The influence of low-temperature on the photochemical activity of chloroplasts and activity of antioxidant enzymes in maize leaves

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Abstract

The effects of low growth temperature on the activities of photosystems (PS) 1 and 2 and antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT) in leaves of various maize inbred and hybrid genotypes (parental lines, F1 hybrids, F2 and backcross generations) were investigated. Considerable decrease of the PS 2 activity (contrary to the activity of PS 1) due to low-temperature stress was observed in the majority of genotypes/generations examined. The GR, APX and SOD activities markedly increased due to chilling, whereas the activity of CAT showed lesser changes which depended on the genotype/generation analysed. The higher susceptibility of the inbred line 2013 to low temperature was transmitted to further generations in case this line was used as the maternal parent. The intraspecific variability in photosynthetic and antioxidant parameters was caused particularly by the dominance (negative or positive), however, the level of the expression of this effect often changed after low-temperature stress and was probably the cause of the increase in the positive F1 heterosis observed in this case. Other genetic effects (*e.g*. the additivity, and particularly the additive or dominant maternal effects) were also found to contribute to the intraspecific variability in parameters analyzed. The dominant maternal effects possibly played an important role in maintaining positive heterosis in F2 generation.

Additional key words: additivity, cold stress, dominance, heterosis, intraspecific variability, maternal effects, photosynthesis, photosystems 1 and 2.

Introduction

Temperature is one of the most important environmental factors affecting plants. Plant response to low temperature involves structural and functional changes on the level of cellular organelles, whole cells, tissues and plants. Photosynthetic processes are usually the first to be influenced by chilling temperatures (Hallgren and Öquist 1990, Baker *et al.* 1994, Foyer *et al.* 2002). Low temperature markedly affects photosynthetic capacity, particularly in chilling-sensitive plants (Nie *et al.* 1992, Haldimann 1999, Yamori *et al.* 2006). It modifies the lipids and some other components of chloroplast membranes and these changes lead to the decrease of membrane fluidity followed by the changes of membrane functions (Long *et al*. 1983, Kościelniak *et al*. 1996). However, the major effect of low temperature on photosynthetic activity is due to the decrease of CO_2 fixation as a consequence of the decrease in enzymatic activities. These changes are accompanied by the over-energization of PS 2 and PS 1 during irradiance, which results in the photoinhibition, in the changes of the assembly of pigment-protein complexes, in the formation of reactive oxygen species and in the overall reduction of

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Abbreviations: *ANOVA* - analysis of variance; APX - ascorbate peroxidase; CAT - catalase; F1, F2 - the first/second filial generation; GR - glutathione reductase; PAR - photosynthetically active radiation; PS - photosystem; RH - relative humidity; SEM - standard error of mean; SOD - superoxide dismutase; XTT - 3´-{1-[(phenylamino)carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate.
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electron-transport chain activity (Greer and Hardacre 1989, Krause 1994, Sonoike 1999, Aroca *et al.* 2001, Lidon *et al.* 2001). Although PS 2 is probably more sensitive to low-temperature damage (Haldimann 1997), PS 1 is also not fully immune to the photodamage during low-temperature conditions; however, the damage is in this case probably caused by different mechanisms (Sonoike 1999, Kudoh and Sonoike 2002).

The plants have developed various protective systems to minimize stressful effects of low temperature (Hodges *et al.* 1997, Foyer *et al.* 2002, Takac 2004, Zou *et al.* 2006, Chinnusamy *et al.* 2007, Kosová *et al.* 2007, Sunkar *et al.* 2007), that include the activation of antioxidant systems protecting plants against reactive oxygen species. Several authors reported that chilling-resistant species have more efficient antioxidant systems compared to the sensitive ones (Jahnke *et al.* 1991, Walker and McKersie 1993, Wise 1995, Leipner *et al.* 1999). In maize, antioxidant enzymes were not significantly affected by chilling in the dark (Anderson *et al.* 1995). However, under irradiance the low temperature induced increase in APX and SOD activities (Massacci *et al.* 1995, Hodges *et al.* 1997). CAT is also sensitive to adverse conditions including chilling under light and, regardless of the chilling tolerance of the respective species, its activity was found to decrease in suboptimum temperatures in leaves of various plants (Leipner *et al.* 1999, Scebba *et al.* 1998, Kingston-Smith *et al.* 1999, Shen *et al.* 1999). On the other hand, GR activity was elevated in maize lines during low temperature treatment (Hodges *et al.* 1997, Leipner *et al.* 1999). The responses of antioxidant enzyme activities to cold are thus evidently dependent on the plant species, the severity and duration of the chilling stress and also on the relative units used for expressing the enzyme activity.

The considerable inter- and intraspecific variability has been found for various photosynthetic traits depending on plant susceptibility or tolerance to low temperature (*e.g.* Greer and Hardcare 1989, Aguilera *et al.* 1999, Aroca *et al.* 2001, Lidon *et al.* 2001, Bertamini *et al.* 2007). The same applies for the variability in the activity of antioxidant enzymes, though the number of studies dealing with this topic is lower (*e.g.* Kocsy *et al.* 1997, Leipner *et al.* 1999, Aroca *et al.* 2001). Maize is a chilling sensitive species with a little capacity for acclimation to low temperatures, which is, nevertheless, often cultivated in areas subject to suboptimum or variable temperatures. The thorough understanding of its response to low temperature is essential for the production of new cold-tolerant genotypes (Greer and Hardacre 1989, Verheul *et al.* 1996, Fracheboud *et al.* 1999, Leipner *et al.* 1999, Revilla *et al.* 2000, Sowinski *et al.* 2005). Unfortunately, physiological, biochemical and particularly genetical mechanisms responsible for the greater tolerance to chilling displayed by some maize genotypes are still poorly understood. The differences in the response of inbred and hybrid genotypes to low temperature and the changes in genetic determination of the photosynthetic parameters under chilling conditions have been only rarely studied (Fracheboud *et al.* 1999, Körnerová and Holá 1999, Holá *et al.* 2003), the influence of the parental genotype on the performance of the progeny regarding these characteristics has, to our knowledge, never been studied. No information exists in this area also in case of the activities of antioxidant enzymes. Thus, the objectives of this work were to study the photochemical efficiency of chloroplasts and the activity of antioxidant enzymes in inbred and hybrid maize genotypes grown under optimum/suboptimum temperatures, and to perform the genetic analysis of these parameters aimed at ascertaining the genetic mechanisms that participate in their inheritance. Particular attention was paid to the possible influence of inbred or hybrid maternal genotype on the response of its progeny.

