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Effect of Cerium on Photosynthetic Pigments and Photochemical Reaction Activity in Soybean Seedling Under Ultraviolet-B Radiation Stress

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Abstract Effects of cerium (Ce) on photosynthetic pigments and photochemical reaction activity in soybean (*Glycine max* L.) under ultraviolet-B (UV-B) radiation stress were studied under laboratory conditions. UV-B radiation caused the decrease in chlorophyll content, net photosynthetic rate, Hill reaction activity, photophosphorylation rate and Mg^{2+}-ATPase activity. Ce (III) (20 mg L^{-1}) could alleviate UV-B-induced inhibition to these photosynthetic parameters because values of these photosynthetic parameters in Ce (III) + UV-B treatment were obviously higher than those with UV-B treatment alone. Dynamic changes of the above photosynthetic parameters show that Ce (III) could slow down the decrease rate of these photosynthetic parameters during a 5-day UV-B radiation and quicken the restoration during recovery period. The final restoration degree of five parameters mentioned above in leaves exposed to low level of UV-B radiation (0.15 W m^2) was higher than that exposed to high level (0.45 W m^2). Correlating net photosynthetic rate with other four parameters, we found that the regulating mechanisms Ce (ΠI) on photosynthesis under various level of UV-B radiation were not the same. The protective effects of Ce (III) on photosynthesis in plants were influenced by the intensity of UV-B radiation.

Keywords Cerium (III) · *Glycine max* seedling · Photochemical reaction activity · Photosynthetic pigments · Protective effect · UV-B radiation

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Introduction

Enhanced ultraviolet-B (UV-B; 280~315 nm) radiation has potentially deleterious consequence for agricultural production and natural plant ecosystems [1, 2] although neutral and positive effects have also been reported [3]. Negative consequence of excessive UV-B radiation on plants are: decreasing crop yields [4], lowering growth rate, damaging photosystem II (PS II) and photosystem I (PS I), reducing chlorophyll and carotenoids content, degrading the D1 and D2 proteins of PS II, disturbing activities of carboxylating enzymes, and damaging DNA and chloroplast ultrastructure [5, 6]. However, studies on the ecological protection of plant from UV-B stress are comparatively few [7] despite increasing concerns of enhanced UV-B exposure due to changes in the UV-B filtering ozone layer in the upper atmosphere. Efforts for searching suitable measures to protect plants from UV-B stress seem timely.

Rare earth elements (REEs), extensively used as biological regulator for crops in China since the 1970s, comprise a group of 17 elements with very similar chemical and physical properties. Even though no proof shows that REEs are essential for plant, numerous applications suggest that suitable dosage REEs can accelerate plant growth, increase production and quality [8], and improve the resistance of plants to a variety of external stresses [9] such as acid rain [10], heavy metal [11], and ozone [12]. In our previous study [13], we found that REEs could alleviate the UV-B-induced inhibition on growth of rape (*Brassica juncea* L.) and soybean (*Glycine max* L.) seedling. In addition, the improvement of photosynthesis regulated by REEs is one of the direct causes preventing plants growth from inhibiting under UV-B stress [14]. However, it is not clear for the underlying mechanisms where cerium (Ce) (III) efficiently improves photosynthetic function in plants under UV-B stress.

Cerium (III) is one of light REEs, used as the main component of commercial REEs regulator [15]. Hill reaction is a key reaction in photosynthesis, directly taking part in the transform process from light energy to electron energy and providing electron and proton for ATP and NADP formation [16]. Photophosphorylation (PP) is the reaction that generates ATP. The rate of PP can reflect activities of photochemical reactions in chloroplast, which is directly restricted by Mg^{2+}-ATPase activity [17]. In this paper, we studied changes of photosynthetic pigments content, Hill reaction activity (HRA), PP rate, and Mg^{2+}-ATPase activity, and relations among these important photosynthetic parameters in soybean seedlings treated with Ce (III) and UV-B radiation, for clarifying the regulating mechanism of Ce (III) on photosynthesis of soybean seedling under UV-B stress.

