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Research Article

Bridging a Gap between Cr(VI)-Induced Oxidative Stress and Genotoxicity in Lettuce Organs after a Long-Term Exposure

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Chromium (Cr) contamination in arable soils and irrigating water remains a priority, particularly due to the challenges posed to crop production and food safety. Long-term Cr(VI) effects remain less addressed than short-term ones, particularly regarding organ-specific genotoxic profiles. Here we used the crop *Lactuca sativa* growing in a protected horticultural system and irrigated for 21 days with Cr(VI) (up to 200 mg/L). Besides the oxidative stress, the genotoxicity was evaluated. Shoots and roots showed distinctive oxidative stress status and genotoxic effects, in a dose-dependent manner. While 50 mg/L stimulated antioxidant activities and no major genotoxic effects were found, plants exposed to ≥150 showed an increase of oxidative disorders, together with cytostatic and DNA damage effects, and some mitotic impairment. Leaves showed less oxidative signs at 50 mg/L, while at 150/200 mg/L the antioxidant battery was stimulated. In Cr treated plants, the highest dose increased the DNA damage, reinforcing the idea that DNA breaks were related to mitotic disorders in higher doses. In conclusion, long-term exposure data show a highly responsive root, with a quadratic response meaning higher defenses at lower Cr doses, and higher oxidative and DNA damage and cytostatic effect at a higher dose.

1. Introduction

Chromium contamination of soils and irrigation water remains a matter of concern [1], with increased health risks in humans due to the food-chain contamination. A fertilizer plant site usually has a soil contamination of ~700 mg/Kg and in industrial/tannery effluents/soils may reach 5000–45,000 mg/Kg (reviewed by Shahid et al. [1]). Chromium's most stable oxidation forms are Cr(III) and Cr(VI), both having different mobility, bioavailability, and toxicity [2]. The physiological mechanisms of Cr transport in the plant through root absorption point to several ways of Cr input. Cr transport promotes gaps in other types of nutrient absorption and water [3]. Most studies on Cr-plant interactions have addressed Cr transfer from soil to plant and Cr hyperaccumulation for phytoremediation purposes (e.g., [4]) and morphophysiological and biochemical responses (e.g., root surface area, growth, morphology, photosynthesis, water/nutrient interactions, and transpiration) (e.g., [1, 5]). Cr accumulation promotes an impairment of photosynthesis in the chloroplasts [3]; thus, these effects promoted a reduction of plant biomass [6].

Besides the direct interaction of Cr with nucleic acids (e.g., promoting DNA-DNA cross-links), it has also been demonstrated that Cr(VI) may increase the generation of reactive oxygen species (ROS), leading to indirect cytotoxic effects [7–10]. The correlation of the antioxidant status with genotoxic damage remains a matter of debate. Besides,

sequestration and/or chelation, a major strategy to limit these oxidation impacts, involve an antioxidant strategy that includes the antioxidant enzymes. Enzymes such as superoxide dismutase (SOD) or catalase (CAT) are essential in ROS molecules scavenging, as SOD is responsible for catalysing the conversion of superoxide radicals to H_2O_2 and CAT [as well as ascorbate peroxidase (APX) and peroxidases that use guaiacol as substrate (GPX)] has an important role in controlling H_2O_2 levels [11, 12]. Some studies on Cr-induced toxicity already reported a decrease in these antioxidant enzymes as a result of exposure to high Cr concentrations, while in lower doses their activity was higher, suggesting that some plants can be Cr-tolerant [e.g., Rice *(Oryza sativa)*, Sunflower (*Helianthus annuus* L.), Rapeseed (*Brassica napus* L.), and Indian mustard (*Brassica juncea* L.)] [13–16]. When the activity of these antioxidant enzymes is not sufficient to combat excessive ROS generation, oxidative processes of lipids, proteins, and nucleic acids occur.

