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Function of silica bodies in the epidermal system of rice (*Oryza sativa* L.): testing the window hypothesis

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Abstract

Silicon has been considered to be important for normal growth and development of the rice plant (*Oryza sativa* L.). To investigate the physiological function of deposited silica in rice leaves, the hypothesis that silica bodies in the leaf epidermal system might act as a 'window' to facilitate the transmission of light to photosynthetic mesophyll tissue was tested. The silica content of leaves increased with supplied silicon and was closely correlated with the number of silica bodies per unit leaf area in the epidermal system. There was a significant difference in silica deposition and formation of silica bodies between Si-treated and non-treated leaves; silicon was polymerized inside the silica cells and bulliform cells of the epidermis, in Si-treated leaves. Although the 'windows' were only formed in leaves with applied silicon, optical properties of leaf transmittance, reflectance and absorptance spectra in Si-treated and non-treated leaves were almost equal. Furthermore, light energy use efficiency and quantum yield of Si-treated leaves were less than in leaves not containing silica bodies. Thus, silica bodies, at least based on the data, do not function as windows in rice leaves.

Key words: Silicon, window hypothesis, rice, optical property, quantum yield.

Introduction

The silica content of higher plants varies significantly among species. Species which deposit a large quantity of silica in aerial parts have been termed silica-accumulator plants (Takahashi and Miyake, 1977). In these plants, the accumulated silica can contribute 5–20% to the shoot's dry weight (Lewin and Reimann, 1969). In the absence of silica, the shoots have either stunted or suppressed growth (Werner and Roth, 1983). Thus, silicon has been considered to be important for normal growth and development of shoots.

In Japan, since the 1950s, silica in the form of slag has been widely used as a siliceous fertilizer for rice plants, and it has been shown that application of slag to rice paddies significantly increases dry matter production and grain yield of rice. Silicon has been reported to benefit rice in a number of ways: (1) it increases canopy photosynthesis as a result of keeping leaf blades erect (Ishizuka, 1971); (2) it increases resistance to certain insects and disease-causing fungi and bacteria (Takijima *et al.*, 1949; Ota *et al.*, 1957); (3) it reduces accumulation of toxic concentrations of Mn^{2+} and other heavy metals; (4) it increases the oxidizing power of roots (Okuda and Takahashi, 1964) ; and (5) it reduces cuticular transpiration and improves water use efficiency (Yoshida *et al.*, 1959; Mitsui and Takatoh, 1959). In spite of numerous investigations on Si application effects, there is no evidence that silicon plays a direct role in the metabolism of rice or other higher plants which accumulate it in considerable quantities.

Typically, silica is deposited in rice plants in the form of silica bodies, which are formed in epidermal cells, silica cells and bulliform cells (Kaufman *et al.*, 1981). Based on histological studies on grass leaves, Kaufman *et al.* (1979), Lau *et al.* (1978) and Takeoka *et al.* (1979) have postulated that the silica bodies would increase rice growth as a result of increasing photosynthetic rate by facilitating the transmission of light through the epidermal system to photosynthetic mesophyll (in leaves) or cortical tissue (in stems) below and inside. According to this 'window' hypothesis, leaves with a high density of silica bodies may show greater photosynthetic light energy use

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efficiency, and hence greater photosynthetic production. Furthermore, leaves with low or high densities of silica bodies may show different optical properties or photochemical activity, including light transmittance, reflectance and quantum yield for CO_2 uptake. However, no attempt has been made to quantify the possible effects of silicon on rice leaves by actual measurement of parameters related to photochemical or optical properties.

In this paper, data are presented on the experimental testing of the 'window' hypothesis by examination of the optimal properties of light absorptance, transmittance, and reflectance spectra. Photochemical parameters are also analysed, including light energy use efficiency and quantum yield for CO_2 uptake based on absorbed photon flux densities.

Materials and methods

Plant materials and growth conditions

Rice plants (*Oryza sativa* L., cv. Koshihikari) were water-cultured in a greenhouse using Yoshida's nutrient solution (Yoshida *et al.*, 1976) containing 0, 20, 40 and 100 ppm of SiO_2, respectively. The culture solution was renewed once a week, and pH of the solution was adjusted to 5.0 with 1 N NaOH and 1 N HCl. Silicon content of leaves and the culture solution was determined by gravimetric procedures and colorimetric molybdenum blue methods (Yoshida *et al.*, 1976), respectively. Four replicate plants were prepared for each measurement of silicon content.

