ISSN 1001—0742
Journal of Environmental Sciences Vol. 18, No. 1, pp. 125—129, 2006 CN 11—2629/X

Article ID: 1001-0742(2006)01-0125-05 CLC number: X503.23; S124 Document code:A

Effects of cerium on growth and physiological mechanism in plants under enhanced ultraviolet-B radiation

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Abstract: Effect of cerium (Ce^{3+}) on the growth, photosynthesis and antioxidant enzyme system in rape seedlings (*Brassica juncea* L.) exposed to two levels of UV-B radiation (T_1: 0.15 W/m^2 and T_2: 0.35 W/m^2) was studied by hydroponics under laboratory conditions. After 5 d of UV-B treatment, the aboveground growth indices were obviously decreased by 13.2%—44.1%(T_1) and 21.4%—49.3% (T_2), compared to CK, and except active absorption area of roots, the belowground indices by 14.1%—35.6%(T_1) and 20.3%—42.6% (T_2). For Ce+UV-B treatments, the aboveground and belowground growth indices were decreased respectively by 4.1%—23.6%, 5.2% —23.3%(Ce+T_1) and 10.8%—28.4%, 7.0%—27.8%(Ce+T_2), lower than those of UV-B treatments. The decrease of growth indices appeared to be the result of changes of physiological processes. Two levels of UV-B radiation induced the decrease in chlorophyll content, net photosynthesis rate, transpiration rate, stomatal conductance and water use efficiency by 11.2%—25.9%(T_1) and 20.9%— 56.9%(T_2), whereas increase in membrane permeability and activities of antioxidant enzymes including superoxide dismutase(SOD), catalase (CAT) and peroxidase (POD) by 6.9%, 22.8%, 21.5%, 9.5%(T_1) and 36.6%, 122.3%, 103.5%, 208.9%(T_2), respectively. The reduction of the photosynthetic parameters in Ce+UV-B treatments was lessened to 3.2%—13.8%(Ce+T_1) and 4.9%—27.6%(Ce+T_2), and the increase of membrane permeability and activities of antioxidant enzymes except POD in the same treatments were lessened to 2.4%, 8.4%, 6.6%(Ce+T_1) and 30.1%, 116.7%, 75.4%(Ce+T_2). These results indicate that the regulative effect of Ce on photosynthesis and antioxidant enzymatic function is the ecophysiological basis of alleviating the suppression of UV-B radiation on growth of seedlings. Furthermore, the protective effect of Ce on seedlings exposed to T_1 level of UV-B radiation is superior to T_2 level.

Keywords: cerium(Ce^{3+}); UV-B radiation; rape; growth; photosynthesis; antioxidant enzyme system

Introduction

Negative impact of ultraviolet-B (UV-B, 280—320 nm) radiation on biological organisms have been well known, and were predicted to be worse with increase of UV-B influences. There are a large number of reports about the effects of UV-B radiation on visual symptoms, leaf ultrastructure and anatomy, photosynthetic pigments, UV-B absorbing compounds, photosynthesis, growth and development, yield and protective mechanisms of plants (Kakani *et al.*, 2003), providing abundant information to access the damage of UV-B radiation on plants. Noticeably, the studies on the ecological protection on plant against damage of UV-B radiation are relatively few.

Since 1970s, the rare earths (RE) have been extensively used as micro-fertilizes on crop yields in China. It has already been proved that RE can improve the production and quality of crops and alleviate the crisis of food supplies (Guo, 1999). Recently, some Chinese researchers paid more attention on the efficiency of RE on enhancing plant resistance to stresses and improving their tolerance to environmental pollution, with the purpose of exploiting the new application function of RE in agriculture and also lightening the damage of environmental pollution to agricultural ecosystem and its production. Some studies have reported that RE lightens the injury of acid rain, heavy metal and ozone to crops (Zhou *et al.*, 2004), but none of these relates to UV-B radiation.

