www.czasopisma.pan.pl PAN POLSKA AKADEMIA NAUK www.journals.pan.pl

ACTA BIOLOGICA CRACOVIENSIA Series Botanica 53/1: 20–25, 2011
DOI: 10.2478/v10182-011-0003-6

INFLUENCE OF MANGANESE DEFICIENCY ON METAL ION UPTAKE, ANTIOXIDANT DEFENSE MECHANISM AND LIPID PEROXIDATION LEVELS IN *MENTHA PIPERITA* LEAVES

NILGÜN CANDAN AND LEMAN TARHAN*

Chemistry Department, Dokuz Eylul University, Buca, 35160 Izmir, Turkey

Received April 24, 2010; revision accepted April 14, 2011

We investigated the antioxidant defense mechanism, metal uptake and lipid peroxidation (LPO) levels at different leaf positions in *Mentha piperita* L. grown in Mn^{2+}-deficient and control conditions. Under manganese deficiency the activity of superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GuaPOX) and the content of ascorbate, chlorophyll, and carotenoid under Mn^{2+} deficiency were significantly lower than in the control for all leaf positions. SOD activity correlated positively with Mn^{2+} uptake. Fe^{2+} uptake was inhibited by Mn^{2+} deficiency. During early stages of Mn^{2+} deficiency, *M. piperita* leaves showed relatively more antioxidant activity and lower LPO. Towards the final stages of the treatment period, comparatively lower SOD, CAT and GuaPOX activity and higher LPO levels accelerated the senescence process.

Key words: Antioxidant defense system, lipid peroxidation, *Mentha piperita*, Mn^{2+} deficiency, metal uptake.

INTRODUCTION

Manganese (Mn^{2+}) is an essential plant micronutrient which is involved in the activation of many enzymes in plant systems, mostly in oxidation-reduction, decarboxylation and hydrolytic reactions (Marchner, 1995a). Incorporation of Mn^{2+} by cells is essential, particularly in photosynthesis, where Mn^{2+} plays a critical role as an accumulator of positive charge equivalents in a reaction catalyzed in photosystem II (Marschner, 1995b). Mn^{2+} is also important in the synthesis of riboflavin, ascorbic acid and carotene.

Plant species differ considerably in the levels of their normal or adequate Mn^{2+} leaf concentrations (30–500 mg $Mn^{2+}kg^{-1}$ dry mass, Clarkson, 1988). Absorption of Mn^{2+} by plant roots depends on the ability of the plants to transfer the metal across the soil-root interface and on the available amount of Mn^{2+} in nutrient (Farasova and Beinrohr, 1998). Regulation within the plant involves precisely targeted transport from the macro-level of the tissue to the micro-level of the cell and organelles. The cellular concentration of Mn^{2+} needs to be finely controlled. Any change away from the threshold level can lead to toxic or deficiency effects (Lidon and Teixeira, 2000, Henriques, 2003, Boojar and Goodarzi, 2008, Hauck et al., 2002, Hauck et al., 2003, Lei et al., 2007).

Mn^{2+} deficiency is a widespread plant nutritional disorder in agriculture. It is difficult to overcome because Mn^{2+} is rapidly oxidized when supplemented artificially in fertilizers. Selection and breeding of plant genotypes tolerant to micronutrient deficiency is a sustainable way to enhance productivity. The success of such an approach depends upon a better understanding of the mechanisms governing tolerance to nutrient deficiency. There is increasing evidence that much of the harm to plants that is due to various environmental stresses is associated with oxidative damage through direct or indirect formation of reactive oxygen species (ROS). The ROS, including superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\cdot}OH$) and singlet oxygen ($^1O_2$), are inevitable byproducts of cell metabolism. These ROS attack lipids, proteins and nucleic acids, causing lipid peroxidation, protein

*e-mail: leman.tarhan@deu.edu.tr

Abbreviations: APX – ascorbate peroxidase; CAT – catalase; GuaPOX – guaiacol peroxidase; MDA – malondialdehyde; LPO – lipid peroxidation; ROS – reactive oxygen species; 6-OHDA – 6-hydroxydopamine; SOD – superoxide dismutase; TBA – thiobarbituric acid.

