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RESEARCH ARTICLE

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Effects of exogenous spermidine on the photosynthesis of *Cucumis sativus* L. seedlings under rhizosphere hypoxia stress

Abstract With water culture, this paper studied the effects of exogenous spermidine (Spd) on the net photosynthetic rate (P_n), intercellular CO_2 concentrations (C_i), stomatal conductance (G_s), transpiration rate (T_r), apparent quantum yield (Φ_c), and carboxylation efficiency (*CE*) of cucumber seedlings under hypoxia stress. The results showed that P_n decreased gradually under the hypoxia stress, and reached the minimum 10 days later, which was 63.33% of the control. Compared with that of the hypoxia-stressed plants, the P_n 10 days after the application of exogenous Spd increased by 1.25 times. A negative correlation ($R^2 = 0.473–0.7118$) was found between P_n and C_i, and G_s and T_r changed in wider ranges, which decreased under the hypoxia-stress, but increased under the hypoxia-stress plus exogenous Spd application. There was a significant positive correlation between G_s and T_r ($R^2 = 0.7821–0.9458$), but these two parameters had no significant correlation with P_n. The hypoxia stress induced a decrease of Φ_c and *CE* by 63.01% and 72.33%, respectively, while the hypoxia stress plus exogenous Spd application made Φ_c and *CE* increase by 23% and 14%, respectively. The photo-inhibition of cucumber seedlings under hypoxia stress was mainly caused by non-stomatal inhibition, while the exogenous Spd alleviating the hypoxia stress by repairing photosynthesis systems.

Keywords hypoxia stress, exogenous spermidine, photosynthesis, *Cucumis sativus* L.

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1 Introduction

Soilless culture has been the core technique of industrialized high-efficiency agriculture. Compared with traditional soil cultivation, soilless culture can regulate the environmental conditions of the root system according to the growth and development of the crop. Therefore, soilless culture can yield high quality horticultural products. On the other hand, soilless culture has been the most effective method to solve the more and more serious problems in protected horticulture such as soil pollution, soil secondary salinization and continuous cropping obstacles. In soilless culture, the dissolved oxygen concentration in the nutrient solution is lower than that in hydroponics and the dissolved oxygen is often consumed quickly by crops. In substrate culture, root mat is often formed, which may aggravate the oxygen deficiency of the root system. Therefore, hypoxia stress exists widely in soilless culture. Hypoxia stress has been a limitation in popularizing soilless culture (Avijie et al., 2002; Guo, 2003).

The application of exogenous regulators has been a convenient and effective method to enhance the stress-tolerance of crops (Wang et al., 2005a). Polyamines (PAs) are a kind of secondary metabolite produced in the nitrogen metabolism in living organisms. The major forms of PAs are putrescine (Put), spermidine (Spd), spermine (Spm) and cadarerine (Cad). PAs were reported to be involved in plant responses to the biotic and abiotic stress (Elisabeth and Martin-Tanguy, 1995; He et al., 2002). PAs can repair the lamella structure of chloroplasts in stressed plants, and protect the photosynthetic organ (Drolet et al., 1986). Therefore, PAs can help maintain normal photosynthesis.

Photosynthesis is fundamental to crop yields. Since each stress can influence plant photosynthetic capacity, many researchers have focused on the photosynthetic physiology of stressed plants. The mechanisms of photosynthesis inhibition are different between species and stresses (Cornic, 2000; Jie et al., 2001; Ma et al., 2005; Zhang et al., 2003). The early response to oxygen deficiency during waterflooding is reported to be stoma closure, which may result in a rapid decrease of photosynthetic rate (Yu and Tang, 2001). In the present experiment, the effects of exogenous Spd on photosynthetic parameters of cucumber seedlings under hypoxia stress were investigated. Our objective was to understand the physiological function of Spd in the tolerance of cucumber to the rhizophere hypoxia stress, and to provide a theoretical basis to mitigate the damage of hypoxia stress in soilless culture.

