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3D Reconstruction of Chloroplasts and their Ultrastructure using Ultra-Thin-Serial-Sections

By

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With 2 Tables and 5 Figures

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Summary

PERKTOLD A., ZELLNIG G., GUTTENBERGER H. & GAILHOFER M. 1998. 3D reconstruction of chloroplasts and their ultrastructure using ultra-thin-serial-sections. – Phyton (Horn, Austria) 38 (1): 159–165, 2 tables, 5 figures. – English with German summary.

Reconstruction of fine structures of plant organelles can not be done applying light microscopy. The resolution of this technique is too poor, therefore Transmission Electron Microscopy (TEM) has to be used. However, it is impossible to evaluate larger parts of the cells or whole cell organelles because specimens are too thin for these topics. This is also the reason why computer-tomography is impossible. Therefore series of ultra-thin-sections were used. Black and white images of TEM-preparations were transformed into pixel-images with a scanner for further analysis with a computer. After this procedure the images were transformed into vector-graphs. Now it was possible to reconstruct organelles in a 3D view and to calculate areas and volumes.

Illuminated spinach leaves were used as objects for evaluation. The results of the 3D reconstruction of spinach chloroplasts with their ultrastructures and the calculations of the volumes of their components are shown in this paper.

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Zusammenfassung

PERKTOLD A., ZELLNIG G., GUTTENBERGER H. & GAILHOFER M. 1998. 3D Rekonstruktion von Chloroplasten und deren Ultrastruktur mittels Ultradünn-Serienschnitten. – Phyton (Horn, Austria) 38 (1): 159–165, 2 Tabellen, 5 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Lichtmikroskopische Methoden haben für eine Rekonstruktion von Feinstrukturen pflanzlicher Organellen eine zu geringe Auflösung. Transmissions-Elektronenmikroskopie (TEM) hat eine genügende Auflösung, aber die Erforschung größerer Abschnitte oder gar ganzer Organellen ist aufgrund der sehr dünnen Schnitt-Präparate für diese Technik nicht möglich. Wegen der geringen Schnittdicke ist auch eine Computer-Tomographie unmöglich. Daher wurden in dieser Arbeit Serien von Ultradünnschnitten hergestellt. Von den Schnittserien wurden mittels TEM Schwarz-Weiß-Bilder hergestellt, die über einen Flachbettscanner in einen Computer eingescannt wurden. Die daraus resultierenden Pixelbilder wurden vektorisiert und anhand dieser Vektorgrafiken 3D Rekonstruktionen von Organellen erstellt, Volumina und Flächen berechnet.

Als Untersuchungsmaterial dienten konstant gezogene und nach Belichtung geerntete Spinatblätter. Die Ergebnisse der 3D Rekonstruktion von den Mesophyll-Chloroplasten und ihren Feinstrukturen und deren Volumsberechnungen werden in dieser Arbeit vorgestellt.

Introduction

Transmission electron microscopy (TEM) is usually used to examine ultrastructures of plant cells (SITTE 1991, KLEINIG & SITTE 1992). However, using this technique it is not possible to investigate whole cell-organelles. This is due to the size of organelles, e.g. chloroplasts usually have a diameter of about 4–8 µm. Therefore, ultrathin-sections with an average thickness of about 80–100 nm represent only a very small part of an organelle and calculations of areas or volumes of organelles and fine structures are not possible or provide uncertain data. In contrast, the resolution of the light microscopic technique is too poor for an evaluation of ultrastructures. 3D reconstructions in connection with TEM and serial sections were rarely done till now (see e.g. SJOSTRAND 1958, STEVENS 1977, KOSTER & al. 1996). Because of the little knowledge about the spatial relations in organelles on ultrastructural level, we investigated the 3D view of spinach chloroplasts based on TEM serial sections and computer reconstructions in order to obtain more detailed informations about the structural relations in the whole organelles. Additionally, surface- and volume-data were calculated.

Materials and Methods

Plant Material and Culture

Spinach plants (*Spinacia oleracea* L. "Medania") were cultivated under reproducible conditions for 21 days in an environmental simulating chamber (Oeko-

phyt, Heraeus Inc.). Day – night rhythm was 12 hours (light intensity: 800 µmol photons per m^2 and second, relative humidity 70%, temperature 20° C). The spinach leaves were harvested after 10 hours of illumination. The leaves were 2 to 4 cm long. Altogether 12 series were investigated. For the 3D reconstructions 3 series out of these were used.

