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Correlations between calcium oxalate crystals and photosynthetic activities in palisade cells of shade-adapted *Peperomia glabella*

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ABSTRACT. Each photosynthetic palisade cell in the leaves of shade-adapted *Peperomia glabella* contains a druse calcium oxalate crystal which we hypothesize is involved in dispersing light to the chloroplasts. The effect of light intensity on druse size, number and position, relative to growth and phototsynthesis was determined. *Peperomia glabella* grew best at 50-100 μE m^{-2} s^{-1}, and at 300-400 μE m^{-2} s^{-1} had smaller leaves with considerable yellowing. Plants grown under lower light had well developed chloroplasts while at 300-400 μE m^{-2} s^{-1} the chloroplasts accumulated plastoglobuli and showed thylakoid swelling. Chlorophyll content, chlorophyll a/b, and photosynthetic rate decreased with increasing light intensity. Druse crystals were produced in palisade cells under all light conditions but crystal diameter changed, being greatest at 100 μE m^{-2} s^{-1} and decreasing with higher light. The position of the crystals also changed with light intensity. Under 50 and 100 μE m^{-2} s^{-1} the crystals were predominantly located at the bottom or middle of cells while at 300 and 400 μE m^{-2} s^{-1} they were at the top of cells. The data indicate an adaptive role of calcium oxalate crystals in photosynthesis in *Peperomia*.

Keywords: Calcium oxalate; Crystals; *Peperomia*; Photosynthesis; Shade plants.

INTRODUCTION

Peperomia is a large genus of plants primarily occurring in the understory of tropical rainforests. The genus includes species adapted to low light and with a mix of photosynthetic mechanisms (C_3, CAM, and CAM-cycling) (Virzo et al., 1983; Sipes and Ting, 1985; Patel and Ting, 1987; Holthe et al., 1992; Ting et al., 1994; Helliker and Martin, 1997). Some species have a "leaf window" which is a colorless multiple epidermis that interfaces with a chlorophyll-rich palisade parenchyma layer (Gibeaut and Thomson, 1989a, b). The window tissue stores water, and in CAM species organic acids, but also is thought to function in enhancing photosynthesis by permitting light to penetrate to the underlying chlorenchyma (Kaul, 1977). The palisade parenchyma cells have been shown to have most of the C_3 photosynthetic machinery (Nishio and Ting, 1987).

A number of *Peperomia* species contain druse crystals within the underlying palisade parenchyma cells (Schürhoff, 1908; Horner, 1976; Franceschi and Horner, 1980; Gibeaut and Thomson, 1989a,b). Our observations of six different species indicate the crystals are restricted to the palisade cells and they are not related to photosynthesis type as both C_3 and CAM species contain crystals (Kuo-Huang and Franceschi, unpublished results). Calcium oxalate crystals are conspicuous in many plant species and tissues (Arnott and Pautard, 1970; Gallagher, 1975; Franceschi and Horner, 1980; Horner and Wagner, 1995; Ku-Huang, 1990; Kuo-Huang et al., 1994; Webb, 1999; Wu and Kuo-Huang, 1997), commonly formed in cells specialized for crystal production, and their widespread occurrence has raised the question of their functional significance. Most studies have focused on their role in calcium regulation as high capacity calcium sinks to remove excess calcium (Frank, 1972; Zindler-Frank, 1975; Franceschi and Horner, 1979; Borchert, 1985, 1986; Franceschi, 1989; Kuo-Huang and Zindler-Frank, 1998; Kostman and Franceschi, 2000; Volk et al., 2002; Wu et al., 2006). The observation that calcium oxalate crystals in *Peperomia* are specifically produced in the photosynthetic palisade cells rather than in cells specialized for crystal formation indicates a function other than calcium regulation in these species. Schürhoff (1908), noting that crystals were associated with the thin layer of photosynthetic tissue

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in *Peperomia* leaf, suggested that they might be involved with the photosynthetic process. This possibility has also been mentioned in reviews on calcium oxalate function (Arnott and Pautard, 1970; Franceschi and Horner, 1980), but no one has ever examined this further.

As an understory plant *Peperomia* is likely to be exposed to primarily low light but also sunflecks of varying intensity and duration. The window tissue will maximize transmission of light into the photosynthetic tissue layer. The palisade cell chloroplasts are distributed to the anticlinal surface and bottom of the cells while the adaxial end is clear of chloroplasts. We hypothesize that the calcium oxalate crystals can act to distribute light entering the palisade cell towards the chloroplasts along the wall of the cell. The crystals are all of the druse type, which is a spherical conglomerate of multiple facets (see Franceschi and Horner, 1980). This crystal structure would be ideal for diffraction or reflection of light around the entire cell, and under high light conditions (prolonged sunflecks) the crystals could scatter some of the light back into the window tissue to avoid photodamage to the low light-adapted palisade chloroplasts. The purpose of this study was to examine the relationship of druse crystals to photosynthesis in *Peperomia*. We determined if crystal size and number, or cellular features changed when plants were grown under a range of light conditions. After screening a number of species we chose *Peperomea glabella*, a C_3 species, for our studies. The results support a role of *Peperomia* druse crystals in photosynthesis.

