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METHODS

A Method for Calculating the Volume and Surface Area in Rice Mesophyll Cells

O. L. Burundukova*, Yu. N. Zhuravlev*, N. V. Solopov*, and V. I. P'yankov†**

**Biology and Soil Institute, Far Eastern Branch, Russian Academy of Sciences, pr. Stoletiya Vladivostoka 159, Vladivostok, 690022 Russia; fax: 7 (4232) 31-0193; e-mail: burundukova@ibss.dvo.ru*

***Faculty of Biology, Ural State University, Yekaterinburg, Russia*

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Abstract—A method was developed for determining the surface area and volume of rice mesophyll cells of elaborate configuration. The method was employed to calculate these indices in several types of rice mesophyll cells found in seventy samples (53 species) of diverse origin coming from Japan, China, Korea, India, Nepal, Australia, France, Italy, Uzbekistan, Afghanistan, and Krasnodar and Primorskii regions. The cultivars of diverse geographic origin varied in cell shape and size due to the number, size, and arrangement of chloroplasts. When the volumes and surface areas of leaf mesophyll cells were compared using the method reported herein and a simple empirical model of the cell as a single ellipsoid, the two methods produced relatively similar data for cell volume; however, the surface area calculated by the former method was about two times larger than in the latter case. The method described in this paper allows for accurate calculations of the volume and surface area of rice mesophyll cells when data are available on the cell shape and linear dimensions and the number of chloroplasts per cell.

Key words: Oryza sativa - mesophyll - quantitative anatomy - leaf structure - photosynthesis

INTRODUCTION

Assessing the quantitative indices of leaf mesophyll structure is essential for understanding the organization of plant photosynthetic apparatus and evaluating plant potential productivity and adaptation to environmental conditions. In the 1970s, Mokronosov [1–3] developed a cohesive anatomical and physiological approach towards the study of quantitative indices of leaf phototrophic tissues; later, this approach was defined as the mesostructure of photosynthetic apparatus. The approach integrates the morphophysiological characteristics that describe the structure of leaf, chlorenchyma, and mesophyll cells concurrently with their functional state. With this purpose, one must assess both the regular leaf indices (area, thickness, and chloroplast number per cell) and the derived indices of assimilatory tissues, such as surface area, cell and chloroplast volume, and the cumulative cell and chloroplast surface areas per leaf unit area. Measuring the surface area in mesophyll cells is essential for evaluating the functional properties of the photosynthetic apparatus because of gas exchange through these surfaces between the leaf and the ambient atmosphere. The total surface area of mesophyll cells per leaf unit area, sometimes defined as a cell membrane index, A_{mes}/A [2, 4, 5], is an integral characteristic of assimilatory tissue development and was shown to closely correlate with mesophyll CO_2 conductivity and the rate of photosynthesis [5–9]. This index was employed in comparative studies of productivity potentials in crop cultivars, such as wheat [10] and rice [11] and also desert pasture species [12].

Several current approaches to calculating the total intraleaf surface area are workable only for plant species with regular cell shapes and are based on approximations on the basis of simple geometric forms [1, 9]. Some researchers attempted to determine the surface areas and volumes of irregular mesophyll cells, e.g., in wheat, as the sum total of individual geometric elements—cell faveoles [10, 13, 14]. In the case of rice mesophyll, the cell shape was approximated by a cylinder with an irregular basis [15]. All these approaches for calculating the three-dimensional indices of grass cells are time-consuming and produce fairly accurate results.

Among the grass species, rice (*Oryza sativa* L.) is a unique plant as far as the functional properties and structure of leaf mesophyll are concerned. Rice is known to have the highest number of stomata (1200 per mm^2) [16] and the smallest cells and chloroplasts among the crop plants [17, 18], with cells and chloroplasts packed at the highest level in very thin (70 to 140 μm) leaf blades [11, 19]. Tsunoda [20] argues that rice possesses unique properties for leaf ventilation providing for low resistance to CO_2 diffusion (0.7–

†Deceased.