Soft X-ray analysis for determination of silica body distribution in whole leaves

Soft X-ray analysis of silica body distribution in rice leaves was carried out according to previously described methods (Takeoka *et al.*, 1983). The adjacent parts of leaves used for the photosynthetic measurement were fixed in FAA (formalin–acetic acid–ethyl alcohol) for 5 d, then washed, pressed and dried. The dried leaf specimens were irradiated on packed soft X-ray film by the micro source of X-rays emitted by the SOFTEX machine (SOFTEX, model CMB), using 10 keV at 5 mA for 30 s. The film was then developed and printed. Silica density (number cm^{-2}) was determined by counting the silica bodies in the four selected portions of each leaf of the Si treatment using photographs. Four replicated plants were prepared for the analysis.

Scanning electron microscopy (SEM) for analysis of silica bodies

Specimens of leaves fixed in FAA solution were dehydrated through an ethanol series, cut with a sharp razor blade, then freeze-dried and gold-coated. The ultrastructure of silica bodies was examined with a SEM (JEOL, model JSM-T200) at an accelerating voltage of 25 keV.

Gas-exchange measurement

The rate of CO_2 uptake was measured on the youngest fully expanded leaves using an open infrared gas analysis system described by Takeda *et al.* (1978). A Yokogawa infrared gas analyser (IR21) was used in differential mode for the measurements. Light was provided by a 100 W high-pressure metal halide lamp (Toshiba, D400) fitted with an aluminium reflector in conjunction with glass and flowing water filters. Incident light values were measured with a spectroradiometer (Yellow Springs, 65A) and silicon photodiode energy sensor (Advantist, 6551 probe). Leaves were first exposed to a photosynthetically active radiation (*PAR*) of 500 μmol m^{-2} s^{-1} until the photosynthetic rate had achieved a steady-state. Subsequently, light intensity was lowered in nine steps, ending in total darkness.

Light energy use efficiency and quantum yield for CO_2 uptake

Light energy use efficiency was calculated from the slope of the linear relationship between absorbed light intensity and net photosynthetic rate. The linear slope was assessed at absorbed light intensities ranging from 0 to 276.9 μmol m^{-2} s^{-1} (400–700 nm). Quantum yield for CO_2 uptake (ϕ) was calculated as

$$\phi = \frac{\text{number of absorbed } CO_2 \text{ molecules per unit leaf area}}{\text{number of absorbed photons of } PAR \text{ per unit leaf area}} \qquad (1)$$

Optical properties of light absorptance, transmittance, and reflectance spectra

Leaf transmittance and reflectance of freshly cut leaf discs were measured with a silicon photodiode energy sensor (Advantist, 6551 probe) described by Yoshida *et al.* (1990*a*, *b*). Transmittance (T) was measured by attaching a leaf to the sensor and measuring at 10 nm wavelength intervals between 400–700 nm. Reflectance (R) was measured relative to the reflectance of a solid MgO standard (100% reflectance). Illumination with a monochromatic light beam incident at 10° to the surface of the sample was provided by a metal halide lamp (Toshiba, D400) attached to a grating monochrometer (Nikon, P-250). The light reflected from leaf or standard was detected by the energy sensor at a given angle ($\theta°$) between 0–90° to the incident light path. $R(\lambda)$ was integrated as

$$R(\lambda) = 2\pi r^2 \int_0^{90} I(\theta) \sin\theta \, d\theta / I_0(\lambda) \qquad (2)$$

where $I_0(\lambda)$ is the incident light intensity and $I(\theta)$ is the reflecting light intensity detected at $\theta°$ to the incident light path. Absorptance (A) was calculated by subtraction, namely

$$A = 1 - \{T(\lambda) - R(\lambda)\}. \qquad (3)$$

From the basic relationship between energy (E), wavelength (λ), and frequency of light (ν) given by $E(\lambda) = nh\nu$, absorbed photon number (n) was culculated as

$$n(\lambda) = E(\lambda)A(\lambda)/h\nu. \qquad (4)$$

Three replicate plants were prepared for each measurement of optical properties of light absorptance, transmittance, and reflectance spectra.