MATERIALS AND METHODS

Plant material

Peperomia glabella L. plants were obtained from a commercial nursery and acclimated for two weeks in a growth chamber at 26°C with 16 h day length under wide spectrum fluorescent lights (approximately 100 μE m^{-2} s^{-1}). The plants were randomly divided into five groups which were placed in the growth chamber at different heights to give light intensities at the top of the plants of 50, 100, 200, 300 and 400 μE m^{-2} s^{-1}. Leaves that developed during growth under each light intensity were used for analysis. Immunolabeling of six different *Peperomia* species for Rubisco and PEP carboxylase (see protocols in Voznesenskaya et al., 1999) indicated that *P. glabella* had very little PEP carboxylase and was a C_3 species.

Light microscopy

Fresh free-hand sections were used for initial comparison of the leaf anatomical features. Sections were viewed with partially crossed polarizing filters for visualization of the birefringent crystals. Resin embedded samples were used for higher resolution observation. Small pieces (1 mm^2) from the same region of leaves from each treatment were fixed overnight at 4°C in 2.5% (v/v) glutaraldehyde and 2% (v/v) paraformaldehyde in 50 mM Pipes buffer (pH 7.2). The specimens were post-fixed overnight in 1% (w/v) osmium tetroxide in sodium phosphate buffer (50 mM, pH 7.2), rinsed with buffer, dehydrated in an acetone series, and embedded in Spurr resin (Spurr, 1969). For light microscopy, sections 1 μm thick were cut on glass knives, dried onto gelatin coated slides and stained with Stevenel's blue (Del Cerro et al., 1980). A drop of immersion oil was placed on the section, a cover slip applied, and images of the sections captured on an Olympus BM2 microscope.

For determining crystal densities and sizes, leaves were cleared of chlorophyll and other pigments by soaking in 70% acetone for 2-3 days. The leaves were rehydrated, infiltrated with glycerol, and examined with an Olympus BM2 microscope by using crossed polarizing filters. The crystals were counted within two 0.15 mm^2 areas of three leaves of each treatment. The diameter of the crystals was measured on 30 crystals along a line on the image of each leaf. A total of 90 crystals were measured per treatment.

Resin sections were used to determine location of crystals within the palisade cells. Crystal location was categorized into upper, middle and lower positions (thirds) within the palisade cells. Crystals within 30 randomly selected cells were examined in each leaf. A total of 90 cells were examined per treatment.

Scanning electron microscopy

For scanning electron microscopy (SEM) analysis of crystal structure, leaf samples were fixed as for light microscopy. After washing and dehydration with an alcohol series, samples were freeze fractured in liquid nitrogen, critical point dried from CO_2, and sputter coated with gold prior to examination on a Hitachi S-570 SEM.

Transmission electron microscopy

Leaf samples prepared for light microscopy were also used for transmission electron microscopy (TEM). Thin sections were made using a diamond knife on a Reichard Ultramicrotome and picked up onto 200 mesh formvar coated nickel grids. Sections were stained with saturated uranyl acetate and 1% (w/v) $KMnO_4$ and observed with a JEOL 1200EX TEM.

Photosynthetic rate and chlorophyll contents

Five mature leaves from plants under each light intensity were analyzed for O_2 evolution using a Hansatech CB1D oxygen electrode apparatus. For each leaf sample the O_2 evolution rate was measured at five levels of light intensity: 50, 100, 200, 300 and 400 μE m^{-2} s^{-1}. The O_2 evolution of mature leaves of rice (*Oryza sativa*) under 1200 μE m^{-2} s^{-1} was also measured as a reference and to ensure that the O_2 apparatus was operating properly.

For chlorophyll analysis, leaves were ground and extracted in 95% ethanol. The total chlorophyll content and chlorophyll a/b ratio were determined by absorbance at 665 and 649 nm using a Perkin-Elmer 552A UV/VIS spectrophotometer. Chlorophyll a and b levels were calculated from the absorbance following the protocol of Wintermans and De Mots (1965).

RESULTS

Effect of light intensity on growth

Peperomia glabella is well adapted to grow under low light (see Figure 1A). Optimum growth in terms of leaf size, chlorophyll content and branching was observed with a light intensity of 100 μE m^{-2} s^{-1}, or approximately 5% of full sunlight. The plants also grew well at 50 μE m^{-2} s^{-1}, although they had fewer branches and their leaves were thinner than leaves grown under 100 μE m^{-2} s^{-1}. At 200 μE m^{-2} s^{-1} the leaves were smaller and some slight yellowing was apparent, and plants grown at 300 and 400 μE m^{-2} s^{-1} had leaves that showed considerable or complete yellowing, respectively (Figure 1A).

General leaf anatomy and light intensity

The leaves of *P. glabella* are differentiated into four distinct tissue layers: upper multiple epidermis ("window tissue" or water storage tissue), a one-cell layered palisade parenchyma, spongy mesophyll, and lower epidermis (Figure 1B). The palisade cells are much darker green than the spongy mesophyll (Figure 1B). Each palisade cell generally contains a single druse crystal (Figure 1B and 1C) in the vacuole, although occasionally more than one crystal is present. The druse is a roughly spherical crystal conglomerate of multiple facets (Figure 1D). Crystals were only found in the palisade cells.

