Chloroplast ultrastructural development in vascular bundle sheath cells of two different maize (*Zea mays* L.) genotypes

A. Vičánková[1, 2], J. Kutík[1]

[1]*Faculty of Science, Charles University in Prague, Czech Republic*
[2]*Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

ABSTRACT

The leaves of maize have two photosynthesizing tissues with two types of chloroplasts, mesophyll cells (MC) and vascular bundle sheaths cells (BSC). The development of chloroplasts in BSC was followed by transmission electron microscopy and point counting method in the middle part of the third leaf of maize plants. From young (Y) to mature (M) leaves, volume density of photosynthetic membrane system (thylakoids) increased, to senescing (S) leaves it did not significantly change. During the whole leaf ontogeny, small thylakoid appression regions (grana) were present in BSC chloroplasts, currently assumed to be agranal. From M to S leaves, volume density of starch inclusions strongly decreased and that of plastoglobuli strongly increased.

Keywords: chloroplast; ultrastructure; C_4 photosynthesis; electron microscopy; *Zea mays* L.

Chloroplasts are cell organelles of photosynthesis. They are, therefore, the most important type of plastids. Higher plant plastids have been studied for a very long time (see e.g. Virgin and Egnéus 1983, Ryberg et al. 1993, Hudák 1997). Chloroplast ultrastructural development during leaf ontogeny was reviewed by Kutík (1998). Photosynthesis is the primary metabolic process, which is important, by fixation of inorganic carbon from CO_2, for life on our planet (e.g. Lawlor 2001). Plants with so-called C_4 photosynthesis have CO_2 fixation compartmentalized between two types of chloroplasts. Most of C_4 plants have typical Kranz (wreath) leaf anatomy. Two types of photosynthetic tissue surround vascular bundles: the bundle sheath cells (BSC) and the mesophyll cells (MC). Maize is a typical C_4 plant of so-called NADP-malic enzyme (NADP-ME) subgroup (Hudák 1997). Chloroplasts are located centrifugally in the bundle sheath cells. They have usually almost no grana (regions of appressed thylakoids) or have small grana composed of two to four thylakoids only (whereas MC chloroplasts have large grana usually from many thylakoids) but they contain usually large inclusions of reserve polysaccharide, starch (which is almost absent in MC chloroplasts). Brangeon (1973a, b) followed differentiation of dimorphic (BSC and MC) chloroplasts of young, growing maize leaves. Differentiation of MCs and BSCs was followed in maize also by molecular approaches (e.g. Furumoto et al. 2000). Kutík et al. (1999) studied stereologically the development of MC chloroplasts during ontogeny of the third leaf of maize plants in genotypes differing in photosynthetic (photochemical) activity. The aim of our work was to evaluate stereologically (for the first time, as far as we know) the development of BSC chloroplasts during ontogeny of the same leaves.

MATERIAL AND METHODS

Maize (*Zea mays* L.) plants in two parent lines, CE813 and CE829, and their hybrids, CE813×CE829 and CE829×CE813, were used in the experiments. They were cultivated in a growth chamber at temperature 25/16°C, air humidity 60 to 80%, and irradiance of 500 μmol/m^2/s. Samples for ultrastructural study were taken from the middle part of the third leaf of maize plants (coleoptile numbered as leaf zero) at the age of the plants of 13, 27, 41 days after sowing, i.e., from young (Y, growing), mature (M, not more growing) and senescing (S, yellowing) leaves. They were always taken from

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. J13/98113100004.

