Mineral nutrient imbalance, total antioxidants level and DNA damage in common bean (*Phaseolus vulgaris* L.) exposed to heavy metals

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Published online: 11 August 2013

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Abstract The present study aimed to analyze the biological effects induced by bioaccumulation of metals in common bean (*Phaseolus vulgaris* L.). Effects of mineral nutrient imbalance, total antioxidants level and DNA damage induced by accumulation of heavy metals, were investigated in bean seedlings treated with two selected metal concentrations for 7 days. Metal content is analyzed by inductively coupled plasma – atomic emission spectrometer (ICP-AES), for total antioxidants level assessment the Ferric-Reducing Antioxidant Power (FRAP) assay is used and Random Amplified Polymorphic DNA (RAPD) method was applied for investigation of DNA damages. The increasing metal concentration in the treatment medium changed synchronously metal content in samples, and decreased total antioxidant activity in all samples with exception only for samples treated with Ni and Cd. The obtained “DNA fingerprints” demonstrated that the increasing metal concentrations induced changes in RAPD profiles (disappearance and/or appearance of bands in comparison with untreated control samples). The highest number of missing bands was observed in samples treated with zinc (total 4 bands) and nickel (total 4 bands) at both concentrations. These results suggested that mineral nutrient imbalance is involved in changes of antioxidant levels and DNA damages of the seedlings, which may help to understand the mechanism of metal toxicity in plants.

Keywords Genotoxicity · RAPD · FRAP · Heavy metals · *Phaseolus vulgaris*

Introduction

Heavy metal stress in all living organisms often results in the production of reactive oxygen species (ROS), which are relatively reactive compared to molecular oxygen and thus potentially toxic (Cargnelutti et al. 2006; Van Assche and Clijsters 1990). A regulated balance between oxygen radical production and destruction is achieved by the plant antioxidative system that includes enzymatic molecules such as superoxide dismutase (SOD; enzyme classification [EC] number 1.15.1.1), ascorbate peroxidase (APX; EC 1.11.1.11), nonspecific peroxidases (POX; EC 1.11.1.7), and catalases (CAT; EC 1.11.1.6) and various antioxidants of low molecular mass (e.g., α -tocopherol and β -carotene) (Van Assche and Clijsters 1990; Dixit et al. 2001). The majority of the antioxidant activity of plants is due to the flavones, isoflavones, anthocyanin, coumarin lignans, catechins and isocatechins (Aqil et al. 2006). Because dietary plants contain several hundred different antioxidants, it would be useful to know the total concentration of electron-donating antioxidants (i.e. reductants) in individual items (Halvorsen et al. 2002). There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods have been substantially investigated and reported in the literature by various authors (Miller et al. 2000a, b). Tolerance to heavy-metal stress has been correlated with efficient antioxidative defense system, as shown by many authors (Van Assche and Clijsters 1990; Dixit et al. 2001; Verma and Dubey 2003). The amount of well-known antioxidants, such as α -tocopherol, vitamin C and β -carotene in