The aim of this study was to investigate the effect of RE on growth and physiological mechanism in rape seedlings exposed to UV-B radiation. Rape is one of important oil crops planted in a large area of China. In plants, growth and photosynthesis, stomatal behavior and transpiration are major targets for UV-B radiation, which have been pinpointed (Teramura and Sullivan, 1994). In addition, photosynthesis is the basis of plants growth and development, and antioxidant enzymes have the capability of scavenging reactive oxidative oxygen caused by stresses, both of which play important roles in life circle of plants. Therefore, it is necessary to discuss the effect of RE on growth, photosynthesis and antioxidant enzyme system in crops exposed to UV-B radiation, providing the basic data to exploit the application of RE in agriculture, and also pointing out a new direction to study the

Foundation item: The National Natural Science Foundation of China (No. 20471030; 30570323) and the Foundation of State Planning Committee(No. GFZ040628); *Corresponding author

ecological protection on the crops against the damage of UV-B radiation.

1 Material and methods

1.1 Plant culture

The rape (*Brassica juncea* L.) seeds of "Shilifeng" were sterilized for 10 min by $HgCl_2$ (0.1%), and washed three times with deionized water. After being soaked for 4 h, seeds were placed in the dish underlaid with three pieces of filter paper and 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 8 klx (12 h/d) which were aired twice every day. When the first leaf was developed, the seedlings were cultured in Arnon+Hoagland solution. The nutrient solution was renewed every 3 d for the pH stabilization. The seedlings with age in 5 weeks were treated by RE and UV-B radiation.

1.2 Treatments

The optimum concentration of $CeCl_3$ solution was 12 mg/L, which was determined in the pre-experiments. The $CeCl_3$ solution was sprayed evenly on the leaves until drops began to fall. The same amount of distilled water was applied to another set as the control (CK). After 48 h, half of seedlings pretreated with Ce were placed under ultraviolet lamps.

Enhanced UV-B radiation was performed with 40 W UV-B lamps (produced by Nanjing Lamp Factory, China) hanged perpendicularly over the plants. The levels of UV-B radiation were 0.15 W/m^2 and 0.35 W/m^2 (Liang *et al.*, 2004), which were determined by ultraviolet radiac (made by Photoelectricity Instrument Factory of Beijing Normal University). Seedlings were irradiated for 5 h from 10:00 to 15:00, for a total of 6 d. The height of lamps over the plants was adjusted to maintain consistent radiation intensity.

There were 6 sample sets in our experiments: control (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 0.35 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 0.35 W/m^2 UV-B radiation). There were 3 replicates for each set and 3 pots per treatment.

1.3 Determination

The physiological indices were measured once after being treated by Ce and UV-B radiation. Chlorophyll was extracted in 80% acetone. The extract was centrifuged at 5300 × g for 10 min and analyzed spectrophotometrically (S24 spectrophotometer, Shanghai, China) at 646 and 663 nm. Chlorophyll was calculated per unit fresh weight according to Lichtenthaler and Wellburn (1983). Membrane permeability (E) was measured according to Ref. (Zhang, 1990). Net photosynthesis rate (Pn), transpiration rate (En), leaf conductance to water vapour (Gs) and intercellular CO_2 concentration (Ci) were measured under the ambience at 25℃ with a portable photosynthetic system (CIRAS-1, PP Systems, UK). PFD were 300 μmol/(m^2·s), and there were 320 μl/L CO_2 provided by the CIRAS-1 photosynthesis system. Water utilizing efficiency (WUE) was expressed with the ratio of Pn to En.

Enzyme extraction: usually 0.5 g leaf materials were homogenized in 5 ml 150 mmol/L phosphate buffer (pH 7.4) containing 5 mmol/L EDTA. The homogenate was centrifuged at 18000 g for 15 min. The supernatant obtained was used for enzyme assays. All operations were carried out at 4℃.

Enzymes assays: SOD was assayed by photochemical method described by Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme resulting in 50% inhibition of the rate of *p*-nitroblue tetratzolium chloride reduction at 560 nm. CAT was assayed by the method of Dhindsa *et al.* (1981). The enzymatic activity was expressed by H_2O_2 mg/(gFw·min). POD activity was measured using a modification of the method of Macadam *et al.* (1992). The reaction mixture, total volume 3 ml, containing 0.1 ml of enzyme extract, 12 mmol/L H_2O_2 and 7.2 mmol/L guaiacol in 50 mmol/L phosphate buffer, pH 5.8, was kept at 35℃ for 5 min, then the absorbance at 470 nm was measured. The absorbance unit of enzyme activity is min^{-1}·g^{-1}Fw.

