BIOLOGIA PLANTARUM 52 (3): 413-422, 2008

REVIEW

# Production of reactive oxygen species and development of antioxidative systems during *in vitro* growth and *ex vitro* transfer

P. BAŤKOVÁ[1,2], J. POSPÍŠILOVÁ[1]* and H. SYNKOVÁ[1]

*Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Na Karlovce 1a, CZ-16000 Prague, Czech Republic*[1]
*Charles University Prague, Faculty of Science, Viničná 5, CZ-12844 Prague, Czech Republic*[2]

## Abstract

*Ex vitro* transfer is often stressful for *in vitro* grown plantlets. Water stress and photoinhibition, often accompanying the acclimatization of *in vitro* grown plantlets to *ex vitro* conditions, are probably the main factors promoting production of reactive oxygen species (ROS) and in consequence oxidative stress. The extent of the damaging effects of ROS depends on the effectiveness of the antioxidative systems which include low molecular mass antioxidants (ascorbate, glutathione, tocopherols, carotenoids, phenols) and antioxidative enzymes (superoxide dismutase, ascorbate peroxidase, catalase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase). This review is focused on ROS production and development of antioxidative system during *in vitro* growth and their further changes during *ex vitro* transfer.

*Additional key words*: ascorbate, ascorbate peroxidase, carotenoids, catalase, dehydroascorbate reductase, glutathione, glutathione reductase, malondialdehyde, peroxidase, superoxide dismutase.

## Introduction

Within the last four decades plant micropropagation has developed from a laboratory curiosity to a real industry. Nevertheless, its widespread use is restricted by the formation of plantlets of abnormal morphology, anatomy and physiology induced by special conditions during *in vitro* culture, *e.g.*, high air humidity, decreased air turbulence, low irradiance, low $CO_2$ concentration during light period, cultivation media supplemented with sugars and growth regulators (for review see, *e.g.*, Pospíšilová *et al*. 1992, 1997, 2005, 2007, Buddendorf-Joosten and Woltering 1994, Desjardins 1995, Kozai and Smith 1995, Kubota *et al*. 1997). After *ex vitro* transfer, these plantlets might be easily impaired by sudden changes in environmental conditions. Low air humidity and high irradiance belong to the most harmful ones.

A common feature of the imposition of environmental stresses is the increased rate of production of reactive oxygen species (ROS; *i.e.* hydrogen peroxide, superoxide radicals, singlet oxygen, hydroxyl radicals). The most important sources of ROS are chloroplasts, mitochondria, peroxisomes, and the cytosol (*e.g*. Miszalski *et al*. 2007). In chloroplasts, one of the sources of ROS production is direct electron flow to oxygen (Mehler reaction). Moreover, during photorespiration $H_2O_2$ generation occurs at the step of glyoxylate formation from glycolate (Levine 1999). In mitochondria, ROS production occurs mainly at two sites of the electron transport chain: NAD(P)H dehydrogenases and the cytochrome $bc_1$ complex (Ślezak *et al*. 2007). Although ROS are inevitable byproducts of aerobic metabolism, they cause lipid peroxidation and consequently membrane injuries, protein degradation, enzyme inactivation, damage of DNA. Therefore their production and removal must be controlled (*e.g*. Hernández *et al*. 2006, Hadži-Tašković

*Received* 7 December 2007, *accepted* 31 January 2008
*Abbreviations*: ABA - abscisic acid; APX - ascorbate peroxidase (EC 1.11.1.11); Car - carotenoids; CAT - catalase; DHAR - dehydroascorbate reductase (EC 1.8.5.1); $GA_3$ - gibberellic acid; GR - glutathione reductase (EC 1.6.4.2); LOX - lipoxygenase (EC 1.13.11.12); MDA - malondialdehyde; MDHAR - monodehydroascorbate reductase (EC 1.6.5.4), PEG - polyethylene glycol; POX - peroxidase (EC 1.11.1.7); ROS - reactive oxygen species; SA - salicylic acid; SOD - superoxide dismutase (EC 1.15.1.1).
*Acknowledgements*: The authors acknowledge the financial support of the Grant Agency of the Czech Republic (grant No. 522/07/0227)
* Corresponding author; fax: (+420) 224310113, e-mail: pospisilova@ueb.cas.cz

Šukalović and Vuletić 2007, Shao *et al*. 2008). The extent of the damaging effects of ROS depends on the effectiveness of the antioxidative systems which include low molecular mass antioxidants (ascorbate, glutathione, tocopherols, carotenoids, phenols) as well as several antioxidative enzymes (superoxide dismutase, SOD, ascorbate peroxidase, APX, catalase, CAT, glutathione reductase, GR, monodehydroascorbate reductase, MDHAR, dehydroascorbate reductase, DHAR) (*e.g*. Hernández *et al*. 2006, Zlatev *et al*. 2006). SODs catalyze dismutation of superoxid radical $O_2^{\bullet -}$ to $H_2O_2$ and $O_2$ at the site of its production. SODs are distributed in all cellular compartments, FeSOD in chloroplasts, MnSOD in mitochondria and peroxisomes, and Cu/ZnSOD in cytosol and chloroplasts. APX, MDHAR, DHAR and GR form so-called ascorbate-glutathione cycle which converts $H_2O_2$ to water and recycle ascorbate and glutathione. CAT also uses $H_2O_2$ as a substrate in peroxisomes. PODs catalyse various reactions where $H_2O_2$ is used as one of their substrates including cell wall lignification (*e.g*. Lee *et al*. 2007).

It is well known that many different stresses, for example high irradiance (Hernández *et al*. 2006, Lambreva *et al*. 2006), drought (Sharma and Dubey 2004, 2005, Zhang *et al*. 2004, Agarwal *et al*. 2005, Jain *et al*. 2006, Zlatev *et al*. 2006, Fazeli *et al*. 2007), salinity (Agarwal and Pandey 2004, Koca *et al*. 2006, Mandhania *et al*. 2006, Niknam *et al*. 2006, Xu *et al*. 2007), high temperature (Ali *et al*. 2005a), heavy metals (Gajewska *et al*. 2006, Scebba *et al*. 2006, Qureshi *et al*. 2007, Sharma and Dubey 2007, Romanowska *et al*. 2008), or aluminum (Liu *et al*. 2008, Shamshi *et al*. 2008) cause ROS production and promote activities of antioxidative enzymes. Nevertheless, stress-induced activities of antioxidative enzymes are species, cultivar and age dependent (Dertinger *et al*. 2003, Khanna-Chopra and Selote 2007). On the other hand, the effects of elevated $CO_2$ concentration, limited water supply and temperature during alfalfa regrowth were not related to significant and general changes in oxidative stress or antioxidant capacity (Erice *et al*. 2007). Nevertheless, plants with higher content of antioxidants, either constitutive or induced, are usually better adapted to stress.

However, ROS at low concentrations can be signals that switch on developmental programs or regulate physiological processes as cell wall loosening required for cell elongation, modulation of cytosol $Ca^{2+}$ concentration, senescence, adaptation to abiotic stresses or resistance to pathogens (Papadakis and Roubelakis-Angelakis 2002, Gechev *et al*. 2005, Procházková and Wilhelmová 2007, Ślesak *et al*. 2007, Vilela *et al*. 2007). ROS have been implicated as second messengers in several plant hormone responses (Kwak *et al*. 2006). Signalling mediated by ROS involves G-proteins, MAP kinases and protein Tyr phosphatases (Zhang *et al*. 2006, Shao *et al*. 2008). In addition to concentration, the balance between their positive and negative effects is influenced by the physiological and developmental status of tissues (Obert *et al*. 2005).