Materials and methods

Plants: Three parental inbred lines of *Zea mays* L. (2013, CE704, CE810), their F1 hybrids (2013×CE810, CE810×2013, CE704×CE810, CE810×CE704; the first genotype of the cross being the maternal parent), F2 generations (self-pollinated F1 hybrids 2013×CE810 and CE704×CE810) and backcrosses of either of the parents on these F1 hybrids were used for the measurements of the photosynthetic efficiency of isolated chloroplasts and the activities of antioxidant enzymes. The seeds of inbred lines and F1 hybrids were obtained from the Maize Breeding Station CEZEA (Čejč, Czech Republic). The parental lines were selected from the wider range of genotypes on the basis of their contrast in some yield and photosynthetic parameters. The necessary hybridisation (for obtaining the F2 and the backcross generations which were necessary for the quantitative genetic analysis) was carried out at the experimental parcels of the Genetic Garden, Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague. The kernels of all genotypes/generations were sown into the pots filled with garden soil, and the seedlings were cultivated in a glasshouse under optimum temperature conditions (day/night temperature 24 - 27/16 - 20 °C, 60 - 80 % RH, no additional irradiance). 12 d after sowing, one half of plants was left to grow in optimum conditions (control plants), the second half (stressed plants) was grown in a non-heated glasshouse (day/night temperature of 14 - 18/0 - 5 °C) for 26 d. Two sets of experiments were made (from the end of March to the end of April), each in four replications.

Photochemical activity of isolated chloroplasts: Leaf tissue (2 - 3 g) was taken from the middle part of the youngest fully developed leaf (usually the third one from the top) of 10 - 15 maize plants per each replication. Mesophyll chloroplasts were isolated from the leaf tissue

using standard techniques after homogenisation of leaf sample (Holá *et al*. 2003, 2007). Their photochemical activity was measured polarographically (Clark type oxygen electrode, *Theta´90*, Prague, the Czech Republic) as the increase (PS 2) or decrease (PS 1) of oxygen amount in the suspensions of isolated chloroplasts after their irradiation (170 W m^{-2} PAR) and addition of the necessary electron donors, acceptors and other cofactors (for more details see Holá *et al*. 2003, 2007).

The activity of antioxidant enzymes: The response of maize antioxidant system to low-temperature was determined as the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR). The leaves were homogenised (Procházková and Wilhelmová 2004), and enzymatic activities in the extracts from homogenate were determined either spectrophotometrically (SOD, APX, GR) (*Hitachi UV 3300*, Tokyo, Japan) or polarographically (CAT; Del Río *et al*. 1977) using the liquid-phase oxygen electrode (*LD1*, *Hansatech Instruments*, King's Lynn, U.K.). Tho 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. The APX activity was determined as a decrease in absorbance at 290 nm due to ascorbate oxidation (Nakano and Asada 1981), the GR activity was determined as a decrease in absorbance at 340 nm due to oxidation of NADPH (Schaedle and Bassham 1977). The soluble protein content was determined by the Bradford method (1976) with standard curves prepared using bovine serum albumin.

Statistical and genetic analysis: The means ± SEM of all parameters were computed from eight values representing the eight independent replicates. The differences between genotypes/generations or between control and stressed plants were analysed by two- and one-way *ANOVA* with interactions and Tukey-Kramer´s non-parametric test using *CoStat* computer programme, version *6.204* (*CoHort Software*, Monterey, CA, USA).

Quantitative genetic analysis aimed at the detection of several types of genetic effects (additivity, dominance, maternal effects) was based on the modified model of Eberhart and Gardner (1966). This analysis is based on the presumption that the mean values of the measured characteristics (as determined for the respective genotypes/generations) can be expressed by linear combination of parameters representing individual genetic effects (see Table 1 for the appropriate equations). Four possible models were consecutively used: the first one included only the additive genetic effects (besides the parameter representing general mean), in the second model the dominant genetic effects were also included, the third one further added the additive maternal genetic effects into the equations, and the last model included all possible combinations of genetic parameters (Table 1). The values of the respective genetic parameters were always estimated from these equations by the weighted least squares method and the goodness-of-fit for each model was tested by the χ^2 test. When the χ^2 value for the simplest model (*i.e*. the one with the additivity only) exceeded the critical value ($P = 0.05$), this model was rejected and the second model (*i.e*. including the dominance) was used for the estimation of the genetic parameters. In the event that even this model was not appropriate, the third model was used, *etc*. The final values of the genetic parameters were estimated always from the best-fitting model and their statistical significancy was tested by *t*-tests. The computer programme *CBE3* from the *Software Package CBE*, version *4.0* (Research Institute of Animal Production, Prague, Czech Republic), was used for this analysis.

Table 1. The equations representing the mean values of the measured characteristics as determined for the respective genotypes/generations, based on the linear combination of parameters representing individual genetic effects. *m* - general mean of the respective characteristic (across all generations analysed), *a* - additive effect of the respective parental inbred line (as indicated by lower index), *d* - dominance effect of the specific combination of the respective parental inbred lines, *Ma* - additive maternal effect of the respective parental inbred line, *Md* - additive maternal effect of the specific combination of the respective parental inbred lines.

