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Effect of calcium on uptake of cadmium in maize seedlings

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ABSTRACT

Cadmium is the highest heavy metal in terms of damage to plant growth. Its toxicity and alleviation by calcium in 1-week-old maize seedlings were studied. The seedlings were treated with cadmium at concentrations of 100 µM for six days. Application of 10 and 20 µM calcium was able to alleviate cadmium toxicity through some mechanisms. The alleviatation showed by calcium adding to cadmium treatment through declining in lipid peroxidation, anthocyanin concentration, proline content and cadmium uptake and rising in leaf chlorophyll and cartenoid compared to cadmium treatment, significantly.

Key words: Cadmium toxicity, Calcium alleviation, Cation uptake, Lipid peroxidation, Anthocyanin, chloroplast Pigments.

INTRODUCTION

Cadmium (Cd) is a non-essential element and the highest heavy metal in terms of damage to plant growth. Its uptake and accumulation in plants poses a serious health thread to humans and living cells via the food chain (Shah and Dubey, 1998; Stohs et al., 2000). Harmful effects of Cd^{2+} might be explained by its ability to inactivate enzymes possibly through reaction with the SH-groups of proteins (Gouia et al., 2000). This highly toxic heavy metal is an associated with industrial processes such as metal plating and the production of nickel-cadmium batteries, pigments, plastics, and other synthetics.

Cd toxicity in cells contains disruption of photosynthesis electron chain in PSII, decreasing enzymatic and non-enzymatic antioxidants, alter enzymes structure by interaction with sulphydryl groups or by replacement with metals in metaloproteins, lipid peroxidation and membranes damage, inhibition of ATPase activity, disruption of channels and transporters, induction of reactive oxygen species (ROS) (Asada and Takashi, 1987, Van Asche and clijesters, 1990). Plants exposed to Cd stress show significantly variation in electron transport in both chloroplasts and mitochondria (Prasad et al. 2001, Shah *et al.* 2001, Zhang et al. 2005). The uptake of Cd ions seems to be in competition for the same transmembrane carriers with nutrients such as K, Ca, Mg, Fe, Mn, Cu, Zn, Ni **(**Korshunova et al., 1999; Connolly et al., 2002; Bernard et al., 2004).

The rate of germination, fresh and dry mass of maize seedlings were increased as calcium (Ca) was added to the nutrient solution, which contained different levels of Cd^{2+}. This effect was alleviated by Ca^{2+} addition. Cd^{2+} content in seedlings was increased as the Cd^{2+} concentration in medium was increased and decreased sharply as Ca^{2+} was present in the culture medium (El-Enany, 1995). The addition of $CaCl_2$ to cdmium-stress common bean plants improved the stem fresh weight, root length, number of flowers and number of pods per plant (Ismail, 2008). In addition, Ca reduced the uptake of Cd and caused a modest reduction in Cd toxicity (Gipps and Coller, 1982).

The main objective of the present study was to investigate the effect of Cd in relation to the influence of Ca on some biochemical responses and uptake of maize seedlings.

MATERIAL AND METHODS

Plant materials and treatments

Seeds of *Zea mays* (var KSC.704) were surface sterilized by using 20-min incubation in 5% (w/v) sodium hypochlorite. After three washes with distilled water, seeds were germinated for 48 h at 24°C and then transferred to pots containing a mixture of sand and perlite (1/1, v/v) and irrigated with nutrient solution (as mgl^{-1}; KNO_3, 1000; $Ca(H_2PO_4)_2$, 250; $MgSO_4.7H_2O$, 250; H_3BO_3, 2.3; $MnCl_2.4H_2O$, 1.8; $ZnSO_4.7H_2O$, 0.22; $CuSO_4.5H_2O$, 0.08; H_2MoO_4, 0.02; FeEDTA, 6.92). The seedlings were grown for 7 days in a greenhouse in which growth conditions were 16 h light (maximum intensity of full sunlight was about 2000 µmol m^{-2} s^{-1}) and 8 h dark, an average minimum temperature of 18 °C and an average maximum temperature of 28°C, the mean humidity of 60%. Then heavy metal was carrying out during six days. Two concentrations of Cd (CdN_2O_6) were applied in solution (100µM without Ca and 50µM with Ca). In addition, Ca (CaN_2O_6) was used in 10 and 20 µM. The fully developed leaf was used as a source material for biochemical analysis and uptake measurement.

