

ORIGINAL RESEARCH ARTICLE

Effects of continuous light deprivation on cucumber growth and anti-oxidant enzyme activities in solar greenhouse in winter

Yu Xiong¹, Jiamin Diao¹, Xiaoping Xue^{2*}, Xuemei Lv³, Jibo Zhang²

¹ Collaborative Innovation Center on Forecast and Evaluation of Meteorological Disasters, Nanjing University of Information Science & Technology, Nanjing 210044, China.

² Shandong Climate Center, Jinan 250031, Shangdong, China. E-mail: xpdhy@163.com

³ Linyi Meteorological Bureau, Linyi 276000, Shangdong, China.

ABSTRACT

Cucumber Variety 'Drite L108' (*Cucumis sativus* L. Cv. Derit L108) was selected as the test material. In the solar greenhouse, different days (1, 3, 5, 7, 9 d) of light (PAR < 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and normal light conditions were designed with shading nets to observe the growth indexes of cucumber plants and the changes of antioxidant enzyme activities in leaves. The results showed that: (1) continuous low light increased the SPAD (relative chlorophyll) value of cucumber leaves and decreased the net photosynthetic rate. The longer the continuous low light days are, the smaller the net photosynthetic rate of cucumber leaves and the worse the photosynthetic recovery ability would be. (2) The plant height, stem diameter and leaf area per plant were lower than CK, and the above indexes could not return to the normal level after 9 days of normal light recovery; the yield and marketability of cucumber fruit decreased under continuous low illumination. (3) The activities of SOD (superoxide dismutase) and POD (peroxidase) in cucumber leaves increased, the activities of CAT (catalase) first increased and then decreased, and the content of MDA (malondialdehyde) continued to increase. The longer the days of continuous light keep, the more seriously the cucumber leaves were damaged by membrane lipid peroxidation. After continuous light for more than 7 days, the metabolic function of cucumber leaves was difficult to recover to the normal level.

Keywords: Oligarchy; Solar Greenhouse; Cucumber; Morphological Index; Output; Enzymatic Activity

ARTICLE INFO

Received: 7 February 2020
Accepted: 2 April 2020
Available online: 16 April 2020

COPYRIGHT

Copyright © 2020 by author(s).
Trends in Horticulture is published by
EnPress Publisher LLC. This work is li-
censed under the Creative Commons At-
tribution-NonCommercial 4.0 International
License (CC BY-NC 4.0).
<https://creativecommons.org/licenses/by-nc/4.0/>

1. Introduction

Cucumber (*Cucumis sativus* L.) is a vegetable species with the largest protected cultivation area in China, belonging to Cucurbitaceae melon. It likes temperature and humidity, avoids high temperature, and has high requirements for light intensity^[1,2]. More and more people pay attention to the nutritional value, health care function and beauty function of the cucumber^[3], which plays an important role in the vegetable production in green house^[4]. The long-term observation shows that the lack of light caused by snow, fog and haze has become the most important meteorological disaster in the production of greenhouse cucumber, which often leads to the difficulty of temperature rising in the solar greenhouse and damage to greenhouse crops. With the increase of the frequency of haze days, lack of sunshine will further seriously threaten the sustainable development of facility agriculture^[5-7].

Leaves are the main photosensitive organ of the plant and also the part sensitive to light. Weak light can directly affect the development and photomorphogenesis of leaves, resulting in the decline of photosynthetic capacity of leaves^[8-10]. In recent years, there have been some

reports on the effects of facility environment on photosynthetic and physiological and ecological characteristics of cucumber. At present, the research conclusions are relatively consistent. It is believed that weak light reduces the net photosynthetic rate of crops, thus affecting the formation of dry matter. Ody^[11] found that weak light decreased the net photosynthetic rate of plants, and the decreasing range was affected by other environmental factors such as temperature, CO₂ concentration and relative humidity. Other studies suggest that low light and low temperature not only affect the activity of PSI, but also the activity of PSII^[12,13]. Qian *et al.*^[14] studied the changes of active oxygen metabolism in pepper seedlings, and showed that, within a certain range, adversity stress will increase the activity of antioxidant enzymes in leaves, so as to achieve the purpose of eliminating excessive active oxygen free radicals. Many domestic scholars have also recognized that low light stress has become the most common limiting factor in the production of solar greenhouses. It is accompanied by low temperature, which brings serious harm to the production of facility agriculture. Many research reports on the impact of low light and low temperature on crops have achieved a lot. Chen *et al.*^[15] used the artificial climate box to study the changes of chlorophyll content, leaf area, photosynthesis and enzyme activity of cucumber seedlings, and believed that the light intensity played a leading role in the growth of cucumber. Ai *et al.*^[16-19] showed that the growth rate of cucumber plants was significantly slowed down under low light and sub optimal temperature stress, and the photosynthetic function of leaves could basically return to normal within 7 days after stress. The research of Ma *et al.*^[20] on cucumber showed that the POD activity of cucumber was significantly enhanced under weak light environment, the MDA content was increased in varying degrees, and the number of enzyme bands was significantly increased. Yang *et al.*^[21] believed that the production rate of superoxide free radicals and the content of H₂O₂ and MDA of cucumber plants decreased under weak light environment. Zhou *et al.*^[22] took the facility cultivated cucumber "Jinchun 3" as the test material, and found that the activities of SOD and

POD increased during the low temperature and weak light treatment, and the activities of antioxidant enzymes recovered to the control level during the recovery process. POD activity increased rapidly after 5 days of low light treatment, decreased during recovery, and MDA continued to accumulate. Related studies showed that the activities of membrane protective enzymes in leaves were sensitive to the stress of membrane lipid peroxidation in plants.

In view of the impact of a meteorological disaster such as low temperature and lack of light on facility agriculture, previous studies mainly focused on the impact on the growth and development of vegetables in the greenhouse. For cucumber, more studies have been carried out on the effects of parameters such as photosynthesis and chlorophyll fluorescence characteristics of leaves at seedling stage, while few studies have been reported on the effects of continuous low light on the growth and yield of cucumber at flower and fruit stage. In the actual production, the meteorological conditions outside the greenhouse affect the photosynthesis, organ dry matter accumulation and distribution, yield and quality formation of cucumbers by affecting the photosynthetic effective radiation and temperature in the greenhouse. At the same time, because the influence of meteorological conditions on the microclimate in the greenhouse has a certain lag, the meteorological factors inside and outside the greenhouse in the early stage also have a certain restrictive effect on it. Domestic and foreign related research mainly focused on the impact of constant light intensity on the physiological characteristics of potted cucumber seedlings by using the artificial climate chamber. There is a certain gap between the experimental environment and the actual greenhouse environment, and the application of the experimental results is limited. Therefore, this study used the shading net to simulate the continuous low light environment in the sunlight greenhouse, and conducted experiments in the flowering and fruiting period of cucumbers to study the effects of continuous low light on the growth and antioxidant enzyme activities of cucumbers in the sunlight greenhouse, in order to provide theo-

retical reference for the cultivation environment management of cucumbers in the greenhouse.

