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Acclimation to irradiance in seedlings of three tropical rain forest *Garcinia* species after simulated gap formation

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Abstract

We investigated the acclimation of seedlings of three tropical rain forest sub-canopy *Garcinia* species (*G. xanthochymus*, *G. cowa*, and *G. bracteata*) after transfer from 4.5 (LI) to 40 % (HI) sunlight and 12.5 (MI) sunlight to HI (LH_1 and LH_2 denoting transfer from LI to HI and MI to HI transfer, respectively). The changes of chlorophyll (Chl) fluorescence, net photosynthetic rate (P_N), dark respiration rate (R_D), Chl content per unit area (Chl_{area}), leaf mass per unit area (LMA), and seedling mortality were monitored over two months after transfer. These parameters together with leaf anatomy of transferred and control seedlings (kept in LI, MI, and HI) were also examined after two months. No seedlings died during the two months. F_v/F_m, P_N, and Chl_{area} of the transferred seedlings decreased in the first 3 to 12 d. LH_1 leaves showed larger reduction in F_v/F_m (>23 % *vs.* <16 %) and slower recovery of F_v/F_m than LH_2 leaves. P_N started to recover after about one week of *I* transfer and approached higher values in all *G. cowa* seedlings and *G. xanthochymus* LH_1 seedlings than those before the transfer. However, P_N of *G. bracteata* seedlings approached the values before transfer. The final P_N values in leaves of transferred *G. xanthochymus* and *G. cowa* seedlings approached that of leaves kept in HI, while the final P_N values of transferred leaves of *G. bracteata* were significantly lower than that of leaves grown under HI ($p<0.05$). R_D of *G. xanthochymus* LH_1 seedlings and all *G. cowa* seedlings increased and approached the value of the seedlings in HI. The final Chl_{area} of both *G. xanthochymus* and *G. cowa* approached the values before transfer, but that of *G. bracteata* did not recover to the level before transfer. The final Chl_{area} of all transferred seedlings was not significantly different from that of seedlings in HI except that *G. cowa* LH_1 seedlings had higher Chl_{area} than that in HI. LMA decreased within 2 d and then increased continuously until about 30 d and approached the value under HI. Spongy/palisade mesophyll ratio decreased after transfer because of the increase in palisade thickness. Leaf thickness did not change, so LMA increase of transferred seedlings was mainly due to the increase of leaf density. Thus the mature leaves under LI and MI of *G. xanthochymus* and *G. cowa* are able to acclimate to HI by leaf physiological and anatomical adjustment, while *G. bracteata* had limited ability to acclimate to HI.

Additional key words: chlorophyll fluorescence; epidermis; leaf thickness; photoinhibition; photosynthesis; respiration; species differences; spongy/palisade ratio; stomata density.

Introduction

Human disturbance and natural gap formation in tropical rainforests are often associated with the sudden exposure of many forest understorey seedlings to high irradiance (*I*). Leaves of understorey seedlings are adapted to very low *I*, relatively high and constant air humidity, and slightly lower but relatively constant temperature compared to those in high *I* microhabitats such as canopy gaps (Zhang *et al.* 2000, Szarzynsk and Anhuf 2001). They usually have a low photon energy utilization and dissipation capacities, but a high chlorophyll (Chl) content per unit mass so as to maximize the photon absorption (Lee *et al.* 1990, Cao 2000, Feng *et al.* 2004). Consequently, after irradiation, the leaves of these seedlings usually suffer from severe photoinhibition of photosynthesis

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(Björkman 1981, Critchley and Smillie 1981, Oberbauer and Strain 1985). This photoinhibition is associated with reductions in quantum yield, photochemical efficiency, and capacity of photosynthesis, and acceleration of non-photochemical quenching (Demmig *et al.* 1987).

The morphological and physiological acclimation of plants to different *I* (Lee *et al.* 1997, Logan *et al.* 1998, Smith *et al.* 1998, Poorter 1999) and short-term acclimation after a sudden change in *I* over a duration of several h or d (Langenheim *et al.* 1984, Öquist *et al.* 1992, Kitao *et al.* 2000) have often been studied. After gap formation, acclimation of the understorey plants to the new environment may take much longer time (several weeks or even months). Before the emergence of new leaves, acclimation of previous leaves developed in shade is important. In some species, fully developed shade leaves acclimated to increase in *I* by improving photosynthetic capacity (Powles 1984, Lovelock *et al.* 1994, Clearwater *et al.* 1999, Sailaja and Rama Das 2000, Yang *et al.* 2001), and even by increasing leaf thickness (Kamaluddin and Grace 1992). However, mature leaves of *Alocasia macrorrhiza* did not acclimate completely to an *I* increase or decrease (Sims and Pearcy 1991, 1992). Shortly after exposure, pre-existing shade leaves rely largely on biochemical and physiological acclimation, but in the long time, new leaves are produced, which are in structural and functional equilibrium with the new environment (Bazzaz 1996). Kursar and Coley (1999) reported that *Hybanthus prunifolius* dropped shade leaves and made new leaves with higher photosynthetic rate shortly after being transferred from low to high *I*. Lovelock *et al.* (1998) reported that some species with long-lived leaves are more tolerant to abrupt increase in *I*. As a whole, we have a limited understanding of dynamic acclimation of low *I* (LI) leaves when transferred to a high *I* (HI). Moreover, when a small gap forms in tropical rainforests, the trees near it fall more easily later to form a larger gap. How the seedlings in a small gap respond to a larger gap formation is less studied.