Further, ROS induce accumulation of stress hormones, such as salicylic acid and ethylene (for review see, Shao *et al*. 2008). On the other hand, abscisic acid (ABA) can affect ROS production and ROS are secondary messengers in many ABA signalling pathways including antioxidative defence induction (Jiang and Zhang 2002, 2004, Xiong *et al*. 2006, Zhang *et al*. 2006). Cytokinins were also reported to modulate antioxidative system and P*ssu-ipt* transgenic tobacco plants with increased cytokinin content exhibited elevated activities of antioxidant enzymes (Synková *et al*. 2006). The increased activities of SOD, CAT, APX, GPX and DHAR were responsible for the delay of osmotic stress induced senescence in transgenic ($P_{SAG12}$-*IPT*) gerbera plants (Lai *et al*. 2007).

This review aims to illustrate still fragmentary knowledge about occurrence of oxidative stress and development of antioxidative system during *in vitro* culture and acclimatization to *ex vitro* conditions. The papers dealing with *in vitro* production of antioxidants for pharmaceutical purposes are not included.

## *In vitro* culture

***In vitro* growth and development:** For cultivation of plantlets *in vitro*, addition of phytohormones into media is often essential. The role of ROS in plant growth and development is therefore further substantiated by the interplay of ROS with a number of phytohormones (Gechev *et al*. 2005). Therefore the study of the ROS production, oxidative stress and the efficiency of antioxidants during different stages of direct organogenesis or somatic embryogenesis is of increasing interest.

It was hypothesized that plant recalcitrance might be associated with ROS production and oxidative stress during *in vitro* culture (*e.g*. Benson 2000, Papadakis and Roubelakis-Angelakis 2002, Dutta Gupta and Datta 2003/4). This suggestion was confirmed by experiments with *Daucus carota* where application of lipid peroxidation products 4-hydroxy-2-nonenal or malondialdehyde (MDA) inhibited proliferation and embryogenesis (Adams *et al*. 1999). Further, addition of antioxidants polyvinylpolypyrrolidone and dithiotreitol to *Pinus virginiana* cultures inhibited tissue necrosis and improved callus formation, shoot differentiation and growth, and rooting (Tang *et al*. 2004a). ROS formation during *in vitro* cultivation of flax was dependent on concentrations of naphthalene acetic acid and benzylaminopurine and the number of embryo-like structures was positively correlated while number of roots was negatively correlated with ROS formation. Moreover, both anti-oxidative compound desferrioxamine (inhibitor of Fenton reaction) and pro-oxidative compound 4-hydroxy-2-nonenal inhibited the formation of shoots

and roots (Obert *et al*. 2005). Also in *Gladiolus*, an addition of natural antioxidants such as glutathione, α-tocopherol and ascorbate stimulated shoot organogenesis. However, these antioxidants inhibited somatic embryogenesis in the same plant species, the frequency of somatic embryogenesis was even increased by addition of $H_2O_2$. In the same cultures, SOD activity decreased while CAT and POX activities increased during shoot formation while SOD activity increased and CAT and POX activities decreased during somatic embryogenesis (Dutta Gupta and Datta 2003/4). CAT and SOD activities were twofold higher in *Quercus robur* plantlets than in seedlings. Further, their activities and occurrence of different isoforms varied in different plantlet organs and in particular CAT-2 isoform was activated in the basal callus of rooted microshoots (Racchi *et al*. 2001). In *Pinus strobus* direct adventitious shoot formation induced by thidiazuron was connected with gradual decrease in activities of POX and CAT (Tang and Newton 2005). Application of polyamines recovered browning tissue of *Pinus virginiana* into normal callus by increasing the activities of APX, GR and SOD and by decreasing lipid peroxidation (Tang *et al*. 2004b). The lower POX activity in combination with higher auxin/cytokinin ratio made *Schlumbergera* more recalcitrant for adventitious shoot formation than *Rhipsalidopsis* (Sriskandarajah *et al*. 2006). On the other hand, difficult-to-root species *Grevillea petrophioides* showed higher total POX activity at the time point of adventitious root formation than easy-to-root species *Grevillea rondeu*. In addition, basic POX isoforms were more prominent in *G. petrophioides* while acidic isoforms in *G. rondeu* (Ludwig-Muller 2003).

*In vitro* culture can be a convenient tool to study switch from vegetative to reproductive phase. Gibberellic acid ($GA_3$) and sucrose in appropriate concentrations were essential for *in vitro* flowering of *Spathiphyllum*. Flowering plants had higher content of glutathione and GR, APX, MDHAR and POX activities in comparison with non-flowering plants (Dewir *et al*. 2007). The authors suggested that oxidative stress during period of $GA_3$ treatment promoted glutathione synthesis and *Spathiphyllum* flowering.

SOD isozyme markers have been successfully used to determine the genetic stability of tissue cultured *Cordyline terminalis* clones (Ray *et al*. 2006).

**Hyperhydricity:** High air humidity has been considered as the most important factor responsible for reduced transpiration, $CO_2$ and $O_2$ exchange, and excessive water accumulation. To explain anatomical and physiological disorders accompanying hyperhydricity in *in vitro* cultures, a research has been also addressed on relationship between hyperhydricity and oxidative stress. The time course of $H_2O_2$ generation in hyperhydric tissues of carnation microshoots confirmed narrow connection between hyperhydricity and oxidative stress in this species (Saher *et al*. 2004). In hyperhydric carnation leaves, increased MDA content and total POX activity was also observed (Olmos *et al*. 1997, Saher *et al*. 2004). However, this increase in POX activity was due to increase in the activity of basic isoforms while activity of acidic isoforms and in consequence lignification was reduced (Olmos *et al*. 1997). An oxidative stress characterized by markedly increased content of MDA and activity of lipoxygenase (LOX) were found in hyperhydric shoots of *Euphorbia millii*. At the same time, these plantlets reduced oxidative stress by increased activities of SOD, POX and CAT. The activities of enzymes of ascorbate-glutathione cycle (APX, GR, MDHAR and DHAR) were also increased indicating a crucial role of elimination of $H_2O_2$ from plant cells (Dewir *et al*. 2006). Increased SOD and CAT activities in hyperhydric tobacco leaves were observed by Piqueras *et al*. (1998). Similarly, higher activities of SOD, CAT, APX and GR were found in hyperhydric leaves than in healthy leaves of apple regenerants grown in bioreactor (Chakrabarty *et al*. 2006). In contrast, a hyperhydricity in liquid-cultured *Narcissus* induced by growth retardant ancymidol was connected with decreased activities of APX and CAT and increased initiation of meristematic centers (Chen and Ziv 2001). In hyperhydric shoots of *Prunus avium*, $H_2O_2$ was accumulated due to increased SOD activity and decreased activities of POX, APX, CAT, DHAR, MDHAR and GR (Franck *et al*. 1995). In micropropagated *Dianthus*, the prevention of hyperhydricity by bottom cooling decreased $H_2O_2$ production, lipid peroxidation (MDA content), and SOD and CAT activities (Saher *et al*. 2005b). Addition of rare earth elements La, Ce and Nd into medium reduced hyperhydricity in *Lepidium meyeni* shoots and enhanced activities of POD, CAT, APX, SOD, MDHAR, and GR (Wang *et al*. 2007).

In contrast, Saher *et al*. (2005a) found induction of the oxidative pentose phosphate and fermentative pathways in carnation hyperhydric leaves. According to their opinion, hypoxia stress was the main factor affecting metabolism of hyperhydric leaves.