Lipid peroxydation measurement

Lipid peroxidation was assayed using 0.3 g of leaf tissue after homogenization in 10 ml of 0.1% (w/v) trichloroacetic acid (TCA). Then the mixture centrifuged at 10000 g for 15 minutes and vortexed 1 ml of the supernatant with 4 ml of 20% (w/v) TCA containing 0.5% (w/v) 2-thiobarbituric acid (TBA). The solution was heated for 30 minutes at 95°C. The samples were cooled on ice for 5 min and then recentrifuged for 10 minutes at 10000 g. The absorbance of the samples was measured at 535 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm (Heath and Packer, 1968). The level of lipid peroxidation products in roots and shoots was expressed as ìmol of malondialdehyde (MDA) per g^{-1} FW. The MDA concentration was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} (Lozano-Rodriguez, 1997).

Anthocyanin, Proline and pigments measurement

To determine the concentration of anthocyanin, 0.1 g fresh leaves were taken and extracted in 15 ml glass centrifuge tubes containing 10 ml of acidified methanol (methanol: HCl, 99: 1, v:v) and kept over night in the dark. The samples were brought up to volume, and the absorbance at 550 nm was determined. Anthocyanin concentration was calculated using an extinction coefficient of 33000 mol^{-1} cm^{-1} according to Wanger (1979). Free proline in 0.1 g of leaf samples were measured according to Bates (1973). Leaf chlorophyll and cartenoid were extracted by acetone and measured spectrophotometrically using Arnon's equation (Arnon, 1959).

Cadmium uptake

For determination of Cd the harvested plant samples were rinsed with deionized water, oven dried at 70°C for 48 h. The dried material ashed at 550 °C for 24 h. The ash residue was incubated with 65% HNO3 for 4 h. Then, HNO3 was added until a clear solution was obtained. Cd was quantified using an atomic absorption spectrophotometer (Varian, spectra AA-220 model) at 228.8 wavelenth according to Wickliff method (1980).

Statistical analysis and computations

All experiment set-ups were randomized complete block with three replicates each. Raw data were imported to Microsoft Excel program for calculations and graphical representation. SPSS version 17.0 program was used for analysis of variance and comparison of means by Duncan's method at $P < 0.05$.

RESULTS

Effect on Lipid peroxidation

Lipid peroxidation of maize seedling leaves increased under Cd treatment significantly (at $P < 0.05$). MDA enhanced 2.7 times compare to control but Ca alleviated the Cd effect. Leaves lipid peroxidation under Ca treatment without Cd were similar to control (Fig. 1).

Effect on anthocyanine, proline and pigments content

Cd increased anthocyanin content of leaves but Ca alleviated its effect significantly (at $P < 0.05$). This alleviatation enhanced when Ca concentration increase from 10 to 20 micro molar. Ca without Cd did not change the anthocyanin

