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Diurnal variation of chloroplast fine structures of spinach

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Abstract. Complete chloroplasts and their fine structures were quantitatively analysed by means of ultrathin serial sections and digital image analysis in order to obtain precise ultrastructural data about diurnal adaptations of the organelles. During the daily course the average chloroplast volume increased from 31 μm^3 in the morning to 44 μm^3 in the evening. In the same time the absolute volumes of starch, thylakoids and plastoglobuli also increased, though to different extents. These observed differences in the diurnal behaviour of this organelles are essential for a precise evaluation of naturally occurring or stress induced changes in chloroplasts.

Keywords: Chloroplasts, serial sections, ultrastructure, volume, spinach

Introduction

Chloroplasts are highly specific organelles of plant cells carrying out several important biosynthetic processes involved in photosynthesis. Investigations of their complex fine structural internal organisation need the use of ultrathin sections and transmission electron microscopy. Applying this technique usually leads to a characterization of the condition of cell organelles in a descriptive manner only. Different environmental factors are known to affect the ultrastructure of cell organelles in general, but plastids were investigated most intensively because alterations or damages were noted very early and/or mainly in these organelles (FORSCHNER & al. 1989, RANTANEN & al. 1994, HOLOPAINEN & al. 1996). However, the evaluation of the condition of this organelle usually based on the information of a limited number of ultrathin sections. Exact quantitative data resulting from ultrastructural analyses are still rare though quantitative morphological analyses proved to be more reliable and highly valuable for a more detailed assessment of cellular changes or ultrastructural adaptations of organelles (ZELLNIG & PERKTOLD 1999, WHEELER & FAGERBERG 2000, REY & al. 2000, GRIFFIN & al. 2001, ROZAK & al. 2002).

In this research complete mesophyll chloroplasts of young spinach leaves were investigated by means of ultrathin serial sectioning, electron microscopy and digital image analysis in order to register the daily periodic ultrastructural adjustments in the architecture and the amount of structures inside the organelle.

Material and methods

Pot cultures of *Spinacia oleracea* L. were grown under defined conditions in a climate chamber with a temperature of 23°C during daytime and 17°C overnight with an average relative humidity of 60 %. The photosynthetic active radiation (PhAR) was adjusted at a level of 900 μmol m^{-2} s^{-1}, distance was 1.5 m. Twilight started at 8 a.m. in 30 minutes from 0–900 μmol m^{-2} s^{-1} and the light decreased at 8 p.m.

Sections were taken from the middle part of young leaves (size about 4 cm) at 07.00 h (early morning), 13.00 h (midday) and 19.00 h (evening). The sections were fixed in 3 % cacodylate buffered glutaraldehyde, pH 7.2, for 90 min. After rinsing in buffer the sections were postfixed in 1 % OsO_4 for 90 min, dehydrated in a graded series of ethanol followed by propylenoxide and embedded in Agar 100 epoxy resin.

Serial ultrathin sections (100 nm) were cut with a Reichert Ultracut S ultramicrotome, stained with lead citrate and uranyl acetate and viewed with a Philips CM 10 transmission electron microscope.

3D measurements

Preliminary investigations are essential in order to exclude 3D reconstructions of untypical chloroplasts. Therefore two leaves were taken from two selected plants of every sample. A ribbon of about 80 serial sections of palisade parenchyma cells was cut of every leaf. TEM micrographs were used for 2D measurements. Four arbitrary selected sectioned areas of chloroplasts in section 1, 30, and 60 of every ribbon (= 12 areas per ribbon, 48 areas per sample) were investigated by TEM and measured by computer to get characteristic data of fine structures. The results of these preliminary investigations served for the selection of the sample with the most characteristic chloroplasts according to the calculated mean values for each sample.

The TEM micrographs were digitised by a scanner and the sectioned areas of interest were measured with an image analysis system (Optimas 6.5., Bio Scan) and exported to the software program Excel. The volumes were calculated by the sum of sectioned areas multiplied by the thickness of the sections. The digitised micrographs were pixel images, which were transformed semi-automatically by a computer program (Corel Trace 10), or by hand (Corel Draw 10), into vectorgraphics. 3D reconstructions were created by the program Carrara Studio 1.0 (Softline). For each sample 6 complete palisade parenchyma chloroplasts and details of them were measured and 3D reconstructed.

The statistical analyses were performed by using the software package Origin (OriginLab Corporation, USA). Differences between morning and evening samples were evaluated using two sample t-test.

Results

The investigated chloroplasts show a homogeneous distribution of thylakoids and plastoglobuli without any noticeable diurnal variation, starch is present in form of a varying number of grains in the single chloroplasts (Fig. 1). During the day the total chloroplast volume increases from 31 μm^3 in the morning samples to 44 μm^3 in the evening. This increase is associated with an increase in the absolute values of all internal chloroplast structures (cf. Fig. 2). A similar trend is also present, when the data are related to relative values. In this case the starch content increases significantly from 12 % of the chloroplast volume in the morning to 27 % in the evening. This change is connected with a decrease in the thylakoid and stroma volumes and a significant increase in the total volume of the plastoglobuli in the evening samples (cf. Fig. 3).

