PHOTOSYNTHETICA 41 (3): 429-442, 2003

Photosynthetic parameters of maize (*Zea mays* L.) inbred lines and F_1 hybrids: their different response to, and recovery from rapid or gradual onset of low-temperature stress

D. HOLÁ*, K. LANGROVÁ**, M. KOČOVÁ, and O. ROTHOVÁ

Department of Genetics and Microbiology, Faculty of Science, Charles University, Viničná 5, CZ 128 44 Praha 2, Czech Republic

Abstract

The activity of photosystems (PS) 1 and 2, together with the content and ratio of photosynthetic pigments, were measured in three inbred lines and two F_1 hybrids of maize (*Zea mays* L.), grown in either optimum or low temperature (LT) conditions. The ability of chilling-stressed plants to deal with the negative effects of long-term exposure to LT and to recover the efficiency of photosynthetic apparatus after their return to optimum temperatures was examined during spring and autumn seasons. The aim was to analyse the possible differences between the rapid and gradual onset of LT on the response of young maize plants to chilling stress. The distinctive superiority of hybrids over their parental lines, found during the exposure of maize plants to LT, was not always retained after the return of chilling-stressed plants to optimum growth conditions. The response of individual genotypes to chilling stress, as well as their ability to recover the photosynthetic efficiency from the cold-induced damage, strongly depended also on the duration and the rapidity of the onset of LT.

Additional key words: carotenoids; chlorophylls; genotypes; Hill reaction; intra-specific variability; photosystem 1 and photosystem 2.

Introduction

Cultivation of crop plants in temperate climates is often negatively affected by various environmental factors. Species originating from the tropics or subtropics, *e.g.* maize, rice, cucumber, tomato, bell pepper, cotton, *etc.* are particularly susceptible to cold and can be sometimes totally destroyed by sudden and unexpected onset of low temperature (LT). This applies not only for the temperature decrease below zero point, which in plants induces freezing stress (associated with the formation of ice crystals in cells or intercellular space), but also for the temperatures between 0–15 °C, generally considered sufficient to induce chilling stress (McKersie and Leshem 1994a,b, Hudák and Salaj 1999).

Plant response to LT includes *e.g.* modification of various cellular structures (particularly biomembranes and cytoskeleton), together with changes of cellular pH, a decrease in the activity of various enzymes associated with photosynthesis and other metabolic pathways, and a decrease in the efficiency of cellular and whole-plant transport have been observed in various plant species (Čiamporová and Trgiňová 1999, Hudák and Salaj 1999, Sowinski *et al.* 1999, Kratsch and Wise 2000). Photosynthetic apparatus, due to its localisation in chloroplasts, is particularly susceptible to negative effects of LT. This is partly because chloroplasts contain extensive system of inner thylakoid membranes, composed largely of lipids with unsaturated fatty acids, partly because the side-product of photosynthesis, molecular oxygen, can be

Received 27 March 2003, *accepted* 18 August 2003.
*Author for correspondence; fax: +420 221951724, e-mail: *danahola@cesnet.cz*
**Present address*: Center for Cancer Cytogenetics, Institute of Clinical Biochemistry and Laboratory Diagnostics, General Faculty Hospital and 1st Medical Faculty, Charles University, U nemocnice 2, CZ 128 08, Praha 2, Czech Republic.
Abbreviations: ANOVA – analysis of variance, Car – carotenoids; Chl – chlorophyll; DCMU – 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; DCPIP – 2,6-dichlorophenol indophenol; F_1 – the first filial generation; LHC – light-harvesting complexes; PAR – photosynthetically active radiation; PS – photosystem; RC – reaction centre; ROS – reactive oxygen species.
Acknowledgements: The authors are grateful to Dr. Jaroslav Poruba from CEZEA Breeding Station in Čejč, Czech Republic for supply of maize seeds, to Dr. Roman Krejčí from the Department of Genetics and Microbiology, Faculty of Science, Charles University for providing us with the statistical programme performing Scheffé's non-parametric tests, and to our students Hana Ždánská and Klára Bajerová for technical help. This study was supported by grants No. 522/01/0846 and 522/02/D174 of the Grant Agency of the Czech Republic, and No. J13/98:113100003 from the Ministry of Education, Youth, and Sports of the Czech Republic.

easily converted into dangerous ROS which can oxidise photosynthetic pigments, proteins, and lipids of thylakoid membranes and thus induce their degradation (Wise 1995, Huner *et al.* 1998, Sonoike 1998, Biswal and Biswal 1999, Allen and Ort 2001).

The primary effect of chilling stress on photosynthetic activity is probably associated with the decrease of the efficiency of CO_2 fixation (due to increased degradation and/or decreased activity of several enzymes participating in the Calvin-Benson or Hatch-Slack cycles, as well as stomatal closure) and the changes in the formation and redistribution of various sugars (Brüggemann *et al.* 1994, Kingston-Smith *et al.* 1997, Zervoudakis *et al.* 1998, Pietrini *et al.* 1999, Caemmerer and Quick 2000, Sage and Pearcy 2000, Savitch *et al.* 2000, Sundar and Reddy 2000). This decrease of CO_2 fixation is accompanied by lower need for energy and reducing power (ATP and NADPH formed during the primary processes of photosynthesis in thylakoid membranes). This results in the over-reduction of photosynthetic electron-transport chain and the formation of strong oxidants, which are potentially dangerous both to chloroplasts and other cellular compartments (Biswal and Biswal 1999, Allen and Ort 2001, Aroca *et al.* 2001).

The damage to the components of photosynthetic apparatus induced by LT can be further increased if plants are subjected to chilling stress in the light. The mechanisms of chilling-induced photoinhibition have been thoroughly studied and the photoinhibition of both PS2 and PS1 has been described as the consequence of plant exposure to moderate or even week irradiance at chilling temperatures (*e.g.* Greer and Hardacre 1989, Sonoike 1998, 1999, Aguilera *et al.* 1999, Venema *et al.* 2000, Lidon *et al.* 2001, Pocock *et al.* 2001, Kudoh and Sonoike 2002). Under such conditions, the photodamage to both photosystems can occur rather rapidly and some of its symptoms persist even after the end of photoinhibitory and/or chilling treatment (Nie *et al.* 1995, Savitch *et al.* 2001, Kudoh and Sonoike 2002).

The majority of studies dealing with the effect of chilling on photosynthesis has been performed on plants subjected to chilling for rather short periods of time (h to d), and during both day and night. To minimise the possible influence of other environmental factors, such as irradiance, soil and air moisture, *etc*. plants have been usually grown in growth chambers or controlled-environment cabinets in precisely defined, fixed conditions, and regularly supplied with optimum nutrient solution (or even grown in hydroponical cultures) (*e.g.* Haldimann 1996, 1999, Gesch and Heilman 1999, Aroca *et al.* 2001, Kudoh and Sonoike 2002, Rapacz and Hura 2002). This type of experiments brings much useful information about plant response to chilling stress (particularly at cellular and sub-cellular level). Unfortunately, application of data obtained by such experiments to plants grown in natural and agricultural habitats can be associated with various problems (Verheul *et al.* 1996). Studies on plants subjected to LT in the field or in the glasshouse (cultivation of plants in a glasshouse usually better corresponds to the natural conditions than cultivation in a growth chamber) are therefore necessary for the true understanding of the effects of cold on the efficiency of photosynthesis *in natura* (Verheul *et al.* 1996, Aguilera *et al.* 1999, Leipner *et al.* 1999).

There are several reasons for the occurrence of some discrepancies between the results of the field and growth-chamber studies of plants subjected to chilling temperatures. In temperate climates, plants usually experience much longer periods of LT (*i.e.* weeks or even months) compared to short chilling treatments accomplished in most (but not all) growth-chamber studies. Moreover, these LT periods are often characterised by a strong decrease of temperature during night, but by an equally strong temperature increase during the day. This happens particularly if the cold period is accompanied by sunny days, as it is often in the spring, when the effect of LT on the development of plants can be damaging at the most. Thus, plants grown in the field conditions in temperate climates need to deal both with their exposure to chilling temperatures during the night (when no photoinhibition can occur), and with substantial alterations of temperature during the light period. Their ability to rapidly recover their photosynthetic apparatus from the damage caused by chilling stress is therefore equally important for their optimum development, as is their efficiency in dealing with the stress itself (Nie *et al.* 1995, Aguilera *et al.* 1999, Allen and Ort 2001, Aroca *et al.* 2001).

