Plant Growth Regul (2009) 57:193–202
DOI 10.1007/s10725-008-9336-x

ORIGINAL PAPER

Effect of exogenous hydrogen peroxide on enzymatic and nonenzymatic antioxidants in leaves of young pea plants treated with paraquat

Irina Moskova · Dessislava Todorova · Vera Alexieva · Sergei Ivanov · Iskren Sergiev

Received: 14 August 2007 / Accepted: 28 September 2008 / Published online: 18 October 2008

Abstract The effects of exogenously applied hydrogen peroxide on the antioxidant system of pea plants were investigated. Ten-day-old pea seedlings were sprayed with 2.5 mM H_2O_2 and 24 h later with 0.2 mM PQ. Samples were taken 0, 2 and 5 h after the start of illumination. The protective effect of H_2O_2 was evaluated by monitoring of parameters related to the damage caused by PQ. The treatment with PQ led to a severe leakage of electrolytes from leaf tissues. Malondialdehyde level increased in PQ treated plants, but remained unchanged in H_2O_2 pre-treated ones after 5 h of illumination. Increased catalase and glutathione-S-transferase activity was observed in pea plants treated with H_2O_2 and PQ. Ascorbate peroxidase activity decreased significantly after paraquat application, but pre-treatment with H_2O_2 prevented ascorbate peroxidase inhibition to some extent. Increased guaiacol peroxidase activity was detected after H_2O_2 application. PQ application caused a drastic decline in the levels of thiol-group bearing compounds, reduced glutathione and ascorbate, while the quantity of oxidized glutathione and dehydroascorbate were increased. The results presented on changes in enzymatic and nonenzymatic antioxidants suggest that preliminary H_2O_2 application to pea plants treated with PQ, alleviates the toxic effects of the herbicide.

Keywords Antioxidants · Hydrogen peroxide · Paraquat · *Pisum sativum* · Stress markers

Abbreviations

APX	Ascorbate peroxidase
AsA	Ascorbic acid
DHA	Dehydroascorbic acid
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione-S-transferase
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
PQ	Paraquat
ROS	Reactive oxygen species
SOD	Superoxide dismutase

Introduction

Oxidative stress emerges as a result of unfavorable environmental conditions leading to generation of reactive oxygen species (ROS), such as superoxide radicals (O_2^-), hydroxyl radicals (HO), and hydrogen peroxide (H_2O_2), which are highly detrimental to the cells. Similarly, oxidative stress occurs in response to pathogen attack and during the natural processes of senescence. It is a widespread phenomenon and leads

I. Moskova (✉) · D. Todorova · V. Alexieva · S. Ivanov · I. Sergiev
Acad. M. Popov Institute of Plant Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113 Sofia, Bulgaria
e-mail: imoskova@bio21.bas.bg

to undesirable events in plants such as reductions in growth, yield and crop quality.

ROS are highly reactive towards membrane lipids, proteins and nucleic acids. They are key factors causing rapid cell damage under stress (Hariyadi and Parkin 1993; O'Kane et al. 1996; Prasad 1996), but also play a signaling role interrelating different stimuli and respective responses (Desikan et al. 2004). Among all ROS, hydrogen peroxide is the most appropriate molecule for this function because of its longer half-life and higher stability.

It is known that the accumulation of hydrogen peroxide is an early, common response to various stress factors, such as pathogen attack, wounding, drought, extreme temperatures, physical and chemical shock, UV-irradiation (Doke et al. 1994; Foyer and Noctor 2000). A stress environment leads to rapid synthesis of H_2O_2 in chloroplasts and other cell organelles and in the apoplast (Foyer et al. 1997).

The generation of ROS and especially H_2O_2 is acknowledged as a signal for activation of plant defense mechanisms under biotic and abiotic stress (Doke et al. 1994; Prasad et al. 1994; Foyer et al. 1997). It is assumed that the different forms of ROS interact with molecular targets, which are sensitive to changes in the ROS concentration within and outside the cells and thus transform this information into alterations in gene expression (Laloi et al. 2004).

It was found that application of H_2O_2 at low concentrations could induce stress tolerance in plants. The preliminary H_2O_2 treatment of *Arabidopsis* or tobacco protects the plants from oxidative damages due to high light intensity (Karpinski et al. 1999; Gechev et al. 2002). Tolerance to low positive temperatures was demonstrated after treatment with low concentrations of H_2O_2 in maize seedlings, *Phalaenopsis* and *Vigna radiata* (Prasad et al. 1994; Yu et al. 2002, 2003), and similarly treated potato nodal explants were found to be resistant to high temperature (Foyer et al. 1997; Lopez-Delgado et al. 1998). In these cases it was established that the pre-treatment with H_2O_2 causes alterations in the activity of several antioxidant enzymes and/or the levels of some antioxidants such as glutathione.

