Toxic Effect of Cadmium and Catalase Activity in the Corms of *Crocus sativus* L.

M. Hadizadeh
Institute of Biochemistry and Biophysics
University of Tehran
13145 Tehran
Iran

E. Keyhani
Laboratory for Life Sciences
19979 Tehran
Iran

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**Abstract**

Cadmium is a highly carcinogenic heavy metal and a known contaminant of today’s industrial world. In this research we report its effect on rooting and on the activity of catalase, a major antioxidant enzyme, in *Crocus sativus* L. corms cultivated for 3, 6 and 9 days in distilled water and in water supplemented with 0, 0.5, 2, 5, 20, 50 mg/l cadmium (Cd\(^{2+}\)). Over 9 days cultivation, root elongation was unaffected in 0.1 mg/l Cd\(^{2+}\), moderately inhibited in 0.5 and 2 mg/l Cd\(^{2+}\) and drastically inhibited in 5-20 mg/l Cd\(^{2+}\); no root elongation was observed after one day cultivation in 50 mg/l Cd\(^{2+}\). However, exposure to Cd\(^{2+}\) triggered an increase in catalase activity after 3 days cultivation. In corms grown for 3 days in distilled water a 60 % increase in catalase activity compared to dormant corms was observed. This increase was brought to 130 % in corms cultivated in 0.1 mg/l Cd\(^{2+}\) and to 110 %, 90 % and 65 % in corms cultivated in 0.5, 2-20 and 50 mg/l Cd\(^{2+}\), respectively. Thus, in up to 0.5 mg/l Cd\(^{2+}\), the increase in catalase activity doubled compared to the control in response to metal-induced oxidative stress; in higher Cd\(^{2+}\) concentrations, the catalase activity would itself be hampered by the metal, resulting in a more moderate increase. In contrast to corms grown for 3 days in the presence of increasing Cd\(^{2+}\) concentrations, in corms exposed for 6 days to Cd\(^{2+}\) catalase activity remained close to that found in corms grown in distilled water for 6 days. After 9 days cultivation, an increase in catalase activity compared to the control was again observed in the presence of Cd\(^{2+}\), but the activity remained lower than in dormant corms. Our investigation showed the importance of catalase in controlling Cd\(^{2+}\) toxicity in *Crocus sativus* L. corm.

**INTRODUCTION**

Cadmium is a widespread environmental pollutant with high toxicity to human, animals and plants (Sanita di Toppi and Gabbrielli, 1999). Cadmium is released into the environment by industrial processes and phosphate fertilizers (Wagner, 1993). In 1993, the International Agency for Research on Cancer classified cadmium among the known human carcinogens. Cadmium is absorbed by inhalation and ingestion and has a very long biological half-life (>25 years). It induces tumorigenesis in lung, prostate, kidney and stomach (Waalkes et al, 1992; Boffetta, 1993; Beyersmann and Hechtenberg, 1997).

Studies carried out in different plant species have revealed that cadmium affects various physiological and biochemical processes in plants. Cadmium accumulation causes reductions in photosynthesis (Somasekaraiah et al, 1992), growth inhibition, decreased uptake of nutrient elements (Gussarason et al, 1996; Sanita di Toppi and Gabbrielli, 1999) and inhibition of various enzyme activities such as the enzymes involved in CO\(_2\) fixation (Greger and Ögren, 1991; De Filippis and...
Ziegler, 1993). Among other effects, Cd$^{2+}$ produces oxidative stress possibly by generating free radicals and reactive oxygen species (ROS) in plant and animal cells (Hendry et al, 1992; Olmos et al, 2003). However, unlike other heavy metals such as copper and iron, Cd$^{2+}$ is, according to some authors, a non-redox metal unable to participate in Fenton-type reactions (Stochs and Bagchi, 1995; Sanita di Toppi and Gab brielli, 1999). On the other hand, Cd$^{2+}$ has been shown to produce free radicals in vitro in the presence of H$_2$O$_2$, although to a much lesser extent than Fe$^{2+}$ or Cu$^{2+}$ (Keyhani et al, 2006). Moreover, Cd$^{2+}$ in high concentrations, can also damage irreversibly proteins in vitro (Keyhani et al, 2003).

ROS are highly cytotoxic and react with lipids, proteins and nucleic acids and cause lipid peroxidation, membrane damage, enzymes inactivation. Thus their levels within plant cells must be controlled by enzymatic and non-enzymatic antioxidant defense systems.

Superoxide radicals generated in plant cells are converted to H$_2$O$_2$ by the action of superoxide dismutase. H$_2$O$_2$ is in turn reduced to H$_2$O either by catalase or by the ascorbate-glutathione cycle. Catalase (EC 1.11.1.6) has the advantage to catalyze the dismutation of H$_2$O$_2$ into H$_2$O and O$_2$ without requiring an additional source of reducing power as all peroxidases do.

