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Photosynthetic responses of *Chrysanthemum morifolium* to growth irradiance: morphology, anatomy and chloroplast ultrastructure

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Abstract

Seedlings of *Chrysanthemum,* cultivar 'Puma Sunny', were grown under a range of shading regimes (natural full sunlight, 55, 25, and 15% of full sunlight) for 18 days. Here, we characterized effects of varying light regimes on plant morphology, photosynthesis, chlorophyll fluorescence, anatomical traits, and chloroplast ultrastructure. We showed that leaf color was yellowish-green under full sunlight. Leaf area, internode length, and petiole length of plants were the largest under 15% irradiance. Net photosynthetic rate, water-use efficiency, PSII quantum efficiency, and starch grain were reduced with decreasing irradiance from 100 to 15%. Heavy shading resulted in the partial closure of PSII reaction centers and the CO_2 assimilation was restricted. The results showed the leaves of plants were thinner under 25 and 15% irradiance with loose palisade tissue and irregularly arranged spongy mesophyll cells, while the plants grown under full sunlight showed the most compact leaf palisade parenchyma. Irradiance lesser than 25% of full sunlight reduced carbon assimilation and led to limited plant growth. Approximately 55% irradiance was suggested to be the optimal for *Chrysanthemum morifolium*.

Additional key words: chlorophyll; dry mass; photochemical efficiency; photosystem; gas exchange.

Introduction

Plants respond to shading by either acclimation or by avoidance. The process of acclimation is typically achieved by increasing the leaf area (LA) and/or reducing the ratio of chlorophyll (Chl) *a* to Chl *b* (Evans and Poorter 2001). Avoidance involves a repositioning of leaves (Ballare 1999). Low light intensity inhibits plant growth and productivity by depressing gas exchange (Zavala and Ravetta 2001, Gregoriou *et al.* 2007) and induces an increase in LA. Low irradiance can also induce a shift in the allocation of dry matter towards the extension of leaf surface area (Cavagnaro and Trione 2007). The photochemical quenching coefficient (q_P), effective quantum yield of PSII photochemistry (Φ_{PSII}), and the electron transport rate (ETR) are all down-graded in *Capsicum* seedlings exposed to low intensity light (Mu *et al*. 2007). Both q_P and ETR are reduced in heavily shaded *Tetrastigma hemsleyanum* plants (Dai *et al*. 2009). Excessive light intensity can also inhibit CO_2 assimilation (Guidi *et al*. 2000, Dai *et al*. 2009). In wheat, a number of indicators of photosynthetic efficiency are at suboptimal levels under full sunlight (Chen *et al*. 2011). In tomato, nonphotochemical quenching (NPQ) is enhanced by excessive light intensity, with some of the light energy being probably dissipated in the form of heat (Han *et al*. 2010).

Light is the environmental variable, which most influences phenotypes, and a process of acclimation to different light levels is highly correlated with changes in

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Abbreviations: C_i, – intercellular CO_2 concentration; Chl – chlorophyll; g_s – stomatal conductance; DAT – days of treatment; DM – dry mass; *E* – transpiration rate; ETR – electron transport rate; F_m – maximal fluorescence yield of the light-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximal quantum yield of PSII photochemistry; LA – leaf area; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; Φ_{PSII} – effective quantum yield of PSII photochemistry; q_P – photochemical quenching coefficient; SLA – specific leaf area; WUE – water-use efficiency.
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leaf mass per unit of area (Élder *et al*. 2003). Leaves exposed to high light intensities, generally present an increase in a number of cell layers in palisade parenchyma, and consequently in mesophyll thickness (Buisson and Lee 1993). This was confirmed by Élder *et al*. (2003), in *Tradescantia pallida*, where leaves of plants kept under shaded conditions were thinner and with reduced specific leaf mass in comparison to those kept under sunny conditions.

Chrysanthemum (*Chrysanthemum morifolium*) is an ornamental species native to China. Like many ornamentals, exposure to sub- or superoptimal light intensity reduces the quality of plants. Defining the optimum light regime for its growth and flowering is therefore a commercially important goal. Here, we described a set of experiments in which chrysanthemum plants were exposed to a range of incident light intensities. The aim of this work was to study the shade acclimation and adaptation for high light, then determine the optimum light intensity. The effects of four light illumination levels were evaluated on morphology (leaf color and specific leaf area, SLA), physiological parameters (photosynthesis and chlorophyll fluorescence), anatomy (longitudinal section), and microstructure (mesophyll cell).