Light level had several anatomical effects. The thickness of the window tissue as a percent of total leaf cross sectional thickness was 43 ± 4% for plants grown at 50 μE m^{-2} s^{-1} and increased to 54 ± 3% in the leaves of plants grown under 400 μE m^{-2} s^{-1}. There were also some slight changes in the size of the palisade and spongy mesophyll cells, but were not quantified in this study (see Figure 2).

The morphology and distribution of chloroplasts in the chlorenchyma were strongly influenced by light intensity. Plants grown under lower light (50 and 100 μE m^{-2} s^{-1}) had rounder and larger chloroplasts compared to those grown under higher light (Figure 2, see insets). Starch was abundant at 50 and 100 μE m^{-2} s^{-1}, but was essentially absent at 300 and 400 μE m^{-2} s^{-1} (see Figure 2, but also examined with PAS staining; data not shown). At the two lowest light levels the chloroplasts of the palisade tissue are distributed along the anticlinal walls and lower part of the cells, with the grana stacks oriented with their surfaces facing the vacuole or cell wall (Figure 3), while in spongy mesophyll, the chloroplasts are located mostly along upper and lower sides of the cells (Figure 2A and 2B). For plants grown above 200 μE m^{-2} s^{-1}, the chloroplasts are located predominantly along the anticlinal periphery of both palisade and spongy mesophyll cells (Figure 2C and 2D), and the grana stacks have less strict orientation (Figure 3H).

Calcium oxalate crystals were produced under all light conditions (Figure 2), although there were changes in size and distribution. The overall structure of the crystals did not appear to be affected by light intensity (Figure 3). The crystals always appeared as multifaceted conglomerates, although the size of the individual facets became smaller at light levels of 200 μE m^{-2} s^{-1} and above (Figure 3). The crystals facets were always surrounded by a membrane. While there was material associated with the crystal surfaces (Figure 3), no connection between the crystal and the tonoplast was observed.

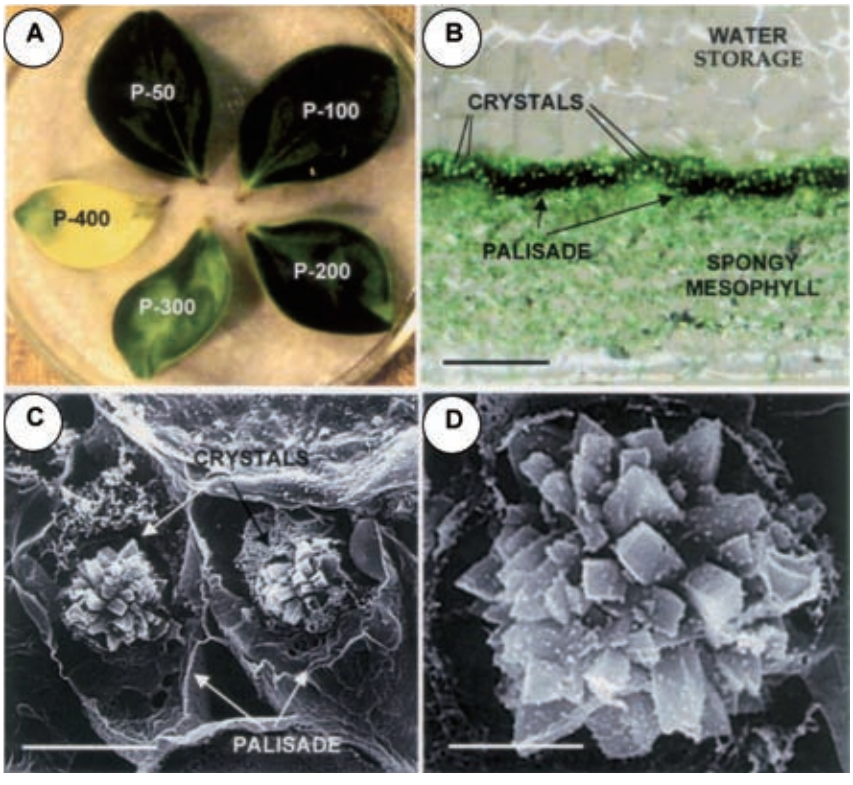

Figure 1. General features of *Peperomia glabella* leaf anatomy. A, Effect of light intensity on leaf size and chlorophyll content. Leaves are of the same age and taken from plants grown at the light level indicated on the leaf (in μE m^{-2} s^{-1}). Plants grown at 300 and 400 μE m^{-2} s^{-1} show severe photobleaching; B, A free-hand cross section taken from a fresh leaf. Four distinct tissues are present: a multiple epidermis called window tissue and involved in water storage, a palisade cell layer of dark green cells, a spongy mesophyll containing less chlorophyll, and the lower epidermis. Calcium oxalate crystals (bright spots) are only found in the palisade cells. Bar = 200 μm; C, A scanning electron microscope view of two palisade cells that have been cleaved open to reveal the druse calcium oxalate crystals. Generally, only one crystal is formed in each palisade cell. Bar = 10 μm; D, A high magnification image of a druse crystal. The crystal is a conglomerate of multiple facets that radiate out from a central core. Bar = 3 μm.