Materials and Methods

Plant Material and Growth Conditions

Soybean (*G. max*) seeds of “Kennong 18”, a variety of the soybean planted widely in eastern China, were supplied by Xishan Seed Company (Wuxi, China). They were surface disinfected for 10 min with $HgCl_2$ (0.1%), and washed three times with deionized water. After being soaked for 4 h, the seeds were placed in a dish under-laid with three pieces of filter paper and germinated in the incubator at 25±1°C. When the length of the hypocotyls were about 2 cm, the seedling was transplanted in plastic pots (diameter 10 cm, five plants per pot) filled with Hoagland solution in a growth room with irradiance 300 μmol photons m^{-2} s^{-1} photosynthetically active radiation (400~700 nm), at 30°C and 70% relative air humidity during day time, and at 25°C and 80% relative humidity at night. The day and night cycle was 12/12 h. When the third leaf of plants was developed completely, the seedlings were treated by Ce (III) and UV-B radiation.

Ce (III) Treatment

In a pre-experiment (results not shown here), we used $CeCl_3$ solution at different concentrations of 10, 15, 20, 25, and 30 mg L^{-1} to treat soybean leaves. Net photosynthetic rate was the highest in leaves treated with 20 mg L^{-1} $CeCl_3$, which was taken as the optimum concentration for our further experiments. The $CeCl_3$ solution (20 mg L^{-1}) was sprayed evenly on the leaves until drops began to fall. The same amount of deionized water was applied to another set as the control. After 48 h, seedlings pretreated with Ce (III) or deionized water were placed under ultraviolet lamps ($CeCl_3$ application done only once before UV-B treatment).

UV-B Radiation Treatment

Enhanced UV-B radiation was performed with 40 W UV-B lamps (Nanjing Lamp Factory, Nanjing, China) hanged perpendicularly over the plants. The levels of UV-B radiation were 0.15 W m^2 and 0.45 W m^2, which were determined by ultraviolet radiac (Photo-electricity Instrument Factory of Beijing Normal University, Beijing, China). Seedlings were irradiated by UV-B lamps for 5 h from 10:00 to 15:00 for 5 days. The height of lamps over the plants was adjusted to maintain consistent radiation intensity.

There were six experimental sets in our experiments: control (sprayed with deionized water), Ce (IΠ) (sprayed with 20 mg L^{-1} $CeCl_3$ solution), T_1 (irradiated with 0.15 W m^{-2} UV-B radiation), T_2 (irradiated with 0.45 W m^{-2}UV-B radiation), Ce (IΠ) + T_1 (sprayed with 20 mg $L^{-1}$$CeCl_3$ solution and then exposed to 0.15 W m^{-2}UV-B radiation), and Ce (IΠ) + T_2 (sprayed with 20 mg $L^{-1}$$CeCl_3$ solution and then exposed to 0.45 W m^{-2}UV-B radiation). Each set was done in triple.

Determination

Net Photosynthesis Rate Measurement

Net photosynthesis rate (Pn) was measured with a portable gas exchange system (CIRAS-1, PP Systems International Ltd., UK) under the cultured condition of soybean seedling at 25±2°C, CO_2 concentration 340 μmol mol^{-1} and photosynthetically active photon flux density 500 μmol m^{-2} s^{-1}.

Photosynthetic Pigments Content Determination

Chlorophyll and carotenoids were extracted with 80% acetone. The extract was centrifuged at 5,300×*g* for 10 min and analyzed spectrophotometrically at 646 and 663 nm for chlorophyll and at 470 nm for carotenoids [18].

Photochemical Reaction Activities Determination

Chloroplasts were isolated from fresh, prechilled soybean leaves with STN solution (0.4 M sucrose and 10 mM NaCl in 20 mM Tris–HCl buffer, pH 7.5) at 4°C according to previously reported methods [19]. HRA was determined as the rate of 2,6-dichlorophenolindophenol (purchased from Sigma Chemical Co., St. Louis, MO) photoreduction using spectrophotometer at 600 nm [20]. The PP rate was measured from a decrease of phosphorus (Pi) content in isolate chloroplast using spectrophotometer at 660 nm [21]. Irradiance for determining decrease rate of Pi was 200 W m^{-2}, t 20±1°C. The activity of Mg^{2+}-ATPasae was measured according to the method reported by Maylan et al. [22].

All photosynthetic parameters determination were done on the third pair of leaflets (counting from below), and measured once every other day for a total of six times.

Statistical Analysis

Data in tables and figures are expressed as mean values ± SE. from five replicates. The statistical significance of differences among means was tested by one-way ANOVA, followed by Fisher's protected least significance difference test at a 5% significance level.

