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Sequential effects of cadmium on genotoxicity and lipoperoxidation in *Vicia faba* roots

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Abstract Kinetics of stress responses to Cd exposure (50, 100 and 200 μM) expanding from 12 to 48 h were studied in roots of hydroponically cultivated-*Vicia faba* seedlings. The heavy metal induced toxicity symptoms and growth arrest of *Vicia* roots gradually to the Cd concentration and duration of the treatment. The intracellular oxidative stress was evaluated with the H_2O_2 production. The H_2O_2 content increased gradually with the sequestered Cd and root growth inhibition. Lipid peroxidation—evidenced by malondialdehyde (MDA) content and Evans blue uptake—and genotoxicity—evidenced by mitotic index (MI) and micronuclei (MCN) values—were concomitantly investigated in root tips. By 12 h, root meristematic cells lost 15% of their mitotic activity under 50 or 100 μM Cd treatment and 50% under 200 μM Cd treatment and led cells with MCN, while the MDA content and Evans blue absorption were not affected. The loss of membrane integrity occurred subsequently by 24 h. The increase in MDA content in root cells treated with 50, 100 and 200 μM Cd was significantly higher than the control. By 48 h, the MDA content increased 134, 178 or 208% in root cells treated with 50, 100 and 200 μM Cd, respectively. The Evans blue absorption was also affected by 24 h in roots when treated with 200 μM Cd and gradually increase by 48 h with the Cd concentration of the treatment. The decrease of mitotic activity triggered by 12 h was even higher by 24 h and the MI reduced to 44, 56 or 80% compared to the control in the three different Cd concentrations tested. The different kinetics of early in vivo physiological and cytogenetic responses to Cd might be relevant to the characterization of its toxicity mechanisms in disrupting primarily the mitosis process.

Keywords Cadmium (Cd) · Genotoxicity · H_2O_2 production · Lipid peroxidation · *Vicia faba*

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Introduction

Cadmium (Cd) is one of the most toxic environmental and industrial pollutants. With a long biological half life, its concentration can reach 345 mg kg^{-1} in contaminated soils (White and Claxton 2004; He et al. 2005). The ability of plants to uptake and accumulate heavy metals makes Cd a potential threat to human health through its entrance into the food-chain.

As yet, it has been demonstrated that Cd has no biological function in plants. The mechanisms by which plants cope with its excess have been largely studied and reviewed (DalCorso et al. 2008; Verbruggen et al. 2009). Cd is suspected to exert its toxic action on cells through oxidative damage. The transition metal is unable to directly generate reactive oxygen species (ROS) via biological redox reactions with molecular oxygen because of its redox potential (−820 mV), however it can indirectly favor the production of different ROS. It has been reported that Cd causes a series of three waves of active oxygen species generation, first the NADPH oxidase-dependent accumulation of hydrogen peroxide, followed by the accumulation

of superoxide anions in mitochondria, and finally fatty acid hydroperoxide, as detected in tobacco cells (Garnier et al. 2006). The enhanced level of lipid peroxidation and hydrogen peroxide concentration in plant tissues are the major indicators of Cd-induced oxidative stress (Dixit et al. 2001). The peroxidation of unsaturated lipids can be monitored by the malondialdehyde (MDA) content which is the ultimate product of lipid peroxidation damage by free radicals. As reported earlier, MDA might be highly affected by Cd (Zhang et al. 2009).

The Cd-induced DNA damage lies in the fact that overproduction of ROS could induce genotoxicity events (Lin et al. 2007). Oxidative DNA damages can produce a multiplicity of modifications in DNA including base and sugar lesions, strand breaks, DNA–protein crosslinks and base-free sites (Gebicki and Gebicki 1999; Roldán-Arjona and Ariza 2009). Besides direct oxidation, DNA bases may be also indirectly damaged through reaction with reactive products generated by ROS attack to lipids. Lipid peroxidation-induced aldehydes are mutagenic molecules and create MDA–guanines adducts (Mancini et al. 2006; Roldán-Arjona and Ariza 2009).