Materials and methods

Plant growth and shading treatment: The chrysanthemum (*Chrysanthemum morifolium*) cultivar 'Puma Sunny' was obtained from the *Chrysanthemum Germplasm Resource Preserving Centre*, Nanjing Agricultural University. Uniform cuttings were taken from mother plants and propagated in plug filled with a 1:1 mixture of peat and perlite. About 15 d later, the rooted seedlings were transplanted into 4-L pots containing a 2:1:1 mixture of garden soil, vermiculite, and perlite with no added fertilizer, and then they were randomly assigned to four irradiance levels. These levels were: full sunlight (excess light), 55% of incident light (optimal), 25% of incident light (suboptimal light), 15% of incident light (severely suboptimal light), respectively. Shading was performed in net-house (2.5 m high, 4 m long, and 4 m wide) covered with various thicknesses of commercial black shading nets. Plants were subjected to different irradiances for 18 d, starting on 9 June 2012. Each treatment involved 60 pots. Diurnal variations of PPFD (wavelengths of 400–700 nm) under four light conditions were frequently monitored with external quantum sensor connected to photosynthesis measuring system (*Li-6400XT*, *Li-Cor*, Lincoln, NE, USA), and displayed in Fig. 1. Average canopy temperatures under three types of shading and full-sun treatment were always within <1.0°C of each other, and no consistent differences in temperature were detected between treatments. The average minimum/maximum temperature, photoperiod (day/night). and air humidity were 30/21°C, 14/10 h, and 70%, respectively. All plants received natural rainfall and additional water as needed. Leaf discs (0.76 cm in diameter) were collected between 10:30 and 12:00 h after 0, 4, and 18 d of the treatment (DAT), snap-frozen in liquid N_2, and stored at –80°C until required.

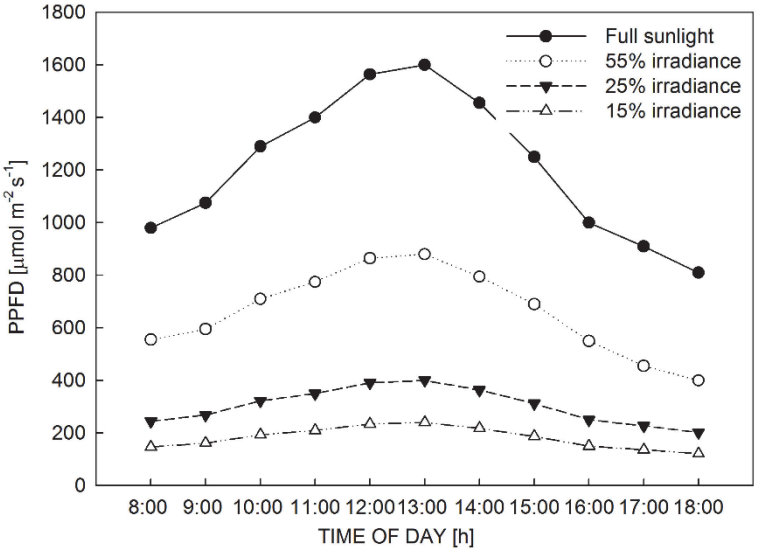

Fig. 1. Curves of diurnal variation of photosynthetic photon flux density (PPFD) under full sunlight, 55%, 25%, and 15% irradiance (wavelengths of 400–700 nm).

Petiole length, internode length and SLA: The first most fully expanded leaves from the apex of the shoot were obtained from each plant. The petiole length and internode was measured by microcalliper, then the LA was measured with *Li-3100C* area meter (*Li-Cor*, Lincoln, NE, USA), and the leaves were dried at 80°C for 48 h and weighed. SLA was calculated as LA/dry mass (DM).

Pigments: Total chlorophyll (Chl), Chl *a* and Chl *b*, and Chl *a/b* were determined spectrophotometrically in 80% acetone from three replicates per treatment using *Ultrospec 3300 pro* (*Ultrospec*, USA), following the method of Lichtenthaler (1987). Each replicate consisted of four leaf discs all sampled from a single leaf.

Photosynthetic parameters and Chl fluorescence: The net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) were measured using a *Li-6400* portable photosynthesis system (*Li-Cor*, Lincoln, NE, USA, soft version: *OPEN6.1.4.*, light source: *6400-02B*). Water-use efficiency (WUE) was calculated as P_N/E. The data were recorded between 10:30 and 12:00 h. During measurements, leaf temperature, external CO_2 concentration, and PPFD were maintained at 35 ± 0.5°C, 385 ± 10 μmol(CO_2) mol^{-1}, and 1,000 μmol m^{-2} s^{-1}, respectively. For the Chl fluorescence assay, maximum efficiency of PSII photochemistry (F_v/F_m) was measured at dawn, and the same leaves were later exposed to sun light, inserted into the measurement chamber, and illuminated with actinic light (PPFD of 1,000 μmol m^{-2} s^{-1}). Steady-state fluorescence yield (F_s), minimal fluorescence yield of the light-adapted state (F_0'), and maximal fluorescence yield of the light-adapted state (F_m') values in the light-adapted state were determined. The q_P, was calculated as $(F_m'–F_s)/(F_m'–F_0')$, the thermal energy dissipation as $F_m/(F_m' – 1)$ (Bilger and Björkman 1990), the Φ_{PSII} as $(F_m' – F_s)/F_m'$, the F_v'/F_m' as $(F_m' – F_0')/F_m'$ (Genty *et al*. 1989), and the ETR = PPFD × Φ_{PSII} × 0.84 × 0.5 (Krall and Edwards 1992). There were three repetitions per treatment (one leaf per repetition).