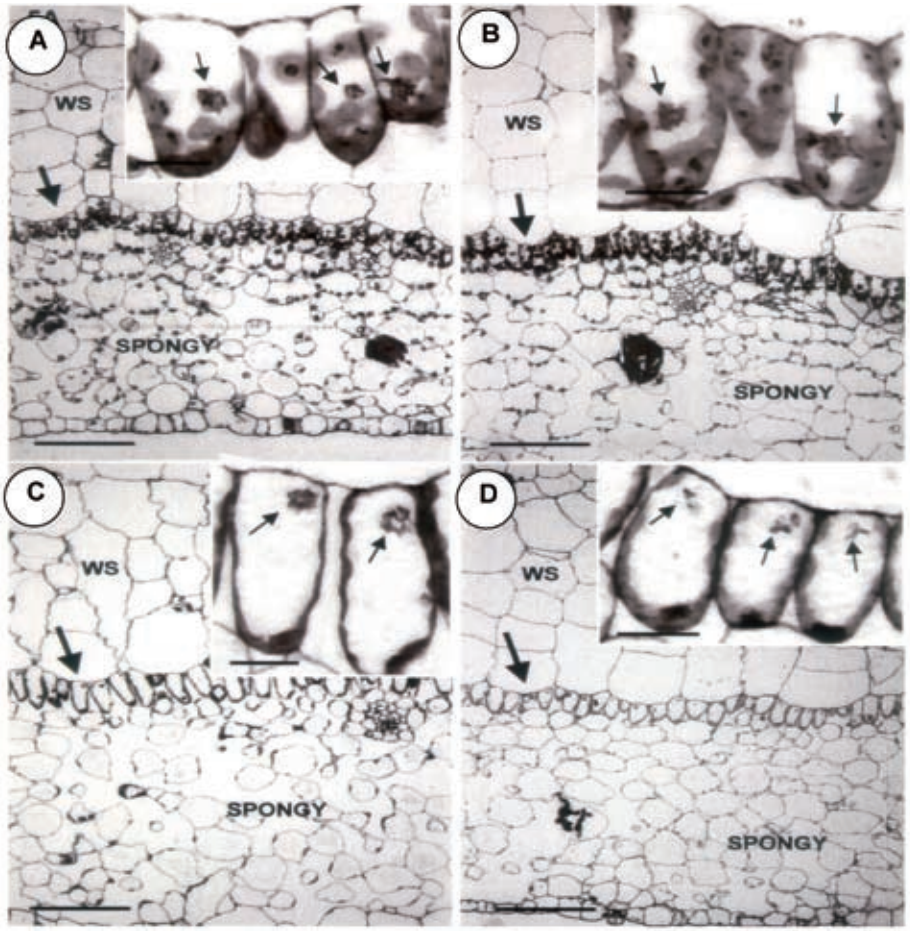

Figure 2. Light microscopy of the anatomy of leaves from plants grown at different light intensities (P-50, etc.). Insets show higher magnification of the palisade cells. A, 50 μE m^{-2} s^{-1}. The cells of the palisade layer (large arrow) contain relatively large chloroplasts and the crystals are displaced towards the middle or bottom of the palisade cells (arrows in inset); B, 100 μE m^{-2} s^{-1}. Palisade cells and crystal position are similar to that in plants grown at 50 μE m^{-2} s^{-1}; C, 300 μE m^{-2} s^{-1}. Palisade cells are longer and the chloroplasts are smaller. The crystals (arrows in inset) are now displaced to the top of the palisade cells; D, 400 μE m^{-2} s^{-1}. Chloroplasts are very reduced in the palisade layer and spongy mesophyll. The crystals are still displaced to the top of the cells but they are small. Bars are 100 μm on low magnification pictures and 20 μm on insets.

In contrast to the stability of crystal structure, chloroplasts in the palisade cells changed considerably in response to changing light intensity. At 50 μE m^{-2} s^{-1}, the chloroplasts had numerous small grana stacks, multiple starch grains, and only a few very small plastoglobuli (Figure 3B). At 100 μE m^{-2} s^{-1}, the grana stacks were fewer but much larger (Figure 3D), while other features were similar. At 200 μE m^{-2} s^{-1}, the grana stacks show some disruption and there are numerous large plastoglobuli (Figure 3F). At 300 and 400 μE m^{-2} s^{-1}, the chloroplasts showed considerable thylakoid swelling and grana stacks were poorly defined (Figure 3H). These observations are consistent with the observation of whole leaf coloration indicating loss of chlorophyll at higher light intensities.

Effect of light on chlorophyll and photosynthetic rate

In *P. glabella*, both the chlorophyll content and chlorophyll a/b ratio were sensitive to light intensity. The total chlorophyll content of leaves grown under lower light (50 and 100 μE m^{-2} s^{-1}) was about 41.5 μg cm^{-2} (compared to 62.9 ± 16.8 μg cm^{-2} for rice leaf, plant grown under 200-300 μE m^{-2} s^{-1}) and decreased significantly with increasing light intensity (Figure 4A). The chlorophyll a/b ratio in leaves grown under lower light intensities was about 2.2 (compared to 3.8 ± 0.4 for rice leaf) and decreased as the light intensity increased (Figure 4B). This change in chlorophyll a/b ratio was due to a decrease in chlorophyll a with increasing light intensity (data not shown). It is possible related to different decay rates of chlorophyll a and b.

