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## Studies on variation of phytochelatin 2 content and *Metallothionein 2* gene expression in presence of cadmium stress in *Plantago ovata* forsk

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### Abstract

Heavy metal toxicity poses a harmful threat to plants. Cadmium is a non-essential heavy metal which enters plant cell via soil as a result of human malpractices, pollution and industrial works and causes severe damage to plants. Our study focuses on the effects of Cadmium stress in the plant *Plantago ovata* (*P. ovata*) by observing the content of Phytochelatin 2 (PC<sub>2</sub>) and the expression of *Metallothionein 2* (MT2) gene, both metal chelating agents. We observed the impact of Cadmium stress on phenotypic changes of *P. ovata* and performed Reverse Phase HPLC (RP-HPLC) analysis to observe the changes in PC<sub>2</sub> content when exposed to an increasing concentration of heavy metal stress. For MT2 gene expression, we performed the Reverse Transcription Polymerase Chain Reaction (RT-PCR) technique. In case of PC<sub>2</sub> we observed gradual increase in its content with response to heavy metal stress whereas it was observed that there is a considerable rise and a sudden fall in expression levels of MT2 gene when exposed to increasing levels of Cadmium stress. The results suggest that although both PC<sub>2</sub> and MT2 are able to combat heavy metal stress, PC<sub>2</sub> does so more effectively when compared to MT2.

**Keywords:** *Plant tissue culture, Phytochelatin 2, Heavy metal stress, Metallothionein 2, RP-HPLC*

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## 1. Introduction

*Plantago ovata*, also known as desert Indian wheat or Isabgol, is a medicinal plant originally found in the Mediterranean regions and Western Asia. It is used as a nutritional fiber. *P. ovata* is a member of the

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Plantaginaceae family and is a short annual herb. It is usually 10-45 cm in height (Karimzadeh and Omidbaigi 2004).

*P. ovata* has medicinal properties because it contains mucilage, albuminous matter, fatty oil, Aucubin (inactive glucoside) as well as a plantiose sugar. The seed husks of this plant can help stop diarrhoea as they can absorb and retain water molecules (Mohebbi and Maleki, 2010). Moreover, *P. ovata* seed husks are known to lower the progress of obesity, high blood pressure, and endothelial dysfunction (Galisteo et al., 2005). Earlier works indicate it can lower plasma lipid levels by changing liver cholesterol metabolism and bile acid metabolism (Romero et al., 2002).

The term heavy metal essentially means any metallic element having a high density that is toxic even at low concentrations (Tchounwou et al., 2012). Although there are many essential low molecular weight heavy metals like Zn, Co, Cu, Ni, Mn, Mo, and Fe, most other heavy metals are non-essential. Most heavy metals are toxic, their toxicity depending on the type of organism that is exposed and the concentration of the heavy metal. Among all of the heavy metals, the most toxic are Pb, Hg, and Cd, which essentially use the existing cellular mechanism to enter an organism. Heavy metal contamination in soil is caused by activities like industries, mining, fertilizers, pesticides, urban and semi-urban sewages, and also metallurgical smelters. (Lin and Aarts, 2012).

Among them, cadmium (Cd) exposure is dangerous as it causes severe damage to the lungs, kidneys, and bones in human beings. But Cd exposure not only affects humans and other animals, it affects plants as well. Cd is not essential to plants, although they are very easily taken up by the plants. Due to excess heavy metal exposure plants show reduced root length, chlorosis, morphological changes, and many other complications often leading to the death of the plant (Yadav, 2010; and Lin and Aarts, 2012). Cd exposure in particular, is dangerous as Cd acts as an enzyme inhibitor, causes degradation of the mitochondria and also changes in the chromosome, which therefore leads to complications in cellular growth and division (Das et al., 1997). So the ways in which a plant protects itself from heavy metal exposure are important.

Earlier literature reveals that plants have adapted a variety of mechanisms to combat heavy metal exposure, like ligand specific chelation and sequestration of the metals. The most widely documented and characterized metal-binding plant ligands are Phytochelatins (PCs) and Metallothioneins (MTs). Metallothioneins are polypeptides that are gene encoded whereas Phytochelatins are peptides that are enzymatically coded. Both of them are abundant in Cysteine residues (Cobbett and Goldsbrough, 2002).

