

Light-sensitivity of chlorophyll formation in the leaves of *Ficus microcarpa* cv. Golden-leaves

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Abstract. We compared the influence of light intensity and illumination time on the chlorophyll (Chl) and carotenoid content, and on the Chl *a/b* and carotenoid/Chl ratios of leaves from the same shoot of normal and of Golden-leaves fig (*Ficus microcarpa* cv. Golden-leaves). Golden-leaves fig possesses light-sensitive leaves with various amounts of Chl and carotenoids and various ratios of Chl *a/b* and carotenoids/Chl under the same illumination. It appears that the deficiency of Chl is not directly associated with the loss of carotenoid, but the alteration of Chl *a/b* ratios might be related to that of the carotenoids/Chl ratio. It is likely that the relationship between synthesis of Chl *b* and carotenoids is more intimate than that of Chl *a* and carotenoids. The unique characteristics of Golden-leaves fig makes this plant a useful tool for studying the photobiochemistry and photomorphogenesis of higher-plant chloroplasts.

Keywords: Carotenoid; Chlorophyll; Golden-leaves fig; Illumination duration; Illumination intensity.

Introduction

Chl-deficient mutants have been widely used to study the biosynthetic pathway of Chl and the biogenesis of the photosynthetic apparatus in higher plants (Somerville, 1986). These mutants have been reported in barley, maize, pea, sweetclover, wheat, *Chlamydomonas reinhardtii* (see references in Markwell et al., 1986), rice (Terao et al., 1985), soybean (Droppa et al., 1988), sugar beet (Abadia et al., 1985), and *Arabidopsis thaliana* (Hirono and Redei, 1963). They are generally divided into two groups: Chl *b*-lacking mutants, which contain no detectable Chl *b*, and Chl *b*-reduced mutants, which contain reduced amounts of this pigment (King, 1991). Some of these mutants are sensitive to light (Hopkins et al., 1980; Markwell et al., 1986; Allen et al., 1988; Greene et al., 1988) and others are sensitive to temperature (Markwell et al., 1986). Among the temperature-sensitive mutants, fewer mutants were found to be sensitive to light intensity and/or photoperiod (Yang et al., 1993). Temperature sensitivity has been proposed to be a general phenomenon among the Chl-deficient mutants (Yang et al., 1990; Markwell and Osterman, 1992) that may attributable to blocked Mg-chelatase activity (Falbel and Staehelin, 1994). According to their response to the growth temperature, Chl-deficient mutants can be placed within two major biochemical phenotypes: 1) those mutants that adjust only their Chl content and 2) those that simultaneously adjust Chl content and Chl *a/b* ratio (Yang et al., 1993).

In a previous study, Golden-leaves fig was found to be sensitive to temperature (Chen and Yang, 1995) and to

possess light-sensitive leaves containing various amounts of Chl and having various Chl *a/b* ratios on the same shoot under the same illumination (Yang et al., unpublished data). In this report, we further study these traits and show that this plant is sensitive to intensity and duration of illumination.

Materials and Methods

Normal and Golden-leaves (*Ficus microcarpa* cv. Golden-leaves) fig plants about one-year old and 50-cm tall were purchased from a local nursery. Plants were grown in sterile soils in a controlled-environment growth chamber with a 12/12 h photoperiod, 25°C temperature, 60–70% relative humidity, and various illumination intensities.

Following the extraction of liquid-nitrogen-frozen leaves with 80% (v/v) acetone, Chl concentration and Chl *a/b* ratios were determined according to the method of Porra et al. (1989). The concentration of total carotenoids was measured by the method of Kirk and Allen (1965). Room temperature absorbance was obtained with a Hitachi U2000 UV-visible spectrophotometer.

Three determinations were made for each experiment and similar patterns were obtained. Only one of the patterns is presented in this paper.

Results and Discussion

The various developmental stages of the leaves of Golden-leaves fig can be classified by their color (Chen and Yang, 1995). The new and yellow leaves on the top are in the young stage (the first four or five leaves), yel-

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low-green leaves are in the greening stage (the sixth to eighth or ninth), the green or dark green leaves are in the mature and old stages. The color is reversible between conditions of restrictive (high) and nonrestrictive (low) illumination intensity.

