Journal of Soil Science and Plant Nutrition, 2014, 14 (2), 447-458

Sensitivity of *Allium cepa* and *Vicia faba* towards cadmium toxicity

S. K. Arya*, A. Mukherjee

*Deparment of Botany, University of Calcutta , Kolkata-700019. India. *Corresponding author: shashiarya03@gmail.com*

Abstract

Sensitivity of common onion (*Allium cepa* L.) and faba bean (*Vicia faba* L.) to cadmium (Cd) stress was investigated using genotoxicity endpoints. Simultaneously, the antioxidative stress enzymes (guaiacol peroxidise and catalase) and lipid peroxidation [malonaldehyde (MDA) content] were investigated in the plants exposed to Cd in solution. The endpoints screened for genotoxicity included chromosomal aberrations, micronuclei frequency and mitotic frequency. Additionally comet assay was performed for DNA fragmentation. Evan's blue uptake by the root meristems as a cell death parameter served as indicator of cytotoxicity. The results indicated that exposure to Cd induced dose-dependent increase in chromosomal aberrations, DNA fragmentation and micronucleus frequency in both *A.cepa* and *V. faba*. The enzymes guaiacol peroxidase (GPX) and catalase (CAT) activities increased in both the plants and were higher in *V. faba*. A positive correlation between the activity of GPX and CAT and MDA content was recorded at all concentrations of Cd. Regarding sensitivity, our results showed that *V. faba* is more sensitive plant than *A. cepa* towards Cd –induced toxicity. Higher genotoxicity level correlated to the increased level of oxidative stress in root tissues. This was likely to be an important cause of *Vicia* plant to be less tolerant than *Allium*.

Keywords: Antioxidant enzymes, cadmium, chromosomal aberrations, comet assay, lipid peroxidation, micronucleus, mitotic frequency

1. Introduction

Vegetables are the staple food for most of the world and play an important role in human diet. Among these vegetables, onions are of great importance and a natural part of the daily diet for most of the world's population. Common red onion (*Allium cepa* L.) is a vegetable of great economic importance grown all over the world (Mogren *et al*., 2007). In recent years, the consumption of faba beans - *Vicia faba* L. has increased, as they are rich source of protein, vitamin and minerals (Duc, 1997). Although *V. faba* are consumed less in western countries, it is one of the main source of protein and energy in Africa, Asia and Latin America.

The heavy metals like cadmium (Cd) and other pollutants in agricultural soils have led to bioaccumulation of various toxicants in food crops; (Nagajyoti *et al*., 2010)

that are easily absorbed by soil and accumulated in different plant parts such as root, stem and leaf. Cd induces clastogenic and genotoxic disturbance in plants and inhibits root growth and cell division in different plants (Fojtova and Kovarik, 2000). Available reports confirm that Cd induces oxidative burst via the generation of reactive oxygen species (ROS), such as H_2O_2, O_2.- radicals, and .OH radicals as well as disturbance to antioxidative systems (Romero-Puertas *et al*., 2004). The Cd-induced genotoxicity events have been reported in *Vicia* (Unyayar *et al*., 2006; Zhang *et al.*, 2009) and *Allium* species (Unyayar *et al*., 2006). The karyological characteristics i.e. low number and long chromosomes, make the observations of nucleus damage substantially easy. The International Program on Chemical Safety (IPCS) and the World Health Organization (WHO) (Ma, 1999) prefer *V. faba* and *A. cepa* seedlings for screening and monitoring of genotoxic agents and mitotic studies (Gopalan, 1999).

The aim of the present work was to determine the sensitivity of the two plants *V. faba* and *A. cepa* towards Cd- induced genotoxicity. Chromosomal aberrations, micronucleus formation and DNA breaks are regarded as the indicators of genotoxicity. Since oxidative stress is implicated in the production of such damages, we have carried experiments to compare the sensitivity of *Allium* and *Vicia* performed at the same time and under same conditions. Additionally, antioxidant enzyme activities and cytotoxic effects of Cd were assessed.

2. Materials and Methods

2.1. Chemicals

Cadmium chloride (CAS No 35658-65-2), N, N-dimethylformamide, hydrogen peroxide (H_2O_2), glacial acetic acid, mercuric chloride, disodium EDTA, sodium chloride (NaCl), sodium hydroxide (NaOH), trizma base and hydrochloric acid (HCl) was purchased from Merck India Ltd, Mumbai, India. Evan's blue, thiobarbituric acid, trichloroacetic acid, ethanol, magnesium chloride ($MgCl_2$), polyvinyl pyrrolidone (PVP) and orcein was purchased from Himedia, India Ltd. Ethyl methane sulphonate (EMS), ethidium bromide and low melting point agarose (LMPA) were purchased from Sigma-Aldrich Co. (USA). Guaiacol was purchased from SRL, Mumbai, India.