PL ISSN 0001-5296

denaturation and DNA mutation (Bowler et al., 1992). Under physiological conditions the production and destruction of ROS is regulated well in the cell metabolism by the antioxidant defense system. Under stress conditions, however, the formation of these radicals can exceed the amount present under physiological conditions, thus creating oxidative stress.

Among the antioxidant enzymes, superoxide dismutase (SOD) catalyzes dismutation of the superoxide anion (O_2.-) into hydrogen peroxide, while catalase (CAT) and peroxidases (AsA-dep and GuaPOX) detoxify H_2O_2. Other antioxidants such as ascorbate, carotenoids, polyphenols and flavonoids scavenge ROSs.

Though much work has been done on production of oxidants and antioxidants during senescence, little information is available on the actual role of oxidative stress and antioxidants in relation to the progression of leaf senescence under Mn^{2+} deficiency over time (Prochazkova et al., 2001; Li et al., 2005; Lei et al., 2007; Lehner et al., 2008).

In this study we (1) examined the activity of antioxidant enzymes (SOD, CAT, AsA-dep, GuaPOX) and the levels of chlorophyll, carotenoid, ascorbate, and membrane LPO at different leaf positions of *Mentha piperita*, (2) measured Mn^{2+}, Cu^{2+}, Zn^{2+} and Fe^{2+} content at leaf 6 of *M. piperita*, and (3) determined antioxidant enzyme activity and LPO levels at leaf 6 under Mn^{2+}-deficient conditions over time.

MATERIALS AND METHODS

PLANT CULTURE CONDITION

Seeds of *Mentha piperita* L. were disinfected with 10% H_2O_2 for 20 min, washed thoroughly with distilled water and germinated between wet paper towels at 25°C in the dark for 3 days. Seedlings were grown in a growth chamber under white fluorescent light (Philips, irradiance 750 μmol m^{-2} s^{-1}) with a 16 h photoperiod at 25/20°C (day/night) and 65±5% relative humidity. The seedlings were grown in Hoagland and Arnon solutions (1.18 μM Mn^{2+}) until the first 8 leaves leafed out (1953). The nutrient solution contained per liter: KNO_3 (1.02 g); $Ca(NO_3)^2$ (0.492 g); $NH_4H_2PO_4$ (0.23 g); $MgSO_4.7H_2O$ (0.49 g); H_3BO_3 (2.66 mg); $MnCl_2.4H_2O$ (1.81 mg); $CuSO_4.5H_2O$ (0.08 mg); $ZnSO_4.7H_2O$ (0.22 mg); $H_2MoO_4.H_2O$ (0.09 mg) and 0.5% $FeSO_4.7H_2O$ (0.6 mL). These solutions were permanently aerated and renewed 3 to 4 times a week to minimize pH shift and nutrient depletion. The seedlings were then transferred to nutrient solution, which included 0 μM Mn^{2+} as deficiency, 1.18 μM Mn^{2+} as the control.

The direction of leaf positions was counted downward (from leaf position 2 to 8). At harvest, 14-day-old leaves were weighed and used for preparation of extracts for enzyme analysis. Extracts of *M. piperita* leaves were prepared for enzyme determinations.

