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The mitigating role of environmental factors in seedling injury and chill-dependent depression of catalase activity in maize leaves

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Abstract

In pot experiments performed on maize seedlings chilled at 5 °C, leaf injury was diminished by the application of elevated temperature (1 or 5 h at 15 or 20°C ,"warm breaks" treatment) in a dose-dependent manner. The lower the injury count, the higher the catalase (CAT) activity. In a separate experiment, the application of 100 % relative humidity also protected the plants from chilling injury and water loss, increased their gas exchange and variable to maximum chlorophyll fluorescence ratio (F_v/F_m), but did not influence CAT activity. Another protective environmental factor, elevated atmospheric CO_2 concentration [700 μmol(CO_2) mol^{-1}(air)] diminished CAT activity inhibition, but only in plants of chilling-resistant cultivar. The positive impact of specific environmental factors accompanying chilling is not obviously related to the suppression of the inhibition of CAT activity, although the enzyme is considered as chilling-sensitive.

Additional key words: air humidity, chilling stress, chlorophyll fluorescence, elevated CO_2, photosynthesis, *Zea mays*.

Introduction

The physiological basis of the chilling-sensitivity of maize has been studied for many years and the tolerance of this species has been gradually improved through various ways and methods (for a review, see Marocco *et al.* 2005). Chilling influences plants directly *via* membrane dysfunction or indirectly, *e.g.* by maintaining transpiration, whereas the hydraulic conductivity of roots is impaired, which results in the wilting of seedlings (Wilson 1976, Rodriguez and Davies 1982). The other indirect effect of low non-freezing temperature is the imbalance between naturally formed reactive oxygen species (ROS) and antioxidants (for a review, see Bączek-Kwinta *et al.* 2005). One such antioxidant – catalase (CAT), the enzyme decomposing H_2O_2, is extremely sensitive to chilling (Feierabend *et al.* 1992). The diurnal cycle of its activity consists of enzyme photoinactivation, proteolytic degradation and subsequent reconstitution (Feierabend and Engel 1986). As recovery processes are impaired by temperature depression, the accumulation of H_2O_2 in cells occurs, promoting uncontrolled oxidation of biomolecules which finally leads to cell death (Prasad *et al.* 1994a,b). It has been established that overproduction of ROS and/or inactivation of the antioxidant system is closely linked to the negative impact of low temperature on plants of tropical origin (Wise 1995, Prasad 1997, Pastori and Foyer 2002).

Under natural conditions found in cool temperate climatic zones, the impact of chilling on plants is usually modulated, *e.g.* by periodically elevated temperature (Skrudlik *et al.* 2000) or air humidity (Janowiak and Markowski 1994). Atmospheric CO_2 concentration increases globally and may rise locally (Ainsworth and Long 2005), and its influence on both C_3 and C_4 plants have been intensively studied (Nátr *et al.* 1996, Ziska and Bunce 1999, Bączek-Kwinta and Kościelniak 2003, Lambreva *et al.* 2006). All these circumstances led us to study the influence of these climatic factors on chilling injury of maize seedlings and CAT activity in their leaves. The choice of this antioxidant was based on its role in the defence system against oxidative stress. Additionally, since catalase is susceptible to unfavourable conditions (as shown earlier), it was borne in mind that the activity of this enzyme may be considered as a good molecular marker of chilling stress.

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Abbreviations: CAT - catalase; CR, CS - chilling-resistant and chilling-sensitive, respectively; E - transpiration rate; EL - electrolyte leakage, F_v/F_m - variable to maximum fluorescence ratio; P_N - net photosynthetic rate; RH - relative humidity.

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Materials and methods

Plants and cultivation: Two maize (*Zea mays* L.) genotypes chilling-resistant KOC 9431 (CR) and chilling-sensitive hybrid K103 × K85 (CS) were used (*Nasiona Kobierzyc*, Kobierzyce, Poland). The susceptibility of seedlings to low temperature was previously estimated in greenhouse experiments (Bączek-Kwinta and Kościelniak 2003).