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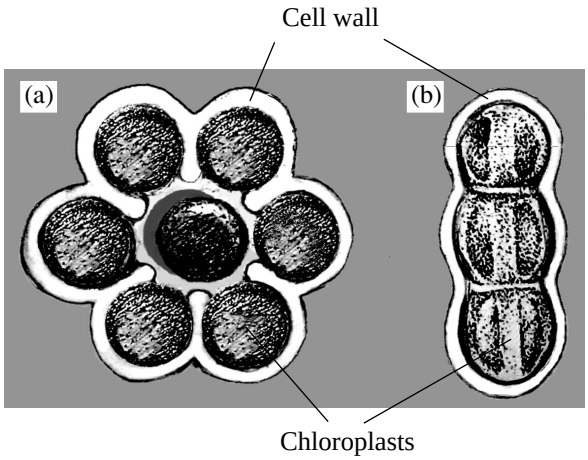


Fig. 1. Rice mesophyll cell.
(a) Frontal view at the cross-section; (b) side view at the tangential section.

0.8 s/cm) [21] and a high photosynthetic rate (up to 60 mg CO_2/dm^2 h) [20].

Rice leaf mesophyll has an elaborate structure [15, 17, 20, 22]. Cells are flattened and densely packed, and the tangential leaf section looks like an isopalisade mesophyll pattern. At the cross section, mesophyll cells are rounded or oval, with numerous outgrowths and folds at their periphery resulting in an elaborate lobate pattern (Fig. 1). The number of such outgrowths is irregular and cultivar-dependent [11, 19]. Because such a morphological pattern is difficult to fit into a simple geometric model, it is not easy to calculate the cell surface area and volume.

The objective of our study was to develop a new way of calculating cell volume and surface area in rice mesophyll using experimental observations and simulation models. In addition, our goal was to analyze the variation of mesophyll cells in rice cultivars of diverse geographical origin; to work out their typology; and to calculate, using the new method, the functionally important indices of leaf mesophyll cells.

MATERIALS AND METHODS

Field experiments. Our studies were carried out in the Primorskii Branch of the Rice Research Institute (Novosel'skoe village, Primorskii region). The collection of rice cultivars was grown in small field plots [23], in a stand of 330 plants/m^2 as recommended by the current technology [24, 25]. The collection comprised 70 forms of diverse geographic origin, including cultivars coming from Japan, China, Korea, India, Nepal, Cameroon, Australia, Hungary, France, Italy, Uzbekistan, Afghanistan, and Krasnodar and Primorskii regions.

The analysis of leaf mesophyll structure. Grown upper leaves on the main shoots at the flowering stage were used for our study. The fragments sampled from the middle part of leaf blades were fixed in 3.5% glutaraldehyde solution in phosphate buffer, pH 7.4. Each sample represented five plants of a particular rice cultivar. Cell linear dimensions and chloroplast numbers were determined following leaf maceration by short heating in 50% KOH at 80–90°C. Macerates were photographed using a camera mounted on an MBI-15 microscope (LOMO, Russia) and a Mikrat-300 film. Cell linear dimensions (length and width) were measured using the photographs thus obtained. To determine cell thickness, we used the photographs of leaf tangential sections. Chloroplast numbers were counted in leaf macerates directly under a microscope or on the microphotographs. We could not obtain squashed preparations usually used for counting photographs [26] because of the small size and strong walls in rice cells.

RESULTS AND DISCUSSION

Cell types in rice leaf mesophyll. When studying leaf macerates in the 70 rice cultivars and forms of diverse geographic origin, we found a wide variation in cell shape and size (Fig. 2). By comparing the characteristic cell and plastid morphology in various rice forms, we discerned several regular characteristics. (1) Cells were very small and densely packed with chloroplasts, which were practically in close contact. The chloroplast numbers correlated with cell lengths. Such correlation was at its highest (r = 0.85) in the cell samples comprising plastids of similar or identical sizes and much lower in the total sample (r = 0.40). (2) Rice cells were shaped as thin oval sheets. The number of plastids observed visually at the frontal cell surface was one-half of the total chloroplast number per cell (N), because the upper layer masked the lower one. (3) The chloroplasts at the cell periphery were placed strictly pairwise, one over another. Each pair was bordered with a cell-wall loop (Fig. 1). These outgrowths determined the characteristic cell shape. (4) Cell-wall outgrowths were in close contact with plastids, practically fitting each pair of peripheral chloroplasts; therefore, the sizes of cell outgrowths depended on the sizes of peripheral plastids.

