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Ameliorated Effect of Shading Treatment in Strong Light Period on Senescence and Photosynthesis in Cucumber 'Jinchun No. 5' Seedlings

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Abstract

To gain a physiological understanding of the effects of light intensity on the cucumber *(Cucumis sativus* L.), we subjected 'Jinchun No. 5' seedlings to shading treatment in three plots with light transmission of 100% (unshaded), 80%, and 60% in strong light periods for 45 days, and examined active oxygen species, senescence indicators and photosynthetic properties on the 15th, 30th, and 45th days of shading treatment respectively. The amounts of active oxygen species superoxide anions and hydrogen peroxide in leaves fell with decreasing light intensity on each of the 15th, 30th, and 45th days of shading treatment respectively. The amounts of chlorophyll and protein also decreased and that of malondialdehyde increased — all indicators of senescence — with decreasing light intensity on each of the 15th, 30th, and 45th days of shading treatment respectively. The photosynthetic property stomatal conductance in the 80% and 60% transmission plots exceeded that in the 100% transmission plot on the 15th and 30th days of shading treatment. No significant difference was seen in sub-stomatal CO_2 concentration between all three light transmission plots. Both transpiration and net photosynthesis rates rose with decreasing light intensity. These results suggest that the shading treatment during strong light periods may suppress the generation and accumulation of active oxygen species, inhibit senescence and also ameliorate photosynthesis in cucumber 'Jinchun No. 5' seedlings.

Discipline: Horticulture
Additional key words: active oxygen species, malondialdehyde, net photosynthesis rate, protein, transpiration rate

Introduction

Senescence and photosynthetic properties in plants are inherently programmed but largely controlled by environmental conditions. The cucumber (*Cucumis sativus* L.) is a C_3 plant native to subtropical rain forest regions, the appropriate light intensity for which is generally regarded as 700–1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [11]. However, the daytime (10:00 am – 2:00 pm) intensity of sunlight from spring to fall, which includes periods of strong light, is at least 1,500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ outdoors and at least 1,100 $\mu molmol \cdot m^{-2} \cdot s^{-1}$ in a greenhouse[13]. The cucumber is affected by high temperatures, strong light, and water stress when cultivated from spring to fall compared to cultivation in winter/spring, meaning a considerably shortened harvest period and a substantial decline in yield and quality.

It has been reported that high temperature stress leads to increased generation and accumulation of active oxygen species, significantly accelerated senescence, mildly affected CO_2 intake and water absorption, and reduced photosynthesis in cucumber leaves[19]. If shading treatment was carried out in periods of strong light, to what extent does the level of active oxygen species vary in cucumber leaves; how much is senescence, which plays a prominent role in productivity, inhibited; and how is photosynthesis, which depends on light energy, affected? These basic physiological data are considered capable of contributing to the cultivation of cucumber cultivars resistant to strong light and physiological research into strong light injury.

In this study, we investigated the amount of active oxygen species superoxide anions ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2); the amounts of chlorophyll, protein, and malondialdehyde (MDA) as indicators of senescence[9]; and the photosynthetic properties of stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i), transpiration rate

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(E), and the net photosynthesis rate (A) in the leaves of 'Jinchun No. 5' cucumber seedlings subjected to shading with light transmittance of 100% (unshaded), 80%, and 60% for 45 days.

Materials and methods

1. Material under test

Cucumber 'Jinchun No. 5' seedlings were grown in a plastic greenhouse from June 2nd, 2008 and then pots (10 cm diameter by 12 cm tall) containing a culture medium of 3:1:1 (v/v/v) soil, peat, and vermiculite.

2. Shading

Seedlings with one true leaf were subjected to shading for 45 days from June 17th in a greenhouse with 83% light transmission. Using black cheesecloth, three plots were prepared with 100% (unshaded), 80% and 60% light transmission. Thirty pots (plants) were placed in each treatment plot, the space around each of which was 25×100 cm, i.e. 4 plants·m^{-2}. The peak photosynthetic photon flux density (PPFD) was measured 9 times every 5 days with a portable photosynthesis system (LC*i*-002/B, ADC BioScientific Ltd., Hertfordshire, U.K.). On clear days during the shading treatment, the maximum PPFD at 100%, 80%, and 60% light transmission plots was 1105–1365, 857–1099, and 612–908 µmol·m^{-2}·s^{-1}, respectively. During the shading treatment, there were 34 clear days, 7 cloudy days, and 4 rainy days. The air temperature during the shading treatment was recorded with an automatic thermometer. The average temperatures at the 100%, 80%, and 60% light transmission plots were 27.5, 27.0, and 26.5℃, respectively.

