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The Effect of Elevated Ca^{2+} Concentrations on Cadmium-Induced Acceleration of Leaf Senescence

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ABSTRACT: The present study is aiming at assessing the influence of Cd^{2+} on dark-induced senescence of excised *Tropaeolum majus* leaves. Besides determining the effects of Cd^{2+} applied individually on leaf senescence, its interactive effects with elevated Ca^{2+} is also determined. The experimental design involved incubation of excised *T. majus* leaves in dark in the absence (control) and presence of Cadmium, Ca^{+} either individually or in combination for 7 days. At the end of treatment chlorophyll levels, lipid peroxidation and K^{+} leakage from leaves were measured. The findings from present study reveal that the leaf senescence acceleration by the influence of Cadmium and Ca^{2+}.

Keywords: Senescence; malondialdehyde; chlorophyll and environmental stresses.

INTRODUCTION: Leaf senescence is accelerated due to various environmental stresses such as extreme temperature, drought, nutrient deficiency, ozone, insufficient light, darkness and pathogen attack (Buchanan-Wollaston, 1997). It proceeds in an age-dependent manner and also occurs because of the accumulation of heavy metals. Prominent symptoms of leaf senescence include yellowing which is a reflection of physiological and biochemical changes such as drop in chlorophyll content decrease in photosynthetic activity, degradation of RNA and proteins (Barth *et al.*, 2006). Accompanying the senescence, anthocyanins are formed in vacuoles of some leaves imparting them red and purple shades.

Programmed cell death (PCADMIUM) in plants is described by two ways namely, senescence and cell death associated with hypersensitive response (HR). There are large differences in the rate of senescence in plant systems. Senescence is particularly very rapid in some cases e.g. flower petals of *Ipomoea tricolor.* In this case, the petals beginsenescing just after the flower has been open. In other cases, the senescence takes longer time period for completion. For example, *Pinus longaeva* leaves have a life span of 45years.

The genes which were involved in gluconeogenesis and chlorophyll breakdown were related to senescence enhanced cDNAs (Smart *et al.*, 2006). In artificially induced senescence of barley, the gene expression was studied by invitro translation and mRNA hybridization with cDNA clones. There was a rapid chlorophyll loss when detached barley leaves were incubated in dark. Six cDNA clones were derived from three different transcripts which were classified according to the expression of mRNAs. In detached leaves, the transcripts were induced by abscisic acid and inhibited by kinetin. They were also introduced by osmotic stress, injuries but not detected in naturally senesced leaves (Becker and Apel, 2004).

MATERIAL AND METHODS: Seeds of garden nasturtium (*Tropaeolum majus* L.) were taken from the botanical garden, Indian Institute of Advanced Studies, Shimla (H.P). Seeds were sown in pots filled with garden soil. The pots were kept in a naturally lit glass house where they were watered at regular intervals. The mature and fully expanded uniform leaves were chosen for experiments described in this study. The leaves were excised by severing the petiole and immediately floated on distilled water and taken in glass tray to avoid the entry of air in the transpiration stream.

The excised leaves as described above were floated on the solution (25 ml) of desired concentrations of effector substances in glass petridishes. The effectors included Cadmium sulphate, $CdSO_4$ (The Central Drug

House (P), Bombay-Delhi): Dissolved in distilled water and Calcium chloride and $CaCl_2$ (Ranbaxy Lab. Ltd. S.A.S. Nagar, Punjab): Dissolved in distilled water.

Desired concentrations were achieved in the bathing solution through appropriate dilutions of the stock solutions of effector substances. Leaves simultaneously floated on distilled H_2O constituted the control. The length of petiole of excised leaves was adjusted to ensure that leaves floated freely on the solution. The petridishes were wrapped with carbon papers and kept in incubator (25±1°C) for 7 days. After 7 days incubation in dark, the leaves were subjected to the measurement of various biochemical parameters.

Biochemical parameters:

Estimation of chlorophyll: The leaf tissue was homogenized with 80% acetone, centrifuged at 5,000 rpm for 5 min and the final volume of supernatant made 5 ml with 80% acetone. The absorbance of extract was read at 663 and 645 nm. The amount of total chlorophyll, Chl. a and Chl. b was estimated by using equations (Harborne, 1973).

$$\textbf{Chl a (mg/g)} = \frac{12.3\,A_{663} - 0.86\,A_{645}}{a \times 1000 \times W} \times V$$

$$\textbf{Chl b (mg/g)} = \frac{19.3\,A_{645} - 3.6\,A_{663}}{a \times 1000 \times W} \times V$$

Where A_{645} and A_{663} are the absorbance at 645 and 663 nm, V is the volume in ml, a is the length of path of light (1cm) and W is the fresh weight of tissue in gram.

