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# Recovery of maize (*Zea mays* L.) inbreds and hybrids from chilling stress of various duration: Photosynthesis and antioxidant enzymes

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## Summary

The differences between two maize (*Zea mays* L.) inbred lines and their F1 hybrids in their response to chilling periods of various duration (1, 2, 3 or 4 weeks) and subsequent return to optimum temperatures were analysed by the measurement of the photosystem (PS) 1 and 2 activity, the photosynthetic pigments' content and the activity of antioxidant enzymes. The PS2 activity and the chlorophyll content decreased in plants subjected to 3 or 4 weeks of chilling, but not in those subjected to 1 or 2 weeks of chilling. This decrease was more pronounced in inbreds compared to their hybrids. The activity of superoxide dismutase did not much change with the increasing length of chilling period in the inbreds but decreased in the hybrids, the glutathione reductase activity increased in both types of genotypes but more in the inbred lines, while for ascorbate peroxidase and catalase the changes in parents-hybrids relationship did not show any specific trend. The PS1 activity and the carotenoids' content was not much affected.


*Abbreviations:* APX, ascorbate peroxidase; Car, carotenoids; CAT, catalase; Chl, chlorophyll; DCMU, 3-(3′, 4′-dichlorophenyl)-1, 1-dimethylurea; DCPIP, 2, 6-dichlorophenolindophenol; F1, the first filial generation; GR, glutathione reductase; HRA, Hill reaction activity; LHC, light-harvesting complexes; PAR, photosynthetically active radiation; PS, photosystem; RH, relative humidity; ROS, reactive oxygen species; SOD, superoxide dismutase; XTT, 3′-{1-[(phenylamino)carbonyl]-3, 4-tetrazolium}-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate

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## Introduction

Many agronomically important plants of tropical or subtropical origin, including maize (*Zea mays* L.), are exposed to low temperatures during their cultivation in temperate climate. They usually encounter these temperatures during spring as young plantlets, i.e. in the developmental stages particularly susceptible to chilling stress. Moreover, they experience not only chilling, but considerable day/night temperature fluctuations as well, because at this time of the year low temperatures most frequently occur at night or early morning and the temperature during the day often strongly increases. Low temperatures experienced during late spring can be particularly harmful to plants due to relatively long periods of daylight occuring at these times; it has been repeatedly shown that the chilling in the light is more stressful to plants compared to the chilling in the dark (Aguilera et al., 1999; Gray et al., 2003; Janda et al., 1994, 1996; Kudoh and Sonoike, 2002; Lidon et al., 2001; Nie et al., 1995; Pocock et al., 2001; Sonoike, 1998, 1999; Szalai et al., 1996, 1997; Tambussi et al., 2004; Venema et al., 2000).

In maize, the symptoms of chilling stress include a decrease of (a) plant growth rate and leaf elongation (Ben-Haj-Salah and Tardieu, 1995; Sowinski et al., 2005; Verheul et al., 1996), (b) uptake of water and nutrients by roots (Aroca et al., 2003; Sowinski and Królikowski, 1995), (c) various transport processes (Sowinski, 1995; Sowinski et al., 1998, 1999), (d) stomatal conductance (Aroca et al., 2003; Janda et al., 1998) and (e) photosynthetic performance (Aroca et al., 2001, 2003; Foyer et al., 2002; Fryer et al., 1995; Haldimann, 1997; Janda et al., 1998; Leipner et al., 1999), an increase in the production of reactive oxygen species (ROS) (Foyer et al., 2002) as well as of enzymatic and non-enzymatic antioxidants (Aroca et al., 2003; Foyer et al., 2002; Kocsy et al., 1997; Leipner et al., 1999; Skrudlik et al., 2000), or changes in membrane properties (Filek and Koscielniak, 1995; Pinhero et al., 1997), cellular and sub-cellular structure (Kutík et al., 2004; Pinhero et al., 1999), leaf anatomy (Sowinski et al., 2001), etc. However, the response of various genotypes to low temperatures can – often rather strongly – differ. The genotypes denoted as chilling-tolerant usually show a lower decrease in the rates of photosynthetic electron transport (Fracheboud et al., 1999; Haldimann, 1997; Ribas-Carbo et al., 2000; Verheul et al., 1995) and photosynthetic carbon metabolism (Pietrini et al., 1999), a lesser degradation of photosynthetic pigments and a different carotenoid composition (Aroca et al., 2001; Haldimann, 1997, 1999; Leipner et al., 1999), different values of the activities and/or content of several protective antioxidants (Aroca et al., 2001; Hodges et al., 1997; Horváth et al., 2002; Leipner et al., 1999; Pinhero et al., 1997), a greater accumulation of abscisic acid in leaves (Aroca et al., 2003; Janowiak et al., 2003) or a more efficient exchange of photosynthetic metabolites between mesophyll and bundle sheath cells (Sowinski et al., 2001, 2003). They are also able to better recover from chilling stress compared to the chilling-sensitive genotypes (Aguilera et al., 1999; Aroca et al., 2001; Haldimann, 1999).

