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Effect of cerium on photosynthetic characteristics of soybean seedling exposed to supplementary ultraviolet-B radiation

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Abstract: Effects of cerium (Ce^{3+}) on photosynthetic characteristics were investigated by hydroponics under laboratory conditions when soybean seedlings were exposed to two levels of supplementary UV-B radiation. UV-B radiation badly inhibited the photosynthesis in soybean seedling, leading to a reduction in net photosynthetic rate (Pn), Hill reaction activity, light saturated photosynthetic rate (Ps) and apparent quanta yield (AQY), as well as the CO_2 and light saturated photosynthetic rate (Pm) and carboxulation efficiency (CE). On the contrary, Ce obviously promoted the photosynthesis of plants by increasing Hill reaction activity, accelerating electron transport and photophosphorylation, and enhancing carboxylation efficiency. For Ce+UV-B treatments, the values of photosynthetic parameters were still lower than those of the control, but obviously higher than those of UV-B treatment. The results indicated that Ce alleviated the inhibition of UV-B radiation on the photosynthesis in soybean seedling to a certain extent. In correlating of Pn with Hill activity, AQY and CE, we found that the changes of photosynthetic rate were mainly influenced by the regulating effect of Ce on Hill activity and AQY at low level (0.15 W/m^2) of UV-B radiation, but were dominated by the regulating effect of Ce on CE at high level (0.45 W/m^2). Thus, Ce could regulate many aspects in photosynthesis of soybean seedling under UV-B stress. The regulating mechanism was close related with the dosage of UV-B radiation.

Keywords: apparent quanta yield; carboxylation efficiency; *Glycine max*; Hill activity; rare earths; UV-B radiation

Introduction

Extensively used as micro-fertilizers for crops in China since the 1970s (Guo, 1998; Huang *et al.*, 2005), rare earths (RE) can accelerate growth, promote production and quality, and improve the resistance of plants to adversity. An and Wang (1994) studied the effects of O_3 on wheat growth and the prevention of RE to ozone injury. They suggested that RE could increase chlorophyll content, enhance photosynthetic capacity, decrease the permeability of membrane, and therefore protect plants from O_3. Yan *et al.* (1999) investigated the protective effect of RE on growth and physiological metabolism of wheat under acid rain stress. In addition, Huang *et al.* (2000) reported that Ce can alleviate the inhibition of acid rain on germination and growth of barley due to scavenging excessive free radicals induced by acid rain and accelerating the synthesis of chlorophyll and the growth of roots. Jia *et al.* (1997) and Hu *et al.* (2001) reported that RE can reduce enrichment of heavy metal in cabbage, tomato, rice and wheat. In our previous study, we found that Ce can alleviate the inhibition of UV-B radiation on the growth of plants and that the improvement of photosynthesis regulated by Ce is one of the direct causes preventing rape seedlings diathesis from worsening under UV-B radiation (Liang *et al.*, 2006b), although the underlying mechanisms are not completely clear. On the one hand, the mechanisms about the damage of UV-B radiation on photosynthesis is complicated, such as inactivation of photosystem II (PS II), degradation of the D1 and D2 proteins of PS II, decrease of thylakoid membrane integrity, inactivity of Rubisco and other enzymes, reduction of chlorophyll and carotenoids content, down-regulation of photosynthetic genes and changes in chloroplast ultrastructure etc. (Jansen *et al.*, 1998). On the other hand, the mechanism of RE improving photosynthetic capacity and alleviating the injury of stresses on photosynthesis is also complicated. For example, Ce can increase chlorophyll content, maintain the structure of chloroplast, accelerate photochemical reaction and activate the enzyme system in dark reaction (Huang *et al.*, 2005). Thus, we should investigate the regulating effect of Ce on each important step of photosynthetic process in plants exposed to UV-B radiation to make clear the underlying mechanism of that Ce efficiently improve the photosynthetic function in plants.