2. Materials and methods

2.1 Test design

The test was conducted in the solar greenhouse of the Facility Agrometeorological Test Station in Linyi City, Shandong province from December 2015 to may 2016. The greenhouse has a top height of 4.6 m, a width of 10.0 m and a length of 68.0 m. The greenhouse is covered with a polyethylene drip free film with a film thickness of 0.6 mm and a light transmittance of 75%. The cucumber variety is “Dreit L108”. On December 25, 2015, cucumber seedlings with strong and consistent growth were selected for planting. The ridge width was 95 cm, the walkway was 80 cm, the row spacing was 50 cm, the plant spacing was 31 cm, and the planting density was $4.42 \text{ plants} \cdot \text{m}^{-2}$. The seedlings were planted slowly and covered with plastic film. The field management was carried out according to the requirements of high-yield cultivation. The observation in the early stage of the test shows that the photosynthetic effective radiation in the solar greenhouse is $100\text{--}200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in cloudy (snowy) weather. Therefore, the test is covered with a shading net (not covered in cloudy weather, one layer covered in cloudy weather, and two layers covered in sunny days) to simulate the continuous overcast and low illumination environment, so as to keep the photosynthetic effective radiation in the greenhouse below $200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. At the flowering and fruiting stage of the cucumber (February 20, 2016), six plots were set in the greenhouse, each treated with five ridges, and six consecutive days of low light treatment were set: 0, 1, 3, 5, 7, and 9d, represented by CK, T1, T3, T5, T7, and T9 respectively. After the low light treatment, the normal light recovery test was continued in the greenhouse. The recovery period was 9d, and the test lasted for one month. The monthly yield of cucumber plants was measured. The pre-test results conducted in the early stage of the test and the previous research results^[23] show that the impact of short-term shading (<7 d) on protected crops can recover rapidly after the normal lighting conditions are restored, and

can basically return to the normal level within a week. Therefore, the test will not take samples for the recovered treatment. The greenhouse shall be ventilated in time during the low illumination treatment to ensure that the temperature in the greenhouse is not higher than $28 \text{ }^{\circ}\text{C}$.

2.2 Project measurement and method

2.2.1 Meteorological data measurement

The meteorological data in the solar greenhouse is automatically collected by the data collector (WatchDog 2000, USA), which includes the air temperature and canopy relative humidity at 1.5 m. The acquisition frequency is once every 10 s, and the average value every 30 min is stored.

During the test in 2016, the changes of the external daily average temperature and air relative humidity of the solar greenhouse are shown in **Figure 1**, and the changes of the internal daily average temperature and air relative humidity of the solar greenhouse are shown in **Figure 2**. From the day of treatment (February 20, 2016) to the end of recovery period (March 9, 2016), measuring the light intensity of each treatment with a handheld digital illuminometer (Lux Meter AR813A, HK), and conducting it on the day when the shading net is hung and at 10:00-11:00 on each sampling day (after uncovering the net). It can be seen in **Figure 3** for the daily variation of outdoor and treated canopy light intensity from February 20 to March 9, 2016.

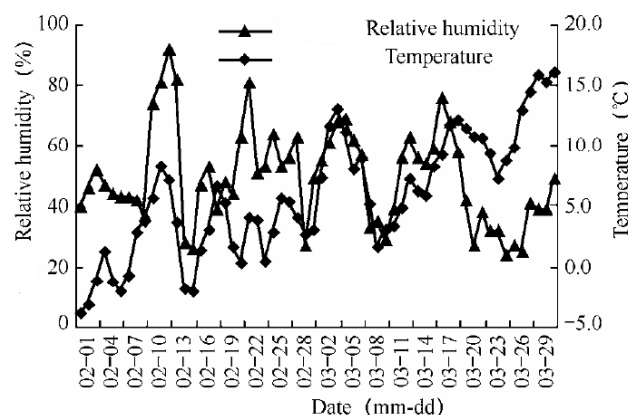


Figure 1. Changes of the daily average temperature and air relative humidity outside the greenhouse during the experiment period.

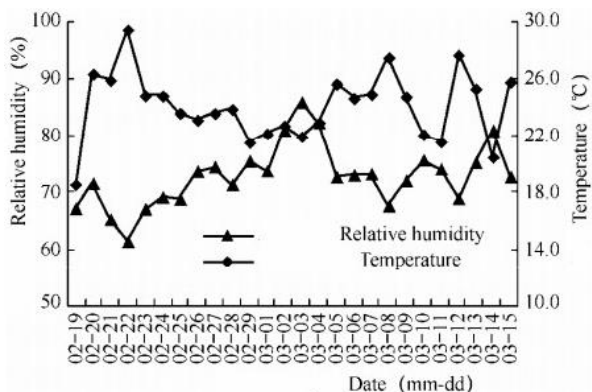


Figure 2. Changes of the daily average temperature and air relative humidity inside the greenhouse during the experiment period.

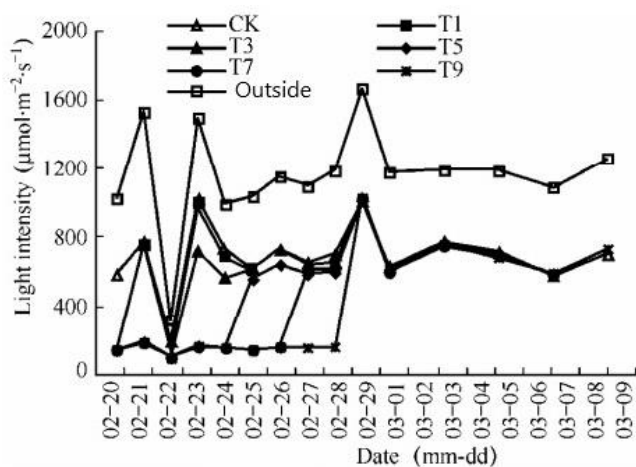


Figure 3. Changes of the light intensity outside and at the top of the canopy during the experiment period

Note: T1, T3, T5, T7 and T9 is low irradiation treatments inside greenhouse for 1 day, 3 days, 5 days, 7 days and 9 days, respectively. CK is normal irradiation

2.2.2 Determination of photosynthetic parameters of leaves

(1) Net photosynthetic rate

Using Li-6400 portable photosynthesis measurement system (LI-COR Biosciences Inc., USA) measures the net photosynthetic rate (P_n , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) of leaves from 9:00 to 11:00 on the day of the end of the low light treatment, and once every other day after the recovery period. Plants with uniform growth were selected for each treatment for repeated determination for 3 times, and the average value was taken.

(2) Chlorophyll

SPAD-502 chlorophyll meter (Soil and Plant Analyzer Development, Japan) was used to measure the SPAD value of leaves at different leaf positions from 9:00 to 11:00, which was measured at the

same time with the photosynthetic rate. Three plants with uniform growth were randomly selected for each treatment. Each plant measured the expanded leaves from the top to the bottom. Each leaf was measured for 6 times, and the average value was taken.

