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Short-term effect of elevated CO_2 concentration and high irradiance on the antioxidant enzymes in bean plants

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Abstract

The effect of short-term exposure to elevated CO_2 concentration and high irradiance on the activity of superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidases (GPX) and catalase (CAT), and on the extent of the lipid peroxidation was studied in bean (*Phaseolus vulgaris* L.) plants. Plants were exposed for 4 d (8 h a day) to irradiance of 100 (LI) or 1000 (HI) μmol m^{-2} s^{-1} at ambient (CA, 350 μmol mol^{-1}) or elevated (CE, 1300 μmol mol^{-1}) CO_2 concentration. Four-day exposure to CE increased the leaf dry mass in HI plants and RuBPC activity and chlorophyll content in LI plants. Total soluble protein content, leaf dry matter and RuBPC activity were higher in HI than in LI plants, although the HI and CE increased the contents of malonyldialdehyde and H_2O_2. Under CA, exposure to HI increased the activity of APX and decreased the total SOD activity. Under CE, HI treatment also activated APX and led to reduction of both, SOD and GPX, enzymes activities. CE considerably reduced the CAT activity at both irradiances, possibly due to suppressed rate of photorespiration under CE conditions.

Additional key words: ascorbate peroxidase, catalase, guaiacol peroxidase, lipid peroxidation, *Phaseolus vulgaris*, RuBPC, superoxide dismutase.

Introduction

Even under optimal conditions many metabolic processes in plants produce active oxygen species and most of them are driven by or associated with events dependent on irradiance (Foyer *et al*. 1994). Under high irradiance photosynthesis is unable to utilize all the energy absorbed by the light-harvesting pigments and the absorption of excess energy can be deleterious since it can potentially result in production of singlet oxygen, superoxide and H_2O_2 (Logan *et al*. 1998b, Asada 1999). It has been shown that almost all of the chloroplastic H_2O_2 is derived from superoxide formed by the univalent transfer of electrons to oxygen from the electron acceptor of photosystem 1, ferredoxin (Asada and Takahashi 1987). Since ferredoxin normally passes its electrons to NADP, the amount of superoxide formed in this side reaction is dependent on the activity of the Calvin-Benson cycle and the supply of CO_2 (Bowler *et al*. 1992). Photorespiration and mitochondrial respiration participate in production of active oxygen forms, both processes dependent on the photosynthetic rate and availability and demand of photosynthates. To prevent oxidative damages all plants possess a well-developed antioxidant system, including low molecular mass antioxidants (ascorbate, glutathione, α-tocopherol) and enzymes scavenging reactive oxygen species (like superoxide dismutase, SOD, ascorbate, APX, guaiacol peroxidases, GPX, or catalase, CAT). It has often been reported that the activity of the antioxidant enzymes and leaf antioxidant content increase as a result of high irradiance (Grace and Logan 1996, Logan *et al*. 1998b) in order to prevent damage caused by active oxygen species. It was also supposed that the status of the antioxidant system is controlled by the extent of oxidative stress (Polle *et al*. 1997, Pritchard *et al*. 2000).

In the last two centuries the emission of CO_2 has increased placing the terrestrial plants in a changed environment. Elevated CO_2 concentration, (CE) usually increases the rate of photosynthesis in many C3 species by increasing the intercellular CO_2 concentration,

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Abbreviations: APX - ascorbate peroxidase; CAT - catalase; d.m. - dry mass; f.m. - fresh mass; GPX - guaiacol peroxidases; HI - high irradiance; LI - low irradiance; MDA - malonyldialdehyde; NBT - nitroblue tetrazolium; PAGE - polyacrylamide gel electrophoresis; PQ - plastoquinone pool; PS 2 - photosystem 2; Q_B - the second quinone acceptors of PS2; RuBP - ribulose-1,5-bisphosphate; RuBPC - ribulose-1,5-bisphosphate carboxylase; SOD - superoxide dismutase; TBA - thiobarbituric acid; TCA - trichloroacetic acid.