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dietary plants has been measured in detail. However, recent data may suggest that a relatively small part of the antioxidants in most dietary plants is contributed by the well-known antioxidants (Paganaga et al. 1999). Thus, the total amount of electron-donating antioxidants (i.e. reductants), derived from combinations of individual antioxidants that occur naturally in foods, may be a better concept than individual dietary antioxidants (Halvorsen et al. 2002). One of methods used to assess the total antioxidant capacity of plants is the Ferric-Reducing Antioxidant Power (FRAP) assay of Benzie and Strain 1996. There are several reasons for using FRAP assay: (i) The FRAP assay is the only assay that directly measures antioxidants or reductants in a sample; (ii) the other assays, but not the FRAP assay, use a lag phase type of measurement; (iii) pretreatment is not required, stoichiometric factors are constant and linearity is maintained over a wide range (Halvorsen et al. 2002). A lot of information is available on the effect of metals on various antioxidant processes in plants (Van Assche and Clijsters 1990; Verma and Dubey 2003; Hou et al. 2007; Radić et al. 2009; Israr et al. 2006). One possible mechanism, in which elevated concentrations of heavy metals may damage plant tissues, is the stimulation of free radical production by imposing oxidative stress (Foyer et al. 1997). Heavy metals like copper and iron can be toxic because of their participation in redox cycles producing hydroxyl radicals ($\text{OH}^\bullet$) which are extremely toxic to living cells (Stoch and Bagchi 1995). By contrast with those metals, Cd is a non-redox metal that is strongly phytotoxic and causes growth inhibition and plant death, produces alterations in the functionality of membranes, damage the photosynthetic apparatus (Quariti et al. 1997; Sidlecka and Baszynsky 1993). Heavy metals also induce several cellular stress responses and damage to different cellular components such as membranes, proteins and DNA. Metals constitute one of the major groups of genotoxic environmental pollutants possessing serious threat to human as well as environmental wellbeing. Genomic protection of our biota from environmental or global pollution is the key for conservation of Earth's biodiversity. Recently, advances in molecular biology have led to the development of a number of selective and sensitive assays for DNA analysis in ecogenotoxicology. Advantages of measuring effects of genotoxic chemicals directly on DNA are mainly related to the sensitivity and short response time. DNA based techniques like Random Amplified Polymorphic DNA (RAPD) is used to evaluate the variation at the DNA level and can clearly be shown when comparing DNA fingerprints from untreated and treated individuals to genotoxic agents (Savva 1998; Atienzar and Jha 2006; Enan 2006; Kekec et al. 2010).

Common bean (*Phaseolus vulgaris* L., Fabaceae) was chosen as the object of this study because it is a widespread crop plant and is frequently used as a model plant. The objective of the present study was to investigate the changes of mineral nutrients and potential genotoxicity, and thus

explore the possible direct DNA damages and changes in endogenous antioxidants level in the plant. To evaluate the capacity of the tolerance mechanisms of plants to metal contamination in the environment, bean seeds were exposed to two different concentrations of heavy metals for 7 days.

Materials and methods

Plant material and growth conditions

The common bean, *Phaseolus vulgaris* L. was used as plant material. This plant is exposed to heavy metals in its natural environment as a result of various human activities. Bean is a diploid ($2n=22$) and it has been widely used in physiological and molecular analysis in toxicology (Enan 2006; Kekec et al. 2010). Seeds were surface-sterilized with 75 % (v/v) ethanol for 5 min, followed by 10 % (m/v) sodium hypochlorite for 10 min. Thereafter, they were thoroughly rinsed with tap water and then soaked for 1 h in distilled water at 25 °C. The seeds were germinated in a sterile glass jar with water-saturated cotton at 25 ± 2 °C under dark condition and left to grow until the roots reached 3–5 mm in length. Subsequently, five plant seeds were transferred to sterile vitro containers containing liquid medium (Murashige and Skoog 1962) (untreated control treatment) or supplemented with $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$, NiSO_4 , $\text{Cd}(\text{NO}_3)_2$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ as treatment solutions (metal enriched). The seeds were treated with solutions of heavy metals at concentrations of 150 and 350 mg l^{-1} for 7 days. In ecotoxicological testing, it is always very important to use doses of the studied substance that are not lethal but that are sufficiently high to enable the observation and characterization of a specific reaction of the biological system to the tested stressor. After treatment, bean seeds were frozen at -20 °C until analysis. Element analysis, FRAP analysis and DNA extraction were performed.

Growth inhibitory test

Growth inhibitory test on root length was performed with the above bean seeds exposed to 150 and 350 mg l^{-1} of selected metals, for 6 days respectively. Twenty seeds were placed in Petri plates ($90 \times 15 \text{ mm}^2$) containing filter paper soaked with basal liquid medium (for the control) or treatment metal enriched solutions. Petri plates were incubated in a growth chamber at temperature of 23 ± 1 °C and a 15-hour-day-9-hour night photoperiod with a light intensity $190 \mu\text{Em}^{-2} \text{ s}^{-1}$. After 6 days of incubation, the root length were measured (Liu et al. 2005). Inhibitory Rate (IR, %) was calculated by the formula $(1-x/y) \times 100$, where “x” was average value detected in samples (treated with different metals) and “y” was one detected in the control sample.

