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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

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Abstract

Differences in ultrastructural parameters of mesophyll cell (MC) chloroplasts, contents of photosynthetic pigments, and photochemical activities of isolated MC chloroplasts were studied in the basal, middle, and apical part of mature or senescing leaf blade of two maize genotypes. A distinct heterogeneity of leaf blade was observed both for structural and functional characteristics of chloroplasts. In both mature and senescing leaves the shape of MC chloroplasts changed from flat one in basal part of leaf to nearly spherical one in leaf apex. The volume density of granal thylakoids decreased from leaf base to apex in both types of leaves examined, while the amount of intergranal thylakoids increased in mature leaves but decreased in senescing leaves. The most striking heterogeneity was found for the quantity of plastoglobuli, which strongly increased with the increasing distance from leaf base. The differences in chloroplast ultrastructure were accompanied by differences in other photosynthetic characteristics. The Hill reaction activity and activity of photosystem 1 of isolated MC chloroplasts decreased from leaf base to apex in mature leaves. Apical part of senescing leaf blade was characterised by low contents of chlorophyll (Chl) *a* and Chl *b*, whereas in mature leaves, the content of Chls as well as the content of total carotenoids (Car) slightly increased from basal to apical leaf part. This was reflected also in the ratio Chl (*a*+*b*)/total Car; the ratio of Chl *a*/*b* did not significantly differ between individual parts of leaf blade. Both genotypes examined differed in the character of developmental gradient observed along whole length of leaf blade.

Additional key words: chloroplast development; chloroplast dimensions; electron microscopy; Hill reaction activity; peripheral reticulum; photosynthesis; photosystem 1 activity; plastoglobuli; thylakoids.

Introduction

Changes in chloroplast structural or functional characteristics during the development of leaf blade have been often studied in grasses (*e.g.*, *Triticum*, *Hordeum*, *Zea*). A gradient of chloroplasts with respect to their developmental stage has been usually found along whole length of developing leaf blade, with the youngest ones in basal part and mature, photosynthetically fully active chloroplasts in leaf apex. The various stages of chloroplast development can be characterised by changes in chloroplast ultrastructure, especially as regards their inner membrane system (see Kutík 1998 for review). Basal part of young leaves usually contains plastids with prolamel-

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Abbreviations: BSC – bundle sheath cells; Car – carotenoids; CCU – chlorophyll content unit; Chl – chlorophyll; DCMU – 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; DCPIP – 2,6-dichlorophenol-indophenol; DMA – dry matter expressed per leaf area unit; DMU – dry matter unit; ES – experimental set; HRA – Hill reaction activity; LAU – leaf area unit; L/W – chloroplast cross section length to width ratio; MC – mesophyll cells; P – plastoglobuli; PAR – photosynthetically active radiation; PR – peripheral reticulum; PS – photosystem; S – stroma; SI – starch inclusions; TG – granal thylakoids; TI – intergranal thylakoids; TT – total thylakoids.
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lar bodies, which are subsequently transformed into immature, pre-granall chloroplasts with a simple thylakoid system. In some cases, these young plastids contain starch inclusions and can be therefore denoted as amyloplasts (Wellburn *et al.* 1982, Chonan *et al.* 1991). Degradation of starch and synthesis of various components of photosynthetic apparatus is typical phenomenon associated with the development of immature plastids into mature, photosynthetically fully active chloroplasts found usually in parts of leaf blade more distant from leaf base (Wellburn *et al.* 1982). Transcription activity of chloroplast genes as well as the content of cpDNA increases from basal to middle part of developing leaves of some plant species, whereas in other species a further increase from middle to apical part has been also observed (Lawrence and Possingham 1986). In C4 plants, which are characterised by typical dimorphism of mesophyll (MC) and bundle sheath (BSC) chloroplasts, the diversification of chloroplast ultrastructure begins at leaf apex and gradually continues with the chloroplast development toward leaf base (Rascio *et al.* 1984, Nishioka *et al.* 1993, and others).

The majority of these studies, however, concentrated on the young leaves, whose growth is still unfinished. This is why we tried to find out whether the ultrastructural changes associated with the developmental gradient observed in young leaves are conserved also in mature, non-growing, or even senescing leaves. To our knowledge, such heterogeneity of chloroplast ultrastructure with respect to various parts of mature or senescing leaf blade has not yet been studied. Gradients in anatomical characteristics (*e.g.*, stomata density or epidermal cell size) on mature leaf blade area are known for a long time (for review see, *e.g.*, Tichá 1985). Several studies dealing with the differences in net photosynthetic rate, contents of photosynthetic pigments, or activities of pigment-protein complexes of thylakoid membranes with respect to heterogeneity of mature (or, in some cases, senescing) leaf blade were also published. The amount of photosynthetic pigments and pigment-protein complexes of thylakoid membranes usually increases with the increasing distance from leaf base. This applies also for the activity of these complexes and the net photosynthetic rate (Wellburn *et al.* 1982, Lebedev *et al.* 1986, Bredenkamp and Baker 1988, Davies *et al.* 1989, 1990, Hew *et al.* 1998, and others). For comprehensive review on various changes in photosynthetic characteristics associated with leaf development see, *e.g.*, Čatský and Šesták (1997) or Šesták and Šiffel (1997).