Determination of lipid peroxidation (MDA contents): Lipid peroxidation was estimated from accumulated malondialdehyde (MDA) contents following the method described by Dhindsa *et al*. (1981). In brief, the plant tissue was homogenized with 2 ml of 0.1% TCA and homogenate centrifuged at 10,000 rpm for 10 min. Two ml of supernatant was reacted with 4 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA and kept at 95ºC in a water bath for 1 hour. After this, the reaction mixture was cooled in ice for 2 min to terminate the reaction. Absorbance was read at 532 nm. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. MDA contents were calculated using extinction coefficient value of 155 $mM^{-1}cm^{-1}$ (Heath and Packer, 1968).

Determination of K^+ leakage: Potassium (K^+) released from the senescing leaves in the bathing medium was estimated at the end of experiment with a flame photometer (Corning 400, U.K.). The instrument was appropriately calibrated with a concentration series of K^+ (as KCl) prior to measuring the K^+ contents.

RESULTS AND DISCUSSION:

Effect of Cd^{2+} on senescence of excised *Tropaeolummajus* leaves: Effect of Cd^{2+} on senescence of excised leaves of *Tropaeolummajus* was assessed by incubating the leaves in dark for 7 days on solutions containing $CdSO_4$ in a range of 0-10 µM and measuring the chlorophyll content, lipid peroxidation and K^+ leakage. Due to 7 days dark incubation, 20% loss of chlorophyll was observed as compared to the initial (0 day) level (1.028 mg Chl. g^{-1} leaf fresh weight). Treatment of leaves with $CdSO_4$ solution led to a concentration – dependent loss of chlorophyll contents (enhancement of senescence). After 7 days dark incubation 45, 67 and 80% loss of chlorophyll was evident as compared to control (Fig.1 and 2).

The loss of chlorophyll due to $CdSO_4$ solution treatment was accompanied by elevated lipid peroxidation, measured as MDA contents. As the $CdSO_4$ solution concentration is increased leaf MDA contents increased progressively. A 1.8-, 2.4-and 3.0-fold increase in MDA content was observed at 1, 5 and 10 µM Cd; respectively. Cd induced acceleration of leaf senescence was reflected in enhanced K^+ leakage in the incubated medium. At 1 µM Cd, 30% increase in K^+ leakage was observed which increased to 2.7-and 3.5-fold over control value at 5 and 10 µM Cd, respectively.

The Cd-induced acceleration of chlorophyll loss was accompanied by correspondingly increased lipid peroxidation (MDA contents) and K^+ leakage from the leaves. These two parameters constitute the reliable indices of membrane integrity and functions. The fact that Cd-induced an increase in both suggests that Cd-dependent membrane damage in excised leaves might account for accelerated leaf senescence to a large extent. The Cd-induced reactive oxygen species (ROS) generation might have triggered the observed enhancement of lipid peroxidation. Cd is a non-redox element and therefore unable to initiate lipid peroxidation on its own like redox elements e.g., Cu. Instead, Cd relies upon activation of enzymes such as lipoxygenase. The lipoxygenase enzyme activity was increased due to cadmium toxicity (Sanita di Toppi and Gabbrielli, 1999). MDA is a product of lipid peroxidation and an indicator of free radical production

and tissue damage. In addition to stimulating ROS generation, Cd also modifies the activities of antioxidative enzymes and levels of antioxidant metabolites (Schutzendubel and Polle, 2002; Sharma and Dietz, 2009). The cadmium toxicity induced a decline in GSH content of leaf discs that was associated with decreased GR (glutathione reductase) activity (Rauser *et al.*, 1995; and Dixit *et al.*, 2001).

