DOI: 10.1007/s11099-015-0179-1 PHOTOSYNTHETICA 54 (2): 251-258, 2016

Effects of low irradiation on photosynthesis and antioxidant enzyme activities in cucumber during ripening stage

Z.Q. YANG+, C.H. YUAN, W. HAN, Y.X. LI, and F. XIAO

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

Abstract

In order to investigate the effects of low irradiation (LI) on cucumber (*Cucumis sativus* L. cv. Jinyou 35) during a ripening stage, our experiment was carried out in a climate chamber. Two levels of PAR were set for plants: normal irradiation [NI, 600 μmol(photon) m^{-2} s^{-1}] and low irradiation [LI, 100 μmol(photon) m^{-2} s^{-1}], respectively. The experiments lasted for 9 d; then both groups of plants were transferred under NI to recover for 16 d. The plants showed severe chlorosis after the LI treatment. Chlorophyll (Chl) *a*, initial slope, photosynthetic rate at saturating irradiation (P_{max}), light saturation point, maximal photochemical efficiency of PSII (F_v/F_m), electron transport rate of PSII (ETR), soluble protein content, and catalase (CAT) activity in cucumber leaves decreased under LI stress, while Chl *b*, carotenoids, light compensation point, nonphotochemical quenching (q_N), superoxide dismutase (SOD), and malondialdehyde (MDA) exhibited an increasing trend under LI. After 16 d of recovery, values of P_{max}, F_v/F_m, ETR, q_N, SOD, CAT, MDA, and soluble protein were close to those of the control after one, three, and five days of the LI treatment, while those kept under LI for 7 and 9 d could not return to the control level. Therefore, 7 d of LI stress was a meteorological disaster index for LI in cucumber at the fruit stage.

Additional key words: chlorophyll fluorescence; lipid peroxidation; low light intensity; P_N/PPFD response curve.

Introduction

Cucumber is a sun plant sensitive to low light during ripening stage. Light is the main meteorological element for plant growth and development (Scholes *et al*. 2011). Therefore, it can provide decision support for the optimization of greenhouse light management by studying the leaf photosynthetic characteristics, Chl fluorescence parameters, and antioxidant enzyme activities of cucumber under LI treatment. Plenty of studies have demonstrated that LI seriously affects crop physiological characteristics, growth, development, fruit quality, *etc.* (Boardman 1977, Barreiro *et al.* 1992, Tinoco-Ojanguren *et al*. 1992, Bailey *et al*. 2001, Hanba *et al*. 2002, Sui *et al*. 2008, Chen *et al*. 2014). LI may block optoelectronic transport in photosystems, reduce carbon assimilation enzyme activity, and change antioxidant enzyme activities (Allen and Ort 2001). Ren *et al.* (2002) have reported that SOD activity of tomato leaves decreased under LI stress. LI also reduced F_v/F_m, ETR, and initial fluorescence (F_0) of bunchgrass, spinach, *Riccia fluitans*, *Perilla*, and *Primula nutans* (Caldwell *et al*. 1983, Ernstsen *et al*. 1999, Andersen and Pedersen 2002, Miko *et al.* 2004, Shen *et al.* 2008). The study has reported that LI stress significantly reduced Chl contents in leaves of *Arabidopsis thaliana* (Tanaka and Tanaka 2005), henbit genus (Haliapas *et al.* 2008), and rice (Yamazaki 2010). In addition, LI stress could increase plant height and leaf area, but reduce the number of plant shoots, leaves, flower buds, leaf thickness, and the yield (Schultz and Matthews 1993, Potter *et al.* 1999, Correll and Weathers 2001, Shen *et al*. 2002, Barisic *et al*. 2006,

Received 14 April 2015, *accepted* 11 September 2015, *published as online-first* 10 October 2015.
+Corresponding author; e-mail: yzq6751@163.com
Abbreviations: AQE – apparent quantum efficiency; Car – carotenoids; CAT – catalase; Chl – chlorophyll; ETR – electron transport rate; F_0 – initial fluorescence; F_0' – minimal fluorescence yield of the light-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry; g_{smax} – maximum of stomatal conductance; *k* – curve convexity; LCP – light compensation point; LI – low irradiation; LSP – light saturation point; MDA – malondialdehyde; NI – normal irradiation; P_{max} – photosynthetic rate at irradiation saturation; P_N – net photosynthetic rate; q_N – nonphotochemical quenching coefficient; R_D – dark respiration rate; ROS – reactive oxygen species; SOD – superoxide dismutase; α – initial slope.
Acknowledgements: We thank to the programs of the National Natural Science Foundation of China (Grant No. 41475107) and National Science & Technology Support Program during the Twelfth Five Year Plan Period (Grant No. 2014BAD10B07).

Baltzer and Thomas 2007, Seidlova *et al*. 2009, Hou *et al.* 2010).

In our study, we aimed to investigate the effects of irradiation stress and recovery on the leaf photosynthetic characteristics, Chl fluorescence parameters, and antioxidant enzyme activities of cucumber at a fruit stage. We analyzed the changes of various parameters during a stress and recovery stage with the aim to find out a meteorological disaster index for LI in cucumber. Two hypotheses were tested: (*1*) based on the fact that photosynthesis was hampered under LI conditions, photosynthetic rate at irradiation saturation (P_{max}) should decline with the duration of LI; and (*2*) cucumber plants, which underwent a certain time under the LI treatment, should recover under NI.

Materials and methods

Experimental design: The experiment was carried out from October 2013 to June 2014 in the climate chamber of Nanjing University of Information Science and Technology, China. In this study, irradiance of 100 μmol(photon) m^{-2} s^{-1} was applied as low light (LI) stress to cucumber according to Guo *et al*. (2012) and Liang *et al.* (2010). The irradiation level during the stress and recovery stage was as follows:

Treatment	LI treatment duration [d]	Irradiation [μmol(photon) m^{-2} s^{-1}]	
		Stress stage	Recovery stage
CK	0	600	600
S1	1	100	600
S3	3	100	600
S5	5	100	600
S7	7	100	600
S9	9	100	600

The plants of all treatments were recovering for 16 d. During the LI and recovery treatment, the photoperiod was set to 12/12 h day/night (day from 07:00–19:00 h), temperature of the day and night was set to 25 and 18°C, respectively, and the relative humidity was set at 75%. The plants were irrigated as needed; the application of fertilizer was 80 kg(urea) ha^{-1}, 75 kg(superphosphate) ha^{-1}, and 60 kg(KCl) ha^{-1}.

