Effect of UV-B radiation treatments on growth, physiology and antioxidant systems of cucumber seedlings in artificial climate chamber

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Abstract: Ultraviolet radiation (UV-B) radiation is a key environmental signal for plant growth and development. An excess or lack of UV-B can affect plant resistance, yield and quality. However, the appropriate dose of UV-B for cucumber seedlings growth in plant factories is not well understood. In this study, the effect of different doses of UV-B radiation on the growth, physiology and antioxidant systems of cucumber seedlings in an artificial climate chamber was studied. The results showed that UV-B radiation effectively inhibited the elongation of cucumber seedlings by 4.2%-32.0% and decreased soluble protein content in cucumber leaves by 14.2%-28.2%. 3.33 μmol/(m^2·s) UV-B promoted stem diameter growth, soluble sugar content, total ascorbic acid and the superoxide dismutase, peroxidase and catalase activities in cucumber leaves by 13.6%-22.3%, 22.7%-56.7%, 16.9%-23.2%, 23.8%-25.9%, 34.1%-50.4% and 27.4%-36.4%, respectively. However, this UV-B dose had no influence on the net photosynthetic rate of cucumber leaves. Therefore, we conclude that 3.33 μmol/(m^2·s) UV-B is beneficial for growth and increases the resistance of cucumber seedlings in an artificial climate chamber. This study is hoped to provides a theoretical basis for cucumber and other seedling growth under UV-B treatments.

Keywords: photosynthesis; ultraviolet radiation; growth; cucumber seedlings; H_2O_2; antioxidant enzyme system; ascorbic acid; artificial climate chamber

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0 Introduction

Light is necessary for plant life, serving as both an energy source for photosynthesis and a signal for development and growth. Furthermore, light quality is a crucial variable for the growth and development of plants, and among the different light wavelengths, UV-B (280 315 nm) is a particularly important environmental factor.

Many studies have reported that excess UV-B has a negative effect on the growth and physiological metabolism of crops, including corn, wheat, rice, and soybeans[1-3]. However, due to the covering materials (e.g., plastic film or glass) used in different light shields and filters (photosynthetic active radiation transmittance is 80-90% and UV-B transmittance is 15-30%), standard light components are not sufficient for greenhouse vegetable cultivation[4-5]. Under such circumstances, vegetable can display excessive growth[6], weak growth, poor resistance[7], low yield[8] and poor quality[8-10].

Recently, several studies have examined the effects of supplemental UV-B on vegetable quality under greenhouse conditions. These studies showed that UV-B can induce secondary metabolites, which are beneficial for both plants and humans. Wang et al. reported that 0.22 μmol/(m^2·s) of supplemental UV-B radiation could improve fruit quality in tomatoes in winter plastic greenhouses[4]. Luthriaand Krizek reported that phenolic acid content in tomatoes was approximately 20% higher under +UV conditions compared with UV conditions[11]. Chen et al. reported that 6.91-10.37 μmol/(m^2·s) supplemental UV-B radiation could increase ascorbic acid (AsA) content in pakchoi leaves[8]. Hou et al. reported the effect of 4.15 μmol/(m^2·s) UV-B on photosynthesis and antioxidant enzyme activity in cucumber seedlings[12-13]. Sun et al. reported the effect of 0.86 and 4.15 μmol/(m^2·s) UV-B on the growth and photosynthesis of cucumber seedlings, but the results were not consistent with those of previous reports[12,14].

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Plant factories are believed to solve the problems of cultivated land limitation and enable vegetable to grow all year. Plant factories can be established by controlling temperature, nutrient supply, light quality and other factors. At present, red light and blue light are widely used in plant factories[15-16]. However, the effect of different doses of UV-B radiation on the growth and antioxidant systems of cucumber seedlings in plant factories has been poorly described.

In the present study, we determined the effects of different doses of UV-B radiation on the growth, physiology and antioxidant systems of cucumber seedlings, and we aimed to determine the dose of UV-B that is beneficial for cucumber seedling growth in plant factories.

1 Materials and methods

1.1 Plant materials and growth conditions

Cucumber seeds (*Cucumis sativus* L. cv. Chinese long 9930) were germinated on September 28, 2015. The germinated seeds were transferred to a greenhouse at the Institute of Vegetables and Flowers at the Chinese Academy of Agricultural Sciences (Beijing, China, 39.9°N, 116.5°E). Uniform seedlings were transplanted into 7 L pots filled with commercial substrate (Shandong, China) when the third leaf had expanded. When the sixth leaf had expanded, the plants were moved to a controlled chamber with a 14 h photoperiod, 28/20 ℃, 60% relative humidity and 120 μmol/(m^2·s) photon flux density (400-700 nm) supplemented with high-pressure sodium lamps from 6:00 AM to 8:00 PM. The experiment was a completely randomized block design with three replicates. Except for the treatment content, the same local management practices were applied in all treatments.