Caryopses, conditioned in 50 % Thiuram, were sown into plastic pots (volume 2 dm^3; 10 seeds per pot) with a mixture of peat, organic soil and sand (3:1:1, v/v). Plants were grown in air-conditioned greenhouse with an ambient CO_2 concentration of *ca*. 350 μmol $mol(air)^{-1}$, day/night temperature 20/17 °C, relative air humidity (RH) *ca*. 60 %, and 16-h photoperiod (natural irradiance and additional irradiance during cloudy days, in order to maintain the minimal value of photosynthetic photon flux density (PPFD) at the level of 500 μmol(photon) m^{-2} s^{-1}.

Chilling stress: When plants reached the phase of the fully developed 3rd leaf, chilling (5 or 7 °C; further details below) was performed in plant growth chambers.

In the experiment on the effect of "warm breaks", the control group was chilled continuously at 5 °C and RH of 60 %, whereas 4 separate groups of chilled seedlings (also at 5 °C and RH of 60 %) were subjected to 15 or 20 °C for 1 or 5 h at the beginning of each day. The samples were taken for analyses before chilling, and on the 4th and 8th day of stress treatment (in each case before a "warm break" application).

In a separate experiment, elevated air humidity (100 %) was obtained by pumping the cooled (7 °C) air saturated with water vapour into the experimental chamber, whereas control plants were kept at 60 % RH. All parameters of the experimental microclimate were continuously monitored. Samples were taken before chilling, and on the 4th day of chilling.

In the study on the impact of elevated CO_2 concentration on chilled plants (the third experiment), the pots containing the seedlings of both genotypes were divided into groups subjected to elevated CO_2 concentration [700 μmol(CO_2) mol^{-1}(air)] and ambient [350 μmol(CO_2) mol^{-1}(air)], placed in plastic containers with a volume of *ca*. 1 m^3, and exposed to 7 °C for 8 d (RH 60 %). In order to prevent the temperature rising, cooled (7 °C) air was pumped into each container. In the experiments with a modified atmosphere, the minimal temperature maintainable was 7 °C. All parameters of the experimental microclimate were continuously monitored. CO_2 concentration in the chambers was measured by infrared gas analysers (*LCA-2*; *Analytical Development Co*., Hoddesdon, UK). Samples were collected before chilling and on the 8th day of chilling.

Electrolyte leakage (EL) was measured according to the procedure of Markowski and Skrudlik (1995), on 4 leaf discs (area of 0.75 cm^2), using a conductometer with automatic temperature compensation (*CC-315*, *Elmetron*, Zabrze, Poland). Plant material was washed with deionized water, closed in tubes with 15 cm^3 of deionized water and shaken for 24 h. Conductivity was measured (L_{s1}), then samples were boiled at 100 °C for 15 min, shaken for 24 h and the assay was repeated (L_{s2}). To eliminate the effect of the boiling on the tubes, the conductivity of deionized water was also measured, both at the beginning of the analysis (L_{w1}) and during the second analysis of plant samples (L_{w2}). Electrolyte leakage was calculated as a percentage of total electrolyte content according to the equation:

$$EL\ [\%] = [(L_{s1} - L_{w1})/(L_{s2} - L_{w2})] \cdot \times 100.$$

Catalase activity (CAT; EC 1.11.1.6) was determined on the dialysed crude extracts of leaves. Samples taken for analysis were frozen in liquid nitrogen, and stored at -80 °C until preparation. Tissue was homogenized in a mortar and a pestle, in a pre-cooled extraction buffer (50 mM potassium phosphate, pH 7.0) containing 0.1 mM of EDTA and 0.5 % of bovine serum albumin (BSA) (all chemicals *Sigma Aldrich*, Poznań, Poland). The homogenate was centrifuged at 10 000 *g* for 3 min, at 4 °C. The supernatant was dialysed at 4 °C for 6 h (in darkness), in a dialysis tubing cellulose membrane (*Sigma Aldrich*), against a potassium phosphate buffer (50 mM, pH 7.0) containing 0.1 mM of EDTA. Dialysates were kept in Eppendorf tubes on ice, in darkness. Analytical procedure relied on the monitoring of the disappearance of H_2O_2 at 240 nm (Aebi 1984; spectrophotometer *LKB Biochrom Ultrospec II*, Cambridge, UK).

Soluble protein content was measured spectrophotometrically on the same supernatant following a method developed by Bradford (1976), using BSA for calibration.