2.2.3 Determination of morphological index and yield

Three cucumber plants with uniform growth were randomly selected for destructive sampling in each treatment. The plant height of the sample plants was measured with a meter ruler with an accuracy of 1 cm, the leaf length and width were measured with a scale with an accuracy of 1 mm and a measuring range of 30 cm, and the stem diameter was measured with a vernier caliper with an accuracy of 0.02 mm.

Leaf length: The length of the main vein of cucumber leaves.

Leaf width: The length of cucumber leaves perpendicular to the widest part of the main vein.

According to the standard of agrometeorological observation specifications, 10 cucumbers were selected for each treatment and collected at a fixed plant. They were collected every three days, and the yield structure items such as single fruit weight, actual number of fruits per plant, number of secondary fruits, number of diseased fruits, vertical diameter of fruits, horizontal diameter of fruits and so on were recorded.

2.2.4 Determination of antioxidant enzyme activity

(1) Preparation of enzyme solution

Cucumber plants with the same growth were randomly selected from each treatment. Three functional leaves were collected and quickly frozen in liquid nitrogen and stored in the freezer layer. During determination, we weigh 0.4 g fresh sample, add phosphoric acid buffer ($0.05\text{ mol}\cdot\text{L}^{-1}$, $\text{pH} = 7.8$) for ice bath grinding, freeze and centrifuge at $0\text{ }^{\circ}\text{C}$ for 20 min (10,500 rpm), and store it in cold storage.

(2) Determination of SOD activity

For the determination of SOD, refer to Li's nitrogen blue tetrazole (NBT) method for colorimetry

at 560 nm^[24]. The calculation formula of SOD enzyme activity (units g⁻¹ FW) is

$$SOD \text{ total activity} = \frac{(A_{ck} - A_E) \times V}{0.5 \times A_{ck} \times W \times V_t} \quad (1)$$

Where, W is the sample weight (g); V is the total volume of the V_t sample solution (ml); V_t is the amount of enzyme solution (ml); A_{CK} is the absorbance of the A_E control tube, and A_E is the absorbance of the sample tube.

(3) Determination of POD activity

The determination of POD refers to the colorimetry of Li's guaiacol method in the spectrophotometer at 470 nm, and the calculation formula of ($\Delta OD_{470} \cdot \text{min}^{-1} \cdot \text{g}^{-1}\text{FW}$) pod enzyme activity is

$$POD \text{ total activity} = \frac{\Delta A \times V}{W \times V_t \times t} \quad (2)$$

Where, A is the absorbance value of the sample tube; W is the fresh sample weight (g), V is the total volume of the sample solution (ml), V_t is the amount of enzyme solution used during determination (mL), and t is the reaction time.

(4) Determination of CAT activity

For the ($\Delta OD_{240} \cdot \text{min}^{-1} \cdot \text{g}^{-1}\text{FW}$) determination of CAT activity, refer to Li's method for colorimetry in the spectrophotometer at 240 nm. The calculation formula of cat enzyme activity is

$$CAT \text{ total activity} = \frac{\Delta A \times V}{W \times V_t \times t} \quad (3)$$

Where, A is the absorbance value of the sample tube, W is the fresh sample weight (g), V is the total volume of the sample solution (mL), V_t is the amount of enzyme solution used during determination (mL), and t is the reaction time.

(5) Determination of MDA activity

For the ($\mu\text{mol} \cdot \text{g}^{-1}\text{FW}$) determination of MDA activity, refer to the thiobarbituric acid colorimetry of Li at three wavelengths of 600, 532 and 450 nm^[22].

$$MDA \text{ total activity} = \frac{[6.54 \times (A_{532} - A_{450}) - 0.56 \times A_{450}] \times V}{W} \quad (4)$$

Where, A_{600} , A_{532} the A_{450} are absorbance values of the sample at 600 nm, 532 nm and 450 nm

respectively. W is the weight of the fresh sample (g), and V is the total volume of the sample solution (mL).

(6) Determination of soluble protein content

The content of soluble protein was determined by Coomassie Brilliant Blue-250 method.

2.3 Data processing

Microsoft Excel 2007 and SPSS20.0 software were used for statistical analysis and chart drawing.

3. Results and analysis

3.1 Effects of continuous low illumination on photosynthetic parameters of cucumber leaves in greenhouse

It can be seen from **Figure 4(a)** that the leaf greenness value (SPAD value) has been at a relatively low level throughout the analysis period without the treatment of low light (CK), and remained within the range of 42.9–44.0. On the first day after the continuous low light treatment, except for the T1 treatment, the SPAD value of cucumber leaves was significantly higher than that of CK, and the longer the duration is, the higher the value is, indicating that the low light treatment for 3–9 d would increase the SPAD value of leaves. It can be seen from the change curve of leaf greenness value in the recovery period after the end of each low light treatment that there are obvious peaks on the second day of recovery, and the longer the low light treatment keep, the higher the rebound peak is. The peak value of T9 treatment is the largest with an increase of 9.5% compared with CK. From the 3rd day, SPAD value gradually decreased, and reached the same level as CK on the 3rd–9th day (T1 recovered for 3 d, T3 recovered for 5 d, T5–T9 recovered for 9 d). It can be seen that continuous low illumination can significantly improve the leaf greenness in a short time. After the treatment of different days, the leaf greenness values measured on other recovery days were significantly higher than that of CK, except within 3 days after T1 treatment and 3 and 4 days after T3 treatment; and the longer the treatment time is, the greener the leaves are in the recovery period.

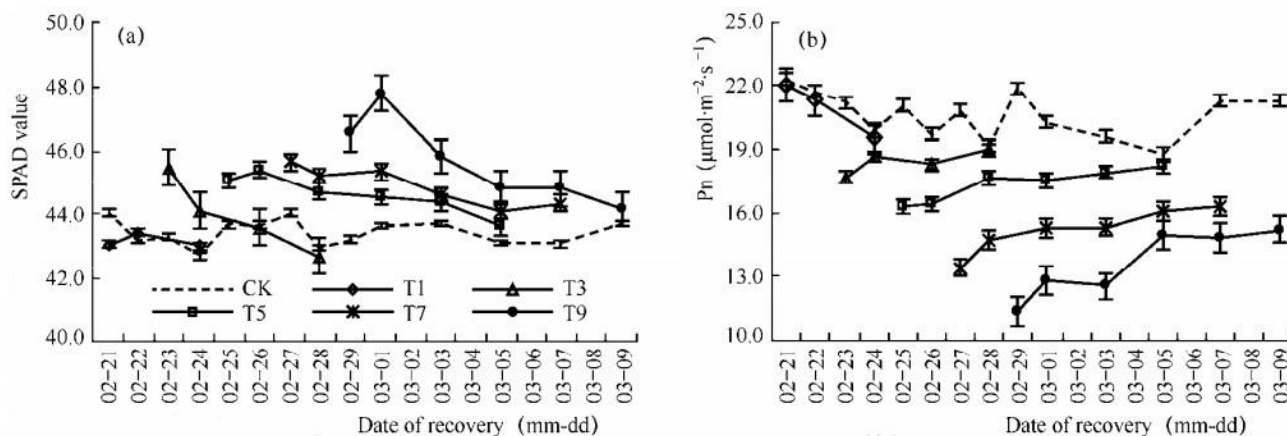


Figure 4. The variation of SPAD value (a) and net photosynthetic rate (b) of cucumber leaves during recovery period after different days of low irradiation

Note: the bar is MSE. The same as below.