Results and discussion

Localization, distribution, and ultrastructural characteristics of silica bodies

X-ray analysis with the SEM is useful for the purpose of locating silica in all the different kinds of epidermal idioblasts (specialized cell types that make up the epidermal system) that accumulate it. From silica X-ray maps and corresponding secondary electron images obtained with SEM, accurate analysis of silica cell frequency and morphological characteristics of the cells can be made.

Silica cells and cork cells are generally found in rows over veins. In the cross-section of Si-treated leaf (Plate 1), these cells were found to occur in the epidermal layer above and below the vascular bundles. The bulliform cells were seen only in the upper epidermal layer between the vascular bundles. Silicon was polymerized as $SiO_2.nH_2O$ inside the silica cells and bulliform cells in the Si-treated leaf (Plate 2A), but non-treated leaves had no silica bodies in those cells (Plate 2B). In this analysis, it also became apparent that the cell walls of the long epidermal cells in the Si-treated leaves undulated and were thicker than in non-treated leaves. Those results are consistent with the analyses of Kaufman *et al.* (1985). Since the polymerized silicic acids in the cells bind strongly with cellulose, forming a silico-cellulose membrane, these walls become silicified as well as lignified at maturity (Lewin and Reimann, 1969; Schwarz, 1973). The higher frequency of silicification sites (e.g. silica cells and bulliform cells) could be responsible for even greater mechanical strength in rice leaves. Cross-section of Si-treated (Plate 2A) and non-treated (Plate 2B) leaves did not show any definite pattern differences in the arrangement of mesophyll cells.

Amorphous silica gel ($SiO_2.nH_2O$) a typical form of silicon accumulated in rice leaves (Kaufman *et al.*, 1981), has very high X-ray absorptivities. Thus, soft X-ray analysis allows the determination of the distribution and relative frequency of silica bodies in bulliform cells in epidermal systems in whole leaf samples (Takeoka *et al.*, 1983; Kaufman *et al.*, 1985). Plate 3 shows sample micrographs obtained by soft X-ray irradiation for selected portions of leaf blades grown under different SiO_2 conditions. Table 1 shows the density of silica bodies and total SiO_2 content in these leaves. The density of silica bodies in bulliform cells, as revealed by soft X-ray analysis, increases with an increase of silica content in leaves. Silica content was parallel with the frequency of silicified silica cells and bulliform cells (Fig. 1). There was a significantly high correlation ($P<0.01$) between the density of silica bodies (SB) and silica content (SC) in leaves, giving the following regression: $SB = -231.1 + 0.00029\ SC$.

Optical properties of absorptance, transmittance and reflectance spectra, and light energy use efficiency and quantum yield for CO_2 uptake

From the window hypothesis, the fact that the outer epidermis of Si-treated leaves had more silica bodies suggests that light absorptance of mesophyll tissues would be increased due to increasing light transmissivity of the epidermis and light scattering within the leaf (Kaufman *et al.*, 1985). In addition, this effect may cause a functional change in photochemical reactions of Si-treated leaves adapted for the predicted higher light intensity within the leaf (Terashima and Takenaka, 1990). In the present experiment, however, optical properties of reflectance, transmittance, and absorptance spectra were almost equal between the Si treatments except above 700 nm (Fig. 2);

