# Leaf Functional Anatomy in Relation to Photosynthesis[1]

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Rubisco is a large enzyme with a molecular mass of approximately 550 kD. The maximum rate of $CO_2$ fixation (i.e. ribulose-1,5-bisphosphate [RuBP] carboxylation) at $CO_2$ saturation is only 15 to 30 mol $CO_2$ $mol^{-1}$ Rubisco protein $s^{-1}$ at 25°C. Affinity to $CO_2$ is also low, and the $K_m$, $K_c$, at 25°C in the absence of oxygen is comparable to the $CO_2$ concentration in water equilibrated with air containing 39 Pa $CO_2$ (approximately 390 $\mu$L $L^{-1}$), 13 $\mu$M. Moreover, RuBP carboxylation is competitively inhibited by RuBP oxygenation, which is the primary step of the energy-wasting process, photorespiration. If the $CO_2$ concentration in the chloroplast stroma is low, the carboxylation rate will decrease while the oxygenation rate will increase. Under such conditions, light energy and other resources, including nitrogen and water, are all wasted, eventually leading to a decrement of fitness of the plants. From these data, we may consider that structural features of the leaf contributing to the maintenance of the high $CO_2$ concentration in the chloroplast stroma may have been selected during evolution.

In this Update, we focus on the key structural features that affect $CO_2$ concentration in the chloroplast stroma. First, we analyze the conductance for $CO_2$ diffusion from the substomatal cavity to the chloroplast stroma (mesophyll conductance [$g_m$], also called internal conductance). Because the low $g_m$ limits photosynthesis, the mesophyll surface area exposed to the intercellular spaces ($S_{mes}$, mesophyll surface area exposed to intercellular spaces per unit leaf area) should be maximized to increase the area for $CO_2$ dissolution and the effective pathway for $CO_2$ diffusion, and thereby photosynthesis. Second, we analyze the light environment within a leaf, because, for maximizing photosynthesis, light should be delivered to all the chloroplasts in the leaf, distributing along the cell walls. In relation to the light environment within a leaf, we also point out some technical problems in measuring photosynthetic parameters. Third, the movement and positioning of cellular organelles are discussed. Finally, we discuss the urgent need for ecologically relevant developmental and cell biological studies that clarify the mechanisms that are responsible for structural and cell biological features in nature.

## THE NATURE OF MESOPHYLL DIFFUSION CONDUCTANCE

During photosynthesis, $CO_2$ diffuses from ambient air through stomata to the intercellular spaces. Then, the $CO_2$ dissolves in the cell wall water and diffuses across the cell wall, plasma membrane, cytosol, chloroplast envelope, and stroma to Rubisco (Fig. 1). $CO_2$ concentration in the chloroplast can be estimated in several ways (Pons et al., 2009). One of the most reliable methods is the simultaneous measurement of gas exchange and stable carbon (C) isotope discrimination. This method relies on the discrimination against $^{13}CO_2$ by Rubisco. In an open system, with unlimited supply of $^{12}CO_2$ and $^{13}CO_2$, Rubisco will preferentially fix the lighter molecule, $^{12}CO_2$. In a closed system, Rubisco will eventually fix both $^{12}CO_2$ and $^{13}CO_2$ until no $CO_2$ is left. The leaf is intermediate between these two extremes. Thus, the ratio $^{13}CO_2/^{12}CO_2$ of $CO_2$ fixed by a leaf is considerably greater than the ratio in the ambient air. Technically, the ratios of $^{13}CO_2$ to $^{12}CO_2$ in the air incoming to and outgoing from an assimilation chamber are measured with a mass spectrometer, a tunable laser diode absorption spectroscope, or a cavity ring-down spectroscope (Pons et al., 2009).

It has been established that the photosynthetic limitation by $g_m$ expressed on a leaf area basis is in the same order as that by stomatal conductance (conductance for $CO_2$ diffusion from the air outside the leaf to the substomatal cavity; Terashima et al., 2006; Flexas et al., 2008; Evans et al., 2009; Niinemets et al., 2009a). The representative values for $CO_2$ concentrations in the substomatal cavity ($C_s$), bulk intercellular space ($C_i$), and chloroplast stroma ($C_c$) in actively photosynthesizing leaves in ambient air containing $CO_2$ at 39 Pa, expressed as ratios, are $C_s/C_a$ = 0.60 to 0.85 (even as

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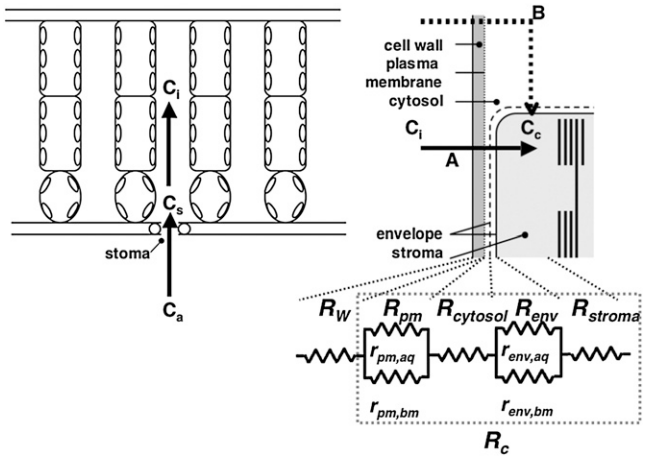