Microscopy: For the observation of leaf anatomy, 0.5 × 0.5 cm segments were fixed in formalin:alcohol:glacial acetic acid (90:5:5, v/v/v) for at least 24 h when cut from plants. The material was then dehydrated through a graded series of ethanol (from 30 to 100%, 30 min each step), cleared in xylene, and embedded in paraffin wax. Sections, 8 μm thick, were obtained using a rotary microtome (*RM2016*, *Laica*, Germany), and let dry on slides overnight at 37°C. After removing wax in xylene, the tissue sections were hydrated through an ethanol series and stained in 0.1% toluidine blue for 20 min, mounted on an *OLYMPUS BX53* microscope (*Olympus*, Japan). For observation of mesophyll cells, the sampled leaves were immediately fixed in 2.5% (v/v) glutaraldehyde (0.1 mol L^{-1} phosphate buffer, pH 7.2) for at least 48 h, then the samples were immersed in 1% (v/v) osmium acid, embedded in resin, and ultrasectioned for inspection by transmission electron microscopy (*H7650*, *Hitachi*, Tokyo, Japan).

Statistical analysis: Results were expressed as mean ± standard error. Statistical analysis was conducted by the one-way analysis of variance (*ANOVA*) using *SPSS v.17.0* (*SPSS Inc*., Chicago, IL, USA) software, and *Duncan*'s multiple range tests was employed to detect differences between means (with P set at 0.05).

Results

Plant growth and leaf morphology: The level of irradiance significantly affected plant growth and leaf morphology. On the fourth DAT, differences between full sunlight and shade treatments were not obvious in leaf morphology and root growth (Fig. 2*C*,*D*). By 18 DAT, the leaves of shaded plants became dark-green colored, compared to the yellowish-green leaves of the plants grown in full sunlight (Fig. 2*E*). Shading to 25% and 15% irradiance caused an increase in internode length, petiole length, and LA. SLA increased with the degree of shading, particularly at 15% irradiance (201% compared with full sunlight treatment) (Table 1).

Chl content: Total Chl, Chl *a*, as well as the Chl *b*, fell in plants exposed to full sunlight at both 4 and 18 DAT (Fig. 3*A–C*). In the most heavily shaded plants, the content of Chl, Chl *a*, Chl *b*, and the Chl *a/b* were unaffected at 4 DAT, but by 18 DAT, the Chl *a* content in particular decreased by 67% (Fig. 3*B*).

Photosynthesis: Both P_N and WUE under 55% irradiance were the highest at 18 DAT, while they were all the lowest under 15% irradiance (Fig. 4*A*,*E*). The g_s values under full sunlight and 15% irradiance were always lower than those of 25% and 55% irradiance-treated plants (Fig. 4*B*). The hightest g_s and C_i were observed in the plants under 25% and 15% irradiance, respectively, at 18 DAT (Fig. 4*B*,*C*). E varied significantly with variations in light intensity, the value at 15% irradiance was the lowest one (Fig. 4*D*).

Chl fluorescence: By 18 DAT, the F_v/F_m, Φ_{PSII}, and ETR of plants exposed to full sunlight were all lower than their equivalents grown under the 55% irradiance treatment (Fig. 5*A*,*D*,*E*). NPQ of the plants grown under full sunlight kept increasing and was higher than that in the shaded plants (Fig. 5*C*). The q_P, Φ_{PSII}, and ETR of the plants decreased with decreasing irradiance from 55% to 15% (Fig. 5*B*,*D*,*E*).

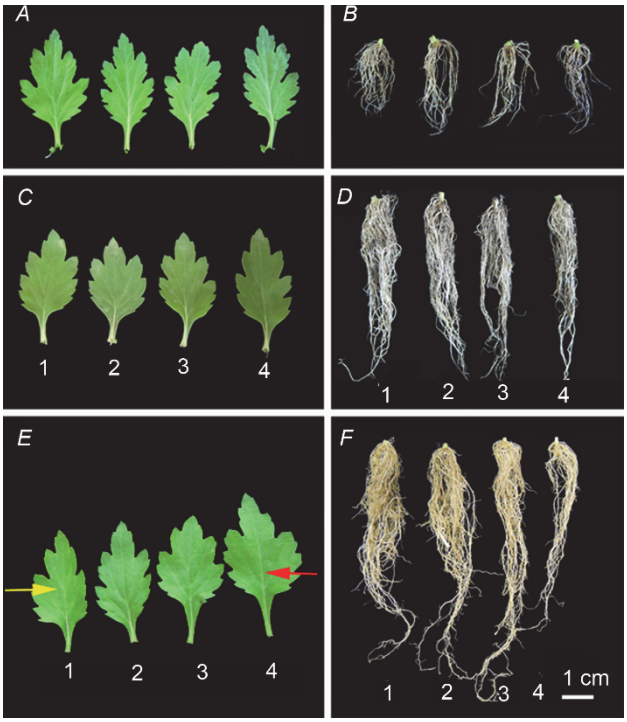

Fig. 2. The appearance of leaves and roots of chrysanthemum plants exposed to full sunlight (1), 55% (2), 25% (3), and 15% irradiance (4) at zero (*A*, *B*), four (*C*, *D*) and 18 days (*E*, *F*). Etiolation of the leaf and enlargement of the leaf surface are marked by, respectively, a yellow and a red arrow. Scale bar (*A*–*F*) 1 cm.