1.2 Experimental design

After the cucumber seedlings were moved to a controlled chamber under 14 h photoperiod, 28/20 ℃, 60% relative humidity and 120 μmol/(m^2·s) photon flux density (400-700 nm) supplemented with high-pressure sodium lamps from 6:00 AM to 8:00 PM for three days (on October 26, 2015), they were exposed to biologically effective UV-B irradiance for 4 h (11:00 AM-2:00 PM). The experiments included 5 different doses: 0(CK), 1.67(T1), 3.33(T2), 5.01(T3) and 6.67(T4) μmol/(m^2·s) (Table 1). The supplemental UV-B was applied using Philips TL20 W/01 RS tubes (311-313 nm spectrum peak, Philips) (Fig.1). The tubes were suspended at different distances above the plant canopy so that the doses of UV-B radiation could be adjusted every 3 days. Samples were collected on 0 d (October 26, 2015), 7 d (November 2, 2015), 14 d (November 9, 2015), 21 d (November 16, 2015) and 28 d (November 23, 2015) after the treatment.

1.3 Growth parameters

The growth parameters of 4 independent cucumber seedlings for each treatment were determined on 0, 7, 14, 21 and 28 d after the start of the treatment[17]. Plant height was measured from the base of the stem to the tip of the stem. Stem diameter was measured at the base of the stem using a caliper. The second, third and fourth leaves counted from bottom to top were selected to determine leaf photosynthesis parameters using a LI-6400 portable photosynthesis system (Li-Cor 6400XT, Lincoln, NE, USA). Measurements were taken in a controlled chamber within a 2 h interval (08:00-10:00), and red and blue LEDs were selected as the light source. The set values of photosynthetic photon flux density, CO_2 concentration (Ca), air temperature, relative humidity (RH) and air flow rate inside sample chamber were 800 μmol/(m^2·s), 400 μmol/s, 28 ℃, 60% and 400 μmol/s, respectively.

Table 1 Experimental treatments

Treatment	Supplemental UV-B dose/ (μmol·(m^{-2}·s^{-1}))	Supplemental UV-B time (hour per day)
CK	0	4 (11:00 AM-2:00 PM)
T1	1.67	4 (11:00 AM-2:00 PM)
T2	3.33	4 (11:00 AM-2:00 PM)
T3	5.01	4 (11:00 AM-2:00 PM)
T4	6.67	4 (11:00 AM-2:00 PM)

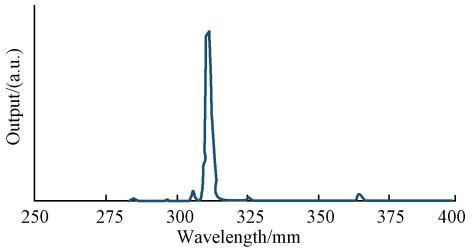


Fig.1 Spectrum of the Philips TL20 W/01 RS tubes

The dry weight of 3 independent cucumber seedlings for each treatment was determined 28 d after the start of the treatment[18]. Plant samples were dried in an oven at 105 ℃ for at least 30 min and then stored at 80 ℃ for at least 4 days before being weighed.

1.4 Physiological parameter measurements

For measurement of physiological responses, three fully expanded leaves were collected from each plant, and the leaves of three plants were mixed as one replicate. The collected leaves were measured immediately and stored at 4 ℃ for no more than 24 h. Soluble protein concentration was determined using a Coomassie brilliant blue staining protein assay kit (Suzhou, China). Soluble sugar, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content were measured according to the methods of Wang[6].

1.5 Antioxidant system measurements

For analysis of antioxidant system responses, the sampling method is consistent with 2.4. The collected leaves were immediately frozen in liquid nitrogen and stored at -80 ℃ until analysis. Superoxide dismutase (SOD) activity was assayed using the nitro blue tetrazolium (NBT) inhibition protocol designed by Wang[6]. Peroxidase (POD) activity was determined using a POD assay kit (Suzhou, China). Catalase (CAT) activity was determined using a CAT assay kit (Suzhou, China). Total AsA content was assayed using the spectrophotometric method described by Wang[6].

