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Ultrastructure and dimensions of chloroplasts in leaves of three maize (*Zea mays* L.) inbred lines and their F_1 hybrids grown under moderate chilling stress

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Abstract

Influence of moderate chilling stress on vascular bundle sheath cell (BSC) and especially mesophyll cell (MC) chloroplasts of mature maize leaves was studied by electron microscopy and stereology. Plants of two inbred lines of maize, differing in their photosynthetic activity, and their F_1 hybrids were cultivated during autumn in heated or unheated glasshouse. Generally, chilling temperatures resulted mainly in the decrease in stereological volume density (VD) of both granal and intergranal thylakoids of MC chloroplasts, while the ratio of granal to all thylakoids (granality) was less affected. The VD of peripheral reticulum and plastoglobuli usually increased after cold treatment of plants. The volume of MC chloroplasts usually increased under chilling stress, the shape of the chloroplasts changed only slightly. The ultrastructure of chloroplasts differed between individual genotypes; chilling-stressed hybrid plants showed positive heterosis particularly in the granal thylakoids' VD of MC chloroplasts.

Additional key words: bundle sheath cells; electron microscopy; genotypes; heterosis; intra-specific variability; mesophyll cells; peripheral reticulum; plastoglobuli; stereology; thylakoids.

Introduction

Chloroplasts are of prime importance in the study of plant response to various stress factors including low temperature. The changes in their ultrastructure are usually the earliest visible symptoms of stress-induced injury in plant cells. These changes often include chloroplast swelling, depletion of starch, formation of peripheral reticulum, thylakoid dilatation, and reduction of thylakoid granal stacks (their three-dimensional structure is still a matter of debate, see Arvidsson and Sundby 1999, Mustárdy and Garab 2003), and increase in the number and size of plastoglobuli (*e.g.* Mostowska 1997, Biswal and Biswal 1999, Čiamporová and Trgiňová 1999, Hudák and Salaj 1999, Kratsch and Wise 2000).

Maize (*Zea mays* L.) is an ideal model plant for the study of chloroplast ultrastructure. It possesses "dimorphic" chloroplasts (in MCs and BSCs) typical for plants with C_4 type of photosynthesis that differ not only in the type of photosynthetic reactions occurring there but also in their size, ultrastructure, and contents of various photosynthetic proteins (*e.g.* Hudák 1997). As a plant with 18–20 distinctive leaf insertion levels and well characterised developmental stages (Ritchie *et al.* 1993) it can be with

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Abbreviations: BSC – bundle sheath cell; F_1 – the first filial generation, MC – mesophyll cell, VD – volume density.
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advantage used for the study of leaf age-specific differences in the chloroplast ultrastructure and photosynthetic processes (Čatský and Šesták 1997). Moreover, maize belongs to the grass family and as such displays a distinctive developmental heterogeneity in structural and functional characteristics of chloroplasts along whole length of leaf blade (such heterogeneity was observed not only in young leaves, but in mature and senescing leaves as well, see Kutík *et al.* 2001).

In a temperate climate, maize plants, which originate from tropics, often suffer from chilling stress. This type of stress usually occurs when the air temperature is substantially lower than the temperature optimum for given plant species but does not yet decrease below zero. For maize, the 14–15 °C is usually cited as the upper margin of chilling stress-inducing temperature (*e.g.* Haldimann 1996, Verheul *et al.* 1996, Lidon *et al.* 2001). As maize is one of the world's most important crops, the agricultural losses due to chilling stress can be very substantial. This is why various aspects of chilling stress have been studied in this plant species. A great number of these studies focused on photosynthesis and photosynthetic organelles—chloroplasts (*e.g.* Robertson *et al.* 1993, Čiamporová and Trgiňová 1996, Verheul *et al.* 1996, Pinhero *et al.* 1999, Pastori *et al.* 2000, Fracheboud *et al.* 2002). However, substantially less studies deal with the differences between maize hybrids (now almost exclusively used in the commercial cultivation of this crop species) and their parental inbred lines in the response of photosynthetic apparatus to chilling stress (Fracheboud *et al.* 1999, Du *et al.* 1999, Körnerová and Holá 1999, Holá *et al.* 2003) and to our knowledge no one has as yet tried to analyse these differences with respect to chloroplast ultrastructure.

The ultrastructure of MC and BSC chloroplasts was studied in two maize (*Zea mays* L.) hybrid combinations, 2013×CE810 and CE704×CE810. Each hybrid combination comprised of the respective parental inbred lines and their reciprocal F_1 hybrids. Parental line 2013 is characterized by good photosynthetic and yield performance in the field conditions, parental line CE810 shows also good photosynthetic performance but rather delayed reproductive stage and poor seed yield, and parental line CE704 is generally characterized by low stature and poor photosynthetic performance. All four F_1 hybrids, and especially CE704×CE810 and its reciprocal hybrid, display a positive heterotic effect in photosynthesis when grown in the field conditions (Holá *et al.* 1999).

Thus, the aim of the present work was to study the influence of chilling stress on ultrastructure of photosynthetic organelles in maize inbred lines and their F_1 hybrids. Particular attention was paid to the possibility of the occurrence of positive heterotic effect in chloroplast ultrastructural parameters that could be associated with the better ability of hybrids to withstand chilling conditions, observed on this material in some functional aspects of photosynthetic apparatus (Körnerová and Holá 1999, Holá *et al.* 2003).

Materials and methods

The seeds of all genotypes were obtained from Maize Breeding Station CEZEA in Čejč (Czech Republic). Plants were cultivated in planting dishes with soil, placed at first in heated glasshouse (24–27/16–20 °C day/night) till the appearance of the first leaf (*i.e.* 9–10 d from the date of sowing). After that, seedlings were divided into two groups; one remained in heated glasshouse (control), the other group was transferred to another, unheated glasshouse (chilling stress). Their cultivation then proceeded for four weeks in October, during which period the temperature in unheated glasshouse gradually decreased from approx. 25 to 18 °C during day and from 13 to 8 °C during night (*i.e.* conditions of moderate chilling stress). Plants were well watered with tap water, the relative humidity in both glasshouses was kept between 70–100 %, and no additional irradiance was applied.