Photosynthesis as measured by O_2 evolution was higher (6-10 μmol m^{-2} s^{-1}) in leaves of plants grown under lower light intensities (50 and 100 μE m^{-2} s^{-1}) and was reduced (less than 6 μmol m^{-2} s^{-1}) in leaves grown under light intensities of 200 μE m^{-2} s^{-1} and greater (Figure 5). For plants grown at a given light intensity, the photosynthetic rates of the leaves increased with increasing light intensity. But

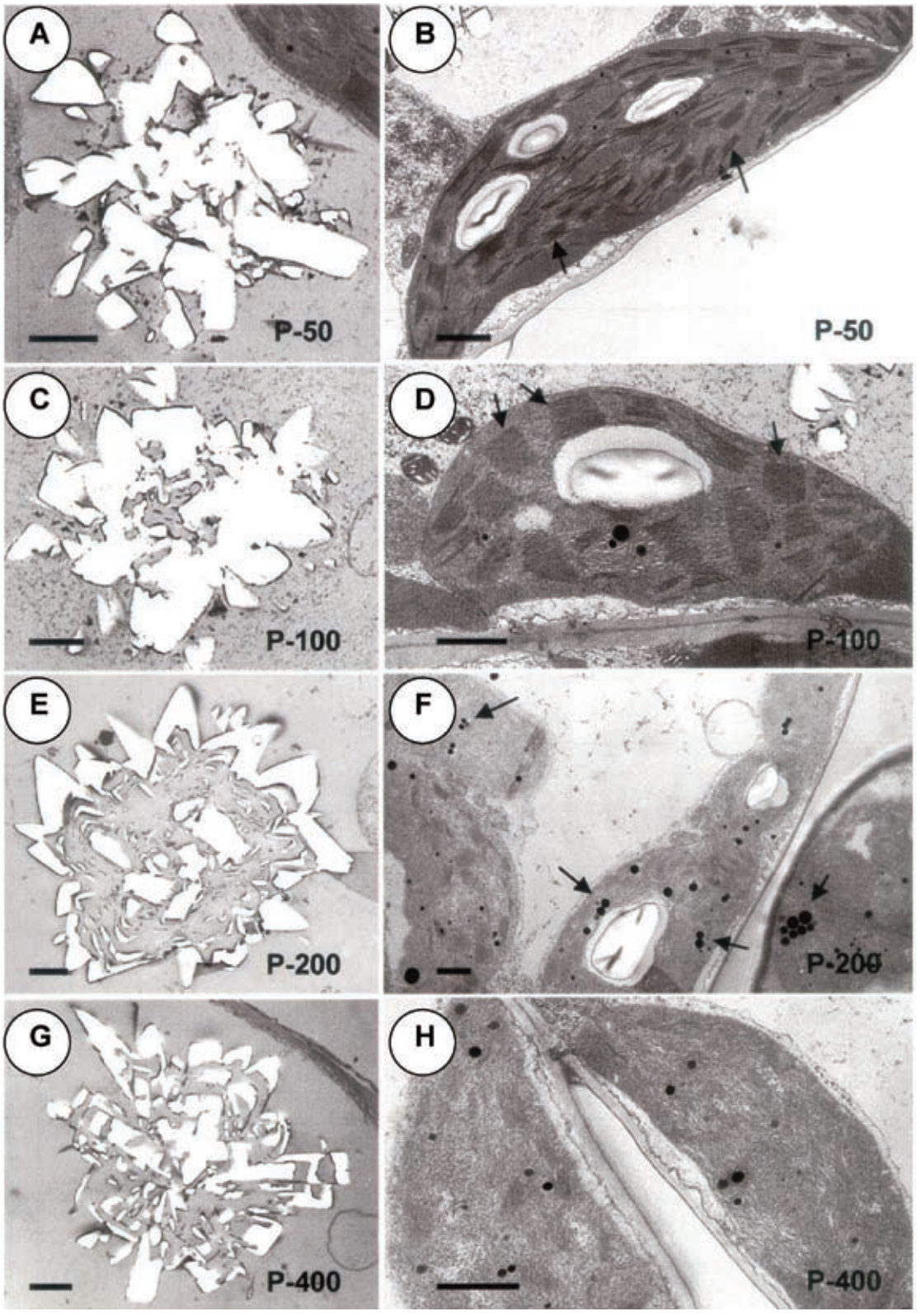

Figure 3. Transmission electron microscopy of the crystals and chloroplasts of plants grown at different light intensities (P-50 etc). All bars = 1 μm. A and B, 50 μE m^{-2} s^{-1}. The sections demonstrate the druse is made of a mass of individual facets (clear spaces in section). The chloroplasts are well developed with many small grana stacks (arrows) and some starch; C and D, 100 μE m^{-2} s^{-1}. Crystal morphology has not changed but the chloroplasts have larger grana stacks (arrows); E and F, 200 μE m^{-2} s^{-1}. This crystal has a slightly modified structure with rings of facets. The chloroplasts have disrupted thylakoids as well as some distinct grana stacks, and there is accumulation of lipid drops (arrows); G and H, 400 μE m^{-2} s^{-1}. The crystal is smaller and has multiple small facets. The chloroplasts have slightly swollen thylakoids and the grana stacks are poorly defined. Note that starch is absent.