The seedlings were harvested at the day 3 after physiological indices being measured. The roots volume was measured following the Reference(Zhang, 1990). Length of stem, plant and main root was directly measured by a ruler. Leaf area was determined by transparent graph paper method (Liu *et al.*, 1998). After above measurements, all parts of each plant were collected, dried for 12 h at 80℃ in an oven, and weighed. All data were analyzed with *LSD* test (P<0.05).

2 Results and discussion

2.1 Effects of Ce on growth of rape seedlings under UV-B radiation

2.1.1 Effects on aboveground growth of rape seedlings

As shown in Table 1, the plant height, stem length, leaf number, total leaves area, leaves and stem fresh (dry) weight in Ce treatment were all superior to those in CK. The increased extent of above indices was about 9.7%—38.8%. However, the 8 indices in T_1 and T_2 treatments were decreased by 13.2% — 44.1% and 21.4% —49.3% respectively, compared with those of CK. In addition, total damage area of leaves of treatment T_2 was larger than that of treatment T_1. For treatments Ce+UV-B, the 9 indices were all inferior to those of CK, but superior to those of treatments UV-B. The reduction was about 2.8% — 23.6% (Ce +T_1) and 6.5% —28.4% (Ce+T_2) respectively, lower than the reduction in UV-B treatments. Furthermore, there were no significant differences in aboveground growth indices except total area of leaves between treatments Ce+T_1 and CK. The results indicate that the damage of plant by UV-B radiation can be alleviated by Ce, and the regulating effect of Ce on plants exposed to low level of UV-B radiation is more obvious than to high level of UV-B radiation.

2.1.2 Effects on belowground growth of rape seedlings

Root is one of the important organs in plants, and the shape and development influences the uptake of mineral elements and water of plants. The indices of root with 6 treatments are shown in Table 1. For treatment Ce, the root volume, fresh and dry weight, total assimilating area, active area and main root length were all increased by about 5.4% —26.8%, compared to those of CK. However, except total assimilating area and active area, the root indices of rape seedlings with treatments T_1 and T_2 were all obviously lower than those of control. The reduction in root indices of treatments T_1 and T_2 were up to 14.1% —35.6% and 20 .3% —42.6% respectively. Although the values of root indices in treatment Ce+T_1 were lower than those in CK, they were obviously higher than those in treatment T_1. In addition, there were no obvious differences in main root length, dry weight, total assimilating area and active area between treatments Ce+T_1 and CK. The phenomenon suggests that the injury of UV-B to roots can be alleviated to a certain extent by Ce. For Ce+T_2 treatment, the root