PCs are a group of structures which have increasing repeats of the  $\gamma$ -GluCys dipeptide followed by a Gly residue at the end, that is  $(\gamma\text{-GluCys})_n\text{-Gly}$ , where n is generally in the range of 2 to 11. PCs are found in many different organisms. PCs are related structurally to glutathione (GSH;  $\gamma\text{-GluCysGly}$ ), and it has been established by different reports that GSH is the substrate for PC biosynthesis. (Cobbett, 2000)

MTs have a molecular weight between 4-8 kDa and they can co-ordinate metal ions. According to earlier classification, there are 4 MT families (15 families according to Mir et al., 2004) spanning several groups of organisms. MTs bind metals by forming mercaptide bonds via their cysteine residues. The MTs look like a dumbbell-shaped structure owing to the presence of two metal-binding, cysteine-rich domains (Cobbett and Goldsbrough, 2002).

In this study, we observe the phenotypic alterations and the changes in PC<sub>2</sub> content and MT<sub>2</sub> gene expression on the application of Cd stress, which is a non-essential heavy metal.

## 2. Materials and methods

### 2.1. Plant tissue culture

For plant tissue culture, all the seeds of *P. ovata* Forsk were obtained from Gujarat, India. Imbibition of the seeds overnight in autoclaved distilled water was carried out. Surface sterilization of the seeds was done by 10% sodium hypochlorite (NaOCl) for duration of 20 min with continuous shaking. The seeds were then washed with autoclaved distilled water 5 times for 5 min each in order to remove any trace of leftover bleach. They were then transferred aseptically to a germination medium containing 3% sucrose (w/v) [SRL, Mumbai, India] and 0.9% agar (w/v) [SRL, Mumbai, India]. The separate doses of Cadmium, in the form of Cadmium Chloride (CdCl<sub>2</sub>) added were 10  $\mu$ M, 40  $\mu$ M, 80  $\mu$ M and a set of media without any treatment was maintained as control

(Figure 1A). The doses were repeated in triplicates. The essential germination environment maintained was a temperature between 22-25 °C, a relative humidity of 55-60% and illumination at 1500 flux for 16/8 h duration of light/dark photo period. The above conditions followed for plant tissue culture was adapted from previously published works of Das and Sen Raychaudhuri (2001). The lethal dose 50 or LD<sub>50</sub> of CdCl<sub>2</sub> was found out to be 200 μM. These above mentioned specific doses of CdCl<sub>2</sub> were chosen after determining the LD<sub>50</sub> of CdCl<sub>2</sub> and only the doses well below the LD<sub>50</sub> which were sub lethal were taken.

## 2.2. Phenotypic alterations

To ascertain the stress mediated response of CdCl<sub>2</sub> on *P. ovata*, in terms of phenotypic alterations, the root and shoot length of seedlings that were 7 days old were measured in cm (Figure 1B). The outcome was demonstrated as mean ± standard error of mean (SEM).

## 2.3. Extraction of PC<sub>2</sub>

The plants, with the above mentioned concentrations of CdCl<sub>2</sub> and without treatment which was treated as control, were allowed to grow for 7 days in culture. Then 100mg fresh weight (FW) of shoot tissue for each dose were measured and subsequently crushed in liquid nitrogen. Then, 0.1% Trifluoro acetic acid (TFA) was added to each sample and homogenized. After that, centrifugation at 4 °C at 10,000 rpm for 10 min was carried out to extract PC<sub>2</sub> based on the method of Sneller et al. (1999) with slight modifications. The extracted samples were filtered using a syringe filter (Acrodisc) and stored at -80 °C.

## 2.4. RP- HPLC

We performed RP-HPLC in a Shimadzu Prominence series HPLC system to estimate PC<sub>2</sub> content in the samples. The separation was carried out using a Luna 5 C18 RP column (250 × 4.6 mm particle size 5 μ, Phenomenex). The mobile phase used was a gradient of two solutions, solution A (0.1% TFA) and solution B (80% Acetonitrile + 20% of 0.1% TFA). The flow rates of pump A was 1 ml/ min and that of pump B was 0.1 ml/min. The samples were run for about 10 min each at 214 nm wavelength. There was a 20-min interval for washing the column between two consecutive runs. At a temperature of 25 ± 2 °C the column was maintained.

Standard run of PC<sub>2</sub> was performed using commercially available PC<sub>2</sub> and concentrations of 40 μg/ml, 120 μg/ml, 160 μg/ml and 200 μg/ml were prepared from the stock solution. Using these different concentrations, a standard curve of PC<sub>2</sub> was prepared.

## 2.5. Total RNA extraction

100 mg FW of shoot tissues were measured and taken from each of the doses of 7-day old *P. ovata* plants with the above mentioned concentrations of CdCl<sub>2</sub> as well as no treatment (taken as control). After that, total RNA extraction was carried out using the Invitrogen RNA kit. The RNA was stored at -80 °C after extraction process was complete. To ascertain the pureness of the extracted total RNA, a spectrophotometric analysis was carried out using a JASCO V-630 UV-Vis Spectrophotometer at 260 nm wavelength. The concentration of the RNA extracted was determined by the optical density using a formula.