The susceptibility to chilling stress and the ability of plants to recover can considerably differ not only between various plant species but also between individual genotypes of one species. Some genotypes are less sensitive to LT and can better adapt to stress than others. This resistance to chilling stress can result either from the lower sensitivity of individual components of photosynthetic apparatus to photo-oxidative damage (Fracheboud *et al.* 1999, Leipner *et al.* 1999, Ribas-Carbo *et al.* 2000), or from increased activity and/or synthesis of protective antioxidants, photosynthetic pigments, various proteins, or other compounds with protective function (Haldimann 1998, 1999, Iannelli *et al.* 1999, Leipner *et al.* 1999, Aroca *et al.* 2001).

Most studies dealing with genetic variability in photosynthetic characteristics in plants stressed by LT are based on the examination of genotypes whose susceptibility to cold is already known, and on the comparison of biochemical or physiological processes occurring in their cells or tissues (*e.g.* Greer and Hardacre 1989, Janda *et al.* 1998, Haldimann 1998, 1999, Aguilera *et al.* 1999, Fracheboud *et al.* 1999, Iannelli *et al.* 1999, Leipner *et al.* 1999, Koroleva *et al.* 2000, Ribas-Carbo *et al.* 2000, Aroca *et al.* 2001, Liu *et al.* 2001, Rapacz and Hura 2002). The possibility of the inheritance of this sensitivity/resistance of photosynthetic apparatus to chilling stress, and the relationship between parents and their

hybrids, has rarely been analysed (Du *et al.* 1999, Fracheboud *et al.* 1999). In our previous study (Körnerová and Holá 1999), we found that hybrid genotypes of maize are generally characterised by a lesser damage to photosynthetic apparatus caused by long-term chilling stress (lower decrease of the Chl content and of the PS2 activity) compared to their parental inbred lines. However, nothing was known about the ability of maize inbreds and hybrids to recover the original efficiency of photosynthetic apparatus after the return of chilling-stressed plants to optimum growth conditions. The present study was therefore aimed at the analysis of potential hybrid-inbred differences in such a recovery. We examined also the effect of the rapidity of the onset of LT and of the duration of chilling stress on the intra-specific variability in selected photosynthetic parameters.

Materials and methods

Plants and experimental design: The activities of PS1 and PS2, together with the contents and ratios of photosynthetic pigments, were measured in two hybrid combinations of maize (*Zea mays* L.), CE704×CE810 and 2013×CE810, grown in two temperature treatments. Each hybrid combination comprised of the respective parental inbred lines and their F_1 hybrid; the susceptibility of all inbred lines to chilling stress was more or less similar. Each genotype in each part of the experimental series was represented by 120-160 plants. Both hybrid combinations were analysed independently in two experimental series: the first (spring series) was performed from March to April, the second (autumn series) took place during October to November. Each experimental series was divided into two parts: "stress" and "recovery".

Maize seeds were obtained from the breeding station CEZEA in Čejč (Czech Republic). At the beginning of the first part of each experimental series ("stress"), they were planted to low dishes with soil and placed in a glasshouse at optimum temperature conditions (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 (each group containing approximately one half of the original plants). One group was transferred to another glasshouse with LT (14-18/0-5 °C day/night), the other was left at the original growth conditions. The relative humidity in both glasshouses was kept between 70–100 %, no additional irradiance was applied. Plants were then left to grow for 25–26 d, at the end of which period the photosynthetic parameters were measured during four consecutive days.

In the second part of each experimental series ("recovery"), the plants originally grown at LT conditions were replanted from low dishes to pots with soil and again divided into two groups. One group was left in the glasshouse with LT while the other was transferred back into optimum temperature. They were kept there for another 18 d, till their developmental stage (determined by number of leaves) was similar to the developmental stage of control plants at the end of the first part of experimental series, and the second 4-d block of measurements was then performed.

Isolation of mesophyll chloroplasts: The first fully developed leaf counting from the vegetative top was used for the measurement of photosynthetic parameters. The leaf blade was cut into small pieces, immediately immersed in 40 cm^3 of isolation medium (0.4 M sucrose, 0.05 M $MgCl_2$, 0.05 M Tris-HCl, pH 7.0) pre-cooled to 0–4 °C, and homogenised for 18 s in *Thurmix 302* homogeniser (*MPW*, Poland; maximum rotations). To get a sufficient amount of the leaf tissue, 10–15 plants were usually needed. The homogenate was filtered through 8 layers of gauze and the filtrate centrifuged at 1 000× *g* for 10 min. The resulting pellet was re-suspended in a small volume (approx. 1 cm^3) of re-suspension medium (0.4 M sucrose, 0.006 M $MgCl_2$, 40 % glycerol, 0.05 M Tris-HCl, pH 7.0). The suspensions, containing mostly (95–98 %) mesophyll chloroplasts with broken envelope membranes, were stored in the dark at 0 °C till the measurement of PS1 and PS2 activities. To obtain chloroplasts with maximum photochemical activity, each of the above steps was performed at 0 °C. The content of chlorophyll (Chl) *a*+*b* in the suspensions was determined spectrophotometrically in 80 % aqueous acetone (Porra *et al.* 1989); the final concentration was about 1–2 kg m^{-3}.

PS1 and PS2 activities were measured polarographically (Clark type oxygen electrode, *Theta '90*, Czech Republic) as the amount of oxygen formed or (in case of PS1 activity) consumed by the suspensions of isolated chloroplasts irradiated by "white light" (170 W m^{-2} PAR) after the addition of artificial electron acceptors or donors. The measurement chamber was made according to Bartoš *et al.* (1975). The reaction medium for the measurement of PS1 activity contained 0.4 M sucrose and 0.05 M phosphate buffer (pH 6.5), 0.15 mM DCPIP reduced by 1 mM sodium ascorbate (artificial donor of electrons), and 0.1 mM methyl viologen (electron acceptor). Fresh solutions of ascorbate and methyl viologen were prepared for each day of experiments. The inhibitor of PS2 activity was 0.1 mM DCMU. A minimum amount of crystalline catalase and 0.5 mM NH_4Cl were also added to the reaction medium prior to measurements. The PS2 activity was measured as Hill reaction activity; the reaction medium was identical to the isolation medium and 7 mM $K_3[Fe(CN)_6]$ was added as an artificial electron acceptor in this case. The v/v ratio of the chloroplast suspensions to the reaction medium was 1/100. A magnetic stirrer stirred the reaction mixtures in the measurement

chamber. A constant temperature of 25 °C was maintained during all measurements. Each genotype/treatment in each experimental day was measured two to four times.

Content of photosynthetic pigments was determined in six leaf discs, each corresponding to 0.5 cm^2, which were cut into small pieces, put into 10 cm^3 of N,N-dimethylformamide and stored in a dark and cool place for 3 d. Chl *a*, Chl *b*, and total Car contents in the extracts were then determined spectrophotometrically (Porra *et al*. 1989, Wellburn 1994). Each genotype/treatment was represented by three samples in each experimental day.

Selected morphological parameters: The plant development was examined by the measurement of lengths of individual internodes between leaves that had a visible ligule, and by the determination of the number and length of these leaves. These measurements were performed regularly in 7-d intervals on six randomly selected plants from each genotype/treatment during each experimental series.

Statistical analysis: The average values characterising each genotype/treatment on each experimental day were used for the statistical analysis of photosynthetic parameters. The differences between the plants grown in optimum or LT conditions, between the spring and autumn series, or between genotypes of each hybrid combination (taken as a whole), and the appropriate interactions between these sources of variation were analysed by two-way or three-way ANOVA with interactions. The statistical significance of the differences between individual genotypes (each part of experimental series and each temperature treatment represented by separate analysis), as well as the differences between "stress" and "recovery" or between plants grown in optimum or LT conditions (when analysed individually for each genotype), was determined by Scheffé's non-parametric test.

Results

Differences between plants grown in optimum or LT conditions: Analysis of the photochemical activities of isolated mesophyll chloroplasts and the contents of photosynthetic pigments in leaves of maize plants stressed or non-stressed by LT confirmed our previous findings that chilling stress negatively affects the activity of PS2 and the Chl content, but shows either no effect or even a positive effect on the activity of PS1 and total Car content (Körnerová and Holá 1999). The differences between plants grown in optimum and LT conditions were usually

Table 1. The differences between plants grown in optimum and low-temperature conditions in selected photosynthetic parameters of two maize hybrid combinations (CE704×CE810 and 2013×CE810). Each genotype in each experimental series and each season were analysed separately. The statistical significance (p) as determined by Scheffé's test is shown. ND – test could not be performed due to absence of some data.