It can be seen from **Figure 4(b)** that the net photosynthetic rate (PN) of CK leaves has been at a relatively high level in the whole analysis period, and remained in the range of 18.8–22.2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The net photosynthetic rate of cucumber leaves decreased gradually with the increase of continuous few days. The net photosynthetic rate of cucumber leaves decreased by 1.0% after 1 day of light treatment; the net photosynthetic rate of cucumber leaves was 15.21 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 28.6% lower than CK, indicating that the net photosynthetic rate of leaves decreased after more than 3 days of low light treatment. It can be seen from the change curve of leaf net photosynthetic rate in the recovery period after the completion of each low light treatment that after the restoration of normal light, the net photosynthetic rate of cucumber leaves treated with T3, T5, T7 and T9 gradually increased, and the shorter the duration of low light treatment is, the smoother the rising curve would be. The net photosynthetic rate of leaves could reach CK level after T3 treatment restored for 5 days and T5 treatment restored for 9 days. The net photosynthetic rate of cucumber leaves in T7 and T9 treatments did not return to CK level after 9 days of recovery, which was 23.5% and 28.6% lower than CK, respectively.

2.2 Effects of continuous low illumination on morphological indexes of the cucumber in greenhouse

It can be seen from **Figure 5** that the plant height and stem diameter the cucumber in each treatment continued to increase throughout the analysis period, and the plant height and stem diameter of each low illumination treatment were lower than that of CK. There was no significant difference in plant height and stem diameter of cucumber compared with CK after 1–5 days of low light exposure. The plant height and stem diameter of cucumber were 8.1% and 11.9% lower than those of CK for 7 days, while the plant height and stem diameter of cucumber were 9.6% and 21.4% lower than those of CK for 9 days. It can be seen that the growth of plant height and stem diameter of cucumber plants slowed down after continuous thinning for more than 7 days. During the recovery period after the completion of the low light treatment, the growth rate of plant height and stem diameter of each low light treatment increased gradually. For the treatment of less than 5 days, the plant height and stem diameter of the treatment of T7 and T9 could return to the normal growth level after returning to normal light for 9 days, while the plant height and stem diameter of the treatment of T7 and T9 were still lower than the CK level after returning to normal light for 9 days, indicating that the growth of plant height and stem diameter of cucumbers under low light for more than 7 days was inhibited, and it was difficult to recover in a short time.

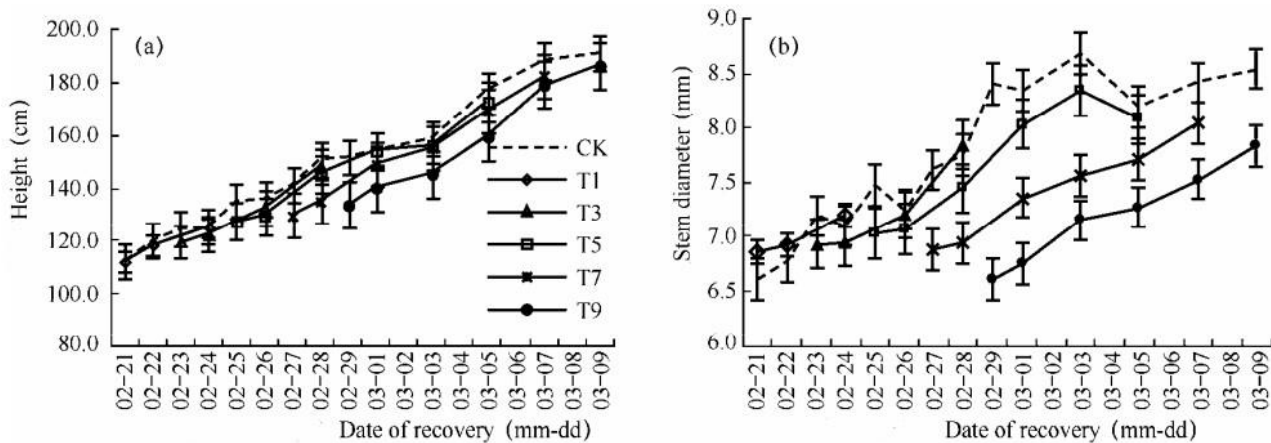


Figure 5. Variation of height (a) and stem diameter (b) during recovery period after different days of low irradiation.

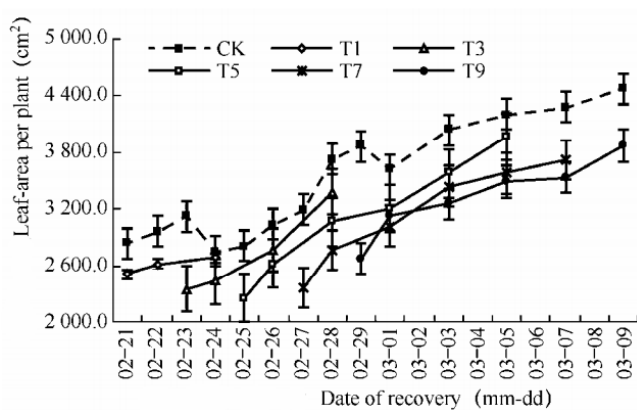


Figure 6. Variation course of leaf area per plant during recovery period after different days of low irradiation.

It can be seen from **Figure 6** that in the treatment without low light, the leaf area per plant of cucumber continued to increase throughout the analysis period, and the leaf area per plant of cucumber in each treatment with low light also continued to increase, but both were lower than CK. The leaf area per plant of cucumber was 2,219.8 cm² with last 9 days low illumination, which decreased by 28.0% compared with CK. After returning to normal light, the growth rate of leaf area of cucumber plants in each treatment was increased. After the recovery period (9 d), the growth rate of leaf area per plant of T1, T3 and T5 treatments was close to the level of CK, while that of T7 and T9 treatments was still 13.1% and 11.2% lower than that of CK, respectively, indicating that the impact of continuous light exposure for more than 7 days on the growth of cucumber leaf area was difficult to return to the normal level in a short time.

2.3 Effects of continuous low illumination on antioxidant enzyme activities of cucumber

leaves in greenhouse

As shown in **Figure 7(a)**, SOD activity in cucumber leaves in greenhouse maintained a good increasing trend with the increase of continuous low illumination time. The SOD activity of cucumber leaves was the highest after 7 days of light exposure, reaching 233.2 U·g⁻¹ (FW), which increased 42.6% compared with the 163.5 U·g⁻¹ of CK treated. The activity of SOD in leaves treated with low light for 9 days decreased slightly to 228.6 U·g⁻¹ compared with that treated with 7 days, and increased by 26.1% compared with CK.

