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Cadmium-induced antioxidant status and sister-chromatid exchanges in *Vicia faba* L.

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Abstract: The effects of cadmium (Cd) on antioxidant responses and sister chromatid exchanges (SCE) were studied in *Vicia faba* L. The activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), and the levels of ascorbate (ASC), glutathione (GSH), hydrogen peroxide, and malondialdehyde (MDA, indicator of lipid peroxidation) were measured. Genotoxicity was evaluated by measuring the changes in the mitotic index (MI) and sister chromatid exchanges (SCE). Increased activities of SOD and CAT were observed in plants treated with 50 and 100 µM Cd. It was found that APX activity increased remarkably with 50 µM Cd, but no significant changes were found in GR activity in any of the treatment groups. These changes in antioxidant enzyme activities were negatively correlated with lipid peroxidation. However, a positive correlation between the increase of H2O2 levels and Cd concentration occurred with all levels of Cd. A significant increase in the malondialdehyde (MDA) level was only observed at the highest (200 µM) Cd concentration. This finding suggests that membrane damage did not occur in moderate Cd frequency. SCE increased significantly in high Cd concentration (200 µM), while MI decreased. The results suggest that exposure of high Cd concentrations is cytotoxic as well as genotoxic for *Vicia faba* L. and also suggest that the 2 phenomena are related.

Key words: Cadmium, antioxidant system, sister-chromatid exchange, *Vicia faba*, genotoxicity

Vicia faba L.'da kadmiyumun uyardığı antioksidan düzeyi ve kardeş-kromatid değişimi

Özet: Kadmiyumun antioksidanın savunma ve kardeş kromatid değişimi üzerine etkileri *Vicia faba* L.'de çalışıldı. Superoksit dismutaz (SOD), katalaz (KAT), askorbat peroksidaz (AP) ve glutatyon redüktaz (GR) aktiviteleri; askorbat (AS), glutatyon (GSH), hidrojen peroksit ve malondialdehit (MDA, lipid peroksidasyonunun indikatörü) seviyeleri ölçüldü. Genotoksik etki mitotik indeks (MI) ve kardeş kromatid değişimi (KKD)'ndeki değişiklikler ölçülerek değerlendirildi. Bitkilere 50 ve 100 µM Cd uygulandığında KAT ve SOD aktivitesinde bir artış olduğu gözlendi. Çalışmada, 50 µM Cd uygulanan bitkilerde AP aktivitesinde belirgin bir artış belirlenirken, uygulama gruplarının hiç birinde GR aktivitesinde anlamlı değişiklikler belirlenmedi. Antioksidan enzim aktiviteleri ve lipid peroksidasyonu arasında negatif bir ilişki olduğu gözlendi. Bunun tersine, tüm Cd uygulamalarında H2O2 seviyelerindeki artış ve Cd konsantrasyonları arasında pozitif bir korelasyon olduğu belirlendi. Malondialdehit (MDA) seviyesinin sadece en yüksek Cd konsantrasyonunda (200 µM) arttığı gözlendi. Bu sonuç, orta düzeyde Cd konsantrasyonunda yüksek antioksidan yanıtların koruyucu bir etkisinin sonucu olarak membran hasarının olmadığını gösterir. Yüksek Cd konsantrasyonunda (200 µM) MI azalırken, KKD frekansı belirgin bir şekilde artmıştır. Sonuçlarımız yüksek konsantrasyonlarda Cd'a maruz kalmanın *Vicia faba* L. için genotoksik olduğu kadar sitotoksik de olduğunu ve bu etkilerin birbiriyle ilişkili olduğunu göstermiştir.

