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Surface density and volume density measurements of chloroplast thylakoids in maize (*Zea mays* L.) under chilling conditions

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Abstract

Measurements of ultrastructural characteristics of chloroplast thylakoids are important for studies of ontogenic or ecological limitations of leaf photosynthetic functions. Most frequently, volumetric proportion of thylakoids in the chloroplast is measured; however, such measurement does not provide a direct information about the surface area of thylakoids which is most important from the functional point of view. Therefore, we adapted the stereological method using "local vertical windows" for estimating thylakoid surface area in the chloroplast volume and compared thus obtained surface density results with results of conventional volume density measurements. The methods were tested in the study of chloroplast ultrastructure in the leaves of plants of two maize (*Zea mays* L.) hybrid combinations, 2013×CE810 and CE704×CE810, developing in control and chilling conditions. Correlation analysis revealed a tight relationship between the granal/intergranal thylakoid surface density and volume density results, both indicating that under chilling conditions the development of the system of thylakoids in maize leaves is suppressed, while the difference is more pronounced in CE704 than in CE810 genotype, known to have a better photosynthetic performance.

Additional key words: appressed and non-appressed thylakoids; comparison of methods; granal and intergranal thylakoids; hybrids; stereology; temperature; vertical sections.

Introduction

Ultrastructure of chloroplasts, organelles of photosynthesis in plants, has not been fully revealed yet. The main attention is paid to thylakoids, *i.e*. the system of photosynthetic membranes playing a key role in photosynthesis. In vascular plants, these sack-like membranous formations are differentiated into stacked (appressed, *i.e*. granal, G) and unstacked (non-appressed, *i.e*. intergranal, IG) thylakoids. G and IG thylakoids differ in photochemical functions and supramolecular structure (Allen and Forsberg 2001). However, this differentiation is not static, changing dynamically, which depends mainly on irradiation. Contemporary models of chloroplast thylakoids, demonstrating namely the nature of connections between G and IG thylakoids and mechanism of grana stacking/unstacking, were presented by Arvidsson and Sundby (1999) and Mustárdy and Garab (2003).

Measurements of ultrastructural characteristics of chloroplast thylakoids are important for studies of ontogenic (during leaf or plant development) or ecological limitations of leaf photosynthetic functions (Kutík *et al.* 1995, 1999, 2004, Kutík 1998). In general, quantitative description of chloroplast thylakoids can help reveal the relationships between structure and function of photosynthetic apparatus in different physiological conditions.

First quantitative measurements and three-dimensional (3-D) reconstructions of chloroplast ultrastructure emerged already in the sixties of the 20th century as soon as it was feasible thanks to the development of electron

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microscopy. Parameters such as "number" of grana per chloroplast or thylakoids per granum were measured and 3-D models of chloroplasts were introduced (Wehrmayer 1965, Paolillo and Falk 1966). Since seventies and eighties, stereological methods have been applied, estimating area and/or volume densities of different chloroplast compartments (Gamaleï and Kulikov 1978, Fagerberg 1983, Kutík *et al.* 1984).

Stereological methods (Weibel 1979, Gundersen and Jensen 1987, Kubínová 1993, 1994, Howard and Reed 1998) enable to study 3-D cell ultrastructural characteristics, such as volume densities (*i.e*. volumetric proportions), from practically two-dimensional (2-D) ultrathin sections for TEM (Loud 1987, Mayhew 2000, Morris 2000). For measuring thylakoid ultrastructural characteristics, mainly stereological point-counting and other methods leading to the estimation of volume densities, such as volumetric proportion of G and IG thylakoids in the chloroplast, are most frequently used (*e.g*. Gamaleï and Kulikov 1978, Vapaavuori *et al.* 1989, Kutík and Bergmannová 1991, Wheeler and Fagerberg 2000, Fagerberg and Bornman 2005). Such methods are efficient and easy to apply but provide only indirect information about the surface area of thylakoids, which is important from the physiological point of view because "light reactions" of photosynthesis are localised in thylakoid membranes. To our knowledge, the relationships between surface area and volume measurements have not been tested yet, *e.g*. how tight is the relationship between the ratio of the volume of grana to the chloroplast volume and the ratio of the surface area of granal thylakoid membranes to the chloroplast volume. The correlation between these two parameters is expected but it should be verified.