four plants. For each plant, five chloroplasts were evaluated. Leaf samples were double fixed with glutaraldehyde and osmic acid, then embedded into Spurr's low viscosity resin via propylene oxide after dehydration in graded ethanol series, see Kutík et al. (2001). Ultrathin sections were contrasted in saturated solution of uranyl acetate in 70% ethanol (for 20 min) and in lead citrate solution after Reynolds (1963), (for 20 min). Chloroplast ultrastructure was evaluated using transmission electron microscope PHILIPS EM 300. Quantitative evaluation of chloroplast ultrastructure was realized by point counting method, using morphometric grids laid on positive photographic images of chloroplasts. This method allows estimating three-dimensional characteristics of given body (e.g. a chloroplast) from its sections. Volume densities (relative partial volumes, if chloroplast volume = 100%, see Gundersen and Jensen 1987) of granal and intergranal thylakoids, peripheral reticulum, starch inclusions, plastoglobuli and stroma were estimated. Area, length and width of chloroplast cross sections in bundle sheaths cells were followed by the system for image processing and analysis LUCIA version 3.52 (Laboratory Imaging Ltd., Czech Republic). Statistical significance of all ontogenic and genotypic differences found was determined by ANOVA software and *t*-test.

RESULTS AND DISCUSSION

The ultrastructure and size of BSC chloroplasts (Figure 1) changed notably during the leaf ontogeny. From Y to M leaves the volume density of thylakoids increased, from M to S leaves it did not change significantly. Small grana were identifiable during whole leaf ontogeny, the lowest level of granality was reached in BSC chloroplast thylakoid system of mature leaves where compartmentalization of CO_2 fixation between MCs and BSCs worked probably best. Presence of the grana with high activity of photosystem two splitting water and producing oxygen is unfavourable for CO_2 fixation, see also Nishioka et al. (1993). From M to S leaves the amount of starch strongly decreased and the volume density of plastoglobuli increased. Both are the signs of chloroplast senescence, as was reviewed by Kutík (1998). Y and M leaves chloroplasts were larger comparing with S leaves chloroplasts. There were small differences only between BSC chloroplasts of the leaves of CE813 and CE829 inbred lines of the same age (Tables 1 and 2).

The volume density of BSC chloroplasts in the cells of mature leaves (data not shown) did not differ between both inbred lines. Comparison between BSC chloroplasts ultrastructure in mature leaves of reciprocal hybrids (Table 1) showed that