The Cd-induced genotoxic events have been well studied in *Vicia* species (Ünyayar et al. 2006; Zhang et al. 2009). *Vicia* karyological characteristics i.e. low number and long chromosomes, make the observations of nucleus damage substantially easy. This species is also highly sensitive to heavy metals with formation of extra-nuclear DNA entity (micronucleus) in mitotic cells. Micronuclei are formed by exclusion of whole chromosomes or chromatin fragments during cell division (Krishna and Hayashi 2000; Çava and Ergene-Gözükara 2003). Micronucleus formation is the most effective and simplest indicator of DNA damages. They are easily detected in vitro using root tip micronucleus assay (Ma et al. 1995; Türkoğlu 2007; Abdel Migid et al. 2007).

Many reports showed the deleterious action of Cd on mitotic activity resulted in the progressive reduction of root growth rate. Cd provokes clearly the appearance of chromosome aberrations in the apical meristems and inhibition of cell enlargement in elongation zones (Liu et al. 2003/2004; Fusconi et al. 2006, 2007). Since its oxidative stress inducing-action produce chromosome breaks, the potential toxicity of the ion itself lead to aneugenicity. Its direct interaction with the multi-cysteine protein such as tubulin, or indirect action on calmodulin-dependent motor proteins make this ion a potential microtubule architecture disrupter generating chromosome migration impairment in mitotic cells (Voutsinas et al. 1997; Fusconi et al. 2007).

The aim of the work was to determine sequentially the lipid and DNA damages triggered by Cd with regards to metal and H_2O_2 intracellular contents. This deleterious phenomenon imposed us to assess the kinetics responses of hydroponic-cultivated *Vicia faba* roots to Cd treatment. Our results showed that the genotoxicity events at chromosomal level occurred soon after Cd exposure (by 12 h) and prior to lipid peroxidation. The sequential events involved in the loss of plasma membrane integrity and disruption of cell division might shed light on the mechanisms of Cd toxicity.

Materials and methods

Plant material and treatments

Seeds of *V. faba* var. Aguadulce were surface sterilized with 10% sodium hypochlorite, rinsed several times with water and placed on moistened filter paper at 25°C for 4–5 days. The germinated seeds were then transferred in hydroponic conditions as described by Souguir et al. (2008). In order to determine growth disruption threshold under Cd stress in *Vicia,* we observed plant responses in a range of Cd concentrations and incubation time. Moreover, by using a transient period we were able to determine the early responses of the plant towards the Cd pollutant. The hydroponic nutrient solution was supplemented with 50, 100 or 200 µM $CdCl_2$ at 12-day-old seedlings for 12, 24 or 48 h. The primary root length was measured with a decimeter scale.

Cadmium content in roots

Root tissues were ground and digested in 65% nitric acid (1 ml per 0.1 g of dry matter). The digested material was resuspended in distilled water and Cd contents were determined using a Perkin Elmer atomic absorption spectrophotometer.

Membrane damages

Cell integrity was measured independently on three *Vicia* root tips. The first centimeter of the primary roots was incubated in 0.025% Evans Blue (m/v) for 30 min, rinsed for 15 min and squashed in 800 µl solution containing 50% MeOH (v/v) and 1% sodium dodecyl sulphate (SDS). The root extracts were then incubated for 15 min at 50°C, the supernatant was clarified by centrifugation at 14,000 rpm for 15 min and the absorbance measured at 600 nm.

Lipid peroxidation

Lipid peroxidation was estimated using MDA content evaluation (Chaoui et al. 1997). Root tissues (1 g) were homogenized in 10% (w/v) TCA (10 ml) and centrifuged at 10,000 rpm for 10 min. An equal volume of 10%

trichloroacetic acid (TCA) solution containing 0.5% 2-thiobarbituric acid (TBA) was added to the supernatant. The sample was incubated at 95°C for 30 min, cooled quickly in an ice-bath and centrifuged at 10,000 rpm for 15 min. The absorbance was measured at 532 nm and corrected for nonspecific absorbance at 600 nm. The concentration of MDA was calculated using 155 mM^{-1} cm^{-1} as extinction coefficient.