Paraquat is a bipyridilium contact herbicide, which by diverting electrons from Photosystem I forms bipyridilium radicals. The bipyridilium radicals, by reaction with oxygen produce superoxide radicals, and this is followed by chain reactions leading to the generation of other reactive forms of oxygen. Paraquat inhibits CO_2 fixation in plants, but its rapid herbicidal action is attributed mainly to generation of ROS (Harvey and Fraser 1980), and thus the biomembranes are subjected to free radical attack (Farrington et al. 1973). The double-bonds of biomembrane unsaturated fatty acids are the primary targets for ROS, which initiates chain reactions of lipid peroxidation followed by destruction and disorder in the normal membrane permeability (Szigetti et al. 2001; Cobb 1992). Numerous hypotheses have been evolved on possible reasons for PQ resistance. Inhibited translocation, binding of PQ to the cell wall, its sequestration to vacuoles, or enhanced activity of oxygen radical detoxifying enzymes (Norman et al. 1993; Hart and DiTomaso 1994; Yu et al. 2004) were proposed as possible mechanisms of resistance.

In our previous investigation (Moskova et al. 2007), we established that pre-treatment of young pea plants with low concentrations of hydrogen peroxide decreased the damage caused by paraquat.

Here we report the changes in some parameters related to paraquat toxicity and the alterations of the antioxidant system in H_2O_2 pre-treated plants.

Materials and methods

Seedlings of *Pisum sativum* L. were grown on a half-strength Hoagland-Arnon nutrition medium in a growth chamber under the following conditions-12/12 h photoperiod; light intensity 70 μmol m^2 s^{-1}; temperature 25 ± 1°C.

Ten-day-old seedlings were sprayed with an aqueous solution of H_2O_2 (2.5 mM) containing 0.1% Tween 80, and 24 h later, part of the plants were sprayed with 0.2 mM aqueous solution of paraquat containing 0.1% Tween 80. Hydrogen peroxide- or PQ-non-treated plants (as well as the controls), were sprayed with water containing 0.1% Tween 80 at each chemical application stage. Paraquat treatment was carried out after the end of the light phase in the photoperiod cycle, thus giving a 12 h of incubation period during which the herbicide effects are usually weak (Chang and Kao 1997).

Samples for measurement were taken at the end of the dark phase (0 h), 2 h after the beginning of illumination when there were still no visible herbicide

effects, and 5 h after beginning of illumination when the first visible symptoms of injury were observed.

Biochemical analyses were carried out on fresh plant material which was immediately extracted and assayed according to the appropriate methods listed below. Lipid peroxidation was estimated by the amount of malondialdehyde (MDA), a product of unsaturated fatty acid peroxidation. MDA concentration was measured by the method of Kramer et al. (1991). Membrane permeability of leaves was measured by electrolyte leakage (Palta et al. 1977). Leaves were cut and placed in tubes containing distilled water and after 24 h at room temperature the conductivity of the solution was measured. After twofold refreezing the conductivity was measured again. The results are presented as a percentage of the total conductivity of solutions after double freezing and unfreezing. Hydrogen peroxide was measured spectrophotometrically according to Alexieva et al. (2001). The free thiol-groups were determined according to Ellman (1959) with modifications by Edreva and Hadjiiska (1984). Glutathione content was measured according to Gronwald et al. (1987), and ascorbate content was measured as described by Yabuta et al. (2007). For determination of enzymatic activities, leaves were homogenized at 4°C in 100 mM K-phosphate buffer (pH 7.5), containing 2 mM DTT and 2 mM EDTA. The homogenate was centrifuged at 19,000*g* for 30 min to yield a crude enzyme extract. Catalase activity was determined by the method of Aebi (1984); peroxidase activity according to Dias and Costa (1983), using guaiacol as an external donor of electrons; superoxide dismutase (SOD) activity according to Beauchamp and Fridovich (1971); glutathione-S-transferase (GST) activity by the method of Gronwald et al. (1987); ascorbate peroxidase (APX) activity according to Guo et al. (2007). Soluble protein was determined by the dye-binding technique (Bradford 1976) using bovine serum albumin as a protein standard.

The experiments were performed three times in three replicates. Data presented are mean values with standard errors (±SE). Different letters indicate significant differences between treatments for the respective measurement point according to Duncan's multiple range test at $P < 0.05$.

Results

Stress marker content

It was found that at the end of the dark period (0 h) as well as 2 h after the beginning of illumination there were no significant difference between the levels of malondialdehyde in treated and control plants (Fig. 1a). After 5 h of illumination the amount of malondialdehyde in paraquat treated plants increased by up to 42% in relation to the control, but remained near the control level in H_2O_2 pretreated plants.

Treatment with hydrogen peroxide alone did not alter the membrane permeability during the whole period (0, 2, and 5 h), but the plants treated with paraquat showed considerably increased leakage of electrolytes (Fig. 1b): 14% (0 h), 44% 2 h after

