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Effect of partial shading on the photosynthetic apparatus and photosystem stoichiometry in sunflower leaves

J. YAMAZAKI+ and Y. SHINOMIYA

Department of Biology, Faculty of Science, Toho University, Miyama 2-2-1, Funabashi, Chiba 274-8510, Japan

Abstract

The partial shading effect on the photosynthetic apparatus of the sunflower (*Helianthus annuus* L.) was examined by monitoring oxygen evolution, maximum quantum yield of PSII photochemistry in dark-adapted leaves (F_v/F_m), the chlorophyll (Chl) concentrations and the Rubisco contents, and leaf mass per area (LMA) at the leaf level and by determining the concentrations of cytochrome (Cyt) *f* and the reaction centres of photosystem (PS) I and PSII at the thylakoid level. In this experiment, partial shading was defined as the shading of 2nd leaves with shade cloths, and the whole treatment was defined as the covering of the whole individuals with shade cloths. In the leaf level responses, oxygen evolution, LMA, Chl concentrations and Rubisco contents decreased in all shade treatments administered for six days. F_v/F_m remained constant irrespective of the shade treatments. On the other hand, in the thylakoid-level responses, the concentrations of the thylakoid components per unit Chl and the stoichiometry of the two photosystems showed no statistical difference among the shade treatments. The data obtained from the present study indicate that the partial shading affected the leaf-level responses rather than the thylakoid-level responses. The light received at the lower leaves might serve as a factor in the regulation of the leaf properties of the upper leaves due to the whole plant photosynthesis, while this factor did not have an effect at the thylakoid level.

Additional key words: antenna size; leaf properties; partial shading; photosystem stoichiometry; sunflower.

Introduction

In upright herbaceous plants, since leaves that develop at the top of a canopy receive full sunlight, the lower leaves receive very weak light. Accordingly, the light intensity decreases exponentially from the uppermost to the bottom layer due to the mutual shading of leaves (Monsi and Saeki 1953, Yamazaki *et al*. 1999).

A previous report demonstrated that an excessive amount of the proteins related to CO_2 fixation is degraded rapidly (Yamazaki *et al*. 1999), whereas the proteins involved in the harvesting and utilization of light effectively remain in weak light (Yamazaki 2010a). In addition, a decline in the Chl *a*/*b* ratio indicates a more rapid degradation of the reaction centre complexes of the photosystems than of the light-harvesting Chl-protein complexes in PSII (LHCII) (Kura-Hotta *et al*. 1987, Leong and Anderson 1986). It is well known that the light intensity modulates not only the Chl *a*/*b* ratios but also contents of the thylakoid components and the PSII/PSI ratios (Leong and Anderson 1986, De la Torre and Burkey 1990, Melis and Harvey 1981, Burkey and Wells 1996, Yamazaki 2010a). Our previous reports on the modulation of the photosystem stoichiometry have proposed a compensatory system for adjusting the light absorption balance between the two photosystems depending on light intensity (Yamazaki *et al*. 1999, Yamazaki 2010a, b). Thus, the leaves exposed to weak light retain the capacity to drive photosynthesis maximally under light-limiting conditions. These reports

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+Corresponding author; phone & fax: +81-47-472-5330, e-mail: junya@bio.sci.toho-u.ac.jp
Abbreviations: C_{550} – electrochromic band shift of pheophytin in the PSII reaction centre complex; Chl – chlorophyll; Cyt – cytochrome; DCIP – 2,6-dichlorophenol indophenol; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_m – maximum fluorescence yield in the dark-adapted leaves; F_o – minimal Chl fluorescence yield in the dark adapted leaves; F_v – variable fluorescence yield in the dark-adapted leaves; F_v/F_m – maximum quantum yield of PSII photochemistry in dark-adapted leaves; LMA – leaf mass per area; P_{700} – the reaction center complex in photosystem I; PART – partial shading treatment; PPFD – photosynthetic photon flux density; PS – photosystem; Q_A – primary quinone acceptor of PSII; WHOLE – whole shading treatment.
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indicate that a plant has the plasticity to adjust its photosynthetic apparatus by optimizing its photosynthetic activity in response to the appearance of mutual shading leaves that are inevitably associated with plant growth.

As stated above, since lower leaves are shaded by developing upper leaves, their photosynthesis is limited due to the weak light conditions, leading to the degradation of photosynthesis-related proteins. Nitrogen released from the degradation proteins in the bottom-layer leaves (*i.e.*, source organ) was remobilized to the upper developing leaves (*i.e.*, sink organ) (Mae and Ohira 1981). There are several reports of experiments regarding the sink-source balance using whole-plant shading, sink removal model and single-rooted model plants (Mondal *et al*. 1978, Crafts-Brandner and Egli 1987, Pons and Pearcy 1994, Sawada *et al*. 2001). Pons and Pearcy (1994) examined a correlation between photosynthetic activities and the leaf nitrogen partition and revealed that the nitrogen exported from the leaf came more at the expense of compounds that make up the photosynthetic capacity than at the expense of those involved in photon absorption, resulting in a change in the partitioning of nitrogen within the photosynthetic apparatus. In addition, Hirose and Werger (1987) indicated that the photosynthetic activities were maximized when nitrogen was partitioned along a gradient from high to low light intensity rather than partitioned uniformly. Moreover, Yano and Terashima (2001) partially shaded plants and elucidated that the differentiation of new leaves was regulated by the light received at mature leaves, whereas that of chloroplasts was regulated by the light received at the apex.