Cr(VI) genotoxicity in plants is far less known than on animals or microorganisms. Studies are restricted to short-term exposures (1 h–96 h), and few address the plant's profile after long-term exposure. Short-term exposures to $Na_2Cr_2O_7$ reduced mitosis while inducing chromosomal aberrations in *Pisum* (≤80 ppm) [17]. Also, chromosome fragmentation/aberration and mitotic abnormalities including lagging were reported in *Hordeum vulgare* (<0.5 mM) [18]. Using only 1 h–24 h exposure, Huang et al. [19] also found the disturbance on the up/down levels of transcripts in *Oryza sativa*. In a longer-term exposure, we have demonstrated that *Pisum sativum* exposed to Cr(VI) doses had cell cycle impairment and DNA fragmentation. Micronuclei formation is intimately associated with clastogenicity [20]. In the last years, comet assay and flow cytometry (FCM) have provided several sensitive and robust parameters that may complement the mitotic and chromosomal classical data and the quantitative genetic analyses.

This study used the crop *Lactuca sativa* under a simulated protected culture system to evaluate the effects of a long-term exposure to Cr(VI) on the antioxidant status and genotoxic disorders in both organs (roots and shoots). For that, the antioxidant enzymatic battery was analyzed, together with flow cytometric, micronuclei, mitotic aberrations, and comet assays.

2. Materials and Methods

Growth Conditions and Cr Content. Seedlings with 15 days old of *Lactuca sativa* L. (cv. "Povoa," Viveiros do Litoral, Aveiro, Portugal) were grown in pots with a peat : vermiculite (1 : 3 v/v) mixture, in a greenhouse with 430 μmol m^{-2} s^{-1} light intensity, a 16 h photoperiod, and 23 ± 2°C. During 21 days, plants were irrigated with 15 mL of 1/2 strength Hoagland's solution (pH 5.8) with 0, 50, 150, and 200 mg/L of Cr(VI) supplied by K_2CrO_4 (Sigma-Aldrich, USA). After that, plants' length was measured and plants were analyzed for chlorosis and necrotic spots. Roots were washed in a 5% $Ca(NO_3)_2$ followed by water to remove adsorbed Cr. For total Cr quantification, weighted fresh roots and leaves were dried at 60°C and then treated according to Azevedo et al. [21]. Elemental Cr content was analyzed by ICP-AES (Jobin Ivon JY70 Plus, France). *L. sativa* plants were used to assess the phytotoxicity of Cr, as this species is recommended as an ISO models for toxicological assays.

Oxidative Stress and Cell Membrane Permeability. Frozen leaf and root samples were ground in 0.1 M potassium phosphate buffer (pH 7.8), 1% polyvinylpyrrolidone, 5 mM Na_2EDTA, and 0.2% Triton X-100. After centrifugation (8000 ×g, 15 min), the supernatant was used for enzymatic assessment and soluble protein quantification (MicroTotal Protein Kit, Sigma-Aldrich, USA). APX, GPX, CAT, and SOD activity and glutathione reductase (GR) activities were determined as described by Silva et al. [22] and Mariz-Ponte et al. [12]. Lipid peroxidation was assessed by the malondialdehyde (MDA) method and the membrane stability was assessed by the membrane permeability (CMP) method [12, 23].

Flow Cytometry. Clastogenicity and changes in ploidy level and cell cycle progression were evaluated in roots and leaves, by flow cytometry [24]. Nucleus suspensions were obtained in woody plant buffer. To the nucleus suspensions, 50 μg mL^{-1} propidium iodide and 50 μg mL^{-1} RNAse were added. At least 5000 nuclei were analyzed per sample in a flow cytometer EPICS-XL Coulter Electronics (USA) with an argon laser (15 mW, 488 nm), and data were analyzed with a SYSTEM II software (v. 3.0, Beckman Coulter®). Nucleus populations in phases G_0/G_1, S, and G_2 and changes in cell cycle progression were analyzed. The Index of Cell Proliferation (%IP) was determined according to the following equation: $\%IP = (\%S + \%G_2)/(\%G_0/G_1 + \%S + \%G_2)$, and the S phase fraction (SPF) was also calculated as $\%SPF = \%S/(\%G_0/G_1 + \%S + \%G_2)$.