Formula used: RNA concentration (μg/mL) = A<sub>260</sub> × 40 × dilution factor. (Absorbance at 260 nm = A<sub>260</sub>). Since we used 1 μL of total RNA extract and 299 μL of nuclease free water, the dilution factor should be 300 in this case.

## 2.6. RT-PCR

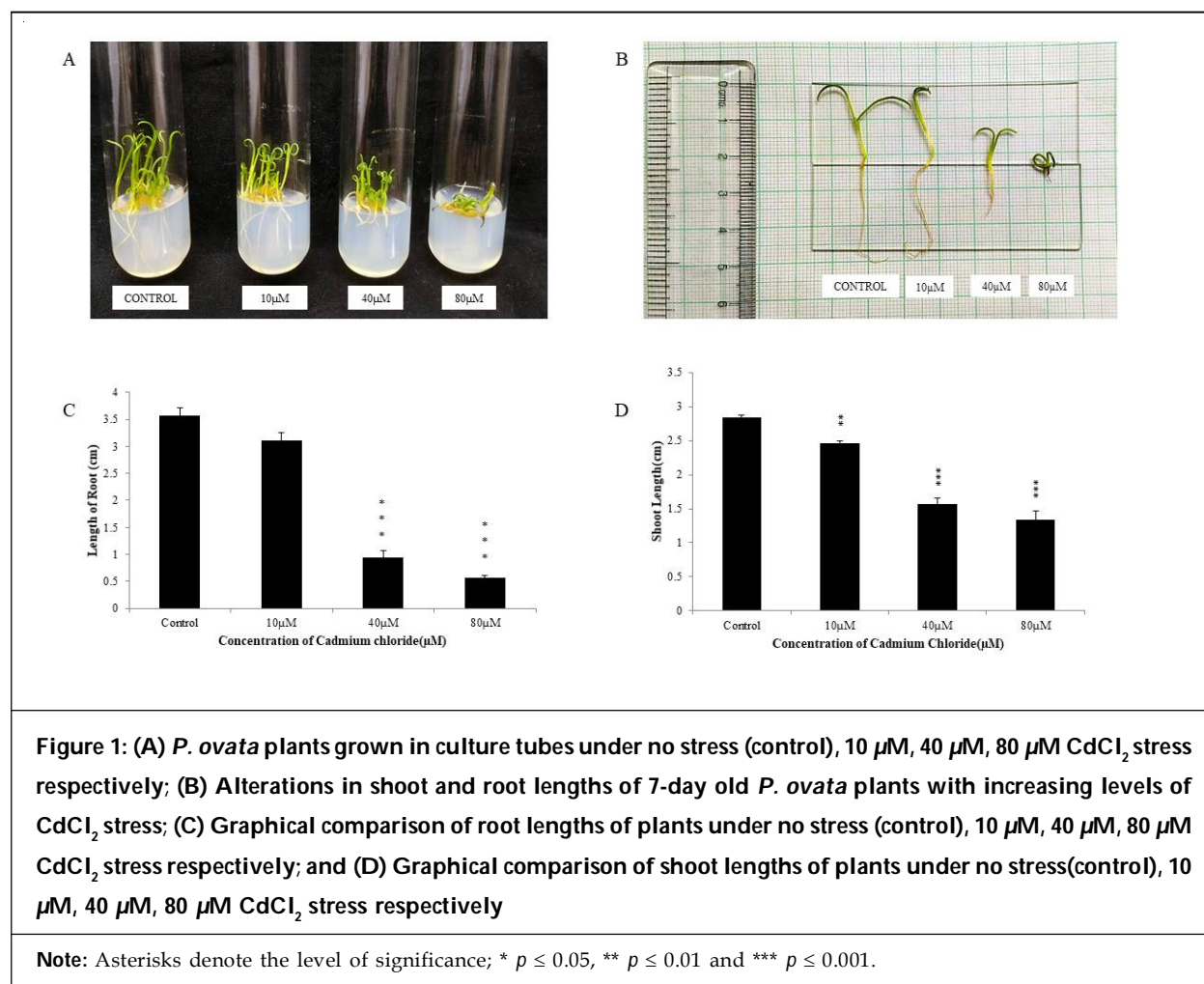
Reverse Transcription Polymerase Chain Reaction (RT-PCR) was carried out for the expression analysis of *MT2* gene in untreated as well as treated *P. ovata* samples under CdCl<sub>2</sub> stress using QIAGEN One Step RT-PCR kit. The samples contained 2 μg concentration of RNA in each reaction mixture. Forward and reverse primers of *MT2* gene respectively were: 5' ATGICTTGCTGCAACGGAAACT 3', 5' CTATTTGCAATTGCATGGATTG 3'. Forward and reverse primers of *β-actin* gene respectively were: 5' ATCATGAAGTGTGATGTTGA 3', 5' ACCTTAATCTTCATGCTGCT 3'. The RT-PCR amplification reactions of *MT2* gene were as follows: The reverse transcription was carried out at 50 °C for 30 min followed by initial denaturation at 95 °C for 15 min. This was followed by 35 cycles of denaturation at 94 °C for 1 min; annealing at 53.5 °C for 1 min and extension at 72 °C for 1 min 30 sec. The final extension was carried out at 72 °C for 10 min.