The goal of the present study was to elucidate the effects of partial shading on the single-leaf-level and the thylakoid-level responses in sunflower leaves. For this purpose, we monitored oxygen evolution and F_v/F_m, determined the Chl and Rubisco contents and LMA at the leaf level, and performed spectrophotometric measurements to determine the thylakoid components and the relative antenna sizes of PSII at the thylakoid level. Using the results obtained, we considered whether partial shading regulates the leaf level or the thylakoid level acclimation.

Materials and methods

Plant materials and growth conditions: Sunflower seeds (*Helianthus annuus* L., Sakata Seed Co., Yokohama, Japan) were purchased from a local market and germinated on a moistened paper towel in a plastic tray at 25°C in darkness for 2 d. One germinated seed was planted in a pot (120 mm diameter, 150 mm height) containing vermiculite. Plants were grown in the open air under a transparent plastic cover on the campus of Toho University, Funabashi, Japan (35°41'N, 140°02'E; 20 m a.s.l.) for *ca.* four weeks under natural sunlight conditions [during summer at the campus, average irradiance cycle, *ca*. 9 h light/16 h dark; daily mean temperature, 23°C; average relative humidity, 70%; maximal PPFD, 1,800 μmol(photon) m^{-2} s^{-1}], watered daily, and fertilized with a nutrition solution (1:1,000 *HYPONeX® 5–10–5, HYPONeX Japan Corp*., Osaka, Japan) once a week. Leaves were numbered in the order of their development excluding the dicots, and the fully expanded 2nd and 3rd leaves were used for all analyses. The shade cloths did not change the light quality but decreased the photosynthetic photon flux density (PPFD) (Yamazaki 2010b). Light intensity was determined with a quantum sensor (*LI-190*; *Li-Cor*, Lincoln, NE, USA). In this experiment, when the 2nd leaves were fully expanded, the plants were divided into three equal groups, and each group was placed under a different light treatment as follows for four more days: no shade cloth (control – CONT; received at 100% full sunlight), five layers of shade cloth upon the 2nd leaves, and the 3rd leaves received full sunlight (partial shading – PART; 3rd leaves received 100% full sunlight and 2nd leaves received 4% relative to full sunlight), five layers of shade cloth covered the whole seedling (whole shading treatment – WHOLE; 4% relative to full sunlight) (Fig. 1).

Photosynthetic characteristics of leaves were measured with a gas-phase leaf-disk oxygen electrode (*LD2/3*; *Hansatech Co*., UK) at 25°C. Leaves were harvested between 10:00 and 11:00 h (local time) and immediately measured. The gas phase consisted of air containing 4% CO_2. A saturating actinic light [2,000 μmol(photon) m^{-2} s^{-1}] was provided by a 100-W halogen lamp. Leaf area was estimated using a custom-made program that involved inputting leaf shapes into a computer with an image scanner. After the photosynthesis measurement, Chl *a* fluorescence was measured with a *PAM* fluorometer (*MINI-PAM*; *Walz*, Effeltrich, Germany) at room temperature and under ambient CO_2 concentration after

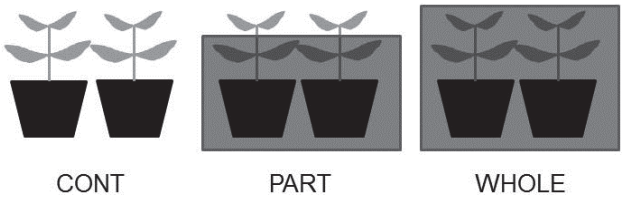

Fig. 1. Experimental design of partial shading effect. No shade cloth [control – CONT; received at 1,800 μmol(photon) m^{-2} s^{-1} defined as 100% full sunlight], five layers of shade cloth upon the 2nd leaves and the 3rd leaves received full sunlight [partial shading – PART; 3rd leaves received at 100% full sunlight and 2nd leaves at 4% relative to full sunlight; *i.e.*, *ca.* 40 μmol (photon) m^{-2} s^{-1}], five layers of shade cloth covered upon whole individuals (whole shading treatment – WHOLE; 4% relative to full sunlight).

the leaves were dark-adapted for at least 30 min. The fluorometer was connected to a computer with data acquisition software (*WinCont 1.60*, *Walz*, Effeltrich, Germany). The maximal quantum yield of PSII in the dark-adapted leaves was calculated as $F_v/F_m = (F_m - F_o)/F_m$. F_o was measured by switching on the modulated measuring light at 0.6 kHz. F_m was measured at 20 kHz with an 800 ms pulse of 8,000 μmol(photon) m^{-2} s^{-1} of halogen light. The standard Chl fluorescence nomenclature was followed as described by van Kooten and Snel (1990).

Leaf properties: For the determination of dry mass and leaf mass per area (LMA), leaves were dried at 80°C for 48 h. For Chl and Rubisco determination, leaf segments were homogenized in cold buffer containing 100 mM phosphate buffer (pH 7.0), 10 mM NaCl, 10 mM $MgCl_2$, 5 mM iodoacetic acid, 1 mM phenylmethylsulfonyl fluoride, 1% (w/v) soluble polyvinyl pyrrolidone, and 20 mM sodium ascorbate. A portion (0.2 ml) of the homogenates was used for Chl determination. Chl was extracted with 80% (v/v) aqueous acetone and quantified spectrophotometrically (Porra *et al*. 1989). The rest of the homogenate was centrifuged at 35,000 × *g* for 30 min, and the supernatant was used for the determination of the large subunit of Rubisco. The supernatant was denatured with 2.5% (w/v) SDS, 8 M urea, and 5% (v/v) 2-mercaptoethanol for 2 h at room temperature, and then 5 μl of the sample solution were applied to 15% acrylamide gel. Electrophoresis was carried out according to the buffer system of Laemmli (1970). The gels were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 dissolved in 15% methanol and 7% acetic acid for 1 h and, after destaining, were then analysed with *ImageJ* software (NIH, http://rsb.info.nih.gov/ij/). A calibration curve for the determination of Rubisco contents was constructed from the BSA standard.

