Photosynthetic rate (oxygen production) was measured in the field on sunny days with a LI-6400 (Li-Cor, USA). The temperature control of the LI-6400 was set to track the ambient air temperature. Chlorophyll (Chl) fluorescence characteristics were measured in the field on sunny days with a field portable, pulse amplitude, modulated fluorometer (PAM-2100, Walz, Effeltrich, Germany). All measurements were taken on the lamina, midway between the base and the tip of mature leaves. The minimal (dark, Fo) fluorescence yield were obtained with weak modulated light (0.04 μmol m^{-2} s^{-1}) and the maximal (Fm) with a 2 s pulse of saturated light (6000 μmol m^{-2} s^{-1}). The actinic light intensity was 280 μmol m^{-2} s^{-1}. The maximal photochemical efficiency of PSII (Fv/Fm) was expressed as the ratio of variable fluorescence (Fv) to maximum yield of fluorescence (Fm). Photochemical quenching (qP) and non-photochemical quenching (qN) were calculated as qP = (Fm'-Fs)/(Fm'-Fo) and qN = 1-(Fm'-Fo)/(Fm-Fo), according to Schreiber *et al.*[15], respectively. The efficiency of energy conversion in PSII (FPSII) was calculated as (Fm'-Fs)/Fm' (Fs = stationary level of fluorescence emission, Fm' = maximum fluorescence during illumination)[16]. The chlorophyll content of leaves was assessed with a SPAD-502 chlorophyll meter (Minolta, Osaka, Japan). The plant height was measured with a ruler. Stem diameter was determined with a vernier caliper.

All of the measurements were performed 5 times and the means and calculated standard deviations (SD) are reported.

RESULTS AND DISCUSSION

As shown in Fig. 1, *L. glaber* seedlings showed significant growth reductions in response to all simulated acid rain treatments. Growth parameters such as plant height and stem diameter of *L. glaber* decreased with the increasing acidity of simulated acid rain. On the other hand, *S. superba* showed no significant growth reductions under simulated acid rain stress. The plant height and stem diameter of *S. superba* plants were even slightly increased in response to simulated acid rain (pH 4.0) when compared to the control rain (pH 5.6). Several studies also have revealed that *S. superba* is a tolerant species to simulated acid rain[14]. These results suggest that *L. glaber* was more susceptible than *S. superba* when exposed to acid rain.

The changes in plant growth of *L. glaber* and *S. superba* seedlings due to simulated acid rain could be a consequence of reduced photosynthesis. The changes in net photosynthetic rate (P_n) of *L. glaber* and *S. superba* leaves exposed to simulated acid rain are shown in Fig. 2. In *L. glaber* seedlings, P_n expressed on leaf area basis was significantly reduced over the range of the acidities examined and the reduction increased with increasing acidity of the simulated acid rain. Compared to control trees, P_n in *L. glaber* exposed to simulated acid rain (pH 2.5, 4.0) decreased by 29 and 10 %, respectively. The decreased photosynthetic rate was also found in simulated acid rain treated *Pinus densiflora* and *Cucumis sativus*[8]. However,