The experiments aimed at the detailed dissection of chilling tolerance mechanisms are usually done with a few model inbred lines (Aroca et al., 2001, 2003; Haldimann, 1997, 1999; Janda et al., 1998; Kocsy et al., 1997; Leipner et al., 1999; Ribas-Carbo et al., 2000) but some authors examined also F1 hybrids and found that these hybrids display a greater tolerance to chilling and/or better recovering ability compared to their inbred parents (Aguilera et al., 1999; Fracheboud et al., 1999; Hardacre and Eagles, 1989; Holá et al., 2003; Körnerová and Holá, 1999; Kutík et al., 2004). This better adaptability of hybrids to unfavourable environmental conditions could be one of the possible causes of so-called positive heterotic effect, i.e. the better performance of hybrids over their parents, manifested today in various yield and morphological traits by the absolute majority of commercially grown maize cultivars.

The majority of these studies has been performed on plants cultivated in growth chambers under steady environmental conditions. The temperatures chosen as the stress-inducing ones either vary between day and night only minimally (in the range of 1–4 °C) (Pinhero et al., 1997, 1999; Janda et al., 1998; Haldimann, 1999; Pietrini et al., 1999) or do not vary at all (Aroca et al., 2001, 2003; Haldimann, 1997; Hodges et al., 1997; Janda et al., 1998; Kocsy et al., 1997; Ribas-Carbo et al., 2000). The application of the data obtained by such experiments to plants grown in natural and agricultural habitats (which experience substantial alterations of temperature during the day/night) can be therefore associated with various problems (Verheul et al., 1996). Moreover, the duration of chilling treatment is usually either very short (in the range of several hours (Haldimann, 1997; Janda et al., 1998) or – maximally 5, d (Aroca et al., 2001, 2003; Fracheboud et al., 1999; Hodges et al., 1997; Kocsy et al., 1997; Pinhero et al., 1997; Ribas-Carbo et al., 2000)) or rather long (30 and more days in the acclimation experiments (Janda et al., 1998; Leipner et al., 1999; Haldimann, 1999)) and

the physiological parameters are measured mostly during or immediately after (1–5 d) the end of the chilling treatment. The differences between chilling-tolerant and –sensitive genotypes in their response to low-temperature stress of medium duration and particularly in their recovering ability after transfer to optimum conditions for more than several days have been rarely examined (Hodges et al., 1997; Pietrini et al., 1999), and to our knowledge there has been as yet no examination of the relationship between F1 hybrids and parental lines in this respect.

Our study was therefore aimed at the analysis of possible differences between maize hybrids and their inbred parents in their response to (and their ability to recover from) chilling periods of various duration and day/night temperature range. These differences were followed by monitoring plant development and morphology, selected photosynthetic parameters and activities of antioxidant enzymes.