Comet Assay. Root and leaf DNA fragmentation was quantified by the comet assay [25]. Tissues were sliced in 0.4 M Tris buffer, pH 7.5. 50 μL of nucleoids and 50 μL of 1% LMPA were spread on slides with a 1% NMPA layer and treated with alkaline buffer (0.30 M NaOH, 1 mM EDTA, pH > 13). Electrophoresis (30 min) took place at 0.74 V cm^{-1} at 4°C. Slides were neutralized with 0.4 M Tris pH 7.5 and stained with ethidium bromide. Comets were analyzed with a fluorescence microscope (Nikon Eclipse 80i; excitation filter: 510–560 nm; barrier filter: 590 nm). The %DNA in the tail (%TDNA) and the tail length (TL) were calculated with the CASP v1.2.2 software.

Micronuclei and Mitotic Aberrations. As micronuclei and mitotic aberrations need mitotic cells, only root meristems were used. Root tips were collected and stained with propidium iodide [26]. Slide preparations were observed under 1000x magnification using a fluorescence microscope (Nikon Eclipse 80i; excitation filter of 510–560 nm; barrier filter of 590 nm). To calculate the micronuclei (%MN), 200 cells were scored [26]. For mitotic aberrations, meristematic tissues were collected and stained with 2% acetic orcein. Samples were observed with a Nikon Eclipse 80i microscope, with a NIS-Elements F 3.00, SP7 software.

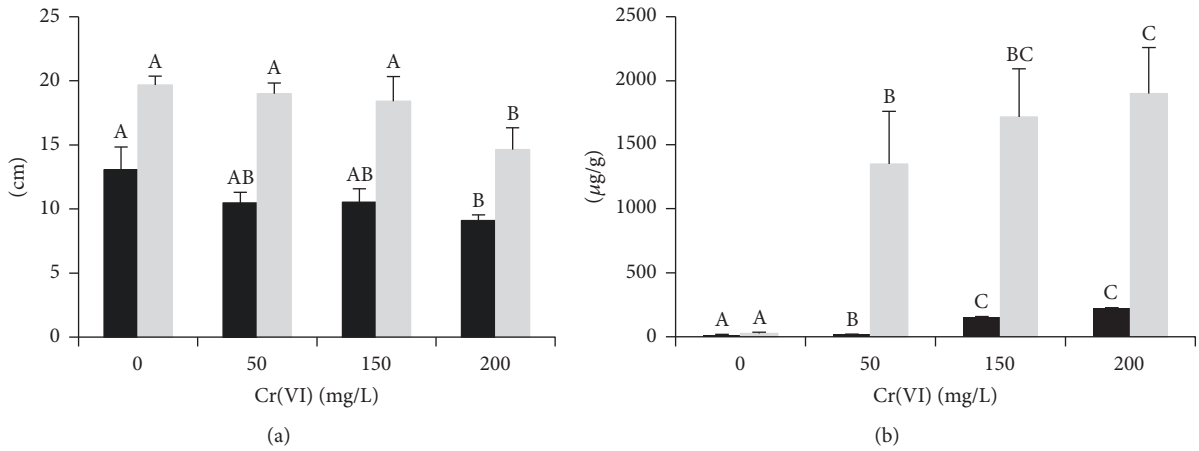

Figure 1: Cr(VI) effect on lettuce shoot (black bars) and root (grey bars) (a) growth (cm) and (b) Cr accumulation (μg/gDW). Different letter means significant differences relative to the control ($p < 0.05$).

Statistical Analysis. Sampling of ~six plants was used as individual or as a pool. Data were analyzed by one-way ANOVA ($p < 0.05$), followed by a Holm-Sidak test ($p < 0.05$) to evaluate the significance of differences in the parameters, and transformed to achieve normality. If required the non-parametric test, Kruskal–Wallis one-way ANOVA by ranks, was used. Statistical analyses were performed with Sigma Plot 11.0 (Systat Software Inc., Germany).