To understand the fate of seedlings and dynamic change of forests after gap formation or expansion, it is necessary to compare the responses among shade-tolerant species when exposed from LI to HI. Comparing differences among the species belonging to the same genus can minimize the effect caused by genetic difference. In the present study, we chose three shade-tolerant congeneric tree species, *Garcinia xanthochymus, G. cowa*, and *G. bracteata* that are common to tropical rainforests in the Xishuangbanna region, southern Yunnan, southwestern China, to investigate their responses to irradiance and simulated gap formation and expansion. We addressed the following two questions: (*1*) Can the mature leaves of the three *Garcinia* species grown in low and intermediate *I* acclimate to HI exposure? (*2*) How do the three species differ in the acclimation to *I* transfer? To answer these questions, seedling mortality, net photosynthetic rate (P_N), dark respiration rate (R_D), Chl fluorescence, Chl_{area}, and leaf mass per unit area (LMA) were monitored over two months after the *Garcinia* seedlings that were raised under 4.5 (LI) or 12.5 (MI) % of full sun were transferred to 40 % (HI) of full sun.

Materials and methods

This study was conducted at Xishuangbanna Tropical Botanical Garden (21°56′N, 101°15′E, 600 m alt.), Chinese Academy of Sciences, Southwest China. The climate of this region is dominated by the southwest monsoon arising from the Indian Ocean. The mean annual temperature is 21.7 °C and the mean temperature of the coldest month (January) is 15.6 °C (Liu and Li 1996). Annual mean precipitation is 1 557 mm, 83 % of which falls in the period from May to October. The minimum monthly rainfall is 9.4 mm (February).

The seedlings of *Garcinia xanthochymus* Hook. f. *ex* Anders., *G. cowa* Roxb., and *G. bracteata* C.Y. Wu & Y.H. Li were used. These species are late-successional sub-canopy trees and often co-occur in the tropical rainforests in the Xishuangbanna region (Liu 1987). Timbers of these species are used for industry and architecture. *G. xanthochymus* and *G. cowa* dominate in sub-canopy, while *G. bracteata* is scattered in the sub-canopy. Fruits of *G. cowa* are food of several animals in the tropical rainforests (Liu *et al.* 2002). The three species can germinate in shade environments and their regeneration can come from the seedlings pre-existing in shade. Field observation discovered that most seedlings of the three *Garcinia* species grow slowly, producing two opposite leaves rhythmically, and the young leaves need more than one month to be mature (the author's observation), and the leaves have life-spans of >4 y (the author's observation).

Seeds of these *Garcinia* species were collected randomly from different mother trees from different forests in Xishuangbanna and were germinated in a LI seedbed filled with sand. One hundred twenty seedlings with two to four leaves per species were transplanted into pots (12 000 cm^3, 0.35-m-tall) containing forest soil obtained from a forest, with one seedling per pot. Fifty seedlings per species were placed in LI and MI, respectively, and 20 seedlings per species in HI. The shading was provided by neutral density shade net. A slow-release chemical fertilizer (N : P : K = 10 : 10 : 5, *Xiangfeng*, Yunnan, China) was applied at 5 g per pot every 30 d. The pots were watered 3–4 times per week to maintain the soil moisture. To minimize the effects of micro-environmental heterogeneity within the shade plots, the pots were rotated randomly every 14 d. The daily maximum photosynthetic photon flux density (PPFD) of full sun averaged 2 012 μmol m^{-2} s^{-1}, which was recorded with quantum sensors (*190SA, Li-Cor*, Lincoln, NE, USA).

