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The uptake of cadmium by *Allium cepa* var. *agrogarum* L. and its effects on chromosome and nucleolar behavior in root tip cells

Absorción de cadmio por *Allium cepa* var. *agrogarum* L. y sus efectos en el comportamiento cromosómico y nucleolar en células del ápice radical

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Abstract. *Allium cepa* var. *agrogarum* L. seedlings are sensitive to Cd stress. We used fluorescence imaging to indicate that Cd^{2+} was localized in cytoplasm in the epidermis of the basal parts of root and vascular tissues after Cd treatment. The nucleoli and the cell walls were the first storage sites of Cd^{2+}. When Cd exposure was prolonged, severe irregularly-shaped nuclei were induced. We used silver nitrate staining to analyze the effects of different concentrations (1–300 μM) of cadmium chloride on chromosome, nucleolus and nucleolus organizer regions (NORs) in root tip cells. Cd^{2+} induced c-mitosis, chromosome bridges, chromosome stickiness and micronuclei. More than 100 μM Cd^{2+} could induce nucleolar material extruded from the nucleus into the cytoplasm. The abnormal phenomena of the nucleolar cycle during mitosis induced by Cd stress contained the nucleoli, which did not exist normally and melted gradually. More silver-stained particles were seen on chromosomes at the metaphase; more silver-stained particulates were localized on the sticky chromosomes, chromosome bridges and cytoplasm. Nucleolar reconstruction was inhibited at the telophase, NORs were localized on chromosomes of c-mitosis. The LysoTracker red stained results indicated that autophagy was active. The number of autolysosomes in roots of Cd-treated plants was more than that on control plant.

Keywords: Cadmium (Cd); *Allium cepa* var. *agrogarum* L.; Chromosome aberration; Nucleoli; Nucleolar organizing region; Autophagy.

Resumen. Las plántulas de *Allium cepa* var *agrogarum* L. son sensibles al estrés por cadmio. Usamos imágenes de fluorescencia para indicar que el Cd^{2+} se encontró en el citoplasma en la epidermis de las partes basales de la raíz y tejidos vasculares luego del tratamiento con Cd^{2+}. El nucléolo y las paredes celulares fueron los primeros sitios de almacenaje de Cd^{2+}. Cuando la exposición con Cd^{2+} fue prolongada, se indujeron núcleos con formas severamente irregulares. Usamos el teñido con nitrato de plata para analizar los efectos de diferentes concentraciones (1-300 μM) de cloruro de cadmio sobre los cromosomas, nuleolus y regiones de organización de nucleolus (NORs) en las células de los ápices radicales. El Cd^{2+} indujo c-mitosis, puentes cromosómicos, cromosomas pegagosos y micronuleolos. Más de 100 μM Cd^{2+} pudo inducir que el material nucleolar saliera del núcleo hacia el citoplasma. El fenómeno anormal del ciclo nucleolar durante la mitosis inducido por el estrés por Cd contuvo al nucléolo, el cual no existió normalmente y se derritió gradualmente. Se observaron más partículas teñidas de plata en los cromosomas en la metafase; se localizaron más partículas teñidas de plata en los cromosomas pegajosos, puentes de cromosomas y citoplasma. La reconstrucción nucleolar fue inhibida en la telofase, NORs se localizaron en los cromosomas en la c-mitosis. El número de autolisosomas en las raíces de las plantas tratadas con Cd fue mayor a aquel en las plantas control.

Palabras clave: Cadmio (Cd); *Allium cepa* var. *Agrogarum* L.; Aberración cromosómica; Nucleolo; Región de Organización Nucleolar; Autofagia.

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INTRODUCTION

The cadmium (Cd) level of soil and aquatic environments is increasing with intensive anthropogenic activities, including industrial, agricultural and/or urban development (Li et al., 2012). The Cd^{2+} can be taken up by roots and accumulate at high concentrations in plant tissues (Irfan et al., 2014). Accumulation of Cd may be toxic for all biology. It may limit plant growth and induce numerous physiological and metabolic disturbances, both at the whole plant and cellular levels. Rates of photosynthesis, leaf chlorosis, growth, and water and nutrient uptake can be inhibited, finally leading to cell death (Howladar, 2014).