Parameter	Genotype	stress				recovery			
		CE704×CE810		2013×CE810		CE704×CE810		2013×CE810	
		Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
PS1	P_1	0.741	0.685	0.027	0.025	0.016	0.642	ND	0.127
	P_2	0.701	0.377	0.996	0.044	0.740	0.399	ND	0.681
	F_1	0.027	0.052	0.189	0.001	0.313	0.961	0.546	0.657
PS2	P_1	0	0.008	0	0.022	0.001	0	ND	0
	P_2	0	0.078	0	0.517	0.003	0	ND	0
	F_1	0.003	0	0.006	0.005	0.001	0	0,005	0
Chl *a*	P_1	0	0.269	0	0.082	0	0	ND	0.086
	P_2	0	0.004	0	0.744	0.001	0	ND	0.010
	F_1	0	0.253	0	0.012	0.001	0	0	0.012
Chl *b*	P_1	0	0.030	0	0.016	0	0	ND	0.306
	P_2	0	0.001	0	0.328	0	0	ND	0.004
	F_1	0	0.085	0	0.030	0.002	0	0	0.013
Car	P_1	0.783	0.449	0.002	0.006	0.021	0.120	ND	0.476
	P_2	0.108	0.573	0.089	0.431	0.438	0.010	ND	0.688
	F_1	0.192	0.013	0.033	0.002	0.206	0.147	0.018	0.641
Chl *a/b*	P_1	0.032	0.016	0.004	0.222	0.002	0.610	ND	0.039
	P_2	0.007	0.003	0.005	0.001	0.526	0.085	ND	0.796
	F_1	0.140	0.007	0	0.028	0.004	0.014	0.018	0.373
Chl/Car	P_1	0	0	0.001	0.001	0	0	ND	0
	P_2	0	0	0.001	0.002	0	0	ND	0
	F_1	0	0.001	0	0.001	0	0	0	0

statistically significant; this applies to both hybrid combinations as well as to all experimental blocks (Table 1).

Treatment usually had no effect on the activity of PS1 during the first part of the spring experimental series ("stress"), with the exception of inbred line 2013 which showed a significant decrease of this parameter due to chilling stress. Contrary to it, the PS1 activity of chloroplasts isolated from the stressed plants of both hybrids was slightly higher compared to the plants grown in optimum conditions, but the difference was statistical ly significant only in the case of CE704×CE810 hybrid (Fig. 1*AC*). The increased values of this parameter were found also during the first part of the autumn experimental series; this increase was most pronounced in the hybrid combination 2013×CE810 (Fig. 1*B,D*). The second part of both experimental series ("recovery") was characterised by the absence of any differences in the activity of PS1 between plants placed in optimum and LT conditions (Fig. 1*A–D*). The only exception was a significant decrease observed in inbred line CE704 during the spring series. However, as the plants of both parental lines of 2013×CE810 hybrid combination did not survive past the first part of this series, the possible differences between both temperature treatments could not be in this case determined (Table 1).

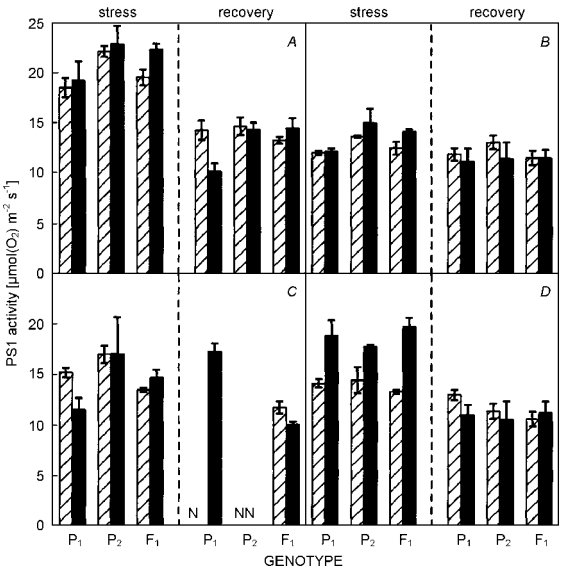

Fig. 1. The effect of low growth temperature on photosystem (PS) 1 activity in two maize hybrid combinations during the first ("stress") and second ("recovery") parts of the spring (*A, C*) or autumn (*B, D*) experimental series. Hybrid combination CE704×CE810 (*A, B*) comprised of the parental lines CE704 (P_1) and CE810 (P_2) and their F_1 hybrid CE704×CE810. Hybrid combination 2013×CE810 (*C, D*) comprised of 2013 (P_1) and CE810 (P_2) parental lines and their F_1 hybrid 2013×CE810. *Hatched bars* represent plants continually grown in ("stress") or returned to ("recovery") optimum temperature conditions, *solid bars* represent plants stressed by low growth temperature. The letter N means that the plants of the respective genotype did not survive past the first part of the experimental series. Means ± SEM.

The changes in the activity of PS2 due to LT treatment of plants showed lesser complexity (Table 1). The values of this parameter strongly and significantly decreased in all genotypes of both hybrid combinations in the first part of the spring experimental series, as well as in the second part of both spring and autumn series (Fig. 2*A–D*). On the other hand, the first part of the autumn experimental series was characterised by an equally distinctive increase of the PS2 activity, observed again in all genotypes of both hybrid combinations (Fig. 2 *B,D*).

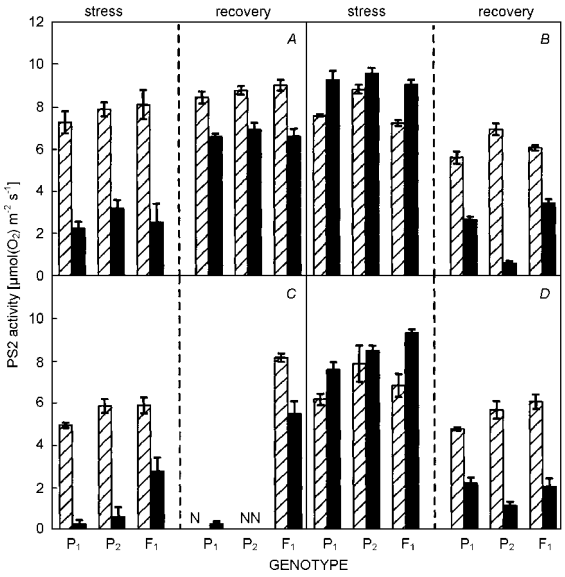

Fig. 2. The effect of low growth temperature on photosystem (PS) 2 activity in two maize hybrid combinations during the first ("stress") and second ("recovery") parts of the spring (*A, C*) or autumn (*B, D*) experimental series. For explanation of hybrid combinations, type of bars, and letter N see Fig. 1. Means ± SEM.

The changes in Chl *a* and *b* contents were similar to those described above for the PS2 activity. These parameters significantly decreased in the plants subjected to LT treatment compared to the non-stressed ones during the first part of the spring experimental series and in all "recovery" blocks (Figs. 3 and 4*A–D*, Table 1). However, the increase in these parameters during the first part of the autumn series was not as pronounced as the increase in the PS2 activity; it was statistically significant only in both hybrids and 2013 inbred line. Actually, the content of both Chls (especially Chl *b*) in the other two inbred lines due to LT treatment significantly decreased even in these experimental blocks (Figs. 3 and 4*B,D*, Table 1).

The situation for total Car content was different. This parameter was not much affected by chilling stress and the differences between temperature treatments of plants

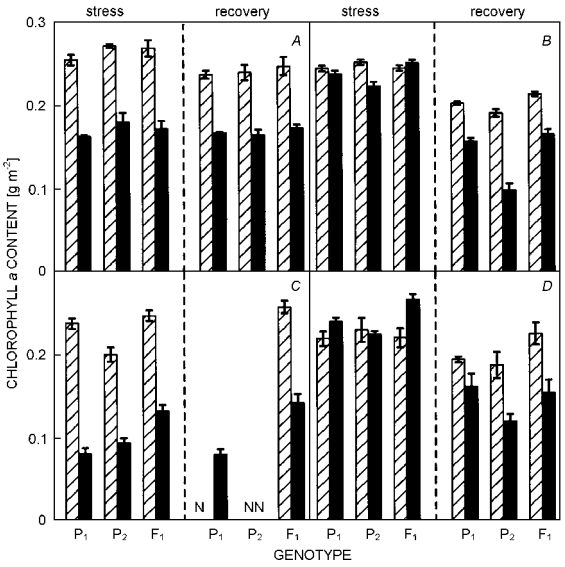

Fig. 3. The effect of low growth temperature on the chlorophyll *a* content in two maize hybrid combinations during the first ("stress") and second ("recovery") parts of the spring (*A, C*) or autumn (*B, D*) experimental series. For explanation of hybrid combinations, type of bars, and letter N see Fig. 1. Means ± SEM.