2.2. Plant materials and treatments

Equal sized bulbs were chosen from a population of a local red variety of the common onion, *Allium cepa* L.(2n =16).The onions were positioned for germination directly on autoclaved sand taken in earthen pots (Mukherjee and Gichner, 2009) at room temperature under a 12 h light/ dark cycle. Seeds of *V. faba* purchased from Institute of Agriculture Sciences, Banaras Hindu University, Varanasi, India were sterilized in 0.1% (w/v) $HgCl_2$ for 8 min. The seeds were washed with tap water and soaked for 18 h in dark. Seeds were allowed to germinate on moistened filter paper at 25 ^{0}C for 4-5 days in petriplates. To get a luxuriant biomass of secondary roots, excised primary root tips of *V faba* were transferred to plastic vials containing tap water. To ensure equivalent available Cd uptake by the roots, root biomass taken for Cd- treatment at similar concentration in *A.cepa* and *V. faba* was same. Roots of both plants were immersed in plastic vials containing 30 ml of a defined concentration of $CdCl_2$ (50, 100, 200 µM) and EMS (2 mM) dissolved in distilled water for 2 h in the dark at 22 ^{0}C. Negative (distilled water) control was included in the experiment. After a recovery period of 24 h, the roots of *A. cepa* and *V. faba* were used for comet assay and other tests as applicable.

2.3. Analysis of Cadmium

Atomic absorption spectrophotometer was used to analyse the Cd content in root tissue of *A. cepa* and *V. faba*. After treatment root tissues were oven dried, ground and digested with concentrated HNO_3:$HClO_4$ (1:3). Digested samples were filtered and diluted with redistilled water to 25 ml. Cd concentrations were determined according to the standard method

of the American Public Health Association (1998) using atomic absorption spectrometry (AAS4136A, Electronic Corporation of India Limited, Hyderabad, India). Acid matrix blanks were included to assess possible Cd contamination.

2.4. Cell death measurement and visualization

To detect cytotoxicity intact roots (length ~ 2cm) of control and treated samples were stained in aqueous solution of Evan's Blue [0.25% (w/v)] for 30 min (Baker and Mock, 1994). Five stained root tips were transferred to 1 ml of N, N-dimethylformamide for 1 h at room temperature. The absorbance of Evan's blue was measured at 600 nm (Beckman Coulter DU730 Lifescience UV/ Vis spectrophotometer).

2.5. Extraction and estimation of antioxidative enzymes and MDA content

300 mg of fresh root tissues of control and treated samples were homogenized in 900 µL extraction buffer [50 mM Tris-HCl pH 7.8 containing 1M EDTA, 1mM MgCl2, 1.5% PVP (w/v)]. The homogenates were centrifuged at 15000 rpm for 20 min at 4 °C. The supernatants were collected and kept at -20 ºC for further use to analyze the GPX and CAT activities (Meng *et al*., 2007). The protein content in the enzyme extracts was estimated according to the method of Lowry *et al*. (1951) using BSA as a standard.

Catalase (CAT) activity was determined following the method of Aebi (1974) with some modifications. The assay mixture in total volume of 1.5 ml contained 1 ml of 100mM potassium phosphate buffer (pH 7), 0.4 ml 200 mM H_2O_2 and 0.1 ml enzyme extract. The decrease in absorbance was measured at 240 nm (ε= 0.036 mM^{-1} cm^{-1}) spectrophotometrically (Beckman Coulter DU730 Lifescience UV/ Vis spectrophotometer). Enzyme activity was expressed as unit min^{-1} mg^{-1} protein.

Guaiacol peroxidase (GPX) activity was assayed according to the method of Hemeda and Klein (1990). 6 ml of reaction mixture contained 1 ml of 1% guaiacol (v/v), 1 ml of 1% H_2O_2 (v/v) and 4 ml of 50 mM potassium phosphate buffer (pH 6.1). 10 µl enzyme extract was added to reaction mixture. The increase in absorbance due to oxidation of guaiacol (ε = 26.6 mM^{-1} cm^{-1}) at 470 nm was monitored in Beckman Coulter DU730 Lifescience UV/ Vis spectrophotometer. Enzyme activity was expressed as unit min^{-1} mg^{-1} protein.

Extent of lipid peroxidation in terms of MDA formation was determined in roots of control and treated plants following the method of Karabal *et al.* (2003) with few modifications. 0.2 g fresh root tissues of control and treated samples were homogenized in 1 ml of 5% (w/v) trichloroacetic acid (TCA) in an ice bath. Homogenates were centrifuged at 12000 rpm for 15 min at room temperature. 100-µl supernatant was added to equal volume of 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) TCA. The mixtures were heated at 95 ºC for 25 min and cooled immediately. Subsequently, the mixtures were centrifuged at 10000 rpm for 5 min and absorbance of the supernatant was measured at 532 nm. MDA content (ε = 1.56 x10^5 $M^{-1}cm^{-1}$) was calculated and expressed in µM/ g of fresh weight.