## Material and methods

### Plant material and growth conditions

Seeds of two maize inbred lines (2013 and CE810) and their reciprocal F1 hybrids (2013 × CE810, CE810 × 2013) were planted to low pots with soil and placed in a glasshouse (60–80% RH, no additional irradiance) under optimum temperature conditions (the ''warm'' glasshouse) for 12 d (till the appearance of the first leaf). Plants were then divided into five groups. 80 plants of each genotype were left in the warm glasshouse for 4 weeks as a control (Treatment 1). Remaining 320 plants of each genotype were transferred to another glasshouse with low-temperature conditions during night (the ''cold'' glasshouse) and subsequently returned to the warm glasshouse for various periods: (a) 1 week of chilling and 3 weeks at optimum temperatures (Treatment 2), (b) 2 weeks of chilling and 2 weeks at optimum temperatures (Treatment 3), c) 3 weeks of chilling and 1 week at optimum temperatures (Treatment 4), and d) 4 weeks of chilling (Treatment 5). Temperatures in both glasshouses were recorded in 1 h intervals during the whole growth of plants and their weekly averages and the daily mean temperature (average over 24 h) are shown in Fig. 1. Experiments were made from the end of February to the beginning of April, in four replicates. All measurements were carried out on 40d-old plants, and the youngest fully developed leaves, i.e. the third one for the Treatments 1, 2, 3 and 4, or the second one for the Treatment 5 were used for the preparation of samples.

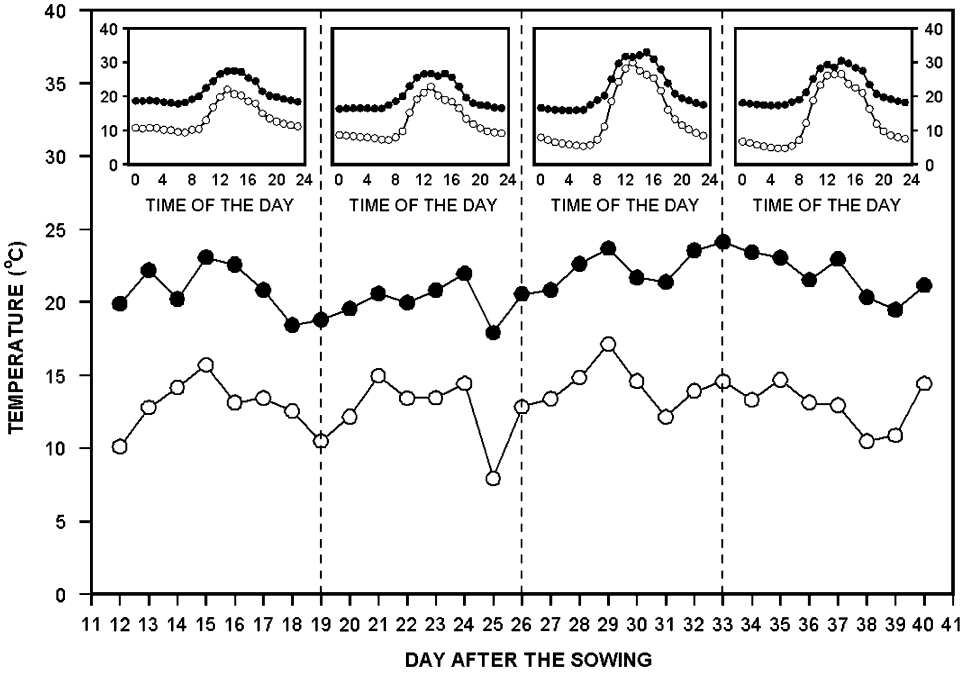


**Figure 1.** The daily mean air temperatures (average over 24 h) during whole period of plant cultivation, as recorded in the warm (black circles) or cold (white circles) glass houses. The inset graphs show the temperature time-course during 24 h (Middle European time) in the first, second, third or fourth week after the beginning of the low-temperature treatment (average over 7 d).

### Electron transport activities in isolated mesophyll chloroplasts

Mesophyll chloroplasts were isolated from the middle third of leaf blade using the procedure described by Holá et al. (2003). The Hill reaction activity (HRA) and the PS1 activity were measured polarographically (Clark type oxygen electrode, Theta' 90, Czech Republic) as the amount of oxygen formed or (in case of the PS1 activity) consumed by the suspensions of isolated chloroplasts irradiated with "white light" (170 W $m^{-2}$ PAR) after the addition of artificial electron acceptors or donors. 7 mM $K_3[Fe(CN)_6]$ was used as an electron acceptor in case of HRA measurements, and 0.25 mM DCPIP (reduced by 10 mM sodium ascorbate) as an electron donor, 0.1 mM methylviologen as an electron acceptor and 0.01 mM DCMU as a PS2 activity inhibitor in case of PS1 activity measurements. Each genotype/treatment in each replicate of experiment was measured two to four times and the mean values were used for the statistical analyses.