**Figure 1.** $CO_2$ diffusion from air outside the leaf to the chloroplast stroma. *C* denotes $CO_2$ concentration as follows: $C_a$, ambient air; $C_s$, substomatal cavity; $C_i$, bulk intercellular space; $C_c$, chloroplast stroma. The left panel shows a cross section of a leaf; the right panel shows a closeup of part of a chloroplast appressed against the plasma membrane; and the bottom panel shows mesophyll conductance per unit chloroplast surface area ($g_m/S_c$). $CO_2$ flux via route B in the right panel is negligible compared with that via route A because diffusion of inorganic C in the medium at pH 7.4 is very slow. Thus, virtually all the inorganic C is transported across the cell wall and cytosol facing chloroplasts. $g_m/S_c$ is the inverse of mesophyll resistance/$S_c$, which is expressed as an array of resistances. These resistances are divided into wall resistance ($R_w$) and cellular resistance ($R_c$). $R_{cytosol}$, Cytosol resistance; $R_{env}$, chloroplast envelope resistance; $R_{pm}$, plasma membrane resistance; $R_{stroma}$, stromal resistance. Resistances across the membranes are expressed as the parallel pathways through aquaporin ($r_{pm,aq}$ and $r_{env,aq}$) and bulk membrane ($r_{pm,bm}$ and $r_{env,bm}$). Redrawn from Terashima et al. (2006) with some modifications.

low as 0.12 with tightly closed stomata), $C_i/C_s = 0.90$ to 0.99, and $C_c/C_i = 0.50$ to 0.80. In this Update, we do not examine stomatal conductance (for review, see Evans and Loreto, 2000). The $CO_2$ drawdown from the substomatal cavity to the bulk intercellular spaces is small, in particular in thin leaves with large fractions of intercellular air space and/or in amphistomatous leaves (for review, see Terashima et al., 2006).

### Diffusion of Inorganic C in the Liquid Phase

$CO_2$ in the intercellular spaces dissolves in the apoplastic water of the cell wall and diffuses across the cell membrane, cytosol, chloroplast membranes, and stroma to Rubisco. According to Henry's law, $CO_2$ concentration in the liquid phase, $[CO_2]$, is proportional to the partial pressure of $CO_2$ in the adjacent gas phase. At 25°C, $[CO_2]$ in the water equilibrated with the air containing 39 Pa $CO_2$ is 13 $\mu$M. $[CO_2]$ is not affected by pH, whereas $[HCO_3^-]$ increases with the increase in pH. Assuming that $[HCO_3^-]$ is in complete equilibrium with $[CO_2]$, the ratio of the concentrations can be calculated using the Henderson-Hasselbalch equation:

$$\log \frac{[HCO_3^-]}{[CO_2]} = \mathrm{pH} - \mathrm{pK_a} \quad (1)$$

With $pK_a$ of 6.2, $[HCO_3^-]/[CO_2]$ at pH 5 (cell wall) is 0.063, at pH 7.4 (cytosol) is 15.8, and at pH 8.0 (chloroplast stroma in the light) is 63.1. $pK_a$ for $HCO_3^-/CO_3^{2-}$ is 10.25, so that $[CO_3^{2-}]$ is negligible under physiological conditions. $pK_a$ is affected by various factors including ionic strength and solute species (Stumm and Morgan, 1996). Here, we adopted the value for an ionic strength of 200 mM (Nobel, 2004). $CO_2$ diffusion in the liquid phase is slower than that in the gas phase by 4 orders of magnitude: diffusion coefficient of $CO_2$ in air and water ($D_w$) at 25°C are $1.56 \times 10^{-5}$ and $1.7 \times 10^{-9}$ $m^2$ $s^{-1}$, respectively. The diffusion coefficient of $HCO_3^-$ in water is 56% of that for $CO_2$ (Evans et al., 2009). Thus, we may express the diffusion coefficient for the total inorganic C as $D_w \times (1 + 0.56\,\kappa)$, where $\kappa$ is $[HCO_3^-]/[CO_2]$. If the activity of carbonic anhydrase is high enough, $\kappa$ may approach the fully equilibrated value calculated by Equation 1.

### Cell Wall Conductance

In the cytosol at pH 7.4, diffusion of inorganic C can be 9.85 (=1 + 0.56 × 15.8) times faster than that of $CO_2$. Even when diffusion of both dissolved $CO_2$ and $HCO_3^-$ is considered, diffusion of inorganic C in the liquid phase is still very slow compared with that in the gas phase. Then, the most effective diffusion pathway through the cytosol is the shortest one, across the chloroplast envelopes and cell walls adjacent to the intercellular air space (compare routes A and B in the right panel in Fig. 1). Thus, the chloroplast surface area would be important. Actually, $g_m$ for the plants of the same functional group (annuals, broad-leaved deciduous trees, broad-leaved evergreen trees, etc) is roughly proportional to the cumulated surface area of chloroplasts facing intercellular spaces divided by leaf surface area (one side), $S_c$ (Evans and Loreto, 2000; Terashima et al., 2006). The slope, $\Delta g_m/\Delta S_c$, however, differs among the functional types and is highest in annual herbs, intermediate in deciduous broad-leaved trees, and lowest in evergreen broad-leaved trees.

