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# Compensatory growth and photosynthetic responses of *Pharbitis purpurea* seedlings to clipped cotyledon and second leaf

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## Abstract

Leaf tissue damaging to seedlings can limit their subsequent growth, and the effects may be more extensive. Compensatory photosynthesis responses of the remnant cotyledon and primary leaf of *Pharbitis purpurea* to clipping and the effect of clipping on seedling growth were evaluated in a pot-cultivated experiment. Three treatments were conducted in the experiment, which were clipped cotyledon (CC), clipped second leaf (CL), and control group (CG). The area, thickness, mass, and longevity of the remaining cotyledon of CC exhibited over-compensatory growth. In contrast, seedlings of CC had under-compensatory growth in seedling height, root length, seedling mass, and root to shoot ratio. However, the traits of remnant cotyledon and seedling in CL treatment exhibited equal-compensatory growth. Net photosynthetic rate of the cotyledon of CC was significantly higher than those of CL and CG treatments, and the diurnal changes in photosynthetic rates showed significantly different patterns which were unimodal curve (CC) and bimodal curve (CL and CG), respectively. There was no significant difference between CL and CG treatment. Net photosynthetic rate of the primary leaf of CL was significantly higher than that of CG treatment. However, the photosynthetic rates of primary leaves of CL and CG treatments showed similar photosynthetic patterns characterized by a bimodal curve. *P. purpurea* seedlings used a compensatory growth strategy in the remaining cotyledon or the primary leaf to resist leaf loss and minimize any adverse effects.

*Additional key words*: clipping; compensatory growth; photosynthesis; primary leaf; remnant cotyledon; seedling.

## Introduction

The seedling stage is the most vulnerable phase of a plant life history (Armstrong and Westoby 1993, Bonfil 1998, Fenner and Thompson 2005, Harper 1977, Leishman and Westoby 1994, Westoby *et al.* 1992). The energy and nutrients needed for the growth and development of species without endosperm (*i.e.*, most dicotyledons) are mainly supported by cotyledons in the early developmental stages before true leaves emerge. Cotyledons are the first leaves of a plant, which play an important role in seedling development (Hanley and May 2006, Kitajima 2003, Zhang *et al.* 2008). Cotyledons provide a major proportion of matter needed for seedling growth until the first true leaf becomes a significant exporter of photosynthates. The contribution of cotyledon photosynthesis for early seedling growth has been emphasized by several authors (Ampofo *et al.* 1976, Burzyński and Zurek 2007, Harris *et al.* 1986, Kitajima 1992). In addition, primary leaf also contributes to seedling development (Ferraro and Oesterheld 2002, Hikosaka *et al.* 2005).

In natural conditions, it is a common phenomenon that partial or entire leaves are defoliated by insects, herbivores or factitious clipping, often leading to reduced fitness (Belsky 1986, Ferraro and Oesterheld 2002, Painter and Belsky 1993, Van Staalduinen and Anten 2005). Both cotyledons and primary leaves are critical photosynthetic organs at the seedling stage. Damage to either or both of them may negatively influence post-seedling development (García-Cebrián *et al.* 2003,

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*Abbreviations*: CC – clipped cotyledon; CL – clipped second leaf; CG – control group; $C_i/C_a$ – intercellular $CO_2$/ambient $CO_2$; $E$ – transpiration rate; $g_s$ – stomatal conductance; $P_N$ – net photosynthetic rate; RH – relative humidity in the chamber; R/S – root/shoot ratio; $T_{air}$ – temperature in the chamber.

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Hanley and May 2006), limiting seedling growth and ultimately reducing the biomass of seedlings (Boege 2005, Evans 1991, Holland *et al.* 1996, McNaughton 1983, 1986). However, clipping may promote plant growth by stimulating photosynthesis, altering biomass allocation and increasing growth rate (Anten and Ackerly 2001, Hilbert *et al.* 1981, Meyer 1998a, Oesterheld and McNaughton 1991, Zhao *et al.* 2008). Several studies found that the increased photosynthetic rate was a possible mechanism for compensation (Dyer 1991, Meyer 1998b, Senock *et al.* 1991, Thomson *et al.* 2003, Zhao *et al.* 2008). Previous studies have reported that plants can often compensate for biomass losses by defoliation; these studies have typically focused on grasses (Ferraro and Oesterheld 2002, Del-Val and Crawley 2005, Van Staalduinen and Anten 2005). Only scant evidence has indicated compensatory responses of dicotyledonous species (Mabry and Wayne 1997, Moriondo *et al.* 2003, Thomson *et al.* 2003); comparatively little is known about how this process occurs during their seedling stage by cotyledon or leaf damage, however.