3. Sampling

Five of the 30 plants in each shading plot were randomly selected and the second and third true leaves of each plant were sampled at about 10:30 am on the 15th, 30th, and 45th days of shading treatment respectively to analyze the active oxygen species and indicators of senescence. The second and third true leaves were immediately finely chopped, 2 g from each plant was weighed, they were frozen in liquid nitrogen and then stored at –80℃.

4. Measurement and analysis

The amount of $O_2^{\cdot-}$ was determined in accordance with the method used by Li et al. (2002)[8]. The 2 g samples were homogenized, extracted in 10 mL 0.2 N $HClO_4$ and centrifuged for 10 min at 15,000 × g. The supernatant was adjusted to pH 7.5 with 4 N KOH and recentrifuged for 10 min at 15,000 × g. After 1.5 mL of the supernatant had been applied to an AG1-X8 column (1.0 cm i.d. × 5 cm, Bio-Rad), the column was eluted withtrh 6 mL distilled water. The collected flow-through (0.8 mL) was then mixed with 0.2 mL 4 mM hydroxylammonium chloride ($NH_2OH{\cdot}HCl$), incubated for 30 min at 25℃, then 1 mL 20 mM sulfanilic acid and 1 mL 10 mM N-(1-naphthyl)ethylenediamine dihydrochloride were added, and the resulting solution was developed for 20 min at 25℃. Subsequently, 1 mL trichloroacetic acid (w/v) was added, centrifuged for 10 min at 3,000 × g, and the absorbance of the supernatant was measured at a wavelength of 545 nm. Calibration was established with $NaNO_2$. The $O_2^{\cdot-}$ concentration was calculated based as $NH_2OH + 2O_2^{\cdot-} + H^+ \rightarrow NO_2^- + H_2O_2 + H_2O$.

The amount of H_2O_2 was determined in accordance with the method used by Okuda et al. (1991)[16]: 0.8 mL 12.5 mM 3-dimethylaminobenzoic acid (adjusted with 0.375 M phosphate buffer at pH 6.5), 160 µL 1.4 mM 3-methyl-2-benzo-thiazolinone hydrazone, and 40 µL 0.25 units peroxidase were added to a 2 mL flow-through collected in the analysis of the amount of $O_2^{\cdot-}$, incubated for 10 min at 25℃, and absorbance was measured at a wavelength of 590 nm.

The amount of chlorophyll was determined in accordance with the method used by Arnon (1949)[1]. The 2 g samples were homogenized and extracted in a small amount of quartz sand plus calcium carbonate and 10 mL acetone until they turned white. After the volume of the samples had been fixed at 25 mL with 80% acetone (v/v), the samples were centrifuged for 10 min at 3,000 × g. The supernatant was diluted with 80% acetone, and absorbances A_{450} and A_{600} were measured at wavelengths of 450 and 600 nm, respectively. The amount of chlorophyll was also calculated with the formula, chlorophyll (µg·mL^{-1}) = $20.21 \times A_{645} + 8.02 \times A_{663}$.

The amount of protein was determined in accordance with the method used by Lowry et al. (1951)[12], using bovine serum albumin as a standard protein.

The amount of MDA was determined in accordance with the method used by Heath and Packer (1968)[7]. The 2 g samples were homogenized and extracted in 12 mL 10% trichloroacetic acid and centrifuged for 10 min at 12,000 × g. The supernatant (2 mL) was mixed with 2 mL 0.6% thiobarbituric acid and incubated for 30 min in a boiling water bath. After cooling, the solution was centrifuged for 10 min at 15,000 × g, and the supernatant absorbances A_{450}, A_{532}, and A_{600} were measured at wavelengths of 450, 532, and 600 nm, respectively. The amount of MDA was calculated with the formula, MDA (µmol·L^{-1}) = $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$.