Gas exchange measurements: Net photosynthetic rate (P_N) and transpiration rate (E) were measured on attached leaves at 20 °C, ambient CO_2 concentration of 350 μmol mol^{-1}, irradiance *ca*. 800 μmol m^{-2} s^{-1} and RH of *ca*. 50 %, using the infrared gas analyser (*LCA-2*) operated as the open system with the leaf chamber *PLC(N)*.

Chlorophyll *a* fluorescence was measured at 20 °C using the plant stress meter (*BioMonitor AB*, Umeå, Sweden). The parameter F_v/F_m was obtained after dark adaptation for 15 min. The saturating irradiance was 600 μmol m^{-2} s^{-1}. Leaf area was measured photometrically using a CCD camera.

Statistics: All the assays were performed on the 3rd leaf of individual plant (biological replicate). Samples were collected at the beginning of the day. Measurements and analyses were performed in 7 - 10 biological and 2 - 3 analytical replicates. The statistical significance of the

treatment was evaluated by analysis of variance (*ANOVA*). This was preceded by the Kolmogorov-Smirnov test to analyse the data distribution, and followed by the comparison of the differentiation of means by the Duncan multiple range test (n = 3) or the Student t-test (n = 2).

Results

"Warm breaks" treatment: On the 4th day of the experiment, the leaves of CR plants subjected to continuous chilling were not injured, contrary to the CS plants, which revealed a rapid increase in EL when compared to the day 0 (Fig. 1*A*). However, this was suppressed by "warm breaks" treatment. The leaves of plants subjected to 1-h warming, irrespective of the warming temperature, revealed lower EL values than those chilled continuously. 5-h warming allowed the plants to avoid the injury, as was indicated by the EL values on the level of day 0. Prolonged stress conditions (8 d) strongly affected the plants of both genotypes (EL of approx. 50 % of total electrolyte content), but "warm break" treatments diminished their injury, and the main regulating factor was the length of the warm break, as was in the case of shorter chilling stress (4 d).

The pattern of changes in CAT activity modulated by warming treatments was usually opposite to that of EL, which means that the lower the level of injury, the higher the enzyme activity. On the 4th day, the CAT activity was diminished by continuous chilling in both hybrids (Fig. 1*B*), but all implemented "warm breaks" elevated the activity to the level obtained before chilling. On the 8th day, CAT activity drastically dropped in response to continuous chilling, but the application of "warm breaks" suppressed this, and the longer and the warmer the "warm break", the higher the activity detected.

There was no clear relationship between CAT activity and protein content, and the influence of chilling itself on the protein pool was also not obvious (Fig. 1*C*). Interestingly, it was not altered by continuous chilling, neither on the 4th day nor on the 8th. On the 4th day, it was