2.6. Genotoxicity assay

For genotoxicity, the following endpoints- mitotic index (MI), chromosomal aberrations (CAs) and micronuclei (MN) were assessed in *A. cepa* and *V. faba* root tip cells. Root tips (1cm) from control and treated samples were excised and fixed immediately in Carnoy's fluid (ethanol: glacial acetic acid 3:1). Root tips were hydrolyzed and stained in warm 2% aceto-orcein stain (aceto-orcein: 1N HCl 9:1) and squashed in 45% acetic acid. Slides were prepared and coded following the method of Mukherjee and Sharma (1988). Approximately 3000 cells per concentration were scored under a binocular light microscope (Japan, Olympus BX51) at 40X magnification.

MI was determined by counting the number of mitotic cells among the total amount of scored cells per treatment (including control). Mean values for each endpoint were calculated and statistically correlated. The methods of Navarrete *et al*. (1997), Mukherjee and Gichner (2009) were followed to detect DNA damage in the roots of *A. cepa* and *V. faba*. Roots of control and treated samples were harvested, washed and blotted dry. All the procedures were performed under dim light and in cooling condition. Nuclei were isolated by chopping the roots with fresh blade in 60 mm petriplates containing 400 µl of 400 mM Tris buffer (pH 7.4). The slides were prepared as described by Navarrete *et al.* (1997) and placed in a horizontal gel tank (Life Technologies, California, USA) containing alkaline electrophoresis buffer (300 mM NaOH and 1 mM EDTA; pH > 13) for 20 min at 40 ^{o}C. This was followed by electrophoresis at 26 V and 300 mA current for 20 min (*A. cepa*) and 15 min (*V. faba*). Slides were neutralized in 400 mM Tris pH 7.4 for 5 min and finally washed in water. Subsequently, the slides were stained with EtBr (20 µg/ ml) and rinsed in water. Stained slides were scored (25 x 3 per sample) and photographed (100X) using image analysis system (Kinetic imaging; Andor Technology, Nottingham, UK) attached to a fluorescence microscope (Leica, Wetzlar, Germany). Images were analysed by Komet 5.5 software.

2.7. Statistical analyses

Sigma Stats.3 software (SPSS Inc., Chicago, Illinois, USA) was used for all statistical analysis using one-way ANOVA test and the level of significance was established at 0.05.

3. Results

3.1. Cd content estimation

Cd analysis in root tissues of *A.cepa* and *V. faba* is presented in Table 1. Cd content detected correlated with the Cd concentration in solution and expressed, as µg g^{-1} DW (dry weight). In control set, Cd content was below detectable level in both the plants.

3.2. Measurement of cell death

$CdCl_2$ exposure induced cell death in root cells of both *A. cepa* and *V. faba* (Figure 1). A ~ 2 - 3.5 fold increase in Evan's blue uptake was observed in *A. cepa* and *V. faba*, indicating cytotoxic effect of Cd on plants.

3.3. Examination of antioxidant enzyme activities and MDA concentration

Figure 2A shows GPX activities in the roots of *A. cepa* and *V. faba*, exposed to different concentrations of Cd. GPX activity was highest (92.81 U min^{-1} mg^{-1} protein) at 100 µM of Cd in *A. cepa*; whereas in *V. faba*, the highest activity (179.13 U min^{-1} mg^{-1} protein) was at 200 µM.

Information on CAT activity is given in Figure 2B. In *V. faba* increase in CAT activity was significantly ($p \leq 0.05$) higher over control and other treatment groups. The values were ~ 29 fold higher than control. In *A cepa*, the values of CAT activity increased from 32.63 U min^{-1} mg^{-1} protein in control to a value of 167.00 U min^{-1} mg^{-1} protein at highest Cd concentration.

The effects of Cd on MDA concentration are presented in Figure 3. Lipid peroxidation expressed in terms of MDA formed in root tissues of *A. cepa* and *V. faba* increased significantly ($p \leq 0.05$) with increasing concentration of Cd. The MDA contents in *V. faba* were significantly ($p \leq 0.05$) higher at all concentrations over the treatment groups in *A. cepa*.

3.4. Genotoxicity assay

Mitotic index and micronuclei frequency in *A. cepa* and *V. faba* are presented in Figure 4. With increasing Cd concentration, the mitotic index decreased significantly ($p \leq 0.05$) whereas the frequency of cells with MN increased in both *A. cepa* and *V. faba*. A similar trend was noted in the plants exposed to the positive control EMS (2mM).

Table1. Cd content in roots of *A. cepa* and *V. faba* treated with cadmium chloride (0, 50, 100 and 200 µM). Each value is the mean of three individual samples ± SD; *Significant compared to control at $p \leq 0.05$.