Results

Effects of Ce (III) and UV-B Radiation on Photosynthetic Pigments

Changes of photosynthetic pigments content in leaves of soybean seedlings in six treatments after 5 days UV-B radiation are given in Table 1. Compared with that of the control, supplementary UV-B radiation caused 22.2% (T_1) and 42.8% (T_2) decrease in total Chl, and the decrease degree was evidently related to the irradiation intensity of UV-B. In addition, the degree of Chl*a* decrease was higher than that of Chl*b*, and Chl*a*/Chl*b* ratio decreased as well. A similar trend was observed in carotenoid (Car) content. Contrarily, Chl*a*, Chl*a*/Chl*b* and total Chl in Ce (III) treatment were all higher than those of the control. The change of Car content in Ce (III) treatment was not significant. For Ce (III) + UV-B treatments, all values were lower than those of the control, but obviously higher than those of UV-B treatments without Ce (III).

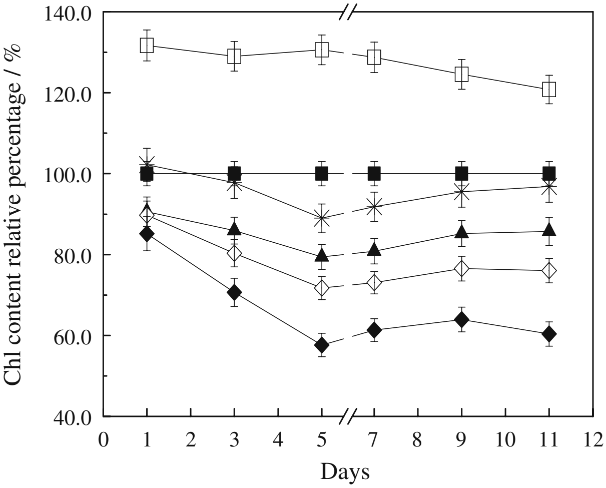
Dynamic changes of total Chl content in leaves during stress and recovery periods are shown in Fig. 1. UV-B radiation kept Chl content continually decreasing (day 1 through day 5). The decrease degree of Chl in T_2 treatment was higher than that in T_1 treatment,

Table 1 Effects of Ce (III) and UV-B radiation on photosynthetic pigments in soybean seedling

Treatments	Control	Ce (III)	T_1	T_2	Ce (III) + T_1	Ce (III) + T_2
Chl*a* ($mg\ g^{-1}FW$)	2.52±0.02 b (100.0)	3.40±0.09 a (134.9)	1.87±0.03 c (74.2)	1.48±0.04 e (58.9)	2.20±0.01 b (86.6)	1.72±0.03 d (68.3)
Chl*b* ($mg\ g^{-1}FW$)	0.91±0.04 b (100.0)	1.11±0.02 a (121.9)	0.80±0.01 c (89.0)	0.70±0.01 d (76.6)	0.81±0.03 c (90.1)	0.73±0.01 d (80.2)
Chl*a*/Chl*b*	2.76 b (0.0)	3.04 a (11.0)	2.32 c (−16.1)	2.14 d (−22.5)	2.72 b (−1.5)	2.35c (−14.7)
Total Chl ($mg\ g^{-1}FW$)	3.43±0.01 b (100.0)	4.51±0.03 a (131.4)	2.67±0.05 d (77.8)	1.96±0.09 e (57.2)	3.01±0.06 c (87.7)	2.45±0.09 d (71.3)
Car ($mg\ g^{-1}FW$)	0.32±0.02 a, b (100.0)	0.33±0.04 a (110.0)	0.25±0.03 c (78.1)	0.19±0.02 d (59.7)	0.29±0.01 b (91.7)	0.26±0.01 c (81.2)

Data are expressed as mean values ± SE from five replicates. Value in bracket is relative percentage (the ratio of absolute value to the control). Values with different letters are significantly different at $P<0.05$

Fig. 1 Dynamic effects of Ce (III) and UV-B radiation on total Chl content in soybean leaves. Chl content analyses were done at 1, 3, and 5 days during UV-B stress period and at 7, 9, and 11 days during recovery period. Data points represent relative percentage ± SE of five replicates. *Filled squares*, control; *empty squares*, Ce (III); *filled triangles*, T_1; *filled diamonds*, T_2; *asterisks*, Ce (III) + T_1; *empty diamonds*, Ce (III) + T_2