Table1 Effects of Ce on growth of rape seedlings under UV-B radiation stress

Treatment	CK	Ce	T_1	T_2	Ce+T_1	Ce+T_2
Plant height, cm	19.31±0.16^{b} (100.0)	22.22±0.18^{a} (115.1)	15.58±0.10^{d} (80.7)	14.59±0.09^{e} (75.6)	17.67±0.08^{b} (91.5)	16.89±0.20^{c} (87.5)
Shoot length, cm	5.36±0.06^{b} (100.0)	5.97±0.03^{a} (111.3)	4.49±0.03^{d} (83.3)	4.03±0.01^{e} (75.3)	5.17±0.06^{b} (96.5)	4.78±0.06^{c} (89.2)
Leaf number, plant^{-1}	6.0±0.4^{ab} (100.0)	6.5±0.6^{a} (109.7)	5.2±0.1^{bc} (86.8)	4.7±0.3^{c} (78.6)	5.3±0.2^{bc} (89.6)	5.0±0.1^{bc} (84.7)
Total leaf area, cm^2/plant	50.75±1.91^{b} (100.0)	70.07±1.69^{a} (138.8)	28.20±0.96^{d} (55.9)	25.58±0.36^{d} (50.7)	38.50±1.55^{c} (76.4)	36.10±1.30c (71.6)
Total damage leaf area, cm^2/plant	0.00^{c} (0.0)	0.00^{c} (0.0)	3.89±0.33^{b} (13.8)	6.85±0.81^{a} (26.8)	1.09±0.17^{c} (2.8)	2.86±0.16^{b} (7.9)
Shoot fresh weight, g/plant	0.320±0.011^{b} (100.0)	0.401±0.011^{a} (124.6)	0.253±0.010^{c} (78.5)	0.226±0.008^{d} (70.0)	0.309±0.006^{b} (95.9)	0.262±0.005^{c} (81.4)
Shoot dry weight, g/plant	0.026±0.001^{b} (100.0)	0.034±0.001^{a} (131.7)	0.021±0.001^{c} (82.1)	0.019±0.001^{d} (73.1)	0.024±0.001^{b} (94.2)	0.021±0.001^{c} (84.1)
Leaves fresh weight, g/plant	2.258±0.065^{b} (100.0)	2.816±0.014^{a} (124.7)	1.449±0.097^{cd} (64.1)	1.182±0.011^{d} (52.3)	1.980±0.073^{b} (87.7)	1.683±0.025^{c} (74.6)
Leaves dry weight, g/plant	0.183±0.010^{b} (100.0)	0.207±0.007^{a} (113.4)	0.118±0.008^{c} (64.4)	0.089±0.008^{d} (48.8)	0.153±0.005^{b} (83.8)	0.133±0.002^{c} (72.6)
Root length, cm	9.62±0.13^{a} (100.0)	10.14±0.18^{a} (105.4)	8.26±0.17^{d} (85.9)	7.67±0.13^{c} (79.7)	9.12±0.09^{ab} (94.8)	8.95±0.13^{bc} (93.0)
Root volume, cm^{-3}	0.48±0.02^{b} (100.0)	0.61±0.01^{a} (126.8)	0.36±0.01^{d} (75.3)	0.31±0.01^{e} (64.9)	0.41±0.02^{c} (85.2)	0.37±0.01^{d} (76.5)
Total absorbing area of root, cm^2	3.13±0.01^{a} (100.0)	3.17±0.02^{a} (101.3)	3.00±0.01^{a} (95.8)	3.00±0.03^{a} (95.7)	3.06±0.01^{a} (97.8)	3.03±0.01^{a} (96.6)
Active area of root, cm^2	1.55±0.02^{a} (100.0)	1.79±0.06^{b} (115.4)	1.51±0.03^{a} (97.3)	1.51±0.01^{a} (97.2)	1.53±0.03a (98.6)	1.52±0.02a (98.2)
Root flesh weight, g/plant	0.255±0.011^{b} (100.0)	0.321±0.004^{a} (125.7)	0.164±0.003^{cd} (64.4)	0.146±0.010^{d} (57.4)	0.196±0.014^{c} (76.7)	0.184±0.004c (72.2)
Root dry weight, g/plant	0.031±0.001^{b} (100.0)	0.037±0.002^{a} (118.1)	0.023±0.001^{c} (73.1)	0.019±0.002^{d} (63.6)	0.027±0.001^{b} (87.7)	0.023±0.001^{c} (75.2)

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

indices, except total assimilating area and active area, were distinctly inferior to those of CK, but obviously superior to those of treatment T_2. The results showed that the regulating effect of Ce can alleviate the injury of UV-B radiation to roots, and also such regulating effect is related to stress intensity.