Photosynthetic pigment content: The colorimetric method was used. The contents of Chl *a*, Chl *b*, and carotenoids (Car) in cucumber leaves were determined by the method of Abbasi *et al*. (2008). The 5^{th}–8^{th} function leaves from the top of a plant were collected and placed into the 96% ethanol for 48 h until the pigments in the leaves were completely extracted. The exact concentrations were measured using spectrophotometer (*Cary 50 UV-VIS*, *Varian*, Victoria, Australia) at 663, 646, and 470 nm, respectively. The Chl *a*, Chl *b*, and carotenoids were calculated according to Wellburn (1994).

Photosynthetic parameters: The irradiation response curves were measured between 09:00 and 11:00 h with photosynthesis system *LI-6400* (*LI-COR Inc.*, USA). The 5^{th}–8^{th} function leaves from the top of a plant were measured. Temperature in the leaf chamber was set at 25°C and CO_2 concentration was controlled at 390 μmol(CO_2) mol^{-1}. Photosynthetic active radiation was set as 2,000; 1,800; 1,500; 1,000; 800, 500, 200, 100, 50, 20, and 0 μmol(photon) m^{-2} s^{-1}, respectively, and the photosynthetic rates (P_N) were determined under different PAR levels. Three repetitions were measured for each treatment once every two days during the LI stress and recovery period. P_N/PPFD curves were modeled by fitting nonrectangular hyperbola to data as described by Prioul and Chartier (1977):

$$P_N = \frac{\alpha\,\mathrm{PPFD} + P_{max} - \left[(\alpha\,\mathrm{PPFD} + P_{max})^2 - 4k\,\alpha\,\mathrm{PPFD}\,P_{max}\right]^{0.5}}{2k} - R_D$$

where α is the initial slope or apparent photosynthetic quantum yield (P_N/PPFD at low PPFD); PPFD is the photosynthetic photon flux density [μmol(photon) m^{-2} s^{-1}]; P_{max} is the photosynthetic rate at irradiation saturation [μmol(CO_2) m^{-2} s^{-1}]; *k* is the curve convexity (dimensionless); and R_D is the dark respiration rate [μmol(photon) m^{-2} s^{-1}].

Chl fluorescence: The fully expanded penultimate leaf was selected and Chl fluorescence parameters were determined with a portable fluorimeter (*FMS 2*, *Hansatech*, UK) once every 4 d for each treatment during the recovery period. Fluorescence parameters represented the maximum quantum yield of PSII photochemistry (F_v/F_m), nonphotochemical quenching coefficient (q_N), and electron transport rate (ETR). Firstly, the irradiation adaptation fluorescence parameters, F_m', F_0', and F_s, were determined under actinic irradiation of 600 μmol(photon) m^{-2} s^{-1}, then the dark-adaptation fluorescence parameters, F_m and F_0, were determined after the leaves were dark-adapted for 30 min. Measurements were repeated three times for each treatment. F_v/F_m, q_N, and ETR were calculated according to Zhang and Gao (1999).

Antioxidant enzyme activities in cucumber leaves were determined once every 3 d for each treatment during the recovery period. The 5^{th}–8^{th} functional leaves were picked from the top of plants between 9:00 and 10:00 h and immediately frozen in liquid nitrogen, then stored in low temperature freezer (–40°C). SOD (EC 1.15.1.1) was determined according to the method of Rabinowitch and Sklan (1980); one unit of the SOD activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of nitroblue tetrazolium (NBT) reduction measured at 560 nm per min (*UV-1800*, *Shimadzu*, Japan). Activity was determined using the extinction coefficient

of 6.39 mM^{-1} cm^{-1} and calculated per fresh mass (FM). CAT (EC 1.11.1.6) activity was measured *via* decomposition of H_2O_2 followed directly by decrease in absorbance at 240 nm. One unit of CAT was the amount of enzyme which decomposed 1 μmol H_2O_2 per min at 25°C (Abei 1984). CAT activity was calculated per g of FM. Each result was the mean of three replications.

Lipid peroxidation was estimated in terms of the MDA content. The content of MDA was determined according to Zhao et al. (1991). Fresh leaves (1.0 g) were ground in 10% trichloroacetic acid and then centrifuged at 3,000 × g for 10 min. Two mL of the supernatant were mixed with 2 mL of 0.6% thiobarbituric acid (TBA) and incubated for 30 min at 100°C to develop the (TBA)2-MDA adduct. The mixture was cooled rapidly in an ice bath. After centrifugation at 5,000 × g for 10 min, the absorbance was measured (*UV-1800, Shimadzu*, Japan). Lipid peroxidation was expressed as μmol g–1(FM) by using the following formula: MDA = 6.45(A532 – A600) – 0.56A450, where A532, A600, and A450 refer to the absorbance measured at wavelength of 450, 532, and 600 nm, respectively.

Soluble protein: Plant tissues were ground in liquid N_2, with 1 mL g^{-1} of 0.1 M sodium phosphate buffer (pH 7.0). The homogenates were centrifuged at 15,000 × *g* for 15 min. All steps were performed at 4°C. The protein concentration was determined in the supernatant according to Bradford (1976), using a UV/VIS spectrophotometer (*Hitachi U-2000*, Japan) at 595 nm.

Statistical analysis: Differences between NI and LI for the photosynthetic parameters, Chl fluorescence parameters, Chl content, antioxidant enzyme activities and lipid peroxidation were tested by a one-way analysis of variance (*ANOVA*) using the statistical software SPSS 16.0 (*SPSS Inc*., Chicago, IL, USA); the treatment means were compared by using *Duncan*'s multiple range test at $P \leq 0.05$.