Cd^{2+} entry to root cells is the first key process for phytotoxicity. Once taken up into the apoplast, Cd^{2+} enters the root cells, is further uploaded into the xylem, and transported to the leaves with the transpiration stream. Cytology evidence in support of this idea is still lacking. Excess Cd^{2+} may firstly cause root browning, twisting, reduction or disappearance of lateral roots (Liu et al., 1995; Fusconia et al., 2006). This may be due to reduction of mitotic and nucleoli activity in apical meristems (Liu et al., 2003/2004; Fusconia et al., 2006; Garaj-Vrhovac et al., 2013). Cell elongation in the extension regions has also been inhibited. Along with the decreased mitotic activity in the apical meristems, mitotic aberrations appear, consisting in C-mitoses, anaphase bridges, chromosome stickiness, vagrant chromosomes and micronuclei (Liu et al., 2003/2004; Fusconia et al., 2006), apoptosis and necrosis (Behboodi & Samadi, 2004). Accordingly, plants produce different types of peptidic defenses against heavy metals. Autophagy is suggested to play a variety of roles in plant metabolism and development, and responses of plants to biotic and abiotic stress factors, including viral infection, oxidative stress, salt stress, and drought stress (Kuzuoglu-Ozturk et al., 2012). Cd stress, as a major abiotic stress factors, can reduce the yield of crop plants by more than 50%. Therefore, understanding the plant tolerance to Cd stress is a major concern of the scientific community.

The effects of Cd^{2+} on meristem activity change in relation to the plant species. A number of plant species have been utilized as an experimental system to investigate physiological, metabolic and molecular aspects of Cd administration, such as *Populus* × *canescens* (He et al., 2011), *garlic* (Liu et al., 2003/2004), barley (Akhtera et al., 2014), and *Arabidopsis* (Ager et al., 2002). *Arabidopsis*, tobacco and rice are the most widely studied plant species for both monitoring and understanding the molecular basis of Cd stress. In this study, we used seedlings of *Allium cepa* var. *agrogarum* L., which were sensitive to Cd stress. Evaluations included the way that Cd^{2+} enters the root cells, the toxic effects of Cd on chromosomes, nucleolus and the nucleolar disruption on meiotic cells of the root tip. Additionally, we determined the autophagy in root cells induced by Cd stress using LysoTracker_red staining. These results will provide valuable information for future research.

MATERIALS AND METHODS

Plant growth conditions and cadmium treatment. Well-developed onion bulbs were used. Before use, the loose outer scales were removed and the dry bases were scraped to expose the root primordial (Qin et al., 2010). Bulbs were thereafter immersed in tap water. Onion roots were allowed to grow for 3 days at room temperature. The tap water was exchanged every 12 hours. Produced roots of approximately 1.5-2 cm length were transferred into Petri dishes containing salt solutions of $CdCl_2$ at various concentrations (0 μM, 1 μM, 10 μM, 100 μM and 300 μM) during 24 h, 48 h, 72 h and 96 h. All solutions were changed regularly every 24 h.

Fluorescence localization of Cd in the root apex. The Cd^{2+} Probe Leadmium Green AM dye (Invitrogen, USA) was used to investigate the distribution of Cd in roots of onion plants pretreated with 100 μM Cd during 24, 48, 72 and 96 h. A stock solution of Leadmium Green AM was made by adding 50 μL of DMSO to one vial of the dye. This stock solution was then diluted with 1:10 of 0.85% NaCl. Roots were immersed in 20 mmol/L Na_2-EDTA for 15 min and then rinsed three times with deionized water. The washed roots and hand-cut sections were immersed in the stained solution for 2 h in the dark and then washed three times for 10 min with 0.85% NaCl. Samples were observed using a confocal laser scanning microscope (ECLIPSE 90i, Japan) with excitation at 488 nm and emission at 500-550 nm, and serial confocal optical sections were taken. Images were analyzed using the Adobe Photoshop CS4.