The seedlings of *P. purpurea*, an annual, epigeal, dicotyledonous species that often grows in frequently disturbed environments such as farmlands, fallow fields or roadsides, generally tolerate damage to leaf tissue, which is probably an important factor contributing to their ability for compensatory growth. Yet we still know little about the compensatory mechanisms by which the seedling of dicotyledonous species can tolerate such environmentally imposed constraints.

In this study, we compared the effects of clipped cotyledon or second leaf on the photosynthesis (remnant cotyledon and primary leaf) and seedling development, to determine if remnant cotyledon and primary leaf could develop compensatory strategies against clipping by morphological and physiological responses to minimize the effects of defoliation on seedling growth.

## Materials and methods

**Study site and material**: A pot experiment was performed in the summer (July) of 2008 in the grassland ecosystem experimental station of Northeast Normal University (44°30'–44°45'N, 123°31'–123°56'E). This area is of typical mesothermal monsoon climate with plain topography in the southern of the Songnen plain (altitude: 137.8–144.8 m). The mean annual temperature is 4.6–6.4°C. The annual accumulated temperature is 2,545–3,374°C, frost-free period is 136 ± 16 d. The mean annual rainfall is about 400–500 mm which is mainly concentrated from June to August and accounts for more than 60% of the rainfall throughout the year. The annual evaporation capacity is 2–3 times more than the rainfall.

Seeds of *P. purpurea* were collected during autumn 2007 from the Songnen plain and stored in cloth bags at room temperature (20 ± 2°C).

**Experimental design**: Pots (20 cm in diameter and 25 cm high) were filled with a soil-sand mixture (1:1, v/v). Twenty seeds were sown in each pot, then covered with soil to the required depth of 2 cm and cultivated in the open air. Irrigation was provided when needed. Seedlings were thinned to five per pot after cotyledon emergence. Three treatments were conducted in the experiment, which were CC: a piece of cotyledon was clipped with scissors after emergence; CL: the second leaf was clipped with scissors after appearance; CG: control group (no clipping) (Fig. 1). There were 5 replicated pots for every treatment, and 5 individual plants per pot were regarded as one replicate. The removed cotyledons and leaves were labeled and dried at 65°C for 48 h to determine the biomass. Net photosynthetic rate ($P_N$), stomatal conductance ($g_s$), intercellular CO$_2$/ambient CO$_2$ ($C_i/C_a$), temperature in the chamber ($T_{air}$) and relative humidity (RH) in the chamber of the remaining cotyledon in the CC, CL, and CK treatments and the primary leaf in the CL and CG treatments were measured with *Li-6400* portable photosynthesis system (*LI-COR*, Lincoln, NE, USA). Diurnal changes in $P_N$ were taken at 2-h intervals from 06:00 to 18:00 used natural sunlight for one day which was a sunny day after the appearance of the third true leaf appearance. The CO$_2$ curve of $P_N$ was detected by using CO$_2$ injector, and started at the ambient CO$_2$ concentration of 380 μmol mol$^{-1}$ and the photosynthetically active radiation of 1,200 μmol m$^{-2}$ s$^{-1}$. The leaf chamber was equilibrated for at least 15 min in order to reach a steady state. The CO$_2$ concentration was then decreased stepwise to 200, 100, and 50 μmol mol$^{-1}$ and then increased stepwise from 380 to 600, 800; 1,200 and 1,600 μmol mol$^{-1}$. The light curve of $P_N$ was detected by using LED stimulative light, ten intensities were applied: 0, 50, 100, 200, 400, 600, 800; 1,200; 1,600; and 2,000 μmol m$^{-2}$ s$^{-1}$, the CO$_2$ concentration was set at 380 μmol mol$^{-1}$. Each sample leaf was given a minimum of 2 min to stabilize to each new increasing light level before measurements were taken. All measurements were taken between 08:30 and 10:30 on the day on which diurnal

Fig. 1. The mode of clipping treatment. CG – no clipping as control group; CC – a piece of cotyledon was clipped with scissors after emergence; CL – the second true leaf was clipped with scissors after appearance.