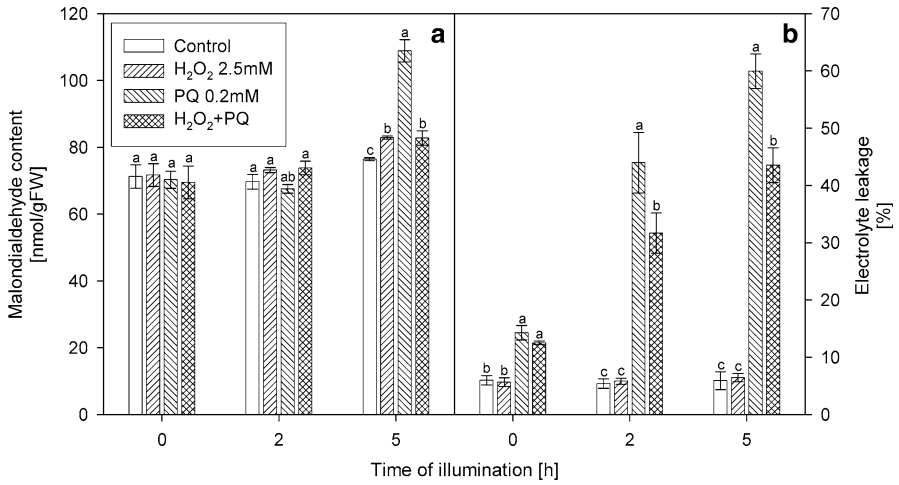

Fig. 1 Malondialdehyde content (**a**), and leakage of electrolytes (**b**), in pea plants treated with H_2O_2 and paraquat. Data are mean values ± SE. Different letters indicate significant differences between treatments at $P < 0.05$

illumination, and 60% after 5 h. Pretreatment with H_2O_2 prevented that induced by paraquat leakage of electrolytes to some extent.

Endogenous hydrogen peroxide and enzyme activities

Application of hydrogen peroxide did not markedly influence endogenous H_2O_2 concentration. On the other hand, paraquat treatment led to a significant decrease in endogenous hydrogen peroxide in both variants—PQ-only treated and pretreated with hydrogen peroxide plants (Fig. 2a). This occurred during the dark phase (0 h) and after illumination hydrogen content was not increased as expected. The activity of superoxide dismutase (Fig. 2b) was not influenced by H_2O_2 treatment but in the variants with paraquat application a decrease in the activity of SOD during the light period (2, 5 h) was detected.

At the end of the dark phase (0 h) catalase activity (Fig. 2c) increased slightly after H_2O_2 application. Treatment with PQ alone caused an inhibition of catalase activity, and the pretreatment with H_2O_2 lessened the negative effect of the herbicide to some extent. Marked alterations in catalase activity were not found during the illumination phase, with the exception of the variant of combined treatment with H_2O_2 and PQ where a significant increase was observed after 5 h of illumination.

Treatment with hydrogen peroxide provoked an increase in guaiacol peroxidase activity (Fig. 2d) during the whole period studied as compared to the control. The herbicide applied alone caused a considerable inhibition of peroxidase activity, especially during the dark phase. However, under light conditions an increase in enzymatic activity was observed. Pretreatment with hydrogen peroxide mitigated the effects of PQ to some extent and at the end of the experimental period peroxidase activity reached the control level.

Hydrogen peroxide treatment caused an inhibition of ascorbate peroxidase as compared to the control.

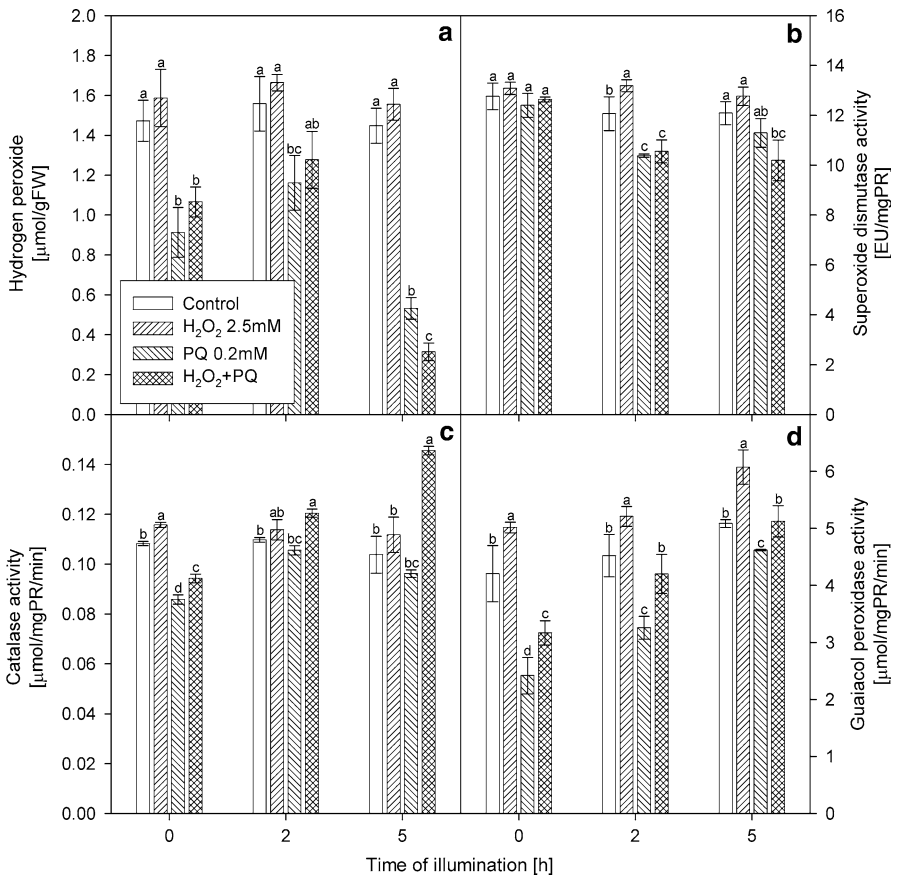

Fig. 2 Hydrogen peroxide content (**a**), activity of superoxide dismutase (**b**), catalase (**c**) and guaiacol peroxidase (**d**) in pea plants treated with H_2O_2 and paraquat. Data are mean values ± SE. Different letters indicate significant differences between treatments at $P < 0.05$

Paraquat also inhibited APX activity but to a much higher degree than H_2O_2, and at the end of the experiment it was 2.4% of the control. This trend was initiated during the dark phase and the effect of the herbicide was enhanced by illumination. On the other hand pretreatment with H_2O_2 preserved APX activity in PQ-treated plants to some extent.