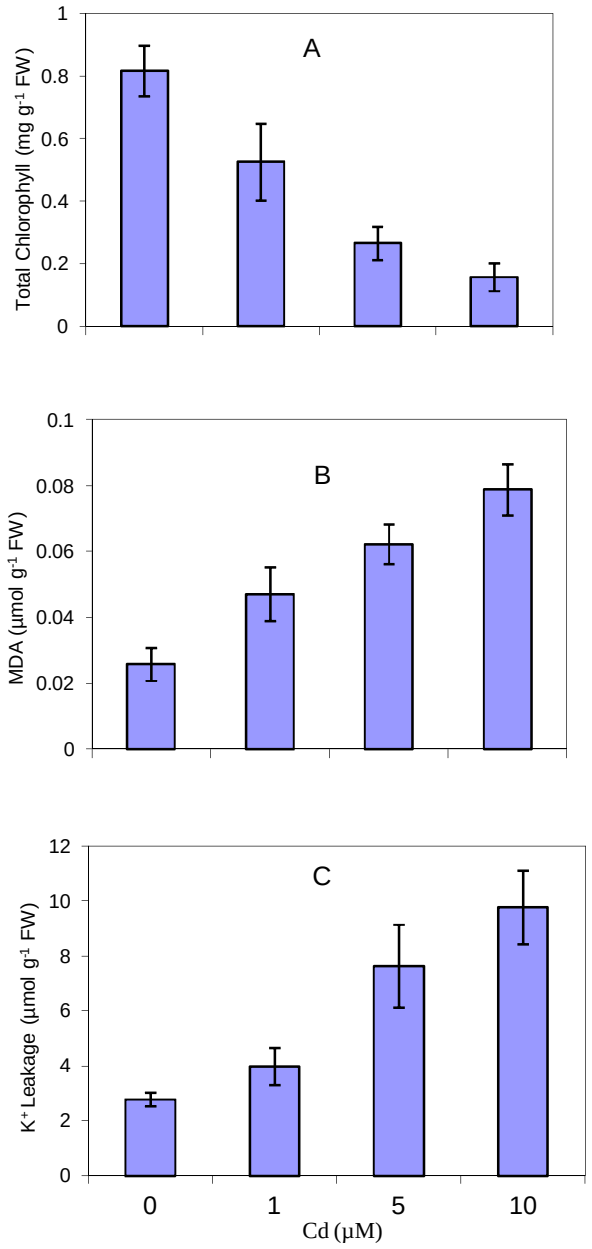

Figure 1: Effect of Cd on chlorophyll content (A), MDA content (B) and K^+ leakage (C) in excised *Tropaeolum majus* leaves.

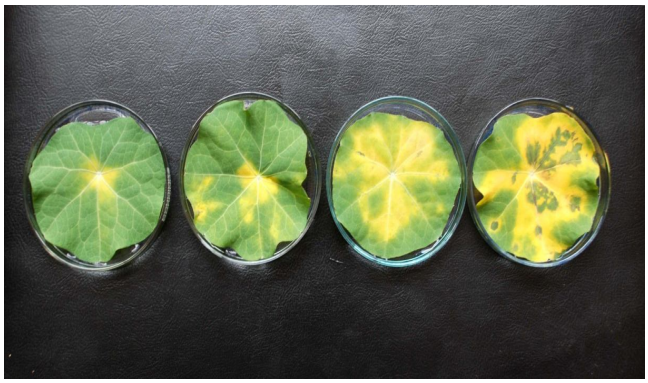

Figure 2: Effect of Cd on senescence of excised *Tropaeolum majus* leaves in dark (7 days).

Ca^{2+} – Cd^{2+} Interaction: There is sufficient evidence suggesting the involvement of Ca^{2+} uptake pathways in Cd uptake. In view of this, the effect of elevated Ca^{2+} concentrations on Cd-induced acceleration of leaf senescence was examined. It was expected that increasing Ca^{2+} concentrations would antagonize the Cd effects. However, this was not found to be the case. Treatment with Ca^{2+} alone slightly enhanced the rate of senescence of excised leaves of *Tropaeolum majus* incubated in dark for 7 days. Thus, there was 46% loss of total chlorophyll content relative to initial levels (1.028 mg Chl. g^{-1} leaf present) in 7 days dark incubated *Tropaeolum majus* leaves. After 7 days, chlorophyll content of calcium-treated leaves was 95, 86 and 76% of control at 10, 50 and 100 µM Ca, respectively (Fig. 3). Cd strongly accelerated the loss of chlorophyll from excised leaves of *Tropaeolum majus.* Leaves which were treated with 5 µM Cd lost 50% chlorophyll content as compared to control. Simultaneously applied Ca^{2+} further increased the Cd effect in chlorophyll degradation, particularly at higher concentrations. Thus, chlorophyll levels in Cd treated leaves were 50, 51, 43 and 33% of control at 0, 10, 50 and 100 µM Ca, respectively (Fig. 3 and 4).

There was a slight increase in MDA contents and K^+ leakage due to individual application of Ca (0-100 µM). Cd (5 µM) induced enhancement of MDA contents and K^+ leakage increased further when Ca was simultaneously applied particularly at higher concentrations. Thus, MDA accumulation rates were increased from 2.6-fold due to Cd (5 µM) alone to 3.4-fold when 100 µM Ca was also present. Similarly K^+ leakage increased from 1.7-fold (Cd alone) to 2.6-fold (Cd, 5 µM + Ca^{2+} 100 µM).