Element analysis of the bean seeds

Seeds (treated and untreated samples), were de-frozen and dried in oven (Instrumentaria/Zagreb ST-01/02) on 105 °C for 1 h. The hulls were removed to prevent increasing of results from metals deposited on the external hull of the seed and then seeds were grind with pestle until homogeny mixture was obtained. 0.5 g were weighed and placed into PTFE vessels with 7 ml HNO₃ (69 % Merck, Tracepur) and 1 ml H₂O₂ (30 %, m/V; Merck), mixture was left at room temperature for 1 h and then mineralized by microwave (MARS CEM XP 1,500) with two steps procedure at 200 °C. Digests were filtered on filter paper (Munkteit), quantitatively transferred in 25 ml calibrated flasks, diluted with demineralized water and analyzed by ICP-AES (Varian715-ES) for selected metals. All results were calculated on a dry weight basis (mg Kg⁻¹ dw).

FRAP assay

Seeds (treated and untreated samples), were de-frozen and grind with pestle with certain amount of water to obtain a proper viscosity for pipetting. Methanol (9 ml) was added to 1 ml of this homogenate, and the samples were mixed and extracted for 4 h at room temperature by shaking vigorously in shaker (Vibromix 312 EVT, Tehnica). The sample suspensions were centrifuged (10,000 g for 15 min), supernatant was filtered through Whatman No.1 filter paper and the filtrate stored at -20 °C till analysis. Samples were analyzed for total antioxidants by FRAP assay of Benzie and Strain 1996, using microplate reader (ChemWell) at 600 nm. The antioxidant status is expressed as $\mu\text{mol FeSO}_4 \text{ l}^{-1} \pm \text{SD}$ from three measurements.

RAPD amplification methods

DNA extractions were performed using REDEExtract-N-Amp Seed PCR Kit (Sigma-Aldrich) following the instructions of the manufacturer. Extract is stored at 2–8 °C until use. PCR reactions were performed in reaction mixtures of 20 μl containing 10 ng of genomic DNA, 0.4 μM primer (Sigma-Aldrich) and 10 μl REDEExtract-N-Amp PCR Reaction Mix. The REDEExtract-N-Amp PCR Reaction Mix is a ready mix containing buffer, salts, dNTPs, and REDTaq DNA polymerase. Sequence (5'→3') for primer (with 60–70 % GC content) used is GGTGCGGGAA; Amplifications were performed in a DNA thermocycler (Mastercycler personal, Eppendorf). After amplification, electrophoretic separation of RAPD reaction products was performed in 2 % (w/v) agarose (Agarose 1,000; Invitrogen) using a TBE (Tris/borate/EDTA) buffer system (1×TBE=90 mM tris base, 90 mM boric acid and 2 mM EDTA). DNA bands were stained with ethidium bromide for 10 min, visualized and photographed under UV light (Biometra).

Statistics

The marked changes observed in RAPD profiles (disappearance and/or appearance of bands in comparison with untreated control samples) were evaluated. The presence and absence of each band was determined by making a binary matrix (1 for band presence and 0 for absence) for every sample. Numerical analysis based on banding pattern obtained from treated samples was compared with the untreated sample (control) via hierarchical cluster analysis. A dendrogram was constructed by the between-groups linkage method using NTSYSps (Numerical Taxonomy and Multivariate Analysis System) program with SAHN module (Rohlf 1994).

Genomic template stability (GTS, %) was calculated as following:

$$\text{GTS} = (1 - a/n) \times 100$$

Where, a is the total number of polymorphic bands detected in each metal treated sample and n the number of total bands registered in the control sample. Polymorphism in RAPD profiles included disappearance of a normal band and appearance of a new band in comparison to the control.