changes in $P_N$ were determined. Throughout all measurements, $T_{air}$ and RH were steady at mean values of 27 ± 2°C and 50 ± 8% at all light levels or $CO_2$ concentrations. Measurements were repeated five times for each sample leaf, for one sample leaf per pot, and the averages of 5 replicates (5 pots) were used in the data analysis. Plants of all treatments were harvested when approximately 50% of cotyledons of controls died. Three pots (15 seedlings in total) of each treatment were harvested randomly, and the other 2 pots (10 seedlings in total) were retained to determine longevity of cotyledons. The final harvest of each treatment was carried out when the cotyledons died. The height and root length of harvested seedlings were measured with a ruler, cotyledon thickness was measured with thickness tester (*Peacock Inc.*, Japan), and leaf area was measured with *Sigmascan* software program (*Systat Software Inc.*, Richmond, California, USA). In addition, plant roots in each pot were screened out of the soil using a 2-mm sieve. After drying at 65°C for 48 h, each fraction was weighed.

**Statistical data analysis**: All experiments were based on five replicated measurements. Data were analyzed by one-way analysis of variance (*ANOVA*) using the statistical software *SPSS 13.0* (*SPSS Inc.*, Chicago, IL, USA). The treatment mean values were compared by *post hoc* least significant difference (LSD) test. The term significant indicates differences for which $P \le 0.05$.

## Results

The cotyledon longevity, area, thickness and mass of different treatments were similar among treatments (Fig. 2). The greatest values occurred in CC, and they were significantly higher than those of CL and CG, indicating that remnant cotyledons of CC had over-compensatory growth responses to clipping. There was no significant difference between CL and CG, indicating CL treatment induced an equal-compensatory growth for remnant cotyledons. In contrast, the seedling mass and root-shoot ratio of CC were significantly lower than those of CL and CG, and there was no significant difference between CL and CG (Table 1), which indicated an under-compensatory and equal-compensatory growth response to CC and CL treatments, respectively. Seedling size was measured in terms of seedling height and root length. Seedling height and root length of CC were significantly lower than those of CL and CG, but there was no significant difference between CL and CG (Table 1), which also indicated an under-compensatory and equal-compensatory growth response to CC and CL treatments, respectively.

Table 1. Seedling height, root length, seedling mass, and R/S of *P. purpurea* under three clipping treatments. The values are means ± SE ($n = 3$). Values with the same letter are not statistically significant at $P<0.05$. CC – clipped cotyledon; CL – clipped second leaf; CG – control group; R/S – root/shoot ratio.

| Treatment | Seedling height [cm] | Root length [cm] | Seedling mass [g] | R/S |
|---|---|---|---|---|
| CC | $11.97 \pm 0.94^b$ | $20.39 \pm 2.55^b$ | $0.32 \pm 0.05^b$ | $0.35 \pm 0.021^b$ |
| CL | $14.79 \pm 1.23^a$ | $23.48 \pm 2.86^a$ | $0.40 \pm 0.04^a$ | $0.47 \pm 0.009^a$ |
| CG | $15.30 \pm 0.85^a$ | $24.07 \pm 2.58^a$ | $0.42 \pm 0.06^a$ | $0.49 \pm 0.018^a$ |

Diurnal changes in $P_N$ of cotyledons showed significantly different patterns between clipped cotyledon (CC) and unclipped cotyledon (CL and CG) treatments (Fig. 3*A*). The results showed that diurnal changes in $P_N$ of CL and CG showed typical bimodal curves, and the peak values occurred at approximately 11:00 and 14:00, which were 15.67 and 15.89 μmol $m^{-2}$ $s^{-1}$, respectively. CC showed a unimodal curve with a maximum value (19.64 μmol $m^{-2}$ $s^{-1}$) at noon. There were no significant differences of $g_s$ among various treatments before 10:00. From 10:00 to 14:00, the daily change of $g_s$ in CC treatment increased significantly, and it was slightly higher than those of $g_s$ in CL and CG treatments. The daily average value of $g_s$ of all treatments decreased after 14:00 (Fig. 3*B*). The daily courses of *E* were similar, and they did not exhibit significant differences among all treatments (Fig. 3*C*). $C_i/C_a$ did not differ significantly among all treatments. They decreased in the period of 07:00-13:00; however, they increased after 15:00 (Fig. 3*D*).