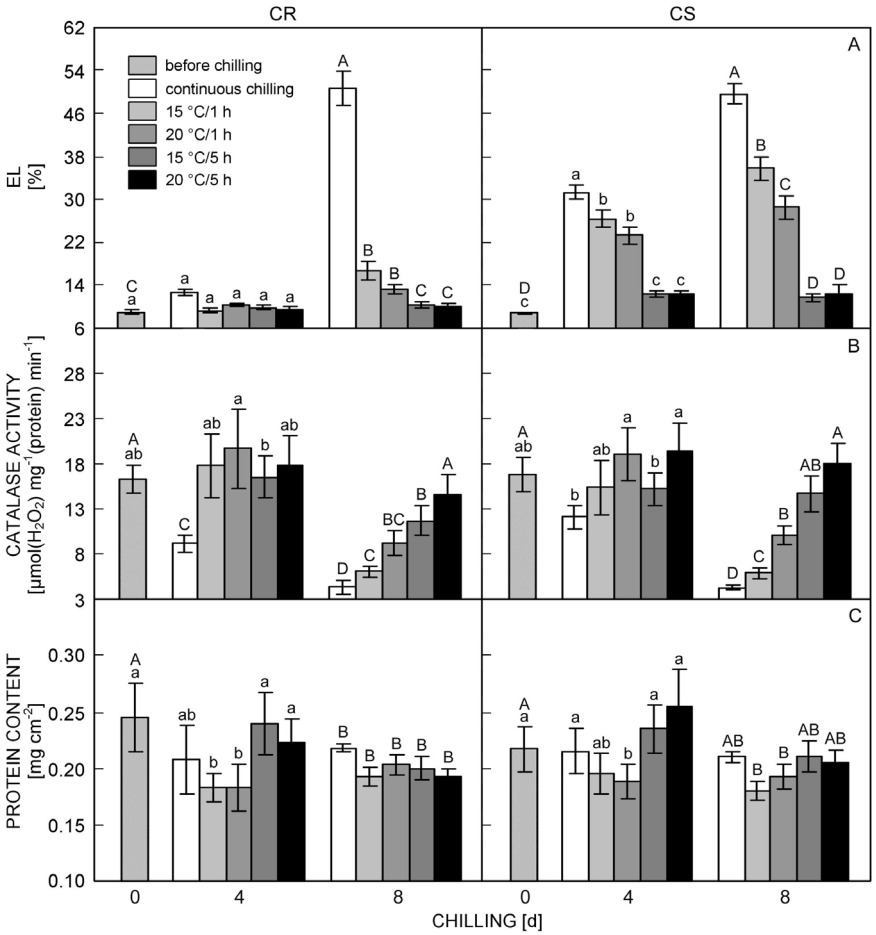

Fig. 1. The influence of continuous chilling and periodical warming during chilling, on electrolyte leakage from the leaves of maize hybrids, specific catalase activity and protein content. Means denoted with the same letters do not differ significantly within the hybrid and the day of chilling (Duncan's multiple range test, P = 0.05, n = 10, third leaf in each case).

Table 1. The influence of elevated relative air humidity during chilling (4 d, 7 °C) on net photosynthetic rate (P_N), maximal photochemical efficiency of PS 2 (F_v/F_m) and leaf water content. Means of 7 - 10 plants (third leaf in each case) ± SE. * - differences significant at $P = 0.05$, according to Student *t*-test.

Parameter	CR		CS	
	RH 100 %	RH 65 %	RH 100 %	RH 65 %
P_N [µmol m^{-2} s^{-1}]	9.26 ± 0.73*	6.63 ± 0.69*	9.62 ± 0.87*	5.86 ± 0.52*
F_v/F_m	0.571 ± 0.059*	0.462 ± 0.038*	0.549 ± 0.070*	0.378 ± 0.022*
Water content [%]	93.2 ± 0.39*	90.7 ± 0.45*	91.8 ± 0.62*	88.3 ± 0.40*