2 Materials and methods

2.1 Plant materials and treatments

This experiment was conducted from March to June in 2004. In this study, the cucumber cultivar Jinchun No.2 was used as plant material. The cucumber seedlings were grown in natural daylight in a glasshouse at Nanjing Agricultural University. Day temperatures ranged from 25°C to 30°C, with night temperatures from 16°C to 20°C. When the third true leaf expanded, seedlings in uniform size were transplanted to flumes filled with half-strength Hoagland nutrient solution. Three days later, when the cucumber seedlings were fully acclimated in control conditions, they were treated as follows: Normal culture as control (CK), with dissolved oxygen (DO) of nutrient solution kept about 8 $\text{mmol}\cdot\text{L}^{-1}$ by vigorous aeration with an air pump; I. Hypoxia treatment, with the DO kept at $(1 \pm 0.5)\ \text{mmol}\cdot\text{L}^{-1}$ by a DO analyzer (QUANTUM-25); II. Spd treatment, with the cucumber seedlings under hypoxia stress sprayed with 20 mL of 1 $\text{mmol}\cdot\text{L}^{-1}$ Spd on leaves per plant at 8:00 and 18:00 everyday. The photosynthetic parameters of the cucumber seedlings were determined once every two days. After 10 days of treatment, both apparent quantum yield (Φ_c) and carboxylation efficiency (*CE*) were determined. Thirty plants of cucumber seedlings were grown in each treatment, and were analyzed by random sampling with three replicates. The whole experiment was repeated three times, with 14 days for each treatment.

2.2 Determination methods

Photosynthetic parameters of cucumber seedlings were determined with the portable photosynthetic analyzer (Li-6400, Li-Cor Inc, USA). The net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r) and intercellular CO_2 concentrations (C_i) were measured between 10:00 and 12:00, using the third true leaf of the cucumber seedling. The open-air pathway was used, and the rate of air current was about 500 $\text{mL}\cdot\text{min}^{-1}$. The temperature of the leaf chamber was controlled at 25°C, the air relative humidity was about 50%, the ambient CO_2 concentration was adjusted to $(380 \pm 10)\ \mu\text{mol}\cdot\text{mol}^{-1}$, and the photo flux density (PFD) was maintained at 900 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with a man-made light source. After the selected leaf was acclimated to the constant conditions for 5 min, three plants of each treatment were measured and each leaf was given three replicates. Finally, the mean value was calculated.

Both apparent quantum yield (Φ_c) and carboxylation efficiency (*CE*) were determined according to the method described by Xu (1999) with minor modifications. Φ_c was measured as the following. The ambient CO_2 concentration was maintained at 400 $\mu\text{mol}\cdot\text{mol}^{-1}$. The PFD of the man-made light source was adjusted to 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at first, then decreased to 500, 300, 150, 120, 90, 60 and 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ successively. The measurements were carried out by an auto-program of light curve, with the data recorded once every 3 min. Then, the light response curve was given with PFD as abscissa axis and P_n as vertical axis. By regression method, its initial slope was viewed as Φ_c. *CE* was measured as follows: The PFD of the man-made light source was maintained at 900 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. In a man-made CO_2 steel cylinder, the CO_2 concentration was adjusted to 250 $\mu\text{mol}\cdot\text{mol}^{-1}$ at first, then decreased to 250, 200, 150, 100 and 50 $\mu\text{mol}\cdot\text{mol}^{-1}$ successively. The measurements were carried out by ~ Ci auto-program, with the data recorded once every 3 min. Then, the CO_2 response curve was given with CO_2 concentration as abscissa axis and P_n as vertical axis. By regression method, its initial slope was viewed as *CE*.

2.3 Statistical analysis

All statistical tests were performed using the computer software Microsoft Excel and SAS. Means were compared using Duncan's multiple-range test. Significant levels were reported as $P < 0.05$.

3 Results

3.1 Effect of PFD on P_n of cucumber seedlings

The light saturation point of cucumber seedling was 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 1). When PFD was below 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, a linear relation was found between P_n and PFD ($Y = 0.0249X–4.2088$; $R^2 = 0.9105$). For cucumber seedlings, the dark respiration rate was 4.2088 $\mu\text{mol}\ CO_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the light compensation point was 169.0281 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The results showed that it is suitable to adjust the fixed light source to 900 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

3.2 Effects of exogenous Spd on P_n, G_s, T_r and C_i of cucumber seedlings under the hypoxia stress

P_n of cucumber seedlings began to decrease two days after the hypoxia stress, reached to the minimum 10 days later by 63.33% of the control, then the P_n appeared