The aim of this work was therefore to determine possible differences in ultrastructure of chloroplasts in three parts of mature and senescing leaf blade of maize, and to analyse the relationship between these structural parameters and various photosynthetic characteristics of mesophyll chloroplasts. Another object of our study was to ascertain whether the genotypic variability found for many photosynthetic characteristics reflects not only in chloroplast development during leaf ontogeny (Kutík *et al.* 1999) but also in the differences of chloroplast structural and functional parameters along the length of leaf blade.

Materials and methods

Plants: The ultrastructural characteristics of MC chloroplasts together with their photochemical activity and the contents of photosynthetic pigments in leaves were studied in two maize (*Zea mays* L.) genotypes: inbred line 2023 and its F1 hybrid 2023×CE810. Two sets of experiments (*i.e.*, ES 1 and ES 2) were performed with similar experimental pattern. Seeds, obtained from Maize Breeding Station CEZEA in Čejč (Czech Republic), were sown to low planting dishes with soil and placed in the growth chamber (*Klimabox RK1-007, Kovodružstvo Slaný*, Czech Republic) at day/night regime 16/8 h, irradiance 470/0 (ES 1) or 230/0 (ES 2) μmol m^{-2} s^{-1} (PAR), temperature 25/16 °C, and relative air humidity 70/80 %. 50-60 plants represented each genotype.

The mature, non-growing third leaf (numbering from coleoptile as the leaf 0) has been studied in both experimental sets. In ES 1, plants were 27-28 d old; however, since their leaves have already started to show various symptoms of senescence, the age of plants used for ES 2 was lowered to 23-24 d, and the irradiance was also reduced. The leaves were taken three hours after beginning of light period, the midrib was excised, and the leaf blade was cut into three parts of approximately equal length, referred to as 'basal', 'middle', and 'apical' part. The samples for the analysis of MC chloroplast structure and function, the photosynthetic pigment contents, and the dry matter were prepared from each third of leaf blade.

Chloroplast ultrastructure: Four plants of each genotype were used for electron microscopic and stereological evaluation following the standard procedure described in Kutík *et al.* (1999). This procedure consisted of double fixation of leaf blade samples with glutaraldehyde followed by osmic acid treatment, their dehydration through ethanol series, and embedding into Spurr's low viscosity resin. Chloroplast ultrastructure was evaluated on transverse ultrathin sections of embedded objects contrasted with uranyl acetate solution, followed by lead citrate solution treatment. The transmission electron microscope *Philips EM 300* (the Netherlands) was used at primary magnifications of about 7 000×. On electron microphotographs, at final magnifications of about 30 000×, chlo-

roplast cross section's length and width were determined together with the volume densities of individual chloroplast compartments. Relative partial volumes of granal and intergranal thylakoids, peripheral reticulum, starch inclusions, plastoglobuli, and stroma (including the space between the outer and inner envelope membranes) were evaluated using morphometric grids with regularly distributed points. Five chloroplasts were analysed for each leaf sample.

Photochemical activity of isolated chloroplasts: The isolation of MC chloroplasts from three parts of maize leaf blade was performed as described in Körnerová and Holá (1999). 18 to 20 plants were usually needed to get sufficient amount of leaf tissue. Photochemical activity of isolated chloroplasts was measured polarographically as Hill reaction activity (HRA) and photosystem 1 (PS1) activity, *i.e.*, the amount of oxygen formed (or, in case of PS1 activity, consumed) by the suspensions of isolated chloroplasts irradiated by "white light" (800 μmol m^{-2} s^{-1}, PAR) after the addition of artificial electron acceptors or donors. In case of HRA, 7 mM $K_3[Fe(CN)_6]$ served as artificial electron acceptor whereas 0.15 mM reduced DCPIP as electron donor, 0.1 mM methylviologen as electron acceptor, and 0.1 mM DCMU as PS2 activity inhibitor were used for measurement of PS1 activity. Each sample was measured four to eight times and the values were expressed per leaf area unit (LAU), dry matter unit (DMU), or chlorophyll content unit (CCU), and time unit.

Contents of photosynthetic pigments: Six leaf discs, each corresponding to 0.5 cm^2, were cut into small pieces which were put into 10 cm^3 of N,N-dimethylformamide and stored in a dark and cool place. After 2 d, Chl *a*, Chl *b*, and total carotenoids (Car) content in the extracts was determined spectrophotometrically (*Spekol 211*, *Carl Zeiss*, Jena, Germany) (Porra *et al.* 1989, Wellburn 1994). Each genotype/part of leaf blade was represented by six samples and the values were expressed per LAU or DMU. The Chl (*a*+*b*) content and the ratios of Chl *a*/*b* and Chl/Car were also evaluated.

Dry matter expressed per leaf area unit (DMA): Eight samples, each containing six 0.5 cm^2 leaf discs (usually 1 to 2 discs per one plant), were taken from each part of leaf blade analysed. They were fully dried and their mass was determined on analytical balance (*Sartorius*, Germany) with precision of 0.03 mg.