In measurements of thylakoid surface area, usually model-based approach was applied: Wheeler and Fagerberg (2000) used such approach assuming thylakoids are formed by disks. Albertsson and Andreasson (2004) also assumed that grana are made from circular disks. This assumption corresponds roughly to reality but it is not precise: The ideas on thylakoid appearance in 3-D are changing and still developing (for contemporary models of chloroplasts see Arvidsson and Sundby 1999, Mustárdy and Garab 2003, Wildman *et al.* 2004) and so rigid assumptions about their shapes are tricky. Moreover, some degree of irregularities in shapes must be expected, irrespective of the chloroplast 3-D model accepted. Design-based stereological approach is more suitable in such situation as it provides unbiased results, independently of the real shape of grana. However, a random orientation of test probes is required during the measurement when the intersections of test lines with the thylakoid surface are counted (Weibel 1979). If electron micrographs of thin chloroplast sections are used for the measurement, two stereological approaches can theoretically be applied, *i.e*. orientator principle (Mattfeldt *et al.* 1990) or method of vertical sections (Baddeley *et al.* 1986): The orientator method would require applying the orientation of thin sections of chloroplasts that would be random in 3-D. However, such sections would cross the thylakoid membranes at different angles which would make their correct detection difficult as in electron micrographs the membranes are clearly seen only when cut perpendicularly to their surface. On the other hand, this fact can turn into an advantage when the method of vertical sections is applied, where sections of the object parallel to a fixed (but arbitrarily chosen) axial direction and randomly rotated around this vertical axis are used. This method was exploited by Stolz *et al.* (1989) who estimated the surface density of G and total thylakoids in lettuce chloroplasts assuming thylakoids are parallel in entire chloroplast sections. This is not always the case; therefore we used a special modification of the method, using "local vertical windows" in the present study. For testing this approach we chose mesophyll cell (MC) chloroplasts of maize plants, cultivated under normal or chilling conditions, already evaluated by a standard procedure using point counting (Kutík *et al.* 2004). From this study volume densities of G and IG thylakoids were available, so it was possible to compare our new surface density results with the published results coming from these more conventional measurements.

Materials and methods

We used material from our previous study (Kutík *et al.* 2004) where the ultrastructure of mesophyll cell chloroplasts was studied in two maize (*Zea mays* L.) hybrid combinations, 2013×CE810 and CE704×CE810. Each hybrid combination comprised the respective parental inbred lines and their reciprocal F_1 hybrids. Parental line CE810 shows better photosynthetic performance than the CE704 one. In our present study, only plants from these two parental lines were used.

The seeds of all genotypes were obtained from Maize Breeding Station CEZEA in Čejč (Czech Republic). Plants were cultivated in planting dishes with soil, placed at first in a heated glasshouse (24–27/16–20 °C, day/night) till the appearance of the first leaf (*i.e.* 9–10 d from the date of sowing). After that, seedlings were divided into two groups; one remained in the heated glasshouse (control), the other group was transferred to another, unheated glasshouse (chilling stress). Their cultivation then proceeded for four weeks in October. During this period the temperature in the unheated glasshouse gradually decreased from approx. 25 to 18 °C during day and from 13 to 8 °C during night. Plants were well watered with tap water, the relative humidity in both glasshouses was kept between 70–100 %, and no additional irradiation was applied.

The plants developing under chilling conditions were

usually smaller and had fewer and shorter leaves compared with the control ones (Holá *et al.* 2003). For this reason, the fourth leaves (counting from the bottom, numbered from coleoptile as leaf zero) were usually sampled from the control plants, whereas the samples representing the stressed plants were taken from their third leaves. In all cases, these leaves were mature (fully developed, not growing further). Four plants (*i.e.* four leaves) were examined in each variant.

Small pieces of leaf (*ca*. 4 mm^2) were cut from the middle third of the leaf blade, between the edge and middle vein of the leaf. Samples for the transmission electron microscopy were prepared according to a standard procedure as described in Kutík *et al.* (1999). They were double-fixed with glutaraldehyde followed by osmic acid, dehydrated through an ethanol series, and embedded into Spurr´s low viscosity resin. For an orientation in the objects and their light-microscopic examination, semithin sections of embedded objects were stained by toluidine blue solution.