Thylakoid membrane preparation: The thylakoid membranes of sunflower leaves were isolated as described in Yamazaki *et al*. (1999). In brief, leaves were homogenized in a cold buffer that contained 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$, and 50 mM HEPES-NaOH (pH 7.5) for 30 s with a Waring blender. The homogenate was filtered through two layers of *Miracloth* (*Calbiochem*, USA), and the filtrate was centrifuged at 250 × *g* for 5 min. The supernatant was centrifuged at 5,500 × *g* for 15 min, and the precipitate was suspended in the medium just described.

Electron transport activities: The rates of evolution or consumption of oxygen at light-saturation were measured polarographically with a Clark-type oxygen electrode at 25°C in a basal medium that contained 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$, and 50 mM HEPES-NaOH (pH 7.5). PSII activity was measured by adding 1 mM phenyl-*p*-benzoquinone (P*p*BQ) to a basal reaction medium. PSI activity was determined in the presence of 10 μM DCMU, 500 μM DCIP, 1 mM methyl viologen, and 2 mM sodium ascorbate. All mixtures contained 20 mM HCl-methylamine as an uncoupler, and thylakoid membranes were adjusted to 11 μM Chl. Chl was determined by the method of Porra *et al*. (1989).

Spectrophotometrical measurements for determination of PSI and PSII reaction centre concentrations and antenna size heterogeneity: The concentrations of P_{700}, C_{550}, and Cyt *f* were measured with a spectrophotometer (*Model 556*, *Hitachi*, Tokyo, Japan) in accordance with Yamazaki *et al*. (1999). The two types of PSII reaction centres, PSIIα and PSIIβ, were estimated by analysing the growth of the area over the fluorescence induction curves in the presence of DCMU with a hand-made fluorometer as described in Yamazaki *et al*. (1999).

Statistical analyses: A one-way analysis of variance followed by mean separation using *Scheffe*'s test was performed using software (*KaleidaGraph ver. 4.1*, *Hulinks*, Tokyo, Japan).

Results

Effects of partial shading on the leaf properties: To examine whether the leaf properties were regulated by partial shading, some parameters were determined in leaves that had been kept under various shade treatments for 6 d.

The leaf oxygen evolution measured under saturating light (Fig. 2*B*) and the Rubisco contents (Fig. 3*H*) remained relatively constant under all light conditions in the 3rd leaves, whereas all treatments showed a drastic decrease in the 2nd leaves (Figs. 2*A*, 3*G*). Chl concentrations were preserved under CONT and the 3rd leaves in PART, while the shade treatment enhanced the degradation of Chl (Fig. 3*C*,*D*). On the other hand, there were no remarkable changes observed in the decline of F_v/F_m (Fig. 2*C*,*D*) or the Chl *a*/*b* ratios (Fig. 3*E*,*F*) in either the 2nd or 3rd leaves over 6 d irrespective of irradiance. LMA, which is an index of the leaf morphological properties related to light acclimation, was measured to determine whether the partial shading affected a single leaf. As shown in Fig. 3*A*,*B*, LMA under the received full sunlight condition (CONT and the 3rd leaves in PART) remained in both the 2nd and 3rd leaves for 6 d. In contrast, LMA decreased gradually under the shade treatment (the 2nd leaves in PART and WHOLE) (Fig. 3*A*,*B*). These results indicate that the partial shading affected the leaf level acclimation.

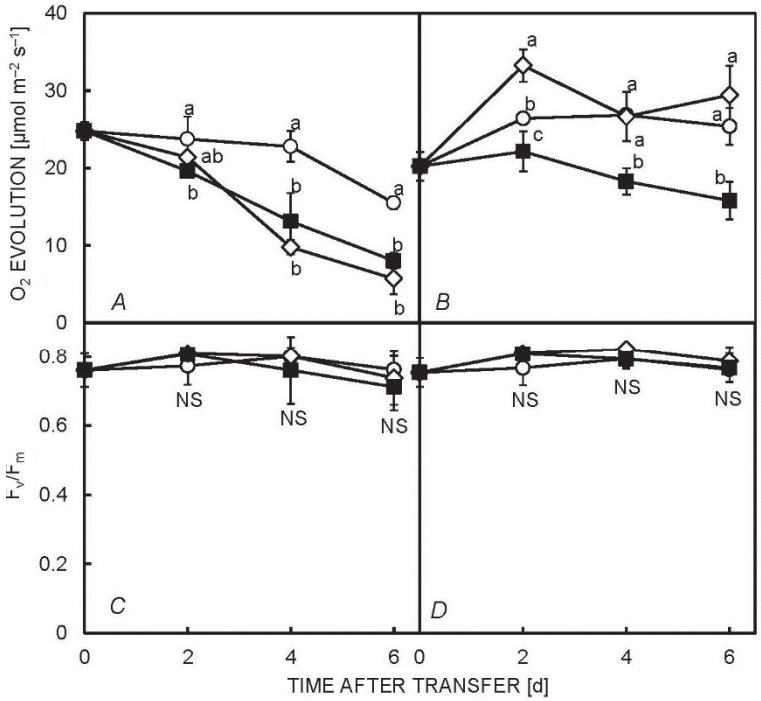

Fig. 2. Time courses of the photosynthetic properties in leaves of the 2nd and the 3rd leaves incubated for six days under different treatments. *A*,: the light-saturated photosynthesis, *C*,*D*: the maximal quantum yield of PSII photochemistry in the dark-adapted leaves (F_v/F_m). Left side (*A*,*C*) and right side (*B*,*D*) showed the 2nd and 3rd leaves, respectively. *Open circles*, *open diamonds* and *closed squares* represent the CONT, PART, and WHOLE treatments for 6 days, respectively. Means are shown and the bars indicate SD (n = 5). *Different letters* indicate significant differences between the treatments in the mean values (P<0.05) according to *Scheffe*'s multiple comparison tests. NS – not significant.