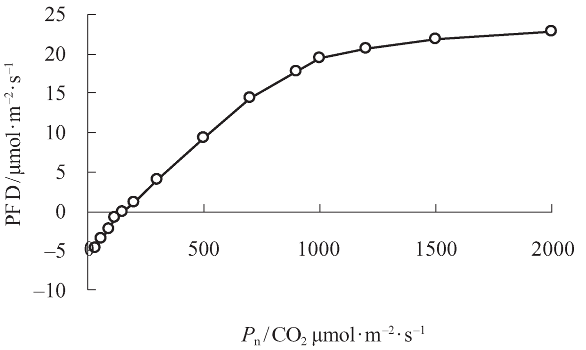

Fig. 1 Light response curve of net photosynthetic rate (P_n) with photo flux density (PFD) of cucumber seedlings

constant and was lower than that of the control significantly (Fig. 2). On the 14th day of treatment, the P_n of cucumber seedlings under the hypoxia stress was 61.83% of the control. Compared with the cucumber seedlings under the hypoxia stress only, the P_n of cucumber seedlings treated by the exogenous Spd combined with the hypoxia stress changed with a similar tendency to that of the hypoxia stress. However, it was significantly higher than that of the hypoxia stress, reaching the minimum on the 10th day by 1.25 times of that under the hypoxia stress, with 1.28 times of that under the hypoxia stress 14 days later. The results showed that the hypoxia stress decreased the P_n of cucumber seedlings, while the foliar spraying with exogenous Spd induced an increase in the P_n of cucumber seedlings under the hypoxia stress.

The G_s of cucumber seedlings changed in a wider range during the period of treatment (Fig. 2). Compared with the control, the G_s of cucumber seedlings under hypoxia stress decreased obviously, reaching the minimum on the 6th day by 63.96% of the control. Then, the G_s increased slightly, maintaining a lower level than that of the control significantly. Also, the G_s reached 82.32% of the control 14 days after treatment. Compared with the cucumber seedlings under the hypoxia stress only, spraying the exogenous Spd induced a minor increase in the G_s of cucumber seedlings under the hypoxia stress. The G_s of cucumber seedlings treated with the exogenous Spd combined with the hypoxia stress reached the minimum on the 6th day by 1.32 times of that of seedlings treated with the hypoxia stress only, by 1.18 times of the seedlings under the hypoxia stress 14 days after the application of exogenous Spd. The results showed that the hypoxia stress reduced the G_s of cucumber seedlings significantly, while spraying the exogenous Spd could improve the G_s of cucumber seedlings under hypoxia stress.

T_r of cucumber seedlings had a similar tendency to G_s under hypoxia stress (Fig. 2). The correlation coefficients R^2 of three treatments were 0.9458, 0.7821 and 0.8844, respectively. The T_r of cucumber seedlings changed in a wider range during the whole treatment. Under the hypoxia stress, T_r decreased obviously, reached the minimum by 63.61% of the control, then increased maintaining a lower level than that of the control significantly. The T_r of cucumber seedlings reached 87.43% of the control 14 days after the hypoxia stress. Compared with the cucumber seedlings treated with the hypoxia stress only, the exogenous Spd could induce an increase in the T_r of cucumber seedlings under the hypoxia stress. The T_r reached the minimum 6 days after the application of exogenous Spd by 1.24 times of that under the hypoxia stress only, and was 1.12 times of that under the hypoxia stress 14 days after the application of exogenous Spd. The results showed that the T_r of cucumber seedlings under the hypoxia stress decreased more significantly than that of the control, while the foliar spraying with Spd could improve the T_r of cucumber seedlings under the hypoxia stress.

A negative correlation was found between C_i and P_n of cucumber seedlings, and the correlation coefficients R^2 of three treatments were 0.642, 0.7118 and 0.473, respectively (Fig. 2). Under hypoxia stress, C_i of cucumber seedlings began to increase two days later, reaching the maximum by 1.31 times of the control, then decreasing gradually at a higher level than that of the control, and finally reaching 1.27 times of that of the control 14 days after the hypoxia stress. Compared with the cucumber seedlings under the hypoxia stress only, the application of exogenous Spd could decrease the C_i slightly, reaching the minimum 14 days later by 93.07% of the control. The results showed that the C_i of cucumber seedlings increased more significantly than that of the control under the hypoxia stress, while the foliar spraying with exogenous Spd could decrease the C_i of cucumber seedlings under the hypoxia stress.