The analysis of shape and size in mesophyll cells from the collection of rice cultivars under study showed that their diversity depended on the number, size, and arrangement of chloroplasts in these cells. We classified rice mesophyll cells into several types based on the following criteria (Figs. 2, 3). (1) The total plastid number per cell varying from 10 to 48 (N/2 from 5 to 24). (2) Variation in cell shape at the same number of plastids due to (a) the number of chloroplast rows at the frontal surface (most often, three, and sometimes, four rows); (b) developmental pattern of the terminal cell segments: one or two chloroplasts at the extended cell butt or two and one on different butts; in some cells, the butt lacked any extensions and chloroplasts. (3) Variation in plastid size in the same plastid. Most cells comprised chloroplasts of the same size, and it was this cell

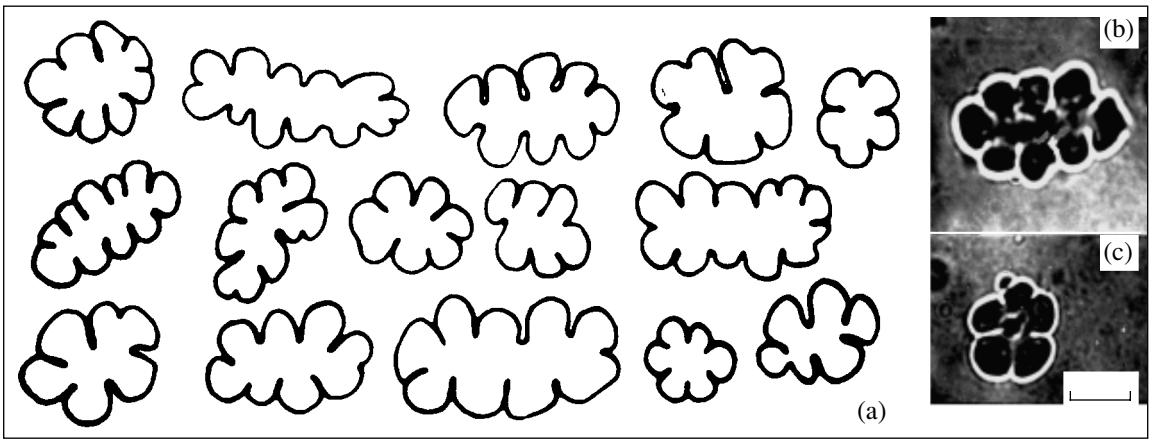


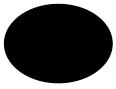
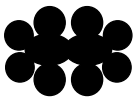
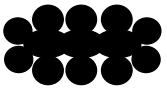
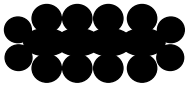
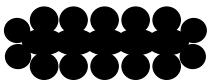
Fig. 2. Cell diversity in shape and size.
(a) Frontal profiles in various rice cultivars (cell contours drawn from the microphotographs made with leaf macerates); (b and c) the examples of cell photographs; bar is 5 μm.

pattern that was employed as a model for further serial computations.

By screening leaf macerates from diverse rice cultivars, we discerned about 15 basic cell types, with their morphology dependent on the total number of chloroplasts and their arrangement (Figs. 2, 3). These cell types served as the basis for geometric models used to simulate mesophyll cell shapes and calculate cell parameters. The data thus obtained were compared to the simple model for cell types with the visually observed plastid numbers *N*/2 of 7, 10, 13, 16, and 19, where the cell volume and surface area were calculated from the formula for a triaxial ellipsoid, with cell length, width, and thickness for its axes.

Rice cell model. The geometric model was developed by computer simulation from the geometric bodies of lower volumes transecting at random. In the case of rice, such a model is represented by a series of ellipsoids with their centers in one plane. The ellipsoids overlap at any depth, and their projections—ellipses—are shown on the computer monitor. Developing the model with a cell shape editing program included the following steps: (a) the number of ellipses was specified equal to the number of chloroplasts (*N*/2) visually

An example of calculating the surface area and volume of rice cells varying in chloroplast number by using the simple ellipsoid model and the comprehensive model built from the intersecting ellipsoids