Anahtar sözcükler: Kadmiyum, antioksidan sistem, kardeş kromatid değişimi, *Vicia faba*, genotoksik etki

Introduction

Cadmium (Cd) is a toxic trace pollutant for humans, animals, and plants. It enters the environment mainly from industrial processes and phosphate fertilizers and then is transferred to the food chain (1). The International Agency for Research on Cancer (IARC) classified cadmium as a carcinogen of category 1 (2). In plants Cd inhibits the activities of several photosynthetic enzymes, particularly those involved in the Calvin cycle and chlorophyll biosynthesis (3). Cd can cause oxidative stress by inducing the generation of reactive oxygen species (ROS), such as H_2O_2, $O_2^{\cdot-}$ radicals, and $^{\cdot}OH$ radicals as well as disturbances in the antioxidative systems for the detoxification of ROS (4). Cd is a redox-inactive metal that could weaken the antioxidative protection via depletion of glutathione and/or via generation of ROS (5). The ROS can cause damage to lipids (lipid peroxidation) (6,7,8), proteins, and DNA (6). Gichner et al. (9) showed that Cd at low concentrations induced lesions on DNA. The toxicity of Cd in plants has been widely documented (1,3,7). However, the relationship between Cd toxicity and oxidative reactions has been studied in more detail in the animals and microorganisms. In human keratinocyte HaCaT cell line, Cd led to the inhibition of SOD, CAT, and GPX activities as well as to the induction of GSH (6). In mice, Cd causes inhibition of SOD activity (10). Yang et al. (11) showed that Cd caused time and dose dependent cellular damage in human fetal lung fibroblasts. The damage was mostly like the result of lipid peroxidation. Increased CAT and SOD activities were shown to reduce lipid peroxidation and Cd-induced cell injury.

Superoxide dismutase (SOD) (E.C.:1.15.1.1), ascorbate peroxidase (APX) (E.C.:1.11.1.11), glutathione reductase (GR) (E.C.:1.6.4.2), and catalase (CAT) (E.C.:1.11.1.6) are key antioxidant enzymes that modulate the concentration of reactive oxygen species (ROS), such as H_2O_2, $O_2^{\cdot-}$ radicals, and $^{\cdot}OH$ radicals (4). Superoxide dismutase is the first line of defense against the ROS. It catalyses the dismutation of superoxide radicals to H_2O_2 and oxygen. H_2O_2 is then decomposed by catalase (CAT) and the ascorbate (ASC)-glutathione (GSH) cycle localized in different cell compartments (12). ASC-GSH cycle plays a central role in preventing oxidative damage in cells subjected to abiotic or biotic stresses by equilibrating the redox status (12). ASC is the major primary antioxidant reacting directly with ROS ($OH^{\cdot}$, $O^{\cdot}_2$, and $^1O_2$). It also acts as a secondary antioxidant preventing membrane damage (5). Glutathione is the predominant non-protein thiol and phytochelatin precursor. It functions as a redox buffer (13). In pea plants, Cd treatment caused a decrease in total glutathione (GSH+GSSG) and ASC content (1).

Sister chromatid exchange (SCE) is a reciprocal exchange between 2 chromatids of a metaphase chromosome that can be recognized after an asymmetric substitution of the chromatids with bromodeoxyuridine (BrdU). These exchanges presumably involve DNA breakage and reunion and SCE analysis affords an excellent opportunity for cytological detection of DNA interchange. An increased frequency of SCEs is considered to be an indicator of persistent DNA damage (14). Under natural conditions, SCE occurs spontaneously in human, animal, and plant cells (15,16). However, environmental stress factors can increase SCE frequency (16). Several studies have shown that Cd produces DNA strand breaks, DNA-protein cross-links, oxidative DNA damage, chromosomal aberrations, dysregulation of gene expression resulting in enhanced proliferation, depressed apoptosis, and/or altered DNA repair (17). As depicted by many researchers, it is indicated that the SCE frequency increased in peripheral blood lymphocytes of peoples exposed to occupational or environmental Cd (18,19). Sivikova and Dianowsky (20) reported that the in vivo effects of feeding of metal-containing emissions from refining plants caused the induction of SCE and mitotic delay in cultured sheep lymphocytes.

We previously showed that high concentrations of cadmium induced membrane damage and MN formation in *Vicia faba* (7). Sister chromatid exchange (SCE) is a simple, sensitive, and rapid indicator for evaluating the genotoxic potential of a variety of mutagenic and carcinogenic agents (21). Under natural conditions, SCE occurs spontaneously in human, animal, and plant cells (15,16). However, environmental stress factors can increase SCE frequency (16). Currently, in plants the effects of Cd exposure on SCE is not well known.

The main goal of this study was to determine the phytotoxic effect of Cd on antioxidant status and sister chromatid exchanges in *Vicia faba*.