ENZYME ACTIVITY ASSAYS

Leaf material (1 g, without main midribs) was homogenized in 4 ml 20 mM phosphate buffer (pH 7.4) containing 50 mM β-mercaptoethanol. The homogenate was filtered and then centrifuged at 15,000 × g for 15 min. The supernatant was used for enzyme analysis. All operations (before enzyme determination) were performed at 0 to 4°C. β-mercaptoethanol was not included in the homogenization buffer system for determination of GuaPOX activity and LPO levels. Superoxide dismutase (EC 1.15.1.1) activity was determined according to Crosti et al. (1987). One unit of enzyme activity is defined as 50% inhibition of 6-hydroxydopamine (6-OHDA) autoxidation under assay conditions. Different forms of SOD were identified using KCN and/or H_2O_2. KCN inhibits Cu/ZnSOD but does not affect MnSOD nor FeSOD, while H_2O_2 inactivates Cu/ZnSOD and FeSOD without affecting MnSOD (Yu and Rengel, 1999). Ascorbate-dependent peroxidase activity (EC 1.11.1.11) was measured according to Nakano and Asada (1981) by monitoring the rate of ascorbate oxidation at 290 nm (E = 2.8 $mM^{-1}cm^{-1}$). The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 1 mM H_2O_2, 0.25 mM AsA and the enzyme sample. There was no change in absorption in the absence of AsA in the test medium. For guaiacol-dependent peroxidase (EC 1.11.1.7) activity, the reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.05% guaiacol, 10 mM H_2O_2 and enzyme. Activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation (E = 26.6 $mM^{-1}cm^{-1}$) (Nakano and Asada, 1981). Catalase (EC 1.11.1.6) activity was assayed in a reaction mixture containing 25 mM phosphate buffer (pH 7.0) 10.5 mM H_2O_2 and enzyme in 25 mM phosphate buffer (pH 7.0). Decomposition of H_2O_2 was followed at 240 nm (E = 39.4 $mM^{-1}cm^{-1}$) (Aebi, 1983).

ANALYTICAL METHODS

The concentrations of the metals were measured by inductively coupled plasma spectroscopy (ICP Model-8410, Labtam, Australia). To determine metal content in *M. piperita*, 1 g wet weight of leaves was digested in 2 ml nitric acid followed by 2 ml perchloric acid. All glassware and apparatus were washed with ultrapure water and 0.1 M HNO_3 before use. Concentrations of chlorophyll (*a* + *b*) and carotenoids in leaves were measured as described by Lichtenthaler and Wellburn (1983) after extraction

TABLE 1. Activity of SOD, CAT and POD enzymes, levels of LPO, Chl and Car, and Chl/Car ratio, at different leaf positions of *M. piperita* on day 14.

		Leaf Position				
	Mn^{2+} (µM)	2	4	6	8	LSD (0.05)
SOD (IU/mg)	1.18*	38.1	41.5	48.0	41.1	1.8
	0	9.3	9.6	19.3	10.3	
CAT(IU/mg)	1.18	14.7	15.6	16.0	14.0	0.8
	0	4.8	5.2	5.6	2.7	
AsA (mM)	1.18	10.6	12.3	14.7	11.2	2.0
	0	9.1	10.4	12.5	10.7	
APX (IU/mg)	1.18	3.2	3.6	4.9	2.6	0.3
	0	4.5	4.6	6.5	4.5	
GuaPOX (IU/mg)	1.18	7.4	7.8	7.9	7.0	0.2
	0	1.0	1.3	1.5	0.9	
LPO (nmol MDA/g)	1.18	5.6	5.2	4.6	4.8	1.3
	0	8.0	8.0	7.5	9.0	
Chl (µg cm^{-2})	1.18	24.3	25.2	26.5	24.1	3.0
	0	18.3	19.0	22.0	17.9	
Car (µg cm^{-2})	1.18	3.0	3.1	3.3	3.0	0.3
	0	2.5	2.6	2.9	2.8	
Chl / Car	1.18	8.0	8.1	8.0	8.0	0.6
	0	7.3	7.3	7.7	6.4	

* Control

with 80% acetone. The absorbance of the pigment extract was measured at 470, 646 and 663 nm. The content of Chl *a*, Chl *b* and carotenoids were estimated with the experimental equations described by Lichtenthaler and Wellburn (1983). Ascorbate concentrations were immediately determined with 2,4-dinitrophenylhydrazine (Nino and Shaw, 1976). The sample absorbance was measured at 460 nm. Lipid peroxidation was estimated based on thiobarbituric acid (TBA) reactivity. Samples were evaluated for malondialdehyde (MDA) production using a spectrophotometric assay for TBA. The extinction coefficient of 153 $mM^{-1}cm^{-1}$ at 532 nm for the chromophore was used to calculate the MDA-like TBA produced (Buege and Aust, 1978). Protein content was determined according to Bradford (1976) using bovine serum albumin (BSA) as standard.