The g_s, C_i, E, and A photosynthetic properties for the second and third true leaves of each of the 5 plants were measured with a portable photosynthesis system (LCi-002/B, ADC BioScientific Ltd., Hertfordshire, U.K.) before

the above-described sampling took place. Photosynthesis measurements, repeated 3 times each for the second and third leaves, were taken at about 10:00 am at each shading plot. The concentration of CO_2 was 374–392 μM and the leaf chamber area was 6.25 cm^2. The average PPFDs for the 100%, 80%, and 60% light transmission plots at the time of photosynthesis measurements were 568, 480, and 359 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively, on the 15th day; 636, 482, and 405 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively, on the 30th day; and 615, 507, and 368 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively, on the 45th day.

Results

1. Active oxygen species

The level of $O_2^{\cdot -}$ fell with decreasing light intensity on the 15th and 30th days of shading treatment, and on the 45th day the 60% plot was lower than the 80% and 100% plots (Fig. 1). The level of H_2O_2 fell with decreasing light intensity on the 15th and 30th days of shading treatment, and on the 45th day the 80% and 60% plots were lower than the 100% plot.

2. Senescence indicators

With decreasing light intensity, although the amounts of chlorophyll and protein rose, the amount of MDA fell on each of the 15th, 30th, and 45th days of shading treatment respectively (Fig. 2).

3. Photosynthetic properties

The photosynthetic property g_s in the 80% and 60% light transmission plots exceeded that of the 100% plot on each of the 15th and 30th days of shading treatment (Fig. 3). No significant difference was seen in C_i between the three light transmission plots on any of the shading treatment days. *E* rose with decreasing light intensity on the 15th and 30th days of shading treatment, and on the 45th day the 60% plot was higher than the 100% plot. *A* rose with decreasing light intensity, and on the 45th day the 80% and 60% plots were higher than the 100% plot.

Discussion

The appropriate light intensity for the growth and development of the cucumber is generally regarded as 700–1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [11]. However, the appropriate figure is less than this for seedlings. The maximum light intensities in this study were 1235 ± 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the 100% (unshaded) light transmission plot, 978 ± 121 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the 80% plot, and 760 ± 148 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the 60% plot.

1. Active oxygen species

A majority of the chlorophyll in the chloroplasts harvests light to capture light energy, but captures too much light energy when exposed to strong light. When this occurs, the excess electrons generated by the photosystem II from the water splitting are not used in the CO_2 fixation reaction and reduce oxygen through photosystem I, generating more $O_2^{\cdot -}$ [2, 5]. Meanwhile, H_2O_2 in the cells is converted from $O_2^{\cdot -}$ by SOD, and more H_2O_2 is generated by the oxidation reaction of glycolic acid through the in-

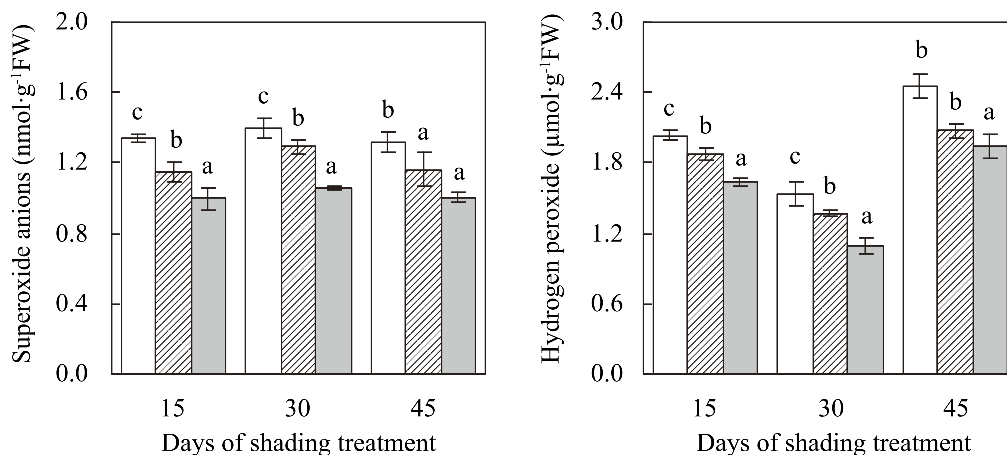

Fig. 1. Effect of shading treatment on superoxide anions and hydrogen peroxide in leaves of cucumber 'Jinchun No. 5' seedlings

□: 100% sunlight transmittance (ST) (unshaded), ▨: 80% ST, ■: 60% ST. Different letters indicate mean separation by Tukey's HSD test, 5% level of significance. Vertical bars represent standard error of the mean (n=5).