Materials and methods

Plant cultivation

Commercially provided, equally-sized, and healthy *V. faba* L. seeds were suspended in test tubes containing 0.5 N Hoagland's nutrient solution (22). When the newly emerged roots were at 1.00-2.00 cm in length, 10 seedlings for each treated group were selected and transferred into a container (10 cm tall, 30–20 cm) with 0.5 N Hoagland's nutrient solution at 26/22 °C (day/night) temperature on 65% ± 5% relative humidity in a growth chamber with 480 μmol/m^2 per second photons (day/night 14/10 h) for 10 days. Then seedlings were transferred into a container containing 50, 100, and 200 μM $CdNO_3$ (Merck, purity = 99%) for 48 h. Leaves used in biochemical analyses were flash-frozen in liquid N_2 and stored at -70 °C.

Enzyme assay

Enzyme extraction

Fresh leaves from each treatment were homogenized in a pestle and mortar with 5 mL of 0.1 M potassium phosphate buffer (pH 6.8) containing 100 mg PVP and 0.1 mM EDTA. The homogenate was centrifuged at 16,000 *g* for 5 min and the supernatant was immediately used for analyzing SOD, CAT, and GR.

SOD assay

SOD activity was detected according to the modified method of Beyer and Fridowich (23). Reaction mixtures contained 200 μL of enzyme extract, 100 μL of 5 mM nitro blue tetrazolium (NBT), 150 μL of 0.1 mM riboflavin, 200 μL of 0.25 M methionine, and 1 mL of 200 mM sodium carbonate; these reagents were prepared with 0.1 M potassium phosphate buffer (pH 7.5) except riboflavin, which was prepared with ultrapure water. One unit of SOD activity was defined as the amount of enzyme that was required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm using a UV-VIS spectrophotometer (Perkin-Elmer Lamda EZ-210).

CAT assay

Catalase activity (Catalase; EC 1.11.1.6) was determined by a spectrophotometer in a reaction mixture containing 2.8 mL of 50 mM potassium phosphate buffer (pH 7); 80 μL of 0.5 M H_2O_2 was prepared immediately before the analyse, and then 120 μL enzyme extract was added. CAT activity was measured by monitoring the decrease in absorbance at the rate of decomposition of H_2O_2 at 240 nm, as described by Aebi (24). The activity was determined by the reduction of the absorbance in 30 s.

GR assay

GR (Glutathione reductase; EC 1.6.4.2) activity was detected according to Carlberg and Mannervik (25). Reaction mixture contained 1.5 mL 0.1 M phosphate buffer, 150 μL of 200 mM oxidized glutathione (GSSG), 150 μL of 2 mM NADPH, 1 mL pure water, 200 μL enzyme extract. GR activity was measured by following the change in 340 nm as oxidized glutathione (GSSG)-dependent oxidation of NADPH.

APX assay

Fresh leaves from each treatment were homogenized in a pestle and mortar with 1.5 mL of extraction buffer containing 200 mM HEPES (pH 7.8), 2 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT and 4 mM sodium ascorbate, 2.5 mL of 50 mM potassium phosphate buffer, 25 μL of 500 μM ascorbate, and 50 μL of 1 mM H_2O_2. The homogenate was centrifuged at 16,000 *g* for 5 min and the supernatant was immediately used for analysis. APX (Ascorbate peroxidase; EC 1.11.1.11) activity was detected at 290 nm as a consequence of ascorbate oxidation (26) and the activity was calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1} for ascorbate at 290 nm.

Antioxidant assay

Ascorbate assay

Frozen leaf material (1 g) was homogenized in 0.1 M cooled sodium acetate buffer (pH 3). Homogenate was centrifuged at 7000 *g* for 5 min at 4 °C and the supernatant was collected for analysis of ascorbate (ASC). Total ASC was determined through a reduction of dehydroascorbate to ascorbate by dithiothreitol. Chromatographic separation was performed using an Agilent 1100 HPLC analytical system. Total ASC was separated on a C18 column (250 × 4.6 mm) using a solvent of 0.1 M sodium ascorbate buffer, pH 5 with an isocratic flow of 1.2 $mL.min^{-1}$. The eluates were monitored by an UV detector at 264 nm (27).