3. Results

Plant Growth and Total Cr Content. At the end of exposure some plants treated with 150 and 200 mg/L developed reddish leaves. While growth decreased and senescence in some leaves was evident, after this long-term exposure, survival rates were not affected (~100%). Figure 1 shows how Cr(VI) affected plant growth, with an evident decrease of both root and shoot length with Cr doses. As expected, the lowest shoot length (69% of the control) and root length (74% of the control) were achieved in plants exposed at 200 mg/L (Figure 1(a)). The elemental content of Cr increased in a linear way in both organs ($R^2 = 0.90$ for shoots and $R^2 = 0.83$ for roots) but the increase was much higher ($p < 0.05$) in roots. The absolute accumulation per gDW in leaves was <10x lower than in roots (Figure 1(b)). For the highest exposure, the accumulation of Cr was ~18x in shoots compared with the 75x increment of Cr in roots.

Oxidative Status. Leaves and roots showed different enzymatic profiles, with the roots being more susceptible to peroxidation ($p < 0.05$ for 150 mg/L and 200 mg/L) and to damage in cell membranes damage ($p < 0.05$ for ≥50 mg/L) (Table 1). In leaves, significant lipid peroxidation and increased cell membrane permeability were only significant for 200 mg/L. GPX decreased in both organs for higher doses, while it increased in a quadratic manner ($p < 0.05$) in roots exposed to 50 mg/L. APX increased in shoots exposed to Cr ($p < 0.05$) and in a quadratic manner in roots (increased at 50 mg/L ($p < 0.05$), decreasing thereafter). CAT and GR showed a general trend to increase in higher doses of Cr in leaves and roots ($p < 0.05$ for 200 mg/L). SOD activity increased in shoots reaching maximum values at 150 mg/L, while in roots it followed a quadratic response of maximum values at 50 mg/L.

Flow Cytometry. No significant changes were found in the complexity and size of nuclei. The control histograms presented FPCV values lower than 4%, an indicator of the reliability of the technique and protocol used. To score for clastogenic damage, the %FPCV of the G_0/G_1 peaks were analyzed, with no detectable variations (Table 2).

No ploidy abnormalities (e.g., aneuploidy and polyploidy) were induced by the doses of Cr(VI) tested, and while no changes in the cell cycle dynamics were detected in leaves, roots showed some cytostatic effects. Cr(VI) induced a blockage of the S phase ($p < 0.05$ for the highest concentration), with consequent trends of decreasing the nuclei populations at G_0/G_1 and G_2 (Table 2). Consequently, the root SPF and PI showed a trend to increase with Cr doses.

Comet Assay. Cr(VI) exposure increased DNA damage in leaves at higher concentrations (both TD and TM; 150 mg/L and 200 mg/L) ($p < 0.05$). Roots exposed to Cr(VI) showed a heterogeneity in the results, with higher damage induced in root cells exposed to 50 mg/L of Cr(VI) ($p > 0.05$) (Figure 2).

Mitotic Aberrations and Micronuclei. Micronuclei were absent in control roots and appeared only occasionally (app. 1/1000) in 50 mg/L exposed roots, but their occurrence increased significantly in roots exposed to higher Cr(VI) concentrations (Table 3). Contrarily, the mitotic index (%MI) showed a general trend for a decrease with the increase of Cr(VI), with significant differences for the higher Cr(VI) concentrations. Other occurrences related to mitotic abnormalities increased in the roots exposed to the highest concentration (Table 3).

4. Discussion

Principle Component Analysis. The PCA analyses showed that the analyzed parameters had different correlations in

Table 1: Cr(VI) effect on lettuce shoot and root oxidative activity. Different letters indicate for the same parameter significantly different means ($p < 0.05$). Chromium (Cr), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), malondialdehyde (MDA), and membrane permeability method (CMP).

