Herbivorous insect causes deficiency of pigment-protein complexes in an oval-pointed cecidomyiid gall of Machilus thunbergii leaf

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Abstract. This research compared the chlorophyll biosynthetic and degradation pathways, pigment-protein complexes, and thylakoid morphology of a mature oval-pointed cecidomyiid gall and the infected leaf of host plant Machilus thunbergii Sieb & Zucc (Lauraceae). The mature gall always possesses far less photosynthetic pigment than the infected leaf. The content of anthocyanin and tannin of the gall are much higher than in the infected leaf. Both the mole percent of porphyrin and the ratio of pheophytin/chlorophyllide are much different between the gall and infected leaf, suggesting their chlorophyll biosynthetic and degradation pathways are much different. While the infected leaf may take the degradation pathway of chlorophyll → pheophytin → pheophorbide as the major route, the cecidomyiid gall may take chlorophyll → chlorophyllide → pheophorbide as the major route. The infected leaf still possesses the CPI and CPII pigment-protein complexes fractionated by Thornber system, or the A1, AB1, AB2, AB3 pigment-protein complexes fractionated by the MARS system while the mature gall contains only CPII or AB3. Electron microscopy demonstrated that the mature gall has normal grana and thylakoid morphology. It is still unknown whether the unique deficiency of pigment-protein complexes is ubiquitous and how the cecidomyiid insects cause the deficiency of some pigment-protein complexes.

Keywords: Cecidomyiid gall; Herbivorous insect; Machilus thunbergii leaf; Pigment-protein complexes; Thylakoid.

Introduction

Whether induced by viruses, bacteria, fungi, nematodes, mites, or insects via developmental inhibition, differentiation, growth, or suppression of host plant tissues, gall is a well-known type of plant structure and growth form. Multiple changes in response to gall inducers have been found in host plant tissues. These include changes in pH, polarity, nuclear and nucleolar hypertrophy, excess free amino acids and sugars, the presence of hydrolytic enzymes such as amylase and protease, and others (Mani, 1992; Rohfritsch, 1992).

Although all plant organs are subject to insect galls, more than 75% occur on plant leaves (Dreger-Jauffret and Shorthouse, 1992; Yang and Tung, 1998). While much attention has been focused on the morphology and anatomy of insects or their induced galls (Meyer 1987; Dreger-Jauffret and Shorthouse, 1992; Williams, 1994), relatively little work has been done on the chloroplast of galls. The limited reports available about gall chloroplasts all concern the morphology of thylakoid membrane distributed in the stroma (Rey, 1973, 1974 and 1992). The few studies on gall-former impacts on host photosynthesis that exist do not suggest any general trends, because they report a range of effects from negative to positive (Andersen and Mizell, 1987; Fay et al., 1993; Bagatto et al., 1996; Larson, 1998). It seems that no researcher has studied the biochemical features of thylakoid membrane in the gall chloroplast.

In this study, we therefore analyze for the first time the biochemical composition of pigment-protein complexes of thylakoid membrane isolated from the two cecidomyiid gall chloroplasts of M. thunbergii Sieb & Zucc. (Lauraceae) mature leaf. A unique pattern of pigment-protein complex different from normal chloroplast was discovered in the gall chloroplast.

Materials and Methods

Plant and Gall

The mature oval-pointed cecidomyiid galls (Figure 1) residing on the lower epidermis of Machilus thunbergii Sieb & Zucc. (Lauraceae) mature leaf was collected from Chung-Cheng Mountain of the Yang-Ming Shan National Park in northern Taiwan. The mature galls were detached from the infected mature leaf, and the surrounding healthy leaf tissue was trimmed to avoid contamination.
Pigment Analysis

Following extraction of liquid-nitrogen frozen mature leaf or gall with 80% acetone, the concentrations of chlorophyll, carotenoid, and three porphyrins—i.e. protoporphyrin IX; Mg-protoporphyrin IX, and protochlorophyllide—were determined according to the methods of Porra et al. (1989), Jasper (1965), and Kahn et al. (1976), respectively. Chlorophyllide and pheophytin was determined as described by Holden (1961). Absorbance and room temperature absorption spectra of pigment-protein complexes were obtained with a Hitachi U2000 UV-visible spectrophotometer.