Preparation for light microscopy. The roots of fifteen seedlings per treatment were cut and fixed in freshly prepared 3:2 (v/v) 95% ethanol/99.8% acetic acid for 1 h at room temperature, and hydrolyzed in 5:3:2 (v/v/v) hydrochloric acid/ethanol/acetic acid for 5 min at 60 °C. Chromosome aberrations were evaluated on squashes of root apexes stained with the Carbol Fuchsin solution following Li (1989) and Liu (1995). Nucleolus aberration were evaluated on squashes of root apices which were squashed in 45% acetic acid, then dried and stained with silver nitrate (Liu & Jiang 1995).

LysoTracker staining. Root tips (about 1–2 cm in length) were collected from control and 100 μM Cd-treated onion bulbs, and incubated in distilled water containing 100 μM E64d (Sigma) cysteine protease inhibitor at room temperature for 16 h. After removal of E64d, root tips were stained with LystoTracker Red in 10 mM Hepes-Na (pH 7.5) for 2 h, and were fixed in a solution containing 50 mM Na phosphate, 5 mM EGTA, 3.7% formaldehyde and 0.02% NaN_3, as described by Kuzuoglu-Ozturk et al. (2012). The stained root tips were observed using a confocal microscope (ECLIPSE 90i, Japan).

RESULTS

Localization of Cd in roots. Almost no green fluorescence was observed in the control roots (Fig. 1 A1-A3, Fig. 2a). In roots treated with 100 μM Cd^{2+} for 24 h, a very weak fluorescence was observed in meristematic or cortical parenchyma of the basal parts of the root (Fig. 1 B1-B3). The fluorescence in vascular tissues became brighter, and moved directionally along vascular tissues far away from the root tips after exposure of 48 and 72 h (Fig. 1 C1-C3 and D1-D3). As Cd exposure was prolonged to 96 h, a greater intensity of fluorescence was observed in all root apexes (Fig. 1 E1-E3).

Nucleoli appear blue after the reaction with DAPI in the same single optical section obtained with the confocal microscope. Cd was localized in the cytoplasm of the epidermis (Fig. 2 b1, b3 and b4) of the basal parts of the roots and vascular tissues (Fig. 2 b1, b2 and b3) in the 96 h treatment. The nucleoli were also storage sites of Cd (Fig. 2 c, d).

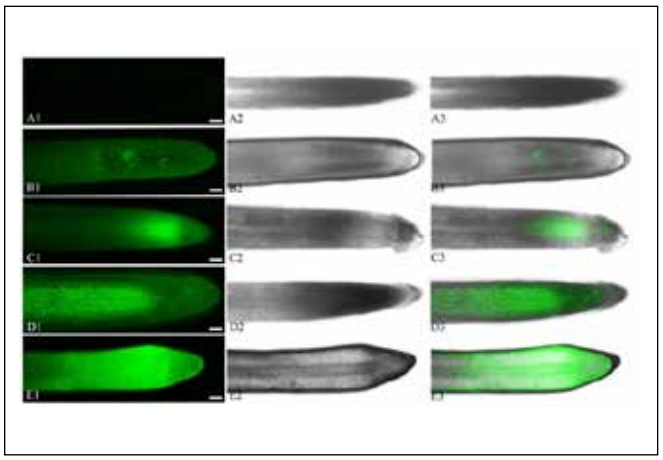

Fig. 1. Localization of Cd in roots of *Allium cepa* var. *agrogarum* L. exposed to 100 μM Cd. Roots from plants pre-treated with Cd for 0 h (A1-A3), 24 h (B1-B3), 48 h (C1-C3), 72 h (D1-D3) and 96 h (E1-E3) were loaded into Leadamium Green AM dye for 2 h. Green fluorescence represents the binding of the dye to Cd. Scale = 200 μm.

Fig. 1. Localización de Cd en raíces de *Allium cepa* var. *agrogarum* L. expuestas a 100 μM Cd. Las raíces de plantas pre-tratadas con Cd durante 0 h (A1-A3), 24 h (B1-B3), 48 h (C1-C3), 72 h (D1-D3) y 96 h (E1-E3) fueron sumergidas en el colorante Leadamium Green AM durante 2 h. La fluorescencia verde representa la unión del colorante al Cd. Escala = 200 μm.