Hydrogen peroxide applied alone did not alter the activity of glutathione-S-transferase (Fig. 3a). Paraquat caused an increase in GST activity after 5 h of illumination. However, the combined treatment caused a significant increase in the activity of GST 2 h after the beginning of illumination, and this tendency was maintained till the end of the experiment.

Content of non-enzymatic compounds

Treatment of plants with H_2O_2 increased the amount of free thiol-group containing compounds (Fig. 3b). On the other hand, the herbicide caused a decrease in the content of thiol-group containing compounds, which occurred during the dark phase and continued to diminish considerably till the end of the experiment. Pretreatment with H_2O_2 followed by application of PQ alleviated the herbicide effect slightly, and after 5 h in light, thiol group content was double that observed in plants treated with herbicide alone.

Hydrogen peroxide by itself caused a slight increase in reduced glutathione (Fig. 3c) after 5 h of illumination. Paraquat treatment markedly decreased GSH content and this low level of GSH was maintained during the whole period. Combined application of both compounds resulted in higher GSH content compared to that measured after herbicide application alone, especially after 5 h of illumination. In contrast to GSH, a significant increase in oxidized glutathione was detected after PQ application (Fig. 3d). Treatment with H_2O_2 had no effect on GSSG content. There was a decline in the paraquat-induced augmentation of GSSG only after 5 h of illumination.

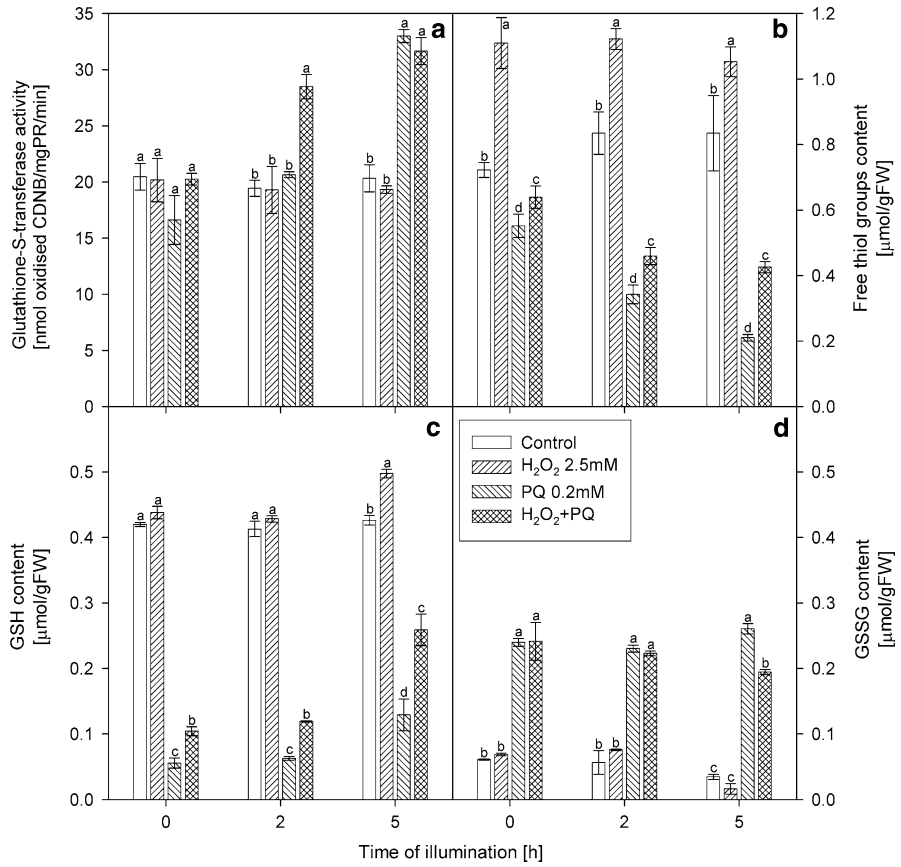

Fig. 3 Glutathione-S-transferase activity (**a**), content of free thiol groups (**b**), reduced glutathione (**c**) and oxidized glutathione (**d**) in pea plants treated with H_2O_2 and paraquat. Data are mean values ± SE. Different letters indicate significant differences between treatments at $P < 0.05$

Table 1 Ascorbate peroxidase activity, ascorbate, and dehydroascorbate content in pea plants treated with 2.5 mM H_2O_2 and 0.2 mM Paraquat (PQ)