Chloroplast ultrastructure was evaluated on ultrathin sections of the samples contrasted with uranyl acetate solution followed by lead citrate treatment. The transmission electron microscopes *Philips EM 300* and later *Philips EM 268 Morgagni* (*Philips*, the Netherlands) were used at primary magnifications of about 7 000×.

In our previous study (Kutík *et al.* 2004), the volume densities (volumetric proportions) of G and IG thylakoids were estimated from electron micrographs by a point-counting method (Weibel 1979) using stereological grids with regularly distributed test points. Five chloroplast sections were evaluated per sample, making a total of 20 MC chloroplasts analysed for each experimental variant.

In our present study of the relationships between surface area and volume measurements of chloroplast ultrastructure, ten MC chloroplast sections per leaf in each sample cut in an isotropic random direction from the above material were used. Whereas in our previous study (Kutík *et al.* 2004) more or less medial sections were chosen, random ones were taken now, *i.e.* some of them were also peripheral (Fig. 1*A,D*). For the measurement of G and IG thylakoid surface densities the method of "local vertical windows" suggested by Baddeley *et al.* (1986) was applied. This method is a modification of the method of vertical sections when the object surface area is estimated from the so called vertical sections, *i.e.* planar sections of the object parallel to a fixed (but arbitrarily chosen) axial direction and randomly rotated around this vertical axis, by counting intersection points between the object surface and a special test system consisting of cycloids. If the structure under study is organized in such manner that from its sections it is possible to detect the angle at which the structure surface was cut, the method of "local vertical windows" can be applied. This is the case of thylakoids that are in relatively large regions of chloroplast parallel to each other. If we choose the direction of the vertical axis perpendicular to the thylakoid membranes we know that in a randomly cut section of a chloroplast the "local vertical windows" correspond to those regions on chloroplast electron micrographs where thylakoid membranes form well focussed parallel lines (Fig. 1). In our measurement of G and IG thylakoid surface densities we outlined the "local vertical windows" in the micrographs of chloroplast sections first (Fig. 1) and then superimposed the cycloid test system combined with a point test system (Fig. 2). The surface density of G (IG) thylakoids in the chloroplast [μm^{-1}], $S_{V,thyl}$ was then estimated by the formula:

$$estS_{V,thyl} = 2 \cdot \frac{p}{l} \cdot \frac{I_{thyl}}{P_{chl}} \quad (1)$$

where p/l is the ratio of the test points of the point grid to the length of the cycloid test system, I_{thyl} is the number of intersections between the G (IG) thylakoid surface and the cycloid test lines counted in all "local vertical windows" analyzed in the given leaf, and P_{chl} is the number of test points falling into all "local vertical windows" outlined in the chloroplast sections in the given leaf. Note that in grana there are two thylakoid membranes appressed against each other, therefore two intersections with cycloid test lines should be counted as shown in Fig. 2.

The ratio of G thylakoid surface area to the surface area of IG thylakoids was calculated by the ratio of surface densities of G and IG thylakoids. The surface area of all thylakoids in 1 μm^3 of chloroplast, *i.e.* the surface density of G and IG thylakoids in the chloroplast [μm^{-1}], was estimated by Eq. (1) with I_{thyl} denoting the number of intersections of cycloid test lines with both G and IG thylakoid surface.

The volume density of grana (IG thylakoids) in the chloroplast, $V_{V,thyl}$, was estimated by a point-counting method (Weibel 1979) applied to the analyzed "local vertical windows":

$$estV_{V,thyl} = \frac{P_{thyl}}{P_{chl}} \quad (2)$$

where P_{thyl} is the number of test points falling within the G (IG) thylakoids counted in all "local vertical windows" analyzed in the given leaf. Analogously, the volume density of all thylakoids in the chloroplast was estimated.

Further, the surface area of G (IG) thylakoids in the G (IG) thylakoid volume [μm^{-1}] and were estimated, using again Eq. (1) with P_{thyl} instead of P_{chl}, the surface area of all thylakoids in the thylakoid volume [μm^{-1}].

Statistical significance of differences in MC chloroplast parameters between plants cultivated under stress or control conditions, as well as between individual genotypes was tested by two-sample unequal variance Student's *t*-test. Further, Spearman correlation was applied to evaluate relationships between volume based and surface area based chloroplast thylakoid parameters, *i.e.* volume density of G (IG, all) thylakoids in the chloroplast *versus* surface density of G (IG, all) thylakoids in the chloroplast.