We studied the response of important photosynthetic net parameters to Ce and UV-B treatments, including net photosynthetic rate (Pn), Hill reaction activity, light saturated photosynthetic rate (Ps), CO_2 and light saturated photosynthetic rate (Pm), apparent quanta yield (AQY) and carboxulation efficiency (CE). Soybean is an important oil crop all the world and one of the sensitive crops to elevated UV-B radiation. We studied the relation among the changes of the photosynthetic parameters in soybean seedlings treated by Ce and UV-B, and consequently estimated the regulating mechanism of Ce on photosynthesis of soybean seedling under UV-B radiation stress.

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1 Materials and methods

1.1 Plant material and growth conditions

Soybean (*Glycine max* L. merr.) seeds of "kennong 18" were sterilized for 10 min by $HgCl_2$ (0.1%), washed with deionized water, soaked for 10 h and then germinated in the incubator at (25±1)℃. When the length of hypocotyl was about 2 cm, seedlings were transplanted in plastic pots (diameter 10 cm, five plants per pot) filled with deionized water under the illumination of 8000 lx (12 h/d). When the first leaf was developed, the seedlings were cultured in Arnon+Hoagland solution which was renewed every 3 d for the pH stabilization. When the third leaf was developed completely, the seedlings were treated by Ce and UV-B radiation.

1.2 Treatment

The optimum concentration of $CeCl_3$ solution was 20 mg/L, which was determined in the pre-experiments (results not shown here). The spraying method was described by previous report (Liang *et al.*, 2006b).

Half of seedlings pretreated with Ce were irradiated at levels of 0.15 W/m^2 and 0.45 W/m^2 UV-B radiation from 9:00 to 15:00, for a total of 5 d as previously reported (Liang *et al.*, 2006b).

There were 6 sample sets in our experiments: CK (sprayed with deionized water), Ce (sprayed with $CeCl_3$ solution), T_1 (irradiated with 0.15 W/m^2 UV-B), T_2 (irradiated with 0.45 W/m^2 UV-B), Ce+T_1 (sprayed with $CeCl_3$ solution and then exposed to 0.15 W/m^2 UV-B radiation) and Ce+T_2 (sprayed with $CeCl_3$ solution and then exposed to 0.45 W/m^2 UV-B radiation). There were 3 replicates for each set.

1.3 Determination

Hill reaction was analyzed according to the procedures described by Arnon (1949). Hill reaction activity was determined as the rate of 2,6-dichloro-phenolindophenol (2,6-DCPIP) photoreduction (Trebst, 1972) using spectrophotometer at 600 nm.

Net photosynthesis rate (Pn) was measured with a portable photosynthetic system (CIRAS-1, PP Systems, UK) under the cultured condition of soybean seedling at (25±2)℃, CO_2 concentration 340 μmol/mol and photosynthetically active photon flux density (PPFD) 600 μmol/(m^2·s).

The Pn-PPFD response curve was measured under a predetermined set of PPFD levels (0, 50, 100, 200, 300, 400, 600, 1000 and 1400 μmol/(m^2·s) at a CO_2 concentration of 340 μmol/mol and (25±2)℃. The saturated part of the Pn-PPFD response curve and the value of Ps are attained. The initial slope of the Pn-PPFD is apparent quanta yield (AQY).

The Pn-Ci response curve was made under the conditions of (25±2)℃, PPFD value (1000 μmol/(m^2·s)) and CO_2 concentration (varying from 0—1400 μmol/mol)). The initial slope of the saturated part of the Pn-Ci response curve, i.e. carboxylation efficiency (CE), and the value of the CO_2 and light saturated photosynthetic rate (Pm) were achieved.

The photosynthetic parameters mentioned above were measured once every other day, for a total of 6 times.

1.4 Statistical processing of data

Three replicated measurements of the parameters above were carried out for each treatment. The statistical significances of differences among all treatments were analyzed with LSD test (P<0.05).