The plants developing under chilling conditions were usually smaller and had fewer and shorter leaves compared with the control ones (Holá *et al.* 2003). For this reason, the fourth leaves (counting from the bottom, numbered from coleoptile as leaf zero) were usually sampled from the control plants, whereas the samples representing the stressed plants were taken from their third leaves. In all cases, these leaves were mature (fully developed, not growing further). According to Bongard-Pierce *et al.* (1996), maize leaves from the first to the fourth all have juvenile features (wax on the surface, no trichomes) and they all are formed in embryo (Vega *et al*. 2002). Four plants (*i.e.* four leaves) were always examined for each variant.

Small pieces (approx. 4 mm^2) were cut from the middle third of the leaf blade, between the edge and middle vein of the leaf. Samples for transmission electron microscopy were prepared according to the standard procedure (Kutík *et al.* 1999). They were double-fixed with glutaraldehyde followed by osmic acid, dehydrated through an ethanol series, and embedded into Spurr's low viscosity resin. For an orientation in the objects and their light-microscopic examination, semi-thin sections of embedded objects were stained by toluidine blue solution. The number of MCs forming "outer ring" of vascular bundle and a number of chloroplast cross sections in these cells were evaluated using a light microscope (*Olympus BX 40*, *Olympus*, Japan, at a magnification of 600×, the objective lens 60×).

Chloroplast ultrastructure was evaluated on transverse ultrathin sections of the objects contrasted with uranyl acetate solution followed by lead citrate treatment. The transmission electron microscopes *Philips EM 300* and later *Philips EM 268 Morgagni* (*Philips*, The

Netherlands) were used at primary magnifications of about 7000×. On electron microphotographs, the length and width of nearly median cross sections of MC chloroplasts were determined at final magnifications of about 30 000×. The approximate chloroplast volume was evaluated from these parameters as the volume of circular ellipsoid. On the same micrographs, the volume densities (relative partial volumes) of main chloroplast compartments, *i.e.* granal and intergranal thylakoids, peripheral reticulum, starch inclusions, plastoglobuli, and stroma (including also the small periplastidial space between the outer and the inner chloroplast envelope membrane) were evaluated using stereological grids with regularly distributed points. For each sample, five chloroplast sections were evaluated, making a total of 20 MC chloroplasts analysed for each experimental variant.

Statistical significance of differences in MC chloroplast parameters between plants cultivated under stress or control conditions, as well as between individual genotypes was tested by Scheffé's test using the 5 % level of statistical significance as the critical one.

Results

Anatomical structure typical for maize leaves ("Kranz anatomy" of C_4 plants, *i.e.* vascular bundles surrounded by parenchymatous bundle sheaths and then by MCs) was apparent on semithin leaf cross sections (*ca*. 1 μm thick), both in control and chilling-stressed plants. The number of MCs forming "outer ring" of leaf vascular bundle was usually 13–14; it did not much differ between the control plants and the plants grown in chilling conditions. No apparent differences were found in the number of chloroplasts (or, more precisely, chloroplast cross sections) per MC cross section, either; on average, five chloroplast cross sections were observable in one MC on semi-thin leaf cross sections.

Chloroplasts were present in both MCs and BSCs in all plants examined. In BSC chloroplasts, prevailing absence of larger starch inclusions was conspicuous both in stressed and control plants. The ultrastructure of these chloroplasts was characterised by many non-appressed thylakoids with more or less developed small rudimentary grana, more or less pronounced peripheral reticulum and some plastoglobuli (Fig. 1). Small starch inclusions were present mostly in leaves of some control plants (see Table 1). In MC chloroplasts, the ultrastructural differences between stressed and control plants were more pronounced, particularly concerning the quantity and differentiation pattern of thylakoids, eventually the extent of peripheral reticulum (see Fig. 2).

The scarcity of starch inclusions and apparent similarity of the BSC chloroplasts of all studied plants were the reasons why we decided to stereologically analyse only the ultrastructure of MC chloroplasts. The results of this stereological evaluation of the MC chloroplasts dimensions and ultrastructure are presented in Figs. 3–5. Chilling stress resulted in a considerable decrease of both granal (appressed) and intergranal (non-appressed) thylakoid VD. However, granality, *i.e.* the ratio of granal to all thylakoid VD, was not much affected by chilling. On the other hand, the VD of peripheral reticulum and that of plastoglobuli usually considerably increased due to chilling stress (with the exception of CE810×CE704 hybrid). The changes in the size of MC chloroplasts were also observed after cold treatment of plants; all genotypes examined (with the exception of CE810) displayed a significant increase in MC chloroplast size compared to the control plants. MC chloroplast shape, described by length/width ratio of chloroplast cross sections, also changed from rather flat in the control plants to more round in the plants subjected to chilling stress, but these differences were usually statistically non-significant.