1.6 Statistical analysis

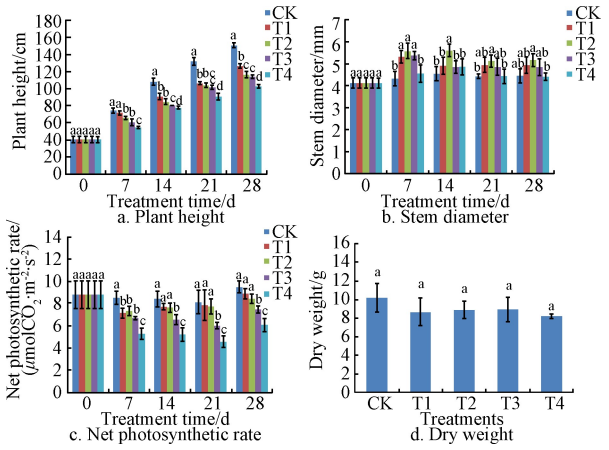
The data were analyzed using the statistical analysis software program SPSS. Statistically significant differences among means were determined using Duncan's multiple range test at a significance level of $P<0.05$.

2 Results

2.1 Effects of different doses of UV-B radiation on the growth characteristics of cucumber seedlings

The growth characteristics of the cucumber seedling under different doses of UV-B radiation are shown in Fig 2. plant height decreased as UV-B radiation dose and treatment time increased. Specifically, plant height decreased by 4.2%-16.0%, 12.2%-22.6%, 18.9%-24.0% and 27.0%-32.0% for T1, T2, T3 and T4, respectively (Fig. 2a). Stem diameter in the T2 group was significantly increased from days7 to 28 and was significantly increased on day 7 for T1 and T3 (Fig.2b).

We found that the net photosynthesis in cucumber leaves under low and medium doses of UV-B radiation (1.67 or 3.33 μmol/(m^2·s)) did not change significantly between days 14 and 28, whereas photosynthesis was significantly reduced under high doses of UV-B radiation (5.01 or 6.67 μmol/(m^2·s)) between days 7 and 28 (Fig.2c). However, the effects of different doses of UV-B radiation on the dry weight of the cucumber seedlings were minimal (Fig.2d).



Note: Mean values with the same letters are not significantly different using Duncan's multiple range test at $P<0.05$, the same below.

Fig.2 Effects of different UV-B radiation doses on plant height, stem diameter, net photosynthetic rate and dry weight

2.2 Effects of different doses of UV-B radiation on the physiological characteristics of cucumber seedlings

The physiological characteristics of the cucumber seedlings under different doses of UV-B radiation are shown in Figure 3. The results demonstrated that the soluble protein content of cucumber seedling leaves exposed to UV-B radiation decreased (Fig.3a). The level of soluble sugar in the T2 treatment group was higher than the control from day 7 to 28, whereas the level of soluble sugar for the T4 treatment group was lower than the control from day 7 to 28. In addition, the soluble sugar content of the T3 treatment group was higher than the control from day 7 to 14, although it was lower than the control on day 28. Overall, these results indicate that 3.33 μmol/(m^2·s) UV-B radiation is beneficial for the accumulation of soluble sugars in cucumber seedling leaves.

The levels of H_2O_2 and MDA showed similar trends (Fig.3c, d). The content of H_2O_2 in T1, T3 and T4 was significantly higher than that in the control during the whole treatment period, and the content of T2 H_2O_2 was not significantly different from that of CK. In addition, the content of H_2O_2 in T4 was significantly higher than that in other treatments. In plants, MDA is the most abundant aldehydic lipid breakdown product. The level of MDA in T3 and T4 was higher than that in the control, and the level of MDA in T2 was lower than that in the control on day 7. The level of MDA in T1, T2, T3 and T4 was significantly higher than that in the control on day 14. The level of MDA in T3 and T4 was significantly higher than that in the control, and the content of MDA in T2 was not significantly different from that in the control from day 21 to 28 (Fig.3d).

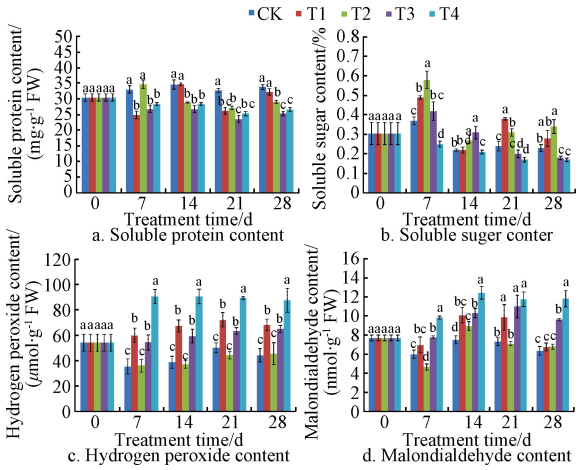


Fig.3 Effects of different UV-B radiation doses on soluble content, soluble sugar content, hydrogen peroxide content and malondialdehyde content

2.3 Effects of different doses of UV-B radiation on the antioxidant systems of cucumber seedlings

We found that UV-B radiation could change superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities in cucumber seedling leaves (Fig.4).