The products of RT-PCR procedure were run on 1.5% agarose gel with 20% Ethidium Bromide (EtBr) of 1mg/ml added to stain and visualized using the BioRad gel documentation software. ImageJ software was used to analyze the expression profile densitometrically from the band intensity of the gels. The expression of *MT2* was compared with the endogenous gene  *$\beta$ -actin* which was taken as control.

Stages	Temperature	Duration	
Reverse transcription	50 °C	30 min	
Initial denaturation	95 °C	15 min	
Denaturation	94 °C	1 min	} 35 cycles
Annealing	53.5 °C	1 min	
Extension	72 °C	1 min 30 sec	
Final extension	72 °C	10 min	

## 2.7. Statistical analysis

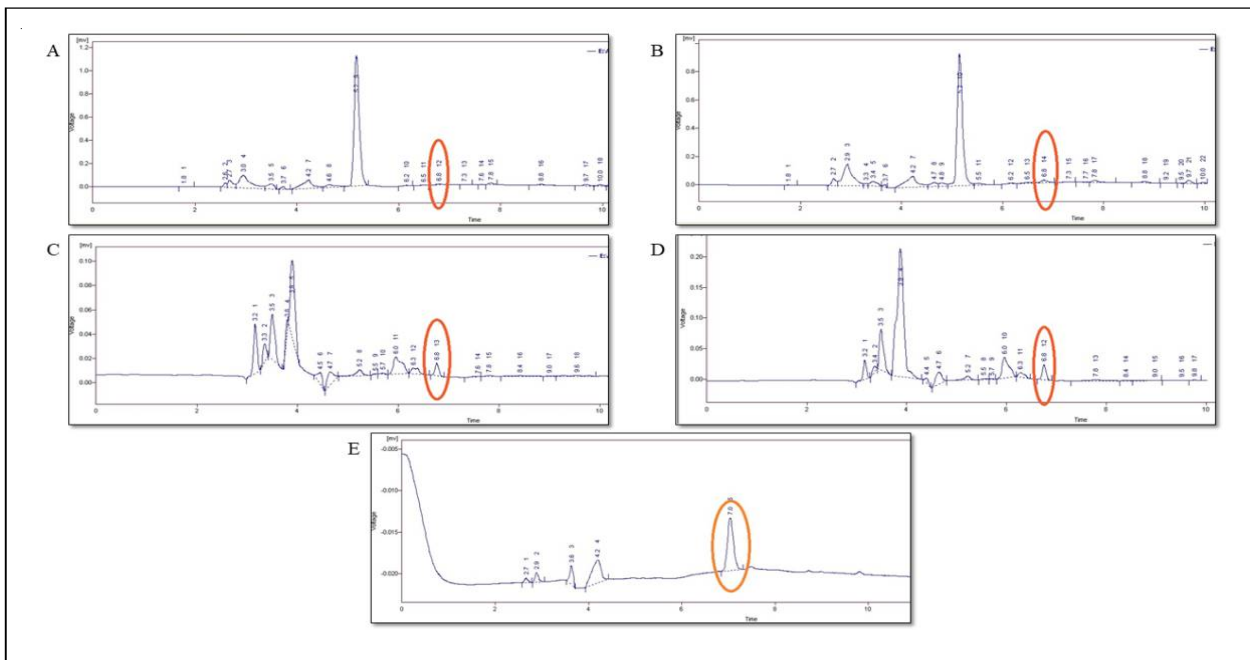
The experimental data was calculated and represented as mean  $\pm$  standard error of the mean (SEM). The data was evaluated by calculating the variance (ONE WAY ANOVA) and the means were compared by Student's *t*-test using KyPlot (version 2.0).  $p \leq 0.05$  differences in the data were considered statistically significant.