After three-month growth under the three *I*, most of the seedlings in MI and HI had produced at least eight mature leaves. Both *G. cowa* and *G. bracteata* in LI had six leaves, while *G. xanthochymus* in LI just had two to four leaves. Forty seedlings per species in LI and MI, respectively, were transferred to HI except that only 14 seedlings (with 4 leaves) of *G. xanthochymus* in LI were transferred to HI. LH_1 and LH_2 denote LI→HI and MI→HI transfer hereafter. This experiment was done in the season with a heavy fog every morning, which disappeared at about 11: 30–12: 00. No rain fell during the experimental period. The transferred seedlings were watered and fertilized following the method mentioned above.

Measurements of leaf Chl fluorescence and gas exchange: Three to four LH_1 and LH_2 seedlings of each species were chosen for measurements of Chl fluorescence, P_N, and R_D. An uppermost, fully developed leaf was labelled in each seedling and monitored over two months (from 9 November 2002 to 10 January 2003). Leaf Chl fluorescence at dawn was measured by a portable pulse-modulated fluorometer (*FMS 2*, *Hansatech*, Norfolk, UK). After leaves were dark-adapted for 30 min, the minimum fluorescence yield (F_0) was measured under a weak modulating beam, and then a saturating pulse (5 000 μmol m^{-2} s^{-1}, 0.8 s) was used to determine the maximum fluorescence yield (F_m). When the fluorescence yield dropped from F_m to close to F_0, an "actinic light" source (400 μmol m^{-2} s^{-1}) was switched on for 150 s, and steady-state fluorescence yield (F_s) was determined. Then, the saturating beam was used again to measure F_m'. The maximum photochemical efficiency of photosystem 2 (PS2) was calculated as: $F_v/F_m = (F_m - F_0)/F_m$. The non-photochemical quenching coefficient was calculated as: $NPQ = F_m/F_m' - 1$ (Bilger and Björkman 1990).

The same leaves as for monitoring Chl fluorescence were used for the measurements of P_N and R_D. Seedlings were irradiated by natural *I* (about 800 μmol m^{-2} s^{-1} under HI) for 20–30 min after fog disappeared, then the P_N at 800 μmol m^{-2} s^{-1} was measured by a portable photosynthetic system (*LI-6400, Li-Cor*, Lincoln, NE, USA), at the CO_2 concentration of about 365 μmol mol^{-1}, the leaf temperature of 25 °C. P_N was recorded when it was stable. R_D was measured after the seedlings were dark-adapted for more than 1 h in the morning.

Leaf Chl_{area} and LMA were monitored for two months in other transferred seedlings than those used for Chl fluorescence and gas-exchange measurement. Leaves were collected from 3–4 seedlings at a time at 09:00–10:00, and one uppermost fully expanded leaf was collected per seedling. They were immediately put into liquid nitrogen and later stored at –70 °C. Leaf discs were taken by a borer and the area of the samples was determined according to the diameter of the borer. Half of leaf samples were used for Chl_{area} determination with a spectrophotometer (*Shimadzu UV 2501*, Kyoto, Japan). Chl was extracted with 80 % acetone, and Chl content was calculated following Arnon (1949). The other half of leaves was dried at 80 °C for 48 h for LMA determination. Chl_{area} and LMA of the LH_1 leaves of *G. xanthochymus* were not analyzed because of the lack of leaf samples.

Seedling mortality and leaf morphological changes: The mortality, leaf shedding, and senescence of transferred seedlings were examined during the two months after transfer. Change of angle between leaf surface and main stem, and leaf bleaching were observed at 08:00–09:00 every 3 d.

The leaf anatomy was examined for seedlings grown under LI, MI, and HI and for LH_1 and LH_2 seedlings after two-month-transfer. Stomatal density was determined from the transparent nail polish prints of the ad- and abaxial leaf surfaces. The stomatal densities were averaged from counts of 10 microscopic fields of 0.228 9 mm^2 on each leaf print. Hand-cut transverse sections were taken from the midst part of a leaf. Thickness of leaf epidermis and cuticle, and palisade and spongy mesophyll were determined with a microscope (*Leica*, Wetzlar, Germany) with a 40× objective and a calibrated ocular micrometer. Leaf samples were taken from 3–4 seedlings. Analysis of variance (ANOVA) and *t*-test were employed to determine the effect of *I* treatments.