Given that $g_m$ is proportional to $S_c$, we can analyze $g_m/S_c$ (mol $m^{-2}$ $s^{-1}$) as the inverse of a series of resistances, analogous to an electrical circuit (Fig. 1). Let us divide these resistances into the wall resistance ($R_w$; in $m^2$ $s^{-1}$ $mol^{-1}$) and cellular resistance ($R_c$; in $m^2$ $s^{-1}$ $mol^{-1}$) and assume that $R_w$ is proportional to cell wall thickness ($\delta_w$; in m). In Figure 2, $g_m/S_c$ is plotted against the thickness of the mesophyll cell wall. The curve with the highest determination coefficient ($r^2 = 0.74$) is:

$$g_m/S_c = 1/\left(3.36 \times 10^8 \times \delta_w + 27.9\right) \quad (2)$$

When we assume that $R_c$ is little different among the species and the cell wall thickness is 0.1 $\mu$m (typical for annual herbs), $R_w$ and $R_c$ are 33.6 and 27.9 $m^2$ $s^{-1}$

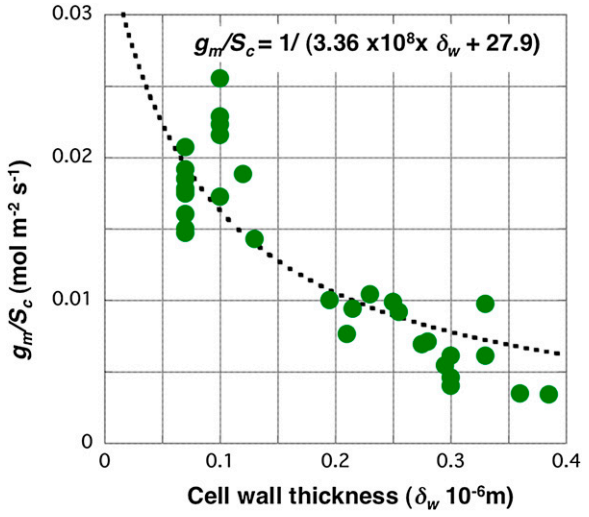


**Figure 2.** Mesophyll conductance per unit chloroplast surface area ($g_m/S_c$) plotted against the thickness of mesophyll cell wall ($\delta_w$). Data are from Terashima et al. (2006) and Evans et al. (2009). An equation fitted to the data was $g_m/S_c = 1/(R_w + R_c) = 1/(\overline{R}_w \times \delta_w + R_c)$, where $\overline{R}_w$ is the intrinsic wall resistance (m s$^{-1}$ mol$^{-1}$) and only $\delta_w$ was variable. The curve with the highest determination coefficient ($r^2$ = 0.74) is $g_m/S_c = 1/(3.36 \times 10^8 \times \delta_w + 27.9)$.

mol$^{-1}$. Thus, the cell wall resistance is responsible for about half the total mesophyll resistance. The equation also indicates that, with the increase in cell wall thickness, $g_m/S_c$ decreases and the contribution of cell wall resistance to the total resistance increases. This probably explains why the slopes of $\Delta g_m/\Delta S_c$ are lower for trees.

Based on Niinemets and Reichstein (2003), Nobel (2004), and Evans et al. (2009), wall conductance may be expressed as:

$$g_w/S_c = \frac{p \times D_c \times (1 + \alpha \times \kappa) \times H \times P}{\tau \times \delta_w} \quad (3)$$

where $p$ is the porosity (m$^3$ m$^{-3}$), $D_c$ is the diffusion coefficient of $CO_2$ in water (m$^2$ s$^{-1}$), $\alpha$ is the ratio of diffusion coefficient of $HCO_3^-$ in water to $D_c$ (=0.56), $H$ is the Henry's law constant in mol m$^{-3}$ Pa$^{-1}$, $P$ is the atmospheric pressure in Pa, $\tau$ is tortuosity (m m$^{-1}$), and $\delta_w$ is cell wall thickness in m. $p$ expresses how much space in the cell wall is used for $CO_2$ diffusion, while $\tau$ expresses how much the path is lengthened or tortured by the cell wall. $H$ is defined as:

$$H = C_l/(P \times C_g) \quad (4)$$

where $C_l$ is the $CO_2$ concentration in the liquid phase in mol m$^{-3}$ and $C_g$ is the $CO_2$ concentration in the gas phase in mol $CO_2$ mol$^{-1}$. Thus, $P \times C_g$ denotes partial pressure of $CO_2$ in Pa.

We may estimate an effective $p/\tau$ from the best-fit curve in Figure 2. Because pH in the apoplastic water is moderately acidic, let us assume that inorganic C in the cell wall is exclusively $CO_2$. When $D_c$, $H \times P$, and $\kappa$, are substituted with $1.7 \times 10^{-9}$ m$^2$ s$^{-1}$, 33.9 mol m$^{-3}$, and 0, respectively, $p/\tau$ is calculated to be 0.052 (i.e. much less than the values that have been assumed, such as 0.3; Nobel, 2004). When nitrate is abundant in the xylem sap and/or the soil is dry, the pH of the apoplast water could increase up to neutral levels (Jia and Davies, 2007). Then, diffusion of $HCO_3^-$ should be considered. Because porosity and tortuosity would also change with pH due to the protonation or ionization of chemical groups in polysaccharides and proteins present in the cell wall, effects of pH on wall conductance should be studied.