2 Results and discussion

2.1 Effect of Ce on photosynthesis in soybean seedlings under supplementary UV-B radiation

Pn is used as an index indicating the state of photosynthesis. As shown in Table 1, compared with that of CK, Pn in UV-B treatment was obviously decreased and the decrease rate of Pn was evidently related to the irradiation intensity of UV-B, while Pn in Ce treatment was distinctly promoted. Pn in Ce+UV-B treatments was still lower than that of CK, but markedly higher than that of UV-B treatments. According to the results of previous experiments (Liang *et al.*, 2006b), Ce can improve photosynthetic function of plants by increasing stomatal conductance, reducing the resistance to CO_2 transfer, and compensating the decrease of Pn due to the damage of UV-B radiation on photosynthetic apparatus.

Hill reaction activity could reflect the integrality of structure function PS II and the efficiency of electron transport. UV-B radiation caused a decrease in Hill activity by reducing electron transfer rate of PS II and limiting photophosphorylation, so as to influence photosynthesis process (Table 1). However, Ce can improve the activity of Hill reaction because Ce can efficiently scavenge excessive O_2^- · and protect the structure and function of chloroplast from active oxygen (Guo *et al.*, 2000). The Hill activity in Ce+UV-B treatments was lower than that of CK, but higher than that in UV-B treatments, similar to changes of Pn. It was suggested that Ce could alleviate the damage of UV-B radiation on Hill activity, and thus lighten the inhibition of UV-B radiation on Pn.

Both Ps and AQY can directly indicate the state of photosynthetic organs. UV-B radiation resulted in a reduction in Ps and AQY and there was a positive correlation between the decrease range and the intensity of UV-B radiation. The simultaneous decrease in Ps and AQY is the prominent characteristic of photoinhibition when photosynthetic organs were damaged (Xu *et al.*, 1992). For Ce treatment, Ps and AQY were simultaneously increased. In addition, Ps and AQY in Ce+UV-B treatments were still lower than those of CK, but

Table 1 Effect of Ce on photosythetic parameters in soybean seedling under UV-B radiation stress

Treatment	Pn, μmol/(m²·s)	Hill reaction rate, μmol (2,6-DCIP)/(mg chl·h)	Ps, μmol/(m²·s)	AQY, mol/mol	Pm, μmol/(m²·s)	CE, μmol/(m²·s)
CK	5.4±0.2ᵇ (100.0)	44.52±2.31ᵃ (100.0)	6.5±0.2ᵇ (100.0)	0.051±0.004ᵇ (100.0)	10.7±0.01ᵇ (100.0)	0.028±0.001ᵇ (100.0)
Ce	6.3±0.1ᵃ (116.6)	51.21±2.49ᵇ (115.1)	7.3±0.1ᵃ (112.6)	0.058±0.001ᵃ (114.1)	13.6±0.02ᵃ (127.1)	0.032±0.002ᵃ (116.4)
T_1	2.7±0.1ᵈ (49.1)	28.51±1.02ᵈ (64.1)	3.7±0.1ᵈ (56.9)	0.024±0.002ᵈ (47.1)	8.3±0.02ᶜ (77.6)	0.021±0.002ᶜ (74.5)
T_2	1.2±0.2ᶠ (22.7)	21.70±0.18ᵉ (48.7)	1.2±0.2ᶠ (18.5)	0.007±0.003ᵉ (13.1)	4.5±0.03ᵉ (42.1)	0.007±0.004ᶜ (23.6)
Ce+T_1	3.9±0.2ᶜ (71.8)	32.30±0.40ᶜ (72.5)	4.3±0.2ᶜ (66.2)	0.035±0.001ᶜ (68.5)	9.1±0.01ᵇ (85.0)	0.024±0.002ᵇᶜ (85.5)
Ce+T_2	2.1±0.1ᵉ (38.7)	27.36±0.72ᵈ (61.5)	2.8±0.1ᵉ (43.1)	0.017±0.004ᵈ (34.6)	7.2±0.01ᶜᵈ (67.3)	0.015±0.003ᵈ (54.5)

Note: Values followed by the same letter within a column are not significantly different ($P<0.05$)

obviously higher than those in UV-B treatments. It suggested that Ce could improve the light-use efficiency of plants under adverse conditions and meet energy need for restoration, so as to alleviate the damage of UV-B radiation on photosynthetic organs (Table 1).