***In vitro* selection of NaCl tolerant plants:** *In vitro* selection is one of the recent methods applied to speed up development of tolerant crops. Among many papers determining survival and growth of calli or regenerants during their adaptation to osmotic or salt stress, few focused also on co-occurring oxidative stress. For example, increase in NaCl concentration from 150 to 250 mM resulted in significant increases in superoxide radical production in cotton callus tissue. This triggered increase in activities of antioxidative enzymes CAT, POD, APX and GR (Vital *et al*. 2008). However, when the cotton calli were pre-treated with superoxide radical scavenger N-acetyl L-cysteine upregulation of enzyme activities was inhibited. Therefore the authors suggested that ROS are involved in "turning on" antioxidative defence (Vital *et al*. 2008). Both ABA-dependent and ABA-independent pathways in the upregulation of antioxidative enzymes during NaCl stress were suggested (Bueno *et al*. 1998, Bellaire *et al*. 2000).

Increased activities of antioxidative enzymes and their

mRNA levels were found in number of cultures subjected to salt stress (Gossett *et al.* 1994, Bueno *et al*. 1998, Molina *et al*. 2002, Ślesak and Miszalski 2003, Molassiotis *et al*. 2006b, Niknam *et al*. 2006, Erturk *et al*. 2007, Lu *et al*. 2007). A significant increase in SOD, CAT, APX and GR activities under 75 or 150 mM NaCl was found in callus of the salt-tolerant cotton cultivar, but not in callus of salt-sensitive cultivar (Gossett *et al*. 1994). NaCl tolerant lines of *Chrysanthemum morifolium* developed *in vitro* through *in vitro* mutagenesis or a stepwise increase in NaCl concentration exhibited significant increase in SOD, APX and GR activities, and the former also higher contents of carotenoids and ascorbate (Hossain *et al*. 2006, 2007). Similarly, increased capacity for ROS scavenging by increased activities of SOD, APX, CAT, GR and glutathione transferase was found in NaCl tolerant tomato calli (Rodríguez-Rosales *et al*. 1999). NaCl and drought tolerance was also increased by over-expression of rice Cu/ZnSOD in chloroplasts of tobacco using *Agrobacterium*-mediated transformation (Badawi *et al*. 2004). The increase in content of ascorbic acid was observed in salt tolerant potato cell lines (Queirós *et al*. 2007). Exogenous proline was more effective than glycinebetaine in maintaining the activity of enzymes of ascorbate-glutathione cycle in NaCl-stressed tobacco cell suspension culture (Hoque *et al*. 2007).

In *in vitro* grown *Mesembryanthemum crystallinum*, NaCl induced shift of its carbon assimilation mode from $C_3$ to CAM pathway, increase in MnSOD, FeSOD and Cu/ZnSOD activities (Ślesak *et al*. 2003), and induction of new MnSODII isoform in roots (Ślesak and Miszalski 2003). Tomato cells adapted to NaCl contained a lower concentration of salicylic acid (SA), and activities of LOX and MnSOD, but higher GR and APX activities than unadapted cells. Moreover, these enzyme activities were differently affected by short-term NaCl stress or SA application in NaCl-adapted and unadapted cells (Molina *et al*. 2002). In tobacco BY-2 cell culture, not only NaCl but also polyethylene glycol (PEG) treatment led to an increase in SOD, CAT and APX activities, whereas GR activity remained unchanged (Bueno *et al*. 1998). PEG treatment increased content of MDA and activity of SOD in calli of two rice genotypes and higher growth in more tolerant genotype was accompanied not only with higher water potential and solute accumulation but also with higher SOD activity (Chandrasekhara Reddy *et al*. 2004). PEG-induced increase in MDA content and activities of SOD, CAT, APX, POX and GR has been found recently in sweet cherry (Sivritepe *et al*. 2008).

In contrast, exogenous $H_2O_2$ increased salt resistance in calli of *Populus euphratica* by increasing K/Na ratio, which was dependent on the increased plasma membrane H+-ATPase activity (Zhang *et al*. 2007).

**Effects of different metals:** *In vitro* cultures are also good models for studying effect of different metals from their deficiency to toxicity. In connection with this, oxidative stress and antioxidants were also followed.

In strawberry culture, addition of silver nitrate inhibited ethylene production and increased contents of chlorophyll and soluble protein and activities of SOD, POX and CAT (Qin *et al*. 2005). The addition of calcium was found important for induction of somatic embryogenesis in *Eucalyptus urophylla* connected with increased contents of proteins and sugars and POX activity (Arruda *et al*. 2000). In peach rootstock culture, iron deficiency caused a reduction of chlorophyll and carotenoid content and CAT and SOD activities (Lombardi *et al*. 2003).

Increasing boron concentrations from 0.1 to 6.0 mM in apple rootstocks enhanced LOX activity, lipid peroxidation, $H_2O_2$ accumulation and SOD and POD activities (Molassiotis *et al*. 2006a).

In *Saccharum officinarum* calli grown under 0.5 or 1 mM $CdCl_2$ a rapid increase of CAT activity was detected (14-fold higher after 15 d) while SOD activity did not exhibit any major variation (Fornazier *et al*. 2002). In sunflower calli, low concentration of Cd (5 μM) increased CAT and POX activities, but higher concentrations (50 and 500 μM) decreased activities of both enzymes. However, in calli which were able to survive at 50 μM Cd for 6 months, CAT and POX activities were higher than in control calli (Azevedo *et al*. 2005). In coffee cell suspension cultures, 0.5 mM Cd induced lipid peroxidation, increased activities of CAT, GR and SOD while decreased APX activity (Gomes *et al*. 2006).

Contents of antioxidants and activities of antioxidative enzymes increased after addition of Hg up to 4 μM concentration and then severely declined at 50 μM Hg (Israr and Sahi 2006).

Exposure of suspension culture of *Panax ginseng* to 50 μM Cu resulted in strong growth inhibition and oxidative stress (accumulation of $H_2O_2$, MDA, increased LOX activity). Ascorbate and glutathione were oxidized to dehydroascorbate and glutathiondisulfide. SOD activity was increased (mainly due to induction of FeSOD) while CAT and POX activities were inhibited (Ali *et al*. 2006b). On the contrary, *Prunus cerasifera* grown *in vitro* tolerated Cu up to 50 μM concentration. Its ability to tolerate this rather high Cu concentration was partially due to induction of SOD and CAT gene expression and in consequence increased SOD and CAT activities (Lombardi and Sebastiani 2005).

In suspension cultures of *Coffea arabica*, addition of $NiCl_2$ into medium induced rapid accumulation of Ni in cells and increase in activities of SOD, CAT, APX, POX and GR (Gomes *et al*. 2006). Lipid peroxidation and alterations in antioxidative enzymes were the main responses of coffee cell suspension also to application of selenite (Gomes *et al*. 2007).

**Effect of other environmental factors:** Sugars in the medium are not only sources of energy and carbon skeleton for *in vitro* grown plants but also signalling molecules. Therefore it is not surprising that they can also affect development of antioxidative systems. It was found

that the SOD activity of *Trifolium repens* was affected by the type of explant as well as presence or absence of sucrose, glucose, fructose and maltose in medium. In cultures derived from cotyledon explants MnSOD activity was highest in medium with sucrose, fructose or maltose, Cu/ZnSODI activity was highest on medium with glucose and fructose while FeSOD and Cu/ZnSODII activities were highest in the absence of sugars. In contrast, in cultures derived from hypocotyl explants MnSOD and FeSOD activities were similar for all tested media and Cu/ZnSODI and Cu/ZnSODII activities were lowest on medium with sucrose (Ślesak *et al*. 2006). Embryo axes isolated from germinating lupine seeds and cultivated *in vitro* on medium without sucrose showed anatomical and physiological features of sucrose starvation. In these cultures SOD activity was higher while CAT and POX activities lower than in cultures with 60 mM sucrose (Morkunas *et al*. 2003). Under chilling stress, the rate of lipid peroxidation and SOD activity in *in vitro* grown potato genotypes were dependent on the content of intracellular sugars (Deryabin *et al*. 2007).