The nucleoli and the cell walls were the first storage sites of Cd^{2+} (Fig. 3a and Fig. 4a). Severe plasmolysis occurred in cortical cells treated with 100 μM Cd^{2+} for 48 h (Fig. 3b). Cd^{2+} accumulated in the nucleoli and whole cytoplasm (Fig. 3c and Fig. 4b). As Cd exposure was prolonged to 96 h, severe irregularly-shaped nuclei were induced, which would lead to cell's death (Fig. 3d and Fig. 4c).

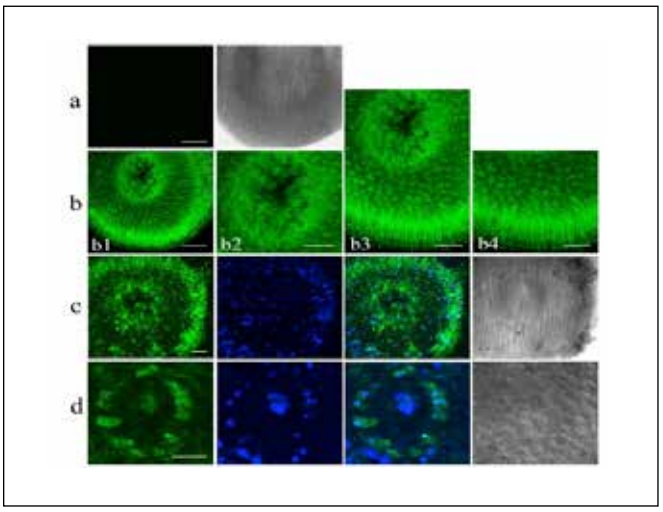

Fig. 2. Fluorescence images of Cd in roots of *Allium cepa* var. *agrogarum* L. exposed to 100 μM Cd for 96 h. Green fluorescence represents the binding of the dye to Cd, blue fluorescence represents the nucleoli. Scale = 100 μm

Fig. 2. Imágenes de fluorescencia de Cd en raíces de *Allium cepa* var. *agrogarum* L. expuestas a 100 μM Cd durante 96 h. La fluorescencia verde representa la unión del colorante a Cd, la fluorescencia azul representa el nucleolo. Escala = 100 μm

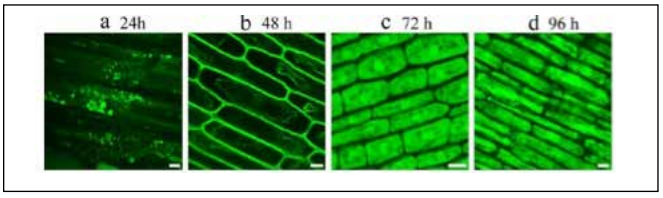

Fig. 3. Fluorescence images of Cd in root cells of *Allium cepa* var. *agrogarum* L. Roots from plants pre-treated with Cd for 24 h (a), 48 h (b), 72 h (c) and 96 h (d) were loaded into Leadamium Green AM dye for 2 h. Green fluorescence represents the binding of the dye to Cd. Scale = 200 μm.

Fig. 3. Imágenes de fluorescencia del Cd en células radicales de *Allium cepa* var. *agrogarum* L. Las raíces de plantas pre-tratadas con Cd durante 24 h (a), 48 h (b), 72 h (c) and 96 h (d) fueron sumergidas en el colorante Leadamium Green AM durante 2 h. La fluorescencia verde representa la unión del colorante al Cd. Escala = 200 μm.

Effects of Cd on chromosome aberration. Low concentrations of Cd (10–100 μM) induced included C-mitosis (Fig. 5a, b and c), and chromosome bridges at anaphase (Fig. 5 d-f). Three hundred μM Cd^{2+} induced severe toxic effects on chromosome, including chromosome stickiness (Fig. 5 g, h) and micronuclei (Fig. 5 i).