The $P_N$ of remnant cotyledons showed certain trends under various $CO_2$ concentration or light intensity treatments; $P_N$ increased with the increase of $CO_2$ concentration or light intensity, and the $P_N$ of CC were all significantly greater than those of CL and CG treatments (Fig. 4*A*,*B*). The $P_N$ of remnant cotyledons of CC was significantly greater than that of CL and CG treatments when $CO_2$ concentration was greater than 100 μmol $mol^{-1}$, and there was no significant difference between CL and CG treatments (Fig. 4*A*). The $P_N$ of remnant cotyledons of CC significantly increased from 200 μmol $m^{-2}$ $s^{-1}$ to 800 μmol $m^{-2}$ $s^{-1}$, then stabilized, but that of CL and CG treatments increased significantly from 400 μmol $m^{-2}$ $s^{-1}$ to 800 μmol $m^{-2}$ $s^{-1}$, and then stabilized in spite of increased light intensity. There was no significant difference between CL and CG treatments (Fig. 4*B*).

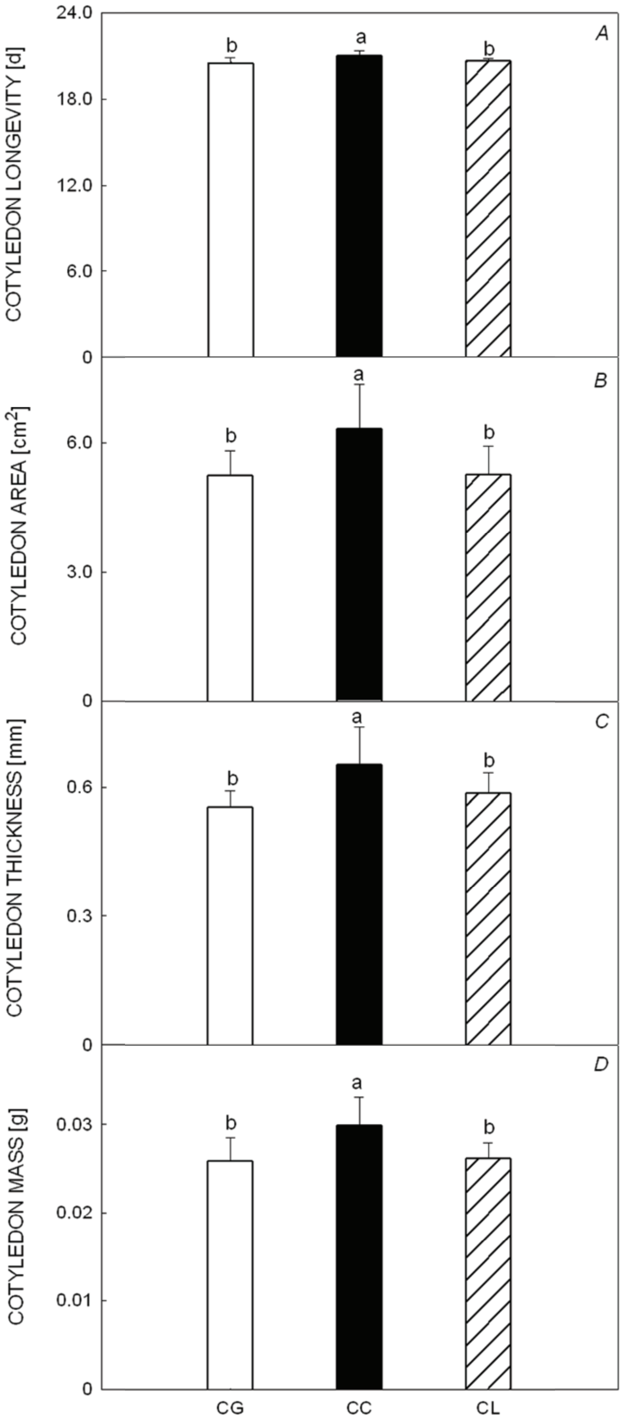

Fig. 2. Compensatory growth of *A*: cotyledon longevity, *B*: cotyledon area, *C*: cotyledon thickness, *D*: cotyledon mass of *P. purpurea* under clipped cotyledon (CC), clipped second leaf (CL) and control group (CG) treatments. The values are means ± SD ($n$ = 3). Values with the same letter are not statistically significant at $P$<0.05.