Chl Formation and Phyllotaxy

To examine the Chl and carotenoid contents, and the Chl *a/b* and carotenoid/Chl ratios, thirteen leaves in the young, greening, and old developmental stages were collected from the top section of the same shoot of normal and of Golden-leaves figs, grown in a growth chamber for 3 weeks under a photonflux of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. The Chl content increased in both genotypes as the leaves aged (Figure 1). While the Chl content of Golden-leaves fig increased from about $150 \mu\text{g g}^{-1}$ fresh leaf in the young stage to about $1,200 \mu\text{g g}^{-1}$ in the old stage, the Chl content of normal fig increased from about $550 \mu\text{g g}^{-1}$ to $1,700 \mu\text{g g}^{-1}$. The greening leaves of both genotypes accumulated large amounts of Chl. The synthesis of Chl in the greening stage exceeded its degradation, but these two processes balanced out in the mature and old stages.

While the changing Chl content in both genotypes displayed a similar pattern, the Chl *a/b* ratio followed a different pattern (Figure 2). The Chl *a/b* ratio of normal fig remained constant (3:1) during the development of the first thirteen leaves, suggesting that the synthesis and deg-

radation of Chl *a* and *b* were well balanced. The Chl *a/b* ratio of Golden-leaves fig, on the other hand, increased rapidly from approximately 3:1 to 7:1 during the young stage, then as leaves were greening, declined gradually to a ratio similar to that in normal leaves. In a separate study, the Chl content gradually increased leaf-by-leaf during the greening stage, but the Chl *a/b* ratio decreased steadily (Yang et al., unpublished data). In Golden-leaves fig, the accumulation of Chl *a* gradually exceeded that of Chl *b* leaf-by-leaf during the young stage, causing a gradual increase in Chl *a/b* ratio. During the greening stage, the accumulation of Chl *b* exceeded Chl *a*, resulting in a gradual decrease in the ratio; The synthesis and degradation of Chl *a* and *b* were well balanced in the mature and old stages, resulting in a constant Chl *a/b* ratio.

Carotenoid Formation and Phyllotaxy

In both genotypes, the accumulation pattern of carotenoid differed from that of Chl (Figures 1 and 3). The carotenoid content increased as the leaf aged. The carotenoid/Chl ratio in normal fig remained almost constant during all developmental stages. The carotenoid/Chl ratio in Golden-leaves fig increased gradually from about 1.8:1 to about 2.4:1 during the young stage, then as the leaves were greening, declined to approximately unity—similar to that in normal fig. It appears, that the accumulation of Chl is not directly associated with that of carotenoid. This

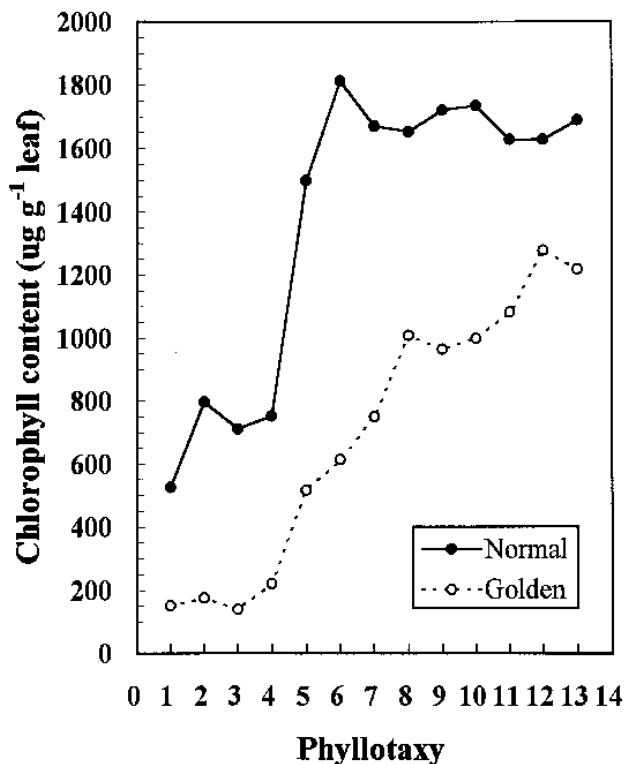


Figure 1. Changing Chl content in various developmental stages of normal and Golden-leaves figs. Plants were grown in a growth chamber for 4 weeks at 25°C with a photonflux of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 60–70% relative humidity.