### Cytosol Conductance and Stromal Conductance

Cytosol conductance and stromal conductance can be also analyzed using Equation 3. The cytosol between the plasma membrane and the chloroplast envelope is usually very thin, typically 0.1 $\mu$m or less (Evans et al., 2009). Under steady-state conditions in the light, chloroplasts are anchored to the plasma membrane by actin filaments, which effectively minimize the cytosol thickness (for review, see Takagi et al., 2009). In the cytosol, $p/\tau$ approaches 1.0 and $\kappa$ is around 9.85. Consequently, for the layer of cytosol with the same thickness as the cell wall, the cytosol conductance would be more than 100-fold greater than the wall conductance.

For the chloroplast stroma, typically 2 $\mu$m thick, $p/\tau$ may be much smaller than 1.0 because of abundant Rubisco, other proteins, and thylakoids. Effects of viscosity can also be included in the $p/\tau$ value. If the activity of carbonic anhydrase is high, $1 + 0.56\kappa$ will approach 36 at pH 8.0. Assuming a $p/\tau$ value of 0.5, then the conductance of 2-$\mu$m-thick stroma is 18 times that of a 0.1-$\mu$m-thick cell wall. The actual conductance is likely to be greater than this estimation because the effective thickness of the stroma could be roughly half if Rubisco molecules are distributed evenly throughout the stroma. On the other hand, if the $p/\tau$ value is much lower because of higher viscosity in the stroma, pH is more neutral in low light, and/or if the activity of carbonic anhydrase is not sufficiently high, then the stromal conductance can be smaller. Otherwise, these rough estimations indicate that both the cytosol conductance and the stromal conductance are much greater than the cell wall conductance.

### Plasma Membrane Conductance and Chloroplast Envelope Conductance

Because the membrane is hydrophobic, the inorganic C that is transported across membranes (the plasma membrane and the inner membrane of the chloroplast envelope) is mainly $CO_2$. For the plasma membrane, the majority of $CO_2$ transported from the cell wall across the membrane is subject to conversion to $HCO_3^-$ because pH in the cytoplasm is around 7.4.

This conversion is catalyzed by carbonic anhydrases located in the plasma membrane and/or the cytosol. In the case of the chloroplast inner envelope membrane, conversions of $HCO_3^-$ to $CO_2$ and of $CO_2$ to $HCO_3^-$, respectively, occur on the outer and inner sides of the membrane. Involvement of the carbonic anhydrase isozymes in different cellular components (Fabre et al., 2007) is crucial for an optimal transfer efficiency of $CO_2$.

Another important topic related to the transport of $CO_2$ across the membranes is the involvement of aquaporins. Changes in the aquaporin content per unit leaf area by genetic transformation techniques confirmed that $g_m$ changes in accordance with the abundance of aquaporin protein expressed (Hanba et al., 2004; Flexas et al., 2006; for review, see Katsuhara et al. 2008). Aquaporins may be involved in $CO_2$ transfer in the plasma membrane as well as in the chloroplast envelope (Uehlein et al., 2008). It is necessary to further analyze mesophyll conductance in relation to the functions of aquaporins. Because aquaporin is not an appropriate term for proteins that transfer $CO_2$, and because aquaporins that transfer $CO_2$ would cooperate with carbonic anhydrases at the membrane-liquid phase interfaces, the term "cooporin" has been proposed (Terashima et al., 2006).

In summary, the magnitude of limitation of photosynthesis by $g_m$ is much greater than had been thought. $CO_2$ diffusion across cell walls and membranes is identified to be the major limiting step. Because $g_m$, in particular wall conductance, plays an important role in differentiating plant functional types according to leaf photosynthetic capacity (Terashima et al., 2006; Evans et al., 2009; Niinemets et al., 2009a), we suggest that this important topic requires high priority in future studies. Although we express the conductance for $CO_2$ diffusion by a one-dimensional model, $C_c$ in the leaf would differ considerably. We discuss this problem later.

Given that $g_m$ is an important limiting factor of photosynthesis, it is very problematic to analyze gas-exchange data assuming that $C_i$ is equal to $C_c$ (this assumes the infinite $g_m$!). The spurious effects of this assumption have been repeatedly pointed out. In particular, estimation of kinetic parameters of Rubisco without taking $g_m$ into account should be avoided (for review, see Niinemets et al., 2009b).