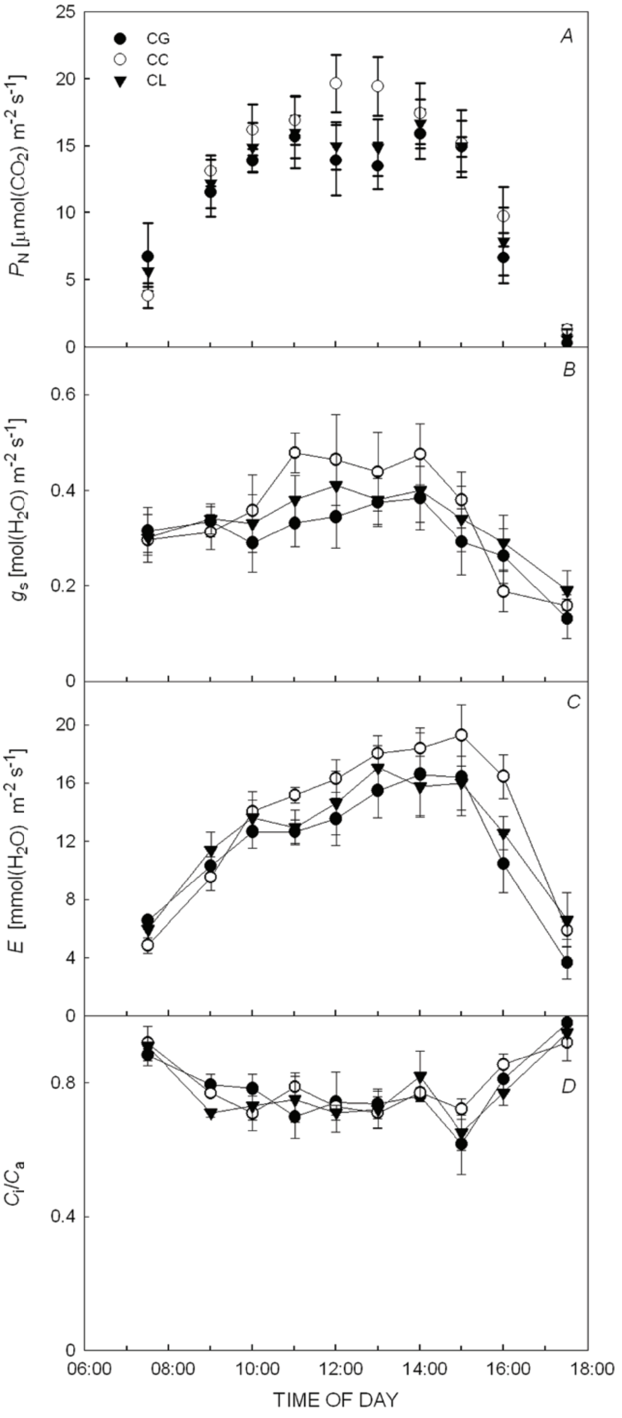

Fig. 3. Diurnal changes in the *A*: net photosynthetic rate ($P_N$), *B*: stomatal conductance ($g_s$), *C*: transpiration rate ($E$) and *D*: intercellular CO$_2$/ambient CO$_2$ ($C_i/C_a$) of remnant cotyledon of *P. purpurea* under clipped cotyledon (CC), clipped second leaf (CL) and control group (CG) treatments. The values are means ± SD ($n$ = 5).

Diurnal changes in $P_N$ of the primary leaf showed similar patterns between clipped leaf (CL) and unclipped leaf (CG) treatments (Fig. 5*A*). The results showed typical bimodal curves, with peaks occurring at approximately 11:00 and 15:00, and the minimum value occurring at 13:00. The $P_N$ of CL was slightly higher than that of CG. The trends of $g_s$, $E$ and $C_i/C_a$ were similar between CL and CG treatments, indicating that clipping did not have significant effects on $g_s$, $E$ and $C_i/C_a$ (Fig. 5*B*,*C*,*D*). However, clipping significantly affected $P_N$ when $CO_2$ or light was provided. The $P_N$ of CL was significantly higher than that of CG treatment when $CO_2$ concentration was greater than 100 μmol $mol^{-1}$ and light intensity was greater than 200 μmol $m^{-2}$ $s^{-1}$ (Fig. 6*A*,*B*).