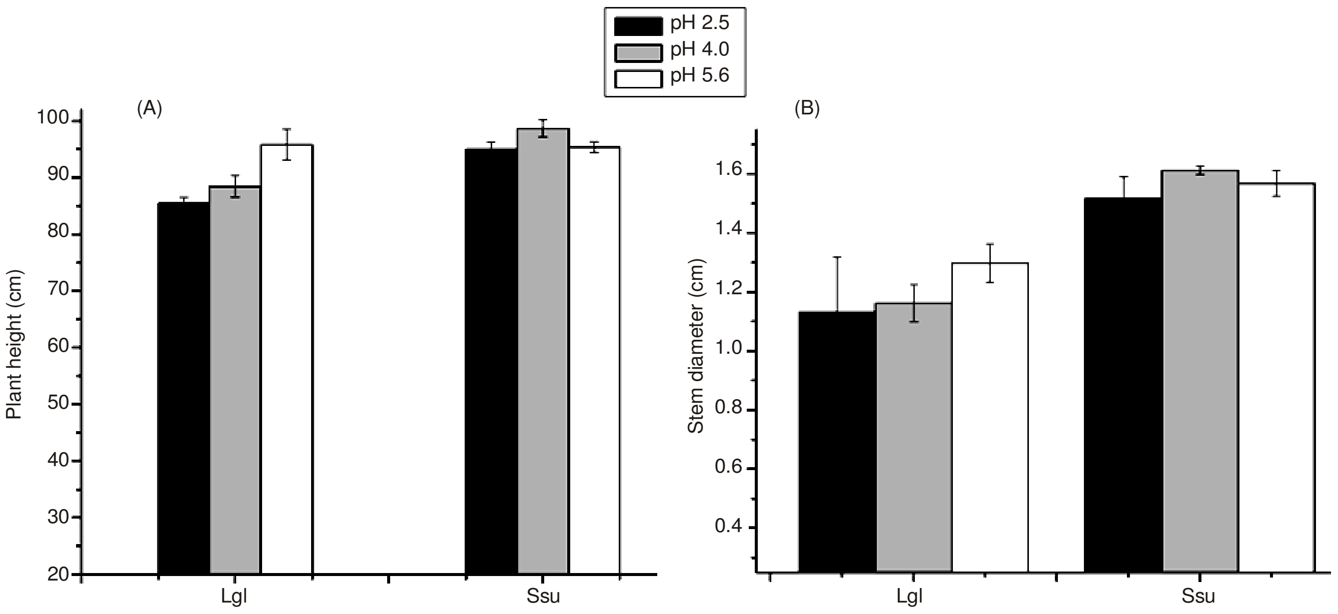

Fig. 1. Changes of plant height (A) and stem diameter (B) in *L. glaber* (Lgl) and *S. superba* (Ssu) leaves under simulated acid rain stress. Values are mean values ± SD, n = 5

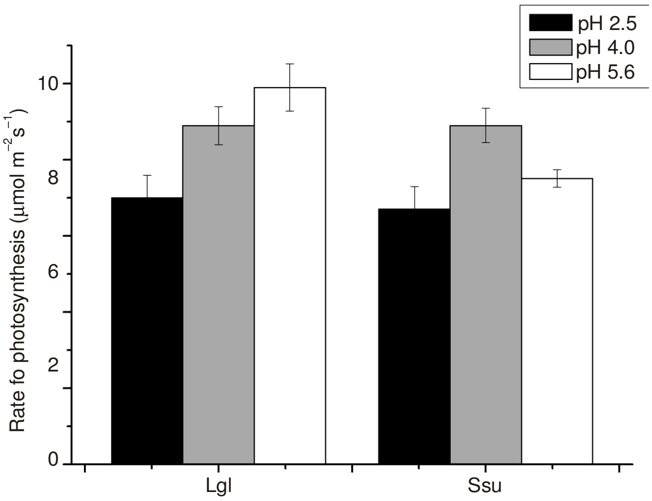

Fig. 2. Changes of net photosynthetic rate (Pn) in *L. glaber* (Lgl) and *S. superba* (Ssu) leaves under simulated acid rain stress. Values are mean values ± SD, n = 5

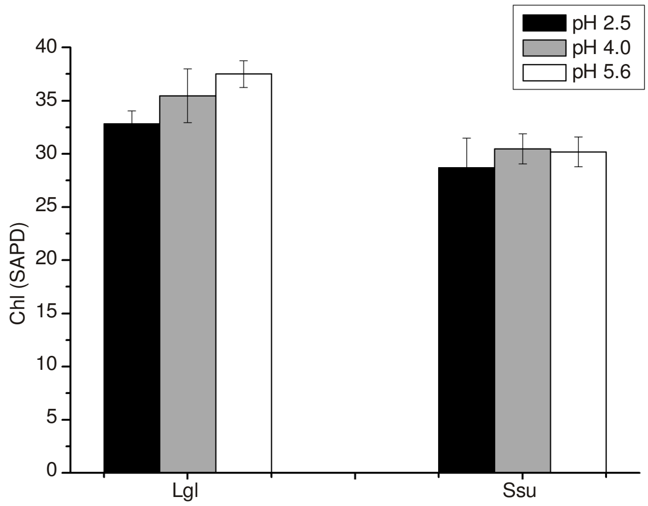

Fig. 3. Changes of chlorophyll content (SAPD) in *L. glaber* (Lgl) and *S. superba* (Ssu) leaves under simulated acid rain stress. Values are mean values ± SD, n = 5

increased P_n in *S. superba* was observed when exposed to pH 4.0 compared to pH 5.6, indicating that slight simulated acid rain increased the photosynthetic rate of *S. superba*. *S. superba* had a higher photosynthetic capacity than *L. glaber* under acid rain stress.

In both plants, simulated acid rain with a pH of 2.5 caused a larger decrease of chlorophyll content than simulated acid rain with a pH of 4.0 (Fig. 3). The decrease of the chlorophyll content in the leaves of *L. glaber* was higher than that in the leaves of *S. superba*. In *S. superba*, the chlorophyll content did not show significant change in simulated acid rain with a pH of 4.0. However, chlorophyll degradation, as was observed during simulated acid rain (pH 2.5) treatment in *L. glaber* and *S. superba* (Fig. 3), has been characterized as a long-term stress event. The lower photosynthetic capacity in plants might be linked to the reduced chlorophyll content in the stressed plants[17].