	0 h	2 h	5 h
Ascorbate peroxidase activity (μmol $mgProt^{-1}$ min^{-1})			
Control	5.66 ± 0.38[a]	6.94 ± 0.20[a]	6.20 ± 0.14[a]
H_2O_2 2.5 mM	4.54 ± 0.20[b]	4.26 ± 0.07[b]	3.83 ± 0.24[b]
PQ 0.2 mM	1.94 ± 0.06[d]	1.36 ± 0.05[c]	0.15 ± 0.01[c]
H_2O_2 + PQ	3.81 ± 0.05[c]	3.99 ± 0.09[b]	4.21 ± 0.22[b]
Ascorbate content (nmol/gFW)			
Control	682.57 ± 12.90[a]	730.82 ± 21.15[a]	697.84 ± 40.18[a]
H_2O_2 2.5 mM	333.04 ± 8.30[b]	353.86 ± 5.95[b]	294.46 ± 7.16[b]
PQ 0.2 mM	1.79 ± 0.55[d]	1.94 ± 0.88[c]	1.56 ± 0.38[c]
H_2O_2 + PQ	90.37 ± 4.83[c]	201.71 ± 4.55[b]	228.57 ± 10.11[b]
Dehydroascorbate content (nmol/gFW)			
Control	93.81 ± 4.15[b]	96.07 ± 1.95[b]	103.70 ± 3.5[b]
H_2O_2 2.5 mM	59.24 ± 3.93[d]	62.94 ± 3.10[d]	55.34 ± 0.98[c]
PQ 0.2 mM	135.13 ± 2.85[a]	187.09 ± 6.44[a]	166.07 ± 7.10[a]
H_2O_2 + PQ	77.95 ± 1.65[c]	84.38 ± 0.88[b]	52.84 ± 2.41[c]

Data are mean values ± SE. Different letters indicate significant differences between treatments at $P < 0.05$

A twofold decrease in ascorbate content after treatment with hydrogen peroxide (Table 1) was observed, and paraquat applied alone caused a drastic fall in the ascorbate pool. Similarly to AsA, application of H_2O_2 diminished dehydroascorbate content, while paraquat provoked an increase in the dehydroascorbate level. In both cases this was observed during the dark phase and the effects persisted till the end of the experiment. On the other hand pretreatment with H_2O_2 prevented ascorbate decline and dehydroascorbate rise caused by paraquat to some extent.

Discussion

Malondialdehyde production, as well as the enhanced leakage of electrolytes from plant tissues are the result of oxidative damage and imbalance in biomembrane permeability, and thus they are considered to be sensitive stress markers reflecting numerous unfavorable environmental factors including herbicides (Kunert and Dodge 1989; Foyer et al. 1994). We observed that there were no substantial changes in the content of malondialdehyde during the dark phase and after 2 h of illumination, but 3 h later there was an increase only in paraquat treated plants. In contrast, the leakage of electrolytes was already initiated before the light period, it was sharply enhanced by the second hour and continued to rise due to paraquat action. Since MDA is one of the bioproducts of membrane oxidation and does not represent the overall membrane imbalance the observed disparity between changes in MDA content and electrolyte leakage is not unexpected. Similar membrane disorders provoked by paraquat were reported in rice plants by Guo et al. (2007). The restoration to the control level of malondialdehyde and electrolyte leakage decrease observed in pea pretreated with 2.5 mM H_2O_2 followed by paraquat is an indication that hydrogen peroxide lessened herbicide damage. This is in conformity with our observation that plants pretreated with H_2O_2 and sprayed with paraquat had higher survival rates compared to those treated with PQ only (Moskova et al. 2007).

Since in the present experimental model, treatment with a naturally-occurring constituent (H_2O_2) has been investigated, the issue we were interested in was whether exogenously applied hydrogen peroxide will affect endogenous H_2O_2 concentration. It should be noted that no changes in endogenous H_2O_2 were detected immediately after the treatment with hydrogen peroxide and this tendency lasted till the end of

the experiment (data not shown). This is in accordance with the observation by Yu et al. (2003) of lack of change in the endogenous H_2O_2 content in *Vigna radiata* after treatment with hydrogen peroxide. In contrast, paraquat application resulted in a decrease in hydrogen peroxide content especially after 5 h of exposure to light. It suggests that PQ treatment provokes a chemical environment facilitating reactions which produce hydroxyl radicals and this results in a concomitant decrease of hydrogen peroxide content. For example Shen et al. (1999) showed that in cucumber cultivars, treated with chilling temperature, H_2O_2 content decreased significantly accompanied by a marked increase in highly reactive hydroxyl radical generation.

Similarly to other ROS, the endogenous concentration per se of H_2O_2 depends on the balance between the rates of its generation (as a metabolite of the normal physiological processes) versus its enzymatic and non-enzymatic utilization or decomposition. In this relation the activities of SOD, catalase, ascorbate peroxidase and guaiacol peroxidase were also evaluated. A minor increase in the activity of SOD was detected in the H_2O_2-treated plants, while the application of paraquat led to a slight inhibition of the total superoxide dismutase activity under light conditions. Most probably different isoforms of SOD were influenced by the herbicide—Iturbe-Ormaetxe et al. (1998) established that paraquat inhibited Fe-SOD isoforms in pea plants, but did not affect Mn-SOD and CuZn-SOD isoforms. Catalase, guaiacol peroxidase and ascorbate peroxidase are key enzyme systems utilizing hydrogen peroxide in plants, and play important roles in the protection of plants against oxidative damage (Asada and Badger 1984; Puntarulo et al. 1988, 1991). We observed that paraquat slightly inhibited catalase and more significantly, guaiacol peroxidase activity, but ascorbate peroxidase was most substantially affected by the herbicide. On the other hand, treatment with H_2O_2 did not influence catalase, but caused some inhibition of ascorbate peroxidase, and increased the activity of peroxidase. In all these enzyme systems pretreatment with hydrogen peroxide opposed to some extent the effects of subsequently applied herbicide, especially ascorbate peroxidase. Similar effects of paraquat and pretreatment with 2-aminoethanol were reported by Masher et al. (2005) in barley.

