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ROLE OF CALCIUM IN ANTAGONIZING CADMIUM INDUCED HEAVY METAL TOXICITY IN MUNGBEAN SEEDLINGS

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ABSTRACT
Exogenous application of calcium plays an important role in antagonizing deleterious effects of cadmium induced toxicity in mungbean seedlings. An interaction of the two ions prevent losses in root and shoot growth and chlorophyll pigments, thus alleviating Cd induced heavy metal toxicity. Calcium plays a significant role in alleviating Cd induced toxicity probably restoring uptake of water through vascular tissues, chlorophyll biosynthesis and accumulation of biomass. The presence of Cd in the root tissue surroundings has its residual effects particularly when calcium is used in lower concentrations. The efficacy of Ca^{+2} is more with higher doses (80 µM) in an amalgam and plays a significant role in neutralizing Cd toxicity.

Keywords: *Ionic Interaction, Amalgum, Legumes, Phytotoxicity, Seed Germination*

INTRODUCTION
Heavy metals are amongst important environmental pollutants and their induced toxicity is a serious social concern for our environment (Tran and Popova, 2013). Uptake and subsequent accumulation of heavy metals such as cadmium is deleterious for both plants and animals. Compared with other metals, Cd has a higher tendency to accumulate in plant tissues (Kabata-Pendias and Pendias, 1992; Lux *et al*., 2010). Cadmium being a non-essential element negatively affects plant growth and development (OSHA, 2004). Due to its higher solubility in water and more toxicity the pollutant gains more significance (Pinto *et al*., 2004). This metal ion enters agricultural soils from pesticides, industrial effluents, phosphate fertilizers and atmospheric deposition which finally lead to transport of this heavy metal to the food chain (Jain *et al*., 2007). It affects many physiological processes, such as, membrane functions by changing the fatty acid composition of the lipids (Djebali *et al*., 2005), nitrogen metabolism (Chaffei *et al*., 2003), oxidative stress through increased proteolytic degradation (Romero-Puertas *et al*., 2002) and lipid peroxidation (Sandalio *et al*., 2001).

Plant alters various mechanisms in response to stimuli to adapt in the new environment (Reddy *et al*., 2011). Abiotic stresses like salt, metal, water and heat induce changes in calcium levels (Takano *et al*., 1997) which plays an important role in stress tolerance of plants (Tuteja and Sopory, 2008; Mansoor and Baig, 2014). Calcium is a ubiquitous signaling molecule and changes in cytosolic Ca^{+2} are involved in plant responses to various stimuli (Komatsu *et al*., 2007). Cd uptake was significantly reduced by calcium in Asiatic calms (Qiu *et al*., 2005). Ca^{+2} pre-exposure played a role in mitigating severity of Cd-induced toxicity in *Synechogobius hasta* (Song *et al*., 2013). Increased calcium levels in the soil limits absorption of heavy metals (like cadmium and lead) and stimulates biomass productivity in *Salix viminalis* (Mleczek *et al*., 2011).

The transport of Ca^{+2} can be competitively impeded or displaced by other elements, especially bivalent cations such as heavy metal Cd^{2+} ions which are toxic to plants. Uptake of Cd^{2+} ions competes with the active trans-membrane carriers such as Ca, Mg, Mn, Cu, and Zn (Korshunova *et al*., 1999; Connolly *et al*., 2002). The coexistence of essential and non-essential elements in the ecosystem leads to additive, antagonistic and/or synergistic interactions (Siedlecka, 1995).

Looking at the importance of antagonistic interaction of ions, it was thought worthwhile to study the role of Ca^{+2} in alleviating Cd^{2+} induced toxicity affecting leaf physiology and growth of mungbean seedlings.

MATERIALS AND METHODS

Seeds of munbean (*Vigna radiata* L. Wilczek *cv.* mung 666 were procured from Punjab Agriculture University, Ludhiana, Punjab and inoculated with standard rhizobial broth cultures. Seeds were surface sterilized with 0.1% $HgCl_2$ (Mercuric Chloride) for 2 minutes and washed thoroughly with distilled water before germination in petri-plates at 28±2°C in the presence of 60 µM cadmium ($CdSO_4$) alone and in presence of 20, 60 and 80µM calcium ($CaCl_2$) in laboratory under control conditions (13 h light and 11 h dark cycle). The seedlings irrigated with distilled water only served as control. The morphological and physiological data was noted for the growth of root and shoots, analysis of leaf chlorophyll parameters on 10^{th} day of seedling after sowing.

Chlorophyll parameters like Chl a, Chl b and Carotenoids were assayed by following method of Arnon (1949). The chlorophyll was extracted with 80% acetone repeatedly to ensure complete extraction and the absorbance was read at 645nm, 663nm and 665nm against 80% acetone. Phytotoxicity index was calculated according to Chou and Muller, 1972.