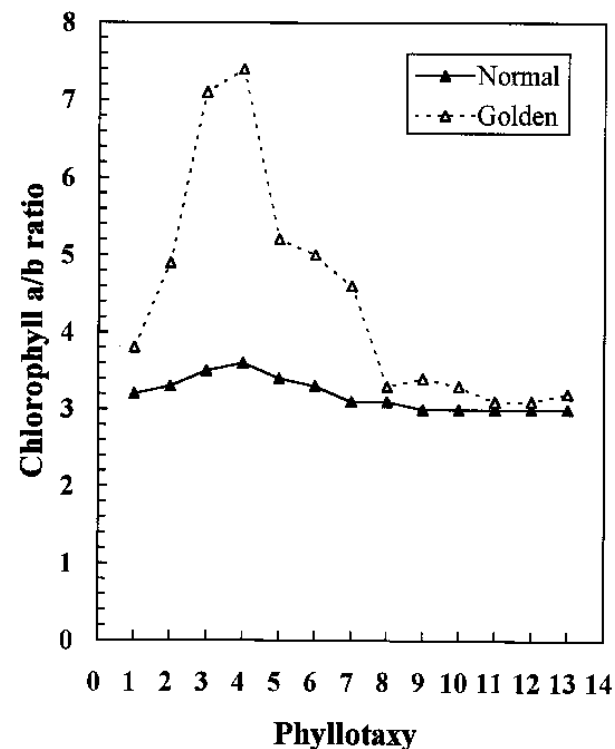


Figure 2. Alteration of Chl *a/b* ratio in various developmental stages of normal and Golden-leaves figs. Plants were grown under the same conditions described for Figure 1.

finding contrast with the findings of Mayfield and Taylor (1984), which indicate that the deficiency in carotenoids may cause a decrease in Chl content. It appears, however, that in both genotypes, the change in Chl *a/b* ratios is related to that of carotenoid/Chl ratios on the same shoot (Figures 2 and 4).

Light Intensity and Chl Formation

To examine the relationship between illumination intensity and pigment formation, normal and Golden-leaves figs were grown for 3 weeks under photonfluxes of 50, 100, 300, and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The fifth leaf (which was in the greening stage) of each shoot was harvested, and Chl and carotenoids contents, and Chl *a/b* and carotenoid/Chl ratios were determined. In normal fig, the Chl content and Chl *a/b* ratio remained constant as the illumination intensity increased. In Golden-leaves fig, the Chl content decreased from 1,300 $\mu\text{g g}^{-1}$ fresh leaf to approximately 50 μg and the Chl *a/b* ratio increased from 3:1 to 9:1 or higher (Figures 5 and 6). In Golden-leaves fig, the greater the illumination intensity, the less the Chl content but the larger the Chl *a/b* ratio. This suggests that illumination intensity has a negative influence on the synthesis or accumulation of Chl, with Chl *b* biosynthetic machinery being more seriously damaged than that of Chl *a*, resulting in a gradual increase in Chl *a/b* ratio.

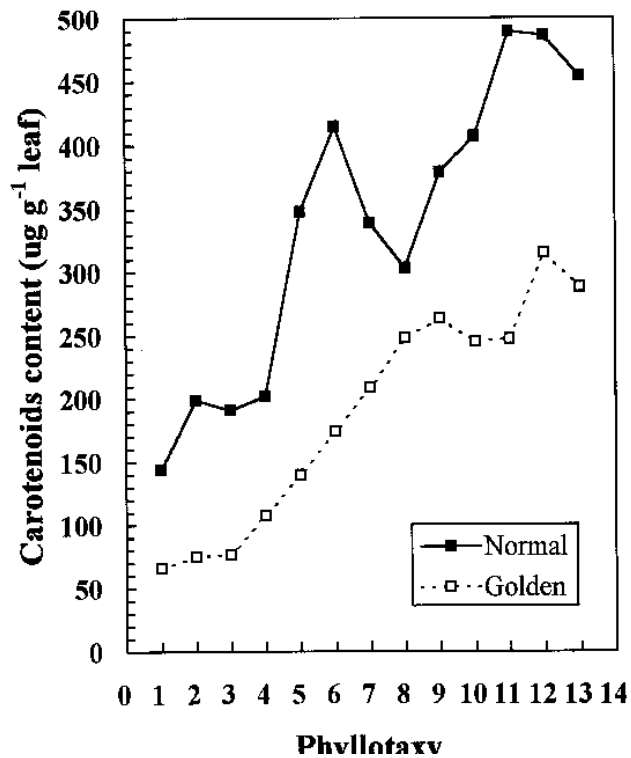


Figure 3. Changing carotenoid content in various developmental stages of normal and Golden-leaves figs. Plants were grown under the same conditions described for Figure 1.

