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Effects of Smoke-Water on Photosynthetic Characteristics of *Isatis indigotica* Seedlings

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Abstract

Smoke-water (SW) had been reported to improve the growth of *Isatis indigotica*, a Chinese medicinal plant. However, there were very few reports on the mechanism of smoke-water improving plant growth. In this study the effects of smoke-water on the photosynthetic characteristics of *I. indigotica* seedlings were investigated for the purpose of understanding the mechanism behind this improved plant growth. The results showed that net photosynthetic rate (P_n) was increased by smoke-water, reaching a maximum on 15, 5 and 15 d after treatment with smoke-water at dilutions of 1:500, 1:1000 and 1:2000 respectively. Transpiration rate (T_r) and stomatal conductance (G_s) both showed similar trends to P_n, however, intercellular CO_2 concentration (C_i) was decreased with smoke-water treatment. The F_v/F_m was not significantly influenced by smoke-water treatment. The ΦPSII was markedly promoted with the application of smoke-water (1:1000) compared with the control and the coefficient of photochemical quenching (qP) showed a similar trend to ΦPSII. However the coefficient of non-photochemical quenching of chlorophyll (NPQ) was decreased with treatment of smoke-water. These findings indicate that smoke-water treatment induce an increase in photosynthesis and suggest the main factors leading to this might be the improved stomatal conductance and the enhanced level of the photochemical efficiency of PSII in leaves.

Keywords: *Isatis indigotica*, smoke-water, gas-exchange, chlorophyll fluorescence

1. Introduction

It has been reported that smoke plays an important role in many aspects of plant biology such as seed germination, plant growth and flowering (Kulkarni, Ascough, & Van Staden, 2008). Smoke treatments stimulate seed germination of more than 1200 species (Brown & Botha, 2004; Dixon, Merritt, Flematti, & Ghisalberti, 2009), and also markedly improve plant growth in certain cases. It's been demonstrated that soaking in smoke-water enhance the vigour of *Zea mays* seedling (Sparg, Kulkarni, & Van Staden, 2006). The yield of crop has been enhanced with the treatment of smoke-water, including okra, tomato and onion (Kulkarni, Ascough, & Van Staden, 2007; Kulkarni, Ascough, & Van Staden, 2008; Kulkarni, Ascough, Verschaeve, Baeten, Arruda, & Van Staden, 2010; Aremu, Bairu, Finnie, & Van Staden, 2012). The great potential of smoke treatments for the use in horticulture and agriculture has been shown. However few studies on the mechanism of how smoke-water improving plant growth have been reported.

Isatis indigotica is one of the most popular Traditional Chinese medicine (TCM), which plays an important role in keeping people's health, and is used as anti-leukemia, antipyretic and anti-influenza agents (Liu, Huang, Lin, Wu, & Lin, 2005; Kunikata, Tatefuji, Aga, Iwaki, Ikeda, & Kurimoto, 2000; Mak, Leung, Wei, Shena, Wonga, Leungd, & Funge, 2004). Due to its medicinal importance, the demand for this herb has improved the cultivation practices of *I. Indigotica*. Our research group has reported that the growth of *I. indigotica* seedlings was improved by the treatment with smoke-water (Zhou, Van Staden, Guo, & Huang, 2011). However, few studies on the mechanism of smoke-water improving the growth of seedlings have been previously reported. Photosynthetic characteristics are important indicators which reflect the accumulation of plant biomass. Thus, examining the effects of smoke-water on the parameters of photosynthetic characteristics will lead to a greater understanding of the mechanism of smoke-water in improving plant growth. Therefore this study was carried out to explore the mechanism of how smoke-water improves plant growth by examining the photosynthetic gas-exchange characteristics and chlorophyll fluorescence for the purpose of providing a theoretical basis for the application of smoke-water in the cultivation of medicinal plants.

2. Materials and Methods

Seeds of *I. indigotica* were sown in a mixture of garden soil and river sand and incubated at 25±1°C. After the third leaf developed uniform seedlings were transplanted into plastic pots filled with quartz sand and half-strength Hoagland's solution (HS) was used as growth medium (Zhou, Van Staden, Guo, & Huang, 2011). The seedlings were used for trial after emergence of the sixth leaf, HS containing smoke-water with dilutions of 1:500, 1:1000 and 1:2000 were applied and an equivalent amount of HS was used as control (100 ml per pot). There were 10 replicates for each treatment and the quartz sand was re-moistened with 50 ml HS once a week. Seedlings were incubated at 25/22°C (day/night) under a 14 h photoperiod with 280 $\mu mol/m^2 \cdot s$ at a relative humidity of 65%. Smoke-water was prepared as described by Van Staden et al. (2004), *Platanus orientalis* and *Platanus occidentalis* were used as plant material.