Cr(VI) (mg/L)	GPX (nKat.mg TSP^{-1})	APX (nKat.mg TSP^{-1})	CAT (nKat.mg TSP^{-1})	GR (nKat.mg TSP^{-1})	SOD (nKat.mg TSP^{-1})	MDA MDA equivalents (nmol mL^{-1} $mgFM^{-1}$)	CMP (%MD)
				Shoot			
0	0.58 ± 0.12^{c}	37.73 ± 4.61^{a}	1.91 ± 0.32^{a}	2.42 ± 0.10^{a}	982.47 ± 477.15^{a}	0.67 ± 0.34^{a}	14.37 ± 3.46^{a}
50	0.23 ± 0.10^{b}	46.84 ± 0.94^{ab}	2.40 ± 0.13^{a}	1.85 ± 0.83^{a}	2062.51 ± 347.36^{b}	0.93 ± 0.17^{a}	19.37 ± 2.89^{a}
150	0.36 ± 0.03^{b}	47.89 ± 1.29^{b}	2.36 ± 0.35^{ab}	2.82 ± 0.62^{a}	4252.10 ± 266.38^{d}	2.36 ± 0.25^{a}	29.00 ± 7.20^{a}
200	0.13 ± 0.02^{a}	48.00 ± 2.63^{b}	5.67 ± 1.21^{b}	3.36 ± 1.86^{b}	3029.70 ± 97.89^{c}	3.10 ± 0.37^{b}	74.13 ± 11.60^{b}
				Root			
0	1.72 ± 0.49^{b}	33.46 ± 0.89^{a}	12.5 ± 2.24^{a}	14.10 ± 3.81^{a}	1057.84 ± 672.24^{a}	0.71 ± 0.24^{a}	12.60 ± 0.37^{a}
50	2.15 ± 0.63^{c}	55.05 ± 2.06^{b}	18.94 ± 4.92^{a}	24.29 ± 5.06^{ab}	2349.10 ± 432.61^{b}	1.15 ± 0.48^{a}	19.75 ± 3.86^{b}
150	0.85 ± 0.03^{a}	37.39 ± 1.30^{a}	12.30 ± 2.16^{a}	17.06 ± 4.21^{ab}	453.03 ± 39.10^{a}	2.72 ± 0.69^{cd}	22.38 ± 5.32^{b}
200	0.91 ± 0.03^{a}	33.04 ± 3.35^{a}	62.28 ± 2.64^{b}	24.50 ± 1.09^{b}	725.25 ± 38.61^{a}	3.46 ± 0.12^{d}	89.81 ± 2.85^{c}