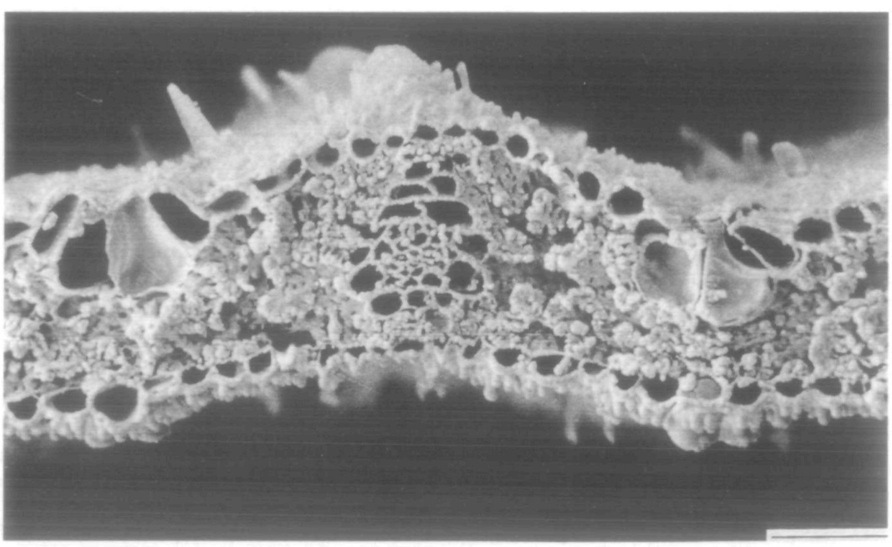

Plate 1. Scanning electron micrograph of a transverse fracture of a Si-treated leaf. Scale bar = 50 μm.

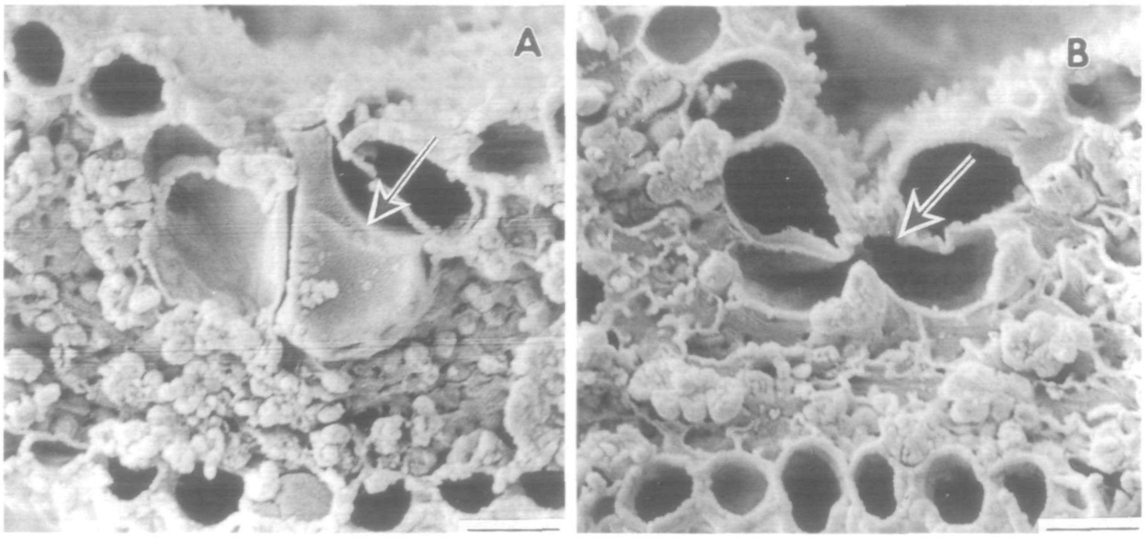

Plate 2. Scanning electron micrographs of transverse fractures of Si-treated (A) and non-treated (B) leaves. Silica bodies are seen in bulliform cells in Si-treated leaves (A, arrow), but not in non-treated leaves (B, arrow). Scale bar = 20 μm.