Preparation and TEM microscopy

1–3 mm^2 preparations of spinach leaves were fixed in 3% glutaraldehyde with 0.06 mol phosphate buffer at pH 7.2 for 2 hours at 4° C. After rinsing in buffer they were postfixed in 1% osmium tetraoxide, dehydrated in a graded series of ethanol and embedded in Agar 100 resin. Series of ultra-thin sections (100 nm) were cut with an ultramicrotome (Ultracut S, Reichert Inc.), put on single-slot grids and stained with lead citrate and uranyl acetate. Micrographs were taken on a CM 10 transmission electron microscope (Philips Inc.). From 3 investigated series 29–58 ultra-thin-sections were made.

3D reconstruction, area and volume estimations

TEM images were scanned with a resolution of 300 dpi by a MFS-6000 CX scanner (Mustek Inc.). The images were enhanced (JÄHNE 1989) and evaluated by the image analysis software Optimas 4.02 (Optimas Inc.). The pixel images were transferred into vector graphs (FRANKEN 1991) using Corel Trace 6.0 and Corel Draw 6.0 (Corel Inc.). The single drawings of the sections were arranged one on top of the other. This was done by using the edges of the sections. 58 sections were used for 3D reconstruction. 7362 single areas were transferred into vector graphs and their areas estimated.

3D reconstructions of complete and incomplete chloroplasts were done by a modified procedure according to ECKHART (VOGLER 1984, PERKTOLD 1997).

Evaluation of different areas was done with Optimas. The volumes were calculated by means of the section thickness (100 nm).

For printing a Tek Phaser 440 printer (Tek Inc.) was used.

Results and Discussion

Observation of unfixed, living preparations of the spinach mesophyll showed chloroplasts with photosynthetic starch. Neither thylakoids nor the stroma of the chloroplasts are visible in the light microscope. In general, TEM images compared with observations using the light microscope showed the same picture: Chloroplasts with different numbers of starch grains are located near the cell wall directly under the plasmalemma (Fig. 1). Of course, much more details are evident now. Between the single starch grains both thylakoids and stroma of the chloroplasts are visible. Additionally, a lot of plastoglobuli occurring in two forms – electron-dense and lightened – are also evident.

Our 3D reconstructions of the investigated different light-determined spinach plastids allow an insight into the arrangement of the structural components. Besides, different systems (structures) can be faded out or

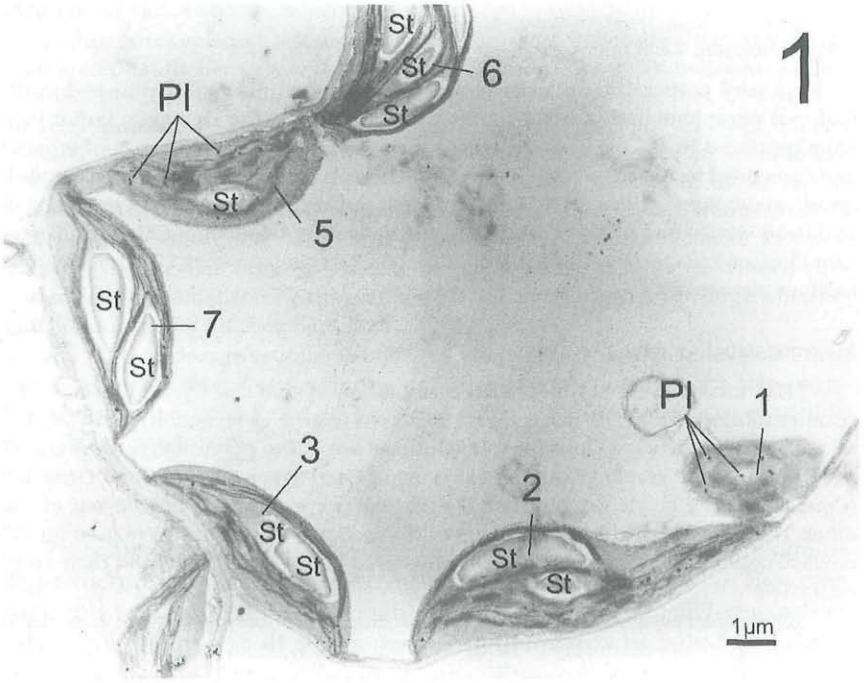


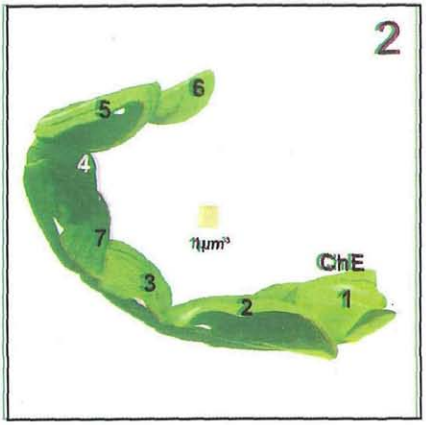
Fig. 1. TEM-micrograph of a spinach mesophyll cell used for 3 D reconstruction. Scale bar = 1 µm.