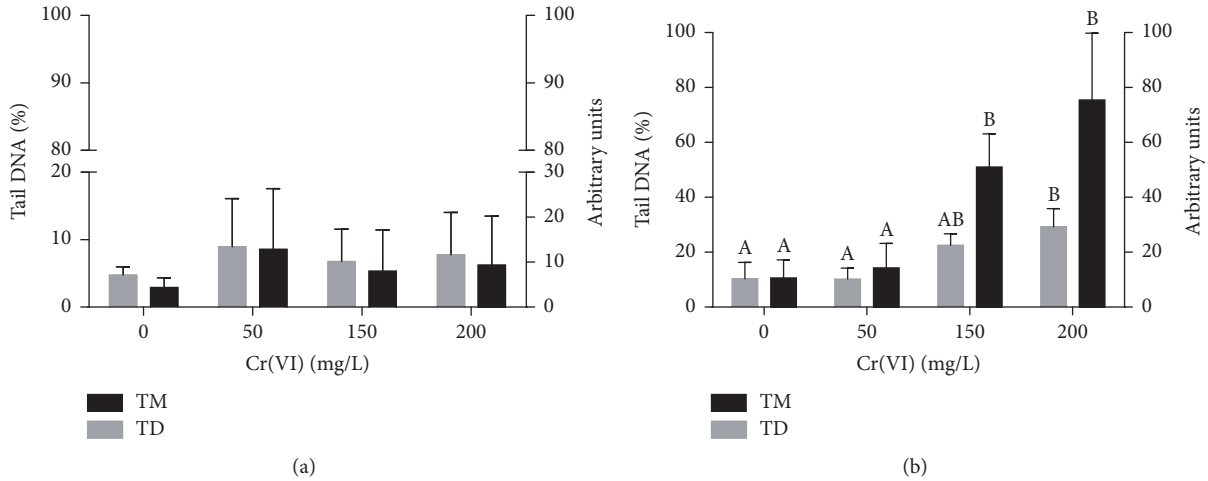

Figure 2: Tail DNA (TD) (%) and tail moment (TM) in (a) roots and (b) leaves exposed to Cr(VI). Different letters indicate for the same parameter significantly different means between control and stressed individuals ($p < 0.05$).

Table 2: Flow cytometry data for roots of *L. sativa* L. plants exposed to Cr(VI). Values are presented as the mean ± coefficient of variation. Different letters indicate for the same parameter significantly different means ($p < 0.05$).

Cr(VI) (mg/L)	Ploidy/abn	%FPCV	%G0/G1	%S	%G2	%IP
			Shoots			
0	2n/No	2.68 ± 0.58[a]	89.34 ± 3.65[a]	5.23 ± 1.87[a]	5.43 ± 0.65[a]	0.11
50	2n/No	2.95 ± 0.62[a]	89.25 ± 3.96[a]	5.72 ± 1.03[a]	5.03 ± 0.45[a]	0.11
150	2n/No	2.89 ± 4.02[a]	88.56 ± 4.02[a]	6.01 ± 1.11[a]	5.43 ± 0.72[a]	0.12
200	2n/No	2.74 ± 0.43[a]	88.24 ± 2.84[a]	6.15 ± 1.57[a]	5.61 ± 0.59[a]	0.13
			Roots			
0	2n/No	3.37 ± 0.74[a]	87.62 ± 4.25[a]	6.51 ± 2.22[a]	4.07 ± 0.94[a]	0.12
50	2n/No	3.31 ± 0.87[a]	85.96 ± 1.43[a]	7.60 ± 1.38[ab]	4.33 ± 1.58[a]	0.13
150	2n/No	2.97 ± 0.37[a]	82.72 ± 4.52[a]	9.81 ± 4.24[ab]	5.31 ± 0.94[a]	0.17
200	2n/No	3.11 ± 0.49[a]	84.84 ± 1.46[a]	9.67 ± 1.29[b]	3.90 ± 0.76[a]	0.15

Table 3: Micronuclei and mitotic index and abnormalities in roots of *L. sativa* L. exposed to Cr(VI). Values are presented as the mean ± standard deviation. Different letters indicate for the same parameter significantly different means ($p < 0.05$). Image: microphotograph of an abnormal metaphase in roots (bar = 20 μm).

Cr(VI) (mg/L)	Micronuclei (n/1000)	MI%	Mitotic abnorm. (n/1000)	
0	Nd (0)	15 ± 5[a]	Nd (0)	
50	1 ± 1[a]	19 ± 6[a]	Nd (0)	
150	5 ± 4[ab]	9 ± 5[ab]	5 ± 3	
200	9 ± 5[b]	7 ± 3[b]	8 ± 3	

leaves versus roots (Figures 3(a) and 3(b)). For roots, PC1 explained 58.8% of the variance, and PC2 explained 26.9% of the variance. In the root PCA, a clear separation between the control (top left quadrant), negatively correlating with most oxidative and genotoxic data, and the other conditions was evident. Also, the population 50 mg/L scores at the down left quadrant positively correlated with GPX, APX, and SOD and with %FPCV and cell cycle parameters (Figure 3(a)). The concentration 150 mg/L scored at the top right positively correlating with a G_2 accumulation/delay and negatively with all the parameters positively correlating with 50 mg/L. Ranking in the right down quadrant, the 200 mg/L samples positively correlated with multiple parameters for peroxidation, membrane degradation, mitotic disorders, and micronuclei.