Acid rain may affect photosynthesis through altered leaf chemistry and morphology, cellular pH balance, carbon partitioning, chloroplast membrane integrity and stomatal conductance[4,18]. The decreased P_n in *L. glaber* may be due to an increased intracellular accumulation of H^+ contained in acid rain; this probably led to uncoupled electron transport and insufficient accumulation of ATP and NADPH[19]. This was confirmed by Chl fluorescence analysis which showed that acid rain treatment induced a significant decrease in Fv/Fm and ΦPSII (Fig. 4). Damage to photosynthesis system was reflected in the depression of maximal photochemical efficiency Fv/Fm. As shown in Fig. 4, Fv/Fm values in *L. glaber* decreased for all simulated acid rain treatments, compared with the controls. The lower Fv/Fm observed was mostly a result of decline in Fm (values not shown), indicating that an impaired capacity for Q_A reduction and increased non-radioactive energy

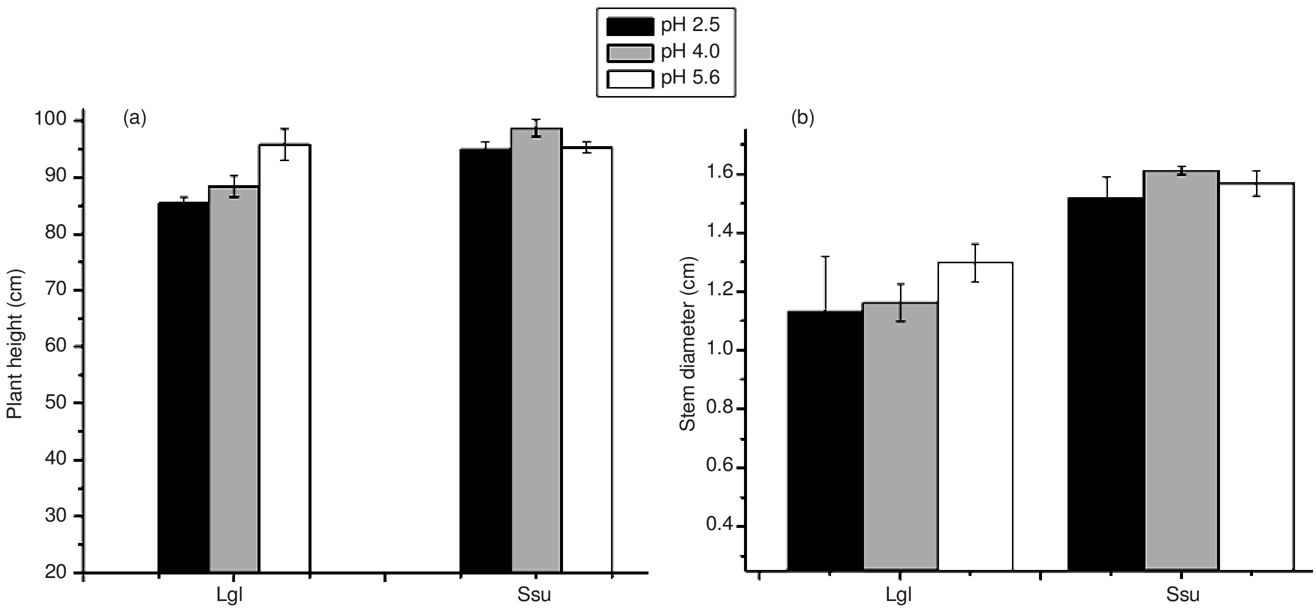

Fig. 4. Changes of chlorophyll fluorescence parameters in *L. glaber* (Lgl) and *S. superba* (Ssu) leaves under simulated acid rain stress. Values are mean values ± SD, n = 5

dissipation upon exposure to simulated acid rain. Simulated acid rain also decreased FPSII, suggesting that electron transport rate in PSII was inhibited in *L. glaber*. In *S. superba*, the Fv/Fm and FPSII values were decreased under simulated acid rain (pH 2.5), but increased under simulated acid rain (pH 4.0), suggesting that slight simulated acid rain promoted photochemical efficiency and electron transport rate of *S. superba* seedlings. Our results in *S. superba* seedlings contradict with the results reported by Liu *et al.*[14]. The P_n, Fv/Fm and FPSII values of *S. superba* were all reduced under simulated acid rain treatments (pH 2.5 and 4.0), compared with the controls[14]. The different responses of *S. superba* plants to acid rain stress might be due to different plant developmental stages, growth conditions, duration and strength of the applied stress. It was reported that qP can be used as a measure of the proportion of closed PSII centers and qN reflects the capacity of plants to carry out non-radiative dissipation of excess energy[20]. Our results showed a decrease in qP and an increase in qN in the simulated acid rain-treated leaves of *L. glaber* and *S. superba*, indicating that simulated acid rain-treated leaves of both trees had higher capacities for dissipating excess energy non-radiatively (Fig. 4).

Conclusion

To conclude, our results showed that acid rain had a negative effect on the photosynthesis, chlorophyll content (SPAD) and growth of *L. glaber* seedlings. The changes in Fv/Fm and ΦPSII in simulated acid rain treatments may be a secondary effect of damage by acidity. However, in *S. superba* seedlings exposed to simulated acid rain, SPAD, Fv/Fm, FPSII, qP, plant height and stem diameter were all in the order of moderate acid rain > control > heavy acid rain, suggesting that moderate acid rain slightly promoted *S. superba* photosynthesis and growth. These results suggested that the effects of simulated acid rain on two wood species in southern China. *L. glaber* was more susceptible to acid rain, compared to *S. superba.*

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