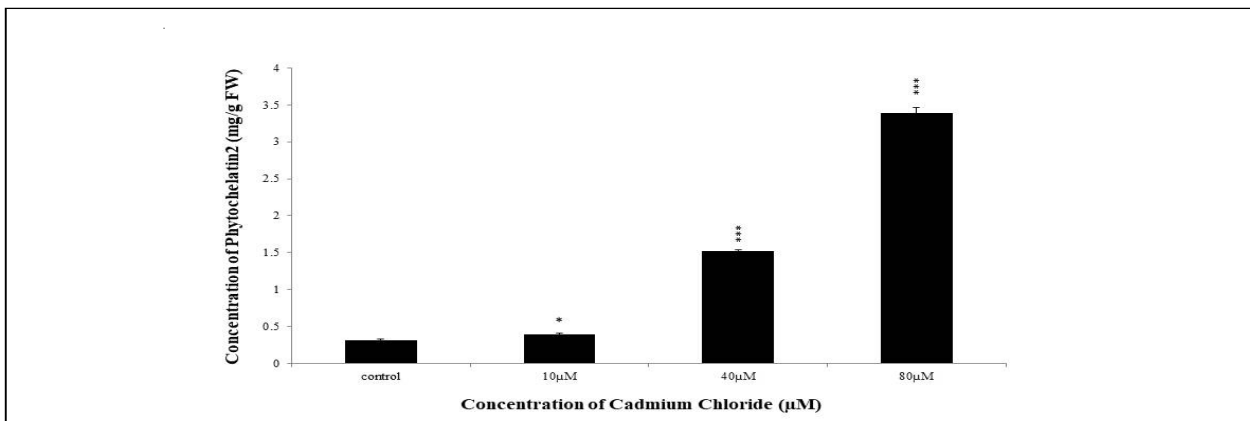
### 3. Results

Root measurements of 7-day old *P. ovata* plants showed significant changes ( $p \leq 0.001$ ) in case of 40  $\mu\text{M}$  (3.82-fold decrease) and 80  $\mu\text{M}$  (6.29-fold decrease) doses of  $\text{CdCl}_2$  with respect to control, whereas no significant change was observed in case of the 10  $\mu\text{M}$  dose with respect to control, although the root length decreases in this case as well (Figure 1C). In case of shoot measurements, we noticed significant decrease in lengths of all three doses of 10  $\mu\text{M}$  (1.14-fold decrease), 40  $\mu\text{M}$  (1.80-fold decrease) and 80  $\mu\text{M}$  (2.12-fold decrease) with respect to control (Figure 1D).

From the standard run of  $\text{PC}_2$  we observed a signature peak around 7 min. (Figure 2E). The peak of  $\text{PC}_2$  in the samples was observed around 6.8 min. The amount of  $\text{PC}_2$  increases gradually from the untreated sample (taken as control) with respect to increment in dose concentration of  $\text{CdCl}_2$ . The area under the peaks was used to quantify the amount of  $\text{PC}_2$ . In case of  $\text{CdCl}_2$  stress of 10  $\mu\text{M}$ , 40  $\mu\text{M}$  and 80  $\mu\text{M}$  samples, the levels increased by 1.25 fold, 4.9-fold and 10.91-fold respectively with respect to untreated samples (Figures 2A, 2B, 2C, 2D). A graphical representation of the data obtained is given in Figure 3.