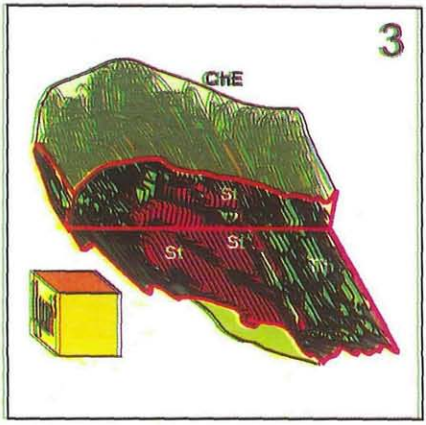
Fig. 2. 3D reconstruction of serial-sectioned chloroplasts of the cell in Fig. 1. Only the envelopes (green) of the chloroplasts are shown.

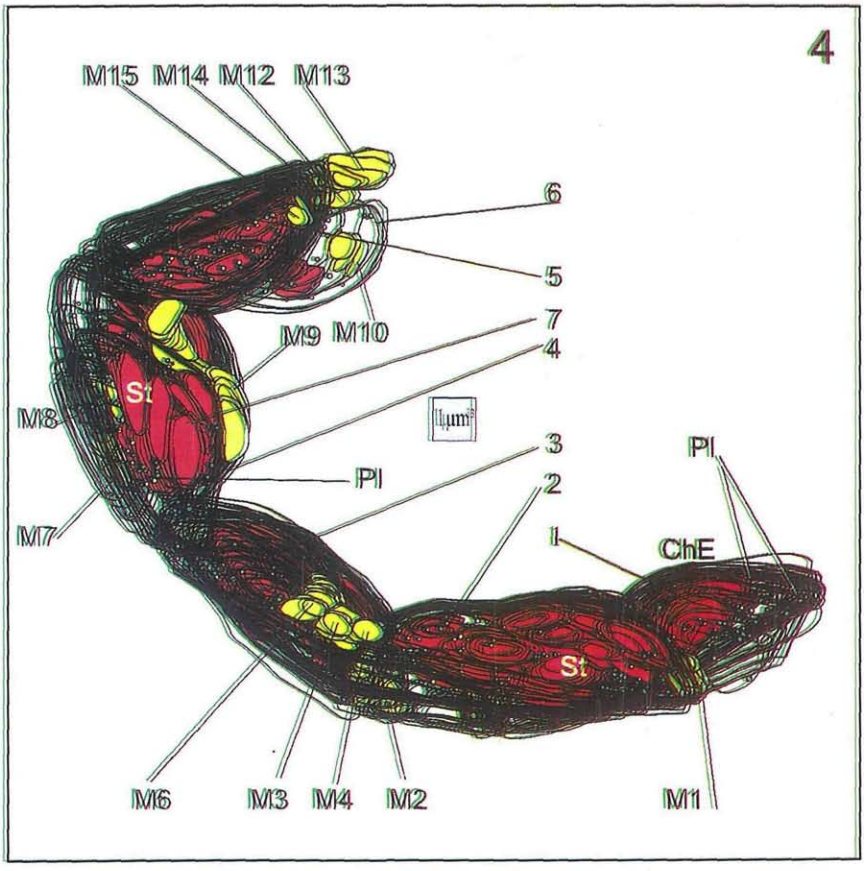
Fig. 3. 3D reconstruction of chloroplast 5 in Figs. 1, 2 with geometrically opened (sectioned) front part. The envelope of the chloroplast is transparent, thylakoids and starch grains (red) are visible.

Fig. 4. 3D wire-model of serial sectioned chloroplasts of Fig. 1. Plastoglobuli (light blue) and starch grains (red) are visible inside the chloroplasts. Mitochondria (yellow) lie adjacent to the chloroplasts.

Abbreviations: 1–7: number of the chloroplasts; ChE: chloroplast envelope; M1-M15: number of the mitochondria (yellow); Pl: plastoglobuli (light blue); St: starch grains (red); Th: thylakoids.







viewed from various sides for a better visualization of the desired structures. Using this method the sections of the chloroplasts of Fig. 1 can be assembled after serial sectioning and now a close contact between the organelles, which is missing in the single section of Fig. 1, can be seen (Fig. 2, 4). This close arrangement is better visible with the reconstruction of the chloroplast envelopes only (Fig. 2). The advantage of the selection of the interesting structures is obvious. Contrary, a transparent depiction of the chloroplast envelope (Fig. 3) or the wire model of the outer membranes of the chloroplasts (Fig. 4) allow an insight into the organelle with emphasis on desired substructures. Reconstructions with geometrically sectioned organelles like Fig. 3 show a well-developed thylakoid system (cf. MENKE 1962), which spreads all over the chloroplast and sometimes even betweeen the starch grains, in all investigated series.