Results

Photosynthetic pigment content: As shown in Table 1, the content of Chl *a* in cucumber leaves gradually declined during the LI treatment. Compared with the control, the content of Chl *a* decreased only slightly after the S1 and S3 treatments, but decreased by 10.1, 18.0, and 25.3% under the S5, S7, and S9 treatment, respectively. The contents of Chl *b* and Car under LI showed a trend of rising with the treatment duration, and those were all significantly higher than the control, for example, the contents of Chl *b* and Car for S9 treatment increased by 15.7% and 30.5% respectively, compared with the control. The Chl *a*/*b* ratio decreased under LI gradually during the experiment, and varied from 2.14 to 3.02. The Chl *a*/*b* ratio for S1 treatment was 3.02, which was not significantly different from the control, while those of all other LI treatments were significantly lower than that of the control. Severe chlorosis was observed after the LI treatment and enhanced with the treatment duration.

Photosynthetic parameters: P_{max} of cucumber leaves declined under LI with the duration of the experiment (Table 2). P_{max} was significantly lower at different days of the LI treatments compared with the control, while the apparent quantum efficiency (AQE) showed a declining trend. AQE was also significantly lower at different days of the LI treatments compared with the control except for S1. LSP of cucumber leaves was reduced under the LI stress with the stress duration, *e.g.*, it was only 645.3 and 485.6 μmol m^{-2} s^{-1} under S7 and S9 treatments, respectively, which was significantly lower than that of the control. The change of LCP was in contrast to LSP; that of all LI treatments was significantly higher than that of the control. The change of LCP was in contrast to LSP; that of all LI treatments was significantly higher than that of the control. The LCP of S7 and S8 treatments enhanced the LCP of cucumber leaves by 62.8% and 68.9%, respectively. The maximum of stomatal conductance (g_{smax}) also decreased, and that for all LI treatments were significantly higher than that of the control.

The recovery capability for photosynthesis in cucumber leaves significantly differed in dependence on the LI treatments (Fig. 1). P_{max} for S1, S3, and S5 treatment reached the control level after 6, 10, and 16 d of recovery, respectively, while that of the S7 treatment was only 53.0% of the control after 16 d of recovery, and that of the S9 treatment was only 13.8% of the control after 16 d of recovery, P_{max} of cucumber leaves changed little with the duration of recovery.

Chl fluorescence parameters: F_v/F_m of cucumber leaves under different LI treatments was lower than that of the control (Table 3). F_v/F_m decreased to 89.0, 79.2, 63.4, 54.9, and 50.0% of the control under S1, S3, S5, S7, and S9 treatment, respectively. q_N rose during the LI stress stage; it increased by 7.4, 37.0, 59.3, 92.5, and 107.4% for S1, S3, S5, S7, and S9 treatment, respectively, as compared with the control. ETR decreased under different LI with increasing time by 14.9, 31.3, 43.8, 59.4, and 60.3%, compared with the control, under S1, S3, S5, S7, and S9 treatment, respectively.

F_v/F_m of the S1, S3, and S5 treatments returned to the control level after 12–16 d of recovery treatment, while that of the S7 and S9 treatment were reduced by 27.4 and 48.8%, respectively, compared with the control after 16 d of the recovery treatment. After 16 d of recovery, q_N of the S1, S3, and S5 treatments were close to the control level and that of the S7 and S9 treatment were 14.8 and 48.1% higher than that of the control, respectively. ETR

Table 1. Effects of low-light stress on contents of photosynthetic pigments in cucumber leaves. *Small letters* indicate significance of $P<0.05$ by *Duncan*'s test. All parameters are expressed on dry mass basis, results are presented as mean ± SD (n = 3). Car – carotenoids; Chl – chlorophyll; CK – control. S1, S3, S5, S7, S9 – 1, 3, 5, 7, and 9 d of the low-irradiation treatment, respectively.

Treatment	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Car [mg g^{-1}(FM)]	Chl *a*/*b*
CK	8.75 ± 0.76^a	2.80 ± 0.18^c	0.72 ± 0.05^c	3.05 ± 0.34^a
S1	8.61 ± 0.91^a	2.89 ± 0.24^b	0.79 ± 0.06^b	3.02 ± 0.42^a
S3	8.38 ± 0.78^a	2.92 ± 0.21^b	0.82 ± 0.07^b	2.86 ± 0.22^b
S5	7.86 ± 0.62^b	3.02 ± 0.26^a	0.88 ± 0.07^a	2.60 ± 0.33^b
S7	7.17 ± 0.58^b	3.11 ± 0.41^a	0.91 ± 0.06^a	2.33 ± 0.32^c
S9	6.54 ± 0.45^c	3.24 ± 0.33^a	0.94 ± 0.08^a	2.14 ± 0.32^c

Table 2. Effects of low-light stress on photosynthetic parameters of cucumber leaves. *Small letters* indicate significance of $P<0.05$ by *Duncan*'s test. All parameters are expressed on dry mass basis, results are presented as mean ± SD (n = 3). P_{max} – maximum of net photosynthetic rate; AQE – apparent quantum efficiency; LSP – light saturation point; LCP – light compensation point; g_{smax} – maximum of stomatal conductance. CK – control. S1, S3, S5, S7, S9 – 1, 3, 5, 7, and 9 d of the low-irradiation treatment, respectively.