3.3 Effects of exogenous Spd on Φ_c of cucumber seedlings under hypoxia stress

A linear correlation was found between P_n and the PFD of cucumber seedlings in each treatment (Fig. 3). The correlation equations were as follows.

For CK treatment, the equation was $Y = 0.0292X - 4.9311$ ($R^2 = 0.9916$).

For I treatment, the equation was $Y = 0.0184X - 3.1666$ ($R^2 = 0.9728$).

For II treatment, the equation was $Y = 0.0226X - 3.766$ ($R^2 = 0.9717$).

The Φ_c of cucumber seedlings under the hypoxia stress was 63.01% of the control, and the foliar spraying with exogenous Spd can improve the Φ_c by 23%.

3.4 Effects of exogenous Spd on *CE* of cucumber seedlings under hypoxia stress

A linear correlation was found between P_n and C_i of cucumber seedlings in each treatment (Fig. 4). The correlation equations were as follows.

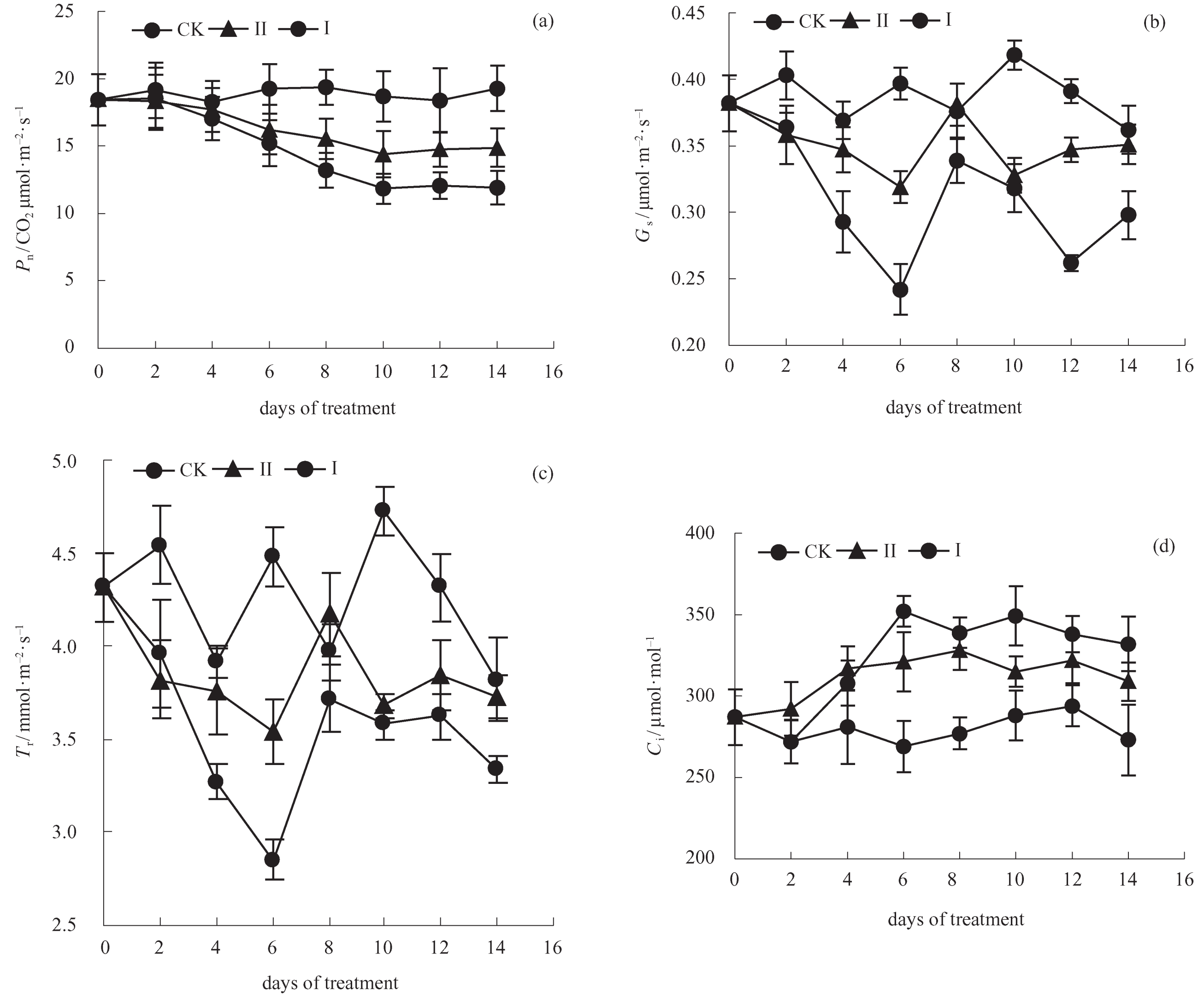

Fig. 2 Effects of exogenous Spd on P_n, G_s, T_r and C_i of cucumber seedling under the rhizosphere hypoxia stress

For CK treatment, the equation was $Y = 0.0907X - 3.8389$ ($R^2 = 0.9874$).

For I treatment, the equation was $Y = 0.0656X - 4.6781$ ($R^2 = 0.9788$).

For II treatment, the equation was $Y = 0747X - 4.2845$ ($R^2 = 0.9927$).

The *CE* of cucumber seedlings under the hypoxia stress was 72.33% of the control, and the foliar spraying with exogenous Spd can improve the *CE* by 14%.

4 Discussion

