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Cadmium Toxicity in Maize Seedlings: Changes in Antioxidant Enzyme Activities and Root Growth

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Abstract: In acid soils worldwide cadmium toxicity is a major factor limiting plant growth. The harmful effect of cadmium is initially expressed as a reduction in growth followed by several other secondary responses. In this study, some of the toxic effects of Cd^{+2} like induction of oxidative stress were investigated. The effect of metal ion on the root growth was considered in maize plants. Maize (*Zea mays* L.) seeds were sterilized with 2.5% sodium hypochlorite solution for 15 min and washed thoroughly with distilled water. These seeds then germinated in petri dish (20 cm) containing distilled water at 37°C in the dark. After a 1 day incubation, uniformly germinated seeds were selected and transferred to Petri dishes (9.0 cm) containing filter paper moistened with 10 mL of distilled water. Each Petri dish contained 12 germinated seeds. Each treatment was replicated 4 times. The germinated seeds were allowed to grow at 27°C in darkness and 5 mL of test solution was added to each Petri dish in the second day. The test solution contained 0, 0.25, 0.5, 0.75, 1, 3 and 5 mM $CdCl_2$. Cadmium treatments, increased GPX and APX activities in root in the presence of 0.25, 0.5, 0.75 mM concentrations, but their activities were constant in 1, 3 and 5 mM. Increased concentrations of $CdCl_2$ from 0.25 to 5 mM decreased root length progressively. However, no reduction of shoot length by $CdCl_2$ was observed.

Key words: Cadmium, *Zea mays*, Oxidative stress, Root growth

INTRODUCTION

Cd is a highly toxic and persistent environmental poison for plants and animals (Di Toppi and Gabbrielli, 1999). Cd interferes with many cellular functions mainly by complex formation with side groups of organic compounds such as proteins resulting in inhibition of essential activities. Although the mechanisms of cytoplasmic toxicity are identical in all organisms, different plant species and varieties show a wide range of plasticity in Cd tolerance, reaching from the high degree of sensitivity of most plants on the one hand to the hyperaccmulating phenotype of some tolerant higher plants on the other hand (McGrath *et al*., 2001). On an expanded concentration scale, even sensitive species vary considerably in their response to Cd. For example pea (*Pisum sativum*) is considerably more sensitive to Cd than barley (*Hordeum vulgare* cv Gerbel), which still grows well at concentrations above 10 mM under nutrient rich conditions. Cd induces genetic and biochemical changes in plant metabolisms that are related to general and Cd-specific stress responses (Blinda *et al*., 1997). Cd tolerance is correlated with intracellular compartmentalization and hence specific transport processes that allow the toxic effects of low Cd levels to decrease at least (Brune and Dietz, 1995; Gonzalez *et al*., 1999). The activation of the cellular antioxidant metabolism belongs to the general stress responses induced by heavy metals (Dietz *et al*., 1999). Although an active antioxidative metabolism does not represent a Cd tolerance mechanism in a strict sense, it is beneficial for plant performance under heavy metal stress. Inadequate activities of antioxidant defense systems cause oxidative damage, lipid peroxidationand membrane leakage in plants exposed to Cu, to Fe and also to Cd.

Cellular damage caused by free radicals might be reduced or prevented by a protective metabolism involving antioxidative enzymes such as SOD, APX, GR, CAT and GPX catalyzes the dismutation of two molecules of superoxide into oxygen and H_2O_2. APX reduces H_2O_2 to water, with ascorbate as electron donor (Asada, 1992). GR plays a part in the control of endogenous H_2O_2 through an oxido-reduction cycle involving glutathione and Ascorbate (Foyer and Halliwell, 1976; Smith *et al*., 1989). CAT and GPX are implicated in removal of H_2O_2. It has been reported that Cd increases the activities of an tioxidative enzymes such as SOD (Chongpraditrum *et al*., 1992; Rama Devi and Prasad, 1998), GPX (Karataglis *et al*., 1991), CAT (Rama Devi and Prasad, 1998) and APX (Rama Devi and Prasad, 1998). It

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is well known that CAT and APX play an important role in preventing oxidative stress by catalyzing the reduction of H_2O_2 (Weckx and Clijsters, 1996). Rama Devi and Prasad (1998) found that CAT and APX activities were increased by Cd, suggesting that excess Cd may increase the production of H_2O_2. Hydrogen peroxide is a necessary substrate for the cell wall stiffening process catalyzed by cell wall GPX (Elstner and Heupel, 1976; Hohl *et al*., 1995; Schopfer, 1996), which is considered to be one of the mechanisms resulting in growth inhibition (Fry, 1986).

Cd was found to produce oxidative stress (Hendry *et al*., 1992; Somashekaraiah *et al*., 1992), but in contrast to other heavy metals such as Cu, it does not seem to act directly on the production of oxygen reactive species (via Fenton and/or Haber-Weiss reactions) (Salin, 1988). As it was previously observed for other stresses, activation or inhibition of antioxidative enzymes depends not only on stress intensity and duration but also on the tissue type and the age of the plant (Sgherri *et al*., 2001).