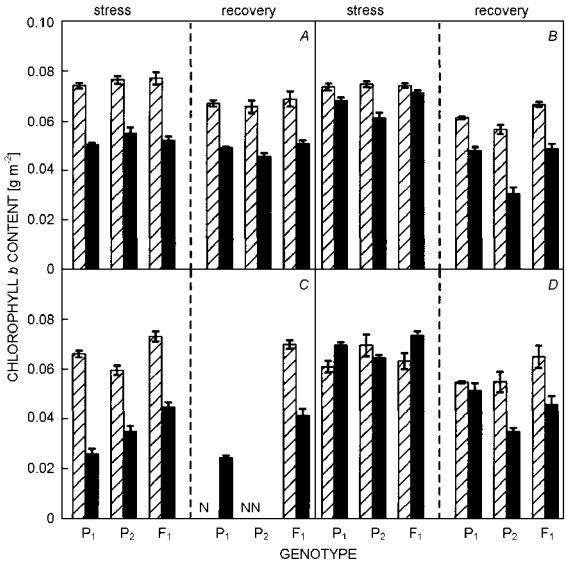

Fig. 4. The effect of low growth temperature on the chlorophyll *b* content in two maize hybrid combinations during the first ("stress") and second ("recovery") parts of the spring (*A, C*) or autumn (*B, D*) experimental series. For explanation of hybrid combinations, type of bars, and letter N see Fig. 1. Means ± SEM.

were often statistically insignificant (Table 1). Some negative changes due to LT treatment were observed in hybrid combination 2013×CE810 during the first part of the spring series (Fig. 5*C*). In the autumn series the content of total Car in leaves of the stressed plants increased (the increase was again most pronounced in both hybrids and 2013 inbred line) (Fig. 5*B,D*). During the "recovery", the content of total Car in leaves of the plants grown in LT, compared to the plants grown in optimum temperature, either slightly decreased or did not differ at all (Fig. 5, Table 1).

The differences in the Chl and total Car contents observed between both temperature treatments were reflected also by the differences in the ratios of Chl *a/b* and Chl/Car. While Chl/Car ratios for the plants grown in LT were invariably lower than those for optimum temperature, the ratio of Chl *a/b* decreased during the spring experimental series and increased or did not change during the autumn series (Table 1).

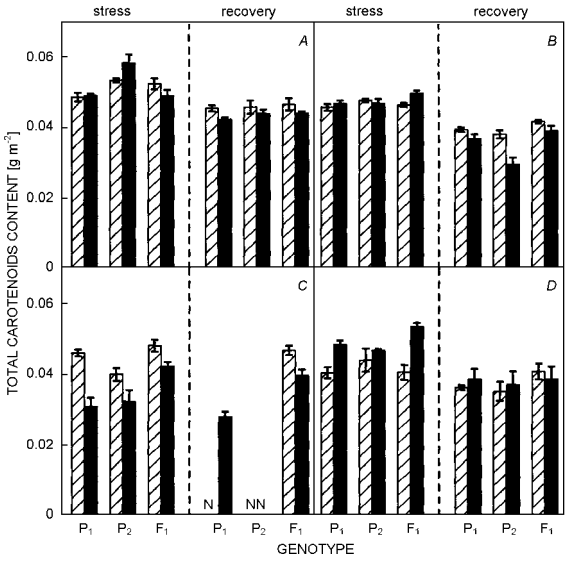

Fig. 5. The effect of low growth temperature on the content of total carotenoids in two maize hybrid combinations during the first ("stress") and second ("recovery") parts of the spring (*A, C*) or autumn (*B, D*) experimental series. For explanation of hybrid combinations, type of bars, and letter N see Fig. 1. Means ± SEM.

Finally, LT stress negatively affected also the morphology of plants. The cold-stressed plants were smaller and more compact compared to those grown in (or returned to) optimum temperature (except for 2013×CE810 hybrid in the second part of the autumn experimental series) as shown by the number and length of their internodes (Fig. 6). They had fewer leaves that were much shorter, rather rounded, and the ratio of leaf width to length was higher than in the non-stressed plants (data not shown).

Differences between spring and autumn seasons were confirmed by statistical analysis, which clearly showed—with some minor exceptions—that photosynthetic parameters of plants subjected to LT treatment at the beginning of spring or during autumn did not respond to stress in the same way. In addition to the significant differences between seasons as well as between temperature

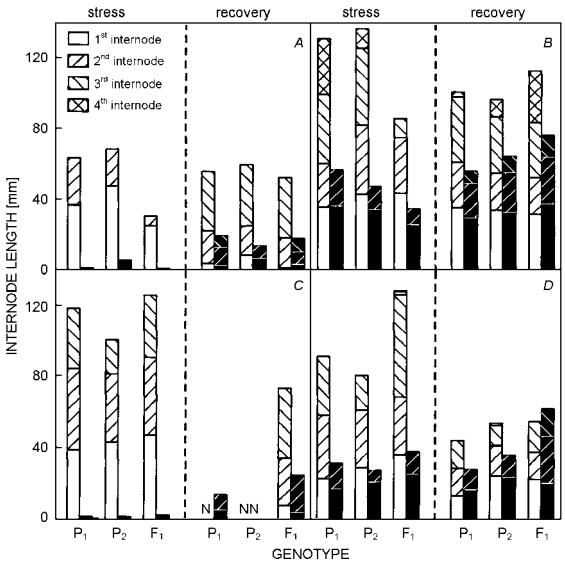

Fig. 6. The effect of low growth temperature on the length of internodes in two maize hybrid combinations during the first ("stress") and second ("recovery") parts of the spring (*A, C*) or autumn (*B, D*) experimental series. For explanation of hybrid combinations, type of bars, and letter N see Fig. 1. Means ± SEM.

treatments, highly significant interactions between these two sources of variation were usually also found when data from both seasons were analysed together (Table 2). This applied namely for the first part of the experimental series, *i.e.* "stress". As the optimum-temperature conditions were more or less similar during both seasons, it means that the reaction of plants to the conditions in LT glasshouse differed in both seasons. During the spring experimental series, the onset of LT (which at night and in the morning did not exceed 5 °C) was rapid and negatively affected plants almost from the beginning of their development. Contrary to it, the autumn experimental series were characterised by gradual decrease of night temperatures that were not as low as during spring (Fig. 7). This was reflected in the higher values of the activity of PS2 and Chl content, as well as in the greater number and length of internodes observed in the LT-stressed plants during the first part of the autumn experimental series, as compared to the spring experimental blocks (Figs. 2, 3, 4, and 6).

In the "recovery" experimental blocks, the situation reversed. By that time, the autumn plants that were left at LT were now subjected to much lower night temperatures than before (Fig. 7*B,D*). On the other hand, the gradual onset of spring slowly increased the day temperatures in non-temperate glasshouse almost to the values maintained for the optimum-temperature grown plants; however, the night and morning temperatures still stayed below 5 °C (Fig. 7*A,C*). The values of most photosynthetic parameters in cold-stressed plants during the "recovery" were therefore usually slightly (or, as in case of the PS2 activity, rather considerably) higher in spring than in autumn. However, the visual appearance of plants did not parallel this observation and the stressed plants, whose size in the first part of spring was extraordinarily minor, did not grow much larger even during the second part of this series. Actually, in 2013×CE810 hybrid combination, both parental lines did not survive the return to optimum-temperature conditions and one of them, CE810, was completely destroyed by the continuation of chilling stress (Fig. 7*C*).

Differences between genotypes: Although the changes in the activities of PS1 and PS2 and in the contents of photosynthetic pigments in leaves of plants stressed by LT usually followed some general rule of decrease or increase, significant differences between genotypes in this response also existed, as shown by the two-way or three-way ANOVA (Tables 3 and 4). The presence of statistically significant interactions between genotypes and temperature treatments was confirmed for the contents of Chl *a* and Chl *b* (with the exception of the first part of the spring experimental series in CE704×CE810 hybrid combination), often also for the content of Car and Chl/Car ratio, and in some cases also for the activity of PS2. This phenomenon usually reflected the better ability of both F_1 hybrids to deal with the negative consequences of chilling stress.

Photosynthetic apparatus in leaves of 2013×CE810

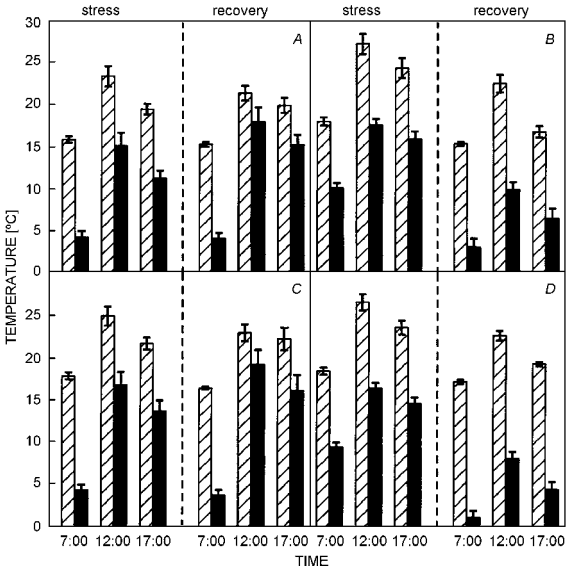

Fig. 7. The mean air temperature during the first ("stress") and second ("recovery") parts of the spring (*A, C*) or autumn (*B, D*) experimental series, during which the photosynthetic parameters of the hybrid combination CE704×CE810 (*A, B*) and 2013×CE810 (*C, D*) of maize were analysed. *Hatched bars* represent the values measured in the temperate glasshouse (optimum temperature conditions), *solid bars* represent the values measured in the non-temperate glasshouse (low-temperature conditions). Means ± SEM.