### Photosynthetic pigments' content

Six leaf discs (diameter 5 mm) from the middle third of leaf blade were put into 10 mL of *N*,*N*-dimethylformamide and stored in a refrigerator for 7 d; during this time the extracts were vortexed at regular intervals. The content of chlorophylls (Chl) *a* and *b* and total carotenoids (Car) in the extracts was then determined spectrophotometrically (Porra et al., 1989) (Anthelie 2 Advanced, Secomam, France). Each genotype/treatment was represented by three samples for each experimental replicate and the mean values were used for the statistical analyses.

### Antioxidant enzymes' activities

Two gram of the leaves were homogenized in extraction medium (0.1 M Tris, 0.001 M dithiothreitol, 0.001 M ethylenediaminetetraacetic acid disodium salt, 1% Triton X-100, 0.005 M ascorbate, pH 7.8). After 2 min ultrasound treatment in an ice bath, the samples were incubated in ice for 30 min, then they were centrifuged for 10 min, 20 000 × g at 2 °C (Heraeus 700, Osterode, Germany). For superoxide dismutase (SOD), the samples were desalted on Sephadex G-25. The supernatant was frozen in liquid nitrogen and stored at −70 °C for further analysis (Procházková and Wilhelmová, 2004). The activities of antioxidant enzymes were measured spectrophotometrically (Hitachi U 3300, Tokyo, Japan) at 25 °C. Total SOD activity was measured according to (Ukeda et al., 1997) with XTT. One unit of SOD activity was defined as an amount of enzyme necessary to produce a 50% inhibition of the XTT reduction rate. Ascorbate peroxidase (APX) activity was determined as a decrease in absorbance at 290 nm due to ascorbate oxidation (Nakano and Asada, 1981). Glutathione reductase (GR) activity was determined as a decrease in absorbance at 340 nm due to oxidation of NADPH (Schaedle and Bassham, 1977). Catalase (CAT) activity was estimated polarographically (Del Río et al., 1977) using a liquid-phase oxygen electrode (Hansatech Instruments, King's Lynn, Great Britain). The soluble protein content was determined by the Bradford (1976) method with standard curves prepared using bovine serum albumin.

### Statistical analysis

Two-way analysis of variance with interactions and Tukey's HSD tests were used to determine the statistical significance of the differences between treatments (T), genotypes (G) or the interaction G × T (CoStat, CoHort Software, USA).

## Results

### Photosynthetic characteristics

The exposure of plants to chilling temperatures for 1 week, followed by their subsequent return to optimum temperatures for 3 weeks, i.e. Treatment 2, did not decrease either HRA, PS1 activity (Fig. 2), or the content or ratios of Chl and Car (Fig. 3). Actually, in some cases the values of these characteristics were slightly, though non-significantly, higher compared to those measured in the control plants. No significant differences between the control plants and the plants subjected to Treatment 3 (2 weeks of chilling, 2 weeks at optimum temperatures) were usually found, either (Figs. 2 and 3). Longer exposure to chilling followed by shorter time for recovery (Treatment 4) significantly decreased the content of Chl in all genotypes except CE810 (Fig. 3), the Chl/Car ratio and the HRA in 2013 inbred line (Figs. 2 and 3), and increased the Chl *a*/*b* ratio in F1 hybrids (Fig. 3). Further decrease of the Chl content and the Chl/Car ratio in all genotypes, the decrease of the HRA in both inbred lines, and the increase of the Chl *a*/*b* ratio in F1 hybrids was observed in the plants subjected to continuous chilling, i.e. Treatment 5