Damage of Cd to nucleus. In the normal interphasic nucleus of a meristematic cell, nucleus appear as a well-defined structure, containing 1-3 nucleoli in the control nucleus (Fig. 6a, Fig. 7a). Figure 6b shows nucleolus enlargement, and some tiny silver-stained particulates which were induced in the nu-

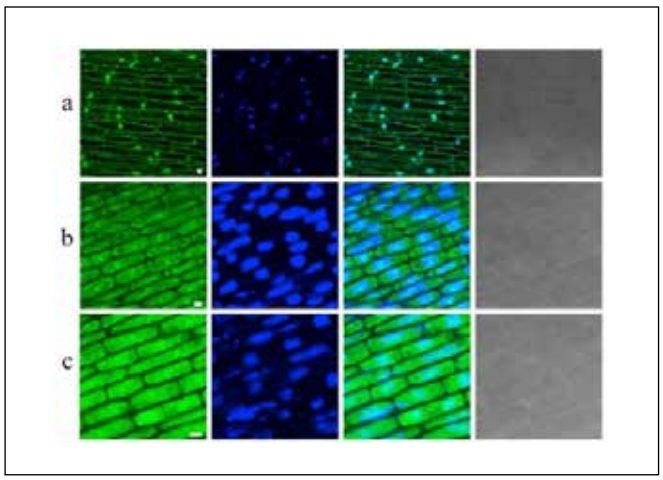

Fig. 4. Fluorescence images of Cd in root cells of *Allium cepa* var. *agrogarum* L. Roots from plants pre-treated with Cd for 24 h (a), 72 h (b) and 96 h (c). Green fluorescence represents the binding of the dye to Cd, and blue fluorescence represents the nucleoli. Scale = 10 μm.

Fig. 4. Imágenes de fluorescencia del Cd en células radicales de *Allium cepa* var. *agrogarum* L. Raíces de plantas pre-tratadas con Cd por 24 h (a), 72 h (b) and 96 h (c). La fluorescencia verde representa la union del colorante al Cd, y la fluorescencia azul representa al nucleolo. Escala = 10 μm.

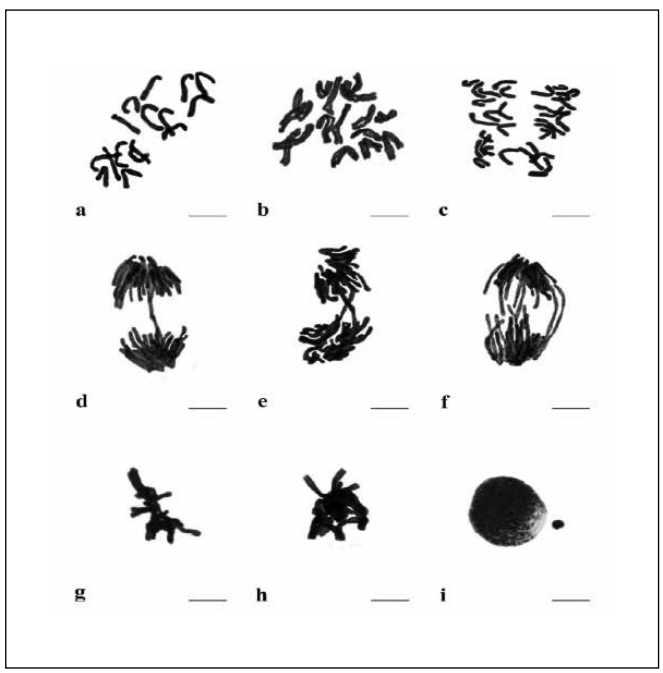

Fig. 5. Effects of Cd on root tip cell division on *Allium cepa* var. *agrogarum* L. (a-c) C-mitosis; (d-f) Chromosome bridges; (g-h) Chromosome stickiness, and (i) Micronuclei. Scale = 10 μm.