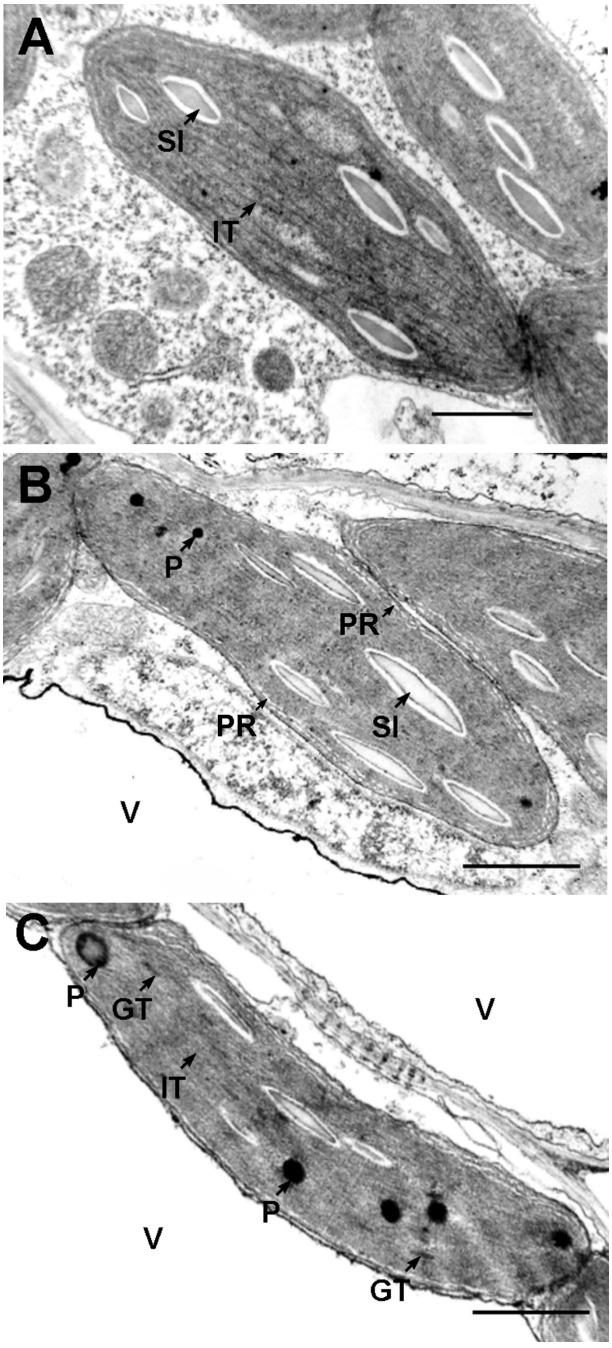

Figure 1. Transmission electron micrographs of chloroplast cross sections taken from the third leaf bundle sheath cells of maize inbred line CE813: (A) young leaf, (B) mature leaf, (C) senescing leaf; the chloroplasts contain many intergranal (nonappressed) thylakoids (IT) and several small grana (GT); peripheral reticulum (PR) is also seen in the chloroplasts; in senescing leaves the starch inclusions (SI) are smallest and plastoglobuli (P) largest; in B and C cell vacuoles (V) are also seen; bar = 1 μm

CE813×CE829 chloroplasts possessed significantly less thylakoids, but at the same time much more starch than CE829×CE813 ones (determined by *t*-test).

Table 1. Structural characteristics (volume densities of chloroplast compartments in % – granal thylakoids, intergranal thylakoids, all thylakoids, peripheral reticulum, plastoglobuli, starch inclusions and stroma) and dimensions of bundle sheath cell chloroplasts cross sections – chloroplast cross section area (μm^2), chloroplast cross section length (μm), chloroplast cross section width (μm) – ($\bar{x} \pm s_{\bar{x}}$) in young, mature and senescing leaves of maize in inbred lines CE813, CE829 and in mature leaves of maize in hybrids CE813×CE829 and CE829×CE813

	Young leaves		Mature leaves				Senescing leaves	
	CE813	CE829	CE813	CE829	CE813× CE829	CE829× CE813	CE813	CE829
Granal thylakoids (%)	4.36 ± 0.48	4.51 ± 0.53	3.60 ± 0.32	4.58 ± 0.69	3.18 ± 0.32	5.03 ± 0.49	5.32 ± 0.88	7.36 ± 0.80
Intergranal thylakoids (%)	22.09 ± 1.31	20.43 ± 1.25	31.13 ± 1.82	30.47 ± 1.46	21.16 ± 2.60	34.65 ± 2.60	30.24 ± 1.97	30.37 ± 2.03
All thylakoids (%)	26.45 ± 1.44	24.94 ± 1.42	33.73 ± 2.00	35.04 ± 1.81	24.33 ± 1.55	40.12 ± 2.80	35.56 ± 2.38	37.73 ± 2.32
Peripheral reticulum (%)	4.77 ± 0.26	6.71 ± 0.47	6.13 ± 0.86	8.61 ± 0.86	6.12 ± 0.49	8.64 ± 2.36	6.48 ± 0.58	4.72 ± 0.87
Plastoglobuli (%)	0.19 ± 0.06	0.09 ± 0.06	0.79 ± 0.13	0.87 ± 0.21	0.69 ± 0.16	0.97 ± 0.20	2.20 ± 0.22	3.18 ± 0.43
Starch inclusions (%)	5.58 ± 0.71	6.77 ± 0.66	5.01 ± 0.60	6.89 ± 1.00	15.06 ± 1.49	2.25 ± 0.37	1.54 ± 0.29	0.54 ± 0.22
Stroma (%)	63.02 ± 1.42	61.50 ± 1.80	54.35 ± 2.00	49.99 ± 8.10	53.82 ± 2.3	48.04 ± 3.02	54.23 ± 2.29	55.36 ± 2.48
Chloroplast cross section area (μm^2)	7.9 ± 0.51	6.49 ± 0.40	7.74 ± 0.45	8.93 ± 0.39	8.93 ± 0.88	6.44 ± 0.39	5.63 ± 0.28	6.81 ± 0.48
Chloroplast cross section length (μm)	5.20 ± 0.22	5.66 ± 0.31	6.08 ± 0.20	7.04 ± 0.40	6.47 ± 0.35	6.49 ± 0.30	5.30 ± 0.17	5.04 ± 0.17
Chloroplast cross section width (μm)	1.99 ± 0.09	1.49 ± 0.06	1.71 ± 0.07	1.74 ± 0.13	1.79 ± 0.10	1.27 ± 0.27	1.33 ± 0.06	1.74 ± 0.10