Photosynthetic inhibition of stress includes stomatal and non-stomatal limitations in higher plants. On the one hand, the stoma closure in the stressed plant may induce a decrease in the C_i, blocking the CO$_2$ transport to the chloroplast, resulting in a photosynthetic inhibition, which is named as the stomatal limitation (Berry and Downton, 1982). On the other hand, the photosynthetic inhibition in the stressed plant may result from the non-stomatal factors such as blocking the CO$_2$ diffusion in mesophyll cells, reducing the CO$_2$ solubility, decreasing both the affinity of RuBP carboxylase to CO$_2$ and the stability of photosynthetic apparatus, which is named as the non-stomatal limitation (Xu et al., 1987). For the stressed-plant, when both C_i and P_n decreased with a similar trend while the stomatal limitation value (*L*s) increased at the same time, the photosynthetic inhibition could be caused mainly by reducing the G_s. When the P_n decreased, the C_i increased and the *L*s decreased, the photosynthetic inhibition may be caused mainly by non-stomatal factors (Berry and Downton, 1982).

For the stressed-plant, the mechanism of photosynthetic inhibition may change with species and stress,

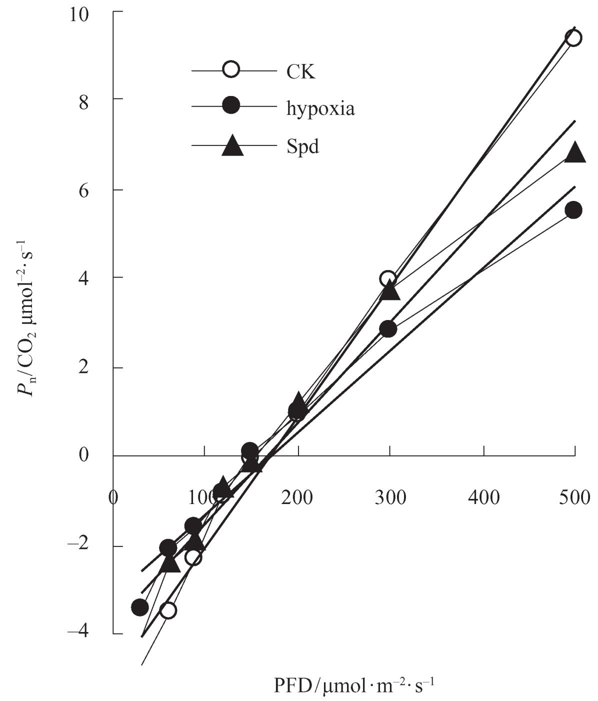

Fig. 3 Effects of exogenous Spd on apparent quantum yield (Φ_c) of cucumber seedling under the rhizosphere hypoxia stress