STATISTICAL ANALYSIS

The Tukey test for multiple comparisons was used to verify the significance of results. Values are means of three separate experiments. Pearson's correlations were used to compare substrates and enzymes.

RESULTS

As seen in Table 1, SOD, CAT and PODs activity and AsA levels reached maximum at leaf 6 and decreased beyond that (leaf position 8) ($p<0.05$). There was a very close positive relationship between Mn^{2+} concentration and the activity of SOD ($r = 0.805$, $p<0.05$). CAT and GuaPOX activity, AsA, chlorophyll, and carotenoid content in Mn^{2+}-deficient conditions were significantly lower, while APX activity was higher than in the control ($p<0.05$). Chlorophyll/carotenoid ratios in Mn^{2+}-deficient conditions were changed, between 7.3 ± 0.1 and 6.4 ± 0.1. Membrane LPO levels under Mn^{2+} deficiency increased along the stem versus the control. In contrast to the variation of antioxidant enzyme activity, LPO level reached minimum at leaf 6 and increased with age.

Since enzyme activity was highest and the LPO level was at minimum in leaf 6, this position was used in further stages of the experiment.

Cu^{2+}, Zn^{2+}, Fe^{2+} and Mn^{2+} levels were assessed at leaf 6 of *M. piperita* on the 14th day. As seen in Table 2, Mn^{2+} and Fe^{2+} uptake by *M. piperita* grown in Mn^{2+}-deficient conditions decreased by ~3.3- and ~2.8-fold, respectively. Cu^{2+} and Zn^{2+} levels did not change significantly with Mn^{2+} deficiency.

TABLE 2. Mn^{2+}, Fe^{2+}, Zn^{2+} and Cu^{2+} concentrations in leaf 6 of *M. piperita*. Each data point represents the mean of three replicates

Mn^{2+} (mM)	Mn^{2+}	Fe^{2+}	Zn^{2+}	Cu^{2+}
	(mg g^{-1} DW)			
0	26.6	32.4	42.0	14.0
0,12	35.4	42.8	43.6	14.5
1.18*	87.8	92.8	45.2	15.0
LSD (0.05)	1.9	1.6	2.7	2.3

* Control

Changes in SOD activity in leaf 6 of *M. piperita* plants grown with Mn^{2+} supply decreasing from 1.18 to 0 μM were assessed with respect to time. SOD activity decreased sharply with Mn^{2+} deficiency. SOD activity in control conditions (1.18 μM) did not change significantly over time ($p>0.05$) (Fig. 1). In the treatments with 0.118–0 μM Mn^{2+}, SOD activity in *M. piperita* leaves dropped continuously during days 1 to 17, and the reduction became significant between days 1 and 6. There was a positive correlation between SOD activity and Mn^{2+} concentration ($r = 0734$, $p<0.05$).

CAT activity showed a similar trend in leaf 6 of *M. piperita* in the 0.590 μM Mn^{2+} treatment ($p>0.05$) (Fig. 2). It progressively decreased under different Mn^{2+} concentrations up to day 12 and did not differ significantly between treatments thereafter. CAT activity and Mn^{2+} concentration correlated positively with time ($r = 0.545$, $p<0.05$).

As seen from Figure 3, APX activity under Mn^{2+} deficiency reached maximum, exceeding the level in the control, between days 10 and 12. APX activity was below control levels in the rest of the treatment period ($p>0.001$). GuaPOX activity in Mn^{2+}-deficient conditions was below that of the control throughout the treatment period (Fig. 4). It decreased up to day 12, and did not change significantly thereafter ($p>0.001$). GuaPOX activity correlated positively with Mn^{2+} concentration ($r = 0.844$, $p<0.05$).