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enhancing carboxylation efficiency and reducing photorespiration (Sharkey 1985, Bowes 1993, Drake *et al*. 1997). Plants grown under CE showed increased resource efficiency (water, light and nitrogen efficiency) as compared with plants grown under CO_2 concentration of about 360 μmol mol^{-1} (CA) (Drake *et al*. 1997). It was supposed that plants grown under CE possessed an enhanced capability to withstand photooxidative stress (Schwanz and Polle 2001) or suffer less than plants grown under CA from intrinsic oxidative stress (Polle *et al*. 1997, Azevedo *et al*. 1998). The response of the antioxidant system to CE differs between species and different genotypes, and depends on treatment duration and other growing conditions. Reduction in SOD (Schwanz *et al*. 1996a,b, Polle *et al*. 1997) and CAT (Thibaud *et al*. 1995, Polle *et al*. 1997, Azevedo *et al*. 1998, Schwanz and Polle 2001) activities in plants grown under CE has been reported. In contrast, CE had no effect on the activities of CAT in orange, oak and pine leaves (Schwanz *et al*. 1996a,b) and of SOD in tobacco, wheat and pea plants (Havir and McHale 1989, Rao *et al*. 1995, Thibaud *et al*. 1995). Di Toppi *et al*. (2002) found no differences between CAT, SOD and APX activities in CE and CA plants. Pritchard *et al*. (2000) reported a significant reduction in the activities of SOD, CAT, GPX, APX, glutathione peroxidase and glutathione reductase in soybean plants under CE. Several mechanisms concerning the role of CE for the reduction of the amount of active oxygen species have been proposed: *1*) suppressed rates of photorespiration, as a result of both diminished rate of the RuBPC oxygenase reaction or reduced RuBP content (Polle *et al*. 1993, Azevedo *et al*. 1998); *2*) decrease of the electron leakage from photosystem 1 to oxygen, as a result of diminished rates of the Mehler reaction (Polle 1996, Schwanz *et al*. 1996b).

Taking the available data into account it may be summarised that high irradiance (HI) and CE may exert an opposite effect on the production of reactive oxygen species. In this respect we aimed to evaluate the effect of combined action of HI and CE on the antioxidant system and the degree of oxidative damage in been plants. The plants were exposed to CE for a relatively short period (4 d) but it was long enough to alter the leaf metabolism, expressed as changes in net photosynthetic rate, starch and pigment content (Lambreva *et al*. 2005).

Materials and methods

Bean (*Phaseolus vulgaris* L. cv. Cheren Starozagorski) plants were grown as hydroponic culture, three plants per darkened glass pot of 1.5 dm^3 volume. Plants were cultivated in a growth chamber at air temperature of 24 ± 2 °C, irradiance of 100 μmol m^{-2} s^{-1} ("white" fluorescence lamps), 12-h photoperiod and relative humidity of about 60 %.

Nineteen-day-old plants (with developed first trifoliate leaves) were moved to a chamber under treatment conditions: temperature about 23 °C, relative humidity 64 ± 3 %, low irradiance (LI, 100 μmol m^{-2} s^{-1}) or high irradiance (HI, 1000 μmol m^{-2} s^{-1}), CO_2 concentration 350 μmol mol^{-1} (CA) or 1300 μmol mol^{-1} (CE). Each experimental group consisted of nine plants in three different pots. The treatment duration was 8 h per day for 4 d (8 h light/16 h dark). The night temperature was 19 ± 1 °C. Fully expanded first trifoliate leaves were used for all the measurements.

The average fresh mass (f.m.) per unit area was estimated from 8 - 10 leaf discs with area of 2.8 cm^2. The dry mass (d.m.) was obtained from leaf discs kept in a drying oven at 105 °C to constant mass. An 80 % acetone extract of the pigments was obtained from approximately 0.1 g (f.m.) in dim light at 4 °C. A small amount of $MgSO_4$ (0.1 mg cm^{-3}) was added to the final extracts to minimize conversion of chlorophylls to phaeophytins. The concentration of photosynthetic pigments was estimated according to Lichtenthaler (1987). Total protein content of the extracts was determined by the method of Bradford (1976), using bovine serum albumin as a standard. For determination of RuBPC (EC 4.1.1.39) activity, leaf tissue (0.5 g f.m.) was homogenised on ice with 3 cm^3 cold extraction buffer, containing 50 mM HEPES-NaOH, 0.5 mM K_2HPO_4, 0.33 M sorbitol, 2 mM KNO_3, 2 mM EDTA, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 20 mM NaCl and 200 mM Na-isoascorbate, pH 7.6. The homogenate was filtered through four layers of cheesecloth, centrifuged at 12 000 *g* for 30 min and the supernatant was used directly for enzyme assay. RuBPC activity was assayed from the activated crude preparation by following the incorporation of $NaH^{14}CO_3$ into acid stable products as described by Popova *et al*. (1988). The reaction mixture contained in 50 mM HEPES (pH 8, NaOH): 20 μM $MgCl_2$, 1 μM dithiothreitol (DTT), 20 μM $NaHCO_3$ (containing 1.48 MBq, specific radioactivity 0.38 MBq μmol^{-1}) in volume 1 cm^3. The reaction, carried out at 25 °C, was initiated by addition of 2 μmol RuBP and stopped after 1 min with 6 M HCl. The amount of fixed $^{14}CO_2$ was measured using a liquid scintillation counter (*SL 4000*, *Intertechnique*, Plaisir, France).