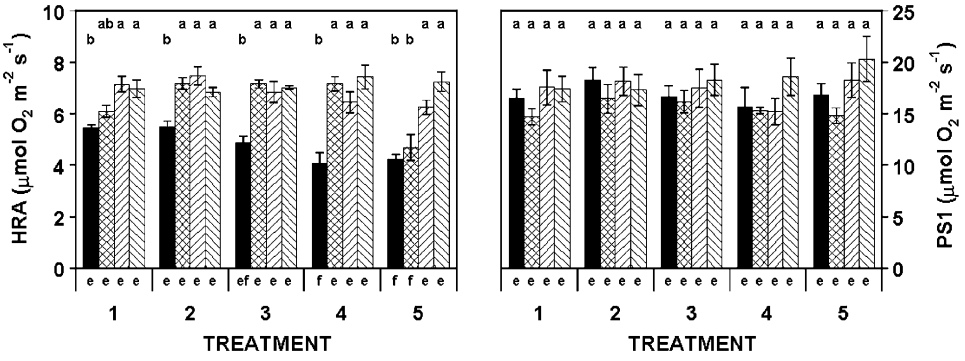


**Figure 2.** Hill reaction activity (HRA) and Photosystem 1 activity (PS1) in mesophyll chloroplasts isolated from leaves of two maize inbred lines 2013 (solid bars) and CE810 (crosshatched bars) and their reciprocal F1 hybrids 2013 × CE810 (/ hatched bars) and CE810 × 2013 (\ hatched bars). Plants were subjected either to (a) 4 weeks at optimum (O) conditions (Treatment 1), (b) 1 week at low-temperature conditions (L) followed by 3 weeks at O (Treatment 2), (c) 2 weeks at L followed by 2 weeks at O (Treatment 3), (d) 3 weeks at L followed by 1 week at O (Treatment 4), or (e) 4 weeks at L (Treatment 5). Means ± SEM ($n = 4$) are shown. Letters **a**–**d** at the top of graphs denote the statistical significance (Tukey's HSD test) of the differences between individual genotypes for each temperature treatment, letters **e-i** at the bottom of graphs denote the statistical significance of the differences between temperature treatments for each genotype examined (only those marked with the different letters differ significantly at $P < 0.05$).

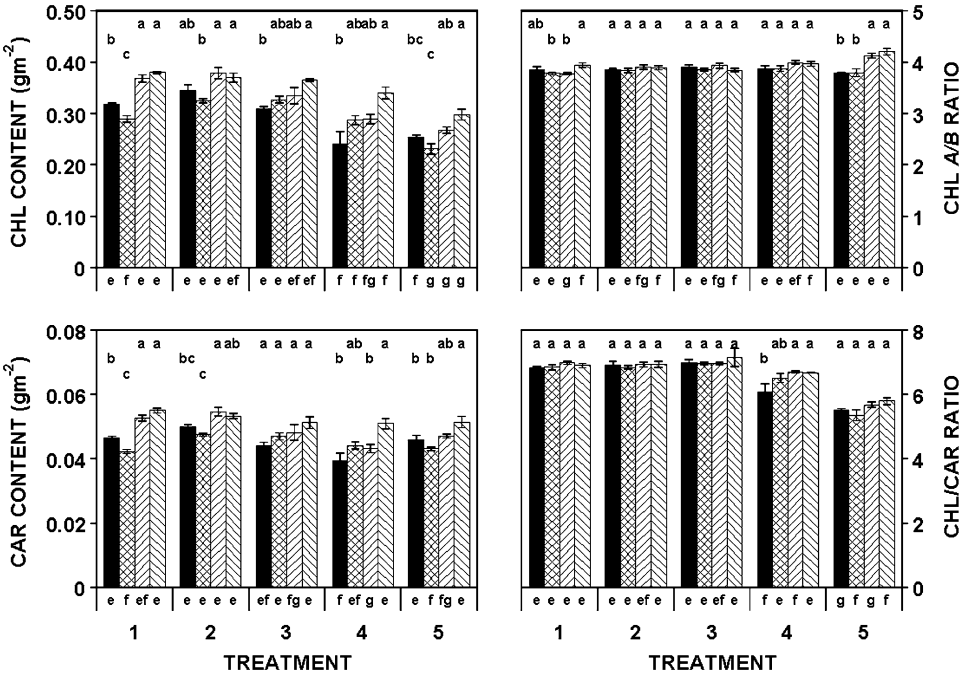


**Figure 3.** Content of total chlorophyll (Chl), total carotenoids (Car), and the ratio of chlorophylls *a*/*b* and total chlorophyll/total carotenoids in leaves of two maize inbred lines 2013 (solid bars) and CE810 (crosshatched bars) and their reciprocal F1 hybrids 2013 × CE810 (/ hatched bars) and CE810 × 2013 (\ hatched bars). For description see legend to Fig. 2.

(Figs. 2 and 3). On the other hand, these plants displayed slightly, though non-significantly higher PS1 activity compared to other groups (Fig. 2).