Fig. 5. Efectos del Cd en la división celular de los ápices radicales en *Allium cepa* var. *agrogarum* L. que incluyó C-mitosis (a-c); puentes cromosómicos (d-f); cromosomas pegajosos (g-h), y Micronúcleos (i). Escala = 10 μm.

cleus of the root tips treated with 10 μM Cd for 24 h. More tiny, silver-stained particulates accumulated in the nucleus at greater Cd concentrations and increasing treatment times (Fig. 6c). Three hundred μM Cd induced that the nucleolar material extruded from the nucleus into the cytoplasm (Fig. 6d), where the nucleolar material disintegrated into different-size silver-stained particulates after 72-96 h of treatment (Fig. 6 e-k). The phenomenon was observed in both the short and rounded meristem cells, and the long and oblong root cap cells. Finally, the silver-stained material in the cytoplasm increased progressively, and aggregated into irregular shapes (Fig. 6 e-l).

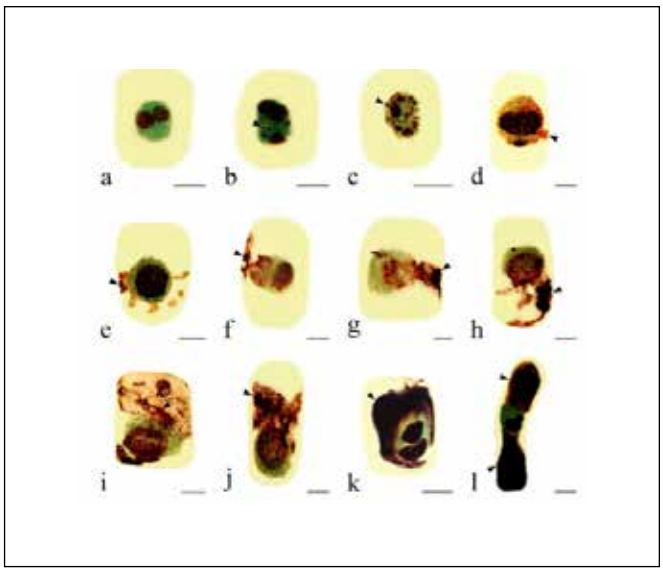

Fig. 6. Effects of different concentrations of Cd on nucleoli in root tip cells of *Allium cepa* var. *agrogarum* L. (Arrowheads show silver-stained materials). (a) Control cells; (b) 10 μM Cd, 24 h; (c) 100 μM Cd, 48 h; (d) 300 μM Cd, 48 h; (e-h) 300 μM Cd, 72 h; (i-k) 300 μM Cd, 96 h; (l) 300 μM Cd, 96 h.

Fig. 6. Efectos de diferentes concentraciones de Cd en el nucleolo de células de apices radicales de *Allium cepa* var. *agrogarum* L. (las flechas indican los materiales teñidos con plata). (a) Células control; (b) 10 μM Cd, 24 h; (c) 100 μM Cd, 48 h; (d) 300 μM Cd, 48 h; (e-h) 300 μM Cd, 72 h; (i-k) 300 μM Cd, 96 h; (l) 300 μM Cd, 96 h.

Effects of Cd on nucleolar organizing regions (NORs). Nucleoli in interphase nuclei of the control cells had many regions impregnated by silver ions, indicating that they were made of nucleolar material (Fig. 3a). Some of them were strikingly larger, but this may be due to the joining of smaller formations. The stained NORs were active during the previous interphase. The reason why these regions are silver-stained in some organisms and not in others is still unknown. Between prometaphase and metaphase, the nucleoli gradually disappeared (Fig. 7e). A terminal NOR in both sister chromatids of a single homologous chromosome of an autosome pair was observed (Fig. 7f). At the metaphase, the NOR was

more strongly stained (Fig. 7f). At the anaphase, two NORs migrated with the sister chromatids and linked to the spindle fibers to be pulled to the cell poles (Fig. 7g). We also saw that the nucleolus at telophase can form at each of these regions (Fig. 7 h-i). Compared to the control group, the abnormal phenomena of the nucleolar cycle during mitosis was induced by Cd stress. The abnormal phenomena containing the nucleoli did not exist normally and melted gradually (Fig. 7j). More silver-stained particles were seen on chromosomes at the metaphase (Fig. 7k), which also localized on the sticky chromosomes (Fig. 7l), and chromosome bridges and cytoplasm (Fig. 7 m-n); nucleolar reconstruction was inhibited at the telophase (Fig. 7o), and NORs were localized on chromosomes of c-mitosis (Fig. 7p).