Genotype/generation	
2013	$m + a_{2013} + Ma_{2013}$
CE704	$m + a_{CE704} + Ma_{CE704}$
CE810	$m + a_{CE810} + Ma_{CE810}$
2013×CE810	$m + ½\ a_{2013} + ½\ a_{CE810} + d_{2013\times CE810} + Ma_{2013}$
CE704×CE810	$m + ½\ a_{CE704} + ½\ a_{CE810} + d_{CE704\times CE810} + Ma_{CE704}$
CE810×2013	$m + ½\ a_{2013} + ½\ a_{CE810} + d_{2013\times CE810} + Ma_{CE810}$
CE810×CE704	$m + ½\ a_{CE704} + ½\ a_{CE810} + d_{CE704\times CE810} + Ma_{CE810}$
F2 $_{(2013\times CE810)\times(2013\times CE810)}$	$m + ½\ a_{2013} + ½\ a_{CE810} + ½\ d_{2013\times CE810} + ½\ Ma_{2013} + ½\ Ma_{CE810} + Md_{2013\times CE810}$
F2 $_{(CE704\times CE810)\times(CE704\times CE810)}$	$m + ½\ a_{CE704} + ½\ a_{CE810} + ½\ d_{CE704\times CE810} + ½\ Ma_{CE704} + ½\ Ma_{CE810} + Md_{CE704\times CE810}$
B (2013×CE810)×2013	$m + ¾\ a_{2013} + ¼\ a_{CE810} + ½\ d_{2013\times CE810} + ½\ Ma_{2013} + ½\ Ma_{CE810} + Md_{2013\times CE810}$
B (CE704×CE810) ×CE704	$m + ¾\ a_{CE704} + ¼\ a_{CE810} + ½\ d_{CE704\times CE810} + ½\ Ma_{CE704} + ½\ Ma_{CE810} + Md_{CE704\times CE810}$
B (2013×CE810)×CE810	$m + ¼\ a_{2013} + ¾\ a_{CE810} + ½\ d_{2013\times CE810} + ½\ Ma_{2013} + ½\ Ma_{CE810} + Md_{2013\times CE810}$
B (CE704×CE810)×CE810	$m + ¼\ a_{CE704} + ¾\ a_{CE810} + ½\ d_{CE704\times CE810} + ½\ Ma_{CE704} + ½\ Ma_{CE810} + Md_{CE704\times CE810}$

Results

The cultivation of maize plants in low-temperature resulted in the changes of photochemical activities of isolated mesophyll chloroplasts as well as the activities of antioxidant enzymes. Nevertheless, the type and the magnitude of these changes depended both on the genotype and the parameter analysed. Low-temperature stress caused the considerable decrease of PS 2 activity in the majority of genotypes (Fig. 1). The differences between stressed and non-stressed plants were statistically significant in all inbred lines. Similar decrease of PS 2 activity was found also in the F1 hybrids 2013×CE810 and CE704×CE810 and in all F2 and backcross generations. The susceptibility to low temperature was particularly marked in the inbred line 2013 and the significant decrease of the PS 2 activity was observed also in the F1 hybrid 2013×CE810, where the line 2013 was used as the maternal genotype. This negative response to cold was retained in the further generations (F2, backcrosses) as well (Fig. 1). On the other hand, the plants of F1 generation with inbred line CE810 used as the maternal parent were tolerant or less susceptible to low temperature and this was true also for the further generations of the CE704×CE810 hybrid combination. The PS2 activity of F1 hybrids CE810×2013 and CE810×CE704 even increased during stress conditions, although this increase was statistically significant only in

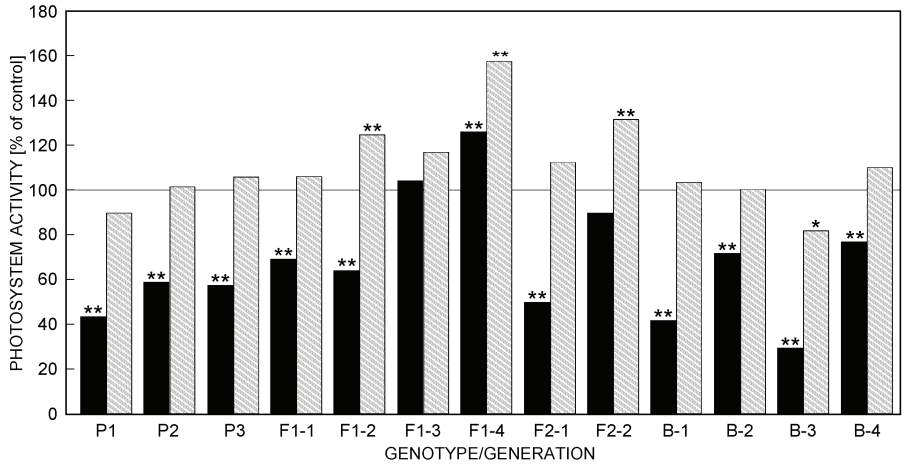

Fig 1. The photosystem 2 (*solid bars*) and photosystem 1 (*hatched bars*) activities in mesophyll chloroplasts isolated from leaves of several genotypes/generations of maize grown in chilling conditions. The percentages of the mean value of the respective characteristics recorded under chilling conditions relative to the appropriate mean value under control conditions are shown (n = 8). The statistical significance of the differences between the means recorded under chilling *vs.* control conditions is indicated by * and ** symbols (0.05 and 0.01 levels of statistical significance, respectively). P1, P2, P3 - inbred lines 2013, CE704 and CE810, respectively; F1-1 to F1-4 - F1 hybrids 2013×CE810, CE704×CE810, CE810×2013 and CE810×CE704, respectively; F2-1 and F2-2 - F2 generation, *i.e.* self-pollinated 2013×CE810 and CE704×CE810 F1 hybrids, respectively; B-1 to B-4 - backcrosses of either of the parents on the F1 hybrids, *i.e.* (2013×CE810)×2013, (CE704×CE810)×CE704, (2013×CE810)×CE810 and (CE704×CE810)×CE810, respectively.