The photosynthetic starch and the plastoglobuli can be made much more accessible with the fading out of thylakoid-membranes (Fig. 4). Now three to nine starch grains per chloroplast can exactly be counted. Besides, the shape of the starch can be determined, which occurs a little flatten. Inside the chloroplasts the plastoglobuli are more or less evenly distributed. The number of plastoglobuli increases with the age of the spinach leaves. In adult leaves 800–1500 plastoglobuli may occur in one chloroplast, in developing leaves less plastoglobuli are found (LICHTENTHALER 1969). The diameter of the plastoglobuli usually lies in the range of about 40–130 nm (LICHTENTHALER 1969, SITTE 1991). However, our 3D reconstructions of chloroplasts of developing spinach leaves confirmed the above mentioned data and showed a number of 68–349 plastoglobuli per chloroplast, with diameters between 40 and 100 nm (Table 1).

Table 1

Number of plastoglobuli per chloroplast and section. Chloroplasts of Figs. 1, 2.

Chloroplast number	Number of sections	Total number of plastoglobuli per chloroplast	Plastoglobuli per chloroplast and section
1	45	349	7.8
2	47	304	6.5
3	55	263	4.8
4	31	171	5.5
5	58	240	4.1
6	8	68	8.50
7	25	129	5.2

Moreover, the 3D reconstruction provides not only better information about the investigated organelle itself, but also enables an exact investigation of associations to other organelles or subcellular structures.

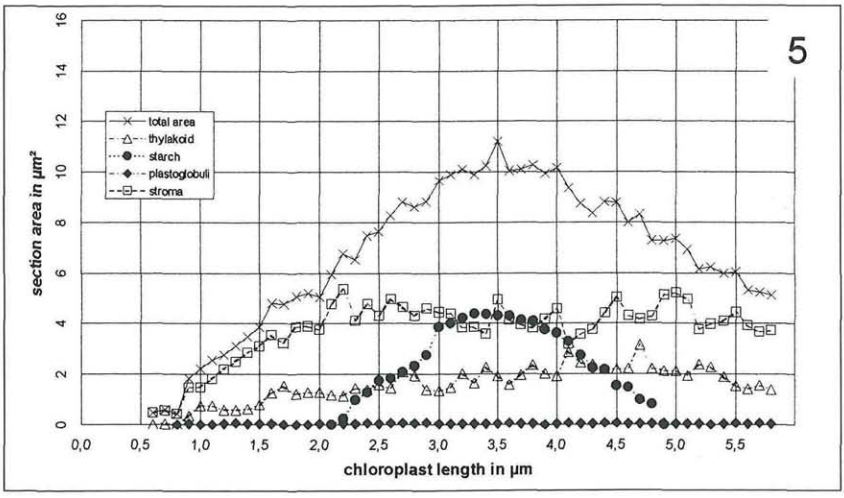


Fig. 5. Areas of the different structural components within chloroplast 5 of Figs. 1, 2.

This kind of information is often lost with the routinely investigation of ultrathin sections. Such associations can be seen very illustrative in Fig. 4, with many mitochondria lying adjacent to the chloroplasts. This close contact is probably induced by the photorespiration (cf. PERKTOLD & al. 1997).

An additional advantage of this method is the possibility of the calculation of areas and volumes of different substructures of organelles like chloroplasts. This can be done for every section (Fig. 5) or the complete organelle (Table 2). Our data demonstrate, that the percentage of the area of the chloroplast components is changing during the sectioned chloroplast (Fig. 5). This is evident especially for the relative share of starch. Therefore, the calculation of the percentage of volumes of the components leads to more relevant data (Table 2).

Table 2

Volumes of structural components of chloroplast 5 of Figs. 1, 2.

	Volumes				
	Chloroplast	Thylakoids	Starch	Plastoglobuli	Stroma
μm^3	35.6	8.3	7.3	0.2	19.8
%	100	23	21	0.4	55.6

In summary, our 3D reconstructions of serial sectioned chloroplasts provide a better knowledge about the architecture and the distribution of fine structures including areas and volumes. The reconstructions are made

of real existing cell structures and are not models based on statistical possibilities.

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Recensiones

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Der Autor nahm das Wort Einführung im Untertitel dieses Bandes ernst und wendet sich tatsächlich an den nicht mykologisch Vorgebildeten. So stehen am An-