The inbred line 2013 was characterized by the lowest HRA values among all four genotypes; this applied for all temperature treatments but was particularly marked in Treatments 4 and 5. In these two groups F1 hybrids displayed positive heterotic effect (Fig. 2). The same generally applied for the content of Chl, though the inbred line CE810 shared with the 2013 the lowest Chl content in several temperature treatments (Fig. 3). Both F1 hybrids displayed also higher Car content compared to their parents, but these differences were not always

statistically significant (Fig. 3). No specific trend for genotypical differences was found either for the Chl *a*/*b* ratio, the Chl/Car ratio or the PS1 activity; actually, these parameters differed between genotypes only exceptionally (Figs. 2 and 3).

### Activities of antioxidant enzymes

The control plants (Treatment 1), particularly 2013 and 2013 × CE810, usually showed relatively low activity of both APX and GR compared to other treatments. Their SOD and CAT activities were rather high, particularly in 2013 × CE810 F1 hybrid (Fig. 4). The highest values of both APX and GR activities were (again with the exception of inbred line CE810) observed in the continually chilled plants, i.e. Treatment 5. On the other hand, the plants subjected to this treatment showed either lower SOD activity compared to control (F1 hybrids) or did not differ from the control plants nor from other treatments (inbred lines) (Fig. 4). The activity of CAT was low in CE810 and 2013 × CE810, but did not decrease in 2013 and CE810 × 2013 genotypes (Fig. 4). As for the other three temperature treatments, the activities of SOD and CAT usually did not much differ among them (though some decreasing trend from Treatment 1–4 could be found for F1 hybrids). However, we observed a positive dependence of the APX and GR activities on the length of chilling treatment in inbred line 2013. In F1 hybrids, the activity of GR in the plants subjected to Treatment 3 was higher compared to those subjected to Treatments 1, 2 or 4, and the APX activity of the plants subjected to Treatment 2 was higher compared to Treatments 1, 3 or 4. The differences between Treatments 1 and 4 in inbred line CE810 were less pronounced (Fig. 4).

F1 hybrids were generally characterized by lower activity of APX compared to their parental lines in all treatments examined. The inbred line CE810 showed the highest activity of this enzyme in Treatments 1 and 2, but line 2013 surpassed it in Treatments 3, 4 and 5 (Fig. 4). Similar changes in the relationship between this line and the other genotypes, i.e. the rise of the 2013 values with the increasing length of chilling treatment, were observed for the GR activity (Fig. 4). Rather interesting positive relationship between F1 hybrid and its maternal parent (i.e., 2013 × CE810 and 2013, or CE810 × 2013 and CE810) could be observed both for SOD and CAT activities, and to a slight degree, for GR activity as well (Fig. 4).

## Discussion

The exposure of maize plants to low temperature has a negative effect on plant development, morphology and physiology, even if its duration is rather short. With prolonged chilling, plants can