Statistical treatment: Statistical significance of differences in photosynthetic characteristics between various parts of leaf blade as well as between genotypes or experimental sets was tested by one- or two-way analysis of variance followed by Scheffe's non-parametric test, using the 5 % level of statistical significance as the critical one.

Results

Differences between experimental sets: The habit and growth pattern of plants was similar in both experimental sets. At the beginning of measurement of photosynthetic characteristics the plants usually had four leaves, but the fourth leaf was not yet fully developed. The third leaf, used for the analysis of structural and functional characteristics of MC chloroplasts, was therefore the youngest one whose growth has been already completed. Its external appearance, however, strongly differed between both experimental sets. In ES 1, the apical part showed yellow or yellow-green colour and (in 2023×CE810) its extreme tip began to dry. Yellowing of leaf blade as the visible symptom of senescence was also apparent in the middle part; it was more accentuated in the hybrid compared to the parental genotype. In ES 2, on the other hand, the entire leaf was green, photosynthetically fully active, in both genotypes studied.

The external differences between experimental sets in the third leaf appearance were accompanied by the differences in photosynthetic characteristics. With the exception of total Car content, the volume density of starch inclusions in MC chloroplasts and the DMA, the values of all other structural and functional characteristics examined significantly differed between ES 1 and ES 2 (Table 1). The MC chloroplasts analysed in ES 2 showed greater volume density of both granal and intergranal thylakoids, less plastoglobuli, and smaller peripheral reticulum compared to ES 1. They were also more flat, as shown by greater value of their length-to-width ratio (Figs. 1 and 2). These structural differences were accompanied by the differences in photochemical activity (both HRA and PS1) and Chl content: the values of these characteristics were higher in ES 2 (Figs. 3 and 4).

The behaviour of ES 1 and ES 2 was not identical, as proven by the statistically significant interaction between genotypes and experimental sets (Table 1). Whereas in ES 2 the hybrid showed lesser volume density of intergranal thylakoids compared to its parent (Fig. 2*B*), the differences between both genotypes in ES 1 were not statistically significant (Fig. 1*B*). Certain variation was found also for granal thylakoids and plastoglobuli (especially in basal and middle parts of leaf blade), as well as for the content of photosynthetic pigments, ratio Chl/Car, and DMA. The most notable differences between both sets of experiments in parent and hybrid behaviour were recorded for the volume density of peripheral reticulum(Figs. 1*D*, 2*D*) and PS1 activity (Figs. 3*B*, 4*B*).

With the exception of HRA and PS1 activity expressed per CCU, Chl/Car and Chl *a/b* ratios, DMA and some ultrastructural characteristics of MC chloroplasts (volume density of starch inclusions, stroma or granal thylakoids), the interaction between experimental sets and parts of leaf blade was also statistically significant (Table 1). This indicated that the differences in structural and functional characteristics of MC chloroplasts between the examined three parts of leaf blade, found in ES 1, were unlike those observed in ES 2.

Table 1. The differences between experimental sets (ES), genotypes (G), and various parts of leaf blade (LP) in selected structural and functional photosynthetic characteristics of maize leaves. Both experimental sets were analysed together. The statistical significances for individual components of variation are shown.

Characteristic	ES	G	LP	ES×G	ES×LP	G×LP	ES×G×LP
	Photochemical activities of mesophyll chloroplasts						
HRA (LAU)	0	0.01	0.03	0.65	0.02	0.14	0.63
HRA (DMU)	0	0	0	0.23	0	0.06	0.60
HRA (CCU)	0	0.07	0.34	0.17	0.22	0.07	0.57
PS1 (LAU)	0	0.27	0.02	0.02	0.01	0.71	0.54
PS1 (DMU)	0	0.29	0	0.77	0	0.83	0.54
PS1 (CCU)	0	0	0.29	0	0.52	0.60	0.70
	Contents of photosynthetic pigments						
Chl (*a*+*b*) (LAU)	0	0	0.01	0.03	0	0.48	0.55
Chl (*a*+*b*) (DMU)	0	0	0	0.20	0	0	0.11
Chl *a* (LAU)	0	0	0.02	0.04	0	0.56	0.61
Chl *a* (DMU)	0	0	0	0.62	0	0.23	0.60
Chl *b* (LAU)	0	0	0	0.01	0	0.20	0.31
Chl *b* (DMU)	0	0	0	0.12	0	0	0.06
Car (LAU)	0.11	0.96	0	0.35	0	0.33	0.84
Car (DMU)	0.17	0	0	0.04	0.02	0.65	0.96
Chl *a/b*	0.01	0.45	0.56	0.58	0.73	0.82	0.86
Chl/Car	0	0	0	0	0.09	0	0.04
	Ultrastructure of mesophyll chloroplasts						
TG	0	0.41	0	0.02	0.07	0.14	0.16
TI	0.01	0.01	0.21	0	0	0.55	0.49
TT	0	0.04	0	0.88	0.03	0.37	0.38
P	0	0	0	0.03	0	0	0.01
SI	0.27	0	0.31	0.23	0.11	0.52	0.88
PR	0	0	0.85	0	0	0.07	0.13
S	0	0.90	0.03	0.75	0.52	0.26	0.21
L/W	0	0	0	0.50	0.04	0.06	0.50
	Dry matter expressed per leaf area unit						
DMA	0.63	0	0	0.01	0.47	0.19	0.51

Differences between genotypes: Plants of the 2023× CE810 genotype were slightly higher and had longer leaves compared to 2023 in both experimental sets. Structural and functional characteristics of MC chloroplasts, as well as the contents of photosynthetic pigments and DMA usually differed between those genotypes. In some cases (especially in ES 1), this difference depended also on the examined part of leaf blade, as proven by the statistically significant interaction between genotypes and leaf parts (Tables 1 and 2).