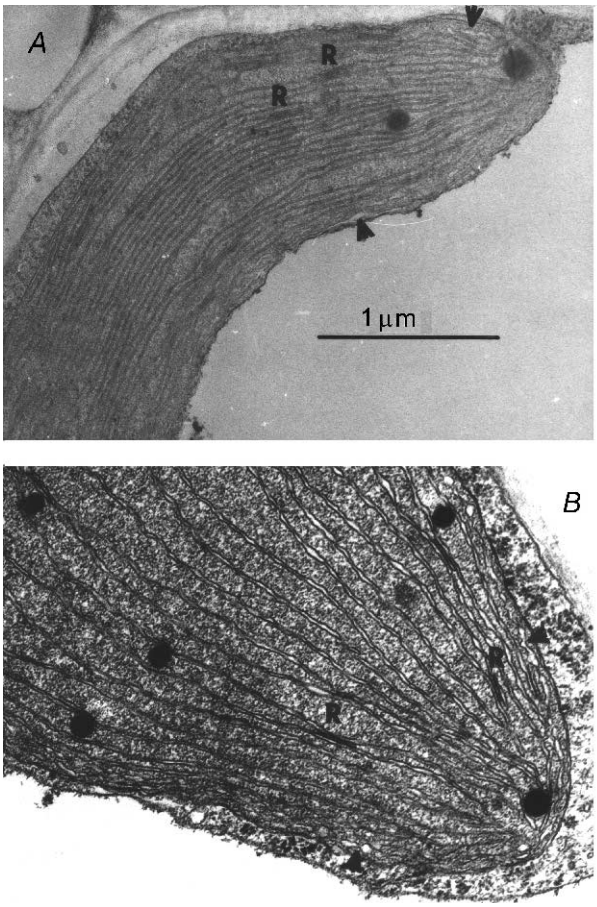

Fig. 1. (*A*) BSC chloroplast of CE704 control plant without starch inclusions, with many non-appressed thylakoids, rudimentary grana (R), peripheral reticulum (*arrowheads*) and two plastoglobuli. (*B*) BSC chloroplast of CE704 chilling-stressed plant with slightly dilated non-appressed thylakoids, small rudimentary grana (R), peripheral reticulum (*arrowheads*), and several plastoglobuli. *Bar* = 1 μm (for both parts).

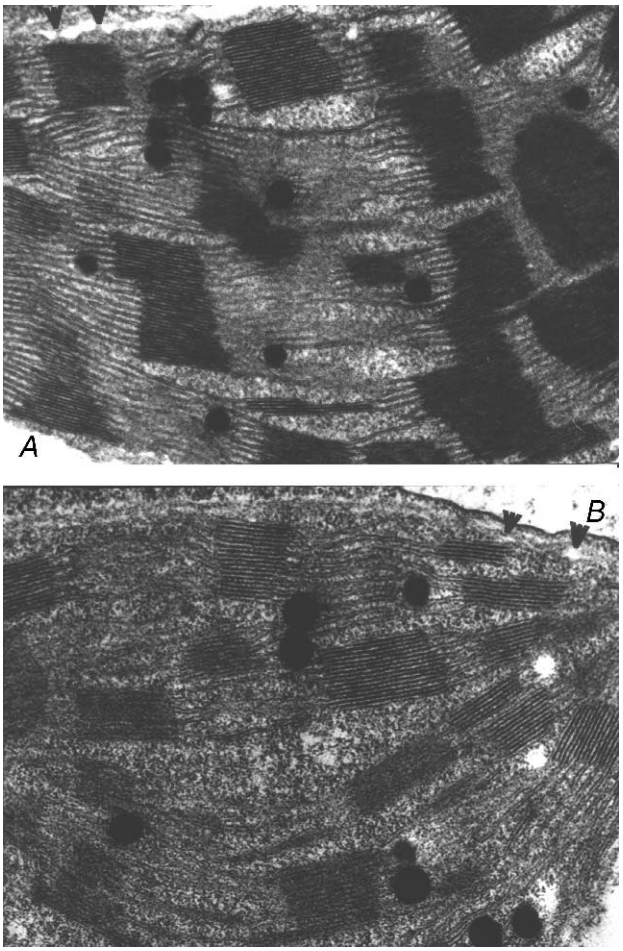

Fig. 2. (*A*) MC chloroplast of CE704 control plant with well developed system of grana and intergranal thylakoids. Several plastoglobuli are seen, as well as peripheral reticulum (*arrowheads*). (*B*) MC chloroplast of CE704 chilling-stressed plant with less-developed system of thylakoids compared to the control plant, several plastoglobuli and peripheral reticulum (*arrowheads*). The magnification is the same as in Fig. 1.

Statistical significance of the differences found between MC chloroplast characteristics of the chilling-stressed and control plants of both hybrid combinations is shown in Table 2. The differences between VD of granal thylakoids in chilling-stressed plants compared to the control ones were highly significant in all genotypes of the hybrid combination 2013×CE810 and in both parental lines of the hybrid combination CE704×CE810. Concerning intergranal thylakoids, the values of statistical significance for these differences were usually higher (*i.e.* lower significance) compared to those for granal thylakoids; significant differences were found for all three inbred lines and CE704×CE810 hybrid but not for other hybrids examined. The differences in the VD of all thylakoids (granal plus intergranal ones) usually followed the pattern observed for granal thylakoids, whereas the differences in granality were mostly non-significant. The increase in the VD of peripheral reticulum observed in the cold-stressed plants was statistically significant only in the hybrid combination 2013×CE810 (with the exception of F_1 hybrid 2013×CE810), whereas in the CE810×CE704 hybrid the values of this parameter significantly decreased. As for plastoglobuli VD, the differences between cold-stressed and control plants were highly significant in all genotypes of the hybrid combination 2013×CE810, but non-significant in the second hybrid combination (with the exception of its parental line CE810). The differences in the stroma VD were usually significant in all genotypes examined (more "free" stroma in chilling-stressed plants).