Mitotic activity and aberrations

To monitor the genotoxic effects of Cd on *V. faba* root cells, we incubated 12, 24 or 48 h seedlings in 50, 100 or 200 μM Cd. The primary root tips were cut and placed overnight in the Carnoy fixation solution containing ethanol and glacial acetic acid (3:1) at 4°C and then stored in 70% ethanol in the dark. After repeated washings in distilled water, root tips were hydrolyzed with 1 N HCl for 10 min. The root cap was removed and root meristematic tissues were stained with orcein, squashed on slides and examined under Zeiss microscope. The mitotic index (MI) was determined by counting the number of mitotic cells among the total amount of scored cells (~3,000) per root. Micronuclei (MCN) frequency was calculated from the number of MCN scored divided by the total cells scored and expressed in terms of MCN per 1,000 interphase cells. Three root meristems were stained per replica. Therefore the analysis was conducted on an average of 9,000 cells per treatment. MI and MCN as well as aberrant mitosis frequencies were measured on the same slide. The following criteria for MCN analysis were used in *V. faba* root tip cells (Tolbert et al. 1992). MCN should: (i) be almost one-third the diameter of the main nucleus; (ii) be on the same plane focus; (iii) have a chromatin structure similar to that of the main nucleus; (iv) be smooth, oval or round shape; and (v) be clearly separated from the main nucleus.

H_2O_2 determination

The intracellular H_2O_2 content was determined according to Sergiev et al. (1997). Roots tissues (500 mg) were homogenized at 4°C with 5 ml 0.1% (w/v) trichloroacetic acid and centrifuged at 12,000 rpm for 15 min. An aliquot (0.5 ml) of the supernatant was added to a solution containing 10 mM potassium phosphate buffer (pH 7.0) (0.5 ml) and 1 M potassium iodide (1 ml). The absorbance was measured at 390 nm and the content of H_2O_2 was calculated based on a standard curve using gradual H_2O_2 concentrations.

Statistical analysis

The results presented are the values ± standard error obtained from at least three replicates. Significant differences between treated and control plants are determined using ANOVA test ($P < 0.05$).

Results

Cd induces root toxicity symptoms

By using a transient period we were able to determine the early physiological responses of *Vicia* plants towards the Cd pollutant. Since high Cd concentration (>200 μM) and prolonged incubation of Cd treatment (>48 h) resulted in unhealthy plants with thick and short roots unsuitable (data not shown) for cytological analysis, we conducted experiments under moderated Cd stress and shorter periods of incubation. Therefore the metallic treatment was conducted over a transient period (up to 48 h) on *V. faba* seedlings by adding a range of Cd concentrations (50–200 μM) in hydroponic media. Cd incubation for 48 h induced the well known high toxicity symptoms on *Vicia* roots consisting in strong brownishing, and thickening growth and branching inhibition (Fig. 1). Early brownishing effects were observed even at the lowest concentration tested at 48 h of incubation. The lateral roots were also affected in terms of size and color (Fig. 1). Root growth arrest was insignificant during 12 h incubation in the three metallic concentrations (50, 100 or 200 μM) (Fig. 2). Nevertheless longer period of Cd exposure (24 or 48 h) showed a significant arrest of root growth. A dramatic decrease of the root primary axis—60% on average—was detected in 200 μM Cd treated seedlings.

Vicia roots accumulated highly the metal. Cd in shoot tissues was dramatically lower (25–50 times less) than the one measured in root tissues during the treatment (data not

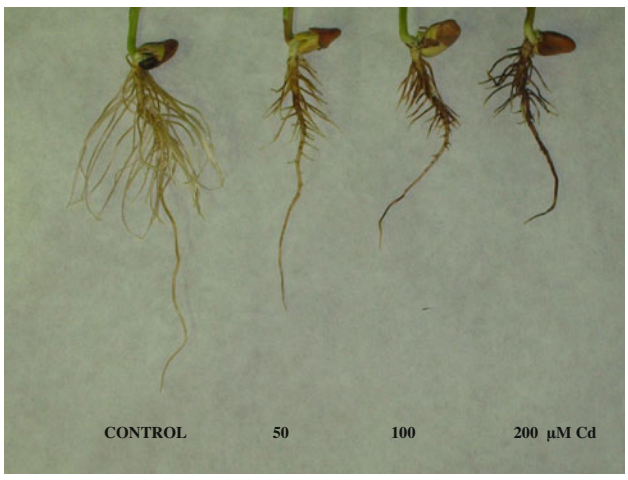

Fig. 1 Toxicity symptoms in roots of hydroponically cultivated seedlings of *V. faba* var. Aguadulce treated with different concentrations of Cd for a period of 48 h