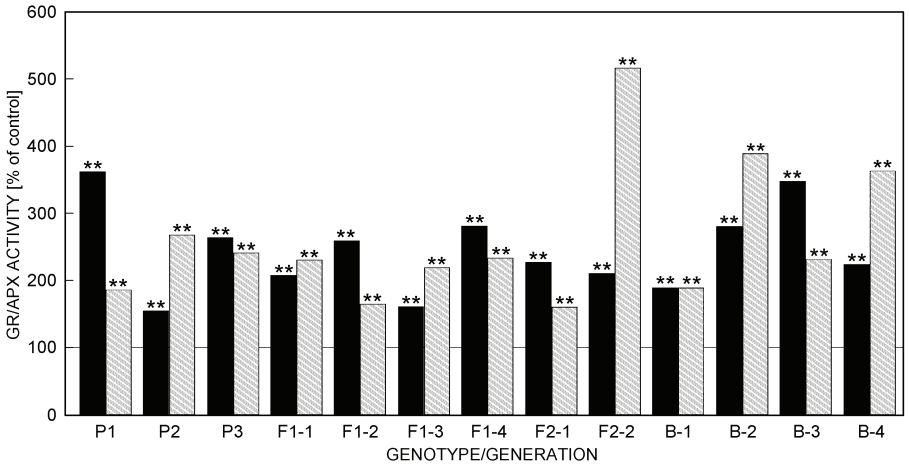

Fig 2. The glutathione reductase (*solid bars*) and ascorbate peroxidase (*hatched bars*) activities in leaves of several genotypes/ generations of maize grown in chilling conditions. For legend see Fig. 1.

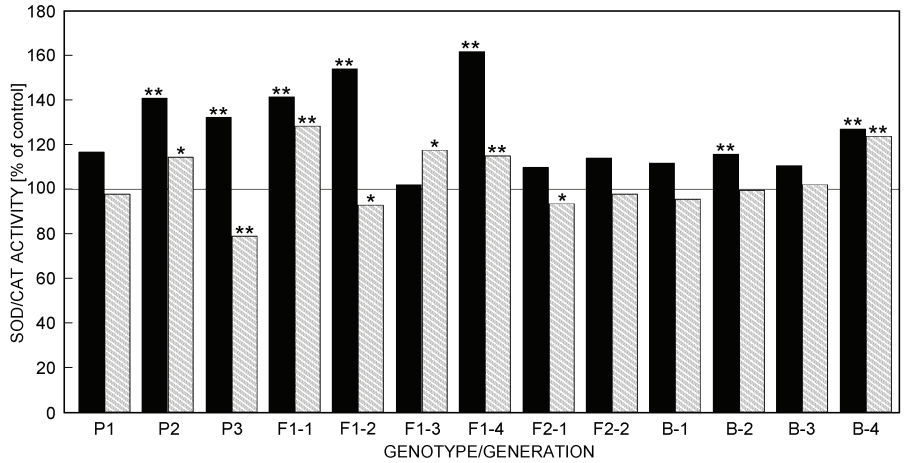

Fig 3. The superoxide dismutase (*solid bars*) and catalase (*hatched bars*) activities in leaves of several genotypes/generations of maize grown in chilling conditions. For legend see Fig. 1.

Table 2. The mid-parent heterosis (% of parental mean) in the photochemical activities of PS 1 and PS 2 or the activities of SOD, APX, GR and CAT as expressed in F1 and F2 generations of maize grown in optimum and low-temperature.

	Genotype/generation	PS 2	PS 1	SOD	APX	GR	CAT
Control plants	2013×CE810	116.59	98.16	119.46	74.19	140.37	89.90
	CE704×CE810	103.11	94.32	86.22	108.80	75.93	96.10
	CE810×2013	124.61	109.90	141.38	69.31	136.35	105.53
	CE810×CE704	79.24	69.90	88.55	80.38	59.37	95.94
	F2 $_{(2013\times CE810)\times(2013\times CE810)}$	109.68	90.68	124.15	117.13	131.24	105.24
	F2 $_{(CE704\times CE810)\times(CE704\times CE810)}$	91.04	91.88	100.99	48.03	79.71	114.81
Stressed plants	2013×CE810	183.09	109.57	128.63	78.92	87.29	143.30
	CE704×CE810	101.84	110.28	104.51	74.10	111.56	89.60
	CE810×2013	293.45	135.05	109.88	70.26	65.67	154.15
	CE810×CE704	153.91	103.25	112.58	77.52	94.72	110.87
	F2 $_{(2013\times CE810)\times(2013\times CE810)}$	123.80	107.06	103.76	86.83	89.08	122.32
	F2 $_{(CE704\times CE810)\times(CE704\times CE810)}$	125.85	114.50	90.63	102.19	95.08	112.70

case of CE810×CE704 (Fig. 1). Contrary to the considerable decrease of the PS 2 activity, no significant decrease of PS 1 activity (with only one exception) was observed. Actually, the PS1 activity increased in the chilling-stressed plants, especially in the hybrid combination CE704×CE810, where the differences between stressed and non-stressed plants were often statistically significant (Fig. 1).

Suboptimum temperature caused also considerable changes in the activities of antioxidant enzymes. The GR, APX and SOD activities increased in all genotypes and generations examined during low-temperature stress and the differences between stressed and non-stressed plants were always (GR, APX) or often (SOD) statistically significant (Figs. 2, 3). The activity of CAT generally showed lesser changes due to low temperature compared to the other enzymes. Both significant decrease (*e.g.* in CE810, CE704×CE810) and increase (*e.g.* in CE704, 2013×CE810, CE810×CE704) was found for CAT activity in stressed plants compared to the control ones (Fig. 3).