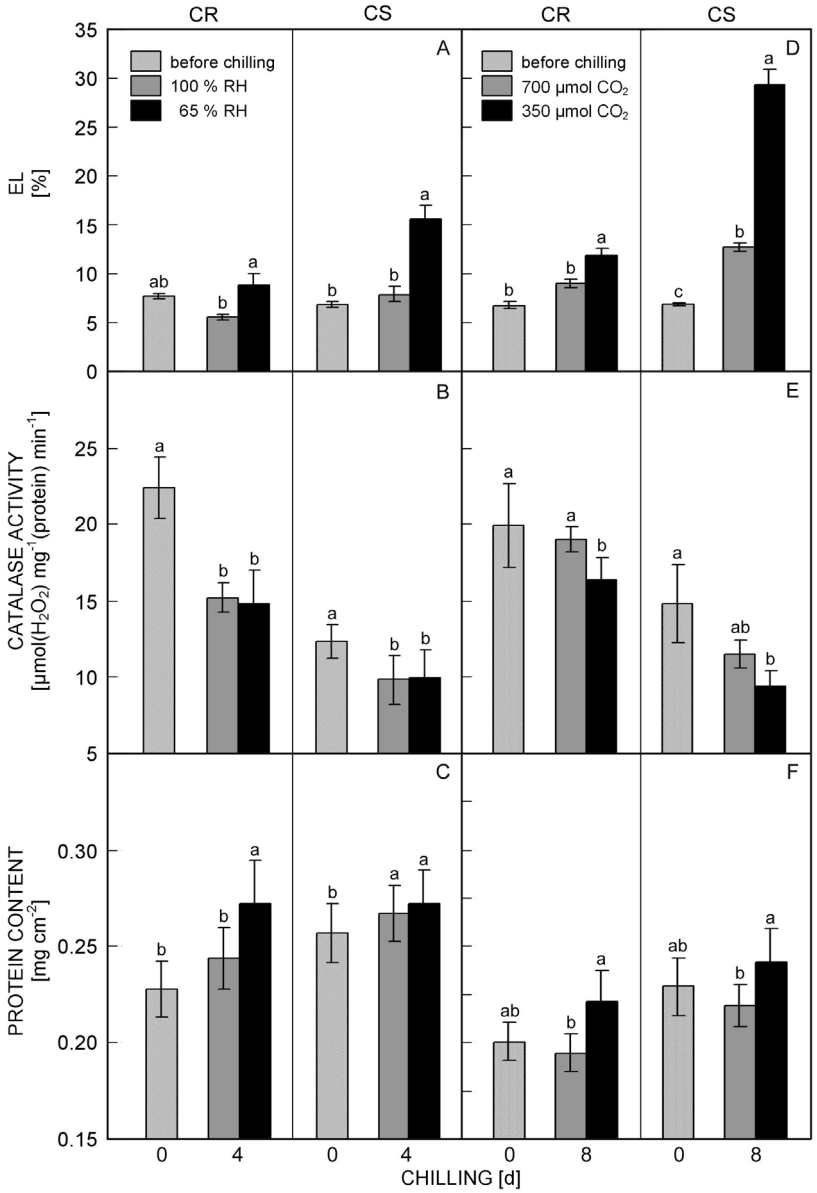

Fig. 2. The influence of elevated relative air humidity (RH; *A-C*) and elevated CO_2 concentration (*D-F*) during chilling, on electrolyte leakage from the leaves of maize hybrids, specific catalase activity and protein content. Means denoted with the same letters, do not differ significantly within the hybrid (Duncan's multiple range test, $P = 0.05$; $n = 10$ plants, third leaf in each case).

rather diminished in the leaves of plants subjected to 1 h warming, and did not alter in the specimens warmed for 5 h. Also the results obtained on the 8th day were not unified, which means, all warmed CR specimens had lowered protein content in relation to that obtained on day 0, but the CS ones only in the case of a 1-h warm break.

Elevated air humidity (RH): The application of 100 % RH during a 4-day chilling period at 7 °C, fully protected the CS plants from chilling injury (Fig. 2*A*). As was observed in the experiment upon periodical warming (Fig. 1*A*), the CR seedlings were not injured in this treatment. No changes in CAT activity were observed (Fig. 2*B*), although there was a difference in protein content between CR plants kept at 65 and 100 % RH (Fig. 2*C*). When analyzing the response of gas exchange (Table 1), we established that 100 % RH increased the net photosynthetic rate (P_N) of both genotypes. F_v/F_m was also increased by the treatment to a similar extent in both genotypes, although F_v/F_m before chilling was slightly lower in CS plants. The leaf water content of all plants grown at 100 % RH was higher than of those grown at 65 % RH, and only a little difference (*ca*. 2 %) between CR and CS genotypes was noticed.

Elevated atmospheric CO_2: The protective influence of CO_2 concentration elevated to 700 μmol mol^{-1} on the leaves of seedlings chilled at 7 °C for 8 d (Fig. 2*D*) was noticed. In this case, the EL of stressed CR plants was at the same level as obtained before chilling. When grown at an ambient CO_2 concentration, the EL of the CR was slightly increased, and CS leaves showed visible lesions (data not shown) accompanied by an EL of approx. 30 %. In this experiment, the duration of chilling was 8 d, but the degree of injury was generally lower than in the warm breaks treatment due to the higher stress temperature (7 °C instead of 5 °C). CAT activity revealed a tendency (significant in CR only) to be less inhibited in the leaves of seedlings grown at 700 μmol(CO_2) mol^{-1} than in those kept at an ambient CO_2 concentration (Fig. 2*E*). The changes in CAT activity and the protein content (Fig. 2*F*) showed the reverse patterns.

Elevated CO_2 also allowed the CR plants to prevail photosynthesis over respiration, unlike seedlings of this genotype grown in an ambient atmosphere, as well as all of the CS specimens, which switched their net CO_2 influx into respiration (Table 2). There was no influence of CO_2 concentration on E, but the stimulatory effect on the maximal photochemical efficiency of CR was established (higher F_v/F_m value). Additionally, although these values were generally low in all plants (which resulted from chilling period longer than in previous experiment), they were higher in seedlings of the CR than CS genotype, as was previously noticed.