Cell indices		Simple ellipsoid model			Model of intersecting ellipsoids		
size, μm	chloroplast number	pattern	volume, μm^3	surface area, μm^2	pattern	volume, μm^3	surface area, μm^2
Length 12 Width 12 Thickness 7	7		523.15	339.07		460.90	517.78
Length 16 Width 12 Thickness 7	10		698.21	427.37		652.52	744.23
Length 20 Width 12 Thickness 7	13		872.55	518.10		795.22	931.33
Length 24 Width 12 Thickness 7	16		1046.30	610.32		953.83	1123.82
Length 28 Width 12 Thickness 7	19		1220.69	703.53		1123.16	1313.72

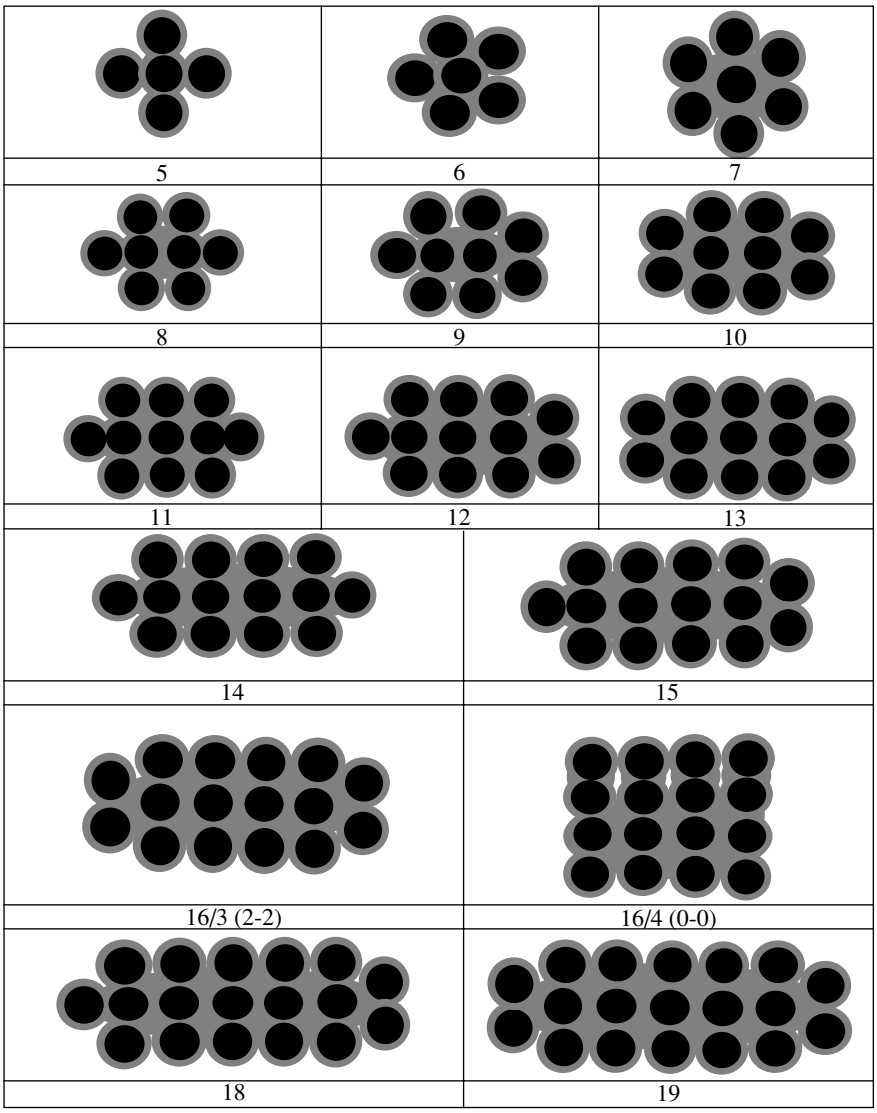


Fig. 3. Cell types accommodating the various chloroplast numbers.
Shown are the chloroplast numbers ($N/2$) seen at cell frontal projection. In addition, for the cell types with equal chloroplast numbers ($N/2 = 16$), the number of chloroplast rows is given following the slash and the chloroplast numbers at the cell end view are given in parentheses.

observed in the cell frontal view; (b) ellipses were shifted to match cell shape and chloroplast arrangement; (c) by changing the size of the ellipses, the model was tailored to fit the cell image in the best possible way. The table illustrates the computer models developed for $N/2$ of 7, 10, 13, 16, and 19.