Pm expresses the rate of electron transport and activity of photophosporylation, i.e. photosynthetic capacity (Xu, 2002). Compared with CK, Pm in UV-B radiation treatment was decreased while that in Ce treatment was obviously increased. Pm in Ce+UV-B treatments was still lower than that of CK but much higher than that of UV-B treatments. Furthermore, that of Ce+T_1 treatment was close to the level of CK. The results indicate that Ce efficiently alleviated the inhibition of UV-B on photosynthetic capability by accelerating the activity of Mg^{2+}-ATPase in membrane of chloroplast and promoting the rate of Hill reaction under adverse conditions (Chen *et al.*, 2001). CE is used as an index of active ribulose 1,5-bisphosphate carboxylase/ oxygenase (Rubisco), because there is a positive correlation between CE and the activity of RuBPcase (Allen *et al.*, 1998). CE in UV-B treatment was decreased, probably because UV-B radiation can inhibit the activity of RuBPcase, prevent the carboxylation reaction, and restrain Pn (Šprtová *et al.*, 1999). CE in Ce treatment was evidently increased. For Ce+UV-B treatments, CE was lower than that of CK, but higher than that in UV-B treatments, similar to changes of all indices mentioned above. It means that Ce can replace Mg^{2+} bonds to oxygen atom of RuBPcase and promote the activity of RuBPcase (Chen *et al.*, 2000).

2.2 Dynamic effects of Ce on photosynthesis in soybean seedlings under UV-B radiation stress

2.2.1 Dynamic effect on Pn

As shown in Fig.1a, Pn in Ce treatment was higher than that of CK throughout the experiment. It indicated that Ce could efficiently promote photosynthesis and keep Pn at high level, and accelerate growth of plant (Liang *et al.*, 2006b). Change extent of Pn was larger in UV-B treatments than in Ce+UV-B treatments although the change pattern was similar under the two given conditions. During 6—11 d, Pn in UV-B treatments was slowly increased because defense systems were induced to participate restoration of plant. The reason for increase of Pn in Ce+UV-B treatments was because Ce directly protected photosynthetic organs from UV-B radiation by eliminating excessive free radicals (Liang *et al.*, 2006b) and simulated the accumulation of UV-B absorbing compounds such as carotenoids, flavonoids etc. (Liang *et al.*, 2006a).

2.2.2 Dynamic effect on Hill reaction activity

The changes of Hill reaction activity in six treatments are shown in Fig.1b. Under UV-B stress, changes of Hill reaction activity were similar to those of Pn, but the change range of the former was lower than that of the latter in each measurement. This difference between Hill activity and Pn indicated that the decrease of the activity of Hill was not only a factor bringing about the reduction of Pn under UV-B radiation, and that the stomatal limitation may be responsible for the decrease in net CO_2 assimilation in the UV-B treatment of 5 d (Sharma *et al.*, 1998). For Ce treatment, the activity of Hill reaction was increased during the whole experiment compared with that of CK, but such increase range was lower than that of Pn in same treatment. It showed that the regulating effect of Ce on the activity of Hill reaction was inferior to that on Pn. For Ce+UV-B treatments, change extent of the Hill activity was lower than that of UV-B treatments because Ce could alleviate the damage of UV-B radiation on Hill activity. The reduced rate of Pn was initially high and then low, contrary to that of Hill activity, although the change