As elevated CO_2 concentration altered the pattern of the photosynthetic parameters, it was intriguing to find whether it influenced plant biomass, and therefore leaf area was measured. It was established that the leaves of plants grown at elevated CO_2 were *ca*. 3 cm^2 larger than those grown in an ambient atmosphere, irrespective of the hybrid (Table 2). The leaves were also larger than before chilling (data not shown), so this effect cannot be considered as an artifact. Similarly to P_N and F_v/F_m, which were higher in the case of CR seedlings, this genotype had larger leaves than CS and the difference was *ca*. 12 cm^2.

Table 2. The influence of elevated CO_2 concentration during chilling (8 d, 7 °C) on net photosynthetic rate (P_N), transpiration rate (E), maximal photochemical efficiency of PS 2 (F_v/F_m) and leaf area. Means of 7 - 10 plants (third leaf in each case) ± SE. * - differences significant at $P = 0.05$, according to Student *t*-test.

Parameter	CR		CS	
	700 μmol mol^{-1}	350 μmol mol^{-1}	700 μmol mol^{-1}	350 μmol mol^{-1}
P_N [μmol m^{-2} s^{-1}]	1.19 ± 0.25	-0.13 ± 0.01*	-0.10 ± 0.01	-0.31 ± 0.01 n.s.
E [mmol m^{-2} s^{-1}]	0.10 ± 0.01	0.03 ± 0.01n.s.	0.08 ± 0.01	0.10 ± 0.01 n.s.
F_v/F_m	0.354 ± 0.031	0.229 ± 0.064*	0.194 ± 0.043	0.171 ± 0.051 n.s.
Leaf area [cm^2]	38.2 ± 1.54	35.5 ± 2.21*	25.4 ± 1.36	23.5 ± 0.91*

Discussion

The chilling temperature, which affects maize, ranges from 0 to 17 °C, and its influence depends on accompanying irradiance (Marocco *et al.* 2005). The temperatures applied in the described experiments (5 and 7 °C), are classified as strong chilling, and one can suppose that they influence the plants to a similar extent. However, the difference in 2 °C is big enough to change the degree of injury, as the EL values indicated. Temperature regimes classified as mild stress and severe stress applied in various laboratories, encounter additional difficulties in direct comparison to their influence on physiological processes (Bączek-Kwinta *et al*. 2005). Differential experimental conditions, the taking of samples at different stages in the experiment, various studied parameters, the reference parameters (such as protein content) and other factors, are also a problem in the examination of the tendency of studied characteristics in case of senescence and other stresses (Procházková and Wilhelmová 2007).

The susceptibility to a given temperature varies according to the genotype: CR plants revealed a more elastic response, as they also had the ability to reduce the extent of their injury at 100 % RH and elevated CO_2 concentration. The CS genotype utilized only "warm breaks", albeit less effectively than CR one.

The importance of environmental factors accompa-

nying a chilling was based on their natural occurrence in various regions. Warm breaks treatment mimicked the periods of the temperature increase during the sunny days. This experiment clearly showed that the higher temperature (20 *vs.* 15 °C) and the longer the period of warming (5 h *vs.* 1 h), the lower the chilling injury, and a thermal shock did not occur, but the protection did. A similar effect was obtained by Skrudlik *et al*. (2000) on three chilling-sensitive species: maize (inbred lines), tomato, and soybean. This implies the agronomical significance of such periodical warming for plant survival and the yield, yet the possible mechanism still needs to be explained. It may be related to changes in lipid composition toward fatty acid unsaturation. It was shown in our laboratory that in microsomal fractions obtained from maize leaves chilled at 5 °C for 4 d, oleic acid increases (Filek and Kościelniak 1996). Similarly, Moon *et al.* (1995), Kaniuga *et al.* (1998), and Pinhero *et al.* (1999) emphasized the increase in lipid unsaturation as a possible reason for prevention of degradation of membrane structures.

Interestingly, although CAT has been considered as being strongly sensitive to chilling, the pattern of changes in its activity was not a replica of that of EL in this experiment. The response of CAT activity in the leaves of plants chilled for 4 d was similar in both genotypes. The higher temperature, irrespective of the "warm break" duration, the higher the enzyme activity. Long-term response (assayed after 8 d) was different, and similar to the effect of the "warm breaks" on EL – the longer (5 h) and the warmer (20 °C) "warm break" the higher the CAT activity. It is likely that during the warming phase, the recovery processes of catalatic protein were improved (Streb and Feierabend 1995), and the activity of catalase-degrading proteases was diminished (Wise 1995). However, this had a cumulative rather than a direct effect.