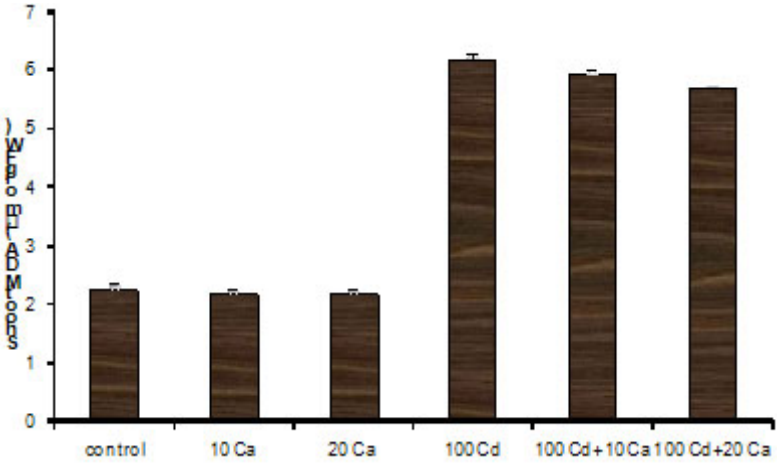
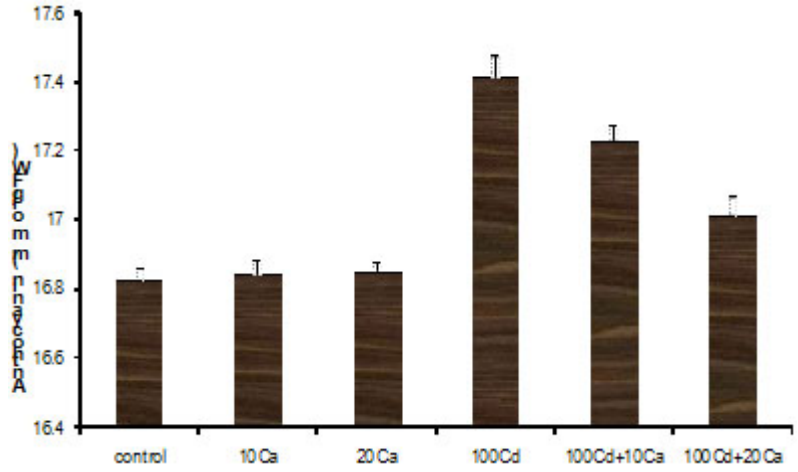


Fig. 1:



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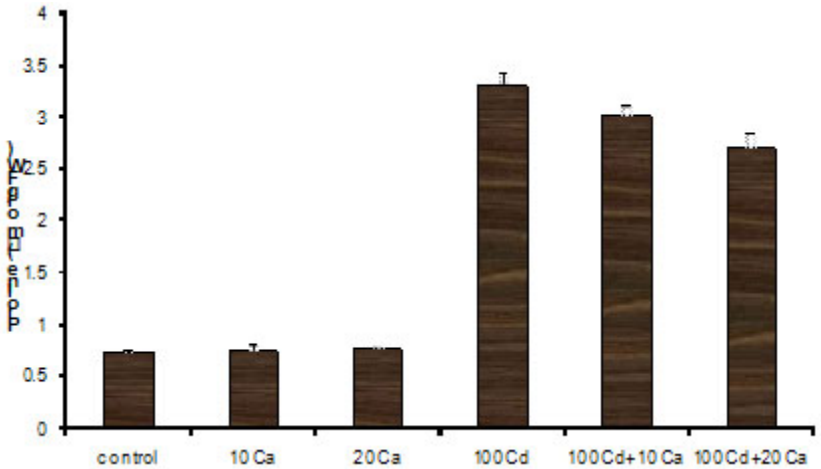

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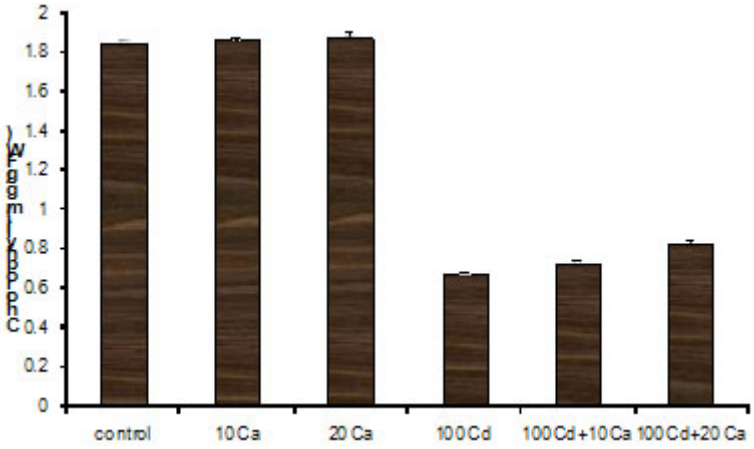