RESULTS AND DISCUSSION

Results and Observations

Leaf Pigments: Mungbean seeds were allowed to germinate in the presence of 60µM Cd alone and in combination with Ca 20, 60, 80µM. The morphological observations on the growth of seedlings revealed interaction of both the ions in an amalgam. Total chlorophyll content of leaves decreased significantly in the presence of heavy metal Cd (both Chl a & Chl b= -69%). The presence of Ca^{+2} in amalgam surprisingly prevented this loss of chlorophyll content. Such losses in the green pigment were least with higher doses of calcium. A linear and positive correlation was observed between chlorophyll content and calcium concentration. Chlorophyll a (-45.1, -5.6, -4.2%) and chlorophyll b (-43.5, -4.3, +8.7%) losses reduced by using Cd+Ca20, Cd+Ca60, Cd+Ca80, respectively and the pigments were almost near to unstressed seedlings particularly in case of Cd_{60}+ Ca_{80} treatment. The toxicity effect of Cd treatment almost got neutralized with $Cd_{60\ \mu M}$ and Ca_{80} amalgam (Figure 1).

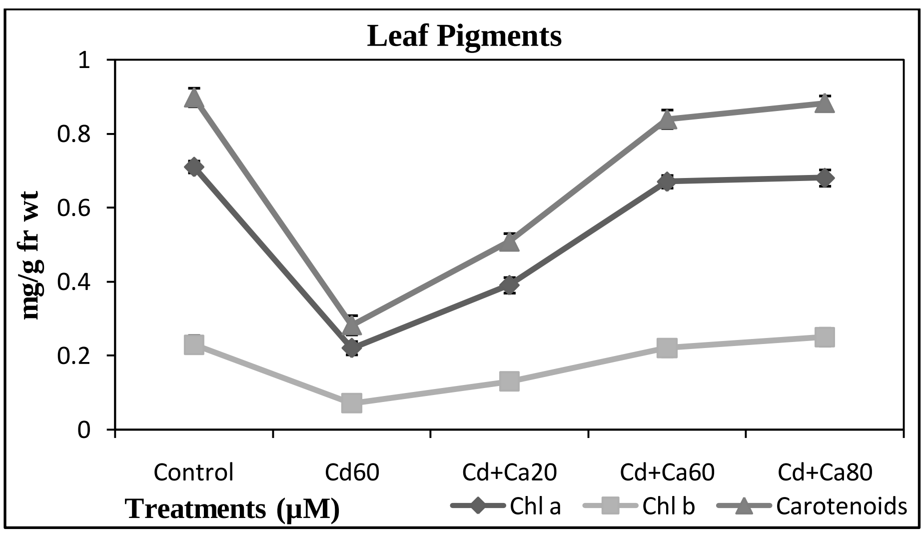

Figure 1: Effect of Cadmium alone and in combination with Calcium on different leaf pigments in mungbean seedlings

Another chlorophyll pigment molecule, carotenoid was to its maximum level in control seedlings followed by a sharp and drastic decline (-68%) in presence of Cd_{60} treatment. Similarly as in case of chlorophyll a and b, loss of carotenoids was checked with an amalgam of Cd and Ca ions together. The efficacy of the amalgam was more when ratio of Ca^{+2} was higher (losses in pigment lowered to -43.4, -6.6, -1.7% in comparison to control with Cd+Ca20, Cd+Ca60, Cd+Ca80, respectively). Loss of pigment was almost negligible by maintaining level as in unstressed seedlings with Cd_{60}+Ca_{80} treatment (Figure 1).

***Root and Shoot Length*:** The growth of mungbean seedlings reduced in comparison to control with heavy metal Cd60 application. Retardation in the length of roots and shoots was 29-30%. Calcium played a significant role in alleviating effect of cadmium by lowering retardation to -29.0, -20.3, -11.6% (roots) and -20.2, -14.2, -5.5% (shoots) using Cd+Ca (20, 60, 80uM) amalgum. The efficacy of Ca^{+2} was more when used in higher doses (80 µM). The reduction in fresh mass of roots was comparatively more than the above ground parts probably absorption of the heavy metal affecting uptake of water and other minerals (Figure 2).

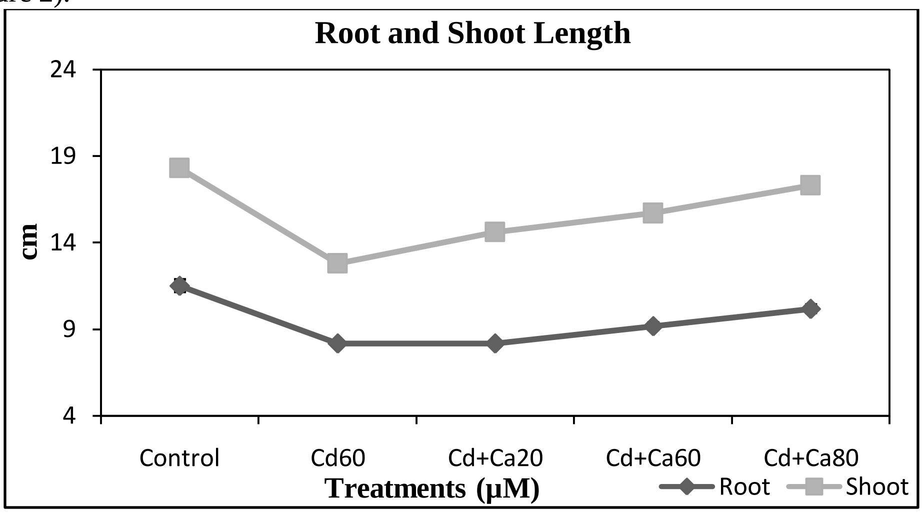

Figure 2: Effect of Cadmium alone and in combination with Calcium on root and shoot length of mungbean seedlings