Statistical analysis of the differences between individual genotypes of plants stressed or non-stressed by chilling temperatures is shown in Tables 3 and 4. Generally, these differences were usually more pronounced in the stressed plants compared to those grown in optimum temperature, and in the hybrid combination CE704×CE810 compared to the other hybrid combination analysed. MC chloroplasts of F_1 hybrid CE810×CE704 grown in optimum temperature (Table 3) showed significantly lower VD of all thylakoids compared to CE704 inbred line (as well as to the mean value of both parental lines),

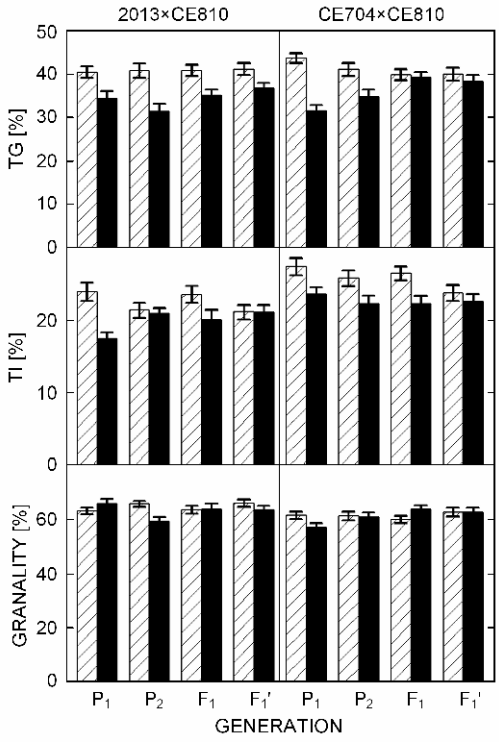

Fig. 3. The volume densities of granal (TG) and intergranal (TI) thylakoids and the granality (*i.e.* the ratio of granal to all thylakoids) in mesophyll cell chloroplasts of two maize hybrid combinations, 2013×CE810 and CE704×CE810, grown in either optimum temperature (*hatched bars*) or chilling (*solid bars*) conditions. P_1 = 2013 or CE704 inbred line, P_2 = CE810 inbred line, F_1 = 2013×CE810 or CE704×CE810 F_1 hybrid, F_1' = CE810×2013 or CE810×CE704 F_1 hybrid, respectively. Means ± SEM.

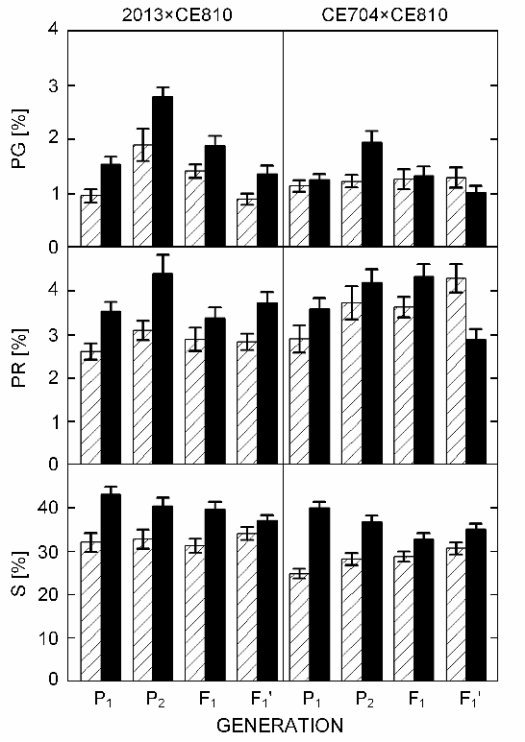

Fig. 4. The volume densities of plastoglobuli (PG), peripheral reticulum (PR), and stroma (S) in mesophyll cell chloroplasts of two maize hybrid combinations, 2013×CE810 and CE704×CE810, grown in either optimum temperature (*hatched bars*) or chilling (*solid bars*) conditions. P_1 = 2013 or CE704 inbred line, P_2 = CE810 inbred line, F_1 = 2013×CE810 or CE704×CE810 F_1 hybrid, F_1' = CE810×2013 or CE810×CE704 F_1 hybrid, respectively. Means ± SEM.

and higher VD of peripheral reticulum and stroma compared to CE704. They were probably smaller compared to CE810 inbred line, as inferred from the results of statistical analysis of MC chloroplast volume. Significant difference in MC chloroplast volume was found also between CE810 and CE704 inbred lines.

In the cold-stressed plants of the CE704×CE810 hybrid combination, the situation was somewhat different (Table 3). MC chloroplasts in leaves of the chilling-stressed plants of both F_1 hybrids were characterised by significantly higher VD of granal and all thylakoids compared to both CE704 inbred line and the parental mean (the only exception was the difference in the all thylakoids VD between CE810×CE704 hybrid and the parental mean, that was statistically non-significant). F_1 hybrid CE704×CE810 stressed by chilling displayed also significantly higher granality and lower VD of stroma in MC chloroplasts than CE704 inbred line. Its reciprocal hybrid showed under these conditions significantly lower VD of plastoglobuli and peripheral reticulum compared to both CE810 inbred line and the parental mean. The difference in the VD of peripheral reticulum between both F_1 hybrids of this hybrid combination stressed by chilling was also statistically significant.

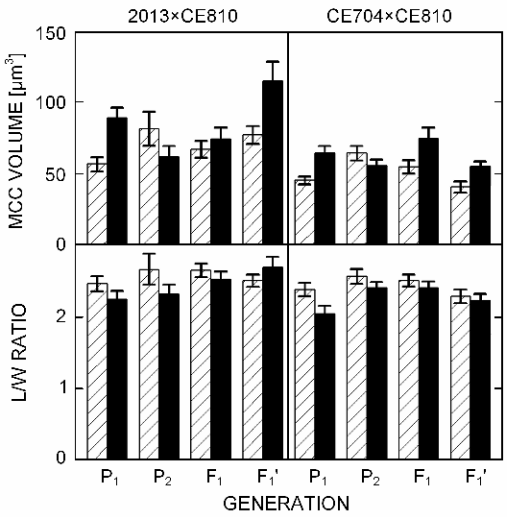

Fig. 5. The approximate volume of mesophyll cell chloroplasts (MCC) and their length to width (L/W) ratio determined on their nearly median cross sections in two maize hybrid combinations, 2013×CE810 and CE704×CE810, grown in either optimum temperature (*hatched bars*) or chilling (*solid bars*) conditions. P_1 = 2013 or CE704 inbred line, P_2 = CE810 inbred line, F_1 = 2013×CE810 or CE704×CE810 F_1 hybrid, F_1' = CE810×2013 or CE810×CE704 F_1 hybrid, respectively. Means ± SEM.