The present investigation was designed to study the change in lipid peroxidation, antioxidative enzyme activities, H_2O_2 level and cell wall GPX activity in Cd-stressed roots of rice seedling and their relation with root growth inhibition.

MATERIALS AND METHODS

Maize (*Zea mays* L.) seeds were sterilized with 2.5% sodium hypochlorite solution for 15 min and washed thoroughly with distilled water. The seeds then germinated in Petri dish (20 cm) containing distilled water at 37°C in the dark. After a 1 day incubation, uniformly germinated seeds were selected and transferred to Petri dishes (9.0 cm) containing filter paper moistened with 10 mL of distilled water. Each Petri dish contained 12 germinated seeds. Each treatment was replicated 4 times. The germinated seeds were allowed to grow at 27°C in darkness and 5 ml of test solution was added to each Petri dish in the second day. The test solution contained 0, 0.25, 0.5, 0.75, 1, 3, 5 mM $CdCl_2$. All experiments described here, were performed three times.

When the maize seedlings were harvested (after 5 days), the root system of each seedling was separated from the shoot and the fresh weights were measured, then dry weights were determined after the preparations were dried for 48 h at 70°C. The length of roots and shoots were measured by a ruler.

For extraction of antioxidative enzymes, roots were homogenized with 0.1 M sodium phosphate buffer (pH 6.8) in a chilled mortar and pestle. The homogenate was centrifuged at 12,000 g for 20 min and the resulting supernatant was used for determination of the enzyme activity. The whole extraction procedure was carried out at 4°C. APX and GR were assayed as described previously (Chang and Kao, 1998). CAT activity was measured according to the method of Chen and Maehly (1959). GPX activity was determined according to the method of Upadhyaya *et al*. (1985) and also we measured APX activity according to the method of Azada and Chen (1989).

RESULTS

Figure 1 shows the effect of $CdCl_2$ on the root growth of maize seedlings. Increasing concentrations of $CdCl_2$ from 0 to 5 mM decreased root length, progressively. However, no reduction of shoot length by $CdCl_2$ was observed (Fig. 2). The differential effect of Cd on root and shoot growth could be accounted by the fact that Cd is accumulated mainly in roots and to a minor extent in shoots (Fernandes and Henriques, 1991).

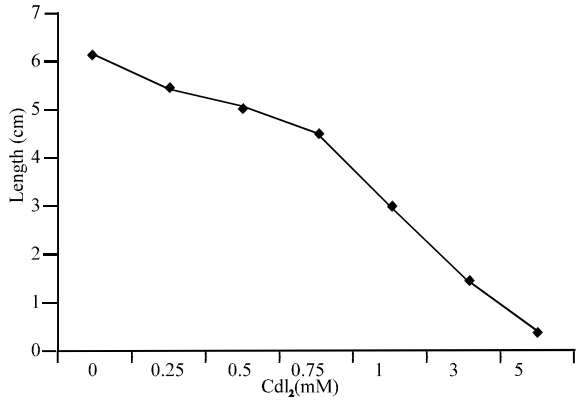

Fig. 1: Effect of $CdCl_2$ on the root growth of maize seedlings. Seedling growth was measured after 5 days of treatment

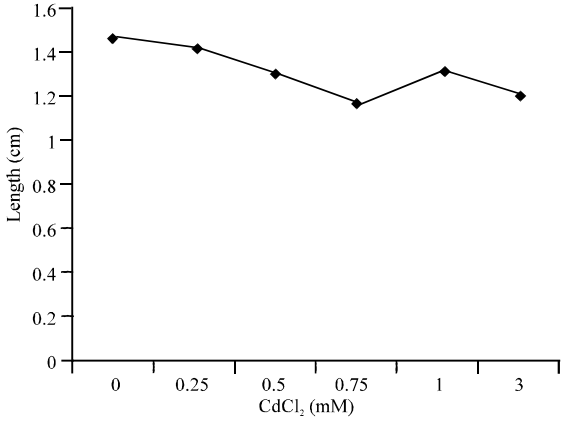

Fig. 2: Effect of $CdCl_2$ on the shoot growth of maize seedlings. Seedling growth was measured after 5 days of treatment

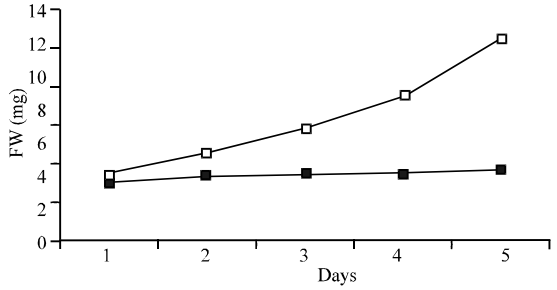

Fig. 3: Time course of $CdCl_2$ effect on root fresh weight of maize seedlings. Maize seedlings were treated with distilled water or 0.75 mM $CdCl_2$. (■ = H_2O, □ = 1 mM $CdCl_2$)