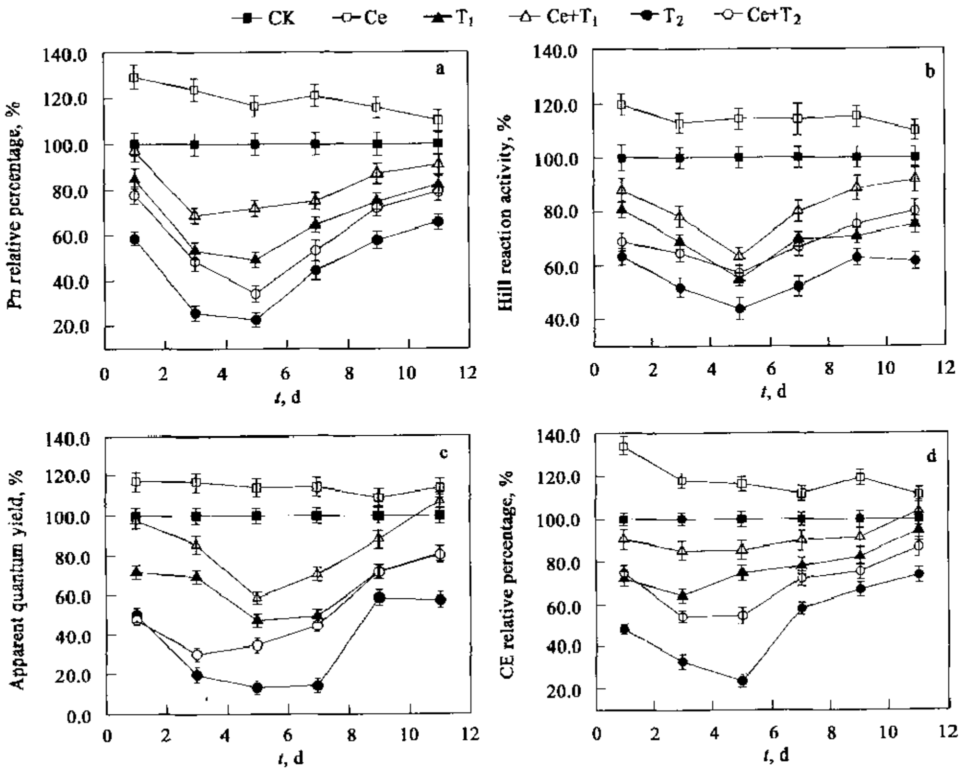

Fig.1 Effects of Ce on Pn (a), Hill reaction activity (b), AQY (c) and CE (d) in soybean seedling under UV-B stress

pattern of two parameters was similar. Such phenomenon was likely caused by the difference in the regulating mechanisms of Ce on Pn and Hill activity beside reasons mentioned above.

2.2.3 Dynamic effect on AQY

Fig.1c shows the dynamic changes of AQY in 6 treatments for 11 d. For Ce treatment, the change pattern of AQY and Pn was similar, and both values were higher than those of CK. The increase of AQY meant that Ce could promote the absorption of light and the transform of energy, accelerate electron transport rater, and keep Pn at high level by making photosynthetic apparatus in a better order. Furthermore, the changes of AQY in UV-B and Ce+UV-B treatments resembled those of Pn, but the decreased range of AQY was higher than that of Pn during 5 d of UV-B treatment, and lower during 6—11 d. It can be concluded that there was difference between the sensibility of AQY and Pn to UV-B radiation. The result that the decreased extent of AQY in Ce+UV-B treatment was lower than that of UV-B treatment demonstrates that Ce could maintain the state of photosynthetic apparatus, promote the AQY, and benefit to keep Pn under UV-B radiation stress.