Results and discussion

Phaseolus vulgaris L. (common bean) represent the most important source of protein for low-income populations in Latin America and in Africa, and Brazil is its largest producer and consumer worldwide. It is widely grown in developed countries, and is the second most important legume crop, after soybean, consumed worldwide (Broughton et al. 2003). The phytotoxicity of metal-enriched solutions, before and after plant exposure, was assessed using 6-day root growth inhibitory test (results in Table 1). Treatment of bean seeds with 150

Table 1 Growth inhibitory test on root length of plant seeds exposed to selected metals

Metal	Metal concentration (mg l ⁻¹)	Inhibitory Rate (%)
Cd	150	38
	350	47
Cu	150	18
	350	30
Mn	150	18
	350	50
Ni	150	44
	350	59
Pb	150	68
	350	71
Zn	150	14
	350	28

and 350 mg l⁻¹ of toxic metals (CuSO₄ · 5H₂O, MnSO₄ · H₂O, Pb(NO₃)₂, NiSO₄, Cd(NO₃)₂ and ZnSO₄ · 7H₂O) for 7 days, resulted with an increase in the contents of Cu, Mn, Pb, Ni, Cd and Zn and changes in the total antioxidant level. Results from elemental analysis are presented in Fig. 1. Results for total antioxidants level in samples treated with toxic metals and untreated sample (control) obtain with FRAP assay in μmol FeSO₄ l⁻¹ are presented in Table 2.

As shown in Fig. 1, varying amounts of metal contents were noted. Contents of metals in seeds changed synchronously with increasing metal exposition in all samples. Samples exposed to 350 mg l⁻¹ of toxic metals, accumulated large amounts of metals, level was approximately from 23.18 times higher (for Mn) to 17840 times higher than the control (for Cd). For samples exposed to 150 mg l⁻¹ of toxic metal salts, was observed also higher Cd accumulation, 5,100 times higher than the control. The results presented in Fig. 1, shows that Pb is metal that is less accumulate by seeds of the investigated plant. This fact is previously noted in other similar studies. Pb uptake studies in plants have demonstrated that plant roots have an ability to up take significant quantities of Pb while there is restriction for its translocation to above ground parts (Lane and Martin 1977; Baker et al. 1994; Kumar et al. 1995). Pb moves predominantly into the root apoplast and accumulates near the endodermis which acts as a partial barrier to the movement of Pb between the root and shoot. In the study of Verma and Dubey 2003, in rice (*Oryza sativa* L.) seedlings raised in sand cultures in nutrient medium containing 500 and 1,000 μM Pb(NO₃)₂ for 10 and 20 days, root growth was reduced up to 42 % compared with 25 % reduced shoot growth and also absorbed Pb was to 3.3 times higher in roots compared to shoots. The content of Pb in different plant organs tends to decrease in order: roots>leaves>stem>inflorescence>seeds; and can vary with plant species (Antosiewicz 1992; Yoon et al. 2006). In other study is proposed that inhibition of shoot growth in corn seedlings is not a consequence of Pb accumulation but due to unknown

Fig. 1 Elemental analysis in bean seedlings after treatment with appropriate toxic metals at concentrations of 150 and 350 mg l⁻¹ for 7 days (in mg Kg⁻¹ dry mass)

signal induced in roots as a response to Pb exposure (Malkowski et al. 2002). Plant biochemical tolerance to Pb is related to capacity to restrict Pb to the cell walls, synthesis of osmolytes and activation of the antioxidant defense system (Sharma and Dubey 2005). This is in correlation with results for high GTS for the samples treated with Pb and the lowest FRAP value obtained in this study. Previously was published that high Pb concentration also induces oxidative stress by increasing the production of ROS in plants (Reddy et al. 2005).