Glutathione-S-transferases are multifunctional enzymes involved in detoxification of a number of xenobiotics and endogenous reactive metabolites. We observed that H_2O_2 did not influence the activity of GST, but paraquat caused a considerable increase after 5 h of illumination. On the other hand, the combined treatment of H_2O_2 and paraquat showed a positive effect on GST activity after 2 h in light. Since there is no direct evidence that paraquat is detoxified by conjugation with glutathione via GST, the observed increase in the activity of glutathione-S-transferase might be attributed to its peroxidase activity. Cummins et al. (1999) reported that glutathione-S-transferases can function as glutathione peroxidases in plants treated with herbicides causing oxidative stress, and such activity is of importance for the survival of the resistant species.

Compounds bearing a free thiol group, such as low molecular cell metabolites like glutathione, as well as a number of enzymes which are active only in a reduced state play a key role in important cellular functions, and the massive oxidation of—SH groups could be regarded as an aspect of oxidative toxicity (Haugaard 2000). The relationship between the—SH state in plants and their resistance to various stress factors is well known. Glutathione is a major fraction of the—SH pool in the cells (Alsher et al. 1997). Additionally, glutathione along with ascorbate are key components of the AsA-GSH cycle and play a pivotal role in the defense systems of plants as antioxidants and GSH particularly in detoxification of xenobiotics via GST (Marrs 1996). The changes in free thiol-group containing compounds and reduced glutathione followed a comparable pattern the application of H_2O_2 caused an increase, and paraquat sharply decreased GSH content. In contrast, GSSG was significantly augmented by the herbicide. The pretreatment with H_2O_2 prevented to some extent that caused by paraquat transition of GSH into GSSG. It is well documented that the glutathione pool in plants can be influenced by a number of stress factors such as drought, herbicides, heavy metals etc., and some authors considered the accumulation of GSH to be a prerequisite for plant stress resistance (Foyer et al. 1997; Loggini et al. 1999; Kocsy et al. 2000; Tari et al. 2002). On the other hand, Yu et al. (2002) showed that GSH accumulation was substantial but not the only factor for H_2O_2-induced cold tolerance of *Vigna radiata* L. plants.

Similarly to glutathione, we observed a drastic depletion of the ascorbate pool after paraquat application. According to the data presented here, the disturbance of glutathione and ascorbate pools takes place during the dark phase, indicating that the herbicide causes considerable damage to the components of the AsA-GSH cycle prior to exposure in light. This cannot be attributed to the primary mode of action of paraquat, but evidence exists that the herbicide also causes injuries in darkness (Marino et al. 2006).

Several authors reported that hydrogen peroxide pretreatment gives protection in plants subjected to various stressors (Yu et al. 2003; Gechev et al. 2002). However, the exact mechanism of the protective action of low H_2O_2 doses against different unfavorable factors (including herbicides) is not yet elucidated. In a previous study we established that H_2O_2 pretreatment prevented the inhibition of photosynthesis and chlorophyll loss provoked by PQ, and the plants showed higher survival rates (Moskova et al. 2007). The results presented here demonstrate that the negative effect of PQ, which was considerable in relation to AsA and GSH content, was eliminated to a great extent by H_2O_2 pretreatment. The application of H_2O_2 prior to PQ treatment influenced the activities of defense enzymes and especially of APX. One of the possible mechanisms explaining the protective action of H_2O_2 against PQ toxicity could be related to processes of oxidative coupling of ferulate and tyrosine residues of cell wall proteins and cell wall polysaccharides which form intra- and interchain bridges. These covalent cross-links between wall polymers are a physiologically significant strategy contributing to the termination of wall extensibility, wall strengthening, and the process catalyzed by peroxidase and H_2O_2 is intensified by various types of stress (Fry 1986; Liyama et al. 1994; Edreva 1996). Another explanation of an H_2O_2 protective mechanism could be the influence of AsA-GSH cycle components on the cellular redox state. GSH has been considered as an inducer of defense genes (Wingate et al. 1988), and changes in cell thiol-disulphide status provide redox signals leading to gene expression alterations (Foyer et al. 1997). In the present study with the model system described above, this assumption could also be considered. However additional investigations to confirm such a possibility are required. Most probably, hydrogen peroxide used in the present model system acts as a mild stressor hardening treated pea plants.