No significant differences in VD of either granal, intergranal, or all thylakoids, or in granality and peripheral reticulum VD were found in plants of the hybrid combination 2013×CE810; this applied both to control and chilling-stressed plants (Table 4). The only differences between genotypes of this hybrid combination, that were statistically significant in the control plants, were those in plastoglobuli VD, observed between inbred lines CE810 and 2013 or CE810×2013 hybrid. They were retained even under chilling conditions, when CE810 line showed significantly higher value of this parameter compared to all three remaining genotypes of the 2013×CE810 hybrid combination. F_1 hybrid CE810×2013 stressed by chilling was also characterised by significantly lower stroma VD compared to both CE810 inbred line and the parental mean, and greater volume of MC chloroplasts compared to CE810, parental mean and 2013×CE810 hybrid.

Discussion

A study of chloroplast ultrastructure in higher plants is based on the evaluation of randomly chosen individual chloroplasts in more-or-less randomly selected photosynthesizing cells of individual plants. This approach is,

naturally, different from the measurement of mean specimens from the leaf material, usually used in biochemical and biophysical studies of photosynthesis. It means that chloroplasts subjected to stereological evaluation have to be selected very carefully, as the structure (and function) of these organelles can be strongly affected by factors such as plant and leaf age, leaf insertion level, or cell position in individual cell layers or on the leaf blade (see Kutík 1998, Kutík *et al.* 1999, 2001). In our study of chloroplast ultrastructure in maize leaves under moderate chilling, we tried to minimize this chloroplast heterogeneity as best as we could. Thus, the leaf insertion on plant, ontogenic state of the leaf, and the part of the leaf blade used were, as far as possible, the same throughout all experiments. Diurnal variability of chloroplast ultrastructure (see Vičánková *et al.* 2002) was also respected: the specimens were taken always at the same time, *i.e.* in the morning, 2–3 h after sunrise. Moreover, chloroplast cross sections close to the median and with well-observable thylakoids, *i.e.* those sectioned roughly perpendicularly to the prevailing plane of thylakoid membranes, were always evaluated. Our analysis should therefore give sufficiently representative results concerning the quantitative changes in chloroplast ultrastructure that are due to chilling stress.

Firstly, let us briefly consider BSC chloroplast ultrastructure. The scarcity or absence of starch inclusions in BSC chloroplasts, observed both in control and chilling-stressed plants, was probably caused by relative shortage of sun radiation during plant cultivation in autumn. A slight tendency for the presence of more starch under control conditions was apparent. However, differences between individual BSC chloroplasts and individual plants in this parameter were frequently conspicuous. The BSC chloroplasts under chilling conditions had usually lesser amount of unstacked thylakoids than those of control plants and they were frequently somewhat dilated, which is common phenomenon under stress (Kratsch and Wise 2000).

Table 1. Semi-quantitative description of the occurrence of starch inclusions (SI) in bundle sheath cell chloroplasts in leaves of two maize hybrid combinations, 2013×CE810 and CE704×CE810, grown in either optimum (control) or chilling conditions. F_1 = 2013×CE810 or CE704×CE810 F_1 hybrid, F_1' = CE810×2013 or CE810×CE704 F_1 hybrid. + frequently occurring SI, ± rarely occurring SI, - no SI.

	2013×CE810				CE704×CE810			
	2013	CE810	F_1	F_1'	CE704	CE810	F_1	F_1'
Control	±	-	-	-	±	±	-	±
Chilling-stressed	-	-	-	-	-	+	-	-

Table 2. The differences between plants grown in optimum and chilling temperature in ultrastructural parameters (volume densities of main chloroplast compartments) and dimensions of mesophyll cell chloroplasts in leaves of two maize hybrid combinations, 2013×CE810 and CE704×CE810. F_1 = 2013×CE810 or CE704×CE810 F_1 hybrid, F_1' = CE810×2013 or CE810×CE704 F_1 hybrid. The statistical significances (*p*) as determined by Scheffé's test are shown.

	2013×CE810				CE704×CE810			
	2013	CE810	F_1	F_1'	CE704	CE810	F_1	F_1'
Granal thylakoids	0.008	0	0.004	0.022	0	0.007	0.721	0.427
Intergranal thylakoids	0	0.754	0.057	0.986	0.018	0.030	0.006	0.436
All thylakoids	0	0.002	0.001	0.031	0	0	0.015	0.159
Granality	0.201	0.002	0.920	0.234	0.040	0.838	0.066	0.988
Peripheral reticulum	0.003	0.011	0.179	0.006	0.099	0.348	0.064	0.001
Plastoglobuli	0.003	0.014	0.037	0.016	0.453	0.005	0.797	0.231
Stroma	0	0.012	0.001	0.453	0	0	0.034	0.020
Chloroplast volume	0	0.165	0.498	0.015	0.002	0.188	0.032	0.008
Length to width ratio	0.177	0.176	0.397	0.257	0.026	0.233	0.405	0.666

For more detailed analysis of chloroplast ultrastructure and dimensions, we have concentrated on MC chloroplasts. The choice of MC chloroplasts for very laborious and time-consuming stereological evaluation was substantiated, along with our own experience, also by their greater susceptibility to chilling compared to BSC chloroplasts as reported by Slack *et al*. (1974). As far as we know, quantitative data comparable with those obtained by us are not currently available. Generally, our results are in good agreement with those presented in older papers dealing with the influence of chilling stress on chloroplast ultrastructure (as reviewed by Hudák and Salaj 1999 or Kratsch and Wise 2000). We recorded, to a various extent, particularly the thylakoid swelling and distortion or the lesser development of thylakoid membranes (flexibility of granal structure must play a role here, see Arvidsson and Sundby 1999, Mustárdy and