Glutathione assay

The total glutathione (reduced form GSH and oxidized form GSSG) was extracted from frozen leaves according to Hawrylak and Szymanska (28). The sample was homogenized in a cold mortar in 2 vol/g 5% 5-sulfosalicilic acid (standard) just before the measurement. Then, 700 µL freshly made buffer (pH 7.5) composed of 143 mM sodium phosphate buffer, 6.3 mM Na_4-EDTA (Merck), 0.248 mg/mL NADPH (Sigma), 100 µL of 6 mM DTNB (Sigma), and 175 µL ultrapure water was warmed in a water bath at 30 °C for 15 min. After homogenization of the leaves, the sample was centrifuged twice at 10,000 *g* for 10 min. The supernatant was used to measure the total GSH content; 25 µL of the supernatant and 5 µL of glutathione reductase from baker's yeast 168 U/mg protein (Sigma) were added to initiate the assay. One minute later spectrophotometric measurements were made at 412 nm. Total GSH content was found from the standard curve made for the reduced form of glutathione (L-GSH, Sigma).

Malonyldialdehyde (MDA) assay

Level of lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) according to Ohkawa et al. (29). Half grams of leaf tissues from control and treated plants were cut into small pieces and homogenized by the addition of 1 mL of 5% trichloroacetic acid (TCA) solution. The homogenates were then transferred into fresh tubes and centrifuged at 12,000 *g* for 15 min at room temperature. Equal volumes of supernatant and 0.5% thiobarbituric acid (TBA) in 20% TCA solution (freshly prepared) were added into a new tube and incubated at 96 °C for 25 min. The tubes were transferred into an ice bath and then centrifuged at 10,000 *g* for 5 min. The absorbance of the supernatant was recorded at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. Twenty percent TCA solution prepared in 0.5% TBA was used as the blank. MDA content was determined using the extinction coefficient of 155 $mM^{-1}cm^{-1}$and expressed as the MDA content in µM g^{-1} FW.

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) levels were measured according to Velikova et al. (30) with minor modifications. Leaf tissues (500 mg) were homogenized in ice bath with 5 mL 2% (w:v) TCA. The homogenate was centrifuged at 12,000 *g* for 15 min and the supernatant was used for H_2O_2 assay. H_2O_2 was quantified with a Bioxytech H_2O_2-560 assay kit (OXIS International, Inc., USA), which is based on the oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}) by hydrogen peroxide under acidic condition. H_2O_2 content was given on a standard curve.

Sister chromatid exchange (SCE) analysis

SCE was assayed according to Gomez-Arroyo et al. (31) with minor modifications. Healthy and equal-sized *V. faba* seeds were germinated between 2 cotton and paper layers soaked with distilled water. The primary roots that reached 2-3 cm were incubated for 20 h in a solution containing 100 µM 5-bromo-2'-deoxyuridine (BrdU), 0.1 µM 5-fluorodeoxyuridine (FdU), and 5 µM uridine (Urd). After rinsing in distilled water for 3 h, roots were transferred into a container containing 50, 100, and 200 µM $CdNO_3$ for 48 h. The double-distilled water treatment was used as the negative control whereas the positive control was 5 mg/mL cyclophosphamide solution. The roots were then left in fresh solutions of BrdU, FdU, and Urd for a second replicate cycle (for 20 h). The treatments were carried out in the dark at 20 °C. The meristems were cut and treated with colchicine (0.05%) for 3 h and stained using the Feulgen differential technique described by Tempelaar et al. (32) with the following minor modifications. Root cuttings after fixing in glacial acetic acid for 1 h were transferred into ethanol/acetic acid (3:1) solution at -20 °C for 2 days. Then they were fixed in 70% ethanol for 15 min and hydrolyzed in 5N HCl for 80 min. After being rinsed in double-distilled water 3 times, they were stained with Feulgen for 12 min in the dark, and then treated with pectinase and cellulase (2.5% each) solution dissolved in 0.01 M citrate buffer (pH 4.7) for 15 min at 28 °C. After being washed with 45% acetic acid for 10 min, they were transferred to 70% cold ethanol for 30 min. The root tips were pounded to a paste using 45% acetic acid. The slides were prepared from suspensions made from this paste. Before scoring, slides were coded. SCEs were scored in 20 metaphase cells for each of the replicate experiments.