## WHY DOES LEAF THICKNESS INCREASE WITH LIGHT AVAILABILITY?

Leaves need a considerable amount of Rubisco per leaf area for photosynthesis, because Rubisco has a low catalytic rate. Given that $g_m$ is finite and not large, as analyzed above, $C_c$ decreases with the increase in the concentration of Rubisco per unit $S_c$ (Fig. 3). Because the affinity of Rubisco to $CO_2$ is poor ($K_c$ is large), with the decrease in $C_c$, the carboxylation rate decreases and the rate of energy-wasting photorespiration increases. Moreover, as a result of a lower total energy use by the carboxylation and oxygenation at the low $C_c$, chloroplasts are more liable to be over-reduced and photoinhibited. Thus, Rubisco/$S_c$ should be kept small to keep $C_c$ at high levels. Figure 3 also shows that, for the similar Rubisco/$S_c$, $C_c$ would be lower in tree species that have lower $g_m/S_c$ than in annual herbs (Evans et al., 2009; Niinemets et al., 2009a). Then, Rubisco/$S_c$ may be lower in tree species to suppress the decrease in $C_c$. However, probably due to scarcity of the data, we cannot see this trend in Figure 3. Alternatively, the very low $CO_2$ may have imposed selection pressure to Rubisco in tree species. Actually, the average specificity factor of Rubisco, $V_c \times K_o/V_o \times K_c$ (where $V_c$ and $V_o$ are the maximum activities of carboxylation and oxygenation and $K_o$ is the $K_m$ for oxygen of Rubisco), is highest in evergreen trees and lowest in annual herbs (Galmés et al., 2005; A. Makino, personal communication).

To realize higher photosynthetic capacities on a leaf area basis ($P_{max}$, maximum photosynthetic rate at light saturation), sun leaves should have more Rubisco per unit leaf area than shade leaves. If leaves have more Rubisco, they should also have greater $S_{mes}$ that are prerequisite for larger $S_c$. Because neither the cell diameter nor the cell density differs greatly between sun and shade leaves of the same species, a larger $S_{mes}$ can be attained by increasing the cell height. This may explain why sun leaves have to be thicker than shade leaves (Terashima et al., 2001).

The importance of the increasing $S_c$ for increasing $P_{max}$ in shaded leaves on exposure to high growth light has been shown by a series of elegant studies (Oguchi et al., 2003, 2005, 2006). In shade leaves of *Chenopodium album*, part of the mesophyll surface area was unoccupied by chloroplasts and $S_c/S_{mes}$ was around 0.78. When these shade plants were exposed to high light, the $S_{mes}$ was unchanged but $S_c$ increased. $S_c/S_{mes}$ attained around 0.96 by exposure for 2 weeks. $P_{max}$ increased proportionally with the increase in $S_c$ (Oguchi et al., 2003). By cutting of a tall dominant tree in a deciduous broad-leaved forest, the saplings of deciduous tree species on the forest floor were suddenly exposed to high light. In *Betula ermanii*, *Kalopanax pictus*, *Magnolia obovata*, and *Quercus crispula*, $S_c/S_{mes}$ and $P_{max}$ increased, while $S_{mes}$ were unchanged, as in *C. album*. Interestingly, leaves of three *Acer* (maple) species showed elongation of mesophyll cells, leading to increases in $S_{mes}$. $S_c$ and $P_{max}$ also increased accordingly (Oguchi et al., 2005, 2006). The leaves of species such as *Fagus crenata*, which have neither mesophyll surfaces unoccupied by chloroplasts nor the ability to increase $S_{mes}$, failed to increase $P_{max}$ (Oguchi et al., 2005). In the study with *C. album*, Rubisco/$S_c$ showed surprisingly constant values around 0.3 $\mu$mol $m^{-2}$ across different light environments (Oguchi et al., 2003).

Figure 4 illustrates several strategies for increasing mesophyll surface area. For example, Sage and Sage (2009) showed that the highly lobed cells (armed cells)

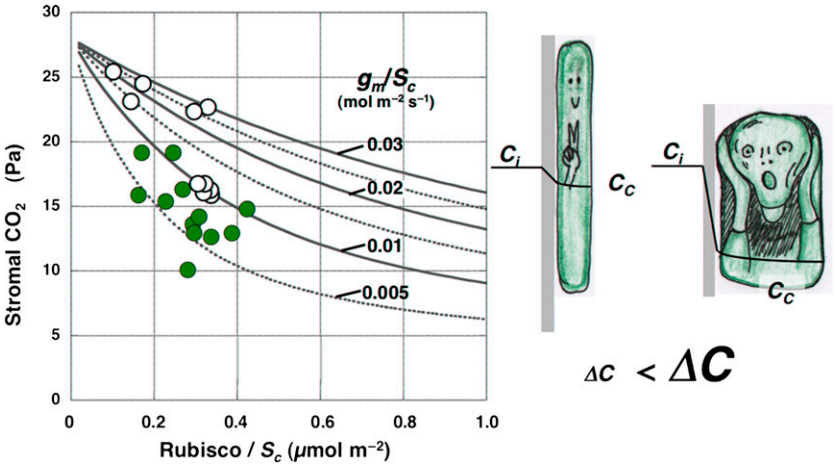


**Figure 3.** $C_c$ plotted against Rubisco content per $S_c$. Theoretical lines are drawn based on the kinetic parameters of rice Rubisco at 25°C (Makino et al., 1985) assuming that $C_i$ is 28 Pa. White and black circles indicate annual herbs and broad-leaved trees (deciduous and evergreen trees are included). Data are from Hanba et al. (1999), Evans and Loreto (2000), Miyazawa and Terashima (2001), Oguchi et al. (2003), Terashima et al. (2006), and D. Tholen (unpublished data). For the same $g_m/S_c$, $C_c$ in the chloroplast with a greater Rubisco/$S_c$ is lower than that in thin chloroplast with a lower Rubisco/$S_c$. There may be some decline in $C_c$ within a chloroplast. $\Delta C$ in the cartoon denotes $C_i - C_c$.

in rice (*Oryza sativa*) can achieve a very high $S_{mes}$ and $S_c$. For other strategies to increase $S_{mes}$ and their possible advantages and disadvantages, see Terashima et al. (2001, 2006).

