

MAIN POINTS OF THE PHD THESIS ENTITLED

**PYSIOLOGICAL CHANGES INDUCED BY CADMIUM
IN MAIZE**

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Introduction

Heavy metals such as cadmium enter the environment mainly as the result of human activities. The heavy metal pollution of the soil is an increasingly serious problem nowadays and is aggravated by the fact that heavy metals accumulated in plants may directly or indirectly be consumed by humans. Cadmium causes numerous physiological changes in plants, such as growth inhibition, changes in the water and ion metabolism, photosynthesis inhibition, changes in enzyme activity, and the formation of free radicals.

In the course of cadmium stress, large quantities of phytochelatins appear in maize within a short time. These are thus useful, early warning signals of heavy metal stress. In general, cadmium is accumulated mainly in the roots of maize, so the binding and detoxification of cadmium is localised in the roots. It is possible that phytochelatins and their compartmentalisation are in the first line of defence. The second line of defence is provided by other systems, such as exclusion and immobilisation mechanisms in the roots, stress proteins, antioxidant enzymes and salicylic acid.

The examination of compounds capable of enhancing plant stress tolerance is of great importance from both the theoretical and practical point of view. The fact that salicylic acid is able to increase plant resistance was first demonstrated in the case of biotic stress effects. Studies on the action mechanism of salicylic acid revealed that it binds to catalase, thus inhibiting the activity of the enzyme. It is possible that the hydrogen peroxide accumulating due to catalase inhibition acts as a signal transducer mediating the effect of salicylic acid and initiating acclimatisation processes resulting in greater stress tolerance. However, salicylic acid probably influences the metabolism at numerous points rather than having one major site of action. Recently it has been found to play a role in an increasing number of abiotic stress responses.

Aims

The research aimed to reveal what defence mechanisms were induced in maize plants after cadmium treatment and whether there was any difference in the responses of leaves and roots to cadmium treatment. The work concentrated on the accumulation of the endogenous signal transduction molecule salicylic acid during the response of maize to cadmium stress. It was aimed to carry out the following investigations:

- In order to detect the oxidative stress induced by cadmium, changes in the values of three stress markers (chlorophyll content, lipid peroxidation and the chlorophyll-*a* fluorescence induction parameter) were monitored.
- In order to trace changes in membrane permeability during cadmium treatment, changes in the fatty acid composition, double bond index and saturation percentage of various membrane fractions were measured.
- Studies on the antioxidant defence system involved the determination of changes in the activity of the enzymes catalase, ascorbate peroxidase, glutathione-S-transferase, glutathione reductase and guaiacol peroxidase.
- Changes were recorded in the quantities of S-containing compounds. This included the induction of glutathione and its precursors (cysteine and γ -glutamyl cysteine), the induction of the synthesis of phytochelatins, which are useful as early warning signals of heavy metal stress, and the tissue-specificity of phytochelatin synthase. A correlation was sought between the *in vivo* phytochelatin level and changes in the *in vitro* activity of phytochelatin synthase.
- The accumulation of the free and conjugated forms of salicylic acid was monitored after cadmium treatment. The quantitative analysis of other possible precursors aimed to reveal the pathway of salicylic acid synthesis in maize during cadmium stress.

Material and methods

Plant material, plant growth, cadmium treatment parameters

The maize (*Zea mays* L., hybrid Norma) plants were grown in the phytotron of the Agricultural Research Institute in Martonvásár as described by Pál et al. (2005).

In the cadmium treatments the nutrient solution of 10-day-old seedlings was exchanged for solutions containing 1, 10, 25 and 50 µM concentrations of Cd(NO₃)₂. Cadmium treatment was continued for 7 days, after which analyses were carried out on the third fully developed leaf and on the roots.

Measurement of chlorophyll content

A SPAD-502 chlorophyll meter was used to record the total chlorophyll content. Measurements were made on the 1st, 4th and 7th days of cadmium treatment.

Determination of lipid peroxidation

The determination of lipid peroxidation is based on the spectrophotometric measurement of the malondialdehyde content, as described by Thomas et al. (2004). Samples were taken on the 1st, 4th and 7th days of cadmium treatment.

Measurement of chlorophyll fluorescence induction

The ΔF/Fm' chlorophyll fluorescence induction parameter was measured using a PAM-2000 fluorometer on the 7th day of cadmium treatment, as described by Janda et al. (1994).