Treatment	P_{max} [μmol m^{-2} s^{-1}]	AQE	LSP [μmol m^{-2} s^{-1}]	LCP [μmol m^{-2} s^{-1}]	g_{smax} [mmol m^{-2} s^{-1}]
CK	16.9 ± 0.6^a	0.051 ± 0.004^a	1424.3 ± 88.6^a	32.8 ± 2.7^d	1.190 ± 0.031^a
S1	15.4 ± 1.2^b	0.047 ± 0.004^a	1232.5 ± 89.6^b	38.4 ± 4.4^c	0.773 ± 0.023^b
S3	13.1 ± 1.0^c	0.032 ± 0.003^b	1142.6 ± 78.4^b	44.6 ± 3.6^b	0.438 ± 0.016^c
S5	9.7 ± 0.8^d	0.022 ± 0.002^c	855.7 ± 67.2^c	46.3 ± 4.7^b	0.227 ± 0.013^d
S7	5.9 ± 0.4^e	0.017 ± 0.001^d	645.3 ± 54.4^d	53.2 ± 5.2^a	0.171 ± 0.009^e
S9	3.4 ± 0.2^f	0.018 ± 0.001^d	585.6 ± 43.8^e	55.4 ± 6.2^a	0.151 ± 0.008^e

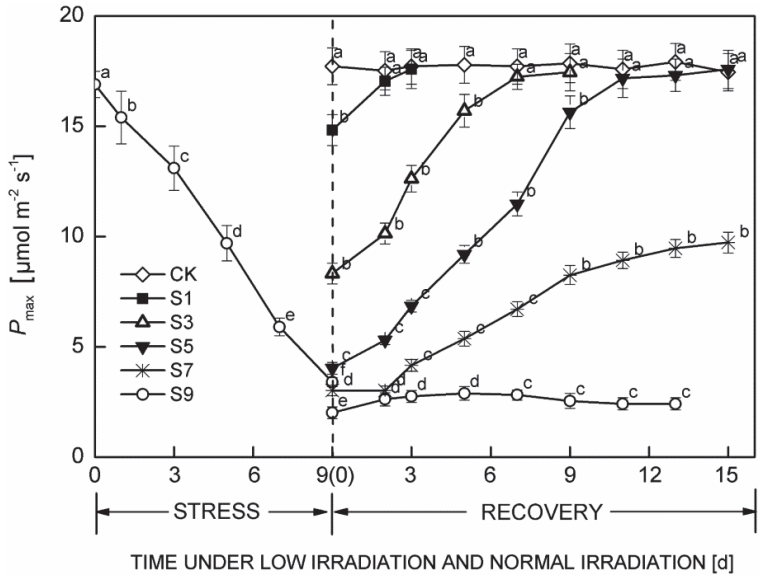

Fig.1. Photosynthetic rate at irradiation saturation (P_{max}) in cucumber leaves during the stress and recovery phase. Error bars represent SD, n = 3. CK – control. S1, S3, S5, S7, S9 – 1, 3, 5, 7, and 9 d of the low-irradiation treatment, respectively.

of the S1 treatment reached the control level after 16 d of the recovery, but that of S3, S5, S7, and S9 was 8.4, 12.9, 29.2, and 60.0% lower than that of the control, respectively.

Antioxidant enzyme activities, MDA and soluble protein: As shown in Fig. 2, SOD under LI increased first and then declined. The highest SOD activity was found in the S5 treatment. The activities of SOD in all LI treatments were significantly higher than that of the control. The activity of CAT was reduced under LI stress; it decreased by 12.4, 15.0, 23.3, 34.7, and 53.8% under S1, S3, S5, S7, and S9, respectively, compared with the control. MDA always increased under LI stress. There was no significant difference in the MDA content between the S1 and S3 treatment, but both treatments were significantly higher than that of the control. The contents of MDA in the S7 and S9 treatment were 68.9 and 98.8%

Table 3. Chlorophyll fluorescence parameters of cucumber at different stages under low-irradiation and recovery treatments. *Small letters* indicate significance of $P<0.05$ by *Duncan*'s test. Results are presented as mean ± SD ($n = 3$). F_v/F_m – maximal quantum yield of PSII photochemistry; q_N – nonphotochemical quenching coefficient; ETR – electron transport rate. CK – control. S1, S3, S5, S7, S9 – 1, 3, 5, 7, and 9 d of the low-irradiation treatment, respectively.

	Treatment	Time under normal irradiation of 600 μmol(photon) m^{-2} s^{-1} [d]				
		0	4	8	12	16
F_v/F_m	CK	0.82 ± 0.05[a]	0.81 ± 0.04[a]	0.84 ± 0.04[a]	0.83 ± 0.04[a]	0.84 ± 0.05[a]
	S1	0.73 ± 0.04[a]	0.75 ± 0.03[b]	0.77 ± 0.03[b]	0.82 ± 0.04[a]	0.83 ± 0.05[a]
	S3	0.65 ± 0.03[b]	0.56 ± 0.03[c]	0.59 ± 0.03[c]	0.74 ± 0.05[b]	0.82 ± 0.06[a]
	S5	0.52 ± 0.03[c]	0.52 ± 0.04[c]	0.54 ± 0.04[c]	0.72 ± 0.05[b]	0.80 ± 0.05[a]
	S7	0.45 ± 0.01[d]	0.46 ± 0.02[d]	0.47 ± 0.02[d]	0.54 ± 0.02[c]	0.61 ± 0.03[b]
	S9	0.41 ± 0.01[d]	0.43 ± 0.02[d]	0.44 ± 0.02[d]	0.45 ± 0.01[d]	0.43 ± 0.01[c]
q_N	CK	0.27 ± 0.03[c]	0.25 ± 0.02[c]	0.25 ± 0.02[c]	0.26 ± 0.03[c]	0.27 ± 0.04[b]
	S1	0.29 ± 0.05[c]	0.33 ± 0.03[c]	0.25 ± 0.03[c]	0.28 ± 0.01[b]	0.26 ± 0.04[b]
	S3	0.37 ± 0.02[b]	0.42 ± 0.05[b]	0.37 ± 0.05[b]	0.33 ± 0.02[b]	0.29 ± 0.02[b]
	S5	0.43 ± 0.05[b]	0.48 ± 0.07[b]	0.45 ± 0.07[b]	0.36 ± 0.03[a]	0.29 ± 0.05[b]
	S7	0.52 ± 0.05[a]	0.58 ± 0.05[a]	0.54 ± 0.05[a]	0.48 ± 0.05[a]	0.31 ± 0.04[b]
	S9	0.56 ± 0.02[a]	0.57 ± 0.08[a]	0.54 ± 0.08[a]	0.45 ± 0.04[a]	0.40 ± 0.05[a]
ETR	CK	41.6 ± 4.7[a]	43.9 ± 4.3[a]	43.9 ± 4.3[a]	43.3 ± 6.9[a]	44.8 ± 5.7[a]
	S1	35.4 ± 5.7[b]	34.9 ± 6.2[b]	35.9 ± 6.2[b]	41.4 ± 4.8[a]	42.5 ± 3.6[a]
	S3	28.6 ± 3.5[c]	27.2 ± 3.2[b]	29.2 ± 3.2[b]	39.4 ± 4.1[a]	41.0 ± 5.3[a]
	S5	23.4 ± 3.7[c]	19.5 ± 2.8[c]	23.5 ± 2.8[c]	35.5 ± 3.6[b]	39.0 ± 4.4[b]
	S7	16.9 ± 2.2[d]	13.5 ± 2.6[d]	18.5 ± 2.6[d]	25.6 ± 1.7[c]	31.7 ± 2.2[c]
	S9	16.5 ± 1.7[d]	14.8 ± 1.8[d]	15.1 ± 1.8[d]	17.5 ± 1.4[c]	17.9 ± 1.8[d]