Elevated $CO_2$ concentration (from 0.03 to 0.5, 1, 2 and 5 %) in bioreactor with root suspension cultures of *Echinacea angustifolia* reduced superoxide anion accumulation, MDA content and LOX activity. Simultaneously, contents of glutathione and ascorbate and activities of APX, DHAR and MDHAR gradually increased while maximum SOD and CAT activities were observed at 0.5 % $CO_2$ (Ali *et al*. 2006a).

Somatic embryos of *Eleutherococcus senticosus* were grown in bioreactor under different temperature (12, 16, 24 and 30 °C) for 45 d and maximum growth and production of secondary metabolites was achieved at 24 °C. Low and especially high temperature induced oxidative stress (increased production of $H_2O_2$, MDA content and LOX activity). On the other hand, activities of antioxidative enzymes (SOD, CAT, DHAR, MDHAR, GR, APX) were increased only at low temperatures (Shohael *et al*. 2006b). Somatic embryos of the same plant species were also grown under different light sources. Higher $H_2O_2$ and MDA contents and LOX, SOD, CAT and MDHAR activities was observed at red radiation compared to dark-grown embryos. On the other hand, maximum APX activity and biomass accumulation was found under fluorescent tubes (Shohael *et al*. 2006a).

*Ribes* genotypes with different survival response following cryopreservation showed differences in content in antioxidants. Higher accumulation of antioxidants during cold acclimation and their persistence during recovery was found in more tolerant genotypes (Johnston *et al*. 2007).

## *Ex vitro* transfer

*Ex vitro* transfer of *in vitro* grown plantlets is often accompanied by water stress and/or photoinhibition (*e.g*. Semorádová *et al*. 2002, Carvalho *et al*. 2006). These stresses might be the main factors promoting production of reactive oxygen species and in consequence oxidative stress. Therefore, for successful *ex vitro* transfer, sufficient content of non-enzymatic antioxidants as well as activities of antioxidative enzymes formed during previous *in vitro* growth are very important. No less important are changes in antioxidants induced after *ex vitro* transfer, however, little is still known about these changes.

The extremely short life time of ROS makes the study of their production *in planta* very difficult. Nevertheless, Vilela *et al*. (2007), using diaminobenzidine and nitroblue tetrazolium together with high-resolution imaging, detected ROS accumulation in the first days after *ex vitro* transfer of grapevine followed by gradual decrease to levels comparable in greenhouse grown plants. While superoxide radical was uniformly distributed, $H_2O_2$ was preferentially accumulated in veins and stomatal guard and surrounding cells. Transient increase in $H_2O_2$ content together with reversible photoinhibition was observed also in grapevine leaves during *ex vitro* acclimatization under irradiance fourfold higher than during *in vitro* growth. Concomitantly, upregulation of APX, DHAR, MDHAR, GR, SOD and CAT activities and expression of associated genes were observed (Carvalho *et al*. 2006).

During *ex vitro* acclimatization of *Spathiphyllum* and *Calathea* plants, CAT activity increased, reaching a maximum 4 weeks after transplantation, while SOD activity reached maximum in the 24$^{th}$ week (Van Huylenbroeck *et al*. 1998). In *Calathea* plants, also activity of GR increased during first 3 weeks of acclimatization while activities of APX and POX later on (Van Huylenbroeck *et al*. 1997). In *Spathiphyllum* leaves, only one band corresponding to MnSOD was detected initially and a second MnSOD band appeared after 12 weeks. *Calathea* leaves showed more SOD isozymes whose relative contribution to the total activity changed with time. Also a new MnSOD band appeared only during the 3$^{rd}$ week of acclimatization (Van Huylenbroeck *et al*. 1998). In the same plant species, the changes in antioxidative enzyme activities after *ex vitro* transfer were dependent on irradiance. SOD activity was not changed at low irradiance while it at first decreased and then increased under high irradiance. CAT activity increased after transplantation more at low than at high irradiance. The highest activity of APX for plants grown at medium and high irradiance was measured at day 14 and 35, respectively. GR activity also increased considerably and DHAR activity decreased but no clear dependences of activities of these enzymes on irradiance were found (Van Huylenbroeck *et al*. 2000). Acclimatization of *Phalaenopsis* plantlets to *ex vitro* conditions was also affected by irradiance (Ali *et al*. 2005b). The highest irradiance used (300 μmol $m^{-2}$ $s^{-1}$) induced photoinhibition (characterized by reduced variable to maximum

fluorescence ratio) and oxidative stress (increased LOX activity and MDA content). Regarding antioxidative enzymes, SOD and CAT activities in leaves increased during acclimatization more under high than under intermediate (160 μmol $m^{-2}$ $s^{-1}$) and low (60 μmol $m^{-2}$ $s^{-1}$) irradiances. In contrast, DHAR and MDHAR activities increased at low and intermediate irradiance but decreased at high irradiance. No significant change in GR activity was found at high and intermediate irradiance, though it decreased at low irradiance (Ali *et al*. 2005b).

Grapevine and chestnut plantlets were acclimatized under four treatments combining two irradiances and two $CO_2$ concentrations and variation in glutathione pools and parameters of chlorophyll fluorescence were monitored (Carvalho and Amâncio 2002). Grapevine revealed a better adaptation to *ex vitro* conditions without photoinhibition symptoms under high irradiance and both species exhibited benefit from increased $CO_2$ concentration. Photoinhibition in chestnut under high irradiance was accompanied with increased content of reduced glutathione and increased GR activity.

In tobacco plants, antioxidative enzyme activities were affected by $CO_2$ concentration during previous *in vitro* cultivation and after *ex vitro* transplantation as well as by ABA application (Synková and Pospíšilová 2002). The highest CAT activity in plants acclimatized to *ex vitro* conditions for 4 weeks was found in those grown *in vitro* under higher $CO_2$ concentration in ventilated *Magenta* boxes (M-plants) and treated immediately after transfer by ABA. Also SOD activity was higher in M-plants than in plants grown *in vitro* under lower $CO_2$ concentration in tightly closed glass vessels (G-plants). In contrast, GR activity was double in G-plants in comparison with that in M-plants, but this difference disappeared after ABA application. The POX activity was also mostly higher in G-plants than in M-plants. ABA application caused decrease in activities of GR, MnSOD and POD. Elevated $CO_2$ concentration during acclimatization increased POX and SOD activities (Synková and Pospíšilová 2002).

For acclimatization of *Spathiphyllum* plantlets *Perlite* with nutrient solution of different osmotic potential was used (Dewir *et al*. 2005) and the moderate osmotic stress induced down-regulation of photosynthesis and increased activities of CAT, APX, POD, GR and MDHAR.

During *ex vitro* acclimatization of *Doritaenopsis* plantlets, POX activity was not affected by relative humidity but increased at low temperature together with decreased chlorophyll content and photosynthetic efficiency (Jeon *et al*. 2006).

For successful *ex vitro* acclimatization another important roles of PODs have been suggested. In connection with their possible participation in lignin formation or auxin metabolism, these enzymes have been proposed as biochemical markers of the rooting (*e.g*. Syros *et al*. 2004). In fact, both *in vitro* or *ex vitro* rooting microshoots of *Gardenia jasminoides* showed a clear relationship between rooting and POX activity: POX activity was lowest in the inductive phase, reached a maximum during root initiation and decreased during further root growth (Hatzilazarou *et al*. 2006).

## References


Adams, L.K., Benson, E.E., Staines, H.J., Bremmer, D.H., Millan, S., Deighton, N.: Effect of the lipid peroxidation products 4-hydroxy-2-nonenal and malondialdehyde on the proliferation and morphogenetic development of *in vitro* plant cells. - J. Plant Physiol. **155**: 376-386, 1999.

Agarwal, S., Pandey, V.: Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. - Biol. Plant. **48**: 555-560, 2004.