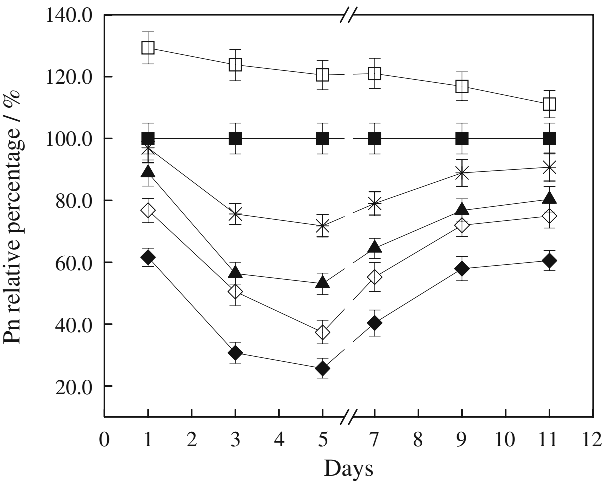


showing an obvious dosage-related effect. During the recovery period (day 6 through day 11), Chl content in leaves with UV-B treatment was increased gradually, measured at day 9 was already significantly higher than that at day 5 ($P<0.05$). For Ce (III) treatment, Chl content was kept at higher level compared with that of the control. Change trends of Chl in Ce (III) + UV-B treatment and UV-B treatment were alike, whereas the decreased degree of Chl in Ce (III) + UV-B treatment was lower than that in UV-B treatment during the same period.

Effects of Ce (III) and UV-B Radiation Photosynthetic Rate

Dynamic changes of photosynthetic rate (Pn) in six treatments (Fig. 2) were similar to those of Chl (Fig. 1). The decrease degree of Pn in UV-B-treated leaves was higher than that of Chl in the same treatment and period. For Ce (III) treatment, Pn was higher than that of the control throughout the experiment though its increase degree lowered slowly (29~11%).

Fig. 2 Dynamic effects of Ce (III) and UV-B radiation on net photosynthetic rate (Pn) in soybean leaves. Pn analyses were done at 1, 3, and 5 days during UV-B stress period and at 7, 9, and 11 days during recovery period. Data points represent relative percentage ± SE of five replicates. *Filled squares*, control; *empty squares*, Ce (III); *filled triangles*, T_1; *filled diamonds*, T_2; *asterisks*, Ce (III) + T_1; *empty diamonds*, Ce (III) + T_2



Changes of Pn in Ce (III) + UV-B treatments and UV-B treatments were similar, whereas the decreased degree of Pn in Ce (III) + UV-B treatments was lower during the same period.

Effects of Ce (III) and UV-B Radiation Photochemical Reaction Activities

UV-B radiation caused decrease in HRA, PP rate and Mg^{2+}-ATPase activity by 35.4%, 26.4%, 23.5% for T_1 treatment and 51.5%, 52.1%, 51.5% for T_2 treatment, respectively (Table 2). The decrease degree of HRA was distinctly higher than that of PP rate and Mg^{2+}-ATPase activity in T_1 treatment. Nevertheless, the decrease degree of three parameters in T_2 treatment was similar. The three parameters in Ce (III) treated leaves were all higher than those in the control, similar to changes of photosynthetic pigments. The three parameters in Ce (III) + UV-B treatment were lower than those in the control, but obviously higher than those in UV-B treatments.

Dynamic changes of HRA, PP rate and Mg^{2+}-ATPase in leaves with the same treatment were similar (Fig. 3). During the stress period, the decrease degree of HRA was higher than that of PP rate and Mg^{2+}-ATPase in leaves exposed to low level of UV-B radiation (T_1). In leaves exposed to high level of UV-B radiation (T_2), the decrease degree of HRA was higher than that of PP rate and Mg^{2+}-ATPase activity at day 1, but was similar to that of PP rate and Mg^{2+}-ATPase activity at days 3 and 5. The decreased degree of the three parameters in Ce (III) + UV-B-treated leaves were all lower than those in UV-B-treated leaves at the same time. In addition, the decrease degree of the three parameters in Ce (III) + T_1 treatment was lower than that in Ce (III) + T_2 treatment. During the recovery period, the restoration degree of the three parameters in Ce (III) + UV-B treatment was higher than that in UV-B treatment. In addition, the final restored degree of the three parameters in Ce (III) + UV-B treatment was different, which is related to the regulating mechanisms of Ce (III).