Figure 5 shows that LPO activity, unlike SOD, CAT, GuaPOX activity, correlated negatively with Mn^{2+} concentration ($r = -0.797$, $p<0.05$). LPO levels in leaf 6 decreased up to day 6 and increased continuously thereafter in all the manganese-deficiency treatments ($p<0.001$).

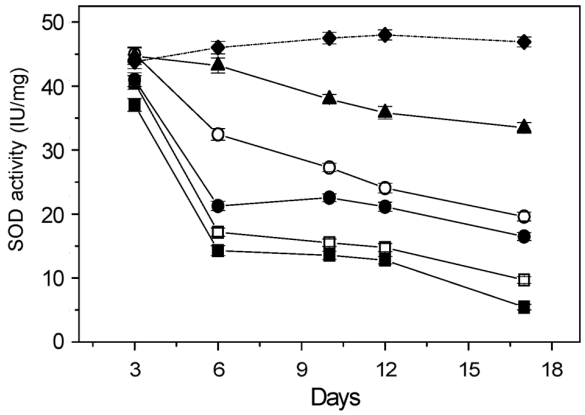

Fig. 1. SOD activity in leaf 6 of *M. piperita* in Mn^{2+} deficiency conditions over time: (-■-) 0 μM, (-□-) 1.18 10^{-2} μM, (-●-) 2.36 10^{-2} μM, (-O-) 1.18 10^{-1} μM, (-▲-) 5.9 10^{-1} μM, and (-♦-) 1.18 μM Mn^{2+}. Each data point represents the mean of three replicates.

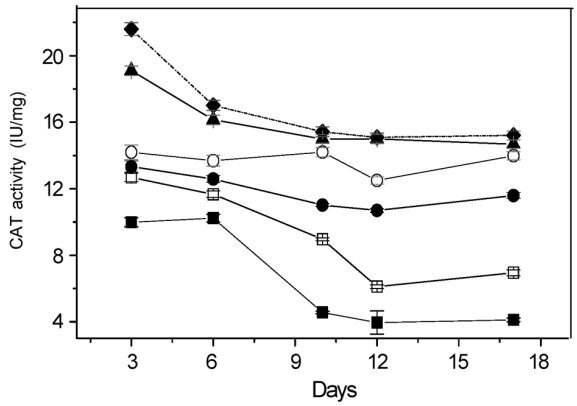

Fig. 2. CAT activity in leaf 6 of *M. piperita* in Mn^{2+} deficiency conditions over time: (-■-) 0 μM, (-□-) 1.18 10^{-2} μM, (-●-) 2.36 10^{-2} μM, (-O-) 1.18 10^{-1} μM, (-▲-) 5.9 10^{-1} μM, and (-♦-) 1.18 μM Mn^{2+}. Each data point represents the mean of three replicates.

DISCUSSION

Manganese is a transition metal, and Mn^{2+} stress is a major factor limiting plant growth (Hauck et al., 2003; Shi et al., 2005). The molecular, physiological and genetic mechanisms of Mn^{2+} tolerance in plants are insufficiently known (Santandrea et al., 2000).

In this study we found that SOD activity correlated positively with Mn^{2+} concentration. These results were coherent with the presence of MnSOD in *M. piperita* leaves because SOD activity was insensitive to both KCN and H_2O_2 in the crude extract. The MnSOD activity of *M. piperita* leaves was 50% of total SOD activity. MnSOD and FeSOD activity decreased, while Cu/Zn SOD activity remained unaffected by Mn^{2+} deficiency (data not shown). In higher plants, MnSOD is widely distributed in chloroplasts. This may imply that leaf chloroplasts/mitochondria are the major site of superoxide radical formation under stress condi-