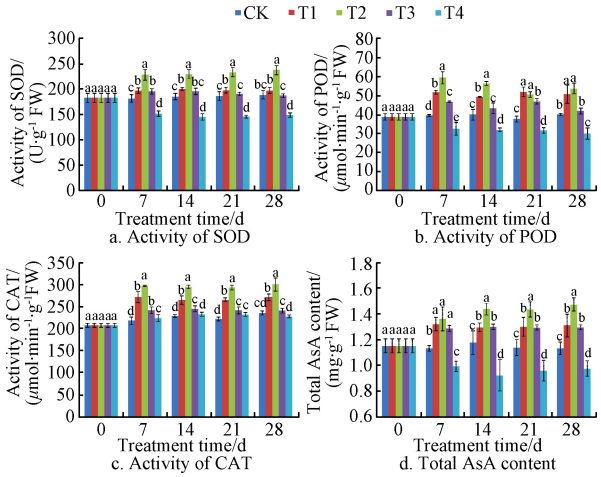


Fig.4 Effects of different UV-B radiation doses on activity of SOD, POD, CAT and total AsA content

SOD, POD and CAT activities in the T1, T2 and T3 treatment groups were markedly increased compared with the control on day 7, whereas SOD and POD activities in theT4 treatment group were significantly decreased on day 7(Fig.4a, b). By contrast, SOD, POD and CAT activities in seedlings exposed to the low and medium doses of UV-B radiation (T1 and T2) increased, while the 5.01 μmol/(m^2·s) UV-B treatment (T3) resulted in no significant changes. The 6.67 μmol/(m^2·s) UV-B radiation treatment decreased SOD and POD activities in leaves compared with the control. Furthermore, total AsA content in the cucumber seedling leaves was significantly higher in the T1, T2 and T3 treatment groups compared with the control. However, at the highest dose of UV-B radiation, the total AsA content decreased significantly.

3 Discussion

Several studies have shown that UV-B radiation inhibits plant elongation[19]. As expected, the elongation of cucumber seedlings was effectively inhibited by UV-B radiation in our study. The effects of UV-B radiation on plant stem diameters[20], net photosynthesis[21] and dry weight[22] have also been reported. We also found that medium intensity UV-B (3.33 μmol/(m^2·s)) favored stem diameter growth, whereas high intensity UV-B (5.54 or 6.67 μmol/(m^2·s)) led to decrease net photosynthesis significantly. By contrast, low and medium intensity UV-B (1.67 or 3.33 μmol/(m^2·s)) did not affect net photosynthesis. We found low, medium and high intensity UV-B radiation did not affect the dry weight of the plants, we speculate that there was no significant difference due to too short treatment time.

UV-B radiation can damage biological macromolecules such as proteins and DNA[23-25]. In our study, the results showed that UV-B radiation reduced soluble protein content in cucumber leaves. Previous studies have also indicated that UV-B radiation can reduce soluble sugar content in plant tissues. We found that soluble sugar content increased under low and medium intensity UV-B radiation (1.67 or 3.33 μmol/(m^2·s)) and decreased under the higher intensity UV-B treatments (particularly the 6.67 μmol/(m^2·s) UV-B treatment).

Jenkins reported that UV-B promoted the synthesis of H_2O_2 in plants[26]. Our results showed that three doses of UV-B radiation induced H_2O_2, synthesis, except for the medium intensity UV-B (3.33 μmol/(m^2·s)). We hypothesized that UV-B could increase H_2O_2 content. However, 3.33 μmol/(m^2·s) activated antioxidant systems and increased SOD, POD, CAT activities, and thus, H_2O_2 content under 3.33 μmol/(m^2·s) UV-B showed no differences compared to the control. In addition, we found that the SOD, CAT and POD enzyme activities increased significantly under 3.33 μmol/(m^2·s) UV-B. MDA levels are an indicator of the extent of membrane lipid peroxidation in plants. In general, MDA content in most UV-B radiation-treated seedlings was higher than in the control group, with the exception of the T2 group (3.33 μmol/(m^2·s) UV-B treatment), consistent with the changes in H_2O_2 content. These results indicated that membrane damage occurred in cucumber seedlings under 1.67, 5.01 and 6.67 μmol/(m^2·s) UV-B.