H_2O_2 content was determined according to Alexieva *et al*. (2001) and malonyldialdehyde (MDA) was measured as described in Heath and Packer (1968). Leaf tissues (0.3 g f.m.) were homogenized in a mortar on ice with 5 cm^3 0.1 % (m/v) trichloroacetic acid (TCA), centrifuged at 10 000 *g* for 20 min at 4 °C. The reaction mixture for the H_2O_2 assay contained 0.5 cm^3 of the supernatant, 0.5 cm^3 50 mM sodium phosphate buffer (pH 7.8) and 1 cm^3 1 M KI. The absorbance was measured at 390 nm and the concentration of H_2O_2 was calculated using a standard curve obtained with known H_2O_2 concentrations. 0.5 cm^3 of the supernatant was

added to 1 cm^3 0.5 % (m/v) thiobarbituric acid (TBA) in 20 % (m/v) TCA. After 30 min incubation in boiling water, the mixture was cooled on ice bath and kept at a room temperature. The absorbance at 532 nm and 600 nm was measured in *Specol 11* (*Carl Zeiss*, Jena, Germany). The amount of the MDA-TBA complex was calculated using a coefficient of absorbance of 155 $mM^{-1}cm^{-1}$.

For enzyme extraction four to five leaves were chosen from different plants of each experimental group, immediately frozen in liquid nitrogen and stored at -72 °C for further analysis of superoxide dismutase (SOD, EC 1.15.1.1) and guaiacol peroxidases (GPX, EC 1.11.1.7) activity. The activities of catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11) were determined in extracts obtained from unfrozen leaves.

For the measurement of SOD, GPX and CAT activities 1 g of leaf powder (leaf tissue in the case of CAT) was homogenized in a mortar on ice with 5 cm^3 50 mM potassium phosphate buffer (pH 7.0), containing 0.1 mM EDTA and 2 % (m/v) *PolyClar*. The homogenates were filtered through *Miracloth* and centrifuged at 4 °C for 30 min at 12 000 *g*.

The extracts used for SOD activity measurement were dialysed for 24 h against half-strength extraction buffer and centrifuged for 10 min at 12 000 *g*. SOD activity was determined according to the method of Beauchamp and Fridovich (1971), based on the SOD ability to inhibit the nitro blue tetrazolium (NBT) reduction in the riboflavin/methionine system. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 0.053 mM NBT, 10 mM methionine, 0.0053 mM riboflavin and an appropriate aliquot of enzyme extract, final volume of 1.5 cm^3. The reaction was started by switching on the light and allowed to run for 6 min. One unit of SOD activity was defined as the amount of enzyme required to cause 50 % inhibition of the reduction of NBT to formazan as monitored at 560 nm.

GPX activity was measured by following the absorbance change at 420 nm due to guaiacol oxidation (Bakardjieva *et al*. 1996). The reaction was initiated by addition of 6 mM H_2O_2 and followed for 5 min in the reaction mixture contained 100 mM Na_2HPO_4/citric acid buffer (pH 4.7), 2 mM guaiacol and 0.1 cm^3 extract in a 5 cm^3 volume. CAT activity was determined by following the consumption of H_2O_2 at 240 nm for 60 s (coefficient of absorbance of 40 mM^{-1} cm^{-1}) according to Aeby (1984) in reaction mixture containing 10 mM potassium phosphate buffer (pH 7.0), 30 mM H_2O_2 and leaf extract in a 2.3 cm^3 volume.