Mole Percent of Porphyrins

The mole percent of individual porphyrin is defined as (protoporphyrin IX or Mg-protoporphyrin IX or protochlorophyllide)/([protoporphyrin IX + Mg-protoporphyrin IX + protochlorophyllide] × 100%.

Secondary Metabolite Analysis

The content of anthocyanin, flavonoid, and tannin were determined as previously described by Mancinelli et al. (1975), Geisman (1955), and Hagerman and Butler (1978), respectively. Their contents are expressed in terms of optical density per gram of fresh leaf.

Pigment-Protein Complexes

Thylakoid membranes isolated from both leaf and detached galls were analyzed for constituent pigment-protein complexes by solubilization with SDS and electrophoresis on Thornber and MARS fractionation gel systems. The system of Thornber resolves two pigment-protein complexes, termed CPI and CPII, in addition to a zone of free pigment. The MARS system resolves four pigment-protein complexes, named A1, AB1, AB2, and AB3, besides a free pigment zone. Pigment-protein complexes were excised from the gel, and their absorption spectra in the gel slices were determined (Markwell, 1986).

Electron Microscopy

The inner part of gall and central part of leaf were collected and cut into small cubes in fixation buffer containing 2.5% glutaraldehyde. After incubation at 4°C for 2 h in 0.1 M cacodylate buffer (pH 7.0) containing 2.5% glutaraldehyde, the samples were washed three times in plain buffer, postfixed in 1% osmium tetraoxide for 2 h, dehydrated through an ethanol series, infiltrated and embedded in Spurr’s resin (Spurr, 1969), and then polymerized at 70°C for 8 h. Gold sections were collected and stained with ethanol uranyl acetate and lead citrate. The thylakoid morphology was examined with a Philips CM 100 transmission electron microscope at 75kV.

Results and Discussion

Photosynthetic Pigments

Compared with the infected leaf, all chlorophyll-related pigments relevant to photosynthesis in the insect-induced gall—such as chlorophyll, protoporphyrin IX, Mg-protoporphyrin IX, protochlorophyllide, pheophytin, and chlorophyllide—drastically decreased by approximately 17-50 fold while carotenoid declined by about 28 fold (Tables 1 and 2). The discrepancy in the chlorophyll a/b ratios between leaf and gall suggests that the mature oval-pointed cecidomyiid galls synthesize relatively more chlorophyll b than chlorophyll a, or degrade chlorophyll a relatively faster than chlorophyll b, therefore causing the decrease of their chlorophyll a/b ratio. Conversely, even though both chlorophyll and carotenoid content decreased, the discrepancy in the carotenoid/chlorophyll ratios suggests that the mature oval-pointed cecidomyiid galls synthesize relatively more carotenoid than chlorophyll or degrade chlorophyll relatively faster than carotenoid.

While the mole percent of total porphyrins, extracted from the leaf of M. thunbergii is comparable to other plants, i.e. approximately 49%, 33%, and 18%, respectively, those of the mature oval-pointed cecidomyiid gall are about 64%,
The increase in the mole percent of protoporphyrin IX is accompanied by a decrease in that of protochlorophyllide while that of Mg-protoporphyrin IX remains at a similar level. The data imply that the chlorophyll biosynthetic capacity of gall is much different from that of the infected leaf. It seems that most protochlorophyllide synthesized in the gall is very quickly transformed into chlorophyll, causing the high percentage of protoporphyrin IX and low percentage of protochlorophyllide.

Pheophytin and chlorophyllide are the catalytic products of Mg-dechelatase and chlorophyllase, respectively, both using chlorophyll as substrate. Both compounds are further transformed directly into pheophorbide. Pheophytin and chlorophyllide contents in the gall also drastically decreased by 46 and 17 fold, respectively, when compared with infected leaf (Table 1). The ratio of pheophytin/chlorophyllide in the infected leaf was approximately 2.7 fold greater than in the gall. The data reveals a great difference between the chlorophyll degradation pathway of the mature cecidomyiid gall and that of the infected leaf. The chlorophyll degradation of the gall may take the pathway of chlorophyll→chlorophyllide→pheophorbide as the major route, and the pathway of chlorophyll→pheophytin→pheophorbide as the minor route. The chlorophyll degradation of the infected leaf is in contrast to that of gall.