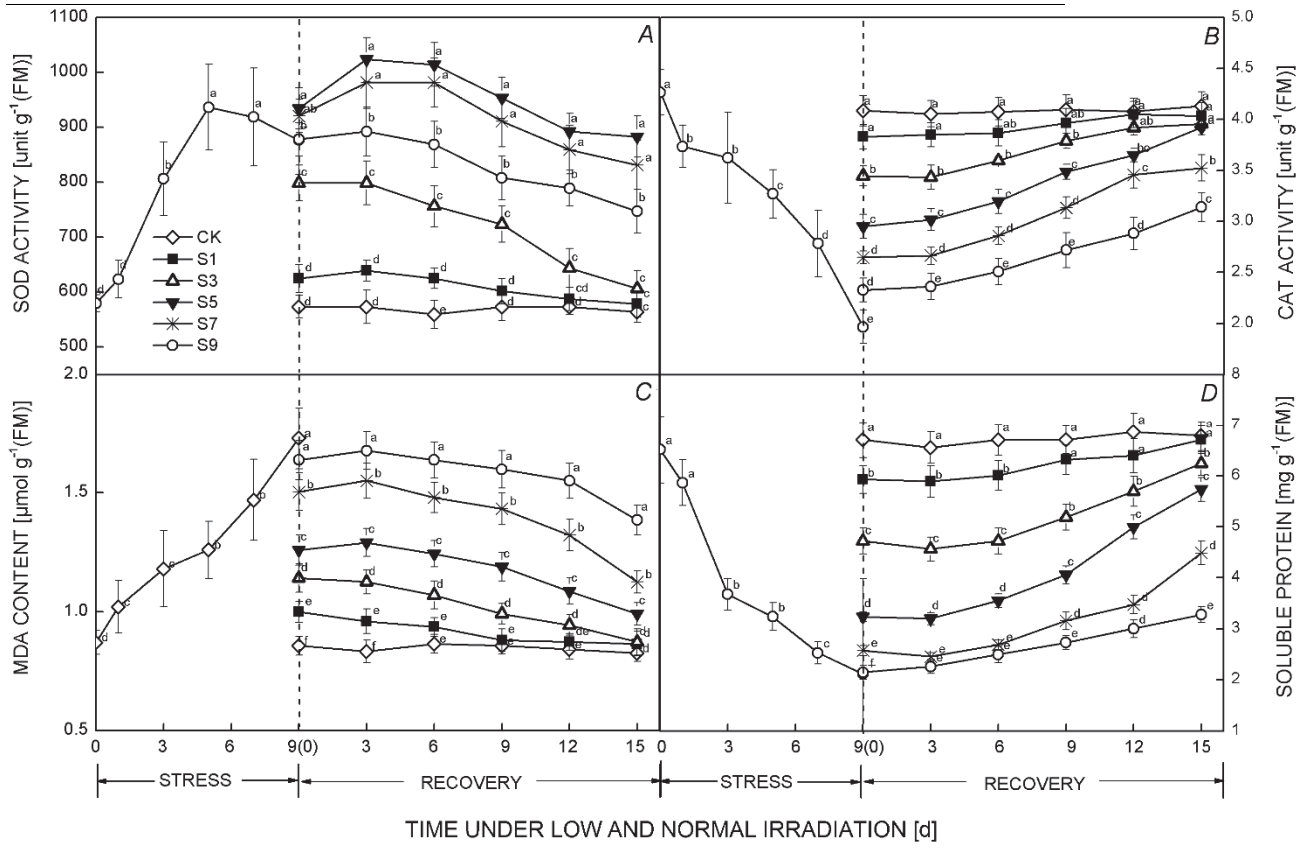

Fig. 2. Superoxide dismutase (SOD) activity (*A*), catalase (CAT) activity (*B*), malondialdehyde (MDA) content (*C*), and soluble protein content (*D*) in cucumber leaves during the stress and the recovery phase. Error bars represent SD, $n = 3$. CK – control. S1, S3, S5, S7, S9 – 1, 3, 5, 7, and 9 d of the low-irradiation treatment, respectively.

higher than that of the control, respectively. The contents of soluble protein in cucumber leaves showed the same trend as that of CAT. The contents of soluble protein were significantly lower after the LI treatment than that of the control with an exception of S1. The content of soluble protein of the S7 and S9 treatment were 61.2 and 67.4% lower than that of the control, respectively.

The activities of SOD and CAT as well as the contents of MDA and soluble proteins of the S1and S3 treatment could recover to the control level (Fig. 2). For the S5 treatment, the activity of CAT and the content of MDA reached the control level after 16 d of recovery, while the activity of SOD and soluble protein content could not recover completely. However, for the S7 and S9 treatment, the activities of SOD, CAT, contents of MDA and soluble protein could not return to the control values. After 16 d of the recovery treatment, the activity of SOD and content of MDA was 31.6 and 66.7% higher, respectively, after the S9 treatment than those of the control, while the activity of CAT and the content of soluble protein was 24.5 and 51.6% lower than those of the control, respectively.