The analysis of the intraspecific variability in photosynthetic and antioxidant parameters was performed both for non-stressed and stressed plants. The results showed greater variability between genotypes in the activities of antioxidant enzymes than in photochemical activities, regardless of temperature conditions. The most pronounced differences were observed between the parental line 2013 (with rather low values of measured parameters) and other genotypes or generations. Comparison of parental lines and F1 hybrids grown in optimum temperature revealed the presence of the positive mid-parent heterosis for some parameters (the PS 2, SOD and GR activities). This effect was more pronounced in hybrids derived from the parental lines 2013 and CE810 compared to the CE704×CE810 hybrid combination. The values of the heterotic effect did not much decrease in the F2 generation; indeed, in some cases (the SOD, CAT activities) this generation showed higher heterosis than the appropriate F1 hybrid (Table 2). Low temperature caused considerable increase of the heterotic effect particularly in the PS 2, PS 1, SOD and CAT activities. The values of the heterosis in the F2 generation derived from the 2013×CE810 hybrid usually did not exceed those in the F1 generation proper, but the reverse was true

Table 3. Genetic analysis of the photochemical activities of PS 1 and PS 2 in mesophyll chloroplasts isolated from leaves of maize grown in optimum or low-temperature. The results of χ^2 tests of the goodness-of-fit of genetic models with various combinations of parameters representing individual genetic effects are shown in the upper part of the table. Based on these tests, the most appropriate genetic model was chosen and the estimates of genetic effects from this model ± their SEM are shown together with their statistical significance (* - significant at $P = 0.05$, ** - significant at $P = 0.01$, ns - non-significant). m - general mean across all examined genotypes/generations, a - additive genetic effect, d - dominance genetic effect, Ma - additive maternal genetic effect, Md - dominance maternal genetic effect (indexes indicate the parental genotype, or (in case of the dominance effects) the parental combination, the respective genetic parameter is relevant to.

		PS 2 activity control	stressed	PS 1 activity control	stressed plants
χ^2 tests (individual effects)	m, a	42.77 **	152.74 **	95.03 **	74.73 **
	m, a, d	17.88 **	61.20 **	39.98 **	43.70 **
	m, a, d, Ma	9.82 ns	31.59 *	23.08 *	31.53 *
	m, a, d, Ma, Md		6.60 ns	9.28 ns	6.71 ns
Values of parameters	m	6.202 ± 0.146 **	3.288 ± 0.338 **	16.609 ± 0.458 **	16.356 ± 0.751 **
	a_{2013}	-0.103 ± 0.363 ns	0.463 ± 0.616 ns	0.233 ± 1.038 ns	2.337 ± 1.238 ns
	a_{CE704}	-0.450 ± 0.453 ns	-0.006 ± 0.606 ns	-2.832 ± 1.123 **	-3.221 ± 1.282 **
	a_{CE810}	0.553 ± 0.285 ns	-0.458 ± 0.429 ns	2.599 ± 0.771 **	0.884 ± 0.870 ns
	$d_{2013\times CE810}$	1.011 ± 0.257 **	2.598 ± 0.598 **	-0.365 ± 0.953 ns	2.347 ± 1.418 ns
	$d_{CE704\times CE810}$	-0.492 ± 0.381 ns	2.188 ± 0.570 **	-2.726 ± 0.851 **	2.726 ± 1.158 *
	Ma_{2013}	-0.862 ± 0.334 **	-1.422 ± 0.536 **	-1.249 ± 0.987 ns	-4.140 ± 1.270 **
	Ma_{CE704}	1.102 ± 0.396 **	0.050 ± 0.548 ns	2.633 ± 0.897 **	2.941 ± 1.040 **
	Ma_{CE810}	-0.241 ± 0.234 ns	1.372 ± 0.355 **	-1.414 ± 0.600 *	1.199 ± 0.761 ns
	$Md_{2013\times CE810}$		-1.929 ± 0.391 **	-1.750 ± 0.589 **	-2.871 ± 0.770 **
	$Md_{CE704\times CE810}$		0.160 ± 0.352 ns	1.968 ± 0.946 *	1.385 ± 0.770 ns

Table 4. Genetic analysis of the activities of antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT) of maize grown under optimum or low temperature conditions. For legend see Table 3. In the case of the APX activity (control plants), the GR activity (stressed plants) and the CAT activity, no model was valid for the estimation of genetic effects and the data are therefore not shown.

		SOD activity control	stressed	APX activity stressed	GR activity control
χ^2 tests (individual effects)	m, a	57.27 **	22.13 *	32.78 **	34.43 **
	m, a, d	21.79 **	12.02 ns	4.50 ns	19.36 *
	m, a, d, Ma	16.27 *			7.42 ns
	m, a, d, Ma, Md	6.80 ns			
Values of parameters	m	32.373 ± 1.134 **	40.968 ± 1.021 **	305.536 ± 11.845 **	23.321 ± 1.252 **
	a_{2013}	3.385 ± 2.270 ns	-0.914 ± 1.658 ns	-0.209 ± 13.032 ns	-0.317 ± 2.6885 ns
	a_{CE704}	-0.105 ± 2.184 ns	1.451 ± 1.406 ns	-2.643 ± 21.106 ns	1.539 ± 2.326 ns
	a_{CE810}	-3.279 ± 1.569 *	-0.537 ± 1.197 ns	2.434 ± 12.872 ns	-1.856 ± 1.812 ns
	$d_{2013\times CE810}$	7.935 ± 1.935 **	7.756 ± 2.972 **	-55.848 ± 16.731 **	8.472 ± 2.500 **
	$d_{CE704\times CE810}$	-2.721 ± 1.792 ns	3.665 ± 1.772 *	-78.376 ± 16.172 **	-4.543 ± 1.932 *
	Ma_{2013}	-2.573 ± 1.965 ns			-3.732 ± 2.529 ns
	Ma_{CE704}	0.290 ± 1.590 ns			4.403 ± 1.595 **
	Ma_{CE810}	2.283 ± 1.138 *			-0. 671 ± 1.342 ns
	$Md_{2013\times CE810}$	3.270 ± 1.592 *			
	$Md_{CE704\times CE810}$	3.031 ± 1.225 **			

(with the exception of the SOD and GR activities) for the self-pollinated CE704×CE810.