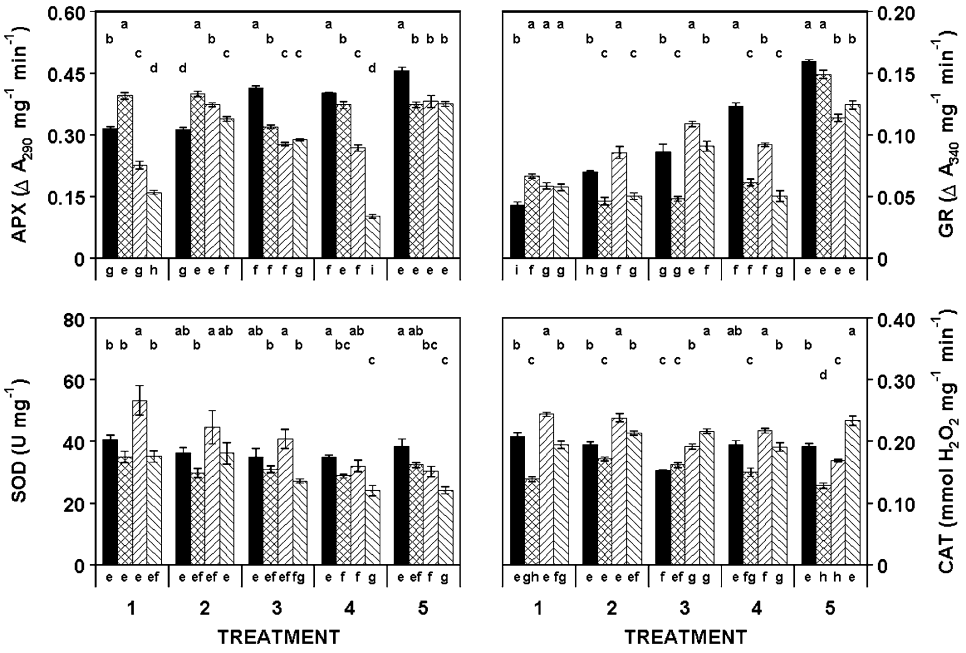


**Figure 4.** Activity of ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) in leaves of two maize inbred lines 2013 (solid bars) and CE810 (crosshatched bars) and their reciprocal F1 hybrids 2013 × CE810 (/ hatched bars) and CE810 × 2013 (\ hatched bars). For description see legend to Fig. 2.

either suffer substantial damage (often strongly hampering their viability and reproduction ability) or acclimate to the unfavourable conditions. However, even in the latter case a possibility still remains that after the rise of the temperature, the plants will not be able to restore the functionality of their metabolism to a prior-to-chilling level, or will actually suffer from this ''favourable'' temperature change more than from the chilling itself.

The response of various maize genotypes to low temperature often strongly differs and various physiological, biochemical or anatomical causes of this intraspecific variability have been described. Heterosis (i.e. hybrid vigor), the well-known phenomenon in this species, can also be at least partly attributed to the lower sensitivity of hybrids to chilling and other unfavourable environmental factors. However, the exact physiological or biochemical causes of this better tolerance displayed by hybrids are mostly unknown. As low temperature affects mainly leaves with photosynthetically active chloroplasts, the tolerance to chilling, as well as the good recovery of plants from it, could result either from the lower sensitivity of photosynthetic apparatus, or from the increased activity/synthesis of various protective compounds. We have therefore studied these parameters in two inbreds and their reciprocal F1 hybrids that were exposed as very young seedlings to chilling periods of various duration followed by subsequent return to optimum temperatures.

The inbred line 2013 was the most affected among all examined genotypes, both in photosynthetic characteristics and antioxidant enzymes' activities. Its PS2 activity was severely affected by cold and the plants of this line were able to withstand well only 1 week of chilling. Even after 2 weeks under the optimum temperature conditions this line retained high activity of APX and GR, which is an indirect evidence for an increased production of highly damaging ROS. It is probable that at least some mesophyll chloroplasts – even in the leaves that continued their development after the end of the chilling treatment – could not in this inbred line recover from the damage caused by exposure of plants to chilling. Nie et al. (1995) showed that such heterogeneity in the response of mesophyll cells to a change in temperature from low to optimum values actually exists and is probably caused by the restrictions in the synthesis of various thylakoid proteins.

The second inbred line examined, CE810, was able to withstand the transfer to optimum conditions rather well; even 1 week in warm temperatures was sufficient for the complete recovery of its PS2 activity from the chilling-caused damage. The activities of antioxidant enzymes in plants subjected to temperature change did not differ from the control and the need for deactivation of ROS was thus probably much lesser than in 2013. However, the continuous chilling not followed by the transfer of plants to optimum conditions was still harmful for this inbred line.

The best ability to deal both with the chilling itself and the return to optimum conditions was displayed by F1 hybrids. It further confirmed our previous findings of the distinctive superiority of hybrids over their parental lines, found during the examination of the effect of long-term exposure of maize plants to low temperatures (Holá et al., 2003). This superiority could – in case of the hybrid plants returned to optimum growth conditions – be related to the slightly delayed development of their leaves (which would therefore have a greater chance to develop in the optimum temperatures compared to both parental lines), but as it was displayed also by the continually chilled plants, it is more probable that chloroplasts of the hybrids were less damaged or better able to adjust to unfavourable conditions. The fact that the activities of both PS2 and PS1 in all cold-stressed hybrids did not differ from the control plants clearly shows that these important components of photosynthetic electron-transport chain were damaged neither by the exposure of plants to low temperature nor by their return to the optimum conditions. The need for the detoxification of ROS was also less in hybrids compared to their parental lines, as seen from the values of the APX activity (and GR and SOD activities in the long-term-chilled plants). Thus, it can be concluded that the better tolerance of F1 hybrids to low temperature is probably caused by the lower sensitivity of the individual components of their photosynthetic apparatus, which results in the lower production of ROS and the lower need for the mobilization of protective antioxidant enzymes.