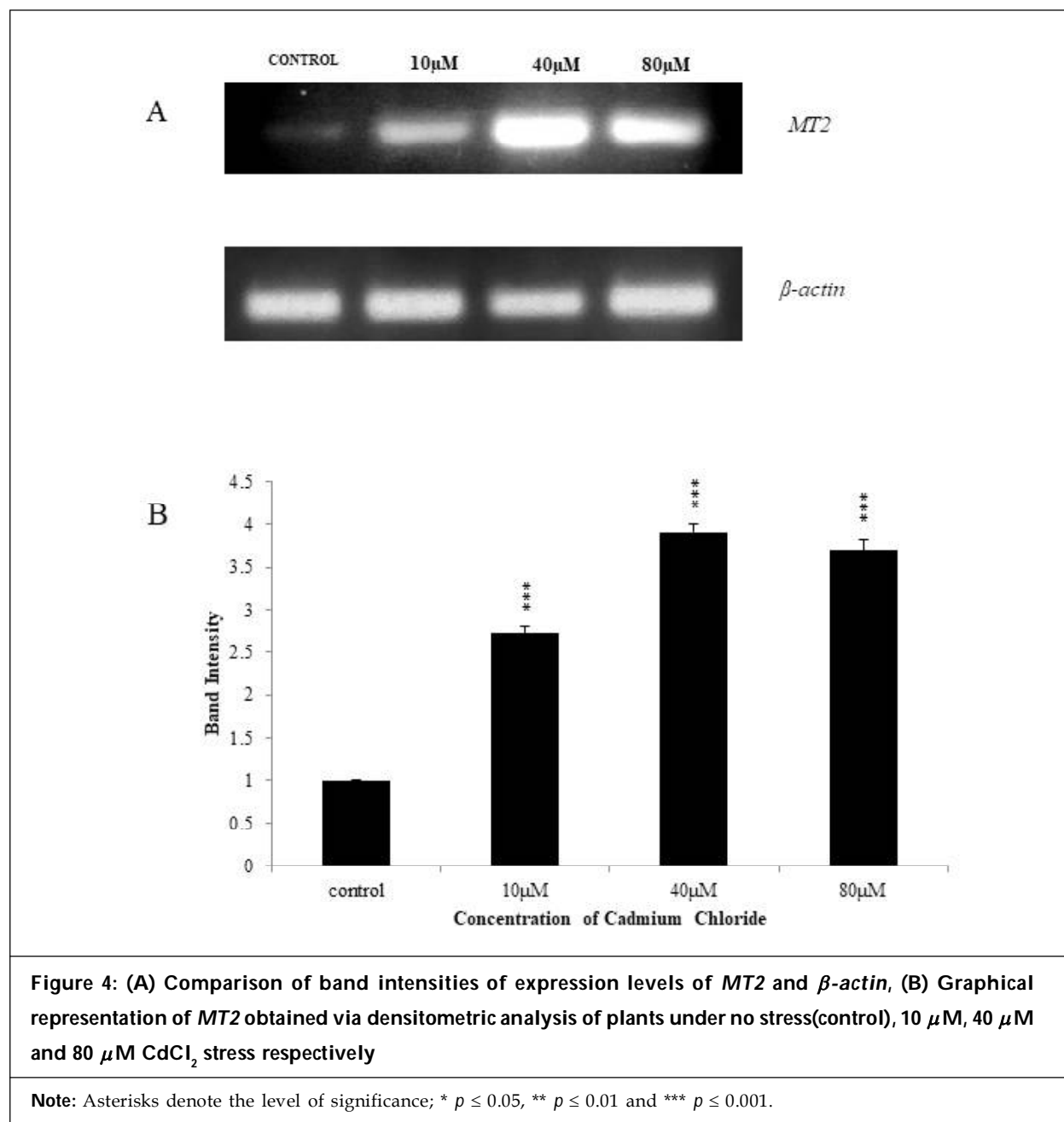
**Figure 2: (A) Chromatogram of sample without any treatment (Control), (B) Chromatogram of sample with 10  $\mu\text{M}$  dose of  $\text{CdCl}_2$ , (C) Chromatogram of sample with 40  $\mu\text{M}$  dose of  $\text{CdCl}_2$ , (D) Chromatogram of sample with 80  $\mu\text{M}$  dose of  $\text{CdCl}_2$ , (E) Chromatogram of commercial  $\text{PC}_2$  sample.**



**Figure 3: Graphical representation of  $\text{PC}_2$  concentrations (mg/g fresh weight) obtained by RP-HPLC analysis with respect to different doses of  $\text{CdCl}_2$**

**Note:** Asterisks denote the level of significance; \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$  and \*\*\*  $p \leq 0.001$ .

The RT-PCR products run on 1.5% agarose gel and from the densitometric analysis of the band intensities (Figure 4A), we saw an increase in the expression levels of *MT2* from control gradually up to 40  $\mu\text{M}$   $\text{CdCl}_2$  and then a comparative decrease in the expression of 80  $\mu\text{M}$   $\text{CdCl}_2$ . There was a 2.73-fold, 3.91-fold and 3.69-fold increase in case of 10  $\mu\text{M}$ , 40  $\mu\text{M}$  and 80  $\mu\text{M}$   $\text{CdCl}_2$  dose with respect to untreated samples (Figure 4B). In case of  $\beta$ -actin gene expression, it was observed there were no significant changes in the expression levels of the doses of 10  $\mu\text{M}$ , 40  $\mu\text{M}$  and 80  $\mu\text{M}$   $\text{CdCl}_2$  stress with respect to untreated samples.