As shown in **Figure 7(b)**, the POD activity in cucumber leaves gradually increased with the increase of days of light deprivation. POD activity in cucumber leaves increased significantly after 3 days of low light treatment. POD activity in cucumber leaves treated with T5 and T7 was 12.7 and 15.7 U·min⁻¹·g⁻¹, respectively increased by 96.6% and 127.4% compared with CK. The POD activity of cucumber leaves reached the maximum after 9 days of continuous low light, which was 16.1 U·min⁻¹·g⁻¹, an increase of 74.7% compared with CK.

It can be seen from **Figure 7(c)** that cat activity in cucumber leaves of all treatments increased first and then decreased in the whole process of low light treatment. The activity of CAT in cucumber leaves increased by 34.4% and reached to 28.2 U·min⁻¹·g⁻¹ after 3 days of light deprivation. The CAT activity in cucumber leaves reached a peak of 43.3 U·min⁻¹·g⁻¹ after 7 days of continuous low light, which increased by 98% compared with CK. The CAT activity of cucumber leaves treated with

T9 was slightly lower than that of T7, which was $40.7 \text{ U}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$, and increased by 76.8% compared with CK. It can be seen from the **Figure 7(d)** that the content of MDA in greenhouse cucumber leaves of all treatments gradually increased under continuous low light, and the growth rate of MDA de-

creased after 9 days of continuous low light. Compared with CK, the content of MDA in cucumber leaves treated with T7 and T9 increased by 90.1% and 96.8% respectively, reaching 4.26 and $4.37 \mu\text{mol}\cdot\text{g}^{-1}$.

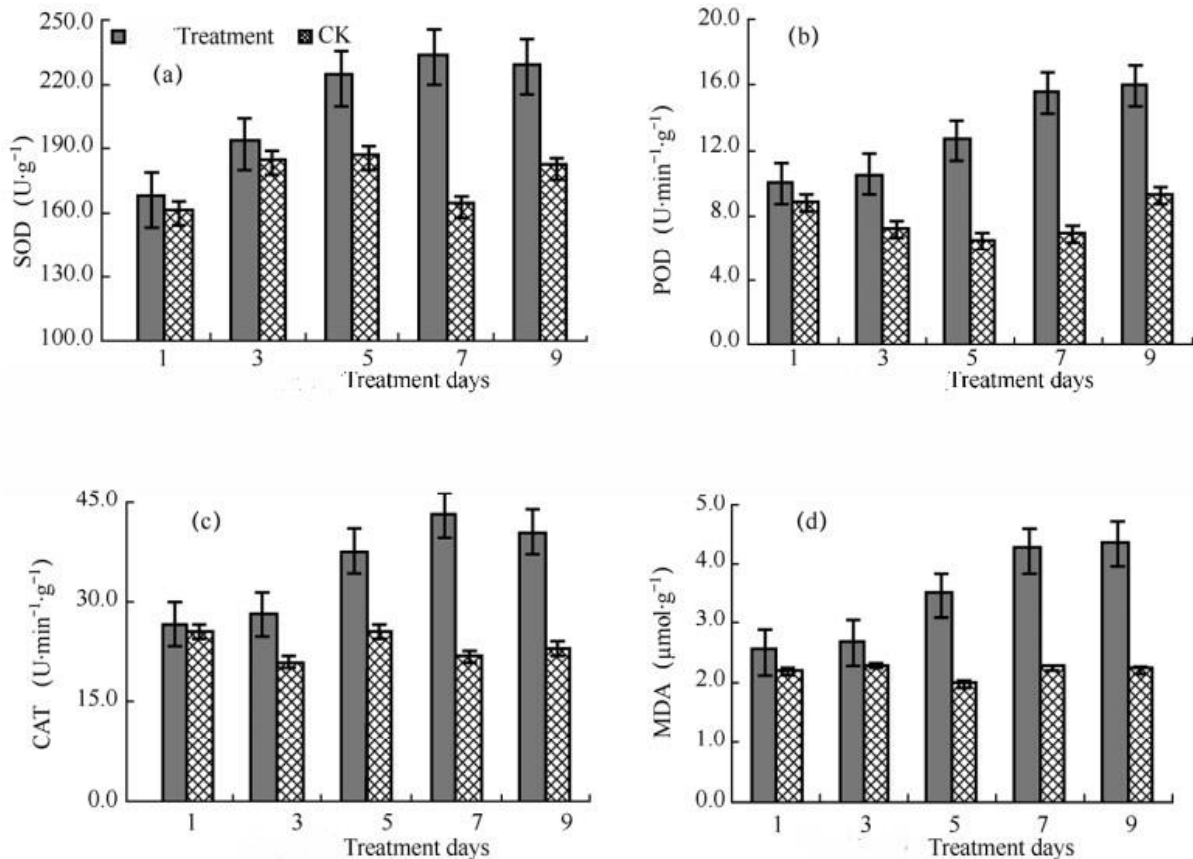


Figure 7. Variation of antioxidant enzyme activities of cucumber leaves in greenhouse after different days of low irradiation.

Figure 8(a), 8(b), 8(c) and **8(d)** show the changes of SOD, POD, CAT activities and MDA content in cucumber leaves during the recovery period. It can be seen from the figure that the activities of SOD, POD, CAT and the content of MDA in cucumber leaves in greenhouse gradually decreased after the restoration of normal light. The activities of the three enzymes in cucumber leaves after 1 day and 3 days of continuous low light could quickly recover to CK level after the restoration of light.

The cucumber leaves after 5 days of low light treatment gradually returned to normal level after 9 days of normal light treatment. The activity of protective enzymes and MDA content in cucumber leaves after 7 days of continuous low light treat-

ment also decreased gradually during the recovery period, but it was difficult to recover to the level before low light treatment within 9 days. At the end of recovery, the activities of three enzymes were 206.3 , 10.8 , $32.4 \text{ U}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ and the content of MDA was $3.0 \mu\text{mol}\cdot\text{g}^{-1}$ in cucumber leaves after 9 days of continuous light deprivation, which increased by 16.6%, 38.6%, 29.2% and 62.2% respectively compared with CK. The results showed that the protective enzyme activity and MDA content in cucumber leaves were significantly affected by continuous low light for more than 7 days, and it was difficult to return to the normal level in a short time.