Concentration of CdCl2 (µM)	Cd content in *A. cepa* roots (µgg-1DW)	Cd content in *V. faba* roots (µgg-1DW)
0	BDL	BDL
50	0.052±0.02*	0.102±0.02*
100	0.098±0.01*	0.153±0.02*
200	0.139±0.01*	0.192±0.02*

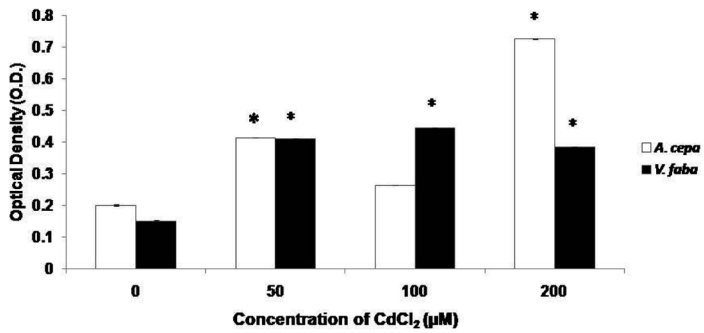


Figure 1. Bar graph showing release of Evan's blue into N, N- dimethyl formamide solution representing induction of cell death induced by cadmium chloride in *Allium cepa* and *Vicia faba* root tissues, using Evan's blue stain; Each value is the mean of three individual samples ± SD; *Significant compared to control at $p \leq 0.05$.

The inhibition of cell division was more pronounced in *A. cepa* than that in *V. faba* roots. In *A. cepa* compared to control, at the highest concentration (200 µM) there was a ~61% reduction in divisional frequency and ~42% reduction in *V. faba*. Such mitotic inhibition reflects toxic effect of the test compound. The number of cells with MN was more or less similar to that observed in *V. faba* and *A. cepa.* The values ranged between 9.7-10.4% in *A. cepa* and 7.54-10.78% in *V. faba* was found at varying concentration of Cd.

Concomitant to MN formation, the chromosomal aberrations were also observed in the root meristematic cells. Figure 5 represents the chromosomal aberrations recorded at different mitotic stages e.g. anaphase- telophase bridges, chromosome laggards and chromosome breaks. Figure 6 represents total aberrations of *A. cepa* and *V. faba* root cells. Compared to control, a total chromosomal aberration recorded at the highest concentration of Cd was 14.24% in *A. cepa* and 23.27% in *V. faba*.

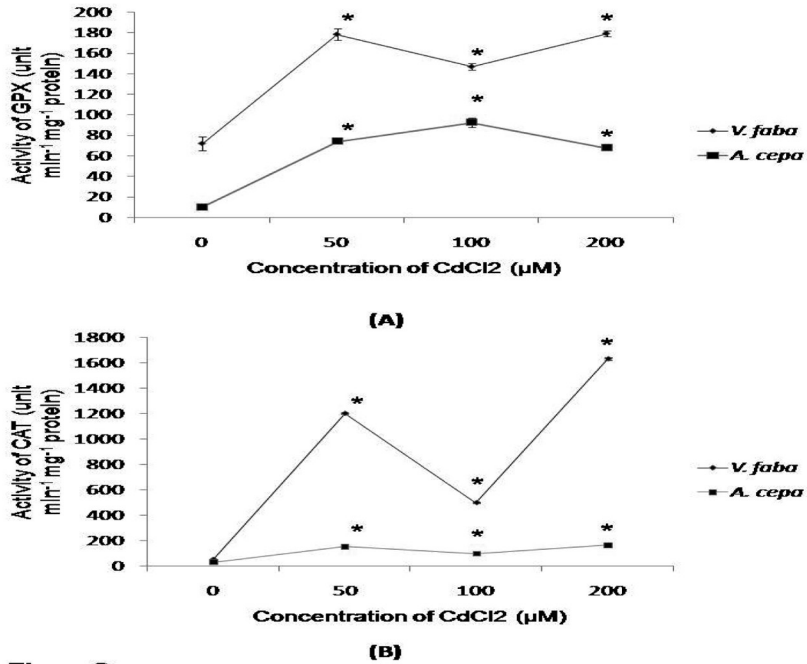


Figure 2. (A) Activity (unit min^{-1} mg^{-1} protein) of Guaiacol peroxidase in roots of *A. cepa* and *V. faba* treated with cadmium chloride; *Significant at $p \leq 0.05$. (B) Activity (unit min^{-1} mg^{-1} protein) of catalase in roots of *A. cepa* and *V. faba* treated with cadmium chloride; *Significant at $p \leq 0.05$.

