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Plant Organelles Analyzed by Ultrathin Serial - Sections

By

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Summary

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Plastids and mitochondria of spinach leaves and spruce needles and roots were serial sectioned, 3D reconstructed and area as well as volume data calculated. Chloroplasts of spinach and spruce are similar in their volume and they show only small differences in their internal structures. Contrary, root leucoplasts are considerably smaller and the relative share of thylakoids and plastoglobuli is very low. In the investigated cells different formed mitochondria occur, their volume and surface data are similar in both plants.

Introduction

Cell organelles like plastids or mitochondria play a key role in plant metabolism. Because of their small size (1-6µm), transmission electron microscopy (TEM) proved to be very suitable for the evaluation of such organelles. The structural conditions routinely are investigated on a limited number of ultrathin sections. However, these sections represent only a small part of the organelles, which makes an evaluation of structural continuities impossible. This disadvantage can be avoided by using computer assisted reconstructions of serial ultrathin sections. The result of the image analysis are real 3D images of selected structures on a high level of resolution with area and volume data. In this paper, plastids and mitochondria of two different plants are investigated and compared with respect to their structure and area/volume data.

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Material and Methods

Needles and roots of *Picea abies* (L.) Karst. and leaves of *Spinacia oleracea* L. were fixed and embedded. Series of 80 nm ultrathin sections were transferred to single slot grids, stained and viewed with a TEM (cf. PERKTOLD & al. 1998). 3D reconstruction based on a method similar to that described by STEVENS 1977. In brief, micrographs of selected structures were scanned with 300 dpi resolution and imported as TIFF files into a computer. The stored images were prepared by an image analysis system (Optimas 4.02, BioScan). Then, profiles of selected structures were traced by hand with the mouse using Corel Draw 7.0, and greyscales were transformed to individual colors. Parallel projection was used for the reconstruction of 3D images (Fig. 1). Area measurements were done with Optimas, volumes calculated by means of the section thickness.

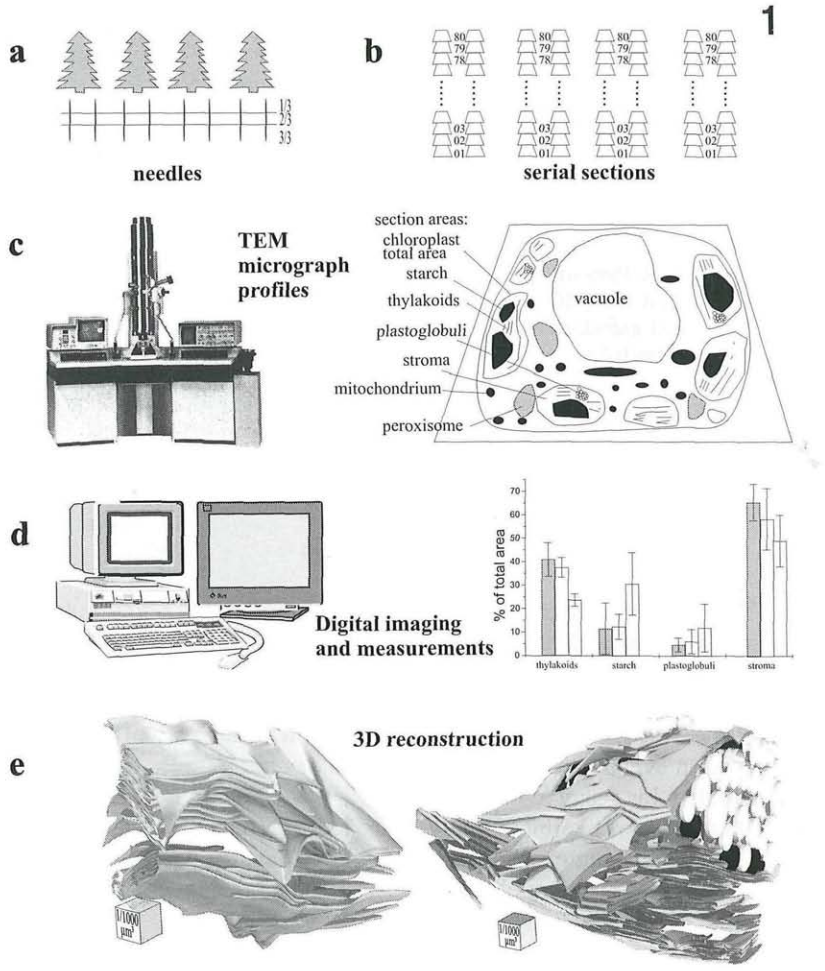
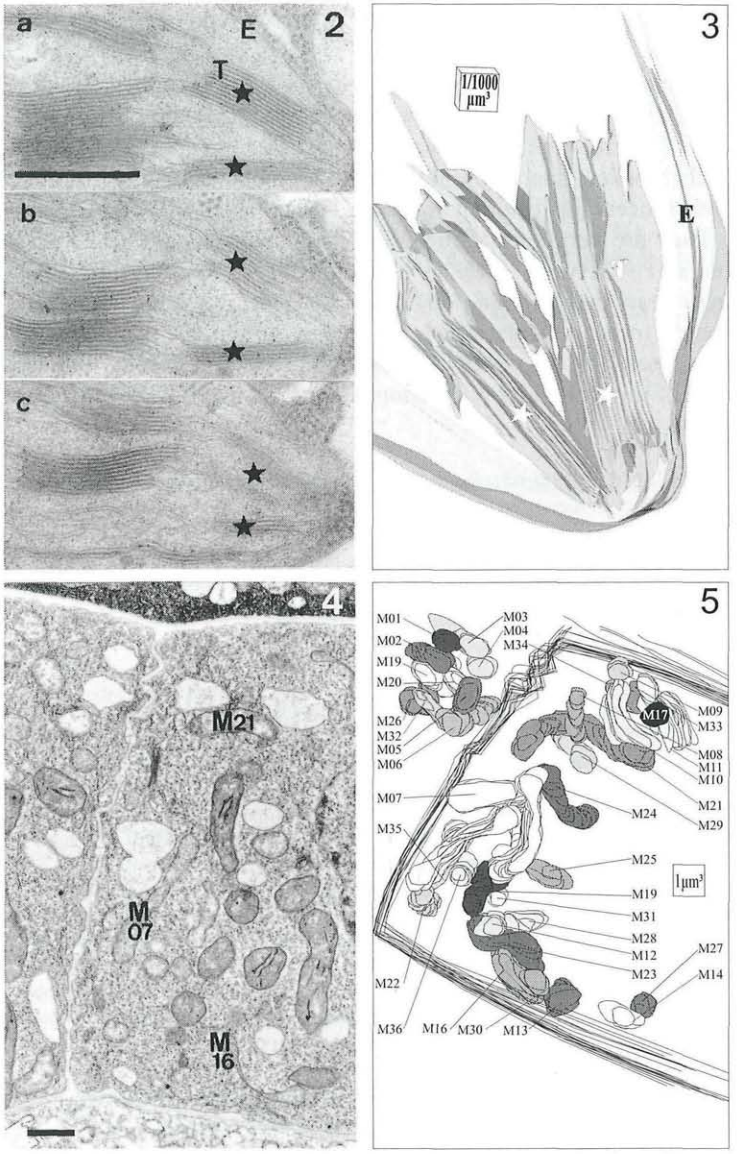