This results for plant Pb accumulation are in correlation with results obtained for IR on root growth for investigated metals, where Pb is metal where highest IR was noted (Table 1). With this test, heavy metals phytotoxicity was put into the evidence and some findings from this test were similar to those reported by different authors (Wong and Bradshaw 1982; Benzarti et al. 2008) who found that the order of heavy metal toxicity for root growth in *T. caerulea* was as follows: Cu>Ni>Mn>Pb>Cd>Zn>Al>Hg>Cr>Fe. In other study, Wang and Zhou 2006, confirmed high inhibition of root elongation by direct contact with Cd ions, similar to the obtained result here with 38 and 47 % IR in samples treated with Cd in two different concentrations.

RAPD profile generated by treated samples was different from those obtained using control DNA. The RAPD profile obtained from used oligonucleotide primer is presented in Fig. 2.

Events observed following the metal exposure were a variation in the disappearance and appearance of new bands. The samples treated with 350 mg l⁻¹ of toxic metals yielded a large number of new fragments (total 11) compared with total number of new fragments (total 5) at 150 mg l⁻¹. Similarly, the total number of disappeared fragments was 7 at 350 mg l⁻¹, whereas at 150 mg l⁻¹, the number of disappeared bands was 5 bands. The highest number of missing bands was observed in samples treated with zinc (total 4 bands) and nickel (total 4 bands) at both concentrations. The highest number of appearance of new fragments (total 3) is recorded in DNA samples treated with copper at concentration of 350 mg l⁻¹. In total, 64 bands were scored from which 43.75 % were polymorphic. Polymorphism (P, in %) was calculated as following:

$$P = [(a + b)/c] \cdot 100$$

Where, *a* is the number of new bands detected in samples (different from the control), *b* is the number of disappeared bands and *c* is the total number of scored bands.

Dendrogram (Fig. 3) constructed using NTSYSps program, showed that the control group merged with group treated with MnSO₄ · H₂O (150 mg l⁻¹) in a separate cluster. These groups are linkage with all other samples treated with metals at concentrations 150 mg l⁻¹ and Pb(NO₃)₂ and

Table 2 Total antioxidants level in bean seedlings obtained with FRAP assay after treatment with appropriate toxic metals at concentrations of 150 and 350 mg l⁻¹ for 7 days (in $\mu\text{mol FeSO}_4 \text{ l}^{-1} \pm \text{SD}$)

Metal	Metal salt used	FRAP values in bean seedlings (in $\mu\text{mol FeSO}_4 \text{ l}^{-1}$)	
		Treated at 150 mg l ⁻¹	Treated at 350 mg l ⁻¹
control	/	92.98 $\pm$ 0.2	
Cu	CuSO ₄ ·5H ₂ O	74.2 $\pm$ 0.26	80.34 $\pm$ 0.34
Mn	MnSO ₄ ·H ₂ O	75.98 $\pm$ 0.17	85.98 $\pm$ 0.18
Pb	Pb(NO ₃) ₂	70.86 $\pm$ 0.34	70.52 $\pm$ 0.51
Ni	NiSO ₄	98 $\pm$ 0.2	110.15 $\pm$ 0.13
Cd	Cd(NO ₃) ₂	113.94 $\pm$ 0.22	95.06 $\pm$ 0.31
Zn	ZnSO ₄ ·7H ₂ O	86.98 $\pm$ 0.19	56.96 $\pm$ 0.24

Cd(NO₃)₂ at concentrations at 350 mg l⁻¹. Finally, samples treated with metals at concentrations 350 mg l⁻¹ together with NiSO₄ at 150 mg l⁻¹, clustered alone and differentiate as samples for which the highest genotoxic effects are noted. Results for GTS, are presented at Table 3.