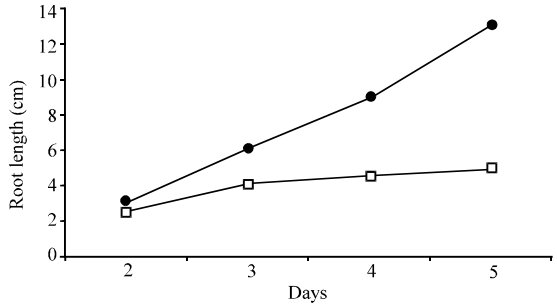

Fig. 4: Time course of $CdCl_2$ effect on root length of maize seedlings. Maize seedlings were treated with distilled water or 0.75 mM $CdCl_2$ (■ = H_2O, □ = 1 mM $CdCl_2$)

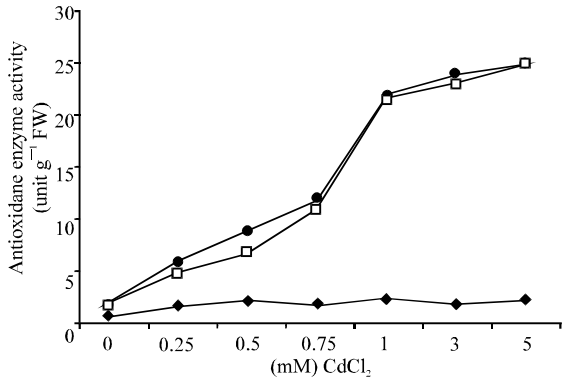

Fig. 5: The effects of Cd treatment on the activity of APX, GPX and CAT in the roots of maize seedlings treated with $CdCl_2$ (●: APX activity, ◆ GPX activity, □: CAT activity)

Figure 3 and 4 show the time courses of the effect of $XDXI_2$ (mM) on root length and root fresh weight. As judged by root length and root fresh weight, the reduction of root growth was evident 2 days after the treatment.

Figure 5 demonstrates that $CdCl_2$ treatment resulted in a significant increase in APX and GPX activities in roots maize seeding treated with $CdCl_2$ had no effect on the activity of CAT in roots of maize seeding. Similar results were obtained when enzyme activities were expressed on the basis of dry weight (data not shown).

DISCUSSION

Cadmium is a non-essential element that negatively affects plant growth and development. It is released into the environment by power stations, heating systems, metal-working industries or urban traffic. It is widely used in electroplating, pigments, plastic stabilizers and nickel-cadmium batteries (Di Toppi and Gabbrielli, 1999). It is recognized as an extremely significant pollutant due to its high toxicity and large solubility in water (Pinto *et al*., 2004).

Several studies have suggested that an oxidative stress could be involved in Cd toxicity, by either inducing oxygen free radical production, or by decreasing enzymatic and non-enzymatic antioxidants (Somashekaraiah *et al*., 1992; Shaw, 1995; Gallego *et al*., 1996; Sandalio *et al*., 2001; Balestrasse *et al*., 2001; Fornazier *et al*., 2002; Cho and Seo, 2005). The accelerated senescence observed in nodules of soybean plants treated with Cd has been attributed to the oxidative stress generated by the metal (Balestrasse *et al*., 2004).

In most environmental conditions, Cd enters first the roots and consequently they are likely to experience Cd damage first (Di Toppi and Gabbrielli, 1999).

The protective mechanisms adapted by plants to scavenge free radicals and peroxides include several antioxidative enzymes such as SOD, APX, GR, CAT and GPX. The antioxidative enzymes are important components in preventing the oxidative stress in plants as is based on the fact that the activity of one or more of these enzymes is generally increased in plants when exposed to stressful conditions (Allen, 1995). Overexpression of genes encoding these enzymes in several transgenic plant species conferring protection against free radicals has also been demonstrated (Allen, 1995). In the present study, Cd treatment resulted in an increase in the activities of APX and GPX (Fig. 5), which can be considered as an indirect evidence for enhanced production of free radicals under Cd stress. The increase of APX and GPX has been reported with Cd (Metwally *et al*., 2003; Karataglis *et al*., 1991; Rama Devi and Prasad, 1998). However, Mazhoudi *et al*. (1997) reported that CAT activities were not affected by Cd. We also found no change in CAT activity (Fig. 5). Such a variation in

response of these enzymes to Cd stress could be due to the variability of plant species in producing free radicals (Mazhoudi *et al*., 1997). Thus, the increase in the activities of APX and GPX by Cd (Fig. 5) suggests increased production of H_2O_2.

If H_2O_2 plays an important role in the cell wall stiffening process, it is expected that H_2O_2 would inhibit root growth. Thus, $CdCl_2$-induced inhibition in root growth of maize seedlings.

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