Leaf anatomy: Irradiance had a significant effect on leaf structure. The palisade cell of the plants exposed to full sunlight ranked more neatly and compact than that under the shading treatments (Fig. 6*F*–*I*). The palisade cells of plants exposed to 25% and 15% irradiance were irregular and the number of spongy cells was smaller than that of the plants exposed to full sunlight and 55% irradiance. The spongy cell spaces of leaves under 25% and 15% irradiance were also larger than those under full sunlight and 55% irradiance (Fig. 6*D*,*E*,*H*,*I*).

Mesophyll cell structure: Both chloroplast size and shape were influenced by the level of incident light. The number of chloroplasts and starch grains within each mesophyll cell were both lowered by low light conditions (Fig. 7). Abundant starch grain emerged in the chloroplasts of the plants exposed to full sunlight (Fig. 7*A*). The chloroplasts of mesophyll cell kept under 15% irradiance became elongated and the cell possessed thinner cell walls (Fig. 7*D*).

Discussion

Shading affects a series of plant characteristics, such as height, internode length, leaf size, color, shape, and overall development (Quero *et al*. 2006, Cavagnaro and Trione 2007, Dai *et al*. 2009, Craven *et al*. 2010, Deng *et al*. 2012a). SLA varies as a consequence of differences in cell mass and cell wall thickness. These characteristic develops later in the plant's life as a product of the plant's ontogeny and its growth environment (Castro-Díez *et al*. 2000).

Table 1. Internode and petiole length and LA of chrysanthemum plants exposed to full sunlight, 55, 25, and 15% irradiance at zero, 4, and 18 days. Data are given as means ± SE (n = 5). Within a column, values marked by a *different letter* differ significantly from one another (P<0.05).

Treatment [d]	Irradiance [%]	Internode length [mm]	Petiole length [mm]	LA [cm^2]	SLA [cm^2 g^{-1}]
0	Full sunlight	8.0 ± 0.53^a	5.7 ± 0.71^a	5.7 ± 0.71^a	194.9 ± 20.4^a
	55%	8.4 ± 0.55^a	5.5 ± 0.66^a	5.5 ± 0.66^a	192.9 ± 16.3^a
	25%	8.3 ± 0.35^a	6.1 ± 0.59^a	6.1 ± 0.59^a	194.1 ± 19.4^a
	15%	8.6 ± 0.43^a	5.9 ± 0.44^a	5.9 ± 0.44^a	196.0 ± 21.0^a
4	Full sunlight	10.2 ± 0.87^a	6.0 ± 0.55^a	6.0 ± 0.55^a	151.2 ± 17.8^c
	55%	10.8 ± 0.62^a	5.6 ± 0.68^a	5.6 ± 0.68^a	194.9 ± 18.2^b
	25%	10.6 ± 1.60^a	6.4 ± 0.91^a	6.4 ± 0.91^a	197.3 ± 17.6^b
	15%	11.2 ± 1.94^a	7.0 ± 0.85^a	7.0 ± 0.85^a	259.9 ± 13.5^a
18	Full sunlight	11.3 ± 0.66^b	6.1 ± 0.78^b	6.1 ± 0.78^b	142.5 ± 19.6^c
	55%	11.6 ± 0.91^b	6.3 ± 0.87^b	6.3 ± 0.87^b	185.2 ± 20.6^b
	25%	13.5 ± 1.00^b	6.7 ± 0.69^b	6.7 ± 0.69^b	195.0 ± 20.7^b
	15%	17.6 ± 1.78^a	7.9 ± 0.94^a	7.9 ± 0.94^a	296.6 ± 14.1^a