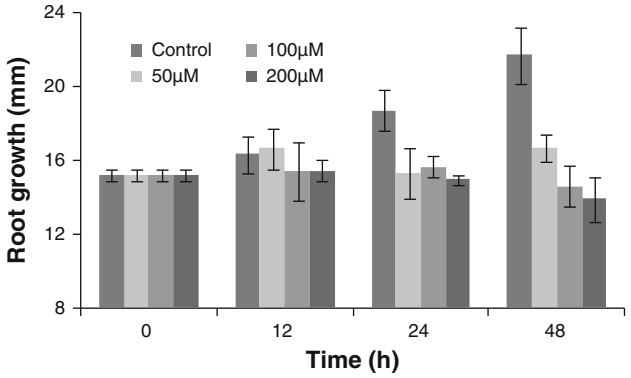

Fig. 2 Root growth consisting in measuring primary root length of hydroponically cultivated seedlings of *V. faba* var. Aguadulce treated with different concentrations of Cd. Data represent mean and ±SD of five independent experiments

shown) suggesting low translocation of the metal to the aerial part. Cd accumulated proportionally to the exogenously supplied concentration in roots and this was related to the prolonged period of the metallic treatments (Table 1). The results showed that the root growth reduction was independent to the Cd intracellular concentrations in *Vicia* plants (Table 1) at the beginning of the experiment (12 h). An apparent delay was observed between the Cd content detected in the treated seedlings and the root growth inhibition. Cd content in 12 h treated roots might not be high enough to significantly inhibit root elongation. With longer treatments (>12 h), gradually accumulated Cd might affect root elongation.

Kinetic of membrane damages and H_2O_2 production

We measured H_2O_2 accumulation in *Vicia* roots over the 48 h of Cd treatment (Table 2). High H_2O_2 overproduction in the root cells was observed within 12 h. The oxidative burst was triggered in all three metallic treatments and increased during the entire period of the treatment. At the end of the metallic treatment, 50 µM Cd induced moderately

Table 1 Cd content in roots of hydroponically cultivated *Vicia* plants exposed for 12, 24 and 48 h to different concentrations of exogenous Cd

Cd content (µg · g^{-1} DW)			
Exposure	12 h	24 h	48 h
Control	30.5 ± 2.6	31.5 ± 10.4	34.3 ± 6.5
$CdCl_2$ (µM)			
50	427.5 ± 27.9***	529.8 ± 33.8***	745.7 ± 60.5***
100	710.7 ± 79.5**	946.3 ± 48.1***	1262.2 ± 82.2***
200	1050.5 ± 90.9**	1467.6 ± 133.9***	2194.5 ± 411.9**

Data represent mean ± SD of triplicates

** $P < 0.01$, *** $P < 0.001$ compared to control

Table 2 H_2O_2 accumulation in roots of hydroponically cultivated *Vicia* plants treated for 12, 24 and 48 h with different concentrations of Cd

H_2O_2 accumulation (µmol · g^{-1} FW)			
Exposure	12 h	24 h	48 h
Control	0.037 ± 0.02	0.061 ± 0.01	0.084 ± 0.02
$CdCl_2$ (µM)			
50	0.091 ± 0.01**	0.102 ± 0.01***	0.109 ± 0.03***
100	0.101 ± 0.01**	0.163 ± 0.02***	0.279 ± 0.03***
200	0.165 ± 0.02***	0.252 ± 0.04***	0.359 ± 0.07***

Data represent mean ± SD of triplicates

** $P < 0.01$, *** $P < 0.001$ compared to control

H_2O_2 production (130% higher than the control) while 100 and 200 µM Cd induced a dramatic increase of the root H_2O_2 content (333 and 428% higher than the control, respectively).

A major effect of heavy metal toxicity is the chemical modification of membrane lipids triggered by the oxidative stress. We investigated a biomarker indicative to lipid peroxidation (LPO) status of the cells and measured MDA content that is the major product of LPO (Fig. 3). The pattern of MDA accumulation in treated seedlings was inversed compared to the one observed for root growth. The MDA content was not significantly affected within 12 h independently to the exogenous Cd concentration. A significant increase was apparent within 24 h and MDA accumulation reached the highest level at the end of the treatment. At 48 h, the MDA content increased 134, 178 or 208% in root cells treated with 50, 100 or 200 µM Cd, respectively.