The Correlation between Pn and Other Photosynthetic Parameters in Six Treatments

The correlation coefficients between Chl content, HRA, PP rate, Mg^{2+}ATPase activity, and Pn were established in Table 3. We found that the correlation coefficients of Pn-Chl and Pn-HRA in T_1 and Ce (III) + T_1 treatments were higher than those in T_2 and Ce (III) + T_2 treatments, whereas the correlation coefficients of Pn-PP rate and Pn- Mg^{2+}ATPase activity in T_1 and Ce (III) + T_1 treatments were lower than those in T_2 and Ce (III) + T_2 treatments.

Table 2 Effects of Ce (III) and UV-B radiation on photochemical reactions activity in soybean seedling

Treatments	Control	Ce (III)	T_1	T_2	Ce (III) + T_1	Ce (III) + T_2
HRA (μmol 2,6-DCIP mg^{-1}chl h^{-1})	50.02±1.66 a (100.0)	60.07±1.45 b (120.1)	32.31±1.14 d (64.6)	24.26±1.01 e (48.5)	36.56±1.05 c (73.1)	30.66±2.29 d (61.3)
PP rate (μmol ATP mg^{-1}Chl h^{-1})	18.27±0.21 a (100.0)	22.87±0.13 b (125.2)	13.47±0.69 d (73.6)	8.76±0.23 e (47.9)	15.46±0.33 c (84.6)	12.22±0.52 d (66.9)
Mg^{2+}-ATPase activity (μmol Pi mg^{-1}Chl h^{-1})	178.85±3.03 b (100.0)	217.48±2.20 a (121.6)	136.86±1.14 d (76.5)	86.74±5.51 f (48.5)	150.41±4.74 c (84.1)	109.45±8.09 e (61.2)

Data are expressed as mean values ± SE from five replicates. Value in bracket is relative percentage (the ratio of absolute value to the control). Values with different letters are significantly different at $P<0.05$