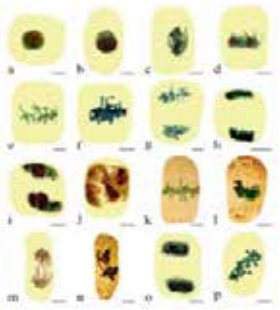

Fig. 7. Effects of Cd on Nucleolar Organizing Regions (NORs) in root tip cells of *Allium cepa* var. *agrogarum* L. during mitosis. (Arrowheads show NORs). (a-d) NORs are organized in control cells. (e-p) NORs are irregular in Cd^{2+}-treated cells. Scale bar = 10 μm.
Fig. 7. Efectos del Cd en Regiones de Organización Nucleolar (NORs) en células de ápices radicales de *Allium cepa* var. *agrogarum* L. durante la mitosis (las flechas indicant NORs). (a-d) Las NORs están organizadas en células control; (e-p) las NORs son irregulares en las células tratadas con Cd^{2+}. Escala de la barra = 10 μm.

Autophagy is induced by Cd stress in wheat roots. In the presence of E64d, LysoTracker red produced autophagosomes (a punctate staining pattern) in both control and stressed plants, indicating that autophagy was constitutively active in *Allium cepa* var. *agrogarum* L. (Fig. 8). The number of autolysosomes in roots of Cd-treated plants was more than that of control plants (Fig. 8). The number of autolysosomes in roots also increased after exposure to 100 μM Cd from 48 to 96 h (Fig. 8).

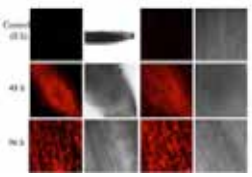

Fig. 8. Autolysosomes were observed in LysoTracker Red-stained, Cd-treated root tips of *Allium cepa* var. *agrogarum* L. Autophagosomes were observed in control (a) and Cd-treated (b) root samples for 48 h (b) and 96 h (c). Results were reproduced in three independent experiments using three or more plants in each experiment. Scale = 10 μm.
Fig. 8. Autolisosomas en ápices radicales de of *Allium cepa* var. *agrogarum* L. tratados con Cd y teñidos de rojo en LysoTracker. Se observaron autofagosomas en muestras radicales control (a) y tratadas con Cd (b) por 48 h (b) y 96 h (c). Los Resultados se reprodujeron en tres experimentos independientes usando tres o más plantas en cada experimento. Escala = 10 μm.

DISCUSSION

Cadmium is taken up by roots and accumulated in the upper part of the plants, thereby affecting crop yield and threatening food safety (Zhao et al., 2006). Many studies evaluated the potential of Cd uptake in an attempt to explain the differences in shoot Cd accumulation between varieties (Zhao et al., 2002). The efficiency of root-to-shoot translocation is theoretically dependent on four processes (Lu et al., 2008): (i) symplastic uptake by roots; (ii) root sequestration: cell walls protect plants from uptake of Cd into the cytosol. The cell walls may act as a barrier to cytosolic Cd uptake; (iii) xylem uploading, and (iv) xylem downloading and uptake of metals by foliar cells. Our results support this view. More Cd^{2+} was observed in the vascular cylinder of root tips as time from Cd^{2+}-treatment increased. This indicated that Cd^{2+} was rapidly transported into the vascular tissues via the symplastic pathway, and then became available for subsequent transport to more distal positions in the root zones. The nucleoli and the cell walls were the first storage sites for Cd^{2+}.