Reactive oxygen species (ROS), such as H_2O_2, are important signal molecules that induce multiple responses, both biotic and abiotic, in plants following environmental stress[27-28]. Following an increase in the ROS concentration, the antioxidant system will be activated in plants to eliminate excessive ROS. However, when the ROS levels are too high, these molecules will cause irreversible damage to plants[29-30]. In the present study, the activities of the SOD, CAT and POD enzymes, which are involved in the antioxidant defense system, increased significantly under 1.67 and 3.33 μmol/(m^2·s) UV-B radiation. Similarly, AsA, a non-enzymatic antioxidant molecule, also showed increased levels under 1.67, 3.33 and 5.01 μmol/(m^2·s) UV-B radiation. By contrast, the activities of the antioxidant enzymes and AsA levels decreased under 6.67 μmol/(m^2·s) UV-B, this perhaps indicating that the antioxidant system had broken down. In summary, our results suggest that cucumber seedlings are not injured by 3.33 μmol/(m^2·s) UV-B and in fact show increased resistance based on the induction of secondary metabolites and activation of the antioxidant system. In addition, 1.67 μmol/(m^2·s) UV-B caused repairable damage to cucumber seedlings. By contrast, we observed clear evidence of damage in plants treated with high intensity UV-B.

In general, we found that low and medium intensity UV-B treatments are beneficial to plant growth due to enhanced resistance, whereas high intensity UV-B can damage macromolecules such as proteins and nucleic acids. Therefore, it is necessary to provide a suitable dose of UV-B radiation for vegetable production under greenhouse conditions.

In this report, the effects of different doses UV-B on the physiological characteristics of cucumber seedlings were studied. A further study could assess the effects of long-term UV-B on the yield and quality of cucumbers and determine the appropriate UV-B intensity for different cucumber growing periods. These findings are of important practical value. And how does UV-B activate the antioxidant system (such as ascorbic acid metabolism) in leaves, whether it is related to reactive oxygen species, and we are doing further research.

4 Conclusions

UV-B is a key environmental signal for plant growth and development. An excess or lack of UV-B can affect plant resistance. However, the appropriate dose of UV-B for cucumber seedlings growth in plant factories is not well understood. The major aim of the current study was to determine the suitable UV-B doses for cucumber growth in a plant factory. This study showed that the growth of cucumber seedlings is promoted by 3.33 μmol/(m^2·s) UV-B, and 3.33 μmol/(m^2·s) UV-B increased the antioxidase activities and AsA content of cucumber seedlings. By contrast,

these characteristics were inhibited by 6.67 μmol/(m^2·s) UV-B. Furthermore, 3.33 μmol/(m^2·s) UV-B had no significant influence on H_2O_2 and MDA content in cucumber leaves, whereas 5.01 or 6.67 μmol/(m^2·s) UV-B led to an increase in MDA and H_2O_2 levels. The results of this study indicate that 3.33 μmol/(m^2·s) UV-B promoted the growth and improved the stress resistance of cucumber plants in a plant factory.

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UV-B 对人工气候室内黄瓜苗期生长、生理及抗氧化系统的影响

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摘 要：紫外线（UV-B）是植物生长发育的关键信号因子。过量或缺少 UV-B 都会影响作物的抗性、产量和品质。 然而，目前植物工厂中适宜黄瓜生长的 UV-B 强度尚不明确。以黄瓜（*Cucumis sativus* L.）苗期植株为材料，研究不同强度 UV-B 对人工气候室内黄瓜苗期植株生长、生理和抗氧化系统的影响。结果表明：与对照相比，UV-B 处理黄瓜植株高度降低 4.2%～32.0%，叶片中可溶性蛋白含量降低 14.2%～28.2%。3.33 μmol/(m^2·s) UV-B 处理植株茎粗增加 13.6%～22.3%，叶片中可溶性糖的含量增加 22.7%～56.7%，同时激活抗氧化系统，超氧化物歧化酶（SOD）、过氧化物酶（POD）、过氧化氢酶（CAT）活性分别提高 16.9%～23.2%，23.8%～25.9%，34.1%～50.4%，抗坏血酸含量增加 27.4%～36.4%。由此可知，3.33 μmol/(m^2·s) UV-B 有利于人工气候室中黄瓜苗期植株的生长发育、抗氧化酶活性提高及抗氧化物质生成。

关键词：光合作用；紫外光；生长；黄瓜植株；过氧化氢；抗氧化酶系统；抗坏血酸；人工气候室