A Cellstat program for computing rice cell surface area and volume. The Cellstat program was created to develop three-dimensional simulation models of rice cells from variable ellipsoid numbers, store and edit the models, and calculate cell surface areas and volumes. The program runs in three modes: individual, serial, and automatic. In the first case, the model simulates any cell shape using its image; this model provides the most precise results, yet it is the most laborious. The serial mode makes it possible to compute the data for numerous samples, and this particular technique was used for rice cultivars. A two-step procedure is employed for computing cell volumes and surface areas. First, the basic cell types are discerned in the particular cell series under study to build the typical simulation models; in this way, one may disregard inconsiderable variations in cell shapes and greatly enhance the calculations of the investigated parameters. At the second step, using macerate microphotographs of the particular leaf sample, cell types are defined, their linear dimensions are measured, and chloroplast numbers calculated. The data are tabulated and entered into a computer, and the computed data on cell volumes and surface areas are added to the same table. Under the automatic mode, the simulation runs automatically from digitized micro-

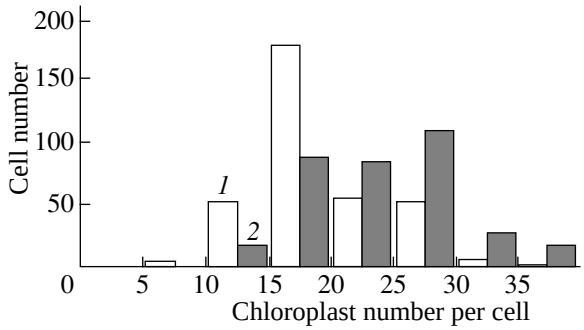


Fig. 4. Frequency distribution of cells with different chloroplast numbers per cell in two groups of rice cultivars in (*1*) the Northern and (*2*) the Southern zones. The Northern zone included the cultivars from the Primorskii region and North Japan and Korea; the Southern zone comprised the cultivars from France, the Krasnodar region, and Afghanistan.

scopic images of real cells. This mode was used to supplement the serial mode with a limited number of cells under study.

Computing cell surface area and volume. Calculations were made by numerical integration using the formulas for ellipsoids transecting at random.

The particular cell model consists of N transecting ellipsoids with their centers at points (x_i, y_i, z_i) and semiaxes a_i, b_i, c_i, where $i = 1\ldots N$. Cell volume is calculated from the following equation:

$$V = \sum_{i=1}^{N} a_i b_i c_i \int_0^1 dr \int_0^{\pi} d\theta \int_0^{2\pi} d\varphi \frac{r^2 \sin\theta}{W_i(r, \theta, \varphi)}.$$

Here, $W_i(r, \theta, \varphi)$ is the function introduced to exclude repeated accounting for transecting regions of ellipsoids; it equals the number of ellipsoids, with internal current integration site (r, θ, φ).

$$W_i(r, \theta, \varphi) = \sum_{j=1}^{N} \Theta(1 - (D_{xij}^2 + D_{yij}^2 + D_{zij}^2)),$$

where

$$D_{xij} = \frac{(x_i - x_j - r\cos\theta\cos\varphi)}{a_j},$$

$$D_{yij} = \frac{(y_i - y_j - r\cos\theta\sin\varphi)}{b_j},$$

$$D_{zij} = \frac{(z_i - z_j - r\sin\theta)}{c_j}.$$

A $\Theta(t)$-stepwise function:

$$\Theta(t) = \begin{cases} 1, & t \geq 0, \\ 0, & t < 0. \end{cases}$$

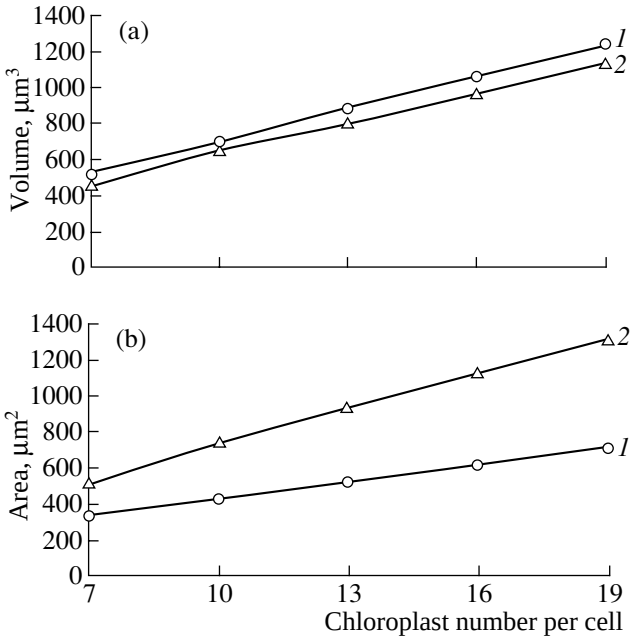


Fig. 5. (a) Volume and (b) surface area of rice mesophyll cells calculated using (*1*) the simple ellipsoid model and (*2*) the comprehensive model for the cells with varying chloroplast numbers.

For calculating the cell surface area, we used the following equation:

$$S = \sum_{i=1}^{N} \int_0^{\pi} d\theta \int_0^{2\pi} d\varphi Q_i(\theta, \varphi) \sqrt{E_i(\theta, \varphi) + F_i(\theta)},$$

where

$$E_i(\theta, \varphi) = c_i^2 \sin^4\theta (a_i^2 \sin^2\varphi + b_i^2 \cos^2\varphi),$$

$$F_i(\theta) = a_i^2 b_i^2 \sin^2\theta \cos^2\theta,$$

$$Q_i(\theta, \varphi) = \prod_{j \neq i} \Theta(D_{xij}^2 + D_{yij}^2 + D_{zij}^2 - 1).$$

Here, $\prod$ is the operator of the product and the function $Q_i(\theta, \varphi)$ is introduced to signify that the current point (θ, φ) is located inside i-th cell. $Q_i(\theta, \varphi) = 0$ when the point is inside at least one of $k \neq i$ ellipsoids and equals 1 in the other case.

The numerical algorithm was realized in a computer program, which made it possible to develop, edit, and store cell models and to calculate cell volumes and surface areas. The table illustrates some examples of the models developed for rice cells containing the various numbers of chloroplasts. The models were developed using the real cell types (Fig. 3) found when screening the 70 rice cultivars and forms of diverse geographic origin. The chloroplast numbers in mesophyll cells (N) in this collection varied from 10 to 48. To realize the model, we used half-numbers ($N/2$) shown in Fig. 3.

Plants with *N*/2 of 18 to 24 were encountered most frequently. In addition, we found that cell types were related to the geographic origin of rice cultivars. To illustrate, in the cultivars from the Eastern regions, especially from their Northern locations (Korea, Primorskii region, and Japan), we observed larger chloroplasts and fewer plastids per cell than in their counterparts in the Southern and Eastern regions (Fig. 4). Thus, the most characteristic chloroplast numbers were 14–18 (*N*/2 = 7–9) in the cultivars from Northern Japan grown on Hokkaido and 20–22 (*N*/2 = 10–11) in the Korean cultivars. In rice cultivars grown in Central Asia (Uzbekistan, Afghanistan, Krasnodar region, and the Mediterranean countries), cells usually comprised 24 to 26 chloroplasts (*N*/2 = 12–13).

Figure 5 presents the results of calculating rice cell volumes and surface areas using our method and the revolving ellipsoid formula. To illustrate the technique, we simulated the cells with *N*/2 equal to 7, 10, 13, 16, and 19 and chloroplast diameter of 4 μm (cell linear dimensions and models are shown in the table). A comparison of the two calculation methods showed that cell surface areas and volumes were different in the two cases. While cell volumes computed using the two models differed inconsiderably (by ca. 10%), the surface area indices differed quite significantly, and this difference increased in larger cells and with higher chloroplast numbers. With a higher chloroplast number per cell, the cell wall becomes more irregular and lobate, and, hence, its plasma membrane is expanded. The cell surface area computed with the elaborate model exceeds the index calculated from the single ellipsoid model by 50% at 20 chloroplasts per cell and by 100% at the maximum number of chloroplasts.

Thus, the calculation of the cell surface area using the simple model disregarding the irregularities of cell surface results in considerable methodical errors. An accurate assessment of the mesophyll cell surface is of substantial importance for evaluating plant photosynthetic and production potentials.

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