Agarwal, S., Sairam, R.K., Srivastava, G.C., Meena, R.C.: Changes in antioxidant enzymes activity and oxidative stress by abscisic acid and salicylic acid in wheat genotypes. - Biol. Plant. **49**: 541-550, 2005.

Ali, B.M., Hahn, E.-J., Paek, K.-Y.: Effect of temperature on oxidative stress defence systems, lipid peroxidation and lipoxygenase activity in *Phalaenopsis*. - Plant Physiol. Biochem. **43**: 213-223, 2005a.

Ali, B.M., Hahn, E.-J., Paek, K.-Y.: Effects of light intensities on antioxidant enzymes and malondialdehyde content during short-term acclimatization on micropropagated *Phalaenopsis* plantlet. - Environ. exp. Bot. **54**: 109-120, 2005b.

Ali, B.M., Hahn, E.-J., Paek, K.-Y.: Antioxidative responses of *Echinacea angustifolia* cultured roots to different levels of $CO_2$ in bioreactor liquid cultures. - Enzyme microb. Technol. **39**: 982-990, 2006a.

Ali, B.M., Hahn, E.-J., Paek, K.-Y.: Copper-induced changes in the growth, oxidative metabolism, and saponin production in suspension culture roots of *Panax ginseng* in bioreactors. - Plant Cell Rep. **25**: 1122-1132, 2006b.

Arruda, S.C.C., Souza, G.M., Almeida, M., Gonçalves, A.N.: Anatomical and biochemical characterization of the calcium effect on *Eucalyptus urophylla* callus morphogenesis *in vitro*. - Plant Cell Tissue Organ Cult. **63**: 143-154, 2000.

Azevedo, H., Pinto, C.G.G., Santos, C.: Cadmium effects in sunflower: membrane permeability and changes in catalase and peroxidase activity in leaves and calluses. - J. Plant. Nutr. **28**: 2233-2241, 2005.

Badawi, G.H., Yamauchi, Y., Shimada, E., Sasaki, R., Kawano, N., Tanaka, K., Tanaka, K.: Enhanced tolerance to salt stress and water deficit by overexpressing superoxide dismutase in tobacco (*Nicotiana tabacum*) chloroplasts. - Plant Sci. **166**: 919-928, 2004.

Bellaire, B.A., Carmody, J., Braud, J., Gossett, D.R., Banks, S.W., Cran Lucas, M., Fowler, T.E.: Involvement of abscisic acid-dependent and-independent pathways in the upregulation of antioxidant enzyme activity during NaCl stress in cotton callus tissue. - Free Rad. Res. **33**: 531-545, 2000.

Benson, E.E.: Do free radicals have a role in plant tissue culture recalcitrance? - *In Vitro* cell. dev. Biol. Plant. **36**: 163-170, 2000.

Buddendorf-Joosten, J.M.C., Woltering, E.J.: Components of

the gaseous environment and their effects on plant growth and development *in vitro*. - Plant Growth Regul. **15**: 1-16, 1994.

Bueno, P., Piqueras, A., Kurepa, J., Savouré, A., Verbruggen, N., Van Montagu, M., Inzé, D.: Expression of antioxidant enzymes in response to abscisic acid and high osmoticum in tobacco BY-2 cell cultures. - Plant Sci. **138**: 27-34, 1998.

Carvalho, L.C., Amâncio, S.: Antioxidant defence system in plantlets transferred from *in vitro* to *ex vitro*: effects of increasing light intensity and $CO_2$ concentration. - Plant Sci. **162**: 33-40, 2002.

Carvalho, L.C., Vilela, B.J., Vidigal, P., Mullineaux, P.M., Amâncio, S.: Activation of the ascorbate-glutathione cycle is an early response of micropropagated *Vitis vinifera* L. explants transferred to *ex vitro*. - Int. J. Plant Sci. **167**: 759-770, 2006.

Chakrabarty, D., Park, S.Y., Ali, M.B., Shin, K.S., Paek, K.Y.: Hyperhydricity in apple: ultrastructural and physiological aspects. - Tree Physiol. **26**: 377-388, 2006.

Chandrasekhara Reddy, P., Halesh, G.K., Najranabhaiah, S.N.: Effect of PEG stress on growth and associated physiological and biohemical changes in selected and non selected upland rice. - Indian J. Plant Physiol. **9**: 413-418, 2004.

Chen, J., Ziv, M.: The effect of ancymidol on hyperhydricity, regeneration, starch and antioxidant enzymatic activities in liquid-cultured *Narcissus*. - Plant Cell Rep. **20**: 22-27, 2001.

Dertinger, U., Schaz, U., Schulze, E.-D.: Age-dependence of the antioxidative system in tobacco with enhanced glutathione reductase activity or senescence-induced production of cytokinins. - Physiol. Plant. **119**: 19-29, 2003.

Deryabin, A.N., Sin′kevich, M.S., Dubinina, I.M., Burakhanova, E.A., Trunova, T.I.: Effect of sugars on the development of oxidative stress induced by hypothermia in potato plants overexpressing yeast invertase gene. - Russ. J. Plant Physiol. **54**: 32-38, 2007.

Desjardins, Y.: Photosynthesis *in vitro* - on the factors regulating $CO_2$ assimilation in micropropagation systems. - Acta Hort. **393**: 45-61, 1995.

Dewir, Y.H., Chakrabarty, D., Ali, M.B., Hahn, E.J., Paek, K.Y.: Effects of hydroponic solution EC, substrates, PPF and nutrient scheduling on growth and photosynthetic competence during acclimatization of micropropagated *Spathiphyllum* plantlets. - Plant growth Regul. **46**: 241-251, 2005.

Dewir, Y.H., Chakrabarty, D., Ali, M.B., Hahn, E.J., Paek, K.Y.: Lipid peroxidation and antioxidant enzyme activities of *Euphorbia millii* hyperhydric shoots. - Environ. exp. Bot. **58**: 93-99, 2006.

Dewir, Y.H., Chakrabarty, D., Ali, M.B., Sing, N., Hahn, E.-J., Paek, K.-Y.: Influence of $GA_3$, sucrose and solid medium/bioreactor culture on *in vitro* flowering of *Spathiphyllum* and association of glutathione metabolism. - Plant Cell Tissue Organ Cult. **90**: 225-235, 2007.

Dutta Gupta, S., Datta, S.: Antioxidant enzyme activities during *in vitro* morphogenesis of gladiolus and the effect of application of antioxidants on plant regeneration. - Biol. Plant. **47**: 179-183, 2003/4.

Erice, G., Aranjuelo, I., Irigoyen, J.J., Sánchez-Díaz, M.: Effect of elevated $CO_2$, temperature and limited water supply on antioxidant status during regrowth of nodulated alfalfa. - Physiol. Plant. **130**: 33-45, 2007.

Erturk, U., Sivritepe, N., Yerlikaya, C., Bor, M., Ozdemir, F., Turkan, I.: Response of the cherry rootstock to salinity *in vitro*. - Biol. Plant. **51**: 597-600, 2007.

Fazeli, F., Ghorbanli, M., Niknam, V.: Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. - Biol. Plant. **51**: 98-103, 2007.

Fornazier, R.F., Ferreira, R.R., Pereira, G.J.G., Molina, S.M.G., Smith, R.J., Lea, P.J., Azevedo, R.A.: Cadmium stress in sugar cane callus cultures: Effect on antioxidant enzymes. - Plant cell tissue Organ cult. **71**: 125-131, 2002.

Franck, T., Kevers, C., Gaspar, T.: Protective enzymatic systems against activated oxygen species compared in normal and vitrified shoots of *Prunus avium* L. raised *in vitro*. - Plant Growth Regul. **16**: 253-256, 1995.