Effects of partial shading on the concentrations of thylakoid components: C_{550}, which is the electrochromic band shift of pheophytin in the PSII reaction centre complex, is known to be proportional to the amount of PSII reaction centre complex estimated from the Q_A photooxidation (McCauley and Melis 1986). Under the shade treatment, the ratio of mmol C_{550} to mol Chl in the 2nd and 3rd leaves gradually decreased from 3.0 and 3.3 on day 0 to 2.2 and 2.5 on day 4, respectively (Fig. 4*A*,*B*). Irrespective of the shade treatments, the concentrations of P_{700} determined on the basis of Chl in the 2nd leaves were approximately 2.0–2.4 mmol P_{700} per mol Chl, whereas that in the 3rd leaves decreased from 2.8 on day 0 to 2.4 on day 4 (Fig. 4*C*,*D*). The Cyt *f* concentrations per unit of Chl in the 2nd and 3rd leaves decreased from 2.4 and 2.7 on day 0 to 2.2 and 2.2 on day 4, respectively (Fig. 4*E*,*F*). It has been recognized that the degradation of Cyt *f* occurs in dark treatments or under natural shade conditions (Leong and Anderson 1984, Yamazaki *et al*. 1999a). The concentrations of Cyt *f* resembled those of PSII under all shade treatments. Thus, the results for sunflower leaves indicated that the behaviour of the concentrations of Cyt *f* resembled that one in rice leaves (Yamazaki 2010a).

Effects of partial shading on the electron transport activities: PSII activity determined from H_2O to P*p*BQ under different shading treatments remained constant both in the 2nd and 3rd leaves (P>0.05; Fig. 5*A*,*B*). Similarly, PSI activity determined from DCIP/Ascorbate to methylviologen showed no statistical difference in either the 2nd or 3rd leaves among the treatments (P>0.05; Fig. 5*C*,*D*).

Effects of partial shading on the PSII antenna heterogeneity: In terms of both function and antenna size, the PSII reaction centres are heterogeneous (Melis and Homann 1975, Melis 1991, Lazár 1999). One has a large antenna and it is functional in electron transport, and the other has a small antenna and it is unable to transfer electrons from Q_A^- to Q_B. The former is defined as PSIIα, and the latter as PSIIβ. Thus, the relative abundance of the PSII reaction centres can be kinetically distinguished as functional or nonfunctional in electron transport by measuring the rate constant of the photoreduction of Q_A through the growth of the area over the fluorescence induction curve. The relative abundance of PSIIα in the 2nd and 3rd leaves was, on average, 59% and 50%, respectively, of the total PSII centres on day 0 (Fig. 6*A*,*B*). The relative abundance of PSIIα centres remained constant in the 2nd leaves and decreased to 40% in the 3rd leaves irrespective of treatment over four days of incubation. In contrast, the relative amount of the PSIIβ centres remained constant in the 2nd leaves and increased to 60% in the 3rd leaves (Fig. 6*C*,*D*). Kα and Kβ are defined as the rate constants of the PSIIα and PSIIβ centres, respectively, and these rate constants are proportional to the size of each antenna (Melis and Homann 1975, Yamazaki *et al*. 1999). Kα increased slightly under PART and WHOLE treatments, while Kβ remained constant irrespective of the irradiance (Fig. 6*E*–H). These results obtained from the sunflower leaves indicated that the proportion of the PSIIα and PSIIβ centres and the antenna size of PSIIα were not regulated by irradiance.

The ratio of PSII to PSI reaction centres indicated approximately one in the shade treatment (Figs. 7*A*,*B*).