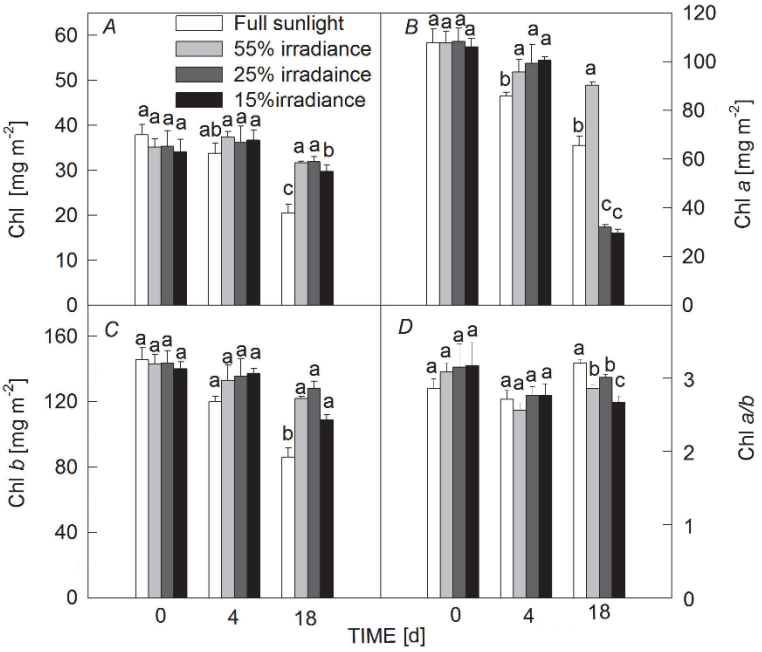

Fig. 3. Leaf chlorophyll (Chl, *A*), chlorophyll *a* (Chl *a, B*), chlorophyll *b* (Chl *b, C*) content, and a ratio of chlorophyll *a/b* (Chl *a/b*, *D*) of chrysanthemum seedlings exposed to 100%, 55%, 25%, and 15% sunlight for zero, four, and 18 days. Bars represent means ± SE (n = 5). Means differing significantly from one another (P<0.05) within a given shading treatment are indicated by different lower case letters.

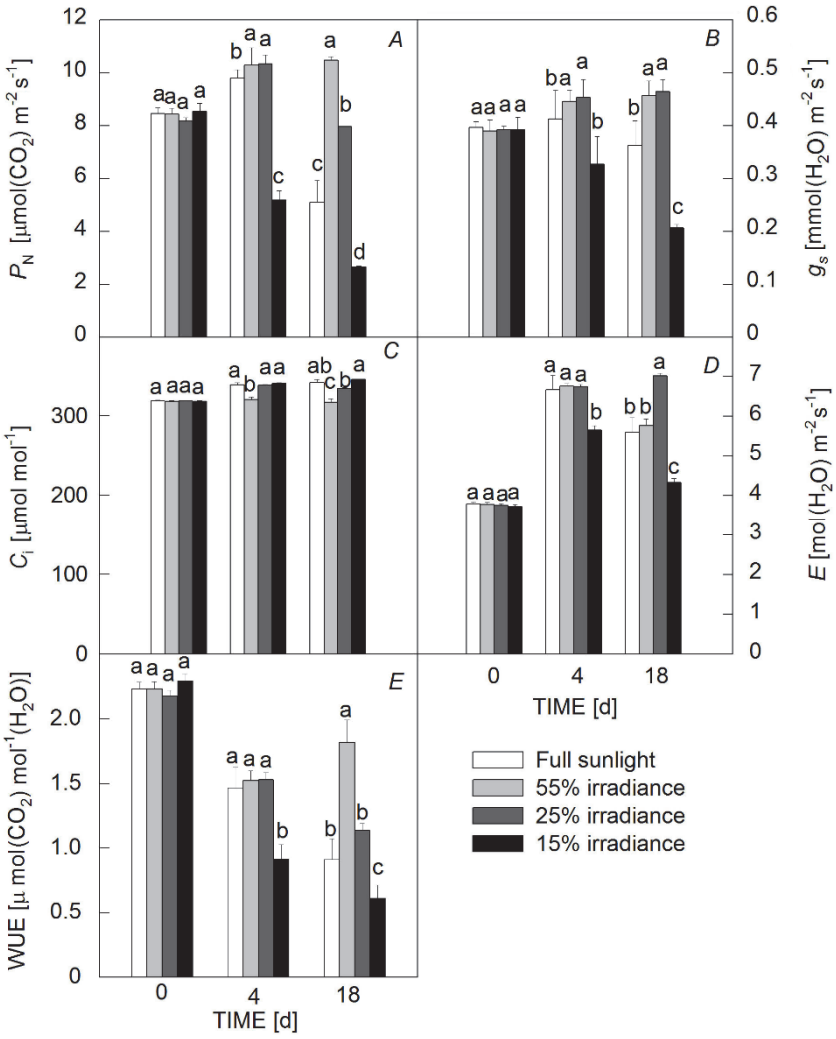

Fig. 4. Net photosynthetic rate (P_N, *A*), stomatal conductance (g_s, *B*), intercellular CO_2 concentration (C_i, *C*), transpiration rate (*E*, *D*), and water-use efficiency (WUE, *E*) in the leaves of chrysanthemum plants exposed to full sunlight, 55%, 25%, and 15% irradiance for zero, four, and 18 days. Bars represent means ± SE (n = 3).Values marked by a different letter indicate that the full sunlight and the shading treatments differ significantly from each other (P<0.05).