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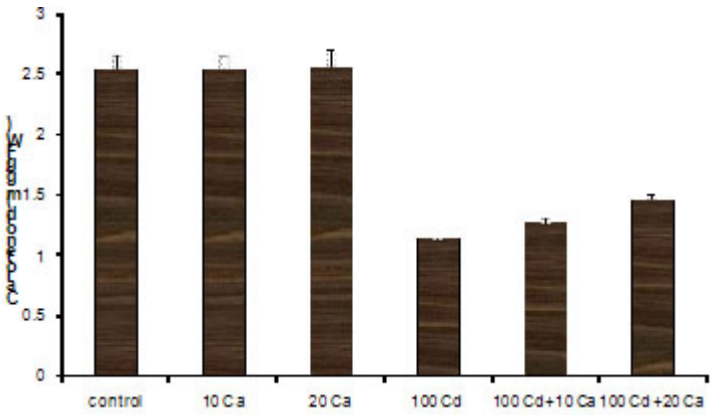

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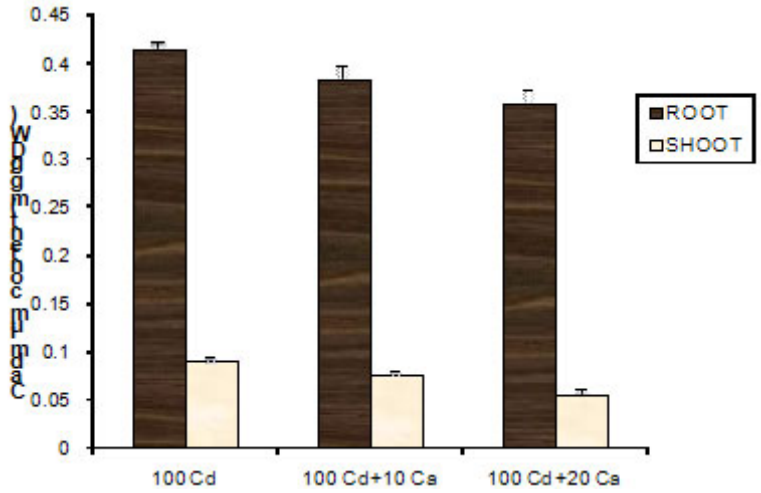

Fig. 6:

content (figure 2). Figure three shows that the amounts of leaf free proline in control and Ca treatments plants were about 0.77 µmol gFW^{-1}. Cd increased that by 4.2 times but alleviated significantly (at $P < 0.05$) after Ca treatments. As shown in figure four and five, leaf chlorophyll and cartenoid contents decreased with Cd treatment significantly (at $P < 0.05$) but Ca alleviated Cd effect slightly.

Effect on cadmium uptake

Cadmium contents of shoot and root decreased with calcium treatment significantly (at $P < 0.05$). The cadmium content of shoot under 100ìM of CdN_2O_6 treatment was 4.7 times regard to root sample (Fig. 6).

DISCUSSION

As the result shows, Cd influence some physiological parameters but Ca can alleviate the toxicity of Cd and reduced Cd uptake in maize. Based on the MDA levels in the present study, lipid peroxidation of leaves increased with the Cd addition and then decreased after intraction with Ca, and this is in accordance with earlier studies (Gallego et al., 1996; Balstrasse et al., 2001; Shah et al., 2001; Suzuki, 2005). An enhanced content of MDA in leaves of maize exposed to Cd indicates that the heavy metal may have caused oxidative stress and membrane damage (Lozano-Rodriguez et al., 1997; Cui1 and Wang, 2006). Leaf anthocyanin increased 3.38 % under Cd treatment compare to control. This may related to anthocyanine role as antioxidant and inhibitor of lipid peroxidation (Cho et al., 2003; Gabrielska and Oszmianski, 2005; Gulcin et al., 2005; Brown and Kelly, 2007). Anthocyanin is also a metal chelators..... Interestingly, the anthocyanin content de-creased with Ca application combined with heavy metal addition.