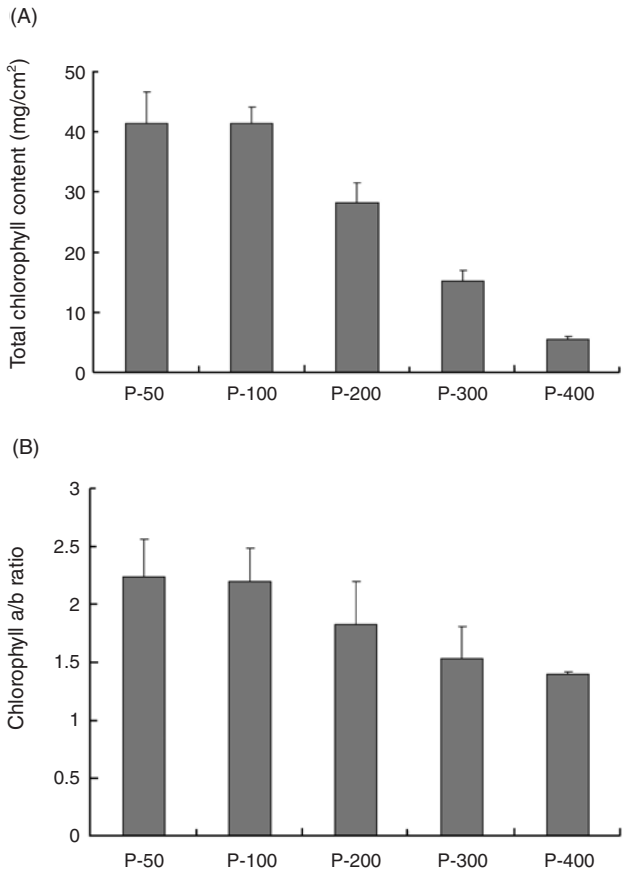

Figure 4. Effect of light intensity (μE m^{-2} s^{-1}) during growth on chlorophyll in *P. glabella* leaves. A, Total chlorophyll levels decline significantly at light above 100 μE m^{-2} s^{-1}; B, Chlorophyll a/b ratio decreases with increasing light intensity. Bars give the mean ± SD.

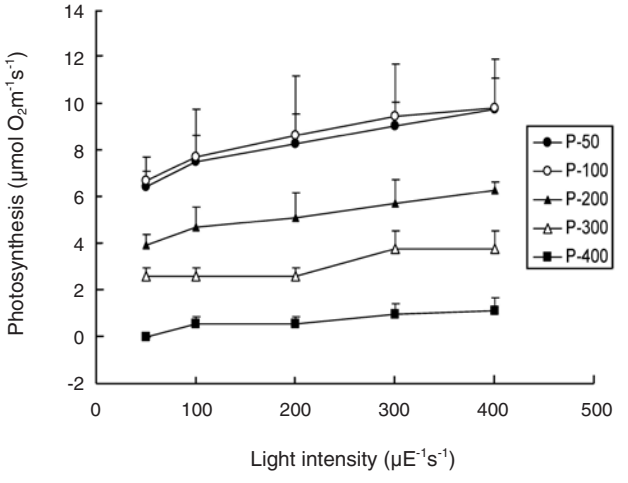

Figure 5. Effect of light intensity (μE m^{-2} s^{-1}) during growth on photosynthesis rate (mean ± SD) as measured by O_2 evolution. Leaves from plants grown at each of 5 different light intensities were measured at 5 different light levels. The results indicate this plant is adapted to low light levels and its photosynthetic machinery is damaged by prolonged exposure to light levels higher than 100 μE m^{-2} s^{-1}.

the increase in photosynthesis was greater (steeper slope) in the leaves of plants grown under 50 and 100 μE m^{-2} s^{-1} than that of plants grown under higher light intensities (Figure 5). For comparison, the photosynthetic O_2 evolution rate for rice leaves under a light intensity of 1200 μE m^{-2} s^{-1} was about 14 ± 3 μmol m^{-2} s^{-1}.

Effect of light on palisade crystals

Under all light conditions used for growth, crystals were only formed in palisade cells. There was not a statistical difference in the number of crystals formed under the different light conditions (Figure 6A). Crystal density (number per unit area) in the leaves of plants grown under different light intensities increased slightly from 3,800 to 4,280 mm^{-2} leaf area (Figure 6A). While crystal number did not change significantly, there was a difference in the size of the crystals within the palisade cells in plants grown at different light levels, which ranged from 5.2 to