Acknowledgments This work was supported by Project INI-14/2005 (PISA) funded by the National Council for Scientific Research of Republic Bulgaria.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126. doi:10.1016/S0076-6879(84)05016-3

Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 24:1337–1344. doi:10.1046/j.1365-3040.2001.00778.x

Alsher RG, Donahue JL, Cramer C (1997) Reactive oxygen species and antioxidants: relationships in green cells. Physiol Plant 100:224–233. doi:10.1111/j.1399-3054.1997.tb04778.x

Asada K, Badger M (1984) Photoreduction of $^{18}O_2$ with concomitant evolution of $^{18}O_2$ in intact spinach chloroplasts: evidence for scavenging of hydrogen peroxide by peroxidase. Plant Cell Physiol 25:1169–1179

Beauchamp C, Fridovich I (1971) Superoxide dismutase improved assay and an assay applicable to acrylamide gels. Anal Biochem 44:276–287. doi:10.1016/0003-2697(71)90370-8

Bradford M (1976) A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. doi:10.1016/0003-2697(76)90527-3

Chang CJ, Kao C (1997) Paraquat toxicity is reduced by polyamines in rice leaves. Plant Growth Regul 22:163–168. doi:10.1023/A:1005890312557

Cobb A (1992) Herbicides and plant physiology. Chapman & Hall, London ISBN 0 412 43860 7, 176pp

Cummins I, Cole D, Edwards R (1999) A role for glutathione transferases functioning as glutathione peroxidases in resistance to multiple herbicides in black-grass. Plant J 18:285–292. doi:10.1046/j.1365-313X.1999.00452.x

Desikan R, Cheung M, Clarke A, Golding S, Sagi M, Fluhr R et al (2004) Hydrogen peroxide is a common signal for darkness- and ABA-induced stomatal closure in *Pisum sativum*. Funct Plant Biol 31:913–920. doi:10.1071/FP04035

Dias A, Costa M (1983) Effect of low salt concentrations on nitrate reductase and peroxidase of sugar beet leaves. J Exp Bot 34:537–543. doi:10.1093/jxb/34.5.537

Doke N, Miura Y, Leandro M, Kawakita K (1994) Involvement of superoxide in signal transduction: responses to attack by pathogens, physical and chemical shocks, and UV radiation. In: Foyer CH, Mullineaux PM (eds) Causes of photooxidative stress and amelioration of defence systems in plants. CRC, Boca Raton, pp 177–197

Edreva A (1996) Responses of tobacco to stress factors. Biotechnol Biotechnol 10:106–113

Edreva A, Hadjiiska E (1984) On the determination of the content of sulphydryl (thiol) groups in plant material (in Bulgarian). Fiziol Rast X(3):73–82

Ellman G (1959) Tissue sulphydryl groups. Arch Biochem Biophys 82:70–75. doi:10.1016/0003-9861(59)90090-6

Farrington JA, Ebert M, Land E, Fletcher K (1973) Bipyridylium quaternary salts and related compounds. V. Pluse radiolysis studies of the reaction of paraquat radical with oxygen implication for the mode of action of bipyridyl herbicides. Biochim Biophys Acta 314:372–381. doi:10.1016/0005-2728(73)90121-7

Foyer C, Noctor G (2000) Oxygen processing in photosynthesis: regulation and signaling. New Phytol 146:359–388. doi:10.1046/j.1469-8137.2000.00667.x

Foyer C, Lelandais M, Kunet K (1994) Photooxidative stress in plants. Physiol Plant 92:696–717. doi:10.1111/j.1399-3054.1994.tb03042.x

Foyer C, Lorez-Delgao H, Dat J, Scott I (1997) Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signaling. Physiol Plant 100:241–254. doi:10.1111/j.1399-3054.1997.tb04780.x

Fry S (1986) Cross-linking of matrix polymers in the growing cell walls of angiosperms. Annu Rev Plant Physiol 37:165–186. doi:10.1146/annurev.pp.37.060186.001121

Gechev T, Gadjev I, Van Breusegem F, Inze D, Dukiandjiev S, Toneva V et al (2002) Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes. Cell Mol Life Sci 59:708–714. doi:10.1007/s00018-002-8459-x

Gronwald J, Fuerst P, Eberlein C, Egli M (1987) Effect of herbicide antidotes on glutathione content and glutathione S-transferase activity of sorghum shoots. Pestic Biochem Physiol 29:66–76. doi:10.1016/0048-3575(87)90085-X

Guo Z, Huang M, Lu S, Yaqing Z, Zhong Q (2007) Differential response to paraquat induced oxidative stress in two rice cultivars on antioxidants and chlorophyll *a* fluorescence. Acta Physiol Plant 29:39–46. doi:10.1007/s11738-006-0007-0

Hariyadi P, Parkin K (1993) Chilling-induced oxidative stress in cucumber (*Cucumis sativus* L. cv. Calipso) seedlings. J Plant Physiol 141:733–738

Hart J, DiTomaso J (1994) Sequestration and oxygen radical detoxification as mechanisms of paraquat resistance. Weed Sci 42:277–284

Harvey B, Fraser T (1980) Paraquat tolerant and susceptible perennial ryegrasses: effects of paraquat treatment on carbon dioxide uptake and ultrastucture of photosynthetic cells. Plant Cell Environ 3:107–117

Haugaard N (2000) Reflections on the role of the thiol group in biology. Ann NY Acad Sci 899:148–158

Iturbe-Ormaetxe I, Escuredo P, Arrese-Igor C, Becana M (1998) Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiol 116:173–178. doi:10.1104/pp.116.1.173

Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux P (1999) Systematic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. Science 284:654–657. doi:10.1126/science.284.5414.654

Kocsy G, Szalai G, Vagujfalvi A, Stehli L, Orosz G, Galiba G (2000) Genetic study of glutathione accumulation during cold hardening in wheat. Planta 210:295–301. doi:10.1007/PL00008137