Garab 2003), the disappearing or absence of starch inclusions, and the increase in the size of chloroplast peripheral reticulum and in the number and size of plastoglobuli. The increase in peripheral reticulum probably increases the surface area of the transport-limiting chloroplast inner membrane in response to the reduction of metabolite transport caused by chilling stress. The increase in the volume density of plastoglobuli is another indicator of stress or senescence (Hudák and Salaj 1999, Kratsch and Wise 2000). As concerns chloroplast stroma, we found a significant increase of its VD in all genotypes of both hybrid combinations examined. This was partly caused by the decrease in thylakoid VD, but the increase in the whole chloroplast volume was also observed. However, this volume was calculated only approximately, taking chloroplasts as symmetric circular ellipsoids, which is not quite true, as seen from electron micrographs. The shape of the chloroplasts generally changed under chilling stress slightly towards more rounded one, which agrees well with the chloroplast swelling observed by other authors (Kratsch and Wise 2000). All these changes are typical for chloroplasts of plants subjected to low temperature for longer periods (Rogers *et al.* 1977, Hudák and Salaj 1999, Sopher *et al.* 1999), which was also our case.

The electron micrographs of dimorphic chloroplasts of maize grown at 25 or 14 °C, published by Robertson *et al*. (1993), resemble our micrographs from control or cold-stressed maize plants, showing also mostly quantitative, not qualitative differences in chloroplast ultrastructure between both temperature variants. According to these authors, as well as to Nie and Baker (1991) or Nie *et al.* (1995), the content of majority of thylakoid proteins as well as the content of chlorophylls are significantly reduced under chilling conditions. This reduction can reach such a level that in a certain fraction of leaf cells, the dimorphic chloroplasts completely lack several thylakoid proteins. Such cells are not able to synthesize these proteins even after transfer of plants to optimum temperature, which can negatively affect chloroplast functions and plant photosynthetic performance (Nie *et al.* 1995).

Table 3. The differences between individual genotypes in ultrastructural parameters (volume densities of main chloroplast compartments) and dimensions of mesophyll cell chloroplasts in leaves of maize hybrid combination CE704×CE810 grown in either optimum (control) or chilling conditions. P_1 = CE704 inbred line, P_2 = CE810 inbred line, F_1 = F_1 hybrid CE704×CE810, F_1' = F_1 hybrid CE810×CE704, ØP = parental mean. The statistical significances (p) as determined by Scheffé's test are shown.

		P_1-P_2	F_1-F_1'	F_1-P_1	F_1'-P_1	F_1-P_2	F_1'-P_2	F_1-ØP	F_1'-ØP
Control	Granal thylakoids	0.609	1.000	0.278	0.309	0.941	0.958	0.522	0.569
	Intergranal thylakoids	0.769	0.380	0.944	0.138	0.977	0.630	1.000	0.215
	All thylakoids	0.183	0.608	0.109	0.003	0.995	0.453	0.468	0.023
	Granality	1.000	0.664	0.920	0.958	0.951	0.929	0.907	0.918
	Peripheral reticulum	0.338	0.541	0.453	0.028	0.997	0.668	0.882	0.107
	Plastoglobuli	0.982	0.999	0.953	0.916	0.999	0.993	0.980	0.951
	Stroma	0.360	0.793	0.225	0.026	0.993	0.627	0.586	0.095
	Chloroplast volume	0.020	0.136	0.455	0.900	0.461	0.002	1.000	0.064
	Length to width ratio	0.614	0.444	0.834	0.915	0.981	0.244	0.993	0.466
Chilling-stressed	Granal thylakoids	0.458	0.979	0.004	0.012	0.186	0.370	0.010	0.036
	Intergranal thylakoids	0.851	0.998	0.851	0.927	1.000	0.998	0.966	0.995
	All thylakoids	0.821	0.994	0.023	0.046	0.189	0.298	0.027	0.059
	Granality	0.456	0.976	0.041	0.114	0.631	0.864	0.119	0.301
	Peripheral reticulum	0.473	0.005	0.289	0.362	0.988	0.013	0.619	0.036
	Plastoglobuli	0.127	0.604	0.990	0.790	0.061	0.001	0.581	0.038
	Stroma	0.432	0.702	0.005	0.106	0.253	0.869	0.016	0.295
	Chloroplast volume	0.696	0.089	0.599	0.681	0.094	1.000	0.168	0.913
	Length to width ratio	0.085	0.645	0.082	0.614	1.000	0.656	0.506	1.000

In our laboratory, Körnerová and Holá (1999) and Holá *et al*. (2003) studied the effect of low growth temperature on photochemical activities of isolated MC chloroplasts and photosynthetic pigment contents on the same maize inbred lines and F_1 hybrids as those used in our present work. However, they examined mostly the effect of more severe chilling (in the spring season) and they did not follow the ultrastructure of chloroplasts. Their results showed a considerable decrease in the contents of chlorophylls in all genotypes studied, the decrease in PS2 activity in the inbreds but usually not in the hybrids, and the increase or no changes in PS1 activity and total carotenoid content under these conditions. When analyzing the effect of moderate chilling on the above mentioned parameters, they found a statistically significant increase both in PS2 and PS1 activity (Holá *et al.* 2003, Tichá *et al.* 2003). This is surprising especially as we found that the VD of granal and, to a lesser extent, also of intergranal thylakoids in MC chloroplasts of the same plants clearly and significantly decreases with moderate chilling stress. The PS2 is localized mainly in the stacked

thylakoid membranes and thus should be particularly susceptible to the chilling stress, so we would expect the decrease in granal thylakoids' VD accompanied by the decrease in PS2 activity. A possible explanation of this discrepancy lies in the nature of stress: our experiments were made in autumn when plants were exposed to low temperature almost exclusively during the night. The chilling stress was thus not accompanied by high irradiances that would eventually lead to the photoinhibition or irreversible photo-oxidative damage to both photosystems. The function of these complexes was therefore probably not much impaired (though their amount could be, as inferred from the decrease of thylakoids' VD). However, a more detailed study of the changes in thylakoid protein amount in plants stressed by moderate or severe chilling is clearly indicated.