Results

Changes in Chl fluorescence characteristics, P_N, and R_D: The leaves grown in LI and MI prior to the seedling transfer to HI had a high F_v/F_m value of 0.85. F_v/F_m decreased continuously on the first 5–7 d after the seedling transfer (Fig. 1), and the extent of this decrease was *G. bracteata* > *G. xanthochymus* > *G. cowa.* F_v/F_m in LH_1 seedlings decreased (31.4, 29.4, and 23.3 % decrease in *G. bracteata, G. xanthochymus*, and *G. cowa*, respectively) more strongly than that of LH_2 seedlings (16.2, 14.7, and 12.8 % decrease correspondingly). F_v/F_m of LH_1 and LH_2 seedlings began to recover from about 7 and 5 d, respectively, and the recovery continued till 3 weeks or so after transfer. After two months, F_v/F_m values were still significantly lower ($p<0.05$) than those before transfer except for *G. cowa* LH_2 seedlings. F_v/F_m of *G. bracteata* LH_1 seedlings did not recover to 0.80. NPQ increased in the first 20 d after transfer and then became stable (Fig. 1). NPQ in the LH_1 seedlings of *G. xanthochymus* and *G. bracteata* increased more strongly than that of the other transferred seedlings. After two months, the final NPQ values of transferred seedlings were significantly higher than those in HI ($p<0.05$).

The trend of decrease in P_N on leaf area basis after the seedling transfer was similar to that of F_v/F_m. P_N decreased over the first several d and then started to recover from about 7 and 4 d after transfer in LH_1 and LH_2 seedlings, respectively, and the recovery continued till about 30 and 20 d in LH_1 and LH_2 seedlings, respectively, for the three species (Fig. 2). The final P_N values were significantly higher ($p<0.05$) than those before transfer in *G. xanthochymus* LH_1 and all *G. cowa* seedlings. The P_N values of *G. cowa* increased by 101.4 and 63.5 % in LH_1 and LH_2 seedlings, respectively, approaching that of the seedlings under HI. In contrast, P_N increased only 38.8 and 31.5 % for *G. bracteata* LH_1 and LH_2 seedlings, respectively, and they were significantly lower than that of seedlings of the same species under HI ($p<0.05$).

After the seedling transfer, R_D increased continuously till about 30 d (Fig. 2). For *G. xanthochymus* LH_1 seedlings and all *G. cowa* seedlings, the final R_D values attained that of seedlings under HI, while for *G. xanthochymus* LH_2 seedlings and all *G. bracteata* seedlings, the final R_D values were still significantly lower than that of the seedlings in HI ($p<0.05$).

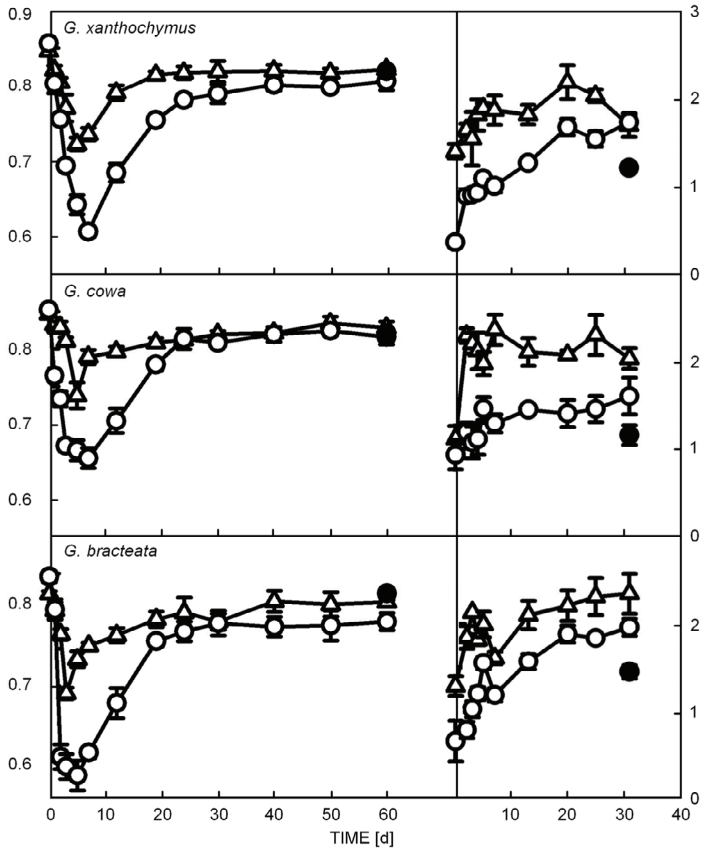

Fig. 1. Changes in (*left*) maximum photochemistry efficiency of photosystem 2 (F_v/F_m) and (*right*) non-photochemical quenching efficiency (NPQ) at dawn in three *Garcinia* species after irradiation. Means ± SE of 3–4 replicate measurements in 3–4 seedlings. Δ transferred from 12.5 % (MI) to 40 % (HI) of full sun; ○ transferred from 4.5 % (LI) to HI; ● under HI.