## LIGHT ABSORPTION BY THE LEAF

For efficient use of the light energy, light needs to be delivered to all the chloroplasts distributed along the cell surfaces throughout the leaf. Leaves, therefore, have to fulfill two contrasting requirements: to absorb much light, and to deliver light to all the chloroplasts. Red and blue light is largely absorbed by chloroplasts located near the illuminated surface of the leaf, because chlorophylls preferentially absorb these wavelengths. Light penetrating deeper inside the leaf is mostly green. Chlorophylls are less efficient in absorbing green light. However, multiple scattering increases the path length of light such that leaves absorb 80% of the green light incident on the leaves (for review, see Terashima et al., 2009). The cell shape of the spongy tissue enhances light scattering. There are few comparative studies on in situ absorption coefficients between the palisade and spongy tissues. In *Spinacia oleracea*, the difference in the in situ absorption coefficient between these tissues was small (Vogelmann and Evans, 2002), while in other species, including *Camellia japonica*, *Helianthus annuus*, and *Antirrhinum majus*, considerable differences were reported (Terashima et al., 2009; Brodersen and Vogelmann, 2010). The question of whether the irregular shape of spongy tissue plays a role in the efficient absorption of green light should be addressed in more species.

It has been argued that the spongy tissue may facilitate lateral $CO_2$ diffusion from substomatal cavities. However, in many herbaceous species, lateral diffusion can also occur in the palisade tissue through gaps between the lateral walls of the neighboring cells. Such herbaceous species tend to have stomata with substomatal cavities on the adaxial leaf surfaces as well, and the size of the cavities is sufficient for $CO_2$ homogenization (Parkhurst, 1977). Thus, this casts doubt on the hypothesis that the function of the spongy tissue is related to the facilitation of lateral $CO_2$ diffusion. Instead, the effects on the lengthening of the light path may be more important.

It is well established that there is a vertical gradient in chloroplast properties within a leaf. Several theoretical models of this phenomenon have been developed (for review, see Terashima et al., 2009). According to one of the theories, the maximum photosynthetic efficiency will be realized if the photosynthetic capacity is proportional to light absorption at each point. However, the gradient of the absorption profile is generally steeper than that of the photosynthetic capacity (for review, see Terashima et al., 2009). The reason for this imperfect matching is not clear. Certainly, the abaxial leaf surfaces also receive some reflected light from the surroundings, which would affect the gradient of photosynthetic capacity. However, a more likely explanation is that the dynamic range for light acclimation of chloroplasts is limited: chloroplasts cannot fully acclimate to very high or very low light.

When the leaf is illuminated with white light from the adaxial side, the chloroplasts located near the adaxial leaf surfaces are light saturated at lower incident irradiances than the chloroplasts near the abaxial surface, because of the imperfect matching of the profiles of photosynthetic capacity and light absorption. When the upper chloroplasts are light saturated, green light that can penetrate deep is more effective in driving photosynthesis of chloroplasts in the deep part. We assessed the efficiency of green light by the differential quantum yield method (Terashima et al., 2009). Figure 5 gives an outline of this method and typical results.

Once absorbed, green light efficiently drives photosynthesis. This has been shown by the conventional quantum yield measurements with monochromatic lights (for review, see Terashima et al., 2009). However, the differential quantum yield measurements revealed that the average quantum yield of green monochro-

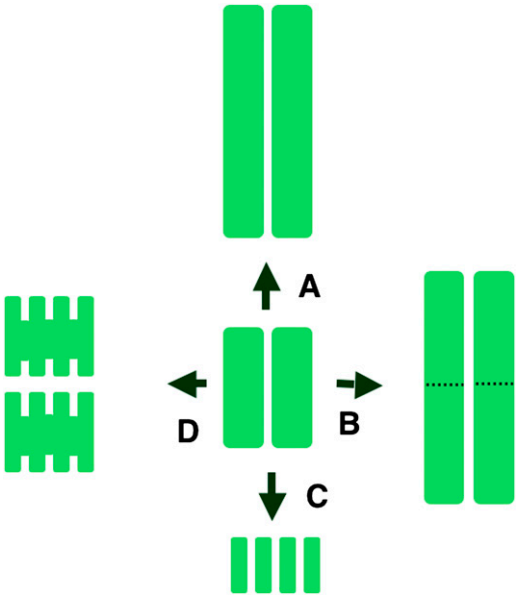


**Figure 4.** Various strategies to increase mesophyll surface areas. A, Cell elongation. B, Cell elongation accompanied by cell division. C, Decrease in cell size. D, Armed cells of grass species having lobes. For cells having the same cell walls of equal thickness, the tissue with smaller cells is mechanically tougher (Terashima et al., 2001). The leaves with larger cells would expand faster. On the other hand, these would inevitably be thicker to have enough $S_{mes}$ to accommodate chloroplasts, in which case the diffusion in the intercellular spaces becomes significant. Such leaves, mostly in annual herbs, tend to have stomata on both epidermises (Terashima et al., 2006). The acclimation of photosynthetic properties of chloroplasts to the local light environment may be more precise in cells in B than in those in A. In grass leaves, armed cells have large cell surface areas. In rice, almost all the cell surfaces facing the intercellular spaces are occupied by chloroplasts. Mitochondria are located in the capsule made by chloroplasts. For more insightful discussion for grass leaves, see Sage and Sage (2009).