Gas-exchange parameters and chlorophyll fluorescence of seedlings were determined at 9:00 am to 11:30 am on 0, 1, 3, 5, 7, 11 and 15 d following treatment. Photosynthesis system (Li-6400, USA) was used to measure the P_n, T_r, C_i and G_s at leaf temperature of 25°C and constant CO_2 level of 380 $\mu mol\ mol^{-1}$. Chlorophyll fluorescence was measured using a portable chlorophyll fluorometer (Opti-Science, USA). Minimum fluorescence (F_o) and maximum fluorescence (F_m) were obtained by imposing a 1s saturating flash to the leaf. The steady-state fluorescence (F_s) was obtained on light-acclimated leaf by imposing the same saturating flash. A second saturating pulse of white light was imposed to determine the maximum fluorescence level (F_m') in the light-adapted state. The actinic light was turned off and the minimal fluorescence level in the light-adapted state (F_o') was obtained by illuminating the leaf with far-red light. The maximum potential photochemical efficiency of PSII was calculated as $F_v/F_m = (F_m - F_o)/F_m$; the actual efficiency of PSII (ΦPSII) was expressed as $\Phi PSII = (F_m' - F_s)/F_m'$ (Genty, Briantais, & Baker, 1989); the coefficient of photochemical quenching (qP) and the coefficient of non-photochemical quenching of chlorophyll (NPQ) were estimated as $qP = (F_m' - F_s)/(F_m' - F_o')$, $NPQ = F_m/F_m' - 1$ (Baker, 2008; Demmig, Adams, Barker, Logan, & Bowling, 1996).

Statistical analysis was conducted using SPSS 13.0 software. Data were subjected to one-way analysis of variance (ANOVA) and differences were considered significant at $p < 0.05$.

3. Results and Discussion

As shown in Table 1, P_n of the seedlings was enhanced and reached the maximum values with the treatment of smoke-water at 1:500, 1:1000 and 1:2000 on 15, 5 and 15 d after treatment, which were estimated to be 14.34%, 17.14% and 9.63% higher than the control, respectively. T_r and G_s showed similar trends to P_n. T_r for smoke-water at 1:500 and 1:1000 was significantly improved by smoke-water treatment at 1:500 and 1:1000 on 3, 5, 7, 11 and 15 d. C_i was decreased by the treatment of smoke-water, which reached a minimum on days 15, 11, and 15 after the treatment with smoke-water at dilutions of 1:500, 1:1000, and 1:2000, respectively. Enhancing gas exchange, photochemical activities and CO_2 fixation can induce an increase in photosynthesis. In this study treatment with smoke-water, especially at the dilution of 1:1000, increased of P_n, T_r and G_s of *I. indigotica* seedlings, which suggests that the treatment of smoke-water likely could enhance the stomatal opening. Thus the induction of stomatal opening by smoke-water treatment may be one of the mechanisms for increasing in photosynthesis observed.

The effects of smoke-water on chlorophyll fluorescence parameters of *I. indigotica* seedlings are presented in Figure 1. The value of F_v/F_m was not enhanced by smoke-water treatment (Figure 1A). The ΦPSII was markedly enhanced by smoke-water at 1:1000 compared to the control (Figure 1B). Maximum values of ΦPSII for smoke-water at 1:1000 were recorded on 1 d after treatment. The qP showed a similar trend to the ΦPSII, however, the NPQ was markedly decreased by the treatment of smoke-water (Figure 1D). Chlorophyll fluorescence measurements are regarded as a sensitive diagnostic technique for determining the effects of environmental factors on photosynthesis (Yang, Yi, & Prasad, 2009). Photochemical reactions, fluorescence (non-photochemical) and heat dissipation are three ways by which the light energy absorbed by chlorophyll molecules is consumed. F_v/F_m, ΦPSII, and qP are used to describe photochemical-quenching parameters, while NPQ for non-photochemical-quenching parameter (Genty, Briantais, & Baker, 1989). In this study ΦPSII, qP and NPQ fluctuated simultaneously, suggesting the absorbed energy was redistributed in the different ways. ΦPSII and qP were increased by smoke-water treatment, indicating that much of the energy is used for the photosynthetic electron transfer. This implies that smoke-water treatment enhances P_n by exploiting the harvested light energy more efficiently in photosynthesis.

Table 1. Effects of smoke-water on P_n, T_r, C_i, and G_s of *I. indigotica* seedlings on 0, 1, 3, 5, 7, 11 and 15d after treatment (mean ± SD). Different letters indicate significant differences at $p<0.05$ when compared with the control according to the Duncan's multiple range test