Secondary Metabolites

Many secondary metabolites are involved in the defense system of plants (Harborne, 1988; Mazza and Miniami, 1993). The mature infected leaf of *M. thunbergii* contains high amounts of flavonoid, no anthocyanin, and very low amounts of tannin. However, the insect-induced galls contain much less flavonoid and much more anthocyanin and tannin (Table 3). The (anthocyanin+tannin)/flavonoid ratio of the mature leaf is 0.007, and that of the insect-induced gall is 0.286. The data suggest that the insect-induced gall may induce the infected leaf to synthesize less photosynthetic pigments to save energy in order to produce more compounds for the plant defense system protecting the insects in the gall.

**Pigment-Protein Complexes**

The Thornber electrophoretic system shows that the infected leaf of *M. thunbergii* contains both the CPI and CPII pigment-protein complexes usually found in higher plants, whereas the mature insect-induced gall contains only CPII complex (Figure 2A). CPII contains only chlorophyll a and is derived from PSI, whereas CPIII contains both chlorophyll a and b and is derived from the light-harvesting complex of PSII (LHCII) (Markwell, 1986). By using the MARS electrophoretic system, only one (AB3) of the three chlorophyll b-containing pigment-protein complexes (AB1, AB2, and AB3) present in the thylakoid membranes of normal higher plant leaf is detectable in the insect-induced gall, which is also deficient in A1 (Figure 2B). A1 is a constituent part of the core of PSI and contains almost no chlorophyll b. AB1, AB2, and AB3, containing either chlorophyll a or chlorophyll b, are derived from LHCII (Markwell, 1986).

![Figure 2](image-url)
The room temperature absorption spectra of the pigment-protein complexes of the infected leaf or the cecidomyiid gall fractionated by the Thornber (Figure 3) and MARS electrophoretic systems are similar to those published for leaves of other higher plants (Figure 4). The spectra patterns of the cecidomyiid gall are the same as those of mungbean testa. The pigment-protein complex pattern of the insect gall is the same as that of mungbean testa, and neither of them is found in the normal chloroplast (Yang et al., 1995). This deficiency of pigment-protein complexes in the insect-induced gall derived from the infected *M. thunbergii* leaf and in the non-leaf green tissue of mungbean testa is an interesting coincidence. However, the chlorophyll a/b ratio of the insect-induced gall, about 2.2, is close to that of leaf while that of mungbean testa, about 0.7, is much lower.

Insect-induced galls are transformed from leaves infected by insect and contain abnormal pigment-protein complex compositions of PSI and PSII. The pigment-protein complexes of gall are not remnant components during gall formation because some are missing throughout the life of the gall.

Many chlorophyll-deficient mutants have been reported in barley, pea, maize, wheat, sweetclover, rice, soybean, sugar beet, *Arabidopsis thaliana*, *Chlamydomonas* and other plants (Yang et al., 1993). Except for three (Nakatani and Baliga, 1985; Quijja et al., 1988; Yang and Chen, 1996), all chlorophyll-deficient mutants reveal reduction in chlorophyll content, a higher ratio of chlorophyll a/b, an immature ultrastructure of thylakoid membrane, marked changes in pigment-protein complexes, and general sensitivity to temperature, light intensity, and photoperiod (Yang et al., 1993). Insect-induced galls in this study or mungbean testa may be recognized as a kind of chlorophyll-deficient mutant of leaf or a non-leaf green tissue with abnormal morphology. However, while the chlorophyll a/b ratios of the insect-induced gall and mungbean testa are below the average, between 2.5 and 3.0 of leaf; those of chlorophyll-deficient mutants are higher than 4.0 (Yang et al., 1993). The incomplete organization of PSI and PSII may affect the gall photosynthetic function of light-harvesting, energy transfer, and photochemical energy conversion performed in pigment-protein complexes.

It is widely accepted that the LHCII pigment-protein complex regulates the formation of grana in the thylakoid membrane; that is, no normal grana are formed if no LHCII complex is assembled in the chloroplast (Allen, 1992; Bennett, 1991). However, the electron microscopy which followed showed that this may not be the case.

**Figure 3.** Room temperature absorption spectra of pigment-protein complexes from thylakoid membranes of the mature oval-pointed cecidomyiid gall and the infected leaf of *M. thunbergii*, fractionated by Thornber electrophoretic systems. A, B, and C are CPI, CPII, and FP of leaf, respectively; D and E are CPII and FP of gall, respectively.