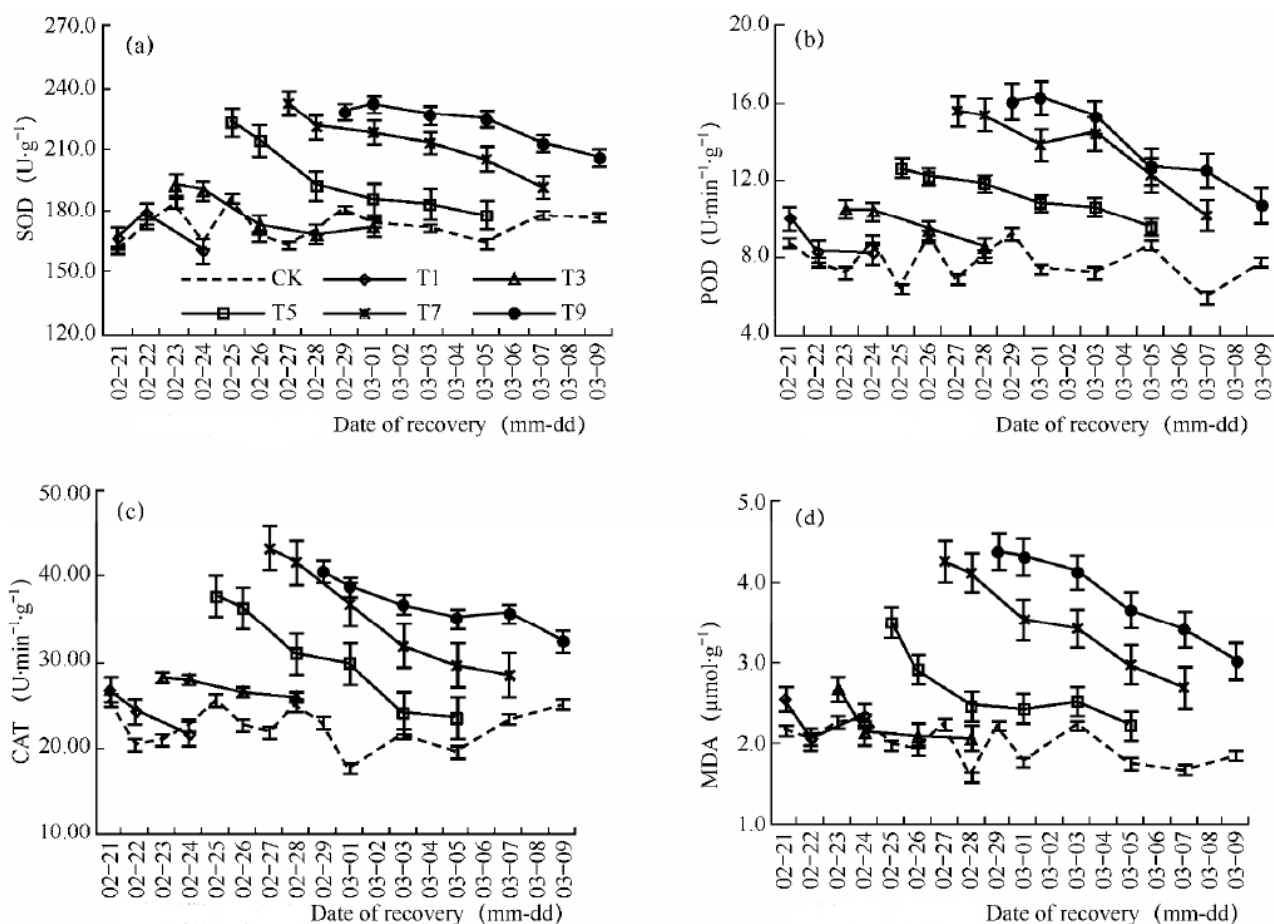


Figure 8. Changes of the antioxidant enzyme activities of cucumber leaves of different treatments after low irradiation treating.

2.4 Effect of continuous low illumination on soluble protein content in cucumber leaves in greenhouse

As shown in Figure 9, the content of soluble protein in cucumber leaves gradually decreased with the continuous increase of days of light deprivation. The content of soluble protein in cucumber leaves treated with low light for 1 d and 3 d decreased by 5.3% and 13.9% respectively compared with CK. In T5 treatment, the soluble protein content in cucumber leaves decreased significantly, reaching $407.8 \text{ mg} \cdot \text{g}^{-1}$ at the end, which decreased by 30.4% compared with CK. The content of soluble protein in cucumber leaves of T7 treatment was $414.9 \text{ mg} \cdot \text{g}^{-1}$, which was slightly higher than that of T5 treatment. The content of soluble protein in cucumber leaves treated with T9 decreased the most, only $320.4 \text{ mg} \cdot \text{g}^{-1}$, which was

43.1% lower than that of CK. After returning to normal light, the soluble protein content in cucumber leaves increased continuously. T1 and T3 treatment quickly returned to normal level. After 9 days of recovery from T5 treatment, its content reached $558.6 \text{ mg} \cdot \text{g}^{-1}$, only decreased by 8.9% compared with CK, and basically returned to the normal level. During the recovery period of T7 and T9 treatment, the content of soluble protein in cucumber leaves continued to increase, which were $502.1 \text{ mg} \cdot \text{g}^{-1}$ and $489.2 \text{ mg} \cdot \text{g}^{-1}$ respectively after 9 days of recovery, but they did not reach the level of CK, which decreased by 12.3% and 18.5% respectively compared with the level of CK. The results showed that the increase of soluble protein content in cucumber leaves caused by continuous low light for more than 7 days was difficult to return to the normal level in a short time.

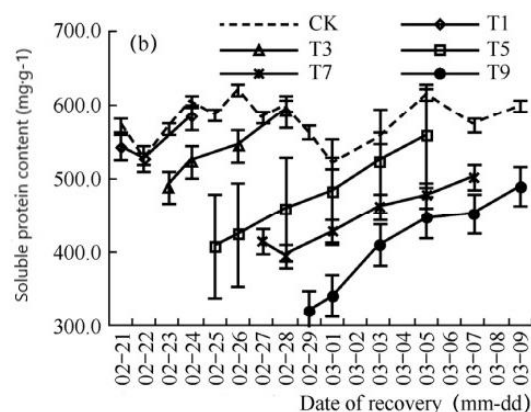
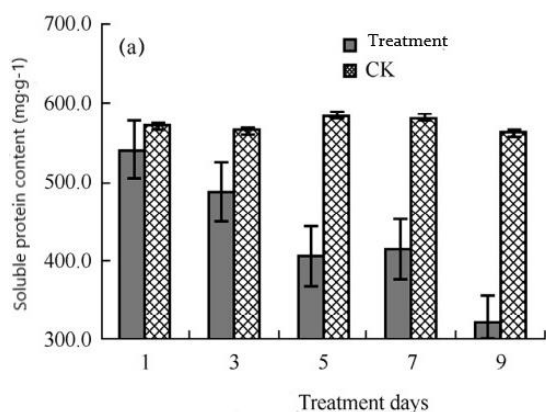


Figure 9. Variation course of soluble protein in cucumber leaves in greenhouse after different days of low irradiation.

2.5 Effects of continuous low illumination on cucumber yield and marketability

In the test, the yield of a single plant was measured after 1 month of low light treatment, and the yield was converted into the yield of the current month after 1 month of continuous yield measurement. The results are shown in **Figure 10**. It can be seen from the figure that the cucumber plant yield under normal light is the largest, reaching to 28,900.5 kg·hm⁻². The yield of cucumber decreased under low illumination, and the longer the days of low illumination keep, the lower the yield is. There was no significant difference among the treatments when the number of days of light exposure was less than 3 days. The monthly yield of cucumber decreased significantly when the number of days of light was more than 5 days, and there were significant differences among the different treatments. The plant yield of cucumber treated with T9 was the smallest, which is 18,100.5 kg·hm⁻², 37.3% lower than that of CK.