P_n (μm·m^{-2} s^{-1})	**0d**	**1d**	**3d**	**5d**	**7d**	**11d**	**15d**
Control	4.98±0.19a	4.80±0.16a	4.82±0.13b	4.90±0.18c	4.89±0.25c	5.03±0.20b	5.09±0.19c
SW（1:500）	5.02±0.15a	4.98±0.17a	5.09±0.14a	5.55±0.13b	5.40±0.18a	5.10±0.10b	5.82±0.16a
SW（1:1000）	5.01±0.23a	4.90±0.14a	4.98±0.18b	5.74±0.15a	5.71±0.24a	5.64±0.21a	5.71±0.13b
SW（1:2000）	5.00±0.18a	4.77±0.19a	4.79±0.16b	5.05±0.14c	5.07±0.13b	5.24±0.25b	5.58±0.23b
T_r (mm·m^{-2}s^{-1})	**0d**	**1d**	**3d**	**5d**	**7d**	**11d**	**15d**
Control	0.87±0.05a	0.84±0.08a	0.93±0.13b	0.95±0.17b	0.87±0.19c	0.81±0.15b	0.92±0.14b
SW（1:500）	0.81±0.03a	0.87±0.08a	1.48±0.11a	1.15±0.11a	1.11±0.17a	1.31±0.28a	1.15±0.17a
SW（1:1000）	0.89±0.02a	0.91±0.09a	1.02±0.12b	1.30±0.25a	1.26±0.06a	1.34±0.25a	1.12±0.16a
SW（1:2000）	0.86±0.03a	0.80±0.09a	0.92±0.14b	0.83±0.09b	1.05±0.09b	0.82±0.35b	0.81±0.23b
C_i (μmol·mol^{-1})	**0d**	**1d**	**3d**	**5d**	**7d**	**11d**	**15d**
Control	251.00±12.83a	240.83±32.10a	253.17±27.47a	254.13±30.01a	245.83±15.7a	256.17±22.13a	249.17±27.53a
SW（1:500）	248.67±31.82a	232.50±25.32a	209.17±33.05b	218.33±25.93b	216.67±26.6b	212.03±35.62b	207.83±34.20b
SW（1:1000）	244.83±26.48a	248.17±23.18a	225.33±23.04b	213.80±23.57b	217.00±27.3b	212.22±21.01b	212.67±31.35b
SW（1:2000）	243.17±27.19a	242.50±23.45a	225.50±25.63b	245.30±28.05a	221.83±23.9b	247.83±14.39a	217.67±21.62b
G_s (mol·m^{-2}s^{-1})	**0d**	**1d**	**3d**	**5d**	**7d**	**11d**	**15d**
Control	0.05±0.007a	0.04±0.002a	0.04±0.003c	0.04±0.002b	0.04±0.008b	0.05±0.010b	0.05±0.010b
SW（1:500）	0.05±0.002a	0.04±0.001a	0.06±0.009a	0.06±0.008a	0.05±0.009a	0.06±0.008a	0.06±0.017a
SW（1:1000）	0.05±0.009a	0.05±0.007a	0.06±0.007a	0.06±0.005a	0.06±0.016a	0.07±0.019a	0.05±0.008a
SW（1:2000）	0.04±0.002a	0.05±0.005a	0.05±0.005b	0.06±0.010a	0.04±0.015b	0.05±0.010b	0.05±0.009b

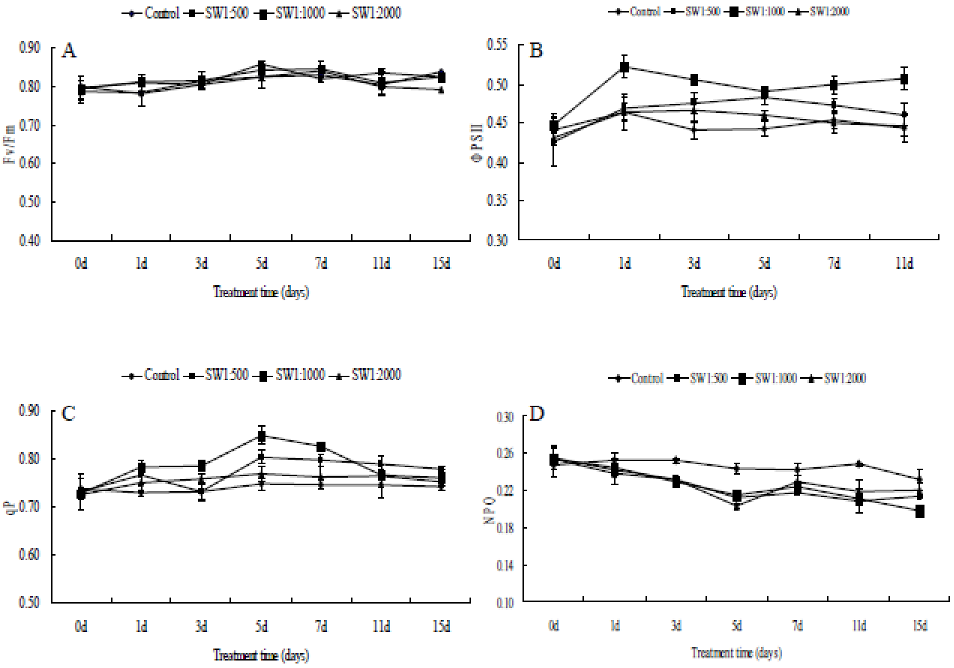

Figure 1. Effects of smoke-water on F_v/F_m (A), ΦPSII (B), qP (C), and NPQ (D) of *I. indigotica* seedlings on 0d, 1d, 3d, 5d, 7d, 11d and 15d after treatment

In conclusion, the photosynthesis of *I. indigotica* seedlings could be enhanced with the treatment of smoke-water. Smoke-water enhances P_n by inducing of stomatal opening and exploiting the harvested light energy more efficiently in photosynthesis.

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