Dimorphic chloroplasts of maize differentiate gradually from identical plastids in very young leaves (Brangeon 1973a, b). Our results demonstrate also the presence of small thylakoid grana even in BSC chloroplasts of mature and senescing maize leaves. As in MC chloroplasts of maize (Kutík et al. 1999), BSC chloroplast ultrastructure changes substantially during whole leaf development. Steady increase of the volume density of plastoglobuli (containing probably lipoid degradation products of thylakoid membranes, see Kutík 1998) is also connected with leaf ageing. These developmental changes in BSC chloroplasts are analogical to differences found in MC chloroplasts of the same leaves of different age (Kutík et al. 1999), as well as to the differences found in MC chloroplasts of mature maize leaves in various parts of the leaf blade with the oldest cells at the top of the blade and the youngest ones at the base (Kutík et al. 2001). Stereology enables us to evaluate chloroplast ultrastructure in a simple and complex way (compared to, e.g. measuring of appressed and nonappressed thylakoid membranes length or counting grana or number of thylakoids in the grana). The results of our work confirm the dynamic nature of chloroplast dimorphism (BSC chloroplasts versus MC chloroplasts) in the leaves of maize. This dimorphism is developed best in mature leaves, which are the main source of photosynthates.

Table 2. Levels of statistical significance of differences in structural characteristics (granal thylakoids, intergranal thylakoids, all thylakoids, peripheral reticulum, starch inclusions, plastoglobuli and stroma) and dimensions (chloroplast cross section area, chloroplast cross section length and chloroplast cross section width) of BSC chloroplasts from analysis of variance followed by ANOVA test in young, mature and senescing leaves of maize of inbred lines CE813 and CE829

	CE813 (young/ mature/ senescing)	CE829 (young/ mature/ senescing)	Young (CE813/ CE829)	Mature (CE813/ CE829)	Senescing (CE813/ CE829)
Granal thylakoids	0.143	0.006	0.835	0.029	0.094
Integral thylakoids	0	0	0.365	0	0.964
All thylakoids	0.005	0	0.46	0	0.518
Peripheral reticulum	0.127	0.003	0.001	0.009	0.101
Plastoglobuli	0	0	0.246	0.72	0.05
Starch inclusions	0	0	0.227	0	0.009
Stroma	0.002	0.001	0.511	0.173	0.74
Chloroplast cross section area	0	0	0.036	0.007	0.04
Chloroplast cross section length	0.005	0	0.234	0.219	0.286
Chloroplast cross section width	0	0.138	0	0.094	0.001

Acknowledgements

Electron microscopic studies described were performed at the laboratory of the State Institute of Health in Prague, headed by Dr. D. Hulínská. The possibility to work there and kind advice given by Ing. M. Doubek are gratefully acknowledged.

REFERENCES

Brangeon J. (1973a): Compared ontogeny of the two types of chloroplasts of *Zea mays*. Journal of Microscopy, *16:* 233–242.

Brangeon J. (1973b): Effect of irradiance on granal configurations of *Zea mays* bundle sheath chloroplasts. Photosynthetica, *7:* 365–372.