For leaves, the PC1 explained 65.1% of the variance, and PC2 explained 21.6% of the variance (Figure 3(b)). Similar to roots, control is isolated (left down quadrant), negatively correlating with most oxidative and genotoxic parameters. Samples exposed to 150 and 200 mg/L show more similar

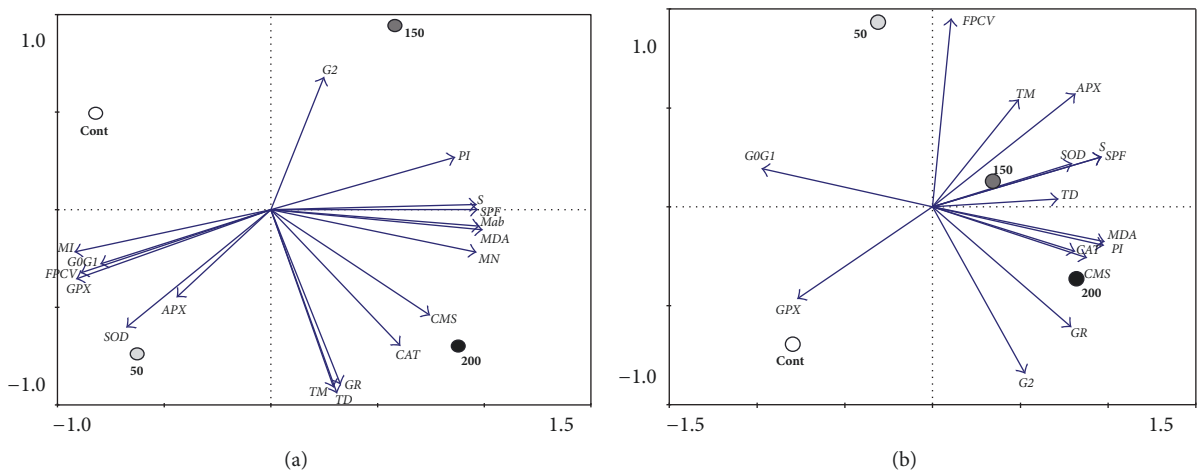

Figure 3: PCA analyses of the oxidative stress and genotoxic effects of Cr(VI) in plants: (a) roots and (b) leaves.

profiles, yet distinctive, as 150 mg/L correlated more with antioxidant and comet parameters, and 200 mg/L correlated with peroxidation and cell membrane degradation.

5. Discussion

Compared with animals and bacteria, a gap of information on Cr effects in plants still persists, and even less is known regarding the plants' response after chronical exposures. The observed reduction of the growth of shoots and roots was negatively correlated with the Cr content in those organs. This is in line with the literature, where Cr-associated growth reduction has been addressed and was also associated with a declined photosynthetic activity and nutritional disturbances for this and other species [3, 27].

As a redox metal, Cr can directly generate ROS such as $O_2^{-\bullet}$, H_2O_2 (a second messenger) $OH^{\bullet}$ and $^1O_2$, and oxidative injury via the Haber-Weiss and Fenton reactions, resulting in cell homeostasis disruption [3]. In this work, we elucidate how a long-term exposure to Cr(VI) leads to discriminative responses according to the organ and dose. We also evidence that the antioxidant enzymes are not stimulated similarly in the organs. Moreover, in roots, their response is variable, with all enzymes being particularly stimulated at 50 mg/L, and while some decreased thereafter, others (e.g., CAT) continued to be stimulated with Cr concentration. It should be stressed that 50 mg/L of Cr/VI) stimulated the antioxidant enzymatic battery and no major damage was visualized (e.g., genotoxic and or membrane permeability). Other doses seemed to already compromise some of these enzymes activities. Literature has shown that the antioxidant response to Cr exposure is complex depending on the dose, period, species, and parameters analyzed. For example, in maize plants exposed to ≤300 mM, H_2O_2, lipid peroxidation, and SOD and GPX activities increased regarding the controls [28], but *Camellia sinensis* L. showed lower enzymatic activity (decreased SOD, peroxidase, and CAT) [29]. Contrarily to 50 mg/L, the 150 mg/L exposed roots showed an evident increase of peroxidation and of the loss of membrane integrity. Besides being an indicator highly correlated with lipid peroxidation (as observed here) and eventually with cell death, the increase of CMP is also particularly relevant because, being the absorption of Cr facilitated by a carrier membrane, the increased accumulation of ROS will also compromise this membrane transport [28]. Besides, at the high Cr concentration, there is a correlation with a decrease of the mitotic index, supporting that the cell cycle progression is reduced or delayed. This is also supported by the blockages/delays found in the high Cr doses, as demonstrated by the increase of numbers in S phase and SPF. The decreased root growth due to Cr toxicity could be due to inhibition of root cell division/root elongation or extension of the cell cycle in roots [30, 31], as plant growth depends on both cell division and cell elongation. It is also evidenced here that Cr differently affected the plant phenology, again depending on the organ and dose.