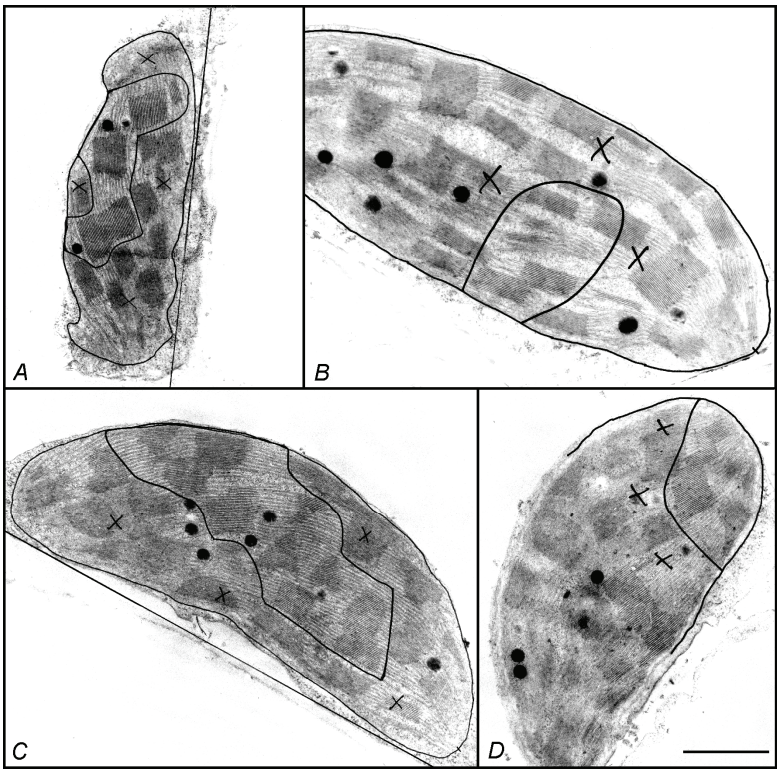

Fig. 1. Electron micrographs of mesophyll cell chloroplasts from two maize (*Zea mays* L.) genotypes, CE704 (*A, C*) and CE810 (*B, D*) developed under chilling (*A, B*) and normal (*C, D*) conditions. The delineated regions are "local vertical windows" where all thylakoid membranes are cut perpendicularly. Non-evaluated areas of the chloroplast section are marked by "X". Scale = 1 μm.

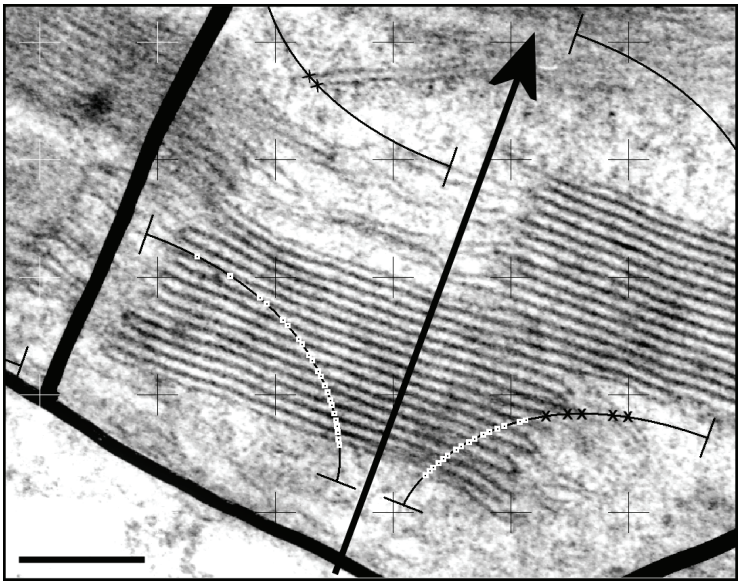

Fig. 2. Cycloid and point test system superimposed on the chloroplast section shown in Fig. 1*B*. Intersections of cycloids with granal thylakoid membranes are marked by white squares with black centres (I = 38) and intersections with intergranal thylakoid membranes by black crosses (I = 7). Number of test points falling into the "local vertical window" in this field of view is equal to 23. *The arrow* shows the vertical direction. Scale = 200 nm.