Regardless of the significant influence of growth temperature on the values of the photosystem and antioxidant enzyme activities, the changes in the genetic determination of these parameters were less pronounced. The detected intraspecific variability was probably caused particularly by the expression of dominance (Tables 3,4). In case of the PS 2 activity, the presence of the positive dominance was ascertained for the hybrid combination 2013×CE810, both in control and stressed plants, and its expression increased during stress. Under

these conditions, the positive overdominance was found also for the CE704×CE810 combination. Contrary to this, the negative overdominance was significant in case of the PS 1 activity in control plants of the hybrid combination CE704×CE810, which, however, changed to the positive overdominance in plants stressed by low temperature (Table 3). Intraspecific variability of antioxidant enzyme activities was also determined by the presence of the positive (SOD, GR) or negative (APX, GR) dominance effects (Table 4). Additive genetic effect (positive for the parental line CE810, negative for the line CE704) was important in the genetic determination of the PS 1 activity (Table 3). The influence of this genetic effect on the variability in antioxidant enzyme activities was mostly non-significant (Table 4). Both positive and negative additive maternal effects were also found to contribute to the intraspecific variability in the PS 2 and PS 1 activities, but (with only two exceptions) they were not detected for the APX, GR or SOD activities. Finally, the expression of the positive or negative dominant maternal effects also affected the genetic variability in some parameters (the PS 2, PS 1 and SOD activities) (Tables 3,4). Generally, the changes both in the type and the level of the expression of individual genetic effects together with the changes of their statistical significance were observed for the majority of parameters examined.

Discussion

Photosynthetic processes are usually among the first to be negatively influenced by low temperature. However, the primary photochemical reactions which take place in the chloroplast thylakoid membranes are usually not directly influenced by this stress (Huner *et al.* 1998, Sonoike 1999) and the main cause of the decrease in their efficiency is usually the photooxidation of photosynthetic apparatus, induced by the combination of low temperature and irradiance. The fact that PS 2 is more sensitive to low-temperature compared to PS 1 is generally (though not universally) accepted (Greer and Hardacre 1989, Haldimann 1997, Fracheboud *et al.* 1999, Leipner *et al.* 1999, Körnerová and Holá 1999, Holá *et al.* 2003, 2007). The decrease of the PS 2 activity observed by us, and no changes or even increase in the PS 1 activity in plants subjected to low temperature, thus agree well with the most of other studies (Greer and Hardacre 1989, Haldimann 1997, Fracheboud *et al.* 1999, Leipner *et al.* 1999), as well as with our previous results (Körnerová and Holá 1999, Holá *et al.* 2003, 2007).

The observed increase of some antioxidant enzyme activities in plants grown under low temperature also agrees with the observations of several authors (*e.g.* Kubo *et al.* 1999, Aroca *et al.* 2001, Van Heerden and Krüger 2002, Takac 2004, Holá *et al.* 2007). Nevertheless, the extent of response was different depending on the individual enzymes. The most pronounced and significant increase was observed for the APX and GR activities. Both these enzymes apparently represented the most efficient defense mechanisms in chloroplasts of our stressed plants. They play an important role in the deactivation of hydrogen peroxide in chloroplast thylakoid membranes or stroma through ascorbate-glutathione cycle (Asada 1992, Kubo *et al.* 1999, Aroca *et al.* 2001, Van Heerden and Krüger 2002). On the other hand, CAT, which ensures a similar function as APX in plant cells, showed only minor and seldom increases of its activity due to chilling. The susceptibility of CAT activity to stress conditions has been proven previously (Feierabend *et al.* 1992, Kubo *et al.* 1999, Schmidt *et al.* 2006). Nevertheless, we have found slight elevation of CAT activity in the CE704 inbred line and the F1 hybrids 2013×CE810, CE810×2013, and CE810×704, as well as in the backcross generation (CE704×CE810)×CE810, displaing their better tolerance to cold. The increases of SOD activities were less striking but were observed in all genotypes, which also agrees with some previous observations (Aroca *et al.* 2001, Takac 2004).

Our main attention in this study was, however, not aimed at the simple response of the examined parameters to low temperature, but at the genotypic variability in these parameters and at the changes of their genetic determination during low temperature stress. We have observed the greatest decrease of both PS 2 and PS 1 activity due to low temperature in the inbred parental line 2013. This genotype also showed the highest increase in the GR activity but the lowest APX and SOD activities among parental genotypes when subjected to stress conditions. When this genotype was used as the maternal one for the F1 hybridisation (2013×CE810), our results have manifested the negative influence of this line on the performance of progeny and this negative effect could also explain the lower activities obtained in the backcross and F2 generations of the 2013×CE810 hybrid combination under the low-temperature stress. This was further confirmed by our findings of the presence of negative maternal effects both of additive (*i.e.* referring to the inbred line 2013) and dominance (*i.e.* referring to the hybrid 2013×CE810) type. Detailed genetic analysis of our results has revealed the significant influence of these effects particularly on photochemical activity. Inbred lines showing similar characteristics would not be thus much suitable for the breeding programmes aimed at the further improvement of maize tolerance to low temperature. On the other hand, inbred line CE810, though itself only of average tolerance to chilling, showed good possibilities for further breeding when used as a maternal parent, and the positive additive maternal effect associated with this genotype expressed itself particularly in the inheritance of photosynthetic parameters under conditions of suboptimum temperature.