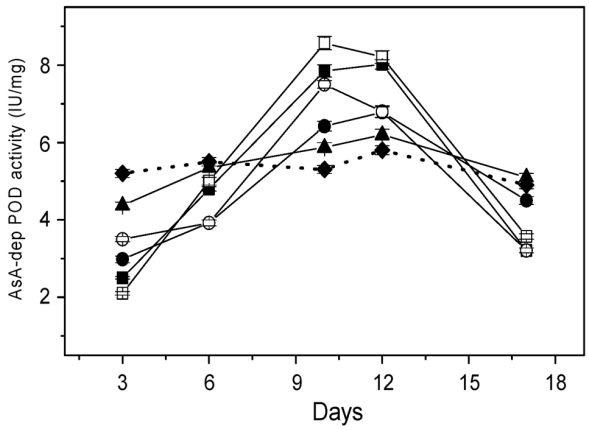

Fig. 3. APX activity in leaf 6 of *M. piperita* in Mn^{2+} deficiency conditions over time: (-■-) 0 μM, (-□-) 1.18 10^{-2} μM, (-●-) 2.36 10^{-2} μM, (-O-) 1.18 10^{-1} μM, (-▲-) 5.9 10^{-1} μM, and (-♦-) 1.18 μM Mn^{2+}. Each data point represents the mean of three replicates.

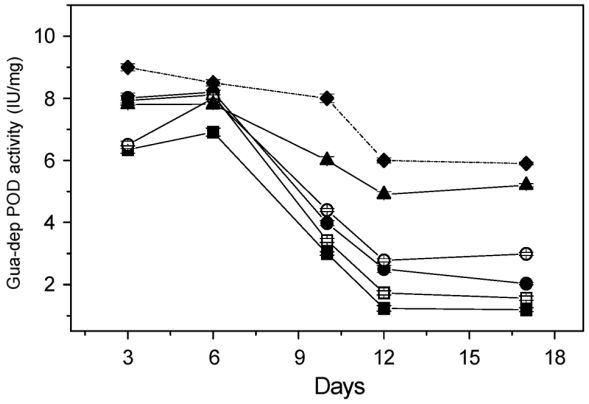

Fig. 4. GuaPOX activity in leaf 6 of *M. piperita* in Mn^{2+} deficiency conditions over time: (-■-) 0 μM, (-□-) 1.18 10^{-2} μM, (-●-) 2.36 10^{-2} μM, (-O-) 1.18 10^{-1} μM, (-▲-) 5.9 10^{-1} μM, and (-♦-) 1.18 μM Mn^{2+}. Each data point represents the mean of three replicates.

tions in *Mentha piperita*, and consequently that MnSOD is the major isoform responsible for superoxide radical scavenging during stress (Lidon and Teixeira, 2000).

We found that chlorophyll content was significantly reduced by Mn^{2+} deficiency, indicating the critical role of Mn^{2+} ions as a cofactor in photosynthetic light-dependent reactions. Lower chlorophyll/carotenoid ratios are an indicator of senescence, stress and damage to the plant and the photosynthetic apparatus, expressed by faster breakdown of chlorophylls than carotenoids (Lichtenthaler and Buschmann, 2001). Ascorbate has a central role in the detoxification of ROS in plant cells under various stress conditions. *M. piperita* leaves responded to Mn^{2+} deficiency with a remarkable decrease in ascorbate content. This

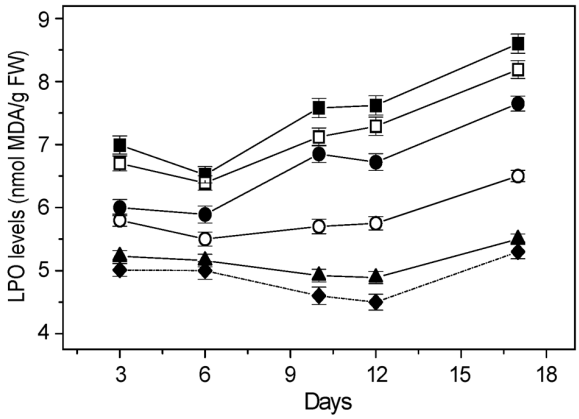

Fig. 5. LPO levels in leaf 6 of *M. piperita* in Mn^{2+} deficiency conditions over time: (-■-) 0 μM, (-□-) 1.18 10^{-2} μM, (-●-) 2.36 10^{-2} μM, (-O-) 1.18 10^{-1} μM, (-▲-) 5.9 10^{-1} μM, and (-♦-) 1.18 μM Mn^{2+}. Each data point represents the mean of three replicates.

decrease might be explained by increases in lipid peroxidation (Tab. 1).