Discussion

Photosynthesis is one of the most important physiological activities in plants and is a fundamental way for plant to synthesize organic matter and obtain energy. The photosynthetic process is highly sensitive to any change in the environment (Yin *et al.* 2006). Leaf Chl is an important component in plant physiological acclimation to different light intensities (Caesar 1989, Feng *et al.* 2004). We showed that the content of Chl *a* and the Chl *a*/*b* ratio in cucumber leaves became reduced, while Chl *b* and Car increased significantly under LI treatments. This was similar to the result of Lichtenthaler *et al.* (1982), who showed a consistent and dramatic decrease of Chl *a*/*b* with a decrease of growth irradiance. The primary reason may be that the LI stress caused disorder of chloroplast ultrastructure and aggravated its degradation, leading to reduction of the Chl content.

P_{max}, which was consistent with Chl, decreased under LI in accordance with Shen *et al.* (2008). The reason may be that low light availability resulted in significant reduction of a carbon gain in the plants. Parameters, such as α and LSP also decreased, while LCP increased during the LI treatment; all species made photosynthetic and respiratory adjustments from strong to medium or to weak light (Thompson *et al.* 1992, James *et al.* 2000), which resulted in a lower LCP, while the change of LSP was dependent on plant species. The stomata regulate the exchange between carbon and water loss through the cuticle and are sensitive to light intensity. The g_{smax} decreased during the LI treatment. Yu *et al.* (2004) also showed that g_s decreased with the decline of light intensity.

F_v/F_m of cucumber plant decreased under LI in accordance with Li *et al.* (2003), indicating that the activity of PSII was inhibited under LI. ETR decreased, while q_N increased, implying that plants efficiently dissipated energy trapped at PSII in the form of heat which protected plants against photoinhibition.

Antioxidative systems play a major role in protecting plants from negative effects of reactive oxygen species (ROS). The antioxidant enzymes SOD and CAT play a vital role in scavenging these destructive oxidant species. This study showed that LI significantly enhanced the SOD activity and MDA content in cucumber leaves, inhibited CAT activity, and reduced the soluble protein content. In this study, in short-term (less than 7 d) LI treatments, the SOD activity increased, mainly because the plants started self-protection mechanisms in order to adapt to external environment and reestablish the balance between production and scavenging of ROS through regulation of antioxidant enzyme activities (Sun *et al.* 2003). The reason might be that metabolic disorders caused by LI resulted in an increase of ROS. The source of ROS might be the photosynthetic electron transport chain, photorespiration, and induced NADPH oxidase (Song *et al.* 2007). The chloroplast is a central switch of the plant's response to light stress. LI exposure in a temperature-dependent manner stimulates the accumulation of ROS in chloroplasts (Crosatti *et al.* 2013). The ROS then regulate the antioxidant enzyme activities. In order to prevent the harmful effects of ROS, plants have evolved a complex antioxidant system, *e.g.* the increase of SOD activity (Trotta *et al.* 2014). But in long-term LI stress treatments, the antioxidant enzymes were inhibited beyond the adaptation and self-protection capacity of plants (Scalet *et al.* 1995, Yang *et al.* 2002, Zhou *et al.* 2003). The activity of CAT was reduced with the prolonged duration of the LI treatment, indicating that the activity of enzyme declined dramatically. This was similar to results of Zhou *et al.* (2004). Keles and Öncel (2002) argued that severe stress conditions, which caused loss of CAT activity, could induce the activities of Mehler reaction enzymes. It is likely that the enhanced ROS accumulation did not keep pace with the rate of oxygen reduction since increased MDA, H_2O_2, and $O_2^{\cdot -}$ concentrations were observed throughout our experiment.

Lipid peroxidation could be identified as a basic cell membrane reactive damage (Mohammad *et al.* 2005, Liu *et al.* 2006). MDA increased during LI stress implying that the cell membrane system of the plants under long-term LI suffered more serious cell damage. The content of soluble protein in cucumber leaves decreased under LI, indicating that senescence of cucumber leaves aggravated the decrease. In fact, severe chlorosis was observed under the LI treatment, the leaf chlorosis was enhanced with the duration of LI stress.

After 16 d of the recovery treatment under normal irradiation, P_{max}, F_v/F_m, and ETR of the cucumber plants

under short-term LI stress could be equal to that of the control. However, after longer exposure (more than 7 d) to LI, P_{max} tended to be stable after 16 d of normal irradiation. Meanwhile, F_v/F_m and ETR could not recover to the control level, indicating that the long-term LI caused irreversible damage to PSII reaction centres, which was consistent with the results of Sun *et al.* (2008) and Vieira *et al.* (2009). What is more, SOD and MDA were much higher under the long-term LI treatment than those of the control, while CAT and soluble protein were much lower than the control after 16 d of recovery, indicating that the ability of cucumber leaves to prevent the oxidation of cell membrane systems by ROS was reduced greatly, therefore leaf senescence started and the cell membrane system suffered serious damage. In conclusion, we showed that Chl *a*, α, P_{max}, LSP, F_v/F_m, ETR, soluble protein content, and CAT activity in cucumber leaves exhibited a declining trend during LI stress, while Chl *b*, Car, LCP, q_N, and MDA increased. For short-term (less than 7 d) LI stress, the photosynthetic capacity of cucumber plants could completely recover after 16 d of normal irradiation. But after 7 d of LI stress, the photosynthetic capacity decreased significantly and could not recover, indicating that 7 d of LI treatment caused an irreversible damage to photosystems that could not be repaired. Therefore, 7 d of LI stress treatment is a critical meteorological index for cucumber at the fruit stage.

References

Abbasi W.M., Ahmed N., Zaki M.J., Shaukat, S. S.: Effect of *Barleria acanthoides* Vahl. on root-knot nematode infection and growth of infected okra and brinjal plants. – Pak. J. Bot. **40**: 2193-2198, 2008.

Aebi H.: Catalase *in vitro*. – Methods Enzymol. **105**: 121-126, 1984.