Cell kinetic parameter-Mitotic index

Mitotic index (MI) was determined by counting the number of cells in metaphase stage among the total amount of scored cells per seedlings. MI was scored using 3000 cells of 3 separate seedlings for each treatment and control groups. Each experiment was run with 3 replications.

Statistical analysis

Each treatment was replicated 3 times for statistical validity. Data were evaluated by analysis of variance using SPSS for Windows. Multiple comparisons were performed by the least significant difference (LSD) test. $P < 0.05$ was considered as the level of significance.

Results

Effects of Cd^{2+} on the activities of antioxidant enzymes

To determine the antioxidant responses to Cd treatment, changes in antioxidant enzyme activity was measured in the leaf tissue as described in "Materials and methods". Figure 1 shows the responses of SOD, APX, GR and CAT enzymes to 50, 100, and 200 μM Cd.

SOD activity increased considerably in all Cd treated plants (Figure 1a). The difference between control and treatment groups was significant ($P < 0.05$). However, the SOD activity between lower and higher concentrations of Cd treated groups did not show a significant difference. Leaves of plants stressed

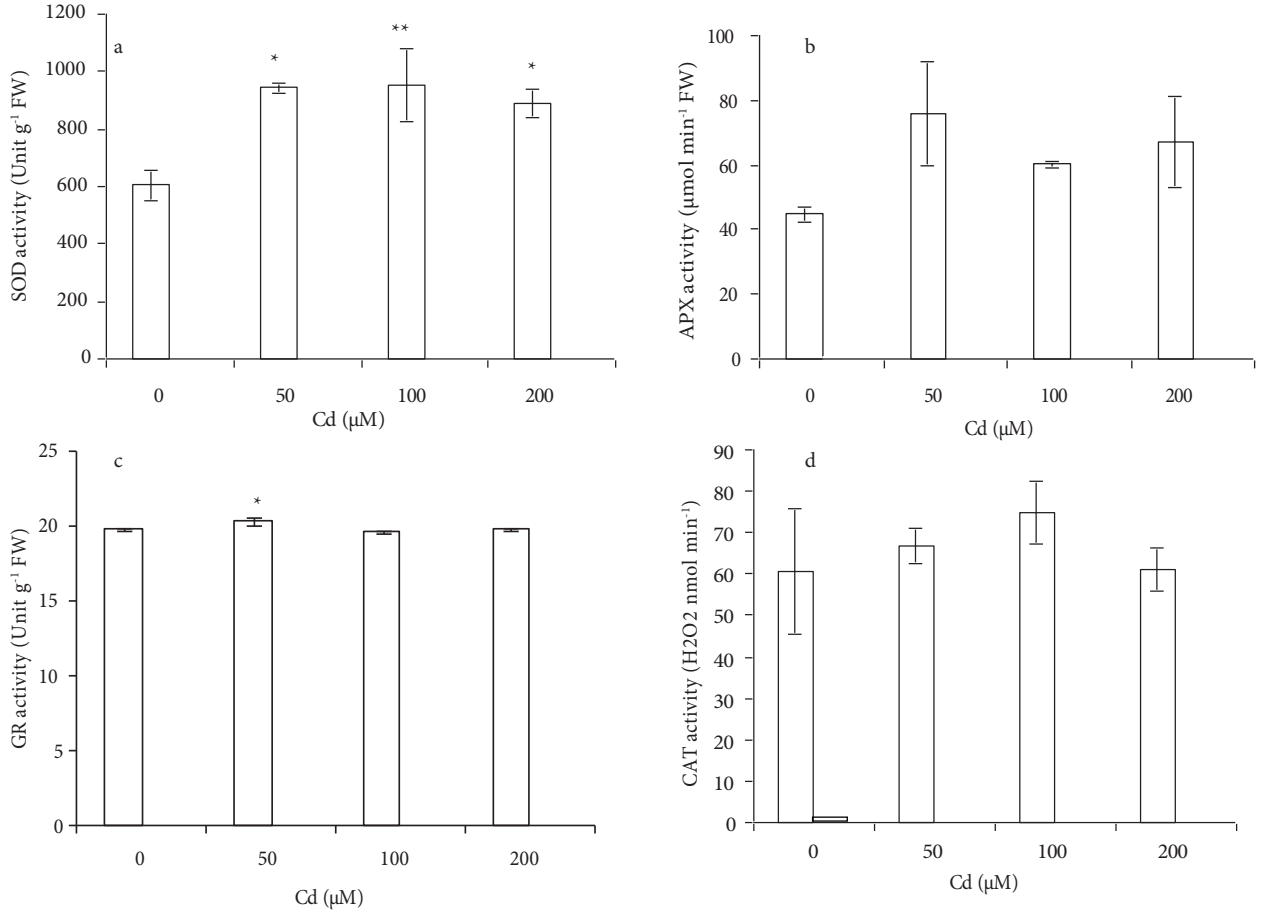

Figure 1. Activities of SOD (a), CAT (b), GR (c), and APX (d) in *V. faba* exposed to Cd. Each data point represents the mean of 3 replicates and its standard error.
* $P < 0.05$, ** $P < 0.01$ compared with negative control.