## Discussion

The development of photosynthetic organs during leaf ontogeny is worthy of attention both from theoretical and practical points of view (Kutik 1985, 1997). A suitable object for such studies are cotyledons due to their short and distinct life span (Tsukaya *et al.* 1994, Wilhelmová *et al.* 1997). In our research, the remnant cotyledon longevity of CC treatment was prolonged 1.9% and 2.4% beyond that of CL and CG treatments (Fig. 2*A*), respectively. This increased cotyledon longevity would ultimately lead to more photosynthate accumulation. In addition, the remnant cotyledons had an increased capacity for achieving their potential area (Fig. 2*B*), a result similar to studies on younger leaves (Rawson *et al.* 1980, Rawson and Turner 1982). Therefore, increased longevity and photosynthesizing area of the remnant cotyledon may be considered as a strategy to maximize the photosynthetic compensation for the clipped cotyledon. Plant seedlings by their very nature are small and often lose aboveground tissue due to various biotic and abiotic factors (Wallace and Eigenbrode 2002, Kitajima 2003, Hanley and Fegan 2007), which may have significant positive or negative effects on subsequent plant growth and establishment (Mabry and Wayne 1997, Moriondo *et al.* 2003, Hanley and May 2006). The traits of remnant cotyledon (such as longevity, area, thickness, and mass) exhibited over-compensatory growth during the stage of cotyledon development (Fig. 2), signifying compensatory growth as a strategy to tolerate clipping (Ruiz-R *et al.* 2008). Despite the strong compensatory response of the remaining cotyledon, overall plant growth was reduced in clipped cotyledon treatment (under-compensatory growth) but not in clipped leaf treatment (equal-compensatory growth) (Table 1). One possible explanation for the diminution of the growth response is that the photosynthetic compensation of the remaining cotyledon was lower than that provided by the storage material when a minimal amount of leaf tissue (*i.e.*, one cotyledon) was removed. On the other hand, the photosynthesis of a cotyledon is more critical to survival and development than that of a primary leaf during this period. These observations might form the basis of a mechanism of defoliation avoidance for dicotyledonous species. Similar results have been observed in other pasture species (Barthram 1997, Zhao *et al.* 2008). Therefore, even after the development of the first leaf, damage to cotyledons may affect seedling survival and development, which can be fatal (Hanley and May 2006, Kitajima 2003, Sork 1987). The results of this short-term study may not directly predict post-plant growth for the long term, but early clipping of cotyledon and leaf may have lasting impacts on seedling growth. Overall, both photosynthetic organs (cotyledons and true leaves) appeared to meet the energy demands of seedlings to a large degree at the seedling stage.

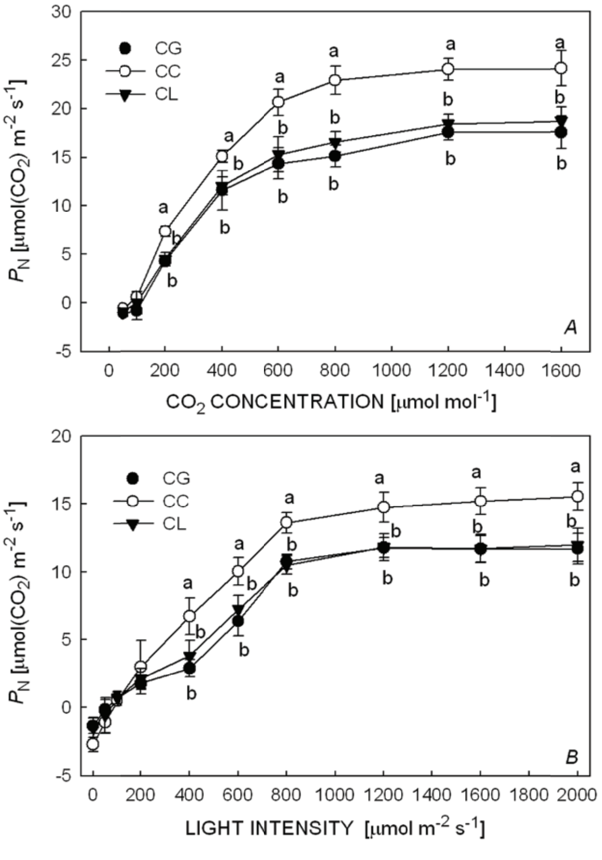

Fig. 4. Changes in the net photosynthetic rate ($P_N$) of remnant cotyledon of *P. purpurea* under clipped cotyledon (CC), clipped second leaf (CL), and control group (CG) treatments with different *A*: $CO_2$ concentration and *B*: light intensity. The values are means ± SD ($n$ = 5). Values with the same letter are not statistically significant at $P<0.05$.