matic light in white light would be comparable to that of red monochromatic light, even when the data were plotted against the incident irradiance. This finding highlights the importance of green light. It is probable that land plants may have been using chlorophylls that strongly absorb red and blue light but only weakly absorb green light, because such a system allows the delivery of light not only to chloroplasts near the irradiated surface but to those deep in the leaf. Given that the dynamic range of acclimation of chloroplasts to irradiance is limited, black leaves that equally absorb light at each wavelength would use light less efficiently at high irradiances than green leaves.

In most optics/photosynthesis studies, collimated light is used. However, in nature, leaves also receive diffuse light and/or collimated light from oblique direction(s). Diffuse light is transmitted less than collimated light (Brodersen and Vogelmann, 2010). When the collimated light hits the leaf obliquely, transmittance also decreases. Therefore, when a significant part of the light is noncollimated, even less light would penetrate deeply into the leaf and green light would be an even more important driver of photosynthesis.

The discrepancy between the profiles of light absorption and photosynthetic capacity in the leaf causes several serious practical problems in measurements of photosynthetic parameters. In many leaf photosynthesis studies, it is implicitly assumed that the leaf behaves like one big chloroplast: all chloroplasts respond to light in accordance with the solution for optimum photosynthesis. In most leaves, this is not the case. Most commercially available fluorometers use red or blue light as the measuring beam that tends to be absorbed by chloroplasts near the leaf surface. Photosynthesis by these surface chloroplasts would be light saturated at lower incident irradiances than chloroplasts deeper into the leaf. The quantum yield of PSII in the light estimated by conventional fluorometry, therefore, is underestimated (Evans, 2009). By contrast, the PSI measurement at 830 nm samples the whole leaf, because this wavelength is only weakly absorbed. The estimation of cyclic electron flow around PSI could be overestimated because of the

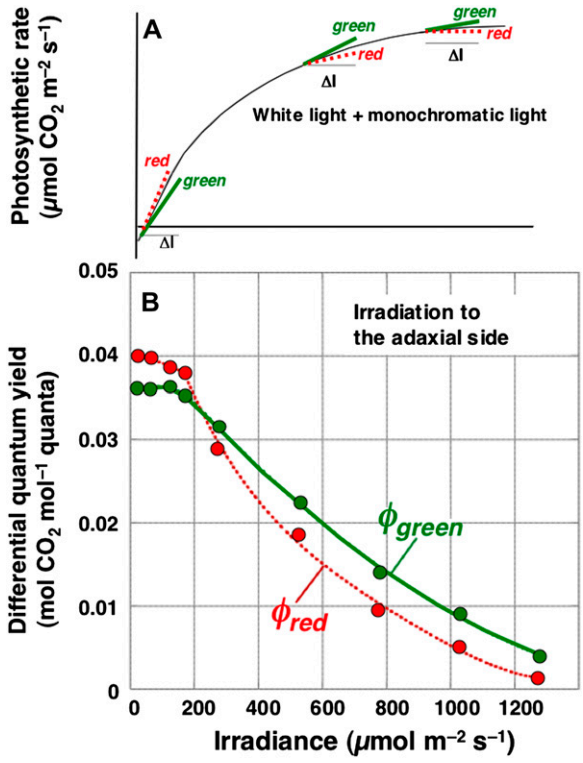


**Figure 5.** Differential quantum yield method (A) and differential quantum yield of green and red monochromatic light in white light in a *H. annuus* leaf (B). The quantum yield of a given monochromatic light is usually measured at low irradiances in the absence of background light. In the differential quantum yield method, weak monochromatic light is added to the various white irradiances. When the upper chloroplasts are light saturated, green light can drive photosynthesis in chloroplasts located deep in the leaf, where additional red or blue light fails to penetrate, instead being dissipated as heat. For details, see Terashima et al. (2009).

underestimation of linear electron flow rate based on the PSII quantum yield measurement. The degree of photoinhibition of PSII assessed by fluorometry may not represent the PSII of the whole leaf (for review, see Terashima et al., 2009).

It should also be stressed here that, when the degree of light saturation differs between the chloroplasts near the irradiated surface and those located deep in the leaf, drawdown of $CO_2$ would also differ. When photosynthesis in the chloroplasts near the leaf surface is light saturated while that in the chloroplasts in the deep part of the leaf is not, it is highly probable that the drawdown of $CO_2$ concentration is smaller in the deep chloroplasts. Obviously, this situation affects the estimation of $g_m$. Care should be taken when $g_m$ is estimated.