Table 2. The differences between seasons (S) and plants grown in optimum or low-temperature conditions (T) in selected photosynthetic parameters of two maize hybrid combinations (CE704×CE810 and 2013×CE810). Values from both seasons (*i.e.* spring and autumn) were analysed together for each experimental series. Each genotype was analysed separately. The statistical significance (*p*) for individual components of variation is shown. ND – analysis could not be performed due to absence of some data.

Parameter	Genotype	stress						recovery					
		CE704×CE810			2013×CE810			CE704×CE810			2013×CE810		
		S	T	S×T	S	T	S×T	S	T	S×T	S	T	S×T
PS1	P_1	0	0.689	0.789	0.009	0.594	0.001	0.457	0.025	0.095	ND	ND	ND
	P_2	0	0.384	0.793	0.671	0.422	0.428	0.055	0.361	0.580	ND	ND	ND
	F_1	0	0.003	0.372	0.002	0	0.001	0.011	0.478	0.436	0.235	0.446	0.756
PS2	P_1	0	0.001	0	0	0	0	0	0	0.032	ND	ND	ND
	P_2	0	0	0	0	0.001	0	0	0	0	ND	ND	ND
	F_1	0	0.007	0	0	0.523	0	0	0	0.642	0	0	0.121
Chl *a*	P_1	0	0	0	0	0	0	0	0	0.002	ND	ND	ND
	P_2	0.109	0	0	0	0	0	0	0	0.302	ND	ND	ND
	F_1	0.003	0	0	0	0.001	0	0.008	0	0.067	0.412	0	0,085
Chl *b*	P_1	0	0	0	0	0	0	0.009	0	0.058	ND	ND	ND
	P_2	0.232	0	0.051	0	0	0.003	0	0	0.183	ND	ND	ND
	F_1	0	0	0	0.001	0.002	0	0.334	0	0.949	0.976	0	0.164
Car	P_1	0.020	0.453	0.720	0.003	0.050	0	0	0.007	0.768	ND	ND	ND
	P_2	0	0.193	0.075	0.003	0.341	0.059	0	0.007	0.056	ND	ND	ND
	F_1	0.055	0.937	0.021	0.261	0.048	0	0.001	0.052	1.000	0.181	0.076	0.306
Chl *a/b*	P_1	0.189	0.567	0.001	0.069	0.002	0.061	0	0.015	0.087	ND	ND	ND
	P_2	0.087	0.830	0	0.001	0.008	0	0	0.119	0.595	ND	ND	ND
	F_1	0.775	0.864	0.007	0	0.002	0	0	0.441	0	0.011	0.040	0.576
Chl/Car	P_1	0	0	0	0	0	0	0.006	0	0.004	ND	ND	ND
	P_2	0	0	0	0	0	0.001	0.003	0	0.228	ND	ND	ND
	F_1	0	0	0	0	0	0	0.034	0	0.004	0,006	0	0.010

Table 3. The differences between genotypes (G) and plants grown in optimum or low-temperature conditions (T) in selected photosynthetic parameters of two maize hybrid combinations. Each experimental series in each season was analysed separately. The statistical significance (*p*) for individual components of variation is shown. ND – analysis could not be performed due to absence of some data.

Hybrid	Parameter	Spring						Autumn					
		stress			recovery			stress			recovery		
		G	T	G×T	G	T	G×T	G	T	G×T	G	T	G×T
CE704×CE810	PS1	0.026	0.168	0.628	0.034	0.118	0.014	0.013	0.066	0.491	0.702	0.364	0.758
	PS2	0.374	0	0.731	0.416	0	0.505	0.001	0	0.087	0	0	0
	Chl *a*	0.099	0	0.930	0.446	0	0.913	0.044	0.007	0.001	0	0	0
	Chl *b*	0.135	0	0.591	0.107	0	0.822	0.009	0	0.003	0	0	0.005
	Car	0.001	0.631	0.044	0.501	0.021	0.848	0.154	0.121	0.091	0	0	0.049
	Chl *a/b*	0.492	0	0.759	0.015	0.002	0.350	0.029	0	0.396	0.826	0.940	0.006
	Chl/Car	0	0	0.006	0.010	0	0.052	0	0	0	0	0	0
2013×CE810	PS1	0.096	0.561	0.343	ND	ND	ND	0.874	0	0.268	0.536	0.405	0.484
	PS2	0.001	0	0.041	ND	ND	ND	0.023	0.001	0.164	0.095	0	0.013
	Chl *a*	0	0	0.005	ND	ND	ND	0.178	0.012	0.036	0.033	0	0.277
	Chl *b*	0	0	0.003	ND	ND	ND	0.470	0.041	0.018	0.009	0	0.030
	Car	0.001	0	0.084	ND	ND	ND	0.369	0	0.038	0.444	0.752	0.698
	Chl *a/b*	0.001	0	0.266	ND	ND	ND	0.008	0.183	0.009	0.624	0.060	0.091
	Chl/Car	0.489	0	0.509	ND	ND	ND	0.041	0	0.765	0	0	0

hybrid, as inferred from the activity of both photosystems and the contents of photosynthetic pigments, was much less damaged due to chilling stress in comparison to both parental lines of this hybrid combination. The differences between hybrid and parents were statistically significant (data not shown). This applied for the "stress" blocks of both spring and autumn experimental series. During the autumn "recovery", the differences were less pronounced and especially 2013 inbred line showed similar response to LT as its F_1 hybrid.

Table 4. The differences between genotypes (G), seasons (S), and plants grown in optimum or low-temperature conditions (T) in selected photosynthetic parameters of two maize hybrid combinations. The data from both seasons (*i.e.* spring and autumn) were analysed together for each experimental series. The statistical significance (*p*) for individual components of variation is shown. ND – analysis could not be performed due to absence of some data.

Hybrid	Parameter	stress						recovery					
		G	S	T	G×S	G×T	S×T	G	S	T	G×S	G×T	S×T
CE704×CE810	PS1	0.001	0	0.030	0.590	0.411	0.753	0.095	0.003	0.097	0.410	0.103	0.778
	PS2	0.039	0	0	0.305	0.886	0	0.101	0	0	0.122	0.002	0
	Chl *a*	0.113	0	0	0.065	0.248	0	0	0	0	0	0.016	0.115
	Chl *b*	0.158	0	0	0.023	0.314	0	0	0	0	0.001	0.022	0.826
	Car	0.001	0	0.283	0.005	0.562	0.736	0.002	0	0	0.004	0.323	0.182
	Chl *a/b*	0.065	0.071	0.774	0.661	0.873	0	0.049	0	0.016	0.182	0.517	0.020
	Chl/Car	0	0	0	0.544	0	0	0	0.670	0	0	0	0.015
2013×CE810	PS1	0.217	0.048	0.013	0.089	0.233	0.001	ND	ND	ND	ND	ND	ND
	PS2	0	0	0	0.159	0.007	0	ND	ND	ND	ND	ND	ND
	Chl *a*	0	0	0	0.135	0.040	0	ND	ND	ND	ND	ND	ND
	Chl *b*	0.001	0	0	0.023	0.206	0	ND	ND	ND	ND	ND	ND
	Car	0.001	0	0.464	0.047	0.040	0	ND	ND	ND	ND	ND	ND
	Chl *a/b*	0	0	0	0.060	0.251	0	ND	ND	ND	ND	ND	ND
	Chl/Car	0.411	0	0	0.391	0.629	0	ND	ND	ND	ND	ND	ND

Table 5. The differences between both parts of experimental series (*i.e.* "stress" and "recovery") in selected photosynthetic parameters of two maize hybrid combinations (CE704×CE810 and 2013×CE810). Each genotype in each temperature treatment and each season was analysed separately. The statistical significance (*p*) as determined by Scheffé's test is shown. ND – test could not be performed due to absence of some data.