We also found that the positive dominance is the most important genetic effect determining heterosis in photosynthetic characteristics in the F1 generation, which

agrees well with results of other studies made on non-stressed maize plants (Mifflin and Hageman 1966, Crosbie *et al.* 1978, Baer and Schrader 1985, Mehta *et al.* 1992, Holá *et al.* 1999, Körnerová and Holá 1999). The increase of heterosis, observed during low temperature stress for some parameters, could be explained by the change of dominance level or the restriction of negative dominance. The expected significant decrease of heterotic effect in F2 generation did not usually occur, which was probably caused by the manifestation of the positive dominant maternal effect. Thus, it seems that the maternal effects can easily compensate for the diminishion of the dominance expected from the changes in genotypic composition from F1 to F2 (or backcross) generations, and can significantly contribute to the preservation of good tolerance to low temperature even in further generations of maize hybrids.

We can conclude that prior to the inclusion of some particular genotype in the breeding programme aimed at the improvement of chilling tolerance, it should be always specified first, whether this genotype can be used regardless of the direction of crossing or as the maternal or paternal parent only. A detailed genetic analysis of several progeny generations is always useful for such specification and both the chloroplast efficiency and antioxidant enzyme activities are suitable and well measurable parameters for these studies.

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.

Anderson, M.D., Prasad, T.K., Stewart, C.R.: Changes in isozyme profiles of catalase, peroxidase, and glutathione-reductase during acclimation to chilling in mesocotyls of maize seedlings. - Plant Physiol. **109**: 1247-1257, 1995.

Asada, K.: Ascorbate peroxidase: a hydrogen peroxide scavenging enzyme in plants. - Physiol. Plant. **85**: 235-241, 1992.

Aroca, R., Irigoyen, J.J., Sánchez-Diaz, 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.

Baer, G.R., Schrader, L.E.: Inheritance of DNA concentration, and cellular contents of soluble protein, chlorophyll, ribulose bisphosphate carboxylase, and pyruvate, Pi dikinase activity in maize leaves. - Crop Sci. **25**: 916-923, 1985.

Baker, N.R., Farage, P.K., Stirling, C.M., Long, S.P.: Photoinhibition of crop photosynthesis in the field at low temperature. - In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis: From Molecular Mechanisms to the Field. Pp. 349-363. Bios Scientific Publ., Oxford 1994.

Bertamini, M., Zulini, L., Muthuchelian, K., Nedunchezhian, N.: Low night temperature effects on photosynthetic performance in two grapevine genotypes. - Biol. Plant. **51**: 381-385, 2007.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Chinnusamy, V., Zhu, J., Zhu, J.-K.: Cold stress regulation of gene expression in plants. - Trends Plant Sci. **12**: 444-451, 2007.

Crosbie, T.M., Mock, J.J., Pearce, R.B.: Inheritance of photosynthesis in a diallel among eight maize inbred lined from Iowa Stiff Stalk Synthetic. - Euphytica **27**: 657-664, 1978.

Del Río, L.A., Ortega, M.G., Lopez, A.L., Gorge, J.L.: A more sensitive modification of the catalase assay with Clark oxygen electrode. Application to the kinetic study of the pea-leaf enzyme. - Anal. Biochem. **80**: 409-415, 1977.

Eberhart, S.A., Gardner, C.O.: A general model for genetic effects. - Biometrics **22**: 864-881, 1966.

Feierabend, J., Schaan, C., Hertwig, B.: Photoinactivation of catalase occurs under both high- and low-temperature stress conditions and accompanies photoinhibition of photosystem II. - Plant Physiol. **100**: 1554-1561, 1992.

Foyer, C.H., Vanacker, H., Gomez, L.D., Harbinson, J.: Regulation of photosynthesis and antioxidant metabolism in maize leaves at optimal and chilling temperatures: review. - Plant Physiol. Biochem. **40**: 659-668, 2002.

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.

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

Haldimann, P.: Chilling-induced changes to carotenoid composition, photosynthesis and the maximum quantum yield of photosystem II photochemistry in two maize genotypes differing in tolerance to low temperature. - J. Plant Physiol. **151**: 610-619, 1997.

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.

Hallgren, J., Öquist, G.: Adaptation to low temperature. - In: Alscher R.G., Cumming J.R. (ed.): Stress Responses in Plants: Adaptation and Acclimation Mechanisms. Pp. 265-293. Wiley-Liss, New York 1990.

Hodges, D.M., Andrews, C.J., Johnson, D.A., Hamilton, R.I.: Antioxidant enzyme responses to chilling stress in differentially sensitive inbred maize lines. - J. exp. Bot. **48**: 1105-1113, 1997.

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

Holá, D., Kočová, M., Rothová, O., Wilhelmová, N., Benešová, M.: Recovery of maize (*Zea mays*. L.) inbreds and hybrids from chilling stress of various duration: photosynthesis and antioxidant enzymes. - J. Plant Physiol. **164**: 868-877, 2007.