Gajewska, E., Skłodowska, M., Słaba, M., Mazur, J.: Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. - Biol. Plant. **50**: 653-659, 2006.

Gechev, T., Gadjev, I., Dukiandjiev, S., Minkov, I.: Reactive oxygen species as signalin molecules controlling stress adaptation in plants. - In: Pessarakli, M. (ed.): Handbook of Photosynthesis, Second Edition, Revised and Expanded. Pp. 209-218. Marcel Dekker, New York 2005.

Gomes, R.A., Jr., Gratão, P.L., Gaziola, S.A., Mazzafera, P., Lea, P.J., Azevedo, R.A.: Selenium-induced oxidative stress in cofee cell suspension cultures. - Funct. Plant Biol. **34**: 449-456, 2007.

Gomes, R.A., Moldes, C.S., Delite, F.S., Gratão, P.L., Mazzafera, P., Lea, P.J., Azevedo, R.A.: Nickel elicits a fast antioxidant response in *Coffea arabica* calls. - Plant Physiol. Biochem. **44**: 420-429, 2006.

Gomes, R.A., Jr., Moldes, C.S., Delite, F.S., Pompeu, G.B., Gratão, P.L., Mazzafera, P., Lea, P.J., Azevedo, R.A.: Antioxidant metabolism of coffee cell suspension cultures in response to cadmium. - Chemosphere **65**: 1330-1337, 2006.

Gossett, D.R., Millhollon, E.P., Cran Lucas, M., Banks, S.W., Marney, M.-M.: The effect of NaCl on antioxidant enzyme activities in callus tissue of salt-tolerant and salt-sensitive cotton cultivars (*Gossypium hirsutum* L.). - Plant Cell Rep. 13: 498-503, 1994.

Hatzilazarou, S.P., Syros, T.D., Yupsanis, T.A., Bosabalidis, A.M., Economou, A.S.: Peroxidases, lignin and anatomy during *in vitro* and *ex vitro* rooting gardenia (*Gardenia jasminoides* Ellis) microshoots. - J. Plant Physiol. **163**: 827-836, 2006.

Hadži-Tašković Šukalović, V., Vuletić, M.: The relationship between respiration rate and peroxidase activities in maize root mitochondria. - Biol. Plant. **51**: 297-302, 2007.

Hernández, J.A., Escobar, C., Creissen, G., Mullineaux, P.M.: Antioxidant enzyme induction in pea plants under high irradiance. - Biol. Plant. **50**: 395-399, 2006.

Hoque, M.A., Banu, M.N.A., Okuma, E., Murata, Y.: Exogenous proline and glycinebetaine increase NaCl-induced ascorbate glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco bright Yellow-2 suspension-cultured cells. - J. Plant Physiol. **164**: 1457-1468, 2007.

Hossain, Z., Mandal, A.K.A., Datta, K.S., Biswas, A.K.: Development of NaCl-tolerant strain in *Chrysanthemum morifolium* Ramat. through *in vitro* mutagenesis. - Plant Biol. **8**: 450-461, 2006.

Hossain, Z., Mandal, A.K.A., Datta, K.S., Biswas, A.K.: Development of NaCl-tolerant line in *Chrysanthemum morifolium* Ramat. through shoot organogenesis of selected callus line. - J. Biotechnol. **129**: 658-667, 2007.

Israr, M., Sahi, S.V.: Antioxidative responses to mercury in the cell cultures of *Sesbania drummondii*. - Plant Physiol.

Biochem. **44**: 590-595, 2006.
Jain, M., Nandwal, A.S., Kundu, B.S., Kumar, B., Sheoran, I.S., Kumar, N., Mann, A., Kukreja, S.: Water relations, activities of antioxidants, ethylene evolution and membrane integrity of pigeonpea roots as affected by soil moisture. - Biol. Plant. **50**: 303-306, 2006.
Jeon, M.-W., Ali, M.B., Hahn, E.-J., Paek, K.-Y.: Photosynthetic pigments, morphology and leaf gas exchange during *ex vitro* acclimatization of micro-propagated CAM *Doritaenopsis* plantlets under relative humidity and air temperature. - Environ. exp. Bot. **55**: 183-194, 2006.
Jiang, M., Zhang, J.: Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. - J. exp. Bot. **53**: 2401-2410, 2002.
Jiang, M.-Y., Zhang, J.-H.: Abscisic acid and antioxidative defense in plant cells. - Acta bot. sin. **46**: 1-9, 2004.
Johnston, J.W., Harding, K., Benson, E.E.: Antioxidant status and genotypic tolerance of Ribes *in vitro* cultures to cryopreservation. - Plant Sci. **172**: 524-534, 2007.
Khanna-Chopra, R., Selote, D.S.: Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than -susceptible wheat cultivars under field conditions. - Environ. exp. Bot. **60**: 276-283, 2007.
Koca, H., Ozdemir, F., Turkan, I.: Effect of salt stress on lipid peroxidation and superoxide dismutase and peroxidase activities of *Lycopersicon esculentum* and *L. pennellii*. - Biol. Plant. **50**: 745-748, 2006.
Kozai, T., Smith, M.A.L.: Environmental control in plant tissue culture - general introduction and overview. - In: Aitken-Christie, J., Kozai, T., Smith, M.L. (ed.): Automation and Environmental Control in Plant Tissue Culture. Pp. 301-318. Kluwer Academic Publishers, Dordrecht 1995.
Kubota, C., Fujiwara, K., Kitaya, Y., Kozai, T.: Recent advances in environment control in micropropagation. - In: Goto, E., Kurata, K., Hayashi, M., Sasa, S. (ed.): Plant Production in Closed Ecosystems. Pp. 153-169. Kluwer Academic Publishers, Dordrecht 1997.
Kwak, J.M., Nguyen, V., Schroeder, J.I.: The role of reactive oxygen species in hormonal responses. - Plant Physiol. **141**: 323-329, 2006.
Lai, Q.-X., Bao, Z.-Y., Zhu, Z.-J., Qian, Q.-Q., Mao, B.-Z.: Effect of osmotic stress on antioxidant enzymes activities in leaf discs of $P_{SAG12}$-*IPT* modified gerbera. - J. Zhejiang Univ. Sci. B **8**: 458-464, 2007.
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.
Lee, B.-R., Kim, K.-Y., Jung, W.-J., Avice, J.-C., Ourry, A., Kim, T.-H.: Peroxidases and lignification in relation to the intensity of water-deficit stress in white clover (*Trifolium repens* L.). - J. exp. Bot. **58**: 1271-1279, 2007.
Levine, A.: Oxidative stresses as a regulator of environmental responses in plants. - In: Lerner, H.R. (ed.): Plant Responses to Environmental Stresses. From Phytohormones to Genome Reoganization. Pp. 247-264. Marcel Dekker, New York - Basel 1999.
Liu, Q., Yang, J.L., He, L.S., Li, Y.Y., Zheng, S.J.: Effect of aluminum on cell wall, plasma membrane, antioxidants and root elongation in triticale. - Biol. Plant. **52**: 87-92, 2008.
Lombardi, L., Sebastiani, L.: Copper toxicity in *Prunus cerasifera*: growth and antioxidant enzyme responses of *in vitro* grown plants. - Plant Sci. 168: 797-802, 2005.
Lombardi, L., Sebastiani, L., Vitagliano, C.: Physiological, biochemical, and molecular effects of *in vitro* induced iron deficiency in peach rootstock Mr.S2/5. - J. Plant Nutr. **26**: 2149-2163, 2003.
Lu, S., Peng, X., Guo, Z., Zhang, G., Wang, Z., Wang, C., Pang, C., Fan, Z., Wang, J.: *In vitro* selection of salinity tolerant variants from triploid bermudagrass (*Cynodon transvaalensis* × *C. dactylon*) and their physiological responses to salt and drought stress. - Plant cell Rep. **26**: 1413-1420, 2007.
Ludwig-Muller, J.: Peroxidase isoenzymes as markers for the rooting ability of easy-to-root and difficult-to-root *Grevillea* species and cultivars of *Protea obtusifolia* (*Proteaceae*). - *In vitro* cell. dev. Biol. Plant. **39**: 377-383, 2003.
Mandhania, S., Madan, S., Sawhney, V.: Antioxidant defense mechanism under salt stress in wheat seedlings. - Biol. Plant. **50**: 227-231, 2006.
Miszalski, Z., Kornas, A., Gawronska, K., Ślesak, I., Niewiadomska, E., Kruk, J., Christian, A.L., Fischer-Schliebs, E., Krisch, R., Lüttge, U.: Superoxide dismutase activity in $C_3$ and $C_3$/CAM intermediate species. - Biol. Plant. **51**: 86-92, 2007.
Molina, A., Bueno, P., Marín, M.C., Rodríguez-Rosales, M.P., Belver, A., Venema, K., Donaire, J.P.: Involvement of endogenous salicylic acid content, lipoxygenase and antioxidant enzyme activities in the response of tomato cell suspension cultures to NaCl. - New Phytol. **156**: 409-415, 2002.
Molassiotis, A.N., Sotiropoulos, T., Tanou, G., Diamantidis, G., Therios, I.: Boron-induced oxidative damage and antioxidant and nucleolytic responses in shoot tip culture of the apple rootstock EM 9 (*Malus domestica* Borkh). - Environ. exp. Bot. **56**: 54-62, 2006.
Molassiotis, A.N., Sotiropoulos, T., Tanou, G., Kofidis, G., Diamantidis, G., Therios, I.: Antioxidant and anatomical responses in shoot culture of the apple rootstock MM 106 treated with NaCl, KCl, mannitol or sorbitol. - Biol. Plant. **50**: 61-68, 2006.
Morkunas, I., Garnczarska, M., Bednarski, W., Ratajczak, E., Waplak, S.: Metabolic and ultrastructural responses of lupine embryos axes to sugar starvation. - J. Plant Physiol. **160**: 311-319, 2003.
Niknam, V., Razavi, N., Ebrahimzadeh, H., Sharifizadeh, B.: Effect of NaCl on biomass, protein and proline contents, and antioxidant enzymes in seedlings and calli of two *Trigonella* species. - Biol. Plant. **50**: 591-596, 2006.
Obert, B., Benson, E.E., Millam, S., Preťová, A., Bremner, D.H.: Moderation of morphogenetic and oxidative stress responses in flax *in vitro* cultures by hydroxynonenal and desferrioxamine. - J. Plant Physiol. **162**: 537-547, 2005.
Olmos, E., Piqueras, A., Martínez-Solano, J.R., Hellín, E.: The subcellular localization of peroxidase and the implication of oxidative stress in hyperhydrated leaves of regenerated carnation plants. - Plant Sci. **130**: 97-105, 1997.
Papadakis, A.K., Roubelakis-Angelakis, K.A.: Oxidative stress could be responsible for the recalcitrance of plant protoplasts. - Plant Physiol. Biochem. **40**: 549-559, 2002.
Piqueras, A., Han, B.H., Van Huylenbroeck, J.M., Debergh, P.C.: Effect of different environmental conditions *in vitro* on sucrose metabolism and antioxidant enzymatic activities in cultured shoots of *Nicotiana*. - Plant Growth Regul. **25**: 5-10, 1998.
Pospíšilová, J., Čatský, J., Šesták, Z.: Photosynthesis in plants cultivated *in vitro*. - In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 525-540. Marcel Dekker, New York