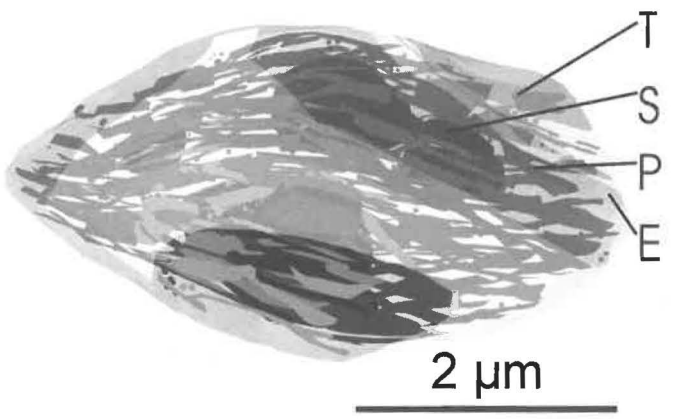

Figure 1: Three-dimensional reconstruction of a spinach palisade parenchyma chloroplast showing the thylakoid system (T), starch grains (S), plastoglobuli (P) and the envelope (E). Sample taken at 07.00 h.

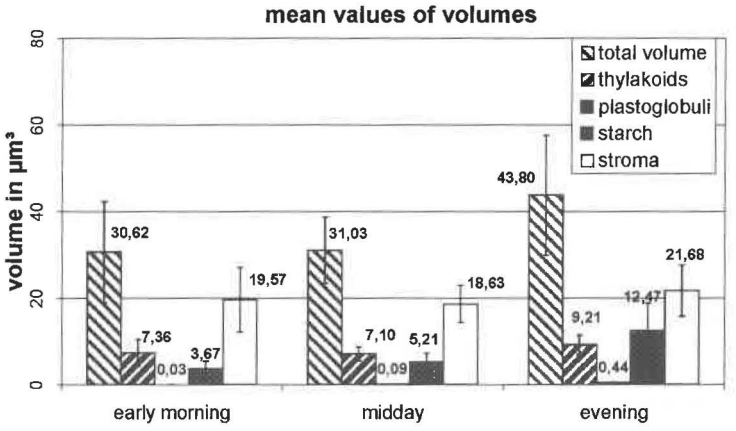

Figure 2: Mean volumes and standard deviation of complete palisade parenchyma chloroplasts and their internal fine structures during the daily course (n = 6 for each sampling time).

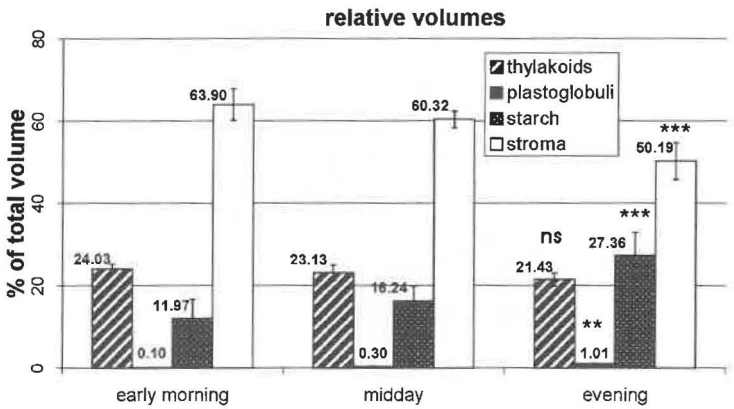

Figure 3: Mean values and standard deviation of the relative values of fine structures of chloroplasts during the daily course. Significance was calculated from randomly sectioned chloroplasts for the morning and evening samples (n = 48 for each sample).

*, ** and *** indicate values that differ significantly from the control at $P < 0.05$, $P < 0.001$, and $P < 0.0001$, respectively; $P > 0.05$ not significant (ns).

Discussion

The investigated spinach chloroplasts clearly showed diurnal fine structural adaptations and changes. During the day the starch content increased due to the photosynthetic CO_2 fixation resulting in the synthesis of transitory starch inside the chloroplasts. The higher starch content is the main reason for a significant increase in the volumes of the chloroplasts in the evening, though all other structures also showed increasing values. Interestingly, two-dimensional measurements of spinach chloroplasts did not reveal a significant diurnal net change in the grana size and number (Rozak & al. 2002). The thylakoid membrane is a very dynamic system even in mature chloroplasts, rapidly adapting to changes in light conditions or performing long term adaptation due to a number of environmental factors (cf. Vothknecht & Westhoff 2001). Our investigations demonstrated changes in the thylakoid system during the day, which are supposed to be caused not only by an increasing light intensity. An adaptation to changes in light intensity were already reported for the thylakoid surface area in sunflower (Wheeler & Fagerberg 2000) and the grana size and number in spinach chloroplasts (Rozak & al. 2002). These changes occurred within minutes, thus light intensity is not supposed to be the main reason for the observed increase in the thylakoid system in the evening samples. In addition to the thylakoid system also the plastoglobuli content showed a more than tenfold increase in the evening samples. Plastoglobuli are regarded as a kind of storage particles for thylakoid components being involved in the formation and degeneration of the thylakoid membrane (cf. Kessler & al. 1999). A diurnal change in the plastoglobuli content and a simultaneous increase of both the thylakoid membranes and the plastoglobuli during the day has not been reported yet and has to be investigated in more detail in further experiments.

Conclusion

Palisade parenchyma chloroplasts of spinach show quantitative changes and adaptations of their fine structures during the daily course, which have to be considered in connection with investigations and measurements of this organelle.

Acknowledgement

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