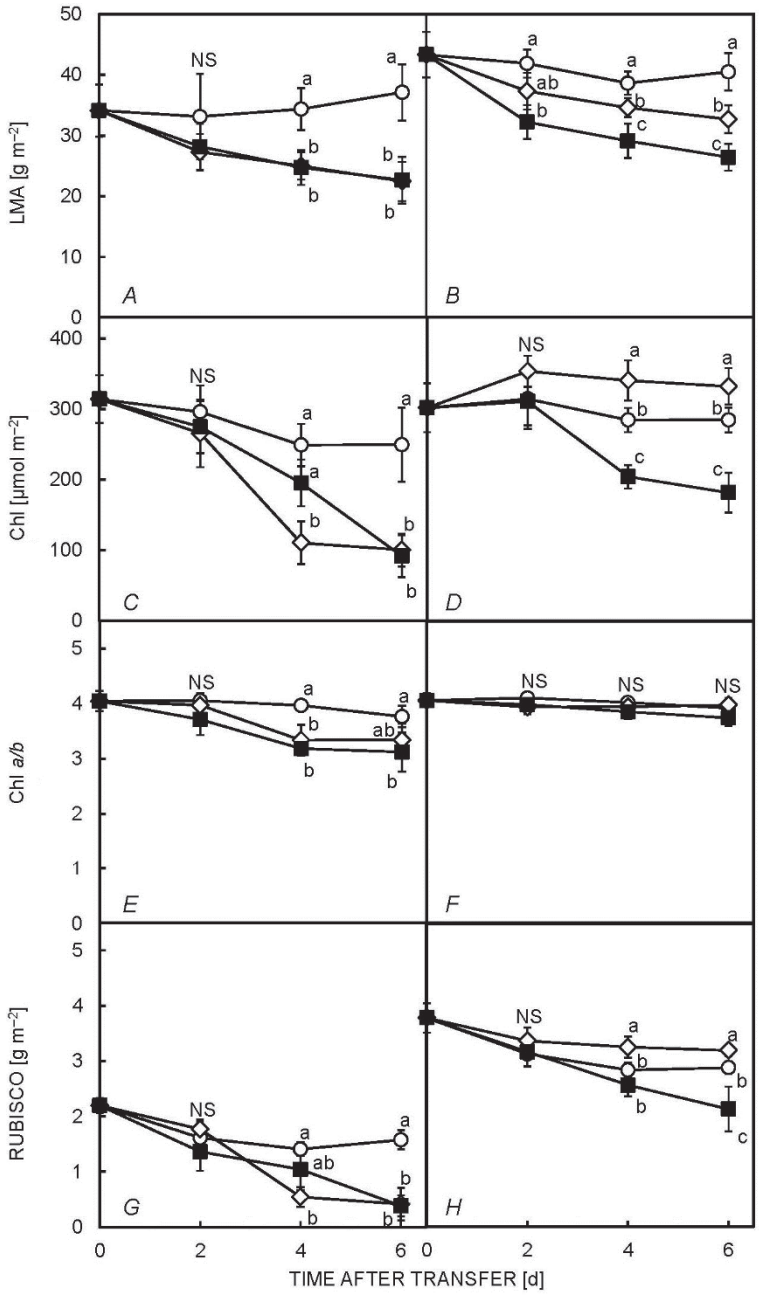

Fig. 3. Time courses of leaf properties in leaves of the 2nd and the 3rd leaves incubated for 6 days under different treatments. *A*,*B*: leaf mass per area (LMA), *C*,*D*: Chl concentrations, *E*,*F*: the Chl *a*/*b* ratio and *G*,*H*: Rubisco contents. Left side (*A*,*C*,*E*,*G*) and right side (*B*,*D*,*F*,*H*) showed the 2nd and the 3rd leaves, respectively. Symbols are the same as in Fig. 2. Means are shown and the bars indicate SD (n = 5). *Different letters* indicate significant differences between the treatments in the mean values (P<0.05) according to *Scheffe*'s multiple comparison tests. NS – not significant.

Therefore, when only the PSII centres that were active in electron transport were taken into account, the PSIIα/PSI ratio was 0.74 in the 2nd leaves and 0.60 in the 3rd leaves on day 0 (Fig. 7*C*,*D*). Nevertheless, there were no statistical differences among the treatments (P>0.05), the percentage of PSIIα was prone to decreasing from day 0 to day 4 under shaded conditions (PART and WHOLE) in the 2nd leaves and under all treatments in the 3rd leaves. Thus, although the imbalance in light absorption of the two photosystems would be partly compensated for by the modulation of the antenna size of PSIIα centres.

Discussion

The effects of partial shading on the leaf level and the thylakoid level in sunflower leaves were investigated in the present study.

LMA decreased in the 3rd leaves in PART and WHOLE conditions because of the shading in the 2nd leaves (Fig. 3*A*,*B*), suggesting that shade treatment of the lower leaves regulated the morphological properties of the upper leaves. Hikosaka (1996) pointed out if the plants were covered as the whole individuals, the growth of the upper leaves (*i.e.*, the sink organ) is inhibited.