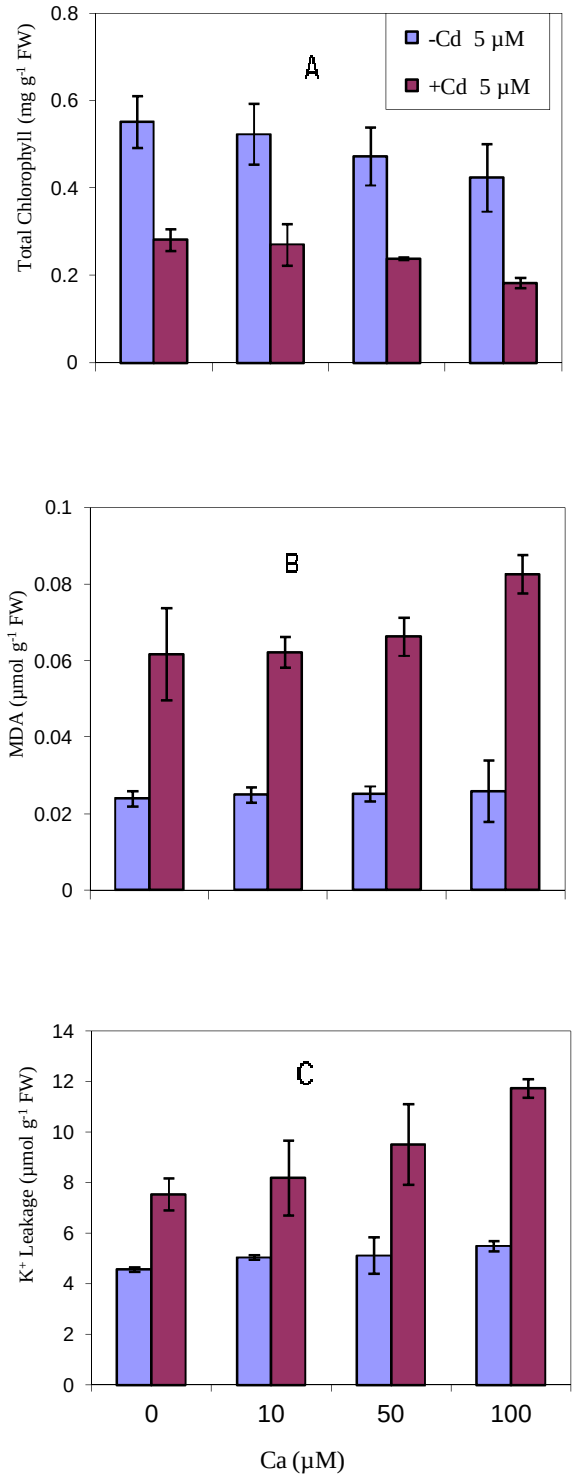

Figure 3: Interactive effects of Ca and Cd on chlorophyll content (A), MDA content (B) and K^+ leakage (C) in excised *Tropaeolum majus* leaves.

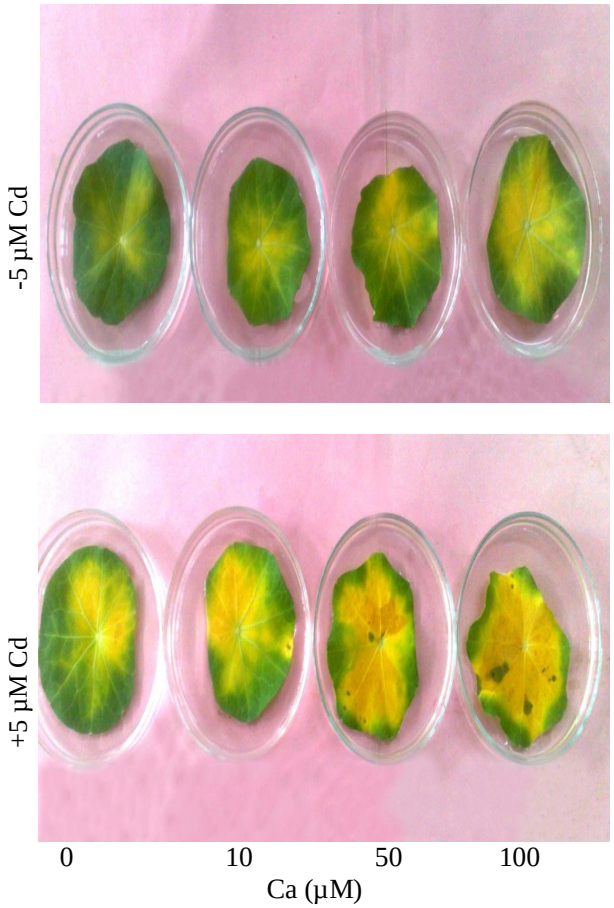

Figure 4: Interactive effects of Ca and Cd on senescence of excised *Tropaeolum majus* leaves in dark (7 days).

CONCLUSION: The findings from present study reveal that the leaf senescence accelerating influence of Cd and Ca^{2+}.

Ca^{2+} applied individually did not affect much the leaf senescence of excised *T. majus* leaves but when applied with Cd, it potentiated the Cd effect on chlorophyll loss, MDA contents and K^+ leakage.

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