The molecular details of Mn^{2+} metabolism and biochemical consequences of deficiency are still under investigation in relation to Fe metabolism. In our study, Fe^{2+} content in leaf 6 under Mn^{2+} deficiency decreased as much as 2.9-fold (Tab. 2). Allen et al. (2007) reported that Mn^{2+} deficiency induces secondary Fe deficiency, probably as a result of reduction of uptake of Fe (catalyzing the Haber Weiss reaction) in order to avoid more oxidative stress due to the reduced activity of MnSOD. The loss of MnSOD and FeSOD activity presumably is a consequence of the deficit of manganese (and secondary Fe deficiency), which is a necessary cofactor. Iron is a cofactor of important antioxidant enzymes such as CAT and PODs, which are involved in maintaining the balance of the redox state. Despite the significant decline in the activity of SOD, CAT and GuaPOX, *M. piperita* leaves showed irregular changes in APX activity in response to Mn^{2+} deficiency. The irregular changes in APX activity might indicate preferential synthesis/activation of this enzyme by *Mentha* plants to counteract oxidative stress. The decreases of MnSOD and FeSOD activity mentioned above were not compensated by increased CAT and GuaPOX activity, resulting in an increase of lipid peroxidation in Mn^{2+}-deficient conditions (Tab. 1). This situation might be attributed to a serious imbalance in ROS antioxidant defense systems.

During the treatment period, SOD, CAT and GuaPOX activity correlated positively with decreasing Mn^{2+} concentration ($r = 0.922$, $r = 0.575$ and $r = 0.630$) (Figs. 1–5). Only APX activity showed an increasing trend during the treatment period. The different affinities of APX (μM range) and CAT (mM range) for H_2O_2 suggest that they belong to two dif-

ferent classes of H_2O_2-scavenging enzymes (Mittlet, 2002). APX might be responsible for fine modulation of ROS, whereas CAT might be responsible for the removal of excess ROS during stress.

The balance between SOD and CAT or PODs activity in cells is crucial in setting the steady-state level of superoxide radical and hydrogen peroxide (Bowler, 1997). During early stages of Mn^{2+} deficiency, *Mentha piperita* leaves showed relatively more antioxidant activity and lower lipid peroxidation levels. Towards the final stages of the treatment period, comparatively lower SOD, CAT and GuaPOX activity and higher LPO levels accelerated the senescence process.

REFERENCES

Aebi HE. 1983. Catalase, In: Bergmeyer HU [eds.], *Methods of Enzymatic Analysis*, 273–286, vol. 3, Verlag Chemice, Deerfield Beach.

Allen MD, Kropat J, Tottey S, del Campo JA, and Merchant SS. 2007. Manganese deficiency in *Chlamydomonas* result in loss of photosystem II and MnSOD function, sensitivity to peroxides, secondary phosphorous and iron deficiency. *Plant Physiology* 14: 263–277.

Boojar MMA, and Goodarzi F. 2008. Comparative evaluation of oxidative stress status and manganese availability in plants growing on manganese mine. *Ecotoxicology Environmental Safety* 71: 692–699.

Bowler C. 1997. Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. *European Molecular Biology Organization Journal* 10: 1723–1737.

Bowler C, van Montagu M, Inze D. 1992. Superoxide dismutase and stress tolerance. *Annual Rewiew of Plant Physiology and Plant Molecular Biology* 43: 83–116.

Bradford MM. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248–254.

Buege JA, and Aust SD. 1978. Microsomal lipid peroxidation. *Methods in Enzymology* 52: 464–478.