In ES 1, the parent and its hybrid did not show statistically significant differences either in the amount of granal, intergranal, or total thylakoids, or in the volume density of chloroplast stroma. They differed, however, in some minor chloroplast compartments. The hybrid was characterised by greater volume density of plastoglobuli and starch inclusions per MC chloroplast, and lower amount of peripheral reticulum (with the exception of the middle part of leaf blade) compared to the parental genotype (Figs. 1D,E). The shape of MC chloroplasts in 2023×CE810 was also more flat, especially in basal or middle part of leaf blade (Fig. 1F). In ES 2, on the other hand, the shape of MC chloroplasts in both parent and hybrid was similar, rather flat (Fig. 2F). The differences between genotypes in the volume density of plastoglobuli and starch inclusions were similar to those found in ES 1 (Fig. 2E). As for peripheral reticulum, the hybrid displayed noticeably greater amount of this chloroplast component compared to 2023 in this set of experiments (Fig. 2D). Inversely, the parental line showed significantly more intergranal thylakoids compared to its hybrid (Fig. 2B).

Comparison of parent and hybrid genotype with respect to the photochemical activity of isolated MC chlo-

roplasts showed that 2023 displayed higher HRA values compared to 2023×CE810 in all three parts of leaf blade examined (Figs. 3*A*, 4*A*). This difference was especially pronounced in ES 1. The same phenomenon was recorded for PS1 activity in this experimental set, while in ES 2 the relationship between both genotypes was reverse (Figs. 3*B*, 4*B*).

Higher content of Chls *a* and *b* and higher Chl/Car ratio in 2023 compared to 2023×CE810 was found in both sets of experiments (Figs. 3*D,E*, 4*D,E*). However, only in ES 1 the differences were statistically significant for both types of Chl content expression (LAU or DMU); they were also more pronounced than in ES 2. No significant differences between genotypes were found for total Car content (with the exception of Car per DMU in ES 2) or for the Chl *a/b* ratio (Figs. 3*F*, 4*F*). As for DMA, greater values in the hybrid genotype compared to the parental line were observed only in ES 2 (Fig. 4*C*).

Table 2. The differences between genotypes (G) and various parts of leaf blade (LP) in selected structural and functional photosynthetic characteristics of maize leaves. Each experimental set was analysed separately. The statistical significances for individual components of variation are shown.

Characteristic	First experimental set			Second experimental set		
	G	LP	G×LP	G	LP	G×LP
	Photochemical activities of mesophyll chloroplasts					
HRA (LAU)	0	0.66	0.31	0.22	0.04	0.32
HRA (DMU)	0.01	0.47	0.26	0.01	0.01	0.20
HRA (CCU)	0.02	0.44	0.37	0.78	0.27	0.18
PS1 (LAU)	0	0.14	0.29	0.11	0.04	0.64
PS1 (DMU)	0	0.03	0.08	0.70	0.01	0.69
PS1 (CCU)	0.77	0.03	0.90	0.01	0.49	0.65
	Contents of photosynthetic pigments					
Chl (*a*+*b*) (LAU)	0	0	0.04	0.07	0.01	1.00
Chl (*a*+*b*) (DMU)	0	0	0.04	0	0	0
Chl *a* (LAU)	0	0.01	0.06	0.09	0.02	1.00
Chl *a* (DMU)	0	0	0.04	0	0	0.83
Chl *b* (LAU)	0	0	0.01	0.03	0	0.98
Chl *b* (DMU)	0	0	0.04	0	0	0
Car (LAU)	0.31	0.06	0.57	0.63	0	0.53
Car (DMU)	0.12	0	0.71	0.01	0	0.80
Chl *a/b*	0.41	0.50	0.74	0.89	0.91	0.99
Chl/Car	0	0.01	0.01	0	0.12	0.26
	Ultrastructure of mesophyll chloroplasts					
TG	0.07	0.01	0.64	0.19	0	0.01
TI	0.70	0	0.26	0	0	0.88
TT	0.35	0	0.93	0.06	0.05	0.06
P	0	0	0	0.01	0	0.44
SI	0.04	0.03	0.53	0	0.71	0.79
PR	0.04	0	0	0	0	0.98
S	1.00	0.50	0.44	0.88	0.03	0.06
L/W	0	0	0.03	0.10	0	0.34
	Dry matter per leaf area unit					
DMA	0.50	0	0.92	0	0	0.05

Differences between parts of leaf blade: In both experimental sets, the differences between individual parts of leaf blade were statistically significant for most characteristics examined (Tables 1 to 3). Certain general observations were made, concerning mainly various structural characteristics, DMA, or the contents of photosynthetic pigments.