1997.

Pospíšilová, J., Dodd, I.C.: Role of plant growth regulators in stomatal limitation to photosynthesis during water stress. - In: Pessarakli, M. (ed.): Handbook of Photosynthesis, Second Edition, Revised and Expanded. Pp. 811-825. Marcel Dekker, New York 2005.

Pospíšilová, J., Solárová, J., Čatský, J.: Photosynthetic responses to stresses during *in vitro* cultivation.- a review. - Photosynthetica **26**: 3-18, 1992.

Pospíšilová, J., Synková, H., Haisel, D., Semorádová, Š.: Acclimation of plantlets to *ex vitro* conditions: effects of air humidity, irradiance, $CO_2$ concentration and abscisic acid. - Acta Hort. **748**: 29-38, 2007.

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

Qin, Y.H., Zhang, S.L., Zhang, L.X., Zhu, D.Y., Syed, A.: Response of *in vitro* strawberry to silver nitrate ($AgNO_3$). - HortScience **40**: 747-751, 2005.

Queirós, F., Fidalgo, F., Santos, I., Salema, R.: *In vitro* selection of salt tolerant cell lines in *Solanum tuberosum* L. - Biol. Plant. **51**: 728-734, 2007.

Qureshi, M.I., Abdin, M.Z., Qadir, S., Iqbal, M.: Lead-induced oxidative stress and metabolic alterations. - Biol. Plant. **51**: 121-128, 2007.

Racchi, M.L., Bagnoli, F., Balla, I., Danti, S.: Differential activity of catalase and superoxide dismutase in seedlings and *in vitro* micropropagated oak (*Quercus robur* L.) - Plant Cell Rep. **20**: 169-174, 2001.

Ray, T., Saha, P., Roy, S.C.: Commercial production of *Cordyline terminalis* (L) Kunth. from shoot apex meristem and assessment for genetic stability of somaclones by isozyme markers. - Scientia Hort. **108**: 289-294, 2006.

Rodríguez-Rosales, M.P., Kerkeb, L., Bueno, P., Donaire, J.P.: Changes induced by NaCl in lipid content and composition, lipogenase, plasma membrane $H^+$-ATPase and antioxidant enzyme activities of tomato (*Lycopersicon esculentum* Mill.) calli. - Plant Sci. **143**: 143-150, 1999.

Romanowska, E., Wróblewska, B., Drożak, A., Zienkiewicz, M., Siedlecka, M.: Effect of Pb ions on superoxide dismutase and catalase activities in leaves of pea plants griown in high and low irradiance. - Biol. Plant. **52**: 80-86, 2008.

Saher, S., Fernández-García, N., Piqueras, A., Hellín, E., Olmos, E.: Reducing properties, energy efficiency and carbohydrate metabolism in hyperhydric and normal carnation shoots cultured *in vitro*: a hypoxia stress? - Plant Physiol. Biochem. **43**: 573-582, 2005a.

Saher, S., Piqueras, A., Hellin, E., Olmos, E.: Hyperhydricity in micropropagated carnation shoots: the role of oxidative stress. - Physiol. Plant. **120**: 1152-161, 2004.

Saher, S., Piqueras, A., Hellin, E., Olmos, E.: Prevention of hyperhydricity in micropropagated carnation shoots by bottom cooling: implications of oxidative stress. - Plant Cell Tissue Organ Cult. **81**: 149-158, 2005b.

Scebba, F., Arduini, I., Ercoli, L., Sebastiani, L.: Cadmium effects on growth and antioxidant enzymes activities in *Miscanthus sinensis*. - Biol. Plant. **50**: 688-692, 2006.

Semorádová, Š., Synková, H., Pospíšilová, J.: Responses of tobacco plantlets to changes of irradiance during transfer from *in vitro* to *ex vitro* conditions. - Photosynthetica **40**: 605-614, 2002.

Shamsi, I.H., Wei, K., Zhang, G.P., Jilani, G.H., Hassan, M.J.: Interactive effects of cadmium and aluminum on growth and antioxidative enzymes in soybean. - Biol. Plant. **52**: 165-169, 2008.