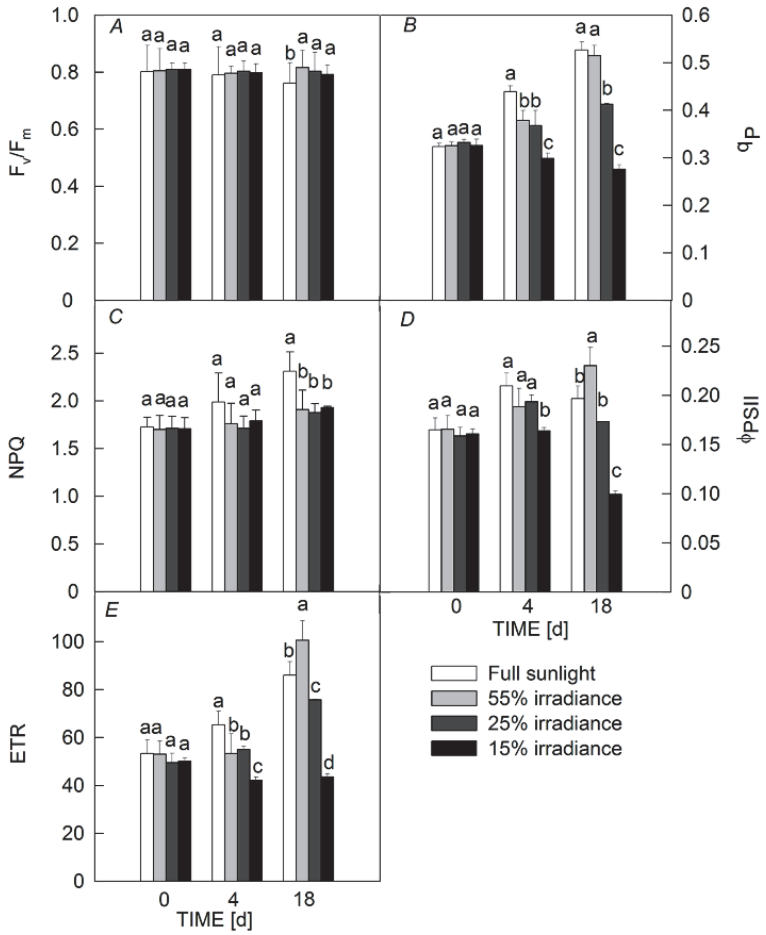

Fig. 5. Maximum efficiency of PSII photochemistry (F_v/F_m, *A*), photochemical quenching coefficient (q_P, *B*), nonphotochemical quenching (NPQ, *C*), effective quantum yield of PSII photochemistry (Φ_{PSII}, *D*), and electron transport rate (ETR, *E*) in the leaves of chrysanthemum exposed to full sunlight, 55%, 25%, and 15% irradiance for zero, four, and 18 d. Bars represent the mean ± SE (n = 3). Values marked by a different letters indicate that the full sunlight and the shading treatments differ significantly from each other (P<0.05).

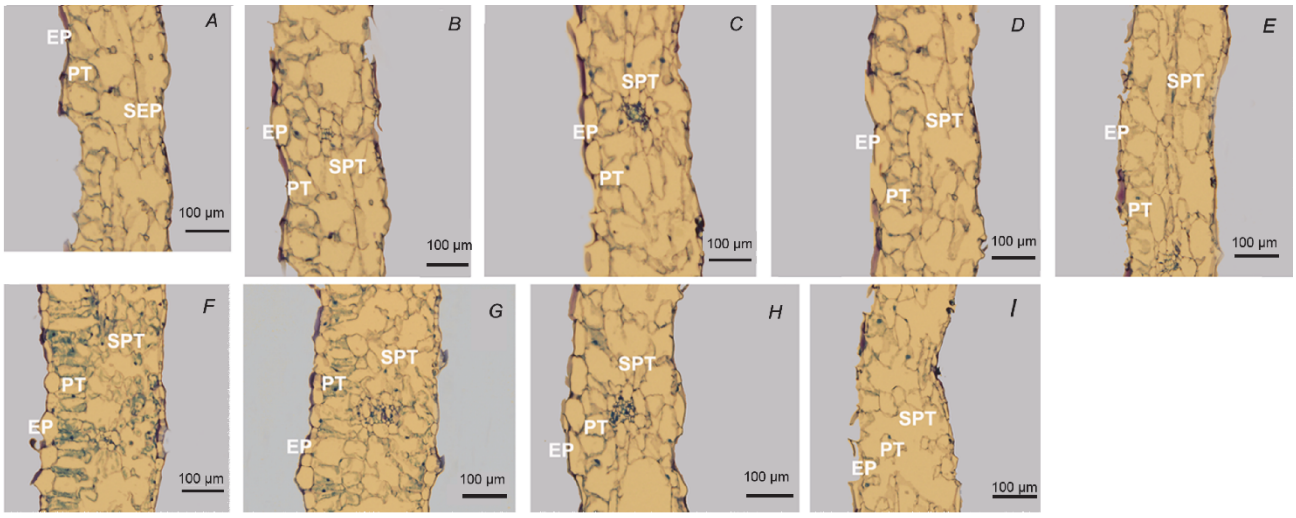

Fig. 6. The longitudinal section of leaves of chrysanthemum exposed to full sunlight (*A*, *B*, *F*), 55% (*C*, *G*), 25% (*D*, *H*), and 15% irradiance (*E*, *I*) for zero (*A*), four (*B–E*), and 18 days (*F–I*). EP: epidermal; PT: palisade tissue; SPT: spongy tissue. (Bars = 100 µm)