APX activity was determined in extracts prepared through homogenization of 0.5 g leaf tissue in 2.5 cm^3 50 mM potassium phosphate buffer (pH 7.0), containing 1 mM EDTA, 2.5 mM ascorbate and 4 % (m/v) *PolyClar*. The homogenates were filtered through *Miracloth* and the activity was measured by following the oxidation of ascorbate at 290 nm, according to Amako *et al*. (1994), for 60 s in 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate (coefficient of absorbance of 2.8 mM^{-1} cm^{-1}), 0.1 mM H_2O_2 and leaf extract in volume of 2.3 cm^3. The reduction of ascorbate content in the reaction mixture caused by ascorbate oxidase (EC 1.10.3.3) and non-enzymatic oxidation were taken into account.

GPX and SOD isoforms were separated by 7.5 % polyacrylamide gel electrophoresis (PAGE) at pH 8.3. Bands with SOD activity in the gels were visualized according to the procedure described by Beauchamp and Fridovich (1971). The different SODs (Cu,Zn-SOD, Mn-SOD, Fe-SOD) were determined using specific inhibitors – KCN and H_2O_2. After electrophoretic separation and before staining the gels were incubated for 5 min in either 3 mM KCN solution or 3 mM H_2O_2 solution. GPX isoenzyme pattern was visualized by incubating the gel in guaiacol-benzidine solution for 5 min and subsequently adding of H_2O_2. The CAT isoforms were separated by native PAGE in 6 % gel, and visualized by consecutively incubation of the gels in H_2O_2 and mixture of $FeCl_3 . 6\,H_2O$ and $K_3[Fe(CN)_6]$. The stained gels were scanned and the images were evaluated by *Sigma Gel* software.

The current data are means of 4 - 6 replications measured for each average sample. To define the differences between groups the nonparametric equivalents to analysis of variance were used (Kruskal-Wallis analysis of ranks and median test). The pair-wise Mann-Whitney U test was performed at level of significance of 0.05. The data for the H_2O_2 and MDA content are means of 6 homogenizations from different plants, each in two replications. For the physiological parameters the number of the individual determinations varied between 3 and 8. For the overall mean comparison between treatments for these parameters, analysis of variance (factorial *ANOVA*) was applied.

Results and discussion

Our previous results showed that 4 d treatment of bean plants with various combinations of CO_2 concentration and irradiance leads to changes in the structure and functions of the photosynthetic apparatus (Lambreva *et al*. 2005). With the present work we continued our investigation, including evaluation of the changes in the activities of antioxidant enzymes and oxidative status of bean plants subjected to CE for a relatively short period of treatment.

The HI plants showed significantly higher rate of investment in leaf dry matter and in leaf soluble protein expressed per leaf area as well as higher RuBPC activity than LI plants (Table 1). Previous studies of the influence of irradiance on the leaf morphology in different species demonstrated that the acclimation to HI produces thicker leaves with mesophyll cells of greater size and number

Table 1. Physiological parameters of bean plants subjected for 4 d to CA (350 μmol mol^{-1}) or CE (1300 μmol mol^{-1}) at LI (100 μmol m^{-2} s^{-1}) or HI (1000 μmol m^{-2} s^{-1}). Means ± SE, n = 8 for f.m. and Chl (a+b); n = 6 for d.m. and protein content; n = 3 for Rubisco activity. Lower case letters group the samples with non-significant differences (P < 0.05).

Parameters	LI		HI	
	CA	CE	CA	CE
Fresh mass [g dm^{-2}]	1.33 ± 0.08a	1.37 ± 0.06a	1.45 ± 0.06ab	1.63 ± 0.07b
Dry mass [g dm^{-2}]	0.10 ± 0.01a	0.09 ± 0.01a	0.19 ± 0.02b	0.24 ± 0.01c
Chl (a+b) [mg dm^{-2}]	2.00 ± 0.10a	2.40 ± 0.10b	2.20 ± 0.10ab	2.30 ± 0.10ab
Protein [mg dm^{-2}]	24.00 ± 2.00a	27.00 ± 2.00a	46.00 ± 3.00b	48.00 ± 3.00b
RuBPC activity [μmol (CO_2) g^{-1}(f.m.)]	1.50 ± 0.20	3.20 ± 0.20	4.50 ± 0.40	4.30 ± 0.30