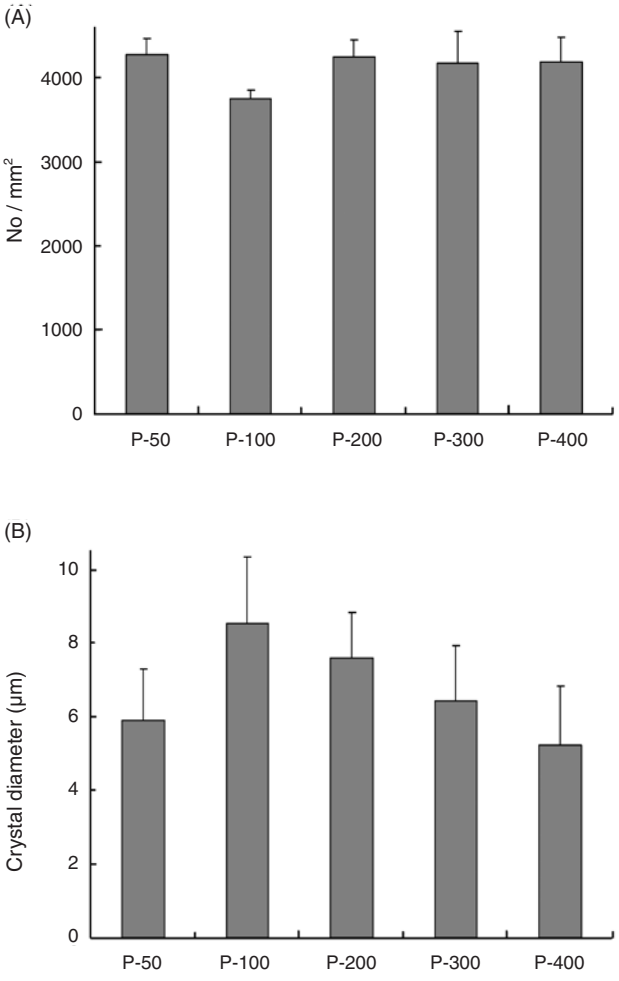

Figure 6. Druse crystal number and size in relation to light intensity (indicated as μE m^{-2} s^{-1}). Bars represent the mean ± SD calculated from 6 areas located in 3 different leaves. A, Crystal number remains relatively constant regardless of light intensity the plants were grown under; B, Crystal size changes in relation to light intensity during growth. The crystals are smaller at the two extremes of our experimental light conditions.

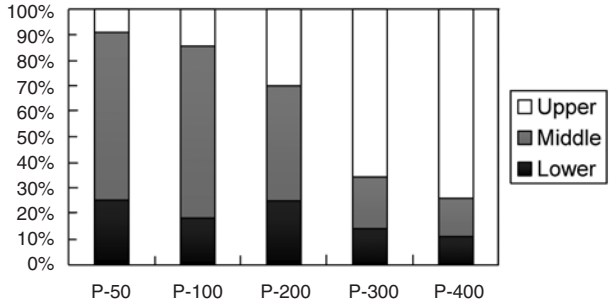

Figure 7. Position of druse crystals in palisade cells in relation to light intensity (indicated as μE m^{-2} s^{-1}). Bars represent the position of the crystal within the cell as a percent of all the cells counted. At low light levels the crystals are primarily in the middle to lower part of the cell while at higher light they are at the top of the cell.

8.5 μm diameter with increasing light intensity. Crystal diameter was greatest in the leaves of plants grown at 100 μE m^{-2} s^{-1} and decreased dramatically in plants grown at higher light intensities (Figure 6B). There was also a slight decrease in the size of crystals in plants grown at the lowest light level of 50 μE m^{-2} s^{-1}.

The crystals are located in the vacuoles of the palisade cells (see Figure 2) and there was a clear change in the position of the crystals within the vacuole relative to light intensity. Under low light levels (50 and 100 μE m^{-2} s^{-1}) the crystals were predominantly (two thirds) found at the bottom or middle of the palisade cells while at higher light levels (300 or 400 μE m^{-2} s^{-1}) they were predominantly at the top of the palisade cells (Figure 7). Plants grown at 200 μE m^{-2} s^{-1} showed a crystal distribution pattern that was intermediate to that found in plants grown at lower and higher light levels (Figure 7). This distribution pattern is also illustrated visually in the insets in Figure 2.

DISCUSSION

Measurement of photosynthetic rates in *Peperomia glabella* under different light intensities demonstrates that this C_3 species is adapted for growth under very low light. The large and abundant grana stacks seen under optimal growth are typical of shade adapted leaves (Boardman, 1977). It appears that this plant cannot effectively adapt to light levels above about 200 μE m^{-2} s^{-1}. At 300-400 μE m^{-2} s^{-1} the leaves have obvious visual signs of photobleaching, which is supported by analyses showing loss of chlorophyll and greatly reduced photosynthetic capacity. Even at 200 μE$m^{-2}s^{-1}$ the chloroplasts begin to show signs of light damage, such as accumulation of lipids and thylakoid swelling (Björkman et al., 1972; Lichtenthaler et al., 1981; Pearcy and Franceschi, 1986), and these symptoms are severe at 300 and 400 μE m^{-2} s^{-1}. Given these observations, it is interesting to note that plants grown at a low light level show increased short term photosynthesis rate at light intensities that cause severe damage during long term growth. It is likely that this is an adaptation to the constantly changing light conditions under the canopy and that anatomical features of the leaves are involved in the short term acclimation to light fluctuations. These anatomical features include presence of a window tissue, having the preponderance of chlorophyll in the palisade layer directly beneath the window tissue, and the presence of a refractive crystal of calcium oxalate at the center of each primary photosynthetic cell.