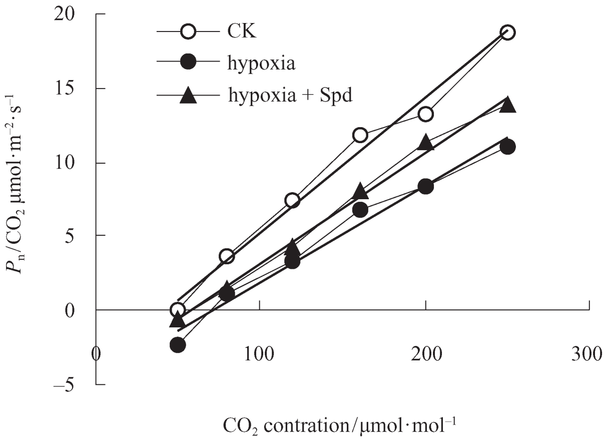

Fig. 4 Effects of exogenous Spd on carboxylation efficiencies (*CE*) of cucumber seedling under the rhizosphere hypoxia stress

and the importance of stomatal limitation or non-stomatal limitation would change during the period of stress. It was reported that the P_n of *Capsicum fructescens* L. decreased consistently with the C_i under a high-temperature stress such as 35°C–40°C, while under the stress of 45°C–50°C, the P_n decreased obviously, accompanied by a minor change in the C_i under the 45°C stress, and by an increase in the C_i under the 50°C stress. The results indicated that photosynthetic inhibition was caused mainly by both the non-stomatal limitation under a serious high-temperature stress and the stomatal limitation under a lower high-temperature stress (Wu et al., 2001). The stoma closure in water-flooding-sensitive plants was one of the earliest responses to the oxygen-deficiency stress, which could induce a rapid decrease in photosynthesis. Furthermore, the later decrease of photosynthesis may be related to the carboxylase activity, chlorisis, senescence and abscission of leaves, resulting in a growth inhibition or plant death (Wang et al., 2002; Yu and Tang, 2001). In the present experiment, a linear correlation was found between G_s and T_r. P_n decreased as both G_s and T_r decreased. No obvious linear correlation was found between P_n and G_s; however, a significant negative correlation was found between P_n and C_i. The results showed that the photo-inhibition of cucumber seedlings under the hypoxia stress was mainly caused by non-stomatal limitation, and the stoma closure was a minor factor. The results proved that cucumber was a kind of tolerant plant to the hypoxia stress (Guo and Tachibana, 1997).

Spd could combine with negative charged groups of amine and sub-amine in nucleic acid, protein and phospholipid by non-covalent bond such as ionic bond, hydrogen bond and water estranging function, and stabilize the thylakoid structure and involve membrane construction. Therefore, Spd can inhibit the peroxidation of membrane lipid and the hydrolysis of membrane protein. Furthermore, Spd can regulate plant physiological activities and functions by protecting green leaves, and control the metabolism to improve the tolerance of plants to stresses (Drolet et al., 1986; Zhao et al., 2000). Under the hypoxia stress, normal aerobic respiration of plants was inhibited, while the lactate accumulation produced by anaerobic respiration caused cytoplasm acidification, viewed as a major cause of injury of most plant roots under anoxia (Guo, 2003). The exogenous Spd could mitigate the damage of hypoxia stress to cucumber seedlings roots, and enhance the endogenous PAs level (Wang et al., 2005c). PAs could improve H$^+$-ATPase activity on the plasma membrane of cucumber roots, and benefit to transport H$^+$ from the cytoplasm to outside of cells or to vacuoles, so PAs could alleviate H$^+$ accumulation caused by the hypoxia stress and mitigate the damage of hypoxia stress (Wang et al., 2005b). In the present experiment, the exogenous Spd application increased the P_n of cucumber seedlings under the hypoxia stress, decreased C_i, and increased Φ_c and *CE* significantly. The results indicated that the exogenous Spd application was an effective pathway to maintain the photosynthesis of cucumber leaves under the hypoxia stress, by stabilizing the photosynthetic enzyme pH and enhancing the carboxylase activity.

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