(Lichtenthaler 1985, Chow *et al*. 1988, Sims and Pearcy 1992, Logan *et al*. 1998b). HI bean plants showed about 2-fold higher (d.m.)/(f.m.) ratio than LI, regardless of the treatment CO_2 concentration, but small HI-induced changes in the (f.m.) per unit leaf area were observed. The leaf (f.m.) dm^{-2} was the most stable parameter (Table 1) and did not differ significantly depending on the treatment conditions. In addition, the difference in leaf relative water content between the variants was less that 5 % (data not shown). The effect of CE on some physiological parameters of bean plants (Table 1) was different at the two irradiance regimes: (1) (d.m.) dm^{-2} significantly increased in the leaves of HI plants, probably due to the increased CE-induced starch accumulation; (2) chlorophyll content increased in LI plants. The main Calvin cycle's enzyme was activated by CE only in LI plants, but no alterations in leaf soluble protein content in any of the variants were found.

Among the major sources of H_2O_2 in plants are the Mehler reaction and the photorespiration, both tightly connected with the rate of photosynthesis. In our study HI treatment provoked a significant increase (with about 200 %) in the H_2O_2 content (Fig. 1). Reduction in the maximal quantum yield of PS 2 photochemistry (F_v/F_m) and reduction of the electron transport rate in HI-exposed bean plants were also observed (Lambreva *et al*. 2004) as a result of changes in the acceptor side of PS 2 (Bertamini and Nedunchezhian 2004) caused by high excitation pressure. The excess energy may be dissipated in different ways, including production of active oxygen species. In spite of the much greater amount of H_2O_2 in HI plants compared to LI plants, there was no evidence for the presence of metabolic disorder caused by oxidative stress, taking into account the rate of photosynthesis (Lambreva *et al*. 2005), leaf dry mass, soluble protein and chlorophyll contents (Table 1).

Lipid peroxidation is often used as a marker for oxidative damage. Its extent increases in unfavourable environment such as drought (Sofo *et al*. 2004), high temperature (Jiang and Huang 2001), as well as in the case of ethylene-induced senescence (Hodges and Forney

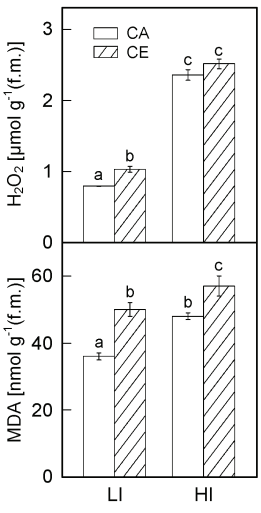

Fig. 1. Content of H_2O_2 and MDA in the leaves of bean plants after 4 d exposure to LI or HI irradiance at CA or CE. Means ± SE, n = 6. Lower case letters group the samples with non-significant differences at P < 0.05. Abbreviations are as in Table 1.

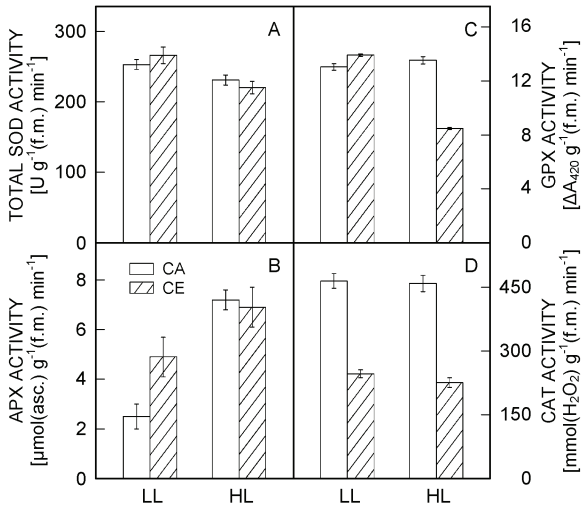

Fig. 2. Activity of total superoxide dismutase (*A*), ascorbate peroxidase (*B*), guaiacol peroxidases (*C*) and catalase (*D*) in leaves of bean plants after 4 d exposure to LI or HI at CA or CE. Means ± SE, n = 4 - 6. One unit of SOD was defined as the amount of enzyme necessary to produce a 50 % inhibition of the NBT reduction. Abbreviations are as in Table 1.