with 50 μM Cd showed higher APX activity when compared to the other Cd treatments, however no significant difference was observed between control and any of the Cd treatment groups (Figure 1b). No change in GR activity was observed after the Cd treatments (Figure 1c). Low concentrations of Cd caused an increase in the CAT activity compared with the corresponding controls (Figure 1d). The greater increase was observed in plants treated with 100 μM Cd whereas 200 μM Cd treatment did not cause any significant changes in the enzyme activity.

Effects of Cd $^{2+}$ on the level of antioxidants, H_2O_2, and MDA

In order to identify the changes in the levels of antioxidant compounds, ASC and GSH levels were measured. There have been indications that ASC and GSH were involved in cytoprotection against cadmium toxicity. Our results showed that ASC content increased by approximately 90% in 100 μM and 200 μM Cd treated-leaves compared with control ($P < 0.001$) (Figure 2a). The increase in the ASC level was less in 50 μM Cd treated plants compared to the other Cd treatments. The total GSH levels were significantly increased after all Cd treatments ($P < 0.001$) (Figure 2b). There was a positive correlation between Cd concentration and the increase in GSH content.

MDA is the final product of membrane lipid peroxidation and increases when plants are subjected to oxidative stress. MDA content significantly increased in 200 μM Cd treated-leaves, compared with control ($P < 0.001$) while it slightly increased in 100 μM Cd (Figure 2c).

H_2O_2, a product of superoxide dismutase reaction, showed a significant increase in Cd-treated-*V.faba*, compared with control ($P < 0.001$) (Figure 2d). On the other hand, the level of H_2O_2 in Cd-treated plants decreased with the increase of Cd concentration.