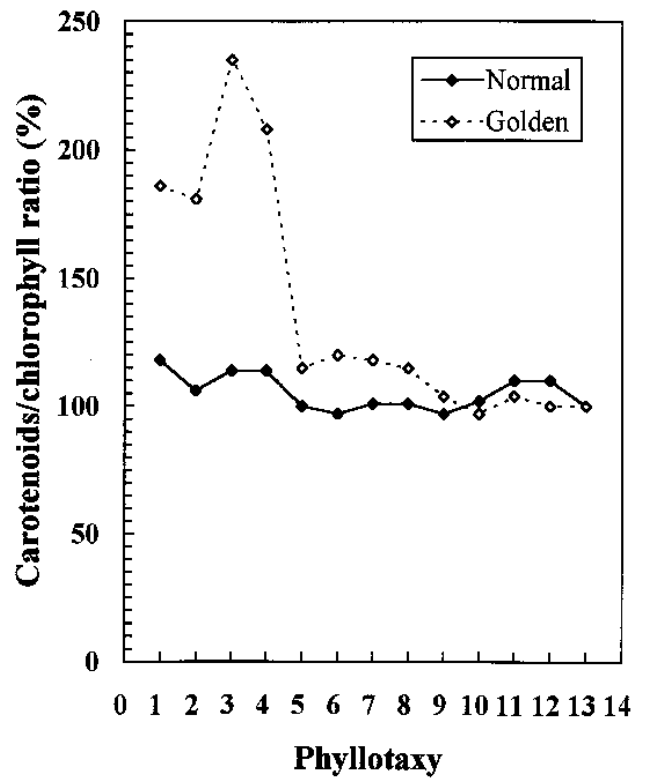


Figure 4. Alteration of carotenoid/Chl ratio in various developmental stages of normal and Golden-leaves figs. Plants were grown under the same conditions described for Figure 1.

Light Intensity and Carotenoid Formation

As light intensity increased from 50 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the carotenoid content of normal fig remained about 340 $\mu\text{g g}^{-1}$ fresh leaf, whereas that of Golden-leaves fig decreased from 280 to 80 $\mu\text{g g}^{-1}$ (Figure 7). Meanwhile, the relative carotenoid/Chl ratio of normal fig remained about unity at all illumination intensities, but that of Golden-leaves fig increased 11.5 fold (Figure 8). As described above, it appears that the accumulation of Chl is not directly associated with the formation of carotenoid, although the change in Chl *a/b* ratio might be related to carotenoid/Chl ratio.

Duration of Illumination and Chl Formation

To examine the relationship between the duration of illumination and pigment formation, normal and Golden-leaves fig plants were grown for the indicated duration in a photonflux of 50 or 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The second and ninth leaf of each shoot were harvested and the Chl *a/b* ratios determined (Figures 9 and 10). Regardless of illumination intensity, the Chl *a/b* ratio of young leaves of both genotypes was higher than that of the corresponding old leaves. While the Chl *a/b* ratio of young leaves of Golden-leaves fig fluctuated between 4:1 and 8.5:1 under high illumination intensity, they fluctuated between 3.5:1 and 4.8:1 under low light intensity. The difference between the Chl *a/b* ratios of young and old leaves of Golden-leaves fig was greater than that between those of