Changes in Chl_{area} **and LMA**: After the seedling transfer, Chl (*a*+*b*) content per unit area continued to decrease till about 12 d. Then it began to recover, and the recovery continued till about 30 d (Fig. 3). After two months, leaf Chl_{area} of transferred seedlings attained the values before transfer, except that of *G. bracteata* was significantly lower than that before transfer ($p<0.05$). Chl *a*/*b* of the three species did not change significantly (data not shown).

LMA decreased within the first 2 d after transfer and then increased gradually till about 15 d in *G. bracteata* and till 30 d in *G. xanthochymus* and *G. cowa* (Fig. 3). After two months, LMA of all transferred seedlings approached that of seedlings in HI.

Seedling mortality and leaf morphological changes: During the two months, mortality of transferred seedlings, leaf shedding, apparent senescence of the mature leaves, and new leaves were not observed in all transferred seedlings. Leaf angle change was not observed in all mature leaves of transferred seedlings, but from about 15 d the uppermost young leaves of *G. cowa* LH_1 seedlings tilted towards the main stem to reduce the absorption of photons. The young leaves of *G. bracteata*

LH_1 seedlings stopped growing and their final size was about half of those matured in LI before transfer. Moreover, in LH_1 seedlings of the three species, Chl development was inhibited in most young leaves that appeared light-green till the end of the experiment.

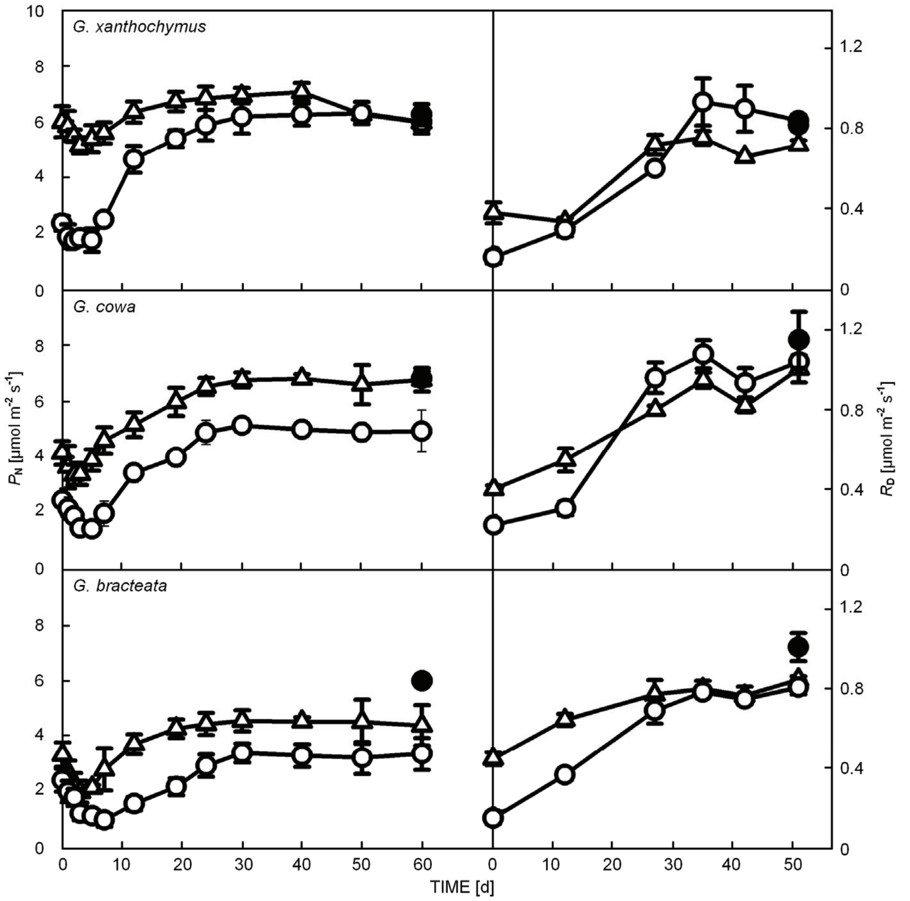

Fig. 2. Changes in (*left*) net photosynthetic rate, P_N at 800 μmol m^{-2} s^{-1} and (*right*) leaf dark respiration rate (R_D) in the three *Garcinia* species after irradiation. The legends are the same as in Fig. 1.

The three species had stomata only on abaxial leaf surfaces. Before transfer, *G. bracteata* had the lowest leaf thickness, while *G. cowa* had the lowest spongy/palisade ratio. The leaf thickness did not increase after transfer, but the spongy/palisade thickness ratio of all *G. cowa* seedlings and *G. bracteata* LH_1 seedlings significantly decreased ($p<0.05$) because of the increase in the length of palisade cells and decrease in the thickness of spongy mesophyll (Table 1).