Parameter	Genotype	Optimum temperature conditions				Low-temperature conditions			
		CE704×CE810		2013×CE810		CE704×CE810		2013×CE810	
		Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn
PS1	P_1	0.020	0.807	ND	0.133	0.004	0.467	0.007	0.005
	P_2	0	0.441	ND	0.088	0.004	0.153	ND	0.007
	F_1	0	0.373	0.072	0.013	0.001	0.023	0.002	0.001
PS2	P_1	0.092	0.001	ND	0.002	0	0	0.942	0
	P_2	0.066	0.002	ND	0.062	0	0	ND	0
	F_1	0.262	0.001	0.002	0.277	0.006	0	0.022	0
Chl *a*	P_1	0.057	0	ND	0.038	0.065	0	0.905	0.003
	P_2	0.016	0	ND	0.097	0.243	0	ND	0
	F_1	0.185	0	0.340	0.792	0.987	0	0.512	0.001
Chl *b*	P_1	0.006	0	ND	0.041	0.253	0	0.797	0.002
	P_2	0.011	0	ND	0.052	0.013	0	ND	0
	F_1	0.076	0.001	0.284	0.751	0.569	0	0.377	0
Car	P_1	0.081	0.002	ND	0.056	0	0.001	0.399	0.021
	P_2	0.008	0	ND	0.081	0.002	0	ND	0.044
	F_1	0.048	0.001	0.550	0.938	0.033	0.001	0.274	0.007
Chl *a/b*	P_1	0.147	0.382	ND	0.282	0.046	0.010	0.433	0.130
	P_2	0.102	0.871	ND	0.023	0.044	0.001	ND	0.876
	F_1	0.078	0.082	0.001	0.669	0.464	0.010	0	0.055
Chl/Car	P_1	0.272	0.013	ND	0.370	0	0	0.672	0.002
	P_2	0.078	0.002	ND	0.538	0.001	0	ND	0
	F_1	0.056	0.116	0.007	0.263	0.002	0	0.548	0

The activity of PS1 in leaves of the F_1 hybrid CE704×CE810 also reacted better to the LT conditions compared to its parents (Fig. 1) but the differences were statistically insignificant. As for the PS2 activity and the content of photosynthetic pigments, the response of inbred line CE704 and hybrid CE704×CE810 to chilling stress was rather similar (particularly in the autumn series); these genotypes were not as negatively affected as CE810 inbred line (Figs. 2 to 5). These differences between CE704 or CE704×CE810 and CE810 were often statistically significant (data not shown).

The different response of individual genotypes to

spring or autumn growth was also confirmed. In both hybrid combinations, statistically significant interactions between genotypes and seasons were found for Chl *b* and Car contents in the first part of experimental series, and in the second part of experimental series they were detected in the CE704×CE810 hybrid (the "recovery" block in the other hybrid combination could not be analysed) for the Chl *a* content (Table 4).

The ability of plants to recover from LT stress: The last part of our study was aimed at the analysis of the ability of photosynthetic apparatus in inbreds and F_1 hybrids of maize subjected to either rapid or gradual onset of LT to recover from chilling stress after the return of plants to optimum temperature. The values of these parameters in "recovery" and "stress" experimental blocks were compared by Scheffé's tests (Table 5).

The changes in temperature during the spring and autumn experimental series, described above, were reflected also in the ability of plants to recover from chilling stress. The exceptional shortness of the first internode, displayed by the plants originally subjected to rapid onset of LT during the first part of the spring series, did not change even after their transfer to optimum temperature, but the second and third internodes, which appeared during the subsequent period, had fairly normal length and the plants showed good ability to fully recover from chilling stress (Fig. 6*A,C*). This was accompanied also by the almost total recovery of photosynthetic apparatus as inferred from the parameters measured. The activity of PS2 in leaves of the plants transferred back to optimum temperature conditions did not differ from the values measured in the non-stressed plants during the first part of the spring series; actually, they were even higher (Fig. 2*A,C*). The contents of both Chl *a* and Chl *b* in "recovered" plants were slightly lower compared to the optimum-temperature grown plants from the first part of experimental series. However, this applied for the inbred lines only; both F_1 hybrids were able to increase the content of these pigments to the original values (Figs. 3 and 4*A,C*). The activity of PS1 as well as the content of Car (with the exception of 2013×CE810 hybrid) did not reach the values originally measured in the non-stressed plants of the first part of the experimental series (Figs. 1 and 5*A,C*).

Contrary to this, the recovery of photosynthetic apparatus in the leaves of plants subjected originally to gradual onset of LT during the first part of the autumn series was not as complete as in spring. Though the growth and development of plants transferred from low to optimum temperatures was accelerated in comparison to those left in the non-temperate glasshouse, the original height of the control plants from the first part of this series was not achieved (with the exception of CE704×CE810 hybrid which grew very well) (Fig. 6*B,D*). The values of all photosynthetic parameters measured were usually also lower in the "recovered" plants compared to those measured in leaves of the non-stressed plants from the first part of this series. The notable exception was 2013×CE810 hybrid which recovered almost fully (Figs. 1 to 5*B,D*).

Comparison of the first and second parts of experimental series in the plants grown continually at LT enabled us also to determine the effect of the length of LT period and/or plant age on photosynthetic parameters. Continuation of the chilling stress during the autumn experimental series (together with the further gradual decrease of temperature) negatively affected all photosynthetic parameters studied but not the morphology of plants, while the prolonged period of exposition of plants to LT during spring had negative effect only on the activity of PS1 and Car content in CE704×CE810 hybrid combination; it even actually promoted the activity of PS2 (Figs. 1 to 6, Table 5).

Discussion

The exposure of thermophilic plants, including maize, to LT induces various changes in the structure and function of photosynthetic apparatus. Although the chilling stress primarily affects the efficiency of CO_2 fixation, further step in plant response to LT is usually the decrease of activity of photosynthetic electron-transport chain (or at least the decrease of the activity of its individual components) localised in thylakoid membranes. If the chilling stress is combined with high or even moderate irradiance, this decrease can be associated with the induction of photoinhibition (usually regarded as a reversible process) or irreversible photo-oxidative damage to photosynthetic pigments, proteins, and lipids of thylakoid membranes. PS2 is generally more susceptible both to photoinhibition and photooxidation as compared to PS1. This can be caused by several factors. The accumulation of ATP in chloroplast stroma due to the decreased efficiency of CO_2 fixation leads to the decrease in the activity of thylakoid ATPase and to the decrease of lumenal pH (Sonoike 1998, 1999), which induces conformational changes of the PS2 complex. The life-time of strong oxidants $P680^+$ and Tyr_Z^+ is thus prolonged and the probability of oxidative damage to the pigments (particularly Chl *a* and β-carotene) and proteins of PS2, as well as to the thylakoid lipids in the proximity of this complex, increases (Wise 1995, Minkov *et al.* 1999, Xu and Shun 1999, Lidon *et al.* 2001). D1 protein of PS2 seems to be most affected by chilling stress in spite of its fast turnover, which has been generally attributed both to the decrease in the expression of its gene and to the reduced fluidity of thylakoid membranes which slows down the migration of damaged D1 proteins from appressed to non-appressed regions of

thylakoids and thus impedes their replacement in PS2 by newly synthesised, functional ones (Allen and Ort 2001).

The greater susceptibility of PS2 to chilling stress found in the majority of studies agrees well with the results of our work. The experiments on maize plants grown at the beginning of spring, *i.e.* a season characterised by considerably LT which usually do not rise above 15 °C for the main part of both night and day, clearly showed that the activity of PS2 in chloroplasts isolated from leaves of the chilling-stressed plants is much lower compared to the optimum-temperature grown plants. The exact cause of this decrease is difficult to determine; however, as the return of stressed plants to optimum temperature resulted in total recovery of the PS2 efficiency, the possibility of occurrence of irreversible photo-oxidative damage to this pigment-protein complex can be probably rejected. Two other potential causes of the observed changes in the PS2 activity still remain: the reversible decrease of the number of functional PS2 complexes associated with photoinhibition, or the acclimation of PS2 to unfavourable conditions caused by the several weeks long plant exposure to cold.

The acclimation to cold has been observed in various plant species after their long-term exposure to chilling temperatures, *e.g.* in winter cereals or other chilling-resistant species such as spinach or potato (*e.g.* Öquist *et al.* 1993, van Wijk and van Hasselt 1993, Hurry *et al.* 1994, Venema *et al.* 2000). It has been also observed in chilling-sensitive species including maize (Leipner *et al.* 2000, Venema *et al.* 2000, Liu *et al.* 2001, Savitch *et al.* 2001, Rapacz and Hura 2002). It can be associated with (*1*) lower energy input into primary photosynthetic processes caused by the decrease in the size of light-harvesting antennae (Gesch and Heilman 1999), (*2*) better dissipation of excess excitation energy as heat with the aid of xanthophyll cycle (Venema *et al.* 2000, Liu *et al.* 2001), (*3*) the use of alternative sinks (*e.g.* photorespiration or dark respiration) for ATP and NADPH produced by primary photosynthetic reactions (Ribas-Carbo *et al.* 2000), or (*4*) greater production and/or activity of enzymatic and non-enzymatic antioxidants and other protective compounds (Hurry *et al.* 1994, Aroca *et al.* 2001). The first two mechanisms should result in the decrease of the efficiency of photosynthetic electron-transport processes.