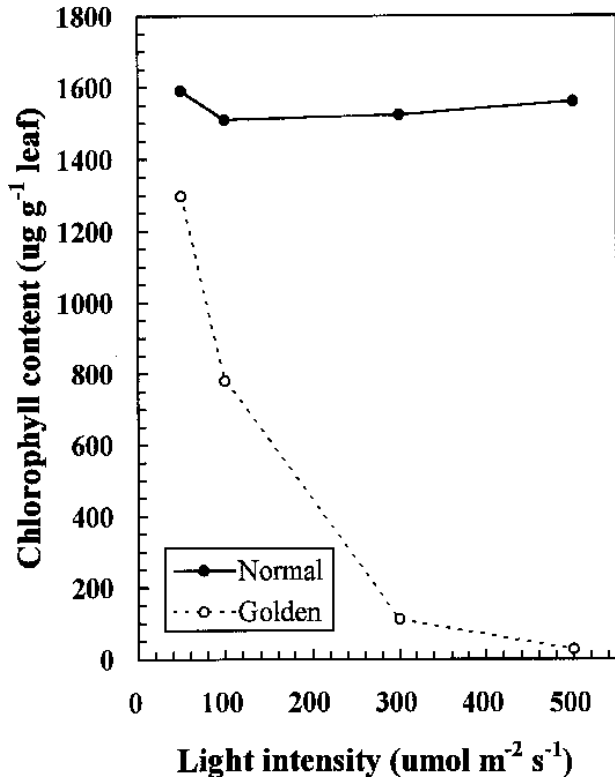


Figure 5. Influence of illumination intensity on Chl content in young leaves of normal and Golden-leaves figs. Plants were grown in a growth chamber for 3 weeks at 25°C, the indicated illumination intensity, and 60–70% relative humidity .

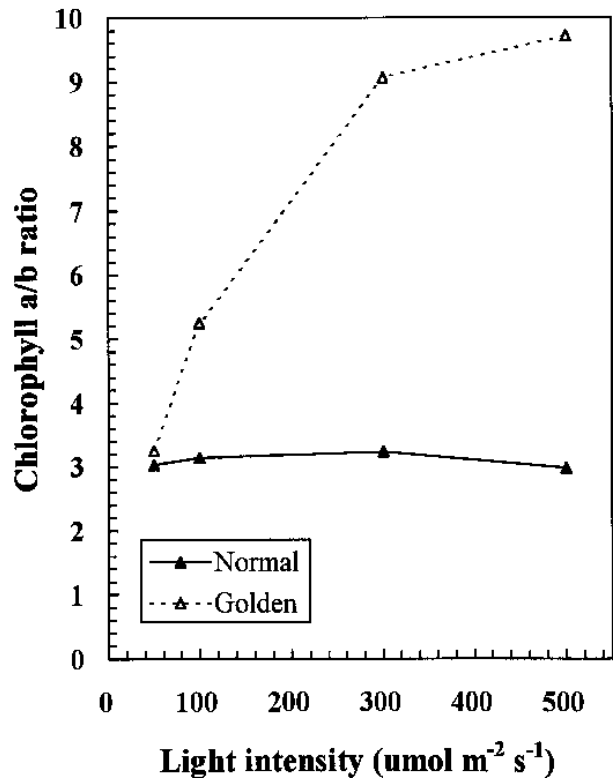


Figure 6. Influence of illumination intensity on Chl a/b ratio in young leaves of normal and Golden-leaves figs. Plants were grown under the same conditions described for Figure 5.

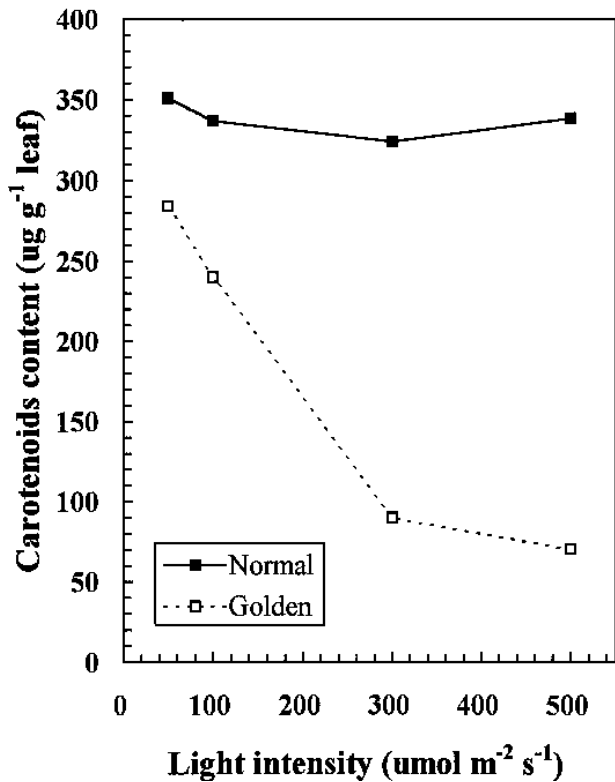


Figure 7. Influence of illumination intensity on carotenoid content in young leaves of normal and Golden-leaves figs. Plants were grown under the same conditions described for Figure 5.