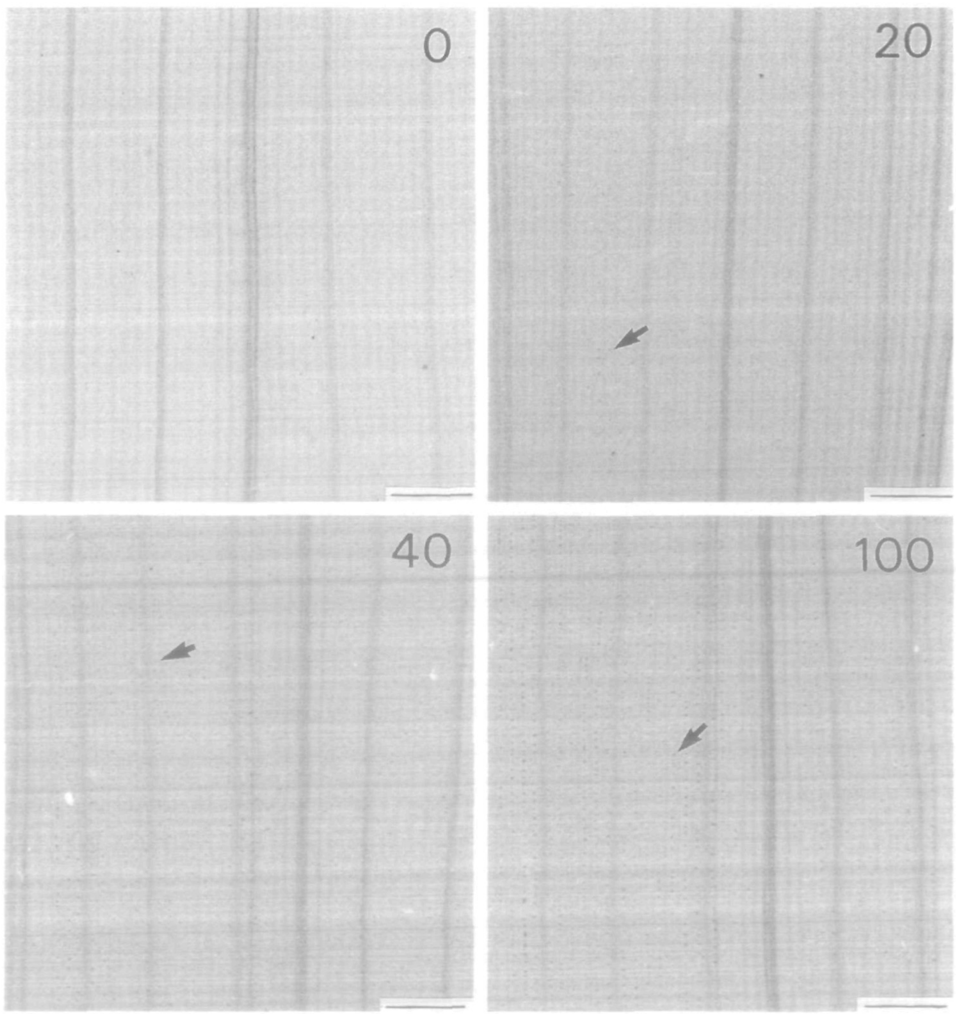

Plate 3. Photographs of soft X-ray pictures of rice leaf blades grown under different SiO_2 conditions, illustrating distribution and relative frequency of occurrence of silica bodies in bulliform cells (arrows). Numbers on these photographs indicate SiO_2 concentration in nutrient solutions. Scale bar = 1 mm.

Table 1. *SiO_2 content and number of silica bodies in rice leaves grown under different SiO_2 conditions*

Data represent the means (±SE) of four replicates.

Applied SiO_2 (ppm)	SiO_2 content (% of dry weight)	Density of silica bodies (number cm^{-2})
0	0.29±0.06	0
20	3.06±0.16	296±149
40	5.56±0.69	2715±431
100	12.60±0.38	3338±583

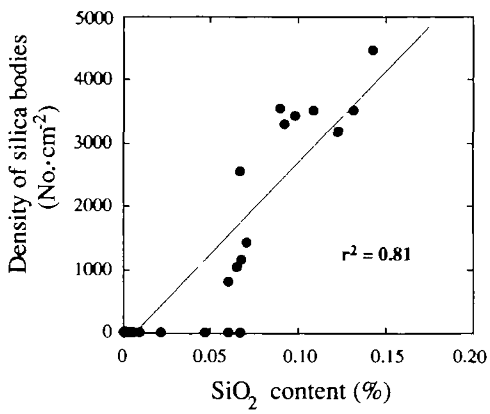

Fig. 1. Relationship between the density of silica bodies and SiO_2 content of leaves. Linear regression equation: $y=-231.1+0.00029x$. r^2: correlation coefficient ($P<0.01$).

and leaf absorptance at 400 nm, 500 nm, and 600 nm was slightly lower in Si-treated (100 ppm) leaves than in non-treated (0 ppm) leaves (Table 2). Furthermore, light energy use efficiency and quantum yield of Si-treated leaves were actually slightly lower than in those not containing silica bodies (Table 3). These results indicate that silica bodies did not increase the amount of light transmitted through the epidermal system to photosynthetic mesophyll tissue and that there was no functional change in photochemical reactions in Si-treated leaves.