creased photorespiration of the peroxisomes, due, in turn, to strong light[15]. In other words, the avoidance of strong light results in the reduced generation of active oxygen species in plant leaf cells due to excess light energy and photorespiration. These are considered the primary reasons for the decreased amounts of $O_2^{\cdot-}$ and H_2O_2 in the

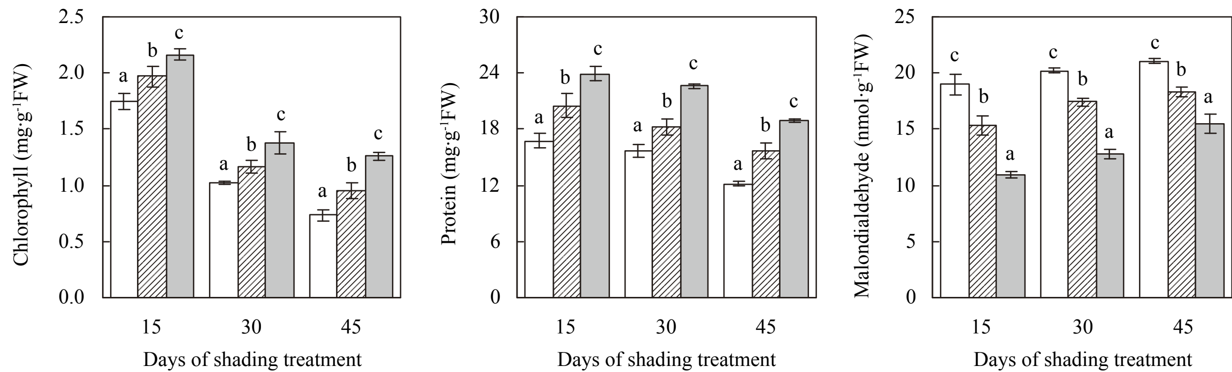

Fig. 2. Effect of shading treatment on chlorophyll, protein, and malondialdehyde in leaves of cucumber 'Jinchun No. 5' seedlings

□: 100% sunlight transmittance (ST) (unshaded), ▨: 80% ST, ■: 60% ST. Different letters indicate mean separation by Tukey's HSD test, 5% level of significance. Vertical bars represent standard error of the mean (n=5).