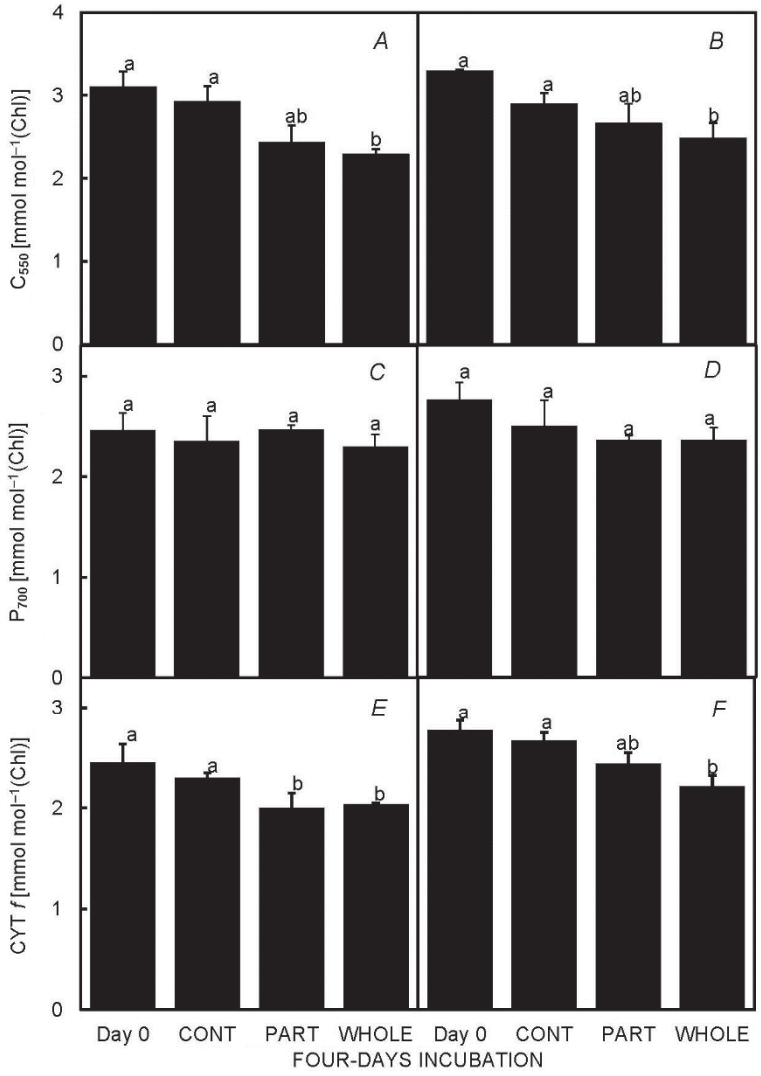

Fig. 4. Electron transport activities in leaves of the 2nd and the 3rd leaves under different light treatments over four days of incubation. *A*,*B*: PSII electron transport activities [μmol O_2 mg^{-1}(Chl) h^{-1}], *C*,*D*: PSI electron transport activities [μmol O_2 mg^{-1}(Chl) h^{-1}]. Left side (*A*,*C*) and right side (*B*,*D*) showed the 2nd and the 3rd leaves, respectively. Bars indicate SD (n = 5). *Letters* indicate significant differences in the mean values (P<0.05) according to *Scheffe*'s multiple comparison tests.

Under WHOLE conditions, since the photosynthesis would be performed insufficiently, the substrate for the constructive respiration also gained insufficiently for the plant growth. As a result, the plants are inhibited in their growth by end-product inhibition (Layne and Flore 1995, Sawada *et al*. 2001). Therefore, the shaded leaves decreased in LMA, while the 3rd leaves in PART increased in LMA. Furthermore, Yano and Terashima (2001) have proposed that there are signal transduction mechanisms from the shade leaves to the developing upper leaves.

The measured parameters except for the Chl *a*/*b* ratios and F_v/F_m showed that a notable feature of the decline kinetics of these parameters in the shading treatments was the occurrence of an initial long lag (Figs. 2*A*,*B*; 3*C*,*D*,*G*,*H*). A long delay in the dark degradation of Chl lasting less than one day was observed in detached leaves in some cases (Thimann *et al*. 1977, Okada *et al*. 1992), but the lag in the previous study (Yamazaki 2010a) lasted until the 2nd day. The fact that this lag was shorter than that observed in the previous study may be the result of an artificially enhanced degradation in the detached leaves. Although Chl is stable even under low irradiance (Okada and Katoh 1998, Yamazaki 2010a), the retardation of Chl concentration was not observed in either the 2nd leaves or in the 3rd leaves (Fig. 3*C*,*D*). Since the Rubisco contents underwent rapid degradation except under the natural light condition in the 2nd leaves (Fig. 3*G*), there was a correlation between the degradation of Rubisco and a decline in the photosynthetic activities (Figs. 2*A*, 3*G*). In contrast, the 3rd leaves showed that the retarding effect of irradiance on Rubisco contents was lower than that on the breakdown of Chl (Figs. 2*D*,*H*). Furthermore, the Chl concentration increased in the 3rd leaves treated under PART conditions. These results show the remobilization of resources involved in the breakdown of Chl and photosynthesis-related proteins from the source to the sink organ.

The light-saturated rate of photosynthesis decreased during the shading treatment, but was somewhat faster than the rate at which the Rubisco contents decreased (Figs. 2*A*, 3*G*), whereas the Chl concentrations declined

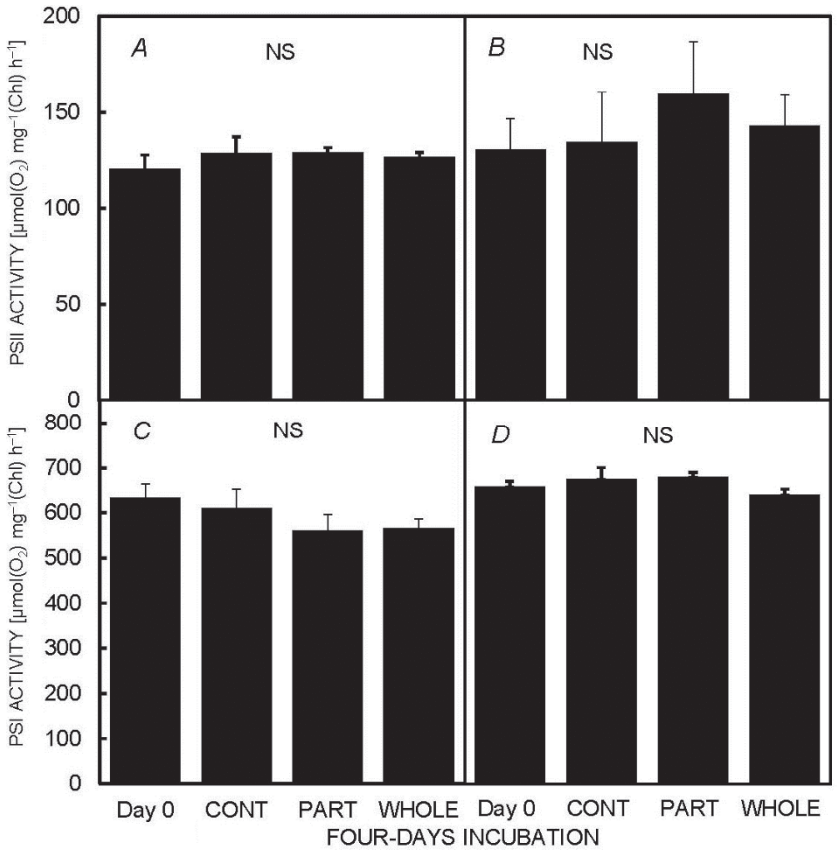

Fig. 5. Levels of thylakoid components in leaves of the 2nd and the 3rd leaves under different light treatments over 4 d of incubation. *A*,*B*: Levels of C_{550} [mmol mol^{-1}(Chl)], *C*,*D*: levels of P_{700} [mmol mol^{-1}(Chl)], *E*,*F*: levels of Cyt *f* [mmol mol^{-1}(Chl)]. Left side (*A*,*C*,*E*) and right side (*B*,*D*,*F*) showed the 2nd and the 3rd leaves, respectively. Bars indicate SD (n = 5). *Letters* indicate significant differences in the mean values (P<0.05) according to *Scheffe*'s multiple comparison tests. NS – not significant.