Lipid extraction and fatty acid analysis

Lipid extraction, the separation of the lipid fractions, and the methyl esterisation and GC analysis of the fatty acids were carried out as described by Pál et al. (2007). Samples were taken on the 7th day of cadmium treatment.

Determination of cysteine, γ-glutamylcysteine and glutathione content

The thiol compounds were analysed by HPLC according to the method of Kocsy et al. (2004). Samples were taken on the 1st, 4th and 7th days of cadmium treatment.

Extraction of antioxidant enzymes and measurements of their activity

Enzyme extraction and the spectrophotometric determination of the activity of the various antioxidant enzymes took place as reported by Janda et al. (1999). Sampling was carried out on the 4th and 7th days of cadmium treatment.

Isolation of phytochelatin synthase and measurement of its activity

The HPLC analysis of the *in vivo* phytochelatin level and the *in vitro* determination of phytochelatin synthase activity were carried out according to Chen et al. (1997). Samples were taken on the 1st, 4th and 7th days of cadmium treatment.

Extraction and quantitative analysis of salicylic acid

The HPLC analysis of salicylic acid was carried out as described by Meuwly and Métraux (1993). Samples were taken on the 4th and 7th days of cadmium treatment.

Statistical analysis

The data are the means of ten replications in the case of chlorophyll-*a* fluorescence induction measurements and chlorophyll content and of five replications for the determination of malondialdehyde content. For the HPLC analyses, enzyme activity data are the means of 3–5 measurements, while 3 measurements were averaged for the GC analysis. The two-sample Student's t-test was applied to determine significance.

Results and Conclusions

- In the course of the work it was demonstrated that under the given experimental conditions the leaves and roots of maize plants reacted differently to cadmium treatment.
- In the leaves cadmium induced oxidative stress, accompanied by reductions in the chlorophyll content and in the chlorophyll-*a* induction parameter and by an increase in the malondialdehyde content, while no symptoms of oxidative stress could be detected in the roots.

- It was shown that in response to cadmium treatment there was a reduction in the quantity of short-chain saturated fatty acids in the phosphatidyl-glyceride and phosphatidyl-ethanolamine fractions in the leaves of maize, and in all the lipid fractions tested in the roots, accompanied by an increase in the ratio of unsaturated fatty acids with longer chains. Membrane fluidity rises proportionately with the quantity of polyunsaturated fatty acids, which could be responsible for the rapid uptake and translocation of cadmium in maize. The less intensive increase in the unsaturation of the lipid fractions at higher cadmium concentrations could be the result of cadmium inhibiting the increase in membrane fluidity.
- The results indicated that the defence mechanisms induced by cadmium treatment were also activated in different ways and to different extents in the leaves and roots of maize.
- In the leaves the oxidative stress induced by cadmium activated the glutathione reductase and guaiacol peroxidase enzymes, while no antioxidant enzyme induction was observed in the roots.
- Cadmium treatment had no pronounced effect on the glutathione content of the leaves, but there was a time- and concentration-dependent decline in the glutathione level in the roots during cadmium treatment.
- In response to cadmium treatment there was a rise in the *in vivo* phytochelatin (PC2) level in maize roots, which was correlated with a drastic reduction in the glutathione level in the roots. The higher phytochelatin level may be responsible for the efficient detoxification and vacuolar compartmentalisation of cadmium in the roots.
- Compared to the control, there was a reduction in the *in vitro* phytochelatin synthase activity in the roots, which could be attributed to the rapid accumulation of phytochelatins. The results confirmed the hypothesis that the phytochelatin synthase enzyme was constantly present in the roots, while in the leaves the presence of cadmium was required before synthesis was induced.

- It was demonstrated for the first time that cadmium induces the accumulation of both the free and bound forms of benzoic acid, salicylic acid and *o*-hydroxycinnamic acid in maize leaves, and that the enhanced synthesis of salicylic acid probably takes place via benzoic acid. In the roots the 50 µM treatment led to a drastic rise in the level of free *o*-hydroxycinnamic acid and to a slight reduction in the bound benzoic acid level. In the present case, the accumulation of salicylic acid recorded in the leaves can be regarded as part of the oxidative stress caused by cadmium rather than as a defence mechanism, as it played no role in the induction of the other defence mechanisms investigated.
- As the accumulation of *o*-hydroxycinnamic acid in both the leaves and roots was independent of salicylic acid synthesis, this could be part of the antioxidant defence mechanism.

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