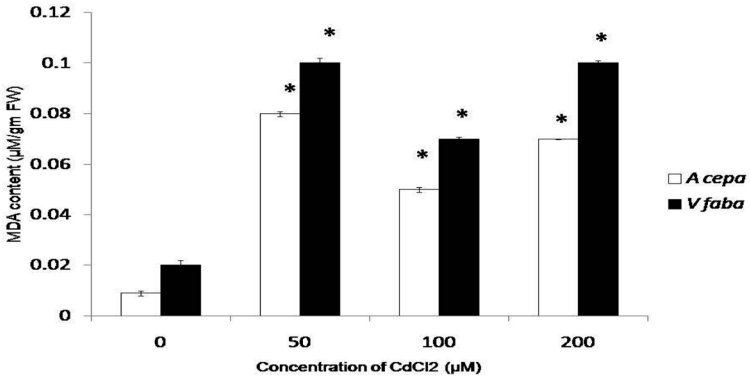


Figure 3. MDA content in root tissues of *A. cepa* and *V. faba* treated with cadmium chloride. Each value is the mean of three individual samples ± SD; *Significant at $p \leq 0.05$.

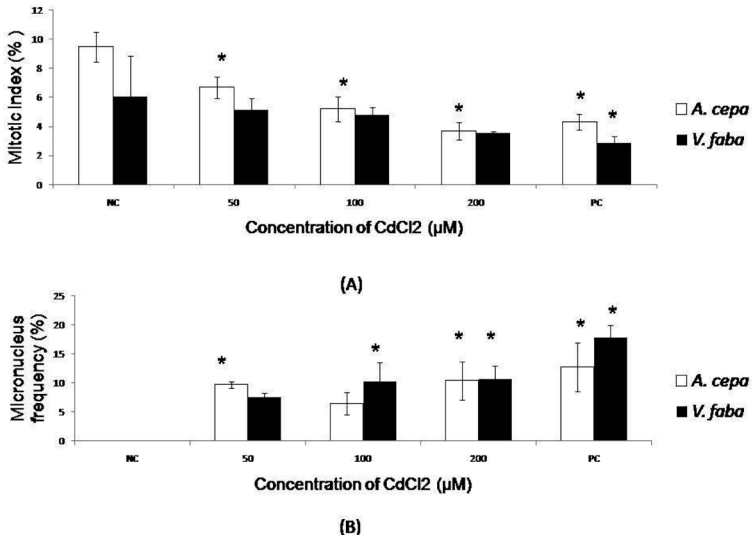


Figure 4. Mitotic index (A) and micronucleus frequency (B) values in *A. cepa* and *V. faba* root tips exposed to cadmium chloride. Values are mean of three samples. The symbol (*) among treatments indicate significant differences at $p \leq 0.05$ [NC, negative control; PC, positive control (2mM EMS)].

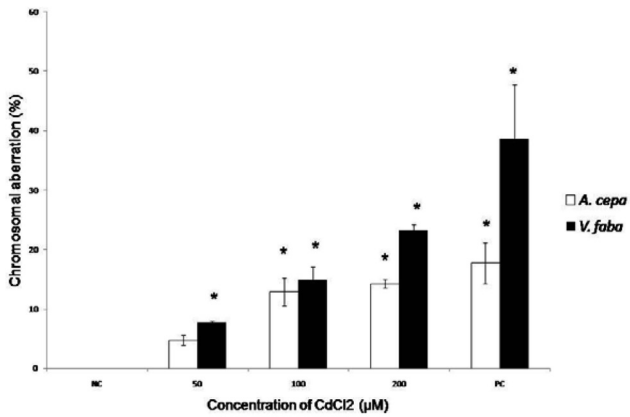


Figure 5. Chromosomal aberrations in root tip cells of *A. cepa* and *V. faba.* Data are presented as the mean ± SD (n = 3); *Significant at $p \leq 0.05$; NC- negative control, PC- positive control (2mM EMS).

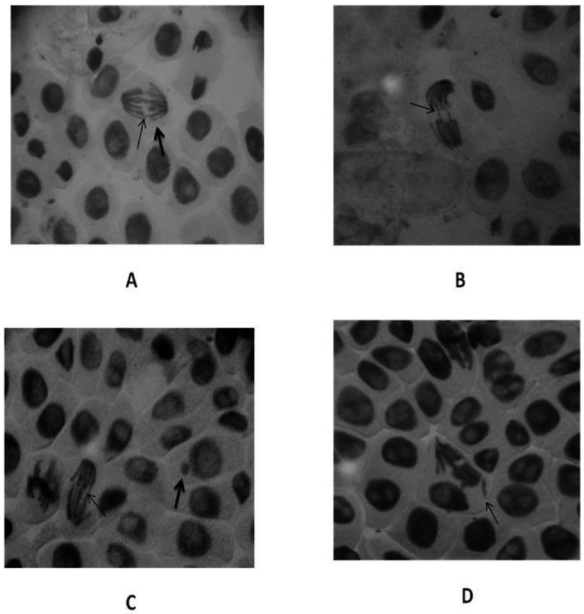
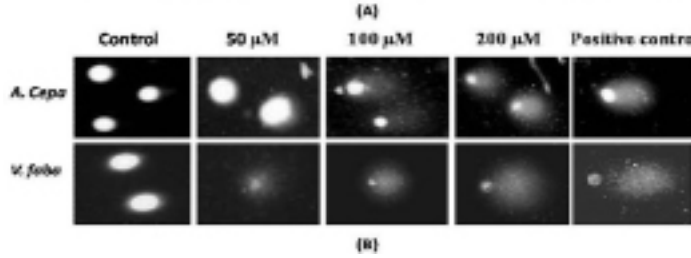