Optical properties correlated with photosynthetic light responses are generally determined by several leaf parameters, such as light transmittance and absorptance of leaves, and light scattering inside leaves. The anatomical/morphological properties of leaves is one of the important factors affecting these parameters (Terashima and Takenaka, 1990). Rice leaves, however, generally have a high degree of uniformity in mesophyll arrangement and receive light on both leaf surfaces. In the present study, silica had no effect on the anatomical properties inside the leaf, the cross-sections of the leaves with different silica contents not showing any definite pattern differences in arrangement of mesophyll cells (Plate 2A, B). In addition, cell wall thickness, which generally accounts for the reduction in the light transmitivity through the epidermis, was higher in Si-treated leaves than in non-treated leaves (Plate 2A, B; Kaufman *et al.*, 1985). From these results,

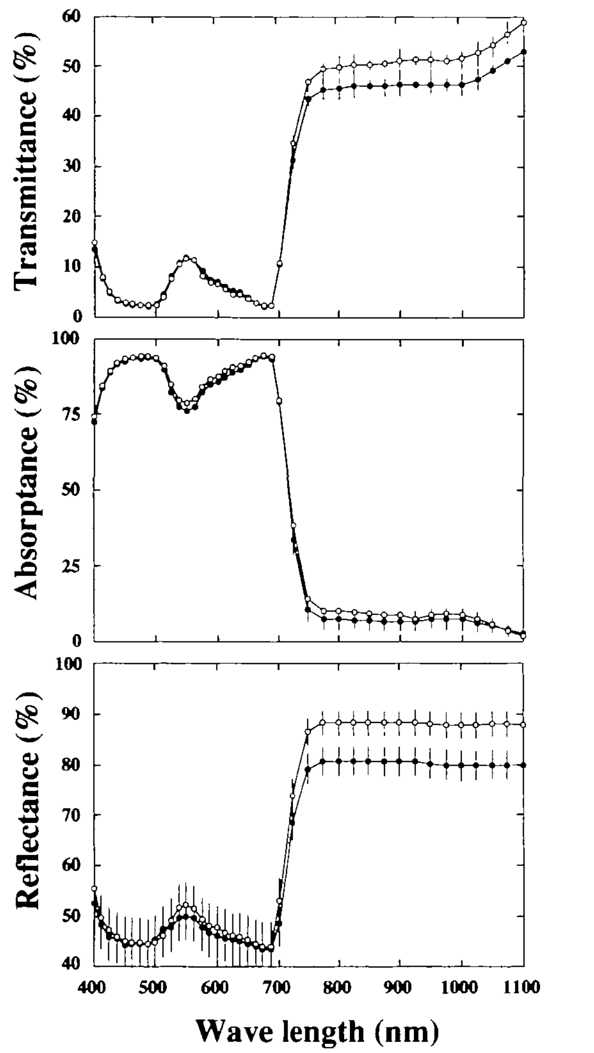

Fig. 2. Optical properties of Si-treated (●) and non-treated (○) rice leaves. Results are expressed as mean values±SE for the three individual experiments.

Table 2. *Effects of SiO_2 application on light absorptance at wavelengths of 400, 500, 600, and 700 nm in rice leaves*

Data represent the means±SE of four replicates. Values in columns followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

Applied SiO_2 (ppm)	Absorptance (%) at each wavelength			
	400 nm	500 nm	600 nm	700 nm
0	74.7±0.3 a	93 7±0.1 a	87.4±0.5 a	80.0±0.0 a
20	70.7±0.6 b	92.4±0.3 b	84.6±0.2 b	77.8±0.9 a
40	74.0±0.4 ac	93.5±0.1 a	87.3±0.4 a	80.1±0.1 a
100	72.1±1.1 bc	92.8±0.3 b	85.8±0.6 b	79 8±1.0 a

there is little possibility that epidermal cell silicon deposits influence optical properties and light environment within the leaf. It was concluded, therefore, that silica bodies do not function as 'windows' in rice leaf epidermal systems.

Table 3. *Effects of SiO_2 application on the light energy use efficiency and quantum yield of rice leaves*

Applied SiO_2 (ppm)	Light energy use efficiency (μmol CO_2 μmol^{-1} quanta)	Quantum yield (mol CO_2 mol^{-1} quanta)
0	0.033	0.080
20	0.024	0.058
40	0.025	0.062
100	0.032	0.078

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