2.2 Effects of Ce on photosynthesis in rape seedlings exposed to UV-B radiation

As shown in Table 2, chlorophyll content, net photosynthesis rate (Pn), transpiration rate (En), stomatal conductance to water vapour (Gs) and intercellular CO_2 concentration (Ci) were higher in leaves treated with Ce, compared with those of CK. The increase in Pn was related to the rise in Gs and Ci, because the latter provided the former with ample material used in carbon assimilation process. As increase rate of En was similar with that of Pn, Ce had no obvious effect on instantaneous WUE. Except Ci, the photosynthetic indices of rape seedlings exposed to UV-B radiation decreased by 11.2% —25.9% (T_1) and 20.9% —56.9% (T_2) respectively. However, the decreased rate of five indices in treatment T_1 was smaller to those in treatment T_2. Analysing the change of Ci in leaves exposed to UV-B radiation, we presume that Ci was increased due to the depressed photosynthesis in leaves and guard cells. The decrease in solute of guard cell led to the restrained effect on stomatal conductance and the decrease in Gs, preventing the rising of En. As En decreased less than Pn, UV-B radiation reduced instantaneous WUE. Except Ci, the five indices in treatments Ce+UV-B were distinctly superior to those in treatments UV-B. In addition, there were no differences in five photosynthetic parameters between Ce+T_1 and CK. The explanation to this phenomenon is that the increase in Ci and Gs compensates the decrease in Pn resulting from damage of UV-B radiation on photosynthetic tissue. As the decline in En was also less than Pn, WUE of treatments Ce+UV-B was still lower than that of CK. Analysis of variance significance shown in Table 2 indicates that Ce has better protective effect on plants exposed to low UV-B radiation than to high UV-B radiation.

Table 2 Effects of Ce on photosynthesis in rape seedlings under UV-B radiation stress

Treatment	CK	Ce	T_1	T_2	Ce+T_1	Ce+T_2
Chl, mg/g	1.62 ± 0.09^{b}	1.85 ± 0.06^{a}	1.32 ± 0.05^{de}	1.16 ± 0.01^{e}	1.55 ± 0.04^{bc}	1.38 ± 0.03^{cd}
	(100.0)	(114.2)	(81.7)	(71.8)	(95.8)	(85.1)
Pn, μmol/(m^2·s)	4.5 ± 0.1^{b}	5.7 ± 0.4^{a}	3.3 ± 0.1^{e}	1.9 ± 0.1^{d}	3.9 ± 0.1^{bc}	2.8 ± 0.2^{e}
	(100.0)	(126.4)	(74.1)	(43.1)	(86.2)	(72.4)
Ci, μl/L	184 ± 5^{a}	208 ± 6^{a}	234 ± 4^{b}	239 ± 3^{b}	240 ± 4^{b}	243 ± 5^{b}
	(100.0)	(112.6)	(127.2)	(126.8)	(129.8)	(132.1)
G_s, mmolH_2O/(m^2·s)	124 ± 6^{b}	142 ± 3^{a}	108 ± 2^{c}	68 ± 4^{d}	128 ± 1^{b}	118 ± 6^{bc}
	(100.0)	(112.6)	(84.0)	(68.0)	(103.2)	(95.1)
En, mmolH_2O/(m^2·s)	1.03 ± 0.06^{b}	1.26 ± 0.06^{a}	0.85 ± 0.04^{bc}	0.55 ± 0.01^{d}	0.98 ± 0.05^{b}	0.8 ± 0.01^{c}
	(100.0)	(122.9)	(82.4)	(54.0)	(95.6)	(78.0)
WUE, μmol/mmolH_2O	4.37 ± 0.08^{a}	4.52 ± 0.12^{a}	3.88 ± 0.06^{a}	3.45 ± 0.14^{b}	3.98 ± 0.12^{a}	3.5 ± 0.05^{ab}
	(100.0)	(103.3)	(88.8)	(79.1)	(91.1)	(80.1)

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

2.3 Effects of Ce on antioxidant enzyme system in rape seedlings exposed to UV-B radiation

Membrane permeability(E%) is used as an index of lipid peroxidation in plants under adverse environmental conditions. Superoxide dismutase (SOD), catalase(CAT) and peroxidase(POD) are three groups of enzymes that effectively scavenge radicals in the cells and prevent excessive radicals induces the lipid peroxidation and the degradation of a variety of important biological molecules such as nucleic acids, proteins etc. The changes of membrane permeability and antioxidant enzymes in 6 treatments were shown in Table 3. For Ce treatment, there was a reduction in E% and an increase in the activities of three antioxidant enzymes, compared to CK. The results indicated that the stability of membrane was increased and the scavenging radical capacity was promoted due to Ce influencing the structure of membrane and improving the activity of antioxidant enzymes. The four indices mentioned above were all increased in treatments UV-B, and the increased rate of T_1 treatment was smaller than that of T_2 treatment. Overall, the sequence of three enzymes activity in treatment T_1 was SOD>CAT>POD, but in treatment T_2 was POD >SOD >CAT. We presumed that the sensitivity of three enzymes to UV-B radiation was different (SOD>CAT>POD), and catalysis function of POD in treatment T_2 changed from scavenging radicals to participating in the process of cell senescence (Zhang, 1994). The increase of E% in UV-B treatments indicated that although the activity of three antioxidant enzymes was increased due to their