Figure 6. Photomicrographs showing chromosomal aberration in root tip cells of *A. cepa* and *V. faba* treated with cadmium chloride (Magnification= 400X) (A) Chromosomal break (↑) and bridge (↑) (B) Chromosome bridge (↑) (C) Micronucleus (↑) and bridge (↑) (D) Chromosomal laggard (↑)

Concentration (µM)	% Tail DNA	
	A. cepa	*V. faba*
0	2.19 ± 1.32	31.05 ± 2.03
50	11.92 ± 0.64	73.43 ± 3.62
100	16.73 ± 0.83	83.72 ± 0.6
200	21.02 ± 1.12	89.21 ± 0.42
EMS (2mM)	26.45± 1.88	93.21± 0.59

(A)



(B)

Figure7. (A) Comet parameter % tail DNA indicating of extent of DNA fragmentation induced by cadmium chloride in root nuclei of *A. cepa* and *V. faba;* (B) Representative images of *damaged nuclei (100X) in A. cepa and V. faba roots induced by cadmium chloride*

The chromosomal aberrations recorded in the positive control EMS (2mM) were 17.75 and 38.58% in *A. cepa* and *V. faba* respectively. Concerning the genotoxicity parameters (MI, micronuclei frequency and CA), it was found that *V. faba* was more sensitive than *A. cepa* when exposed to EMS and to $CdCl_2$.

The DNA migrations (comet assay) in root meristematic samples are shown in Figure 7. In both the plants, the values of tail DNA percent increased significantly ($p \leq 0.05$) in a dose- dependent manner and was comparable to that induced by EMS (2 mM). The control values of DNA damage as measured by percent tail DNA was considerably high in *V. faba* (31.05%) than that in *A. cepa* (2.19%). Since the comet assay was performed under same conditions, it is possible to conclude that *V. faba* is more sensitive to the $CdCl_2$ exposure than *A. cepa*.

4. Discussion

The primary objective of this study was to assess the sensitivity of different endpoints measured in *V. faba* and *A. cepa* plants exposed to $CdCl_2$ in solution. The research aimed to determine whether, through measuring different endpoints simultaneously in the same protocol (cytotoxicity, MDA content, activity of antioxidative enzymes- guaiacol peroxidase and catalase, chromosomal aberrations, micronuclei formation and DNA damage) it is possible to demonstrate that changes detected can be associated with the sensitivity of the plants.

The decrease in cell division and the increase in Evan's blue uptake observed in the root cells of both *A. cepa* and *V. faba* indicate the cytotoxicity of $CdCl_2$ on the plants. The chromosomal aberrations, micronuclei frequency and DNA breaks demonstrate that Cd had a genotoxic effect. Considering the endpoints performed *V. faba* appeared to be more sensitive than *A. cepa* when exposed to Cd in solution. The total chromosomal aberrations, micronuclei frequency and DNA migration (% tail DNA) recorded were of higher values in *Vicia*. The antioxidant enzyme activities – GPX, CAT and lipid peroxidation expressed in terms of MDA formed were higher in *Vicia* than in *Allium.* Sensitivity to Cd toxicity has been reported among different plant species (Unyayar *et al*., 2006). $CdCl_2$ is known to be highly available for plant and to induce micronucleus in *V. faba* and *A. cepa* (Seth *et al.*, 2008). Seth *et al*. (2008) suggested that exposure to Cd prevented cells entering cell division phases, which then resulted in a decrease in MI. Additionally, the primary action of Cd on the mitotic spindle promoted spindle-related abnormalities such as laggard chromosomes and bridges during cell division, which has been reported in our findings. The mitotic depression caused by heavy metals is found to prevent a number of cells entering the prophase thereby blocking the mitotic phase of the cell cycle.