(somewhat more slowly than the photosynthetic activity Figs. 2*A*, 3*D*). Pons and Pearcy (1994) found that photosynthetic nitrogen use efficiency increased during the shading treatment, which was for the most part due to the decrease in leaf nitrogen content, to some extent to the decrease in respiration rate, and only in small part due to the change in the partitioning of nitrogen within the photosynthetic apparatus. Therefore, the partition of nitrogen in the leaf acted to maximize the photosynthesis and to maintain the whole plant photosynthesis.

Many investigations have reported that the irradiance modulates the concentrations of the thylakoid components. The concentrations of the PSII reaction centre complex were slightly influenced by light intensity (Fig. 4*A*,*B*), while those of the PSI reaction centre complex showed no statistical change (Fig. 4*C*,*D*)(Leong and Anderson 1984, Lee and Whitmarsh 1989, De la Torre and Burkey 1990, Burkey and Wells 1996), suggesting that the components decreased in parallel with the Chl concentration. As a result, the PSII/PSI ratios did not show any statistical change among the shade treatments (Fig. 7*A*,*B*). This finding indicates that the stoichiometry of PSII and PSI was not modulated by the partial shading.

The observed declines in the concentrations of Cyt *f* indicated that the thylakoid components were influenced by light intensity (Figs. 4*E*,*F*), as plants exposed to weak light intensity had lower concentrations of Cyt *f* than plants exposed to high light intensity (Leong and Anderson 1984, De la Torre and Burkey 1990, Murchie and Horton 1998, Yamazaki 2010b). Chow and Anderson (1987) suggested that the Cyt b_6/f complex and PQ limit photosynthesis. However, the electron transport activities of PSI and PSII did not change irrespective of the shade treatment (Fig. 5). This indicates that the overall photosynthetic pathway was limited not by the concentrations of the intermediate carrier but by the Rubisco contents. These valuable results indicate that the partial shading had a stronger effect on the leaf level than on the thylakoid level.

The decline in the Chl *a*/*b* ratio can be interpreted as an acclimative response of leaves to low-light conditions (Leong and Anderson 1984). Yamazaki *et al*. (1999) have indicated that there is a positive correlation between the Chl *a*/*b* ratio and the PSII/PSI ratio, and that a decrease in the Chl *a*/*b* ratios is consistent with an increase in the PSIIα centres. In this study, the Chl *a*/*b* ratios of the shaded 2nd leaves decreased, whereas those of the 3rd leaves remained fairly constant (Fig. 3*E*,*F*). In a parallel result, the abundance of PSIIα remained constant in the 2nd leaves, while it decreased in the 3rd leaves (Fig. 6*A*,*B*). These results suggest that the 2nd leaves might extend their antenna sizes to provide more effective light capture under weak light conditions, suggesting that the PSIIα centres under the PART and WHOLE conditions were partly compensated for by an increase in the size of the antennae attached to the PSIIα