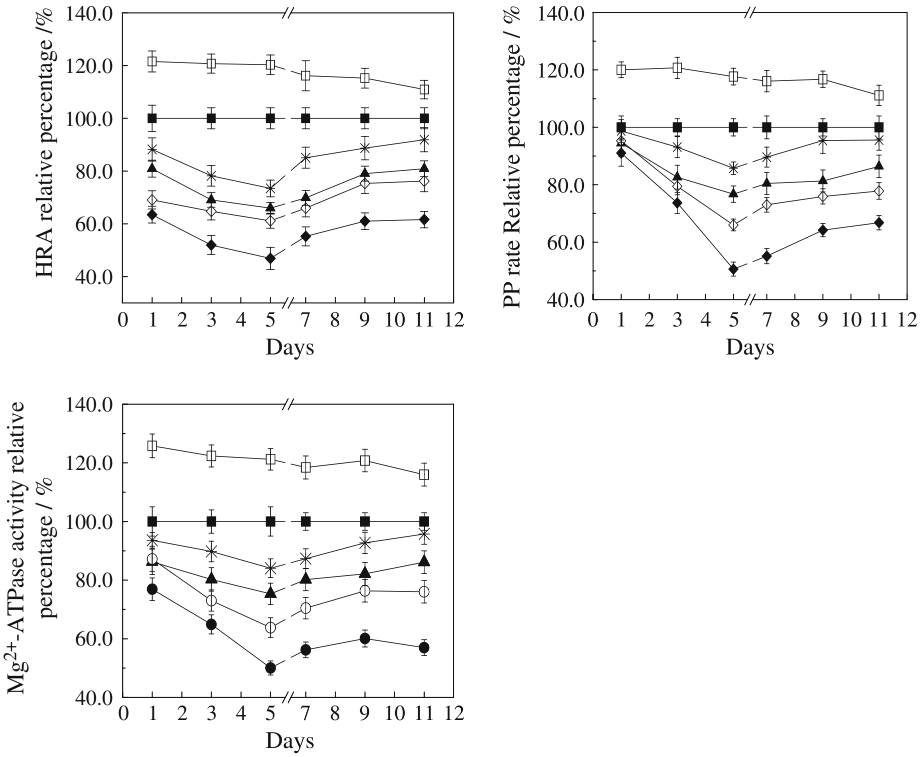


Fig. 3 Dynamic effects of Ce (III) and UV-B radiation on photosynthetic reactions activity in soybean leaves. HRA, PP rate and Mg^{2+}-ATPase activity analyses were done respectively at 1, 3, and 5 days during UV-B stress period and at 7, 9, and 11 days during recovery period. Data points represent relative percentage ± SE of five replicates. *Filled squares*, control; *empty squares*, Ce (III); *filled triangles*, T_1; *filled diamonds*, T_2; *asterisks*, Ce (III) + T_1; *empty diamonds*, Ce (III) + T_2

Discussion

Chl*a*, Chl*b*, Chl*a*/Chl*b*, and total Chl in leaves exposed to UV-B radiation were decreased, resulting from light degradation induced by UV-B radiation [23] and Chl synthesis inhibition [24]. Another cause was reduction in Car content that is a kind of UV-B-absorbing compound and can protect Chl from degrading caused by excessive light energy [25]. All parameters of photosynthetic pigments in Ce (III) + UV-B treatment were

Table 3 The correlation coefficients between Chl content, HRA, PP rate, Mg^{2+}ATPase activity, and Pn

Treatment	T_1	Ce (III) + T_1	T_2	Ce (III) + T_2
Pn-Chl content	0.932**	0.853*	0.437	0.634
Pn-HRA	0.968**	0.922**	0.855*	0.826*
Pn-PP rate	0.617	0.677	0.984**	0.937**
Pn-Mg^{2+}ATPase activity	0.633	0.872*	0.917**	0.980**

*P<0.05; **P<0.01

significantly higher than those in UV-B treatment, showing that Ce (III) could alleviate the UV-B-induced Chl degradation, to maintain the capacity of Chl for absorbing and using light energy in plants under adverse conditions. The reason that rare earth ions can alleviate the UV-B-induced damage on Chl is because rare earth ion can increase Chl content by effectively accelerating chloroplast protein synthesis, postponing chloroplast senescence [26], catalyzing the transform from original Chl to Chl [27]. Indirect cause is that the amount of UV-B radiation reaching chloroplast is reduced by UV-B-absorbing compound (flavonoids, carotenoid, etc.) content increased by rare earth iron, hence enhancing their absorbency [28].

Changes of photochemical reaction activities show that the decrease degree of HRA was distinctly higher than that of PP rate and Mg^{2+}-ATPase activity in T_1 treatment, indicating that HRA was more sensitive to UV-B radiation than PP rate and Mg^{2+}-ATPase activity. Nevertheless, the decrease degree of the three parameters in T_2 treatment was similar. The difference in changes of the three parameters between T_1 treatment and T_2 treatment showed that the damage mechanisms at different level UV-B radiation on the three parameters were not the same. We previously reported that apparent quanta yield is the main limiting factor to Pn in soybean seedling exposed to low level of UV-B radiation [14]. Consequently, it can be inferred that quick decrease in HRA in leaves exposed to low level of UV-B radiation was mainly resulted from UV-B-induced inhibition in light energy transformation and electron transfer. In photosynthetic reactions, light capture, transform, and transfer all happen on thylakoid membrane [29]. Directly acting on H_2O_2, high level of UV-B radiation induces excessive free radicals generation resulting in thylakoid membrane lipid peroxidation and membrane liquidity reduced [30, 31]. In this paper, the similar and simultaneous decrease in HRA, PP rate as well as Mg^{2+}-ATPase activity in leaves exposed to high level of UV-B radiation was closely related to UV-B-induced lipid peroxidation and inactivation of functional sites in thylakoid membrane. From changes of three parameters in Ce (III) + UV-B treatment, we found that Ce (III) could alleviate UV-B-induced inhibition on HRA, PP rate, and Mg^{2+}-ATPase activity, and so lighten the injury of photosynthesis caused by UV-B radiation. Chen et al. [32] found that lanthanum (La) can promote PP rate in tobacco chloroplast. They proved that the improvement of photosynthetic capacity is closely correlated with the acceleration of the coupling between electron transfer and PP in tobacco leaves treated with La. In our previous study [33, 34], we found that Ce (III) can enhance the abilities of defense enzymes to scavenge excessive active oxygen induce by UV-B radiation in plants. Hence, it can be inferred that Ce (III) could alleviate the threat of excessive active oxygen to photosynthetic organs in plants exposed to UV-B radiation, and consequently could maintain photosynthetic capacity in plants exposed to UV-B radiation. In addition, Wang et al. [35] suggested that Ce (III) scavenge O_2^- and protect chloroplast from active oxygen damage, similar to the characteristics of superoxide dismutase. They described the mechanism as follows:

$$Ce^{3+} + \cdot O_2^- \rightarrow Ce^{4+} + O_2$$
$$Ce^{4+} + \cdot O_2^- \rightarrow Ce^{3+} + H_2O_2$$

Analyzing the changes of HRA, PP rate and Mg^{2+}-ATPase activity in Ce(III) + UV-B treatment, we inferred that the direct effect of Ce (III) was increasing HRA, accelerating PP rate, and quickening the whole process of light energy transform in leaves exposed to UV-B radiation. The indirect effect of Ce (III) was scavenging O_2^- and preventing UV-B-induced thylakoid membrane lipid peroxidation.

Dynamic changes of Chl content, Pn, HRA, PP rate, and Mg^{2+}-ATPase activity in leaves with the same treatment were similar. For Ce (III) treatment, the five photosynthetic parameters were higher than those of the control throughout the experiment though the increase degree lowered slowly. The phenomenon suggested that Ce (III) efficiently promoted photosynthesis whereas the promotion effect was restricted by Ce (III) treating time, which is consistent with previous reports [36–38]. For UV-B treatment, decrease degree of five parameters was gradually increased during stress period and related closely to irradiation intensity, showing the obvious time-related effect and dose-related effect. The decrease degree of Pn was higher than that of Chl in the same treatment and period, suggesting that Pn was more sensitive to the UV-B-induced inhibition, and that the decrease in Pn was not only resulted from Chl degradation. In addition, the sensibilities of HRA, PP rate, and Mg^{2+}-ATPase activity to UV-B radiation were different, and the damage mechanisms of UV-B radiation on the three parameters were not the same. The correlation coefficients of Pn-Chl, Pn-HRA, Pn-PP rate, and Pn-Mg^{2+}-ATPase activity in T_1 and T_2 treatments further proved that the change of Pn had the same direction with that of Chl content and HRA at low level of UV-B radiation whereas changes of Pn was similar with that of PP rate and Mg^{2+}ATPase activity at high level of UV-B radiation. Damage mechanisms of UV-B radiation on photosynthesis were influenced by the dosage of UV-B radiation. During the recovery period, five parameters in UV-B treatment increased gradually, indicating photorepair realized in the plant [39], and the final recovery degree was inversely correlated with stress intensity. For Ce (III) + UV-B treatment, Ce (III) could slow down the decrease degree of five photosynthetic parameters during 5-day UV-B radiation, and quicken the restoration rate during recovery period (day 6 through day 11). Moreover, the final restoration degree of each parameter in plant exposed to low level of UV-B radiation was higher than that exposed to high level of UV-B radiation. Analyzing the correlation coefficients of Pn-Chl, Pn-HRA, Pn-PP rate, and Pn-Mg^{2+}-ATPase activity in Ce (III) + T_1 and Ce (III) + T_2 treatment, we found that the change of Pn had the same direction with that of Chl content and HRA in T_1 and Ce (III) + T_1 treatments, while the change of Pn was similar with that of PP rate and Mg^{2+}ATPase activity in T_2 and Ce (III) + T_2 treatments. The result indicate the regulating mechanisms Ce (III) on photosynthesis in soybean seedlings exposed to various level of UV-B radiation were not the same.

Ce (III) could alleviate UV-B-induced inhibition on photosynthesis by regulating photosynthetic pigment content and photochemical reaction activities. Regulating mechanisms of Ce (III) on these photosynthetic parameters in leaves were influenced by the intensity of UV-B radiation. Photosynthesis was a particularly complicated reaction in plants. Thus, roles of Ce (III) to alleviate UV-B-induced inhibition on photosynthetic capacity in plants are deserved to study further, not only because of scientific importance but also application potentials in agriculture.

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