The molecular mechanism responsible for the genotoxicity of Cd remains largely unclear, it may involve either direct or indirect interaction of Cd with DNA (Valverde *et al.*, 2001). In *Vicia* / tobacco the Cd, induced DNA damage observed in the acellular comet assay after treatment of isolated nuclei without cytosol supports the assumption that the DNA damage induced by Cd is preferentially mediated indirectly (Gichner *et al*., 2004). Apparently, the metabolic products of Cd induced stress in the cytosol i.e. ROS, mainly participate in the production of DNA damage, and the direct binding of Cd with nuclear DNA is of less importance. This metal depletes the cell's major antioxidants, particularly thiol-containing antioxidants and enzymes and thus may cause an increase in production of ROS such as the hydroxyl radical, the superoxide radical or hydrogen peroxide. Enhanced generation of ROS can overwhelm the cell's intrinsic antioxidant defences, and result in a condition known as "oxidative stress". Cells under oxidative stress display various dysfunctions due to lesions caused by ROS to lipids, proteins and DNA (Romero-Puertas *et al.*, 2004). This is in disagreement with the report of Hossain and Huq (2002) according to them Cd

may directly bind with DNA possibly at G, A and T bases. Cd toxicity enhances lipid peroxidation in plant cells, reflected by increased MDA concentration (Chaoui *et al.*, 1997). This suggests that Cd indirectly leads to production of superoxide radicals, resulting in increased lipid peroxidative products and high oxidative stress in *V. faba* than *A. cepa*. Lipid peroxidation induced by Cd may be due to the attack of free radicals on the fatty acid component of membrane lipids. Excessive generation of MDA may be responsible for the higher DNA damage in the treated root cells. This was observed in our study. The enhanced level of lipid peroxidation is one of the major indicators of Cd induced oxidative stress (Chaoui *et al.*, 1997). Cell death or the senescence as evaluated by Evan's blue test is also high in *V. faba* plants (Figure 1). This could be attributed to the induction of high MDA content or lipid peroxidation suggestive of high production of ROS leading to cell death in *V. faba* making it more sensitive towards Cd. Moreover, the loss of membrane integrity, a primary marker of loss of cell viability is in accordance with the observed peroxidation of lipids and is consistent with the increase of cell senescence and death, because of higher exposure to Cd. In the present study, Cd induced lipid peroxidation, resulted in ROS formation. In reponse to a defensive mechanism, antioxidative enzymes, GPX and CAT were induced correspondingly for removing free radicals and scavenging ROS. The significant responses of GPX and CAT activities in *A. cepa* and *V. faba* suggested antioxidative enzymatic system might play an important role in the resistance of plant to Cd stress. Several researchers reported that the increase of antioxidant enzyme activities in response to Cd and other heavy metal exposure might be taken as evidence for an enhanced detoxification capacity of *V. faba* (Cordova Rosa *et al*., 2003), *A. cepa* (Fatima and Ahmad, 2005) and *Allium sativum* (Xu *et al.*, 2008) plants towards ROS that might be generated in stress conditions. It has already been reported that *A. cepa* contains phenol compounds, particularly flavonols like Quercetin-4' glucoside, Quercetin-3, 4' diglucoside etc. which are potential free radical scavenger that might aid tolerance to this plant (Caridi *et al.*, 2007; Prakash *et al.*, 2007). The results in the present study are in accordance with the earlier reports with characteristic differences in Cd accumulation when comparing one vegetable to another, following the order: legumes (Leguminosae) < melon vegetables (Cucurbitaceae) < alliums (Amaryllidaceae and Liliaceae) < root vegetables (Umbelliferae and Cruciferae) < kail vegetables (Cruciferae) < solanaceous vegetables (Solanaceae) < leafy vegetables (Cruciferae, Compositae and Chenopodiaceae). As the plant *V. faba* belongs to Fabaceae or Leguminosae, its place in relation to Cd tolerance comes last.

5. Conclusion

This study accommodated the detailed cytotoxic and genotoxic effects of Cd in higher plants that are also important vegetables. Based on the results of the present study we can conclude that *Vicia* is more sensitive to Cd exposure and caution should be exercised while growing this plant on Cd-contaminated sites. Considering the myriad of endpoints screened, *V. faba* was found to be more sensitive than *A. cepa.* Additional studies on the molecular mechanism that confers such sensitivity should be conducted.

Acknowledgements

The authors are thankful to UGC [F.4-2/2006 (BSR)/ 13- 445/ 2011 (BSR), dt 12/9/2011] for the financial support.

Reference

Aebi, H. 1974. Catalase; Methods in enzymatic analysis. In: H.U. Bergmeyer (eds), Academic press, New York, pp 673–684.

American Public Health Association (APHA). 1998. Standard Methods for the Examina- tion of Water and Wastewater, 20th ed. Washington, DC.

Baker, C.J., Mock, N.M. 1994. An improved method for monitoring cell death in cell suspension and leaf disc assays using Evan's blue. Plant Cell Tissu. Org. Cult. 39, 7–12.

Caridi, D., Trenerry, V.C., Rochfort, S., Duong, S., Laugher, D., Jones, R. 2007. Profiling and quantifying quercetin glucosides in onion (*Allium cepa* L.) varieties using capillary zone electrophoresis and high performance liquid chromatography. Food Chem. 105, 691–699.

Chaoui, A., Mazhoudi, S., Ghorbal, M.H., El Ferjani, E. 1997. Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). Plant Sci. 127,139–147.