Discussion

Physiological acclimation: The dynamic trends of F_v/F_m in LH_1 and LH_2 leaves of the three *Garcinia* species were similar (Fig. 1). F_v/F_m decreased for 5–7 d after transfer and did not recover completely after two months except for *G. cowa* LH_2 seedlings. This revealed an irreversible loss of photochemical efficiency (Björkman and Demmig 1987), *i.e.* chronic photoinhibition occurred in the three *Garcinia* seedlings after irradiation. This result was consistent with those reported by Kamaluddin and Grace (1992) and Lovelock *et al.* (1994). Mulkey and Pearcy (1992) considered that the effect of long time direct sunlight combined with high leaf temperature was the most likely cause of irreversible loss in photochemistry after irradiation. Long *et al.* (1994) suggested that the long-term photoinhibition in the exposed seedlings is likely to be associated with a lack of increase in photon energy dissipation capacity. We found that the decline of F_v/F_m in the first days after seedling transfer was mainly caused by both F_0 increase and F_m decrease (data not shown). Decrease of F_v/F_m was the largest in *G. bracteata* and smallest in *G. cow*a among the three species, indicating that *G. bracteata* suffered the most severe photoinhibition. When exposed to higher irradiance, *G. cowa* can more easily survive the new environment than *G. xanthochymus* and *G. bracteata*. Similarly, LH_1 seedlings experienced more severe photoinhibition than LH_2 seedlings which indicated that the seedlings grown in LI were inhibited more than those in MI. NPQ is now believed to be the most important pathway to dissipate energy (Demmig-Adams and Adams 1992). The increase in NPQ in all *Garcinia* species after irradiation (Fig. 1) indicated that energy dissipation is important for the recovery of F_v/F_m, especially for the LH_1 seedlings that had higher NPQ than LH_2 seedlings. The increase in NPQ of the LH_1 seedlings of *G. xanthochymus* and *G. bracteata* was more than that of *G. cowa* LH_1 seedlings, indicating that *G. cowa* may have higher ability of photon energy use.

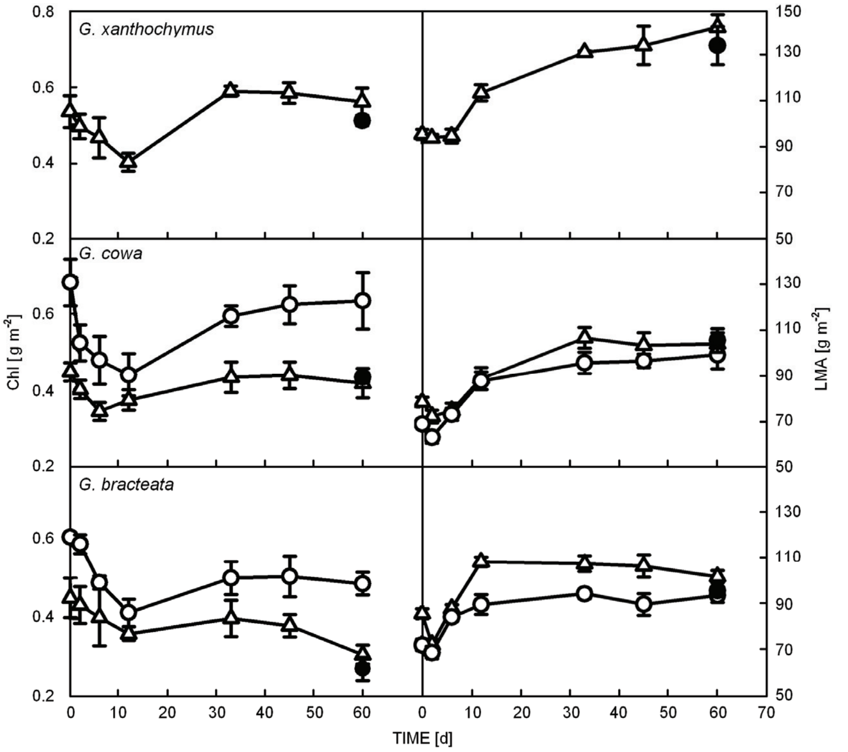

Fig. 3. Changes in (*left*) leaf chlorophyll (Chl) content per unit area and (*right*) leaf mass per unit area (LMA) in three *Garcinia* species. The legends are the same as in Fig. 1.