#### 4. Discussion

Cadmium is a heavy metal that poses a threat to human health. Damages in kidney, bone, and lungs have been known to have occurred due to cadmium exposure. Heavy metal accumulation also takes a heavy toll on plants. Wojcik and Tukiendorf (2004) demonstrated that Cd stress leads to diminished biomass in *Arabidopsis thaliana*. Also, the leaves showed signs of chlorosis and even necrosis. In a study by Mohanpuria et al. (2007), the authors reported decrease in protein and chlorophyll content whereas lipid peroxidation levels elevated due to Cd stress. To combat these accumulative effects, plants deploy both Phytochelatins and Metallothioneins, which are metal chelators of plants, effective in combating heavy metal stress.

Experiments by Chen *et al.* (2003), showed that Cd exposure slowed down the rates of germination and growth of radish and carrot roots, and this hindrance increased with increasing Cd levels. In our study we observed gradual decrease in both root and shoot lengths of *P. ovata* plants with respect to an increase in Cd concentrations (10  $\mu\text{M}$ , 40  $\mu\text{M}$  and 80  $\mu\text{M}$ ) as compared to untreated samples.

Our study aimed at viewing the contents of PC<sub>2</sub> in shoot tissues of *P. ovata* when exposed to gradually increasing concentrations of Cd stress. We observed that the amounts of PC<sub>2</sub> were gradually elevated as Cd concentration levels increased. These observations are similar to those observed in other plants. In one study, the researchers observed the levels of PC<sub>2</sub> and Phytochelatin 3 (PC<sub>3</sub>) elevated with response to increasing amounts of Cd concentration up to a certain level, after which it diminished in water plant *Hydrilla verticillata*. (Tripathi *et al.*, 1996). Similarly, in case of plants like *Bacopa monnieri* L, researchers found gradual elevation in amounts of PC<sub>2</sub> as well as PC<sub>3</sub> when exposed to increasing amounts of Cd stress concentration in leaves, but a gradual rise followed by a fall in levels of both PC<sub>2</sub> and PC<sub>3</sub> was observed in the roots (Mishra *et al.*, 2006). The plant *Carthamus tinctorius* also showed increased PC<sub>2</sub> and PC<sub>3</sub> levels in both roots and leaves in presence of Cd, however the levels were comparatively higher in case of roots (Namdjoyan *et al.*, 2012).

In a study of a mangrove species *Bruguiera gymnorrhiza* by Huang and Wang (2009), the expression of MT2 gene for 3 days and 7 days old plants elevated to different levels of Pb concentration and then declined considerably. These plants also showed different tolerance levels for heavy metals Zn and Cu as well. As shown in another study, the presence of Zn metal in plants leads to activation of expression of MT2 up to a certain threshold level (800  $\mu\text{M}$ ) to which the plant can endure Zn stress, after that the expression levels of MT2 decline upon further elevation of stress (Pramanick *et al.*, 2017). These reports corroborate our finding that showed that expression of MT2 gene in *P. ovata* increased gradually up to 40  $\mu\text{M}$  Cd stress (with respect to untreated samples), but then declined considerably at a higher concentration of 80  $\mu\text{M}$  Cd. The concentrations of withstanding Zn stress are relatively higher as Zn is an essential heavy metal as compared to Cd.

## 5. Conclusion

We observed from this study that CdCl<sub>2</sub> stress increased the levels of PC<sub>2</sub> considerably for all doses of CdCl<sub>2</sub> when compared to untreated control. This indicates activation of PC<sub>2</sub> in response to heavy metal chelation. On the other hand, the MT2 expression in presence of CdCl<sub>2</sub> stress resulted in a gradual increase initially but then an eventual drop was observed. This could be explained due to the fact that *P. ovata* plants can withstand the concentration of non-essential heavy metals like Cd up to a certain threshold limit, after which it becomes toxic to the plant. In our case the threshold limit was 40  $\mu\text{M}$  of Cd stress, beyond which the expression of MT2 declines. Our study also suggests that PC<sub>2</sub> is a more effective metal chelator which combats heavy metal stress even at higher concentrations in comparison with MT2.