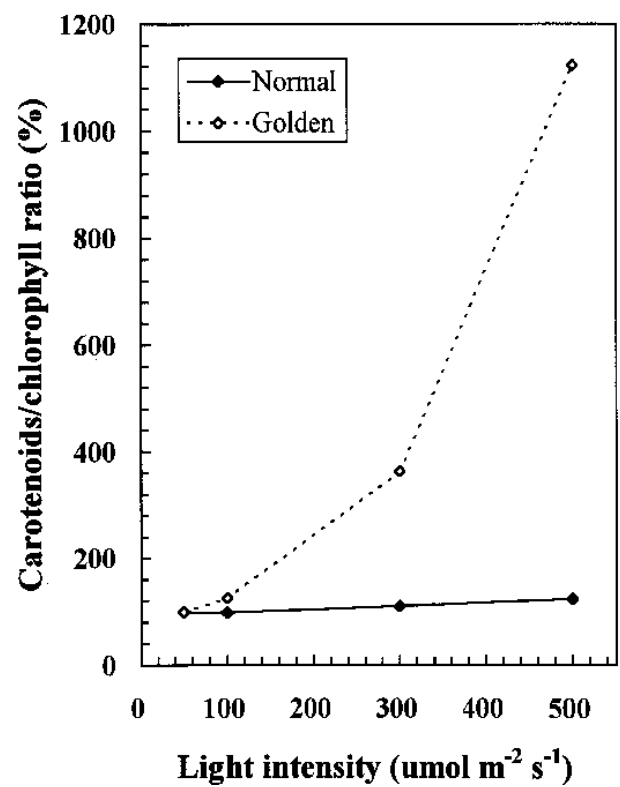


Figure 8. Influence of illumination intensity on carotenoid/Chl ratio in young leaves of normal and Golden-leaves figs. Plants were grown under the same conditions described for Figure 5.

young and old normal fig leaves, especially under high illumination intensity, irrespective of the duration of illumination. Under low illumination or shade conditions the young leaf of Golden-leaves fig was sensitive to light during the first 3 weeks and then behaved similar to the others (Figure 10). Under a photonflux of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$, the young leaf of normal fig was sensitive to light after 3 weeks of treatment. The Chl *a/b* ratios were never higher than 4.3:1, but the ratio in old leaf remained nearly constant at approximately 3:1. The Chl *a/b* ratio in old leaves of Golden-leaves fig began to increase after 3 weeks of treatment, while that in young leaves began to change after 1 week or less (Figure 9). These results indicate that duration of illumination influenced Golden-leaves fig much more than it did normal fig—the leaves of Golden-leaves fig were much more sensitive to light than were those of normal fig. This further suggests that high illumination intensity partially inhibits Chl biosynthetic capacity, especially that of Chl *b*, resulting in a low Chl content and a high Chl *a/b* ratio.

The leaves on the same shoot of *F. microcarpa* cv. Golden-leaves contained different quantities of Chl and had different Chl *a/b* ratios according to their developmental stage, and the Chl content and Chl *a/b* ratio could be regulated by temperature (Chen and Yang, 1995). In this report, we showed that the accumulation of Chl is

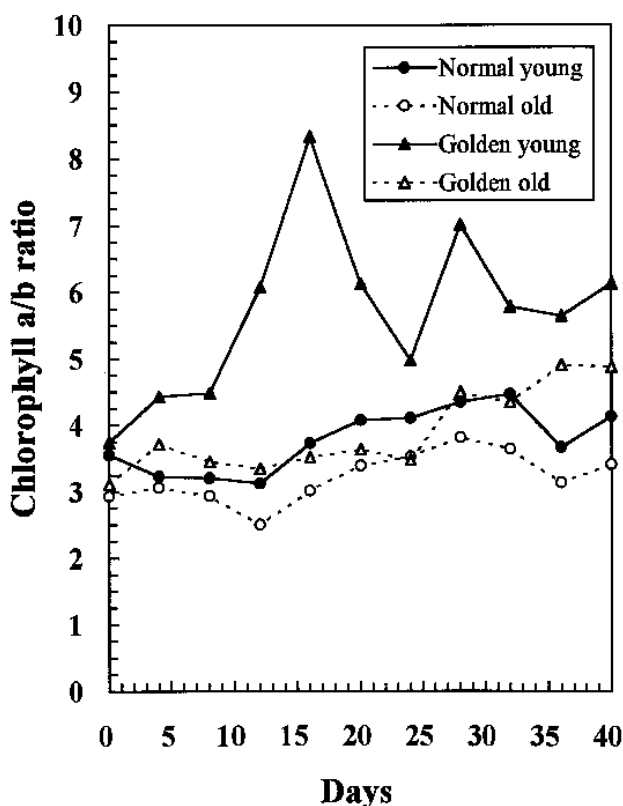


Figure 9. Influence of illumination time on Chl *a/b* ratios in the young and old leaves of normal and Golden-leaves figs grown at 25°C under a high light condition ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$) with 60–70% relative humidity.