Results

Results of the stereological evaluation of maize mesophyll cell chloroplasts under cold stress and in control conditions are summarized in Table 1. Volume densities of all thylakoids were similar to the results in Kutík *et al.* (2004, see Fig. 3, CE704×CE810, and Table 2, CE704×CE810). Statistical significance of the differences between cold stress variant and the control one was higher for the genotype CE704 than for CE810 in Kutík *et al.* (2004) as well as in the present results. For CE810, the difference in volume density of grana did not achieve the 5 % level of significance.

In accordance with the results for volume densities, the surface densities of all thylakoids in the chloroplast were significantly higher in control than in cold stressed chloroplasts for both genotypes. Again, the difference was more pronounced in CE704 than in CE810. Situation was similar for granal thylakoids, but the difference was not significant in the case of CE810. As to the intergranal

Table 1. Parameters of chloroplast ultrastructure in two maize (*Zea mays* L.) genotypes, CE810 and CE704, developed under normal (810 K, 704 K) and chilling (810 S, 704 S) conditions and their comparisons with respect to variant or growing conditions. Means ± SD ($n = 4$). *significant at $p \leq 0.05$, **significant at $p \leq 0.01$, ns – not significant.

	810 K	810 S	704 K	704 S	810 K× 810 S	704 K× 704 S	810 K× 704 K	810 S× 704 S
Ratio of G thylakoid surface area to the IG thylakoid surface area	1.88±0.34	2.59±0.63	2.27±0.53	1.90±0.26	ns	ns	ns	ns
Volume density of grana in chloroplast	0.41±0.02	0.38±0.04	0.42±0.04	0.31±0.02	ns	**	ns	*
Volume density of IG thylakoids in chloroplast	0.25±0.01	0.20±0.01	0.27±0.03	0.22±0.02	**	*	ns	ns
Volume density of all thylakoids in chloroplast	0.66±0.01	0.58±0.05	0.69±0.04	0.54±0.04	*	**	ns	ns
Surface density of G thylakoids in chloroplast [μm^{-1}]	41.74±5.19	38.70±3.58	49.39±6.59	31.21±2.32	ns	**	ns	*
Surface density of IG thylakoids in chloroplast [μm^{-1}]	22.48±1.73	15.55±3.21	22.14±3.16	16.54±1.61	*	*	ns	ns
Surface density of all thylakoids in chloroplast [μm^{-1}]	64.22±4.51	54.26±4.15	71.52±6.78	48.50±2.26	*	**	ns	ns
Surface area of G thylakoids in the G thylakoid volume [μm^{-1}]	102.30±13.00	102.30±6.82	117.20±9.74	99.47±8.06	ns	*	ns	ns
Surface area of IG thylakoids in the IG thylakoid volume [μm^{-1}]	91.24±9.21	76.55±17.5	84.25±17.80	75.39±11.60	ns	ns	ns	ns
Surface area of all thylakoids in the thylakoid volume [μm^{-1}]	97.90±5.93	93.28±6.32	104.20±10.90	89.25±2.11	ns	ns	ns	ns

thylakoids, their surface area per cubic micrometer of chloroplast volume was again significantly higher in control than in stressed variant for both genotypes.

A tight relationship between the ratio of the volume of grana to the chloroplast volume and the ratio of the surface area of grana to the chloroplast volume was confirmed also by the Spearman correlation analysis where the correlation coefficient $r = 0.867$ was calculated ($p \leq 0.001$). A significant correlation was found also for the ratio of the volume of IG thylakoids to the chloroplast volume and the ratio of the surface area of IG thylakoids to the chloroplast volume ($r = 0.508$, $p \leq 0.05$). The same holds for the surface area and volume measurements if all (both G and IG) thylakoids are taken into account ($r = 0.949$, $p \leq 0.001$).

We did not find any significant differences between stress and control conditions concerning the ratio of G thylakoid surface area to the surface area of IG thylakoids in both genotypes studied, although in genotype CE704 this ratio was slightly higher in control than in chilling variant, while the opposite was true for genotype CE810. The variance in this parameter among individual leaves was high, so if more leaves and chloroplasts were evaluated the difference between the control and chilling variant might prove to be significant.