The increased relative humidity (100 % RH) improved seedlings by increasing their P_N and F_v/F_m probably due to increased water content in the leaves leading to less injury of cell membranes than in plants grown at 65 % RH (as implied from the reduced EL values under 100 % RH). This allowed to maintain the proper water flux, which is usually facilitated by water channels (aquaporins; Aroca *et al.* 2005). Also CO_2 enrichment decreased the injury and increased the F_v/F_m of both genotypes at the same temperature regime. P_N was affected by this treatment only in the CR hybrid, but E was not altered. Similar results were obtained by Bertamini *et al*. (2007) on plants of two grapevine genotypes. Additionally, the increase of leaf area resulting from the influence of elevated CO_2 concentration, probably means increased production of photosynthates and/or their utilization for biomass production. Inhibition of dark respiration should also be considered, although Ziska and Bunce (1999) in their work on four C_4 species proved, that the biomass increment at elevated CO_2 is caused by increased P_N, and not by a reduction of carbon loss at night. Additionally, the studies of Pitterman and Sage (2001) on C_4 grass species showed, that low temperature-induced limitations in RuBPCO activity was overcome by elevated atmospheric CO_2. Elevated CO_2 also increased CAT activity but only in the CR genotype. However, one must take into account that catalase exists in different isoforms located in various organelles (Prasad *et al.* 1994a,b), and the total activity does not reflect the changes in their individual activities. Additionally, their susceptibility to chilling might be different (Anderson *et al.* 1995).

Since the maize leaf area is rather big (almost 40 cm^2) whereas leaf lamina thickness is *ca.* 10 - 200 μm (Stoyanova *et al.* 2002), the calculation of soluble protein content per leaf area unit was far better than per fresh mass unit, which changes during the stress due to the different water content in leaves. In our studies, CAT activity did not correspond to protein content, although minor exceptions were observed. The absence of the link between these two parameters may be explained in different ways. Firstly, CAT protein is only a part of the total soluble protein pool. Secondly, chilling-dependent inactivation of CAT does not imply degradation of the enzymatic protein only, but both the inactivation of its heme moieties and/or proteolytic degradation (Streb and Feierabend 1995). Moreover, total CAT activity was measured, although this did not reflect the changes in the CAT pool, as it was noticed earlier. Taken together, catalase activity assayed in a crude extract should not be regarded as a universal chilling stress marker, contrary to EL. On the other hand, it was distinct that the protective factors implemented in the described experiments often altered the pools of protein. In their abundance newly synthesised, stress-related proteins may be found (Wahid and Close 2007).

References

Aebi, H.: Catalase *in vitro*. - Methods Enzymol. **105**: 121-126, 1984.

Ainsworth, E.A., Long, S.P.: What have we learned from 15 years of free air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. - New Phytol. **16**: 351-372, 2005.

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.

Aroca, R., Amodeo, G., Fernández-Illescas, S., Herman, E.M., Chaumont, F., Chrispeels, M.J.: The role of aquaporins and membrane damage in chilling and hydrogen peroxide induced changes in the hydraulic conductance of maize roots. - Plant Physiol. **137**: 341-353, 2005.

Bączek-Kwinta, R., Kościelniak, J.: Anti-oxidative effect of elevated CO_2 concentration in the air on maize hybrids subjected to severe chill. - Photosynthetica **41**: 161-165, 2003.

Bączek-Kwinta, R., Niewiadomska, E., Miszalski Z.: Physiological role of reactive oxygen species in chill-sensitive plants. - Phyton **45**: 25-37, 2005.

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

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

Feierabend, J., Engel, S.: Photoinactivation of catalase *in vitro* and in leaves. - Arch. Biochem. Biophys., **251**: 567-576. 1986.

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.

Filek, M., Kościelniak, J.: The effect of chilling of maize shoots and roots on the electric potential, composition of fatty acids and the ATPase activity in the non-chilled organs of seedlings. - J. Agron. Crop Sci. **177**: 261-267, 1996.