Kramer G, Norman H, Krizek D, Mirecki R (1991) Inflence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. Phytochemistry 30:2101–2108. doi:10.1016/0031-9422(91)83595-C

Kunert K, Dodge A (1989) Herbicide-induced radical damage and antioxidative systems. In: Target sites of herbicide action. CRC Press Inc., Boca Raton, FL, pp 45–63

Laloi C, Apel K, Danon A (2004) Reactive oxygen signaling: the latest news. Plant Biol 7:323–328

Liyama K, Lam T, Stone B (1994) Covalent cross-links in the cell wall. Plant Physiol 104:315–320

Loggini B, Scartazza A, Brugnol E, Navari-Izzo F (1999) Antioxidative defence system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiol 119:1091–1099. doi:10.1104/pp.119.3.1091

Lopez-Delgado H, Dat J, Foyer C, Scott I (1998) Induction of termotolerance in potato microplants by acetylsalicyclic acid and H_2O_2. J Exp Bot 49:713–720. doi:10.1093/jexbot/49.321.713

Marino D, Gonzalez E, Arrese-Igor C (2006) Drought effects on carbon and nitrogen metabolism of pea nodules can be mimicked by paraquat: evidence for the occurrence of two regulation pathways under oxidative stress. J Exp Bot 57:665–673. doi:10.1093/jxb/erj056

Marrs K (1996) The functions and regulation of glutathione S-transferases in plants. Annu Rev Plant Physiol Plant Mol Biol 47:127–158. doi:10.1146/annurev.arplant.47.1.127

Masher R, Fisher S, Scheiding W, Neagoe A, Bergmann H (2005) Exogenous 2-aminoethanol can diminish paraquat induced oxidative stress in barley (*Hordeum vulgare* L.). Plant Growth Regul 45:103–112. doi:10.1007/s10725-005-0401-4

Moskova I, Todorova D, Alexieva V, Sergiev I (2007) Hydrogen peroxide pretreatment alleviates paraquat injuries in pea (*Pisum sativum* L.). Compt Rend Acad Bulg Sci 60(10):1101–1106

Norman M, Fuerst E, Smeda R, Vaughn K (1993) Evaluation of paraquat resistance mechanisms in Conyza. Pestic Biochem Physiol 46:236–249. doi:10.1006/pest.1993.1055

O'Kane D, Gill V, Boyd P, Burdon R (1996) Chilling, oxidative stress and antioxidant responses in *Arabidopsis thaliana* callus. Planta 198:371–377. doi:10.1007/BF00620053

Palta JP, Levit J, Stadelman E (1977) Freezing injury in onion bulb cells. Evaluation of the conductivity method and analysis of ion and sugar efflux from injured cells. Plant Physiol 60:393–397

Prasad T (1996) Mechanisms of chilling-induced oxidative stress injury and tolerance in developing maize seedlings: changes in antioxidant system, oxidation of proteins and lipids, and protease activities. Plant J 10:1017–1026. doi:10.1046/j.1365-313X.1996.10061017.x

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

Puntarulo S, Sanchez R, Bovieris A (1988) Hydrogen peroxide metabolism in soybean embryonic axes at the onset of germination. Plant Physiol 86:626–630

Puntarulo S, Galleano M, Sanchez R, Bovieris A (1991) Superoxide anion and hydrogen peroxide metabolism in

embryonic axes during germination. Biochim Biophys Acta 1074:277–283

Shen W, Nada K, Tachibana S (1999) Oxygen radical generation in chilled leaves of cucumber (*Cucumis sativus* L.) cultivars with different tolerances to chilling temperature. J Jpn Soc Hortic Sci 68:780–787

Szigetti Z, Racz I, Lasztity D (2001) Paraquat resistance of weeds—the case of *Conyza canadensis* (L.). Cronq Z Naturforsch 56c:319–328

Tari I, Szalai G, Lorincz Z, Balint A (2002) Changes in thiol content in roots of wheat cultivars exposed to copper stress. Biol Plant 45:255–260. doi:10.1023/A:1015105025080

Wingate V, Lawton M, Lamb C (1988) Gutathione massive and selective induction of plant defense genes. Plant Physiol 87:206–210

Yabuta Y, Mieda T, Rapolu M, Nakamura A, Motoki T, Maruta T, Yashimura K, Ishikawa T, Shigeoka S (2007) Light regulation of ascorbate biosynthesis is dependent on the photosynthetic electron transport chain but independent of sugars in *Arabidopsis*. J Exp Bot 58:2661–2671. doi:10.1093/jxb/erm124

Yu C-W, Murphy T, Sung W-W, Lin C-H (2002) H_2O_2 treatment induces glutathione accumulation and chilling tolerance in mung bean. Funct Plant Biol 29:1081–1087. doi:10.1071/PP01264

Yu C-W, Murphy T, Lin C-H (2003) Hydrogen peroxide-induced chilling tolerance in mung bean mediated through ABA-independent glutathione accumulation. Funct Plant Biol 30:955–963. doi:10.1071/FP03091

Yu Q, Cairns A, Powles S (2004) Paraquat resistance in population of Lolium rigidum. Funct Plant Biol 31:247–254. doi:10.1071/FP03234