Table 4. The differences between individual genotypes in ultrastructural parameters (volume densities of main chloroplast compartments) and dimensions of mesophyll cell chloroplasts in leaves of maize hybrid combination 2013×CE810 grown in either optimum (control) or chilling conditions. P_1 = 2013 inbred line, P_2 = CE810 inbred line, F_1 = F_1 hybrid 2013×CE810, F_1' = F_1 hybrid CE810×2013, ØP = parental mean. The statistical significances (*p*) as determined by Scheffé's test are shown.

		P_1-P_2	F_1-F_1'	F_1-P_1	F_1'-P_1	F_1-P_2	F_1'-P_2	F_1-ØP	F_1'-ØP
Control	Granal thylakoids	0.998	0.999	0.997	0.989	1.000	0.999	1.000	0.994
	Intergranal thylakoids	0.466	0.512	0.997	0.383	0.602	0.999	0.935	0.747
	All thylakoids	0.888	0.892	1.000	0.894	0.885	1.000	0.974	0.979
	Granality	0.561	0.598	0.997	0.464	0.695	0.999	0.957	0.788
	Peripheral reticulum	0.487	0.998	0.855	0.924	0.924	0.855	1.000	1.000
	Plastoglobuli	0.006	0.254	0.372	0.996	0.314	0.003	1.000	0.128
	Stroma	0.994	0.780	0.994	0.903	0.954	0.974	0.970	0.919
	Chloroplast volume	0.164	0.837	0.823	0.325	0.620	0.982	0.997	0.870
	Length to width ratio	0.778	0.907	0.820	0.997	1.000	0.876	0.968	0.989
Chilling-stressed	Granal thylakoids	0.613	0.876	0.993	0.989	0.441	0.114	0.736	0.236
	Intergranal thylakoids	0.119	0.921	0.342	0.383	0.944	1.000	0.918	0.516
	All thylakoids	0.997	0.713	0.602	0.894	0.728	0.157	0.555	0.057
	Granality	0.069	1.000	0.870	0.464	0.328	0.374	0.950	0.972
	Peripheral reticulum	0.237	0.870	0.988	0.924	0.124	0.473	0.462	0.938
	Plastoglobuli	0	0.170	0.522	0.996	0.003	0	0.577	0.002
	Stroma	0.811	0.058	0.652	0.903	0.993	0.028	0.841	0.001
	Chloroplast volume	0.244	0.029	0.726	0.325	0.840	0.002	0.999	0.012
	Length to width ratio	0.985	0.806	0.506	0.997	0.729	0.218	0.500	0.074

Čiamporová and Trgiňová (1996, 1999) compared cold resistant and sensitive genotype of maize and observed the increase of plastoglobuli in MC chloroplasts in both genotypes but a disruption in thylakoid arrangement (empty regions in chloroplast stroma) only in the sensitive genotype. According to them, the changes in the amount and organization of thylakoid membranes and the associated changes of chloroplast stroma VD depend not only on temperature treatment but also on the examined genotype. We also recorded genotypic differences in several ultrastructural parameters of MC chloroplasts that were more pronounced in chilling-stressed plants. The most interesting finding was that F_1 hybrids showed lower decrease in thylakoid VD (especially in granal thylakoids) due to chilling stress, compared to their parental inbred lines. This agrees well with positive heterosis observed in F_1 generation in the activity of PS2 (Holá *et al.* 2003). MC chloroplasts in leaves of stressed plants of F_1 hybrids usually showed also lower increase in the VD of plastoglobuli and they were not as rounded as the parental ones; may be they were less negatively affected by chilling.

In conclusion, we found that the ultrastructure, shape, and dimensions of "dimorphic" maize chloroplasts, especially MC ones, quantitatively change even under moderate chilling. These changes depend rather strongly on maize genotype; in many cases, positive heterosis in chloroplast ultrastructure occurs in F_1 hybrid plants concerning their power of resistance against chilling stress.

References

Arvidsson, P.-O., Sundby, C.: A model for topology of the chloroplast thylakoid membrane. – Aust. J. Plant Physiol. **26**: 687-694, 1999.

Biswal, B., Biswal, U.C.: Photosynthesis under stress: stress signals and adaptive response of chloroplasts. – In: Pessarakli, M. (ed.): Handbook of Plant and Crop Stress. 2nd Ed. Pp. 315-336. Marcel Dekker, New York – Basel 1999.

Bongard–Pierce, D.K., Evans, M.M.S., Poethig, R.S.: Heteroblastic features of leaf anatomy in maize and their genetic regulation. – Int. J. Plant Sci. **157**: 331-340, 1996.

Čatský, J., Šesták, Z.: Photosynthesis during leaf development. – In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 633-660. Marcel Dekker, New York – Basel – Hong Kong 1997.

Čiamporová, M., Trgiňová, I.: Ultrastructure of chloroplasts in leaves and of plastids in root tips of two maize lines differing in chilling tolerance. – Biológia (Bratislava) **51**: 441-447, 1996.

Čiamporová, M., Trgiňová, I.: Modifications of plant cell ultrastructure accompanying metabolic responses to low temperatures. – Biológia (Bratislava) **54**: 349-360, 1999.