Being a way of qualitative measurement of genotoxic effects, GTS is related to the level of DNA damage and also efficacy of DNA repair and replication (Atienzar et al. 1999; Liu et al. 2005; Cenkci et al. 2010; Erturk et al. 2012). GTS was used for comparing the changes in RAPD profiles, where generally in this study, GTS values tended to decrease with increasing concentrations of metal treatments. The values are in the range of “no changes” in the RAPD profile (GTS 100 %) for treatment with Mn 150 mg l⁻¹ (placed in the same cluster with control sample, Fig. 3) to GTS 0 % for samples treated with Ni and Zn in concentration 350 mg l⁻¹. The results for Zn treatment correspond to the results obtain in the study of Erturk et al. 2013. In this study the authors determine genotoxic effects of boron and zinc on *Zea mays* seedlings using RAPD analysis, and for GTS they obtain values in the range of 14.3 to 100 % for selected concentrations of 5, 10, 20 or 40 mM zinc used. The same group of authors in another study (Erturk et al. 2012) determinates effects of Ni in different concentrations (5, 10, 20

or 40 mM) on the GTS values in *Zea mays* seedlings and the obtained values are in the range from 11.1 to 100 % in comparison with the control sample. Another study (Taspinar et al. 2011) also shows that after Cd treatment, GTS and RAPD profiles changes and increased Cd concentrations caused decreasing GTS value and increasing polymorphism values. Similar results for GTS were obtained in the study of Liu et al. from 2005, where also effect of cadmium pollution in barley (*Hordeum vulgare*) seedlings is investigated using RAPD analysis. These genomic instabilities in RAPD patterns reflect DNA damaged induced by genotoxins as heavy metals are. These changes are consistent with electrophoretic analysis. It is considered that the alterations in RAPD profiles due to genotoxic agents reflect the changes in GTS.

The ability of plants to increase antioxidative protection to combat negative consequences of heavy metal stress appears to be limited. There are a lot of studies showed that exposure to elevated concentrations of redox reactive metals resulted in decreased and not in increased activities of antioxidative defense system besides fact that heavy metal toxicity is reported to increase the activity of enzymes (Nagajyoti et al. 2010)). This fact is also valid for bean, *Phaseolus vulgaris* L., as previously shown in studies of many authors

Fig. 2 RAPD profiles of bean seedlings treated with metals at 150 mg l⁻¹ (Cu1, Cd1, Ni1, Pb1, Mn1 and Zn1) and 350 mg l⁻¹ (Cu3, Cd3, Ni3, Pb3, Mn3 and Zn3), compared with untreated sample – control (C); M is DNA marker;

Fig. 3 Dendrogram based on DNA polymorphism among bean seedlings exposed to 150 and 350 mg l⁻¹ of toxic metals compared to untreated control sample

(Shainberg et al. 2000; Chaoui et al. 1997; Weckx and Clijster 1996). Results obtained from FRAP assay in this study, shows that heavy metals induces oxidative stress in experimental model system which is evident from antioxidant levels (in $\mu\text{mol FeSO}_4 \text{ l}^{-1}$), as in all cases levels for total antioxidant activity in samples treated with metals in both concentrations are lower than total antioxidant level in control sample, with exceptional for samples treated with Ni and Cd as non-redox metals. In average, the samples treated with metals in concentrations 150 mg l⁻¹ shows for 17.2 % lower antioxidant activity from the control sample, while for the samples treated with 350 mg l⁻¹ this percentage is 21.23 (Table 4). As FRAP assay measures only non-enzymatic

(reductants) antioxidants in the sample, there is an interesting relationship among metal content and obtained FRAP value, valid for all investigated metals which are redox metals. There is a study on strawberry cultivars where results shows that samples possessed low FRAP values, have a relatively high polyphenol content (Wu et al. 2004). This variable correlations can be explained by a range of influencing factors, including the diversity among samples, variable reaction kinetics, presence and activity of enzymes responsible for mediating oxidative stress, (Jaio and Wang 2000; Wu et al. 2004; Ozgen et al. 2006) and etc. In the samples treated with Ni and Cd as non-redox metals, correspondingly to previously published facts we detected high levels of

Table 3 Genomic template stability

Genomic template stability											
Cu150	Cu350	Cd150	Cd350	Ni150	Ni350	Pb150	Pb350	Mn150	Mn350	Zn150	Zn350
75 %	25 %	50 %	50 %	25 %	0 %	75 %	50 %	100 %	25 %	25 %	0 %

Table 4 Differences in total antioxidants level in bean seedlings obtained with FRAP assay after treatment with appropriate toxic metals at concentrations of 150 and 350 mg l⁻¹ for 7 days