The window tissue in *Peperomia* would satisfy a need for water storage capacity in these mostly epiphytic plants while maximizing collection of light and its transmission to the photosynthetic palisade cell layer (Krulik, 1980), which are the primary photosynthetic cells in this plant. Having most of the chlorophyll in a single cell layer reduces the problem of light attenuation if the light had to move through multiple photosynthetic cell layers. The palisade cell crystals are an interesting structural feature that is shown here to vary in position with changing light intensity, and may play a unique, though indirect role in photosynthesis. We feel the primary function of the crystals is to disperse the light coming into the top of the cell so that all the chloroplasts of the cell, which are primarily lined up above each other along the anticlinal sides, are exposed to a roughly equal amount of light. Horner (1976) noted the grana "are usually oriented perpendicular to imaginary radii drawn from the center of the crystals". We also observed that the chloroplast grana are oriented towards the vacuole (or wall) of the cell. Reflection of light off the crystal surfaces towards the grana stacks would help to maximize light capture/utilization, especially at lower light levels.

A role of crystals in photosynthesis in *Peperomia* is difficult to demonstrate directly. However, our observations strongly support such a hypothesis. The crystals are only formed in the palisade cells, which make up the primary photosynthetic tissue. The only type of crystal formed is druse, which has multiple, radial oriented facets and the potential reflective properties that would help disperse light to the surrounding chloroplasts. The number of crystals is held constant in the palisade layer regardless of light level, although crystal size changes. This would indicate that the crystals are required by these primary photosynthetic cells. Even at higher light levels where starch is absent and photoassimilates are limited, resources are still put into production of calcium oxalate, though carbon limitation may result in reduction of size of the crystals at light intensity above or below optimum levels for growth.

A surprising observation was that the position of the druse crystals within the palisade cells was altered in response to growth under different light levels. This further supports our hypothesis of a role of the crystals in the photosynthesis process. At low light levels the crystals were mostly at the middle or bottom of the cells. This would help to ensure distribution of the limited light to the chloroplasts towards the bottom half of the cells, and help maximize light capture. At an intermediate light level,

where light would more easily penetrate deeper into the palisade cells, the crystals were at the middle and top half of the cell. At high light levels the crystals were primarily at the top of the palisade cells. Under high light the crystals at the upper surface of the cell may act to reflect part of the light back into the window tissue and thus provide some protection of the shade adapted chloroplasts from photo-damage by high light. Changing the position of the druse crystal within the palisade cell appears to provide a means for roughly regulating the amount of light entering the cell and how that light is distributed within the cell.

Calcium oxalate crystals are a common component of many plant species, but the regulation of their formation and the various functions they play in the plant are still being resolved. They are clearly involved in bulk regulation of calcium in some plants, defense in other plants, and possibly mechanical properties as well (reviewed by Arnott and Pautard, 1970; Horner and Franceschi, 1980; Webb, 1999). We suggest that crystals originally evolved as a means to remove excess calcium as a physiologically and osmotically inactive precipitate, at a minimum cost of carbon since oxalic acid has only two carbons but two carboxylic acid groups. Over time, plants have evidently modified crystal form and patterns of distribution for secondary functions such as defense (i.e. larger crystals with pointed and/or barbed tips). Here we provide evidence that in some plants calcium oxalate crystals have also been utilized as part of the overall photosynthetic process. It will be interesting to survey other low light and "window plants" to see how widespread this phenomenon is.

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耐陰植物玲瓏椒草 (*Peperomia glabella*) 之柵狀葉肉細胞內的草酸鈣晶體與光合作用力的相關性

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耐陰植物玲瓏椒草 (*Peperomia glabella*) 每個柵狀光合葉肉細胞內均具有一個晶簇狀的草酸鈣晶體，我們假設其與光線的散射到葉綠體有關。本研究針對光線強度對晶體之大小、個數與位置，以及相對於植株生長與光合作用的效應加以測試。玲瓏椒草最適宜生長的光強度為 50-100 μE m^{-2} s^{-1}，而在 300-400 μE m^{-2} s^{-1} 光強度生長的植株則葉子較小且有明顯黃化的現象。植株生長於光強度較弱的環境下可生成正常的葉綠體，然而在 300-400 μE m^{-2} s^{-1} 光強度生長的植株生成的葉綠體則會累積葉綠體油滴且呈現類囊膜膨脹的現象。隨著光線強度的增加，則葉綠素含量、葉綠素 a 與 b 的比、以及光合作用率則隨之降低。在所有之測試的光強度下，柵狀葉肉細胞內均會產生晶簇狀的草酸鈣晶體，但所形成晶體的直徑並不相同，在 100 μE m^{-2} s^{-1} 光強度下其直徑最大，而在光線較強時，晶體的直徑則減小。晶體在柵狀光合葉肉細胞內的相關位置亦隨著光強度而改變，在 50 與 100 μE m^{-2} s^{-1} 的光強度下，晶體在柵狀細胞內的位置主要位於細胞的基部或是細胞的中央部位，然而在 300 與 400 μE m^{-2} s^{-1} 的光強度下，晶體在細胞內的位置則明顯的主要位於細胞的頂部。本研究結果顯示玲瓏椒草葉片之光合作用的進行，其柵狀細胞內的草酸鈣晶體扮演著相對的適應性。

關鍵詞：草酸鈣；晶體；椒草；光合作用；耐陰植物。