irritability to UV-B radiation, excessive radicals accumulated and lipid peroxidation happened. Compared to UV-B treatments, E% and the activity of SOD and CAT in Ce+UV-B treatments were lower, and POD activity was higher due to the change of its function. The results showed that the injury of free radicals to cell membrane was effectively alleviated due to Ce effectively regulating the function of defense enzymes. Analysis of variance significance shown in Table 3 similarly indicated that Ce has better regulating effect on plants exposed to low level of UV-B radiation than to high level.

Table 3 Effects of Ce on membrane permeability and activities of antioxidant enzymes in rape seedling under UV-B radiation

Treatment	CK	Ce	T_1	T_2	Ce+T_1	Ce+T_2
E, %	22.38 ± 0.95^{c} (0.0)	17.57 ± 1.13^{d} (-4.8)	29.29 ± 1.65^{b} (6.9)	58.80 ± 1.94^{a} (36.6)	24.74 ± 1.39^{bc} (2.4)	52.50 ± 0.97^{a} (30.1)
POD, A_{470}, min/g FW	0.22 ± 0.01^{d} (100.0)	0.26 ± 0.01^{c} (118.7)	0.24 ± 0.00^{cd} (109.5)	0.67 ± 0.05^{b} (308.9)	0.22 ± 0.00^{d} (101.2)	0.77 ± 0.05^{a} (356.4)
CAT, H_2O_2mg/(g Fw·min)	618.00 ± 17.37^{c} (100.0)	699.57 ± 4.19^{d} (113.2)	750.61 ± 6.95^{c} (121.5)	1257.69 ± 9.83^{a} (203.5)	658.57 ± 9.69^{dc} (106.6)	1083.97 ± 29.46^{b} (175.4)
SOD, U/g FW	623.82 ± 24.10^{d} (100.0)	731.11 ± 13.75^{c} (117.2)	765.98 ± 9.67^{bc} (122.8)	1386.82 ± 13.59^{a} (222.3)	676.09 ± 17.63^{c} (108.4)	817.61 ± 32.81^{b} (216.7)

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

3 Conclusions

Ce can alleviate the restraining effect of UV-B radiation during the growth of rape seedlings, and has better protective effect on rape seedlings exposed to T_1 level of UV-B radiation than to T_2 level. The evidences of the above conclusion were that the decreased rate of aboveground and belowground growth indices in treatments Ce+T_1 and Ce+T_2 was lower than that in treatments T_1 and T_2.

The decreased rate of photosynthetic parameters in treatments Ce+T_1 and Ce+T_2 was respectively around 3.2%—13.8% and 4.9%—27.6%, lower than that in treatments T_1 and T_2 between 11.2%—25.9% and 20.9%—56.9%, too. These data indicate that Ce can reduce the change extent of Chl content, Pn, En, Gs and WUE in leaves exposed to UV-B radiation, and its regulating effect on photosynthesis may be one of main causes that reduce the decrease in growth of rape seedlings exposed to UV-B radiation.

Membrane permeability and the activity of antioxidant enzymes except POD in treatments Ce+UV-B were all lower than those in treatments UV-B. This phenomenon suggests that injury of free radicals to cell membrane is effectively alleviated by Ce regulating the function of defense enzymes. Under high level (T_2) of UV-B radiation, the catalysis function of POD changed from scavenging radicals to participating in the process of cell senescence, and therefore E% in treatment Ce+T_2 was larger than that in treatment Ce+T_1. Similarly, Ce has better protective effect on antioxidant enzyme system exposed to T_1 level of UV-B radiation than to T_2 level. We deduced that the Ce can regulate the activities of defense enzymes and maintain membrane stability, which was the basis of improving the resistance of crops to UV-B radiation.

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(Received for review May 8, 2005. Accepted June 21, 2005)