Both cotyledons and true leaves are photosynthetic organs. Leaf area loss is a typical damage resulting from insects, herbivores, and factitious clipping (Lee and Bazzaz 1980, Moles and Westoby 2004). Kitajima (2003) suggested that loss of photosynthetic cotyledons will have more impact than loss of reserve cotyledons. Our results showed that without a cotyledon, the $P_N$ of the remnant cotyledon could increase in response to clipping. A similar conclusion was drawn by Meyer (1998a) in goldenrod (*Solidago altissima*). One reason for induced compensatory photosynthesis could be that more chlorophyll is synthesized within remnant leaves (Martens and Trumble 1987). There were significantly different

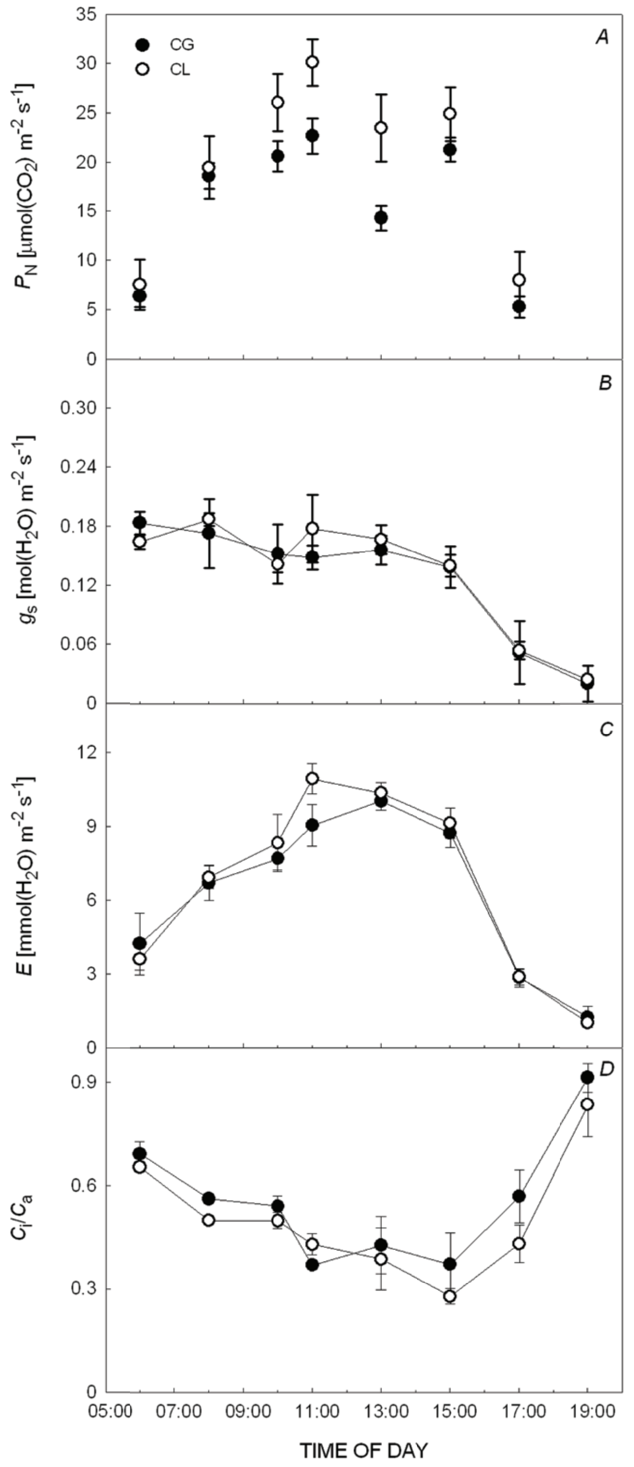

Fig. 5. Diurnal changes in the *A*: net photosynthetic rate ($P_N$), *B*: stomatal conductance ($g_s$), *C*: transpiration rate ($E$) and *D*: intercellular CO$_2$/ambient CO$_2$ ($C_i/C_a$) of primary leaf of *P. purpurea* under clipped second leaf (CL) and control group (CG) treatments. The values are means ± SD ($n$ = 5).