Furumoto T., Hata S., Izui K. (2000): Isolation and characterization of cDNAs for differentially accumulated transcripts between mesophyll cells and bundle sheath strands of maize leaves. Plant and Cell Physiology, *41:* 1200–1209.

Gundersen H.J.G., Jensen E.B. (1987): The efficiency of systematic sampling in stereology and its prediction. Journal of Microscopy, *147:* 229–263.

Hudák J. (1997): Photosynthetic apparatus. In: Pessarakli M. (ed.): Handbook of photosynthesis. Marcel Dekker, New York, Basel, Hong Kong: 27–48.

Kutík J. (1998): The development of chloroplast structure during leaf ontogeny. Photosynthetica, *35:* 481–505.

Kutík J., Holá D., Vičánková A., Šmídová M., Kočová M., Körnerová M., Kubínová L. (2001): The heterogeneity of structural and functional photosynthetic characteristics of mesophyll chloroplasts in various parts of mature or senescing leaf blade of two maize (*Zea mays* L.) genotypes. Photosynthetica, *39:* 497–506.

Kutík J., Kočová M., Holá D., Körnerová M. (1999): The development of chloroplast ultrastructure and Hill reaction activity during leaf ontogeny in different maize (*Zea mays* L.) genotypes. Photosynthetica, *36:* 497–507.

Lawlor D.W. (2001): Photosynthesis. 3rd edition. BIOS Scientific Publishers Limited, Oxford.

Nishioka D., Brisibe E.A., Miyake H., Taniguchi T. (1993): Ultrastructural observations in the suppression of granal development in bundle sheath chloroplasts of NaDP-ME type C_4 monocot and dicot species. Japanese Journal of Crop Science, *62*: 621–627.

Reynolds E.S. (1963): The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. The Journal of Cell Biology, *17*: 208–212.

Ryberg H., Ryberg M., Sundquist C. (1993): Plastid ultrastructure and development. In: Sundquist C., Ryberg M. (eds.): Pigment – Protein complexes in plastids: Synthesis and assembly. Academic Press, San Diego, New York, Boston, London, Sydney, Tokyo, Toronto: 25–62.

Virgin H.I., Egnéus H.S. (1983): Control of plastid development in higher plants. In: Shropshire W., Mohr H. (eds.): Photomorphogenesis. Springer Verlag, Berlin, Heidelberg, New York, Tokyo: 289–311.

Recieved on June 7, 2004

ABSTRAKT

Vývoj ultrastruktury chloroplastů v buňkách pochev cévních svazků dvou různých genotypů kukuřice (*Zea mays* L.)

Listy kukuřice tvoří dvě fotosyntetická pletiva s dvěma typy chloroplastů, mezofylové buňky a buňky pochev cévních svazků. Vývoj chloroplastů v buňkách pochev cévních svazků byl sledován pomocí transmisní elektronové mikroskopie a bodové stereologické metody ve střední části čepele třetího listu rostlin kukuřice. Objemová hustota tylakoidů (fotosyntetického membránového systému) stoupla v průběhu dospívání listů, v průběhu stárnutí listů se průkazně nezměnila. Během celé listové ontogeneze jsme sledovali přítomnost gran (na sebe přitisklých tylakoidů) v chloroplastech v buňkách pochev cévních svazků, považovaných běžně za agranální. Objemová hustota škrobových inkluzí v chloroplastech výrazně klesla a objemová hustota plastoglobulů výrazně stoupla v průběhu senescence listů.

Klíčová slova: chloroplast; ultrastruktura; C_4 fotosyntéza; elektronová mikroskopie; *Zea mays* L.

Corresponding author:

Mgr. Anna Vičánková, Ústav experimentální botaniky, Akademie věd České republiky, Rozvojová 135, 165 00 Praha 6, Česká republika
phone: + 420 220 390 402, fax: + 420 220 390 419, e-mail: vicankova@ueb.cas.cz