Phytotoxicity Index: Induced toxicity of the heavy metal was measured for both above and underground parts. PI was calculated as 29-30% with Cd exposure of the seedlings. Interaction of two ions Cd+Ca (Cd+Ca 20, 60, 80uM) reduced toxicity index to 28.9, 20.3, 60.0% (roots) and 20.0, 14.5, 5.44% (shoots), respectively. Calcium, thus, played a significant role in mitigating heavy metal Cd induced stress. The lowering of phytoxicity index was more rapid and effective in case of above ground parts (shoots) indicating that the presence of heavy metal Cd in near vicinity of root tissues did not allow early recovery specifically when Ca is used in lower concentrations in the amalgam (Figure 3).

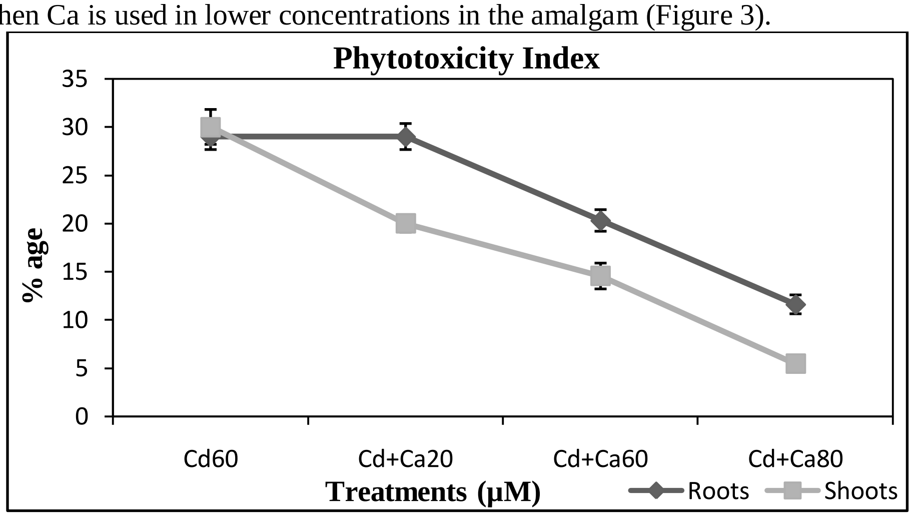

Figure 3: Effect of Cadmium alone and in combination with Calcium on phytotoxicity index (PI) in root and shoots of mungbean seedlings

Discussion

Growth parameters are sensitive to the presence of heavy metals in higher plants (Arun *et al.,* 2005). Our studies have clearly illustrated that cadmium retard growth of the plants. Calcium played a significant role in alleviating heavy metal Cd induced toxicity. Roots being the first organs receiving the Cd^{+2} are affected more subsequently leading to its accumulation (Drazkiewicz *et al*., 2003). Cd toxicity in plants disturbs mineral-nutrient homeostasis (Kinraide, 1998). Cadmium uptake and accumulation in oilseed rape was dependent on calcium nutrition ending up with more uptakes when calcium not present in the environment (Wan *et al.,* 2011). Cadmium and calcium ions interact influencing each other's accumulation in roots and stem of Norway spruce (Osteras and Gregar, 2006).

General symptoms like chlorosis of the foliage were reported with cadmium. The determination of total chlorophyll is a reliable marker of cadmium toxicity in higher plants (Krupa *et al*., 1996). Cadmium inhibits chlorophyll content in several plant systems (Singh *et al.,* 1988; Parekh *et al.,* 1990; Vassilev *et al.,* 1998). In addition to inhibition of chlorophyll biosynthesis, chlorophyll degradation, disorganization of chloroplasts, a decreased number of photosynthetic membranes and oxidative stress can also be the reasons for reduced chlorophyll content (Rascio *et al*., 2008; Gonclaves *et al.*, 2009). Cadmium treated soybean seedlings showed reduced net photosynthetic rate, chlorophyll content and stomatal conductance (Xue *et al.,* 2013). Calcium plays a significant role in alleviating heavy metal Cd induced toxicity probably restoring uptake of water through vascular tissues, chlorophyll biosynthesis and accumulation of biomass. The efficacy of Ca^{+2} was more when used in higher doses (80 µM) in an amalgam. Lowering of Phytoxicity index indicates that the presence of Cd in the surroundings of root tissues did not allow early recovery when Ca is used in lower concentrations in an amalgam.

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