Contrarily to our chronical exposure, several studies have been conducted with germinating/seedlings, while, like in another study, a tendency to occur a cytostatic effect is evident for high doses, which was supported by the decrease in MI in a dose-dependent way. For example, Fozia et al. [32] suggested that the decrease in root length of plants exposed to Cr was due to a *"cell cycle extension triggered by Cr toxicity."* Also, Cr reduced root growth of *Amaranthus* species, which was associated with the inhibition of cell division and oxidative stress [33, 34]. For *L. sativa*, Cr exposure showed a decrease of roots and aerial part length in high concentrations [6]. The Cr dose influenced the cell cycle dynamics, which was related to the DNA clastogenicity/fragmentation and with the delay in the cell cycle progression. In roots, a blockage at S (at expenses of mostly G_0/G_1) was seen for ≥150 mg/L, suggesting that cells suffer an arrest during/after S synthesis, preventing the cell from entering cell division thus limiting mitosis. This hypothesis was accompanied by the increase of comet assay parameters, supporting that higher Cr doses induced breaks in the genetic material as seen for other species [20]. Huang et al. [19] showed in tomato plants that during Cr-24 h exposure around 2,097 genes were more

responsive than those responsive to 1 h exposure and that some of those were involved in DNA repair. In roots, in the PCA right half where 150 and 200 mg/L are ranking, there is an evident correlation with S delay, seen in the SPF and PI, together with a correlation with degradative events such as the mitotic abnormalities, micronuclei, and CMP. Samples exposed to 200 mg/L also correlate more with both %TDNA and TL, suggesting higher damage in this concentration. Cr may interact directly with nucleic acids, forming DNA-DNA cross-links and DNA-protein [35]. On the other hand, ROS-induction by Cr may contribute to damage in DNA and fragmentation, specially targeting bases such as guanine, promoting fragmentations and mutations [36]. Also, Cr-induced chromosomal abnormalities will lead to mitotic disorders, thus increasing the micronuclei formation. Abnormalities, such as chromosomal bridge, may be due to sticky characteristics of chromosomes, which compromises their migration towards the cell poles during anaphase [37]. Also, Cr may bind the tubulin carbonyl groups, compromising its structure and function and thus affecting also cell division (e.g., loss of microtubule of spindle fibers).

As recently stressed by Shahid et al. [1] the Cr-induced toxic effects (cytotoxic, genotoxic, and mutagenic) vary according to the plant and organ, so Cr-induced toxicity remains an area to be explored.

6. Conclusions

In conclusion, DNA damage induced by metal stress may be originated by indirect processes, such as the increase of ROS or by direct interaction of the metal with DNA [18, 36] that may lead to breaks and mutations. This is the most comprehensive evidence in long-term Cr-exposed plant genotoxic effects using multiparametric data (flow cytometry, comet, and mitotic parameters), where it is also demonstrated that the response is dependent on the organ and dose. Comparing shoots and roots, these parameters show often more dramatic responses in roots, where defense batteries are particularly active at low Cr doses in roots, and degradation endpoints are evident for higher doses.

Data Availability

The data of this study is available upon request sent to the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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