Increasing of free proline under heavy metal stress have been reported by others (Zengin and Munzuroglu, 2005; Choudhary *et al.*, 2007; Schat et al. 2008). Proline has been attributed to up-regulation of Ä^1-pyrroline-5-carboxylate synthetase encoding gene expression (Hong et al., 2000) and decrease in proline consumption (Raymond and Smirnoff, 2002). Proline accumulation may serve as a means of osmotic adjustment and storing carbon and nitrogen when stress leads to slower growth (Bohnert and Jensen, 1996). Increase in both proline and MDA content with increasing heavy metal concentration is indicative of a correlation between free radical generation and proline accumulation (Choudhary et al., 2007). Exposure to heavy metals, especially Cd (Barcelo et al., 1986), is known to disturb the plant water balance. Proline accumulation in plants under Cd stress is induced by a Cd-imposed decrease of the plant water potential, and the functional significance of this accumulation would lie in its contribution to water balance maintenance; proline-mediated alleviation of water deficit stress could substantially contribute to Cd tolerance (Costa and Morel, 1994; El-Enany and Issa**,** 2001). Proline increases the stress tolerance of plants through such mechanisms as osmoregulation, protection of enzymes against denaturation, and stabilization of protein synthesis (Kuznetsov and Shevyakova, 1997). Due to high concentrations of Ca around Ca channels, Cd uptake was possibly decreased by competition for metal ion influx (Suzuki, 2005) and therefore proline content will decrease. In addition, proline is a metal chelators.

Proline and Phh of cells...

The present results showed that Cd toxicity decreased the total chlorophyll and cartenoid contents of the leaves of maize seedlings as the other reports in *Brassica napus* (Larsson et al. 1998; Baryla et al. 2001) *Phaseolus vulgaris* (Zengin and Munzuroglu, 2005), *Azolla imbricate* (Dai et al., 2006), and *Bacopa monnieri* (Shukla et al., 2007). Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity (parekh, 1990). Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Cd was reported to affect chlorophyll biosynthesis and inhibit protochlorophyll reductase and aminolevulinic acid synthesis (Stobart et al., 1985). Cd induces oxidative stress and the first defensive mechanism is the scavenging of activated oxygen species at the sites where they are generated, especially chloroplasts where Cd accumulates. The chlorophyll and carotenoid decreases at relatively low Cd

concentration can be indicative of damage at the chloroplast level (Lagriffoul et al., 1998) for example, thylakoid membrane and photosynthesis electron transport chain (Clijsters and van Assche, 1985; Prasad and Zeeshan, 2005; Kalaji and Lobota, 2007). In this study, the damage of 100ìM Cd on chlorophyll and carotenoid contents are more than the Ca alleviation.

As figure 6 shows Cd content in root is higher than shoot, which is similar to previous researches (Blum, 1997; Liang et al., 2005). Cd is easily taken up by roots, accumulates in root, and shoots plants. Accumulation of Cd in plant tissues can be toxic at a cellular level limiting growth and development. Preventation of Cd uptake by plant roots is, therefore, an important strategy to minimize the adverse biological effects of Cd (McLauglin et al., 1999). Cation ion reduced Cd absorption significantly in this study and in other studies (El-Enani, 1997; Adiloglu et al., 2005). One reasons for Ca ion alleviatation of Cd toxicity is the displacement of cell-surface toxic cations by Ca. Since plasma membrane surface are usually negatively charged, high level Ca^{2+} would reduce cell-surface negativity and alleviate the harmfulness of cationic toxicants (Kinraide et al. 1998). The other proposed mechanism is the uptake of Cd through calcium channels to mimic Ca (Perfus-Barbeoch et al. 2002). There is a report that the Ca channel blockers, diltiazem, verapmil, nifedipine and nitrendipine (Blazka and Shaikh 1991) inhibit the uptake of Cd. In addition, the biological activity of heavy metals can be markedly affected by the presence of metal chelators, which may reverse their toxicity. Several studies indicated that Ca could reduce heavy metal toxicity by forming complex compounds with them, which are then, either eliminated or unable to cross biological membranes (Khalil, 1994 and 1996).

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