Shao, H.-B., Chu, L.-Y., Lu, Z.-H., Kang, C.-M.: Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. - Int. J. biol. Sci. **4**: 8-14, 2008.

Sharma, P., Dubey, R.S.: Ascorbate peroxidase from rice seedlings: properties of enzyme isoforms, effects of stresses and protective role of osmolytes. - Plant Sci. **167**: 541-550, 2004.

Sharma, P., Dubey, R.S.: Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. - Plant Growth Regul. **46**: 209-221, 2005.

Sharma, P., Dubey, R.S.: Involvement of oxidative stress and role of antioxidative defence system in growing rice seedlings exposed to toxic concentrations of aluminum. - Plant Cell Rep. **26**: 2027-2038, 2007.

Shohael, A.M., Ali, M.B., Yu, K.-W., Hahn, E.-J., Paek, K.-Y.: Effect of light on oxidative stress, secondary metabolites and induction of antioxidant enzymes in *Eleutherococcus senticosus* somatic embryos in bioreactor. - Process Biochem. 41: 1179-1185, 2006a

Shohael, A.M., Ali, M.B., Yu, K.-W., Hahn, E.-J., Paek, K.-Y.: Effect of temperature on secondary metabolites production and antioxidant enzyme activities in *Eleutherococcus senticosus* somatic embryos. - Plant cell Tissue Organ Cult. **85**: 219-228, 2006b.

Sivritepe, N., Erturk, U., Yerlikaya, C., Turkan, I., Bor, M., Ozdemir, F.: Response of the cherry rootstock to water stress induced *in vitro*. - Biol. Plant. **52**: 573-576, 2008.

Ślesak, I., Hałdaś, W., Ślesak, H.: Influence of exogenous carbohydrates on superoxide dismutase activity in *Trifolium repens* L. explants cultured *in vitro*. - Acta biol. cracov. **48**: 93-98, 2006.

Ślesak, I., Libik, M., Karpinska B., Karpinski, S., Miszalski, Z.: The role of hydrogen peroxide in regulation of plant metabolism and cellular signalling in response to environmental stresses. - Acta. biochim. polon. **54**: 39-50, 2007.

Ślesak, I., Libik, M., Miszalski, Z.: Superoxide dismutase activity in callus from $C_3$-CAM intermediate plant *Mesembryanthemum crystallinum*. - Plant Cell Tissue Organ Cult. **75**: 49-55, 2003.

Ślesak, I., Miszalski, Z.: Superoxide dismutase-like protein from roots of the intermediate $C_3$-CAM plant *Mesembryanthemum crystallinum* L. in *in vitro* culture. - Plant Sci. **164**: 497-505, 2003.

Sriskandarajah, S., Prinsen, E., Motyka, V., Dobrev, P.I., Serek, M.: Regenerative capacity of cacti *Schlumbergera* and *Rhipsalidopsis* in relation to endogenous phytohormones, cytokinin oxidase/dehydrogenase, and peroxidase activities. - J. Plant Growth Regul. **25**: 79-88, 2006.

Synková, H., Pospíšilová, J.: *In vitro* precultivation of tobacco affects the response of antioxidative enzymes to *ex vitro* acclimation. - J. Plant Physiol. **159**: 781-789, 2002.

Synková, H., Semorádová, Š., Schnáblová, R., Witters, E., Hušák, M., Valcky, R.: Cytokinin-induced activity of antioxidant enzymes in transgenic P*ssu-ipt* tobacco during plant ontogeny. - Biol. Plant. **50**: 31-41, 2006.

Syros, T., Yupsanis, T., Zafiriadis, H., Economou, A.: Activity and isoforms of peroxidases, lignin and anatomy, during adventitious rooting in cuttings of *Ebenus cretica* L. - J. Plant Physiol. **161**: 69-77, 2004.

Tang, W., Harris, L.C., Outhavong, V., Newton, R.J.: Antioxidants enhance *in vitro* plant regeneration by inhibiting the accumulation of peroxidase in Virginia pine (*Pinus virginiana* Mill.) - Plant Cell Rep. **22**: 871-877,

2004a.

Tang, W., Newton, R.J.: Peroxidase and catalase activities are involved in direct adventitious shoot formation induced by thidiazuron in eastern white pine (*Pinus strobus* L.) zygotic embryos. - Plant physiol. Biochem. **43**: 760-769, 2005.

Tang, W., Newton, R.J., Outhavong, V.: Exogenously added polyamines recover browning tissues into normal callus cultures and improve plant regeneration in pine. - Physiol. Plant. **122**: 386-395, 2004b.

Van Huylenbroeck, J.M., Piqueras, A., Debergh, P.C.: Effect of light intensity on photosynthesis and toxic 02 scavenging enzymes during acclimatization of micropropagated *Calathea*. - Phyton 37: 283-290, 1997.

Van Huylenbroeck, J.M., Piqueras, A., Debergh, P.C.: The evolution of photosynthetic capacity and the antioxidant enzymatic system during acclimatization of micropropagated *Calathea* plants. - Plant Sci. **155**: 59-66, 2000.

Van Huylenbroeck, J.M., Van Laare, I.M.B., Piqueras, A., Debergh, P.C., Bueno, P.: Time course of catalase and superoxide dismutase during acclimatization and growth of micropropagated *Calathea* and *Spathiphyllum* plants. - Plant Growth Regul. **26**: 7-14, 1998.

Vilela, B.J., Carvalho, L.C., Ferreira, J., Amâcio, S.: Gain of function of stomatal movements in rooting *Vitis vinifera* L. plants: regulation by $H_2O_2$ is independent of ABA before the protruding of roots. - Plant Cell Rep. **26**: 2149-2157, 2007

Vital, S.A., Fowler, R.W., Virgen, A., Gossett, D.R., Banks, S.W., Rodriguez, J.: Opposite roles for superoxide and nitric oxide in the NaCl stress-induced upregulation of antioxidant enzyme activity in cotton callus tissue. - Environ. exp. Bot. **62**: 60-68, 2008.

Wang, Y.-L., Wang, X.-D., Zhao, B., Wang, Y.-C.: Reduction of hyperhydricity in the culture of *Lepidium meyenii* shoots by the addition of rare earth elements. - Plant Growth Regul. **52**: 151-159, 2007.

Xiong, Y.-C., Xing, G.-M., Gong, C.-M., Li, F.-M., Wang, S.-M., Li, Z.-X., Wang, Y.-F.: Dual role of abscisic acid on antioxidative defense in grass pea seedlings (*Lathyrus sativus* L.). - Pakistan J. Bot. **38**: 999-1014, 2006.

Xu, C.-M., Zhao, B., Wang, X.-D., Wang, Y.-C.: Lanthanum relieves salinity-induced oxidative stress in *Saussurea involucrata*. - Biol. Plant. **51**: 567-570, 2007.

Zhang, A., Jiang, M., Zhang, J., Tan, M., Hu, X.: Mitogen-activated protein kinase is involved in abscisic acid-induced antioxidant defense and acts downstream of reactive oxygen species production in leaves of maize plants. - Plant Physiol. **141**: 475-487, 2006.

Zhang, F., Guo, J.-K., Yang, Y.-L., He, W.-L., Zhang, L.-X.: Changes in the pattern of antioxidant enzymes in wheat exposed to water deficit and rewatering. - Acta Physiol. Plant. **26**: 345-352, 2004.

Zhang, F., Wang, Y., Yang, Y., Wu, H., Wang, D., Liu, J.: Involvement of hydrogen peroxide and nitric oxide in salt resistance in the calluses from *Populus euphratica*. - Plant Cell Environ. **30**: 775-785, 2007.

Zlatev, Z.S., Lidon, F.C., Ramalho, J.C., Yordanov, I.T.: Comparison of resistance to drought of three bean cultivars. - Biol. Plant. **50**: 389-394, 2006.