The shape of MC chloroplasts changed from rather flat one in basal part of the leaf to more rounded one in leaf apex (Figs. 1*F*, 2*F*). The difference in this parameter between middle and apical part of leaf blade was statistically significant in ES 1 only (Table 3). In this experimental set, many MC chloroplasts in leaf apex showed various symptoms of advanced senescence, *e.g.*, thylakoid breakdown or envelope membrane rupture. This was characteristic especially for the hybrid 2023×CE810.

The volume density of plastoglobuli increased from middle to apical part of leaf (Figs. 1*E*, 2*E*). The amount of starch did not change with increasing distance from

Table 3. Differences between basal (B), middle (M), and apical (A) parts of leaf blade in selected structural and functional photosynthetic characteristics of maize genotypes 2023 (I) and 2023×CE810 (H). Each experimental set was analysed separately. The statistical significances as determined by Scheffe' test are shown.

Characteristic	First experimental set						Second experimental set					
	B–M		B–A		M–A		B–M		B–A		M–A	
	I	H	I	H	I	H	I	H	I	H	I	H
	Photochemical activities of mesophyll chloroplasts											
HRA (LAU)	0.95	0.16	0.76	0.06	0.61	0.50	0.01	0.70	0	0.73	0.07	1.00
HRA (DMU)	0.86	0.96	0.40	0.48	0.64	0.60	0.01	0.47	0	0.38	0.07	0.97
HRA (CCU)	0.93	0.12	0.73	0.15	0.55	0.95	0.09	0.99	0.03	0.97	0.29	0.93
PS1 (LAU)	0.37	0.33	0.95	0.13	0.49	0.58	0.63	0.21	0.44	0.06	0.91	0.40
PS1 (DMU)	0.45	0.21	0.09	0.56	0.26	0.58	0.29	0.12	0.17	0.04	0.82	0.28
PS1 (CCU)	0.22	0.31	0.99	0.89	0.25	0.20	1.00	0.82	0.99	0.13	0.99	0.22
	Contents of photosynthetic pigments											
Chl (*a*+*b*) (LAU)	0.70	0.47	0.91	0.01	0.49	0	0	0.38	0	0.27	0	0.92
Chl (*a*+*b*) (DMU)	0.01	0.07	0.02	0.93	0.50	0.09	0.04	0.10	0	0.09	0.01	0.02
Chl *a* (LAU)	0.72	0.51	0.89	0.01	0.49	0	0	0.41	0	0.30	0	0.94
Chl *a* (DMU)	0.01	0.07	0.02	0.94	0.44	0.09	0	0.23	0	0.12	0.13	0.74
Chl *b* (LAU)	0.61	0.38	0.98	0.02	0.53	0.01	0	0.25	0	0.15	0.06	0.86
Chl *b* (DMU)	0.01	0.07	0.01	0.85	0.87	0.10	0.02	0.09	0	0.09	0.01	0.01
Car (LAU)	0.44	0.16	0.77	0.15	0.78	0.99	0	0.17	0	0.07	0	0.52
Car (DMU)	0	0.02	0.01	0.01	0.79	0.91	0	0.11	0	0.04	0.07	0.35
Chl *a*/*b*	0.61	0.87	0.97	0.98	0.73	0.95	0.98	1.00	0.92	0.99	0.83	0.99
Chl/Car	0.81	0.69	0.95	0	0.95	0.01	0.75	0.72	0.42	0.24	0.21	0.50
	Ultrastructure of mesophyll chloroplasts											
TG	0.99	0.72	0.06	0.60	0.08	0.20	0	0.77	0	0	0.90	0
TI	0.14	0.04	0.02	0	0.66	0.34	0.32	0.82	0	0	0.01	0.03
TT	0.52	0.65	0	0.03	0.04	0.22	0.98	0.09	0.07	0.62	0.11	0.46
P	0.33	0.12	0	0	0	0	0.10	0.57	0	0	0	0
SI	0.76	0.92	0.02	0.56	0.10	0.81	0.14	0.88	1.00	0.97	0.14	0.76
PR	0.81	0	0.03	0.27	0.12	0.01	0.04	0.16	0.03	0.10	1.00	0.97
S	0.79	0.93	0.21	0.99	0.55	0.97	0.04	0.93	0.53	0.08	0.36	0.16
L/W	0	0	0	0	0.05	0	0	0	0	0	0.57	0.54
	Dry matter per leaf area unit											
DMA	0.01	0.11	0.01	0.10	0.96	0.99	0.04	0.02	0.03	0.01	0.84	0.49

leaf base; the same applied to the volume density of chloroplast stroma except for the statistically significant difference between basal and middle part observed in 2023 in ES 2 (Table 3, Figs. 1*C*, 2*C*). The volume density of granal thylakoids slightly diminished from basal to apical part of leaf blade. The lowest amount of granal thylakoids was observed in leaf apex, while the difference between basal and middle part was usually less pronounced (Figs. 1*A*, 2*A*). However, there were some differences between experimental sets or genotypes (Table 3). As regards intergranal thylakoids, the values clearly differed between experimental sets. In ES 1, the basal part of leaf was characterised by the highest volume density, while in ES 2 it displayed the lowest volume density of these thylakoids (Figs. 1*B*, 2*B*). The MC chloroplasts in this part of leaf blade also showed the lowest (in ES 1) or the highest (in ES 2) content of peripheral reticulum (Figs. 1*D*, 2*D*), respectively.