Samples treated with heavy metals	Differences in total antioxidants level in bean (%) compared to control sample (100 % total antioxidants)	
	150 mg l ⁻¹	350 mg l ⁻¹
Cu	20.4	14.0
Mn	18.28	7.5
Pb	23.7	24.7
Ni	105.4	118.3
Cd	122.6	102.2
Zn	6.4	38.7
Average for all samples:	17.2 %	21.23 %

FRAP values (Quariti et al. 1997). There is correlation between results obtain for Ni and Cd in our study (high FRAP values, highest number of missing bands in RAPD profile and lowest GTS) with a study from 2010 (Wan-Ibrahim et al. 2010) where author's associated a high antioxidant level in edible plants with high genotoxic properties.

In eco-genotoxicology, RAPD technique, as PCR based technique, has been successfully used to detect DNA damage and mutations in plants induced by various types of toxic chemicals (Enan 2006; Kekec et al. 2010; Cenkci et al. 2009). The RAPD, PCR based assay described here is fast, reliable, and easy to conduct in any laboratory for assessment of environmental hazardous metals on plants. Each fragment in RAPD is derived from a region of the genome that contains two short segments in inverted orientation on opposite strands that are complementary to the primer and sufficiently close together for the amplification process (Hon et al. 2003). These unique bands clearly differentiated the samples treated with different metals, and would be act as marker for assessment of environmental doses of these metals. Generally, based on results from this study, the low element contents may lead to the low level of genotoxic effects of bean seedlings. In our study, the number of lost bands was found higher than that of extra bands. The disappearance of normal bands may be related to the DNA damage (e.g. single-strand breaks, double-strand breaks, modified or oxidized bases, bulky adduct), point mutations and/or complex chromosomal rearrangements induced by genotoxic chemicals (Atienzar and Jha 2006). The highest number of disappeared bands that was observed with zinc and nickel at both concentrations suggests that these metals maybe cause one of these changes to DNA of the treated plants that consequently resulted in the disappearance of DNA bands. Nickel's genotoxic effect was previously examined on the somatic cells of *Vicia faba*, and it revealed that significantly inhibited the mitotic index and induced frequent chromosomal and mitotic aberrations in a

dose-dependent manner (Chandra et al. 2004) as also in the study of Al-Qurainy 2009, where similar results were obtain. The appearance of new bands was also detected. New RAPD amplification products may be related to mutations (new annealing events), large deletions (bringing to pre-existing annealing site closer), and/or homologous recombination (two sequences that match the sequences of primer). The highest number of new appeared bands that was observed with copper at 350 mg l⁻¹, suggests that this metal probably cause mutations on genomic level in high doses. In the study, previously described by Rank and Nielsen 1998, wastewater sludge's which were heavy metals contaminated, *Allium cepa* was analyzed for genotoxicity, and it was found that these heavy metals (Pb, Ni, Cr, Zn, Cu) induced significant chromosome aberrations. Nickel and zinc were metals for which we detect the highest level of DNA damage (GTS only 25 % at concentration of 150 mg l⁻¹ for both metals), and for nickel we noted increasing level of total antioxidants in samples which probably is associated with non-redox activity of Ni.

It may be noted that the nutrient imbalance leads to DNA damages, mutations on genomic level in case of *P. vulgaris* L. and also effects plant antioxidative defense system which contributed to the toxic effects in plants exposed to the high metal concentrations.

Conclusions

In summary, this study has shown that heavy metals can decrease total antioxidants level and induce DNA damage in plants. The obtained unique bands clearly differentiated the samples treated with different metals, and would be act as marker for assessment of environmental doses of these metals. The changes occurring in RAPD profiles of the plant, can be successfully used as a sensitive tool for detecting metal-induced DNA damage and showed potential as a reliable assay for genotoxicity. Genomic targets of metals exposure should further be assessed with systematic sequencing to make RAPD-PCR assay a quantification method rather than a qualification method.

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