With respect to chrysanthemum, shading avoidance responses have not been reported before. Of our interest was to explore how the plant behaved under different shading regimes. Shading to 25% and 15% irradiance caused increase in LA (Table 1); it was consistent with shading to 50% and 20% irradiance that induced enlargement of LA in jasmine (Deng *et al*. 2012b), and that of 33% and 25% irradiance that induced increase in LA of *Tetrastigma hemsleyanum* Diels et Gilg (Dai *et al*. 2009). Previous studies have shown that shade-adapted species,

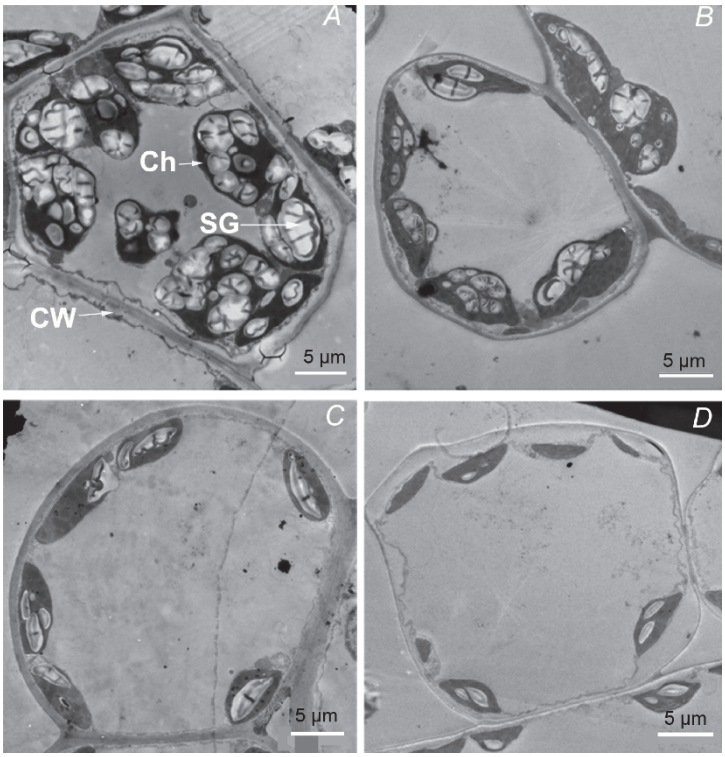

Fig. 7. The chloroplast ultrastructure observed in leaves of chrysanthemum exposed to full sunlight (*A*), 55% (*B*), 25% (*C*), and 15% irradiance (*D*) after 18 d. *Abbreviations*: Ch – chloroplast; CW – cell wall; SG – starch grain. (Bars = 5 μm)

Dieffenbachia longispatha, displays a modest capacity for photosynthetic acclimation to increase LA (Skillman *et al*. 2005). Low irradiance can induce a shift in the allocation of dry matter towards the extension of leaf surface area (Cavagnaro and Trione 2007); the same conclusion was obtained in our experiment, where SLA of the shaded plants was larger than that under full sun (Table 1).

With respect to leaf color, the leaves produced by the plants grown under full sunlight were yellowish-green (Fig. 2*E*), which was consistent with results obtained for *Fagus crenata* (Naramoto *et al*. 2006), tropical pioneer species (Favaretto *et al*. 2011), *Tetrastigma hemsleyanum* Diels et Gilg (Dai *et al*. 2009), and jasmine (Deng *et al*. 2012b). The results were consistent with the conclusion that the etiolation emergence under full sunlight was photoinhibition-induced by excessive irradiance (Osmond 1994).

Chl content is a critical determinant for the rate of photosynthesis (Garty *et al*. 2001). Here, the leaves of plants grown under full sunlight showed less Chl than those grown under 55% irradiance, showing that full sunlight intensity impaired the photosynthetic apparatus. The higher Chl content was observed in the 25% and 55% irradiance-treated plants and it might at least partially explain the higher photosynthetic rate in leaves of these plants, which is consistent with the previous reports (Dai *et al*. 2009, Deng *et al*. 2012a). The Chl content in the leaves of plants grown under 15% irradiance decreased significantly (Fig. 3*A*), which implies that the reduced Chl content was probably associated with increase in SLA. The Chl *a/b* ratio is one of the most important indexes and reflects the ability of adaptation to low light (Wherley *et al*. 2005). Shading decreased the Chl *a/b* ratio in our experiment (Fig. 3*D*), in pioneer and late-successional tropical tree species (Favaretto *et al*. 2011), and in jasmine (Deng *et al*. 2012a). The Chl *a/b* ratio in shaded leaves was reduced, which occurred mainly due to a significant decrease in the Chl *a* content, caused most likely by changes in the organization of light-harvesting complexes (Schiefthaler *et al*. 1997). The decreased Chl *a/b* ratio was suitable for plants grown under low light. Low light of 25% irradiance did not restrict growth, but full sunlight and 15% irradiance affected growth negatively by 18 DAT.