Similar to F_v/F_m, Chl_{area} declined after transfer, and the minimum values were reached around 12 d (Fig. 3), by then F_v/F_m and P_N had increased for more than 5 d (Figs. 1 and 2). This result agreed with the fact that Chl bleaching occurred after a certain degree of photoinhibition (Powles 1984). The final Chl_{area} of *G. xanthochymus* and *G. cowa* attained the values before transfer, but it was significantly lower than that value before transfer for *G. bracteata*, consistent with the more severe photoinhibition of the latter species. The recovery of Chl_{area} was slower than that of F_v/F_m and P_N, indicating that Chl recovery could depend on F_v/F_m and P_N recovery.

The trend of P_N change after the seedling exposure was similar to those of F_v/F_m and Chl_{area} (Figs. 1-3). Photoinhibition and leaf bleaching may be the main causes of the suppression of P_N. The final P_N after two-month acclimation was higher than that before transfer for *G. cowa* and *G. xanthochymus* or recovered to the value before transfer for *G. bracteata* (Fig. 2), although F_v/F_m and Chl_{area} did not recover completely (Figs. 1 and 3). The increase in P_N can be caused by increasing activity of ribulose-1,5-bisphosphate carboxylase/oxygenase in chloroplast, or number and volume of chloroplasts, or combined impact of them. Avalos and Mulkey (1999) suggested that P_N acclimation seems to depend on accumulation of photosynthetic enzymes in the mature leaves after irradiation. The rearrangement of chloroplasts in palisade cells to cover more cell membrane area was reported in *Chenopodium album* (Oguchi *et al.* 2003). The increase in P_N of *G. bracteata* was the smallest among the three species, consistent to its severe photoinhibition, stronger increase in NPQ, and leaf bleaching after irradiation (Fig. 1). With P_N increase, R_D increased significantly in transferred leaves (Fig. 2), which may reflect the higher cost of maintaining the photosynthetic apparatus and increased investment in photo-protective mechanisms in open environments (Demmig-Adams and Adams 1992, Chazdon *et al.* 1996). Comparatively, *G. bracteata* increased P_N less and thus had limited photon use efficiency in HI. This is in agreement with its severer photoinhibition and leaf bleaching after irradiation (Figs. 1 and 3).

Table 1. Stomatal density, thickness of leaf, palisade and spongy mesophyll, and adaxial and abaxial epidermis, and spongy/palisade ratio in the seedlings of three *Garcinia* species (*G. xanthochymus = G.x., G. cowa = G.c., G. bracteata = G.b.*) grown under three irradiances, *I* (LI, MI, HI) and plants transferred from LI to HI (LH_1) or MI to HI (LH_2). *The different small and capital letters* indicate significant differences in the means of the same traits among light regimes (*ANOVA*, $p<0.05$) and between the transferred and control seedlings (LI or MI) of the same species, respectively. Means ± 1 SE. $n = 3–5$.

Species	*I*	Stomata density [No mm^{-2}]	Thickness [µm] leaf	epidermis adaxial	abaxial	palisade	Spongy	Spongy/palisade ratio
G..x.	LI	148±6a	360.4±15.7 a	21.7±2.2a	16.9±1.5a	41.3±3.1a	281.7±16.2a	6.85±0.21a
	MI	161±3aA	401.6±5.3 abA	28.2±2.6aA	21.9±3.1aA	67.3±9.0abA	285.9±0.9aA	4.32±0.56bA
	HI	184±8bA	413.3±9.6 b	24.7±1.4aA	18.1±3.2aA	88.1±0.7bA	282.2±15.3aA	3.20±0.15bA
	LH_2	161±2A	405.6±10.3A	25.0±0.5A	13.1±0.7A	73.1±9.0A	299.7±16.1A	4.29±0.79A
G. c.	LI	85±12aA	358.8±9.5a	22.5±1.4aA	16.7±0.8aA	62.5±2.6aA	268.2±2.2aA	4.31±0.20aA
	MI	92±3aA	373.6±12.5aA	27.2±2.0abA	19.2±2.1aA	78.9±1.8bA	239.8±10.8bA	3.04±0.07bA
	HI	112±1b	436.0±9.9b	30.7±1.6b	18.9±2.3a	114.7±4.2c	270.0±15.7ab	2.37±0.20c
	LH_1	85±2A	376.9±20.7A	29.7±2.7B	21.9±1.7B	107.2±6.2B	227.1±20.3B	2.11±0.07B
	LH_2	91±2A	363.1±3.1A	28.5±1.4A	18.9±0.7A	98.9±7.3B	207.9±9.7B	2.14±0.27B
G..b.	LI	158±12aA	239.8±6.6aA	21.1±2.5aA	16.4±1.5aA	38.6±1.5aA	170.0±5.8aA	4.41±0.17aA
	MI	195±11abA	284.5±5.1bA	25.6±1.9aA	15.6±2.9aA	54.3±8.4abA	186.5±2.9aA	3.62±0.62abA
	HI	233±8bA	295.6±19.3bA	26.9±5.8aA	9.9±0.4bA	64.7±4.9bA	181.8±2.7aA	2.85±0.23bcA
	LH_1	165±15A	272.0±14.8A	18.9±1.1A	14.2±2.2A	58.5±5.5B	179.1±12.9A	3.11±0.36B
	LH_2	214±7A	279.2±11.7A	22.5±2.68A	12.5±0.8A	56.7±2.4A	187.8±6.9A	3.32±0.10A