Cordova Rosa, E.V., Valgas, C., Souza-Sierra, M.M., Correa, A.X.R., Radetski, C.M. 2003. Biomass growth, micronucleus induction, and antioxidant stress enzyme responses in *Vicia faba* exposed to cadmium in solution. Environ. Toxicol. Chem. 22, 645–649.

Duc, G. 1997. Faba bean (*Vicia faba* L.). Field Crops Res. 53, 99–109.

Fatima, R.A., Ahmad, M. 2005. Certain antioxidant enzymes of *Allium cepa* as biomarkers for the detection of toxic heavy metals in wastewater. Sci. Total Environ. 346, 256- 273.

Fojtova, M., Kovarik, A. 2000. Genotoxic effect of cadmium is associated with apoptotic changes in tobacco cells. Plant Cell Environ. 23, 531–537.

Gichner, T., Patkov´a, Z., Sz´akov´a, J., Demnerova, K. 2004. Cadmium induces DNA damage in tobacco roots, but no DNA damage, somatic mutations or homologous recombination in tobacco leaves. Mutat. Res. 559, 49–57.

Gopalan, H.N.B. 1999. Ecosystem health and human wellbeing: the mission of the International Program on Plant Bioassays. Mutat. Res. 426, 99–102.

Hemeda, H.M., Klein, B.P. 1990. Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. J. Food Sci. 55,184-185.

Hossain, Z., Huq, F. 2002. Studies on the interaction between Cd^{2+} ions and DNA. J. Inorg. Biochem. 90, 85–96.

Karabal, E., Yucel, M., Oktem, H.A. 2003. Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity. Plant Sci. 164, 925–933.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. J. Biol Chem. 193, 265–75.

Ma, T.H. 1999. The International Program on Plant Bioassays Collaborative Studies with Plant Systems. Mutat. Res. 426, 97–98.

Meng, Q., Zou, J., Zou, J., Jiang, W., Liu, D. 2007. Effect of Cu^{2+} concentration on growth, antioxidant enzyme activity and malondialdehyde content in garlic (*Allium sativum* L.). Acta Biol. Craco. Bot. 49, 95-101.

Mogren, L.M., Olssen, M.E., Gertsson, U.E. 2007. Effects of cultivar, lifting time and nitrogen fertiliser level on quercetin content in onion (*Allium cepa* L.) at lifting. J Sci. Food Agri. 87, 470–476.

Mukherjee, A., Gichner, T. 2009. Plant bioassays: comet assay in higher plants. In: D. A. Sampietro and S.S. Narwal (eds). Research Methods in Plant Sciences: Allelopathy. Studium Press, LLC. Houston,Texas, USA. pp: 97-108.

Mukherjee, A., Sharma, A. 1988. Effects of cadmium and selenium on cell division and chromosomal aberrations in *Allium sativum.* Water Air Soil Poll. 37, 433–438.

Nagajyoti, P.C., Lee, K.D., Sreekanth, T.V.M. 2010. Heavy metals, occurrence and toxicity for plants. Environ. Chem. Lett. 8, 199−216.

Navarrete, M.H., Carrera, P., de Miguel, M., de la Torre, C. 1997. A fast comet assay variant for solid tissue cells. The assessment of DNA damage in higher plants. Mutat. Res. 389, 271–277.

Prakash, D., Singh, B.N., Upadhyay, G. 2007. Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). Food Chem. 102, 1389–1393.

Romero-Puertas, M., Rodriguez-Serrano, M., Corpas, F.J., Gomez, M., del Rio, L.A. 2004. Cadmium-induced subcellular accumulation of O^{2-} and H_2O_2 in pea leaves. Plant Cell Environ. 27, 1122-1134.

Seth, C.S., Misra, V., Chauhan, L.K.S., Singh, R.R. 2008. Genotoxicity of cadmium on root meristem cells of *Allium cepa*: cytogenetic and Comet assay approach. Ecotoxicol. Environ. Safety. 71, 711-716.

Unyayar, S., Celik, A., Cekic, F.O., Gozel, A. 2006. Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. Mutagenesis. 21, 77–81.

Valverde, M., Trejo, C., Rojas, E. 2001. Is the capacity of lead acetate and cadmium chloride to induce genotoxic damage due to direct DNA-metal interaction?. Mutagenesis. 16, 265–270.

Xu, P., Zou, J., Meng, Q., Zou, J., Jiang, W., Liu, D. 2008. Effects of Cd^{+2} on seedling growth of garlic (*Allium sativum* L.) and selected physiological and biochemical characters. Bioresource Technol. 99, 6372-6378.

Zhang, S., Zhang, H., Qin, R., Jiang, W., Liu, D. 2009. Cadmium induction of lipid peroxidation and effects on root tip cells and antioxidant enzyme activities in *Vicia faba* L. Ecotoxicol. 18, 814-823.