Low Cd concentrations (50 or 100 µM) did not affect significantly the Evans blue absorption within the 12 or 24 h treatment (Fig. 4). However, prolonged incubation

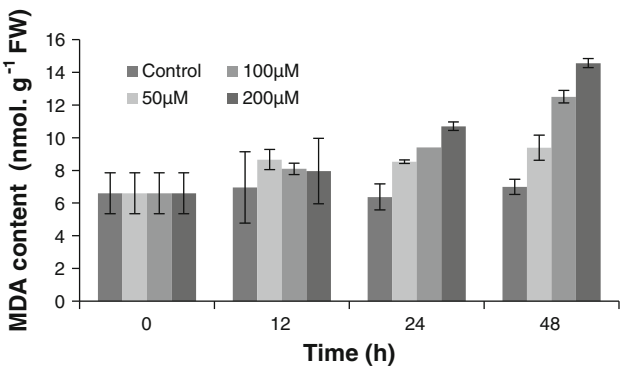

Fig. 3 Kinetic of MDA accumulation in roots of hydroponically cultivated seedlings of *V. faba* var. Aguadulce treated with different concentrations of Cd. Data represent means and ±SD of three independent experiments

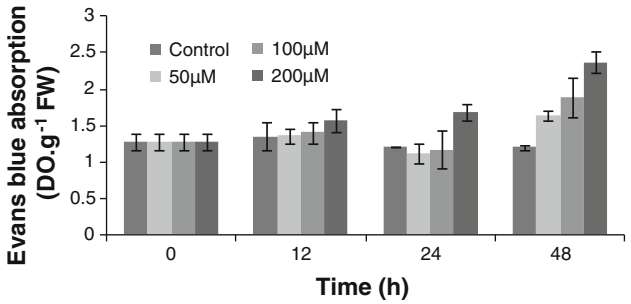

Fig. 4 Plasma membrane integrity as determined by Evans blue absorption of root extracts obtained from hydroponically cultivated seedlings of *V. faba* var. Aguadulce treated with different concentrations of Cd. Data represents means and ±SD of three independent experiments

periods (48 h) induced higher losses of plasma membrane integrity. The highest Cd concentration affected significantly the Evans blue absorption within 24 h treatment. Nevertheless, the apparent loss of plasma membrane integrity was detected in roots treated for 48 h at 200 µM Cd.

Kinetic of genotoxicity activity

Figure 5 depicts the effect of Cd treatments on MI. The MI was affected drastically as early as 12 h after the onset of the heavy metal treatment for all three Cd concentrations. The root meristems lost 15% of their mitotic activity under 50 or 100 µM Cd treatment and 50% under 200 µM Cd treatment. For the rest of the period treatment, the MI was drastically affected. Cd reduced severely mitotic activity at 48 h incubation. MI reduced to 44, 56 or 80% compared to the control in the three different Cd concentrations tested (50, 100 or 200 µM, respectively). These results show that the reduction on MI was proportional to Cd concentration and incubation periods.

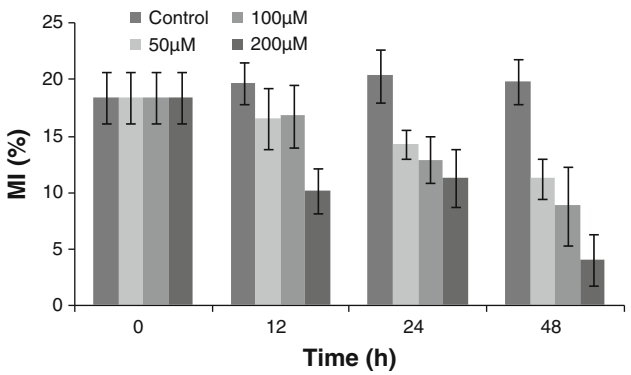

Fig. 5 MI in root meristems of hydroponically cultivated seedlings of *V. faba* var. Aguadulce treated with different concentrations of Cd. Data represents means and ±SD of three independent experiments