Clarkson DT. 1988. The uptake and translocation of manganese by plant roots. In: Graham RD, Hannam RJ, Uren NC [eds.], *Manganese in Soil and Plants*, 101–111. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Crosti N, Serviden TJ, Bajer N, and Serra A. 1987. Modification of 6-hydroxydopamine technique for the correct determination of superoxide dismutase. *Journal of Clinical Chemistry and Clinical Biochemistry* 25: 265–272.

Farasova A, and Beinrohr E. 1998. Metal-metal interaction in accumulation of V, Ni, Mo, Mn, and Cu in under and above ground parts of *Sinapis alba*. *Chemosphere* 36: 1305–1317.

Hauck M, Paul A, Gross S, and Raubuch M. 2003. Manganese toxicity in epiphytic lichens: chlorophyll degradation and interaction with iron and phosphorus. *Environmental and Experimental Botany* 49:181–191.

Hauck M, Paul A, Mulack C, Fritz E, and Runge M. 2002. Effects of manganese on the viability of vegetative diaspore of the epiphytic lichen *Hypogymnia physodes*. *Environmental and Experimental Botany* 47: 127–142.

Henriques FS. 2003. Gas exchange, chlorophyll *a* fluorescence kinetics and lipid peroxidation of pecan leaves with varying manganese concentrations. *Plant Science* 165: 239–44.

Hoagland DR, and Arnon DI. 1953. The water culture method for growing plants without soil. California Agriculture Experimental State University of California, Berkeley Circle, 347, 12–19.

Lei Y, Korpelainen H, and Li C. 2007. Physiological responses-to high Mn concentrations in two contrasting *Populus cathayana* populations. *Chemosphere* 68: 686–694.

Lehner A, Mamadou N, Poels P, Côme D, Bailly C, and Corbineau F. 2008. Changes in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during ageing in wheat grains. *Journal of Cereal Science* 47: 555–565.

Li L, Ng TB, Gao W, Li W, Fu M, Niu S, Zhao M, Chen L, and Liu F. 2005. Antioxidant activity of gallic acid from rose flowers in senescence accelerated mice. *Life Science* 77: 230–240.

Lichtenthaler HK, and Buschmann C. 2001. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. *Current Protocols Food Analytical Chemistry* F4.3.1–F4.3.8.

Lichtenthaler HK, and Wellburn AR. 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochemical Society Transactions* 11: 591–592.

Lidon FC, and Teixeira MG. 2000. Oxygen radical production and control in the chloroplast of Mn-treated rice. *Plant Science* 152: 7–15.

Marschner H. 1995a. *Mineral Nutrition of Higher Plants*, 2nd ed. Academic Press, New York.

Marschner H. 1995b. *Functions of mineral nutrients: Micronutrients, Mineral Nutrition of Higher Plants*, 324–338. Academic Press, New York.

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* 7: 405–410.

Nakano Y, and Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiology* 22: 867–880.

Nino HV, and Shaw W. 1976. Determination of ascorbic acid. In: Tietz NW [ed.], *Vitamins Fundamental of Clinical Chemistry*, 542–550. WB Saunders Co., Philadelphia.

Prochazkova D, Sairam RK, Srivastava GC, and Singh DV. 2001. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. *Plant Science* 161: 765–771.

Santandrea G, Pandolfini T, and Bennici A. 2000. A physiological characterization of Mn-tolerant tobacco plants selected by in vitro culture. *Plant Science* 150: 163–170.

Shi Q, Bao Z, Zhu Z, He Y, Qian Q, and Yu J. 2005. Silicon-mediated alleviation of Mn toxicity in *C. sativus* in relation to activities of superoxide dismutase and ascorbate peroxidase. *Phytochemistry* 66: 1551–1559.

Yu Q, Rengel Z. 1999. Micronutrient deficiency influences plant growth and activities of superoxide dismutases in narrow-leafed lupins. *Annals of Botany* 183: 175–182.