Morphological and anatomical acclimation: LMA of LI and MI leaves changed greatly after transfer to HI (Fig. 3). LMA decreased within 2 d after transfer and then continuously increased until about 30 d in *G. xanthochymus* and *G. cowa* and 10 d in *G. bracteata*. Kamaluddin and Grace (1992) found LMA in *Bischofia javanica* continuously increased after being transferred from low to high *I*. Why did LMA decrease within 2 d in our study? During the study period, fog disappeared at about 11:30–12:00 and then a high *I* to 800 µmol m^{-2} s^{-1} was received suddenly at noon in HI plot, which led to photoinhibition of the seedlings that were just transferred from LI and MI. Thus whole-plant carbon fixation was negatively affected at the first day after transfer and saccharides were consumed through R_D. Thus, decrease of LMA in the first 2 d in the exposed seedlings was probably caused by the reduction of starch and sugar in the leaves (Björkman 1981). Although the leaf thickness did not increase, the final LMA of transferred leaves reached that of leaves originally grown in HI. Kamaluddin and Grace (1992) considered the increase in LMA after seedling exposure might be caused by an increase in leaf density. The accumulation of starch, sugars, and inorganic solutes (Björkman 1981), and the loss of water may be the cause of leaf density increase.

The leaves grown in LI of the three species were almost horizontally extended. This is an adaptive strategy to grow in LI by maximizing photon interception of leaves. After irradiation, the mature leaves did not change angle, but the young leaves of *G. cowa* LH_1 seedlings moved upwards to reduce photon absorption. Other studies have also found that change in leaf angle close to vertical orientation protected leaves from absorbing excess *I* when exposed to high *I* (Ludlow and Björkman 1984) and improve water use efficiency (Chiariella *et al.* 1987). The mature leaves of the three *Garcinia* species were not able to change their orientation and thus suffered from the excess *I* after irradiation. After irradiation, the three *Garcinia* species may mainly depend on the mature leaves to maintain carbon gain balance before new leaves were well-developed. Sims and Pearcy (1992) also found that young leaves of *Alocasia macrorrhiza* grown in shade did not acclimate completely when transferred to full sun until the new leaf had developed.

Lovelock *et al.* (1994) have suggested that the species having rhythmic growth pattern like *Barringtonia* species producing rosettes of leaves may result in slow growth and reduce competitive abilities compared to continuously growing species. Under all of the three growth *I* levels, the three *Garcinia* species grew slowly and produced opposite leaves rhythmically. In the transferred seedlings, new leaves did not appear in two months, which led to a slow whole-plant acclimation to new *I* environment. It indicated the three *Garcinia* species were not mainly dependent on producing new leaves and shedding mature leaves in a short time to acclimate to new high *I*. That is, for the three *Garcinia* species with long-lived leaves, the main strategy to acclimate to irradiation was to increase the tolerance of the mature leaves. Lovelock *et al.* (1998) reported that the species with long-lived leaves were more tolerant of an increase in *I* than the species with short-lived leaves. This characteristic is not helpful for the three *Garcinia* species to compete with other fast-growing species when a gap forms or expands.

No death of the transferred seedlings occurred during the study period, indicating the HI alone was not fatal for the three *Garcinia* species. Turner (1990) reported higher mortality of shade suppressed seedlings during dry season, and Lovelock *et al.* (1994) also found that although tropical tree seedlings commonly experience photoinhibition after gap formation, the seedlings can usually survive unless other stresses co-occur at the same time. Variation in soil conditions and nutrient availability within open areas affected seedling acclimation response after logging (Scholes *et al.* 1997). In our study, the transferred seedlings were well watered and fertilized, which alleviated the stresses compared with those in natural gaps. Although the three *Garcinia* species are all sub-canopy trees, they differentiated in physiological and anatomical acclimation to the *I* transfer to some degree. *G. bracteata* was the most vulnerable to HI exposure. *G. cowa* showed a high ability of acclimation to *I*. For all of the three species, the LH_1 seedlings were more vulnerable to *I* than LH_2 seedlings, indicating that gap expansion has smaller adverse effect than gap formation on the pre-existing seedlings.

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