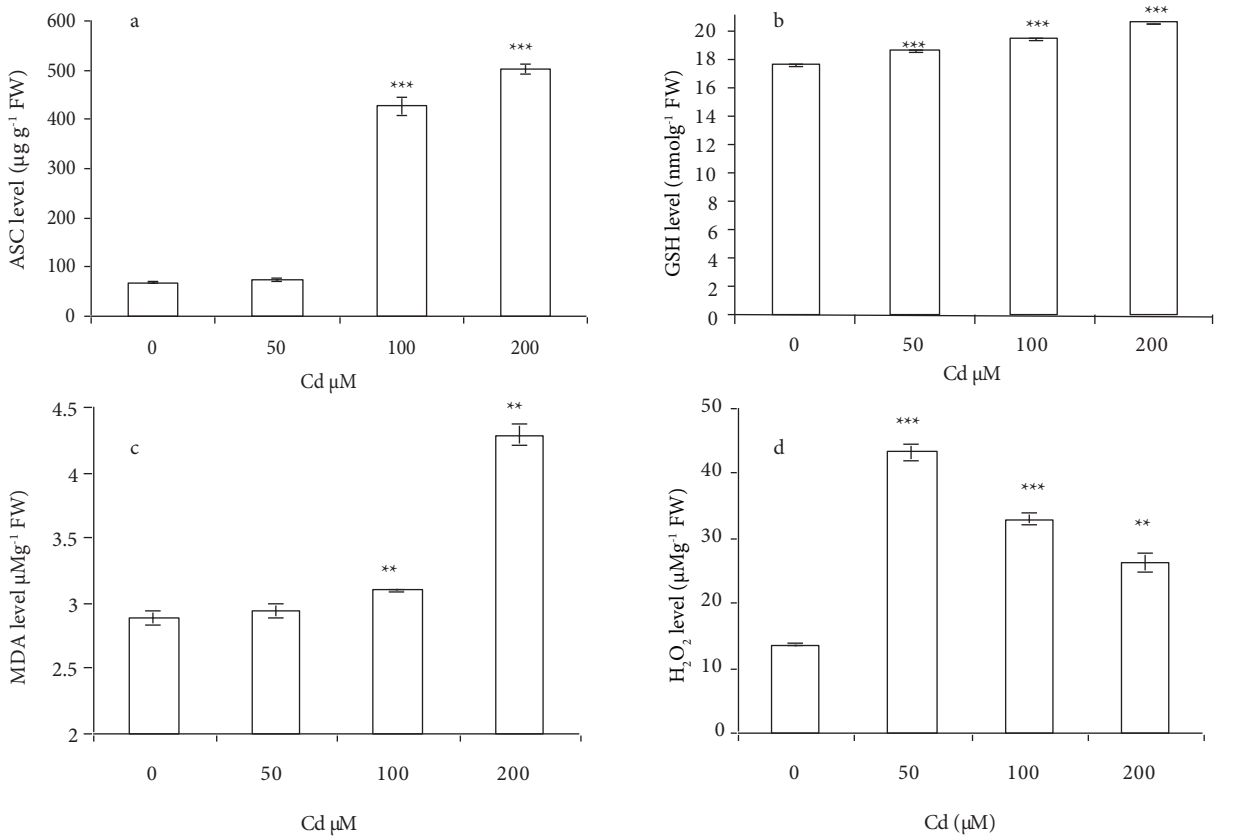

Figure 2. Levels of ASC (a), GSH (b), MDA (c), and H_2O_2 (d) in *V.faba* exposed to Cd. Each data point represents the mean of 3 replicates and its standard error.
** $P < 0.01$, ** $P < 0.001$ compared with negative control.

Effects of Cd $^{2+}$ on the SCE frequency and MI values

Exposure to Cd caused mitotic delay and decreased MI in *V. faba* roots (Table). MI decreased with the increase of Cd concentrations and showed differences among Cd treatments and the negative control ($P < 0.001$). The lowest MI was obtained in 200 μM Cd treated-plants.

The genotoxicity of Cd was evaluated by the SCE assay using *V. faba* root tips (Table). A significant increase in the frequency of SCE in the *V. faba* root cells exposed to 100 and 200 μM Cd ($P < 0.01$ and $P < 0.001$, respectively) was observed. Results were consistent with the results of mitotic inhibition.

Discussion

In our study, Cd toxicity caused antioxidative responses at 50 and 100 μM concentrations via increasing CAT, SOD, and APX activities. There were negative relationships between SOD, CAT, APX, and lipid peroxidation or MDA levels. This may indicate that high activities of antioxidant enzymes could reduce membrane damages by eliminating ROS. Several researchers reported that the increase of antioxidant enzyme activities in response to Cd exposure might be taken as evidence for an enhanced detoxification capacity of *V. faba* (33) and *Allium sativum* (34) plants towards ROS that might be generated in stress conditions. On the other hand, no change in the GR activity was observed after Cd treatment. Exposure to the high concentration (200 μM) of Cd is apparently linked to a reduction of antioxidant activities, whereas exposure to low doses (50 μM and/or 100 μM) is associated with enhanced levels of all antioxidant enzymes probably due to the induction of an adaptive response. Higher concentrations of H_2O_2 level is observed in Cd-treated plants when compared to the control plants while a decrease in the H_2O_2 level is noted when the Cd concentration increased. Depletion of H_2O_2 in the Cd treated plants appears to be a consequence of induced H_2O_2 scavenging machinery. If this radical is not scavenged by antioxidants, it disturbs vital biomolecules (33). It is suggested that CAT and APX may play the most important role in H_2O_2 detoxification (3,4). Romero-Puertas et al. (1) demonstrated that the CAT mRNA by the metal treatment could be induced by the higher H_2O_2 production in pea leaves. Cd-dependent H_2O_2 production of *V. faba* is in agreement with the results obtained with other species subjected to different Cd treatments (5).