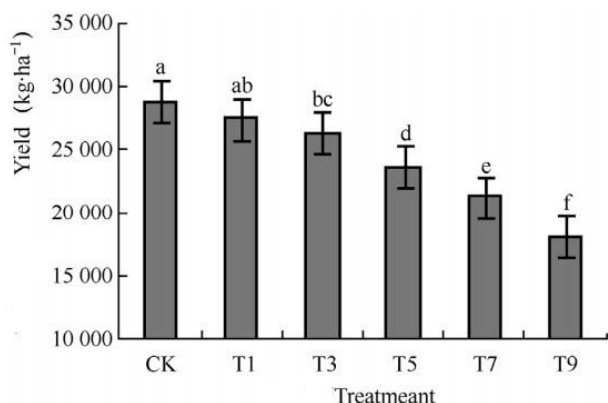


Figure 10. Comparison of cucumber yield under different treatment condition.

Note: lowercase indicates the difference significance among treatments at 0.05 Level.

The appearance quality of cucumber, including shape, color, size and defects, is an important part of cucumber quality, which directly affects sales. The cucumber fruits of each treatment were classified into three grades according to their shape, color, size and defects. The classification results are shown in **Table 1**. The first grade fruit should have the characteristics of long rod shape, straight, vertical diameter of more than 27cm, uniform size, dark green and glossy peel, medium and uniform prickles size, and no belly, sharp mouth, bee waist and other defects. It can be seen from **Table 1** that the ratio of the first grade fruit to the total harvest will gradually decrease, the ratio of the third grade fruit will gradually increase, and the bad fruit ratio will also gradually increase with the increase of the number of light days, the change rule of secondary fruit was not obvious. The fruit began to appear gray mold symptoms after the light exposure lasted for more than 5 days. The flower pedicel softened in the form of water stains, and the surface was densely covered with gray fog. The longer the light exposure time was, the more the bad fruits were. The bad fruit rate of cucumber fruit treated with T9 reached 25%.

Table 1. Percent of fruits on different levels under low irradiation (%)

Treatment	1st class	2nd class	3rd class	Bad fruit
CK	88.0	8.0	4.0	0.0
T1	78.6	21.4	0.0	0.0
T3	75.0	20.0	5.0	0.0
T5	70.0	6.7	10.0	13.3
T7	58.3	12.5	12.5	16.7
T9	45.0	20.0	20.0	25.0

3. Conclusion and discussion

Continuous lack of light makes it difficult for

the temperature in the solar greenhouse to rise, which often leads to sub optimal temperature stress in weak light. Cucumber is very sensitive to weak light and low temperature in the flowering and fruiting period, and photosynthesis is inhibited. Previous studies have shown that the SPAD value of leaves is in direct proportion to the chlorophyll content. Therefore, the SPAD value can reflect the changes of chlorophyll content in cucumber leaves caused by low light stress^[25,26]. The results showed that the SPAD value of cucumber leaves increased, the leaf color deepened, the net photosynthetic rate decreased and the photosynthetic capacity weakened under continuous low illumination. The net photosynthetic rate of cucumber leaves gradually increased, the photosynthetic capacity gradually recovered, and the SPAD value decreased after removing the light stress. The photosynthetic recovery ability of cucumber leaves was weakened after 7 days of continuous low light, and it was difficult to recover to the normal level in a short time. The plant height, stem diameter and leaf area per plant of cucumber were significantly lower than those of CK, and the longer the days of continuous low light is, the greater the impact on the appearance of cucumber plant and the worse the recovery ability is. According to the appearance quality of cucumber, the proportion of bad fruit increased with continuous low illumination, and the longer the low illumination time is, the greater the rate of bad fruit would be. The low illumination with different duration days caused the reduction of cucumber single fruit weight and yield per plant in different degrees, resulting in the decline of yield. The longer the low illumination is duration days, the smaller the cucumber yield is.

The effect of low light stress on cucumber leaves is to affect the enzyme system by affecting the activity of enzymes on the membrane, so as to accumulate reactive oxygen species, increase the degree of membrane lipid peroxidation in cucumber leaves, and damage the membrane lipids, thus affecting the metabolic process in the body. SOD, POD and CAT are collectively referred to as active oxygen scavengers. SOD is the first line of defense of plant antioxidant system, which can convert ac-

tive oxygen into H_2O_2 and O_2 , and then remove H_2O_2 in the body through POD and CAT, effectively prevent the interaction between active oxygen and H_2O_2 , so as to maintain the metabolic balance of active oxygen in the body^[27,28]. MDA is one of the final products of membrane lipid peroxidation, which can inhibit protein synthesis^[29], and its content can better identify the damage degree of cucumber leaf cell membrane and the strength of stress response^[30]. The results of this study showed that continuous low illumination increased the activities of SOD and POD in cucumber leaves, increased the activities of cat first and then decreased, and increased the content of MDA continuously, which was consistent with the results of Hu *et al.*^[31,32]. The results showed that low temperature and weak light could induce the increase of SOD and POD activities, and to some extent, prevent or reduce membrane lipid peroxidation damage, so as to protect the photosynthetic membrane of cucumber leaves from damage, which may be a protective response of cucumber to poor light and temperature environment. With the continuous increase of days of low light, CAT activity decreased gradually, which may be a protective measure to stimulate the resistance mechanism in leaves and improve the stress resistance of plants by partially blocking CAT activity in leaves. The content of MDA in leaves increased with the continuous increase of days of low light, which reflected that the degree of leaf damage was greater with the increase of days of low light. After the restoration of normal light conditions, the MDA content in leaves continued to decrease, the activities of SOD, POD and CAT in T1, T3 and T5 cucumber leaves quickly returned to normal levels, and the metabolic function was restored. All the indexes of cucumber leaves could not return to the normal level in a short time after continuous low light for more than 7 days.

By studying the effects of different continuous low light days on photosynthesis and antioxidant enzyme activities of cucumber leaves at flowering and fruiting stage in solar greenhouse, it was confirmed that continuous low light increased SPAD value of cucumber leaves, decreased net photosynthetic rate, inhibited plant growth, and decreased

fruit yield and marketability. The activities of SOD and POD increased, the activities of CAT increased first and then decreased, and the content of MDA increased continuously. The number of days of continuous low light was less than 7 days, which affected the photosynthesis and antioxidant enzyme activities of cucumber leaves, but could recover quickly under normal light and temperature conditions. The activities of photosynthesis and antioxidant enzymes in leaves were difficult to recover to normal level in a short time after continuous low light for more than 7 days. Therefore, continuous low light for 7 days could be used as the critical value of low light disaster in actual production. However, from a large number of inconsistent and even contradictory results, it can be seen that the mechanism of plant resistance to low temperature and weak light is quite complex. The characteristics of resistance to low temperature and weak light in the whole growth and development stage of cucumber under the actual production conditions of greenhouse and the impact of low temperature and weak light on the yield and quality of cucumber still need to be further studied.

Conflict of interest

The authors declared no conflict of interest.