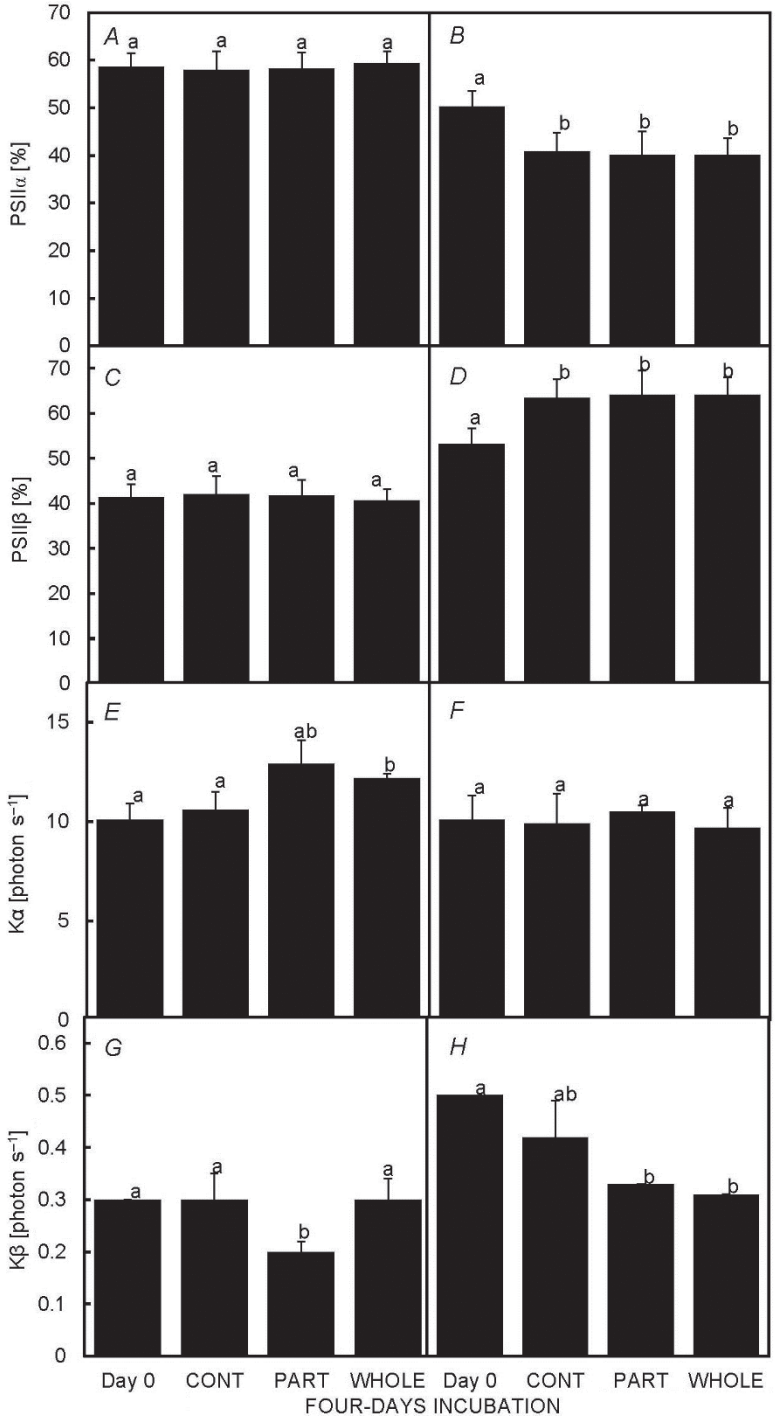

Fig. 6. Relative abundances of PSIIα and PSIIβ and the rate constants of PSIIα and PSIIβ in leaves of the 2nd and the 3rd leaves under different light treatments over four days of incubation. *A*,*B*: relative abundances of PSIIα [%],*C*,*D*: relative abundances of PSIIβ [%], *E*,*F*: the rate constants of PSIIα (Kα [photon s^{-1}]) and *G*,*H*: the rate constants of PSIIβ (Kβ [photon s^{-1}]). Left side (*A*,*C*,*E*,*G*) and right side (*B*,*D*,*F*,*H*) showed the 2nd and the 3rd leaves, respectively. Bars indicate SD (n = 5). *Letters* indicate significant differences in the mean values (P<0.05) according to *Scheffe*'s multiple comparison tests.

centres rather than by the abundance of the PSIIα centres (Fig. 6*E*,*F*). In addition, we speculate that based on the data of the antenna size heterogeneity, this type of regulation minimally mitigated the imbalance of light absorption between the two photosystems under weak light conditions (Fig. 7*C*,*D*).

In conclusion, the present study clearly demonstrated that the partial shading of lower leaves affected the nonshaded upper leaves based on the leaf-level responses obtained from morphological observations, whereas the partial shading had no effect on the thylakoid-level responses obtained from physiological and biochemical observations. The light received at the lower leaves might serve as a factor in the regulation of the leaf properties of the upper leaves due to the whole plant photosynthesis, while this factor did not have an effect at the thylakoid level.

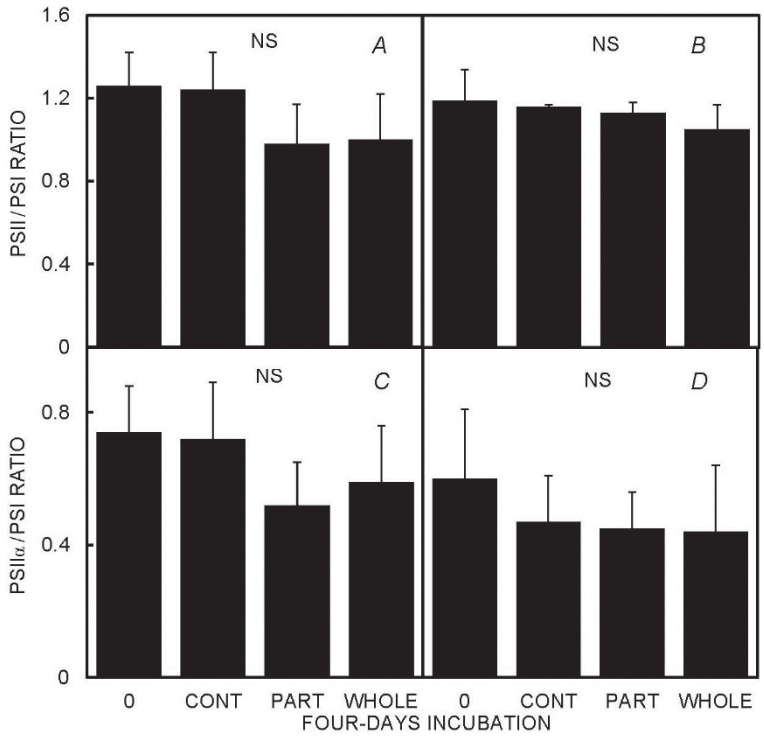

Fig. 7. Stoichiometries of PSII and PSI, and comparisons of the energy absorbed balances in leaves of the 2nd and 3rd leaves under different light treatments over four days of incubation. *A*,*B*: PSII/PSI ratios, *C*,*D*: PSIIα/PSI ratios. Left side (*A*,*C*) and right side (*B*,*D*) showed the 2nd and the 3rd leaves, respectively. Bars indicate SD (n = 5). *Letters* indicate significant differences in the mean values (P<0.05) according to *Scheffe*'s multiple comparison tests. NS – not significant.

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