An interesting relationship between the parents and hybrids was found for the GR, SOD and CAT activities; the activity of these enzymes in hybrids showed a direct dependence on the performance of their maternal parent. A care should be thus taken in the breeding programmes aimed at the improvement of maize tolerance to low temperatures, whether the desirable parent is used as the maternal or paternal one, as this can have important consequences on the protective processes occuring in the chloroplasts.

As regards the differences in the changes of photosynthetic parameters and antioxidant enzymes' activities caused by the different length of the plant exposure to low temperatures, it was

evident that the shorter the duration of the exposure of the developing leaf to low temperatures, the lesser the possibility of the irreversibility of this damage. Two weeks, or even one week (depending on the genotype) of subsequent growth under optimum conditions were quite sufficient for the complete recovery of the efficiency of the PS2 and the Chl content in the third leaf, though it started to develop in the chilling conditions. The activities of antioxidant enzymes in the leaves also did not differ much from the values measured in control plants providing that plants were returned to optimum temperatures after chilling (Treatments 2–4). Actually, the activities of antioxidant enzymes can adapt to temperature of environment quite quickly in a range from 1 d to 1 week (Aroca et al., 2001; Leipner et al., 1999; Skrudlik et al., 2000). However, the inbred line 2013 and its hybrid 2013 × CE810 displayed higher values of GR activity and APX activity compared to control plants even in these cases, which shows that the need for the increased activity of ascorbate-glutathione cycle in these plants still persisted.

Sowinski et al. (2005) worked with maize grown under continuous chilling conditions until the third leaf stage and then transferred to optimum conditions. They observed a complete recovery of the PS2 photosynthetic efficiency only from the seventh leaf upwards. The discrepancy between their and our results could be explained by various factors. These authors, as well as Nie et al. (1995) who also observed only the incomplete recovery of the photosynthetic electron transport after transfer of 40 d-chilled plants to optimum temperatures, measured the photosynthetic efficiency on the already fully developed leaf whereas in our case this leaf in the time of plant transfer to optimum conditions has not yet finished its development. Haldimann (1996) found that the immature leaves are able to better cope with the transfer to optimum temperatures compared to the mature, fully developed ones.

Another reason for the difference between our results and those of Sowinski et al. (2005) could be that in their case, chilling was continuous (i.e. even during the day) and plants were therefore exposed to the joint effect of low temperature and light, which could induce stronger damage. Moreover, towards the afternoon the temperatures in our non-tempered glasshouse increased to the more favourable range. Skrudlik et al. (2000), who studied the effect of the interruption of chilling treatment by several hours of non-chilling temperatures on the maximum quantum yield of PS2, found that even 4-h break in the chilling treatment has a favourable effect on the recovery of this parameter. The electrolyte leakage and the intensity of net photosynthesis, as well as other parameters, have been also lower in plants stressed by chilling only during the night period, compared to the continually-stressed ones (Markowski and Skrudlik, 1995; Skrudlik et al., 2000). It seems that the differences between the response of the plants exposed to constant low temperature (i.e. cultivated in the growth chambers or controlled-environment cabinets) and those subjected to chilling in the field or in the conditions more resembling the natural conditions that plants encounter in temperate zones of Europe (as was our case) can be rather strong. We should be therefore always extremely careful when applying the results obtained from the growth chamber-grown plants to the field-grown ones.

In conclusion, the results of our work showed that some genotypes of maize can very well withstand even long-term chilling without irreversible damage to their photosynthetic apparatus, supposing that the temperature conditions are such as those naturally occuring in the middle Europe during early spring (i.e. not the continuous day-and-night chilling simulated in many experiments with plants cultivated in the grown chambers) and that they can fully recover from the consequences of such a chilling if a period of at least 1 week of warm temperatures follows. In this capacity, the hybrids are clearly superior to their inbred parents, and this superiority is probably caused by the lower sensitivity of their photosynthetic apparatus and the lesser production of ROS resulting in the lower overall damage to chloroplasts.

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