Further, there were no significant differences in the surface area of all thylakoids in the thylakoid volume for both genotypes studied. In fact, this means that the mean distance between the thylakoid membranes was not changing. The same was true for G and IG thylakoids taken separately, with a sole exclusion of stacked thylakoids in CE704 genotype where this characteristic was significantly higher in control in comparison with stress, whereas in CE810 the respective values were almost identical. It means that granal thylakoids are less tightly stacked in CE704 under chilling conditions when compared with control.

Discussion

The presented method using "local vertical windows" (Baddeley *et al.* 1986) proved to be suitable for chloroplast thylakoid surface area estimation. This design-based stereological method is more appropriate than model-based methods (Wheeler and Fagerberg 2000, Rozak *et al.* 2002, Albertsson and Andreasson 2004) as different models of chloroplasts exist and real structures always deviate to some extent from the models. Moreover, the "local vertical windows" method exploits the fact that in electron micrographs the membranes are clearly seen only when cut perpendicularly to their surface, which makes it easy to find those regions in the chloroplast sections where the thylakoid membranes are cut vertically. Thus it was feasible to estimate surface densities of thylakoid membranes, whether concerning G, IG, or both G and IG thylakoids in the chloroplast. Moreover, if the total volume of chloroplast is measured (*e.g.* by the Cavalieri principle, see Gundersen and Jensen 1987), it is possible to estimate also the total surface area of G, IG, or all thylakoids in the chloroplast.

Our results indicate that under chilling the development of the system of thylakoids in maize leaves is suppressed, while the difference is more pronounced in CE704 than in CE810 genotype. Similar results, based on measurements of volume densities only, were obtained in Kutík *et al.* (2004). Our present results allow to refine the differences in the chilling effect in the two genotypes under study: In CE704 genotype, which is more sensitive to chilling, granal thylakoids were affected more than intergranal ones, while in CE810 genotype the effect of chilling was more pronounced for intergranal thylakoids. It is possible to speculate about different composition of thylakoid membranes in both maize genotypes, *e.g.* various proportion of photosystems one and two in them, and their different sensitivity to chilling.

The similarity between results for volume densities in our present and previous (Kutík *et al.* 2004) studies demonstrates that the results of our measurements are consistent, even though the sampling of chloroplast sections and evaluated regions were different: In the previous study, nearly medial chloroplast sections were evaluated while in the present study "vertical windows" in randomly cut sections (which means that sections were cut in different positions within the chloroplast, *i.e.* some of them were nearly medial, while other ones were closer to the periphery) were measured. This consistency in results, which can be explained by the fact that the system of thylakoid membranes is relatively homogeneously distributed within the chloroplast, shows that both types of measurement are reasonable. The approach, presented in this study, provides more complex quantitative data on the thylakoid ultrastructure but it is more laborious and time consuming than the approach based on measurements of volume densities (Kutík *et al.* 2004). Taking into account that high correlation was shown between the surface and volume densities of thylakoids in the chloroplast, *i.e.* in parameters of interest in the comparisons of different variants in the present study, it is possible to substitute more tedious surface area measurements by easier volume estimation in such case. In general, it can be recommended to test the correlation between surface area and volume measurements on a small sample first and then, if this is verified, apply the faster and more efficient measurement of volume densities. However, in cases where the mean distance between neighbouring thylakoid membranes is different in different variants, the substitution of surface densities by volume densities would not be appropriate as larger surface area of thylakoids would not mean automatically their larger volume and *vice versa*.

The method using "local vertical windows" is applied to electron micrographs of ultra-thin sections which is a standard technique used in studies of chloroplast ultrastructure. If 3-D images of thicker sections, such as those obtained by up-to-date but expensive transmission electron microtomography methods (Shimoni *et al.* 2005) are available, the efficient stereological fakir method (Kubínová and Janáček 1998) could be applied. Moreover, if the automatic segmentation of thylakoid membranes in such 3-D image data is feasible, fast automatic digital methods for surface area estimation could be applied (Kubínová *et al.* 1999). Chloroplast grana, their arrangement, and changes under *in vivo* conditions can be studied by confocal laser scanning microscopy (Mehta *et al.* 1999, Wildman *et al.* 2004); however, the resolution is not sufficient to examine surfaces of thylakoid membranes.