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## References

- Chen, Y.X., He, Y.F., Luo, Y.M., Yu, Y.L., Lin, Q. and Wong, M.H. (2003). [Physiological mechanism of plant roots exposed to cadmium. \*Chemosphere\*, 50\(6\), 789-793.](#)
- Cobbett, C. and Goldsbrough, P. (2002). [Phytochelatin and metallothioneins: roles in heavy metal detoxification and homeostasis. \*Annu. Rev. Plant Biol.\*, 53\(1\), 159-182.](#)
- Cobbett, C. (2000). [Phytochelatin and their roles in heavy metal detoxification. \*Plant Physiol.\* Jul; 123\(3\), 825-32.](#)
- Das, P., Samantaray, S. and Rout, G.R. (1997). [Studies on cadmium toxicity in plants: a review. \*Environ. Pollut.\*, 98\(1\), 29-36.](#)
- Das (Pal), M. and Sen Raychaudhuri, S. (2001). [Enhanced development of somatic embryos of \*Plantago ovata\* Forsk. By additives. \*In Vitro Cell.Dev.Biol.-Plant\*, 37, 568-571.](#)
- Galisteo, M., Sainchez, M., Vera, R., Gonzalez, M., Anguera, A., Duarte, J. and Zarzuelo, A. (2005). [A diet supplemented with husks of \*Plantago ovata\* reduces the development of endothelial dysfunction,](#)

- hypertension, and obesity by affecting adiponectin and TNF- $\alpha$  in obese Zucker rats. *J. Nutr.*, 135(10), 2399-2404.
- Huang, G.Y. and Wang, Y.S. (2009). Expression analysis of type 2 metallothionein gene in mangrove species (*Bruguiera gymnorhiza*) under heavy metal stress. *Chemosphere*, 77(7), 1026-1029.
- Karimzadeh, G. and Omidbaigi, R. (2004). Growth and Seed Characteristics of Isabgol (*Plantago ovata* Forsk) as influenced by some Environmental Factors. *JAST*. 6 (3), 103-110.
- Lin, Y.F. and Aarts, M.G. (2012). The molecular mechanism of zinc and cadmium stress response in plants. *Cell. Mol. Life Sci.*, 69(19), 3187-3206.
- Mir, G., Domènech, J., Huguët, G., Guo, W. J., Goldsbrough, P., Atrian, S. and Molinas, M. (2004). A plant type 2 metallothionein (MT) from cork tissue responds to oxidative stress. *J. Exp. Bot.*, 55(408), 2483-2493.
- Mishra, S., Srivastava, S., Tripathi, R. D., Govindarajan, R., Kuriakose, S. V. and Prasad, M. N. V. (2006). Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. *Plant Physiol. Biochem.*, 44(1), 25-37.
- Mohebbi, M. and Maleki, A. (2010). Effect of water stress on some seed characteristics of isabgol (*Plantago ovata* Forsk) in Zanjan (Iran). *Adv. Environ. Biol.*, 10-14.
- Namdjoyan, S., Namdjoyan, S. and Kermanian, H. (2012). Induction of phytochelatin and responses of antioxidants under cadmium stress in safflower (*Carthamus tinctorius*) seedlings. *Turk. J. Bot.*, 36(5), 495-502.
- Mohanpuria, P., Rana, N.K. and Yadav, S.K. (2007). Cadmium induced oxidative stress influence on glutathione metabolic genes of *Camellia sinensis* (L.) O. Kuntze. *Environ Toxicol.* 2007 Aug; 22(4), 368-374.
- Pramanick, P., Chakraborty, A. and Sen Raychaudhuri, S. (2017). Phenotypic and biochemical alterations in relation to MT2 gene expression in *Plantago ovata* Forsk under zinc stress. *Biometals*, 30(2), 171-184.
- Romero, A.L., West, K.L., Zern, T. and Fernandez, M.L. (2002). The seeds from *Plantago ovata* lower plasma lipids by altering hepatic and bile acid metabolism in guinea pigs. *J. Nutr.*, 132(6), 1194-1198.
- Sneller, F. E. C., Noordover, E. C., Ten Bookum, W. M., Schat, H., Bedaux, J. J. and Verkleij, J. A. (1999). Quantitative relationship between phytochelatin accumulation and growth inhibition during prolonged exposure to cadmium in *Silene vulgaris*. *Ecotoxicology*, (8), 167-175.
- Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K. and Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Exp Suppl.*; 101:133-164.
- Tripathi, R.D., Rai, UN., Gupta, M. and Chandra, P. (1996). Induction of phytochelatin in *Hydrilla verticillata* (L) Royle under cadmium stress. *Bull. Environ. Contam. Toxicol.*, 56(3), 505-512.
- Wojcik, M. and Tukiendorf, A. (2004). Phytochelatin synthesis and cadmium localization in wild type of *Arabidopsis thaliana*. *Plant Growth Regul.*, 44(1), 71-80.
- Yadav, S.K. (2010). Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatin in heavy metal stress tolerance of plants. *S. Afr. J. Bot.*, 76(2), pp.167-179.

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