As the LHCs of thylakoid membranes are unique in their ability to bind Chl *b*, we can expect that diminishing the antennae should result in the increase of Chl *a*/*b*. However, in our experiments the opposite was true and Chl *a/b* in leaves of the chilling-stressed plants grown at the beginning of spring significantly decreased. Hence probably the number or size of antennae complexes relative to PS2 reaction centres did not change as a consequence of cold stress. Naturally, the Chl *a/b* ratio does not inform about the organisation of LHC. Nie *et al.* (1995) found that the proteins of the LHC associated with PS2 occur mainly in monomeric form in maize plants grown for 4 weeks at 14 °C, whereas on transfer of these plants to 25 °C for 3 or 6 d the ratio of the oligomeric to monomeric LHC forms increases. Thus, the possibility that the decrease in the activity of PS2, observed in our study in the first part of the spring experimental series, could be associated with lower stability of the oligomeric forms of the antennae still remains.

The decrease in the Chl *a/b* ratio observed in chilling-stressed plants during the spring experimental series can be explained by another phenomenon as well. Several authors reported that chilling temperatures (especially chilling in the light) could lead to the irreversible degradation of the PS1 complex (Sonoike 1998, 1999, Minkov *et al.* 1999, Kudoh and Sonoike 2002). This complex is characterised by high Chl *a/b* ratio and its proteins and/or Chl *a* molecules bound to them, if degraded due to chilling-induced photo-oxidation, cannot be so easily replaced as D1 protein of the PS2 complex. Though we usually did not find significant changes in the activity of PS1 in plants stressed by cold at the beginning of spring, prolonged exposure of plants to LT during this season resulted in a marked decrease of PS1 activity in most genotypes studied. Moreover, when the chilling-stressed plants were returned to optimum temperature, their ability to recover the activity of this pigment-protein complex was much lower compared to the recovery of PS2, and the values of this parameter did not reach the original level found in the plants continually grown at optimum temperatures. Thus, a certain fraction of PS1 complexes in leaves developed in LT conditions could indeed be irreversibly damaged by chilling stress and could not be repaired even after the return of plants to optimum conditions. Several authors have shown that a "patchiness effect" (*i.e.* the presence of cells lacking certain thylakoid proteins in close neighbourhood of relatively normal cells in the mesophyll tissue) occurs in chilling-stressed maize and that this heterogeneity remains even after the plants are transferred to optimum-temperature (Robertson *et al.* 1993, Nie *et al.* 1995). However, the potential influence of such inability to repair PS1 complexes on the effectivity of net photosynthesis could not be great, as in our case the plants transferred from low to optimum temperatures resumed almost normal growth rate.

The above interpretation of the results of our spring experiments with cold-stressed plants brings one question: If the long-term exposure of maize plants to chilling temperatures induces the irreversible degradation of PS1 and (possibly) reversible damage to PS2, why we did not observe similar response in plants grown in the autumn experimental series? We think that the reason lies in the different structure of chilling stress during these seasons. While the onset of LT at the beginning of spring was rapid and plants were immediately subjected to potentially damaging growth conditions, the temperature decrease in autumn was more gradual and during the first part of the autumn experimental series even the night temperatures did not decline below 10 °C. This gradual decline of temperatures might allow plants to better pre-

pare for the unfavourable conditions. Though the development of the LT grown plants during the autumn experimental series was also slowed down compared to those grown at optimum temperature, this reduction of the growth rate was not as marked as during the spring series, and the synthesis and re-distribution of photosynthates was therefore probably also less affected. The over-reduction of the thylakoid electron-transport chain thus did not need to occur. It is even possible that the activity of other processes, associated with the use of ATP and NADPH synthesised during primary photochemical reactions, increased, which would explain the increase in both PS1 and PS2 activities observed during the first part of the autumn experimental series in the chilling-stressed plants.

The occurrence of LT mainly during night could also contribute to the absence of any significant damage to the thylakoid components of photosynthetic apparatus in the autumn experimental series, as the chilling stress was not accompanied by high irradiances. Moreover, the chilling-protective mechanisms, including both an increase in the activity of some anti-oxidative enzymes and the greater efficiency in the conversion of violaxanthin to zeaxanthin, were also activated. This was shown in some experiments with the same material, which were obtained during our study of the photochemical activity of chloroplasts (Tichá *et al.* 2002, D. Haisel and N. Wilhelmová, personal communication). However, the prolongation of chilling stress had markedly negative effect on all photosynthetic parameters studied, and the recovery of the photosynthetic apparatus after returning the chilling-stressed plants to optimum temperature was also not complete. We believe that this was associated with the greater intensity of plant growth during the first part of the autumn experimental series, as compared to the spring-grown ones. Similar results were found by Rapacz and Hura (2002) who showed that the intense elongation growth rate of two cultivars of oilseed rape negatively affects the ability of plants to recover their photosynthetic capacity after return of cold-acclimated plants to optimum temperatures.

Both hybrid genotypes studied were generally characterised by greater values of photosynthetic parameters compared to their parental inbred lines. This phenomenon could be observed particularly in 2013×CE810 hybrid which exceeded its parents not only in the ability to deal with chilling stress itself, but also in the ability to recover the efficiency of photosynthetic apparatus after the return to optimum growth conditions (as seen in the spring experimental series when the parental lines 2013 and CE810 did not survive such return). However, the difference between this hybrid and its parental line 2013 in the recovery of photosynthetic parameters was almost eliminated during the autumn experimental series. Similarly, CE704×CE810 hybrid, though also better than its parental lines (particularly CE810) in the response to chilling stress, did not much differ in the ability to recover the efficiency of photosynthetic apparatus after the return to optimum temperatures. Hence the distinctive superiority of hybrids over their parental lines, found during the exposure of maize plants to LT (Fracheboud *et al.* 1999, Körnerová and Holá 1999), is not always retained after the return of chilling-stressed plants to optimum growth conditions. The response of individual genotypes to chilling stress, as well as their ability to recover some components of photosynthetic apparatus from the cold-induced damage, strongly depends also on the duration and the rapidity of the onset of LT.

References

Aguilera, C., Stirling, C.M., Long, S.P.: Genotypic variation within *Zea mays* for susceptibility to and rate of recovery from chill-induced photoinhibition of photosynthesis. – Physiol. Plant. **106**: 429-436, 1999.

Allen, D.J., Ort, D.R.: Impacts of chilling temperatures on photosynthesis in warm-climate plants. – Trends Plant Sci. **6**: 36-42, 2001.

Aroca, R., Irigoyen, J.J., Sánchez-Díaz, M.: Photosynthetic characteristics and protective mechanisms against oxidative stress during chilling and subsequent recovery in two maize varieties differing in chilling sensitivity. – Plant Sci. **161**: 719-726, 2001.

Bartoš, J., Berková, E., Šetlík, I.: A versatile chamber for gas exchange measurements in suspensions of algae and chloroplasts. – Photosynthetica **9**: 395-406, 1975.

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.

Brüggemann, W., Klaucke, S., Maas-Kantel, K.: Long-term chilling of young tomato plants under low light. V. Kinetic and molecular properties of two key enzymes of the Calvin cycle in *Lycopersicon esculentum* Mill. and *L. peruvianum* Mill. – Planta **194**: 160-168, 1994.

Caemmerer, S. von, Quick, W.P.: Rubisco: Physiology *in vivo*. – In: Leegood, R.C., Sharkey, T.D., Caemmerer, S. von (ed.): Photosynthesis: Physiology and Metabolism. Pp. 85-113. Kluwer Academic Publishers, Dordrecht – Boston – London 2000.

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.

Gesch, R.W., Heilman, J.L.: Responses of photosynthesis and phosphorylation of the light-harvesting complex of Photosystem II to chilling temperature in ecologically divergent cultivars of rice. – Environ. exp. Bot. **41**: 257-266, 1999.

Greer, D.H., Hardacre, A.K.: Photoinhibition of photosynthesis and its recovery in two maize hybrids varying in low temperature tolerance. – Aust. J. Plant Physiol. **16**: 189-198, 1989.

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

Haldimann, P.: Low growth temperature-induced changes to pigment composition and photosynthesis in *Zea mays* genotypes differing in chilling sensitivity. – Plant Cell Environ. **21**: 200-208, 1998.

Haldimann, P.: How do changes in temperature during growth affect leaf pigment composition and photosynthesis in *Zea mays* genotypes differing in sensitivity to low temperature? – J. exp. Bot. **50**: 543-550, 1999.