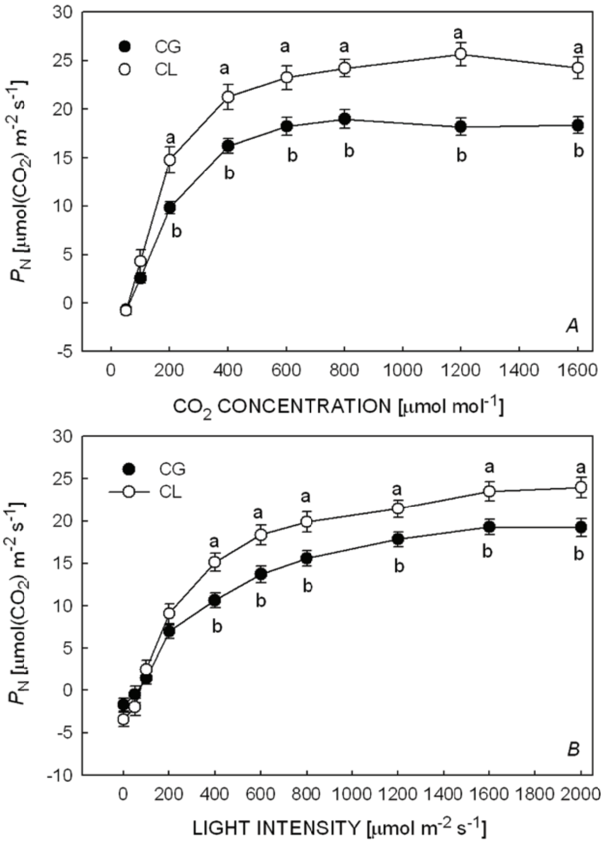

Fig. 6. Changes in the net photosynthetic rate ($P_N$) of primary leaf of *P. purpurea* under clipped second leaf (CL) and control group (CG) treatments with *A*: different CO$_2$ concentration and *B*: light intensity. The values are means ± SD ($n$ = 5). Values with the same letter are not statistically significant at $P$<0.05.

patterns between clipped cotyledon (CC) and unclipped cotyledon (CL and CG) treatments; photosynthetic compensation occurred during the period of 11:00–14:00 (Fig. 3*A*), the high-temperature period during the day. The results of $g_s$ and $E$ demonstrated that photosynthetic compensation could conceivably have been attributed to water because the cotyledonary transpiration area of clipped treatment (CC) was two-fold smaller than that of unclipped cotyledon treatments (CL and CG). Under the same amount of water absorbed by the root system, the amount of water lost through $E$ of clipped treatment (CC) was lower than that of unclipped cotyledon treatments (CL and CG). Therefore, the photosynthesis of the remaining cotyledon of clipped treatment was not decreased by stomatal closure, while photosynthesis of unclipped cotyledon treatments was decreased through stomatal closure to prevent water loss. Taken together, we predict that the unimodal curve of clipped treatment might not occur in some species in arid habitats, and the bimodal curve of unclipped cotyledon treatment might not occur in some species in wetland habitats. Furthermore, the results of $P_N$ of the remnant cotyledon under various CO$_2$ concentration or light intensity treatments also exhibited compensatory photosynthesis.

In addition, we found that not only remnant cotyledon exhibited compensatory photosynthesis, but the $P_N$ of primary leaf was also stimulated and increased following the clipping of second leaf (Fig. 5*A*). Nevertheless, the photosynthetic pattern of primary leaf did not change as markedly as that of remnant cotyledon. It is clear from this study that the slight difference of $P_N$ between natural conditions (Figs. 3*A*, 5*A*) and simulated conditions (Figs. 4, 6) might be influenced by $E$ and $g_s$, which were also verified in photosynthetic compensation of primary leaf. Although the effect of clipping second leaf on primary leaf was small because leaf area removed was small relative to the primary leaf area, it can also stimulate photosynthesis of primary leaf. This might be a specific compensatory mechanism of primary leaf to adapt a clipped new leaf. Mabry and Wayne (1997) suggested that plants can restore growth depending on the compensatory photosynthesis of remnant leaves. There is little evidence to justify changes of primary photosynthesis after such clipping treatment. Compensation is increasingly seen as an important way in which plants combat defoliation (Hidding *et al.* 2009, Moriondo *et al.* 2003, Noy-Meir 1993).

In conclusion, *P. purpurea* seedlings used a compensatory growth strategy in the remaining cotyledon or the primary leaf to resist leaf loss and minimize any adverse effects of defoliation. The photosynthetic responses of plant to defoliation suggest a trade-off between acclimation ability and plasticity, and should constitute an important future direction for research into the photosynthetic response to leaf loss.

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