Ali M.B., Hahn E.J., Paek K.Y.: Effects of light intensities on antioxidant enzymes and malondialdehyde content during short-term acclimatization on micropropagated *Phalaenopsis* plantlet. – Environ. Exp. Bot. **54**: 109-120, 2005.

Allen D.J., Ort D.R.: Impacts of chilling temperatures on photosynthesis in warm climate plants. – Trends Plant Sci. **6**: 36-42, 2001.

Andersen T., Pedersen O.: Interactions between light and CO_2 enhance the growth of *Riccia fluitans*. – Hydrobiologia **477**: 163-170, 2002.

Bailey S., Walters R.G., Jansson S. *et al.*: Acclimation of *Arabidopsis thaliana* to the light environment: the existence of separate low light and high light responses. – Planta **213**: 794-801, 2001.

Baltzer J.L., Thomas S.C.: Physiological and morphological correlates of whole-plant light compensation point in temperate deciduous tree seedlings. – Oecologia **153**: 209-223, 2007.

Barišić N., Stojković B., Tarasjev A.: Plastic responses to light intensity and planting density in three *Lamium* species. – Plant Syst. Evol. **262**: 25-36, 2006.

Barreiro R., Guiamet J.J., Beltrano J. *et al.*: Regulation of the photosynthetic capacity of primary been leaves by the red: far-red ratio and photosynthetic photon flux density of incident light. – Physiol. Plantarum **85**: 97-101, 1992.

Boardman N.K.: Comparative photosynthesis of sun and shade plants. – Annu. Rev. Plant Physiol. **28**: 355-377, 1977.

Bradford M.M.: A rapid and sensitive method for the determination of microgram quantities of protein utilizing the principle of protein-dye binding. – Anal. Biochem. **72**: 248-255, 1976.

Bunce J.A.: Responses of stomatal conductance to light, humidity, and temperature in winter wheat and barley grown at three concentrations of carbon dioxide in the field. – Global Change Biol. **6**: 371-382, 2000.

Caesar J.C.: Effect of simulated shade radiation quality on the chlorophyll content of long and short shoot early leaves of birch (*Betula pendula* Roth.). – Photosynthetica **23**: 126-129, 1989.

Caldwell M.M., Dean T.J., Nowak R.S. *et al.*: Bunchgrass architecture, light interception, and water-use efficiency: assessment by fiber optic point quadrats and gas exchange. – Oecologia **59**: 178-184, 1983.

Correll M.J., Weathers P.J.: Effects of light, CO_2 and humidity on carnation growth, hyperhydration and cuticular wax development in a mist reactor. – In Vitro Cell. Dev-Pl. **37**: 405-413, 2001.

Crosatti C., Rizza F., Badeck F.W. *et al.*: Harden the chloroplast to protect the plant. – Physiol. Plantarum. **147**: 55-63, 2013.

Dong C., Fu Y.M., Liu G.H. *et al.*: Low light intensity effects on the growth, photosynthetic characteristics, antioxidant capacity, yield and quality of wheat (*Triticum aestivum* L.) at different growth stages in BLSS. – Adv. Space Res. **53**: 1557-1566, 2014.

Ernstsen J., Woodrow I.E., Mott K.A.: Effects of growth-light quantity, growth-light quality and CO_2 concentration on Rubisco deactivation during low PFD or darkness. – Photosynth. Res. **61**: 65-75, 1999.

Feng Y.L., Cao K.F., Zhang J.L.: Photosynthetic characteristics, dark respiration, and leaf mass per unit area in seedlings of four tropical tree species grown under three irradiances. – Photosynthetica **42**: 431-437, 2004.

Guo S.H., Xue L., Rou Z. *et al.*: Photosynthetic response of four species seedlings to low temperature stress. – J. South China Agr. Univ. **33**: 373-376, 2012.

Haliapas S., Yupsanis T.A., Syros T.D. *et al.*: *Petunia× hybrida* during transition to flowering as affected by light intensity and quality treatments. – Acta Physiol. Plant. **30**: 807-815, 2008.

Hanba Y.T., Kogami H., Terashima I.: The effect of growth irradiance on leaf anatomy and photosynthesis in *Acer* species differing in light demand. – Plant Cell Environ. **25**: 1021-1030, 2002.

Hou J.L., Li W.D., Zheng Q.Y. *et al.*: Effect of low light intensity on growth and accumulation of secondary metabolites in roots of *Glycyrrhiza uralensis* Fisch. – Biochem. Syst. Ecol. **38**: 160-168, 2010.

Keleş K., Öncel I.: Response of antioxidative defense system to temperature and water stress combinations in wheat seedlings. – Plant Sci. **163**: 783-790, 2002.

Kirschbaum M.U., Ohlemacher C., Küppers O.M.: Loss of quantum yield in extremely low light. – Planta **218**: 1046-1053, 2004.

Li X.G., Meng Q.W., Jiang G.Q. *et al.*: The susceptibility of cucumber and sweet pepper to chilling under low irradiance is related to energy dissipation and water-water cycle. – Photosynthetica **41**: 259-265, 2003.

Liang F., Zheng C.S., Sun X.Z. *et al.*: [Effects of low temperature- and weak light stress and its recovery on the photosynthesis and chlorophyll fluorescence parameters of cut flower chrysanthemum.] – Chin. J. Appl. Ecol. **21**: 29-35, 2010. [In Chinese]

Lichtenthaler H.K., Kuhn G., Prenzel U. *et al.*: Adaptation of chloroplast-ultrastructure and of chlorophyll- protein levels to high-light and low-light growth conditions. – Z. Naturforsch. C **37**: 464-475, 1982.

Liu P., Yang Y.S., Xu G.D. *et al.*: Physiological response of rare and endangered seven-son-flower (*Heptacodium miconioides*) to light stress under habitat fragmentation. – Environ. Exp. Bot. **57**: 32-40, 2006.

Potter T.I., Rood S.B., Zanewich K.P.: Light intensity, gibberellin content and the resolution of shoot growth in *Brassica*. – Planta **207**: 505-511, 1999.