The aim of this work was to evaluate the effect of cadmium on rooting and on the activity of catalase in *Crocus sativus* L. corm.

**MATERIALS AND METHODS**

**Corms and Cultivation Media**

Saffron plant corms were obtained from the University of Tehran farm located in Karaj, near Tehran. Corms were depleted from their sheathing leaves, cleaned from any dirt particle and cultivated in distilled water and in water supplemented with various concentrations of cadmium sulfate.

**Extract Preparation**

Extracts from saffron (*Crocus sativus* L) corms were prepared as previously described (Keyhani et al, 2000). Briefly, cleaned corms were homogenized in 0.1 M phosphate buffer, pH 7.0. After centrifugation at 3000 g for 10 min, a pellet was obtained which was discarded and the supernatant was recentrifuged at 35000 g for 30 min. A clear, transparent supernatant termed “crude extract” was obtained and used for our studies. Protein concentration was determined by the Lowry method.

**Enzymatic Activity Assay**

Catalase activity was measured as described in (Keyhani et al, 2002) by following the dismutation of H$_2$O$_2$ spectrophotometrically using an extinction coefficient for H$_2$O$_2$ at 240 nm of 27 M$^{-1}$cm$^{-1}$. One unit was defined as the amount of enzyme decomposing 1µmol H$_2$O$_2$ per minute. All assays were done at room temperature (~ 22-25° C) with an ultrospec 3000 UV/visible spectrophotometer from Pharmacia Biotech; the reaction mixture consisted of 1 ml 0.1 M citrate-phosphate-borate buffer, pH 7.00, containing H$_2$O$_2$ in final concentration of 15 to 75 mM, and 20 µl corm extract.

**RESULTS AND DISCUSSION**

**Root Elongation**

Figure 1 shows *Crocus sativus* L. corms and roots gross morphology after 8 days cultivation in selected cadmium concentrations. Compared to the control, root
growth was considerably slowed in the presence of 5 mg/l Cd\(^{2+}\) and further inhibited in higher Cd\(^{2+}\) concentrations namely 20 mg/l and 50 mg/l.

To monitor the effect of Cd\(^{2+}\) on root elongation, the length of individual roots was measured at days 1, 2, 6 and 9 of cultivation in the various Cd\(^{2+}\) concentrations and the average root length was calculated for each condition; the results are reported in Figure 2.

Root elongation was unaffected in corms exposed to 0.1 mg/l Cd\(^{2+}\) for up to 9 days. For corms exposed to 0.5-2 mg/l Cd\(^{2+}\), the average root length was unchanged compared to the control after 1 day cultivation but was diminished by 50 % compared to the control after 2 days cultivation, by 40 % compared to the control after 6 days cultivation and, respectively, by 10 % and 24 % compared to the control after 9 days cultivation in 0.5 mg/l Cd\(^{2+}\) and 2 mg/l Cd\(^{2+}\).

For corms exposed to 5 mg/l Cd\(^{2+}\), the average root length was still the same as that of the control after 1 day cultivation, but was decreased by 66 % compared to the control after 2 days cultivation, by 72 % compared to the control after 6 days cultivation and by 76 % compared to the control after 9 days cultivation.

For corms exposed to 20-50 mg/l Cd\(^{2+}\), root length was reduced by 50 % compared to the control after 1 day cultivation, by, respectively, 83 % and 85 % compared to the control after 2 days cultivation, 89 % and 93 % compared to the control after 6 days cultivation, and by 90 % and 95 % compared to the control after 9 days cultivation. Thus root elongation was severely inhibited in 20-30 mg/l Cd\(^{2+}\).

**Root Number**

Figure 3 shows the variation of the average root number per saffron corms cultivated for 1, 2, 6 and 9 days in the presence of increasing cadmium concentrations. As a whole, no significant changes with respect to corms cultivated in distilled water were observed in the average root number.

**Catalase Activity**

Catalase activity was measured in extracts from corms cultivated for 3, 6 and 9 days in distilled water and in water supplemented with 0.1, 0.5, 2, 5, 20, 50 mg/l Cd\(^{2+}\).

Figure 4A shows that corms that were cultivated for 3 days in distilled water displayed a 60 % increase in catalase activity compared to dormant corms. However, exposure to Cd\(^{2+}\) triggered a larger increase in catalase activity. This increase was brought to 130 % in corms cultivated in 0.1 mg/l Cd\(^{2+}\) and to 110 %, 90 % and 65 % in corms cultivated in 0.5, 2-20 and 50 mg/l Cd\(^{2+}\), respectively. Thus, in up to 0.5 mg/l Cd\(^{2+}\), the increase in catalase activity doubled compared to the control in response to metal-induced oxidative stress; in higher Cd\(^{2+}\) concentrations the catalase activity would itself be hampered by the metal, resulting in a more moderate increase.