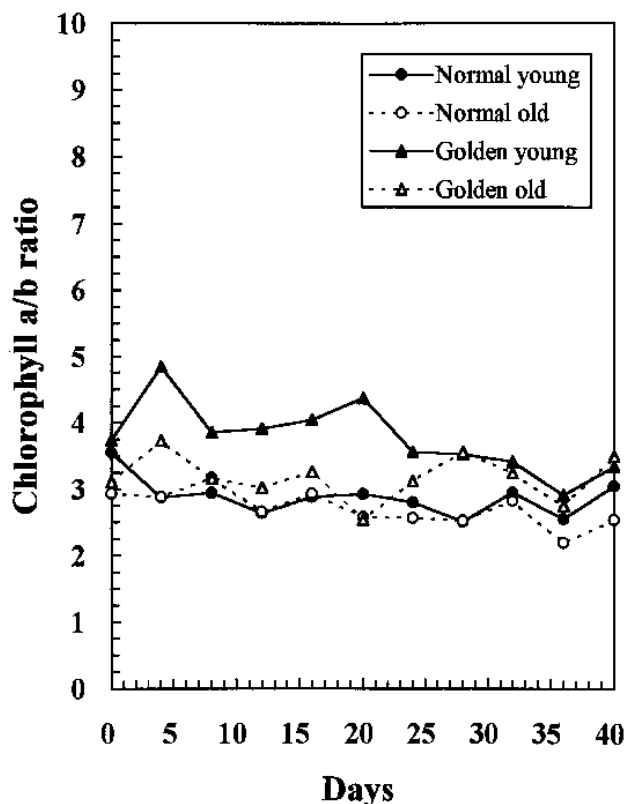


Figure 10. Influence of illumination time on Chl *a/b* ratios in the young and old leaves of normal and Golden-leaves figs grown at 25°C under a weak light condition ($50 \mu\text{mol m}^{-2} \text{s}^{-1}$) with 60–70% relative humidity.

sensitive to illumination intensity and duration, and that in the leaves of Golden-leaves fig, the changes in Chl *a/b* ratio might be directly related to the carotenoid/Chl ratio. The leaves of Golden-leaves fig became Chl-deficient under intense illumination, and regained complete Chl biosynthetic capacity after sufficient exposure to dim or low illumination. Chl-deficient mutants of algae and higher plants have been widely used in the study of photosynthetic apparatus and Chl biosynthesis (Somerville, 1986). Regardless of species, Chl-deficient mutants produce a constant amount of Chl and have a constant Chl *a/b* ratio in all parts of the plant in their mature stage. The leaves of Golden-leaves fig, however, display unique characteristics. By taking advantage of their light sensitivity, the leaves of Golden-leaves fig could be made Chl-deficient by exposing them to a restrictive illumination intensity for a sufficient duration. The unique ability to change biochemical characteristics in response to the illumination intensity or duration suggests that Golden-leaves fig can serve as a useful system for studying the photobiochemistry and photomorphogenesis of higher plant chloroplasts.