No statistically significant differences in photochemical activities of MC chloroplasts were found in ES 1 (Table 3). However, in ES 2, the basal part of leaf blade in the parental line displayed the lowest HRA compared to the middle or apical part (Fig. 2*A*). The differences in PS1 activity were usually statistically insignificant in either experimental set (Table 3).

The contents of Chls *a* and *b* and total Cars in fully developed, non-senescing leaves (*i.e.*, those analysed in ES 2) increased from basal to apical part of leaf blade (Fig. 4*D-F*). However, the differences between leaf parts were statistically significant in the parental line only (Table 3). No differences between various parts of leaf blade in Chl or Car content per LAU were observed in senescing leaves (ES 1) of 2023 genotype, but the apex of 2023×CE810 leaves showed significantly lower contents of Chls compared to their basal or middle part (Table 3, Fig. 3*D,E*). When the content of photosynthetic pigments was expressed per DMU, the results were similar to those found in ES 2 (Table 3). The Chl/Car ratio or Chl *a*/*b* ratio did not differ between individual parts of leaf blade in either of genotypes examined (Table 3). The highest DMA was found for leaf base both in 2023 and 2023×CE810 (Figs. 3*C*, 4*C*).

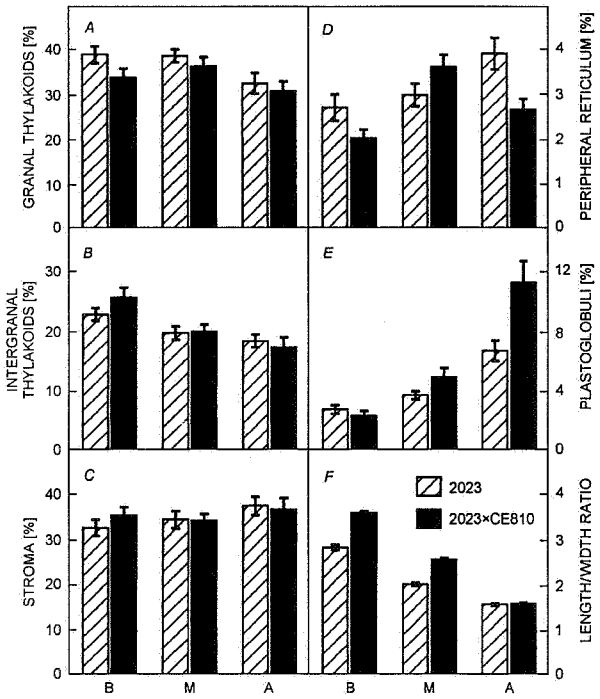

Fig. 1. Structural characteristics of mesophyll chloroplasts in basal (B), middle (M), and apical (A) part of senescing leaf blade (experimental set 1) of two maize genotypes (*hatched bars* – 2023, *solid bars* – 2023×CE810). Means ± standard error of mean (SEM).

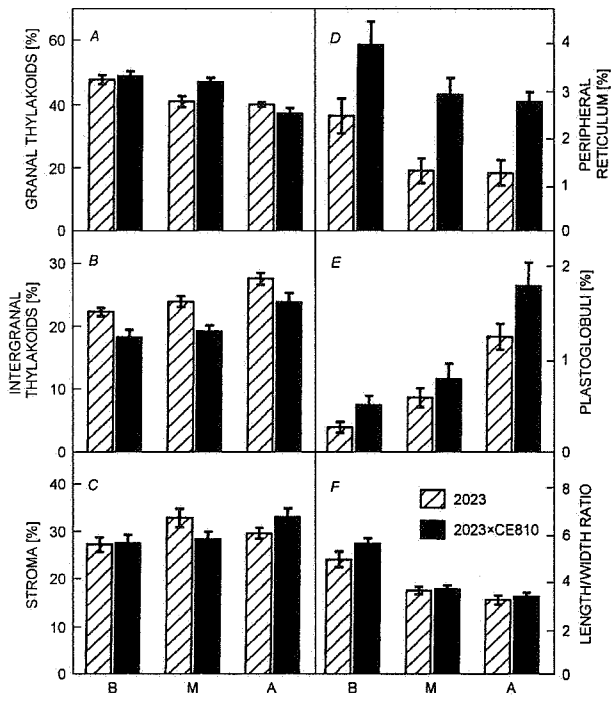

Fig. 2. Structural characteristics of mesophyll chloroplasts in basal (B), middle (M), and apical (A) part of mature leaf blade (experimental set 2) of two maize genotypes (*hatched bars* – 2023, *solid bars* – 2023×CE810). Means ± standard error of mean (SEM).