Du, Y.-C., Nose, A., Wasano, K.: Effects of chilling temperature on photosynthetic rates, photosynthetic enzyme activities and metabolite levels in leaves of three sugarcane species. – Plant Cell Environ. **22**: 317-324, 1999.

Fracheboud, Y., Haldimann, P., Leipner, J., Stamp, P.: Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.). – J. exp. Bot. **50**: 1533-1540, 1999.

Fracheboud, Y., Ribaut, J.-M., Vargas, M., Messmer, R., Stamp, P.: Identification of quantitative trait loci for cold-tolerance of photosynthesis in maize (*Zea mays* L.). – J. exp. Bot. **53**: 1967-1977, 2002.

Haldimann, P.: Effect of changes in growth temperature on photosynthesis and carotenoid composition in *Zea mays* leaves. – Physiol. Plant. **97**: 554-562, 1996.

Holá, D., Kočová, M., Körnerová, M., Sofrová, D., Sopko, B.: Genetically based differences in photochemical activities of isolated maize (*Zea mays* L.) mesophyll chloroplasts. – Photosynthetica **36**: 187-197, 1999.

Holá, D., Langrová, K., Kočová, M., Rothová, O.: Photosynthetic parameters of maize (*Zea mays* L.) inbred lines and F1 hybrids: their different response to, and recovery from rapid or gradual onset of low-temperature stress. – Photosynthetica **41**: 429-442, 2003.

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

Hudák, J., Salaj, J.: The effect of low temperatures on the structure of plant cells. - In: Pessarakli, M. (ed.): Handbook of Plant and Crop Stress. 2nd Ed. Pp. 441-464. Marcel Dekker, New York – Basel 1999.

Körnerová, M., Holá, D.: The effect of low growth temperature on Hill reaction and photosystem 1 activities and pigment contents in maize inbred lines and their F_1 hybrids. – Photosynthetica **37**: 477-488, 1999.

Kratsch, H.A., Wise, R.R.: The ultrastructure of chilling stress. – Plant Cell Environ. **23**: 337-350, 2000.

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

Kutík, J., Holá, D., Vičánková, A., Šmídová, M., Kočová, M., Körnerová, M., Kubínová, L.: 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, 2001.

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

Lidon, F.C., Loureiro, A.S., Vieira, D.E., Bilho, E.A., Nobre, P., Costa, R.: Photoinhibition in chilling stressed wheat and maize. – Photosynthetica **39**: 161-166, 2001.

Mostowska, A.: Environmental factors affecting chloroplasts. – In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 407-426. Marcel Dekker, New York – Basel – Hong Kong 1997.

Mustárdy, L., Garab, G.: Granum revisited. A three-dimensional model – where things fall into place. – Trends Plant Sci. **8**: 117-122, 2003.

Nie, G.Y., Baker, N.R.: Modifications to thylakoid composition during development of maize leaves at low growth temperatures. – Plant Physiol. **95**: 184-191, 1991.

Nie, G.Y., Robertson, E.J., Fryer, M.J., Leech, R.M., Baker, N.R.: Response of the photosynthetic apparatus in maize leaves grown at low temperature on transfer to normal growth temperature. – Plant Cell Environ. **18**: 1-12, 1995.

Pastori, G., Foyer, C.H., Mullineaux, P.: Low temperature-induced changes in the distribution of H_2O_2 and antioxidants between the bundle sheath and mesophyll cells of maize leaves. – J. exp. Bot. **51**: 107-113, 2000.

Pinhero, R.G., Paliyath, G., Yada, R.Y., Murr, D.P.: Chloroplast membrane organization in chilling-tolerant and chilling-sensitive maize seedlings. – J. Plant Physiol. **155**: 691-698, 1999.

Ritchie, S.W., Hanway, J.J., Benson, G.O.: How a corn plant develops. – Spec. Rep. No. 48. Iowa State University Extension, Ames 1993.

Robertson, E.J., Baker, N.R., Leech, R.M.: Chloroplast thylakoid protein changes induced by low growth temperature in maize revealed by immunocytology. – Plant Cell Environ. **16**: 809-818, 1993.

Rogers, R.A., Dunn, J.H., Nelson, C.J.: Photosynthesis and cold hardening in *Zoysia* and bermudagrass. – Crop Sci. **17**: 727-732, 1977.

Slack, C.R., Roughan, P.G., Bassett, H.C.M.: Selective inhibition of mesophyll chloroplast development in some C_4-pathway species by low night temperature. – Planta **118**: 57-73, 1974.

Sopher, C.R., Krol, M., Huner, N.P.A., Moore, A.E., Fletcher, R.A.: Chloroplastic changes associated with paclobutrazol-induced stress protection in maize seedlings. – Can. J. Bot. **77**: 279-290, 1999.

Tichá, I., Haisel, D., Holá, D., Kočová, M., Kutík, J., Rothová, O., Wilhelmová, N.: Chloroplast structure and functions as affected by chilling temperatures. – In: ELSO 2003 Proceedings. P. 127. Dresden 2003.

Vega, S.H., Sauer, M., Orkwiszewski, J.A.J., Poethig, R.S.: The *early phase change* gene in maize. – Plant Cell **14**: 133-147, 2002.

Verheul, M.J., Picatto, C., Stamp, P.: Growth and development of maize (*Zea mays* L.) seedlings under chilling conditions in the field. – Eur. J. Agron. **5**: 31-43, 1996.

Vičánková, A., Holá, D., Kočová, M., Wilhelmová, N., Haisel, D., Kutík, J.: Changes in structural and photochemical characteristics of maize chloroplasts during diurnal cycle and along the leaf blade. – In: Roubelakis-Angelakis, K.A. (ed.): 13th Congress of the Federation of European Societies of Plant Physiology. Book of Abstracts. P. 483. FESPP, Herakleion 2002.