2.2.4 Dynamic effect on CE

As shown in Fig.1a and Fig.1d, the dynamic tendencies of Pn and CE in six treatments were similar. Moreover, the change range of CE was lower than that of Pn in the same measurement at low level of UV-B radiation (T_1 and Ce+T_1 treatments) while the change extent of CE was similar to that of Pn at high level (T_2 and Ce+T_2 treatments). It presented that there was the dose response relation between irradiation intensity and damage degree, and that the mechanisms under which UV-B radiation inhibiting CE and Pn are various. Other than stomatal limitation, the limitation of decomposition due to degradation or inactivation of enzymes of the Calvin cycle might be responsible for the decrease in Pn at high level of UV-B radiation (Sharma *et al.*, 1998). The difference in the decrease rate of CE between Ce+UV-B and UV-B treatments indicated that Ce could alleviate the inhibition of UV-B radiation on CE during a long period. As for Ce+UV-B treatments, the decreased rate of CE was lower than that of Pn in the same measurement and the recovery degree of the former was obviously higher than that of the latter, which might be due to the different regulating effect of Ce on CE and Pn besides factors mentioned above.

2.3 Correlation between Hill reaction rate, AQY, CE and Pn in six treatments

The correlation coefficients between Pn and other photosynthetic parameters were established to investigate the effect of Ce on photosynthesis under UV-B stress. Table 2 shows that the correlation coefficients of Pn-Hill activity and Pn-AQY in T_1 and Ce+T_1 treatments were higher than those in T_2 and Ce+T_2 treatments while the correlation coefficients of Pn-CE in T_1 and Ce+T_1 treatments were lower than those in T_2 and Ce+T_2 treatments. The results suggested that the changes of photosynthetic parameters in plants treated with Ce and UV-B radiation were influenced by many factors such as the tolerance of plant to different intensity of UV-B radiation and the damaging mechanism of various dosage of UV-B. To summarize, the damage mechanism of UV-B radiation is closely related to the dosage of UV-B radiation. Pn was mainly influenced by changes of Hill activity and AQY at low level of UV-B radiation while closely related to changes of CE at high level of UV-B radiation. On the other hand, the regulating mechanisms under which Ce efficiently alleviated the inhibition of UV-B on Pn were not the same when soybean seedlings were exposed to different level of UV-B radiation, which depended on the intensity of UV-B radiation.

Table 2 Correlation coefficients between AQY, Hill reaction rate, CE and Pn

Treatment	T_1	Ce+T_1	T_2	Ce+T_2
Pn-AQY	0.986**	0.932*	0.878	0.856
Pn-Hill activity	0.955**	0.918*	0.851	0.810
Pn-CE	0.648	0.861	0.905*	0.969**

Notes: *$P<0.05$; **$P<0.01$

3 Conclusions

$CeCl_3$ at the concentration of 20 mg/L could improve such photosynthetic parameters as Pn, Hill activity, Ps, AQY, Pm and CE. Furthermore, it could alleviate the inhibition of UV-B radiation on photosynthetic capacity resulted from reduction in Hill activity, quanta yield, photophosphorylation and the activity of RuBPcase to a certain extent.

The dynamic changes of Pn, Hill activity, AQY and CE in six treatments suggest that Ce could slower the decrease rate of these four photosynthetic parameters during 5 d of UV-B radiation, quicken the restoration rate during 6—11 d. Moreover, the final restoration degree of each index in plant exposed to low level of UV-B radiation is higher than that exposed to high level.

The correlation between Hill activity, AQY, CE and Pn indicate that Pn was mainly influenced by Hill activity and AQY at low level of UV-B radiation (T_1 and Ce+T_1 treatments) while was primarily restricted by the changes of CE at high level of UV-B radiation (T_2 and Ce+T_2 treatments). It could be inferred that there were many regulating aspects improved by Ce in photosynthetic system. Moreover, the regulating mechanisms of Ce on various sites of photosynthetic process were different. Thus, the mechanisms under which Ce regulated each step of photosynthesis and the reasons in which Ce improve photosynthetic capacity in plants exposed to UV-B radiation were deserved to investigate further.

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