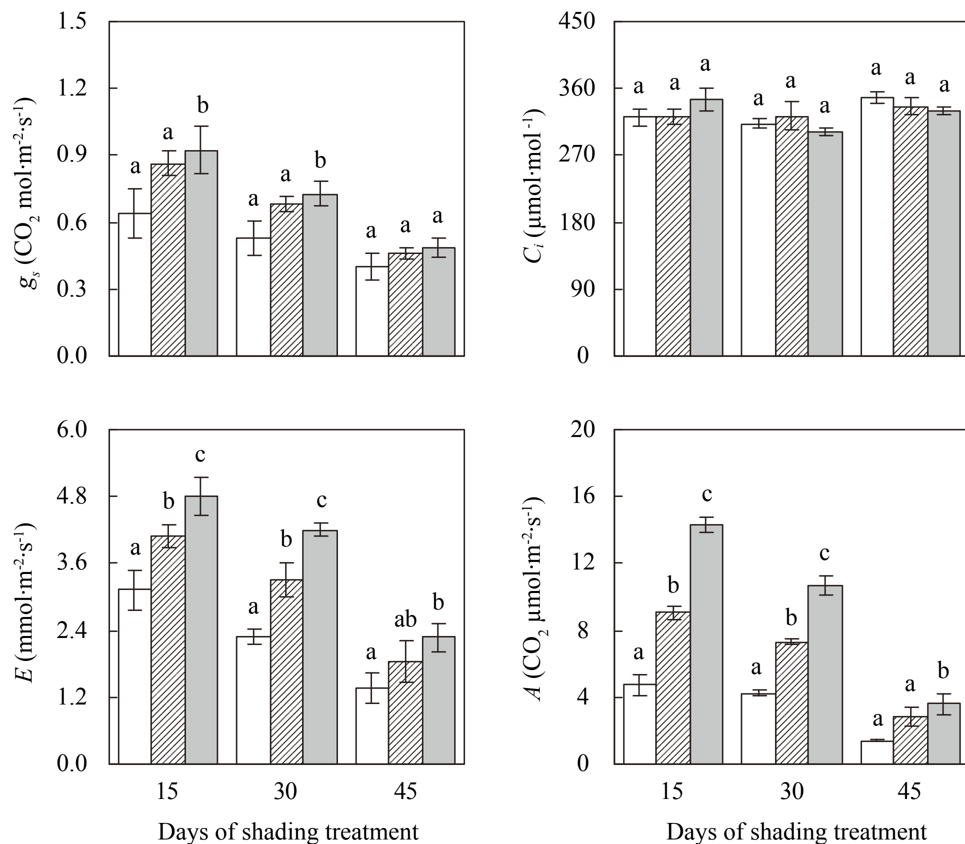

Fig. 3. Effect of shading treatment on stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i), transpiration rate (E), and net photosynthesis rate (A) in leaves of cucumber 'Jinchun No. 5' seedlings

□: 100% sunlight transmittance (ST) (unshaded), ▨: 80% ST, ■: 60% ST. Different letters indicate mean separation by Tukey's HSD test, 5% level of significance. Vertical bars represent standard error of the mean (n=5).

cucumber 'Jinchun No. 5' seedlings with decreasing light intensity during the shading treatment in this experiment (Fig. 1). Decreased $O_2^{\cdot -}$ and H_2O_2 in the leaves due to shading has also been reported in tomatoes (C_3 plant) and woody plants in subtropical rain forests[10, 18].

2. Senescence indicators

Leaf senescence in cucumbers is largely influenced by environmental conditions, and is far more rapid during the high temperatures and strong light of the spring to fall cultivation period compared to winter. Leaf senescence in the 'Jinchun No. 5' cucumber seedlings slowed with decreasing light intensity in this experiment, as indicated by the senescence indicators (Fig. 2) — the amounts of chlorophyll, protein and MDA. These results show that a large leaf area in cucumber 'Jinchun No. 5' seedlings is susceptible to strong light and demonstrate the inhibitory effect of shading on senescence. It is clear from these results that high temperatures as well as strong light accelerate senescence in the leaves of cucumber 'Jinchun No. 5' seedlings[19].

Oxidative damage to chlorophyll, proteins, and membrane lipids due to numerous active oxygen species is the main cause of leaf senescence accelerated by environmental stress[4, 9, 14]. Likewise, leaf senescence in the cucumber 'Jinchun No. 5' seedlings that slowed with decreasing light intensity (Fig. 2) is considered involved in the decline in active oxygen species in the leaf cells due to shading (Fig. 1), as indicated by the results of this experiment.

3. Photosynthetic properties

As photosynthetic activity depends on CO_2 from the stomata and intercellular spaces, and H_2O absorbed through transpiration, g_s, C_i, and E are regarded as photosynthetic properties, and directly influence the net photosynthesis rate. Each of the photosynthetic properties g_s, C_i, and E in the 'Jinchun No. 5' cucumber seedlings were higher in the 80% and 60% light transmission plots in this experiment compared to the 100% plot, and both E and A were higher in the 60% plot than the 80% plot (Fig. 3). These results demonstrate the ameliorated effect of shading on the photo-inhibition of photosynthesis in cucumber 'Jinchun No. 5' seedlings during periods of strong light. Meanwhile, the fact that the effect of shading on C_i is not seen is considered attributable to the increase in CO_2 consumption due to a rise in the net photosynthesis rate, which, in turn, is caused by an increase in g_s.

The results of this experiment (Figs. 1, 2 and 3) suggest that the reduction of A under this strong light is caused by photo-inhibition from the accumulation of active oxygen species, and the reduced photosynthetic function arising from senescence, as well as reduced CO_2 intake and water absorption[3, 6, 14, 17]. This differs from the minimal involvement of g_s and E in the reduction in A in the cucumber 'Jinchun No. 5' seedlings due to high temperature stress[19].

Conclusion

In this study we demonstrated how the generation and accumulation of the active oxygen species $O_2^{\cdot -}$ and H_2O_2 was inhibited in cucumber 'Jinchun No. 5' seedlings due to shading in periods of strong light. The ameliorated effect of shading on senescence was also borne out in the altered amounts of chlorophyll, protein, and MDA. Also, we propose that the ameliorated effect of shading on photosynthesis decrease in cucumber 'Jinchun No. 5' seedlings, is due to the photo-inhibition of active oxygen species, the reduced photosynthetic function, and the increase in g_s and E associated with senescence. These physiological data can then be used to cultivate a cucumber cultivar resistant to strong light, and facilitate physiological research into strong light injury.

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