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

stress. - Photosynthetica **41**: 429-442, 2003.
Huner, N.P.A., Öquist, G., Sarhan, F.: Energy balance and acclimation to light and cold. - Trends Plant Sci. **3**: 224-230, 1998.
Jahnke, L.S., Hull, M.R., Long, S.P.: Chilling stress and oxygen metabolizing enzymes in *Zea mays* amd *Zea diploperennis*. - Plant Cell Environ. **14**: 98-104, 1991.
Kingston-Smith, A.H., Harbinson, J., Foyer, C.H.: Acclimation of photosynthesis, H_2O_2 content and antioxidants in maize (*Zea mays*) grown at sub-optimal temperatures - Plant Cell Environ. **22**: 1071-1083, 1999.
Kocsy, G., Owttrim, G., Brander, K., Brunold, C.: Effect of chilling on diurnal rhythm of enzymes involved in protection against oxidative stress in a chilling-tolerant and a chilling-sensitive maize genotype. - Physiol. Plant. **99**: 249-254, 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 F1 hybrids. - Photosynthetica **37**: 477-488, 1999.
Kościelniak, J., Markowski, A., Skrudlik, G., Filek, M.: Effects of some periods of variable daily exposure to temperatures of 5 and 20 °C on photosynthesis and water relations in maize seedlings. - Photosynthetica **32**: 53-61, 1996.
Kosová, K., Vítámvás, P., Prášil, I.T.: The role of dehydrins in plant response to cold. - Biol. Plant. **51**: 601-617, 2007.
Krause, G.H.: Photoihibition induced by low temperatures. - In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis. Pp. 331-348. Bios Scientific Publ., Oxford 1994.
Kubo, A., Aono, M., Nakajima, N., Saji, H., Tanaka, K., Kondo, N.: Differential responses in activity of antioxidant enzymes to different environmental stress in *Arabidopsis thaliana*. - J. Plant Res. **112**: 279-290, 1999.
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., 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.
Long, S.P., East, T.M., Baker, N.R.: Chilling damage to photosynthesis in young *Zea mays* L. Effect of light and temperature variation on photosynthetic CO_2 assimilation. - J. exp. Bot. **34**: 177-188, 1983.
Massacci, A., Iannelli, M.A., Pietrini, F., Loreto, F.: The effect of growth at low-temperature on photosynthetic characteristics and mechanisms of photoprotection of maize leaves. - J. exp. Bot. **46**: 119-127, 1995.
Mehta, H., Sarkar, K.R., Sharma, S.K.: Genetic analysis of photosynthesis and productivity in corn. - Theor. appl. Genet. **84**: 242-255, 1992.
Mifflin, B.J., Hageman, R.H.: Activity of chloroplasts isolated from maize and their F1 hybrids. - Crop Sci. **6**: 185-187, 1966.
Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. - Plant Cell Physiol. **22**: 867-880, 1981.
Nie, G.Y., Long, S.P., Baker, N.R.: The effect of development at sub-optimal growth temperatures on photosynthetic capacity and susceptibility to chilling-dependent photo-inhibition in *Zea mays*. - Physiol. Plant. **85**: 554-560, 1992.
Procházková, D., Wilhelmová, N.: Changes in antioxidative protection in bean cotyledons during natural and continuous irradiation-accelerated senescence. - Biol. Plant. **48**: 33-39, 2004.
Revilla, P., Malvar, R.A., Cartea, M.E., Butron, A., Ordas, A.: Inheritance of cold tolerance at emergence and during early season growth in maize. - Crop Sci. **40**: 1579-1585, 2000.
Scebba, F., Sebastini, L., Vitagliano, C.: Changes in activity of antioxidative enzymes in wheat (*Triticum aestivum*) seedlings under cold acclimation. - Physiol. Plant. **104**: 747-752, 1998.
Schaedle, M., Bassham, J.A.: Chloroplasts glutathione reductase. - Plant Physiol. **59**: 1011-1012, 1977.
Shen, W.Y., Nada, K., Tachibana, S.: Oxygen radical generation in chilled leaves of cucumber (*Cucumis sativus* L.) cultivars with different tolerance to chilling temperatures. - J. jap. Soc. hort. Sci. **68**: 780-787, 1999.
Schmidt, M., Grief, J., Feierabend, J.: Mode of translational activation of the catalase (cat1) mRNA of rye leaves (*Secale cereale* L.) and its control through blue light and reactive oxygen. - Planta **223**: 835-846, 2006.
Sonoike, K.: The different rates of chilling temperatures in the photoinhibition of photosystem I and photosystem II. - J. Photochem. Photobiol. B. Biol. **48**: 136-141, 1999.
Sowinski, P., Rudzinska-Langwald, A., Adamczyk, J., Kubica, W., Fronk, J.: Recovery of maize seedling growth, development and photosynthetic efficiency after initial growth at low temperature. - J. Plant Physiol. **162**: 67-80, 2005.
Sunkar, R., Chinnusang, V., Zhu, J., Zhu, J.-K.: Small RNAs as big players in plant abiotic stress responses and nutrient deprivation. - Trends Plant Sci. **12**: 301-309, 2007.
Takac, T.: The relationship of antioxidant enzymes and some physiological parameters in maize during chilling. - Plant Soil Environ. **50**: 27-32, 2004.
Ukeda, H., Maeda, S., Ishii, T., Sawamura, M.: Spectrophotometric assay for superoxide dismutase based on tetrazolium salt 3′-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate reduction by xantine-xantine oxidase. - Anal. Biochem **251**: 206-209, 1997.
Van Heerden, P.D.R., Krüger, H.J.G.: Separately and simultaneously induced dark chilling and drought stress effect on photosynthesis, proline accumulation and antioxidant metabolism in soybean. - J. Plant Physiol. **159**: 1077-1086, 2002.
Verheul, M.J., Picatto, C., Stamp, P.: Growth and development of maize (*Zea mays* L.) seedlings under chilling conditions in the field. - Eur. J. Agron. **5**: 31-43, 1996.
Walker, M.A., McKersie, B.D.: Role of the ascorbate-glutathione antioxidant system in chilling resistance of tomato - J. Plant Physiol. **141**: 234-239, 1993.
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.
Yamori, W., Suzuki, K., Noguchi, K., Nakai, M., Terashima, I.: Effects of Rubisco kinetics and Rubisco activation state on the temperature dependence of the photosynthetic rate in spinach leaves from contrasting growth temperatures. - Plant Cell Environ. **29**: 1659-1670, 2006.
Zou, H., Wu, Z., Yang, Q., Zhang, X., Cao, M., Jia, W., Huang, C., Xiao, X.: Gene expression analyses of *Zm Pti1*, encoding a maize Pti-kinase, suggest a role in stress signaling. - Plant Sci. **171**: 99-105, 2006.