Prioul J.L., Chartier P.: Partitioning of transfer and carboxylation components of intracellular resistance to photosynthetic CO_2 fixation: a critical analysis of the methods used. – Ann. Bot-London **41**: 789-800, 1977.

Rabinowitch H.D., Sklan D.: Superoxide dismutase: A possible protective agent against sunscald in tomatoes (*Lycopersicon esculentum* Mill.). – Planta **148**: 162-167, 1980.

Ren H., Huang W., Zhang F.: [Effects of low temperature and poor light on some physiological parameters of tomato.] – J. China Agr. Univ. **7**: 95-101, 2002. [In Chinese]

Scalet M., Federico R., Guido M.C. *et al.:* Peroxidase activity and polyamine changes in response to ozone and simulated acid rain in Aleppo pine needles. – Environ. Exp. Bot. **35**: 417-425, 1995.

Seidlova L., Verlinden M., Gloser J. *et al.*: Which plant traits promote growth in the low-light regimes of vegetation gaps? – Plant Ecol. **200**: 303-318, 2009.

Shen H.H., Tang Y.H., Muraoka H. *et al.*: Characteristics of leaf photosynthesis and simulated individual carbon budget in *Primula nutans* under contrasting light and temperature conditions. – J. Plant Res. **121**: 191-200, 2008.

Shen W.Q., Zhang G.P., Xi L.F. *et al.*: Effects of light and temperature conditions on flowering in Mat Rush. – J. Zhejiang Univ. Sci. **3**: 611-616, 2002.

Scholes G.D., Fleming G.R., Olaya-Castro A. *et al.*: Lessons from nature about solar light harvesting. – Nat. Chem. **3**: 763-774, 2011.

Schultz H.R., Matthews M.A.: Xylem development and hydraulic conductance in sun and shade shoots of grapevine (*Vitis vinifera* L.): evidence that low light uncouples water transport capacity from leaf area. – Planta **190**: 393-406, 1993.

Song L.L., Zhang Q.: Reactive oxygen gene network of plants and its regulation. – Chin. Bull. Life Sci. **19**: 346-352, 2007.

Sui X.L., Mao S.L., Wang L.H. *et al.*: [Effects of low temperature on photosynthesis of sweet pepper under low light.] – Acta Agric. Nucl. Sin. **22**: 880-886, 2008. [In Chinese]

Sun G.C., Zeng X.P., Zhao P. *et al.*: [Photosynthesis and free radical yield of *Litchi chinensis* leaves under increased CO_2 partial pressure in atmosphere.] – Chin. J. Appl. Ecol. **14**: 331-335, 2003. [In Chinese]

Sun S., Zhang L.T., Wang J.X. *et al.*: [Effects of low temperature and weak light on the functions of photosystem in *Prunus armeniaca* L. leaves in solar greenhouse.] – Chin. J. Appl. Ecol. **19**: 512-516, 2008. [In Chinese]

Tanaka R., Tanaka A.: Effects of chlorophyllide *a* oxygenase overexpression on light acclimation in *Arabidopsis thaliana*. – Photosynth. Res. **85**: 327-340, 2005.

Thompson W.A., Huang L.K., Kriedemann P.E.: Photosynthetic response to light and nutrients in sun-tolerant and shade-tolerant rainforest trees. II. Leaf gas exchange and component processes of photosynthesis. – J. Plant Physiol. **19**: 19-42, 1992.

Tinoco-Ojanguren C., Pearcy, R.W.: Dynamic stomatal behavior and its role in carbon gain during lightflecks of a gap phase and an understory *Piper* species acclimated to high and low light. – Oecologia **92**: 222-228, 1992.

Trotta A., Rahikainen M., Konert G. *et al.*: Signaling crosstalk in light stress and immune reactions in plants. – Philos. T. R. Soc. B **369**: doi: 10.1098/rstb.2013.0235, 2014.

Vieira S., Calado R., Coelho H. *et al.*: Effects of light exposure on the retention of kleptoplastic photosynthetic activity in the sacoglossan mollusc *Elysia viridis*. – Mar. Biol. **156**: 1007-1020, 2009.

Wellburn A.R.: The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids using various solvents with spectrophotometers of different resolutions. – Plant Physiol. **144**: 307-313, 1994.

Yamazaki J.Y.: Is light quality involved in the regulation of the photosynthetic apparatus in attached rice leaves? – Photosynth. Res. **105**: 63-71, 2010.

Yang G.D., Zhu Z.J, Ji Y.M.: Effect of light intensity and magnesium deficiency on chlorophyll fluorescence and active oxygen in cucumber leaves. – J. Plant Nutr. Fertil. Sci. **8**: 115-118, 2002.

Yin C.Y., Berninger F., Li C.Y.: Photosynthetic responses of *Populus przewalski* subjected to drought stress. – Photosynthetica **44**: 62-68, 2006.

Yu Q., Zhang Y.Q., Liu Y.F. *et al.*: Simulation of the stomatal conductance of winter wheat in response to light, temperature and CO_2 changes. – Ann. Bot-London **93**: 435-441, 2004.

Zhang S., Gao R.: Diurnal changes of gas exchange, chlorophyll fluorescence, and stomatal aperture of hybrid poplar clones subjected to midday light stress. – Photosynthetica **37**: 559-571, 1999.

Zhao W.J., Xu C.C., Zou Q. *et al.*: [Improvements of method for measurement of malondialdehyde in plant tissues.] – Plant Physiol. Commun. **30**: 207-210, 1991. [In Chinese]

Zhou Y.H., Yu J.Q., Huang L.F. *et al.*: The relationship between CO_2 assimilation, photosynthetic electron transport and water-water cycle in chill-exposed cucumber leaves under low light and subsequent recovery. – Plant Cell Environ. **27**: 1503-1514, 2004.

Zhou Y.H., Yu J.Q., Qian Q.Q. *et al.*: [Effects of chilling and low light on cucumber seedlings growth and their antioxidative enzyme activities.] – Chin. J. Appl. Ecol. **14**: 921-924, 2003. [In Chinese]