2000) or herbicide (diquat) treatment (Beligni and Lamattina 2002). The MDA content, as an indirect measure of lipid peroxidation, increased in HI-exposed plants by about 30% in comparison with LI plants (Fig. 1). The lesser effect of HI on the MDA content, in contrast with the HI-induced increase of H_2O_2 content, indicates that the potential of the HI-treated bean plants to prevent H_2O_2-provoked damages was not exceeded.

A similar increase of MDA content was observed as a result of CE under both irradiances and of H_2O_2 content in LI plants. The cause of such increase is not clear but it could be connected with other sites of active oxygen metabolism, as mitochondria or peroxisomes.

At HI the total SOD activity decreased (Fig. 2*A*), irrespective of the CO_2 concentration. This finding differs from some of the observations demonstrating an activation of SOD in plants grown at full sunlight in comparison to shaded plants (Logan *et al*. 1998a,b) or in wheat seedlings transferred from low to high irradiance (Mishra *et al*. 1995). Procházková and Wilhelmová (2004) attributed the reduction of SOD activity in continuously illuminated bean cotyledons to strong increase in production of H_2O_2. It might be supposed that the HI-provoked decrease in SOD activity in bean plants was not caused by increased H_2O_2, because some enzymes more sensitive to H_2O_2 (these of Calvin cycle according to Casano *et al*. (1997)) were not affected (Lambreva *et al*. 2005). The activity of SOD did not change after CO_2 treatment as it was reported in other studies for tobacco (Havir and McHale 1989) or wheat plants (Rao *et al*. 1995). Three SOD bands were determined as one Mn-SOD (Fig. 3, *band A*) and two Cu,Zn-SODs (Fig. 3, *bands B and C*). We did not find any Fe-SOD. These identifications are in agreement with Kono *et al*. (1979) and Pitcher *et al*. (1992). The activities of bands A (Mn-SOD) and C (Cn,Zn-SOD) did not vary among the variants. The activity of band B (Cn,Zn-SOD) was about 10 % lower in HI conditions under both CO_2 concentrations (data not shown) and most probably caused the decrease in total SOD activity observed in leaf extracts of HI-exposed plants.

The ascorbate peroxidase (APX) is a major part of the complimentary SOD/APX system detoxifying the reactive oxygen species. HI increased APX activity (Fig. 2*B*). By using electron transport inhibitors acting before or after plastoquinone (Q_B/PQ) Karpinski *et al*. (1997) suggested that the HI-induced synthesis of cytosolic APX mRNA was associated with increased reduction rate of Q_B/PQ. They were also able to provoke such synthesis by H_2O_2 (Karpinski *et al*. 1999) and concluded that the transcription of leaf cytosolic APX genes could be controlled by a combination of redox signals from Q_B/PQ and from H_2O_2. In our experiment the increased APX activity in the HI exposed plants might be a result of an increased reduction rate of electron carriers in the electron transport chain (Lambreva *et al*. 2004) as well as an increased concentration of H_2O_2.

CE had no significant effect on the APX activity as it was reported previously by Schwanz and Polle (2001) for poplar, Di Toppi *et al*. (2002) for herbaceous plants and Rao *et al*. (1995) for wheat plants. In contrast Pritchard *et al*. (2000) found reduction in APX activity as well as in other antioxidant enzymes in two soybean genotypes grown at CE.

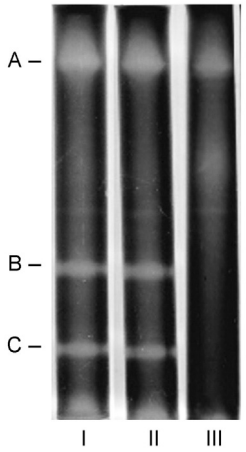

Fig. 3. SOD isoenzymes pattern in first trifoliate leaves of untreated 22-d-old bean plants. After electrophoretic separation and before staining the gels were soaked for 5 min in: *line I* - control; *line II* - 3 mM H_2O_2; *line III* - 3 mM KCN and the bands were determined as follow: *band A* - Mn-SOD; *bands B* and *C* - Cu,Zn-SODs.