Janowiak, F., Markowski, A.: Changes in leaf water relations and injuries in maize seedlings induced by different chilling conditions. - J. Agron. Crop Sci. **172**: 19-28, 1994.

Kaniuga, Z. Sączyńska, V., Miśkiewicz, E., Garstka, M.: Galactolipase activity but not the level of high-melting point phosphatidylgycerol is related to chilling tolerance in differentially sensitive *Zea mays* inbred lines. - Plant Cell Rep. **17**: 897-901, 1998.

Lambreva, M, Christov, K., Tsonev, T.: Short-term effect of elevated CO_2 concentration and high irradiance on the antioxidant enzymes in bean plants. - Biol. Plant. **50**: 617-623, 2006.

Markowski, A., Skrudlik, G.: Electrolyte leakage, ATP content in leaves and intensity of net photosynthesis in maize seedlings at permanent or different daily exposure to low temperature. - J. Agron. Crop Sci. **175**: 109-117, 1995.

Marocco, A., Lorenzoni, C., Fracheboud, Y.: Chilling stress in maize. - Maydica **50**: 571-580, 2005.

Moon, B.Y., Higashi, S.I., Gombos, Z., Murata, N.: - Unsaturation of the membrane lipids of chloroplasts stabilizes the photosynthetic machinery against low-temperature photoinhibition in transgenic tobacco plants. - Proc. nat. Acad. Sci. USA **92**: 6219-6223, 1995.

Nátr, L., Driscoll, S., Lawlor, DW.: The effect of increased CO_2 concentration on the dark respiration rate of etiolated wheat seedlings. - Cereal Res. Commun. **24**: 53-59, 1996.

Pastori, G.M., Foyer, Ch.H.: Common components, networks, and pathways of cross-tolerance to stress. The central role of "redox" and abscisic acid-mediated controls. - Plant Physiol. **129**: 460-468, 2002.

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

Pittermann, J., Sage, R.F.: The response of the high altitude C4 grass *Muhlenbergia montana* (Nutt.) A.S. Hitch. to long- and short-term chilling. - J. exp. Bot. **357**: 829-838, 2001.

Prasad, T.K.: Role of catalase in inducing chilling tolerance in pre-emergent maize seedlings. - Plant Physiol. **114,** 1369-1376, 1997.

Prasad, T.K., Anderson, M.B., Martin, B.A., Stewart, C.R.: Evidence for chilling-induced oxidative stress in maize and a regulatory role for hydrogen peroxide. - Plant Cell **6**: 65-74, 1994a.

Prasad, T.K., Anderson, M.B., Stewart, C.R.: Acclimation, hydrogen peroxide and abscisic acid protect mitochondria against irreversible chilling injury in maize seedlings. - Plant Physiol. **105**: 619-627, 1994b.

Procházková, D., Wilhelmová, N.: Leaf senescence and activities of the antioxidant enzymes. - Biol. Plant. **51**: 401-406, 2007.

Rodriguez, J.L., Davies, W.J.: The effects of temperature and ABA on stomata of *Zea mays* L. - J. exp. Bot. **33**: 977-987, 1982.

Skrudlik, G., Bączek-Kwinta, R., Kościelniak, J.: The effect of short warm breaks during chilling on photosynthesis and the activity of antioxidant enzymes in plants sensitive to chilling. - J. Agron. Crop Sci. **184**: 233-240, 2000.

Stoyanova, D., Tchakalova, E., Yordanov, I.: Influence of different soil moisture on anatomy of maize leaves and ultrastructure of chloroplasts. - Bulg. J. Plant Physiol. **28**: 11-20, 2002.

Streb, P., Feierabend, J.: Oxidative stress responses accompanying photoinactivation of catalase in NaCl-treated rye leaves. - Bot. Acta **109**: 125-132, 1995.

Wahid, A., Close, T.J.: Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. - Biol. Plant. **51**: 104-109, 2007.

Wilson, J.M.,: The mechanism of chill-and drought-hardening of *Phaseolus vulgaris* leaves. - New Phytol. **76**: 257-270, 1976.

Wise, 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.

Ziska, L.H., Bunce, J.A.: Effect of elevated carbon dioxide concentration at night on the growth and gas exchange of selected C_4 species. - Aust. J. Plant Physiol **26**: 71-77, 1999.