Fig.1. Schematic graph of the procedure for the 3D analysis of organelles (a-e).



Figs. 2,3. Mesophyll cell chloroplast of *Spinacia*. Serial TEM-micrographs of a part of the thylakoid system (Fig. 2; Scale bar = 0.5µm), representing section number 1 (a), 4 (b) and 6 (c), and 3D reconstruction (Fig. 3). The stars indicate the corresponding membranes. E: chloroplast. envelope; T: thylakoids.

Fig. 4. TEM-micrograph of *Picea* root parenchyma cells with different formed mitochondria (M07, M16, M21).

Fig. 5. 3D reconstruction (35 serial sections) of altogether 36 mitochondria (M01-M36) in the cells of Fig. 4. Scale bar = 1µm.

Results and Discussion

3D analysis of plastids showed that the chloroplast of spinach (Figs. 2,3) and spruce do not differ very much in the measured parameters apart from a higher percentage of plastoglobuli in spruce chloroplasts. Leucoplasts of spruce roots are significantly smaller than chloroplasts and they contain mainly stroma (Table 1). Mitochondria occur in different forms in spruce (Figs. 4,5) and mainly oval in spinach. They are very similar regarding their mean volume (0.29-0.36μm^3) and surface area (1.9–2.1μm^2). Besides an improved registration of associations and distances, these basic data on plastids and mitochondria complete 2D morphometric measurements, which are recently used for a better quantification of cellular damages (PALOMÄKI 1995). Additionally they provide information on the distribution of mitochondrial populations in one plant cell (STICKENS & VERBELEN 1996).

Table 1. Chloroplasts of spinach leaves (A) and spruce needles (B); leucoplasts of spruce roots (C). n = number of measured plastids.

sample	n	average volume [μm^3]	average total area [μm^2]	thylakoids %	starch %	plastoglobuli %	stroma %
A	7	50	8.3	24	15	0.5	60.5
B	9	60	9.1	18	11	5.0	66.0
C	5	1.7	1.3	5.5	0	1.2	93.3

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