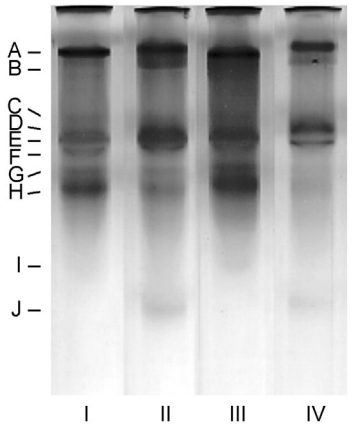

Fig. 4. GPX isoenzymes pattern in first trifoliate leaves of bean plants exposed for 4 d to: LI and CA - *line I*; HI and CA - *line II*; LI and CE- *line III* and HI and CE - *line IV*. Eight different bands (*A*-*J*) with GPX activity were visualized. Abbreviations are as in Table 1.

Guaiacol peroxidases (GPX) may also participate in the scavenging of H_2O_2. Eight different bands with GPX activity were visualized in leaf extracts (Fig. 4). A new band appeared in extracts of HI-treated plants at both CO_2 concentrations, marked as band J (Fig. 4). In plants exposed to LI, CE slightly increased the GPX activity and conversely reduced it in HI-exposed plants. Schwanz *et al*. (1996a) have reported for unchanged peroxidase activity in orange trees long-term acclimated to CE and reduction in activity of this enzyme in sun-leaves in

comparison to shade acclimated leaves. Pritchard *et al*. (2000) have observed a decrease of GPX activity in two different genotypes of soybean plants and Di Toppi (2002) found no changes in two herbaceous species, in both cases plants were grown under CE for a long time (comparable to vegetation period).

The CAT removes H_2O_2 produced in peroxisomes during photorespiration and detoxifies H_2O_2 formed as a result of mitochondrial electron transport (Willekens *et al*. 1995). Two bands with CAT activity were visualised after PAGE (data not shown). It was reported that CAT activity was higher in sun- in comparison to shade-leaves of orange trees (Schwanz *et al*. 1996a) or in HI- that in LI-acclimated plants (Logan *et al.* 1998b), both in field experiments. Schwanz *et al*. (1996a) speculated that enhanced HI tolerance might be associated with enhanced CAT activity, while Logan *et al*. (1998b) attributed HI-induced activation of CAT to the elevation of the leaf temperature that occurs in the field grown plants, presupposing that as the temperature increased the photorespiration and/or the mitochondrial respiration also increased. We observed no increase in CAT activity of HI compared to LI bean plants (Fig. 2*D*), as it was reported by Grace and Logan (1996) in a growth chamber study where air temperature was kept constant.

The response of the CAT activity to CE differs among species and treatments. Growth of plants at CE reduces the activity of CAT in spruce (Havir and McHale 1989), beech (Polle *et al*. 1997), tobacco (Polle *et al*. 1993), pea (Thibaud *et al*. 1995) and soybean (Pritchard *et al*. 2000). Azevedo *et al*. (1998) found a rapid increase of CAT activity in barley leaves after transferring the plants from CE to CA. In orange, oak and pine leaves, however CE did not affect CAT activity (Schwanz *et al*. 1996a,b). CAT activity in bean plants grown for 4 d at CE was about 2 times lower than that in CA grown plants. The response of this enzyme to CE might be ascribed to lower demand to scavenge the photorespiratory generated H_2O_2 in the peroxisomes, as it is generally believed that the photorespiration is negligible once the photosynthesis is saturated by high CO_2 concentration (Long 1991, Drake *et al*. 1997).

In summary, short consecutive exposures (4 d, 8 h per day) to CE did not alter considerably the activity of the main antioxidant enzymes of bean plants under LI. The plants subjected to combined action of HI and CE showed higher APX activity and reduced SOD and GPX activities when compared to plants kept under LI and CA. HI-treatment provoked an increase of H_2O_2 and MDA contents and CE alleviate this effect. We suppose that the CO_2-induced increase of H_2O_2 and MDA contents was presumably consequence of intensified mitochondrial metabolism. CAT activity was reduced at CE exposed plants under both LI and HI, most probably as a result of diminished Rubisco oxygenase reaction.

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