After 6 days cultivation, the amount of catalase detectable in corms diminished under all conditions. For corms cultivated in distilled water, a 20 % decrease in the catalase activity compared with dormant corms was observed; this represented also a 50 % decrease compared to the enzymatic activity detectable after 3 days cultivation. Exposure to 0.1 to 50 mg/l Cd\(^{2+}\) had no significant effect on the catalase activity detectable in corms after 6 days cultivation. Some fluctuation was observed with varying Cd\(^{2+}\) concentrations, but the enzymatic activity detected remained lower than in dormant corms. (Fig.4B).

Corms grown for 9 days in distilled water displayed a 50 % decrease in catalase activity compared to dormant corms. Exposure to 0.1 mg/l Cd\(^{2+}\) resulted in a 30 % increase in catalase activity but this was still lower than the activity in dormant
corms. The amount of catalase detected in corms exposed to higher Cd\(^{2+}\) concentrations (0.1< [Cd\(^{2+}\)] <2 mg/l) decreased and finally, at 5-50 mg/l Cd\(^{2+}\) dropped back to activities similar to those found in the control corms cultivated in the absence of Cd\(^{2+}\) (Fig.4C).

Cd\(^{2+}\) has been shown to inhibit growth in various plants (Godbold and Hüttermann, 1985; Arisi et al, 2000). In the present study, we investigated the effect of cadmium on rooting and the activity of catalase in *Crocus sativus* L. corms. We showed that root length in corms grown for 2, 6 and 9 days was unaffected in 0.1 mg/l Cd\(^{2+}\) but that it would progressively slow down as Cd\(^{2+}\) concentration increased from 0.5 to 50 mg/l; up to 95% inhibition of root elongation occurred at 50 mg/l Cd\(^{2+}\). In contrast to root length, in corms exposed to increasing Cd\(^{2+}\) concentrations, the average root number was not significantly affected as compared with controls.

In this study, catalase activity was also determined in corms cultivated in the presence of 0.1 to 50 mg/l Cd\(^{2+}\) for 3, 6, and 9 days. Catalase is a key enzyme in protecting cells against oxidative stress, catalyzing the dismutation of H\(_2\)O\(_2\) into H\(_2\)O and O\(_2\). In corms grown for 3 days in the presence of 0.1 to 50 mg/l Cd\(^{2+}\), catalase activity exhibited a 50% increase compared to the control in lower Cd\(^{2+}\) concentrations (0.1-0.5 mg/l) but in higher concentrations (2-50 mg/l), the increase in catalase activity was more moderate: a 25% increase was observed in 2-20 mg/l Cd\(^{2+}\) and a 10% increase was observed in 50mg/l Cd\(^{2+}\).

In contrast to the results obtained after 3 days cultivation, the catalase activity detectable in corms grown for 6 days was comparable to the control in all Cd\(^{2+}\) concentrations. After 9 days cultivation up to 30% increase in catalase activity was observed in corms cultivated in the presence of Cd\(^{2+}\).

**CONCLUSION**

Root elongation was severely inhibited for *Crocus sativus* L. corms cultivated in 5-50 mg/l Cd\(^{2+}\) while root number remained unaffected.

The effect of cadmium on catalase activity in corms grown for 3 days was stimulatory while no effect was observed in corms grown for 6 days. In corms grown for 9 days, stimulation was observed at 0.1-2 mg/l Cd\(^{2+}\) and a gradual decrease in the catalase activity was observed of 5-50 mg/l Cd\(^{2+}\). Different cadmium effects on catalase activity may be due to the following reasons: 1) Exposure to cadmium for a long period (6 or 9 days) may inhibit the synthesis of catalase or inactive and/or down regulate the enzyme. 2) Isoenzyme pattern of catalase may change after a given period of exposure to cadmium.

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Fig. 1 Saffron corm cultivated for 8 days in double distilled water (Control), or in distilled water containing either 5 mg/l cadmium sulfate, 20 mg/l cadmium sulfate, or 50 mg/l cadmium sulfate. Note the drastic inhibition in root elongation and the appearance of brown discoloration on the roots and corms caused by exposure to Cd$^{2+}$ and indicative of stress.
Fig. 2. Average root length after 1, 2, 6, and 9 days cultivation in the presence of increasing cadmium concentrations.

Fig. 3. Average root number per corm after 1, 2, 6, and 9 days cultivation in the presence of increasing cadmium concentrations.
Relative Catalase Activity (% Activity in Dormant Corms)

A

B

C