Whether model-based, design-based stereological or automatic image analysis techniques are applied, it is always necessary to follow proper sampling and perform sufficient number of measurements. In this study we evaluated regions (*i.e.* "local vertical windows") in 10 sections of chloroplasts per leaf. This is not a very large sample; however, the reasonable variance in the measured parameters among leaves within a variant indicated that the sample was sufficiently large for our purpose. If more subtle differences in chloroplast ultrastructure are to be detected, a higher number of leaves and chloroplasts sampled for measurement should be analyzed. Further, it is necessary to consider possible bias due to technical processing of the tissues under study. Similarly to each standard electron microscopic study, fixed and embedded material was analyzed. Of course, the system of chloroplast thylakoids may react quickly to changing irradiance during fixation, as described by Rozak *et al.* (2002). Therefore, we took care to standardize the fixation procedure as best as possible. We assumed that possible deformations of chloroplast ultrastructure followed the same pattern in all variants under study.

In conclusion, we showed that the presented

stereological methods for measuring surface area and other characteristics of chloroplast thylakoid membranes represent a useful and feasible tool, yielding reliable results and being applicable to current electron micrographs of chloroplasts sections.

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Volume 24 of the well known series "Advances in Photosynthesis and Respiration" has a subtitle naming the key enzyme of this system. This is similar to the title of previous volume 22 that deals with photosystem 2 (PS2) and its key enzyme is water:plastoquinone oxidoreductase (for review see Photosynthetica **45**: 120, 2007). The volume 22 is by only about 5 % thicker than the reviewed volume 24 is, even if one usually thinks that PS2 is studied much more often than photosystem 1 (PS1).

The huge amounts of pages show that all aspects of the photosystems are analysed in detail. Nevertheless, I wonder what part of this collection of recent information will be valid after say 10 years. In any case, it will remain a valid thesaurus of findings or theories, because such voluminous books are rarely read cover to cover. This book series stresses the increasing importance of genetics in photosynthesis research: volume 24 presents, in addition to the traditional Subject Index, also three special indices: Organism Index, Mutant Index, and Gene and Gene Product Index.

Forty chapters of volume 24 were written by 79 authors, most of them being leading scientists in universities and institutes all over the world, including also the recently often neglected Russia. The four introductory chapters deal with the history of studying this part of photosynthetic electron transfer apparatus. They were written by pioneers of this research such as Anthony San Pietro, Richard Malkin, Bacon Ke, Paul Mathis, and Kenneth Sauer. The story started with three papers published in 1951 and I read the one prepared by Daniel Arnon and presented in Nature probably as a first paper dealing with photosynthesis soon after its appearance.

The following 36 chapters are divided into ten parts. The part entitled "Molecular Architecture" contains four chapters on structure, function, and regulation of PS1 in cyanobacteria, algae, and higher plants, on association of PS1 with the light-harvesting complex (LHC) 2, and with stromal subunits. Part III deals with pigment-protein interactions, accessory chlorophyll proteins, LHC1, and acceptor and donor sides of PS1. Two chapters of Part IV describe ultrafast optical spectroscopy (including the respective techniques) of PS1 and its long-wavelength chlorophylls. Next part (2 chapters) is on genetic manipulation of ligands, quinones, Fe-S centres, plastoquinone, and α-tocopherol.

Part VII includes eight chapters on spectroscopic studies of cofactors (P700, phylloquinones, donors, acceptors, and intermediates), showing not only results but also information on research methods (EPR, FTIR, TRSS, measurement of membrane potentials, *etc*.). Six chapters of part VII (Kinetics of Electron Transfer) deal with components of the transfer path and with the respective enzymes and intermediates (ferredoxin, flavodoxin, thioredoxin, plastocyanin, cytochromes, *etc.*). Assembly and turnover of PS1, iron-sulfur clusters, the reducing side, and their genetic basis is the next main topic.

Modelling of processes and units is dealt with in almost every chapter of the book. Four chapters especially on modelling are collected in Part IX; they deal with thermodynamics of PS1, its optical spectra and light harvesting, and with reaction centres. Part X describes cyclic electron transfer around PS1, and photoinhibition and protection against it. The final part is on evolution of PS1 and its components (cytochrome and plastocyanin).

Similar to other volumes of this series, also this one deserves to be on bookshelves of every laboratory interested in photosynthesis research. Those who will buy the electronic version will easily find references to the source articles. Comparison of colour figures on plates and black-and-white ones in the text shows how their instructiveness can be improved by using colour print.

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