The ASC-GSH cycle is another important antioxidant mechanism involved in H_2O_2 detoxification. ASC-GSH cycle is composed of APX and CAT, and by GSH and ASC (1,11). APX uses ascorbate as the electron donor for the reduction of this radical. This enzyme is activated in water-water and glutathione-ascorbate cycles (1). In our experiment after the treatment with high Cd, the level of total ASC was elevated, suggesting the involvement

Table. MI and frequency of SCE in *V. faba* after 48 h $CdNO_3$ treatments. Each data point represents the mean of 3 replicates and its standard error.

Test substance	Concentration	SCEs/metaphase (mean ± SE)	MI (mean ± SE)
Negative Control (Hoagland's nutrient Solution)	0	6.26 ± 0.29	11.80 ± 0.11
Cadmium nitrate (μM)	50	6.63 ± 0.3	9.70 ± 0.26***
	100	7.43 ± 0.04**	7.16 ± 0.32***
	200	7.90 ± 0.07***	1.16 ± 0.03***
Positive control (Cyclophosphamide, μg/mL)	5	12.11 ± 0.06***	2.36 ± 0.31***

** ($P < 0.01$) and *** ($P < 0.001$) compared with negative control.

of ASC in ROS detoxification. Participation of ASC in the non-enzymatic quenching of ROS in Cd-treated barley was reported (5). GSH serves as a precursor in the phytochelatin biosynthesis (5,1). It is also a hydrogen (H) donor for the GR (5). Several levels of metal-induced depletion of GSH have been reported in different plant species (1). In the present study, GSH increased with increasing Cd concentrations. The abiotic stress-induced increase in GSH level may reflect an adaptive capacity of cells to resist to high level of Cd exposure. Nzengue et al. (6) reported increase of GSH in Cd exposured-human HaCaT cells and also Kocsy et al. (12) reported increase of GSH in water stressed-maize. Lipid peroxidation is linked to the activity of antioxidant enzymes, such as SOD, APX, GR, and CAT. Increased antioxidant enzyme activity enhances oxidative stress tolerance, which causes a decrease in MDA (7). We observed low MDA levels when antioxidant enzyme activity was high. Several researchers also reported that there is a relationship between MDA and antioxidant enzyme activity (7,33,35). In a previous study, it was mentioned that Cd treatment caused a decrease in mitotic index (MI) (7,36). High Cd concentration might delay mitosis by damaging the transport mechanism because of high lipid peroxidation. The increase of SCE frequency was observed in human (16) and in *V. faba* (31) after treatment with cadmium. In the present study, 100 and 200 mM Cd treatment caused an increase in SCE frequency whereas low Cd concentration caused no effect. This might be linked to the increase of antioxidant enzyme activities and constant MDA levels in low Cd concentration when compared with control. Cadmium intoxication induces cell death (4). Cd affects several cellular compartments by inducing mitochondrial dysfunction and ROS, oxidatively generated damage to DNA, membranes and proteins (1), and a decrease in cellular antioxidants (4). Intracellular signaling as well as apoptotic pathways is clearly impaired after Cd exposure. Concerning Cd-induced mutagenesis, it appears that Cd has cytotoxic effects inducing DNA damage and inhibiting its repair (6). It is indicated that high cytotoxic Cd concentrations are associated with strand break in DNA, chromosomal aberations, and SCE and DNA-protein binding failures in several types of mammalian cells (37)

In conclusion, our results suggest that Cd is both cytotoxic and genotoxic in *V. faba* L. Moreover, antioxidative enzymes (SOD, CAT, APX, and GR) and antioxidants (ASC and GSH) may play important roles in the protection of Cd-exposed *V. faba* from genotoxic effects of this metal. Further studies of the mechanism(s) of Cd-induced SCEs in relation to the responses of the antioxidative systems may allow new insights into how the mutagenic effects of Cd may be modified by plant adaptation mechanisms in Cd contaminated environments.

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