References

- Kong H. Characteristics and pollution-free cucumber cultivation techniques of cucumber. *Modern Agricultural Science and Technology* 2013; (23): 94–95.
- Shao H. Characteristics and greenhouse cultivation techniques of cucumber. *Modern Agricultural Science and Technology* 2012; (23): 92–93.
- Guan Y, Chen Q, Pan J, *et al.* Construction of a BAC library from cucumber (*Cucumis sativus* L.) and identification of linkage group specific clones. *Progress in Natural Science* 2008; 18(2): 143–147.
- Liu G, Zhao J, Xue T, *et al.* Study on breeding and cultivation techniques of a new mini fruit cucumber ‘Chunqiu Xianfeng No.5’. *Northern Horticulture* 2014; 24(18): 175–177.
- Li C, Guo J. Effect of continuous fog and haze of agricultural production facilities in Tianjin 2013–2014 winter. *Science and Technology of Tianjin Agriculture and Forestry* 2014; (3): 36–37.
- Lidon FC, Loureiro AS, Vieira DE, *et al.* Photoinhibition in chilling stressed wheat and maize. *Photosynthetica* 2001; 39(2): 161–166.
- Mao Y. Influence of haze on facility agriculture and coping strategy. *Journal of Henan Agricultural Sciences* 2014; 43(7): 76–79.
- Routaboul JM, Fischer SF, Browse J. Trienoic fatty acids are required to maintain chloroplast function at low temperatures. *Plant Physiology* 2000; 124(4): 1697–1705.
- Vijayan P, Browse J. Photoinhibition in mutants of *Arabidopsis* deficient in thylakoid unsaturation. *Plant Physiology* 2002; 129(2): 876–885.
- Li X, Bi Y, Zhao S. Effects of short-term chilling stress on the photosystems and chloroplast ultrastructure in sweet pepper. *Scientia Agricultura Sinica* 2005; 38(6): 1226–1231.
- Ody Y. Effects of light intensity, concentration and leaf temperature on gas exchange of strawberry plants-feasibility studies on enrichment in Japanese conditions. *Acta Horticulturae* 1997; 439: 563–573.
- Shen J, Terashima I, Katoh S. Cause for dark, chilling-induced inactivation of photosynthetic oxygen-evolving system in cucumber leaves. *Plant Physiology* 1990; 93(4): 1354–1357.
- Sonoike K. Various aspects of inhibition of photosynthesis under light/chilling stress: “Photoinhibition at chilling temperatures” versus “chilling damage in the light”. *Journal of Plant Research* 1998; 111: 121–129.
- Qian Z, Ding L, Cao S. Di wen xie po dui la (tian)jiao mo zhi guo yang hua shui ping ji bao hu mei huo xing de ying xiang (Chinese) [Effect of temperature stress on pepper seedling lipid peroxidation level and protective enzyme activity]. *Acta Horticulturae Sinica* 1994; 21(2): 203–204.
- Chen Q, Zhang F, Wang Y, *et al.* Influence of critical low temperature and poor light on photosynthesis characters and enzyme variance of cucumber. *Acta Agriculturae Boreali-Sinica* 2003; 18(4): 31–34.
- Ai X, Ma X, Yu L, *et al.* Effect of long-term suboptimal temperature and short-term low temperature under low light density on cucumber growth and its photosynthesis. *Chinese Journal of Applied Ecology* 2004; 15(11): 2091–2094.
- Ai X, Wang X, Guo Y, *et al.* Effects of suboptimal temperature and low temperature under low light intensity on stomatal characteristics and chloroplast ultrastructure of cucumber seedlings. *Scientia Agricultura Sinica* 2006; 39(10): 2063–2068.
- Ai X, Zhang Z, He Q, *et al.* Changes in ecological factors and their effects on photosynthesis of cucumber leaves (*Cucumis sativus* L.) in heliogreenhouse. *Chinese Journal of Applied and Environmental Biology* 2002; 8(1): 41–46.
- Wang S, Cao W, Wang Q, *et al.* Positional distribution of leaf color and diagnosis of nitrogen nutrition in rice plant. *Scientia Agricultura Sinica* 2002; 35(12): 1461–1466.
- Ma D, Pang J, Huo Z, *et al.* Effect of low light on photosynthesis and membrane-lipid peroxidation of *Cucumis sativus* seedling. *Acta Agriculturae Universitatis Henanensis* 1998; 32(1): 1–3.

21. Yang G, Zhu Z. Effects of magnesium deficiency on growth and active oxygen scavenging system in cucumber under different light intensities. *Acta Horticulturae Sinica* 2001; 28(5): 430–434.
22. Zhou Y, Yu J, Qian Q, *et al.* Effects of chilling and low light on cucumber seedlings growth and their antioxidative enzyme activities. *Chinese Journal of Applied Ecology* 2003 14(6): 921–924.
23. Yang Z, Yuan C, Han W, *et al.* Effects of low irradiation on photosynthesis and antioxidant enzyme activities in cucumber during ripening stage. *Photosynthetic* 2016; 54(2): 251–258.
24. Li H. Principles and techniques of plant physiological biochemical experiment. Beijing: Higher Education Press; 2000.
25. Kumagai E, Araki A, Kubota F. Correlation of chlorophyll meter readings with gas exchange and chlorophyll fluorescence in flag leaves of rice (*Oryza sativa* L.) *Plant. Plant Production Science* 2009; 12: 50–53.
26. Lin F, Qiu L, Deng J, *et al.* Investigation of SPAD meter-based indices for estimating rice nitrogen status. *Computers and Electronics in Agriculture* 2010; 71(Suppl 1): 60–65.
27. Green BR, Durnford DG. The chlorophyll-carotenoid proteins of oxygenic photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 1996; 47: 685–714.
28. Kornyejev D, Logan BA, Payton P, *et al.* Enhanced photochemical light utilization and decreased chilling-induced photoinhibition of photosystem II in cotton overexpressing genes encoding chloroplast-targeted antioxidant enzymes. *Physiologia Plantarum* 2001; 113(3): 323–331.
29. Cao X. Mo zhi guo yang hua dui xi bao yu ji ti de zuo yong (Chinese) [The role of membrane lipid peroxidation on the cell and body]. *Progress in Biochemistry and Biophysics* 1986; (2): 17–21.
30. Feng X, Yu X, Guo H, *et al.* Effect of low temperature stress on the protective-enzyme activity of grafted cucumber seedling and own-root cucumber seedling. *Journal of Shandong Agricultural University: Natural Sciences* 2002; 33(3): 302–304.
31. Hu W, Wu Y, Zeng J, *et al.* Chill-induced inhibition of photosynthesis was alleviated by 24-epibrassinolide pretreatment in cucumber during chilling and subsequent recovery. *Photosynthetica* 2010; 48: 537–544.
32. Rossa MM, de Oliveira MC, Okamoto OK, *et al.* Effect of visible light on superoxide dismutase (SOD) activity in the red alga *Gracilariopsis tenuifrons* (Gracilariales, Rhodophyta). *Journal of Applied Phycology* 2002; 14: 151–157.