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.

Huner, N.P.A., Öquist, G., Sarhan, F.: Energy balance and acclimation to light and cold. – Trends Plant Sci. **3**: 224-230, 1998.

Hurry, V.M., Malmberg, G., Gardeström, P., Öquist, G.: Effects of a short-term shift to low temperature and of long-term cold hardening on photosynthesis and ribulose-1,5-bisphosphate carboxylase/oxygenase and sucrose phosphate synthase activity in leaves of winter rye (*Secale cereale* L.). – Plant Physiol. **106**: 983-990, 1994.

Iannelli, M.A., van Breusegem, F., van Montagu, M., Inze, D., Massacci, A.: Tolerance to low temperature and paraquat-mediated oxidative stress in two maize genotypes. – J. exp. Bot. **50**: 523-532, 1999.

Janda, T., Szalai, G., Ducruet, J.-M., Páldi, E.: Changes in photosynthesis in inbred maize lines with different degrees of chilling tolerance grown at optimum and suboptimum temperatures. – Photosynthetica **35**: 205-212, 1998.

Kingston-Smith, A.H., Harbinson, J., Williams, J., Foyer, C.H.: Effect of chilling on carbon assimilation, enzyme activation, and photosynthetic electron transport in the absence of photoinhibition in maize leaves. – Plant Physiol. **114**: 1039-1046, 1997.

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.

Koroleva, O.Y., Krause, G.H., Brüggemann, W.: Effects of long-term chilling under excessive light on xanthophyll cycle activity and non-photochemical fluorescence quenching in *Lycopersicon* genotypes. – J. Plant Physiol. **156**: 341-349, 2000.

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

Kudoh, H., Sonoike, K.: Irreversible damage to photosystem I by chilling in the light: cause of the degradation of chlorophyll after returning to normal growth temperature. – Planta **215**: 541-548, 2002.

Leipner, J., Basilides, A., Stamp, P., Fracheboud, Y.: Hardly increased oxidative stress after exposure to low temperature in chilling-acclimated and non-acclimated maize leaves. – Plant Biol. **2**: 243-251, 2000.

Leipner, J., Fracheboud, Y., Stamp, P.: Effect of growing season on the photosynthetic apparatus and leaf antioxidative defenses in two maize genotypes of different chilling tolerance. – Environ. exp. Bot. **42**: 129-139, 1999.

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

Liu, P., Meng, Q., Zou, Q., Zhao, S., Liu, Q.: Effects of cold-hardening on chilling-induced photoinhibition of photosynthesis and on xanthophyll cycle pigments in sweet pepper. – Photosynthetica **39**: 467-472, 2001.

McKersie, B.D., Leshem, Y.Y.: Chilling stress. – In: McKersie, B.D., Leshem, Y.Y. (ed.): Stress and Stress Coping in Cultivated Plants. Pp. 79-103. Kluwer Academic Publishers, Dordrecht – Boston – London 1994a.

McKersie, B.D., Leshem, Y.Y.: Freezing stress. – In: McKersie, B.D., Leshem, Y.Y. (ed.): Stress and Stress Coping in Cultivated Plants. Pp. 104-131. Kluwer Academic Publishers, Dordrecht – Boston – London 1994b.

Minkov, I.N., Jahoubjan, G.T., Denev, I.D., Toneva, V.T.: Photooxidative stress in higher plants. – In: Pessarakli, M. (ed.): Handbook of Plant and Crop Stress. 2nd Ed. Pp. 499-525. Marcel Dekker, New York – Basel 1999.

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.

Öquist, G., Hurry, V.M., Huner, N.P.A.: Low-temperature effects on photosynthesis and correlation with freezing tolerance in spring and winter cultivars of wheat and rye. – Plant Physiol. **101**: 245-250, 1993.

Pietrini, F., Iannelli, M.A., Battistelli, A., Moscatello, S., Loreto, F., Massacci, A.: Effects on photosynthesis, carbohydrate accumulation and regrowth induced by temperature increase in maize genotypes with different sensitivity to low temperature. – Aust. J. Plant Physiol. **26**: 367-373, 1999.

Pocock, T.H., Hurry, V., Savitch, L.V., Huner, N.P.A.: Susceptibility to low-temperature photoinhibition and the acquisition of freezing tolerance in winter and spring wheat: the role of growth temperature and irradiance. – Physiol. Plant. **113**: 499-506, 2001.

Porra, R.J., Thompson, W.A., Kriedemann, P.E.: Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. – Biochim. biophys. Acta **975**: 384-394, 1989.

Rapacz, M., Hura, K.: The pattern of changes in photosynthetic apparatus in response to cold acclimation and de-acclimation in two contrasting cultivars of oilseed rape. – Photosynthetica **40**: 63-69, 2002.

Ribas-Carbo, M., Aroca, R., Gonzáles-Meier, M.A., Irigoyen, J.J., Sánchez-Díaz, M.: The electron partitioning between the cytochrome and alternative respiratory pathways during chilling recovery in two cultivars of maize differing in chilling sensitivity. – Plant Physiol. **122**: 199-204, 2000.

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.

Sage, R.F., Pearcy, R.W.: The physiological ecology of C_4 photosynthesis. – In: Leegood, R.C., Sharkey, T.D., Caemmerer, S. von (ed.): Photosynthesis: Physiology and Metabolism. Pp. 497-532. Kluwer Academic Publishers, Dordrecht – Boston – London 2000.

Savitch, L.V., Barker-Astrom, J., Ivanov, A.G., Hurry, V.,

Öquist, G., Huner, N.P.A., Gardeström, P.: Cold acclimation of *Arabidopsis thaliana* results in incomplete recovery of photosynthetic capacity, associated with an increased reduction of the chloroplast stroma. – Planta **214**: 295-303, 2001.

Savitch, L.V., Harney, T., Huner, N.P.A.: Sucrose metabolism in spring and winter wheat in response to high irradiance, cold stress and cold acclimation. – Physiol. Plant. **108**: 270-278, 2000.

Sonoike, K.: Various aspects of inhibition of photosynthesis under light/chilling stress: "Photoinhibition at chilling temperatures" *versus* "Chilling damage in the light". – J. Plant Res. **111**: 121-129, 1998.

Sonoike, K.: The different roles of chilling temperatures in the photoinhibition of Photosystem I and Photosystem II. – J. Photochem. Photobiol. B **48**: 136-141, 1999.

Sowinski, P., Dalbiak, A., Tadeusiak, J., Ochodzki, P.: Relations between carbohydrate accumulation in leaves, sucrose phosphate synthase activity and photoassimilate transport in chilling treated maize seedlings. – Acta Physiol. Plant. **21**: 375-381, 1999.

Sundar, D., Reddy, A.R.: Low night temperature-induced changes in photosynthesis and rubber accumulation in guayule (*Parthenium argentatum* Gray). – Photosynthetica **38**: 421-427, 2000.

Tichá, I., Kutík, J., Kosová, K., Holá, D., Kočová, M., Rothová, O., Langrová, K., Ždánská, H., Bajerová, K., Haisel, D., Wilhelmová, N., Mýtinová, Z.: Chloroplast response to cold stress in hybrids and inbreds of maize. – In: Roubelakis-Angelakis, K.A. (ed.): Book of Abstracts. 13th Congress of the Federation of European Societies of Plant Physiology. P. 647. University of Crete, Heraklion 2002.

van Wijk, K.J., van Hasselt, P.R.: Kinetic resolution of different recovery phases of photoinhibited photosystem II in cold-acclimated and non-acclimated spinach leaves. – Physiol. Plant. **87**: 187-198, 1993.

Venema, J.H., Villerius, L., van Hasselt, P.R.: Effect of acclimation to suboptimal temperature on chilling-induced photodamage: comparison between a domestic and a high-altitude wild *Lycopersicon* species. – Plant Sci. **152**: 153-163, 2000.

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.

Wellburn, A.R.: The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. – Plant Physiol. **144**: 307-313, 1994.

Wise, R.R.: Chilling-enhanced photooxidation: The production, action and study of reactive oxygen species produced during chilling in the light. – Photosynth. Res. **45**: 79-97, 1995.

Xu, Q., Shen, Y.K.: Light stress: Photoinhibition of photosynthesis in plants under natural conditions. – In: Pessarakli, M. (ed.): Handbook of Plant and Crop Stress. 2nd Ed. Pp. 483-497. Marcel Dekker, New York – Basel 1999.

Zervoudakis, G., Angelopoulos, K., Salahas, G., Georgiou, C.D.: Differences in cold inactivation of phosphoenolpyruvate carboxylase among C_4 species: The effect of pH and of enzyme concentration. – Photosynthetica **35**: 169-175, 1998.