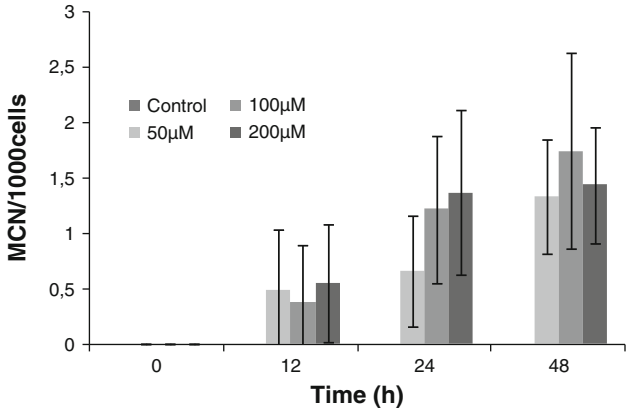

Fig. 6 MCN frequency in root meristems of hydroponically cultivated seedlings of *V. faba* var. Aguadulce treated with different concentrations of Cd. Data represents means and ±SD of three independent experiments

The abnormalities observed during mitotic activity were also evaluated with micronucleus (MCN) detection. Figure 6 displays the MCN frequency recorded in *Vicia* root meristems. The frequency of MCN formation enhanced significantly in Cd-treated root tips. MCN induction was detected significantly ($P < 0.05$) within 12 h and turn to be proportional to the time of incubation since the highest frequency of cells with MCN was detected at the end of the treatment. The lower MCN formation detected at 48 h incubation in the presence of 200 µM Cd could be attributed to the dramatic loss of mitotic activity (20% of the control mitotic activity) in this heavy-metal treatment condition.

Concomitant to MCN formation, the chromosomal aberrations were also observed in the root meristematic cells. The genotoxic activity of Cd is shown on micrographs of *V. faba* root cells. Figure 7 depicts MCN and different type of chromosomal aberrations induced by Cd in the root tips. Various chromosomal abnormalities recorded at different mitotic stages e.g., double MCN, sticky chromosomes, anaphase bridges, chromosome laggards and chromosome breaks were all induced by Cd treatments.

Discussion

Plant growth and architecture is based on two interactive parameters: cellular division and enlargement. The first is generally detected in apical meristems. The latter is usually manifested either by topical or throughout cellular enlargement at different sites of the plant body. Both rely on proteins confronting the mitotic apparatus and the cytoskeleton.

Our results confirmed the high sensitivity of *V. faba* to Cd. The root growth reduction was observed at ≥50 µM,