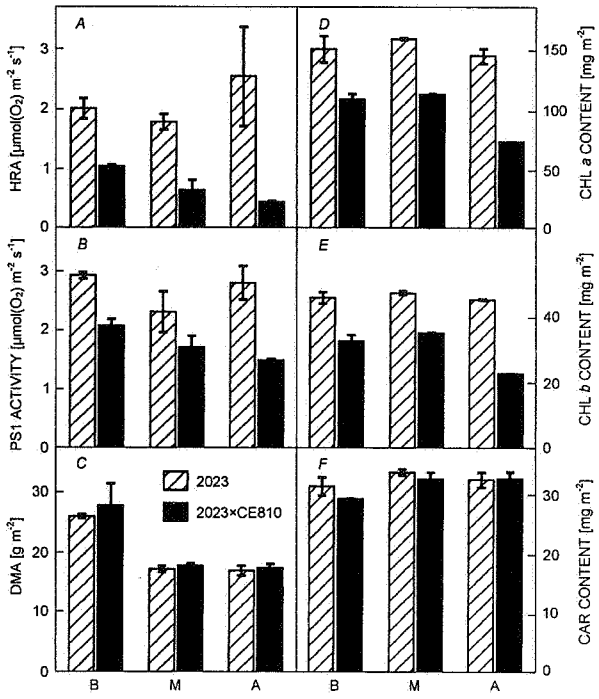

Fig. 3. Photochemical activities of mesophyll chloroplasts, contents of photosynthetic pigments, and dry matter expressed per leaf area unit (DMA) in basal (B), middle (M), and apical (A) part of senescing leaf blade (experimental set 1) of two maize genotypes (*hatched bars* – 2023, *solid bars* – 2023×CE810). Means ± standard error of mean (SEM).

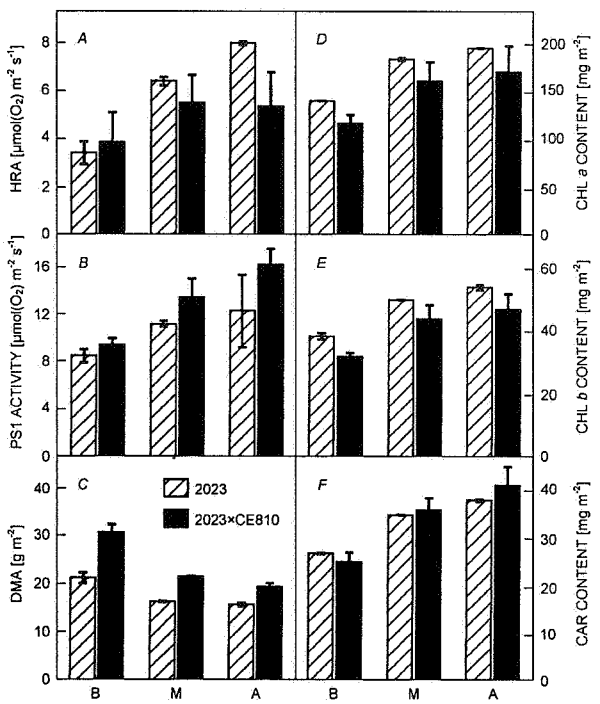

Fig. 4. Photochemical activities of mesophyll chloroplasts, contents of photosynthetic pigments, and dry matter expressed per leaf area unit (DMA) in basal (B), middle (M), and apical (A) part of mature leaf blade (experimental set 2) of two maize genotypes (*hatched bars* – 2023, *solid bars* – 2023×CE810). Means ± standard error of mean (SEM).

Discussion

Our study of MC chloroplasts in maize leaves showed that the chloroplast structural and functional characteristics in various parts of leaf blade were heterogeneous both in mature and senescing leaves. The chloroplasts from leaf apex were characterised by various parameters usually associated with advanced development, while those localised in basal part of leaf blade were clearly younger. This observation applied to both mature and senescing leaves. In both types of leaves, an increasing volume density of plastoglobuli was observed from basal to apical part of leaf blade: the changes were more abrupt in senescing leaves. This increase in plastoglobuli number or volume (or both) is a sign of advancing chloroplast senescence, as the products of breakdown of thylakoid lipids are probably accumulated in this compartment (Kutík *et al.* 1993, Ram *et al.* 1994, Kutík 1985, 1998, Hudák 1997).

Another symptom associated with development of mature chloroplasts and their gradual conversion to gerontoplasts, or senescing chloroplasts, is the change of chloroplast shape and dimensions (Somersalo and Aro 1987, Hashimoto *et al.* 1989, Chonan *et al.* 1991, Ono *et al.* 1995, Kutík 1998, Kutík *et al.* 1999). Senescing leaves of maize examined in our study contained smaller chloroplasts in mesophyll cells compared to non-senescing, mature leaves. A gradual transformation of flat chloroplasts found in leaf base to more rounded ones in middle or apical part of leaf blade was also observed. This phenomenon is characteristic for advanced developmental stage of these organelles (Kutík *et al.* 1999). In senescing leaves, MC chloroplasts of leaf apex were often deformed, with ruptured envelope and broken thylakoid membranes. These chloroplasts could be regarded as the final stage in the chloroplast development (Hudák 1997).