The uptake of Cd^{2+} in the root induced chromosomal aberrations and mitotic abnormalities. The most common aberrations observed in our treatments were c-mitosis, anaphase

bridges, stickiness and micronuclei. The frequency of stickiness was greater than the other aberrations for Cd stress. The micronucleus was the most prominent among these aberrations. Stickiness is considered as a chromatid type aberration, and it is attributed to the effect of environmental pollutants on degredation or depolymerization of chromosomal DNA on DNA condensation and on entanglement of insertions between chromosomes (Türkoğlu, 2012). The stickiness, as an irreversible chromosome abnormality, commonly gives rise to cell death. Heavy metals have been reported to induce stickiness, and the results of this study are in agreement with those obtained after treating the different materials with different metals (Gabara et al., 1995; Fusconiet et al., 2007; Türkoğlu, 2012). Micronuclei were observed after Cd treatments. The increase in micronucleus implies that it causes several chemical damages to DNA (Gabara et al., 1995; Fusconiet et al., 2007; Türkoğlu, 2012). Micronucleus can be derived from acentric fragments involving clastogenic activity, or from entire chromosomes involving an aneugenic activity. This means that Cd is clastogens that induce chromosome breaks and/or aneugens explaining lagging chromosomes. Similarly, micronuclei were recorded by many investigators following treatments with different metals (Gabara et al., 1995; Fusconiet et al., 2007; Türkoğlu, 2012). In addition to the above-mentioned type of abnormalities, anaphase bridges and c-mitosis were also observed in the present investigation. The bridges noticed in the cells were probably formed by breakage and fusion of chromosomes and chromatids. The occurence of C-mitosis indicated that Cd caused inhibition of spindle formation similar to the effect of colchicine. These results agree with those of many research groups that examined the effects of different chemicals on different plant materials (Gabara et al., 1995; Fusconiet et al., 2007; Türkoğlu, 2012).

Within the nuclear organelle, the nucleolus is the largest and most conspicuous part and it is also the site of NORs containing rDNA genes (Miller et al., 1976; Teerarak et al., 2009). A wide variation in number and location of NORs was closely related to Cd stress. The nucleolar behavior during the different stages of meiosis was similar to that in other species (Zhang et al., 2009). Based on the analysis of proteins and nucleolar activity, several authors hypothesized that by suppressing the synthesis of rDNA in late prophase, transcription factors such as UBF, SL1 and topo I and several pol I subunits remain associated with the mitotic NORs, and the remaining nucleolar antigens (pol I subunits, rRNA processing and ribosome assembly components) move from the DFC and GC out of the nucleolus, toward the nuclear envelope along defined paths within the nucleoplasm. By the metaphase, the antigens have reaccumulated in the perichromosomal sheath (Sirri et al., 2008). Despite that NORs labeled in meiotic metaphasic chromosomes have a smaller size compared to mitotic labeling, the amount of nucleolar material that remains associated with NORs during meiosis may be less than in mitosis, a fact that may be associated with differences in nucleolar dynamics in different phases of the cell cycle of these cells. In the anaphase, when rDNA transcription is reactivated, proteins associated with the perichromosomal regions and the transcribed snoRNAs and partially processed pre-rRNA detached from the chromosome periphery, moving to the cytoplasm where they associated with larger particles called "nucleolus-derived foci" (NDF) (Dundr et al., 2000). As the cells proceeded through the telophase, the number of NDFs decreased and other particles (i.e., the PNB) appeared in the cell. These subsequently fuse with the chromosomal NORs of the mitotic telophase and earlier interphase cells (Dundr et al., 2000). The abnormal phenomena of the nucleolar cycle during mitosis were observed after Cd treatment. These abnormal phenomena may be due to severe toxic effects of Cd on the nucleus and the nucleolus in root tip cells of *Allium cepa* var. *agrogarum* L.

Recently, some reports regarding plant autophagy have suggested a role for autophagy in plant responses to salt and osmotic stress (Liu et al. 2009; Kuzuoglu-Ozturk et al., 2012). The number of LysoTracker red-stained autophagosomes increased in the roots of Cd-treated seedlings, which indicated that autophagy is induced by Cd stress. It has been shown that salt and osmotic stresses induce autophagy in *Arabidopsis* and wheat plants (Liu et al., 2009; Kuzuoglu-Ozturk et al., 2012). Our data support the view that autophagy might play a positive role in plant responses to Cd stress.

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