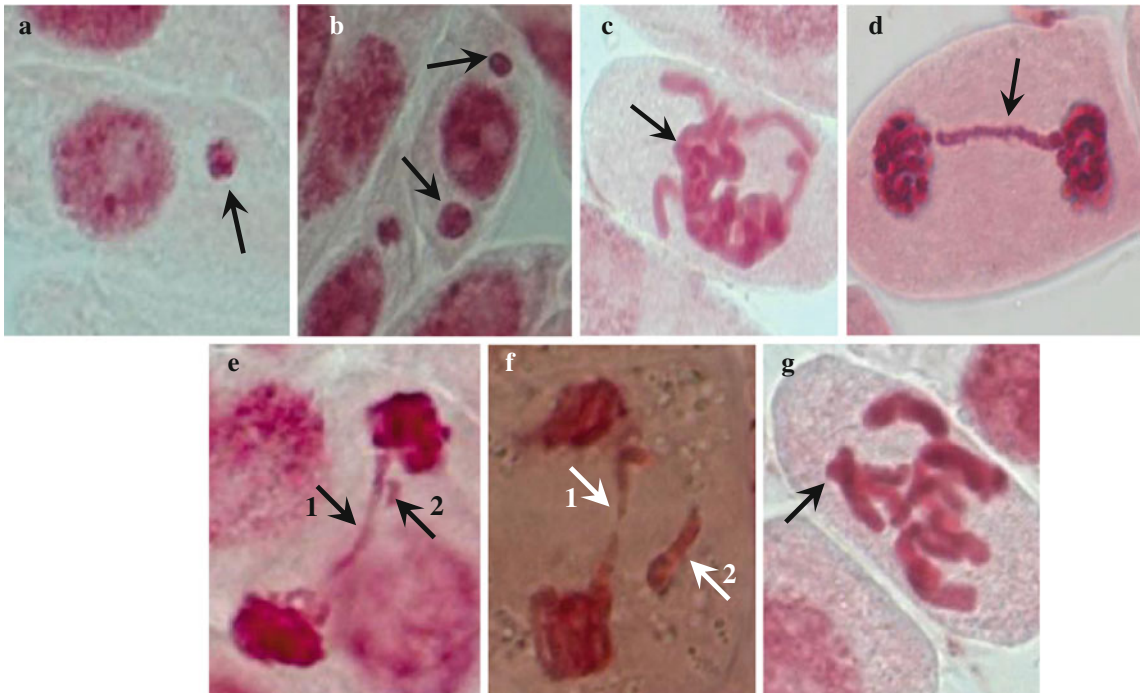

Fig. 7 Cytological analysis of chromosomal aberrations in *Vicia* root meristems of hydroponically cultivated seedlings of *V. faba* var.Aguadulce treated for 24 h (**b**) and 48 h (**a**, **c**–**g**) with 50 µM Cd (**a**, **b**, **e**, **f**) and 200 µM Cd (**c**, **d**, **g**). **a** Micronuclei in interphase; **b** cell with two micronucleus; **c** sticky chromosome; **d** chromosome bridge in anaphase; **e** chromosome bridge in anaphase (*1*), chromosome break (*2*); **f** chromosome bridge in anaphase (*1*), isolated chromosome (*2*); **g** laggard chromosome in metaphase

24 h after Cd treatment and the metal retarded the root growth and changed the architecture. The brownishing of primary and lateral roots were related to ROS production within the root cells. Primary axis and lateral roots become shorter and larger than those in controls. These data are in agreement with previous results showing a positive relation between the rate of root lengthening and both the length of the apical meristems and root diameter. Indeed, in slowly growing roots, cell differentiation occurs closer to the apical meristem than in faster growing ones giving rise to shorter meristems (Fusconi et al. 1994) and the enlargement of the root diameter just above the root tip is manifested by radial cell expansion (Voutsinas et al. 1997).

The reduction of root lengthening is also related to the decrease of apical meristem activity and cell elongation. Mitotic index reduction is proportionally related to Cd concentrations (Liu et al. 2003/2004). In our experiment, MI as the result of meristem activity was measured at successive time in root cells during the Cd treatment. In *Vicia*, meristematic cell division might be poorly involved in root lengthening. This is obvious at the beginning of the experiment (12 h). Indeed, MI analyses indicated that Cd (200 µM) induced a drop of MI as early as 12 h after the start of the Cd treatment while the root lengthening was not as yet affected by the Cd treatment. Since the plasma membrane integrity monitored by MDA content and Evans blue uptake was not significantly altered at this period of Cd treatment, it suggests that root lengthening was carried out mainly by cell elongation at this period of treatment (12 h). However, a small but detectable drop in size was manifested in higher Cd treatment.

Higher Cd concentration and longer treatment period decelerated the MI proportionally to the root growth inhibition and induced loss of plasma membrane integrity. After 24 h, high Cd concentration (200 µM) led to 50% of MI values on average compared to the control, a significant increase of MDA content (40% compared to the control) and Evans blue absorption (30% compared to the control). These data suggest that root growth reduction might be then the result of the combined effect of lower meristematic activity and changes in the physiological characteristics as determined by MDA and membrane integrity.

Vicia roots were affected by Cd treatment since this organ sequestered highly the metal proportionally to the exogenous Cd and significantly increased intracellular H_2O_2 content. The Cd-inducing lipid peroxidation is well documented (Singh et al. 2006; Tamás et al. 2008) and the positive correlation between lipid peroxidation status and genotoxicity has been demonstrated earlier (Mayer et al. 2000). Ünyayar et al. (2006) showed the lipid peroxidation and genotoxicity of Cd ions in *V. faba*. In our study, the short-term kinetic of metallic treatment on hydroponically cultivated *V. faba* plants allowed to analyze the sequential events underlining both phenomena. Under our