Certain interesting differences in the organisation of thylakoid membranes were also found in MC chloroplasts from various parts of leaf blade. Contrary to the increase of size and amount of granal thylakoids often described for young, growing leaves (Kutík 1998), we observed a slightly decreasing gradient from leaf base to apex for chloroplasts both in mature and senescing leaves. This is in good agreement with general trend depicted in many other developmental studies: once the mature, photosynthetically fully active chloroplasts with large grana are formed, the size of this chloroplast compartment slowly decreases, thylakoid membranes dilate and finally break up (Kutík *et al.* 1988). It can be therefore concluded that the chloroplasts of leaf apex analysed in our study are characterised by more advanced developmental stage compared to those from basal part of leaf blade. As for intergranal thylakoids, our observation that even in mature, fully developed and non-growing leaves their amount increases with the increasing distance from leaf base is fairly interesting. In senescing leaves, the volume density of both granal and intergranal thylakoids decreased with the increasing distance from leaf base which shows that MC chloroplasts have already undertaken further step toward their senescence.

No significant changes associated with the heterogeneity of leaf blade were found in the volume of chloroplast stroma. The same applied to the amount of starch inclusions, which was extremely small; the differences between various parts of leaf blade in the volume density of this chloroplast compartment were usually statistically insignificant. It might be interesting to compare the number and size of starch inclusions along the whole leaf blade in MC and BSC chloroplasts: the ultrastructure of BSC chloroplasts from our experiments is presently evaluated and preliminary results of this analysis have already been published (Vičánková *et al.* 2000). We found interesting differences for the peripheral reticulum: its amount in mature leaves decreased with the increasing distance from leaf base, but in senescing leaves a reverse trend was observed. The role of peripheral reticulum in chloroplasts is still far from being fully solved; it is presumed that this compartment (often found in C4 plants) is involved in various metabolic and transport processes not directly associated with photosynthesis (*e.g.*, Hudák 1997). We can therefore only speculate that the intensity of metabolite efflux from chloroplasts in leaf apex of senescing leaves increased before their final degradation.

The heterogeneity in MC chloroplast ultrastructural parameters observed in various parts of leaf blade was in some cases also accompanied by differences in other photosynthetic characteristics. The content of Chls in mature leaves increased from leaf base to apex, while in senescing leaves this trend was reverse, which is in accord with the findings of other authors (Davies *et al.* 1989, 1990). The content of total Cars increased from basal to middle part of mature, non-senescing leaf blade; no further changes were detected either from middle to apical part of mature leaf blade or in senescing leaves, similarly to Wellburn *et al.* (1982). Lower Chl *a/b* ratio in senescing leaves compared to mature ones could be attributed to more abrupt decrease in the amount of Chl *a*, but no differences in this parameter along the length of leaf blade were detected. The slower decrease in the content of Chl *b* is rather interesting because this pigment is associated with light-harvesting complexes, important in thylakoid stacking (Jackowski and Kluck 1993). On the other hand, Chl *a* is bound in large quantities to PS1 complexes, the number of which strongly decreases during leaf senescence (Lebedev *et al.* 1986), while PS2 or light-harvesting complexes account for much lesser amount of Chl *a*. These results are thus in good agreement with our findings that the reduction in PS1 activity observed in senescing leaves compared to mature ones was more abrupt than the reduction in HRA (which is a measure of PS2 activity). As for various parts of leaf blade, the decrease of photochemical activities of MC

chloroplasts observed from basal to apical part of senescing leaf blade was not statistically significant, due probably to rather large residual variation in those characteristics. On the other hand, an increase both in HRA and (though insignificant) in PS1 activity was observed with an increasing distance from leaf base in mature leaves, similarly to the findings of Webber *et al.* (1986) or Bredenkamp and Baker (1988) in *Triticum*.

The differences in structural and functional photosynthetic characteristics observed between both examined genotypes, as well as the presence of statistically significant interaction between genotypes and leaf parts or genotypes and experimental series can be attributed to the faster development of the hybrid compared to its parental inbred line. The lower content of Chls, lower photochemical activity of isolated MC chloroplasts, greater volume density of plastoglobuli and starch inclusions, more developed peripheral reticulum in mature leaves—all these parameters can be associated with more advanced developmental stage of the 2023×CE810 genotype. The apparent discrepancy in observation that MC chloroplasts of both genotypes contain similar amount of thylakoid membranes while the activities of PS1 and PS2 in senescing leaves are lower in the hybrid than in the parent, can be explained by different methods of evaluation of these parameters. While for the assessment of volume density of individual chloroplast compartments, only undamaged chloroplasts were used, the chloroplasts for measurements of photochemical activity were isolated from much larger part of leaf blade which could contain both functional and partly impaired organelles. This could have great influence especially on HRA or PS1 activity expressed per LAU or DMU.

Our study shows that the developmental gradient of chloroplasts along the whole leaf blade, observed previously for various plant species, is conserved also in mature, non-growing leaves of maize as well as in the senescing ones. Our observation of the ultrastructural changes of mesophyll chloroplasts found from basal to apical part of mature and senescing maize leaves prove for the first time the existence of such gradient in this species. The differences in chloroplast ultrastructure in various parts of leaf blade are accompanied by the changes of photochemical activity and contents of photosynthetic pigments as well, and depend strongly both on the developmental stage of leaf and on the genotype examined.

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