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Literature Cited

- Abadia, J., R. E. Glick., S. E. Taylor., N. Terry, and A. Melis. 1985. Photochemical apparatus organization in the chloroplasts of two *Beta vulgaris* genotypes. *Plant Physiol.* **79**: 872–878.
- Allen, K. D., M. E. Duysen, and L. A. Staehelin. 1988. Biogenesis of thylakoid membranes is controlled by light intensity in the conditional chlorophyll b-deficient CD3 mutant of wheat. *J. Cell. Biol.* **107**: 907–919.
- Chen, H. Y. and C. M. Yang. 1995. Temperature-sensitivity of chlorophyll expression in the leaves of *Ficus microcarpa* cv. Golden-leaves. *Proc. Natl. Sci. Coun. B. ROC* **19**(3): (in press).
- Droppa, M., M. I. Ghirardi., G. Horvath, and A. Melis. 1988. Chlorophyll b deficiency in soybean mutants. II. Thylakoid membrane development and differentiation. *Biochim. Biophys. Acta* **932**: 138–145.
- Falbel, T. G. and L. A. Staehelin. 1994. Characterization of a family of chlorophyll-deficient wheat (*Triticum*) and barley (*Hordeum vulgare*) mutants with defects in the magnesium-insertion step of chlorophyll biosynthesis. *Plant Physiol.* **104**: 639–648.
- Greene, B. A., D. A. Allred., D. T. Morishige, and L. A. Staehelin. 1988. Hierarchical response of light harvesting chlorophyll-proteins in a light-sensitive chlorophyll b-deficient mutant of maize. *Plant Physiol.* **87**: 357–364.
- Hirono, Y. and G. P. Redei. 1963. Multiple allelic control of chlorophyll b level in *Arabidopsis thaliana*. *Nature* **197**: 1324–1325.
- Hopkins, W. G., D. B. Hayden, and M. G. Neuffer. 1980. A light-sensitive mutant in maize (*Zea mays* L.) I. Chlorophyll, chlorophyll-protein and ultrastructural studies. *Zeit. Pflanzenphysiol.* **99**: 417–426.
- King, J. 1991. The chlorophyll b-deficient mutants. In J. King (ed.), *The Genetic Basis of Plant Physiological Processes*. Oxford University Press, Inc., pp. 153–166.
- Kirk, J. T. and R. L. Allen. 1965. Dependence of pigment synthesis on protein synthesis. *Biochem. Biophys. Res. Commun.* **21**: 523–530.
- Markwell, J. P., S. J. Danko, H. Bauwe, J. C. Osterman, H. J. Gorz, and F. A. Haskins. 1986. A temperature-sensitive chlorophyll b-deficient mutant of sweetclover (*Melilotus alba*). *Plant Physiol.* **81**: 329–334.
- Markwell, J. P. and J. C. Osterman. 1992. Occurrence of temperature-sensitive phenotypic plasticity in chlorophyll-deficient mutants of *Arabidopsis thaliana*. *Plant Physiol.* **98**: 392–394.
- Mayfield, S. P. and W. C. Taylor. 1984. Carotenoid-deficient maize seedlings fail to accumulate light-harvesting chlorophyll a/b binding protein (LHCP) mRNA. *Eur. J. Biochem.* **144**: 79–84.
- Porra, R. T., W. A. Thompson, and P. E. Kriedelman. 1989. Determination of accurate extractions and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentrations of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta* **975**: 384–394.
- Somerville, C. R. 1986. Analysis of photosynthesis with mutants of higher plants and algae. *Annu. Rev. Plant Physiol.* **37**: 467–507.
- Terao, T., A. Yamashita, and S. Katoh. 1985. Chlorophyll b-deficient mutants of rice. II. Antenna chlorophyll a/b-proteins of photosystem I and II. *Plant Cell Physiol.* **26**: 1369–1377.
- Yang, C. M., J. C. Hsu, and Y. R. Chen. 1993. Light- and temperature-sensitivity of chlorophyll-deficient and virescent mutants. *Taiwania* **38**: 49–56.
- Yang, C. M., J. C. Osterman, and J. P. Markwell. 1990. Temperature sensitivity as a general phenomenon in a collection of chlorophyll-deficient mutants sweetclover (*Melilotus alba*). *Biochem. Genet.* **28**: 31–40.

黃金榕葉葉綠素形成的光敏感性

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本研究比較光強度及照光時間對正常榕及黃金榕同樹枝上葉的葉綠素及類胡蘿蔔素含量，葉綠素 a/b 比及類胡蘿蔔素／葉綠素比的影響。黃金榕擁有對光敏感的葉子。在相同光照下，同樹枝上的黃金榕葉的色素含量，葉綠素 a/b 比及類胡蘿蔔素／葉綠素比都不相同。葉綠素的缺失似乎不直接伴隨著類胡蘿蔔素的缺失；但是，葉綠素 a/b 比的變化可能和類胡蘿蔔素／葉綠素比的變化有關聯。葉綠素 b 的合成與類胡蘿蔔素的關係可能比葉綠素 a 的合成與類胡蘿蔔素的關係更密切。黃金榕葉的獨特特性可能使此植物成為研究高等植物葉綠體光生化學及光形態發生的良好材料。

關鍵詞：類胡蘿蔔素；葉綠素；黃金榕；照光時間；光強度。