experimental conditions, the H_2O_2 content increased significantly as early as 12 h after the Cd treatment that was proportional to the Cd sequestrated in the roots. At this time point, the metal and/or H_2O_2 concentrations did not lead to lipid peroxidation since the MDA content was not significantly affected by the Cd treatment. However, the MI drops radically proportionally to the intracellular Cd ions/H_2O_2 concentrations concomitantly to MCN detection. Taken together, the primary effect of intracellular Cd or H_2O_2 increase is detected on the mitotic activity. The further Cd treatment i.e., 24 and 48 h, led to a dramatic decrease of MI values and related MCN production. The MDA content started to increase proportionally to the Cd concentration and/or H_2O_2 production. The Cd-induced oxidative damage of lipids leading to the loss of plasma membrane integrity was further confirmed by root extract absorption of Evans Blue (Yamamoto et al. 2001). The uptake of the non-permeable dye (Evans blue) into the *Vicia* roots was significantly detected after 24 h for the high Cd-concentrated treatment (200 µM) and increase proportionally to the Cd/H_2O_2 concentrations at 48 h supporting the substantial loss of membrane integrity at the end of the treatment. These data clearly demonstrated sequentially the Cd-induced deleterious effects in *Vicia* roots. The genotoxicity events occurred soon after Cd exposure (12 h) and prior plasma membrane integrity and the lipid peroxidation.

The plasma membrane may be regarded as the first "living" structure that is the target for heavy metal toxicity. Tolerance mechanisms may be involved in the protection of plasma membrane integrity and/or the action of a repairing system after damage to restore metal homeostasis in the root cells either by preventing or reducing entry into the cell or through efflux mechanisms. According to Hall (2002), this repairing mechanism could involve heat shock proteins or metallothioneins. In *Vicia*, roots might activate such repair mechanisms. During the initial stages of the heavy metal treatment (12 h), the plasma membrane integrity remained unaltered. Accordingly, previous work demonstrated the overexpression of protecting proteins (MT2 and Hsp70) after the 12 h of Cd treatment (unpublished data).

Recent results have shown that transient heavy metal treatments might result into DNA damages as cytologically measured with MCN formation (Souguir et al. 2008). A spectrum of structural and numerical abnormalities of chromosomes was detected in *V. faba* meristematic cells after the Cd treatment. As suggested by Souguir et al. (2008), these chromosomal abnormalities reflect clastogenic and aneugenic actions of mutagen agents with formation of MCN. In our experiments, the induction of micronucleated cells indicates a potential phenomenon for both clastogenicity and aneugenicity. The lipid peroxidation events detected subsequently to the genotoxic ones might not be related. The mitotic abnormalities might not be attributed to high lipid peroxidation damaging transport mechanism, as it has been suggested by Ünyayar et al. (2006). In that respect, ROS and/or Cd ions could rapidly (in 12 h) affect DNA/chromosomal movement and trigger genotoxicity events. There are several preliminary evidences about the effect of these two deleterious factors on mitosis and MCN production, and their potent target. As a Ca^{2+} ion analog, Cd^{2+} could enter the cell through Ca channels in the plasma membrane and bind to Ca^{2+} binding proteins such as calmodulin. Ca^{2+} and calmodulin have been implicated in several aspects of cytoskeleton organization and cell division apparatus in plants. Kao et al. (2000) have shown that the kinesin-like calmodulin-binding protein, a motor protein with microtubule-binding activity is highly regulated by Ca^{2+}. These events could represent the initial steps triggering a cascade of sequential phenomena (Liu et al. 2003/2004; Clemens 2006) in *Vicia* meristem roots explaining the mitotic abnormalities caused by Cd itself such as chromosome bridges, chromosome stickiness and breaks. Other report has shown that dysfunction of the spindle is mainly due to the reactivity of the metal ions with thiol groups of tubulin in which the cysteine residues are actively involved in regulating microtubule-assembly dynamics (Chaudhuri et al. 2001). Since Cd^{2+} has a high affinity for sulfhydryl groups (Seregin and Ivanov 2001), the aneuploidy phenomenon observed in *Vicia* root meristems could result from the high sensitivity of the mitotic apparatus to this ion. Early Cd genotoxicity-related events may be also attributed to excessive generation of ROS, as early as 12 h after exposure that contribute to the increase in DNA/chromosome damages (Cordova Rosa et al. 2003; Lin et al. 2007).

This study accommodated the collection of more detailed information about various effects of Cd in higher plants. Additional studies affecting chromosomes should be conducted to understand aneuploid and clastogenic cell productions in Cd stressed-plants directly or indirectly through the H_2O_2 generation.

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