**Figure 4.** Room temperature absorption spectra of pigment-protein complexes from thylakoid membranes of the mature oval-pointed cecidomyiid gall and the infected leaf of *M. thunbergii*, fractionated by MARS electrophoretic systems. A, B, C, D, and E are A1, AB1, AB2, AB3, and FP of leaf, respectively; F, G, and H are A2, AB3, and FP of gall, respectively.
Ultrastructural studies showed that the chloroplast of either the mature *M. thunbergii* Sieb & Zucc leaf or the insect-induced gall has normal grana and thylakoid morphology, and they are the same as those of other higher plants (Figure 5). The deficiency in pigment-protein complexes does not cause significant abnormality of grana stacking in the insect-induced gall chloroplasts. This is much different from the chlorophyll-deficient mutants of higher plants. Rey (1973, 1974 and 1992) reported that the chloroplasts in the gall of *Pontania proxima* infected willow leaf never contain starch, but a bundle of tubules appears in their stroma, very often isolated in a stretched lobe.

However, the characteristics of deficiency in pigment-protein complexes of the insect-induced gall do not match the criteria of chlorophyll-deficient mutants of higher plants. The loss of LHCII pigment-protein complexes such as AB1 and AB2 does not affect the grana stacking in the gall chloroplast. Therefore, factors other than LHCII may be involved in the grana stacking (Yang and Chen, 1996).

In this report, we have examined several biochemical characteristics of the mature cecidomyiid insect-induced gall collected from the mature infected leaf of *M. thunbergii* and have shown that: (1) most protochlorophyllide synthesized in the gall is very quickly transformed into chlorophyll, causing the high percentage of protoporphyrin IX and the low percentage of protochlorophyllide; (2) while the infected leaf may take the degradation pathway of chlorophyll→opheophytin→pheophorbide as the major route, the cicedomyiid gall may take chlorophyll→chlorophyllide→pheophorbide as the major route; (3) the insect-induced gall lacks the pigment-protein complex CPI of PSI and is totally deficient in the pigment-protein complexes A1, AB1 and AB2 of PSII; (4) the insect-induced gall may induce infected leaf to produce more anthocyanin and tannin to protect the galling insects; and (5) the insect-induced gall contains lower amounts of LHCII complex, but contains normal grana stacking and thylakoid morphology.

However, it is still unknown (1) how widespread the deficiency phenomena of pigment-protein complexes is in other insect galls; (2) how the galling insects shut down or slow down the chlorophyll biosynthetic machinery; (3) how the galling insects induce the lack of some pigment-protein complexes; (4) how the galling insects trigger or initiate the synthesis of anthocyanin and tannin; and (5) what the physiology of the deficiency of some pigment-protein complexes is.

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**Literature Cited**


植食性昆蟲癭蝨誘發紅楠葉片癭蝨色素蛋白複合體之缺失

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本研究比較紅楠（Machilus thunbergii）葉片及其上成熟癭蝨癭蝨之葉綠素生合成與崩解途徑、色素蛋白複合體及類囊膜形態。癭蝨的光合色素含量遠低於被感染葉，但花青素及葉綠素含量則相反。比林百分比及 pheophytin/chlorophyllide 比值在癭蝨及被感染葉則相差甚大，此顯示它們的葉綠素生合成與崩解途徑有很大不同。當被感染葉以 chlorophyll→pheophytin→pheophorbide 為主要途徑時，癭蝨癭蝨則可能走 chlorophyll→chlorophyllide→pheophorbide 為主要途徑。當被感染葉擁有 Thornber 系統中的 CPI 及 CPII 二種色素蛋白複合體，及 MARS 系統中的 A1、AB1、AB2 及 AB3 四種色素蛋白複合體時，成熟癭蝨則分別只含有 CPII 及 AB3 而已。電子顯微鏡顯示，成熟癭蝨仍擁有正常摺疊的葉綠素（grana）及類囊膜形態。迄今仍未知曉此種色素蛋白複合體缺失現象是否為普遍現象，且不知癭蝨昆蟲如何導致寄主葉缺少某些色素蛋白複合體。

關鍵詞：植食性昆蟲；色素蛋白複合體；癭蝨昆蟲；紅楠葉；類囊膜。