The main biological significance of photosynthesis is to fix carbon and water in order to synthesize carbohydrates and produce oxygen. Nonoptimal light intensity is well known to reduce plant growth (Gregoriou *et al*. 2007, Chen *et al*. 2011). The highest P_N recorded here was found in the plants exposed to 55% irradiance (Fig. 4*A*). The markedly reduced CO_2-assimilation rate that was recorded for the plants exposed to both full sunlight and 15% irradiance, which occurred due to nonstomatal limitations as the intercellular CO_2 concentration in the leaves was not lower than that of 55% irradiance-treated plants (Fig. 4*C*). At the same time, the value of g_s decreased significantly in the plants grown under full sunlight (Fig. 4*B*). Under high light, the reduced g_s and E implied the plants closed stomata due to light saturation and in order to decrease water loss (Dai *et al*. 2009), but WUE value was lower than that under the 55% irradiance treatment. This suggested that stomata closure at high irradiance reduced other physiological indexes more than transpiration.

Plants subjected to high-irradiance stress, typically show lower F_v/F_m values than that of nonstressed plants (Li *et al*. 2010, Fu *et al*. 2012). F_v/F_m of leaves in the plants grown under full sunlight fell below 0.8, a sign that photoinhibition occurred (Valladares *et al*. 2002, Naramoto *et al.* 2006). In this experiment, the plants grown under full sunlight exhibited lower F_v/F_m than those shaded ones (Fig. 5*A*). The F_v/F_m value changed slightly under 55%, 25%, and 15% irradiance, probably because that the photosynthetic reaction center works well in a certain range of light intensity. Our results confirmed reports in jasmine (Deng *et al*. 2012b) and lettuce (Fu *et al*. 2012). Light energy absorbed by Chl molecules in a leaf can have different fate: it can be used to drive photosynthesis (photochemistry), excess energy can be dissipated as heat, or it can be re-emitted as Chl fluorescence, and all three processes occur in competition (Maxwell and Johnson 2000, Fu *et al*. 2012). The q_P, an indicator of the proportion of PSII reaction centers, which are open (Maxwell and Johnson 2000), can be reduced by heavy shading (Mu *et al*. 2007, Dai *et al.* 2009). The same observations was made in the present experiments (Fig. 4*B*). This shows that a significant difference in the electron transport rate in PSII can occur when plants are grown under various shade treatments. The NPQ represents the energy which cannot be utilized for transport of photosynthetic electrons and is dissipated harmlessly as heat energy (Müller *et al*. 2001, Veres *et al*. 2006). The higher NPQ in the full sunlight-treated plants showed that the energy absorbed was much higher than that needed for photochemical utilization, which might cause photoinhibition (Fig. 5*C*). Φ_{PSII} can be downregulated in two ways: first, thermal dissipation of excitation energy, secondly, closure of the PSII reaction centers (Genty *et al*. 1990). In the present study, Φ_{PSII} in leaves under full sunlight, 15% and 25% irradiance was lower than that under 55% irradiance (Fig. 5*D*). So, it appears that the reduction in Φ_{PSII} caused by exposure to full sunlight was due to the former pathway, while the reduction induced by shading was due to the latter.

Leaves exposed to high light intensities generally present an increase in the number of cell layers in palisade parenchyma, and consequently in mesophyll thickness (Kubínova 1991), which was confirmed by Élder *et al*. (2003). In our experiment, the number of cell layers in palisade parenchyma under full sunlight was not higher than that under shading conditions (Fig. 6), but plants exposed to full sunlight exhibited more compact and longer palisade parenchyma. These results are related with those found by Dimassi-Theriou and Bosabalidis (1997) in kiwifruit, where parenchyma thickness increased when leaves were subjected to high light. The reduction in palisade parenchyma length by shading (Fig. 6*H*,*I*) resulted in better light penetration to the chloroplasts (Evans 1999). Such a thickness reduction and the increasing individual LA could be a leaf adaptation mechanism to low light conditions in order to enhance light capture.

An increased accumulation of photoassimilate can lead to fall in the rate of CO_2 assimilation (Goldschmidt *et al*. 1992). The content of starch was higher under full sunlight than that in the shaded leaves (Fig. 7), as it has been similarly observed in sunflower (Maria *et al.* 2006). Han and Chen (2008) showed that high amount of starch in the leaf can affect the structure and function of the chloroplasts, thus, the more abundant starch grain in chloroplast of leaves under full sunlight might act as an important inducing factor for photoinhibition.

Conclusion: Full sunlight proved not to be optimal, contrary to 55% irradiance, where plants were morphologically superior and showed a higher photosynthetic capacity. Photoinhibition was associated with the excessive accumulation of starch grains. Plants responded to low light by their acclimation and modulated their development by enlarging LA, closing PSII reaction centres, and reducing palisade parenchyma length.

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