## ORGANELLE MOVEMENT

Chloroplast movements in response to light have been studied intensively. In weak light, chloroplasts move so as to maximize light absorption, whereas in strong light, they move to minimize it. These movements (Kadota et al., 2009) as well as anchoring (Kadota and Wada, 1992) are both mediated by actin filaments (Takagi et al., 2009). Phototropins 1 and 2 have been identified as the photoreceptors responsible for weak and strong light responses, respectively (Wada et al. 2003). It is worth pointing out that phototropins are involved in various phenomena closely related to photosynthesis, such as phototropism, leaf flattening, stomatal opening, and chloroplast movement. Nuclei also show an avoidance movement from strong light, which is mediated by phototropin 2, whereas the weak-light response is absent (Iwabuchi et al., 2007).

It has recently been shown that chloroplast movement causes changes in $S_c$ and proportional changes in $g_m$ (Tholen et al., 2008). In Arabidopsis (*Arabidopsis thaliana*) grown at moderate light, irradiation with strong light causes decreases in $S_c$. These leaves were not typical sun leaves, and cell surfaces parallel to the light direction were less abundant than those perpendicular to the light direction. Avoidance of photodamage occurred at the expense of decreases in $g_m$. In addition, movement of chloroplasts in the upper part of the leaf would allow greater light penetration to deep parts within the leaf. While reducing $g_m$ of the upper cells, this would enhance whole leaf light use efficiency.

Mitochondria and peroxisomes are involved in photorespiration together with chloroplasts. Mitochondria also dissipate excessive reducing power generated by chloroplasts in excess light (Noguchi and Yoshida, 2008). Recently, it has been shown that mitochondria (Islam et al., 2009) and peroxisomes (Mano et al., 2002) also move with chloroplasts in the light. For mitochondria, blue light receptors, possibly phototropins, may be involved in the light-induced movement with chloroplasts (Islam et al., 2009).

The arrangement of mitochondria, peroxisomes, and chloroplasts in the cell has been attracting attention. $CO_2$ is released from mitochondria during photorespiration and respiration. Refixation of $CO_2$ by Rubisco will be more efficient if mitochondria are sitting inside a capsule made of chloroplasts covering cell surfaces. In rice leaves, Sage and Sage (2009) found that sheet-like extensions of chloroplasts cover almost all the surfaces of plasma membranes facing intercellular spaces and that mitochondria are almost exclusively distributed within the chloroplast capsule. This arrangement would also facilitate the refixation of $NH_3$ released in the course of photorespiration. Although reassimilation of ammonia is costly (using ATP and ferredoxin), the loss of ammonia is more costly, particularly in plants that take up nitrate or obtain ammonium via nitrogen-fixing symbiosis.

Cell morphogenesis and organelle positioning clearly require further investigations in relation to photosynthesis. Concerning $NH_3$ trapping, it may be worth mentioning that thick mesophyll cell walls found in plants surviving in stressful environments could serve as a good gas trap.

## HOW DO LEAVES REGULATE MESOPHYLL SURFACE AREA?

Thick leaves having extensive $S_{mes}$ are advantageous to realize high photosynthetic rates in high-light environments. An ecotype of the Japanese beech *F. crenata*, on the Pacific side of Japan, prepares all leaves in its winter buds. Both the number of leaves and the cell layers in the palisade tissue can be counted in overwintering buds. Whether the leaves unfold as sun leaves or shade leaves is determined in the previous year. When such sun leaves on the shoot are artificially heavily shaded, winter buds prepare shade leaves with one cell-layered palisade tissue. The structure of the current-year leaves in this late successional species is determined to a large extent by the light environment or photosynthetic productivity of the previous year (Uemura et al., 2000). On the other hand, in several temperate evergreen species, leaf development depends on the average irradiance level (Niinemets et al., 2004), suggesting greater anatomical plasticity. The capacity for adjustment to new light environments depends on the stage of leaf development. Young developing leaves can acclimate to the new light conditions, while older leaves with partly or entirely lignified cell walls can only partly acclimate to altered light conditions (Yamashita et al., 2002).

When leaves develop successively, developing young leaves are covered by older leaves and may be unable to directly sense the light environment outside the bud. Using *C. album* plants, Yano and Terashima (2001) found that the number of cell layers in the palisade tissue of developing young leaves was determined depending on the light environment of mature leaves, irrespective of the light conditions of

the developing young leaves. When mature leaves were exposed to strong light, periclinal divisions of palisade-tissue precursor cells occurred in young leaves that produced two cell layers of palisade tissue, whereas when mature leaves were kept in weak light, such periclinal divisions did not occur.

Stomatal density of developing leaves may also be regulated by the conditions of mature leaves (e.g. $CO_2$ or light; Lake et al., 2001). Molecular mechanisms for these systemic regulations are unknown. However, such mechanisms may play an important role in allowing a leaf to function efficiently immediately after unfolding.

## CONCLUDING REMARKS

We have argued several structural features that appear to be highly important for efficient leaf photosynthesis. Nevertheless, it is still essential to assess their functional roles quantitatively. It is also necessary to assess such relationships across a variety of plant species and to determine how the structure-function relationships vary depending on the ecological strategy of the species, such as the position in succession and plant functional type (Hallik et al., 2009a, 2009b).

Further clarifications of the mechanisms that are responsible for these adaptive morphogenesis and organellar movements are strongly awaited. Such developmental and/or cell biological studies should be made together with actual measurements of photosynthetic parameters. Overall, we believe that it is highly relevant to promote ecologically meaningful developmental studies and cell biological studies through the close collaboration of molecular physiologists, cell biologists, and ecophysiologists.

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